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The systematic position of the Hypopterygiaceae (Bryopsida) inferred from *rps*4 gene sequences

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Abstract — Phylogenetic relationships of the Hypopterygiaceae within the Bryidae were assessed by comparison of DNA sequences of the *rps*4 gene of the chloroplast genome. A total of 26 taxa were examined in this study. 20 taxa were newly sequenced for this analysis. The phylogenetic trees obtained from maximum parsimony (MP) and maximum likelihood (ML) methods were consistent in the main clades. *Cyathophorum bulbosum* (Hedw.) Müll.Hal. and *Cyathophorum adiantum* (Griff.) Mitt. are separated from the rest of the Hypopterygiaceae. The remaining genera of the Hypopterygiaceae form a monophyletic clade sister to the "pleurocarpous" mosses, contradicting a proposed placement within the Bryales.

Bryopsida / Hypopterygiaceae / chloroplast DNA / *rps*4 gene / molecular systematics / cladistic analysis / phylogeny

INTRODUCTION

The Hypopterygiaceae Mitt. s.l. (Kruijer, 1990) are a small family of "pleurocarpous" mosses, comprising 21 species in 7 genera: *Canalohypopterygium* W. Frey & Schaepe, *Catharomnion* Hook. f. & Wilson, *Cyathophorum* P. Beauv., *Dendrocyathophorum* Dixon, *Dendrohypopterygium* (Hedw.) Kruijer, *Hypopterygium* Brid. and *Lopidium* Hook. f. & Wilson. They have mainly a Gondwana distribution and usually occur in humid forests of warm temperate to tropical areas (Kruijer, 2002).

In the past, the Hypopterygiaceae were regarded as a monophyletic group belonging to the Hookeriales (e.g. Kindberg, 1901; Fleischer, 1908; Brotherus, 1925). During the last thirty years the classification and phylogenetic status of the Hypopterygiaceae has been questioned. Based on morphological characters the family was discarded and the genera formerly belonging to the Hypopterygiaceae were transferred to the Daltoniaceae and Hookeriaceae (both in the Hookeriales, Crosby, 1974). Buck and Vitt (1986) placed the Hypop-

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terygiaceae within the Bryales. Buck (1987) transferred *Cyathophorum* and *Cyathophorella* Broth. to the Hookeriaceae and maintained *Hypopterygium, Lopidium* and *Catharomnion* in the Hypopterygiaceae within the Bryales. In contrast, Kruijer (1997) included all the former genera in the Hypopterygiaceae, but questioned the systematic position of *Cyathophorum bulbosum* (Hedw.) Müll.Hal. A new classification of mosses (Buck & Goffinet, 2000), which takes into account recent morphological data as well as new implications of molecular data (e.g. Buck *et al.*, 2000), maintained the Hypopterygiaceae, comprising the genera *Canalohypopterygium, Catharomnion, Hypopterygium* and *Lopidium,* within the Hookeriales, whereas the genera *Cyathophorella, Cyathophorum* and *Dendrocyathophorum* were shifted to the Hookeriaceae.

Most recently Kruijer (2002) published his extensive taxonomic and phylogenetic study on the Hypopterygiaceae. Based on cladistic studies of morphological characters he proposed a monophyletic status of the seven genera of the Hypopterygiaceae. He merged the genus *Cyathophorella* with *Cyathophorum*. Furthermore, two species of the genus *Hypopterygium*, *H. filiculiforme* (Hedw.) Brid. and *H. arbuscula* (P. Beauv.) Brid. were transferred to the newly described genus *Dendrohypopterygium* (*D. filiculiforme* (Hedw.) Kruijer, *D. arbuscula* (Brid.) Kruijer).

Based on sequences of the *rsp*4 gene, as a good marker for phylogenetic studies (e.g. Goffinet *et al.*, 2001), we reinvestigated the Hypopterygiaceae using representatives of the Bryales, Splachnales, Hookeriales and Hypnales, in order to provide new molecular based evidence for its systematic position.

Recent cladistic studies based on morphological (Hedenäs, 1996), molecular (Buck *et al.*, 2000) and combined data sets (De Luna *et al.*, 1999) only addressed the relationships within "pleurocarpous" mosses in general and were performed using only a limited sample of genera of the Hypopterygiaceae s.l. (*Hypopterygium arbuscula, Hypopterygium tamariscinum* (Hedw.) Brid., *Cyathophorum bulbosum*). Furthermore, cladistic analyses where all genera of the Hypopterygiaceae were investigated used solely morphological data (Kruijer, 2002), or the molecular based data focussed on interfamilial relationship within the Hypopterygiaceae s.l. (Stech *et al.*, 1999, 2002).

This is the first study addressing the systematic position of the Hypopterygiaceae within the subclass Bryidae based on an extensive generic sampling of the family using sequences of the *rps*4-gene.

MATERIALS AND METHODS

Plant materials

Overall, we included 26 species in our analysis. For twenty species the *rps*4 sequences were newly obtained (Tab. 1, no. 1-20). In addition we used the sequences of six species from the GenBank (Tab. 1, no. 21-26). Voucher information, GenBank accession numbers and the current systematic position (Buck & Goffinet, 2000) for all specimens used in the phylogenetic analysis are presented in Table 1.

DNA methods

Total DNA was extracted from herbarium material (0.1-0.3 g) and ground to a fine powder in liquid nitrogen by the CTAB procedure (Doyle & Doyle, 1990). The DNA, dissolved and diluted in TE buffer, was used for amplification reactions. The amplification primers used were those described by Nadot (1994) with the forward primer located on position 1 to 17 in the *rps*4-gene. Amplification was done using the Ready TO GO PCR beads (Amersham Pharmacia Biotech) in a Stratagene gradient Robo Cycler.

A typical amplification assay included an initial denaturation (4 min, 94°C) followed by 30 cycles with 1 min denaturation at 94°C, 1 min annealing at 54 °C and 2 min extension at 72°C, with a final elongation period of 8 min at 72°C. Three independent PCR reactions were pooled and separated on 1% agarose gels (Sambrook & Russel, 2001). Bands of the expected size were excised from the gels and purified with the extraction kit (Machery-Nagel AG) according to the manufacturer's instructions.

The isolated bands of the PCR product were cloned using a Topo TA cloning kit (Invitrogen). Double stranded fluorescence sequencing from 3-5 positive clones containing the expected fragment was performed using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) with the plasmid primers in order to acquire the sequence of the complete PCR-product. Sequencing products were analysed on an ABI 377 DNA sequencing system (Applied Biosystems). Start and stop codon for the *rps*4 gene were identified according to Ohyama *et al.* (1984). Alternatively, the purified PCR products from the species were sequenced (*Cyathophorum adiantum,* (Griff.) Mitt. *Dendrocyathophorum decolyi* (Broth. ex M.Fleisch.) Kruijer and *Schimperobryum splendidissimum* (Mont.) Marg.) using the manual radioactive or the automated technique described above. In the latter case, the same primers as for PCR amplification were used.

Data analysis

Two different data sets were used in the analysis. The first data set consisted of 26 representative species of six different orders. The analysis of this data set was addressed to the systematic position of the Hypopterygiaceae s.l. within the Bryidae using *Timmia austriaca* Hedw. (Timmiales) and *Funaria hygrometrica* Hedw. (Funariales) as outgroup taxa. The analysis of the second data set comprises nine species of the Hypopterygiaceae identified as a monophyletic group and four outgroup taxa. It was addressed to gain information on the intrafamiliar relationship within the Hypopterygiaceae.

For the alignment, the multicolour Alignment Editor Align32 (Hepperle, 1997) was used. The sequences were aligned manually. The data sets were reduced to 576 base pairs in order to enable a sensible alignment with the partial sequences from the databank and *Schimperobryum splendidissimum.*

Phylogenetic tree construction was done with PAUP* 4.0b8 (Swofford, 2001) using the two optimality criteria maximum parsimony (MP) and maximum likelihood (ML). Phylogenetic reconstruction was carried out for the first data set (26 taxa) according to the MP principle as a "heuristic" search where all most parsimonious trees (MULPARS) were saved, branch swapping by tree bisection and reconnection (TBR) and random taxon addition (1000 replicates). All characters were equally weighted, character states were treated as unordered, and gaps as missing data. For the second data set (13 taxa), comprising thirteen taxa, a "branch

Tab. 1. Systematic position (Buck & Goffinet, 2000), Voucher information, GenBank Accession no., gene length and GC-content. Superscript number in front of the species refers to the herbarium where the voucher is deposited: [1] Herbarium R. Blöcher, Botanisches Institut der Universität Bonn; [2] DNA collection of Prof. I. Capesius, Botanisches Institut der Universität Heidelberg, now with Prof. Frahm, Botanisches Institut der Universität Bonn; [3] Voucher specimen held by M. Stech and T. Pfeiffer, Systematische Botanik und Pflanzengeographie, Freie Universität Berlin. The systematic position of the genera of the Hypopterygiaceae s.l. is in accordance with Kruijer (1995). The taxonomy of the Hypopterygiaceae follows Kruijer (2002).

	Taxon	*Family (order)*	*Voucher or Reference no*	*GenBank Accession No*	*Sequence length [bp]*	*GC-content [%]*	*country of origin*
1	[1] *Bryum capillare* Hedw	Bryaceae (Bryales)	*Blöcher 990530/1* (det. R. Blöcher	AJ269691	592	28.5	Germany
2	[1] *Canalohypopterygium tamariscinum* (Hedw.) Kruijer	Hypopterygiaceae (Hookeriales)	Frahm 9-1 (det. J.-P. Frahm)	AJ269694	592	27.2	New Zealand
3	[1] *Catharomnion ciliatum* (Hedw.) Wilson	Hypopterygiaceae (Hookeriales)	*W. Frey & T. Pfeiffer 98-Z 132 B* (det. W. Frey & T. Pfeiffer)	AJ269695	592	26.2	New Zealand
4	[3] *Cyathophorum adiantum* (Griff.) Mitt. (syn. *Cyathophorella tonkinensis* (Broth. & Paris) Broth.)	Hypopterygiaceae (Hookeriales)	*Yamaguchi* s.n. (det. T. Yamaguchi)	AJ315872	592	26.7	Japan
5	[1] *Cyathophorum bulbosum* (Hedw.) Müll.Hal.	Hypopterygiaceae (Hookeriales)	*Frahm 1-1a* (det. J.-P. Frahm)	AJ269693	592	26.7	New Zealand
6	[3] *Dendrocyathophorum decolyi* (Broth. ex M.Fleisch.) Kruijer	Hypopterygiaceae (Hookeriales)	*Matsui* s.n. (det.T. Matsui)	AJ271645	592	25.2	Japan
7	*Dendrohypopterygium arbuscula* (Brid.) Kruijer (syn. *Hypopterygium arbuscula* (P. Beauv.) Brid.)	Hypopterygiaceae (Hookeriales)	*Frey & Frey 95-17* (det. H. & W. Frey)	AJ252293	592	26.9	Chile
8	*Dendrohypopterygium filiculiforme* (Hedw.) Kruijer (syn. *Hypopterygium filiculiforme* (Hedw.) Brid.)	Hypopterygiaceae (Hookeriales)	Frahm 31-17 (det. J. D. Kruijer, 1999)	AJ252290	592	26.7	New Zealand
9	[2] *Funaria hygrometrica* Hedw.	Funariaceae (Funariales)	*Capesius 95-07* (det. M. Stech)	AJ250120	589	29.7	Germany
10	[1] *Hookeria lucens* (Hedw.) Sm.	Hookeriaceae (Hookeriales)	*Blöcher 980328/1* (det. R. Blöcher)	AJ269689	592	27.4	Germany

11	[2] *Hylocomium splendens* (Hedw.) B.S.G.	Hylocomiaceae (Hypnales)	*Stech 951203* (det. M. Stech)	AJ250457	592	27.9	Germany
12	[2] *Hypnum cupressiforme* Hedw.	Hypnaceae (Hypnales)	*Capesius 95-12* (det. M. Stech)	AJ269690	592	27.9	Germany
13	[1] *Hypopterygium didictyon* Müll.Hal.	Hypopterygiaceae (Hookeriales)	*Frahm 9-7* (det. J. D. Kruijer)	AJ252292	592	27.5	New Zealand
14	[3] *Hypopterygium tamarisci* (Sw.) Brid. ex Müll.Hal.	Hypopterygiaceae (Hookeriales)	*O'Shea 99E28a* (det. B. J. O'Shea)	AJ252291	592	27.2	South Africa
15	[1] *Leucodon sciuroides* Hedw.	Leucodontaceae (Hypnales)	*Blöcher 961115/7* (det. R. Blöcher)	AJ269688	592	27.2	Germany
16	[1] *Lopidium concinnum* (W.Hook.) Wilson	Hypopterygiaceae (Hookeriales)	*Frahm 1-1b* (det. J.-P. Frahm)	AJ252289	595	27.6	New Zealand
17	[3] *Lopidium struthiopteris* (Brid.) M.Fleisch.	Hypopterygiaceae (Hookeriales)	*O'Shea 99C14b* (det. B.J. O'Shea)	AJ252288	595	27.4	South Africa
18	[1] *Neckera crispa* Hedw.	Neckeraceae (Hypnales)	*Blöcher 990705/2* (det. R. Blöcher)	AJ269692	592	28.2	Ireland
19	[1] *Schimperobryum splendidissimum* (Montagne) Margadant	Hookeriaceae (Hookeriales)	*Blöcher 01-09-1* (det. R. Blöcher)	AJ315873	573		Chile
20	[1] *Splachnum sphaericum* Hedw.	Splachnaceae (Splachnales)	*Blöcher 990707/1* (det. R. Blöcher)	AJ250183	592	28.0	Austria
21	*Timmia austriaca* Hedw.	Timmiaceae (Timmiales)	*Schofield 98363*	AF223035	see Goffinet & Cox (2000)		see Goffinet & Cox (2000)
22	*Adelothecium bogotense* (Hampe) Mitt.	Adelotheciaceae (Hookeriales)	*Buck 26301*	AF143073	see Buck *et al.* (2000)		see Buck *et al.* (2000)
23	*Garovaglia elegans* (Dozy & Molk.) Bosch & Lac.	Garovagliaceae (Hookeriales)	*Streimann 40482*	AF143017	see Buck *et al.* (2000)		see Buck *et al.* (2000)
24	*Neorutenbergia usagarae* (Dixon) Bizot & Pócs	Rutenbergiaceae (Hypnales)	*Pócs et al. 88110/A*	AF143019	see Buck *et al.* (2000)		see Buck *et al.* (2000)
25	*Ptychomnion aciculare* (Brid.) Mitt.	Ptychomniaceae (Hookeriales)	*Hiscox 3*	AF143015	see Buck *et al.* (2000)		see Buck *et al.* (2000)
26	*Fontinalis dalecarlica* Bruch & Schimp.	Fontinalaceae (Hypnales)	*Allen 20153* (MO)	AF143064	see Buck *et al.* (2000)		see Buck *et al.* (2000)

and bound" search was performed with an initial upper bound of 158 and by adding the furthest sequence. This upper bound was previously detected by a heuristic search as score of the most parsimonious tree (MPT). Bootstrap values were generated with 1000 replicates using a heuristic search with random taxon addition (100 replicates) and the same options for each data set as above in effect. For all generated maximum parsimony trees the consistency index (CI) and retention index (RI) as well as tree length were calculated. Branch support (Bremer, 1994) was estimated for the consensus tree (data set one) and the MPT (data set two) with the program AutoDecay vers. 4.0 (Eriksson, 1999) using the same settings as in the parsimony analyses. The likelihood for the gamma shape parameter (data set one: 0.833655; data set two: 0.67071) and the base frequencies (data set one: A=0.40758, C=0.15394, G=0.14233, T=0.28977; data set two: A=0.40900, C=0.14191, G=0.13519, T=0.31390) were computed from one of the fifteen most-parsimonious-trees (tree No. 15, chosen at random) generated by the maximum parsimony criterion for the first data set, and for the second data set from the single most parsimonious tree. Six substitution types were assumed according to the results in table 2. The values were then used as settings under the likelihood criterion. The substitution rate-matrix parameters were estimated via maximum likelihood. These settings correspond to the general time reversible model (GTR). In the heuristic search, the furthest sequence was added and branches were swapped by the TBR option. To test the phylogenetic signals in the data set, the g_1 statistics of the distribution of 100,000 random trees was calculated (Hillis & Huelsenbeck 1992) using the "RANDTREES" option in PAUP. MEGA (Kumar *et al.*, 1993) was used to determine the substitution rates (estimated as pairwise comparisons) and the base composition.

RESULTS

Sequence analysis

The PCR amplification resulted in a single product for the *rps*4 gene for all twenty sequences. For nineteen species (Tab. 1, no. 1-18, 20) the sequences obtained ranged from position 18 to the stop codon of the *rps*4-gene. The length of these sequences ranged from 589 bp in *Funaria hygrometrica* to 595 bp in *Lopidium concinnum* (W.Hook.) Wils. and *Lopidium struthiopteris* (Brid.) M.Fleisch. The remaining sixteen species revealed 592 bp each. The GC content was highest in *Funaria hygrometrica* (29.7 %) and lowest in *Catharomnion ciliatum* (Hedw.) Wilson (26.2 %) (Tab. 1). The base composition was biased to high AT values. The alignment in both data sets consists of 576 base pairs. The data set comprising 26 species has 108 informative positions, while the one with the 13 species has only 54 (Tab. 2). The average value of the ts/tv ratios for the complete set of 26 species was 2.8, for the set of 13 species it was 3.8. The ratios obtained by pairwise species comparisons varied greatly within each data set, resulting in high standard deviations (Tab. 2). One tree was retained under the likelihood criterion with the best score of 2893.08718 for the first data set and with 1604.91524 for the second. The length variation of a random sample of

Tab. 2. Number of sites, sequence variation, base composition, substitution rates and values of the tree statistics for the two data sets.

	Dataset 1 (26 species incl. 2 outgroup taxa)	*Dataset 2 (13 species incl. 4 outgroup taxa)*
Sites in alignment	576	576
g_1-value	−0.878	−0.933
Sequence variation:		
Constant	363	467
Uninformative	105	55
Informative	108	54
Average base composition:		
A	0.40	0.40
C	0.14	0.13
G	0.14	0.14
T	0.32	0.33
AT/GC	72/28	73/27
Substitution rate:		
Transition-transversion ratio	2.8 (± 1.7)	3.8 ± (2.0)
AG	16.4	14.1
TC	10.7	8.9
AT	3.4	2.1
AC	4.5	2.6
TG	2.3	1.4
CG	1.8	1.0
Tree statistics:		
Most parsimonious trees	15	1
Tree length	413	158
RI	0.574	0.669
CI	0.630	0.753

100,000 trees shows a left-skewed frequency curve (data set one: g_1= –0.878; data set two: g_1= –0.933). These results suggest that both data sets contain a phylogenetic signal (Hillis & Huelsenbeck, 1992).

The values for the branch support range from 0 to 24 in the first data set (Fig. 1). Most of the clades have a branch support of 1. The highest values are those of the clade *Ptychomnion aciculare* (Brid.) Mitt.-*Garovaglia elegans* (Dozy

& Molk.) Bosch & Lac. with 24, and the *Lopidium*-clade with 10. In the second data set (Fig. 4) the values for branch support range from 1 to 10.

Phylogenetic analysis

The MP analyses resulted in fifteen most parsimonious trees (MPTs) for the first data set (26 species) and one MPT for the second data set (13 species). One tree was obtained from the ML analysis of each data set. Both of these trees supported the main clades obtained in the MP trees and are therefore not depicted separately.

Analysis of the data set with 26 species using Funaria hygrometrica *and* Timmia austriaca *Hedw. as outgroup taxa*

The strict consensus tree of the 15 MPTs (Fig. 1) shows *Bryum capillare* Hedw., as a representative of the Bryales, and *Splachnum sphaericum* Hedw. (Splachnales), separated from the "pleurocarpous" clade A, with a high bootstrap support (branch support 4, Fig. 1). The close affinity of *Cyathophorum bulbosum* and *Cyathophorum adiantum* in this study is expressed by high bootstrap values (branch support 3) as shown in Fig. 1. *Cyathophorum bulbosum* and *Cyathophorum adiantum* appear either at a basal position of the Hypopterygiaceae (clade B, Fig. 2) or as sister group to both clade B and the representatives of the Hookeriales and Hypnales (clade C, Fig. 3). In none of the optimal trees *Cyathophorum* was placed sister to clade C. The remaining 9 species of Hypopterygiaceae appear as a monophyletic group (clade B) sister to clade C which contains the representative species of the Hookeriales and Hypnales as well as taxa which in recent studies (see discussion) were linked with the Hypopterygiaceae s.l.

Clade B was investigated further in order to obtain some information on the intrafamiliar relationship of the Hypopterygiaceae.

Analysis of the data set with 9 species of Hypopterygiaceae and four species as outgroup

The 9 species of Hypopterygiaceae form a well-supported monophyletic group (94%, branch support 5) in our analysis (Fig. 4). They are separated into two sister clades: clades G and H. Clade G (Fig. 4) comprises *Dendrohypopterygium arbuscula*, *Dendrocyathophorum decolyi*, *Canalohypopterygium tamariscinum* (Hedw.) Kruijer and *Catharomnion ciliatum*. Clade H (Fig. 4) consists of *Hypopterygium didictyon* Müll.Hal. and *Hypopterygium tamarisci* (Sw.) Brid. ex Müll.Hal. (clade F), the two *Lopidium* species (clade E) and *Dendrohypopterygium filiculiforme*. The latter species occupies a basal position within clade H. Clade H was not recovered in the ML analysis due to the fact that *Lopidium concinnum* and *L. struthiopteris* appeared as the most basal taxa of the nine species of the Hypopterygiaceae (results not shown). The close relationships of the sister taxa *Hypopterygium didictyon* and *H. tamarisci* (clade F), as well as *Lopidium concinnum* and *L. struthiopteris* (clade E) and *Canalohypopterygium tamariscinum* and *Catharomnion ciliatum* (clade D), are well supported by high bootstrap and branch support values (Fig. 4). The basal position of

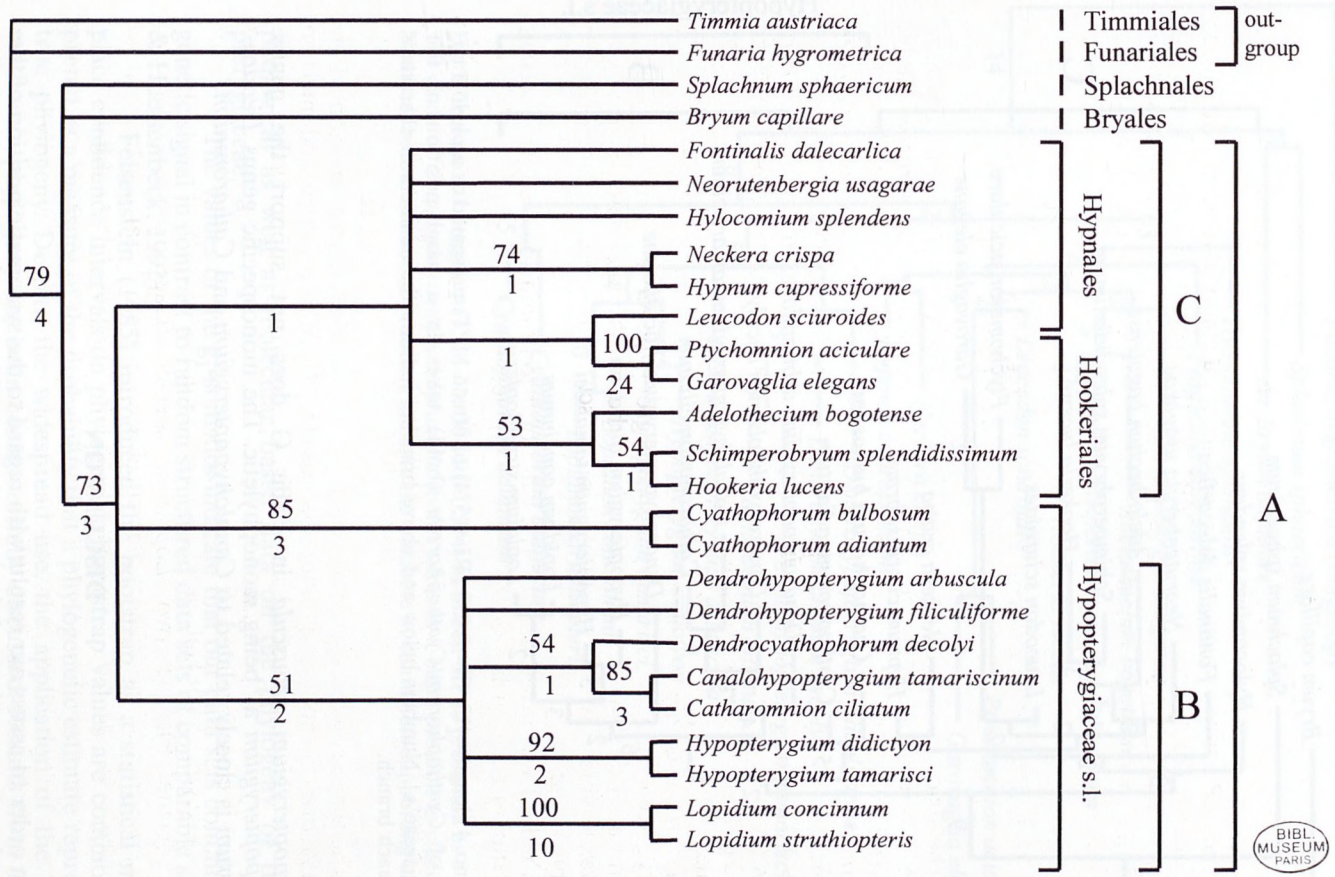

Fig. 1. Strict consensus of fifteen MPTs found by a heuristic search based on *rps*4 gene sequences of 26 species of the Hypopterygiaceae, Funariales, Timmiales, Bryales, Splachnales, Hookeriales, and Hypnales (in total 576 characters, including 108 informative characters). Numbers above branches are bootstrap values (1000 replicates). Numbers below branches are branch support values. *Timmia austriaca* and *Funaria hygrometrica* were defined as outgroup taxa.

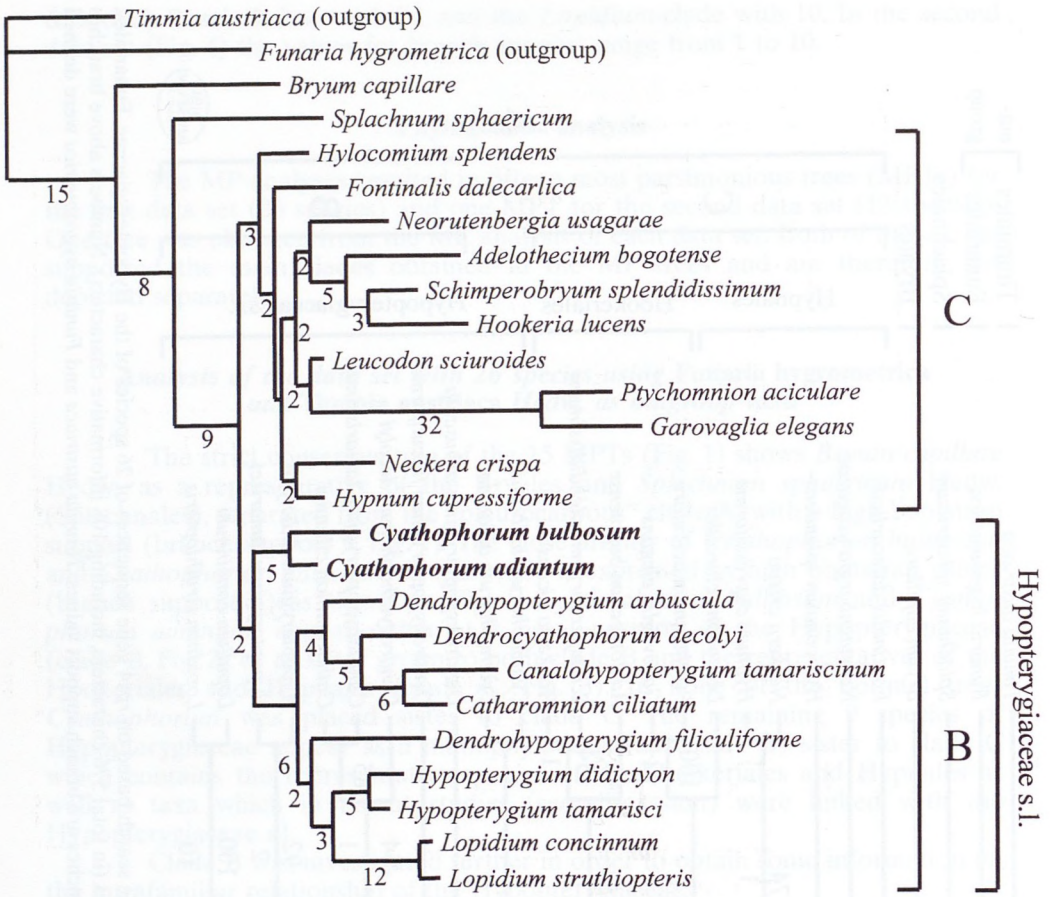

Fig. 2. Tree no. 4 (length=413, CI= 0.630 , RI=0.574) of fifteen MPTs presented as a phylogram. The clade of *Cyathophorum*/*Cyathophorella* (bold) takes in a basal position of the Hypopterygiaceae s.l. Numbers below and above branches indicate the numbers of characters supporting each branch.

Dendrohypopterygium arbuscula in clade G does not support the genus *Dendrohypopterygium* as being monophyletic. The monospecific genus *Dendrocyathophorum* is closely related to *Canalohypopterygium* and *Catharomnion*.

DISCUSSION

In order to assess our results with regard to the systematic position of the Hypopterygiaceae we estimated the g_1-statistics (Hillis & Huelsenbeck, 1992), the bootstrap values (Felsenstein, 1985), and the branch support (Bremer, 1994). Most important, we discuss our results from the cladistic analyses with existing mor-

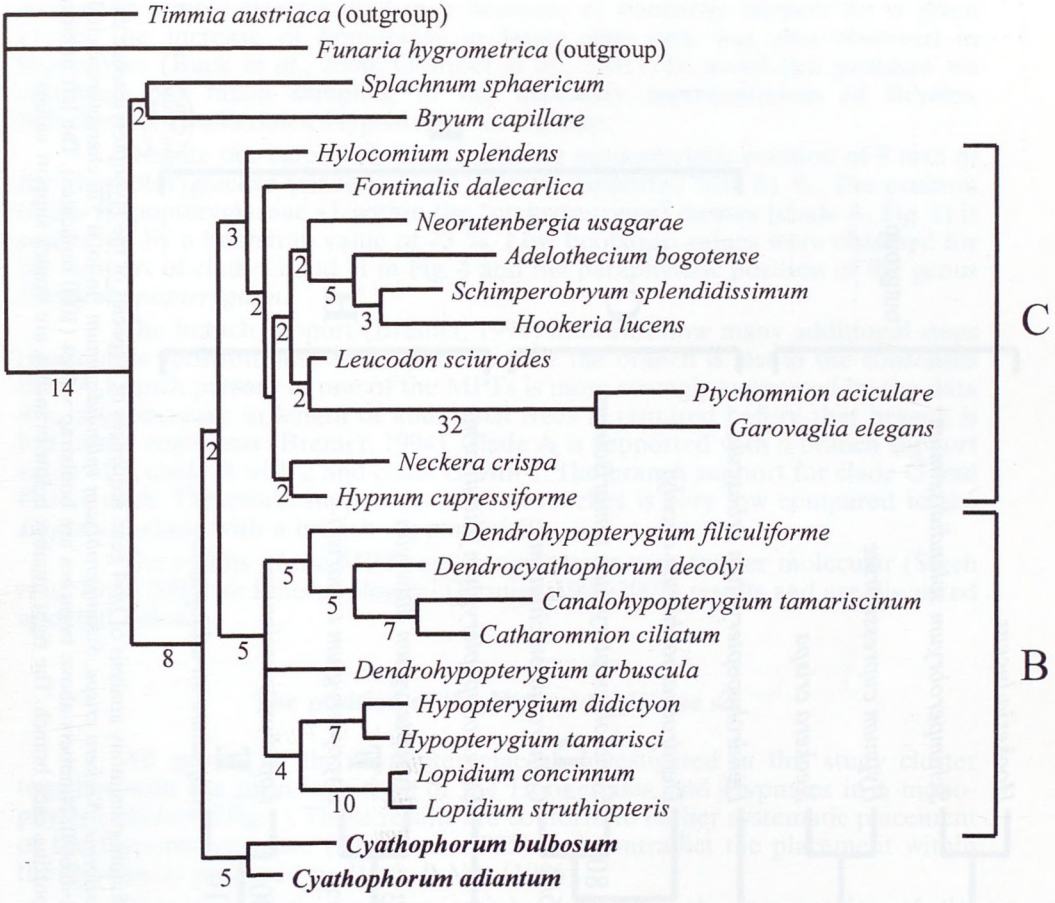

Fig. 3. Tree no. 10 (length=413, CI= 0.630 , RI=0.574) of fifteen MPTs presented as a phylogram. The clade of *Cyathophorum*/*Cyathophorella* (bold) takes in a basal position to clades B and C. Numbers below and above branches indicate the numbers of characters supporting each branch.

phological as well as molecular based data on the phylogeny of the Hypopterygiaceae.

The values of the g_1-statistics indicate that our data sets contain a phylogenetic signal in contrast to random structured data sets of comparable size (Hillis & Huelsenbeck, 1992).

Felsenstein (1985) introduced the bootstrap as a statistical method to place confidence intervals on phylogenies. Bootstrap values are commonly interpreted as a measure of the probability that a phylogenetic estimate represents the true phylogeny. Despite the widespread use, the application of the bootstrap method in phylogeny has been controversially discussed. Results from Sanderson & Donoghue (1989) indicated that if more taxa are added to an existing data set, the characters become prone to homoplasy. This results in a decrease of the consistency index (CI) value. Sanderson & Wojciechowski (2000) observed that an

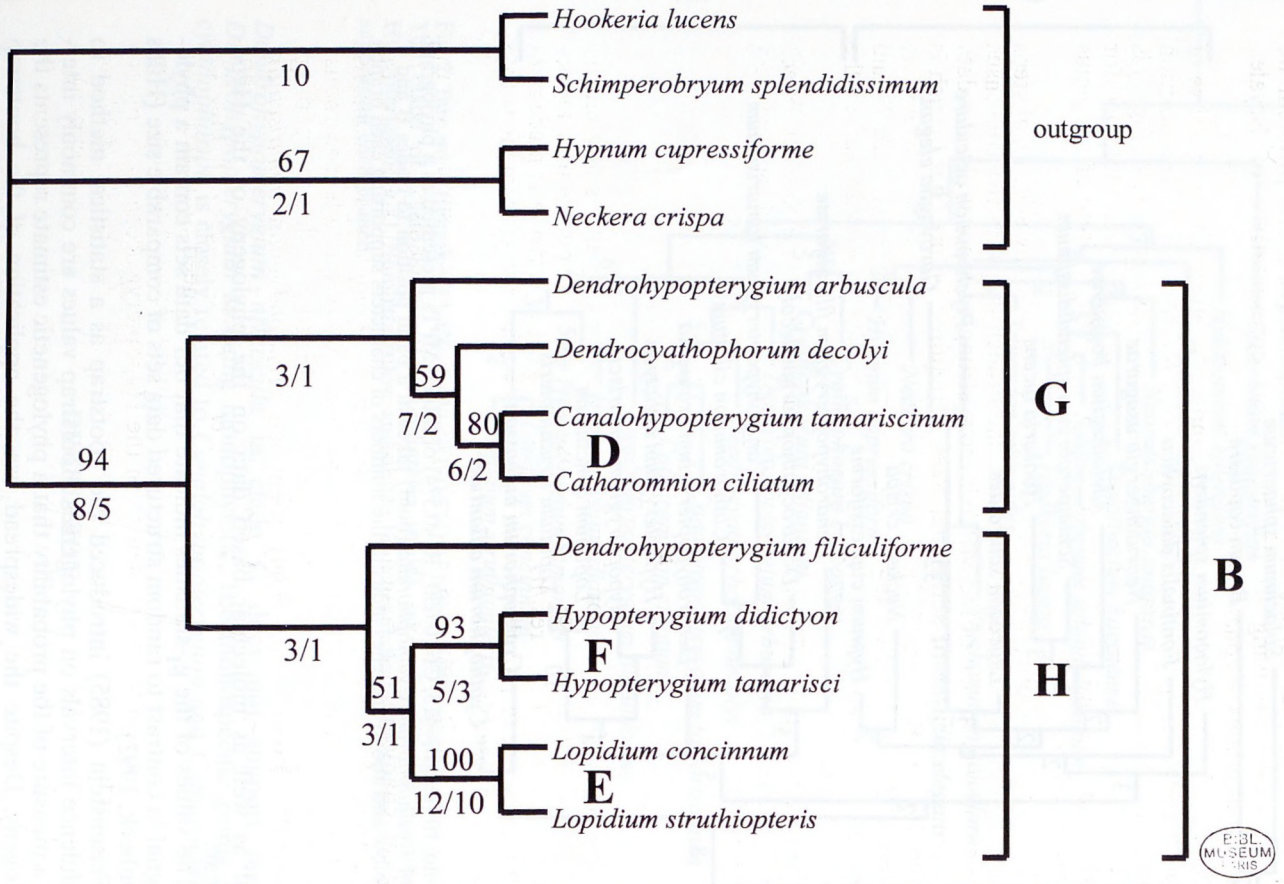

Fig. 4. Single MPT found by branch & bound search maximum parsimony analysis of *rps*4 gene sequences of 9 species of the Hypopterygiaceae and four outgroup species: *Hookeria lucens, Hypnum cupressiforme, Neckera crispa, Schimperobryum splendidissimum* (in total 576 characters, including 55 informative characters, length=158, CI=0.753 , RI=0.669). Numbers above branches are bootstrap values (1000 replicates). The left number below branches, indicate the numbers of characters supporting each branch. The right number below branches, are branch support values

increasing sample size resulted in a decrease of bootstrap support for a given group. The increase of homoplasy in larger data sets was also observed in bryophytes (Buck *et al.*, 2000; Goffinet *et al.*, 2001). To avoid this problem we restricted our taxon sampling to the necessary representatives of Bryales, Splachnales, Hookeriales, Hypnales as an ingroup.

Despite our careful choice of taxa the monophyletic position of 9 taxa of the Hypopterygiaceae (clade B, Fig. 1) is only supported with 51 %. The position of the Hypopterygiaceae s.l. within the "pleurocarpous" mosses (clade A, Fig. 1) is supported by a bootstrap value of 73 %. Low bootstrap values were obtained for the support of clade G and H in Fig. 4 and the paraphyletic position of the genus *Dendrohypopterygium*.

The branch support (Bremer, 1994) indicates how many additional steps (nucleotide substitutions) are required before the branch is lost in the consensus tree. A branch present in one of the MPTs is more strongly supported by the data if a large increase in length of additional trees is required before that branch is lost in the consensus (Bremer, 1994). Clade A is supported with a branch support value of 3, clade B with 2 and clade C with 1. The branch support for clade G and H is 1 each. Therefore, support for these branches is very low compared to the *Lopidium*-clade with a branch support of 10.

The results of the MPTs are in agreement with former molecular (Stech *et al.* 1999, 2002) and morphological (Kruijer, 1995-2002) results and are discussed in detail below.

The position of the Hypopterygiaceae s.l.

All genera of the Hypopterygiaceae investigated in this study cluster together with the representatives of the Hookeriales, and Hypnales in a monophyletic clade A (Fig.1). These results are conform to earlier systematic placement of the Hypopterygiaceae (Brotherus, 1925) and contradict the placement within the Bryales as proposed by Buck & Vitt (1986).

Recent cladistic analyses mainly covering only few species of the Hypopterygiaceae came up with the following results: In the cladistic analyses of morphological characters by Hedenäs (1996) the investigated species of the Hypopterygiaceae (*Dendrohypopterygium arbuscula, Hypopterygium tamarisci* and *Cyathophorum bulbosum*) appear at a basal position of the "pleurocarpous" orders. A cladistic analysis of *rbc*L sequences in combination with a morphological data set (De Luna *et al.*, 1999), identified *Hypopterygium tamariscinum* (Hedw.) Brid. as a sister taxon of *Hookeria acutifolia* Hook. & Grev. and *Fontinalis dalecarlica* Bruch & Schimp. Buck *et al.* (2000) suggested that the Hypopterygiaceae together with the Rutenbergiaceae may need to be accommodated in a new order. In contrast, Kruijer (2002) in his detailed account of the Hypopterygiaceae, found the seven genera of the Hypopterygiaceae to be monophyletic and sister to a clade consisting of *Achrophyllum dentatum* Hook.f. & Wilson (Daltoniaceae) and *Calyptrochaeta apiculata* Hook.f. & Wilson (Hookeriaceae).

In our study six of the genera of the Hypopterygiaceae appeared in a distinct clade B (Figs 1-4) as sister to the representatives of the Hookeriales and Hypnales (clade C, Figs 1-3). Clade C includes taxa recently considered as allied to the Hypopterygiaceae: e.g. Ptychomniaceae (*Ptychomnion aciculare*), Rutenbergiaceae (*Neorutenbergia usagarae* (Dixon) Bizot & Pócs), Fontinalaceae (*Fontinalis dalecarlica*) and Hookeriales (e.g. *Hookeria lucens* (Hedw.) Sm.). Our

study revealed no evidence that any of these taxa are closely related to the Hypopterygiaceae.

The cladistic analyses by Hedenäs (1996) using morphological characters revealed an ambiguous position of *Cyathophorum bulbosum*, as this species appeared to be related either to the Hookeriaceae or Ptychomniaceae depending on the characters used. In our study, *Cyathophorum bulbosum* and *Cyathophorum adiantum* are at a basal position close to the members of Hypopterygiaceae (clade B) but no evidence could be obtained for a closer relationship to any of the taxa in clade C.

Cyathophorella and *Cyathophorum* were formerly regarded as closely related taxa and delimitated from the remaining genera based on a suite of characters including their short seta, unbranched stems and weak costa (Brotherus, 1925; Fleischer, 1908; Miller, 1971; Whittemore & Allen, 1989). Based on these and other characters, these two genera were accommodated into their own tribe within the family Hypopterygiaceae (Fleischer, 1908; Brotherus, 1925) whereas more recent studies appointed the two genera the rank of a family Cyathophoraceae (Miller, 1971; Whittemore & Allen, 1989).

Based on Kruijer's cladistic studies (Kruijer, 2002) where the taxa of *Cyathophorum* and *Cyathophorella* appeared monophyletic, the species of *Cyathophorella* have been transferred into the genus *Cyathophorum*. According to Kruijer (2002) the genus *Cyathophorum*, as sister to the *Lopidium*-clade, takes in a terminal position within the Hypopterygiaceae.

In our study the genus *Cyathophorum* represented by *Cyathophorum bulbosum* and *Cyathophorum adiantum* (syn. *Cyathophorella tonkinensis* (Broth. & Paris) Broth.) was also found to be monophyletic. The genus *Cyathophorum* appeared either basal to the clade B, suggesting a monophyly of the Hypopterygiaceae s.l. (Fig. 2) or basal to clade B and C (Fig. 3). No evidence was obtained for a closer relationship of these taxa to the representatives of the Hookeriaceae (*Hookeria lucens* and *Schimperobryum splendidissimum*) as suggested by Buck & Goffinet (2000).

In our study *Cyathophorum bulbosum* and *Cyathophorum adiantum* are in a basal position close to the members of Hypopterygiaceae (clade B, Figs 1-3) but separated from *Dendrocyathophorum*. These three genera are regarded as closely related within the Hookeriaceae (Buck & Goffinet, 2000). Kruijer (2002) obtained *Dendrocyathophorum* in a clade together with *Cyathophorum* but separated by the genus *Lopidium*. Also the cladistic analysis of molecular data from Stech *et al.* (2002) suggested a separation of *Dendrocyathophorum* from the genus *Cyathophorum*.

The intrafamiliar relationships within the Hypopterygiaceae (clade B)

The two species of *Hypopterygium* investigated in our study compose a monophyletic group. *Hypopterygium didictyon* and *H. tamarisci* form a well supported clade (F, Figs 1, 2) sister to the *Lopidium*-clade (E).

In our study *Dendrohypopterygium arbuscula* is the basal taxon in clade G (Fig. 2). *Dendrohypopterygium filiculiforme* appears as sister group to the *Hypopterygium*-clade (F) and to both *Lopidium* species (clade E) at a basal position of clade H. These results are in accordance with early systematic studies (e.g. Brotherus, 1925). The new genus *Dendrohypopterygium* also appeared paraphyletic in Kruijer's (2002) cladistic studies. The outstanding position of the South American endemic *Dendrohypopterygium arbuscula* (syn. *Hypopterygium arbus-*

cula (P. Beauv.) Brid.) was already pointed out by Kindberg (1901), Brotherus (1925) and Fleischer (1908). Also the special status of the New Zealand endemic *Dendrohypopterygium filiculiforme* (syn. *Hypopterygium filiculiforme* (Hedw.) Brid.) was recognised quite early and the species received a monotypic status (Kindberg, 1901; Fleischer, 1908). Molecular data of the *trn*L-Intron and ITS region (Stech *et al.* 1999; Stech *et al.*, 2002) revealed no evidence of a monophyletic status of the genus *Dendrohypopterygium*.

Noticeable relationships appear also within clade G. The close relationship between *Canalohypopterygium tamariscinum* and *Catharomnion ciliatum* (clade D) is resolved in all phylogenetic reconstructions and is supported by high bootstrap values. Earlier investigations based on morphological and anatomical characters support these results (Kruijer, 1995, 2002). The occurrence of the internal stem channels together with bristle-like short branches (Reimers, 1953; Frey *et al.*, 1983) are regarded as synapomorphies in these two species (Stech *et al.*, 1999). Furthermore, the sister position of *Dendrocyathophorum decolyi* to *Canalohypopterygium*/*Catharomnion* as revealed in this study is in correspondence with Whittemore & Allen (1989) who placed the genus *Dendrocyathophorum* within the Hypopterygiaceae close to *Canalohypopterygium tamariscinum* because of the similar internal stem channels in both taxa. Additionally, the independent status of *Canalohypopterygium tamariscinum*, formerly in the genus *Hypopterygium* (Frey & Schaepe, 1989), found to be distinct from the other species in *Hypopterygium*, in our study is also supported by studies using the *trn*L-intron (Stech *et al.* 1999).

Within clade H, the close relationship of *Lopidium concinnum* and *L. struthiopteris* (clade E) as revealed in this study fits well with the phylogenetic results of the cladistic analysis of morphological data (Kruijer, 2002) as well as with systematic studies using molecular data (Stech *et al.*, 1999).

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