



First insights on the biogeographical history of *Phlegmariurus* (Lycopodiaceae), with a focus on Madagascar

Lucie Bauret^{a,*,1}, Ashley R. Field^{b,1}, Myriam Gaudeul^a, Marc-André Selosse^{a,c}, Germinal Rouhan^a

^a Institut Systématique Evolution Biodiversité (ISYEB), Muséum national d'Histoire naturelle, CNRS, Sorbonne Université, EPHE, 57 rue Cuvier, CP39, 75005 Paris, France

^b Queensland Herbarium, Department of Environment and Science, Australian Tropical Herbarium, James Cook University, PO Box 6811, Cairns, Qld 4878, Australia

^c Department of Plant Taxonomy and Nature Conservation, University of Gdansk, ul. Wita Stwosza 59, 80-308 Gdansk, Poland



ARTICLE INFO

Keywords:

Biogeography
Molecular phylogeny
Lycophytes
Fossil
Southern Hemisphere
Western Indian Ocean

ABSTRACT

We explored the biogeographical history of a group of spore-bearing plants focusing on *Phlegmariurus* (Lycopodiaceae), a genus of lycophytes comprising ca. 250 species. Given its wide distribution in the Southern Hemisphere, *Phlegmariurus* provides a good model to address questions about the biogeographical processes underlying southern distributions, notably in Madagascar and surrounding islands, also called the Western Indian Ocean (WIO). Our aims were (i) to discuss the systematics of the Malagasy species in the light of molecular phylogenetic results, (ii) to provide the first dating analysis focused on *Phlegmariurus* and (iii) to understand the relative role of vicariance, dispersal and diversification in the origin of the Malagasy *Phlegmariurus* species.

The phylogenetic relationships were inferred based on three plastid DNA regions (*rbcL*, *trnH-psbA* and *trnL + trnL-trnF*) and on a dataset comprising 93 species, including 16 Malagasy species (80% of the total Malagasy diversity for the genus). Our results highlighted the need to combine Malagasy *Huperzia* species in *Phlegmariurus*, as well as the polyphyly of widely distributed species: *Phlegmariurus phlegmaria*, *P. squarrosus* and *P. verticillatus* with the Malagasy species not belonging with the types of *P. phlegmaria* or *P. squarrosus*. This led us to propose new circumscriptions of *Phlegmariurus* species, especially in the WIO.

Our dating analysis, relying on fossil calibrations, showed that *Phlegmariurus* would have originated in the Late Cretaceous and diversified in the Early Eocene. The biogeographical analysis highlighted uncertainties about the biogeographical origins of *Phlegmariurus*: the genus would have started to diversify in an ancestral range covering at least the Neotropics and Australasia. Hypotheses on the biogeographical history of *Phlegmariurus* were discussed, especially the roles of long distance dispersal, migration via Antarctica and via the Boreotropics. Six long distance dispersal events over the last 40 Ma would explain the Malagasy species diversity of *Phlegmariurus*, in combination with an important in situ diversification starting in the Miocene.

1. Introduction

Madagascar is a large 590,000 km² continental island of the Western Indian Ocean (WIO) renowned for its species richness and high level of endemism. It is considered to be one of earth's important biodiversity hotspots (Myers et al., 2000) with ca. 11,000 species of vascular plants, among which 82% are endemic (Callmander, 2011). Approximately 600 species of ferns and lycophytes are estimated to inhabit Madagascar, whereas 800 are estimated in Africa (Moran, 2008). The level of endemism is also relatively high with 45% endemic (Rakotondrainibe, 2003), more than the Galapagos (7%) (Moran, 2008), or New Caledonia (38%) (Morat et al., 2012; Munzinger et al.,

2016). The high species richness and level of endemism raise questions about the origin of the Malagasy lineages and the evolutionary processes leading to such diversity.

Madagascar has a Gondwanan origin, having separated from Africa 165 Ma and from India 95–84 Ma (McLoughlin, 2001) so ancient vicariant origins are plausible hypotheses to explain the unique divergent biota of Madagascar (Yoder and Nowak, 2006). However, the rise of molecular dating has highlighted more recent Cenozoic origins for most of the Malagasy lineages, suggesting that dispersal would be the main biogeographical process explaining the presence of modern Malagasy lineages (Yoder & Nowak, 2006; Agnarsson and Kuntner, 2012). Madagascar's biogeographical affinities have been found to be the highest

* Corresponding author at: Institut Systématique Evolution Biodiversité (ISYEB), Sorbonne Université, MNHN, CNRS, EPHE, 57 rue Cuvier, CP39, 75005 Paris, France.

E-mail address: lu.bauret@gmail.com (L. Bauret).

¹ The authors contributed equally.

with the nearest continent Africa for animals and for plants (Yoder & Nowak, 2006; Buerki et al., 2013) even though its separation from India is more recent than from Africa. In agreement with the prediction of the Theory of Island Biogeography (MacArthur and Wilson, 1967), Africa was found as a source of dispersal for numerous taxa towards Madagascar (e.g. Agnarsson and Kuntner, 2012; Bacon et al., 2016; Blair et al., 2015; Forthman and Weirauch, 2016; Zhou et al., 2012). However, long distance dispersal events (LDD) for plants and animals were also hypothesized from tropical Asia (Federman et al., 2015; Forthman and Weirauch, 2016; Warren et al., 2010) and in fewer events from the Neotropics, particularly for plants (Janssens et al., 2016; Smedmark et al., 2014). Studies on ferns have highlighted that the Neotropics are an important source pool of species diversity for Madagascar in vascular spore-bearing plants (e.g. Bauret et al., 2017a,b; Rouhan et al., 2004). Evidence of African dispersal events were also found (Korall and Pryer, 2014; Sessa et al., 2017), as well as Asian affinities (e.g. Lehtonen et al., 2010). LDD from the Neotropics to Madagascar has also been found in bryophytes (Scheben et al., 2016) but no biogeographical analyses focused on Malagasy lycophytes have been done so far.

Madagascar is also well known to be a place of diversification of its fauna and flora, notably thanks to its isolation and its past and current habitat heterogeneity (Janssen et al., 2008; Vences et al., 2009; Wood et al., 2015). Phylogenetic studies showed that dispersal events followed by diversification in situ is a major process in the generation of Malagasy endemic fern flora (Bauret et al., 2017a,b; Chao et al., 2014; Hennequin et al., 2017; Janssen et al., 2008; Labiak et al., 2014). It could be informative to test the monophyly of Malagasy lycophytes to observe if all the vascular spore-bearing plants are capable of such diversifications in Madagascar.

Phlegmariurus Holub is a pantropical genus of lycophytes in the family Lycopodiaceae, including about 250 species (PPGI, 2016), occurring in wet tropical forested and montane habitats of tropical regions. Two primary growth forms occur, hanging epiphytes in mesic forests and erect terrestrials in high montane non-forested areas, with the terrestrials being considered a derivation from ancestrally epiphytic species (Fig. 1; Field et al., 2016; Wikström et al., 1999). *Phlegmariurus* is a monophyletic genus, forming with *Huperzia* and *Phylloglossum* the Huperzioidae subfamily (Fig. 2B, Field et al., 2016).

The biogeographical history of lycophytes remains poorly understood with few studies being available. Worldwide biogeographical analyses were done recently on the cosmopolitan *Selaginella* (Klaus et al., 2016), revealing a Devonian origin in Euramerica and a diversification coinciding with the formation of Pangea, as well as on *Isoetes* focused on the Neotropics (Pereira et al., 2017). Pereira et al. (2017) showed that the earliest diversification of *Isoetes* took place after the breakup of Pangea but that most of the diversity has arisen at the Cretaceous-Palaeogene boundary. Another study on *Isoetes* was performed by Kim and Choi (2016) but on a dataset focused on North Pacific species. Huge uncertainties remain about the age of divergence of *Phlegmariurus*, with estimates in a large range of ages from 351 to 167 Ma (Larsén and Rydin, 2016; Wikström and Kenrick, 2001). These studies were however not focused on *Phlegmariurus*. A large diversification of *Phlegmariurus* has been recovered for the Neotropics and the Eastern Paleotropics (Asia, South-East Asia, Melanesia, Australasia and Pacific islands) (Field et al., 2016) but low sampling in the Western Paleotropics (Africa, Madagascar, Mascarenes, Seychelles, Comoros) leaves the overall pattern of diversification in the Paleotropics poorly understood. Indeed, only four Western Paleotropical species have previously been sampled out of the 27 estimated in this region (Table 1).

The Western Paleotropical region is outstanding in harboring one of the world's highest proportions of endemic *Phlegmariurus* with 90% of species being endemic. Madagascar harbors 20 out of the 27 species estimated in the Western Paleotropics and 14 of these are endemic to Madagascar (Table 1). The Malagasy species of *Phlegmariurus* exhibit a spectrum of morphological diversity capturing most of the forms found in epiphytic *Phlegmariurus* globally (Fig. 1). Therefore, based on

morphological evidence only, it is difficult to interpret if the Malagasy *Phlegmariurus* result from multiple dispersal events to Madagascar that have converged geographically or if these species have stemmed from an endemic diversification, adaptively exploring congruent habitats to those found elsewhere and thereby repeating the spectrum of adaptive morphologies.

The Western Paleotropical region is also unique for the genus *Phlegmariurus* as several species exhibit unusual apomorphies, in particular triangular shoots (Fig. 1, E,G) and fimbriate leaf margins (Fig. 1, I). Since the latter character is only found in two endemic species to Madagascar (*P. pecten* and *P. gagnepainianus*), those species were kept separate by Herter (1909) and Nessel (1939) in *Urostachys* (Pritz.) Herter subgenus *Eurostachys* Herter section *Selaginurus* Herter, suggesting an affinity with species now placed in the genus *Huperzia* s.s. rather than the genus *Phlegmariurus*. They were later placed in *Huperzia* subgenus *Tardieublottia* by Holub (1991) suggesting they were distantly related to all other species. Most recently, they were placed in *Phlegmariurus* Holub by Field and Bostock (2013), based on their lack of bulbils and relatively unornamented weakly foveolate isotetrahedral spore type. As they have never been placed in a molecular phylogenetic analysis, their evolutionary position remains unclear.

Our study aims to improve the systematics of the Malagasy *Phlegmariurus* and to unravel the biogeographical history of *Phlegmariurus*, especially regarding Madagascar. The precise goals are (i) to test the monophyly of Malagasy species and to uncover their phylogenetic relationships; (ii) to date Malagasy *Phlegmariurus* lineage (s) and (iii) to infer the biogeographical history of Malagasy species of the genus. As we investigated the biogeographical history of the Malagasy lineages in a worldwide framework, we also aim to discuss the biogeographical history underlying the pantropical distribution of *Phlegmariurus*.

2. Material and methods

2.1. Taxonomic sampling

We gathered the largest dataset on *Phlegmariurus* so far, including 135 specimens representing 93 ingroup species. We included 60 species from the worldwide phylogeny of Field et al. (2016) to provide a global framework plus a new dataset mostly focused on the WIO, especially Madagascar: 73 new specimens were added, representing 33 species (among which 28 were sequenced for the first time). Compared to the most recent phylogenetic study (Field et al., 2016), the entire dataset increased the Malagasy diversity from two to 16 species or 80% of the estimated diversity, including 9 out of the 14 Malagasy endemics (Table 1). Two outgroups were used: *Huperzia australiana* (Herter) Holub and *Phylloglossum drummondii* Kunze, known to form the sister-group to *Phlegmariurus* (Field et al., 2016; Ji et al., 2008; Wikström and Kenrick, 2001). This sampling was modified for dating and biogeography. Vouchers details and GenBank accession numbers are reported in Appendix 1.

2.2. DNA extraction, amplification and sequencing

DNA extraction was performed from silica-dried leaf material or, when such material was not available, from herbarium specimens. The Qiagen DNeasy Plant Mini Kit (Valencia, California, USA) was used and we modified the protocol for herbarium specimens by adding 30 μ L proteinase K (20 mg/mL) and 30 μ L beta-mercaptoethanol for the initial lysis step, which was carried out at 42 °C during 24 h.

We amplified three plastid regions, the gene *rbcl*, *trnL* intron plus *trnL-trnF* spacer (*trnL-trnLF*) and the intergenic spacer *trnH-psbA*. All PCRs were carried out in 25 μ L containing 1 \times PCR buffer, 2.5 mM MgCl₂, 250 μ M of each dNTP, 1 M betaine, 0.4 μ M of each primer, 0.75 U Taq polymerase (Taq CORE kit; MP Biomedicals, Illkirch, France), and 1 μ L of template, non-diluted genomic DNA. Primer



Fig. 1. A–F: Habitat diversity of *Phlegmariurus* in Madagascar, (A) *Phlegmariurus gnidioides*; (B) *Phlegmariurus lecomteanus*; (C) *Phlegmariurus megastachyus*; (D) *Phlegmariurus obtusifolius*; (E) *Phlegmariurus obtusifolius*, (F) *Phlegmariurus ophioglossoides*; G–I: diversity of microphyll shapes: (G) in *Phlegmariurus obtusifolius*, (H) *Phlegmariurus lecomteanus* and (I) *Phlegmariurus pecten*. Photos: G. Rouhan (D, E, F), L. Bauret (A–C, G–I).

sequences and thermal cycling conditions are reported in Table 2. PCR products were checked on a 1% agarose gel and sequenced in both directions by Eurofins (Evry, France) using the amplification primers and additional internal primers for *rbcL* (Table 2). DNA strands were edited and assembled in Sequencher 4.9 (Gene Codes Corporation) and the resulting consensus sequences were submitted to GenBank (accession numbers in Appendix 1).

2.3. Phylogenetic analyses

The sequences were aligned with Muscle 3.8.425 (Edgar, 2004), and the resulting alignments were checked by eye and revised manually. After analysis of each marker independently and control of conflicts

between the topologies, the data matrix was built by concatenation of the three alignments with Sequence Matrix 1.7.8 (Vaidya et al., 2011). Each DNA region constituted an independent partition in the final dataset. Gaps were treated as missing data.

Phylogenetic trees were inferred based on Maximum Likelihood (ML) and Bayesian Inference (BI) approaches, as respectively implemented in RAxML-HPC2 8.2.6 (Stamatakis, 2014) and MrBayes 3.2.6 (Ronquist et al., 2012). The analyses were conducted on the CIPRES science gateway (Miller et al., 2010).

For the BI, we used a Metropolis-coupled Markov chain Monte Carlo method implemented in MrBayes 3.2.6 (Ronquist et al., 2012). For each region, a reversible jump MCMC (rjMCMC, Huelsenbeck et al., 2004) was used with MrBayes to find the best suited model of nucleotide

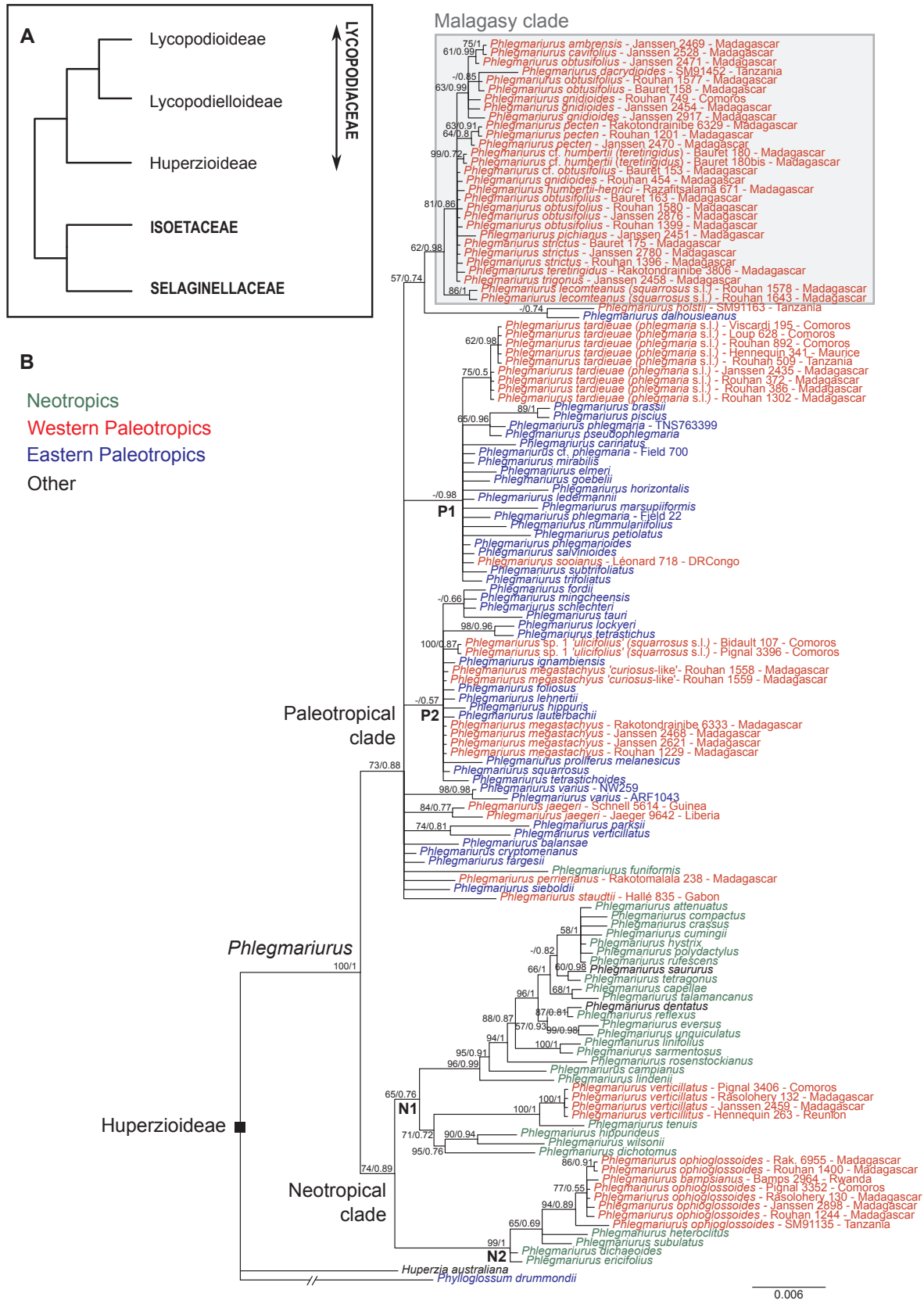


Fig. 2. (A) Simplified tree of Lycopodiaceae, based on Field et al. (2016). (B) Majority rule consensus tree of *Phlegmariurus* estimated by Bayesian inference on the combined cpDNA dataset (*rbL*, *trnH-psbA*, *trnL-trnLF*), with support values from the Bayesian inference and Maximum likelihood methods at each node (BS/PP). A dash (–) stands for BS < 50 or a non-retrieved node. A color code indicates the region of sampling: green for the Neotropics, red for the Western Paletropics, blue for the Eastern Paletropics and black for other regions (temperate North Hemisphere or wide distribution covering several regions). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
 African and Western Indian Ocean Huperzioidae considered in this study, their distribution and systematic notes. Species sampled are in bold, with an asterisk for the sampled localities in our molecular study.

	Madagascar	Mascarenes	Seychelles	Comoros	Continental Africa	Notes
<i>P. acutifolius</i> (Desv. ex Poir.) A.R. Field & Testo		1	1		1	= <i>Lycopodium acutifolium</i> and <i>Huperzia epiceifolium</i> , in Roux (2009) = <i>Phlegmariurus squarrosus</i> in Mascarenes and Seychelles, in Field & Bostock (2013)
<i>P. ambrensis</i> (Rakotondr.) A.R. Field & Bauret	1*					Only known from type collection. Possibly conspecific with <i>Phlegmariurus megastachyus</i>
<i>P. cavifolius</i> (C. Chr.) A.R. Field & Bostock	1*	1				
<i>P. curiosus</i> (Herter) A.R. Field & Bostock	1					
<i>P. dacrydioides</i> (Baker) A.R. Field & Bostock	1			1*		
<i>P. gagepauianus</i> (Herter) A.R. Field & Bostock	1					
<i>P. gnidioides</i> (L.f.) A.R. Field & Bostock	1*	1	1	1*	1	Also known in French Southern and Antarctic Lands = <i>Huperzia squarrosa</i> in Madagascar, in Rakotondrainibe & Jouy (2015) = <i>Huperzia hildebrandtii</i> , in Roux (2009) Only known from type specimen.
<i>P. hildebrandtii</i> (Herter) A.R. Field & Bauret	1					
<i>P. holstii</i> (Hieron.) A.R. Field & Bostock					1*	
<i>P. humbertii</i> (Nessel) A.R. Field & Bostock	1*					
<i>P. humbertii-henrici</i> (Herter) A.R. Field & Bostock	1*					
<i>P. lecomiteanus</i> (Nessel) A.R. Field & Bostock	1*					
<i>P. megastachyus</i> (Baker) A.R. Field & Bostock	1*					
<i>P. obtusifolius</i> (P. Beauv.) A.R. Field & Bostock	1*	1				
<i>P. ophioglossoides</i> (Lam.) A.R. Field & Bostock	1*	1	1	1*	1*	
<i>P. pecten</i> (Baker) A.R. Field & Bostock	1*					
<i>P. perrierianus</i> (Tardieu) A.R. Field & Bostock	1*					
<i>P. phlegmaria</i> (L.) T. Sen & U. Sen					?	Also known in Asia*, Australasia*
<i>P. pichianus</i> (Tardieu) A.R. Field & Bostock	1*					Taxonomic revision needed, possibly several species under the name, type from Mauritius, also known from French Southern and Antarctic Lands*
<i>P. saururus</i> (Lam.) B. Øllg.		1		?	1	
<i>P. standtii</i> (Nessel) A.R. Field & Bostock					1*	
<i>P. strictus</i> (Baker) A.R. Field & Bostock	1*					
<i>P. tardieuana</i> (Herter) A.R. Field & Testo	1*	1*		1*	1*	= <i>Huperzia phlegmaria</i> var. <i>tardieuana</i> in Roux (2009) = <i>Huperzia phlegmaria</i> in Africa and Mauritius in Roux (2009)
<i>P. trigonus</i> (C. Chr.) A.R. Field & Bostock	1*					Segregated from <i>Phlegmariurus squarrosus</i> in Comoros, morphologically close to <i>P. ulicifolius</i> (India)
<i>P. sp1 "ulicifolius"</i> (Sw.) S.R. Ghosh	1*			1*		
<i>P. verticillatus</i> (L.f.) A.R. Field & Testo	1*	1*		1*	1	
<i>P. xiphophyllus</i> (Baker) A.R. Field & Bostock	1					<i>Huperzia</i> s.s., in Field et al. (2016) <i>Huperzia suberecta</i> in Roux (2009) Presence in the tropics doubtful. Several literature records but no specimen
<i>H. suberecta</i> (Lowe) Tardieu	?	?	?	?	?	27 species estimated for the Western Palearctic
Total	20	8	3	5	9	

Table 2

PCR primers sequences and thermal cycling conditions; primers only used as internal primers for sequencing are marked with an asterisk (*).

DNA region	Primer name	Literature reference	Primer sequence (5'–3')	Thermal cycling conditions
<i>rbcl</i>	ESRBCL1F	Schuettpelz and Pryer (2007)	ATG TCA CCA CAA ACG GAG ACT AAA GC	5 min 94 °C/40 × (30 s 94 °C/45 s 45–50 °C/2.5 min 72 °C)/10 min 72 °C
	ESRBCL1361R	Schuettpelz and Pryer (2007)	TCA GGA CTC CAC TTA CTA GCT TCA CG	
	ESRBCL628F*	Schuettpelz and Pryer (2007)	CCA TTY ATG CGT TGG AGA GAT CG	
	ESRBCL654R*	Schuettpelz and Pryer (2007)	GAA RCG ATC TCT CCA ACG CAT	
<i>trnH-psbA</i>	trnH2	Tate (2002)	CGC GCA TGG TGG ATT CAC AAT CC	5 min 94 °C/40 × (30 s 94 °C/45 s 48 °C/45 s 72 °C)/10 min 72 °C
	psbAF	Sang et al. (1997)	GTT ATG CAT GAA CGT AAT GCT C	
<i>trnL-trnLF</i>	c	Taberlet et al. (1991)	CGA AAT CGG TAG ACG CTA CG	5 min 94 °C/40 × (30 s 94 °C/30 s 48–50 °C/45 s 72 °C)/10 min 72 °C
	Fern-1	Trewick et al. (2002)	ATT TGA ACT GGT GAC ACG AG	

substitution and we allowed the rjMCMC to move across models with +I+Γ. Two independent but parallel analyses using ten million generations each were conducted, with four chains and a temp set as 0.05 in *mcmc* command. The trees were sampled every 1000 generations to obtain 10,000 sampled trees. We used Tracer 1.6.0 (Rambaut and Drummond, 2013) to check the output parameter estimates through time, and to estimate the point of convergence to the stationary distribution of the two runs: the 25% initial trees were discarded as burn-in. A 50% majority-rule consensus was computed based on the post-burn-in trees with average branch lengths and posterior probability (PP) estimates for all nodes.

The ML analysis was performed with RAXML-HPC2 8.2.6 (Stamatakis, 2014), using the same three partitions as in MrBayes. Parameters for the RAXML analysis were determined on the CIPRES interface: we used the GTRGAMMA model of nucleotide substitution, performed 1,000 rapid bootstrap (BS) replicates and searched for the best-scoring ML tree.

The BI 50% majority-rule consensus tree and ML best tree were visualized in FigTree 1.4.2 (Rambaut, 2014).

2.4. Fossil calibration and divergence time estimation

The dataset was modified to perform the dating analyses by first reducing the matrix to 96 specimens representing the 91 sampled *Phlegmariurus* species. We kept one sample per species or one per lineage when the species were retrieved as polyphyletic. We then added 22 outgroup species for dating and biogeographical analyses: *Phylloglossum drummondii*, 16 *Huperzia* species, *Lycopodiella inundata* (L.) J. Holub from the Lycopodielloideae (*sensu* PPGI, 2016), *Diphazium jussiaei* (Desv. ex Poir.) Rothm. from the Lycopodioidae (*sensu* PPGI, 2016), *Isoetes flaccida* Shuttlew. and two *Selaginella* species.

Although fossils of vegetative structures have been ascribed to the genus *Huperzia* s.l. (meaning *Huperzia* and *Phlegmariurus*), they could not be confidently placed within the Huperzioidae and used as reliable calibrations points. Morphological characters of *Huperzia* s.l. have generally been considered to be plesiomorphic, leading analyses to place fossils of *Huperzia* s.l. at a basal position within lycophytes

(Bateman, Kenrick, & Rothwell, 2007; Xue, 2011). This basal position is in contradiction with molecular based phylogenies in which *Huperzia* s.l. is found nested in Lycopodiaceae and exhibit singular vegetative synapomorphies that could be preserved in fossils (Field et al., 2016). Especially, cortical root emergence is a synapomorphy of Huperzioidae and bulbils in the axils of sporophylls is a synapomorphy of *Huperzia* (Field et al., 2016), but these features are presently unknown in the fossil record. Vegetative bulbils are present in the Pragian *Hueberia zhichangensis* Yang, Li & Edwards (Xue, 2013; Yang et al., 2009), but this species exhibits anisotomous branching, rounded sporangia and bulbils that are not associated with sporophylls, contrasting with *Huperzia* which exhibit isotomous branching, and reniform sporangia and bulbils that are strictly associated with sporophylls.

Huperzioidae are confidently known from spore fossils similar to modern spores of *Phlegmariurus phlegmaria* (L.) Holub and *Huperzia selago* (L.) Bernh., from the fossil record of New Zealand and Australia (Cieraad & Lee, 2006). The spore fossil *Foveotriletes lacunosus* Partridge is a *Phlegmariurus phlegmaria* type spore that has a relatively continuous record of presence in New Zealand and Australia from the Chatian Oligocene (28.1 Ma) to present day (Cieraad and Lee, 2006). However, both *Phylloglossum drummondii* and all species of *Phlegmariurus* share the *Phlegmariurus phlegmaria* spore type, and it is therefore not possible to place *Foveotriletes lacunosus* in either position as a calibration point. The spore fossil *Foveotriletes palaequestrus* is a *Huperzia selago* type spore that is reported to have a relatively continuous record of presence in New Zealand and Australia from the Lutetian Eocene (47.8 Ma), merging with present day *Huperzia australiana* (Cieraad and Lee, 2006). Some doubts exist as to the earliest record of *F. lacunosus* spores (Cieraad and Lee, 2006) and furthermore it is not possible to determine if it should be placed with *Huperzia australiana* or elsewhere within *Huperzia* as all species share the *Huperzia selago* spore type.

To fill this gap in useable Huperzioidae fossils, we calibrated our analysis with four reliable outgroup fossils, summarized in Table 3. The maximum height of our lycophytes tree was defined on the occurrences of the earliest lycophytes, like *Asteroxylon mackiei* (Kidston and Lang, 1920), *Hueberia zhichangensis* (Bateman et al., 2007; Xue, 2013; Yang et al., 2009) and *Zhenglia radiata* (Hao et al., 2006). These fossils were

Table 3

Calibrated nodes for the dating analysis with BEAST.

Node calibrated	Uniform prior values (Ma)	Fossils	Stratigraphic occurrence age (Ma)
Tree height	382.7–410.8	Earliest lycophytes fossils, e.g. <i>Asteroxylon mackiei</i> (Kidston and Lang, 1920), <i>Hueberia zhichangensis</i> (Bateman et al., 2007; Xue, 2013; Yang et al., 2009) and <i>Zhenglia radiata</i> (Hao et al., 2006)	Lower Devonian, Pragian, 407.6–410.8
<i>Isoetes-Selaginella</i> divergence	382.7–410.8	<i>Yuguangia ordinata</i> Hao et al. (2007)	Middle Devonian, Givetian, 382.7–387.7
<i>Selaginella</i> crown node	330.9–410.8	<i>Selaginella resimus</i> Rowe (1988)	Carboniferous, Visean, 330.9–346.7
Lycopodielloideae-Lycopodioidae divergence	190.8–410.8	<i>Retitriletes</i> spp. Bomfleur et al. (2014)	Lower Jurassic, Sinemurian, 190.8–199.3

dated in the Pragian Devonian (407.6–410.8 Ma, Lower Devonian) and the lower limit of this period was used to constrain the maximum age of lycophytes. The lycophyte *Baragwanathia longifolia* has been reported as occurring as early as the Silurian, but doubts remain for some authors on the earliest stratigraphic occurrence (Hueber, 1992), so we kept the more reliable Pragian age.

The minimum age of divergence between the Isoetales and the Selaginellales was defined with the occurrence of *Yuguangia ordinata*, placed by a cladistic analysis in the clade including *Isoetes* and *Selaginella* (Hao et al., 2007). This fossil was found in the Givetian (382.7–387.7 Ma, Middle Devonian) and the upper limit of the period was used as minimum age and the maximum tree height as the maximum age.

We calibrated the minimum age of the *Selaginella* crown node with *Selaginella resimus*, a fossil already used in Klaus et al. (2016) for their *Selaginella* dating and occurring in the Visean (330.9–346.7 Ma, Carboniferous). We defined the upper limit of the Visean as the minimum age.

The minimum age of divergence between Lycopodielloideae and Lycopodiodeae was defined on the oldest confident occurrence of fossils assigned to this node with *Retitriletes* spp. (Bomfleur et al., 2014), in the Sinemurian (190.8–199.3 Ma, Lower Jurassic). The upper stratigraphic limit was chosen as the minimum age. *Retitriletes* is a spore genus of isotetrahedral spores with distinct reticulate ornamentation present on the distal surface that makes it closely comparable with the reticulate distal surface structure in the *Lycopodium clavatum* spore group that is a synapomorphy of modern Lycopodiodeae species (but lost in *Pseudolycopodium* Holub and *Pseudodiphazium* Holub) (Juhász, 1975; Field et al., 2016).

We performed the dating analyses using BEAST v2.4.2 (Drummond et al., 2012) on the CIPRES science gateway, the same partitions and research of models of nucleotide substitution used for MrBayes analysis were defined. The package RBS 1.3.1 was installed in BEAST to implement the rjMCMC research. We used an uncorrelated lognormal relaxed clock with a Birth-Death tree prior and a random starting tree. The fossil calibration points were all set following a uniform prior with values summarized in Table 3. We conducted four runs of 200 million generations with random seed values and sampled every 20,000 generations to obtain 10,000 sampled trees. Tracer 1.6.0 was used to confirm the convergence of the estimated parameters and to define the burn-in at 10%. The post burn-in trees from the four runs were combined using LogCombiner 2.4.2, the maximum clade credibility trees using TreeAnnotator v2.4.2 and visualized with the associated chronogram using FigTree 1.4.2 (Rambaut, 2014).

2.5. Ancestral area estimation

To reconstruct the biogeographical history, we estimated the ancestral ranges using the R package BioGeoBEARS (Biogeography with Bayesian Evolutionary Analysis in R Scripts, Matzke, 2013) in R studio 1.0.44 (RStudio Team, 2015). It implements in a likelihood framework three commonly used methods: Dispersal–Extinction–Cladogenesis (DEC; Ree and Smith, 2008), Dispersal–Vicariance Analysis (DIVA; Ronquist, 1997; DIVALIKE in BioGeoBEARS) and Bayesian Inference for Discrete Areas (BayArea; Yu et al., 2015; BAYAREALIKE in BioGeoBEARS). Three analyses using each method (DEC, DIVALIKE, BAYAREALIKE) were performed plus three including the additional parameter *j* (DEC+*j*, DIVALIKE+*j*, BAYAREALIKE+*j*). The '*j*' parameter adds the process of a founder event speciation, when a daughter lineage jumps to a new range outside the range of its ancestor. The Akaike Information Criterion (AIC) was then used to select the best-suited model, and to infer the ancestral ranges along the phylogeny of *Phlegmariurus*.

Eight areas were defined to code the distribution of the species (see map, Fig. 3): a large and mostly temperate Northern hemisphere area, including North America, Iceland, Europe, Macaronesian Islands and

temperate Asia (up to 25°N); a temperate to boreal circumpolar area above 40°S, including Southern South America, Tristan da Cunha archipelago, French Southern and Antarctic lands (Crozet, Kerguelen, Amsterdam) and Macquarie Island; the Neotropics (30°N to 40°S); continental and tropical Africa; Madagascar; Mascarenes, Comoros and Seychelles archipelagos; tropical Asia, including the continental part (up to 25°N), Sri Lanka, Taiwan and Southeast Asia (Malaysia, Philippines and Indonesian islands West of Lydekker's line); Melanesia-Australasia-Oceania (later referred as Australasia). Each sample was coded following the distribution of the species (except for geographic polyphyletic lineages of *Phlegmariurus squarrosus*), based on type citations, herbarium occurrences observed in the herbaria AAU, B, BHCB, BONN, BR, BRI, CANB, CNS, HO, JCT, KLU, P, PR, PRC, QRS, SING, TUD and US (herbarium codes following The Index Herbarium, Thiers) as well as flora and personal observations (A.R. Field). The maximum number of areas coded was set to four as it was the maximum number of areas coded for a tip.

3. Results

3.1. Phylogeny

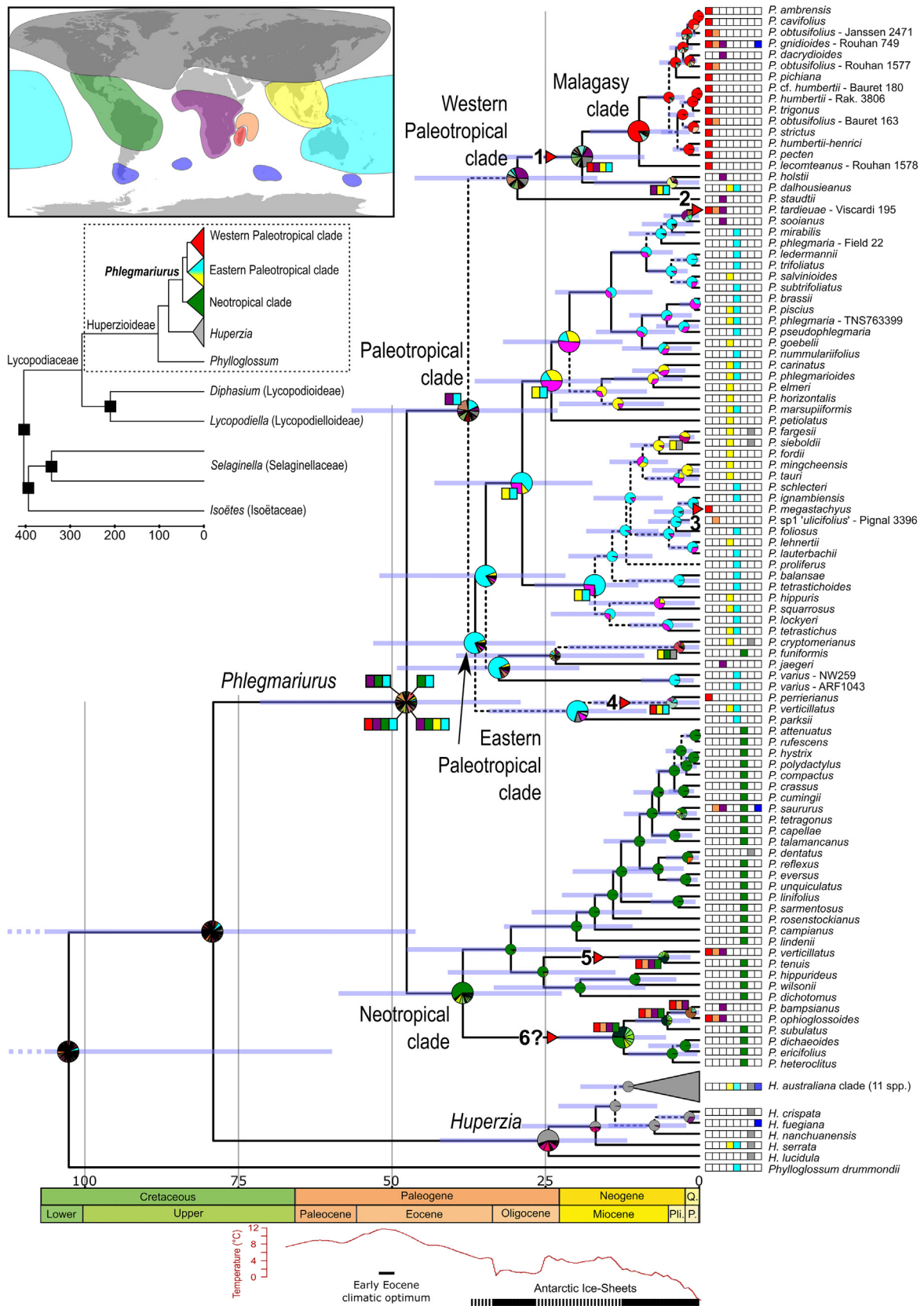
3.1.1. Overall topology

The combined dataset was composed of 137 specimens and 2890 characters; summary statistics of the dataset are available in Table 4. BI and ML topologies were similar with a well-supported *Phlegmariurus* clade (BS = 100; PP = 1) nesting two clades with less support (Fig. 2). Firstly, the Neotropical clade (BS = 74; PP = 0.89) included mostly Neotropical species and two Paleotropical lineages: (1) the Western Paleotropical *Phlegmariurus verticillatus* (in clade N1) and (2) one lineage including the African species *Phlegmariurus bampsianus* and the Western Paleotropical *P. ophioglossoides* (in clade N2). The Macaronesian *P. dentatus* was also retrieved in the Neotropical clade as well as the wide-ranging *P. saururus*. Secondly, the Paleotropical clade (BS = 73; PP = 0.88) nested the remaining two thirds of the sampled diversity with predominantly Paleotropical species and including only one Neotropical species, *P. funiformis* (Fig. 2). Overall the topology inferred was similar to the most recent worldwide phylogenetic study on *Phlegmariurus* (Field et al., 2016) but the addition of numerous Western Paleotropical species resulted in a completely new clade (Malagasy clade), included in the Paleotropical clade (Fig. 2). The newly sampled Western Paleotropical species were inserted in eight distinct positions, both in the Malagasy clade and across Neotropical and Eastern Paleotropical clades (Fig. 2). Within the Paleotropical clade, several species were arranged in a polytomy or among one of three Paleotropical clades, the Malagasy, P1 and P2 clades.

3.1.2. Composition of the two major clades

The Neotropical clade included two clades, N1 (*P. attenuatus* to *P. dichotomus*; BS = 65; PP = 0.76; Fig. 2) and N2 (*P. ophioglossoides* to *P. ericifolius*; BS = 99; PP = 1), with both clades including species from Western Paleotropics. All six species within N2 are hanging epiphytes with markedly heterophyllous bifurmic shoots with leafy fertile shoots terminated by funiforme-filiforme fertile spikes, whereas clade N1 includes a spectrum of heterophyllous to homophyllous hanging epiphytes, facultative epiliths and erect terrestrials.

This arrangement is mirrored in the Paleotropical clade which includes two morphologically equivalent clades both including species from the Western Paleotropics. All of the species within the moderately supported clade P1 (*P. tardieuae* to *P. trifoliatus*, BS < 50; PP = 0.98, Fig. 2) are strongly heterophyllous bifurmic hanging epiphytes with leafy fertile shoots and funiforme to filiform fertile spikes and by comparison the poorly supported clade P2 (*P. fordii* to *P. tetrastichoides*, BS < 50; PP = 0.57, Fig. 2) includes a spectrum of heterophyllous to homophyllous hanging epiphytes, facultative epiliths and facultative terrestrials.



(caption on next page)

Fig. 3. Chronogram resulting from the BEAST analysis for Huperzioideae and ancestral areas estimated by BioGeoBEARS (DEC+j model). Horizontal blue bars correspond to 95% HPD intervals of the median ages. Dashed branches represent non-supported clades (PP < 0.5). Next to terminals, colored squares depict biogeographical distribution considering eight areas: temperate Northern Hemisphere (grey), circumpolar (dark blue), Neotropics (green), continental and tropical Africa (violet), Madagascar (red), Mascarenes-Comoros-Seychelles islands (orange), tropical Asia (yellow), Australasia-Oceania (light-blue). Colored pies represent the ancestral areas estimated at nodes. When associated to a pie chart section, colored squares represent the combination of areas covered by an estimated range (for the sake of clarity, only the most likely ranges were depicted). These combinations do not necessarily represent the most likely ancestral range estimated at a node. A reduced topology represents the entire BEAST chronogram, simplified in Huperzioideae, with black squares depicting the four fossil calibration points. The stratigraphy follows the 2017 International Chronostratigraphic Chart, the global temperature and the climatic events were mapped following Zachos et al. (2001). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 4

Statistics on the three separate plastid DNA regions and the combined dataset.

	Number of sequences	Aligned length (in base pairs)	Percent of variable characters	Percent of informative characters
<i>rbcl</i>	88	1349	11.0	5.9
<i>trnH-psbA</i>	102	297	28.6	15.2
<i>trnL/trnL-trnF</i>	99	1244	32.2	19.1
Whole matrix	137	2890	22.0	12.5

3.1.3. Composition of the Malagasy clade

This study was the first to recover a clade comprised entirely of Western Paleotropical species (Malagasy clade, Fig. 2) with 11 out of the 20 species estimated in Madagascar (including the enigmatic *P. pecten*). However, most of the phylogenetic relationships within the Malagasy clade were unresolved. In our phylogeny, *P. pecten* was inserted among *P. gnidioides* and *P. obtusifolius*, confirming it was a *Phlegmariurus* and not a *Huperzia* s.s. The most recently described species of *Huperzia* from Madagascar, *H. ambrensis* Rakotondr. and *H. teritrigida* Rakotondr. were also inserted in this clade of *Phlegmariurus* (Fig. 2). The Malagasy clade included many morphologically dissimilar WIO endemics such as *P. ambrensis*, *P. cavifolius*, *P. lecomteanus*, *P. obtusifolius*, *P. pecten*, *P. pichianus* which repeat the morphological spectrum of *Phlegmariurus* found elsewhere in the Paleotropics. This clade included both heterophyllous hanging epiphytes, homophyllous epiliths and facultative terrestrials. The morphologically singular species *P. gnidioides* and *P. obtusifolius* were each recovered as non-monophyletic or unresolved. Two large robust nearly homophyllous hanging epiphytes, the tropical east African endemic *P. holstii* and the Asian-Oceanian *P. dalhousieanus* were placed as sister to the Malagasy clade, each inserted on a long branch (Fig. 2).

3.1.4. Focus on the Western Paleotropical species

The phylogenetic hypotheses inferred were not fully resolved to species level and many closely related species were placed in polytomies, and where structure was recovered, several species were polyphyletic (Fig. 2). *Phlegmariurus bampsianus* was retrieved nested in a clade among *P. ophioglossoides* collections (= *P. rubricus sensu Tardieu-Blot, 1970*; and *P. rubricus + P. ophioglossoides sensu Field & Bostock, 2013*) (clade N2, Fig. 2). *Phlegmariurus verticillatus* s.l. was polyphyletic with specimens sampled from the Mascarenes type population of *P. verticillatus* inserted in the Neotropical clade with Malagasy and Comoros specimens, whereas specimens sampled from the Asian region were inserted in the Paleotropical clade (Fig. 2). Also, *P. squarrosus* s.l. is polyphyletic, being inserted in three places in the tree recovered, in the Malagasy clade and in two positions in P2 clade. These collections were re-identified in the strict sense as *P. lecomteanus* from Madagascar (Malagasy clade), *P. sp1 'ulcifolius'* from Comoros (P1 clade) and *P. squarrosus* from the Oceanian region (P1 clade). *Phlegmariurus lecomteanus* is readily distinguished from *P. squarrosus* by having a red stem wall and non-twisted leaves (Fig. 1, H) but is otherwise divergent from the Malagasy species it is related to. Similarly, *P. phlegmaria* s.l. (e.g. Chinnock, 1998; Roux, 2009) was also recovered as polyphyletic but all collections were inserted in the P1 clade. All *P. phlegmaria* group species from Western Paleotropics formed an endemic clade that is re-identified in the strict sense as *P. tardieuae* (Fig. 2). Several species were

not retrieved in the Malagasy, P1 or P2 clades of the Paleotropical clade, notably *P. cryptomerianus*, *P. jaegeri*, *P. parksii* a species with *P. verticillatus*-like morphology and *P. perrierianus* a species with a *P. phlegmaria*-like morphology (Fig. 2).

3.2. BEAST topology and divergence time estimates

Huperzia and *Phlegmariurus* were found in the same clade (PP = 0.87, Fig. 3) and strongly supported as monophyletic respectively (PP = 1). *Phylloglossum drummondii* was retrieved as a sister species to the *Huperzia-Phlegmariurus* clade (PP = 1). Within *Phlegmariurus*, the topology did not contradict the MrBayes topology and brought more resolution, however it was often weakly supported in several clades, in particular in the Paleotropical clade.

Based on the four calibration points derived from fossils, our analysis estimated that Lycopodiaceae have originated in the Lower Devonian at 404.1 Ma (95% Highest Posterior Density (HPD): 390.7–410.8), and to have started to diversify during the Permian at 273.6 Ma (HPD: 213.6–344.2) when Huperzioideae diverged from a lineage including Lycopodielloideae and Lycopodioideae. *Phylloglossum* would have diverged from *Phlegmariurus-Huperzia* 102.6 Ma (HPD: 59.8–160.3) in the Lower Cretaceous, whereas *Phlegmariurus* would be estimated to diverge from *Huperzia* during the Upper Cretaceous 79.1 Ma (HPD: 46.2–122; Fig. 3). These two genera would have started their diversification during the Eocene at 47.6 Ma (HPD: 29.1–71.5) for *Phlegmariurus* and more recently during the Oligocene at 24.5 Ma (HPD: 11.7–42.2) for *Huperzia*.

3.3. Ancestral area estimation

The biogeographical model DEC+J conferred the highest likelihood on the data and the best AIC (LnL = -289.7; AIC = 585.4; followed by DEC model with LnL = -296.3; AIC = 596.6) and the inferred ranges were plotted on the BEAST chronogram (Fig. 3).

Uncertainties between estimated ranges were found at the *Phlegmariurus* crown node, as well as the node of divergence between *Huperzia* and *Phlegmariurus*. Indeed, numerous ranges were found with small probabilities ($p < 0.06$, Appendix 2). Most likely, *Huperzia* started to diversify in the temperate North Hemisphere ($p = 0.55$) and *Phlegmariurus* in an uncertain and wide range involving Neotropical and Paleotropical regions: the four most likely inferred ranges were Africa-Neotropics-Australasia ($p = 0.10$), Madagascar-Africa-Neotropics-Australasia ($p = 0.09$), Neotropics-Australasia ($p = 0.08$) and Africa-Neotropics-Asia-Australasia ($p = 0.07$).

Six range expansions by long distance dispersal would explain the species diversity of *Phlegmariurus* in Madagascar for the last ca. 30 Ma,

but with uncertainties as wide ancestral ranges were estimated (except LDD3): from Africa 26.6–19.1 Ma but with Asian-Australasian uncertainties (LDD1), most likely from Africa 2.0–0.0 Ma (LDD2), from Australasia 1.0 Ma (LDD3), from Australasia 19.8–4.4 Ma but with an Asian uncertainty (LDD4) and from the Neotropics 25.3–5.7 Ma (LDD5) and 38.5–12.3 Ma (LDD6) but with Western Paleotropical uncertainties. Concerning LDD6, it is uncertain if the dispersal event to Madagascar occurred between 38.5–12.3 Ma or 12.3–5.2 Ma. A diversification starting exclusively in Madagascar was estimated in the Malagasy clade ca. 10 Ma. It was the only diversification estimated in Madagascar for extant *Phlegmariurus*, and so for Huperzioideae.

4. Discussion

4.1. Systematics

The phylogeny presented here provides evidence to address several systematics issues in *Phlegmariurus* of the Western Paleotropics. Firstly, we establish the placement of *P. pecten* in the Malagasy clade of Paleotropical *Phlegmariurus* and we reject the need for its placement in the subgenus *Tardieublotia* Holub *sensu* Holub (1991) or *Huperzia* *s.s.* (*sensu* Herter, 1909; Nessel, 1939). The unusual fringed leaf margins of *P. pecten* (and its close relative or synonym *P. gagnepainianus*) (Fig. 1, I) appears to be an apomorphy not found elsewhere in the genus *Phlegmariurus* and is not indicative of a relationship with *Huperzia*.

Secondly, we show that the recently described species *H. ambrensis* Rakotondr. and *H. teretirigida* Rakotondr. are inserted in the Malagasy clade diversification of *Phlegmariurus*, so we propose a combination in *Phlegmariurus*. We also synonymise *H. teretirigida* with *Phlegmariurus humbertii* (Nessel) A.R.Field & Bostock based on examination of the types. Both *H. ambrensis* and *H. teretirigida* were correctly placed in the *P. gnidioides* group by Rakotondrainibe and Jouy (2015) and are well supported by morphological similarity as they share slightly enrolled leaf margins with many species in this group.

Thirdly, based on our results we corroborate the findings of Wikström et al. (1999) and Field et al. (2016) that *P. phlegmaria* s.l., *P. squarrosus* s.l., and *P. verticillatus* are polyphyletic taxa. We prefer a classification that separates the Eastern Paleotropical *P. squarrosus* from *P. sp1 'ulicifolius'* and *P. lecomteanus*, and we define *P. tardieuae* as an endemic Malagasy species, distinct from the Western Paleotropical-Asian *P. phlegmaria*. We also separate the Asian-Oceanian *P. verticillatus* from the Western Paleotropical type population of *P. verticillatus* s.s. which is nested in the Neotropical clade.

Future sampling in the Malagasy region should focus on obtaining *P. xiphophyllus*, which is the only Malagasy member of the Indian-Asian *P. hamiltonii* group (H2, Øllgaard, 1987) and potentially represents another LDD to Madagascar, from the Himalayan region. It should also focus on relocating the long-missing *P. hildebrandtii* which is of uncertain affinity and possibly related to the *P. reflexus* group from the Neotropics (Øllgaard, 1989). Future sampling in the African region should focus on bifurcated species occurring in Africa such as *P. afro-montanus* and *P. ellenbeckii*. Based on their morphology it is expected that these species belong to the Neotropical clade bifurcated clade including *P. ophioglossoides* and their inclusion in future sampling could ascertain whether they represent a single or multiple trans-Atlantic dispersals to Africa. Future sampling across the Atlantic and Indian Oceanic regions should include the many disparate and disjunct populations of the morphologically heterogeneous *P. saururus* to ascertain if this species is the result of a single or several long-distance dispersals from the Andean region.

4.2. Age estimates

Our study provided the first dating analysis focused on *Phlegmariurus*, showing that despite a Cretaceous divergence, most of the extant diversity has arisen from diversifications starting during the

Eocene. Huperzioideae were already estimated to have diverged 167 Ma and diversified 62 Ma by a study focused on *Isoetes* (Larsén and Rydin, 2016). These ages are younger than our results (273.6 Ma and 102.6 Ma, respectively) and could be explained by several reasons such as an under-sampling, also discussed by Linder et al. (2005) and Testo and Sundue (2016), or a different priors setting (e.g. Warnock et al., 2014). Our results are however much younger than ages estimated by Wikström and Kenrick (2001), who found respectively 351 Ma and 265 Ma based on a nonparametric rate smoothing analysis and *rbcL* sequences alone. They also estimated the divergence between the Neotropical and Paleotropical clade at 184 Ma, against 47.6 Ma in our results. It is however hard to define without additional analyses why they found substantially older ages, with many parameters being different between their analysis and ours, such as the calibrations used and the species and markers sampled. The use of different analytical methods has also showed significant differences in molecular dating (Grimm et al, 2015).

4.3. Biogeographical history of *Phlegmariurus*: three hypotheses

Phlegmariurus is a genus distributed across the Southern Hemisphere but its species diversity is geographically clustered in three major clades, the Neotropical clade, the Western and the Eastern Paleotropical clades, which would have respectively started to diversify into the Neotropics, Africa, and Australasia since the middle of the Eocene. Our biogeographical analysis failed to estimate with confidence the ancestral area of *Phlegmariurus*, as well as its ancestral area of diversification. Given these uncertainties, three biogeographical processes need to be examined to explain the current, mostly Southern distribution of *Phlegmariurus*.

4.3.1. The direct dispersal hypothesis

Dispersal is a hypothesis often used to explain the distribution of spore-bearing plants (Moran, 2008; Norhazrina et al., 2016; Wolf et al., 2001) and biogeographical connections in the Southern Hemisphere have been shown to be correlated with circumaustral wind currents for many taxa of lycophytes, ferns or mosses (Munoz et al., 2004; Parris, 2001; Sanmartín and Ronquist, 2004). If dispersal events cannot be excluded to explain the current distribution of *Phlegmariurus* across continents, circumaustral wind currents could not have been involved in the biogeographical history of *Phlegmariurus* before the Oligocene. Indeed, a circumpolar circulation would have established only after the separation of Australia and Antarctica ca. 35 Ma and the Drake passage opening ca. 30 Ma (McLoughlin, 2001; Sanmartín et al., 2007).

4.3.2. A southern origin hypothesis via Antarctica

Before being the iced continent we know today, Antarctica was covered by subtropical to temperate forests (Cantrill et al., 2013; Poole et al., 2003; Pross et al., 2012) and provided a bridge between South America and Australia, allowing species to be widely distributed across these three landmasses. It was also a bridge for migration or stepping-stone dispersal (Winkworth et al., 2015), which has been hypothesized for other vascular spore-bearing plants like ferns (Korall and Pryer, 2014; Labiak et al., 2014). Our age estimates are compatible with the hypothesis of an Antarctica connection, with the *Phlegmariurus* crown node dated to ca. 50 Ma. Antarctica was not an area coded in our biogeographical analysis since no species is currently known from this area, but an equivalent range covering the Neotropics and Eastern Paleotropics was retrieved among the most likely ancestral area at crown node of *Phlegmariurus*, suggesting an ancestral distribution including Antarctica. The Antarctica connection would have progressively declined following the global cooling after the Early Eocene climatic optimum and would have been definitively disrupted with the ice-sheet formation ca. 30 Ma (Fig. 3). This disruption could have then isolated populations from the Neotropics and the Paleotropics, leading to distinct, geographically structured lineages. However, this hypothesis

could not completely explain our results, because Africa was separated from Antarctica between 165 and 132 Ma and from South America 135–105 Ma (McLoughlin, 2001).

Fossils proved the presence of vascular spore-bearing plants in Antarctica before ice-sheets formation (Cantrill et al., 2013), but no fossils confirm that *Phlegmariurus* was in Antarctica. Furthermore, *Phlegmariurus* is the sister lineage to *Huperzia*, which mostly diversified in the Northern Hemisphere, rather suggesting a shared history between Southern and Northern lineages.

4.3.3. A northern origin hypothesis via the Boreotropics

A tropical region spanning North America and Eurasia existed during the Cretaceous, at a time when they were geographically closer, facilitating dispersal between the continents that shared numerous taxa within the Boreotropics (Morley, 2003; Wolfe, 1975). The decrease of the global temperature at the Eocene/Oligocene boundary (Zachos et al., 2001) fragmented the Boreotropical forest belt by forcing the frost-sensitive flora to migrate southward (Morley, 2003). Several authors explained amphipacific or amphiatlantic distributions by the disruptions of the Boreotropics (e.g. Antonelli et al., 2009; Couvreur et al., 2011; Hennequin et al., 2010; Liu et al., 2013; Manns et al., 2012; Smedmark et al., 2014; Wei et al., 2015), notably explaining disjunction between temperate and tropical regions (Weeks et al., 2014). Applied to *Phlegmariurus*, this hypothesis implies that the ancestor of modern *Phlegmariurus* was a northern lineage in the Boreotropics. The divergence ca. 50 Ma (HPD: 29.1–71.5) of localized lineages in the Neotropics and the Paleotropics would be explained by southward migrations in less connected continents, facilitating speciation.

Observations about the ancestral ecology of lycophytes could also support the hypothesis of a northern origin of *Phlegmariurus*. It is likely that lycophytes have a mesic origin as they exhibit physiological features such as passive stomatal control that limit their capacity to ameliorate water loss (Brodrigg and McAdam, 2011; McAdam and Brodrigg, 2012). Moreover, an ancestral terrestrial habitat was reconstructed for Lycopodiaceae, with a shift in *Phlegmariurus* that would have started to diversify as epiphytic (Field et al., 2016), so *Phlegmariurus* appears to occupy a relatively xeric biome compared to other living and fossil lycophytes. Diversification linked to epiphytism during Cenozoic was also retrieved in ferns, and is likely correlated with the development of modern tropical forests (Schuettelpelz and Pryer, 2009) and epiphytism acquisition could be an “enabler” trait mentioned by Donoghue and Edwards (2014) to facilitate a biome shift. *Phlegmariurus* may have originated from northern temperate terrestrial ancestors like its sister group *Huperzia*, but that the modern species started to diversify as epiphytes in tropical forests.

4.4. Biogeographical history of Malagasy *Phlegmariurus* species

Our sampling greatly improved the number of Malagasy species included in a phylogenetic study on *Phlegmariurus*, increasing from two (Field et al., 2016) to 16 species the sampling and so the understanding on the biogeographical history of the group. We estimated at least six LDD from the last 30 Ma to explain the origin of the Malagasy *Phlegmariurus*, as well as a diversification in Madagascar starting around 10 Ma.

4.4.1. Dispersal in the Southern Hemisphere

Several sources of dispersal to Madagascar were highlighted by our results, all being an area or a combination of areas from the Southern Hemisphere that confirms the biogeographical connections among southern regions.

One LDD from Australasia to Madagascar (LDD3) was estimated, and one from Asia and/or Australasia (LDD4), suggesting that spores could have followed westward winds across the Indian Ocean, like those of the Indian monsoon of winter. The initiation of Asian monsoon is subject to controversy, but the associated winds would have already

been established during the late Miocene (Gupta et al., 2015; Jacques et al., 2011; Passey et al., 2009), which is compatible with our age estimates. Such dispersal events have also been hypothesized for angiosperms (e.g. Federman et al., 2015; Wikström et al., 2010) and for ferns (Chao et al., 2014).

A precise area of origin for LDD2, 5 and 6 cannot be estimated due to the large distribution of the Malagasy species that make plausible first dispersal events to Africa or other WIO islands before Madagascar. However, LDD5 and LDD6 highlighted connection between Western Paleotropical areas and the Neotropics, retrieved for other vascular spore-bearing plants like ferns (Bauret et al., 2017a,b; Labiak et al., 2014; Rouhan et al., 2004, 2007) and to a lesser extent for angiosperms (e.g. Buerki et al., 2013; Janssens et al., 2016) or bryophytes (Scheben et al., 2016). LDD2 confirms a biogeographical connection between Western Paleotropics and Australasia, previously discussed.

The role of Africa in the biogeographical history of Malagasy *Phlegmariurus* is ambiguous. In the Western Paleotropical clade, if the ancestral range estimates showed a diversification starting in Africa, the distribution in the Eastern Paleotropics of the sister lineage to the Malagasy clade adds uncertainties on the area of origin of the Malagasy clade. Africa was however estimated as the second most likely area at the Malagasy stem node, suggesting that Africa could be the area of origin of this Malagasy lineage. Our analysis also relied on a non-exhaustive sampling of the global species diversity. Numerous species from Asia and Australasia remain to be included in a phylogeny and we observed that LDD from these regions to Madagascar are plausible for *Phlegmariurus*. About LDD2, the distribution of *Phlegmariurus tardieuae* in all the WIO prevents a clear estimate of the biogeographical origin of the dispersal event to Madagascar, which could be Africa or other WIO islands.

To conclude, the study of *Phlegmariurus* brought new evidence about the existence of biogeographical connections between Western Paleotropical regions, especially Madagascar, and the other continents of the Southern Hemisphere. *Phlegmariurus* also tends to follow a pattern retrieved in ferns, with an ambiguous role of Africa and other islands of the WIO, but with evidence of trans-oceanic dispersal with other continents. Moreover, studies highlighted the importance of diversification for animals and plants in Madagascar, even for highly dispersive spore-bearing plants like ferns, and the process was also retrieved in lycophytes with *Phlegmariurus*. Overall, multiple LDD could be inferred from our results to explain the Malagasy diversity but diversification is also major process having generated from a single colonization of the Western Palaeotropics at least 11 species out of the 20 estimated in Madagascar (Table 1).

4.4.2. Diversification: an important driver of Malagasy diversity

The diversification of the Malagasy clade is the most localized ever documented for *Phlegmariurus* and even for Huperzioidae, showing Madagascar as a great catalyst of diversification even for highly dispersive plants. Species in the Malagasy clade showed little molecular divergence in the sequenced markers compared to their broad morphological and ecological heterogeneity, which could reflect recent adaptations to different niches. The Malagasy clade species occupy similar geographic distribution in Madagascar, mostly in the centre region occupied by evergreen forests (Rakotondrainibe and Jouy, 2015; Tardieu-Blot, 1971), with an exception for *P. ambrensis*, which is endemic to the Montagne d’Ambre (Rakotondrainibe and Jouy, 2015). Differences in habitat, growth forms or morphology have been observed after adaptive radiation for angiosperms (Givnish, 2010) and for Malagasy animals (Wirta et al., 2010), suggesting that the Malagasy species diversity of *Phlegmariurus* may have arisen from adaptive radiation. Non-adaptive radiations are rather linked to allopatric speciation and without ecological divergence (Rundell and Price, 2009).

4.5. Nomenclatural combinations

Two additional nomenclatural combinations and a new synonym are recognised for the *Phlegmariurus* of Madagascar:

Phlegmariurus ambrensis (Rakotondr.) A.R.Field & Bauret **comb. nov.**

≡ *Huperzia ambrensis* Rakotondr. Candollea 70(1): 50. 2015 [30 Jun 2015]. Type: Madagascar, Antsiranana, Parc National de la Montagne d'Ambre, 23Jul1992, *F.Rakotondrainibe 1793* (holotype: P00100305!).

Phlegmariurus hildebrandtii (Herter) A.R.Field & Bauret **comb. nov.**

≡ *Lycopodium hildebrandtii* Herter in Bot. Jahrb. Syst. 43 (Beibl. 98): 40. 1909. *Urostachys hildebrandtii* (Herter) Herter ex Nessel, Bärlappgewächse: 28. 1939. *Huperzia hildebrandtii* (Herter) Tardieu, Adansonia ser. 2, 10: 18. 1970. Type: Madagascar, Antananarivo, Ouest Imerina, Andrangoloaka, Nov1880, *Hildebrandt 4152* (lectotype designated by Tardieu, 1971: B; isolectotypes: BM000785227!, K000351212!, P00226994!, P00466631!, P00466632!).

Phlegmariurus humberitii (Nessel) A.R.Field & Bostock, PhytoKeys 20: 41. 2013.

≡ *Urostachys humberitii* Nessel, Repert. sp. Nov. Regni Veg. 48:170. 1940. Type: Madagascar, Quellgebiet des mangoroflusses, 350 m alt. 7Aug1909. *J.Kiese* [s.n.] (holotype: BONN-Nessel-445a !).

≡ *Huperzia teretirigida* Rakotondr. Candollea 70(1): 53. 2015. **syn. nov.** Type: Madagascar, Antananarivo, Anjozorobe, forêt d'Andranomay, 19Dec1996, *F.Rakotondrainibe 3806* (holotype: P00835663 !).

Acknowledgements

Collecting permits in Madagascar were granted by Madagascar National Parks and the Ministère de l'Environnement, de l'Ecologie et des Forêts. We are also grateful, for field assistance, to CNRE-Madagascar, MBG-Madagascar and CNDRS-Comoros. We thank all the collectors of samples used in this study, and F. Rakotondrainibe for her help in the P Herbarium. We acknowledge funding from the ATM MNHN 'Emergence des clades, des biotes et des cultures', ATM MNHN 'Taxonomie moléculaire, DNA Barcode & gestion durable des collections' and the FRB project 'Connaître pour conserver: le patrimoine naturel caché des Comores'. This project was also supported by the 'Service de Systématique Moléculaire' (UMS2700 MNHN/CNRS). ARF's contribution to this research was supported by (i) an Australian Biological Resource Study grant RFL215-34; (ii) an IPID4all Germany Academic Exchange, Technische Universität Dresden Graduate Academy Project 2015_43 and (iii) a Queensland Smithsonian Fellowship 2017. All molecular work done at the MNHN was performed at the BoEM laboratory. We would like to thank the anonymous reviewer for the comments which helped to improve our manuscript.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.ymp.2018.05.004>.

References

Agnarsson, I., Kuntner, M., 2012. The generation of a biodiversity hotspot: biogeography and phylogeography of the western Indian Ocean islands. In: Ananthawat-Jónsson, K. (Ed.), Current Topics in Phylogenetics and Phylogeography of Terrestrial and Aquatic Systems. InTech, pp. 33–82. <http://doi.org/10.5772/38958>.

Antonelli, A., Nylander, J.A.A., Persson, C., Sanmartin, I., 2009. Tracing the impact of the Andean uplift on Neotropical plant evolution. Proc. Natl. Acad. Sci. 106, 9749–9754. <http://dx.doi.org/10.1073/pnas.0811421106>.

Bacon, C.D., Simmons, M.P., Archer, R.H., Zhao, L.-C., Andriantiana, J., 2016. Biogeography of the Malagasy Celastraceae: multiple independent origins followed by widespread dispersal of genera from Madagascar. Mol. Phylogenet. Evol. 94, 365–382. <http://dx.doi.org/10.1016/j.ymp.2015.09.013>.

Bateman, R.M., Kenrick, P., Rothwell, G.W., 2007. Do eligulate herbaceous lycopsids

occur in Carboniferous strata? *Hestia eremosa* gen. et sp. nov. from the Mississippian of Oxroad Bay, East Lothian, Scotland. Rev. Palaeobot. Palynol. 144, 323–335. <http://dx.doi.org/10.1016/j.revpalbo.2006.08.002>.

Bauret, L., Gaudel, M., Sundue, M.A., Parris, B.S., Ranker, T.A., Rakotondrainibe, F., Hennequin, S., Ranaiivo, J., Selosse, M.-A., Rouhan, G., 2017a. Madagascar sheds new light on the molecular systematics and biogeography of grammitid ferns: new unexpected lineages and numerous long-distance dispersal events. Mol. Phylogenet. Evol. 111, 1–17. <http://dx.doi.org/10.1016/j.ymp.2017.03.005>.

Bauret, L., Rouhan, G., Hirai, R.Y., Perrie, L.R., Prado, J., Salino, A., Senterre, B., Shepherd, L.D., Sundue, M.A., Selosse, M.-A., Gaudel, M., 2017b. Molecular data, based on an exhaustive species sampling of the fern genus *Rumohra* (Dryopteridaceae), reveal a biogeographic history mostly shaped by dispersal and several cryptic species in the widely distributed *Rumohra adiantiformis*. Bot. J. Linn. Soc. 185, 463–481. <http://dx.doi.org/10.1093/botlinnean/box072>.

Blair, C., Noonan, B.P., Brown, J.L., Raselimanana, A.P., Vences, M., Yoder, A.D., 2015. Multilocus phylogenetic and geospatial analyses illuminate diversification patterns and the biogeographic history of Malagasy endemic plated lizards (Gerrhosauridae: Zonosaurinae). J. Evol. Biol. 28, 481–492. <http://dx.doi.org/10.1111/jeb.12586>.

Bomfleur, B., Schöner, R., Schneider, J.W., Viereck, L., Kerp, H., McKellar, J.L., 2014. From the transantarctic basin to the ferrar large igneous province—new palynostratigraphic age constraints for triassic-jurassic sedimentation and magmatism in East Antarctica. Rev. Palaeobot. Palynol. 207, 18–37. <http://dx.doi.org/10.1016/j.revpalbo.2014.04.002>.

Brodribb, T.J., McAdam, S.A.M., 2011. Passive origins of stomatal control in vascular plants. Science 331, 582–585. <http://dx.doi.org/10.1126/science.1197985>.

Buerki, S., Devey, D.S., Callmander, M.W., Phillipson, P.B., Forest, F., 2013. Spatio-temporal history of the endemic genera of Madagascar. Bot. J. Linn. Soc. 171, 304–329. <http://dx.doi.org/10.1111/boj.12008>.

Callmander, M.W., 2011. The endemic and non-endemic vascular flora of Madagascar updated. Plant Ecol. Evol. 144, 121–125. <http://dx.doi.org/10.5091/plevevo.2011.513>.

Cantrill, D.J., Tosolini, A.-M.P., Francis, J.E., 2013. Paleocene flora from Seymour Island, Antarctica: revision of Dusén's (1908) angiosperm taxa. Alcheringa: Australas. J. Palaeontol. 37, 366–391. <http://dx.doi.org/10.1080/03115518.2013.764698>.

Chao, Y.S., Rouhan, G., Amoroso, V.B., Chiou, W.L., 2014. Molecular phylogeny and biogeography of the fern genus *Pteris* (Pteridaceae). Ann. Bot. 114, 109–124. <http://dx.doi.org/10.1093/aob/mcu086>.

Chinnock, R.J., 1998. Lycopodiaceae. Flora of Australia. 66–85.

Cieraad, E., Lee, D.E., 2006. The New Zealand fossil record of ferns for the past 85 million years. New Zeal. J. Bot. 44, 143–170. <http://dx.doi.org/10.1080/0028825X.2006.9513015>.

Couvreux, T.L.P., Pirie, M.D., Chatrou, L.W., Saunders, R.M.K., Su, Y.C.F., Richardson, J.E., Erkens, R.H.J., 2011. Early evolutionary history of the flowering plant family Annonaceae: steady diversification and boreotropical geodispersal. J. Biogeogr. 38, 664–680. <http://dx.doi.org/10.1111/j.1365-2699.2010.02434.x>.

Donoghue, M.J., Edwards, E.J., 2014. Biome Shifts and Niche Evolution in Plants. Annu. Rev. Ecol. Syst. 45, 547–572. <http://dx.doi.org/10.1146/annurev-ecolsys-120213-091905>.

Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A., 2012. Bayesian Phylogenetics with BEAUti and the BEAST 1.7. Mol. Biol. Evol. 29, 1969–1973. <http://dx.doi.org/10.1093/molbev/mss075>.

Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32, 1792–1797. <http://dx.doi.org/10.1093/nar/gkh340>.

Federman, S., Dornburg, A., Downie, A., Richard, A.F., Daly, D.C., Donoghue, M.J., 2015. The biogeographic origin of a radiation of trees in Madagascar: implications for the assembly of a tropical forest biome. BMC Evol. Biol. 15, 216. <http://dx.doi.org/10.1186/s12862-015-0483-1>.

Field, A.R., Bostock, P.D., 2013. New and existing combinations in Palaeotropical *Phlegmariurus* (Lycopodiaceae) and lectotypification of the type species *Phlegmariurus phlegmaria* (L.) T. Sen & U. Sen. PhytoKeys 51, 33–51. <http://dx.doi.org/10.3897/phytokeys.20.4007>.

Field, A.R., Testo, W., Bostock, P.D., Holtum, J.A.M., Waycott, M., 2016. Molecular phylogenetics and the morphology of the Lycopodiaceae subfamily Huperzioidae supports three genera: *Huperzia*, *Phlegmariurus* and *Phylloglossum*. Mol. Phylogenet. Evol. 94, 635–657. <http://dx.doi.org/10.1016/j.ymp.2015.09.024>.

Forthman, M., Weirauch, C., 2016. Phylogenetics and biogeography of the endemic Madagascar millipede assassin bugs (Hemiptera: Reduviidae: Ectrichodinae). Mol. Phylogenet. Evol. 100, 219–233. <http://dx.doi.org/10.1016/j.ymp.2016.03.011>.

Givnish, T.J., 2010. Ecology of plant speciation. Taxon 59, 1329–1366. <http://dx.doi.org/10.2307/20774033>.

Grimm, G.W., Kapli, P., Bomfleur, B., McLoughlin, S., Renner, S.S., 2015. Using more than the oldest fossils: dating Osmundaceae with three Bayesian clock approaches. Syst. Biol. 64, 396–405. <http://dx.doi.org/10.1093/sysbio/syu108>.

Gupta, A.K., Yuvaraja, A., Prakasam, M., Clemens, S.C., Velu, A., 2015. Evolution of the South Asian monsoon wind system since the late Middle Miocene. Palaeogeogr. Palaeoclimatol. Palaeoecol. 438, 160–167. <http://dx.doi.org/10.1016/j.palaeo.2015.08.006>.

Hao, S., Wang, D., Wang, Q., Xue, J., 2006. A new lycopsid, *Zhenglia radiata* gen. et sp. nov., from the Lower Devonian Posongchong Formation of southeastern Yunnan, China, and its evolutionary significance. Acta Geol. Sin. - English Ed. 80, 11–19. <http://dx.doi.org/10.1111/j.1755-6724.2006.tb00789.x>.

Hao, S.G., Xue, J.H., Wang, Q., Liu, Z.F., 2007. *Yuguangia ordinata* gen. et sp. nov., a new lycopsid from the middle devonian (late givetian) of Yunnan, China, and its phylogenetic implications. Int. J. Plant Sci. 168, 1161–1175. <http://dx.doi.org/10.1086/520727>.

- Hennequin, S., Hovenkamp, P., Christenhusz, M.J.M., Schneider, H., 2010. Phylogenetics and biogeography of *Nephrolepis* – a tale of old settlers and young tramps. *Bot. J. Linn. Soc.* 164, 113–127. <http://dx.doi.org/10.1111/j.1095-8339.2010.01076.x>.
- Hennequin, S., Rouhan, G., Salino, A., Duan, Y.-F., Lepeigneux, M.-C., Guillou, M., Ansell, S., Almeida, T.E., Zhang, L.-B., Schneider, H., 2017. Global phylogeny and biogeography of the fern genus *Ctenitis* (Dryopteridaceae), with a focus on the Indian Ocean region. *Mol. Phylogenet. Evol.* 112, 277–289. <http://dx.doi.org/10.1016/j.ympev.2017.04.012>.
- Herter, G., 1909. Beiträge zur kenntnis der kattung *Lycopodium*. Studien über dieuntergattung *Urostachys*. *Bot. Jahrb. Syst.* 43, 1–56.
- Holub, J., 1991. Some taxonomic changes within Lycopodiales. *Folia Geobot. Phytotaxon.* 26, 81–94. <http://dx.doi.org/10.1007/BF02912943>.
- Hueber, F.M., 1992. Thoughts on the early lycopsids and zosterophylls. *Ann. Missouri Bot. Gard.* 79, 474. <http://dx.doi.org/10.2307/2399751>.
- Huelsenbeck, J.P., Larget, B., Alfaro, M.E., 2004. Bayesian phylogenetic model selection using reversible jump Markov Chain Monte Carlo. *Mol. Biol. Evol.* 21, 1123–1133. <http://dx.doi.org/10.1093/molbev/msh123>.
- Jacques, F.M.B., Guo, S.X., Su, T., Xing, Y.W., Huang, Y.J., Liu, Y.S., Ferguson, D.K., Zhou, Z.K., 2011. Quantitative reconstruction of the Late Miocene monsoon climates of southwest China: a case study of the Lincang flora from Yunnan Province. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 304, 318–327. <http://dx.doi.org/10.1016/j.palaeo.2010.04.014>.
- Janssen, T., Bystriakova, N., Rakotondrainibe, F., Coomes, D., Labat, J.N., Schneider, H., 2008. Neoenemism in Madagascan scaly tree ferns results from recent, coincident diversification bursts. *Evolution (N.Y.)*, 62, 1876–1889. <http://doi.org/10.1111/j.1558-5646.2008.00408.x>.
- Janssens, S.B., Groeninckx, I., De Block, P.J., Verstraete, B., Smets, E.F., Dessein, S., 2016. Dispersing towards Madagascar: biogeography and evolution of the Madagascan endemics of the Spermaceae tribe (Rubiaceae). *Mol. Phylogenet. Evol.* 95, 58–66. <http://dx.doi.org/10.1016/j.ympev.2015.10.024>.
- Ji, S.-G., Huo, K.-K., Wang, J., Pan, S.-L., 2008. A molecular phylogenetic study of *Huperziaceae* based on chloroplast *rbcL* and *psbA-trnH* sequences. *J. Syst. Evol.* 46, 213–219. <http://dx.doi.org/10.3724/SP.J.1002.2008.07036>.
- Juhász, M., 1975. *Lycopodiaceae* spores from Lower Cretaceous deposits of Hungary. *Acta Biol. Szeged* 21, 21–34.
- Kidston, R., Lang, W.H., 1920. On old red sandstone plants showing structure, from the Rhynie Chert bed, Aberdeenshire. Part III. *Asteroxylon mackiei*. *Trans. R. Soc. Edinburgh* 643–680.
- Kim, C., Choi, H.-K., 2016. Biogeography of North Pacific *Isoetes* (Isoetaceae) inferred from nuclear and chloroplast DNA sequence data. *J. Plant Biol.* 59, 386–396. <http://dx.doi.org/10.1007/s12374-016-0123-3>.
- Klaus, K.V., Schulz, C., Bauer, D.S., Stützel, T., 2016. Historical biogeography of the ancient lycophyte genus *Selaginella*: early adaptation to xeric habitats on Pangea. *Cladistics* 1–12. <http://dx.doi.org/10.1111/cla.12184>.
- Korall, P., Pryer, K.M., 2014. Global biogeography of scaly tree ferns (Cyatheaceae): Evidence for Gondwanan vicariance and limited transoceanic dispersal. *J. Biogeogr.* 41, 402–413. <http://dx.doi.org/10.1111/jbi.12222>.
- Labiak, P.H., Sundue, M.A., Rouhan, G., Hanks, J.G., Mickel, J.T., Moran, R.C., 2014. Phylogeny and historical biogeography of the lastreopsid ferns (Dryopteridaceae). *Am. J. Bot.* 101, 1207–1228. <http://dx.doi.org/10.3732/ajb.1400071>.
- Larsén, E., Rydin, C., 2016. Disentangling the phylogeny of *Isoetes* (Isoetales), using nuclear and plastid data. *Int. J. Plant Sci.* 177, 157–174. <http://dx.doi.org/10.1086/684179>.
- Lehtonen, S., Tuomisto, H., Rouhan, G., Christenhusz, M.J.M., 2010. Phylogenetics and classification of the pantropical fern family Lindsaeaceae. *Bot. J. Linn. Soc.* 163, 305–359. <http://dx.doi.org/10.1111/j.1095-8339.2010.01063.x>.
- Linder, H.P., Hardy, C.R., Rutschmann, F., 2005. Taxon sampling effects in molecular clock dating: an example from the African Restionaceae. *Mol. Phylogenet. Evol.* 35, 569–582. <http://dx.doi.org/10.1016/j.ympev.2004.12.006>.
- Liu, X.-Q., Ickert-Bond, S.M., Chen, L.-Q., Wen, J., 2013. Molecular phylogeny of *Cissus* L. of Vitaceae (the grape family) and evolution of its pantropical intercontinental disjunctions. *Mol. Phylogenet. Evol.* 66, 43–53. <http://dx.doi.org/10.1016/j.ympev.2012.09.003>.
- MacArthur, R.H., Wilson, E.O., 1967. *The Theory of Island Biogeography*. Princeton University Press.
- Manns, U., Wikström, N., Taylor, C.M., Bremer, B., 2012. Historical biogeography of the predominantly neotropical subfamily Cinchonoideae (Rubiaceae): into or out of America? *Int. J. Plant Sci.* 173, 261–286. <http://dx.doi.org/10.1086/663971>.
- Matzke, N.J., 2013. BioGeoBEARS: BioGeography with Bayesian (and Likelihood) Evolutionary Analysis in R Scripts.
- McAdam, S.A.M., Brodribb, T.J., 2012. Fern and lycophyte guard cells do not respond to endogenous abscisic acid. *Plant Cell* 24, 1510–1521. <http://dx.doi.org/10.1105/tpc.112.096404>.
- McLoughlin, S., 2001. The breakup history of Gondwana and its impact on pre-Cenozoic floristic provincialism. *Aust. J. Bot.* 49, 271–300. <http://dx.doi.org/10.1071/BT00023>.
- Miller, M.A., Pfeiffer, W., Schwartz, T., 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. 2010 Gatew. Comput. Environ. Work. GCE 2010, pp. 1–8. <http://doi.org/10.1109/GCE.2010.5676129>.
- Moran, R.C., 2008. Diversity, biogeography, and floristics. In: Ranker, T.A., Haufler, C.H. (Eds.), *Biology and Evolution of Ferns and Lycophytes*. Cambridge University Press, pp. 367–394.
- Morat, P., Jaffré, T., Tronchet, F., Munzinger, J., Pillon, Y., Veillon, J.-M., Chalopin, M., Birnbaum, P., Rigault, F., Dagostini, G., Tinél, J., Lowry, P.P., 2012. Le référentiel taxonomique Florical et les caractéristiques de la flore vasculaire indigène de la Nouvelle-Calédonie. *Adansonia* 34, 179–221. <http://dx.doi.org/10.5252/a2012n2a1>.
- Morley, R.J., 2003. Interplate dispersal paths for megathermal angiosperms. *Perspect. Plant Ecol. Evol. Syst.* 6, 5–20.
- Munoz, J., Felicísimo, A.M., Cabezas, F., Burgaz, Á.R., Martínez, I., 2004. Wind as a Long-Distance Dispersal Vehicle in the Southern Hemisphere. *Science*. 304, 1144–1147. <http://dx.doi.org/10.1126/science.1095210>.
- Munzinger, J., Morat, P., Jaffré, T., Gábelé, G., Pillon, Y., Tronchet, F., Veillon, J.-M., Chalopin, M., 2016. FLORICAL: checklist of the vascular indigenous flora of New Caledonia. vers. 22.IV.2016. < <http://www.botanique.nc/herbier/florical> > .
- Myers, N., Mittermeiera, R., Mittermeier, C.G., da Fonseca, G.A.B., Kent, J., 2000. Biodiversity hotspots for conservation priorities. *Nature* 403, 853–858. <http://dx.doi.org/10.1038/35002501>.
- Nessel, H., 1939. Die Bärlappgewächse (Lycopodiaceae). *Jena*.
- Norhazrina, N., Wang, J., Hagborg, A., Geffert, J.L., Mutke, J., Gradstein, S.R., Baselga, A., Vanderpoorten, A., Patiño, J., 2016. Tropical bryophyte floras: a homogeneous assemblage of highly mobile species? Insights from their spatial patterns of beta diversity. *Bot. J. Linn. Soc.* <http://dx.doi.org/10.1111/boj.12495>.
- Øllgaard, B., 1989. Index of the Lycopodiaceae volume 34 of Biologiske skrifter, in: Roy. Dan. Acad. Sci. Lett.
- Øllgaard, B., 1987. A revised classification of the Lycopodiaceae s.l. *Oper. Bot.* 92, 153–178.
- Parris, B.S., 2001. Circum-Antarctic continental distribution patterns in pteridophyte species. *Brittonia* 53, 270–283. <http://dx.doi.org/10.1007/BF02812702>.
- Passes, B.H., Ayliffe, L.K., Kaakinen, A., Zhang, Z., Eronen, J.T., Zhu, Y., Zhou, L., Cerling, T.E., Fortelius, M., 2009. Strengthened East Asian summer monsoons during a period of high-latitude warmth? Isotopic evidence from Mio-Pliocene fossil mammals and soil carbonates from northern China. *Earth Planet. Sci. Lett.* 277, 443–452. <http://dx.doi.org/10.1016/j.epsl.2008.11.008>.
- Pereira, J.B.S., Labiak, P.H., Stützel, T., Schulz, C., 2017. Origin and biogeography of the ancient genus *Isoetes* with focus on the Neotropics. *Bot. J. Linn. Soc.* 185, 253–271. <http://dx.doi.org/10.1093/botlinnean/box057>.
- Poole, I., Menega, A.M.W., Cantrill, D.J., 2003. Valdivian ecosystems in the Late Cretaceous and Early Tertiary of Antarctica: further evidence from myrtaceous and eucryphiaceous fossil wood. *Rev. Palaeobot. Palynol.* 124, 9–27. [http://dx.doi.org/10.1016/S0034-6667\(02\)00244-0](http://dx.doi.org/10.1016/S0034-6667(02)00244-0).
- PPGI, 2016. A community-derived classification for extant lycophytes and ferns. *J. Syst. Evol.* 54, 563–603. <http://dx.doi.org/10.1111/jse.12229>.
- Pross, J., Contreras, L., Bijl, P.K., Greenwood, D.R., Bohaty, S.M., Schouten, S., Bendle, J.A., Röhl, U., Tauxe, L., Raine, J.I., Huck, C.E., van de Fliedert, T., Jamieson, S.S.R., Stickley, C.E., van de Schootbrugge, B., Escutia, C., Brinkhuis, H., Brinkhuis, H., Escutia Dotti, C., Klaus, A., Fehr, A., Williams, T., Bendle, J.A.P., Bijl, P.K., Bohaty, S.M., Carr, S.A., Dunbar, R.B., González, J.J., Hayden, T.G., Iwai, M., Jimenez-Espejo, F.J., Katsuki, K., Soo Kong, G., McKay, R.M., Nakai, M., Olney, M.P., Passchier, S., Pekar, S.F., Pross, J., Riesselman, C.R., Röhl, U., Sakai, T., Shrivastava, P.K., Stickley, C.E., Sugisaki, S., Tauxe, L., Tuo, S., van de Fliedert, T., Welsh, K., Yamane, M., 2012. Persistent near-tropical warmth on the Antarctic continent during the early Eocene epoch. *Nature* 488, 73–77. <http://doi.org/10.1038/nature11300>.
- Rakotondrainibe, F., 2003. Checklist of the pteridophytes of Madagascar. In: Goodman, S., Benstead, J. (Eds.), *The Natural History of Madagascar*. University of Chicago Press, pp. 295–313.
- Rakotondrainibe, F., Jouy, A., 2015. Nouveautés dans le Genre *Huperzia* Bernh. (Lycophytes, Lycopodiaceae) à Madagascar. *Candollea* 70, 49–56. <http://dx.doi.org/10.15553/c2015v701a5>.
- Rambaut, A., 2014. FigTree 1.4. 2 software. Institute of Evolutionary Biology, Univ. Edinburgh.
- Rambaut, A., Drummond, A., 2013. Tracer 1.6. University of Edinburgh, Edinburgh, UK.
- Ree, R.H., Smitha, S., 2008. Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Syst. Biol.* 57, 4–14. <http://dx.doi.org/10.1080/10635150701883881>.
- Ronquist, F., 1997. Dispersal-vicariance analysis: a new approach to the quantification of historical biogeography. *Syst. Biol.* 46, 195–203. <http://dx.doi.org/10.1093/sysbio/46.1.195>.
- Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* 61, 539–542.
- Rouhan, G., Dubuisson, J.-Y., Rakotondrainibe, F., Motley, T.J., Mickel, J.T., Labat, J.N., Moran, R.C., 2004. Molecular phylogeny of the fern genus *Elaphoglossum* (Elaphoglossaceae) based on chloroplast non-coding DNA sequences: Contributions of species from the Indian Ocean area. *Mol. Phylogenet. Evol.* 33, 745–763. <http://dx.doi.org/10.1016/j.ympev.2004.08.006>.
- Rouhan, G., Hanks, J.G., McClelland, D., Moran, R.C., 2007. Preliminary phylogenetic analysis of the fern genus *Lomariopsis* (Lomariopsidaceae). *Brittonia* 59, 115–128. [http://dx.doi.org/10.1663/0007-196X\(2007\)59\[115:PPAOTF\]2.0.CO;2](http://dx.doi.org/10.1663/0007-196X(2007)59[115:PPAOTF]2.0.CO;2).
- Roux, J.P., 2009. Synopsis of the Lycopodiophyta and Pteridophyta of Africa. Madagascar and Neighbouring Islands, South African National Biodiversity Institute.
- Rundell, R.J., Price, T.D., 2009. Adaptive radiation, nonadaptive radiation, ecological speciation and nonecological speciation. *Trends Ecol. Evol.* 24, 394–399. <http://dx.doi.org/10.1016/j.tree.2009.02.007>.
- Sanmartín, I., Ronquist, F., 2004. Southern hemisphere biogeography inferred by event-based models: problems versus animal patterns. *Syst. Biol.* 53, 216–243. <http://dx.doi.org/10.1080/10635150490423430>.
- Sanmartín, I., Wannertorp, L., Winkworth, R.C., 2007. West Wind Drift revisited: testing for directional dispersal in the Southern Hemisphere using event-based tree fitting. *J. Biogeogr.* 34, 398–416. <http://dx.doi.org/10.1111/j.1365-2699.2006.01655.x>.
- Scheben, A., Bechteler, J., Lee, G.E., Pócs, T., Schäfer-Verwimp, A., Heinrichs, J., 2016.

- Multiple transoceanic dispersals and geographical structure in the pantropical leafy liverwort *Ceratolejeunea* (Lejeuneaceae, Porellales). *J. Biogeogr.* 1–11. <http://dx.doi.org/10.1111/jbi.12779>.
- Schuettpelz, E., Pryer, K.M., 2007. Fern phylogeny inferred from 400 leptosporangiate species and three plastid genes. *Taxon* 56, 1037–1050. <http://dx.doi.org/10.2307/25065903>.
- Schuettpelz, E., Pryer, K.M., 2009. Evidence for a Cenozoic radiation of ferns in an angiosperm-dominated canopy. *Proc. Natl. Acad. Sci. USA* 106, 11200–11205. <http://dx.doi.org/10.1073/pnas.0811136106>.
- Sessa, E.B., Juslén, A., Våre, H., Chambers, S.M., 2017. Into Africa: Molecular phylogenetics and historical biogeography of sub-Saharan African woodferns (*Dryopteris*). *Am. J. Bot.* 104, 477–486. <http://dx.doi.org/10.3732/ajb.1600392>.
- Smedmark, J.E.E., Razafimandimbison, S.G., Wikström, N., Bremer, B., 2014. Inferring geographic range evolution of a pantropical tribe in the coffee family (Lasiantheae, Rubiaceae) in the face of topological uncertainty. *Mol. Phylogenet. Evol.* 70, 182–194. <http://dx.doi.org/10.1016/j.ympev.2013.09.007>.
- Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30, 1312–1313.
- Tardieu-Blot, M.-L., 1970. A propos des Lycopodiales de la région Malgache. *Adansonia* ser.2 10, pp. 15–22.
- Tardieu-Blot, M.-L., 1971. Lycopodiacees-Huperziacees. In: Humbert, H. (Ed.), *Flore de Madagascar et Des Comores*. Muséum national d'Histoire naturelle, Paris.
- Testo, W., Sundue, M.A., 2016. A 4000-species dataset provides new insight into the evolution of ferns. *Mol. Phylogenet. Evol.* 105, 200–211. <http://dx.doi.org/10.1016/j.ympev.2016.09.003>.
- Vaidya, G., Lohman, D.J., Meier, R., 2011. SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics* 27, 171–180. <http://dx.doi.org/10.1111/j.1096-0031.2010.00329.x>.
- Vences, M., Wollenberg, K.C., Vieites, D.R., Lees, D.C., 2009. Madagascar as a model region of species diversification. *Trends Ecol. Evol.* 24, 456–465. <http://dx.doi.org/10.1016/j.tree.2009.03.011>.
- Warnock, R.C.M., Parham, J.F., Joyce, W.G., Lyson, T.R., Donoghue, P.C.J., 2014. Calibration uncertainty in molecular dating analyses: there is no substitute for the prior evaluation of time priors. *Proc. R. Soc. B Biol. Sci.* 282, 20141013. <http://dx.doi.org/10.1098/rspb.2014.1013>.
- Warren, B.H., Strasberg, D., Bruggemann, J.H., Prys-Jones, R.P., Thébaud, C., 2010. Why does the biota of the Madagascar region have such a strong Asiatic flavour? *Cladistics* 26, 526–538. <http://dx.doi.org/10.1111/j.1096-0031.2009.00300.x>.
- Weeks, A., Zapata, F., Pell, S.K., Daly, D.C., Mitchell, J.D., Fine, P.V.A., 2014. To move or to evolve: contrasting patterns of intercontinental connectivity and climatic niche evolution in Terebinthaceae (Anacardiaceae and Burseraceae). *Front. Genet.* 5, 1–20. <http://dx.doi.org/10.3389/fgene.2014.00409>.
- Wei, R., Xiang, Q., Schneider, H., Sundue, M.A., Kessler, M., Kamau, P.W., Hidayat, A., Zhang, X., 2015. Eurasian origin, boreotropical migration and transoceanic dispersal in the pantropical fern genus *Diplazium* (Athyriaceae). *J. Biogeogr.* 42, 1809–1819. <http://dx.doi.org/10.1111/jbi.12551>.
- Wikström, N., Avino, M., Razafimandimbison, S.G., Bremer, B., 2010. Historical biogeography of the coffee family (Rubiaceae, Gentianales) in Madagascar: case studies from the tribes Knoxieae, Naucleaeae, Paederieae and Vanguerieae. *J. Biogeogr.* 37, 1094–1113. <http://dx.doi.org/10.1111/j.1365-2699.2009.02258.x>.
- Wikström, N., Kenrick, P., 2001. Evolution of Lycopodiaceae (Lycopsidea): estimating divergence times from *rbcL* gene sequences by use of nonparametric rate smoothing. *Mol. Phylogenet. Evol.* 19, 177–186. <http://dx.doi.org/10.1006/mpev.2001.0936>.
- Wikström, N., Kenrick, P., Chase, M., 1999. Epiphytism and terrestrialization in tropical *Huperzia* (Lycopodiaceae). *Plant Syst. Evol.* 218, 221–243. <http://dx.doi.org/10.1007/BF01089229>.
- Winkworth, R.C., Hennion, F., Prinzing, A., Wagstaff, S.J., 2015. Explaining the disjunct distributions of austral plants: the roles of Antarctic and direct dispersal routes. *J. Biogeogr.* 42, 1197–1209. <http://dx.doi.org/10.1111/jbi.12522>.
- Wirta, H., Viljanen, H., Orsini, L., Montreuil, O., Hanski, I., 2010. Three parallel radiations of Canthonini dung beetles in Madagascar. *Mol. Phylogenet. Evol.* 57, 710–727. <http://dx.doi.org/10.1016/j.ympev.2010.08.013>.
- Wolf, P.G., Schneider, H., Ranker, T.A., 2001. Geographic distributions of homosporous ferns: does dispersal obscure evidence of vicariance? *J. Biogeogr.* 28, 263–270. <http://dx.doi.org/10.1046/j.1365-2699.2001.00531.x>.
- Wolfe, J.A., 1975. Some aspects of plant geography of the northern hemisphere during the late cretaceous and tertiary. *Ann. Missouri Bot. Gard.* 62, 264–279. <http://dx.doi.org/10.2307/2395198>.
- Wood, H.M., Gillespie, R.G., Griswold, C.E., Wainwright, P.C., 2015. Why is Madagascar special? The extraordinarily slow evolution of pelican spiders (Araneae, Archaeidae). *Evolution (N.Y.)*, 69, 462–481. <http://doi.org/10.1111/evo.12578>.
- Xue, J., 2011. Phylogeny of Devonian Lycopsidea Inferred from Bayesian Phylogenetic Analyses. *Acta Geol. Sin. – English Ed.* 85, 569–580. <http://dx.doi.org/10.1111/j.1755-6724.2011.00452.x>.
- Xue, J.-Z., 2013. New material of *Hueberia zhichangensis* Yang, Li & Edwards, a basal lycopsid from the Early Devonian of Yunnan, China. *Neues Jahrb. für Geol. und Paläontologie - Abhandlungen* 267, 331–339. <http://doi.org/10.1127/0077-7749/2013/0318>.
- Yang, N., Li, C.S., Edwards, D., 2009. *Hueberia zhichangensis* gen. et sp. nov., an Early Devonian (Pragian) Plant From Yunnan, China. *Palynology* 33, 113–124. <http://dx.doi.org/10.2113/gspalynol.33.1.113>.
- Yoder, A.D., Nowak, M.D., 2006. Has vicariance or dispersal has vicariance been the predominant force in biogeographic Madagascar? Only time will tell. *Annu. Rev. Ecol. Evol. Syst.* 37, 405–431. <http://dx.doi.org/10.2307/annurev.ecolsys.37.091305.30000016>.
- Yu, Y., Harris, A.J., Blair, C., He, X., 2015. RASP (Reconstruct Ancestral State in Phylogenies): a tool for historical biogeography. *Mol. Phylogenet. Evol.* 87, 46–49. <http://dx.doi.org/10.1016/j.ympev.2015.03.008>.
- Zachos, J., Pagani, M., Sloan, L., Thomas, E., Billups, K., 2001. Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science* 292, 686–693. <http://dx.doi.org/10.1126/science.1059412>.
- Zhou, L., Su, Y.C.F., Thomas, D.C., Saunders, R.M.K., 2012. “Out-of-Africa” dispersal of tropical floras during the Miocene climatic optimum: Evidence from *Uvaria* (Annonaceae). *J. Biogeogr.* 39, 322–335. <http://dx.doi.org/10.1111/j.1365-2699.2011.02598.x>.