



First Miocene fossils of Vivianiaceae shed new light on phylogeny, divergence times, and historical biogeography of Geraniales

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Received 2 December 2011; revised 23 March 2012; accepted for publication 23 March 2012

The origin of Geraniales (approximately 900 species in three families: Geraniaceae, Melianthaceae, and Vivianiaceae) is traced back to the Cretaceous of Gondwana, yet their geotemporal history is largely unknown because of a limited fossil record and incomplete phylogenies. In the present study, we provide the first fossil record of Vivianiaceae and a highly resolved molecular phylogeny for all extant Geraniales genera. Our results support the hypothesis that five (instead of three) families should be recognized in the order Geraniales: Francoaceae A. Juss. (*Francoa*, *Greyia*, *Tetilla*), Geraniaceae Juss. (*Erodium*, *Geranium*, *Monsonia*, *Pelargonium*), Hypseocharitaceae Wedd. (monogeneric), Melianthaceae Horan. (*Bersama*, *Melianthus*), and Vivianiaceae Klotzsch (*Balbisia*, *Rhynchotheca*, *Viviania*). The four major lineages (i.e. Geraniaceae, Francoaceae + Melianthaceae, Hypseocharitaceae, Vivianiaceae) all originated within a narrow time frame during the Eocene (36.9–49.9 Mya) based on the five fossil calibration points. The divergence of most of the extant genera occurred much later, from the Miocene onwards. The South American–South African disjunction in Francoaceae apparently goes back to long distance dispersal with an estimated divergence time of the lineages in the Middle Miocene [11.2 (5.9–17.7) Mya]. Diversification in *Melianthus* appears to be much more recent than previously assumed [starting approximately 3.4 (1.9–5.2) Mya rather than approximately 8–20 Mya]. However, divergence of the Andean *Hypseocharis* lineage [36.9 (31.9–42.8) Mya] significantly predates the main Andean uplift: Current distributions likely go back to northward migrations and subsequent extinctions in Patagonia. Similarly, *Rhynchotheca*, *Balbisia*, and *Viviania* have a current southern distribution limit > 10°N of the fossil finds, indicating a massive northward displacement. The present evidence suggests that niche conservatism likely played a major role in the historical biogeography of Geraniales. © 2012 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2012, **107**, 67–85.

ADDITIONAL KEYWORDS: fossil pollen grains – molecular clock – paleobiogeography – time estimates.

INTRODUCTION

Geraniales are included within the rosoid clade (approximately 70 000 species), one of the largest but least-resolved clades of angiosperms (APG III, 2009).

Although Geraniales likely originated during the Cretaceous (Wang *et al.*, 2009), only few fossil remains have been discovered to date, leaving the chronology of divergence events and the historical biogeography of this order largely unknown. The delimitation and circumscription of Geraniales has changed considerably over the years, although molecular studies have provided a progressively more refined view (Chase *et al.*, 1993; Price & Palmer, 1993; Savolainen *et al.*,

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Table 1. Overview over current Geraniales classification and distribution

Family	Genera	Number of species	Distribution
Geraniaceae	<i>Hypseocharis</i>	6	Argentina to Peru
Geraniaceae	<i>Erodium</i>	80	subcosmopolitan
Geraniaceae	<i>Geranium</i>	430	subcosmopolitan
Geraniaceae	<i>Monsonia</i>	40	Africa (+ Madagascar, south-west Asia)
Geraniaceae	<i>Pelargonium</i>	280	Africa (+ Madagascar, south-west Asia, Australia, New Zealand)
Melianthaceae	<i>Bersama</i>	8	Africa
Melianthaceae	<i>Melianthus</i>	8	Southern Africa
Melianthaceae	<i>Greyia</i>	3	Southern Africa
Melianthaceae	<i>Francoa</i>	1 or 2	Chile
Melianthaceae	<i>Tetilla</i>	1	Chile
Vivianiaceae	<i>Balbisia</i>	8	Argentina/Chile to Peru
Vivianiaceae	<i>Wendtia</i>	3	Argentina/Chile
Vivianiaceae	<i>Rhynchotheca</i>	1	Peru, Ecuador
Vivianiaceae	<i>Viviania</i>	6	Chile to Brazil

Families and genera *sensu* APG III (2009). Species numbers and distribution from Albers & van der Walt (2007), Linder (2007) and Weigend (2007).

2000; Wang *et al.*, 2009). Geraniales in their most recent circumscription comprise the families Geraniaceae, Melianthaceae, and Vivianiaceae (APG III, 2009) and are tentatively accepted as sister group to the Myrtales (Zhu *et al.*, 2007; Wang *et al.*, 2009; APG III, 2009; Qiu *et al.*, 2010). Geraniales are termed 'a poorly known order' (APG III, 2009), and especially Melianthaceae are very incompletely understood. APG III (2009) includes *Bersama*, *Greyia*, and *Melianthus* in Melianthaceae, plus 'monogeneric' Francoaceae (i.e. *Francoa*). However, Francoaceae also includes the Patagonian genus *Tetilla* DC. (Linder, 2007), not mentioned in APG III (2009). Several phylogenetic studies of individual genera have been published, most notably *Erodium* and *Geranium* (Fiz *et al.*, 2006, 2008), *Melianthus* (Linder *et al.*, 2006), *Monsonia* (Touloumenidou, Bakker & Albers, 2007), and *Pelargonium* (Bakker *et al.*, 2000; Bakker, Breman & Merckx, 2006). Nevertheless, phylogenetic relationships within Geraniales are still poorly resolved, especially with regard to the incompletely known South American taxa, for which only incomplete (*Hypseocharis*, most *Viviania* and *Balbisia*) or no (*Tetilla*) molecular data have been published. Overall, the order comprises approximately 900 species in a total of 13 genera (Table 1) but there has been no attempt made so far at resolving relationships within Geraniales based on a complete sampling of genera.

Geraniales have a clear centre of distribution in the Southern Hemisphere; eight of its genera are restricted to the southern part of South America or Africa. Another three genera have their centres of

diversity in South America or Africa, whereas only two genera (*Geranium*, *Erodium*) are more diverse in the northern than in the southern hemisphere. The South American genera *Francoa* and *Tetilla* are restricted to the western Patagonian forests, a region that is home to several ancient and distinct lineages such as *Gomortega* and *Atherospermataceae* (Renner & Chanderbali, 2000; Renner, Foreman & Murray, 2000). *Hypseocharis*, universally placed as the sister group of Geraniaceae s.s. and often considered as separate family Hypseocharitaceae Wedd. (Fiz *et al.*, 2008), is essentially high Andean, ranging from central Argentina to northern Peru (Slanis & Grau, 2001) at elevations of > 2000 m in the southern part of its range and at elevations of up to 4000 m in the northern part.

Vivianiaceae are essentially southern South American: *Viviania s.l.* (Weigend, 2007) is largely restricted to central and southern Chile, where the genus is most diverse in the Mediterranean climate zone; only a single species is found in southern Brazil and adjacent Uruguay, Paraguay, and Argentina. *Viviania* is restricted to at least seasonally moist habitats, with some species in permanently wet forests (e.g. *Viviania elegans*; Lefor, 1975; Jørgensen & Yanez León, 1999; Weigend, 2005). *Balbisia s.l.* (Weigend, 2005) is largely restricted to semi-arid to arid habitats on both sides of the Andes but has a single species ranging into the moister parts of the Patagonian steppe (*Balbisia gracilis*). Several species (*Balbisia meyeniana*, *Balbisia verticillata*, *Balbisia microphylla*, *Balbisia stichkinii*) are restricted to the extremely arid northern Atacama desert in northern Chile and southern

Peru (Weigend, 2011). All South American genera of Geraniales thus include at least some species in the south temperate and Mediterranean zones of Chile and Argentina. Only the morphologically distinct *Rhynchotheca spinosa* represents an exception being restricted to moist scrub forests and the margins of cloud forests in the Central and Northern Andes at elevations of approximately 3000–3500 m.

South American Geraniales show a highly stratified distribution pattern with extant lineages restricted to the Mediterranean, warm-temperate, subtropical, and Andean regions. This invites a study of their geographical hierarchies and a comparison of divergence time estimates of lineages to the estimated ages of their current habitats. Several recent studies have attempted to correlate ages of lineages with the ages of their respective habitats (Luebert *et al.*, 2011). Others have provided new insights into the geological processes and geotemporal trajectory of Andean uplift (Hoorn *et al.*, 2010), confirming that elevations of > 2000 m in the Central Andes have become available approximately 10 Mya. The recent origin and consequently ongoing speciation in high Andean habitats is reflected in the consequently ongoing and rapid speciation in animals and plants (Kadereit & von Hagen, 2003; Hughes & Eastwood, 2006; Weir, 2006). So far, phylogenetic reconstructions of Andean plant groups have mostly concentrated on holarctic floristic elements, which entered the Andean chain from the North (e.g. *Gentianella*: von Hagen & Kadereit, 2001; *Ribes*: Weigend, Motley & Mohr, 2002; *Halenia*: Kadereit & von Hagen, 2003; *Valeriana*: Bell & Donoghue, 2005; *Lupinus*: Hughes & Eastwood, 2006). These evidently play an important role, although some recent studies also show that southern elements may likewise contribute to the present-day composition of the Andean flora (*Malesherbia*: Gengler-Nowack, 2002; *Chaetanthera*: Hershkovitz *et al.*, 2006; *Paranepheliinae*: Soejima *et al.*, 2008; *Heliotropium*: Luebert *et al.*, 2011). The present study investigates the phylogeny and historical biogeography of South American Geraniales, another group with a centre of diversity in southern South America but several high Andean representatives towards the Equator.

The stem node of Geraniales has been estimated to be 83–89 Mya (Anderson, Bremer & Friis, 2005) or 99–109 Mya (Wang *et al.*, 2009), and the crown node to be 80–86 Mya (Anderson *et al.*, 2005) or 88–101 Mya (Wang *et al.*, 2009). However, the fossil record of Geraniales is poor and largely restricted to Geraniaceae, and options for internal calibration in dating studies are consequently limited. Fossil pollen from the Late Miocene of Spain assigned to subgroups of *Erodium* and *Geranium* have been used to estimate divergence times (Bakker *et al.*, 2004; Fiz *et al.*, 2006;

Fiz-Palacios *et al.*, 2010). This evidence consistently points to a diversification in the Late Miocene to Pliocene for all four genera. Increasing aridity, establishment of winter-rainfall regimes, and dispersal into more inclement climates are invoked as causes of these diversification events (Fiz *et al.*, 2008; Fiz-Palacios *et al.*, 2010).

In the present study, we report the discovery of novel, well-preserved Geraniales fossil pollen from Patagonia (southern Argentina). Pollen morphology of Geraniaceae and Vivianiaceae has been intensively but not comprehensively studied (Erdtman, 1952; Bortenschlager, 1967; Heusser, 1971; Lefor, 1975; Markgraf & D'Antoni, 1978). The unusual pantoporate pollen in Vivianiaceae provides a notable diagnostic apomorphy for this family compared to the other families in the Geraniales, which consistently have triaperturate pollen. The occurrence of highly characteristic pantoporate pollen in Vivianiaceae makes their recognition easy (Borsch & Barthlott, 1998). Their high degree of diagnostic micromorphological diversification further permits assignment of these fossil pollen to individual lineages (i.e. species or species groups).

Recent studies by one of us (L.P.) led to the discovery of fossil pollen that can be clearly assigned to Vivianiaceae. The present study illustrates the fossil pollen grains and places them in the context of extant Vivianiaceae. A more evenly and densely sampled phylogeny of the genera of the order Geraniales is presented, with a particular focus on enhanced sampling of Vivianiaceae, which is the most poorly known group in the order. Based on these new fossils and a more complete phylogeny, we attempt to estimate divergence times for lineages within Vivianiaceae and other subgroups of Geraniales to shed light on geotemporal patterns of diversification across Geraniales as a whole.

MATERIAL AND METHODS

FOSSIL DATING AND CALIBRATION

Most of the fossil Geraniales-bearing strata were dated by means of magnetostratigraphy, stratigraphic correlation, or biostratigraphy. Slides containing fossil specimens are housed in the palynological collection of the Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia': BAPal 6011; BAPal 6017; BaPal ex CIRGEO 940; BaPal ex CIRGEO 955a. Coordinates shown in figure explanations of the light microscopy (LM)-illustrated fossil specimens refer to England Finder for accurately providing the position of an area of interest on a specimen slide. Figure 1 shows the fossil localities in relation to the distribution of the extant representatives of the families.

For purposes of morphological comparison, pollen grains of 28 extant Vivianiaceae species were

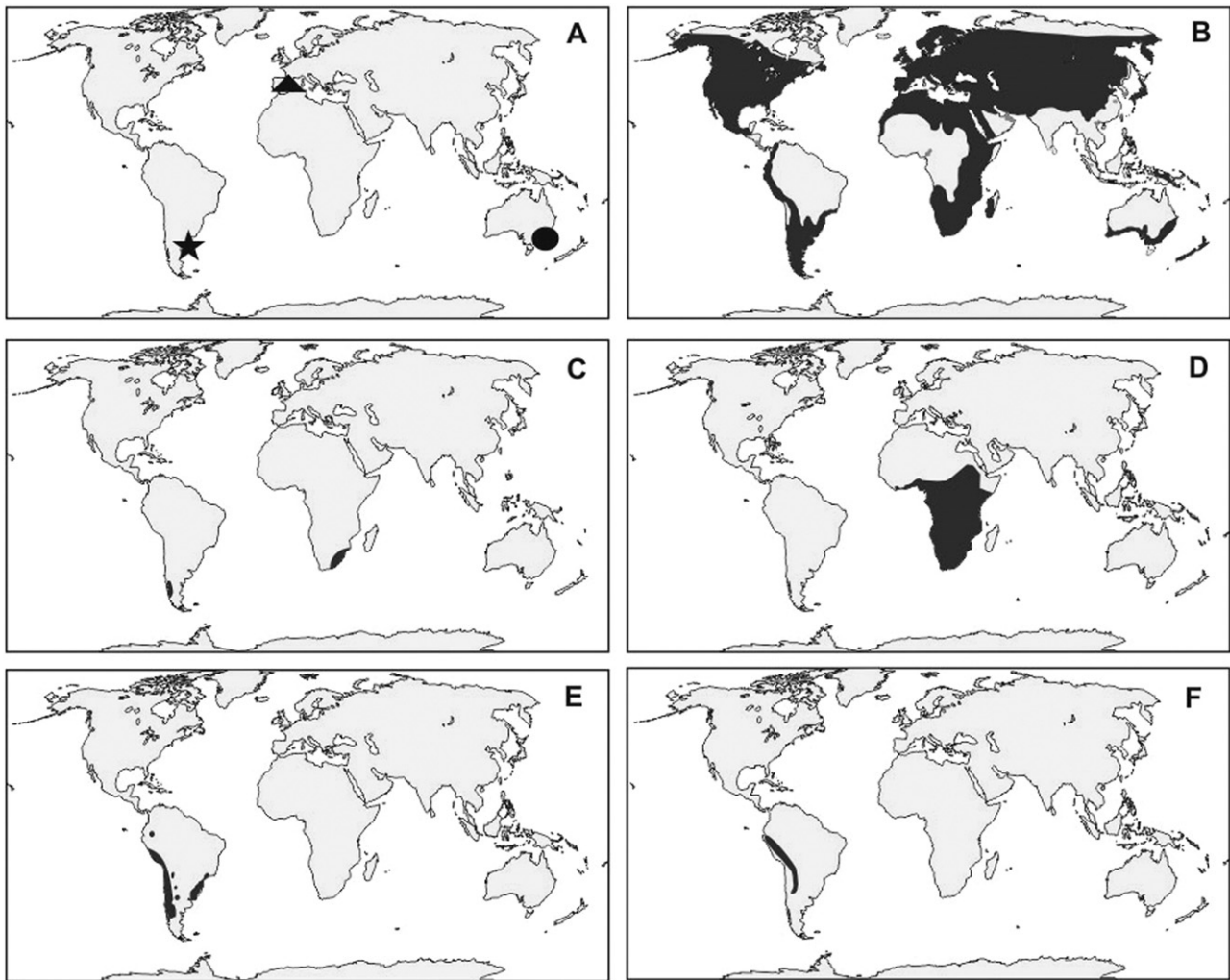


Figure 1. Global maps showing fossil and extant Geraniales distribution. A, fossil-bearing localities. Circle, *Pelargonium* type from the Oligocene of Australia. Triangle: *Erodium* subg. *Barbata* and *Geranium* subg. *Robertium* from the Late Miocene of Spain. Star: *Viviania marifolia* type, *Viviania albiflora* type, *Balbisia* sect. *Wendtia* type, *Balbisia* type and *Rhynchotheca* type from the Miocene of eastern Patagonia (Argentina). B, C, D, E, F, extant distribution of the (B) Geraniaceae, (C) Francoaceae, (D) Melianthaceae, (E) Vivianiaceae, and (F) Hypseocharitaceae.

obtained (for a list of specimens examined, see the Appendix, Doc. A1), representing *Balbisia*, *Viviania*, and *Rhynchotheca* (*sensu* the generic circumscriptions of Weigend, 2007). Recent pollen samples were acetolysed according to the technique of Erdtman (1952). For LM, pollen slides were prepared by mounting pollen directly in glycerol jelly. Size measurements were based on 20 pollen grains per sample. For scanning electron microscopy (SEM), samples were mounted on aluminium stubs. After sputter coating, pollen grains were observed and photographed with a LEO VP 430 SEM at 15 kV. Descriptive terminology of the pollen is carried out *sensu* Borsch & Barthlott (1998) and Punt *et al.* (2007). A list of voucher specimens for pollen samples is given in the Appendix (Table A1).

For dating, a total of eight different Geraniales fossil pollen types were considered in the present study (Figs 2, 3; Table 2), including five newly-discovered fossils of Vivianiaceae (for a detailed description, see the Supporting information, Appendix S1). The absolute time used to calibrate nodes of the phylogenetic tree was derived from the age of the upper boundary of the narrowest stratigraphic interval, to which the oldest fossil species was assigned (*sensu* Magallón & Castillo, 2009). *Tricolporopollenites pelargoniooides* is the oldest morphotype assigned to Geraniales and unique to *Pelargonium* of the Geraniaceae (Martin, 1973; Müller, 1981). This fossil species is consistently recorded from the Oligocene to the Pliocene (28.4 ± 0.1 Mya) of Australia (Macphail, 1999) and was used as constraint for the stem node of

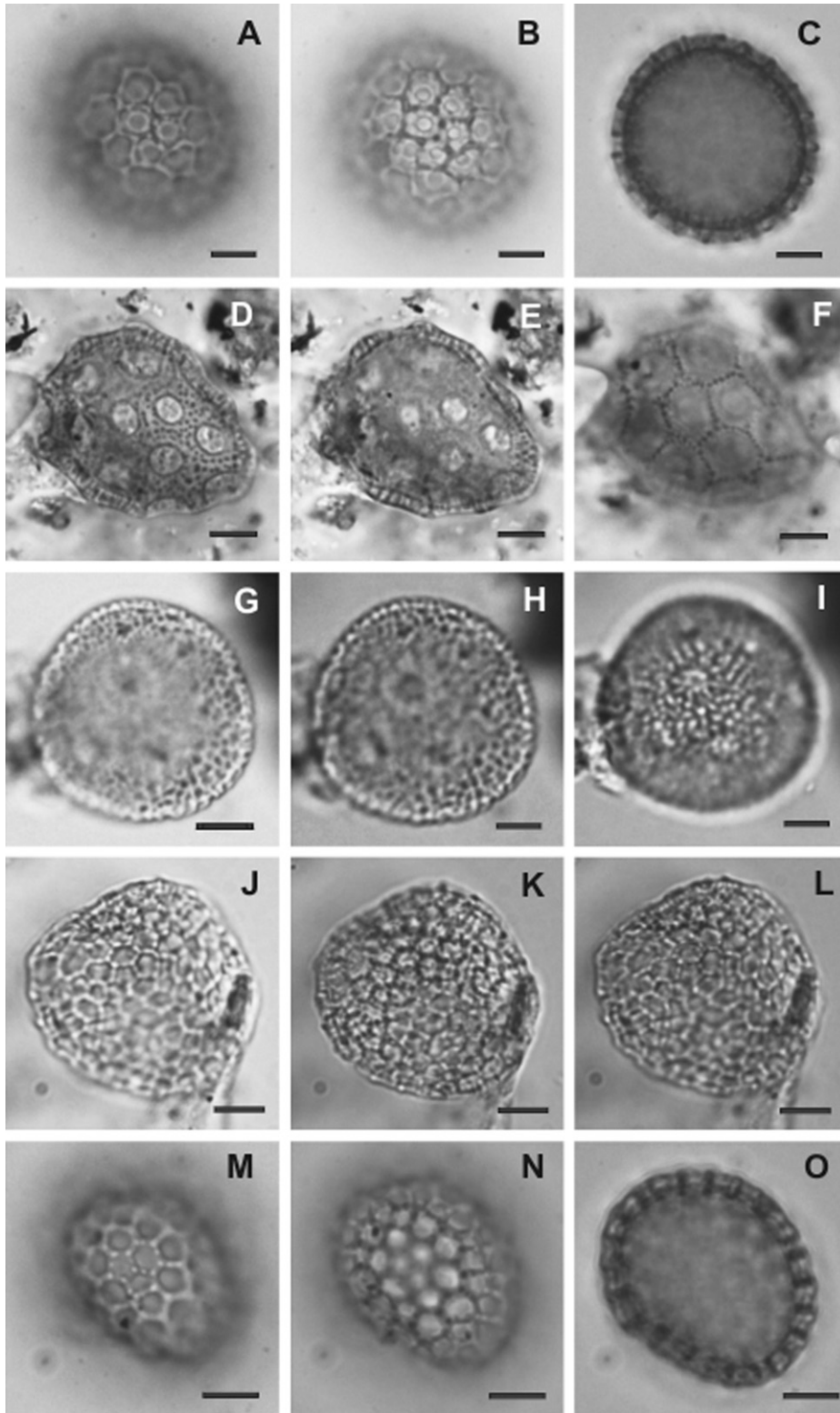


Figure 2. Fossil pollen grains assigned to Vivianiaceae (light micrographs). Each taxon is followed by sample catalogue numbers. Stage coordinates refer to the England finder (in parenthesis). Scale bar = 5 μ m. A, B, C, *Viviania marifolia* type BAPal 6017 (G53/G54). D, E, F, *Viviania albiflora* type BAPal 6011 (E39/E40). G, H, I, *Rhynchotheca* type BaPal ex CIRGEO 940 (G37-1). J, K, L, *Balbisia* sect. *Wendtia* type BaPal ex CIRGEO 955a (O31). M, N, O, *Balbisia* sect. *Balbisia* type BAPal 6013 (Q36-4).

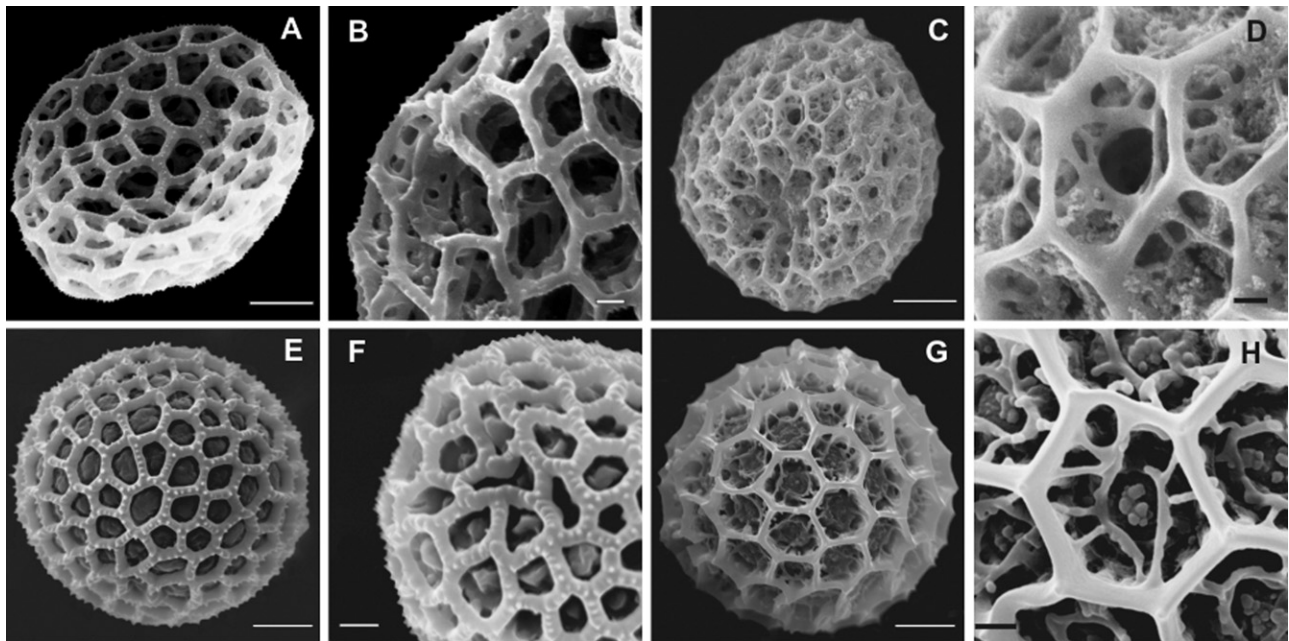


Figure 3. Scanning electron micrographs of the fossil pollen grains (A–D) and their closest living morphological analogues (E–H). A, general view of *Balbisia* sect. *Wendtia* type. B, details of *Balbisia* sect. *Wendtia* type showing the incomplete microechinate tectum. C, D, *Viviania marifolia* type. C, general view of the slightly corroded specimen. D, details of the specimen in (C) showing rodlet-like elements connecting distal and proximal parts of the mesoporia. E, *Balbisia calycina* Sleumer 246. F, *Balbisia calycina* Mulgura de Romero 4119. G, *Viviania ovata* Boelcke 13740. H, *Viviania marifolia* Ackermann 543. Scale bar = 5 µm in (A), (C), (E), and (G); 1 µm in (B), (D), (F), and (H).

Table 2. Fossil pollen record of Geraniales

Fossil taxon	Minimum age (Mya)	Assigned to modern group	Locality	Reference
<i>Tricolporopollenites pelargonioides</i> Martin	28.4 ± 0.1	<i>Pelargonium</i> type	Australia	Martin (1973); Macphail (1999)
<i>Balbisia</i> sect. <i>Wendtia</i> type	15.97 ± 0.05	<i>Balbisia aphanifolia</i> , <i>Balbisia calycina</i> , <i>Balbisia gracilis</i>	Patagonia (Chenque Formation)	Present study
<i>Rhynchotheca</i> type	15.97 ± 0.05	<i>Rhynchotheca spinosa</i>	Patagonia (Chenque Formation)	Present study
<i>Balbisia</i> sect. <i>Balbisia</i> type	10 ± 0.3	<i>Balbisia weberbaueri</i> , <i>Balbisia microphylla</i> , <i>Balbisia miniata</i> , <i>Balbisia peduncularis</i>	Patagonia (Puerto Madryn Formation)	Present study
<i>Viviania marifolia</i> type	10 ± 0.3	<i>Viviania crenata</i> , <i>Viviania marifolia</i> , <i>Viviania ovata</i>	Patagonia (Puerto Madryn Formation)	Present study
<i>Viviania albiflora</i> type	10 ± 0.3	<i>Viviania albiflora</i>	Patagonia (Puerto Madryn Formation)	Present study
<i>Erodium</i> sp.	7.246 ± 0.005	<i>Erodium</i> subg. <i>Barbata</i>	Spain	Van Campo (1989); Fiz <i>et al.</i> (2008)
<i>Geranium</i> cf. <i>lucidum</i>	7.246 ± 0.005	<i>Geranium</i> subg. <i>Robertium</i>	Spain	Van Campo (1989); Fiz <i>et al.</i> (2008)

Pelargonium. Other fossil types assigned to Geraniaceae are *Erodium* subg. *Barbata* and *Geranium* subg. *Robertium*, recorded from the Late Miocene of Spain (7.246 ± 0.005 Mya; Van Campo, 1989; Fiz

et al., 2008). They were used as crown node constraints for *Geranium*, respectively, *Erodium*. The most complete fossil assemblage of Geraniales comes from the Miocene of eastern Patagonia (Argentina),

representing the first record of Vivianiaceae. These are *Balbisia* sect. *Wendtia* type and *Rhynchotheca* type from the early Middle Miocene (15.97 ± 0.05 Mya) and *Balbisia* sect. *Balbisia* type, *Viviania marifolia* type, and *Viviania albiflora* type from the Late Miocene (10 ± 0.3 Mya) Scasso *et al.* (2001). The *Balbisia* sect. *Wendtia* type was selected as fossil constraint for the *Balbisia* crown node, representing one of the two extant lineages (sect. *Wendtia*). The two *Viviania* type fossils were used as crown node constraints for *Viviania*, representing both of the extant lineages. *Balbisia* sect. *Balbisia* type was not used because it is considerably younger than the other crown node fossil of *Balbisia* (*Wendtia* type). The *Rhynchotheca* type was not used either as fossil constraint because it represents a monotypic genus and could not make a sensible contribution to the dating effort.

MOLECULAR ANALYSIS

All genera of Geraniales were included in the phylogenetic analysis (the currently recognized families and genera are summarized in Table 1). For the larger genera *Erodium*, *Geranium*, and *Pelargonium*, the major lineages retrieved in published phylogenetic studies were used to represent their diversity (Bakker *et al.*, 2000, 2006; Fiz *et al.*, 2006, 2008). Sequences of *Greyia*, *Hypseocharis*, and all Vivianiaceae were newly generated for this study, and those of *Bersama*, *Erodium*, *Geranium*, *Melianthus*, and *Pelargonium*, plus outgroup taxa, were mostly obtained from GenBank (vouchers and GenBank numbers are listed in the Appendix, Table A1). The final data matrix comprised 63 accessions corresponding to 57 species. In two cases, doubtful segregates were included (*Balbisia reynoldsii* as doubtfully distinct from *B. gracilis* as well as *Francoa sonchifolia* as doubtfully distinct from *Francoa appendiculata*). Based on recent studies (Zhu *et al.*, 2007; Wang *et al.*, 2009; APG III, 2009; Qiu *et al.*, 2010), three representatives of Myrtales were used as outgroups. In several cases, especially in *Balbisia* and *Viviania*, we failed to sequence the *trnL-trnF* spacer (*trnL-F*), so that the phylogeny includes nine taxa with missing data for *trnL-F*. The final matrix included 54 sequences of *trnL-F* and 63 sequences of the Internal Transcribed Spacer (ITS) region. For the dated phylogeny, only the 54 accessions sequenced for both loci were used.

DNA extraction, polymerase chain reaction, purification, and sequencing followed standard protocols (Gottschling & Hilger, 2001). The same primers were used for amplification and sequencing. The *trnL-F* sequences were amplified with primers C and F *sensu* Taberlet *et al.* (1991), the primers P5 and P4 of White *et al.* (1990) were used for ITS. Cycle sequencing was

carried out with the BigDye Terminator, version 1.1 Cycle Sequencing Kit (Perkin Elmer) on an Applied Biosystems 3130xl Genetic Analyser.

The initial sequence data were edited using CHROMASPRO, version 1.33 (Technelysium Pty Ltd, 2003–2005). The sequences were separately aligned in two partitions by using MAFFT, version 6.624b (Kato *et al.*, 2005; Kato & Toh, 2008; <http://mafft.cbrc.jp/alignment/software/index.html>) and were concatenated afterwards. Phylogenetic analyses were run using resources of the Leibniz Rechenzentrum (LRZ, Munich; linux cluster HLRB-II) and of the SGI system (Zuse Institute Berlin, ZIB) of the North German High Performance Computer (HLRN). For Maximum Likelihood (ML), RAXML, version 7.0.4 (Stamatakis, 2006; <http://sco.h-its.org/exelixis/software.html>) using the GTR + CAT substitution model was used to search for the best-scoring ML tree and a rapid bootstrap analysis of 1000 nonparametric replicates under the partition data mode. Bayesian phylogenetic analysis was performed using MrBayes, version 3.1.2 (Ronquist & Huelsenbeck, 2003; <http://mrbayes.sourceforge.net/download.php>) under the GTR + Γ substitution model and the random-addition-sequence method with ten replicates. We ran two independent analyses of four chains (one cold and three heated) with 20 000 000 cycles, sampled every 1000th iteration, with an appropriate burn-in (10%, after checking convergence and sufficiency of statistical values using TRACER, version 1.5; <http://tree.bio.ed.ac.uk/software/tracer/>). Statistical support values (BPP, Bayesian posterior probabilities; LBS, ML bootstrap support) were drawn on the Bayesian 50% majority-rule consensus tree. Gaps were always treated as missing data.

The phylogeny was dated with BEAST, version 1.6.1 (Drummond & Rambaut, 2007), with settings recommended for interspecific data that might or might not satisfy the molecular clock. A Yule branching process with lognormal priors was adopted using the five calibration points specified above. For the GTR + Γ substitution model with four discrete categories, we applied a relaxed molecular clock with a lognormal distribution of rate changes. The unweighted pair group method with arithmetic mean was used to construct a starting tree, and the final topology was estimated by combining three independent chains each of 70 000 000 generations, sampling every 10 000th iteration. TRACER, version 1.5 was used to evaluate effective sample sizes values and to confirm adequate combining of the Markov chain Monte Carlo chains with an appropriate burn-in (10%). Because age estimates may be highly sensitive to inadequate sampling of the outgroup (Linder, 2007), we included more sequences from the Crossosomatales, Fagales, and Myrtales in the dating analysis.

RESULTS

POLLEN MORPHOLOGY IN VIVIANIACEAE

Pollen grains of Vivianiaceae are apolar, spheroidal, and pantoporate, and easily distinguishable from the remaining tri-aperturate Geraniales. Pantoporate pollen superficially similar to that of Vivianiaceae occurs in Amaranthaceae, Zygophyllaceae, Caryophyllaceae, and Convolvulaceae, although it differs in exine sculpture, size, and pore numbers (Borsch & Barthlott, 1998). Most Vivianiaceae are metareticulate (deeply recessed pores and narrow mesoporia), with the exception of *R. spinosa*, *V. albiflora*, and *V. elegans*. The pore number ranges from 15 (*V. albiflora*) to approximately 180 (*Balbisialcalycina*). Pore diameter is almost uniform on individual grains, except in *V. albiflora*. Operculate pollen grains occur in *R. spinosa* and *V. elegans*. Pore membrane is psilate or covered by ektexinous bodies. The mesoporia are approximately equal in width on individual grains and are flat or strongly vaulted, depending on the species. The most distal parts of the mesoporia form an angular, semi-angular, or rounded side. The mesoporia are simplicolumellate or pluricolumellate; columellae are consistently cylindrical, and the tectum is complete or incomplete and recessed in the vertical parts of the mesoporia or confined to the most distal parts of the mesoporia. Microspines are common in many species and are arranged in one (*B. calycina*, *Viviania tenuicaulis*) or two (*V. albiflora*) rows or are evenly distributed (*V. elegans*). In some species, they are only present at the conjunction points of the mesoporia (*V. marifolia*). The fossil specimens described and illustrated in the Supporting information (Appendix S1), as well as in Figures 2, 3, closely correspond to extant *B. aphanifolia*, *B. weberbaueri*, *R. spinosa*, *V. albiflora*, and *V. marifolia*, representing the most complete fossil assemblage of Geraniales.

MOLECULAR PHYLOGENY

The *trnL-F*/ITS-dataset provides a well-resolved backbone phylogeny for Geraniales (Fig. 4), with

Geraniaceae (1.00BPP, 100LBS) as sister group to the remaining families (1.00BPP, 95LBS). The genera of Geraniaceae are retrieved in the well-established relationships with South American *Hypseocharis* (1.00BPP, 100LBS) sister to the rest, and the core-Geraniaceae (1.00BPP, 100LBS) in the sequence (*Pelargonium*, (*Monsonia*, (*Erodium*, *Geranium*))). The clades comprising *Bersama* and *Melianthus* (i.e. Melianthaceae Horan.), *Francoa*, *Greyia*, and *Tetilla* (Francoaceae A. Juss., syn. Greyiaceae Hutch.) as well as *Balbisial*, *Rhynchotheca*, and *Viviania* (Vivianiaceae Klotzsch) are all retrieved with high support (1.00BPP, >95LBS). The relationships between these clades are not fully resolved, and they are retrieved either in the order (Melianthaceae, (Francoaceae, Vivianiaceae)) or ((Melianthaceae, Francoaceae), Vivianiaceae). South African *Greyia* is consistently retrieved as sister to South American *Francoa* and *Tetilla* (1.00BPP, 99LBS). Within Vivianiaceae, all three genera receive high statistical support (1.00BPP, 100LBS), with a possible sister group relationship between *Balbisial* and *Rhynchotheca* (.97BPP). Within *Balbisial*, *Balbisial* sect. *Wendtia* (1.00BPP, 100LBS) and sect. *Balbisial* (incl. *Balbisial miniata* from sect. *Tricarpellatae* Desc., O'Don. & Lourt.: 1.00BPP, 100LBS) are robustly supported as sister clades. In *Viviania*, two clades are also retrieved, one corresponding to *Viviania* in the strictest sense (incl. *V. marifolia* and allies: 1.00BPP, 100LBS), the other to *Cissarobryon* (*V. elegans*) and *Caesarea* (*V. albiflora*; 1.00BPP, 88LBS).

DIVERGENCE TIMES

The presence of fossil pollen that can be unambiguously assigned to extant species/species groups in both *Balbisial* and *Viviania* provides direct evidence that the divergence of these genera into their extant clades has already taken place at or before 10 Mya. Five fossil calibration points are used to calibrate the phylogeny of Vivianiaceae and the remaining Geraniales; three in Geraniaceae and two in Vivianiaceae. A

Figure 4. Phylogeny of Geraniales based on internal transcribed spacer (ITS) and *trnL-F*. Diversification of the Geraniales into four well-supported lineages: Bayesian 50% majority-rule consensus tree of 59 members of the Geraniales (including 68 new sequences) as inferred from the combined ITS-*trnL-F* dataset (974 parsimony-informative positions). Clades at the family level are indicated, and branch lengths are drawn to scale (with the scale bar indicating the number of nt substitutions per site). Numbers on branches are statistical support values to clusters on the right of them (above: Bayesian posterior probabilities, values < 0.90 are not shown; below: Maximum Likelihood bootstrap support values, values < 50 are not shown), maximal statistical support values are indicated by asterisks. The tree is rooted with members of the Myrtales. Genus abbreviations: Ba., *Balbisial*; Be., *Bersama*; C., *Combretum*; E., *Erodium*; Fr., *Francoa*; Fu., *Fuchsia*; Ge., *Geranium*; Gr., *Greyia*; H., *Hypseocharis*; L., *Lythrum*; Me., *Melianthus*; Mo., *Monsonia*; R., *Rhynchotheca*; V., *Viviania*; numbers behind terminals: KXXXXX = DNA-numbers Kew, BXXX = DNA-numbers Berlin; all others are GenBank-numbers.

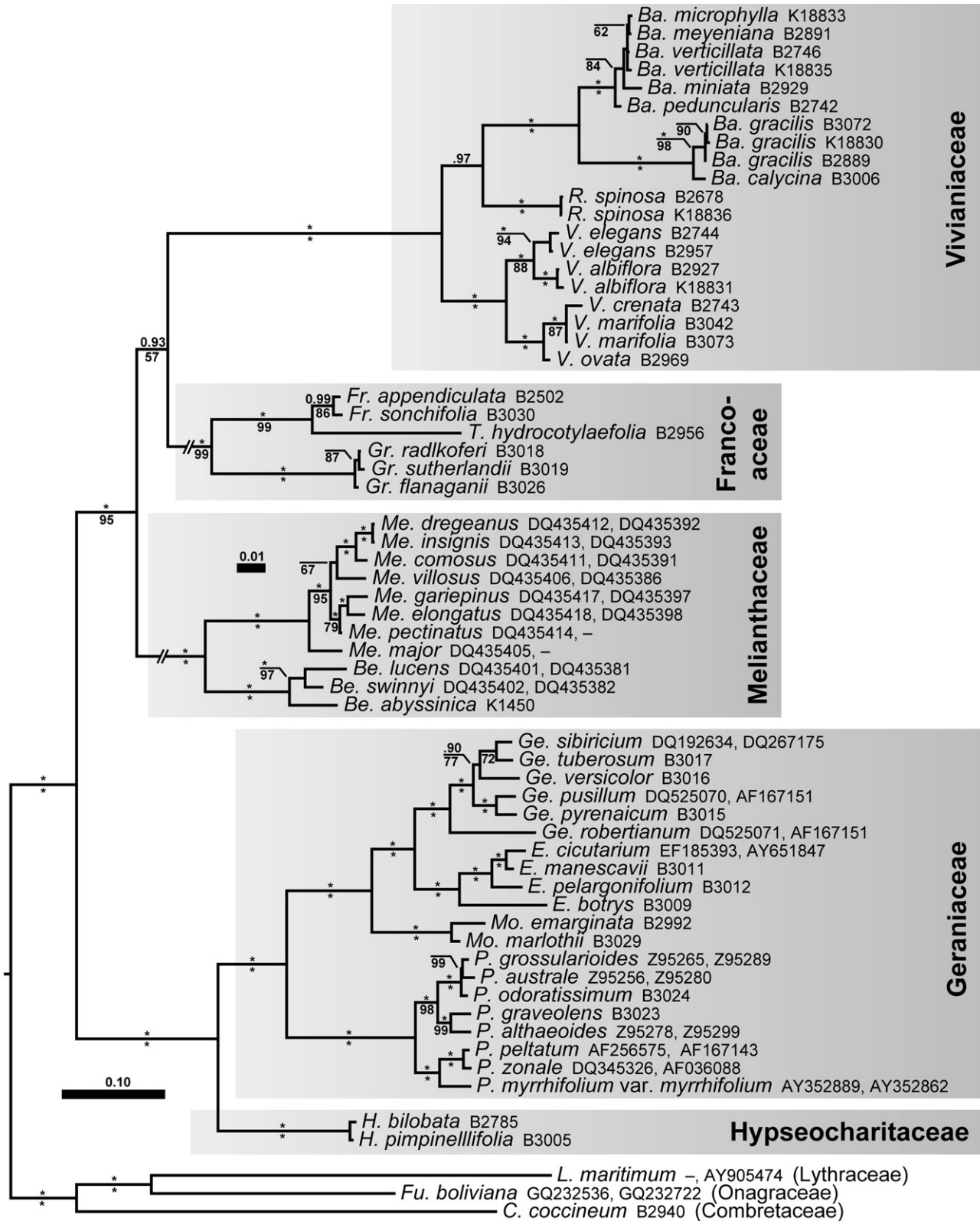


Figure 5. Chronogram of Geraniales diversification. Dated phylogeny of the Geraniales as inferred from the combined ITS-*trnL-F* dataset. Ultrametric maximum clade credibility tree with node ages from the Bayesian uncorrelated lognormal analysis. Nodes with fossil age constraints identified as circles in dark grey (lognormal prior distribution). Small black circles indicate nodes that have been constrained to monophyly, whereas the white circle represents the age of the *Rhynchotheca* fossils not used for calibration. Median rate is given in units of substitutions per million years (including 95% confidence intervals). Absolute ages are in million years, and epochs are indicated (Plc, Pliocene; Q, Quaternary). Genus abbreviations: Ba., *Balbisia*; Be., *Bersama*; Bt., *Betula*; C., *Combretum*; D., *Decodon*; E., *Erodium*; Fr., *Francoa*; Fu., *Fuchsia*; Ga., *Galpinia*; Ge., *Geranium*; Gr., *Greyia*; H., *Hypseocharis*; K., *Koehneria*; La., *Lawsonia*; Lo., *Lopezia*; Ly., *Lythrum*; Me., *Melianthus*; Mo., *Monsonia*; N., *Nesaea*; O., *Ostrya*; Rh., *Rhynchotheca*; Ri., *Ribes*; Sp., *Staphylea*; Su., *Stachyurus*; V., *Viviania*.

time scale for the evolution of the order Geraniales based on a Bayesian dating analysis is depicted in Figure 5 (all EES values >200). The four major lineages (i.e. Geraniaceae, Francoaceae + Melianthaceae, Hypseocharitaceae, Vivianiaceae) all originated within a narrow time frame: 36.9–49.9 (31.9–58.9) Mya. Based on these data the morphologically closely allied western Andean (Atacama) species in *Balbisia* sect. *Balbisia* diversified only since the Pleistocene, approximately 2.0 (0.8–3.5) Mya and south-central Chilean *V. marifolia* and *V. ovata* in the Late Pliocene, approximately 3.1 (1.3–5.2 Mya). *Rhynchotheca*, the only Central Andean genus of Geraniales, diverged from the *Viviania* lineage around the Oligocene–Miocene boundary at approximately 23.1 (17.4–29.5) Mya. An early divergence of the *Rhynchotheca* is plausible based on the fossil finds of *Rhynchotheca*-type pollen of approximately 16 Mya (Table 2).

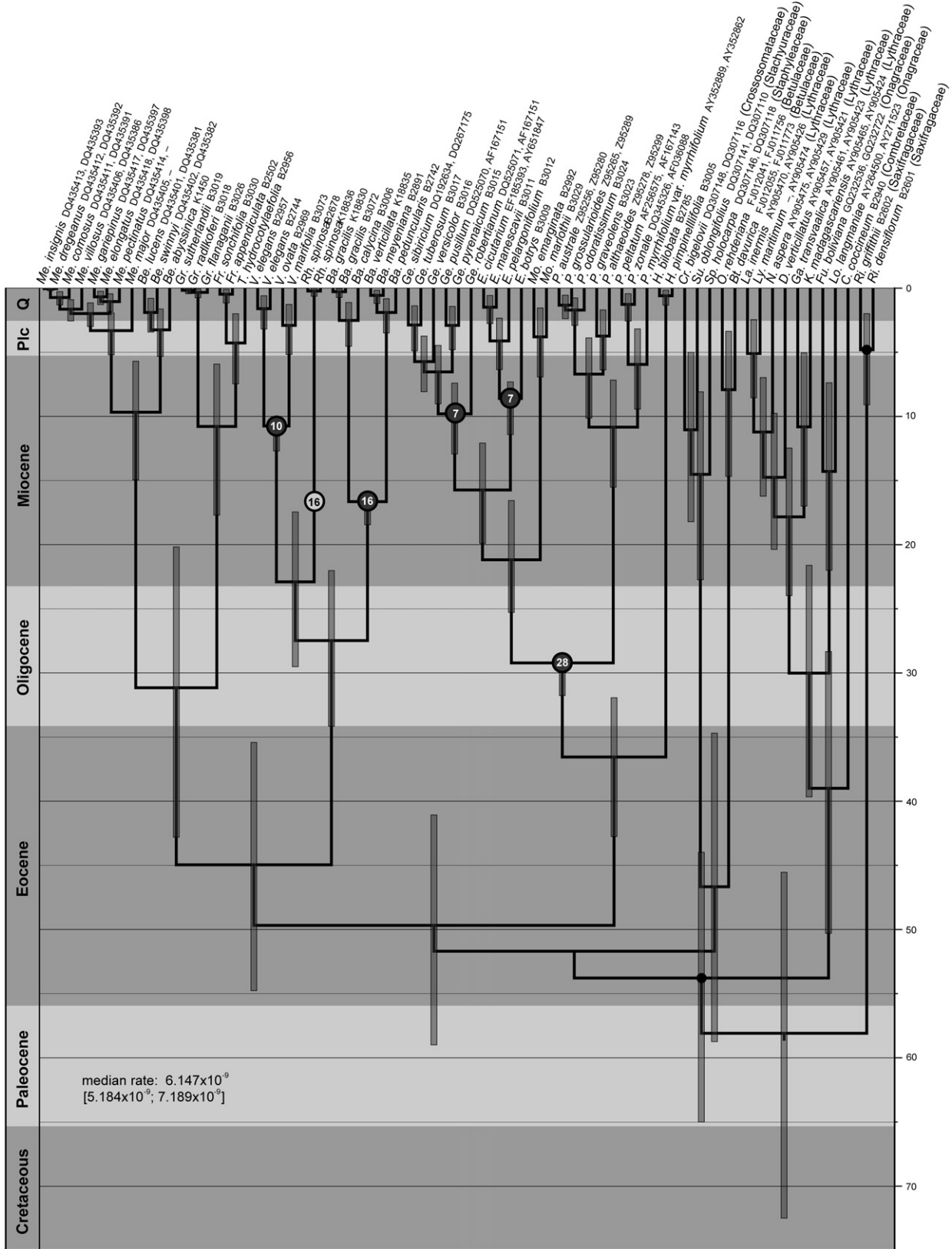
South African *Greyia* and South American *Francoa* + *Tetilla* diverged from each other only in the Middle Miocene at approximately 11.2 (5.9–17.7) Mya. The diversification of the extant species of *Greyia* is very recent and is placed into the Pleistocene at approximately 0.4 (0.07–0.8) Mya. Morphologically highly divergent *Francoa* and *Tetilla* appear to have diverged in the late Pliocene (approximately 4 Mya). Similarly, *Bersama* and *Melianthus* diverged in the late Miocene, approximately 10 (5.7–14.9) Mya, and the basal split between *Melianthus major* and the remainder of the genus is retrieved at the end of the Miocene [approximately 3.4 (1.9–5.2) Mya]. The main diversification of *Melianthus* is dated to the Lower Pleistocene at approximately 2.0 (1.2–2.9) Mya. Conversely, the extant genera of Geraniaceae appear to have diverged much earlier, with (1) the split between *Hypseocharis* and the remainder of the family in the Middle Eocene at approximately 36.9 (31.9–42.8) Mya; (2) the divergence of *Pelargonium* Middle Eocene at approximately 29.6 (28.4–31.8) Mya; and (3) the divergence of the major lineages in *Erodium*, *Geranium*, and *Pelargonium* in the Miocene at approximately 8.9–11.0 (7.2–15.5) Mya.

DISCUSSION

PHYLOGENETIC RELATIONSHIPS AND CLASSIFICATION

The combined marker analysis retrieves a largely resolved and well-supported phylogeny for the group under study. The relationships retrieved within Geraniaceae correspond to those found by Fiz *et al.* (2008). The sister group relationship between *Hypseocharis* and the remaining Geraniaceae is, moreover, confirmed with the inclusion of a second species of this genus. Francoaceae (*Francoa*, *Greyia*, and *Tetilla*) are retrieved as a South American–South African disjunct group. The close relationship between *Francoa* and *Greyia* retrieved by molecular data confirms the close association previously postulated based on anatomical and ontogenetic data (Ronse Decraene & Smets, 1999). The APG III (2009) treatment of Geraniales could thus be improved by reducing Melianthaceae to *Bersama* and *Melianthus* and redefining Francoaceae to include *Francoa*, *Greyia*, and *Tetilla*. Recognition of the genus *Wendtia* in APG III (2009) is rejected because the two species of “*Wendtia*” included (i.e. *B. miniata* and the type species *B. gracilis*) are retrieved on different clades of *Balbisia*, as would be expected from morphology (Weigend, 2007). The recognition of Ledocarpaceae and Vivianiaceae, as two distinct but morphologically very similar families with two genera and one genus, respectively, is possible, although superfluous, and we argue for the recognition of a single family Vivianiaceae including all three genera *Balbisia*, *Rhynchotheca*, and *Viviania* (Weigend, 2007). Conversely, the inclusion of *Hypseocharis* in Geraniaceae does not appear warranted, in view of its discordant fruit, seed, floral, and vegetative morphology (Slanis & Grau, 2001), and the recognition of this distinct clade as a monogeneric family, Hypseocharitaceae, appears justified.

Melianthaceae *s.l.* (Linder, 2007; corresponding to our Francoaceae and Melianthaceae) comprises a morphologically heterogeneous assemblage that is doubtfully monophyletic when Vivianiaceae are excluded. This leaves two main options for classification: to include the morphologically highly divergent Vivianiaceae in a very broadly defined Melianthaceae



(together with Francoaceae) or to segregate Francoaceae from Melianthaceae s.s. Given both the degree of morphological coherence and the ages of the clades, subdivision of Geraniales into five families appears to be the most defensible option: Hypseocharitaceae Wedd. (monogeneric), Geraniaceae Juss. (*Erodium*, *Geranium*, *Monsonia*, *Pelargonium*), Melianthaceae Horan. (*Bersama*, *Melianthus*), Francoaceae A.Juss. (*Francoa*, *Greyia*, *Tetilla*), and Vivianiaceae Klotzsch (*Balbisia*, *Rhynchotheca*, *Viviania*).

DIVERGENCE TIMES

Age estimates for several clades have shown that most disjunctions between South America and Africa must go back to long-distance dispersal (Renner *et al.*, 2010), which is probably by far the most common cause for southern hemisphere disjunctions in plants (Sanmartín & Ronquist, 2004). The Geraniales, with several clades (above the generic level) disjunct between South America and South Africa, appear to show the same pattern. Numerous hypotheses of long-distance dispersal events in Geraniaceae have been compiled for *Erodium*, *Geranium*, and *Pelargonium* by Fiz *et al.* (2006, 2008); Fiz-Palacios *et al.*, 2010, and it is thus not surprising that the earlier evolutionary history of the group involved additional dispersal events. Diaspores for most genera in Geraniales are illustrated in Figure 6. Capsules in Vivianiaceae and *Hypseocharis* are incompletely and tardily dehiscent (Fig. 6A, B, C) to indehiscent (Fig. 6E). The seeds, even if released from the capsule, are large, heavy, and poorly adapted to long-distance dispersals. All these genera have more or less continuous ranges, and there is no evidence for long distance dispersal. This is a stark contrast to the small, spindle-shaped seeds of Francoaceae (*Francoa*, *Greyia*, *Tetilla*; Figs. 6N, O), which can likely be dispersed over larger distances by wind. This emphasizes the plausibility of a dispersal event in Francoaceae between South America and Africa, as implied by our dated phylogeny. The only weakly differentiated mericarps of *Rhynchotheca* (Fig. 6F) are too heavy for wind-dispersal and are probably dispersed by mammals rather than birds because they lack any specialized structures for attachment to feathers. Conversely, the more highly specialized mericarps of most Geraniaceae (excl. *Hypseocharis*) appear to be particularly suited to long distance dispersal by, for example, birds, which is borne out by multiple intercontinental dispersal events as documented by Fiz *et al.* (2006, 2008) and Fiz-Palacios *et al.* (2010).

Our analysis is based on an independent set of multiple calibration points (four new Vivianiaceae fossils reported in the present study and three previously reported pollen fossils of Geraniaceae) and

arrives at divergence times that are considerably younger than those found in previous studies (Wikström, Savolainen & Chase, 2001; Fiz *et al.*, 2008). Stem group ages for *Erodium*, *Geranium*, and *Monsonia* are estimated at 26–34 Mya in Fiz *et al.* (2008b), whereas we find an age of 15.8–21.1 Mya. Fiz *et al.* (2008) estimated a divergence of *Hypseocharis* from Geraniaceae at approximately 55 Mya, which is much older than the age of 36.9 (31.9–42.8) Mya in the present study. Wikström *et al.* (2001) estimated an age of 59–67 Mya for the divergence of *Bersama* from *Greyia*, whereas our data indicates an age of approximately 31.4 (20.2–42.8) Mya.

Similarly, the divergence times estimated by Linder *et al.* (2006) within *Melianthus* and for the split between *Bersama* and *Melianthus* are much older than the ages obtained in the present study, with the split between *Bersama* and *Melianthus* in the late Oligocene (approximately 27 Mya; our estimate is approximately 10 Mya). The entire diversification of *Melianthus* was considered by Linder *et al.* (2006) to have taken place during the Miocene (approximately 8–20 Mya). Our results indicate that diversification started with the split between *M. major* and the remainder of the genus in the Late Pliocene, approximately 3.4 (1.9–5.2) Mya, and the main diversification of *Melianthus* is then placed in the Early Pleistocene at approximately 2.0 (1.2–2.9) Mya. The inference of Linder *et al.* (2006) is based on a single secondary calibration point, namely the age limits taken from Wikström *et al.* (2001) for the divergence times of *Bersama* and *Greyia*. As previously demonstrated by Linder *et al.* (2006), age estimates may be highly sensitive to inadequate sampling of the outgroup, and these factors likely explain the divergent time estimates found by Linder *et al.* (2006) for *Bersama* and *Melianthus*.

Linder *et al.* (2006) discussed the likely influence of paleoclimatic changes on the diversification of *Melianthus*. In their analysis, diversification in South African *Greyia* and *Melianthus* occurred in the late Miocene, a period of minor tectonic uplift in eastern South Africa and incipient aridification from a humid, tropical climate. In our analysis, extant species would have arisen much more recently, after a period of accelerated, major tectonic uplift and accelerated aridification in the Late Pliocene and Pleistocene from approximately 3.4 Mya onwards. This may be more plausible in terms of eco-geographical isolation of the extant and morphologically weakly differentiated species in *Greyia* and *Melianthus*.

HISTORICAL BIOGEOGRAPHY

The Patagonian fossil pollen specimens, representing all five extant lineages in *Balbisia*, *Rhynchotheca*,

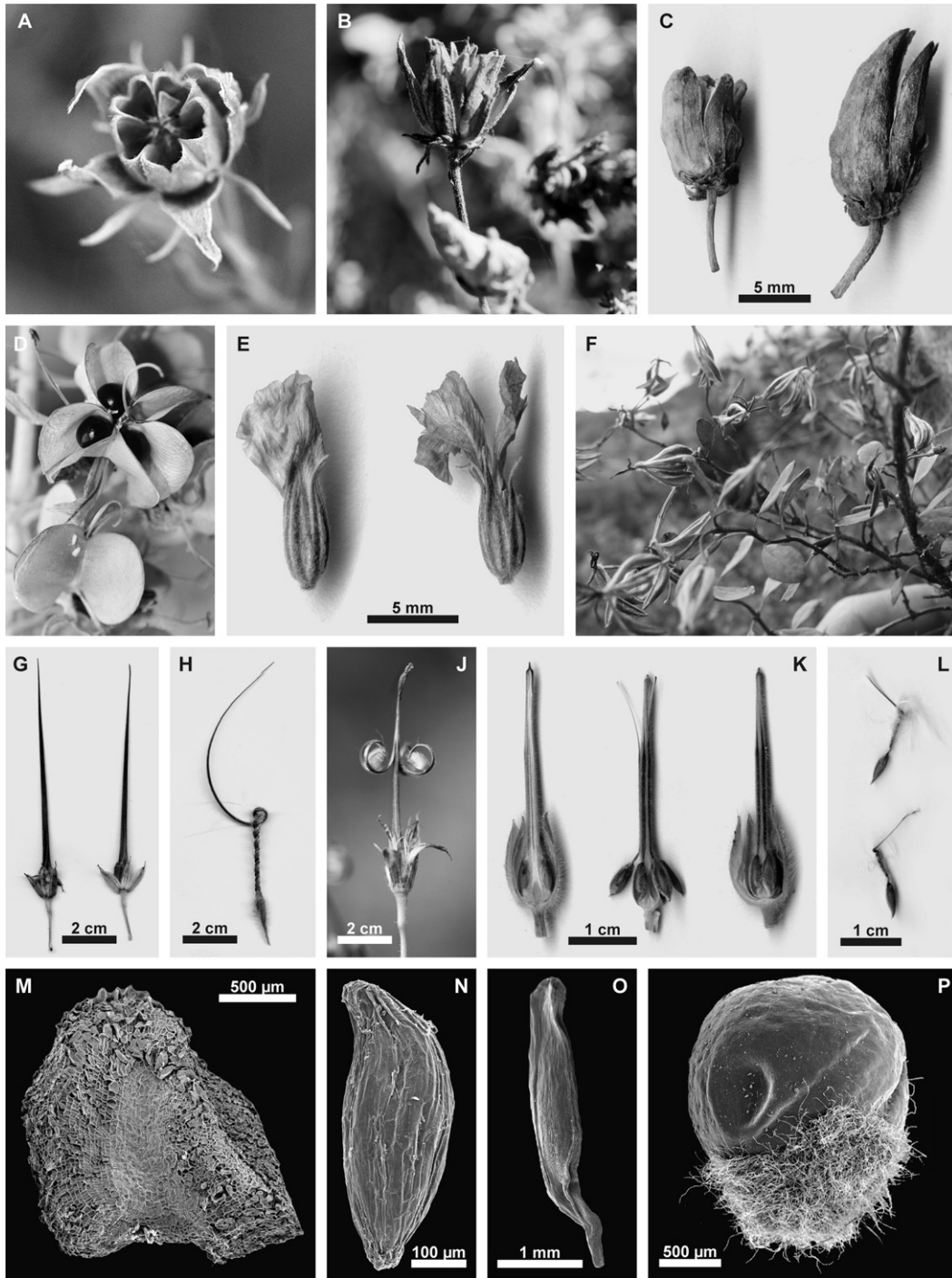


Figure 6. Fruits and diaspores of Geraniales. A, B, ventricidal capsule with partial desiccation of *Balbisia verticillata* (Weigend s.n.; B). C, ventricidal capsule with partial desiccation *Hypseocharis biloba* (Ortuño 1631; B). D, ventricidal capsule of *Melianthus pectinatus*, with large, shiny seed (Weigend 9164; B). E, dry flower with seeds firmly enclosed as fruit of *Viviania marifolia* (Weigend 9352; B). F, fruiting branch of *Rhynchotheca spinosa* with ovary falling into five one-seeded mericarps (Weigend 9107; B). G, H, fruit and mericarpid of *Erodium* with long, later spirally twisted 'awn' (Weigend 9303; B). I, mature fruit of *Geranium versicolor* with seeds already expelled (Weigend 9313; B). K, L, fruit and mericarps of *Pelargonium capitatum* (not vouchered, Botanischer Garten Berlin Dahlem). M, large, angular seed of *Hypseocharis biloba* (Ortuño 1631; B). N, small, wind-dispersed, spindle-shaped seed of *Greyia flanaganii* (Weigend 9298; B). O, small, wind-dispersed, spindle-shaped seed of *Tetilla hydrocotylaefolia* (Eggli et al. 3132; P) large, round seed of *V. albiflora* with hair tuft at funicular pole (Krapovickas & Cristobal 419777; F).

and *Viviania*, come from an area where extant species of all five lineages are absent. The geographically closest occurrence of a lineage represented in the Puerto Madryn and Chenque formation fossil records is *B. gracilis* from Patagonian Chile at a latitude of 41°S (i.e. almost at the same latitude the fossils come from but on the much moister western side of Patagonia). The closest region, where extant representatives of at least three of the five lineages represented in the fossil record (*B.* sects *Balbisia* and *Wendtia*, *Viviania s.s.*) co-occur, is Coquimbo in north-central Chile, at approximately 30°S (Zuloaga, Morrone & Belgrano, 2008): This is more than 10°N of the fossil assemblage in an essentially subtropical to Mediterranean climate. The lineage represented by the fourth pollen fossil, 'Caesarea' (i.e. *V. albiflora*), is now restricted to northern Argentina, Uruguay, and Brazil, with a southern distribution limit at approximately 33°S, whereby extant taxa of this lineage also have a southern distribution limit approximately 10°N of their Late Miocene records.

The *Rhynchotheca* lineage, which had already diverged from *Balbisia* and was present in Patagonia approximately 16 Mya, migrated even further north and now has its southern limit at approximately 15°N (i.e. 16°N of its Miocene habitat). Miocene paleoclimates are considered to have been relatively warm and only seasonally dry, with low trees and shrubs dominating eastern Patagonia (Barreda & Palazzesi, 2007). Early Miocene (23–16 Mya) pollen and spore assemblages indicate a sub-humid, temperate to warm–temperate climate, whereas conditions changed to warm but seasonally dry by the Late Miocene (9–11 Mya; Palazzesi, Barreda & Tellería, 2009). The climatic conditions prevailing in Patagonia around the time from which our fossils date thus closely paralleled the climatic conditions, in which the bulk of the extant species of the four genera are found. This northward displacement thus appears to have been triggered by tectonic (Andean uplift) and global paleoclimatic (Antarctic ice-sheet development) events during the Oligocene–Miocene, leading to cooler and more arid conditions throughout the Patagonian landscapes (Zachos *et al.*, 2001; Blisniuk *et al.*, 2005). These events had less severe effects at lower latitudes, opening new suitable habitats further north. The northward displacement of the various lineages of South American Geraniales, and the colonization of the Andes, are likely the result of niche conservatism in the sense of limited climatic tolerance and climate tracking (Wiens & Graham, 2005). Niche conservatism has likely played a major role in the patterns of plant distribution over time ranges of tens of millions of years (Crisp *et al.*, 2009), and the historical biogeography of South American Geraniales is just one example of an apparently quite general

phenomenon. Other South American angiosperm groups have consequently followed similar migratory routes, such as *Schlechtendalia* (Asteraceae), nowadays also recorded 10°N of its Miocene fossil locality (Palazzesi *et al.*, 2009).

At least two now exclusively Andean lineages (*Hypseocharis* and *Rhynchotheca*) are estimated to have arisen a very long time before Andean orogeny provided the elevations and climatic conditions they currently inhabit. The Central Andes had only reached approximately a third of their current elevation 20 Mya and only half of their current elevation approximately 10–15 Mya (Gregory-Wodzicki, 2000; Graham, 2009). The currently high-Andean *Hypseocharis*-lineage, 36.9 (31.9–42.8) Mya, far predates even the early phases of Andean uplift to relevant elevations. Páramo communities, where the main distribution of the genus now lies (at least in the northern part of the range), are believed to have come into existence as recently as 3.5 Mya (Graham, 2009), and a relatively recent northward expansion into the Andes has to be assumed.

Similarly complex patterns of lineages much older than the Andean elevations they currently inhabit are found in Asteraceae (HersHKovitz *et al.*, 2006; Palazzesi *et al.*, 2009) and Ranunculaceae (Emadzade *et al.*, 2010), and these may derive from similar biogeographic histories. It must be assumed that these lineages inhabited (then warm-temperate) southern South America in the Miocene and migrated northwards as the Andean uplift provided seasonally arid and temperate climatic conditions in areas previously characterized by wet-tropical conditions. Increasing aridity and increasingly lower temperatures subsequently led to the disappearance of these lineages from Patagonia.

ACKNOWLEDGEMENTS

We would like to express our sincere gratitude to Mark Chase and Lazlo Cziba (Jodrell Laboratory, Kew Gardens, UK) for helping out with DNA-isolates; and to Alfredo Grau (Universidad de Tucuman, Argentina), Tobias Kern and Teresa Ortuñez (Berlin), Fernando Zuloaga (Darwinion, Argentina), Michail Belov (Talca, Chile), and Alicia Marticorena, Roberto Rodriguez, Götz Palfner, and Carlos Baeza (Universidad de Concepción, Chile), for helping with the tedious accumulation of the required plant material. We thank H. H. Hilger (Berlin) for the use of equipment and staff and botconsult GmbH (Berlin) for helping to fund the field studies. We further gratefully acknowledge the helpful suggestions of four anonymous reviewers, which greatly improved the manuscript. We are grateful for funding provided for field studies and study abroad by a scholarship by the

German Academic Exchange Service (DAAD) to L.P., and a grant by CONICET to V.B. and L.P. (PIP 0342).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Description of the fossil Vivianiaceae-pollen from Patagonia.

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APPENDIX

DOC. A1. VOUCHER FOR THE POLLEN STUDIES

Vouchers for pollen studies of Ledocarpaceae and allied families: *Balbisia aphanifolia* (Griseb.) Hunz. & Ariza, *R. Kiesling* 6645 (IBODA), *B. calycina* (Griseb.) Hunz. & Ariza, *M. E. Múlgura de Romero* 4119 (IBODA), *H. Sleumer* 246 (B); *B. gracilis* (Meyen) Hunz. & Ariza, *A. Burkart* 19824 (IBODA); *B. meyeniana* Klotzsch, *K. Fiebrig* 3094 (B), *M. Ackermann & F. Cáceres* H. 658 (M, B, HUSA, USM); *B. microphylla* (Phil.) Reiche, *E. Werdermann* 1063 (IBODA, B, F, SI); *B. miniata* (I.M. Johnst.) Descole & O'Donnell & Lourteig, *R. Kiesling* 9463 (IBODA), *B. E. Leuenberger et al.* 4195 (B); *B. peduncularis* (Lindl.) D. Don, *E. Werdermann* 800 (IBODA, B, E, F, SI), *M. Quezada & E. Ruiz* 193 (CONC), *M. Quezada & E. Ruiz* 383 (CONC), *C. Aedo* 6846 (CONC); *B. stitchkinii* Ricardi, *Villagrán* 32987 (CONC), *M. Ricardi et al.* 36407 (CONC); *B. verticillata* Cav., *N. Dostert & F. Cáceres-H.* 1020 (B), *M. Weigend & N. Dostert* 97/13 (F, M); *Bersama abyssinica* Fresen., *Polhill & Paulo* 1659 (B), *R. Koelker* 01 (B); *Francoa sonchifolia* Cav., *Gartenherbarbeleg* 36921, *Beurton* 3/2002 (B); *Greyia radlkoferi* Szyszyl., *Gartenherbarbeleg* 45227, *Leuenberger* 8.4.2008 (B); *G. sutherlandii* Hook. & Harv., *M. Weigend* 9168 (B); *Hypseocharis pimpinellifolia* Remy, *K. Fiebrig* 2626 (B); *Melanthus major* L., *Gartenherbarbeleg* 34732, *Leuenberger* 1996 (B); *M. villosus* Bolus, *M. Weigend* 9163 (B); *Rhynchotheca spinosa* Ruiz & Pav., *D. N. Smith* 6611 (USM); *Tetilla hydrocotylaefolia* DC., *U. Eggli et al.* 3132 (B); *Viviania albiflora* (Cambess.) Reiche, *V. Solís Neffa* 888 (IBODA), *F. O. Zuloaga* 8193 (IBODA), *G. Herter* 99150 (B), *B. Rambo* 43528 (B), *B. Rambo* 51647 (B), *V. crenata* (Hook.) G. Don, *F. Schlegel* 1677 (B), *R. A. Philippi s.n.* (B); *V. elegans* (Kunze ex Poepp.) Reiche & Johow, *M. K. Arroyo et al.* 996032 (CONC); *S. Teillier & C. Márquez* 4910 (CONC); *V. marifolia* Cav., *T. S. Tombesi* 216 (IBODA), *W. Schwabe s.n.* (B) *V. marifolia* Cav., *M. Ackermann* 543 (B, M); *V. ovata* Phil., *O. Boelcke* 13740 (IBODA), *C. Marticorena & O. Mathei* 715 (B, CONC), *V. tenuicaulis* Barnéoud in Gay, *C. Marticorena et al.* 1581 (CONC), *M. Ricardi & C. Marticorena* 4515/900 (CONC).

Table A1. Vouchers for the DNA-sequences

Species	Voucher	Herbarium	Country of origin	DNA-number	ITS genbank nr.	trnL-F genbank nr.
<i>Balbisia calycina</i> (Griseb.) Hunz. & Ariza	A. Grau s.n.	LIL	Argentina	BSB 3006	HE795044	HE795452
<i>Balbisia gracilis</i> (Meyen) Hunz. & Ariza	M. Weigend <i>et al.</i> 5817	B	Argentina	KEW 18830	HE795045	HE795453
<i>Balbisia gracilis</i> (Meyen) Hunz. & Ariza (= <i>Wendtia reynoldsii</i> Endl.)	J. Grau 2936	M	Chile	BSB 2889	HE795046	–
<i>Balbisia gracilis</i> (Meyen) Hunz. & Ariza (= <i>Wendtia reynoldsii</i> Endl.)	M. Weigend 9439	BSB	Chile	BSB 3072	HE795047	HE795454
<i>Balbisia meyeniana</i> Klotzsch	M. Weigend & M. Ackermann 9266	B, USM, HUSA,	Peru	BSB 2891	HE795048	HE795455
<i>Balbisia microphylla</i> Reiche	Eggli, U. <i>et al.</i> 2735	B	Chile	KEW 18833	HE795049	–
<i>Balbisia miniata</i> (I.M. Johnston) Descole, O'Donnell & Lourteig	Leutenberger <i>et al.</i> 4195	B	Argentina	BSB 2929	HE795050	–
<i>Balbisia peduncularis</i> D. Don	M. Quezada & R. Ruiz 193	M	Chile	BSB 2742	HE795051	HE795456
<i>Balbisia verticillata</i> Cav.	G. Beck <i>et al.</i> 22108	M	Chile?	BSB 2746	HE795052	–
<i>Balbisia verticillata</i> Cav.	Weigend, M. <i>et al.</i> 7312 B	B	Peru	KEW 18835	HE795053	HE795457
<i>Bersama lucens</i> Szyszyl.	Knox 2569	K	Africa	Genbank	DQ435401.1	DQ435381
<i>Bersama abyssinica</i> Fresen.			Africa	KEW 1450	HE795054	HE795458
<i>Bersama swinnyi</i> E. Phillips			Africa	Genbank	DQ435402	DQ435382
<i>Combretum coccineum</i> Lam.			Africa	BSB 2940	HE795055	HE795459
<i>Erodium botrys</i> (Cav.) Bertol.			Europe	BSB 3009	HE795056	HE795460
<i>Erodium cicutarium</i> (L.) L'Hér.	<i>Weigend s.n.</i>	BSB	Europe	Genbank	EF185393	AY651847
<i>Erodium manescavi</i> Coss.	M. Weigend		Europe	BSB 3011	HE795057	HE795461
<i>Erodium pelargoniflorum</i> Boiss. & Heldr.	M. Weigend 9312		Europe	BSB 3012	HE795058	HE795462
<i>Franseria appendiculata</i> Cav.	Chase 2502	K	w. Asia	BSB 2502	HE795059	HE795463
<i>Franseria sonchifolia</i> Cav.	M. Weigend 9353	B	Chile	BSB 3030	HE795060	HE795464
<i>Fuchsia boliviana</i> Carrière			Chile	Genbank	GQ232536	GQ232722
<i>Geranium pusillum</i> L.	M. Weigend 9308		Europe	Genbank	DQ525070	AF167151
<i>Geranium pyrenaicum</i> Burm.f.			Europe	BSB 3015	HE795061	HE795465
<i>Geranium robertianum</i> L.			Europe	Genbank	DQ525070	AF167151
<i>Geranium sibiricum</i> L.			Europe	Genbank	DQ192634	DQ267175
<i>Geranium tuberosum</i> L.	n.v.		Europe	BSB 3017	HE795062	HE795466
<i>Geranium versicolor</i> L.	M. & K. Weigend 9313		Europe	BSB 3016	HE795063	HE795467
<i>Greyia flanaganii</i> Bolus	M. Weigend 9298		RSA	BSB 3026	HE795064	HE795468
<i>Greyia radlkoferi</i> Szyszyl.	BGBM – voucher fehlt!		RSA	BSB 3018	HE795065	HE795469
<i>Greyia sutherlandii</i> Hook. & Harv.	M. Weigend 9168		RSA	BSB 3019	HE795066	HE795470
<i>Hypseocharis bilobata</i> Killip	Chase 2785	K	Bolivia	BSB 2785	HE795067	HE795471
<i>Hypseocharis pimpinellifolia</i> Remy	A. Grau s.n.	LIL	Argentina	BSB 3005	HE795068	HE795472
<i>Lythrum hyssopifolia</i> L.			Argentina	Genbank	AY905428.1	–
<i>Lythrum maritimum</i> Cham. & Schltdl.			Argentina	Genbank	–	AY905474.1
<i>Melianthus comosus</i> Vahl			RSA	DQ435411	DQ435391	DQ435391
<i>Melianthus dregeanus</i> Sond.			RSA	Genbank	DQ435412	DQ435392
<i>Melianthus elongatus</i> Wijnands			RSA	Genbank	DQ435418	DQ435398

Melianthus gariepinus Merxm. & Roessler				RSA						DQ435417		DQ435397
Melianthus insignis Kuntze				RSA						DQ435413		DQ435393
Melianthus major L.				RSA						DQ435405		-
Melianthus pectinatus Harv.				RSA						DQ435414		-
Melianthus villosus Bolus				RSA						DQ435406		DQ435386
Monsonia emarginata L'Hér.				RSA						HE795069		HE795473
Monsonia marlothii (Engl.) F. Albers				RSA		B				HE795070		HE795474
Pelargonium grossularifolium Salisb.		Gartenherbarbeleg 28138		RSA		B				Z95265		Z95289
Pelargonium myrrhifolium (L.) L'Hér. var. myrrhifolium				RSA						AY352889		AY352862
Pelargonium althaeoides L'Hér.				RSA						Z95278		Z95299
Pelargonium australe J. Jacq.				Australia						Z95256		Z95280
Pelargonium graveolens L'Hér.				RSA						HE795071		HE795475
Pelargonium odoratissimum (L.) L'Hér.		n.v.		RSA						HE795072		HE795476
Pelargonium peltatum (L.) L'Hér.		M. Weigend 9299		RSA						AF256575		AF167143?
Pelargonium zonale (L.) L'Hér.				RSA						DQ345326		AF036088 ??
Rhynchotheca spinosa Ruiz & Pav.		M. Weigend 9107		Ecuador		BSB, M				HE795073		HE795477
Tetilla hydrocotylaeifolia DC.		M. Weigend <i>et al.</i> 5413		Peru		B				HE795074		HE795478
Viviania albiflora (Cambess.) Reiche		T. Kern & M. Belov 21		Chile		CONC				HE795077		HE795481
Viviania albiflora (Cambess.) Reiche		Krapovickas & Cristóbal 41977		Brazil		F				HE795078		
Viviania crenata (Hook.) G. Don		R. Wasum 100		Brazil		B				HE795079		
Viviania elegans (Kunze ex Poepp.) F. Meigen		C. Jiles 5556		Chile		M				HE795080		
Viviania marifolia Cav.		K. H. & W. Reehinger 63128		Chile		M				HE795081		HE795482
Viviania ovata Phil.		M. Mihoc <i>et al.</i> 7273		Chile		CONC				HE795082		HE795483
Crossosoma bigelovii S. Watson		M. Weigend 9352		Chile		BSB				HE795083		HE795484
Ribes densiflorum Phil.		M. Weigend 9353		Chile		BSB				HE795084		
Ribes griffithii Hook.f. & Thomson		M. Rosas 2159		Chile		CONC (169872)				HE795085		HE795485
Lopezia langmaniae Miranda		Ickert-Bond 1833		USA		F				DQ307116		DQ307148
Koehneria madagascariensis (Baker) S. A. Graham, H. Tobe & Baas		P. Brownless <i>et al.</i> 945		India		E				HE795075		HE795479
Galpinia transvaalica N.E.Br.		ACE324		Mexico		E				HE795076		HE795480
Decodon verticillatus Elliott		Breedlove 32300		n. ind.		CAS				AY264500		AY271523
Nesaea aspera Koehne		D'Arcy & Rakotozfy 15317		n. ind.		MO				AY905465		AY905424
Lawsonia inermis L.		Balsinhas 3263		n. ind.		MO				AY905461		AY905423
Betula davurica Pall.		Graham 917		n. ind.		MO				AY905457		AY905421
Ostrya rehderiana Chun		Drummond 11446		n. ind.		MO				AY905475		AY905429
Staphylea holocarpa Hemsl.		Correll 45915		n. ind.		TEX				AY905470		AY905426
Stachyurus oblongifolius F. T. Wang & Tang		Tibet 218		n. ind.		n. ind.				FJ012055		FJ011773
		Wen 5085		n. ind.		n. ind.				FJ012041		FJ011756
		Wen 5740		n. ind.		F				DQ307146		DQ307118
		Zhu J-11		n. ind.		PE				DQ307141		DQ307110