



Phylogenetic systematics, morphological evolution, and natural groups in neotropical *Phlegmariurus* (Lycopodiaceae)



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ARTICLE INFO

Keywords:

Clubmosses
Lycophytes
Morphology
Neotropics
Phylogenetics
Synapomorphy

ABSTRACT

The Neotropical clade of the lycophyte genus *Phlegmariurus* is comprised of an estimated 150 described species and exhibits exceptional morphological and ecological diversity. Because of their simple morphology, frequent convergent evolution, and the recentness of the group's diversification, the delimitation of species and species groups has remained challenging. Here, we present a robustly supported phylogeny of Neotropical *Phlegmariurus* based on six chloroplast markers and ca. 70% of known species, and use ancestral character state reconstruction to investigate morphological evolution in the clade, and define natural species groups. The Neotropical species of *Phlegmariurus* form a clade that also includes a small number of Afro-Madagascan species. A morphologically and ecologically variable group of species from southeastern Brazil form a monophyletic group and represent a parallel radiation to principally Andean lineages. Species groups in Neotropical *Phlegmariurus* that were previously recognized based on morphology are not monophyletic. We find support for 11 morphologically cohesive and well-supported species groups. Morphological homoplasy is common in *Phlegmariurus* and complicates infrageneric classification of the Neotropical taxa. Our results provide a useful framework for identifying species groups and understanding patterns of morphological evolution in Neotropical *Phlegmariurus*. The radiation of the Brazilian species remains poorly understood and requires further study.

1. Introduction

With an estimated 250 species, *Phlegmariurus* is by far the most species-rich genus in the clubmoss family Lycopodiaceae (PPG I, 2016). *Phlegmariurus* is widely distributed in tropical and subtropical regions across the world and is mostly closely related to the temperate genus *Huperzia* and the monotypic *Phylloglossum* of Australia and New Zealand (Wikström and Kenrick, 1997; Field et al., 2016); together, these three genera comprise Lycopodiaceae subfamily Huperzioidae (Wagner and Beitel, 1992; Øllgaard, 2015; PPG I, 2016). In addition to its remarkable species richness, *Phlegmariurus* also is characterized by morphological and ecological diversity that is unparalleled among extant lycophyte genera. *Phlegmariurus* species occupy an elevational amplitude—from sea level to at least 5000 m above sea level—that is among the broadest of any genus of vascular plants, and are prominent

both in montane forests and alpine grasslands. The numerous and disparate epiphytic, terrestrial, and rupicolous niches occupied by members of the genus apparently have driven the exceptional morphological differentiation observed in the group; *Phlegmariurus* species vary conspicuously in growth habit, size, leaf shape, phyllotaxy, and extent of fertile-sterile leaf dimorphy (Fig. 1).

The morphological variability of *Phlegmariurus* has presented a challenge to taxonomic work in the genus, from generic circumscription itself to the resolution of species complexes. Until recently, taxonomists (Holub, 1985; Øllgaard, 1987, 1989a, 1990, 1992; Øllgaard and Windisch, 1987) generally treated members of *Phlegmariurus* within a broadly construed *Huperzia* due to their close morphological similarity. However, this taxonomic scheme became inconsistent with that applied to the rest of the family—thirteen genera are now recognized among Lycopodiaceae subfamilies Lycopodioidae and

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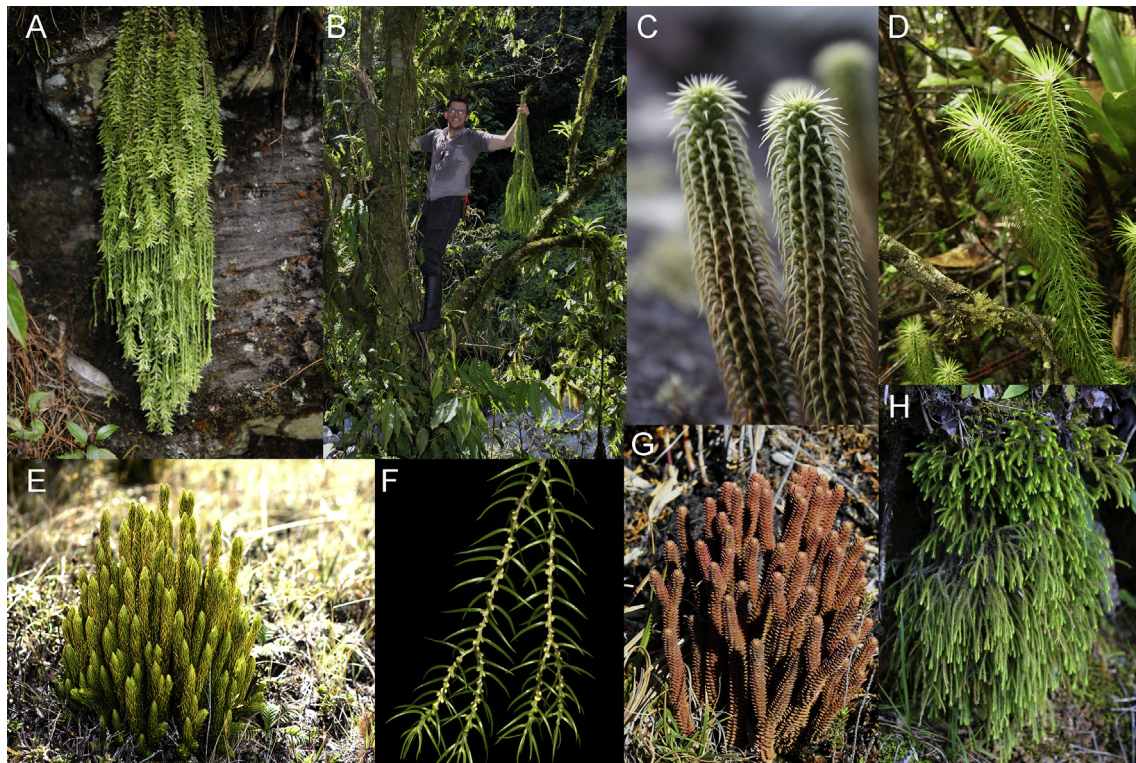


Fig. 1. Representative diversity of Neotropical *Phlegmariurus*. A. *P. myrsinites* (*Phlegmariurus aqualupianus* group), B. *P. taxifolius* (*Phlegmariurus taxifolius* group), C. *P. mooreanus* (*Phlegmariurus ruber* group), D. *P. hippurideus* (*Phlegmariurus dichotomus* group), E. *P. talamancanus* (*Phlegmariurus crassus* group), F. *P. linifolius* (*Phlegmariurus linifolius* group), G. *P. brevifolius* (*Phlegmariurus crassus* group), H. *P. acerosus* (*Phlegmariurus acerosus* group). Photos credits: A, B, D: Michael Sundue; C, F, H: Fernando Matos; E, G: Weston Testo.

Lycopodielloideae—and molecular phylogenetic studies (Wikström and Kenrick, 1997; Field et al., 2016) indicated the likely paraphyly of *Huperzia* s.l. with respect to *Phylloglossum*. Thus, the tropical members of this assemblage were transferred to *Phlegmariurus* in a series of recent papers (Øllgaard, 2012a, 2012b; Field and Bostock, 2013; Arana, 2016; Field et al., 2016).

To segregate the diversity of *Phlegmariurus* into morphologically cohesive groups, Øllgaard (1987) proposed (then within *Huperzia*) 21 species groups based on a suite of morphological characteristics, primarily pertaining to growth habit and leaf morphology. Øllgaard (1987) stressed that these species groups were informal and that the affinities of many species remained uncertain; he later (Øllgaard, 1992) dissolved one of these groups and transferred its constituent taxa into two other groups. Under this modified informal infrageneric classification scheme, 20 species groups are recognized, 11 of which occur in the Neotropics. Subsequent molecular phylogenetic studies (Wikström and Kenrick, 1997; Wikström et al., 1999; Wikström and Kenrick, 2000; Field et al., 2016) revealed the presence of two major clades in *Phlegmariurus*—one Neotropical, the other Paleotropical—thus indicating that the pantropical species groups proposed by Øllgaard were polyphyletic and that the similarity of Neotropical and Paleotropical representatives of these groups resulted from convergent evolution. Field et al. (2016) also suggested that some species groups within the Neotropical clade of *Phlegmariurus* may also not be monophyletic; they concluded that morphological convergence was “common among species that occupy similar niches.”

Morphological variability and apparently rampant convergence also represent significant impediments to species circumscription in the genus. In the Paleotropical clade, the widespread and polymorphic *Phlegmariurus phlegmaria* is the most conspicuous example of the phenomenon; this name is applied to a non-monophyletic assemblage of poorly differentiated taxa sporadically distributed from western Africa to Oceania (Field et al., 2016). Among the Neotropical species, a large number of new species—many morphologically cryptic—have been

described in the past four decades in the course of extensive study by Benjamin Øllgaard (Øllgaard, 1982, 1988, 1989b, 1993, 1995, 2003, 2015, 2016a, 2016b; Øllgaard et al., 2018). Despite these efforts, resolution of several widespread species complexes (e.g., *Phlegmariurus brevifolius*, *P. crassus*, *P. taxifolius*) remains intractable and the existence of numerous species with unusual range disjunctions (e.g., Guatemala/Mexico and northern/central Andes in both *Phlegmariurus hartwegianus* and *P. amentaceus*) suggests that additional cryptic species remain undetected. The challenges facing advancement of a cohesive taxonomy for Neotropical *Phlegmariurus* were summarized by Øllgaard (1992), who noted that “few selective forces seem to be operating on the morphological features that are used for recognition and identification. The species therefore are often difficult to define.”

In this work, we contribute to the systematics of Neotropical *Phlegmariurus* by presenting a robustly supported and densely sampled phylogeny of the clade and using that phylogeny to evaluate patterns of morphological evolution in the group and to examine standing taxonomic hypotheses. We test the monophyly of the informal species groups proposed by Øllgaard (1987, 1992), revise them when necessary, and attempt to characterize morphological synapomorphies of these groups.

2. Materials and methods

2.1. Taxon sampling

In order to focus on the phylogenetic relationships of the Neotropical clade of *Phlegmariurus*, we sampled 106 of the estimated 150 described species in the clade, including representatives of all 11 of Øllgaard’s (1987, 1992) Neotropical species groups. Outgroups were sampled from Paleotropical *Phlegmariurus* (nine species), the remaining Lycopodiaceae subfamily Huperzioidae genera *Huperzia* (three species) and *Phylloglossum* (one species), and *Lycopodium clavatum*. Voucher information and sequence accession numbers are available in

Table 1

Nucleotide substitution model parameters for each marker used, primers used for PCR and sequencing, and PCR conditions. PCR conditions include time in seconds on the top row and temperature in degrees Celsius on the bottom row of each cell.

Marker	Aligned length	% missing	Best model	Primers	PCR conditions denat./anneal/exten
<i>petG-trnP</i>	656	9%	TIM1 + I + G	GATGTAGCGCAGCTTGGTAGC (f), ATGGTTGAAGCTCTTATCCGG (r)	45/45/60 94/55/72
<i>psbA-trnH</i>	419	23%	K80 + G	GTTATGCATGAACGTAATGCTC (f), CGCGCATGGATTACAATCC (r)	45/45/60 94/48/72
<i>rbcl</i>	1417	56%	TIM1 + I + G	ATGTCACAAACGGAGACTAAAGC (f), TCAGGACTCCACTTACTAGCTTACG (r)	45/45/90 94/53/72
<i>rps4-trnS</i>	657	19%	GTR + I + G	ATGTCCCGTTATCGAGGACCTC (f), TTACCGAGGGTTCCGAATCCCTC (r)	45/45/60 94/55/72
<i>trnL</i>	510	22%	F81 + G	CGAAATCGGTAGACGCTACG (f), GGGGATAGAGGGACTTGAAC (r)	45/45/60 94/52/72
<i>trnL-trnF</i>	862	30%	GTR + I + G	GGTTCAAGTCCCTCTATCCC (f), ATTTGAAGTGGTGACACGAG (r)	45/45/60 94/55/72

Appendix A.

2.2. DNA extraction, PCR, and sequencing

Total genomic DNA was extracted from silica-dried leaf material or herbarium specimens using a standard CTAB extractions protocol (Doyle and Doyle, 1987). PCR was performed in 25 μ L mixtures of 12.5 μ L Bullseye Taq mix (MIDSCI, St. Louis), 9 μ L water, 1.25 μ L each of 10 mM forward and reverse primers, and 1 μ L of 10–20 ng \times μ L⁻¹ DNA. Six regions of chloroplast DNA (*rbcl*, *psbA-trnH*, *rps4-trnS*, *trnL*, *trnL-trnF*, and *trnP-petG*) were amplified; amplification primers and reaction conditions are provided in Table 1. PCR products were purified using shrimp alkaline phosphatase and subsequently diluted to 2 ng \times μ L⁻¹. DNA sequencing was performed in both forward and reverse directions using BigDye chemistry on an ABI 3730xl DNA analyzer at Genewiz, South Plainfield, New Jersey, USA and at Australian Genome Research Facility, University of Queensland, Australia. Sequences were assembled in Geneious 10.0.3 (Biomatters, Ltd.) and were visually inspected and manually edited as needed.

2.3. Phylogenetic analyses

Sequences for each region were aligned using the MAFFT (Katoh et al., 2002) plugin in Geneious. We used jModelTest2 (Darriba et al., 2012) to select the optimal nucleotide substitution model for each region using the corrected Akaike Information Criterion. Maximum likelihood (ML) phylogenetic analyses were performed using RAxML 8.2.10 (Stamatakis, 2006) implemented in the Cipres Science Gateway portal (Miller et al., 2010). The GTR+ Γ substitution model was employed across all partitions; 1000 ML bootstraps were performed, followed by search for the single highest-likelihood tree. Bayesian Inference (BI) of phylogeny was performed using MrBayes 3.2.6 (Ronquist and Huelsenbeck, 2003) implemented using the Cipres portal (Miller et al., 2010). Substitution models were employed per our jModelTest2 output and the MCMC analysis was performed with four chains run for 20 million generations, sampled every 5000 generations. The resulting log files were inspected for convergence and adequate sampling using Tracer 1.6 (Drummond and Rambaut, 2007); the first 10% of trees were discarded as burn-in. A majority rule consensus tree was generated from the remaining 3600 trees. Support values provided were BI posterior probabilities (PP) and ML bootstrap (BS) percentages.

2.4. Morphological analyses

To reconstruct the evolution of morphological characters in Neotropical *Phlegmariurus*, trait measurements and observations were obtained from descriptions in the literature (Øllgaard, 1988, 1995; Mickel and Smith, 2004), herbarium specimens, and observations of

plants in the field. We scored six traits that Øllgaard (1987, 1992) proposed as taxonomically informative for defining species groups: growth habit, stem thickness, stem coloration, leaf margin shape, fertile/sterile leaf dimorphism, and leaf length/width ratio. A brief description of the variation of these characters among Neotropical *Phlegmariurus* and of the character state scoring scheme used is provided here; a complete trait matrix is provided in Appendix B.

Roughly equal numbers of terrestrial and epiphytic species are known in Neotropical *Phlegmariurus*, and growth habit has been widely used to delimit major species groups in the genus. This trait was scored as binary; though we acknowledge some species occasionally exhibit both growth habits, these are exceptions and those species were scored for the growth habit they commonly exhibit. Stem thickness varies conspicuously across Neotropical *Phlegmariurus*, ranging from less than 1 mm thick in some delicate epiphytic species to more than 1 cm thick in some terrestrial taxa. We scored this trait as continuous, and used mean values of stem excluding leaves for all taxa. In some species of *Phlegmariurus*, the stems are often completely or partially reddish in color; this has been used to help characterize some species groups. We scored this trait as binary; species were scored as having reddish stems when this character state is at least commonly encountered. Although most Neotropical *Phlegmariurus* have smooth leaf margins, some species possess leaf margins that are prominently toothed; the presence of toothed leaves is a defining characteristic of Øllgaard's *P. reflexus* species group. We scored this trait as binary; species with rugose or weakly toothed leaf margins were scored as having smooth leaf margins, as these character states often intergrade within species. Although the highly-specialized strobili present in genera in the Lycopodiaceae subfamilies Lycopodioideae and Lycopodielloideae are absent in *Phlegmariurus*, some species do possess fertile leaves that differ conspicuously from their sterile leaves in size, shape, and phyllotaxis. This fertile-sterile leaf dimorphism has been used to characterize the *H. phlegmaria* species group. We scored this trait as binary, and species with weak reduction in leaf size across the length of their shoots were considered to be monomorphic. The relative length and width of leaves varies remarkably among Øllgaard's Neotropical species groups, and in some cases (e.g., the *H. brevifolia* and *H. verticillata* groups) was proposed as a primary defining characteristic. This trait was scored as continuous, and the mean values of length and width from leaves at the middle of the shoot axis were used to calculate the ratio.

Character reconstruction was carried out in R using the package 'phytools' (Revell, 2012). Discrete characters were reconstructed under a continuous-time Markov chain model (Lewis, 2001) using the "ace" function from the 'ape' package (Paradis et al., 2004) and the phytools function 'lik.anc'; continuous traits were reconstructed using the phytools functions 'fastAnc' and 'contMap'. For all reconstructions, the BI majority rule consensus tree was used; for visualization purposes, the tree was ultrametricized using the 'compute.brln' function in 'ape'.



Fig. 2. Best-scoring ML phylogeny of Neotropical *Phlegmariurus*. Support values above branches are Bayesian posterior probabilities (PP); values below branches are ML bootstrap percentages (BS). Asterisks indicate PP = 1.0 and BS = 100%; branches without support values indicate nodes with PP < 0.5 or BS < 50%. Dashed branches have branch lengths that are scaled down by a factor of 10 for visualization purposes.

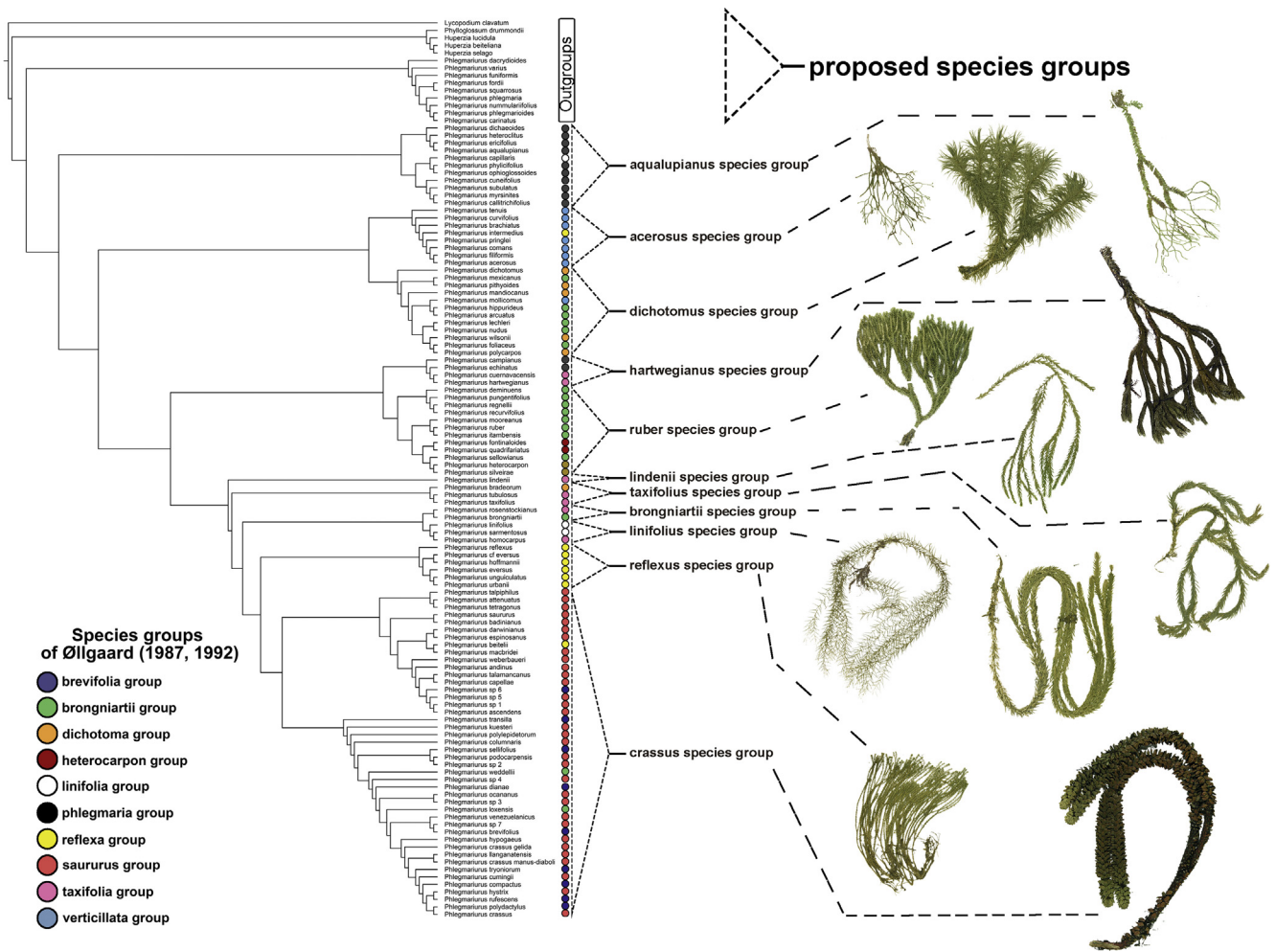


Fig. 3. Species groups described by Øllgaard (1987, 1992) and those proposed here mapped onto our best-scoring ML phylogeny of Neotropical *Phlegmariurus*.

3. Results

3.1. Phylogeny and species groups

We found *Phlegmariurus* to be strongly supported (BS 100%, PP 1.0) as monophyletic and sister to a clade comprising *Huperzia* and *Phylloglossum* (Fig. 2) with no difference recovered between ML and BI analyses. Statistical support for the position of *Phylloglossum* was moderate (BS 77%, PP 0.82). Within *Phlegmariurus*, two principal clades were recovered, one primarily consisting of Neotropical species and the other nearly entirely Paleotropical. Among Neotropical *Phlegmariurus*, we recovered eleven clades that we treat as distinct species groups (Fig. 3). Most of these clades are strongly supported (BS > 90%, PP > 0.95) and many are largely, but not entirely, consistent with Øllgaard’s species groups. Sister to the rest of Neotropical *Phlegmariurus* is a clade (the *Phlegmariurus aqualupianus* group) that includes most of the Neotropical members of Øllgaard’s *Huperzia phlegmariia* group and a single Paleotropical species, *Phlegmariurus ophioglossoides*. At the next divergence, two clades are strongly supported as sister to the remainder of the species; one of these clades corresponds to the Neotropical members of Øllgaard’s *Huperzia verticillata* group, the other includes narrow-leaved members of Øllgaard’s *Huperzia brongniartii* group intermixed with his *Huperzia dichotoma* group. We name these clades as the *Phlegmariurus acerosus* and *Phlegmariurus dichotomus* groups, respectively. Subsequently, we recover two clades as sister to the remainder of the taxa, with moderate support (BS 74%, PP 0.83). One clade is comprised of a small number of robust, thick-leaved, primarily epiphytic species; we refer to this as the *Phlegmariurus hartwegianus*

group. The other clade is comprised of a morphologically disparate assemblage of species that are mostly endemic to southeastern Brazil, the *Phlegmariurus ruber* group. At the next divergence within the tree, we recover a single taxon, *Phlegmariurus lindenii*, as sister to the remainder of the species, though support for the position of this taxon is low (BS 58%, PP 0.77). We maintain this morphologically isolated taxon as its own group—the *Phlegmariurus lindenii* group. The next clade is a small group of epiphytic species allied to *Phlegmariurus taxifolius*, which we call the *Phlegmariurus taxifolius* group. We split the next clade recovered in our analyses into two species groups: one consisting of two morphologically similar species from high-elevation forests in the Andes, the other comprised of the widespread *Phlegmariurus linifolius* and allied species. We refer to these as the *Phlegmariurus brongniartii* group and the *Phlegmariurus linifolius* group, respectively. The clade comprising these two species groups is sister to a large group of terrestrial species, in which we recover two well supported clades. One is primarily comprised of pioneer species of mid- to high-elevation exposed habitats and corresponds to Øllgaard’s *Huperzia reflexa* group; we refer to it as the *Phlegmariurus reflexus* group. The other group is by far the largest species group clade resolved in our analyses and includes a diverse assemblage of taxa that are restricted to páramos and similar alpine habitats primarily in Central America and the northern and central Andes. This clade corresponds closely to Øllgaard’s *Huperzia brevifolia* and *Huperzia saururus* groups, but also includes some species previously assigned to the *Huperzia brongniartii* and *Huperzia reflexa* groups. Though this clade is well supported as monophyletic, relationships among the many species that belong to it are not well supported and genetic differentiation among many species is weak. We

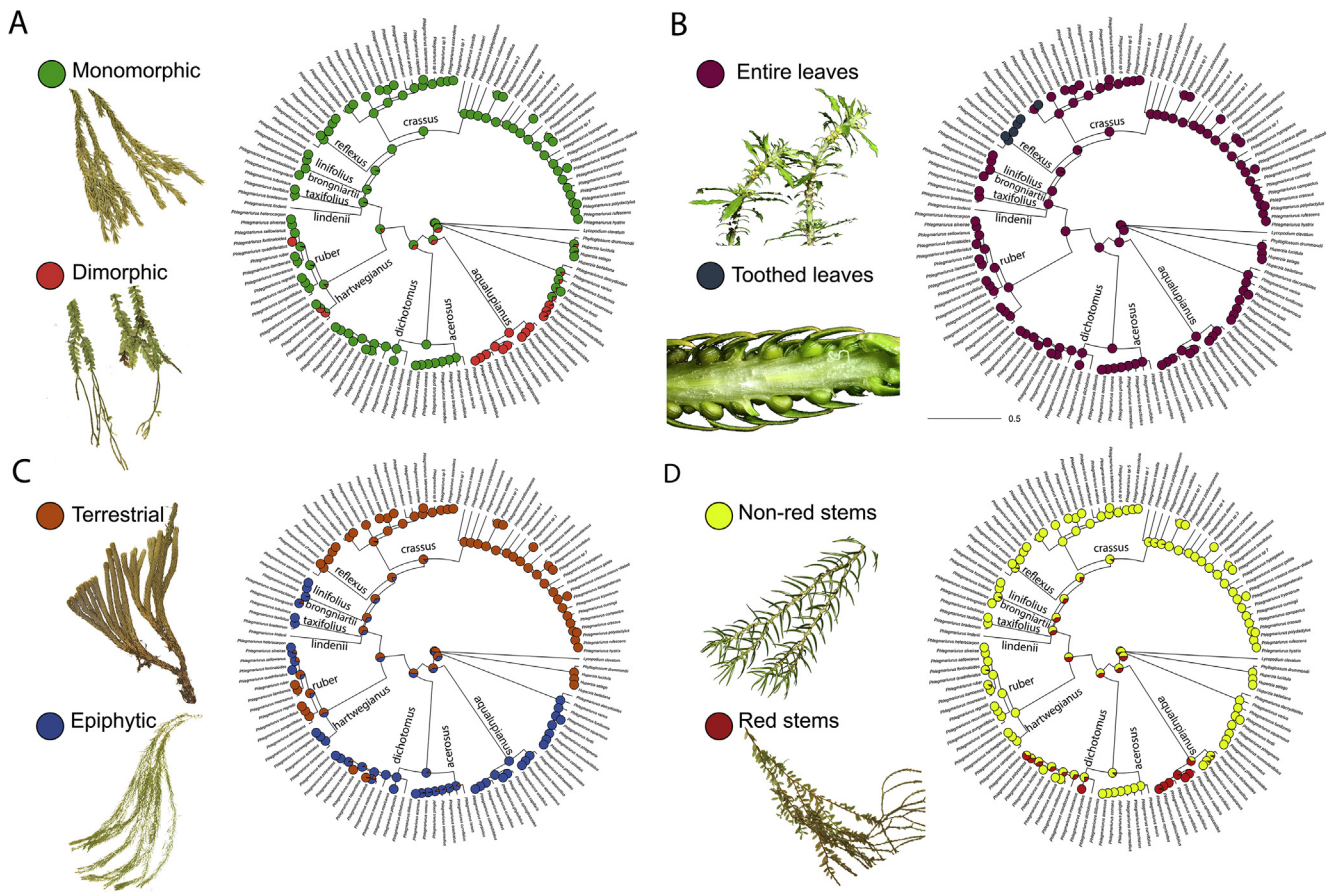


Fig. 4. Phylogenetic reconstruction of discrete morphological traits in Neotropical *Phlegmariurus*. Pie charts represent probability of each character state at the corresponding node, as inferred under maximum-likelihood using the *Mk1* model of evolution. Traits are: A. fertile-sterile leaf dimorphy, B. leaf margin toothiness, C. growth habit, D. stem coloration.

refer to this large assemblage of species as the *Phlegmariurus crassus* group, after the most widespread and polymorphic species in the group.

3.2. Morphological evolution

3.2.1. Fertile/sterile leaf dimorphy

The most probable ancestral condition of leaf dimorphy for Neotropical *Phlegmariurus* is monomorphic (Fig. 4A), with transitions to dimorphy in the *Phlegmariurus aqualupianus*, *Phlegmariurus hartwegianus*, and *Phlegmariurus ruber* groups. Several parallel transitions to dimorphy are inferred to have occurred in the Paleotropical clade of *Phlegmariurus*.

3.2.2. Leaf margin

The ancestral leaf margin condition is reconstructed as smooth, and transitions to toothed margins occurred in the *Phlegmariurus reflexus* group and within the *Phlegmariurus crassus* group (Fig. 4B).

3.2.3. Growth habit

Terrestrial growth is ancestral within Lycopodiaceae subfamily Huperzioidae, but the ancestral growth habit of both *Phlegmariurus* and for the Neotropical clade is ambiguous (Fig. 4C). Following the evolution of epiphytism in Neotropical *Phlegmariurus*, we infer that reversals to terrestrial growth have occurred several times, including at least two times each in the *Phlegmariurus dichotomus* group, once in the *Phlegmariurus acerosus* group and in the *Phlegmariurus ruber* group, and a single time in the ancestral lineage shared by the *Phlegmariurus reflexus* and *Phlegmariurus crassus* groups.

3.2.4. Stem coloration

The most probable ancestral stem coloration is green/yellow (Fig. 4D); hence, reddish stem coloration has evolved numerous times among Neotropical *Phlegmariurus*, including in the *Phlegmariurus aqualupianus* group, the *Phlegmariurus dichotomus* group, the *Phlegmariurus ruber* group, and the *Phlegmariurus crassus* group.

3.2.5. Stem thickness

Stem thickness varied considerably across Neotropical *Phlegmariurus*, but was generally stable within species groups (Fig. 5A). A dramatic reduction in stem thickness occurred in the *Phlegmariurus aqualupianus*, *Phlegmariurus acerosus*, and *Phlegmariurus linifolius* groups, and a general increase in stem thickness characterizes the *Phlegmariurus crassus* group.

3.2.6. Leaf length/width ratio

Leaf length/width ratio increased dramatically in the *Phlegmariurus dichotomus* group and to a lesser extent in the *Phlegmariurus acerosus* and *Phlegmariurus linifolius* groups (Fig. 5B). Significant reduction in leaf length/width ratio occurred in the *Phlegmariurus aqualupianus* group (especially in *P. dichaeoides* and allied species) and in the *Phlegmariurus crassus* group.

4. Discussion

4.1. Phylogenetic systematics

Our results corroborate the findings of Wikstrom and Kenrick (1997) and Field et al. (2016) regarding the phylogeny of the Lycopodiaceae subfamily Huperzioidae and are consistent with the generic

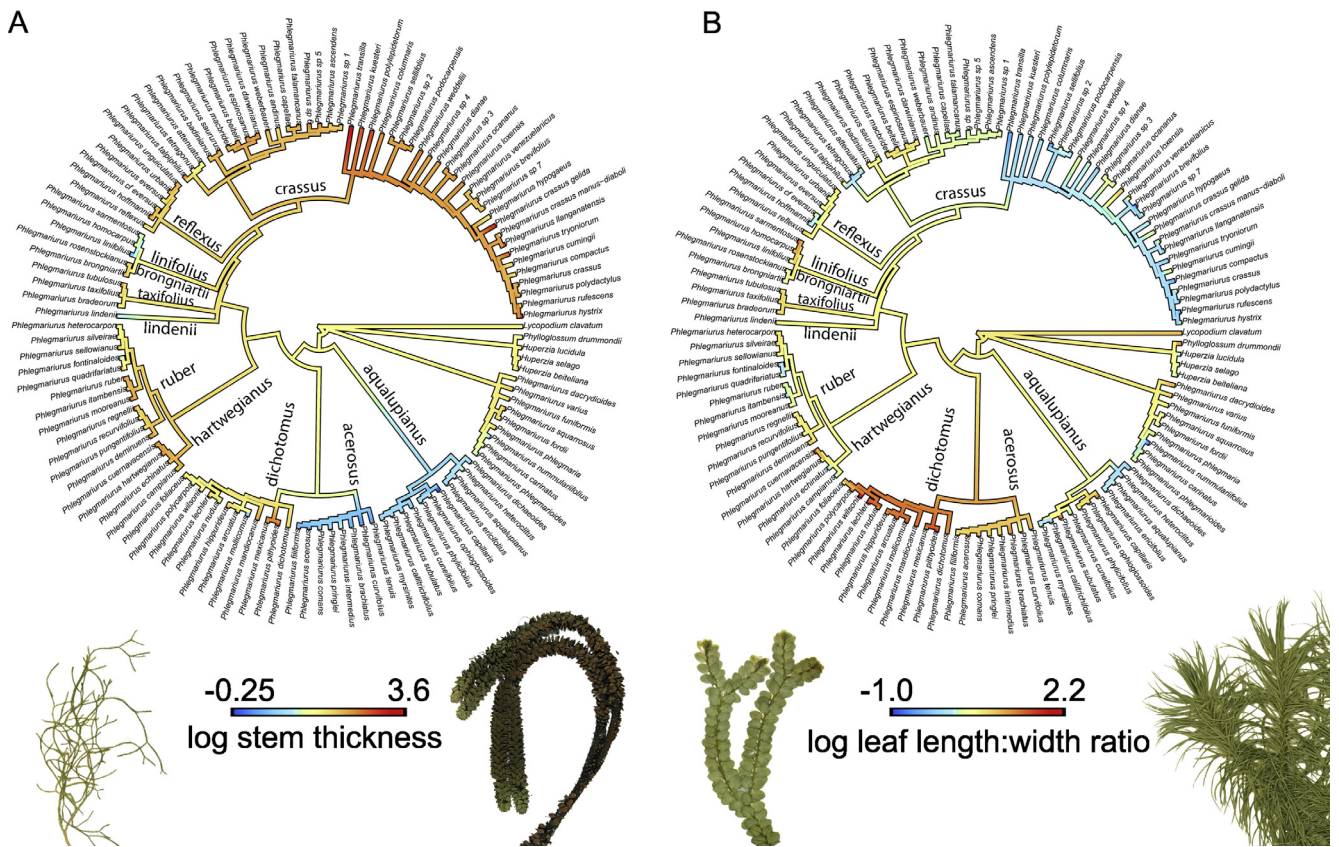


Fig. 5. Phylogenetic reconstruction of continuous morphological traits in Neotropical *Phlegmariurus* as inferred under maximum-likelihood and mapped with continuous change along branches, following log-transformation. Traits are: A. stem thickness, B. leaf length/width ratio.

taxonomic scheme used by most workers in the group in recent years (Øllgaard, 2012a,b; Field and Bostock, 2013, PPG 1, 2016). As found in earlier phylogenetic studies (Wikström and Kenrick, 1997, 2000; Field et al., 2016), our analyses indicate that *Phlegmariurus* is divided into two principal clades: one Palearctic and the other Neotropical (Fig. 2). As discussed extensively in several earlier phylogenetic studies (Wikström et al., 1999; Wikström and Kenrick, 2000; Field et al., 2016), this deep split between Palearctic and Neotropical lineages means that the existence of morphologically similar species in the Old World and New World tropics is due to dramatic convergence among species adapted to similar habitats in each region. Only a few species violate this biogeographic pattern: the widespread Neotropical epiphyte *Phlegmariurus funiformis* is a member of the Palearctic clade, the African and Malagasy species *Phlegmariurus ophioglossoides* is a member of the Neotropical *Phlegmariurus aqualupianus* group, and *Phlegmariurus saururus*—a member of *Phlegmariurus crassus* group—is known from alpine grasslands in Andean South America, South Atlantic islands, southern Africa, Madagascar, and the Mascarenes.

Our findings also provide insight into patterns of morphological evolution within the Neotropical clade of *Phlegmariurus* and allow for the morphology-based classification system of Øllgaard (1987, 1992) to be evaluated within a phylogenetic framework. Although our taxonomic sample (106/ca. 150 species) is not exhaustive and thus we are unable to comment on the affinities of some species, our analyses generally resolved major groups with strong support. Of the 11 Neotropical species groups recognized by Øllgaard (1992), we find support for recognizing several (e.g., *Phlegmariurus acerosus* and *Phlegmariurus reflexus* groups) with minimal modifications. In several other cases (e.g. *Phlegmariurus hartwegianus* and *Phlegmariurus lindenii* groups) we find support for the distinctness of lineages that Øllgaard (1987, 1992) had placed in other species groups but recognized as being at best weakly allied to those groups.

There are also several cases in which the topology of our phylogeny departs significantly from Øllgaard's species group classification. With respect to these, the non-monophyly of some of previously recognized species groups appears to reflect a major feature in the evolutionary history of Neotropical *Phlegmariurus*: adaptive radiations that occurred in parallel in the Andes and in southeastern Brazil, producing groups of evolutionarily distinct but morphologically similar taxa.

The most notable example of this pattern is our finding of a monophyletic clade that includes the *Phlegmariurus ruber* group, which together comprise an estimated 20 species (12 sampled here) that are largely endemic to southeastern Brazil (some species extend to other regions of Brazil and the Guayanas). This group is an ecologically and morphologically heterogeneous assemblage of species—it includes both pendulous epiphytes and robust terrestrial taxa—and its members were assigned to three different species groups by Øllgaard (1987, 1992) on account of this variation. This presence of a morphologically diverse, monophyletic clade of Brazilian endemics was first reported in a recent phylogenetic study of Brazilian *Phlegmariurus* by Gissi (2017) and indicates that an adaptive radiation occurred in the Atlantic forest and *campos rupestres* of southeastern Brazil, in parallel with the primary Andean radiation of the group. Further study is needed to better understand the evolutionary history of this group, especially with respect to the narrow endemism found in many species.

We also find the *Huperzia brongniartii* group to be highly polyphyletic. Øllgaard (1992) defined this group primarily by their terrestrial growth habit (in most species), linear entire leaves, and “bottle-brush-like” growth habit, but noted that the species group was variable and suggested that several species might be allied to the *Huperzia dichotoma*, *Huperzia saururus*, and *Huperzia taxifolia* groups. Our results corroborate these concerns and indicate that members of the *Huperzia brongniartii* group belong to four different clades: (1) a group of species with long, linear leaves from upper elevations forests in the Andes (e.g.,

P. hippurideus, *P. lechleri*, *P. arcuatus*) and one species from southeastern Brazil (*P. nudus*) belong to the *Phlegmariurus dichotomus* group; (2) a group of more coriaceous-leaved terrestrial species from mostly exposed montane habitats in southeastern Brazil (e.g. *P. mooreanus*, *P. itambensis*, *P. deminuens*) belong to the endemic *Phlegmariurus ruber* group; (3) members of this group from alpine habitats in the Andes (e.g., *P. loxensis*, *P. weddellii*) belong to the *P. crassus* group; and (4) *P. brongniartii* itself is allied only to *P. rosenstockianus*, the two of which form the *P. brongniartii* group as it is recognized here.

A similarly heterogeneous group in Øllgaard's classification scheme is the *Huperzia taxifolia* group, in which he includes 12 slender to robust, primarily epiphytic species. We sampled eight species from this group; only *Phlegmariurus tubulosus* (and *Phlegmariurus bradeorum*, treated by Øllgaard (1992) in the *Huperzia dichotoma* group) are closely allied to *Phlegmariurus taxifolius*. The remaining species either are allied to the *Phlegmariurus hartwegianus* group (*P. cuernavacensis*, *P. hartwegianus*), the *Phlegmariurus linifolius* group (*P. homocarpus*), or represent isolated lineages in the Neotropical (*P. lindenii*, *P. rosenstockianus*) or Paleotropical (*P. funiformis*) clades. The loose affinities of some of these taxa to *P. taxifolius* were noted by Øllgaard (1992); thus, the finding that some species, such as *P. lindenii* and *P. funiformis*, are unrelated should not be surprising. As noted by Øllgaard (1992), hybridization and polyploidy appear to have played an important role in the evolution of this group; if this is the case, additional study including data from biparentally inherited nuclear genes may improve our understanding of the relationships among these species and further alter circumscription of this group.

4.2. Circumscription of species groups

In light of the results of our phylogenetic analyses and morphological character reconstructions, we can now provide an overview of the species groups we have delimited in this study, including characterization of their morphology, general ecology, distributions, and estimated species richness. We emphasize that these groups are informal and that further study will likely result in their revision; they are described here to detail our current understanding of evolutionary relationships among Neotropical *Phlegmariurus* as working hypotheses for future study.

4.2.1. *Phlegmariurus aqualupianus* group

This species group corresponds with the *Huperzia myrsinites* and *Huperzia aqualupianus* subgroups of the *Huperzia phlegmaria* group (Øllgaard, 1992). Members of this group are characterized by their pendulous epiphytic growth habit and prominent fertile/sterile leaf dimorphism (except the monomorphic *Phlegmariurus capillaris*; Fig. 4A). Reddish stems and leaf bases appears to be a synapomorphy for the group of species closely allied to *Phlegmariurus phyllicifolius*, which corresponds to Øllgaard's *Huperzia myrsinites* subgroup (Fig. 4D). The *Phlegmariurus aqualupianus* group is widespread in the Neotropics; the Andean cordillera appears to have been an important barrier to dispersal in this group, especially in the group of species allied to *Phlegmariurus aqualupianus*. Gissi (2017) found that two Brazilian endemics, *P. bififormis* and *P. erythrocaulon*, belong in this group; the presence of these species in southeastern Brazil is likely due to diversification following a single dispersal event from the Andes. This group includes approximately 16 species.

4.2.2. *Phlegmariurus dichotomus* group

This species group includes Øllgaard's *Huperzia dichotoma* group (excluding *P. bradeorum*) and members of the *Huperzia brongniartii* group. Terrestrial species and both pendulous and erect epiphytes are known; the group is best characterized by spreading, filiform to linear leaves (Fig. 5B). Several species possess red coloration on their stem at the bases of their leaves (Fig. 4D); this character appears to have evolved multiple times in the clade. Several species in this group (e.g.,

P. dichotomus, *P. hippurideus*, and *P. wilsonii*) are very widespread, though some narrowly distributed species (e.g., *P. foliaceus*, *P. lechleri*, and *P. nudus*) exist. Perhaps a dozen species belong to this group.

4.2.3. *Phlegmariurus acerosus* group

This species group is perhaps the most morphologically uniform among Neotropical *Phlegmariurus*. Members of this group are slender to extremely delicate, with narrow shoots (Fig. 5A) and very narrow leaves (Fig. 5B) that are monomorphic or nearly so (Fig. 4A). Most species are pendulous epiphytes, although the group includes a facultative (*P. comans*) and an obligate (*P. intermedius*) terrestrial species. *Phlegmariurus intermedius* is an atypical member of this group on account of its relatively robust shoots, terrestrial growth habit, and lanceolate leaves. We suspect that this species may have arisen following hybridization between a member of this group—perhaps *P. comans*—and *P. pungentifolius* or another member of the *P. ruber* species group. *Phlegmariurus mollicomus* and *P. sarmentosus*, which were included in the *Huperzia verticillata* group by Øllgaard (1987, 1992) do not belong here. Altogether, this group comprises approximately 10 species.

4.2.4. *Phlegmariurus ruber* group

This species group is a heterogeneous assemblage of terrestrial and epiphytic taxa. *Phlegmariurus ruber* possesses brilliantly red stems and leaves; red stem coloration appears to be an autapomorphy in this case (Fig. 4D). Clear morphological synapomorphies for this group are lacking, but the species are geographically cohesive. Members of this group are endemic to southeastern Brazil, and most have small geographic ranges and occupy narrow ecological niches (Almeida et al., in prep.). Members of this group represent a significant adaptive radiation in Brazil that parallels the Andean radiation with respect to the ecological and morphological diversity displayed by the constituent taxa. Terrestrial members of this group were treated within the *Huperzia brongniartii* group by Øllgaard (1987, 1992) but as discussed previously, are not closely related to other members of that group. Additional study may result in further subdivision of this group; however, we prefer a conservative circumscription until more taxa are sampled. An estimated 20 species belong to this group.

4.2.5. *Phlegmariurus hartwegianus* group

This species group is an assemblage of robust epiphytic and terrestrial species from high-elevation habitats, with representatives distributed from Mexico to Ecuador and northern Peru. Both monomorphic and strikingly dimorphic species are included in this group; they are united by their generally large size, robust shoots (Fig. 5A), and thickly herbaceous to coriaceous leaves. Monomorphic members of this group were placed in the *Huperzia taxifolia* group by Øllgaard (1987, 1992); dimorphic species were treated as members of the *Huperzia phlegmaria* group, but were not thought to be closely related to each other. Two rare species not sampled by us (*P. pruinatus* and *P. robustus*) probably belong here. Five to seven species belong to this group.

4.2.6. *Phlegmariurus lindenii* group

The sole representative of this species group is *Phlegmariurus lindenii*, which is a pendulous epiphyte restricted to high elevation woodlands in Colombia and Ecuador. This species was tentatively placed in the *Huperzia taxifolia* group by Øllgaard (1987, 1992), who commented on its morphologically distinctness and suggested that it may be most closely related to *Phlegmariurus macgregorii*, which occurs in similar habitats in New Guinea. The affinities between these species remain untested, as we did not have material of the New Guinean plant.

4.2.7. *Phlegmariurus taxifolius* group

This species group is more narrowly circumscribed than Øllgaard's *Huperzia taxifolia* group, and excludes *P. cuernavacensis*, *P. funiformis*, *P.*

hartwegianus, *P. homocarpus*, *P. lindenii*, and *P. rosenstockianus*. Remaining in this group are *P. taxifolius*, *P. tubulosus*, and *P. bradeorum*. *Phlegmariurus bradeorum* is an erect epiphyte with narrower leaves than the other members of this group; it may represent a cross between *P. taxifolius* or a related species and a member of the *P. dichotomus* group. *Phlegmariurus taxifolius* is one of the most widespread and variable species in the genus, and many heterotypic synonyms have been published. Further study may demonstrate that some of these are sufficiently distinct to warrant recognition, though a significant portion of the observed variation appears to be in response to environmental conditions. Hybridization and polyploidy appear to be common in the *P. taxifolius* group and may have played an important role in its evolutionary history. Probable hybrids between *P. taxifolius* and *P. linifolius* were reported by Øllgaard (1988), and intermediates with *P. homocarpus* are also known. The Costa Rican endemic *Phlegmariurus oellgaardii* may be an allopolyploid derived from a cross between *P. taxifolius* and *P. tubulosus*; it is morphologically intermediate to these species and possesses large spores (Øllgaard, 1995; Rojas, 2005). Overall, species in this group are characterized by their epiphytic growth habit (Fig. 4C), monomorphic to gradually dimorphic shoots (Fig. 4A) and relatively narrow, lanceolate leaves (Fig. 5B). Two other species not sampled here (*P. sotae* and *P. killipii*) may belong here. The total number of species in this group is uncertain, perhaps six.

4.2.8. *Phlegmariurus linifolius* group

This group represents a modification of Øllgaard's (1987, 1992) *Huperzia linifolia* group, in which he placed pendulous epiphytes with narrow stems and alternate, subpetiolate, falcate-ascending leaves. We exclude *Phlegmariurus capillaris*, which Øllgaard (1992) considered close to *Phlegmariurus linifolius*; this species belongs instead to the red-stemmed clade of the *Phlegmariurus aqualupianus* group. Included in this group is *Phlegmariurus sarmentosus* (which Øllgaard (1992) considered allied to the *Huperzia verticillata* group) and *Phlegmariurus homocarpus*, a member of the *Huperzia taxifolia* group that Øllgaard (1992) noted was difficult to differentiate from some material of *P. linifolius*. *Phlegmariurus linifolius* is a widespread and very polymorphic species, with four geographically and morphologically distinct varieties currently recognized. Our sampling is insufficient to robustly evaluate the relationships among these varieties, and additional study is needed to determine the species' monophyly. With these changes in the group's circumscription considered, we define it with a modified set of the characters used by Øllgaard (1987, 1992): epiphytic growth (Fig. 4C), narrow stems (Fig. 5A), homophyllous shoots (Fig. 4A), and falcately ascending leaves. Circumscription of this group is likely to change as additional species are sampled. Species richness is at least six and likely higher.

4.2.9. *Phlegmariurus brongiartii* group

The circumscription of this group differs from the *Huperzia brongiartii* group of Øllgaard, which was rather broadly construed and included a diverse assemblage of species, nearly all of which are assigned to other groups in the present work. Here, we include two species: *Phlegmariurus brongiartii* and *Phlegmariurus rosenstockianus*, both of which are restricted to high-elevation forests in the northern and central Andes. Both species are rather robust plants with monomorphic shoots (Fig. 4A) that differ most notably by growth habit (Fig. 4C); *P. brongiartii* is a terrestrial species, whereas *P. rosenstockianus* is typically epiphytic. Morphologically intermediate specimens that may represent hybrids between these species have been reported (Øllgaard, 1988).

4.2.10. *Phlegmariurus reflexus* group

Included here are terrestrial species with spreading to reflexed, lanceolate to linear leaves (Fig. 5B) with (in most species) toothed margins (Fig. 4B). Most species are pioneer species in exposed medium- to high-elevation habitats; this habit is particularly characteristic of the most common and widespread species, *Phlegmariurus reflexus*.

Delimitation of some species is problematic, especially *P. reflexus*, which is highly variable and appears to hybridize frequently, including with species as distantly related as *P. linifolius* (Øllgaard, 1985). Sampling of *P. reflexus* across its range (W. Testo, unpublished data) indicate that this species is monophyletic, but a broader sampling of related taxa, such as *Phlegmariurus acifolius* and *Phlegmariurus sintenisii*, is needed to confirm this hypothesis. We recover *Phlegmariurus eversus* as polyphyletic, with Costa Rican specimens being allied to the Costa Rican endemic *Phlegmariurus hoffmannii* and Ecuadorean material related to *Phlegmariurus urbanii* and *Phlegmariurus unguiculatus*. The specimens of Costa Rican *P. eversus* included in this study were collected at localities where both *P. hoffmannii* and *P. reflexus* occur; they may possibly represent hybrids between these two species. Costa Rican *P. eversus* appears to be intermediate between *P. hoffmannii* and Costa Rican *P. reflexus* with respect to stem thickness (Fig. 5A), phyllotaxy, leaf margin toothiness, and stem thickness (Fig. 5B); further study is needed to test this hypothesis of a hybrid origin.

Our analyses indicate that *Phlegmariurus beitelii*, which was placed in the *Huperzia reflexa* group by Øllgaard (1987, 1992) belongs instead to the *Phlegmariurus crassus* species group. Other robust, high-elevation species treated in the *Huperzia reflexa* group by Øllgaard (1987, 1992), including *Phlegmariurus riobambensis* and *P. sieberianus*, may also belong to the *Phlegmariurus crassus* species group. *Phlegmariurus intermedius* was placed in the *Huperzia reflexa* group, but our analyses resolve it with the *Phlegmariurus acerosus* group (see discussion under that group). With these species excluded, we conservatively estimate that ten species belong to this group, though taxonomic revision may lead to more species being recognized.

4.2.11. *Phlegmariurus crassus* group

This group comprises mostly robust terrestrial species that are adapted to growth in open habitats above treeline. It is by far most diverse in the páramos of the northern Andes, though some species are found in similar habitats in Central America, Mexico, Hispaniola, and southeastern Brazil. The diversification of this group is clearly linked to the Andean orogeny, and its remarkable species richness appears to be due to its successful invasion of novel habitats that formed within the past few million years. Many species have exceedingly small ranges, reflecting the patchiness of suitable habitat in the topographically complex Andes. Since their formation, fluctuations in climate repeatedly altered the extent and connectivity of páramos (van der Hammen, 1974); this dynamic history appears to have permitted intermittent gene flow between otherwise isolated populations of species in this group. Due to this and other factors, many species appear to be weakly differentiated, and species delimitation is problematic (Øllgaard, 1988, 1992). The recentness of divergence and minimal differentiation among species in this group is reflected in the short branch lengths and low support values we recover within this clade.

Additional sequence data from more variable markers is needed to better resolve relationships among members of this group; however, we can draw some important conclusions. First, we recover two large clades with moderate support (BS 67%, PP 0.84) that are somewhat distinct in their morphology and ecology. One clade is comprised of relatively unspecialized taxa such as *P. andinus*, *P. capellae*, and *P. webbaueri* (Fig. 2). The other clade consists of species that appear more specialized for growth in exposed alpine habitats, such as *P. brevifolius*, *P. hypogaeus*, and *P. talpiphilus*. Further study is needed to better understand the morphological and ecological differences between these clades.

We do not find support for the recognition of Øllgaard's *Huperzia brevifolia* and *Huperzia saurus* groups (Fig. 3). We find that the character used to define these groups—leaf length/width ratio—varies dramatically across the *Phlegmariurus crassus* group, and species with broadly lanceolate to orbicular leaves—formerly the *Huperzia brevifolia* group—are interspersed among more narrow-leaved species in our phylogeny (Fig. 5B). The environmental factors acting on leaf shape in

this group are yet unknown, though their general growth form appears to an adaptation to intense radiation and freezing experienced in the alpine habitats they occupy (Øllgaard, 1992).

Several species in this group for which we were able to sample multiple accessions appear to be polyphyletic. The most prominent example is *Phlegmariurus crassus*, which is perhaps the most widespread and variable species in this group and includes three varieties: *P. crassus* var. *crassus*, *P. crassus* var. *gelida*, and *P. crassus* var. *manus-diaboli*. We find that none of these three varieties are closely related to each other, and that different accessions of *P. crassus* var. *crassus* do not group together, though we note that support values within the clade generally are low. While accessions from Ecuador and Costa Rica seem closely related and may be conspecific, two collections from southern Mexico belong to a different clade altogether. These plants belong to the clade of less-specialized taxa and appear allied to *P. capellae* and *P. talamancanus*. Similarly, we find that *P. brevifolius* is not monophyletic as currently circumscribed: whereas Ecuadorean material is closely allied to *P. sellifolius* and *P. rufescens*, Costa Rican material appears to be rather distantly related. Increased sampling of these and related taxa is needed to improve our understanding of species boundaries in this group; sequence data from additional, variable markers should prove particularly insightful.

Reticulate evolution in this group is poorly documented but probably common. As in most *Phlegmariurus*, hybrids between members of this group appear to have normal spores and may be at least partly fertile, making their detection difficult. Some species (e.g., *P. tryoniorum*, Øllgaard 2016b; *P. polydactylus*, Øllgaard, 1988) appear to be of hybrid origin, but these hypotheses remain untested. As has been the case in several other Lycopodiaceae genera (Wagner et al., 1985; Stoor et al., 1996; Aagaard et al., 2009; Hanušová et al., 2014), characterizing the history of reticulate evolution in this and other groups of *Phlegmariurus* may prove to be an important step in resolving standing taxonomic problems.

This species group is characterized by a terrestrial growth habit (Fig. 4C), thick stems (Fig. 5A), monomorphic leaves (Fig. 4A), and (in most species) entire or weakly toothed leaf margins (Fig. 4B). Two traits that have been used to help delimit species in this group, the extent of developmental shoot differentiation and presence of air-filled cavities in leaf bases, appear to be highly homoplastic, but were not scored in this study. Because species in this group are so difficult to define, the total number of species is highly uncertain, but almost certainly greater than 60.

5. Conclusions

Phlegmariurus is by far the most species-rich genus in the Lycopodiaceae, and exhibits exceptional morphological and ecological diversity, especially in the Neotropical clade. Despite extensive taxonomic study, relationships between many species and groups of species have remained uncertain, especially among high-elevation Andean taxa. This work provides a phylogenetic framework in which these relationships and patterns of morphological evolution in Neotropical *Phlegmariurus* can be better understood; we hope that the findings presented here will facilitate ongoing taxonomic work and inform future efforts towards a robust and stable infrageneric classification of *Phlegmariurus*. Our results largely corroborate the standing hypotheses about species groups made by Øllgaard (1987, 1992) but also provide new insights into evolutionary history of Neotropical *Phlegmariurus*, including the documentation of parallel adaptive radiations in the Andes and southeastern Brazil, and evidence suggesting the occurrence of hybridization between morphologically disparate, distantly related species. This study also highlights the need for additional research to improve resolution of species relationships—especially in the *Phlegmariurus crassus* group—and examine the importance of reticulate evolution as a driver of diversification in the genus. Finally, although only few species in our study were included with several accessions, our

finding of several polyphyletic species adds to the growing body of evidence that species diversity within the genus may be considerably underestimated due to the limited number of morphological traits as well as convergence (Øllgaard, 1992; Field et al., 2016).

Declaration of interests

None.

Acknowledgements

Michael Sundue, Bianca Canestraro, Alexandre Salino, Fernando Matos, Dirk Karger, Ivan Jimenez, and Carl Rothfels collected material that was important to completing this work. Ingi Agnarsson, Cathy Paris, and Jill Preston provided helpful comments on an earlier draft of this manuscript. W.T. is thankful to Art Gilman for helpful discussions about the Lycopodiaceae.

A. Voucher information and sequence accession numbers

Data are formatted as follows: taxon, voucher, trnP-petG, psbA-trnH, rbcL, rps4, trnL, trnL-trnF.

Huperzia beiteliana, Testo 847 (VT), MG560280, MG560384, MG560492, MG560556, MG560642, MG560705; *Huperzia lucidula*, Renzaglia 3200 (UC), NC006861, NC006861, NC006861, NC006861, NC006861, NC006861; *Huperzia selago*, Wikström 36 (S), *, DQ464210, AB574636, *, AJ224592, AJ224592; *Lycopodium clavatum*, Matos 2462 (MEXU), *, KT749941, AB574626, *, *, *, *Phlegmariurus acerosus*, Testo 664 (VT), MG560291, MG560393, *, MG560563, *, MG560715; *Phlegmariurus andinus*, Renvoize 5012 (GH), MG560292, MG560394, *, MG560564, MG560648, MG560716; *Phlegmariurus aqualupianus*, Sundue 3302 (NY), MG560293, MG560395, *, MG560565, MG560649, MG560717; *Phlegmariurus arcuatus*, Øllgaard 8592 (AAU), *, *, *, *, *, MG560718; *Phlegmariurus ascendens*, Øllgaard 8600 (AAU), MG560294, MG560396, *, MG560566, MG560650, MG560719; *Phlegmariurus attenuatus*, Testo 232 (VT), *, KT749924, KT634232, *, AJ224573, AJ224573; *Phlegmariurus badinianus*, Almeida 3382 (VT), MG560301, MG560402, MG560502, MG560572, MG560654, MG560728; *Phlegmariurus beitelii*, Beitel 85176 (NY), MG560295, MG560397, *, *, *, *, *Phlegmariurus bradeorum*, Kluge 1362 (UC), MG560296, *, *, *, *, MG560720; *Phlegmariurus brevifolius*, Øllgaard 8469 (AAU), MG560297, MG560398, MG560498, MG560567, MG560651, MG560721; *Phlegmariurus brongniartii*, Øllgaard 2432 (AAU), *, *, *, *, *, MG560722; *Phlegmariurus callitrichifolius*, Holm-Nielsen 3988 (AAU), MG560298, MG560399, MG560499, MG560568, MG560652, MG560723; *Phlegmariurus campianus*, Øllgaard 74513 (AAU), *, *, *, MG560569, AJ224586, MG560724; *Phlegmariurus capellae*, Rangel et al. 11205 (US), MG560299, MG560400, MG560500, MG560570, MG560653, MG560725; *Phlegmariurus capillaris*, Testo 125 (VT), MG560300, MG560401, MG560501, MG560571, *, *, *Phlegmariurus carinatus*, Field & Field 969 (cult.), *, GU592495, DQ464229, *, *, *, *Phlegmariurus cf. brevifolius*, Testo 1033 (VT), *, *, *, *, *, MG560726; *Phlegmariurus* sp nov 1, Testo 846 (VT), MG560352, MG560468, MG560534, MG560616, MG560685, MG560727; *Phlegmariurus cf. eversus*, Testo 658 (VT), *, MG560417, *, *, *, *, *Phlegmariurus colummnaris*, Øllgaard 91000 (AAU), MG560302, MG560403, MG560503, MG560573, MG560655, MG560729; *Phlegmariurus comans*, Canestraro 841 (MBM), *, *, *, *, *, MG560730; *Phlegmariurus compactus*, Øllgaard 59554 (AAU), *, *, *, *, *, AJ224571, AJ224571; *Phlegmariurus crassus*, Testo 230 (VT), MG560303, MG560404, *, *, *, MG560731; *Phlegmariurus crassus* var. *gelida*, Øllgaard 10077 (AAU), MG560304, MG560405, *, *, MG560656, MG560732; *Phlegmariurus crassus* var. *manus-diaboli*, Øllgaard 10058 (AAU), MG560406, *, *, *, MG560657, MG560733; *Phlegmariurus cuernavacensis*, Banda 6 (MEXU), MG560305, MG560407, MG560504, MG560574, MG560658, MG560734; *Phlegmariurus cumingii*, Øllgaard 100836 (AAU), *, *, Y07930, *

AJ224578, AJ224578; *Phlegmariusus cuneifolius*, Testo 127 (VT), MG560306, MG560408, MG560505, MG560575, MG560659, MG560735; *Phlegmariusus curvifolius*, Sundue 3206 (VT), MG560307, MG560409, MG560506, MG560576, MG560660, MG560736; *Phlegmariusus dacrydioides*, Kessler 14538 (Z), MG560308, MG560410, *, MG560577, *, MG560737; *Phlegmariusus darwinianus*, Hamilton & Holligan 1243 (GH), *, MG560411, *, *, *, *; *Phlegmariusus deminuens*, Salino 15658 (VT), MG560309, MG560412, MG560507, MG560578, *, MG560738; *Phlegmariusus diana*, Testo 977 (HUA), MG560310, MG560413, MG560508, MG560579, MG560661, MG560739; *Phlegmariusus dichaeoides*, Testo 701 (VT), MG560311, MG560414, *, MG560580, *, *; *Phlegmariusus dichotomus*, Testo 1230 (VT), *, JQ663808, *, *, AJ224567, MG560740; *Phlegmariusus echinatus*, Wurdack 1707 (NY), MG560312, MG560415, *, MG560581, MG560662, MG560741; *Phlegmariusus ericifolius*, Wikström 286 (S), *, *, *, AJ224587, AJ224587; *Phlegmariusus espinosanus*, Lewis & Klitgaard 3128 (GH), MG560313, MG560416, MG560509, MG560582, MG560663, MG560742; *Phlegmariusus eversus*, Rothfels 3574 (DUKE), MG560314, MG560418, *, MG560583, *, MG560743; *Phlegmariusus filiformis*, Field & Field 1027 (BRI), MG560315, MG560419, *, MG560584, *, MG560744; *Phlegmariusus foliaceus*, Testo 1000 (PMA), *, *, *, *, MG560745; *Phlegmariusus fontinaloides*, Canestraro 703 (MBM), *, *, *, *, MG560746; *Phlegmariusus fordii*, Anon. 763058 (TNS), *, DQ464215, AB574630, *, AJ224548, AJ224548; *Phlegmariusus funiformis*, Testo 704 (VT), MG560316, MG560420, *, MG560585, *, MG560747; *Phlegmariusus hartwegianus*, Holm-Nielsen 6362 (AAU), MG560318, MG560422, *, *, *, MG560749; *Phlegmariusus hellwigii*, Sundue 3601 (VT), MG560364, MG560475, *, MG560626, *, MG560795; *Phlegmariusus heterocarpon*, Pereira 428 (VT), MG560319, MG560423, MG560510, MG560587, MG560664, MG560750; *Phlegmariusus heteroclitus*, Lehnert 1851 (VT), MG560320, MG560424, MG560511, *, MG560665, AJ224588; *Phlegmariusus hippurideus*, Testo 151 (VT), *, MG560425, MG560512, MG560588, MG560666, MG560751; *Phlegmariusus hoffmannii*, Testo 698 (VT), MG560426, *, MG560513, *, MG560667, MG560752; *Phlegmariusus homocarpus*, Testo 580 (VT), MG560321, MG560427, MG560514, MG560589, MG560668, MG560753; *Phlegmariusus hypogaeus*, Sundue 2597 (VT), MG560322, MG560428, MG560515, MG560590, MG560669, MG560754; *Phlegmariusus hystrix*, Wikström 294 (S), *, *, *, *, AJ224574, AJ224574; *Phlegmariusus intermedius*, Almeida & Field 4578 (BHCB), MG560429, *, MG560516, *, *, *, *; *Phlegmariusus itambensis*, Almeida 4315 (BHCB), MG560430, *, MG560517, *, *, *, *; *Phlegmariusus kuesteri*, Øllgaard 9568 (AAU), MG560323, MG560431, MG560518, MG560591, MG560670, MG560755; *Phlegmariusus lechleri*, Vargas 16767 (US), MG560325, MG560433, *, MG560593, *, *; *Phlegmariusus lindenii*, Sundue 2603 (VT), MG560326, MG560434, *, MG560594, *, MG560757; *Phlegmariusus linifolius* var. *linifolius*, Testo 795 (VT), MG560327, MG560435, *, MG560595, MG560671, MG560758; *Phlegmariusus linifolius* var. *tenuifolius*, Sundue 3253 (NY), MG560436, *, MG560519, *, MG560672, MG560759; *Phlegmariusus llanganatensis*, Øllgaard 38748 (AAU), MG560328, MG560437, *, MG560596, *, *; *Phlegmariusus loxensis*, Øllgaard 74255 (AAU), MG560329, MG560438, *, *, *, *; *Phlegmariusus macbridei*, Jorgensen 92770 (AAU), MG560330, MG560439, *, MG560597, MG560673, MG560760; *Phlegmariusus mandiocanus*, Tressens 6067 (NY), MG560331, MG560440, *, MG560598, *, MG560761; *Phlegmariusus mexicanus*, Daniel 9880 (MEXU), MG560332, MG560441, *, MG560599, MG560674, MG560762; *Phlegmariusus mollicomus*, Lellingner 1074 (US), *, MG560442, *, *, *, *; *Phlegmariusus mooreanus*, Almeida 4421 (CNS), *, MG560443, MG560520, *, *, *; *Phlegmariusus myrsinites*, Testo 881 (MEXU), MG560333, MG560444, MG560521, MG560600, MG560675, *; *Phlegmariusus nudus*, Almeida 3377a (VT), MG560334, MG560445, MG560522, MG560601, MG560676, MG560763; *Phlegmariusus nummulariifolius*, Sundue 3854 (VT), *, JQ663824, *, *, AJ224552, AJ224552; *Phlegmariusus ocananus*, Dorr 8657 (US), MG560335, MG560446, MG560523, MG560602, MG560677, MG560764; *Phlegmariusus phlegmaria*, Wikström 160 (S), MG560448, *, *, MG560603, *, MG560766; *Phlegmariusus phlegmarioides*, Field et al. 1005 (BRI), *, JQ663838, AJ133896, *, AJ224554, AJ224554; *Phlegmariusus phyllicifolius*, Sundue 781 (NY), MG560337, MG560449, MG560524, MG560604, MG560678, MG560767; *Phlegmariusus pithyoides*, Castillo-Hernandez 357a (MEXU), MG560339, MG560452, MG560525, MG560606, MG560679, MG560770; *Phlegmariusus podocarpensis*, Øllgaard 74255a (AAU), *, MG560453, *, *, *, MG560771; *Phlegmariusus polycarpus*, Chase 84263 (GH), MG560340, MG560454, MG560526, MG560607, *, MG560772; *Phlegmariusus polydactylus*, Øllgaard 8792 (AAU), *, *, *, *, AJ224575, AJ224575; *Phlegmariusus polylepidetorum*, Harling and Andersson 13359 (AAU), MG560341, MG560455, *, *, *, *; *Phlegmariusus pringlei*, Sundue 3454 (VT), MG560342, MG560456, MG560527, MG560608, MG560680, MG560773; *Phlegmariusus pungentifolius*, Almeida 3327 (VT), MG560343, MG560457, MG560528, MG560609, MG560681, MG560774; *Phlegmariusus quadrifariatus*, Pereira 264 (VT), MG560344, MG560458, *, MG560610, *, MG560775; *Phlegmariusus recurvifolius*, Almeida 4425 (CNS), *, MG560459, MG560529, *, *, *, *; *Phlegmariusus reflexus*, Testo 800 (VT), MG560345, MG560460, MG560530, MG560611, MG560682, MG560776; *Phlegmariusus regnellii*, Almeida 4290 (CNS), *, MG560461, MG560531, *, *, *; *Phlegmariusus rosenstockianus*, Rothfels 3548 (DUKE), MG560346, MG560462, *, MG560612, *, MG560777; *Phlegmariusus ruber*, Almeida 3333 (VT), *, MG560463, MG560532, *, *, *; *Phlegmariusus rufescens*, Holm-Nielsen 5914 (AAU), *, *, *, *, AJ224576, AJ224576; *Phlegmariusus sarmenosus*, Øllgaard 100816 (AAU), *, *, *, *, AJ224584, AJ224584; *Phlegmariusus saururus*, Arana s.n. (VT), MG560347, *, MG560533, MG560613, MG560683, MG560778; *Phlegmariusus sellifolius*, Øllgaard 2932 (AAU), MG560348, MG560464, *, *, *, MG560779; *Phlegmariusus sellowianus*, Canestraro 712 (MBM), MG560349, MG560465, *, MG560614, MG560684, MG560780; *Phlegmariusus silveirae*, Pereira 428b (VT), MG560350, MG560466, *, *, *, MG560781; *Phlegmariusus cf capellae*, Testo s.n. (HUA), MG560351, MG560467, *, MG560615, *, MG560782; *Phlegmariusus* sp nov 2, Testo 968 (HUA), MG560353, MG560469, *, MG560617, *, MG560785; *Phlegmariusus* sp nov 3, Testo 973 (HUA), MG560354, *, *, MG560618, *, MG560786; *Phlegmariusus* sp nov 4, Testo 966 (HUA), MG560355, *, *, MG560619, *, MG560787; *Phlegmariusus* sp nov 5, Testo 969 (HUA), MG560356, *, *, MG560620, *, MG560788; *Phlegmariusus* sp nov 6, Testo 967 (HUA), MG560357, *, *, *, *, MG560789; *Phlegmariusus* sp nov 7, Testo 971 (HUA), MG560358, *, *, *, *, *; *Phlegmariusus squarrosus*, Field et al. 748 (BRI), *, JQ663809, DQ464235, *, AJ224557, AJ224557; *Phlegmariusus subulatus*, Navarrete 3040 (AAU), MG560359, MG560470, MG560535, MG560621, MG560686, MG560790; *Phlegmariusus talamancanus*, Testo 171 (VT), MG560360, MG560471, MG560536, MG560622, *, MG560791; *Phlegmariusus talpiphilus*, Sundue 822 (NY), MG560361, MG560472, MG560537, MG560623, MG560687, MG560792; *Phlegmariusus taxifolius*, Matos 2468 (MEXU), MG560362, MG560473, MG560538, MG560624, MG560688, MG560793; *Phlegmariusus tenuis*, Lehnert 2311 (VT), MG560363, MG560474, MG560539, MG560625, MG560794, *; *Phlegmariusus tetragonus*, Rothfels 3512 (DUKE), MG560365, MG560476, *, MG560627, *, MG560796; *Phlegmariusus tetrastichus*, Testo s.n. (VT), MG560366, MG560477, *, MG560628, *, MG560797; *Phlegmariusus transilla*, Holm-Nielsen 28688 (AAU), MG560367, MG560478, *, *, MG560689, MG560798; *Phlegmariusus tryoniorum*, Testo 773 (VT), MG560368, MG560479, MG560540, MG560629, MG560690, MG560799; *Phlegmariusus tubulosus*, Testo 989 (PMA), MG560369, MG560480, MG560541, MG560630, MG560691, MG560800; *Phlegmariusus unguiculatus*, Rothfels 3715 (DUKE), MG560370, MG560481, MG560542, MG560631, MG560692, MG560801; *Phlegmariusus urbanii*, Øllgaard 58592 (AAU), MG560371, MG560482, MG560543, MG560632, MG560693, MG560802; *Phlegmariusus varius*, Field & Field 1043 (BRI), *, JQ663831, JQ679089, *, *, *; *Phlegmariusus venezuelanicus*, Stergios 20572 (UC), MG560372, MG560483, MG560544, MG560633, MG560694, MG560803;

Phlegmariurus verticillatus, Wikström et al. 156 (S), *, *, AJ133897, *, AJ224561, AJ224561; *Phlegmariurus weberbaueri*, Madsen 86424 (AAU), MG560373, MG560484, MG560545, MG560634, MG560695, MG560804; *Phlegmariurus weddellii*, Øllgaard 38245 (AAU), MG560374, MG560485, *, *, *, *; *Phlegmariurus wilsonii*, Testo 1159 (JAUM), MG560375, MG560486, MG560546, MG560635, MG560696, MG560805; *Phylloglossum drummondii*, Crane s.n. (S), *, *, KU295021, *, AJ224593, AJ224593;

B. Character state scoring used in ancestral character state reconstruction analyses

Data are presented as follows: taxon, growth habit (0 = terrestrial, 1 = epiphytic), stem thickness (in mm), stem coloration (0 = yellow, 1 = red), leaf margin (0 = smooth, 1 = toothed), leaf dimorphy (0 = monomorphic, 1 = dimorphic), leaf length/width ratio.

Huperzia beiteliana, 0, 2.3, 0, 0, 0, 3.8; *Huperzia lucidula*, 0, 2.3, 0, 1, 0, 3.5; *Huperzia selago*, 0, 2.0, 0, 0, 0, 4.5; *Lycopodium clavatum*, 0, 2.0, 0, 0, 1, 10.2; *Phlegmariurus acerosus*, 1, 0.6, 0, 0, 0, 16.7; *Phlegmariurus andinus*, 0, 2.5, 0, 0, 0, 4.3; *Phlegmariurus aqualupianus*, 1, 1.3, 0, 0, 1, 3.8; *Phlegmariurus arcuatus*, 0, 2.5, 0, 0, 0, 20; *Phlegmariurus ascendens*, 0, 2.5, 0, 0, 0, 3.8; *Phlegmariurus attenuatus*, 0, 1.8, 0, 1, 0, 4.4; *Phlegmariurus badinianus*, 0, 2.5, 0, 0, 0, 4.8; *Phlegmariurus beitelii*, 0, 3.5, 0, 0, 0, 7.8; *Phlegmariurus brachiatus*, 1, 0.7, 0, 0, 0, 13.8; *Phlegmariurus bradeorum*, 1, 1.8, 0, 0, 0, 7.4; *Phlegmariurus brevifolius*, 0, 3.5, 0, 0, 0, 0.8; *Phlegmariurus brongniartii*, 0, 3.3, 0, 0, 0, 3.8; *Phlegmariurus callitrichifolius*, 1, 1, 1, 0, 1, 1.6; *Phlegmariurus campianus*, 1, 3.0, 0, 0, 1, 3.6; *Phlegmariurus capellae*, 0, 2.8, 0, 0, 0, 4.0; *Phlegmariurus capillaris*, 1, 0.4, 1, 0, 0, 13.6; *Phlegmariurus carinatus*, 1, 1.7, 0, 0, 0, 4.0; *Phlegmariurus cf eversus*, 0, 2.5, 0, 1, 0, 5.3; *Phlegmariurus columnaris*, 0, 4.0, 0, 0, 0, 2.8; *Phlegmariurus comans*, 0, 0.9, 0, 0, 0, 11; *Phlegmariurus compactus*, 0, 2.5, 0, 0, 0, 1.6; *Phlegmariurus crassus*, 0, 4.0, 0, 0, 0, 4.0; *Phlegmariurus crassus gelida*, 0, 7.0, 0, 0, 0, 4.4; *Phlegmariurus crassus manus-diaboli*, 0, 2.0, 0, 0, 0, 4.5; *Phlegmariurus cuernavacensis*, 1, 4.0, 0, 0, 0, 30; *Phlegmariurus cumingii*, 0, 2.3, 0, 0, 0, 4.3; *Phlegmariurus cuneifolius*, 1, 0.8, 1, 0, 1, 3.8; *Phlegmariurus curvifolius*, 1, 0.6, 0, 0, 0, 6.0; *Phlegmariurus dacrydioides*, 1, 1.9, 0, 0, 0, 10; *Phlegmariurus darwinianus*, 0, 4.0, 0, 0, 0, 5.5; *Phlegmariurus deminuens*, 0, 4.0, 0, 0, 0, 9.6; *Phlegmariurus diana*, 0, 3.5, 0, 0, 0, 2.2; *Phlegmariurus dichaeoides*, 1, 1, 0, 0, 1, 2.1; *Phlegmariurus dichotomus*, 1, 2.5, 0, 0, 0, 14.7; *Phlegmariurus echinatus*, 1, 3.0, 0, 0, 1, 3.1; *Phlegmariurus ericifolius*, 1, 1, 0, 0, 1, 3.2; *Phlegmariurus espinosanus*, 0, 4.0, 0, 0, 0, 8.6; *Phlegmariurus eversus*, 0, 2.0, 0, 1, 0, 5.3; *Phlegmariurus filiformis*, 1, 0.8, 0, 0, 0, 4.3; *Phlegmariurus foliaceus*, 1, 3.0, 1, 0, 0, 4.6; *Phlegmariurus fontinaloides*, 1, 0.9, 1, 0, 1, 2.0; *Phlegmariurus fordii*, 1, 1.9, 0, 0, 0, 5.0; *Phlegmariurus funiformis*, 1, 2.3, 0, 0, 0, 6.4; *Phlegmariurus hartwegianus*, 1, 4.0, 0, 0, 0, 5.7; *Phlegmariurus heterocarpon*, 1, 1.8, 0, 0, 0, 8.6; *Phlegmariurus heteroclitus*, 1, 1.4, 1, 0, 1, 2.7; *Phlegmariurus hippurideus*, 0, 3.3, 0, 0, 0, 13.6; *Phlegmariurus hoffmannii*, 0, 4.0, 0, 1, 0, 2.0; *Phlegmariurus homocarpus*, 1, 1.8, 0, 0, 0, 9.6; *Phlegmariurus hypogaeus*, 0, 2.3, 1, 0, 0, 3.3; *Phlegmariurus hystrix*, 0, 7.5, 0, 0, 0, 4.3; *Phlegmariurus intermedius*, 0, 1.3, 0, 0, 0, 8.3; *Phlegmariurus itambensis*, 0, 2.5, 0, 0, 0, 2.3; *Phlegmariurus kuesteri*, 0, 5.5, 0, 0, 0, 2.3; *Phlegmariurus lechleri*, 0, 3.0, 0, 0, 0, 25.4; *Phlegmariurus lindenii*, 1, 1.3, 0, 0, 0, 5.9; *Phlegmariurus linifolius*, 1, 0.8, 0, 0, 0, 5.4; *Phlegmariurus llanganatensis*, 0, 7.0, 0, 0, 0, 1.8; *Phlegmariurus loxensis*, 0, 4.0, 0, 0, 0, 8.4; *Phlegmariurus macbridei*, 0, 3.0, 0, 0, 0, 7.1; *Phlegmariurus mandiocanus*, 1, 3.5, 1, 0, 0, 17.9; *Phlegmariurus mexicanus*, 1, 3.8, 1, 0, 0, 13.5; *Phlegmariurus mollicomus*, 1, 0.8, 0, 0, 0, 20; *Phlegmariurus mooreanus*, 0, 4.3, 0, 0, 0, 5.0; *Phlegmariurus myrsinites*, 1, 1, 0, 0, 1, 3.8; *Phlegmariurus nudus*, 0, 1.7, 0, 0, 0, 13.6; *Phlegmariurus nummularifolius*, 1, 1.2, 0, 0, 1, 1.2; *Phlegmariurus ocananus*, 0, 4.0, 0, 0, 0, 4.0; *Phlegmariurus ophioglossoides*, 1, 1.5, 0, 0, 1, 10; *Phlegmariurus phlegmaria*, 1, 2.5, 0, 0, 1, 7.0; *Phlegmariurus phlegmarioides*, 1, 1.3, 0, 0, 1, 4.5; *Phlegmariurus phyllicifolius*, 1, 1, 1, 0, 1, 4.6; *Phlegmariurus pthyoides*, 1, 5.0, 1, 0, 0, 28.9;

Phlegmariurus podocarpensis, 0, 3.5, 0, 0, 0, 3.1; *Phlegmariurus polycarpus*, 1, 1.3, 0, 0, 0, 17.8; *Phlegmariurus polydactylus*, 0, 3.0, 1, 0, 0, 2.2; *Phlegmariurus polylepidetorum*, 0, 6.0, 0, 0, 0, 3.3; *Phlegmariurus pringlei*, 1, 1, 0, 0, 0, 8.8; *Phlegmariurus pungentifolius*, 0, 3.5, 0, 0, 0, 5.8; *Phlegmariurus quadrifariatus*, 1, 1.8, 0, 0, 1, 4.8; *Phlegmariurus recurvifolius*, 0, 2.4, 0, 0, 0, 6.5; *Phlegmariurus reflexus*, 0, 2.3, 0, 1, 0, 8.0; *Phlegmariurus regnellii*, 0, 4.0, 0, 0, 0, 6.0; *Phlegmariurus rosenstockianus*, 1, 2.5, 0, 0, 0, 4.2; *Phlegmariurus ruber*, 0, 4.0, 1, 0, 0, 6.0; *Phlegmariurus rufescens*, 0, 2.5, 0, 0, 0, 1.2; *Phlegmariurus sarmentosus*, 1, 1.3, 0, 0, 0, 20; *Phlegmariurus saurus*, 0, 4.0, 0, 0, 0, 7.3; *Phlegmariurus sellifolius*, 0, 3.5, 0, 0, 0, 1.3; *Phlegmariurus sellowianus*, 0, 3.0, 0, 0, 0, 5.5; *Phlegmariurus silveirae*, 1, 1.8, 0, 0, 0, 7.2; *Phlegmariurus* sp nov 1, 0, 3.0, 0, 0, 0, 4.8; *Phlegmariurus* sp nov 2, 0, 4.0, 0, 0, 0, 5.2; *Phlegmariurus* sp nov 3, 0, 3.5, 0, 0, 0, 4.6; *Phlegmariurus* sp nov 4, 0, 3.5, 0, 0, 0, 3.5; *Phlegmariurus* sp nov 5, 0, 4.0, 0, 0, 0, 4.0; *Phlegmariurus* sp nov 6, 0, 4.2, 0, 0, 0, 3.8; *Phlegmariurus* sp nov 7, 0, 2.8, 0, 0, 0, 4.3; *Phlegmariurus squarrosus*, 1, 3.6, 0, 0, 0, 4.3; *Phlegmariurus subulatus*, 1, 1, 1, 0, 1, 16.7; *Phlegmariurus talamancanus*, 0, 4.0, 0, 0, 0, 3.8; *Phlegmariurus talpiphilus*, 0, 4.0, 0, 0, 0, 4.7; *Phlegmariurus taxifolius*, 0, 1.8, 0, 0, 0, 8.2; *Phlegmariurus tenuis*, 0, 0.6, 0, 0, 0, 10; *Phlegmariurus tetragonus*, 0, 1.5, 0, 1, 0, 1.4; *Phlegmariurus transilla*, 0, 13.5, 1, 0, 0, 1.5; *Phlegmariurus tryoniorum*, 0, 5.0, 0, 0, 0, 2.2; *Phlegmariurus tubulosus*, 1, 4.0, 0, 0, 0, 8.0; *Phlegmariurus unguiculatus*, 0, 3.3, 0, 1, 0, 7.6; *Phlegmariurus urbanii*, 0, 3.5, 0, 1, 0, 4.7; *Phlegmariurus varius*, 1, 2.7, 0, 0, 1, 8.5; *Phlegmariurus venezuelanicus*, 0, 3.0, 0, 0, 0, 4.5; *Phlegmariurus weberbaueri*, 0, 3.0, 0, 0, 0, 3.8; *Phlegmariurus weddellii*, 0, 4.5, 0, 0, 0, 4.3; *Phlegmariurus wilsonii*, 1, 1.8, 1, 0, 0, 35.0; *Phylloglossum drummondii*, 0, 1.5, 0, 0, 1, 13.0.

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