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

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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 c. 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

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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.


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 & Eldénäs, 2007; Pillon et al., 2009; Bauret et al., 2017; Crowd, Myers & Cellinese, 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, 2010). Species are terrestrial, epilithic or epiphytic, and the genus is morphologically characterized by a combination of several features (Fig. 1): creeping rhizomes with a dorsal leaf arrangement; elongate ventral meristele; lanceolate

<table>
<thead>
<tr>
<th>Table 1. Distribution of the seven <em>Rumohra</em> spp.</th>
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<tbody>
<tr>
<td><strong>Western Indian Ocean – Africa</strong></td>
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<tr>
<td><strong>R. adiantiformis</strong></td>
</tr>
<tr>
<td><strong>R. berteroana</strong></td>
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<tr>
<td><strong>R. glandulosissima</strong></td>
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<tr>
<td><strong>R. linearisquamata</strong></td>
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<tr>
<td><strong>R. lokohensis</strong></td>
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<tr>
<td><strong>R. madagascarica</strong></td>
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<tr>
<td><strong>R. quadrangularis</strong></td>
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</table>

*Unsampled localities for the widely distributed _R. adiantiformis_.

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to delorate, 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).

*Rumohra* belongs to Dryopteridaceae subfamily Elaphoglossoideae (PPGI, 2016) or Polypodiaceae subfamily Dryopteridioideae (Christenhusz & Chase, 2014). With the genera *Megalastrum* Holttum (91 species; Moran, Prado & Sundue, 2014, b), *Lastreopsis* Ching (16 species; Labiak et al., 2015) and *Parapolystichum* (Keyserl.) Ching (28 species; Labiak et al., 2015; 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. berteroana* (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 widespread 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.

**MATERIAL AND METHODS**

**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 S1.

**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 µL proteinase K (20 mg/mL) and 30 µL 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*trnF). All PCRs were carried out in a 25 µL volume containing 1× PCR buffer, 2.5 mM

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**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. berteroana* 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.

MgCl₂, 250 μM each dNTP, 1 M betaine, 0.4 μM each primer, 0.75 U Taq polymerase (Taq CORE kit; MP Biomedicals, Illkirch, France) and 1 μL 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 \textit{atpA}, \textit{atpB}, \textit{rbcL} and \textit{trnG-trnR} (Table 2). DNA strands were edited and assembled in Sequencher 4.9 (Gene

<table>
<thead>
<tr>
<th>DNA region</th>
<th>Primer name</th>
<th>Literature reference</th>
<th>Primer sequence (5’ to 3’)</th>
<th>Thermal cycling conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{atpA}</td>
<td>ESTRNR46F</td>
<td>Schuettpelz, Korall &amp; Pryer (2006)</td>
<td>GTATAGGTTCAARTCCTATTGGACG</td>
<td>5 min 94 °C/40× (30 s 94 °C/1 min 50 °C/2 min 72 °C/10 min 72 °C)</td>
</tr>
<tr>
<td>\textit{atpB}</td>
<td>ESATPA535F</td>
<td>Schuettpelz et al. (2006)</td>
<td>ACAGCGAGTCTAGGACGATAG</td>
<td>72 °C/10 min 72 °C</td>
</tr>
<tr>
<td>\textit{atpB}</td>
<td>ESATPA856F*</td>
<td>Schuettpelz et al. (2006)</td>
<td>CGAGAACGAGTTCTCG</td>
<td>72 °C</td>
</tr>
<tr>
<td>\textit{atpB}</td>
<td>ESATPA877R*</td>
<td>Schuettpelz et al. (2006)</td>
<td>CATCCTCCGGATATGCTTCG</td>
<td>72 °C</td>
</tr>
<tr>
<td>\textit{rbcL}</td>
<td>ESATPB172F</td>
<td>Schuettpelz &amp; Pryer (2007)</td>
<td>AATTTACTTTGAGTTCAACAAT</td>
<td>5 min 94 °C/40× (30 s 94 °C/1 min 50 °C/2 min 72 °C/10 min 72 °C)</td>
</tr>
<tr>
<td>\textit{atpB}</td>
<td>ESATPE45R</td>
<td>Schuettpelz &amp; Pryer (2007)</td>
<td>ATCCCAACWATTCGATTWGGAG</td>
<td>50 °C/2 min 72 °C/10 min</td>
</tr>
<tr>
<td>\textit{atpB}</td>
<td>ESATPA1163F*</td>
<td>Schuettpelz et al. (2006)</td>
<td>ATGGCGAGGRAATTTCCGAGAT</td>
<td>72 °C</td>
</tr>
<tr>
<td>\textit{atpB}</td>
<td>ESATPA877R*</td>
<td>Schuettpelz et al. (2006)</td>
<td>CATTAYATGCGTGGAGAGATCG</td>
<td>72 °C</td>
</tr>
<tr>
<td>\textit{rbcL}</td>
<td>ESATPB910R*</td>
<td>Schuettpelz &amp; Pryer (2004)</td>
<td>TTCCTGYARAGANCCCATTTCTG</td>
<td>72 °C</td>
</tr>
<tr>
<td>\textit{atpB-}rbcL</td>
<td>atpB 672F</td>
<td>Wolf (1997)</td>
<td>ACACCTWAGAGGGCTCCCGATCAA</td>
<td>5 min 94 °C/40× (30 s 94 °C/30 s 50 °C/1.5 min 72 °C/10 min 72 °C)</td>
</tr>
<tr>
<td>\textit{atpB-}rbcL</td>
<td>rbcL 49R</td>
<td>Wolf (1997)</td>
<td>CACCAGCTTTGACCACCTCAA</td>
<td>72 °C/10 min 72 °C</td>
</tr>
<tr>
<td>\textit{rps4-trnS}</td>
<td>rps4-3r.f</td>
<td>Skog et al. (2004)</td>
<td>AGTTGGTAGTTGCTGAGTAT</td>
<td>5 min 94 °C/40× (30 s 94 °C/30 s 50 °C/45 s 72 °C/10 min 72 °C)</td>
</tr>
<tr>
<td>\textit{rps4-trnS}</td>
<td>trnS-r</td>
<td>Smith &amp; Cranfill (2002)</td>
<td>TACCGAGGTTCGAATC</td>
<td>5 min 94 °C/40× (30 s 94 °C/30 s 50 °C/45 s 72 °C/10 min 72 °C)</td>
</tr>
<tr>
<td>\textit{trnG-trnR}</td>
<td>TRNG1F</td>
<td>Nagalingum, Schneider &amp; Pryer (2007)</td>
<td>GCGGGATAGTTAGTGTTGGA</td>
<td>5 min 94 °C/40× (30 s 94 °C/1 min 50 °C/1.5 min 72 °C/10 min 72 °C)</td>
</tr>
<tr>
<td>\textit{trnG-trnR}</td>
<td>TRNR22R</td>
<td>Nagalingum et al. (2007)</td>
<td>CTATCCAGTTAGCGATCGAG</td>
<td>50 °C/1.5 min 72 °C/10 min</td>
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<tr>
<td>\textit{trnG-trnR}</td>
<td>TRNG43F1*</td>
<td>Nagalingum et al. (2007)</td>
<td>TGATGCAGCTTCCGATTCG</td>
<td>72 °C/10 min 72 °C</td>
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<tr>
<td>\textit{trnG-trnR}</td>
<td>TRNG68R*</td>
<td>Nagalingum et al. (2007)</td>
<td>GGCGGAATGAAGCCGACCATCA</td>
<td>72 °C</td>
</tr>
<tr>
<td>\textit{trnH-psbA}</td>
<td>psbH2</td>
<td>Tate (2002)</td>
<td>GGCOCATGTGAGTTCCACAATTC</td>
<td>5 min 94 °C/40× (30 s 94 °C/45 s 48 °C/45 s 72 °C/10 min 72 °C)</td>
</tr>
<tr>
<td>\textit{trnH-psbA}</td>
<td>psbAF</td>
<td>Sang, Crawford &amp; Stuessy (1997)</td>
<td>GTTATGATGAAGCAGTAATGCTC</td>
<td>72 °C/10 min 72 °C</td>
</tr>
<tr>
<td>\textit{trnL/trnL-}trnF</td>
<td>f</td>
<td>Taberlet et al. (1991)</td>
<td>ATTTGAACCTGTTGACACGAG</td>
<td>5 min 94 °C/40× (30 s 94 °C/30 s 50 °C/45 s 72 °C/10 min 72 °C)</td>
</tr>
<tr>
<td>\textit{trnL/trnL-}trnF</td>
<td>Fern-1</td>
<td>Trewick et al. (2002)</td>
<td>GGCAGCCCCCARATTCGAGRAACC</td>
<td>72 °C/10 min 72 °C</td>
</tr>
</tbody>
</table>

*Primers only used as internal primers for sequencing.
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, Lohman & Meier, 2011). Each region constituted an independent partition in the final data-set, 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; Huaelsenbeck, 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 2500 (25%) trees as burn-in, and pooled the 7500 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 GTR+ΓA model of nucleotide substitution. We performed 1000 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).

**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).

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+Γ 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 (Schuettpelz & Pryer, 2009; Testo & Sundue, 2016). Testo & Sundue (2016) reported older ages for numerous nodes and explained the contrast between their estimates and those of Schuettpelz & Pryer (2009) by differences in the fossil calibrations and statistical methods used, notably the use of penalized likelihood by Schuettpelz & 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 Schuettpelz & Pryer, 2009; node 284) for lastreopsids, and 52.5 Mya (based on Testo & Sundue, 2016) or 40.5 Mya (based on Schuettpelz & 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).
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 & Rodríguez, 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.

Results

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; Fig. 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. madagascarica* (Bonap.) Tardieu]; (2) the Neotropics [*R. quadrangularis* (Fée) Table 3. Statistics for the eight plastid DNA regions and combined dataset obtained for *Rumohra*

<table>
<thead>
<tr>
<th>DNA Region</th>
<th>Number of sequences</th>
<th>Aligned length (in base pairs)</th>
<th>Percentage of variable characters</th>
<th>Percentage of potentially informative characters</th>
</tr>
</thead>
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<td>58</td>
<td>6926</td>
<td>14.1</td>
<td>9.1</td>
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</table>

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).

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. berteroana* samples) and an Australasian subclade (with *R. adiantiformis* samples only; both subclades with BS = 100; PP = 1).

*Rumohra adiantiformis* was grossly polyphyletic, being recovered in eight lineages (Fig. 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. berteroana, BP = 94 and PP = 1) or unresolved (R. glandulosissima, R. linearisquamata, R. quadrangularis).

**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 (Fig. 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.

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 (Fig. 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* (Fig. 4).

**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 \( P = 0.22 \); Fig. 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.

Focusing on clade B, the DEC+j analysis inferred an Australasian ancestral area as the most probable (crown node; \( P = 0.60 \); Fig. 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 (Fig. 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).
DISCUSSION

Our molecular phylogenetic results supported the evolutionary distinctiveness of three (out of seven) Rumohra spp.: the Malagasy endemics R. lokohensis and R. madagascarica and the Juan Fernández endemic R. berteroana. 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. berteroana based on a restricted sampling (i.e. one
Several LDD events from a Neotropical ancestral area. The circum-austral distribution of the genus, involving biogeographical scenario to explain the current, mostly 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.

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 sympathy, 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 & Eldénäs, 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 triangulifera (Boeckeler) C.B. Clarke (Britton, Hedderson & Verboom, 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 sympathy, they are easy to distinguish based on the shape and size of their scales on the rhizomes and costae (Fig. 1G, I; Rakotondrainibe, 2010), 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 (Fig. 1H; Sundue et al., 2013).

**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).

**Hybridization hypothesis**

Hybridization is now accepted as a major evolutionary mechanism in vascular plants (Abbott, Barton & Good, 2016), and especially in ferns (Barrington, Haufer & Werth, 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.

**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, Islam & Ranker, 2010; Bauret et al., 2017), bolbitidioids (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.

**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 (sympleiomorphies). 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 widespread 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.

**HISTORICAL BIOGEOGRAPHY OF RUMOHRA**

**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 lastreopсид 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 lastreopсид 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 Lastreopsideae, 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 circum-austral 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.

**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; Fig. 5), which occurred c. 9.8 and 9.4 Ma, 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 Ma (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; Fig. 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 Ma, i.e. after complete Antarctic glaciation (which dates back to 14–4 Ma; 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-antarctic area (Muñoz et al., 2004). Therefore, spores most probably travelled eastward through the Atlantic and Indian Oceans (LDD4; Fig. 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. berteroana, 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 Ma (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).

Focus on the Malagasy species hotspot

The two transatlantic eastward LDD events (LDD2 and LDD3), which occurred c. 10 Ma, 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 & Kunstner, 2012; Buerki et al., 2013) and with the African origin reported for many Malagasy angiosperms (e.g. Bartish et al., 2011; Calcino, Teruel & Downie, 2015; Bacon et al., 2016; Janssens 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; Fig. 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., 2007), 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, 2010). Species are commonly found in sympathy, without intermediate morphological forms that would indicate the occurrence of interspecific crosses. Whether speciation took place in sympathy or, more likely, whether sympatric sites result from secondary contact between previously, allopatrically derived species remains to be determined.

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REFERENCES


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Rambaut A. 2014. FigTree 1.4.2. Program distributed by the author. Available at: http://tree.bio.ed.ac.uk/software/figtree

Rambaut A, Suchard MA, Xie D, Drummond AJ. 2014. Tracer v1.6. Program distributed by the authors. Available at: http://beast.bio.ed.ac.uk/Tracer


**SUPPORTING INFORMATION**

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Appendix S1.** Herbarium vouchers and GenBank accession numbers. Newly generated sequences are indicated with an asterisk.