Explaining the 'anomalous' distribution of *Echinodium* (Bryopsida: Echinodiaceae): Independent evolution in Macaronesia and Australasia

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Abstract

The peculiar disjunction between Macaronesia and Australasia of the morphologically isolated pleurocarpous moss genus *Echinodium* is one of the most prominent questions in bryology. *Echinodium* as traditionally circumscribed comprises six extant species, four restricted to the Macaronesian archipelagos and two confined to the Australasian/Pacific regions. Molecular phylogenetic analyses based on plastid *trnL*$_{UAA}$ intron and nuclear ribosomal ITS sequences indicate that *Echinodium* is polyphyletic and split into three groups. Three of the four Macaronesian species (*E. spinosum* and the single island endemics *E. renauldii* and *E. setigerum*) are closely related to each other and treated as *Echinodium* s.str. (Echinodiaceae). Further clarification of the relationships of *Echinodium* s.str. with *Orthostichella*, a segregate of Lembophyllaceae, is needed. The remaining Macaronesian species, *E. prolixum*, is transferred to *Isothecium* (Lembophyllaceae); this systematic position is also strongly supported by leaf characters. The two Australasian species, *E. hispidum* and *E. umbrosum*, are molecularly unrelated to the Macaronesian species and are transferred to *Thamnobryum* in the Neckeraceae. While the molecular data suggest that the peculiar distribution pattern of '*Echinodium*' is an artefact, the striking morphological similarity observed in Macaronesian and Australasian species cannot be dismissed. Possible explanations are: (i) parallel morphological evolution of the '*Echinodium* habit' in Macaronesia and Australasia, or (ii) retention of a set of plesiomorphic characters in non-related groups in relict habitats, the Macaronesian laurel forest and the austral temperate rain forests, respectively. Of these hypotheses, the evolutionary parallelism hypothesis seems more plausible for several reasons, which are discussed.
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Keywords: Biogeography; Echinodiaceae; ITS; Molecular relationships; Pleurocarpous mosses; *trnL*$_{UAA}$ intron

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Introduction

The Echinodiaceae are a small family of pleuro-carpous mosses, comprising only one genus, *Echinodium* Jur., with six extant species (Churchill 1986). *Echino-dium* shows one of the most peculiar disjunct distribu-tion patterns (Fig. 1), which puzzled bryologists for more than a century. Four species are restricted to the Macaronesian archipelagos, *E. prolixum* (Mitt.) Broth. to the Azores and Madeira, *E. spinosum* (Mitt.) Jur. to Madeira and Canary Is., *E. renauldii* (Card.) Broth. endemic to the Azores, and *E. setigerum* (Mitt.) Jur. endemic to Madeira. The remaining two species are confined to the Australasian region, *E. hispidum* (Hook. f. & Wils.) Reichardt in Eastern Australia, New Zealand and New Caledonia, and *E. umbrosum* (Mitt.) A. Jaeger in New Zealand, reaching northwards to Fiji (Churchill 1986). A separate, nowadays extinct species, *E. savicziae* A. Abr. & I. Abr., was described based on fossils from the Pliocene of Georgia (Abramov and Abramov 1958) and the Miocene of Poland (Lancucka-Srodoniowa 1996). These and further fossil finds from the Eocene in Baltic and Saxon amber (Frahm 2004) as well as from the Pleistocene of Tasmania (Jordan and Dalton 1995) indicate an evolutionary old age and a wider distribution of the genus *Echinodium* in the Tertiary (Fig. 1). This is supported by the predominant occurrence of the extant *Echinodium* species in relict forest types, the laurel forest (Laurisilva) in Macarone-sia, a presumed remnant of the Tertiary flora (e.g., Capelo et al. 2005), and southern temperate rain forests to semi-tropical forests in Australasia. Because of their limited distribution areas all four Macaronesian species are either considered rare or threatened in European or worldwide red lists (ECCB 1995; IUCN 2002), with *E. renauldii* and *E. setigerum* classified as ''Vulnerable'' by IUCN (2002).

The *Echinodium* species have been well characterised morphologically and ecologically (e.g., Churchill 1986; Hedenäs 1992; Gabriel 1994; Dierßen 2001; Fontinha et al. 2001, 2006). The genus *Echinodium* is easily recognized by the combination of rather stiff, irregularly pinnate or subdendroid plants, generally long subulate and plicate leaves with partially to fully bistratose
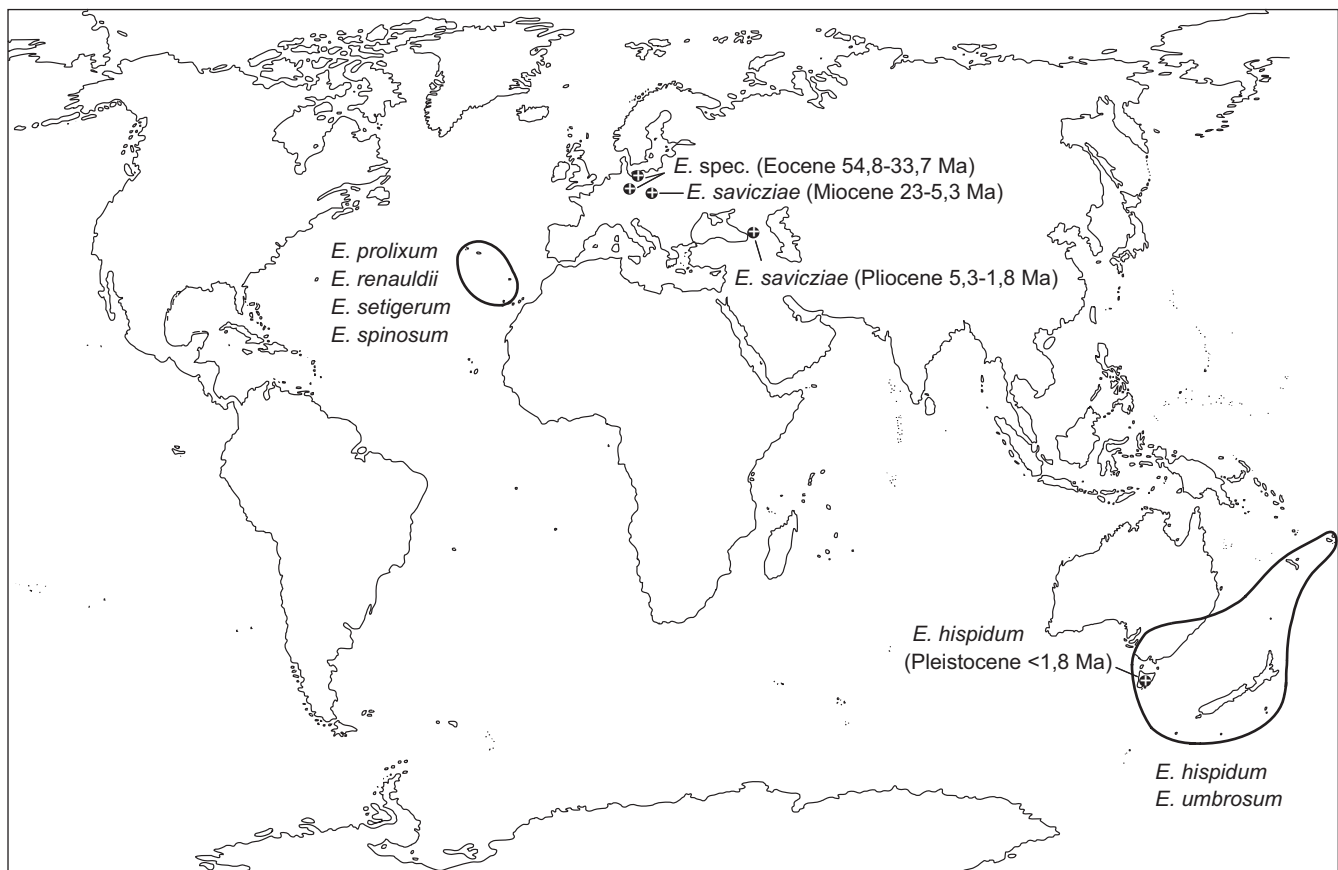

Fig. 1. Extant disjunct distribution of the moss genus *Echinodium* (bold lines), with names of the species occurring on the Macaronesian islands and in the Australasian/Pacific regions, respectively, as well as locations and ages (in million years before present) of fossil finds assigned to *Echinodium*.

margins, percurrent to excurrent costae, and short leaf cells. Despite this characteristic 'Echinodium habit', Churchill (1986) pointed out that the subulate, plicate leaves and bistratose margins are the only morphological synapomorphies, and hypothesised that the species could belong to different genera. Furthermore, the systematic position of Echinodiaceae within the pleurocarpous mosses remains ambiguous. Echinodiaceae have been placed near various pleurocarpous families in the former Isobryales (e.g., Fleischer 1909–1923; Brotherus 1925; Walther 1983) or Hypnobryales (especially the Thuidiaceae; e.g., Robinson 1971; Vitt 1984; Buck and Vitt 1986). Interestingly, none of these authors questioned the monophyly of the family Echinodiaceae or its taxonomic rank. First doubts on the systematic position and rank were put forward by Hedenäs (1992) in his flora of Madeiran pleurocarpous mosses, where he included the Macaronesian Echinodium species in Lembophyllaceae. Echinodium hispidum was resolved as sister to Limbella tricostata (Sull.) Müll. Hal. ex E.B. Bertram (Neckeraceae) in the cladistic analyses of morphological characters by Hedenäs (1995), but relationships of these two species to other isobryalean taxa remained ambiguous.

Based on molecular phylogenetic analyses, the two main pleurocarpous orders (Isobryales/Neckerales/Leucodontales and Hypnobryales/Hypnales) were merged into one, Hypnales (Buck et al. 2000). Resolving familial relationships among pleurocarpous mosses with confidence, however, remains a challenging task (see, e.g., Goffinet and Buck 2004). Echinodiaceae have not been challenged on molecular grounds, as only one species (E. umbrosum) has been included in previous molecular studies of pleurocarpous mosses (Buck et al. 2000; De Luna et al. 2000). These studies were based on different combinations of plastid DNA regions, rps4 and trnL-F (Buck et al. 2000) or rps4, trnL-F and rbcL (De Luna et al. 2000), respectively, and indicated a closer relationship of Echinodium umbrosum to either Thamnobryum (Buck et al. 2000) or Lembophyllum (De Luna et al. 2000). However, Thamnobryum was missing in the analyses of De Luna et al. (2000), and the trees in both studies lacked significant support for most clades.

The present study aims at providing the first comprehensive molecular investigation of the enigmatic genus Echinodium. Based on sequences of the plastid trnL_{UAA} intron and the more variable internal transcribed spacers (ITS) 1 and 2 of the nuclear ribosomal DNA (nrDNA), we aim (i) to test the monophyly of Echinodium and to evaluate the significance of the morphological characters used to define the genus, (ii) to clarify systematic and evolutionary relationships of the Echinodium species, and (iii) to develop hypotheses that could explain the peculiar extant distribution pattern.

Material and methods

Plant material

Material of the Macaronesian Echinodium species was collected during field trips to the Azores and Madeira by the authors and by T. Pfeiffer (Greifswald). Material of E. hispidum was collected by J.-P. Frahm (Bonn) in New Zealand in the course of the BRYO AUSTRAL project. In addition, herbarium material of Echinodium from B, LISU, MADJ, and the private herbarium of A. Schäfer-Verwimp (Herdwangen-Schönach) was used. Voucher information and GenBank accession numbers for the Echinodium samples and further newly sequenced samples are listed in Table 1. Sequences from other pleurocarpous species were either taken from our previous studies or from GenBank (detailed information is available upon request), resulting in a data matrix comprising 62 specimens.

DNA extraction, PCR and sequencing

All plant material was thoroughly cleaned with distilled water and partly by ultrasonic treatment. Extraction of DNA, PCR amplification and manual or automated sequencing of the plastid trnL_{UAA} intron and the nuclear ribosomal ITS1-5.8S-ITS2 region was performed as described in Quandt et al. (2007) and Sim-Sim et al. (2005).

Alignment, sequence analysis and phylogenetic reconstructions

Based on the criteria laid out in Kelchner (2000) and Quandt and Stech (2005), the DNA sequences were manually aligned using PhyDE® v0.983 (Müller et al. 2006). Phylogenetic reconstructions using maximum parsimony (MP) or maximum likelihood (ML) principles were performed with PAUP 4.0b10 (Swofford 2002). Three species of the Hookeriales were used as outgroup representatives, according to the sister group relationship of Hookeriales and Hypnales inferred in recent molecular analyses (e.g., Buck et al. 2000). Heuristic searches under parsimony were implemented using random sequence addition with 10 replicates and employing the default settings otherwise. In addition, the data set was analyzed using a simple indel coding (SIC) strategy (Simmons and Ochoterena 2000) as implemented in SeqState (Müller 2004a). SeqState generates a ready-to-use nexus file containing the sequence alignment with an automatically generated indel matrix appended. This file can be either executed directly in PAUP or submitted to PRAP (Müller 2004b) for ratchet and decay analyses. Heuristic bootstrap searches under parsimony were performed with 1000

Table 1. Voucher information, geographic origin, and GenBank accession numbers of the *Echinodium* specimens and other newly sequenced samples

Taxon	Geographic origin	Voucher no. (herbarium location)	Accession no.	
			trnL	ITS
Echinodium hispidum (Hook. f. & Wils.) Reichardt	New Zealand 1	Frey & Frey 94-162 (Frey)	EU434007	EU477599
E. hispidum (Hook. f. & Wils.) Reichardt	New Zealand 2	Frahm 31-11 (Frey)	EU434009	EU477601
E. hispidum (Hook. f. & Wils.) Reichardt	New Zealand 3	Vitt 2710 (B)	EU434008	EU477600
E. prolixum (Mitt.) Broth.	Azores 1	sub Sérgio 11810 (LISU)	EU434003	EU477595
E. prolixum (Mitt.) Broth.	Azores 2	Gabriel RG-011204/2 (LISU)	EU434006	EU477598
E. prolixum (Mitt.) Broth.	Madeira 1	Sim-Sim et al. Lev. 109 (LISU)	EU434004	EU477596
E. prolixum (Mitt.) Broth.	Madeira 2	Stech et al. 04-192 (L)	EU434005	EU477597
E. renauldii (Card.) Broth.	Azores 1	Pfeiffer 2003-98 (Pfeiffer)	EU434013	EU477605
E. renauldii (Card.) Broth.	Azores 2	Gabriel RG-011204/1 (LISU)	EU434014	EU477606
E. setigerum (Mitt.) Jur.	Madeira	Lobo & Santos 20.12.04 (MADJ)	EU434015	EU477607
E. spinosum (Mitt.) Jur.	Madeira 1	Stech et al. 04-128 (L)	EU434011	EU477603
E. spinosum (Mitt.) Jur.	Madeira 2	Sim-Sim et al. s.n. (LISU)	EU434012	EU477604
E. umbrosum (Mitt.) A. Jaeger	?	(GenBank)	AF161137	AY999172
E. umbrosum var. *glauco-viride* (Mitt.) Churchill	Norfolk Is.	Streimann 49634 (Schäfer-Verwimp)	EU434010	EU477602
Neckera pennata Hedw.	?	B300274526 (B)	EU503108	EU503112
N. platyantha (Müll. Hal.) Paris	Rwanda	Pócs 6557 (Frey)	EU503109	EU503113
Thamnobryum alleghaniense (Müll. Hal.) Nieuwl.	USA	Miller 14949 (BONN, dupl. NYS)	EU503110	EU503114
Th. fasciculatum (Sw. ex Hedw.) I. Sastre	Colombia	B300265177 (B)	EU503111	EU503115

replicates, 10 random addition cycles per bootstrap replicate and the same options in effect. Decay values as further measurement of support for the individual clades were obtained using PRAP in combination with PAUP, employing the following ratchet settings: 10 random addition cycles of 200 iterations each with 25% upweighting of the characters in the iterations.

Maximum likelihood analyses were executed assuming a general time reversible model (GTR + Γ + I), and a rate variation among sites following a gamma distribution (four categories represented by mean). GTR + Γ + I was chosen as the model that best fits the data by Modeltest v3.6 (Posada and Crandall 1998) employing the Windows® interface MTgui (Nuin 2005). The settings proposed by Modeltest v3.6 were executed in PAUP 4.0b10. For the combined data set the following settings were used: BaseFreq = (0.2053 0.2743 0.2806), Nst = 6, Rmatrix = (1.0000 3.3710 0.8191 0.8191 4.4120), Shape = 0.6679, and Pinvar = 0.3827. Likelihood bootstrap searches were performed with 500 replicates using the 'fast bootstrap' option (bootstrapping without branch-swapping).

For further measurement of clade support posterior probabilities were calculated using MrBayes V3.1 (Huelsenbeck and Ronquist 2001). As in the ML analysis, the GTR model of nucleotide substitution was employed, assuming site-specific rate categories following a gamma distribution. The a priori probabil-

ities supplied were those specified in the default settings of the program. Posterior probability (PP) distributions of trees were created using the Metropolis-coupled Markov chain Monte Carlo (MCMCMC) method and following the search strategies suggested by Huelsenbeck et al. (2001, 2002). To allow for possible deviating substitution models for the different regions, the data set was divided into four partitions. Four runs with four chains (10⁶ generations each) were run simultaneously, with the temperature of the single heated chain set to 0.2. Chains were sampled every 10 generations and the respective trees written to a tree file. Calculations of the consensus tree and of the posterior probability of clades were performed based upon the trees sampled after the chains converged (we used 25% as default). Consensus topologies and support values from the different methodological approaches were compiled and drawn using TreeGraph 1.0 (Müller and Müller 2004).

Results

Sequence characterisation and alignment

Features characteristic for the pleurocarpous Hypnales, such as low sequence variation and a short middle part of the intron (stem-loop region P8 in the respective secondary structure; cf. Quandt and Stech 2005)

were also observed in all *Echinodium* species. The length of the *trnL* intron in *Echinodium* was either 265 nucleotides (nt) (*E. hispidum*, *E. prolixum*, *E. umbrosum*), the typical length among pleurocarpous mosses, or 254 nt (*E. renauldii*, *E. setigerum*, *E. spinosum*), i.e., the latter species were characterised by an 11 nt deletion located in P8. The *trnL* intron alignment of the 62 compiled sequences of pleurocarpous mosses comprised 357 positions.

In bryophytes, the nuclear ribosomal spacers (ITS1 and ITS2) are generally more variable than the *trnL* intron, with a higher degree of length variation (e.g., Stech et al. 2003; Vanderpoorten et al. 2006). In *Echinodium*, sequence lengths of the ITS1-5.8S-ITS2 region ranged from 674 nt (*E. renauldii*, *E. setigerum*, *E. spinosum*) to 690 nt (*E. prolixum*) and 701 nt (*E. hispidum*, *E. umbrosum*), respectively. The ITS sequence alignment of all included pleurocarpous mosses comprised 1317 positions.

Of the 1674 positions in the combined *trnL*/ITS alignment, 445 (26.6%) were variable, and 277 (16.5%, or 62.2% of the variable positions) were parsimony-informative. The simple indel coding approach yielded another 269 parsimony-informative characters, resulting in a total of 546 parsimony-informative characters.

Phylogenetic analysis

The MP analysis employing the parsimony ratchet retained 6 trees (length 1091, CI 0.529, RI 0.708, RC 0.374). After inclusion of the indel matrix, four trees were retained (length 1863, CI 0.575, RI 0.732, RC 0.421), of which the strict consensus is shown in Fig. 2, with bootstrap support values (BS) and decay indices (DI) indicated on the branches. Values above the branches are based upon the sequence matrix only, whereas the values below were obtained when the indel matrix was included in the analyses. In the ML analysis, three optimal trees were found (ln $L = -8073.31622$), which were identical except for slight variation within the poorly resolved branch of *Echinodium renauldii*, *E. setigerum*, and *E. spinosum*. One of these trees is shown in Fig. 3, with ML bootstrap values indicated above the branches and significant (≥ 94) posterior probabilities (PP) below. For most branches no or only slight differences in PP values were observed when the indel matrix was included in the analysis. However, three branches were only significantly supported without

indel coding, whereas eight branches received significant PP values only with indel coding.
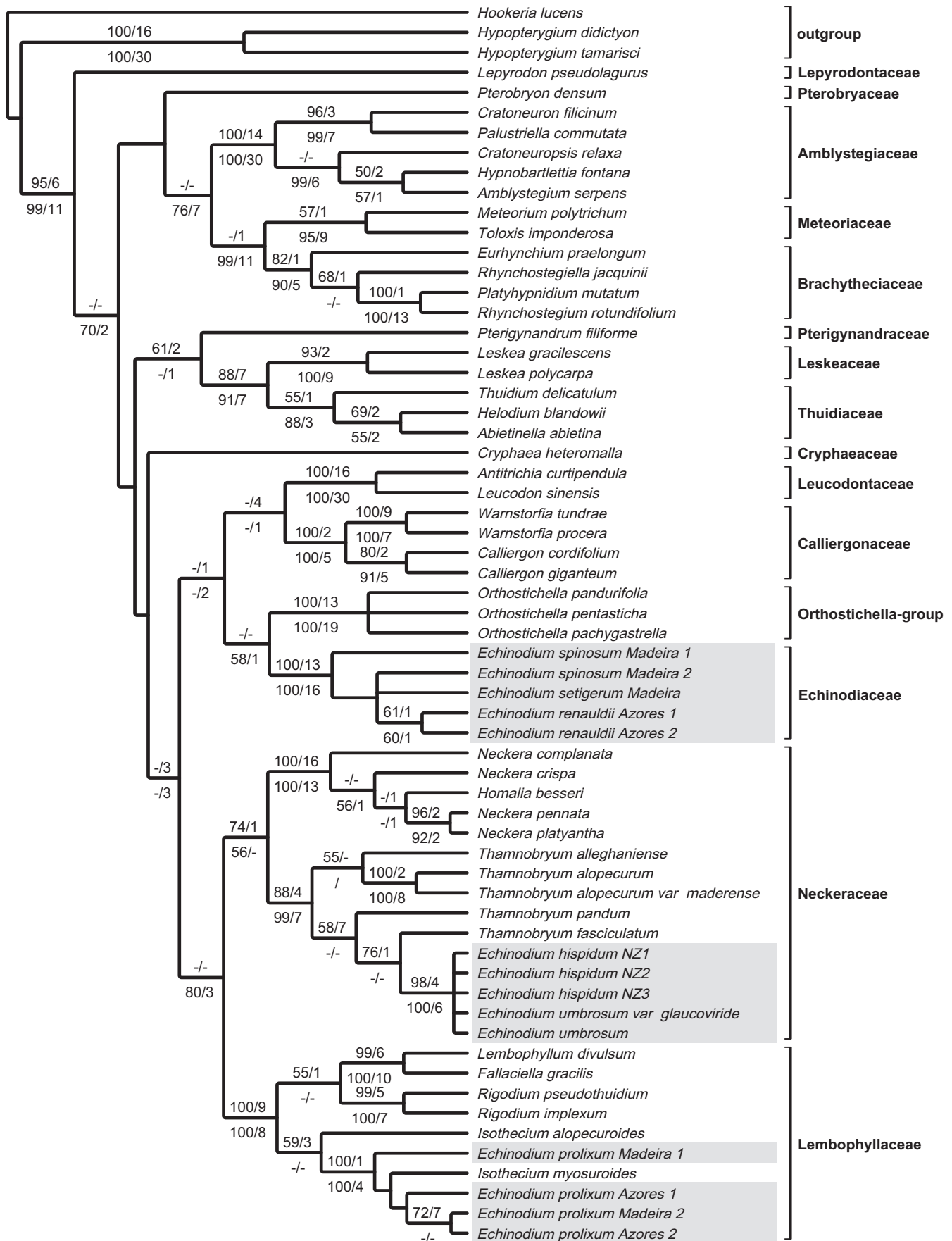
In all analyses the *Echinodium* species are divided into three separate clades. The Macaronesian species *E. renauldii*, *E. setigerum*, and *E. spinosum* are closely related to each other (99–100% BS, DI 13–16, PP 100) (Figs. 2 and 3), but relationships to other genera and families are ambiguous. A sistergroup relationship with *Orthostichella* is indicated in the parsimony analysis with the indel matrix included, but weakly supported (58% BS; Fig. 2). The remaining Macaronesian species, *E. prolixum*, is nested within *Isothecium* in the well-supported Lembophyllaceae clade. In particular, a clade comprising *E. prolixum* and *I. myosuroides* Brid. receives maximal BS and PP support. The closely related samples of *E. hispidum* and *E. umbrosum* (95–98% BS, DI 4–6, PP 100) are nested within *Thamnobryum* in the Neckeraceae. Their sistergroup relationship with *T. pandum* (Hook. f. & Wilson) I.G. Stone & G.A.M. Scott from New Zealand and *T. fasciculatum* (Sw. ex Hedw.) I. Sastre from the Neotropics is less well supported.

Discussion

The molecular data provide clear evidence for polyphyly of *Echinodium* and thus support the suspicion of Churchill (1986) that some *Echinodium* species might belong to other genera. Three molecular groups are significantly supported: the first comprises three of the four Macaronesian species (*E. renauldii*, *E. setigerum*, and *E. spinosum*), the second the remaining Macaronesian species *E. prolixum* (together with *Isothecium myosuroides*), and the third the Australasian species *E. hispidum* and *E. umbrosum*. Similar results were obtained by a preliminary analysis of a smaller data set using *rbcL* sequences (data not shown).

The species of the first group are closely related, not only at the molecular level, but also morphologically (cf. Table 2). In particular *E. spinosum* and *E. setigerum* are sometimes difficult to distinguish at first sight (Fontinha et al. 2001). Furthermore, all three species are largely confined to shady and humid narrow valleys of the laurel forest; *E. renauldii* was also collected at lower altitudes at cave entrances (González-Mancebo et al. 1991). Concerning the substrates, *E. spinosum* occasionally occurs on soil or as an epiphyte, whereas *E. renauldii* and *E. setigerum* are strictly epilithic except

Fig. 2. Strict consensus of four most parsimonious trees showing relationships of the genus *Echinodium* (in grey shading) within the pleurocarpous moss order Hypnales. Combined analysis of plastid *trnL* intron and nuclear ribosomal ITS sequences evaluated with PAUP 4.0b10, employing the parsimony ratchet and simple indel coding according to Simmons and Ochoterena (2000). Bootstrap support values and decay indices indicated on branches. Values above branches based upon sequence matrix only; values below branches obtained when indel matrix was included.

for one collection of the former species on a stem base. On Madeira island, *E. spinosum* and especially *E. setigerum* are linked to the *Clethro arboreae–Ocoteetum foetentis* vascular plant community (Capelo et al. 2000), the latter being restricted to few valleys of well-conserved laurel forest. Possibly, *E. renauldii* and *E. setigerum* evolved from *E. spinosum* under specific environmental conditions in epilithic microhabitats in the laurel forests of Madeira and the Azores, respectively. As *Echinodium spinosum* is the type species of the genus, and because no close relationships with other included pleurocarpous genera and families were observed in the molecular trees (Figs. 2 and 3), the three species represent what remains of *Echinodium* (Echinodiaceae). Their unsupported sistergroup relationship with *Orthostichella* in the parsimony analysis (Fig. 2) needs further investigation of a larger set of pleurocarpous taxa. *Orthostichella* is a segregate of Lembophyllaceae that was placed in Neckeraceae in a recent morphological revision (Allen and Magill 2007), but probably constitutes a separate family according to the molecular data.

Echinodium prolixum is distinguished from the other Macaronesian (and Australasian) species not only at the molecular level, but also morphologically by the leaf shape, the costa ending noticeably before the leaf apex (cf. Table 2), and by its wider ecological amplitude. In Madeira and the Azores, it is the most abundant species of the genus and occurs on several substrates, such as rock, soil, and bark (see, for example, Gabriel and Bates 2005). In Madeira, *E. prolixum* can be found in the laurel forest, in heathlands at higher elevations (*Vaccinio padifoli–Ericetum maderinicolae* and *Polysticho falcinelli–Ericetum arboreae* vascular plant communities; Capelo et al. 2000) as well as in semi-natural forest types. For this species, the affinity with the Lembophyllaceae proposed by Hedenäs (1992) is well supported by the present molecular analyses. By noting morphological similarities between high mountain phenotypes of *E. prolixum* and *Isothecium holtii* Kindb. in Madeira, Hedenäs (1992) also provided morphological support for the transfer of *E. prolixum* to *Isothecium*, which is made here (see Taxonomy section). Thus, the evolutionary history of *E. prolixum* has to be discussed in the course of phylogenetic analyses of *Isothecium* and the Lembophyllaceae in general. However, recent molecular data were partly incongruent with the morphological circumscription of *Isothecium* species (Draper et al. 2007), and further molecular analyses including *E. prolixum* have to be carried out to infer its affinities and taxonomic status.

The position of the Australasian species *E. hispidum* and *E. umbrosum* within the *Thamnobryum* clade (Figs. 2 and 3) is in accordance with the molecular study of Buck et al. (2000), which, however, included only *E. umbrosum*, but is more difficult to interpret on morphological grounds. A comparison of gametophytic characters indicates that *E. hispidum* and *E. umbrosum* are somewhat intermediate between *Echinodium* s.str. and *Thamnobryum* (Table 2). Ecologically, *E. hispidum* does share with many *Thamnobryum* spp. the preference for wet and aquatic habitats. At least for this species, the long subulate leaves with partially to fully bistratose margins enhancing mechanical stability may be an adaptation to aquatic environments, a phenomenon also found in *Thamnobryum*, e.g., in the Madeiran endemic *T. fernandesii* Sérgio (cf. Hedenäs 1992). However, as with *E. prolixum*, the affinities of *E. umbrosum* and *E. hispidum* have to be treated in the context of a more detailed phylogenetic analysis of the Neckeraceae, which is beyond the scope of this paper.

The present molecular study bears important consequences for the understanding of the evolutionary history of *Echinodium* and the explanation of the extant distribution pattern. Churchill (1986) noted that the Macaronesian–Australasian disjunct distribution of *Echinodium* as a natural taxon would be exceptionally noteworthy, but that questions over the monophyly of the genus meant that biogeographic conclusions should be treated with caution. In fact, the polyphyly of *Echinodium* indicated by the present molecular data clearly suggests that the distribution pattern based on the morphology of Macaronesian and Australasian plants is an artefact of independent evolutionary events. Nevertheless, the novel occurrence of a suite of morphological characters not typical for taxa included in well-supported clades amongst the Hypnalean pleurocarps requires some explanation. This could imply either that a set of plesiomorphic characters (irregularly pinnate to subdendroid branching, subulate leaves with percurrent to excurrent costae, short leaf cells) was retained in unrelated groups in relict habitats in Macaronesia and Australasia, or that parallel morphological evolution of the 'Echinodium habit' took place in both geographic regions. Of these hypotheses, the evolutionary parallelism scenario seems more plausible for several reasons. Firstly, the genus *Bescherellia* of the Hypnodendrales is morphologically very similar to *Echinodium*, except that the costa is differentiated in cross-section, and also occurs in similar habitats. However, *Bescherellia* is regarded as quite derived within the hypnodendroid pleurocarps, which are sister to the homocostate pleurocarps to which the Hypnales belong (cf. Bell et al. 2007). Secondly, according to molecular dating (Newton et al. 2007), the diversification of major lineages within the Hypnales leading to extant genera had already taken place by the Early to Late Cretaceous (mainly between ca. 130 and 80 mya) and therefore much earlier than the oldest fossil records of *Echinodium* (cf. Fig. 1). Furthermore, none of the families that may represent basal lineages in the Hypnales according to molecular phylogenies, e.g.,
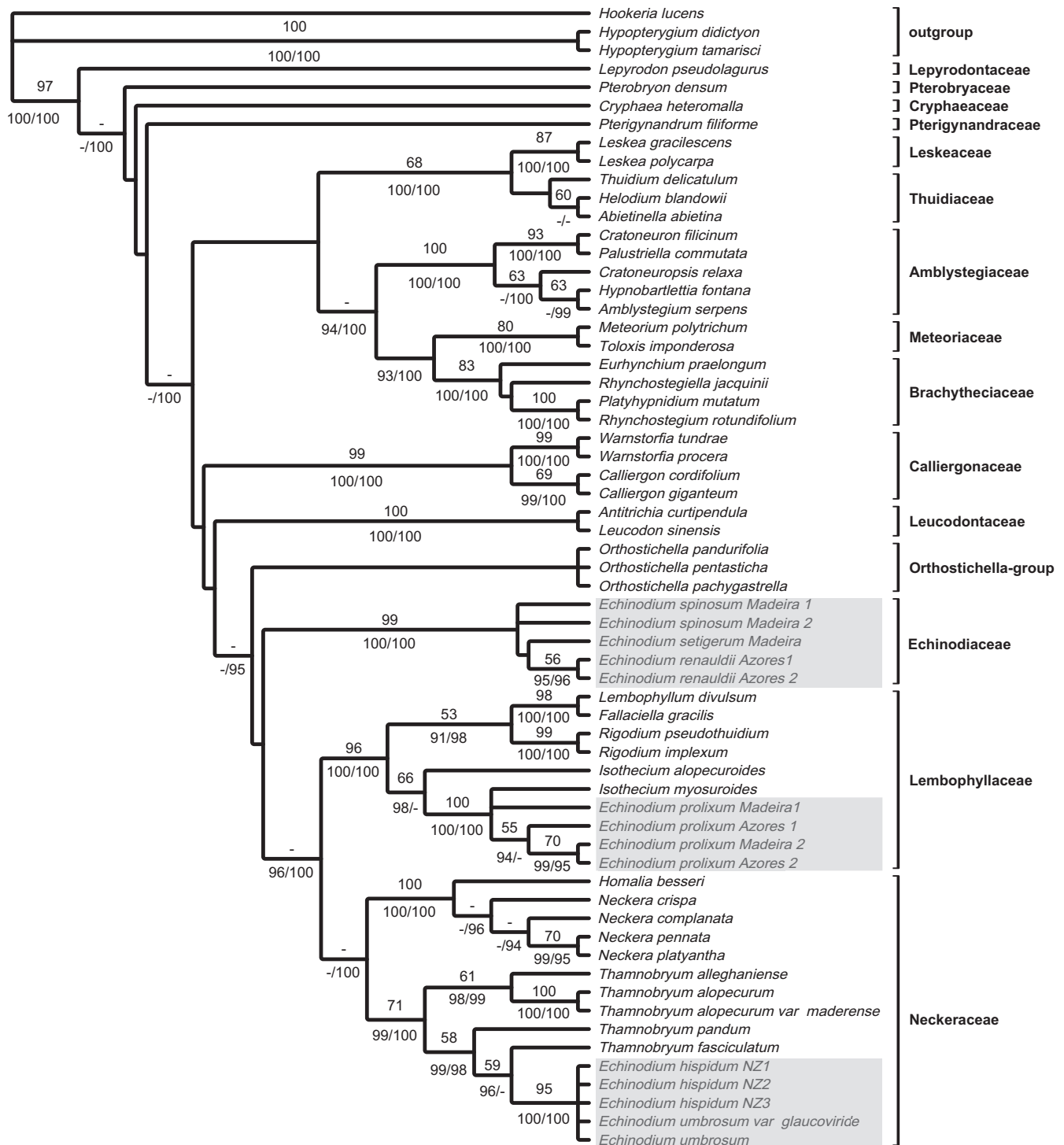
Fig. 3. One out of three optimal maximum likelihood trees (GTR + Γ + I model) based on combined plastid *trnL* intron and nuclear ribosomal ITS sequences, showing relationships of the genus *Echinodium* (in grey shading) within the pleurocarpous moss order Hypnales. Likelihood bootstrap values indicated above branches; significant (≥94) posterior probabilities (without/with simple indel coding) below branches.

Fontinalaceae, Fabroniaceae, Plagiotheciaceae, or Stereophyllaceae (e.g., Buck et al. 2000; Tsubota et al. 2004; Ignatov et al. 2007), show the combination of characters attributed to the 'Echinodium habit'. Thirdly, the *Echinodium* fossils from Tasmania are of only

Pleistocenic age, and at least the extant Australasian *Echinodium* species could be the result of a rather recent parallel evolution in aquatic environments. However, as they are related with *Thamnobryum* according to the present molecular data, *E. hispidum* and *E. umbrosum*,

Table 2. Comparison of gametophytic characters in *Echinodium*, *Isothecium*, and *Thamnobryum*

Taxon character	*Echinodium* s.str. (*E. renauldii*, *E. setigerum*, *E. spinosum*)	*Echinodium hispidum*/ *E. umbrosum*	*Thamnobryum* spp.	*Echinodium prolixum*	*Isothecium* spp.
Branching of secondary stems	Irregularly pinnate or sub-dendroid	Irregularly pinnate	Irregularly pinnate to often frondose or dendroid	Irregularly pinnate or sub-dendroid	Irregularly pinnate or sub-dendroid
Leaf shape (leaves of secondary stems)	Subulate from ovate or triangular base	Broadly linear-lanceolate or subulate from ovate or triangular base	Ovate to lanceolate	Broadly lanceolate to ovate-triangular	Broadly ovate to long-triangular
Leaf plication	Plicate	Plicate or indistinct	Not plicate	Plicate	Not plicate
Upper leaf margins	Entire (awn toothed in *E. spinosum*)	Entire or serrulate	Often serrate	Serrulate	Serrulate
Leaf lamina	Margins bi- or pluristratose, other lamina uni- or bistratose	Margins bi- or pluristratose, other lamina uni- or bi- to pluristratose	Uni- to variously bi- or pluristratose	Sometimes small portions bistratose	Unistratose
Shape of median lamina cells	Isodiametric to oblong	Isodiametric to oblong	Isodiametric to rhomboidal	Rhomboidal to linear	Rhomboidal to linear
Ornamentation of median lamina cells	Smooth	Smooth or papillose	Smooth or papillose	Smooth	Smooth or abaxially prorate
Alar cells	In small and often auriculate group	Not differentiated	Few, not sharply delimited	Basal and alar cells similarly differentiated (in stem leaves alar groups more similar to *Isothecium*)	In indistinct auriculate group
Costa	Long-excurrent	Percurrent to long-excurrent	Subpercurrent	Ending in upper 1/3 of leaf, well below leaf apex	Ending at mid-leaf or in upper half of leaf

like *Thamnobryum* in general, probably represent the most primitive elements within Neckeraceae (e.g., Enroth 1994).

Taxonomy

Isothecium prolixum (Mitt.) Stech, Sim-Sim, Tangney & D. Quandt, comb. nov.

Basionym: *Leskea prolixa* Mitt. Journal of the Proceedings of the Linnean Society 8: 7. 1. 1864.
Echinodium prolixum (Mitt.) Broth. Die Natürlichen Pflanzenfamilien I(3): 1217. 1909.

Thamnobryum hispidum (Hook. f. & Wilson) Stech, Sim-Sim, Tangney & D. Quandt, comb. nov.

Basionym: *Hypnum hispidum* Hook. f. & Wilson. London Journal of Botany 3: 552. 1844.

Echinodium hispidum (Hook. f. & Wilson) Reichardt. Reise der Österreichischen Fregatte Novara um die Erde 1(3): 127. 1870.

Thamnobryum umbrosum (Mitt.) Stech, Sim-Sim, Tangney & D. Quandt, comb. nov.

Basionym: *Leskea umbrosa* Mitt. Journal of the Proceedings of the Linnean Society 4: 92. 1859.
Echinodium umbrosum (Mitt.) A. Jaeger. Bericht über die Thätigkeit der St. Gallischen Naturwissenschaftlichen Gesellschaft 1876–1877: 314 (Gen. Sp. Musc. 2: 1132). 1878.

Thamnobryum umbrosum var. *glauco-viride* (Mitt.) Stech, Sim-Sim, Tangney & D. Quandt, comb. nov.

Basionym: *Hypnum glaucoviride* Mitt. Handbook of the New Zealand Flora 473. 1867.

Echinodium umbrosum var. *glauco-viride* (Mitt.) S.P. Churchill. Journal of Bryology 14: 129. 1986.

Acknowledgements

References

Abramov, A.L., Abramov, I.I., 1958. On certain genetic relations among the fossil mosses of the flora of Duab (Abkhazia). Bot. Zhurn. USSR 43, 1018–1024.

Allen, B.H., Magill, R.E., 2007. A revision of *Orthostichella* (Neckeraceae). Bryologist 110, 1–45.

Brotherus, V.F., 1925. Musci (Laubmoose), 2. Hälfte. In: Engler, A., Prantl, K. (Eds.), Die Natürlichen Pflanzenfamilien. Engelmann, Leipzig, pp. 1–542.

Bell, N.E., Quandt, D., O'Brien, T.J., Newton, A.E., 2007. Taxonomy and phylogeny in the earliest diverging pleurocarps: square holes and bifurcating pegs. Bryologist 110, 533–560.

Buck, W.R., Vitt, D.H., 1986. Suggestions for a new familial classification of pleurocarpous mosses. Taxon 35, 21–60.

Buck, W.R., Goffinet, B., Shaw, A.J., 2000. Testing morphological concepts of orders of pleurocarpous mosses (Bryophyta) using phylogenetic reconstructions based on *trnL-trnF* and *rps4* sequences. Mol. Phyl. Evol. 16, 180–198.

Capelo, J., Costa, J.C., Lousã, M., Fontinha, S., Jardim, R., Sequeira, M., Rivas-Martínez, S., 2000. Vegetação da Madeira (Portugal). I. Aproximação à tipologia fitossociológica. Silva Lus 7, 257–282.

Capelo, J., Sequeira, M., Jardim, R., Mesquita, S., Costa, J.C., 2005. The vegetation of Madeira Island (Portugal). A brief overview and excursion guide. Quercetea 7, 95–122.

Churchill, S.P., 1986. A revision of *Echinodium* Jur. (Echinodiaceae: Hypnobryales). J. Bryol. 14, 117–133.

De Luna, E., Buck, W.R., Akiyama, H., Arikawa, T., Tsubota, H., Gonzalez, D., Newton, A.E., Shaw, A.J., 2000. Ordinal phylogeny within the Hypnobryalean pleurocarpous mosses inferred from cladistic analyses of three chloroplast DNA sequence data sets: *trnL-F*, *rps4*, and *rbcL*. Bryologist 103, 242–256.

Dierßen, K., 2001. Distribution, ecological amplitude and phytosociological characterization of European bryophytes. Bryophyt. Bibl. 56, 1–289.

Draper, I., Hedenäs, L., Grimm, G.W., 2007. Molecular and morphological incongruence in European species of *Isothecium* (Bryophyta). Mol. Phyl. Evol. 42, 700–716.

ECCB, 1995. Red Data Book of European Bryophytes. European Committee for Conservation of Bryophytes, Trondheim.

Enroth, J., 1994. On the evolution and circumscription of the Neckeraceae (Musci). J. Hattori Bot. Lab. 76, 13–20.

Fleischer, M., 1909–1923. Die Musci der Flora von Buitenzorg. E.J. Brill, Leiden.

Fontinha, S., Sim-Sim, M., Sérgio, C., Hedenäs, L., 2001. Briófitos endémicos da Madeira. In: DRAmb SRA (Ed.), Biodiversidade Madeirense: Avaliação e Conservação. Madeira, Portugal, pp. 1–51.

Fontinha, S., Sim-Sim, M., Lobo, C., 2006. The bryophytes of the Laurisilva of Madeira. Guide to Some Species. SRARN, SPNM, e FFCUL e CEBVFCUL, Portugal.

Frahm, J.-P., 2004. A new contribution to the moss flora of Baltic and Saxon amber. Rev. Palaeobot. Palynol. 129, 81–101.

Gabriel, R., 1994. Briófitos da Ilha Terceira (Açores). Ecologia, Distribução e Vulnerabilidade de Espécies Seleccionadas. Thesis, Universidade dos Açores.

Gabriel, R., Bates, J.W., 2005. Bryophyte community composition and habitat specificity in the natural forests of Terceira, Azores. Plant Ecol. 177, 125–144.

Goffinet, B., Buck, W.R., 2004. Systematics of the Bryophyta (mosses): from molecules to a revised classifcation. In: Goffinet, B., Hollowell, V., Magill, R. (Eds.), Molecular Systematics of Bryophytes. Monogr. Syst. Bot. Missouri Bot. Gard. 98, 205–239.

González-Mancebo, J.M., Losada-Lima, A., Hérnandez-Garcia, C.D., 1991. A contribution to the floristic knowledge of caves on the Azores. Mém. Biospéol. 17, 219–226.

Hedenäs, L., 1992. Flora of Madeiran pleurocarpous mosses (Isobryales, Hypnobryales, Hookeriales). Bryophyt. Bibl. 44, 1–165.

Hedenäs, L., 1995. Higher taxonomic level relationships among diplolepidous pleurocarpous mosses – a cladistic overview. J. Bryol. 18, 723–781.

Huelsenbeck, J.P., Ronquist, F., 2001. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.

Huelsenbeck, J.P., Ronquist, F., Nielsen, R., Bollback, J.P., 2001. Bayesian inference of phylogeny and its impact on evolutionary biology. Science 294, 2310–2314.

Huelsenbeck, J.P., Larget, B., Miller, R.E., Ronquist, F., 2002. Potential applications and pitfalls of Bayesian inference of phylogeny. Syst. Biol. 51, 673–688.

Ignatov, M.S., Gardiner, A.A., Bobrova, V.K., Milyutina, I.A., Huttunen, S., Troitsky, A.V., 2007. On the relationships of mosses of the order Hypnales, with special reference to taxa traditionally classified in the Leskeaceae. In: Newton, A.E., Tangney, R.S. (Eds.), Pleurocarpous Mosses, Systematics and Evolution. Syst. Assoc. Spec. Vol. 71, 177–213.

IUCN, 2002. The 2000 IUCN World Red List of Bryophytes. International Union for the Conservation of Nature (IUCN). ⟨http://www.artdata.slu.se/guest/SSCBryo/WorldBryo.htm⟩.

Jordan, G.J., Dalton, P.J., 1995. Mosses from Early Pleistocene sediments in western Tasmania. Alcheringa 19, 291–296.

Kelchner, S.A., 2000. The evolution of non-coding chloroplast DNA and its application in plant systematics. Ann. Missouri Bot. Gard. 87, 482–498.

Lancucka-Srodoniowa, M., 1996. Tortonian flora from the "Gdow Bay" in the South of Poland. Acta Palaeobot. 7, 1–135.

Müller, K., 2004a. SeqState – primer design and sequence statistics for phylogenetic DNA data sets. Appl. Bioinformatics 4, 65–69.

Müller, K., 2004b. PRAP – calculation of Bremer support for large data sets. Mol. Phyl. Evol. 31, 780–782.

Müller, J., Müller, K., 2004. TreeGraph: automated drawing of complex tree figures using an extensible tree description format. Mol. Ecol. Notes 4, 786–788.

Müller, K., Müller, J., Neinhuis, C., Quandt, D., 2006. PhyDE – Phylogenetic Data Editor, v0.983. Program distributed by the authors. ⟨http://www.phyde.de⟩.

Newton, A.E., Wikström, N., Bell., N., Forrest, L.L., Ignatov, M.S., 2007. Dating the diversification of the pleurocarpous mosses. In: Newton, A.E., Tangney, R.S. (Eds.), Pleurocarpous Mosses, Systematics and Evolution. Syst. Assoc. Spec. Vol. 71, 337–366.

Nuin, P.A.S., 2005. Mtgui – A Simple Interface to ModelTest. Program distributed by the author. University of Toronto. ⟨http://www.genedrift.org/mtgui.php⟩.

Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Quandt, D., Stech, M., 2005. Molecular evolution and secondary structure of the chloroplast trnL intron in bryophytes. Mol. Phyl. Evol. 36, 429–443.

Quandt, D., Bell, N., Stech, M., 2007. Unravelling the knot: Pulchrinodaceae, fam. nov. (Bryales). In: Kürschner, H., Pfeiffer, T., Stech, M. (Eds.), A Festschrift to Wolfgang Frey – a scientist's life between 'deserts' and 'rain forests'. Nova Hedwigia Beih. 131, 21–39.

Robinson, H., 1971. A revised classification for the orders and families of mosses. Phytologia 21, 289–293.

Simmons, M.P., Ochoterena, H., 2000. Gaps as characters in sequence-based phylogenetic analyses. Syst. Biol. 49, 369–381.

Sim-Sim, M., Stech, M., Esquível, M.G., Figueiredo, A.C., Costa, M.M., Barroso, J.G., Pedro, L.G., Fontinha, S., Lobo, C., 2005. Plagiochila spinulosa (Dicks.) Dumort. (Plagiochilaceae, Hepaticophytina) in Madeira Island – morphological, phytochemical, and molecular evidence. J. Hattori Bot. Lab. 98, 131–147.

Stech, M., Quandt, D., Lindlar, A., Frahm, J.-P., 2003. The systematic position of Pulchrinodus inflatus (Pterobryaceae, Bryopsida) based on molecular data. Studies in austral temperate rain forest bryophytes 21. Aust. Syst. Bot. 16, 561–568.

Swofford, D.L., 2002. PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods), version 4.0b10. Sinauer Associates Inc., Sunderland, MA.

Tsubota, H., De Luna, E., González, D., Ignatov, M.S., Deguchi, H., 2004. Molecular phylogenetics and ordinal relationships based on analyses of a large-scale data set of 600 rbcL sequences of mosses. Hikobia 14, 149–170.

Vanderpoorten, A., Goffinet, B., Quandt, D., 2006. Utility of the internal transcribed spacers of the 18S-5.8S-26S nuclear ribosomal DNA in plant systematics. In: Sharma, A.K., Sharma, A. (Eds.), Plant Genome: Biodiversity and Evolution, vol. 2B. Science Publishers, Enfield, NH, pp. 385–407.

Vitt, D.H., 1984. Classification of the Bryopsida. In: Schuster, R.M. (Ed.), New Manual of Bryology, vol. 2. Hattori Botanical Laboratory, Nichinan, pp. 696–759.

Walther, K., 1983. Bryophytina. Laubmoose. In: Gerloff, J., Poelt, J. (Eds.), Syllabus der Pflanzenfamilien, 13. Aufl., Gebrüder Bornträger, Berlin, pp. 1–108.