Molecular Phylogenetics and Evolution 94 (2016) 635–657

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Molecular phylogenetics and the morphology of the Lycopodiaceae subfamily Huperzioideae supports three genera: *Huperzia*, *Phlegmariurus* and *Phylloglossum* ☆

Ashley R. Field [a,b,c,*], Weston Testo [d], Peter D. Bostock [b], Joseph A.M. Holtum [c,e], Michelle Waycott [c,f]

[a] Australian Tropical Herbarium, PO Box 6811, Cairns, QLD 4878, Australia
[b] Queensland Herbarium, Department of Science, Information Technology and Innovation, Toowong, QLD 4066, Australia
[c] College of Marine and Environmental Sciences, James Cook University, Douglas, QLD 4811, Australia
[d] Department of Plant Biology, University of Vermont, 111 Jeffords Hall, 63 Carrigan Drive, Burlington, VT 05405, USA
[e] Smithsonian Tropical Research Institute, PO Box 0843-0392, Balboa, Ancon, Panama
[f] School of Earth and Environmental Sciences, The University of Adelaide and State Herbarium of South Australia, Adelaide, SA 5001, Australia

ARTICLE INFO

Article history:
Received 13 July 2015
Revised 27 September 2015
Accepted 28 September 2015
Available online 19 October 2015

Keywords:
Lycopodiaceae
Phlegmariurus
Phylloglossum
Huperzia
Tassel-fern
Club-moss

ABSTRACT

The generic classification of huperzioid Lycopodiaceae was tested using Bayesian inference and Maximum likelihood phylogenetic analyses of DNA sequences from four chloroplast loci for 119 taxa and optimisation of 29 morphological characteristics onto the phylogeny. Consistent with previous studies, the subfamilies Lycopodioideae and Huperzioideae are monophyletic and diagnosable by synapomorphies that correlate with differences in their life-histories. Within the Huperzioideae, the monophyly of the widely adopted genus *Huperzia* (excl. *Phylloglossum*) is poorly supported. Three clades of huperzioid Lycopodiaceae were recovered in all analyses of molecular data: *Phylloglossum drummondii*, *Huperzia sensu stricto* and *Phlegmariurus sensu lato*. These clades are strongly supported by morphological characters, including differences in spores, gametophytes, sporophyte macro-morphology, as well as growth habit and life-histories. Our findings indicate that either a one-genus (*Huperzia s.l.*) or a three-genus (*Phylloglossum*, *Huperzia s.s.* and *Phlegmariurus s.l.*) classification of huperzioid Lycopods are equally supported by molecular evidence, but a two-genus system (*Huperzia s.l.* + *Phylloglossum*) is not. We recommend recognising three genera in the huperzioid Lycopodiaceae, as this classification best reflects evolutionary, ecological, and morphological divergence within the lineage.

Crown Copyright © 2015 Published by Elsevier Inc. All rights reserved.

1. Introduction

Lycopods, class Lycopodiopsida, represent an early diverging group of vascular plants with an ancient and diverse fossil record over 420 million years (Bateman, 1996; Bateman et al., 1992, 2007; Garrat, 1984; Rickards, 2000; Ruggiero et al., 2015). They dominated earth's flora in size and diversity through the Devonian and Carboniferous periods but were largely superseded by ferns, conifers and angiosperms and now represent less than 1% of the world's flora (Kenrick and Davis, 2004; White, 1986). There are three extant orders in the class Lycopodiopsida, the heterosporous Isoetales that includes the family Isoetaceae with ~150 spp. and the Selaginellales which includes the family Selaginellaceae with ~700 spp. and the homosporous Lycopodiales which represents the family Lycopodiaceae with 200–500 spp. (Øllgaard, 1987; Ruggiero et al., 2015). Of these lineages, the Lycopodiaceae occupies the widest range of habitats and exhibits a diversity of life forms that include small deciduous semi-aquatics, vines, robust scrambling and clump-forming terrestrials and pendent epiphytes (Fig. 1). Not surprisingly, the generic classification of Lycopodiaceae has been contentious, ranging from a two to a sixteen-genus system (Christenhusz et al., 2011; Field and Bostock, 2013; Holub, 1985a, 1991b; Nessel, 1939; Øllgaard, 1987). Most Lycopodiaceae species have been re-classified into different genera several times, leading to uncertainty about their most appropriate generic identification. The epiphytic huperzioid species for example have been variously placed in the genera *Lycopodium* L., *Urostachys* Herter, *Huperzia* Bernh. and *Phlegmariurus* Holub.

Molecular phylogenetic studies have provided evidence for deep Palaeozoic divergence between lycopodioid and huperzioid clades of Lycopodiaceae and of extant genera dating back to the

☆ This paper was edited by the Associate Editor Elizabeth Zimmer.

* Corresponding author at: Australian Tropical Herbarium, PO Box 6811, Cairns, QLD 4878, Australia.
E-mail address: ashley.field@jcu.edu.au (A.R. Field).

http://dx.doi.org/10.1016/j.ympev.2015.09.024
1055-7903/Crown Copyright © 2015 Published by Elsevier Inc. All rights reserved.



Fig. 1. Diversity of habits and habitats in the family Lycopodiaceae. Subfamily Huperzioideae: (A) *Huperzia australiana*, Australia, A.Field photo; (B) *Phlegmariurus phlegmarioides*, Australia, A.Field photo; (C) *Phlegmariurus dalhousieanus*, Australia, A.Field photo; (D) *Phlegmariurus hypogaeus*, Ecuador, M.Sundue photo; (E) *Phylloglossum drummondii*, Australia, W.Archer photo; (F) *Phlegmariurus treitubensis*, Brazil, T.Almeida photo. Subfamily Lycopodioideae: (G) *Pseudodiphasium volubile*, Papua New Guinea, M.Sundue photo; (H) *Dendrolycopodium obscurum* United States of America, M.Sundue photo; (I) *Spinulum annotinum*, United States of America, M.Sundue photo; (J) *Palhinaea cernua*, Australia, A.Field photo; (K) *Diphasiastrum digitatum*, United States of America, M.Sundue photo; (L) *Lycopodiastrum casuarinoides*, Malaysia, M.Sundue photo.

Table 1
Summary statistics for the four data alignments used to investigate the phylogeny of the Lycopodiaceae.

Matrix	Matrix length	Parsimony-informative characters	Substitution model
*rbc*L	1351	366	GTR + G
*trn*L + *trn*L-*trn*F	1406	387	HKY + G
trnH-psbA	406	195	GTR + G

Mesozoic (Ji et al., 2008; Wagner and Beitel, 1992; Wikström, 2001; Wikström and Kenrick, 1997, 2001; Yatsentyuk et al., 2001). Within the huperzioid clade, molecular studies show an affinity between the tiny *Phylloglossum drummondii* Kunze and the genus *Huperzia* (Wikström and Kenrick, 1997) and a single origin of epiphytism with distinct Neotropical and Palaeotropical subclades in the epiphytic clade (Wikström and Kenrick, 2000). Based on interpretation of the studies published so far, we consider that evidence for the monophyly of *Huperzia s.l.* but excluding *Phylloglossum* is not adequate to justify its acceptance.

Numerous comprehensive morphological and anatomical studies of Lycopodiaceae are available that variously group the species in differing generic classifications (Bower, 1885; Breckon, 1974; Breckon and Falk, 1974; Bruce, 1976a; Hackney, 1950; Holloway, 1935; Holub, 1964; Øllgaard, 1975, 1979b, 1987, 1989a, 2012; Wagner and Beitel, 1992; Whittier, 2006; Whittier and Braggins, 1992, 2000; Whittier and Storchova, 2007; Wilce, 1972). In summary, shoot branching patterns, stele type, spore shape and surface ornamentation, gametophyte type and fertile/sterile microphyll dimorphy have been consistently used to characterise major lineages; however, these traits have not yet been fully explored within the context of molecular-derived phylogenetic hypotheses. We consider that the optimisation of morphological characters onto phylogenetic trees is essential for (1) testing existing classifications (2) identifying and placing in a cladistic framework morphological features found in fossils that could be used as calibration points for future estimation of clade ages and (3) translating the recovered phylogenetic hypotheses into a revised morphology-based classification.

The aim of this study is to reconstruct phylogenetic relationships in huperzioid Lycopodiaceae based on molecular evidence and investigate the relationships with morphological and anatomical characters. Our study expands the sampling of Huperzioid Lycopodiaceae from 26 species in the most recent published study (Ji et al., 2008) to 86 species. It also expands sampling further into Malesia and Oceania including previously unsampled morphological groups. This is the first molecular phylogenetic study presented that maps and appraises the morphological characteristics used to underpin existing classifications. In particular, we aim to examine the support for three available generic classifications of Huperzioideae: 1: *Huperzia sensu lato* (Christenhusz et al., 2011); 2: *Huperzia* + *Phylloglossum* (Øllgaard, 1987) and 3: *Huperzia* + *Phylloglossum* + *Phlegmariurus* (Field and Bostock, 2013; Øllgaard, 2012, 2015; Wagner and Beitel, 1992; Zhang, 2004; Zhang and Kung, 1998, 1999, 2000). We also characterise patterns of divergence for the Lycopodiaceae as a whole and provide further insight into the important innovations that underlie the ecological diversity that is characteristic of this family.

Table 2
Phenotypic characters used for phylogenetic analysis and character mapping of the Lycopodiaceae. Further discussion of characters is provided in Appendix B.

Life cycle
1. 0 = homosporous; 1 = heterosporous

Spores
2. 0 = foveolate-fossulate; 1 = scabrate; 2 = rugate; 3 = bacculate; 4 = reticulate
3. 0 = spore ornamentation on distal surface only; 1 = spore surface ornamentation on distal and proximal surfaces
4. 0 = spores with convex margins in polar view; 1 = spores with concave margins in polar view and corners truncate

Gametophytes
5. 0 = gametophyte without paraphyses among gametangia; 1 = gametophyte with paraphyses among gametangia
6. 0 = gametophyte germination subterranean in the dark; 1 = gametophyte germination surficial in the light
7. 0 = gametophyte growth axis vertical with a radial cross section; 1 = gametophyte growth axis horizontal with a dorsiventral cross section
8. 0 = gametophyte holomycotrophic; 1 = gametophyte trophy facultative; 2 = gametophyte autotrophic

Sporophyte anatomy
9. 0 = eligulate; 1 = ligulate
10. 0 = protostele; 1 = polystele
11. 0 = roots adventitious; 1 = roots rhizophorous; 2 = roots rhizomorphous
12. 0 = actinostelic; 1 = pseudosolenostelic; 1 = pseudosolenostelic; 2 = plectostelic in prostrate branches and actinostelic in aerial branches
13. 0 = stem stele 1/2–1/3 stem diameter and root emergence non-corticular; 1 = stem stele 1/4–1/20 stem diameter and root emergence corticular
14. 0 = mucilage duct in sporophyll base absent; 1 = mucilage duct in sporophyll base present.
15. 0 = sporangial valve cell walls thick and lignified; 1 = sporangial valve cell walls thin and non-lignified
16. 0 = sporangial cell walls sinuate; 1 = sporangial cell walls non-sinuate

Sporophyte morphology
17. 0 = shoot elongate and branched; 1 = shoot reduced, unbranched, microphylls tufted
18. 0 = unbranched; 1 = shoots isotomously branched; 2 = shoots anisotomously branched
19. 0 = new shoots and roots emerging from stem bases forming a tuft; 1 = new shoots and roots arising adventitiously from laterally spreading branches
20. 0 = shoots evergreen, lacking an underground tuber; 1 = shoots deciduous dying back to an underground tuber
21. 0 = fertile shoot sessile; 1 = fertile shoot on leafy peduncle; 2 = fertile shoot on leafless peduncle
22. 0 = sporophylls paleate; 1 = sporophylls peltate
23. 0 = sporophylls evergreen; 1 = sporophylls deciduous following dehiscence of sporangia
24. 0 = sporangial stalk absent; 1 = sporangial stalk present
25. 0 = sporophyllous shoots not bulbiferous; 1 = sporophyllous shoots bulbiferous
26. 0 = lycophylls flat; 1 = lycophylls terete
27. 0 = isophyllous; 1 = weakly to strongly anisophyllous but lycophylls not ordered into specialised ranks; 2 = trimorphic lycophylls with one dorsal and one ventral rank of narrow lycophylls and two lateral ranks of broad lycophylls; 3 = dimorphic lycophylls with broad dorsal lycophylls in 2 ranks and narrow ventral lycophylls in 2–3 ranks; 4 = flattened isophyllous shoots achieved via broad compressed lateral leaf bases; 5 = trimorphic lycophylls with 1–2 dorsal ranks and one ventral rank of narrow lycophylls and 2 lateral ranks of broad compressed subopposite lycophylls
28. 0 = branching inclinate anisotomous; 1 = branching flabellate anisotomous

Habit
29. 0 = aquatic; 1 = terrestrial; 2 = epiphytic

2. Materials and methods

2.1. Taxon sampling

DNA, herbarium vouchers and living tissue for morphological character analysis were collected from the wild for broad spectrum Lycopodiaceae. This study increased the sampling of Huperzioid Lycopoidaceae to 84 species, adding 67 new sequences and 55 new species. DNA accessions not made by the authors were obtained from numerous field collectors and from GenBank. Representatives were sampled from all previously reported clades within the Lycopodiaceae and from as many informal *Huperzia*

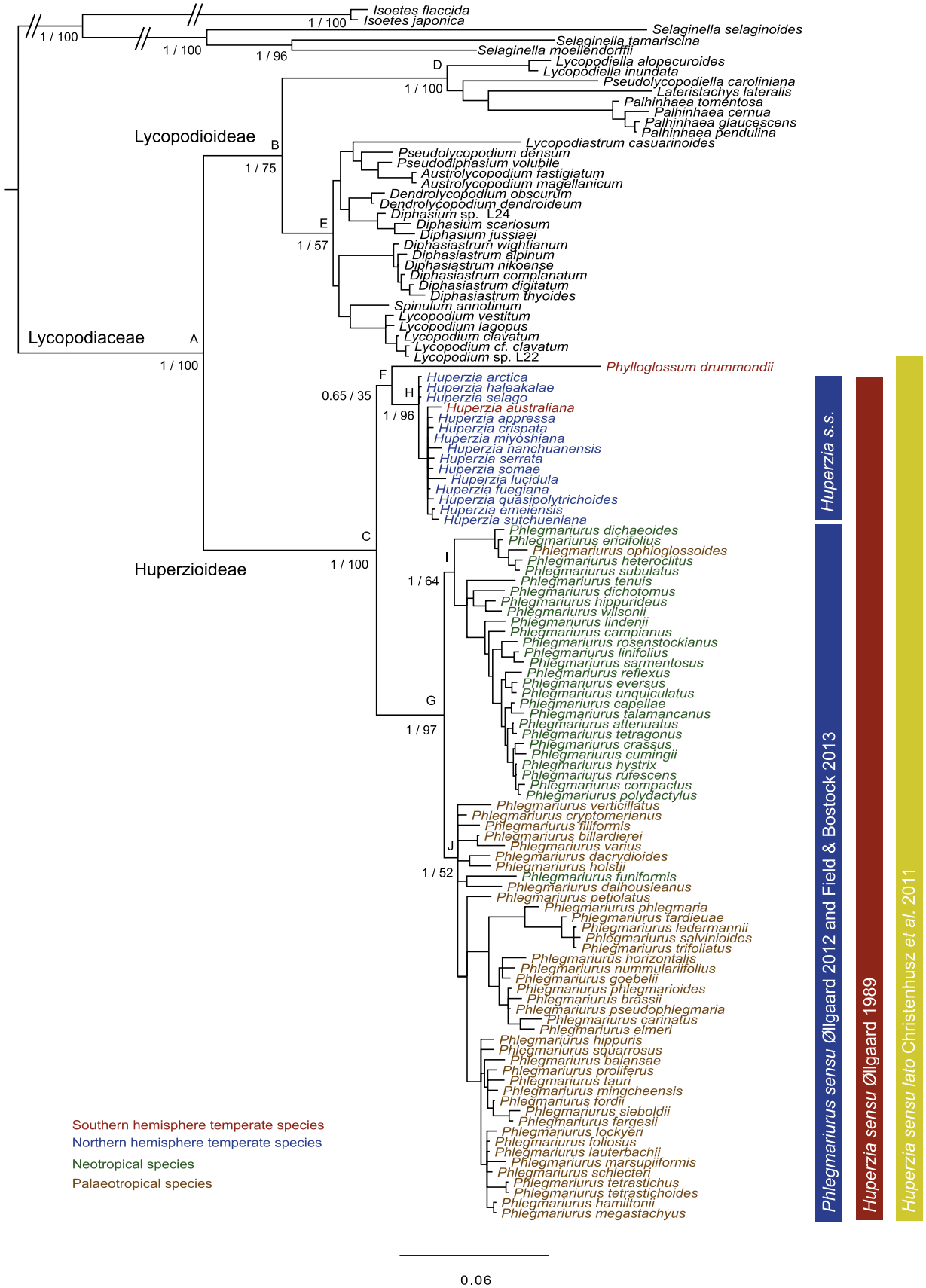


Fig. 2. 50% majority rule Bayesian inference phylogram of the Lycopodiaceae rooted with Isoetaceae and Selaginellaceae based on DNA sequences of the chloroplast loci *rbc*L, *trn*L, *trn*L-*F* and *trn*H-*psb*A. Nodes mentioned in the text are lettered A–J and support values for these nodes are shown below the node as posterior probabilities of the Bayesian analysis/bootstrap values of the Maximum likelihood analysis. The scale bar represents substitutions per site. Northern hemisphere temperate Huperzioid species are labelled in blue, Southern hemisphere temperate species in red, Neotropical species in green and Palaeotropical species in brown. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

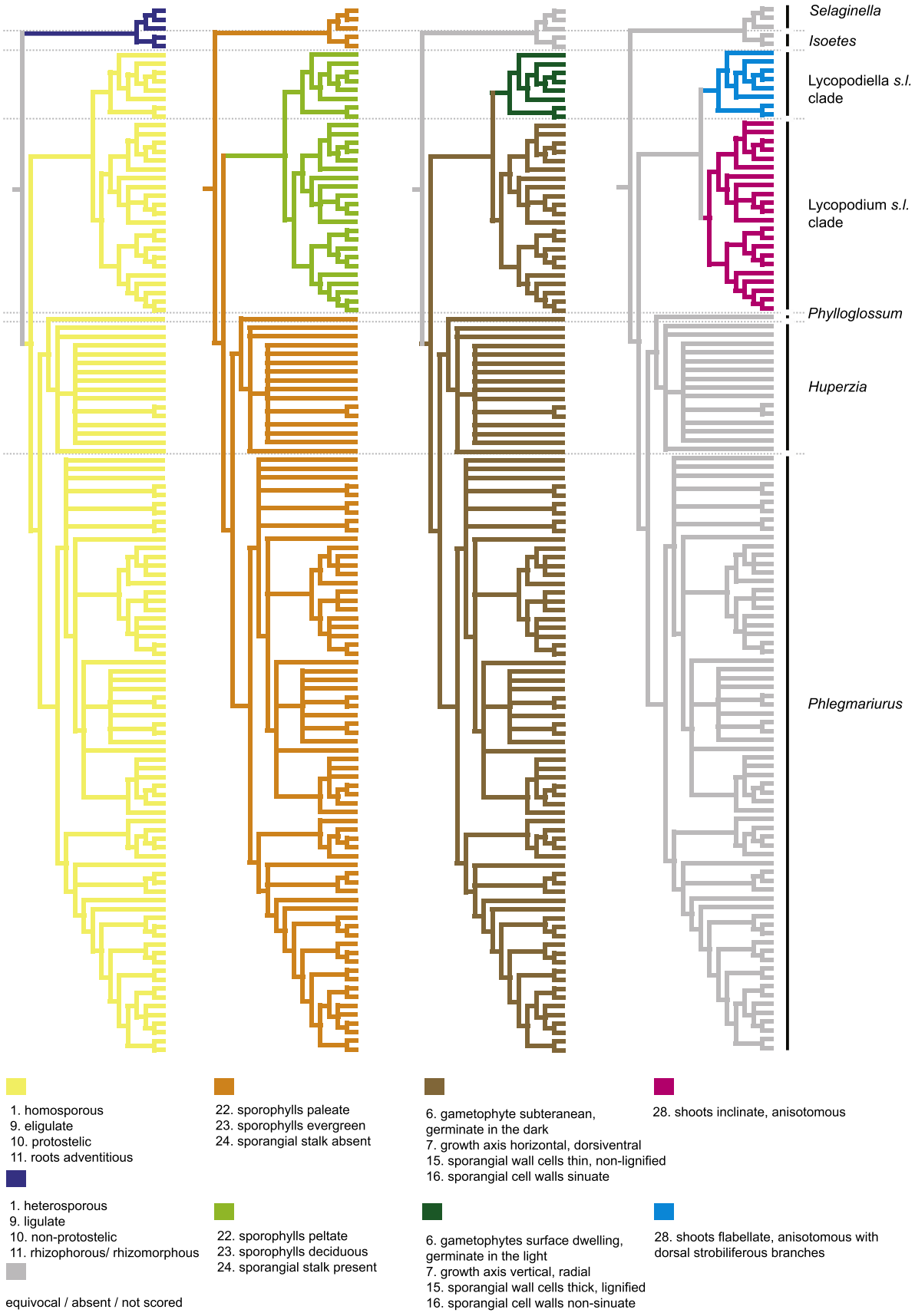


Fig. 3. Synapomorphies of Lycopodiaceae, Lycopodioideae and Lycopodioid genera mapped onto 50% majority rule Bayesian inference phylogram of the Lycopodiopsids with Isoetaceae and Selaginellaceae based on DNA sequences of the chloroplast loci *rbc*L, *trn*L, *trn*L-*F* and *trn*H-*psb*A. Characters listed 1–29 are summarised in Table 1 and described in Appendix B. The morphological data matrix is presented in Appendix C.



Fig. 4. Synapomorphies of Huperzioideae, *Huperzia*, *Phlegmariurus* and *Phylloglossum* mapped onto 50% majority rule Bayesian inference phylogram of the Lycopodiopsids with Isoetaceae and Selaginellaceae based on DNA sequences of the chloroplast loci *rbc*L, *trn*L, *trn*L-*F* and *trn*H-*psb*A. Characters listed 1–29 are summarised in Table 1 and described in Appendix B. The morphological character matrix is presented in Appendix C.

groups and geographic areas as were available spanning 22 countries (Appendix A). The identity of herbarium vouchers for sequences obtained from GenBank were checked when possible but many cited vouchers could not be found. One hundred and nineteen taxa were sampled in total, including 114 species of Lycopodiaceae and a small representation of the outgroup families Selaginellaceae (3 species) and Isoetaceae (2 species) (Appendix A).

2.2. Molecular marker selection

Molecular markers were chosen based on previous phylogenetic studies of the Lycopodiaceae (Wikström and Kenrick, 1997, 2001; Wikström, 2001; Wikström and Kenrick, 2001; Yatsentyuk et al., 2001; Ji et al., 2008). Four chloroplast loci were sampled, using previously published primers: *rbc*L, *trn*L, *trn*L-F and *trnH-psbA* (Ji et al., 2008; Kress et al., 2005; Taberlet et al., 1991; Wikström and Kenrick, 1997; Wolf et al., 2005). The *rbc*L region was sequenced using the primers *rbc*La-F and *rbc*La-R as shown in Wikström and Kenrick (1997). The *trn*L and *trn*L-F regions were sequenced together using the c (B49317) and f (A50272) primers (B49317–A50272) shown in Taberlet et al. (1991), or using the c and d (A49855) primers and the e (B49873) and f primers if the entire c to f sequence could not be generated. The *trnH-psbA* region was sequenced using the primers *psbA3*_F and *trnHf*_05 as shown in Ji et al. (2008). Some loci could not be generated for silica-dried and in some cases from fresh collections leading to gaps in the overall data matrix.

2.3. Molecular methods

DNA was extracted from fresh or silica-dried lycophylls using a QIAGEN DNEasy Plant Mini Kit protocol (QIAGEN, 2006) wherein fresh tissue was ground directly into the buffer or using a standard CTAB extraction (Doyle and Doyle, 1987).

PCR reactions (total volume of 25 μl) contained 16.9 μl of molecular grade H_2O, 2.5 μl of Bioline 10 X Buffer, 2 μl of 25 mM $MgCl_2$, 0.4 μl of 10 mM dNTPs, 1 μl of each 10 mM primer, 0.2 μl of 5 unit per μl Bioline Taq Polymerase and 1 μl of 5–10 ng per μl DNA. Amplification of gene regions was performed using PCR as follows: *trn*L and *trn*L-*trn*F—94 °C 5 min, 35 × 94 °C 1 min, 50 °C 30 s, 72 °C 1 min, 1 × 72 °C 10 min. All remaining loci—94 °C 5 min, 30 × 94 °C 1 min, 55 °C 30 s, 72 °C 1 min, 1 × 72 °C 10 min.

Sequencing reactions (total volume of 10 μl) contained 4 μl of Dyenamic ET terminator DNA sequencing chemistry (GE Biosciences) and 15–20 ng of purified PCR product diluted in molecular grade H_2O. Sequences were generated for both forward and reverse strands and used the same primer pair as for PCR reactions. Sequencing reactions were run on an MJ Research DNA Engine Tetrad 2 Peltier Thermal Cycler following manufacturer's instructions. DNA fragment analysis following DNA sequencing reactions was carried out on a MegaBACE 1000 (GE Biosciences) at the James Cook University Genetic Analysis Facility, Townsville Queensland Australia or on a ABI 3730XL (Applied Biosystems) at Beckman Coulter Genomics, Danvers, Massachusetts, USA.

Sequences were assembled using Geneious 8.04 (Biomatters, Ltd.) and were visually inspected and edited by hand where necessary. Alignment was performed for each marker individually using the MAFFT plugin (Katoh et al., 2002). Matrices from each locus were concatenated in Geneious for all downstream analyses.

2.4. Phylogenetic analyses

Bayesian inference phylogenetic analyses were performed using MrBayes 3.2.2 (Huelsenbeck and Ronquist, 2001). Models of character evolution for each locus were chosen based on the corrected Akaike Information Criterion (AICc) using jModelTest2 (Darriba et al., 2012; Huelsenbeck and Ronquist, 2001; Lewis, 2001). The data partitions analysed and the models chosen are presented in Table 1.

A Metropolis-coupled Markov Chain Monte Carlo was performed running four simultaneous chains (three heated and one cool) for 10 million generations sampling every 1000th generation. The first 25% of the trees was discarded as a burn-in. A fifty-percent majority rule consensus tree was generated from the 7500 trees retained.

Maximum likelihood analyses were performed using RAxML v. 8.1.11 (Stamatakis, 2014) using the CIPRES Science Gateway (Miller et al., 2015). Tree search analyses were run on the locus-partitioned dataset using the GTR + gamma model of nucleotide substitution; 500 rapid bootstrap replicates were performed.

2.5. Morphological and anatomical methods

Twenty-nine traits were scored for character mapping (Table 2). Characters were chosen based on use in previous classifications, reviews and cladistic analyses (Bower, 1885; Breckon, 1974; Breckon and Falk, 1974; Bruce, 1976a; Hackney, 1950; Holloway, 1935; Holub, 1964; Øllgaard, 1975, 1979b, 1980, 1987, 1989a; Wagner and Beitel, 1992; Whittier, 2006; Whittier and Braggins, 1992, 2000; Whittier and Storchova, 2007; Wilce, 1972) supplemented by examination of 886 living and preserved specimens of 125 Lycopodiaceae species. Characters were chosen where they were non-plastic within taxa sampled but showed consistent variation between taxa sampled. Huperzine alkaloids compositions were found to be too variable within species and within samples to be useful for analysis (Goodger et al., 2008; Lim et al., 2009, 2010).

Anatomical characters of the shoots and roots were examined using an Olympus CX31 light microscope to view hand-cut phloroglucinol–HCl stained transverse sections of fresh or alcohol fixed shoots. Microscopic surface morphology characters of the spores were examined using a JEOL JSM-5410LV scanning electron microscope in the Advanced Analytical Centre at James Cook University. Gametophyte characters were scored from Whittier *loc. cit.* and supplemented with fresh collections where available. Habit characters were scored from field and herbarium specimens and also from literature (Øllgaard, 1987, 1989, 1992b, 2015; Salino et al., 2013). Non-molecular characters were mapped onto the BI tree using MacClade v. 4.08 (Maddison and Maddison, 2008).

3. Results

3.1. Summary of phylogenetic data

Summary statistics and data coverage of the phylogenetic datasets are presented in Table 1. Data matrices for molecular characters and phylogenetic trees from mixed-model multi-locus analyses and from analyses of individual loci were submitted to treeBASE (submission ID 18266). The data matrix for morphological characters is presented in Appendix C. Where nodes of the phylogeny are discussed in the results support values are given in the text as Posterior Probabilities (PP) from Bayesian analyses and Bootstrap Values (BS) from Maximum likelihood analyses using the format PP/BS.

3.2. Monophyly of the Lycopodiaceae

The monophyly of the family Lycopodiaceae is strongly supported by all analytic methods across concatenated and single locus datasets (Fig. 2 clade A, 1/100). Morphological synapomorphies for the

Lycopodiaceae include homospory, presence of a protostele, absence of rhizophorous or rhizomorphous roots and absence of a ligule (Fig. 3). The Lycopodiaceae are comprised of two clades (Fig. 2 clades B and C) that correspond to the subfamilies Lycopodioideae (Fig. 2 clade B, 1/75) and Huperzioideae (Fig. 2 clade C, 1/100) *sensu* Wagner and Beitel (1992). This is the most consistently supported division within the Lycopodiaceae.

3.3. Monophyly of the Lycopodioideae and its genera

The Lycopodioideae is monophyletic, with reasonable support (Fig. 2 clade B, 1/75). Morphological synapomorphies for Lycopodioideae include anisotomous heteroblastic branching, determinate strobili, a broad stem stele with absence of corticular root emergence, deciduous sporophylls with a mucilage duct present in the sporophyll base, new shoots arising adventitiously from lateral branches, peltate or nearly peltate sporophylls, presence of a sporangial stalk (even if minutely so), reticulate—rugate spores, erect gametophytes and the absence of paraphyses among gametangia (Fig. 3). They are the only clade to have members with a plectostele in lateral branches, but this character is not present in all stems of members at all stages of development (Fig. 3).

The lycopodioid clade includes two subclades that correspond to the genera *Lycopodium* (Fig. 2 clade E, 1/57) and *Lycopodiella* (Fig. 2 clade D, 1/100) *sensu* Øllgaard (1987) or the subfamilies Lycopodioideae and Lycopodielloideae *sensu* Øllgaard (2012). These two groups differ by the presence of pedunculate strobili with modified sporophylls in the lycopodioid genera and the presence of surficial, photosynthetic gametophytes among the lycopodielloid genera (Fig. 3). The segregate genera *Lycopodiella*, *Palhinhaea*, *Austrolycopodium*, *Dendrolycopodium*, *Diphasium*, *Diphasiastrum* and *Lycopodium* are all recovered as monophyletic (Fig. 2 clade D and E).

The two types of branching patterns found in lycopodioid species correspond with the two primary lycopodioid subclades. Inclinate isotomous branching is a synapomorphy for the *Lycopodium s.l.* clade including *Austrolycopodium* + *Dendrolycopodium* + *Diphasium* + *Diphasiastrum* + *Lycopodium s.s.* + *Lycopodiastrum* + *Pseudodiphasium* + *Pseudolycopodium* + *Spinulum* (Fig. 3). This corresponds with the genus *Lycopodium sensu* Øllgaard (1987) or subfamily Lycopodioideae *sensu* Øllgaard (2012). Laterally spreading flabellate sterile branches with dorsally positioned fertile branches is a synapomorphy for the *Lycopodiella s.l.* clade including *Lateristachys* + *Lycopodiella* + *Palhinhaea* + *Pseudolycopodiella* (Fig. 3). Together this corresponds directly with the genus *Lycopodiella sensu* Øllgaard (1987) or subfamiliy Lycopodielloideae *sensu* Øllgaard (2012). Trimorphic lycophylls with one dorsal and one ventral rank of narrow lycophylls and two lateral ranks of broad lycophylls is a synapomorphy of the *Diphasiastrum* clade (Fig. 3). Dimorphic lycophylls with broad dorsal lycophylls in two ranks and narrow ventral lycophylls in two to three ranks was an autapomorphy of *Diphasium*. Isophyllous or anisophyllous but unranked lycophylls is recovered as plesiomorphic and present in all other Lycopodiaceae sampled.

Spore surface ornamentation differs between the clades. Reticulate spores are a synapomorphy of the lycopodioid genera *Austrolycopodium* + *Diphasium* + *Diphasiastrum* + *Lycopodium s.s.* + *Pseudodiphasium* + *Spinulum*. The scabrate spore condition of *Lycopodiastrum casuarinoides* and baculate spores of *Pseudolycopodium densum* are derived from reticulate spore sculpture (Fig. 3). The rugate spore condition is a synapomorphy of all lycopodielloid genera *Lycopodiella* + *Palhinhaea* + *Pseudolycopodiella*. The absence of spore surface sculpture on the proximal surface of some lycopodioid species is homoplasious, arising several times in different subclades.

3.4. Monophyly of the Huperzioideae and its genera

The Huperzioideae is monophyletic, with strong support (Fig. 2 clade C, 1/100) and is supported by multiple synapomorphies including isotomous primary branching, an extremely narrow stem stele (less than ¼ stem diameter) with corticular emergence of roots (evident in mature stems), new shoots arising axillary from tufted shoot bases, tufted root system, paleate sporophylls, evergreen sporophylls lacking a mucilage duct in the sporophyll base, strictly reniform axillary sporangia, absence of a sporangial stalk, foveolate-fossulate spores, dorsiventral gametophytes with a horizontal growth axis, and the presence of paraphyses among the gametangia (Fig. 4). Isotomous branching is inferred as a synapomorphy because Lycopodioideae, Selaginellaceae and Isoetaceae possess anisotomous branching or are unbranched.

Phylloglossum and two subclades are recovered within the huperzioid clade (Fig. 2 clade C). The genus *Huperzia* as broadly circumscribed by Øllgaard (1987) is paraphyletic with respect to *Phylloglossum* (Fig. 2). Although the pattern of relationships between the three huperzioid subclades varies between analyses, the clades themselves are consistently recovered (Fig. 2 clade H, 1/96 and G, 1/97) and are well supported by morphological synapomorphies (Fig. 4). These clades directly correspond with the three genera outlines in Sections 3.5–3.7 (Øllgaard, 2012; Field and Bostock, 2013).

3.5. Huperzia s.s. (Fig. 2 clade H, 1/96)

The *Huperzia s.s.* clade is recovered with strong molecular support. This clade equates to *Huperzia selago* group *sensu* Øllgaard (1987) or *Huperzia* subgenus *Huperzia sensu* Holub. This clade is unique in bearing reproductive bulbils, having a derived spore type with ornamentation on both surfaces and spores with concave lateral margins and truncate corners (Fig. 4).

3.6. Phylloglossum (Fig. 2)

Phylloglossum has many autapomorphies, is positioned on a long branch (Fig. 2) and its position varies between analyses as either sister to *Phlegmariurus*, sister to *Huperzia* or sister to both. *Phylloglossum* is unique in being annual, having a leafless pedunculate strobilus, a nearly cylindrical stem stele, an underground tuber, and (usually) unbranched shoots and roots (Fig. 4). When shoot and root branching does occur (rarely) it is isotomous. Corticular emergence of roots in second-season grown that is characteristic of the huperzioid clade was not observed in the short-lived annual stems available.

3.7. Phlegmariurus s.l. (Fig. 2 clade G, 1/97)

The *Phlegmariurus* clade is consistently recovered in all analyses of all datasets. This clade directly corresponds with all *Huperzia* groups except the *Huperzia selago* group *sensu* Øllgaard (1987) or *Huperzia* subg. *Subselago* Holub. *Phlegmariurus* differs from *Huperzia s.s.* by lacking bulbils and by having spores lacking the character combination described above and differs from *Phylloglossum* by having sessile fertile shoots and lacking the deciduous habit and underground tuber (Fig. 4). Although it is defined by an absence of synapomorphies of other clades, it is the most strongly supported genus-level clade in our molecular phylogeny (Fig. 2 clade G).

Within the *Phlegmariurus* clade are two diverse clades, the Neotropical clade (Fig. 2 clade I, 1/64) and the Palaeotropical clade (Fig. 2 clade J, 1/52). Very few of the sampled species occur in one region but belong to the alternate clade (Fig. 2 see taxon colours). Two exceptions among sampled species are *Phlegmariurus ophioglossoides*, which occurs in the African region but is placed

in the Neotropical clade, and *Phlegmariurus funiformis*, which is widely distributed in Central and South America but is placed in the Palaeotropical clade.

3.8. Neotropical Phlegmariurus (Fig. 2 clade I)

Our analyses indicate relationships generally consistent with those recovered by Wikström et al. (1999) and Wikström and Kenrick (2000) in their studies of growth habit evolution among Neotropical *Phlegmariurus* (treated therein as *Huperzia*) and provide support for most of the species groups proposed by Øllgaard (1987, 1992) that we sampled. The earliest-divergent Neotropical lineage is comprised of epiphytic species that possess fertile–sterile leaf dimorphy and correspond to Øllgaard's (1987, 1992) "*Huperzia phlegmaria* group", which our analyses and previous studies (Wikström et al., 1999; Wikström and Kenrick, 2000) show to be polyphyletic, with representatives in both the Neotropical and Palaeotropical clades. Though taxon sampling is limited, we recover a single clade that includes narrow-leaved species of Øllgaard's "*Huperzia dichotoma* group" (*P. dichotomus* and *P. wilsonii*) and "*Huperzia brongniartii* group" (*P. hippurideus*). Broader sampling of the Neotropical *Phlegmariurus* clade (W.L. Testo, unpublished data) indicate that some narrow-leaved species treated by Øllgaard in the "*Huperzia brongniartii* group", including *P. hippurideus*, *P. mexicanus*, and *P. lechleri*, are allied to members of the "*Huperzia dichotoma* group" and not closely related to *P. brongniartii* or other members of the group. We recover a grade of morphologically disparate species allied to the widespread epiphytes *P. linifolius* and *P. taxifolius* as sister to the exceptionally diverse clade of terrestrial species. Within the clade of terrestrial species, a assemblage of pioneer species (corresponding to Øllgaard's "*Huperzia reflexa* group") is sister to a collection of highly specialised alpine species which correspond to Øllgaard's "*Huperzia saururus*" and "*Huperzia brevifolia*" species groups.

3.9. Palaeotropical Phlegmariurus (Fig. 2 clade J)

The present analyses provide limited resolution of the species relationships within the Palaeotropical *Phlegmariurus* clade. This clade includes two subclades supported in Bayesian analyses but with lower support in Maximum likelihood analyses. They are the *P. squarrosus* clade and the *P. phlegmaria* clade. It also includes a suite of atypical early diverging species that do not have any close relatives among the species sampled here. Early divergent species include the broadest spectrum of morphological forms ranging from *P. dalhousieanus* group which are massive, homophyllous, glaucous species to *P. filiformis* group which are plants that are wholly filiform and occur in cloud forests of the Indo-Pacific island regions, and *P. varius* which is a phenotypically plastic species that is a facultative epiphyte, lithophyte or terrestrial.

The Palaeotropical *P. squarrosus* subclade includes two morphological groups that are recovered as polyphyletic in the phylogenetic tree with generally very low support. They are species with squarrose to foliose shoots and squarrose to foliose strobili (the *P. squarrosus* group), and species with squarrose to foliose shoots with funiforme terete or quadrangular strobili (the *P. proliferus* group). Both groups range from Madagascar to Oceania and are usually subcanopy epiphytes and epiliths. The Palaeotropical *P. phlegmaria* subclade includes species with foliose shoots with filiforme multibranched strobili (Fig 3 clade J, *P. phlegmaria* to *P. elmeri*). This clade ranges from Madagascar to Oceania includes a variety of montane and lowland rainforest canopy epiphytes. Within the *P. phlegmaria* clade are two subclades, a clade of species with petiolate lycophylls that are related to the type *P. phlegmaria* and a clade of species with sessile lycophylls that are related to *P. phlegmarioides*. The nominate species *P. phlegmaria* is a non-monophyletic assemblage of cryptic species with morphologically distinct species such as *P. salvinioides* and *P. subtrifoliatus* being embedded among forms hitherto grouped under *P. phlegmaria*.

4. Discussion

4.1. Monophyly of the Lycopodiaceae

The Lycopodiaceae have been found to be monophyletic in all molecular and morphological phylogenetic studies presented to date (Ji et al., 2008; Wikström and Kenrick, 1997, 2001; Yatsentyuk et al., 2001). Within the Lycopodiaceae are two deeply diverged major clades that correspond directly with the subfamilies Lycopodioideae and Huperzioideae *sensu* Wagner and Beitel (1992) (Ji et al., 2008; Wikström and Kenrick, 1997, 2001; Yatsentyuk et al., 2001). These two clades are supported by synapomorphies in sporophyte branching pattern, gametophyte type and spore type. Although the decision of taxonomic rank is an arbitrary one, we consider that recognition of these clades at subfamily rank *sensu* Wagner and Beitel (1992), rather than as families *sensu* Rothmaler (1944) is the most practical and stable approach.

4.2. Subfamily Lycopodioideae

Our phylogenetic hypothesis supports a view that the Lycopodioideae (*Austrolycopodium* + *Dendrolycopodium* + *Diphasium* + *Diphasiastrum* + *Lycopodium s.s.* + *Lycopodiastrum* + *Pseudodiphasium* + *Pseudolycopodium* + *Spinulum* + *Lateristachys* + *Lycopodiella* + *Palhinhaea* + *Pseudolycopodiella*) constitute a monophyletic group characterised primarily by heteroblastic shoot formation, a morphological feature that has important ecological implications. This growth habit allows these taxa to spread laterally via derived indeterminate sterile lateral branches and to produce determinate fertile aerial branches, which facilitate spore dispersal. The spreading growth habit, especially their ability for direct root development characteristic of lycopodioid taxa may have played an important role in enabling these taxa to form large and long lived clonal colonies and to successfully dominate semi-wetland terrestrial environments.

This subfamily occurs on all continents except Antarctica and it attains its highest diversity in montane tropical regions and subtropical regions in mesic high-light environments on oligotrophic soils. A higher diversity of Lycopodioid species and genera appear to occur in the northern hemisphere than the southern hemisphere; however, detailed biogeographic analyses should await improved sampling and incorporation of species distribution data.

4.3. Subfamily Huperzioideae

We propose the view that an important innovation of the Huperzioideae is provided by homoblastic tufted shoots and corticular root emergence that enable these plants to dominate the immediately local environment and spread by sexual and asexual dispersal, rather than by clonal growth. Corticular root emergence enables a densely tufted root system, a characteristic of huperzioid taxa that enables these plants to build up a large localised root mass (absent in Lycopodioideae) and thereby improve drought resilience. This may have allowed these taxa to occupy seasonally drier habitats than are available to most lycopodioid species and especially to occupy the epiphytic habitat.

4.4. Phylloglossum

The generic placement of *Phylloglossum drummondii* is enigmatic from a morphological perspective (Øllgaard, 1989). It has

previously been interpreted as combining the unique characteristics of several genera from both subfamilies, i.e. the photosynthetic gametophytes of *Lycopodiella s.l.*, the pedunculate strobili of *Lycopodium s.l.*, the absence (now known to be incorrect) of paraphyses among the gametangia found in *Lycopodium s.l.* and *Lycopodiella s.l.*, the foveolate-fossulate spore ornamentation found in *Huperzia* and *Phlegmariuus* and presence of perine found in *Lycopodium s.l.* and *Lycopodiella s.l.* (Bower, 1885; Breckon, 1974; Breckon and Falk, 1974; Hackney, 1950; Holloway, 1935; Øllgaard, 1987; Whittier and Braggins, 1992). *Phylloglossum* also exhibits autapomorphies that have made it difficult to interpret, especially its highly reduced nearly cylindrical protostele and its unbranched shoot morphology with globular or pouched tubers. These characters are not known elsewhere among living or fossil Lycopodiopsida. Molecular phylogenetic evidence has shown that *Phylloglossum drummondii* has affinities with the huperzioid genera (Wikström and Kenrick, 1997), a placement that has since been corroborated by reinterpretation of its gametophytes as bearing paraphyses (Whittier, 2006; Whittier and Braggins, 2000; Whittier and Storchova, 2007) and by evidence presented here that its pedunculate strobili are not homologous with those found in *Lycopodium s.s*. The affinity of *Phylloglossum* with huperzioid genera is again strongly supported by molecular and morphological lines of evidence in our study. Its phylogenetic placement confirms that the two-genus taxonomy (*Huperzia s.l.* + *Phylloglossum*) is non-monophyletic. We interpret the unusual morphology of *Phylloglossum* as a product of its adaption to annually seasonal temperate wetlands. The cytology of *Phylloglossum* needs close re-investigation. The only report found in literature is from Blackwood (1953), which reports 246 bivalents and 11 univalents suggestive of a hybrid origin but with parents unknown (Tindale and Roy, 2002).

4.5. Huperzia

Huperzia s.s is well supported by spore characteristics and by its unusual vegetative bulbils which are detachable anisotomous branchlets formed in sporophyll axils. The vegetative bulbils of *Huperzia* enable them to establish satellite colonies, a unique method of vegetative propagation among lycophytes in which detached genetically identical colonies can be dispersed. This is considered to be an advantageous feature for a long-lived and terrestrial plant that cannot spread by lateral runners like its Lycopodioid counterparts. Diversity in the genus *Huperzia* is concentrated in northern hemisphere temperate and subtropical regions with relatively few outlying species in the tropics or southern hemisphere, however more detailed sampling and species distribution data are required to test the hypothesis that it has a northern hemisphere origin. Chromosome numbers are variable with $2n$ ranging from 90 to over 400. A hypothetical base chromosome number for *Huperzia s.s.* of $x = 11$ was proposed by Tindale and Roy (2002) and although this is the most common base number there are numerous exceptions (Wagner, 1992).

Species within this clade are, for the most part, poorly differentiated morphologically and are typically small, terrestrial, plants distributed in forests and alpine habitats throughout the north-temperate region, with some representatives in high-altitude tropical floras and very few in south-temperate regions.

4.6. Phlegmariurus

The remarkable evolutionary success of *Phlegmariurus* is almost certainly linked to their adaptation to the epiphytic niche and secondary terrestrialization among high-elevation Neotropical species. These shifts in growth habit are unique among lycophytes and the associated burst of diversification in this group parallels the exceptional species richness displayed by many epiphytic fern and angiosperm lineages (Gentry and Dodson, 1987). Sporophyte features that require further study are corticular emergence of roots to develop a localised tree-anchoring root mass and the apparent enlargement and ramification of fertile zones in epiphytic *Phlegmariurus* compared with terrestrial *Phlegmariurus*, an innovation that hypothetically may increase spore production as an adaptation to the epiphytic niche. Further study is needed to explore the possibility that the branched morphology of the independent gametophyte phase in this genus may have been a important innovation underlying epiphytism in this group, as has been shown for ferns (Watkins and Cardelús, 2012; Watkins et al., 2007).

Phlegmariurus has undergone a significant radiation and comprises a broad spectrum of specialised morphologies that are associated with particular epiphytic, lithophytic or terrestrial habits. Species sharing massive and robust, brush-like, flattened, reduced or thread-like forms are not clearly related to each other, with morphological convergence being common among species that occupy similar niches. This has implications for species enumeration and species level taxonomy. Several broad sense species concepts such those hitherto adopted for *P. phlegmaria* and *P. filiformis* are polyphyletic and therefore a more strict sense approach to the species level taxonomy of these species may need to be adopted.

The Palaeotropical clade of *Phlegmariurus* is the most geographically widespread clade of *Phlegmariurus* spanning from West Africa to the Eastern Pacific Islands. It does not appear to have an obvious centre of endemism or diversity with species located in high numbers in offshore Oceanic islands as well as large mainland floras. The Palaeotropical *Phlegmariurus* appears to be a radiation comprising approximately 100 species with most of those species belonging to a small number of species complexes. Palaeotropical *Phlegmariurus* are primarily pendent epiphytes of tropical rainforest with few terrestrials or facultative species. Drivers of speciation and radiation in the Palaeotropical *Phlegmariurus* remain poorly understood.

The Neotropical clade of *Phlegmariurus* is the most morphologically and ecologically diverse lineage within the Lycopodiaceae, comprising approximately 150 species that occupy a broad array of epiphytic and terrestrial habitats. This clade appears to have recently undergone a rather remarkable radiation in concert with the uplift of the Andes Mountains (Wikström et al., 1999); at least half of the species in this group occur in high-elevation disturbed sites and páramo grasslands that formed within the past five million years. Because of the young age of this group, many members of the páramo-endemic *Phlegmariurus crassus* and *Phlegmariurus brevifolius* groups are thought to be in the process of speciation and remain poorly differentiated.

A hypothetical base chromosome number for *Phlegmariurus* is $x = 17$ (Tindale and Roy, 2002) but there are many exceptions (Wagner, 1992). Chromosome counts for *Phlegmariurus* are generally very high, ranging from $n = 136$ to $n = c.$ 278 for *P. tetrastichoides* as *H. prolifera* (Tindale and Roy, 2002). An extensive survey of *Phlegmariurus* cytology is needed to ascertain the role of polyploidy and hybridization in the diversity of the many species complexes in the genus.

4.7. Nomenclatural combinations

Several nomenclatural combinations for the genus *Phlegmariurus* remained outstanding prior to this publication. At the time of publication of Field and Bostock (2013) these taxa were either poorly known, the types had not been located, or they were considered synonyms of other taxa. These nomenclatural combinations are presented below:

***Phlegmariurus acutifolius* (Desv. *ex* Poir.) A.R. Field & Testo comb. nov.** Basionym: *Lycopodium acutifolium* Desv. *ex* Poir.,

Encycl. suppl. 3:599. 1813 [1814]. Type: Mauritius. *s.c. s.n.* (holo: P00466615). Synonyms: *Lycopodium epiceifolium* Desv. *ex* Poir., Encycl. suppl. 3:559. 1813 [1814]. *Huperzia epiceifolia* (Desv. *ex* Poir.) Trevis., Atti Soc. Ital. Sci. Nat. 17: 248. 1874. Type: Mauritius. *s.c. s.n.* (holo: P00466614).

***Phlegmariurus divergens* (Alderw.) A.R. Field & Testo comb. nov.** Basionym: *Lycopodium divergens* Alderw., Malayan fern allies 45. 1915. Type: Java n.d. *Blume s.n.* (holo: L0544695, L0544696).

***Phlegmariurus filicaulon* (Copel.) A.R. Field & Testo comb. nov.** Basionym: *Lycopodium filicaulon* Copel., Philipp. J. Sci. 60:100, t. 2. 1936**.** Synonyms: *Urostachys copelandii* Herter, Index Lyc. 57. 1949. *Huperzia copelandii* (Herter) Holub, Folia Geobot. Phytotax. 26(1):92, 1991. Type: Lake Luralu [Loloru], Koniguru, Solomon Islands, 11 August 1930, *S.F. Kajewski 2069* (holo: PNH [destroyed 1945]; iso: BISH1000365, GH, MICH1287174, BM0016727, NY00127350, UC574158, BRI-AQ0149708, P01219325).

***Phlegmariurus haeckelii* (Herter) A.R. Field & Testo comb. nov.** Basionym: *Lycopodium haeckelii* Fedde Repert 5:22. 1908. *Urostachys haeckelii* (Herter) Herter ex Nessel, Bärlappgewachse 138. 1939. *Huperzia haeckelii* (Herter) Holub, Folia Geobot. Phytyax. 20: 73. 1985. Type: Tahiti [French Polynesia], 1850 *Ribourt* (P).

***Phlegmariurus hippuris* (Poir.) A.R. Field & Testo comb. nov.** Basionym: *Lycopodium hippuris* Desv. ex Poir., Encycl. suppl. 3: 559 1813 [1814]. *Huperzia hippuris* (Poiret) Trevisan; *Urostachys hippuris* (Poir.) Herter. Type: Java (P).

***Phlegmariurus ignambiensis* (Compton) A.R. Field & Testo comb. nov.** Basionym: *Lycopodium ignambiense* Compton, J. Linn. Soc. 45: 458. 1922. Synonyms: *Huperzia ignambiensis* (Compton) Folia Geobot. Phytotax. 26(1): 92. 1991. Type: Ignambi [New Caledonia] alt 2500 ft, 08 August 1914, *R.H. Compton 1689* (holo: BM01038078).

***Phlegmariurus kajewskii* (Copel.) A.R. Field & Testo comb. nov.** Basionym: *Lycopodium kajewskii* Copel., J. Arnold. Arbor. 12: 48.1931. Type: Vanikoro, Santa Cruz Islands, 6 November 1928, *S. F. Kajewski 573* (holo: UC422659, iso: US00134331, MO-022131, MICH1287172, BRI-AQ326730, GH021588).

***Phlegmariurus magnusianus* (Herter) A.R. Field & Testo comb. nov.** Basionym: *Lycopodium magnusianum* Herter, Hedwigia 49:91. 1909. *Huperzia magnusiana* (Herter) Holub, Geobot. Phytotax. 20(1):74. 1985. Type: Camp Keithley, Lake Lanao, Mindanao, Philippines, April 1907, *M.S. Clemens Phil. Bur. Sci. # 36936-2* (iso: F0077346, MICH1287170, US00811493).

***Phlegmariurus melanesicus* (Brownlie) A.R. Field & Testo comb. nov.** Basionym: *Lycopodium melanesicum* Brownlie Nova Hedwigia Beih. 55: 20. 1977. Synonym: *Huperzia melanesica* (Brownlie) Holub Folia Geobot. Phytotax. 26(1)92. 1991. Type: Mt Victoria, Viti Levu July 1975 *S. Vodonaivalu 18784* (holo: CHR00341168).

***Phlegmariurus mirabilis* (Willd) A.R. Field & Testo comb. nov.** Basionym: *Lycopodium mirabile* Willd., Sp. pl. 5:11. 1810. Synonym: *Huperzia mirabilis* (Willd.) Holub, Folia Geobot. Phytotax. 26 (1):93. 1991. Type: Dillen (1741) *Historia Muscorum* (p. 450 tab. 61 fig. 5, D & E.!) [*non* B-Willd. no. 19340]. Heterotypic synonyms: *Phlegmariurus macrostachys* (Hook. *ex* Spring) N.C. Nair & S.R. Ghosh, J. Econ. Taxon. Bot. 12(1):194. 1988. Synonyms: *Huperzia macrostachys* (Hook. *ex* Spring) Holub, Geobot. Phytotax. 20(1): 74. 1985. Type: Neel Gherries [Nilgiri Hills], India. *F. Adams s.n.* (syn: K *n.v.*). Ceylon [Sri Lanka]. *Walker s.n.* (syn: K *n.v.*). Ceylon [Sri-Lanka] *Wight s.n.* (syn: K *n.v.*).

***Phlegmariurus nilagiricus* (Spring) A.R. Field & Testo comb. nov.** Basionym: *Lycopodium nilagiricum* Spring, Bull. Acad. Roy. Sci. Belg. 8:517. 1841. Synonym: *Huperzia nilagirica* (Spring) Dixit, J. Bombay Nat. Hist. Soc. 77(3):541. 1981. Type: India. *Perottet* (holo: G-Deless.).

***Phlegmariurus obovalifolius* (Bonap.) A.R. Field & Testo comb. nov**. Basionym: Lycopodium obovalifolium Bonap. Notes Pteridol. 14: 190. 1923. Type: Annam [Vietnam] Dr. Sallet s.n. (P).

***Phlegmariurus oceanianus* (Herter) A.R. Field & Testo comb. nov.** Basionym: *Lycopodium oceanianum* Herter, Bot. Jahrb. Syst. 43: Beibl. 98:52. 1909. Synonym: *Huperzia oceaniana* (Herter) Holub, Folia Geobot. Phytotax. 20:75. 1985. Type: syn: Aneitum, New Hebrides [Aneityum Island, Vanuatu] 1859 *s.c. s.n.* (P *n.v.*); syn: Ualau (P *n.v.*).

***Phlegmariurus pachyphyllus* (Kuhn ex Herter) A.R. Field & Testo comb. nov.** Basionym: Lycopodium pachyphyllum Kuhn ex Herter, Bot. Jahrb. Syst. 43: Beibl. 98:51. 1909; Huperzia pachyphylla (Kuhn) Holub, Geobot. Phytotax. 20(1):75. 1985. Type: Madagascar. J.M. Hildebrandt 4141 (holo: B200085521; iso: B200085522, B200085523, P00466624, P00466625, P00466626).

***Phlegmariurus pseudophlegmaria* (Kuhn) A.R. Field & Testo comb. nov.** Basionym: *Lycopodium pseudophlegmaria* Kuhn, Forschungsr. Gazelle 4: 16. 1889; *Urostachys pseudophlegmaria* (Kuhn) Herter *ex* Nessel, Bärlappgewächse 237. 1939; *Huperzia pseudophlegmaria* (Kuhn) Holub, Folia Geobot. Phytotax. 20:76. 1985; Syntypes: *F.-C. Naumann*, Fiji Islands (B?), Fiji Islands 1860 *B. Seemann 702* P06243932, K00695363; Insel Ovalau Oct 1954 *Milne & Macgillivray s.n.* P06243931. Heterotypic synonym: *Urostachys kandavuensis* Nessel, Repert. Spec. Nov. Regni Veg. 39:67. 1935; *Huperzia kandavuensis* (Nessel) Holub, Geobot. Phytotax. 20(1): 74. 1985. Type: Fiji. *A.C. Smith 142* (holo: NY00127357).

***Phlegmariurus societensis* (J. Moore) A.R. Field comb. nov.** Basionym: *Lycopodium societense* J. Moore, Bernice P. Bishop Mus. Bull. 102: 12. 1933. Type: Raiatea, 23 Mar 1927, *J.W. Moore 686* (holo: BISH1000377; iso: RSA0000003, MIN1001727, BISH1000375, BISH100376, BISH100378, BISH100379, U0007448).

***Phlegmariurus subfalciformis* (Alderw.) A.R. Field & Testo comb. nov.** Basionym: *Lycopodium subfalciforme* Alderw., Bull. Jard. Bot. Buitenzorg. ser. 2, 28:44. 1918. Synonym: *Huperzia subfalciformis* (Alderw.) Holub, Geobot. Phytotax. 20(1): 77. 1985. Type: Papua New Guinea *Branderhorst 24* (syn: BO *n.v.*, BM001044020, B200119453).

***Phlegmariurus tardieuae* (Herter) A.R. Field & Testo comb. nov.** Basionym: *Urostachys tardieuae* Herter, Notul. Syst. (Paris) 15:355. 1958. Synonyms: *Huperzia tardieuae* (Herter) Holub Folia Geobot. Phytotax. 26(1):93. 1991. *Huperzia phlegmaria* (L.) Rothm. var. *tardieuae* (Herter) Tardieu, Adansonia ser. 2, 10(1): 18. 1970. Type: from Madagascar [Farafananga], 13 Nov 1881, *s.c. s.n.* (P00466621).

***Phlegmariurus tauri* (Herter) A.R. Field & Testo comb. nov.** Basionym: *Lycopodium apiculatum* Spring [Miq., Pl. Jungh. 3: 272 (1854) (non Poiret 1814)] = *Urostachys tauri* Herter [Index Lyc. 85 (1949)]. *Huperzia tauri* (Herter) Holub [Folia Geobot. Phytotax. 26 (1):92 (1991). Lectotype: Pangerango, Java, *Junghuhn 274* (selected by F. Badré, Lejeunia n. ser. 109:4. 1983.) (isolecto: LG, K, L0057314, L0057315).

***Phlegmariurus verticillatus* (L.f.) A.R. Field & Testo comb. nov.** Basionym: *Lycopodium verticillatum* L.f., Suppl. Pl. 448. 1782. Synonyms: *Huperzia verticillata* (L.f.), *Plananthus verticillatus* (L.f.) P. Beauv.; *Stachygynandrum verticillatum* (L.f.) P. Beauv. Trevisan; *Urostachys verticillatus* (L.f.) Herter. Type: Sonnerat per Thouin, Insula Bourbon [Reunion] (holo: SBT).

***Phlegmariurus warneckei* (Herter *ex* Nessel) A.R. Field & Testo comb. nov.** Basionym: *Urostachys warneckei* Herter *ex* Nessel [Bärlappgewächse 213. 1939, *nom. inval.*] Revista Sudamer. Bot. 6:166, t. 13, f. 64. 1940. *Huperzia warneckei* (Herter ex Nessel) Pic. Serm. Webbia 23:163. 1968. 1968 Type: Cameroon, Baume Bamenda, *Joh Greven* 1909 (holo: BONN-Nessel).

5. Conclusion

Based on the phylogenetic hypotheses presented, we reject a two-genus system (2: *Huperzia* + *Phylloglossum* because *Phylloglossum* is embedded in this definition of *Huperzia*. In comparison, both the one-genus classification 1: *Huperzia sensu lato* and the

three-genus system 3: *Huperzia* + *Phylloglossum* + *Phlegmariurus* reflect monophyletic groups. The choice between these systems becomes largely a question of rank and the nomenclature of both are available. Of these two classifications, we believe that the three-genus system better represents the morphological diversity and the deep divergence and habitat specific radiation of huperzioid Lycopodiaceae than does one genus.

A broadly defined *Huperzia s.l.* is nomenclaturally simple, however it does not account for the deep divergences and the resultant genus *Huperzia s.l.* becomes defined only by micro-morphological synapomorphies (gametophytes with paraphyses among their gametangia) which are of limited use in the field for use on sporophytes. One genus does not account for ecological and geographic disparity among the major huperzioid lineages.

We prefer a three-genus classification (*Huperzia* + *Phylloglossum* + *Phlegmariurus*) because it reflects the deep divergences among huperzioid Lycopodiaceae and it takes into account the divergent life-history strategies that occur among the genera. This three-genus system is corroborated by several synapomorphies that are clearly observable in the field and correlates with a temperate terrestrial radiation (*Huperzia s.s.*), a tropical predominantly epiphytic radiation (*Phlegmariurus*) and a southern temperate semi-aquatic relic (*Phylloglossum*). As such, we believe this taxonomic approach is the most practical and useful to botanists and best reflects our current understanding of the evolutionary history of the huperzioid Lycopodiaceae.

Acknowledgments

This research was supported by the Queensland Government *Growing the Smart State PhD Funding Program*, a James Cook University *School of Marine and Tropical Biology* teaching stipend and an Australian Biological Resource Study National Taxonomy Research Grant. Thank you to Holly Field and the Field, Kupke, Rawlins and Wilkinson families for assisting to maintain the living lycopod collection and to all field collectors and photographers of samples used in this study.

Appendix A

GenBank sequence accessions and collection details for all Lycopodiopsids analysed. Generic classification of Lycopodiaceae follows Øllgaard (2012) and Field and Bostock (2013) and nomenclatural abbreviations follow *International Plant Names Index*. Each species is listed as *Genus*, *species* (basionym authority) combination authority and each collection is listed as LOCATION *Collector & number* (Herbarium) followed by the GenBank accessions for *rbc*L, *trn*L-*trn*L-*F* and *psbA-trnH*. – represents missing data. * indicates new data.

Genus species **(Basionym authority) Combination authority** LOCATION *Collector & number* (Herbarium)	*rbc*L	*trn*L-*trn*L*F*	*psbA-trnH*
Austrolycopodium fastigiatum **(R. Br.) Holub**			
NEW ZEALAND *N. Wikstrom 246* (S)	AJ133252	AJ224595	–
Austrolycopodium magellanicum **(P. Beuv.) Holub**			
ECUADOR *B. Øllgaard 100621* (AAU)	AJ133251	AJ224594	–
Dendrolycopodium dendroideum **(Michx.) A. Haines**			
CANADA *N. Cavallin & T. Elliott 2012-408* (–)	KM212142	–	
USA *W. Testo 405* (AAU, TAIF, VT)	–	–	KT749937*
Dendrolycopodium obscurum **(L.) A. Haines**			
UNITED STATES OF AMERICA *T. Eriksson 666* (GH)	Y07935	AJ224598	–
JAPAN – (TNS:765137)	–	–	AB575310
Diphasiastrum alpinum **(L.) Holub**			
SWEDEN *T. Eriksson 699* (S)	AJ133250	AJ224599	–
Diphasiastrum complanatum **(L.) Holub**			
JAPAN – (TNS)	AB574627	–	AB575304
Diphasiastrum digitatum **(A. Braun) Holub**			
CANADA *A. Por & J. Gerrath AP326-b*	HQ590169	–	–
USA *W. Testo 404* (AAU, TAIF, VT)	–	–	KT749936*
Diphasiastrum nikoense **(Franchet & Savat.) Holub**			
JAPAN – (TNS)	AB574633	–	AB575309
Diphasiastrum thyoides **(Willd.) Holub**			
COSTA RICA – *LA8276* (–)	–	–	GQ429143
Diphasiastrum wightianum **(Wall. ex Hook. & Grev.) Holub**			
MALAYSIA *N. Wikstrom, H-E Wanntorp 137* (S)	AJ133254	AJ224600	–
PAPUA NEW GUINEA *M. Sundue 3705* (BISH, VT)	–	–	KT749939*
Diphasium jussiaei **(Desv. ex Poir.) Rothm.**			
ECUADOR *B. Øllgaard 100615* (AAU)	AJ133256	AJ133264	–
ECUADOR *C. Rothfels 3601* (DUKE, QCA)	–	–	KT749946*
Diphasium scariosum **(G. Forst.) Rothm.**			
MALAYSIA *N. Wikstrom, H-E Wanntorp 136* (S)	AJ133255	AJ133263	–
PAPUA NEW GUINEA *M. Sundue 3628* (BISH, VT)	–	–	KT749943*
Huperzia appressa **(Desv.) Á. Löve & D. Löve**			
CHINA *S.L. Pan WJ0409048* (SHMU)	DQ464220	–	DQ464203
Huperzia arctica **(Tolm.) Sipliv.**			
NORWAY – (–)	–	GQ245031	–
Huperzia australiana **(Herter) Holub**			
AUSTRALIA *A.R. Field et al. ARF1041* (BRI)	JQ513908*	JQ679088*	JQ520368*

Appendix A (*continued*)

Genus species **(Basionym authority) Combination authority** LOCATION *Collector & number* (Herbarium)	*rbc*L	*trn*L-*trn*LF	*psbA-trnH*
Huperzia crispata **(Ching) Ching**			
CHINA *S.L. Pan WJ0509023* (SHMU)	DQ464221	–	DQ464204
Huperzia emeiensis **(Ching & H.S. Kung) Ching & H.S. Kung**			
CHINA *S.L. Pan WJ409023* (SHMU)	DQ464222	–	DQ464205
Huperzia fuegiana **(Roiv.) Holub**			
CHILE Larrain 36361 (NY)	–	–	KT749932*
Huperzia haleakalae **(Brack.) Holub**			
NORWAY – (–)	–	GQ245032	–
CANADA *C. Rothfels 4706* (DUKE)	–	–	KT749935*
Huperzia lucidula **(Michx.) Trevis.**			
CANADA *J. Gerrath & A. Porr JAG607* (–)	HQ590135	–	HQ596728
UNITED STATES OF AMERICA *T. Eriksson 664* (GH)	–	AJ224591	–
Huperzia miyoshiana **(Makino) Ching**			
JAPAN – (TNS:765256)	DQ464225	–	–
CANADA *C. Rothfels 4483* (DUKE)	–	–	KT749934*
Huperzia nanchuanensis **(Ching & H.S. Kung) Ching & H.S. Kung**			
CHINA *S.L. Pan WJ409101* (SHMU)	DQ464226	–	DQ464209
Huperzia quasipolytrichoides **(Hayata) Ching**			
PAPUA NEW GUINEA *M. Sundue 3692* (BISH, NY)	–	–	KT749933*
Huperzia selago **(L.) Bernh. ex Schrank & Mart.**			
SWEDEN *N. Wikstrom 36* (S)	Y07934	AJ224592	–
JAPAN – (TNS:765813)	–	–	AB575312
Huperzia serrata **(Thunb. ex Murray) Trevis.**			
CHINA *S.L. Pan WJ409041* (SHMU)	DQ464228	–	–
JAPAN – (TNS:763064)	–	–	AB575313
Huperzia somae **(Hayata) Ching**			
TAIWAN – (TNS:776522)	AB574640	–	AB575316
Huperzia sutchueniana **(Herter) Ching**			
CHINA *S.L. Pan JSG0504192*	–	–	DQ464212
Isoetes flaccida **A. Braun**			
UNITED STATES OF AMERICA – (WTU)	GU191333	GU191333	GU191333
Isoetes japonica **A. Braun**			
JAPAN – (TNS:763864)	AB574658	–	AB575325
JAPAN *S. Tsugaru and T. Sawada 33184* (TNS)	–	JN578364	–
Lateristachys lateralis **(R. Br.) B. Øllg.**			
AUSTRALIA *Bostock et al. s.n.* (BRI)	AJ133262	AJ224609	–
Lycopodiastrum casuarinoides **(Spring) Holub**			
MALAYSIA *N. Wikstrom, H-E Wanntorp 134* (S)	AJ133248	AJ224603	–
JAPAN – (TNS:762656)	–	–	AB575302
Lycopodiella alopecuroides **(L.) Cranfill**			
ECUADOR *B. Øllgaard 100822* (AAU)	Y07937	AJ224604	–
Lycopodiella inundata **(L.) Holub**			
SWEDEN *H.E. Waantorp & N. Wikstrom s.n.* (S)	Y07938	AJ224605	AB575308
Lycopodium annotinum **L.**			
SWEDEN *N. Wikstrom 37* (S)	AJ133247	GQ245104	–
JAPAN – TNS:765139	–	–	AB575301
Lycopodium clavatum **L.**			
FINLAND *Muola s. n.* (–)	DQ026595	GQ245106	–
JAPAN – (TNS:762554)	–	–	AB575303
Lycopodium japonicum **Thunb.**			
CHINA – (PS1298MT01)	–	–	GQ435342
Lycopodium lagopus **(Laest. ex C. Hartm.) G. Zinserl. ex Kuzen.**			
USA *W. Testo 597* (VT)	–	–	KT749938*
Lycopodium vestitum **Desv. ex Poiret**			
ECUADOR *N. Wikstrom 278* (S)	AJ133257	AJ224602	–
Lycopodium **cf** ***clavatum*** **L.**	–	–	KT749940*
PAPUA NEW GUINEA *M. Sundue 3716* (BISH, VT)			
Palhinhaea cernua **(L.) Carv. Vasc & Franco**			
MALAYSIA *N. Wikstrom & H-E Wanntorp 144* (S)	AJ133258	AJ224608	–
CHINA – (PS1296MT02)	–	–	GQ435340

(*continued on next page*)

Appendix A (*continued*)

Genus species **(Basionym authority) Combination authority** LOCATION *Collector & number* (Herbarium)	*rbc*L	*trn*L-*trn*LF	*psbA-trnH*
Palhinhaea glaucescens **(C. Presl) Holub**			
ECUADOR *B. Øllgaard 100602a* (AAU)	AJ133260	AJ224606	–
Palhinhaea pendulina **(Hook) Holub**			
ECUADOR *B. Øllgaard 100602b* (AAU)	AJ133259	AJ224607	–
Palhinhaea tomentosa **(Alderw.) Holub**			
PAPUA NEW GUINEA *M. Sundue 3718* (BISH, VT)	–	–	KT749942*
Phlegmariurus attenuatus **(Spring) B. Øllg.**			
ECUADOR *N. Wikstrom 300* (S)	–	AJ224573	–
COSTA RICA *W. Testo 167* (VT)	KT634232*	–	KT749924*
Phlegmariurus balansae **(Herter) A.R. Field & Bostock**			
NEW CALEDONIA *N. Wikstrom 243* (S)	–	AJ224553	–
Phlegmariurus billardierei **(Spring) A.R. Field & Testo**			
NEW ZEALAND *N. Wikstrom 259* (S)	AJ133894	AJ224564	–
Phlegmariurus brassii **(Copel.) A.R. Field & Bostock**			
PAPUA NEW GUINEA *A.R. Field et al. 89*	–	–	JQ520372*
Phlegmariurus campianus **(B. Øllg.) B. Øllg.**			
ECUADOR *B. Øllgaard 100612* (AAU)	X98282	AJ224586	–
ECUADOR *B. Øllgaard 75413* (AAU, QCA, VT)	–	–	KT749931*
Phlegmariurus capellae **(Herter) B. Øllg.**			
ECUADOR *B. Øllgaard 100835* (AAU)	–	AJ224570	–
ECUADOR *B. Øllgaard 8597* (AAU, VT)	–	–	KT749929*
Phlegmariurus carinatus **(Desv.) Ching**			
AUSTRALIA *A.R. Field & H.R. Field 969*	DQ464229	JQ679090*	JQ520363*
Phlegmariurus compactus **(Hook) B. Øllg.**			
ECUADOR *N. Wikstrom 271* (S)	–	AJ224571	–
Phlegmariurus crassus **(Humb. & Bonpl. ex Willd.) B. Øllg.**			
ECUADOR *B. Øllgaard 100832* (AAU)	–	AJ224572	–
COSTA RICA *W. Testo 230* (AAU, VT)	–	–	KT749923*
Phlegmariurus cryptomerinus **(Maxim.) Satou**			
JAPAN – (TNS:763172)	AB574628	–	AB575305
Phlegmariurus cumingii **(Nessel) B. Øllg.**			
ECUADOR *B. Øllgaard 100836* (AAU)	Y07930	AJ224578	–
Phlegmariurus dacrydioides **(Baker) A.R. Field & Bostock**			
MADAGASCAR *S. Mantelow 91452*	–	AJ224562	–
Phlegmariurus dalhousieanus **(Spring) A.R. Field & Bostock**			
AUSTRALIA *A.R. Field & H.R. Field 766* (BRI)	–	–	JQ520367*
Phlegmariurus dichaeoides **(Maxon) B. Øllg.**			
PANAMA *J. Aranda & J.A.M. Holtum s.n.* (BRI)	–	–	JQ663815*
COLOMBIA *M. Sundue 3229* (COL, NY, VT)	–	KT634236*	–
Phlegmariurus dichotomus **(Jacq.) W.H. Wagner**			
MEXICO *Eliasson 538* (S)	–	AJ224567	–
MEXICO *G. Wrinkle s.n.* (BRI)	–	–	JQ663808*
Phlegmariurus elmeri **(Herter) A.R. Field & Bostock**			
PHILIPPINES *A.R. Field et al. 816* (BRI)	–	–	JQ663836*
Phlegmariurus ericifolius **(C. Presl) B. Øllg.**			
ECUADOR *N. Wikstrom 286* (S)	–	AJ224587	–
Phlegmariurus eversus **(Poir.) B. Øllg.**			
ECUADOR *B. Øllgaard 100620* (AAU)	–	AJ224579	–
ECUADOR *H.v.d. Werf et al. 19606* (MO)	–	–	JQ663825
Phlegmariurus fargesii **(Herter) Ching**			
JAPAN *Yahara et al. 398* (TNS)	AB574629	–	AB575306
Phlegmariurus filiformis **(Sw.) W.H. Wagner**			
AUSTRALIA *A.R. Field & H.R. Field 1027* (BRI)	–	–	JQ663839*
Phlegmariurus foliosus **(Copel.) A.R. Field & Bostock**			
FIJI *A.R. Field ex R. Hilder ARF019* (BRI)	–	–	JQ663817*
Phlegmariurus fordii **(Baker) Ching**			
JAPAN – (TNS:763058)	AB574630	AJ224548	AB575307
Phlegmariurus funiformis **(Cham. ex Spring) B. Øllg.**			
ECUADOR *Asplund 19290* (S)	–	AJ224560	–
PANAMA *J. Aranda & J.A.M. Holtum* (BRI)	–	–	JQ663832*
Phlegmariurus goebelii **(Nessel) A.R. Field & Bostock**			
MALAYSIA *A.R. Field et al. 2742* (BRI)	–	–	JQ663822*

Appendix A (*continued*)

Genus species **(Basionym authority) Combination authority** LOCATION *Collector & number* (Herbarium)	*rbc*L	*trn*L-*trn*LF	*psbA-trnH*
Phlegmariurus hamiltonii **(Spreng.) Á. Löve & D. Löve**			
MYANMAR *A.R. Field ex D.J. Liddle s.n.* (BRI)	–	–	JQ663840*
Phlegmariurus heteroclitus **(Desv. ex Poir.) B. Øllg.**			
ECUADOR *N. Wikstrom 283* (S)	–	AJ224588	–
ECUADOR *M. Lehnert 1851* (VT)	KT634230*	–	KT749920*
Phlegmariurus hippurideus **(Christ) B. Øllg.**			
ECUADOR *B. Øllgaard 100619* (AAU)	Y07931	AJ224566	–
COSTA RICA *W. Testo 151* (AAU, VT)	–	–	KT749922*
Phlegmariurus hippuris **(Desv. ex Poiret) A.R. Field & Testo**			
INDONESIA *N. Wikstrom et al. 157* (S)	AJ133895	AJ224550	–
Phlegmariurus holstii **(Hieron.) A.R. Field & Bostock**			
MADAGASCAR *S. Mantelow 91163* (UPS)	–	AJ224563	–
Phlegmariurus horizontalis **(Nessel) A.R. Field & Bostock**			
INDONESIA *H.-E. Waantorp s.n.* (S)	–	AJ224559	–
Phlegmariurus hystrix **(Herter) B. Øllg.**			
ECUADOR *N. Wikstrom 294* (S)	–	AJ224574	–
Phlegmariurus lauterbachii **(E. Pritz. ex K. Schum. & Lauterb.) A.R. Field & Bostock**			
PAPUA NEW GUINEA *A.R. Field ex D.J. Liddle 770* (BRI)	–	–	JQ663820*
Phlegmariurus ledermannii **(Herter) A.R. Field & Bostock**			
AUSTRALIA *A.R. Field & H.R. Field 1001* (BRI)	–	–	JQ663816*
Phlegmariurus lindenii **(Spring) B. Øllg.**			
ECUADOR *B. Øllgaard 100831* (AAU)	–	AJ224585	–
ECUADOR *M. Sundue 2603* (QCA, VT)	–	–	KT749927*
Phlegmariurus linifolius **(L.) B. Øllg.**			
ECUADOR *S. Dalstrom et al.* 1928 (UPS)	Y07932	AJ224569	–
ECUADOR *H. van-derWerf et al. 19390* (MO)	–	–	JQ663821
Phlegmariurus lockyeri **(D. Jones & B. Gray) A.R. Field & Bostock**			
AUSTRALIA *A.R. Field & H.R. Field 1025* (BRI)	–	AJ224551	JQ663828*
Phlegmariurus marsupiiformis **(D. Jones & B. Gray) A.R. Field & Bostock**			
AUSTRALIA *A.R. Field & H.R. Field 1023* (BRI)	–	–	JQ663811*
Phlegmariurus megastachyus **(Baker) A.R. Field & Bostock**			
MADAGASCAR *H. van-derWerf et al.* (MO)	–	–	JQ663833*
Phlegmariurus mingcheensis **Ching**			
CHINA *S.L. Pan WJ112127* (SHMU)	DQ464232	–	DQ464216
Phlegmariurus nummulariifolius **(Blume) Ching**			
INDONESIA *N. Wikstrom 166* (S)	AJ224552	–	–
PAPUA NEW GUINEA *P.I. Forster & D.J. Liddle s.n.* (BRI)	–	–	JQ663824*
Phlegmariurus ophioglossoides **(Lam.) A.R. Field & Bostock**			
TANZANIA *S. Manktelow 91135* (UPS)	–	AJ224590	–
Phlegmariurus petiolatus **(C. B. Clarke) H. S. Kung & Li Bing Zhang**			
CHINA *S.L. Pan WJ107181* (SHMU)	DQ464233	–	DQ464217
Phlegmariurus phlegmaria **(L.) T. Sen & U. Sen**			
JAPAN – (TNS:763399)	AB574635	–	AB575311
INDONESIA *N. Wikstrom 160* (S)	–	AJ224558	–
Phlegmariurus phlegmarioides **(Gaudich.) A.R. Field & Bostock**			
NEW CALEDONIA *N. Wikstrom et al. 238* (S)	AJ133896	AJ224554	–
AUSTRALIA *A.R. Field et al. 1005* (BRI)	–	–	JQ663838*
Phlegmariurus polydactylus **(B. Øllg.) B. Øllg.**			
ECUADOR *B. Øllgaard* 100834 (AAU)	–	AJ224575	–
Phlegmariurus proliferus **(Blume) A.R. Field & Bostock**			
INDONESIA *N. Wikstrom 161* (S)	–	AJ224547	–
Phlegmariurus pseudophlegmaria **(Nessel) A.R. Field & Testo**			
FIJI *A.R. Field ex R. Hilder 632* (BRI)	–	–	JQ663837*
Phlegmariurus reflexus **(Lam.) B. Øllg.**			
ECUADOR *B. Øllgaard 100617* (AAU)	–	AJ224581	–
MEXICO *M. Sundue 3089* (AAU, MEXU, VT)	KT634233*	–	KT749925*
Phlegmariurus rosenstockianus **(Herter) B. Øllg.**			
ECUADOR *J. Branbyge 42178* (S)	–	AJ224583	–
ECUADOR *Sperling and Bleiweiss 5137* (AAU)	–	–	KT749930*

(*continued on next page*)

Appendix A (*continued*)

Genus species **(Basionym authority) Combination authority** LOCATION *Collector & number* (Herbarium)	*rbc*L	*trn*L-*trn*LF	*psbA-trnH*
Phlegmariurus rufescens **(Hook.) B. Øllg.**			
ECUADOR *B. Øllgaard 100833* (AAU)	–	AJ224576	–
Phlegmariurus salvinioides **(Hert.) Ching**			
MALAYSIA *A.R. Field et al. 51* (BRI)	–	–	JQ663819*
Phlegmariurus sarmentosus **(Spring) B. Øllg.**			
ECUADOR *B. Øllgaard 100816* (AAU)	–	AJ224584	–
Phlegmariurus schlechteri **(E. Pritz.) A.R. Field & Bostock**			
NEW CALEDONIA *P.D. Ziesing et al. 63* (CBG)	–	–	JQ663823*
Phlegmariurus sieboldii **(Miq.) Ching**			
JAPAN *Yahara et al. 176* (TNS)	AB574638	–	AJ224549
Phlegmariurus squarrosus **(G. Forst.) Á. Löve & D. Löve**			
CHINA *S.L. Pan WJ207111* (SHMU)	DQ464235	–	–
MALAYSIA *N. Wikstrom 143* (S)	–	AJ224557	–
AUSTRALIA *A.R. Field et al. 748* (BRI)	–	–	JQ663827*
Phlegmariurus subulatus **(Desv. ex Poir.) B. Øllg.**			
ECUADOR *B. Øllgaard 100618* (AAU)	–	AJ224589	–
Phlegmariurus talamancanus **(B. Øllg.) B. Øllg.**			
COSTA RICA *W. Testo 171* (AAU, VT)	KT634231*	KT634234*	KT749921*
Phlegmariurus tardieuae **(Herter) A.R. Field & Testo**			
MADAGASCAR *H. van derWerf et al. 12764* (MO)	–	–	JQ663812*
Phlegmariurus tauri **(Herter) A.R. Field & Testo**			
INDONESIA *N. Wikstrom et al. 151* (S)	–	AJ224556	–
Phlegmariurus tenuis **(Humb. & Bonpl. Ex Willd.) B. Øllg.**			
ECUADOR *B. Øllgaard 100817* (AAU)	–	AJ224568	–
Phlegmariurus tetragonus **(Hook. & Grev.) B. Øllg.**			
ECUADOR *N. Wikstrom 301* (S)	–	AJ224577	–
Phlegmariurus tetrastichoides **(A.R. Field & Bostock) A.R. Field & Bosock**			
AUSTRALIA *A.R. Field & H.R. Field 1018 (BRI)*	–	–	JQ663834*
Phlegmariurus tetrastichus **(Kunze) A.R. Field & Bostock**			
PAPUA NEW GUINEA *A.R. Field ex G. Stocker 815* (BRI)	–	–	JQ663842*
Cultivated *W. Testo s.n.* (VT)	–	KT634235*	–
Phlegmariurus trifoliatus **(Copel.) A.R. Field & Bostock**			
FIJI *A.R. Field ex R. Hilder 984* (BRI)	–	–	JQ663814*
Phlegmariurus unguiculatus **(B. Øllg.) B. Øllg.**			
ECUADOR *N. Wikstrom 264* (S)	–	AJ224582	–
Phlegmariurus varius **(R. Br.) A.R. Field & Bostock**			
AUSTRALIA *A.R. Field & H.R. Field 1043* (BRI)	JQ679089*	JQ672503*	JQ663831*
Phlegmariurus verticillatus **(L.f.) A.R. Field & Testo**			
INDONESIA *N. Wikstrom et al. 156* (S)	AJ133897	AJ224561	–
Phlegmariurus wilsonii **(Underw. & F.E. Lloyd) B. Øllg.**			
ECUADOR *B. Øllgaard et al. 100611* (AAU)	Y07933	AJ224565	–
ECUADOR *B. Øllgaard et al. 1700* (AAU, VT)	–	–	KT749928*
Phylloglossum drummondii **Kunze**			
AUSTRALIA *Crane s.n.* (S)	Y07939	AJ224593	XXXXX*
Pseudodiphasium volubile **(G. Forst.) Holub**			
MALAYSIA *N. Wikstrom et al. 130* (S)	AJ133253	AJ224596	–
PAPUA NEW GUINEA *M. Sundue 3614* (BISH, VT)			KT749944
Pseudolycopodiella caroliniana **(L.) Holub**			
JAPAN – (TNS:743681)	AB574623	AJ133266	–
Pseudolycopodium densum **(Rothm.) Holub**			
NEW CALEDONIA *N. Wikstrom 242* (S)	AJ133249	AJ224597	–
Selaginella moellendorffii **Hieron.**			
– (–)	HM173080	HM173080	HM173080
Selaginella selaginoides **(L.) P. Beauv. ex Mart. & Schrank**			
CANADA *Therrien s.n.* (KANU)	AF419048	–	–
SWITZERLAND *M. Sundue s.n.* (VT)	–	KT634237*	KT749945*
Selaginella tamariscina **(P. Beauv.) Spring**			
UNKNOWN *O. Shurba 5157* (S)	AJ295861	–	KJ025044

Appendix B

Description of morphological and anatomical characters used in phylogenetic analysis of Huperzioid Lycopodiaceae.

B.1. Life cycle

1. Life cycle: The presence or absence of a homosporous or heterosporous life cycle is a well-examined synapomorphy of the Lycopod families Lycopodiaceae, Selaginellaceae and Isoetaceae. This character was determined by the presence or absence of micro and mega sporangia (and micro and mega spores) in the axils of sporophylls of the strobilus. This character has two discrete and qualitative states defined as follows: (0) plants homosporous, sporangia bisexual; (1) plants heterosporous, male and or female sporangia present.

B.2. Spore shape and surface sculpture

The spores of all Lycopodiaceae are trilete tetrahedral with recognisable proximal and distal surfaces. The proximal surface faced the other three spore cells in the tetrad and it bears three roughly triangular flattened faces that are divided by a trilete scar referred to as the laesurae. The distal surface faced outwards when the spores were in the tetrad and it is roughly hemispherical. The scar between the proximal and distal surfaces is referred to as the margo. This general spore shape and pattern was present in Lycopsid and Lycopsid like fossils at least as early as lower Devonian but early fossils are generally relatively simple in shape and surface ornamentation and lack obvious synapomorphies that can be associated with extant groups. The shape in polar-view and the surface ornamentation of Lycopodiaceae spores have been use to defined generic and sectional segregates within the Lycopodiaceae (Boivin, 1950; Breckon, 1974; Freeberg, 1957; Øllgaard, 1987; Wagner and Beitel, 1992; Wilce, 1972). These characters are particularly useful because spores within Lycopodiaceae are durable and do not appear to be phenotypically plastic.

2. Spore distal surface sculpture: Five types of surface sculpture have been reported: foveolate-fossulate spores are reported for the Huperzioid genera *Huperzia* Bernh., *Phylloglossum* Kunze and *Phlegmariurus* Holub, rugulate spores for the Lycopodioid genus *Lycopodiella* Holub *sensu* Øllgaard (1987) and reticulate spores for the most species of the Lycopodioid genus *Lycopodium* L. *sensu* Øllgaard (1987). Baculate spores have so far only been reported for a single species, *Pseudolycopodium densum* (Rothm.) Holub and scabrate spores for a single species *Lycopodiastrum casuarinoides* (Spring) Holub, both of these spore types may be derived from the reticulate type (Wikström and Kenrick, 2001). This character is qualitative with five discrete states defined as follows *sensu* Wilce (1972): (0) distal surface sculpture foveolate-fossulate; (1) scabrate; (2) rugate; (3) bacculate; (4) reticulate.

3. Spore proximal surface sculpture: This character is discrete and qualitative with two states defined as follows: (0) spore proximal surfaces sculptured; (1) spore proximal surfaces smooth.

4. Spore outline in polar view: Two polar-view spore-outline shapes have been reported: spores with a sub-triangular to rounded outline with straight to convex lateral margins and acute to rounded corners have been reported for the majority of Lycopodiaceae genera whereas spores with concave lateral margins and truncate corners has been reported for species of *Huperzia* Bernh *sensu* Wagner and Beitel (1992). Although Wagner and Beitel (1992) scored margins and corners as separate characters, they are dependent upon each other and are combined in our study. This character is qualitative and discrete with two states defined as follows: (0) spore margins convex to straight and corners acute to rounded; (1) spores margins concave and corners truncate.

B.3. Gametophytes

The gametophytes of Lycopodiaceae are free-living bisexual organs that bear antheridia and archegonia in a cap on their upper surface. A historical study by Bruchmann (1898) recognised five gametophyte types based on their trophic requirements, presence of paraphyses, branching patterns and germination positions. Among these were two trophic-groups, autotrophic surface living gametophytes and holomycotrophic subterranean gametophytes. Autotrophy and mycoheterotrophy was subsequently reported to be phenotypically plastic (Freeberg, 1957; Freeberg and Wetmore, 1957), an experimental finding that was later refuted (Bruce, 1976b). Experimental work on *in vitro* gametophytes, largely pioneered by Whittier et al. *loc cit.*, has established that the forms are generally non-plastic and that gametophytes are either holomycotrophic from germination to sexual maturity, mycotrophic as juvenile and autotrophic when sexually mature or completely autotrophic.

5. Gametophyte paraphyses: The presence or absence of paraphyses in between the gametangia on the dorsal surface cap of the gametophyte has been considered an important character that distinguishes between *Huperzia sensu* Øllgaard (1987) which is reported to have paraphyses and all other Lycopodiaceae which are reported to lack paraphyses. Early reports of the gametophyte of *Phylloglossum drummondii* suggested it lacked paraphyses making it consistent with the gametophytes of Lycopodioid genera but Whittier and Braggins (2000) have shown that paraphyses are present in *Phylloglossum drummondii* when the gametophytes are mature making it consistent with Huperzioid genera. This character is discrete and qualitative with two states defined as follows: (0) paraphyses present; (1) paraphyses absent.

6. Gametophyte germination position: The surface or subterranean position of Lycopodiaceae gametophytes was used as a character (39) by Wagner and Beitel (1992) and has been discussed in several taxonomic investigations of the family (Boivin, 1950; Bruce, 1976b). Studies of germination requirements of Lycopodiaceae gametophytes by Whittier et al. have shown that the gametophyte germination position is linked to spore germinate in the light or dark (Bruce, 1976b; Wagner and Beitel, 1992; Whittier, 1998, 2006; Whittier and Braggins, 1992, 2000; Whittier and Storchova, 2007). The genera *Huperzia*, *Phlegmariurus* and *Phylloglossum* and *Lycopodium s.l.* germinate only when subject to a dark pre-treatment and their gametophytes are subterranean, whereas *Lycopodiella s.l.* germinates only when exposed to light and its gametophytes develop on the surface of the substrate. This character has been scored separately from Char. 1 because the states are independent in *Phylloglossum drummondii* which exhibits dark + subterranean germination but develops photosynthetic lobes upon emergence at the substrate surface (Whittier and Braggins, 1992, 2000). This character is discrete and qualitative with two states defined as follows: (0) spore germinating following a period of dark and gametophyte subterranean; (1) spore germinating in the light and gametophyte wholly surface-living.

7. Gametophyte growth axis orientation: The growth axis and shape in cross section of gametophytes was determined from microscopical examination of field-collected gametophytes for Australian Lycopodiaceae or from literature reports (Bruce, 1976b; Whittier, 1998, 2006; Whittier and Braggins, 1992; Whittier and Storchova, 2007). This character has been scored separately from germination position and trophic source because the states are independent in *Phylloglossum, Lycopodium s.l.* and *Lycopodiella s.l.* The additional character of gametophyte branching used by Wagner and Beitel (1992) was not used because it

appeared to be plastic within gametophyte colonies belonging to the same species, i.e. they could be both unbranched and branched at maturity. This character is discrete and qualitative with two states defined as follows: (0) gametophyte growth axis horizontal with a dorsiventral cross section; (1) gametophyte growth axis vertical with a radial cross section.

8. Gametophyte type: This character is discrete and qualitative with three states defined as follows: (0) gametophytes holomycotrophic; (1) gametophytes initially mycotrophic becoming autotrophic upon emergence at substrate surface; (2) gametophytes autotrophic.

B.4. Sporophyte anatomy

9. Presence or absence of a ligule: The presence of a ligule is considered to be a synapomorphy of the Isoetaceae and Selaginellaceae being absent in the Lycopodiaceae. This character was defined as discrete with two qualitative states defined as follows: (0) ligule absent; (1) ligule present.

10. Shoot stele type: The type of shoot stele present in all Lycopodiopsid stems is a protostele which has xylem more or less confined to the central core of the stem with phloem arranged around it. Lycopodiopsida protosteles lack the pith core, arrangement of xylem and phloem in concentric cylinders and the leaf-gap found in the Siphonosteles of Monilophytes and Spermatophytes. Within the Lycopodiopsida there are three general patterns present; the solitary protostele of the Lycopodiaceae, the polystele of Selaginellaceae and the amorphous Isoetalean stele of the Isoetaceae. This character is discrete with three qualitative states defined as follows: (0) protostelic; (1) polystelic; (2) Isoetalean stele.

11. Root emergence type: The roots of Lycopodiopsida emerge in three different ways. The family Lycopodiaceae is considered to have adventitious roots which emerge directly from the stem stele either along the stem or via the stem base (see Character 13), the family Selaginellaceae are considered to have rhizophores which are root like stem projections emerging from the lower surface of the shoot-stem and the Isoetaceae are considered to have a rhizomorphous mass from which roots emerge in a tuft. This characters has discrete with three qualitative states defined as follows: 0 = roots adventitious; 1 = roots rhizophorous; 2 = roots rhizomorphous.

12. Protostele type: Within the Lycopodiaceae two general patterns have been reported, actinosteles, which appear as radial (sometimes highly asymmetric and variable along the stem) masses when viewed in cross section, and plectosteles, which appear as multiple parallel bands when viewed in cross section (Finger et al., 2011; Jones, 1905; Øllgaard, 1987). The genera *Huperzia* and *Phlegmariurus* are actinostelic throughout whereas the genera *Lycopodium s.l.* and *Lycopodiella s.l.* are plectostelic in lateral branch modules and actinostelic in aerial branch modules. The stele shape found in *Phylloglossum* is difficult to interpret and has been suggested to have some similarities to a siphonostele. This character included two qualitative discrete states defined as follows: (0) basal module of stem bearing an actinostelic protostele; (1) basal module of stem bearing a plectostelic protostele.

13. Shoot stele diameter and corticular root emergence: In addition to stele type, previous authors have described categorical differences in the diameter of the stele with respect to stem size, with two non-intergrading categories reported (Hill, 1914; Øllgaard, 1979a). In *Huperzia s.l.* the stele is very narrow and occupies a small area of the stem, contrasting with *Lycopodium* and *Lycopodiella s.l.* in which the stele is much broader, occupying almost half of the stem diameter. This character appears to be strictly linked with corticular emergence of roots as the broad cortex is occupied by roots in these species. Corticular emergence of roots occurs when new roots emerge directly from the stem stele at a point along the stem and then travel down through the cortex inside the stem to emerge in a tuft at the base of the stem. The ratio of stele diameter to stem diameter and the type of root emergence was determined by light microscope examination of hand cut phloroglucinol–HCl stained transverse sections of stems harvested from the base of the first stem module of mature cultivated or field collected sporophytes. This character was defined as discrete with two qualitative states as follows: (0) stem stele ½–1/3 stem diameter and root emergence non-corticular; (1) stem stele ¼–1/20 stem diameter and root emergence corticular.

14. Mucilage duct in sporophyll base: The presence or absence of mucilage ducts in the sporophyll base at the junction of the stem was investigated by Bruce (1976a,b) and used as a character by Wagner and Beitel (1992) (Bruce, 1976a,b, Øllgaard 1987 and Wagner and Beitel 1992). The distribution of this character among genera was scored from Bruce (1976) and Wagner and Beitel (1992) for *Lycopodium s.l.* and *Lycopodiella s.l.* and from live cultivated material for *Huperzia s.l.* This character is generally dependent on sporophylls being peltate but is independent in *Pseudodiphasium volubile* (G.Forst.) Holub. This character was defined with two discrete states: (0) mucilage duct in sporophyll base absent; (1) mucilage duct in sporophyll based present.

15. Sporangial valve wall cell lignification: The thickness and lignification of cell walls in sporangial valve cells was investigated by Øllgaard (1975) who considered there were two major types of cell walls, with thick and lignified sporangial valve cell walls present in *Huperzia s.l.* and *Phylloglossum* and thin non-lignified cell walls present in *Lycopodium s.l.* and *Lycopodiella s.l.* This character was scored *sensu* Øllgaard (1975) and Øllgaard (1987) with two qualitative states as follows: (0) sporangial valve walls thick and lignified; (1) sporangial valve walls thin and unlignified.

16. Sporangial valve wall cell shape: The shape of the cell walls in the sporangial valves was investigated by Øllgaard (1975) who considered there were differences in the wall shapes with *Huperzia s.l., Phylloglossum* and *Lycopodium s.l.* exhibiting sinuate cell walls whereas *Lycopodiella s.l.* exhibited straight cell walls. This character was scored *sensu* Øllgaard (1975) and Øllgaard (1987) with two discrete qualitative states as follows: (0) sporangial cell walls sinuate; (1) sporangial cell walls non-sinuate.

B.5. Sporophyte morphology

Multiple genera and subfamilies have been recognised based upon sporophyte characteristics, with habit, branching pattern and morphology of lycophylls and sporophylls playing key roles in previous and current classifications of the Lycopodiaceae (Herter, 1949a, 1949b, 1950; Holub, 1964, 1975, 1983, 1985b, 1991a; Øllgaard, 1975, 1979b, 1987; Øllgaard, 1992a; Wagner and Beitel, 1992). In contrast to previous studies, the fertile zone of all species is referred to as a strobilus because the developmental origin is the same regardless of whether or not the fertile shoot is clearly differentiated from the non-fertile zone. Macromorphological characters were examined from field collected, live cultivated and herbarium vouchers without magnification, or by using a low power stereo-microscope.

17. Shoot elongation: Lycopodiopsids generally have shoots with sub-whorls of ranked lycophylls spaced along an elongate stem. Two exceptions have been reported, all members of the family Isoetaceae and the monospecific *Phylloglossum* in the Lycopodiaceae, both of which have clustered lycophylls on a short shoot. This shoot form has been traditionally interpreted as reduced (Bower, 1885; Huang and Chengzhong, 2010; Kenrick and Crane, 1997; Wikström and Kenrick, 1997). This discrete and qualitative character was defined as follows: (0) lycophylls borne on an elongate shoot (space visible between alternating lycophyll

sub-whorls); (1) lycophylls borne on reduced shoot appearing tufted (no space visible between sub-whorls of lycophylls).

18. Shoot branching pattern: The branching pattern, in particular the symmetry of branching, has been explored for a broad spectrum of Lycopodiaceae species and has been defined as a key character in the recognition of genera (Øllgaard, 1979b, 1987). Three major branching patterns have been described, branching absent, isotomous branching, and anisotomous branching. Isotomous shoots form following equal branching of shoot apical meristems, whereas anisotomous shoots form as a result branching of shoot apical meristems where the subsequent meristems are unequal in size (Imaichi, 2008; Imaichi and Hiratsuka, 2007; Troll, 1937). Homology between these states has been inferred in all studies of the Lycopodiaceae and is consistent with their ontogenic origin. Anisotomous branching has been described as including two subtypes, anisotomous with an inclinate orientation and anisotomous with flabellate horizontal branching and inclinate branches arising dorsally from horizontal shoots (Øllgaard, 1979b, 1987). Difficulty was encountered in differentiating between these subtypes in some incomplete specimens so they were grouped into a single state for anisotomy. This character was defined as discrete and qualitative with three states as follows: (0) shoots unbranched; (1) shoots isotomously branched; (2) shoots anisotomously branched.

19. Auxiliary branches: The point of origin of new auxiliary branches has received relatively little attention in taxonomic investigation of the Lycopodiaceae. Observation of cultivated material suggested the presence of two non-plastic types of new shoot emergence, new shoots arising solely from the axils of basal lycophylls to form a locally tufted plant, and new shoots arising from the axils of sporophylls along modified lateral shoots to form a spreading non-tufted plant. A third type of shoot origin is described for *Phylloglossum* which has solitary shoots that arise from an underground tuber (Bower, 1885; Breckon, 1974; Chinnock, 1998; Huang and Chengzhong, 2010). This character was defined as discrete and qualitative with three states follows: (0) new shoots arising from an underground tuber like stem; (1) new shoots arising from the axils of stem base lycophylls thereby forming a tuft of shoots; (2) new shoots arising from the axils of lateral stem lycophylls thereby forming a laterally spreading colony.

20. Shoots deciduous: Shoot persistence was determined from personal observation of field and cultivated plants and from herbarium records. This character is discrete and qualitative with two states defined as follows: (0) shoots evergreen/persistent; (1) shoots deciduous/ephemeral.

21. Strobilus peduncle: The presence or absence of a peduncle at the base of the strobilus was used to define genera in the classification of Øllgaard (1987) and was used as a character by Wagner and Beitel (1992) who recognised two states, 'stalked' and 'unstalked'. It has generally been presumed that the sessile strobilus is the plesiomorphic state (Wagner and Beitel, 1992) being present in Selaginellaceae, Isoetaceae and most fossil Lycopodiopsids (Kenrick and Davis, 2004). There are two anatomical origins for the strobilus peduncle observed in the Lycopodiaceae, the strobilus peduncle form present in *Phylloglossum* occurs as a result of elongation of sterile section of the stem without lycophylls or sporophylls (Bower, 1885; Huang and Chengzhong, 2010; Øllgaard, 1987) whereas the strobilus present in *Lycopodium sensu* Øllgaard (1987) occurs as a result of reduction of the lycophylls along the pedunculate section of the stem (Øllgaard, 1987). This character was therefore defined to include three discrete qualitative states as follows: (0) peduncle absent; (1) leafless strobili peduncle present; (2) leafy strobilus peduncle present.

22. Sporophyll attachment type: The attachment type of the sporophyll was used as a character to define both genera and subgenera in the classification of Øllgaard (1987) who recognised two states, paleate and peltate. The states were redefined by Wagner and Beitel (1992) as 'basal' and 'pseudopeltate-peltate' (Char 22). This character was defined as having two discrete qualitative states as follows: (0) sporophylls paleate (basally attached and not peltate); (1) sporophylls peltate.

23. Sporophyll type persistence: The deciduousness or persistence of sporophylls following maturation and dehiscence of sporangia has previously been considered a synapomorphy of *Lycopodium* and *Lycopodiella sensu* Øllgaard (1987). Two discrete qualitative states are included in this character defined as follows: (0) evergreen persistent following dehiscence of sporangia; (1) deciduous ephemeral, senescing following dehiscence of sporangia.

24. Sporangial stalk: The presence of absence of a sporangial stalk was used as a character in the classifications of Øllgaard (1987) and the cladistic study of Wagner and Beitel (1992) and is considered to be characteristic of *Lycopodiella s.l.* This character is discrete and qualitative with two states defined as follows: 0 = sporangial stalk absent; 1 = sporangial stalk present.

25. Strobili bulbils: The presence or absence of bulbils has been used in taxonomic delineation in several classifications of the Lycopodiaceae. Strobilus bulbils are small bilaterally-shaped axillary side branchlets with an abscission layer at their base that can detach and form new roots and establish as independent plants (Gola, 2008; Holloway, 1917; Ma et al., 1998). Their presence, symmetry and the presence of an abscission layer were scored as separate characters by Wagner and Beitel (1992) but they are dependent and are here considered to be developments relating to a single character. The presence or absence of bulbils in the axils of sporophylls was determined from examination sporophytes in the field or herbarium specimens. This character was defined as having two discrete qualitative states as follows: (0) strobili not bulbiferous; (1) strobili bulbiferous. Anisotomous side-branchlets that superficially resembled bulbils were twice recorded in epiphytic species but they were not scored as bulbils as they lacked an abscission layer and could not be attached as autonomous plantlets.

26. Lycophylls flat or terete: The lycophylls of Lycopodiopsids are generally flat in cross section as found in *Selaginella* and the majority of Lycopodiaceae. Terete lycophylls have been recorded for a number of Lycopodiopsida including *Isoetes* and *Phylloglossum* but their frequency among other Lycopodiopsida has been poorly recorded (Bower, 1885). The presence or absence of terete lycophylls in Lycopodiaceae was determined from microscopical examination of unstained transverse sections of fresh living material, with the exception of *Phylloglossum*, which was examined from a specimen preserved in alcohol. This character was defined as discrete and qualitative with two states as follows: (0) lycophylls not terete; (1) lycophylls terete.

27. Isophylly/anisophylly: Isophylly and homophylly were defined as separate characters by Øllgaard (1987). Isophylly occurs when all of the lycophylls attached at one point are equal and anisophylly when the lycophylls attached at one point are not equal. Isophylly has traditionally being considered to be the plesiomorphic state for Lycopodiaceae (Øllgaard, 1987) but it should be noted that Selaginellaceae, one of the sisters to the Lycopodiaceae, is anisophyllous. Øllgaard (1987) considered five different states of anisophylly were present in the Lycopodiaceae. In the study of Wagner and Beitel (1992) the independent forms of anisophylly were grouped into one homoplasious character. Following Øllgaard (1987), six discrete qualitative character states were scored for this study: (0) isophyllous; (1) weakly to strongly anisophyllous but lycophylls not ordered into specialised ranks; (2) trimorphic lycophylls with one dorsal and one ventral rank of narrow lycophylls and two lateral ranks of broad lycophylls; (3) dimorphic lycophylls with broad dorsal lycophylls in 2 ranks and narrow ventral lycophylls in 2–3 ranks (*Diphasium* Presl ex Rothm.); (4)

flattened isophyllous shoots achieved via broad compressed lateral leaf bases (*Lycopodiastrum* Holub); (5) trimorphic lycophylls with 1–2 dorsal ranks and one ventral rank of narrow lycophylls and 2 lateral ranks of broad compressed subopposite lycophylls (*Pseudodiphasium* Holub).

28. Branching inclinate anisotomous or branching flabellate anisotomous. Forms of anisotomous branching were reviewed by Øllgaard (1979a,b) who characterised inclinate anisotomous branching which was used as a defining characterising of *Lycopodium sensu* Øllgaard (1987) and flabellate anisotomous branching which was used as a defining characteristic of *Lycopodiella sensu* Øllgaard (1987). Following Øllgaard (1979a,b) two character states for anisotomous branching were scored: (0) inclinate anisotomous branching and (1) flabellate anisotomous branching. No state was scored for taxa that lacked anisotomous branching.

29. Habit aquatic, terrestrial or epiphytic. Lycopods occupy a broad range of habitats with *Isoetes* being mostly aquatic, *Selaginella* being mostly terrestrial and the Lycopodiaceous genera being found in aquatic, terrestrial and epiphytic habitats. Although habit is not generally used as a synapomorphy, the unidentified underlying morphological, anatomical and life-history traits that drive habit preference are important innovations. Aquatic habit was defined as growing primarily in a wetland of wholly aquatic area that is submerged by water for at least part of the year. Terrestrial was defined as growing on the ground in soil and not submergend in a wetlands and not growing on another plant or a rock surface. Epiphytic was defined as growing upon another plant, especially a tree, or upon the soil less surface of rocks (epilithic). Three states were scored (0) aquatic, (1) terrestrial and (2) epiphytic.

Appendix C. Morphological data matrix

Character	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
Austrolycopodium fastigiatum	0	4	0	0	0	0	1	0	0	0	0	2	0	1	0	0	0	1	1	0	1	1	1	1	0	0	0	0	1
Austrolycopodium magellanicum	0	4	0	0	0	0	1	0	0	0	0	2	0	1	0	0	0	1	1	0	1	1	1	1	0	0	0	0	1
Dendrolycopodium dendroidium	0	4	1	0	0	0	1	0	0	0	0	2	0	1	0	0	0	1	1	0	1	1	1	1	0	0	0	0	1
Diphasiastrum alpinum	0	4	1	0	0	0	1	0	0	0	0	2	0	1	0	0	0	1	1	0	1	1	1	1	0	0	2	0	1
Diphasiastrum complanatum	0	4	1	0	0	0	1	0	0	0	0	2	0	1	0	0	0	1	1	0	1	1	1	1	0	0	2	0	1
Diphasiastrum digitatum	0	4	1	0	0	0	1	0	0	0	0	2	0	1	0	0	0	1	1	0	1	1	1	1	0	0	2	0	1
Diphasiastrum nikoense	0	4	1	0	0	0	1	0	0	0	0	2	0	1	0	0	0	1	1	0	1	1	1	1	0	0	2	0	1
Diphasiastrum thyoides	0	4	1	0	0	0	1	0	0	0	0	2	0	1	0	0	0	1	1	0	1	1	1	1	0	0	2	0	1
Diphasiastrum wightianum	0	4	1	0	0	0	1	0	0	0	0	2	0	1	0	0	0	1	1	0	1	1	1	1	0	0	2	0	1
Diphasium jussiaei	0	4	0	0	0	0	1	0	0	0	0	2	?	1	0	0	0	1	1	0	1	1	1	1	0	0	3	0	1
Diphasium scariosum	0	4	0	0	0	0	1	0	0	0	0	2	?	1	0	0	0	1	1	0	1	1	1	1	0	0	3	0	1
Huperzia appressa	0	0	1	1	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	?	1
Huperzia arctica	0	0	1	1	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	?	1
Huperzia australiana	0	0	1	1	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	?	1
Huperzia crispata	0	0	1	1	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	?	1
Huperzia emeiensis	0	0	1	1	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	?	1
Huperzia fuegiana	0	0	1	1	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	?	1
Huperzia haleakalae	0	0	1	1	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	?	1
Huperzia lucidula	0	0	1	1	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	?	1
Huperzia miyoshiana	0	0	1	1	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	?	1
Huperzia nanchuanensis	0	0	1	1	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	?	1
Huperzia quasipolytrichoides	0	0	1	1	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	?	1
Huperzia selago	0	0	1	1	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	?	1
Huperzia serrata	0	0	1	1	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	?	1
Huperzia somae	0	0	1	1	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	?	1
Huperzia sutchueniana	0	0	1	1	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	?	1
Isoetes flaccida	1	?	?	0	?	?	?	?	1	0	2	?	?	?	?	?	1	?	?	0	0	0	0	?	0	1	0	?	?	0
Isoetes japonica	1	?	?	0	?	?	?	?	1	0	2	?	?	?	?	?	1	?	?	?	0	0	0	0	0	1	0	?	?	0
Lycopodiastrum casuarinoides	0	1	0	0	0	0	1	0	0	0	0	2	0	1	0	0	0	1	1	0	1	1	1	1	0	0	0	0	1
Lycopodiella alopecuroides	0	2	0	0	0	1	0	2	0	0	0	2	0	1	1	1	0	1	1	0	0	1	1	1	0	0	0	1	1
Lycopodiella inundata	0	2	0	0	0	1	0	2	0	0	0	2	0	1	1	1	0	1	1	0	0	1	1	1	0	0	0	1	1
Lycopodiella lateralis	0	2	0	0	0	1	0	2	0	0	0	2	0	1	1	1	0	1	1	0	0	1	1	1	0	0	0	1	1
Lycopodium annotinum	0	4	1	0	0	0	1	0	0	0	0	2	0	1	0	0	0	1	1	0	1	1	1	1	0	0	0	0	1
Lycopodium cf clavatum	0	4	1	0	0	0	1	0	0	0	0	2	0	1	0	0	0	1	1	0	1	1	1	1	0	0	0	0	1
Lycopodium clavatum	0	4	1	0	0	0	1	0	0	0	0	2	0	1	0	0	0	1	1	0	1	1	1	1	0	0	0	0	1
Lycopodium sp. L22	0	4	1	0	0	0	1	0	0	0	0	2	0	1	0	0	0	1	1	0	1	1	1	1	0	0	0	0	1
Diphasium sp. L24	0	4	0	0	0	0	1	0	0	0	0	2	0	1	0	0	0	1	1	0	1	1	1	1	0	0	3	0	1
Lycopodium lagopus	0	4	1	0	0	0	1	0	0	0	0	2	0	1	0	0	0	1	1	0	1	1	1	1	0	0	0	0	1
Dendrolycopodium obscurum	0	4	1	0	0	0	1	0	0	0	0	2	0	1	0	0	0	1	1	0	1	1	1	1	0	0	0	0	1
Lycopodium vestitum	0	4	1	0	0	0	1	0	0	0	0	2	0	1	0	0	0	1	1	0	1	1	1	1	0	0	0	0	1
Palhinhaea cernua	0	2	0	0	0	1	0	2	0	0	0	2	0	1	1	1	0	1	1	0	0	1	1	1	0	0	0	1	1
Palhinhaea glaucescens	0	2	0	0	0	1	0	2	0	0	0	2	0	1	1	1	0	1	1	0	0	1	1	1	0	0	0	1	1
Palhinhaea pendula	0	2	0	0	0	1	0	2	0	0	0	2	0	1	1	1	0	1	1	0	0	1	1	1	0	0	0	1	1

Appendix C (*continued*)

Character	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
Palhinhaea tomentosa	0	2	0	0	0	1	0	2	0	0	0	2	0	1	1	1	0	1	1	0	0	1	1	1	0	0	0	1	1
Phlegmariurus attenuatus	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	1
Phlegmariurus balansae	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus billardierei	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus brassii	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	1
Phlegmariurus campianus	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus capellae	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	1
Phlegmariurus carinatus	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus compactus	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	1
Phlegmariurus crassus	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	1
Phlegmariurus cryptomerianus	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus cumingii	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	1
Phlegmariurus dacrydioides	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus dalhousieanus	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus dichaeoides	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus dichotomus	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus elmeri	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus ericifolius	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus eversus	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	1
Phlegmariurus fargesii	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus filiformis	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus foliosus	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus fordii	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus funiformis	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus goebelii	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus hamiltonii	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus heteroclitus	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus hippurideus	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	1
Phlegmariurus hippuris	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus holstii	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus horizontalis	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus hystrix	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	1
Phlegmar. pseudophlegmaria	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus lauterbachii	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus ledermannii	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus lindenii	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus linifolius	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus lockyeri	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus marsupiiformis	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus megastachyus	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus mingcheensis	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus nummulariifolius	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus ophioglossoides	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus petiolatus	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus phlegmaria	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus phlegmarioides	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus polydactylus	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	1
Phlegmariurus proliferus	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus reflexus	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	1
Phlegmariurus rosenstockianus	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus rufescens	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	1
Phlegmariurus salvinioides	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus sarmentosus	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus schlecteri	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus sieboldii	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus squarrosus	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus subulatus	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus talamancanus	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	1
Phlegmariurus tardieuae	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus tauri	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2

(*continued on next page*)

Appendix C (*continued*)

Character	0 1	0 2	0 3	0 4	0 5	0 6	0 7	0 8	0 9	1 0	1 1	1 2	1 3	1 4	1 5	1 6	1 7	1 8	1 9	2 0	2 1	2 2	2 3	2 4	2 5	2 6	2 7	2 8	2 9
Phlegmariurus tenuis	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus tetragonus	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	1
Phlegmariurus tetrastichoides	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus tetrastichus	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus trifoliatus	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	2
Phlegmariurus unquiculatus	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	1
Phlegmariurus varius	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	1
Phlegmariurus verticillatus	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	1
Phlegmariurus wilsonii	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	1
Phylloglossum drummondii	0	0	0	0	1	0	0&1	0&1&2	0	0	0	1	1	0	0	0	1	0	0	1	2	0	0	0	0	1	0	?	0
Pseudodiphasium volubile	0	4	1	0	0	0	1	0	0	0	0	2	0	1	0	0	0	1	1	0	1	1	1	1	0	0	0	0	1
Pseudolycopodiella caroliniana	0	2	0	0	0	1	0	2	0	0	0	2	0	1	1	1	0	1	1	0	0	1	1	1	0	0	0&1	1	1
Pseudolycopodium densum	0	3	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	1	1	0	0	1	1	1	0	0	0	0	1
Selaginella moellendorffii	1	?	?	0	?	?	?	?	1	1	1	?	?	?	?	?	0	?	1	?	0	0	0	0	0	0	?	?	?	1
Selaginella selaginoides	1	?	?	0	?	?	?	?	1	1	1	?	?	?	?	?	0	?	1	?	0	0	0	0	0	0	?	?	?	1
Selaginella tamariscina	1	?	?	0	?	?	?	?	1	1	1	?	?	?	?	?	0	?	1	?	0	0	0	0	0	0	?	?	?	1

Appendix D. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2015.09.024.

References

Bateman, R.M., 1996. An overview of lycophyte phylogeny. In: Camus, J.M., Gibby, M., Johns, R.J. (Eds.), Pteridology in perspective. Royal Botanic Gardens, Kew, pp. 405–417.

Bateman, R.M., DiMichelle, W.A., Willard, D.A., 1992. Experimental cladistic analyses of anatomically preserved arborescent lycopsids from the Carboniferous of Euramerica: an essay in palaeobotanical phylogenetics. Ann. Mo. Bot. Gard. 79, 500–550.

Bateman, R.M., Kenrick, P., Rothwell, G.W., 2007. Do eligulate herbaceous lycopsids occur in Carboniferous stata? *Hestia eremosa gen. et sp. nov.* from the Mississippian of Oxroad Bay, East Lothian, Scotland. Rev. Palaeobot. Palynol. 144, 323–335.

Blackwood, M., 1953. Chromosomes of *Phylloglossum drummondii* Kunze. Nature 172, 591–592.

Boivin, B., 1950. The problem of generic segregates in the form-genus *Lycopodium*. Am. Fern J. 40, 32–41.

Bower, F.O., 1885. On the development and morphology of *Phylloglossum drummondii*. Philos. Trans. R. Soc. Lond. 176, 665–678.

Breckon, G.J., 1974. External spore morphology and taxonomic affinities of *Phylloglossum drummondii* Kunze (Lycopodiaceae). Am. J. Bot. 61, 481–485.

Breckon, G.J., Falk, R.H., 1974. External spore morphology and taxonomic affinities of *Phylloglossum drummondii* Kunze (Lycopodiaceae). Am. J. Bot. 61, 481–485.

Bruce, J.G., 1976a. Development and distribution of mucilage canals in *Lycopodium*. Am. Fern J. 63, 481–491.

Bruce, J.G., 1976b. Gametophytes and subgeneric concepts in *Lycopodium*. Am. J. Bot. 63, 919–924.

Bruchmann, H., 1898. Uber die Prothallien un Keimpflanzen mehrerer europäischer Lycopodien. Gotha.

Chinnock, R.J., 1998. Lycopodiophyta. In: McCarthy, P.M. (Ed.), Ferns, Gymnosperms and Allied Groups. ABRS/CSIRO, Canberra, pp. 66–85.

Christenhusz, M.J.M., Zhang, X.-C., Schneider, H., 2011. A linear sequence of extant families and genera of lycophytes and ferns. Phytotaxa 19, 7–54.

Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. JModelTest 2: more models, new heuristics and parallel computing. Nat. Methods 9, 772-772.

Doyle, J.J., Doyle, J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19, 11–15.

Field, A.R., Bostock, P.D., 2013. New and existing combinations in Palaeotropical Phlegmariurus (Lycopodiaceae) and lectotypification of the type species Phlegmariurus phlegmaria (L.) T. Sen & U. Sen. Phytokeys 20, 33–51.

Finger, A., Kettle, C.J., Kaiser-Bunbury, C.N., Valentin, T., Doudee, D., Matatiken, D., Ghazoul, J., 2011. Back from the brink: potential for genetic rescue in a critically endangered tree. Mol. Ecol. 20, 3773–3784.

Freeberg, J.A., 1957. The apogamous development of sporelings of *Lycopodium cernum* L., *L. complanatum* var. *flabelliforme* Fernald and *L. selago* L. in vitro. Phytomorphology 7, 217–229.

Freeberg, J.A., Wetmore, R.H., 1957. Gametophytes of *Lycopodium* as grown in vitro. Phytomorphology 7, 04–217.

Garrat, M.J., 1984. The appearance of *Baragwanathia* (Lycophytina) in the Silurian. Bot. J. Linn. Soc. 89, 355–358.

Gentry, A.H., Dodson, C.H., 1987. Diversity and biogeography of neotropical vascular epiphytes. Ann. Mo. Bot. Gard. 74, 205–233.

Gola, E., 2008. Reproductive strategies of *Huperzia*. In: Szczesniak, E., Gola, E. (Eds.), Club mosses, horsetails and ferns in Poland. Polish Botanical Society & Institute of Plant Biology, University of Wroclaw, Wroclaw, pp. 5–14.

Goodger, J.Q.D., Whincup, A.L., Field, A.R., Holtum, J.A.M., Woodrow, I.E., 2008. Variation in huperzine A and B in Australasian *Huperzia* species. Biochem. Syst. Ecol. 36, 612–618.

Hackney, F.M.V., 1950. A review of contribution to the knowledge of *Phylloglossum drummondii* Kunze. Proc. Roy. Soc. New South Wales 75.

Herter, G., 1949a. Systema Lycopodiorum. Revista Sudamericana de botánica 8, 67–86.

Herter, G., 1949b. Index Lycopodiorum. Herbarium Herter, Montevideo, Uruguay.

Herter, G., 1950. Systema Lycopodiorum. Revista Sudamericana de botánica 8, 93–116.

Hill, J.B., 1914. The anatomy of six epiphytic species of *Lycopodium*. Bot. Gaz. 58, 61–85.

Holloway, J.E., 1917. Studies of the New Zealand species of the genus *Lycopodium*: Part II Methods of vegetative reproduction. Trans. New Zealand Inst. 49, 80–93.

Holloway, J.E., 1935. The gametophyte of *Phylloglossum drummondii*. Ann. Bot. 49, 513–519.

Holub, J., 1964. *Lycopodiella*, novy rod radu Lycopodiales. Preslia 36, 16–22.

Holub, J., 1975. *Diphasiastrum*, a new genus in Lycopodiaceae. Preslia 36, 16–22.

Holub, J., 1983. Validation of generic names in Lycopodiaceae: with a description of a new genus *Pseudolycopodiella*. Folia Geobotanica et Phytotaxonomica 18, 439–442.

Holub, J., 1985a. Transfers of *Lycopodium* species to *Huperzia* – with a note on generic classification in Huperziaceae. Folia Geobot. Phytotx. 20, 67–80.

Holub, J., 1985b. Transfers of *Lycopodium* species to *Huperzia*: with a note on generic classification in Huperziaceae. Folia Geobot. Phytotx. 20, 67–80.

Holub, J., 1991a. Some taxonomic changes within Lycopodiales. Folia Geobot. Phytotx. 26, 80–93.

Holub, J., 1991b. Some taxonomic changes within the Lycopodiales. Folia Geobot. Phytotx. 26, 81–94.

Huang, J., Chengzhong, H., 2010. Population structure and genetic diversity of *Huperzia serrata* (Huperziaceae) based on amplified fragment length polymorphism (AFLP) markers. Biochem. Syst. Ecol. 38, 1137–1147.

Huelsenbeck, J.L., Ronquist, F., 2001. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17, 745–755.

Imaichi, R., 2008. Meristem organisation and organ diversity. In: Ranker, T.A., Haufler, C.H. (Eds.), Biology and Evolution of Ferns and Lycophytes. Cambridge University Press, Cambridge, pp. 75–103.

Imaichi, R., Hiratsuka, R., 2007. Evolution of shoot apical meristem structures in vascular plants with respect to plasmodesmatal network. Am. J. Bot. 94, 1911–1921.

Ji, S.-G., Hou, K.-K., Wang, J., Pan, S.-L., 2008. A molecular phylogenetic study of Huperziaceae based on chloroplast *rbc*L and psbA-trnH sequences. J. Syst. Evol. 46, 213–219.

Jones, C.E., 1905. The morphology and anatomy of the stem of the genus *Lycopodium*. Trans. Linnaean Soc. Lond., ser 2 7, 15–36.

Katoh, K., Misawa, K., Kuma, K., Miyata, T., 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res. 30, 3059–3066.

Kenrick, P., Crane, P.R., 1997. The Origin and Early Diversification of Land Plants: A Cladistic Study. Smithsonian Institute, Washington.

Kenrick, P., Davis, P., 2004. Fossil Plants. Smithsonian Books, Washington.
Kress, W.J., Wurdack, K.J., Zimmer, E.A., Weigt, L.A., Janzen, D.H., 2005. Use of DNA barcodes to identify flowering plants. Proc. Natl. Acad. Sci. U.S.A. 102, 8369–8374.
Lewis, P.O., 2001. A likelihood approach to estimating phylogeny from discrete morphological character data. Syst. Biol. 50, 913–925.
Lim, W.H., Goodger, J.Q.D., Field, A.R., Holtum, J.A.M., Woodrow, I.E., 2009. Australasian *Huperzia* as potential sources of Huperzine A and B. Planta Medica 75, 1081-1081.
Lim, W.H., Goodger, J.Q.D., Field, A.R., Holtum, J.A.M., Woodrow, I.E., 2010. Huperzine alkaloids from Australasian and southeast Asian *Huperzia*. Pharm. Biol. 48, 1073–1078.
Ma, X.-Q., Jiang, S.-H., Zhu, D.-Y., 1998. Alkaloid patterns in *Huperzia* and some related genera of *Lycopodiaceae Sensu Lato* occuring in CHina and their contribution to classification. Biochem. Syst. Ecol. 26, 723–728.
Maddison, D.R., Maddison, W.P., 2008. Macclade 4: Analysis of Phylogeny and Character Evolution Version 4.08a. Sinauer Associates, Sunderland, Massachusetts.
Miller, M.A., Schwartz, T., Pickett, B.E., He, S., Klem, E.B., Scheuermann, R.H., Passarotti, M., Kaufman, S., O'Leary, M.A., 2015. A RESTful API for access to phylogenetic tools via the CIPRES science gateway. Evol. Bioinform. 11, 43–48.
Nessel, H., 1939. Die Bärlappgewächse. G.Fischer, Jena.
Øllgaard, B., 1975. Studies in the Lycopodiaceae I. Observations of the structure of the sporangium wall. Am. Fern J. 65, 19–27.
Øllgaard, B., 1979a. Studies in Lycopodiaceae 2. Branching patterns and infrageneric groups of *Lycopodium sensu lato*. Am. Fern J. 69, 49–61.
Øllgaard, B., 1979b. Studies in the Lycopodiaceae II. The branching patterns and infrageneric groups of *Lycopodium sensu lato*. Am. Fern J. 69, 49–61.
Øllgaard, B., 1980. Problems of classification in Lycopodiaceae. Biol. J. Linn. Soc. 14, 451-451.
Øllgaard, B., 1987. A revised classification of the Lycopodiaceae s.l. Oper. Bot. 92, 153–178.
Øllgaard, B., 1989. New taxa and combinations of Venezuelan Lycopodiaceae. Am. Fern J. 79, 151–154.
Øllgaard, B., 1989a. Index of the Lycopodiaceae volume 34 of Biologiske skrifter. Roy. Dan. Acad. Sci. Lett.
Øllgaard, B., 1992a. Neotropical Lycopodiaceae – an overview. Ann. Missouri Bot. Gard. 79, 687–717.
Øllgaard, B., 2012. New combinations in neotropical Lycopodiaceae. Phytotaxa 57, 10–22.
Øllgaard, B., 2015. Six new species and some nomenclatural changes in neotropical Lycopodiaceae. Nordic J. Bot. 33, 186–196.
QIAGEN, 2006. DNEasy Plant Mini Kit DNA extraction protocol. QIAGEN.
Rickards, R.B., 2000. The age of the earliest club mosses: the Silurian *Baragwanathia* flora in Victoria, Australia. Geol. Mag. 137, 207–209.
Rothmaler, W., 1944. Pteridophyten-Studien. Feddes Repert. 54, 55–82.
Ruggiero, M.A., Gordon, D.P., Orrell, T.M., Bailly, N., Bourgoin, T., Brusca, R.C., Cavalier-Smith, T., Guiry, M.D., Kirk, P.M., 2015. A higher level classification of all living organisms. PLoS One 10, 60.
Salino, A., Mota, N.F.D., Almeida, T.E., 2013. Lycophytes and monilophytes in Rio Preto State Park, Minas Gerais, Brazil. Acta Bot. Bras. 27, 252–263.
Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30, 1312–1313.
Taberlet, P., Gielly, L., Pautou, G., Bouvet, J., 1991. Universal primers for amplification of 3 noncoding regions of chloroplast DNA. Plant Mol. Biol. 17, 1105–1109.
Tindale, M.D., Roy, S.K., 2002. A cytotaxonomic survey of the Pteridophyta of Australia. Aust. Syst. Bot. 15, 839–937.
Troll, W., 1937. Vergleichende Morphologie der höhren Pflanzen. Gebröder Borntraeger, Berlin.
Wagner, F.S., 1992. Cytological problems in *Lycopodium sens. lat*. Ann. Missouri Bot. Gard. 79, 718–729.
Wagner, W.H., Beitel, J.M., 1992. Generic classification of modern North American Lycopodiaceae. Ann. Missouri Bot. Gard. 79, 676–686.
Watkins, J.E., Cardelús, C.L., 2012. Ferns in an angiosperm world: cretaceous radiation into the epiphytic niche and diversification on the forest floor. Int. J. Plant Sci. 173, 695–710.
Watkins, J.E., Mack, M.K., Mulkey, S.S., 2007. Gametophyte ecology and demography of epiphytic and terrestrial tropical ferns. Am. J. Bot. 94, 701–708.
White, M.E., 1986. The Greening of Gondwana. Reed, Sydney.
Whittier, D.P., 1998. Germination of spores of the Lycopodiaceae in axenic culture. Am. Fern J. 88, 106–113.
Whittier, D.P., 2006. Gametophytes of four tropical, terrestrial *Huperzia* species (Lycopodiaceae). Am. Fern J. 96, 54–61.
Whittier, D.P., Braggins, J.E., 1992. The young gametophyte of *Phylloglossum* (Lycopodiaceae). Ann. Missouri Bot. Gard. 79, 730–736.
Whittier, D.P., Braggins, J.E., 2000. Observations on the mature gametophyte of *Phylloglossum* (Lycopodiaceae). Am. J. Bot. 87, 920–924.
Whittier, D.P., Storchova, H., 2007. The gametophyte of *Huperzia selago* in culture. Am. Fern J. 97, 149–154.
Wikström, N., 2001. Diversification and relationships of extant homosporous Lycopods. Am. Fern J. 91, 150–165.
Wikström, N., Kenrick, P., 1997. Phylogeny of Lycopodiaceae and the relationships of *Phylloglossum drummondii* Kunze based on *rbc*L sequences. Int. J. Plant Sci. 158, 862–871.
Wikström, N., Kenrick, P., 2000. Phylogeny of epiphytic *Huperzia* (Lycopodiaceae): paleotropical and neotropical clades corroborated by *rbc*L sequences. Nordic J. Bot. 20, 165–171.
Wikström, N., Kenrick, P., 2001. Evolution of Lycopodiaceae (Lycopsida): estimating divergence times from *rbc*L gene sequences by use of nonparametic rate smoothing. Mol. Phylogenet. Evol. 19, 177–186.
Wikström, N., Kenrick, P., Chase, M., 1999. Epiphytism and terrestrialization in tropical *Huperzia* (Lycopodiaceae). Plant Syst. Evol. 218, 221–243.
Wilce, J.H., 1972. Lycopod spores, 1. General spore patterns and the generic segregates of *Lycopodium*. Am. Fern J. 52, 65–79.
Wolf, P.G., Karol, K.G., Mandoli, D.F., Kuehl, J., Arumuganathan, K., Ellis, M.W., Mishler, B.D., Kelch, D.G., Olmstead, R.G., Boore, J.L., 2005. The first complete chloroplast genome sequence of a lycophyte, *Huperzia lucidula* (Lycopodiaceae). Gene 350, 117–128.
Yatsentyuk, S., Valiejo-Roman, K.M., Samigullin, T.H., Wikstrom, N., Troitsky, A.V., 2001. Evolution of Lycopodiaceae inferred from spacer sequencing of chloroplast rRNA geners. Russ. J. Genet. 37, 1274–1280.
Zhang, L.-B., 2004. Huperziaceae. In: Wu, C.-Y. (Ed.), Flora Reipublicae Popularis Sinicae. Science Press, Beijing, pp. 1–54.
Zhang, L.-B., Kung, H.S., 1998. A taxonomic study of Huperzia Berhn. (s. str.) sect. Huperzia in China. Acta Phytotax. Sinica 36, 521–529.
Zhang, L.-B., Kung, H.S., 1999. On the taxonomy of Phlegmariurus (Herter) Holub (Huperziaceae) sect. Huperzioides H. S. Kung et L. B. Zhang (sect. nov.) in China. Acta Phytotax. Sinica 37, 40–53.
Zhang, L.-B., Kung, H.S., 2000. Two sections of Phlegmariurus (Herter) Holub (Huperziaceae) from China. Acta Phytotax. Sinica 38, 23–29.