



HAL
open science

How to settle in Madagascar? Towards a better understanding of the biogeographical history of the Malagasy ferns.

Lucie, Anaïs Bauret

► To cite this version:

Lucie, Anaïs Bauret. How to settle in Madagascar? Towards a better understanding of the biogeographical history of the Malagasy ferns.. Biodiversity. Université Pierre et Marie Curie - Paris VI, 2017. English. NNT: 2017PA066464 . tel-01794017

HAL Id: tel-01794017

<https://theses.hal.science/tel-01794017v1>

Submitted on 17 May 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Université Pierre et Marie Curie

Ecole doctorale 227

« Sciences de la Nature et de l'Homme : écologie et évolution »

UMR 7205 ISYEB : Institut de Systématique, Evolution, Biodiversité

How to settle in Madagascar? Towards a better understanding of the biogeographical history of the Malagasy ferns.

Par Lucie BAURET

Thèse de doctorat de Systématique et Evolution

Dirigée par :

Marc-André SELOSSE, Professeur au Muséum national d'Histoire naturelle, HDR
Germinal ROUHAN, Maître de conférences au Muséum national d'Histoire naturelle
Myriam GAUDEUL, Maître de conférences au Muséum national d'Histoire naturelle

Présentée et soutenue publiquement le Mardi 5 Décembre 2017

Devant un jury composé de :

Eric QUEINNEC	Professeur, UPMC	Président du jury
Petra KORALL	Associate Professor, Uppsala University	Rapportrice
Guillaume BESNARD	Chargé de Recherche, CNRS	Rapporteur
Valérie PONCET	Chargée de Recherche, IRD	Examinatrice
Marc-André SELOSSE	Professeur, MNHN	Directeur de thèse
Germinal ROUHAN	Maître de Conférences, MNHN	Directeur de thèse



Except where otherwise noted, this work is licensed under
<http://creativecommons.org/licenses/by-nc-nd/3.0/>

A mes parents,
ma famille,
mes ami-e-s,
mes collègues,

merci pour votre soutien.

Acknowledgements - Remerciements

Ce travail de trois ans n'aurait pas été possible sans la contribution précieuse de nombreuses personnes, sur plusieurs plans qu'ils soient professionnels ou personnels.

Je remercie premièrement mes encadrants et mon directeur de thèse, Germinal, Myriam et Marc-André, pour m'avoir donné cette opportunité unique qu'est le doctorat au Muséum. J'aurai vécu une expérience de trois ans avec vous, non sans difficultés, mais qui aura été professionnellement très enrichissante. Je mentionnerai tout particulièrement Germinal, que je remercie pour avoir partagé avec moi son expérience du terrain à Madagascar.

To the jury members, thank you very much to have accepted my invitation. Especially, I would like to thank the reviewers for their work on my manuscript.

Je remercie également le ministère de l'Enseignement Supérieur et de la Recherche, pour sa dotation ayant financé mes trois années de recherche à l'Université Pierre et Marie Curie, au sein de laquelle j'aurai passé huit ans de vie académique.

Ce doctorat n'aurait jamais eu lieu sans l'aval de mon laboratoire, l'Institut de Systématique, Evolution, Biodiversité, et de l'Ecole Doctorale 227. Je remercie également les membres de mon comité de thèse pour le temps qu'ils m'ont accordé, leur écoute et leurs conseils.

Pour l'organisation de ma mission de terrain à Madagascar, je remercie les collègues du Missouri Botanical Garden et tout particulièrement Christian Camara, Faranirina Lantoarisoa, Patrice Antilahimena, Désiré Ravelonarivo, Charles Rakotovao, Jean-Ba, ainsi que Vololotahiana Razafindrahaja, du Parc Botanique et Zoologique de Tsimbazaza, pour leur aide précieuse. Madagascar est un pays extraordinaire, mais il le serait moins sans ces habitants et ses experts naturalistes. Je remercie également nos guides, avec une mention particulière pour Edmond au Marojejy. Cette mission n'aurait jamais eu lieu sans l'aval de Madagascar National Park, que je remercie pour la confiance que l'institution accorde aux chercheurs vazaha. Cette mission fut financée par l'ATM « Emergence des clades, des biotes et des cultures », sous la coordination de Stéphane Peigné, que je remercie chaleureusement.

Merci également aux responsables et aux personnels des laboratoires BoEM et SSM, au sein desquels mes travaux moléculaires ont été effectués.

-

Many thanks to the numerous collaborators on this project, especially:

Ashley, thank you very much for your contagious passion about *Phlegmariurus* and our enthusiastic discussions in Washington, Paris and on the internet. It was a great pleasure to meet you. I hope that we will continue our Malagasy *Phlegmariurus* adventure and that we will find new opportunities to work on exciting projects after my PhD.

Michael Sundue, Barbara Parris and Tom Ranker who I met with great pleasure in Washington during the *Next Generation Pteridology* conference, thank you for your

knowledge and your data of grammitid ferns. I was glad to publish my first article of thesis with you.

My work also relied on the contribution of many people who collected ferns all around the world and who have provided precious collections, especially Samuli Lehtonen, Leon Perrie, André L. de Gasper and their collaborators, Regina Hirai, Jefferson Prado, Alexandre Salino, Bruno Senterre, Lara Shepherd, Michael Sundue, and also France Rakotondrainibe, Germinal Rouhan, Thomas Janssen and all the collectors who transmitted a precious trace of the natural heritage of Madagascar.

-

Je remercie également l'ensemble de mes collègues de l'unité, de l'équipe et de l'herbier, en particulier :

Catherine, je t'avais déjà mentionnée dans mon mémoire de Master 2 mais tu mérites bien de nouveaux remerciements pour m'avoir transmis la fibre botanique, il y maintenant cinq ans. Les souvenirs de cette mission bryo-ptéridologique à Madagascar sont impérissables et nourriront pour encore longtemps ma passion pour les petites (et plus grandes) plantes à spores. Je te remercie également pour ton amitié, ton soutien durant la thèse, tes relectures ainsi que tes conseils.

Pour ta prestation magique du cœlacanthe dans mon amphitheâtre de L1 (qui a marqué toute une génération d'étudiants !), mais surtout pour la confiance que tu m'as accordée concernant tes projets scientifiques, je te remercie Jean-Yves. Durant ces trois années de thèse, tu t'es montré disponible pour discuter science mais pas que ! Tu es même venu me donner quelques distractions botaniques, pour mon plus grand bonheur.

Chère France, ta connaissance de la flore ptéridologique de Madagascar a nourri nombre de nos discussions, mais tu as aussi été une bonne oreille pour d'autres sujets plus personnels et je te remercie d'avoir été à l'écoute malgré quelques divergences d'opinion sur d'autres sujets.

Merci Visotheary, d'être venu discuter avec moi après ma présentation, nos discussions m'ont aidé à redémarrer durant ma 2^{ème} année de thèse, alors que ça n'allait pas très fort. J'espère bien que nous arriverons un jour à faire quelque chose de ces données !

Florian, je te remercie également, pour avoir accepté d'être dans mon comité, mais aussi pour tes conseils professionnels et parfois plus personnels concernant la gestion de ma thèse.

Merci à Sabine pour ses conseils (en particulier dans la recherche de post-doc !) et sa bibliothèque biogéographique, ainsi qu'à Timothée pour ces siné-anecdotes et ces encouragements ; je remercie Hubert pour ces passages « discrets » dans le bureau des étudiants, Véronique pour nos salutations matinales et son attention, Serge pour sa bienveillance envers les étudiants, Didier pour nos causeries du samedi, Mathieu, Claudia, Liliane, Cécile, Marc et Fabrice pour leur bonne humeur en toute circonstance.

Merci à tous les collègues du bureau Cosson, anciens et actuels. Mentions spéciales à Anaëlle et Lauriane (vous me manquez les filles !).

Je remercie également la communauté en ligne des phylogénéticiens et biogéographes, pour leurs blogs, forums, conseils et tutoriels qui m'ont bien aidés durant ces trois années.

-

Car mes activités associatives ont fait partie intégrante de mon doctorat à l'UPMC et au MNHN, je remercie également les étudiants des associations B'DEM et Timarcha, ceux qui se sont impliqués mais également ceux qui font vivre ces associations en participant à leurs évènements. Au B'DEM, mentions spéciales à Camille, Lauriane, Antoine (tu pourras me voler une bière, promis), Agathe, Baptiste, Corentin et les faisans, on sait tous que notre bureau était le meilleur de tous ! Vous êtes également nombreux à remercier à Timarcha, Catherine et Mathilde (encore elles !), Valentin, Paul, Camille et tant d'autres !

-

Merci à tous les ami-e-s du Muséum et d'ailleurs et aux camarades de master, vous êtes très nombreux à remercier pour avoir fait vivre mon passage au Muséum ! Quelques mentions spéciales tout de même :

Mathilde, ma voisine, collègue de bureau, de Timarcha, compagne de déjeuners, de soirées et de vie quotidienne jusqu'à ces contrées lointaines que nous avons visitées ensemble. Ces années de thèse n'ont pas été des plus faciles pour nous deux, mais je suis heureuse de les avoir passées à tes côtés.

Charlène, ta bonne humeur et ton caractère enjoué et curieux de tout font de toi une personne avec qui il est toujours agréable de discuter. Tu es également une personne attentionnée, qui s'est toujours montrée à l'écoute durant ma thèse.

Meggy, t'es une vraie bourrique mais c'est comme ça qu'on t'aime alors ne change pas. Merci à toi pour ta petite surprise de fin de thèse, ça m'a bien fait plaisir ! Tes délires permettent de décrocher un peu du Muséum et ça fait du bien.

Merci à la promo BEVT « *Taxus baccata* », avec laquelle j'espère garder des liens pour encore longtemps. Mention spéciale à Florian, Fred, Laura et Oriane, qui sont pour certains déjà trop loin ! RDV à Mada en 2018 ?

Paul-Ange, merci pour le soutien logistique infailible sur la YBR. Et dans la famille « Motardes du Muséum », je voudrais saluer Perrine ! V.

Enfin, merci à tous les FT, sympathisants, arrangeoirs, faisans, ENFP et tardigrades, pour avoir été vous-mêmes, tous autant plus fous les uns que les autres. Mon passage au Muséum n'aurait certainement pas été le même sans vous. Nos diverses activités ont déjà commencé à nous séparer (on n'en a même déjà perdu quelques-uns dans l'hémisphère sud !), mais la cladistique parviendra toujours à nous rassembler, quelque part, un jour, 42.

-

Je remercie mes nombreux enseignants pour m'avoir porté jusqu'ici, et qui m'ont supporté dans les classes, bus scolaires et amphithéâtres. Merci en particulier aux responsables pédagogiques et enseignants du Master SEP et BEVT.

-

Enfin, je remercie du fond du cœur ma famille, avec une pensée particulière pour mes parents à qui je dédie ce travail. Vous m'avez toujours soutenue dans mes choix professionnels, même si vous n'étiez pas très rassurés à l'idée de me voir partir dans ces lointaines forêts tropicales. Durant ma thèse, vous avez été mon meilleur soutien, grâce à votre écoute et vos conseils, surtout dans les moments les plus difficiles. Une pensée également à ma petite mamie, toujours aussi émerveillée par mes aventures académiques. Et bon anniversaire à Alfred Grimal !



Contents

GENERAL INTRODUCTION	1
Chapter I - Madagascar sheds new light on the molecular systematics and biogeography of grammitid ferns: new unexpected lineages and numerous long-distance dispersal events	23
CHAPTER II - Molecular data, based on an exhaustive species sampling of the fern genus <i>Rumohra</i> (dryopteridaceae), reveal a biogeographical history mostly shaped by dispersal and several cryptic species in the widely distributed <i>Rumohra adiantiformis</i>	53
CHAPTER III - Biogeographical history of Blechnoideae in Madagascar: connection with tropical regions of the Southern Hemisphere and two in situ diversifications highlighted.....	77
CHAPTER IV - Biogeographical history of <i>Lindsaea</i> and <i>Odontosoria</i> (Lindsaeaceae) in Madagascar: at least two dispersal events from tropical Asia and one in situ diversification highlighted.....	95
CHAPTER V - First insights on the biogeographical history of <i>Phlegmariurus</i> (Lycopodiaceae), with a focus on Madagascar	111
CHAPTER VI - Overview of the biogeographical history of the Malagasy ferns	135
CONCLUSION.....	153
REFERENCES.....	155
SUPPLEMENTARY DATA.....	179
RESUME LONG EN FRANÇAIS	229
ABSTRACT	234
RESUME.....	234

General introduction

In the middle of 18th century, about 10,000 plant species were known by the scientific community (Linnaeus, 1753). A report from the Kew Royal Botanic Gardens evaluates today to more than 369,000 the number of flowering plants, with around 2,000 plant species described annually (Willis, 2017). Around half of this diversity is estimated to occur on 2.3% of the terrestrial surface, in 'biodiversity hotspots' (Myers *et al.*, 2000). To define an area as a hotspot, 0.5% or 1,500 endemic plants have to be listed in an area under threat that have lost 70% or more of its primary vegetation. Today, 35 biodiversity hotspots are defined (Myers *et al.*, 2000; Mittermeier *et al.*, 2004; Williams *et al.*, 2011) and on the basis of the number of endemic plants, the five most important ones are the Tropical Andes, Sundaland, the Mediterranean Basin, Madagascar and the Indian Ocean Islands, and the Brazil's Atlantic Forest. Why is biodiversity so localized to these precise areas and what are the underlying evolutionary, ecological and biogeographical processes behind?

Out of the 35 biodiversity hotspots (Myers *et al.*, 2000; Mittermeier *et al.*, 2004, 2011; Williams *et al.*, 2011), nine are exclusively composed of islands (Caribbean, Wallacea, Philippines, East Melanesian Islands, Japan, Madagascar and the Indian Ocean Islands, New Caledonia, New Zealand and Polynesia-Micronesia). They correspond to a very small area (ca. 10% of all hotspots) but to 21% of the species diversity of all hotspots, highlighting disequilibrium between the surface and the number of species on continents vs. islands.

Islands have played a crucial role in understanding the evolutionary mechanisms in nature, in particular the Moluccas islands in Indonesia and the Galápagos, where Alfred Russel Wallace and Charles Darwin respectively observed an extraordinary biodiversity that influenced their research (Darwin 1859, Wallace 1876). Islands provide exceptional study models by their isolation and their relative simplicity compared to continents, and permit easier developments of models to be later applied on more complex systems (Emerson, 2002; Losos & Ricklefs, 2009; Warren *et al.*, 2015; Santos, Field, & Ricklefs, 2016; Crawford & Archibald, 2017). Moreover, islands vary in their geologic origin, topology, size and localization in diverse bioclimatic regions. The existence of numerous islands provides replicates for evolutionary scientists to test hypotheses about how evolutionary, geologically or spatial processes have shaped biodiversity. However, despite 150 years of island biogeography, the island systems remain a source of many questions about the evolutionary processes: in the study of global diversity patterns, the 'study of relative roles of spatial, historical and ecological processes in driving taxonomic, phylogenetic and functional diversity patterns of island systems' was notably among the first priority issues of the scientific community in island biogeography (Patiño *et al.*, 2017).

1. MADAGASCAR

1.1. CHARACTERISTICS

Madagascar and the Western Indian Ocean islands are located South East of the African coast (Figure 1). It includes Madagascar, the fourth biggest island in the world with ca. 590,000 km² (equal to Spain and Portugal together), the Mascarenes (Réunion, Maurice, Rodrigues; 4,575 km²), the Comoros archipelago (1,862 km²), the Seychelles archipelago (454 km²) and the Scattered Islands (48 km²).

Thereafter, Madagascar and the Indian Ocean islands will be named 'Western Indian Ocean' (WIO) and 'MCS islands' will refer to the Mascarenes, Comoros and Seychelles archipelagos.

Madagascar nests an extraordinary biodiversity that remains poorly known and estimation of species diversity is regularly revised (Goodman & Benstead, 2003, 2005; Callmander, 2011). The endemism is also remarkable, 86% of 5,800 macroinvertebrate species are endemic, but also 84% of 879 vertebrate species and several taxa are endemic (e.g. Malagasy non-flying mammals, Plecoptera, Megaloptera, Ephemera, terrestrial Gastropoda and scorpions, Goodman & Benstead, 2005). About vascular plants, more than 10,000 species of angiosperms are referenced in the *Catalogue of the Vascular Plants of Madagascar*, of which 84% are endemic, as well as four families and 310 of the 1,730 genera (Callmander, 2011; Buerki *et al.*, 2013).

The ecosystem diversity is also high. Madagascar is remarkable by its humidity gradient increasing from West to East, driven by two main factors: the humid winds coming from the Indian Ocean, and a mountain barrier running along almost all the eastern coast, holding these winds and leading to rainfalls (Figure 2A-B). Tropical rainforests are found all along the eastern coast in the humid and subhumid zones. The high central plateau of Madagascar is characterized by a subhumid climate and a dry climate is found along the Western coast. The South, where is found a subarid climate, is less influenced by this gradient (Figure 2B).

The species and ecologic diversity encountered in Madagascar could be explained by the past geographic, geologic and climatic history of Madagascar (Yoder & Nowak, 2006; Agnarsson & Kuntner, 2012). The island was formerly part of Gondwana, a supercontinent of the Southern Hemisphere from the Paleozoic to Mesozoic (McLoughlin, 2001; Seton *et al.*, 2012). The Gondwana break-up started in the beginning of Jurassic with the separation of Antarctica, Africa-South America and Madagascar-India (165-162 Ma, Figure 3). Africa and South America separated from each other during Lower Cretaceous. Madagascar was geologically isolated after its separation with India at the end of Cretaceous (95-84 Ma). At this time, Madagascar was most likely covered by an arid forest, similar to the forest found today in the South (Buerki *et al.*, 2013). From the Paleocene, the Madagascar northward migration allowed the island to leave this arid climatic zone, now restricted to the extreme South of the island (Wells, 2003). Moreover, the humid winds from the Indian Ocean, formerly stopped by India, could have started to increase the humidity from 60-35 Ma, leading to the gradient of humidity still observed today (Wells, 2003). Beside these tectonic events, volcanic activities and other geologic events have also shaped the Malagasy landscape, notably the northern Montagne d'Ambre or the Western karstic Tsingy of Bemahara that offer plenty of original habitats.

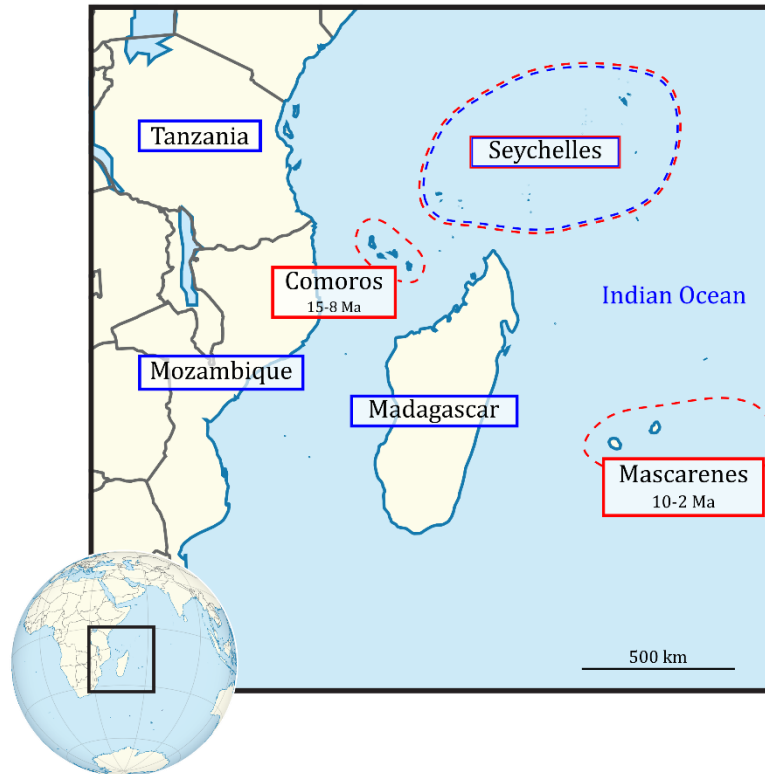


Figure 1. Map of the Western Indian Ocean islands and Eastern Africa. Areas resulting from the break-up of the paleo-continent Gondwana are illustrated in blue and oceanic islands in red, associated with their age of origin.

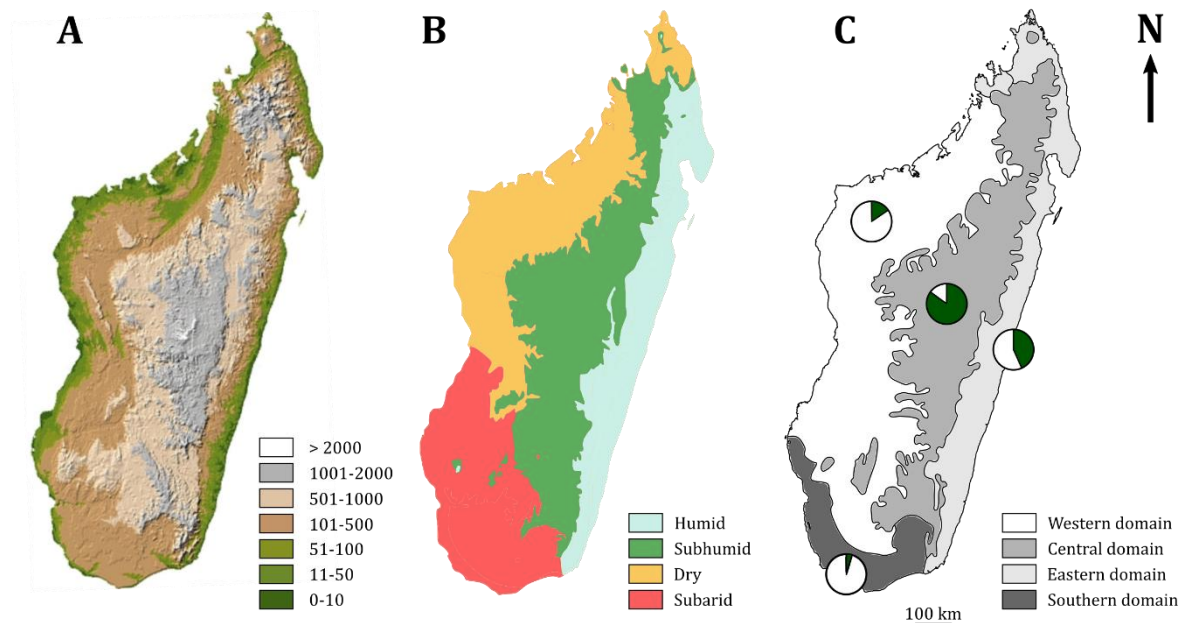


Figure 2. A. Topographic map of Madagascar (source: Wikipédia). B. Bioclimates by Cornet (1974), map modified from www.mobot.org/mobot/research/madagascar/gazetteer/. C. Phylogeographic domains from White (1983). Pie charts represent in dark green the percent of the total diversity of pteridophytes found in the different domains, from Rakotondrainibe (2003a).

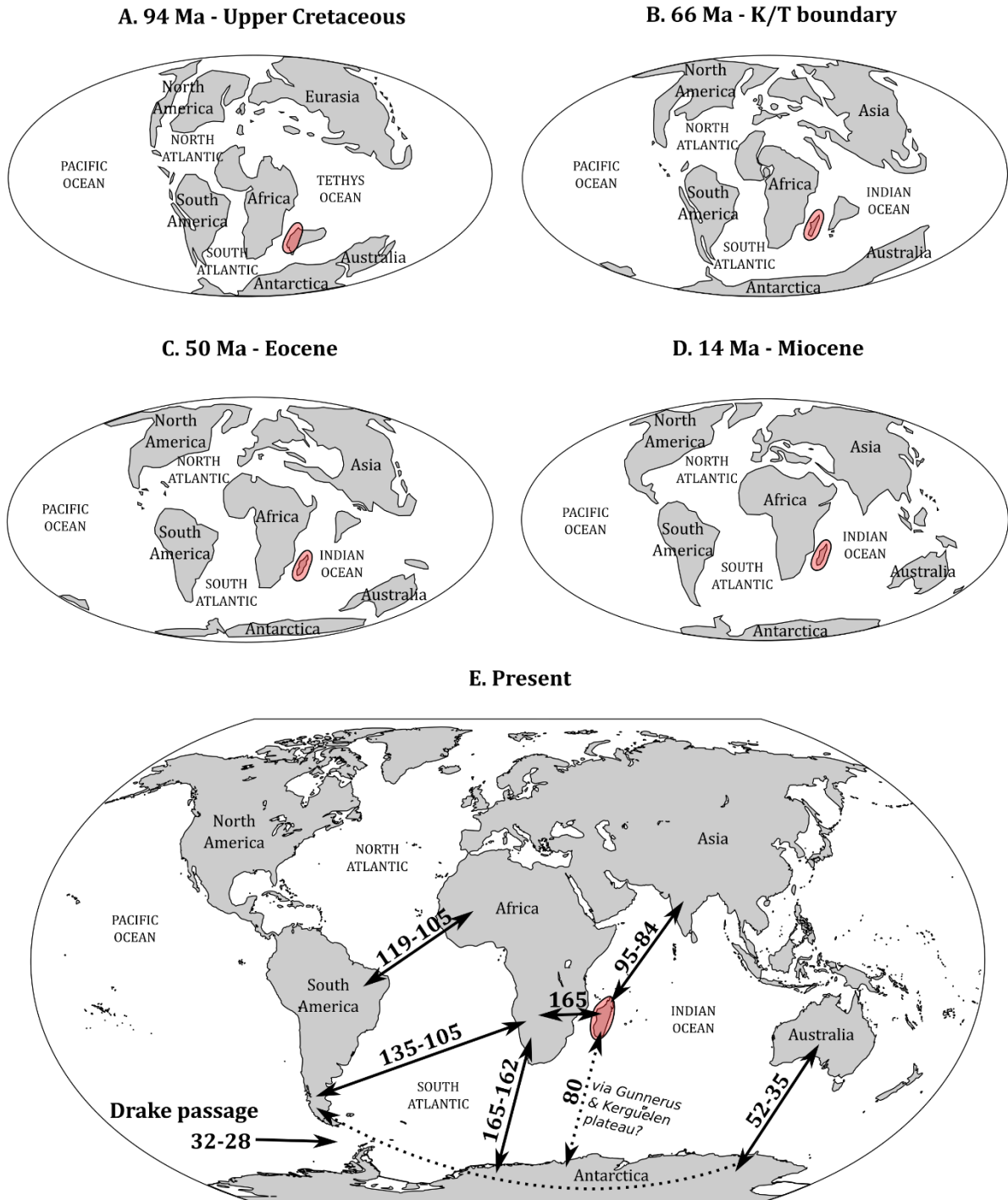


Figure 3. A-D: Spatio-temporal history of the continents. Paleomaps modified from PALEOMAP project, Scotese (2001). **E.** Modern positions of the continents (source: Wikimedia), with age of Gondwana break-ups between modern lands from McLoughlin (2001). Madagascar is indicated in red.

1.2. BIOGEOGRAPHY OF MALAGASY SPECIES

1.2.1. *A complex biogeographical history*

The modern assemblage of Malagasy species is most likely the result of a complex biogeographical history, involving many biogeographical processes like Gondwanan vicariance, dispersal from various source pools, anagenesis, cladogenesis and extinction (Yoder & Nowak, 2006; Agnarsson & Kuntner, 2012). However, compared to oceanic islands like Hawaii or the Galapagos, the biogeography of species of continental islands like Madagascar and the relative importance of these processes remain poorly studied.

When Madagascar became isolated at the end of Cretaceous, it favored an independent evolution of its biome and vicariance due to the break-up of Gondwana. Vicariance can occur when a population is divided (in two or more) subsequently to the formation of a barrier like a mountain or a continental break-up. If the organisms fail to maintain sufficient gene flow, isolation will result in independent evolution on each side of the barrier and so to vicariant speciation. This isolation is a main factor in the biogeographical history of Malagasy species, giving the original biodiversity we know today and represented by some iconic endemic groups like the lemurs, or by the absence of others like the salamanders (Yoder & Nowak, 2006). Vertebrate fossils shared between Madagascar, India and South America however suggest that a terrestrial connection remained between these lands after the Gondwanan break-up until 80 Ma, via the Gunnerus and Kerguelen Plateau and Antarctica (Yoder & Nowak, 2006), but some authors have doubts about this connection (Ali & Krause, 2011).

Reviews on the biogeographical history of Malagasy biotas based on molecular studies reach a common conclusion: Cenozoic dispersal events have mostly shaped the modern assemblage of Malagasy taxa (Masters, De Wit, & Asher, 2006; Yoder & Nowak, 2006; Agnarsson & Kuntner, 2012; Samonds *et al.*, 2012; Buerki *et al.*, 2013). Based on phylogenies and the distribution of Malagasy sister taxa, reviews on the flora and the fauna showed diverse biogeographical affinities but especially that the nearest continent, Africa, would be the main source pool for Madagascar (Yoder & Nowak, 2006; Agnarsson & Kuntner, 2012). This observation is concordant with the Theory of Island Biogeography for which the main species source pool for an island would be the nearest continent (MacArthur & Wilson, 1967). Among the angiosperms, on the basis of floristic affinities, this tendency was well retrieved with 81% of the Malagasy non-endemic species also found in Africa and 39% shared only with Africa (Callmander, 2011). A study on the Malagasy endemic genera of angiosperms also retrieved a main African biogeographical affinity (Buerki *et al.*, 2013).

However, Africa is not the only species source pool for Madagascar. In one of the reviews that showed a major African influence (Yoder & Nowak, 2006), 31% of all the biogeographical affinities were found with Asia (once all the Asian subdivisions are gathered), against 38% for Africa (flora plus fauna). The Asian biogeographical affinities became even more important than the African ones when only the vertebrates were studied (32% for Asia, 29% for Africa). Biogeographical affinities between Asia and Madagascar could be explained by vicariance, winds across Indian Ocean (monsoons, South Indian Ocean gyre), stepping-stone dispersal events by the Seychelles since its

separation from Madagascar 65 Ma (McLoughlin, 2001), by the drifting Indian landmass and by a chain of stepping-stone islands that formed between India and Madagascar during the last 34 Ma (Warren *et al.*, 2010; and references therein).

The third main area of origin by dispersal found in the literature is South America. For the angiosperm endemic genera, it was found in fourth position with 7.7% of biogeographical affinity, behind Africa (22.4%), Southeast Asia (9.1%) but before India (6.2%) or Australasia (5.6%, Buerki *et al.*, 2013). Neotropics are however largely behind if all the Asian affinities are combined (22.8%, India-Asia-Southeast Asia-Australasia, against 7.7%). Biogeographical affinities with the Neotropics were also retrieved by Yoder & Nowak (2006) for plants and animals, but with higher biogeographical affinities for plants.

1.2.2. Diversification: generation of a regional source pool

Biologists have also examined Madagascar as a model system to study diversification (Yoder & Nowak, 2006; Vences *et al.*, 2009; Schneider *et al.*, 2010; Agnarsson & Kuntner, 2012). The geographical isolation, the habitat heterogeneity and the climatic fluctuations could be main drivers that explain diversification in Madagascar (Vences *et al.*, 2009; Schneider *et al.*, 2010). Diversification is also correlated to habitats, high elevation range and habitat heterogeneity being drivers of species richness and in situ diversification (e.g. Hortal *et al.*, 2009; Stein, Gerstner, & Kreft, 2014).

It was widely assumed that islands are sinks for biodiversity, and that island lineages never colonize a continental area (Heaney, 2007). However, Madagascar has played a role of source pool, firstly for the other islands in the WIO but also for Africa. Strong biogeographical affinities with the Mascarenes, Seychelles and Comoros archipelagos were shown based on the study of sister taxa (Yoder & Nowak, 2006; Buerki *et al.*, 2013) and biogeographical analyses have also provided a growing body of evidences based on estimation of the ancestral areas (e.g. Krüger, Razafimandimbison, & Bremer, 2012; Strijk *et al.*, 2012; Bukontaite *et al.*, 2015; Bacon *et al.*, 2016; Kainulainen *et al.*, 2017). Moreover, dispersal hypotheses out of WIO were also documented to Asia in animals like beetles (Bukontaite *et al.*, 2015) and in angiosperms (Bacon *et al.*, 2016).

In the Yoder & Nowak's analysis of the Malagasy sister taxa (2006), only one taxon of non-seed plants was included (*Plagiochila*, a Marchantiophyta genus), highlighting the obvious lack of data for spore-bearing plants. Malagasy ferns have been poorly studied compared to angiosperms and were not mentioned in reviews (Yoder & Nowak, 2006; Agnarsson & Kuntner, 2012). Until 2014, tree ferns Cyatheaceae were the only ferns on which a biogeographical analysis focused on Madagascar was done, showing a biogeographical history dominated by multiple African colonizations followed by radiation (Janssen *et al.*, 2008). However, there is a growing body of phylogenetic evidences showing other biogeographical patterns for Malagasy ferns, maybe driven by a higher connectivity among the southern continents, especially between Madagascar and the Neotropics (Rouhan *et al.*, 2004, 2007a).

2. INTRODUCTION TO FERNS

2.1. SYSTEMATICS

Polypodiopsida, or 'ferns', form a monophyletic group in the tracheophytes (vascular plants) and they are often called in the literature together with lycophytes under the names 'seed-free vascular plants', 'pteridophytes' or 'ferns and ferns allies', but these terms refer to non-monophyletic taxa as they name the tracheophytes without the seed-plants (Figure 4). Ferns would represent 90% of extant pteridophyte species diversity.

Since 2016, the pteridologists community has reached a consensus on the classification of ferns, led by the 'Pteridophyte Phylogeny Group' (PPGI, 2016). This consensus will be followed in the present thesis and the term 'fern' and 'Polypodiopsida' will be considered as equal and will designate a tracheophyte clade including the Equisetidae (horsetails), Ophioglossidae (whisk ferns, moonworts, adder's-tongue ferns), Marattidae, and Polypodiidae (leptosporangiates). Polypodiidae includes the most part of the diversity like the royal ferns, the filmy ferns (Figure 5D), the aquatic ferns (Figure 5G), the tree ferns (Figure 5J) or the polypody. Around 12,000 pteridophyte species are estimated, and classified into 337 genera and 51 families (PPGI, 2016). It is comparatively small face to the flowering plants diversity of 369,000 species and some authors explain it by: i) high rates of gene flow, thanks to the wind-mediated spores that prevents speciation; ii) plasticity (whereas angiosperms easily form ecotypes); and iii) lack of sexual selection that would be a key driver of radiation in angiosperms (Givnish, 2010; Patiño *et al.*, 2014; Ranker & Sundue, 2015).

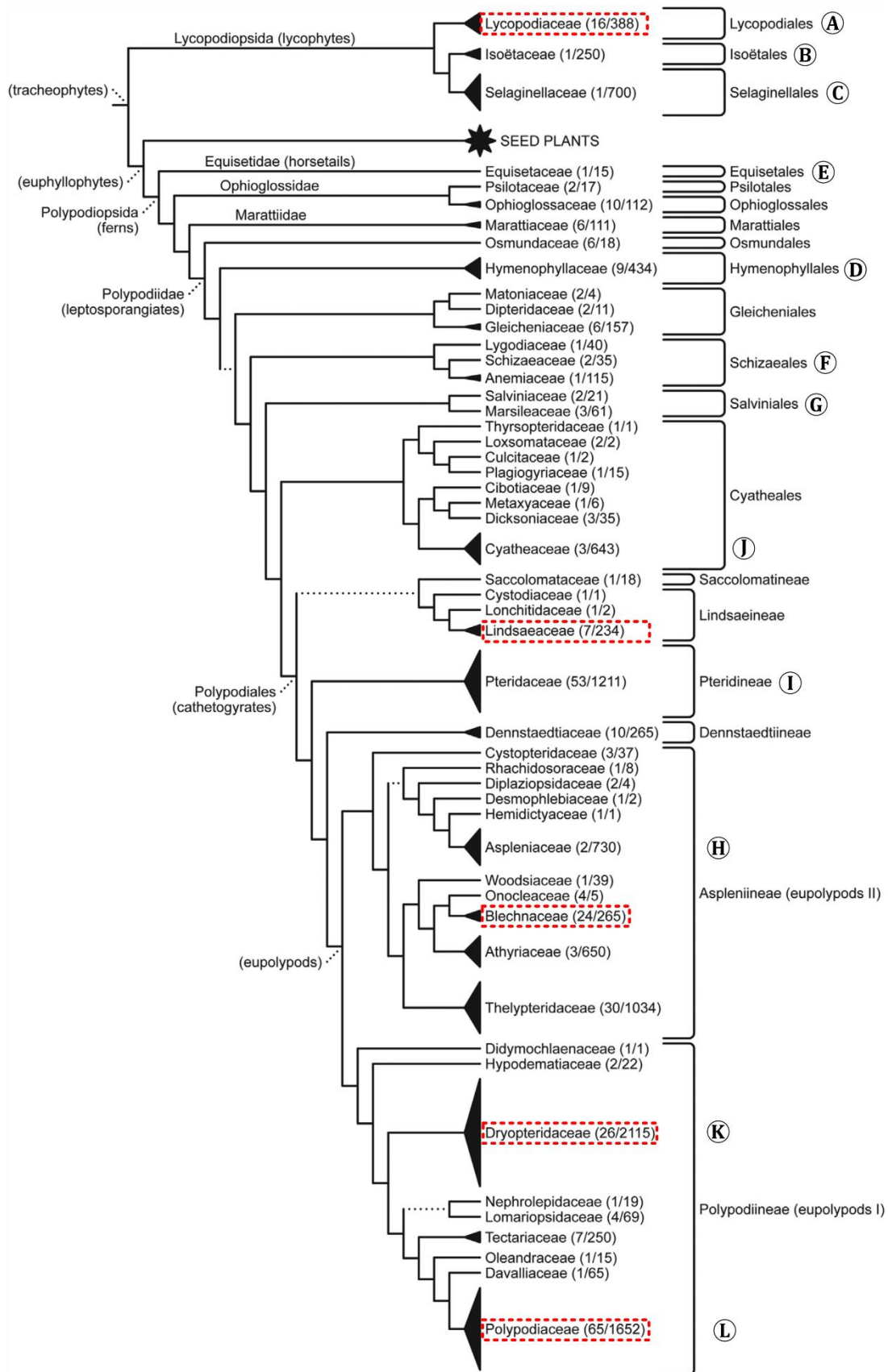


Figure 4. Summary tracheophyte phylogeny modified from PPGI (2016), number of genera and species estimated by family. Families of the taxa studied in this thesis are highlighted by red boxes. Letters A to L, next to taxon names, refer to illustrations in Figure 5.

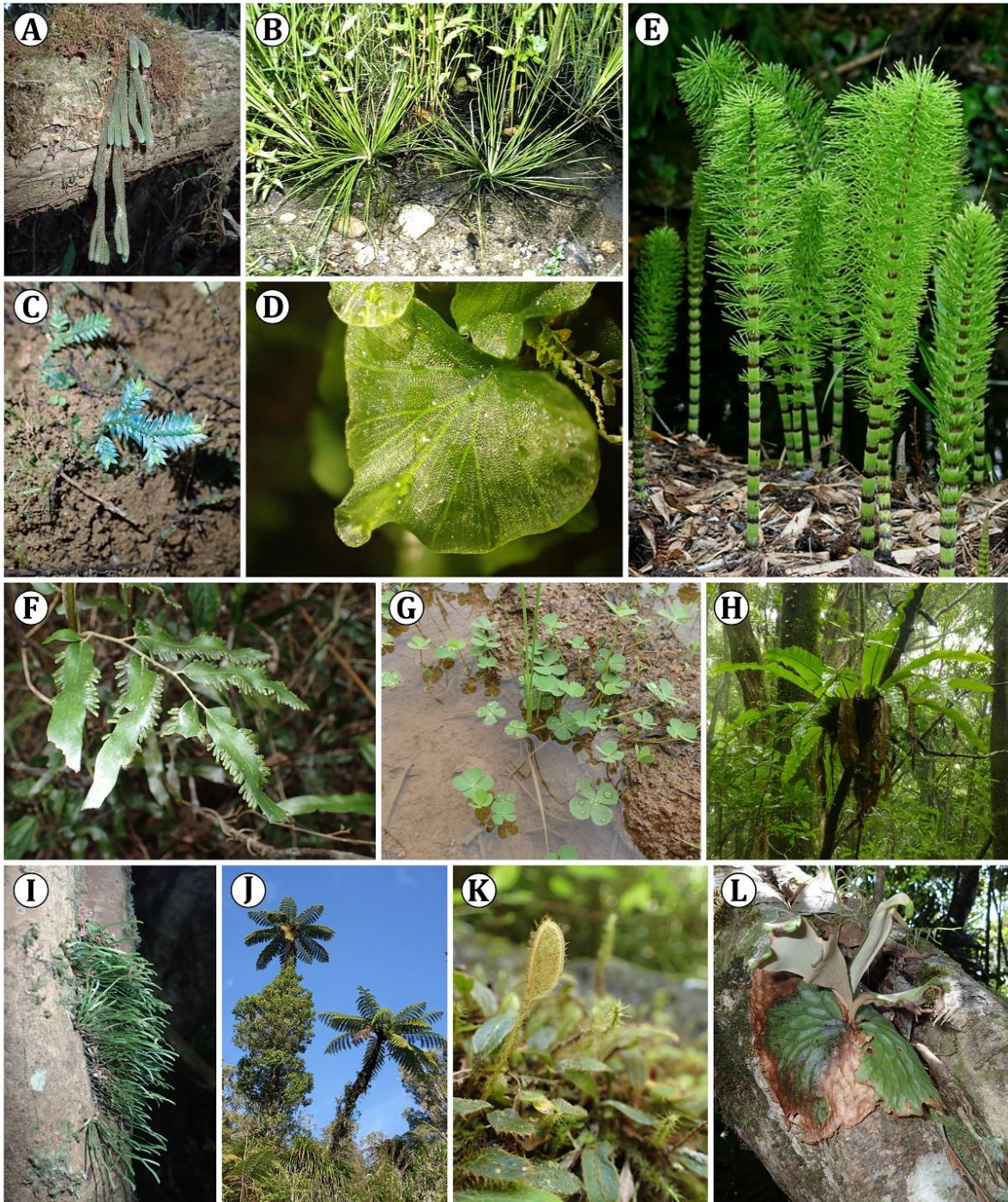


Figure 5. Lycophytes and ferns diversity. A. *Phlegmariurus pecten*, B. *Isoetes engelmannii*, C. *Selaginella* sp., D. *Didymoglossum cuspidatum*, E. *Equisetum* sp., F. *Lygodium lanceolatum*., G. *Marsilea quadrifolia*, H. *Asplenium nidus*, I. *Monogramma graminea*, J. *Cyatheaceae*, K. *Elaphoglossum spatulatum*, L. *Platycerium* sp. (Photos: L.Bauret : A, C, D, F-I, J, L ; Wikipedia Commons: B, E, J).

2.2. LIFE CYCLE AND DISPERSAL

Ferns are spore-bearing plants with a life cycle alternating between a haploid and a diploid generation. The gametophyte produces the gametes (sperm and egg cell) and represents the haploid generation. It is unisexual or bisexual. A humid environment is necessary for fecundation, the sperm being dependent on water to swim from antheridia to encounter the egg cell in the archegonia. The diploid embryo will form a sporophyte, the spore-bearer and dominant generation (in most cases), morphologically characterized by a frond (lamina plus petiole) and a rhizome (Figure 6). This life cycle (Figure 6) is one of the possible sexual mating systems in homosporous ferns. Crossing between gametes from two different gametophytes was also documented, like apomixis (alternation of generations without fertilization) and apogamy (sporophytes from somatic cells) from gametophytes or sporophyte buds (Sheffield, 2008; Sessa, Testo, & Watkins, 2016).

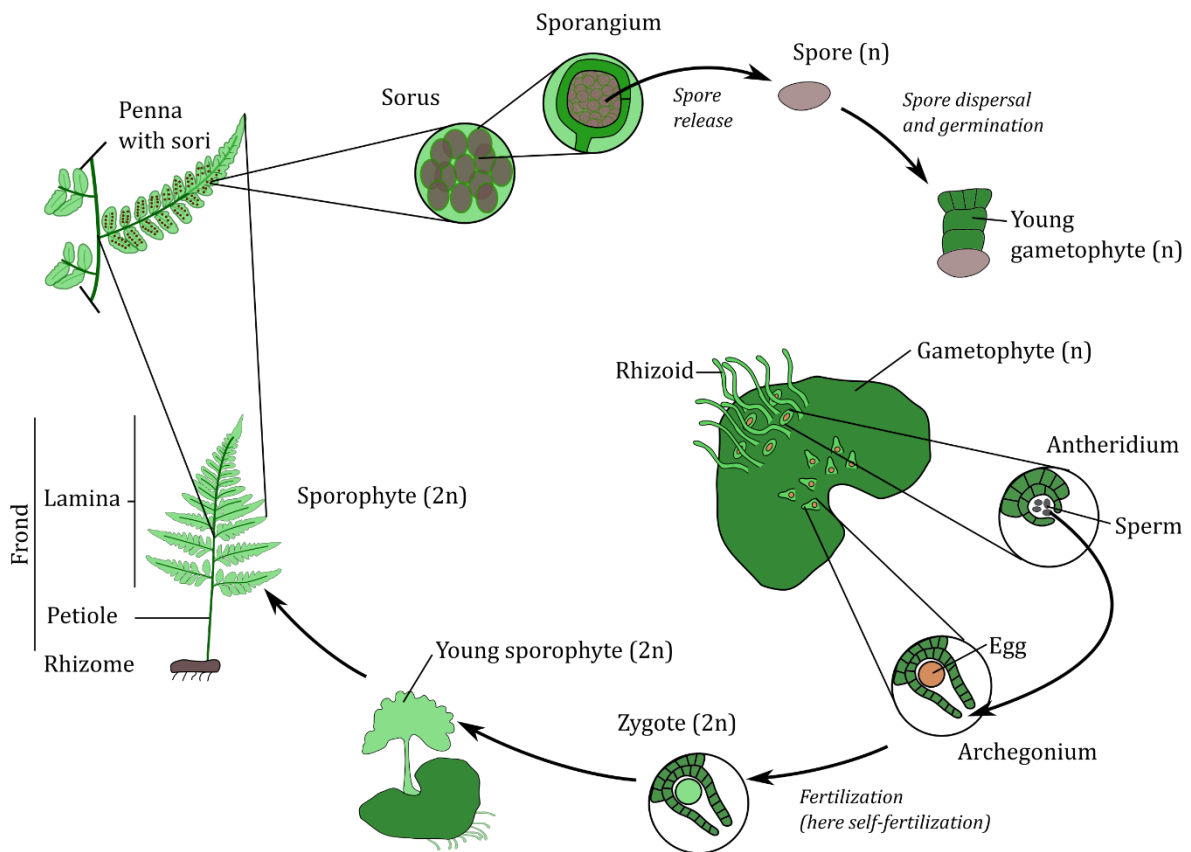


Figure 6. Life cycle of ferns showing gametophyte selfing, a sexual mating system where a single spore germinates into a bisexual gametophyte that produces both eggs and sperm, partially redrawn from Sessa, Testo, & Watkins (2016).

Spores are also involved in the dispersal of ferns. The first step of a dispersal event is spore shooting by the sporangium, a structure consisting of a capsule measuring about 0.25 mm in diameter, and in which the spores grow (Moran, 2004). The process is fast and impressive, and can be summarized as a catapult: 1) the physical and chemical properties of water allow bending the sporangium cell wall, slowly opening it and exposing the spores (Figure 7a-c); 2) when the bending reaches a critical point, all the accumulated

pressure is suddenly released (8d), shooting the spores out of the sporangium, and the sporangium retrieves its initial position (8e).

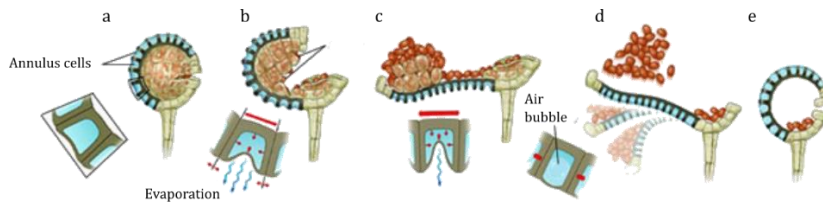


Figure 7. Stages of spore shooting by a sporangium. (Modified from T. Moore's drawings).

Some authors reported presence of spores on animals (Lewis *et al.*, 2014), but their small size (15-150 μm) allow them to be transported by wind (Tryon, 1986). Atmospheric samplings at high altitudes, notably jet streams, have revealed the presence of fern spores resisting low temperatures and UV radiations (Moran, 2008; and references therein). The importance of wind for spore-bearing plants have notably been showed in the Southern Hemisphere (Munoz *et al.*, 2004), in particular for ferns (Parris, 2001; Sundue, Rouhan, & Moran, 2010b) to explain wide distributions of species or higher taxa. In fact, the great dispersal ability of ferns has even made some authors to consider that the dispersal ability as a variable or as a limiting factor can be eliminated in the study of fern biogeography (Tryon, 1972).

These high dispersal abilities are retrieved in the biogeography of ferns that on average represent 3.6% of the flora on continents but 15.3% on islands (Kreft *et al.*, 2010) and they are overrepresented on remote islands by representing 16-60% of the flora (Moran, 2008). A lower endemism compared to angiosperms was also noticed, with for example 89% of endemic angiosperms in Hawaii, against 76% for ferns (Ranker, Gemmill, & Trapp, 2000; Ranker, 2016). In the literature, these observations are explained by the high vagility of spores (Smith, 1972; Moran, 2008).

If spores provide dispersal abilities to ferns, some gametophytic morphologies or habitats could provide supplementary advantages. For example, a disharmony was observed in island flora in favor of epiphytic species with gametophytic gemmae (tissue fragments that could develop into a new gametophyte), which would increase success of colonization (Dassler & Farrar, 2001). Even continental islands like Madagascar displays the same disharmony compared to Africa (Dassler & Farrar, 2001).

Even if the important role of dispersal in fern biogeography is undeniable, there is still a debate to understand the relative importance of intrinsic and extrinsic factors, dispersal and vicariance, in shaping the ranges of ferns (Wolf, Schneider, & Ranker, 2001; Haufler, 2007).

2.3. WORLDWIDE DISTRIBUTION OF FERN SPECIES

Fern species can be terrestrial (e.g. Figure 5J), aquatic (Figure 5G), epilithic (Figure 5K) or epiphytic (e.g. Figure 5A) and are found on every continent except Antarctica. The species diversity follows a strong latitudinal and altitudinal gradient: highest species richness is retrieved between the tropics and in mountains, and the Neotropics and Southeast Asia nest the highest species diversity, with respectively ca. 3,500 and 4,500 fern and lycophyte species (Figure 8).

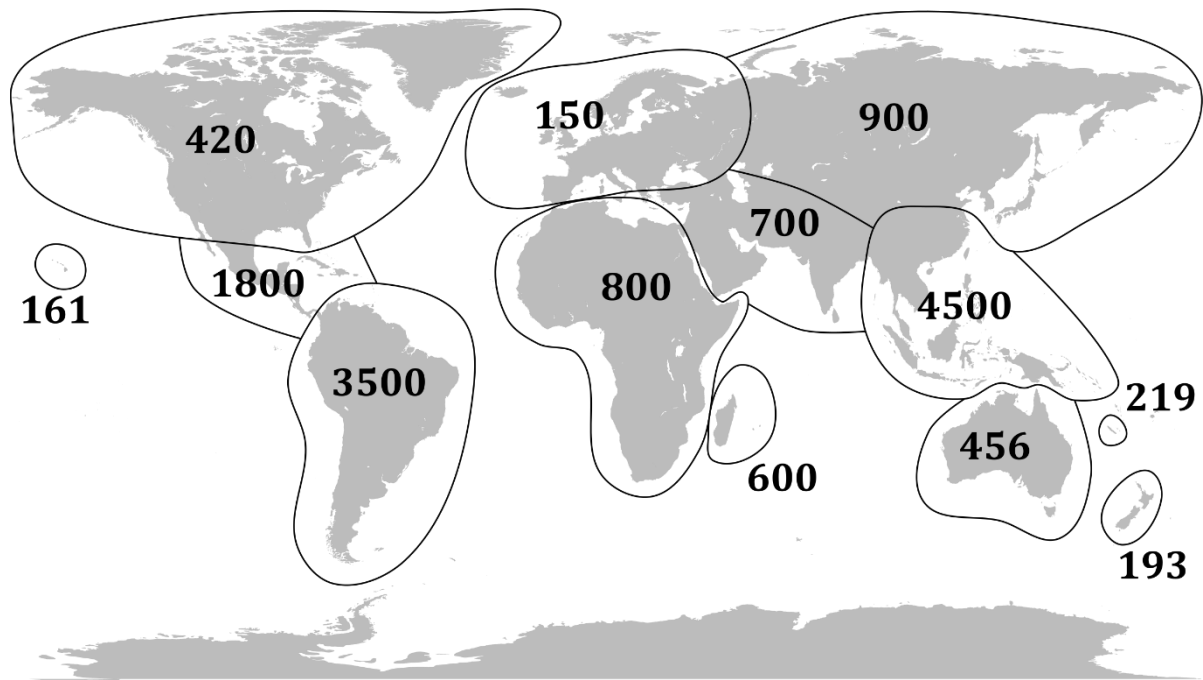


Figure 8. Ferns and lycophytes diversity in the world. Redrawn from Moran (2008).

2.4. ORIGIN AND PERIODS OF SPECIES DIVERSIFICATION

Leptosporangiate ferns would have originated during the Carboniferous (Galtier & Scott, 1985) and would have undergone successive radiations: an initial radiation in the Carboniferous (giving rise to several, now extinct families), a second in the late Paleozoic and early Mesozoic (first diverging families of living leptosporangiate ferns like Hymenophyllaceae, Cyatheaceae or Salviniaceae), and a third one in the Cretaceous giving rise to most families represented in the Paleogene (Rothwell & Stockey, 2008; Schuettpelz & Pryer, 2009). The last radiation, occurring during the Cenozoic, coincide with the proliferation of angiosperm forests that would have offered new habitats, both on forest undergrounds and in forest canopies as epiphytes. However, no evidence of differential rates of diversification among terrestrial and epiphytes was found (Schuettpelz & Pryer, 2009; Testo & Sundue, 2016).

In contrast to the common idea that ferns are ‘old plants’, being wrongly called ‘living-fossils’ by the general public, the modern fern species arose quite recently during the Cenozoic compared to their deep phylogenetic roots in the Carboniferous. This relative youth has obvious consequences on our understanding of their biogeographical history.

3. FERNS OF MADAGASCAR

3.1. SYSTEMATICS AND ECOLOGY

The study of the Malagasy fern diversity started from the end of the 19th century, notably thanks to Baker and Bonaparte (e.g. Baker, 1887; 1915-25), on the basis of collection from famous explorers and naturalists (e.g. Commerson, Boivin, Humblot, Hildbrandt, Perrier de la Bâthie, Decary or Humbert). Major works were provided by Christensen (1932) who listed and described more than 500 Malagasy fern species, and twenty-years after by Tardieu-Blot who published the *Flore de Madagascar et des Comores* (1951-71)

under the direction of Humbert, in the Muséum national d'Histoire naturelle (MNHN). Today, the research on the Malagasy fern flora is still mainly led by researchers at the MNHN, especially by Rakotondrainibe and Rouhan who also have been contributing to enrich the fern herbarium collection from Madagascar over the past 30 years. In 2003, 586 species and varieties of pteridophytes representing 106 genera were estimated (Rakotondrainibe, 2003a), but a new estimation would be closer to 700 species (Rakotondrainibe, pers. com.).

The Malagasy ferns are terrestrial, epilithous, epiphyte or aquatic, and a species is most often characterized by only one of these habitats (Rakotondrainibe, 2003b). One genus, *Lygodium*, is lianescent (Figure 8F). Studies on ecological gradients in four Malagasy forests showed a climatic influence, especially a moisture effect on ferns: the percent of epiphytes decreased with the water deficit and increased with the elevation. Furthermore, 43.3% and 85.0% of the total diversity is found respectively in the Eastern and Central domains of Madagascar (Figure 2C; Rakotondrainibe, 2003b). A high number of species in the Central domain are endemic to this part of the island (37.4%), probably because of the important altitudinal zonation and the moisture. The dry to subarid Western and Southern domains are comparatively poor (with respectively 15.4% and 4.6% of the total diversity represented).

Based on Rakotondrainibe's checklist (2003a), 45.2% of the Malagasy pteridophytes are endemic (against 84.0% for angiosperms; Callmander, 2011). Moreover, no higher taxon is endemic to Madagascar (genus or family). The most diversified genera are *Asplenium* (65 species; 35.4% of endemic species and varieties), *Cyathea* (43 spp.; 90.7% of endemism, it is the highest endemism level observed for Malagasy ferns at a generic level) and *Elaphoglossum* (34 spp.; 58.8% of endemism).

The *Catalogue of the Plants of Madagascar* (www.tropicos.org/Project/Madagascar; accessed 26th June, 2017) counts today 721 accepted pteridophytes species and varieties, classified in 120 genera. Therefore, the database includes 135 more pteridophytes species and varieties, and 30 additional genera compared to the Rakotondrainibe's checklist. Forty genera (ca. 33%) are represented in Madagascar by one non-endemic species and five by one endemic species. The increasing number of species is a sign of recent improvements in the knowledge of Malagasy diversity (e.g. Rouhan, Rakotondrainibe, & Moran, 2007b; Janssen & Rakotondrainibe, 2008; Rakotondrainibe, 2010a), but these numbers should be treated carefully as a lot of information remains to be checked in the database. The increasing use of molecular markers has notably permitted the circumscription of species or higher taxa hardly distinguishable on the basis of their morphology, notably in ferns (e.g. Ranker *et al.*, 2004; Rothfels *et al.*, 2008). However, the systematics of Malagasy ferns was poorly studied with the aid of molecular data (Rouhan *et al.*, 2004, 2007a, 2012).

3.2. BIOGEOGRAPHICAL HISTORY OF THE MALAGASY FERNS: CLUES FROM DIFFERENT DATA

Several sources of information can be used to understand the biogeography of the Malagasy ferns: fossils, floristic affinities, phylogenies, dating analyses and ancestral area estimates.

3.2.1. *A poorly known fossil record*

Paleobotanical data on Malagasy ferns are scarce and restricted to Appert's studies about the Jurassic and Cretaceous (Appert, 1973, 2010). A temperate fern flora was present in Late Jurassic in the Manamana region (South-West), with Schizeaceae, Gleicheniaceae, Matoniaceae and Dipteridaceae (Appert, 1973). A study of macrofossils from the Early Cretaceous (133-129 Ma) was conducted in the Manja region (South-West; Appert, 2010). It revealed a flora dominated by Bennettiales (cycadeoid fossil plants) but ferns were also found, representing several families: Marattiaceae, Osmundaceae, Matoniaceae, Dipteridaceae, Gleicheniaceae, Hymenophyllaceae, Dicksoniaceae and Polypodiaceae s.l. This assemblage suggests a warm, probably tropical and seasonally humid climate, probably moderated by the proximity of the sea. Wild fires are also suggested, due to the presence of charred plant debris.

Therefore, the biogeographical history of the Malagasy ferns cannot be understood on the basis of the fossil record only. The Appert's contributions however highlighted a bioclimatic turnover, from temperate fern species in the Late Jurassic to species of warm and humid climate.

3.2.2. *Floristic affinities*

On the basis of the *Catalogue of the Plants of Madagascar* (www.tropicos.org/Project/Madagascar; accessed 26th June, 2017), 18% of the Malagasy pteridophytes were shared with the whole Western Paleotropics (WIO plus Africa), 11% with other WIO islands, 12% with Africa and at least 14% were shared with other tropical regions (Figure 9A). Therefore, at least 41% of the non-endemic Malagasy species show affinities with another Western Paleotropical region. Floristic affinities with distant areas were also highlighted in previous floristic surveys with the Neotropics (Moran & Smith, 2001) or tropical Asia (Kramer, 1972).

In lycophytes (Figure 9B), very close results were found with 55% of endemism, 15% of the Malagasy lycophytes being distributed in Madagascar and Africa, 10% in the WIO, 10% being pantropical, 8% being distributed in all the Western Paleotropics and 2% being shared with the Neotropics. These results suggest that ferns and lycophytes may have similarities in their historical biogeography, leading to similar floristic affinities. This could result from shared intrinsic factors (dispersal by spores, ecological preferences). By contrast, angiosperms showed a higher level of endemism (84%; Figure 9C), highlighting the dissimilarities between seed and spore-bearing plants.

At a regional scale, the Malagasy ferns endemic to the WIO were mostly shared with the Mascarenes (43% of the non-endemic Malagasy species shared only with the Mascarenes), then with the Comoros-Mascarenes (20%), and with the Comoros (16%, Figure 9D). Malagasy angiosperms however showed the highest floristic affinity with the Comoros (50%), then the Mascarenes (31%), and relatively few species are distributed in Madagascar and two other WIO archipelagos, suggesting less exchange between the WIO

islands compared to ferns (Figure 9E). The higher affinities found with the Comoros rather than with the Mascarenes in angiosperms may be correlated to ecological factors, like lower elevation range in the Comoros (ferns being especially diversified in the mountainous rainforests of Madagascar, Figure 2).

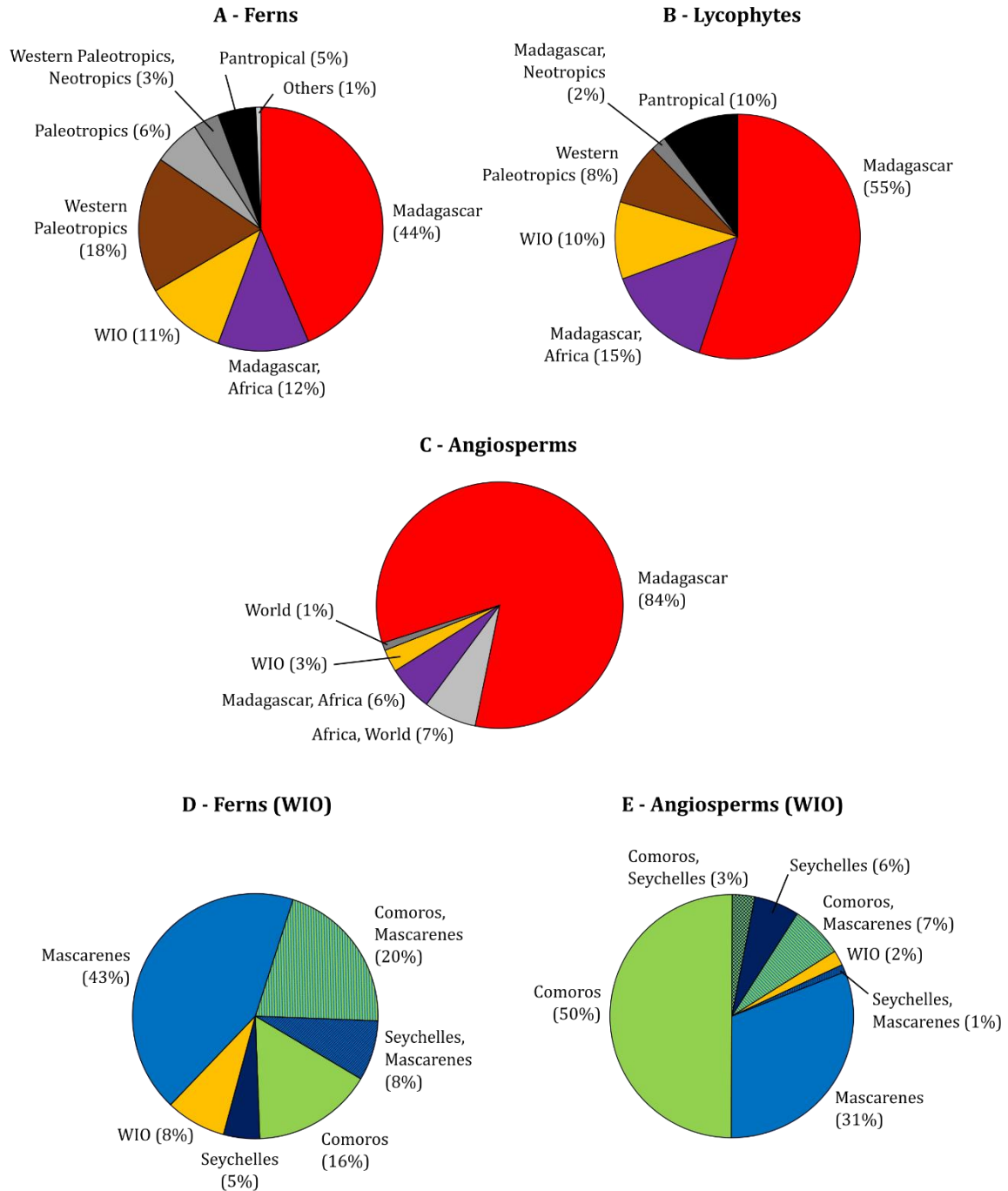


Figure 9. Distribution of the Malagasy ferns, lycophytes and angiosperms. Distribution of A. Malagasy ferns, based on a data retrieval of 579 accepted species from the *Catalogue of the Plants of Madagascar* (26th June, 2017), B. Malagasy lycophytes, based on a data retrieval of 48 accepted species from the *Catalogue of the Plants of Madagascar* (26th June, 2017), C. Malagasy angiosperms (10,319 spp.), modified from Callmänder (2011), D. non-endemic Malagasy ferns shared only with the WIO islands (63 spp.), based on the same data retrieval as B., E. non-endemic Malagasy angiosperms shared only with the WIO islands (285 spp.), modified from Callmänder (2011).

3.2.3. *Molecular phylogenies, dating and ancestral area estimates*

In historical biogeography, methods rely on the idea that the past biogeographical distribution can be inferred from the current distribution of species and their phylogenetic relationships (Ronquist & Sanmartín, 2011). Additionally, time-calibrated molecular phylogenies combined to biogeographical analyses allow inferences of the biogeographical history of taxa: the areas of origin and the underlying processes like vicariance, dispersal, diversification, and the timing of these processes.

Rouhan *et al.* (2004) were the first to make inferences about the areas of origin of Malagasy ferns on the basis of phylogenetic relationships estimated from molecular data (Table 1). Their work on *Elaphoglossum* suggested that at least 13 long-distance dispersal events (LDD) occurred between the Neotropics and the Western Paleotropics, notably Madagascar. Such Neotropical connections were notably confirmed in *Lomariopsis* (Rouhan *et al.*, 2007a). The first biogeographical analysis including estimates of ancestral areas focused on Malagasy ferns was conducted on Cyatheaceae. It brought evidences for three independent dispersal events from African ancestors during the Miocene, all followed by radiation in Madagascar (Janssen *et al.*, 2008; Korall & Pryer, 2014).

Since then, numerous phylogenies were published but often relied on a poor sampling of the worldwide and/or Malagasy species diversity, making sometimes hazy to infer the areas of origin. Moreover, the sampling of widely distributed species often blurred the inferences that could be more easily retrieved on the basis of the sampling of Malagasy endemics. In Table 1, the studies including Malagasy endemics were recorded, as well as the most likely ancestral range of the Malagasy lineage (estimated by analyses or inferred by hand).

Neotropical origins hypothesized by Rouhan *et al.* (2004, 2007a) were observed at least in grammitid ferns (Sundue *et al.*, 2014). If this study relied on a poor sampling of the Malagasy species diversity, it however highlighted that multiple trans-Atlantic dispersal events occurred from the last 15 Ma from the Neotropics to the Western Paleotropics. About Africa, tree ferns would not be the only ferns to have colonized Madagascar from this continent, as other dispersal events from African ancestors were inferred in *Parapolystichum* and *Dryopteris* over the last 25 Ma (Labiak *et al.*, 2014; Sessa *et al.*, 2017). Biogeographical affinities were also found with the Eastern Paleotropics (Asia, Southeast Asia to Australasia and the Pacific) in Lindsaeaceae and Hymenophyllaceae, but only Chao *et al.* (2014) clearly inferred Asian dispersal events by ancestral areas estimates over the last 10 Ma.

In Hymenophyllaceae, the old divergence of the family (ca. 280 Ma, Schuettpelz & Pryer, 2009) can also allow vicariance hypotheses subsequently to tectonic events, as the separation of Madagascar from India dated to ca. 90 Ma (McLoughlin, 2001). Therefore, dating analyses remain to be done to know if the age of the Malagasy lineages coincide with this event. Most of the families distributed in Madagascar have appeared after the isolation of Madagascar (Schuettpelz & Pryer, 2009), so the biogeographical origins of Malagasy ferns would be mainly explained by dispersal events. The ages of dispersal recorded in Table 1 notably show that all the events that have been inferred so far would postdate the end of Paleogene (< 25 Ma). Dating analyses are for now too scarce to know if this tendency can be generalised, but these results highlighted that factors could have

been limiting in the colonization of Madagascar by vascular spore bearing plants. Finally, in situ diversifications were observed in at least 10 genera, suggesting that the process would be a driver of Malagasy species diversity.

Some biogeographical origins remained unknown as they cannot be directly inferred from the phylogeny and the distribution, due to the diversity of the distribution of Malagasy sister lineages (e.g. *Platyserium*), lack of resolution (e.g. *Bolbitis*) or widely distributed Malagasy species (e.g. *Dryopteris*, Table 1). Also, ancestral areas estimates could not lead to clear results (e.g. *Pteris*, Table 1).

	Area(s) of origin	Age (when dating analysis)	Diversification in situ?	References
<i>Elaphoglossum</i> Dryopteridaceae	Neotropics, unknown	-	Yes, but not in all the lineages	Rouhan <i>et al.</i> (2004)
<i>Platyserium</i> Polypodiaceae	Unknown	-	Yes	Kreier & Schneider (2006)
<i>Lomariopsis</i> Lomariopsidaceae	Neotropics	-	Yes	Rouhan <i>et al.</i> (2007a)
<i>Cyathea</i> s.l. Cyatheaceae	Africa	< ca. 10 Ma	Yes	Janssen <i>et al.</i> (2008) Korall & Pryer (2014)
<i>Hymenophyllum</i> Hymenophyllaceae	Asia s.l., unknown	-	No	Hennequin <i>et al.</i> (2010a)
<i>Bolbitis</i> Dryopteridaceae	Unknown	-	No	Moran, Labiak, & Sundue (2010)
<i>Lindsaea</i> Lindsaeaceae	Asia s.l.	-	Yes, but not in all the lineages	Lehtonen <i>et al.</i> (2010)
<i>Odontosoria</i> Lindsaeaceae	Asia s.l.	-	No	Lehtonen <i>et al.</i> (2010)
Grammitid ferns Polypodiaceae	Neotropics	-	No	Rouhan <i>et al.</i> (2012)
<i>Crepidomanes</i> Hymenophyllaceae	Asia s.l., unknown	-	No	Dubuisson <i>et al.</i> (2013)
<i>Osmolindsaea</i> Lindsaeaceae	Asia s.l.	-	Yes	Lehtonen <i>et al.</i> (2013)
<i>Pteris</i> Pteridaceae	Asia s.l., unknown	< ca. 25 Ma	Yes, but not in all the lineages	Chao <i>et al.</i> (2014)
<i>Parapolystichum</i> Dryopteridaceae	Africa	< ca. 25 Ma	Yes	Labiak <i>et al.</i> (2014)
<i>Triplophyllum</i> Tectariaceae	Unknown	-	No	Moran <i>et al.</i> (2014a)
Grammitid ferns Polypodiaceae	Neotropics, unknown	< 15 Ma	No	Sundue <i>et al.</i> (2014)
<i>Anemia</i> Anemiaceae	Unknown	-	No	Labiak, Mickel, & Hanks (2015a)
<i>Arthropteris</i> Arthropteridaceae	Unknown	-	No	Zhang & Zhang (2015)

<i>Polystichum</i> Dryopteridaceae	Unknown	< 20 Ma	No	Le Péchon <i>et al.</i> (2016)
<i>Deparia</i> Athyriaceae	Unknown	< ca. 10 Ma	Yes	Kuo <i>et al.</i> (2016)
<i>Ctenitis</i> Dryopteridaceae	Neotropics and/or La Réunion	< ca. 10 Ma	Yes	Hennequin <i>et al.</i> (2017)
<i>Dryopteris</i> Dryopteridaceae	Africa unknown	< ca. 5 Ma	No	Sessa <i>et al.</i> (2017)
<i>Pyrrosia</i> Polypodiaceae	Unknown	-	No	Zhou <i>et al.</i> (2017)
<i>Abrodictyum</i> Hymenophyllaceae	Asia s.l.	-	Yes	Dubuisson <i>et al.</i> (in Press)
<i>Trichomanes</i> Hymenophyllaceae	Unknown	-	Yes	Dubuisson <i>et al.</i> (in Press)

Table 1. Area of origin and age (when available) of Malagasy fern lineages recorded from studies including Malagasy endemics (in chronological order of publication). A in situ diversification was recorded when at least two Malagasy endemics were retrieved sister species.

4. AIMS AND GLOBAL APPROACH

The aim of this thesis was to understand how the Malagasy fern flora has originated, by unraveling its biogeographical history. Especially, some questions were at the heart of this thesis.

- *What are the biogeographical origin(s) of Malagasy ferns and their relative importance?*

Most of the Malagasy lineages would have arrived from dispersal, due to the young age of divergence of the modern ferns. Therefore, what are the areas of origin of these dispersal events and their relative importance? As previously highlighted, the Theory of Island Biogeography predicts that the nearest continent would be the main source pool of species for an island. However, studies proved that further sources like the Neotropics and tropical Asia could be sources as well. These observations firstly highlighted that Madagascar could be highly connected with distant areas in the Southern Hemisphere and secondly called into question the importance of the nearest continent Africa in the biogeographical history of the Malagasy ferns.

- *How old are the Malagasy ferns?*

After its isolation from the Gondwana, many fern lineages would have colonized and diversified on the island, but the rate of colonization could have stayed steady over time or could have varied. Do the first fern colonizations coincide with events like the establishment of the Malagasy rainforests, the onset of wind currents between some source regions and Madagascar or events in the other tropical regions of the world?

4.1. MATERIAL

Five groups were analyzed: the grammitid ferns (Polypodiaceae), *Rumohra* Raddi (Dryopteridaceae), Blechnoideae (Blechnaceae), *Lindsaea* Dryand. (Lindsaeaceae) and the lycophyte genus *Phlegmariurus* Holub. (Lycopodiaceae) (Table 2). These taxa were chosen on several criteria:

- their species diversity in Madagascar and especially the relative number of endemic species compared to the whole Malagasy diversity –as non-endemics could add uncertainties in the inference of the biogeographical history;
- the availability of a worldwide phylogenetic study including a representative sampling of the global distribution and species diversity;
- the existence of a recent taxonomic treatment;
- the availability in the Paris herbarium of silica-dried samples for most Malagasy taxa, allowing DNA extraction (although a field trip was performed, in 2015, to complete this sampling as much as possible).

In total, these five groups include 84 of the ca. 600 Malagasy species and 50 endemics (Table 2).

Taxon	Worldwide species diversity	Number of Malagasy Non-endemic / Endemic species	Distribution	Worldwide phylogenetic study
Grammitid ferns (Polypodiaceae)	900 ¹	31 / 13	Pantropical, circumaustral	Sundue <i>et al.</i> (2014)
<i>Rumohra</i> Raddi (Dryopteridaceae)	7 ²	4 / 3	Neotropics, Western Paleotropics, Australasia, circumaustral	-
Blechnoideae (Blechnaceae)	239 ³	15 / 8	Cosmopolite	De Gasper <i>et al.</i> (2016a)
<i>Lindsaea</i> Dryand. (Lindsaeaceae)	150 ⁴	14 / 12	Pantropical	Lehtonen <i>et al.</i> (2010)
<i>Phlegmariurus</i> Holub. (Lycopodiaceae)	250 ⁵	20/14	Pantropical, circumaustral	Field <i>et al.</i> (2016)

Table 2. Worldwide and Malagasy diversity of the studied taxa and worldwide phylogeny of reference. References: ¹ Perrie & Parris (2012), ² Rakotondrainibe (2010b), Sundue, Hirai, & Prado (2013), ³ de Gasper *et al.* (2016b), ⁴ Lehtonen *et al.* (2010), ⁵ PPGI, (2016).

Biogeographical analyses have to rely on a clear taxonomy, that's why taxa were chosen if a recent taxonomic treatment was available. In this dissertation, the evolutionary concept species was adopted (Simpson, 1951; Wiley, 1978; Mayden, 1997), implying that a species is a group of populations that share a common ancestor and maintains its identity from other such groups, whether or not the morphology has changed during the process. The taxonomic treatments available relied on morphological traits alone, yet morphology can be misleading and molecular data revealed systematic issues, especially in taxa known for their homoplastic features like in grammitid ferns (Ranker *et al.*, 2004). A first step of the work was to test the monophyly of species, to ensure that they formed evolutionary distinct entities from other such groups in the biogeographical analyses.

4.2. METHODS

For each group, the following approaches were adopted:

A first step was to sample the Malagasy species and to obtain the plastid DNA sequences of the markers used in the worldwide phylogeny of reference. When it was possible, duplicates per species were sampled to test the monophyly of species. The final datasets were composed of two parts: sequences from the worldwide phylogeny of reference and newly acquired sequences, from Madagascar, but also from other WIO islands and Africa, to complete as much as possible the worldwide framework.

Secondly, the species phylogenetic relationships were inferred on the basis of two statistical methods: Maximum Likelihood (ML) and Bayesian Inference (BI). The obtained phylogenies relied on the broadest taxonomic sampling available.

Thirdly, a dating analysis was conducted on a reduced sampling, by keeping one sample per species or one sample per lineage in case of non-monophyletic species (assuming that a polyphyletic species could represent distinct evolutionary lineages not defined by morphological approaches). Divergence time estimates were calculated using a Bayesian approach implemented in BEAST.

Finally, the ancestral ranges were estimated using statistical biogeographical models, depending on the taxonomic group (see the following Chapters).

4.3. ORGANIZATION OF THE DISSERTATION

The first chapters of the present thesis aimed to document the biogeographical history of the grammitid ferns, *Rumohra*, Blechnoideae, *Lindsaea* (together with *Odontosoria*) and *Phlegmariurus*. They are followed by an overview of the biogeographical history of the Malagasy ferns (Chapter VI).

Chapter I - Grammitid ferns

These ferns are renowned for their homoplastic morphological features, highlighted by molecular phylogenetic analyses (e.g. Ranker *et al.*, 2004). However, the taxonomy of Malagasy species was poorly studied on the basis of molecular data and their sampling could reveal systematic issues.

Moreover, floristic and biogeographical affinities between the Neotropics and Madagascar were observed (Moran & Smith, 2001; Sundue *et al.*, 2014), but remained to be confirmed.

This work is published in *Molecular Phylogenetic and Evolution*:

Bauret L, Gaudeul M, Sundue MA, Parris BS, Ranker TA, Rakotondrainibe F, Hennequin S, Ranaivo J, Selosse MA, Rouhan G. 2017. Madagascar sheds new light on the molecular systematics and biogeography of grammitid ferns: New unexpected lineages and numerous long-distance dispersal events. *Molecular Phylogenetics and Evolution* 111: 1–17.

Chapter II - *Rumohra*

Rumohra was a good candidate to study the biogeographical connections between continents of the Southern Hemisphere. Two centres of diversity are observed in the genus, in the Neotropics and in Madagascar, which nest three endemic species each. In contrast, the widespread *Rumohra adiantiformis* is retrieved in the Neotropics, Western Palearctica, Australasia and temperate circumastral regions.

The manuscript has been accepted in the *Botanical Journal of the Linnean Society*:

Bauret L, Rouhan G, Hirai RY, Perrie LR, Prado J, Salino A, Senterre B, Shepherd LD, Sundue MA, Selosse MA, Gaudeul M. Molecular data, based on an exhaustive species sampling of the fern genus *Rumohra* (Dryopteridaceae), reveal a biogeographical history mostly shaped by dispersal and several cryptic species in the widely distributed *R. adiantiformis*. *Botanical Journal of the Linnean Society* in Press.

Chapter III - Blechnoideae

Blechnoideae is another taxon showing a most diverse southern distribution and provided a good replicate to understand the biogeographical history of a taxon distributed in Madagascar and in other regions of the Southern Hemisphere.

The group was subject to recent taxonomic re-delimitations in agreement with a molecular phylogeny (de Gasper *et al.*, 2016a,b), by the re-assignment of *Blechnum* s.l. species in several genera, including the Malagasy species (although poorly sampled in the phylogeny). The monophyly of these new genera was here tested.

Chapter IV - *Lindsaea* and *Odontosoria*

Whereas connections between the Neotropics and Madagascar were highlighted, few evidences of Asian dispersal were provided by biogeographical analyses. The Malagasy species of *Lindsaea* and *Odontosoria* were good candidates, as close biogeographical affinities with Asia were evidenced by phylogenetic analyses (Lehtonen *et al.*, 2010). However, the phylogenetic position of the newly sampled *L. marojejensis* sp. nov. was unknown, and no dating analyses focused on *Lindsaea* and *Odontosoria* were done.

Chapter V - *Phlegmariurus*

This chapter was focused on *Phlegmariurus*, a genus of lycophytes widely distributed in the Southern Hemisphere. It provided a replicate in an independent lineage of vascular spore-bearing plants. Malagasy angiosperms showed important differences, in their biogeography (main African origin and high level of endemism) compared to preliminary results on ferns. These differences were notably explained by ferns vagility, related to spores, but could other vascular spore-bearing plants have experienced similar biogeographical histories as ferns?

In addition, some Malagasy *Phlegmariurus* species show strong morphological convergences with Neotropical and Asian lineages, but also singular morphological traits. The study of the biogeography was completed by a discussion on the systematics of these intriguing species.

This chapter will be submitted to *Molecular Phylogenetics and Evolution*.

Bauret L, Field AR, Gaudeul M, Selosse MA, Rouhan G. First insights on the biogeographical history of *Phlegmariurus* (Lycopodiaceae), with a focus on Madagascar. In prep.

Chapter VI - Overview

This chapter included an overview of the biogeographical history of the Malagasy ferns, based on the results presented in the previous chapters and the literature. It aimed to present a first insight on the biogeographical history of the Malagasy ferns. What are the biogeographical origins? How old are the Malagasy ferns? A biogeographical scenario was also proposed, which has to be confirmed by more data on Malagasy ferns,

Chapter I - Madagascar sheds new light on the molecular systematics and biogeography of grammitid ferns: New unexpected lineages and numerous long-distance dispersal events

Lucie Bauret ^{a,b}, Myriam Gaudeul ^a, Michael A. Sundue ^c, Barbara S. Parris ^d, Tom A. Ranker ^e, France Rakotondrainibe ^a, Sabine Hennequin ^b, Jaona Ranaivo ^f, Marc-André Selosse ^{a,g}, Germinal Rouhan ^a

^a Muséum national d'Histoire naturelle, Sorbonne Universités, Institut de Systématique, Evolution, Biodiversité (UMR 7205 CNRS, MNHN, UPMC, EPHE), Herbar national, 16 rue Buffon CP39, F-75005 Paris, France

^b Université Pierre et Marie Curie, Sorbonne Universités, Institut de Systématique, Evolution, Biodiversité (UMR 7205 CNRS, MNHN, UPMC, EPHE), 57 rue Cuvier CP48, F-75005 Paris, France

^c The Pringle Herbarium, Department of Plant Biology, The University of Vermont, 27 Colchester Ave., Burlington, VT 05405, USA

^d Fern Research Foundation, 21 James Kemp Place, Kerikeri, Bay of Islands 0230, New Zealand

^e Department of Botany, University of Hawai'i at Mānoa, 3190 Maile Way, Honolulu, HI 96822, USA

^f Centre National de Recherches sur l'Environnement, 34 rue Rasamimanana Fiadanana, BP 1739, 101 Antananarivo, Madagascar

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

Accepted, 3 March 2017, in *Molecular Phylogenetics and Evolution*.

Available online: <https://doi.org/10.1016/j.ympev.2017.03.005>

ABSTRACT

Based on a worldwide phylogenetic framework filling the taxonomic gap of Madagascar and surrounding islands of the Western Indian Ocean (WIO), we revisited the systematics of grammitid fern species (Polypodiaceae). We also investigated the biogeographic origin of the extant diversity in Madagascar and estimated the relative influence of vicariance, long-distance dispersals (LDD) and in situ diversification.

Phylogenetic inferences were based on five plastid DNA regions (*atpB*, *rbcL*, *rps4-trnS*, *trnG-trnR*, *trnL-trnF*) and the most comprehensive taxonomic sampling ever assembled (224 species belonging to 31 out of 33 recognized grammitids genera). 31 species from Madagascar were included representing 87% of the described diversity and 77% of the endemics. Our results confirmed a Paleotropical clade nested within an amphi-Atlantic grade. In addition, we identified three new major clades involving species currently belonging to *Grammitis* s.l., *Ctenopterella* and *Enterosora*. We resolved for the first time *Grammitis* s.s. as monophyletic, and *Ctenopterella* (newly tested here) and *Enterosora* as polyphyletic. The Neotropical genus *Moranopteris* was shown to also occur in Madagascar through a newly discovered species. Most importantly, we suggest a >30% inflation of the species number in the WIO due to the hidden diversity in >10 cryptic lineages, best explained by high morphological homoplasy.

Molecular dating and ancestral areas reconstruction allowed identifying the Neotropics as the predominant source of LDD to the African-WIO region, with at least 12 colonization events within the last 20 Ma. Repeated eastward migrations maybe explained by transoceanic westerly winds transporting the dust-like spores. Tropical Asia s.l. would also have played a (minor) role through one dispersal event to Madagascar at the end of the Oligocene. Last, within the complex Malagasy region made of a mosaic of continental and oceanic islands located close to the African continent, we showed that contrary to theoretical expectations and empirical evidence in angiosperms, Africa does not act as a dispersal source and Madagascar seems to have a more important influence on the regional dynamics: we observed both in situ species diversification and dispersal out of Madagascar. This influence also extends beyond the region, since one dispersal event probably originated from Madagascar and reached the Subantarctic island of Amsterdam.

KEYWORDS

Biogeography, Ferns, Long-distance dispersal, Madagascar, Phylogeny, Western Indian Ocean

1. INTRODUCTION

Madagascar is a continental island of the Western Indian Ocean (WIO) known to host an extraordinary biodiversity, with about 12,000 vascular plant species (Callmender, 2011), and listed as one of the world's hotspots of biodiversity (Myers *et al.*, 2000). Eighty-three percent of vascular plants, 52% of birds and 86% of invertebrates are endemics, and this proportion reaches 100% for terrestrial Gastropoda and non-volant Mammalia (Goodman & Benstead, 2005). However, this biological diversity is still under-documented and the evolutionary processes explaining its emergence are poorly known, since only a few phylogenetic studies are available. In particular, assessing the relative contributions of vicariance due to plate tectonics, trans-oceanic long-distance dispersal (LDD) and in situ species diversification remains a central question.

Investigating the origin and evolutionary history of Malagasy lineages is challenging because Madagascar is not strongly isolated from the East African coast, and is also surrounded by several archipelagos. Thus, Madagascar must be studied in the broader context of the WIO, involving a complex geologic history of continental and volcanic islands (Agnarsson & Kuntner, 2012). Most importantly, Madagascar was once part of the supercontinent Gondwana that began to breakup 155–118 Ma, with the separation of the America/Africa block from the Madagascar/India/Australia/ Antarctica block. America and Africa split shortly after (110 Ma). Later on (118–84 Ma), India/Seychelles broke away from Madagascar before colliding with Asia 50 Ma, followed by the separation of the Seychelles Plateau (100–95 Ma; Seward, Grujic, & Schreurs, 2004). In addition, the WIO is characterized by two volcanic archipelagos, the Comoros and Mascarenes, which respectively emerged within the last ca. 15 Ma (Emerick & Duncan, 1982; Nougier, Cantagrel, & Karche, 1986) and 8 Ma (McDougall & Chamalaun, 1969). Whereas some studies underlined the impact of continental drift on the distribution of species occurring in Madagascar, others have more recently emphasized a greater role of LDD during the Cenozoic period (Yoder & Nowak, 2006; Renner *et al.*, 2010; Warren *et al.*, 2010; Agnarsson & Kuntner, 2012; Buerki *et al.*, 2013; Bacon *et al.*, 2016). At least, the role of dispersal is undisputable in the origin of lineages on the recent volcanic islands surrounding Madagascar.

In this context, ferns are a very interesting biological model to study the biogeographic origin of the Malagasy and WIO diversity. As many as 600 fern species are listed in Madagascar, among which ca. 45% are endemic (Rakotondrainibe, 2003a; Goodman & Benstead, 2005; Roux, 2009). This proportion is high, although not as high as for flowering plants and some other groups cited above, probably because ferns dust-like spores have stronger dispersal abilities that facilitate species arrival and ultimately slow down the speciation process (Smith, 1972). Spore dispersal is assumed to occur mostly through air currents, although dispersal mediated by bats (Sugita *et al.*, 2013) or birds (Lewis *et al.*, 2014) has been documented. This high capacity of spore movement, combined to the relatively recent origin of extant fern lineages (especially in the species-rich Polypodiales) since the Cretaceous (<150 Ma; Schuettpelz & Pryer, 2009; Rothfels *et al.*, 2015), suggests a major influence of LDD compared to Gondwanan vicariance in the origin of the African-WIO groups. In particular, connections between the Neotropics and Madagascar were previously highlighted based either on floristic affinities (Moran & Smith, 2001) or on

molecular phylogenetic analyses. Among the latter studies, LDD between the two areas were hypothesized for numerous *Elaphoglossum* lineages (Rouhan *et al.*, 2004; Vasco, Moran, & Rouhan, 2009), and in the genera *Lomariopsis* (Rouhan *et al.*, 2007a), *Stenogrammitis* (as *Lellingeria* in Labiak, Sundue, & Rouhan, 2010), *Leucotrichum* (Rouhan *et al.*, 2012) and other Polypodiaceae (Janssen, Kreier, & Schneider, 2007). Transatlantic LDD was also inferred based on dated phylogenies in *Alsophila* (Janssen *et al.*, 2008) and *Parapolytichum* (Labiak *et al.*, 2014). Claims for Asiatic origins have also been documented, highlighting dispersal routes from Asia e.g. in the genera *Pteris* (Chao *et al.*, 2014), *Nephrolepis* (Hennequin *et al.*, 2010b), and *Deparia* (Kuo *et al.*, 2016). At a more restricted scale, data on ferns are almost completely lacking within the African-WIO region, although a few insights from *Platyserium* (Kreier & Schneider, 2006) and *Alsophila* (Janssen *et al.*, 2008) might support continental Africa as a source of dispersal to Madagascar. This trend, implying migration across the Mozambique Channel, is strongly supported in other plant groups (Agnarsson & Kuntner, 2012).

Our study focused on grammitid ferns that had long been considered as a family, 'Grammitidaceae', until they were shown to form a clade nested within the larger Polypodiaceae family using molecular data (Schneider *et al.*, 2004; Ranker *et al.*, 2004; Sundue *et al.*, 2014). Comprising around 1500 species, Polypodiaceae is the second largest family of ferns. The estimated 900 species of grammitid ferns (Perrie & Parris, 2012) belong to 33 genera, many of which were described in the last decade based on molecular phylogenetic studies that revealed extensive polyphyly as a recurrent issue resulting from morphological homoplasy (Ranker *et al.*, 2004; Sundue, Islam, & Ranker, 2010a). Grammitid ferns are mostly found as epiphytes in tropical montane forests, and distributed in two main phytogeographic zones (Parris, 2003): (i) around 400 species in the Neotropics, tropical Africa, and the WIO (including Madagascar, Mascarenes, Comoros and Seychelles); and (ii) around 500 species in tropical Asia s.l., including Malesia and the Pacific Ocean islands. The Neotropics and the WIO were grouped in the same phytogeographic zone because they share several taxa: the genera *Alansmia*, *Ceradenia*, *Cochlidium*, *Enterosora*, *Grammitis*, *Leucotrichum*, *Melpomene*, *Stenogrammitis*, and *Zygophlebia*, and even some species (*Alansmia elastica*, *Cochlidium serrulatum* and *Melpomene flabelliformis*). In contrast, only a few taxa are known to be shared by the two phytogeographic zones: (i) *Ctenopterella* is mainly distributed in Malesia and the South-Pacific Ocean islands, but also in the WIO although its monophyly has never been tested; (ii) *Stenogrammitis* is mainly Neotropical but also distributed in Africa, the WIO, Hawaii and the South-Pacific Ocean islands; (iii) *Notogrammitis* has a circumaustral distribution, from Australia and New Zealand to South Africa and South America.

A recent phylogenetic and biogeographic study showed that grammitids evolved from a Neotropical ancestor between the end of the Eocene and beginning of the Paleocene (45–37 Ma; Sundue *et al.*, 2014). In agreement with the distribution patterns and delimitation of the two phytogeographic zones, the inferred phylogeny exhibited a clear geographic pattern: a single clade from tropical Asia s.l. (comprising Australia and Melanesia) was nested in a Neotropical grade, which also included the WIO species. This result was explained by one LDD from the Neotropics to tropical Asia s.l. at the end of the Paleocene, and repeated LDD from the Neotropics to the WIO since molecular dating suggested relatively recent diversification and allowed excluding vicariance hypotheses linked to

Gondwana breakup. However, the sampling of Sundue *et al.* (2014) included only eight species occurring in the WIO, so that many questions remain about the systematics and biogeography of grammitid species in this area.

For the WIO and Africa, Roux (2009) listed 51 species of grammitid ferns belonging to nine genera, and in particular 31 species in Madagascar including 13 endemics (Table 1; Figure 1). However, taking into account several taxa awaiting description based on morphological evidence, the actual diversity could reach >40 species in Madagascar alone. In addition, generic delimitations have to be revised for some genera already identified as non-monophyletic at the worldwide scale (especially *Grammitis* and *Zygophlebia*), and may also have to be for some others (in particular *Ctenopterella*, which is shared between Tropical Asia s.l. and the WIO; Table 1).

Genus	Worldwide estimated diversity (number of species)	Number of species recorded in the WIO region [and in Madagascar only]	Number of species recorded in Africa	Comments (at the worldwide scale)
<i>Alansmia</i>	26	1 [1]	1	
<i>Ceradenia</i>	73	5 [2]	2	
<i>Cochlidium</i>	18	1 [1]	1	
<i>Ctenopterella</i>	24	3 [3]	2	monophyly never tested
<i>Enterosora</i>	11	2 [2]	2	monophyletic but nested in <i>Zygophlebia</i>
<i>Grammitis</i>	26	12 [10]	7	polyphyletic, and <i>Grammitis</i> s.s. paraphyletic with respect to <i>Cochlidium</i>
<i>Leucotrichum</i>	6	1 [1]	0	
<i>Melpomene</i>	29	1 [1]	1	
* <i>Moranopteris</i>	30	0	0	monophyly to be tested given the newly discovered species in Madagascar
<i>Stenogrammitis</i>	31	3 [3]	7	
<i>Zygophlebia</i>	20	7 [7]	3	paraphyletic with respect to <i>Enterosora</i>

Table 1. Grammitid genera occurring in the Western Indian Ocean (WIO, incl. Madagascar) and Africa, based on Roux (2009) updated with more recent genera recognized, and corrected for distributions of some species. *Only known from the Neotropics to date, but a new species was recently discovered in Madagascar.

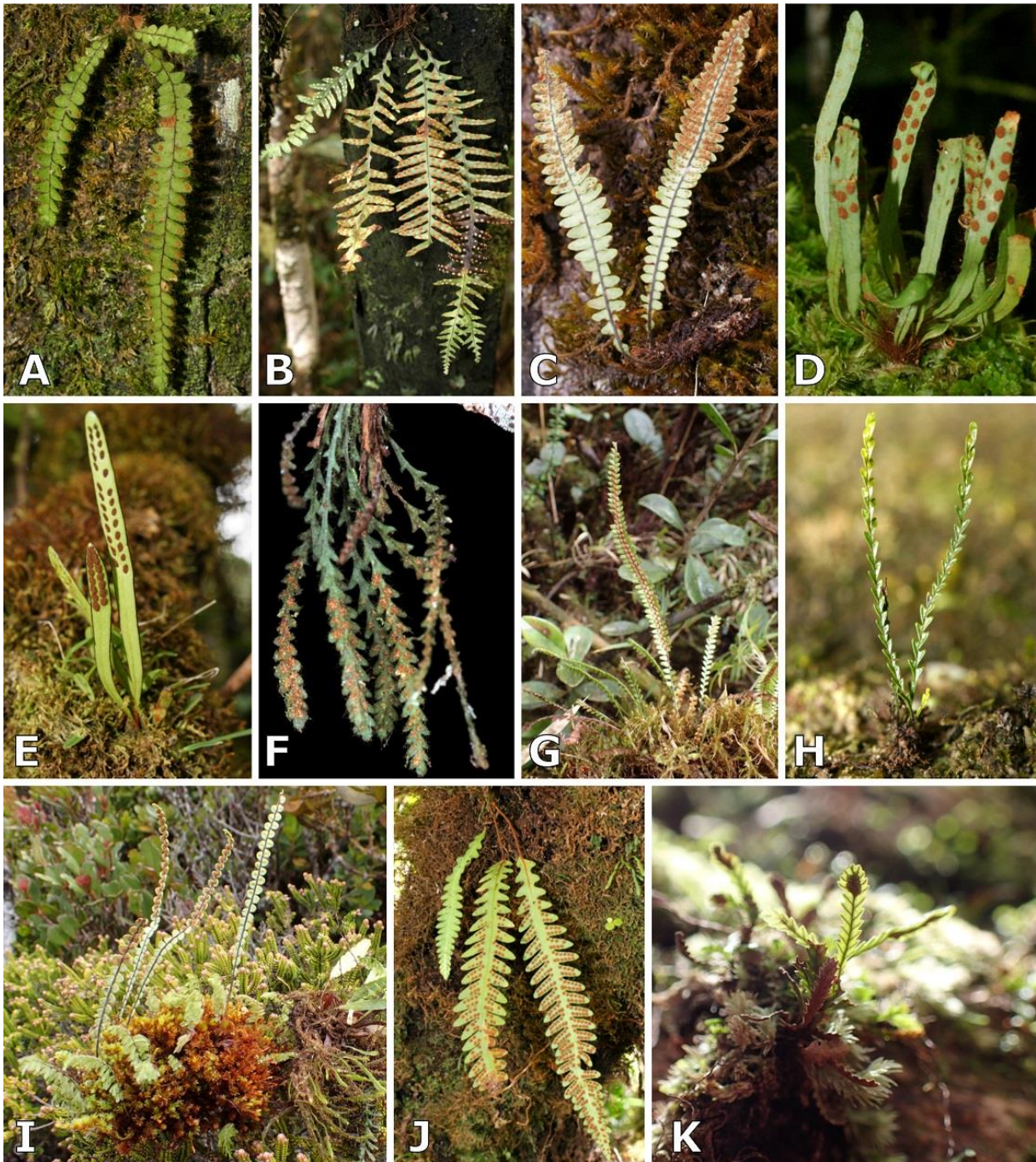


Figure 1. Morphology and habit of the 11 genera distributed in Madagascar: (A) *Alansmia elastica* (Rouhan et al. 1138); (B) *Ceradenia deltodon* (Rouhan et al. 1350); (C) *Ctenopterella zenkeri* (Rouhan et al. 1347); (D) *Enterosora sprucei* (Rouhan et al. 1227); (E) *Grammitis copelandii* (Rouhan et al. 1190); (F) *Leucotrichum madagascariense* (Rakotondrainibe 6957); (G) *Moranopteris* sp. 1 (Bauret et al. 107); (H) *Stenogrammitis hildebrandtii* (Rouhan et al. 1375); (I) *Melpomene flabelliformis* (Bauret et al. 105); (J) *Zygophlebia goodmanii* (Rouhan et al. 1382); (K) *Cochlidium serrulatum* (Bauret et al. 7). Photos: G. Rouhan; except G, I, K: L. Bauret.

Based on an extended sampling and using phylogenetic inference, molecular dating and ancestral area estimation, we aim at improving the systematics of the Malagasy and other WIO grammitids, better understanding their biogeographic history, and shedding light on the evolutionary mechanisms that led to their emergence. More specifically, our goals are: (i) to further test the monophyly of grammitid genera, particularly those diversified in the WIO, namely *Grammitis*, *Zygophlebia* and for the first time *Ctenopterella*; (ii) more

broadly, to improve the systematics of grammitid ferns by delimiting species as monophyletic groups; (iii) to test the global biogeographic scenario proposed by Sundue *et al.* (2014); (iv) to infer the biogeographic origin of the Malagasy and other WIO species (i.e., did they emerge after dispersal events from the Neotropics only, as suggested by Sundue *et al.* (2014)? How many such events explain the extant diversity in the area?); (iv) to assess to what extent dispersal occurred and the predominant migration routes at the regional scale, in the WIO; and v) to check whether diversification occurred within the WIO.

2. MATERIALS AND METHODS

2.1. TAXONOMIC SAMPLING

The sampling was composed of two parts: the first one, based on the worldwide grammitid phylogeny of Sundue *et al.* (2014), included 199 samples representing 180 grammitid species and provided a global framework. The second one was focused primarily on the WIO and Africa, and even more specifically on Madagascar: 193 new samples represented 61 species (Appendix 1.1). In total, the ingroup comprised 396 samples representing 224 species, including 36 species from Africa and WIO islands, of which 31 occur in Madagascar (taking into account a few undescribed species), and 12 of which are endemic (representing 87% of the described species, and ca. 77% of the described endemics). A total of 31 out of 33 recognized grammitid genera were represented (*Archigrammitis* Parris and *Luisma* M.T.Murillo & A.R.Sm. were lacking) and one type species (*Tomophyllum subsecundodissectum*) was newly sampled, for a total of 21 type species (Appendix 1.1). Five outgroups were added, belonging to four other Polypodiaceae genera that belong to the sister clade of grammitids (Sundue, Testo, & Ranker, 2015): *Microgramma* C.Presl, *Niphidium* J.Sm., *Polypodium* L. and *Serpocaulon* A.R.Sm. Voucher information is listed in Appendix 1.1.

2.2. DNA SEQUENCING

Total genomic DNA was extracted using the Qiagen DNeasy Plant Mini Kit (Valencia, California, USA) either from silica-dried leaves whenever available, or from herbarium specimens. The manufacturer's protocol was followed but slightly modified for herbarium samples, adding 30 mL proteinase K (20 mg/mL) and 30 mL beta-mercaptoethanol for the initial lysis step, which was carried out at 42°C during 24 h on a tipping plate.

The polymerase chain reaction (PCR) was used to amplify five plastid DNA regions: the two coding genes *atpB* and *rbcL*, and the three non-coding intergenic spacers: *rps4-trnS*, *trnG-trnR* and *trnL-trnF*. PCR were carried out in a 25 µL volume containing 1x PCR buffer, 2.5mM MgCl₂, 250 µM of each dNTP, 1M betaine, 0.4 µM of each primer, 0.75 U Taq polymerase (Taq CORE kit; MP Biomedicals, Illkirch, France), and 0.75 µL of template DNA. Primer sequences and thermal cycling conditions are reported in Table 2. The resulting PCR products were checked on a 1% agarose gel and sequenced in both directions at the Centre National de Séquençage (Evry, France), using the amplification primers and additional, internal primers for *atpB*, *rbcL* and *trnG-trnR* (Table 2). Forward and reverse DNA strands were assembled in Sequencher 4.9 (GeneCodes Corporation,

Ann Arbor, Michigan, USA). The newly obtained consensus sequences were submitted to GenBank (accession numbers listed in Appendix 1.1).

2.3. PHYLOGENETIC ANALYSES

The sequences were aligned with the MAFFT plugin (Kato & Toh, 2010) in Jalview 2.8.2 (Waterhouse *et al.*, 2009) using the default parameters. The alignments were then checked and revised manually when necessary. For each DNA region, the most suitable model of nucleotide substitution was determined with MrModelTest 2.3 (Nylander, 2004), following the Akaike Information Criterion. A data matrix was then built by concatenation of all five regions using Sequence Matrix 1.7.8 (Vaidya, Lohman, & Meier, 2011), each DNA region constituting a distinct partition in the final dataset.

Phylogenetic trees were inferred based on two probabilistic methods: Maximum Likelihood (ML) and Bayesian Inference (BI), as respectively implemented in RAxML-HPC2 8.2.6 (Stamatakis, 2014) and MrBayes 3.2.3 (Ronquist *et al.*, 2012). Gaps were treated as missing data and analyses were performed on the CIPRES science gateway (Miller, Pfeiffer, & Schwartz, 2010). Parameters for the RAxML analysis were determined on the CIPRES interface. We used the GTRGAMMA+I model of nucleotide substitution for each partition, performed 1000 rapid bootstrap (BS) replicates and searched for the best-scoring ML tree. In MrBayes, we conducted two independent but parallel analyses with four chains each (one cold and three incrementally heated at a temperature of 0.1). The BI analyses were run for 15 million generations each, sampled every 1500 generations, and performed with the GTR+I+C model of nucleotide substitution for each partition and parameters unlinked. Tracer 1.6.0 (Rambaut & Drummond, 2013) was used to check that effective sample sizes were higher than recommended (>200) and that the two runs had converged to the stationary distribution. The 25% initial trees were discarded as burn-in, the post-burn-in trees (15,000 total trees) were pooled and a 50% majority-rule consensus was computed with average branch lengths and posterior probability (PP) estimates for all nodes. This tree and the best ML tree were visualised with FigTree 1.4.2 (Rambaut, 2014).

DNA region	Primer name	Literature reference	Primer sequence (5' to 3')	Thermal cycling conditions
<i>atpB</i>	ESATPB172F	Schuettpelz & Pryer (2007)	AAT GTT ACT TGT GAA GTW CAA CAA T	5 min 94°C / 40 × (30s 94°C / 1 min 50°C / 2.5 min 72°C) / 10 min 72°C
	ESATPE45R	Schuettpelz & Pryer (2007)	ATT CCA AAC WAT TCG ATT WGG AG	
	q OR 1163F*	Wolf (1997)	ATG GCA GAA TRT TTC CGA GAT RTY A	
	ATPB910R*	Pryer <i>et al.</i> (2004)	TTC CTG YAR AGA NCC CAT TTC TGT	
<i>rbcL</i>	ESRBCL1F	Schuettpelz & Pryer (2007)	ATG TCA CCA CAA ACG GAG ACT AAA GC	5 min 94°C / 40 × (30s 94°C / 45s 50°C / 2.5 min 72°C) / 10 min 72°C
	ESRBCL1361R	Schuettpelz & Pryer (2007)	TCA GGA CTC CAC TTA CTA GCT TCA CG	
	ESRBCL628F*	Schuettpelz & Pryer (2007)	CCA TTY ATG CGT TGG AGA GAT CG	
	ESRBCL654R*	Schuettpelz & Pryer (2007)	GAA RCG ATC TCT CCA ACG CAT	
<i>trnG-trnR</i>	TRNG1F	Nagalingum, Schneider, & Pryer (2007)	GCG GGT ATA GTT TAG TGG TAA	5 min 94°C / 40 × (30s 94°C / 1 min 50°C / 1.5 min 72°C) / 10 min 72°C
	TRNR22R	Nagalingum, Schneider, & Pryer (2007)	CTA TCC ATT AGA CGA TGG ACG	
	TRNG43F1*	Nagalingum, Schneider, & Pryer (2007)	TGA TGC GGG TTC GAT TCC CG	
	TRNG63R*	Nagalingum, Schneider, & Pryer (2007)	GCG GGA ATC GAA CCC GCA TCA	
<i>trnL-trnF</i>	f	Taberlet <i>et al.</i> (1991)	ATT TGA ACT GGT GAC ACG AG	5 min 94°C / 40 × (30s 94°C / 30s 50°C / 1.5min 72°C) / 10min 72°C
	Fern-1	Taberlet <i>et al.</i> (1991)	GGC AGC CCC CAR ATT CAG GGR AAC C	
<i>rps4-trnS</i>	rps4-3r.f	Smith & Cranfill (2002)	AGT TGT TAG TTG TTG AGT AT	5 min 94°C / 40 × (30s 94°C / 30s 50°C / 45 s 72°C) / 10 min 72°C
	trnS-r	Smith & Cranfill (2002)	TAC CGA GGG TTC GAA TC	

Table 2. PCR primers sequences and thermal cycling conditions; primers used as internal primers for sequencing only are marked with an asterisk (*)

2.4. DIVERGENCE TIME ESTIMATION

Divergence time estimation was performed on the CIPRES science gateway using BEAST 1.8.0 (Drummond *et al.*, 2012). Our biogeographic analyses were performed at the species level and focused on Madagascar. Therefore, given that the BEAST outputs were used as inputs for the estimation of ancestral areas, we reduced our ingroup sampling to 149 samples representing 139 species for the dating analysis. For genera that included Malagasy species, we kept one sample per species that was retrieved as monophyletic and as many samples as retrieved lineages when species were poly- or paraphyletic. For genera without any Malagasy species, we retained at least two samples of two distinct species including the type species of the genus whenever available, and the species that was recovered as the most early-diverging lineage (in order not to underestimate the diversification date of the genus). For genera with no resolution on the crown node, we kept all the sampled species branched on the polytomous node.

Partitions and models of nucleotide substitution were the same as for the BI. An uncorrelated lognormal relaxed clock was used, with a Yule tree prior (Gernhard, 2008) and a random starting tree. Based on a large-scale, fossil-based study on leptosporangiate ferns (Schuettpelez & Pryer, 2009), we used four secondary calibration points corresponding to four well-supported clades within the Polypodiaceae (nodes 367, 368, 378, 382 in Schuettpelez & Pryer, 2009; figured as black squares on Figure 4) that were also used by Sundue *et al.* (2014). These points were modelled by normal distribution priors on crown nodes, with 10% standard deviation (S.D.) in all cases: (1) the age of the grammitid clade was set to 31.2 Ma (S.D. = 3.1); (2) the age of the Neotropical clade including *Mycopteris-Stenogrammitis* was set to 23.3 Ma (S.D. = 2.3); (3) the age of the clade including the Paleotropical clade plus *Moranopteris*, was set to 23.4 Ma (S.D. = 2.3); and (4) the diversification of the genus *Serpocaulon*, in the outgroup, was dated to 15.5 Ma (S. D. = 1.5). The prior for ucl.d.mean was defined by continuous-time Markov chain (CTMC) Rate Reference, as advised when no prior knowledge is available (Ferreira and Suchard, 2008). Two analyses of 20 million generations each were performed with tree and parameters sampling every 2000 generations. Tracer 1.6.0 was used to define burn-in length and the first 1000 trees (10%) were discarded for each run. We used LogCombiner v1.8.0 to combine results from the two runs and TreeAnnotator v1.8.0 (Drummond *et al.*, 2012) to compute the maximum clade credibility tree. FigTree 1.4.2 (Rambaut, 2014) was used to visualize the maximum clade credibility tree and the associated chronogram.

2.5. ANCESTRAL AREAS ESTIMATION

We used both the statistical Dispersal-Vicariance Analysis S-DIVA (Yu, Harris, & He, 2010) and the Bayes-Lagrange S-DEC (Beaulieu, Tank, & Donoghue, 2013) models implemented in the RASP software (Yu *et al.*, 2015) to estimate ancestral ranges at all nodes. Nine biogeographic regions were defined (see Figure 2): (A) Madagascar; (B) Mascarenes (including Réunion, Mauritius, and Rodrigues; but no grammitid species is known from Rodrigues), Comoros, and Seychelles (regions A and B together form the WIO); (C) Continental Africa except North Africa; (D) Neotropics; (E) Tropical Asia s.l. (India to South-East Asia and Japan, North Australia and Melanesia except Fiji); (F) Other Pacific Ocean islands (Fiji, Micronesia, French Polynesia, Hawaiian Islands); (G) Temperate

circumastral region, south of 28°S latitude; (H) South Atlantic Islands (Saint Helena); and (I) North America. Geographic distributions were coded for all species, based on Sundue *et al.* (2014), and on herbarium specimens (mostly from the Paris Herbarium) and personal observations for all other samples. The taxonomic identification of all herbarium specimens was checked. We considered polyphyletic species as distinct lineages and coded as many distinct distributions as retrieved lineages. The maximal number of ancestral areas was set to two and, owing to the high wind-dispersal ability of fern spores over long distances, we considered that dispersal probability was not correlated to geographic distance. The frequencies of an ancestral range at a node were averaged over 100 random, post burn-in trees taken from the BEAST output. The condensed tree was the maximum clade credibility tree estimated in BEAST.

3. RESULTS

3.1. PHYLOGENETIC ANALYSES

Our combined DNA sequence matrix of the five plastid regions was composed of 401 specimens and 5593 base pairs. Table 3 reports the percentage of samples successfully sequenced and other statistics, for each DNA region and the combined alignment. The phylogenetic hypotheses produced by the ML analysis and BI resulted in very similar topologies, with a few minor, not statistically supported differences. Consequently, only the BI topology is summarized in Figure 2 (see Appendix 1.2 for the full tree with all terminals).

Both the ML analysis and BI supported grammitid ferns as a strongly-supported clade (BS = 100; PP = 1; Figure 2). The resulting phylogeny was almost fully resolved at the species level. Most nodes were strongly supported, especially those supporting genera (BS = 95 and/or PP = 0.95; Figure 2), although more moderate support values were retrieved for the backbone nodes and some nodes within clade-1 (Figure 2). The inferred topology almost fully agreed with the most recent phylogenetic study on the group (Sundue *et al.*, 2014), with two notable exceptions: we recovered *Grammitis* s.s. as monophyletic (BS = 100; PP = 1), and three new clades were supported (highlighted as grey-filled triangles, Figure 2) involving species currently classified in *Grammitis*, *Ctenopterella* and *Enterosora* (as detailed later).

Figure 2 – Majority rule consensus phylogram of grammitids recovered from the BI based on the combined dataset (*atpB*, *rbcL*, *trnG-trnR*, *trnL-trnF* and *rps4-trnS*), and figured as a summary phylogeny (infrageneric branches are shown in Appendix 1.2). Generic lineages are represented by triangles (whose heights are proportional to the numbers of samples included), with names of genera preceded by colored squares figuring their geographic distribution (see map) and followed by (number of species analyzed / number of samples included). Non-monophyletic genera are indicated in bold and their names are followed by symbols to show the distinct lineages of a given genus. The three grey-filled triangles are the three newly evidenced clades, and circled numbers (1 to 3) correspond to clades detailed in Figure 3. Unless mentioned next to the nodes, support values were BS \geq 95 and PP \geq 0.95.

The phylogeny was geographically structured, with a Palearctic clade (BS = 78; PP = 0.86) nested within a large grade (Figure 2). The Palearctic clade included 15 genera mostly distributed in tropical Asia s.l. and the Pacific Ocean islands. Notably, the Palearctic clade also comprised eight species from the WIO and Africa, belonging to *Ctenopterella* and *Grammitis* and all together forming clade-3 (BS = 100; PP = 1; Figure 2). This clade is a newly recovered one for grammitids. The relationships between clade-3, *Chrysogrammitis*, and the rest of the Palearctic clade were not resolved.

The rest of the tree consisted of a large grade, hereafter called amphi-Atlantic grade. It included 17 genera showing either an exclusively Neotropical distribution for six genera, or a distribution on both sides of the Atlantic Ocean (including Africa and the WIO) for 10 genera (with the exceptions of *Stenogrammitis* and *Grammitis* s.s., which include a few additional species from the Pacific Ocean islands, tropical Asia s.l. or the circumaustral region; and *Adenophorus* restricted to Hawaii; Figure 2).

Out of the 31 genera that we sampled, monophyly could not be assessed for *Acrosorus* and *Lomaphlebia* (represented by only one species each). Twenty-two genera were recovered as monophyletic, including *Moranopteris* that was exclusively described from the Neotropics to date and that was also represented in this study by an undescribed, endemic species to Madagascar (*Moranopteris* sp. 1). By contrast, seven genera were not recovered as monophyletic: *Ceradenia*, *Ctenopterella*, *Enterosora*, *Grammitis*, *Oreogrammitis*, *Radiogrammitis* and *Zygophlebia* (indicated in bold, Figure 2). In particular, *Grammitis*, *Ctenopterella* and *Enterosora* were retrieved as highly polyphyletic: *Grammitis* was retrieved in the clade-1 of the amphi-Atlantic grade (as *Grammitis* s.s.), but also as four distinct lineages within the Palearctic clade (two lineages in clade-3, one clade sister-group to *Notogrammitis*, and *G. stenophylla* as sister lineage to the *Acrosorus-Ctenopterella denticulata* clade; Figures 2 and 3). *Ctenopterella* species formed three distinct lineages within the Palearctic clade: *C. denticulata* was included in the clade *Acrosorus-Grammitis stenophylla* clade, *C. lasiostipes* and two other undescribed species were sister-group to *Grammitis-Notogrammitis*, and two species were included in clade-3; Figures 2 and 3). *Enterosora* was retrieved as four lineages belonging to the amphi-Atlantic grade: two species were sister-group to *Adenophorus* in clade-1 and, in clade-2, two distinct lineages were nested within *Zygophlebia* (counting four and one species, respectively) and one species within *Ceradenia* (Figure 3).

Our molecular sampling included multiple individuals per species, especially for those from the WIO, and also allowed the identification of several cases of polyphyly at the

species level. Twenty- eight species were thus recovered as non-monophyletic (Appendix 1.2). They belonged to 15 genera and included 12 species from the WIO and Africa (Table 4). These 12 non-monophyletic species were mostly found in *Grammitis*, with four non-monophyletic species in *Grammitis* s.s. (highlighted in distinct colors; Figure 3, clade-1; Table 4) and six non-monophyletic species which could be classified in one to several new genera in order to accommodate their placement in clade-3 (Figure 3; Table 4).

DNA region	Number of sequences	Aligned length (in base pairs)	Number of variable characters	Number of informative characters
<i>atpB</i>	339 (85%)	1272	436 (34%)	342 (27%)
<i>rbcL</i>	366 (91%)	1240	482 (39%)	340 (27%)
<i>rps4-trnS</i>	307 (76%)	767	505 (66%)	422 (55%)
<i>trnG-trnR</i>	271 (67%)	1671	937 (56%)	747 (45%)
<i>trnL-trnF</i>	360 (90%)	643	431 (67%)	316 (49%)
Combined dataset	401 (100%)	5593	2791 (50%)	2167 (39%)

Table 3. Statistics on the five separate plastid DNA regions and the combined dataset

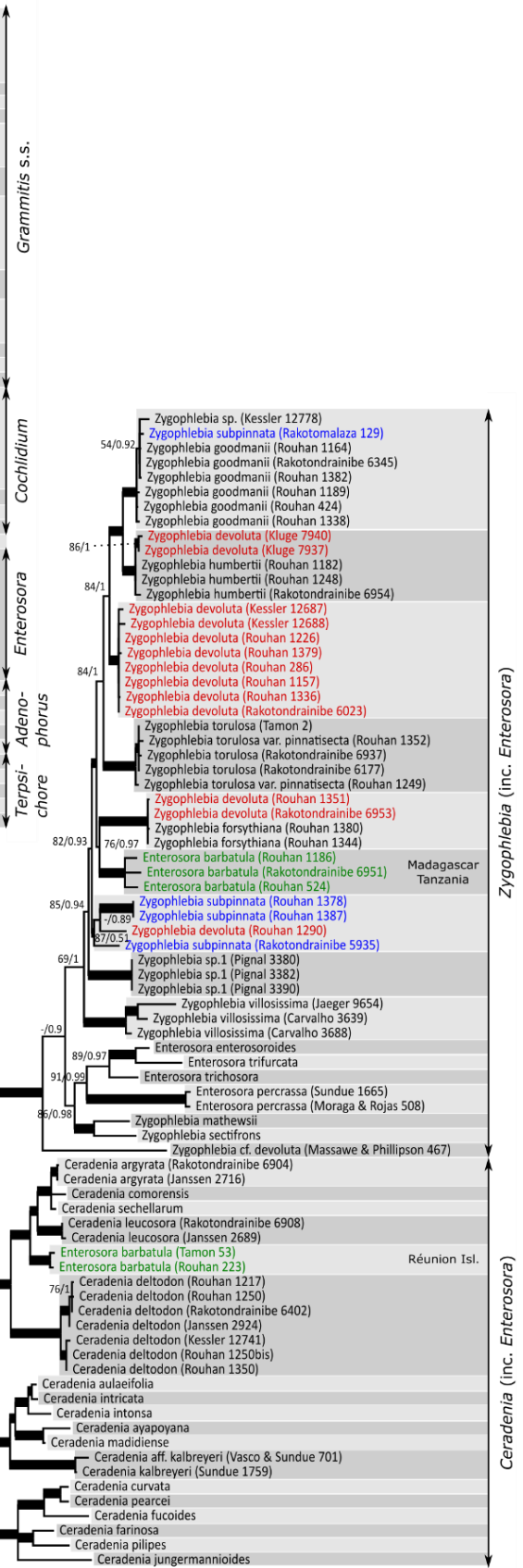
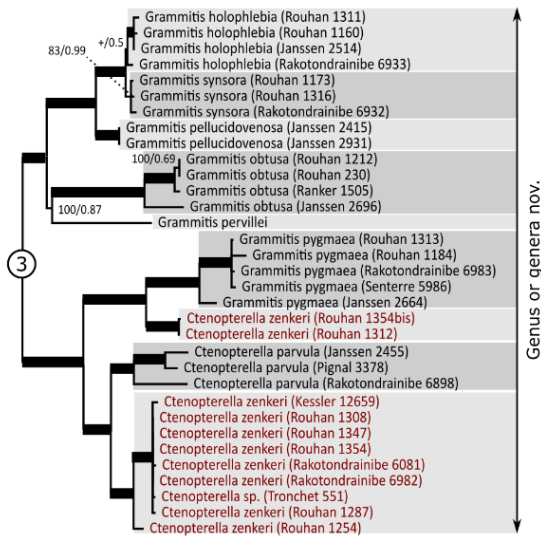
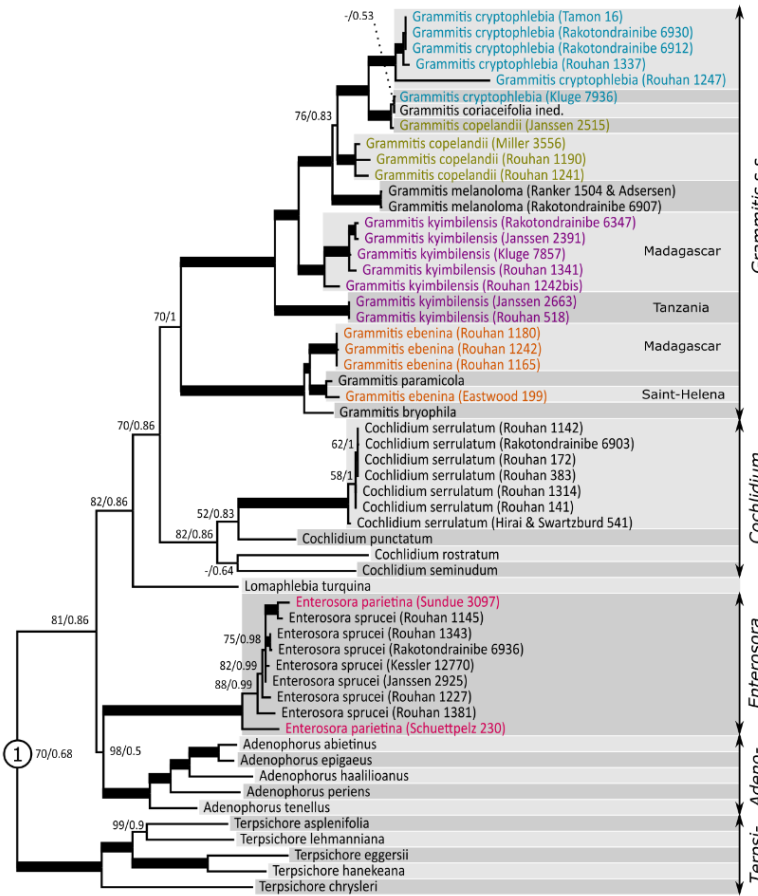


Figure 3 – Detailed phylograms of clades 1 to 3. Unless mentioned next to the nodes, support values were BS \geq 95 and PP \geq 0.95, thick branches indicate BS \geq 95 and PP \geq 0.95. Polyphyletic species are indicated in color. Vouchers are indicated when several samples of the same species were sequenced, and localities specified are discussed in the text.

3.2. DIVERGENCE TIME AND ANCESTRAL AREAS ESTIMATIONS

3.2.1. Divergence time estimation

Molecular dating showed that grammitid ferns diverged from other Polypodiaceae about 39.3 Ma (stem age; 95% Highest Posterior Density (HPD) interval: 31.2–50.6) and started to diversify at least 34.1 Ma (crown age; 95% HPD: 28.3–40.2) at the end of the Eocene (Figure 4A). The Paleotropical clade was dated to 24.9 Ma for the stem age (95% HPD: 19.6–28.1; Figure 4B).

Divergence dates (stem ages) of generic lineages ranged between 13.1 Ma (divergence between *Acrosorus* and *Ctenopterella denticulata*; 95% HPD: 9.0–18.1; Figure 4B) and 32.1 Ma (divergence between *Terpsichore* and its sister-clade; 95% HPD: 26.5–38.7; Figure 4A), while their diversification (crown) ages ranged between 2.0 and 14.6 Ma for *Xiphopterella* and *Ascogrammitis* respectively (Figure 4B). In fact, diversification could also have occurred earlier: several new genera will have to be newly described based on this study (see Table 3 and Discussion) and as a result, the potential genus corresponding to clade-V would have started to diversify 21.1 Ma.

3.2.2. Ancestral areas estimation

S-DIVA and S-DEC analyses produced very similar results. When they differed, S-DEC inferred slightly broader ancestral areas (i.e., including an additional region, but with a low probability), but the areas inferred by S-DIVA were also always the most probable ones inferred by S-DEC (results not shown). The only notable difference was an ancestral area covering both Madagascar and the Neotropics for the crown node of *Stenogrammitis* and its next outer node by S-DEC, vs. Madagascar only by S-DIVA (Figure 4B).

Grammitid ferns originated and diversified in the Neotropics (Figure 4A) and a Neotropical origin was also inferred for all the generic lineages of the amphi-Atlantic grade, with two exceptions: the ancestral area of *Adenophorus* was inferred in the Neotropics and the Pacific Ocean islands, while *Stenogrammitis* would have originated in the Neotropics and Madagascar.

Species	Figure	Monophyletic	Taxonomic issues	Taxonomic novelties suggested
<i>Ctenopterella parvula</i>	Figure 2 Figure 3, clade-3	yes	belongs to clade-3, a newly recovered clade for grammitids	should possibly be placed in a new genus if the type species of the genus (<i>C. blechnoides</i>) belongs to a distinct clade
<i>Ctenopterella zenkeri</i>		no	i/ belongs to clade-3, a newly recovered clade for grammitids ii/ cryptic species (diphyletic)	i/ should possibly be placed in a new genus if the type species of the genus (<i>C. blechnoides</i>) belongs to a distinct clade ii/ to be further investigated
<i>Enterosora barbatula</i>	Figure 3, clade-2	no	cryptic species, diphyletic: a/ La Réunion (nested within <i>Ceradenia</i>) b/ Madagascar-Africa (nested within <i>Zygophlebia</i>)	a/ to be combined with <i>Ceradenia</i> b/ 1 new species for Madagascar and Africa (type of <i>E. barbatula</i> being from La Réunion) to be described under <i>Zygophlebia</i>
<i>Enterosora sprucei</i>	Figure 2 Figure 3, clade-1	no	i/ belongs to a newly recovered clade (<i>E. sprucei</i> / <i>E. parietina</i>) for grammitids ii/ paraphyletic with respect to <i>E. parietina</i> -Sundue 3097	i/ <i>E. sprucei</i> / <i>E. parietina</i> should possibly be placed in a new genus if the type species of the genus (<i>E. campbellii</i>) belongs to a distinct clade ii/ to be synonymized with <i>E. parietina</i> (earliest species name)
<i>Grammitis copelandii</i>	Figure 3, clade-1	no	cryptic species, diphyletic	to be further investigated
<i>Grammitis cryptophlebia</i>		no	cryptic species, diphyletic	to be further investigated
<i>Grammitis ebenina</i>		no	cryptic species, diphyletic: a/ St-Helena b/ Madagascar	a/ remains as <i>G. ebenina</i> (type specimen from St Helena) b/ 1 new species, endemic to Madagascar
<i>Grammitis kyimbilensis</i>		no	cryptic species, diphyletic a/ Tanzania b/ Madagascar	a/ remains as <i>G. kyimbilensis</i> (type specimen from Tanzania) b/ 1 new species, endemic to Madagascar
<i>Grammitis holophlebia</i>	Figure 2	yes	belongs to clade-3, a newly recovered clade for grammitids	to be placed in a new genus
<i>Grammitis obtusa</i>	Figure 3, clade-3	yes	belongs to clade-3, a newly recovered clade for grammitids	to be placed in a new genus

<i>Grammis pellucidovenosa</i>		yes	belongs to clade-3, a newly recovered clade for grammitids	to be placed in a new genus
<i>Grammitis pervillei</i>		untested (1 sample included)	belongs to clade-3, a newly recovered clade for grammitids	to be placed in a new genus
<i>Grammitis pygmaea</i>		yes	belongs to clade-3, a newly recovered clade for grammitids	to be placed in a new genus
<i>Grammitis synsora</i>		yes	belongs to clade-3, a newly recovered clade for grammitids	to be placed in a new genus
<i>Moranopteris</i> sp. 1	Appendix 1.2	untested (1 sample included)	genus known only from the Neotropics, to date	1 new species endemic to Madagascar
<i>Stenogrammitis oosora</i>	Appendix 1.2	no	paraphyletic	to be further investigated (paraphyly being not well supported, this species might eventually be resolved as monophyletic)
<i>Stenogrammitis</i> sp. 1		no	undescribed species, paraphyletic with respect to <i>S.</i> sp. 2	1 new species for Amsterdam Island (but further investigation should assess its conspecificity with <i>S.</i> sp. 2)
<i>Stenogrammitis</i> sp. 2		untested (1 sample included)	undescribed species (analyzed as <i>S. reunionensis</i> Parris ined. in Ranker <i>et al.</i> , 2010)	1 new species for Madagascar and La Réunion (but further investigation should assess its conspecificity with <i>S.</i> sp. 1)
<i>Zygophlebia devoluta</i>	Figure 3, clade-2	no	cryptic species, polyphyletic (4 lineages)	to be further investigated
<i>Zygophlebia goodmanii</i>		no	cryptic species, paraphyletic with respect to <i>Z. subpinnata</i> -MA8 and <i>Z.</i> sp. MA38	to be further investigated
<i>Zygophlebia</i> sp. 1		yes	undescribed species	1 new species endemic to the Comoros
<i>Zygophlebia subpinnata</i>		no	cryptic species, polyphyletic (3 lineages)	to be further investigated

Table 4. Taxonomic issues at the species level for African and WIO grammitid fern taxa, raised by the molecular phylogenetic results; taxonomic novelties accommodating these results are suggested whenever possible. Non-monophyletic species are indicated in color (same colors as in Figure 3). For some species, several issues emerged and were distinguished by i/ and ii/, while the distinct lineages of non-monophyletic species were distinguished by a/ and b/.

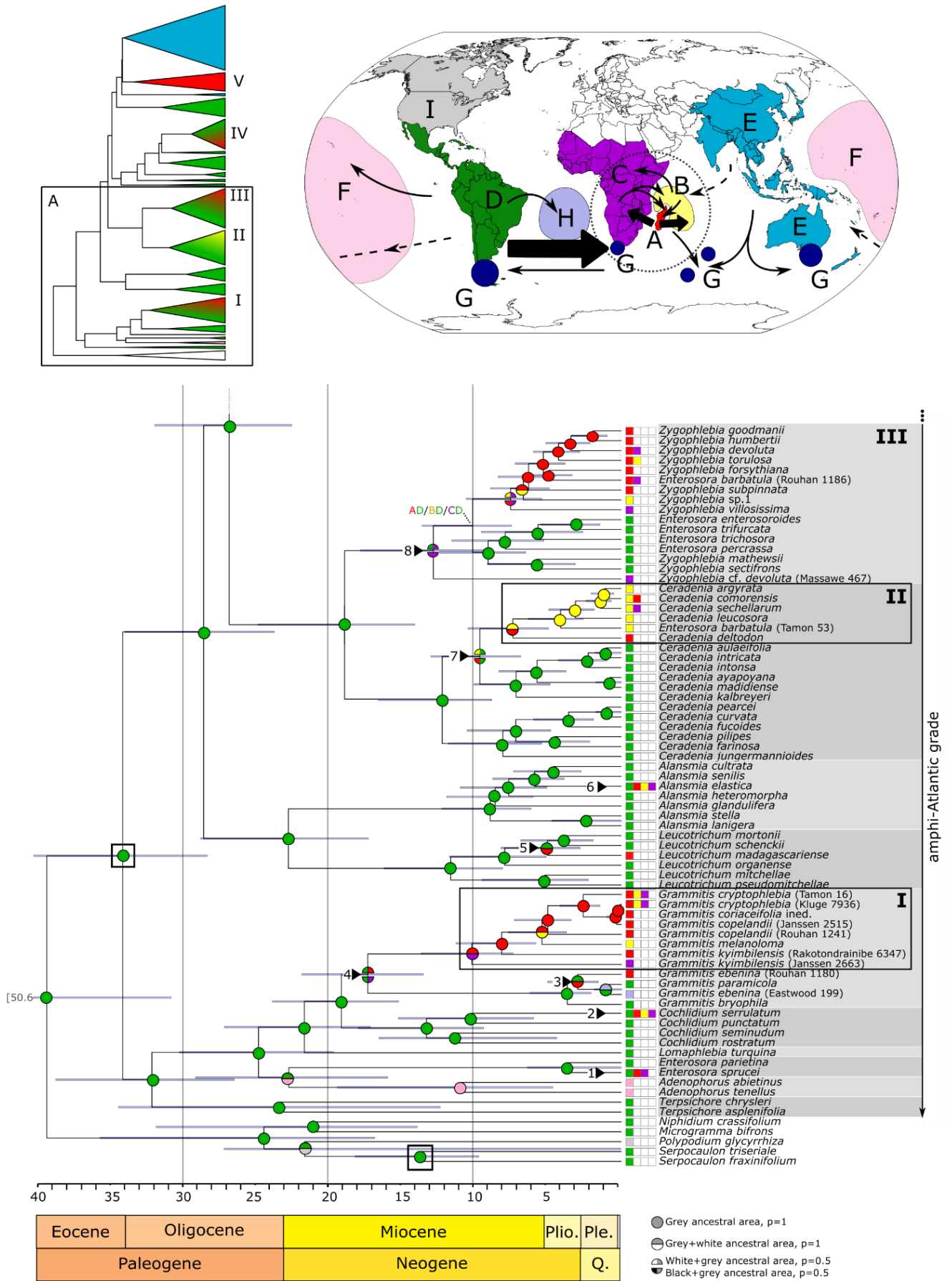
The Palearctic clade originated in tropical Asia s.l. and the Neotropics (Figure 4B). Within this clade, all genera have an exclusively tropical Asian origin, except *Notogrammitis* (for which the circumaustral area was also inferred) and clade-V if considered as a new genus (for which Madagascar was also inferred).

3.2.3. Focus on Madagascar, surrounding islands and Africa

The lineages occurring in this region were dispersed throughout the phylogeny. They originated by 13 LDD events dated from the end of the Oligocene or more recently (indicated by black arrows on branches; Figure 4): 12 from the Neotropics and one from tropical Asia s.l., leading to clade-V. However, this latter event was not statistically supported since the node grouping the African-WIO clade-V with the rest of the Palearctic clade (excluding the Asian genus *Chrysogrammitis*) had PP = 0.45. Five LDD were inferred to Madagascar (black arrows for LDD-3, LDD-5, LDD-10, LDD-12 and LDD-13; Figure 4). These events resulted in three species endemic to Madagascar: *Grammitis ebenina*-Rouhan1180 (LDD-3), *Leucotrichum madagascariense* (LDD-5) and *Moranopteris* sp. 1 (LDD-12). The two other dispersal events to Madagascar were at the origin of the genus *Stenogrammitis* (LDD-10) and of clade-V (LDD-13 from Asia). One LDD only reached Africa (LDD-8). Seven other LDD gave rise to lineages distributed in several areas, with no possibility to infer whether one or several dispersal events occurred, and which area(s) was(were) colonized at first (LDD-1, 2, 4, 6, 7, 9 and 11). Only one transatlantic LDD from Madagascar back to the Neotropics was suggested, within the genus *Stenogrammitis*.

At a regional scale, several dispersal events were also inferred in all directions between Madagascar, Africa and the neighbouring islands, but most of them originated from Madagascar to give rise to *Grammitis melanoloma* endemic to Réunion, and for others to expand their original ranges (*Grammitis cryptophlebia*, *Zygophlebia devoluta*-Rouhan1379, *Zygophlebia torulosa*, *Enterosora barbatula*-Rouhan1186, *Stenogrammitis oosora*, *Grammitis synsora*, *Grammitis pygmaea*, *Ctenopterella zenkeri* and *Ctenopterella parvula*). In two cases, dispersal originated in other WIO islands: *Ceradenia sechellarum* dispersed to Africa, and *Ceradenia comorensis* to Madagascar. Last, only one dispersal was inferred from Africa towards Madagascar and the other WIO islands, which gave rise to clade-III (*Zygophlebia-Enterosora*; Figure 4).

The analysis suggested that out of the 13 LDD that gave rise to lineages of the WIO and Africa, five were followed by species diversifications in the region (clades I to V, Figure 4).



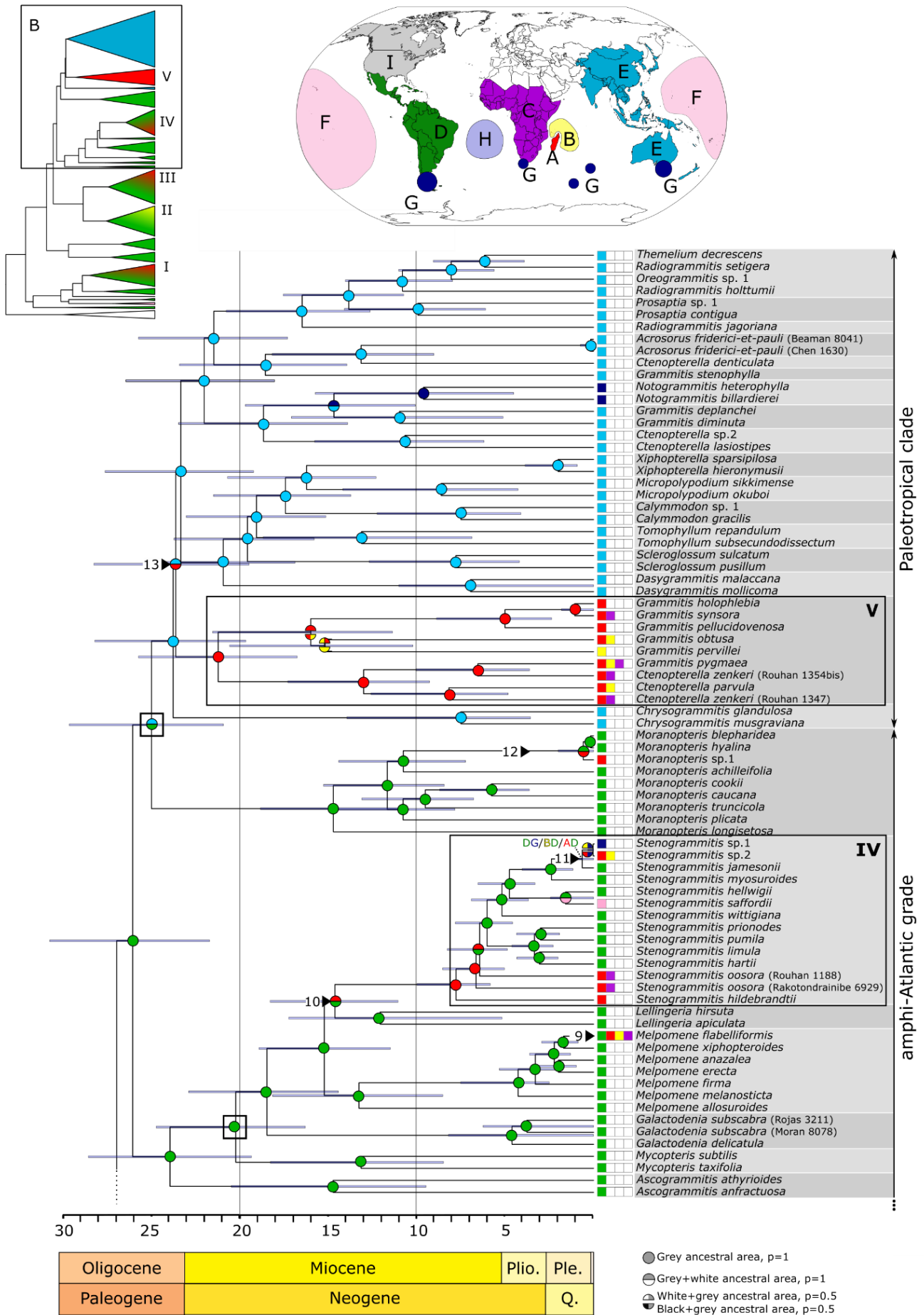


Figure 4 – Molecular dating estimates (represented on the maximum clade credibility tree) and ancestral areas reconstruction inferred by BEAST and S-DIVA, respectively, on the reduced ingroup sampling of 149 samples. The four calibrated nodes are surrounded by squares and horizontal blue bars correspond to 95% HPD intervals of the median divergence dates. Next to terminals, colored squares depict geographic distribution considering nine areas: (A) Madagascar; (B) Mascarenes, Comoros and Seychelles; (C) Africa; (D) Neotropics; (E) Tropical Asia s.l.; (F) Pacific Ocean islands; (G) Temperate circumastral region; (H) South Atlantic Islands; (I) North America. At each node, colored pies represent the inferred ancestral distribution. A two-color but one-block pie stands for a distribution covering two areas, inferred with probability $p = 1$; a two-block pie indicates that two alternative distributions were inferred, with $p = 0.5$ each (see the graphic legend). Black triangles on branches indicate the 13 LDD events inferred from other areas than A, B and C to at least one of them. Clades I to V are those that diversified in Africa and WIO. Inferred dispersals are reported on the map, with line thickness proportional to the relative number of events (see Discussion).

4. DISCUSSION

Our phylogenetic analyses included 25% of all grammitid species and especially filled major gaps in the Malagasy and other WIO taxa (e.g. 87% of the described Malagasy diversity and 77% of the known endemics are now included vs. 22% and 0%, respectively in Sundue *et al.*, 2014). This allowed improving our understanding of both the worldwide systematics and the biogeographic history of the group, as hereafter discussed.

4.1. SYSTEMATIC IMPLICATIONS

Our results generally supported those previously obtained with a more limited sampling (Sundue *et al.*, 2014): grammitid ferns formed a strongly supported clade, and the same global topology was recovered although support values of deeper nodes were more moderate. In addition, 22 genera were confirmed as monophyletic and four as polyphyletic (*Grammitis*, *Oreogrammitis*, *Radiogrammitis* and *Zygophlebia*; the assessment was not possible for *Lomaphlebia* and *Acrosorus*, represented by only one species each, and for *Luisma* and *Archigrammitis* still to be sampled; Figure 2).

Besides these, our results revealed six new major findings: (i) *Grammitis* s.s. was resolved as monophyletic; (ii) all other species of *Grammitis* s.l. were retrieved across four lineages, reinforcing the polyphyly of this genus; (iii) for the first time, *Ctenopterella* (newly tested here) and *Enterosora* were recovered as polyphyletic; (iv) three new major clades were supported (grey-filled triangles 1, 2, and 3, in Figure 2), involving species currently classified in *Grammitis* s.l., *Ctenopterella* and *Enterosora*; (v) three new species, suspected based on morphological evidence, were confirmed as distinct molecular lineages and will be described elsewhere (*Moranopteris* sp. 1 as endemic to Madagascar, whereas the genus was known only from the Neotropics to date; *Stenogrammitis* sp. 1 as endemic to Amsterdam Island, which is the new southernmost occurrence for the genus; and *Zygophlebia* sp. 1 as endemic to the Comoros); and (vi) a high level of cryptic species was suggested. Some of these findings being related to each other, they are hereafter discussed by genus or new major clade.

4.1.1. *Grammitis* s.s. is monophyletic

Grammitis s.s. was newly resolved as monophyletic and sister-clade to *Cochlidium* (Figure 2), whereas it was previously interpreted as paraphyletic with respect to *Cochlidium* (Sundue *et al.*, 2014). Our result is in agreement with morphological

observations, which suggested that all the ca. 20 species showing distinct, blackish sclerotic lamina margins (e.g. *G. copelandii*; Figure 1E) were close relatives (Parris, 2007). All such species are indeed placed within *Grammitis* s.s. in our molecular phylogeny, and we also anticipate that the type species of the genus (*G. marginella*, not included here) belongs to this group. However, *G. cryptophlebia* and *G. coriaceifolia* ined. are also both nested within *Grammitis* s.s. based on molecular data, although their laminae do not exhibit blackish margins (Figure 3, clade-1). It suggests that this character was lost at least once during the evolution of the clade. Consequently, the blackish sclerotic margin adds to the long list of homoplastic morphological characters in the evolution of grammitids (Ranker *et al.*, 2004), which resulted in the recent description of many genera.

4.1.2. *Grammitis* s.l. and *Ctenopterella* are highly polyphyletic, and lead to the identification of two new major clades

Apart from the *Grammitis* s.s. clade, other *Grammitis* species were retrieved in four lineages (Figure 2) that are consequently in need of new generic concepts. These phylogenetic placements were described previously for *G. stenophylla* (sister lineage to *Acrosorus* and *Ctenopterella denticulata*), and for the three-species clade sister to *Notogrammitis* (Perrie and Parris, 2012; Sundue *et al.*, 2014). In addition, we identified two new lineages nested within clade-3 (Figure 2).

The latter clade-3 is new for the grammitid phylogeny. Exclusively composed of African and WIO species, it was unexpected due to its singular placement within the large Paleotropical clade otherwise only composed of Asian and Oceanian taxa, and due to its grouping of six species of *Grammitis* with two species of *Ctenopterella* (Figures 2 and 3). Lamina dissection was commonly used to distinguish *Grammitis* (simple, undivided laminae; Figure 1E) from *Ctenopterella* (pinnatisect laminae; Figure 1C) but the grouping of species from the two genera, in clade-3, supports leaf dissection as homoplastic as evidenced within other grammitid genera like *Ceradenia* (Ranker *et al.*, 2004). This explains why the modern generic classification of grammitids is rather based on characters of the rhizomes, rhizome scales and frond hairs (Parris, 2009; Sundue, 2010; Sundue *et al.*, 2010a). However, many of these morphological characters are variable among the species clustered in clade-3 (e.g. rhizome symmetry is either dorsiventral or radial, rhizomes scales are concolorous or bicolor, and clathrate or not). Clade-3 could nevertheless be supported by the presence of hydathodes, hypothesizing one reversion in the anhydathodous *G. obtusa* (Figure 3). This clade could be treated as one or several new genera distinct from *Grammitis*, which should ultimately be restricted to *Grammitis* s.s. Another possibility would be to accommodate the six species of *Grammitis* of clade-3 in an expanded concept of *Ctenopterella*. However, the placement of *Ctenopterella* in the whole tree is uncertain because the type species of the genus (*C. blechnoides*) has never been sampled and moreover, we recovered the three other sampled species of *Ctenopterella* as another distinct, new clade of the grammitid phylogeny (Figure 2).

Of this new *Ctenopterella* lineage, retrieved as sister to the clade formed by *Notogrammitis* and three species of *Grammitis* (Figure 2), two species are endemic to New Caledonia (*C. lasiostipes* and *Ctenopterella* sp. 1) and the third, *Ctenopterella* sp. 2, occurs in eastern Papua New Guinea, Solomon Islands and Vanuatu (Parris unpubl.; Appendix 1.2). We speculate that most, if not all other species from Oceania and South-eastern Asia

(including the type species *C. blechnoides*) could belong here, i.e., 16 out of the ca. 21 species in the genus (Parris, 2009, 2012). In contrast, the four *Ctenopterella* species from Africa and the WIO (Parris, 2012) likely all cluster in clade-3 (as observed for the two species included here, *C. zenkeri* and *C. parvula*).

4.1.3. *Enterosora* is highly polyphyletic, notably revealing a new major grammitid clade

Enterosora was here recovered as polyphyletic for the first time (Figure 2). Although the genus was previously hypothesized as paraphyletic with respect to *Zygophlebia* (Sundue *et al.*, 2010a), it was recently supported as monophyletic (Sundue *et al.*, 2014). Our extended sampling included nine out of ca. 17 *Zygophlebia* species plus seven out of 11 *Enterosora* species, and led to two findings.

First, the close relationship between these two genera was confirmed, two lineages of *Enterosora* being nested within *Zygophlebia* (Figure 3, clade-2). The lack of morphological apomorphy supporting *Enterosora* (Ranker *et al.*, 2004; Sundue, 2010) argues for including the five concerned species (*E. enterosoides*, *E. trifurcata*, *E. trichosora*, *E. percrassa* and *E. barbatula*) into *Zygophlebia* (the additional segregate lineage of *E. barbatula* from Réunion Island, retrieved within *Ceradenia*, is discussed in the next section, 4.1.4).

Second, the two species *E. parietina* and *E. sprucei* formed a new clade, placed as sister-group to *Adenophorus* (Figures 2 and 3, clade-1). This grouping is meaningful since *E. parietina* and *E. sprucei* are morphologically most closely related to each other within the genus. It is also in agreement with Bishop and Smith (1992), who underlined that the affinity of *E. sprucei* (as *E. gilpinae*) and *E. parietina* to other species of *Enterosora* was not obvious, especially considering unique characters like medial soral placement and the number of annulus cells in the sporangia. All these characters should help to define a new genus accommodating this new clade. The type species of the genus, *E. campbellii*, has not been sampled yet, however we expect it to reside within clade-2 based on its assemblage of morphological characters. Within the new clade, *E. parietina* and *E. sprucei* were not monophyletic with respect to each other, in agreement with Bishop and Smith (Bishop & Smith, 1992) who regarded them as conspecific in the Neotropics (as *E. parietina*). In fact, our results even support recognition of only one species on both sides of the Atlantic Ocean, as suggested by Parris (Parris, 2002, 2005) and contrary to Bishop and Smith (1992) who distinguished *E. parietina* in the Neotropics from *E. gilpinae* in Africa and Madagascar.

4.1.4. High level of cryptic species as revealed by a dense sampling on Madagascar

Our sampling, especially focused on Madagascar and other WIO islands and Africa, included at least two individuals from each of 30 species from this geographical region. This allowed assessment of monophyly at the species level. Based on our molecular data, a total of 12 species were revealed as non-monophyletic, although we could not identify morphological characters to differentiate the distinct lineages recovered for each nominal species (Table 4, Appendix 1.2). Therefore, nine of these species are best interpreted as cryptic species (Bickford *et al.*, 2007). The three remaining ones (*Stenogrammitis oosora*, *Stenogrammitis* sp. 1, and *Enterosora sprucei*) deserve further investigation since they

could actually turn out to represent a single species each (see Table 4). In three cases of polyphyly (*Grammitis ebenina* (Figure 3, clade-1), *Grammitis kyimbilensis* (Figure 3, clade-1) and *Enterosora barbatula* (Figure 3, clade-2), the segregate lineages occurred in disjunct geographic areas. This provides an additional line of evidence to describe new species (respectively, two new species for Madagascar and one for Madagascar/Africa) that would accommodate the segregate, allopatric species with monophyly (Table 4). By contrast, for the six other cryptic species, only an in-depth study of integrative taxonomy combining molecular data, morphology, anatomy, cytometry and cytology might help to identify discriminating characters. Given that most grammitid ferns are epiphytes, a possibility of differential host-plant specificity should also be explored.

We do not know the evolutionary processes underlying cryptic speciation, but the apparent morphological similarity must be either a plesiomorphy due to shared ancestry (morphological stasis), or a homoplastic result of parallel or convergent evolution. Morphological stasis is rather expected to result from non-ecological vicariant speciation, e.g. when the topography is complex and populations are isolated (Britton, Hedderson, & Anthony Verboom, 2014). In contrast, parallel or convergent evolution usually suggests the influence of selection, e.g. to adapt to extreme environmental conditions (Bickford *et al.*, 2007) such as high elevations for many grammitid ferns (Parris, 2009; Sylvester, Sylvester, & Kessler, 2014). We tend to favour the latter mechanism given the high dispersal capacities of ferns and the well-known homoplastic nature of several morphological traits in this group (Ranker *et al.*, 2004). Hybridization might also be at the origin of some cryptic species (Mallet, 2007) and is thought to be especially frequent in ferns compared to angiosperms (Barrington, Haufler, & Werth, 1989; Haufler, 2008). Hybrids are often postulated based on morphological intermediary between the two putative parents, but they sometimes retain the overall morphology of only one parent. In such a case, the comparison of plastid- and nuclear-based phylogenetic analyses may eventually provide evidence of such reticulate evolution, and explain the incongruence between morphological and molecular plastid data.

Our results showed that the identification of morphologically cryptic lineages increased the number of sampled species from 36 to >50 in the African-WIO region (some cryptic species have to be confirmed, and their exact number cannot be firmly established). Whatever the evolutionary origins of the cryptic species, failure to distinguish them greatly underestimates species richness (Gill *et al.*, 2016), and consequently the impact of habitat destruction that is already a very serious threat in the Malagasy region (Harper *et al.*, 2007). The inclusion of several samples per a priori delimited species is therefore crucial in a conservation perspective, to uncover the possible hidden but threatened Malagasy diversity.

Finally, taking into account all our phylogenetic findings, we suggest that the African-WIO region harbours >68 grammitid species (>50 analyzed here, plus 18 non-sampled species) instead of the 51 previously listed (Roux, 2009); that is, we document a >30% increase in the species richness of the region. Although some taxa are still missing and the extinction of some lineages may bias the inferences as in all biogeographic studies, our extended sampling provides valuable insights into the biogeographic events and evolutionary processes underlying the emergence of the species in the region.

4.2. BIOGEOGRAPHIC HISTORY OF GRAMMITID FERNS, AND ORIGINS OF THE AFRICAN-WIO SPECIES

4.2.1. *At the worldwide scale: A Neotropical origin, followed by numerous LDD events*

At the global scale, as in Sundue *et al.* (2014), our results supported the Neotropical origin of grammitids and the divergence and start of diversification of the group were dated to the Eocene and early Oligocene (39 and 34 Ma, respectively). This relatively recent timing confirms that dispersal, rather than vicariance linked to continental fragmentation, explains species distribution patterns. The high dispersal ability of dust-like spores has been previously invoked to explain LDD in fern lineages of diverse ages and in different areas (Hennequin *et al.*, 2010b; Labiak *et al.*, 2010, 2014; Lu *et al.*, 2012; Chao *et al.*, 2014; Chen *et al.*, 2015; Wei *et al.*, 2015; Le Péchon *et al.*, 2016). In addition, epiphytism with occurrence of long-lived and slow growing gametophytes, as observed in grammitids (Stokey & Atkinson, 1958; Parris, 1990), may allow to delay sexual reproduction until a second gametophyte germinates nearby and confer an additional advantage for successful colonization (Dassler & Farrar, 2001; Moran & Smith, 2001; Sessa, Testo, & Watkins, 2016).

4.2.2. *Origin of the African-WIO grammitids: A clearly predominant role of the Neotropics, with one possible migration from tropical Asia s.l*

Based on the phylogenetic pattern composed of ‘a monophyletic tropical Asian clade nested within a primarily Neotropical and African grade’, Sundue *et al.* (2014) inferred the expansion and diversification of grammitids throughout the tropics. From the Neotropics, they inferred at least six dispersal events to Madagascar, two to the Hawaiian Islands in the Pacific, and only one to tropical Asia s.l. where the group underwent a striking species diversification (referred to as the Paleotropical clade, emerging in the late Oligocene 25 Ma). From tropical Asia s.l., they showed that the group further dispersed towards the Hawaiian Islands on the one hand, and to the circumaustral region on the other hand.

In contrast to those previous findings, we recovered the existence of an African-WIO clade nested within the Paleotropical clade, and composed of species sampled for the first time (clade V, Figure 4). This suggests the occurrence of a newly inferred dispersal from Tropical Asia s.l. to the African-WIO region (see map, Figure 4), which would be the oldest LDD event to this region observed in grammitids, at the end of the Oligocene. However, this inference remains to be confirmed since the branching pattern of the African-WIO clade with the rest of the Paleotropical clade was not well-supported statistically. Therefore, we cannot fully exclude that dispersal actually occurred from the Neotropics to Africa-WIO, and either later on from Africa-WIO to Tropical Asia s.l., or independently from the Neotropics to Tropical Asia s.l.

In spite of this new finding, our study strengthened the scenario of Sundue *et al.* (2014) of repeated dispersals from the Neotropics to the African-WIO region (Figure 4): while they inferred at least six such events, our data showed that at least 12 (occurring within the last 20 Ma and including species of 10 distinct genera) must actually be hypothesized to explain the current species richness of the region. This was in line with the observed distribution patterns: indeed, no genus is shared between the African-WIO region and

Tropical Asia s.l. (only *Ctenopterella* and *Grammitis* were thought to be shared but turned out to be polyphyletic, with distinct lineages in both regions), whereas 11 genera are shared between the African-WIO region and the Neotropics. We do not exclude that the generic affinities between the Neotropics and the African-WIO region could be even greater since two genera were discovered recently in Madagascar, namely *Leucotrichum* (Rouhan *et al.*, 2012) and *Moranopteris* (this study), which were previously considered endemic to the Neotropics. At the species level, even four species (*A. elastica*, *C. serrulatum*, *E. sprucei* and *M. flabelliformis*) are shared: this co-occurrence in the Neotropics and WIO may be explained by recent dispersal events (<4 Ma; and, therefore, insufficient time for geographically disjunct populations to diverge genetically) or by repeated dispersals, causing recurrent gene flow and preventing genetic differentiation.

Another eastward transatlantic migration of grammitid ferns is exemplified by the sister relationships between the Neotropical *G. paramicola* and *G. ebenina*, which is endemic to the mid-South Atlantic Ocean island of St. Helena, although this island is geographically closer to Africa (Figure 4). A similar closer evolutionary relationship between species from St. Helena and the Neotropics has been reported in the genus *Elaphoglossum* (Vasco *et al.*, 2009).

In contrast to these 12 eastward dispersal events, only one back-dispersal from the African-WIO region to the Neotropics was inferred, in *Stenogrammitis* (clade-IV; Figure 4). An additional such westward back-dispersal may have occurred in the *Zygophlebia-Enterosora* clade, where the African *Zygophlebia* cf. *devoluta* was retrieved as basal to a dichotomy of a Neotropical and an African-WIO clades: this pattern suggested either two independent dispersal events from the Neotropics to Africa-WIO, or one migration followed by a back-dispersal. In any case, transatlantic migrations appear highly skewed in their directions, with a predominant eastward direction.

The much higher affinity and closer phylogenetic relationships of grammitid ferns of the WIO with those of the Neotropics, compared to those of Tropical Asia s.l., may be due neither to ecological specificities – in all regions, most species occur in mountain forests – nor to lower dispersal abilities of taxa occurring in Tropical Asia s.l. – most of which have large distribution areas. Therefore, we rather hypothesize a higher potential for dispersal from the Neotropics than from Tropical Asia s.l. due to environmental factors, even if the Neotropics do not seem to be a major area in the biogeographic history of other Malagasy vascular plants: in their broad-scale survey, Buerki *et al.* (2013) identified only two undisputable cases of American ancestry for Malagasy angiosperms (in *Haematodendron*, Myristicaceae; and *Tsoala*, Solanaceae). More recently, Smedmark *et al.* (2014) and Janssens *et al.* (2016) provided two additional examples of such dispersal from the Neotropics to Africa-WIO in *Saldinia* and *Astiella* and closely-related genera, respectively (both Rubiaceae). However, this pattern remains scarce in flowering plants. In contrast, it has been inferred in several fern groups (see Introduction). In some of them (e.g. *Parapolystichum* and *Lastreopsis*; Labiak *et al.*, 2014) connections between the Neotropics and the WIO could be facilitated by overland dispersal events via Antarctica, which were possible until the Late Cretaceous (Buerki *et al.*, 2013; Yoder and Nowak, 2006). However, it cannot be invoked in grammitids, whose stem lineage is younger (here dated to the Eocene at the earliest). Therefore, the most likely hypothesis for the strong

link between the Neotropical and the WIO grammitids involves transoceanic LDD. Their highly predominant occurrence in the eastward direction could be due to transport of spores through winds blowing from the west, either surface winds over the Central Atlantic Ocean (10°S–5°N; even if these are subject to significant year-to-year changes), or the jet streams always blowing from the west and that may extend across the entire Atlantic basin (Renner, 2004). In fact, prevailing westerly trade winds existing at even higher latitudes (30–60°S latitude) could also have contributed to skewed dispersals (Munoz *et al.*, 2004), as suggested for many other fern genera with a circumaustral distribution including Madagascar (Parris, 2001; Sundue, Rouhan, & Moran, 2010b; Labiak *et al.*, 2014). Such a very southern trajectory is likely to reach Madagascar and the other WIO islands through the counter clockwise gyre over the South Atlantic Ocean, moving over the ocean south of Africa and arriving in Madagascar, as modelled for spores of *Ophioglossum reticulatum* (Meza Torres *et al.*, 2015).

4.2.3. At the regional African-WIO scale: Madagascar, rather than Africa, acts as a major source of dispersal

Many species co-occur in Africa, Madagascar or the other WIO islands: 20 species are shared by at least two of these areas, out of the 51 species recorded in the overall region (Roux, 2009). In agreement with these shared distribution patterns, species that are endemic to one territory were most often retrieved as sister- species to others distributed in nearby areas, showing a high level of dispersal at this regional scale.

Strikingly, we inferred only one possible dispersal event from Africa towards Madagascar and the other WIO islands, which gave rise to clade-III (Figure 4). In all other cases, the origin of dispersal within the region appeared to be either Madagascar or the other WIO islands. Therefore, Africa does not seem to be a source region for grammitid species in the WIO, contrary to what could be expected based on the Theory of Island Biogeography (MacArthur & Wilson, 1967) and empirical evidence for most of the angiosperms studied (Yoder & Nowak, 2006; Buerki *et al.*, 2013; Janssens *et al.*, 2016; Bacon *et al.*, 2016). Actually, there is a growing body of evidence against the paradigm postulating that continents and islands respectively act as sources and sinks of species diversity (Heaney, 2007), and an increasing awareness of the importance of islands as cradles of diversity themselves: it has been documented for the WIO islands (Agnarsson & Kuntner, 2012; Krüger, Razafimandimbison, & Bremer, 2012) and for the Macaronesian islands (Patiño *et al.*, 2015). Although the observed patterns may be overestimated due to missing African species in our study (we included 14 out of 26 African species), grammitid ferns corroborate the latter scenario, and provide another example contradicting the traditional view. This was also suggested by the higher species diversity described from Madagascar compared to continental Africa, which harbour ca. 31 and 26 species, respectively, although Africa is 50 times larger in area. Such a lower taxonomic diversity in Africa compared to Madagascar was also observed in many other fern genera (Kornas, 1993; Roux, 2009). It may be due either to the extinction of lineages after much more severe droughts there than in Madagascar during the Late Miocene and Pleistocene (Morley, 2000; Aldasoro, Cabezas, & Aedo, 2004), which led to a decline of African rainforests (Kornas, 1993; Morley, 2000; Linder, Pennington, & Schneider, 2014), or to the lack of extensive mountain ranges (Moran, 1995), or most probably to several intricate, both historical and more recent causes (Couvreur, 2015).

From Madagascar and/or the neighbouring islands, dispersal also occurred over larger geographic distances, as evidenced by the colonization of the Subantarctic island of Amsterdam by the genus *Stenogrammitis*: the phylogeny showed that the Neotropical *S. jamesonioides* was sister-species to a clade formed by two species, one from Madagascar and Réunion, and the other from Amsterdam. The most likely biogeographic scenario involves a dispersal event from the Neotropics to Madagascar and/or Réunion, later followed by dispersal to Amsterdam (Figure 4). Whereas Sundue *et al.* (2014) suggested that circumaustral taxa only originated in Tropical Asia, our results therefore suggest that the WIO area also played a role in the range expansion of grammitids to this temperate area.

4.3. THE OCCURRENCE OF POST-DISPERSAL DIVERSIFICATION

In total, at least 13 LDD events may explain the origin of the >50 species (including cryptic lineages) sampled in Africa, Madagascar, and other WIO islands. In particular, five LDD gave rise to diversifications counting six to 14 species each. Species diversification probably proceeds at a reduced pace in ferns compared to flowering plants, because of their strong dispersal capacities that slow down genetic differentiation (Gillespie *et al.*, 2012). We actually noticed that diversification occurred in the most ancient lineages, suggesting that the time elapsed since the colonization of a given lineage may be involved in its diversification. Species diversification was probably favoured by the environmental diversity within Madagascar: with its very diverse ecological conditions, the island is known to host spectacular species radiations (Vences *et al.*, 2009; Soulebeau *et al.*, 2015). At the regional scale, the insular system and resulting relative isolation of populations probably also facilitated species diversification, in spite of dispersal. The evolutionary processes involved in such diversification will be investigated in further studies.

Acknowledgments

We thank Drs. B. Senterre, D. Middleton and S. Lindsay for providing leaf material; T. Cheminel and I. Fabre for their help in the lab; and Dr. M. S. Vorontsova and another, anonymous reviewer for their valuable comments on an earlier version of the manuscript. 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 (especially Y. Ibrahim). 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 network 'Bibliothèque du Vivant' funded by the CNRS, the MNHN, the INRA and the CEA (Centre National de Séquençage). All molecular work was performed at the BoEM laboratory of the MNHN.

Chapter II - Molecular data, based on an exhaustive species sampling of the fern genus *Rumohra* (Dryopteridaceae), reveal a biogeographical history mostly shaped by dispersal and several cryptic species in the widely distributed *Rumohra adiantiformis*

Lucie Bauret^{1,2*}, Germinal Rouhan¹, Regina Y. Hirai³, Leon Perrie⁴, Jefferson Prado³, Alexandre Salino⁵, Bruno Senterre^{6,7}, Lara Shepherd^{4,8}, Michael Sundue⁹, Marc-André Selosse^{1,10} and Myriam Gaudeul¹

¹ Muséum national d'Histoire naturelle, Sorbonne Universités, Institut de Systématique, Evolution, Biodiversité (UMR 7205 CNRS, MNHN, UPMC, EPHE), Herbar national, 16 rue Buffon CP39, F-75005 Paris, France

² Université Pierre et Marie Curie, Sorbonne Universités, Institut de Systématique, Evolution, Biodiversité (UMR 7205 CNRS, MNHN, UPMC, EPHE), 57 rue Cuvier CP48, F-75005 Paris, France

³ Instituto de Botânica, Caixa Postal 68041, CEP 04045-972 São Paulo, SP, Brazil

⁴ Museum of New Zealand Te Papa Tongarewa, PO Box 467, Wellington, New Zealand

⁵ Departamento de Botânica, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627, Pampulha, 31270-901 Belo Horizonte, Brazil

⁶ Evolutionary Biology & Ecology, CP 160/12, Université Libre de Bruxelles, 50 Av. F. Roosevelt, 1050, Bruxelles, Belgique

⁷ Natural History Museum, P.O. Box 720, Victoria, Mahé, Seychelles

⁸ School of Biological Sciences, Victoria University of Wellington, PO Box 600, Wellington, New Zealand

⁹ The Pringle Herbarium, Department of Plant Biology, The University of Vermont, 27 Colchester Ave., Burlington, VT 05405, USA

¹⁰ Department of Plant Taxonomy and Nature Conservation, University of Gdańsk, ul. Wita Stwosza 59, 80-308 Gdańsk, Poland

Accepted, 15 September 2017, in the *Botanical Journal of the Linnean Society*.

Not yet available online, however a doi was attributed: 10.1093/botlinnean/box072

ABSTRACT

Rumohra is a fern genus comprising seven species, three in South America, three in Madagascar and one (*R. adiantiformis*) which is widely distributed across the Southern Hemisphere. Our goals were to assess species delimitation based on molecular data and to infer the biogeographical history that led to such contrasting distributions among species. We sampled all *Rumohra* spp., with 46 samples including 28 *R. adiantiformis* accessions from 14 regions, and sequenced eight plastid DNA regions: *atpA*, *atpB*, *atpB-rbcL*, *rbcL*, *rps4-trnS*, *trnG-trnR*, *trnH-psbA* and *trnL/trnL-trnF*. The resulting phylogenetic trees showed *R. adiantiformis* to be polyphyletic, with at least six lineages found in distinct geographical regions. Given the apparent absence of distinctive morphological characters among lineages, they are best understood as cryptic species. Such genetically distinct but morphologically similar populations may result from a recurrent history of hybridization, morphological convergence or (most probably) morphological stasis. Molecular dating and ancestral area estimations showed that *Rumohra* diverged from the genus *Megalastrum* c. 46.4 Mya in the Neotropics, and started to diversify 11.2 Mya. Its biogeographical history was probably shaped by seven long-distance dispersal (LDD) events including three initial events from the Neotropics to southern Africa, the Malagasy region and southern South America. The Australasian lineage resulted from a LDD from southern South America, and the three species endemic to Madagascar diversified in situ.

KEYWORDS

Madagascar, molecular phylogeny, pteridophytes, southern Hemisphere, species concept, systematics, western Indian Ocean

1. INTRODUCTION

Delimiting species is an essential prerequisite for studies of ecology and evolution and the implementation of appropriate conservation measures. Beyond morphological investigations, molecular markers have greatly improved our ability to circumscribe taxonomic units in recent decades, leading to a so-called integrative taxonomy (Rouhan & Gaudeul, 2014). Molecular markers are especially valuable in groups with few morphological attributes and in groups comprising morphologically similar populations that sometimes occur across large geographical areas. In the latter case, a widely distributed morphologically defined species might consist of a single interconnected gene-pool (e.g. if dispersal is frequent), or may be composed of several evolutionarily independent lineages. Indeed, large geographical distances can lead to reduced gene exchange among populations, resulting in neutral genetic divergence under the action of genetic drift. Nevertheless, such genetic divergence is not necessarily accompanied by morphological changes (morphological stasis). Alternatively, morphological similarity in widely distributed taxa can result from convergence towards traits that confer a selective advantage and that are therefore independently selected in lineages that are not closely related (Patterson & Givnish, 2002). The discovery of cryptic species (distinct species that are, or have been, erroneously classified under one species name because of their similar morphology, but are genetically distinct; Bickford *et al.*, 2007) is probably still limited by the low variability of molecular markers, especially in plants, and by the sampling schemes adopted, which often consist of a single or few individual/accession(s) per morphologically recognized species. However, a growing number of studies provide evidence for cryptic species in various plant groups (Bickford *et al.*, 2007; see also, e.g. Hedenäs & Eldenäs, 2007; Pillon *et al.*, 2009; Crawl, Myers, & Cellinese, 2017; Bauret *et al.*, 2017). Genetic data can also provide insight into the evolutionary (phylogenetic) relationships among taxa and their diversification history, providing hypotheses to explain the observed species distributions and morphological disparity or, by contrast, morphological uniformity.

Rumohra Raddi is a small fern genus of seven, mostly tropical species. It contains one species with populations spread across the whole Southern Hemisphere [*R. adiantiformis* (G.Forst) Ching], whereas the remaining six species are restricted to two centres of endemism (Table 1): South America (three endemics; Sundue, Hirai, & Prado, 2013) and Madagascar (three endemics; Rakotondrainibe, 2010b). Species are terrestrial, epilithic or epiphytic, and the genus is morphologically characterized by a combination of several features (Figure 1): creeping rhizomes with a dorsal leaf arrangement; elongate ventral meristele; lanceolate to deltate, two- to three- (to four-) pinnate laminae; decurrent leaf margins forming erect wings along the axes; and peltate indusia. Because the architecture of the lamina is fairly uniform across the genus, species are mostly distinguished based on their rhizome habit, rhizome scales and lamina indument (e.g. sessile and/ or stipitate glands).

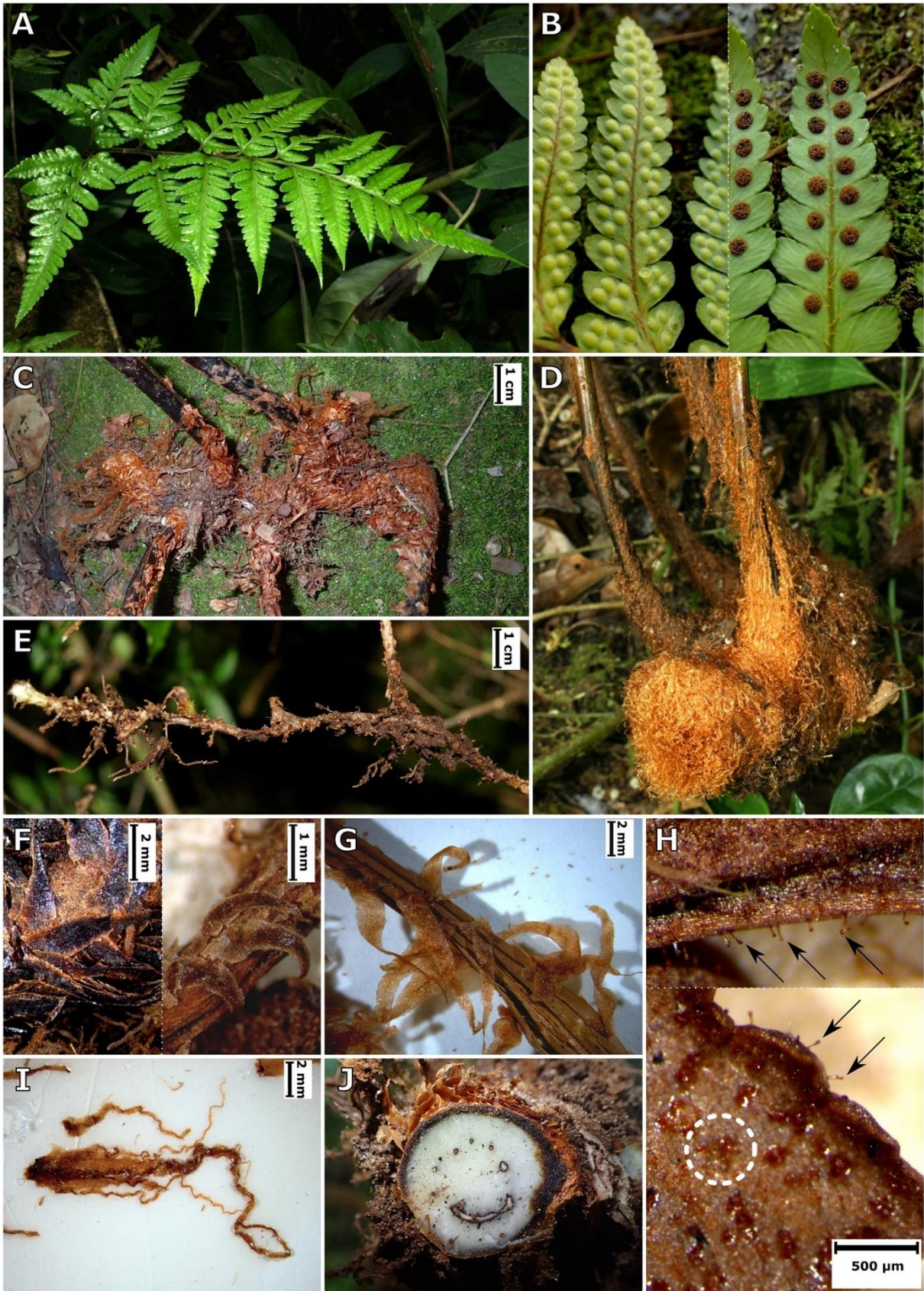


Figure 1. Morphology of *Rumohra* spp. (A) *R. adiantiformis* lamina, Rouhan *et al.* 1148, Madagascar. (B) *R. adiantiformis* sori, left: young sori with indusia, right: mature sori with indusia either shrivelled or fallen, Bauret *et al.* 83, Madagascar. (C) *R. adiantiformis* rhizome, Bauret *et al.* 83, Madagascar. (D) *R. linearisquamata* rhizome, Rouhan *et al.* 1395, Madagascar. (E) *R. lokohensis* rhizome, Rouhan *et al.* 1167, Madagascar. (F) *R. berteroaana* indument, left: rhizome scales, right: petiole scales, Gay s.n., Juan Fernández, Chile. (G) *R. adiantiformis* petiole scales, which are identical to those of the rhizome, Rouhan *et al.* 1148, Madagascar. (H) *R. glandulosissima* lamina glands: glandular hairs indicated by arrows, one shiny dot-like gland circled, Glaziou 11696, Brazil. (I) *R. linearisquamata* rhizome and petiole scales, Rakotondrainibe *et al.* 4068, Madagascar. (J) *R. adiantiformis* rhizome meristele, Bauret *et al.* 83, Madagascar. Photographs: L. Bauret, except A, D, E: G. Rouhan.

Rumohra belongs to Dryopteridaceae subfamily Elaphoglossoideae (PPGI, 2016) or Polypodiaceae subfamily Dryopteridoideae (Christenhusz & Chase, 2014). With the genera *Megalastrum* Holttum (91 species; Moran, Prado, & Sundue, 2014b,c), *Lastreopsis* Ching (16 species; Labiak *et al.*, 2015b) and *Parapolystichum* (Keyserl.) Ching (28 species; Labiak *et al.*, 2015b; Sundue & Testo, 2016), *Rumohra* forms a monophyletic group of 142 species referred to as lastreopsid ferns (Labiak *et al.*, 2014). To date, no phylogenetic study has focused on *Rumohra*. The relationships of two species [*R. berteroaana* (Colla) R.Rodr.R. (endemic to the Juan Fernández Islands, Chile) and *R. adiantiformis* (circum-austral)] have been analysed. These species were recovered as forming a group that is sister to *Megalastrum* (Labiak *et al.*, 2014). Based on sampling from three localities of *R. adiantiformis*, Labiak *et al.* (2014) also revealed that this wide-spread species may contain more than one lineage. The systematics of *Rumohra* may thus require revision, and *R. adiantiformis* is a candidate with which to investigate the alternative evolutionary causes of morphologically similar and widespread populations: morphological convergence vs. morphological stasis.

Besides its systematics, the distribution of *Rumohra* raises questions regarding the biogeographical history that has led to such contrasting distributions among species, in terms of spatial extent and geographical location, with three species endemic to South America, three endemic to Madagascar and one widespread, circum-austral species. Labiak *et al.* (2014) highlighted the importance of both vicariance and dispersal in the diversification history of lastreopsids and favoured several long-distance dispersal (LDD) events as the most likely explanation for the expansion and diversification of the *Megalastrum*–*Rumohra* clade between the Eocene and Oligocene. This is congruent with the well-known abilities of ferns to disperse easily across long distances owing to their dust-like meiospores (Barrington, 1993; Moran & Smith, 2001; Wolf, Schneider, & Ranker, 2001). Nevertheless, this scenario remained tentative for *Rumohra*, due to limited sampling.

In this study, we focused on *Rumohra* and, based on an exhaustive species sampling and extensive within- species sampling for the widely distributed *R. adiantiformis*, we inferred the molecular phylogeny of the genus in a spatiotemporal framework. Our goals were: (1) to assess the monophyly of the genus and its species; (2) more specifically, to identify whether *R. adiantiformis* represents a single taxonomic unit or several distinct but morphologically similar evolutionary units (cryptic species); and (3) to infer the biogeographical history of the group.

	Western Indian Ocean - Africa	America	Australasia	Other
<i>R. adiantiformis</i>	Madagascar, Mascarenes, Comoros, Seychelles, Southern Africa	Neotropics, Galapagos*, Southern South America, Falkland Islands, West Indies	Australia, Papua New Guinea, New Zealand (type locality)	Tristan da Cunha, South Indian Ocean Islands, Bermuda*
<i>R. berteroana</i>	-	Juan Fernández Islands	-	-
<i>R. glandulosissima</i>	-	Neotropics (Brazilian Atlantic Rainforest)	-	-
<i>R. linearisquamata</i>	Madagascar	-	-	-
<i>R. lokohensis</i>	Madagascar	-	-	-
<i>R. madagascarica</i>	Madagascar	-	-	-
<i>R. quadrangularis</i>	-	Neotropics (Brazilian Atlantic Rainforest)	-	-

Table 1. Distribution of the seven *Rumohra* spp. *Unsampled localities for the widely distributed *R. adiantiformis*.

2. MATERIAL AND METHODS

2.1. TAXONOMIC SAMPLING

Our study relied on an exhaustive sampling of *Rumohra* species diversity, with 46 specimens representing the seven known species. As *R. adiantiformis* is widespread throughout the tropics, and is mostly of circum-austral distribution, we sampled this species in 14 representative, disjunct localities to cover its large distribution (Table 1). Twelve species were added as outgroups, including five species of the two most closely related genera to *Rumohra*, namely *Megalastrum* and *Lastreopsis* (Labiak *et al.*, 2014). Vouchers details are provided in Supporting Information, Appendix 2.

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. We used the Qiagen DNeasy Plant Mini Kit (Valencia, CA, USA), and 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 for 24 h. Eight plastid DNA regions were amplified by PCR: the

genes *atpA*, *atpB* and *rbcl* and the non-coding regions *atpB-rbcl*, *rps4-trnS*, *trnG-trnR*, *trnH-psbA* and the *trnL* intron plus the *trnL-trnF* intergenic spacer (*trnL/trnL-trnF*). All PCRs were carried out in a 25 μ L volume containing 1 \times PCR buffer, 2.5 mM MgCl₂, 250 μ M each dNTP, 1 M betaine, 0.4 μ M each primer, 0.75 U Taq polymerase (Taq CORE kit; MP Biomedicals, Illkirch, France) and 1 μ L non-diluted genomic DNA. Primer 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 *atpA*, *atpB*, *rbcl* and *trnG-trnR* (Table 2). DNA strands were edited and assembled in Sequencher 4.9 (Gene Codes Corporation, Ann Arbor, MI, USA) and the 292 newly obtained consensus sequences were submitted to GenBank (Supporting Information, Appendix 2).

2.3. PHYLOGENETIC ANALYSES

Consensus sequences were automatically aligned in Muscle 3.8.425 (Edgar, 2004), and the eight resulting alignments were checked by eye and revised manually. After analysis of each marker independently to check the absence of supported conflicts between the estimated topologies, a data matrix was built by concatenation of all eight DNA regions using Sequence Matrix 1.7.8 (Vaidya *et al.*, 2011). Each region constituted an independent partition in the final dataset, and gaps were treated as missing data.

Bayesian inference (BI) and maximum likelihood (ML) approaches were used to infer phylogenetic relationships and were conducted on the CIPRES science gateway (Miller, Pfeiffer & Schwartz, 2010). For the BI, we used a Metropolis-coupled Markov chain Monte Carlo (MCMC) method implemented in MrBayes 3.2.6 (Ronquist *et al.*, 2012). For each region, a reversible jump MCMC (rjMCMC; Huelsenbeck, Larget, & Alfaro, 2004) was used to find the most appropriate model of nucleotide substitution. We allowed the rjMCMC to move across models with +I+ Γ . Two independent, but parallel, analyses of three million generations each were conducted, with four chains (one cold and three incrementally heated at a temperature of 0.1) sampled every 300 generations to obtain 10,000 sampled trees. We used Tracer 1.6.0 (Rambaut *et al.*, 2014) to check the output parameter estimates through time, the convergence of the two runs to the same stationary distribution and the burn-in length. We discarded the first 2,500 (25%) trees as burn-in, and pooled the 7,500 remaining trees in a 50% majority-rule consensus with average branch lengths and posterior probability (PP) estimates for all nodes.

The ML analysis was performed using RAxML-HPC2 8.2.6 (Stamatakis, 2014), using the same eight partitions as in MrBayes and with the GTRGAMMA+I model of nucleotide substitution. We 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. DIVERGENCE TIME ESTIMATION

We performed the dating analyses on a reduced data- set of 26 samples representing all seven *Rumohra* spp. and 12 outgroups. We kept one sample per species or per lineage in the case of *R. adiantiformis* (keeping two samples for the lineage including specimens from South Africa and Amsterdam Island; see Results).

DNA region	Primer name	Literature reference	Primer sequence (5' to 3')	Thermal cycling conditions
<i>atpA</i>	ESTRNR46F	Schuettpelz, Korall, & Pryer (2006)	GTA TAG GTT CRA RTC CTA TTG GAC G	5 min 94°C / 40 × (30 s 94°C / 1 min 50°C / 2 min 72°C) / 10 min 72°C
	ESATPA535F	Schuettpelz, Korall, & Pryer (2006)	ACA GCA GTA GCT ACA GAT AC	
	ESATPA856F*	Schuettpelz, Korall, & Pryer (2006)	CGA GAA GCA TAT CCG GGA GAT G	
	ESATPA877R*	Schuettpelz, Korall, & Pryer (2006)	CAT CTC CCG GAT ATG CTT CTC G	
<i>atpB</i>	ESATPB172F	Schuettpelz & Pryer (2007)	AAT GTT ACT TGT GAA GTW CAA CAA T	5 min 94°C / 40 × (30 s 94°C / 1 min 50°C / 2 min 72°C) / 10 min 72°C
	ESATPE45R	Schuettpelz & Pryer (2007)	ATT CCA AAC WAT TCG ATT WGG AG	
	atpB 1163F*	Wolf (1997)	ATG GCA GAA TRT TTC CGA GAT RTY A	
	ATPB910R*	Pryer <i>et al.</i> (2004)	TTC CTG YAR AGA NCC CAT TTC TGT	
<i>rbcl</i>	ESRBCL1F	Schuettpelz & Pryer (2007)	ATG TCA CCA CAA ACG GAG ACT AAA GC	5 min 94°C / 40 × (30 s 94°C / 45 s 50°C / 2.5 min 72°C) / 10 min 72°C
	ESRBCL1361R	Schuettpelz & Pryer (2007)	TCA GGA CTC CAC TTA CTA GCT TCA CG	
	ESRBCL628F*	Schuettpelz & Pryer (2007)	CCA TTY ATG CGT TGG AGA GAT CG	
	ESRBCL654R*	Schuettpelz & Pryer (2007)	GAA RCG ATC TCT CCA ACG CAT	
<i>atpB-rbcl</i>	atpB 672F	Wolf (1997)	ACA CTW AGA GGR GCT CCC GTA TCA A	

	rbcl 49R	Wolf (1997)	CAC CAG CTT TGA ATC CAA CAC TTG C	5 min 94°C / 40 × (30 s 94°C / 30 s 50°C / 1.5 min 72°C) / 10 min 72°C
<i>rps4-trnS</i>	rps4-3r.f	Skog <i>et al.</i> (2004)	AGT TGT TAG TTG TTG AGT AT	5 min 94°C / 40 × (30 s 94°C / 30s 50°C / 45 s 72°C) / 10 min 72°C
	trnS-r	Smith & Cranfill (2002)	TAC CGA GGG TTC GAA TC	
<i>trnG-trnR</i>	TRNG1F	Nagalingum, Schneider, & Pryer (2007)	GCG GGT ATA GTT TAG TGG TAA	5 min 94°C / 40 × (30 s 94°C / 1 min 50°C / 1.5 min 72°C) / 10 min 72°C
	TRNR22R	Nagalingum, Schneider, & Pryer (2007)	CTA TCC ATT AGA CGA TGG ACG	
	TRNG43F1*	Nagalingum, Schneider, & Pryer (2007)	TGA TGC GGG TTC GAT TCC CG	
	TRNG63R*	Nagalingum, Schneider, & Pryer (2007)	GCG GGA ATC GAA CCC GCA TCA	
<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, Crawford, & Stuessy (1997)	GTT ATG CAT GAA CGT AAT GCT C	
<i>trnL/trnL-trnF</i>	f	Taberlet <i>et al.</i> (1991)	ATT TGA ACT GGT GAC ACG AG	5 min 94°C / 40 × (30 s 94°C / 30 s 50°C / 45 s 72°C) / 10 min 72°C
	Fern-1	Trewick <i>et al.</i> (2002)	GGC AGC CCC CAR ATT CAG GGR AAC C	

Table 2. PCR primers sequences and thermal cycling conditions.

Divergence time estimates were calculated using BEAST 2.4.2 (Bouckaert *et al.*, 2014) on the CIPRES science gateway. Partitions were the same as for the BI and ML phylogenetic analyses, and nucleotide substitutions followed a +I+G reversible-jump model as implemented in the RBS 1.3.1 BEAST package.

The uncertainty about the placement of fossils of Dryopteridaceae previously led authors not to rely on these fossils (Collinson, 2001; Sessa, Zimmer, & Givnish, 2012; Labiak *et al.*, 2014), but a recently described fossil unambiguously assigned to *Elaphoglossum* Schott ex J.Sm. was dated to 15–20 Mya (early Miocene) and could provide an external calibration point (Lóriga *et al.*, 2014). This fossil was not assigned to any section of *Elaphoglossum*, and could even belong to an extinct lineage of the genus. We therefore imposed the age constraint at the node between *E. decoratum* (Kunze) T.Moore and *E. amygdalifolium* (Mett. ex Kuhn) Christ, the latter being sister to the rest of the genus (Rouhan *et al.*, 2004; Lóriga *et al.*, 2014). The age of this node was modelled by a uniform prior from 15 Mya to infinity.

We also used secondary calibration points from two large-scale studies on the timing of the diversification of leptosporangiate ferns (Schuettzel & Pryer, 2009; Testo & Sundue, 2016). Testo & Sundue (2016) reported older ages for numerous nodes and explained the contrast between their estimates and those of Schuettzel & Pryer (2009) by differences in the fossil calibrations and statistical methods used, notably the use of penalized likelihood by Schuettzel & Pryer (2009) vs. an uncorrelated lognormal relaxed clock in their study. We chose to run two separate analyses to successively adopt two calibration points from these two studies, to consider all the available data to date and to compare their influence on dating estimates in *Rumohra*: the ages of the lastreopsids and *Rumohra* stem nodes were modelled by normal prior distributions with means of 116.7 Mya (based on Sundue & Testo, 2016) or 67.0 Mya (based on Schuettzel & Pryer, 2009; node284) for lastreopsids, and 52.5 Mya (based on Testo & Sundue, 2016) or 40.5 Mya (based on Schuettzel & Pryer, 2009; node 288) for *Rumohra*. Standard deviations were set to 10% of the mean estimates.

An uncorrelated lognormal relaxed clock was used, with a birth–death tree prior and a random starting tree. For each analysis, two independent runs of 100 million generations with random seed values were conducted, sampled every 10,000 generations to obtain 10,000 sampled trees. Using Tracer 1.6.0 (Rambaut *et al.*, 2014), we checked the convergence of the two runs and set the burn-in length to 10%. We combined the runs with LogCombiner 2.4.2, computed the maximum clade credibility tree with TreeAnnotator 2.4.2, and visualized the maximum clade credibility tree and the associated chronogram in FigTree 1.4.2 (Rambaut, 2014).

2.5. BIOGEOGRAPHICAL ANALYSES

To explore the biogeographical history of *Rumohra*, we estimated ancestral range variation along the resulting chronogram, using the R package BioGeoBEARS (Biogeography with Bayesian Evolutionary Analysis in R Scripts; Matzke, 2013) in R studio 1.0.44 (R Studio Inc., Boston, MA, USA). In a likelihood framework, BioGeoBEARS implements three commonly used methods: dispersal–extinction–cladogenesis (DEC; Ree & Smith, 2008), dispersal–vicariance analysis (DIVA; Ronquist, 1997; referred to as

DIVALIKE in BioGeoBEARS) and Bayesian inference for discrete areas (BayArea; Landis *et al.*, 2013; referred to as BAYAREALIKE in BioGeoBEARS). We performed three analyses using each of these methods (DEC, DIVALIKE, BAYAREALIKE), plus three implementing the additional free parameter *j* (DEC+*j*, DIVALIKE+*j*, BAYAREALIKE+*j*) that stands for the additional process of a founder event speciation, when a daughter lineage disperses to a new range outside the range of its ancestor. These '+*j*' models infer ancestral areas at each node and, for example, the DEC+*j* model has been shown to perform significantly better than the DEC model for island groups and intercontinental distributions (Matzke, 2014). We then selected from among the six models the one that was best-suited to model our data based on the Akaike information criterion (AIC). The selected model was used to infer the relative probabilities of ancestral ranges along the phylogeny.

We also performed biogeographical stochastic mapping (BSM) implemented in BioGeoBEARS (Matzke, 2016). BSM simulates biogeographical histories (called 'realizations') given the phylogenetic topology (here, the one inferred by BEAST) and the best-suited biogeographical model identified under the AIC. Averaging over many realizations will result in the same ancestral range probabilities as those calculated under the likelihood model. As a BSM realization constitutes one of many possible exact histories, biogeographical events can be objectively hypothesized and counted from it. We performed 100 realizations of BSM to observe the most commonly retrieved biogeographical histories, successively considering the two most likely origins inferred for *Rumohra*, i.e. a Neotropical origin and an Australasian origin.

Geographical distributions were coded as combinations of eight areas: Neotropics; southern (temperate) South America; southern Africa; Comoros; Madagascar; Mascarenes and Seychelles; southern Indian Ocean islands; and Australia–New Zealand–Papua New Guinea (referred to as Australasia). We coded the distributions of whole species (and not only the localities of the included specimens), except for *R. adiantiformis*. This species was polyphyletic (see Results) and we therefore coded the locality of each sampled specimen. Geographical distributions were based on herbarium specimens (checked for taxonomic identifications), floras (Marticorena & Rodriguez, 1995; McCarthy, 1998; Labiak *et al.*, 2014) and personal observations of the authors. The maximum range size was set to two areas, as it is the maximum number of areas today covered by terminal taxa.

3. RESULTS

3.1. PHYLOGENETIC RELATIONSHIPS

We obtained a combined data matrix of 58 specimens and 6926 characters (Table 3). The topologies inferred by the BI and ML analyses were similar and both strongly supported the monophyly of *Rumohra* (BS = 100; PP = 1), with *Megalastrum* as sister genus (BS = 99; PP = 1; Figure 2).

Rumohra was divided into two supported clades. In clade A (BS = 90; PP = 1), four strongly supported geographical subclades (forming a polytomy) grouped samples from: (1) the western Indian Ocean [WIO, including *R. adiantiformis*, *R. linearisquamata* Rakotondr., *R. lokohensis* Tardieu and *R. madagascariensis* (Bonap.) Tardieu]; (2) the Neotropics

[*R. quadrangularis* (Fée) Brade, *R. glandulosissima* Sundue & J.Prado and *R. adiantiformis*]; (3) South Africa; and (4) South Africa and Amsterdam Island, the two latter subclades including only *R. adiantiformis* samples. Clade B (BS = 100; PP = 1) encompassed a southern South American subclade (with *R. adiantiformis* and *R. berteroaana* samples) and an Australasian subclade (with *R. adiantiformis* samples only; both subclades with BS = 100; PP = 1).

	Number of sequences	Aligned length (in base pairs)	Percent of variable characters	Percent of informative characters
<i>atpA</i>	33	948	1.8	1.7
<i>atpB</i>	36	1275	1.9	1.6
<i>atpB-rbcL</i>	36	629	3.5	3.2
<i>rbcL</i>	47	1308	18.8	10.9
<i>rps4-trnS</i>	47	548	40.5	26.8
<i>trnG-trnR</i>	41	961	27.3	17.8
<i>trnH-psbA</i>	36	437	3.4	3.2
<i>trnL/trnL-trnF</i>	46	820	20.7	12.1
Combined dataset	58	6926	14.1	9.1

Table 3. Statistics on the eight plastid DNA regions and combined dataset obtained for *Rumohra*.

Rumohra adiantiformis was grossly polyphyletic, being recovered in eight lineages (Figure 2). However, taking into account the lack of resolution in clade A and weak support of the *R. adiantiformis*–*R. linearisquamata* clade (BS < 50; PP = 0.66), the results showed that *R. adiantiformis* formed at least six geographically structured and supported lineages distributed in the Comoros, WIO excluding the Comoros, Neotropics, South Africa and Amsterdam Island [hypothesizing that subclades (3) and (4) in fact group together], southern South America, and Australasia. The other six species were either monophyletic (*R. lokohensis*, BS = 66 and PP = 0.76; *R. madagascarica*, BP = 86 and PP = 1; and *R. berteroaana*, BP = 94 and PP = 1) or unresolved (*R. glandulosissima*, *R. linearisquamata*, *R. quadrangularis*).

3.2. DIVERGENCE TIME ESTIMATES

Trees obtained from the two dating analyses, based on calibration points from Testo & Sundue (2016) and Schuettpelz & Pryer (2009), respectively, were similar for both the topology and the node support values (Figure 3). They showed slightly better resolution in clade A (PP = 0.99) than the BI and ML trees estimated in MrBayes and RAxML: a subclade grouped the South African and Amsterdam Island samples of *R. adiantiformis* (PP = 0.88), and was sister to the Neotropical subclade (including *R. quadrangularis*, *R. glandulosissima* and *R. adiantiformis*; PP = 1) with PP = 0.71.

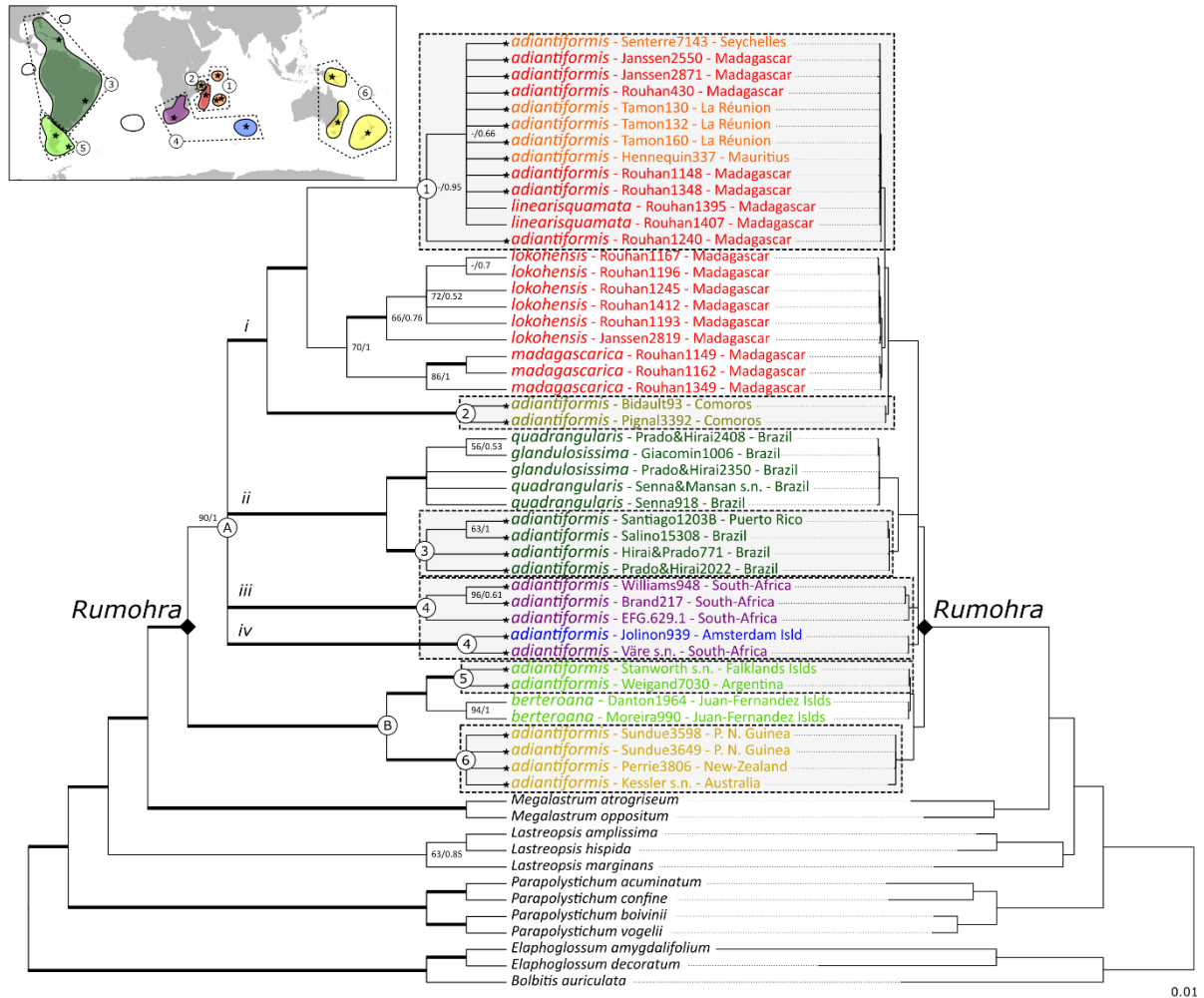


Figure 2. Majority rule consensus tree for the genus *Rumohra* estimated by Bayesian inference on the combined plastid DNA dataset (cladogram on the left, phylogram on the right), with support values from the Bayesian inference and maximum likelihood method. Unless mentioned next to the nodes, support values are posterior probabilities (PP) ≥ 0.95 and bootstraps (BS) ≥ 95 (also highlighted by thick branches). A dash (-) indicates BS < 50 or a non-retrieved node. The samples of *R. adiantiformis* are highlighted by asterisks and the geographical lineages including specimens of *R. adiantiformis* are numbered and bordered with a dashed line on the insert map. Colours represent the areas coded in the biogeographical analysis: Neotropics (dark green); southern South America (light green); southern Africa (purple); Comoros (olive); Madagascar (red); Mascarenes and Seychelles (orange); southern Indian Ocean islands (blue); and Australasia (yellow). Top left corner: distribution map of the genus *Rumohra*. Colours represent the areas coded in the biogeographical analysis, while asterisks indicate *R. adiantiformis* sample localities and dashed lines and numbers correspond to the lineages of *R. adiantiformis*. Scale bar is for branch lengths of the phylogram (substitutions/site).

Molecular dating analyses suggested that *Rumohra* diverged from its sister genus *Megalastrum* c. 46.4 Mya [95% highest posterior density (HPD): 38.0–54.6] or 30.7 Mya (95% HPD: 24.6–36.8) based on calibration points from Testo & Sundue (2016) and Schuettpelz & Pryer (2009), respectively (Figure 4). Diversification of extant *Rumohra* spp. would have started much more recently, during the Miocene, either 11.2 (95% HPD: 8.3–14.4) or 7.2 Mya (95% HPD: 5.1–9.2). Despite these differences among age estimates based on the two calibration sources, the 95% HPD overlapped for all nodes in *Rumohra* (Figure 4).

3.3. ANCESTRAL AREAS ESTIMATION

We identified DEC+j as the best fitting model under the AIC (LnL = -54.61; AIC = 115.2), followed by the DIVALIKE+j (LnL = -55.81; AIC = 117.6) and BAYAREALIKE+j (LnL = -58; AIC = 122). The DEC+j model estimated the Neotropics as the most probable ancestral area for the lineage leading to extant *Rumohra* spp. (stem node, P = 0.48; followed by Australasia with P = 0.22; Figure 3). However, Australasia and the Neotropics were inferred with nearly equal probabilities at the *Rumohra* crown node (P = 0.30 and 0.29, respectively). When performing 100 BSM realizations, we counted 37 realizations with a Neotropical origin for the *Rumohra* lineage (stem node), which showed an ancestral range at the crown node also in the Neotropics for 16 of them. An Australasian origin for *Rumohra* (stem node) was inferred for 24 realizations out of 100, all showing an ancestral range at the crown node also in Australasia.

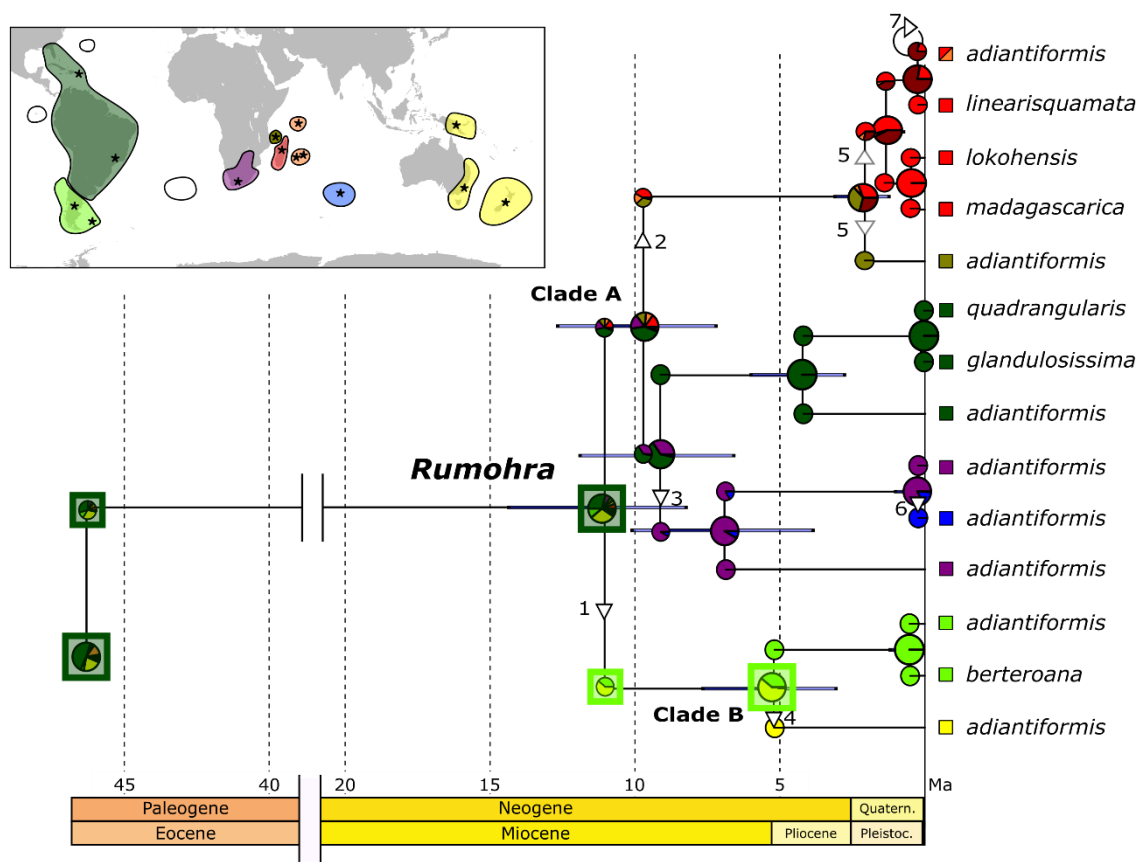


Figure 3. Chronogram resulting from the BEAST analysis for the genus *Rumohra*, and ancestral areas estimated by BioGeoBEARS (DEC+j model) and biogeographical stochastic mapping (BSM). Horizontal blue bars correspond to 95% HPD intervals of the median ages. Coloured pies represent the ancestral areas estimated at nodes, with the following colour code: Neotropics (dark green); southern South America (light green); southern Africa (purple); Comoros (olive); Madagascar (red); Mascarenes and Seychelles (orange); southern Indian Ocean islands (blue); and Australasia (yellow). The area covering Madagascar, Mascarenes and Seychelles appears in dark red. For the stem and crown nodes of the genus and for the crown node of clade B, coloured squares around pie charts depict the most likely ancestral areas as inferred through the BSM realizations considering a Neotropical origin of *Rumohra*. White arrows on branches indicate the seven long-distance dispersal (LDD) events inferred in the biogeographical history of the genus. Top left corner: distribution map of the genus *Rumohra*. Colours represent the areas coded in the biogeographical analysis and asterisks indicate *R. adiantiformis* sample localities.

Focusing on clade B, the DEC+j analysis inferred an Australasian ancestral area as the most probable (crown node; $P = 0.60$, Figure 3), whereas a southern South America area was less probable ($P = 0.39$). However, the BSM realizations with a Neotropical stem for the genus, which were the most abundant (see above), were mostly followed by inferences of the Neotropics at the crown node of *Rumohra* (16 out of 37 realizations), which in turn showed 11 out of 16 realizations (69%) with a southern South America ancestral area for clade B.

Based on the DEC+j and BSM results that both suggested a Neotropical origin for the stem lineage of the genus, *Rumohra* would have dispersed three times (Figure 5): to southern South America 11.2 Mya (LDD1); to Comoros or Madagascar 9.8 Mya (LDD2); and to southern Africa 9.4 Mya (LDD3). Later on, additional dispersal events must also have occurred: from southern South America to Australasia 5.3 Mya (LDD4); from the Comoros to Madagascar or from Madagascar to the Comoros 2.1 Mya (LDD5); from southern Africa to southern Indian Ocean islands 0.3 Mya (LDD6); and from Madagascar to Mascarenes/Seychelles (< 0.3 Mya; LDD7).

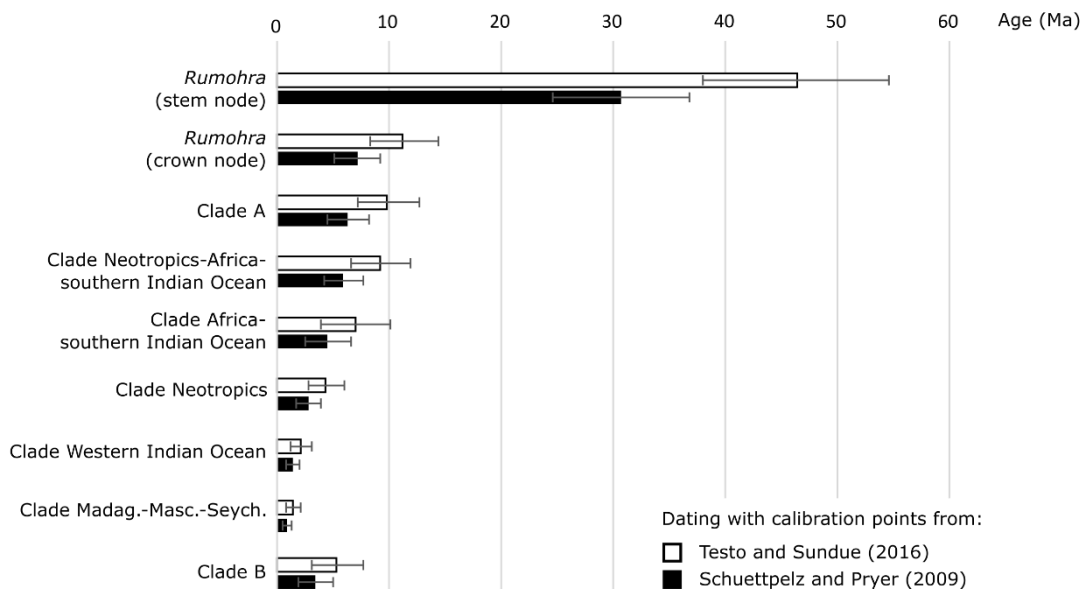


Figure 4. Comparison of median ages (in My) obtained for the main nodes of the phylogenetic tree for *Rumohra* based on calibration points taken from Testó & Sundue (2016; in white) and Schuettpelz & Pryer (2009; in black). Error bars represent the 95% HPD intervals. WIO, western Indian Ocean area.

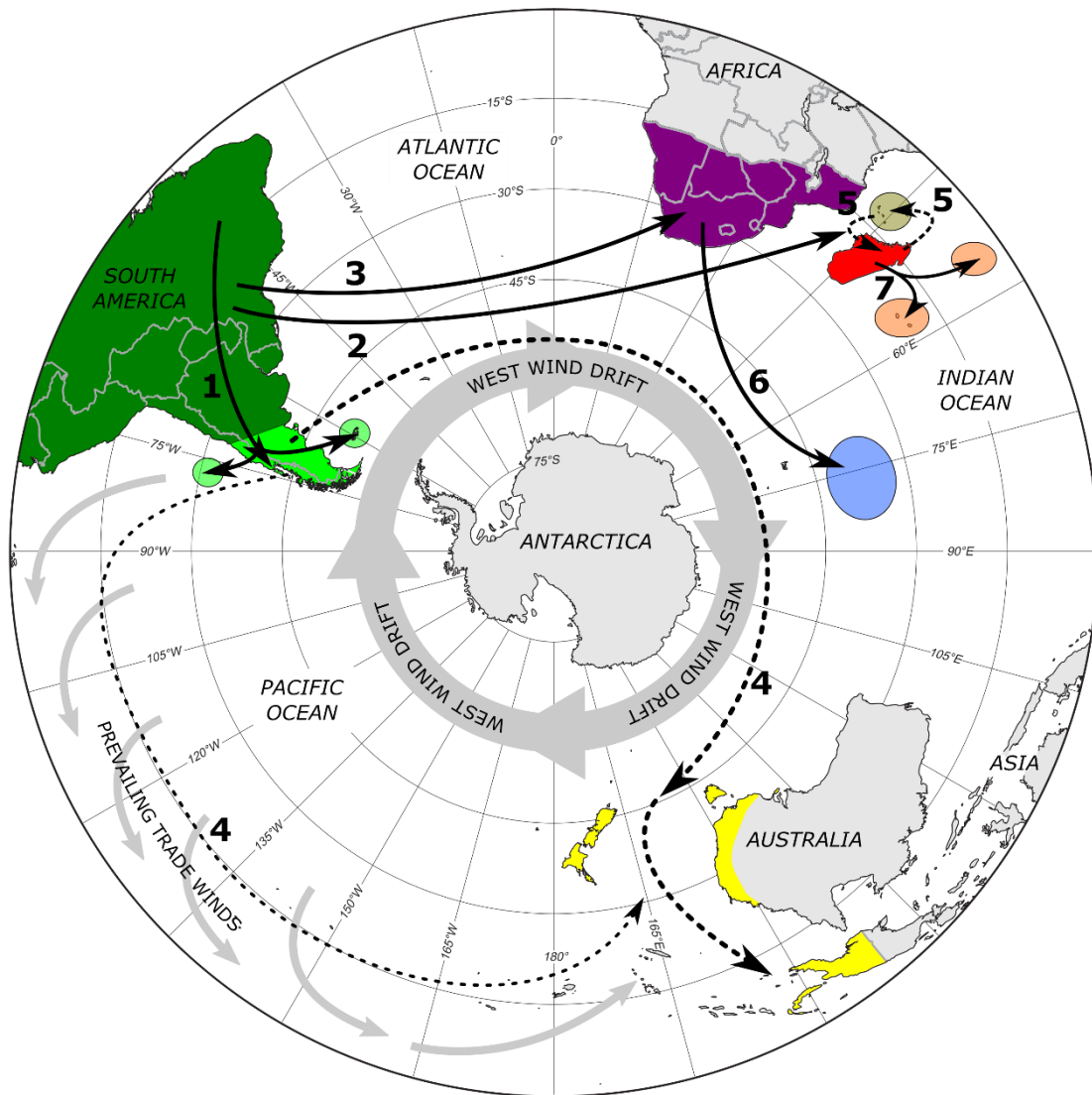


Figure 5. Circum-austral map showing the seven long-distance dispersal (LDD) events inferred in the biogeographical history of the genus *Rumohra*. Area colours and LDD numbers are the same as in Figures 2 and 3. For LDD4 and LDD5, dotted lines indicate alternative directions of dispersal.

4. DISCUSSION

Our molecular phylogenetic results supported the evolutionary distinctiveness of three (out of seven) *Rumohra* spp.: the Malagasy endemics *R. lokohensis* and *R. madagascariensis* and the Juan Fernández endemic *R. berteroi*. The monophyly of three other species (the Malagasy *R. linearisquamata* and the Neotropical *R. quadrangularis* and *R. glandulosissima*) remained uncertain because of low resolution. Importantly, we observed that the only widely distributed species, *R. adiantiformis*, was polyphyletic, with the spatial distribution of its phylogenetic lineages exhibiting a clear geographical structure. This polyphyly was in agreement with, and further strengthened, the pattern detected by Labiak *et al.* (2014), who revealed the paraphyly of *R. adiantiformis* with

respect to *R. berteriana* based on a restricted sampling (i.e. one *R. berteriana* and three *R. adiantiformis* samples from Madagascar, Brazil and Australia).

In addition, our results suggest that although *Rumohra* originated c. 46.4 Mya, its diversification started only 11.2 Mya and most extant species/line-ages evolved < 5 Mya (based on calibration points from Testo & Sundue, 2016; and assuming that all lineages of the genus are still represented today). We propose a biogeographical scenario to explain the current, mostly circum-austral distribution of the genus, involving several LDD events from a Neotropical ancestral area.

4.1. POLYPHYLY OF *R. ADIANTIFORMIS*: IMPLICATIONS FOR SYSTEMATICS AND CONSERVATION

Based on sampling that includes all known regions where *R. adiantiformis* occurs, we detected at least six (and up to eight) distinct lineages in this species (the exact number remains uncertain owing to the lack of resolution on deep nodes in the phylogeny). Despite this polyphyletic pattern, no clear morphological or anatomical characters that distinguish among these lineages of *R. adiantiformis* have been found to date (L. Bauret, pers. obs.). Based on current knowledge, lineages of *R. adiantiformis* therefore do not fit the early morphological or 'typological' species concept (Darwin, 1859). In addition, we do not know whether

R. adiantiformis lineages would maintain their molecular distinctness in sympatry, i.e. whether they are reproductively isolated and fit the concept of 'biological' species sensu Mayr (1942). Nevertheless, it is now commonly accepted that species do not have to be fully isolated reproductively, a phenomenon that sometimes leads to (at least transiently) paraphyletic species (De Queiroz, 2007; Der *et al.*, 2009; Vanderpoorten & Shaw, 2010). Furthermore, the lineages of *R. adiantiformis* appear to fit the current most commonly used 'evolutionary' species concept, according to which a species consists of a group of populations that share a common ancestor and evolve independently from other such groups (Simpson, 1951; reviewed by Mallet, 2007; De Queiroz, 2007). Therefore, given the absence of well-established morphological disparity and although this result should be checked with other molecular (in particular, nuclear) markers, *R. adiantiformis* is best understood as a complex of cryptic species (Bickford *et al.*, 2007). Cryptic species are increasingly being discovered with the more widespread use of molecular tools, especially in morphologically simple organisms, such as non-vascular plants, that lack obvious characters (see, e.g. Hedenäs & Eldenäs, 2007, for a case study in mosses). Cryptic species have also been detected in ferns (e.g. Bauret *et al.*, 2017), underlining the importance of analysing numerous samples per morphologically delimited species. In addition to further genetic investigations, it would be useful to determine whether a combination of characters (as opposed to a single one) could distinguish among lineages by adopting a morphometric approach, such as successfully applied on the sedge *Tetraria triangularis* (Boeckeler) C.B. Clarke (Britton *et al.*, 2014).

In such an evolutionary framework, our result should therefore lead to the recognition of at least five additional species. The type specimen of *R. adiantiformis* was collected in New Zealand (Nicolson & Fosberg, 2003), and the name would therefore have to be retained for the lineage observed in New Zealand, Papua New Guinea and Australia, which are the only Australasian areas where *R. adiantiformis* occurs. Other species would have to be

recognized to account for plants growing in: (1) the Neotropics (Brazil to West Indies); (2) southern South America (Argentina to the Falkland Islands); (3) Madagascar, Seychelles, Reunion and Mauritius; (4) the Comoros archipelago; and (5) southern Africa and the southern Indian Ocean islands (Amsterdam and St. Paul). The recognition of such species has an obvious consequence and importance in terms of conservation planning. Indeed, species are the fundamental units for biodiversity assessments and management and there would be an increase from one widely distributed species (the former *R. adiantiformis*) to six species with necessarily smaller distribution areas (the most extreme case being the species/lineage restricted to the Comoros). The newly recognized species would be more susceptible to habitat destruction and extinction, and would consequently deserve more careful consideration and potential protection. The observed lineages represent distinct evolutionary units and should therefore be conserved as much as possible, as the extinction of one of them would result in the extinction of its unique genetic background and associated adaptive potential (Bickford *et al.*, 2007).

From a practical point of view, there is currently no clear diagnostic morphological character to distinguish among lineages of *R. adiantiformis*. It could therefore be advocated that this will cause difficulties for identification in the field and application of conservation measures but, based on our data, the lineages appear geographically disjunct. Before the completion of further in-depth morpho-anatomical studies, species identification could thus rely on geographical provenance by field practitioners. As a consequence, if the current result was also supported by nuclear data, six species should be recognized. This will enable both a more adequate conservation management (since each species will represent an evolutionary independent entity), and further studies on the diversification and evolutionary history of the group.

Rumohra linearisquamata and *R. adiantiformis* were not separated based on our plastid dataset and additional molecular markers should be considered to assess the existence of two distinct clades. However, although these two species share many morphological characters and occur in sympatry, they are easy to distinguish based on the shape and size of their scales on the rhizomes and costae (Figure 1G, I; Rakotondrainibe, 2010b), and their recognition as two distinct entities therefore appears justified. Similarly, *R. quadrangularis* and *R. glandulosissima* (Sundue *et al.*, 2013) were not resolved in the plastid phylogenetic tree, but *R. glandulosissima* clearly differs morphologically by having densely glandular indumenta on all parts of the leaf, with two kinds of glandular hairs (Figure 1H; Sundue *et al.*, 2013).

4.2. POLYPHYLY OF *R. ADIANTIFORMIS*: WHAT EVOLUTIONARY SCENARIO(S)?

Cryptic species are often (incorrectly) assumed to be recently diverged, which would explain why they would not have had sufficient time to diverge morphologically (Bickford *et al.*, 2007). However, in *Rumohra*, some morphologically differentiated species have evolved more recently than some lineages of *R. adiantiformis* and this explanation therefore does not hold. We propose three possible (and mutually non-exclusive) scenarios to explain the polyphyly of *R. adiantiformis*: (1) a complex history of recurrent hybridization events in the genus; (2) morphological convergence among distinct *R. adiantiformis* lineages; and (3) morphological stasis, i.e. the sharing of ancestral morphological characters among lineages of *R. adiantiformis* (symplesiomorphies).

4.2.1. *Hybridization hypothesis*

Hybridization is now accepted as a major evolutionary mechanism in vascular plants (Abbott, Barton, & Good, 2016), and especially in ferns (Barrington *et al.*, 1989; Sigel, 2016). The resulting reticulate evolution often leads to the formation of species complexes, within which cryptic species have been repeatedly detected (e.g. Paris, Wagner, & Wagner, 1989; Adjie *et al.*, 2007, and references therein; Pillon *et al.*, 2009; Crowl *et al.*, 2017). Organelles are maternally inherited in ferns (Adjie *et al.*, 2007; Sigel, 2016, and references therein), and our phylogenetic tree based on plastid data therefore provides information on the history of the maternal component of the genome in *Rumohra*. Our resulting topology may suggest that (at most) one lineage of *R. adiantiformis* represents the ‘true’ *R. adiantiformis* stem line, whereas others (or possibly all of them) would have resulted from recurrent hybridization events with distinct species as the maternal parent. Each hybrid lineage would thus display either the plastid haplotype of the (possibly now extinct) maternal ancestor or a haplotype that is more or less closely related to the haplotype of another *Rumohra* sp., reflecting ancient hybridization events and subsequent genetic differentiation from the maternal parent. In all cases, genetic relatedness between lineages of *R. adiantiformis* and other *Rumohra* spp. is correlated with geographical proximity, making the hybridization hypothesis plausible. Such a hypothesis of multiple hybrid origins was suggested for other species, e.g. in *Polypodium* L. and *Pteridium caudatum* (L.) Maxon, to explain the placement of some species accessions in two parts of phylogenetic trees based on plastid data (Der *et al.*, 2009; Sigel, Windham, & Pryer, 2014). Nevertheless, two observations make this scenario unlikely. First, although all *Rumohra* spp. were represented by several samples, no other species displays the signature of hybridization events. Second, putative hybrids would always display the morphology of *R. adiantiformis* and *R. adiantiformis* would always have acted as the paternal parent; although highly asymmetric patterns of hybrid formation have been observed, for example in the *Asplenium nidus* L. complex (Yatabe *et al.*, 2009) and in *Dryopteris* Adans. (Xiang *et al.*, 2000; Testo, Watkins, & Barrington, 2015), we consider this unlikely. A nuclear-based phylogenetic analysis would bring crucial information to confirm or reject the possible history of reticulate evolution in *Rumohra*.

4.2.2. *Morphological convergence hypothesis*

Another possible explanation for the striking polyphyly of *R. adiantiformis* is morphological convergence among lineages, i.e. the parallel or convergent evolution of a suite of derived characters. Homoplasy of morphological character evolution has previously been shown in ferns, at a broad scale in eupolypods II (Sundue & Rothfels, 2014) and in smaller groups such as the grammitids (Ranker *et al.*, 2004; Sundue *et al.*, 2010a; Bauret *et al.*, 2017), bolbitidoids (Moran, Labiak, & Sundue, 2010), cheilanthoids (Rothfels *et al.*, 2008; Grusz & Windham, 2013) and in other spore-bearing vascular plants (Lycopodiaceae, Field *et al.*, 2016; Selaginellaceae, Weststrand & Korall, 2016). Such observed homoplasy has led to numerous taxonomic changes since the advent of molecular methods in systematics. It can result from morphological convergence, especially when species that are not closely related occur in similar but extreme environments. A character conferring a selective advantage can indeed evolve repeatedly (see, e.g. Patterson & Givnish, 2002). However, *R. adiantiformis* occurs in disparate environments (e.g. in Brazil, from evergreen rainforest to sea-side vegetation where it

grows in the full sun among the dunes; Sundue *et al.*, 2013) and can grow terrestrially, as a climber or an epiphyte. Therefore, morphological convergence towards traits adapted to a particular environment appears unlikely.

4.2.3. Morphological stasis hypothesis

The third scenario that we hypothesize to explain the polyphyly of *R. adiantiformis* is morphological stasis among lineages, i.e. shared ancestral characters (symplesiomorphies). *Rumohra adiantiformis* displays broad ecological amplitude and high morphological diversity (Sundue *et al.*, 2013). As a consequence, our inability to identify diagnostic morphological characters among lineages may be due to their morphological diversity, which does not lead to the emergence of particular morphotype(s) in any geographical region or habitat, rather than to the absence of variation. We suggest that from a common ancestor, which was morphologically close to extant *R. adiantiformis* and exhibited extensive morphological diversity, the six other species differentiated locally and evolved towards contrasting morphologies, whereas the ancestral morphology was retained in lineages dispersed throughout the tropics. Such a mechanism was suggested by Darwin (1859), who postulated that wide-spread and highly variable species were most likely to give rise to new species compared to more narrowly distributed, less variable species. It would also be congruent with the fact that *R. adiantiformis* is present in all regions where other species have evolved, and would correspond to the budding speciation mode proposed by Mayr (1954) and later developed in light of molecular phylogenetics (e.g. Vanderpoorten & Shaw, 2010). In parallel to this occasional pattern of morphological divergence, lineages of *R. adiantiformis* most probably differentiated genetically as a result of low gene exchange due to spatial separation and the action of genetic drift. Despite the high dispersal capacities of ferns, we indeed inferred only a single successful colonization to each coded geographical region.

Nonetheless, although we tend to favour this third scenario to explain the polyphyly of *R. adiantiformis*, morphological convergence and morphological stasis might be involved, depending on the character considered, and the impact of hybridization events also cannot be excluded.

4.3. HISTORICAL BIOGEOGRAPHY OF *RUMOHRA*

4.3.1. A genus of late Palaeogene, Neotropical origin

All age estimates along the chronogram for *Rumohra* differed based on whether the calibration points were taken from older dates obtained on the large-scale fern phylogenetic analysis of Testo & Sundue (2016), or younger dates obtained by Schuettpelz & Pryer (2009). Thus, our results suggested an origin of *Rumohra* (stem node) between c. 46.4 and 30.7 Mya. The latter age is similar to the age of 28.8 Mya found by Labiak *et al.* (2014) in their broad analysis across the lastreopsid ferns, which was also calibrated based on the analysis of Schuettpelz & Pryer (2009). Their slightly lower age estimate could result from their more restricted sampling, which included only two *Rumohra* spp. compared to our exhaustive sampling of seven species (e.g. Linder, Hardy, & Rutschmann, 2005). Despite the differences observed depending on the calibration points, all age estimates were consistent with the biogeographical history proposed here

and we only report estimates based on Testo & Sundue (2016) in the text below, for the sake of clarity.

Based on our results, the austral genus *Rumohra* started to diversify during the late Miocene. Among lastreopsid genera, it was thus the last to diversify, in agreement with Labiak *et al.* (2014) even when considering the relatively older age estimates in our study. At that time, the southern continents resulting from the breakup of Gondwana were well separated from each other (New Zealand, Australia, South America and Antarctica became isolated from one another during the late Cretaceous and Tertiary; Sanmartín & Ronquist, 2004). Therefore, contrary to the early range expansion and subsequent divergence of Lastreopsids, which could have been influenced by an Antarctic land bridge that persisted between Australia and southern South America until c. 40 Mya (Labiak *et al.*, 2014), the diversification history of *Rumohra* is too recent, even when taking 95% HPDs into account, to explain its southern circumaustral distribution by geological vicariance events. Thus, the geographical disjunctions in *Rumohra* must rather be explained by transoceanic LDD events.

The ancestral area for the crown node of *Rumohra* was uncertain based on DEC+j, given the equal probabilities assigned to the Neotropics and Australasia. However, *Rumohra* most probably diverged from its sister genus *Megalastrum* in the Neotropics (based on both DEC+j and stochastic mapping at the stem node of *Rumohra*) and, considering this Neotropical origin, stochastic mapping also recovered the Neotropics as the centre of origin of the genus (crown node). Therefore, we favour a scenario in which the diversification started in the Neotropics and resulted in the two main clades of the genus.

4.3.2. A late Miocene diversification, mostly shaped by three major LDD events

Clade A diversified in situ, in the Neotropics, giving rise to a clade of three species (*R. quadrangularis*, *R. glandulosissima* and *R. adiantiformis*), but it also dispersed twice independently to southern Africa and the western Indian Ocean (Madagascar or Comoros), respectively. These two LDD events (LDD2 and LDD3; Figure 5), which occurred c. 9.8 and 9.4 Mya, are best interpreted as trans-Atlantic LDD events of spores, since the involved lineages clearly postdate the separation of Africa and Madagascar from all other southern continents (especially Antarctica and South America) c. 165–105 Mya (McLoughlin, 2001). Spores were probably transported eastward via the West Wind Drift, a wind current initiated around Antarctica after the opening of the Drake Passage between South America and Antarctica in the Oligocene (Sanmartín, Wanntorp, & Winkworth, 2007).

From the Neotropical ancestor of the genus, clade B probably evolved by an initial dispersal event to southern South America reaching as far as the Falkland Islands (LDD1; Figure 5), as supported by stochastic mapping. Then, there was a disjunction between southern South America (*R. adiantiformis* and *R. berteroana*) and Australasia (*R. adiantiformis*). Similar disjunctions have been observed in many other plant groups and three potential dispersal routes were proposed to explain them: a direct transoceanic dispersal; dispersal through intermediate stepping-stone(s); or an out-of-Antarctica scenario involving an origin in Antarctica followed by a bidirectional dispersal (Winkworth *et al.*, 2015). In *Rumohra*, the disjunction was established <5.3 Mya, i.e.

after complete Antarctic glaciation (which dates back to 14–4 Mya; Zachos *et al.*, 2001; Siegert *et al.*, 2008), forcing the South American ancestor to a direct transoceanic dispersal route to Australasia. If considering the shortest route between South America and Australasia, which is across the Pacific Ocean, this LDD event would have occurred westward in an opposite direction to the West Wind Drift. As analysed by Sanmartín *et al.* (2007), such an apparent westward direction has in fact frequently been inferred in various plant groups, but it does not exclude the influence of the West Wind Drift: in particular, spore dispersal patterns are indeed more influenced by wind connectivity than by geographical distance in the circum-austral area (Muñoz *et al.*, 2004). Therefore, spores most probably travelled eastward through the Atlantic and Indian Oceans (LDD4; Figure 5), under the action of the West Wind Drift, instead of the shortest westward route across the Pacific Ocean. Alternatively, prevailing trade winds blowing at lower latitudes from the western coast of South America to Australasia (Gillespie *et al.*, 2012) or birds through ectozoochory (Lewis *et al.*, 2014) may be involved.

The clade B lineage that remained in southern South America includes *R. berteriana*, which is endemic to the Juan Fernández archipelago. This species formed recently (c. 590,000 years ago) and probably resulted from anagenetic speciation after short range dispersal of the southern South American lineage of *R. adiantiformis*. The timing of this speciation event is congruent with the date of emergence of these islands < 5 Mya (Stuessy *et al.*, 1984). Anagenesis is an important model of speciation on oceanic archipelagos, accounting for 36% of the endemic plant species diversity in the Juan Fernández archipelago (Stuessy *et al.*, 2006), and it has been shown to be even more important in spore-producing plants than in seed plants in several other archipelagos (Patiño *et al.*, 2014).

4.4. FOCUS ON THE MALAGASY SPECIES HOTSPOT

The two transatlantic eastward LDD events (LDD2 and LDD3), which occurred c. 10 Mya, resulted in a clade comprising all Malagasy, Comorian and other western Indian Ocean lineages on the one hand, and in a clade consisting of a continental African (and southern Indian Ocean) lineage of *R. adiantiformis* on the other hand. Therefore, African and western Indian Ocean lineages of *Rumohra* are not closely related despite their geographical proximity. This is in contrast to the numerous biotic affinities found in other groups of plants and animals (Agnarsson & Kuntner, 2012; Buerki *et al.*, 2013) and with the African origin reported for many Malagasy angiosperms (e.g. Bartish *et al.*, 2011; Calvino, Teruel, & Downie, 2015; Janssens *et al.*, 2016; Bacon *et al.*, 2016). As opposed to the apparent absence of dispersal between continental Africa and the western Indian Ocean area, we inferred at least one LDD event between Madagascar and the Comoros (LDD5; Figure 5; the direction remains unknown because of the uncertain ancestral area estimation), one LDD event from Madagascar to Mascarenes/ Seychelles (LDD7) and one LDD event from continental Africa to the southern Indian Ocean islands (LDD6; both LDD6 and LDD7 events were inferred within lineages of *R. adiantiformis*).

The LDD event inferred from the Neotropics to the Malagasy region (LDD2) reinforces the importance of the dispersal events between these two regions for the origin of the fern flora of Madagascar, as already shown in other fern groups such as *Elaphoglossum* (≥ 13 events; Rouhan *et al.*, 2004), or the grammitid ferns (five or more events; Bauret *et al.*,

2017). However, in *Rumohra*, the Malagasy hotspot (comprising three endemic species and at least one *R. adiantiformis* lineage) resulted from a single successful long-distance colonization with subsequent diversification on the island, as otherwise only found in *Lomariopsis* Fée (Rouhan *et al.*, 2007a), *Ctenitis* (C.Chr.) C.ChR. (Hennequin *et al.*, 2017) and some grammitid fern genera (Sundue *et al.*, 2014; Bauret *et al.*, 2017). The mechanisms involved in the diversification of *Rumohra* in Madagascar are unknown. Nowadays, most extant species do not display any obvious difference in their ecological preferences and all occur in rainforests. *Rumohra madagascariensis*, however, provides a probable case of ecological speciation, since it is only observed as an epiphyte on species of *Pandanus Parkinson* (G. Rouhan, pers. obs.; Rakotondrainibe, 2010b). Species are commonly found in sympatry, without intermediate morphological forms that would indicate the occurrence of interspecific crosses. Whether speciation took place in sympatry or, more likely, whether sympatric sites result from secondary contact between previously, allopatrically derived species remains to be determined.

ACKNOWLEDGMENTS

We thank P. Danton, M. Kessler, N. Rendell, E. Santiago Valentin, A. Stanworth, J.M. Tamon, and H. Väre for providing leaf material. We thank the Editor and two anonymous reviewers for helpful comments on an earlier version of the manuscript. 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 (especially Y. Ibrahim). 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), and the network 'Bibliothèque du Vivant' funded by the CNRS, the MNHN, the INRA and the CEA (Centre National de Séquençage). All molecular work was performed at the BoEM laboratory of the MNHN.

Chapter III - Biogeographical history of Blechnoideae in Madagascar: connection with tropical regions of the Southern Hemisphere and two in situ diversifications highlighted

1. INTRODUCTION

The subcosmopolitan family Blechnaceae comprises around 270 species and three subfamilies: Blechnoideae, Stenochlaenoideae and Woodwardioideae. Most of the species diversity is found in Blechnoideae, with 18 genera and about 240 species (PPGI, 2016). The family is recognizable in the field by linear sori that are parallel and adjacent to midveins, introrse indusia, and vascular bundles forming a ring in the petiole cross section. All the species are terrestrial, except the New Caledonian *Oceaniopteris francii* (Rosenst.) Gasper & Salino that is aquatic. In Madagascar, all the Blechnoideae species have entire to pinnate fronds (Figure 1; except *Lomaridium bifforme* that can be pinnate-pinnatifid), and dimorphic fronds with the fertile fronds being more or less reduced to the sori (Figure 1C, D, F, G).

De Gasper *et al.* (2016b) proposed the most recent classification of Blechnaceae, in which the large genus *Blechnum* was split into 16 genera, all classified in Blechnoideae together with *Sadleria* Kaulf. and *Brainea* J.Sm. This revised classification was based both on morphology and molecular data provided by the most comprehensive phylogeny so far, including a sampling of half of the world's species diversity, mostly from the two diversity centres, the Neotropics and Australasia (de Gasper *et al.*, 2016a). The three subfamilies were retrieved monophyletic, with Blechnoideae sister group to Stenochlaenoideae, and all the 24 genera were also monophyletic as the large non-monophyletic *Blechnum* s.l. was circumscribed in smaller monophyletic genera. In Madagascar, the most recent taxonomic treatment based on morphology estimated 15 *Blechnum* s.l. species (Rakotondrainibe *et al.*, 2013). Applying the latest classification of Blechnoideae, the 15 Malagasy species belong to five genera: *Austroblechnum* Gasper & V.A.O.Dittrich, *Blechnum* L., *Lomaridium* C.Presl, *Lomariocycas* (J.Sm.) Gasper & A.R. Sm. and *Parablechnum* C.Presl. (Table 1). Only five out of the 15 Malagasy species were included in the study of de Gasper *et al.* (2016a): *Lomaridium bifforme*, *Blechnum punctulatum*, *Blechnum australe*, *Lomariocycas tabularis* and *Parablechnum marginatum*, so the genus assignments of the other ten species remain to be tested by molecular data.

The origin of Blechnaceae was dated to Late Cretaceous by two large-scale, fossil-based studies on leptosporangiate ferns (Schuettpelz & Pryer, 2009; Testo & Sundue, 2016), but the biogeographical history of the family remains unknown. The pantropical distribution of Blechnoideae raises the question of how such a large distribution could be explained, especially since some genera are restricted to small areas (like the Hawaiian endemic *Sadleria*) whereas others are widely distributed (like *Parablechnum* in the tropics). The subfamily is particularly diversified in the Neotropics and Australasia (de Gasper *et al.*, 2016a,b), but it is also distributed in Africa and Western Indian Ocean (WIO), southern

South America, Tristan da Cunha and in the French Southern Lands. The overall distribution is therefore almost entirely in southern regions, and suggests important biogeographical connections between the Neotropics and Australasia in the history of Blechnoideae. Floristic affinities were retrieved between these two regions in numerous genera (Moreira-Muñoz, 2007), and phylogenetic studies have unravelled the history of Neotropical-Australasian connections, attributing some to long-distance dispersal and others to trans-Antarctic routes that persisted until the Eocene (Sanmartín & Ronquist, 2004; Chacón *et al.*, 2012; Winkworth *et al.*, 2015). Antarctica was indeed ice-free and probably covered by near-tropical forests until Early Eocene (Pross *et al.*, 2012). The trans-Antarctica connection stopped after the opening of South Tasman Sea and Drake passage about 30-35 Ma, leading to the isolation of Antarctica and its cooling, as well as to the establishment of circumaustral currents (Sanmartín *et al.*, 2007; Yang, Galbraith, & Palter, 2014).

The classification of Malagasy species in five monophyletic genera implies that the Malagasy diversity have originated from multiple distinct biogeographical events (at least four events, as *Blechnum* and *Austroblechnum* were retrieved sister by de Gasper *et al.*, 2016b). The family being dated to Late Cretaceous, hypotheses of vicariance linked to the Gondwanan break-up are unlikely because Madagascar was already isolated at this time (McLoughlin, 2001). Therefore, the biogeographical history of Malagasy lineages of Blechnoideae is likely to be dominated by dispersal. The species composition of Malagasy Blechnoideae shows affinities with the nearest continent Africa, with four species distributed in both areas (Table 1). Nevertheless, six Malagasy species belong to two mostly Neotropical genera (*Lomariocycas* and *Blechnum*) and floristic affinities were highlighted with Neotropical *Lomariocycas* and *Lomaridium* species (Moran & Smith, 2001).

This chapter aims to study the systematics and biogeographical history of Malagasy Blechnoideae, and specifically: 1) to test the monophyly of Blechnoideae genera distributed in Madagascar and to confirm the classification of de Gasper *et al.* (2016a) with additional specimens; 2) to test the monophyly of Malagasy species; 3) to estimate the age of Malagasy lineages, and 4) to infer the biogeographical origins of the Malagasy lineages in the global framework of the Blechnoideae biogeographical history.

Species	Distribution	Notes
<i>Austroblechnum bakeri</i> (C.Chr.) Gasper & V.A.O.Dittrich	WIO, Africa	Combination in <i>Cranfillia</i> ? (see Results)
<i>Austroblechnum integrifrons</i> (Bonap. ex Rakotondr.) Gasper & V.A.O.Dittrich	Endemic to Madagascar	Combination in <i>Lomaridium</i> ? (see Results)
<i>Blechnum australe</i> L.	WIO, Neotropics, Tristan da Cunha archipelago	
<i>Blechnum punctulatum</i> Sw.	WIO, Africa	
<i>Lomaria inflexa</i> Kunze	Endemic to Africa	
<i>Lomaridium attenuatum</i> (Sw.) Gasper & V.A.O.Dittrich	WIO (excluding Madagascar), Africa	
<i>Lomaridium biforme</i> (Baker) Gasper & V.A.O.Dittrich	Endemic to Madagascar	
<i>Lomaridium bonapartei</i> (Rakotondr.) Gasper & V.A.O.Dittrich*	Endemic to Madagascar	
<i>Lomaridium simillimum</i> (Baker) Gasper & V.A.O.Dittrich	WIO	Combination confirmed by this study
<i>Lomaridium xiphophyllum</i> (Baker) Gasper & V.A.O.Dittrich	Endemic to Madagascar	Combination confirmed by this study
<i>Lomariocycas decrescens</i> (Rakotondr.) Gasper & A.R.Sm.	Endemic to Madagascar	
<i>Lomariocycas longepetiolata</i> (Tardieu) Gasper & A.R.Sm.	Endemic to Madagascar	Combination confirmed by this study
<i>Lomariocycas longipinna</i> (Rakotondr.) Gasper & A.R.Sm.	Endemic to Madagascar	Combination confirmed by this study
<i>Lomariocycas madagascariensis</i> (Tardieu) Gasper & A.R.Sm.	Endemic to Madagascar	Combination confirmed by this study
<i>Lomariocycas tabularis</i> (Thunb.) Gasper & A.R.Sm.	WIO, Africa, Neotropics	
<i>Parablechnum capense</i> (Burm.f.) Gasper & Salino	Africa (records from Asia or the Pacific region refer most probably to <i>P. vestitum</i>)	
<i>Parablechnum marginatum</i> (Kuhn) Gasper & Salino	WIO	Combination of the Malagasy variety confirmed by this study

Table 1. Blechnoideae species occurring in tropical Africa and Western Indian Ocean (including Madagascar), distribution based on Roux (2009) and Rakotondrainibe *et al.* (2013) and taxonomic notes on the basis of my results. Sampled species are in bold.

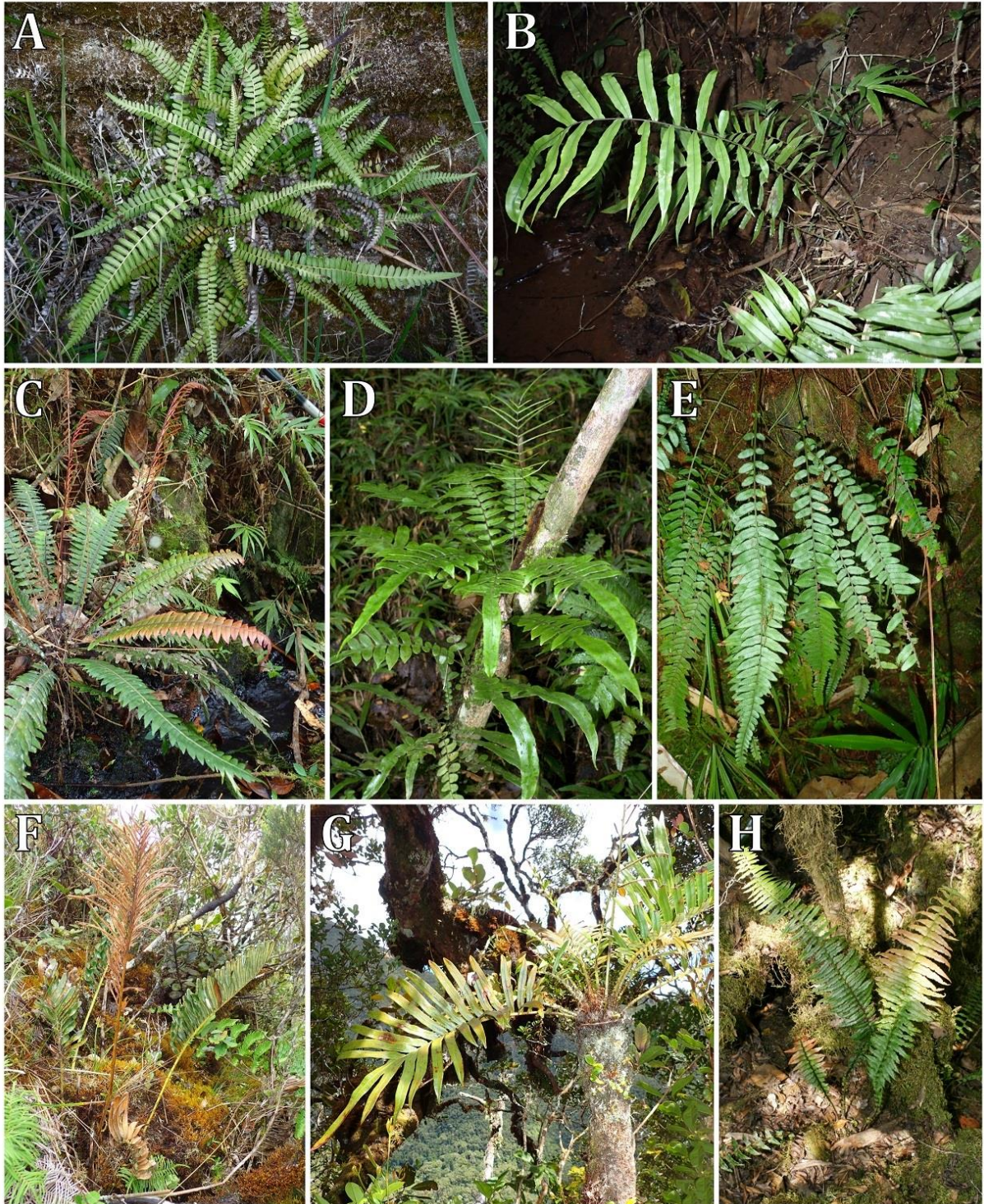


Figure 1. Diversity of Blechnoideae in Madagascar. (A) *Blechnum australe*, Rouhan *et al.* 1416; (B) *Lomaridium xiphophyllum*, no voucher; (C) *Austroblechnum bakeri*, Rouhan *et al.* 1625; (D) *Lomaridium simillimum*, Rouhan *et al.* 1370; (E) *Lomaridium attenuatum*, no voucher; (F) *Lomariocycas longepetiolata*, Bauret *et al.* 101; (G) *Lomariocycas longipinna*, Rouhan *et al.* 1585; (H) *Blechnum punctulatum*, Rouhan *et al.* 1405. Photos: G. Rouhan (A, D, H), L. Bauret (B, C, E-G).

2. MATERIAL AND METHODS

2.1. SAMPLING

The sequence matrix from de Gasper *et al.* (2016a) was combined with my dataset, which was mostly focused on Madagascar and included 49 specimens representing 16 species. Ten species were never sampled so far, among which eight Malagasy taxa (Appendix 3). The whole dataset included 13 species from Africa and WIO (76% of the diversity for this region, and 87% of the Malagasy diversity) and about 62% of the Blechnoideae diversity was included. Eighteen species from the two other subfamilies and four from closely related families were added as outgroups. The whole dataset included 164 species and 202 specimens (Appendix 3).

2.2. DNA EXTRACTION, AMPLIFICATION AND SEQUENCING

The protocols for DNA extraction, amplification and sequencing followed those of Chapter II. Four plastid DNA regions were amplified by PCR and sequenced: the two genes *rbcl* and *rps4*, and two non-coding regions *rps4-trnS* and the *trnL* intron plus the *trnL-trnF* intergenic spacer (*trnL/trnL-trnF*). For *rps4* and *rps4-trnS* that were amplified and sequenced together, I used the primers *rps4* 5'-ATG TCS CGT TAY CGA GGA CCT-3' (Small *et al.*, 2005) and *trnS-r* 5'-TAC CGA GGG TTC GAA TC-3' (Smith & Cranfill, 2002) and the following thermal cycling conditions: 5 min 94°C / 5 × (30 s 94°C / 30 s 65-55°C / 1 min 30 s 72°C) / 35 × (30 s 94°C / 30 s 55°C / 1 min 30 s 72°C) / 10 min 72°C. For the first five cycles, 65-55°C stands for a temperature that was increased by 2.5° at each cycle.

2.3. PHYLOGENETIC ANALYSES

The analyses followed the protocol detailed in Chapter II about alignments, matrix concatenation and phylogenetic approaches: Maximum Likelihood (ML) and Bayesian Inference (BI). Four partitions were defined by DNA regions: *rbcl*, *rps4*, *rps4-trnS* and *trnL/trnL-trnF*. The parameters were set as in Chapter II, except for the MrBayes analysis; the runs comprised five million generations and were sampled every 500 generations to obtain 10,000 sampled trees, with a temperature of chains set to 0.05.

2.4. AGE ESTIMATES

A reduced dataset of 170 samples was used to perform the dating analysis, representing all 164 species, outgroups included. One sample per species was kept, except in case of statistically supported paraphyly and polyphyly. I followed the same protocol as in Chapter II, using BEAST 2.4.2 (Bouckaert *et al.*, 2014) on the CIPRES science gateway (Miller *et al.*, 2010) and with the specific calibration points mentioned below.

I used a primary calibration point provided by a fossil assignable to *Onoclea sensibilis* (Onocleaceae; Rothwell & Stockey, 1991), previously used for dating analyses by Schuettpelz & Pryer (2009) and Testo & Sundue (2016) and dated to the Paleocene. Therefore, the stem age of this species was calibrated using a uniform prior with a minimum age of 56 Ma. In addition, three secondary calibration points were modeled by normal prior distributions with means from Testo & Sundue (2016) and standard deviations (S.D.) of 10% of the mean values: tree height (85.9 Ma; S.D. = 8.6), Blechnoideae stem node (59.5 Ma; S.D. = 6.0) and Blechnoideae crown node (55.8 Ma; S.D. = 5.6). The more comprehensive sampling (both in terms of markers and taxonomic diversity) led

me to favor their results, as calibration sources, relatively to those of Schuettpeltz & Pryer (2007).

An uncorrelated lognormal relaxed clock was used, with a Birth-Death tree prior and a random starting tree. For each analysis, two independent runs of 100 million generations with random seed values were conducted, sampled every 10,000 generations to obtain 10,000 sampled trees.

2.5. BIOGEOGRAPHICAL ANALYSES

The BioGeoBEARS R package was used to estimate the ancestral areas (Matzke, 2013), as in Chapter II. Six biogeographical models were considered: DEC, DIVALIKE, BAYAREALIKE, and the same models implementing the additional parameter *j*, which stands for the founder event speciation. The Akaike Information Criterion (AIC) was then used to select the best model among the six.

I coded species distributions by combinations of eight areas: (A) Madagascar, (B) Mascarenes-Comoros-Seychelles, (C) Africa, (D) Neotropics, (E) Asia-Southeast Asia, (F) Australasia-Oceania (latter referred as Australasia), (G) North America-Europe, and (H) a circumaustral region including the southern South America, Tristan da Cunha archipelago and French Southern Lands (see map of Figure 3). The entire distribution of species was coded, i.e. not only the sampling localities, based on herbarium collections, floras and the GBIF database when necessary. Two species were however coded differently: for *Parablechnum montbrisonis*, the coding followed the distribution of its two varieties that were found paraphyletic; and for *Lomaridium attenuatum*, a clade nesting Mascarenes samples was inferred and coded in the area Mascarenes-Comoros-Seychelles (see Results). The maximum range size was set to five areas, as it is the maximum number of areas coded for a terminal taxon.

3. RESULTS

3.1. PHYLOGENETIC ANALYSES

The final matrix included 202 specimens and 3493 characters (Table 2). The topologies inferred by the MrBayes and raxML analyses showed that Blechnaceae and the three subfamilies were monophyletic (Figure 2A). Similar intergeneric relationships were found between the two analyses, with an exception for *Diploblechnum* that was retrieved sister group to *Parablechnum* in raxML results (not shown). The Blechnoideae genera were monophyletic, except: i) *Oceaniopteris* that was found paraphyletic with respect to *Doodia* (Figure 2C; but with low support values, BS<50, PP=0.77); ii) *Parablechnum*, as *P. chauliodontum* was nested within *Lomaridium* (Figure 2A); and iii) *Austroblechnum* retrieved as three non-closely related lineages: nineteen out of the 21 sampled species of *Austroblechnum* formed a clade (Figure 2B), but *A. integrifrons* was found in *Lomaridium* (Figure 2A) and *A. bakeri* was nested in *Cranfillia* (Figure 2B).

DNA region	Number of sequences (on the total number of samples)	Aligned length (base pairs)	Percent of variable characters	Percent of informative characters
<i>rbcL</i>	194 (96%)	1252	29.6	23.5
<i>rps4</i>	177 (88%)	570	45.4	31.4
<i>rps4-trnS</i>	175 (87%)	542	62.0	48.3
<i>trnL/trnL-trnF</i>	176 (87%)	1129	55.4	42.6
Combined dataset	202 (100%)	3493	45.5	34.8

Table 2. Matrix statistics.

At species level, the results showed that the Blechnoideae species from Madagascar were nested in five genera: *Blechnum*, *Cranfillia*, *Lomaridium*, *Lomariocycas* and *Parablechnum*, but none of them belong to *Austroblechnum* s.s. (*Austroblechnum andinum*-*Austroblechnum doodioides* clade; Figure 2B). The monophyly of several Malagasy species remained uncertain despite the sampling of multiple individuals, due to a lack of resolution. It was the case i) in *Lomaridium* (Figure 2A) for *L. biforme* and *L. xiphophyllum*; and ii) in *Lomariocycas* (Figure 2C) for *L. madagascariensis*. The paraphyly of several species was suggested with medium to high statistical supports: *Lomaridium attenuatum*, *Lomaridium simillimum* (Figure 2A), the widespread *Blechnum australe* (Figure 2B), *Lomariocycas longipinna* and *Parablechnum marginatum* (Figure 2C). *Lomariocycas tabularis* was also retrieved paraphyletic but the position of the specimen 'Hennequin R105' was barely supported (BS=63, PP=0.55; Figure 2C). Two species and a variety were found monophyletic: *Austroblechnum bakeri*, *Blechnum punctulatum* (BS=100; PP=1; Figure 2B) and *Parablechnum marginatum* var. *humbertii* (BS=100; PP=1; Figure 2C). The monophyly of *Parablechnum marginatum* var. *marginatum* was not tested, due to the sampling of one specimen only, which was found sister group to Neotropical *Parablechnum* species.

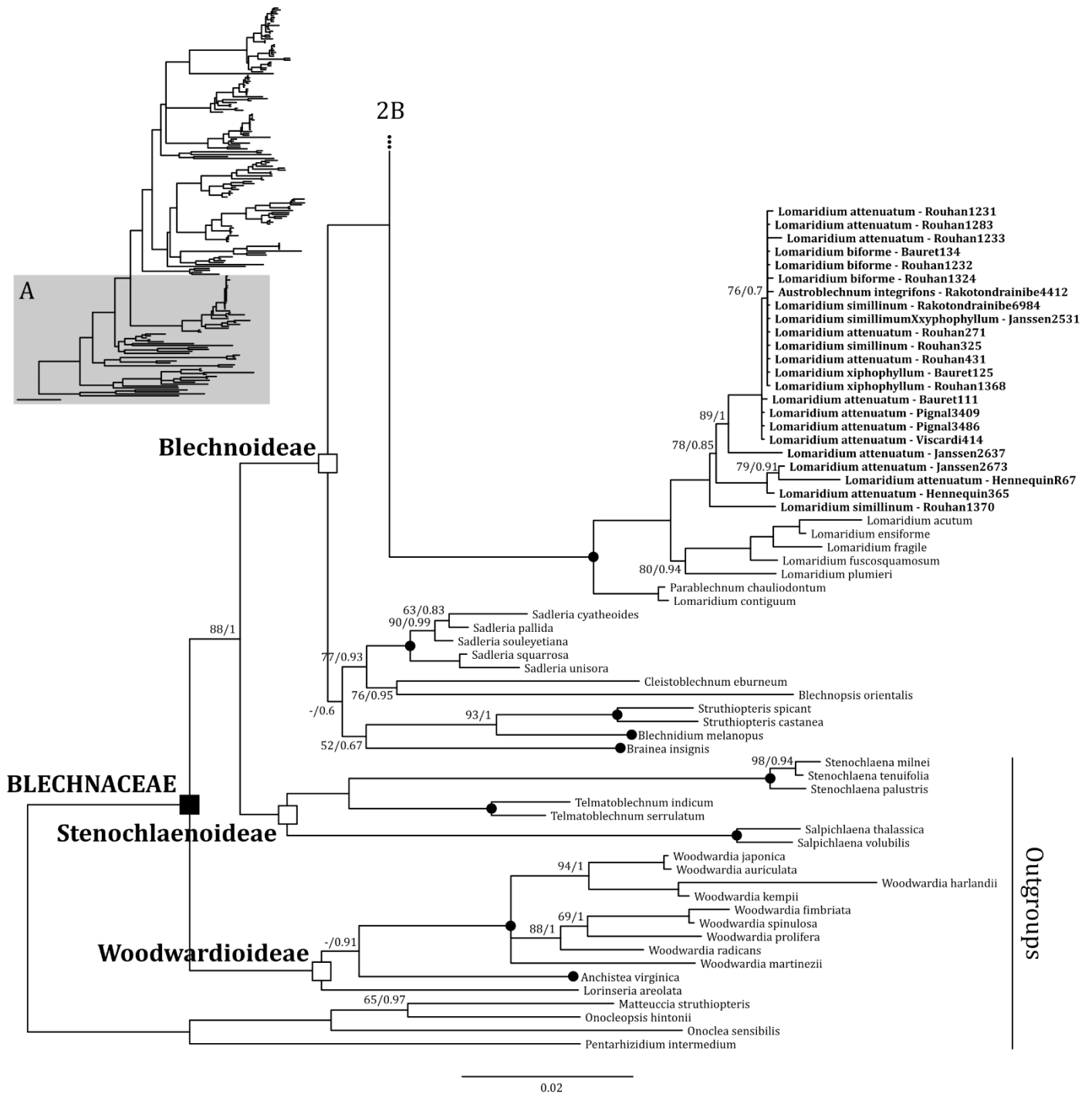
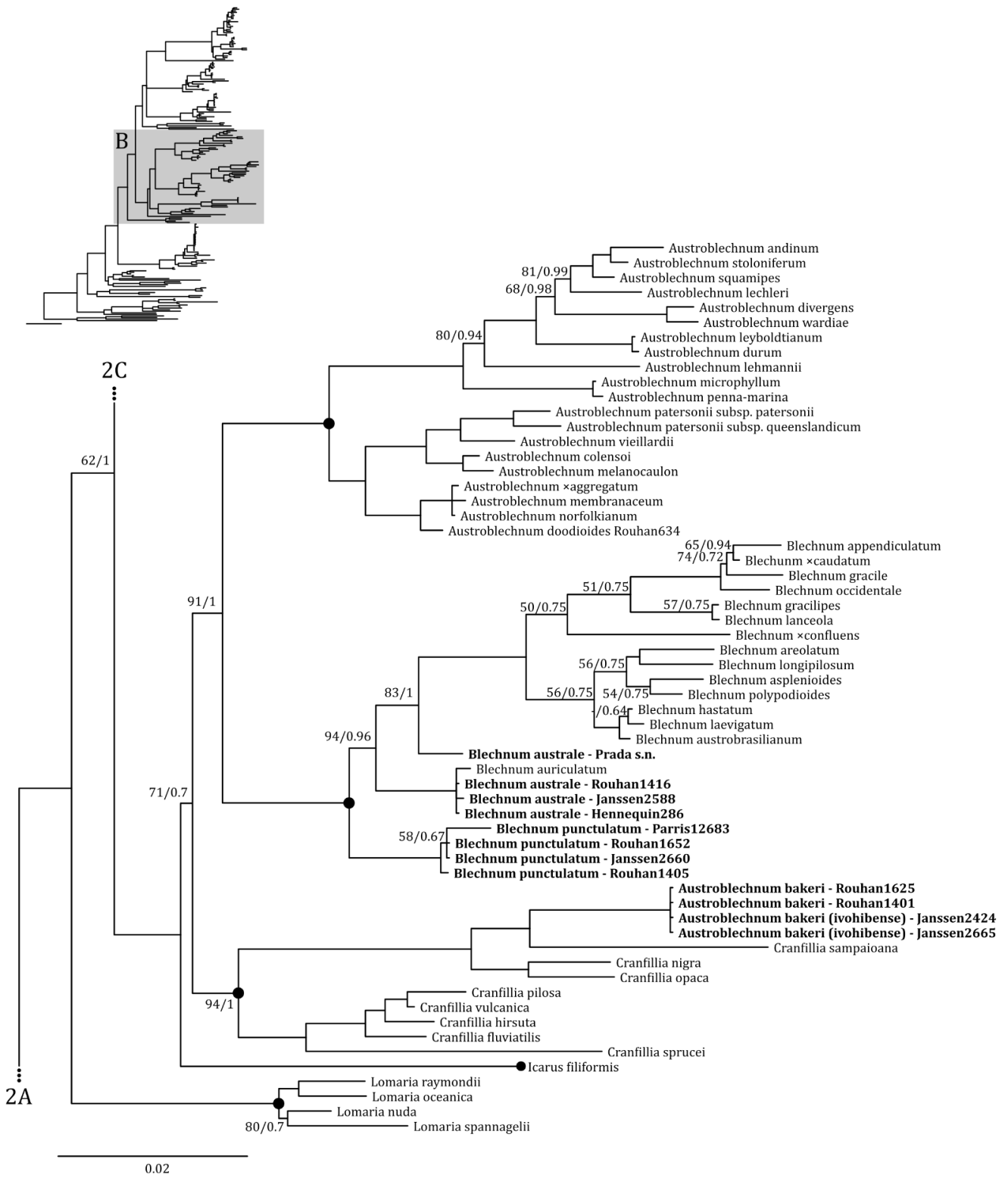
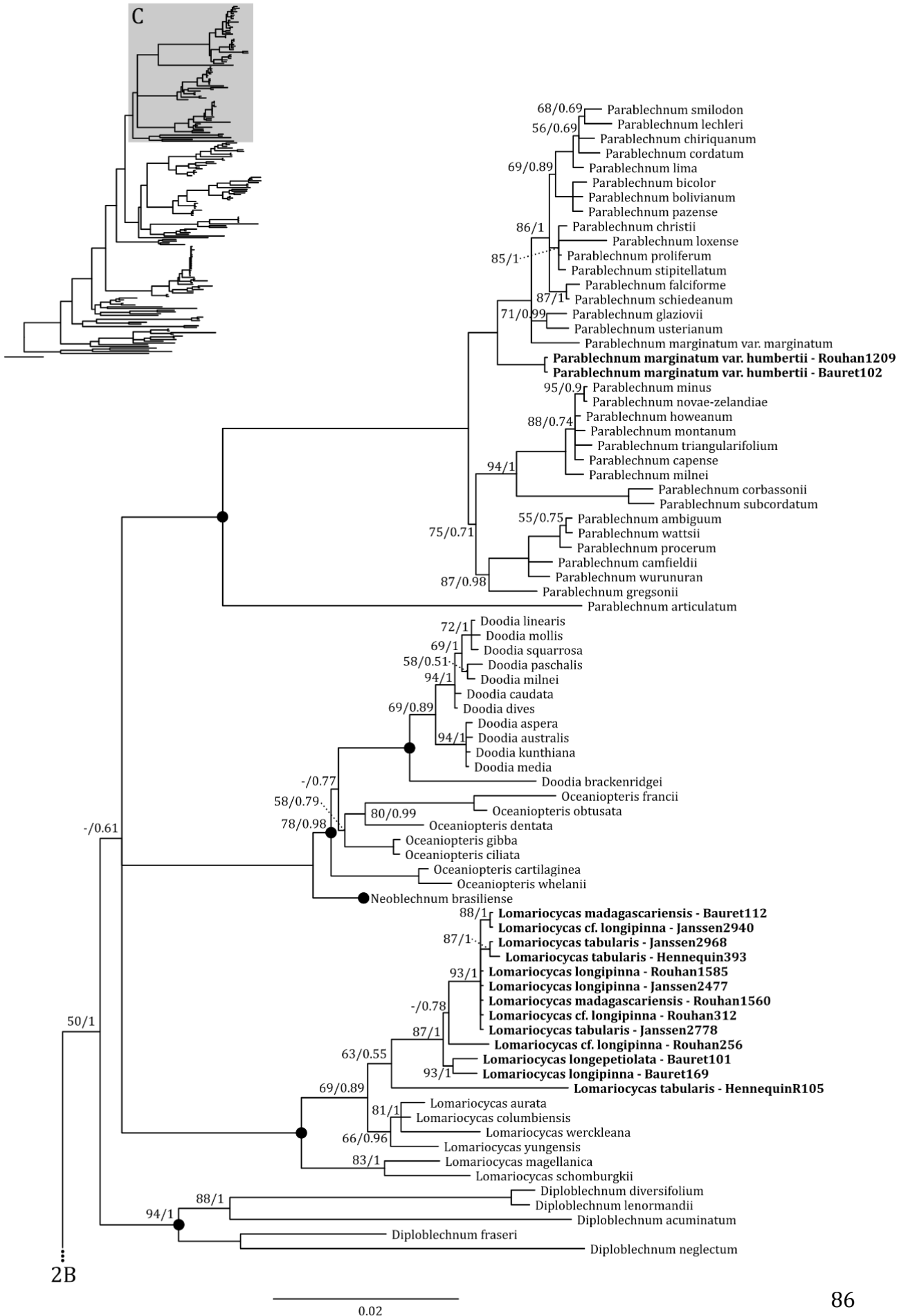


Figure 2. Majority rule consensus tree of Blechnaceae estimated by Bayesian inference on the combined chloroplast DNA dataset, with support values from the Bayesian inference and Maximum likelihood methods. Unless mentioned next to the nodes, support values were posterior probabilities (PP) ≥ 0.95 and bootstraps (BS) ≥ 95 . A dash (-) stands for BS < 50 or a non-retrieved node. Blechnoideae Malagasy species are in bold. Black dots stand for genera crown nodes (at tip when the genus is monotypic). White squares are placed at the subfamily crown nodes, and a black square for the family crown node.





3.2. BIOGEOGRAPHICAL HISTORY

Based on AIC, the DEC model was inferred as the most appropriate (AIC=561.6, Table 2), but the Likelihood Ratio Test (LRT) showed that the null hypothesis could not be rejected (p -value>0.1; H0: model with and without +j process confer equal likelihoods on the data). I therefore considered the results based on both the DEC and DEC+j models, and observed that the most likely ancestral ranges inferred were the same.

Biogeographical model	LnL	AIC	LRT p -value
DEC	-278.8	561.6	0.69
DEC+J	-278.7	563.4	
DIVALIKE	-305.4	614.8	0.40
DIVALIKE+J	-305	616.1	
BAYAREALIKE	-357.5	719	1.6e-18
BAYAREALIKE+J	-319	643.9	

Table 3. Biogeographical models tested, with log-likelihoods and AIC values, and the p -value of the LRT between nested models. Models selected for estimating the biogeographical history are in bold.

Blechnoideae would have diverged from their sister group during Early Paleocene (64.1 Ma; Figure 3A, Table 4), most likely in a wide ancestral range covering the Neotropics and the Eastern Paleotropics (Asia-Southeast Asia and Australasia), and would have started to diversify in the Eastern Paleotropics during the Early Eocene (55.4 Ma).

Three range expansions from Australasia to the Neotropics were suggested, in *Lomaria*, *Austroblechnum-Blechnum* and *Parablechnum-Diploblechnum-Doodia-Oceaniopteris-Neoblechnum-Lomariocycas* lineages (Figure 3A). Other range expansions resulted in ancestral wide ranges, and making difficult the inference of a clear biogeographical history.

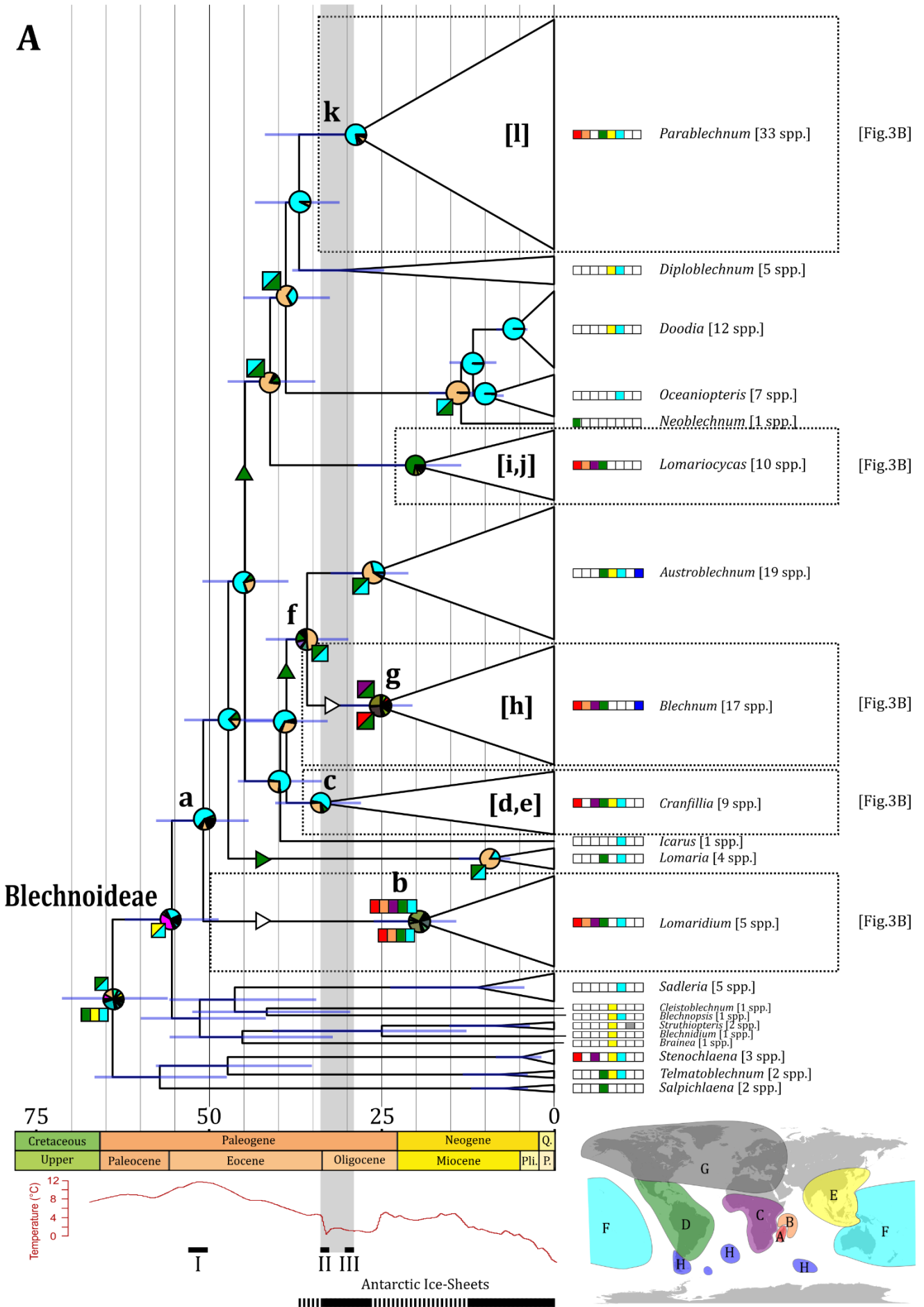
Nevertheless, at least five range expansions to Madagascar can be hypothesized (Figure 3B, Table 4): i) between 50.9-19.7 Ma [between nodes a and b]; ii) most likely between 34.0-18.8 Ma [c-d]; iii) one between 35.8-25.9 Ma [f-g] or two between 25.9-23.1 [g-h] and 25.9-0.0 Ma [g-*Blechnum punctulatum*]; iv) between 9.6-3.8 Ma [i-j]; and v) between 29.0-12.1 Ma [k-l]. Another range expansion could be estimated, for *Blechnum australe* (Prada s.n.), but the lineage was only known from a southern South American specimen. Neither precise ages of divergence nor precise areas of origin could be inferred for these Malagasy lineages, due to the number of areas estimated in the ancestral ranges (Figure 3B, Table 4).

Node	Age (Ma)	95% Highest Posterior Density (Ma)	Most likely ranges
Blechnoideae stem	64.1	56.1-71.4	DEF: 0.22 DF:0.15
Blechnoideae crown	55.4	48.7-62.3	EF: 0.39 F: 0.25
a	50.9	44.3-57.7	F: 0.58 DF: 0.10
b	19.7	14.2-26.2	ABCDF: 0.27 ABDF: 0.26 ADF: 0.14

c	34.0	28.0-40.5	F: 0.59 DF: 0.25
d	18.8	14.1-24.3	CDF: 0.24 ADF: 0.23 ACDF: 0.19 DF: 0.16
e	12.9	8.7-17.8	ACD: 0.44 CD: 0.29 AD: 0.27
f	35.8	29.9-41.8	DF: 0.49 D: 0.19 AF: 0.10 DF: 0.10
g	25.9	20.5-31.6	AD: 0.25 CD: 0.25
h	23.1	18.1-28.4	AD: 0.20 CD: 0.20 D: 0.10
i	9.6	6.4-13.5	D: 0.38 AD: 0.15 ABCD: 0.12
j	3.8	2.2-5.8	ABCD: 0.43 ABD: 0.19 ACD: 0.19 AD: 0.18
k	29.0	22.6-35.8	F: 0.78
l	12.1	9.0-15.5	ABDF: 0.46 ADF: 0.17 ABF: 0.15

Table 4. Age and ancestral ranges estimated by the DEC model. (A) Madagascar, (B) Mascarenes-Comoros-Seychelles, (C) Africa, (D) Neotropics, (E) Asia-Southeast Asia, (F) Australasia-Oceania (referred as Australasia in the text), (G) North America-Europe, and (H) a circumaustral region including the southern South America, Tristan da Cunha archipelago and the French Southern Lands. Only ranges with associated probabilities higher than 0.10 are indicated.

Four diversifications were estimated to have started in ranges including Madagascar, the Neotropics and other Paleotropical regions at nodes b, d (or e) and l. A diversification in *Blechnum* would have started in a range covering Madagascar and the Neotropics at node g (or h) and in *Lomariocycas* in a range covering the Neotropics, Africa and the WIO at node j. Diversification estimated in Madagascar alone were only found at *Lomariocycas madagascariensis* and *L. longepetiolata*-Bauret 101 stem nodes (Figure 3B). DIVA analysis (not selected based on AIC) however retrieved Madagascar as the only area of diversification for two clades nesting Western Paleotropical species in *Lomaridium* (*L. attenuatum*-Rouhan1283-*L. simillimum*-Rouhan1370) and *Lomariocycas* (node j).



B

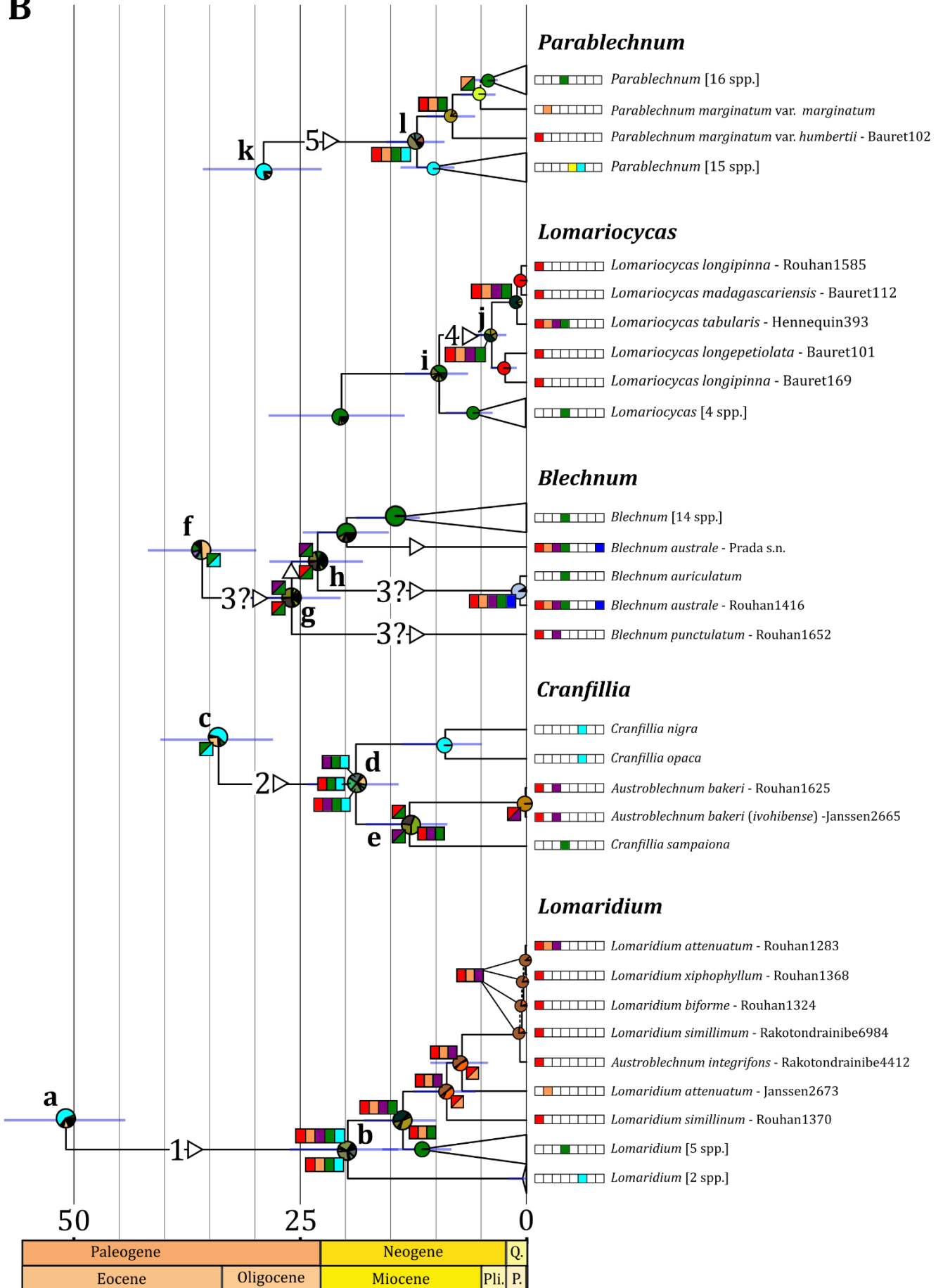


Figure 3. A. Simplified chronogram resulting from the BEAST analysis on Blechnoideae and ancestral areas estimated by BioGeoBEARS (DEC model). Dot-lined squares indicate genera for which details are available in Figure 3B. Number of sampled species per genera is shown between square brackets at tips (size of the triangle proportional to the number of sampled species). Horizontal blue bars correspond to 95% HPD intervals of the median ages. Colored squares at tips represent the current distributions of the genera, with the following color code: (A) Madagascar (red); (B) Mascarenes-Comoros-Seychelles (orange); (C) Africa (purple); (D) Neotropics (green); (E) Asia (yellow); (F) Australasia (light blue); (G) North America and Europe (grey) and (H) southern temperate circumastral regions (dark blue). For wide ranges (covering several coded areas), their composition is detailed by colored squares next to the corresponding pie section. For the sake of clarity, only the distribution of the most likely ranges were depicted, but details are available in Table 4. Green arrows on branches indicate range expansions from Australasia to the Neotropics and white arrows show range expansions from/to wide ranges, not allowing clear inferences about the biogeographical history of the Malagasy lineages (see text). The stratigraphy follows the 2017 International Chronostratigraphic Chart, the global temperature and tectonic events were mapped following Zachos *et al.* (2001): (I) Early Eocene Climatic Optimum, (II) Tasmania-Antarctic passage opens, (III) Drake passage opens. The vertical grey bar corresponds to the disconnection between South America and Australasia via Antarctica. B. Detailed chronograms and ancestral areas estimated for the five Malagasy lineages of Blechnoideae.

4. DISCUSSION

4.1. SYSTEMATICS ISSUES ABOUT MALAGASY SPECIES

The phylogenetic placement of the Malagasy species supports the classification of de Gasper *et al.* (2016b), except for *Austroblechnum bakeri* retrieved nested within *Cranfillia* and *Austroblechnum integrifrons* within *Lomaridium*. Several hypotheses could explain these results: wrong genus assignation and misleading genetic processes like hybridization.

The taxonomic identification of our four samples of *Austroblechnum bakeri* was checked, based on the morphology-based identification key of de Gasper *et al.* (2016,a,b). The identification actually led to the genus *Cranfillia*, into which the samples were phylogenetically retrieved (Figure 2B), rather than to *Austroblechnum*. A combination of *A. bakeri* into *Cranfillia* should therefore be published.

Austroblechnum integrifrons is morphologically well distinct from all other Malagasy Blechnoideae species by being the only one with entire fertile fronds (Rakotondrainibe *et al.*, 2013). The key to Blechnaceae genera (de Gasper *et al.*, 2016b) correctly assigns the species to *Austroblechnum*, even if the pinnae has to be considered as adnate to reach the assignation. Furthermore, the species does not match the description of *Lomaridium*, into which it was retrieved in the phylogeny, since this genus is characterized by a long creeping rhizome and the absence of hydathodes, whereas *A. integrifrons* has a short-creeping to erect rhizome and distinct hydathodes (Rakotondrainibe *et al.*, 2013). Only one sample of *Austroblechnum integrifrons* was successfully sequenced, and additional specimens will be necessary to confirm the phylogenetic position of this taxon. Sequencing a nuclear marker would also allow testing if the specimen(s) or the species - like other species retrieved non-monophyletic - has a hybrid origin (see also Chapter II, discussion on the hybridization hypothesis).

Only two Malagasy Blechnoideae species (out of 15) and a variety were retrieved monophyletic. The lack of resolution was important in most clades of Malagasy species (e.g. *Lomariocycas*, Figure 2C), precluding the test of monophyly for three species, and six other species were retrieved paraphyletic (e.g. *Lomariocycas longipinna*, Figure 2C). Interestingly, we detected a possible case of cryptic species in *Lomaridium* since a clade nesting the three *Lomaridium attenuatum* specimens from Mascarenes (Janssen 2673, Hennequin R67, Hennequin 365; Figure 2A), was sister-group to the remaining samples of the species, collected in Comoros and Africa.

4.2. AN UNCERTAIN MALAGASY BIOGEOGRAPHICAL HISTORY

The biogeographical history of the Malagasy Blechnoideae could not be very precisely documented, but our results nevertheless provide some information and suggest numerous biogeographical connections between southern regions.

At least three range-expansions were inferred from Australasia to explain the Neotropical distribution of *Lomaria*, *Austroblechnum-Blechnum* and *Parablechnum-Diploblechnum-Doodia-Oceaniopteris-Neoblechnum-Lomariocycas* lineages (Figure 3A). Australasia and South America were connected via Antarctica until the end of Eocene (McLoughlin, 2001) and such overland migrations via Antarctica were already hypothesized in both directions for ferns in Lastreopsids (Labiak *et al.*, 2014), Cyatheaceae (Korall & Pryer, 2014) and for many other plant lineages and animals (Sanmartín & Ronquist, 2004; Bartish *et al.*, 2011; Winkworth *et al.*, 2015). Migration via Antarctica however stopped at Eocene-Oligocene boundary, due to Antarctic ice-sheets formation (Zachos *et al.*, 2001). Therefore, connections between the Neotropics and Australasia after ca. 35 Ma are most likely explained by long distance dispersal events, following the onset of circumaustral winds (Munoz *et al.*, 2004). Hypotheses of trans-Antarctica migrations are compatible with range expansions from Australasia to Neotropics in *Austroblechnum-Blechnum*, *Parablechnum-Lomariocycas* clades and in *Lomaridium*, although a direct dispersal event could also lead to *Lomaridium* (Figure 3).

Hypotheses of Antarctica routes are not compatible with migration to Madagascar as the island was isolated from Antarctica for at least ca. 80 Ma (Yoder & Nowak, 2006). Therefore, the biogeographical history of Malagasy Blechnoideae would be driven by dispersal events from the Neotropics, Australasia, Africa and other WIO islands. Precise areas of origin cannot be inferred from the present results but hypotheses may be proposed in some cases, especially dealing with the Malagasy lineages. In *Lomariocycas*, the number of endemic species in clade j highly suggests a diversification in Madagascar only, and so a direct dispersal from the Neotropics to Madagascar between 9.6-3.8 Ma.

4.3. IN SITU DIVERSIFICATION

In situ diversifications would explained the Malagasy endemic diversity in *Lomariocycas* (clade j) and in *Lomaridium* (*L. attenuatum*-Rouhan1283-*L. simillimum*-Rouhan1370 clade), giving rise to four and five species respectively. *Lomariocycas* and *Lomaridium* seem to have colonized Madagascar and diversified by retaining their original ecological preferences. Malagasy *Lomariocycas* species are terrestrial with an erect rhizome (often sub-arborescent, Figure 1G) and occur between 800-1900 meters of altitude and mostly found in ericoid and summit forests (Rakotondrainibe *et al.*, 2013), as well as the

Neotropical species like *Lomariocycas yungensis* (e.g. >2000 meters in Bolivia, Giacosa, 2010). Within *Lomaridium*, the species display the same ecological preferences, growing as epiphytes or hemi-epiphytes (Figure 1D,E), rarely epilithes, in a wide range of altitudes (often higher than 700 m), in Madagascar (Rakotondrainibe *et al.*, 2013), the Neotropics (Dittrich *et al.*, 2017) and in Australasia (Brownlie, 1969).

Chapter IV - Biogeographical history of *Lindsaea* and *Odontosoria* (Lindsaeaceae) in Madagascar: at least two dispersal events from tropical Asia and one in situ diversification highlighted

1. INTRODUCTION

Lindsaeaceae include seven genera and around 230 species distributed in the Neotropics and the Paleotropics, *Lindsaea* being the most diversified genus with 180 estimated species (Lehtonen *et al.*, 2010; PPGI, 2016). Lindsaeaceae species are either terrestrial or epiphyte, and characterized by marginal or sub-marginal sori that open towards the margin at the extremity of veins. Furthermore, many species have dimidiate pinnae, giving them an *Adiantum*-like appearance (Figure 1A).

The lack of clear morphological features to segregate the Lindsaeaceae genera has led to several classifications before the current consensus (Lehtonen *et al.*, 2010; PPGI, 2016). The latest and most comprehensive molecular phylogenetic study on Lindsaeaceae showed that *Lindsaea* was sister-clade to a clade nesting three genera: *Nesolindsaea*, *Osmolindsaea* and *Tapeinidium* (Lehtonen *et al.*, 2010). However, another analysis also showed *Odontosoria* as the sister genus to *Lindsaea* (Lehtonen, Wahlberg, & Christenhusz, 2012), suggesting that the phylogenetic relationships between all these genera could be sensitive to the dataset and the methods used. Lehtonen *et al.* (2010) also showed that *Odontosoria* was still difficult to define morphologically: the small number of sporangia per sorus was the only supporting character, but not shared by all the species. For *Lindsaea*, several supporting morphological characters were found to discriminate the genus from others, such as short stipes, abaxially keeled rachis, broad rachis sulcus and a vein order of two.

Lindsaea is the most diversified Lindsaeaceae genus in the Western Paleotropics (Africa-Western Indian Ocean) with 14 species all present in Madagascar, followed by *Odontosoria* with four species (Table 1). Numerous collections from recent field works in Madagascar provide material for molecular studies to test the taxonomy based on morphology, by adopting the evolutionary species concept applied in the previous chapters (Simpson, 1951). In particular, the molecular phylogenetic results will be discussed in the light of an ongoing taxonomic study based on morphological characters (Rakotondrainibe, pers. com.). The sampling of Lehtonen *et al.* (2010) already included two specimens of *L. blotiana* and *L. millefolia* but the monophyly of numerous Malagasy species remained untested. The ongoing taxonomic revision by Rakotondrainibe also hypothesized synonymies that could be tested on the basis of molecular data (Table 1).

This work will provide the first biogeographical analysis on Lindsaeaceae, with a focus on the Malagasy species of *Lindsaea* and *Odontosoria*. The species diversity in Madagascar would be explained by dispersal rather than Gondwanan origins, as *Lindsaea* diverged from *Odontosoria* between ca. 45-70 Ma (Lehtonen *et al.*, 2012; Testo & Sundue, 2016), after the isolation of Madagascar ca. 90 Ma (McLoughlin, 2001). Christensen (1932) found

that the Eastern Paleotropics (Asia to Australasia and the Pacific) have the strongest phytogeographic affinities with the Malagasy species of Lindsaeaceae. These affinities were retrieved by Lehtonen *et al.* (2010): in their results, the Malagasy species were nested in clades of the Eastern Paleotropics, whereas all the Neotropical species were nested in two other clades with no Paleotropical species, suggesting that dispersal events from Eastern Paleotropics (rather than Neotropics) would explain the presence of *Lindsaea* species in Madagascar. Such affinities with the Eastern Paleotropics were also retrieved in *Odontosoria*, as the Malagasy endemic *O. melleri* was found sister species to Eastern Paleotropical species. Within the Western Paleotropics, relatively few exchanges are expected due to the high proportion of Malagasy endemics in *Lindsaea* (12 out of the 14 Malagasy species, Table 1).

This chapter aims 1) to test the monophyly of the Malagasy species in the light of a current taxonomic revision (Rakotondrainibe, com. pers.), 2) to estimate the age of the Malagasy lineages, and 3) to infer the biogeographical history of the Malagasy species of *Lindsaea* and *Odontosoria*.

Species	Distribution	Synonymy to test
<i>Lindsaea blotiana</i> K.U.Kramer	Endemic to Madagascar	
<i>Lindsaea decaryana</i> (C.Chr.) Tardieu	Endemic to Madagascar	
<i>Lindsaea ensifolia</i> Sw.	WIO, Southeast Asia, Australasia-Oceania	
<i>Lindsaea flabellifolia</i> (Baker) Kuhn	Endemic to Madagascar	= <i>L. millefolia</i> syn. nov. (Rakotondrainibe, com. pers.)
<i>Lindsaea goudotiana</i> (Kunze) Mett. ex Kuhn	Endemic to Madagascar	= <i>L. subtilis</i> syn. nov. (Rakotondrainibe, com. pers.)
<i>Lindsaea grandiareolata</i> (Bonap.) K.U.Kramer	Endemic to Madagascar	
<i>Lindsaea heterophylla</i> Dryand.	WIO, Southeast Asia	
<i>Lindsaea humbertii</i> Tardieu	Endemic to Madagascar	
<i>Lindsaea leptophylla</i> Baker	Endemic to Madagascar	= <i>L. coursii</i> syn. nov. (Rakotondrainibe, com. pers.)
<i>Lindsaea madagascariensis</i> Baker	Endemic to Madagascar	
<i>Lindsaea marojejyensis</i> Rakotondr. sp. nov. ined.	Endemic to Madagascar	
<i>Lindsaea odontolabia</i> (Baker) K.U.Kramer	Endemic to Madagascar	
<i>Lindsaea oxyphylla</i> Baker	Endemic to Madagascar	
<i>Lindsaea plicata</i> Baker	Endemic to Madagascar	
<i>Odontosoria afra</i> (K.U. Kramer) J.P.Roux	Endemic to Africa	
<i>Odontosoria africana</i> F.Ballard	Endemic to Africa	
<i>Odontosoria chinensis</i> (L) J.Sm.	WIO, Africa, Southeast Asia, Australasia-Oceania	
<i>Odontosoria melleri</i> (Flook. ex Baker) C.Chr.	Endemic to Madagascar	

Table 1. *Lindsaea* and *Odontosoria* species occurring in Africa and Western Indian Ocean (WIO), species distribution, and hypothesized synonymies based on an ongoing morphological taxonomic revision (Rakotondrainibe, com. pers.). Species sampled for the molecular study are in bold.



Figure 1. Diversity of *Lindsaea* and *Odontosoria* species in Madagascar. (A) *Lindsaea blotiana*, Rouhan 1199; (B) *Lindsaea marojejyensis* sp. nov. ined., Rouhan 1191; (C) *Lindsaea goudotiana*, Rouhan 1156; (D) *Odontosoria chinensis*, Rouhan 1152; (E) *Odontosoria melleri*, Rouhan 1359, plus detail of a pinna. Photos: G. Rouhan.

2. MATERIAL AND METHODS

2.1. TAXONOMIC SAMPLING

The sampling was composed of two parts: one from the worldwide phylogenetic study of Lindsaeaceae by Lehtonen *et al.* (2010), and one from new specimens recently collected in Madagascar. All four Western Paleotropical species of *Odontosoria* and 11 out of 14 Afro-Malagasy species of *Lindsaea* were sampled (Table 1, Appendix 4). For *Lindsaea*, 27 new specimens were added compared to the previous phylogenetic study (Lehtonen *et al.*, 2010), as well as the new species *L. marojejyensis* sp. nov. ined. Also, ten species were added as outgroups for calibration needs (see 1.4 Divergence time estimation). The whole dataset included 178 specimens representing 149 species. Voucher details are available in Appendix 4.

2.2. DNA EXTRACTION, AMPLIFICATION AND SEQUENCING

The protocols for DNA extraction, amplification and sequencing followed those of Chapters II and III. Five plastid regions were sequenced, the two coding regions *rpoC1* and *rps4*, and the three non-coding regions *rps4-trnS*, *trnH-psbA* and the *trnL* intron plus the *trnL-trnF* intergenic spacer (*trnL/trnL-trnF*). For *rpoC1*, specific primers were used: LP1 5'-TAT GAA ACC AGA ATG GAT GG-3' and LP5 5'-CAA GAA GCA TAT CTT GAS TYG G-3' (Chase *et al.*, 2007) with the following thermal cycling conditions: 5 min 94°C / 40 × (30 s 94°C / 30 s 48°C / 45 s 72°C) / 10 min 72°C.

2.3. PHYLOGENETIC ANALYSES

The phylogenetic analyses followed the protocol detailed in Chapter II about alignments, matrix concatenation and phylogenetic approaches (ML, BI). The five plastid markers were concatenated but formed five independent partitions in the MrBayes and raxML analyses. The parameters were set following the Chapter II, except for the MrBayes analysis; the runs were conducted on five million generations and sampled every 500 generations to obtain 10,000 sampled trees.

2.4. DIVERGENCE TIME ESTIMATION

The dating analysis was performed on a reduced dataset including one sample per species, or per lineage when species were retrieved non-monophyletic. I followed the same protocol as developed in Chapter II, using BEAST 2.4.2 (Bouckaert *et al.*, 2014) on the CIPRES science gateway and with the specific calibration points mentioned below.

Two fossils identified as Lindsaeaceae have already been used for calibration in the literature: a root system and a leaf, both from the Upper Cretaceous, but the authors could not assign them to one of the extant genera (Schneider & Kenrick, 2001; Regalado *et al.*, 2017). We combined these fossil calibration points with secondary calibrations from a large-scale dating analysis (Testo & Sundue, 2016).

The age of the Lindsaeaceae stem node was modeled by a uniform prior from 100.5 Ma to infinity, based on the stratigraphic occurrences of the two fossils in the Upper Cretaceous (Schneider & Kenrick, 2001; Regalado *et al.*, 2017). The age of divergence between *Odontosoria* and *Lindsaea* was modeled by a normal prior distribution with a mean of 71.1 Ma, the crown node age of *Odontosoria* with a mean of 61.2 Ma and the stem node

age of the *Lindsaea* Neotropical clade with a mean of 32.0 Ma based on Testo & Sundue (2016). Standard deviations were set to 10% of the mean estimates.

An uncorrelated lognormal relaxed clock was used, with a Birth-Death tree prior and a random starting tree. For each analysis, two independent runs of 100 million generations with random seed values were conducted and sampled every 10,000 generations to obtain 10,000 sampled trees.

2.5. BIOGEOGRAPHICAL ANALYSES

The ancestral ranges were estimated using the R package BioGeoBEARS (Matzke, 2013) in R studio 1.0.44 (RStudio Team, 2015). I conducted six analyses, successively using the models DEC, DEC+j, DIVALIKE, DIVALIKE+j, BAYAREALIKE, BAYAREALIKE+j (see Material and Methods in Chapter II for details). Among these six models, the most adequate to reconstruct the biogeographical history was selected based on the Akaike Information Criterion (AIC).

Six areas were defined to code the geographic distributions of species: (A) Madagascar, (B) Mascarenes-Comoros-Seychelles, (C) Africa, (D) Neotropics, (E) Asia-Southeast Asia, and (F) Australasia-Oceania (later referred as Australasia). The entire species distribution was coded (not only the voucher localities) based on herbarium collections, floras, personal communications (Rakotondrainibe) and the GBIF database when necessary. The maximum range size was set to five areas, as it is the maximum number of areas covered by an extant taxon.

3. RESULTS

3.1. PHYLOGENETIC RESULTS

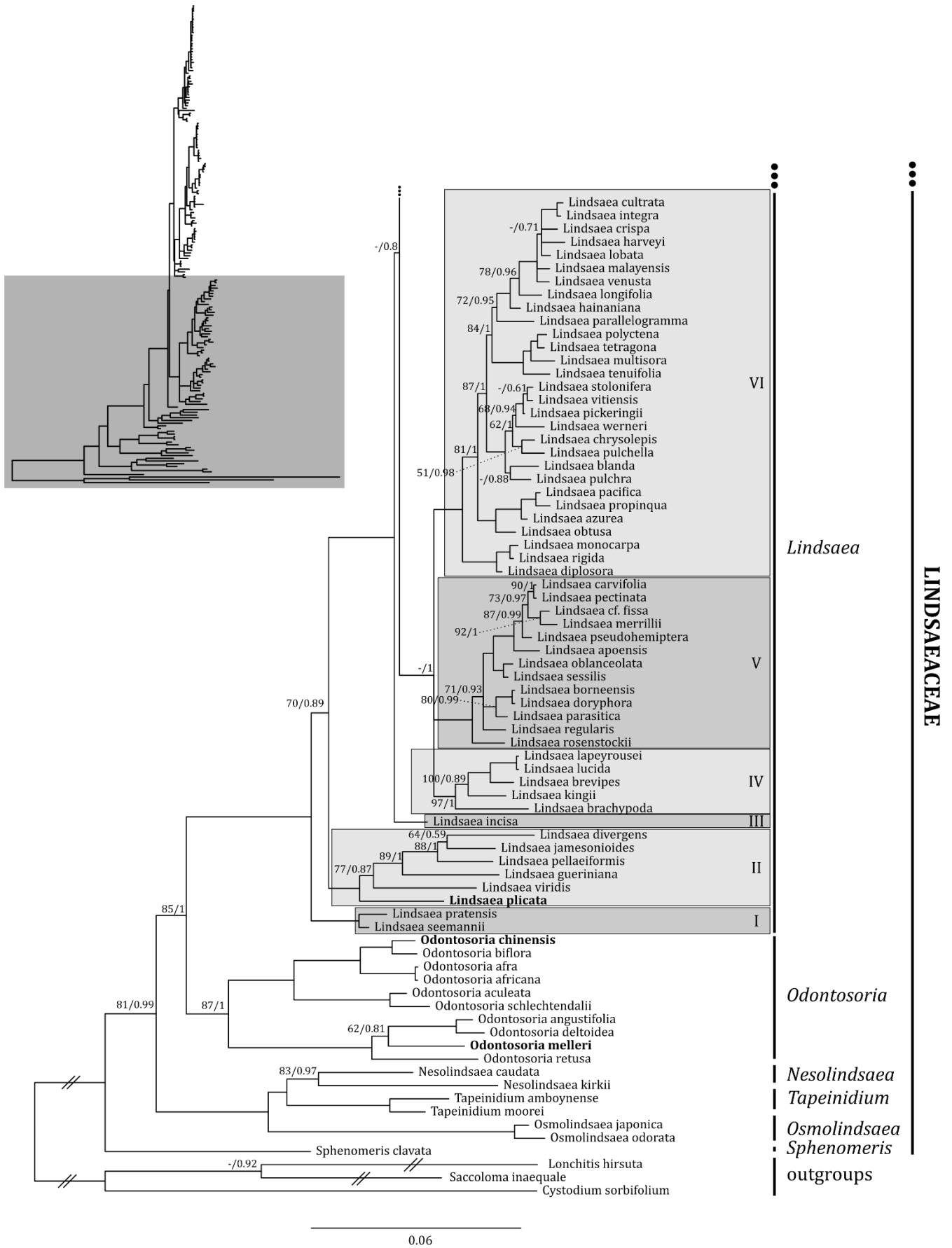
The whole matrix included 178 specimens and 3314 characters (Table 2). MrBayes and RaxML topologies both supported the monophyly of *Lindsaea* (Bootstrap (BS)=100; Posterior Probability (PP)=1) and *Odontosoria* (BS=87; PP=1), which were retrieved sister groups (BS=85; PP=1; Figure 2A). With the other genera *Nesolindsaea*, *Tapeinidium*, *Osmolindsaea* and *Sphenomeris*, they formed the well-supported clade Lindsaeaceae (BS=100; PP=1; Figure 2A). The clade annotations of Lehtonen *et al.* (2010) were transposed on Figure 2, to facilitate comparison with their retrieved topology.

DNA region	Number of sequences (on the total number of sequences)	Aligned length (base pairs)	Percent of variable characters	Percent of informative characters
<i>rpoC1</i>	117 (66%)	732	28.8	21.6
<i>rps4</i>	90 (51%)	577	43.8	26.5
<i>rps4-trnS</i>	89 (50%)	502	37.1	25.5
<i>trnH-psbA</i>	166 (93%)	674	45.3	28.2
<i>trnL/trnL-trnF</i>	175 (98%)	829	51.5	40.4
Combined dataset	178 (100%)	3314	41.7	29.1

Table 2. Matrix statistics

The two Malagasy species of *Odontosoria* were retrieved in two clades (Figure 2A), with *O. chinensis* sister-group to the Asian-Australasian *O. biflora* (BS=100; PP=1), and *O. melleri* sister-group to the Asian clade formed by *O. angustifolia* and *O. deltoidea*, however with weak support (BS=62; PP=0.81). Within *Lindsaea*, the Malagasy species were nested in two clades: in clade II, with the endemic *L. plicata* sister-group to a clade of Asian species (Figure 2A; BS=100; PP=1), and in clade IX (BS=87; PP=1; Figure 2B) with ten out of the 11 sampled Malagasy species of *Lindsaea*. In clade IX, a polytomy was found between three subclades: two clades including Malagasy species, poorly supported based on ML (BS≤60, PP≥0.85), and a third one that nested Asian-Australasian species but not statistically supported (BS<50; PP=0.59). At least nine species would have originated from a single diversification in the *L. flabellifolia*-*L. oxyphylla* subclade (BS=54; PP=0.85; Figure 2B).

Within clade IX (Figure 2B), *L. blotiana* samples were found mixed with *L. flabellifolia* samples (BS=90; PP=1). *L. goudotiana* was proved monophyletic, well-supported (BS=1; PP=1), and sister-group to the *L. flabellifolia*-*L. blotiana* clade (BS=1; PP=1). *Lindsaea leptophylla*, *L. madagascariensis* and *L. marojejyensis* specimens were all included in the same clade but with low support (BS=60; PP=0.81) and the three species were not monophyletic. A similar result was observed in the clade nesting *L. heterophylla*, *L. grandiareolata* and *L. oxyphylla* (BS<50; PP=0.79). *Lindsaea heterophylla* was polyphyletic, with a sample forming a clade with *L. grandiareolata* and *L. oxyphylla* (BS<50; PP=0.88) and another sample forming a clade with two *L. ensifolia* samples (BS=60; PP=0.99). The polyphyly of *L. heterophylla* was however poorly or not supported in ML (BS≤60).



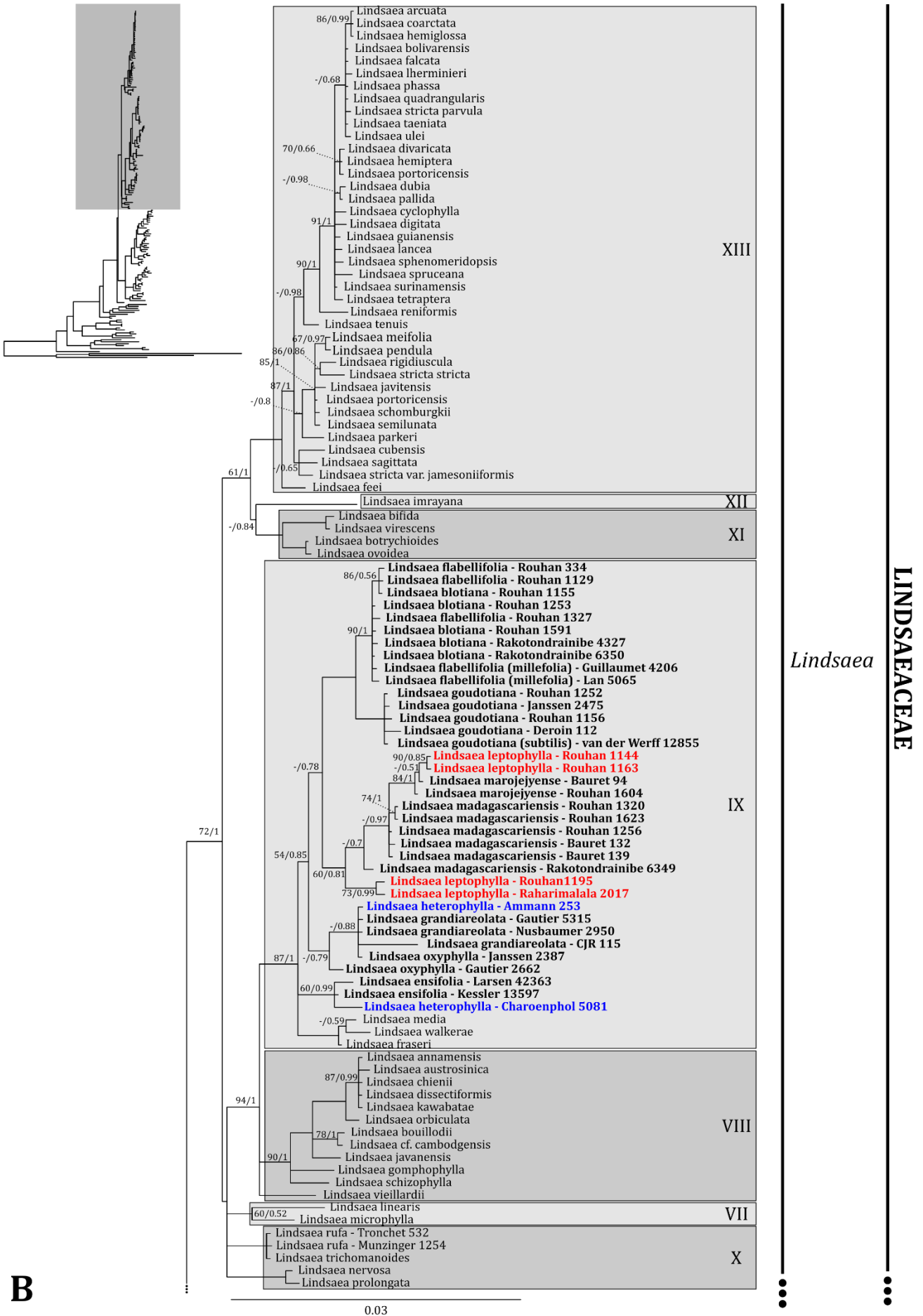


Figure 2. Majority rule consensus tree of *Lindsaea* and *Odontosoria* estimated by Bayesian inference on the combined chloroplast DNA dataset, with support values from the Bayesian inference and Maximum likelihood methods. Unless mentioned next to the nodes, support values were posterior probabilities (PP) ≥ 0.95 and bootstraps (BS) ≥ 95 . A dash (-) stands for BS < 50 or a non-retrieved node. The Malagasy species are in bold and Malagasy species retrieved polyphyletic are colored (but not all the non-monophyletic species).

3.2. AGE AND BIOGEOGRAPHICAL ORIGIN OF MALAGASY LINEAGES

The DEC model was selected based on the AIC value (Table 3), but the Likelihood Ratio Test (LRT) showed that the null hypothesis cannot be rejected (p -value > 0.1 ; H_0 : models with and without +J process confer equal likelihoods on the data). So, both DEC and DEC+J results were examined but the same ancestral ranges were inferred as most likely.

Biogeographical model	LnL	AIC	LRT p -value
DEC	-224.5	452.9	1
DEC+J	-224.5	454.9	
DIVALIKE	-245.6	495.2	1
DIVALIKE+J	-245.6	497.2	
BAYAREALIKE	-244	492.1	0.11
BAYAREALIKE+J	-242.8	491.6	

Table 3. Biogeographical models tested, with log-likelihoods, AIC values and the p -value of the LRT between nested models. Models selected for estimating the biogeographical history are in bold.

Based on these inferences, *Lindsaea* and *Odontosoria* diverged during the Upper Cretaceous in Australasia (Table 4). Extant species of *Lindsaea* started to diversify during the Middle Eocene (45.8 Ma; HPD: 37.5-55.4) and the two most likely ancestral ranges of diversification inferred were Australasia-Neotropics ($p=0.20$) and Australasia ($p=0.17$; Figure 3). An ancestral range covering both the Neotropics and Australasia was also retrieved as the most likely at nodes g-i between Eocene and early Miocene. Clades VII, VIII, IX and X would have originated in Australasia during the Miocene (Figure 3, nodes k-m).

Node	Age (Ma)	95% Highest Posterior Density (Ma)	Most likely ranges (DEC)
a- Divergence of <i>Lindsaea</i> and <i>Odontosoria</i>	77.3	67.2-87.5	F: 0.32
b- <i>Odontosoria</i> crown node	65.4	55.0-76.7	F: 0.27
c	36.5	27.8-47.0	ACDEF: 0.13 ABDEF: 0.11 BCDEF: 0.11
d	25.0	15.6-34.7	AF: 0.49 F: 0.28 AEF: 0.11 EF: 0.1
e- <i>Lindsaea</i> crown node	45.8	37.5-55.4	DF: 0.20 F: 0.17 ADEF: 0.16 ADF: 0.13 AF: 0.10
f	34.8	27.4-43.7	AEF: 0.50 AF: 0.38

g	41.0	32.2-50.2	DF: 0.52 D: 0.38
h	27.9	22.5-33.6	DF: 0.65 F: 0.34
i	25.8	20.7-32.1	DF: 0.67 F: 0.32
n	9.9	7.12-12.7	F: 0.40 AF:0.26
o	8.6	6.6-11.6	F: 0.50 AEF: 0.10

Table 4. Age and ranges estimated at nodes by DEC model. (A) Madagascar, (B) Mascarenes-Comoros-Seychelles, (C) Africa, (D) Neotropics, (E) Asia-Southeast Asia and (F) Australasia-Oceania (referred as Australasia in the text). Only ranges with associated probabilities higher than 0.10 are indicated.

The Malagasy diversity of *Lindsaea* would be explained by at least two dispersal events: one between 34.0-45.8 Ma from an unknown area as wide ancestral ranges were retrieved (L1, nodes e-f, Table 4) and one from Australasia between 8.6-9.9 Ma (L2). The latter was followed by an in situ diversification in Madagascar that started 6.9 Ma (node p). Two dispersal events could explain the Malagasy diversity of *Odontosoria*, one from an unknown area, most likely between 36.5-64.4 Ma (O1, nodes b-c) and one from Australasia between 25.0-64.4 Ma (O2, nodes b-c, Table 4). Clade c was estimated to initially originate from Australasia but no range was clearly estimated at crown node; the two most likely ancestral ranges covered Madagascar and four other areas (C or B, D, E and F, Table 4). Therefore, the dispersal event to Madagascar may have originated from any of these other areas.

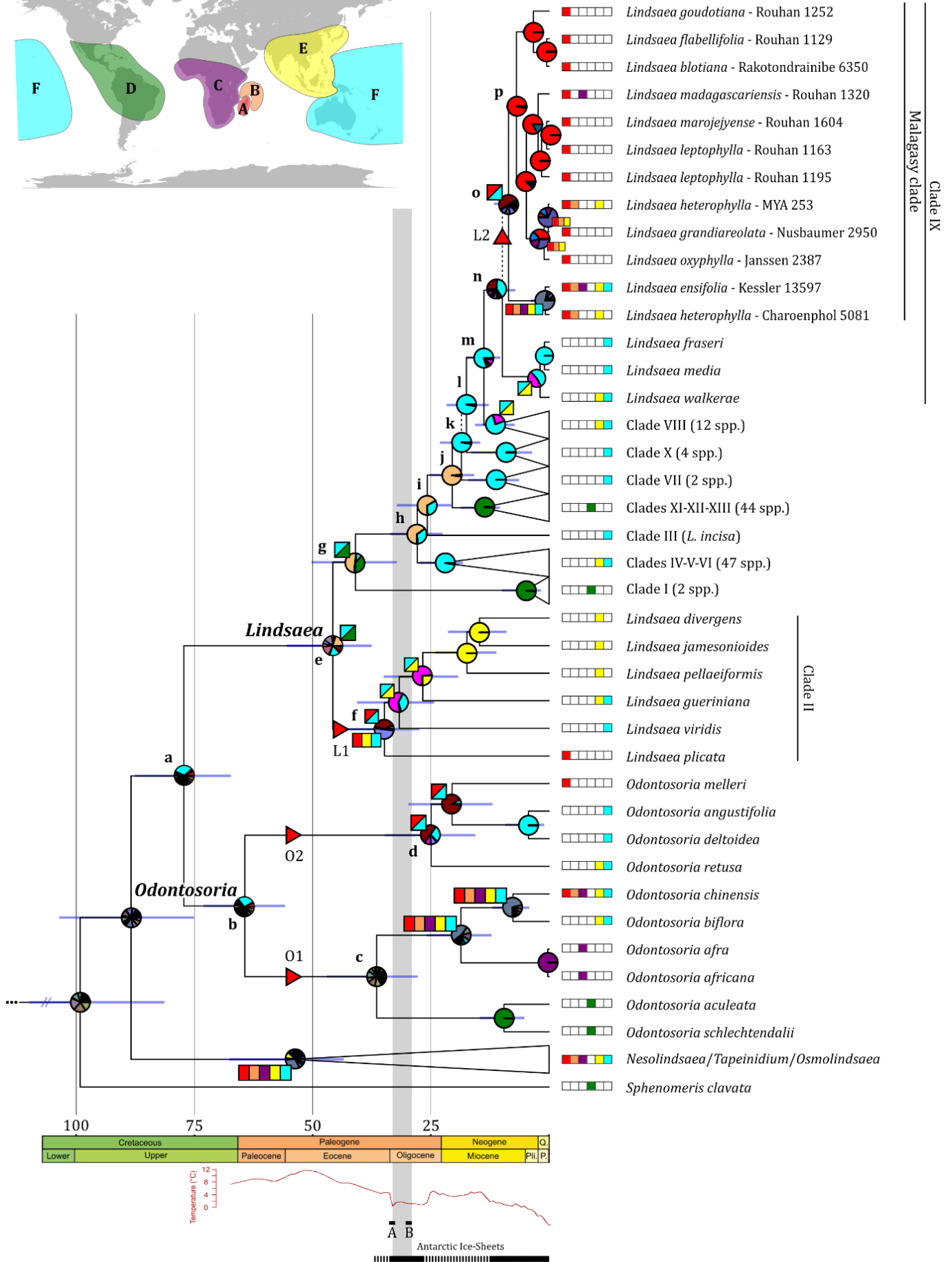
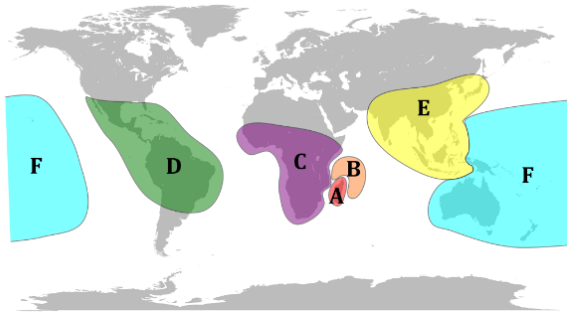


Figure 3. Chronogram resulting from the BEAST analysis on *Lindsaea* and *Odontosoria*, and ancestral areas estimated by BioGeoBEARS (DEC model). Horizontal blue bars correspond to 95% HPD intervals of the median ages. Dotted branches indicate not-supported relationships (PP<50). Colored squares at tips represent the current distribution, with the following color code: (A) Madagascar (red), (B) Mascarenes-Comoros-Seychelles (orange), (C) Africa (purple), (D) Neotropics (green), (E) Asia-Southeast Asia (yellow), and (F) Australasia-Oceania (light blue, referred in the text as Australasia). For wide ranges (covering several coded areas), their composition is detailed by colored squares next to the corresponding pie section. For the sake of clarity, only the distribution of the most likely ranges were depicted, but details about nodes probabilities are available in Table 4. Red arrows on branches indicate hypothesized dispersal events to Madagascar. The map represents the areas coded in the biogeographical analysis. The stratigraphy follows the 2017 International Chronostratigraphic Chart, the global temperature and tectonic events were mapped following Zachos *et al.* (2001), with (A) Tasmania-Antarctic passage opens and (B) Drake passage opens.

4. DISCUSSION

4.1. SYSTEMATICS

In *Lindsaea*, the tree topology obtained was globally congruent with the one found by Lehtonen *et al.* (2010): the same phylogenetic relationships were retrieved with good support for clades I to VI, no resolution was found between clades VII, VIII-IX and X and the relationships between clades XI, XII and XIII remained unresolved due to low support in both studies (Figure 2B). Within *Odontosoria*, I found the same phylogenetic results as Lehtonen *et al.* (2010) and the position of *O. melleri* remain uncertain due to the lack of good supports.

Within *Lindsaea*, the synonymy of *Lindsaea millefolia* under *L. flabellifolia* proposed by Rakotondrainibe (com. pers.) cannot be confirmed by the present results: they were found in the same clade but without resolution, suggesting that the two species are phylogenetically close but not necessarily conspecific. In the same clade, my results did not discriminate *Lindsaea flabellifolia* from *L. blotiana*, like Lehtonen *et al.* (2010). Based on their morphology, these species are however well segregated with for example *Lindsaea flabellifolia* having linear ultimate free divisions with one vein connecting one sorus whereas *L. blotiana* has branching veins connecting two or more sori per ultima division.

The *Lindsaea subtilis* sample was retrieved in the *L. goudotiana* clade, supporting the need to place *L. subtilis* under synonymy, as it was suggested by morphological observations (Rakotondrainibe, com. pers.). Kramer (1972) distinguished these species by the width of the ultimate free division and the shape of the segment. However, ongoing taxonomic works on *Lindsaea* show that the genus displays a continuum of sizes and shapes of the segments (Rakotondrainibe, com. pers.), making hazy taxonomic distinctions based on these characters.

With the synonymy of *Lindsaea subtilis* under *L. goudotiana*, the latter was the only monophyletic species retrieved by my phylogenetic analyses. For some species, there was not enough resolution to assess monophyly, suggesting that the markers could be not enough variable at this taxonomic scale (e.g. *L. flabellifolia* and *L. blotiana*). Also, several genetic processes can be misleading in the recognition of species and their relationships (Naciri & Linder, 2015), notably hybridization that is a process largely recognized for ferns (Barrington *et al.*, 1989; Haufler, 2008; Sigel, 2016) and previously suggested in

Lindsaea (Lehtonen *et al.*, 2010). This could notably explain the polyphyly of *L. leptophylla* and *L. heterophylla*.

4.2. BIOGEOGRAPHICAL HISTORY

4.2.1. *Disjunction between the Paleotropics and the Neotropics*

Ancestral ranges covering both the Paleotropics and the Neotropics were estimated at deep nodes in *Lindsaea* and *Odontosoria* (e.g. nodes g to j, Figure 3), although the lineages diversified in only one of the two regions. Such disjunctions in the Southern Hemisphere are usually explained by Gondwanan vicariance, direct dispersal or Antarctica routes (Sanmartín & Ronquist, 2004; Winkworth *et al.*, 2015).

As developed in Chapter III for Blechnoideae, the biogeographical history of *Lindsaea* and *Odontosoria* could be explained by migration via Antarctica and/or direct dispersal mediated by circumaustral wind currents. Compatible ancestral ranges with an Antarctica distribution were estimated in *Lindsaea* at nodes e to g, and eventually at nodes h and i when the 95% HPD is taken into account. The independent diversification in the Neotropics and Paleotropics could be therefore explained by an isolation of the two regions after ice-sheets formation at the Eocene/Oligocene boundary (Figure 3). In *Odontosoria*, the wide ranges estimated at node c included the Neotropics and Eastern Paleotropical regions, but also Western Paleotropical areas, suggesting that an ancestral Antarctica distribution does not completely explain the present results and suggest that other processes like dispersal could be involved. After ca. 35-30 Ma, connections between the Neotropics and the Paleotropics in *Lindsaea* are more likely explained by dispersal events from Australasia to the Neotropics rather than disjunctions of wide ranges consequently to vicariant events.

4.2.2. *Biogeographical history of the Malagasy species*

Four dispersal events most likely explain the distribution of *Lindsaea* and *Odontosoria* in Madagascar during the Cenozoic (O1, O2, L1, L2; Figure 3), since Madagascar was isolated from other landmasses since the Upper Cretaceous (McLoughlin, 2001). At least two direct dispersal events between Australasia and Madagascar were suggested by the results (O2, L2; Figure 3). For ferns, dispersal events between these two regions were previously reported, but only during the Neogene and Quaternary (Hennequin *et al.*, 2010b; Chao *et al.*, 2014; Le Péchon *et al.*, 2016; Kuo *et al.*, 2016; Sessa *et al.*, 2017). They were hypothesized to be linked to the onset of Asian monsoons. The age of initiation of Asian monsoons is subject to controversy but the associated winds would have been established at the latest during late Miocene (Passey *et al.*, 2009; Jacques *et al.*, 2011; Gupta *et al.*, 2015). The dispersal event O2 was estimated during Paleogene and would may rather be explained by stepping-stone dispersal via the drifting India (Warren *et al.*, 2010).

For L1, the exact area of origin of the dispersal event remains doubtful, due to wide ranges covering different areas estimated at nodes e and f. Moreover, the position of clade I in the BEAST analysis, as sister group to all other *Lindsaea* species, was poorly supported (PP=0.63) and this clade was instead found at the crown node of *Lindsaea* by MrBayes and raxML analyses (Figure 2A). Therefore, the biogeographical origins of clade II and

Lindsaea plicata remain uncertain. About O1, a range covering multiple areas was inferred at node c and made it impossible to infer the area of origin of the Malagasy lineage.

With at least ten species that would have originated from in situ diversification, that is, 71% of the *Lindsaea* species diversity in Madagascar, diversification would be an important process to explain the diversity of the genus in Madagascar. Such diversification in Madagascar was also documented in previous chapters of this thesis, in genera of the grammitid ferns and Blechnoideae, in *Rumohra*, but also in *Phlegmariurus*.

All extant *Lindsaea* species of the Malagasy clade (included in clade IX) occur in tropical rainforests, mostly in the centre of the island (Tardieu-Blot, 1958). *Lindsaea* species are usually terrestrial, and epiphytes are only retrieved in clades V, VI, and in the Malagasy clade (Lehtonen *et al.*, 2010). Therefore, speciation of some Malagasy *Lindsaea* may have been caused by adaptation to the epiphytic habitat. *Lindsaea goudotiana* and *L. leptophylla* are specialized epiphytes with host specificity, respectively on tree ferns and palm stipes, and their distant phylogenetic position suggests that epiphytism is most probably a convergence. Other species are occasionally epiphytes, like *L. blotiana*, *L. flabellifolia* or *L. madagascariensis*, but they are found close to the ground or on fallen branches/trunks, suggesting a slightly modified habitat compared to other fully terrestrial *Lindsaea* species.

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

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

^a Muséum national d'Histoire naturelle, Sorbonne Universités, Institut de Systématique, Evolution, Biodiversité (UMR 7205 CNRS, MNHN, UPMC, EPHE), Herbar national, 16 rue Buffon CP39, F-75005 Paris, France

^b Université Pierre et Marie Curie, Sorbonne Universités, Institut de Systématique, Evolution, Biodiversité (UMR 7205 CNRS, MNHN, UPMC, EPHE), 57 rue Cuvier CP48, F-75005 Paris, France

^c Queensland Herbarium, Department of Science and Innovation; Australian Tropical Herbarium, James Cook University, POBox 6811, Cairns, Qld 4878, Australia.

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

*the authors contributed equally

Article in preparation, to be submitted to *Molecular Phylogenetics and Evolution*.

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 of 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 that includes 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*. 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 Early Eocene. The biogeographical analysis highlighted uncertainties concerning 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 the Boreotropics. Six long distance dispersal events over the last 40 Ma would explain the Malagasy species diversity of *Phlegmariurus*, as well as an important in situ diversification starting in Miocene.

KEYWORDS

Biogeography, molecular phylogeny, lycophytes, fossil, Southern Hemisphere, Western Indian Ocean

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 and 95% are angiosperms (Callmander, 2011). Approximately 600 species of ferns and lycophytes are estimated to inhabit Madagascar, whereas 800 are estimated in all Africa (Moran, 2008). The level of endemism is also relatively high and evaluated to 45.2% of endemism (Rakotondrainibe, 2003a), more than the Galapagos (7%) or New Caledonia (38.2%) (Moran, 2008; Morat *et al.*, 2012; Munzinger *et al.*, 2016). These high species richness and level of endemism raise the question 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 & 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 & Kuntner, 2012). Madagascar's biogeographical affinities have been found to be higher 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 & Wilson, 1967), Africa was found as a source of dispersal for numerous taxa towards Madagascar (e.g. Agnarsson & Kuntner, 2012; Zhou *et al.*, 2012; Blair *et al.*, 2015; Bacon *et al.*, 2016; Forthman & Weirauch, 2016). However, long distance dispersal events (LDD) for plants and animals were hypothesized from tropical Asia (Warren *et al.*, 2010; Federman *et al.*, 2015; Forthman & Weirauch, 2016) or from the Neotropics in plants (Smedmark *et al.*, 2014; Janssens *et al.*, 2016). Studies on ferns have highlighted that the Neotropics are an important source of dispersal for Madagascar in vascular spore-bearing plants (e.g. Rouhan *et al.*, 2004; Bauret *et al.*, 2017). Evidence of African dispersal events were also found (Korall & Pryer, 2014; Sessa *et al.*, 2017), as well as Asian affinities (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 diversification is a major process in the generation of Malagasy endemic fern flora (Bauret *et al.*, 2017, in Press.; Janssen *et al.*, 2008; Chao *et al.*, 2014; Labiak *et al.*, 2014; Hennequin *et al.*, 2017). It could be informative to test the monophyly of Malagasy lycophytes to observe if all the vascular spore-bearing plants have capable of such diversifications in Madagascar.

Phlegmariurus Holub is a pantropical genus of lycophytes 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 (Figure 1; Wikström, Kenrick, & Chase, 1999; Field *et al.*, 2016). *Phlegmariurus* is a monophyletic genus of Lycopodiaceae, forming with *Huperzia* and *Phylloglossum* the Huperzioidae subfamily (Figure 2A, Field *et al.*, 2016).

The biogeographical history of lycophytes is poorly understood and worldwide biogeographical analyses were done only recently on the cosmopolitan *Selaginella* (Klaus *et al.*, 2016), revealing a Devonian origin in Euramerica and a diversification coinciding with the formation of Pangea. On *Isoetes*, such analyses were provided by Kim & Choi (2016) but on a dataset reduced to North Pacific species, and are lacking on Lycopodiaceae genera, like *Phlegmariurus*. Huge uncertainties remain about the age of divergence of *Phlegmariurus*, with estimates in a large range of ages from 351 to 167 Ma (Wikström & Kenrick, 2001; Larsén & Rydin, 2016). 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) in the most comprehensive phylogenetic study (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, Field *et al.*, in prep).

The Western Paleotropical region is outstanding in harboring one of the world's highest proportions of endemic *Phlegmariurus* with 90 % of endemic species (Table 1, Field *et al.*, in prep.). Madagascar harbors 20 out of the 27 species estimated in the Western Paleotropical species and 14 of these are endemic to Madagascar (Table 1, Field *et al.* in prep). The Malagasy species of *Phlegmariurus* exhibit a spectrum of morphological diversity capturing most of the forms found in epiphytic *Phlegmariurus* globally (Figure 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 (Figure 1, E,G) and fimbriate leaf margins (Figure 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 *Tardieablottia* by Holub (1991) suggesting they were distantly related to all other species. Most recently they were placed in *Phlegmariurus* Holub by Field & 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.

	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 comb. ined. (Field <i>et al.</i>, in prep)	1*					
<i>P. cavifolius</i> (C.Chr.) A.R.Field & Bostock	1*	1				
<i>P. curiosus</i> (Herter) A.R.Field & Bostock	1					Only known from type collection. Possibly conspecific with <i>Phlegmariurus megastachyus</i>
<i>P. dacrydioides</i> (Baker) A.R. Field & Bostock					1*	
<i>P. gagnepainianus</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>P. hildebrandtii</i> (Herter) A.F.Field comb. ined. (Field <i>et al.</i> , in prep)	1					= <i>Huperzia squarrosa</i> in Madagascar, in Rakotondrainibe & Jouy (2015). = <i>Huperzia hildebrandtii</i> , in Roux (2009) Only know from type specimen.
<i>P. holstii</i> (Hieron.) A.R.Field & Bostock					1*	
<i>P. humbertii</i> (Nessel) A.R.Field & Bostock	1*					= <i>Huperzia teretirigida</i> in Rakotondrainibe & Jouy (2015)

	Madagascar	Mascarenes	Seychelles	Comoros	Continental Africa	Notes
<i>P. humbertii-henrici</i> (Herter) A.R.Field & Bostock	1*					
<i>P. lecomteanus</i> (Nessel) A.R.Field & Bostock	1*					= <i>Huperzia squarrosa</i> in Madagascar in Rakotondrainibe & Jouy (2015)
<i>P. megastachyus</i> (Baker) A.R.Field & Bostock	1*					
<i>P. obtusifolius</i> (P.Beauv.) A.R.Field & Bostock	1*	1				= <i>Huperzia pachyphylla</i> in Roux (2009) <i>Phlegmariurus pachyphyllus</i> in Field et al. (2016)
<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*					
<i>P. saururus</i> (Lam.) B. Øllg.		1		?	1	Taxonomic revision needed, possibly several species under the name, type from Mauritius Also know from French Southern and Antarctic Lands*
<i>P. staudtii</i> (Nessel) A.R.Field & Bostock					1*	

	Madagascar	Mascarenes	Seychelles	Comoros	Continental Africa	Notes
<i>P. strictus</i> (Baker) A.R.Field & Bostock	1*					
<i>P. tardieuae</i> (Herter) A.R.Field & Testo	1*	1*		1*	1*	= <i>Huperzia phlegmaria</i> var. <i>tardieuae</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*					
<i>P. sp1 "ulicifolius"</i> (Sw.) S.R.Ghosh				1*		Segregated from <i>Phlegmariurus squarrosus</i> in Comoros, morphologically close to <i>P. ulicifolius</i> (India)
<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>H. suberecta</i> (Lowe) Tardieu	?	?	?	?	?	<i>Huperzia s.s.</i> , in Field <i>et al.</i> (2016) <i>Huperzia suberecta</i> in Roux 2009 Presence in the tropics doubtful. Several literature records exist but not subtending specimens have been recovered
Total	20	8	3	5	9	27 species estimated for the Western Paletropics

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.

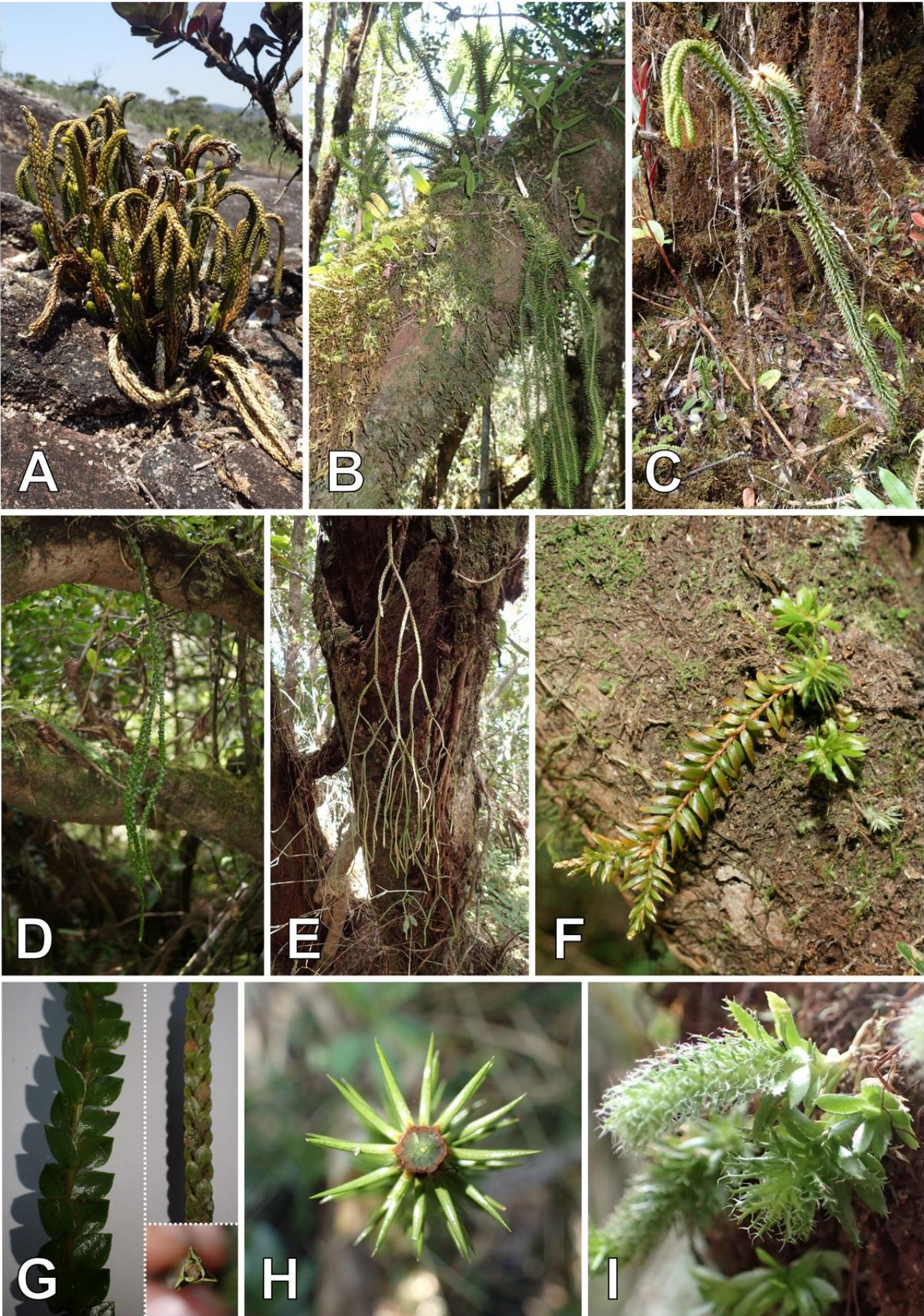


Figure 1. A-F: habitat diversity of *Phlegmariurus* in Madagascar, (A) *Phlegmariurus gnidioides*; (B) *Phlegmariurus lecomteanus*; (C) *Phlegmariurus megastachyus*; (D) *Phlegmariurus obtusifolius*; (E) *Phlegmariurus obtusifolius*, (F) *Phlegmariurus ophioglossoides*; G-I: diversity of microphyll shapes: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

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 Africa and the WIO: 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 13 Malagasy endemics (Table 1). Two outgroups were used: *Huperzia australiana* (Herter) Holub and *Phylloglossum drummondii* Kunze, known to form the sister-group to *Phlegmariurus* (Wikström & Kenrick, 2001; Ji *et al.*, 2008; Field *et al.*, 2016). This sampling was modified for dating and biogeography (see 1.4). Vouchers details and GenBank accession numbers are reported in Appendix 5.

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 hrs.

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 a 25 μ L volume containing 1x PCR buffer, 2.5 mM MgCl₂, 250 μ M of each dNTP, 1M 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 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 5).

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

Table 2. PCR primers sequences and thermal cycling conditions; primers only used as internal primers for sequencing are marked with an asterisk (*)

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 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 1,000 generations to obtain 10,000 sampled trees. We used Tracer 1.6.0 (Rambaut & 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 drumondii*, 16 *Huperzia* species, *Lycopodiella inundata* (L.) J. Holub from the Lycopodielloideae (*sensu* PPGI, 2016), *Diphasium 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 preserve in fossils (Field *et al.*, 2016). In particular, corticular 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 (Yang, Li, & Edwards, 2009; Xue, 2013), but this species exhibits anisotomous branching, rounded sporangia and the 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 species groups 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 & 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 & Lee, 2006). Some doubts exist as to the earliest record of *F. lacunosus* spores (Cieraad & 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 Huperzioideae fossils, we calibrated our analysis with four confident 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 (Kidston & Lang, 1920), *Hueberia zhichangensis* Yang, Li & Edwards (Bateman *et al.*, 2007; Yang *et al.*, 2009; Xue, 2013) and *Zhenglia radiata* Hao, Wang, Wang & Xue (Hao *et al.*, 2006). These fossils were dated in the Pragian Devonian (407.6-410.8 Ma, Lower Devonian) and the lower limit of this stage was used to constrain the maximum age of lycophytes. The lycophyte *Baragwanathia longifolia* Lang & Cookson 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 confident 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 stage 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 Visean as the minimum age.

Node calibrated	Uniform prior values (Ma)	Fossils	Stratigraphic occurrence age (Ma)
Tree height	382.7-410.8	Earliest lycophytes fossils	Lower Devonian, Pragian, 407.6-410.8
<i>Isoetes-Selaginella</i> divergence	382.7-410.8	<i>Yuguangia ordinata</i> Hao <i>et al.</i> (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-Lycopodioideae divergence	190.8-410.8	<i>Retitriletes</i> spp. Bomfleur <i>et al.</i> (2014)	Lower Jurassic, Sinemurian, 190.8-199.3

Table 3. Calibrated nodes for the dating analysis with BEAST

The minimum age of divergence between Lycopodielloideae and Lycopodioideae 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 Lycopodioideae species (but lost in *Pseudolycopodium* Holub and *Pseudodiphasium* Holub) (Juhász, 1975; Field *et al.*, 2016). Modern studies comparing *Retitriletes* and Lycopodioideae spores using scanning and transmission electron microscopies are lacking and it is important to note that several of the species assigned to the genus *Retitriletes* are dissimilar to modern Lycopodioideae (e.g. Hashemi-Yazdi, Sajjadi, & Dehbozorgi, 2015) and the genus is likely non-monophyletic.

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 and RaxML analyses 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 & 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, then to infer the ancestral ranges along the phylogeny of *Phlegmariurus*.

Eight areas were defined to code the distribution of the species (see map, Figure 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), plus 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, BHC, 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. Field). The maximum number of areas coded was set to four as it was the maximum number of area 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 (Figure 2). Firstly, the Neotropical clade (BS=74; PP=0.89) included mostly Neotropical species and two Paleotropical lineages: 1) one including the African species *Phlegmariurus bampsianus* and the Western Paleotropical *P. ophioglossoides* (in N1) and 2) the Western Paleotropical *Phlegmariurus verticillatus* (in N2). The Macaronesian *P. dentatus* was also retrieved in the Neotropical clade as well as the wide-ranged *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* (Figure 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 (Figure 2). The newly sampled Western Paleotropical species were inserted in 8 distinct positions, both in Malagasy clade and across Neotropical and Eastern Paleotropical clades (Figure 2). Within the Paleotropical clade, many of the species were arranged in a polytomy or among one of three Paleotropical clades, the Malagasy, the P1 and the P2 clades.

	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

Table 4. Statistics on the three separate plastid DNA regions and the combined dataset.

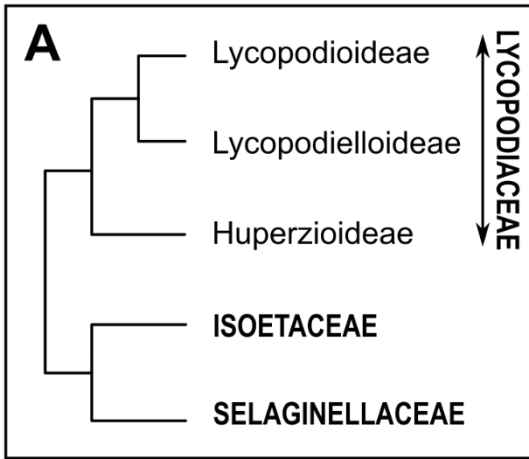
3.1.2. Composition of the two major clades

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

This arrangement is mirrored in the Paletropical clade which includes two morphologically equivalent subclades both including species from the Western Paletropics. All of the species within the moderately supported clade P1 (*P. tardieuae* to *P. trifoliatus*, BS<50; PP=0.98, Figure 2) are strongly heterophyllous biformic 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, Figure 2) includes a spectrum of heterophyllous to homophyllous hanging epiphytes, facultative epiliths and facultative terrestrials.

3.1.3. Composition of the Malagasy clade

This study was the first to recover a clade comprised entirely of Western Paletropical species (Malagasy clade, Figure 2) with 11 out of the 20 species estimated in Madagascar (including the enigmatic *P. pecten*) and out of the 26 estimated in the Western Paletropics. 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. teretirigida* Rakotondr. were inserted in this clade of *Phlegmariurus* (Figure 2). The Malagasy clade included many morphologically dissimilar Western Indian Ocean endemics such as *P. ambrensis* comb. ined., *P. cavifolius*, *P. lecomteanus*, *P. obtusifolius*, *P. pecten*, *P. pichianus* which repeat the morphological spectrum of *Phlegmariurus* found elsewhere in the Paletropics. This clade included both heterophyllous hanging epiphytes, homophyllous epiliths and facultative terrestrials. The morphologically singular species *P. gnidioides* and *P. obtusifolius* in particular 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 (Figure 2).



B

- Neotropics
- Western Paletropics
- Eastern Paletropics
- Other

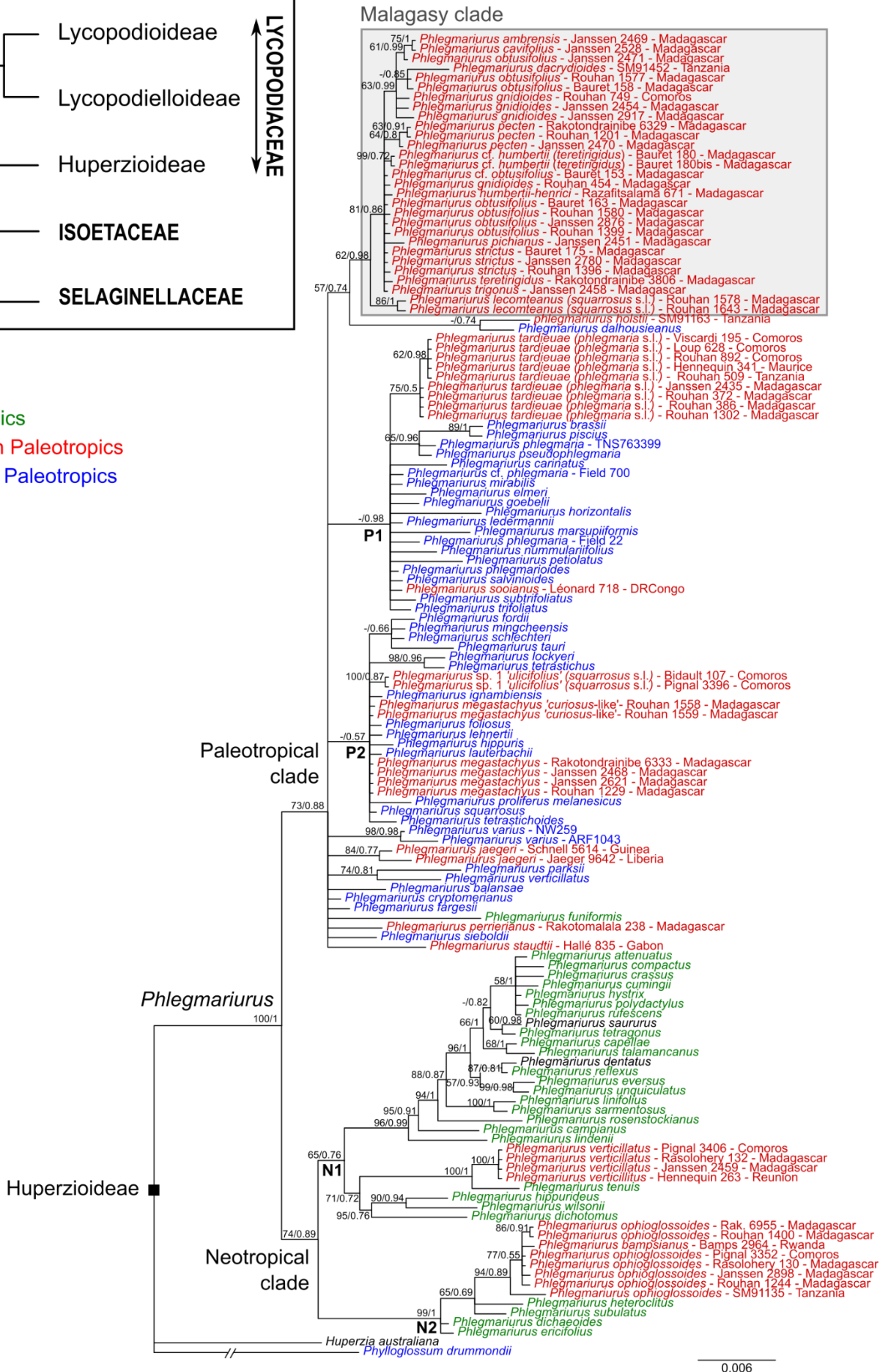


Figure 2. (A) Simplified tree of Lycopodiaceae, based on Field *et al.* (2016). (B) Majority rule consensus tree of *Phlegmariurus* estimated by Bayesian inference on the combined cpDNA dataset (*rbcl*, *trnH-psbA*, *trnL/trnL-trnF*), with support values from the Bayesian inference and Maximum likelihood 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 Paleotropics, blue for the Eastern Paleotropics and black for other regions (temperate North Hemisphere or wide distribution covering several regions).

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 (Figure 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, Figure 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 (Figure 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 (Figure 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* (Figure 2). Several species were not retrieved in the Malagasy, P1 or P2 subclades 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 (Figure 2).

3.1.5. Molecularly based clades versus morphologically defined groups

Many of the 21 intuitive morphological species groups proposed by Øllgaard (H2—H22; 1987, 1989) formed molecular clades. The erect Neotropical terrestrial species of the *P. reflexus* group (H20), the *P. saururus* group (H21) and the *P. brevifolius* group (H22) combined formed a well-supported clade (BS=96; PP=1). Within this clade the *P. saururus* and *P. brevifolius* groups (H21 & 22) intermingled form a clade (BS=66; PP=1) sister to a clade of the *P. reflexus* group (H20) including the Macaronesian *P. dentatus* (BS=57; PP=0.93). The *P. phlegmaria* group (H12a-k) was polyphyletic with members of its subgroups embedded amongst members of several other groups in both the Palaeotropical and Neotropical clades. Species from the *obtusifolius* subgroup (H12g) were embedded among species from the *P. gnidioides* group (H4) and species from the *P. carinatus* group H3 were embedded in the *P. phlegmaria* group (H12) and the *P. squarrosus* group (H13). Members of the *P. hippuris* group (H8) and *squarrosus* group (H13) were intermingled. The *verticillatus* group (H9) was the most markedly polyphyletic with species inserted in five different positions among the species sampled.

Several species included in previous studies were re-identified including *H. billardierii* from Wikström & Kenrick (2000) here re-identified as *P. varius* (R.Br.) A.R.Field & Bostock and a specimen misattributed to *P. hamiltonii* in Field *et al.* (2016) here tentatively re-identified as *P. lenhertii* Testo.

3.2. BEAST TOPOLOGY AND DIVERGENCE TIME ESTIMATES

Huperzia and *Phlegmariurus* were found in the same clade (PP=0.87, Figure 3) and strongly supported as monophyletic respectively (PP=1). *Phylloglossum drumondii* was retrieved sister species to the clade *Huperzia-Phlegmariurus* (PP=1). Within *Phlegmariurus*, the topology did not contradict the MrBayes topology and brought more resolution however 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, when *Phlegmariurus* would be estimated to diverge from *Huperzia* during the Upper Cretaceous 79.1 Ma (HPD: 46.2-122; Figure 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 (Figure 3).

Uncertainties between estimated ranges were found at *Phlegmariurus* crown node, as well as the node at divergence between *Huperzia* and *Phlegmariurus*. Indeed, numerous ranges were found with small probabilities ($p < 0.06$). 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), from Africa to WIO 2.0-0.0 Ma (LDD2), from Australasia to Madagascar 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. A diversification starting exclusively in Madagascar was estimated in the Malagasy clade ca. 10 Ma. It was the only diversification estimated in Madagascar for *Phlegmariurus*, and so for Huperzioideae.

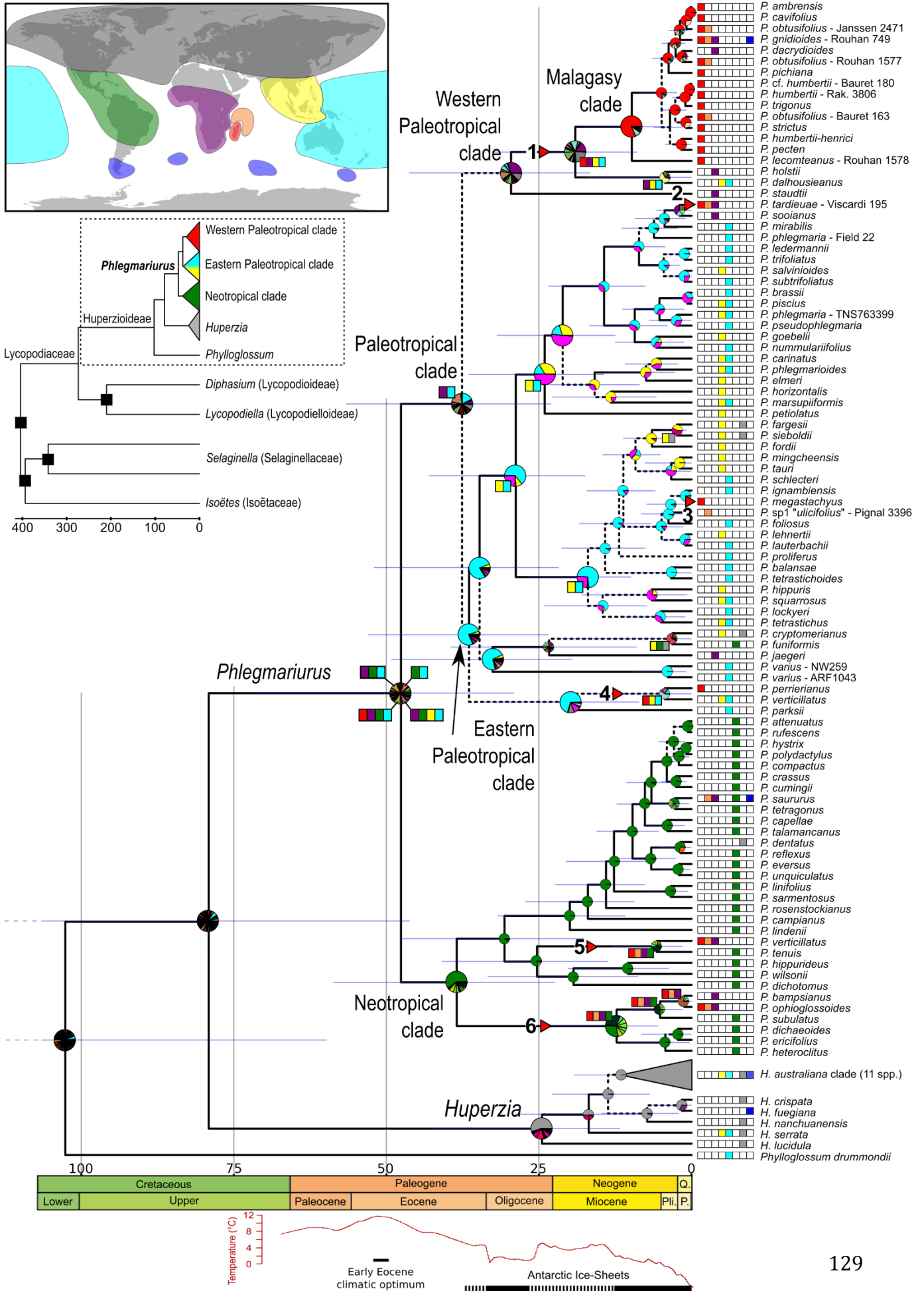
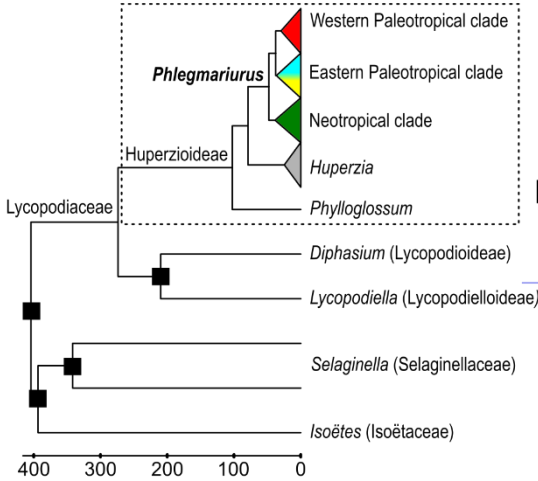
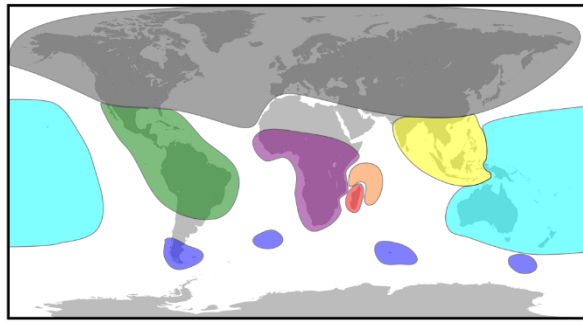


Figure 3. Chronogram resulting from the BEAST analysis for Huperzioideae and ancestral areas estimated by BioGeoBEARS (DEC+j model). Horizontal blue bars correspond to 95% HPD intervals of the median ages. Next to terminals, colored squares depict biogeographical distribution considering eight areas: 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 present next to pie charts, colored squares represent the combination of areas covered by a range. 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).

4. DISCUSSION

4.1. SYSTEMATICS

The phylogeny presented here provides evidence to address several systematics issues in *Phlegmariurus* of Madagascar, Africa and the Western Indian Ocean region. 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 *Tardieublottia* 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*) (Figure 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* and a new nomenclatural combination is provided for *P. ambrensis* (Rakotondr. & Jouy) A.R.Field and *H. teretirigida* is synonymised with the little known species *P. humbertii* (Nessel) A.R.Field & Bostock (Field *et al.*, in prep.). These species were correctly placed in the *gnidioides* group by Rakotondrainibe & 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* that delimits *P. tardieuae* as an endemic Malagasy distinct species from the WIO-Asian *P. phlegmaria* and that separates the Asian-Oceanian *P. verticillatus* from the Western Paleotropical type population of *P. verticillatus* s.s. which is nested in the Neotropical clade. Morphological features that distinguish these taxa are presented in Field *et al.* (in prep).

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 *P. hildebrandtii* which is of uncertain affinity possibly related to the *P. reflexus* group from the Neotropics (Øllgaard, 1989). Future sampling in the African region should focus on bifurmic species occurring in Africa such as

P. afromontanus and *P. ellenbeckii*. Based on their morphology it is expected these species belong to the Neotropical clade bifurmic subclade including *P. ophioglossoides* and their inclusion in future sampling could ascertain whether they represent a single or multiple trans-Atlantic dispersal to Africa. Future sampling across the Atlantic and Indian Oceanic regions should sample 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 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 & Rydin, 2016). These ages are younger than our results (resp. 273.6 Ma and 102.6 Ma) and could be explained by an under-sampling (only two *Phlegmariurus* species), which has already been correlated with younger age estimates (e.g. Linder, Hardy, & Rutschmann, 2005; Testo & Sundue, 2016). Our results are however much younger than ages estimated by Wikström & 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, the species and markers sampled and analytical method used.

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 during the last 40 Ma. 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 (Wolf *et al.*, 2001; Moran, 2008; Norhazrina *et al.*, 2016) and biogeographical connections in the Southern Hemisphere have been shown to be correlated with circumastral wind currents for many taxa of lycophytes, ferns or mosses (Parris, 2001; Sanmartín & Ronquist, 2004; Munoz *et al.*, 2004). If dispersal events cannot be excluded to explain the current distribution of *Phlegmariurus* across continents, circumastral 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 (Poole, Mennega, & Cantrill, 2003; Pross *et al.*, 2012; Cantrill, Tosolini, & Francis, 2013) 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 & 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 by following the global cooling after the Early Eocene climatic optimum and would have been definitively disrupted with the ice-sheet formation ca. 30 Ma (Figure 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 were separated from Antarctica between 165-132 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*, mostly diversified in the Northern Hemisphere, rather suggesting a common 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 (Wolfe, 1975; Morley, 2003). 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 amphi-Pacific or amphi-Atlantic distributions by the disruptions of the Boreotropics (e.g. Antonelli *et al.*, 2009; Hennequin *et al.*, 2010b; Couvreur *et al.*, 2011; Manns *et al.*, 2012; Liu *et al.*, 2013; 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.

Clues 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 (Brodribb & McAdam, 2011; McAdam & Brodribb, 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 (Schuettpelez & Pryer, 2009) and epiphytism acquisition could be an “enabler” trait mentioned by Donoghue & 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 (see 3.3.1).

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 (Passey *et al.*, 2009; Jacques *et al.*, 2011; Gupta *et al.*, 2015), which is compatible with our age estimates.

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 (Rouhan *et al.*, 2004, 2007a; Janssen *et al.*, 2007; Labiak *et al.*, 2014; Bauret *et al.*, 2017) 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 saw that LDD from these regions to Madagascar area plausible for *Phlegmariurus*. About

LDD2, the distribution of *Phlegmariurus tardieuae* in all the Western Indian Ocean avoid a clear estimate of the biogeographical origin of the dispersal event to Madagascar, which could be Africa or other WIO islands.

The study of *Phlegmariurus* brought new evidences about the existence of biogeographical connections between Western Paleotropical regions, in particular 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 evidences 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*. Indeed, if multiple LDD could be inferred from our results to explain the Malagasy diversity, diversification is a major process that have generated in a single event at least 11 species out of the 20 estimated in Madagascar (Table 1).

4.4.2. Diversification: a main driver of Malagasy diversity

The diversification of the Malagasy clade is the most localized ever documented for *Phlegmariurus* and even for Huperzioideae, 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 (see 2.1.5), which could reflect adaptations to different niches. The Malagasy clade species occupy similar geographic distribution in Madagascar, mostly in the centre region occupied by evergreen forests (Tardieu-Blot, 1971; Rakotondrainibe & Jouy, 2015), with an exception for *P. ambrensis*, which is endemic to the Montagne d'Ambre (Rakotondrainibe & 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 & Price, 2009).

The diversification in the Malagasy clade also highlighted morphological convergences, with species from similar habitats sharing similar traits, which supports the hypothesis of adaptation in the Malagasy forests. Species with slender filiform-funifiform shoots (e.g. *P. acerosus*, *P. verticillatus*, *P. filiformis*, *P. pichianus*) tend to occur as hanging epiphytes in cloud forest and upper montane rainforest; species with thick bottlebrush shoots comprised of slender spreading leaves like shoots (e.g. *P. acutifolius*, *P. dichotomus*, *P. lecomteanus*, *P. squarrosus*) tend to occur as mid canopy epiphytes in very wet valley forest; species with broad spreading leaves and markedly biformic shoots (e.g. *P. tardieuae*, *P. phlegmarioides*, *P. obtusifolius*, *P. ophioglossoides*) are generally canopy epiphytes in mid-montane to lowland rainforest, species with thick funifiform shoots of adpressed leaves (e.g. *P. ambrensis*, *P. carinatus*, *P. cavifolius*, *P. tetrastichus*) live in bright light exposed montane habitats and short growing biformic species with lingulate-lanceolate leaves and thick quadrangular or terete fertile species (e.g. *P. gnidioides*, *P. lockyeri*, *P. megastachyus*, *P. varius*) are commonly facultative epiphytes, epiliths or terrestrials on shallow soils.

Chapter VI - Overview of the biogeographical history of the Malagasy ferns

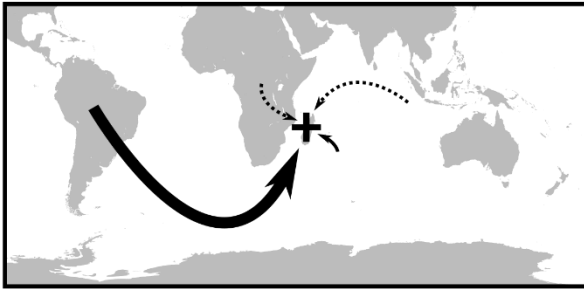
Madagascar, including nearby islands, is a biodiversity hotspot, comprising 2.3% of all plant species worldwide (Myers *et al.*, 2000). It is now widely accepted that Cenozoic dispersal events from Africa have played a great role to explain the presence of living organisms in Madagascar, although dispersal from other further areas and vicariance were also shown (Yoder & Nowak, 2006; Agnarsson & Kuntner, 2012). The Malagasy ferns, however, remained poorly studied.

Fern diversity is extremely high in Madagascar with ca. 600 species among which ca. 45% are endemic to the island (Rakotondrainibe, 2003a), and although evolving on a much larger area, the African fern diversity is barely more diversified with ca. 800 species (Introduction-Figure 8). Floristic affinities show that the Malagasy flora is mostly shared with the other islands of the Western Indian Ocean (WIO) and Africa, but also with further regions in the Neotropics and the Eastern Paleotropics (Asia to Australasia and Pacific; see Introduction). The first molecular phylogenetic analyses focused on Madagascar highlighted multiple dispersal from Africa and the Neotropics, as well as important in situ diversifications that could explain the endemism of Malagasy ferns (Rouhan *et al.*, 2004, 2007a; Janssen *et al.*, 2008). Additional molecular phylogenetic studies confirmed a Neotropical and African biogeographical connection with Madagascar but also showed that the Eastern Paleotropics can play a role in the biogeographical history of the Malagasy ferns (e.g. Chao *et al.*, 2014). The relative importance of these three source pools remain barely known and more data are required to draw a tendency, about the source pools but about the age of colonization as well.

An overview based on the literature and on new data obtained during my thesis will aim at (i) presenting the biogeographical origins of the Malagasy ferns in a spatio-temporal framework, (ii) discussing the factors that could have influenced dispersal, diversification and extinction, and (iii) proposing a summarized scenario of the biogeographical history of the Malagasy ferns.

1. THREE MAIN SPECIES SOURCE POOLS: AFRICA, THE NEOTROPICS AND THE EASTERN PALEOTROPICS

New analyses more focused on Madagascar, in grammitid ferns, *Rumohra*, Blechnoideae, *Lindsaea*, *Odontosoria* and *Phlegmariurus* (Figure 1), confirmed that Malagasy lineages of ferns and lycophytes originated from African, Neotropical and Eastern Paleotropical dispersal events. Additionally, another source pool in the Western Indian Ocean was highlighted (grammitid ferns, Figure 1). Information from the studies developed in Chapters I to V and from the literature was summarized in Table 1. Only the studies providing dating and ancestral area estimates were compiled, to compare with relevance the areas of origin and the age of dispersal to Madagascar.

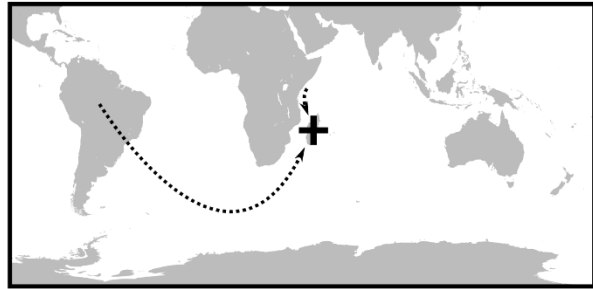


GRAMMITID FERNS - 900/31/13

- LDD to Madagascar: ≥ 14 within the last 20 Ma
- In situ diversification: 5 events (≥ 21 spp.)

Sources of direct dispersal to Madagascar:

- Neotropics (≥ 4 LDD events)
- Western Indian Ocean (1 LDD event)
- Eastern Paleotropics?
- Africa?

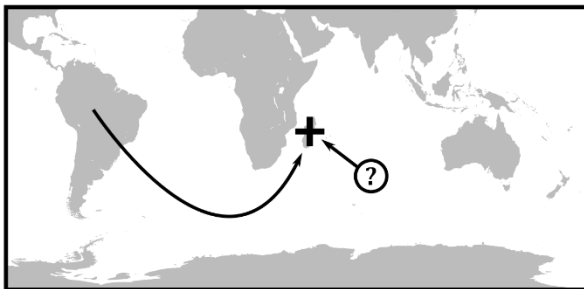


RUMOHRA - 7/4/3

- LDD to Madagascar: 1 ca. 10 Ma
- In situ diversification: 1 event (4 spp.)

Sources of direct dispersal to Madagascar:

- Neotropics or Comoros

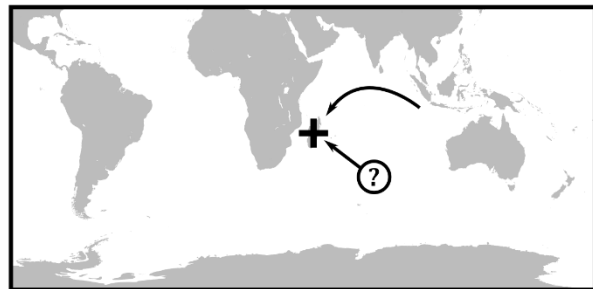


BLECHNOIDEAE - 239/15/8

- LDD to Madagascar: 5 within the last 50 Ma
- In situ diversification: 2 events (9 spp.)

Sources of direct dispersal to Madagascar:

- Neotropics (≥ 1 LDD event)
- Unkwnow (wide ancestral ranges inferred, covering tropical regions of the Southern Hemisphere)

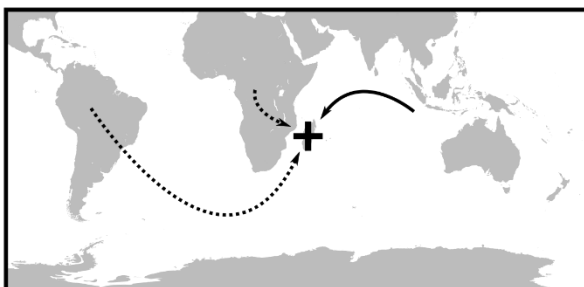


LINDSAEA 150/14/12 & ODONTOSORIA - 23/2/1

- LDD to Madagascar: 4 within the last ca. 65 Ma
- In situ diversification: 1 event (10 spp., *Lindsaea*)

Sources of direct dispersal to Madagascar:

- Australasia (≥ 2 LDD events)
- Unkown (wide ancestral ranges inferred, covering tropical regions of the Southern Hemisphere)



PHLEGMARIURUS - (300/20/14)

- LDD to Madagascar: 6 within the last ca. 40 Ma
- In situ diversification: 1 event (11 spp.)

Sources of direct dispersal to Madagascar:

- Australasia (≥ 1)
- Africa?
- Neotropics?

Figure 1. Summary of the results from the Chapters I to V, focused on the dispersal events to Madagascar (arrows) and in situ diversification (+). Each map represents the results of one chapter, with the name of the taxa, followed by numbers: worldwide species diversity/Malagasy species diversity/Malagasy endemic species. The number of dispersal and diversification events inferred are indicated (with the number of species having diversified in Madagascar), together with the detail of the biogeographical sources. On the maps, arrows with dotted lines represent hypothesized (but uncertain) origins and question marks represent unknown areas of origin of Malagasy fern lineages.

1.1. NEOTROPICS

The Neotropics were already hypothesized to be an important source of diversity for Madagascar, based on floristic and biogeographical affinities (e.g. Moran & Smith, 2001; Rouhan *et al.*, 2004). Chapter I confirmed the importance of eastward trans-Atlantic dispersal in grammitid ferns, by estimating at least four direct LDD to Madagascar (Figure 1, Table 1). Similar results in two genera belonging to two distinct families, *Lomariocycas* (Blechnaceae, Chapter III) and *Rumohra* (Dryopteridaceae, Chapter II, however with uncertainty with Comoros) highlighted that the process was not an exception in the biogeographical history of Malagasy ferns. My results also showed that eastward trans-Atlantic dispersal is a process involved in the biogeographical history of another group of vascular spore-bearing plants, in *Phlegmariurus* (Lycopodiaceae, Chapter V), but the analysis did not allow to infer whether dispersal occurred directly from the Neotropics to Madagascar, or whether there were intermediate stepping-stones (Malagasy species being widespread in the Western Paleotropics, chapter V). Out of the 40 dispersal events recorded in Table 1 (without uncertainties on the estimates), such trans-Atlantic dispersal were retrieved 16 times in the recorded studies, including five direct dispersal events to Madagascar.

All inferred trans-Atlantic dispersal events would be under the influence of the southern Prevailing Westerly winds (also called West Wind Drift, however the name refers more to the oceanic current), which are circumaustral winds connecting southern continents like South America, Africa-Madagascar and Australia (Sanmartín, Wanntorp, & Winkworth, 2007). Moreover, modelling of the trajectory of spores from Brazil showed that an arrival in Madagascar via the South Atlantic Ocean is theoretically possible (Meza Torres *et al.*, 2015) and therefore supports the hypothesis of direct LDD from Neotropics to Madagascar.

1.2. EASTERN PALEOTROPICS

Comprising regions from the tropical continental Asia to Australasia and the Pacific, the Eastern Paleotropics cover areas renowned for their tropical biodiversity (Southeast Asia being one of the richest place of fern diversity, Introduction-Figure 8) that would be biogeographically linked to Western Paleotropics, notably Madagascar (Warren *et al.*, 2010; Buerki *et al.*, 2013). Given the biogeographical affinities between Madagascar and Asia and the important biodiversity, the region was expected to be another important source pool for Madagascar. This was confirmed by data from the literature as well as my results, with 14 dispersal events to the Western Paleotropics, including six direct dispersal events to Madagascar (Tableau 1).

The available data were however too scarce to estimate the relative importance of regions like Southeast Asia, Melanesia or Australasia in the biogeographical history of Malagasy

ferns, as they were not similarly coded in the surveyed studies. Some authors were indeed focused on the Eastern Paleotropics like Chao *et al.* (2014), whereas Hennequin *et al.* (2010b) worked at a global scale and coded the entire region as only one area. However, continental and tropical Asia (Chao *et al.*, 2014) or Australasia (Lindsaeaceae, Chapter III) were inferred as ancestral ranges, suggesting that different inclusive regions of the Eastern Paleotropics could act as species source pools for Madagascar. Dispersal to Madagascar could be explained by direct dispersal thanks to the Asian monsoons that blow from continental Asia to the WIO during winter. Warren *et al.* (2010) also suggested that islands could have been stepping stones for Asian dispersers, as well as the drifting Indian subcontinent after its separation from Madagascar 90 Ma (McLoughlin, 2001).

1.3. AFRICA

The Theory of Island Biogeography (MacArthur & Wilson, 1967) predicts that the nearest continent would be the main species source pool of an island. For Madagascar, its geographic position in the WIO near Africa suggests that this continent should be the main source pool of dispersal. Floristic and biogeographical affinities were found between the continent and Madagascar in angiosperms and ferns (Introduction; e.g. Buerki *et al.*, 2013; Eiserhardt *et al.*, 2011) and some studies found Africa as a source for dispersal to Madagascar (Janssen *et al.*, 2008; Labiak *et al.*, 2014; Sessa *et al.*, 2017). However, the recorded biogeographical results from the literature and the present thesis showed the Neotropics and the Eastern Paleotropics at least as important as Africa, although these two regions are much farther away: respectively five and six direct dispersal from the Neotropics and the Eastern Paleotropics were inferred to Madagascar, against five from Africa (Table 1). Several studies unfortunately relied on incomplete samplings of the African flora that could bring bias in the ancestral area estimates (e.g. Chao *et al.*, 2014).

Several factors could also explain why Africa was not retrieved as the dominant source region compared to the Neotropics and the Eastern Paleotropics: the relative species-richness and size of the species source pools and ecological preferences of ferns for mountainous rainforests.

1.3.1. Species richness and size of the species source pools

The Neotropics, the Eastern Paleotropics (especially Southeast Asia) and Africa represent the most species-rich regions for ferns (Introduction-Figure 8). The Neotropics and Southeast Asia are however four to six times more species-rich than Africa, so they are the most species-rich pools among potential sources. Ferns are dependent on humid environments like rainforests which cover much more surface in the Neotropics and the Eastern Paleotropics than in Africa (Figure 2A). In Africa, rainforests are mostly reduced to an equatorial and subequatorial belt that extends from West Africa into the Congo basin (*sensu* Corlett & Primack, Figure 2A). The distance between Madagascar and the Neotropics or Southeast Asia could be counterbalanced by the abundance of ferns, the species-richness and the surface that would increase the probability of successful dispersal compared to Africa.

One of the most widely cited causes underlying the lower African species diversity is extinction (Couvreur, 2015, and references therein) that occurred during the Cenozoic, due to climatic events. A global cooling after the Early Eocene climatic optimum and the

closure of the Tethys Seaway would have resulted in the global decline of rainforests and Africa would have been more impacted than the other continents (Pan *et al.*, 2006; Morley, 2007; Couvreur, 2015). A warmer and moister climate provided a time of diversification in Africa during the Early Pliocene, but another climate change during the Late Pliocene (3.6–2.6 Ma) triggered a new decrease of rainforests and species extinctions (Morley, 2000). The decrease of tropical rainforests in Africa and extinction of numerous angiosperm lineages could have impacted the African ferns, by decrease and loss of their habitats.

Extinction of African fern lineages could have two consequences: i) less dispersal events to Madagascar, meaning a decrease of the importance of Africa in the biogeographical history of the Malagasy ferns, and ii) biases in biogeographical analyses, in which the African origins cannot be inferred because the parent lineage went extinct. Moreover, studies highlighted that some African fern lineages have originated from Neotropical or Asian ancestors (Janssen, Kreier, & Schneider, 2007; Labiak *et al.*, 2014; Sessa *et al.*, 2017), which could be erroneously inferred as parent of a Malagasy lineage that has originated from the extinct African lineage, reinforcing the apparent role of other tropical regions.

1.3.2. Ecological preferences

If African rainforests represent a relatively small and poor species source pool to Madagascar compare to the Neotropics and the Eastern Paleotropics, the effective African source pool could be even smaller, due to ecological preferences of ferns for mountainous regions (Moran, 2008; Kessler, Karger, & Kluge, 2016), especially dealing with Malagasy ferns.

Among all the African regions, Malagasy ferns show the highest floristic affinities with the mountainous regions of East Africa (Aldasoro, Cabezas, & Aedo, 2004; Liu *et al.*, 2016). In ferns, dispersal events recorded in Table 1 from Africa also show connections with these regions: numerous *Dryopteris* species are distributed in East Africa, notably species found relatively close to the Malagasy endemic *Dryopteris subcrenulata* (Sessa *et al.*, 2017), and in *Cyathea*, the species found sister to the Malagasy lineages are endemic to the eastern African mountains (Edwards, 2005; Korall & Pryer, 2014). The affinities with these regions could be explained by the ecological disequilibrium of the Malagasy fern flora itself, as 85% of the Malagasy ferns occur in the elevated Central phytogeographic domain that covers mountainous regions (Rakotondrainibe, 2003b).

Moreover, the eastern African mountains represent a relatively small source pool compared to all the African tropical rainforests (Figure 2C) or compared to the mountainous regions in the Neotropics and the Eastern Paleotropics (Figure 2A, B), notably listed in the biodiversity hotspots (Myers, 2000).

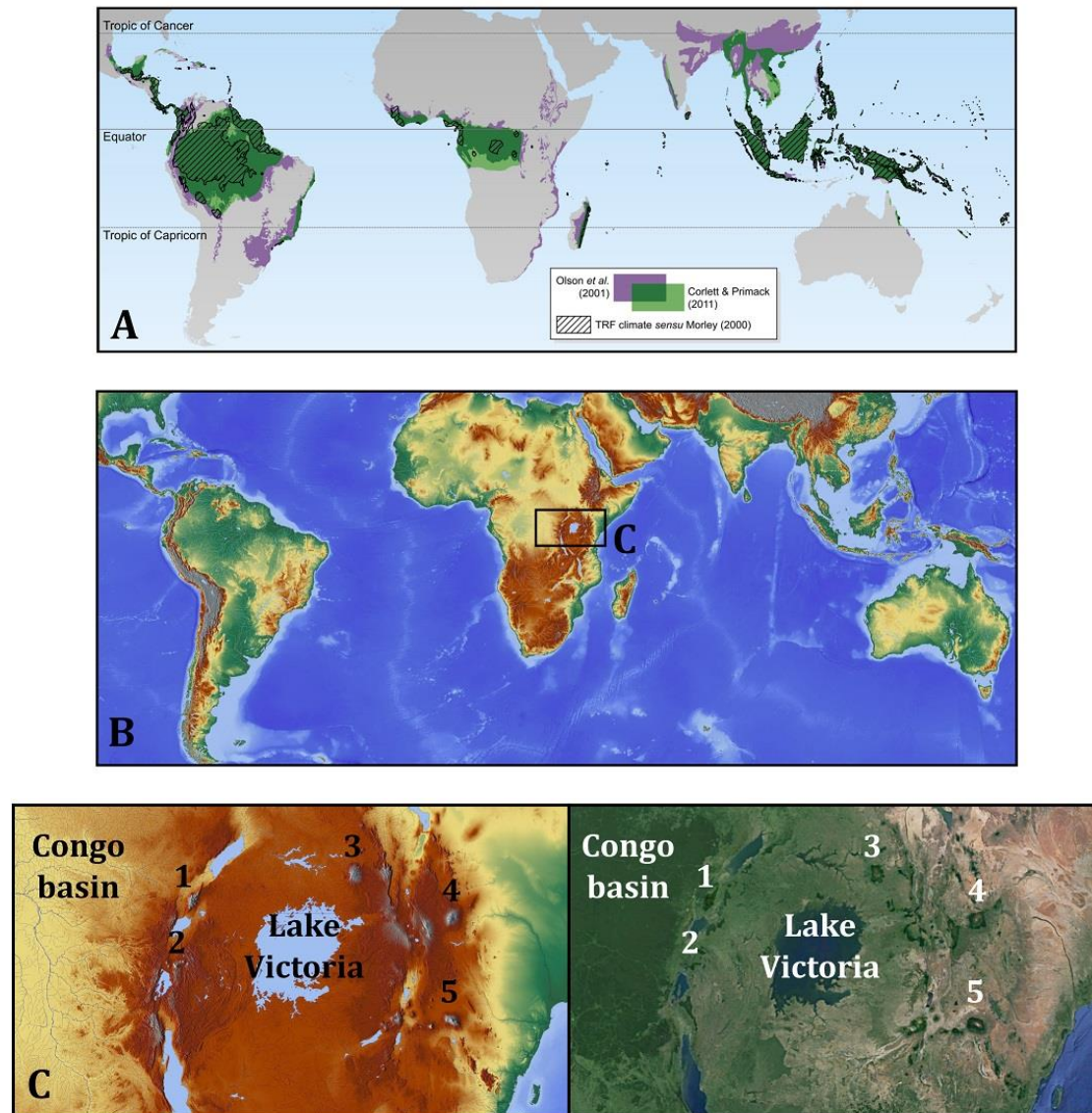


Figure 2. Tropical rainforests and mountains of the world, with a focus on Africa. A. Delimitation of tropical rainforests according to different authors, from Eiserhardt, Couvreur, & Baker (2017). Olson et al's definition often referred to tropical rainforest sensu lato, and includes non-tree rainforest vegetation like the Malagasy high plateau. TRF = tree rainforest. B. Topographic map, the relative altitude being coded by colors: green being the lowest altitudes, dark brown and grey the highest. (Source: pixabay.com). C. Mountainous rainforests of East Africa, around Lake Victoria, left: topographic map following the same color scheme as B, right: satellite view from Google Maps (19th September, 2017), the rainforests being visible by dark green patches around the peaks and in Congo basin, 1: Mount Stanley (5109 m), 2: Mount Karisimbi (4507 m), 3: Mount Elgon (4321 m), 4: Mount Kenya (5199 m) and 5: Mount Kilimanjaro (5895 m).

1.4. THE WESTERN INDIAN OCEAN

A growing body of evidence show that islands are not the sink of biodiversity previously hypothesized, notably for spore-bearing plants (Hutsemékers *et al.*, 2011). In the WIO, Madagascar seems to be a major source of species diversity for the other WIO islands, playing the role of a continental source in angiosperms (e.g. Strijk *et al.*, 2012) but also in ferns, e.g. in grammitid ferns (Chapter I) and in *Ctenitis* (Hennequin *et al.*, 2017).

Surprisingly, a dispersal event from the small WIO islands to Madagascar was inferred in grammitid ferns over the last 1.2 Ma (*Ceradenia comorensis*, Chapter I). Mascarenes, Seychelles and Comoros were coded in a same range that was inferred as the range of origin of this dispersal event, so the island of origin remains unknown, but *Ceradenia comorensis* is only distributed in Madagascar and Comoros, suggesting a dispersal from this archipelago. Other dispersal from the small WIO island could be expected, especially from La Réunion with which Malagasy ferns show high floristic affinities (Introduction-Figure 9).

	Neotropics	Age	Eastern Paleotropics	Age	Africa	Age	Other	Age
Athyriaceae								
<i>Deparia</i> Kuo <i>et al.</i> (2016)							n.a. (?)	≤ 6.7
Blechnaceae								
<i>Lomaridium</i> Chapter III							LDD1 (?)	50.9-19.7
<i>Cranfillia</i> Chapter III							LDD2 (?)	≤ 34.0
<i>Blechnum</i> Chapter III							LDD3 (?) <i>Blechnum australe</i> (polyphyly)	≤ 35.8 n.a.
<i>Lomariocycas</i> Chapter III	LDD4	9.6						
<i>Parablechnum</i> Chapter III							LDD5 (?)	29.0-12.1
Cyatheaceae								
<i>Alsophila</i> Janssen <i>et al.</i> (2008) Korall & Pryer (2014)					<i>A. acutula</i> clade <i>A. lastii</i> clade	ca. 8.0 ca. 5.0		
<i>Cyathea</i> Janssen <i>et al.</i> (2008) Korall & Pryer (2014)					<i>C. andohahelensis</i> clade	ca. 10		
Dryopteridaceae								
<i>Ctenitis</i> Hennequin <i>et al.</i> (2017)	<i>C. cirrhosa</i> - <i>C. sieberiana</i> clade* (?)	10.4-6.1						
<i>Dryopteris</i> Sessa <i>et al.</i> (2017)					<i>D. subcrenulata</i> <i>D. comorensis</i> * <i>D. manniana</i> *	ca. 5.0 ca. 5.0-0.0 ca. 5.0-0.0	<i>D. pentheri</i> (polyphyly) <i>D. squamiseta</i> (?) <i>D. antarctica</i> (polyphyly)	n.a. ≤ ca. 5.0 n.a.
<i>Parapolystichum</i> Labiak <i>et al.</i> (2014)					<i>P. barterianum</i> - <i>P. vogelii</i> clade (<i>Lastreopsis</i> s.l.)	ca. 23.0-7.0		
<i>Polystichum</i> Le Péchon <i>et al.</i> (2016)			<i>P. ammifolium</i> - <i>P. maevaranense</i> clade*	≤ 20.0 (precise age not shown)				

	Neotropics	Age	Eastern Paleotropics	Age	Africa	Age	Other	Age
<i>Pteris</i> Chao <i>et al.</i> (2014)			<i>P. catoptera</i> - <i>P.</i> sp2 clade <i>P. friesii</i> * <i>P. pteridioides</i> * <i>P. vittata</i> *	ca. 5.0 ca. 8.0 ≤ ca. 8.0 ca. 13.0-0.0			<i>P. biaurita</i> (?) <i>P. cretica</i> (?) B2 (?) <i>P. tripartita</i> (?)	ca. 1.0-0.0 ca. 1.0-0.0 ca. 7 ca. 3-0.0
<i>Rumohra</i> Chapter II	LDD2*	9.8						
Lindsaeaceae								
<i>Lindsaea</i> Chapter IV			L2	9.9-8.6			L1 (?)	45.8-35.8
<i>Odontosoria</i> Chapter IV			O2	64.4-25.0			O1 (?)	64.4-36.5
Nephrolepidaceae								
<i>Nephrolepis</i> Hennequin <i>et al.</i> (2010b)			<i>N. biserrata</i> * <i>N. acutifolia</i> * <i>N. abrupta</i> * <i>N. cordifolia</i> *	ca. 5.0-0.0 ca. 3.0-0.0 ca. 22-0.0 ca. 2.0-0.0				
Polypodiaceae								
<i>Alansmia</i> Chapter I	LDD6*	5.9-0.0						
<i>Ceradenia</i> Chapter I	LDD7*	12.2-7.4					<i>Ceradenia comorensis</i> (WIO)	1.2-0.0
<i>Cochlidium</i> Chapter I	LDD2*	10.3-0.0						
<i>Enterosora</i> Chapter I	LDD1*	3.7-0.0						
<i>Grammitis</i> s.s. Chapter I	LDD3 LDD4*	3.7-3.0 19.1-10.2						
Genus nov. (inc. <i>Ctenopterella</i> and <i>Grammitis</i> species) Chapter I			LDD13	23.7-23.5				
<i>Leucotrichum</i> Chapter I	LDD5	8.0-5.1						
<i>Melpomene</i> Chapter I	LDD9*	1.7-0.0						

	Neotropics	Age	Eastern Paleotropics	Age	Africa	Age	Other	Age
<i>Moranopteris</i> Chapter I	LDD12	10.7-0.6						
<i>Stenogrammitis</i> Chapter I	LDD10	15.1-14.6					LDD11 (?)	≤2.4
<i>Zygophlebia</i> Chapter I	Clade III*	12.9-6.7						
Lycopodiaceae								
<i>Phlegmariurus</i> Chapter V	LDD5* LDD6*	25.3-5.7 38.5-12.3	LDD3 LDD4	1.0 19.8-4.4	LDD2*	2.0-0.0	LDD1 (?)	29.6-19.1

Table 1. Dispersal events to a range including Madagascar, recorded from the literature and the present dissertation. The table summarizes a survey in the literature that recorded studies including ancestral areas estimates and dating, it was constructed as followed: 1) each Malagasy lineage that has originated from a dispersal event was recorded in a line of the table, 2) when the dispersal event was already named in the relevant study, the name was kept (e.g. *Moranopteris*: LDD10), if not, the lineage was named on the basis of the associated clade (e.g. *Zygophlebia*: Clade III), 3) when the source of the dispersal was clearly inferred to be Africa, the Neotropics or the Eastern Paleotropics, it was recorded in the relevant column, whereas dispersal events from unknown (?) or other sources were recorded in the last column. However, exception was done in case of uncertainties with other Western Paleotropical areas: the dispersal event was recorded in the relevant column and highlighted by an asterisk (e.g. *Rumohra*: LDD2*, from the Neotropics to Madagascar or Comoros; *Phlegmariurus*: LDD5*, from the Neotropics to Madagascar and other areas of the Western Paleotropics), 4) the age of dispersal was then recorded on the same line: a unique age stands for a lineage that has diverged into Madagascar (e.g. *Phlegmariurus*: LDD3), whereas a range of ages stands when the exact age of dispersal is unknown (e.g. *Phlegmariurus*: LDD2). A minimum age was recorded in case of important uncertainties in the biogeographical history of the Malagasy lineage. Dispersal events were graphically represented in a chronological framework in Figure 3 (except when the source was unknown).

2. CENOZOIC ORIGINS, WITH INTENSIFICATION OF ARRIVALS SINCE MIOCENE

Regarding all the source pools and events recorded in Table 1 and Figure 3, direct dispersal to Madagascar would have occurred over the last ca. 65 Ma to nowadays, highlighting the Cenozoic age of the Malagasy ferns. These relatively young ages compared to the old origin of ferns could be explained by the youth of the studied lineages themselves, combined to geologic or climatic events.

Indeed, a first observation is the relative young age of divergence of the genera recorded in Table 1, the older having diverged ca. 90 Ma (*Nephrolepis*, Hennequin *et al.*, 2010b) and all of them having started their diversification in the last ca. 65 Ma (Figure 3). Moreover, most of these genera started to diversify in the Neotropics, tropical Asia or Africa, leading to a delay before a dispersal to Madagascar occurred, but this delay could also be explained by other limiting factors: the Cenozoic age of the Malagasy rainforest, the onset of some wind currents and the history of the biogeographical sources.

2.1. A CENOZOIC MALAGASY FOREST

It was only after the northward migration of India that humid winds from the Indian Ocean could have reached the eastern coast of Madagascar, allowing the onset of rainforests ca. 60 Ma. A real establishment of those rainforests would have started after a reinforcement of rainfalls ca. 35 Ma (Wells, 2003). The Cenozoic age of the Malagasy rainforests could explain the Cenozoic age of the Malagasy fern flora, as the presence of rainforests (and so the presence of humid environments) in Madagascar is an important prerequisite to fern colonization. However, on the basis of the current data available on the Malagasy fern lineages (Figure 3), it seems that most of them have colonized Madagascar more recently. Out of the 15 direct LDD to Madagascar recorded, at least 11 would have occurred in the last ca. 10 Ma. This tendency was also retrieved for LDD events to Western Paleotropical ranges including Madagascar, with at least 17 out of 23 dispersal events being recorded for the last ca. 10 Ma.

2.2. MID-CENOZOIC WIND CURRENTS

The circumaustral wind current explaining eastward dispersal events (e.g. from the Neotropics to Madagascar) is considered to have started at the Eocene-Oligocene boundary ca. 35-30 Ma ago (Sanmartín *et al.*, 2007). However, the onset of this circulation does not seem to coincide with most of the dispersal events from the Neotropics to Madagascar, which would be more recent (Figure 3). Indeed, despite their Neotropical origin that predates the establishment of a circumaustral circulation, the grammitid ferns, *Lomariocycas* (Blechnaceae) and *Rumohra* (Dryopteridaceae) colonized Madagascar only over the last 10 Ma, suggesting that other factors were limiting.

Considering tropical Asia, however, dispersal events to Western Paleotropics fully coincide with the onset of the Indian monsoons during mid-Miocene (Jacques *et al.*, 2011; Gupta *et al.*, 2015) and only the LDD recorded for *Odontosoria* is even much older (ca. 64-25 Ma). This latter could be explained by extinct wind currents generated by the past geologic and climatic configuration or/and by stepping stone dispersal by Indian during its Northward drifting in the Indian Ocean (Warren *et al.*, 2010).

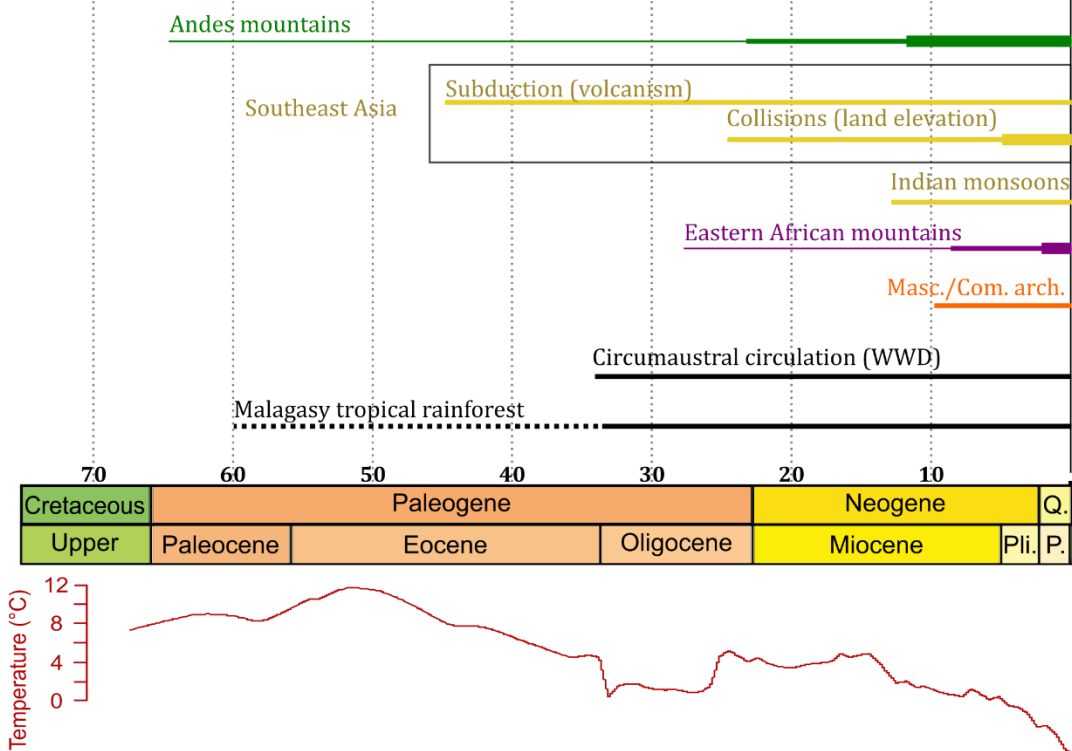
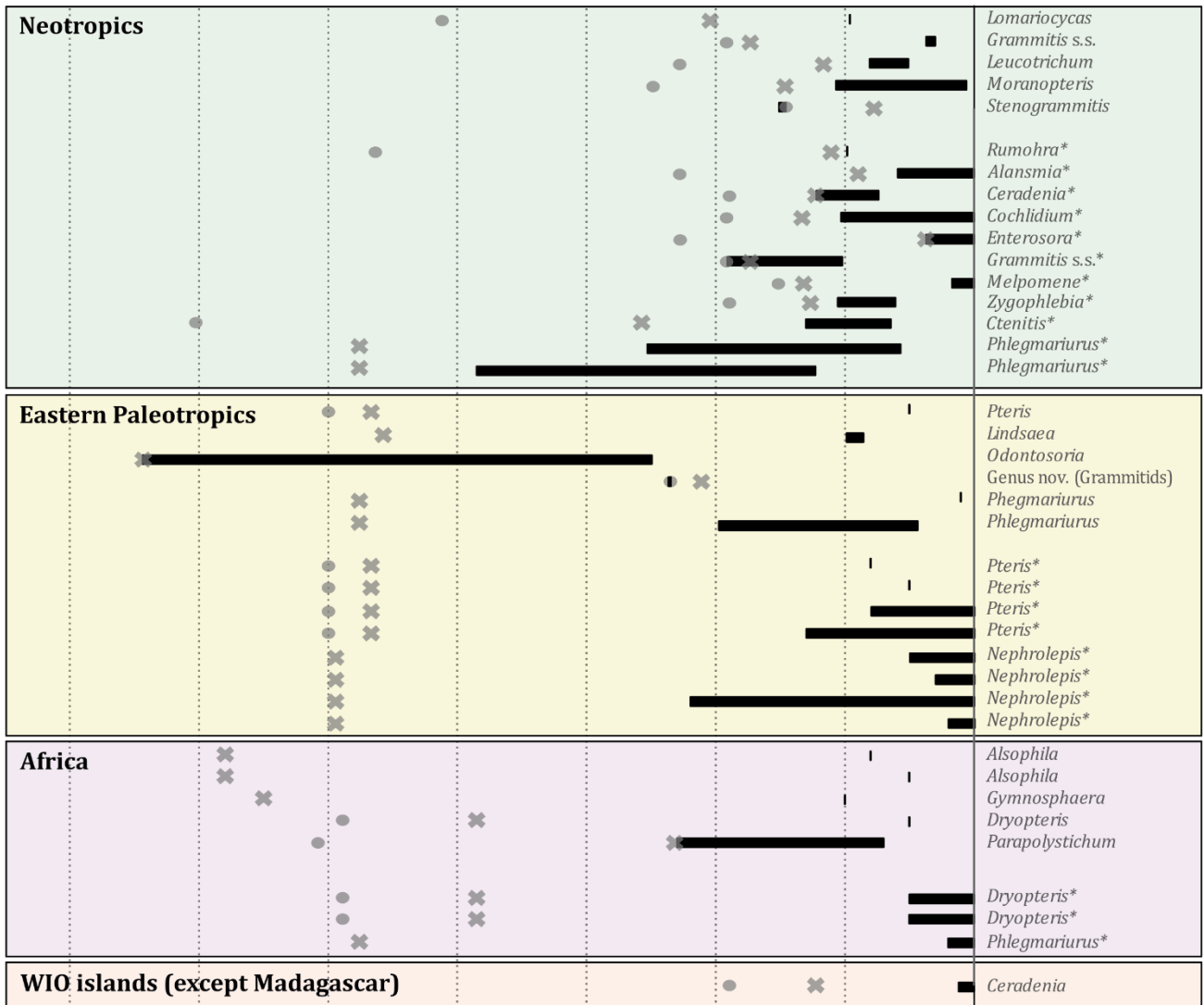


Figure 3. Ages of dispersal events to Madagascar, as recorded in Table 1 from the literature and the present thesis, in relation to the area of origin and climatic and geologic events (at the bottom). One line represents one dispersal event to Madagascar. An asterisk stands for a LDD event to a range including Madagascar and at least another Western Paleotropical area, or when Western Paleotropical areas were found with similar probabilities as Madagascar. On the same line, a grey dot represents the age of divergence of the whole genus and a grey cross its age of diversification (ages of divergence older than 75 Ma were not represented). Climatic and geologic events references were given in the text (see part 2.3). Stratigraphy follows the International Chronostratigraphic Chart v.2017.02 and global temperature was redrawn from Zachos (2001).

2.3. BIOGEOGRAPHICAL HISTORY OF THE SOURCE POOLS: IMPORTANT DIVERSIFICATIONS STARTING DURING THE NEOGENE DUE TO GEOLOGICAL EVENTS

Understanding the biogeographical history of Malagasy ferns requires to know the history of the biogeographical sources. We saw that extinctions in Africa could have decreased the importance of the continent in the biogeographical history of the Malagasy ferns, but other events could also explain the sudden importance of the Neotropics and the Eastern Paleotropics starting from 10 Ma and even why dispersal from Africa occurred so recently.

When some prerequisites elements to fern colonization to Madagascar were established, like the Malagasy rainforest and the circumaustral wind circulation (ca. 35-30 Ma; Wells, 2003), the biogeographical source pools in their modern form did not yet exist, preventing numerous dispersal and colonization of Madagascar. Especially, the tropical mountains that provide rich place of species diversity sharing the same ecological preferences as the extant Malagasy ferns mostly appeared over the last 10 Ma.

In South America, many modern plants and animals have started their diversification with the Andes orogenesis during the Miocene (Hoorn *et al.*, 2010), like ferns in *Polystichum* (McHenry & Barrington, 2014). Diversification in grammitid ferns genera and *Lomariocycas* (Chapter I,III) also coincide with the orogenesis, as the first mountains appeared by the late Oligocene to early Miocene (ca. 23 Ma) with an intensification of the phenomenon during the late middle Miocene (ca. 12 Ma) and early Pliocene (ca. 4.5 Ma, Hoorn *et al.*, 2010).

At the end of the Oligocene (ca. 25 Ma), the orogenesis of Southeast Asian mountains started, due to the collision of the Australian plate with the Pacific plate (Hall, 2009). However, numerous lands emerged later, with for example Sumatra, Java, Sulawesi and the Moluccas emerging since 5 Ma. Another example is the elevation of New Guinea that began only ca. 10 Ma, the present height being reached only ca. 5 Ma (Hall, 2009). The collision and the resultant uplifts have also impacted the regional climate, by holding the moisture from the warm Pacific Ocean. This, in turn, allowed the establishment of rainforests and an increase in diversity during the Miocene in the region (Morley, 2012). Diversification that predate dispersal to Madagascar notably coincide with these geological events in *Lindsaea*, grammitid ferns and *Phlegmariurus*.

Similarly, the mountains of East Africa are relatively young. Firstly, Eastern Arc Mountains blocks have been reactivated by the development of the East African Rift System the last seven million years to form the modern mountains found in Tanzania (Griffiths, 1993). Secondly, numerous volcanoes of the region would have originated over the last eight

million years (Dawson, 1992; Griffiths, 1993). The highest summit of Africa, the Mount Kilimanjaro, notably appeared between 2.5 and 1.9 Ma (Nonnotte *et al.*, 2008). Here again, the emergence of mountains has attracted orographic precipitation and led to the formation of rainforests during the mid-Miocene, which coincides with the ages of tropic-montane and tropic-alpine angiosperms (Linder, Pennington, & Schneider, 2014) and with dispersal events of ferns to Madagascar (Figure 3).

3. MADAGASCAR: AN IMPORTANT ROLE OF IN SITU DIVERSIFICATION

The number of endemic species, as well as the results recorded from the literature and the present thesis (Table 2) highlighted that in situ diversification have played a great role in the origin of Malagasy ferns, in addition to LDD.

Taxa	Number of LDD events to Madagascar	Number of Malagasy species included in the study	N species / LDD event: mean [min-max]
Cyatheaceae Janssen <i>et al.</i> (2008)	3	32	10.7 [5-22]
<i>Ctenitis</i> Hennequin <i>et al.</i> (2017)	1	8	8
Grammitid ferns	14	31	2.2 [1-7]
<i>Rumohra</i>	1	4	4
Blechnoideae	5	13	2.6 [1-5]
<i>Lindsaea</i> - <i>Odontosoria</i>	4	13	3.3 [1-10]
<i>Phlegmariurus</i>	6	16	2.7 [1-11]

Table 2. Number of dispersal events to Madagascar and mean number of Malagasy species per event recorded in ferns and lycophytes, from the present thesis and in studies coding Madagascar as a single area.

Madagascar is an island renowned for the diversification of its flora, thanks to the richness of bioclimates and ecosystems (Yoder & Nowak, 2006). Especially, the eastern mountains would have played a crucial role for the diversification of Malagasy ferns, as tropical mountains are known to be great places of diversification for these plants (Kessler *et al.*, 2016).

Diversification of ferns in Madagascar is poorly understood and only one study focused on this issue. Based on the observation that tree ferns shared similar bioclimatic niches, Janssen *et al.* (2008) hypothesized that the impressive radiations of these ferns were influenced by climatic oscillations that have led to habitat fragmentation as well as shifts in altitudinal gradients. Grammitid genera, *Lomaridium* and *Lomariocycas*, are genera for which the colonization and the diversification in Madagascar were not followed by obvious change in their ecological preferences and in their morphology. Bioclimatic niche analyses would bring more evidences to understand if they could have diversified by following the same process as tree ferns, or if another process of diversification could be highlighted. *Lindsaea* and *Phlegmariurus* both showed signs of adaptive speciation, by the acquired epiphytism in *Lindsaea* species (especially with host specificity, Chapter IV) and by morphological convergences linked to ecological preferences in *Phlegmariurus*

(Chapter V). These observations would suggest that diversification of ferns in Madagascar may have been under the influence of different factors and processes.

4. SCENARIO OF THE BIOGEOGRAPHICAL HISTORY OF THE MALAGASY FERNS: AN ATTEMPT

Based on the current knowledge of the tectonic and climatic history of Madagascar and its species source pools, as well on the results from the present thesis and the literature, a biogeographical history is here proposed (Figure 4A-G). However, this scenario should be tested by more biogeographical analyses on Malagasy ferns.

A- Lower Cretaceous (145-100 Ma)

The break-up of Gondwana has already started, the Indo-Madagascar subcontinent forms an independent land from Africa and vicariance events could have occurred consequently to the separation from Africa (McLoughlin, 2001). A connection with Antarctica could have persisted until the end of Cretaceous (Yoder & Nowak, 2006), despite being contested (Ali & Krause, 2011). Madagascar would be under the influence of a temperate climate (Wells, 2003) but some regions colonized by ferns would be warm, probably tropical and seasonally humid (Appert, 2010).

B- Upper Cretaceous (100-66 Ma)

India and Madagascar separated ca. 90 Ma, but India still blocked the humidity from Indian Ocean and so prevented the establishment of a rainforest during this period in Madagascar. Moreover, the northern part of the island entered a subtropical arid zone due to its northward migration (Wells, 2003), leading to plausible extinction in Malagasy ferns.

The separation with India could have led to vicariant events. However, if Madagascar was under the influence of an arid climate at this time, few species would have been impacted by the separation of Madagascar from India, maybe drought-tolerant species that survived the climate change or species having found refuges. The plausible connection between Madagascar and Antarctica definitively ended (Yoder & Nowak, 2006). Starting from this point, vicariance linked to tectonic events is therefore not possible anymore.

C- Paleocene (66-56 Ma)

The gradual passage of Madagascar through an arid belt probably led to extinctions more to the South of the island and would explain the subsequent dominance of Cenozoic colonization and diversification in the biogeographical history of the Malagasy flora (Wells, 2003). The subarid climate was progressively reduced to the extreme South of Madagascar.

Northward migration of India progressively exposed Madagascar to the humid winds from Indian Ocean, leading to rainfalls on the eastern coasts. The increasing humidity could have allowed the establishment of a humid or sub-humid climate and dispersal from Africa and the drifting India could have consequently occurred, as well as stepping stone dispersal from tropical Asia via India (e.g. *Odontosoria?*, Figure3). Species that would have survived the dry period in refuges during Upper Cretaceous could have started their

spread on the island. However, the rainfalls could be not enough important to allow a real establishment of rainforests (Wells, 2003), so dispersal events probably remained scarce.

On a global scale, catastrophic events combining a meteor impact and intense volcanic activities ca. 65 Ma led to a major biological crisis and ecological changes (e.g. Vellekoop *et al.*, 2017). These events could have deeply impacted the flora of Madagascar (Agnarsson & Kuntner, 2012).

D- Eocene (ca. 56-34 Ma)

Volcanism at Tsaratanana and Manongarivo was reported, forming new massifs in the Northern Madagascar (Wells, 2003), maybe leading to diversification of ferns in Madagascar. However, the tropical rainforests were probably not yet fully established (Wells, 2003), limiting the effect of these volcanoes on fern diversification.

India's rifting ended with its collision with Asia. Dispersal events from Indian seem unlikely as no event was reported (Figure 3) and as no wind current is known between India and Madagascar at this period.

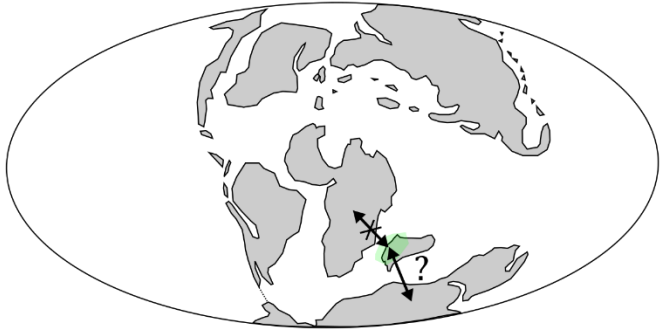
Dispersal between Africa and Madagascar could have occurred, maybe reinforced from Africa during early and middle Eocene thanks to a globally warm temperature coinciding with rainforest expansion (Couvreur, 2015; and references therein).

E- Oligocene (34-23 Ma)

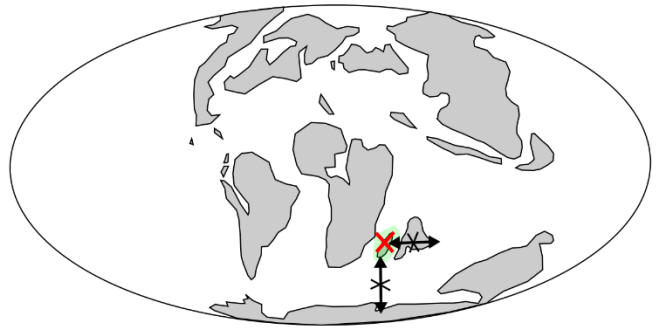
The circumaustral wind circulation started ca. 30-35 Ma thanks to the opening of Drake Passage between South America and Antarctica (Sanmartín *et al.*, 2007), allowing the first dispersal events from the Neotropics to Madagascar (e.g. maybe in *Phlegmariurus*, Figure 3). It would also have reinforced the South Indian Ocean gyre and so the rainfalls on Eastern Malagasy coasts (Wells, 2003). The Malagasy bioclimatic zones have probably reached their current areas, with a humid zone covering all the Eastern part of the island, including fully established tropical rainforests.

The establishment of rainforests would have resulted in intensification of fern colonisation in Madagascar, notably from African dispersal events, and diversification. However, the Eocene/Oligocene abrupt decrease of temperature resulted in the decline of rainforests and in extinctions in Africa (Couvreur, 2015), reducing the dispersal potential from the continent.

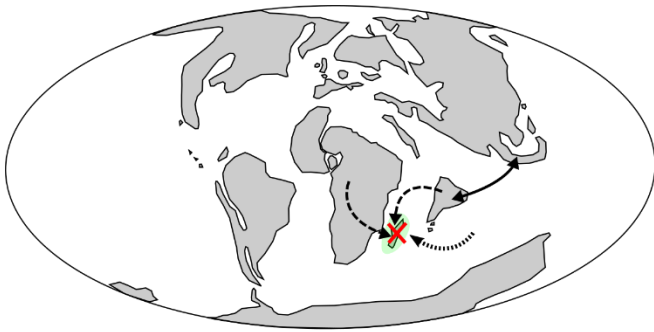
Ankaratra volcanism began (Wells, 2003), leading to a new high peak in Madagascar with an altitude reaching more than 2600 meters (current height) and so to a new plausible place of fern diversification.



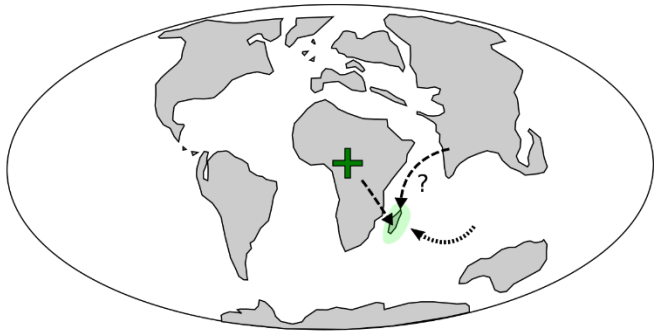
A - Lower Cretaceous (145-100 Ma)



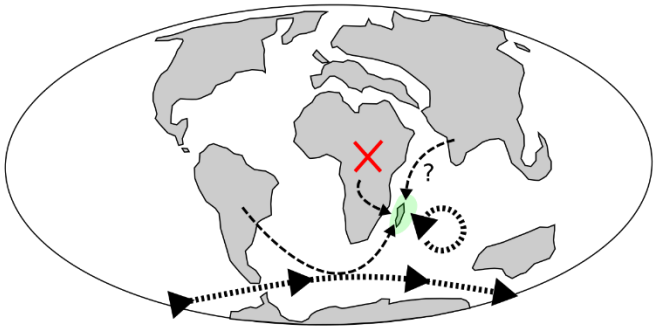
B - Upper Cretaceous (100-66 Ma)



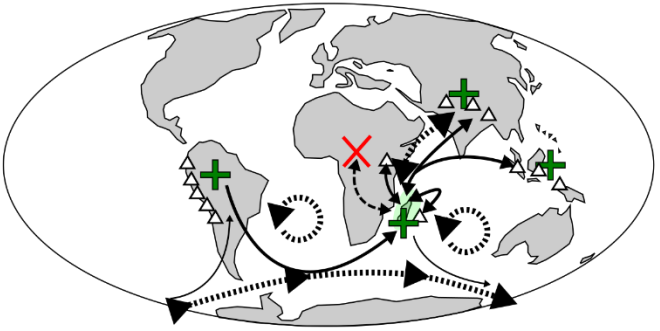
C - Paleocene (66-56 Ma)



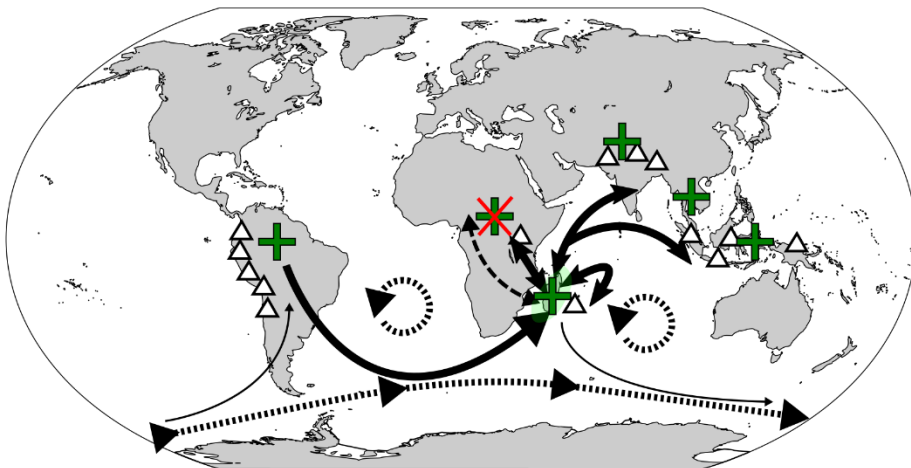
D - Eocene (56-34 Ma)



E - Oligocene (34-23 Ma)



F - Miocene (23-5 Ma)



G - Pliocene to present (5-0 Ma)

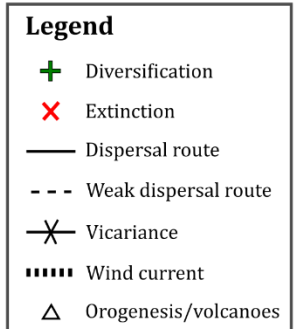


Figure 4. Hypotheses on the biogeographical history of Malagasy ferns, paleomaps modified from PALEOMAP project, Scotese (2001). Each time frame was commented in the text. Malagasy mountains and volcanoes formation were not represented for the sake of clarity, but were mentioned in the text.

F- Miocene (23-5 Ma)

This period would be characterized by an intensification of dispersal to Madagascar, thanks to the formation of species-rich source pools combined to wind circulation.

Firstly, the establishment of Indian monsoons in the middle Miocene has allowed wind-driven dispersal events from Asia and Southeast Asia (Jacques *et al.*, 2011; Gupta *et al.*, 2015). The northward migration of the Australian plate and its collision with the Pacific one have generated multiple volcanoes and the orogenesis of mountains, retaining humid winds from the Pacific (Hall, 2009; Morley, 2012). A rapid uplift of the Tibetan plateau also occurred ca. 15-20 Ma but did not lead to the current high altitudes (Royden, Burchfiel, & van der Hilst, 2008). These events would have provided high elevated rainforests and ferns diversification in the source regions (Wang *et al.*, 2012), and so species-rich source pools connected to Madagascar. Dispersal to the Western Paleotropics, especially to Madagascar, are documented (Figure 3).

Secondly, in the Neotropics, the Andes orogenesis provided mountain peaks (Hoorn *et al.*, 2010) and would be linked to the diversification of ferns (McHenry & Barrington, 2014). Dispersal events from this newly formed species-rich source pool occurred, especially for mountainous species, via circumaustral circulation (e.g. grammitid ferns, Chapter I).

The formation of volcanoes in East Africa the last ca. 8 Ma (Dawson, 1992; Nonnotte *et al.*, 2008) have provided a new source pool of mountainous species for Madagascar, as it was highlighted by dispersal from this region to Madagascar, e.g. in tree ferns and *Dryopteris* (Figure 3).

In Madagascar, modern fern species have started their diversification (e.g. *Lindsaea*, Chapter IV), forming another species-rich source pool in the Southern Hemisphere. Dispersal to other regions would have started to Africa, newly formed archipelagos (Comoros from ca. 8 Ma and Mascarenes from ca. 10 Ma), French Southern and Antarctic Lands, and even the Neotropics (grammitid ferns, Chapter I). Thanks to summer monsoons, dispersal from Madagascar to tropical Asia should be expected either.

G- Pliocene to present (5-0 Ma)

Similar dispersal route and place of diversification are hypothesized during the Pliocene and the Pleistocene, however they were intensified in Asia and in the Neotropics due to last uplifts (Li *et al.*, 1979; Zhou *et al.*, 2006; Hall, 2009; Hoorn *et al.*, 2010).

In Africa, a period of diversification was followed by a dry and cold climate leading to another extinction event (Couvreur, 2015), which could have importantly decreased/blurred the importance of the African source pool for Madagascar. A part of the modern African diversity would be explained by dispersal from Madagascar, especially in Eastern Africa.

Human establishment in Madagascar has led to a drastic decrease of the rainforests, almost 40% of forest cover has been lost from the 1950s to ca. 2000 and 9.1% of species were estimated to have been committed to extinction due to deforestation (Allnutt *et al.*, 2008).

Conclusion

The study of the biogeographical history Malagasy ferns supports the idea that despite the old isolation of Madagascar, the island was colonized recently mostly by dispersal post-dating the geologic isolation of the island. Reviews highlighted that dispersal events mostly originated from African ancestors in animals and angiosperms, the Neotropics and the Eastern Paleotropics playing a minor role in the biogeographical history of these Malagasy taxa. In ferns and lycophytes, the results from the present thesis and the literature so far rejected this tendency and showed equal roles of these source regions. Moreover, dispersal events were especially inferred from the Miocene (< 23 Ma).

Ferns and lycophytes are spore-bearing plants, renowned for their vagility by wind-drifted dispersal. However, these plants are also dependent on humid environments (with rare exceptions). In Madagascar, ferns and lycophytes are mostly retrieved in elevated rainforests. These characteristics have probably influenced the biogeographical history of the Malagasy ferns. Indeed, all the dispersal events recorded coincide with or post-date the establishment of the Malagasy rainforests and wind-currents between Madagascar and its source regions. Dispersal events from Miocene would be linked to orogenesis shaping the source regions and leading to high elevated rainforests, where ferns with ecological preferences similar to the Malagasy species would have diversified.

The hypothesis of links between the establishment of ferns and lycophytes in Madagascar and the establishment of high elevated rainforests in source regions should be further tested. The biogeographical analyses should expand to more fern and lycophyte taxa, to observe if the ecological preference for high elevated rainforests whether or not has been mostly preserved from ancestors or acquired in Madagascar. This hypothesis would be supported if the ecological preferences for elevated rainforests have also driven the biogeographical history of other taxa, like angiosperms. A comparison with other taxa could also help to answer other questions, on the importance of the dispersal abilities for example. Do we observe the same biogeographical histories between highly-dispersive organisms, such as bryophytes, mushrooms, angiosperms with small seeds, lycophytes and ferns?

The present thesis also highlighted that diversifications in situ have generated numerous species in the studied taxa, with means of ca. 2 to 11 species generated by colonization event. Diversification of ferns in Madagascar is poorly understood and only one study focused on the issue so far. Some results have revealed patterns of diversification that should be studied, notably via bioclimatic niche analysis, to understand the factors and the processes underlying Malagasy in situ diversification.

References

- Abbott RJ, Barton NH, Good JM. 2016. Genomics of hybridization and its evolutionary consequences. *Molecular Ecology* 25: 2325–2332.
- Adjie B, Masuyama S, Ishikawa H, Watano Y. 2007. Independent origins of tetraploid cryptic species in the fern *Ceratopteris thalictroides*. *Journal of Plant Research* 120: 129–138.
- Agnarsson I, Kuntner M. 2012. The generation of a biodiversity hotspot: biogeography and phylogeography of the western Indian Ocean islands. In: Anamthawat-Jónsson K, ed. *Current Topics in Phylogenetics and Phylogeography of Terrestrial and Aquatic Systems*. Rijeka: InTech, 33–82.
- Aldasoro JJ, Cabezas F, Aedo C. 2004. Diversity and distribution of ferns in sub-Saharan Africa, Madagascar and some islands of the South Atlantic. *Journal of Biogeography* 31: 1579–1604.
- Ali JR, Krause DW. 2011. Late Cretaceous bioconnections between Indo-Madagascar and Antarctica: refutation of the Gunnerus Ridge causeway hypothesis. *Journal of Biogeography* 38: 1855–1872.
- Allnutt TF, Ferrier S, Manion G, Powell GVN, Ricketts TH, Fisher BL, Harper GJ, Irwin ME, Kremen C, Labat JN, Lees DC, Pearce TA, Rakotondrainibe F. 2008. A method for quantifying biodiversity loss and its application to a 50-year record of deforestation across Madagascar. *Conservation Letters* 1: 173–181.
- Antonelli A, Nylander JAA, Persson C, Sanmartin I. 2009. Tracing the impact of the Andean uplift on Neotropical plant evolution. *Proceedings of the National Academy of Sciences* 106: 9749–9754.
- Appert O. 1973. Die Pteridophyten aus dem Oberen Jura des Manamana. *Schweizerische Paläontologische Abhandlungen* 94: 62.
- Appert O. 2010. Die fossile Makroflora der Unterkreide (Hauterivian) von Manja in Südwest-Madagaskar. Farne und Schachtelhalme (Filicatae und Equisetatae). *Schweizerische Paläontologische Abhandlungen* 129: 79.
- Bacon CD, Simmons MP, Archer RH, Zhao LC, Andriantiana J. 2016. Biogeography of the Malagasy Celastraceae: Multiple independent origins followed by widespread dispersal of genera from Madagascar. *Molecular Phylogenetics and Evolution* 94: 365–382.
- Baker JG. 1887. Further Contributions to the Flora of Madagascar. *Journal of the Linnean Society of London, Botany* 22: 441–472.
- Barrington DS. 1993. Ecological and Historical Factors in Fern Biogeography. *Journal of Biogeography* 20: 275–279.
- Barrington DS, Haufler CH, Werth CR. 1989. Hybridization, Reticulation, and Species Concepts in the Ferns. *American Fern Society* 79: 55–64.

- Bartish IV., Antonelli A, Richardson JE, Swenson U. 2011. Vicariance or long-distance dispersal: historical biogeography of the pantropical subfamily Chrysophylloideae (Sapotaceae). *Journal of Biogeography* 38: 177–190.
- Bateman RM, Kenrick P, Rothwell GW. 2007. Do eligulate herbaceous lycopsids occur in Carboniferous strata? *Hestia eremosa* gen. et sp. nov. from the Mississippian of Oxroad Bay, East Lothian, Scotland. *Review of Palaeobotany and Palynology* 144: 323–335.
- Bauret L, Gaudeul M, Sundue MA, Parris BS, Ranker TA, Rakotondrainibe F, Hennequin S, Ranaivo J, Selosse MA, Rouhan G. 2017. Madagascar sheds new light on the molecular systematics and biogeography of grammitid ferns: New unexpected lineages and numerous long-distance dispersal events. *Molecular Phylogenetics and Evolution* 111: 1–17.
- Bauret L, Rouhan G, Hirai RY, Perrie LR, Prado J, Salino A, Senterre B, Shepherd LD, Sundue MA, Selosse MA, Gaudeul M. 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*. *Botanical Journal of the Linnean Society* in Press.
- Beaulieu JM, Tank DC, Donoghue MJ. 2013. A Southern Hemisphere origin for campanulid angiosperms, with traces of the break-up of Gondwana. *BMC Evolutionary Biology* 13: 80.
- Bickford D, Lohman DJ, Sodhi NS, Ng PKL, Meier R, Winker K, Ingram KK, Das I. 2007. Cryptic species as a window on diversity and conservation. *Trends in Ecology & Evolution* 22: 148–155.
- Bishop LE, Smith AR. 1992. Revision of the Fern Genus *Enterosora* (Grammitidaceae) in the New World. *Systematic Botany* 17: 345.
- Blair C, Noonan BP, Brown JL, Raselimanana AP, Vences M, Yoder AD. 2015. Multilocus phylogenetic and geospatial analyses illuminate diversification patterns and the biogeographic history of Malagasy endemic plated lizards (Gerrhosauridae: Zonosaurinae). *Journal of Evolutionary Biology* 28: 481–492.
- Bomfleur B, Schöner R, Schneider JW, Viereck L, Kerp H, McKellar JL. 2014. From the transantarctic basin to the ferrar large igneous province-new palynostratigraphic age constraints for triassic-jurassic sedimentation and magmatism in East Antarctica. *Review of Palaeobotany and Palynology* 207: 18–37.
- Bonaparte R. 1915-1925. *Notes ptéridologiques*. Paris: Printed for the Author.
- Bouckaert R, Heled J, Kühnert D, Vaughan T, Wu CH, Xie D, Suchard MA, Rambaut A, Drummond AJ. 2014. BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS Computational Biology* 10: e1003537.
- Britton MN, Hedderson TA, Anthony Verboom G. 2014. Topography as a driver of cryptic speciation in the high-elevation cape sedge *Tetraria triangularis* (Boeck.) C. B. Clarke (Cyperaceae: Schoeneae). *Molecular Phylogenetics and Evolution* 77: 96–109.

- Brodribb TJ, McAdam SAM. 2011. Passive Origins of Stomatal Control in Vascular Plants. *Science* 331: 582–585.
- Brownlie G. 1969. *Flore de la Nouvelle-Calédonie et dépendances. Fasc. 3, Ptéridophytes*. Paris: Muséum national d'Histoire naturelle.
- Buerki S, Devey DS, Callmander MW, Phillipson PB, Forest F. 2013. Spatio-temporal history of the endemic genera of Madagascar. *Botanical Journal of the Linnean Society* 171: 304–329.
- Bukontaite R, Ranarilalaitiana T, Randriamihaja JH, Bergsten J. 2015. In or Out-of-Madagascar? Colonization Patterns for Large-Bodied Diving Beetles (Coleoptera: Dytiscidae). *PLOS ONE* 10: e0120777.
- Callmander MW. 2011. The endemic and non-endemic vascular flora of Madagascar updated. *Plant Ecology and Evolution* 144: 121–125.
- Calvino CI, Teruel FE, Downie SR. 2015. The role of the Southern Hemisphere in the evolutionary history of Apiaceae, a mostly north temperate plant family. *Journal of Biogeography* 43: 398–409.
- Cantrill DJ, Tosolini AMP, Francis JE. 2013. Paleocene flora from Seymour Island, Antarctica: revision of Dusén's (1908) angiosperm taxa. *Alcheringa: An Australasian Journal of Palaeontology* 37: 366–391.
- Chacón J, de Assis MC, Meerow AW, Renner SS. 2012. From East Gondwana to Central America: historical biogeography of the Alstroemeriaceae. *Journal of Biogeography* 39: 1806–1818.
- Chao YS, Rouhan G, Amoroso VB, Chiou WL. 2014. Molecular phylogeny and biogeography of the fern genus *Pteris* (Pteridaceae). *Annals of Botany* 114: 109–124.
- Chase MW, Cowan RS, Hollingsworth PM, Van Den Berg C, Madriñán S, Petersen G, Seberg O, Jørgensen T, Cameron KM, Carine M, Pedersen N, Hedderson TAJ, Conrad F, Salazar GA, Richardson JE, Hollingsworth ML, Barraclough TG, Kelly L, Wilkinson M. 2007. A proposal for a standardised protocol to barcode all land plants. *Taxon* 56: 295–299.
- Chen LY, Grimm GW, Wang QF, Renner SS. 2015. A phylogeny and biogeographic analysis for the Cape-Pondweed family Aponogetonaceae (Alismatales). *Molecular Phylogenetics and Evolution* 82: 111–117.
- Chinnock, RJ. 1998. Lycopodiaceae. *Flora of Australia* 48 : 66-85.
- Christenhusz MJM, Chase MW. 2014. Trends and concepts in fern classification. *Annals of Botany* 113: 571–594.
- Christensen C. 1932. Pteridophyta of Madagascar. *Dansk Botanisk Arkiv*: 1–253.
- Cieraad E, Lee DE. 2006. The New Zealand fossil record of ferns for the past 85 million years. *New Zealand Journal of Botany* 44: 143–170.
- Collinson ME. 2001. Cainozoic ferns and their distribution. *Brittonia* 53: 173–235.

- Cornet A. 1974. *Essai de cartographie bioclimatique à Madagascar. Notice explicative*. Paris: ORSTOM.
- Couvreur TLP. 2015. Odd man out: why are there fewer plant species in African rain forests? *Plant Systematics and Evolution* 301: 1299–1313.
- Couvreur TLP, Pirie MD, Chatrou LW, Saunders RMK, Su YCF, Richardson JE, Erkens RHJ. 2011. Early evolutionary history of the flowering plant family Annonaceae: Steady diversification and boreotropical geodispersal. *Journal of Biogeography* 38: 664–680.
- Crawford DJ, Archibald JK. 2017. Island floras as model systems for studies of plant speciation: Prospects and challenges: Island floras as models of plant speciation. *Journal of Systematics and Evolution* 55: 1–15.
- Crowl AA, Myers C, Cellinese N. 2017. Embracing discordance: Phylogenomic analyses provide evidence for allopolyploidy leading to cryptic diversity in a Mediterranean *Campanula* (Campanulaceae) clade. *Evolution* 71: 913–922.
- Darwin CR. 1859. *On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life* (J Murray, Ed.). London.
- Dassler CL, Farrar DR. 2001. Significance of gametophyte form in long-distance colonization by tropical, epiphytic ferns. *Brittonia* 53: 352–369.
- Dawson JB. 1992. Neogene tectonics and volcanicity in the North Tanzania sector of the Gregory Rift Valley: contrasts with the Kenya sector. *Tectonophysics* 204: 81–92.
- De Queiroz K. 2007. Species Concepts and Species Delimitation. *Systematic Biology* 56: 879–886.
- Der JP, Thomson JA, Stratford JK, Wolf PG. 2009. Global Chloroplast Phylogeny and Biogeography of Bracken (*Pteridium*; Dennstaedtiaceae). *American Journal of Botany* 96: 1041–1049.
- Dittrich VAO, Salino A, Monteiro R, de Gasper AL. 2017. The family Blechnaceae (Polypodiopsida) in Brazil: key to the genera and taxonomic treatment of *Austroblechnum*, *Cranfillia*, *Lomaridium*, *Neoblechnum* and *Telmatoblechnum* for southern and southeastern Brazil. *Phytotaxa* 303: 1–33.
- Donoghue MJ, Edwards EJ. 2014. Biome Shifts and Niche Evolution in Plants. *Annual Review of Ecology, Evolution, and Systematics* 45: 547–572.
- Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012. Bayesian Phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* 29: 1969–1973.
- Dubuisson JY, Rouhan G, Grall A, Hennequin S, Senterre B, Pynee K, Ebihara A. 2013. New insights into the systematics and evolution of the filmy fern genus *Crepidomanes* (Hymenophyllaceae) in the Mascarene Archipelago with a focus on dwarf species. *Acta Botanica Gallica* 160: 173–194.

- Dubuisson JY, Bauret L, Grall A, Rouhan G, Senterre B, Said AH, Pynee K, Ebihara A, Hennequin S. Revision of fern genera *Abrodictyum* C.Presl and *Trichomanes* L. (Hymenophyllaceae, Polypodiidae) from western Indian Ocean, and description of a new *Abrodictyum* species for Madagascar. *Phytotaxa* in Press.
- Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32: 1792–1797.
- Edwards PJ. 2005. Cyatheaceae. In: Beentje HJ, Ghazanfar SA, eds. *Flora of Tropical East Africa*. Royal Botanic Gardens, Kew: 1-15.
- Eiserhardt WL, Rohwer JG, Russell SJ, Yesilyurt JC, Schneider H. 2011. Evidence for radiations of cheilanthoid ferns in the greater cape floristic region. *Taxon* 60: 1269–1283.
- Eiserhardt WL, Couvreur TLP, Baker WJ. 2017. Plant phylogeny as a window on the evolution of hyperdiversity in the tropical rainforest biome. *New Phytologist* 214: 1408-1422.
- Emerick CM, Duncan RA. 1982. Age progressive volcanism in the Comores Archipelago, western Indian Ocean and implications for Somali plate tectonics. *Earth and Planetary Science Letters* 60: 415–428.
- Emerson BC. 2002. Evolution on oceanic islands: Molecular phylogenetic approaches to understanding pattern and process. *Molecular Ecology* 11: 951–966.
- Federman S, Dornburg A, Downie A, Richard AF, Daly DC, Donoghue MJ. 2015. The biogeographic origin of a radiation of trees in Madagascar: implications for the assembly of a tropical forest biome. *BMC Evolutionary Biology* 15: 216.
- Field AR, Bostock PD. 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.
- Field AR, Testo W, Bostock PD, Holtum JAM, Waycott M. 2016. Molecular phylogenetics and the morphology of the Lycopodiaceae subfamily Huperzioidae supports three genera: *Huperzia*, *Phlegmariurus* and *Phylloglossum*. *Molecular Phylogenetics and Evolution* 94: 635–657.
- Forthman M, Weirauch C. 2016. Phylogenetics and biogeography of the endemic Madagascan millipede assassin bugs (Hemiptera: Reduviidae: Ectrichodiinae). *Molecular Phylogenetics and Evolution* 100: 219–233.
- Galtier J, Scott AC. 1985. Diversification of early ferns. *Proceedings of the Royal Society of Edinburgh. Section B. Biological Sciences* 86: 289–301.
- De Gasper AL, Almeida TE, Dittrich VA de O, Smith AR, Salino A. 2016a. Molecular phylogeny of the fern family Blechnaceae (Polypodiales) with a revised genus-level treatment. *Cladistics* 33: 429-446.

- De Gasper AL, Dittrich VADO, Smith AR, Salino A. 2016b. A classification for Blechnaceae (Polypodiales: Polypodiopsida): New genera, resurrected names, and combinations. *Phytotaxa* 275: 191–227.
- Gernhard T. 2008. The conditioned reconstructed process. *Journal of Theoretical Biology* 253: 769–778.
- Giacosa JPR. 2010. *Blechnum yungense* (Pteridophyta, Blechnaceae), una Nueva Especie de Argentina y Bolivia. *Novon: A Journal for Botanical Nomenclature* 20: 68–72.
- Gill BA, Kondratieff BC, Casner KL, Encalada AC, Flecker AS, Gannon DG, Ghalambor CK, Guayasamin JM, Poff NL, Simmons MP, Thomas SA, Zamudio KR, Funk WC. 2016. Cryptic species diversity reveals biogeographic support for the ‘mountain passes are higher in the tropics’ hypothesis. *Proceedings of the Royal Society B: Biological Sciences* 283: 20160553.
- Gillespie RG, Baldwin BG, Waters JM, Fraser CI, Nikula R, Roderick GK. 2012. Long-distance dispersal: A framework for hypothesis testing. *Trends in Ecology and Evolution* 27: 47–55.
- Givnish TJ. 2010. Ecology of plant speciation. *Taxon* 59: 1329–1366.
- Goodman SM, Benstead JP. 2003. *The Natural History of Madagascar*. Chicago, IL: The University of Chicago Press.
- Goodman SM, Benstead JP. 2005. Updated estimates of biotic diversity and endemism for Madagascar. *Oryx* 39: 73–77.
- Griffiths CJ. 1993. The geological evolution of East Africa. In: Lovett JC, Wassez SK, eds. *Biogeography and ecology of the rain forests of eastern Africa*. Cambridge: Cambridge University Press, 9–21.
- Grusz A, Windham M. 2013. Toward a monophyletic *Cheilanthes*: The resurrection and recircumscription of *Myriopteris* (Pteridaceae). *PhytoKeys* 32: 49–63.
- Gupta AK, Yuvaraja A, Prakasam M, Clemens SC, Velu A. 2015. Evolution of the South Asian monsoon wind system since the late Middle Miocene. *Palaeogeography, Palaeoclimatology, Palaeoecology* 438: 160–167.
- Hall R. 2009. Southeast Asia’s changing palaeogeography. *Blumea - Biodiversity, Evolution and Biogeography of Plants* 54: 148–161.
- Hao S, Wang D, Wang Q, Xue J. 2006. A New Lycopoid, *Zhenglia radiata* gen. et sp. nov., from the Lower Devonian Posongchong Formation of Southeastern Yunnan, China, and Its Evolutionary Significance. *Acta Geologica Sinica - English Edition* 80: 11–19.
- Hao S, Xue J, Wang Q, Liu ZF. 2007. *Yuguangia ordinata* gen. et sp. nov., a new lycopoid from the middle Devonian (late Givetian) of Yunnan, China, and its phylogenetic implications. *International Journal of Plant Sciences* 168: 1161–1175.
- Harper GJ, Steininger MK, Tucker CJ, Juhn D, Hawkins F. 2007. Fifty years of deforestation and forest fragmentation in Madagascar. *Environmental Conservation* 34: 325–333.

- Hashemi-Yazdi F, Sajjadi F, Dehbozorgi A. 2015. A new lycophyte miospore species from the Middle Jurassic of Iran. *Revista Brasileira De Paleontologia* 18: 251–260.
- Haufler CH. 2007. Genetics, phylogenetics, and biogeography: Considering how shifting paradigms and continents influence fern diversity. *Brittonia* 59: 108–114.
- Haufler CH. 2008. Species and speciation. In: Ranker TA, Haufler CH, eds. *Biology and Evolution of Ferns and Lycophytes*. Cambridge: Cambridge University Press, 303–331.
- Heaney LR. 2007. Is a new paradigm emerging for oceanic island biogeography? *Journal of Biogeography* 34: 753–757.
- Hedenäs L, Eldenäs P. 2007. Cryptic speciation, habitat differentiation, and geography in *Hamatocaulis vernicosus* (Calliergonaceae, Bryophyta). *Plant Systematics and Evolution* 268: 131.
- Hennequin S, Ebihara A, Dubuisson JY, Schneider H. 2010a. Chromosome number evolution in *Hymenophyllum* (Hymenophyllaceae), with special reference to the subgenus *Hymenophyllum*. *Molecular Phylogenetics and Evolution* 55: 47–59.
- Hennequin S, Hovenkamp P, Christenhusz MJM, Schneider H. 2010b. Phylogenetics and biogeography of *Nephrolepis* – a tale of old settlers and young tramps. *Botanical Journal of the Linnean Society* 164: 113–127.
- Hennequin S, Rouhan G, Salino A, Duan YF, Lepeigneux MC, Guillou M, Ansell S, Almeida TE, Zhang LB, Schneider H. 2017. Global phylogeny and biogeography of the fern genus *Ctenitis* (Dryopteridaceae), with a focus on the Indian Ocean region. *Molecular Phylogenetics and Evolution* 112: 277–289.
- Herter G. 1909. Beiträge zur kenntniis der kattung Lycopodium. Studien über dieuntergattung Urostachys. *Bot. Jahrb. Syst* 43: 1–56.
- Holub J. 1991. Some taxonomic changes within Lycopodiales. *Folia Geobotanica et Phytotaxonomica* 26: 81–94.
- Hoorn C, Wesselingh FP, ter Steege H, Bermudez MA, Mora A, Sevink J, Sanmartin I, Sanchez-Meseguer A, Anderson CL, Figueiredo JP, Jaramillo C, Riff D, Negri FR, Hooghiemstra H, Lundberg J, Stadler T, Sarkinen T, Antonelli A. 2010. Amazonia Through Time: Andean Uplift, Climate Change, Landscape Evolution, and Biodiversity. *Science* 330: 927–931.
- Hortal J, Triantis KA, Meiri S, Thébault E, Sfenthourakis S. 2009. Island Species Richness Increases with Habitat Diversity. *The American Naturalist* 174: E205–E217.
- Hueber FM. 1992. Thoughts on the Early Lycopsids and Zosterophylls. *Annals of the Missouri Botanical Garden* 79: 474.
- Huelsenbeck JP, Larget B, Alfaro ME. 2004. Bayesian phylogenetic model selection using reversible jump Markov Chain Monte Carlo. *Molecular Biology and Evolution* 21: 1123–1133.

- Hutsemékers V, Szovenyi P, Shaw AJ, Gonzalez-Mancebo JM, Munoz J, Vanderpoorten A. 2011. Oceanic islands are not sinks of biodiversity in spore-producing plants. *Proceedings of the National Academy of Sciences* 108: 18989–18994.
- Jacques FMB, Guo SX, Su T, Xing YW, Huang YJ, Liu YS, Ferguson DK, Zhou ZK. 2011. Quantitative reconstruction of the Late Miocene monsoon climates of southwest China: A case study of the Lincang flora from Yunnan Province. *Palaeogeography, Palaeoclimatology, Palaeoecology* 304: 318–327.
- Janssen T, Bystriakova N, Rakotondrainibe F, Coomes D, Labat JN, Schneider H. 2008. Neoendemism in Madagascan scaly tree ferns results from recent, coincident diversification bursts. *Evolution* 62: 1876–1889.
- Janssen T, Kreier HP, Schneider H. 2007. Origin and diversification of African ferns with special emphasis on Polypodiaceae. *Brittonia* 59: 159–181.
- Janssen T, Rakotondrainibe F. 2008. A revision of the indusiate scaly tree ferns (Cyatheaceae, *Cyathea* subgen. *Alsophila* sect. *Alsophila*) in Madagascar, the Comoros and the Seychelles. *Adansonia* 30: 221–374.
- Janssens SB, Groeninckx I, De Block PJ, Verstraete B, Smets EF, Dessein S. 2016. Dispersing towards Madagascar: Biogeography and evolution of the Madagascan endemics of the Spermaceae tribe (Rubiaceae). *Molecular Phylogenetics and Evolution* 95: 58–66.
- Ji SG, Huo KK, Wang J, Pan SL. 2008. A molecular phylogenetic study of Huperziaceae based on chloroplast *rbcL* and *psbA-trnH* sequences. *J. Syst. Evol.* 46: 213–9.
- Juhász, M. 1975. Lycopodiaceae spores from Lower Cretaceous deposits of Hungary. *Acta Biologica Szeged*, 21, 21-34.
- Kainulainen K, Razafimandimbison SG, Wikström N, Bremer B. 2017. Island hopping, long-distance dispersal and species radiation in the Western Indian Ocean: historical biogeography of the Coffeae alliance (Rubiaceae). *Journal of Biogeography*: 1–14.
- Katoh K, Toh H. 2010. Parallelization of the MAFFT multiple sequence alignment program. *Bioinformatics* 26: 1899–1900.
- Kessler M, Karger DN, Kluge J. 2016. Elevational diversity patterns as an example for evolutionary and ecological dynamics in ferns and lycophytes. *Journal of Systematics and Evolution* 54: 617–625.
- Kidston R, Lang WH. 1920. On old red sandstone plants showing structure, from the Rhynie Chert bed, Aberdeenshire. Part III. *Asteroxylon mackiei*. *Transactions of The Royal Society of Edinburgh*: 643–680.
- Kim C, Choi HK. 2016. Biogeography of North Pacific *Isoetes* (Isoëtaceae) inferred from nuclear and chloroplast DNA sequence data. *Journal of Plant Biology* 59: 386–396.

- Klaus K V., Schulz C, Bauer DS, Stützel T. 2016. Historical biogeography of the ancient lycophyte genus *Selaginella*: early adaptation to xeric habitats on Pangea. *Cladistics*: 1–12.
- Korall P, Pryer KM. 2014. Global biogeography of scaly tree ferns (Cyatheaceae): Evidence for Gondwanan vicariance and limited transoceanic dispersal. *Journal of Biogeography* 41: 402–413.
- Kornas J. 1993. The Significance of Historical Factors and Ecological Preference in the Distribution of African Pteridophytes. *Journal of Biogeography* 20: 281–286.
- Kramer KU. 1972. The Lindsaeoid ferns of the Old World: IX Africa and Its Islands. *Bulletin du Jardin botanique national de Belgique / Bulletin van de National Plantentuin van België* 42: 305.
- Kreft H, Jetz W, Mutke J, Barthlott W. 2010. Contrasting environmental and regional effects on global pteridophyte and seed plant diversity. *Ecography* 33: 408–419.
- Kreier HP, Schneider H. 2006. Phylogeny and biogeography of the staghorn fern genus *Platyserium* (Polypodiaceae, Polypodiidae). *American Journal of Botany* 93: 217–225.
- Krüger Å, Razafimandimbison SG, Bremer B. 2012. Molecular phylogeny of the tribe Danaideae (Rubiaceae: Rubioideae): Another example of out-of-Madagascar dispersal. *Taxon* 61: 629–636.
- Kuo LY, Ebihara A, Shinohara W, Rouhan G, Wood KR, Wang CN, Chiou WL. 2016. Historical biogeography of the fern genus *Deparia* (Athuriaceae) and its relation with polyploidy. *Molecular Phylogenetics and Evolution* 104: 123–134.
- Labiak PH, Mickel JT, Hanks JG. 2015a. Molecular phylogeny and character evolution of Anemiaceae (Schizaeales). *Taxon* 64: 1141–1158.
- Labiak PH, Sundue MA, Rouhan G. 2010. Molecular phylogeny, character evolution, and biogeography of the grammitid fern genus *Lellingeria* (Polypodiaceae). *American Journal of Botany* 97: 1354–1364.
- Labiak PH, Sundue MA, Rouhan G, Hanks JG, Mickel JT, Moran RC. 2014. Phylogeny and historical biogeography of the lastreopsid ferns (Dryopteridaceae). *American Journal of Botany* 101: 1207–1228.
- Labiak PH, Sundue MA, Rouhan G, Moran RC. 2015b. New combinations in *Lastreopsis* and *Parapolystichum* (Dryopteridaceae). *Brittonia* 67: 79–86.
- Landis MJ, Matzke NJ, Moore BR, Huelsenbeck JP. 2013. Bayesian Analysis of Biogeography when the Number of Areas is Large. *Systematic Biology* 62: 789–804.
- Larsén E, Rydin C. 2016. Disentangling the Phylogeny of *Isoetes* (Isoetales), Using Nuclear and Plastid Data. *International Journal of Plant Sciences* 177: 157–174.
- Le Péchon T, Zhang L, He H, Zhou XM, Bytebier B, Gao XF, Zhang LB. 2016. A well-sampled phylogenetic analysis of the polystichoid ferns (Dryopteridaceae) suggests a

- complex biogeographical history involving both boreotropical migrations and recent transoceanic dispersals. *Molecular Phylogenetics and Evolution* 98: 324–336.
- Lehtonen S, Tuomisto H, Rouhan G, Christenhusz MJM. 2010. Phylogenetics and classification of the pantropical fern family Lindsaeaceae. *Botanical Journal of the Linnean Society* 163: 305–359.
- Lehtonen S, Tuomisto H, Rouhan G, Christenhusz MJM. 2013. Taxonomic Revision of the Fern Genus *Osmolindsaea* (Lindsaeaceae). *Systematic Botany* 38: 887–900.
- Lehtonen S, Wahlberg N, Christenhusz MJM. 2012. Diversification of lindsaeoid ferns and phylogenetic uncertainty of early polypod relationships. *Botanical Journal of the Linnean Society* 170: 489–503.
- Lewis LR, Behling E, Gousse H, Qian E, Elphick CS, Lamarre JF, Bêty J, Liebezeit J, Rozzi R, Goffinet B. 2014. First evidence of bryophyte diaspores in the plumage of transequatorial migrant birds. *PeerJ* 2: e424.
- Li J, Wen S, Zhang Q, Wang F, Zheng B, Li B. 1979. A discussion on the period, amplitude and type of the uplift of the Qinghai-Xizang Tibetan Plateau. *China Science* 6: 608–616.
- Linder HP, Hardy CR, Rutschmann F. 2005. Taxon sampling effects in molecular clock dating: An example from the African Restionaceae. *Molecular Phylogenetics and Evolution* 35: 569–582.
- Linder HP, Pennington T, Schneider H. 2014. The evolution of African plant diversity. *Frontiers in ecology and evolution* 2: 1–14.
- Linnaeus C. 1753. *Species Plantarum* (L Salvii, Ed.). Stockholm.
- Liu XQ, Ickert-Bond SM, Chen LQ, Wen J. 2013. Molecular phylogeny of *Cissus* L. of Vitaceae (the grape family) and evolution of its pantropical intercontinental disjunctions. *Molecular Phylogenetics and Evolution* 66: 43–53.
- Liu HM, Zhang SZ, Wan T, Kamau PW, Wang ZW, Grall A, Hemp A, Schneider H. 2016. Exploring the pteridophyte flora of the Eastern Afromontane biodiversity hotspot. *Journal of Systematics and Evolution* 54: 691–705.
- Lóriga J, Schmidt AR, Moran RC, Feldberg K, Schneider H, Heinrichs J. 2014. The first fossil of bolbitidoid ferns belongs to the early divergent lineages of *Elaphoglossum* (Dryopteridaceae). *American Journal of Botany* 101: 1466–1475.
- Losos JB, Ricklefs RE. 2009. Adaptation and diversification on islands. *Nature* 457: 830–836
- Lu JM, Wen J, Lutz S, Wang YP, Li DZ. 2012. Phylogenetic relationships of Chinese *Adiantum* based on five plastid markers. *Journal of Plant Research* 125: 237–249.
- MacArthur RH, Wilson EO. 1967. *The theory of island biogeography*. Princeton: Princeton University Press.
- Mallet J. 2007. Hybrid speciation. *Nature* 446: 279–283.

- Manns U, Wikström N, Taylor CM, Bremer B. 2012. Historical biogeography of the predominantly Neotropical Subfamily Cinchonoideae (Rubiaceae): Into or out of America? *International Journal of Plant Sciences* 173: 261–286.
- Martcorena C, Rodriguez R. 1995. *Flora de Chile. Vol. 1, Pteridophyta-Gymnospermae* (C Martcorena and R Rodriguez, eds.). Concepción: Universidad de Concepción.
- Masters JC, De Wit MJ, Asher RJ. 2006. Reconciling the origins of Africa, India and Madagascar with vertebrate dispersal scenarios. *Folia Primatologica* 77: 399–418.
- Matzke NJ. 2013. *BioGeoBEARS: BioGeography with Bayesian (and likelihood) evolutionary analysis in R scripts. R package, v. 0.2.1.* Available at: <https://cran.r-project.org/web/packages/BioGeoBEARS/>
- Matzke NJ. 2014. Model Selection in Historical Biogeography Reveals that Founder-Event Speciation Is a Crucial Process in Island Clades. *Systematic Biology* 63: 951–970.
- Matzke NJ. 2016. Stochastic mapping under biogeographical models. PhyloWiki BioGeoBEARS website. Available at: [http://phylo.wikidot.com/biogeobears#stochastic mapping](http://phylo.wikidot.com/biogeobears#stochastic_mapping) (accessed 19 November 2016).
- Mayden RL. 1997. A hierarchy of species concepts: the denouement in the saga of the species problem. In: Claridge MF, Dawah HA, Wilson MR, eds. *Species. The units of biodiversity*. Springer Netherlands, 381–423.
- Mayr E. 1942. *Systematics and the origin of species from the viewpoint of a zoologist*. New York: Columbia University Press.
- Mayr E. 1954. Change of genetic environment and evolution. *Evolution as a Process*. 157–180.
- McAdam SAM, Brodribb TJ. 2012. Fern and Lycophyte Guard Cells Do Not Respond to Endogenous Abscisic Acid. *The Plant Cell* 24: 1510–1521.
- McCarthy PM. 1998. *Flora of Australia. Volume 48. Ferns, gymnosperms and allied groups*. Melbourne: CSIRO Publishing.
- McDougall I, Chamalaun FH. 1969. Isotopic Dating and Geomagnetic Polarity Studies on Volcanic Rocks from Mauritius, Indian Ocean. *Geological Society of America Bulletin* 80: 1419–1442.
- McHenry MA, Barrington DS. 2014. Phylogeny and biogeography of exindusiate Andean *Polystichum* (Dryopteridaceae). *American Journal of Botany* 101: 365–375.
- McLoughlin S. 2001. The breakup history of Gondwana and its impact on pre-Cenozoic floristic provincialism. *Australian Journal of Botany* 49: 271–300.
- Meza Torres EI, Cerne B, Ulke AG, Morbelli M a. 2015. Distribution of *Ophioglossum reticulatum* L. in South America. A case of long-distance jump dispersal? *International Journal of Biometeorology* 59: 137–150.

- Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *2010 Gateway Computing Environments Workshop, GCE 2010*: 1–8.
- Mittermeier RA, van Dijk PP, Rhodin AGJ, Nash SD. 2004. *Hotspots Revisited: Earth's Biologically Richest and Most Endangered Ecoregions*. Boston: University of Chicago Press.
- Mittermeier RA, Turner WR, Larsen FW, Brooks TM, Gascon C. 2011. *Biodiversity Hotspots* In: FE Zachos, JC Habel, eds. Berlin: Springer.
- Moran RC. 1995. The importance of mountains to Pteridophytes, with emphasis on Neotropical montane forests. In: Churchill SP, Balslev H, Forero E, Luteyn JL, eds. *Biodiversity and Conservation of Neotropical Montane Forest*. New York: The New York Botanical Garden, 359–363.
- Moran RC. 2004. Spore Shooting. *A Natural History of Ferns*. Portland: Timber Press, 24–30.
- Moran RC. 2008. Diversity, biogeography, and floristics. In: Ranker TA, Haufler CH, eds. *Biology and Evolution of Ferns and Lycophytes*. Cambridge: Cambridge University Press, 367–394.
- Moran RC, Labiak PH, Hanks JG, Prado J. 2014a. The phylogenetic relationship of *Tectaria brauniana* and *Tectaria nicotianifolia*, and the recognition of *Hypoderris* (Tectariaceae). *Systematic Botany* 39: 384–395.
- Moran RC, Labiak PH, Sundue MA. 2010. Phylogeny and Character Evolution of the Bolbitidoid Ferns (Dryopteridaceae). *International Journal of Plant Sciences* 171: 547–559.
- Moran RC, Prado J, Sundue MA. 2014b. *Megalastrum* (Dryopteridaceae) in Andean South America, Part I. *American Fern Journal* 104: 109–178.
- Moran RC, Prado J, Sundue MA. 2014c. *Megalastrum* (Dryopteridaceae) in Andean South America, Part II. *American Fern Journal* 104: 181–236.
- Moran RC, Smith AR. 2001. Phytogeographic relationships between neotropical and African-Madagascan pteridophytes. *Brittonia* 53: 304–351.
- Morat P, Jaffré T, Tronchet F, Munzinger J, Pillon Y, Veillon JM, Chalopin M, Birnbaum P, Rigault F, Dagostini G, Tinel J, Lowry PP. 2012. Le référentiel taxonomique Florical et les caractéristiques de la flore vasculaire indigène de la Nouvelle-Calédonie. *Adansonia* 34: 179–221.
- Moreira-Muñoz A. 2007. The Austral floristic realm revisited. *Journal of Biogeography* 34: 1649–1660.
- Morley RJ. 2000. *Origin and evolution of tropical rain forests*. New York: Wiley.
- Morley RJ. 2003. Interplate dispersal paths for megathermal angiosperms. *Perspectives in Plant Ecology, Evolution and Systematics* 6: 5–20.

- Morley RJ. 2007. Cretaceous and tertiary climate change and the past distribution of megathermal rainforests. In: Bush MB, Flenely J, eds. *Tropical rainforest responses to climatic changes*. Chichester: Praxis Publishing, 1–31.
- Morley RJ. 2012. A review of the Cenozoic palaeoclimate history of Southeast Asia. In: Gower D, Johnson K, Richardson J, Rosen B, Ruber L, Williams S, eds. *Biotic evolution and environmental change in Southeast Asia*. Cambridge: Cambridge University Press, 79–114.
- Munoz J, Felicísimo AM, Cabezas F, Burgaz ÁR, Martínez I. 2004. Wind as a Long-Distance Dispersal Vehicle in the Southern Hemisphere. *Science* 304: 1144–1147.
- Munzinger J, Morat P, Jaffré T, Gâteblé G, Pillon Y, Tronchet F, Veillon JM, Chalopin M. 2016. FLORICAL: Checklist of the vascular indigenous flora of New Caledonia.
- Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J. 2000. Biodiversity hotspots for conservation priorities. *Nature* 403: 853–858.
- Naciri Y, Linder HP. 2015. Species delimitation and relationships: The dance of the seven veils. *Taxon* 64: 3–16.
- Nagalingum NS, Schneider H, Pryer KM. 2007. Molecular Phylogenetic Relationships and Morphological Evolution in the Heterosporous Fern Genus *Marsilea*. *Systematic Botany* 32: 16–25.
- Nessel H. 1939. *Die Bärlappgewächse (Lycopodiaceae)*. G Fisher, ed. Jena.
- Nicolson DH, Fosberg FR. 2003. The Forsters and the botany of the second Cook Expedition (1772—1775). *Regnum vegetabile* 139: 1729–1798.
- Nonnotte P, Guillou H, Le Gall B, Benoit M, Cotten J, Scaillet S. 2008. New K–Ar age determinations of Kilimanjaro volcano in the North Tanzanian diverging rift, East Africa. *Journal of Volcanology and Geothermal Research* 173: 99–112.
- Norhazrina N, Wang J, Hagborg A, Geffert JL, Mutke J, Gradstein SR, 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. *Botanical Journal of the Linnean Society*.
- Nougier J, Cantagrel JM, Karche JP. 1986. The Comores archipelago in the western Indian Ocean: volcanology, geochronology and geodynamic setting. *Journal of African Earth Sciences (1983)* 5: 135–144.
- Nylander JAA. 2004. MrModeltest ver. 2.3. Program distributed by the author. *Evolutionary Centre, Uppsala Univ.*
- Øllgaard B. 1987. A revised classification of the Lycopodiaceae s.l. *Oper. Bot.* 92: 153–178.
- Øllgaard B. 1989. Index of the Lycopodiaceae volume 34 of Biologiske skrifter. *Roy. Dan. Acad. Sci. Lett.*

- Pan AD, Jacobs BF, Dransfield J, Baker WJ. 2006. The fossil history of palms (Arecaceae) in Africa and new records from the Late Oligocene (28-27 Mya) of north-western Ethiopia. *Botanical Journal of the Linnean Society* 151: 69–81.
- Paris CA, Wagner FS, Wagner WH. 1989. Cryptic Species, Species Delimitation, and Taxonomic Practice in the Homosporous Ferns. *American Fern Journal* 79: 46–54.
- Parris BS. 1990. Grammitidaceae. In: Kramer K, Green PS, eds. *Pteridophytes and Gymnosperms*. Berlin: Springer-Verlag, 153–157.
- Parris BS. 2001. Circum-Antarctic continental distribution patterns in pteridophyte species. *Brittonia* 53: 270–283.
- Parris BS. 2002. New Species and New Combinations in African Grammitidaceae (Filicales). *Kew Bulletin* 57: 423–434.
- Parris BS. 2003. The distribution of Grammitidaceae (Filicales) inside and outside Malesia. *Telopea* 10: 451–466.
- Parris BS. 2005. Grammitidaceae. In: Beentje HJ, Ghazanfar SA, eds. *Flora of Tropical East Africa*. Kew: Royal Botanic Gardens.
- Parris BS. 2009. New genera of Malesian Grammitidaceae (Monilophyta). *Blumea - Biodiversity, Evolution and Biogeography of Plants* 54: 217–219.
- Parris BS. 2012. *Ctenopterella gabonensis*, a new species of grammitid fern (Polypodiaceae) from Gabon, Africa. *Fern Gazette* 19: 89–93.
- Passey BH, Ayliffe LK, Kaakinen A, Zhang Z, Eronen JT, Zhu Y, Zhou L, Cerling TE, 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 and Planetary Science Letters* 277: 443–452.
- Patiño J, Carine M, Fernández-Palacios JM, Otto R, Schaefer H, Vanderpoorten A. 2014. The anagenetic world of spore-producing land plants. *New Phytologist* 201: 305–311.
- Patiño J, Carine M, Mardulyn P, Devos N, Mateo RG, Gonzalez-Mancebo JM, Shaw AJ, Vanderpoorten A. 2015. Approximate Bayesian Computation Reveals the Crucial Role of Oceanic Islands for the Assembly of Continental Biodiversity. *Systematic Biology* 64: 579–589.
- Patiño J, Whittaker RJ, Borges PAV, Fernández-Palacios JM, Ah-Peng C, Araújo MB, Ávila SP, Cardoso P, Cornuault J, de Boer EJ, de Nascimento L, Gil A, González-Castro A, Gruner DS, Heleno R, Hortal J, Illera JC, Kaiser-Bunbury CN, Matthews TJ, Papadopoulou A, Pettorelli N, Price JP, Santos AMC, Steinbauer MJ, Triantis KA, Valente L, Vargas P, Weigelt P, Emerson BC. 2017. A roadmap for island biology: 50 fundamental questions after 50 years of The Theory of Island Biogeography. *Journal of Biogeography* 44: 963–983.
- Patterson TB, Givnish TJ. 2002. Phylogeny, concerted convergence, and phylogenetic niche conservatism in the core Liliales: insights from *rbcl* and *ndhF* sequence data. *Evolution* 56: 233–252.

- Perrie LR, Parris BS. 2012. Chloroplast DNA sequences indicate the grammitid ferns (Polypodiaceae) in New Zealand belong to a single clade, *Notogrammitis* gen. nov. *New Zealand Journal of Botany* 50: 457–472.
- Pillon Y, Hopkins HCF, Munzinger J, Amir H, Chase MW. 2009. Cryptic species, gene recombination and hybridization in the genus *Spiraeanthemum* (Cunoniaceae) from New Caledonia. *Botanical Journal of the Linnean Society* 161: 137–152.
- Poole I, Mennega AMW, Cantrill DJ. 2003. Valdivian ecosystems in the Late Cretaceous and Early Tertiary of Antarctica: further evidence from myrtaceous and eucryphiaceous fossil wood. *Review of Palaeobotany and Palynology* 124: 9–27.
- PPGI. 2016. A community-derived classification for extant lycophytes and ferns. *Journal of Systematics and Evolution* 54: 563–603.
- Pross J, Contreras L, Bijl PK, Greenwood DR, Bohaty SM, Schouten S, Bendle JA, Röhl U, Tauxe L, Raine JI, Huck CE, van de Flierdt T, Jamieson SSR, Stickley CE, van de Schootbrugge B, Escutia C, Brinkhuis H, Brinkhuis H, Escutia Dotti C, Klaus A, Fehr A, Williams T, Bendle JAP, Bijl PK, Bohaty SM, Carr SA, Dunbar RB, González JJ, Hayden TG, Iwai M, Jimenez-Espejo FJ, Katsuki K, Soo Kong G, McKay RM, Nakai M, Olney MP, Passchier S, Pekar SF, Pross J, Riesselman CR, Röhl U, Sakai T, Shrivastava PK, Stickley CE, Sugisaki S, Tauxe L, Tuo S, van de Flierdt T, Welsh K, Yamane M. 2012. Persistent near-tropical warmth on the Antarctic continent during the early Eocene epoch. *Nature* 488: 73–77.
- Pryer KM, Schuettpelz E, Wolf PG, Schneider H, Smith AR, Cranfill R. 2004. Phylogeny and evolution of ferns (monilophytes) with a focus on the early leptosporangiate divergences. *American Journal of Botany* 91: 1582–1598.
- Rakotondrainibe F. 2003a. Checklist of the pteridophytes of Madagascar. In: Goodman S, Benstead J, eds. *The Natural History of Madagascar*. Chicago, IL: University of Chicago Press, 295–313.
- Rakotondrainibe F. 2003b. Diversity, Ecology, and Distribution of the pteridophyte Flora. In: Goodman SM, Benstead JP, eds. *The Natural History of Madagascar*. Chicago, IL: University of Chicago Press, 282–295.
- Rakotondrainibe F. 2010a. Deux espèces nouvelles de *Pseudotectaria* Tardieu sect. *Pseudotectaria* (Pteridophyta, Tectariaceae) de Madagascar et discussion sur les sections *Pseudotectaria* et *Isopinnatae* Holttum. *Adansonia* 32: 31–38.
- Rakotondrainibe F. 2010b. Le genre *Rumohra* Raddi (Pteridophyta, Dryopteridaceae) à Madagascar : une espèce et deux combinaisons nouvelles. *Adansonia* 32: 217–228.
- Rakotondrainibe F, Jouy A. 2015. Nouveautés dans le Genre *Huperzia* Bernh. (Lycophytes, Lycopodiaceae) à Madagascar. *Candollea* 70: 49–56.
- Rakotondrainibe F, Jouy A, Meyer S, Reeb C. 2013. Révision synoptique du genre *Blechnum* L. (Blechnaceae) à Madagascar. *Adansonia* 35: 151–193.

- 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.
- Ranker TA. 2016. What do we know about Hawaiian ferns and lycophytes? *Journal of Systematics and Evolution* 54: 626–637.
- Ranker TA, Gemmill CEC, Trapp PG. 2000. Microevolutionary patterns and processes of the native Hawaiian colonizing fern *Odontosoria Chinensis* (Lindsaeaceae). *Evolution* 54: 828.
- Ranker TA, Smith AR, Parris BS, Geiger JMO, Haufler CH, Straub SCK, Schneider H. 2004. Phylogeny and evolution of grammitid ferns (Grammitidaceae): a case of rampant morphological homoplasy. *Taxon* 53: 415.
- Ranker TA, Sundue MA. 2015. Why are there so few species of ferns? *Trends in Plant Science* 20: 402–403.
- Ree RH, Smith SA. 2008. Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Systematic biology* 57: 4–14.
- Regalado L, Schmidt AR, Müller P, Kobbert MJ, Schneider H, Heinrichs J. 2017. The first fossil of Lindsaeaceae (Polypodiales) from the Cretaceous amber forest of Myanmar. *Cretaceous Research* 72: 8–12.
- Renner SS. 2004. Bayesian analysis of combined chloroplast loci, using multiple calibrations, supports the recent arrival of Melastomataceae in Africa and Madagascar. *American Journal of Botany* 91: 1427–1435.
- Renner SS, Strijk JS, Strasberg D, Thébaud C. 2010. Biogeography of the Monimiaceae (Laurales): A role for East Gondwana and long-distance dispersal, but not West Gondwana. *Journal of Biogeography* 37: 1227–1238.
- Ronquist F. 1997. Dispersal-Vicariance Analysis: A New Approach to the Quantification of Historical Biogeography. *Systematic Biology* 46: 195–203.
- Ronquist F, Sanmartín I. 2011. Phylogenetic Methods in Biogeography. *Annual Review of Ecology, Evolution, and Systematics* 42: 441–464.
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic biology* 61: 539–542.
- Rothfels CJ, Li FW, Sigel EM, Huiet L, Larsson A, Burge DO, Ruhsam M, Deyholos M, Soltis DE, Stewart CN, Shaw SW, Pokorny L, Chen T, dePamphilis C, DeGironimo L, Chen L, Wei X, Sun X, Korall P, Stevenson DW, Graham SW, Wong GKS, Pryer KM. 2015. The evolutionary history of ferns inferred from 25 low-copy nuclear genes. *American Journal of Botany* 102: 1–19.

- Rothfels CJ, Windham MD, Grusz AL, Gastony GJ, Pryer KM. 2008. Toward a monophyletic *Notholaena* (Pteridaceae): Resolving patterns of evolutionary convergence in xeric-adapted ferns. *Taxon* 57: 712–724.
- Rothwell GW, Stockey RA. 1991. *Onoclea sensibilis* in the Paleocene of North America, a dramatic example of structural and ecological stasis. *Review of Palaeobotany and Palynology* 70: 113–124.
- Rothwell GW, Stockey RA. 2008. Phylogeny and evolution of ferns: a paleontological perspective. In: Ranker TA, Haufler CH, eds. *Biology and Evolution of Ferns and Lycophytes*. Cambridge: Cambridge University Press, 332–366.
- Rouhan G, Dubuisson JY, Rakotondrainibe F, Motley TJ, Mickel JT, Labat JN, Moran RC. 2004. Molecular phylogeny of the fern genus *Elaphoglossum* (Elaphoglossaceae) based on chloroplast non-coding DNA sequences: Contributions of species from the Indian Ocean area. *Molecular Phylogenetics and Evolution* 33: 745–763.
- Rouhan G, Gaudeul M. 2014. Plant taxonomy: a historical perspective, current challenges, and perspectives. *Molecular Plant Taxonomy: Methods and Protocols*. Springer, 1–37.
- Rouhan G, Hanks JG, McClelland D, Moran RC. 2007a. Preliminary phylogenetic analysis of the fern genus *Lomariopsis* (Lomariopsidaceae). *Brittonia* 59: 115–128.
- Rouhan G, Labiak PH, Randrianjohany E, Rakotondrainibe F. 2012. Not so neotropical after all: the Grammitid fern genus *Leucotrichum* (Polypodiaceae) is also paleotropical, as revealed by a new species from Madagascar. *Systematic Botany* 37: 331–338.
- Rouhan G, Rakotondrainibe F, Moran RC. 2007b. *Elaphoglossum nidusoides* (Dryopteridaceae), a New Species of Fern from Madagascar with an Unusual Phylogenetic Position in the *Squamipedia* Group. *Systematic Botany* 32: 227–235.
- Roux JP. 2009. *Synopsis of the Lycopodiophyta and Pteridophyta of Africa, Madagascar and neighbouring islands*. South African National Biodiversity Institute.
- Rowe NP. 1988. A herbaceous lycophyte from the lower Carboniferous Drybrook sandstone of the forest of Dean, Gloucestershire. *Palaeontology* 31: 69–83.
- Royden LH, Burchfiel BC, van der Hilst RD. 2008. The Geological Evolution of the Tibetan Plateau. *Science* 321: 1054–1058.
- RStudio Team. 2015. RStudio: Integrated Development Environment for R.
- Rundell RJ, Price TD. 2009. Adaptive radiation, nonadaptive radiation, ecological speciation and nonecological speciation. *Trends in Ecology & Evolution* 24: 394–399.
- Samonds KE, Godfrey LR, Ali JR, Goodman SM, Vences M, Sutherland MR, Irwin MT, Krause DW. 2012. Spatial and temporal arrival patterns of Madagascar's vertebrate fauna explained by distance, ocean currents, and ancestor type. *Proceedings of the National Academy of Sciences* 109: 5352–5357.

- Sang T, Crawford DJ, Stuessy TF. 1997. Chloroplast DNA Phylogeny, Reticulate Evolution, and Biogeography of *Paeonia* (Paeoniaceae). *American Journal of Botany* 84: 1120–1136.
- Sanmartín I, Ronquist F. 2004. Southern hemisphere biogeography inferred by event-based models: plant versus animal patterns. *Systematic biology* 53: 216–243.
- Sanmartín I, Wanntorp L, Winkworth RC. 2007. West Wind Drift revisited: testing for directional dispersal in the Southern Hemisphere using event-based tree fitting. *Journal of Biogeography* 34: 398–416.
- Santos AMC, Field R, Ricklefs RE. 2016. New directions in island biogeography. *Global Ecology and Biogeography* 25: 751–768.
- Scheben A, Bechteler J, Lee GE, Pócs T, Schäfer-Verwimp A, Heinrichs J. 2016. Multiple transoceanic dispersals and geographical structure in the pantropical leafy liverwort *Ceratolejeunea* (Lejeuneaceae, Porellales). *Journal of Biogeography*: 1–11.
- Schneider H, Janssen T, Byrskiakova N, Heinrichs J, Hennequin S, Rakotondrainibe F. 2010. Rapid Radiations and Neoendemism in the Madagascan Biodiversity Hotspot. In: Glaubrecht M, ed. *Evolution in Action*. Berlin: Springer Berlin Heidelberg, 3–15.
- Schneider H, Kenrick P. 2001. An Early Cretaceous root-climbing epiphyte (Lindsaeaceae) and its significance for calibrating the diversification of polypodiaceous ferns. *Review of Palaeobotany and Palynology* 115: 33–41.
- Schneider H, Smith AR, Cranfill R, Hildebrand TJ, Haufler CH, Ranker TA. 2004. Unraveling the phylogeny of polygrammoid ferns (Polypodiaceae and Grammitidaceae): Exploring aspects of the diversification of epiphytic plants. *Molecular Phylogenetics and Evolution* 31: 1041–1063.
- Schuettpelz E, Korall P, Pryer KM. 2006. Plastid *atpA* data provide improved support for deep relationships among ferns. *Taxon* 55: 897–906.
- Schuettpelz E, Pryer KM. 2007. Fern phylogeny inferred from 400 leptosporangiate species and three plastid genes. *Taxon* 56: 1037–1050.
- Schuettpelz E, Pryer KM. 2009. Evidence for a Cenozoic radiation of ferns in an angiosperm-dominated canopy. *Proceedings of the National Academy of Sciences of the United States of America* 106: 11200–11205.
- Scotese CR, 2001. *Atlas of Earth History*, Volume 1, Paleogeography, PALEOMAP Project, Arlington, Texas, 52 pp.
- Sessa EB, Juslén A, Väre H, Chambers SM. 2017. Into Africa: Molecular phylogenetics and historical biogeography of sub-Saharan African woodferns (Dryopteris). *American Journal of Botany* 104: 477–486.
- Sessa EB, Testo WL, Watkins JE. 2016. On the widespread capacity for, and functional significance of, extreme inbreeding in ferns. *New Phytologist* 211: 1108–1119.

- Sessa EB, Zimmer EA, Givnish TJ. 2012. Phylogeny, divergence times, and historical biogeography of New World *Dryopteris* (Dryopteridaceae). *American Journal of Botany* 99: 730–750.
- Seton M, Müller RD, Zahirovic S, Gaina C, Torsvik T, Shephard G, Talsma A, Gurnis M, Turner M, Maus S, Chandler M. 2012. Global continental and ocean basin reconstructions since 200 Ma. *Earth-Science Reviews* 113: 212–270.
- Seward D, Grujic D, Schreurs G. 2004. An insight into the breakup of Gondwana: Identifying events through low-temperature thermochronology from the basement rocks of Madagascar. *Tectonics* 23.
- Sheffield E. 2008. Alternation of generations. In: Ranker TA, Haufler CH, eds. *Biology and Evolution of Ferns and Lycophytes*. Cambridge: Cambridge University Press, 49–74.
- Siegert MJ, Barrett P, DeConto R, Dunbar R, Ó Cofaigh C, Passchier S, Naish T. 2008. Recent advances in understanding Antarctic climate evolution. *Antarctic Science* 20: 313–325.
- Sigel EM. 2016. Genetic and genomic aspects of hybridization in ferns. *Journal of Systematics and Evolution* 54: 638–655.
- Sigel EM, Windham MD, Pryer KM. 2014. Evidence for reciprocal origins in *Polypodium hesperium* (Polypodiaceae): A fern model system for investigating how multiple origins shape allopolyploid genomes. *American Journal of Botany* 101: 1476–1485.
- Simpson GG. 1951. The species concept. *Evolution* 5: 285–298.
- Skog JE, Mickel JT, Moran RC, Volovsek M, Elizabeth A, Zimmer EA. 2004. Molecular studies of representative species in the fern genus *Elaphoglossum* (Dryopteridaceae) Based on cpDNA Sequences *rbcl*, *trnL-F*, and *rps4-trnS*. *International Journal of Plant Sciences* 165: 1063–1075.
- Small RL, Lickey EB, Shaw J, Hauk WD. 2005. Amplification of noncoding chloroplast DNA for phylogenetic studies in lycophytes and monilophytes with a comparative example of relative phylogenetic utility from Ophioglossaceae. *Molecular Phylogenetics and Evolution* 36: 509–522.
- Smedmark JEE, Razafimandimbison SG, 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. *Molecular Phylogenetics and Evolution* 70: 182–194.
- Smith AR. 1972. Comparison of Fern and Flowering Plant Distributions with Some Evolutionary Interpretations for Ferns. *Biotropica* 4: 4–9.
- Smith AR, Cranfill RB. 2002. Intrafamilial Relationships of the Thelypteroid Ferns (Thelypteridaceae). *American Fern Journal* 92: 131–149.
- Soulebeau A, Aubriot X, Gaudeul M, Rouhan G, Hennequin S, Haevermans T, Dubuisson JY, Jabbour F. 2015. The hypothesis of adaptive radiation in evolutionary biology: hard facts about a hazy concept. *Organisms Diversity and Evolution* 15: 747–761.

- Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313.
- Stein A, Gerstner K, Kreft H. 2014. Environmental heterogeneity as a universal driver of species richness across taxa, biomes and spatial scales. *Ecology Letters* 17: 866–880.
- Stokey AG, Atkinson LR. 1958. The gametophyte of the Grammitidaceae. *Phytomorphology* 8: 391–403.
- Strijk JS, Noyes RD, Strasberg D, Cruaud C, Gavory F, Chase MW, Abbott RJ, Thébaud C. 2012. In and out of Madagascar: Dispersal to peripheral islands, insular speciation and diversification of Indian Ocean daisy trees (*Psiadia*, Asteraceae). *PLoS ONE* 7: 18–22.
- Stuessy TF, Foland KA, Sutter JF, Sanders RW, Silva M. 1984. Botanical and geological significance of potassium-argon dates from the Juan Fernandez Islands. *Science* 225: 49–51.
- Stuessy TF, Jakubowsky G, Gomez RS, Pfosser M, Schluter PM, Fer T, Sun BY, Kato H. 2006. Anagenetic evolution in island plants. *Journal of Biogeography* 33: 1259–1265.
- Sugita N, Ootsuki R, Fujita T, Murakami N, Ueda K. 2013. Possible spore dispersal of a bird-nest fern *Asplenium setoi* by Bonin flying foxes *Pteropus pselaphon*. *Mammal Study* 38: 225–229.
- Sundue MA. 2010. A morphological cladistic analysis of *Terpsichore* (Polypodiaceae). *Systematic Botany* 35: 716–729.
- Sundue MA, Hirai RY, Prado J. 2013. *Rumohra glandulosissima* (Dryopteridaceae) a new species from the Atlantic Rainforest, and revision of the species occurring in Brazil. *Systematic Botany* 38: 915–924.
- Sundue MA, Islam MB, Ranker TA. 2010a. Systematics of grammitid Ferns (Polypodiaceae): Using morphology and plastid sequence data to resolve the circumscriptions of *Melpomene* and the polyphyletic genera *Lellingeria* and *Terpsichore*. *Systematic Botany* 35: 701–715.
- Sundue MA, Parris BS, Ranker TA, Smith AR, Fujimoto EL, Zamora-Crosby D, Morden CW, Chiou WL, Chen CW, Rouhan G, Hirai RY, Prado J. 2014. Global phylogeny and biogeography of grammitid ferns (Polypodiaceae). *Molecular phylogenetics and evolution* 81: 195–206.
- Sundue MA, Rothfels CJ. 2014. Stasis and convergence characterize morphological evolution in eupolypod II ferns. *Annals of Botany* 113: 35–54.
- Sundue MA, Rouhan G, Moran RC. 2010b. *Megalastrum* (Dryopteridaceae) of the circumaustral region: Chile, Argentina, and Southern Islands of the Atlantic, Pacific, and Indian Oceans. *Systematic Botany* 35: 461–475.
- Sundue MA, Testo WL, Ranker TA. 2015. Morphological innovation, ecological opportunity, and the radiation of a major vascular epiphyte lineage. *Evolution* 69: 2482–2495.

- Sylvester SP, Sylvester MDP V, Kessler M. 2014. The world's highest vascular epiphytes found in the Peruvian Andes. *Alpine Botany*: 0–4.
- Taberlet P, Gielly L, Pautou G, Bouvet J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109.
- Tardieu-Blot ML. 1951-1971. *Flore de Madagascar et des Comores* (Humbert H, Ed.). Paris: Firmin-Didot.
- Tardieu-Blot ML. 1958. Polypodiacées (sensu lato). In: Humbert H, ed. *Flore de Madagascar et des Comores*. Paris: Firmin-Didot.
- Tardieu-Blot ML. 1970. A propos des Lycopodiales de la région Malgache. *Adansonia* 10: 15–22.
- Tardieu-Blot ML. 1971. Lycopodiacées-Huperziacées. In: Humbert H, ed. *Flore de Madagascar et des Comores*. Paris: Firmin-Didot.
- Tate JA. 2002. Systematics and evolution of *Tarasa* (Malvaceae): an enigmatic Andean polyploid genus. Unpublished thesis, The University of Texas at Austin.
- Testo W, Sundue MA. 2016. A 4000-species dataset provides new insight into the evolution of ferns. *Molecular Phylogenetics and Evolution* 105: 200–211.
- Testo W, Watkins JE, Barrington DS. 2015. Dynamics of asymmetrical hybridization in North American wood ferns: reconciling patterns of inheritance with gametophyte reproductive biology. *New Phytologist* 206: 785–795.
- Thiers B. [continuously updated]. Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium.
- Trewick SA, Morgan-Richards M, Russell S, Henderson S, Rumsey F, Pintér I, Barrett J, Gibby M, Vogel J. 2002. Polyploidy, phylogeography and Pleistocene refugia of the rockfern *Asplenium ceterach*: evidence from chloroplast DNA. *Molecular Ecology* 11: 2003–2012.
- Tryon R. 1972. Endemic areas and geographic speciation in tropical American ferns. *Biotropica* 4: 121.
- Tryon R. 1986. The biogeography of species, with special reference to ferns. *The Botanical Review* 52: 117–156.
- Vaidya G, Lohman DJ, Meier R. 2011. SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics* 27: 171–180.
- Vanderpoorten A, Shaw AJ. 2010. The application of molecular data to the phylogenetic delimitation of species in bryophytes: a note of caution. *Phytotaxa* 9: 229–237.
- Vasco A, Moran RC, Rouhan G. 2009. Circumscription and phylogeny of the *Elaphoglossum ciliatum* group (*E. sect. Lepidoglossa*, Dryopteridaceae) based on cpDNA sequences. *Taxon* 58: 825–834.

- Vellekoop J, Woelders L, Açikalin S, Smit J, van de Schootbrugge B, Yilmaz İÖ, Brinkhuis H, Speijer RP. 2017. Ecological response to collapse of the biological pump following the mass extinction at the Cretaceous–Paleogene boundary. *Biogeosciences* 14: 885–900.
- Vences M, Wollenberg KC, Vieites DR, Lees DC. 2009. Madagascar as a model region of species diversification. *Trends in Ecology and Evolution* 24: 456–465.
- Wallace AR. 1876. *The geographic distribution of animals* (Macmillan, Ed.). London.
- Wang L, Schneider H, Zhang XC, Xiang QP. 2012. The rise of the Himalaya enforced the diversification of SE Asian ferns by altering the monsoon regimes. *BMC Plant Biology* 12: 210.
- Warren BH, Simberloff D, Ricklefs RE, Aguilée R, Condamine FL, Gravel D, Morlon H, Mouquet N, Rosindell J, Casquet J, Conti E, Cornuault J, Fernández-Palacios JM, Hengl T, Norder SJ, Rijdsdijk KF, Sanmartín I, Strasberg D, Triantis KA, Valente LM, Whittaker RJ, Gillespie RG, Emerson BC, Thébaud C. 2015. Islands as model systems in ecology and evolution: prospects fifty years after MacArthur-Wilson. *Ecology Letters* 18: 200–217.
- Warren BH, Strasberg D, Bruggemann JH, Prys-Jones RP, Thébaud C. 2010. Why does the biota of the Madagascar region have such a strong Asiatic flavour? *Cladistics* 26: 526–538.
- Waterhouse AM, Procter JB, Martin DMA, Clamp M, Barton GJ. 2009. Jalview Version 2 - a multiple sequence alignment editor and analysis workbench. *Bioinformatics* 25: 1189–1191.
- Weeks A, Zapata F, Pell SK, Daly DC, Mitchell JD, Fine PVA. 2014. To move or to evolve: contrasting patterns of intercontinental connectivity and climatic niche evolution in “Terebinthaceae” (Anacardiaceae and Burseraceae). *Frontiers in Genetics* 5: 1-20.
- Wei R, Xiang Q, Schneider H, Sundue MA, Kessler M, Kamau PW, Hidayat A, Zhang X. 2015. Eurasian origin, boreotropical migration and transoceanic dispersal in the pantropical fern genus *Diplazium* (Athuriaceae). *Journal of Biogeography* 42: 1809–1819.
- Wells NA. 2003. Geology and Soils. In: Goodman SM, Benstead JP, eds. *The Natural History of Madagascar*. Chicago, IL: University of Chicago Press, 16–51.
- Weststrand S, Korall P. 2016. A subgeneric classification of *Selaginella* (Selaginellaceae). *American Journal of Botany* 103: 2160–2169.
- White, F. 1983. *The vegetation of Africa*. UNESCO, Paris.
- Wikström N, Kenrick P. 2000. Phylogeny of epiphytic *Huperzia* (Lycopodiaceae): paleotropical and neotropical clades corroborated by *rbcL* sequences. *Nordic Journal of Botany* 20: 165–171.

- Wikström N, Kenrick P. 2001. Evolution of Lycopodiaceae (Lycopsidea): estimating divergence times from *rbcL* gene sequences by use of nonparametric rate smoothing. *Molecular phylogenetics and evolution* 19: 177–186.
- Wikström N, Kenrick P, Chase M. 1999. Epiphytism and terrestrialization in tropical *Huperzia* (Lycopodiaceae). *Plant Systematics and Evolution* 218: 221–243.
- Wiley EO. 1978. The Evolutionary Species Concept Reconsidered. *Systematic Zoology* 27: 17–26.
- Williams KJ, Ford A, Rosauer DF, De Silva N, Mittermeier R, Bruce C, Larsen FW, Margules C. 2011. Forests of East Australia: The 35th Biodiversity Hotspot. In: Zachos FE, Habel JC, eds. *Biodiversity Hotspots: Distribution and Protection of Conservation Priority Areas*. Berlin: Springer Berlin Heidelberg, 295–310.
- Willis KJ. 2017. *State of the World's Plants 2017. Report*. Kew: Royal Botanic Gardens.
- Winkworth RC, Hennion F, Prinzing A, Wagstaff SJ. 2015. Explaining the disjunct distributions of austral plants: the roles of Antarctic and direct dispersal routes. *Journal of Biogeography* 42: 1197–1209.
- Wirta H, Viljanen H, Orsini L, Montreuil O, Hanski I. 2010. Three parallel radiations of *Canthonini* dung beetles in Madagascar. *Molecular Phylogenetics and Evolution* 57: 710–727.
- Wolf PG. 1997. Evaluation of *atpB* nucleotide sequences for phylogenetic studies of ferns and other pteridophytes. *American Journal of Botany* 84: 1429–1440.
- Wolf PG, Schneider H, Ranker TA. 2001. Geographic distributions of homosporous ferns: Does dispersal obscure evidence of vicariance? *Journal of Biogeography* 28: 263–270.
- Wolfe JA. 1975. Some Aspects of Plant Geography of the Northern Hemisphere During the Late Cretaceous and Tertiary. *Annals of the Missouri Botanical Garden* 62: 264–279.
- Wood HM, Gillespie RG, Griswold CE, Wainwright PC. 2015. Why is Madagascar special? The extraordinarily slow evolution of pelican spiders (Araneae, Archaeidae). *Evolution* 69: 462–481.
- Xiang L, Werth CR, Emery SN, McCauley DE. 2000. Population-specific gender-biased hybridization between *Dryopteris intermedia* and *D. carthusiana*: Evidence from chloroplast DNA. *American Journal of Botany* 87: 1175–1180.
- Xue J. 2011. Phylogeny of Devonian Lycopsidea Inferred from Bayesian Phylogenetic Analyses (Intergovernmental Panel on Climate Change, Ed.). *Acta Geologica Sinica - English Edition* 85: 569–580.
- Xue J. 2013. New material of *Hueberia zhichangensis* Yang, Li & Edwards, a basal lycopsid from the Early Devonian of Yunnan, China. *Neues Jahrbuch für Geologie und Paläontologie - Abhandlungen* 267: 331–339.

- Yang S, Galbraith E, Palter J. 2014. Coupled climate impacts of the Drake Passage and the Panama Seaway. *Climate Dynamics* 43: 37–52.
- Yang N, Li CS, Edwards D. 2009. *Hueberia zhichangensis* gen. et sp. nov, an Early Devonian (Pragian) plant from Yunnan, China. *Palynology* 33: 113–124.
- Yatabe Y, Shinohara W, Matsumoto S, Murakami N. 2009. Patterns of hybrid formation among cryptic species of bird-nest fern, *Asplenium nidus* complex (Aspleniaceae), in West Malesia. *Botanical Journal of the Linnean Society* 160: 42–63.
- Yoder AD, Nowak MD. 2006. Has Vicariance or Dispersal Has Vicariance Been the Predominant Force in Biogeographic Madagascar? Only Time Will Tell. *Annual Review of Ecology, Evolution, and Systematics* 37: 405–431.
- Yu Y, Harris AJ, Blair C, He X. 2015. RASP (Reconstruct Ancestral State in Phylogenies): A tool for historical biogeography. *Molecular Phylogenetics and Evolution* 87: 46–49.
- Yu Y, Harris AJ, He X. 2010. S-DIVA (Statistical Dispersal-Vicariance Analysis): A tool for inferring biogeographic histories. *Molecular Phylogenetics and Evolution* 56: 848–850.
- Zachos J, Pagani M, Sloan L, Thomas E, Billups K. 2001. Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science* 292: 686–93.
- Zhang LB, Zhang L. 2015. Didymochlaenaceae: A new fern family of eupolypods I (Polypodiales). *Taxon* 64: 27–38.
- Zhou L, Su YCF, Thomas DC, Saunders RMK. 2012. ‘Out-of-Africa’ dispersal of tropical floras during the Miocene climatic optimum: Evidence from *Uvaria* (Annonaceae). *Journal of Biogeography* 39: 322–335.
- Zhou S, Wang X, Wang J, Xu L. 2006. A preliminary study on timing of the oldest Pleistocene glaciation in Qinghai-Tibetan Plateau. *Quaternary International* 154–155: 44–51.
- Zhou XM., Zhang L, Chen CW, Li CX, Huang YM, Chen DK, Lu NT, Cicuzza D, Knapp R, Luong TT, Nitta JH, Gao XF, Zhang LB. 2017. A plastid phylogeny and character evolution of the Old World fern genus *Pyrrosia* (Polypodiaceae) with the description of a new genus: *Hovenkampia* (Polypodiaceae). *Molecular Phylogenetics and Evolution* 114: 271–294.

Supplementary data

APPENDIX 1.1

Vouchers and GenBank accession numbers (grammitid ferns). Newly sequenced samples are in bold. Herbarium acronyms follow the Index Herbarium (<http://sweetgum.nybg.org/ih/>, accessed 19 August 2016).

Taxon	included in BEAST analysis	Voucher	Collection Locality	<i>atpB</i>	<i>rbcL</i>	<i>rps4-trnS</i>	<i>trnG-trnR</i>	<i>trnL-trnF</i>
<i>Acrosorus friderici-et-pauli</i> (Christ) Copel.		Averyanov 3673 (P)	Vietnam	-	-	KY712388	-	KY711717
<i>Acrosorus friderici-et-pauli</i>	x	Beaman 8041 (US)	Sabah, Malaysia	-	KM218753	KM106106	KM105964	KM106047
<i>Acrosorus friderici-et-pauli</i>	x	Chen 1630 (TAIF)	Vietnam	KM218817	KM218751	KM106104	-	-
<i>Acrosorus friderici-et-pauli</i>		Allen 2282 (US)	Peninsular Malaysia	-	KM218752	KM106105	KM105963	KM106046
<i>Adenophorus abietinus</i> (D. C. Eaton) K. A. Wilson	x	Ranker 1100 (COLO)	Hawaii, USA	AF469778	AF468202	-	-	AF469791
<i>Adenophorus epigaeus</i> (L. E. Bishop) W. H. Wagner		Ranker 1103 (COLO)	Hawaii, USA	AF469779	AF468203	-	-	AF469792
<i>Adenophorus haalilioanus</i> (Brack.) K. A. Wilson		Ranker 1561 (COLO)	Hawaii, USA	AF469775	AF468200	-	-	AF469788
<i>Adenophorus periens</i> L. E. Bishop		Ranker 1114 (COLO)	Hawaii, USA	AF469774	AF468199	-	-	AF469787
<i>Adenophorus tenellus</i> (Kaulf.) Ranker	x	Ranker 1352 (COLO)	Hawaii, USA	AF469773	AF468198	-	-	AF469786
<i>Alansmia aff. cultrata</i> (Bory ex Willd.) Moguel & M. Kessler		Sundue 1162 (NY)	Ecuador	GU376477	GU376495	JN654942	JN654955	JN654968
<i>Alansmia cultrata</i>	x	Sundue 1214 (NY)	Colombia	GU376476	GU376496	KM106107	JN654956	JN654969
<i>Alansmia elastica</i> (Bory. ex Willd.) Moguel & M. Kessler		Hennequin et al. 339 (P)	Mauritius	KY711748	KY711922	KY712249	KY712090	KY711575
<i>Alansmia elastica</i>		Kluge 7863 (Z)	Madagascar	KM218858	KM218795	**	KM105965	KM106048
<i>Alansmia elastica</i>	x	Rakotondrainibe 5833 (P)	Madagascar	KY711747	KY711921	KY712248	KY712089	KY711574
<i>Alansmia elastica</i>		Rouhan et al. 1138 (P)	Madagascar	KY711745	KY711919	KY712245	KY712087	KY711572
<i>Alansmia elastica</i>		Rouhan et al. 1150 (P)	Madagascar	KY711746	KY711920	KY712247	KY712088	KY711573
<i>Alansmia elastica</i>		Rouhan et al. 1171 (P)	Madagascar	KY711831	KY712007	KY712323	KY712171	KY711656
<i>Alansmia elastica</i>		Rouhan et al. 1376 (P)	Madagascar	KY711832	KY712008	KY712324	KY712172	KY711657

<i>Alansmia elastica</i>		Senterre 6125 (P)	Seychelles	KY711889	KY712063	KY712381	KY712229	KY711709
<i>Alansmia glandulifera</i> (Rojas) Moguel & M. Kessler	x	Sundue 1765 (NY)	Costa Rica	GU376472	GU376497	JN654944	JN654957	JN654970
<i>Alansmia heteromorpha</i> (Hook. & Grev.) Moguel & M. Kessler	x	Sundue 2609 (NY)	Ecuador	-	KM218803	-	KM105966	-
<i>Alansmia lanigera</i> (Desv.) Moguel & M. Kessler	x	León 3647 UC	Peru	AY459505	AY460672	-	-	GU476718
<i>Alansmia senilis</i> (Fee) Moguel & M. Kessler	x	Sundue 1156 (NY)	Ecuador	GU376474	GU376498	JN654941	JN654954	JN654967
<i>Alansmia stella</i> (Copel.) Moguel & M. Kessler	x	Sundue 1083 (NY)	Ecuador	GU376473	GU376499	JN654939	JN654952	JN654965
<i>Ascogrammitis anfractuosa</i> (Kunze ex Klotzsch) Sundue	x	Lehnert 1035 (UC)	Bolivia	GU476783	GU476853	KM106108	KM105967	GU476675
<i>Ascogrammitis angustipes</i> (Copel.) Sundue		Sundue 1237(NY)	Colombia	KM218837	GU476891	KM106109	KM105968	GU476703
<i>Ascogrammitis athyrioides</i> (Hook.) Sundue	x	Lehnert 261 (UC)	Peru	KM218840	GU476856	KM106110	KM105969	GU476704
<i>Ascogrammitis clathrata</i> (Sundue & M. Kessler) Sundue		Kromer 1237(UC)	Bolivia	KM218838	GU476843	KM106111	KM105970	GU476708
<i>Ascogrammitis clavigera</i> A. R. Sm. ex Sundue		Schneider 2400 (UC)	Venezuela	KM218839	GU476925	KM106112	-	GU476709
<i>Ascogrammitis colombiense</i> Sundue		Dassler 94-7-13-1 (OS)	Colombia	GU476805	GU476827	KM106113	-	GU476711
<i>Ascogrammitis cuencana</i> (Hieron.) Sundue		Lehnert 1164 (GOET)	Ecuador	-	GU476851	KM106114	KM105971	GU476714
<i>Ascogrammitis david-smithii</i> (Stolze) Sundue		Sundue 785 (NY)	Bolivia	GU376639	GU387012	GU387122	GU387205	GU387284
<i>Ascogrammitis dilatata</i> (Sundue & M. Kessler) Sundue		Labiak 4728 UUPCB)	Bolivia	GU376640	GU387033	GU387124	GU387206	GU387285
<i>Ascogrammitis loxensis</i> Sundue		Sundue 1073 (NY)	Ecuador	-	GU386995	GU387125	GU387207	GU387286
<i>Ascogrammitis nana</i> (Sundue & M. Kessler) Sundue		Labiak 4725 (UPCB)	Bolivia	GU376642	GU387031	GU387126	GU387208	GU387287
<i>Ascogrammitis pichincae</i> (Sodirol) Sundue		Wilson 2816a (UC)	Ecuador	-	GU476928	KM106115	-	GU476730
<i>Ascogrammitis pichinchensis</i> (Hieron.) Sundue		Lehnert 1577 (UC)	Ecuador	GU476816	GU476854	KM106116	-	GU476732

<i>Calymmodon clavifer</i> (Hook.) T. Moore		Sundue 2235 (VT)	Sabah, Malaysia	KM218818	KM218804	KM106117	-	KM106049
<i>Calymmodon decipiens</i> Parris ined.		Sundue 2218 (VT)	Sabah, Malaysia	-	KM218805	KM106119	-	KM106051
<i>Calymmodon gracilis</i> (Fee.) Copel.	x	Chiou 97-09-12-01* (TAIF)	Taiwan	AY459451	-	-	-	GU476618
<i>Calymmodon gracilis</i>	x	Kuo 2023* (TAIF)	Philippines	-	-	-	KY712080	-
<i>Calymmodon luerssenianus</i> (Domin) Copel.		Kessler 14264 (VT)	Australia	KM218819	KM218806	KM106118	KM105973	KM106050
<i>Calymmodon mnioides</i> Copel.		Karger 1675 (Z)	West Papua, Indonesia	KM218820	KM218807	KM106120	-	KM106052
<i>Calymmodon sp. 1</i>		Rouhan et al. 601 (P)	Vanuatu	KY711749	KY711923	KY712250	KY712091	KY711576
<i>Calymmodon sp. 1</i>	x	Rouhan et al. 662 (P)	Vanuatu	KY711750	KY711924	KY712251	KY712092	KY711577
<i>Ceradenia argyrata</i> (Bory ex Willd.) Parris	x	Janssen et al. 2716 (P)	La Réunion Island, France	KY711752	KY711926	KY712253	KY712094	KY711579
<i>Ceradenia argyrata</i>		Rakotondrainibe 6904 (P)	La Réunion Island, France	KY711751	KY711925	KY712252	KY712093	KY711578
<i>Ceradenia aulaeifolia</i> L. E. Bishop ex A. R. Sm.	x	Rojas et al. 3232 (CR)	Costa Rica	AY459453	AY460619	KM106122	KM105974	GU476623
<i>Ceradenia ayapoyana</i> M. Kessler & A. R. Sm.	x	Jiménez 1114 (UC)	Bolivia	-	KM218811	KM106123	KM105975	KM106053
<i>Ceradenia comorensis</i> (Bak.) Parris	x	Janssen et al. 2452 (P)	Madagascar	KY711760	KY711935	KY712262	KY712103	KY711588
<i>Ceradenia curvata</i> (Sw.) L. E. Bishop	x	Jiménez 1559 (UC)	Bolivia	KM218821	KM218788	-	KM105976	KM106054
<i>Ceradenia deltodon</i> (Bak.) Parris		Janssen et al. 2924 (P)	Madagascar	-	KY711932	KY712259	KY712100	KY711585
<i>Ceradenia deltodon</i>		Kessler 12741 (UC)	Madagascar	-	-	-	-	KY711565
<i>Ceradenia deltodon</i>		Rakotondrainibe 6402 (P)	Madagascar	KY711757	KY711931	KY712258	KY712099	KY711584
<i>Ceradenia deltodon</i>		Rouhan et al. 1250 (P)	Madagascar	KY711754	KY711928	KY712255	KY712096	KY711581
<i>Ceradenia deltodon</i>	x	Rouhan et al. 1250bis (P)	Madagascar	KY711755	KY711929	KY712256	KY712097	KY711582
<i>Ceradenia deltodon</i>		Rouhan et al. 1350 (P)	Madagascar	KY711756	KY711930	KY712257	KY712098	KY711583
<i>Ceradenia deltodon</i>		Rouhan et al. 1217 (P)	Madagascar	KY711753	KY711927	KY712254	KY712095	KY711580
<i>Ceradenia farinosa</i> (Hook.) L. E. Bishop	x	Neill 11985 (MO)	Ecuador	KM218823	KM218790	KM106124	KM105977	KM106055
<i>Ceradenia fucooides</i> (Christ) L. E. Bishop	x	Sundue 1766 (NY)	Costa Rica	GU476749	GU476907	KM106125	-	GU476625
<i>Ceradenia intonsa</i> L. E. Bishop ex Leon-Parra & Mostacero	x	Sundue 1321 (NY)	Colombia	GU476750	GU476901	KM106126	-	GU476626

<i>Ceradenia intricata</i> (C. V. Morton) L. E. Bishop ex A. R. Sm.	x	Lehnert 1108 (UC)	Bolivia	KM218833	KM218791	KM106127	KM105978	KM106056
<i>Ceradenia jungermanniioides</i> (Klotzsch) L. E. Bishop	x	Smith 2576 (UC)	Costa Rica	AY459454	AY460620	-	-	-
<i>Ceradenia aff. kalbreyeri</i> (Baker) L. E. Bishop		Vasco & Sundue 701 (NY)	Colombia	GU476747	GU476825	KM106121	-	GU476622
<i>Ceradenia kalbreyeri</i>	x	Sundue 1759 (NY)	Colombia	GU476744	GU476905	KM106128	KM105979	GU476617
<i>Ceradenia leucosora</i> (Boj. ex Hook.) Parris		Janssen et al. 2689 (P)	La Réunion Island, France	KY711759	KY711934	KY712261	KY712102	KY711587
<i>Ceradenia leucosora</i>	x	Rakotondrainibe 6908 (P)	La Réunion Island, France	KY711758	KY711933	KY712260	KY712101	KY711586
<i>Ceradenia madidiensis</i> M. Kessler & A. R. Sm.	x	Jiménez 1089 (UC)	Bolivia	KM218834	-	KM106129	-	KM106057
<i>Ceradenia pearcei</i> (Baker) L. E. Bishop	x	Fuentes & Villalobos 13997 (MO)	Bolivia	KM218822	KM218789	KM106130	KM105980	KM106058
<i>Ceradenia pilipes</i> (Baker) L. E. Bishop	x	Rojas et al. 3233 (INB)	Costa Rica	AY459456	AY460622	-	-	GU476620
<i>Ceradenia sechellarum</i> (Bak.) Parris	x	Senterre 6122 (P)	Seychelles	KY711886	KY712060	KY712378	KY712226	KY711706
<i>Chrysogrammitis glandulosa</i> (J. Sm.) Parris	x	Ranker 2195 (COLO)	Sabah, Malaysia	JF514082	JF514014	-	-	JF514048
<i>Chrysogrammitis musgraviana</i> (Baker) Parris		Kessler 12570 (UC)	Sabah, Malaysia	AY459458	AY460624	KM106131	-	GU476630
<i>Chrysogrammitis musgraviana</i>	x	Sundue 2234 (VT)	Sabah, Malaysia	KM218825	KM218797	KM106132	KM105981	KM106059
<i>Cochlidium punctatum</i> (Raddi) L. E. Bishop	x	Silva 3914 (UC)	Brazil	JF514057	JF513987	-	-	GU476631
<i>Cochlidium rostratum</i> (Hook.) Maxon ex C. Chr.	x	Valdespino & Aranda 180 (UC)	Panama	-	AY460626	-	KM105982	-
<i>Cochlidium seminudum</i> (Willd.) Maxon	x	Hill 29102A no voucher***	Dominican Republic	AY459460	AY460627	-	-	KM106060
<i>Cochlidium serrulatum</i> (Sw.) L. E. Bishop		Hirai & Swartzburd 541 (SP)	Brazil	JF514078	JF514010	KM106133	KM105983	JF514044
<i>Cochlidium serrulatum</i>		Rakotondrainibe 6903 (P)	La Réunion Island, France	KY711763	KY711938	KY712265	-	KY711590
<i>Cochlidium serrulatum</i>		Rouhan et al. 1142 (P)	Madagascar	KY711761	KY711936	KY712263	KY712104	KY711589
<i>Cochlidium serrulatum</i>		Rouhan et al. 1314 (P)	Madagascar	KY711762	KY711937	KY712264	KY712105	-
<i>Cochlidium serrulatum</i>		Rouhan et al. 141 (P)	Seychelles	KY711764	KY711939	KY712266	KY712106	KY711591

<i>Cochlidium serrulatum</i>		Rouhan et al. 172 (P)	Mauritius	KY711765	KY711940	KY712267	KY712107	KY711592
<i>Cochlidium serrulatum</i>	x	Rouhan et al. 383 (P)	Madagascar	KY711766	KY711941	KY712268	KY712108	KY711593
<i>Ctenopterella denticulata (Blume) Parris</i>	x	Chen 2010 (TAIF)*	Java, Indonesia	-	-	KM106134	KM105984	-
<i>Ctenopterella denticulata</i>	x	Ranker 2113 (COLO)*	Sabah, Malaysia	JF514081	JF514013	-	-	JF514047
<i>Ctenopterella lasiostipes (Mett.) Parris</i>		Mackee 17342 (P)	New Caledonia, France	KY711896	KY712068	KY712389	KY712231	KY711719
<i>Ctenopterella lasiostipes</i>		Mackee 42028 (P)	New Caledonia, France	KY711898	KY712070	KY712391	-	KY711721
<i>Ctenopterella lasiostipes</i>		Mackee 43573 (P)	New Caledonia, France	-	-	KY712392	-	KY711722
<i>Ctenopterella lasiostipes</i>		Mackee 45654 (P)	New Caledonia, France	KY711899	KY712071	KY712393	-	KY711723
<i>Ctenopterella lasiostipes</i>		Munzinger 1166 (P)	New Caledonia, France	KY711777	KY711952	KY712278	KY712119	KY711604
<i>Ctenopterella lasiostipes</i>	x	Munzinger 1673 (P)	New Caledonia, France	KY711778	KY711953	KY712279	KY712120	KY711605
<i>Ctenopterella lasiostipes</i>		Munzinger 687 (P)	New Caledonia, France	KY711897	KY712069	KY712390	KY712232	KY711720
<i>Ctenopterella parvula (Bory ex Willd.) Parris</i>	x	Janssen et al. 2455 (P)	Madagascar	KY711770	KY711945	KY712272	KY712112	KY711597
<i>Ctenopterella parvula</i>		Pignal et al. 3378 (P)	Comoros	KY711771	KY711946	KY712273	KY712113	KY711598
<i>Ctenopterella parvula</i>		Rakotondrainibe 6898 (P)	La Réunion Island, France	KY711769	KY711944	KY712271	KY712111	KY711596
<i>Ctenopterella sp.</i>		Tronchet 551 (P)	New Caledonia, France	KY711780	KY711955	-	-	-
<i>Ctenopterella sp. 1</i>		Le Rat 912 (P)	New Caledonia, France	KY711891	KY712065	KY712384	-	KY711712
<i>Ctenopterella sp. 1</i>		Mackee 18525 (P)	New Caledonia, France	KY711908	KY712076	KY712401	-	KY711737
<i>Ctenopterella sp. 1</i>		Mackee 6387 (P)	New Caledonia, France	-	-	KY712383	-	KY711711
<i>Ctenopterella sp. 1</i>		Perrie P026561 (WELT)	New Caledonia, France	-	KY711917	-	-	KY711558
<i>Ctenopterella sp. 2</i>	x	Rouhan et al. 618 (P)	Vanuatu	KY711767	KY711942	KY712269	KY712109	KY711594

<i>Ctenopterygia zenkeri</i> (Hieron.) Parris		Kessler 12659 (UC)	Madagascar	-	KY711913	KY712239	KY712083	-
<i>Ctenopterygia zenkeri</i>		Rakotondrainibe 6081 (P)	Madagascar	KY711775	KY711950	-	KY712117	KY711602
<i>Ctenopterygia zenkeri</i>		Rakotondrainibe 6982 (P)	Madagascar	KY711776	KY711951	KY712277	KY712118	KY711603
<i>Ctenopterygia zenkeri</i>		Rouhan et al. 1254 (P)	Madagascar	KY711792	KY711967	KY712291	KY712132	KY711617
<i>Ctenopterygia zenkeri</i>		Rouhan et al. 1287 (P)	Madagascar	KY711860	KY712035	KY712352	KY712200	KY711684
<i>Ctenopterygia zenkeri</i>		Rouhan et al. 1308 (P)	Madagascar	KY711768	KY711943	KY712270	KY712110	KY711595
<i>Ctenopterygia zenkeri</i>		Rouhan et al. 1312 (P)	Madagascar	KY711861	KY712036	KY712353	KY712201	KY711685
<i>Ctenopterygia zenkeri</i>	x	Rouhan et al. 1347 (P)	Madagascar	KY711772	KY711947	KY712274	KY712114	KY711599
<i>Ctenopterygia zenkeri</i>		Rouhan et al. 1354 (P)	Madagascar	KY711773	KY711948	KY712275	KY712115	KY711600
<i>Ctenopterygia zenkeri</i>	x	Rouhan et al. 1354bis (P)	Madagascar	KY711774	KY711949	KY712276	KY712116	KY711601
<i>Dasygrammitis brevivenosa</i> (Alderw.) Parris		Sundue 2206 (VT)	Sabah, Malaysia	KM218829	KM218775	KM106136	KM105986	KM106061
<i>Dasygrammitis brevivenosa</i>		Sundue 2222 (VT)	Sabah, Malaysia	KM218828	KM218774	KM106135	KM105985	-
<i>Dasygrammitis crassifrons</i> (Baker) Parris		Game 95-80 (UC)	Fiji	JF514062	KM218777	KM106137	-	KM106062
<i>Dasygrammitis fuscata</i> (Blume) Parris		Parris 12784 (AK)	Peninsular Malaysia	-	KM218778	KM106138	KM105987	-
<i>Dasygrammitis malaccana</i> (Baker) Parris	x	Parris 12796 (AK)	Peninsular Malaysia	-	KM218779	KM106139	KM105988	-
<i>Dasygrammitis malaccana</i>		Winkler s.n. (P)	Indonesia, Sumatra	-	-	-	-	KY711726
<i>Dasygrammitis mollicoma</i> (Nees & Bl.) Parris	x	Parris 12797 (AK)	Peninsular Malaysia	-	KM218776	-	KM105989	KM106063
<i>Enterosora barbatula</i> (Bak.) Parris	x	Tamon 53 (P)	La Réunion Island, France	KY711781	KY711956	KY712281	KY712122	KY711607
<i>Enterosora barbatula</i>		Rakotondrainibe 6951 (P)	Madagascar	KY711783	KY711958	KY712283	KY712124	KY711609
<i>Enterosora barbatula</i>	x	Rouhan et al. 1186 (P)	Madagascar	KY711782	KY711957	KY712282	KY712123	KY711608
<i>Enterosora barbatula</i>		Rouhan et al. 223 (P)	La Réunion Island, France	KY711784	KY711959	KY712284	-	-
<i>Enterosora barbatula</i>		Rouhan et al. 524 (P)	Tanzania	KY711785	KY711960	KY712285	KY712125	KY711610
<i>Enterosora enterosoroides</i> (Christ) Rojas	x	Sundue 1172 (NY)	Ecuador	GU476756	-	KM106140	-	GU476634
<i>Enterosora parietina</i> (Kl.) L. E. Bishop		Sundue 3097 (VT)	Mexico	-	-	KY712237	-	KY711559

<i>Enterosora parietina</i>	x	Schuettpelz 230 (DUKE)	Ecuador	EF463494	EF463248	-	-	-
<i>Enterosora percrassa</i> (Baker) L. E. Bishop	x	Moraga & Rojas 508 (UC)	Costa Rica	AY459468	AY460635	-	-	GU476636
<i>Enterosora percrassa</i>		Sundue 1665 (NY)	Costa Rica	GU476757	-	KM106141	-	GU476635
<i>Enterosora sprucei</i> (Hook.) Parris	x	Janssen et al. 2925 (P)	Madagascar	KY711790	KY711965	KY712289	KY712130	KY711615
<i>Enterosora sprucei</i>		Kessler 12770 (UC)	Madagascar	-	KY711914	KY712241	KY712084	KY711566
<i>Enterosora sprucei</i>		Rakotondrainibe 6936 (P)	Madagascar	KY711789	KY711964	KY712288	KY712129	KY711614
<i>Enterosora sprucei</i>		Rouhan et al. 1145 (P)	Madagascar	KY711828	KY712004	KY712322	KY712168	KY711653
<i>Enterosora sprucei</i>		Rouhan et al. 1227 (P)	Madagascar	KY711787	KY711962	KY712286	KY712127	KY711612
<i>Enterosora sprucei</i>		Rouhan et al. 1343 (P)	Madagascar	KY711788	KY711963	KY712287	KY712128	KY711613
<i>Enterosora sprucei</i>		Rouhan et al. 1381 (P)	Madagascar	KY711791	KY711966	KY712290	KY712131	KY711616
<i>Enterosora trichosora</i> (Hook.) L. E. Bishop	x	Moran 7569 (NY)	Ecuador	-	GU476920	-	-	GU476637
<i>Enterosora trifurcata</i> (L.) L. E. Bishop	x	Ranker 1608 (COLO)*	Puerto Rico	-	-	KM106142	-	-
<i>Enterosora trifurcata</i>	x	Sundue 1774 (NY)*	Costa Rica	GU476754	GU476909	-	-	GU476632
<i>Galactodenia delicatula</i> (M. Martens & Galeotti) Sundue & Labiak	x	Mickel 4639 (NY)	Mexico	-	-	GU387123	-	KM106064
<i>Galactodenia parrisiae</i> Sundue & Labiak		Monro & Knapp 5721 (NY)	Panama	-	KM218794	-	KM105990	-
<i>Galactodenia subscabra</i> (Klotzsch) Sundue & Labiak	x	Moran 8078 (NY)	Costa Rica	GU476821	GU476860	-	GU387209	GU476739
<i>Galactodenia subscabra</i>	x	Rojas et al. 3211 (UC)	Costa Rica	AY459511	AY460677	-	-	GU476740
<i>Grammitis bryophila</i> (Maxon) F. Seym.	x	Rojas et al. 3240 (UC)	Costa Rica	AF469784	AF468208	KM106143	-	AF469797
<i>Grammitis copelandii</i> Tard.	x	Janssen et al. 2515 (P)	Madagascar	KY711795	KY711970	-	-	-
<i>Grammitis copelandii</i>		Miller 3556 (MO)	Madagascar	-	-	-	-	KY711562
<i>Grammitis copelandii</i>		Rouhan et al. 1190 (P)	Madagascar	KY711793	KY711968	KY712292	KY712133	KY711618
<i>Grammitis copelandii</i>	x	Rouhan et al. 1241 (P)	Madagascar	KY711882	KY712057	KY712374	KY712222	KY711703
<i>Grammitis coriaceifolia</i> ined.	x	Rouhan et al. 1181 (P)	Madagascar	KY711796	KY711971	KY712294	KY712135	KY711620
<i>Grammitis cryptophlebia</i> (Baker) Copel.		Rakotondrainibe 6912 (P)	La Réunion Island, France	KY711885	KY712059	KY712377	KY712225	KY711705
<i>Grammitis cryptophlebia</i>	x	Kluge 7936 (Z)	Madagascar	KM218815	KM218799	KM106144	KM105992	KM106065
<i>Grammitis cryptophlebia</i>		Rakotondrainibe 6930 (P)	Madagascar	KY711800	KY711975	KY712298	KY712139	KY711624

<i>Grammitis cryptophlebia</i>		Rouhan et al. 1247 (P)	Madagascar	KY711797	KY711972	KY712295	KY712136	KY711621
<i>Grammitis cryptophlebia</i>		Rouhan et al. 1337 (P)	Madagascar	KY711827	KY712003	KY712321	KY712167	KY711652
<i>Grammitis cryptophlebia</i>	x	Tamon 16 (P)	La Réunion Island, France	KY711798	KY711973	KY712296	KY712137	KY711622
<i>Grammitis deplanchei (Baker) Copel.</i>	x	Gaudeul 99 (P)	New Caledonia, France	KY711801	KY711976	KY712299	KY712140	KY711625
<i>Grammitis deplanchei</i>		Hodel 1450 (UC)	New Caledonia, France	AY459471	AY460639	KM106145	-	GU476639
<i>Grammitis deplanchei</i>		Munzinger 1242 (P)	New Caledonia, France	KY711802	KY711977	KY712300	KY712141	KY711626
<i>Grammitis deplanchei</i>		Munzinger 1422 (P)	New Caledonia, France	KY711803	KY711978	KY712301	KY712142	KY711627
<i>Grammitis deplanchei</i>		Tronchet 529 (P)	New Caledonia, France	KY711779	KY711954	KY712280	KY712121	KY711606
<i>Grammitis diminuta</i>	x	Papadopulos AP794 (NSW)	Lord Howe Island, Australia	-	JF950810	-	-	JF950911
<i>Grammitis diminuta (Baker) Copel.</i>		Papadopulos AP845 (NSW)	Lord Howe Island, Australia	-	JF950809	-	-	JF950910
<i>Grammitis ebenina (Maxon) Tard.</i>	x	Eastwood 199 (E)	St Helena, United Kingdom	KY711890	KY712064	KY712382	-	KY711710
<i>Grammitis ebenina</i>		Rouhan et al. 1165 (P)	Madagascar	KY711825	KY712001	KY712320	KY712165	KY711650
<i>Grammitis ebenina</i>	x	Rouhan et al. 1180 (P)	Madagascar	KY711804	KY711979	KY712302	KY712143	KY711628
<i>Grammitis ebenina</i>		Rouhan et al. 1242 (P)	Madagascar	KY711805	KY711980	KY712303	KY712144	KY711629
<i>Grammitis holophlebia (Bak.) Copel.</i>		Janssen et al. 2514 (P)	Madagascar	KY711809	KY711984	-	KY712148	KY711633
<i>Grammitis holophlebia</i>	x	Rakotondrainibe 6933 (P)	Madagascar	KY711808	KY711983	KY712306	KY712147	KY711632
<i>Grammitis holophlebia</i>		Rouhan et al. 1160 (P)	Madagascar	KY711807	KY711982	KY712305	KY712146	KY711631
<i>Grammitis holophlebia</i>		Rouhan et al. 1311 (P)	Madagascar	KY711786	KY711961	-	KY712126	KY711611
<i>Grammitis kyimbilensis (Brause ex Brause & hieron.) Copel.</i>		Janssen et al. 2391 (P)	Madagascar	KY711813	KY711988	KY712310	KY712152	KY711637
<i>Grammitis kyimbilensis</i>	x	Janssen et al. 2663 (P)	Tanzania	KY711814	KY711989	KY712311	KY712153	KY711638
<i>Grammitis kyimbilensis</i>		Kluge 7857 (UC)	Madagascar	KY711742	KY711915	KY712238	KY712082	KY711560
<i>Grammitis kyimbilensis</i>	x	Rakotondrainibe 6347 (P)	Madagascar	KY711812	KY711987	KY712309	KY712151	KY711636
<i>Grammitis kyimbilensis</i>		Rouhan et al. 1242bis (P)	Madagascar	KY711806	KY711981	KY712304	KY712145	KY711630

<i>Grammitis kyimbilensis</i>		Rouhan et al. 1341 (P)	Madagascar	KY711794	KY711969	KY712293	KY712134	KY711619
<i>Grammitis kyimbilensis</i>		Rouhan et al. 518 (P)	Tanzania	KY711815	KY711990	KY712312	KY712154	KY711639
<i>Grammitis melanoloma (Cordemoy) Tardieu</i>	x	Rakotondrainibe 6907 (P)	La Réunion Island, France	KY711816	KY711991	KY712313	KY712155	KY711640
<i>Grammitis melanoloma</i>		Ranker & Adsersen 1504 (COLO)	La Reunion	AY459475	AY460643	KM106148	-	GU476641
<i>Grammitis obtusa</i> Willd. ex Kaulf.		Janssen et al. 2696 (P)	La Réunion Island, France	KY711818	KY711993	KY712315	KY712157	KY711642
<i>Grammitis obtusa</i>		Ranker 1505 (COLO)	La Reunion	-	-	-	-	KY711561
<i>Grammitis obtusa</i>		Rouhan et al. 1212 (P)	Madagascar	KY711817	KY711992	KY712314	KY712156	KY711641
<i>Grammitis obtusa</i>	x	Rouhan et al. 230 (P)	La Réunion Island, France	KY711819	KY711994	-	KY712158	KY711643
<i>Grammitis paramicola</i> L. E. Bishop	x	Jiménez & Gallegos (UC)	Bolivia	KM218816	KM218801	KM106149	KM105994	KM106067
<i>Grammitis pellucidovenosa</i> (Bonap.) Ching	x	Janssen et al. 2415 (P)	Madagascar	KY711820	KY711995	-	KY712159	KY711644
<i>Grammitis pellucidovenosa</i>		Janssen et al. 2931 (P)	Madagascar	KY711821	KY711996	-	KY712160	KY711645
<i>Grammitis pervillei</i> (Mett. ex Kuhn) Tard.	x	Senterre 6128 (P)	Seychelles	KY711887	KY712061	KY712379	KY712227	KY711707
<i>Grammitis pseudoaustralis</i> E. Fourn.		McPherson 18253 (MO)	New Caledonia, France	KY711867	KY712042	KY712359	KY712207	-
<i>Grammitis pseudoaustralis</i>		Munzinger 1676 (P)	New Caledonia, France	KY711822	KY711997	KY712316	KY712161	KY711646
<i>Grammitis pseudoaustralis</i>		Tibell 19878 (AK)	New Caledonia, France	-	KM218785	KM106150	-	KM106068
<i>Grammitis pygmaea</i> (Mett. ex Kuhn) Copel.		Janssen et al. 2664 (P)	Tanzania	-	KY712000	KY712319	KY712164	KY711649
<i>Grammitis pygmaea</i>		Rakotondrainibe 6983 (P)	Madagascar	KY711824	KY711999	KY712318	KY712163	KY711648
<i>Grammitis pygmaea</i>	x	Rouhan et al. 1184 (P)	Madagascar	KY711823	KY711998	KY712317	KY712162	KY711647
<i>Grammitis pygmaea</i>		Rouhan et al. 1313 (P)	Madagascar	KY711799	KY711974	KY712297	KY712138	KY711623
<i>Grammitis pygmaea</i>		Senterre 5986 (P)	Seychelles	KY711888	KY712062	KY712380	KY712228	KY711708
<i>Grammitis stenophylla</i> Parris	x	Parris 12576 (AK)	Australia	JX499239	JQ904084	-	-	JQ911714
<i>Grammitis synsora</i> (Bak.) Copel.		Rakotondrainibe 6932 (P)	Madagascar	KY711830	KY712006	-	KY712170	KY711655
<i>Grammitis synsora</i>	x	Rouhan et al. 1173 (P)	Madagascar	KY711826	KY712002	-	KY712166	KY711651

<i>Grammitis synsora</i>		Rouhan et al. 1316 (P)	Madagascar	KY711829	KY712005	-	KY712169	KY711654
<i>Lellingeria apiculata</i> (Kunze ex Klotzsch) A. R. Sm. & R. C. Moran	x	Labiak 4223 (UPCB)	Brazil	GU376573	GU387025	GU387047	GU387133	GU387215
<i>Lellingeria bishopii</i> Labiak		Sundue 1159 (NY)	Ecuador	GU376574	GU387000	GU387048	-	GU387216
<i>Lellingeria flagellipinnata</i> M. Kessler & A. R. Sm.		Labiak 4726 (NY)	Bolivia	GU376582	GU387032	GU387056	GU387142	GU387224
<i>Lellingeria hirsuta</i> A. R. Sm. & R. C. Moran	x	Rojas & Fletes 3145 (UC)	Costa Rica	AY459482	AY460649	-	-	-
<i>Lellingeria humilis</i> (Mett.) A. R. Sm. & R. C. Moran		Sundue & Vasco 1289 (NY)	Colombia	GU476763	GU476897	-	-	GU476649
<i>Lellingeria major</i> (Copel.) A. R. Sm. & R. C. Moran		Sundue 1147 (NY)	Ecuador	GU376592	GU386998	GU387069	GU387155	GU387233
<i>Lellingeria randallii</i> (Maxon) A. R. Sm. & R. C. Moran		Mickel 9495 (NY)	Trinidad	KM218860	GU386992	GU387090	KM105996	GU387253
<i>Lellingeria simacensis</i> (Rosenst.) A. R. SM. & R. C. Moran		Sundue 1169 (NY)	Ecuador	GU376610	GU387001	GU387092	GU387175	GU387255
<i>Lellingeria subsessilis</i> (Baker) A. R. Sm. & R. C. Moran		Sundue 1139 (NY)	Ecuador	GU376613	GU386997	GU387095	GU387178	GU387258
<i>Lellingeria suprasculpta</i> (Christ) A. R. Sm. & R. C. Moran		Lellinger 989 (US)	Costa Rica	GU376614	GU386987	GU387096	GU387179	GU387259
<i>Lellingeria suspensa</i> (L.) A. R. Sm. & R. C. Moran		Sundue 1047 (NY)	Ecuador	GU376618	GU386994	GU387100	GU387183	GU387263
<i>Leucotrichum madagascariense</i> Rakotondr. & Rouhan	x	Rakotondrainibe 6957 (P)	Madagascar	JN654936	JN654924	JN654949	JN654962	JN654975
<i>Leucotrichum mitchelliae</i> (Baker ex Hemsl.) Labiak	x	Breedlove & Almeda 48307 (NY)	Mexico	GU376479	GU376488	JN654937	JN654950	JN654963
<i>Leucotrichum mortonii</i> (Copel.) Labiak	x	Liogier 16026 (NY)	Dominican Republic	GU376478	GU376489	-	-	-
<i>Leucotrichum organense</i> (Gardner) Labiak	x	Labiak 4302 (UPCB)	Brazil	GU376485	GU376492	JN654946	JN654959	JN654972
<i>Leucotrichum pseudomitchelliae</i> (Lellinger) Labiak	x	Rojas 3005 (MO)	Costa Rica	AY459484	AY460652	-	-	-
<i>Leucotrichum schenckii</i> (Hieron.) Labiak	x	Salino 4538 (UC)	Brazil	AY459483	AY460651	-	-	KM106072
<i>Lomaphlebia turquina</i> (Maxon) Sundue & Ranker	x	Sanchez 82061 (HAJB)	Cuba	KM218814	KM218800	-	-	KM106069

<i>Melpomene allosuroides</i> (Rosenst.) A. R. Sm. & R. C. Moran	x	Solomon 1289 (NY)	Bolivia	GU376628	GU387038	GU387109	GU387192	GU387273
<i>Melpomene anazalea</i> Sundue & Lehnert	x	Sundue 1290 (NY)	Colombia	GU476773	GU476898	KM106152	KM105997	GU476662
<i>Melpomene erecta</i> (C. V. Morton) A. R. Sm. & R. C. Moran	x	Nunez 25 (NY)	Bolivia	GU376629	GU387013	GU387110	GU387193	GU387274
<i>Melpomene firma</i> (J. Sm.) A. R. Sm. & R. C. Moran	x	Labiak 4733 (UPCB)	Bolivia	GU376630	GU387035	GU387112	GU387195	GU387276
<i>Melpomene flabelliformis</i> (Poir.) A. R. Sm. & R. C. Moran		Janssen et al. 2669 (P)	Tanzania	KY711836	KY712012	KY712328	KY712176	KY711661
<i>Melpomene flabelliformis</i>		Rakotondrainibe 6906 (P)	La Réunion Island, France	KY711834	KY712010	KY712326	KY712174	KY711659
<i>Melpomene flabelliformis</i>		Rakotondrainibe 6940 (P)	Madagascar	KY711835	KY712011	KY712327	KY712175	KY711660
<i>Melpomene flabelliformis</i>	x	Rouhan et al. 1179 (P)	Madagascar	KY711833	KY712009	KY712325	KY712173	KY711658
<i>Melpomene flabelliformis</i>		Sundue 1303 (NY)	Colombia	GU376631	GU387005	GU387113	GU387196	GU387277
<i>Melpomene melanosticta</i> (Kunze) A. R. Sm. & R. C. Moran	x	Labiak 4156 (NY)	Brazil	GU376633	GU387024	GU387115	-	GU387279
<i>Melpomene moniliformis</i> (Lag. ex Sw.) A. R. Sm. & R. C. Moran		Moraga & Rojas 446 (INB)	Costa Rica	AY459486	AY460654	-	-	GU476664
<i>Melpomene xiphopteridoides</i> (Liebmann) A. R. Sm. & R. C. Moran	x	Sundue 1300 (NY)	Colombia	GU376637	GU387004	GU387119	KM105998	GU387282
<i>Microgramma bifrons</i> (Hook.) Lellinger	x	Neill 8309 (UC)*	Ecuador	-	AY362582	AY362654	-	-
<i>Microgramma bifrons</i>	x	van der Werff 18062 (UC)*	Peru	EF463499	-	-	-	DQ642224
<i>Micropolypodium okuboi</i> (Yatabe) Hayata	x	Parris 12154 (AK)	Japan	JF514064	JF513994	-	-	JF514028
<i>Micropolypodium sikkimense</i> (Hieron.) X. C. Zhang		Miehe 00-093-32 (UC)	Bhutan	JF514068	JF513999	-	-	JF514032
<i>Micropolypodium sikkimense</i>	x	Xian et al. s.n. (UC)	China	JF514087	JF514019	-	-	JF514054
<i>Moranopteris achilleifolia</i> (Kaulf.) R. Y. Hirai & J. Prado	x	Cordeiro & Ribas 1398 (UC)	Brazil	AY459499	AY460666	KM106153	KM105999	-
<i>Moranopteris blepharidea</i> (Copel.) R. Y. Hirai & J. Prado	x	Jiménez 708 (GOET)	Bolivia	JF514065	JF513995	KM106154	KM106000	JF514029
<i>Moranopteris caucana</i> (Hieron.) R. Y. Hirai & J. Prado	x	Lehnert 182 (GOET)	Ecuador	JF514071	JF514002	KM106155	KM106001	JF514035

<i>Moranopteris cookii</i> (Underw. & Maxon ex Maxon) R. Y. Hirai & J. Prado	x	Sundue 1771 (NY)	Costa Rica	JF514076	JF514007	KM106156	KM106002	JF514040
<i>Moranopteris hyalina</i> (Maxon) R. Y. Hirai & J. Prado	x	Lehnert 1426 (GOET)	Ecuador	JF514070	JF514001	KM106157	KM106003	JF514034
<i>Moranopteris longisetosa</i> (Hook.) R. Y. Hirai & J. Prado	x	Lehnert 596 (GOET)	Bolivia	JF514072	JF514003	KM106158	KM106004	JF514036
<i>Moranopteris plicata</i> (A. R. Sm.) R. Y. Hirai & J. Prado	x	Lehnert 929 (UC)	Ecuador	JF514074	JF514005	KM106159	KM106005	JF514038
<i>Moranopteris</i> sp.1	x	Bauret et al. 107 (P)	Madagascar	-	KY711912	-	-	-
<i>Moranopteris truncicola</i> (Klotzsch) R. Y. Hirai & J. Prado	x	Vasco & Sundue 626 (NY)	Colombia	JF514084	JF514016	KM106160	KM106006	JF514051
<i>Mycopteris amphidasyon</i> (Kunze ex Mett.) Sundue		Moran 7646 (NY)	Ecuador	GU476759	GU476922	KM106161	KM106007	GU476638
<i>Mycopteris attenuatissima</i> (Copel.) Sundue		Sundue 1098 (NY)	Ecuador	GU386959	GU476866	-	GU387204	GU476705
<i>Mycopteris leucosticta</i> (J. Sm.) Sundue		Lehnert 1128 (UC)	Ecuador	GU476811	GU476848	KM106162	-	GU476720
<i>Mycopteris longicaulis</i> (Sundue & M. Kessler) Sundue		Jimenez 373 (UC)	Bolivia	GU476813	GU476840	KM106163	-	GU476724
<i>Mycopteris longipilosa</i> Sundue		Sundue & Martin 1033 (NY)	Ecuador	GU476814	GU476861	KM106164	KM106008	GU476726
<i>Mycopteris praeceps</i> (Sundue & M. Kessler) Sundue		Jimenez 2173 (UC)	Bolivia	GU476817	GU476839	KM106165	-	GU476734
<i>Mycopteris aff. subtilis</i> (Kunze ex Klotzsch) Sundue		Sundue & Schuettpelz 1077 (NY)	Ecuador	-	GU476865	KM106166	-	GU476687
<i>Mycopteris subtilis</i>	x	Sundue 1140 (NY)	Ecuador	KY711743	KY711916	KY712244	KY712085	KY711569
<i>Mycopteris taxifolia</i> (L.) Sundue	x	Labiak 4018 (NY)	Brazil	GU476800	GU476914	KM106167	KM106009	GU476699
<i>Niphidium crassifolium</i> (L.) Lellinger	x	Kreier s.n. (GOET)*	cultivated	-	-	EU250358	-	EU250359
<i>Niphidium crassifolium</i>	x	Schuettpelz 209 (DUKE)*	Ecuador	EF463503	EF463254	-	-	-
<i>Notogrammitis cf. angustifolia</i> (Jacq.) Parris		Jolinon 1089 (P)	Amsterdam Island, France	KY711907	KY712075	KY712400	KY712234	KY711735
<i>Notogrammitis angustifolia</i>		Lendemmer 30006 (NY)	Australia	KM218824	KM218770	KM106168	KM106010	KM106074
<i>Notogrammitis angustifolia</i>		Parris 12423 (AK)	New Zealand	JX499252	JQ904083	-	-	JQ904113
<i>Notogrammitis angustifolia</i>		Jolinon 1089 (P)	Amsterdam Island, France	-	-	-	-	KY711736

<i>Notogrammitis billardierei</i> (Willd.) Parris		Brownsey P022138 (WELT)	Australia	JX499250,	JQ904096	-	-	JQ904103
<i>Notogrammitis billardierei</i>	x	Parris 12421 (AK)	New Zealand	AY459469	JQ904094	KM106169	KM106011	JQ904102
<i>Notogrammitis billardierei</i>		Sundue s.n. (VT)	Australia	-	KM218755	KM106170	KM106012	KM106075
<i>Notogrammitis ciliata</i> (Colenso) Parris		Jane s.n. (AK)	New Zealand	JX499249	JQ904097	-	-	JQ904105
<i>Notogrammitis ciliata</i>		Parris 12360 (AK)	New Zealand	JX499247	JQ904078	-	-	JQ904104
<i>Notogrammitis ciliata</i>		Parris 12657 (AK)	New Zealand	JX499248	JQ904095	-	-	JQ904106
<i>Notogrammitis crassior</i> (Kirk) Parris		Boulangeat 11 (P)	Kerguelen Islands, France	KY711810	KY711985	KY712307	KY712149	KY711634
<i>Notogrammitis crassior</i>		Boulangeat 19 (P)	Kerguelen Islands, France	KY711811	KY711986	KY712308	KY712150	KY711635
<i>Notogrammitis crassior</i>		Corner 333 (P)	Malouines, UK	-	-	-	-	KY711734
<i>Notogrammitis crassior</i>		Huntley 721 (P)	Prince Edward Isl., South-Africa	-	-	-	-	KY711733
<i>Notogrammitis crassior</i>		Perrie 3742 (WELT)	New Zealand	JX499241	JQ904077	-	-	JQ904115
<i>Notogrammitis crassior</i>		Quetel & Chambrin 2 (P)	Kerguelen Islands, France	KY711744	KY711918	KY712246	KY712086	KY711571
<i>Notogrammitis givenii</i> (Parris) Parris		Perrie 3842 (WELT)	New Zealand	JX499242	JQ904079	-	-	JQ904110
<i>Notogrammitis heterophylla</i> (Labill.) Parris	x	Parris 12419 (AK)	New Zealand	AY459462	AY460629	-	-	JQ904100
<i>Notogrammitis patagonica</i> (C. Chr.) Parris		Perrie 4760 (WELT)	New Zealand	JX499246	JQ904088	-	-	-
<i>Notogrammitis pseudociliata</i> (Parris) Parris		Perrie 4088 (WELT)	New Zealand	JX499245	JQ904080	-	-	JQ904117
<i>Notogrammitis rawlingsii</i> (Parris) Parris		Young s.n. (AK296949) (AK)	New Zealand	-	JQ904085	-	-	JQ904119
<i>Notogrammitis rigida</i> (Hombron) Parris		Perrie 4857 (WELT)	New Zealand	-	JQ904082	-	-	JQ904121
<i>Oreogrammitis adspersa</i> (Blume) Parris		Sundue 2233 (VT)	Sabah, Malaysia	-	KM218808	KM106171	KM106013	KM106076
<i>Oreogrammitis congener</i> (Blume) Parris		Parris 12782 (AK)	Peninsular Malaysia	KM218845	KM218762	KM106172	KM106014	KY711570
<i>Oreogrammitis congener</i>		Ranker 2126 & Klimas (COLO)	Sabah, Malaysia	-	EF178617	-	KY712081	-
<i>Oreogrammitis forbesiana</i> (W. H. Wagner) Parris		Ranker 1321 (COLO)	Hawaii, USA	AY459472	AY460640	-	-	-

<i>Oreogrammitis graniticola</i> Parris ined.		Sundue 2212 (VT)	Sabah, Malaysia	KM218846	KM218757	-	KM106015	KM106078
<i>Oreogrammitis hookeri</i>		Ranker 1116 (COLO)	Hawaii, USA	AY459473	AY460642	-	-	-
<i>Oreogrammitis locellata</i> (Baker) Parris		Clemens 12380 (UC)	Papua New Guinea	-	KM218763	KM106173	-	KM106079
<i>Oreogrammitis medialis</i> (Bak.) Parris		Ballard 1082 (P)	Sri Lanka	-	-	KY712397	-	KY711730
<i>Oreogrammitis reinwardtioides</i> (Copel.) Parris		Ranker 2228 (COLO)*	Sabah, Malaysia	-	KM218756	KM106174	-	-
<i>Oreogrammitis reinwardtioides</i>		Sundue 2202 (VT)*	Sabah, Malaysia	KM218847	-	-	KM106016	KM106080
<i>Oreogrammitis</i> sp. 1	x	Rouhan et al. 614 (P)	Vanuatu	KY711837	KY712013	KY712329	KY712177	KY711662
<i>Oreogrammitis uapensis</i> (E. D. Br.) Parris		Wood 10825 (US)	Marquesas Islands	-	KM218787	-	KM105995	KM106070
<i>Oreogrammitis ultramaficola</i> Parris ined.		Ranker 2153 (COLO)	Sabah, Malaysia	KM218841	KM218754	KM106151	-	KM106071
<i>Oreogrammitis wurunuran</i> (Parris) Parris		Kessler 14307 (VT)	Australia	KM218842	KM218760	KM106175	KM106017	-
<i>Oreogrammitis wurunuran</i>		Kessler 14251 (VT)	Australia	KM218843	KM218761	KM106176	KM106018	-
<i>Polypodium glycyrrhiza</i> D. C. Eaton	x	Haufler & Mesler s.n. (KANU)	United States	AY459518	-	-	-	GU476671
<i>Prosaptia celebica</i> (Blume) Tagawa & K. Iwats.		Parris 12835 (K)	Philippines	KM218849	KM218786	KM106178	KM106021	-
<i>Prosaptia contigua</i> (G. Forst.) C. Presl	x	Munzinger 1271 (P)	New Caledonia, France	KY711838	KY712014	KY712330	KY712178	KY711663
<i>Prosaptia contigua</i>		Rouhan et al. 669 (P)	Vanuatu	KY711839	KY712015	KY712331	KY712179	KY711664
<i>Prosaptia contigua</i>		Sundue 2219 (VT)	Sabah, Malaysia	KM218850	KM218765	-	KM106022	KM106083
<i>Prosaptia davalliacea</i> (F. Muell. & Baker) Copel.		Sundue 2210 (VT)	Sabah, Malaysia	KM218852	KM218798	KM106179	KM106023	KM106084
<i>Prosaptia maidenii</i> (Watts) Parris		Kessler 14243 (VT)	Australia	-	KM218766	-	KM106024	-
<i>Prosaptia obliquata</i> (Blume) Mett.		Larsen 33479 (P)	Thailand	KY711894	KY712067	KY712387	KY712230	KY711716
<i>Prosaptia obliquata</i>		Karger 166 (Z)	Philippines	KM218854	KM218768	KM106181	KM106026	KM106085
<i>Prosaptia palauensis</i> Hosokawa		Rondeau & Rodda 93-011 (UC)	Palau, Micronesia	-	AY460662	-	KM106027	-
<i>Prosaptia pectinata</i> T.Moore		Averyanov 3376 (P)	Vietnam	KY711893	-	-	-	KY711715
<i>Prosaptia pinnatifida</i> C. Presl		Karger 608 (Z)	Philippines	KM218848	KM218764	KM106177	KM106020	KM106082

<i>Prosaptia serriformis</i> (Wall. ex Hook.) Christ		Middleton 5509 (E)	Thailand	KY711910	KY712078	KY712403	KY712235	KY711740
<i>Prosaptia</i> sp. 1		Flynn 6004 (UC)	Kosrae, Micronesia	AY459493	AY460660	-	KM106019	KM106081
<i>Prosaptia</i> sp. 1	x	Rouhan et al. 605 (P)	Vanuatu	KY711840	KY712016	KY712332	KY712180	KY711665
<i>Prosaptia</i> sp. 2		Sundue 2740 (VT)	Australia	KM218851	KM218767	KM106180	KM106025	-
<i>Prosaptia venulosa</i> (Blume) M. G. Price		Karger 859 (Z)	Philippines	KM218853	KM218769	KM106182	-	KM106086
<i>Radiogrammitis cheesemanii</i> (Parris) Parris		Game 85-53 (UC)	Rarotonga	KM218861	-	KM106183	-	KM106087
<i>Radiogrammitis hirtelloides</i> (Copel.) Parris		Rouhan et al. 615 (P)	Vanuatu	KY711841	KY712017	KY712333	KY712181	KY711666
<i>Radiogrammitis hirtelloides</i>		Rouhan et al. 663 (P)	Vanuatu	KY711842	KY712018	KY712334	KY712182	KY711667
<i>Radiogrammitis holttumii</i> (Copel.) Parris	x	Sundue 2208 (VT)	Sabah, Malaysia	KM218856	KM218771	KM106184	KM106028	EF178653
<i>Radiogrammitis jagoriana</i> (Mett. ex Kuhn) Parris	x	Sundue 2225 (VT)	Sabah, Malaysia	KM218857	-	-	-	KM106103
<i>Radiogrammitis kinabaluensis</i> (Copel.) Parris		Sundue 2211 (VT)	Sabah, Malaysia	KM218855	KM218773	KM106185	-	KM106088
<i>Radiogrammitis multifolia</i> (Copel.) Parris		Tagawa et al. T4797 (US)	Thailand	-	KM218772	KM106146	-	KM106066
<i>Radiogrammitis neocaledonica</i> (Copel.) Parris		Lowry 6815 (P)	New Caledonia, France	KY711904	-	KY712396	-	KY711729
<i>Radiogrammitis neocaledonica</i>		Munzinger 1721 (P)	New Caledonia, France	KY711843	KY712019	KY712335	KY712183	KY711668
<i>Radiogrammitis parva</i> (Brause) Parris		Ranker 1763a (COLO)	Papua New Guinea	AY459476	AY460644	-	-	-
<i>Radiogrammitis setigera</i> (Blume) Parris	x	Li 982 (TAIF)*	Philippines	-	-	-	KM106029	-
<i>Radiogrammitis setigera</i>	x	Karger 813 (Z)*	Philippines	-	KM218759	KM106186	-	KM106089
<i>Scleroglossum pusillum</i>		Parris 12779 (AK)	Peninsular Malaysia	-	KM218812	KM106187	KM106030	KM106090
<i>Scleroglossum pusillum</i> (Blume) Alderw.	x	Middleton 5137 (E)	Thailand	KY711911	KY712079	KY712404	KY712236	KY711741
<i>Scleroglossum sulcatum</i> (Kuhn) Alderw.	x	Bowden-Kerby 24182b (UC)	Pohnpei, Micronesia	AY459498	AY460665	KM106188	-	JF514049
<i>Scleroglossum sulcatum</i> (Kuhn) Alderw.		Whitford 1503 (P)	Philippines	-	-	-	-	KY711738

<i>Scleroglossum wooroonooran</i> (F. M. Bailey) C. Chr.		Kessler 14289 (VT)	Australia	KM218835	KM218809	KM106189	KM106031	KM106091
<i>Scleroglossum wooroonooran</i>		Kessler 14304 (VT)	Australia	KM218836	KM218810	KM106190	KM106032	KM106092
<i>Scleroglossum wooroonooran</i>		Mackee 18207 (P)	New Caledonia, France	-	-	KY712386	-	KY711714
<i>Serpocaulon fraxinifolium</i> (Jacq.) A. R. Sm.	x	Jimenez 3302 (UC)	Bolivia	-	EF551071	EF551096	-	EF551134
<i>Serpocaulon triseriale</i> (Sw.) A. R. Sm.	x	Smith 2902 (UC)	Brazil	-	-	EF551118	-	EF551157
<i>Stenogrammitis hartii</i> (Jenman) Labiak	x	Lellinger 455 (US)	Dominican Republic	GU376583	GU386985	GU387057	GU387143	GU387225
<i>Stenogrammitis hellwigii</i> (Mickel & Beitel) Labiak	x	Mickel & Leonard 4636 (NY)	Mexico	GU376584	GU386990	GU387058	GU387144	GU387226
<i>Stenogrammitis hildebrandtii</i> (Hieron.) Labiak	x	Rouhan et al. 1213 (P)	Madagascar	KY711844	KY712020	KY712336	KY712184	KY711669
<i>Stenogrammitis hildebrandtii</i>		Rouhan et al. 1375 (P)	Madagascar	KY711845	KY712021	KY712337	KY712185	KY711670
<i>Stenogrammitis jamesonii</i> (Hook.) Labiak	x	Sundue 1149 (NY)	Ecuador	GU376596	GU386999	GU387074	GU387160	GU387237
<i>Stenogrammitis limula</i> (Christ) Labiak	x	Sundue 1736 (NY)	Costa Rica	GU476765	GU476903	GU387066	GU387152	GU476651
<i>Stenogrammitis myosuroides</i> (Sw.) Labiak	x	Liogier 4 (NY)	Guatemala	GU376599	GU386988	GU387077	GU387163	GU387240
<i>Stenogrammitis oosora</i> (Baker) Labiak		Cable 77 (K)	Cameroon	GU376600	GU386972	GU387078	GU387164	GU387241
<i>Stenogrammitis oosora</i>	x	Rakotondrainibe 6929 (P)	Madagascar	KY711848	KY712024	KY712340	KY712188	KY711673
<i>Stenogrammitis oosora</i>	x	Rouhan et al. 1188 (P)	Madagascar	KY711846	KY712022	KY712338	KY712186	KY711671
<i>Stenogrammitis oosora</i>		Rouhan et al. 1246 (P)	Madagascar	KY711847	KY712023	KY712339	KY712187	KY711672
<i>Stenogrammitis prionodes</i> (Mickel & Beitel) Labiak	x	Mickel & Beitel 6567 (NY)	Mexico	GU376605	GU386991	GU387086	GU387170	GU387249
<i>Stenogrammitis pumila</i> (Labiak) Labiak	x	Labiak 4015 (UPCB)	Brazil	GU376608	GU387022	GU387089	GU387173	GU387252
<i>Stenogrammitis saffordii</i> (Maxon) Labiak	x	Ranker 1892 (COLO)	Hawaii, USA	EF178645	EF178628	KM106191	-	EF178662
<i>Stenogrammitis</i> sp. 1		Jolinon 912 (P)	Amsterdam Island, France	KY711905	KY712073	KY712398	-	KY711731
<i>Stenogrammitis</i> sp. 1	x	Noël 35 (P)	Amsterdam Island, France	KY711906	KY712074	KY712399	-	KY711732

<i>Stenogrammitis</i> sp. 1		Noël 85 (P)	Amsterdam Island, France	KY711909	KY712077	KY712402	-	KY711739
<i>Stenogrammitis</i> sp. 2	x	Rakotondrainibe 6882 (P)	La Réunion Island, France	KY711849	HQ599526	KY712341	KY712189	GU476655
<i>Stenogrammitis wittigiana</i> (Fee & Glaziou ex Fee) Labiak	x	Labiak 4441 (UPCB)	Brazil	GU376625	GU387029	GU387106	GU387189	-
<i>Terpsichore asplenifolia</i> (L.) A. R. Sm.	x	Moraga & Rojas 506 (MO)	Costa Rica	JF514059	KM218802	KM106192	-	-
<i>Terpsichore chryseri</i> (Proctor ex Copel.) A. R. Sm.	x	Jiménez 1369 (UC)	Bolivia	KM218859	KM218813	-	KM106033	-
<i>Terpsichore eggersii</i> (Baker) A. R. Sm.		Hill 29109 (UC)	Dominican Republic	AF469785	AF468209	-	-	AF469798
<i>Terpsichore hanekeana</i> (Proctor) A.R.Sm.		Ranker 1610 (COLO)	Puerto Rico	AY459503	AY460670	-	-	-
<i>Terpsichore lehmanniana</i> (Hieron.) A. R. Sm.		Wilson 2589 (UC)	Ecuador	AY459506	AY460673	-	-	-
<i>Themelium conjunctisorum</i> (Baker) Parris		Ranker & Trapp 1758 (COLO)	Papua New Guinea	AY459514	AY460680	-	-	-
<i>Themelium decrescens</i> (Christ) Parris	x	Parris 12836 (AK)	Philippines	-	KM218758	KM106193	KM106034	KM106093
<i>Themelium halconense</i> (Copel.) Parris		Sundue 2226 (VT)	Sabah, Malaysia	KM218844	-	KM106194	KM106035	KM106094
<i>Tomophyllum macrum</i> (Copel.) Parris		Karger 165 (Z)*	Philippines	KM218830	-	-	-	-
<i>Tomophyllum macrum</i>		Karger 532 (Z)*	Philippines	-	KM218780	-	-	-
<i>Tomophyllum perplexum</i> (Parris) Parris		Faden 77/12 (P)	Sri Lanka	KY711901	-	-	-	KY711725
<i>Tomophyllum repandulum</i> (Mett.) Parris	x	Onraedt 3747 (P)	Sri Lanka	KY711900	KY712072	-	-	KY711724
<i>Tomophyllum repandulum</i>		Ranker & Trapp 1767 (COLO)	Papua New Guinea	AY459466	AY460633	-	KM106036	-
<i>Tomophyllum secundum</i> (Ridl.) Parris		Ranker 2193 (COLO)	Sabah, Malaysia	KM218832	KM218781	-	KM106037	KM106095
<i>Tomophyllum subsecundodissectum</i> (Zoll.) Parris	x	Mackee 11829 (P)	New Caledonia, France	KY711895	-	-	-	KY711718
<i>Tomophyllum walleri</i> (Maiden & Betche) Parris		Kessler 14250 (VT)	Australia	KM218831	KM218782	KM106196	KM106038	KM106096
<i>Xiphopterella hieronymusii</i> (C. Chr.) Parris	x	Parris 12781 (AK)	Peninsular Malaysia	-	KM218783	KM106197	KM106039	KM106097
<i>Xiphopterella sparsipilosa</i> (Holttum) Parris	x	Parris 12790 (AK)	Peninsular Malaysia	-	KM218784	KM106198	KM106040	KM106098

<i>Zygophlebia devoluta</i> (Baker) Parris		Kluge 7937 (Z)	Madagascar	KM218827	KM218793	KM106199	KM106042	KM106100
<i>Zygophlebia devoluta</i>		Kluge 7940 (Z)	Madagascar	KM218826	KM218792	-	KM106041	KM106099
<i>Zygophlebia devoluta</i>		Kessler 12687 (UC)	Madagascar	-	-	-	-	KY711563
<i>Zygophlebia devoluta</i>		Kessler 12688 (UC)	Madagascar	-	-	KY712240	-	KY711564
<i>Zygophlebia devoluta</i>		Rakotondrainibe 6023 (P)	Madagascar	KY711883	KY712058	KY712375	KY712223	KY711704
<i>Zygophlebia devoluta</i>		Rakotondrainibe 6953 (P)	Madagascar	KY711856	KY712031	KY712348	KY712196	KY711680
<i>Zygophlebia devoluta</i>		Rouhan et al. 286 (P)	Madagascar	KY711857	KY712032	KY712349	KY712197	KY711681
<i>Zygophlebia devoluta</i>		Rouhan et al. 1157 (P)	Madagascar	KY711868	KY712043	KY712360	KY712208	KY711690
<i>Zygophlebia devoluta</i>		Rouhan et al. 1226 (P)	Madagascar	KY711852	KY712027	KY712344	KY712192	KY711676
<i>Zygophlebia devoluta</i>		Rouhan et al. 1290 (P)	Madagascar	KY711853	KY712028	KY712345	KY712193	KY711677
<i>Zygophlebia devoluta</i>		Rouhan et al. 1336 (P)	Madagascar	KY711869	KY712044	KY712361	KY712209	KY711691
<i>Zygophlebia devoluta</i>		Rouhan et al. 1351 (P)	Madagascar	KY711854	KY712029	KY712346	KY712194	KY711678
<i>Zygophlebia devoluta</i>	x	Rouhan et al. 1379 (P)	Madagascar	KY711855	KY712030	KY712347	KY712195	KY711679
<i>Zygophlebia forsythiana</i> (Bak.) Parris		Rouhan et al. 1344 (P)	Madagascar	KY711859	KY712034	KY712351	KY712199	KY711683
<i>Zygophlebia forsythiana</i>	x	Rouhan et al. 1380 (P)	Madagascar	KY711858	KY712033	KY712350	KY712198	KY711682
<i>Zygophlebia goodmanii</i> Rakotondr.		Kessler 12778 (UC)	Madagascar	-	-	KY712242	-	KY711567
<i>Zygophlebia goodmanii</i>		Rakotondrainibe 6345 (P)	Madagascar	KY711862	KY712037	KY712354	KY712202	-
<i>Zygophlebia goodmanii</i>	x	Rouhan et al. 424 (P)	Madagascar	KY711863	KY712038	KY712355	KY712203	KY711686
<i>Zygophlebia goodmanii</i>		Rouhan et al. 1164 (P)	Madagascar	KY711850	KY712025	KY712342	KY712190	KY711674
<i>Zygophlebia goodmanii</i>		Rouhan et al. 1189 (P)	Madagascar	KY711851	KY712026	KY712343	KY712191	KY711675
<i>Zygophlebia goodmanii</i>		Rouhan et al. 1338 (P)	Madagascar	KY711870	KY712045	KY712362	KY712210	KY711692
<i>Zygophlebia goodmanii</i>		Rouhan et al. 1382 (P)	Madagascar	KY711871	KY712046	KY712363	KY712211	KY711693
<i>Zygophlebia humbertii</i> (C. Chr.) Parris	x	Rakotondrainibe 6954 (P)	Madagascar	KY711866	KY712041	KY712358	KY712206	KY711689
<i>Zygophlebia humbertii</i>		Rouhan et al. 1182 (P)	Madagascar	KY711864	KY712039	KY712356	KY712204	KY711687
<i>Zygophlebia humbertii</i>		Rouhan et al. 1248 (P)	Madagascar	KY711865	KY712040	KY712357	KY712205	KY711688
<i>Zygophlebia mathewsii</i> (Kunze ex Mett.) L. E. Bishop	x	Sundue 1119 (NY)*	Ecuador	-	-	-	KM106043	-
<i>Zygophlebia mathewsii</i>	x	Sundue & Vasco 1254 (NY)*	Colombia	KY775306	GU476895	KY775305	-	GU476743

<i>Zygophlebia sectifrons</i> (Kunze ex Mett.) L. E. Bishop	x	Sundue 1757 (NY)	Costa Rica	-	GU476904	KY775304	KM106044	KM106101
<i>Zygophlebia</i> sp. 1	x	Pignal et al. 3380 (P)	Comoros	KY711872	KY712047	KY712364	KY712212	KY711694
<i>Zygophlebia</i> sp. 1		Pignal et al. 3382 (P)	Comoros	KY711873	KY712048	KY712365	KY712213	KY711695
<i>Zygophlebia</i> sp. 1		Pignal et al. 3390 (P)	Comoros	KY711874	KY712049	KY712366	KY712214	KY711696
<i>Zygophlebia</i> cf. <i>devoluta</i>	x	Massawe & Phillipson 467 (MO)	Tanzania	-	KM218796	KM106200	-	KM106102
<i>Zygophlebia subpinnata</i> (Bak.) L. E. Bishop ex Parris		Rakotomalaza et al. 129 (MO)	Madagascar	-	-	KY712243	-	KY711568
<i>Zygophlebia subpinnata</i>		Rakotondrainibe 5935 (P)	Madagascar	KY711876	KY712051	KY712368	KY712216	-
<i>Zygophlebia subpinnata</i>	x	Rouhan et al. 1378 (P)	Madagascar	KY711875	KY712050	KY712367	KY712215	KY711697
<i>Zygophlebia subpinnata</i>		Rouhan et al. 1387 (P)	Madagascar	KY711877	KY712052	KY712369	KY712217	KY711698
<i>Zygophlebia torulosa</i> (Bak.) Parris		Tamon 2 (P)	La Réunion Island, France	KY711878	KY712053	KY712370	KY712218	KY711699
<i>Zygophlebia torulosa</i>		Rakotondrainibe 6937 (P)	Madagascar	KY711881	KY712056	KY712373	KY712221	KY711702
<i>Zygophlebia torulosa</i>		Rakotondrainibe 6177 (P)	Madagascar	KY711884	-	KY712376	KY712224	-
<i>Zygophlebia torulosa</i> var. <i>pinnatisecta</i> <i>ined.</i>		Rouhan et al. 1249 (P)	Madagascar	KY711879	KY712054	KY712371	KY712219	KY711700
<i>Zygophlebia torulosa</i> var. <i>pinnatisecta</i> <i>ined.</i>	x	Rouhan et al. 1352 (P)	Madagascar	KY711880	KY712055	KY712372	KY712220	KY711701
<i>Zygophlebia villosissima</i> (Hook.) L. E. Bishop		Carvalho 3639 (P)	Equatorial Guinea	KY711903	-	KY712395	KY712233	KY711728
<i>Zygophlebia villosissima</i>	x	Carvalho 3688 (P)	Equatorial Guinea	KY711892	KY712066	KY712385	-	KY711713
<i>Zygophlebia villosissima</i>		Jaeger 9654 (P)	Sierra Leone	KY711902	-	KY712394	-	KY711727

* marker(s) from this voucher combined in a unique sequence

** only 122 bp of sequence data were obtained; too short for GenBank to accession; the sequence is provided here:

TGATCATTCACTAAATTAGTAGTTTACTAAAAAGAGTTGGAAATTCCTCGTTTTTTTTTTGAACCGCAATCTAGCTAGATTTTGAATAAAAAGAGGAGAACCTCGG
GTAGGCAAAAATAGAG

*** see Hill 27921, ILLS, ATRC and VT; *C. seminudum* collected from vicinity of Hill 29102A

APPENDIX 1.2

Fully detailed majority rule consensus phylogram of grammitids recovered from the BI based on the combined dataset (*atpB*, *rbcl*, *trnG-trnR*, *trnL-trnF* and *rps4-trnS*). Unless mentioned next to the nodes, support values were BS \geq 95 and PP \geq 0.95. Non-monophyletic species are highlighted in bold and color. Vouchers are indicated when several samples were included for the same species. Genera names are on the right, followed by (number of species analyzed / number of samples included), as in Fig. 1.

Available online:

<http://www.sciencedirect.com/science/article/pii/S1055790317301860?via%3Dihub>

APPENDIX 2

Herbarium vouchers and GenBank accession numbers (*Rumohra*). Newly generated sequences are indicated with an asterisk.

Species	Locality	Collector(s)	<i>atpA</i>	<i>atpB</i>	<i>atpB-rbcL</i>	<i>rbcL</i>	<i>rps4-trnS</i>	<i>trnG-trnR</i>	<i>trnH-psbA</i>	<i>trnL+trnL-trnF</i>
<i>Bolbitis auriculata</i> (Lam.) Alston	Sierra Leone	Fay 1110 (NY)	-	-	-	KJ464421	GU376649	-	-	GU376505
<i>Elaphoglossum amygdalifolium</i> (Mett. ex Kuhn) H. Christ	Costa Rica	Herrera 2063 (NY)	-	-	-	AY534863	AY536173	-	-	AY534845
<i>Elaphoglossum decoratum</i> Maxon	Cultivated at the NYBG	NYBG391/77B (NY)	-	-	-	AY534850	AY536140	-	-	AY534811
<i>Lastreopsis amplissima</i> (C. Presl) Tindale	Brazil	Labiak s.n. (UPCB)	-	-	-	KJ464432	KJ464674	KJ464521	-	KJ464604
<i>Lastreopsis hispida</i> (Sw.) Tindale	New Zealand	Given 11819 (NY)	-	-	-	KJ464446	KJ464691	KJ464533	-	KJ464614
<i>Lastreopsis marginans</i> Sm. & Tindale	Australia	Kessler 14354 (VT)	-	-	-	KJ464450	KJ464695	KJ464537	-	KJ464616
<i>Megalastrum atrogriseum</i> (C. Chr.) A.R. Sm. & R.C. Moran	Costa Rica	Sundue 1779 (VT)	-	-	-	KJ464479	KJ464730	KJ464566	-	KJ464643
<i>Megalastrum oppositum</i> (Holttum) Holttum	Réunion Island	Grangaud 3 (P)	-	-	-	KJ464480	KJ464731	KJ464567	-	KJ464644
<i>Parapolystichum acuminatum</i> (Houlston) Labiak, Sundue & R.C.Moran	Australia	Coveny 10182 (NY)	-	-	-	KJ464430	KJ464671	KJ464518	-	KJ464601
<i>Parapolystichum boivinii</i> (Baker) Rouhan	Madagascar	Rouhan et al. 1356 (P)	-	-	-	KJ464436	KJ464679	KJ464525	-	KJ464608
<i>Parapolystichum confine</i> (Maxon ex C. Chr.) Labiak, Sundue & R. C. Moran	Dominican Republic	Liogier 25604 (NY)	-	-	-	KJ464438	KJ464681	KJ464527	-	KJ464610
<i>Parapolystichum vogelii</i> (Hook.) Rouhan	Equatorial Guinea	Visa 2498 (NY)	-	-	-	KJ464470	KJ464721	KJ464559	-	-
<i>Rumohra adiantiformis</i> (G. Forst.) Ching	Comoros	Bidault 93 (P)	-	*MF157744	*MF157780	*MF157816	*MF157858	*MF157894	*MF157928	*MF157964
<i>Rumohra adiantiformis</i>	South Africa	Brand 217 (NY)	*MF158009	*MF157754	*MF157790	*MF157825	*MF157864	-	*MF157938	*MF157974
<i>Rumohra adiantiformis</i>	South Africa	Euston-Brown EFG.629.1 (n/a)	-	-	-	KP110458	-	-	-	-
<i>Rumohra adiantiformis</i>	Mauritius	Hennequin 337 (P)	*MF158014	*MF157758	*MF157797	*MF157832	*MF157871	*MF157905	*MF157944	*MF157980
<i>Rumohra adiantiformis</i>	Brazil	Hirai & Prado 771 (SP)	*MF158004	*MF157746	*MF157782	*MF157818	*MF157860	*MF157896	-	*MF157966

<i>Rumohra adiantiformis</i>	Madagascar	Jansen & Rouhan 2550 (P)	*MF158007	*MF157752	-	KJ464500	-	KJ464584	*MF157936	KJ464655
<i>Rumohra adiantiformis</i>	Madagascar	Janssen 2871 (P)	-	-	*MF157791	*MF157826	*MF157865	*MF157900	-	*MF157975
<i>Rumohra adiantiformis</i>	Amsterdam Island	Jolinon 939 (P)	-	-	-	*MF157821	*MF157855	-	*MF157932	*MF157969
<i>Rumohra adiantiformis</i>	Australia	Kessler s.n. (VT)	-	-	-	KJ464501	KJ464748	KJ464585	-	-
<i>Rumohra adiantiformis</i>	New Zealand	Perrie 3806 (WELT)	-	*MF157750	-	*MF157823	*MF157862	*MF157898	-	*MF157972
<i>Rumohra adiantiformis</i>	Comoros	Pignal 3392 (P)	*MF158013	*MF157757	*MF157796	*MF157831	*MF157870	*MF157904	*MF157943	*MF157979
<i>Rumohra adiantiformis</i>	Brazil	Prado & Hirai 2022 (NY,SP)	*MF158006	*MF157751	*MF157788	KJ464502	KJ464749	KJ464586	*MF157935	KJ464656
<i>Rumohra adiantiformis</i>	Madagascar	Rouhan et al. 430 (P)	-	-	*MF157792	*MF157827	*MF157866	-	*MF157939	-
<i>Rumohra adiantiformis</i>	Madagascar	Rouhan et al. 1148 (P)	*MF158015	*MF157759	*MF157798	*MF157833	*MF157872	*MF157906	*MF157945	*MF157981
<i>Rumohra adiantiformis</i>	Madagascar	Rouhan et al. 1240 (P)	*MF158016	*MF157760	*MF157799	*MF157834	*MF157873	*MF157907	*MF157946	*MF157982
<i>Rumohra adiantiformis</i>	Madagascar	Rouhan et al. 1348 (P)	*MF158017	*MF157761	*MF157800	*MF157835	*MF157874	*MF157908	*MF157947	*MF157983
<i>Rumohra adiantiformis</i>	Brazil	Salino 15308 (BHCB)	*MF158018	-	*MF157801	*MF157836	*MF157875	*MF157909	-	*MF157984
<i>Rumohra adiantiformis</i>	Puerto Rico	Santiago 1203B (P)	*MF158005	*MF157747	*MF157783	*MF157819	*MF157861	*MF157897	*MF157930	*MF157967
<i>Rumohra adiantiformis</i>	Seychelles	Senterre 7143 (SEY)	-	*MF157749	-	*MF157822	*MF157857	-	*MF157934	*MF157971
<i>Rumohra adiantiformis</i>	Falkland Islands / Islas Malvinas	Stanworth s.n. (P)	*MF158003	*MF157745	*MF157781	*MF157817	*MF157859	*MF157895	*MF157929	*MF157965
<i>Rumohra adiantiformis</i>	Papua New Guinea	Sundue 3598 (VT)	*MF158034	*MF157778	*MF157786	*MF157852	*MF157892	*MF157926	*MF157962	*MF158001
<i>Rumohra adiantiformis</i>	Papua New Guinea	Sundue 3649 (VT)	*MF158035	*MF157779	*MF157787	*MF157853	*MF157893	*MF157927	*MF157963	*MF158002
<i>Rumohra adiantiformis</i>	Réunion Island	Tamon 130 (P)	*MF158010	-	*MF157793	*MF157828	*MF157867	*MF157901	*MF157940	*MF157976
<i>Rumohra adiantiformis</i>	Réunion Island	Tamon 132 (P)	*MF158011	*MF157755	*MF157794	*MF157829	*MF157868	*MF157902	*MF157941	*MF157977
<i>Rumohra adiantiformis</i>	Réunion Island	Tamon 160 (P)	*MF158012	*MF157756	*MF157795	*MF157830	*MF157869	*MF157903	*MF157942	*MF157978
<i>Rumohra adiantiformis</i>	South Africa	Väre s.n. (H)	-	*MF157748	-	*MF157820	*MF157854	-	*MF157931	*MF157968
<i>Rumohra adiantiformis</i>	Argentina	Weigand 7030 (NY)	*MF158008	*MF157753	*MF157789	*MF157824	*MF157863	*MF157899	*MF157937	*MF157973
<i>Rumohra adiantiformis</i>	South Africa	Williams 948 (P)	-	-	-	-	*MF157856	-	*MF157933	*MF157970
<i>Rumohra berteriana</i> (Colla) R. R. Rodriguez	Chile: Juan Fernández archipelago	Danton 1964 (P)	*MF158019	*MF157762	*MF157802	KJ464503	KJ464750	KJ464587	*MF157948	KJ464657
<i>Rumohra berteriana</i>	Chile: Juan Fernández archipelago	Moreira 990 (SGO)	*MF158020	-	*MF157803	*MF157837	*MF157876	*MF157910	*MF157949	*MF157985
<i>Rumohra glandulosissima</i> Sundue & J.Prado	Brazil	Giacomin 1006 (BHCB)	*MF158033	*MF157777	*MF157815	*MF157851	*MF157891	*MF157925	*MF157961	*MF158000

<i>Rumohra glandulosissima</i>	Brazil	Prado & Hirai 2350 (SP)	*MF158021	*MF157763	*MF157804	*MF157838	*MF157877	*MF157911	*MF157950	*MF157986
<i>Rumohra linearisquamata</i> Rakotondr.	Madagascar	Rouhan et al. 1407 (P)	*MF158023	*MF157765	*MF157806	*MF157840	*MF157879	*MF157913	*MF157951	*MF157988
<i>Rumohra linearisquamata</i>	Madagascar	Rouhan et al. 1395 (P)	*MF158022	*MF157764	*MF157805	*MF157839	*MF157878	*MF157912	-	*MF157987
<i>Rumohra lokohensis</i> Tard.	Madagascar	Janssen 2819 (P)	-	*MF157766	-	-	*MF157880	-	*MF157952	*MF157989
<i>Rumohra lokohensis</i>	Madagascar	Rouhan et al. 1167 (P)	*MF158024	*MF157767	*MF157807	*MF157841	*MF157881	*MF157914	*MF157953	*MF157990
<i>Rumohra lokohensis</i>	Madagascar	Rouhan et al. 1193 (P)	*MF158028	*MF157771	*MF157810	*MF157845	*MF157885	*MF157918	*MF157956	*MF157994
<i>Rumohra lokohensis</i>	Madagascar	Rouhan et al. 1196 (P)	*MF158025	*MF157768	*MF157808	*MF157842	*MF157882	*MF157915	*MF157954	*MF157991
<i>Rumohra lokohensis</i>	Madagascar	Rouhan et al. 1245 (P)	*MF158026	*MF157769	*MF157809	*MF157843	*MF157883	*MF157916	-	*MF157992
<i>Rumohra lokohensis</i>	Madagascar	Rouhan et al. 1412 (P)	*MF158027	*MF157770	*MF157784	*MF157844	*MF157884	*MF157917	*MF157955	*MF157993
<i>Rumohra madagascariica</i> (Bonap.) Tard.	Madagascar	Rouhan et al. 1149 (P)	*MF158029	*MF157772	*MF157811	*MF157846	*MF157886	*MF157919	*MF157957	*MF157995
<i>Rumohra madagascariica</i>	Madagascar	Rouhan et al. 1162 (P)	*MF158030	*MF157773	*MF157812	*MF157847	*MF157887	*MF157920	-	*MF157996
<i>Rumohra madagascariica</i>	Madagascar	Rouhan et al. 1349 (P)	*MF158031	*MF157774	*MF157813	*MF157848	*MF157888	*MF157921	*MF157958	*MF157997
<i>Rumohra quadrangularis</i> (Fée) Brade	Brazil	Prado & Hirai 2408 (SP)	*MF158032	*MF157775	*MF157785	*MF157849	*MF157889	*MF157922	*MF157959	*MF157998
<i>Rumohra quadrangularis</i>	Brazil	Senna & Mansan s.n. (HAS)	-	*MF157776	*MF157814	*MF157850	*MF157890	*MF157923	*MF157960	*MF157999
<i>Rumohra quadrangularis</i>	Brazil	Senna 918 (PACA)	-	-	-	-	-	*MF157924	-	-

APPENDIX 3

Herbarium vouchers and GenBank accession numbers (Blechnoideae).

Species name	Voucher	Locality	<i>rbcL</i>	<i>rps4/rps4-trnS</i>	<i>trnL/trnL-trnF</i>
<i>Anchistea virginica</i> (L.) C.Presl	Cranfill s.n. (RBC 606) (UC)	USA	AY137660	AF533857	n.a.
<i>Austroblechnum × aggregatum</i> (Colenso) Gasper & V.A.O.Dittrich	Wilson 68 (MELU)	Australia	KJ170817	KJ170790	KJ170844
<i>Austroblechnum andinum</i> (Baker) Gasper & V.A.O.Dittrich	Salino s.n. (BHCB)	Brazil	KU898604	KU898550	KU925243
<i>Austroblechnum bakeri</i> (C.Chr.) Gasper & V.A.O.Dittrich	Rouhan et al. 1625 (P)	Madagascar	to submit	to submit	to submit
<i>Austroblechnum bakeri</i> (C.Chr.) Gasper & V.A.O.Dittrich	Rouhan et al. 1401 (P)	Madagascar	to submit	to submit	to submit
<i>Austroblechnum bakeri</i> (C.Chr.) Gasper & V.A.O.Dittrich	Janssen 2424 (P)	Madagascar	to submit	to submit	to submit
<i>Austroblechnum bakeri</i> (C.Chr.) Gasper & V.A.O.Dittrich	Janssen 2665 (P)	Tanzania	to submit	to submit	to submit
<i>Austroblechnum colensoi</i> (Hook.f.) Gasper & V.A.O.Dittrich	Perrie 3572 (WELT)	New Zealand	KF975783	KF975739	DQ683379
<i>Austroblechnum divergens</i> (Kunze) Gasper & V.A.O.Dittrich	Funez 1000 (FURB)	Brazil	KU898605	KU898548	KU898664
<i>Austroblechnum doodioides</i> (Brack.) Gasper & V.A.O.Dittrich	Rouhan et al. 634 (P)	Vanuatu	to submit	to submit	to submit
<i>Austroblechnum durum</i> (T.Moore) Gasper & V.A.O.Dittrich	Perrie 4023 (WELT)	New Zealand	KF975788	KF975744	DQ683383
<i>Austroblechnum integrifons</i> (Bonap. ex Rakotondr.) Gasper & V.A.O.Dittrich	Rakotondrainibe 4412 (P)	Madagascar	n.a.	to submit	to submit
<i>Austroblechnum lechleri</i> (T.Moore) Gasper & V.A.O.Dittrich	Larsen 252 (SI)	Chile	KU898608	KU898553	KU898667
<i>Austroblechnum lehmannii</i> (Hieron.) Gasper & V.A.O.Dittrich	Almeida 3374 (BHCB)	Brazil	KU898606	KU898551	KU898665
<i>Austroblechnum leyboldtianum</i> (Phil.) Gasper & V.A.O.Dittrich	Perrie 3295 (WELT)	New Zealand	KF975780	KF975736	DQ683374
<i>Austroblechnum melanocaulon</i> (Brack.) Gasper & V.A.O.Dittrich	Perrie & Brownsey FJ2011182 (WELT)	Fiji	KF975794	KF975750	KF975720
<i>Austroblechnum membranaceum</i> (Colenso ex Hook.) Gasper & V.A.O.Dittrich	Perrie 3475 (WELT)	New Zealand	KF975795	KF975751	DQ683391
<i>Austroblechnum microphyllum</i> (Goldm.) Gasper & V.A.O.Dittrich	Biganzoli 2141 (SI)	Argentina	KU898607	KU898552	KU898666
<i>Austroblechnum norfolkianum</i> (Heward) Gasper & V.A.O.Dittrich	Parris 12358 (WELT)	New Zealand	KF975801	KF975757	DQ683401

<i>Austroblechnum patersonii</i> (R.Br.) Gasper & V.A.O.Dittrich subsp. <i>patersonii</i> Gasper & V.A.O.Dittrich	Wilson 70 (MELU)	Australia	KJ170822	KJ170795	KJ170849
<i>Austroblechnum patersonii</i> (R.Br.) Gasper & V.A.O.Dittrich subsp. <i>queenslandicum</i> (T.C.Chambers & P.A.Farrant) Gasper & V.A.O.Dittrich	Ohlsen BB144 (BRI)	Australia	KJ170823	KJ170796	KJ170850
<i>Austroblechnum penna-marina</i> (Poir.) Gasper & V.A.O.Dittrich	Gasper 3039 (FURB)	Brazil	KU898609	KU898554	KU898668
<i>Austroblechnum squamipes</i> (Hieron.) Gasper & V.A.O.Dittrich	Salino 14737 (BHCB)	Brazil	KU898610	KU898555	KU898669
<i>Austroblechnum stoloniferum</i> (Mett. ex E.Fourn.) Gasper & V.A.O.Dittrich	Campos Salas 12 (MEXU)	Mexico	KU898611	KU898556	KU898670
<i>Austroblechnum vieillardii</i> (Mett.) Gasper & V.A.O.Dittrich	Perrie NC 2012 227 (WELT)	New Caledonia	KF975814	n.a.	KF975730
<i>Austroblechnum wardiae</i> (Mickel & Beitel) Gasper & V.A.O.Dittrich	Salino 15367 (BHCB)	Panama	KU898612	KU898557	KU898671
<i>Blechnidium melanopus</i> (Hook.) T.Moore	Knapp 3355 (P)	Taiwan	KU898627	n.a.	n.a.
<i>Blechnopsis orientalis</i> (L.) C.Presl	Brownsey & Perrie FJ201190 (WELT)	Australia	KF975806	KF975762	KF975726
<i>Blechnum appendiculatum</i> Willd.	Salino 15454 (BHCB)	Panama	KU898613	KU898558	KU898672
<i>Blechnum areolatum</i> V.A.O.Dittrich & Salino	Salino 15184 (BHCB)	Brazil	KU898614	KU898559	KU898673
<i>Blechnum asplenioides</i> Sw.	Salino 14449 (BHCB)	Brazil	KU898615	KU898560	KU898674
<i>Blechnum auriculatum</i> Cav.	Funez 2162 (FURB)	Brazil	KU898616	KU898561	KU898675
<i>Blechnum australe</i> L.	Unknown / Prada s.n. (Unknown / MA)	Argentina / Unknown	AB040557	n.a.	JQ907366
<i>Blechnum australe</i> L.	Rouhan et al. 1416 (P)	Madagascar	to submit	to submit	to submit
<i>Blechnum australe</i> L.	Janssen 2588 (P)	Madagascar	to submit	to submit	to submit
<i>Blechnum australe</i> L.	Hennequin 286 (P)	La Réunion	to submit	to submit	to submit
<i>Blechnum austrobrasilianum</i> de la Sota	Almeida 3372 (BHCB)	Brazil	KU898617	KU898562	KU898676
<i>Blechnum ×caudatum</i> Cav.	Almeida 3371 (BHCB)	Brazil	KU898618	KU898563	KU898677
<i>Blechnum ×confluens</i> Schtdl. & Cham.	Kessler 13457 (LPB)	Bolivia	KU898625	KU898569	KU898682
<i>Blechnum gracile</i> Kaulf.	Jiménez I. 1051 (LPB)	Bolivia	KU898620	KU898565	KU898678
<i>Blechnum gracilipes</i> (Rosenst.) M.Kessler & A.R.Sm.	Jiménez I. 1485 (LPB)	Bolivia	n.a.	n.a.	KU898679
<i>Blechnum hastatum</i> Kaulf.	Zuloaga 11322 (SI)	Argentina	KU898619	KU898564	n.a.
<i>Blechnum laevigatum</i> Cav.	Canestraro 527 (MBM)	Brazil	KU898621	KU898566	n.a.
<i>Blechnum lanceola</i> Sw.	Laurent 275 (BHCB)	Brazil	KU898622	KU898567	KU898680

<i>Blechnum longipilosum</i> V.A.O.Dittrich & Salino	Salino 15570 (BHCB)	Brazil	KU898626	KU898570	KU898683
<i>Blechnum occidentale</i> L.	Funez 548 (FURB)	Brazil	KU898623	KU898568	KU898681
<i>Blechnum polypodioides</i> Raddi	Salino 15334 (BHCB)	Panama	KU898624	KU925241	KU925244
<i>Blechnum punctulatum</i> Sw.	Parris 12683 (WELT)	cult.	KF975811	KF975767	DQ683412
<i>Blechnum punctulatum</i> Sw.	Rouhan et al. 1652 (P)	Madagascar	to submit	to submit	to submit
<i>Blechnum punctulatum</i> Sw.	Rouhan et al. 1405 (P)	Madagascar	to submit	to submit	to submit
<i>Blechnum punctulatum</i> Sw.	Janssen 2660 (P)	Tanzania	to submit	to submit	to submit
<i>Brainea insignis</i> (Hook.) J.Sm.	Averyanov 2702 (P)	Vietnam	KU898628	KU898571	KU898684
<i>Cleistoblechnum eburneum</i> (Christ) Gasper & Salino	LU/Z1 (PYU)	China	JN168003	JN168071	n.a.
<i>Cranfillia fluviatilis</i> (R.Br.) Gasper & V.A.O.Dittrich	Wilson 64 (MELU)	Australia	KJ170818	KJ170791	KJ170845
<i>Cranfillia hirsuta</i> (Rosenst.) Gasper & V.A.O.Dittrich	Perrie NC 2012 196 (WELT)	New Caledonia	KF975792	KF975748	KF975718
<i>Cranfillia nigra</i> (Colenso) Gasper & V.A.O.Dittrich	Perrie 3454 (WELT)	New Zealand	KF975800	KF975756	DQ683399
<i>Cranfillia opaca</i> (Mett.) Gasper & V.A.O.Dittrich	Perrie NC 2012 167 (WELT)	New Caledonia	KF975805	KF975761	KF975725
<i>Cranfillia pilosa</i> (Brack.) Gasper & V.A.O.Dittrich	Perrie & Brownsey FJ2011181 (WELT)	Fiji	KF975809	KF975765	KF975728
<i>Cranfillia sampaioana</i> (Brade) Gasper & V.A.O.Dittrich	Salino 13818 (BHCB)	Brazil	KU898629	KU898572	KU898685
<i>Cranfillia sprucei</i> (C.Chr) Gasper & V.A.O.Dittrich	Jiménez 1743 (LPB)	Bolivia	KU898630	KU898573	KU898686
<i>Cranfillia vulcanica</i> (Blume) Gasper & V.A.O.Dittrich	Perrie 3461 (WELT)	Australia	KF975816	KF975771	DQ683416
<i>Diploblechnum acuminatum</i> (C.T.White & Goy) Gasper & V.A.O.Dittrich	Field et al. s.n. (CNS)	Australia	KJ170839	KJ170812	KJ170866
<i>Diploblechnum diversifolium</i> (Mett.) Gasper & V.A.O.Dittrich	Perrie NC 2012 157 (WELT)	New Caledonia	KF975787	KF975743	KF975716
<i>Diploblechnum fraseri</i> (A.Cunn.) De Vol	Perrie 3434 (WELT)	New Zealand	KF975790	KF975746	DQ683390
<i>Diploblechnum lenormandii</i> (Baker) Gasper & V.A.O.Dittrich	Perrie NC 2012 169 (WELT)	New Caledonia	KF975793	KF975749	KF975719
<i>Diploblechnum neglectum</i> (F.M.Bailey) Gasper & V.A.O.Dittrich	van der Werff 17045 (MO)	Australia	KU898631	KU898574	KU898687
<i>Doodia aspera</i> R.Br.	Perrie and Ohlsen BB113 (MELU)	Australia	KJ170836	KJ170809	KJ170863

<i>Doodia australis</i> Parris	Ohlsen BB151 (MELU)	Australia	KJ170837	KJ170810	KJ170864
<i>Doodia brackenridgei</i> Carruth. ex Seem.	Perrie & al. FJ2011123 (WELT)	Fiji	KF975818	KF975773	KF975732
<i>Doodia caudata</i> (Cav.) R.Br.	Perrie and Ohlsen BB88 (MELU)	Australia	KJ170838	KJ170811	KJ170865
<i>Doodia dives</i> Kunze	Perrie et al. BB24 (BRI)	Australia	KJ170831	KJ170804	KJ170858
<i>Doodia kunthiana</i> Gaudich.	Unknown / WELT P21219	USA	AB040578	n.a.	DQ683424
<i>Doodia linearis</i> J.Sm.	Ohlsen BB147 (MELU)	Australia	KJ170833	KJ170806	KJ170860
<i>Doodia media</i> R.Br.	Perrie NC 2012 120 (WELT)	Australia	KF975819	KF975774	KF975733
<i>Doodia milnei</i> Carruth.	Parris 12689 (WELT)	New Zealand	KF975820	KF975775	DQ683425
<i>Doodia mollis</i> Parris	Parris 12688 (WELT)	New Zealand	KF975821	KF975776	DQ683427
<i>Doodia paschalis</i> C.Chr.	Christenhusz 5047 (P)	Chile (Easter Island)	n.a.	n.a.	KU925245
<i>Doodia squarrosa</i> Colenso	Perrie 3517 (WELT)	New Zealand	KF975822	KF975777	DQ683429
<i>Icarus filiformis</i> (A.Cunn.) Gasper & Salino	Perrie 3459 (WELT)	New Zealand	KF975789	KF975745	DQ683385
<i>Lomaria nuda</i> (Labill.) Willd.	Wilson 69 (MELU)	Australia	KJ170821	KJ170794	KJ170848
<i>Lomaria oceanica</i> (Rosenst.) Gasper & V.A.O.Dittrich	Perrie NC 2012 105 (WELT)	New Caledonia	KF975804	KF975760	KF975724
<i>Lomaria raymondii</i> Gasper & V.A.O.Dittrich	Perrie 4015 (WELT)	New Zealand	KF975786	KF975742	DQ683382
<i>Lomaria spannagelii</i> (Rosenst.) Gasper & V.A.O.Dittrich	Almeida 3387 (BHCB)	Brazil	KU898643	KU898584	KU898698
<i>Lomaridium acutum</i> (Desv.) Gasper & V.A.O.Dittrich	van der Werff 16799 (MO)	Peru	KU898638	KU925242	KU925246
<i>Lomaridium attenuatum</i> (Sw.) Gasper & V.A.O.Dittrich	Pignal 3409 (P)	Comoros	to submit	to submit	to submit
<i>Lomaridium attenuatum</i> (Sw.) Gasper & V.A.O.Dittrich	Pignal 3486 (P)	Comoros	to submit	to submit	to submit
<i>Lomaridium attenuatum</i> (Sw.) Gasper & V.A.O.Dittrich	Bauret et al. 111 (P)	Madagascar	to submit	to submit	to submit
<i>Lomaridium attenuatum</i> (Sw.) Gasper & V.A.O.Dittrich	Rouhan et al. 1231 (P)	Madagascar	to submit	to submit	to submit

<i>Lomaridium attenuatum</i> (Sw.) Gasper & V.A.O.Dittrich	Rouhan et al. 1283 (P)	Madagascar	to submit	to submit	to submit
<i>Lomaridium attenuatum</i> (Sw.) Gasper & V.A.O.Dittrich	Hennequin 365 (P)	Maurice	to submit	to submit	to submit
<i>Lomaridium attenuatum</i> (Sw.) Gasper & V.A.O.Dittrich	Rouhan et al. 1233 (P)	Madagascar	to submit	n.a.	to submit
<i>Lomaridium attenuatum</i> (Sw.) Gasper & V.A.O.Dittrich	Janssen 2673 (P)	La Réunion	to submit	to submit	to submit
<i>Lomaridium attenuatum</i> (Sw.) Gasper & V.A.O.Dittrich	Viscardi 414 (P)	Comoros	to submit	to submit	to submit
<i>Lomaridium attenuatum</i> (Sw.) Gasper & V.A.O.Dittrich	Rouhan et al. 271 (P)	Madagascar	to submit	to submit	to submit
<i>Lomaridium attenuatum</i> (Sw.) Gasper & V.A.O.Dittrich	Rouhan et al. 431 (P)	Madagascar	to submit	to submit	to submit
<i>Lomaridium attenuatum</i> (Sw.) Gasper & V.A.O.Dittrich	Janssen 2637 (P)	Tanzania	to submit	to submit	to submit
<i>Lomaridium attenuatum</i> (Sw.) Gasper & V.A.O.Dittrich	Hennequin R67 (BM)	La Réunion	KF992444	n.a.	n.a.
<i>Lomaridium biforme</i> (Baker) Gasper & V.A.O.Dittrich	Bauret et al. 134 (P)	Madagascar	to submit	to submit	to submit
<i>Lomaridium biforme</i> (Baker) Gasper & V.A.O.Dittrich	Rouhan et al. 1232 (P)	Madagascar	to submit	to submit	to submit
<i>Lomaridium biforme</i> (Baker) Gasper & V.A.O.Dittrich	Rouhan et al. 1324 (P)	Madagascar	to submit	to submit	to submit
<i>Lomaridium chauliodontum</i> (Copel.) Gasper & Salino	Munzinger 1294 (P)	New Caledonia	n.a.	to submit	n.a.
<i>Lomaridium contiguum</i> (Mett.) Gasper & V.A.O.Dittrich	Perrie P026062 (WELT)	New Caledonia	KF975804	KF975760	KF975724
<i>Lomaridium ensiforme</i> (Liebm.) Gasper & V.A.O.Dittrich	Jiménez 697 (LPB)	Bolivia	KU898639	KU898581	KU898694
<i>Lomaridium fragile</i> (Liebm.) Gasper & V.A.O.Dittrich	Salino 15860 (BHCB)	Panama	KU898640	KU898582	KU898695
<i>Lomaridium fuscusquamosum</i> (A.Rojas) Gasper & V.A.O.Dittrich	van der Werff 16823 (MO)	Peru	KU898641	KU898583	KU898696
<i>Lomaridium plumieri</i> (Desv.) Presl.	Pignal 3278 (P)	Brazil	to submit	to submit	to submit
<i>Lomaridium simillimum</i> Xyphophyllum	Janssen 2531 (P)	Madagascar	to submit	to submit	to submit
<i>Lomaridium simillinum</i> (Baker) Gasper & V.A.O.Dittrich	Rakotondrainibe 6984 (P)	Madagascar	to submit	to submit	to submit
<i>Lomaridium simillinum</i> (Baker) Gasper & V.A.O.Dittrich	Rouhan et al. 1370 (P)	Madagascar	n.a.	to submit	to submit
<i>Lomaridium simillinum</i> (Baker) Gasper & V.A.O.Dittrich	Rouhan et al. 325 (P)	Madagascar	to submit	to submit	to submit
<i>Lomaridium xiphophyllum</i> (Baker) Gasper & V.A.O.Dittrich	Bauret et al. 125 (P)	Madagascar	to submit	to submit	to submit
<i>Lomaridium xiphophyllum</i> (Baker) Gasper & V.A.O.Dittrich	Rouhan et al. 1368 (P)	Madagascar	to submit	to submit	to submit
<i>Lomariocycas aurata</i> (Fée) Gasper & A.R.Sm.	Jiménez 1852 (LPB)	Bolivia	KU898633	KU898576	KU898690
<i>Lomariocycas columbiensis</i> (Hieron.) Gasper & A.R.Sm.	van der Werff 16910 (MO)	Peru	KU898634	KU898577	n.a.
<i>Lomariocycas longepetiolata</i> (Tardieu) Gasper & A.R.Sm.	Bauret et al. 101 (P)	Madagascar	to submit	to submit	to submit
<i>Lomariocycas cf. longipinna</i> (Rakotondr.) Gasper & A.R.Sm.	Janssen 2940 (P)	Madagascar	to submit	to submit	to submit
<i>Lomariocycas cf. longipinna</i> (Rakotondr.) Gasper & A.R.Sm.	Rouhan et al. 256 (P)	Madagascar	to submit	to submit	to submit

<i>Lomariocycas cf. longipinna</i> (Rakotondr.) Gasper & A.R.Sm.	Rouhan et al. 312 (P)	Madagascar	to submit	to submit	to submit
<i>Lomariocycas longipinna</i> (Rakotondr.) Gasper & A.R.Sm.	Bauret et al. 169 (P)	Madagascar	to submit	to submit	to submit
<i>Lomariocycas longipinna</i> (Rakotondr.) Gasper & A.R.Sm.	Rouhan et al. 1585 (P)	Madagascar	to submit	to submit	to submit
<i>Lomariocycas longipinna</i> (Rakotondr.) Gasper & A.R.Sm.	Janssen 2477 (P)	Madagascar	to submit	to submit	to submit
<i>Lomariocycas madagascariensis</i> (Tardieu) Gasper & A.R.Sm.	Bauret et al. 112 (P)	Madagascar	to submit	to submit	to submit
<i>Lomariocycas madagascariensis</i> (Tardieu) Gasper & A.R.Sm.	Rouhan et al. 1560 (P)	Madagascar	to submit	to submit	to submit
<i>Lomariocycas magellanica</i> (Desv.) Gasper & A.R.Sm.	Unknown / Prada s.n. (MA)	Argentina / Unkown	AB040559	n.a.	JQ907376
<i>Lomariocycas schomburgkii</i> (Klotzsch) Gasper & A.R.Sm.	Salino 15692 (BHCB)	Brazil	KU898636	KU898579	KU898692
<i>Lomariocycas tabularis</i> (Thunb.) Gasper & A.R.Sm.	Janssen 2778 (P)	Madagascar	to submit	to submit	to submit
<i>Lomariocycas tabularis</i> (Thunb.) Gasper & A.R.Sm.	Janssen 2968 (P)	Madagascar	to submit	to submit	to submit
<i>Lomariocycas tabularis</i> (Thunb.) Gasper & A.R.Sm.	Hennequin 393 (P)	Maurice	to submit	to submit	to submit
<i>Lomariocycas tabularis</i> (Thunb.) Gasper & A.R.Sm.	Hennequin R105 (BM)	Reunion	KF992447	n.a.	n.a.
<i>Lomariocycas werckleana</i> (Christ) Gasper & A.R.Sm.	Salino 15924 (BHCB)	Panama	KU898637	KU898580	KU898693
<i>Lomariocycas yungensis</i> (J.P.Ramos) Gasper & A.R.Sm.	Jiménez 2383 (LPB)	Bolivia	KU898635	KU898578	KU898691
<i>Lorinseria areolata</i> (L.) C.Presl	Cranfill s.n. (RBC 170) (Unknown)	USA	AF425102	AF425155	n.a.
<i>Matteuccia struthiopteris</i> (L.) Tod.	Cranfill s.n. (RBC 460) (UC)	cultivated	U05930	AF425158	KC254425
<i>Neoblechnum brasiliense</i> (Desv.) Gasper & V.A.O.Dittrich	Unknown / Christenhusz 4968 / Prada s.n. (unknown/BM/MA)	Argentina	AB040545	HQ157324	JQ907369
<i>Oceaniopteris cartilaginea</i> (Sw.) Gasper & Salino	Bayly 2182 (MELU)	Australia	KJ170816	KJ170789	KJ170843
<i>Oceaniopteris ciliata</i> (T.Moore) Gasper & Salino	Perrie NC 2012 146 (WELT)	New Caledonia	KF975799	KF975755	KF975722
<i>Oceaniopteris dentata</i> (Brack.) Kuhn	Perrie & Brownsey FJ2011210 (WELT)	Fiji	KF975815	KF975770	KF975731
<i>Oceaniopteris francii</i> (Rosenst.) Gasper & Salino	Christenhusz 6198 (NOU)	New Caledonia	n.a.	n.a.	KU898688
<i>Oceaniopteris gibba</i> (Labill.) Gasper & Salino	Perrie NC 2012 76 (WELT)	New Caledonia	KF975791	KF975747	KF975717
<i>Oceaniopteris obtusata</i> (Labill.) Gasper & Salino	McPherson 18146 (MO)	New Caledonia	KU898632	KU898575	KU898689

<i>Oceaniopteris whelanii</i> (F.M.Bailey) Gasper & Salino	Perrie et al. BB64 (BRI)	Australia	KJ170827	KJ170800	KJ170854
<i>Onoclea sensibilis</i> L.	Weed s.n. (UC)	cultivated	U05936	AF425159	n.a.
<i>Onocleopsis hintonii</i> F.Ballard	Mickel s.n. (UC)	Mexico	U62033	AF425160	n.a.
<i>Parablechnum ambiguum</i> C.Presl	Ohlsen BB233 (MELU)	Australia	KJ170813	KJ170786	KJ170840
<i>Parablechnum articulatum</i> (F.Muell.) Gasper & Salino	Perrie et al. BB36 (MELU)	Australia	KJ170814	KJ170787	KJ170841
<i>Parablechnum bicolor</i> (M.Kessler & A.R.Sm.) Gasper & Salino	Jiménez 942 (LPB)	Bolivia	KU898645	KU898586	KU898700
<i>Parablechnum bolivianum</i> (M.Kessler & A.R.Sm.) Gasper & Salino	Bach 1404 (LPB)	Bolivia	KU898646	KU898587	KU898701
<i>Parablechnum camfieldii</i> (Tindale) Gasper & Salino	Ohlsen BB216 (BRI)	Australia	KJ170815	KJ170788	KJ170842
<i>Parablechnum chiriquanum</i> (Broadh.) Gasper & Salino	Rojas & Gabriel y Galan CR 10419 (Unknown)	Panama	n.a.	n.a.	KM001891
<i>Parablechnum christii</i> (C.Chr.) Gasper & Salino	Salino 15459 (BHCB)	Panama	KU898647	KU898588	n.a.
<i>Parablechnum corbassonii</i> (Brownlie) Gasper & Salino	Perrie NC 2012 14 (WELT)	New Caledonia	KF975785	KF975741	KF975715
<i>Parablechnum cordatum</i> (Desv.) Gasper & Salino	Gasper 3051 (FURB)	Brazil	KU898648	KU898589	KU898702
<i>Parablechnum falciforme</i> (Liebm.) Gasper & Salino	Salino 15430 (BHCB)	Panama	KU898649	KU898590	KU898703
<i>Parablechnum glaziovii</i> (Christ) Gasper & Salino	Salino 15689 (BHCB)	Brazil	KU898650	KU898591	KU898704
<i>Parablechnum gregsonii</i> (Tindale) Gasper & Salino	Ohlsen BB228 (MELU)	Australia	KJ170819	KJ170792	KJ170846
<i>Parablechnum howeanum</i> (T.C.Chambers & P.A.Farrant) Gasper & Salino	Papadopulos et al. (2011) (NSW)	Australia	JF950804	n.a.	JF950940
<i>Parablechnum lechleri</i> (Mett.) Gasper & Salino	Bach 1311 (LPB)	Bolivia	KU898651	KU898592	KU898705
<i>Parablechnum lima</i> (Rosenst.) Gasper & Salino	Bach 1461 (LPB)	Bolivia	KU898652	KU898593	KU898706
<i>Parablechnum loxense</i> (Kunth) Gasper & Salino var. <i>stenophyllum</i> (Klotzsch) Gasper & Salino	Christenhusz 6777 / Gabriel y Galán s.n. (H / MA)	Peru	KJ716414	n.a.	JQ907375
<i>Parablechnum marginatum</i> (Kuhn) Gasper & Salino	Hennequin R76 (BM)	Réunion	KF992446	n.a.	n.a.
<i>Parablechnum marginatum</i> (Kuhn) Gasper & Salino var. <i>humbertii</i> (Tardieu) Gasper & Salino	Rouhan et al. 1209 (P)	Madagascar	to submit	to submit	to submit
<i>Parablechnum marginatum</i> (Kuhn) Gasper & Salino var. <i>humbertii</i> (Tardieu) Gasper & Salino	Bauret et al. 102 (P)	Madagascar	to submit	to submit	to submit
<i>Parablechnum milnei</i> (Carruth.) Gasper & Salino	Perrie & Brownsey FJ2011165 (WELT)	Fiji	KF975796	KF975752	KF975721
<i>Parablechnum minus</i> (R.Br.) Gasper & Salino	Wilson 72 (MELU)	Australia	KJ170820	KJ170793	KJ170847

<i>Parablechnum montanum</i> (T.C.Chambers & P.A.Farrant) Gasper & Salino	Perrie 3498 (WELT)	New Zealand	KF975798	KF975754	KJ187000
<i>Parablechnum novae-zelandiae</i> (T.C.Chambers & P.A.Farrant) Gasper & Salino	Perrie 3129 & Lovis (WELT)	New Zealand	KF975802	KF975758	DQ683403
<i>Parablechnum pazense</i> (M.Kessler & A.R.Sm.) Gasper & Salino	Jiménez I. 751 (LPB)	Bolivia	KU898653	KU898594	KU898707
<i>Parablechnum procerum</i> (G.Forst.) C.Presl	Perrie 3127 (WELT)	New Zealand	KF975810	KF975766	DQ683411
<i>Parablechnum proliferum</i> (Rosenst.) Gasper & Salino	Salino 15534 (BHCB)	Brazil	KU898654	KU898595	KU898708
<i>Parablechnum schideanum</i> (Schltdl. ex C.Presl) Gasper & Salino	Salino 15700 (BHCB)	Panama	KU898655	KU898596	KU898709
<i>Parablechnum smilodon</i> (M.Kessler & Lehnert) Gasper & Salino	Jiménez I. 1679 (LPB)	Bolivia	KU898644	KU898585	KU898699
<i>Parablechnum stipitellatum</i> (Sodiolo) Gasper & Salino	van der Werff 16635 (MO)	Peru	KU898656	KU898597	KU898710
<i>Parablechnum subcordatum</i> (E.Fourn.) Gasper & Salino	McPherson 18139 (MO)	New Caledonia	KU898657	KU898598	KU898711
<i>Parablechnum triangularifolium</i> (T.C.Chambers & P.A.Farrant) Gasper & Salino	Perrie 3416 (WELT)	New Zealand	KF975813	KF975769	DQ683415
<i>Parablechnum usterianum</i> (Christ) Gasper & Salino	Salino 15688 (BHCB)	Brazil	KU898658	KU898599	KU898712
<i>Parablechnum cf. vestitum</i> (Blume) Gasper & Salino	Unknown (Unknown)	New Zealand	AB040547	n.a.	n.a.
<i>Parablechnum wattsii</i> (Tindale) Gasper & Salino	Perrie and Ohlsen BB127 (MELU)	Australia	KJ170826	KJ170799	KJ170853
<i>Parablechnum wurunuran</i> (Parris) Gasper & Salino	Perrie et al. BB34 (MELU)	Australia	KJ170828	KJ170801	KJ170855
<i>Pentarhizidium intermedium</i> (C.Chr.) Hayata	Zhang 3394 (PE)	China	KC254354	KC254505	KC254426
<i>Sadleria cyatheoides</i> Kaulf.	Ornudff 8506 / Ornudff 8506 / Unknown (UC / UC / WELT)	USA	AF425103	AF425156	DQ683431
<i>Sadleria pallida</i> Hook. & Arn.	Ranker 1287 (COLO)	USA	U05943	n.a.	n.a.
<i>Sadleria souleyetiana</i> (Gaudich.) T.Moore	Unknown (Unknown)	USA	AB040591	n.a.	n.a.
<i>Sadleria squarrosa</i> (Gaudich.) T.Moore	Unknown (Unknown)	USA	AB040592	n.a.	n.a.
<i>Sadleria unisora</i> (Baker) W.J.Rob.	Unknown (Unknown)	USA	AB040593	n.a.	n.a.
<i>Salpichlaena thalassica</i> Grayum & R.C.Moran	van der Werff 16278 (MO)	Peru	KU898659	KU898600	KU898713
<i>Salpichlaena volubilis</i> (Kaulf.) J.Sm.	Salino 14313 (BHCB)	Brazil	KU898660	KU898601	KU898714

<i>Stenochlaena milnei</i> Underw.	UC Botanical Garden 55.0076 (UC)	Philippines	AF425104	AF425157	n.a.
<i>Stenochlaena palustris</i> (Burm.f.) Bedd.	Ohlsen and Bayly BB197 (MELU)	Australia	KJ170829	KJ170802	KJ170856
<i>Stenochlaena tenuifolia</i> (Desv.) T.Moore	Schuettpelz 504 (DUKE)	cultivated	EF463163	n.a.	n.a.
<i>Struthiopteris castanea</i> (Makino & Nemoto) Nakai	Ohashi 49965 (LPB)	Japan	KU898661	n.a.	KU898715
<i>Struthiopteris spicant</i> (L.) F.W.Weiss	Windham 3395 / cultivated (DUKE / AK)	cultivated	JF832059	n.a.	DQ683413
<i>Telmatoblechnum indicum</i> (Burm.f.) Perrie	Perrie and Ohlsen BB107 (MELU)	Australia	KJ170830	KJ170803	KJ170857
<i>Telmatoblechnum serrulatum</i> (Rich.) Perrie	Funez 487 (FURB)	Brazil	KU898662	KU898602	KU898716
<i>Woodwardia auriculata</i> Blume	Cranfill s.n. (RBC 650) (UC)	Malaysia	AY137661	n.a.	n.a.
<i>Woodwardia fimbriata</i> Sm.	Unknown (Unknown)	Japan	AB040597	AF533859	n.a.
<i>Woodwardia harlandii</i> Hook.	Unknown (Unknown)	Japan	AB040598	AF533860	n.a.
<i>Woodwardia japonica</i> (L.f.) Sm.	Cranfill 21 / Cranfill 21 / P21222 (UC / UC / WELT)	Japan	AY137664	AF533861	DQ683432
<i>Woodwardia kempii</i> Copel	Cranfill s.n. (RBC 023) (UC)	Taiwan/Chi na	KM60699 2	KM606992	n.a.
<i>Woodwardia martinezii</i> Maxon ex Weath.	Cerón 420 (MEXU)	Mexico	KU898663	n.a.	n.a.
<i>Woodwardia prolifera</i> Hook. & Arn.	Cranfill s.n. (RBC 010) / Cranfill s.n. (RBC 010) / P21332 (UC / UC / WELT)	cultivated	AY137666	AF533864	DQ683433
<i>Woodwardia radicans</i> (L.) Sm.	Cranfill 163 / Cranfill 163 / (UC; UC; WELT)	Spain	AY137667	AF533865	DQ683434
<i>Woodwardia spinulosa</i> M.Martens & Galeotti	Salino 15746 (BHCB)	Mexico	n.a.	KU898603	KU898718

APPENDIX 4

Herbarium vouchers and GenBank accession numbers (Lindsaeaceae).

Species	Voucher	Locality	<i>trnL/trnL-trnF</i>	<i>trnH-psbA</i>	<i>rpoC1</i>	<i>rps4</i>	<i>rps4-trnS</i>
<i>Cystodium sorbifolium</i> (Sm.) J.Sm.	Hoogland 4617 (BM)	Papua New Guinea	GU478726	n.a.	n.a.	n.a.	n.a.
<i>Lindsaea annamensis</i> K.U.Kramer	Averyanov VH2540 (AAU)	Vietnam	GU478804	GU478501	GU478612	n.a.	n.a.
<i>Lindsaea apoensis</i> Copel. in Perkins	Elmer 11565 (U)	Mindanao	GU478865	GU478563	n.a.	n.a.	n.a.
<i>Lindsaea arcuata</i> Kunze	Maas 5032 (U)	Costa Rica	GU478788	GU478506	GU478615	GU478657	GU478359
<i>Lindsaea austrosinica</i> Ching	Averyanov VH4816 (AAU)	Vietnam	GU478801	GU478486	n.a.	n.a.	n.a.
<i>Lindsaea azurea</i> Christ	Cheesman 165 (U)	Papua New Guinea	GU478852	GU478536	n.a.	n.a.	n.a.
<i>Lindsaea bifida</i> (Kaulf.) Mett. ex Kuhn	Lehtonen 552 (TUR)	Brazil	GU478799	GU478485	GU478608	n.a.	n.a.
<i>Lindsaea blanda</i> Mett. ex Kuhn	Palmer 747 (U)	Java	GU478806	GU478525	n.a.	n.a.	n.a.
<i>Lindsaea blotiana</i> K.U.Kramer	Rakotondrainibe 4327 (MO)	Madagascar	GU478814	GU478511	n.a.	n.a.	n.a.
<i>Lindsaea blotiana</i> K.U.Kramer	Rakotondrainibe 6350 (P)	Madagascar	GU478813	GU478510	GU478633	GU478679	GU478381
<i>Lindsaea blotiana</i> K.U.Kramer	Rouhan et al. 1253 (P)	Madagascar	to submit	to submit	to submit	to submit	to submit
<i>Lindsaea blotiana</i> K.U.Kramer	Rouhan et al. 1591 (P)	Madagascar	to submit	to submit	to submit	n.a.	n.a.
<i>Lindsaea blotiana</i> K.U.Kramer	Rouhan et al. 1155 (P)	Madagascar	n.a.	to submit	to submit	to submit	to submit
<i>Lindsaea bolivarensis</i> V.Marcano	Tuomisto 14478 (TUR)	Peru	FJ360992	FJ360902	FJ360947	GU478652	GU478354
<i>Lindsaea borneensis</i> Hook.	Wong 2 (AAU)	Brunei	GU478866	GU478538	n.a.	n.a.	n.a.
<i>Lindsaea botrychioides</i> A.St.-Hil.	Windisch 4987 (AAU)	Brazil	FJ360994	FJ360904	FJ360949	GU478714	GU478416
<i>Lindsaea bouillodii</i> Christ	Nielsen 592 (AAU)	Sarawak	FJ360993	FJ360903	FJ360948	GU478716	GU478418
<i>Lindsaea brachypoda</i> (Baker) Salomon	Balگوoy 1529 (L)	Australia	GU478848	GU478553	n.a.	n.a.	n.a.
<i>Lindsaea brevipes</i> Copel.	Braithwaite 4029 (MO)	Guadalcanal	GU478809	GU478541	GU478627	n.a.	n.a.
<i>Lindsaea carvifolia</i> K.U.Kramer	Mjöberg 9 (BM)	Borneo	GU478860	GU478562	n.a.	n.a.	n.a.

<i>Lindsaea cf. cambodgensis</i> Christ	Iwatsuki 14505 (AAU)	Thailand	GU478840	GU478502	n.a.	n.a.	n.a.
<i>Lindsaea cf. fissa</i> Copel.	Tolentino HAL-049 (U)	Mindoro Island	GU478857	GU478557	n.a.	n.a.	n.a.
<i>Lindsaea chienii</i> Ching	Kramer 7554 (U)	Taiwan	FJ360995	FJ360905	FJ360950	n.a.	n.a.
<i>Lindsaea chrysolepis</i> K.U.Kramer	Croft 216 (L)	New Ireland	GU478850	GU478552	n.a.	GU478709	GU478411
<i>Lindsaea coarctata</i> K.U.Kramer	Tuomisto 14500 (TUR)	Peru	FJ360996	FJ360906	FJ360951	GU478656	GU478358
<i>Lindsaea crispa</i> Baker	Parris 11469 (L)	Borneo	GU478834	GU478526	n.a.	n.a.	n.a.
<i>Lindsaea cubensis</i> Underw. & Maxon	Morton 10035 (BM)	Cuba	GU478795	GU478494	n.a.	GU478687	GU478389
<i>Lindsaea cultrata</i> (Willd.) Sw.	Poulsen 128 (AAU)	Borneo	GU478859	GU478554	n.a.	GU478668	GU478370
<i>Lindsaea cyclophylla</i> K.U.Kramer	Liesner 23788 (MO)	Venezuela	GU478776	GU478483	n.a.	n.a.	n.a.
<i>Lindsaea digitata</i> Lehtonen & Tuomisto	Tuomisto 14470 (TUR)	Peru	EU146057	EU146041	EU146051	GU478698	GU478400
<i>Lindsaea diplosora</i> Alderw.	Nielsen 815 (AAU)	Sarawak	GU478833	GU478535	GU478598	GU478722	GU478424
<i>Lindsaea dissectiformis</i> Ching	Averyanov VH2557 (AAU)	Vietnam	FJ360997	FJ360907	FJ360952	n.a.	n.a.
<i>Lindsaea divaricata</i> Klotzsch	Tuomisto 14553 (TUR)	Peru	EU146052	EU146040	EU146042	GU478697	GU478399
<i>Lindsaea divergens</i> Wall. ex Hook. & Grev.	Abdullah 228 (U)	Malaysia	GU478765	GU478465	n.a.	n.a.	n.a.
<i>Lindsaea doryphora</i> K.U.Kramer	Poulsen 341 (AAU)	Borneo	GU478868	GU478539	GU478636	GU478683	GU478385
<i>Lindsaea dubia</i> Spreng.	Christenhusz 2312 (TUR)	French Guiana	GU478779	GU478493	GU478623	GU478701	GU478403
<i>Lindsaea ensifolia</i> Sw.	Larsen 42363 (AAU)	Thailand	GU478853	GU478522	n.a.	n.a.	n.a.
<i>Lindsaea ensifolia</i> Sw.	Kessler 13597 (GOET)	La Réunion	n.a.	GU478521	GU478606	GU478678	GU478380
<i>Lindsaea falcata</i> (Dryand.) Rosenst.	Tuomisto 14925 (TUR)	Peru	FJ361005	FJ360914	FJ360960	GU478702	GU478404
<i>Lindsaea feei</i> C.Chr.	van der Werff 3418 (U)	Venezuela	FJ361004	FJ360913	FJ360959	GU478689	GU478391
<i>Lindsaea flabellifolia</i> (Bak.) C. Chr	Rouhan et al. 1327 (P)	Madagascar	to submit	to submit	to submit	to submit	to submit
<i>Lindsaea flabellifolia</i> (Bak.) C. Chr	Rouhan et al. 334 (P)	Madagascar	to submit	to submit	to submit	to submit	to submit
<i>Lindsaea flabellifolia</i> (Bak.) C. Chr	Rouhan et al. 1129 (P)	Madagascar	to submit	to submit	to submit	to submit	to submit
<i>Lindsaea flabellifolia (millefolia)</i> (Bak.) C. Chr	Lan 5065 (L)	Madagascar	GU478812	GU478513	n.a.	n.a.	n.a.
<i>Lindsaea flabellifolia (millefolia)</i> (Bak.) C. Chr	Guillaumet 4206 (P)	Madagascar	GU478811	n.a.	n.a.	n.a.	n.a.

<i>Lindsaea fraseri</i> Hook.	Streimann 8951 (L)	Australia	FJ360998	FJ360908	FJ360953	GU478719	GU478421
<i>Lindsaea gomphophylla</i> Baker	Hotta 12856 (U)	Brunei	GU478820	GU478497	n.a.	n.a.	n.a.
<i>Lindsaea goudotiana</i> (Kunze) Mett. ex Kuhn	Deroin 112 (P)	Madagascar	GU478818	n.a.	n.a.	n.a.	n.a.
<i>Lindsaea goudotiana</i> (Kunze) Mett. ex Kuhn	Rouhan et al. 1252 (P)	Madagascar	to submit	to submit	to submit	to submit	to submit
<i>Lindsaea goudotiana</i> (Kunze) Mett. ex Kuhn	Janssen 2475 (P)	Madagascar	to submit	to submit	to submit	to submit	to submit
<i>Lindsaea goudotiana</i> (Kunze) Mett. ex Kuhn	Rouhan et al. 1156 (P)	Madagascar	to submit	to submit	to submit	to submit	to submit
<i>Lindsaea goudotiana (subtilis)</i> (Kunze) Mett. ex Kuhn	van der Werff 12855 (MO)	Madagascar	GU478817	GU478514	GU478595	n.a.	n.a.
<i>Lindsaea grandiareolata</i> (Bonap.) Kramer	Gautier 5315 (P)	Madagascar	to submit	n.a.	to submit	n.a.	n.a.
<i>Lindsaea grandiareolata</i> (Bonap.) Kramer	Nusbaumer 2950 (P)	Madagascar	to submit	n.a.	to submit	n.a.	to submit
<i>Lindsaea grandiareolata</i> (Bonap.) Kramer	CJR (Reeb) 115 (-)	Madagascar	to submit	n.a.	to submit	n.a.	n.a.
<i>Lindsaea gueriniana</i> (Gaudich.) Desv.	Stone 2506 (US)	Solomon Islands	GU478766	GU478467	GU478594	n.a.	n.a.
<i>Lindsaea guianensis</i> (Aubl.) Dryand.	Tuomisto 14927 (TUR)	Peru	FJ360999	FJ360944	FJ360954	GU478700	GU478402
<i>Lindsaea hainaniana</i> (K.U.Kramer) Lehtonen & Tuomisto	Wuzhishan fern survey 533 (MO)	Hainan	GU478807	GU478555	GU478604	GU478671	GU478373
<i>Lindsaea harveyi</i> Carrière	Brownlie 978 (TUR)	Fiji	FJ361000	FJ360909	FJ360955	GU478673	GU478375
<i>Lindsaea hemiglossa</i> K.U.Kramer	Tuomisto 12742 (TUR)	Peru	FJ361001	FJ360910	FJ360956	GU478660	GU478362
<i>Lindsaea hemiptera</i> K.U.Kramer	Tuomisto 13049 (TUR)	Peru	FJ374265	FJ374263	FJ374264	GU478717	GU478419
<i>Lindsaea heterophylla</i> Dryand.	Charoenphol 5081 (AAU)	Thailand	GU478855	n.a.	GU478607	n.a.	n.a.
<i>Lindsaea heterophylla</i> Dryand.	Ammann 253 (P)	Madagascar	to submit	n.a.	to submit	n.a.	n.a.
<i>Lindsaea imrayana</i> (Hook.) Perez	Christenhusz 2826 (TUR)	Dominica	GU478772	GU478435	GU478592	GU478649	GU478351
<i>Lindsaea incisa</i> Prent.	Blake 21210 (MO)	Australia	GU478856	GU478520	n.a.	GU478682	GU478384

<i>Lindsaea integra</i> Holttum	Poulsen 72 (AAU)	Borneo	GU478821	GU478531	n.a.	GU478670	GU478372
<i>Lindsaea jamesonioides</i> Baker	Clemens 30733 (BM)	Borneo	GU478764	GU478466	n.a.	GU478711	GU478413
<i>Lindsaea javanensis</i> Blume	Hennipman 3937 (U)	Thailand	FJ361002	FJ360911	FJ360957	n.a.	n.a.
<i>Lindsaea javitensis</i> Humb. & Bonpl. ex Willd.	Prance 15677 (U)	Brazil	GU478792	GU478490	n.a.	n.a.	n.a.
<i>Lindsaea kawabatae</i> Kurata	Saiki 1093 (Z)	Japan	GU478803	GU478499	GU478596	n.a.	n.a.
<i>Lindsaea kingii</i> Copel.	Braithwaite 4304 (U)	Solomon Islands	GU478831	n.a.	GU478629	GU478681	GU478383
<i>Lindsaea lancea</i> (L.) Bedd. var. <i>lancea</i>	Tuomisto 14466 (TUR)	Peru	FJ361006	FJ360915	FJ360961	GU478651	GU478353
<i>Lindsaea lapeyrousei</i> (Hook.) Baker	Brownlie 1575 (U)	Fiji	FJ361007	FJ360916	FJ360962	GU478680	GU478382
<i>Lindsaea leptophylla</i> Baker	Raharimalala 2017 (MO)	Madagascar	GU478819	GU478509	n.a.	n.a.	n.a.
<i>Lindsaea leptophylla</i> Baker	Rouhan et al. 1144 (P)	Madagascar	n.a.	to submit	to submit	to submit	to submit
<i>Lindsaea leptophylla</i> Baker	Rouhan et al. 1163 (P)	Madagascar	to submit	to submit	to submit	to submit	to submit
<i>Lindsaea leptophylla</i> Baker	Rouhan et al. 1195 (P)	Madagascar	n.a.	to submit	to submit	n.a.	n.a.
<i>Lindsaea lherminieri</i> Fée	Bernard 4775 (P)	Guadeloupe	GU478784	GU478505	n.a.	GU478718	GU478420
<i>Lindsaea linearis</i> Sw.	Braggins 589 (AAU)	New Zealand	FJ361008	FJ360917	FJ360963	GU478721	GU478423
<i>Lindsaea lobata</i> Poir. in Lam.	Alston 16699 (U)	North Moluccas	FJ361009	FJ360918	FJ360964	GU478674	GU478376
<i>Lindsaea longifolia</i> Copel.	Elmer 16151 (U)	Luzon	GU478837	GU478527	n.a.	n.a.	n.a.
<i>Lindsaea lucida</i> Blume	Charoenphol 4999 (AAU)	Thailand	GU478808	GU478542	GU478628	n.a.	n.a.
<i>Lindsaea madagascariensis</i> Baker	Rakotondrainibe 6349 (P)	Madagascar	GU478815	GU478512	GU478634	n.a.	n.a.
<i>Lindsaea madagascariensis</i> Baker	Rouhan et al. 1256 (P)	Madagascar	to submit	to submit	to submit	to submit	to submit
<i>Lindsaea madagascariensis</i> Baker	Rouhan et al. 1320 (P)	Madagascar	to submit	to submit	to submit	to submit	to submit
<i>Lindsaea madagascariensis</i> Baker	Bauret et al. 132 (P)	Madagascar	to submit	to submit	to submit	to submit	to submit
<i>Lindsaea madagascariensis</i> Baker	Bauret et al. 139 (P)	Madagascar	to submit	to submit	to submit	n.a.	n.a.
<i>Lindsaea madagascariensis</i> Baker	Rouhan et al. 1623 (P)	Madagascar	to submit	to submit	to submit	to submit	to submit
<i>Lindsaea malayensis</i> Holttum	Schneider 222 (Z)	Malaysia	GU478858	GU478530	GU478602	GU478672	GU478374
<i>Lindsaea marojejense</i> sp. nov.	Bauret et al. 94 (P)	Madagascar	to submit	to submit	to submit	n.a.	n.a.

<i>Lindsaea marojejense</i> sp. nov.	Rouhan et al. 1604 (P)	Madagascar	to submit	to submit	to submit	to submit	to submit
<i>Lindsaea media</i> R.Br.	van der Werff 11655 (MO)	Australia	GU478843	GU478516	GU478597	n.a.	n.a.
<i>Lindsaea meifolia</i> (Kunth) Mett. ex Kuhn	Liesner 7062 (MO)	Venezuela	GU478783	GU478478	n.a.	n.a.	n.a.
<i>Lindsaea merrillii</i> Copel. ssp. <i>yaeyamensis</i> (Tagawa) K.U.Kramer	Kramer 8005 (U)	Taiwan	GU478863	GU478559	n.a.	n.a.	n.a.
<i>Lindsaea microphylla</i> Sw.	Everist 8062 (AAU)	Thailand	FJ361010	FJ360919	FJ360965	n.a.	n.a.
<i>Lindsaea monocarpa</i> Rosenst. in C.Chr.	Edwards 4414A (L)	Papua New Guinea	FJ361011	FJ360920	FJ360966	n.a.	n.a.
<i>Lindsaea multisora</i> Alderw.	Cicuzza 104 (GOET)	Sulawesi	GU478849	GU478534	GU478631	GU478676	GU478378
<i>Lindsaea nervosa</i> Mett.	McKee 2617 (U)	New Caledonia	GU478829	n.a.	n.a.	n.a.	n.a.
<i>Lindsaea oblanceolata</i> Alderw.	Larsen 42928 (U)	Thailand	FJ361012	FJ360921	FJ360967	GU478685	GU478387
<i>Lindsaea obtusa</i> J.Sm. ex Hook.	Kramer 8004 (U)	Taiwan	GU478823	GU478547	GU478599	n.a.	n.a.
<i>Lindsaea orbiculata</i> (Lam.) Mett. ex Kuhn	Averyanov VH4814 (AAU)	Vietnam	FJ361013	FJ360922	FJ360968	n.a.	n.a.
<i>Lindsaea ovoidea</i> Fée	Hatschbach 7083 (L)	Brazil	FJ361014	FJ360923	FJ360969	GU478713	GU478415
<i>Lindsaea oxyphylla</i> Baker	Gautier 2662 (P)	Madagascar	GU478816	GU478508	n.a.	n.a.	n.a.
<i>Lindsaea oxyphylla</i> Baker	Janssen 2387 (P)	Madagascar	to submit	to submit	to submit	n.a.	n.a.
<i>Lindsaea pacifica</i> K.U.Kramer	Brownlie 979 (U)	Fiji	FJ361015	FJ360924	FJ360970	GU478663	GU478365
<i>Lindsaea pallida</i> Klotzsch	Christenhusz 2518 (TUR)	French Guiana	GU478778	GU478479	GU478624	GU478705	GU478407
<i>Lindsaea parallelogramma</i> Alderw.	Poulsen 342 (AAU)	Borneo	GU478851	GU478533	n.a.	GU478675	GU478377
<i>Lindsaea parasitica</i> (Roxb. ex Griffith) Hieron.	Larsen 42927 (AAU)	Thailand	FJ361016	FJ360925	FJ360971	GU478684	GU478386
<i>Lindsaea parkeri</i> (Hook.) Kuhn	Boudrie 4254 (TUR)	French Guiana	GU478775	GU478492	GU478622	GU478695	GU478397
<i>Lindsaea pectinata</i> Blume	Nielsen 816 (AAU)	Australia	FJ361021	FJ360930	FJ360976	GU478686	GU478388
<i>Lindsaea pellaeiformis</i> Christ	Kjellberg 3561 (BM)	Celebes	GU478767	GU478468	n.a.	n.a.	n.a.
<i>Lindsaea pendula</i> Klotzsch	Kramer 2891 (U)	Suriname	GU478782	GU478477	n.a.	n.a.	n.a.
<i>Lindsaea phassa</i> K.U.Kramer	Tuomisto 14940 (TUR)	Peru	FJ361017	FJ360926	FJ360972	GU478659	GU478361

<i>Lindsaea pickeringii</i> (Brack.) Mett. ex. Kuhn	Brownlie 996 (U)	Fiji	FJ361018	FJ360927	FJ360973	GU478666	GU478368
<i>Lindsaea plicata</i> Baker	Callmander 291 (P)	Madagascar	GU478768	GU478471	GU478593	GU478661	GU478363
<i>Lindsaea polyctena</i> K.U.Kramer	Price 2789 (Z)	Mindanao	GU478838	GU478544	n.a.	n.a.	n.a.
<i>Lindsaea portoricensis</i> Desv.	Christenhusz 3384 (TUR)	Jamaica	FJ361019	FJ360928	FJ360974	n.a.	n.a.
<i>Lindsaea portoricensis</i> Desv.	Tuomisto 13045 (TUR)	Peru	FJ361028	FJ360937	FJ360983	GU478692	GU478394
<i>Lindsaea pratensis</i> Maxon	Croat 90913 (MO)	Ecuador	GU478771	GU478469	n.a.	n.a.	n.a.
<i>Lindsaea prolongata</i> E.Fourn.	Mackee 6609 (P)	New Caledonia	GU478830	GU478519	n.a.	n.a.	n.a.
<i>Lindsaea propinqua</i> Hook. in Night.	Wood 10051 (BISH)	Marquesas	GU478825	GU478537	GU478601	GU478662	GU478364
<i>Lindsaea pseudoheptera</i> (Alderw.) Lehtonen & Tuomisto	Hou 245 (U)	Borneo	GU478861	GU478560	n.a.	n.a.	n.a.
<i>Lindsaea pulchella</i> (J.Sm.) Mett. ex Kuhn	Alston 16692 (U)	North Moluccas	GU478805	GU478528	n.a.	GU478723	GU478425
<i>Lindsaea pulchra</i> (Brack.) Carrière ex Seem.	Brousilie 1049 (U)	Fiji	GU478826	GU478551	n.a.	GU478665	GU478367
<i>Lindsaea quadrangularis</i> Raddi ssp. <i>antillensis</i> K.U.Kramer	Christenhusz 3588 (TUR)	Puerto Rico	FJ361020	FJ360929	FJ360975	GU478653	GU478355
<i>Lindsaea regularis</i> Rosenst.	Jaag 1730 (Z)	Flores	GU478847	GU478564	n.a.	n.a.	n.a.
<i>Lindsaea reniformis</i> Dryand.	Boudrie 4250 (TUR)	French Guiana	GU478777	GU478482	GU478618	GU478704	GU478406
<i>Lindsaea rigida</i> J.Sm.	Tolentino HAL-026 (U)	Mindoro Island	GU478832	GU478549	GU478600	GU478710	GU478412
<i>Lindsaea rigidiuscula</i> Lindm.	Poole 2060 (Z)	Brazil	GU478794	GU478476	GU478621	GU478691	GU478393
<i>Lindsaea rosenstockii</i> Brause	Hoogland 10860 (L)	Papua New Guinea	FJ361023	FJ360932	FJ360978	n.a.	n.a.
<i>Lindsaea rufa</i> K.U.Kramer	Munzinger 1254 (P)	New Caledonia	GU478828	GU478518	n.a.	n.a.	n.a.
<i>Lindsaea rufa</i> K.U.Kramer	Tronchet 532 (P)	New Caledonia	to submit	to submit	to submit	n.a.	n.a.
<i>Lindsaea sagittata</i> Dryand.	Christenhusz 2618 (TUR)	Suriname	FJ361024	FJ360933	FJ360979	GU478688	GU478390
<i>Lindsaea schizophylla</i> (Baker) Christ	Sledge 613 (Z)	Sri Lanka	GU478802	GU478498	n.a.	n.a.	n.a.
<i>Lindsaea schomburgkii</i> Klotzsch	Tuomisto 13044 (TUR)	Peru	FJ361026	FJ360935	FJ360981	GU478694	GU478396

<i>Lindsaea seemannii</i> J.Sm. in Seem.	Hammel 3369 (AAU)	Panama	GU478770	GU478470	n.a.	n.a.	n.a.
<i>Lindsaea semilunata</i> (C.Chr.) C.Chr.	Renz 14307 (U)	Guyana	GU478797	GU478489	GU478619	GU478693	GU478395
<i>Lindsaea sessilis</i> Copel.	Koster 13846 (U)	Papua New Guinea	GU478862	GU478561	n.a.	n.a.	n.a.
<i>Lindsaea sphenomeridopsis</i> K.U.Kramer	Renz 14184 (U)	Guyana	FJ361027	FJ360936	FJ360982	GU478708	GU478410
<i>Lindsaea spruceana</i> Mett. ex Kuhn	Quipuscoa 878 (AAU)	Peru	GU478787	n.a.	n.a.	n.a.	n.a.
<i>Lindsaea stolonifera</i> Mett. ex Kuhn	Braithwaite 4283 (U)	Solomon Islands	GU478827	GU478524	n.a.	n.a.	n.a.
<i>Lindsaea stricta</i> (Sw.) Dryand. var. <i>jamesoniiformis</i> K.U.Kramer	Maas 6460 (U)	Puerto Rico	GU478790	GU478473	GU478611	GU478696	GU478398
<i>Lindsaea stricta</i> (Sw.) Dryand. var. <i>parvula</i> (Fée) K.U.Kramer	Boudrie 4253 (TUR)	French Guiana	GU478789	GU478474	GU478625	GU478690	GU478392
<i>Lindsaea stricta</i> (Sw.) Dryand. var. <i>stricta</i>	Boudrie 4246 (TUR)	French Guiana	GU478791	GU478472	GU478610	GU478650	GU478352
<i>Lindsaea surinamensis</i> Posth.	Granville 15461 (CAY)	French Guiana	GU478785	GU478495	GU478630	GU478703	GU478405
<i>Lindsaea taeniata</i> K.U.Kramer	Tuomisto 14170 (TUR)	Peru	FJ361029	FJ360938	FJ360984	GU478655	GU478357
<i>Lindsaea tenuifolia</i> Blume	Hartley 12334 (AAU)	Papua New Guinea	FJ361030	FJ360939	FJ360985	GU478677	GU478379
<i>Lindsaea tenuis</i> Klotzch	Maguine 53824 (U)	Venezuela	GU478798	GU478491	n.a.	GU478715	GU478417
<i>Lindsaea tetragona</i> K.U.Kramer	Kato 14212 (MO)	Seram	GU478839	GU478543	GU478603	n.a.	n.a.
<i>Lindsaea tetraptera</i> K.U.Kramer	Tuomisto 13048 (TUR)	Peru	FJ361031	FJ360940	FJ360986	GU478699	GU478401
<i>Lindsaea trichomanoides</i> Dryand.	Cameron s.n. (AAU)	New Zealand	FJ361032	FJ360941	FJ360987	n.a.	n.a.
<i>Lindsaea ulei</i> Hieron. ex Christ	Tuomisto 13002 (TUR)	Peru	GU478796	GU478488	GU478616	GU478658	GU478360
<i>Lindsaea venusta</i> Kaulf. ex Kuhn	Sledge 1377 (U)	Sri Lanka	GU478835	GU478550	n.a.	GU478720	GU478422
<i>Lindsaea vieillardii</i> Mett.	McKee 5304 (U)	New Caledonia	GU478844	GU478496	n.a.	n.a.	n.a.
<i>Lindsaea virescens</i> Sw.	Lehtonen 621 (TUR)	Brazil	GU478800	GU478484	GU478609	GU478712	GU478414
<i>Lindsaea viridis</i> Col.	Crookes s.n. (U)	New Zealand	GU478769	GU478503	n.a.	n.a.	n.a.
<i>Lindsaea vitiensis</i> K.U.Kramer	Smith 5918 (U)	Fiji	GU478845	GU478529	n.a.	n.a.	n.a.
<i>Lindsaea walkerae</i> Hook.	Bostock 638 (Z)	Australia	GU478841	GU478517	n.a.	n.a.	n.a.
<i>Lindsaea wernerii</i> Rosenst.	Alston 16860 (U)	North Moluccas	FJ361034	FJ360943	FJ360989	GU478667	GU478369

<i>Lonchitis hirsuta</i> L.	Christenhusz 3508 (TUR)	Puerto Rico	GU478725	GU478429	GU478567	GU478640	n.a.
<i>Nesolindsaea caudata</i> (Hook.) Lehtonen & Christenh.	Ballard 1390 (US)	Sri Lanka	GU478757	GU478446	GU478570	n.a.	n.a.
<i>Nesolindsaea kirkii</i> (Hook. ex Baker) Lehtonen & Christenh.	Kramer 11073 (MO)	Seychelles	GU478758	GU478447	n.a.	n.a.	n.a.
<i>Odontosoria aculeata</i> (L.) J.Sm.	Christenhusz 4242 (TUR)	Puerto Rico	GU478740	GU478463	GU478580	n.a.	n.a.
<i>Odontosoria afra</i> (K.U.Kramer) J.P.Roux	Hess-Wyss s.n. (Z)	Angola	GU478738	GU478454	GU478578	n.a.	n.a.
<i>Odontosoria africana</i> F.Ballard	Lewate 5959 (P)	Burundi	GU478739	GU478455	n.a.	n.a.	n.a.
<i>Odontosoria angustifolia</i> (Bernh.) C.Chr.	Sarasin 65 (Z)	New Caledonia	GU478736	GU478451	n.a.	n.a.	n.a.
<i>Odontosoria biflora</i> (Kaulf.) C.Chr.	Raulerson 5549 (BISH)	Guam	GU478732	GU478453	GU478586	n.a.	n.a.
<i>Odontosoria chinensis</i> (L.) J. Sm.	Bauret et al. 11 (P)	Madagascar	to submit	to submit	to submit	n.a.	n.a.
<i>Odontosoria deltoidea</i> (Copel.) Lehtonen & Tuomisto	Mackee 19315 (P)	New Caledonia	GU478733	n.a.	n.a.	n.a.	n.a.
<i>Odontosoria melleri</i> (Hook.) C. Chr.	Bauret et al. 6 (P)	Madagascar	to submit	to submit	to submit	n.a.	n.a.
<i>Odontosoria retusa</i> (Cav.) J.Sm.	Fallen 620 (MO)	Papua New Guinea	GU478735	GU478450	n.a.	n.a.	n.a.
<i>Odontosoria schlechtendalii</i> (C.Presl.) C.Chr.	Nee 24879 (Z)	Mexico	GU478745	GU478457	GU478585	n.a.	n.a.
<i>Osmolindsaea japonica</i> (Baker) Lehtonen & Christenh.	Huang 329 (MO)	Taiwan	GU478761	GU478433	GU478590	GU478644	GU478346
<i>Osmolindsaea odorata</i> (Roxb.) Lehtonen & Christenh.	Averyanov VH3287 (AAU)	Vietnam	GU478760	GU478431	GU478589	GU478646	GU478348
<i>Saccoloma inaequale</i> (Kunze) Mett.	Tuomisto 14409 (TUR)	Peru	GU478727	GU478428	GU478568	GU478638	n.a.
<i>Sphenomeris clavata</i> (L.) Maxon 1	Christenhusz 4218 (TUR)	Puerto Rico	GU478729	GU478448	GU478587	n.a.	n.a.
<i>Tapeinidium amboynense</i> (Hook.) C.Chr.	Dayton 26238 (BISH)	Babeldaob, Palau	GU478753	GU478441	GU478573	GU478642	GU478344
<i>Tapeinidium moorei</i> (Hook.) Hieron.	Mackee 196 (AAU)	New Caledonia	GU478756	GU478445	n.a.	n.a.	n.a.

APPENDIX 5

Herbarium vouchers and GenBank accession numbers (*Phlegmariurus*).

Taxon	Locality	Collector (Herbarium)	<i>rbcL</i>	<i>trnL-trnL/F</i>	<i>trnH-psbA</i>
<i>Diphasium jussiaei</i> (Desv. ex Poir.) Rothm.	Ecuador	Øllgaard 100615 (AAU)	AJ133256	AJ133264	n.a.
<i>Diphasium jussiaei</i> (Desv. ex Poir.) Rothm.	Ecuador	Rothfels 3601 (DUKE, QCA)	n.a.	n.a.	KT749943
<i>Huperzia appressa</i> (Desv.) Á. Löve & D. Löve	China	Pan WJ0409048 (SHMU)	DQ464220	n.a.	DQ464203
<i>Huperzia arctica</i> (Tolm.) Sipliv.	Norway	– (–)	n.a.	GQ245031	n.a.
<i>Huperzia australiana</i> (Herter) Holub	Australia	Field 1041 (BRI)	JQ513908	JQ679088	JQ520368
<i>Huperzia crispata</i> (Ching) Ching	China	Pan WJ0509023 (SHMU)	DQ464221	n.a.	DQ464204
<i>Huperzia emeiensis</i> (Ching & H.S. Kung) Ching & H.S. Kung	China	Pan WJ409023 (SHMU)	DQ464222	n.a.	DQ464205
<i>Huperzia fuegiana</i> (Roiv.) Holub	Chile	Larrain 36361 (NY)	n.a.	n.a.	KT749932
<i>Huperzia haleakalae</i> (Brack.) Holub	Norway	– (–)	n.a.	GQ245032	n.a.
<i>Huperzia lucidula</i> (Michx.) Trevis.	Canada	Gerrath & A. Porr JAG607 (–)	HQ590135	n.a.	HQ596728
<i>Huperzia lucidula</i> (Michx.) Trevis.	USA	Eriksson 664 (GH)	n.a.	AJ224591	n.a.
<i>Huperzia miyoshiana</i> (Makino) Ching	Canada	Rothfels 4483 (DUKE)	DQ464225	n.a.	n.a.
<i>Huperzia nanchuanensis</i> (Ching & H.S. Kung) Ching & H.S. Kung	China	Pan WJ409101 (SHMU)	DQ464226	n.a.	DQ464209
<i>Huperzia quasipolytrichoides</i> (Hayata) Ching	Papua New Guinea	Sundue 3692 (BISH, NY)	n.a.	n.a.	KT749933
<i>Huperzia selago</i> (L.) Bernh	Norway	Rouhan 1114 (P)	to submit	n.a.	to submit
<i>Huperzia serrata</i> (Thunb. ex Murray) Trevis.	China	Pan WJ409041 (SHMU)	DQ464228	n.a.	n.a.
<i>Huperzia somae</i> (Hayata) Ching	Taiwan	TNS:776522	AB574640	n.a.	AB575316
<i>Huperzia suberecta</i> (Lowe) Tard.	Portugal	Rouhan 1043 (P)	to submit	n.a.	to submit
<i>Huperzia sutchueniana</i> (Herter) Ching	China	Pan JSG0504192	n.a.	n.a.	DQ464212
<i>Isoetes flaccida</i> Shuttlew.	USA	– (WTU)	GU191333	n.a.	n.a.
<i>Lycopodiella inundata</i> (L.) Holub	Sweden	Waantorp & N. Wikstrom s.n. (S)	Y07938	AJ224605	AB575308
<i>Phlegmariurus ambrensis</i> (Rakotondr.) A.R.Field & Bauret comb. ined.	Madagascar	Janssen 2469 (P)	to submit	to submit	to submit
<i>Phlegmariurus attenuatus</i> (Spring) B. Øllg.	Costa Rica	Testo 167 (VT)	KT634232	n.a.	KT749924

<i>Phlegmariurus balansae</i> (Herter) A.R. Field & Bostock	New Caledonia	Wikstrom 243 (S)	n.a.	AJ224553	n.a.
<i>Phlegmariurus bampsianus</i> (Pichi-Serm.) A. R.Field & Bostock	Rwanda	Bamps 2964 (P)	n.a.	n.a.	to submit
<i>Phlegmariurus brassii</i> (Copel.) A.R. Field & Bostock	Papua New Guinea	Field 89 ex Rawlins, O. s.n. (CNS)	n.a.	n.a.	JQ520372
<i>Phlegmariurus campianus</i> (B. Øllg.) B. Øllg.	Ecuador	Øllgaard 100612 (AAU)	X98282	AJ224586	n.a.
<i>Phlegmariurus capellae</i> (Herter) B. Øllg.	Ecuador	Øllgaard 100835 (AAU)	n.a.	AJ224570	n.a.
<i>Phlegmariurus carinatus</i> (Desv.) Ching	Australia	Field 969 (BRI)	DQ464229	JQ679090	JQ520363
<i>Phlegmariurus cavifolius</i> (C.Chr.) A.R.Field & Bostock	Madagascar	Janssen 2528 (P)	to submit	to submit	to submit
<i>Phlegmariurus</i> cf. <i>obtusifolius</i> (P. Beauv.) A.R.Field & Bostock	Madagascar	Bauret et al. 153 (P)	to submit	to submit	to submit
<i>Phlegmariurus</i> cf. <i>phlegmaria</i> (L.) T. Sen & U. Sen	Papua New Guinea	Field 700 ex Mason, M. s.n. (CNS)	n.a.	n.a.	to submit
<i>Phlegmariurus compactus</i> (Hook) B. Øllg.	Ecuador	Wikstrom 271 (S)	n.a.	AJ224571	n.a.
<i>Phlegmariurus crassus</i> (Humb. & Bonpl. ex Willd.) B. Øllg.	Ecuador	Øllgaard 100832 (AAU)	n.a.	AJ224572	n.a.
<i>Phlegmariurus cryptomerinus</i> (Maxim.) Satou	Japan	-- (TNS)	AB574628	n.a.	AB575305
<i>Phlegmariurus cumingii</i> (Nessel) B. Øllg.	Ecuador	Øllgaard 100836 (AAU)	Y07930	AJ224578	n.a.
<i>Phlegmariurus dacrydioides</i> (Baker) A.R. Field & Bostock	Tanzania	Mantelow 91452 (S)	n.a.	AJ224562	n.a.
<i>Phlegmariurus dalhousieanus</i> (Spring) A.R. Field & Bostock	Australia	Field 766 (BRI)	n.a.	n.a.	JQ520367
<i>Phlegmariurus dentatus</i> (Herter) Arana	Portugal	Rouhan et al. 1032 (P)	to submit	to submit	to submit
<i>Phlegmariurus dichaeoides</i> (Maxon) B. Øllg.	Panama	Sundue 3229 (COL, NY, VT)	n.a.	KT634236	n.a.
<i>Phlegmariurus dichotomus</i> (Jacq.) W.H. Wagner	Mexico	Eliasson 538 (S)	n.a.	AJ224567	n.a.
<i>Phlegmariurus elmeri</i> (Herter) A.R. Field & Bostock	Philippines	Field 816 ex Rawlins, O. s.n. (CNS)	n.a.	n.a.	JQ663836
<i>Phlegmariurus ericifolius</i> (C. Presl) B. Øllg.	Ecuador	Wikstrom 286 (S)	n.a.	AJ224587	n.a.
<i>Phlegmariurus eversus</i> (Poir.) B. Øllg.	Ecuador	Øllgaard 100620 (AAU)	n.a.	AJ224579	n.a.
<i>Phlegmariurus fargesii</i> (Herter) Ching	Japan	Yahara et al. 398 (TNS)	AB574629	n.a.	AB575306
<i>Phlegmariurus foliosus</i> (Copel.) A.R. Field & Bostock	Fiji	Field 19 ex Rawlins s.n. (CNS)	n.a.	n.a.	JQ663817
<i>Phlegmariurus fordii</i> (Baker) Ching	Japan	-- (TNS)	AB574630	AJ224548	AB575307
<i>Phlegmariurus funiformis</i> (Cham. ex Spring) B. Øllg.	Panama	Asplund 19290 (S)	n.a.	AJ224560	n.a.

<i>Phlegmariurus gnidioides</i> (L. fil.) A.R.Field & Bostock	Comoros	Rouhan et al. 749 (P)	to submit	to submit	to submit
<i>Phlegmariurus gnidioides</i> (L. fil.) A.R.Field & Bostock	Madagascar	Janssen 2454 (P)	to submit	to submit	to submit
<i>Phlegmariurus gnidioides</i> (L. fil.) A.R.Field & Bostock	Madagascar	Janssen 2917 (P)	to submit	to submit	to submit
<i>Phlegmariurus gnidioides</i> (L. fil.) A.R.Field & Bostock	Madagascar	Rouhan et al. 454 (P)	to submit	n.a.	to submit
<i>Phlegmariurus goebelii</i> (Nessel) A.R. Field & Bostock	Malaysia	Field 2742 (CNS)	n.a.	n.a.	JQ663822
<i>Phlegmariurus heteroclitus</i> (Desv. ex Poir.) B. Øllg.	Ecuador	Lenhert 1851 (VT)	KT634230	n.a.	KT749920
<i>Phlegmariurus hippurideus</i> (Christ) B. Øllg.	Ecuador	Øllgaard 100619 (AAU)	Y07931	AJ224566	n.a.
<i>Phlegmariurus hippuris</i> (Desv. ex Poiret) A.R. Field & Testo	Indonesia	Wikstrom et al. 157 (S)	AJ133895	AJ224550	n.a.
<i>Phlegmariurus holstii</i> (Hieron.) A.R. Field & Bostock	Tanzania	Mantelow 91163 (S)	n.a.	AJ224563	n.a.
<i>Phlegmariurus horizontalis</i> (Nessel) A.R. Field & Bostock	Indonesia	Waantorp s.n. (S)	n.a.	AJ224559	n.a.
<i>Phlegmariurus humbertii</i> (Nessel) A.R.Field & Bostock	Madagascar	Bauret et al. 180 (P)	to submit	to submit	to submit
<i>Phlegmariurus humbertii</i> (Nessel) A.R.Field & Bostock	Madagascar	Bauret et al. 180b (P)	to submit	to submit	to submit
<i>Phlegmariurus humbertii</i> (Nessel) A.R.Field & Bostock	Madagascar	Rakotondrainibe 3806 (P)	to submit	n.a.	to submit
<i>Phlegmariurus humbertii-henrici</i> (Herter) A.R.Field & Bostock	Madagascar	Razafitsalama 671 (P)	to submit	n.a.	to submit
<i>Phlegmariurus hystrix</i> (Herter) B. Øllg.	Ecuador	Wikstrom 294 (S)	n.a.	AJ224574	n.a.
<i>Phlegmariurus ignambiensis</i> (Compton) A.R.Field & Testo	Papua New Guinea	Field 714 ex Clements s.n. (CANBG)	n.a.	n.a.	to submit
<i>Phlegmariurus jaegeri</i> (Herter) A.R.Field & Bostock	Guinea	Schnell 5614 (P)	n.a.	n.a.	to submit
<i>Phlegmariurus jaegeri</i> (Herter) A.R.Field & Bostock	Liberia	Jaeger 9642 (P)	to submit	to submit	to submit
<i>Phlegmariurus lauterbachii</i> (E. Pritz. ex K. Schum. & Lauterb.) A.R. Field & Bostock	Papua New Guinea	Field 770 ex Kenning s.n. (CNS)	n.a.	n.a.	JQ663820
<i>Phlegmariurus lecomteanus</i> (Nessel) A.R.Field & Bostock	Madagascar	Rouhan et al. 1578 (P)	to submit	to submit	to submit
<i>Phlegmariurus lecomteanus</i> (Nessel) A.R.Field & Bostock	Madagascar	Rouhan et al. 1643 (P)	to submit	to submit	to submit
<i>Phlegmariurus ledermannii</i> (Herter) A.R. Field & Bostock	Australia	Field 1001 (BRI)	n.a.	n.a.	JQ663816
<i>Phlegmariurus lehnertii</i> Testo	Burma	Field 851 ex Liddle s.n. (CNS)	n.a.	n.a.	JQ663840
<i>Phlegmariurus lindenii</i> (Spring) B. Øllg.	Ecuador	Øllgaard 100831 (AAU)	n.a.	AJ224585	n.a.
<i>Phlegmariurus linifolius</i> (L.) B. Øllg.	Ecuador	Werff 19390 (MO)	Y07932	AJ224569	n.a.

<i>Phlegmariurus lockyeri</i> (D. Jones & B. Gray) A.R. Field & Bostock	Australia	Field 1025 (BRI)	n.a.	AJ224551	JQ663828
<i>Phlegmariurus marsupiiformis</i> (D. Jones & B. Gray) A.R. Field & Bostock	Australia	Field 1023 (BRI)	n.a.	to submit	JQ663811
<i>Phlegmariurus megastachyus</i> (Bak.) A.R.Field & Bostock	Madagascar	Rakotondrainibe 6333 (P)	to submit	to submit	to submit
<i>Phlegmariurus megastachyus</i> (Bak.) A.R.Field & Bostock	Madagascar	Janssen 2468 (P)	to submit	to submit	to submit
<i>Phlegmariurus megastachyus</i> (Bak.) A.R.Field & Bostock	Madagascar	Janssen 2621 (P)	to submit	to submit	to submit
<i>Phlegmariurus megastachyus</i> (Bak.) A.R.Field & Bostock	Madagascar	Rouhan et al. 1229 (P)	to submit	to submit	to submit
<i>Phlegmariurus megastachyus</i> (Bak.) A.R.Field & Bostock "curiosus-like"	Madagascar	Rouhan et al. 1558 (P)	to submit	to submit	to submit
<i>Phlegmariurus megastachyus</i> (Bak.) A.R.Field & Bostock "curiosus-like"	Madagascar	Rouhan et al. 1559 (P)	to submit	to submit	to submit
<i>Phlegmariurus mingcheensis</i> Ching	China	Pan WJ112127 (SHMU)	DQ464232	n.a.	DQ464216
<i>Phlegmariurus mirabilis</i> (Willd.) A.R.Field & Testo	Australia	Field 717 (BRI)	n.a.	n.a.	to submit
<i>Phlegmariurus nummulariifolius</i> (Blume) Ching	Indonesia_PNGu inea	Forster & Liddle s.n. (BRI)	n.a.	n.a.	JQ663824
<i>Phlegmariurus obtusifolius</i> (P. Beauv.) A.R.Field & Bostock	Madagascar	Bauret et al. 163 (P)	to submit	to submit	to submit
<i>Phlegmariurus obtusifolius</i> (P. Beauv.) A.R.Field & Bostock	Madagascar	Rouhan et al. 1577 (P)	to submit	to submit	to submit
<i>Phlegmariurus obtusifolius</i> (P. Beauv.) A.R.Field & Bostock	Madagascar	Bauret et al. 158 (P)	to submit	n.a.	to submit
<i>Phlegmariurus obtusifolius</i> (P. Beauv.) A.R.Field & Bostock	Madagascar	Rouhan et al. 1580 (P)	to submit	n.a.	to submit
<i>Phlegmariurus obtusifolius</i> (P. Beauv.) A.R.Field & Bostock	Madagascar	Janssen 2471 (P)	to submit	to submit	to submit
<i>Phlegmariurus obtusifolius</i> (P. Beauv.) A.R.Field & Bostock	Madagascar	Janssen 2876 (P)	to submit	to submit	to submit
<i>Phlegmariurus obtusifolius</i> (P. Beauv.) A.R.Field & Bostock	Madagascar	Rouhan et al. 1399 (P)	to submit	n.a.	to submit
<i>Phlegmariurus ophioglossoides</i> (Lam.) A.R. Field & Bostock	Comoros	Pignal 3352 (P)	to submit	to submit	to submit
<i>Phlegmariurus ophioglossoides</i> (Lam.) A.R. Field & Bostock	Madagascar	Rakotondrainibe 6955 (P)	to submit	to submit	to submit
<i>Phlegmariurus ophioglossoides</i> (Lam.) A.R. Field & Bostock	Madagascar	Rasolohery 130 (P)	to submit	to submit	to submit
<i>Phlegmariurus ophioglossoides</i> (Lam.) A.R. Field & Bostock	Madagascar	Janssen 2898 (P)	to submit	to submit	to submit
<i>Phlegmariurus ophioglossoides</i> (Lam.) A.R. Field & Bostock	Madagascar	Rouhan et al. 1244 (P)	to submit	to submit	to submit
<i>Phlegmariurus ophioglossoides</i> (Lam.) A.R. Field & Bostock	Madagascar	Rouhan et al. 1400 (P)	to submit	to submit	to submit
<i>Phlegmariurus ophioglossoides</i> (Lam.) A.R. Field & Bostock	Tanzania	Mantelow 91135 (S)	n.a.	AJ224590	n.a.

<i>Phlegmariurus parksii</i> (Copel.) A.R.Field & Bostock	Vanuatu	Rouhan et al. 606 (P)	to submit	to submit	to submit
<i>Phlegmariurus pecten</i> (Bak.) A.R.Field & Bostock	Madagascar	Rakotondrainibe 6329 (P)	to submit	to submit	to submit
<i>Phlegmariurus pecten</i> (Bak.) A.R.Field & Bostock	Madagascar	Janssen 2470 (P)	to submit	to submit	to submit
<i>Phlegmariurus pecten</i> (Bak.) A.R.Field & Bostock	Madagascar	Rouhan et al. 1201 (P)	to submit	to submit	to submit
<i>Phlegmariurus perrierianus</i> (Tard.) A.R.Field & Bostock	Madagascar	Rakotomalala 238 (P)	n.a.	n.a.	to submit
<i>Phlegmariurus petiolatus</i> (C. B. Clarke) H. S. Kung & Li Bing Zhang	China	Pan WJ107181 (SHMU)	DQ464233	n.a.	DQ464217
<i>Phlegmariurus phlegmaria</i> (L.) T. Sen & U. Sen	Australia	Field 22 (CNS)	n.a.	n.a.	to submit
<i>Phlegmariurus phlegmaria</i> (L.) T. Sen & U. Sen	Japan	-- (TNS)	AB574635	n.a.	AB575311
<i>Phlegmariurus phlegmarioides</i> (Gaudich.) A.R. Field & Bostock	New Caledonia	Wikstrom 238 (S)	AJ133896	AJ224554	n.a.
<i>Phlegmariurus pichianus</i> (Tard.) A.R.Field & Bostock	Madagascar	Janssen 2451 (P)	to submit	to submit	to submit
<i>Phlegmariurus piscius</i> comb. Ined.	Australia	Field 2690 (BRI)	n.a.	n.a.	to submit
<i>Phlegmariurus polydactylus</i> (B. Øllg.) B. Øllg.	Ecuador	Øllgaard 100834 (AAU)	n.a.	AJ224575	n.a.
<i>Phlegmariurus proliferus</i> (Bl.) A.R.Field & Bostock	Vanuatu	Rouhan et al. 607 (P)	to submit	to submit	to submit
<i>Phlegmariurus pseudophlegmaria</i> (Nessel) A.R. Field & Testo	Fiji	Field 632 ex Hilder, R. s.n. (CNS)	n.a.	n.a.	JQ663837
<i>Phlegmariurus reflexus</i> (Lam.) B. Øllg.	Mexico	Sundue 3089 (AAU)	KT634233	n.a.	KT749925
<i>Phlegmariurus rosenstockianus</i> (Herter) B. Øllg.	Ecuador	Branbyge 42178 (S)	n.a.	AJ224583	n.a.
<i>Phlegmariurus rufescens</i> (Hook.) B. Øllg.	Ecuador	Øllgaard 100833 (AAU)	n.a.	AJ224576	n.a.
<i>Phlegmariurus salvinioides</i> (Hert.) Ching	Malaysia	Field 51 ex Rawlins s.n. (CNS)	n.a.	n.a.	JQ663819
<i>Phlegmariurus sarmentosus</i> (Spring) B. Øllg.	Ecuador	Øllgaard 100816 (AAU)	n.a.	AJ224584	n.a.
<i>Phlegmariurus saururus</i> (Lam.) B. Øllg.	Kerguelen	Chambrin 4 (P)	to submit	to submit	to submit
<i>Phlegmariurus schlechteri</i> (E. Pritz.) A.R. Field & Bostock	New Zealand	Field 658 ex Ziesing 63 (CANBG)	n.a.	n.a.	JQ663823
<i>Phlegmariurus sieboldii</i> (Miq.) Ching	Japan	Yahara et al. 176 (TNS)	AB574638	to submit	AJ224549
<i>Phlegmariurus sooianus</i> Lawalrée	Democratic Republic of the Congo	Léonard 718 (P)	n.a.	n.a.	to submit
<i>Phlegmariurus</i> sp1 " <i>ulicifolius</i> "	Comoros	Bidault 107 (P)	to submit	to submit	to submit
<i>Phlegmariurus</i> sp1 " <i>ulicifolius</i> "	Comoros	Pignal 3396 (P)	to submit	to submit	to submit

<i>Phlegmariurus squarrosus</i> (G. Forst.) Á. Löve & D. Löve	China	Pan WJ207111 (SHMU)	DQ464235	n.a.	n.a.
<i>Phlegmariurus staudtii</i> (Nessel) A.R.Field & Bostock	Gabon	Hallé 835 (P)	to submit	to submit	to submit
<i>Phlegmariurus strictus</i> (Baker) A.R.Field & Bostock	Madagascar	Bauret et al. 175 (P)	to submit	to submit	to submit
<i>Phlegmariurus strictus</i> (Baker) A.R.Field & Bostock	Madagascar	Janssen 2780 (P)	to submit	to submit	to submit
<i>Phlegmariurus strictus</i> (Baker) A.R.Field & Bostock	Madagascar	Rouhan et al. 1396 (P)	to submit	n.a.	to submit
<i>Phlegmariurus subtrifoliatus</i> (Brownlie) A.R.Field & Bostock	Vanuatu	Rouhan et al. 595 (P)	to submit	to submit	to submit
<i>Phlegmariurus subulatus</i> (Desv. ex Poir.) B. Øllg.	Ecuador	Øllgaard 100618 (AAU)	n.a.	AJ224589	n.a.
<i>Phlegmariurus talamancanus</i> (B. Øllg.) B. Øllg.	Costa Rica	Testo 171 (AAU)	KT634231	KT634234	KT749921
<i>Phlegmariurus tardieuae</i> (Herter) A.R.Field & Testo	Comoros	Loup 628 (P)	to submit	to submit	to submit
<i>Phlegmariurus tardieuae</i> (Herter) A.R.Field & Testo	Comoros	Rouhan et al. 892 (P)	to submit	to submit	to submit
<i>Phlegmariurus tardieuae</i> (Herter) A.R.Field & Testo	Madagascar	Janssen 2435 (P)	to submit	to submit	to submit
<i>Phlegmariurus tardieuae</i> (Herter) A.R.Field & Testo	Madagascar	Rouhan et al. 372 (P)	to submit	to submit	to submit
<i>Phlegmariurus tardieuae</i> (Herter) A.R.Field & Testo	Madagascar	Rouhan et al. 386 (P)	to submit	to submit	to submit
<i>Phlegmariurus tardieuae</i> (Herter) A.R.Field & Testo	Tanzania	Rouhan et al. 509 (P)	to submit	to submit	to submit
<i>Phlegmariurus tardieuae</i> (Herter) A.R.Field & Testo	Maurice	Hennequin 341 (P)	to submit	to submit	to submit
<i>Phlegmariurus tardieuae</i> (Herter) A.R.Field & Testo	Madagascar	Rouhan et al. 1302 (P)	to submit	to submit	to submit
<i>Phlegmariurus tardieuae</i> (Herter) A.R.Field & Testo	Comoros	Viscardi 195 (P)	to submit	to submit	to submit
<i>Phlegmariurus tauri</i> (Herter) A.R. Field & Testo	Indonesia	Wikstrom N. et al. 151 (S)	n.a.	AJ224556	n.a.
<i>Phlegmariurus tenuis</i> (Humb. & Bonpl. Ex Willd.) B. Øllg.	Ecuador	Øllgaard 100817 (AAU)	n.a.	AJ224568	n.a.
<i>Phlegmariurus tetragonus</i> (Hook. & Grev.) B. Øllg. ECUADOR	Ecuador	Wikstrom, N. 301 (S)	n.a.	AJ224577	n.a.
<i>Phlegmariurus tetrastichoides</i> (A.R.Field & Bostock) A.R.Field & Bostock	Australia	Field 822 (BRI)	n.a.	n.a.	to submit
<i>Phlegmariurus tetrastichus</i> (Kunze) A.R. Field & Bostock	Papua_New_Gui nea	Field 815 ex Stocker s.n. (CNS)	n.a.	613 (116 indels)	n.a.
<i>Phlegmariurus trifoliatus</i> (Copel.) A.R.Field & Bostock	Vanuatu	Rouhan et al. 660 (P)	to submit	to submit	to submit
<i>Phlegmariurus trigonus</i> (C.Chr.) A.R.Field & Bostock	Madagascar	Janssen 2458 (P)	to submit	to submit	to submit
<i>Phlegmariurus unguiculatus</i> (B. Øllg.) B. Øllg.	Ecuador	Wikstrom 264 (S)	n.a.	AJ224582	n.a.
<i>Phlegmariurus varius</i> (R. Br.) A.R. Field & Bostock	Australia	Field 1043 (BRI)	JQ679089	JQ672503	JQ663831

<i>Phlegmariurus varius</i> (R. Br.) A.R. Field & Bostock	New Zealand	Wikstrom 259 (S)	to submit	to submit	n.a.
<i>Phlegmariurus verticillatus</i> (L.f.) A.R. Field & Testo	Madagascar	Rasolohery 132 (P)	to submit	to submit	to submit
<i>Phlegmariurus verticillatus</i> (L.f.) A.R. Field & Testo	Comoros	Pignal 3406 (P)	to submit	to submit	to submit
<i>Phlegmariurus verticillatus</i> (L.f.) A.R. Field & Testo	Madagascar	Janssen 2459 (P)	to submit	to submit	to submit
<i>Phlegmariurus verticillatus</i> (L.f.) A.R. Field & Testo	Reunion	Hennequin 263 (P)	to submit	to submit	to submit
<i>Phlegmariurus verticillatus</i> (L.f.) A.R. Field & Testo	Indonesia	Wikstrom N. et al. 156 (S)	AJ133897	AJ224561	n.a.
<i>Phlegmariurus wilsonii</i> (Underw. & F.E. Lloyd) B. Øllg.	Ecuador	Øllgaard, B. 1700 (AAU)	Y07933	AJ224565	n.a.
<i>Phylloglossum drummondii</i> Kunze	Australia	Waycott & Calladine s.n. (AD)	Y07939	AJ224593	to submit
<i>Selaginella moellendorffii</i> Hieron.	-	- (-)	HM17308 0	n.a.	n.a.
<i>Selaginella selaginoides</i> (L.) P. Beauv. ex Mart. & Schrank	Canada	Therrien s.n. (KANU)	AF419048	n.a.	n.a.

Résumé long en français

La biodiversité est inégalement répartie à la surface de la planète : sur les 369,000 espèces de plantes aujourd'hui recensées, environ la moitié serait présente sur 2,3% de la surface terrestre dans des points chauds de diversité. Ces points chauds sont des écosystèmes menacés par les activités anthropiques et hébergeant une importante biodiversité endémique. Comment peut-on expliquer une telle concentration de biodiversité ? Quels sont les mécanismes évolutifs sous-jacents ?

Les îles ont joué un rôle crucial dans la compréhension des écosystèmes, des espèces et de leur évolution. Elles sont souvent comparées à des laboratoires naturels, fournissant des milieux plus simples à étudier par leur isolement. Madagascar est une île continentale au large de l'Afrique, dans l'Océan Indien, avec les autres îles de l'ouest de l'Océan Indien (OOI), elle est classée parmi les 35 points chauds de biodiversité. Plus de 12 000 espèces de plantes vasculaires y sont recensées, dont plus de 10 000 espèces d'angiospermes et environ 600 espèces de fougères, caractérisées par un endémisme d'environ 45%. L'exceptionnelle biodiversité de Madagascar est notamment expliquée par la diversité de ses écosystèmes, favorisée par un gradient d'humidité augmentant d'ouest en est et généré par les pluies d'origine orographique sur la côte est. Les forêts tropicales humides ne se forment ainsi que le long de la côte est.

Madagascar est d'origine gondwanienne, et s'est séparée de l'Afrique il y a 165 millions d'années (Ma) puis de l'Inde il y a 90 Ma. Pourtant, la flore et la faune semblent être majoritairement plus récentes et issues de dispersions durant le Cénozoïque (ces 66 derniers millions d'années), majoritairement en provenance du continent africain. L'étude de la flore s'étant cependant fondée majoritairement sur les angiospermes, les origines biogéographiques d'autres groupes, comme les fougères, restent mal connues.

Malgré leur origine ancienne de plus de 300 millions d'années, les données disponibles sur les fougères actuelles semblent montrer une colonisation récente de Madagascar, après sa séparation de l'Afrique et de l'Inde. Trois sources de dispersion ont été mises en évidence : les néotropiques, l'Afrique et l'Asie tropicale (au sens large, incluant l'Asie continentale et le sud-est asiatique jusqu'en Australasie), mais sans dominance de l'Afrique par rapport aux autres sources. Cependant, relativement peu de données sont disponibles pour confirmer cette tendance. Quelles sont les sources de dispersion et leur importance relative ? Quel est l'âge des lignées malgaches ? Peut-on corréliser les événements de dispersion avec des événements climatiques et géologiques ayant eu lieu à Madagascar et dans d'autres régions du monde ?

Pour répondre à ces questions, quatre groupes de fougères ont fait l'objet d'une étude biogéographique : les sous-familles de fougères grammitides et blechnoides, les genres *Rumohra* et *Lindsaea-Odontosoria*, ainsi qu'un genre de lycophytes *Phlegmariurus* qui fournit un réplica phylogénétique dans une autre lignée de plantes vasculaires à spores. L'histoire biogéographique des lignées malgaches au sein de ces groupes a été reconstruite, à partir de phylogénies moléculaires mondiales dont l'échantillonnage a été amélioré par l'ajout des espèces malgaches, de datations moléculaires, ainsi que de

l'estimation des aires ancestrales. Les résultats concernant chaque groupe ont été développé dans cinq chapitres qui sont ici brièvement résumés.

Chapitre I – Madagascar apporte de nouveaux éléments à la systématique et la biogéographie des fougères grammitides : découverte de nouvelles lignées, et inférence de nombreux événements de dispersion à longue distance.

Trois nouveaux clades incluant des espèces des genres *Grammitis* s.l., *Ctenopterella* et *Enterosora* ont été observés. Les résultats montrent que *Grammitis* s.s. est monophylétique et que *Ctenopterella* et *Enterosora* sont polyphylétiques. Le genre néotropical *Moranopteris* est désormais connu à Madagascar par la découverte d'une nouvelle espèce sur l'île. Les résultats mettent en évidence plusieurs lignées cryptiques, probablement expliquées par homoplasie morphologique.

Les résultats permettent d'identifier au moins 12 dispersions vers la région Afrique-OOI durant les 20 derniers millions d'années, principalement depuis les néotropiques. L'Asie tropicale s.l. n'aurait joué qu'un rôle mineur via une dispersion à la fin de l'Oligocène. De nombreuses dispersions depuis Madagascar vers d'autres régions (en particulier vers l'Afrique et d'autres îles de l'Océan Indien) et des diversifications in situ sont également à noter.

Chapitre II - Des données moléculaires, basées sur un échantillonnage exhaustif des fougères *Rumohra* (Dryopteridaceae), révèlent une histoire biogéographique marquée par des dispersions à longue distance et plusieurs espèces cryptiques au sein de *Rumohra adiantiformis*.

Rumohra adiantiformis, à large répartition au sein de l'hémisphère sud, est hautement polyphylétique. L'absence de caractères morphologiques distinctifs entre les lignées suggère un complexe d'espèces cryptiques. De telles divergences moléculaires non retrouvées sur le plan morphologique peuvent être expliquées par hybridation, convergence morphologique ou (plus probablement ici) par stase morphologique.

Le genre *Rumohra* aurait divergé de *Megalastrum* dans les néotropiques il y a environ 46 Ma et se serait diversifié il y a 11 Ma environ. Sept dispersions à longue distance expliqueraient la répartition actuelle du groupe dans l'hémisphère sud dont trois depuis les Néotropiques vers l'Afrique, Madagascar et la région australe de l'Amérique du Sud. Les trois espèces endémiques de Madagascar seraient issues d'une diversification in situ durant les deux derniers millions d'années.

Chapitre III – Histoire biogéographique des Blechnoideae à Madagascar : mise en évidence de connexions avec les régions tropicales de l'hémisphère sud et de deux diversifications in situ.

Le placement phylogénétique des espèces malgaches confirme leur récente classification dans les genres *Blechnum*, *Lomaridium*, *Lomariocycas* et *Parablechnum*, mais pas *Austroblechnum*. La polyphylie de *Lomaridium attenuatum*, avec une lignée composée uniquement de spécimens des Mascareignes, suggère la présence d'espèces cryptiques.

L'origine biogéographique des lignées malgaches est rendue confuse par d'importantes connexions biogéographiques entre les régions tropicales de l'hémisphère sud.

Néanmoins, au moins une dispersion depuis les Néotropiques est inférée (il y a 10 Ma) suivie d'une diversification in situ. Au moins quatre autres dispersions durant les 50 derniers millions d'années expliqueraient la présence des Blechnoideae à Madagascar, depuis les Néotropiques, l'Australasie, l'Afrique ou d'autres îles de l'OOI, également suivie d'une diversification in situ pour *Lomaridium*.

Chapitre IV – Histoire biogéographique des *Lindsaea* et *Odontosoria* à Madagascar : au moins deux dispersions depuis l'Asie tropicale, et une diversification in situ.

L'ajout de nouveaux spécimens dans la phylogénie des *Lindsaea* a permis de tester la monophylie de dix des onze espèces malgaches, mais seul *L. goudotiana* est retrouvé monophylétique. *Lindsaea marojejyensis* sp. nov. ined. est incluse dans un clade regroupant toutes les espèces malgaches sauf *L. plicata*.

Similairement aux Blechnoideae, l'origine biogéographique n'a pas pu être définie pour toutes les lignées malgaches. Néanmoins, deux dispersions depuis l'Australasie peuvent être inférées : une entre 25 et 64 Ma et une il y a environ 10 Ma, suivie d'une diversification in situ générant 10 des 11 espèces du genre *Lindsaea* à Madagascar.

Chapitre V – Première étude biogéographique du genre *Phlegmariurus* (Lycopodiaceae), et focus sur Madagascar.

Les résultats des analyses phylogénétiques confirment les combinaisons taxonomiques des espèces malgaches d'*Huperzia* dans le genre *Phlegmariurus*. Par ailleurs, *Phlegmariurus phlegmaria*, *P. squarrosus* and *P. verticillatus* sont polyphylétiques, mettant en évidence des lignées cryptiques, notamment dans l'OOI.

Les analyses de datations révèlent que *Phlegmariurus* aurait divergé d'*Huperzia* durant le Crétacé, la diversification des espèces actuelles commençant durant l'Eocène. L'estimation des aires ancestrales est incertaine quant à l'aire d'origine et de diversification de *Phlegmariurus*. Le genre aurait néanmoins commencé à se diversifier dans une région couvrant au moins les néotropiques et l'australasie. La distribution actuelle du genre pourrait être expliquée par des événements de dispersion longue-distance et des migrations via l'Antarctique ou les boréotropiques. En particulier, six dispersions durant les 40 derniers millions d'années expliqueraient la présence de *Phlegmariurus* à Madagascar, en provenance d'australasie et peut-être des Néotropiques et d'Afrique. Une importante diversification in situ démarrant au Miocène serait à l'origine de plus de la moitié des espèces recensées à Madagascar.

Chapitre VI – Synthèse sur l'histoire biogéographique des fougères de Madagascar

Les données issues de la littérature et de cette thèse confirment que les Néotropiques, l'Afrique et l'Asie tropicale sont toutes trois des régions sources de dispersion vers Madagascar pour les fougères, mais aussi pour les lycophytes. Les connexions entre Madagascar et les sources lointaines sont expliquées par des courants éoliens transportant les spores : les vents circumaustraux connectent notamment les Néotropiques et Madagascar, alors que les vents liés aux moussons, ainsi que les cyclones traversant l'Océan Indien, pourraient transporter les spores depuis l'Asie tropicale s.l.

Jusqu'ici, rien ne permet cependant d'affirmer qu'une de ces sources est dominante. Cette observation peut paraître surprenante vu la proximité géographique de l'Afrique par rapport aux autres sources, suggérant que le continent devrait être une source majoritaire pour Madagascar selon la théorie de la biogéographie des îles. Plusieurs facteurs pourraient expliquer que l'Afrique ne soit pas une source de biodiversité dominante : i) les néotropiques et l'Asie tropicale s.l. sont des sources de biodiversité plus importantes, ce qui compenserait leur éloignement, ii) plusieurs extinctions passées ayant fortement impacté les forêts tropicales africaines pourraient avoir affaibli le rôle de l'Afrique ou biaisé des inférences d'aire ancestrale, et iii) une préférence écologique des fougères pour les forêts tropicales humides d'altitude pourrait avoir réduit les capacités de la source africaine par rapport aux autres régions sources. En effet, à Madagascar, 85% des espèces de fougères sont recensées dans le domaine phytogéographique du centre, notamment caractérisé par ses forêts humides et une altitude >600 m et incluant les plus hauts sommets de l'île (>2000 m).

Les données issues de la littérature et de cette thèse montrent que les fougères auraient colonisé Madagascar à partir du Cénozoïque, la majorité de ces événements étant notamment datés à partir du Miocène (<23 Ma), soit après la formation des forêts tropicales malgaches et de la circulation circumaustrale (permettant des dispersions en provenance des néotropiques). Le démarrage des moussons indiennes au milieu du Miocène, associées à des vents vers Madagascar, coïncide avec les premières dispersions en provenance de l'Asie tropicale s.l. Le nombre important de colonisations à partir du Miocène pourrait être lié à la formation de forêts tropicales d'altitude dans les régions sources à la même période, suite à des orogénèses ou la formation de volcans.

Abstract

Madagascar is a continental island in the Indian Ocean, near African coasts. It is also a biodiversity hotspot, comprising more than 12,000 species of vascular plants and including more than 600 fern species with an endemism reaching the 45%. Comparatively, continental Africa comprises only 800 species. How can such a diversity be explained in Madagascar? What are the biogeographical origins of the Malagasy ferns? Thanks to new data combined to the literature, hypotheses on the biogeographical history of Malagasy ferns are here proposed.

Four fern taxa were newly investigated: grammitid and blechnoid subfamilies, *Rumohra* and *Lindsaea-Odontosoria*, as well as *Phlegmariurus*, a genus of lycophytes considered as a phylogenetic replicate in another vascular spore-bearing plant lineage. The biogeographical history of the Malagasy lineages was inferred, based on worldwide molecular phylogenies completed by Malagasy species, molecular dating and ancestral area estimates.

Despite its Gondwanian origin, ferns and lycophytes would have colonized Madagascar after its isolation, during the Cenozoic (< 66 Ma), from the Neotropics (South America), Africa and tropical Asia s.l. (meaning from continental Asia to Southeast Asia and Australasia). Dispersal events were especially inferred from the Miocene (< 23 Ma). These results could be explained by the combination of events during the Cenozoic (establishment of the Malagasy rainforests, onset of wind currents allowing spore dispersal) and ecological preferences of ferns and lycophytes for elevated tropical rainforests that have established from the Miocene in the source regions.

Résumé

Madagascar est une île continentale de l'Océan Indien, au large de l'Afrique. C'est également un point chaud de biodiversité, hébergeant plus de 12 000 espèces de plantes vasculaires, dont plus de 600 espèces de fougères caractérisées par un endémisme dépassant les 45%. L'Afrique continentale n'héberge en comparaison que 800 espèces. Comment peut-on expliquer une telle concentration de biodiversité à Madagascar ? Quelles sont les origines biogéographiques des fougères malgaches ? L'apport de nouvelles données et la littérature préexistante permettent de proposer ici des hypothèses sur l'histoire biogéographique des fougères de Madagascar.

Quatre groupes de fougères ont fait l'objet d'une étude biogéographique : les sous-familles de fougères grammitides et blechnoïdes, les genres *Rumohra* et *Lindsaea-Odontosoria*, ainsi qu'un genre de lycophytes, *Phlegmariurus*, représentant un réplica dans une autre lignée de plantes vasculaires à spores. L'histoire biogéographique des lignées malgaches au sein de ces groupes a été reconstruite, à partir de phylogénies moléculaires mondiales complétées par les espèces malgaches, de datations moléculaires, ainsi que de l'estimation des aires ancestrales.

Bien que Madagascar soit d'origine ancienne, gondwanienne, les résultats des analyses biogéographiques montrent que les fougères et les lycophytes auraient colonisé Madagascar après son isolement, durant le Cénozoïque (< 66 Ma), depuis les néotropiques (Amérique du Sud) l'Afrique et l'Asie tropicale (au sens large, incluant l'Asie continentale et le sud-est asiatique jusqu'en Australasie). En particulier, de nombreuses dispersions ont été observées à partir du Miocène (< 23 Ma). Ces résultats seraient expliqués par une combinaison d'événements au Cénozoïque (formation des forêts tropicales humides Malgaches, formation de courants éoliens permettant la dispersion par spores) et des préférences écologiques des fougères et des lycophytes pour les forêts tropicales humides d'altitude, s'étant formées à partir du Miocène dans les régions sources.