Phylogenetics and historical biogeography of *Lomaridium* (Blechnaceae: Polypodiopsida)

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Abstract Blechnaceae is a worldwide family of leptosporangiate ferns composed of about 250 species. Most of the species in the family were recognised under a single large genus *Blechnum* until recently, when a new classification proposed the recognition of 24 genera. Given this new systematics of Blechnaceae, which largely resolves the genus-level relationships in the family, there is a need for phylogenetic research to investigate relationships within the majority of the newly proposed genera. In this paper, we unravel the phylogenetic relationships and the historical biogeography of the species of *Lomaridium*, a genus including most of the hemiepiphytic species in the Blechnaceae. Our sampling includes 11 species, which represents 85% of the diversity in the genus and which covers the entire geographic distribution of the group. We constructed two datasets with three plastid markers: one for phylogenetic analyses (maximum likelihood, Bayesian inference) with four outgroups from phylogenetically close genera (*Brainea*, *Lomaria*, *Sadleria*, *Woodwardi*); and a second for molecular dating and historical biogeographic analyses that included a larger set of outgroups so that we could accurately reconstruct ancestral events at the base of *Lomaridium*, under different models. We are able to recognize four highly supported lineages: *L. contiguum* and the *L. schottii*, *L. attenuatum*, and *L. fragile* clades. Our results date the origin of *Lomaridium* at some point during the Paleocene epoch, and the most likely geographic area for its origin is Australia plus tropical Central and South America. Several dispersal events are inferred, all of which are most likely long-distance dispersal events. From Australia, we infer a first dispersal event that brought the ancestor of the extant species *L. contiguum* to New Caledonia. In Central and South America, *Lomaridium* continued to diversify and colonized additional areas, including the Caribbean (*L. binervatum*), some Pacific islands (*L. schottii*), and Africa and Madagascar. While our goal in the current study was not to estimate the biogeographic or diversification history of all Blechnaceae, our analyses do suggest that the early history of the family was complex biogeographically, with extensive long-distance dispersal events. *Lomaridium* exemplifies this high dispersal capacity, as a genus with only a modest number of species that have reached far-flung regions of the globe via numerous long-distance dispersal events.

Keywords *Blechnum*; dispersal; ferns; hemiepiphytes; morphology; plastid DNA

Supplementary Material DNA sequence alignment, and xml file for BEAST analysis along with its output tree file are available in the Supplementary Data section of the online version of this article at http://www.ingentaconnect.com/content/iapt/tax

■ INTRODUCTION

The Blechnaceae is a medium-sized family of leptosporangiate ferns that comprises 220–250 species (Kramer & al., 1990; Perrie & al., 2014). The family is distributed worldwide, but most of the diversity occurs in the Southern Hemisphere, primarily in tropical and subtropical ecosystems, but extending also to temperate-cold habitats (Christenhusz & al., 2011; Rothfels & al., 2012). Blechnaceae is included as a monophyletic family in the most widely accepted fern classifications (Smith & al., 2006; Rothfels & al., 2012; PPG I, 2016). Traditionally, the family contained 9–10 genera, with the large genus *Blechnum* L. comprising almost 80% of the diversity. However, many recent studies focusing on specific groups of species (Rolleri & Prada, 2006; Passarelli & al., 2010; Rolleri & al., 2012, 2013; Dittrich & al., 2015) and all recent phylogenies of the family (Shepherd & al., 2007; Gabriel y Galán & al., 2013; Perrie & al., 2014; Gasper & al., 2017) have shown that there is widespread paraphyly and disparity of characters among *Blechnum* species. As a result, the genus has recently been split into several entities, and a total of 24 genera have been proposed to be recognized in Blechnaceae (Gasper & al., 2016; PPG I, 2016). With these changes, the global systematics of the family appears to be more or less clarified at the genus level, but much work still remains to be done within most of the genera. Many species have not been sampled in a molecular framework, and the lack of detailed morphological information for some taxa prevents proper taxonomic placement, the elucidation of phylogenetic relationships, and the comprehension of their evolutionary significance.

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Lomaridium C.Presl is a recently resurrected genus (Gasper & al., 2016) that is sister to *Lomaria* Willd. plus the members of what have been called Superclades A and B of the Blechnaceae, which are sister to each other (Gasper & al., 2017). *Lomaridium* comprises a group of hemiepiphytic species, climbers by rhizomes in the tropical rainforests, although sometimes juvenile or underdeveloped adult individuals are terrestrial, and rarely individuals can be complete epiphytes. The following characters can generally be found in all species of *Lomaridium* (Figs. 1, 2): rhizome scales ovate, lanceolate or linear-lanceolate, margin denticulate, with acute or filiform apex, bicolorous or tricolorous, rarely concolorous; plants dimorphic; trophophylls deeply pinnatisect or pinnate, with adnate pinnae, lamina base with highly reduced segments; frond apex conform or pinnatifid; glabrous (sometimes with glandular rachises); fertile pinnae contracted, strongly recurved; sori protected by an anatomically complex, "lamina-like" structure; spores with low reticulate perispore (Tryon & Tryon, 1982; Kramer & al., 1990; Passarelli & al., 2010; Prada & al., 2016). A chromosome base number of $x = 29$ has been assigned to the genus (Gasper & al., 2016) but is, as far as we know, derived from counts of only two species (Smith & Foster, 1984; Kurita, 1986).

Following the most recent classification of *Lomaridium* included in the treatment by Gasper & al. (2016), the genus comprises 16 species. In this study, we have deviated from this concept in five cases, the justification of which is set out below.

First, *Lomaridium xiphophyllum* (Baker) Gasper & V.A.O. Dittrich is difficult to segregate from *L. simillimum* (Baker) Gasper & V.A.O.Dittrich. Previous authors (Rakotondrainibe & al., 2013) attributed these problems to intense and frequent hybridization between the two species in nature. In the absence of proper studies devoted to resolving the issue, they decided to maintain the species as distinct. However, in our opinion, it is more reasonable to maintain them as con-specific until further analyses are done to properly decide the status of the two entities; some other authors expressed the same opinion (Schelpe, 1952; Roux, 2009). Second, the original description of *L. bonapartei* (Rakotondr.) Gasper & V.A.O.Dittrich reported a plant that is terrestrial or epilithic, with rhizome not repent and bearing scales with entire margins (Rakotondrainibe & al., 2013). There is no information, molecular or morphological, suggesting that this species could belong to *Lomaridium*. Third, the taxonomic status of *L. dendrophilum* (Sodiro) Gasper & V.A.O.Dittrich is uncertain: Sodiro (1893) mentioned its close resemblance to *L. fragile* (Liebm.) Gasper & V.A.O.Dittrich, but as the type specimen is missing (Navarrete & Pitman, 2003), the acceptance of this entity as a distinct species is uncertain. Fourth, *L. pteropus* (Kunze) Gasper & V.A.O.Dittrich has recently been considered as con-specific with *L. plumieri* (Desv.) C.Presl (Dittrich & al., 2017). Fifth, we considered *Blechnum kunthianum* C.Chr. (for which there is no available combination in *Lomaridium* yet) as a distinct species, following some authorities (Durán, 1997), based on the larger size of all organs and structures compared to *L. fragile*, with which *B. kunthianum* was formerly considered to be con-specific. This difference in size between *L. fragile* (which inhabits Central America and northern areas of South America) and plants from southern latitudes (Argentina, Paraguay, south Brazil) was also noticed by Morton & Lellinger (1967) who suggested that, in case of segregation, the latter should be called *B. kunthianum*. A new combination for this species in *Lomaridium* as *L. angustifolium* comb. nov. is proposed in Appendix 1.

Based on the above, we consider that *Lomaridium* includes the following 13 species: *L. acutum* (Desv.) Gasper & V.A.O.Dittrich, *L. angustifolium* comb. nov., *L. attenuatum* (Sw.) Gasper & V.A.O.Dittrich (includes *B. giganteum* (Kaulf.) Schltdl.), *L. biforme* (Baker) Gasper & V.A.O.Dittrich (includes *B. microbasis* (Baker) C.Chr.), *L. binervatum* (Poir.) Gasper & V.A.O.Dittrich, *L. contiguum* (Mett.) Gasper & V.A.O.Dittrich, *L. ensiforme* (Liebm.) Gasper & V.A.O.Dittrich, *L. fragile*, *L. fuscosquamosum* (A.Rojas) Gasper & V.A.O.Dittrich, *L. nigrocostatum* (A.Rojas) Gasper & V.A.O.Dittrich, *L. plumieri*, *L. schottii* (Colla) Gasper & V.A.O.Dittrich and *L. simillimum*.

Considered as a whole, *Lomaridium* occupies mainly a southern geographical distribution that extends from some Pacific islands (New Caledonia, Lord Howe) to South and Central America (including islands such as the Juan Fernandez Islands) and Central and South Africa (including islands such as Madagascar and Reunion). This distribution suggests an austral origin and subsequent dispersal, as inferred in previous studies of the distribution of Blechnaceae (Chambers & Farrant, 2001), but which has not yet been formally tested.

In the current paper we expand the taxon sampling of previous studies from 7 (Perrie & al., 2014; Gasper & al., 2017) to 11 species, which includes roughly 85% of the diversity of *Lomaridium*. Our aims are to investigate species relationships, and to explore historical biogeographic patterns within the genus.

■ MATERIALS AND METHODS

Materials and datasets. — We constructed two separate datasets, one for the basic phylogenetic analysis, and a second for molecular dating plus biogeographic analyses. In both datasets, 11 species of *Lomaridium* were included (Appendix 2). We lacked suitable material of *L. nigrocostatum* and *L. plumieri*, which prohibited their inclusion. For the phylogenetic analysis, we added four closely related species (Gasper & al., 2017) as outgroups: *Brainea insignis* (Hook.) J.Sm., *Lomaria discolor* (G.Forst.) Willd., *Sadleria cyatheoides* Kaulf. and *Woodwardia radicans* (L.) Sm. For the molecular dating and biogeographic analyses, we included a larger set of outgroups that included representatives of all the closest relatives of *Lomaridium*, from all geographic locations in which these closest relatives occur, so that we could accurately reconstruct ancestral events at the base of *Lomaridium* and avoid biases due to improper outgroup sampling. Gasper & al., (2017) found *Lomaridium* to be sister to a large clade comprised of ((Superclade A, Superclade B) *Lomaria*), with the superclades containing four to six genera each. We included all available sequences of *Lomaria* (4 available/6 total species), and sequences from one genus each in Superclades A and B (*Icarus* Gasper & Salino

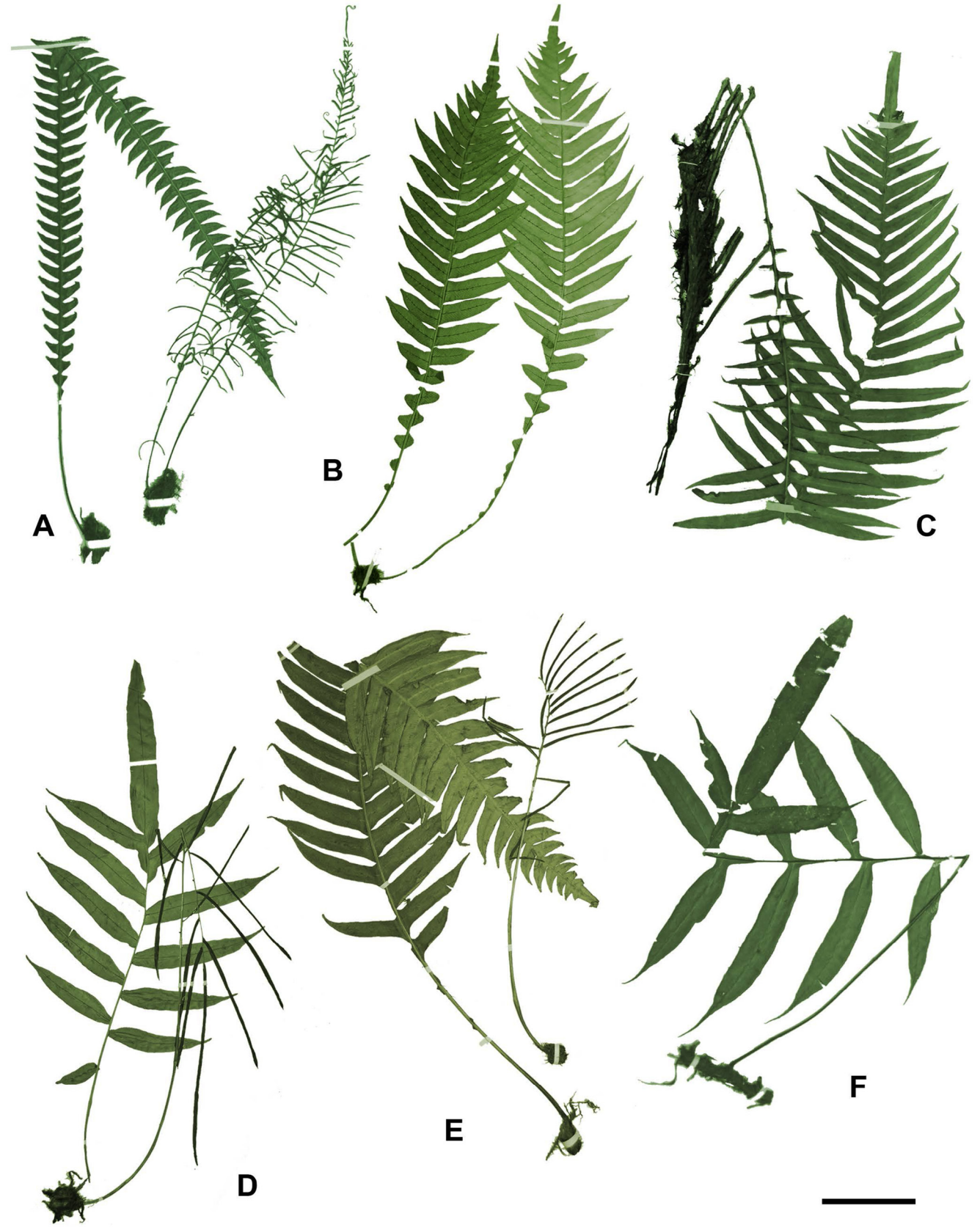


Fig. 1. Representatives of *Lomaridium*. **A,** *L. fragile* (UC 1548973, sub *B. fragile*); **B,** *L. attenuatum* (UC 956249, sub *B. attenuatum*); **C,** *L. angustifolium* (CONC 37734, sub *B. kunthianum*); **D,** *L. simillimum* (UC 1871997, sub *B. simillimum*); **E,** *L. ensiforme* (UC 1525289, sub *B. ensiforme*); **F,** *L. acutum* (UC 1745469, sub *B. acutum*). — Bar = 7 cm in all.

1/1, and *Neoblechnum* Gasper & V.A.O.Dittrich 1/1, respectively). Two clades of three species each are then successively sister to (*Lomaridium* (*Lomaria* (Superclade A, Superclade B))): ((*Cleistoblechnum*, *Blechnopsis*) *Sadleria*), and then ((*Struthiopteris*, *Blechnidium*) *Brainea*). We included all species of these genera for which sequences were available in GenBank, attaining nearly complete sampling: *Blechnidium* T.Moore (1/1), *Blechnopsis* C.Presl (1/2), *Brainea* J.Sm. (1/1), *Cleistoblechnum* Gasper & Salino (1/1), *Sadleria* Kaulf. (5/6), and *Struthiopteris* Scop. (5/5). We also included all four genera and five species of Onocleaceae, the sister family to Blechnaceae, to permit fossil calibration in the molecular dating analysis.

Samples were either collected and maintained in silica gel until DNA extraction, or obtained from herbarium material. Vouchers of the new collections have been included in the MACB herbarium. When necessary, we updated accession names from GenBank to reflect the new classification for Blechnaceae (Gasper & al., 2016).

For the phylogenetic analysis, we constructed a dataset of 59 accessions representing 15 species (Appendix 2). The accessions included partial sequences of three regions of cpDNA: (1) the *trnL* gene and *trnL-trnF* intergenic spacer; (2) *rbcL*; and (3) the *rps4-trnS* intergenic spacer. For the molecular dating analysis, we constructed a dataset of 44 species, each represented by a single accession. In addition to our new sequences for *Lomaridium*, the sampling in this dataset included many more outgroups in Blechnaceae, and these were all accessed from GenBank (Appendix 3). We used only cpDNA, which is maternally inherited in ferns (Vogel & al., 1998), because of our focus on basic phylogenetics and biogeography. While bi-parentally inherited nuclear markers can recover evidence of polyploidy and hybridization if these phenomena have occurred, our goal here was to establish primary relationships between species and to infer historical biogeographic events. Little is known about polyploidy and hybridization in *Lomaridium* (Gasper & al., 2016), and determining whether they have occurred in the genus would be an interesting focus for future studies.

PCR and sequencing. — Total DNA was extracted from dried material (≈20 mg) with a DNeasy Plant Mini Kit (Qiagen, Valencia, California, U.S.A.) following the manufacturer's protocols. PCR was used to amplify three plastid regions that have previously been used in the evaluation of fern species relationships (Li & Lu, 2006; De Groot & al., 2011; Li & al., 2011; Gabriel y Galán & al., 2013).

The PCR reaction protocol was as follows: 5 µl buffer (10×, containing 15 mM $MgCl_2$), 5 µl BSA (2.5 µg/µl), 10 µl sol Q (5×), 3 µl of each primer (5 µM), 1 µl dNTPs (10 mM), 1 U *Taq* polymerase (5 U/µl; HotStarTaqPlus Polymerase, Qiagen) and dH_2O to a final volume of 50 µl with 1–3 µl of DNA template.

The primers used and reaction conditions were the following. For *trnL-trnF*: Fern1/F (Taberlet & al., 1991; Gabriel y Galán & al., 2013); 35 cycles of (1 min 94°C, 1 min 55°C, 1 min 30 s 72°C), followed by 6 min 72°C. For *rps4-trnS*: F/R (Li & Lu, 2006); 38 cycles of (2 min 94°C, 40 s 42°C,

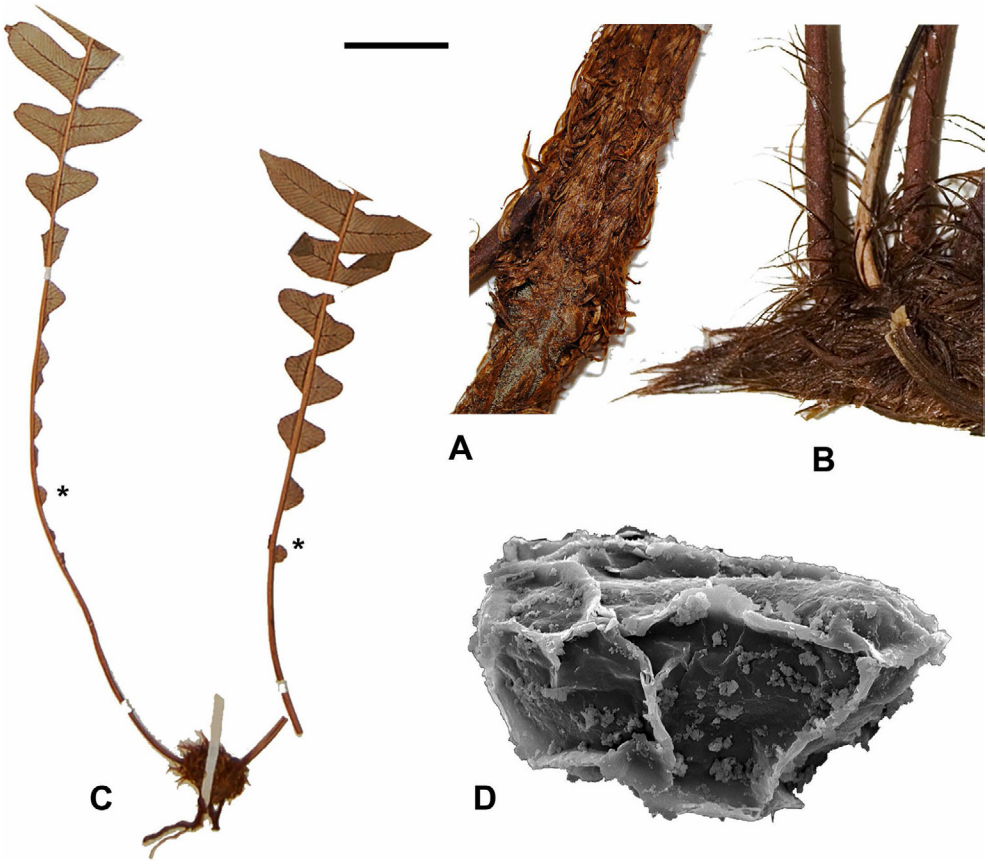


Fig. 2. Morphological features of *Lomaridium*. **A,** Partial portion of the rhizome, with bicolorous scales, *L. acutum* (UC 1793954, sub *B. acutum*); **B,** Partial portion of the rhizome, with bicolorous scales, *L. biforme* (UC 1604665, sub *B. microbasis*); **C,** Petioles and basal part of laminae, with highly reduced, separated lobes (*), *L. attenuatum* (UC 956249, sub *B. attenuatum*); **D,** Spore with low reticulate perispore, *L. fragile* (LIL 409018, sub *B. fragile*). — Bar = 1.5 cm in A & B; 2.5 cm in C; 13 µm in D.

1 min 30 s 72°C), followed by 7 min 72°C. For *rbcL*: 1F/1361R (Schuettpelz & Pryer, 2007): 35 cycles of (45 s 94°C, 1 min 55°C, 2 min 72 °C), followed by 10 min 72°C.

PCR products were checked using 1% agarose gel electrophoresis. After purification (QIAquick PCR Purification kit, Qiagen), samples were sequenced on an ABI3730XL sequencer (Macrogen, Amsterdam, the Netherlands).

Phylogenetic analyses. — We used Geneious R6 (http://www.geneious.com, Kearse & al., 2012) to edit the sequences. Alignments were constructed using the ClustalW v.2.1 algorithm (Larkin & al., 2007), with the following conditions: gap open cost 15 and gap extend cost 6. As chloroplast markers are linked and behave as a single non-recombining marker (Naumann & al., 2011), our three individual markers *trnL-trnF*, *rps4-trnS* and *rbcL* were concatenated in a single dataset that was analysed as a whole. We carried out maximum likelihood (ML) and Bayesian inference (BI) analyses, using models of nucleotide evolution previously identified using jModelTest v.2 (Darriba & al., 2012). ML analyses were conducted with PhyML v.2.2.3 (Guindon & Gascuel, 2003), and BI analyses with MrBayes v.2.0.9 (Ronquist & Huelsenbeck, 2003), using the best models identified for the datasets by jModelTest and the following conditions. For ML: NNI topology search method and a bootstrap analysis for branch support with 500 replicates. For BI: Multiple chains (4), chain length 5,100,000, burn-in length 100,000 and unconstrained branch lengths.

Molecular dating and biogeographic analyses. — We conducted molecular dating analyses using BEAST v.2.4.2. A fossil of *Onoclea sensibilis* L. (Rothwell & Stockey, 1991) was used as the single calibration point in the analysis. A fossil attributed to *Woodwardia* Sm. exists (Collinson, 2001) but was not employed here due to the recent taxonomic changes in Blechnaceae, which make it unclear how this fossil should be placed: it may belong at the base of *Woodwardia* sensu Gasper & al. (2017), the base of subfamily Woodwardioideae, or even the base of all Blechnaceae. Given the more reliable identification of the *Onoclea sensibilis* fossil, we opted to use it as a single calibration point instead. We constrained the node uniting *Onoclea sensibilis* with *Onocleopsis* F.Ballard+*Matteuccia* Tod. to 55.8 mya (million years ago) based on the fossil, using a gamma prior distribution with alpha and beta set to 2.0 and 5.0, respectively, and the offset to the age of the fossil. This centered the bulk of the age distribution for the calibrated node at slightly older than the age of the fossil, and with a tail long enough that the median ages estimated for Onocleaceae in two previous studies (Schuettpelz & Pryer, 2009; Testo & Sundue, 2016) were included in the 95% confidence interval.

We implemented an uncorrelated, lognormal relaxed clock model with a birth-death process tree prior and the best nucleotide substitution model identified for each locus as described above. The analysis was run for 20 million generations, saving trees every 4000 generations and all other parameters every 200 generations. The posterior distribution and estimated sample size (ESS) of all parameters were examined using Tracer v.1.6 (Drummond & Rambaut, 2007), and we determined that the analysis had run for sufficiently long when all ESS values were above 200. We used TreeAnnotator v.2.4.2 (Bouckaert & al., 2014) to combine and summarize a post-burn-in set of trees, compute the 95% highest posterior density intervals (HPD) for all node ages, and generate a maximum clade credibility chronogram for use in ancestral range estimation analyses.

We used BioGeoBEARS (Matzke, 2014) to estimate and compare ancestral ranges under several models: DEC (dispersal-extinction-cladogenesis; Ree & Smith, 2008), DIVA-like (dispersal-vicariance analysis; Yu & al., 2010), and BayArea-like (Landis & al., 2013). Each of these models was tested with and without the "jump dispersal" (j) parameter that is available in BioGeoBEARS, for a total of six models. Likelihood ratio tests were used to identify the model(s) that produced the most likely set of ancestral ranges. The analysis was time-stratified to accommodate changes in proximity of geographic areas over the last 100 million years; for example, the Juan Fernández islands are volcanic in origin and would not have been available as a target for dispersal until within the last 10 million years. We used four time periods in the analysis (100–60 mya, 60–30 mya, 30–10 mya, 10–0 mya). Dispersal parameters between geographic areas in each time slice were based on a survey of relevant studies that have performed similar analyses over similar temporal and spatial scales (e.g., Sessa & al., 2012; Spalink & al., 2016). Ranges of extant taxa were determined from a survey of the literature. We removed the outgroup species belonging to Onocleaceae before running the biogeographic analyses, so that they would not bias the ancestral range estimations at the base of the Blechnaceae ingroup.

■ RESULTS

Phylogenetic relationships in *Lomaridium*. — The combined dataset *trnL-trnF+rsp4-trnS+rbcL* was 2289 nucleotides long. jModelTest identified HKY+G as the best model of evolution for each marker. Maximum likelihood and Bayesian analyses both produced a fully resolved topology. The monophyly of *Lomaridium* was clearly supported, with 100% bootstrap support and a posterior probability of 1.00 (Fig. 3). Almost all major branches within the ingroup were highly supported, ranging from 90% to 100% bootstrap support, and 0.90 to 1.00 posterior probability (Fig. 3).

The molecular dating analysis produced the same topology as the other analyses, and placed the divergence of *Lomaridium* from its sister group, the clade (*Lomaria* (*Icarus filiformis* (A.Cunn.) Gasper & Salino–Superclade A, *Neoblechnum brasiliense* (Desv.) Gasper & V.A.O. Dittrich–Superclade B)) at 63.6 mya (95% HPD is 99.6–34.2 mya) (Fig. 4). Within *Lomaridium*, the first divergence occurred at 41.5 mya (67.2–21.2 mya), and separated *L. contiguum* from the remaining species. The remaining divergence events within the genus occurred over the last ca. 32 million years, with the most recent, between *L. biforme* and *L. simillimum*, at 1.6 mya (5.3 mya to <1000 years ago).

Four clades can be recognized within the ingroup. First, the *Lomaridium contiguum* lineage, which contains the single species *L. contiguum* (Figs. 3, 4). The second clade, supported by a bootstrap value of 99% and a posterior probability of 1.00,

comprises two species, *L. angustifolium* and *L. schottii*, which diverged from each other 18.7 mya (33.8–6.5 mya); their ancestor diverged from the remaining species (except *L. contiguum*) 31.8 mya (52.6–16.7 mya) (Fig. 4). The rest of the species form another large clade with 98% bootstrap support and posterior probability of 1.00 (Fig. 3). Within it, two highly supported branches subtend two additional clades that diverged from one another 23.4 mya (38.2–11.1 mya) (Fig. 4). One is the *L. attenuatum* clade, supported by a bootstrap value of 92% and posterior probability of 0.99, which includes *L. attenuatum*, *L. simillimum* and *L. biforme*, the latter two sister to each other (92% bootstrap value and posterior probability of 0.98) (Fig. 3). The other clade is a larger group of species that we call the *L. fragile* clade, and is supported by 92% bootstrap value and posterior probability of 1.00 (Fig. 3). This clade splits again at 19.5 mya (32.1–8.9 mya) (Fig. 4): one subclade, supported by a bootstrap value of 60% and posterior probability of 0.81, contains *L. fragile* and *L. fuscosquamosum*, and the other, supported by 99% bootstrap value and posterior probability of 1.00, contains *L. acutum*, *L. binervatum* and *L. ensiforme* (Figs. 3, 4).

Biogeography and dispersal of *Lomaridium*. — Molecular dating analyses place the divergence of *Lomaridim* from its sister group at 63.6 mya (99.6–34.2 mya). The DIVALIKE model was the best scoring among the models tested in BioGeoBEARS (LnL = −139.8), and it significantly outperformed the next-best model, DIVALIKE+j (LnL = −146.8, *P*-value = 2.10e-5, d.f. = 1). The model reconstructed the ancestor of *Lomaridium* plus (*Lomaria* (Superclade A, Superclade B)) as having occurred in Australia, with the immediate ancestor of all *Lomaridium* lineages present in both Australia and tropical Central/South America by 41.5 mya (Fig. 4). The next divergence within *Lomaridium* occurred strictly in Central/South America, and five additional dispersal events were reconstructed during the history of the genus (Fig. 4). First, the ancestor of modern *L. contiguum* dispersed from the ancestral range to Micronesia/Polynesia/Melanesia within the last 41.5 myr. Second, the ancestor of *L. schottii* dispersed from Central/South America to the Juan Fernandez Islands within the last 18.7 myr. Third, the ancestor of the *L. attenuatum* clade dispersed to Madagascar by ca. 23.4 mya, with subsequent dispersal to continental Africa by the ancestor of *L. attenuatum* itself within the last 15.4 myr. Finally, the ancestor of *L. binervatum* and *L. acutum* dispersed to the Caribbean region by at least 7.4 mya, where *L. binervatum* remains today (Fig. 4).

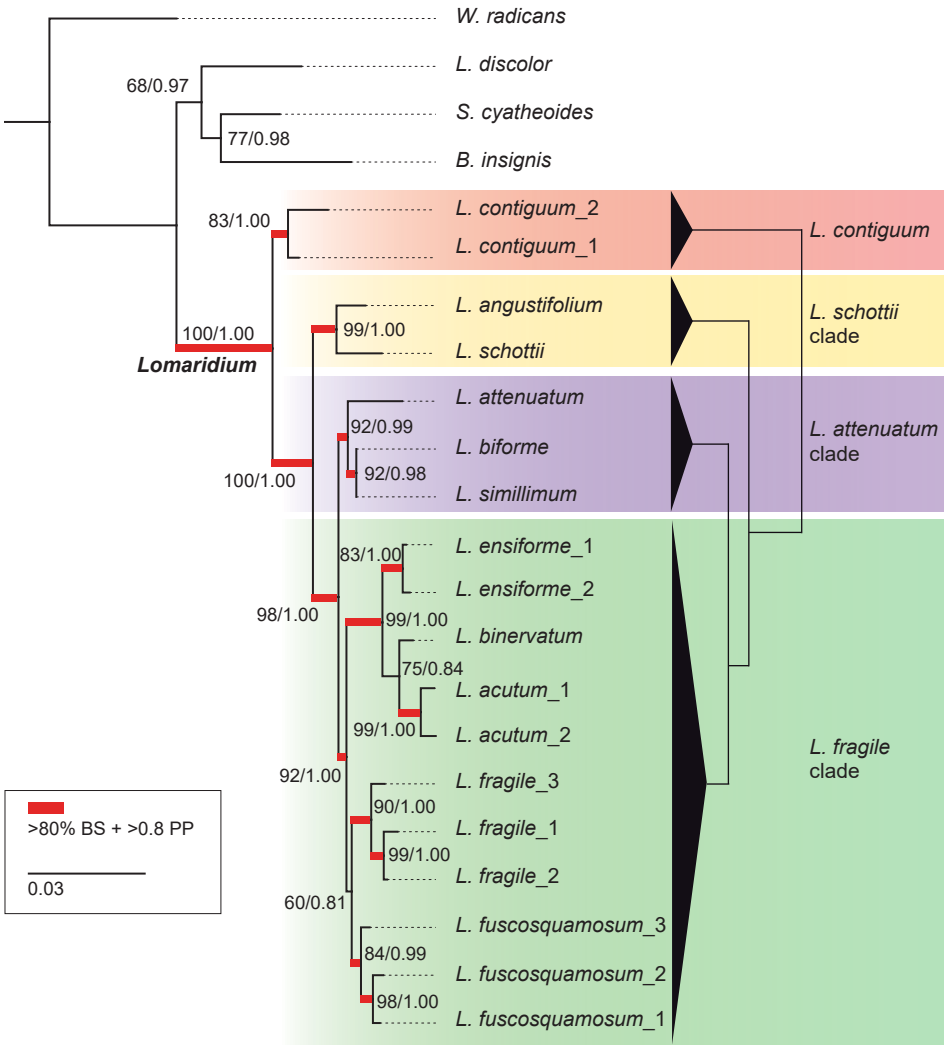


Fig. 3. Phylogram of *Lomaridium* (and outgroups) based on combined *trnL-trnF*, *rps4-trnS* and *rbcL* sequences of 50 accessions. Numbers on branches are bootstrap values and posterior probabilities, respectively. Branches with bootstrap support ≥80 % and posterior probabilities ≥0.80 have been highlighted. Four lineages can be identified in *Lomaridium*: *L. contiguum* and the *L. schottii, L. fragile and L. attenuatum* clades, all supported by high BS and PP values.

■ DISCUSSION

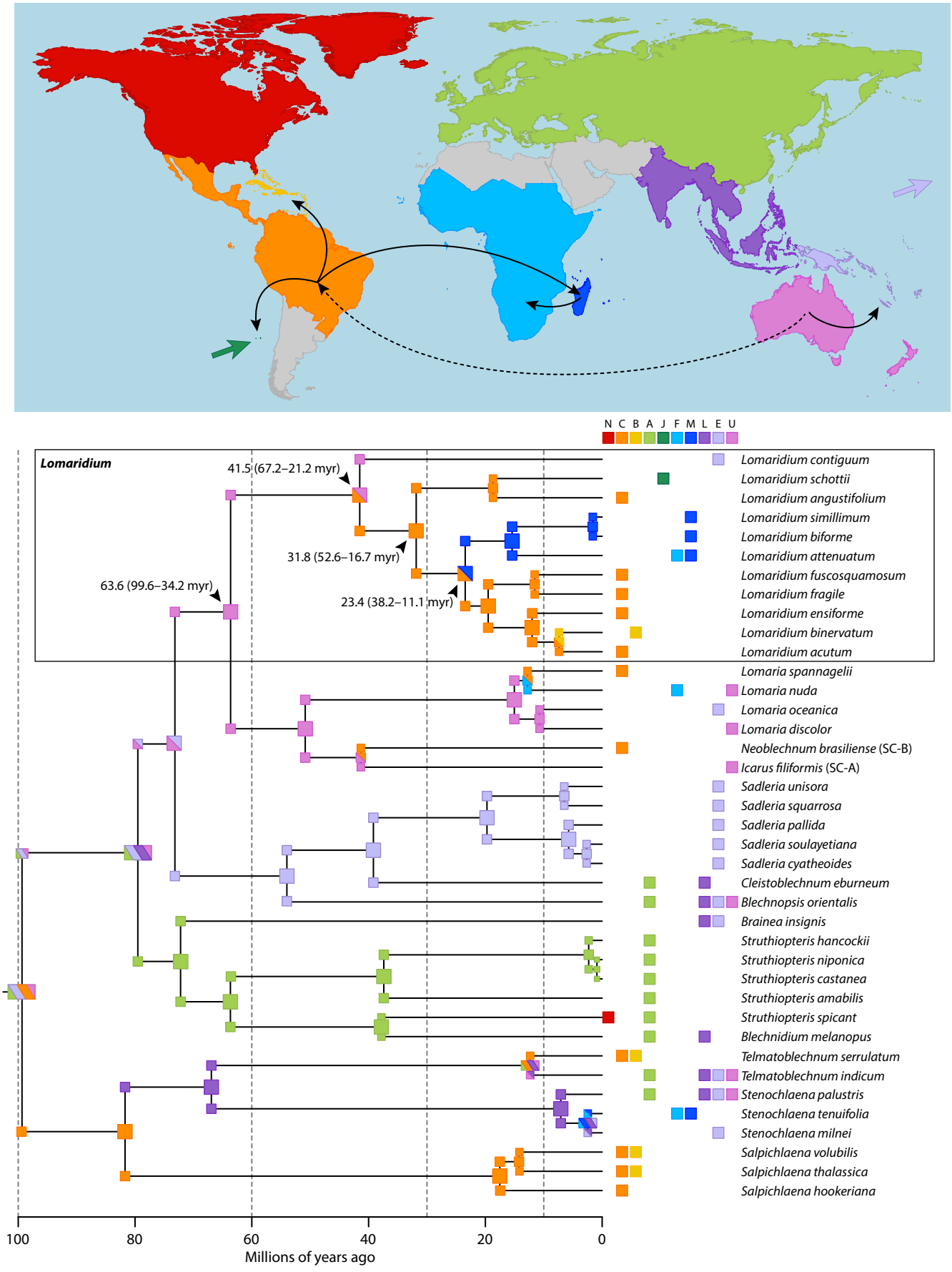
We have studied the phylogeny and historical biogeography of *Lomaridium*, a genus that contains the bulk of the hemiepiphytic species in Blechnaceae. Even though we were not able to include all species considered to belong to the genus, we nonetheless have the largest sampling of the genus of any study to date, and have included at least one representative from each of the geographical regions in which *Lomaridium* occurs. Consequently, we believe we have established the monophyly of the group with greater confidence than in previous studies that were based on much more limited sampling of *Lomaridium* (Gabriel y Galán & al., 2013; Perrie & al., 2014; Gasper & al., 2017).

Our results date the origin of *Lomaridium* at some point during the Paleocene epoch (Paleogene period, Cenozoic era), between ≈63 mya, when a common ancestor with other genera existed, and ≈42 mya, when the first divergence occurred within *Lomaridium* (Fig. 4). The most likely geographical location of the ancestor of all *Lomaridium* is Australia, followed by a dispersal event to Central/South America by 41.5 mya, and subsequent diversification largely in the Neotropics (Fig. 4). The timing agrees with the idea that most of the extant families and genera of leptosporangiate ferns diversified during the Cenozoic (Schuettpelz & Pryer, 2009). Schuettpelz & Pryer (2009) suggested that diversification of ferns in this period was triggered when suitable habitat became available for the evolution of epiphytic and hemiepiphytic forms, i.e., once angiosperm-dominated tropical rainforests with large trees and a year-round humid atmosphere were stabilized as a biome. This habitat is likely to have emerged early in the Cenozoic era (Burnham & Johnson, 2004; Schuettpelz & Pryer, 2009).

During much of its evolutionary history, *Lomaridium* has diversified in the tropical areas of South and Central America, which were treated as one region in our analyses (Fig. 4). One distinct group that arose here is the *L. fragile* clade, which is morphologically well characterised. Our results support the recognition of at least five extant species in this clade: *L. ensiforme*, *L. binervatum*, *L. acutum*, *L. fragile* and *L. fuscosquamosum*, (Figs. 3, 4). The first diversification within this clade seems to have occurred about 19.5 mya (32.0–8.9 mya), separating a lineage that contains *L. fragile* and *L. fuscosquamosum* from another that contains *L. ensiforme*, *L. acutum* and *L. binervatum*, the latter two being sister to each other. *Lomaridium nigrocostatum*, which was not included in our sequencing, appears to be related to this group based on morphology (Rojas-Alvarado, 2006), most likely belonging to the *L. fragile* and *L. fuscosquamosum* subclade. *Lomaridium plumieri* may also be part of the *L. fragile* clade based on its morphology (Dittrich & al., 2017); its most probable relationship is with the group *L. acutum*/*L. ensiforme*/*L. binervatum*. The traditionally recognized entities of the *L. fragile* clade (*L. fragile*, *L. acutum*, *L. binervatum*, *L. ensiforme*) were previously united in the single species *Blechnum binervatum* (Poir.) C.V.Morton & Lellinger, with several subspecies (Morton & Lellinger, 1967; Tryon & Stolze, 1993), which has caused more than a few taxonomic issues. If this former concept should be maintained, a broadly circumscribed, updated *L. binervatum* should also include *L. fuscosquamosum* and possibly *L. nigrocostatum*. This argument seems less reasonable than considering them to be a set of species whose members may have formed several hybrids, as happens for example with *L. fragile* and *L. ensiforme* (Moran, 1995). In addition, our different samples of *L. acutum*, *L. ensiforme*, *L. fragile*, and *L. fuscosquamosum* all form monophyletic species (Fig. 3), thus supporting their recognition as different entities. For some of the species, ecological features also support their separation, such as the different altitudinal preferences of *L. ensiforme* (montane cloud forests) and *L. acutum* (lowland tropical rainforest). If, as we propose, all these species should be maintained as separate named entities, the concept of *L. binervatum* would be restricted to Caribbean plants from the Lesser Antilles and Puerto Rico, which share an apparent morphological apomorphy, concolorous rhizome scales (Morton & Lellinger, 1967). This Caribbean species is inferred to have descended from an ancestor shared with *L. acutum* that occurred in both Central/South America and the Caribbean. These species diverged from each other about 7.4 mya (13.4–2.5 mya; Fig. 4).

From tropical Central and South America, *Lomaridium* has spread and colonized other territories, including Africa, Madagascar, and the Caribbean (the latter region has been coded in our biogeographic analysis separately from Central America; Fig. 4). As the age of the first divergence within the genus is estimated at about 42 myr, it is highly probable that these colonization events have occurred by means of transoceanic spore dispersal, as this time period postdates the separation of the different Gondwanic landmasses. Therefore, vicariance cannot be invoked to explain the current distribution of *Lomaridium*. Long-distance dispersal has been documented for other circum-Antarctic (Parris, 2001) and pantropical fern families and genera (Kato, 1993; Korall & Pryer, 2014), including primarily epiphytes (Schneider & al., 2004a; Hennequin & al., 2010). For the Blechnaceae as a whole, other authors

Fig. 4. Molecular dating and ancestral range reconstruction for *Lomaridium*. The maximum clade credibility chronogram from the BEAST ▶ analysis is shown, with ranges of extant taxa indicated at the tips of the tree, and reconstructed ancestral ranges calculated in BioGeoBEARS under the best-performing model (DIVALIKE) shown at internal nodes. When multi-colored or multi-patterned boxes appear at nodes, that ancestor was assumed to have inhabited a range encompassing multiple areas prior to cladogenesis. Boxes at the corners show ranges immediately following cladogenesis. The map above corresponds to the ranges used, and solid arrows show five dispersal events inferred within *Lomaridium* (the genus is outlined by a box in the phylogeny). The dotted line indicates that the ancestor of all extant *Lomaridium* species was inferred to have a range that included Australia and Central/South America. The vertical dotted lines in the phylogeny indicate the time slices used in the BioGeoBEARS analysis. *Icarus filiformis* and *Neoblechnum brasiliense* represent Superclades A and B of Gasper & al. (2017), respectively, and this is indicated next to their names (SC-A and SC-B).


N C B A J F M L E U
Lomaridium
41.5 (67.2–21.2 myr)
31.8 (52.6–16.7 myr)
63.6 (99.6–34.2 myr)
23.4 (38.2–11.1 myr)
Lomaridium contiguum
Lomaridium schottii
Lomaridium angustifolium
Lomaridium simillimum
Lomaridium biforme
Lomaridium attenuatum
Lomaridium fuscosquamosum
Lomaridium fragile
Lomaridium ensiforme
Lomaridium binervatum
Lomaridium acutum
Lomaria spannagelii
Lomaria nuda
Lomaria oceanica
Lomaria discolor
Neoblechnum brasiliense (SC-B)
Icarus filiformis (SC-A)
Sadleria unisora
Sadleria squarrosa
Sadleria pallida
Sadleria soulayetiana
Sadleria cyatheoides
Cleistoblechnum eburneum
Blechnopsis orientalis
Brainea insignis
Struthiopteris hancockii
Struthiopteris niponica
Struthiopteris castanea
Struthiopteris amabilis
Struthiopteris spicant
Blechnidium melanopus
Telmatoblechnum serrulatum
Telmatoblechnum indicum
Stenochlaena palustris
Stenochlaena tenuifolia
Stenochlaena milnei
Salpichlaena volubilis
Salpichlaena thalassica
Salpichlaena hookeriana
100
80
60
40
20
0
Millions of years ago

have also invoked long-distance dispersal events to explain the distributions of some austral species (Perrie & Brownsey, 2007; Shepherd & al., 2007).

The dispersal of *Lomaridium* appears to have occurred in five different events (Fig. 4). The dispersal of *L. contiguum* from Australia to New Caledonia occurred sometime in the last 41.5 myr, and this also represents the basal-most split in the genus. There are no additional extant taxa closely related to *L. contiguum*, so either additional diversification has not occurred in this area, or other species have arisen and then gone extinct (or possibly escaped detection), leaving *L. contiguum* as an isolated, endemic species.

A second dispersal event involves *Lomaridium schottii*. A divergence event at 31.8 mya separated the *L. schottii* clade, which includes two species, *L. schottii* and *L. angustifolium*, from the remaining species of *Lomaridium*. *Lomaridium angustifolium* has sometimes been considered synonymous with *L. fragile* (Morton & Lellinger, 1967), but our results undoubtedly show the distinctiveness of this entity, which is phylogenetically distant from *L. fragile* in our analyses (Figs. 3, 4). A geographically interesting pattern in this clade is that *L. schottii* is endemic to the Juan Fernández Islands (Chile), a volcanic archipelago with an estimated age of less than 10 myr, with some of the islands being much younger (Rodrigo & Lara, 2014). *Lomaridium angustifolium*, its sister taxon, occurs in the tropical Yungas of South Bolivia and North Argentina. Considering our estimates for the age of the *L. schottii* clade and the geological origin of the Juan Fernández Islands, the most probable evolutionary history involves diversification within mainland South America that was followed by dispersal of the ancestor(s) of *L. schottii* to the Juan Fernández Islands and the emergence there of *L. schottii* itself. This pattern of long-distance dispersal and subsequent speciation is thought to have operated continuously in the Juan Fernández Islands over the last several million years and to have generated a large number of ferns and other vascular plants there, greatly increasing the endemicity level of these islands (Ricci, 1996). We note that there are at least two other examples of Blechnaceae endemic to the Juan Fernández archipelago: *Lomariocycas cycadifolia* (Colla) Gasper & A.R.Sm. and *Cranfillia longicauda* (C.Chr.) Gasper & V.A.O.Dittrich. These two genera also have representatives in mainland South America.

A third dispersal event is estimated to have occurred by about 23.4 mya, in which the ancestor of the *Lomaridium attenuatum* and *L. fragile* clades is inferred to have dispersed from Central/South America to Madagascar. We note however, that an alternative scenario not captured by our analyses seems equally feasible: dispersal to Africa, or Africa plus Madagascar, followed by extinction in Africa as aridity on the continent increased over the last 20 million years. This dispersal event was ultimately followed by the diversification of the *L. attenuatum* clade in Madagascar/Africa, while the *L. fragile* clade descended from an ancestor that remained in the Americas (Fig. 4). The diversification in Madagascar/Africa resulted in the emergence of three extant species: *L. attenuatum*, *L. simillimum*, and *L. biforme*. The latter two are endemic to Madagascar (Rakotondrainibe & al., 2013), and our results show that they are sister species that diverged only very recently, approximately 1.6 mya. *Lomaridium attenuatum* diverged earlier (about 15.4 mya) from the ancestor of these two, and according to our results, spread to tropical and austral areas of the African mainland, and also to other Indian Ocean Islands (Aldasoro & al., 2004). This species is variable in morphology, and some varieties have been proposed, such as var. *giganteum* (*Blechnum attenuatum* var. *giganteum* (Kaulf.) Bonap.), which is described as sometimes having a terrestrial instead of a hemiepiphytic habit (Rakotondrainibe & al., 2013).

The last dispersal event that we infer in *Lomaridium* involves *L. binervatum.* This species is found in the Caribbean, and descended from an ancestor that had arrived in that region by 7.4 mya. Its sister taxon, *L. acutum*, remained in mainland Central/South America.

Several other fern families and genera appear to have trans-Atlantic biogeographical relationships similar to those seen in *Lomaridium*, and with common elements (genera or even species) distributed in both the Americas and Africa. Among these, there is another Blechnaceae species, *Lomariocycas tabularis* (Thunb.) Gasper & A.R.Sm., which has a disjunct distribution in both continents (Moran & Smith, 2001; Rolleri & al., 2013). The biogeographical hypotheses proposed to explain these patterns typically invoke long-dispersal events, mostly from the Neotropics to Africa (Moran & Smith, 2001), which is congruent with our results for *Lomaridium*. Most major diversification within extant ferns has happened far too recently to be explained by Gondwanan vicariance, as discussed above. While it may seem surprising that ferns—with their dust-like spores that are capable of extensive long-distance dispersal—have relatively high levels of geographic structure, ours is not the first study to reach this conclusion. Work on *Dryopteris* Adans. (Sessa & al., 2012), scaly tree ferns (Cyatheaceae) (Korall & Pryer, 2014) and *Diplazium* Sw. (Wei & al., 2015) has also demonstrated substantial geographic structure in ferns, as well as evidence for long-distance dispersal.

Finally, fossil data and historical reconstructions of the Blechnaceae, including our analyses here, place the divergence of subfam. Woodwardioideae (which includes *Woodwardia* Sm., *Anchistea* C.Presl, and *Lorinseria* C.Presl) from the rest of Blechnaceae (subfam. Stenochlaenoideae and subfam. Blechnoideae) in at least the early Paleogene (Collinson, 2001; Cranfill & Kato, 2003; Schneider & al., 2004b), or even as long ago as the Cretaceous (Fig. 4). While we did not include any Woodwardioideae species in our dating analyses, the earliest divergence we recovered within Blechnaceae represents the split between Stenochlaenoideae (*Stenochlaena* J.Sm., *Salpichlaena* J.Sm., *Telmatoblechnum* Perrie & al.) and Blechnoideae (all remaining species in our analysis). This divergence is dated to 99.4 mya, the middle of the Cretaceous (Fig. 4), and the Woodwardioideae are sister to these two groups and so would necessarily have diverged from them before this.

We obtained an age of 128.1 myr (191.1–73.2 myr) for the divergence of Blechnaceae and Onocleaceae (not shown in Fig. 4), which is substantially older than the divergence dates for these two families recovered by Testo & Sundue (2016) or Schuettpelz & Pryer (2009). This may be due in part to

our improved sampling within Onocleaceae. Schuettpelz & Pryer (2009), for example, included only *Onoclea sensibilis* and therefore placed the fossil at the node subtending this taxon plus their sampling of Blechnaceae. This would likely have made ages within Blechnaceae artificially younger, as the crown node of the family would have to be younger than the age of this fossil, 55.8 myr. Given that the dates we obtained are so much older than these other studies, considerable effort should be put towards analysing additional fossils so that more calibration points can be used in future dating analyses of Blechnaceae. The fossil *Woodwardia* discussed above would be an ideal place to begin, and Collinson (2001) lists several other fossils that may belong to Blechnaceae and which could be assessed using the updated classification (Gasper & al., 2016) to place them more confidently within genera or subfamilies of Blechnaceae.

The divergence dates within Blechnaceae are of particular interest for resolving two competing views that have been put forth previously to describe historical movements within the family: Cranfill & Kato (2003) thought that *Woodwardia* (and Onocleaceae, the sister family to Blechnaceae) originated in North America and from there spread towards East Asia and, later, to Europe. Given that the clade (Stenochlaenoideae, Blechnoideae) shares an ancestor with Woodwardioideae, the origin of both should therefore be placed in the northern hemisphere, from which supposedly the different genera spread southward. Today many extant genera and species are austral (Fig. 4). This contrasts with the view of other authors, e.g., Chambers & Farrant (2001), who wrote that:

"Our interpretation of the genus (*Blechnum*) suggests that it is an early group of leptosporangiate ferns (probably late Cretaceous) with a radiate distribution pattern centred on Gondwana, with distinctive but overlapping lines of speciation extending northwards from Antarctica. One of these geographic lines extends through South and Central America to the Caribbean Islands and into the more humid south-eastern areas of North America. Another line extends from southern Africa to central and eastern North Africa. A third line can be traced through some of the subantarctic islands to New Zealand, Tasmania, and the eastern coast of Australia, extending to some of the Pacific Islands of Oceania, and with a branch-line to Malesia."

While our goal in the current study was not to undertake molecular dating or ancestral range estimation analyses for all Blechnaceae, the timing of early diversification events in the family that can be inferred from our analyses suggests that several geographic areas were likely involved in ancestral ranges deep in the family. Further work will be necessary to resolve these two views on the biogeographic history of Blechnaceae as a whole—a northern origin followed by southward expansion, or a southern origin followed by northward expansion—but our results suggest that the early history of the family was complex biogeographically, with extensive long distance dispersal events throughout its history. *Lomaridium* exemplifies this high dispersal capacity, as a genus with only a modest number of species that have reached far-flung regions of the globe following numerous long-distance dispersal events.

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Appendix 1. Nomenclatural combination.

Our results show the distinctiveness of the entity formerly called *Blechnum kunthianum*, which was clearly separated in our topologies from its supposed relative *Lomaridium fragile*. As we consider it a different species, there is a need to give it a new name in *Lomaridium*, which we propose as follows:
Lomaridium angustifolium (Kunth) Vicent & Gabriel y Galán, **comb. nov.** ≡ *Lomaria angustifolia* Kunth in Humboldt & al., Nov. Gen. Sp. 1, ed. qu.: 18. 1816 ('1816') ≡ *Blechnum kunthianum* C.Chr., Index Filic., Suppl. 1906–1912: 16. 1913.

Appendix 2. Source of biological material and molecular data accessions.

Species: sample (if applicable), voucher (location): *trnL-trnF*, *rbcL*, *rps4-trnS*. Accessions refer to GenBank. All accessions have been newly generated for this study except those indicated by *.

Ingroup. ***Lomaridium acutum*** (Desv.) Gasper & V.A.O.Dittrich: sample 1, Peru, *Smith 4451* (UC), KU892709, KU892703, KU892692; sample 2, Ecuador, *A. & L. Fay 3704* (UC), KU892711, KU892706, KU892683. ***Lomaridium angustifolium*** (C.Chr.) Vicent & Gabriel y Galán: Argentina, *C. Prada s.n.* (MA), JQ907373, KU892707, KU892687. ***Lomaridium attenuatum*** (Sw.) Gasper & V.A.O.Dittrich: Reunion Island, *S. Hennequin R67* (BM), –, KF992444*, –. ***Lomaridium biforme*** (Baker) Gasper & V.A.O.Dittrich: Madagascar, *Nakahira & Kato*, unknown voucher, –, AB040561*, –. ***Lomaridium binervatum*** (Poir.) Gasper & V.A.O.Dittrich: Puerto Rico, *Christenhusz & Thomas 3446* (UC), JQ907368, KU892699, KU892689. ***Lomaridium contiguum*** (Mett.) Gasper & V.A.O.Dittrich: sample 1, New Caledonia, *Perrie 2012-31* (WELT), KF975714*, KF975784*, KF975740*; sample 2, New Caledonia, *Munzinger 651* (MO), KU898697*, KU898642*, KU898549*. ***Lomaridium ensiforme*** (Liebm.) Gasper & V.A.O.Dittrich: sample 1, Panama, *Valdespino & Aranda 310* (UC), JQ907371, KU892702, KU892695; sample 2, Costa Rica, *Gabriel y Galán & Puelles 2013-22* (MACB), KU892710, KU892708, KU892693. ***Lomaridium fragile*** (Liebm.) Gasper & V.A.O.Dittrich: sample 1, Costa Rica, *Gabriel y Galán 2008-53* (MACB), JQ907372, KU892704, KU892686; sample 2, Costa Rica, *Gabriel y Galán & A. Rojas 10424* (MACB), KU892712, KU892697, KU892688; sample 3, Panamá, *Salino 15860* (BHCB), KU898695*, KU898640*, KU898582*. ***Lomaridium fuscosquamosum*** (A.Rojas) Gasper & V.A.O.Dittrich: sample 1, Costa Rica, *Gabriel y Galán & A. Rojas 10422* (MACB), KM001898, KU892696, KU892684; sample 2, Costa Rica, *A. Rojas s.n.* (CR), KU892713, KU892701, KU892694; sample 3, Peru, *Van der Werff 16823* (MO), KU898696*, KU898641*, KU898583*. ***Lomaridium schottii*** (Colla) Gasper & V.A.O.Dittrich: Chile, Juan Fernández Islands, *Stuessy & García 1162* (MA), KU892714, KU892698, KU892691. ***Lomaridium simillimum*** (Baker) Gasper & V.A.O.Dittrich: Madagascar, *Nakahira & Kato*, unknown voucher, –, AB040570*, –. — **Outgroup.** ***Brainea insignis*** (Hook.) J.Sm.: Vietnam, *Averyanov 2702* (P), KU898684*, AY137672*, AF533870*. ***Lomaria discolor*** (G.Forst.) Willd.: New Zealand, *Perrie 4015 & Shepherd* (WELT), DQ683382*, KF975786*, KF975742*. ***Sadleria cyatheoides*** Kaulf.: New Zealand (cultivated, original source unknown), *B. Parris s.n.* (WELT), DQ683431*, AB040583*, AF425156*. ***Woodwardia radicans*** (L.) Sm.: Spain, *Gabriel y Galán 122* (MACB), JQ907391*, AY137667*, KU892690.

Appendix 3. Accession information for outgroup taxa included in the molecular dating and biogeographic analyses

Species: *trnL-trnF*, *rbcL*, *rps4-trnS*. Accessions refer to GenBank.

Blechnidium melanopus (Hook.) T.Moore: –, KU898627, –. ***Blechnopsis orientalis*** (L.) C.Presl: KJ398409, KJ398411, KJ398407. ***Brainea insignis*** (Hook.) J.Sm.: KU898684, KU898628, KU898571. ***Cleistoblechnum eburneum*** (Christ) Gasper & V.A.O.Dittrich: –, JN168003, JN168071. ***Icarus filiformis*** (A.Cunn.) Gasper & Salino: see Appendix 1. ***Lomaria discolor*** (J.R.Forst.) Willd.: DQ683382, KF975786, KF975742. ***Lomaria nuda*** (Labill.) Willd.: KJ170848, KJ170821, KJ170794. ***Lomaria oceanica*** (Rosenst.) Gasper & V.A.O.Dittrich: KF975724, KF975804, KF975760. ***Lomaria spannagelii*** (Rosenst.) Gasper & V.A.O.Dittrich: KU898698, KU898643, KU898584. ***Matteuccia struthiopteris*** (L.) Tod.: –, AB232415, AF425158. ***Neoblechnum brasiliense*** (Desv.) Gasper & V.A.O.Dittrich: JQ907369, AB040545, –. ***Onoclea sensibilis*** L.: –, JF832076, AF425159. ***Onocleopsis hintonii*** F.Ballard: –, JF832077, AF425160. ***Pentarhizidium intermedium*** (C.Chr.) Hayata: KC254426, KC254354, KC254505. ***Pentarhizidium orientale*** (Hook.) Hayata: –, JF832079, –. ***Sadleria cyatheoides*** Kaulf.: –, KJ716413, –. ***Sadleria pallida*** Hook. & Arn.: –, AB040588, –. ***Sadleria souleyetiana*** (Gaudich.) T.Moore: –, AB040591, –. ***Sadleria squarrosa*** (Gaudich.) T.Moore: –, AB040592, –. ***Sadleria unisora*** (Baker) W.J.Rob.: –, AB040593, –. ***Salpichlaena hookeriana*** (Kuntze) Alston: –, KJ628825, –. ***Salpichlaena thalassica*** Grayum & R.C.Moran: KU898713, KU898659, KU898600. ***Salpichlaena volubilis*** (Kaulf.) J.Sm.: KU898714, KU898660, –. ***Stenochlaena milnei*** Underw.: –, AF425104, AF425157. ***Stenochlaena palustris*** (Burm.f.) Bedd.: KJ170856, KJ170829, KJ170802. ***Stenochlaena tenuifolia*** (Desv.) T.Moore: –, EF463163, –. ***Struthiopteris amabilis*** (Makino) Ching: –, AB575050, –. ***Struthiopteris castanea*** (Makino) Nakai: –, AB575051, –. ***Struthiopteris hancockii*** (Hance) Tagawa: –, AB575052, –. ***Struthiopteris niponica*** (Kunze) Nakai: –, AB575053, –. ***Struthiopteris spicant*** (L.) Weiss: JQ907386, HQ676498, –. ***Telmatoblechnum indicum*** (Burm.f.) Perrie, D.J.Ohlsen & Brownsey: KJ170857, KJ170830, KJ170803. ***Telmatoblechnum serrulatum*** (Rich.) Perrie, D.J.Ohlsen & Brownsey: KU898716, KU898662, KU898602.