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

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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 rosid 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.,...
Table 1. Overview over current Geraniales classification and distribution

| Family        | Genera       | Number of species | Distribution                                |
|---------------|--------------|-------------------|---------------------------------------------|
| Geraniaceae   | Hypseocharis | 6                 | Argentina to Peru                           |
| Geraniaceae   | Erodium      | 80                | subcosmopolitan                             |
| Geraniaceae   | Geranium     | 430               | subcosmopolitan                             |
| Geraniaceae   | Monsonia     | 40                | Africa (+ Madagascar, south-west Asia)       |
| Geraniaceae   | Pelargonium  | 280               | Africa (+ Madagascar, south-west Asia, Australia, New Zealand) |
| Melianthaceae | Bersama      | 8                 | Africa                                      |
| Melianthaceae | Melianthus   | 8                 | Southern Africa                             |
| Melianthaceae | Greyia       | 3                 | Southern Africa                             |
| Melianthaceae | Francoa      | 1 or 2            | Chile                                       |
| Melianthaceae | Tetilla      | 1                 | Chile                                       |
| Vivianiaceae  | Balbisia     | 8                 | Argentina/Chile to Peru                     |
| Vivianiaceae  | Wendtia      | 3                 | Argentina/Chile                            |
| Vivianiaceae  | Rhynchotheca | 1                 | Peru, Ecuador                               |
| Vivianiaceae  | Viviana      | 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 & Mercxx, 2006). Nevertheless, phylogenetic relationships within Geraniales are still poorly resolved, especially with regard to the completely known South American taxa, for which only incomplete (Hypseocharis, most Viviani 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 & Chandler, 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. *Viviana* is restricted to at least seasonally moist habitats, with some species in permanently wet forests (*e.g.* *Viviana 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 stitchkinii*) 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 Ma. 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 Bernardo 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
obtained (for a list of specimens examined, see the Appendix, Doc. A1), representing *Balbisia*, *Viviania*, and *Rhynchoteca* (*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 pelargonioides* 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

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**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: *Viviana marifolia* type, *Viviana albiflora* type, *Balbisia* sect. *Wendtia* type, *Balbisia* sect. *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.
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 μm. 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).

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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),

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

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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 *Viviana 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 *Viviana*, 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 *Viviana*, 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 (Katoh et al., 2005; Katoh & 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 Geraniaceae. 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 (Balbisia calycina). 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; columnellae 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, Vivania 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. apanifolia, B. weberbaueri, R. spinosa, V. albiflora, and V. marifolia, representing the most complete fossil assemblage of Geraniaceae.

MOLECULAR PHYLOGENY

The trnL-F/ITS-dataset provides a well-resolved backbone phylogeny for Geraniaceae (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. Meliantheaceae Horan.), Francoa, Greyia, and Tetilla (Francoaceae A. Juss., syn. Greyiaceae Hutch.) as well as Balbisia, 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 (Meliantheaceae, Francoaceae, Vivianiaceae) or ((Meliantheaceae, 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 Balbisia and Rhynchotheca (.97BPP). Within Balbisia, Balbisia sect. Wendtia (1.00BPP, 100LBS) and sect. Balbisia (incl. Balbisia 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 Balbisia 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 Geraniaceae; three in Geraniaceae and two in Vivianiaceae. A

Figure 4. Phylogeny of Geraniaceae based on internal transcribed spacer (ITS) and trnL-F. Diversification of the Geraniaceae into four well-supported lineages: Bayesian 50% majority-rule consensus tree of 59 members of the Geraniaceae (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., Balbisia; 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: KXXXX = DNA-numbers Kew, BXXX = DNA-numbers Berlin; all others are GenBank-numbers.

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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 Vivianiaceae 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 De Craeke & 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 Viviani (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 Geraniaceae 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, Viviana).

**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 Geraniaceae, 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 Geraniaceae 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 deshiscence of *Balbisia verticillata* (Weigend *s.n.*; B). C, ventricidal capsule with partial deshiscence *Hypsechris 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 *Visania 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 mericarp of *Erodium* with long, later spirally twisted ‘awn’ (Weigend 9303; B). I, mature fruit of *Geranium versicolor* with seeds already expelled (Weigend 9313; B). J, L, fruit and mericarps of *Pelargonium capitatum* (not vouchered, Botanischer Garten Berlin Dahlem). M, large, angular seed of *Hypsechris 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 hydrocotylaeefolia* (Eggl 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, Vivania 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.

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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. Mülgera 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 3094 (IBODA); M. Ackermann 3094 (IBODA, B, F, SI); B. ricardi (Conc), M. Ackermann 3094 (IBODA, B); B. gracilis (Meyen) Hunz. & Ariza, R. Kiesling 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); Borsama abyssinica Fresen., Polhill & Paulo 1659 (B), R. Koeleber 01 (B); Francoa sonchifolia Cav., Gartenherbarbeleg 36921, Beurton 3/2002 (B); Greyvia radlkoferi Szyszyl., Gartenherbarbeleg 45227, Leuenberger 8.4.2008 (B); G. sutherlandii Hook. & Harv., M. Weigend 9168 (B); Hypecocharis pimpinellifolia Remy, K. Fiebrig 2626 (B); Melianthus 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. Solis Neffia 888 (IBODA), F. O. Zuloaga 8193 (IBODA), G. Herter 99150 (B), B. Rambo 43528 (B), B. Rambo 51647 (B) C. Marticorena et al. 8193 (IBODA), M. Ackermann 543 (B, M); V. ovata Phil., O. Boelcke 13740 (IBODA), C. Marticorena & O. Matthei 715 (B, CONC), V. tenuicaulis Barnéoud in Gay, C. Marticorena et al. 1581 (CONC), M. Ricardi & C. Marticorena 4515/900 (CONC).

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| Species | Voucher | Herbarium | Country of origin | DNA-number | ITS genbank nr. | trnL-F genbank nr. |
|---------|---------|-----------|-------------------|------------|----------------|------------------|
| Balbisia calycina (Griseb.) Hunz. & Ariza | A. Grau s.n. | BSB | Argentina | HE795452 | HE795044 | HE795465 |
| Balbisia gracilis (Meyen) Hunz. & Ariza | M. Weigend et al. 5817 | BSB | Argentina | HE795453 | HE795045 | HE795466 |
| Balbisia gracilis (Meyen) Hunz. & Ariza (= Wendthia gracilis Endl.) | J. Grau 285/46 | BSB | Chile | HE795447 | HE795046 | HE795467 |
| Balbisia meyeniana Klotzsch | M. Weigend & M. Ackermann 9266 | BSB | Chile | HE795455 | HE795048 | HE795468 |
| Balbisia miniata (I.M. Johnst.) Descole | M. Weigend 9439 | K | Argentina | HE795451 | HE795049 | HE795470 |
| Balbisia peduncularis D. Don | M. Weigend 2746 | K | Chile | HE795452 | HE795050 | HE795471 |
| Balbisia verticillata Cav. | G. Beck et al. 22108 | K | Chile | HE795456 | HE795051 | HE795472 |
| Bersama abyssinica Fresen. | Knox 3599 | BSB | Africa | HE795052 | HE795052 | HE795052 |
| Bersama linerifolia D. Don | Knox 4185 | BSB | Europe | HE795053 | HE795053 | HE795053 |
| Bersama lucens Szyszyl. | Knox 3599 | BSB | Africa | HE795054 | HE795054 | HE795054 |
| Combretum coccineum Lam. | M. Weigend s.n. | BSB | Africa | HE795055 | HE795055 | HE795055 |
| Erodium botrys (Cav.) Bertol. | M. Weigend 9303 | K | Europe | HE795056 | HE795056 | HE795056 |
| Erodium cicutarium (L.) L’Hér. | M. Weigend et al. 5756 | BSB | Europe | HE795057 | HE795057 | HE795057 |
| Erodium manescavi Coss. | M. Weigend Europe | BSB | Europe | HE795058 | HE795058 | HE795058 |
| Erodium pelargoniflorum Boiss. & Heldr. | M. Weigend 7312 | BSB | Europe | HE795059 | HE795059 | HE795059 |
| Geranium bolivianum Carrière | M. Weigend 9313 | K | Europe | HE795060 | HE795060 | HE795060 |
| Geranium pusillum L. | M. Weigend 9288 | BSB | Europe | HE795061 | HE795061 | HE795061 |
| Geranium pyrenaicum Burm.f. | M. Weigend 9308 | BSB | Europe | HE795062 | HE795062 | HE795062 |
| Geranium sibiricum L. | M. Weigend 9313 | BSB | Europe | HE795063 | HE795063 | HE795063 |
| Geranium tuberosum L. | M. Weigend 9288 | BSB | Europe | HE795064 | HE795064 | HE795064 |
| Geranium versicolor L. | M. Weigend 9313 | K | Europe | HE795065 | HE795065 | HE795065 |
| Lythrum hyssopifolia L. | M. Weigend 9313 | BSB | Europe | HE795066 | HE795066 | HE795066 |
| Melianthus comosus Vahl | M. Weigend 9288 | BSB | RSA | HE795067 | HE795067 | HE795067 |
| Melianthus dregeanus Sond | M. Weigend 9288 | BSB | RSA | HE795068 | HE795068 | HE795068 |
| Melianthus elongatus Wijnands | M. Weigend 9288 | BSB | RSA | HE795069 | HE795069 | HE795069 |

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| Scientific Name                    | Country | Database Code | Accession Numbers                  |
|-----------------------------------|---------|---------------|-----------------------------------|
| Melianthus gariepinus Merxm. & Roessler | RSA     | Genbank       | DQ435417                          |
| Melianthus insignis Kuntze        | RSA     | Genbank       | DQ435413                          |
| Melianthus major L.               | RSA     | Genbank       | DQ435405                          |
| Melianthus pectinatus Harv.       | RSA     | Genbank       | DQ435414                          |
| Melianthus villosus Bolus         | RSA     | Genbank       | DQ435406                          |
| Monsonia emarginata L'Hér.        | Gartenherbarbeleg 44182 | B | RSA BSB 2992 HE795069 HE795473   |
| Monsonia marlothii (Engl.) F. Albers | Gartenherbarbeleg 28138 | B | RSA BSB 3029 HE795070 HE795474   |
| Pelargonium grossularifolium (L.) L'Hér. var. myrrhifolium | RSA | Genbank | Z95265 Z95289                    |
| Pelargonium altheoides L'Hér.     | RSA     | Genbank       | Z95278 Z95299                      |
| Pelargonium australis J. Jacq.    | Australia | Genbank | Z95256 Z95280                      |
| Pelargonium gravedens L'Hér.      | RSA     | BSB 3023 HE795071 HE795475   |
| Pelargonium odoratissimum (L.) L'Hér. | RSA BSB 3024 HE795072 HE795476   |
| Pelargonium peltatum (L.) L'Hér.  | RSA     | Genbank       | AF265755 AF167143                  |
| Pelargonium zonale (L.) L'Hér.    | RSA     | Genbank       | DQ345326 AF036088 ??             |
| Rhetenhoeca spinosa Ruiz & Pav.   | M. Weigend 9107 BSB M Ecuador BSB 2678 HE795073 HE795477 |
| Rhetenhoeca spinosa Ruiz & Pav.   | M. Weigend et al. 5413 CONC Chile BSB 2956 HE795077 HE795481 |
| Tetilla hydrocotylaefolia DC.     | T. Kern & M. Below 21 Krapovickas & Cristóbal 41977 | F | Brazil 2927-PEG HE795078          |
| Viviania albiapora (Cambess.) Reiche | B. Waasum 100 B KEW 18381 | B | KEW 18381 HE795079                |
| Viviana crenata (Hook.) G. Don    | C. Jiles 5556 M Chile BSB 2743 HE795080                   |
| Viviana elegans (Kunze ex Poepp.) F. Meigen | K. H. & W. Rechinger 63128 M Chile BSB 2744 HE795081 HE795482 |
| Viviana elegans Kunze ex Poepp.   | M. Mihoc et al. 7273 CONC Chile BSB 2957 HE795082 HE795483 |
| Viviana marifolia Cav.            | M. Weigend 9352 BSB Chile BSB 3042 HE795083 HE795484 |
| Viviana marifolia Cav.            | M. Weigend 9353 BSB Chile BSB 3073 HE795084 HE795485 |
| Viviana ovata Phil.              | M. Rosas 2159 CONC (169872) Chile BSB 2969 HE795085 HE795485 |
| Crossosoma a bigelovi S. Watson   | Ickert-Bond 1833 F USA DQ307116 DQ307148                   |
| Ribes densiflorum Phil.           | P. Brownless et al. 945 E Chile B2601 HE795075 HE795479 |
| Ribes densiflorum Phil.           | ACE324 E India B2602 HE795076 HE795480                   |
| Lopezia langmaniæe Miranda       | Breedve 32300 CAS Mexico AY264500 AY271253                 |
| Staphylea helocarpa Hemsl.         | D'Arcy & Rakotozafy 15317 MO n. ind. AY905465 AY905424             |
| Staphylea helocarpa Baker         | S. A. Graham, H. Tobe & Baas | n. ind. | AY905461 AY905423                 |
| Galphinia transvaalica N.E.Br.    | Balsinhas 3263 MO n. ind. AY905461 AY905423                 |
| Decodon verticillatus Elliott     | Graham 917 MO n. ind. AY905457 AY905421                 |
| Nesaea aspera Koehne              | Drummond 11446 MO n. ind. AY905475 AY905429                 |
| Lawsonia inermis L.               | Correll 45915 TEX AY905470 AY905426                 |
| Betula davurica Pall.             | Tibet 218 n. ind. n. ind. FO12055 FO11773                 |
| Ostrya rehderiana Chun             | Wen 5085 n. ind. n. ind. FO12041 FO11756                 |
| Staphylea helocarpa Hemsl.         | Wen 5740 F n. ind. DQ307114 DQ307110                      |
| Staphylea oblongifolius F. T. Wang & Tang | Zhu J-11 PE n. ind. DQ307114 DQ307110                      |