

The systematic position of *Pedinophyllum* (Marchantiophyta: Jungermanniales)

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(Received 12 December 2009, accepted 5 February 2010)

Abstract – Phylogenetic analyses of an *rbcL* sequence dataset indicates that the generitype *Pedinophyllum interruptum* nests within the Plagiochilaceae. Previous reports of *Pedinophyllum truncatum* in the Adelanthaceae or Jamesoniellaceae are likely based on a misidentification of *Syzygiella nipponica*.

Adelanthaceae / Jamesoniellaceae / *Pedinophyllum* / Plagiochilaceae / *rbcL*

INTRODUCTION

The Plagiochilaceae are made up of the species-rich genus *Plagiochila* (Dumort.) Dumort. and several satellite genera such as *Chiastocaulon* Carl, *Dinckleria* Trevis., *Pedinophyllopsis* R.M. Schust. et Inoue, *Pedinophyllum* (Lindb.) Lindb. and *Plagiochilion* S. Hatt. (Crandall-Stotler *et al.*, 2009). However, the circumscription and systematic position of *Pedinophyllum* (Lindb.) Lindb. is still subject to controversy. This genus shares the basally-inflated, laterally-compressed, bilabiate perianths with other representatives of Plagiochilaceae but differs in its autoicous condition. Based on nrITS and cp DNA *rps4* sequences of a single specimen, Groth & Heinrichs (2003), Heinrichs *et al.* (2006) and Hentschel *et al.* (2007) confirmed the placement of the generitype *Pedinophyllum interruptum* (Nees) Kaal. in Plagiochilaceae. A second species, *Pedinophyllum truncatum* (Steph.) Inoue, was identified as an element within the Jamesoniellaceae (= Adelanthaceae, Feldberg *et al.*, 2010) by He-Nygrén *et al.* (2004, 2006) based on a cp DNA *rbcL*, *trnL-F* and *rps4* sequence set. Heinrichs *et al.* (2005) and Hentschel *et al.* (2006) confirmed the position of *Pedinophyllum truncatum* in Adelanthaceae using the same sequences. The presence of the two species in different suborders is surprising considering the alternative treatment of

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the taxa as subspecies of a single species (Inoue, 1958). Crandall-Stotler *et al.* (2009) assumed a misidentification of the *Pedinophyllum truncatum* DNA voucher; however, this hypothesis has not yet been verified using further accessions. Here we produce *rbcL* sequences of further *Pedinophyllum interruptum* specimens, and provide evidence that *Pedinophyllum truncatum* is sometimes confused with members of the *Syzygiella autumnalis* (DC.) Feldberg *et al.*/*S. nipponica* (S.Hatt.) Feldberg *et al.* complex.

MATERIALS AND METHODS

DNA extraction, PCR amplification and sequencing were carried out as described in Hentschel *et al.* (2006). In case no product was detected a second (nested) PCR was performed using the forward primer *rbcL*-210-F and the reverse primer *rbcL*-1200-R (Gradstein *et al.*, 2006). Several new *rbcL* sequences of *Pedinophyllum interruptum* and putative *Pedinophyllum truncatum* were included in an alignment of more than 500 published *rbcL* sequences of liverworts including those from the Adelanthaceae dataset of Feldberg *et al.* (2010). Maximum parsimony analyses allowed for an identification of closely related taxa. Based on these analyses (data not shown) a subset of 62 sequences was compiled for further analyses (Table 1). The alignment was constructed using Bioedit (Hall, 1999). Lacking parts of sequences were coded as “N” (A, C, G or T). Two representatives of Schistochilaceae were designated as outgroups based on the phylogenies of He-Nygrén *et al.* (2006) and Heinrichs *et al.* (2007). Further representatives of Jungermanniales in the circumscription of Heinrichs *et al.* (2005) formed the ingroup. Maximum likelihood (ML) and parsimony (MP) analyses of the dataset were carried out using PAUP* (Swofford, 2003). The MP analysis was performed with the following options implemented: heuristic search mode with 100 random-addition sequence replicates, tree bisection-reconnection branch swapping (TBR), MULTrees option on, and collapse zero-length branches off. All characters were treated as equally weighted and unordered. Bootstrap support (BS) values were estimated by calculating 500 bootstrap replicates (Felsenstein, 1985). To decide on the nucleotide substitution model with the smallest number of parameters that best fits the data the program Modeltest (Posada and Crandall, 1998) was used. The Akaike information criterion (Akaike, 1974) selected the GTR model (Tavaré, 1986) with gamma shape parameter for among site variation and proportion of invariable sites. This model was implemented in ML analyses carried out as heuristic searches. Branching confidence was assessed using 100 bootstrap resamplings in ML analysis using a neighbor joining tree as starting tree and allowing for 2000 rearrangements under the GTR + I + G model. Clade support was also assessed by bootstrapping with 200 replicates, accomplished with GARLI version 0.96 (Zwickl, 2008) using the default search parameters. Bootstrap percentage values above 70 were regarded as good support (Hillis & Bull, 1993).

RESULTS

Three hundred and twenty one of the 1122 aligned characters of the *rbcL* gene were parsimony-informative, 105 autapomorphic and 696 constant. The strict

Table 1: Voucher specimens for molecular sequences. Genbank numbers [rbcL] are included for all sequences used in MP and ML analyses.

Taxon	Voucher	Origin	GenBank Acc.-No.
<i>Adelanthus lindenbergianus</i> (Lehm.) Mitt.	Hyvönen 5637a (H)	Argentina	AY462285
<i>Balanitopsis diplophylla</i> (Hook. f. et Taylor) Mitt.	Engel 20853 (GOET)	New Zealand	DQ312476
<i>Barbitophozia barbata</i> (Schreb.) Loeske	Hentschel Bryo 0753 (GOET)	Bulgaria	DQ312477
<i>Bazzania tricrenata</i> (Wahlenb.) Lindb.	Heinrichs 4388 (GOET)	Austria	AY699990
<i>Brevianthus flavus</i> (Grolle) J.J. Engel et R.M. Schust.	Glenny 8918 (H)	New Zealand	DQ026582
<i>Cephalozia bicuspidata</i> (L.) Dumort.	Piippo 5655 (H)	Finland	AY462291
<i>Cephalozia divaricata</i> (Sm.) Schiffn.	Hentschel Bryo 01159 (GOET)	Germany	DQ312481
<i>Chaetophyllopsis whiteleggei</i> (Carrington et Pearson) Hamlin	Curnow 4804 (H)	Australia	AY462292
<i>Chiaetocaulon dendroides</i> (Nees) Carl	Kurita 84 (HIRO)	Japan	AY699991
<i>Chiloscyphus polyanthos</i> (L.) Corda	He-Nygrén & Piippo 1469 (H)	Finland	AY149851
<i>Cuspidatula contracta</i> (Reinw. et al.) Grolle	Vojtko 9461/A, Z (EGR)	Madagascar	GO900295
<i>Dendromastigophora flagellifera</i> (Hook.) R.M. Schust.	Glenny 8520	New Zealand	AY462294
<i>Dinckleria pleurata</i> (Hook. f. et Taylor) Trevis.	Schäfer-Verwimp & Verwimp 13777 (GOET)	New Zealand	DQ194074
<i>Diplophyllum obtusifolium</i> (Hook.) Dumort.	Wheeler s.n. [ref. no. 351]	USA	AY507397
<i>Eremonotus myriocarpus</i> (Carrington) Pearson	Rothero NH 2116-2410 (GOET)	United Kingdom	EF503674
<i>Geocalyx graveolens</i> (Schrad.) Nees	Meinunger s.n. (JE)	Germany	DQ312490
<i>Gottschelia schizopleura</i> (Spruce) Grolle	Ah Peng R96 (GOET)	Madagascar	FJ984940
<i>Gymnomitron coralloides</i> Nees	Hentschel Bryo 0458 (GOET)	Norway	DQ312491
<i>Harpanthus flotovianus</i> (Nees) Nees	Heinrichs et al. 4390 (GOET)	Austria	DQ312492
<i>Herbertus dicranus</i> (Gottsche) Trevis.	Long 17542	Nepal	AY462300
<i>Heteroscyphus aselliformis</i> (Reinw. et al.) Schiffn.	Gradstein 10240 (GOET)	Indonesia	DQ312493
<i>Jungermannia atrovirens</i> Dumort.	Hentschel & Heinrichs Bryo 01784 (GOET)	Germany	EF503675
<i>Leitocolea collaris</i> (Nees) Jörg.	Hentschel Bryo 0864 (GOET)	Slovakia	DQ312501
<i>Leptocolea pruinosa</i> (Taylor) Spruce	Frahm et al. s.n.	Peru	AY462307
<i>Leptoscyphus amphibolius</i> (Nees) Grolle	Dauphin et al. 1578 (GOET)	Panama	DQ312502
<i>Litochlaena lanceolata</i> Nees	Stotler & Crandall-Stotler 107 (ABSH)	USA	AY507409
<i>Lophozia ventricosa</i> (Dieks.) Dumort.	Heinrichs 3826 (GOET)	Belgium	AY699994
<i>Mastigophora woodsii</i> (Hook.) Nees	Frahm (loan from CANB639918)	Australia	AY462314
<i>Nardia scalaris</i> Gray	Hentschel & Wilson Bryo 01618 (GOET)	Germany	DQ312508

Table 1: Voucher specimens for molecular sequences. Genbank numbers [*rbcL*] are included for all sequences used in MP and ML analyses. (*continued*)

<i>Taxon</i>	<i>Voucher</i>	<i>Origin</i>	<i>GenBank Acc.-No.</i>
<i>Neoscyophus argillaceus</i> (Nees) Grolle	Schäfer-Verwimp & Verwimp 11107	Brazil	DQ312509
<i>Nowellia curvifolia</i> (Dicks.) Mitt.	Burghardt 4493 (GOET)	Mexico	DQ312510
<i>Odontoschisma denudatum</i> (Mart.) Dumort.	Horn 1809 (DUKE)	USA	AY608036
<i>Paraschistochoila tuloides</i> (Hook. f. et Taylor) R.M. Schust.	Glenny 8499	New Zealand	AY462321
<i>Plagiochila asplenitoides</i> (L.) Dumort.	Heinrichs & Groth 4339 (GOET)	Germany	AY699996
<i>Plagiochilon oppositum</i> (Reinw. et al.) S. Hatt.	Schäfer-Verwimp 20935 (GOET)	Indonesia	AY700000
<i>Pedinophyllum interruptum</i> (Nees) Kaal.	Heinrichs et al. 4701 (GOET)	Germany	GU593095
<i>Pedinophyllum interruptum</i>	Gradstein 11067 (GOET)	Germany	GU593096
<i>Scapania nemorea</i> (L.) Grolle	Stotler & Crandall-Stotler s.n. [ref.no. 265]	USA	AY507423
<i>Schiffneria hyalina</i> Steph.	Mizutani 15961	Japan	AY462327
<i>Schistochoila laminigera</i> (Hook. f. et Taylor) A. Evans	Hyvonen 5888	Chile	AY462329
<i>Sphenolobus minutus</i> (Schreb.) Berggr.	Hentschel Bryo 0421 (GOET)	Norway	DQ312475
<i>Solenostoma obovatum</i> (Nees) C. Massal.	Heinrichs et al. 4391 (GOET)	Austria	DQ312499
<i>Syzygiella anomala</i> (Lindenb. et Gottsche) Steph.	Heinrichs et al. K4 (GOET)	Ecuador	GO900328
<i>Syzygiella autumnalis</i> (DC.) Feldberg et al. I	Koponen 55912 (H)	China	AY462303
<i>Syzygiella autumnalis</i> II	Ohnishi 2023 p.p. (HIRO)	Japan	GU593093
<i>Syzygiella autumnalis</i> III	Ohnishi 3452 p.p. (HIRO)	Japan	GU593094
<i>Syzygiella autumnalis</i> IV	Schröder 6713/4 (JE)	Germany	GO900302
<i>Syzygiella autumnalis</i> V	Jedrzeiko & Zarnowiec 151 (JE)	Poland	GO900304
<i>Syzygiella nipponica</i> (S. Hatt.) Feldberg et al. I	Zhu 20060728-14 (HSNU)	China	GO900311
<i>Syzygiella nipponica</i> II	Long 17022 (E)	Nepal	GO900312
<i>Syzygiella nipponica</i> (det. <i>Pedinophyllum truncatum</i>)	Koponen 46768 (H)	China	AY149855
<i>Syzygiella colorata</i> (Lehm.) Feldberg et al.	Holz & Franzaring CH-00-71 (GOET)	Chile	GO900306
<i>Syzygiella concreta</i> (Gottsche) Spruce	Holz & Schäfer-Verwimp CR-99-1142 (GOET)	Costa Rica	GO900334
<i>Syzygiella purpurascens</i> (Steph.) Feldberg et al.	Arts RSA 26/33 (JE)	South Africa	GO900315
<i>Syzygiella rubricaulis</i> (Nees) Steph.	Sauer E 262 (GOET)	Ecuador	GO900357
<i>Tetracymbaliella cymbalifera</i> (Hook. f. et Taylor) Grolle	Pócs et al. 90066/F (H3192330)	Tanzania	DQ026591
<i>Triandrophylum subtrifidum</i> (Hook. f. et Taylor) Fulford et Hatcher	Hyvönen 5334 (H)	Chile	AY462331
<i>Tritomaria quinqueidentata</i> (Huds.) H. Buch	Heinrichs 2978 (GOET)	Germany	AY700003
<i>Wettsteinia inversa</i> (Sande Lac.) Schiffn.	Gradstein 11014 (GOET)	Indonesia	FJ984935

consensus of four maximally parsimonious trees (length 1.672 steps, consistency index excluding uninformative characters 0,31) is depicted in Fig. 1. The two accessions of *Pedinophyllum interruptum* form a monophyletic lineage with a BS of 100. This lineage is nested in the robust Plagiochilaceae (BS 94) represented by *Chiastocaulon*, *Dinckleria*, *Plagiochila* and *Plagiochilion*. Plagiochilaceae are placed in a well supported sister-relationship (BS 94) with a clade including several representatives of Lophocoleaceae (*Chiloscyphus* Corda, *Heteroscyphus* Schiffn., *Leptoscyphus* Mitt., *Tetracymbaliella* Grolle) and *Brevianthus flavus* (Grolle) J.J. Engel et R.M. Schust. of the monogeneric family Brevianthaceae. The Lophocoleaceae/Brevianthaceae clade achieves a BS of 77. The published *rbcL* sequence assigned to *Pedinophyllum truncatum* is nested in a lineage with two accessions of *Syzygiella nipponica* (S. Hatt.) Feldberg et al. with a BS of 99. Two sequences from Japanese specimens identified as *P. truncatum* (“*Syzygiella autumnalis* I and III”) are placed sister to a lineage with three sequences of *Syzygiella autumnalis* (I, IV, V).

The ML analysis yielded a single tree with ln=-9764,2164. The ML topology (Fig. 2) resembles the MP strict consensus topology. Differences are seen only in clades without or with low BS.

DISCUSSION

The present study introduces new DNA vouchers of *Pedinophyllum interruptum*. The position of these accessions in the MP and ML trees (Figs 1, 2) lends strong support to the placement of *Pedinophyllum* in Plagiochilaceae. This position is supported by the “plagiochilid” perianths of *Pedinophyllum*, being laterally compressed and bilabiate, with the mouth truncate and not contracted. *Pedinophyllum truncatum* shares the perianth structure with the generitype (Gao, 2000, p. 262, plate 137) and thus also conforms with the Plagiochilaceae. The DNA voucher Koponen 46768 from China (GenBank no AY462321) was not available for study, however, its position in the robust *Syzygiella nipponica* clade lends support to the hypothesis of Crandall-Stotler et al. (2009) that the assignment to *Pedinophyllum* was based on a misidentification. Sterile material of the *Syzygiella autumnalis*/*S. nipponica* complex may easily be confused with *Pedinophyllum*, as is seen in the position of two further sequences generated from Japanese “*Pedinophyllum truncatum*” specimens that each include a mixture of various liverworts (HIRO, Ohnishi 2022 and 3452). These specimens include single perianth bearing shoots of *Pedinophyllum* and sterile *Syzygiella* shoots. DNA extraction from the *Pedinophyllum* material remained unsuccessful. However, we were able to sequence the sterile *Syzygiella* material (Figs 1, 2, “*S. autumnalis* II, III). This material is nested in a robust lineage of *Syzygiella nipponica* and *S. autumnalis*, and placed sister to *S. autumnalis* s.str. (MP tree, Fig. 1) or nested in the polytomous *S. autumnalis* clade (ML tree, Fig. 2). Fertile material of Jamesonielloideae can easily be separated from Plagiochilaceae by the plicate perianths narrowed towards their mouth.

The *rbcL* sequence of *Brevianthus flavus* of He-Nygrén et al. (2006) places this species within Lophocoleaceae, sister to *Tetracymbaliella*. Engel & Schuster (1982) pointed out a striking resemblance of *Brevianthus* J.J. Engel et R.M. Schust. and *Clasmatocolea* Spruce [= *Chiloscyphus* (Hässel de Menéndez, 1996; Hentschel et al., 2007)] in the Lophocoleaceae. However, based on morphological peculiarities such as lack of underleaves and presence of androecia

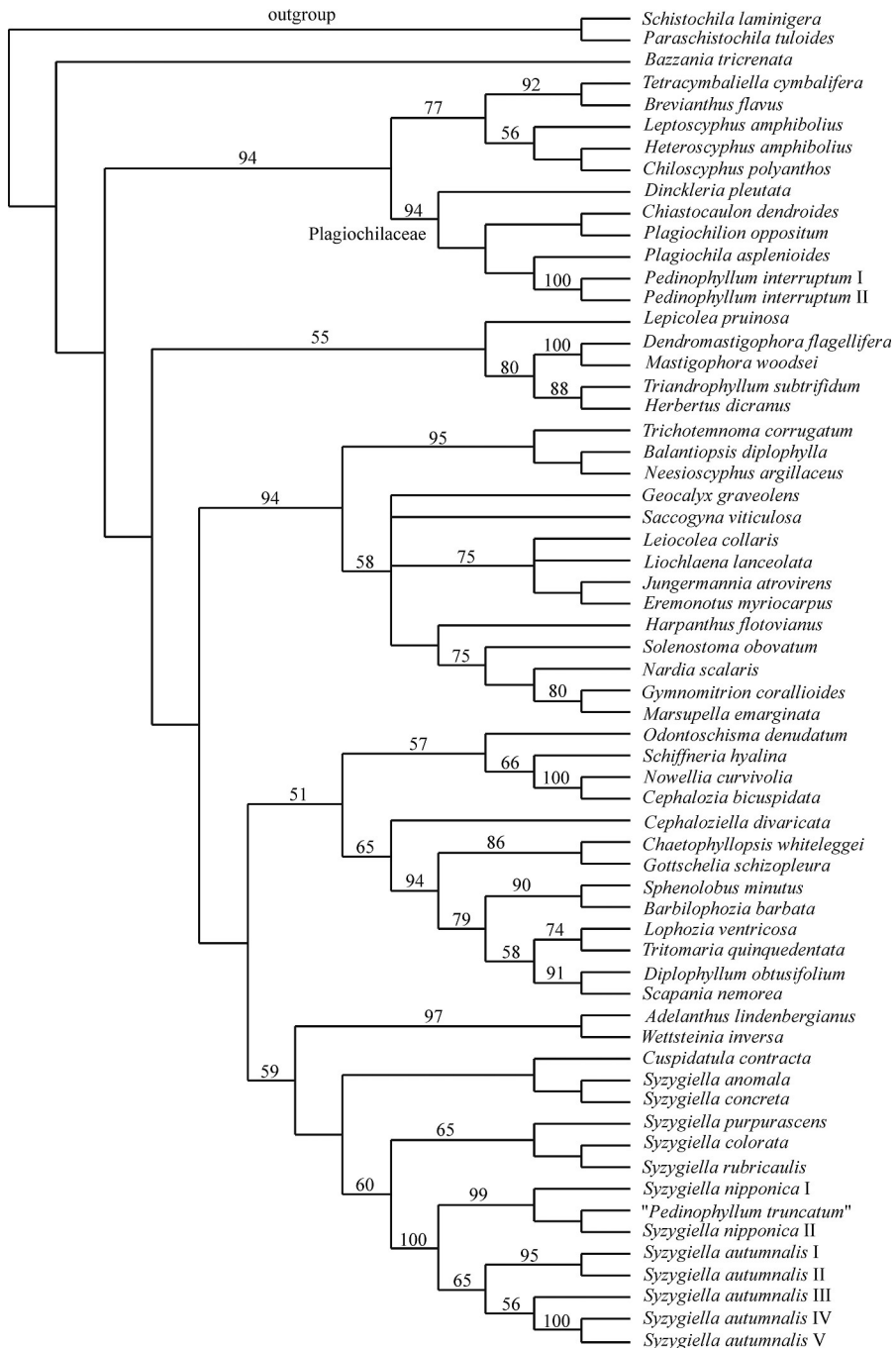


Fig. 1. Strict consensus of four most parsimonious trees with bootstrap percentage values > 50 indicated at branches. *Pedinophyllum interruptum* is placed in the well-supported Plagiochilaceae.

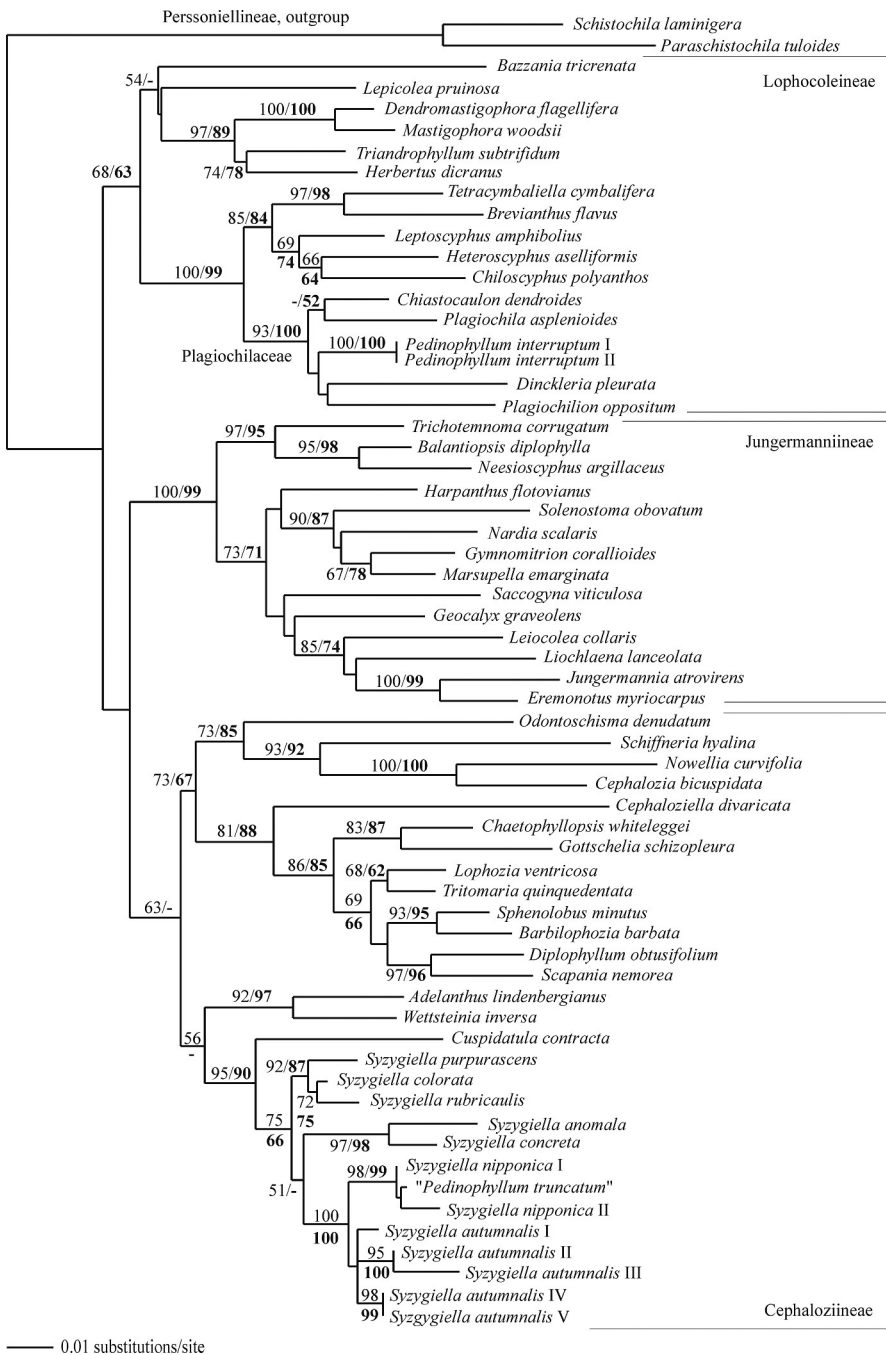


Fig. 2. Optimal tree (ln = -9764.2164) obtained in a maximum likelihood analysis under the GTR + G + I model. Bootstrap percentage values (BPV) > 50 are given at branches with PAUP*-BPV printed in regular letters, but GARLI-BPV in bold.

and perianths on branches lacking normal leaves, Engel & Schuster (1982) proposed a separate family, Brevianthaceae. Generic classification of Lophocoleaceae is subject to controversy (e.g., Hentschel *et al.*, 2007; Crandall-Stotler *et al.*, 2009) and deserves further study. The present investigation strongly supports inclusion of Brevianthaceae in studies aiming at clarifying the global phylogeny of Lophocoleaceae.

Acknowledgements. Financial support by the German Research Foundation (DFG grant HE 3584 / 4 to JH) is gratefully acknowledged. JV acknowledges support from the Ministry of Education of the Czech Republic through project No. 0021620828. RLZ appreciates support from the National Natural Science Foundation of China (NSFC, nos. 30825004, 30670155) and the Program of Shanghai Subject Chief Scientist (no. 08XD14016). We thank the director and curator of the Hiroshima University herbarium (HIRO) for the loan of specimens and the permission to extract DNA, and numerous colleagues for checking their herbaria for recently collected material of *Pedinophyllum truncatum*.

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