Devonian origin and Cenozoic radiation in the clubmosses (Lycopodiaceae)

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(LYCOPODIACEAE)

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**ABSTRACT**

Together with the heterosporous lycophytes, the clubmoss family (Lycopodiaceae) is the sister lineage to all other vascular land plants. Given the family’s important position in the land-plant phylogeny, studying the evolutionary history of this group is an important step towards a better understanding of plant evolution. Despite this, little is known about the Lycopodiaceae, and a well-sampled, robust phylogeny of the group is lacking. The goal of this dissertation is to resolve the relationships among evolutionary lineages in the Lycopodiaceae and provide insight into the timing and drivers of diversification in the family.

First, to place the evolution of the family within a global and historical context, I generated a densely sampled, time-calibrated phylogeny of the family. I sampled 50% of the estimated 400 extant species in the Lycopodiaceae and used eight fossils to calibrate the age of major divergence events in the family and across the land-plant phylogeny. Further, we used a probabilistic biogeographic model to infer the historical biogeography of the family. Together, these analyses indicate that the Lycopodiaceae originated in the late Devonian, began its early diversification in the Carboniferous, and accumulated much of its extant diversity during the Cenozoic. From a geographical perspective, major cladogenesis events in the family’s history appear to be linked to the breakup of the Pangaean and Gondwanan supercontinents, with long-distance dispersal playing a role in the establishment of younger evolutionary lineages.

Second, I examined the drivers of diversification in the species-rich genus *Phlegmariurus* in the Neotropics. This clade includes an estimated 150 species and is most diverse in high-elevation habitats in the tropical Andes of South America. Using a time-calibrated phylogeny of the group and species distribution and niche data, I demonstrate a strong positive association between lineage diversification rates and the mean elevation of species’ distributions as well as a strong negative correlation between diversification rates and the size of species’ ranges. Further, we employ a paleoelevation-dependent diversification model to test for an association between the uplift of the Andes and diversification in the clade and demonstrate that speciation rates in Neotropical *Phlegmariurus* are positively associated with increasing elevations in the Andes.

Third, I use a phylogenetic framework to test the monophyly of morphology-based species groups in Neotropical *Phlegmariurus*. I demonstrate that most groups are not monophyletic, and that convergent evolution is widespread in the genus. We use ancestral character-state reconstruction methods for six morphological traits to elucidate patterns of trait evolution and to circumscribe new species groups. A total of eleven new monophyletic species groups are proposed and defined.
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CHAPTER 1: COMPREHENSIVE LITERATURE REVIEW

ANDEAN GEOGRAPHY AND OROGENY

Spanning more than 67 degrees of latitude and the entire length of the South American continent, the Andes are the most ecologically diverse and species-rich region on Earth (Mittermeier et al., 1999, Myers et al., 2000). Replete with areas of contact with the similarly diverse Amazon basin on their eastern periphery and attaining heights of 7 km, this mountain range is the second highest in the world and presents the most extensive climatic gradient of any terrestrial bioregion. Assembled along this steep temperature and precipitation gradient (Colwell et al., 2008), diverse habitat types are juxtaposed within close geographic proximity throughout the Andes, together, these habitats harbor immense biological diversity — an estimated 45,000 plant species are found in the tropical portion of the Andes; nearly half of these taxa are endemic (Myers et al., 2000). These remarkable levels of richness and endemism make the Andes an exceptional region in which to examine important evolutionary questions about processes of speciation and patterns of diversification; they have been studied extensively in the region, especially among birds (Brumfield and Edwards, 2007; Miller et al., 2008; Sedano and Burns, 2010; DuBay and Witt, 2012; Chaves et al., 2013; McGuire et al., 2014), reptiles (e.g. Schulte et al., 2000; Doan, 2003; Torres-Carvajal, 2007, 2016) and angiosperms (e.g. Emshwiller, 2002; von Hagen and Kadereit, 2003; Hughes and Eastwood, 2006; Scherson et al., 2008; Emadzade et al., 2010; Jabaily and Sytsma, 2010; Sanín et al., 2016; Diazgranados and Barber, 2017).
Most studies of the geology, geography, and biodiversity of the Andes divide the region into three latitudinal segments: the northern Andes, central Andes, and southern Andes. These regions are recognized primarily on the basis of their unique orogenic histories (Graham, 2009) and conveniently correspond roughly to political boundaries, with the northern Andes comprising Venezuela, Colombia, and Ecuador; the central Andes spanning Bolivia and Peru; and the southern Andes running through Argentina and Chile to the southern tip of the continent. The northern and central Andes together comprise a larger, climatically defined sub-region known as the tropical Andes, whereas the southern Andes are primarily temperate. Most of the region’s biodiversity, especially among vascular plants (Myers et al., 2000) is concentrated in the tropical Andes; this review will deal primarily with this region.

Discussion of the patterns of diversification underlying current Andean biodiversity and endemism must be grounded in consideration of the history of mountain-building events. The Andean orogeny exerted a powerful effect on the biogeographic history of the region’s biota by altering climate, changing habitat availability, and alternatingly isolating populations and forming new migration corridors. Recently, renewed study of the processes underlying the uplift of the Andes has improved our understanding of the timing of these events; the findings of these studies are summarized here.

It is generally understood that rise of the Andes occurred as a series of related but independent uplift events progressing in a generally south-to-north direction. Uplift of the central Andes is thought to have begun in the late Cretaceous (~85 MYA) following a series of tectonic collision and subduction events (Gregory-Wodzicki, 2000; Schellart et al., 2010). During the late Cretaceous, the oceanic Farallon Plate began subducting under
the southwestern portion of the North American plate and eventually fragmented into five smaller plates, one of which (the Nazca Plate) would continue to play an important part in the orogeny of the Andes (Sigloch and Mihalynuk, 2013). The Nazca Plate, which split from the remnants of the Farallon Plate around the Oligocene-Miocene boundary (Lonsdale, 2005) began subducting under the South American Plate, accelerating uplift in this region. Recent studies suggest that the initial period of uplift corresponds closely with these geological events, with the Western Cordillera attaining half of its current height around the time of the divergence of the Nazca and Farallon plates (~25 MYA; Gregory-Wodzicki, 2000; Graham et al., 2001; Garzione et al., 2008; Sigloch and Mihalynuk, 2013). Following this initial period of uplift, the continued subduction of the Nazca Plate resulted in accelerated rates of mountain-building in the Miocene and Pliocene, with the central Andes gaining at least 2 km in elevation during this period (Gregory-Wodzicki, 2000; Lonsdale, 2005; Sigloch and Mihalynuk, 2013) and attaining their modern elevation by the late Pliocene (Hooghiemstra and van der Hammen, 2004; Mora et al., 2010).

The orogeny of the northern Andes is more complicated than that of the central Andes and was driven by a different plate subduction event. The Caribbean Plate, a mostly oceanic plate of contested origin (Frisch et al., 1992; Meschede and Frisch, 2002; Kennan and Pindell, 2009) began to drift eastward across the Caribbean basin in the Cretaceous and started the subduction of the South American Plate, causing a series of volcanic events which resulted in the formation of the Aves Islands and the Greater and Lesser Antilles (Iturralde-Vinent and Gahagan, 2002; Garcia-Casco et al., 2008). As this subduction of the Caribbean Plate continued, mountain-building events in northwestern
South America followed, forming the northern Andes (Iturralde-Vinent and Gahagan, 2002). Numerous studies have suggested that the orogenesis of the northern Andes was recent and rapid relative to that of the central Andes; estimates by Gregory-Wodzicki (2000) for the Eastern Cordillera in Colombia indicate that this region attained half of its modern elevation 10 MYA, during the late Miocene. Rapid uplift (~600 m MY\(^{-1}\)) in this region occurred during the early Pliocene (Hooghiemstra and van der Hammen, 2004; Hoorn et al., 2010) until the mountains attained their current elevation around the Pliocene-Pleistocene boundary (2.5 MYA). In both the central and northern Andes, these relatively recent periods of rapid uplift in the Pliocene lead to the appearance of extensive alpine grassland habitats, especially in the northern Andes, about 5-3 MYA (van der Hammen et al., 1973; van der Hammen, 1974).

The uplift of the Andes shifted the climate and ecosystem composition of the South American continent dramatically; these shifts coincided with the most rapid species diversification events known (Madriñan et al., 2013). Prior to the uplift of the Andes, the South American continent was mostly flat and dominated by tropical forest comprised of taxa with Gondwanan affinities (Gentry, 1982; van der Hammen, 1974, 1989). The Andean orogenesis events of the Miocene allowed for the development of montane forests like those which currently dominate middle elevations in the Andes (Simpson, 1983; Hooghiemstra et al., 2006). At this time, mountain development also resulted in greater geographic and temporal variation in regional precipitation, with a general trend towards aridity on the western edge of the Andes and increased precipitation on the eastern face of the Andes by this period (Hartley, 2003; Houston and Hartley, 2003); additional ecoclimatic diversity was generated by later uplift, which resulted in further
elevational stratification of forest types, especially in the northern Andes (van der Hammen, 1974). Rapid elevational turnover in species composition and narrow niche spaces in these habitats are critical in maintaining the region’s unparalleled biodiversity and endemism (Mittermeier et al., 1999; Myers et al., 2000; Swenson et al., 2012). By the late Pliocene (5-3 MYA), the northern Andes had emerged above tree-line, establishing proto-páramo habitats—these expanded considerably during the following 2 MYA (Sklenár et al., 2011) and palynological evidence indicates the development of a modern páramo flora by the Pliocene-Pleistocene boundary (van der Hammen, 1974; Gómez et al., 2007). Subsequent cycles of glaciation altered elevational zonation of vegetation by as much as 2 km and causing isolated páramos to merge for periods of time, only to again be separated during interglacial periods which precipitated upward shifts in the elevational limits of the páramo (van der Hammen, 1974; Hooghiemstra et al., 2006; Rutter et al., 2012; Villota et al., 2012). These patterns of habitat expansion and contraction are thought to have had an important effect on the distribution and diversification of páramo lineages (Hewitt, 2000; Hooghiemstra et al., 2006; Giarla and Jansa, 2015; Riberio et al., 2016), though it is difficult to ascertain the precise impacts of these episodes given their recent occurrence and the uncertain boundaries of these shifts. Along with these eco-climatic factors, basic features of the geography of the Andes have influenced the biogeographic history of South American flora and fauna. The Andean uplift created an effective barrier for east-west dispersal, especially in taxonomic groups with limited dispersibility (Pirie et al., 2006; Miller et al., 2008; Smith et al., 2014; Pérez-Escobar et al., 2017) resulting in isolation of populations which had formerly been widely distributed. This isolation facilitated divergence on either side of this longitudinal barrier
and, in many cases, subsequent radiations in the unique habitats on each side of the Andean cordilleras. Conversely, the Andean uplift facilitated range expansion for some taxa, primarily along north-south habitat corridors. In particular, the formation of contiguous montane habitats by the Miocene is thought to have allowed for intra-Andean north-south migrations and provided extensive novel habitat for temperate taxa, which comprise an estimated 1/3 of extant tropical Andean diversity (Smith and Cleef, 1988; Sklenár et al., 2011).

ORIGINS OF ALPINE ANDEAN TAXA

Numerous studies have investigated the impact of this complex and dynamic geo-climatic history on the diversification and current distribution of Andean taxa. These studies utilize time-calibrated, species-level phylogenies and explicit biogeographic models to infer historical distributions and patterns of migration and have provided insight into both the geographical origin of Andean taxa and the direction of migration following colonization. Here I present the summary of a review of relevant literature in Andean plant biogeography, which is a divided into two general topics: 1) the origin of alpine Andean plant taxa and 2) patterns of intra-Andean migration following colonization. In his seminal work on Andean biogeography, Chardón (1933) presented two hypotheses for colonization of the tropical Andes: 1) long-distance dispersal from temperate regions, and 2) range expansion of lineages already present in adjacent lowland regions. Later, surveys of the taxonomic affinities of Andean plant lineages generated evidence in support for both hypotheses. Studies by Simpson (1975), Cleef (1979) and Smith and Cleef (1988) demonstrated that the affinities of most high-elevation Andean plants are to
lineages that are North or South temperate, or both. They suggested that colonization of
“tropicalalpine” habitats (sensu Smith, 1975) by temperate plant lineages occurred
primarily during the Pleistocene, when dispersal distances to newly formed Andean
alpine habitats were considerably reduced due to climate-driven range shifts among
temperate taxa. Conversely, Gentry (1982) suggested a Gondwanan origin for most South
American plant groups, with lowland taxa radiating into montane regions as they became
available.
More recently, the integration of molecular phylogenetics into biogeographic studies has
provided further insight into the origin of Andean plant lineages and has provided support
for both of Chardón’s (1933) hypotheses. I summarize these studies here, including only
those with sufficient sampling and phylogenetic resolution to securely demonstrate the
study taxa’s biogeographic history.

_Dispersal from temperate regions_

Here I present the 14 examples of prominent high-elevation plant groups in the tropical
Andean which have been demonstrated to have dispersed from temperate regions. Of
these, 10 are derived from north temperate regions whereas four originated in the south
temperate zone.
Summarizing the vascular flora of Andean páramo habitats, Cleef (1979) reported that
30% of all species were derived from North American lineages. More recently, an array
of biogeographic studies have demonstrated the prevalence of north temperate and
especially North American origins among high-elevation Andean taxa; ten of these are presented here.

In a study of the primarily north temperate genus *Erigeron* (Asteraceae), Noyes (2000) used nuclear ITS sequence data to infer the group’s global biogeography and found that the Andean representatives of the group were nested within a clade of North American species. He concluded that the Andean diversity in the genus was likely the product of a recent radiation following long-distance dispersal from North America, likely from Mexico. Similar scenarios involving dispersal by southwestern North American representatives of primarily north temperate lineages have been demonstrated for numerous groups, including: *Cerastium* (Sheen et al., 2004), *Draba* (Koch and Shehbaz, 2002), *Lupinus* (Hughes and Eastwood, 2006), *Polystichum* (McHenry and Barrington, 2014), *Astragalus* (Scherson et al., 2008), *Valeriana* (Bell and Donoghue, 2005), *Gentianella* (von Hagen and Kadereit, 2001), *Halenia* (von Hagen and Kadereit, 2003) and *Ranunculus* (Emadzade et al., 2011). Of these studies, all but that of Koch and Shehbaz (2002) include explicit estimates of dispersal times for their respective lineages, and in all but one case, the relevant dispersal event(s) occurred after the cessation of major Andean uplift and concurrent formation of alpine habitats therein during the Pliocene. Using a simple molecular clock technique to date a three-marker plastid phylogeny of the primarily Holarctic *Cerastium* (Caryophyllaceae), Sheen et al. (2004) estimated the group’s arrival in the Andes at 2.22-1.31 Ma and suggested that the recently developed Isthmus of Panama may have acted as a “stepping stone” for plants migrating from southwestern North America. Using a dated phylogeny inferred from a combined nuclear/chloroplast DNA sequence data set, Scherson et al. (2008) recovered similar
times of arrival (1.89 and 0.98ma) for two dispersal events within *Astragalus* (Fabaceae) into Andean alpine habitats, with rapid subsequent diversification in both cases. In an investigation of the rapid radiation of *Lupinus* (Fabaceae) in the Andes, Hughes and Eastwood (2006) estimated the genus’ arrival in South America from Mexico at 1.76-1.18ma. von Hagen and Kadereit (2001, 2003) utilized a molecular clock on a total evidence phylogeny of the Gentianaceaе genera *Gentianella* and *Halenia* (Gentianaceae) to determine that both genera arrived in the Andes after alpine habitats became available, inferring Pleistocene long-distance dispersal events for both genera. Multiple long-distance dispersal events from North America, Asia, and Europe during the Pleistocene are thought to underlie the diversity of the primarily North-temperate *Ranunculus* (Ranunculaceae) in the southern Andes, as shown by a robust series of analyses by Emadzade et al. (2011).

Building on the earlier work of Cleef (1979) which illustrated the prevalence of North American lineages among high-elevation Andean floras, Smith and Cleef (1988) suggested that south temperate regions—especially southern South America—may have served as even more important sources of diversity, with colonization of Andean alpine habitats occurring during the Pleistocene, after these habitats became established. Here I present five examples from recent studies which support this south temperate-Andean pattern of dispersal.

In a phylogenetic study of the primarily south temperate sedge genus *Oreobolus* (Cyperaceae) based on nuclear ITS sequence data, Chacón et al. (2006) demonstrated that the South American representatives of the genus arrived from Australia 6–5.5 MYA, during the late Miocene. Andersson et al. (2006) utilized a three-gene plastid phylogeny
of the prominent South American cushion-plant genus *Azorella* (Apiaceae) to show that the taxa dispersed northward from temperate South America into the Andes; estimates of the timing of this migration were not produced. A similar origin and pattern of migration is thought to underlie the distribution of the diverse *Calceolaria* (Calceolariaceae) in the Andes. In a pair of studies, Cosacov et al. (2009, 2010) demonstrated that *Calceolaria* migrated northward through South America from an ancestral range in the Patagonian steppe, arriving in the northern Andes approximately 11 MYA with a later divergence between Patagonian and southern Andean taxa occurring at approximately 7.7 MYA. Among the most conspicuous and charismatic constituents of the Andean páramo flora is the bromeliad genus *Puya*, which is distributed along nearly the entire Andean cordillera, with additional species in lowland Chile and páramos of Panama and Costa Rica. Jabaily and Sytsma (2010, 2013) demonstrated that the genus, which diverged from its sister lineage approximately 10 MYA (Givnish et al., 2011) originated in lowland habitats in Chile and subsequently radiated north into the Andes in the mid-Pliocene. In a series of studies on focusing on the centropogonid clade of the hyper-diverse cosmopolitan family Campanulaceae, Lagomarsino and colleagues (Lagomarsino et al., 2014; 2016) demonstrated that group’s remarkable richness in Andean montane habitats was the result of rapid diversification concurrent with the uplift of the Andes; the tropical Andean clades appear to be derived from temperate lineages from the southern Andes. A similar pattern was reported by Sanín et al. (2016) for the palm genus *Ceroxylon*, which is thought to have originated in the Central Andes and radiated northwards during the past 10 million years, with species segregating along elevation-driven climatic gradients.
Within-tropics origins

A series of recent biogeographic studies focusing on high-elevation Andean vascular plants support Chardón’s (1933) hypothesis of colonization of alpine habitats from nearby lowland tropical regions. In a study of the *Oxalis tuberosa* group (Oxalidaceae) which is broadly distributed in high-elevation regions of Central and South America, Emshwiller (2002) utilized a dataset comprised of two nuclear loci to show that the *O.* tuberosa alliance diverged from a clade of species distributed on the eastern foothills of the southern Andes. This phylogeny lacked divergence time estimates, though the author suggested that the group’s radiation likely corresponded with the south-to-north formation of alpine habitats during the Pliocene. The Rubiaceae subfamily Cinchonoideae, a widespread Neotropical clade comprising an estimated 130 species, are thought to have colonized the Andes by gradual migration from the Amazon Basin during the mid-Miocene (Antonelli et al., 2009). Using a phylogeny inferred from one nuclear and four chloroplast markers, they showed that this lineage dispersed from North America to northeastern Amazonia in the early Eocene and subsequently diversified into the Northern Andes during rapid mountain-building in that region ~15 MYA. Diazgranados and Barber (2017) recently demonstrated that the iconic páramo genus *Espeletia* underwent a recent, rapid burst of diversification in the North Andes following the lineage’s divergence from a clade comprised of the genera *Icthyothere* and *Rumfordia*, both of which are widespread in mid-elevation habitats in the Neotropics (Rauscher, 2002).
Several studies have indicated southeastern Brazil as an ancestral area for lineages that subsequently underwent rapid diversification in the high-elevation Andes. Multiple colonization events of montane habitats in the Andes from various regions of Central and South America were inferred in a recent study of the biogeography of Neotropical Gesneriaceae by Perret et al. (2013). They demonstrated that all major clades of high-elevation Andean Gesneriaceae species resulted from independent migration events from Central America, northern Amazonia, and southeastern Brazil primarily in the mid-Miocene, followed by subsequent rapid radiations in the Andes. A southeastern Brazilian origin was also inferred for three high-elevation Andean fern genera. Kreier et al. (2008) demonstrated that the epiphytic genus Serpocaulon (Kreier et al., 2008) which is widespread throughout Central and South America, with most species distributed in the tropical Andes (Smith et al., 2006) originated in montane forests of southeastern Brazil and Bolivia and subsequently radiated northward; however, divergence times were not estimated in that study. In a study of the fern genus Jamesonia, which primarily occupies paramos and other open high-elevation habitats in the Andes, Sanchez-Baracaldo (2004) demonstrated that the genus originated southeastern Brazil and subsequently radiated into the Andes.

PATTERNS OF INTRA-ANDEAN MIGRATION

In addition to elucidating the origin of prominent Andean vascular plant lineages, a central goal of many of the studies discussed herein was to determine patterns of post-colonization diversification within the Andes. A prominent biogeographic hypothesis proposed to explain observed patterns of distribution within the context of the Andean
orogeny is the south-to-north-speciation hypothesis (SNSH) used by Doan et al., (2003) in a study of the lizard genus *Proctoporus*. This hypothesis suggests that species diverged along the Andes in a northerly direction as alpine habitats became available, in concert with the south-to-north progression of mountain uplift. Within a phylogenetic context, this hypothesis indicates that within a given lineage distributed throughout the Andes, the species with the southernmost distribution would be the earliest-divergent, with more derived species being distributed consecutively to the north. Though this model may be overly simple in light of the complexity of Andean geologic history (Torres-Carvajal, 2007) and others have indicated that the original SNSH example (Andean *Proctoporus*) did not diversify in a strictly south-to-north pattern (Castoe et al., 2004; Doan and Castoe, 2005; Goicoechea, 2012) the SNSH presents a reasonable and easily tested hypothesis for the diversification of Andean biota, including vascular plants. Here, patterns of intra-Andean migration inferred from phylogenies of vascular plant clades are discussed with reference to the SNSH.

An array of studies of prominent angiosperm genera and three studies of ferns support Doan’s (2003) intra-Andean diversification hypothesis. *Gentianella* (von Hagen and Kadereit, 2001), *Halenia* (von Hagen and Kadereit, 2003), *Oxalis* (Emshwiller, 2002) and *Valeriana* (Bell and Donoghue, 2005) all followed essentially the same path, dispersing from North America to the south-central Andes in the Plio-Pleistocene and subsequently engaging in a rapid radiation into the northern Andes as new alpine habitats became available. The Andean clade of the fern genus *Polystichum* arrived earlier (mid-Miocene) than the other north temperate lineages discussed here but followed a similar pattern of diversification from the central Andes northward, as montane forest habitats became
available (McHenry and Barrington, 2014). Among genera from south temperate and tropical elements, the general pattern of south-to-north diversification is shared: after arriving in the south-central Andes during the Miocene as a result of either long distance dispersal (e.g. Oreobolus, Jamesonia/Eriosphorus) or incremental range expansion from adjacent low- to mid-elevation regions (e.g. Puya, Serpocaulon, centropogonids, Ceroxylon), these genera diversified into the central and northern Andes during the same period as north temperate taxa, which were altogether absent from the Andes for several million years after the appearance of tropical and south temperate lineages in the region. A north-to-south pattern of diversification is also known from some examples among prominent montane vascular plant groups. Hedyosmum, a genus of montane shrubs and trees in the Chloranthaceae, dispersed from the Northern to the Central Andes several times during the Pliocene and Pleistocene (Antonelli and Sanmartín, 2011). The Andes served as a corridor of suitable habitat for the grass genus Festuca, which dispersed from North America to the Northern Andes, and subsequently dispersed southward to temperate South America, where they underwent a significant radiation (Inda et al., 2008). A similar pattern was reported by Feodorova et al. (2010) for Cleome; a large clade appears to have originated in the Northern Andes and the adjacent Guiana Shield and subsequently migrated southwards into the Central and Southern Andes. Though they did not include a time-calibrated phylogeny in their study, Diazgranados and Barber (2017) demonstrated that early divergent lineages of Espeletia s.l. are distributed in páramos of northwestern Venezuela and northern Colombia, with more recently derived lineages being distributed progressively further south.
A conspicuous feature of many montane vascular plant lineages in the Andes is high species richness in comparison to extra-Andean sister groups (Luebert and Weigand, 2014). Given this remarkable richness and the young age of most Andean lineages, it is not surprising that many of these groups exhibit exceptionally high rates of species diversification. In a study that included 73 dated phylogenies, Madriñan et al. (2013) demonstrated that the mean diversification rate of páramo vascular plant lineages is higher than that obtained from any other biodiversity hotspot on Earth. Using the net diversification rate of Magallón and Sanderson (2001), they reported a mean net diversification rate of 1.36 events MY$^{-1}$ for páramo lineages, compared to a next-highest rate of 1.07 events MY$^{-1}$ for groups from the Mediterranean hotspot. Among Andean páramo lineages, mean diversification rates ranged from 0.18 events MY$^{-1}$ for _Arcytophyllum_ to 5.10 events MY$^{-1}$ for _Aragoa_. Other studies report similarly high rates of diversification; to facilitate cross-study comparison, I report all rates using the zero-extinction rate metric of Magallón and Sanderson (2001). Lagomarsino et al. (2017) reported a rate of 1.15 events MY$^{-1}$ for the centropogonid clade of the Campanulaceae; diversification rates among this clade were dramatically elevated in comparison to the rates observed in closely related clades. Even higher rates were reported for Andean _Lupinus_ (2.52 events MY$^{-1}$; Hughes and Eastwood, 2006), _Astragalus_ (2.05 events MY$^{-1}$; Scherson et al., 2008), and _Gentianella_ (1.48 events MY$^{-1}$; von Hagen and Kadereit, 2001). Though they did not report diversification rates in their study, I calculated a mean
net diversification rate of ~1.05 events MY$^{-1}$ for the fern genus *Jamesonia* from the time-calibrated phylogeny of Sanchez-Baracaldo and Thomas (2014). Sundue et al. (2015) reported a somewhat lower rate of 0.38 events MY$^{-1}$ for *Serpocaulon*, a primarily Andean genus of epiphytic ferns that primarily occupy montane forest habitats. Though estimation of diversification rates from molecular phylogenies remain contentious (Moore et al., 2016; Rabosky et al., 2017), the rate values provided for these groups are likely closer to true values than would be generally expected, as these lineages are young and do not appear to have experienced extensive extinction, which remains the single largest confounding factor in the calculation of these rates (Rabosky, 2010; Beaulieu and O’Meara, 2015). Though these values may be expected to change some as divergence time estimates are revised and methods of inferring diversification rates improve, these findings demonstrate a consistent pattern of strongly elevated diversification rates in high-elevation Andean plant groups in comparison to both related extra-Andean lineages as well as assemblages from other species-rich regions on Earth. Further study of lineage diversification dynamics of high-elevation Andean taxa should provide a more thorough understanding of the tempo of speciation in the region and how rapid diversification of many groups over the past few million years contributed to the establishment of the tropical Andes as the most diverse bioregion on Earth.

CONCLUSIONS

Along with rainforests of the Amazon basin, the Andean cordillera is the defining geographic feature of the South American continent. Moreover, the portion of the mountain range that spans the continent’s tropical latitudes comprise the most biodiverse
region on Earth, with more than 45,000 species of vascular plants and nearly 3400 vertebrate species known in an area covering approximately 1% of Earth’s land area (Myers et al., 2000). Levels of endemism range from 27–28% for birds and mammals to 44% for vascular plants and more than 50% for amphibians; these values are certainly underestimates, given the prevalence of cryptic species among many groups from the tropical Andes and adjacent regions (Giraldo et al., 2008; Funk et al., 2011; Särkinen et al., 2011; Lücking et al., 2014; Gill et al., 2016). Given this outstanding level of biodiversity across an array of taxonomic groups, the region’s complex geography, and its well-documented geologic history, the Andes are an excellent study system for examining the role that mountain-building has played in driving species proliferation.

Biogeographic studies based on dated molecular phylogenies have provided valuable insight into the history of colonization and subsequent range expansion of numerous plant lineages within the Andes. Many prominent Andean plant groups dispersed from temperate regions since the Pliocene; the success of these otherwise temperate groups in the high-elevation Andes appears to be due in part to preadaptation to cold temperature, a lack of which may have precluded many tropical taxa from lower elevations from successfully invading novel alpine habitats as they arose over the past 5 million years (Donoghue, 2008; Edwards and Donoghue, 2013). However, several tropical lineages did successfully radiate into high-elevation habitats in the Andes through gradual migration from low- and mid-elevation tropical forests; epiphytes—which are poorly represented in temperate regions—are prominent this group. Extensive intra-Andean migration following initial colonization events has been recorded for some genera, with many lineages exhibiting a northerly trajectory to their diversification, following the
cordillera’s south-to-north progression of uplift, which provided novel habitats into which these groups could diversify.

Along with resolving the biogeographic histories of high-elevation Andean plant lineages, phylogenetic studies have provided insight into the timing and tempo of their diversification. As expected given the young age of the Andean cordillera, most diversification events in these groups are recent, occurring primarily during the Pleistocene. In addition to being a relatively recent phenomenon, speciation among Andean plant groups has occurred with remarkable frequency, with the páramos of the Northern and Central Andes being the fastest-diversifying ecosystem on Earth (Madriñan et al., 2013). Although the factors responsible for the remarkable rates of diversification observed among high-elevation Andean lineages remain difficult to elucidate, evidence from several recent studies suggests that intermittent isolation of populations due to climatic fluctuations has played an important role in promoting genetic divergence and speciation. The rise of the topographically complex high Andes created a myriad of isolated alpine “sky islands” separated by regions of montane forests. Gene flow between these distant high-elevation habitats is thought to be low during interglacial periods, but their lower elevational limits were repeatedly depressed during glacial periods, increasing connectivity of these alpine habitats and allowing intermittent gene flow among populations that inhabit them. Consequences of this history of incomplete isolation include low genetic divergence and poor morphological differentiation exhibited among many páramo species (Kolář et al., 2016; Vásquez et al., 2016; Diazgranados and Barber, 2017), which pose significant challenges to molecular phylogeneticists and alpha taxonomists alike. Next-generation sequencing approaches have revealed extensive
cryptic species diversity in some clades of high-elevation Andean taxa (Guarnizo et al., 2015; Brehm et al., 2016; Gill et al., 2016); these studies suggest the true species richness of the tropical Andes is much higher than is currently thought.

The exceptional diversity of the Andes has drawn the attention of naturalists for nearly two centuries (Humboldt, 1820; Wallace, 1853; Darwin, 1909; Cuatrecasas, 1934; Gentry, 1982), and for good reason. The region’s unparalleled species richness and high levels of endemism, coupled with its geographic complexity, history of recent uplift, and subsequent climate fluctuations, make it an excellent system for studying rapid evolutionary radiations. Though the evolutionary history of the Andean flora remains poorly understood relative to better-studied biodiversity hotspots, recent phylogenetic studies have elucidated prominent patterns of diversification in the region and provide an increasingly robust framework for evaluating the drivers of exceptional diversification in this landscape. It is within this framework that researchers progress towards a more complete understanding of how the interplay of geologic, climatic, and biotic factors contributed to the establishment of the world’s “hottest” biodiversity hotspot.

**LYCOPODIACEAE IN THE ANDES**

The clubmosses (Lycopodiaceae) provide an excellent group in which to study diversification of an Andean-centered group of spore-dispersed vascular plants, providing a useful point of comparison to the numerous studies on angiosperms. Comprising an estimated 400 species, the Lycopodiaceae are one of three extant lycophyte families, along with the heterosporous lycophyte families Selaginellaceae and Isoetaceae (PPG I, 2016); these three families are sister to the euphyllophyte clade.
Though the Lycopodiaceae is distributed worldwide, the family attains its highest species richness in the Andean region. Eight Lycopodiaceae genera are represented in the Andes; by far the most diverse of these is *Phlegmariurus*, which includes approximately 120 Andean representatives. This exceptionally diverse group forms a monophyletic clade, sister to a principally paleotropical clade of *Phlegmariurus* (Field et al., 2016) and is comprised of species that are remarkably diverse ecologically and morphologically—members of this group range from sea level to at least 5000 m elevation, and include nearly even numbers of epiphytic and terrestrial species.

The exceptional diversity of Andean Lycopodiaceae and especially that of *Phlegmariurus* in the region has led researchers (Ollgaard, 1992; 1995; Field et al., 2016) to hypothesize that the group has undergone a period of recent and rapid diversification in concert with the uplift of the Andes, like that observed in groups covered in this review. The principal goal of the research presented in this dissertation seeks to test this hypothesis and provide a comprehensive understanding of the evolutionary history of the Lycopodiaceae as a whole.
LITERATURE CITED


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CHAPTER 2: DIVERGENCE TIMES AND HISTORICAL BIOGEOGRAPHY OF THE LYCOPODIACEAE

INTRODUCTION

Comprising three extant families (Isoetaceae, Lycopodiaceae, and Selaginellaceae) the lycophytes hold an important phylogenetic position sister to all other vascular plants (Wickett et al., 2014). This ancient lineage had its origins in the Silurian (Garratt et al., 1984; Rickards, 2000) and dominated Earth’s ecosystems through the Carboniferous (DiMichele et al., 2001). Though modern lycophytes’ diversity and ecological prominence pale in comparison to their Paleozoic relatives and they are today overshadowed by seed plants and ferns, understanding the evolutionary history of the group is important because it provides insight into the timing and patterns of early land plant diversification. Recent studies have examined the divergence timing and historical biogeography of the Isoetaceae (Pereira et al., 2017) and Selaginellaceae (Klaus et al., 2016; Weststrand, 2016); however, the only dated phylogeny of the Lycopodiaceae (Wikström and Kenrick, 2001) was published more than 15 years ago and did not include a biogeographical analysis.

Though the Wikström and Kenrick study was an important early application of divergence time estimation and has provided insight into the timing of lycophyte diversification, it does have significant limitations. First, fewer than 10% of described Lycopodiaceae species were included in the phylogeny. Sparse taxon sampling can lead to biased divergence time estimates (Linder et al., 2005; Renner, 2005; Duchêne et al., 2014; Zheng and Wiens, 2015), especially in the absence of abundant fossils for node
calibration, as is the case in the Lycopodiaceae. The potential effects of this limitation on the divergence time estimates of Wikström and Kenrick (2001) are likely significant, as the divergence timing analysis used in that study—penalized likelihood—is particularly sensitive to taxon sampling biases (Linder et al., 2005). Further, the phylogeny of Wikström and Kenrick (2001) was based on a single chloroplast gene, which is insufficiently variable to robustly resolve some relationships in the family and is known to have an anomalously high substitution rate in the Selaginellaceae, one of the sister groups of the Lycopodiaceae. Among-lineage substitution rate heterogeneity poses a significant challenge to divergence time estimation, especially when models that assume some sort of a molecular clock are used (Sanderson, 1997; Welch and Bromham, 2005; Drummond and Suchard, 2010). Since the Wikström and Kenrick study was published, methods that explicitly permit among-lineage rate heterogeneity have been developed (Renner, 2005; Drummond et al., 2006; Drummond and Rambaut, 2007; Drummond and Suchard, 2010); these have provided markedly different estimates of divergence times in numerous clades of organisms (Douzery et al., 2004; Bell et al., 2010; Smith et al., 2010; Magallón et al., 2015; Testo and Sundue, 2016). Given the demonstrated high rates of substitution in *Selaginella* and evidence from molecular branch lengths of similarly elevated rates in some taxa in the Lycopodiaceae itself, we expect that more sophisticated dating analyses will improve estimates of divergence times in the Lycopodiaceae.

The aim of this paper is to infer the evolutionary history of the Lycopodiaceae with improved marker and taxon sampling and more sophisticated analyses than were available to Wikström and Kenrick (2001). We increase sampling within the
Lycopodiaceae to greater than 50% of the family’s diversity, use sequence data from six chloroplast markers, and incorporate eight fossil calibrations to construct our phylogeny and infer divergence times. To understand and mitigate the effects of among-lineage rate heterogeneity on our age estimates, we carry out model comparison between two relaxed molecular clock models implemented in BEAST—an uncorrelated lognormal clock and a random local clock—and compare date estimates obtained using each method. Finally, to place the diversification of the Lycopodiaceae within the context of Earth’s complex geologic and geographic history and to infer the relative roles of vicariance and long-distance dispersal in shaping the modern distribution of the family, the historical biogeography of the family was inferred.

MATERIALS AND METHODS

Taxon sampling

Ingroup sampling included a total of 199 taxa, representing all 16 Lycopodiaceae genera (PPG I, 2016). In some cases, multiple accessions of a given named taxon were included when that species was known to be polyphyletic and comprising multiple cryptic species. Outgroups were selected from the sister families Selaginellaceae and Isoetaceae, as well as representatives of the other major land plant lineages; representatives of outgroups were selected with a focus on capturing the deepest divergences in their corresponding clade.
**DNA extraction, PCR, and sequencing**

Total genomic DNA was extracted from silica-dried leaf material or herbarium specimens using a standard CTAB extraction protocol (Doyle and Doyle, 1987). PCR was performed in 25µL mixtures of 12.5 µL Bullseye Taq mix (MIDSCI, St. Louis), 9 µL water, 1.25 µL each of 10mM forward and reverse primers, and 1 µL of 10–20 ng×µL⁻¹ DNA. Six regions of chloroplast DNA (\textit{rbcL}, \textit{psbA-trnH}, \textit{rps4-trnS}, \textit{trnL}, \textit{trnL-trnF}, and \textit{trnP-petG} IGS) 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×µL⁻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. Sequences were assembled in Geneious 10.0.3 (Biomatters, Ltd.) and were visually inspected and manually edited as needed. Specimen voucher information and GenBank accession numbers are provided in Appendix 1.

**Divergence time analyses**

Sequences for each region were aligned using the MAFFT (Katoh et al., 2002) plugin in Geneious. We used jModelTest 2 (Darriba et al., 2012) on each marker partition to identify best-fitting substitution models for downstream phylogenetic analyses (Table 1). To facilitate computation, we generated a starting tree for all divergence time estimation (DTE) analyses using a Maximum Likelihood (ML) approach implemented in RAxML 8.2.10 (Stamatakis 2006; 2014) implemented in the Cipres Science Gateway portal (Miller et al. (2010). To generate the starting tree, the GTR+Γ substitution model was
employed across all partitions following the suggestion of Stamatakis (2006), and the monophyly of seed plants, ferns, and lycophytes was enforced. The resulting best tree was retained for use in subsequent analyses.

We performed all DTE analyses in BEAST 2.4.5, using the Cipres Science Gateway portal (Miller et al. 2010). For all analyses, the matrix was partitioned by marker, and substitution models were assigned to each partition following from the results of our model test analysis. Because of a priori evidence of significant rate heterogeneity both between the Lycopodiaceae and its sister family Selaginellaceae as well as within the Lycopodiaceae itself, special care was taken to consider the effect of molecular clock models on DTE. We compared the performance of two molecular clock models: an uncorrelated lognormal clock (UCLN) model (Drummond et al., 2006), in which different rates are assigned to each branch from a lognormal distribution of values, and a random local clock (RLC) model (Drummond and Suchard, 2010), which allows for a set of distinct substitution rate regimes to be applied locally across a phylogeny. The UCLN model allows for substitution rates for each branch in the phylogeny to be drawn from a single unimodal prior distribution, whereas the RLC model works by proposing and comparing various regimes of local strict clocks; each of these clocks can arise along any branch in the phylogeny and are applied to a set of descendent branches. Evidence from both real and simulated datasets suggests that RLC models outperform UCLN models when sudden shifts in substitution rates occur across a phylogeny (Dornburg et al., 2011; Crisp et al., 2014), but it may be a poor fit in cases where rate variation is not punctuated along the phylogeny.
For all analyses, a birth-death tree prior was selected with a uniform prior from 0–10 for speciation rate and 0–1 for relative extinction rate, and with starting values of 1.0 for speciation and 0.5 for relative extinction rates. For each analysis, we performed three separate MCMC runs of 100 million generations and sampled every 40,000 generations, resulting in 2500 trees per run and 7500 trees in total. The resulting log files were inspected for convergence and adequate sampling using Tracer 1.6 (Drummond and Rambaut, 2007); effective sample sizes all exceeded 200 and the first 20% of trees were discarded as burn-in. A majority rule consensus tree was generated from the remaining 6000 trees. The fit of alternative clock models was compared by performing path sampling analyses (Baele et al., 2012; 2013) and calculating Bayes factors.

A node-dating approach incorporating eight fossils was used to calibrate the timing of divergences across the land plant phylogeny. To constrain the MRCA of land plants, we took a conservative approach and assigned a uniform distribution from 472–444 MYA, bridging the span of time between the oldest likely land plant fossils (cryptospores from the Middle Ordovician of Argentina; Rubinstein et al., 2010) and the oldest fossils of certain affinity to the embryophyte lineage (trilete spores from the Silurian in Sweden, Steemans et al., 2009). All other fossil calibrations were provided lognormal prior distributions defined by $\mu=3$ and $\sigma=1$ and with the lower bound equal to the age of the fossil. The node marking the divergence of lycophytes and euphyllophytes was constrained at a minimum age of 420 MYA based on the lycophyte *Baragwanathia longifolia*, which dates to the Upper Silurian (Garratt and Rickards, 1987; Hueber, 1992). The node marking the divergence of heterosporous and homosporous lycophytes was
constrained to a minimum age of 385 MYA, based on *Leclergia complexa*, which belongs to the stem lineage subtending Selaginellaceae and Isoetaceae (Meyer-Berthaud et al., 2003). The crown node of the Selaginellaceae was given a minimum age of 311 MYA, based on *Selaginella suissei*, which dates to the mid-Pennsylvanian and clearly belongs to the Selaginellaceae (Thomas, 1997). The euphyllophyte crown node was assigned a minimum age of 385 MYA based on *Ibyka*, dating to the Middle Devonian (Skog and Banks, 1973) which has affinities to the fern lineage (Kenrick and Crane, 1997). The divergence of the Equisetales from the remainder of the ferns was given a minimum age of 346 MYA, based on the occurrence of Equisetalean *Archaeocalamites* in the Lower Carboniferous (Bateman, 1991). The crown node of leptosporangiate ferns was constrained at a minimum of 299 MYA, based on *Grammatopteris*, which belongs to Osmundales (Skog, 2001). The seed plant crown node was given a minimum age of 323 MYA, corresponding to the occurrence of Cordaitalean fossils from near the Lower-Upper Carboniferous boundary, which are interpreted as members of an early divergent gymnosperm lineage, following Crane (1985).

_Ancestral range estimation_

We inferred the biogeographic history of the Lycopodiaceae using the R package BioGeoBEARS (Matzke, 2013) using a dispersal-extinction-cladogenesis (DEC) model (Ree and Smith, 2008) on the maximum credibility tree from our DTE analysis. We ran the analysis with two variants of the DEC model, one with and one without the “jump” parameter \( j \) of Matzke (2014); these models were compared using a log-likelihood ratio
test, and the better-fitting model was selected. Species distributions were determined through examination of floras (Wagner and Beitel, 1993; Ollgaard, 1995; Mickel and Smith, 2004; Wu et al., 2013), taxonomic treatments (Ollgaard, 1992), and examination of herbarium specimens. Nine biogeographic areas were included in the analysis: North America, Central and tropical South America, southeastern Brazil, temperate South America, Eurasia, Africa and Madagascar, Eastern Asia, Southeast Asia, and Oceania. We did not impose any limits on the maximum number of areas that could be occupied by a given taxon, and all combinations of areas were allowed, as one extant species, *Lycopodium clavatum*, occupies all of them. To account for change in geographic proximity of landmasses over time, we incorporated four different dispersal probability matrices in our analysis, with each corresponding to non-overlapping periods of time. The time periods were defined as follows: 0–50 MYA, 50–100 MYA, 100–200 MYA, and more than 200 MYA; these time slices were chosen to reflect major motifs of continental drift, including the disintegration of Pangaea, the subsequent separation of both Laurasia and Gondwana, and the gradual shift of the resulting continents to their current positions.

RESULTS

Divergence time estimation

Our best ML tree had a -lnL of -17097.56 and recovered phylogenetic relationships generally concordant with those previously reported within the Lycopodiaceae and within other major lineages. Relationships among major land plant lineages were also consistent with those recovered in recent large-scale analyses (Magallón et al., 2013; Wickett et al.,
BEAST analyses generally recovered strong support for most backbone nodes in the phylogeny, though support for some relationships among recently diverged lineages in the Lycopodiaceae were weak. Path sampling analyses indicated that the best-fitting clock model was the RLC ($\Delta\text{lnL} = 70.06$, BF = 13.7), and we recovered three distinct substitution rate regimes across the Lycopodiaceae, with mean regime rates ranging from $1.3 \times 10^{-3}$ in *Palhinhaea* to $2.1 \times 10^{-4}$ in *Phlegmariurus*. Apart from the background rate regime of the family, distinct elevated rate regimes were found in subfamily Lycopodielloideae and in *Phylloglossum*.

Divergence date estimates were different under the RLC and UCLN models (Figs. 1, 2). Dates obtained with the RLC model were generally older and had broader confidence intervals, whereas the UCLN model tended to provide the younger ages and the narrower confidence intervals. Major differences in clade age estimates between these models were most evident for the crown age of the Lycopodiaceae, (mean age of 368.4 MYA under the RLC model and 308.8 MYA under the UCLN model) and within the subfamily Huperzioideae. The estimates generated from the best-fitting (RLC) clock model indicate that lycophytes diverged from the euphyllophyte lineage 431.5 MYA [95% HPD 426–440 MYA], that the homosporous and heterosporous lycophyte lineages diverged 403.3 MYA [95% HPD 394–414 MYA], and that the crown age of the Lycopodiaceae was 368.4 MYA [95% HPD 326–389 MYA]. Within the Lycopodiaceae, the mean estimate of the crown age of subfamily Huperzioideae is 199.5 MYA [95% HPD 175–223 MYA], and the divergence of subfamilies Lycopodioidae and Lycopodielloideae is estimated to have occurred at 293.6 MYA [95% HPD 262–320 MYA]. Divergence time estimates for
genera range from 210.4 MYA [95% HPD 202–236 MYA] for the split of
*Lycopodiastrum* from the rest of subfamily Lycopodioideae to 53.1 MYA [95% HPD 35–
65.5 MYA] for the divergence between *Austrolycopodium* and *Pseudodiphasium*.

**Ancestral range estimation**

Comparison of the DEC and DEC + *j* models implemented in BioGeoBEARS indicated
that inclusion of the *j* parameter resulted in a more likely model (DEC lnL= -447.02,
DEC + *j* lnL= -429.03, Δ=35.98, P=0.023). Results obtained with the DEC + *j* model
indicate that the most likely ancestral range of the Lycopodiaceae was North America +
Eurasia + East Asia + Oceania, that the ancestral range of subfamilies Lycopodioideae
and Lycopodielloideae was Oceania, and that of subfamily Huperziioideae was East Asia
+ Oceania. Within the Lycopodioideae, most lineages are widespread in the north
temperate region, though a clade comprising *Pseudolycopodium*, *Pseudodiphasium*, and
*Austrolycopodium* diversified in Oceania, with a subsequent long-distance dispersal event
in *Austrolycopodium* to southern South America. Subfamily Lycopodielloideae appears
to have radiated out of Oceania in the late Mesozoic, with a generally north temperate
affinity among *Lycopodiella* and tropical/austral distributions in *Lateristachys*,
*Palhinhaea*, and *Pseudolycopodiella*. In the Huperzioidae, we recover an ancestral range
of Australasia for *Phylloglossum*, East Asia + tropical Asia for *Huperzia*, and Neotropics
+ East Asia + Africa + tropical Asia + Oceania for *Phlegmariurus*. Several recent long-
distance dispersal events between austral regions in *Huperzia* were recovered, and many
species are shared between North America and East Asia. In *Phlegmariurus*, the
divergence of its two principal clades into the Neotropics and Paleotropics likely
occurred during the lower Cretaceous (102 MYA); several subsequent long-distance dispersal events and inferred to have occurred between these regions.

DISCUSSION

Substitution rate heterogeneity and clock model effects

We found evidence for two shifts in nucleotide substitution rate across the Lycopodiaceae, corresponding to the subfamily Lycopodielloideae and *Phylloglossum*. The shifts in substitution rate observed in these groups appear to be related to a transition in growth strategy, as both taxa are characterized by rapid life cycles in comparison to other members of the family. In particular, members of the subfamily Lycopodielloideae and *Phylloglossum* possess surficial, photosynthetic gametophytes that are fast-growing in comparison to the subterranean gametophytes found in other members of the family. Though elevated rates of substitution are known to occur in groups with fast generation times in plants (Gaut et al., 1992; Clegg et al., 1994; Lanfear et al., 2013), this is the first evidence demonstrating coordinated shifts in substitution rates and gametophyte ecology. As gametophyte growth rates and reproductive biology vary dramatically across both lycophytes (Bruce, 1976; Whittier, 1998) and ferns (Nayar and Kaur, 1971; Whittier, 1981; Raghavan, 1989; Haufler et al., 2016; Sessa et al., 2016), this finding has important implications for understanding the variation in rates of molecular evolution observed across these clades of taxa with independent gametophyte life phases. Further study of the correlation of gametophyte longevity and nucleotide substitution rates is needed, but this effect may explain the patterns of anomalous substitution rates in some fern genera.
with either fast- (e.g., *Ceratopteris*) or slow-growing (e.g., *Botrychium*, *Ophioglossum*) gametophytes.

We demonstrate a significant effect of clock model on estimates of divergence times in the Lycopodiaceae (Figs. 1, 2), with the RLC model outperforming the UCLN model. Better performance of a RLC model is consistent with findings from other groups that include lineages in which punctuated shifts in molecular evolution rates are known, such as the monocot family Xanthorrhoeaceae (Crisp *et al.* 2014) and cetaceans (Dornburg *et al.* 2011). Moreover, the accuracy of dates generated using the UCLN model appears to be poor in regions of the tree that lack a nearby fossil calibration (Fig. 2). Given the strong effect of clock model on divergence time estimates reported here and in other studies (Dornburg *et al.*, 2011; Werthheim, 2012; Crisp *et al.*, 2014; Beaulieu *et al.*, 2015), researchers should be cognizant of potentially misleading inferences of divergence times stemming from ill-fitting clock models, especially if their study group presents *a priori* evidence for among-lineage rate heterogeneity.

*Divergence time estimates*

Considering estimates obtained from the RLC model, our divergence time estimates for the Lycopodiaceae are generally more recent than those reported by Wikström and Kenrick (2001), though our estimates for the initial divergences in the family are similar. Congruence of our estimates and those of Wikström and Kenrick (2001) with respect to deep divergences may be primarily a function of similar fossil calibrations constraining the backbone of both phylogenies; the influence of fossil calibration choice on age estimates deep in the land plant phylogeny has been demonstrated previously (Magallón
and Sanderson, 2005; Yang and Rannala, 2006; Magallón et al., 2013). Among internal nodes of the Lycopodiaceae, which were uncalibrated by Wikström and Kenrick (2001) and constrained only at a single node in our phylogeny, our dates diverge more conspicuously, though direct comparisons are difficult to make due to much denser taxon sampling in our study. Considering the crown ages for major clades that were well-sampled in both studies, we report considerably (ca. 50–90 MY) younger ages for all three subfamilies and most genera. These differences appear to be driven by the dramatic acceleration of substitution rates found in subfamily Lycopodielloideae and *Phylloglossum*, given that major among-lineage heterogeneity in rates of molecular evolution can drive artificially old age estimates under relaxed clock models (Beaulieu et al., 2015).

We place the divergence of the homosporous and heterosporous lycophyes during the early Devonian (median age = 403 MYA). This age estimate is consistent with the hypothesis of a relatively rapid diversification of the major embryophyte lineages over approximately 50-75 million years after the initial colonization of land by plants, which is corroborated both by fossil (Kenrick and Crane, 1997; Crane et al., 2004) and molecular phylogenetic (Bateman et al., 1998; Finet et al., 2010; Magallón et al., 2013; Testo and Sundue, 2016) evidence. Consistent with the findings of Wikström and Kenrick (2001), our estimates of the divergences among subfamilies of the Lycopodiaceae date to the Carboniferous and Permian, indicating that the initial diversification of the family was underway well before the initial breakup of Pangaea at approximately 175 MYA. The divergence of the three ecologically disparate genera comprising the Huperzioideae
(Huperzia, Phlegmariurus, and Phylloglossum) coincided with the breakup of Pangaea, with these three lineages splitting during the lower to middle Triassic. A critical innovation that appears to have been attained during this initial burst of lineage diversification and specialization within the Lycopodiaceae is the evolution of epiphytism in Phlegmariurus, which several analyses have indicated to be the ancestral growth habit of the genus (Field et al., 2016). Assuming that epiphytism evolved in Phlegmariurus coincidentally with the divergence of the genus from the Huperzia/Phylloglossum lineage or shortly thereafter, this marks one of the earliest transitions to epiphytic growth known among vascular plants, and the earliest known record from an extant genus. Other early epiphytes, such as the ophioglossalean fern Botryopteris, grew as trunk epiphytes on Marattielean tree ferns (Rothwell, 1991); the earliest epiphytic Phlegmariurus may have exhibited a similar growth strategy. Given that vascular epiphytes were rare until the late Cretaceous (Benzing, 1990; Taylor et al., 2009; Schuettpelz and Pryer, 2009; Watkins and Cardelús, 2012), the early radiation into and persisting success in the epiphytic niche by Phlegmariurus is remarkable. Altogether, it appears that the early divergence events in the Lycopodiaceae correspond to a partitioning of ecological strategies among the different subfamilies and genera of subfamily Huperzioideae; much of the family’s functional diversity appears to have been attained early in its evolutionary history.

Though the initial diversification of the family occurred during the Carboniferous and Permian, much of the family’s generic and species richness accumulated considerably later. Apart from the early divergences in subfamily Huperzioideae and the establishment of some depauperon lineages (Donoghue and Sanderson, 2015) such as Lycopodiastrum
and *Phylloglossum* by the upper Triassic, most groups diversified primarily during the Cretaceous and Cenozoic, coincident with the rise of modern fern (Schneider et al., 2004; Schuettpelz and Pryer, 2009; Testo and Sundue, 2016; Lehtonen et al., 2017), gymnosperm (Won et al., 2006; Nagalingum et al., 2011; Condamine et al., 2015; Saladin et al., 2017), and angiosperm (Xi et al., 2012; Magallón et al., 2013) lineages. The rapid diversification of some of these groups, such as leptosporangiate ferns (Schneider et al., 2004; Schuettpelz and Pryer, 2009; Watkins and Cardelús, 2012) and the angiosperm order Malpighiales (Davis et al., 2005; Xi et al., 2012) is clearly closely linked to the establishment of angiosperm-dominated rainforests by the early Cretaceous; this appears to be the case for some tropical and subtropical genera such as *Phlegmariurus*, *Palhinhaea*, and *Pseudodiphasium*, but not for other groups, such as *Huperzia*, *Diphasiastrum*, and *Diphasium*, which appear to be adapted primarily to open temperate and tropical alpine habitats. Most species-level divergences are recent; occurring within the last 15 million years.

*Historical biogeography*

The biogeographic history of the Lycopodiaceae is complex, and ancient vicariance events appear to have played the most important role in shaping the group’s distribution (Figs. 3, 4). The initial divergences within the family occurred while Earth’s landmasses were united in Pangaea, when these lineages most likely were widely distributed across the supercontinent. Early divergences within each subfamily correspond with the initial breakup of Pangaea into the Laurasian and Gondwanan landmasses during the early Jurassic; distributions of extant genera in the Lycopodielloideae and Huperzioideae
clearly reflect this pattern (Fig. 4). The primary divergence event in the Lycopodielloideae split *Lycopodiella*, which has a primarily Laurasian distribution, from the principally Gondwanan lineage comprising the genera *Lateristachys*, *Palhinhaea*, and *Pseudolycopodiella*. Similarly, the divergence of the Laurasian genus *Huperzia* from the otherwise Gondwanan Huperzioideae is estimated to have occurred in the mid-Jurassic, while Laurasia and Gondwana were separated yet still proximate. Similar patterns of lineage splitting concordant with the Pangaean breakup have been demonstrated for numerous animal groups (Springer et al., 2003; San Mauro et al., 2005; Wildman et al., 2007), but appear to be exceedingly rare among plants; currently, robust phylogenetic evidence for this pattern is only known from the Cupressaceae (Mao et al., 2012) and Selaginellaceae (Klaus et al., 2016; Weststrand, 2016). In addition to providing a rare example of Pangaean vicariance in a plant family, the geographic fidelity of these deep divergences within the Lycopodiaceae is remarkable given the group’s high potential for long-distance dispersal, which some authors (Wolf et al., 2001; Vanderpoorten et al., 2009) suggest could obscure ancient vicariance in groups of spore-dispersed plants.

The subsequent breakup of Gondwana also had an important role shaping the geographic distribution of *Phlegmariurus*, the most species-rich genus in the Lycopodiaceae (Fig. 4). *Phlegmariurus* comprises two clades of approximately equal size—one nearly entirely Paleotropical, the other almost entirely Neotropical—which diverged from each other an estimated 102 MYA. Our estimate of the timing of this split is much younger than the lower Cretaceous estimate (184 MYA) reported by Wikström and Kenrick (2001) and corresponds closely to the final rifting of South America and Africa (König and Jokat,
Both clades subsequently underwent exceptional species diversification during the Cenozoic, apparently radiating in angiosperm-dominated rainforests like most leptosporangiate fern groups (Schneider et al., 2004; Schuettpelz and Pryer, 2009; Testo and Sundue, 2016). Unlike most emblematic Gondwanan seed plant (e.g., *Podocarpus*, Quiroga et al., 2015; *Nothofagus*, Swenson et al., 2001; Proteaceae, Barker et al., 2007) and fern lineages (e.g., Cyatheaceae, Korall and Pryer, 2013; *Trichomanes*, Dubuisson et al., 2003; Dicksoniaceae, Noben et al., 2017), there is no evidence of migration between South America and Oceania through Antarctica (a connection that persisted until the middle Eocene; Wilf et al., 2013) or of intercontinental long-distance dispersal leading to subsequent diversification events in *Phlegmariurus*.

This study confirms the finding of a recent phylogeny (Gissi, 2017) that documented the presence of an endemic clade of Brazilian species nested within the Neotropical *Phlegmariurus* clade. This group is estimated to have diverged from its closest relatives approximately 37 MYA (Fig. 3), and has subsequently undergone considerable diversification in the mountains and rainforests of southeastern Brazil. Unlike the tropical Andes, which formed primarily since the Miocene (Gregory-Wodzicki, 2000), the mountain ranges of the Brazilian Highlands were established prior to the divergence of this clade, eliminating the possibility that this clade arose following a long-distance dispersal event from the Andes, as has been documented in other plant groups (Safford, 1999; McHenry and Barrington, 2014; Luebert and Weigand, 2014). Instead, it appears that this group radiated into these habitats from adjacent lowland forests and subsequently became adapted to a diversity of available habitat types in the region.
Compared to that of vicariance, the role of long-distance dispersal in shaping the
diversity and distribution of the Lycopodiaceae appears to be minor. Nonetheless, several
long-distance dispersal events have important implications for understanding the
evolutionary history of some genera and warrant discussion. Recent trans-Austral long-
distance dispersal has occurred in both *Austrolycopodium* and *Huperzia* (Fig. 3), fitting a
pattern commonly found in Austral plant groups (Richardson et al., 2004; Barker et al.,
2010; Renner et al., 2010; Noben et al., 2017). Long-distance dispersal is also responsible
for both species (*Phlegmariurus saururus*) and species-pair (*Phlegmariurus phylicifolius*
and *P. ophioglossoides*) range disjunctions between the Neotropics and southern
Africa/Madagascar, as has been reported for numerous fern and lycophyte genera by
Moran and Smith (2001). Interestingly, both *P. phylicifolius* (to at least 19°S) and *P.
saururus* (to at least 31°S) have ranges that extend farther south in the Andes than most
other Neotropical *Phlegmariurus*; these southerly distributions may have permitted their
(or their close relatives’) dispersal to Africa via the prevailing westerly winds at south-
temperate latitudes. Several long-distance dispersal events to southeastern Brazil from the
Andes have occurred since the Miocene; these dispersals did not result in subsequent
radiations (Fig. 3). Long-distance dispersal certainly has played an important role in the
diversification of *Phlegmariurus* in tropical Asia and Oceania (Field et al., 2016), but the
scale of these dispersal events is too fine to be detected in our analyses, as they mostly
have occurred within—rather than between—the areas defined in this study.

This study improves our understanding of the evolutionary history of the Lycopodiaceae
by providing a densely sampled, time-calibrated phylogeny and inference of the family’s
complex biogeographic history. We demonstrate the importance of considering among-lineage rate heterogeneity when estimating lineage divergence times, and provide clade age estimates that differ considerably from previously published estimates. The Lycopodiaceae arose during the Devonian and prominent divergence events in the family appear to be associated with major vicariance events, particularly the breakup of Pangaea and the subsequent rifting of Gondwana; most long-distance dispersal events are recent and have been of limited evolutionary consequence. Finally, though the family is ancient, most of its extant species diversity accumulated during the Cenozoic, coincident with the recent diversification of other major land plant lineages.
Acknowledgments

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CHAPTER 3: PHYLOGENETIC SYSTEMATICS, MORPHOLOGICAL EVOLUTION, AND NATURAL GROUPS IN NEOTROPICAL PHLEGMARIURUS (LYCOPODIACEAE)

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 Huperzioideae (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 perhaps unsurpassed by any other 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. 5).

The morphological variability of *Phlegmariurus* has presented a significant impediment 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 Lycopodioideae and 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 distinct but 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; 1994; 2003; 2015; 2016a; 2016b). 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: “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.

MATERIALS AND METHODS

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*, the remaining Lycopodiaceae subfamily Huperzioideae genera *Huperzia* and *Phylloglossum*, and *Lycopodium clavatum*. Sequence data for six chloroplast markers (*psbA-trnH, rbcL, rps4, trnL, trnL-trnF*, and *trnP-petG*) were obtained from Genbank, based on a recently published time-calibrated phylogeny of the Lycopodiaceae (Testo et al., in press). Voucher information and sequence accessions numbers are available in Appendix 1.

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; 2014) 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 (Huelsenbeck and Ronquist, 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.

*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; 1994; 1995; Mickel and Smith, 2004), herbarium specimens, and observations of plants in the field. We scored six traits that have been proposed as taxonomically informative: growth habit, stem thickness, stem coloration, leaf margin shape, fertile/sterile leaf dimorphy, 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 2.

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 was 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 the 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 Lycopodioidae 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 dimorphy 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 we 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) has been 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.brlen’ function in ‘ape’.

RESULTS

Phylogeny and species groups

We found \textit{Phlegmariurus} to be strongly supported (BS 100%, PP 1.0) as monophyletic and sister to a clade comprising \textit{Huperzia} and \textit{Phylloglossum} (Fig. 6). Statistical support for the position of \textit{Phylloglossum} was moderate (BS 77%, PP 0.82). Within \textit{Phlegmariurus}, two principal clades were recovered, one primarily consisting of Neotropical species and the other nearly entirely Paleotropical. Among Neotropical \textit{Phlegmariurus}, we recovered eleven clades that we treat as distinct species groups (Fig. 7). 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 \textit{Phlegmariurus} is a clade (the \textit{Phlegmariurus aqualupianus} group) that
includes most of the Neotropical members of Øllgaard’s *Huperzia phlegmaria* 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 refer to this large assemblage of species as the *Phlegmariurus crassus* group, after the most widespread and polymorphic species in the group.

*Morphological evolution*

Fertile/sterile leaf dimorphy

The most probable ancestral condition of leaf dimorphy for Neotropical *Phlegmariurus* is monomorphic (Fig. 8A), 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*. 
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. 8B).

Growth habit

Terrestrial growth is ancestral within Lycopodiaceae subfamily Huperzioideae, but the ancestral growth habit of both *Phlegmariurus* and for the Neotropical clade is ambiguous (Fig. 8C). 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.

Stem coloration

The most probable ancestral stem coloration is green/yellow (Fig. 8D); 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.

Stem thickness

Stem thickness varied considerably across Neotropical *Phlegmariurus*, but was generally stable within species groups (Fig. 9A). 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.

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. 9B). 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.

DISCUSSION

Phylogenetic systematics

Our results corroborate the findings of Wikstrom and Kenrick (1997) and Field et al. (2016) regarding the phylogeny of the Lycopodiaceae subfamily Huperziioideae and are consistent with the generic taxonomic scheme used by most workers in the group in recent years (Øllgaard 2012a,b; Field and Bostock, 2013, PPG I, 2016). As found in earlier phylogenetic studies (Wikstrom and Kenrick, 1997; 2000; Field et al., 2016), our analyses indicate that Phlegmariurus is divided into two principal clades: one Paleotropical and the other Neotropical (Fig. 6). Only a few species violate this biogeographic pattern: the widespread Neotropical epiphyte Phlegmariurus funiformis is a member of the Paleotropical 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. As discussed extensively in several earlier phylogenetic studies (Wikstrom et al., 1999; Wikstrom and Kenrick, 2000; Field et al., 2016), this deep split between Paleotropical 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.

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. 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 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 heterogenous 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 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* and 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.
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.

Phlegmariurus aqualupianus group

This species group corresponds with the Huperzia myrsinites and Huperzia aqualupianus subgroups of Øllgaard’s Huperzia phlegmaria group. Members of this group are characterized by their pendulous epiphytic growth habit and prominent fertile/sterile leaf dimorphy (except the monomorphic Phlegmariurus capillaris; Fig. 8A). Reddish stems and leaf bases appears to be a synapomorphy for the group of species closely allied to Phlegmariurus phylicifolius, which corresponds to Øllgaard’s Huperzia myrsinites subgroup (Fig 8D). 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. biformis 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.

*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 best characterized by spreading, filiform to linear leaves (Fig. 9B). Several species possess red coloration on their stem at the bases of their leaves (Fig. 8D); 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.

*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. 9A) and very narrow leaves (Fig. 9B) that are monomorphic or nearly so (Fig. 8A). Most species are pendulous epiphytes, although the group includes a facultative (*P. comans*) and an obligate (*P. intermedius*) rupestral species. *Phlegmariurus intermedius* is an atypical member of this group on account of its relatively robust shoots, rupestral 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.

**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. 8D). 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.

**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. 9A), 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. pruinosus* and *P. robustus*) probably belong here. Five to seven species belong to this group.

**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 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.

**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, the group is characterized by their epiphytic growth habit (Fig. 8C), monomorphic to gradually dimorphic shoots (Fig. 8A) and relatively narrow, lanceolate leaves (Fig. 9B). Several species not included in our study may belong here; they include *P. sotae* and *P. killipii*. The total number of species in this group is uncertain, perhaps six.

*Phlegmariurus linifolius* group

This group represents a modification of Øllgaard’s *Huperzia linifolia* group, in which he placed pendulous epiphytes with narrow stems and alternate, subpetiolate, falcate-ascending leaves. We exclude *Phlegmariurus capilliaris*, which Øllgaard 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 considered allied to the *Huperzia verticillata* group) and
Phlegmariurus homocarpus, a member of the Huperzia taxifolia group that Øllgaard 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. 8C), narrow stems (Fig. 9A), homophyllous shoots (Fig. 8A), 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.

Phlegmariurus brongiartii group

The circumscription of this group differs from the Huperzia brongniartii 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 Andes. Both species are rather robust plants with monomorphic shoots (Fig. 8A) that differ most notably by growth habit (Fig. 8C); P. brongniartii 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).
**Phlegmariurus reflexus** group

Included here are terrestrial species with spreading to reflexed, lanceolate to linear leaves (Fig. 9B) with (in most species) toothed margins (Fig 8B). 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 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. 9A), phyllotaxy, leaf margin toothiness, and stem thickness (Fig. 9B); further study is needed to test this hypothesis of a hybrid origin.

Our analyses indicate that *Phlegmariurus beitelii* species 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.

*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. weberbaueri* (Fig. 6). 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 saururus* groups (Fig. 7). 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 9B). 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 (Billings, 1974; 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. 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 2016; *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. 8C), thick stems (Fig. 9A), monomorphic leaves (Fig. 8A), and (in most species) entire or weakly toothed leaf margins (Fig. 8B). 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.

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. Finally, this study highlights the need for additional research to improve resolution of species relationships—especially among the *Phlegmariurus crassus* group—and examine the importance of reticulate evolution as a driver of diversification in the genus.
LITERATURE CITED


CHAPTER 4: THE RISE OF THE ANDES PROMOTED RAPID DIVERSIFICATION IN NEOTROPICAL PHLEGMARIURUS (LYCOPODIACEAE)

INTRODUCTION

It is well-established that mountains harbor a disproportionate share of Earth’s biodiversity (Humboldt and Bonpland, 1807; Hoorn et al., 2013; Antonelli, 2015; Hughes and Atchison, 2015; Lagomarsino et al., 2016; Xing and Ree, 2017). Though they cover approximately one-eighth of Earth’s land surface, mountains host approximately one-third of terrestrial species (Spehn et al., 2012) and have supported exceptional species radiations (Hughes and Eastwood, 2006; McGuire et al., 2007; Schwery et al., 2015; Lagomarsino et al., 2016; Xing and Ree, 2017) in major lineages spanning the Tree of Life. Given the exceptional species diversity distributed on the world’s mountains, developing a thorough understanding of the factors driving the diversification of biotic lineages in these regions is an important goal of evolutionary biologists. Though methodological advances in comparative phylogenetics and historical biogeography made in recent years have improved our capability to identify the relative roles of biotic and abiotic factors as drivers of evolutionary radiations (Matzke, 2013; Morlon, 2014), our understanding of the timing and mode of diversification in montane systems remains mostly confined to a few groups of organisms.

Among the best geographic regions in which to study the diversification of montane lineages is Andean South America. Of interest for researchers in many groups are the tropical Andes, which span western South America from Venezuela to northern Argentina. This area is the most species-rich region on Earth, with more than 45,000
plant and 3,400 vertebrate species; nearly half of these are found nowhere else on Earth (Myers et al., 2000). The accumulation of this unparalleled diversity is due in large part to major evolutionary radiations that have occurred in a broad array of Andean lineages, including groups of fungi (Leavitt et al., 2012; Lücking et al., 2014), animals (García-Moreno et al., 1999; Doan, et al., 2003; Elias et al., 2009; Chaves et al., 2011), and plants (Hughes and Eastwood, 2006; Antonelli et al., 2009; Luebert and Weigend, 2014; Lagomarsino et al., 2016). These radiations are particularly striking given that most are recent and ongoing, having started during the Pliocene and Pleistocene epochs (Madriñan et al., 2013), in concert with the rapid uplift of the high Andes over the past 10 million years (Gregory-Wodzicki, 2000; Garzione et al., 2008; 2014). Though the timing and geography of the Andean uplift remain topics of considerable debate, it is evident that the Andean orogeny is a highly dynamic phenomenon that, along with climatic fluctuations and biotic interactions, has driven some of the fastest diversification rates known (Madriñan et al., 2013; Lagomarsino et al., 2016).

Though the number of focused studies on the diversification of montane Andean lineages has increased substantially over the past decade, most work has focused on a few groups, such as amphibians (Gonzalez-Voyer et al., 2011; Castroviejo-Fisher et al., 2014), birds (Chaves et al., 2011; Fjeldså et al., 2012; McGuire et al., 2014), and flowering plants (Hughes and Eastwood, 2006; Madriñan et al., 2013; Lagomarsino et al., 2016). In contrast, the evolutionary histories of other prominent tropical Andean groups, such as ferns (Kreier et al., 2008; McHenry and Barrington, 2014; Sánchez-Baracaldo and Thomas, 2014) and lichens (Lücking et al., 2014) have received little attention; others yet, including lycophytes and bryophytes, remain unstudied within a phylogenetic
context. To better understand how the Andean uplift has impacted the diversification of the region’s biota as a whole, increased focus on understudied groups, especially those that differ in life history strategies and dispersal ability, is needed. Here, we address this gap in our knowledge by examining the role of the Andean uplift as a driver of diversification in the species-rich lycophyte genus *Phlegmariurus* (Lycopodiaceae). The Neotropical clade of *Phlegmariurus* represents an excellent study system for examining the relationship between the Andean orogeny and species diversification in spore-dispersed vascular plants. This clade is monophyletic, comprises an estimated 150 species, and includes species that are remarkably diverse morphologically and ecologically (Øllgaard, 1992; Field *et al.*, 2016). Neotropical *Phlegmariurus* is distributed from Florida and Mexico to Argentina and southeastern Brazil and can be found from sea level to at least 5000 m elevation, but is most diverse in the northern Andes (Øllgaard, 1995), where most species are either epiphytes in humid montane forests or clump-forming terrestrial herbs in alpine grasslands. The presence of a large (> 60 species), monophyletic group of terrestrial taxa restricted to young alpine habitats of the Andes and adjacent regions (Øllgaard, 1992) suggests that the group may have undergone a recent and rapid diversification burst, thus providing a useful comparison to angiosperm genera with similar but better-studied evolutionary histories. Using a dated phylogeny, species distribution information, and both climate and geological data, we apply comparative phylogenetic models to investigate the evolutionary history of Neotropical *Phlegmariurus* within the context of the Andean orogeny.
MATERIALS AND METHODS

Taxon sampling and phylogeny

A time-calibrated phylogeny of Neotropical *Phlegmariurus* was obtained by pruning the clade from a larger dated phylogeny of the Lycopodiaceae based on six chloroplast loci and eight fossil calibration published by Testo *et al.* (*in press*). A single African species that belongs to the Neotropical clade (*Phlegmariurus ophioglossoides*) was trimmed, leaving a total of 105 species, representing approximately 70% of known species richness in the group. The final trimmed tree is available in the Supplementary Materials.

Diversification rate estimation

Lineage diversification rates were estimated using BAMM 2.5.0 (Rabosky, 2014), which uses a reverse-jump MCMC to sample a large number of possible diversification regimes from a given time-calibrated phylogeny. The MCMC was run for 100 million generations and sampled every 40,000 generations. Prior values were selected using the ‘setBAMMpriors’ function; we accounted for incomplete taxon sampling by providing a sampling file with estimated sampling proportions for each of the eleven species groups in the clade as defined by Testo *et al.* (*in press*). Post-run analyses were performed using the ‘BAMMtools’ (Rabosky *et al.*, 2014) package in R 3.4.2 (R Core Team, 2014). The initial 10% of the MCMC run was discarded as burn-in, and the remaining data were assessed for convergence and ESS values greater than 200. Net diversification rates were obtained for all taxa in the phylogeny using the ‘getTipRates’ function; these values represent the posterior distribution of rates at the tip of the tree, as described by Rabosky.
To compare diversification rates of species groups in the genus, mean clade rates were extracted using the ‘getCladeRates’ function.

Species distribution data and association with diversification rates

To estimate features of species distributions and to estimate niche space, georeferenced locality data were obtained for 4,887 collections of Neotropical *Phlegmariurus*. Specimen data were obtained from the GBIF and Tropicos databases and from examination of herbarium material at AAU, COL, FMB, GH, HUA, MEXU, NY, PSO, and VT. All collection data were reviewed and both duplicate collections and specimens from beyond the known range of the species that appeared to be misidentified were deleted. Following deletion of erroneous data, 2,974 collections remained, ranging from 2 for *Phlegmariurus tryoniorum* to 324 for *Phlegmariurus reflexus*. In total, we estimated four features of species distributions and niche occupancy: mean elevation, elevational range, species range size, and niche breadth. Mean elevation and elevational range were calculated from values provided on collection labels or estimated using lat/long coordinates and the ‘elevation’ function in R. Range size was estimated by applying a 5 km buffer around each locality point using the ‘gBuffer’ function in the ‘rgeos’ (Bivand *et al.*, 2017) package in R, following the methodology of Anacker and Strauss (2014). To calculate niche breadth, we calculated niche models for each species using locality data and climate layers obtained from the CHELSA (Karger *et al.*, 2017) dataset. Using these data, we extracted four bioclimatic variables that were the strongest uncorrelated predictors of the species environmental niche model: annual temperature, annual precipitation, seasonality of temperature, and seasonality of precipitation. Niche breadth
was calculated using ENMTools (Warren et al., 2010) using Levins’ (1968) B metric. The correlations between these variables and species diversification rates were examined with phylogenetically independent contrasts (Felsenstein, 1985) implemented in the R package ‘ape’ (Paradis et al., 2004); range-size data were log-transformed prior to analysis to overcome their skewed distribution. Trait variables were plotted on the phylogeny of Neotropical *Phlegmariurus* using the ‘plotTree.wBars’ function in ‘phytools’ (Revell, 2012).

*Orogeny-dependent diversification*

We tested for an association between the uplift of the Andes and diversification rates in Neotropical *Phlegmariurus* by comparing a series of time-dependent diversification models that allow speciation and/or extinction to vary with the paleoelevation of the Andes against constant diversification null models. Paleoelevation data were obtained from the list compiled by Lagomarsino et al. (2016); which is based on historical elevation estimates from Garzione et al. (2006; 2008; 2014), Ehlers and Poulsen (2009) and Leier et al., 2013). These data are available in the Supplementary Materials. A smoothing line fitting these data was generated using the R package ‘pspline’ and this line was used to provide the best estimate of Andean paleoelevation at any given time point. A total of eight paleoelevation-dependent diversification models were applied; four with exponential dependency and four with linear dependency. Two null models were also generated: a Yule model and a constant-rate birth-death model. Models were compared and the best-fitting model was selected using the corrected Akaike Information Criterion. These models were implemented in the R package ‘RPANDA’ (Morlon et al.,
2016) and are based on the environment-dependent birth-death model described by Condamine et al. (2013). All models, including parameters and comparison metrics, are provided in Table 2.

To visualize the accumulation of lineage diversity in Neotropical *Phlegmariurus* through time and compare that rate to patterns of lineage accumulation expected under constant-rate null models, a log-lineage-through-time plot (LTT) was generated for the time-calibrated *Phlegmariurus* phylogeny as well as for two simulated trees of the same age and taxon richness. One simulated tree was generated under a Yule model (birth rate = 1, death rate = 0), and another was generated under a birth-death model (birth rate = 1, death rate = 0.5). Simulated trees were generated using the “LTT” function and all LTT plots were generated using the “ltt.plot” function in “ape” (Paradis et al., 2004).

**RESULTS**

Net diversification tip rates varied from 0.097 events MY$^{-1}$ for *Phlegmariurus ericifolius* to 0.308 events MY$^{-1}$ for *Phlegmariurus columnaris*; rates were generally lowest in members of the *Phlegmariurus aqualupianus* group and highest in the *Phlegmariurus crassus* group. The fastest diversifying and most species-rich clades in the genus tend to be present at high elevations (Figs. 10, 11); however, there are exceptions to this pattern. Phylogenetic independent contrasts indicated a significant negative correlation between diversification rates and species range size (Figs. 12A, 13A), nonsignificant correlations between diversification rates and both elevational range and niche breadth (Figs. 12B-C, 13B-C), and a significant positive correlation between diversification rates and mean elevation (Figs. 12D, 13D).
LTT plots indicate that lineage accumulation in Neotropical *Phlegmariurus* occurred at a slower rate than would be expected under either of the constant-rate simulations (Fig. 11) until the late Miocene (ca. 10 MYA), when diversity began to rapidly accumulate. The timing of this shift corresponds closely with our paleoelevation estimates (Fig. 11, green dotted line) of the time point at which the Andes reached the approximate elevation of the modern forest/alpine ecosystem boundary, as well as the crown age of the species-rich alpine *Phlegmariurus crassus* species group (Fig. 11, in red).

Comparison of ten time-dependent diversification models indicated that the best-fit diversification model included a positive, exponential association between speciation rates and the paleoelevation of the Andes, with no extinction (-lnL = -298.362, AICc = 600.84). As shown in Table 2, all paleoelevation-dependent models outperformed both the Yule and birth-death null models.

**DISCUSSION**

With unparalleled species richness and high levels of endemism, the Andes have long been recognized as one of the world’s foremost biodiversity hotspots. In recent years, researchers have sought to understand the exceptional biodiversity of this region by studying the rapid diversification of an array of Andean lineages within a phylogenetic context. Among plants, nearly all studies on Andean radiations have focused on angiosperms (Hughes and Eastwood, 2006; Luebert *et al*., 2011; Antonelli *et al*., 2009; Nürk *et al*., 2013; Luebert and Weigend, 2014; Lagomarsino *et al*., Salariato *et al*., 2016; Diazgranados and Barber, 2017); other groups have received little attention (but see
Kreier et al., 2008; McHenry and Barrington, 2014; Sánchez-Baracaldo and Thomas, 2014). Though extensive study of angiosperms is warranted given their exceptional diversity in the Andes and elsewhere and the phenomenal radiations of some flowering plant groups (Madriñan et al., 2013), studying other plant groups in this context is important in order to generate a more synthetic understanding of the timing and mode of diversification of plants in the Andes. By examining the drivers of diversification in Neotropical Phlegmariurus, we partly address this taxonomic unevenness in our knowledge and provide a first step towards understanding the complex evolutionary history of one of the most diverse groups of spore-dispersed vascular plants in the tropical Andes.

Lineage diversification rates vary widely across Neotropical Phlegmariurus, and these rates are strongly correlated with the size of species ranges and the mean elevation at which species are distributed. Taken together, the fastest diversifying lineages of Neotropical Phlegmariurus tend to comprise species with narrow ranges that are distributed at high elevations (Figs. 10, 12A, D, 13A, D). The pattern of rapid diversification among high-elevation taxa is consistent with the hypothesis of a rapid recent diversification of members of the Phlegmariurus crassus species group in alpine grasslands, primarily in the tropical Andes. These high-elevation habitats only became available for colonization around the Pliocene/Pleistocene boundary (Gregory-Wodzicki, 2000), meaning that the species-rich Phlegmariurus crassus group (Fig. 10), which is specialized to these habitats, must have undergone a recent and rapid diversification.

How Neotropical Phlegmariurus colonized Andean alpine habitats can be inferred from its sister groups. The sister group to the Phlegmariurus crassus group is the
Phlegmariurus reflexus group, which comprises an array of generalist terrestrial species that are common in disturbed mid- and high-elevation terrestrial habitats. These two species groups are in turn sister to the large clade of mostly epiphytic species (Field et al., 2016; Testo et al., in press), suggesting that colonization of Andean alpine habitats likely resulted from range expansion by a lineage that had previously transitioned to terrestrial growth from epiphytism and occupied disturbed habitats near the forest limit. The strong negative correlation between diversification rates and species range size (and negative correlation between range size and mean elevation of species’ distributions) highlights another important feature of this recent diversification: the isolated nature of páramo habitats. Given the complex topography of the Andes and the restriction of alpine habitats to high (generally > 3200 m) elevations, páramos are generally small and scattered on the landscape; thus, they have often been considered habitat islands, surrounded by a “sea” of tropical forest and dry valleys. The patchiness of this habitat type thus promotes speciation, as populations are highly isolated: consequently, species turnover is high across páramos and a large proportion of taxa have narrow ranges. As in other plant groups such as lupines (Hughes and Eastwood, 2006), St. John’s worts (Nürk et al., 2013), and frailejones (Diazgranados and Barber, 2017), recently formed and patchily distributed alpine habitats in the tropical Andes appear to be hotbeds of rapid diversification in Neotropical Phlegmariurus.

We found no evidence of significant associations between diversification rates and elevational range or niche breadth (Figs. 12B-C, 13B-C). These results were surprising, as we anticipated that species with small ranges would also have small elevational ranges and narrow niches. Instead, we found that these attributes were not strongly associated,
and that many species with broad ranges were restricted to rather narrow altitudinal bands and had narrow niches. Conversely, some species with medium-sized to small distributions were found along large elevational gradients and exhibited rather broad niches. Though there are many possible explanations for this pattern, a few scenarios seem likely; we consider those here.

First, given the extremely young ages of most species in the alpine-adapted and rapidly diversifying *Phlegmariurus crassus* clade, these lineages may simply not have persisted long enough to have engaged in niche partitioning and instead occupy the entire available niche space and elevational range available at a local scale. Species in this group therefore are segregated primarily by geographic isolation rather than ecological specialization within a given páramo system; ongoing niche differentiation may contribute to the rather unclear species boundaries in this group. In contrast, species in older, primarily forest-dwelling clades which are generally more well-defined ecologically appear to exhibit strong niche separation, and even taxa with rather broad distributions are specialists with restricted niches, such as high-canopy epiphytes (e.g. *Phlegmariurus pithyoides*) or very humid high-elevation forests (e.g., *Phlegmariurus subulatus*, *P. tenuis*). The occupation of broad geographical distributions while maintaining narrow ecological niches may be possible for these forest-dwelling epiphytic taxa because of the continuity of their preferred habitat types in relatively narrow elevational bands throughout the American tropics, especially in comparison to the patchy distribution of alpine grasslands in the same region. This pattern is doubtlessly complemented by strong stratification within the epiphytic niche, which may allow
several species with distinct niches to persist locally, even on a single host tree (Krömer et al., 2007; Watkins and Cardelús, 2009).

Second, it is almost certain that our metric of niche space incompletely represents the realized niche of the studied species. Although the climate-layer dataset we used appears to outperform earlier datasets, especially in the tropics, where orographic effects have a strong impact on local climate (Karger et al., 2017), global-level climate datasets fail to completely characterize species niches in many cases, especially for habitat specialists and epiphytic taxa (Potter et al., 2013; Dymytrova et al., 2016). Given the affinities of many Phlegmariurus species to distinct microhabitats and the generally patchy distribution of individual plants within seemingly suitable habitat (Øllgaard, 1995), it may be important to include finer-scale microclimate measurements in order to accurately characterize the niches of these taxa. Incorporation of these data may prove especially important in understanding the adaptive radiation of Phlegmariurus in southeastern Brazil (Gissi, 2017), as many of the species comprising this group appear to be highly specialized and narrowly distributed.

Although most studies on the evolution of Andean taxa have recognized the importance of mountain uplift in shaping diversity in these groups, relatively few have explicitly tested for an association between the rise of the Andes and lineage diversification in their study group. We used a paleoenvironmental-dependent diversification model developed by Condamine et al. (2013), recently used in a study of the radiation of Andean bellflowers (Lagomarsino et al., 2016) to detect such an association in Neotropical Phlegmariurus, the presence of which was strongly supported by our analyses (Table 2). The correlation between mountain building and diversification rates in Neotropical
Phlegmariurus is not surprising given the high species richness at high elevations in the Andes, but this insight is important because it represents one of the few attempts to explicitly test for a relationship between lineage diversification and a paleoenvironmental variable. In the case of Neotropical Phlegmariurus, a sustained increase in diversification rates occurred around the late Miocene, coincident with the start of exceptionally rapid uplift in the Andes (Garzione et al., 2008; Hoorn et al., 2010) and the expansion of montane forests in the region. This burst of diversification matches the timing of an important event in the evolutionary history of Neotropical Phlegmariurus: a transition to terrestrial growth in the clade comprising the Phlegmariurus reflexus and Phlegmariurus crassus species groups. Thus, rapid diversification among terrestrial Phlegmariurus within the last 10 million years appears to be driven first by increased ecological opportunity as novel habitats became available, and subsequently by the proliferation of isolated alpine habitats as the uplift continued. Nevertheless, additional factors associated with the Andean uplift and habitat availability that were not detected by our analyses certainly played roles in the recent diversification of Andean Phlegmariurus. Foremost among these is the complex history of fluctuating habitat zonation in the Andes in response to cyclical glaciation during the Quaternary (Simpson, 1975; Van der Hammen and Cleef, 1986; Hooghiemstra and Van der Hammen, 2004). Given the dramatic topography of the high Andes and the rapid turnover of habitats along elevational gradients in the tropics (Janzen, 1967; Colwell et al., 2008), glacial-interglacial cycles had profound impacts on habitat connectivity— and gene flow among populations of organisms—in the high Andes. During periods of glaciation, the lower limits of alpine habitats were depressed, connecting páramos that had previously been isolated and
driving compressions of up to 55% in montane forest habitats (Hooghiemstra and Van der Hammen, 2004); forest limits would expand upwards and páramos would again become isolated as glaciers retreated. An important consequence of this cycle of alternating fusion and fragmentation of alpine habitats is that populations have been in intermittent contact with each other, complicating the history of divergence among young evolutionary lineages in the *Phlegmariurus crassus* species group. Coupled with the slow accumulation of reproductive isolation in pteridophytes (Øllgaard, 1985; Hanušová et al., 2014; Rothfels et al., 2015; Sigel, 2016), this fluctuation in habitat connectivity may explain the weak differentiation observed among páramo-dwelling species of *Phlegmariurus*. Nonetheless, resolving such recent, complex evolutionary scenarios is difficult (Hughes et al., 2006; Hughes et al., 2013), and requires levels of resolution unattainable by datasets typically used in the reconstruction of genus-level phylogenies. Using a large nuclear dataset, Vargas et al. (2017) demonstrated widespread introgression and hybridization in a rapidly diversifying clade of Andean *Diplostephium* (Asteraceae) that was undetected with chloroplast and mitochondrial datasets; similar patterns have been demonstrated previously in rapidly diversifying extra-Andean groups, such as African cichlids (Genner and Turner, 2011; Joyce et al., 2011), North American sedges (Escudero et al., 2014), and Mexican frogs (Streicher et al., 2014). Application of a similar dataset and approaches should prove illuminating for better understanding the dynamics of recent diversification of *Phlegmariurus* and other high Andean groups.
LITERATURE CITED


Gissi DS. 2017. Phylogeny of Phlegmariurus (Lycopodiaceae) focusing on Brazilian endemic species.


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.

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Table 2. Comparison of paleoelevation-dependent diversification models implemented in RPANDA.

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Figure 1. Comparison of divergence time estimates obtained using random local clock and uncorrelated lognormal clock models. Branch colors designate major land plant clades, and colored circles indicate the position of fossil node calibrations.
**Figure 2.** Comparison of crown age distributions from select clades in the Lycopodiaceae. Distributions are kernel-density plots of mean ages obtained across the MCMC runs under each clock model.
**Figure 3.** Historical biogeography of the Lycopodiaceae, as inferred using the DEC + J model implemented in BioGeoBEARS. Colored blocks represent current (at branch tips) and inferred historical (at nodes) distributions of Lycopodiaceae taxa. Arrows represent inferred long-distance dispersal events and are color-coded to the area to which the lineage is inferred to have dispersed.
Figure 4. Major vicariance events and the biogeography of Lycopodiaceae subfamily Huperzioideae from 200 MYA to the present.
**Figure 5.** 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. hippocrepis* (*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.
Figure 6. 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.
Figure 7. Species groups described by Øllgaard (1987, 1992) and those proposed here mapped onto our best-scoring ML phylogeny of Neotropical *Phlegmariurus*. 
**Figure 8.** 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 dimorphism, B. leaf margin toothiness, C. growth habit, D. stem coloration.
Figure 9. Phylogenetic reconstruction of continuous morphological traits in Neotropical *Phlegmariurus* as inferred under maximum-likelihood and mapped with continuous change along branches. Values were log-transformed and traits are: A. stem thickness, B. leaf length/width ratio.
Figure 10. Elevation and diversity in species groups of Neotropical *Phlegmariurus*. Circles represent species groups in the genus, circle size is relative to group species richness. Species group elevation and net diversification rate values are means obtained from all representatives in the group that were included in this study.
Figure 11. Diversification dynamics of Neotropical *Phlegmariurus*. The clade highlighted in red on the time-calibrated phylogeny is the *Phlegmariurus crassus* species group. The green dotted line represents the spline of paleoelevational estimates obtained from geological data, as described in the Methods. The red and blue lines are log-lineage-through-time plots simulated under birth-death and pure birth (Yule) models of diversification, respectively. The yellow line is a log-lineage-through-time plot for the time-calibrated phylogeny of Neotropical *Phlegmariurus* used in this study. The horizontal gray dotted line marks the approximate current elevation of treeline in the tropical Andes.
Figure 12. Phylogeny of Neotropical *Phlegmariurus* with diversification rates and (A) range size, (B) elevational range, (C), niche breadth, and (D) mean elevation provided for each species. Bar lengths represent values for given species attributes; bar color represents species net diversification rate.
**Figure 13.** Association between net diversification rates and (A) range size, (B) elevational range, (C) niche breadth, and (D) mean elevation provided for Neotropical *Phlegmariurus* species. $R^2$ and P-values provided are from phylogenetic independent contrasts.
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Appendix 1.
Vouchers and GenBank accession numbers of specimens used in this study. Accessions are presented as follows: taxon, voucher, \textit{trnP-petG} accession number, \textit{psbA-trnH} accession number, \textit{rbcL} accession number, \textit{rps4} accession number, \textit{trnL} accession number, \textit{trnL-trnF} accession number. Asterisks indicate missing data.

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MG560691, MG560800; Phlegmariurus unguiculatus, Rothfels 3715 (DUKE), MG560370, MG560481,
MG560692, MG560801; Phlegmariurus urbani, Øllgaard 58592 (AAU), MG560371, MG560482, MG560543, MG560632, MG560693, MG560802; Phlegmariurus varius, Field & Field 1043 (BRI), *, JQ663831, JQ679089, *, *, *; Phlegmariurus venezuelanicus, Stergios 20572 (UC), MG560372, MG560483, MG560544, MG560633, MG560694, MG560803; Phlegmariurus verticillatus, Wikströ et al. 156 (S), *, *, AJ133897, *, AJ224561, AJ224561; Phlegmariurus weberbaueri, Madsen 8642 (AAU), MG560373, MG560484, MG560648, MG560545, MG560634, MG560695, MG560804; Phlegmariurus weddelii, Øllgaard 38245 (AAU), MG560374, MG560485, *, *, *, Phlegmariurus wilsonii, Testo 1159 (JAUM), MG560375, MG560486, MG560546, MG560635, MG560696, MG560805; Phyloglossum drummondii, Crane s.n. (S), *, *, KU295021, *, AJ224593, AJ224593; Physcomitrella patens, AP005672, AP005672, AP005672, AP005672, Picea abies, NC021456, NC021456,
NC021456, NC021456, NC021456, Podocarpus lambertii, NC023805, NC023805, NC023805, NC023805, NC023805, NC023805, Polyposium glycyrrhiza, KP136832, KP136832, KP136832, KP136832, KP136832, KP136832, Pseudodiphasium volubile, Sundue 3614 (BISH), MG560376, MG560648, MG560547, MG560546, MG560636, MG560697, MG560806; Pseudolyco-podiella caroliniana, Anon. 743681 (TNS), *, *, AB574623, *, AJ133266, *, Pseudolyco-podium densum, Wikströ 242 (S), *, *
AJ133249, *, AJ224597, *; Psilotum nudum, KC117179, KC117179, KC117179, KC117179, KC117179, KC117179,
Selaginella moellendorffii, FJ755183, FJ755183, FJ755183, FJ755183, FJ755183, FJ755183; Selaginella selaginoides, MG560488, *, MG560548, *, *, MG560807; Selaginella uncinata, AB197035, AB197035, AB197035, AB197035, AB197035, Sphagnum palustre, NC031918, NC031918, NC031918, NC031918, NC031918, NC031918, NC031918, NC031918; Spinulum annotinum, Ebihara s.n. (TNS), *, AB575301, AB574622, *, *, *, Syntrichia ruralis, FJS46412, FJS46412, FJS46412, FJS46412, FJS46412, FJS46412, Welwitschia mirabilis, EU342371, EU342371, EU342371, EU342371, EU342371, EU342371; Zea mays, NC011666, NC011666, NC011666, NC011666, NC011666, NC011666;
Appendix 2.

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 bradecorum,1,1.8,0,0,0,7.4; Phlegmariurus brevilolius,0,3.5,0,0,0,8.8; Phlegmariurus brongniarti,0,3.3,0,0,3.8; Phlegmariurus callitrichifolius,1,1,0,1,1,6; Phlegmariurus campianus,1,3.0,0,1,3.6; Phlegmariurus capellae,0,2.8,0,0,0,4.0; Phlegmariurus capilalaris,1.0,4.1,0,0,0,13.6; Phlegmariurus carinatus,1,1.7,0,0,0,4.0; Phlegmariurus cf.versus,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 dacydioides,1,1.9,0,0,0,10; Phlegmariurus darwinianus,0,4.0,0,0,0,5.5; Phlegmariurus diminuens,0,4.0,0,0,0.9.6; Phlegmariurus dianae,0,3.5,0,0,0.2.2; Phlegmariurus dichaeoides,1,1.0,0,1,2.1; Phlegmariurus dihotomus,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 versus,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 fontinales,1,0.9,1,0,1.2; Phlegmariurus fordi,1,1,9,0,0,0.5.0; Phlegmariurus funiformis,1,2.8,0,0,0.6.4; Phlegmariurus hartwegianus,1,4,0,0,0,0.5; Phlegmariurus heterocarpus,1,1.8,0,0,0.8.6; Phlegmariurus heteroclitus,1,1.4,1,0,1,2.7; Phlegmariurus hippocrepus,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 hypogaueus,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 locensis,0,4.0,0,0,0.8.4; Phlegmariurus macbrellei,0,3.0,0,0,0.7.1; Phlegmariurus mandiocanus,1,3.5,1,0,0,17.9; Phlegmariurus mexicanus,1,3.8,1,0,0,15.3; 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 phlegmarioideus,1,1.3,0,0,1.4.5; Phlegmariurus phylicifolius,1,1.1,0,1,4.6; Phlegmariurus pithyoides,1,5.0,1,0,0.28.9; Phlegmariurus podocarpensis,0,3.5,0,0,0.3.1; Phlegmariurus
polycarpos, 1, 1.3, 0, 0, 0, 17.8; Phlegmariurus polydactylus, 0, 3, 0, 1, 0, 2.2; Phlegmariurus polylepide, 0, 6, 0, 0, 0, 3.3; Phlegmariurus pringlei, 1, 1, 0, 0, 8.8; Phlegmariurus pungentifolius, 0, 3.5, 0, 0, 0, 5.8; Phlegmariurus quadfrifolius, 1, 1.8, 0, 0, 1, 4.8; Phlegmariurus recurvifolius, 0, 2.4, 0, 0, 6.5; Phlegmariurus reflexus, 0, 2.3, 0, 1, 0, 8.0; Phlegmariurus regnellii, 0, 4, 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, 1, 2; Phlegmariurus sarmentosus, 1, 1, 3, 0, 0, 20; Phlegmariurus saururus, 0, 4, 0, 0, 0, 7.3; Phlegmariurus sellifolius, 0, 3.5, 0, 0, 0, 1.3; Phlegmariurus sellowianus, 0, 3, 0, 0, 0, 5.5; Phlegmariurus silveirae, 1, 1, 8, 0, 0, 7.2; Phlegmariurus sp 1, 0, 3, 0, 0, 0, 4.8; Phlegmariurus sp 2, 0, 4, 0, 0, 0, 5.2; Phlegmariurus sp 3, 0, 3.5, 0, 0, 0, 4.6; Phlegmariurus sp 4, 0, 3.5, 0, 0, 0, 3.5; Phlegmariurus sp 5, 0, 4, 0, 0, 0, 4.0; Phlegmariurus sp 6, 0, 4.2, 0, 0, 0, 3.8; Phlegmariurus sp 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, 3.8; Phlegmariurus talpiphilus, 0, 4, 0, 0, 0, 4.7; Phlegmariurus taxifolius, 0, 1.8, 0, 0, 0, 8.2; Phlegmariurus tenuis, 0, 0, 6, 0, 0, 10; Phlegmariurus tetragonus, 0, 1, 5, 0, 1, 0, 1.4; Phlegmariurus transilla, 0, 13.5, 1, 0, 1, 5; Phlegmariurus tryoniorum, 0, 5, 0, 0, 0, 2.2; Phlegmariurus tubulosus, 1, 4, 0, 0, 0, 8.0; Phlegmariurus unguiculatus, 0, 3.3, 0, 1, 0, 7.6; Phlegmariurus urbani, 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, 4.5; Phlegmariurus weberbaueri, 0, 3, 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;