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SYSTEMATIC POSITION OF HABRODON (HABRODONTACEAE, MUSCI) AS INFERRED FROM NUCLEAR ITS1 AND ITS2 AND CHLOROPLAST *trn*L INTRON AND *trn*L-*trn*F SPACER SEQUENCE DATA

СИСТЕМАТИЧЕСКОЕ ПОЛОЖЕНИЕ HABRODON (HABRODONTACEAE, MUSCI) ПО ДАННЫМ АНАЛИЗА ПОСЛЕДОВАТЕЛЬНОСТЕЙ ЯДЕРНОЙ (ITS1, ITS2) И ХЛОРОПЛАСТНОЙ (*trn*L ИНТРОН И *trn*L-*trn*F СПЕЙСЕР) ДНК

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Abstract

Systematic position of the genus *Habrodon* was considered quite differently by various authors – it was placed in Habrodontaceae, Fabroniaceae, Myriniaceae, Leskeaceae, and Pterigynandraceae. The analysis of nucleotide sequence data (chloroplast *trn*L-F region and nuclear ITS1 & ITS2) was undertaken to elucidate *Habrodon* relationshi ps. This analysis revealed quite distinct position of *Habrodon* from *Leskea* and *Myrinia*, and found *Habrodon* within a paraphyletic grade with Plagiotheciaceae (sensu lato, cf. Hedenäs, 1987; Pedersen & Hedenäs, 2002) and *Fabronia* and sometimes also with *Pterigynandrum*. The acceptance of the monogeneric family of Habrodontaceae Schimp. is suggested. Other inferrings from analysis support the segregaion of *Iwatsukiella* from *Habrodon*, but do not support the monophyly of the family Leskeaceae, which seems need to be strongly reconsidered. Pterigynandraceae sensu Buck & Goffinet (2000), represented in our analysis by all five genera (i. e. *Habrodon, Myurella, Heterocladium, Iwatsukiella,* and *Pterigynandrum*) were also found not monophyletic. Topology of tree obtained from molecular data shows certain correlation with some morphological characters, especially pattern of distribution of foliose structures around juvenille branch primordia. Also, *Habrodon* and neighboring members of its paraphyletic grade all have uniqe structure of uniseriate axillary gemmae.

Резюме

Систематические положение монотипного рода *Habrodon* трактовалась разными исследователся крайне неоднозначно – его относили к Habrodontaceae, Fabroniaceae, Myriniaceae, Leskeaceae и Pterigynandraceae. Анализ нуклеотидных последовательностей хлоропластной ДНК (участок *trn*L-F) и ядерной ДНК (ITS1, ITS2) выявил отсутствие родства *Habrodon* с *Leskea* и *Myrinia*, и подтвердил определенную близость *Habrodon* с Plagiotheciaceae (в широком смысле, cf. Hedenäs, 1987; Pedersen & Hedenäs, 2002) и *Fabronia*, и в некоторых вариантах анализа – также с *Pterigynandrum*. Обосновывается предпочтительность выделения рода в самостоятельное семейство (что было предложено еще Шимпером). Данные молекулярного анализа подтверждают правильность выделения из *Habrodon* рода *Iwatsukiella*, но не подтверждают монофилетичности семейства Leskeaceae, объем которого заслуживает серьезного пересмотра. Также, не близкими оказываются представители всех пяти родов, относимых Buck & Goffinet (2000) к Pterigynandraceae (i. e. *Habrodon, Myurella, Heterocladium, Iwatsukiella, и Pterigynandrum*). Обсуждается связь топологии деревьев, полученных на основе молекулярных данных, с некоторыми морфологическими признаками – при этом подтверждается важность признаков ювенильных листьев в основании зачатков веточек. Также для *Habrodon* и ближайших членов парафилетической грады характерно наличие пазушных выводковых тел из 3-6 клеток, расположенных в один ряд, которые известны в порядке Hypnales только у этих групп.

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INTRODUCTION

Until the recent past, the structure of the peristiome was considered to be one the most important character for the classification of pleurocarpous mosses. *Habrodon perpusillus* (De Not.) Schimp., a speices with strongly reduced peristome, was referred to several families, circumscribed for taxa with reduced peristome. However, the differences between these families were not well understood, so *Habrodon* was placed in as many as in five families.

Schimper (1860) originally described the genus *Habrodon* with one speices and put it into the separate family Habrodontaceae. Brotherus (1905-1909) transferred the genus into the family Fabroniaceae, and later (Brotherus, 1925), following Fleischer's (1915) concept of the phylogeny of the Fabroniaceae, he gave *Habrodon* the status of the subfamily Habrodontoideae within the Fabroniaceae. This concept was widely followed in European floras (Smith, 1978; Nyholm, 1960, etc.). Buck (1977) put *Habrodon* in Myriniaceae, but soon afterwards Buck & Crum (1978) revised the traditional concepts of the Fabroniaceae, and found that *Habrodon* fits better to Leskeaceae, as its peristome is reduced. This concept was followed by e. g. Hedenäs (1992) and Corley & al. (1981). Buck and Crum (1978), also segregated the principally Asian *Habrodon leucotrichum* (Mitt.) Perss. and japanese *H. noguchii* (Sak.) Sak. in the new genus *Iwatsukiella*. The latter species was subsequently synonymized with *Pylaisiella subcircinata* (Card.) Iwats. et Nog., leaving *Iwatsukiella* to be monospecific (cf. Crosby & al., 1999).

Later Buck and Crum (1990), revising the subfamily Heterocladioideae of the Thuidiaceae, noted that the gametophytes of the species of *Heterocladium* and *Pterigynandrum* show a remarkable resemblance to three other genera (*Habrodon, Iwatsukiella,* and *Myurella*), which were never previously associated with them. These genera share a similarity in some morphological characters of the gametophytes and distribution, and also most of them have reduced peristomes. Therefore, Buck and Crum placed this united assemblage of the genera in a family of its own, the Pterigynandraceae, considering it to be closely related to the Leskeaceae and Thuidiaceae.

Thus, until now the systematical position of the genus *Habrodon* and its phylogenetic relationships have remained the controversial point in bryophyte taxonomy.

To test the strength of the phylogenetic hypothesis based on morphology and to determine whether they are congruent with other sources of data we explore the information residing in DNA sequences of different genes. Our aim was to obtain sequences for the *trn*L-*trn*F region of the chloroplast DNA (cpDNA) and the sequences of the internal transcribed spacers (ITS1, ITS2) of the nuclear ribosomal transcription unit (nrDNA). The region of the internal transcribed spacers of nrDNA conventionally includes the entire ITS1, 5,8S gene and ITS2 portion of the nrDNA cistron and is one of the most informative molecular markers for phylogenetic analyses at the genus and species levels (Coleman, 2003). Whereas, the chloroplast *trn*L-*trn*F region, especially the *trn*L intron, is used widely for inferring phylogenetic relationships among families and genera of angiosperms (Taberlet & al.,1991), of bryophytes (Stech & al., 2003) and therefore provide another independent marker for phylogenetic reconstruction in our study.

Successful usage of nrITS and *trn*L–*trn*F sequence data for the analysis of phylogeny of pleurocarps at the genus-family level was shown by, i. e., Vanderpoorten & al., (2002a,b), Huttunen & Ignatov (2004).

MATERIAL AND METHODS

Details on species names, references or voucher specimens and GenBank accession numbers are given in Table 1.

Total DNA's were extracted from herbarium specimens using NucleoSpin Plant Kit (Macherey – Nagel). The cpDNA $trnL_{UAA}$ – $trnF_{GAA}$ region, consisting of the intron, 3' exon of $trnL_{UAA}$ gene, *trn*L-*trn*F intergenic spacer (IGS) and part of *trn*F gene, was amplified and sequenced using "c" and "f" primers that correspond to the conserved sequences near 5' and 3' ends of *t*RNA genes (Taberlet & al.,1991). The ITS regions were amplified and subsequently sequenced using the primer pairs "ITS-L" and "ITS-4"; and in some cases also additionally were used internal primers "ITS-3" and "ITS-2" (White & al. 1990).

PCR reactions were done in 25 µl aliquots containing 30 mM Tris-HCl (pH 8,3), 50 mM KCl, 4mM $MgCl_2$, dNTP's (0,3 mM each), 2 units

Taq-polymerase (Sibenzym), 10-15 ng of template DNA and 10pm of each primer. The PCR was run on Tercik thermocycler (DNA technology, Russia) following the protocol: 3 min. 95°C, 30 cycles (50 s 94°C, 40 s 50°C, 60 s 72°C) and 2 min 72°C of extension time. Amplified fragments were visualized on 1% agarose TAE gels, purified using GFX™ PCR DNA and Gel Band Purification Kit (Amersham Biosciences), and sequenced using ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit and Avant 3100 automated DNA sequencer (Applied Biosystems).

The sequence data have been submitted to the DDBJ/EMBL/GenBank database under accession numbers shown in Table 1.

The data matrix was prepared for phylogenetic analyses by manual alignment of the sequences using the SED editor of the VOSTORG package (Zharkikh & al., 1990).

Three methods were implemented for phylogenetic trees reconstruction: Bayesian analysis (BA), neighbor joining (NJ), and parsimony (P) methods.

Initially the computer program Modeltest ver. 3.06 (Posada & Crandall, 1998) was used to determine a best-fit evolutionary model by calculating log likelihood scores for a set of models and performing hierarhical likelihood ratio tests. The resulting best-fit model according to Akaike information criterion (AIC) estimate was the general time-reversible model with invariable sites and a gamma-distributed rate heterogenity parameter (GTR+I+Γ).

Bayesian analysis was executed using MrBayes ver. 3.0B4 computer program (Huelsenbeck and Ronquist, 2001). GTR+I+Γ model of nucleotide substitution with 4 rate categories were used. Seven character partitions were specified: parts of 26S and 5.8S rDNA (112 sites in a total), ITS1 (452 sites), ITS2 (444 sites), *trnL* exon (62 sites), *trnL* intron (332 sites), IGS (68 sites), part of *trnF* (20 sites). Four Metropolis-coupled MCMC chains were run from randomly chosen starting trees for 2000000 generations, trees were saved once every 10 generations, 65000 first trees were ignored and 50% majority-rule consensus tree and bayesian posterior probabilities as branch support values were calculated. Gaps were counted as N base.

A neighbor joining method was employed (Saitou & Nei, 1987) using the TREECON 1.3b package (Van de Peer & De Wachter, 1997). Construction of distance matrices was done using Kimura's two-parameter model distance (Kimura, 1980). Gaps were taken into account using an option treating a row of adjacent gaps as one gap. Support for individual nodes was assessed using a bootstrap resampling procedure with 1000 replicates (Felsenstein, 1985). NJ tree was constructed also using PAUP* 4.0b10 software package (Swofford, 1998) assuming GTR+Γ model of nucleotide substitution and using 1000 bootstrap replicates. In this case gaps are treated as missing data.

Parsimony analysis were performed using the software package PAUP* 4.0b10 (Swofford, 1998) Analyses involved a heuristic search with TBR (tree-bisection-reconnection) branch swapping with MulTrees, and Collapse options in effect. Characters were unordered, no weighting scheme was employed and random sequence addition was used. Gaps were treated as additional character state (fifth base). 200 jackknife resampling were performed with 20% of the data to be deleted for each replicate, and majority rule consensus tree was constructed.

In all trees *Hookeria lucens* was used as an outgroup.

RESULTS

New sequence data for cpDNA $trnL_{UAA}$ – $trnF_{GAA}$ region included *trnL* intron and *trnL* – *trnF* intergenic spacer (IGS) and ITS1-2 region were obtained for thirteen species: *Fabronia ciliaris, Habrodon perpusillus, Haplocladium angustifolium, Heterocladium heteropterioides, Isopterygiopsis muelleriana, Iwatsukiella leucotricha, Leptopterigynandrum austro-alpinum, Leskea polycarpa, Pseudoleskeella nervosa, Myrinia pulvinata, Plagiothecium denticulatum, Pterigynandrum filiforme, Pylaisia polyantha.* For *Thelia asperella* we got the *trn*L-*trn*F sequence, and ITS sequences were obtained from DNA data base. The ITS1 sequence of *Hypnum cupressiforme* was granted to us by S. Huttunen. Other data were taken from the GenBank. Since both ITS and *trn*L-F data were not available for any of *Myurella* species, we used ITS data of *M. sibirica*, and *trn*L-F of *M. tenerrima* as these species are obviously closely related.

The whole alignment of combined sequences from 29 species consists of 1490 sites; among them 560 characters are constant, 436 variable

Table 1. Data on species, specimens used for sequence alalysis and the GenBank accession numbers. The data taken from GenBank are supplemented with the corresponding publications or the author of unpublished data [in square brackets].

Species	Specimen or/and authors of sequence	trnL	Its1	Its2
Amblystegium fluviatile (Hedw.) B. S. G.	Allen 16372 (DUKE) [Vanderpoorten & al., 2002b]	AY009822	AF168154	AF168154
Amblystegium humile (P. Beauv.) Crundw.	Buck 15943 (DUKE) [Vanderpoorten & al., 2002b]	AY009874	AF168165	AF168165
Amblystegium serpens (Hedw.) B. S. G.	Schofield 106313 (DUKE) [Vanderpoorten & al.,2002b]	AY009827	AF168152	AF168152
Calliergon cordifolium (Hedw.) Kindb.	Ireland 24198 (DUKE) [Vanderpoorten & al., 2002b]	AY009836	AF168146	AF168146
Entodon seductrix (Hedw.) Müll. Hal.	Majestyk (unpubl); Chiang (unpubl.)	AY255486	-	AJ288572
**Fabronia ciliaris* Brid.	Russia, Perm Province, Bezgodov 268 (1999)(MHA)	AY527128	AY528883	AY528883
**Habrodon perpusillus* (De Not.) Lindb.	Russia, Adler, M. & E. Ignatov 9.VIII.2002 (MHA)	AY527126	AY528880	AY528880
**Haplocladium angustifolium* (Hampe et Müll. Hal.) Broth.	Japan, Higuchi 13216 (MHA)	AY527129	AY528884	AY528885
Haplocladium virginianum (Brid.) Broth.	Buck 32482 (NY) [Vanderpoorten & al., 2002b]	AF161133	AF168160	AF168160
**Heterocladium heteropterum* (Brid.) B. S. G.	Russia, Caucasus, Akatova 20.VIII.1999 (MHA)	AY527130	AY528894	AY528895
Hookeria lucens (Hedw.) Smith	Cox& al., 2000	AF215906		
Hookeria lucens (Hedw.) Smith	Capesius & Bloecher (unpubl.)		AJ252137	AJ252137
Hypnum cupressiforme Hedw.	Finland, S. Huttunen 1438 (H)	AF397812	AY528888	AF403607
**Isopterygiopsis muelleriana* (Brid.)	Russia, Yakutiya, Ignatov 00-298 (MHA)	AY527138	AY528882	AY528882
**Iwatsukiella leucotricha* (Mitt.) Buck et Crum	Khabarovsk Ter., Ignatov 97-243 (MHA)	AY527132	AF516162	AF516157+
**Leptopterigynandrum austro-alpinum* Müll. Hal.	Russia, Altai, Ignatov 27.VII.1993 (MHA)	AY527133	AF516163	AF516158
Leskea gracilescens Hedw.	Buck 30102 (NY) [Vanderpoorten & al., 2002b]	AF161135	AF176277	AF176277
**Leskea polycarpa* Hedw.	Russia, Moscow Prov., Ignatov 18.VI.1996 (MHA)	AY527134	AY528889	AF516151
**Leskeella nervosa* (Brid.) Loeske	Russia, Kerzhensky Reserve, Ignatov 12.IX.1999 (MHA)	AY527135	AF516167	AF516152
**Myrinia pulvinata* (Wahlenb.) Schimp.	Russia, Ryazan, Ignatov 29.IX.1999 (MHA)	AY527127	AY528886	AY528887
Myurella sibirica (Müll. Hal.) Reim.	Chiang (unpubl.)		AJ288415	AJ277227
Myurella tenerrima (Brid.) Lindb.	Pedersen & Hedenäs (2002)	AF472461		
Neckera pennata Hedw.	Shaw 9354 (DUKE) [Vanderpoorten & al., 2002b]	AF315072	AY009809	AY009809
**Plagiothecium denticulatum* (Hedw.) B. S. G.	Russia, Yakutiya, Ignatov 00-883 (MHA)	AY527131	AY528892	AY528893
Platygyrium repens (Brid.) Schimp.	Buck 33448 (NY) [Vanderpoorten & al., 2002b]	AF161131	AY009798	AY009798
Platydictya jungermannioides (Brid.) Crum	Schofield & al. 101911 (DUKE) [Vanderpoorten & al.,2002b]	AY009857	AF168162	AF168162
**Pterigynandrum filiforme* Hedw.	Altai, Ignatov 4.VII.1991 (MHA)	AY526198	AY528890	AY528891
**Pylaisia polyantha* (Hedw.) Schimp.	Moskva, 10.VII.2003 (MHA)	AY527137	AY528881	AY528881
Rhytidium rugosum (Hedw.) Kindb.	Schofield &al. 98103 (DUKE) [Vanderpoorten & al.,2002b]	AY009849	AY009801	AY009801
Thelia asperella (Schimp.) Sull. et Lesq.	Chiang,T.Y. (unpubl.)		AJ288413	AJ277225
Thelia asperella (Schimp.) Sull. et Lesq.	USA, Tan 92-158 (MHA)	AY527136		
Thuidium delicatulum (Hedw.) B. S. G.	Buck 32594 (NY) [Vanderpoorten & al., 2002b]	AF161132	AF176278	AF176278

characters are parsimony-uninformative, and 494 characters are parsimony-informative. The length of sequences vary from 960 bp (*Leptopterigynandrum austro-alpinum*) to 1067 bp (*Hookeria lucens*). The average nucleotide composition of a combined data set is 25.1% (A), 23.9%(T), 25.5%(C), and 25.5%(G).

The most variable regions are located in ITS1 and ITS2, parts of alignment of these sequences are presented at Fig. 4 (the whole alignment of combined data can be obtained from authors upon request). The observed variability is due mainly to a significant number of indels. The insertions are positioned mainly in a number of «hot spots» and originated either from duplication of short adjacent strethces of bases or intercalation of foreign sequences. In the first case the presence of identical inserts in the same site is not yet an evidence of their origin from a common ancestor. In a result the alignment cannot be considered to be absolutely unambiguous. However several alternative variants tested do not change the topology of phylogenetic trees infered from the data.

The phylogenetic trees constructed by three different methods are presented at Figs. 1-3. The support values are <50% for a number of nodes; BA tree is best supported. The topologies of trees differ in some details while having in common significant features that will be discussed below. The topologies of NJ and BA trees does not differs greatly despite the fact that not optimal evolutionary model was used in the first case.

NJ 50% majority rule consensus tree at fig. 3 has a greater mean bootstrap support value than the similar NJ tree calculated assuming GTR+Γ model of nucleotide substitution (not shown). The topology of these two trees are identical with exception of the relative position of *Leskea polycarpa* and *Haplocladium virginianum* if clades supported by <50% are considered as unresolved.

The three trees differ in a manner of taking into account of gaps: in the BA tree the contribution of gaps is the lowest and in the P tree is the greatest (see Materials and Methods). That may be one reason for the differences between trees.

Parsimony analyses revealed 1 most parsimonious trees with a length of 2443 steps (CI=0.55, RI=0.53, HI=0.45). Jackknife 50% majority rule consesus tree included other groups compatible with this tree (Fig. 3) is 18 steps longer. The next single less parsimonous tree is 2446 steps in a length.

Discussion

The present analysis includes 28 species of Hypnales s. l., the number which is insufficient for immediate systematic re-arrangements. However the results might give a suggestion *pro* or *contra* cases where two or more alternative opinions are already existed.

The genus *Habrodon* was considered quite differently by various authors – it was placed in Habrodontaceae (Schimper, 1860), Fabroniaceae (Brotherus, 1905-1909), Leskeaceae (Buck & Crum, 1978), or Pterigynandraceae (Buck & Crum, 1990). The present analyses revealed the distinct position of *Habrodon* from *Leskea* and *Myrinia*, and found *Habrodon* within a paraphyletic grade, which includes also Plagiotheciaceae (sensu lato, cf. Hedenäs, 1987; Pedersen & Hedenäs, 2002), and *Fabronia*, and, in two of three analyses (Figs. 1-2) also *Pterigynandrum.* However the parsimony analysis, Fig. 3, and portions of alignment, Fig. 4, do not confirm so close position of *Habrodon*+Plagiotheciaceae +*Fabronia* to *Pterigynandrum*.

There are two rare morphological character states shared by *Habrodon* and these neighboring taxa: (1) uniseriate axillary gemmae and (2) lack of pseudoparaphyllia. These character can be commented as follow:

(1) *Habrodon* has peculiar uniseriate gemmae of 3-6 cells, developed in clusters in leaf axils and sometimes in all around the distal part of stem (Fig. 5, see also Ignatova & Ignatov, 2003); these gemmae are similar to those of *Pterigynandrum* and genera of Plagiotheciaceae s. l. (*Isopterygiopsis, Myurella, Orthothecium, Plagiothecium, Platydictya* (and *Bardunovia*, if one would not consider it to be congeneric with *Platydictya,* as suggested by Hedenäs & Pedersen, 2002). The axillary uniseriate gemmae is a rare character in Hypnales s. l., known also only in Sematophyllaceae (but in that family they are long-filamentose, quite different from those of *Habrodon*–Plagiotheciaceae–*Pterigynandrum* type). The gemmae of *Habrodon* are especially similar to those of *Pterigynandrum* – in both they get brownish color of relatively

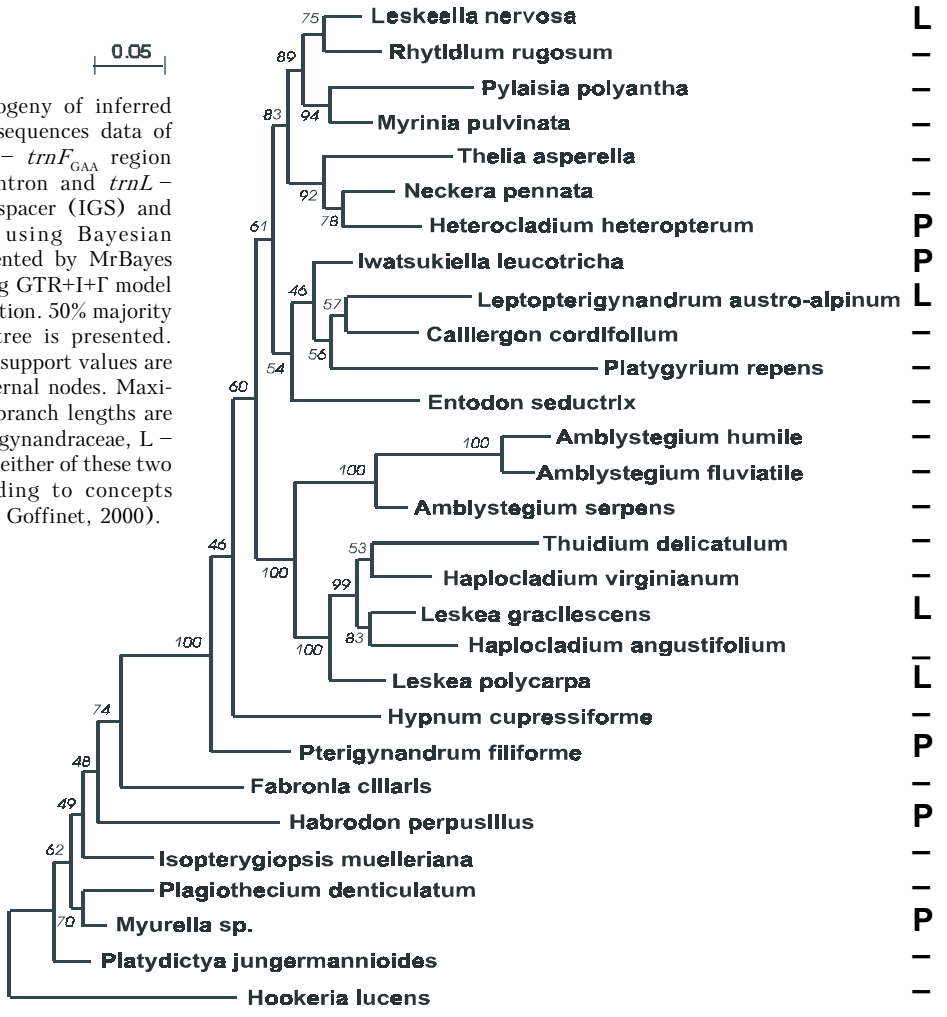

Fig. 1. Phylogeny of inferred from combined sequences data of cpDNA $trnL_{UAA}$ – $trnF_{GAA}$ region included *trnL* intron and *trnL* – *trnF* intergenic spacer (IGS) and ITS1-2 region using Bayesian analysis implemented by MrBayes program assuming GTR+I+Γ model of sequence evolution. 50% majority rule consensus tree is presented. Posterior branch support values are indicated for internal nodes. Maximum likelihood branch lengths are shown. P – Pterigynandraceae, L – Leskeaceae, – – neither of these two families (according to concepts given by Buck & Goffinet, 2000).

thick cell walls with age, whereas gemmae of Plagiotheciaceae are composed of thin-walled cells and are remained usually pure green. However, at earlier stages, gemmae of *Habrodon* and *Pterigynandrum* are very similar to those of Plagiotheciaceae (Fig. 5).

(2) We consider *Habrodon* to be similar to Plagiotheciaceae s. l. and *Fabronia* in the pattern of arrangement of leaf-like structures at early branch initial stage, calling this "pseudoparaphyllia absent". This statement, however, needs an expanded explanation.

There are several approaches to the understanding pseudoparaphyllia. For example, Akiyama & Nishimura (1993) and Enroth (1994) suggested to differentiate them from juvenille branch leaves (=proximal branch leaves, or scaly leaves, or rudimentary leaves). Hedenäs (1995) found no way to consider them separately, as two different organs. The main difference between them, according to Hedenäs (l.c.) may be in the timing of their growth. The case of "pseudoparaphyllia absence", according to Hedenäs, is equivalent of Akiyama & Nishimura' (1993) concave or convex branch primrdium without appendages.

Ignatov & al. (1996) found that in *Orthothecium* there are incised foliose structures at place of branch initial, but after a branch initial starts elongation, all these foliose structures appear on branch and the removal of the latter leaves nothing foliose on stem. This case admits interpretations – where to refer it to "pseudoparaphyllia absent" or "pseudoparaphyllia present". Ignatov & al. (1996) accepted the former, and this approach is followed here also by the following reasons.

Comparing the "typical", clear cases of

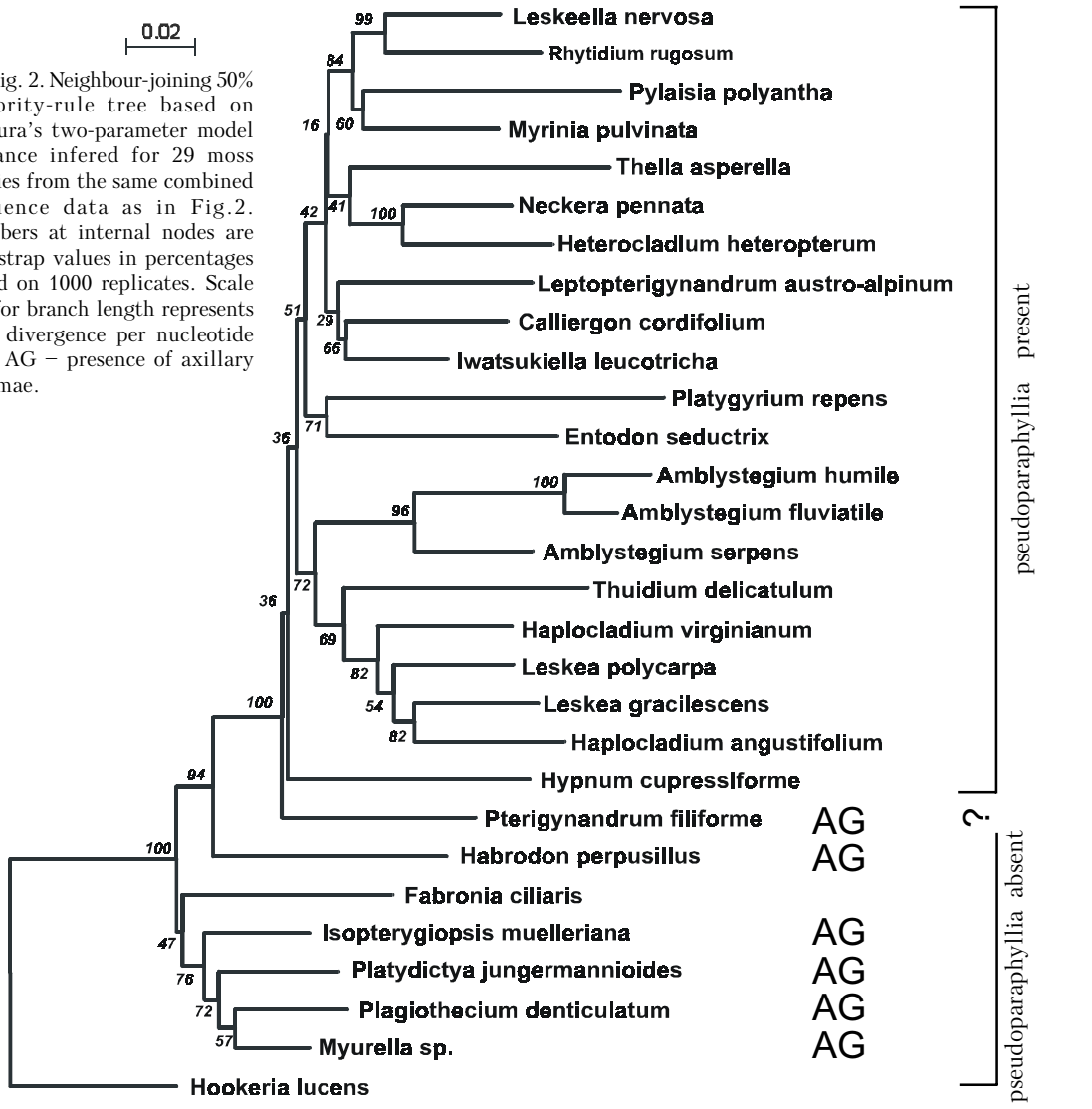

Fig. 2. Neighbour-joining 50% majority-rule tree based on Kimura's two-parameter model distance infered for 29 moss species from the same combined sequence data as in Fig.2. Numbers at internal nodes are bootstrap values in percentages based on 1000 replicates. Scale bar for branch length represents 0.02 divergence per nucleotide site. AG – presence of axillary gemmae.

branch inital with and without pseudoparaphyllia, for example *Hygrohypnum duriusculum* (Fig. 6) and *Plagiothecium laetum* (Fig. 5), it can be easily noticed that juvenille branch leaves in the former appear before the branch is starting elongation, whereas in the latter juvenille branch leaves start development only after branch undergo the elongation.

Typically in Plagiotheciaceae dormant buds are naked, though sometimes (cases of *Plagiothecium nemorale* and most of *Orthothecium* species) have some leaf-like structure at the dormant buds. However in all these latter cases these leaf-like structures shift to the branch after it starts elongation, resulting in nothing remaining on stem. Dormand buds in *Habrodon* (Fig. 5) are usually flat, with one or two narrowly lanceolate to subfilamentose appendages, which are clearly appearing *on* the branch initial when it reaches the stage of low convex raising. Appendages are sitting on rather enlarged cells, contrasted with elongate cells of stem cortex.

Also we think that the case of *Hookeria lucens* (Hedw.) Sm. (Fig. 5), where the first juvenille leaf appears already in the convex initial stage, belong to this "pseudoparaphyllia absent" state. Most of the dormant buds in this species were observed flat and totally leafless.

The lack of pseudoparaphyllia is a rare character among pleurocarps, known, besides the mentioned taxa, for example, in some Sematophyllaceae s. l.

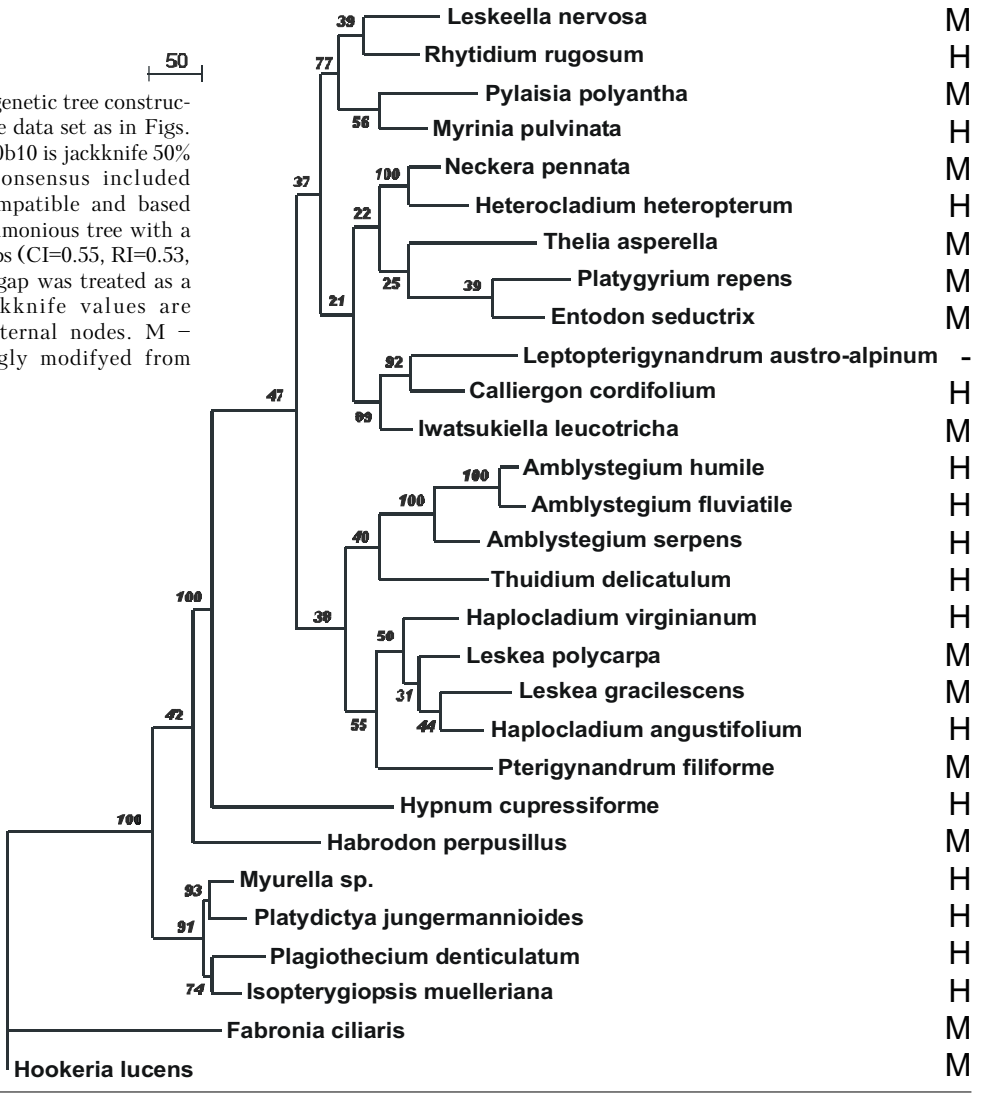

Fig. 3. Phylogenetic tree constructed from the same data set as in Figs. 1-2 by PAUP* 4.0b10 is jackknife 50% majority rule consensus included other groups compatible and based on the most parsimonious tree with a length of 2443 steps (CI=0.55, RI=0.53, HI=0.45). Each gap was treated as a fifth base. Jackknife values are indicated for internal nodes. M – peristome strongly modifyed from hypnoid (H).

Utilizing this approach, the definition of pseudoparaphyllia will be quite narrow – they are foliose structures on stem around branch initial (though ontogenetiaclly closely connected to new branch and often have obvious phyllotaxis, especially definite in Brachytheciaceae and Meteoriaceae, cf. Ignatov 1999). We agree with Hedenäs (1995), that pseudoparaphyllia are not princi pally different from juvenille branch leaves and the timing of growth is their main differential character.

There are cases which remain somewhat uncertain because of difficulties in observation. In *Pterigynandrum* some initials could be certainly interpreted and "pseudoparaphyllia absent" – despite dormand buds at flat stage has many subfilamentose appendages around, all of them are shifted on the branch as soon as it becomes a low convex raising. However sometimes, one largest lanceolate foliose structure is sitting quite apart from the initial and remain on stem, unlike many smaller ones which are shifted (Fig. 5). We refer *Pterigynandrum* to the group of "pseudoparaphyllia absent" (cf. Fig. 2) not without hesitating.

Concluding, *Habrodon's* pattern of early branch development is similar to that of the neigboring taxa in the paraphyletic grade (Figs. 1-2), and it is called here "pseudoparaphyllia absent". The interpretation of this character state is, however, somewhat broader, than that one used by Hedenäs (1995).

* * *

Fig.4. The alignments of parts of ITS1 and ITS2. The numbering is from the start of corresponding regions.

```
ITS1                                      91       101       111       121       131       141       151       161       171       181       191
Iwatsukiella leucotricha                  TCCC-TT---CGT---------CGCG-AGCGAGCG-----------------CGCT-CGGGGCGTCGGG-----------------TTT---CCCAC--GATGCA
Calliergon cordifolium                    TCCT-CT---CGT---------CACG-AGC---------TTCG-TCGC---GGCGAACT-CGTGGCGTGGGG-----------------TTT---CCAC--GATGCA
Thelia asperella                          TCCC-TT---CGT---------CACGTC-CGG---------------------CGCGGCGGAGGGG-----------------AT----CCAC--GATGCC
Myrinia pulvinata                         TCCC-TT---CGT---------CACG-CTCGAG-----CTTG----------CTCT---CGTGGCGTCGGG-----------------TTT---CCAC--GATGCA
Pylaisia polyantha                        TCCC-TT---CGC---------CAA---TCGC------CACG----------CGCG-CGCGGCGTCGGG-----------------TTT---CCAC--GATGAA
Leptopterigynandrum austro-alpinum        TCCC-TT---CGT---------CGCG-AGC---------TTCG-TCCCG-TCACGAACT-CGGGGCGTCGGG-----------------TTT---CCAC--GATGCC
Hypnum cupressiforme                      TCCC-TT---CGT---------CACG-AGC---------------------TCGCT-CGTGGCG-CGGGC-----------------TT----CCAC--CATGCN
Neckera pennata                           TCCC-T----CGT---------CACG---CG---------------------CTGCGG-CGTGGCGTCGGG-----------------TTT---CCACGCGATGCC
Leskeella nervosa                         TCCC-TT---CGT---------CACG-CGCGAG-------TTCG----------CTCGCG-CGCGGCGTGGGG-----------------TTT---CCAC--GATGCG
Heterocladium heteropterum                TCCC-TT---CGT---------CACG-AGCGAGCGAG-TTCG----------CTCGCG-CGCGGCGTCGGG-----------------TTT---CCGCGCGATGCC
Haplocladium virgnianum                   TCCC-TT---CGT---------CACG-GGCGAGCGAG-----CTCGC--TCGTTCGCT-CGCGGCGCGGGG-----------------TTTT----CAC--GATGCG
Leskea polycarpa                          TCCCCTTCG-CGT---------CACG-AGCGAG---------TCGCT-CGCGGCGTGGGG-----------------TTT---CCAC--TATGCA
Platygyrium repens                        GCC--T-CG-CGG---------CGCG-AGCGAG---------CTCC---TCGTGAGTT-CGTGGCGTCGCG----TGGCG-----------------TTT---CCAC--GATGCG
Haplocladium angustifolium                TCCC-TCCGTCGC-------GACACG-AGCGAC---------CTCGC--TCGCTCGTT-CGCGGCGTGGGG-----------------TTT---CCAC--GATGTG
Leskea gracilescens                       TCCCCTTCG-CGT---CACGACACG-AGCGAG---------CTCGC--TTGTTCGTT-CGCGGCGTGGGGGG----------------TTT---CCAC--GATGTG
Rhytidium rugosum                         TCCC-TT---CGT---------CACG-CGCGAG-------TNCG----------CTCGCT-CGCGGCGTTGGG-----------------TTT---CCAC--GATGCA
Pterigynandrum filiforme                  TCCC-TTCGTCGT---------CACG-AGCGTC---CAAA----------CT-CGGGGCGTCGGGGG----------TCCTCTTT----CAC--GATGCA
Thuidium delicatulum                      TCCC-TTCGTCGTCGT-----CACG-AGCGAGC---------TCCGGCTCGTTCGCGGCGCGCG-CGGGG-----------------TGTT---CACCAC--GATGCG
Amblystegium serpens                      TCCCCTTCG-CGT---------CACG-AGCGAG---------------CTCGCTCGCT-CGCGGCGTGGG-----------------TTT---CCAC--TATGCA
Amblystegium fluviatile                   TCCCCT----CGT---------CCCG-AGCGAGC---------------TCGGCTCCCT-CNNGGAGGAGCGGG-----------------TTCACACCAC---ATGCA
Amblystegium humile                       TCCCCT----CGT---------CCCG-AGCGAGC---------------TCGCGCTCCCT-CGCGGNGTCNGCGGG-----------------TTCACACCAC---ATGCA
Hookeria lucens                           TCCC-GA---GGT---------CACG-AGACT----------TCC----------CGCT-CGCGGCTTCCGGGGG-----AAA-----AICT----CAC--GATGCA
Fabronia ciliaris                         TCCC-GA---GAC---------CGCG-AGCTT----CAAA----------CCT-CACGGCGTCGGGGG-----TAAT----CTTT----CAC--GATGCA
Myurella sp.                              TCCC-GA---GGT---------CACG-AGCGT----CAAAA----------CT-CGTGGCGTCGGGGGG----TAAT----TTT-----CAC--GATGCA
Isopterygiopsis muelleriana               TCCC-GA---GGT---------CACG-AGCGTCG---CAA----------CT-CGTGGCGTCGGGGGG----TACC----CTTT----CAC--GATGCA
Platydictya jungermannioides              TCCC-GA---GGT---------CACG-AGCTT----CAAAA----------CT-CGTGGCGTCGGGGG----TAAT----CTTT----CAC--GATGCA
Plagiothecium denticulatum                TCCC-GA---AGT---------CACG-AGCGT----CAAA----------CCT-CGCGGCGTCGGGGG----TACC-CTTTCTTT----CAC--GATGCA
Habrodon perpusillus                      TCCC-GG---GGT---------CACG-AGCGTG---CAAA----------CTCT-CGCGGCGTCGNCAGG----AAAT--CTTCTTT----CAC--GATGTG

ITS2                                      331       341       351       361       371       381       391       401       411       421       431       531
Entodon seductrix                         CC-CAGTCGGTT--GTG-G-----ATCCCTCGTG-CAGGTCGA-CGTCACCGCCCCCC----------------------GTGGCGCGCG-------TCACG
Iwatsukiella leucotricha                  CC-CAGTTGGTT--GTG-G-----TTACCTCATG-CAGGTCGA-GCGCGCC-CCCC----------------------GCGGCCGCG-------TCACG
Calliergon cordifolium                    CC-CAGTTGGTT--GTG-G-----TTACCTCATG-CAGGTCGA-GCGCGCC-CCC-----------------------GTGGCGCGCG-------TCACG
Thelia asperella                          CCCCAGTTGGTC----------CCCCCTCGTG-CAGGTCGA-CCGC-CC-CCC-----------------------------GGCGCGCT-------TCAAG
Myrinia pulvinata                         CC-CAGTTGGTT--GCG-G-----TTACCTCGTGCCAGGTCGA-GCGCGCC-CCC-----------------------GCGGCGCGCG-------TCACG
Pylaisia polyantha                        CC-CAGTTGGCT---TTG-G-----ATACCTCGTG-CAGGTTGA-GCGTGCC-CCTTCAT-----------------------GGGGCACGCG-------TCACG
Leptopterigynandrum austro-alpinum        CC-CAGTTGGTCTCCTCG-G-----TTCCCTCACG-CAGGTCGA-GCGCGCC-CCC-----------------------GCGGCGCGCG-------TCACG
Hypnum cupressiforme                      CCCCAGTCGGTCC--GTG-G-----TTACC-C----CAGGTCGATGCGCGCC-CGC-----------------------GAGGCGCGCG-------TCACG
Neckera pennata                           CC-CAGTTGGTT--GTG-G-----TTACCTCATG-CAGGTCGA-GCGCGCC-CCC-----------------------GCGGCGCGCG-------TCACG
Leskeella nervosa                         CC-CAGTCGGTG---GCG-G-----TTACCTCGTG-CAGGTCGA-GCGCGCC-CCC-----------------------GCGGCGCGCG-------TCACG
Heterocladium heteropterum                CC-CAGTCGGTG---TCG-G----CATTACCTCATG-CAGGTCGA-GCGCGCC-CCC-----------------------GCGGCGCGCG-------TCACG
Haplocladium virgnianum                   CC-CAGTCGGTT--GCGAG-----TTGCCTCATG-CAGGTCGA-GCGTGCC-CCCC----------------------GCGGCGCGCG-------TCACG
Leskea polycarpa                          CC-CAGTCGGTT--GCGAG-----TTGCCTCTTG-CAGGTCGA-GCGCGCC-CCCA----------------------GCGGCGCGCG-------TCACG
Platygyrium repens                        CC-CAGTCGGTT---TCC------CTGGCTCGTG-CAGGTCGA-GCGCGCC-CCC-----------------------GCGGCGCGCG-------TCACG
Haplocladium angustifolium                CC-CAGTCGGTT--GCGAG-----TTGCCTCCTG-CAGGTCGA-GCGCGCC-CCC-----------------------GCGGCGCGCG-------TCACG
Leskea gracilescens                       CC-CAGTCGGTT--GCGAG-----TTGCCTCCTG-CAGGTCGA-GCGCGCCGCCCCCCC-----------------------GCGGCGCGCG-------TCACG
Rhytidium rugosum                         CC-CAGTTGGTT--GTG-G-----TTACCTCGTG-CAGGTCGA-GCGCGCC-CCC-----------------------GCGGCGCGCG-------TCACG
Pterigynandrum filiforme                  CC-CAGTCGGCT--GCGAG-----TTGCCTCCTG-CAGGTCGA-GCGCGCCGCCCCC-----------------------GCGGCGCGCG-------TCACG
Thuidium delicatulum                      CC-CARTCGGTT---GTG-G-----TTACCTCACG-CANGTCGA-GCGCGCC-CCCTGAACGGC----------------------GGCGCGCGCG-------TCACG
Amblystegium serpens                      CC-CAGTTGGCT---CCG-G-----TTACCTCGTG-CAGGTCGG-GCGCGCC-CCCCGAATGGG----------------------GCGGCGCGCGTCACG--TCACG
Amblystegium fluviatile                   CC-CAGTTGGCT---CCGTT-----TTTCCTCGTG-CAGGTCGG-GCGCGCC-CCCCGGATGGG----------------------GCGGCGCGCGTCACG--TCACG
Amblystegium humile                       CC-CAGTTGGCT---A----------TTACCCATG-CAGGTC----CCG--CC-CCC-----------TTGGCGCTC--TCGGGATCGGCGGAAGGGCGCTGCG-------TCACG
Hookeria lucens                           CC-CAGTTGGTC---GT---------TTGCCCCATG-CAGGTC----CCG--CC-CCC-----------TTGGCGCTC--TCGGGATCGGCGGAAGGGCGCTGCG-------TCACG
Fabronia ciliaris                         CC-CAGTTGGTT---G----------TTACCTCATG-CAGGTC---CCG--CC-CCC-----------TCGGCGCTCCCTTCGGGA-CGCGCCAA-GGGGCAGCG-------TCACG
Myurella sp.                              CC-CAGTTGGTT---G----------TTACCTCATG-CAGGTCCGGCCG---C-----------TTGGCGCTC--TTCGGGAGCGC--GAA-GTGGCTGCG-------TCACG
Isopterygiopsis muelleriana               CC-CAGTTGGCT---G----------CTACCTCATG-CAGGTC---CCGCGCC-CCCCC-----TTGCCGCTCC-TCGGGACGC--GAA-CGGGCTGCG-------TCACG
Platydictya jungermannioides              CC-CAGTTGGTT---A----------TTACCCCATG-CAGGTC---CCG--CC-CCC-----------TTGGCGCTCC-TTCGGGAGCGC--GAA-GTGGCGTGCG-----TGTCACG
Plagiothecium denticulatum                CC-CAGTCGGTC---GTT-GGTCG--TTACCTCATG-CAGGTC---CCGGGTC-CCCC----------TTGGCGCTCC-TTCGGGAGCGC--GAA-GTGGCGTGCG-------TCACG
Habrodon perpusillus                      CC-CAGTCGGTT---GTT-G-----TTACCTCGTG-CAGGTC---CCG---C-----------TCGGTGCTCC-TCCGGGAGTGCGCAGA-----GTGCG-------TCACG
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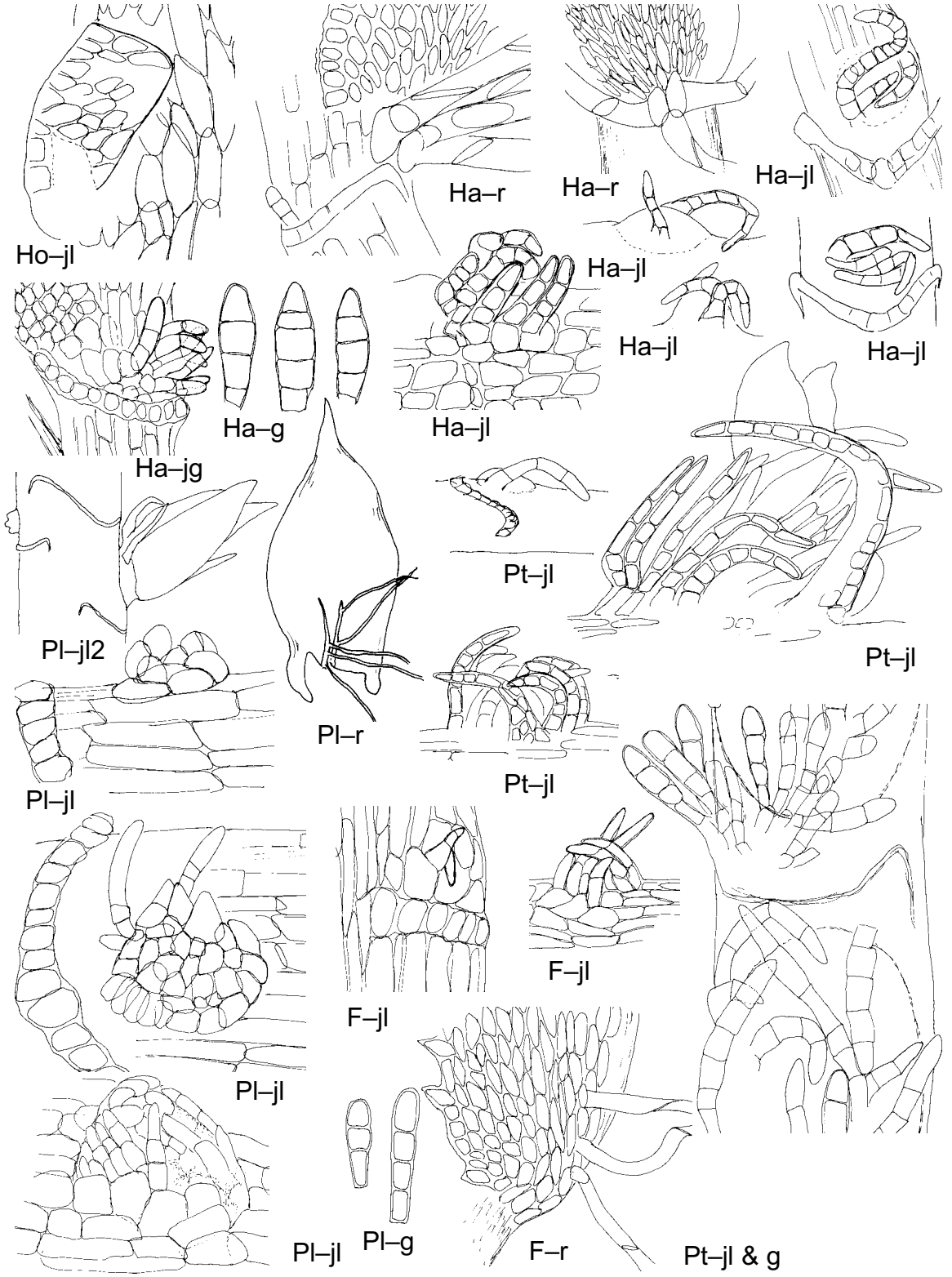

Fig. 5. Axillary gemmae (-g), juvenille gemmae (-jg), rhizoid position (-r) and branch initals showing variation in arrangements of juvenille branch leaves (-jl) in F – *Fabronia ciliaris* (Brid.) Brid.; Ha – *Habrodon perpusillus* (De Not.) Lindb.; Ho – *Hookeria lucens* (Hedw.) Sm.; Pl – *Plagiothecium laetum* B. S. G.; Rt – *Pterigynandrum filiforme* Hedw. All (except Pl-jl2, Pl-r) – ×260; Pl-jl2 – ×60, Pl-r – ×20.

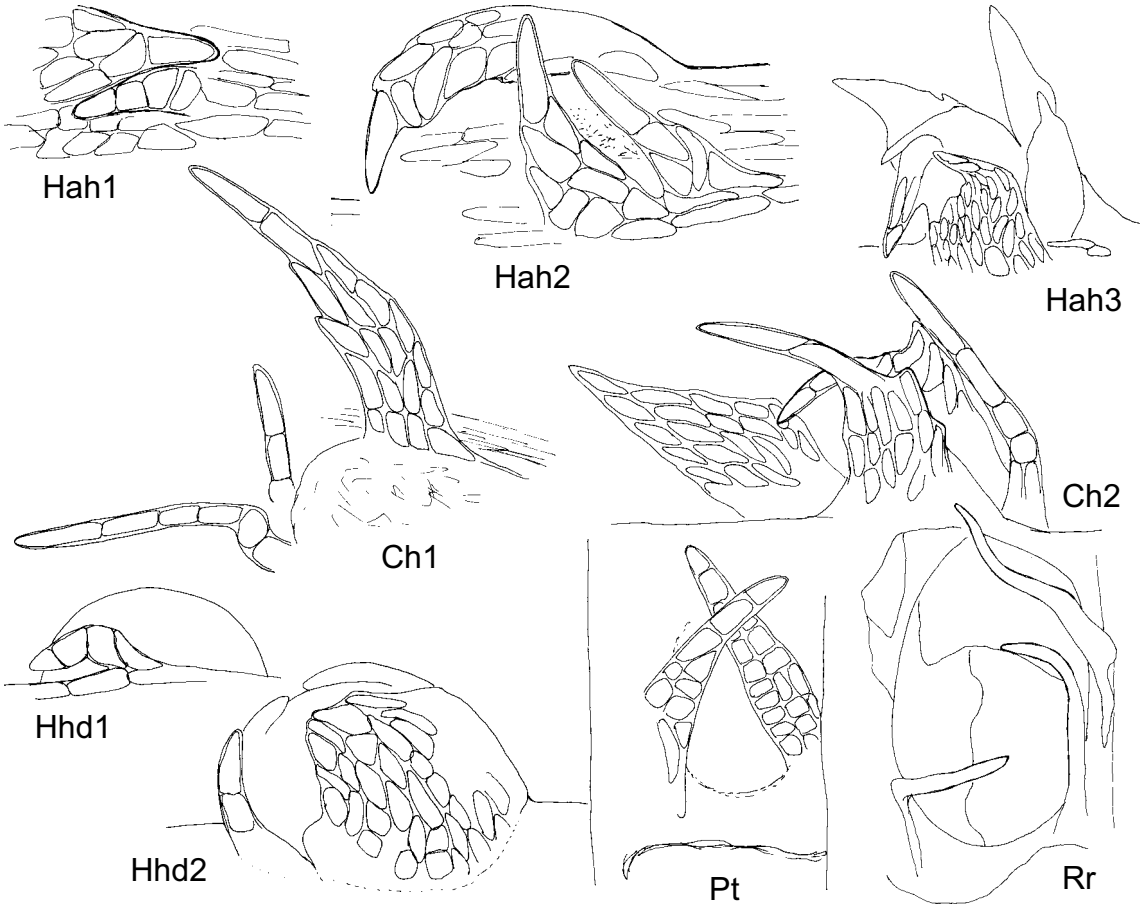

Fig. 6. Branch initials showing variation in arrangements of pseudoparaphyllia (note that they are situated at the certain distance from the raising of initial). Ch – *Campyophyllum halleri* (Hedw.) Fleisch.; Hah – *Hygroamblystegium humile* (P. Beauv.) Vanderpoorten & al.; Hhd – *Hygrohypnum duriusculum* (De Not.) Jamieson; Pt – *Pseudoleskeella tectorum* (Funck ex Brid.) Kindb.; Rr – *Rhytidium rugosum* (Hedw.) Kindb. [stem apex is to the left or to the top of figure]. All (except Rr) – ×320; Rr – ×80.

The peristome of *Habrodon* is strongly modifyed: endostome is almost absent, and exostome teeth are thin, smooth from outside (Ignatova & Ignatov, 2003). These peculiarities where considered taxonomically important in the recent past, but the advances of molecular pleurocarp systematics demonstrate more and more cases of rapid changes in peristome structure in lineages turned to epiphytic growth: *Anacamptodon* in Amblystegiaceae (Vanderpoorten & al., 2002a,b), *Struckia* in Plagiotheciaceae (Pedersen & Hedenäs, 2002), numerous genera in all four subfamilies of Brachytheciaceae (Ignatov & Huttunen, 2002, Huttunen & Ignatov, 2004) and in both Brachytheciaceae, Meteoriaceae and Lembophyllaceae (Huttunen & al., 2004), etc. The almost total reduction of endostome is more rare, but still known in, e. g. *Fabronia, Rhizofabronia, Struckia*, *Neckera, Leucodon, Iwatsukiella,* etc.

As the role of peristomial characters for pleurocarp systematics appear to be animadversed, the families circumscribed mainly using sporophytic characters appear to be in urgent need of reconsideration.

Leskeaceae were found in the present analyses to be polyphyletic (Fig. 1, also 2-3). *Leskea* itself was found most closely related to *Haplocladium*, and actually most differences between these two genera are in sporophytic characters, while paraphyllia and papillosity pattern of laminal cells of them are essentially identical. *Pseudoleskeella* were found unrelated to *Leskea*, but always close to *Rhytidium*, sometimes with very high support (Fig. 2). The monophyly of these two genera is very unexpected, at least

nobody considered them close as relatives before. The morphological synapomorphies of *Rhytidium* and *Pseudoleskeella* are almost none. However one might notice that most differences of the latter are those associated with epiphytism (straight capsule, partial reduction of peristome, small size, short laminal cells). Having in mind trends from *Amblystegium* to *Anacamptodon*, from *Cirriphyllum* to *Clasmatodon*,and partly from *Homalia* to *Neckera*, it would be difficult to find a serious counter-argument against the possible relationship of these two genera. Of course, their relationship must be explored more carefully including many more species before involving into systematics.

Pterigynandraceae sensu Buck & Goffinet (2000) are represented in our analysis by all five genera (i. e. *Habrodon, Myurella, Heterocladium, Iwatsukiella, and Pterigynandrum*). The present analysis does not confirm their monophyly, cf. Figs. 1, also 2-3.

In one case, the similarity in the peristome reduction pattern and in the exostome ornamentation might be considered as a synapomorphism. Two of three present analyses revealed among others a clade composed by *Platygyrium* and *Entodon*. The former genus was usually placed in Hypnaceae, but Brotherus (1923) considered it as a memeber of Entodontaceae. Later Brotherus (1925), placed it back to Hypnaceae, and this concept was almost universally followed throughout 20th century. The outstanding similarity of the two was demonstrated by SEM studies of exostome by Ignatov & al. (1996). At least some of recent rbcL-based analyses also found these two genera rather closely related (Arikawa & Higuchi, 2003) [at least *Platygyrium* is closer to *Entodon*, than to *Pylaisia* and most species of *Hypnum*].

The present analysis also provides an additional evidence in favour of the broad undestanding of Plagiotheciaceae (Hedenäs, 1987; Pedersen & Hedenäs, 2002), a family circumscribed mainly by gametophytic characters.

Among the important morphological synapomorphies of Plagioteciaceae is the attachment of rhizoids in position other than just below leaf insertion (which is most common in pleurocarpous mosses). In Plagiotheciaceae rhizoids are either axillary (*Myurella, Platydictya, Isopterygiopsis*, etc.), or confined to the dorsal surface of costa near leaf base (*Plagiothecium*). The rhizoid position on adaxial costa in *Fabronia* is identical to that in *Plagiothecium,* unlike all other species included in the present analysis where rhizoids are on stem just below the leaf insertion (except *Calliergon* with rhizoids diffusely arranged on stem). Rhizoids in *Habrodon* were found in most common for pleurocaps position (Fig. 5), but Hedenäs (1992) noted that besides this pattern, they sometimes are sitting on dorsal costa. This difference in observations might be a result of a limited number of rhizoids in our material.

CONCLUDING REMARK

The topology of the phylogenetic trees (Figs. 1-3), characteristic indels (Fig. 4), and some rare morphological characters indicate a relationship of *Habrodon* with Plagiotheciaceae, *Fabronia,* and maybe also *Pterigynandrum*. However, all four groups are not forming a clade, and are differently interrelated depending of method of analysis. Unlike other members, *Fabronia* lacks axillary gemmae. Rhizoid position of *Fabronia* and Plagiothecium is different from that in *Habrodon* and *Pterigynandrum*. Differences between *Habrodon* and *Pterigynandrum* are numerous:

Character	*Habrodon*	*Pterigynandrum*
plants	small	medium-sized
leaves	obtuse	acuminate
leaf margin	sinuose	serrate to serrulate
costa	double	double to single
lamina cells	rhombic to ovate	linear-elongate
lamina cells	smooth	papillose
peristome	single	double
exostome	smooth	striolate

Summing up, we suggest better to keep the genus *Habrodon* in the monogeneric family of Habrodontaceae, as was suggested by Schimper (1860).

The present analysis supports the segregaion of *Iwatsukiella* from *Habrodon* (Buck & Crum 1978).

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