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PARAPHYLY OF BRYOPHYTES AND CLOSE RELATIONSHIP OF HORNWORTS AND VASCULAR PLANTS INFERRED FROM ANALYSIS OF CHLOROPLAST rDNA ITS (cpITS) SEQUENCES

ПАРАФИЛЕТИЧНОСТЬ МОХООБРАЗНЫХ И БЛИЗКОЕ РОДСТВО АНТОЦЕРОТОВЫХ И СОСУДИСТЫХ РАСТЕНИЙ: ДАННЫЕ АНАЛИЗА ПОСЛЕДОВАТЕЛЬНОСТЕЙ ХЛОРОПЛАСТНЫХ СПЕЙСЕРОВ рДНК

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Abstract

Phylogenetic analysis of nucleotide sequences of chloroplast rDNA internal transcribed spacer (cpITS) regions (cpITS2-4) of 14 species of liverworts, 4 species of hornworts, 20 species of mosses, 7 species of lycopods and 2 species of algae was carried out. Phylogenetic trees constructed by maximum parsimony, maximum likelihood and neighbour-joining methods indicated that bryophytes are not monophyletic. The cpITS data suggest that the hepatic lineage branches most deeply in the land plant topology and that mosses are monophyletic, forming the sister group of lycopods and hornworts. Within the mosses, a conspicuous deletion with distinct phylogenetic distribution was observed in the cpITS3 region. This deletion is absent in other land plants, including the enigmatic genus *Takakia,* and marks *Takakia* as an evolutionary lineage distinct from mosses.

Резюме

Проведен филогенетический анализ нуклеотидных последовательностей спейсеров хлоропластной рДНК (cpITS2-4) у 14 видов печеночников, 4 видов антоцеротовых, 20 видов мхов, 7 видов плауновидных и 2 видов харовых водорослей. Согласно топологии филогенетических деревьев, реконструированных методами максимального правдоподобия, ближайшего связывания и максимальной экономии, мохообразные не монофилетичны. На кладограммах среди наземных растений первыми отделяются ветви, ведущие к печеночникам. Мхи предстают как монофилетическая группа, сестринская к кладе, объединяющей плауновидные и антоцеротовые. В нуклеотидной последовательности cpITS3 у мхов обнаружена протяженная делеция, отсутствующая у остальных наземных растений, включая *Takakia*.

Introduction

Until recently, bryophytes (plants in which the gametophytic stage dominates during their life cycle) most often were treated as the phylum Bryophyta and subdivided into three classes, Anthocerotae, Hepaticae and Musci. This phylum was generally considered as an evolutionary branch distinct from vascular plants, but beyond that there was little consensus concerning precise evolutionary affinities. In various schemes, bryophytes were viewed as sharing either a common or a different algal ancestor as vascular plants, they were viewed either as descendants or as ancestors of vascular plants. Furthermore,

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some authors defined bryophytes narrowly to include liverworts and mosses only, in which case bryophytes and hornworts were considered as independent branches stemming from a common ancestor with vascular plants. Alternative views hypothesized that two groups diverged early in land plant evolution: one including the hornworts and a separate lineage including mosses and vascular plants. As a further alternative, the various groups of bryophytes were sometimes considered as independent phyla originating by parallel evolution from various ancestors possessing isomorphic change of generation (for reviews of these various evolutionary scenarios, see: Meyer, 1958; Crandall-Stotler, 1984; Smith, 1986; Kenrick & Crane, 1997a).

Recent analysis of morphological and molecular data sets suggest that bryophytes are paraphyletic and placed at the base of land plant clade (Kenrick & Crane, 1997a; Qiu, Palmer, 1999; Mishler, 2000). Currently, many authors favor the notion that bryophytes share a common ancestor with an ancestral charalean stem, whereby the relationships among bryophytes and vascular plants still remain obscure.

The analysis of morphological and biochemical characters alone holds little hope of resolving bryophyte phylogeny. Paleobotanical data from the early phases of land plant diversification are comparatively poor in unambiguous characters and demonstrate rapid morphological diversification of the land flora pioneers. As a consequence, various groups of Devonian plants are difficult to assign unequivocally to either the bryophytes or the vascular plants on the basis of their preserved morphological traits (Edwards & al. 1995; Edwards & al. 1998; Wellman & al., 1998; Edwards & Axe, 2000).

Although DNA-systematics (genosystematics) has been applied to study the problem of early evolution of land plants in the end of the past century, the current data have yet to produce a unanimously supported view of early plant diversification. Molecular data are consistent with at least two competing hypothesis. Under the first view, hornworts would be the most ancient land plants and mosses (or mosses together with liverworts) form a clade sister to vascular plants (Malek & al., 1996; Beckert & al., 1999; Garbary, Renzaglia, 1998; Hedderson & al., 1998; Nishiyama, Kato, 1999; Renzaglia & al., 2000; Nickrent & al., 2000). The second hypothesis suggests that the basal land plant group includes liverworts, whereby the hornworts are the youngest representatives among the bryophytes and share a common ancestor with vascular plants (Lewis & al., 1997; Qiu & al., 1998). The view that hornworts are younger than liverworts and branched off form the main stem of land plant phylogeny before the divergence of mosses and vascular plants (Mishler, Churchill, 1984; Kenrick & Crane, 1997a; Graham & al., 2000) does not find support from recent DNA sequence analyses.

Molecular methods and the analysis of additional markers can help discriminate between these competing hypotheses. To obtain additional information on phylogenetic relationships of the most ancient land plants, we obtained and extended the set of molecular data by analyzed nucleotide sequences from the chloroplast rDNA internal transcribed spacers (cpITS2, cpITS3 and cpITS4), as well as of the 4.5S and 5S rRNA genes located in the same operon.

MATERIALS AND METHODS

DNA was isolated from fresh (in some cases dried) plant tissues. The list of analyzed species and GeneBank accession numbers for the corresponding ITS sequences are given in Table 1.

DNA was isolated by CTAB method (Murray, Thompson, 1980). Amplification and sequencing of the analyzed region was carried out as described earlier (Goremykin & al., 1996; Samigullin & al., 1998). Nucleotide sequences were aligned manually by using Sed program of the Vostorg package (Zharkikh & al., 1990). Sites with dubious positional homology were excluded from phylogenetic analysis. The resulting data matrix was analyzed by programs Tree-puzzle (Strimmer, von Haeseler, 1996) and PAUP* version 4.0b8 (Swofford, 2000).

Maximum parsimony (MP) analysis involved a heuristic search conducted with PAUP* using TBR (tree-bisection-reconnection) branch swapping with character states specified as unordered and equally weighted. 100 random addition replicates were performed. In the parsimony analyses all gaps were treated as missing data.

Bootstrap analyses (Felsenstein, 1985) were performed to assess the degree of support for

particular branches on the trees. Bootstrap values were calculated from 100 replicate analyses with TBR branch swapping and 5 replicates of random addition sequence of taxa. 5000 most parsimonious trees from each replicate were saved.

Maximum likelihood (ML) phylogeny estimation was explored using the Tree-puzzle program. The Tamura-Nei (1993) model of substitutions with eight Gamma rate categories and approximate parameter estimation was used.

Distance trees were calculated using the neighbor-joining (NJ) method (Saitou, Nei, 1987) as it implemented in PAUP*. The Tamura-Nei (1993) model of substitutions was used. Rates across sites were assumed to follow gamma distribution. 1000 bootstrap resamplings were performed. Insertions and deletions were not taken into account.

Results

The set of aligned sequences of cpITS2-4 includes sequences of 20 species of mosses, 14 species of liverworts, 4 species of hornworts, 7 species of lycopods, and 2 species of Charales taken as outgroup. It had 966 sites employed in phylogenetic reconstruction, out of which 356 sites (36.8%) proved to be phylogenetically informative.

A significant number of insertions and deletions (indels) was found in the analyzed sequences. Some of them are rather long and typical of certain groups of species. For example, a deletion of 52 bases common to all jungermannian liverworts is observed in cpITS2. Another deletion common to all mosses was observed in cpITS3. However, this deletion did not include *Takakia* (Fig. 1), which some of the authors include within the mosses. Curiously, cpITS2 is completely deleted from the *Riella* sequence and the 4.5S rRNA gene sequence starts directly after the 23S rRNA coding region.

One of the prerequisites for methods of DNA-based phylogenetic inference to function properly is the homogeneity of the nucleotide composition across sequences. Thus, we had to exclude *Takakia* from our analysis because the nucleotide composition of its ITS2-4 markedly differs from that of other species. Accordingly, phylogenetic trees constructed by NJ method using transversions (Galtier, Gouy, 1995) had a very low resolution. Excluding *Takakia*, the nucleotide composition of cpITS2-4 of the analyzed plants appears to be rather uniform and thus suitable for phylogenetic analysis. Tamura-Nei distances between all the pairs of sequences were lower than 0.5, and transition / transversion ratio was 2.9.

Phylogenetic trees constructed by ML, NJ and MP methods are presented in Figs. 2-5. These topologies are not completely identical, but the relative position of the main bryophyte groups is constant across trees, irrespective of the reconstruction algorithm and the method of tree optimization used.

In all trees, mosses, hornworts and lycopods form a clade with high bootstrap support (94%, 82%, 75% for ML, NJ and MP trees, respectively).

Among mosses the basal position is occupied by *Sphagnum* and *Andreaea*. *Buxbaumia*, *Polytrichales* (*Atrichum*, *Pogonatum*, *Polytrichum*) and *Tetraphis* branch off subsequently. Other mosses form a cluster with a lower bootstrap support (56%, 75%, 66%). Low number of nucleotide substitutions observed inside this cluster do not permit further resolution with this marker.

The clade including hornworts and lycopods appears to be the sister to aforementioned clade (bootstrap support 68%, 88%, 72%). Among hornworts, the basal position is occupied by *Anthoceros* (86%, 100%, 100%), whereby *Notothylas* is the closest to *Phaeoceros* (96%, 77%, 100%).

Marchantialean and jungermannialean liverworts occupy basal positions among land plants in these trees. Relationships of these groups are not well resolves, nor are relationships of lower taxa within these groups with the present data.

Discussion

Morphological characters of recent bryophytes seem to be insufficient for conclusively resolving their phylogeny, *inter alia* because interpretations of the advanced or primitive state of a given character in addition to its phylogenetic weight are very difficult judgements. The derived or primitive nature of the character «gametangia development» could serve as an example. Following Smith (1955) and Schuster (1966), many researchers are prone to think that antheridia and archegonia of *Haplomitrium* developing in one and the same manner represent the most primitive type of

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                    416                                                600
Chara               ---------AC---------- … CAGAA----TCAAAAG-------------------------
Nitella             --------------------- … CAAAA----TCAA-A--------------------------
Marchantia          AACAACTTTACCCTGC----- … CAGGG-TT-TCAA-AGGGG--------TTT---TTTT--CC
Conocephalum        AACAACTTGACCCTGC----- … CAGGG-TTTTCAA-AGGG---------TT----TTTTR--C
Preissia            AACAACTTTACCCTGC----- … CAGGG-TT-TCAA-AGGG---------TT----TTTTGGCC
Riccia              AACAACTTGACCCTGC----- … CAGGG-TT-TCAA-AGG----------CTT---TTTTG-CC
Sphaerocarpos       AACAACTTAACCCTGC----- … CAGGG-CG---AAGAGGG---------TT----CTTTG-CC
Riella              AATAACTTCACCCTGC----- … CAGGG-CG-T-AAGAGGG---------TT----CTTTG-CC
Aneura              -ACGGTT-AGCCCCG------ … CGGGG-C-AT-AGGAGG----------TT----------CC
Pellia              --------------------- … CAGG-----------------------CT----------CC
Ptilidium           AACAGTTTAGCCCTGCC---- … CAGGG-C-AT-GAGAGGG---------TC----ATTTC---
Trichocolea         ---------GCCCTGCC---- … CAGGG-T-AT-AAGAGG----------TT----CCTTC-TT
Blepharostoma       ---------ACCCTGCC---- … CAGGG-T-AT-AAGAGAA---------TC-----CTTC-TT
Ciloscyphus         ---------GCCCTGC----- … CAGGG-C-AT-AAGAGG----------CT----CCTTC-TT
Plagiochila         ---------ACCCTGCC---- … CAGGG-T-AT-AAGAGG----------TT----CCTT--CT
Lophocolea          ---------GCTCTGCC---- … CAGGG-C-AT-AAGAGG----------TT----TGTTT-CT
Calypogeia          ---------GCCCTGCC---- … CGGGG-T-AT-AAGAGG----------TT--------C-TT
Cephalozia          -------NNGCCCTGC----- … CATCA-ANNGGA--AG-----------CT------TAC---
Orthotrichum        AACAG---------------- … ---------------------------TT----CTCTG-CC
Rhodobryum          AACAG---------------- … ---------------------------TT----CTAAT-TC
Schistostega        AACAG---------------- … ---------------------------CT----CTAAT-TC
Homalia             AAAAG---------------- … ---------------------------TT----CTCTG-CC
Climacium           AACAG---------------- … ---------------------------TT----CTCTG-CC
Pleurozium          AACAG---------------- … ---------------------------TT----CTCTG-CC
Hylocomium          AACAG---------------- … ---------------------------TT----CTCTG-CC
Rhytidiadelphus     AACAG---------------- … ---------------------------TT----CTCTG-CC
Ceratodon           AACAG---------------- … ---------------------------CT----CTCTA-CC
Racomitrium         AACAG---------------- … ---------------------------CT----CTCTG-CC
Splachnum           AACAG---------------- … ---------------------------TT----CTCTG-TC
Plagyomnium         AACAG---------------- … ---------------------------TT----CTTTA-CC
Physcomitrium       AACAG---------------- … ---------------------------CT----CTCTG-CC
Funaria             AACAG---------------- … ---------------------------CT----CTCTG-CC
Tetraphis           AACAG---------------- … ---------------------------CT----CTCTG-CC
Polytrichum         AAAAG---------------- … ---------------------------CT----CTCTG-CC
Pogonatum           AAAAG---------------- … ---------------------------CT----CTCTG-CC
Atrichum            AAAAG---------------- … ---------------------------CT----CTCTA-CC
Buxbaumia           AACAA---------------- … ---------------------------CT----CTCTG-CC
Andreaea            AACAG---------------- … ---------------------------------CTCTG-CC
Sphagnum            AATAG---------------- … ---------------------------------CTCTG-CC
Takakia             AAAAGCTCCGCCCCGCC---- … CAGGG------CGGAGG-AGGG-----TT----CTCTG-CC
Anthoceros          AACAAGTCCGCCCTGAC---- … CAGCGG------AGAG-AAGGG-----TT----CTCTG-CC
Notothylas          AACAGCTCTGCCCTGAC---- … CAGGG------CAGAG-AAGGG-----TT----CTCTG-CC
Phaeoceros          AACAGCTCTGCCCTGAC---- … CAGGG------CAGAG-AAGGG-----TT----CTCTG-CC
Megaceros           AACAGCTCCGCCCTGAC---- … CAGGG------CAGAG-AAGGG-----TT----CTCTG-CC
Phylloglossum       AAAAGCTCCGCCCTGCC---- … CAGGG------CATAGG-AGGG-----TT----CTGTG-CC
H. selago           AAAAGCTCCGCCCTGCC---- … CAGGG------CATAGG-AGGG-----TT----CTGTA-CC
H. wilsonii         AAAAGCTCCGCCCCGCC---- … CAGGG-------ATAGG-AGGG-----TT----CTGTG-CC
H. billardieri      AAAAGCTCCGCCCTGCC---- … -AGGG-------ATAGG-AGGG-----TT----CTGTG-CC
H. cumingii         AAAAGCTCCGCCCTGCC---- … -AGGG-------ATAGG-AGGG-----TT----CTGTG-CC
H. phlegmarioides   AAAAGCTCCGCCCTGCC---- … CAGGG------CATAGG-AGGG-----TT----CTGTG-CC
Psilotum            AAGAGCTTCGCCCTGCC---- … CA-GGT-----C---GG-AGGAAGG--TT----CTCTG-TC
Pinus               AAAAGCTCTGCCCTCCC---- … CA--GT------GGAGGGA--------T-----CTCTA-CC
Cycas               AAAAGCTCTGCCCCGCC---- … CAGGG------CGGAGG-AGGG-----TT----CTCTA-CC
Zamia               AAGAGCTCTGCCCCGCC---- … CAGGG------CGGAGG-AGGG-----TT----CTCTA-CC
Gingko              AAAAGCTCTGCCTTGCC---- … CAGGG------CAGAGG-AGGG-----TT----CTCTA-CC
Ephedra             AAAA-------CCTGCC---- … T------------GA---AGGG-----TTCTCCCTCTA-CC
Nymphaea            AAAAGCTCTGCCCTACA---- … CA------------GAGGGAGG-----TT------------
Barclaya            AAAAGCTCTGCCCTACA---- … CA------------GAGGGAGG-----TT------------
Cabomba             AAAAGCTCTGCCCTACA---- … CA------------GAGGGAGG-----TT------------
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Fig. 1. Part of cpITS3 alignment.

gametangia among liverworts. Based upon the possession of *Haplomitrium*-type gametangia, Schuster considered Calobryales as the most primitive group among liverworts (Schuster 1966, 1984). However, he also noted, that "unfortunately, existing morphological evidence can often be read in two directions" (Schuster, 1966, p.257). Indeed, a different point of view on the ontogeny of *Haplomitrium*-type gametangia exists. Goebel (1902) demonstrated the homology of antheridia and archegonia and showed that during the development of the bryophyte archegonium there is a successive sterilization of fertile quadrants of young gametangia.Such a tendency could,in principle, be considered as a reduction (Meyer, 1958), but one also could argue that archegonia mature at an earlier stage of gametangium development than antheridia do. Presumably, progenesis played a more prominent role in the evolution of Calobryales and, according to Gould (1978), r-selection could facilitate progenesis occurring in environments lacking competitors, as could rapidly fluctuating ecological conditions. Notably, the preferred niche of *Haplomitrium hookeri* occurs precisely in such ecological conditions (Baczkiewicz, Szweykowski, 2001). Hence, similarity of antheridia and archegonia in Calobryales considered by Schuster and others as primitive characters could just as easily be the result of the increased rate of gametangium development during which antheridia mature at the same early phase of development as archegonia do. Furthermore, based on Schuster's hypothesis regarding the primitiveness of Calobryales, investigators of spermatogenesis who found an unusually broad spline composed of 57 microtubes in *Haplomitrium*, have initially came to the conclusion that the most primitive type of spermatozoids among liverworts is that of *Haplomitrium* (Carothers, Duckett, 1979; 1980; Duckett & al., 1982). But the same authors subsequently postulated that the most complex blepharoplasts could be found among Marchantiales, whereby "there is nothing in the morphology of spermatozoids which points to *Haplomitrium* as a possible starting point for hepatic evolution" and more probably, *Haplomitrium* might be considered as "the closest living survivor to a missing link between bryophytes and pteridophytes" (Duckett & al., 1983, p. 245).

A further, more recent example demonstrating the difficulty of polarizing morphological characters among bryophytes can be found. Renzaglia & al. (2000) state that "…in hornworts antheridial initial is located at the base of the schizogenous antheridial chamber, and not at the thallus surface as in other bryophytes. Apparently, in hornworts, an evolutionary shift in developmental potential has occurred from epidermal (layer surrounding the external surface) to epithelial (layer surrounding an internal space) cells…" (p. 776). As hornworts do not possess an epithelium proper, it is possible that molecular data suggesting a yet unsubstantiated basal position of hornworts in land plant phylogeny might have weighed more heavily than the widely accepted view that antheridia in hornworts are basically endogenous in origin.

In recent years nucleotide sequences have been used successfully in many studies of plant systematics and phylogenetics. However, the sobering experience of phylogenetic analysis indicates that the trees constructed from molecular data do not tend to differ less than those based on morphology. Such differences in topology of molecular phylogenetic trees can arise due to many factors (Wendel, Doyle, 1998), a detailed discussion of which lies outside the scope of this paper.

The transcribed spacers of chloroplast DNA ribosomal operon are located in inverted repeats and carry a substantial number of polymorphic sites. Their length is moderate, but for one and the same set of species they have twice as many informative sites than the sequences of nuclear 18S rDNA. From time to time it has been stated that such sequences are less informative in molecular phylogenetics (e.g. Kelchner, 2000), especially in the case of higher rank taxa because substitutions in such sequences accumulate faster than in coding sequences and thus saturation is reached at earlier stage of evolution and alignment of such sequences is often hampered by numerous indels. Although it is true that large numbers of indels versus substitutions makes alignment difficult, this does not impair alignment altogether. As to the saturation problem, its limit seems to be higher than it has been suggested earlier (Yang, 1998). Analysis of chloroplast

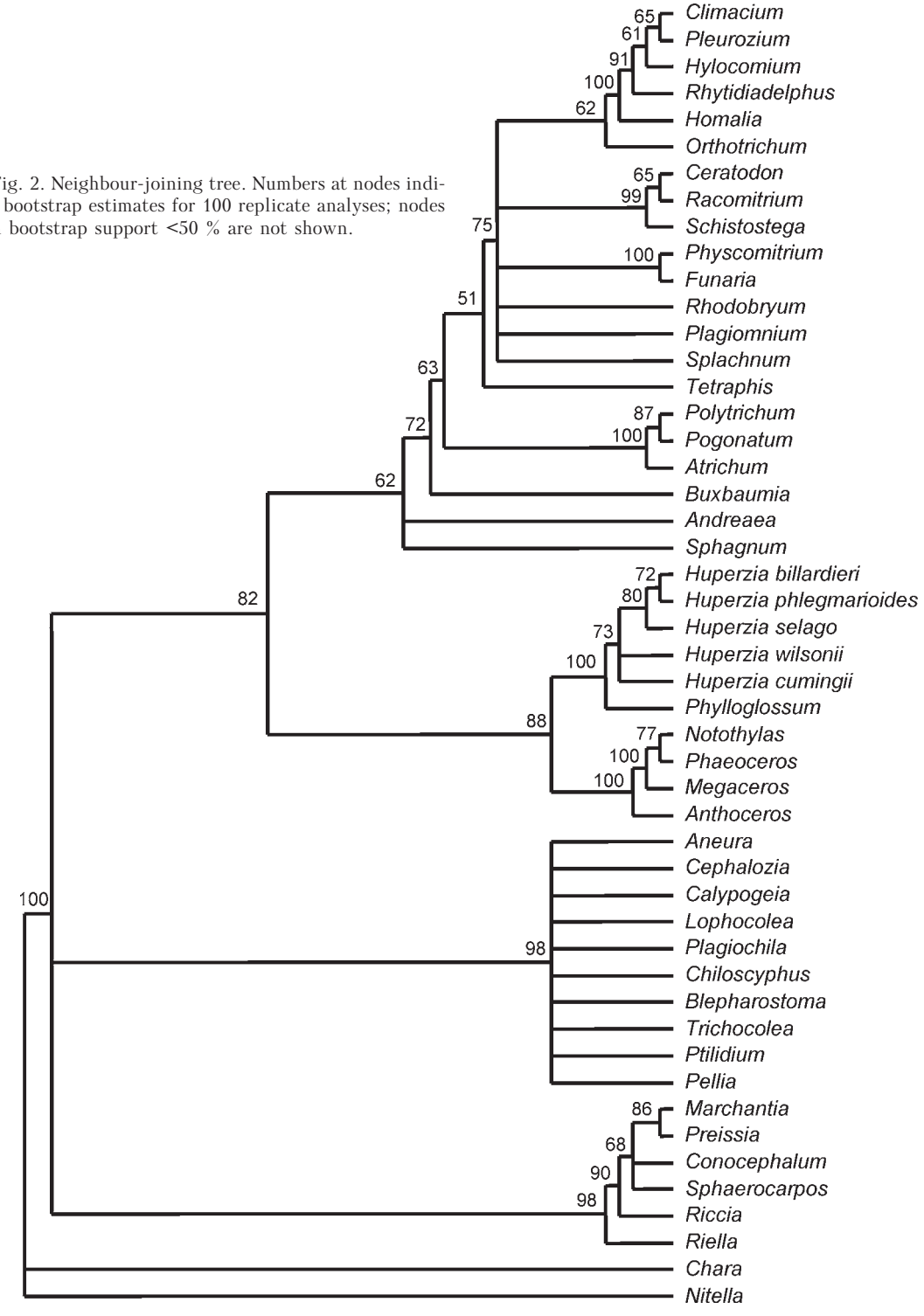

Fig. 2. Neighbour-joining tree. Numbers at nodes indicate bootstrap estimates for 100 replicate analyses; nodes with bootstrap support <50 % are not shown.

rDNA spacer evolution appeared to be informative in the studies of evolution of major taxa of land plants (Goremykin & al., 1996; Samigullin & al., 1998; Antonov & al., 2000), and many of the salient conclusions therein were subsequently substantiated by others.

One of criteria of selection of species analyzed in this study was the homogeneity of

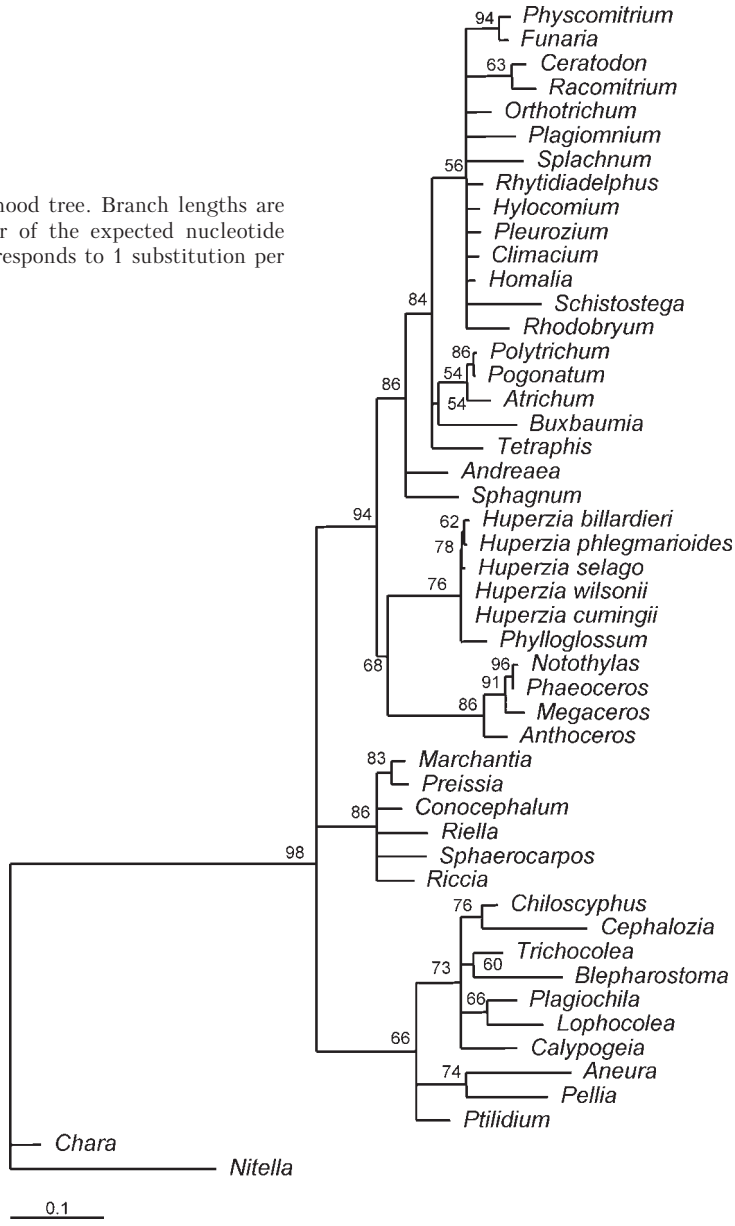

Fig. 3. Maximum likelihood tree. Branch lengths are proportional to the number of the expected nucleotide substitutions, scale bar corresponds to 1 substitution per 10 sites.

cpITS2-4 nucleotide composition. When gymnosperms and angiosperms were added, the relationships of bryophyte groups remain unaltered, but almost all mosses had to be excluded from analysis because of differences in nucleotide composition (data not shown). Uneven taxon sampling may also lead to selection of wrong tree topologies, therefore only lycopods, which are generally accepted as a basal group of monophyletic vascular plants, were studied and the lycopod lineage can be considered as a vascular plants lineage.

Bryophyte paraphyly and basal position of liverworts. Results of cladistic analysis of morphological and molecular data indicate that bryophytes are paraphyletic (see reviews Kenrick & Crane, 1997a; Qiu & Palmer, 1999; Mishler, 2000). The present cpITS sequence analysis also indicates that bryophytes are not monophyletic (Figs 2-5). The topology of trees constructed by NJ, ML and MP with bootstrap resampling methods does not resolve whether liverworts are monophyletic or not (Figs. 2, 3, 5), whereas mosses and hornworts are clearly

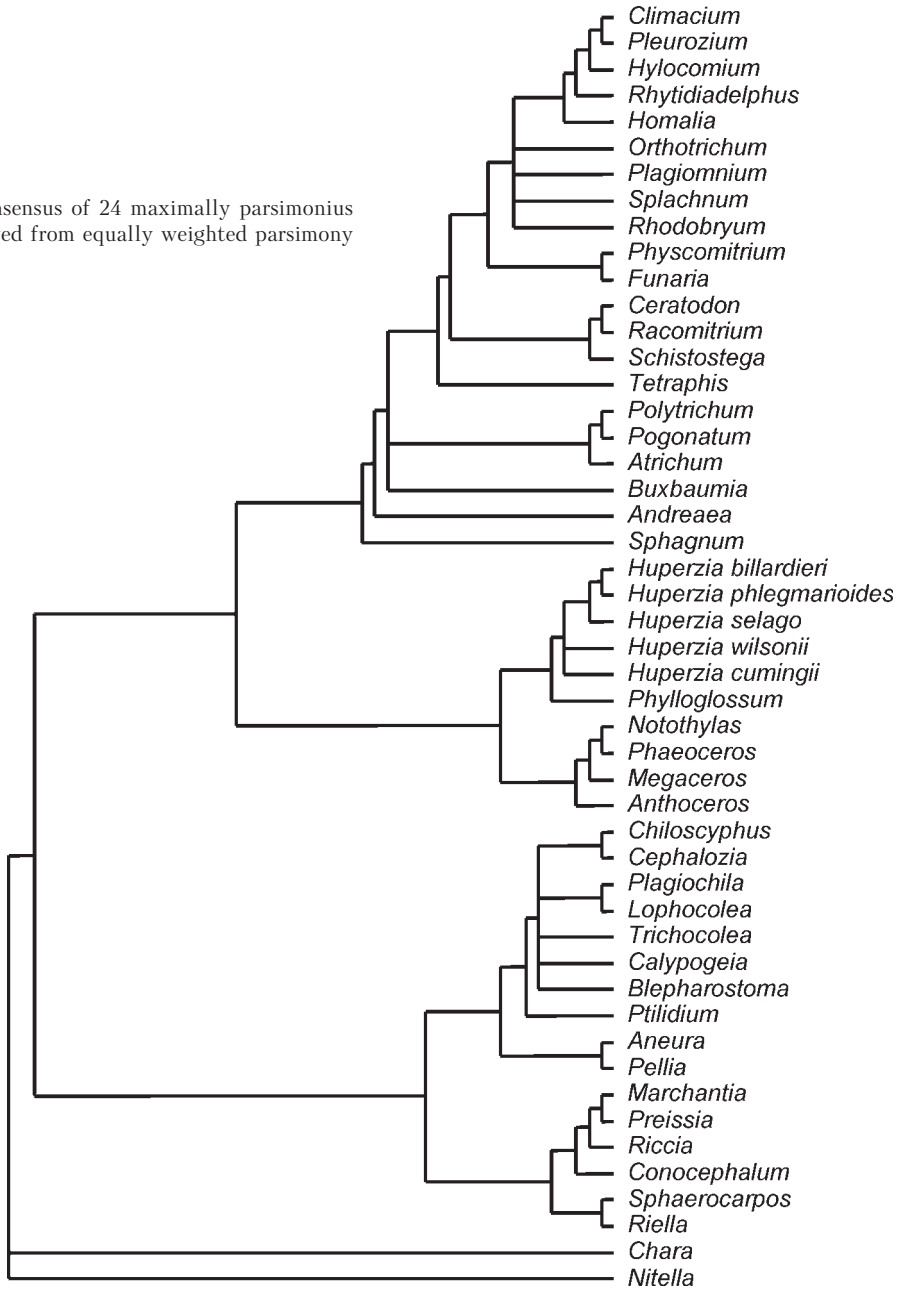

Fig. 4. Strict consensus of 24 maximally parsimonius 1284-step trees derived from equally weighted parsimony analysis.

monophyletic (Figs. 2-5) with this data. In our previous analysis of a smaller number of species, liverworts formed a monophyletic group (Samigullin & al., 1998), whereas the *rbc*L and 18S rDNA analysis indicate that liverworts are paraphyletic (Bopp, Capesius, 1986; Lewis & al., 1997). Anyway, our data, as well as the data obtained in cladistic analysis of morphological characters (Mishler, Churchill, 1984), the studies of 18S rDNA and *rbc*L evolution (Bopp, Capesius, 1986; Lewis & al., 1997) and the study of distribution of three introns in mitochondrial *nad*1 and *cox*3 genes (Qiu & al., 1998) attest that liverworts occupy basal position in land plant phylogeny.

When data obtained in the present study are compared to traditional schemes, one might note that the two lineages branching off first in the land plant tree correspond to Marchantiidae-Jungermanniidae of Schuster's system

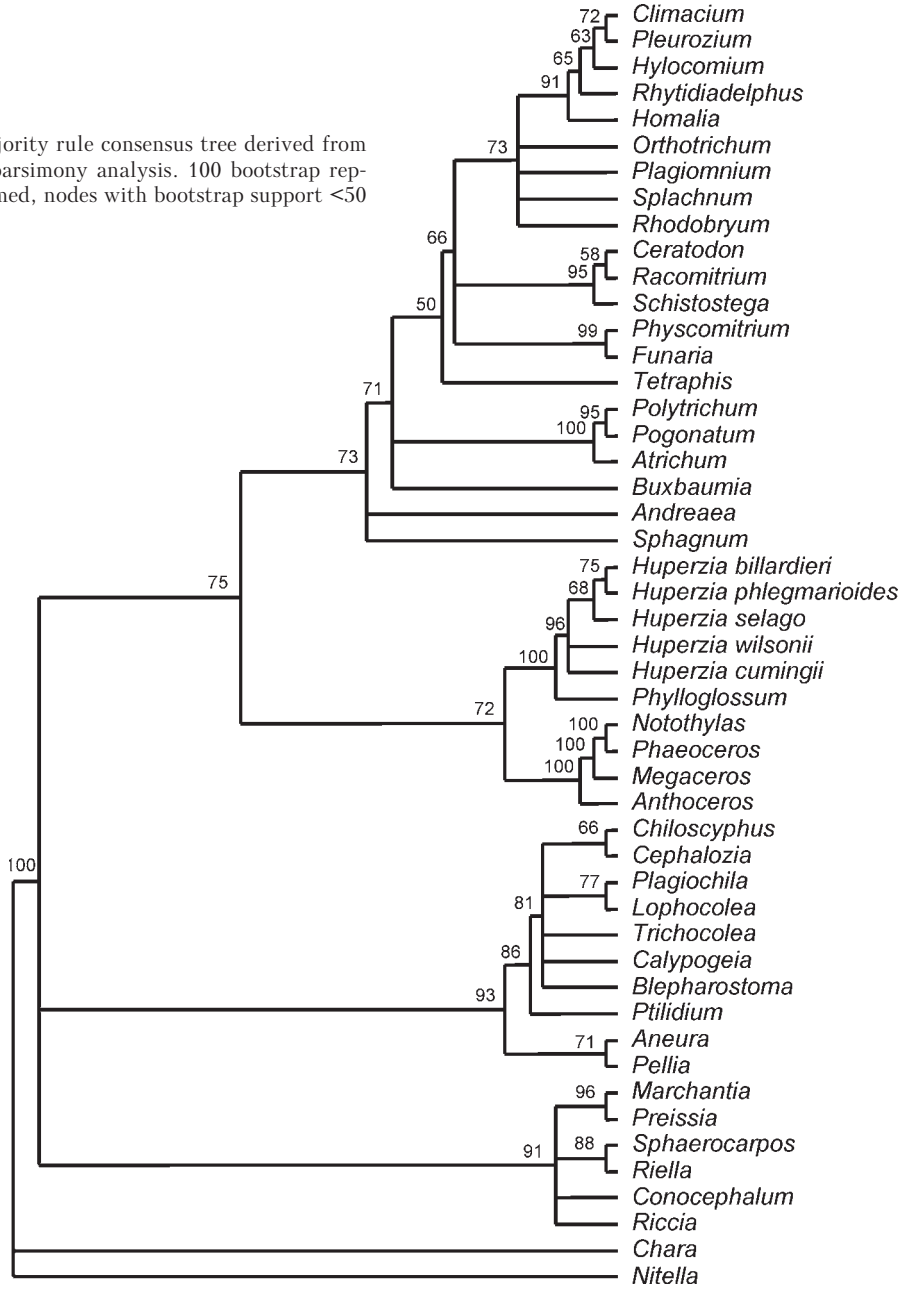

Fig. 5. 50% Majority rule consensus tree derived from equally weighted parsimony analysis. 100 bootstrap replicates were performed, nodes with bootstrap support <50 % are not shown.

(1984), but the relatedness of these branches as well as of genera comprising them cannot be unequivocally determined.

Mosses. Our data do not contradict the subdivision of mosses into four classes, namely Sphagnopsida, Andreaeopsida, Polytrichopsida, Bryopsida (Vitt & al., 1998). Sphagnopsida and Andreaeopsida seem to be the most ancient among them. Again, just as in the case of liverworts, the tree topology does not allow to resolve relationships of classes and orders of mosses.It is worth noticing that analyzing the clade corresponding to the Dicranidae subclass of the system Vitt & al. (1998), i.e. mosses with *Dicranum*-type peristome, we found that one of the gymnostomic mosses, *Schistostega*, joins this group, although its sporoderma characters and mode of spore dispersion is similar to diplolepidous Splachnaceae (Ignatov, Ignatova, 2001). Such a position of *Schistostega* finds added support in the results of mitochondrial *nad2* and *nad5* gene studies (Beckert & al., 1999; 2001).

Takakia. *Takakia* deserves special consideration as its systematic position has long been debated (see Renzaglia & al., 1997). Some authors attribute it to Calobryales (Schuster, 1984), whereas others include *Takakia* in Andreaeopsida (Smith, Davidson, 1993; Vitt & al., 1998). In recent studies, *Takakia* branched off together with *Andreaea* at the base of the moss cluster next to *Sphagnum* (Hedderson & al., 1998), or *Takakia* formed a cluster with *Sphagnum* (Newton & al., 2000). At some schemes, *Takakia* forms an individual branch diverging below *Sphagnum* in the base of the moss cluster (Renzaglia & al., 2000). We found in the *Takakia* cpITS3 sequence a region which is highly similar to that of other land plants, but absent in mosses. Since a multiple origin on the insertion in this region seems unlikely, this deletion in moss cpITS3 might be considered as a synapomorphic character. Hence, if *Takakia* belongs to the mosses, it should occupy the basal position in this group among taxa sampled, branching off below the *Sphagnum* and *Andreaea* lineages. Taking into account that *Takakia* possesses some characters typical of different groups of mosses and of lycopsids, it has been suggested to treat *Takakia* as a monotypic taxon within bryophytes of a high rank equal to that of liverworts, hornworts and mosses (Crandall-Stotler, 1986; Crum, 2001) and our cpITS data are generally consistent with this suggestion.

Hornworts. The position of hornworts in the land plant hierarchy remains unclear. According to *rbcL* analysis and distribution of tree introns in mitochondrial *nad1* and *cox3* genes, liverworts appear as the most basal land plant clade (Lewis & al., 1997; Qiu & al., 1998), whereby hornworts either branch off next to liverworts (Mishler, Churchill, 1984) or form a claster together with vascular plants (Lewis & al., 1997). At the same time, analysis of sequences of *nad5* and *cox3* genes, as well as five sequences of chloroplast genes (*psaA, psaB, psbD, rbcL*, *rpoC2*) and of concatenated sequences of SSU rRNA genes from three cellular compartments and of *rbcL* gene, the basal position is occupied by hornworts, and mosses (or mosses+liverworts) are a sister group to vascular plants (Malek & al., 1996; Beckert & al., 1999; Gabary, Renzaglia, 1998; Hedderson & al., 1998; Nishiyama, Kato, 1999; Renzaglia & al., 2000; Nickrent & al., 2000). As it follows from Figs. 2-5, hornworts seem to be the closest group relative to vascular plants, represented in our analysis by lycopods. Thus our data correspond with the results of an *rbcL* analysis (Lewis & al., 1997) and are consistent with the distribution of three group II introns in mitochondrial *nad1* and *cox3* genes of bryophytes and vascular plants. It was found that the latter are absent in liverworts and green algae (Qiu & al., 1998).

Bryophyte classification. Data obtained in this study of nucleotide sequences of cpITS2-4 spacers and of 4.5S+5S rDNA genes address the classification of plants traditionally related to bryophytes. The solution to this problem depends on the approach to the problem of classification. Traditional systematics is based on Linnean principles differing from cladistics. Trying to adopt the Linnean nomenclature based on predictive and rigid taxa hierarchy to monophyletic clades of phylogenetic systems, some authors introduce taxonomic categories absent in ICBN, e.g., “infradivision”, “plesion”, “cohort” (Kenrick & Crane, 1997b). According to Rasnitsyn (1983), principles of cladism cannot be applied consistently. As Brummit noted, “any attempts to develop a nomenclature for monophyletic taxa (clades) should be completely independent of the Linnean system and not confusable with it” (Brummit, 1999). According to this point of view, Linnean taxonomy and nomenclature are suited to cataloging biodiversity and attempts to fully eliminate paraphyletic taxa from traditional systems result most notably in nomenclature instability. Within the framework of traditional approaches, bryophytes might be considered as taxon corresponding to the concept of monophyletic continuum (Rasnitsyn, 1983) and could be isolated, for example, as a subdivision Bryophytina within division Embryophyta. The differentiating character of this group might be the existence of a sporophyte in their life cycle that is unable to grow and mature independently, that is, requiring support from the gametophyte. Within this taxon, which might have even higher rank, liverworts, hornworts, mosses, takakias might be considered as individual taxa. Relationships between these groups and their link to vascular plants, which are illustrated by different cladograms, might be treated as independent phylogenetic

hypotheses. In any case, independent of classification approach, one cannot but assume that the final task of systematics "is not to group organisms, but to give groups a pithy interpretation, to understand, which evolutionary events they reflect" (Shatalkin, 1988, p.167).

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Table 1. Analyzed species names and GeneBank accession numbers for the corresponding ITS sequences.

Species name	Ac. Number
Chara australis R. Brown	AF033652
Nitella mucronata A. Br.	AF518405
Marchantia polymorpha L.	NC_001319
Conocephalum conicum (L.) Lindb.	AF426184
Preissia quadrata (Scop.) Nees	AF426185
Riccia fluitans L.	AF033633
Sphaerocarpos donnellii Aust.	AF426183
Riella helicophylla Mont.	AF426187
Aneura pinguis L. Dum.	AF033631
Pellia neesiana (Gott.) Limpr.	AF033632
Ptilidium pulcherrimum (G. Web.) Vainio	AF033629
Trichocolea tomentella (Ehrh.) Dum.	AF426186
Blepharostoma trichophyllum (L.) Dum.	AF033627
Chiloscyphus polyanthos (L.) Corda	AF033626
Plagiochila porelloides (Torr. ex Nees) Lindenb.	AF033624
Lophocolea heterophylla (Schrad.) Dum.	AF033625
Calypogeia integristipula Steph.	AF033628
Cephalozia bicuspidata (L.) Dum.	AF033630
Orthotrichum speciosum Nees	AF033639
Rhodobryum roseum (Hedw.) Limpr.	AF033636
Schistostega pennata (Hedw.) Web. et Mohr	AF033641
Homalia trichomanoides (Hedw.) B.S.G.	AF033640
Climacium dendroides (Hedw.) Web. et Mohr	AF033644
Pleurozium schreberi (Brid.) Mitt.	AF033645
Hylocomium splendens (Hedw.) B.S.G.	AF033642
Rhytidiadelphus triquetrus (Hedw.) Warnst.	AF033647
Ceratodon purpureus (Hedw.) Brid.	AF033646
Racomitrium microcarpum (Hedw.) Brid.	AF033649
Splachnum luteum Hedw.	AF426189
Plagiomnium ellipticum (Brid.) T.Kop.	AF426190
Physcomitrium pyriforme (Hedw.) Brid.	AF426191
Funaria hygrometrica Hedw.	AF426192
Tetraphis pellucida Hedw.	AF033643
Polytrichum commune Hedw.	AF518406
Pogonatum urnigerum (Hedw.) P.Beauv.	AF518407
Atrichum undulatum (Hedw.) P.Beauv.	AF033638
Buxbaumia aphylla Hedw.	AF033635
Andreaea rupestris Hedw.	AF033637
Sphagnum palustre L.	AF033648
Takakia lepidoziodes Hatt. et Inoue	AF426188
Anthoceros punctatus L.	AF426180
Notothylas breutelii (Gottsche) Gottsche	AF426181
Phaeoceros carolinianus (Michx.) Prosk.	AF426182
Megaceros tosanus Stephani	AF518408
Phylloglossum drummondii Kuntze	AF338736
Huperzia selago (L.) Bernh.	AF338737
Huperzia wilsonii (Underwood & Lloyd) B. Pllg.	AF338738
Huperzia billardieri (Spring) Trevisan	AF338739
Huperzia cumingii (Nessel) Holub	AF338740
Huperzia phlegmarioides (Gaudich.) Rothm.	AF338745