Biogeographic and cytogenetic evolution of the Alstroemeriaceae/Colchicaceae inferred from multi-locus molecular phylogenies, fluorescent *in situ* hybridization data, and probabilistic models of geographic and chromosome number change

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A mis padres

Preface

Statutory Declaration

Erklärung

Diese Dissertation wurde im Sinne von §12 der Promotionsordnung von Prof. Dr. Susanne S. Renner betreut. Ich erkläre hiermit, dass die Dissertation nicht einer anderen Prüfungskommission vorgelegt worden ist und dass ich mich nicht anderweitig einer Doktorprüfung ohne Erfolg unterzogen habe.

Eidesstattliche Erklärung

Ich versichere hiermit an Eides statt, dass die vorgelegte Dissertation von mir selbstständig und ohne unerlaubte Hilfe angefertigt wurde.

Juliana Chacón Pinilla

München, 25. Juli 2013

Note

In this thesis, I present the results from my doctoral research, carried out in Munich from September 2009 to July 2013 under the guidance of Prof. Dr. Susanne S. Renner. My thesis resulted in five manuscripts, presented in Chapters 2 to 6, of which two have been published (Chapters 2 and 5), two are in review (Chapters 3 and 4), and one has been accepted pending minor revision (Chapter 6). I also gave the presentations listed below. I generated all data and conducted all analyses myself, except for the estimation of ancestral chromosome numbers in Colchicaceae (part of Chapter 6), which was done with help of Dr. Natalie Cusimano, and the identification of fossil leaves from New Zealand (Chapter 3), which was done in collaboration with Dr. John G. Conran (The University of Adelaide, Australia), Dr. Dallas C. Mildenhall (Institute of Geological and Nuclear Sciences, New Zealand), Dr. Jennifer M. Bannister, and Dr. Daphne E. Lee (University of Otago, New Zealand). Writing and discussion involved collaboration with Professor Susanne Renner.

List of publications

Peer-reviewed journal articles

CHACÓN, JULIANA, M. C. ASSIS, A. W. MEEROW, AND S. S. RENNER. 2012. From East Gondwana to Central America: Historical biogeography of the Alstroemeriaceae. *Journal of Biogeography* 39: 1806–1818. (accepted April 13th 2012)

CHACÓN, JULIANA, A. SOUSA, C. M. BAEZA, AND S. S. RENNER. 2012. Ribosomal DNA distribution and a genus-wide phylogeny reveal patterns of chromosomal evolution in *Alstroemeria* (Alstroemeriaceae). *American Journal of Botany* 99: 1501–1512. (accepted 6thAugust 2012)

CHACÓN, JULIANA, N. CUSIMANO, AND S. S. RENNER. 2013. The evolution of Colchicaceae, with a focus on changes in chromosome numbers. *Systematic Botany*. (accepted pending minor revision, 5th July 2013)

CHACÓN, JULIANA AND S. S. RENNER. 2013. Assessing model sensitivity in Likelihood Analyses of Geographic Range Evolution (LAGRANGE): A study

using Colchicaceae and experimental data. *Journal of Biogeography* (manuscript classified as requiring full revision, May 21st 2013).

CONRAN, J. G., J. M. BANNISTER, D. C. MILDENHALL, D. E. LEE, J. CHACÓN, AND S. S. RENNER. Leaves and a flower with *in situ* pollen of *Liliacidites contortus* Mildenh. sp. nov. from the Late Oligocene–Early Miocene of New Zealand. *American Journal of Botany* (in review).

Posters

CHACÓN, J. AND S. S. RENNER. Molecular phylogenetics and biogeography of Alstroemeriaceae, an important clade of the Austral floristic realm. *VW Status Symposium in Evolutionary Biology*. Frauenchiemsee, Germany, May 9 – 12, 2010.

Oral presentations

CHACÓN, JULIANA. AND S. S. RENNER. When do models that account for changing continental connectivities make a difference? An example from the Colchicaceae. *66th International Conference of the International Biogeography Society.* Miami, Florida, USA, January 8–13, 2013.

CHACÓN, JULIANA. History of a pair of clades that parted ways in East Gondwana and then followed non-overlapping trajectories: corms and fire-adaptations in South Africa, the Mediterranean, and Australia vs. hummingbird pollination in South America. *EES LMU Conference 2012*, Ludwig Maximilians University, Munich, Germany, October 4–5, 2012.

CHACÓN, JULIANA. AND S. S. RENNER. Alstroemeriaceaeae, a plant family with an Austral-Antarctic distribution that expanded into tropical latitudes – inferring the when and how. *14th Nordic Meeting on Tropical Botany*. Gothenburg, Sweden, August 6–8, 2012.

CHACÓN, JULIANA, A. VINNERSTEN, AND S. S. RENNER. Nuclear and mitochondrial data tell a new story about genus boundaries and biogeography of the Colchicaceae. *Southern African Society for Systematic Biology 10th Meeting, SASSB X*. Arniston. South Africa, July 16–20, 2012.

CHACÓN, JULIANA, M. CAMARGO DE ASSIS, A. W. MEEROW, AND S. S. RENNER. New insights into the biogeography of the Austral floristic realm from a complete phylogeny for the Alstroemeriaceae. *XVIII International Botanical Congress IBC 2011*. Melbourne, Australia, July 23 – 30 2011.

CHACÓN, JULIANA AND S. S. RENNER. Phylogeny and biogeography of the Alstroemeriaceae, an important clade of the Austral floristic realm. *BioSystematics Berlin 2011*. Berlin, Germany, February 21 – 27, 2011.

Herbaria visited

- München (M), Germany, 2009–2012
- Universidad de los Andes (ANDES), Colombia, August 2010
- Universidad del Valle (CUVC), Colombia, August 2010
- Universidad de Antioquia (HUA), Colombia, August 2010
- Universidad Nacional Sede Medellín (MEDEL), Colombia, August 2010
- Jardín Botánico Joaquín Antonio Uribe (JAUM), Colombia, August 2010
- Herbario Nacional Colombiano (COL), Colombia, September 2010
- Berlin (B), Germany, February 2011
- Sydney (NSW), August 2011
- University of Cape Town (BOL), July 2012
- South African National Biodiversity Institute (NBG), July 2012
- University of Gothenburg (GB), August 2012

Field work

- Colombia, July 2008
- South Africa, July – August 2012

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- Travel to the conference in Berlin by the EES^{LMU} Travel Grant

Contents

Summary

This dissertation addresses two issues of key importance in the evolution and diversification of plants, namely geographic expansion and isolation, and chromosomal change. To study these two topics, I focused on sister families in the Liliales, the Alstroemeriaceae and the Colchicaceae. Specifically, I studied (i) the biogeography of the Alstroemeriaceae using standard methods of historical biogeography, (ii) the biogeography of the Colchicaceae exploring the sensitivity of results to model assumptions, (iii) chromosome evolution in *Alstroemeria* (Alstroemeriaceae) using fluorescent *in situ* hybridization (FISH), and (iv) chromosome number evolution in the Colchicaceae using event-based maximum likelihood models.

The first biogeographic chapter of my thesis focuses on the Alstroemeriaceae, a family of c. 200 species in four genera: *Alstroemeria* and *Bomarea*, with c. 198 species restricted to the Neotropics, *Drymophila*, with two species native to Australia, and *Luzuriaga*, with three species in Chile and one in New Zealand. It is one of 28 flowering plant families shared between South America, New Zealand, and Australia. I investigated its biogeography by means of a molecular phylogeny (resulting from 3130 aligned nucleotides for 125 species, mostly newly sequenced), a Bayesian dating analysis with fossil calibrations, and a parsimony-based ancestral area reconstruction method. As a contribution to the larger question of the origin of the Neotropic biota, I compared key biological traits and diversification times of the Austral-Antarctic families that spread from Patagonia to the equator. The most recent common ancestor of the Alstroemeriaceae-Colchicaceae apparently lived c. 93 million years ago (Ma) in East Gondwana (Australia, Antarctica and India), which was connected to West Gondwana (South America and Africa) via Antarctica. *Alstroemeria* and *Bomarea* diversified in the Miocene (18–11 Ma) during the main uplift of the Andean mountains. Only five of the 28 families, including the Alstroemeriaceae, expanded all the way from Patagonia to Mexico and eastern Brazil. A main dispersal barrier appears to have been the South American Arid Diagonal, an arid belt that crosses South America's Southern Cone from east to west. This zone originated as a result of the Andean uplift c. 16 Ma. The single *Luzuriaga* species living in New Zealand today apparently resulted from a recent (c. 7 Ma) long-distance dispersal event from

Chile, while a leaf of *Luzuriaga* (and an associated flower with *in situ* pollen) from the Early Miocene (23 Ma) of New Zealand represents an extinct relative and constitutes another proof of the biogeographic connections that existed between South America and New Zealand during the Oligocene-Miocene. With colleagues from New Zealand, I became a coauthor on the description of these fossils.

The second biogeographic chapter of this thesis focuses on the Colchicaceae, a family of c. 270 species in 15 genera that occur on all continents except Central and South America. For this analysis, I used a maximum likelihood-based approach of ancestral range evolution implemented in the software LAGRANGE (Ree et al., *Evolution*, 59, 2299–2311, 2005). This parametric method can incorporate information about past dispersal routes through user-defined *a priori* settings. To explore the effects of such *a priori* settings I conducted experiments in LAGRANGE using my Colchicaceae data matrices (6451 aligned nucleotides for up to 83 species, mostly newly sequenced) and artificial data. I found that the use of unconstrained adjacency matrices (which concern permitted/forbidden range connections) and a balanced number of nodes per time slice (i.e., user-defined geologic periods in the past) give the most trustworthy results. The best-fit model and a time-calibrated phylogeny for the Colchicaceae showed that this family diversified in Australia about 75 Ma and then dispersed to southern Africa during the Paleocene–Eocene (c. 62 Ma). The ancestor of the clade comprising the genera *Uvularia* and *Disporum* dispersed to the Eurasian continent and from there to North America (28–16 Ma) via the Bering land bridge. Two expansions out of South Africa occurred during the Miocene: eastwards across the Indian Ocean to Australia (*Wurmbea*), and northwards to the Mediterranean (*Colchicum*). The presence of underground storage stems or corms probably was a key adaptation for surviving the fire regimes that characterize South Africa and Australia since Miocene times.

The first of the two cytological chapters of my thesis focuses on chromosome evolution in *Alstroemeria*, based on a newly generated DNA phylogeny. Although all *Alstroemeria* species counted so far have $n = 8$ chromosomes, even closely related species can differ strikingly in their ribosomal DNA (rDNA). To study this aspect, I mapped the 5S and 18-25S rDNA genes in Brazilian and Chilean alstroemerias by FISH and analyzed the data in a phylogenetic context. The results imply a rapid increase, decrease, or translocation of the ribosomal genes during the evolution of

Alstroemeria. The FISH experiments also revealed telomeric sequences located near the centromeres of *A.* cf. *rupestris*, indicating a possible Robertsonian fusion. This finding suggests that the same mechanism could have occurred during the divergence from the sister genus, *Bomarea*, which has a basic chromosome number $n = 9$, instead of 8 as in *Alstroemeria*.

My second chromosome study focuses on the Colchicaceae, which are characterized by highly variable chromosome numbers and ploidy levels, especially in the genus *Colchicum*. To understand how this diversity arose, my coauthors and I used a maximum likelihood approach to infer ancestral chromosome numbers for clades of interest and the possible events that may explain the observed chromosome number in today's species. We found that a main mechanism of chromosome number evolution in most Colchicaceae clades has been the gain or loss of single chromosomes (dysploidy or aneuploidy). An exception was *Colchicum* in which polyploidization played a major role, presumably as a connection with hybridization.

General Introduction

A wide array of biotic and abiotic factors, interacting with each other over long periods of time, have driven the evolution and diversification of plants in different ecosystems. Over the past 20 years, the rise of molecular-clock dating, in combination with increasingly complex statistical tools, has allowed assessing the plausibility of some of these factors, such as continental drift, the onset of particular types of climates, or the diversification of specialized groups of pollinators, by comparing the temporal coincidence of events. Although temporal and geographical correlations cannot establish causation, they can provide likely explanations that can be tested by comparing multiple clades that experienced the same biotic or abiotic conditions. Like all correlation studies, one will only find associations among factors included in the analysis, and great care must therefore be taken not to overestimate the role of particular factors.

Research focusing on molecular clocks and their application to a wide spectrum of biological questions has nowhere had a greater impact than in historical biogeography. This field has been revolutionized by the ability to infer dates of lineage splits and to reconstruct ancestral areas of clades. There has been a tendency, however, for studies to focus exclusively on continental movement, orogeny, origin of land connections, and different climates as explanations for clade diversification. Other explanatory factors have received little attention, probably because they are more difficult to include in correlation studies than are abiotic factors. For plants, the first of such intrinsic biotic factors coming to mind is chromosomal change, especially polyploidy and other types of changes in the organization of an organism's genome. Such changes were the focus of research during the period of biosystematics (1920 to c. 1960), but were neglected in diversification studies during the beginning of the 21st century. They are currently experiencing a come-back (e.g., Adams et al., 2000; Martínez et al., 2010; Lan and Albert, 2011; Catalán et al., 2012; Cusimano et al., 2012; Weiss-Schneeweiss et al., 2012; Cristiano et al., 2013).

For my doctoral research I decided to bring together the study of historical biogeography and chromosome evolution in a system involving two plant families, using methods from cytogenetics, phylogenetics, molecular clock dating, ancestral character reconstruction, and probabilistic models of chromosome change. My focus

is on the Alstroemeriaceae-Colchicaceae clade in the order Liliales of the monocots. This clade of c. 470 species has an intriguing disjunct distribution (see Appendix 1), with species diversity located either in the Neotropics (Alstroemericeae) or in Africa, Australia, Eurasia, and North America (Colchicaceae). From an evolutionary perspective, the clade is also interesting because of its karyotype characteristics, with a highly dynamic ribosomal DNA in *Alstroemeria* (Alstroemeriaceae) and a great diversity of chromosome numbers (ranging from $2n = 14$ to $2n = 216$) in the Colchicaceae.

In the first part, I focus on the biogeography of the Alstroemeriaceae and the Colchicaceae and use fossil-dated phylogenies (cross-validated by alternative approaches) and ancestral area reconstruction methods to shed light on the species diversification in disjunct geographic areas of the world. In the second part, I focus on chromosome evolution in the two families and use FISH data to infer patterns of chromosome restructuring in *Alstroemeria* and likelihood-based models to estimate ancestral chromosome numbers and chromosome evolution in the Colchicaceae.

Historical biogeography of Alstroemeriaceae and Colchicaceae

Recent methodological progress in biogeography

Biogeography is the study of the distribution of organisms through space and time. While this field of research goes back at least to the 1850s (Alfred Russel Wallace; Moritz Wagner; Charles Darwin), it is only recently that methods have become available that can fully exploit the information relevant to biogeographic history contained in molecular trees. The "classic" quantitative methods, among them Dispersal-Vicariance Analysis (DIVA; Ronquist, 1994, 1996, 1997), rely on parsimony (which minimizes change regardless of the time between splits in the tree) and are therefore unable to incorporate information about relative divergence times contained in the branch lengths of molecular trees. There is also no straightforward way to assess the uncertainty in the biogeographic inference that arises from poorly supported phylogenetic relationships. Since 2005, several methods have been proposed that take into account genetic branch lengths or that integrate over topological uncertainty and branch length uncertainty (Ree et al., 2005; Nylander et

al., 2008; Ree and Smith, 2008; Lamm and Redelings, 2009; Ree and Sanmartín, 2009; Yu et al., 2010).

Parsimony-based dispersal-vicariance analyses using DIVA (Ronquist, 1994, 1996, 1997), S-DIVA (Yu et al., 2010) or Bayes-DIVA (Nylander et al., 2008; Buerki et al., 2010) have the advantage that one does not need to specify model parameters or prior probabilities as one does in Bayesian approaches. The method uses a "cost matrix" that assigns costs of 1 for dispersal and extinction events and no costs for vicariance and within area speciation events, thus favoring vicariance over dispersal (Lamm and Redelings, 2009). DIVA requires a fully resolved topology, while S-DIVA and Bayes-DIVA integrate over Markov chains of trees that differ in poorly supported nodes. All three approaches have the disadvantage that they often lead to unrealistically large ancestral ranges. This is because parsimony tends to underestimate change along branches, which is equivalent to underestimating dispersal and instead favoring widespread ancestors.

The relatively recent Dispersal-Extinction-Cladogenesis (DEC) approach implemented in LAGRANGE, which stands for **L**ikelihood **A**nalysis of **G**eographic **Ran**ge **E**volution (Ree et al., 2005; Ree and Smith, 2008), has the advantage that it incorporates the information contained in branch lengths (the essence of all maximum likelihood approaches). It has the disadvantage, however, that it not only requires a fully bifurcated tree (as does DIVA) but moreover two user-defined matrices. One of these matrices is the "adjacency matrix" (this is how this matrix is called in the online LAGRANGE configurator), where the user defines the range constraints. The adjacency matrix basically defines which area connections are allowed in the model, and it only accepts "0" or "1" (similar to the cost matrix in DIVA). The other is the area-dispersal matrix, where the user defines the values for the dispersal probabilities based on prior notions of the likelihood of dispersal between geographic regions (range expansion) or extinction (range contraction). This matrix accepts probabilities between 0 and 1, and the user can built as many area-dispersal matrices for different periods of time ("time slices") as is deemed appropriate. The assignment of such probabilities thus differs between studies. For example, the probability of dispersal between Australia and South America during the Cretaceous (145–66 Ma), when these landmasses were connected across Antarctica, was assigned $P = 1$ in Buerki et al. (2011), $P = 0.5$ in Mao et al. (2012), and $P = 0.01$ in Nauheimer et al. (2012). With a time-calibrated

tree (a so-called chronogram) and the two required matrices, LAGRANGE can estimate dispersal and extinction rates and probabilities of range inheritance scenarios (Ree and Smith, 2008). This means, however, that this method (DEC modelling) requires many more *ad hoc* parameter values than does DIVA.

LAGRANGE also calculates the global likelihood of a biogeographic hypothesis of range inheritance for a group of taxa given a set of parameter values (Ree et al., 2005), and in principle these likelihoods can be compared when model parameters are changed. A likelihood ratio test, however, cannot be used to compare the likelihood scores between different DEC models because they are not nested (that is, they differ in more than one parameter). Instead, the Akaike information criterion (AIC; Akaike, 1974) provides a way to compare non-nested models. One limitation of the DEC approach – and parametric methods in general – is that the number of biogeographic parameters to estimate from the data increases exponentially with the number of areas, increasing computational time and decreasing the inferential power of the model (Ree and Sanmartín, 2009). DEC also sometimes overestimates the frequency of extinction events (i.e., ancestral ranges that are outside the extant species ranges), owing to dispersal constraints enforced by the model, i.e., the transition probability matrix (Buerki et al., 2010).

Some studies have compared results obtained with DIVA (and its statistical derivatives S-DIVA or Bayes-DIVA) versus the DEC approach. One concerned the genus *Cyrtandra* (Gesneriaceae), which is widespread on oceanic islands in the Pacific (Clark et al., 2008). The only plausible explanation for the observed disjunctions is over-water dispersal (which was indeed inferred), but the study suffered from its sole calibration point being the age of an island. The second study to compare results obtained with DIVA and DEC focused on the Simaroubaceae (Clayton et al., 2009). In their DEC analysis, Clayton et al. used a single transition model with four time slices (between 5 Ma to present, 30 Ma to 5 Ma, 45 Ma to 30 Ma and 70 Ma to 45 Ma) and probabilities between 0 and 1 depending on the closeness of the areas. The authors used the same adjacency (cost) matrix in their DIVA and DEC analyses. The comparison showed that the DEC analysis revealed multiple ranges in younger clades, but was unable to infer events deeper in the phylogeny. DIVA produced similar results when ancestral ranges were restricted to two areas, but even then gave improbably large ancestral ranges at several nodes. A

comparison of Bayes-DIVA and DEC inferences in the Sapindaceae (Buerki et al., 2010) showed that DEC gave reconstructions that were in better agreement with palaeogeographical evidence, but reconstructed ancestral ranges with high levels of uncertainty, probably because of low inferential power when many area transitions are being inferred from a phylogeny with too few nodes (Ree and Sanmartín, 2009). Finally, a study of *Alocasia* (Araceae) that compared results from S-DIVA and DEC found congruence except for contradictions in the deepest nodes, where S-DIVA inferred combined (implausibly large) ancestral areas more often than did DEC, while DEC inferred more dispersal events than did DIVA (Nauheimer et al., 2012).

As explained above, the DEC approach implemented in LAGRANGE requires two user-defined matrices, the adjacency matrix and the area-dispersal matrix. Different area-dispersal matrices can be assigned to different time slices of cladogenesis, as if one were assigning a particular nucleotide substitution model to a period between x and y million years, followed by a different model for the adjacent period of t and z million years. Some studies have assessed model fit by comparing schemes with many or few time slices and/or with different dispersal probabilities. Couvreur et al. (2011) and Baker and Couvreur (2013) compared unconstrained models without time slices to constrained models with 5 time slices. In both studies, the constrained models had higher likelihoods. Mao et al. (2012) compared models with four to eight time slices using dispersal probabilities between 0.1 to 1.0. They found that the eight-time-slice model fit their data best as it had the best likelihood score calculated by LAGRANGE. In a similarly-sized data set, Nauheimer et al. (2012) compared models with three or four time slices, but found that the three-time-slice-model fit best. For a study of the genus *Psychotria* in Hawaii, Ree and Smith (2008) varied the adjacency matrix, and found that a constrained matrix fit the data better. All these studies show the importance of evaluating the effects of the user-defined parameteres when choosing a model to reconstruct the evolution of ancestral ranges in LAGRANGE. Experiments would need to address the effects of changing the number of time slices and thus the nodes falling within each slice. A critical evaluation of the pitfalls and strengths of introducing time slices in DEC analysis will be useful for future studies, since transition probability matrices can be (and have been) used across studies of clades of similar ages and geographic distribution (for example, similar connectivity matrices were used for the cosmopolitan families Sapindaceae and Araceae, which

began to diversify during the Early Cretaceous; Buerki et al., 2011, Nauheimer et al., 2012).

Neotropical biogeography

Studies of the evolution of the Neotropical flora have increased dramatically over the last ten years. This has resulted from a combination of factors, such as the availability of cheaper DNA sequencing, the development of statistical tools and computer platforms, and the rapid development of the relevant earth sciences geology, climatology, and paleontology, which have provided essential data for reconstructing past biological scenarios. As a result, the origins of biodiversity hotspots, such as the Andean mountains in western South America, have become better understood.

The tremendous impact that especially the Andean uplift had in the diversification of plants has been demonstrated in studies of legumes (*Lupinus*: Hughes and Eastwood, 2006; *Amicia, Coursetia*, *Cyathostegia*, *Mimosa*, and *Possonia*: Särkinen et al., 2012), the coffee-family (Rubiaceae: Antonelli et al., 2009) or the *Espeletia* complex (Asteraceae: Rauscher, 2002). All these genera underwent rapid adaptive radiations in response to the new ecological niches created during the Andean uplifting. Páramos offer an amazing example of such radiations. These island-like habitats at high altitudes on the Andes (3000–4800 m) support one of the richest tropical alpine floras in the world (>3,500 species; Luteyn, 1999), but evolved only over the last 3–5 million years (My) of mountain building from both Northern and Southern Hemisphere elements (Gregory-Wodzicki, 2000). Another island-like biome that assembled during the Andean uplift is the seasonally dry tropical forest, a biome restricted to the rain shadowed inter-Andean valleys and the Pacific coast in South America (0–2500m), and which evolved over the past 15 My (Hartley, 2003).

Stable isotope data suggest that the uplift of the Andes occurred in pulses, the most recent one currently dated to 10–6 Ma, and a previous one about 25 Ma (Garzione et al., 2008; Capitanio et al., 2011). Paleoelevation reconstructions indicate that the Altiplano area, which still lay at sea-level at the end of the Cretaceous (Coney and Evenchick, 1994; Sempere et al., 1997), had reached only half of its current elevation when the Late Miocene uplift phase set in (Gregory-Wodzicki, 2000; Garzione et al., 2008). Atmospheric circulation models have recently corroborated the

effects of the Andean uplift on the South American climate (Insel et al., 2010). The development of strong rain shadow effects on the western slopes of the Central Andes in the Altiplano area and on the eastern slopes of the Patagonian Andes caused the establishment of the South American Arid Diagonal (SAAD; Eriksen, 1983; Blisniuk et al., 2005), a belt of dry ecosystems that reaches from the Peruvian and Atacama Desert to the Patagonian steppes, crossing the Andes between 22º and 26°S (Maldonado et al., 2005). In the southern part of the SAAD, the uplift of the Patagonian Andes caused the development of the Monte desert and the Patagonian steppes on the eastern side of the Andes from about 14–15 Ma onward (Blisniuk et al., 2005). These new arid habitats, together with the newly created alpine environments above the timberline in the Andes, provided a unique opportunity for the evolution and diversification of arid-adapted lineages.

The Alstroemeriaceae family

The Andes between the tropics of Capricorn and Cancer are one of five important biodiversity hotspots, with approximately 45,000 vascular plant species, half of which are endemic (Myers et al., 2000). Among the angiosperm families with the highest degree of endemism in the Andean region is the Alstroemeriaceae (Liliales), with c. 80% of its 204 species growing in Andean cloud forests, high-Andean grasslands (páramo and puna) and inter-Andean dry valleys (Hofreiter, 2007). Most species belong to the genus *Bomarea* (120 species) and are distributed from central Mexico to Chile and Argentina, with one species in Brazil. The highest species diversity is found in the northern Andes of Colombia and Ecuador, and in the Central Andes of Peru (Hofreiter and Tillich, 2002; Harling and Neuendorf, 2003; Hofreiter and Rodriguez, 2006; Alzate et al., 2008). Bomareas are predominantly climbers with colorful inflorescences that are hummingbird-pollinated. The second-largest genus is *Alstroemeria* (c. 78 species), which occurs from southern Peru to Patagonia, and is especially diverse in the seasonal Mediterranean steppes of Chile and Argentina (Aagesen and Sanso, 2003), and in eastern Brazil (Assis, 2001). Alstroemerias are erect herbs, which are either bee-pollinated (Chilean species) or humming-bird pollinated (Brazilian species; Buzato et al., 2000 and Appendix 2). Apart from these large Andean groups the two small genera, *Luzuriaga* and *Drymophila*, also belong in the Alstroemeriaceae. *Luzuriaga* has an intriguing disjunct distribution, with three

species in Chile and one in New Zealand (Arroyo and Leuenberger, 1988; Wardle et al., 2001), and *Drymophila* has two species native to eastern Australia and Tasmania (Conran and Clifford, 1998).

Previous molecular phylogenetic studies of the Alstroemeriaceae have been focused either on *Alstroemeria* (Aagesen and Sanso, 2003) or on *Bomarea* (Alzate et al., 2008), while large-scale studies of the Liliales (Chase et al., 1995; Rudall et al., 2000; Vinnersten and Bremer, 2001; Fay et al., 2006; Petersen et al., 2012) have included only one species of *Luzuriaga* and/or one of *Drymophila*. Therefore, neither the mutual monophyly nor the relationships of the four genera were reliably known when I started my doctoral research.

The Austral floristic realm

From a biogeographic perspective, Alstroemeriaceae belong to the Austral floristic realm. This realm is comprised of 15 Southern Hemisphere families that are restricted to South America and Australasia (Takhtajan, 1986; Moreira-Muñoz, 2007). While the discovery of the floristic relationships between southernmost South America and New Zealand goes back to Treviranus (1803), relatively few phylogenetic studies have focused on this realm. Only six of the 15 families have been analyzed with molecular clocks [e.g., Araucariaceae: Liu et al., 2009; Atherospermataceae: Renner et al., 2000; Calceolariaceae: Nylinder et al., 2012; Cunoniaceae: Barnes et al., 2001; Escalloniaceae (*Escallonia*): Zapata, 2013; Nothofagaceae: Knapp et al. 2005; Proteaceae: Barker et al. 2007, Sauquet et al., 2009; Restionaceae: Linder et al., 2003; not yet studied biogeographically: Asteliaceae, Berberidopsidaceae, Centrolepidaceae, Corsiaceae, Donatiaceae, Griseliniaceae, and Stylidiaceae].

Some of the floristic relationships between South America, Australia, and New Zealand are probably due to the break-up of East Gondwana (Antarctica, Australia/New Zealand, Madagascar, and India). For a long period, the closest connection between East Gondwana and West Gondwana was the southern tip of South America, a region that therefore is of great biogeographic interest. Patagonia and Antarctica were connected by land bridges during times of low sea level (Stevens, 1989; Reguero et al., 2002; Cione et al., 2007; Iglesias et al., 2011), and Antarctica and Australia remained connected via the Tasman Rise until the Eocene-Oligocene

boundary (37 Ma). Eocene paleo-temperatures at high southern latitudes, for example, near Seymour Island, off the NE side of the Antarctic Peninsula, indicate a 10°C cooling from the early Eocene climatic optimum (when mean temperatures were about ~15 °C) through the end of the Eocene (minimum ~5°C; Ivany et al., 2008). Much of this cooling took place between 52 and 41 Ma, with conditions continuing to deteriorate more gradually thereafter. However, the Antarctic coastline and the Transantarctic Mts. supported *Nothofagus* forests well into the mid-Miocene (15–13 Ma; Truswell, 1989). The gradual severance of land connections, combined with a drastically changing climate, created the complex background against which the evolution of the 15 seed plant families that define the Austral floristic realm needs to be placed and interpreted.

It is clear, however, that long-distance dispersal also has played an important role in shaping the Austral floristic realm. A recent meta-analysis reported 226 transoceanic dispersal events in vascular plant clades of the southern hemisphere, including the Cape region (Crisp et al., 2009). Indeed, the resurrection of transoceanic dispersal (Muñoz, et al., 2004; Renner, 2005; McGlone, 2005) as an explanation for range disjunctions has become so pervasive that long-distance dispersal now seems a more plausible *a priori* explanation for most disjunctions than continental drift (Christenhusz and Chase, 2012). Nevertheless, there are angiosperm clades that predate the break-up of East Gondwana, and such clades present intriguing puzzles for historical biogeography, requiring careful testing of alternative explanations for geographic range disjunctions.

The split between Alstroemerioideae (*Alstroemeria* and *Bomarea*) and Luzuriagoideae (*Luzuriaga* and *Drymophila*), that is the crown group of Alstroemeriaceae, has been dated to 79 Ma; that between Chilean *Luzuriaga* and Australian/Tasmanian *Drymophila* to 56 Ma (Janssen and Bremer, 2004). Both ages would be sufficiently old for overland dispersal between Australasia and South America during the Upper Campanian to Late Palaeocene, when Antarctica carried tropical vegetation (Axsmith et al., 1998; Poole and Gottwald, 2001) and was home to huge dinosaurs (Agnolin et al., 2010). The above-cited age estimates are based on five *rbcL* sequences of Alstroemeriaceae that were part of a large (800 sequence) molecular dating effort for all monocots (Janssen and Bremer, 2004). Other molecular clock studies of divergence times in monocots have included up to five *rbcL*

sequences of Alstroemeriaceae (*Alstroemeria* + *Luzuriaga*: Bremer, 2000; *Alstroemeria*, *Bomarea*, *Leontochir*, *Drymophila, Luzuriaga*: Janssen and Bremer, 2004; same data re-analyzed: Britton et al., 2007; Anderson and Janssen, 2009). They all inferred ages similar to those quoted above (Alstroemeriaceae crown group: 79 Ma; Tasmanian *Drymophila* vs. Chilean *Luzuriaga*: 56 Ma). However, these ages were obtained with just very few species (see above, section "The Alstroemeriaceae family"), a single chloroplast marker (*rbc*L), or partially wrong topologies (for example, in Bremer, 2000, and in Janssen and Bremer, 2004 *Luzuriaga* is sister to *Colchicum* rather than to *Alstroemeria*).

The discovery of fossil leaves that ressemble living *Luzuriaga* in lake sediments near Otago, New Zealand (J. Conran, personal communication, May 2010), will help to elucidate the geographic disjunctions found in the Alstroemeriaceae as it would constitute the first fossil record for the whole Alstroemeriaceae/Colchicaceae clade (based on the Paleobiology Database, http://paleodb.org, accessed on 21 May, 2013 using the "taxonomic search form" option and the scientific names "Alstroemeriaceae" and "Colchicaceae").

The Colchicaceae family

After mentally leaving the tropical Andes and moving across the Atlantic Ocean to southern Africa, we come to the Greater Cape Floristic Region (Born et al., 2007), another of the world's biodiversity hotspots (Myers et al., 2000). Climatically, it is characterized by winter rainfall. It harbors two vegetation types, the *fynbos* and the succulent Karoo, and is the home of many geophytes (plants with underground storage organs), including the Colchicaceae. At least 80 of that family's 270 species are endemic to the Greater Cape Floristic Region (Nordenstam, 1998; del Hoyo et al., 2009). Colchicaceae are seasonal plants with subterranean storage stems associated with renewal buds (corms or rhizomes; Nordenstam, 1998). A synapomorphy of the family is colchicine, a medicinal alkaloid traditionally used in the treatment of gout, and also in cytogenetics due to its properties as a cell division inhibitor (Vinnersten and Larsson, 2010).

The Colchicaceae are the sister family of the Alstroemeriaceae and have 16 genera (but see the next paragraph and the *Discussion* section about the

circunscription of genera) distributed in Africa, Eurasia, Australia, and North America (Nordenstam, 1998). The strictly African genera are *Baeometra* (1 species), *Camptorrhiza* (2 species), *Hexacyrtis* (1 species), *Ornithoglossum* (8 species), and *Sandersonia* (2 species); the strictly Australian genera are *Burchardia* (6 species), *Kuntheria* (1 species), *Schelhammera* (2 species), and *Tripladenia* (1 species). *Disporum* (20 species) is native to Asia. *Uvularia* (5 species) is restricted to North America. *Colchicum* (c. 100 species) occurs in Eurasia from the Mediterranean to western Asia. Four genera have disjunct geographic distributions: *Iphigenia* (12 species) occurs in Africa, India and Australasia, *Gloriosa* (10 species) in Africa, India, and south-eastern Asia, *Androcymbium* (57 species) in extreme southern and northern portions of Africa, and *Wurmbea* in Australia (c. 30 species) and South Africa (20 species) (Vinnersten and Manning, 2007; del Hoyo and Pedrola-Monfort, 2008; Persson et al., 2011). The closest relatives of the Alstroemeriaceae-Colchicaceae clade are the Petermannianceae (Fay et al., 2006; Petersen et al., 2012), a monotypic family (the only species is *Petermannia cirrosa*) of rhizomatous woody climbers restricted to temperate rainforests in east Australia (Conran and Clifford, 1998).

Previous molecular-phylogenetic work on the Colchicaceae relied on plastid sequences and led to the recognition of six small tribes (Burchardieae, Uvularieae, Tripladenieae, Iphigenieae, Anguillarieae, and Colchiceae) as well as re-circumscription of the genera *Wurmbea* (including *Onixotis* and *Neodregea*), *Colchicum* (including *Androcymbium*, *Bulbocodium*, and *Merendera*), and *Gloriosa* (including *Littonia*) (Vinnersten and Reeves, 2003; Vinnersten and Manning, 2007). A recent phylogenetic study that used chloroplast DNA sequence data recovered the same tribal and generic re-circumscriptions but reverted to treating *Onixotix* and *Neodregea* as separate genera instead of including them in *Wurmbea* (Nguyen et al., 2013).

The taxonomic status of *Androcymbium* and *Colchicum* also is still controversial. A redefinition of the genus *Colchicum* to include *Androcymbium* was proposed by Manning et al. (2007) and was accepted by Persson (2007) and Nguyen et al. (2013), while del Hoyo and Pedrola-Monfort (2008) preferred to treat *Androcymbium* and *Colchicum* as separate genera. A recent phylogenetic analysis of *Colchicum* by Persson et al. (2011), which included molecular, morphological, and

cytogenetic data for 96 of the 100 species, only sampled three species of *Androcymbium* and thus could not test the relationships between the two genera properly.

State of the art of Colchicaceae biogeography

The intriguing distribution of the Colchicaceae, which are found on every continent except Central and South America, and the absence of a fossil record leaves open the question about where Alstromeriaceae and Colchicaceae diverged from each other: (i) The split could have occurred in Australia (with subsequent spread of the ancestor of Alstromeriaceae to South America); (ii) it could have occurred in Antarctica; or (iii) it could have occurred in South America (with subsequent spread of the ancestor of Colchicaceae to Australia and beyond).

By the Turonian (93.9–89.8 Ma), monocots were already relatively diverse as evident from fossil flowers of Triuridaceae (Gandolfo et al., 1998, 2002) and much older (112 Ma old) flowers with associated pollen of Araceae (Friis et al., 2004, 2006, 2011; reviewed in Doyle et al., 2008). A Cretaceous origin of the Alstroemeriaceae/Colchicaceae split was earlier inferred based on an *rbc*L clock (Vinnersten and Bremer, 2001). To understand the geographic unfolding of the Colchicaceae/Alstroemeriaceae clade, the geologic context from the Turonian onwards is required. After Pangea had broken into the two supercontinents Laurasia (comprising North America, Europe and Asia) and Gondwana (South America, Africa, India, Antarctica and Australia; Smith et al., 1994; Scotese, 2001), there was a long period during which epicontinental seaways and intercontinental connections divided it into Euramerica (Europe and eastern North America, linked across the Atlantic) and Asiamerica (Asia and Western North America, linked via the Beringian Land Bridge). With the closing of the Tethys Seaway at the Oligocene/Miocene transition, Africa (part of West Gondwana) approached Europe at Gibraltar and Asia at the Isthmus of Suez, allowing Gondwanan elements to come back in contact with Laurasian ones and causing numerous faunal and floral exchanges among the regions. These connections may have permitted the Colchicaceae to move northwards; this of course needs testing.

Long distance dispersal also is known to have played a role in shaping the distribution of Colchicaceae. For example, the *Colchicum-Androcymbium* clade diverged from is closest relatives in southwestern Africa (Caujapé-Castells et al., 2001, 2002) around 13.4 ± 1.5 million years ago, followed by dispersal west and northward to several arid regions of Africa (del Hoyo et al., 2009). The geographic distribution of *Wurmbea* on separate sides of the Indian Ocean could have resulted from eastward *trans*-oceanic dispersal out-of-southern-Africa to the south-easternmost regions of Australia, by means of the West Wind Drift (Bergh and Linder, 2009). This Antarctic Circumpolar Current that flows from west to east around Antarctica has facilitated the transport of benthic echinoderms between Africa and Australia (Knox, 1980; Waters and Roy, 2004), but its role for the transport of plant parts (floating debris, floating stems, perhaps with seeds or other propagules attached) is poorly understood. Any scenario of ocean rafting also only becomes plausible after the Eocene-Oligocene, once the West Wind Drift became established (Stickley et al., 2004). Lastly, there is no evidence that *Wurmbea* seeds are tolerant to marine salt-water.

Chromosome evolution

Understanding how species interact with each other and when and where the diversification of clades has taken place, provides hints about the process of speciation in plants. A more detailed view can only be achieved by looking at the mechanisms responsible for the reproductive isolation of species. Although the role of chromosomal rearrangements as mechanisms for plant speciation is still debated (Faria and Navarro, 2010) studies of the distribution of ribosomal DNA genes and changes in chromosome numbers have begun to shed light on the evolutionary significance of chromosomal changes (Weiss-Schneeweiss and Schneeweiss, 2013).

Ribosomal DNA evolution in *Alstroemeria*

The chromosomes of *Alstroemeria* have fascinated cytologists for the past 120 years due to their large size and ease of manipulation. The haploid chromosome number of $n = 8$ was reported for the first time by Eduard Strasburger after studying the meiosis of the pollen mother cells of *Alstroemeria chilensis* (Strasburger, 1882). The

karyotype diversity of *Alstroemeria* is homogeneous, with species sharing the same basic chromosome number (*n* = 8) and asymmetric karyotypes (i.e., prevalence of telocentric and subtelocentric chromosomes; Stephens et al., 1993; Buitendijk and Ramanna, 1996; Kamstra et al., 1997; Sanso and Hunziker, 1998; Sanso, 2002; Jara-Seguel et al., 2004). However, much variation in the nuclear genome has been revealed with cytogenetic techniques for estimating the DNA content, identifying C-banding patterns (i.e., centromere- or heterochromatin-banding stain patterns), and localizing ribosomal RNA-specific gene sequences on the chromosomes.

Fluorescent ***i**n **s**itu* **H**ybridization (FISH) of ribosomal genes (rDNA) has been widely used to study the chromosomes of plants and animals. Variations in the number and distribution of the rDNA sites have elucidated evolutionary relationships among taxa and have yielded information on chromosome evolution and genome organization (Shan et al., 2003; Heslop-Harrison and Schwarzacher, 2011). For *Alstroemeria*, studies using FISH have revealed high levels of polymorphism in the rDNA signals of homologous chromosomes (Kamstra et al., 1997; Baeza et al., 2007). Interspecific variation in total chromosome length and C-banding patterns between Chilean and Brazilian species of *Alstroemeria* has also been described (Buitendijk and Ramanna, 1996; Kuipers et al., 2002).

These studies provide evidence that chromosome evolution in *Alstroemeria* has been highly dynamic. The chromosome numbers of the remaining Alstroemeriaceae genera are also known; *Bomarea* has *n* = 9 chromosomes (Sanso and Hunziker, 1998; Palma-Rojas, et al., 2007; Baeza et al., 2008), and *Luzuriaga* and *Drymophila* have *n* = 10 (Conran, 1987; Jara-Seguel et al., 2010). The elements to infer evolutionary trends in *Alstroemeria* chromosome evolution are thus available, but prior to my work the lack of a phylogeny including a representative number of Brazilian and Chilean species, as well as species of the remaining Alstroemeriaceae genera had precluded understanding the karyotype evolution in *Alstroemeria*.

Chromosome number evolution in Colchicaceae

As mentioned in the previous section, chromosome numbers in the Alstroemeriaceae vary between *2n* = 16 to *2n* = 20. Such variation is small compared to that in the sister family Colchicaceae, which has chromosome numbers between *2n* = 14 (e.g.

Uvularia grandiflora; Therman and Denniston, 1984) and *2n* = 216 (e.g. *Colchicum corsicum*; Persson, 2009). In particular, the cytogenetics of the genus *Colchicum* is complex, with different species having variable chromosome numbers and ploidy levels (from tetra- to 24-ploid; Persson et al., 2011). Nordenstam (1998) considered that polyploidy in this genus might be related to the presence of colchicine, an alkaloid known to affect chromosome separation after the anaphase of mitosis. This effect of colchicine was discovered by B. Pernice in 1889, described more fully by Eigsti et al. (1945), and revolutionized cytogenetics because it permitted experimental generation of polyploidy. Generally, changes in chromosome number can been attributed to doubling (polyploidy), chromosome fission (ascending dysploidy) or chromosome fusion (descending dysploidy) (Schubert and Lysak, 2011). Ancient whole-genome duplications have been documented for several monocot lineages (Soltis et al., 2009). Polyploidy is though to promote the ecological diversification of species because it facilitates the adaptation to new environments by generating novel biochemical, physiological, and developmental changes not found in the progenitors (Levin, 1983). For this reason, knowledge about the mechanisms of chromosome number change will improve our understanding of species formation, especially if it is time-explicit (as possible with molecular clock dating).

A new method for inferring ancestral chromosome numbers and possible mechanisms of chromosome evolution (such as end-to-end fusion) has been proposed by Mayrose et al. (2010). It is a probabilistic approach that tries to model chromosome number change along the phylogeny, assuming that those changes are gradual and proportional to time. Thus, a molecular phylogeny (and the associated branch lengths) is needed as well as a list of the observed chromosome numbers in the species included in the phylogeny. The method has been used to reconstruct the ancestral chromosome numbers in the Araceae family, and revealed an ancestral haploid number of x = 16, different from the previously inferred numbers x = 14 or x = 7 (Cusimano et al., 2011). The main mechanism of chromosome evolution in that group appears to be chromosome fusion, rather than polyploidy (Cusimano et al., 2011).

Aim of this study

As explained in the preceding sections, the aim of my thesis was to increase the knowledge about the evolution of the Alstroemeriaceae-Colchicaceae lily clade by studying the molecular phylogenetics and biogeography of the two families at a global scale, and by studying their chromosome evolution at more local scales, namely within *Alstroemeria* and within Colchicaceae. The main questions I wanted to answer were (i) by which routes and when did Alstroemeriaceae and Colchicaceae expand geographically and diversify or suffer extinction, (ii) by which mechanisms did the chromosomes of *Alstroemeria* evolve, and (iii) which types of events best explain the changes in chromosome numbers in Colchicaceae (polyploidy, chromosome fusion, or chromosome breaks) and when and where did these changes occur.

To answer these questions I generated two molecular phylogenies including DNA sequences from the three plant genomes (i.e., chloroplast, mitochondrial and nuclear) for 125 of the 204 Alstroemeriaceae species and for 83 of the 270 Colchicaceae species. For both families, I applied molecular-clock dating with up to four fossil calibrations from the ingroup and from outgroups. For the Alstroemeriaceae, the ancestral areas were inferred using statistical parsimony in S-DIVA (Chapter 2). Possible biogeographic scenarios and the influence of the new *Luzuriaga*-like fossil on inferred divergence times were evaluated with a molecular clock model using alternative calibration nodes (Chapter 3). Ancestral ranges for the Colchicaceae were inferred using the likelihood DEC model in LAGRANGE. I also carried out a sensitivity analysis by experimentally changing key parameters of my DEC model for the Colchicaceae (Chapter 4). The chromosome evolution in *Alstroemeria* was investigated by means of a molecular phylogeny that focused on Brazilian and Chilean species for which karyological information and FISH data where generated and then mapped (Chapter 5). Finally, I used my novel molecular phylogeny for the Colchicaceae as well as a newly generated phylogeny of *Colchicum*, which together with the chromosome numbers reported in the literature were used to infer ancestral chromosome numbers and mechanisms of cytogenetic evolution in this family (Chapter 6).

Chapter 2

FROM EAST GONDWANA TO CENTRAL AMERICA:

HISTORICAL BIOGEOGRAPHY OF THE ALSTROEMERIACEAE

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ORIGINAL ARTICLE

From East Gondwana to Central America: historical biogeography of the Alstroemeriaceae

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ABSTRACT

Aim The Alstroemeriaceae is among 28 angiosperm families shared between South America, New Zealand and/or Australia; here, we examine the biogeography of Alstroemeriaceae to better understand the climatic and geological settings for its diversification in the Neotropics. We also compare Alstroemeriaceae with the four other Southern Hemisphere families that expanded from Patagonia to the equator, to infer what factors may have permitted such expansions across biomes.

Location South America, Central America, Australia and New Zealand.

Methods Three chloroplast genes, one mitochondrial gene and one nuclear DNA region were sequenced for 153 accessions representing 125 of the 200 species of Alstroemeriaceae from throughout the distribution range; 25 outgroup taxa were included to securely infer evolutionary directions and be able to use both ingroup and outgroup fossil constraints. A relaxed-clock model relied on up to three fossil calibrations, and ancestral ranges were inferred using statistical dispersal–vicariance analysis (S-DIVA). Southern Hemisphere disjunctions in the flowering plants were reviewed for key biological traits, divergence times, migration directions and habitats occupied.

Results The obtained chronogram and ancestral area reconstruction imply that the most recent common ancestor of Colchicaceae and Alstroemeriaceae lived in the Late Cretaceous in southern South America/Australasia, the ancestral region of Alstroemeriaceae may have been South America/Antarctica, and a single New Zealand species is due to recent dispersal from South America. Chilean *Alstroemeria* diversified with the uplift of the Patagonian Andes *c.* 18 Ma, and a hummingbird-pollinated clade (*Bomarea*) reached the northern Andes at 11–13 Ma. The South American Arid Diagonal (SAAD), a belt of arid vegetation caused by the onset of the Andean rain shadow 14–15 Ma, isolated a Brazilian clade of *Alstroemeria* from a basal Chilean/Argentinean grade.

Main conclusions Only Alstroemeriaceae, Calceolariaceae, Cunoniaceae, Escalloniaceae and Proteaceae have expanded and diversified from Patagonia far into tropical latitudes. All migrated northwards along the Andes, but also reached south-eastern Brazil, in most cases after the origin of the SAAD. Our results from *Alstroemeria* now suggest that the SAAD may have been a major ecological barrier in southern South America.

Keywords

Ancestral area reconstruction, Andean uplift, Austral–Antarctic families, Australia, East Gondwana, molecular clock, New Zealand, South America.

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INTRODUCTION

As a result of ancient overland connections and similar ecological conditions, the floras of southern South America and Australia/New Zealand share many genera and families (Treviranus, 1803; Takhtajan, 1986; Wardle *et al.*, 2001; Moreira-Muñoz, 2007). Over the past few years, molecular phylogenetic studies have begun to unravel the history of these Austral–Antarctic connections, attributing some to Cretaceous or Palaeogene trans-Antarctic ranges and others to long-distance dispersal (e.g. Renner *et al.*, 2000: Atherospermataceae; Bradford & Barnes, 2001: Cunoniaceae; Knapp *et al.*, 2005: *Nothofagus*; Chacón *et al.*, 2006: *Oreobolus*; Barker *et al.*, 2007: Proteaceae; Cosacov *et al.*, 2009: Calceolariaceae). Trans-Antarctic ranges were possible in the Late Cretaceous when the southern tip of South America was connected to Antarctica (Fig. 3 in Reguero *et al.*, 2002; Fig. 1D in Iglesias *et al.*, 2011), and fossils demonstrate that some groups that had already gone extinct in Southwest Gondwana continued to survive on Antarctica well into the Eocene (Reguero *et al.*, 2002). The Southwest Gondwana floristic province (south of 30° S) spanned two climatic belts, subtropical seasonal dry and warm temperate, while Southeast Gondwana mostly had a warm temperate climate (Iglesias *et al.*, 2011).

Today, 28 flowering plant families are shared between South America, New Zealand and/or Australasia (Appendix S1 in Moreira-Muñoz, 2007; although the Proteaceae are included in the main text of this paper, they were omitted from the Appendix apparently by mistake). Most of them are restricted to cool temperate climates and their ranges do not extend north to equatorial latitudes. This is surprising because at least those that date back to Cretaceous, Palaeocene or Eocene times must have evolved under warm, tropical conditions and one might expect such clades to have expanded their ranges further north. Among the few families that did is the Alstroemeriaceae, on which this study focuses. The Alstroemeriaceae comprises 200 species in four genera – *Bomarea*, with 120 species in Central America and northern-central South America; *Alstroemeria*, with 78 species in southern South America and eastern Brazil; *Luzuriaga*, with three species in Chile and one in New Zealand; and *Drymophila*, with one species in Australia and one in Tasmania. The sister clade of Alstroemeriaceae is the family Colchicaceae, which has 200 species on all continents except South America (and Antarctica), and based on a Liliales-wide analysis, Vinnersten & Bremer (2001) suggested that the Alstroemeriaceae might have entered South America from the south. However, Vinnersten & Bremer's (2001) higher-level analysis included only four of the family's 200 species (one from each genus) and therefore could

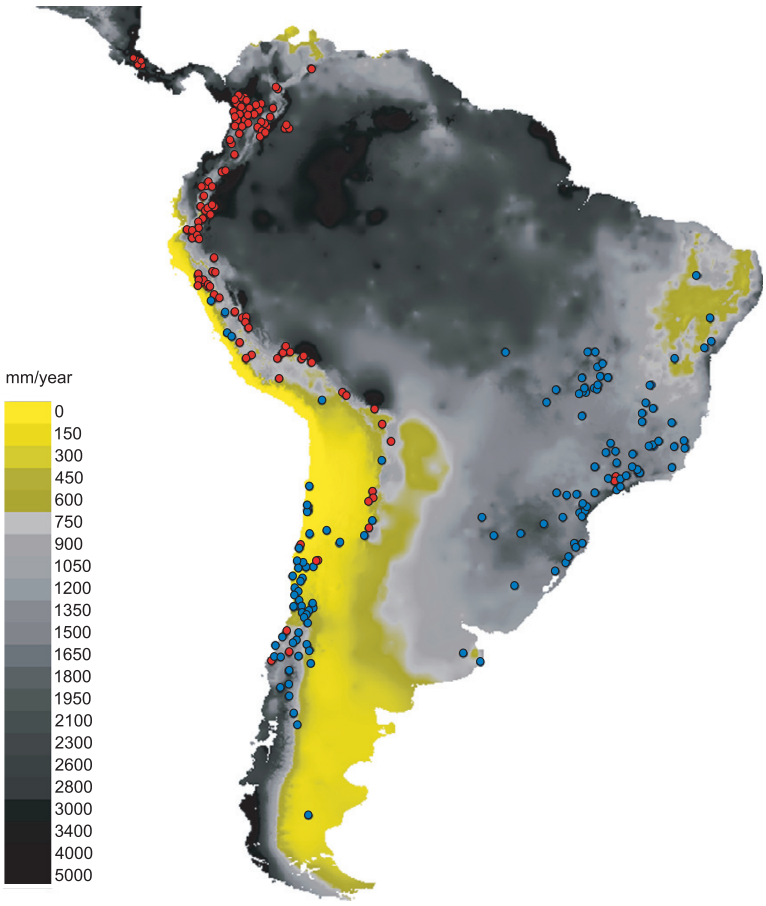

Figure 1 Geographical distribution of *Alstroemeria* (blue dots) and *Bomarea* (red dots) and location of the South American Arid Diagonal (SAAD). Different shading on the map refers to annual mean precipitation in millimetres (lower left inset) obtained from WorldClim – Global Climate Data (http://www.worldclim.org/). The SAAD receives precipitation of < 300 mm year^{-1} (light yellow zone). No species of Alstroemeriaceae occur in the southern SAAD.

not test the monophyly of the genera, nor provide divergence time estimates for clades. Other studies of Alstroemeriaceae also sampled too few species to address questions about the timing of trans-Antarctic connections or possible northward expansion from high to low latitudes (Aagesen & Sanso, 2003; Alzate *et al.*, 2008).

Most species of Alstroemeriaceae occur at elevations of 2500–3800 m in the Andes, and it is clear that the family's diversification has been strongly influenced by the orogeny of the Andean Cordillera (Hofreiter, 2007). The uplift of the Central Andean Plateau occurred in pulses, the most recent of which is currently dated to 6–10 million years ago (Ma) (Garzione *et al.*, 2008; Capitanio *et al.*, 2011), while the Patagonian Andes' main uplift dates to 26–28 Ma (Blisniuk *et al.*, 2005). The rain shadow effects of the latter created the South American Arid Diagonal (SAAD), a narrow area with low precipitation (< 300 mm year^{-1}; the yellow area in Fig. 1) that crosses South America from 2° S in the Gulf of Guayaquil to 52° S bounding the Straits of Magellan (Eriksen, 1983; Blisniuk *et al.*, 2005). Along the western coast of South America, the SAAD spans mainly desert (Moreira-Muñoz, 2011), while towards the east, it spans the seasonally dry Chaco forest and subtropical grasslands (Pennington *et al.*, 2006; Simon *et al.*, 2009; Werneck, 2011). Palynological evidence dates this dry belt to < 16 Ma (Blisniuk *et al.*, 2005). The SAAD is likely to have influenced the geographical expansion and diversification of Alstroemeriaceae, because of its extremely different climate.

Here, we present a comprehensive fossil-calibrated molecular phylogeny of the Alstroemeriaceae and use statistical ancestral area reconstruction to test the hypothesis of Vinnersten & Bremer (2001) that the family's disjunct distribution reflects the break-up of Eastern Gondwana. We also infer the geotemporal patterns of expansion of the Alstroemeriaceae from the southern cone of South America to the equatorial tropics and eastern Brazil. Finally, we compare the patterns and times of diversification, as well as key biological traits, in Austral–Antarctic angiosperm clades that expanded from Patagonia into equatorial habitats, and test the idea that the SAAD may have presented an ecological filter for northwards expansion or may have led to fragmented ranges in clades older than the *c.* 16 million year (Myr) old SAAD.

MATERIALS AND METHODS

Taxon sampling

We sequenced 125 of the 200 species of Alstroemeriaceae, focusing on geographical representativeness, and added 23 species of Colchicaceae plus two species of Campynemataceae as outgroups (Vinnersten & Bremer, 2001). Our sample comprises 63 species of *Alstroemeria* L. (out of *c.* 78 species), 56 species of *Bomarea* Mirb. (out of *c.* 120 species), both species of *Drymophila* R. Br. (*Drymophila cyanocarpa* and *Drymophila moorei*), and the four species of *Luzuriaga* Ruiz & Pav. (*Luzuriaga marginata*, *Luzuriaga parviflora*, *Luzuriaga polyphylla* and *Luzuriaga radicans*). We also included the monotypic segregate genera *Leontochir* R.A. Philippi (found to be nested within *Bomarea* by Aagesen & Sanso, 2003) and *Taltalia* Ehr. Bayer (found to be nested within *Alstroemeria* by Sanso & Xifreda, 2001). For *Alstroemeria*, species concepts followed Bayer (1987) for the Chilean species and Assis (2001) for the Brazilian species. For Ecuadorian *Bomarea*, species concepts followed Harling & Neuendorf (2003); for the remaining *Bomarea*, we followed Hofreiter & Tillich (2002). For 26 species, we included samples from separate locations to test species monophyly. All sampled plant material, with its geographical origin, herbarium voucher specimen, species names and authors, and GenBank accession numbers, is listed in Appendix S1 in Supporting Information.

DNA extraction, amplification and sequencing

Total DNA was extracted from *c.* 0.3 g of dried leaf tissue using the Nucleospin Plant II kit (Macherey-Nagel, Düren, Germany). The resulting DNA was amplified with standard methods. The chloroplast genes *ndh*F, *mat*K and *rbc*L, the mitochondrial *mat*R, and the complete nuclear ribosomal internal transcribed spacer (ITS) were amplified using standard primers. Sequencing relied on the BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Inc., Warrington, UK) and an ABI 3100 Avant capillary sequencer (Applied Biosystems). The ITS region always yielded single bands and unambiguous base calls, and we therefore refrained from cloning. Sequence assembly of forward and reverse strands was carried out with Sequencher (Gene Codes, Ann Arbor, MI, USA) and alignment with MacClade 4.8 (Maddison & Maddison, 2002) or for ITS with mafft 5.64 (Katoh *et al.*, 2005) with manual adjustment. All sequences were blast-searched in GenBank.

Phylogenetic analyses

Tree searches relied on maximum likelihood (ML) as implemented in RAxML (Stamatakis, 2006) using the GTR+G model. FindModel (available from http://hcv.lanl.gov/content/sequence/findmodel/findmodel.html), which implements Posada & Crandall's (1998) Modeltest, selected this as the best fit for both organellar and nuclear sequences. These data partitions were first analysed separately, and in the absence of statistically supported topological conflict (defined as > 80% bootstrap support) were combined. Statistical support for nodes was assessed by 100 ML bootstrap replicates under the same model. We also conducted a Bayesian analysis, using MrBayes 3.2 (Ronquist *et al.*, 2012) with two parallel runs with one cold and four heated chains; the Markov chain had a length of 2 million generations, sampled every 1000 generations. A plot of the generation number against the log-probability of the data was generated in Tracer 1.5 (Rambaut & Drummond, 2007), and the results indicated that convergence was reached after 250,000 generations. The

maximum clade credibility tree was calculated using BayesTrees 1.3 (available from http://www.evolution.reading.ac.uk/BayesTrees.html).

Molecular clock analyses

Molecular clock analyses used either the plastid and mitochondrial genes or all three data partitions (plastid, mitochondrial and nuclear). The dating matrices included 77 of the 153 ingroup and 25 outgroups sequences to avoid zero-length branches (resulting from multiple accessions of the same species or very closely related species), because these are known to cause problems for molecular clocks. The clock model was a Bayesian relaxed clock implemented in beast 1.6.1 (Drummond *et al.*, 2006; Drummond & Rambaut, 2007), using the GTR+G substitution model, a Yule tree prior, and uncorrelated and lognormally distributed rate variation. Markov chain Monte Carlo (MCMC) runs extended for 60 million generations (burn-in 10%), with parameters sampled every 1000 or 2000 generations.

We applied up to four calibration points (three from fossils), each with a normal prior distribution and a 95% confidence interval (CI) as indicated below.

1. The crown node of *Smilax* was set to 46 Ma (standard deviation (SD) 4.5, CI 37.2–54.8 Ma), which represents a conservative minimal age, given that *Smilax*-like fossils are known from the Early/Lower Eocene (48.6–55.8 Ma; Edelman, 1975; Wilf, 2000) and the Middle Eocene (37.2–48.6 Ma; MacGinitie, 1941; Wilde & Frankenhäuser, 1998).

2. The stem age of the monotypic family Rhipogonaceae was set to 51 Ma (SD 1.5, CI 48.5–53.5 Ma) based on leaf macrofossils of *Rhipogonum* from Tasmania dated to 51–52 Ma (Conran *et al.*, 2009a).

3. One run included a *Luzuriaga*–like fossil from the Foulden Maar deposits near Middlemarch, New Zealand, dated to 23 Ma (J. Conran, School of Earth and Environmental Sciences, University of Adelaide, pers. comm., 9 September 2011; also Conran *et al.*, 2009b). The fossil has been assigned to *Luzuriaga* through a parsimony ratchet analysis of 33 morphological characters relating to vegetative (stems, leaves, stomata) and reproductive structures (inflorescences, flowers, fruits, seeds) of eight Alstroemeriaceae species (one *Alstroemeria* and one *Bomarea* species, the two *Drymophila* species, and the four *Luzuriaga* species) (J. Conran, pers. comm., May 2010). This fossil was used to constrain the crown node of the *Drymophila/Luzuriaga* clade to 23 Ma (SD 0.5, CI 22–24 Ma).

4. The root of the tree was constrained to 117 Ma (SD 0.5, CI 116.2–117.8 Ma) based on Janssen & Bremer's (2004) estimate for the crown group of the Liliales, an order represented here by exemplars of seven of the ten families (Appendix S1). Absolute ages for geological periods are from Walker & Geissman (2009), and inferred node ages were checked against estimates from larger monocot data sets that did not use exactly the same fossil constraints as those used here (Janssen & Bremer, 2004).

Ancestral area reconstruction

Species occurrences were compiled from vouchers included in this study (Appendix S1) plus herbarium specimens and the literature (Bayer, 1987; Rodríguez & Marticorena, 1987; Arroyo & Leuenberger, 1988; Conran & Clifford, 1998; Assis, 2001; Wardle *et al.*, 2001; Hofreiter & Tillich, 2002; Harling & Neuendorf, 2003; Hofreiter & Rodriguez, 2006; Hofreiter, 2007; Alzate *et al.*, 2008). For ancestral area reconstruction, we grouped species ranges into seven regions (listed in Table 1), following Weigend (2002) for the subdivision of the Andes into the northern, central and southern Andes. Note that because the seven regions are based on the ranges of modern species, Antarctica is not included, and so cannot be inferred as an ancestral range (see Discussion). The analyses relied on statistical dispersal–vicariance analysis (S-DIVA; Yu *et al.*, 2010) as implemented in rasp 2.0b (Yu *et al.*, 2011). This parsimony-based approach reconstructs ancestral areas

Table 1 Geographical areas used in the biogeographical analyses.

Area code	Description	Circumscription
A	Central America	Sierra Madre Oriental and Occidental in Mexico, mountain range from Guatemala to Panama
B	Northern Andes	Cordilleras Occidental and Central in Colombia, Cordillera Oriental in Colombia and Venezuela, Nudo de los Pastos between southern Colombia and northern Ecuador, where the three cordilleras join into one, Andean mountains in northern Peru including the Amotape–Huancabamba zone as far as *c.* 8.1° S
C	Central Andes	Andean mountains extending south of the Amotape–Huancabamba deflection as far as central Bolivia, at 18° S, including the Altiplano between the eastern and western cordilleras in southern Peru, Bolivia, and northern Argentina/Chile
D	Atacama Desert	Desert area that extends south of the Peru–Chile border to about 30° S, on the western side of the Andes
E	Southern Andes	Andean mountains south of the Central Andes, from southern Bolivia as far as Patagonia in southern Chile and Argentina
F	Eastern Brazil	Area between 0° S and 51° E and 32° S and 53° E in Brazil, including the limits with southern Paraguay and the eastern Uruguay
G	Australasia	South-eastern Australia and Tasmania, New Zealand

based on a sample of trees (in our case, the last 5000 trees of a BEAST run), thereby generating credibility support values for alternative phylogenetic hypotheses. To explore the effects of area constraints, we performed two S-DIVA runs, one that allowed maximally two areas for a clade's ancestral range and a second that did not constrain the maximum number of areas.

Comparison of 'southern-immigrant' Neotropical families

The reviews of Wardle *et al.* (2001) and Moreira-Muñoz (2007) were used to identify angiosperm clades disjunctly distributed between the Neotropics and Australia/New Zealand. For all clades that expanded throughout the South American continent northwards to Central America and/or eastern Brazil, we then compiled information on species diversity, habitats, key biological traits, divergence times and migration direction.

RESULTS

Phylogenetics of the Alstroemeriaceae

The combined matrix of the organellar markers *ndh*F, *rbc*L, *mat*K and *mat*R comprised 2399 aligned nucleotides, representing 153 ingroup and 25 outgroups accessions. The ITS matrix had 731 aligned nucleotides and 85 accessions, of which 72 corresponded to ingroup accessions and 13 to outgroups. Maximum likelihood trees obtained from the organellar and the nuclear data showed no robustly supported incongruence, and analysis of the combined data yielded higher bootstrap values and better resolution at the internal nodes. The 26 species for which more than one individual was sampled were all resolved as monophyletic (Fig. 2).

In the ML tree (Fig. 2), Alstroemerioideae (*Alstroemeria* and *Bomarea*) are sister to Luzuriagoideae (*Luzuriaga* and *Drymophila*) with high bootstrap support (99%). The Brazilian species (42 of 44 species occurring in Brazil were

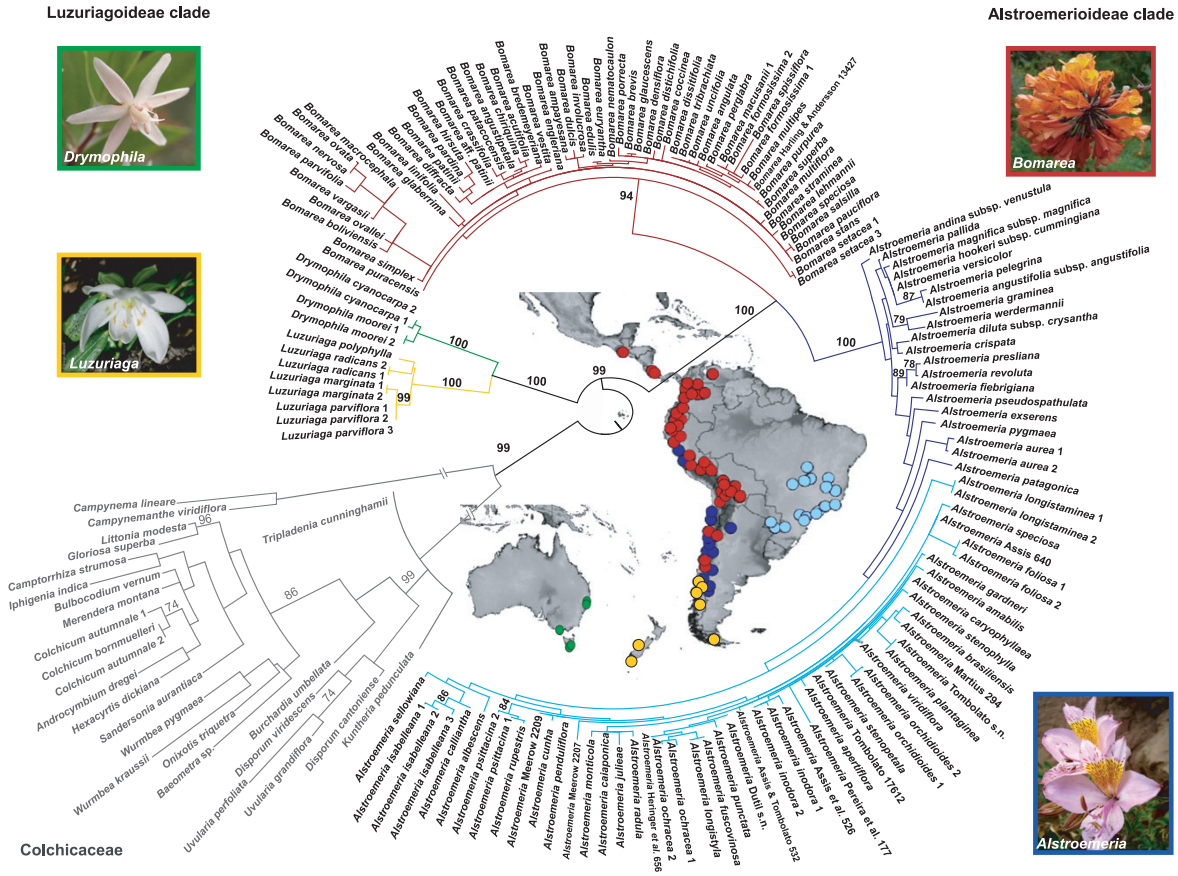

Figure 2 Maximum likelihood phylogram for Alstroemeriaceae based on the combined analysis of plastid, mitochondrial and nuclear sequences (3130 aligned nucleotides). The tree is rooted on the sister clade, Colchicaceae, plus two species of Campynemataceae. Bootstrap support from 100 replicates is shown above branches. The maps show the geographical origin of sequenced plants. Images of typical flowers clockwise from right: *Bomarea multiflora* (S. Madriñán), *Alstroemeria exserens* (E. Olate), *Alstroemeria inodora* (M. C. Assis), *Luzuriaga radicans* (D. Alarcón) and *Drymophila moorei* (J. Bruhl).

sampled) form a monophyletic group that arises from within a Chilean/Argentinean species group (Fig. 2). The Central American species are derived from Colombian *Bomarea* (< 60%), and the family's sole New Zealand species, *L. parviflora*, is embedded among Chilean/Argentinean *Luzuriaga* species (100%, Fig. 2). The results of the Bayesian analysis were congruent with the ML tree, with all early divergences having a high posterior probability (PP > 0.9, Appendix S2).

Divergence times and ancestral area reconstruction

Figure 3 shows a time tree for Alstroemeriaceae and related Liliales obtained from the plastid and mitochondrial matrix, and divergence times relevant to our questions are summarized in Table 2. Appendix S3 shows the times obtained when the nuclear ITS data were added. With ITS included, the inferred divergence times were slightly older. The standard deviations of the uncorrelated lognormal and the coefficient of variation were 0.68 and 0.7 (for plastid plus mitochondrial) and 0.74 and 0.75 (for plastid and mitochondrial plus nuclear ITS), implying no substantial rate heterogeneity among lineages (Drummond & Rambaut, 2007). Effective sample sizes (ESS) were checked in Tracer 1.4.1 (Rambaut & Drummond, 2007) and were all well above 200. Divergence times estimated with and without the *Luzuriaga*-like fossil as a calibration point did not differ significantly (Table 2). Because the nuclear matrix included non-randomly distributed missing data, we focus on the chronogram obtained without the nuclear data (Simmons, 2012).

The most recent common ancestor of Colchicaceae and Alstroemeriaceae (node I in Fig. 3) is placed in the Cretaceous in the southern Andes and Australasia (ancestral area EG) *c.* 93 (73.4–115.8) Ma. The ancestral region of Alstroemeriaceae (node II) is inferred as the southern Andes (but see Discussion), and the split between the *Luzuriaga* clade and the *Alstroemeria* clade is dated to *c.* 57.5 (37.8–77.6) Ma. Extant Alstroemerioideae (node III) began diversifying *c.* 29 (18.2–42.6) Ma, i.e. before the main rise of the Andes, in the central and southern Andes (ancestral area CE). The dry-adapted *Alstroemeria* species of southern Chile (node V) began to diversify *c.* 18.4 (11.2–26.8) Ma, and the Argentinean/Brazilian clade (node VI) dates to 9.2 Ma. *Bomarea* (node VII) began diversifying *c.* 14.3 (7.1–23.1) Ma, that is, before the major uplift of the central Andes (ancestral area C), and reached the northern Andes at *c.* 11–13 Ma (Fig. 3). It then spread north, reaching Central America by the Late Pliocene.

The Luzuriagoideae clade (node IV) is estimated to be *c.* 22 (19–24) Ma in the run in which the *Luzuriaga*-like Miocene fossil from New Zealand is included as a constraint (see Materials and Methods); without this fossil, the same clade dates to *c.* 35.9 (19.5–55.5) Ma (column A in Table 2). The single extant New Zealand species of *Luzuriaga* (*L. parviflora*) is inferred to be *c.* 2.9 (0.4–6.1) Ma (Fig. 3) and the split between the Australian and Tasmanian species of *Drymophila* *c.* 4 (0.7–8.6) Ma.

The results of the two S-DIVA runs with different constraints on the maximal permitted number of ancestral areas are shown in Table 2. With the number of ancestral areas unconstrained, all nodes near the root had multi-region ancestral area reconstructions, which is biologically implausible and an artefact, probably because S-DIVA disregards branch-length information, causing it to underestimate trait changes along long branches, such as those leading to the root.

Comparison of 'southern-immigrant' plant families in the Neotropics

Only five Austral–Antarctic angiosperm families have expanded all the way from Patagonia to Central America or the tropics of north-eastern Brazil. These are the Alstroemeriaceae, Calceolariaceae, Cunoniaceae, Escalloniaceae and Proteaceae, with a total of 670 species (Table 3). Except for Cunoniaceae and Proteaceae, these families are more species-rich in the Neotropics than in Australia or New Zealand. Except for Calceolariaceae and Cunoniaceae, their geographical ranges show clear disjunctions between south-western South America and eastern Brazil, and all five families have species adapted to mountain habitats along the entire Andes. There are no other obvious similarities in pollination or dispersal biology. Escalloniaceae diversified in the Late Cretaceous (*c.* 72 Ma), Proteaceae in the Middle Eocene (*c.* 45 Ma), Cunoniaceae in the Early Oligocene (33.9–28.4 Ma), and Calceolariaceae during the Middle Miocene (*c.* 15 Ma; references in Table 3).

DISCUSSION

Biogeographical history of the Alstroemeriaceae

Of the 28 flowering plant families shared between New Zealand or Australia and South America (Moreira-Muñoz, 2007), most are strictly confined to the cool/temperate zone and never reached the humid tropics. Only five managed to expand from Patagonia north into equatorial latitudes, one of which is the Alstroemeriaceae. Our molecular clock-dated biogeographical analysis supports Vinnersten & Bremer's (2001) hypothesis that the Alstroemeriaceae/Colchicaceae lineage dates back to the Late Cretaceous, a time when Australia, Antarctica and South America were still connected or very close (Reguero *et al.*, 2002; Iglesias *et al.*, 2011). The split between the Australasian/Chilean *Drymophila*/*Luzuriaga* clade and the South American *Alstroemeria*/*Bomarea* clade occurred about 57.5 (37.8–77.6) Ma (Fig. 3), close to the Palaeocene–Eocene Thermal Maximum at 55 Ma (Zachos *et al.*, 2001; Hinojosa & Villagrán, 2005; Iglesias *et al.*, 2011). Subtropical climates at that time extended as far as latitude 30° S, with moisture brought in by the tropical easterlies during the summer and the polar westerlies during the winter (Iglesias *et al.*, 2011). This climate regime, which has no modern analogue, could only exist as long as the Andean Cordillera was too low to cause a strong rain shadow (Hinojosa & Villagrán, 2005). It is plausible that Alstroemeriaceae evolved under this climate

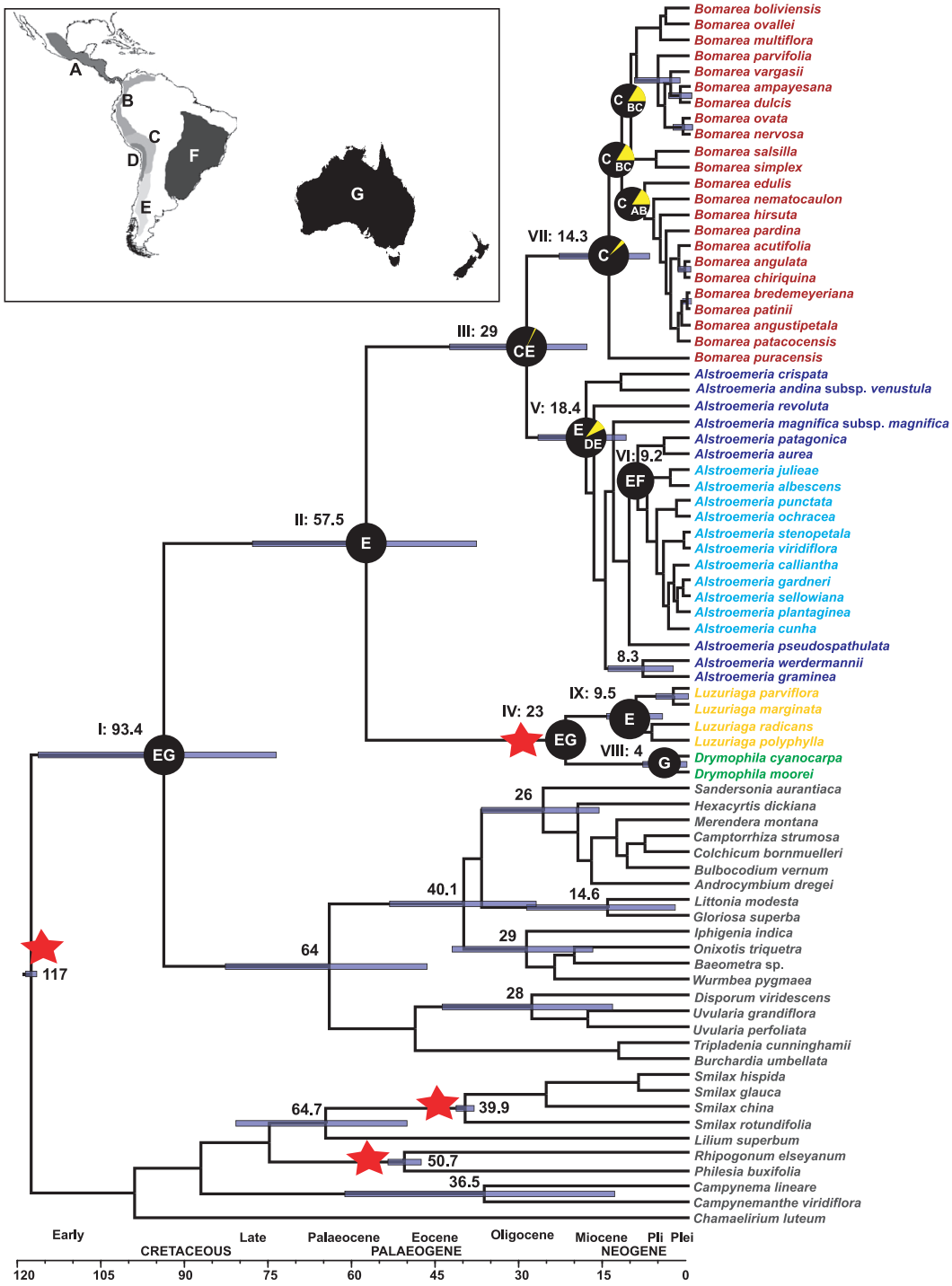

Figure 3 Chronogram for Alstroemeriaceae obtained under a Bayesian relaxed clock model applied to 77 accessions and 2399 aligned nucleotides of chloroplast DNA and mitochondrial DNA sequences. Bars at nodes with > 95% posterior probability indicate the 95% confidence intervals on the estimated times. Numbers above branches are node ages (Ma) and the stars are calibration nodes. Roman numerals correspond to the node numbers in Table 2. The Brazilian clade is shown in light blue. Pie charts at internal nodes represent the probabilities for each alternative ancestral area derived by using statistical dispersal–vicariance analysis (S-DIVA) on 5000 Bayesian trees. Black pies denote nodes with a posterior probability of > 0.8 according to the values shown in Table 2. The geographical areas used in the S-DIVA analyses are shown in the inset.

Table 2 Age estimates and ancestral area reconstructions for the main nodes of Alstroemeriaceae.

Node number	Description	Node age [95% HPD] A	Node age [95% HPD] B	Ancestral area (PP) C	Ancestral area (PP) D
I	Stem Alstroemeriaceae	96.5 [76.8–116.7]	93.4 [73.4–115.8]	EG (1.00)	ABCDEFG (0.6), ABCEFG (0.4)
II	Crown Alstroemeriaceae	64.2 [42.5–86.8]	57.5 [37.8–77.6]	E (1.00)	ABCDEFG (0.3), ABCDEF (0.3) ABCEF (0.2), ABCEFG (0.2)
III	Crown Alstroemerioideae	31.9 [18.5–47.8]	29.0 [18.2–42.6]	CE (0.99), BE (0.01)	ABCDEF (0.5), ABCEF (0.5)
IV	Crown Luzuriagoideae	35.9 [19.5–55.5]	n.a.	EG (1.00)	EG (0.6), G (0.4)
V	Crown *Alstroemeria*	19.7 [11.3–29.5]	18.4 [11.2–26.8]	E (0.88), DE (0.12)	E (1.0)
VI	Stem Brazilian *Alstroemeria*	9.7*	9.2*	EF (1.00)	EF (1.0)
VII	Crown *Bomarea*	15.4 [7.3–25.3]	14.3 [7.1–23.1]	C (0.93), DE (0.05), BC (0.02)	ABCF (0.4), ABCDF (0.2), ABCEF (0.2), ABCDEF (0.2)
VIII	Crown *Drymophila*	4.9 [0.6–11.4]	4.0 [0.7–8.6]	G (1.00)	G (1.0)
IX	Crown *Luzuriaga*	12.4 [4.8–21.4]	9.5 [4.8–14.7]	E (1.00)	E (0.6), EG (0.4)

Column headings: A, age estimates (Ma) with outgroup calibrations only; B, age estimates (Ma) with an additional ingroup calibration from a *Luzuriaga*-like fossil; C, inferred ancestral area with maximum number of areas constrained to two; D, inferred ancestral areas with no constraint on the maximum number of areas. Letter codes for columns C and D follow Table 1.
n.a., not applicable; HPD, highest posterior density interval for the divergence time estimate; PP, posterior probability.
*The confidence interval for this date is below the 95% HPD.

regime. We were unable, however, to reliably infer their area of origin because our seven coded geographical regions are those of extant species. Without fossils from Antarctica, we know of no approach that would permit inference of an Antarctic ancestral area for the family, even though it is possible (even likely given the range of their sister clade) that Alstroemeriaceae originated in Antarctica instead of South America (southern Andes) as inferred here.

The stem group age of the Brazilian *Alstroemeria* clade (*c.* 9.2 Ma, Fig. 3) falls towards the end of a phase of global cooling (Zachos *et al.*, 2001; 10–14 Ma) and pre-dates the expansion of C_4 grasslands in north-western Argentina (Blisniuk *et al.*, 2005; 7–8 Ma). The only dated clade with a similar geographical range in Brazil, Laeliinae orchids, radiated 11–14 Ma (Antonelli *et al.*, 2010), about the same time as the Patagonian/Brazilian *Alstroemeria* (*Alstroemeria aurea*, *Alstroemeria patagonica* and *Alstroemeria pseudospathulata*; Fig. 3; *c.* 13.5 Ma). The arid conditions (i.e. the SAAD) that arose *c.* 16 Ma appear to have had a strong influence on the distribution of *Alstroemeria*. The gap in the distribution of *Alstroemeria* evident in southern South America (light yellow area in Fig. 1) is probably a consequence of the establishment of the arid belt. Based on species ranges and fieldwork, the Alstroemeriaceae specialist A. Hofreiter has hypothesized the importance of the SAAD as an ecological barrier for Alstroemeriaceae (Hofreiter, 2007).

The inferred diversification of the Andean *Bomarea* clade at *c.* 14.3 Ma closely matches the Miocene radiation of the hummingbirds, *c.* 17 Ma (Bleiweiss, 1998). Judging from flower colour, nectar supply, diurnal anthesis, size and orientation, most *Bomarea* species are pollinated by hummingbirds, and this is supported by field observations (Hofreiter & Rodriguez, 2006). Hummingbirds are reliable pollinators at high elevations in the Andes, which may have played a role in the successful spread and diversification of *Bomarea*. Colombia, Ecuador and Peru each have some 30–35 species of *Bomarea*. The Amotape–Huancabamba zone at *c.* 5° S, which is a zone of phytogeographical transition at the border between Ecuador and Peru, is especially rich in endemic species, probably because of its heterogeneity in orographic, microclimatic, geological and edaphic conditions (Weigend, 2002; Richter *et al.*, 2009). Of the four Central American endemic species of *Bomarea* (Hofreiter, 2007), only two are sampled here, which prevents us from inferring when and how often *Bomarea* reached Central America.

The New Zealand leaf fossil resembling *Luzuriaga* (see Materials and Methods) implies that Luzuriagoideae existed in New Zealand around 23 Ma. Like so many other New Zealand clades (Pole, 1994; Landis *et al.*, 2008; Jordan *et al.*, 2010) they then went extinct, perhaps during times of submergence, only to reach New Zealand again by long-distance dispersal from southern Chile (Fig. 3). This would be analogous to the New Zealand Richeeae (Ericaceae), which date to < 7 Ma, yet have New Zealand fossils that are 20–25 Myr old (Jordan *et al.*, 2010).

Characteristics of 'southern immigrant' Neotropical plant clades that diversified into equatorial latitudes

Only five angiosperm families shared between South America, New Zealand and/or Australia have expanded and diversified far into tropical latitudes. These are the Alstroemeriaceae, Calceolariaceae, Cunoniaceae, Escalloniaceae and Proteaceae (Table 3). Together, they comprise 670 species or < 1% of Neotropical plant diversity (assuming a total of 90,000 seed plant species for the Neotropics; Gentry, 1982), and they thus form only a small floristic component compared with northern

Table 3 Angiosperm families disjunct between Australia/New Zealand and the Neotropics that managed to expand throughout South America to the equator, with key aspects of their geographical distribution, habitat, habit, pollination and seed dispersal.

Family	Species in the Neotropics/Australia, New Zealand	Centres of diversity in the Neotropics	Habitat	Habit	Pollinators	Fruit type and seed dispersal	Age of Neotropical clade, entry
Alstroemeriaceae	198/3						Crown node: *c.* 57.5 Ma[1]
Alstroemeria[2]	78	S Chile and SE Brazil	Seasonal Mediterranean steppes, deserts, bushland and gallery forests	Erect herbs	Bumblebees (Chile)[3], hummingbirds (mainly in Brazil)[4]	Explosive capsule; autochory	Crown node: *c.* 18.4 Ma[1]
Bomarea[5]	120	Andean region in S Ecuador/N Peru; a secondary centre of diversity in Costa Rica and N Panama	Cloud forests, páramo, lowland rain forests, deserts	Erect, climbing, and prostrate herbs	Hummingbirds (> 80% of species), occ. bees or moths[6]	Loculicidal capsules or berries; ornithochory[6]	Crown node: *c.* 14.3 Ma[1]
Luzuriaga[7]	3/1	S Chile	Cool humid temperate forests	Climbing and creeping herbs	Mainly flies[8]	Berries; ornithochory[9]	Crown node: *c.* 9.5 Ma[1]
Calceolariaceae (*Calceolaria, Jovellana*)[10]	252 (*Calceolaria:* 250; *Jovellana*: 2)/ (*Jovellana:* 4 in New Zealand)	C Chile and N Peru (S of the Huancabamba deflection)	High Andean grasslands, Andean cloud forests and Patagonian steppes	Herbs and shrubs	Apoidea (oil-collecting bees)	Septicidal and loculicidal capsules; autochory	Age of split from sister clade Gesneriaceae unknown. Split *Calceolaria/Jovellana* 15 (4–27) Ma[11]
Cunoniaceae (mainly *Weinmannia*)[12]	83/197	Andean forests of Ecuador and Colombia; Atlantic rain forests in Brazil (*Lamanonia*, *Weinmannia*, together 10 species, 8 endemic)	Mostly in mountain and lowland forests, but also in temperate rain forests in Chile and Argentina, seasonally dry forests in Brazil, Argentina, and Paraguay	Trees and shrubs	Mainly bees	Dry capsules with winged seeds, autochory	Australian macrofossils of *Weinmannia* and *Eucryphia* (currently disjunct between S. Am. and Australia) dated to the Early Oligocene (28.4–33.9 Ma) and Late Palaeocene (55.8–58.7 Ma), respectively[13]
Escalloniaceae (mainly *Escallonia*)[14]	41/3	Southern Andes (Chile and Argentina), SE Brazil, tropical Andes	Andean mountain forests	Trees and shrubs (annual herbs)	No information	Septicidal capsule, autochory	Split from W Australian sister clade, Eremosynaceae 65 (48–81) Ma and 72 (55–87) Ma (using different priors)[15]

Table 3 Continued

Family	Species in the Neotropics/Australia, New Zealand	Centres of diversity in the Neotropics	Habitat	Habit	Pollinators	Fruit type and seed dispersal	Age of Neotropical clade, entry
Proteaceae[16]	85/1095	C. Am., Andean region in Colombia, Ecuador, and Peru, SE Brazil and Guyana highlands. A secondary centre of diversity in temperate S. Am.	Mostly in mountain forests and S Andean alpine habitats	Perennial shrubs and trees	Very few studies on pollination in the Neotropics	Follicles with winged seeds or one-seeded indehiscent fruits; anemochory, hydrochory, zoochory (fruit bats and rodents)	Calibration stem node *Embothrium* (Australia and Tasmania – S. Am.): *c.* 45.8 Ma; estimated crown ages of clades with transoceanic disjunctions: Node A (Australia & Islands – S. Am.): *c.* 61.2 Ma; Node B (Australia – Australia and Tasmania and S. Am.): *c.* 45.1 Ma[17]

C, central; N, north; S, south; SE, south-east; S. Am., South America; C. Am., Central America.
[1]This study, [2]Assis (2001), [3]Aizen (2001), [4]Buzato *et al.* (2000), [5]Hofreiter (2007), [6]Hofreiter & Rodriguez (2006), [7]Rodríguez & Marticorena (1987), [8]Smith-Ramírez *et al.* (2005), [9]Armesto & Rozzi (1989), [10]Cosacov *et al.* (2009), [11]Renner & Schaefer (2010), [12]Bradford *et al.* (2004), [13]Barnes *et al.* (2001), [14]Stevens (2001 onwards), [15]Bell *et al.* (2010, Appendix S24 therein), [16]Prance *et al.* (2007), [17]Barker *et al.* (2007).

plant immigrants into South America. Comparison of the five families reveals few similarities (Table 3): four entered South America well before the uplift of the Patagonian Andes (26–28 Ma); Alstroemeriaceae *c.* 29 Ma, Escalloniaceae *c.* 72 Ma, Cunoniaceae 28.4–33.9 Ma and Proteaceae *c.* 45 Ma (references in Table 3). Only Calceolariaceae (*c.* 260 species in South America) appear to be younger than the Patagonian Andes, yet managed to expand their range from the southern tip of South America to Mexico (Cosacov *et al.*, 2009). All five lineages migrated northwards, mainly along the Andean chain, and all also adapted to subtropical climates in south-eastern Brazil (Table 3). A fuller understanding of the role of the *c.* 16-Myr-old SAAD (our Fig. 1) as an ecological barrier to northwards expansion, however, will require densely sampled and dated species-level analyses and geographical mapping of many more species ranges.

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SUPPORTING INFORMATION

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

Appendix S1 Species sampled in this study, voucher information, geographical origin and GenBank accession numbers.
Appendix S2 Bayesian tree of the Alstroemeriaceae based on the combined analysis of plastid, mitochondrial and nuclear sequences (3130 aligned nucleotides).
Appendix S3 Age estimates for the main nodes of Alstroemeriaceae based on the analysis of the combined alignment of chloroplast, mitochondrial, and nuclear (internal transcribed spacer) sequences.

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BIOSKETCHES

Juliana Chacón is a botanist interested in the evolution of Neotropical plants; she is currently undertaking a PhD at the University of Munich, focusing on the molecular phylogenetics and biogeography of Alstroemeriaceae and Colchicaceae.

Author contributions: J.C. and S.S.R. conceived the study; J.C., M.C.A. and A.W.M. acquired the data; J.C. carried out the analyses; J.C. and S.S.R. wrote the manuscript; all authors read and approved the final version of the manuscript.

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SUPPORTING INFORMATION

From east Gondwana to Central America: historical biogeography of the Alstroemeriaceae

Juliana Chacón, Marta Camargo de Assis, Alan W. Meerow and Susanne S. Renner

Journal of Biogeography

Appendix S1 Species sampled in this study, voucher information, geographical origin and GenBank accession numbers. Species for which sequences from different plants were combined are marked by an asterisk, and three specimens vouchered by photos are marked by a dagger. Undetermined specimens are identified by a herbarium collection number.

Species name	Voucher	Geographical origin	GenBank accession numbers				
			*ndh*F	*rbc*L	*mat*K	*mat*R	ITS
ALSTROEMERIACEAE							
Alstroemeria albescens M. C. Assis	R. B. Setúbal 204 (ICN)	Brazil, Rio Grande do Sul	JQ404506				JQ405003
Alstroemeria amabilis M. C. Assis	O. S. Ribas *et al*. 3072 (SPF)	Brazil, Paraná	JQ404507				
Alstroemeria andina subsp. *venustula* (Phil.) Ehr. Bayer	DNA sample L. Aagesen C648, source plant: R. Kiesling *et al*. 9216 (SI)	Argentina, San Juan	JQ404508	AY120366	JQ404769	JQ404893	JQ405004
Alstroemeria angustifolia Herb. subsp. *angustifolia*	DNA sample L. Aagesen C435, source plant: Cultivated plant P1995-5003 (C)	Chile, Valparaíso	JQ404509	AY120358	JQ404770	JQ404894	
Alstroemeria apertiflora Baker	T. B. Cavalcanti *et al.* 3664 (CEN)	Brazil	JQ404510				
Alstroemeria Assis & Tombolato 532	M. C. Assis & A. F. C. Tombolato 532 (UEC)	Brazil, Sao Paulo	JQ404571	JQ404700	JQ404812	JQ404934	JQ405050
Alstroemeria Assis 640	M. C. Assis 640 (UEC)	Brazil, Rio de Janeiro	JQ404572	JQ404701	JQ404813	JQ404935	
Alstroemeria Assis *et al.* 526	M. C. Assis *et al.* 526 (IAC,	Brazil, Sao Paulo	JQ404570				JQ405049

	SPF)						
Alstroemeria aurea Graham 1	DNA sample L. Aagesen C81, source plant: L. Aagesen s. n. (BAA)	Argentina, Chubut	JQ404511	AY120359	JQ404771	JQ404895	JQ405005
Alstroemeria aurea Graham 2	F. Meyer s. n.†	Chile		JQ404664	JQ404772	JQ404896	JQ405006
Alstroemeria brasiliensis Spreng.	T. B. Cavalcanti *et al.* 2226 (SPF)	Brazil, Tocantins	JQ404512		JQ404773		JQ405007
Alstroemeria caiaponica Ravenna	P. Fiashi & A. C. Marcato 118 (SPF)	Brazil, Goiás	JQ404513				
Alstroemeria calliantha M. C. Assis	J. Dutilh s. n. (UEC)	Brazil, Sao Paulo	JQ404514				
Alstroemeria caryophyllaea Jacq. 1*	A. F. C. Tombolato 1 (IAC)	Brazil, Sao Paulo	JQ404515				
Alstroemeria caryophyllaea Jacq. 2*	A. F. C. Tombolato 2 (IAC)	Brazil, Sao Paulo	JQ404516	JQ404665	JQ404774	JQ404897	JQ405008
Alstroemeria crispata Phil.	K. H. & W. Rechinger 63671 (M)	Chile, Coquimbo	JQ404517	JQ404666	JQ404775	JQ404898	JQ405009
Alstroemeria cunha Vell.	A. Meerow & A. F. C. Tombolato 2103 (NA)	Brazil, Rio de Janeiro	JQ404518	JQ404667	JQ404776	JQ404899	JQ405010
Alstroemeria diluta subsp. *chrysantha* Ehr. Bayer	J. Grau 2547 (M)	Chile, Coquimbo	JQ404519	JQ404668		JQ404900	
Alstroemeria Dutilh s. n.	J. Dutilh s. n. (UEC)	Brazil, Minas Gerais	JQ404574				JQ405051
Alstroemeria exserens Meyen 1*	E. Olate 2786 (SSUC)	Chile	JQ404521				JQ405012
Alstroemeria exserens Meyen 2*	J. Grau 2926 (M)	Chile, Maule		JQ404670			
Alstroemeria fiebrigiana Kraenzl.	K. Fiebrig s. n. 1903-1904 (M)	Bolivia, Tarija	JQ404522	JQ404671			
Alstroemeria foliosa Mart. 1	A. Meerow & A. F. C. Tombolato 2102 (NA)	Brazil, Rio de Janeiro	JQ404523		JQ404778	JQ404902	JQ405013
Alstroemeria foliosa Mart. 2	M. C. Assis 639 (UEC)	Brazil, Rio de Janeiro	JQ404524	JQ404672	JQ404779	JQ404903	JQ405014
Alstroemeria fuscovinosa Ravenna	A. Rapini *et al.* 620 (SPF)	Brazil, Minas Gerais	JQ404525	JQ404673	JQ404780		

Alstroemeria gardneri Baker	J. B. Pereira *et al.* 174 (CEN)	Brazil, Distrito Federal	JQ404526			JQ405015
Alstroemeria graminea Phil.	DNA sample L. Aagesen C85, source plant: C. Bohlen 1275 (SGO)	Chile, Antofagasta	JQ404527	AY120360	JQ404781	
Alstroemeria Heringer *et al.* 656	E. P. Heringer *et al*. 656 (MO)	Brazil, Distrito Federal	JQ404573	JQ404702	JQ404814	JQ404936
Alstroemeria hookeri subsp. *cummingiana* (Herb.) Ehr. Bayer	DNA sample L. Aagesen C448, source plant: Cultivated plant P1995-5010 (C)	Chile, Coquimbo	JQ404528	JQ404674	JQ404782	JQ404904
Alstroemeria inodora Herb. 1	M. C. Assis 633†	Brazil, Sao Paulo	JQ404529			JQ405016
Alstroemeria inodora Herb. 2	M. C. Assis & A. F. C. Tombolato 531 (SPF)	Brazil, Sao Paulo	JQ404530			JQ405017
Alstroemeria isabelleana Herb. 1	A. F. C. Tombolato & A. Meerow 501 (NA)	Brazil, Santa Catarina	JQ404531	JQ404675	JQ404783	JQ404905 JQ405018
Alstroemeria isabelleana Herb. 2	A. F. C. Tombolato & A. Meerow 495 (NA)	Brazil, Santa Catarina	JQ404532		JQ404784	JQ404906 JQ405019
Alstroemeria isabelleana Herb. 3	M. C. Assis 638 (UEC)	Brazil, Santa Catarina	JQ404533	JQ404676	JQ404785	JQ404907 JQ405020
Alstroemeria julieae M. C. Assis	A. M. Farinaccio *et al*. 316 (SPF)	Brazil, Minas Gerais	JQ404534	JQ404677	JQ404786	
Alstroemeria longistaminea Mart. 1	A. Meerow 2204 (NA)	Brazil, Bahia	JQ404537	JQ404680	JQ404789	JQ404910 JQ405023
Alstroemeria longistaminea Mart. 2	A. Meerow 2205 (NA)	Brazil, Bahia	JQ404538	JQ404681	JQ404790	JQ404911 JQ405024
Alstroemeria longistyla Schenk	M. C. Assis & R. S. Bianchetti 341 (SPF)	Brazil, Distrito Federal	JQ404539			
Alstroemeria magnifica Herb. subsp. *magnifica*	DNA sample L. Aagesen C449, source plant: Cultivated plant P1995-5031 (C)	Chile, Valparaíso	JQ404540	JQ404682	JQ404791	JQ404912 JQ405025
Alstroemeria Martius 294	F. Martius 294 (M)	Brazil, Minas Gerais	JQ404569	JQ404699		JQ404933
Alstroemeria Meerow 2207	A. Meerow 2207 (NA)	Brazil, Mato Grosso do Sul	JQ404567	JQ404697	JQ404810	JQ404931 JQ405047
Alstroemeria Meerow 2209	A. Meerow 2209 (NA)	Brazil, Goiás	JQ404568	JQ404698	JQ404811	JQ404932 JQ405048
Alstroemeria monticola Mart.	N. Hind & L. Queiros 50949	Brazil, Bahia	JQ404541	JQ404683		JQ405026

1*	(SPF)						
Alstroemeria monticola Mart. 2*	R. C. Forzza *et al.* 1181 (SPF)	Brazil, Bahia	JQ404542				
Alstroemeria ochracea M. C. Assis 1	A. F. C. Tombolato & J. M. Leme s. n. (BHCB)	Brazil, Minas Gerais	JQ404543				JQ405027
Alstroemeria ochracea M. C. Assis 2	A. Meerow 2206 (NA)	Brazil, Minas Gerais	JQ404544	JQ404684	JQ404792	JQ404913	JQ405028
Alstroemeria orchidioides Meerow, Tombolato & F. K. Mey. 1	A. Meerow 2201 (FLAS)	Brazil, Goiás	JQ404545	JQ404685	JQ404793	JQ404914	JQ405029
Alstroemeria orchidioides Meerow, Tombolato & F. K. Mey. 2	A. Meerow 2202 (FLAS)	Brazil, Goiás	JQ404546	JQ404686	JQ404794	JQ404915	JQ405030
Alstroemeria pallida Graham 1*	DNA sample L. Aagesen C444, source plant: Cultivated plant P1995-5035 (C)	Chile, Santiago	JQ404547	JQ404687	JQ404795	JQ404916	JQ405031
Alstroemeria pallida Graham 2*	Jiles 6104 (CONC)	Chile					EU159930
Alstroemeria patagonica Phil.	DNA sample L. Aagesen C82, source plant: L. Aagesen s. n. (BAA)	Argentina, Neuquén	JQ404548	AY120362	JQ404796	JQ404917	JQ405032
Alstroemeria pelegrina L.	DNA sample L. Aagesen C437, source plant: Cultivated plant P1995-5037 (C)	Chile, Coquimbo	JQ404549	AY120363	JQ404797	JQ404918	
Alstroemeria penduliflora M. C. Assis	R. Mello-Silva *et al*. 2502 (SPF)	Brazil, Minas Gerais	JQ404550				
Alstroemeria Pereira *et al.* 177	J.B. Pereira *et al.* 177 (CEN)	Brazil, Distrito Federal	JQ404566				
Alstroemeria plantaginea Mart. 1*	A.F.C. Tombolato s. n. (IAC)	Brazil, Sao Paulo	JQ404553				JQ405035
Alstroemeria plantaginea Mart. 2*	M.C. Assis & J. Dutilh 339 (SPF)	Brazil, Sao Paulo	JQ404554				
Alstroemeria presliana Herb.	DNA sample L. Aagesen C80, source plant: L. Aagesen s. n.	Argentina, Neuquén	JQ404555	JQ404690	JQ404800	JQ404921	JQ405036

	(BAA)						
Alstroemeria pseudospathulata Ehr. Bayer	DNA sample L. Aagesen C89a, source plant: C.C. Xifreda & A.M. Sanso 2004 (SI)	Argentina, Neuquén	JQ404556	JQ404691	JQ404801	JQ404922	JQ405037
Alstroemeria psittacina Lehm. 1	M.C. Assis 641 (UEC)	Brazil, Rio Grande do Sul					JQ405038
Alstroemeria psittacina Lehm. 2	DNA sample L. Aagesen C91a, source plant: Quesada s. n. (BA)	Argentina, Buenos Aires	JQ404557	AY120364	JQ404802	JQ404923	JQ405039
Alstroemeria punctata Ravenna	J.B. Pereira *et al*. 176 (CEN)	Brazil, Goiás	JQ404558	JQ404692	JQ404803	JQ404924	JQ405040
Alstroemeria pygmaea Herb.	DNA sample L. Aagesen C79b, source plant: L. Aagesen s. n. (BAA)	Argentina, Tucumán	JQ404559	AY120365	JQ404804	JQ404925	JQ405041
Alstroemeria radula Dusén	A. Meerow & A.F.C. Tombolato 2101 (NA)	Brazil, Rio de Janeiro	JQ404560		JQ404805	JQ404926	JQ405042
Alstroemeria revoluta Ruiz & Pav.	DNA sample L. Aagesen C434, source plant: Cultivated plant P1995-5050 (C)	Chile, Maule	JQ404561	JQ404693	JQ404806	JQ404927	JQ405043
Alstroemeria rupestris M. C. Assis	M.C. Assis 635 (UEC)	Brazil, Minas Gerais	JQ404562	JQ404694	JQ404807	JQ404928	JQ405044
Alstroemeria sellowiana Seub.	A. Meerow 2208 (NA)	Brazil, Santa Catarina	JQ404564	JQ404696	JQ404809	JQ404930	JQ405046
Alstroemeria speciosa M. C. Assis	M.C. Assis 634 (UEC)	Brazil, Sao Paulo	JQ404575	JQ404703	JQ404815	JQ404937	
Alstroemeria stenopetala Seub.	J. B. Pereira *et al.* 175 (CEN)	Brazil, Distrito Federal	JQ404577	JQ404704	JQ404816	JQ404938	JQ405052
Alstroemeria stenophylla M. C. Assis	A.F.C. Tombolato 481†	Brazil, Goiás	JQ404578	JQ404705	JQ404817	JQ404939	JQ405053
Alstroemeria Tombolato 17612	A.F.C.Tombolato 17612 (IAC)	Brazil, Bahia	JQ404565				
Alstroemeria Tombolato s. n.	A.F.C. Tombolato s. n. (IAC)	Brazil, Sao Paulo	JQ404576				
Alstroemeria versicolor Ruiz & Pav.	DNA sample L. Aagesen C447, source plant: Cultivated plant	Chile, Libertador General Bernardo	JQ404580	JQ404707	JQ404819	JQ404941	

	P1995-5053 (C)	O'Higgins					
Alstroemeria viridiflora Warm.	M.C. Assis *et al*. 414 (CEN, SPF)	Brazil, Goiás	JQ404581				JQ405055
Alstroemeria werdermannii Ehr. Bayer	DNA sample L. Aagesen C446, source plant: Cultivated plant P1995-5054 (C)	Chile, Atacama	JQ404582	JQ404708	JQ404820	JQ404942	JQ405056
Bomarea acutifolia (Link. & Otto) Herb.	P. Döbbeler 4284 (M)	Costa Rica, Cartago	JQ404584	JQ404822	JQ404822		
Bomarea aff. *patinii* Baker	J. Chacón 02 (ANDES)	Colombia, Valle del Cauca	JQ404585	JQ404711	JQ404823	JQ404944	
Bomarea ampayesana Vargas	A. Hofreiter 2001/2413 (M)	Peru	JQ404586	JQ404712	JQ404824	JQ404945	JQ405058
Bomarea angulata Benth.	G. Harling & L. Andersson 13180 (GB)	Ecuador, Cañar	JQ404587		JQ404825		
Bomarea angustipetala (Benth.) Baker	A. Diaz 76 (ANDES)	Colombia, Cundinamarca	JQ404588	JQ404713	JQ404826	JQ404946	
Bomarea boliviensis Baker	DNA sample L. Aagesen C75b, source plant: L. Aagesen s. n. (BAA)	Argentina, Jujuy	JQ404589	AY120368	JQ404827	JQ404947	
Bomarea bredemeyeriana Herb. 1*	J.M. Velez-Puerta *et al*. 2339 (MEDEL)	Colombia, Antioquia	JQ404590	JQ404714		JQ404948	
Bomarea bredemeyeriana Herb. 2*	F. Alzate 2897 (HUA)	Colombia					EU159933
Bomarea brevis (Herb.) Baker	B. Ståhl 5415 (GB)	Bolivia, Cochabamba	JQ404591	JQ404715	JQ404828	JQ404949	
Bomarea chiriquina Killip	G. Davidse *et al.* 1584 (GB)	Costa Rica, San José	JQ404592	JQ404716	JQ404829	JQ404950	
Bomarea coccinea (Ruiz & Pav.) Baker	J. Farfán 1070 (MO)	Peru, Cusco	JQ404593	JQ404717	JQ404830		
Bomarea crassifolia Baker	C. García 119 (ANDES)	Colombia, Cundinamarca	JQ404594	JQ404718	JQ404831	JQ404951	
Bomarea densiflora Herb.	M. Weigend *et al*. 2000/737 (M)	Peru, Cajamarca	JQ404595	JQ404719	JQ404832	JQ404952	
Bomarea diffracta Baker 1*	F. Alzate 3400 (HUA)	Colombia, Antioquia	JQ404596	JQ404720	JQ404833	JQ404953	
Bomarea diffracta Baker 2*	F. Alzate 3007 (HUA)	Colombia					EU159937
Bomarea dissitifolia Baker	G. Harling & L. Andersson	Ecuador, Loja	JQ404597	JQ404721	JQ404834	JQ404954	

	13536 (GB)						
Bomarea distichifolia (Ruiz & Pav.) Baker	L. Holm-Nielsen *et al*. 3559 (L)	Ecuador, Loja	JQ404598				
Bomarea dulcis (Hook.) Beauverd	A. Hofreiter 2001/2412 (M)	Peru	JQ404599	JQ404722	JQ404835	JQ404955	JQ405059
Bomarea edulis (Tussac) Herb.	DNA sample L. Aagesen C76a, source plant: L. Aagesen s. n. (BAA)	Argentina, Jujuy	JQ404600	JQ404723	JQ404836	JQ404956	
Bomarea engleriana Kraenzl.	CONC						EU159939
Bomarea euryantha Alzate	HUA						EU159940
Bomarea formosissima (Ruiz & Pav.) Herb. 1	W. Hoffmann 212 (M)	Peru, Cusco	JQ404601	JQ404724	JQ404837	JQ404957	
Bomarea formosissima (Ruiz & Pav.) Herb. 2	T. Hofreiter 2AB3 (M)	Peru, Puno	JQ404602	JQ404725	JQ404838		
Bomarea glaberrima Pax	HUA						EU159941
Bomarea glaucescens (Kunth) Baker 1*	L. Holm-Nielsen & B. Øllgaard 24320 (L)	Ecuador, Pichincha	JQ404603	JQ404726	JQ404839	JQ404958	
Bomarea glaucescens (Kunth) Baker 2*	F. Alzate 2930 (HUA)						EU159942
Bomarea Harling & Andersson 13427	G. Harling & L. Andersson 13427 (GB)	Ecuador, Loja	JQ404629		JQ404859		
Bomarea hirsuta (Kunth) Herb.	F. Alzate 2899 (HUA)	Colombia, Cundinamarca	JQ404604		JQ404840	JQ404959	EU159944
Bomarea involucrosa (Herb.) Baker	M. Weigend & H. Förther 97-608 (M)	Peru, Huancavelica	JQ404605	JQ404727	JQ404841	JQ404960	
Bomarea lehmannii Baker	HUA						EU159946
Bomarea linifolia (Kunth) Baker 1*	G. McPherson 13164 (HUA)	Colombia, Antioquia	JQ404606				
Bomarea linifolia (Kunth) Baker 2*	HUA						EU159947
Bomarea macrocephala Pax	DNA sample L. Aagesen C78b, source plant: L. Aagesen s. n. (BAA)	Argentina, Tucumán	JQ404607	AY120367	JQ404842	JQ404961	
Bomarea macusanii Hofreiter	M. & K. Weigend 2000/197	Peru, Cusco	JQ404608	JQ404728	JQ404843		

& E. Rodr. 1	(M)						
Bomarea macusanii Hofreiter & E. Rodr. 2	M. & K. Weigend 2000/422 (M)	Peru, Cusco	JQ404609	JQ404729	JQ404844		
Bomarea multiflora (L.f.) Mirb. 1*	P.J.M. Maas & L. Cobb 4813 (L)	Ecuador, Pichincha	JQ404610		JQ404845		
Bomarea multiflora (L.f.) Mirb. 2*	HUA						EU159949
Bomarea multipes Benth.	G. Harling 11316 (GB)	Ecuador, Loja	JQ404611	JQ404730	JQ404846	JQ404962	
Bomarea nematocaulon Killip	T. Franke & T. Hofreiter 4/6 (M)	Peru	JQ404612	JQ404731	JQ404847	JQ404963	
Bomarea nervosa (Herb.) Baker	M. Weigend *et al*. 2000/924 (M)	Peru, San Martín	JQ404613	JQ404732	JQ404848	JQ404964	JQ405060
Bomarea ovallei (Phil.) Ravenna	DNA sample L. Aagesen C676, source plant: Cultivated plant s. n. (C)	Chile, Atacama	JQ404614	AY120369	JQ404849	JQ404965	
Bomarea ovata (Cav.) Mirb.	M. Weigend *et al*. 5106 (M)	Peru, Ancash	JQ404615	JQ404733	JQ404850	JQ404966	
Bomarea pardina Herb.	J. Chacón 06 (M), cultivated plant Munich Bot. Gard. 05/3092	Ecuador	JQ404616	JQ404734	JQ404851	JQ404967	
Bomarea parvifolia Baker	M. Weigend *et al*. 7264 (M)	Peru, Lima	JQ404617	JQ404735	JQ404852	JQ404968	
Bomarea patacocensis Herb.	M.A. Bello 090 (ANDES)	Colombia, Cundinamarca	JQ404618	JQ404736	JQ404853	JQ404969	
Bomarea patinii Baker	F. Alzate 2894 (HUA)	Colombia, Cundinamarca	JQ404619	JQ404737	JQ404854	JQ404970	EU159951
Bomarea pauciflora (Kunth) Herb. 1*	J. Betancur & J. Sarmiento 3994 (HUA)	Colombia, Cundinamarca	JQ404620				
Bomarea pauciflora (Kunth) Herb. 2*	HUA						EU159952
Bomarea perglabra Harling & Neuendorf	G. Harling *et al.* 8888 (GB)	Ecuador, Cotopaxi	JQ404621	JQ404738	JQ404855	JQ404971	
Bomarea porrecta Killip	M. Weigend *et al*. 97/245 (M)	Peru, La Libertad	JQ404622				
Bomarea puracensis Alzate	L. Albert *et al*. 4321 (HUA)	Colombia, Cauca	JQ404623				
Bomarea purpurea (Ruiz & Pav.) Herb.	M. Weigend & N. Dostert 97/67a (M)	Peru, Pasco	JQ404624	JQ404739	JQ404856	JQ404972	

Bomarea salsilla (L.) Mirb.	M. Ackermann 545 (M)	Chile, Maule	JQ404625	JQ404740	JQ404857	JQ404973	
Bomarea setacea (Ruiz & Pav.) Herb. 1*	L. Albert & B.A. Stein 6201 (HUA)	Colombia, Antioquia	JQ404626				
Bomarea setacea (Ruiz & Pav.) Herb. 2*	HUA						EU159953
Bomarea setacea (Ruiz & Pav.) Herb. 3	W.D. Rodriguez & J. Rios 6134 (MEDEL)	Colombia, Antioquia	JQ404627				
Bomarea simplex Herb.	S.G. Beck 17544 (M)	Bolivia, La Paz	JQ404628	JQ404741	JQ404858	JQ404974	
Bomarea speciosa Killip	M. Weigend *et al*. 5407 (M)	Peru, Huanuco	JQ404630		JQ404860		
Bomarea spissiflora Harling & Neuendorf	G. Harling & L. Andersson 13030 (GB)	Ecuador, Morona Santiago	JQ404631	JQ404742	JQ404861	JQ404975	
Bomarea stans Kraenzl.	R. Ehrich 187 (M)	Bolivia, Tarija	JQ404632		JQ404862		
Bomarea straminea Killip ex Alzate	HUA						EU159954
Bomarea superba Herb.	M. Weigend *et al*. 2000/730 (M)	Peru, Cajamarca	JQ404633	JQ404743	JQ404863		
Bomarea tribrachiata Kraenzl.	M. Weigend *et al*. 97/445 (M)	Peru, Cajamarca	JQ404634				
Bomarea uncifolia Herb.	U. Molau & B. Eriksen 2982 (GB)	Ecuador, Bolivar	JQ404635	JQ404744	JQ404864	JQ404976	
Bomarea vargasii Hofreiter	M. Weigend *et al*. 5030 (M)	Peru, Ancash	JQ404636	JQ404745	JQ404865	JQ404977	
Bomarea vestita Baker	F. Alzate 2814 (HUA)	Colombia, Risaralda	JQ404637	JQ404746	JQ404866	JQ404978	EU159955
Drymophila cyanocarpa R. Br. 1	C. Helman s. n. (NE)	Tasmania, South Cape Rivulet	JQ404643	JQ404751	JQ404872		
Drymophila cyanocarpa R. Br. 2	G. Jordan s. n. (UTAS)	Tasmania, Fern Tree	JQ404644	JQ404752	JQ404873	JQ404983	
Drymophila moorei Baker 1	D. Crestani 48 (NSW)	Australia, New South Wales	JQ404645	JQ404753	JQ404874	JQ404984	JQ405063
Drymophila moorei Baker 2	P.D. Hind 6021 (NSW)	Australia, New South Wales	JQ404646	JQ404754	JQ404875	JQ404985	
Luzuriaga marginata (Gaertn.) Benth. & Hook.f. 1	M. Gusinde 119 (M)	Chile, Magallanes	JQ404650	JQ404757	JQ404878	JQ404988	JQ405066
Luzuriaga marginata (Gaertn.) Benth. & Hook.f. 2	DNA sample L. Aagesen C74, source plant: L. Aagesen s. n.	Argentina, Río Negro	JQ404651	JQ404758	JQ404879	JQ404989	

	(BAA)						
Luzuriaga parviflora (Hook.f.) Kunth 1	A. Meebold 4332 (M)	New Zealand	JQ404653		JQ404881	JQ404991	
Luzuriaga parviflora (Hook.f.) Kunth 2	U. Schweinfurth 210 (M)	New Zealand, Stewart Island	JQ404654	JQ404760	JQ404882	JQ404992	
Luzuriaga parviflora (Hook.f.) Kunth 3	U. Schweinfurth 603 (M)	New Zealand, South Island	JQ404655	JQ404761	JQ404883	JQ404993	
Luzuriaga polyphylla (Hook.f.) J. F. Macbr.	K.H. & W. Rechinger 63997 (M)	Chile, Aisén	JQ404656	JQ404762	JQ404884	JQ404994	
Luzuriaga radicans Ruiz & Pav. 1	E. Bayer 214 (M)	Chile, Biobío	JQ404657		JQ404885	JQ404995	
Luzuriaga radicans Ruiz & Pav. 2	M. Weigend *et al*. 7024 (K)	Argentina, Chubut		JQ404763	JQ404886	JQ404996	JQ405067
CAMPYNEMATACEAE							
Campynema lineare Labill.	Walsh 3488 (MEL)	Tasmania	AF276013	Z77264			
Campynemanthe viridiflora Baill.	C. Skottsberg 189 (S)	New Caledonia		AJ276349			
COLCHICACEAE							
Androcymbium dregei C. Presl.	Roy. Bot. Gardens Kew DNA Bank 26666, source plant: J. C. Manning 2355, (SANBI LHMS #239)	South Africa	JQ404520	JQ404669	JQ404777	JQ404901	JQ405011
Baeometra sp.	Roy. Bot. Gardens Kew DNA Bank 2222, source plant: M. W. Chase 2222 (K)	Australia, Perth	JQ404638	JQ404747	JQ404867	JQ404979	JQ405061
Bulbocodium vernum L.	J. Chacón 05 (M), cultivated plant Munich Bot. Gard. G/0312	Western Europe	JQ404639	JQ404748	JQ404868	JQ404980	
Burchardia umbellata R. Br.	M.W. Chase 1006 (K)	Australia	Z77266				
Camptorrhiza strumosa (Baker f.) Oberm.	Roy. Bot. Gardens Kew DNA Bank 28317, source plant: SANBI LHMS #484	South Africa	JQ404641		JQ404870		
Colchicum autumnale L. 1*	A. Case 84 (TRT)	Canada	EU044627				
Colchicum autumnale L. 2*	Gulyas 626-23 (DE)	North America					AJ876751

Colchicum bornmuelleri Freyn	Roy. Bot. Gardens Kew DNA Bank 26687, source plant: SANBI LHMS #266	South Africa	JQ404640	JQ404749	JQ404869	JQ404981	JQ405062
Disporum cantoniense (Lour.) Merr.	Roy. Bot. Gardens Kew DNA Bank 18195, source plant: M.W. Chase 18195 (K)	Indonesia	JQ404642	JQ404750	JQ404871	JQ404982	
Disporum viridescens (Maxim.) Nakai	DNA sample L. Aagesen C422, source plant: Cultivated plant S1939-1565 (C)	China	JQ404647	JQ404755	JQ404876	JQ404986	JQ405064
Gloriosa superba L.	A. Case 82 (TRT)	South Africa	EU044626				
Hexacyrtis dickiana Dinter	Roy. Bot. Gardens Kew DNA Bank 26676, source plant: SANBI LHMS #249	South Africa	JQ404648				
Iphigenia indica (L.) A. Gray ex Kunth	M.W. Chase 1028 (K)	India	AY224999				
Kuntheria pedunculata (F. Muell.) Conran & Clifford	P.I. Foster 21749 (BRI)	Australia, Queensland	JQ404649	JQ404756	JQ404877	JQ404987	JQ405065
Littonia modesta Hook.	Roy. Bot. Gardens Kew DNA Bank 26646, source plant: SANBI LHMS #203	South Africa	JQ404652	JQ404759	JQ404880	JQ404990	
Merendera montana (L.) Lange	Roy. Bot. Gardens Kew DNA Bank 1070, source plant: M.W. Chase 1070 (K)	Spain	JQ404658	JQ404764	JQ404887	JQ404997	
Onixotis triquetra (L.f.) Mabb.	Roy. Bot. Gardens Kew DNA Bank 422, source plant: P. Goldblatt s. n. (MO)	South Africa	JQ404659	JQ404765	JQ404888	JQ404998	JQ405068
Sandersonia aurantiaca Hook.	Roy. Bot. Gardens Kew DNA Bank 580, source plant: M.W. Chase 580 (K)	RBG KEW Liv. Coll. 1952-1802	JQ404660	JQ404766	JQ404889	JQ404999	
Tripladenia cunninghamii D. Don 1*	R.C. Coveny & A.J. Whalen 16692 (K)	Australia, New South Wales	AY465655				
Tripladenia cunninghamii D. Don 2*	A. Whelan s. n. (SYD)	Australia		Z77268			
Uvularia grandiflora Sm.	DNA sample L. Aagesen C857,	USA	JQ404661	AB09950.	JQ404890	JQ405000	JQ405069

	source plant: Cultivated plant 1080-4 (C)						
Uvularia perfoliata L.	Roy. Bot. Gardens Kew DNA Bank 494, source plant: M.W. Chase 494 (K)	RBG KEW, Liv. Coll. 1956-55702. Donated by Perry's Hardy Plant Farm	JQ404662	JQ404767	JQ404891	JQ405001	
Wurmbea kraussii Baker	Roy. Bot. Gardens Kew DNA Bank 28321, source: SANBI LHMS #488	South Africa	JQ404663	JQ404768	JQ404892	JQ405002	JQ405070
Wurmbea pygmaea (Endl.) Benth.	A. Case 77 (PERTH)	Australia	AF547012				
LILIACEAE							
Lilium superbum L.	M.W. Chase 112 (NCU)	USA	AY007655	L12682			
MELANTHIACEAE							
Chamaelirium luteum (L.) A. Gray	S.W. Leonard 3198	USA	AY225006	AJ276347			
PHILESIACEAE							
Philesia buxifolia Lam. ex Poir.	M.W. Chase 545 (K)	Chile	AF276014	Z77302			
RHIPOGONACEAE							
Rhipogonum elseyanum F. Muell.	M.W. Chase 187 (NCU)	Australia, Sydney	AF276016	Z77309			
SMILACACEAE							
Smilax china L.	H. Kato s. n. (KYO)	Japan, Pref. Shiga		D28333			
Smilax glauca Walter	M.W. Chase 107 (NCU)	USA		Z77310			
Smilax hispida Muhl. ex Torr	T.J. Givnish s. n. (WIS)	USA	AF276018				
Smilax rotundifolia L.	Uhl 92-07 (BH)	USA	AY465710				

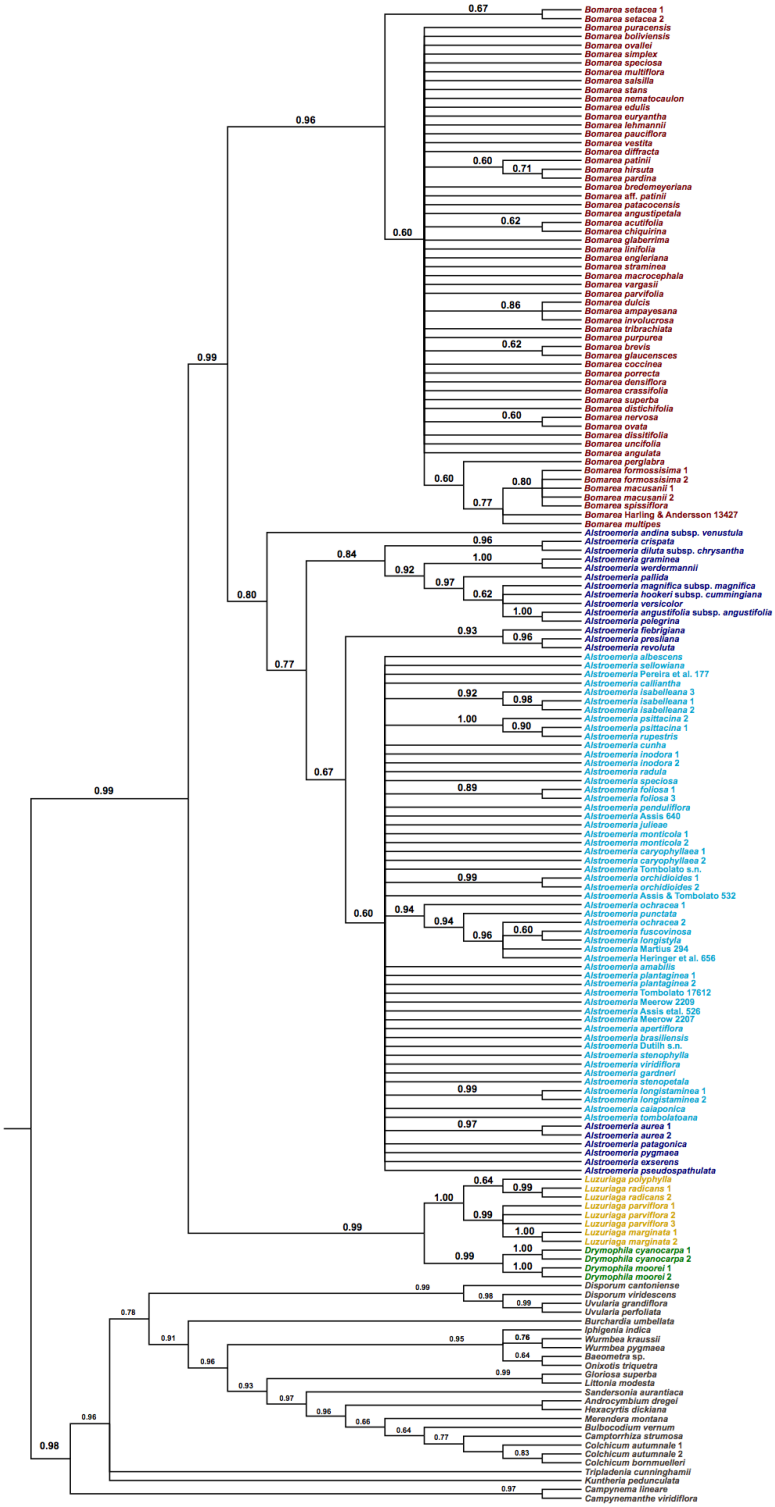
SUPPORTING INFORMATION

From east Gondwana to Central America: historical biogeography of the Alstroemeriaceae

Juliana Chacón, Marta Camargo de Assis, Alan W. Meerow and Susanne S. Renner

Journal of Biogeography

Appendix S2 Bayesian tree of the Alstroemeriaceae based on the combined analysis of plastid, mitochondrial and nuclear sequences (3130 aligned nucleotides). The tree is rooted on the sister clade, Colchicaceae, plus two species of Campynemataceae. Posterior probability values are shown above branches.


Bomarea setacea 1
Bomarea setacea 2
Bomarea puracensis
Bomarea boliviensis
Bomarea ovallei
Bomarea simplex
Bomarea speciosa
Bomarea multiflora
Bomarea salsilla
Bomarea stans
Bomarea nematocaulon
Bomarea edulis
Bomarea euryantha
Bomarea lehmannii
Bomarea pauciflora
Bomarea vestita
Bomarea diffracta
Bomarea patinii
Bomarea hirsuta
Bomarea pardina
Bomarea bredemeyeriana
Bomarea aff. patinii
Bomarea pataconsis
Bomarea angustipetala
Bomarea acutifolia
Bomarea chiquirina
Bomarea glaberrima
Bomarea linifolia
Bomarea englerianа
Bomarea straminea
Bomarea macrocephala
Bomarea vargasii
Bomarea parvifolia
Bomarea dulcis
Bomarea ampayesana
Bomarea involucrosa
Bomarea tribrachiata
Bomarea purpurea
Bomarea brevis
Bomarea glaucensces
Bomarea coccinea
Bomarea porrecta
Bomarea densiflora
Bomarea crassifolia
Bomarea superba
Bomarea distichifolia
Bomarea nervosa
Bomarea ovata
Bomarea dissitifolia
Bomarea uncifolia
Bomarea angulata
Bomarea perglabra
Bomarea formossisima 1
Bomarea formossisima 2
Bomarea macusanii 1
Bomarea macusanii 2
Bomarea spissiflora
Bomarea Harling & Andersson 13427
Bomarea multipes
Alstroemeria andina subsp. venustula
Alstroemeria crispata
Alstroemeria diluta subsp. chrysantha
Alstroemeria graminea
Alstroemeria werdermannii
Alstroemeria pallida
Alstroemeria magnifica subsp. magnifica
Alstroemeria hookerl subsp. cumminglana
Alstroemeria versicolor
Alstroemeria angustifolia subsp. angustifolia
Alstroemeria pelegrina
Alstroemeria flebrigiana
Alstroemeria presiliana
Alstroemeria revoluta
Alstroemeria albescens
Alstroemeria sellowiana
Alstroemeria Pereira et al. 177
Alstroemeria callantha
Alstroemeria isabelleana 3
Alstroemeria isabelleana 1
Alstroemeria isabelleana 2
Alstroemeria psittacina 2
Alstroemeria psittacina 1
Alstroemeria rupestris
Alstroemeria cunha
Alstroemeria inodora 1
Alstroemeria inodora 2
Alstroemeria radula
Alstroemeria speciosa
Alstroemeria follosa 1
Alstroemeria follosa 3
Alstroemeria pendulifiora
Alstroemeria Assis 640
Alstroemeria juliaae
Alstroemeria monticola 1
Alstroemeria monticola 2
Alstroemeria caryophyllaea 1
Alstroemeria caryophyllaea 2
Alstroemeria Tombolato s.n.
Alstroemeria orchidioides 1
Alstroemeria orchidioides 2
Alstroemeria Assis & Tombolato 532
Alstroemeria ochracea 1
Alstroemeria punctata
Alstroemeria ochracea 2
Alstroemeria fuscovinosa
Alstroemeria longistyla
Alstroemeria Martius 294
Alstroemeria Heringer et al. 656
Alstroemeria amabilis
Alstroemeria plantaginea 1
Alstroemeria plantaginea 2
Alstroemeria Tombolato 17612
Alstroemeria Meerow 2209
Alstroemeria Assis etal. 526
Alstroemeria Meerow 2207
Alstroemeria apertiflora
Alstroemeria brasiliensis
Alstroemeria Dutilh s.n.
Alstroemeria stenophylla
Alstroemeria viridiflora
Alstroemeria gardneri
Alstroemeria stenopetala
Alstroemeria longistaminea 1
Alstroemeria longistaminea 2
Alstroemeria caiaponica
Alstroemeria tombolatoana
Alstroemeria aurea 1
Alstroemeria aurea 2
Alstroemeria patagonica
Alstroemeria pygmaea
Alstroemeria exserens
Alstroemeria pseudospathulata
Luzuriaga polyphylla
Luzuriaga radicans 1
Luzuriaga radicans 2
Luzuriaga parviflora 1
Luzuriaga parviflora 2
Luzuriaga parviflora 3
Luzuriaga marginata 1
Luzuriaga marginata 2
Drymophila cyanocarpa 1
Drymophila cyanocarpa 2
Drymophila moorei 1
Drymophila moorei 2
Disporum cantoniense
Disporum viridescens
Uvularia grandiflora
Uvularia perfoliata
Burchardia umbellata
Iphigenia indica
Wurmbea kraussii
Wurmbea pygmaea
Baeometra sp.
Onixotis triquetra
Gloriosa superba
Littonia modesta
Sandersonia aurantiaca
Androcymbium dregei
Hexacyrtis dickiana
Merendera montana
Bulbocodium vernum
Camptorrhiza strumosa
Colchicum autumnale 1
Colchicum autumnale 2
Colchicum bornmuelleri
Tripladenia cunninghamii
Kuntheria pedunculata
Campynema linearе
Campynemanthe viridiflora

SUPPORTING INFORMATION

From east Gondwana to Central America: historical biogeography of the Alstroemeriaceae

Juliana Chacón, Marta Camargo de Assis, Alan W. Meerow and Susanne S. Renner

Journal of Biogeography

Appendix S3 Age estimates for the main nodes of Alstroemeriaceae based on the analysis of the combined alignment of chloroplast, mitochondrial, and nuclear (ITS) sequences. A. Age estimates (Ma) inferred with outgroup calibrations only. B. Age estimates (Ma) inferred with an additional ingroup calibration from a *Luzuriaga*-like fossil; n.a. = not applicable.

Node number	Description	A [95% HPD]	B [95% HPD]
I	Stem Alstroemeriaceae	97.7 [80.5–115.8]	95.0 [76.4–115.4]
II	Crown Alstroemeriaceae	73.2 [50.8–95.0]	66.8 [44.2–88.8]
III	Crown Alstroemerioideae	42.0 [26.2–60.1]	38.9 [24.4–54.6]
IV	Crown Luzuriagoideae	38.1 [18.7–58.6]	n.a.
V	Crown *Alstroemeria*	25.8 [16.3–37.3]	23.5 [14.3–34.2]
VI	Stem Brazilian *Alstroemeria*	20.2 [12.4–28.6]	18.3 [11.3–26.2]
VII	Crown *Bomarea*	18.2 [8.6–31.6]	16.5 [7.5–28.1]
VIII	Crown *Drymophila*	5.8 [0.8–13.1]	4.5 [0.9–9.7]
IX	Crown *Luzuriaga*	12.2 [4.1–22.1]	9.3 [4.1-14.6]

Chapter 3

LEAF FOSSILS OF *LUZURIAGA* AND A MONOCOT FLOWER WITH *IN SITU* *LILIACIDITES CONTORTUS* MILDENH. SP. NOV. POLLEN FROM THE EARLY MIOCENE

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Abstract

Premise of the study: The Foulden Maar lake sediments in Otago, South Island, New Zealand, date to the earliest Miocene and provide an important picture of the diversity of the Australasian biota, paleoecology and climate at a time when New Zealand had a smaller land area than today. The diverse rainforest contains many taxa now restricted to Australia, New Caledonia, or South America. The presence of *Luzuriaga*-like fossils in these deposits is important for understanding Alstroemeriaceae evolution and the biogeography of genera shared between New Zealand and South America.

Methods: Leaves and a flower with in situ pollen that resemble extant *Luzuriaga* are described and placed phylogenetically. Geographic range information and a molecular clock model for the Alstroemeriaceae were used to investigate possible biogeographic scenarios and the influence of the new fossil on inferred divergence times.

Key results: *Luzuriaga peterbannisteri* Conran, Bannister, Mildenh., & D.E.Lee sp. nov. represents the first macrofossil record for Alstroemeriaceae. An associated *Luzuriaga*-like flower with in situ fossil pollen of *Liliacidites contortus* Mildenh. sp. nov. is also described. The biogeographic analysis suggests that there have been several dispersal events across the Southern Ocean for the genus, with the fossil representing a now-extinct ancestral New Zealand lineage.

Conclusions: *Luzuriaga* was present in early Miocene New Zealand, indicating a long paleogeographic history for the genus, and *L. peterbannisteri* strengthens biogeographic connections between South America and Australasia during the Oligo-Miocene.

Keywords: Alstroemeriaceae; biogeography; earliest Miocene; fossil plants; Liliales; monocot; pollen

Introduction

The monocot family Alstroemeriaceae contains four genera and ~200 species (Angiosperm Phylogeny Group, 2009; Chacón et al., 2012). The family is probably best known for the horticulturally important *Alstroemeria* L. (Peruvian Lily), with 78 species, several of them used in the cut-flower trade. Together with *Bomarea* Mirb.

(120 species), these two genera comprise the subfamily Alstroemerioideae, which has a wide distribution in South and Central America (Fig. 1). The second subfamily of Alstroemeriaceae is the Luzuriagoideae, consisting of *Luzuriaga* Ruiz & Pav. with three species in Chile and one in New Zealand, and *Drymophila* R.Br. with one species in Australia and one in Tasmania. The fossil record of the family consists of reports of *Luzuriaga* pollen from the Quaternary of New Zealand (Wardle et al., 2001) and Chile (Ashworth et al., 1991) and a contested association of the auriculate pollen morphotype *Auriculiidites reticulatus* Elsik with some species of *Bomarea* (see Macphail and Partridge, 2012 and references therein).

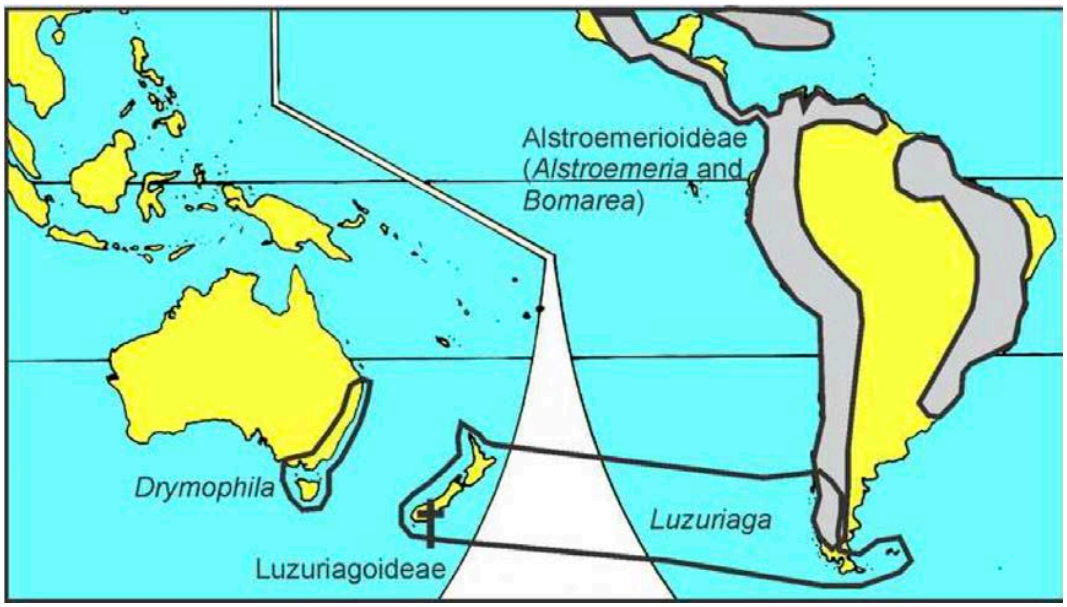


Figure 1. Distribution of extant Alstroemeriaceae and the location of the fossil (†).

Research on macrofossils in New Zealand, mostly leaves with well-preserved distinctive and diagnostic cuticles from *Lagerstätten* deposits and lignites of Late Oligocene and Early Miocene age from Otago and Southland, suggest that many New Zealand plants have been present on the island for at least 25–23 million years and possibly longer (e.g. Lee et al., 2001; 2007b; 2012). For example, there are macrofossils and/or pollen records for nearly all the extant New Zealand conifer genera (Jordan et al., 2011). Forest trees with macrofossil records include species of Cunoniaceae, Elaeocarpaceae, Atherospermataceae, Monimiaceae, Myrsinaceae, Lauraceae and Onagraceae, and when combined with pollen records from the same

sites, this list increases. Deep fossil records are now available for a considerable number of modern New Zealand forest families, including Chloranthaceae, Strasburgeriaceae, Myrtaceae, Proteaceae and others (see Pole, 2008; Lee et al., 2012, and references therein). Similarly, some extant New Zealand monocots now have fossil records extending back to the Late Oligocene–Early Miocene, if not earlier, including Arecaceae (Ballance et al., 1981; Pole, 1993b; Hartwich et al., 2010), Asparagaceae: *Cordyline* Comm. ex R.Br. (unpubl. data), Asteliaceae (Maciunas et al., 2011), Orchidaceae (Conran et al., 2009a), Ripogonaceae (Pole, 1993a), Typhaceae (Pole, 2007), and Xanthorrhoeaceae: *Dianella* Lam. ex Juss. or *Phormium* J.R.Forst. & G.Forst. (Maciunas et al., 2009; Ferguson et al., 2010).

One of the richest Miocene fossil sites is Foulden Maar in Otago, South Island, which to date has yielded a wide range of leaf, flower and fruit taxa (Bannister et al., 2005; Lee et al., 2012). Most of the plant macrofossils are isolated, more-or-less complete, compressed mummified leaves of which about 45% are from the family Lauraceae, including common species with affinities to *Cryptocarya* R.Br.*, Beilschmiedia* Nees, and *Litsea* Lam. (Bannister et al., 2012). The remainder represent a diverse range of families, including Araliaceae, Cunoniaceae, Elaeocarpaceae, Euphorbiaceae, Menispermaceae, Myrsinaceae, Myrtaceae, Proteaceae, and Sterculiaceae (Lee et al., 2012). The site has also yielded over 130 insect fossils (Kaulfuss et al., 2010; 2011), with many leaves showing evidence of insect damage by chewing or leaf mining and some bearing *in situ* scale insects (Harris et al., 2007). Several leaf taxa with prominent domatia, indicating possible associations with beneficial leaf mites, have been described, as well as some plants with conspicuous extra-floral nectaries (Lee et al., 2010).

Although monocot leaf fossils are rare globally, the Foulden site has yielded several types of monocot leaves, including *Astelia* Banks & Sol. ex R.Br.*, Cordyline,* two orchids, *Ripogonum* J.R.Forst & G.Forst., and *Typha* L. (Conran et al., 2009c, 2011). Cuticular analysis showed that the fossil *Astelia* is related to *A. alpina* R.Br. and *A. linearis* Hook.f., but differs from these modern species (Maciunas et al., 2011) for at least 10 features of cuticular morphology. The orchid leaves from Foulden are the oldest unequivocal vegetative orchid fossils (Conran et al., 2009a) and represent two epiphytic genera within subfamily Epidendroideae, *Dendrobium* Sw. and *Earina* Lindl. Preliminary investigations of *Luzuriaga*-like leaves discovered at the site from

2005–2012 suggested that they represent a new species, and here we describe and phylogenetically place these leaves, as well as a *Luzuriaga*-like monocot flower with *in situ* pollen found at the same site.

Materials and methods

Fossil collection and preparation

The specimens were collected from a finely varved, leaf-bearing diatomite in a small mining pit on Foulden Hills Station, near Middlemarch, Otago, registered as I43/f8503 in the New Zealand Fossil Record File administered by the Geological Society of New Zealand. The NZ Map Grid reference is NZMS 260 I43/929166 (45.5271°S, 170.2218°E). The site is described in detail in Bannister et al. (2005), Lee et al. (2007a), and Lindqvist and Lee (2009).

The fossil locality is in the upper part of the Foulden Hills Diatomite (Pole, 1993c, 1996), which was formed in a maar lake that resulted from a short-lived explosive volcanic vent during an early phase of Dunedin Volcanic Group volcanism (Coombs et al., 1986). Based on a palynoflora from the same locality, Couper (in Coombs et al., 1960) suggested a Taranaki Series (Late Miocene) to Waitotaran (Pliocene) age. More recent work on palynofloras of Oligocene and Miocene strata from Otago and Southland (Pocknall and Mildenhall, 1984; Mildenhall and Pocknall, 1989) indicates an Early Miocene age (*Spinitricolpites latispinosus* Zone). This is consistent with a latest Oligocene to Early Miocene date of 23 ± 0.2 million years ago (Ma) radiometric age from the associated volcanics (Lindqvist and Lee, 2009; Kaulfuss et al., 2011), corresponding to the Waitakian Stage in New Zealand (Cooper, 2004).

The fossils were preserved as mummified compressions on light-colored bedding planes dominated by diatom frustules and the leaves and flowers were prepared following the methods outlined in Bannister et al. (2012). In addition, a few in situ pollen grains from the perianth parts were removed using a very fine paintbrush, cleared for a short period in 10% KOH, rinsed in water and mounted in glycerin jelly on a slide for light microscopy and photography. Comparative reference specimens for pollen grains of a range of species from all extant Alstroemeriaceae genera were also prepared.

A palynological preparation was made at GNS Science, Lower Hutt, from a small piece of the diatomite slab on which the flower is preserved. Standard processing techniques were used for the processing of pollen slides (e.g. Moore et al., 1991). Treatment comprised hydrofluoric acid digestion, followed by nitric acid oxidation to remove amorphous organic matter, then sieving to retain the 10–260 µm palynomorph fraction. The organic residue consisted of abundant, well-preserved to semi-degraded plant cuticle, felted amorphous organic matter, and well-preserved pollen and spores.

Fossil pollen grains from the perianth and matching grains from the diatomite were compared to the database of fossil pollen grains from New Zealand (Raine et al., 2011). The coordinates of the type specimens were taken from a Zeiss Axioplan 2 imaging photomicroscope at GNS Science, Lower Hutt, New Zealand. The slides, prefixed by the letter L, are housed in the palynological type collection of GNS Science.

Phylogenetic analysis

To place the fossil phylogenetically, morphological and anatomical characters were used to construct a data matrix for the six extant Luzuriagoideae species (Table 1), based on examination of preserved collections housed at Adelaide University (ADU), Otago Regional Herbarium (OTA), and live specimens in cultivation in Adelaide and the Dunedin Botanic Gardens. Missing data were coded as '?'. Data were also coded for *Alstroemeria* and *Bomarea* (Alstroemerioideae), the sister clade to Luzuriagoideae (Chacón et al., 2012). Information on character states was also obtained from Conover (1983, 1991), Conran (1985, 1987, 1989), Arroyo and Leuenberger (1988), Rodriguez and Marticorena (1988), Bayer (1998b), Conran and Clifford (1998) and Hofreiter and Lyshede (2006) and Hofreiter (2007).

These data were analysed using the parsimony ratchet option (10,000 replicates; random addition; mult*TBR; hold 20 trees; sample 6 characters; all character non-additive) in ASADO version 1.89 (Nixon, 2004). The analyses were run with extant taxa and with the fossil included or excluded, and the robustness of the trees was assessed using both bootstrapping (10,000 reps; 33% resampling) and Bremer decay analysis (20 steps limit) with TNT 1.1 (Goloboff et al., 2008), following Jordan and

Hill (1999) and Conran et al. (2009b). Character state mapping was performed in ASADO using the ACCTRAN option.

Table 1. Morphological and anatomical matrix used for phylogenetic placement of the fossil. Characters and character states: **1**. Stem growth: 0=indeterminate, 1=determinate, 2=annual herbaceous scape; **2**. Stems branching: 0=absent, 1=present; **3**. Leaf margin: 0=smooth, 1=serrulate; **4**. Vein order no.: 0=>4, 1=4, 2=3, 3=2; **5**. Primary vein number: 0=3, 1=5, 2=>5; **6**. Exmedial vein convergence: 0=apical, 1=proximal; **7**. Acropetal weakening: 0=slight, 1=pronounced, 2=very pronounced; **8**. Highest vein orientation: 0=parallel, 1=transverse, 2=random; **9**. Free vein ends: 0=absent, 1=present, 2=rare; **10**. Abaxial periclinal surface: 0=smooth, 1=granulate; **11**. Adaxial papillae: 0=absent, 1=bands, 2=uniform; **12**. Adaxial sinuosity: 0=straight/curved, 1=weak (ht/w <0.5), 2=strong (ht/w ≥0.5); **13**. Abaxial sinuosity: 0=straight/curved, 1=weak, 2=strong; **14**. Stomata sunken: 0=absent, 1=present; **15**. Stomatal papillae: 0=absent, 1=present; **16**. Stomatal contact cells: 0=anomocytic, 1=paracytic, 2=tetracytic, 3=hexacytic; **17**. Adaxial vein cells: 0=undifferentiated, 1=differentiated; **18**. Adaxial vein wall sinuosity: 0=strong, 1=weak, 2=straight/curved; **19**. Abaxial vein wall sinuosity: 0=strong, 1=weak, 2=straight/curved; **20**. Inflorescence branched: 0=present, 1=absent; **21**. Flowers per inflorescence: 0=many, 1=solitary; **22**. Floral bracts: 0=solitary, 1=multiple; **23**. Tepal marcesence: 0=absent, 1=present; 24. Tepals clawed: 0=absent, 1=present; **25**. Tepal color: 0=whitish to pale pink, 1=strongly coloured; **26**. Tepals spotted: 0=absent, 1=present; **27**. Pollen wall: 0=thick, 1=thin; **28.** Pollen exine: 0=coarsely reticulate/foveolate, 1=finely granulate; **29.** Ovary position: 0=superior, 1=inferior; **30**. Style: 0=long, 1=short, 2=sessile; **31**. Stigma: 0=capitate, 1=sessile, 2=trifid; **32**. Placentation: 0=axile, 1=parietal; **33**. Fruit dehiscence: 0=absent, 1=present; **34**. Seed color: 0=brown, 1=pale yellow; **35**. Seed surface: 0=smooth, 1=tuberculate. Polymorphies are indicated as: *=0,1; $=1,2.

Taxon	1				5					10					15					20
Alstroemeria	2	0	*	0	2	0	1	1	0	0	0	2	2	0	0	2	1	2	2	0
Bomarea	2	*	*	0	2	0	1	1	0	0	0	$	2	0	0	2	1	2	2	0
Luzuriaga parviflora	1	0	0	3	0	1	0	0	2	1	1	0	0	1	1	3	1	2	2	1
Luzuriaga marginata	1	0	1	2	1	1	0	1	0	1	0	0	0	1	1	2	1	2	2	1
Luzuriaga polyphylla	1	1	0	2	2	1	0	1	0	0	2	0	0	0	1	2	1	2	2	1
Luzuriaga radicans	1	1	1	1	2	1	0	1	0	1	1	0	0	1	1	2	1	2	2	1
†Luzuriaga peterbannisteri	?	?	0	3	2	1	0	0	0	1	0	0	0	1	1	2	0	2	2	?
Drymophila cyanocarpa	1	1	0	1	0	1	1	1	1	0	0	2	2	0	0	0	0	0	0	1
Drymophila moorei	1	0	0	1	0	1	1	1	1	0	0	2	2	0	0	0	0	0	0	1

	21			25						30					35
Alstroemeria	0	0	0	1	1	1	1	1	1	0	2	1	1	0	1
Bomarea	0	0	0	1	1	1	1	1	1	0	2	1	*	0	1
Luzuriaga parviflora	1	1	1	0	0	0	0	0	0	1	0	0	0	1	0
Luzuriaga marginata	1	1	1	0	0	0	0	0	0	1	0	0	0	1	0
Luzuriaga polyphylla	1	1	1	0	0	0	0	0	0	1	0	0	0	1	0
Luzuriaga radicans	1	1	1	0	0	0	0	0	0	1	0	0	0	1	0
†Luzuriaga peterbannisteri	?	?	1	0	?	?	0	0	0	?	?	?	?	?	?
Drymophila cyanocarpa	1	1	0	0	0	0	0	0	0	1	2	0	0	1	0
Drymophila moorei	1	1	0	0	0	0	0	0	0	1	2	0	0	1	0

Molecular clock analyses

Molecular clock analyses were performed on a reduced DNA sequence matrix of Chacón et al. (2012), which included two species of *Alstroemeria* (*A. aurea* and *A. pelegrina*), two of *Bomarea* (*B. ovata* and *B. salsilla*), all four *Luzuriaga* species, the two *Drymophila* species, and five outgroups, and a combined alignment of 2368 nucleotides from chloroplast, mitochondrial and nuclear DNA. A Bayesian relaxed clock model was run in BEAST v. 1.7.4 (Drummond et al., 2006; Drummond and Rambaut, 2007), with the GTR + G substitution model, a Yule tree prior, and uncorrelated and lognormally distributed rate variation. Markov chains were 10 million generations long, using a burnin of 10%, with parameters sampled every 1000 generations. Effective sample sizes (ESS) were checked in Tracer v.1.5 (Rambaut and Drummond, 2007) and were all above 200.

The calibration points used were as follows: The root of the phylogeny was set to 117 Ma (Standard Deviation [SD] 0.5, Confidence Interval [CI] 116.2–117.8 Ma) based on Janssen and Bremer's (2004) estimate for the crown group of the Liliales. The crown node of *Smilax* L. was always set to 46 Ma (CI 37.2–54.8 Ma), which represents a conservative minimal age, given that *Smilax*-like fossils are known from the Early/Lower Eocene (48.6–55.8 Ma; Edelman, 1975; Wilf, 2000) and the Middle Eocene (37.2–48.6 Ma; MacGinitie, 1941; Wilde and Frankenhäuser, 1998). In one run, *Luzuriaga peterbannisteri* (described in the present study) was placed at the crown node of the genus *Luzuriaga.* In another, it was placed at the crown node of the *L. parviflora* + *L. marginata* clade. In both runs, its age was set to 22.94 ± 1.95 Ma, with a gamma prior distribution (shape 2.0, scale 3.5, and offset=36.3 Ma).

Results

A data matrix with the morphological characters listed in Table 1, but not including the fossil, yielded two equally-parsimonious trees of length 46 steps, Consistency Index (CI) 89, Retention Index (RI) 91 (Fig. 2A), differing only in the species relationships within the terminal clade consisting of *L. marginata* Benth. & Hook.f.*, L. parviflora* Kunth and *L. radicans* Ruiz & Pav. There was strong bootstrap and Bremer support for the Alstroemerioideae and Luzuriagoideae clades, and the majority of the character states along each branch were unique synapomorphies.

Luzuriaga has eight such synapomorphies, including the presence of adaxial papillae (11/1,2) in most species and lack of strongly sinuous anticlinal cell walls (12/0) in all of them, sinuosity being apparently plesiomorphic in the family. There was weak support for the position of *L. polyphylla* J.F.Macbr. as sister to the remainder of the extant *Luzuriaga* species, from which its leaves differ in lacking the finely granulate abaxial periclinal walls (10/1) and sunken stomata (14/1) of the crown lineage.

Incorporation of the fossil into the same data matrix resulted in a single most-parsimonious tree of length 47, CI 87, RI 89 (Fig. 2B). This topology was the same as one of the two extant-only trees, but with the fossil placed as sister in a crown lineage with the extant New Zealand species *L. parviflora*. The position of *L. polyphylla* in the genus received slightly stronger bootstrap and Bremer support, and there was also weak support for the *L. parviflora* + fossil clade. These last two were also linked by the shared possession of only two vein orders (4/3) and parallel orientation of the highest vein order (8/0). The fossil differs from *L. parviflora* in at least five characteristics, notably the lack of undifferentiated cells over the veins (17/0), while *L. parviflora* has only 3 main veins (5/0), the vein ends rarely being free (9/2), stomatal papillae occurring in bands (11/1), and there being six stomatal contact cells (16/3).

Because the molecular analyses of Chacón et al. (2012) showed a different internal topology for the living species of *Luzuriaga*, we mapped the morphological characters (Table 1) onto the molecular tree, with the fossil placed as sister to the rest of *Luzuriaga* (Fig. 2C), and we also performed an analysis in which the molecular tree was constrained to match the morphological tree. Trait optimization in the latter run was significantly worse than in the most parsimonious morphological tree (length 51, CI 80, RI 82). The fossil was differentiated by the homoplasious configuration of its two vein orders (4/3) without transverse or random orientation of the highest order (8/0), whereas extant *Luzuriaga* species all have papillate stomatal bands (11/1) and differentiated epidermal cells over the veins (17/1).

Given these results, we here describe the fossil as a new species of the genus *Luzuriaga*. An associated flower is also described, but as it was not attached to the leaves it is not included explicitly as part of the definition of the taxon. Similarly, *in situ* pollen from the flower is placed into the form genus *Liliacidites* Couper and is

also described as a new species, as it differs from the other morphotaxa in that genus in several features.

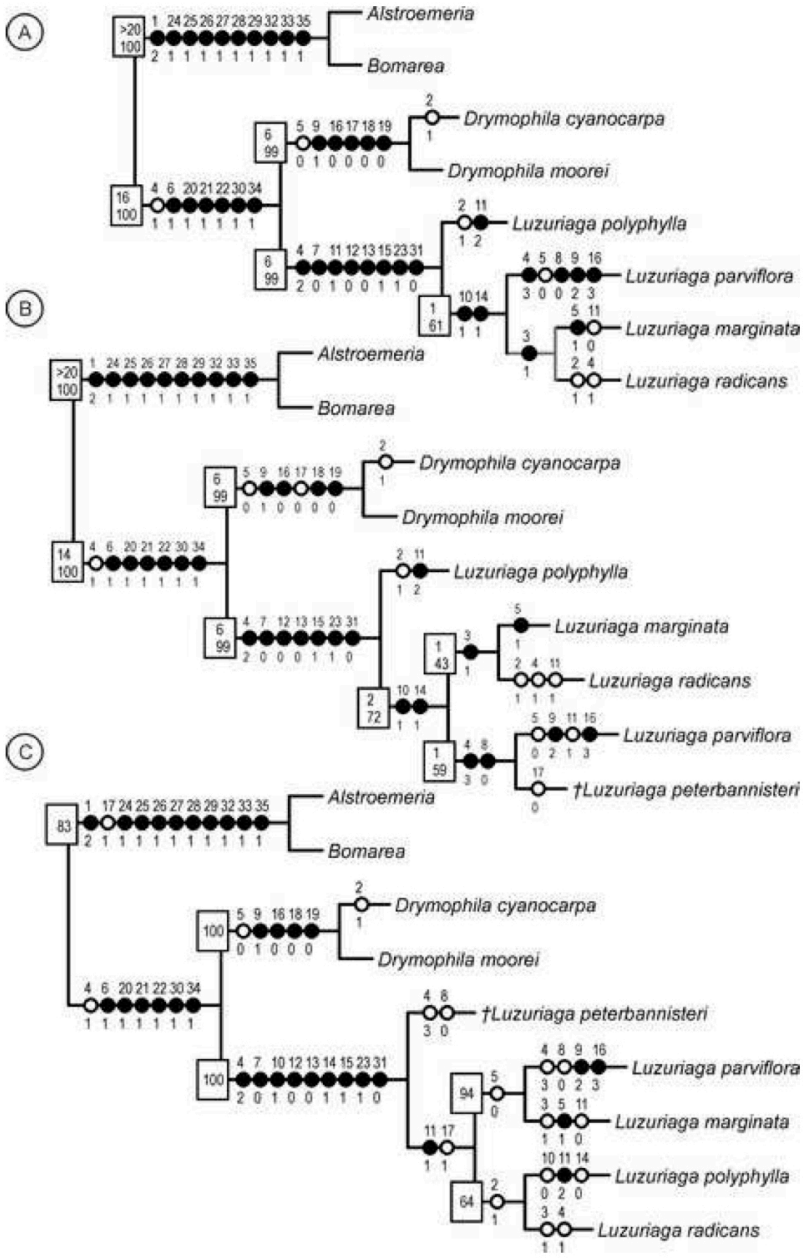


Figure 2. Phylogenetic analysis of extant Alstroemeriaceae taxa in relation to the fossil. *A, B,* Two equally most-parsimonious trees (length 46 steps, *ci* 89, *ri* 91) derived from the data in Table 1 with character states mapped using ACCTRAN. *C,* Character evolution inferred from placement of the fossil as proximal to *Luzuriaga* in a molecular tree derived from the study of Chacón et al. (2012). Numbers in boxes at the nodes are Bremer decay (upper) and bootstrap support values (BS values only for *C*); filled circles are unique synapomorphies, open circles represent homoplasious character states.

Molecular dating

Figure 3 shows the two dated trees (chronograms) obtained with the *Luzuriaga*-like fossil placed either at the crown node of *Luzuriaga* (Fig. 3A) or at the crown node of the *L. marginata* + *L. parviflora* clade (Fig. 3B). In the first case, the standard deviations of the uncorrelated lognormal and the coefficient of variation were 0.64 and 0.62, and in the second case 0.93 and 0.87, implying a slightly higher rate heterogeneity among lineages when the fossil is placed at the crown node of *L. marginata* + *L. parviflora* clade. Divergence times estimated with two different placements of the *Luzuriaga*-like fossil did not differ significantly (Figs. 3A, B). They were also congruent with the dates reported in Chacón et al. (2012), which included a more comprehensive sampling of Alstroemeriaceae and a placement of the *Luzuriaga*-like fossil at the stem node of the *Luzuriaga* clade (Fig. 3 in Chacón et al., 2012).

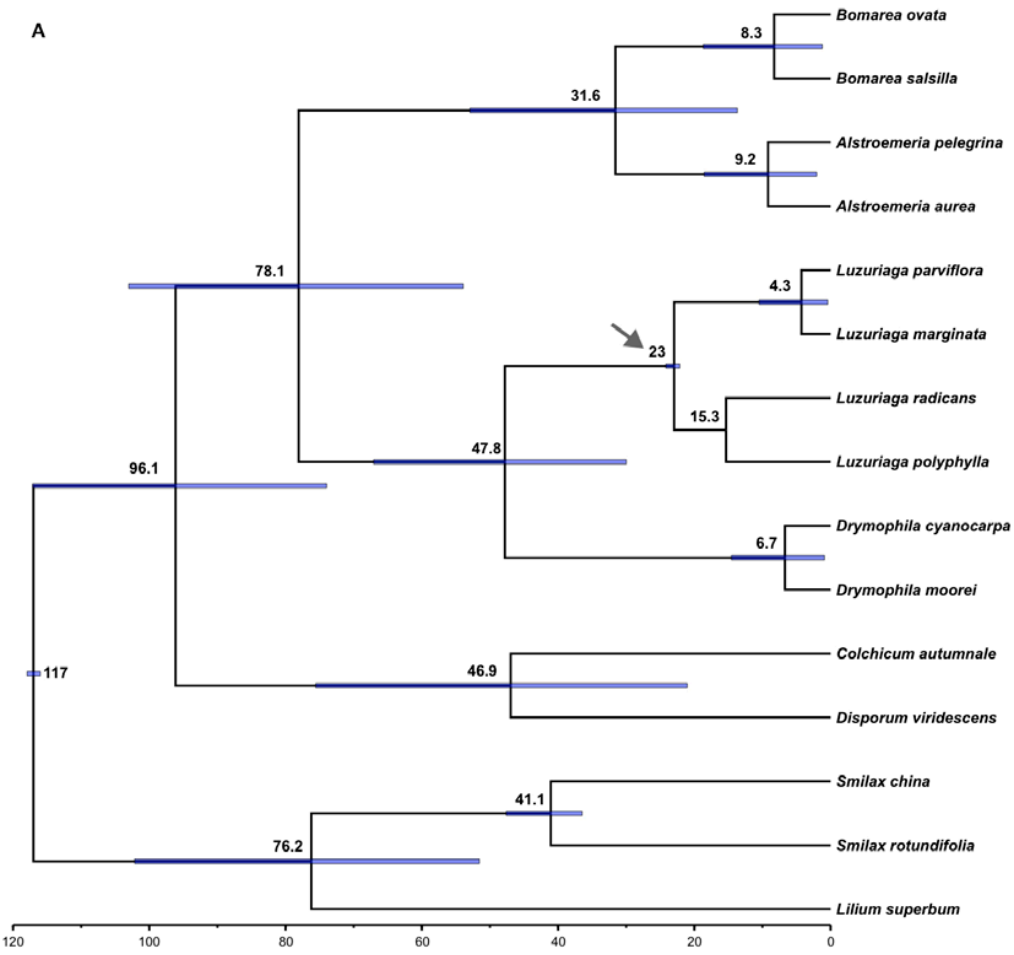
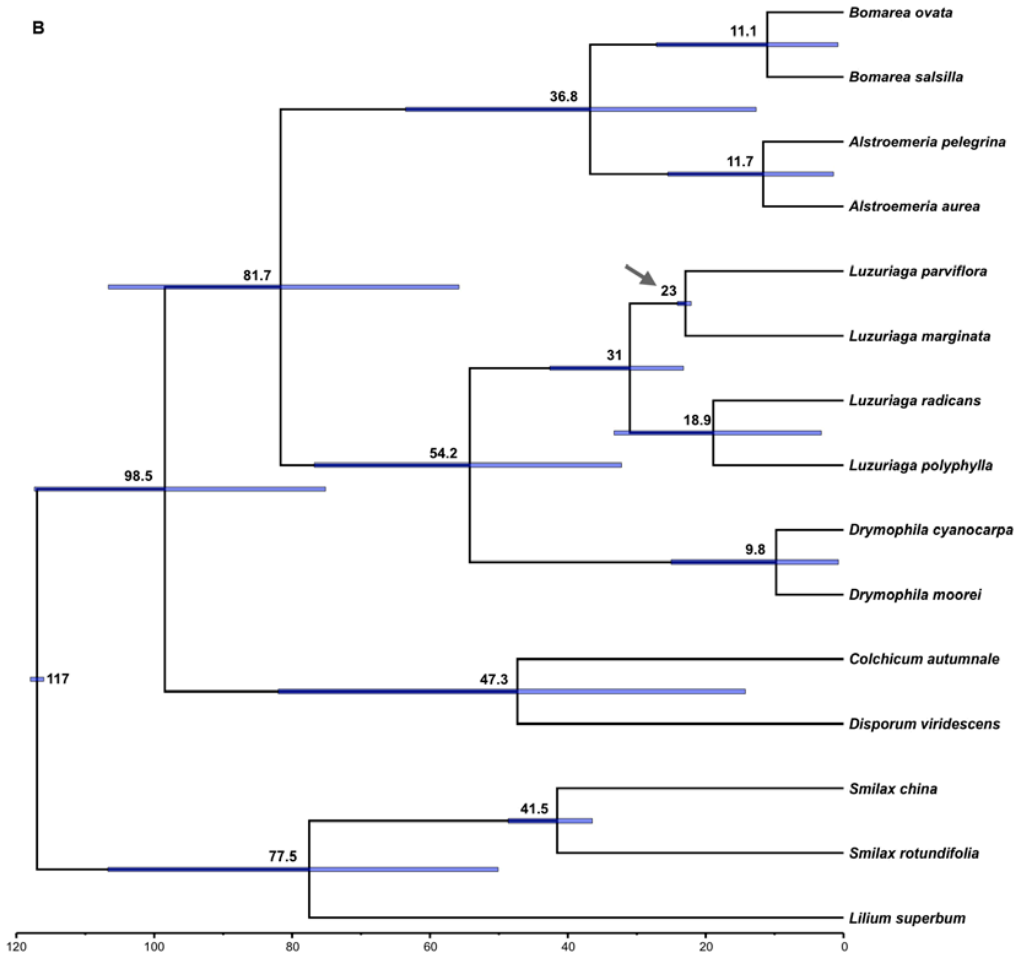


Figure 3. *Continued*



(**Figure 3.** *Continued*) Chronograms of Alstroemeriaceae obtained under relaxed clocks with two different placements of the *Luzuriaga*-like fossil (gray arrow), either at the crown node of *Luzuriaga* (*A*) or at the crown node of the *L. marginata* + *L. parviflora* clade (*B*). Bars at nodes indicate the 95% confidence intervals on the estimated times.

Systematics

Order—Liliales Perleb, 1826

Family—Alstroemeriaceae Dumort., 1829 nom. coms. (incl. Luzuriagaceae Lotsy, 1911)

Subfamily—*Luzuriagoideae* Engl. in Engl. & Prantl, 1887

Tribe—Luzuriageae Benth. et Hook.f., 1883

Genus—*Luzuriaga* Ruiz et Pav., 1802

Species—*Luzuriaga peterbannisteri* Conran, Bannister, Mildenh., et D.E.Lee, sp. nov.

Diagnosis—Leaves ovate, apex bluntly acute, base with a short, conspicuously resupinate petiole. Vein orders two, cross veins absent. Abaxial (upper) epidermal cells anticlinally straight to curved and periclinally finely granulate. Adaxial (lower) surface with slightly sunken stomata spread across the leaf and with no obvious differentiated epidermal cells over the veins.

Etymology—The specific epithet honors Peter Bannister (1939–2008), former Professor of Botany at the University of Otago and collector of the type specimen.

Holotype—FH 437 (OU32666)

Paratypes—FH 187 (OU32416), FH 409 (OU32638), FH739 (OU33216), FH 720 (OU33128).

Type locality—Foulden Maar, Otago, South Island, New Zealand.

Stratigraphic position—Foulden Hill Diatomite.

Age—Latest Oligocene to earliest Miocene (23±0.2 Ma)

Description—Leaves at least 18–36 x 10–12 mm (mean ± SD = 27 ± 13.2 x 14.3 ± 8.6), broadly lanceolate to ovate, resupinate, apex acute to obtuse, base more or less rounded (Fig. 4A, B, E–I); margin minutely serrulate (Figs 4D, 5A); petiole 2–3 mm long, folded (Fig. 4A, E, G); primary venation parallelodromous, lateral primary veins in 3–4 pairs, basal; midrib weakly defined, ~0.25 mm wide at mid-leaf, straight; laterals converging apically, slightly weakening towards the apex, curved; secondaries parallel to the laterals, converging apically; higher vein orders, areoles and cross veins absent (Fig. 4B, C, F). Abaxial (upper) epidermal cells rounded to rectangular, 43–70 x 30–53 µm (56.0 ± 9.1 x 40.5 ± 8.1), thick-walled, randomly oriented, end walls square to oblique, anticlinal walls straight to rounded, periclinal walls finely granulate, cells over veins not differentiated (Fig. 5B, C); adaxial (lower) epidermal cells rounded to slightly rectangular, 28–53 x 20–38 µm (40.0 ± 7.4 x 31.3 ± 5.4), thick-walled, randomly oriented, end walls square to oblique, anticlinal walls straight to rounded, periclinal walls finely granulate, cells over veins not differentiated (Fig. 5D, E); stomata 48–55 x 38–50 µm (51.0 ± 2.1 x 45.8 ± 3.9), mostly tetracytic, sometimes with five contact cells (Fig. 5E), contact cells similar to epidermal cells, 33–50 x 15–35 µm (42.0 ± 5.1 x 26.3 ± 7.3), guard cells 33–38 x 10–15 µm (34.5 ±

2.0 x 11.8 ± 1.7), slightly sunken and partially covered by contact cell flanges (Fig. 5D, E), stomatal density 148–259 mm^{-2} (200.0 ± 39.8).

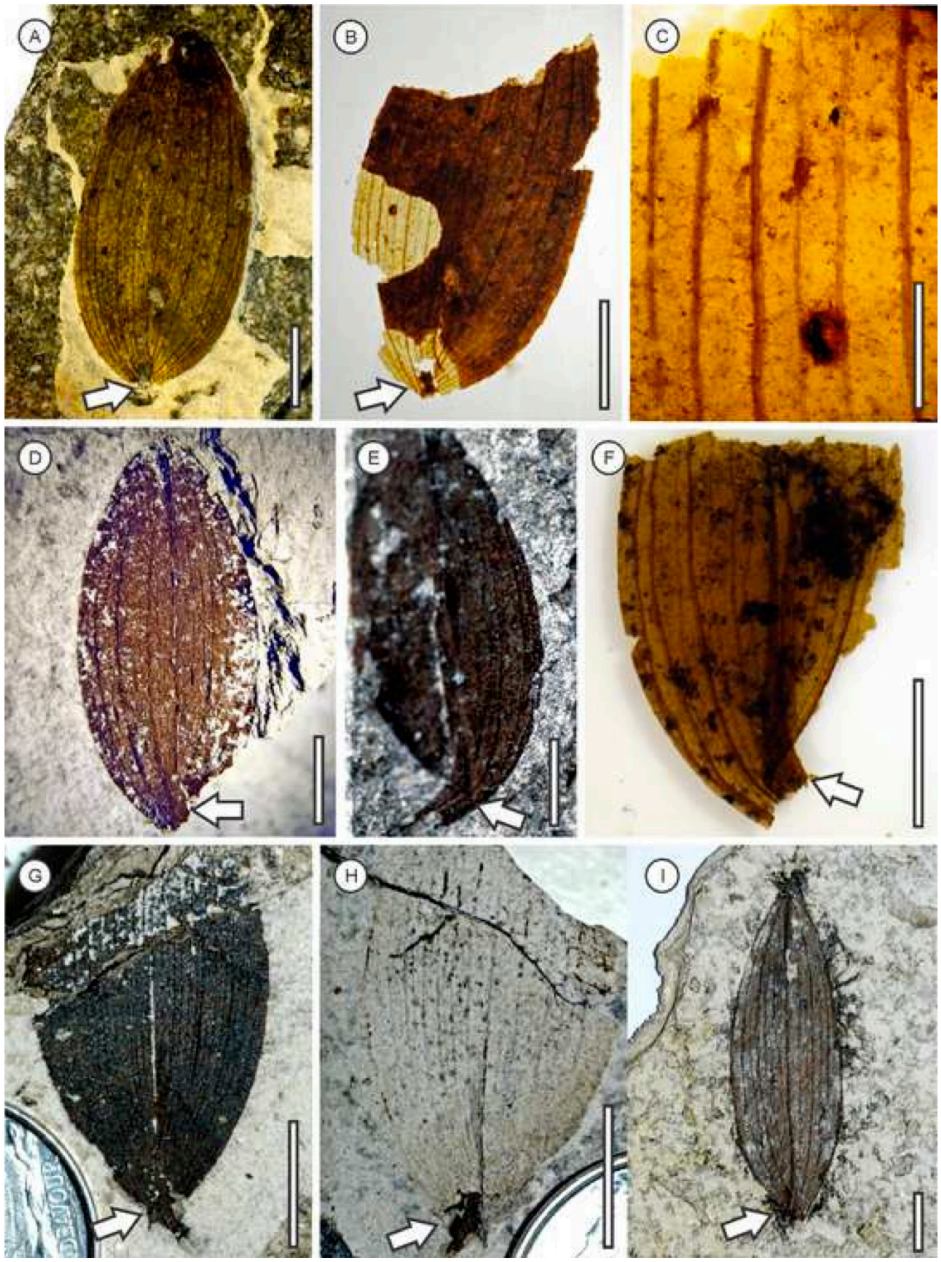


Figure 4. *Luzuriaga peterbannisteri* leaves *A,* Holotype (OU32666) incomplete leaf. *B,* Holotype after removal from matrix with some venation exposed (apparent cross veins are an artefact from an air bubble on the specimen). *C,* Detail of venation on holotype. *D,* Paratype (OU33216). *E,* Paratype (OU32416). *F,* Paratype (OU32416) after removal from matrix. counterpart. *G,* Paratype (OU32638). *H,* Paratype (OU32638A) counterpart. *I,* Paratype (OU33128). Arrows indicate resupinate leaf bases. Scales equal 5 mm in *A, B, D–I*, 1 mm in *C*.

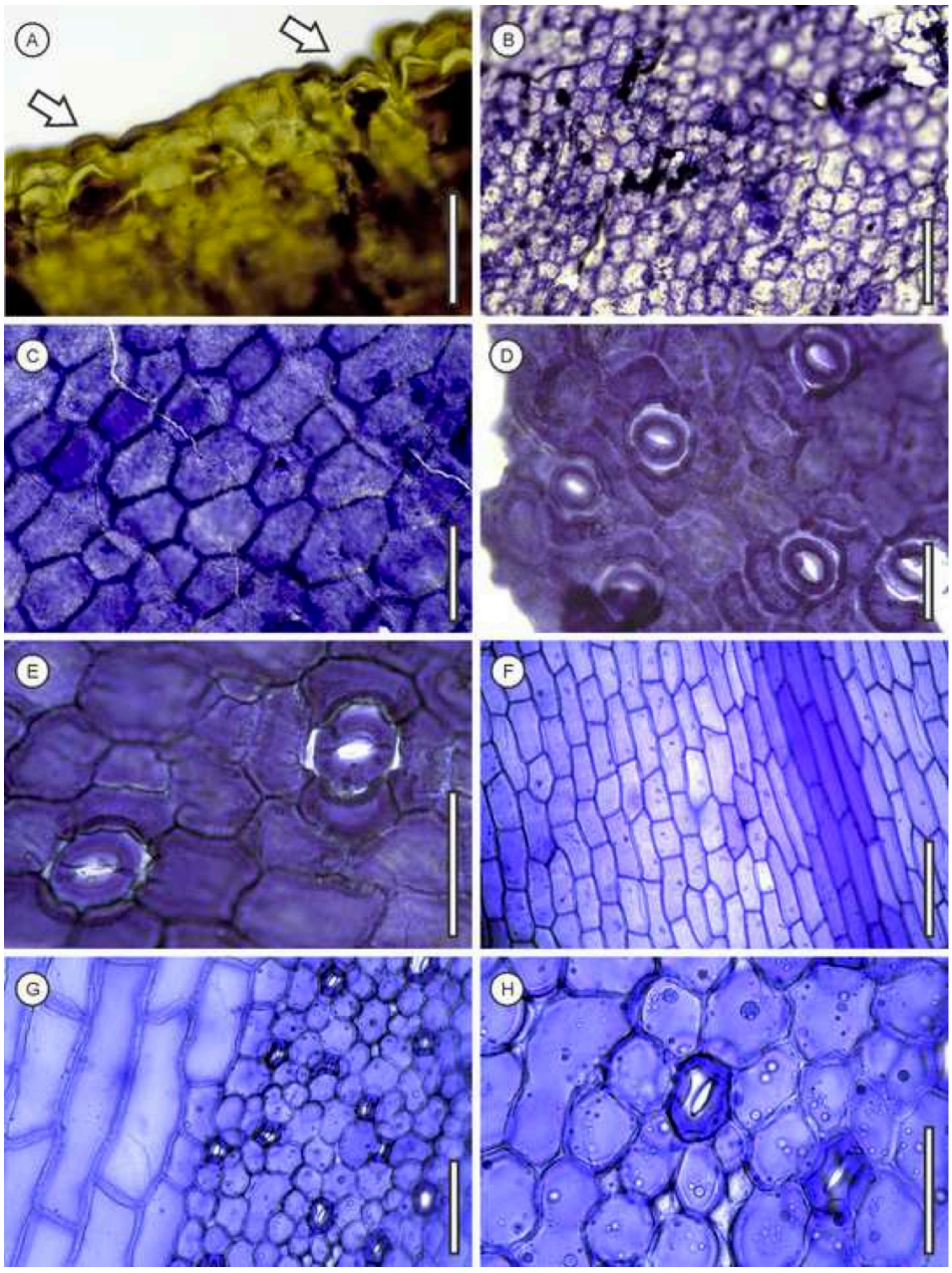


Figure 5. Comparison of *Luzuriaga peterbannisteri* leaf margin and cuticular features (A–E) with extant *L. parviflora* (Mark, s.n., OTA019011) cuticles (F–H). A, Leaf margin showing minute toothing (OU32638). B, Abaxial (upper) cuticle (OU32666). C, Abaxial cuticle detail (OU32638). D, Adaxial (lower) cuticle showing stomata with 4–5 subsidiary cells (OU32416). E, Adaxial stomatal detail showing slightly sunken stomata with flanged subsidiary cells (OU32416). F, Luzuriaga parviflora abaxial (upper) surface showing differentiated vein cells. G, Same showing adaxial (lower) surface with differentiated vein cells and stomata in inter-vein areas. H, Same with details of stomata with 6 subsidiary cells and sunken stomata with flanged subsidiary cells. Scales equal 100 µm in A, B, G, 200 µm in F, and 50 µm in C–E, H.

Parataxon 1—

Specimen examined—FH 695 (OU33103)

***Description*—**Flower 14 mm long, apparently hypogynous; pedicel at least 3.1 x 1.7 mm, tapering apically and articulating along its length (Fig. 6A). Perianth actinomorphic, apparently 6-merous, tepals ovate-lanceolate, 5.5–6.7 x 2.9–3.5 mm, glabrous, margins entire, slightly rounded basally, apex acute to shortly sub-acuminate; tepals apparently not disarticulating separately at abscission. Anthers and ovary not visible.

Pollen morphospecies

Anteturma—Pollenites, R.Potonié, 1931

Turma—*Monosulcates,* Burger, 1994

Genus—*Liliacidites* Couper, 1953

Type species*—Liliacidites kaitangataensis* Couper, 1953

Morphospecies—*Liliacidites contortus* Mildenh. et Bannister sp. nov. (Fig. 6B–F)

Diagnosis—Pollen sub-circular to elongate-spheroidal, large; areolate to finely but irregularly reticulate; exine thin, grains misshapen, split and contorted as a result of splaying out of the thin exines; sulcus appears to be elongate and rounded at ends.

Holotype hic designatus—Slide L24916, coordinates 1085/178 (N-S followed by E-W), England Finder Reading G46/3.

Paratypes—Slide L24916/1, coordinates 1012/194, England Finder Reading F38/2. A clump of about 8 specimens is present.

***Etymology*—**The specific epithet refers to the contorted nature of all the pollen grains found caused by their thin exines which made accurate description of the species difficult.

***Type locality*—**Foulden Maar, Otago, South Island, New Zealand.

Stratigraphic position—Foulden Hill Diatomite.

***Age*—**Latest Oligocene to earliest Miocene (23±0.2 Ma)

Description—Pollen monads sub-circular to elongate-spheroidal, always misshapen, anisopolar, bilaterally symmetrical; monosulcate, sulcus contorted, split in all

specimens seen, probably elongate, rounded at ends, covering at least 2/3 of the distal pole, margins apparently regular in outline, but appears irregular in the holotype; exine thin, structure uncertain, ~1–1.5 µm, very thin endexine, thicker ectexine, tectate, tectum uneven, columellate, simplibaculate, columellae visible in optical section, areolate to finely reticulate, luminae 1 µm wide or less, muri displaying heads of baculae giving a "beaded" appearance, ~1 µm wide or less, reticulum evenly dispersed across distal and proximal surfaces; size 30–44 µm (10 specimens, longest axis measured only).

Comparisons—When compared with the pollen of other Alstroemeriaceae, the grains found on the tepals of the fossil flower (Fig. 6B–F) are a close match to *Luzuriaga* (Fig. 6G) and to a lesser degree *Drymophila* (Fig. 6H), both genera possessing ovoid to slightly plano-convex grains with a weakly developed sulcus, thin exine and finely granulate sexine. In contrast, material from five *Alstroemeria* and seven *Bomarea* species examined at GNS, as well as those described by Erdtman (1952), Schulze (1978), Bayer (1998a; 1998b), and Sarwar et al.(2010), showed that Alstroemerioideae pollen was clearly distinct from Luzuriagoideae. All examined taxa of the former possess ovate to slightly reniform, plano-convex grains with thick-walled exines, a prominent sulcus, and a striate or sub-orbiculoidate, coarse and variably reticulate sexine (Fig. 6I, J). The fossil pollen type, combined with the morphology of the flower on which it was found supports further the identification of the fossil as *Luzuriaga*. No other fossil liliaceous pollen type is close to the morphology expressed by *L. contortus*.

In contrast, the palynomorphs *Liliacidites aviemorensis* McIntyre, *L. bainii* Stover in Stover & Partridge, *L. intermedius* Couper, *L. kaitangataensis* Couper, *L. lanceolatus* Stover in Partridge & Stover and *L. variegatus* Couper are all robust, elliptical in shape, reticulate with larger luminae, and have thicker, clearly layered exines. *Liliacidites perforatus* Pocknall is perforate. The sulcus in these taxa also does not appear to be circular or occupy most of the distal pole, as is apparent with modern New Zealand *Luzuriaga* (Cranwell, 1952; Moar, 1993; Moar et al., 2011); however, the holotype of *L. contortus* does appear to have a rounded sulcus with irregular (disrupted) margins.

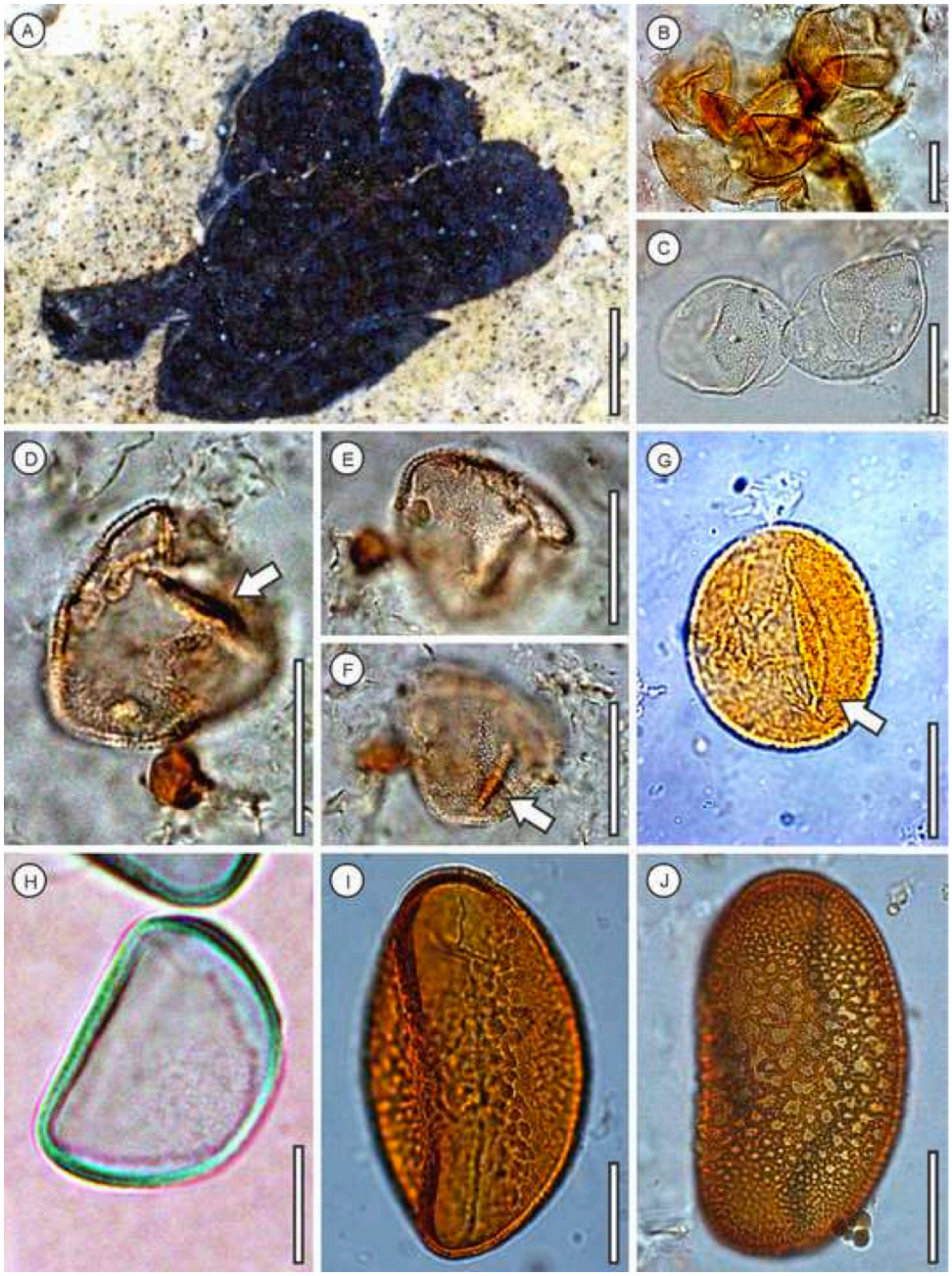


Figure 6. Comparison of Parataxon 1 (OU3103) flower and associated *in situ* pollen of *Liliacidites contortus* (GNS L24916) with extant Alstroemeriaceae pollen grains. *A,* Parataxon 1 flower (partially fragmented) showing pedicellate abscission and lanceolate tepals. (*B–D*) *L. contortus*. *B,* Cluster of grains on surface of cleared tepal. *C–E,* Holotype pollen grain in different planes showing contorted shape and finely reticulate surface and thin exine. *F, Luzuriaga parviflora* pollen (*Mark, s.n.,* OTA019011). *G, Drymophila moorei* Baker pollen (*Conran 1042*, ADU). *H, Alstroemeria stenopetala* Seub. (*Vindob s.n.*, MSB). *H, Bomarea peruviana* Hofreiter (*Weigend et al. 2000/682*, MSB). Scales equal 2 mm in *A,* and 20 μm in *B–I.*

Discussion

Leaves—The resupinate leaves relate the fossil to Alstroemeriaceae (Fig. 7B, E, F, H), for which this is a defining feature (Bayer, 1998b; Conran and Clifford, 1998). Although resupination also occurs in other monocots such as *Geitonoplesium* A.Cunn. ex R.Br. (Xanthorrhoeaceae: Hemerocalliodeae; Clifford et al., 1998), some grasses , and occasionally *Allium ursinum* L., as well as the eudicot *Stylidium pilosum* Labill. (Stylidiaceae) (Goebel, 1920; Troll, 1937–1943; Hill, 1939), the gross morphology, venation and cuticular features of the fossils rule these out as possible relatives.

Within Alstroemeriaceae, Alstroemerioideae have spirally-arranged leaves (Fig. 7H), whereas Luzuriagioideae have two-ranked leaves (Fig. 7A, D, E). The more or less isodiametric adaxial (lower surface) epidermal cells with straight to rounded walls place the fossil with extant *Luzuriaga* species and this is further supported by the slightly sunken stomata (Fig. 5G, H). The absence of cross veins and few vein orders further makes *L. peterbannisteri* resemble a large-leaved version of *L. parviflora* (Fig. 7B), to which it is sister in the phylogenetic analysis (Fig. 2B). However, the fossil differs from all extant *Luzuriaga* species, as they possess differentiated cells over the veins on both surfaces (Fig. 5G, H) and have more or less elongated abaxial (upper surface) epidermal cells (Fig. 5G). These characteristics in combination strongly support the placement of the fossil into the extant genus *Luzuriaga,* but as a new, extinct species.

Flower—Despite the relatively poor state of preservation of the flower, one of the features that separates *Luzuriaga* from the remainder of Alstroemeriaceae is the possession of hypogynous flowers with articulated pedicels (Fig. 7D) and ovate-lanceolate tepals (Fig. 7C). This means that unfertilized flowers fall as a single unit at senescence (Fig. 7A), rather than each tepal abscising separately, as in the other genera. Alstroemerioideae also have epigynous or perigynous flowers and the tepals are usually spathulate and clawed (Fig. 7H–I) (Hofreiter and Rodríguez, 2006), whereas the tepals of *Drymophila* are generally linear-lanceolate (Fig. 7G) (Clifford and Conran, 1987). Compared to *Alstroemeria*, the outer tepals of *Bomarea* are firmer in texture than the inner ones (Hofreiter and Tillich, 2002; our Fig. 7I). As with the leaf characteristics, this set of features supports a placement of the fossil close to, or in *Luzuriaga*; however, as the flower was not associated directly with the leaves of *L.*

peterbannisteri, it is treated here as associated material, rather than included as part of the type description.

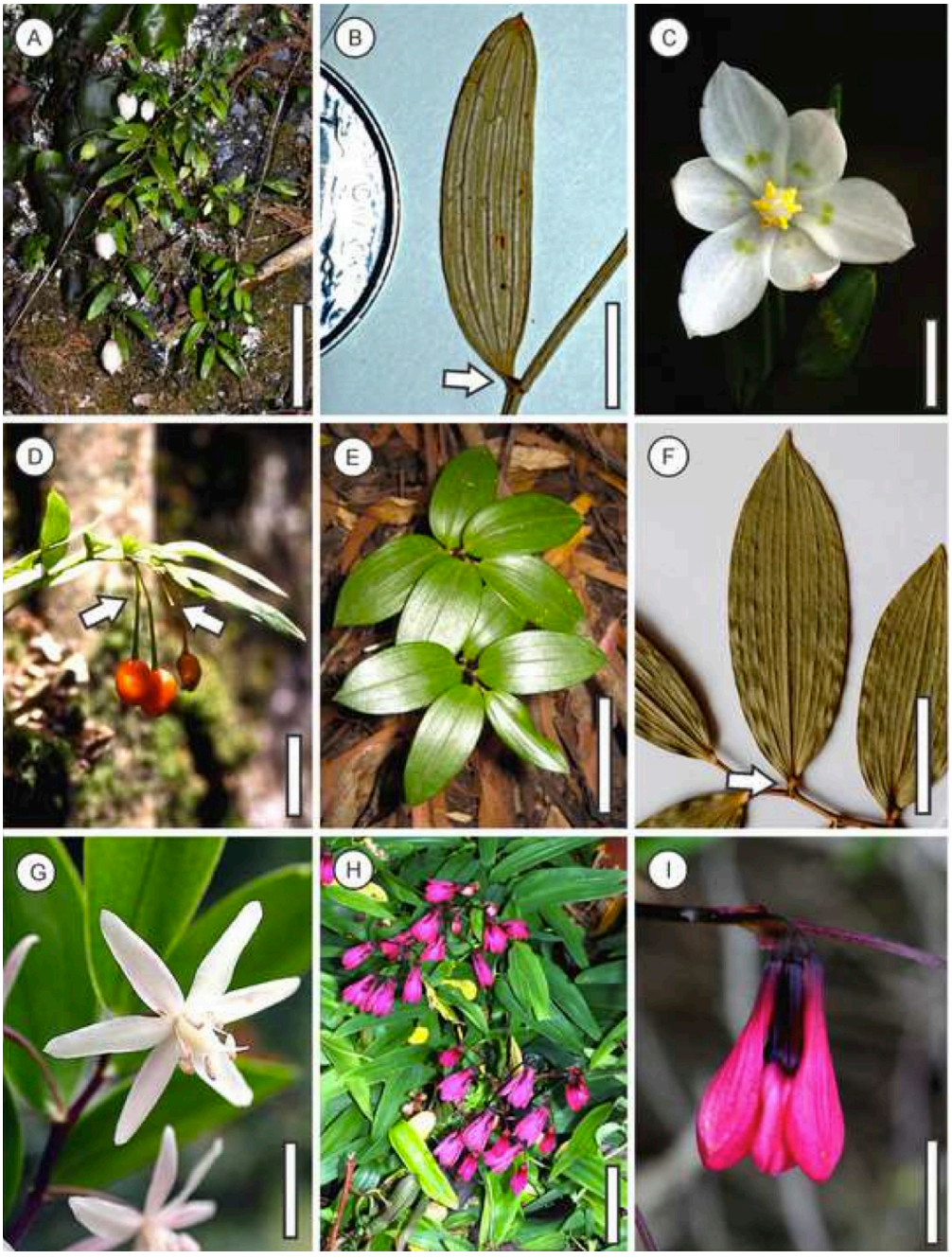


Figure 7. Comparative extant examples to show habit and morphology of the genera of Alstroemeriaceae. *A, Luzuriaga parviflora* in fruit, growing as an epiphyte at Ship Creek near Haast, Westland, New Zealand. *B, L. parviflora* (*Mark, s.n.,* OTA019011) showing resupinate leaf base (arrow) and few vein orders with largely parallel venation. *C, L. parviflora* close up of flower showing slightly oblanceolate tepals (Ship Creek). *D, L. radicans* in fruit with long, articulated pedicels (arrows) growing as an epiphyte near Valdivia, Chile. (*Continued*)

(**Figure 7.** *Continued*) *E, Drymophila moorei* growing as a rhizomatous rainforest floor shrublet at Springbrook, southeastern Queensland, Australia. *F, D. moorei* leaves showing resupinate base (arrow) and parallelodromous venation with prominent cross veins (*Conran 1042*, ADU). *G, D. moorei* close up of axillary flower showing narrow petal that fall individually at senescence, New England National Park, NSW. *H, Bomarea multiflora* Mirb. annual, climbing, herbaceous stems with resupinate leaves and terminal, branched, cymose inflorescences (Dunedin Botanic Gardens, New Zealand). *I, B. multiflora* close up of epigynous flower with spathulate tepals that fall individually (Dunedin Botanic Gardens, New Zealand). Scales *A,* 5 cm, (*B–G, I*) 5 mm, *H,* 2 cm. Photographs J.G. Conran (*A–F, H, I*), J. Bruhl (*G*), used with permission.

Pollen—When compared with the pollen of other Alstroemeriaceae, the grains found on the tepals of the fossil flower (Fig. 6B–D) are a close match to *Luzuriaga* (Fig. 6E) and to a lesser degree *Drymophila* (Fig. 6F); both genera possessing ovoid to slightly plano-convex grains with a weakly developed sulcus, thin exine and finely granulate sexine. In contrast, material from five *Alstroemeria* and seven *Bomarea* species examined at GNS, as well as those described by Erdtman (1952), Schulze (1978) and Bayer (1998a; 1998b), showed that Alstroemerioideae pollen was very distinct from Luzuriagoideae. All examined taxa of the former possess ovate to slightly reniform, plano-convex grains with a thick-walled exine, prominent sulcus, and a striate or sub-orbiculoidate, coarse and variably reticulate sexine (Fig. 6G, H). Schulze (1978) and Sanso and Xifreda (2001) also noted that the pollen of *Alstroemeria* is striato-reticulate (Fig. 6G), whereas that of *Bomarea* is foveolate-reticulate (Fig. 6H). These characteristics, combined with the morphology of the flower on which the pollen was found further support the identification of the fossil as *Luzuriaga,* or at least a member of Luzuriagoideae.

The holotype was selected from dispersed pollen; pollen from the fossil flower of *Luzuriaga* were morphologically identical but none were suitable as a holotype. Many specimens were examined, and 10 were measured to get an idea of the size range; the contortion of the other specimens was too great to estimate original size, and measurements of equatorial v. polar diameters were not possible. The size range estimates also fall within the range of modern New Zealand *Luzuriaga* pollen of ~32 μm (Moar, 1993; Moar et al., 2011).

The very thin exine, relatively large size and compression of the pollen grains make preservation of pollen of this type a rare event. The few dispersed pollen grains found in the Foulden Maar is testament to the comparatively quiet nature of the depositional environment. Even so, all pollen grains are split and broken to varying degrees, including those from the flower itself, and it would be easy to miss the pollen type in any pollen analysis. Quaternary fossil *Luzuriaga* pollen comparable to modern taxa has been reported from New Zealand (Wardle et al., 2001) and Chile (Ashworth et al., 1991).

Macphail and Partridge (2012) recently reported Alstroemeriaceae-like pollen refered to *Auriculiidites* sp. cf. *A. reticulatus* Elsik (1964) from the Eocene of Tasmania. *Auriculiidites* Elsik is a Late Cretaceous–Paleocene pollen morphogenus thought by Elsik and Thanikaimoni (1970) to resemble the auriculate pollen of *Bomarea* subgen. *Bomarea* sect. *Pardinae* M.Neuendorf (1977), in particular, the pollen of *B. lyncina* Herb., a synonym of *B. pardina* (Hofreiter and Rodríguez, 2006). This affinity was challenged by Muller (1981), who noted that the pollen grains differ in size, exine thickness and reticulum type, as well as in the absence of a distinct subdivision between tectum, columellae and nexine seen in the living species. Although noting this problem, Macphail and Partridge (2012) nevertheless regarded the current tropical distribution of living *Bomarea* (120 species, most of them in Peru) as supporting evidence for early Eocene warming at high palaeolatitudes in the Southern Hemisphere. However, even if *Auriculiidites* does represent an ancient Alstroemeriaceae-like plant, it is clearly distant from *Liliacidites contortus*.

Historical biogeography and paleoecology

A biogeography study of the Alstroemeriaceae that included the leaf fossil described here as one calibration point dated the split between the Luzuriagoideae and the Alstroemerioideae to 57.5 (37.8–77.6) Ma, and the *Alstroemeria* and *Bomarea* split to 29 (18.2–42.6) Ma (Chacón et al., 2012). The dates obtained with the alternative placements of the fossil in the present study are congruent and are in agreement with the hypothesis that the fossil represents an extinct lineage of *Luzuriaga* that inhabited New Zealand ca. 23 million years ago. Given that the sister genus *Drymophila* is confined to Australia, it is possible that *Luzuriaga* may have evolved initially in New

Zealand and spread to South America (potentially via Antarctica) before becoming extinct in New Zealand, with a subsequent more recent reintroduction by long distance dispersal.

Luzuriaga in Chile generally behaves as an epiphyte (Fig. 7D) growing on moss-covered tree trunks in wet forests (Hofreiter, 2007) and in New Zealand, the modern *L. parviflora* is either an epiphyte (Fig, 7A; Hofstede et al., 2001), forest floor herb in deep litter, or a plant of swamp edges and moss beds (Robertson et al., 1990). The presence of relatively abundant leaves at Foulden Maar (compared to other fossil taxa) suggests that the plants were growing close to the lake edge, possibly in lake-margin moss beds (J. Conran, unpubl. obs. of extant *Luzuriaga* at Lake Wilkie, Southland, New Zealand). In contrast, *Drymophila* is always a forest floor herb in damp to seasonally dry, cool to warm-temperate forests, where it displays strongly seasonal growth phases (Conran, 1988b).

The South American Alstromerioideae often have showy flowers pollinated by hummingbirds (Fig. 7H, I; Hofreiter and Rodriguez, 2006; Chacon et al., 2012), while Luzuriagoideae have smaller flowers often adapted to bees (Newstrom and Robertson, 2005), paticularly in species with apically porate anthers that can be exploited only by buzz-pollinating female bees (Buchmann, 1983). In southeastern Queensland, *Drymophila moorei* Baker was found to be visited by 20 insect species from 10 families (Conran, 1988a), although the main pollinators for that species appear to be syrphid flies (*Baccha* Fabricius, 1775 sp. and *Betasyrphus serarius* Wiedemann, 1830) and halictid bees (*Lasioglossum* Curtis, 1833 subgen. *Chilalictus* Michener, 1965).

Conclusions

Based on both the fossil record and molecular phylogenetic data, *Luzuriaga* was present in early Miocene New Zealand, indicating a long paleogeographic history for the genus. The new leaf fossil *L. peterbannisteri* strengthens biogeographic connections between South America and Australasia during the Oligo–Miocene, suggesting a possible New Zealand origin with disperal to and then back from South America after local extinction in New Zealand.

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Chapter 4

Assessing model sensitivity in Likelihood Analyses of Geographic Range Evolution (Lagrange): A study using Colchicaceae and experimental data

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Abstract

Aim: Likelihood analyses of ancestral ranges require a parameterized model that consists of a time-calibrated phylogeny, an "adjacency matrix" of allowed or forbidden area connections, and an "area dispersal" matrix with probabilities for discrete periods of time. The approach is implemented in the software LAGRANGE (Ree et al., *Evolution*, 59, 2299–2311, 2005). Because it can incorporate information about past continental positions, the approach has been used in historical biogeographic studies of relatively old clades. Surprisingly, how the number of nodes relative to areas and time periods, and the interactions among input matrices affect parameter estimates have never been evaluated. Here we use the lily family Colchicaceae and artificial data to study the inferential power of Lagrange models.

Location: Africa, Australia, Eurasia, North America, and South America.

Methods: Using eight plastid, mitochondrial, and nuclear DNA regions from 83 of the c. 270 species of Colchicaceae (representing all genera and the entire geographic range) plus 5 outgroups we obtained a well-resolved phylogeny dated with a molecular clock, and assigned the tips to 6 geographic distributions. We then carried out 22 LAGRANGE runs in which we modified the adjacency and dispersal matrices, the latter with 0, 2, or 4 time periods and 1, 3, or 5 dispersal probabilities. For a second data set, more "area switches" were introduced by reassigning tip distributions. Models were compared based on global log-likelihoods.

Results: The adjacency matrix and the number of nodes in a particular time slice determined model fit. For the Colchicaceae, a model with an unconstrained adjacency matrix and 2 time periods had the highest likelihood, with dispersal probability categories having a minor effect. Colchicaceae likely originated in Cretaceous East Gondwana, initially diversified in Australia (c. 75 Ma), reached southern Africa during the Palaeocene-Eocene, and from there extended their range to Southeast Asia (probably through Arabia) and then North America (through Beringia).

Main conclusions: At least in small data sets, the inferential power of LAGRANGE models should always be tested with sensitivity analyses, as carried out here; unconstrained adjacency matrices and high node to area and time period ratios will enhance power.

Keywords: Likelihood models in AAR, chronogram, adjacency matrix, area-dispersal

matrix, model over-parameterization, palaeogeography.

Introduction

The rise of molecular clock dating as a tool in historical biogeographic analysis has been accompanied by the development of new methods of ancestral area reconstruction (AAR). The most sophisticated of these methods, Likelihood Analysis of Geographic Range Evolution (LAGRANGE; Ree et al., 2005; Ree and Smith, 2008) is model-based and has been the method of choice for deep-time biogeographic studies because it allows the incorporation of palaeogeographic data. This is achieved through the combination of four model components: (i) a fully resolved chronogram, (ii) a species distribution matrix, (iii) an adjacency matrix specifying allowed and forbidden ranges, and (iv) an area-dispersal matrix specifying dispersal probabilities. The chronogram provides the time-calibrated nodes and branches for which the probability of ancestor-descendant change is calculated; the discrete states of interest are the range subdivision-inheritance scenarios at the nodes rather than the ranges itself (Ree and Sanmartín, 2009). The likelihood function then integrates over the conditional likelihoods of all ancestral states at every internal node weighted by their prior probability (Ree and Smith, 2008).

As regards the species distribution matrix, users will define areas appropriate for their clade and research question, with the limitation that the number of biogeographic parameters to estimate from the data increases exponentially with the number of areas, decreasing the inferential power of the model (Ree and Sanmartín, 2009). Studies have used three to 15 geographic areas (see Nauheimer et al., 2012: Table 1), seeking a balance between the dispersion of tips across areas (hence the potential inferred "switches" at nodes deep in the tree) and the risk of having many singletons (areas occupied by a single tip taxon). The user-defined adjacency matrix is a presence-absence matrix that defines which ranges are allowed in the model (for example, the combined continent Laurasia but not a combined Asia and Australia); it is equivalent to the cost matrix used in DIVA analyses (Ronquist, 1997). In the area-dispersal matrix, the user specifies values (such as 1, 0.5, 0.01, or 0) for dispersal probabilities between areas based on prior notions about range expansion. An absence of expansion could be just that or could be due to extinction; both are captured by

extremely low dispersal probability values. The dispersal probability matrix is used in the analysis to obtain area-specific scaling factors for the average rate of dispersal. The user can build as many dispersal matrices for different periods of time ("time slices") as deemed appropriate.

The components described above imply that LAGRANGE requires many more *ad hoc* parameter values than other biogeographic methods. Studies using the program have differed considerably both in model details and in the reporting of model parameterization (Nauheimer et al. 2012: Table 1 provides an overview). For example, different studies have left adjacency matrices unconstrained (Carlson et al., 2012) or constrained (Clayton et al., 2009), but without testing how this interacted with probability matrices or how a different treatment would have impacted model likelihood. The probability of dispersal between Australia and South America during the Cretaceous (145–66 Ma) has been assigned $P = 1$ (Buerki et al., 2011: Time slices before 60 and before 80 Ma), $P = 0.5$ (Mao et al., 2012: Time slice between 105–70 Ma), or $P = 0.01$ (Nauheimer et al., 2012: Time slices between 150–90 Ma and 90–30 Ma). The number of probability categories has ranged from five (Mao et al., 2012; $P = 0.1, 0.25, 0.5, 0.75$, and 1) to three (Buerki et al., 2011; $P = 0.01, 0.5$, and 1).

We know of five studies that have used model comparisons to assess model fit to particular data sets. Couvreur et al. (2011) and Baker and Couvreur (2013) compared unconstrained models with zero-time slices to constrained models with 5 time slices. In both studies, the constrained models had higher likelihoods. Mao et al. (2012) compared models with four, five, six, seven, or eight time slices. The migration probabilities ranged from 0.1 for well-separated areas to 1.0 for contiguous landmasses. They found that the eight-time-slice model fit their data best (judged by this model having the best likelihood score as calculated by LAGRANGE). In a similarly-sized data set, Nauheimer et al. (2012) compared models with three or four time slices, but found that the three-time-slice-model fit best. None of these studies varied their adjacency matrices. For a study of the genus *Psychotria* in Hawaii, Ree and Smith (2008) varied the adjacency matrix, and found that a constrained matrix fit the data better (as assessed by the two log-likelihood difference).

Especially in small data sets, i.e., those with few nodes relative to the number of areas and time slices, models may easily become overparameterized, and a study of the inferential power of likelihood models for ancestral area reconstruction seemed

overdue. We decided to investigate the interactions among the input matrices, number of time slices, dispersal probability categories, and node/area/time slice ratio in an empirical data set and an artificial one. The lily family Colchicaceae constitutes a suitable group for this purpose due to its intriguing geographic distribution and moderate size and age. This family of 270 species in 16 genera is distributed in Africa, Eurasia, Australia, and North America, while being notably absent in Central and South America (Fig. 1; see Nordenstam, 1998). Strictly African genera are *Baeometra* (1 species), *Camptorrhiza* (2 species), *Hexacyrtis* (1 species), *Ornithoglossum* (8 species), and *Sandersonia* (2 species); strictly Australian genera are *Burchardia* (6 species), *Kuntheria* (1 species), *Schelhammera* (2 species), and *Tripladenia* (1 species). In Eurasia, *Colchicum* (ca. 100 species) occurs from the Mediterranean to western Asia, and *Disporum* (20 species) is native to Asia. *Uvularia* (5 species) is restricted to North America. Four genera have disjunct geographic distributions: *Iphigenia* (12 species) occurs in Africa, India and Australasia, *Gloriosa* (10 species) in Africa, India, and Southeastern Asia, *Androcymbium* (57 species) in extreme southern and northern portions of Africa and the Mediterranean, and *Wurmbea* in Australia (ca. 30 species) and South Africa (20 species) (Vinnersten and Manning, 2007; del Hoyo and Pedrola-Monfort, 2008; Persson et al., 2011). The sister family of the Colchicaceae are the Alstroemeriaceae, a family of c. 200 species, all in the Neotropics (Fig. 1) except for three in Australia and New Zealand (Chacón et al., 2012).

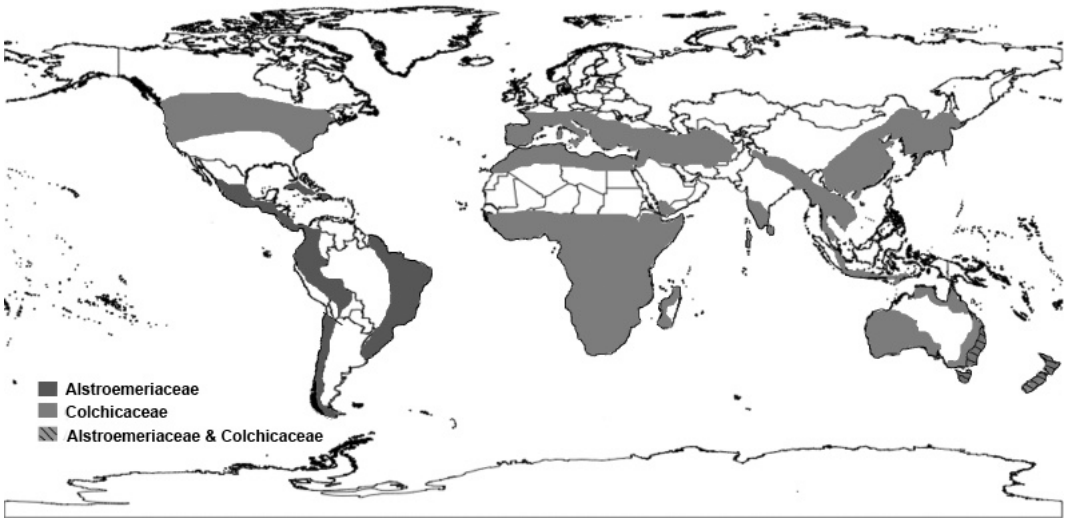

Figure 1. Geographic distribution of the Colchicaceae and their sister family, Alstroemeriaceae.

Previous molecular-phylogenetic work on the Colchicaceae led to the recognition of six small tribes (Burchardieae, Uvularieae, Tripladenieae, Iphigenieae, Anguillarieae, and Colchiceae) as well as the re-circumscription of the genera *Wurmbea* (including *Onixotis* and *Neodregea*), *Colchicum* (including *Androcymbium*, *Bulbocodium*, and *Merendera*), and *Gloriosa* (including *Littonia*) (Vinnersten and Reeves, 2003; Vinnersten and Manning, 2007). The taxonomic status of *Androcymbium* and *Colchicum* has remained controversial. A redefinition of the genus *Colchicum* to include *Androcymbium* was proposed by Manning et al. (2007) and Persson (2007), while del Hoyo and Pedrola-Monfort (2008) preferred to treat *Androcymbium* and *Colchicum* as separate genera. A phylogenetic analysis of *Colchicum*, including 96 of its 100 species, included only three species of *Androcymbium* (Persson et al., 2011), thus could not test mutual monophyly.

The approach taken in this study is to conduct experiments in LAGRANGE with different adjacency matrices, area-dispersal matrices, dispersal probabilities, and time slices using a time-calibrated phylogeny for the Colchicaceae and for a fictitious clade with tips recoded to increase area dispersion across taxa, potentially resulting in more "area switches" at deeper nodes. A critical evaluation of the pitfalls and strengths of maximum likelihood-based ancestral area reconstruction, especially of the use of time slices with different dispersal probability matrices, can be useful for future studies, since matrices can be (and have been) used across studies of clades of similar ages and geographic distribution (for example, similar connectivity matrices were used for various Pinaceae, Sapindaceae, and Araceae; Moore and Donoghue, 2007: Fig. 7; Havill et al., 2008; Buerki et al., 2011, Nauheimer et al., 2012; Lockwood et al., in review).

Materials and methods

Taxon sampling

We obtained DNA sequences from 83 of the c. 270 species of Colchicaceae representing all 16 genera and the geographic range of the family, and added five outgroup species from the Alstroemeriaceae and the Petermanniaceae, the latter a monotypic family (*Petermannia cirrosa*) of rhizomatous woody climbers restricted to

temperate rainforests in east Australia (Conran and Clifford, 1998; Chacón et al., 2012). Our sampling included 19 of the c. 57 species of *Androcymbium* Willd., the only species of *Baeometra* Salisb. ex Endl. (*B. uniflora* (Jacq.) G. J. Lewis), three of the six species of *Burchardia* R. Br., one of the two species of *Camptorrhiza* Hutch., 17 of the c. 100 species of *Colchicum* L., five of the 20 species of *Disporum* Salisb. ex G. Don., three of the 10 species of *Gloriosa* L., the only species of *Hexacyrtis* Dinter (*H. dickiana* Dinter), four of the 12 species of *Iphigenia* Kunth, the only species of *Kuntheria* Conran & Clifford (*K. pedunculata* (F. Muell.) Conran & Clifford), six of the eight species of *Ornithoglossum* Salisb., the only species of *Sandersonia* Hook (*S. aurantiaca* Hook.), one of the two species of *Schelhammera* R. Br., the only species of *Tripladenia* D. Don (*T. cunninghamii* D. Don), three of the five species of *Uvularia* L., and 16 of the c. 50 species of *Wurmbea* Thunb. All sampled material with species names and authors, geographic origin, herbarium voucher specimen, and GenBank accession numbers is listed in Appendix S1 in Supporting Information.

DNA extraction, amplification and sequencing

Total DNA was extracted from 20 mg of dried leaf tissue using the Nucleospin Plant II kit (Macherey-Nagel, Düren, Germany). The concentration and purity of the resulting DNA was measured in a Nanodrop 2000 UV-Vis Spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, USA). The chloroplast genes *ndh*F, *mat*K, *rbc*L, the mitochondrial *mat*R, and the complete nuclear ribosomal internal transcribed spacer (ITS) were amplified using standard methods and universal primers. Additional sequences from the chloroplast regions *atp*B-*rbc*L, *rps16*, and *trn*L-F were obtained from GenBank. The amplified DNA was sequenced using BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Inc., Warrington, UK) and an ABI 3100 Avant capillary sequencer (Applied Biosystems). Sequences were assembled in SEQUENCHER (Gene Codes, Ann Arbor, MI, USA) and aligned in MAFFT 5.64 (Katoh et al., 2005) with manual adjustment in MACCLADE 4.8 (Maddison and Maddison, 2002). All sequences were BLAST-searched in GenBank

Phylogenetic analyses and molecular clock dating

The combined plastid, mitochondrial and nuclear data set comprised 93 taxa (84 ingroup accessions) and 6451 aligned nucleotide regions. A phylogeny from this data set was obtained using Maximum Likelihood (ML) in the software programs RAxML v. 7.0.4 (Stamatakis, 2006) and RAXMLGUI 1.0 (Silvestro and Michalak, 2011) under the GTR + G substitution model. FINDMODEL (http://hcv.lanl.gov/content/sequence /findmodel/findmodel.html), which implements Posada and Crandall's (1998) ModelTest, selected this as the best fit for both the organellar and nuclear data based on the Akaike information criterion. Statistical support for nodes was assessed by 100 ML bootstrap replicates under the same model.

Molecular clock analyses were conducted in BEAST v1.7.4 (Drummond et al., 2006; Drummond and Rambaut, 2007), using the same matrix, except that *Wurmbea glassii* and *Disporum chinense* were excluded because some of their sequences were incomplete. We used a Bayesian relaxed clock under the GTR + G substitution model and a Yule Process tree prior. The length of the Markov Chain Monte Carlo (MCMC) was set to 90 million generations with parameters sampled every 1000 generations and a burnin of 10%. Following Chacón et al. (2012) we applied four calibration points, three of them from fossils. The root of the phylogeny was set to 117 million years ago (Ma) with a normal prior distribution and 95% confidence interval (SD 0.5, CI 116.2–117.8 Ma) based on Janssen and Bremer's (2004) estimate for the crown group of the Liliales. A gamma prior distribution was used for the three fossil calibrations as follows: The crown node of *Smilax* was set to 41 Ma (shape 2.0, scale 3.5, and offset 36.3 Ma), which represents a conservative minimal age, given that *Smilax*-like fossils are known from the Early/Lower Eocene (48.6–55.8 Ma; Edelman, 1975; Wilf, 2000) and the Middle Eocene (37.2–48.6 Ma; MacGinitie, 1941; Wilde and Frankenhauser, 1998). The stem age of the monotypic family Ripogonaceae was set to 51 Ma (shape 2.0, scale 0.6, and offset 50.0) based on leaf macrofossils of *Ripogonum* from Tasmania dated to 51–52 Ma (Conran et al., 2009). The stem node of the *Luzuriaga* clade in the Alstroemeriaceae was set to 22 Ma (shape 2.0, scale 0.3, and offset 21.4 Ma), based on the age of a *Luzuriaga*-like fossil from the Foulden Maar deposits near Otago, New Zealand, dated to c. 23 Ma (J. G. Conran , J. M. Bannister, D. C. Mildenhall, D. E. Lee, J. Chacón, and S. S. Renner, manuscript). Absolute ages for geological periods are from Walker and Geissman (2009), and

estimated node ages were checked against estimates from larger monocot data sets that did not use exactly the same fossil constraints as those used here (Janssen and Bremer, 2004).

Ancestral areas inference for the empirical and an artificial data set, and assessment of model fit

Geographic areas were delimited based on the geographic ranges of the sequenced species of Colchicaceae, Alstroemeriaceae, and Petermaniaceae, with the information coming from herbarium vouchers and taxonomic revisions. The six areas were: A, south to middle Africa; B, Mediterranean region in Europe, northern Africa and Arabian Peninsula; C, Australia and New Zealand; D, Asia and Southeast Asia; E, North America; F, South and Central America.

To study the effect of the different LAGRANGE model components, we designed experiments that modified the adjacency matrix, the number of time slices, and the dispersal probabilities in a hierarchically structured manner, resulting in a total of 22 experiments (11 for the Colchicaceae data set and 11 for the artificial data set). A graphical overview of the experiments is shown in Fig. 2 and their settings and rationale are described below.

For the artificial chronogram, tip nodes were recoded such that both old and young nodes in the tree would be affected: Seven Australian species (*Wurmbea australis, W. biglandulosa, W. centralis, W. dioica, W. murchisoniana, W. pygmaea,* and *W. saccata*) were coded as North American and three Australian species (*Schelhammera undulata, Kuntheria pedunculata,* and *Tripladenia cunninghamii*) as African.

For the Colchicaceae experiments, we used either an unconstrained adjacency matrix in which all range connections were permitted ("1" in all fields of the matrix) or a constrained matrix in which areas connected at least once over the last 120 million years received a value of "1", others a "0." This is based on the assumption that Colchicaceae have a low ability to disperse over non-adjacent areas because their fruits are dry capsules that release the seeds through loculicidal or septicidal dehiscence, with no obvious adaptations to wind dispersal or zoochory (Nordenstam, 1998). For that reason the following ranges were forbidden: Africa-Australia (AC),

Africa-Asia (AD), Europe-Australia (BC), Europe-South America (BF), Australia-North America (CE), Asia-South America (DF). Because Colchicaceae species have relatively narrow ranges (at a continental scale), we limited the maximum number of ancestral areas at nodes to two.

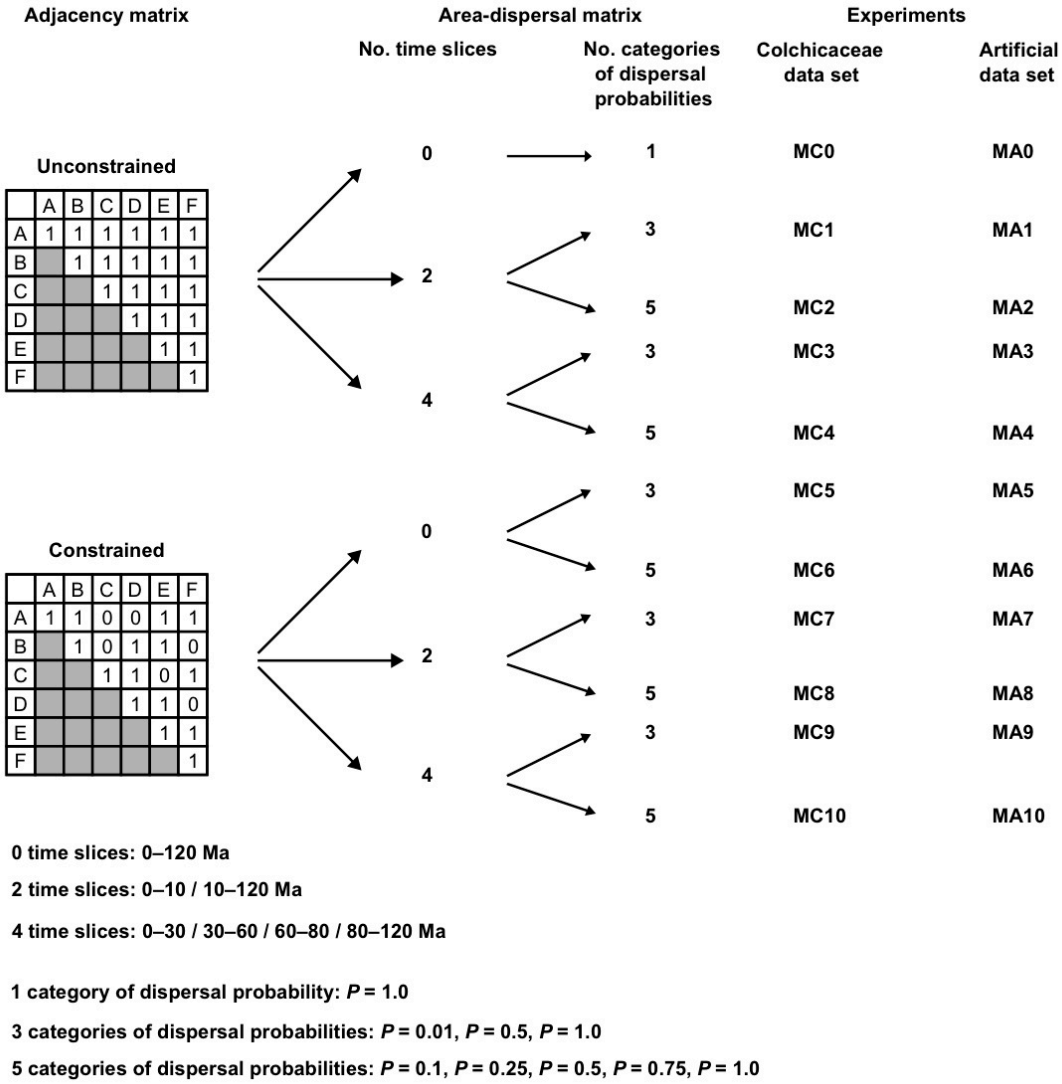

Figure 2. Flow diagram depicting the 22 experiments conducted in LAGRANGE for the empirical data (Colchicaceae; models MC0 to MC10) and the artificial data (MA0 to MA10).

For each adjacency matrix, we then defined three area-dispersal matrices with 0, 2, or 4 time slices. The 0-time-slice scheme comprises the entire time between 120

Ma and the present, with all 83 nodes of the Colchicaceae included (Appendix S2). The 2-time-slice scheme was designed such that similar numbers of nodes were included per time slice. Thus, the time slice between 0–10 Ma contained 39 nodes, and the time slice between 10–120 Ma contained 44 nodes. The 4-time-slice scheme was designed to reflect major palaeogeographic changes during the history of Colchicaceae, which led to a highly unbalanced number of nodes included per slices; 0–30 Ma (collision of the Australian Plate with Eurasia; Antarctic Circumpolar Current established) with 70 nodes, 30–60 Ma with 8 nodes (Drake Passage opens between the Antarctic Peninsula and southern South America; Tethys Sea closes; North Atlantic Land Bridge still available: Tiffney and Manchester, 2001), 60–80 Ma (East Gondwana and West Gondwana still linked across the Antarctic Peninsula) with 3 nodes, and 80–120 Ma (break up of West Gondwana) with 2 nodes.

For each adjacency and area-dispersal matrix combination, we used different numbers of dispersal probability categories, one with only $P = 1.0$, one with $P = 0.01$, 0.5, and 1.0, and one with $P = 0.1$, 0.25, 0.5, 0.75, and 1.0. A low value was given for not connected or not neighboring areas, a medium value for partly connected areas, and a high value for connected or neighboring areas. For the 4-time-slice matrices and three categories of probabilities, we employed the probability values of Buerki *et al.* (2011), while for the five categories of dispersal probabilities we followed Mao *et al.* (2012). For the 2-time-slice matrices, we averaged the probability values of the oldest and youngest bins from these two studies. For 0-time-slice matrices, we used the corresponding adjacency matrices and replaced the zeros either with $P = 0.01$ (for the three categories of dispersal probabilities) or with $P = 0.1$ (for the five categories of dispersal probabilities). The main objective of this strategy was to be able to compare the results (at least somewhat). All area-dispersal matrices used are shown in Appendix S3.

To compare models, we used their global likelihood scores as given by LAGRANGE.

Results

Molecular phylogeny and chronogram of Colchicaceae

The ML analysis resulted in a robust phylogeny with most clades having >80% bootstrap support (Fig. 3). The dated phylogeny obtained from essentially the same data is shown in Fig. 4, and the mean ages for the 31 nodes of particular interest [i.e., the root, the stem and crown groups of genera with more than one species, two nodes within *Androcymbium* (which together with *Colchicum* forms the largest clade), and four nodes within *Wurmbea*, which has c. 30 species in Africa and 20 in Australia] with 95% confidence intervals estimated from a sample of 70,000 trees from the stationary zone of the Bayesian MCMC are shown in Table 1. The most recent common ancestor of the Colchicaceae started diversifying c. 75 (61.9–90.2) Ma (Fig. 4). The Colchicaceae then split in two clades, a North American-Asian clade formed by *Uvularia* and *Disporum*, which diversified c. 28.3 (14.2–44) Ma, and a clade formed by the remaining species, whose most recent common ancestor diversified c. 54.2 (43.1–66.9) Ma. The main divergences in the Colchicaceae go back to the Eocene (at c. 45.8 Ma) with most of the splits occurring within the last 24 Ma (Table 1).

Figure 3. Maximum likelihood phylogeny for the Colchicaceae using combined sequences of eight chloroplast, mitochondrial, and nuclear DNA regions. Circles on branches are shaded to indicate bootstrap support (>50%) as shown in the inset. Ancestral areas were infered for the numbered nodes.

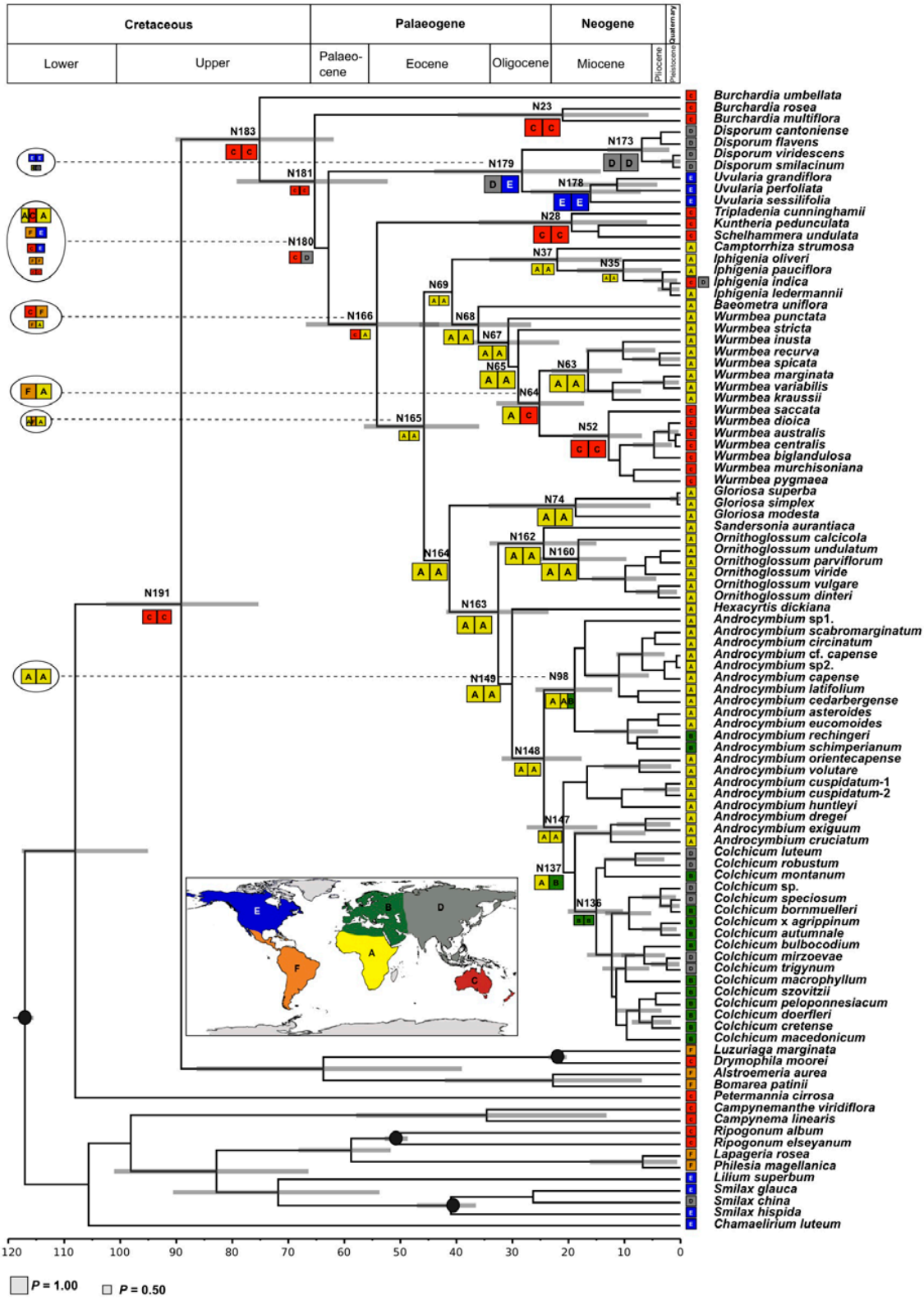

Figure 4. *Continued*

(**Figure 4.** *Continued*) Chronogram for the Colchicaceae inferred from the same data as used in Fig. 3, with 95% confidence intervals for node ages (grey bars) and results of the ancestral area analyses performed in LAGRANGE (coloured squares). Node numbers at branches are the same as in Fig. 3. The ancestral areas obtained with the best-fit model (MC2) are shown in the squares below each node, with square size proportional to the probability of the reconstruction (see Table 1 and scale at the bottom of the figure). Alternative ancestral areas obtained with other models (see Table 3) are shown inside the ovals. The black circles refer to the calibration nodes (Materials and Methods).

Results of the LAGRANGE experiments

The ancestral ranges and probabilities inferred in the 22 LAGRANGE experiments are shown in Appendix S4 and the global likelihood scores (-lnL) in Table 2. A significant difference in likelihood scores was observed between the first five experiments (MC0 to MC4 for the Colchicaceae and MA0 to MA5 for the artificial data set) and the remaining experiments (MC5 to MC10 for the Colchicaceae and MA5 to MA10 for the artificial data set), which had lower likelihoods. Experiments MC0 to MC4 (and MA0 to MA4) used an unconstrained adjacency matrix, while experiments MC5 to MC10 (and MA5 to MA10) used a constrained adjacency matrix (see Fig. 2 for details of each model). This inferior fit of the latter models can also be seen in Fig. 5. The best likelihood score for the Colchicaceae data set was the MC2 model (-lnL = 107.6, Table 2), which used an unconstrained adjacency matrix, 2 time slices, and 5 categories of dispersal probabilities (Fig. 2). The ancestral areas inferred under the best-fit model are shown in the Table 1 and the Fig. 4.

The best-fit model for the artificial data set again was one of the models that used an unconstrained adjacency matrix, namely model MA2, which is equivalent to MC2 for Colchicaceae (see Fig. 2 and Table 2). The worst likelihood scores were obtained with models MC9 and MA9 (for the Colchicaceae and the artificial data set, respectively), which used a constrained adjacency matrix with 4 time slices and 3 categories of dispersal probabilities (Fig. 2).

Table 1. Mean node ages (Ma) and 95% highest posterior density interval (HPD) obtained for the Colchicaceae (see Fig. 3 for the location of nodes). The ancestral areas and the probabilities shown in the right column were inferred with the best-fit model (MC2) in LAGRANGE.

Node	Age (95% HPD)	Ancestral range
N191	89.1 (75.3–102.5)	[C|C] 0.81; [C|CF] 0.12; [C|F] 0.04
N183	75.1 (61.9–90.2)	[C|C] 0.88; [CD|C] 0.08
N181	65.3 (52.3–79.2)	[C|C] 0.52; [C|CD] 0.34; [C|CE] 0.13
N180	62.8*	[C|D] 0.68; [C|E] 0.27; [CD|D] 0.05
N179	28.3 (14.2–44.0)	[D|E] 1.00
N178	16.1 (7.1–26.8)	[E|E] 1.00
N173	6.9 (2.0–13.0)	[D|D] 1.00
N166	54.2 (43.1–66.9)	[C|A] 0.57; [C|C] 0.32; [C|AC] 0.06; [C|D] 0.05
N165	45.8 (35.9–56.5)	[A|A] 0.57; [AC|A] 0.37; [AD|A] 0.05
N164	41.2*	[A|A] 1.00
N163	32.5 (23.6–41.8)	[A|A] 0.99
N162	24.4 (15.0–34.1)	[A|A] 1.00
N160	18.2 (9.6–26.9)	[A|A] 1.00
N149	30.0*	[A|A] 0.97
N148	24.4 (17.6–31.9)	[A|A] 0.75; [AB|A] 0.13; [A|AB] 0.04; [B|AB] 0.04
N147	20.9 (14.8–27.5)	[A|A] 0.68; [AB|A] 0.27
N137	18.8*	[A|B] 0.83; [A|D] 0.16
N136	15.0 (10.2–20.1)	[B|B] 0.58; [BD|B] 0.39
N98	18.9 (12.2–25.9)	[A|AB] 0.86; [A|A] 0.14
N74	18.7 (5.3–34.2)	[A|A] 1.00
N69	40.8*	[A|A] 0.57; [AC|A] 0.22; [A|AC] 0.15; [AD|A] 0.05
N68	36.1 (26.7–46.6)	[A|A] 0.84; [A|AC] 0.16
N67	30.7 (21.7–39.8)	[A|A] 0.81; [AC|A] 0.19
N65	28.9*	[A|A] 0.75; [A|AC] 0.25
N64	25.2 (17.2–32.9)	[A|C] 1.00
N63	16.5 (10.4–23.1)	[A|A] 1.00
N52	12.8 (6.9–19.2)	[C|C] 1.00
N37	22.0 (10.5–34.1)	[A|A] 0.65; [AC|A] 0.25; [AD|A] 0.10
N35	10.2 (3.3–18.5)	[A|A] 0.42; [A|AC] 0.33; [A|AD] 0.24
N28	19.4 (6.0–36.0)	[C|C] 1.00
N23	21.0 (5.7–39.8)	[C|C] 1.00

*The confidence interval for these ages is below the 95%

Table 2. Global maximum likelihood scores at the root node (-lnL) and rates of dispersal and extinction estimated in the LAGRANGE experiments for the Colchicaceae and the artificial data sets (see Fig. 2 for model details). The best-fit model is marked in bold.

Experiments	-lnL	Dispersal	Extinction
MC0	111.4	0.001321	9.269e-09
MC1	108.8	0.00375	2.284e-09
MC2	**107.6**	**0.004123**	**1.632e-09**
MC3	117.7	0.003381	0.001733
MC4	110.5	0.006122	0.001217
MC5	137.4	0.003882	0.003236
MC6	137.3	0.00388	0.003258
MC7	134.8	0.008934	0.003723
MC8	133.1	0.009332	0.003753
MC9	144.2	0.007962	0.004283
MC10	138.4	0.01213	0.003719
MA0	111.2	0.001344	0.0001165
MA1	110.8	0.003564	4.285e-09
MA2	106.5	0.004055	6.518e-09
MA3	119.3	0.003193	0.001573
MA4	109.1	0.006187	0.001033
MA5	129.3	0.003432	0.002793
MA6	129.2	0.003425	0.002792
MA7	129.9	0.007542	0.003
MA8	124.5	0.008061	0.003059
MA9	138.7	0.006593	0.003546
MA10	126.2	0.01054	0.002948

With regard to the dispersal probabilities (dispersal rates), the lowest rate was estimated under models MC0 and MA0 and the highest under model MC10 and MA10 (Table 2). For the extinction rate, the lowest value was estimated under model MC2 and MA2 for the Colchicaceae and the artificial data sets, respectively (Table 2). To identify commonalities in the results from the empirical and artificial data set, we plotted the global likelihood scores, and the dispersal and the extinction rates against each experiment. For both data sets, likelihoods become worse, the more complex the model (Fig. 5), while dispersal and extinction rates increase with the model complexity (Fig. 6).

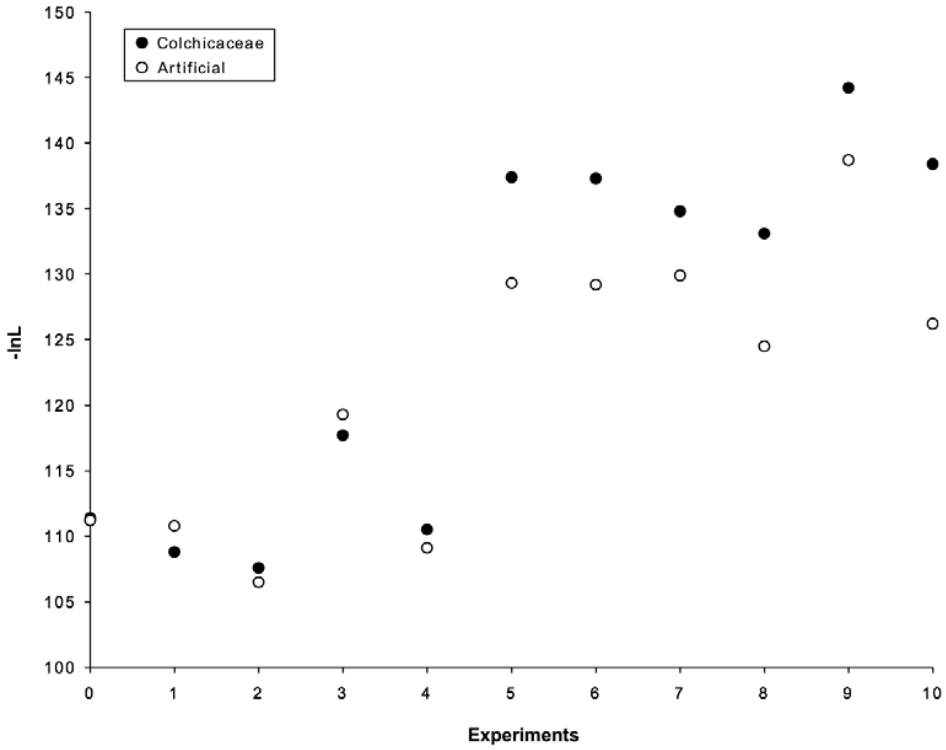

Figure 5. Global likelihood scores obtained in the 11 experiments conducted for the empirical and the artificial data.

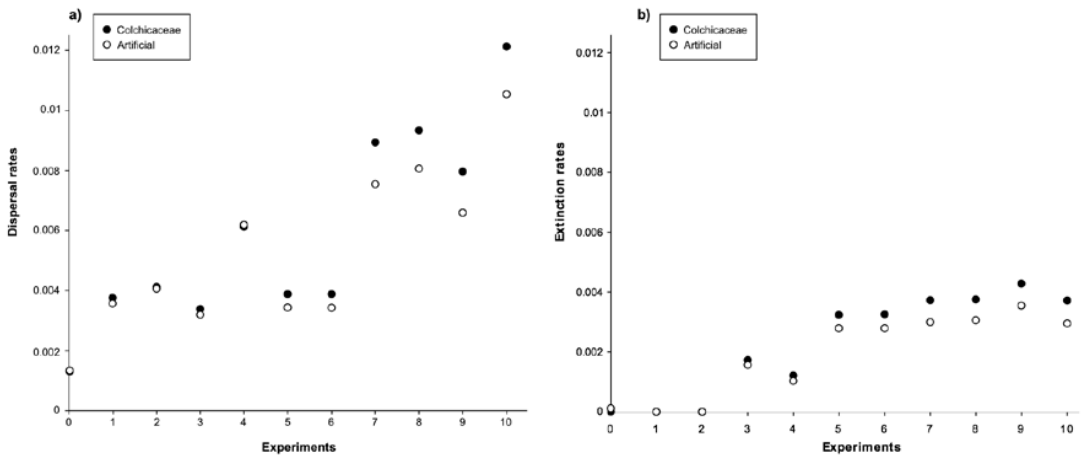

Figure 6. Rates of dispersal (a) and extinction (b) estimated in the Lagrange experiments using the empirical and artificial data.

Ancestral areas reconstructed for many Colchicaceae nodes were unaffected by model choice (Appendix S4), but conflicting reconstructions were obtained for nodes N179, N180, N166, N165, N98, and N64 (Table 3 and Appendix S4-A). The node with the highest number of alternative ancestral areas was N180 (Table 4, Fig. 4). Among models, MC10 was the most ambiguous, with the highest number of alternative ranges estimated at problematic nodes. Models MC0 and MC1 were the least ambiguous (Table 4). The ambiguous results were strikingly concentrated in the model that used the highest number of time slices (Fig. 2). An intriguing result was the inference of ancestral areas involving South America (area F), where no Colchicaceae species occur today (Models MC5, MC6, MC7, MC8, MC9, and MC10, Table 3). For the artificial data set, three problematic nodes were N181, N180, and N98, the most ambiguous model was MA8, and the least ambiguous MA1 (see Appendix S5).

The number of dispersal probability categories affected model likelihood only slightly: In a comparison of models that only differed in this parameter (MC1 *vs*. MC2, or MC7 *vs*. MC8), models with 5 probability categories had better likelihoods than models with three categories. However, only in two cases was this difference >2 likelihood units: Between MC3 (-lnL = 117.7) and MC4 (-lnL = 110.5), and between MC9 (-lnL = 144.2) and MC10 (-lnL = 138.4).

Table 3. Nodes of Colchicaceae for which incongruent ancestral ranges were inferred in different LAGRANGE experiments (see Fig. 4 and Appendix S4). The probability (P) of each ancestral range obtained in the corresponding experiment (third column) is also shown. The probabilities obtained with best-fit model for the Colchicaceae data set (MC2) is highlighted in bold letters.

Node number	Ancestral range (P)	Experiments
N180	[AC|A] (0.82)	MC3
	[F|E] (0.60)	MC9
	[C|D] (**0.68**, 0.39, 0.39, 0.59)	**MC2**, MC5, MC6, MC10
	[C|E] (0.50, 0.48)	MC0, MC1
	[F|F] (0.39)	MC7
	[C|C] (0.34, 0.30)	MC4, MC8

Continued

Table 3 *Continued*

Node number	Ancestral range (P)	Experiments
N179	[D|E] (1.0, 1.0, **1.0,** 0.42, 0.73, 0.73, 0.72, 0.73, 0.56)	MC0, MC1, **MC2**, MC4, MC5, MC6, MC7, MC8, MC10
	[E|E] (0.42)	MC9
	[AD|D] (0.27)	MC3
N166	[C|A] (0.74, 0.80, **0.57**, 0.82, 0.47)	MC0, MC1, **MC2**, MC3, MC4
	[C|F] (0.78, 0.77, 0.61, 0.40, 0.70)	MC5, MC6, MC8, MC9, MC10
	[F|A] (0.42)	MC7
N165	[A|A] (0.74, 0.80, **0.57**, 0.90, 052, 0.67, 0.47, 0.59)	MC0, MC1, **MC2**, MC3, MC4, MC7, MC8, MC9
	[AF|A] (0.57, 0.55, 0.51)	MC5, MC6, MC10
N98	[A|A] (0.88, 0.88, 0.88, 0.88, 0.88, 0.54, 0.87, 0.88)	MC0, MC3, MC4, MC5, MC6, MC7, MC9, MC10
	[A|AB] (0.50, **0.86**, 0.84)	MC1, **MC2**, MC8
N64	[A|C] (1.0, 1.0, **1.0**, 0.75, 0.84)	MC0, MC1, **MC2**, MC3, MC4
	[F|A] (0.89, 0.89, 0.87, 0.87, 0.85, 0.88)	MC5, MC6, MC7, MC8, MC9, MC10

Table 4. Number of alternative ancestral areas obtained in the Lagrange experiments for the problematic nodes of Colchicaceae.

	# Alternative ancestral areas						
Experiment	N180	N179	N166	N165	N98	N64	**Total # alternative areas inferred per experiment**
MC0	2	1	2	2	2	1	**10**
MC1	2	1	2	2	2	1	**10**
MC2	3	1	4	3	2	1	**14**
MC3	4	10	4	3	2	2	**25**
MC4	7	8	4	3	2	2	**26**
MC5	7	5	4	3	2	3	**24**
MC6	7	5	4	3	2	3	**24**
MC7	6	5	5	3	2	3	**24**
MC8	9	5	6	3	2	3	**28**
MC9	6	6	6	4	2	3	**27**
MC10	8	7	7	4	2	3	**31**
Total # of alternative areas inferred per node	**61**	**54**	**48**	**33**	**22**	**25**	

Discussion

Effect of the components of the DEC model

Maximum likelihood-based ancestral area reconstruction (AAR) requires a fully resolved chronogram (Ree and Smith, 2008), and a well-supported phylogeny of Colchicaceae (Fig. 3) therefore was an important basis for this study. The family's long evolutionary history, which spans the geologic periods between the Upper Cretaceous and the Holocene (Fig. 4), and its distribution on several continents also made Colchicaceae a suitable system for assessing the sensitivity of AAR to changing model parameters, especially the use of time periods with different *ad hoc* dispersal probability values.

The results of the 22 experimental runs show that LAGRANGE results are very sensitive to the specification of the user-defined components, especially the adjacency matrix (Table 2; Fig. 2 for model details). In general, the best likelihood scores were obtained with the unconstrained adjacency matrix in which all ranges were allowed, meaning that all rows and columns were multiplied with the area-specific scaling factors from the dispersal probability matrix. Although the same effect was identified for both the Colchicaceae and the artificial data set (Fig. 5), generalization may not be possible. Thus, in the Hawaiian genus *Psychotria*, the more constrained adjacency matrix fit the data better (as assessed by the two log-likelihood difference; Ree and Smith, 2008), perhaps because in the Hawaiian archipelago, with its emersion and submersion of islands, certain islands were only available during discrete time periods. In the case of the Colchicaceae, use of a constrained adjacency matrix likewise implies the *a priori* rejection of the hypothesis of successful long-distance dispersal, which may be less plausible for this study system, given the disjunct ranges of some genera (see *Introduction*; also Fig. 6). We found no significant effect of the number of categories of dispersal probabilities on the results, in agreement with a study of the Annonaceae (Couvreur et al., 2011) in which the use of three or five categories of probabilities also failed to affect results.

The area dispersal probability matrix and the delineation of time slices with varying number of nodes per slice are other important steps in likelihood-based AAR in LAGRANGE. This was clear from the likelihoods of models with four *vs.* two time slices (Table 2 and Fig. 5). The calculation of the global likelihood involves the

estimation of fractional likelihoods at points along branches intersecting the boundaries of a time slice, coupled with the likelihoods of range inheritance scenarios (dispersal or extinction) at lineage divergence points (Ree and Smith, 2008). These calculations apparently become problematic when slices (time periods) contain highly unequal number of nodes (and therefore of potential range change events) contributing to the overall likelihood of the model (Ree and Sanmartín, 2009).

The likelihood calculations in LAGRANGE proceed backwards, from the tips to the root (Ree and Smith, 2008), meaning that the youngest time slice scheme will always contain many more nodes than older time slices (in our 4-time-slice model, it contained >80% of all nodes; Appendix S2), explaining why reconstructions become more ambiguous closer to the root (Tables 3 and 4). Nevertheless, under the best-fit model, most internal nodes of the Colchicaceae had optimal range inheritance scenarios that scored significantly better than any alternative range at that node.

Model complexity always raises the specter of over-parameterization and loss of inferential power. For both the empirical and the artificial data sets, the more complex models were not only the most ambiguous (Table 4) but also contain apparent inaccuracies (MC5 to MC10; Fig. 2 and Table 3), such as inferred ancestral ranges comprising Central and South America (area F, see Fig. 4), for a family absent from both regions. These were the models with the worse likelihood scores (Fig. 5) and, of course, the highest inferred rates of extinction (Fig. 6b). As pointed out by Ree and Sanmartín (2009), an important challenge for all model-based methods is achieving an optimal balance between the complexity and the realism of models against computational feasibility and inferential power.

The biogeography of Colchicaceae based on the best-fit AAR model (MC2)

The common ancestor of Colchicaceae/Alstroemeriaceae likely lived in East Gondwana (75.3–102.5 Ma, Fig. 4) at a time when the connection to West Gondwana was still close and the climate sufficiently warm for dinosaurs and broad-leaved forests to inhabit Antarctica (Poole and Gottwald, 2001; Ezcurra and Agnolín, 2012). After the initial radiation of the Colchicaceae at c. 75 Ma in Australia (Fig. 4), early lineages may have suffered extinction, as indicated by the long length of the branch subtending the *Burchardia rosea* - *B. multiflora* clade, and the paraphyly of this

genus. Range expansion to Africa appears to taken place during the Palaeogene, c. 62.8 Ma (node N180, Fig. 4), at least under the best-fit model (see Table 3). Similar African/Australian disjunctions are known from other families, including Proteaceae (Barker et al., 2007), Restionaceae (Linder et al., 2003, Verboom et al., 2008), Poaceae (*Ehrharta*, Verboom et al., 2003), and Iridaceae (*Patersonia-Geosiris,* Goldblatt et al., 2002), and to our knowledge they are now all attributed to transoceanic dispersal. Colchicaceae then appear to have diversified in southern and central Africa from about 54.2 Ma onward (Table 1). As Africa moved north and the Tethys Sea was closing, the ancestor of the *Disporum*/*Uvularia* clade dispersed to Southeast Asia probably via Arabia and from there to North America (28.3–16.1 Ma, Table 1) via the Bering land bridge.

Several Oligocene and Miocene long-distance dispersal events are inferred to explain the ranges of *Wurmbea*, *Iphigenia,* and *Androcymbium* (Fig. 4). The dispersal of *Wurmbea* eastwards across the Indian Ocean from southern Africa to Australia took place c. 25.2 Ma (Table 1; already suspected by Berg and Linder, 2009). *Androcymbium* dispersed twice from southern Africa to the Mediterranean region in Europe and Northern Africa, once giving rise to the *Colchicum* clade (from c. 18.8 Ma onward), and once resulting in the diversification of *Androcymbium* in Eastern Europe and the Arabian Peninsula (from about 18.9 Ma onward; Fig. 4). Our results contradict findings of three long-distance dispersal events for the South African *Androcymbium,* starting at the end of the Miocene (c. 7 Ma) as a result of a "late Miocene-Pliocene arid track in the east of Africa" (del Hoyo et al., 2009: 848, 857–585). With our denser gene and species sampling of *Colchicum* the diversification of the *Androcymbium-Colchicum* clade is inferred to have started 30–24.4 Ma, during the Oligocene (Table 1), a date closer to the estimated diversification times for other plant lineages of the South African Cape Region, where *Androcymbium* is most diverse.

Conclusions

Our experiments demonstrate the model sensitivity of likelihood-based AAR (also stressed by Ree et al., 2005; Ree and Sanmartín, 2009) and show which of the many user-defined components of the model have the greatest and which the least effect.

For many data sets, the use of constrained adjacency matrices probably is problematic because it denies the possibility of long-distance dispersal, thus increasing potential inaccuracy of inferences. Models with many time slices are problematic because they necessarily will include time periods with few nodes, preventing confident likelihood calculations. We want to stress, however, that a careful likelihood-based AAR still is an excellent use of available plate tectonic knowledge for historical biogeography. For small data sets, model comparisons similar to those done here (but dropping different probability scores, which make no difference) are easily possible and seem the best strategy to fully use *a priori* knowledge and empirical data.

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Supporting information

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

Appendix S1. Species sampled in this study, voucher information, geographic origin, and GenBank accession numbers.

Appendix S2. Time slices schemes with zero time slices (A), two time slices (B), and four time slices (C). The nodes included in each time slice are shown within the circles.

Appendix S3. Area-dispersal matrices used in the experiments

Appendix S4. Ancestral areas and probabilities (*P*) estimated at the nodes of interest in the experiments conducted with the Colchicaceae (A) and the artificial data set (B).

Appendix S5. Number of alternative ancestral areas obtained in the LAGRANGE experiments for the problematic nodes of the artificial data set

Biosketches

Juliana Chacón is an evolutionary botanist; she is currently undertaking a Ph.D. at the University of Munich, focusing on the molecular phylogenetics and biogeography of Alstroemeriaceae and Colchicaceae.

Author contributions: J.C. and S.S.R. conceived the study; J.C. acquired the data; J.C. carried out the analyses; J.C. and S.S.R. wrote the manuscript; both authors read and approved the final version of the manuscript.

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Appendix S1. Species sampled in this study, voucher information, geographical origin and GenBank accession numbers. Species for which sequences from different plants were combined are marked by an asterisk.

Species name	Voucher	Geographical origin	*ndh*F	*rbc*L	*atp*B-*rbc*L	*mat*K	*rps*16	*trn*L-*trn*F	*mat*R	ITS
Colchicaceae										
Androcymbium asteroides Manning & Goldblatt	J.C. Manning 2322 (NBG)	South Africa, Northern Cape Province	X	X	AJ554228	X	AJ551183	AJ551333	X	X
Androcymbium capense (L.) Druce	JBM CAPEHO 2064 (HMiM)	South Africa, Cape Town, Hopefield			EU236983		EU237064			
Androcymbium cedarbergense U. Müll.-Doblies & D. Müll-Doblies	JBM CLANPK 2399 (HMiM)	South Africa, Wuppertal, Clanwilliam			EU236984		EU237065			
Androcymbium cf. *capense* (L.) Druce	J. Chacón 20 (MSB, BOL)	South Africa, Western Cape, Katbakkies	X	X		X			X	X
Androcymbium circinatum Baker	J.C. Manning 2355A (NBG)	South Africa, Northern Cape, Springbok	X	X	AJ554232	X	AJ551187	AJ551335	X	X
Androcymbium cruciatum U. Müll.-Doblies & D. Müll.-Doblies	J.C. Manning 2354 (NBG)	South Africa, Cape Province	X	X	AJ554233	X	AJ551188	AJ551336	X	X
Androcymbium cuspidatum Baker 1	J. Chacón 14 (MSB, BOL)	South Africa, Western Cape, Cederberg Wilderness Area	X	X		X			X	X
Androcymbium cuspidatum Baker 2	J.C. Manning 2359 (NBG)	South Africa	X	X	AJ554234	X	AJ551189		X	X
Androcymbium dregei C. Presl.	J.C. Manning 2355 (NBG)	South Africa, Northern Cape, Springbok	JQ404520	JQ404669	AJ554235	JQ404777	AJ551190	AJ551338	JQ404901	JQ405011
Androcymbium eucomoides (Jacq.) Willd.	P. Goldblatt & J.C. Manning 11390 (NBG)	South Africa, Western Cape, Caledon, Hermanus			AJ554236		AJ551191	AJ551339		
Androcymbium exiguum subsp. *vogelii (U.Müll.-Doblies & D.Müll.-Doblies) U.Müll.-Doblies & D.Müll.-Doblies**	P. Goldblatt & J.C. Manning 11344 (NBG)	Namibia, Spitskop	X	X		X			X	
Androcymbium exiguum subsp. *vogelii (U.Müll.-Doblies & D.Müll.-Doblies) U.Müll.-Doblies & D.Müll.-Doblies**	P. Goldblatt & J.C. Manning 11687 (NBG)	South Africa			AJ554243		AJ551198	AJ551345		
Androcymbium huntleyi Pedrola, Membrives, J.M. Monts. & Caujape	JBM HUNTEK1 2348 (HMiM)	South Africa, Vioolsdrif, Eksteenfontein			EU236998		EU237075	AY622713		
Androcymbium latifolium Schinz	J.C. Manning 2360 (NBG)	South Africa, Nieuwoudtville	X	X	AJ554238	X	AJ551193	AJ551341	X	X
Androcymbium orientecapense U. Müll.-Doblies & D. Müll.-Doblies	J.C. Manning 2323 (NBG)	South Africa, Northern Cape, Hanover	X	X	AJ554240	X	AJ551195	AJ551342	X	X
Androcymbium rechingeri Greuter*	E. Bergmeier s.n. P1998.5234(C)	Greece, Crete	X	X		X				
Androcymbium rechingeri Greuter*	JBM REEL 220.691 (HMiM)	Greece, Crete, Elafonisos			EU237012		EU237089	AY608525		
Androcymbium scabromarginatum Schltr. & K.Krause	J.C. Manning 2635 (NBG)	South Africa, Little Namaqualand	X	X	AJ554241	X	AJ551196	AJ551343	X	X
Androcymbium schimperianum (Hochst.)	A. Vinnersten 113	Yemen			AJ554272		AJ551228	AJ551367		

K.Perss.	(UPS)									
Androcymbium sp1.	J. Chacón 16 (MSB, BOL)	South Africa, Western Cape, Hondsverbrand	X	X		X			X	X
Androcymbium sp.2	J. Chacón 25 (MSB, BOL)	South Africa, Western Cape, Katbakkies	X	X		X			X	X
Androcymbium volutare Burch.	J.C. Manning 2358 (NBG)	South Africa	X	X	AJ554244	X	AJ551199	AJ551346	X	X
Baeometra uniflora (Jacq.) G. J. Lewis*	M.W. Chase 2222 (K)	South Africa	JQ404638	JQ404747		JQ404867			JQ404979	JQ405061
Baeometra uniflora (Jacq.) G. J. Lewis*	P. Goldblatt & J.C. Manning 11393 (NBG)	South Africa			AJ554246		AJ551201			
Burchardia multiflora Lindl.*	J.G. Conran 3045 (PERTH, ADU)	Western Australia, c. 12 km W of Walpole along SW Hwy	X	X		X			X	X
Burchardia multiflora Lindl.*	A. Case 81 (PERTH)	Australia			EU044616			EU044684		
Burchardia rosea Keighery	M.W. Chase 2224 (K)	Australia			AJ554248		AJ551203	AJ560295		
Burchardia umbellata R. Br.*	K. Bremer 3954 (UPS)	Australia					AJ551204	AJ551349		
Burchardia umbellata R. Br.*	J.G. Conran 3094 (AD, ADU)	South Australia, Muddy Flat NFS 5.6 km N of Nangwarry off Riddock Hwy	X	X		X			X	
Camptorrhiza strumosa (Baker f.) Oberm.	J.C. Manning 2994 (NBG)	South Africa, Mpumalanga	JQ404641	AJ554249	AJ554249	JQ404870	AJ551205	AJ551350		
Colchicum autumnale L.	A. Vinnersten 103 (UPS)	Bulgaria	X	X	AJ554251	X	AJ551207	AJ551352	X	X
Colchicum bornmuelleri Freyn	SANBI LHMS #266	South Africa	JQ404640	JQ404749		JQ404869			JQ404981	JQ405062
Colchicum bulbocodium Ker Gawl.*	J. Chacón 05 (M)	Europe	JQ404639	JQ404748		JQ404868			JQ404980	
Colchicum bulbocodium Ker Gawl.*	A. Vinnersten 102 (UPS)	Spain			AJ554247		AJ551202	AJ551348		
Colchicum cretense Greuter	A. Strid 53589 (GB)	Greece, Crete	X	X	JF933961	X	JF934213	JF934464		
Colchicum doerfleri Haláksy	A. Vinnersten 107 (UPS)	Macedonia	X	X	AJ554252	X	AJ551208	AJ551353		X
Colchicum luteum Baker	A. Vinnersten 110 (UPS)	Himalayan region	X	X	AJ554253	X	AJ551209	AJ551354	X	X
Colchicum macedonicum Kosanin	A. Vinnersten 109 (UPS)	Macedonia	X	X	AJ554254	X	AJ551210	AJ551355	X	X
Colchicum macrophyllum B. L. Burtt	A. Vinnersten 106 (UPS)	Greece, Crete	X	X	AJ554255	X	AJ551211	AJ551356	X	X
Colchicum mirzoevae (Gabrieljan) K. Perss.	B. Zhirair GBG 2006-2129 (GB)	Armenia, Ijevan	X	X	JF934007	X	JF934259	JF934510		
Colchicum montanum L.*	J. Chacon 10 (MSB)	France, Nantes	X	X		X			X	X
Colchicum montanum L.*	K. Persson HZ8744 (HMiM)	Spain, Huesca			EU237023		EU237101			
Colchicum peloponnesiacum Rech. f. & P.H. Davis	J. & K. Persson 98-099 (GB)	Greece, Achaea	X	X	JF934016	X	JF934267	JF934519	X	X
Colchicum robustum (Bunge) Stef. (s.str.)*	I.C. Hedge *et al.* 7369 (GB)	Greece	X	X		X				

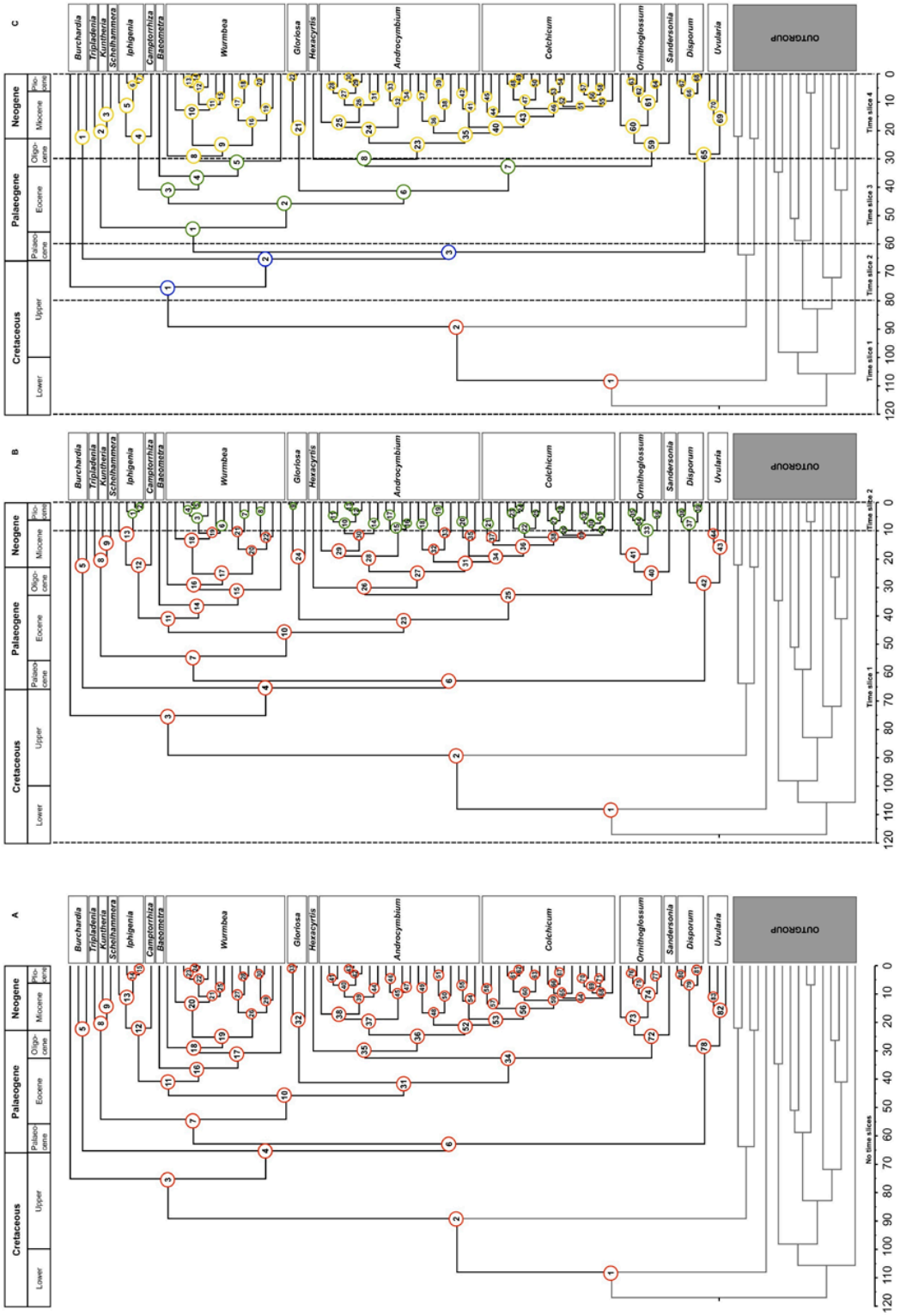
Colchicum robustum (Bunge) Stef. (s.str.)*	K. Persson 2220	Afghanistan, Parwan			EU237024		EU237102	EU237048		
Colchicum sp.	H. Akhani s.n. (MSB)	Iran	X	X		X			X	X
Colchicum speciosum Steven	A. Vinnersten 104 (UPS)	Georgia	X	X	AJ554256	X	AJ551212		X	X
Colchicum szovitzii Fisch. & C.A. Mey. subsp. *szovitsii*	GBG 1983-0244 (GB)	Bulgaria, Stara Zagora	X	X	JF934043	X	JF934291	JF934418		
Colchicum trigynum (Steven ex Adams) Stearn.*	A. Groeger & J. Wainwright Klein 06.15.2 (MSB)	Georgia	X	X		X			X	X
Colchicum trigynum (Steven ex Adams) Stearn.*	J. Cuba 90-088 (GB)	Georgia, Tbilisi			JF934045		JF934045	JF934548		
Colchicum x *agrippinum* Baker	A. Vinnersten 101 (UPS)	Europe	X	X	AJ554250	X	AJ551206	AJ551351	X	X
Disporum cantoniense (Lour.) Merr.	J.G. Conran 3247 (AD, ABG)	Australia, cultivated Adelaide Bot. Gard.	X	X		X			X	X
Disporum chinense (Ker Gawl.) Kuntze	M.W. Chase 18195 (K)	Indonesia, Java	JQ404642	JQ404750		JQ404871			JQ404982	
Disporum flavens Kitag.	A. Vinnersten 118 (UPS)	China	X		AJ554257	X	AJ551213	AJ551357	X	X
Disporum smilacinum A. Gray	Ex hortus Göteborg Bot. Gard. 1986-1068	China	X		AJ554258	X	AJ551214	AJ551358	X	X
Disporum viridescens (Maxim.) Nakai	Cultivated S1939-1565 (C)	China	JQ404647	JQ404755		JQ404876			JQ404986	JQ405064
Gloriosa modesta (Hook.) J.C.Manning & Vinn.*	SANBI LHMS #203	South Africa	X	X		X			X	X
Gloriosa modesta (Hook.) J.C.Manning & Vinn.*	J.E. Roux 37/80 & J.P. Burrows 35 (NBG)	South Africa			AJ554269		AJ551225	AJ551365		
Gloriosa simplex L.*	J.G. Conran 3120D (ABG)	Australia, cultivated Adelaide Bot. Gard.	X	X		X			X	X
Gloriosa simplex L.*	Ex hortus Sydney Bot. Gard.	Tropical Africa			AJ554262		AJ551216	AJ551360		
Gloriosa superba L.	Ex hortus Sydney Bot. Gard.	India	X	X	AJ554261	X	AJ551218	AJ551362	X	X
Hexacyrtis dickiana Dinter	J.C. Manning 2745 (NBG)	South Africa	X	X		X			X	X
Iphigenia indica (L.) A. Gray ex Kunth*	M.W. Chase 1028 (K)	India	AY224999	AJ417893	EU044593		AJ551223	AJ551392		
Iphigenia indica (L.) A. Gray ex Kunth*	J. Russell-Smith & D. Lucas 7304 (NSW)	Australia, Northern territory	X	X						
Iphigenia ledermannii Engl. & K.Krause	M.G. & S.B. Gilbert 2160 (UPS)	Tropical Africa			AJ554265		AJ551221	AJ551394		
Iphigenia oliveri Engl.	M. Thulin & Warfa 5855 (UPS)	Africa			AJ554266		AJ551222	AJ551364		
Iphigenia pauciflora Martelli	M. Thulin *et al.* 3504 (UPS)	Tropical Africa			AJ554268		AJ551224	AJ551393		
Kuntheria pedunculata (F. Muell.)	J.L. Dowe 1085 (JCU,	Australia, Queensland,	X	X		X			X	X

Conran & Clifford	ADU)	Josephine Creek Falls								
Ornithoglossum calcicola Krause & Dinter	H. Kinges 2767 (MSB)	Namibia, Omuramba	X	X		X			X	X
Ornithoglossum dinteri Krause	O.H. Volk 12561 (MSB)	Namibia, Maltahöhe	X	X		X			X	
Ornithoglossum parviflorum B. Nord.	P. Goldblatt & J.C. Manning 11670 (NBG)	South Africa, Cape Province	X	X	AJ554276	X	AJ551232	AJ551370	X	X
Ornithoglossum undulatum Sweet	J.C. Manning 2340 (NBG)	South Africa	X	X	AJ554277	X	AJ551233	AJ551371	X	X
Ornithoglossum viride Aiton	P. Goldblatt & J.C. Manning 11663	South Africa, Cape Province			AJ554278		AJ551234	AJ551372		
Ornithoglossum vulgare B. Nord.*	R.K. Brummitt & R.M. Polhill 13667 (UPS)	Tanzania			AJ554279		AJ551235	AJ551373		
Ornithoglossum vulgare B. Nord.*	W. Giess *et al.* 11089 (MSB)	Namibia, Tsumkwe	X	X		X			X	X
Sandersonia aurantiaca Hook.	Ex hortus NBG s.n.	South Africa	X	X	AJ554280	X	AJ551236	AJ560299	X	X
Schelhammera undulata R. Br.	Ex hortus Mt. Annan Bot. Gard. 923427	Australia	X	X	AJ554281	X	AJ551237	AJ551374	X	X
Tripladenia cunninghamii D. Don*	K. Bremer 3947 (UPS)	Australia			AJ554282		AJ551238	AJ551375		
Tripladenia cunninghamii D. Don*	J.G. Conran 3120C (AD, ABG)	Australia, cultivated Adelaide Bot. Gard.	X	X		X			X	X
Uvularia grandiflora Sm.	A. Vinnersten 112 (UPS)	North America	X	X	AJ554284	X	AJ551240	AJ551377	X	X
Uvularia perfoliata L.	M.W. Chase 494 (K)	USA	JQ404662	JQ404767		JQ404891		AJ560300	JQ405001	X
Uvularia sessilifolia L.*	J. Bright 12403 (UPS)	North America			AJ554286		AJ551242	AJ551378		
Uvularia sessilifolia L.*	R.D. Thomas 51358 (MSB)	USA, Louisiana	X	X		X			X	
Wurmbea australis (R.J.Bates) R.J.Bates	J.G. Conran 3124 (AD, ADU)	South Australia, Battery Track, Mt Remarkable NP at N edge of park	X	X		X			X	
Wurmbea biglandulosa (R. Br.) T. D. Macfarl.	Sheathers GVV 981024	Australia	X	X	AJ554288	X	AJ551244	AJ551380	X	X
Wurmbea centralis T. D. Macfarlane	J. Thompson s.n. (NSW)	South Australia	X							
Wurmbea dioica (R. Br.) F. Muell.*	J.G. Conran 3116 (AD, ADU)	South Australia, Anstey Hill Recreation Reserve above gate #3	X	X		X			X	X
Wurmbea dioica (R. Br.) F. Muell.*	H.I. Aston 606 (UPS)	Australia			AJ554289		AJ551245	AJ551381		
Wurmbea glassii (C.H.Wright) J.C.Manning & Vinn.	J.C. Manning 2168 (NBG)	South Africa, Cape Province			AJ554273		AJ551229	AJ551368		
Wurmbea inusta (Baker) B. Nord	P. Runnalls 591 (NBG)	South Africa, Western Cape, Somerset West	X	X	AJ554291	X	AJ551247	AJ551383	X	X
Wurmbea kraussii Baker	I. Nänni & F. Forest 11230 (NBG)	South Africa	JQ404663	JQ404768	AJ554292	JQ404892	AJ551248	AJ551384	JQ405002	JQ405070

Wurmbea marginata (Desr.) B. Nord.	J.C. Manning 2362A (NBG)	South Africa, Western Cape, Caledon	X	X	AJ554293	X	AJ551249	AJ551385	X	X
Wurmbea murchisoniana T.D.Macfarl.*	A. Case 2 (PERTH)	Australia			EU044604			EU044703		
Wurmbea murchisoniana T.D.Macfarl.*	J.G. Conran 3017 (PERTH)	Western Australia, 200 m S of Murchison R xing	X	X		X			X	X
Wurmbea punctata (L.) J.C. Manning & Vinn.*	J.C. Manning 3364 (NBG)	South Africa, Western Cape, Elandsberg Farm	X	X		X			X	X
Wurmbea punctata (L.) J.C. Manning & Vinn.*	P. Goldblatt & J.C. Manning 11391 (NBG)	South Africa			AJ554274		AJ551230	AJ551369		
Wurmbea pygmaea (Endl.) Benth.*	A. Case 77 (PERTH)	Australia	AF547012							
Wurmbea pygmaea (Endl.) Benth.*	B. Kaspiew 550820	Australia			AJ554297		AJ551253	AJ551389		
Wurmbea recurva B. Nord.	A. Vinnersten s.n. (UPS)	South Africa	X	X					X	X
Wurmbea saccata T. D. Macfarl. & S. J. van Leeuwen	S. van Leeuwen 1674 (NSW)	Western Australia	X	X	AJ554299		AJ551255	AJ551391		
Wurmbea spicata (Burm.f.) T. Durand & Schinz*	P. Goldblatt M. Fay 11021 (K)	South Africa, Western Cape, Piketberg	X							
Wurmbea spicata (Burm.f.) T. Durand & Schinz*	L.K. Jesson 2 (TRT)	South Africa			EU044613			EU044691		
Wurmbea stricta (Burm.f.) J.C.Manning & Vinn.	P. Goldblatt s. n. (MO)	South Africa	JQ404659	JQ404765		JQ404888		AJ560298	JQ404998	JQ405068
Wurmbea stricta (Burm.f.) J.C.Manning & Vinn.*	A. Case 16 (PERTH)	South Africa, Canberra			EU044615					
Wurmbea variabilis B. Nord.	P. Goldblatt 11438	South Africa, Cape Province	X	X	AJ554295		AJ551251	AJ551387	X	X
OUTGROUPS										
Alstroemeriaceae										
Alstroemeria aurea Graham*	L. Aagesen s. n. (BAA)	Argentina, Chubut	JQ404511	AY120359		JQ404771	AY120373		JQ404895	JQ405005
Alstroemeria aurea Graham*	M.C. Sheahan s.n. (K)	Chile			AY699131			AY699225		
Bomarea patinii Baker*	J. Chacón 02 (ANDES)	Colombia, Valle del Cauca	JQ404585	JQ404711		JQ404823			JQ404944	EU159951
Bomarea patinii Baker*	F. Alzate 2894 (HUA)	Colombia, Cundinamarca								EU159951
Bomarea edulis (Tussac) Herb.*	L. Aagesen w/n (SI)	Argentina					AY120390			
Drymophila moorei Baker	D. Crestani 48 (NSW)	Australia, New South Wales	JQ404645	JQ404753		JQ404874			JQ404984	JQ405063
Luzuriaga marginata (Gaertn.) Benth. & Hook.f.*	M. Gusinde 119 (M)	Chile, Magallanes	JQ404650	JQ404757		JQ404878	AY120393		JQ404988	JQ405066
Luzuriaga radicans Ruiz & Pav.*	M.W. Chase 499 (K)				AY699134			AY699162		
Campynemataceae										
Campynema lineare Labill.*	N. G. Walsh 3488 (MEL)	Tasmania	AF276013	Z77264						
Campynema lineare Labill.*	M.F. Duretto, 1842					JN417414				
Campynemanthe viridiflora Baill.	Y. Pillon *et al.* 24 (NOU)	New Caledonia		JN417506		JN417415				
Liliaceae										

Lilium superbum L.*	M.W. Chase 112 (NCU)	USA	AY007655	L12682						
Lilium sp.*	Qiu 96072 (IND)								DQ401403	
Melanthiaceae										
Chamaelirium luteum (L.) A. Gray	S.W. Leonard 3198	USA	AY225006	AJ276347						
Petermanniaceae										
Petermannia cirrosa F. Muell.	S. Frederiksen *et al.* s.n. (C)	Australia	AY465662	AY465714	AY699144					
Philesiaceae										
Lapageria rosea Ruiz & Pav.	J.G. Conran 2999A (ADU)	Cultivated Mt. Lofty Bot. Gard.	X	X		X			X	
Philesia magellanica J.F.Gmel.*	J.G. Conran 3000A (ADU)	Cultivated Mt. Lofty Bot. Gard.	X	X		X			X	
Philesia magellanica J.F.Gmel.*	M.W. Chase 545 (K)	Chile			AY699137			AY699227		
Ripogonaceae										
Ripogonum album R. Br.	J.G. Conran 3120A (AD)	Australia, Cultivated Adelaide Bot. Gard.	X	X		X			X	X
Rhipogonum elseyanum F. Muell.*	M.W. Chase 187 (NCU)	Australia, Sydney	AF276016	Z77309	AY699139			AY699164		
Rhipogonum elseyanum F. Muell.*	J.G. Conran 1045 (ADU, BRI)	Australia, SE Queensland, Lyrebird Ridge, Springbrook Plateau	X	X					X	
Smilacaceae										
Smilax china L.	H. Kato s. n. (KYO)	Japan, Pref. Shiga		D28333						
Smilax glauca Walter	M.W. Chase 107 (NCU)	USA		Z77310						
Smilax hispida Muhl. ex Torr*	T.J. Givnish s. n. (WIS)	USA	AF276018							
Smilax hispida Muhl. ex Torr*	H. Schaefer 2009/107 (unvouchered)	USA		GU945054		GU945048				

Appendix S2. Time slices schemes with zero (a), two (b), and four time slices (c). The nodes included in each time slice are shown within the circles.



Appendix S3. Area dispersal matrices used in the LAGRANGE experiments (models) depicted in Figure 1, with zero-time-slices (120–0 Ma), two-time-slices (0–10, 10–120 Ma), and four-time-slices (0–30, 30–60, 60–80, 80–120 Ma), using different categories of dispersal probabilities (one category: $P = 1.0$; 3 categories: $P = 0.01$, $P = 0.5$, $P = 1.0$; 5 categories: $P = 0.1$, $P = 0.25$, $P = 0.5$, $P = 0.75$, $P = 1.0$). A, South to middle Africa; B, Mediterranean region in Europe and Northern Africa; C, Australia and New Zealand; D, Asia and Southeast Asia; E, North America; F, South America.

Zero-time-slices, 1 category of dispersal probabilities (models MC0 and MA0).

	A	B	C	D	E	F
A	1.0	1.0	1.0	1.0	1.0	1.0
B	1.0	1.0	1.0	1.0	1.0	1.0
C	1.0	1.0	1.0	1.0	1.0	1.0
D	1.0	1.0	1.0	1.0	1.0	1.0
E	1.0	1.0	1.0	1.0	1.0	1.0
F	1.0	1.0	1.0	1.0	1.0	1.0

Zero-time-slices, 3 categories of dispersal probabilities (models MC5 and MA5).

	A	B	C	D	E	F
A	1.0	1.0	0.01	0.01	1.0	1.0
B	1.0	1.0	0.01	1.0	1.0	0.01
C	0.01	0.01	1.0	1.0	0.01	1.0
D	0.01	1.0	0.01	1.0	1.0	0.01
E	1.0	1.0	0.01	1.0	1.0	1.0
F	1.0	0.01	1.0	0.01	1.0	1.0

Zero-time-slices, 5 categories of dispersal probabilities (models MC6 and MA6).

	A	B	C	D	E	F
A	1.0	1.0	0.1	0.1	1.0	1.0
B	1.0	1.0	0.1	1.0	1.0	0.1
C	0.1	0.1	1.0	1.0	0.1	1.0
D	0.1	1.0	0.1	1.0	1.0	0.1
E	1.0	1.0	0.1	1.0	1.0	1.0
F	1.0	0.1	1.0	0.1	1.0	1.0

Two-time-slices, time slice between 0–10 Ma, 3 categories of dispersal probabilities (models MC1, MC7, MA1, and MA7).

	A	B	C	D	E	F
A	1.0	1.0	0.01	1.0	0.01	0.5
B	1.0	1.0	0.01	1.0	0.5	0.01
C	0.01	0.01	1.0	0.5	0.01	0.01
D	1.0	1.0	0.5	1.0	0.5	0.01
E	0.01	0.5	0.01	0.5	1.0	1.0
F	0.5	0.01	0.01	0.01	1.0	1.0

Two-time-slices, time slice between 10–120 Ma, 3 categories of dispersal probabilities (models MC1, MC7, MA1, and MA7).

	A	B	C	D	E	F
A	1.0	0.5	0.5	0.5	0.01	0.5
B	0.5	1.0	0.01	1.0	1.0	0.01
C	0.5	0.01	1.0	0.01	0.01	0.5
D	0.5	1.0	0.01	1.0	1.0	0.01
E	0.01	1.0	0.01	1.0	1.0	0.5
F	0.5	0.01	0.5	0.01	0.5	1.0

Two-time-slices, time slice between 0–10 Ma, 5 categories of dispersal probabilities (models MC2, MC8, MA2, and MA8).

	A	B	C	D	E	F
A	1.0	0.25	0.1	0.1	0.1	0.1
B	0.25	1.0	0.1	1.0	0.1	0.1
C	0.1	0.1	1.0	0.75	0.1	0.1
D	0.1	1.0	0.75	1.0	0.1	0.1
E	0.1	0.1	0.1	0.1	1.0	1.0
F	0.1	0.1	0.1	0.1	1.0	1.0

Two-time-slices, time slice between 10–120 Ma, 5 categories of dispersal probabilities (models MC2, MC8, MA2, and MA8).

	A	B	C	D	E	F
A	1.0	0.75	0.25	0.5	0.5	0.5
B	0.75	1.0	0.1	1.0	0.5	0.25
C	0.25	0.1	1.0	0.25	0.1	0.5
D	0.5	1.0	0.25	1.0	0.5	0.1
E	0.5	0.5	0.1	0.5	1.0	1.0
F	0.5	0.25	0.5	0.1	1.0	1.0

Four-time-slices, time slice between 0–30 Ma, 3 categories of dispersal probabilities (models MC3, MC9, MA3, and MA9).

	A	B	C	D	E	F
A	1.0	1.0	0.5	1.0	0.01	0.5
B	1.0	1.0	0.01	1.0	1.0	0.01
C	0.5	0.01	1.0	0.01	0.01	0.5
D	1.0	1.0	0.01	1.0	1.0	0.01
E	0.01	1.0	0.01	1.0	1.0	1.0
F	0.5	0.01	0.5	0.01	1.0	1.0

Four-time-slices, time slice between 30–60 Ma, 3 categories of dispersal probabilities (models MC3, MC9, MA3, and MA9).

	A	B	C	D	E	F
A	1.0	1.0	0.01	1.0	0.01	0.01
B	1.0	1.0	0.01	1.0	1.0	0.01
C	0.01	0.01	1.0	0.01	0.01	1.0
D	1.0	1.0	0.01	1.0	1.0	0.01
E	0.01	1.0	0.01	1.0	1.0	0.01
F	0.01	0.01	1.0	0.01	0.01	1.0

Four-time-slices, time slice between 60–80 Ma, 3 categories of dispersal probabilities (models MC3, MC9, MA3, and MA9).

	A	B	C	D	E	F
A	1.0	0.01	1.0	0.01	0.01	0.01
B	0.01	1.0	0.01	1.0	1.0	0.01
C	1.0	0.01	1.0	0.01	0.01	1.0
D	0.01	1.0	0.01	1.0	1.0	0.01
E	0.01	1.0	0.01	1.0	1.0	1.0
F	0.01	0.01	1.0	0.01	1.0	1.0

Four-time-slices, time slice between 80–120 Ma, 3 categories of dispersal probabilities (models MC3, MC9, MA3, and MA9).

	A	B	C	D	E	F
A	1.0	0.01	1.0	0.01	0.01	1.0
B	0.01	1.0	0.01	1.0	1.0	0.01
C	1.0	0.01	1.0	0.01	0.01	1.0
D	0.01	1.0	0.01	1.0	1.0	0.01
E	0.01	1.0	0.01	1.0	1.0	0.01
F	1.0	0.01	1.0	0.01	0.01	1.0

Four-time-slices, time slice between 0–30 Ma, 5 categories of dispersal probabilities (models MC4, MC10, MA4, and MA10).

	A	B	C	D	E	F
A	1.0	0.5	0.1	0.1	0.1	0.1
B	0.5	1.0	0.1	1.0	0.1	0.1
C	0.1	0.1	1.0	0.75	0.1	0.1
D	0.1	1.0	0.75	1.0	0.1	0.1
E	0.1	0.1	0.1	0.1	1.0	0.75
F	0.1	0.1	0.1	0.1	0.75	1.0

Four-time-slices, time slice between 30–60 Ma, 5 categories of dispersal probabilities (models MC4, MC10, MA4, and MA10).

	A	B	C	D	E	F
A	1.0	0.75	0.1	0.75	0.5	0.1
B	0.75	1.0	0.1	1.0	0.75	1.0
C	0.1	0.1	1.0	0.1	0.1	0.5
D	0.75	1.0	0.1	1.0	0.5	0.1
E	0.5	0.75	0.1	0.5	1.0	0.25
F	0.1	0.1	0.5	0.1	0.25	1.0

Four-time-slices, time slice between 60–80 Ma, 5 categories of dispersal probabilities (models MC4, MC10, MA4, and MA10).

	A	B	C	D	E	F
A	1.0	0.5	0.25	0.25	0.25	0.5
B	0.5	1.0	0.1	0.5	0.5	0.25
C	0.25	0.1	1.0	0.1	0.1	0.5
D	0.25	0.5	0.1	1.0	0.25	0.1
E	0.25	0.5	0.1	0.25	1.0	0.5
F	0.5	0.25	0.5	0.1	0.5	1.0

Four-time-slices, time slice between 80–120 Ma, 5 categories of dispersal probabilities (models MC4, MC10, MA4, and MA10).

	A	B	C	D	E	F
A	1.0	0.75	0.5	0.5	0.75	1.0
B	0.75	1.0	0.1	0.75	0.75	0.5
C	0.5	0.1	1.0	0.1	0.25	0.75
D	0.5	0.75	0.1	1.0	0.5	0.5
E	0.75	0.75	0.25	0.5	1.0	0.75
F	1.0	0.5	0.75	0.5	0.75	1.0

Appendix S4-A. Ancestral areas and probabilities (P) estimated at the nodes of interest in the experiments conducted with the Colchicaceae data set. The areas with the highest probability (P > 0.5) are shown at the top. The node numbers are shown in Fig. 2.

Node	MC0	MC1	MC2	MC3	MC4	MC5	MC6	MC7	MC8	MC9	MC10
N191	[C|C] 0.80 [C|CF] 0.14 [C|F] 0.03	[C|C] 0.81 [C|CF] 0.12 [C|F] 0.04	[C|C] 0.81 [C|CF] 0.12 [C|F] 0.04	[C|C] 0.80 [C|CF] 0.14 [C|F] 0.03	[C|C] 0.73 [C|CF] 0.19 [C|F] 0.03	[C|C] 0.67 [C|CF] 0.16 [C|F] 0.08 [CF|F] 0.04	[C|C] 0.67 [C|CF] 0.16 [C|F] 0.08 [CF|F] 0.04	[C|C] 0.56 [CF|F] 0.14 [C|CF] 0.10 [F|F] 0.08 [C|F] 0.05 [CF|C] 0.04	[C|C] 0.66 [C|CF] 0.14 [CF|F] 0.06 [C|F] 0.06 [F|F] 0.03	[C|C] 0.57 [C|CF] 0.14 [CF|F] 0.10 [C|F] 0.06 [F|F] 0.05 [CF|C] 0.04	[C|C] 0.62 [C|CF] 0.24 [C|F] 0.07 [CF|F] 0.03
N183	[C|C] 0.88 [CE|C] 0.06 [CD|C] 0.06	[C|C] 0.88 [CE|C] 0.06 [CD|C] 0.05	[C|C] 0.88 [CD|C] 0.08	[C|C] 0.94 [AC|C] 0.05	[C|C] 0.94 [CE|C] 0.03	[C|C] 0.86 [CF|C] 0.07 [CD|C] 0.04	[C|C] 0.86 [CF|C] 0.07 [CD|C] 0.04	[C|C] 0.60 [CF|C] 0.29 [F|C] 0.04 [F|F] 0.03	[C|C] 0.78 [CF|C] 0.13 [CD|C] 0.03 [F|C] 0.01	[C|C] 0.71 [CF|C] 0.17 [F|F] 0.04 [F|C] 0.02 [CD|C] 0.02	[C|C] 0.89 [CD|C] 0.05 [CF|C] 0.04
N181	[C|C] 0.52 [C|CE] 0.24 [C|CD] 0.24	[C|C] 0.50 [C|CE] 0.23 [C|CD] 0.23	[C|C] 0.52 [C|CD] 0.34 [C|CE] 0.13	[C|C] 0.55 [C|AC] 0.37 [C|CD] 0.05	[C|C] 0.70 [C|CE] 0.14 [C|CD] 0.11	[C|C] 0.65 [C|CD] 0.20 [C|F] 0.06 [C|CF] 0.06	[C|C] 0.65 [C|CD] 0.20 [C|F] 0.07 [C|CF] 0.06	[C|F] 0.46 [C|C] 0.21 [C|CF] 0.16 [CF|F] 0.09 [F|F] 0.04	[C|C] 0.51 [C|F] 0.18 [C|CD] 0.12 [C|CF] 0.09 [CF|F] 0.04 [C|D] 0.02	[C|F] 0.59 [C|C] 0.16 [C|CD] 0.09 [CF|F] 0.06 [C|CF] 0.05	[C|C] 0.49 [C|CD] 0.28 [C|F] 0.08 [C|D] 0.08 [C|CF] 0.04
N180	[C|E] 0.50 [C|D] 0.49	[C|E] 0.48 [C|D] 0.47	[C|D] 0.68 [C|E] 0.27 [CD|D] 0.05	[AC|A] 0.82 [CD|D] 0.08 [C|E] 0.04 [C|D] 0.03	[C|C] 0.34 [C|E] 0.23 [C|D] 0.20 [AC|C] 0.06 [CD|D] 0.06 [C|CE] 0.05 [AC|A] 0.03	[C|D] 0.39 [C|C] 0.39 [F|F] 0.05 [CF|F] 0.05 [CF|C] 0.04 [F|E] 0.02 [C|CD] 0.02	[C|D] 0.39 [C|C] 0.38 [F|F] 0.05 [CF|F] 0.05 [CF|C] 0.04 [F|E] 0.03 [C|CD] 0.02	[F|F] 0.39 [CF|F] 0.26 [F|E] 0.18 [C|C] 0.06 [C|D] 0.04 [AF|F] 0.01	[C|C] 0.30 [C|D] 0.24 [F|F] 0.15 [CF|F] 0.11 [F|E] 0.07 [CF|C] 0.03 [D|D] 0.02 [C|CD] 0.02 [F|C] 0.01	[F|E] 0.60 [C|D] 0.12 [CF|F] 0.09 [CD|D] 0.08 [F|F] 0.04 [EF|E] 0.02	[C|D] 0.59 [C|C] 0.09 [D|D] 0.08 [F|E] 0.08 [CD|D] 0.05 [CF|F] 0.03 [F|CF] 0.02 [CF|C] 0.01
N179	[D|E] 1.0	[D|E] 1.0	[D|E] 1.0	[AD|D] 0.27 [D|E] 0.19 [D|D] 0.14 [A|E] 0.13 [D|A] 0.06 [D|AD] 0.05 [A|A] 0.05 [E|E] 0.02 [A|D] 0.01 [A|B] 0.01	[D|E] 0.42 [C|E] 0.31 [C|C] 0.07 [D|C] 0.06 [E|E] 0.04 [D|D] 0.02 [A|E] 0.02 [DE|E] 0.01	[D|E] 0.73 [D|D] 0.10 [DE|E] 0.06 [D|DE] 0.04 [E|E] 0.03	[D|E] 0.73 [D|D] 0.10 [DE|E] 0.06 [D|DE] 0.04 [E|E] 0.03	[D|E] 0.72 [E|E] 0.12 [D|DE] 0.05 [DE|E] 0.05 [E|EF] 0.02	[D|E] 0.73 [D|D] 0.08 [D|DE] 0.05 [DE|E] 0.05 [E|E] 0.05	[E|E] 0.42 [D|E] 0.29 [D|D] 0.10 [E|EF] 0.08 [EF|E] 0.03 [DE|E] 0.03	[D|E] 0.56 [D|D] 0.14 [C|F] 0.06 [DE|E] 0.06 [CD|D] 0.05 [E|E] 0.04 [D|DE] 0.04
N178	[E|E] 1.0	[E|E] 1.0	[E|E] 1.0	[E|E] 0.72 [E|DE] 0.14 [DE|E] 0.07 [E|AE] 0.03	[E|E] 0.92 [E|CE] 0.04	[E|E] 0.93 [E|DE] 0.04	[E|E] 0.93 [E|DE] 0.04	[E|E] 0.96	[E|E] 0.93 [E|DE] 0.04	[E|E] 0.90 [E|DE] 0.04 [DE|E] 0.02	[E|E] 0.86 [E|DE] 0.06 [DE|E] 0.03
N173	[D|D] 1.0	[D|D] 1.0	[D|D] 1.0	[D|D] 0.94 [D|AD] 0.03	[D|D] 0.93 [D|CD] 0.03	[D|D] 0.98	[D|D] 0.99	[D|D] 0.96	[D|D] 0.98	[D|D] 0.91 [D|DE] 0.05	[D|D] 0.96

N166	[C|A] 0.74 [C|C] 0.21	[C|A] 0.80 [C|C] 0.16	[C|A] 0.57 [C|C] 0.32 [C|AC] 0.06 [C|D] 0.05	[C|A] 0.82 [C|D] 0.08 [C|AC] 0.04 [AC|A] 0.03	[C|A] 0.47 [C|C] 0.38 [C|D] 0.06 [C|AC] 0.06	[C|F] 0.78 [CF|F] 0.11 [F|A] 0.06 [F|F] 0.02	[C|F] 0.77 [CF|F] 0.19 [F|A] 0.06 [F|F] 0.02	[F|A] 0.42 [C|F] 0.38 [F|F] 0.09 [CF|F] 0.06 [F|AF] 0.02	[C|F] 0.61 [F|A] 0.17 [CF|F] 0.10 [F|F] 0.04 [D|B] 0.02 [C|C] 0.01	[C|F] 0.40 [F|A] 0.31 [F|F] 0.12 [C|D] 0.07 [CF|F] 0.03 [F|AF] 0.02	[C|F] 0.70 [D|B] 0.06 [CF|F] 0.06 [C|D] 0.04 [F|A] 0.04 [C|C] 0.02 [F|F] 0.02
N165	[A|A] 0.74 [AC|A] 0.25	[A|A] 0.80 [AC|A] 0.19	[A|A] 0.57 [AC|A] 0.37 [AD|A] 0.05	[A|A] 0.90 [AD|A] 0.04 [AC|A] 0.04	[A|A] 0.52 [AC|A] 0.41 [AD|A] 0.05	[AF|A] 0.57 [A|A] 0.29 [A|AF] 0.12	[AF|A] 0.55 [A|A] 0.30 [A|AF] 0.12	[A|A] 0.67 [AF|A] 0.22 [A|AF] 0.11	[A|A] 0.47 [AF|A] 0.34 [A|AF] 0.15	[A|A] 0.59 [AF|A] 0.25 [A|AF] 0.10 [AB|A] 0.03	[AF|A] 0.51 [A|A] 0.30 [A|AF] 0.09 [AB|A] 0.04
N164	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0	[A|A] 0.99	[A|A] 0.92 [AF|A] 0.05	[A|A] 0.91 [AF|A] 0.06	[A|A] 0.93 [AF|A] 0.05	[A|A] 0.90 [AF|A] 0.07	[A|A] 0.93 [AF|A] 0.031	[A|A] 0.92 [AF|A] 0.03
N163	[A|A] 0.99	[A|A] 0.99	[A|A] 0.99	[A|A] 0.99	[A|A] 0.99	[A|A] 0.98	[A|A] 0.98	[A|A] 0.98	[A|A] 0.97	[A|A] 0.98	[A|A] 0.97
N162	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0	[A|A] 0.99	[A|A] 0.99	[A|A] 1.0	[A|A] 0.99	[A|A] 0.99	[A|A] 0.99
N160	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0
N149	[A|A] 0.99	[A|A] 0.98	[A|A] 0.97	[A|A] 0.99	[A|A] 0.98	[A|A] 0.99	[A|A] 0.98	[A|A] 0.98	[A|A] 0.96	[A|A] 0.98	[A|A] 0.97
N148	[A|A] 0.91 [A|AB] 0.05	[A|A] 0.83 [AB|A] 0.07 [A|AB] 0.04	[A|A] 0.75 [AB|A] 0.13 [A|AB] 0.04 [B|AB] 0.04	[A|A] 0.91 [A|AB] 0.05	[A|A] 0.90 [A|AB] 0.06	[A|A] 0.91 [A|AB] 0.06	[A|A] 0.91 [A|AB] 0.06	[A|A] 0.84 [AB|A] 0.07 [A|AB] 0.05	[A|A] 0.74 [AB|A] 0.13 [A|AB] 0.053 [B|AB] 0.04	[A|A] 0.90 [A|AB] 0.06	[A|A] 0.90 [A|AB] 0.07
N147	[A|A] 0.72 [AB|A] 0.21 [AD|A] 0.06	[A|A] 0.70 [AB|A] 0.23 [AD|A] 0.06	[A|A] 0.68 [AB|A] 0.27	[A|A] 0.73 [AB|A] 0.21 [AD|A] 0.06	[A|A] 0.72 [AB|A] 0.26	[A|A] 0.73 [AB|A] 0.26	[A|A] 0.73 [AB|A] 0.26	[A|A] 0.72 [AB|A] 0.28	[A|A] 0.69 [AB|A] 0.30	[A|A] 0.73 [AB|A] 0.26	[A|A] 0.73 [AB|A] 0.27
N137	[A|B] 0.76 [A|D] 0.24	[A|B] 0.77 [A|D] 0.23	[A|B] 0.83 [A|D] 0.16	[A|B] 0.72 [A|D] 0.21 [A|A] 0.04	[A|B] 0.90 [A|D] 0.06	[A|B] 0.90 [A|A] 0.05	[A|B] 0.90 [A|A] 0.05	[A|B] 0.90 [A|A] 0.05	[A|B] 0.90 [A|A] 0.053	[A|B] 0.87 [A|A] 0.07 [AB|B] 0.04	[A|B] 0.89 [A|A] 0.06
N136	[B|B] 0.53 [BD|B] 0.44	[B|B] 0.53 [BD|B] 0.44	[B|B] 0.58 [BD|B] 0.39	[B|B] 0.55 [BD|B] 0.38 [AB|B] 0.02	[B|B] 0.67 [BD|B] 0.30	[B|B] 0.74 [BD|B] 0.22	[B|B] 0.74 [BD|B] 0.22	[B|B] 0.70 [BD|B] 0.26	[B|B] 0.70 [BD|B] 0.26	[B|B] 0.74 [BD|B] 0.21	[B|B] 0.74 [BD|B] 0.21
N98	[A|A] 0.88 [A|AB] 0.11	[A|AB] 0.50 [A|A] 0.49	[A|AB] 0.86 [A|A] 0.14	[A|A] 0.88 [A|AB] 0.11	[A|A] 0.88 [A|AB] 0.11	[A|A] 0.88 [A|AB] 0.12	[A|A] 0.88 [A|AB] 0.12	[A|A] 0.54 [A|AB] 0.45	[A|AB] 0.84 [A|A] 0.16	[A|A] 0.87 [A|AB] 0.11	[A|A] 0.88 [A|AB] 0.11
N74	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0	[A|A] 0.98	[A|A] 0.98	[A|A] 0.98	[A|A] 0.97	[A|A] 0.98	[A|A] 0.98
N69	[A|A] 0.74 [A|AC] 0.13 [AC|A] 0.11	[A|A] 0.79 [A|AC] 0.10 [AC|A] 0.09	[A|A] 0.57 [AC|A] 0.22 [A|AC] 0.15 [AD|A] 0.05	[A|A] 0.92 [AD|A] 0.04	[A|A] 0.54 [A|AC] 0.22 [AC|A] 0.18 [AD|A] 0.05	[A|A] 0.46 [AF|A] 0.32 [A|AF] 0.18	[A|A] 0.48 [AF|A] 0.29 [A|AF] 0.19	[A|A] 0.82 [A|AF] 0.13	[A|A] 0.69 [A|AF] 0.18 [AF|A] 0.09	[A|A] 0.77 [A|AF] 0.14 [AF|A] 0.06	[A|A] 0.48 [A|AF] 0.43 [AF|A] 0.03
N68	[A|A] 0.86 [A|AC] 0.14	[A|A] 0.89 [A|AC] 0.11	[A|A] 0.84 [A|AC] 0.16	[A|A] 0.97	[A|A] 0.77 [A|AC] 0.22	[A|A] 0.80 [A|AF] 0.17	[A|A] 0.80 [A|AF] 0.18	[A|A] 0.87 [A|AF] 0.12	[A|A] 0.81 [A|AF] 0.16	[A|A] 0.86 [A|AF] 0.12	[A|A] 0.56 [A|AF] 0.41
N67	[A|A] 0.82 [AC|A] 0.17	[A|A] 0.85 [AC|A] 0.14	[A|A] 0.81 [AC|A] 0.19	[A|A] 0.98	[A|A] 0.75 [AC|A] 0.25	[A|A] 0.79 [AF|A] 0.20	[A|A] 0.78 [AF|A] 0.21	[A|A] 0.85 [AF|A] 0.15	[A|A] 0.80 [AF|A] 0.19	[A|A] 0.88 [AF|A] 0.11	[A|A] 0.56 [AF|A] 0.43
N65	[A|A] 0.76 [A|AC] 0.24	[A|A] 0.79 [A|AC] 0.21	[A|A] 0.75 [A|AC] 0.25	[A|A] 0.94 [A|AC] 0.06	[A|A] 0.72 [A|AC] 0.28	[A|A] 0.73 [A|AF] 0.25	[A|A] 0.73 [A|AF] 0.26	[A|A] 0.79 [A|AF] 0.20	[A|A] 0.74 [A|AF] 0.24	[A|A] 0.85 [A|AF] 0.15	[A|A] 0.54 [A|AF] 0.45

N64	[A|C] 1.0	[A|C] 1.0	[A|C] 1.0	[A|C] 0.75 [A|A] 0.21	[A|C] 0.84 [A|A] 0.13	[F|A] 0.89 [A|A] 0.05 [F|AF] 0.03	[F|A] 0.89 [A|A] 0.05 [F|AF] 0.03	[F|A] 0.87 [A|A] 0.07 [F|AF] 0.04	[F|A] 0.87 [A|A] 0.06 [F|AF] 0.04	[F|A] 0.85 [A|A] 0.08 [F|AF] 0.04	[F|A] 0.88 [A|A] 0.04 [F|AF] 0.04
N63	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0	[A|A] 0.99	[A|A] 1.0	[A|A] 0.99	[A|A] 0.99	[A|A] 0.99	[A|A] 0.98	[A|A] 0.98	[A|A] 0.99
N52	[C|C] 1.0	[C|C] 1.0	[C|C] 1.0	[C|C] 0.93 [AC|C] 0.05	[C|C] 0.95	[C|C] 0.67 [CF|C] 0.25 [C|CF] 0.08	[C|C] 0.67 [CF|C] 0.25 [C|CF] 0.08	[C|C] 0.68 [CF|C] 0.24 [C|CF] 0.07	[C|C] 0.67 [CF|C] 0.25 [C|CF] 0.07	[C|C] 0.67 [CF|C] 0.24 [C|CF] 0.08	[C|C] 0.66 [CF|C] 0.25 [C|CF] 0.08
N37	[A|A] 0.84 [AC|A] 0.13	[A|A] 0.87 [AC|A] 0.11	[A|A] 0.65 [AC|A] 0.25 [AD|A] 0.10	[A|A] 0.93 [AD|A] 0.05	[A|A] 0.75 [AC|A] 0.18 [AD|A] 0.06	[A|A] 0.65 [AF|A] 0.32	[A|A] 0.68 [AF|A] 0.28	[A|A] 0.96	[A|A] 0.83 [AB|A] 0.08 [AF|A] 0.05	[A|A] 0.91 [AF|A] 0.04	[A|A] 0.93 [AB|A] 0.042
N35	[A|A] 0.74 [A|AC] 0.18 [A|AD] 0.07	[A|A] 0.76 [A|AC] 0.16 [A|AD] 0.08	[A|A] 0.42 [A|AC] 0.33 [A|AD] 0.24	[A|A] 0.81 [A|AD] 0.12 [A|AC] 0.06	[A|A] 0.67 [A|AC] 0.23 [A|AD] 0.10	[A|A] 0.58 [A|AF] 0.40	[A|A] 0.61 [A|AF] 0.36	[A|A] 0.91 [A|AB] 0.08	[A|A] 0.59 [A|AB] 0.32 [A|AF] 0.06	[A|A] 0.80 [A|AB] 0.12 [A|AF] 0.06	[A|A] 0.82 [A|AB] 0.15
N28	[C|C] 1.0	[C|C] 1.0	[C|C] 1.0	[C|C] 0.99	[C|C] 0.99	[C|C] 0.96	[C|C] 0.96	[C|C] 0.87 [CF|C] 0.08	[C|C] 0.92 [CF|C] 0.04	[C|C] 0.82 [CF|C] 0.11 [C|CF] 0.06	[C|C] 0.91 [CD|C] 0.03 [CF|C] 0.02
N23	[C|C] 1.0	[C|C] 1.0	[C|C] 1.0	[C|C] 1.0	[C|C] 1.0	[C|C] 0.99	[C|C] 0.99	[C|C] 0.97	[C|C] 0.98	[C|C] 0.96	[C|C] 0.98

Appendix S4-B. Ancestral areas and probabilities (P) estimated at the 31 nodes of interest in the experiments conducted with the artificial data set. The areas with the highest probability (P > 0.5) are shown on the top. The node numbers are shown in Fig. 2.

Node	MA0	MA1	MA2	MA3	MA4	MA5	MA6	MA7	MA8	MA9	MA10
N191	[C|C] 0.78 [C|CF] 0.13 [C|F] 0.03 [AC|C] 0.02	[C|C] 0.80 [C|CF] 0.12 [AC|C] 0.04	[C|C] 0.79 [C|CF] 0.12 [C|F] 0.04	[C|C] 0.78 [C|CF] 0.14 [AC|C] 0.04	[C|C] 0.69 [C|CF] 0.18 [AC|C] 0.06 [C|F] 0.03	[C|C] 0.51 [C|CF] 0.13 [CF|F] 0.12 [F|F] 0.10 [C|F] 0.06 [CF|C] 0.03	[C|C] 0.51 [C|CF] 0.13 [CF|F] 0.12 [F|F] 0.10 [C|F] 0.06 [CF|C] 0.03	[C|C] 0.48 [CF|F] 0.20 [F|F] 0.10 [C|CF] 0.09 [C|F] 0.05 [CF|C] 0.05	[C|C] 0.51 [CF|F] 0.14 [C|CF] 0.11 [F|F] 0.09 [C|F] 0.05 [CF|C] 0.04 [F|CF] 0.03	[C|C] 0.51 [C|CF] 0.12 [CF|F] 0.10 [F|F] 0.07 [CD|C] 0.07 [C|F] 0.04 [CF|C] 0.04	[C|C] 0.60 [C|CF] 0.23 [C|F] 0.06 [CD|C] 0.05 [CF|F] 0.02
N183	[C|C] 0.75 [AC|C] 0.12 [CE|C] 0.06 [CD|C] 0.06	[C|C] 0.76 [AC|C] 0.23	[C|C] 0.76 [AC|C] 0.13 [CD|C] 0.08	[C|C] 0.87 [AC|C] 0.11	[C|C] 0.83 [AC|C] 0.10 [CE|C] 0.04	[C|C] 0.55 [CF|C] 0.18 [CD|C] 0.11 [F|C] 0.07 [F|F] 0.05	[C|C] 0.55 [CF|C] 0.18 [CD|C] 0.11 [F|C] 0.06 [F|F] 0.05	[C|C] 0.50 [CF|C] 0.40 [F|C] 0.05 [F|F] 0.03	[C|C] 0.51 [CF|C] 0.24 [F|C] 0.08 [CD|C] 0.06 [F|F] 0.04 [F|CF] 0.02	[C|C] 0.60 [CF|C] 0.11 [CD|C] 0.09 [F|F] 0.08 [F|C] 0.06	[C|C] 0.81 [CD|C] 0.11 [CF|C] 0.02
N181	[C|A] 0.47 [C|E] 0.25 [C|D] 0.24	[C|A] 0.98	[C|A] 0.53 [C|D] 0.33 [C|E] 0.12	[C|A] 0.90 [AC|A] 0.04 [C|D] 0.03	[C|A] 0.55 [C|E] 0.16 [C|D] 0.15 [C|C] 0.06 [C|AC] 0.04	[C|D] 0.40 [C|F] 0.31 [CD|D] 0.06 [F|A] 0.06 [CF|F] 0.05 [F|E] 0.04 [F|F] 0.02 [C|CF] 0.02	[C|D] 0.41 [C|F] 0.31 [CD|D] 0.06 [F|A] 0.06 [CF|F] 0.05 [F|E] 0.04 [F|F] 0.02 [C|CF] 0.02	[C|F] 0.78 [CF|F] 0.12 [F|F] 0.04	[C|F] 0.46 [C|D] 0.24 [CF|F] 0.08 [F|E] 0.05 [CD|D] 0.04 [F|A] 0.04 [F|F] 0.03 [C|CF] 0.01	[C|F] 0.41 [C|D] 0.36 [F|E] 0.14 [CF|F] 0.04 [CD|D] 0.03	[C|D] 0.82 [CD|D] 0.06 [C|F] 0.06 [F|E] 0.02
N180	[A|E] 0.43 [A|D] 0.42 [A|A] 0.05 [E|E] 0.03 [D|D] 0.03	[A|D] 0.97	[A|D] 0.53 [A|E] 0.37 [D|D] 0.06	[A|A] 0.60 [A|E] 0.17 [A|D] 0.13 [D|D] 0.03 [AE|E] 0.03	[A|E] 0.33 [A|D] 0.29 [A|A] 0.11 [A|C] 0.08 [D|D] 0.06 [E|E] 0.05 [D|DE] 0.01 [A|AE] 0.01 [E|DE] 0.01	[E|DE] 0.37 [A|F] 0.14 [F|F] 0.13 [F|E] 0.08 [E|D] 0.04 [B|D] 0.04 [A|E] 0.04 [A|A] 0.04 [F|C] 0.03 [E|E] 0.02 [AF|A] 0.01	[E|DE] 0.38 [A|F] 0.14 [F|F] 0.13 [F|E] 0.08 [E|D] 0.04 [B|D] 0.04 [A|E] 0.04 [A|A] 0.04 [F|C] 0.03 [E|E] 0.02 [AF|A] 0.01	[A|F] 0.39 [F|F] 0.34 [F|E] 0.20 [AF|F] 0.02	[A|F] 0.20 [F|F] 0.20 [E|DE] 0.18 [F|E] 0.12 [B|D] 0.07 [A|E] 0.04 [E|E] 0.03 [A|A] 0.03 [E|D] 0.02 [F|C] 0.01 [AF|F] 0.01 [A|AF] 0.01 [D|D] 0.01	[F|E] 0.34 [B|D] 0.23 [E|DE] 0.13 [E|E] 0.08 [A|E] 0.07 [A|F] 0.04 [B|BD] 0.02 [F|F] 0.02 [E|EF] 0.01 [EF|E] 0.01	[E|DE] 0.85 [F|E] 0.03 [B|D] 0.03 [A|F] 0.02 [A|E] 0.02
N179	[D|E] 0.96	[D|E] 1.0	[D|E] 1.0	[D|E] 0.30 [AD|D] 0.19 [D|D] 0.12 [A|E] 0.11 [E|E] 0.09 [D|A] 0.05 [D|AD] 0.04 [A|A] 0.04	[D|E] 0.67 [A|E] 0.07 [C|E] 0.06 [E|E] 0.06 [D|A] 0.04 [D|D] 0.02 [DE|E] 0.02 [D|C] 0.01	[D|E] 0.78 [E|E] 0.09 [DE|E] 0.05 [D|DE] 0.04	[D|E] 0.78 [E|E] 0.09 [DE|E] 0.053 [D|DE] 0.04	[D|E] 0.76 [E|E] 0.12 [D|DE] 0.04 [DE|E] 0.04	[D|E] 0.80 [E|E] 0.07 [D|DE] 0.05 [DE|E] 0.04	[D|E] 0.42 [E|E] 0.32 [D|D] 0.11 [E|EF] 0.04 [DE|E] 0.04 [D|DE] 0.03	[D|E] 0.82 [DE|E] 0.07 [D|DE] 0.04 [E|E] 0.03

				[DE|E] 0.01							
N178	[E|E] 0.99	[E|E] 1.0	[E|E] 1.0	[E|E] 0.79 [E|DE] 0.10 [DE|E] 0.05	[E|E] 0.95	[E|E] 0.98	[E|E] 0.98	[E|E] 0.97	[E|E] 0.97	[E|E] 0.91 [E|DE] 0.04	[E|E] 0.97
N173	[D|D] 0.99	[D|D] 1.0	[D|D] 1.0	[D|D] 0.94 [D|AD] 0.02	[D|D] 0.96	[D|D] 0.98	[D|D] 0.98	[D|D] 0.96	[D|D] 0.97	[D|D] 0.94 [D|DE] 0.03	[D|D] 0.98
N166	[A|A] 0.92 [A|AE] 0.02	[A|A] 0.99	[A|A] 0.90 [A|AD] 0.07	[A|A] 0.94 [A|AE] 0.03	[A|A] 0.86 [A|AD] 0.05 [A|AE] 0.04	[A|A] 0.45 [AE|A] 0.22 [AF|A] 0.12 [A|AE] 0.08 [A|AF] 0.05 [AB|A] 0.02	[A|A] 0.45 [AE|A] 0.22 [AF|A] 0.12 [A|AE] 0.08 [A|AF] 0.05 [AB|A] 0.02	[A|A] 0.59 [AF|A] 0.29 [A|AF] 0.09	[A|A] 0.48 [AF|A] 0.19 [AE|A] 0.13 [A|AF] 0.06 [A|AE] 0.04 [AB|A] 0.04 [A|AB] 0.02	[A|A] 0.35 [A|AE] 0.17 [AF|A] 0.15 [AB|A] 0.10 [A|AF] 0.09 [A|AB] 0.07 [AE|A] 0.04	[A|A] 0.31 [AE|A] 0.30 [A|AE] 0.30 [AF|A] 0.02 [AB|A] 0.01
N165	[A|A] 0.94 [AE|A] 0.02	[A|A] 0.99	[A|A] 0.90 [AD|A] 0.07	[A|A] 0.96	[A|A] 0.91 [AD|A] 0.04 [AE|A] 0.04	[A|A] 0.92 [AF|A] 0.03 [AE|A] 0.03	[A|A] 0.92 [AE|A] 0.03	[A|A] 0.96	[A|A] 0.93 [AE|A] 0.02 [AF|A] 0.01	[A|A] 0.78 [AE|A] 0.15 [AB|A] 0.02	[A|A] 0.80 [AE|A] 0.16
N164	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0	[A|A] 0.98	[A|A] 0.98	[A|A] 0.99	[A|A] 0.98	[A|A] 0.98	[A|A] 0.98
N163	[A|A] 0.99	[A|A] 0.99	[A|A] 0.99	[A|A] 0.99	[A|A] 0.99	[A|A] 0.99	[A|A] 0.99	[A|A] 0.99	[A|A] 0.98	[A|A] 0.99	[A|A] 0.99
N162	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0
N160	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0
N149	[A|A] 0.99	[A|A] 0.98	[A|A] 0.97	[A|A] 0.99	[A|A] 0.98	[A|A] 0.99	[A|A] 0.99	[A|A] 0.98	[A|A] 0.96	[A|A] 0.98	[A|A] 0.98
N148	[A|A] 0.91 [A|AB] 0.05	[A|A] 0.83 [AB|A] 0.07 [A|AB] 0.04	[A|A] 0.75 [AB|A] 0.13 [A|AB] 0.04 [B|AB] 0.04	[A|A] 0.91 [A|AB] 0.05	[A|A] 0.90 [A|AB] 0.06	[A|A] 0.91 [A|AB] 0.06	[A|A] 0.91 [A|AB] 0.06	[A|A] 0.84 [AB|A] 0.07 [A|AB] 0.05	[A|A] 0.75 [AB|A] 0.13 [A|AB] 0.05 [B|AB] 0.04	[A|A] 0.91 [A|AB] 0.06	[A|A] 0.91 [A|AB] 0.06
N147	[A|A] 0.72 [AB|A] 0.21 [AD|A] 0.06	[A|A] 0.70 [AB|A] 0.23 [AD|A] 0.06	[A|A] 0.68 [AB|A] 0.27	[A|A] 0.73 [AB|A] 0.21 [AD|A] 0.06	[A|A] 0.72 [AB|A] 0.26	[A|A] 0.73 [AB|A] 0.27	[A|A] 0.73 [AB|A] 0.27	[A|A] 0.71 [AB|A] 0.28	[A|A] 0.69 [AB|A] 0.31	[A|A] 0.73 [AB|A] 0.26	[A|A] 0.73 [AB|A] 0.27
N137	[A|B] 0.76 [A|D] 0.24	[A|B] 0.77 [A|D] 0.23	[A|B] 0.83 [A|D] 0.16	[A|B] 0.72 [A|D] 0.21 [A|A] 0.04	[A|B] 0.91 [A|D] 0.06	[A|B] 0.91 [A|A] 0.05	[A|B] 0.91 [A|A] 0.05	[A|B] 0.91 [A|A] 0.04	[A|B] 0.91 [A|A] 0.04	[A|B] 0.89 [A|A] 0.06	[A|B] 0.91 [A|A] 0.05
N136	[B|B] 0.53 [BD|B] 0.44	[B|B] 0.53 [BD|B] 0.44	[B|B] 0.58 [BD|B] 0.39	[B|B] 0.55 [BD|B] 0.38 [AB|B] 0.02	[B|B] 0.67 [BD|B] 0.30	[B|B] 0.74 [BD|B] 0.23	[B|B] 0.74 [BD|B] 0.23	[B|B] 0.70 [BD|B] 0.27	[B|B] 0.70 [BD|B] 0.27	[B|B] 0.74 [BD|B] 0.22	[B|B] 0.73 [BD|B] 0.22
N98	[A|A] 0.88 [A|AB] 0.11	[A|AB] 0.50 [A|A] 0.49	[A|AB] 0.86 [A|A] 0.14	[A|A] 0.88 [A|AB] 0.11	[A|A] 0.88 [A|AB] 0.11	[A|A] 0.88 [A|AB] 0.11	[A|A] 0.88 [A|AB] 0.11	[A|A] 0.53 [A|AB] 0.46	[A|AB] 0.84 [A|A] 0.16	[A|A] 0.88 [A|AB] 0.11	[A|A] 0.88 [A|AB] 0.11
N74	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0	[A|A] 0.99	[A|A] 0.99	[A|A] 0.99	[A|A] 0.99	[A|A] 0.99	[A|A] 0.99
N69	[A|A] 0.94 [A|AE] 0.02	[A|A] 0.98	[A|A] 0.90 [AD|A] 0.07	[A|A] 0.96	[A|A] 0.90 [AD|A] 0.04 [A|AE] 0.04	[A|A] 0.95 [AF|A] 0.02	[A|A] 0.95 [AF|A] 0.02	[A|A] 0.98	[A|A] 0.95	[A|A] 0.82 [A|AE] 0.14	[A|A] 0.84 [A|AE] 0.14
N68	[A|A] 0.97	[A|A] 0.99	[A|A] 0.98	[A|A] 0.97	[A|A] 0.94 [A|AE] 0.06	[A|A] 0.97	[A|A] 0.97	[A|A] 0.99	[A|A] 0.98	[A|A] 0.85 [A|AE] 0.14	[A|A] 0.84 [A|AE] 0.14
N67	[A|A] 0.93	[A|A] 0.95	[A|A] 0.94	[A|A] 0.94	[A|A] 0.80	[A|A] 0.95	[A|A] 0.95	[A|A] 0.96	[A|A] 0.96	[A|A] 0.83	[A|A] 0.75

	[AE|A] 0.06		[AE|A] 0.05	[AE|A] 0.06	[AE|A] 0.20					[AE|A] 0.16	[AE|A] 0.25
N65	[A|A] 0.86 [A|AE] 0.13	[A|A] 0.88 [A|AE] 0.12	[A|A] 0.87 [A|AE] 0.12	[A|A] 0.90 [A|AE] 0.09	[A|A] 0.77 [A|AE] 0.23	[A|A] 0.90 [A|AE] 0.09	[A|A] 0.90 [A|AE] 0.09	[A|A] 0.92 [A|AE] 0.07	[A|A] 0.91 [A|AE] 0.08	[A|A] 0.80 [A|AE] 0.18	[A|A] 0.73 [A|AE] 0.27
N64	[E|A] 0.98	[E|A] 1.0	[E|A] 1.0	[E|A] 0.69 [A|A] 0.18 [D|A] 0.04 [B|A] 0.04 [F|A] 0.02	[E|A] 0.85 [A|A] 0.12	[E|A] 0.64 [A|A] 0.3 [AE|A] 0.03	[E|A] 0.64 [A|A] 0.30 [AE|A] 0.03	[E|A] 0.53 [A|A] 0.30 [B|A] 0.07 [F|A] 0.04 [AE|A] 0.03	[E|A] 0.61 [A|A] 0.32 [AE|A] 0.03	[E|A] 0.50 [A|A] 0.26 [B|A] 0.11 [F|A] 0.05 [AE|A] 0.03	[E|A] 0.69 [A|A] 0.25 [AE|A] 0.03
N63	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0	[A|A] 0.99	[A|A] 1.0	[A|A] 0.99	[A|A] 0.99	[A|A] 0.99	[A|A] 0.99	[A|A] 0.99	[A|A] 0.99
N52	[E|E] 0.99	[E|E] 1.0	[E|E] 1.0	[E|E] 0.90 [AE|E] 0.05 [E|AE] 0.01	[E|E] 0.96	[E|E] 0.89 [AE|E] 0.08	[E|E] 0.89 [AE|E] 0.08	[E|E] 0.85 [AE|E] 0.08 [E|AE] 0.02	[E|E] 0.88 [AE|E] 0.09	[E|E] 0.83 [AE|E] 0.07 [BE|E] 0.03 [E|AE] 0.02	[E|E] 0.91 [AE|E] 0.07
N37	[A|A] 0.93 [AD|A] 0.04	[A|A] 0.96	[A|A] 0.82 [AD|A] 0.13	[A|A] 0.98	[A|A] 0.93 [AD|A] 0.05	[A|A] 0.93 [AF|A] 0.06	[A|A] 0.93 [AF|A] 0.05	[A|A] 0.97	[A|A] 0.88 [AB|A] 0.09	[A|A] 0.95	[A|A] 0.97
N35	[A|A] 0.82 [A|AD] 0.09 [A|AC] 0.09	[A|A] 0.84 [A|AD] 0.08 [A|AC] 0.08	[A|A] 0.54 [A|AD] 0.32 [A|AC] 0.14	[A|A] 0.86 [A|AD] 0.09	[A|A] 0.82 [A|AD] 0.10 [A|AC] 0.07	[A|A] 0.82 [A|AF] 0.17	[A|A] 0.82 [A|AF] 0.14	[A|A] 0.91 [A|AB] 0.08	[A|A] 0.62 [A|AB] 0.33 [A|AF] 0.02	[A|A] 0.84 [A|AB] 0.12	[A|A] 0.85 [A|AB] 0.13
N28	[A|A] 1.0	[A|A] 1.0	[A|A] 1.0	[A|A] 0.99	[A|A] 0.99	[A|A] 0.93 [AE|A] 0.02	[A|A] 0.93 [AE|A] 0.02	[A|A] 0.95 [AF|A] 0.03	[A|A] 0.93 [AF|A] 0.02	[A|A] 0.91 [AF|A] 0.03 [AB|A] 0.02	[A|A] 0.91 [AE|A] 0.05
N23	[C|C] 1.0	[C|C] 1.0	[C|C] 1.0	[C|C] 0.99	[C|C] 0.99	[C|C] 0.94 [CF|C] 0.02	[C|C] 0.94 [CF|C] 0.02	[C|C] 0.96	[C|C] 0.94 [CF|C] 0.02	[C|C] 0.91 [CF|C] 0.04	[C|C] 0.96

Appendix S5. Number of alternative ancestral areas obtained in the Lagrange experiments for the problematic nodes of the artificial data set.

	# Alternative ancestral areas			
Experiment	N181	N180	N98	**Total # alternative areas inferred per experiment**
MA0	3	5	2	**10**
MA1	1	1	2	**4**
MA2	3	3	2	**8**
MA3	3	5	2	**10**
MA4	5	9	2	**16**
MA5	8	11	2	**21**
MA6	8	11	2	**21**
MA7	3	4	2	**9**
MA8	8	13	2	**23**
MA9	5	10	2	**17**
MA10	4	5	2	**11**
Total # of alternative areas inferred per node	**51**	**77**	**22**	

Chapter 5

Ribosomal DNA distribution and a genus-wide phylogeny reveal patterns of chromosomal evolution in *Alstroemeria* (Alstroemeriaceae)

Juliana Chacón, Aretuza Sousa, Carlos M. Baeza,

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Ribosomal DNA distribution and a genus-wide phylogeny reveal patterns of chromosomal evolution in *Alstroemeria* (Alstroemeriaceae)[1]

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- *Premise of the study:* Understanding the flexibility of monocot genomes requires a phylogenetic framework, which so far is available for few of the ca. 2800 genera. Here we use a molecular tree for the South American genus *Alstroemeria* to place karyological information, including fluorescent in situ hybridization (FISH) signals, in an explicit evolutionary context.
- *Methods:* From a phylogeny based on plastid, nuclear, and mitochondrial sequences for most species of *Alstroemeria*, we selected early-branching (Chilean) and derived (Brazilian) species for which we obtained 18S-25S and 5S rDNA FISH signals; we also analyzed chromosome numbers, 1C-values, and telomere FISH signals (in two species).
- *Key results:* Chromosome counts for *Alstroemeria* cf. *rupestris* and *A. pulchella* confirm $2n = 16$ as typical of the genus, which now has chromosomes counted for 29 of its 78 species. The rDNA sites are polymorphic both among and within species, and interstitial telomeric sites in *Alstroemeria* cf. *rupestris* suggest chromosome fusion.
- *Conclusions:* In spite of a constant chromosome number, closely related species of *Alstroemeria* differ drastically in their rDNA, indicating rapid increase, decrease, or translocations of these genes. Previously proposed Brazilian and Chilean karyotype groups are not natural, and the $n = 8$ chromosomes in *Alstroemeria* compared to $n = 9$ in its sister genus *Bomarea* may result from a Robertsonian fusion.

Key words: Chilean *Alstroemeria*; Alstroemeriaceae; FISH; 18S-25S rDNA; 5S rDNA; interstitial telomeric sequences; primary chromosomal rearrangements.

Several genomic features are distinctive in monocots compared to dicots, including greater genome size variation and greater flexibility in how DNA is organized into chromosomes (Leitch et al., 2010). A review of monocot genome characteristics based on data for 534 of the ca. 2800 genera revealed that Liliales have a wide range of ploidy levels (up to $22x$) and that they rarely have small chromosomes and small genomes (Leitch et al., 2010). Cytogenetic data for the Liliales, however, are sparse and uneven, and very few clades have been analyzed in a phylogenetic context (e.g., Leitch et al., 2007: Liliaceae).

Among the Liliales families that have fascinated cytogeneticists for a long time are the Alstroemeriaceae, which consist of the neotropical genera *Bomarea*, with 120 species, and *Alstroemeria* with 78; the disjunctly distributed *Luzuriaga*, with three species in Chile and one in New Zealand; and *Drymophila*, with one species in Australia and one in Tasmania. Strasburger (1882) studied male meiosis in *A. chilensis*, with $n = 8$, a number since reported for all 27 species of *Alstroemeria* whose chromosomes have been counted (Appendix S1, see Supplemental Data with the online version of this article). Karyotypes in *Alstroemeria* are asymmetric and bimodal (ca. 15 species have been investigated; Stephens et al., 1993; Buitendijk and Ramanna, 1996; Kamstra et al., 1997; Sanso and Hunziker, 1998; Sanso, 2002; Jara-Seguel et al., 2004; Baeza et al., 2006; Baeza et al., 2010). The karyotypes of the few species of *Bomarea*, *Drymophila*, and *Luzuriaga* that have been studied also are asymmetric and bimodal (Jara-Seguel et al., 2005, 2010; Baeza et al., 2008). All nine *Bomarea* species counted have $n = 9$, while *Luzuriaga* and *Drymophila* species have $n = 10$ (Appendix S1). A summary of the karyotype characteristics of the four genera is shown in Fig. 1.

In spite of the apparently invariable chromosome number, studies using molecular-cytogenetic techniques suggest a dynamic picture of chromosome restructuring in *Alstroemeria*. For example, fluorescence in situ hybridization (FISH) analyses in seven Chilean and Brazilian species revealed high levels of polymorphism in the ribosomal DNA (rDNA) signals of presumed homologous chromosomes (Kamstra et al., 1997; Kuipers et al., 2002; Baeza et al., 2007). Likewise, C-banding and measurements of nuclear DNA content (2C value), PI/DAPI indices, and chromosome arm lengths in 12 Brazilian and Chilean species (five of them the same as studied with FISH) showed large differences in these parameters (Buitendijk and Ramanna, 1996; Buitendijk et al., 1997; Kuipers et al., 2002; the PI/DAPI index reflects differences in the AT/GC ratio: Barow and Meister, 2002).

The aim of the current study is to infer directions of chromosomal evolution in *Alstroemeria* by studying rDNA FISH data in the light of a phylogeny. Specifically, we wanted to test whether Chilean and Brazilian "karyotype species groups" distinguished in earlier studies (Buitendijk et al., 1997; Jara-Seguel et al., 2004) reflect evolutionary homology or are the result

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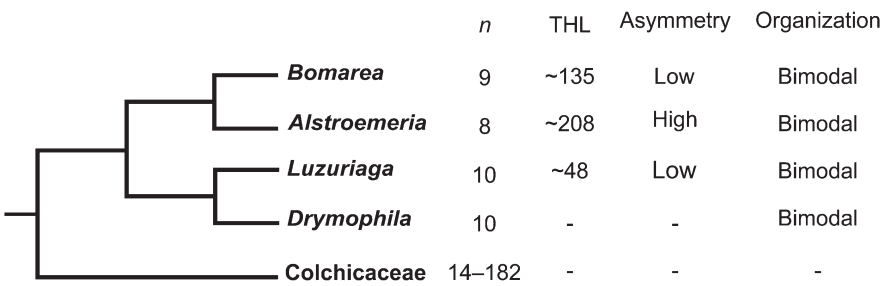

Fig. 1. Molecular phylogeny of the Alstroemeriaceae (simplified from Chacón et al., 2012) showing cytogenetic characteristics, such as the haploid chromosome number (*n*), the total haploid length of all chromosomes (THL in µm), the level of karyotype asymmetry, and karyotype morphology (bimodal = karyotypes comprising two size classes). Information was taken from Conran (1987); Sanso and Hunziker (1998); Sanso (2002); Baeza et al. (2007, 2008), Palma-Rojas et al. (2007); and Jara-Seguel et al. (2010).

of parallel evolution. A division into eastern and western karyotype groups might be inferred from the presence of *Alstroemeria* on both sides of Andes—44 of its 78 species occur in Brazil, 34 in Chile. Starting with a family-wide phylogeny (Chacón et al., 2012), we selected a subset of early-branching and derived *Alstroemeria* species for which FISH data were available (Baeza et al., 2007), and we then undertook additional FISH studies to study ribosomal DNA changes across the genus.

Changes in rDNA can serve to individually characterize chromosomes and to compare them between populations, species, or clades, an approach widely used since the introduction of fluorescence in situ hybridization (Pinkel et al., 1986). Variation in the number and distribution of FISH signals indicates genome reorganization (Hasterok et al., 2006; Heslop-Harrison and Schwarzacher, 2011), and when rDNA variation is analyzed in a phylogenetic context, the direction of karyotypic change can be inferred. Many studies on flowering plants have established the power of the method (Adams et al., 2000: *Aloe*; Ran et al., 2001: *Clivia*; Shan et al., 2003: *Boronia*; Weiss-Schneeweiss et al., 2008: *Hypochaeris*; Garcia et al., 2007: *Artemisia*; Martínez et al., 2010: *Iris*; Fukushima et al., 2011: *Byblis*; Lan and Albert, 2011: *Paphiopedilum*; Catalán et al., 2012: *Brachypodium distachyon*).

MATERIALS AND METHODS

***Taxon sampling*—**For this study, we augmented and modified a phylogeny of *Alstroemeria* so that 16 of the 34 species occurring in Chile and adjacent countries were included, while species from other parts of South America not relevant in the present context were less densely sampled. Three species of *Bomarea* were used as outgroups based on Chacón et al. (2012). All sequenced plant materials with species names and their authors, geographic origin of the sample, herbarium voucher specimen, and GenBank accession numbers are listed in the Table 1, which also gives the geographic origin of the plants used in the FISH analyses. For *A. aurea* and *A. ligtu*, plants from different populations roughly 10–15 km apart were sampled to assess within-species variability. *Alstroemeria aurea* is polymorphic in flower color, which can vary from yellow to red with both colors sometimes in the same inflorescence, and this polymorphism was represented in the sample. *Alstroemeria aurea*, *A. ligtu*, and *A. hookeri* are common in the Biobío region, where the latter grows on the coast and *A. ligtu* in the interior valleys.

***Chromosome preparation*—**Mitotic metaphase chromosomes were prepared from meristematic tissue obtained from root tips. The samples were pretreated in 0.1% colchicine (w/v water) for 3 h at room temperature, fixed in freshly prepared 3:1 (v/v) ethanol–glacial acetic acid at room temperature overnight, and kept at –20°C in this solution. For chromosome preparations, fixed root tips were washed in distilled water, digested with 1% (w/v) cellulase Onozuka-RS (Serva, Heidelberg, Germany), 0.4% (w/v) pectolyase (Sigma-Aldrich, St. Louis, Missouri, USA), and 0.4% (w/v) cytohelicase (Sigma-Aldrich, Missouri, USA) in citric buffer (10 mmol/L, pH 4.8) for 50 min at 37°C. The meristems were dissected and squashed in a drop of 45% acetic acid. Coverslips were removed after freezing in dry ice, and preparations were then air-dried at room temperature. The best slides were selected using phase-contrast microscopy and stored at 20°C prior to fluorescence in situ hybridization (FISH) experiments.

***DNA probes and FISH*—**The following probes were used in the FISH experiments: The 18S-5.8S-25S rDNA unit from *Arabidopsis thaliana* in plasmid pBSK+, labeled with digoxigenin-11-dUTP (Roche Diagnostics, Basel, Switzerland) using a nick translation mix; and the 349-bp fragment of the 5S rRNA gene from *Beta vulgaris* was inserted into pBSK+ (Schmidt et al., 1994), labeled with biotin-16-dUTP (Roche Diagnostics, Basel, Switzerland) using PCR. Additionally, an *Arabidopsis*-like telomeric probe was amplified by PCR according to Ijdo et al. (1991) using the oligomer primers (5′-TTTAGGG-3′)5 and (5′-CCCTAAA-3′)5 and labeled with digoxigenin-11-dUTP using nick translation.

Chromosome and probe denaturation, posthybridization washes, and detection were performed using the methods of Sousa et al. (in press). The hybridization mixtures consisted of 50% (w/v water) formamide, 2× saline sodium citrate (SSC), 10% (w/v) dextran sulfate, and 100–200 ng of labeled probe. The hybridization mix was denatured at 75°C for 10 min and cooled for 10 min on ice. The slides and hybridization mix were denatured for 5 min at 75°C and hybridized for up to 20 h at 37°C. For digoxigenin and biotin detection, slides were incubated in blocking buffer (2% BSA in 2× SSC) for 30 min at 37°C, followed by incubation (1 h, 37°C) with either antiDIG-FITC conjugate (Roche Diagnostics) to detect digoxigenin or streptavidin-Cy3 conjugate (Sigma-Aldrich) to detect biotin. Excess of antibody was removed by washing the slides twice for 7 min in 2× SSC and for 7 min in 2× SSC/0.1% (v/v) Tween 20 at 42°C. Chromosomes were counterstained with diamidino-2-phenylindol (DAPI, 2 µg/mL) and mounted in Vectashield (Vector Laboratories, Burlingame, California, USA). Images were taken with a Leica DMR microscope equipped with a KAPPA-CCD camera and the KAPPA software. For rDNA analyses, a minimum of 10 well-spread metaphases were analyzed for each species. The images were optimized for best contrast and brightness using software Adobe Photoshop CS3 version 10.0 (Adobe Systems, Washington, USA).

***DNA extraction, amplification, and sequencing*—**Total DNA was extracted from ca. 0.3 g of dried leaf tissue using standard methods and the primers referenced in Chacón et al. (2012). Sequencing was performed using BigDye (Applied Biosystems, Warrington, UK) and an ABI 3100 Avant capillary sequencer. The ITS region always yielded single bands and unambiguous base calls, and we therefore refrained from cloning. Sequence assembly of forward and reverse strands was carried out with the program Sequencher (Gene Codes, Ann Arbor, Michigan, USA), and aligned with the program MAFFT v. 6 (Katoh et al., 2002) using the L-INS-i algorithm (Katoh et al., 2005) followed by manual adjustment in the program MacClade v. 4.8 (Maddison and Maddison, 2002) based on the similarity criterion of Simmons (2004). All sequences were BLAST-searched in GenBank.

TABLE 1. Species included in this study, with voucher information, geographic origin, and GenBank accession numbers. The specimens used for the chromosome analyses are in bold font.

Species name	Voucher	Geographic origin	GenBank accession numbers				
			ndhF	*rbcL*	*matK*	*matR*	ITS
Alstroemeria aurea Graham	DNA sample L. Aagesen C81, source plant: L. Aagesen s.n. (BAA)	Argentina, Chubut province, Minas	JQ404511	AY120359	JQ404771	JQ404895	JQ405005
***Alstroemeria aurea* Graham**	**C. Baeza 4193 (CONC)**	**Chile, Biobío region, Ñuble**					
***Alstroemeria aurea* Graham**	**C. Baeza 4201 (CONC)**	**Chile, Biobío region, Biobío**					
***Alstroemeria aurea* Graham**	**C. Baeza 4202 (CONC)**	**Chile, Biobío region, Biobío**					
***Alstroemeria aurea* Graham**	**K. Tremetsberger 1090 (W)**	**Chile, Araucanía region, Cautín**					
Alstroemeria brasiliensis Spreng.	T. B. Cavalcanti et al., 2226 (SPF)	Brazil, Tocantins	JQ404512		JQ404773		JQ405007
Alstroemeria caryophyllaea Jacq.	A. F. C. Tombolato 2 (IAC)	Brazil, Sao Paulo	JQ404516	JQ404665	JQ404774	JQ404897	JQ405008
Alstroemeria crispata Phil.	K. H. and W. Rechinger 63671 (M)	Chile, Coquimbo region, Elqui	JQ404517	JQ404666	JQ404775	JQ404898	JQ405009
Alstroemeria cunha Vell.	A. Meerow and A. F. C. Tombolato 2103 (NA)	Brazil, Rio de Janeiro, Itatiaia	JQ404518	JQ404667	JQ404776	JQ404899	JQ405010
Alstroemeria foliosa Mart.	M. C. Assis 639 (UEC)	Brazil, Rio de Janeiro, Itatiaia	JQ404524	JQ404672	JQ404779	JQ404903	JQ405014
Alstroemeria hookeri Lodd. subsp. *cummingiana* Ehr. Bayer	DNA sample L. Aagesen C448, source plant: Cultivated plant P1995-5010 (C)	Chile, Coquimbo region	JQ404528	JQ404674	JQ404782	JQ404904	
Alstroemeria hookeri* Lodd. subsp. *hookeri	**C. Baeza 4181 (CONC)**	**Chile, Biobío region, Concepción**					
Alstroemeria inodora Herb.	A. Meerow 2207 (NA)	Brazil, Mato Grosso do Sul	JQ404567	JQ404697	JQ404810	JQ404931	JQ405047
Alstroemeria isabelleana Herb.	A. F. C. Tombolato and A. Meerow 501 (NA)	Brazil, Santa Catarina	JQ404531	JQ404675	JQ404783	JQ404905	JQ405018
Alstroemeria kingii Phil.	M. Gomez 211 (CONC)	Chile, Atacama region	JQ404535	JQ404678	JQ404787	JQ404908	JQ405021
Alstroemeria ligtu L. subsp. *simsii* Ehr. Bayer	CONC 166179 (CONC)	Chile, Santiago Metropolitan region	JQ404536	JQ404679	JQ404788	JQ404909	JQ405022
Alstroemeria ligtu* L. subsp. *ligtu	**C. Baeza 4178 (CONC)**	**Chile, Biobío region, Concepción**					
Alstroemeria ligtu* L. subsp. *ligtu	**C. Baeza 4179 (CONC)**	**Chile, Biobío region, Concepción**					
Alstroemeria ligtu* L. subsp. *ligtu	**C. Baeza 4180 (CONC)**	**Chile, Biobío region, Concepción**					
Alstroemeria ligtu* L. subsp. *ligtu	**C. Baeza 4184 (CONC)**	**Chile, Biobío region, Concepción**					
Alstroemeria ligtu* L. subsp. *ligtu	**C. Baeza 4185 (CONC)**	**Chile, Biobío region, Concepción**					
Alstroemeria longistaminea Mart.	A. Meerow 2204 (NA)	Brazil, Bahia	JQ404537	JQ404680	JQ404789	JQ404910	JQ405023
Alstroemeria magnifica Herb. subsp. *magnifica*	DNA sample L. Aagesen C449, source plant: Cultivated plant P1995-5031 (C)	Chile, Valparaíso	JQ404540	JQ404682	JQ404791	JQ404912	JQ405025
Alstroemeria ochracea M. C. Assis	A. Meerow 2206 (NA)	Brazil, Minas Gerais	JQ404544	JQ404684	JQ404792	JQ404913	JQ405028
Alstroemeria orchidioides Meerow, Tombolato & F. K. Mey.	A. Meerow 2201 (FLAS)	Brazil, Goiás	JQ404545	JQ404685	JQ404793	JQ404914	JQ405029
Alstroemeria patagonica Phil.	DNA sample L. Aagesen C82, source plant: L. Aagesen s. n. (BAA)	Argentina, Neuquén province, Catán-Lil	JQ404548	AY120362	JQ404796	JQ404917	JQ405032
Alstroemeria pelegrina L.	DNA sample L. Aagesen C437, source plant: Cultivated plant P1995-5037 (C)	Chile, IV Region	JQ404549	AY120363	JQ404797	JQ404918	
***Alstroemeria pelegrina* L.**	**INIA s.n. (INIA)**	**Chile, V Region, Playa Quintay**					
Alstroemeria philippii Baker subsp. *albicans* Muñoz-Schick	ULS 10251 (ULS)	Chile, IV Region, Isla Damas	JQ404551	JQ404688	JQ404798	JQ404919	JQ405033
Alstroemeria philippii Baker subsp. *philippii*	CONC 166170 (CONC)	Chile, III Region, Punta Lobos	JQ404552	JQ404689	JQ404799	JQ404920	JQ405034
Alstroemeria presliana Herb.	DNA sample L. Aagesen C80, source plant: L. Aagesen s. n. (BAA)	Argentina, Neuquén, Minas, Lagunas de Epulafquen	JQ404555	JQ404690	JQ404800	JQ404921	JQ405036
Alstroemeria presliana* Herb. subsp. *presliana	**C. Baeza 4192 (CONC)**	**Chile, VIII Region, Ñuble, Termas de Chillán**					

TABLE 1. Species included in this study, with voucher information, geographic origin, and GenBank accession numbers. The specimens used for the chromosome analyses are in bold font. Continued.

Species name	Voucher	Geographic origin	GenBank accession numbers				
			ndhF	*rbcL*	*matK*	*matR*	ITS
Alstroemeria pseudospathulata Ehr. Bayer	DNA sample L. Aagesen C89a, source plant: C. C. Xifreda and A. M. Sanso 2004 (SI)	Argentina, Neuquén, Chos-Malal	JQ404556	JQ404691	JQ404801	JQ404922	JQ405037
Alstroemeria psittacina Lehm.	DNA sample L. Aagesen C91a, source plant: Quesada s. n. (BA)	Argentina, Buenos Aires	JQ404557	AY120364	JQ404802	JQ404923	JQ405039
Alstroemeria pulchella L. f.	**J. Chacon 12 (MSB)**	**Brazil, cultivated at Munich Botanical Garden**	JX418005	JX418007	JX418009	JX418010	JX418012
Alstroemeria punctata Ravenna	J. B. Pereira et al., 176 (CEN)	Brazil, Goiás	JQ404558	JQ404692	JQ404803	JQ404924	JQ405040
Alstroemeria pygmaea Herb.	DNA sample L. Aagesen C79b, source plant: L. Aagesen s. n. (BAA)	Argentina, Tucumán, Trancas	JQ404559	AY120365	JQ404804	JQ404925	JQ405041
Alstroemeria radula Dusén	A. Meerow and A. F. C. Tombolato 2101 (NA)	Brazil, Rio de Janeiro, Itatiaia	JQ404560		JQ404805	JQ404926	JQ405042
Alstroemeria revoluta Ruiz & Pav.	DNA sample L. Aagesen C434, source plant: Cultivated plant P1995-5050 (C)	Chile, VII Region, Pte. Loncomilla	JQ404561	JQ404693	JQ404806	JQ404927	JQ405043
Alstroemeria rupestris M. C. Assis	M. C. Assis 635 (UEC)	Brazil, Minas Gerais	JQ404562	JQ404694	JQ404807	JQ404928	JQ405044
***Alstroemeria* cf. *rupestris* Jacq.**	**J. Chacon 11 (MSB)**	**Brazil, cultivated at Munich Botanical Garden**	JX418004	JX418006	JX418008		JX418011
Alstroemeria schizanthoides Grau	CONC 166190 (CONC)	Chile, III Region, Embalse Santa Juana	JQ404563	JQ404695	JQ404808	JQ404929	JQ405045
Alstroemeria sellowiana Seub.	A. Meerow 2208 (NA)	Brazil, Santa Catarina	JQ404564	JQ404696	JQ404809	JQ404930	JQ405046
Alstroemeria speciosa M. C. Assis	M. C. Assis and A. F. C. Tombolato 532 (UEC)	Brazil, Sao Paulo	JQ404571	JQ404700	JQ404812	JQ404934	JQ405050
Alstroemeria stenopetala Schenk	J. B. Pereira et al., 175 (CEN)	Brazil, Distrito Federal	JQ404577	JQ404704	JQ404816	JQ404938	JQ405052
Alstroemeria stenophylla M.C.Assis	A. F. C. Tombolato 481*	Brazil, Goiás	JQ404578	JQ404705	JQ404817	JQ404939	JQ405053
Alstroemeria umbellata Meyen	CONC 166195 (CONC)	Chile, Santiago metropolitan region, Embalse El Yeso	JQ404579	JQ404706	JQ404818	JQ404940	JQ405054
Alstroemeria viridiflora Ravenna	A. Meerow 2209 (NA)	Brazil, Goiás	JQ404568	JQ404698	JQ404811	JQ404932	JQ405048
Alstroemeria zoellneri Ehr. Bayer	CONC 166184 (CONC)	Chile, Valparaíso region, Parque Nacional La Campana	JQ404583	JQ404709	JQ404821	JQ404943	JQ405057
Bomarea ampayesana Vargas	A. Hofreiter 2001/2413 (M)	Peru	JQ404586	JQ404712	JQ404824	JQ404945	JQ405058
Bomarea dulcis Beauverd	A. Hofreiter 2001/2412 (M)	Peru	JQ404599	JQ404722	JQ404835	JQ404955	JQ405059
Bomarea patinii Baker	F. Alzate 2894 (HUA)	Colombia, Cundinamarca	JQ404619	JQ404737	JQ404854	JQ404970	EU159951

* Specimen vouchered by photos

***Phylogenetic analyses*—**Tree searches relied on maximum likelihood (ML) (Felsenstein, 1973) as implemented in the programs RAxML v. 7.0.4 (Stamatakis, 2006) and RAxMLGUI 1.0 (Silvestro and Michalak, 2011) using the GTR + G substitution model. FindModel (http://hcv.lanl.gov/content/sequence/findmodel/findmodel.html), which implements Posada and Crandall's (1998) ModelTest, selected this as the best fit for both the organellar and nuclear data based on the Akaike information criterion (Akaike, 1974). Statistical support for nodes was assessed by 100 ML bootstrap replicates (Felsenstein, 1985) under the same model. The alignment and inferred phylogeny are available in TreeBase (http://www.treebase.org, submission ID 12675). A Bayesian Markov chain Monte Carlo (MCMC) analysis (Yang and Rannala, 1997) of the same data relied on the program MrBayes v. 3.2 (Ronquist et al., 2012), using two parallel runs with one cold and four heated chains; the Markov chain had a length of 2 million generations, sampled every 1000th generations. Two separate runs were performed. A maximum clade credibility tree was obtained using BayesTrees 1.3 (available from http://www.evolution.reading.ac.uk/BayesTrees.html).

RESULTS

***Phylogeny of the genus Alstroemeria*—**The plastid, mitochondrial, and nuclear markers were successfully amplified for all ingroup and outgroup accessions. The combined matrix of the organellar regions *ndhF*, *rbcL*, *matK*, and *matR* comprised 2333 aligned nucleotides, and the nuclear ITS matrix comprised 729 aligned nucleotides. Maximum likelihood trees obtained from the organellar and the nuclear data showed no robustly supported incongruence (>75% ML bootstrap support; online Appendix S2 shows both trees), and analysis of the combined data yielded higher bootstrap values and better resolution. The results of the Bayesian analyses were congruent with the ML analyses, and posterior probability values for many nodes were high (≥0.97; Fig. 2).

The 37 species and subspecies of *Alstroemeria* selected to represent the genus fall into two clades that are sister to each other. One is a group of seven species distributed in northern and central Chile (clade a in Fig. 2) including *A. hookeri*, *A. magnifica* subsp. *magnifica*, and *A. pelegrina*. The other (clade b) comprises all remaining species of the genus, which are distributed in central and southern Chile, Argentina, and Brazil. It includes *A. presliana* and *A. ligtu*, which are closely related, the more distant *A. aurea*, and a Brazilian clade (c) to which *Alstroemeria*

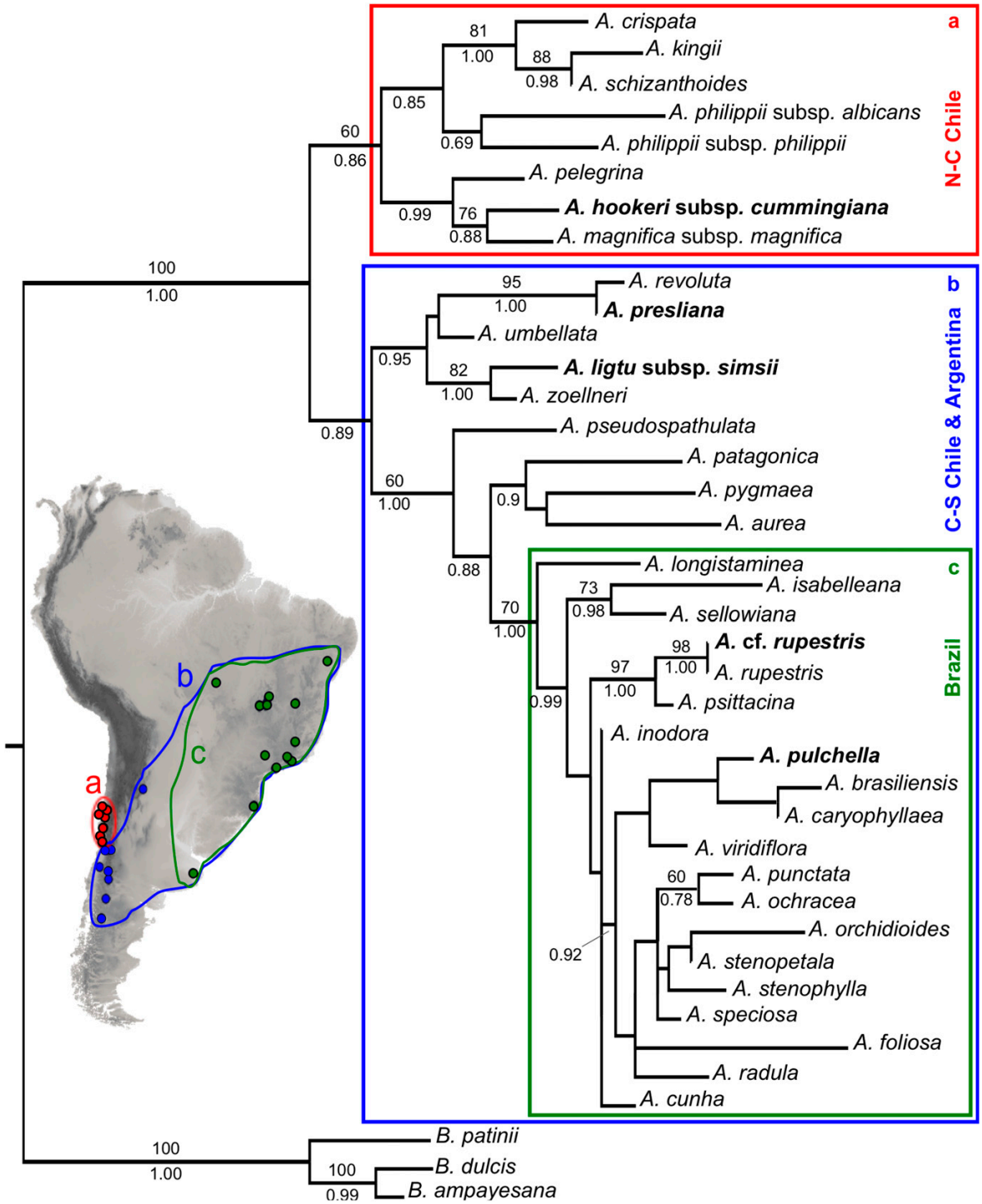

Fig. 2. Maximum likelihood phylogram of the genus *Alstroemeria* based on the combined analysis of plastid, mitochondrial, and nuclear sequences (3062 aligned nucleotides). The tree is rooted on the *Alstroemeria* sister clade, *Bomarea*. Maximum likelihood bootstrap support from 100 replicates is shown above branches, and posterior probability from a Bayesian analysis of the same data below branches. The boxes indicate clades discussed in the text. The map shows the geographic origin of the plants sequenced for the phylogeny, color-coded by clade. The five species with molecular cytogenetic data are in boldface.

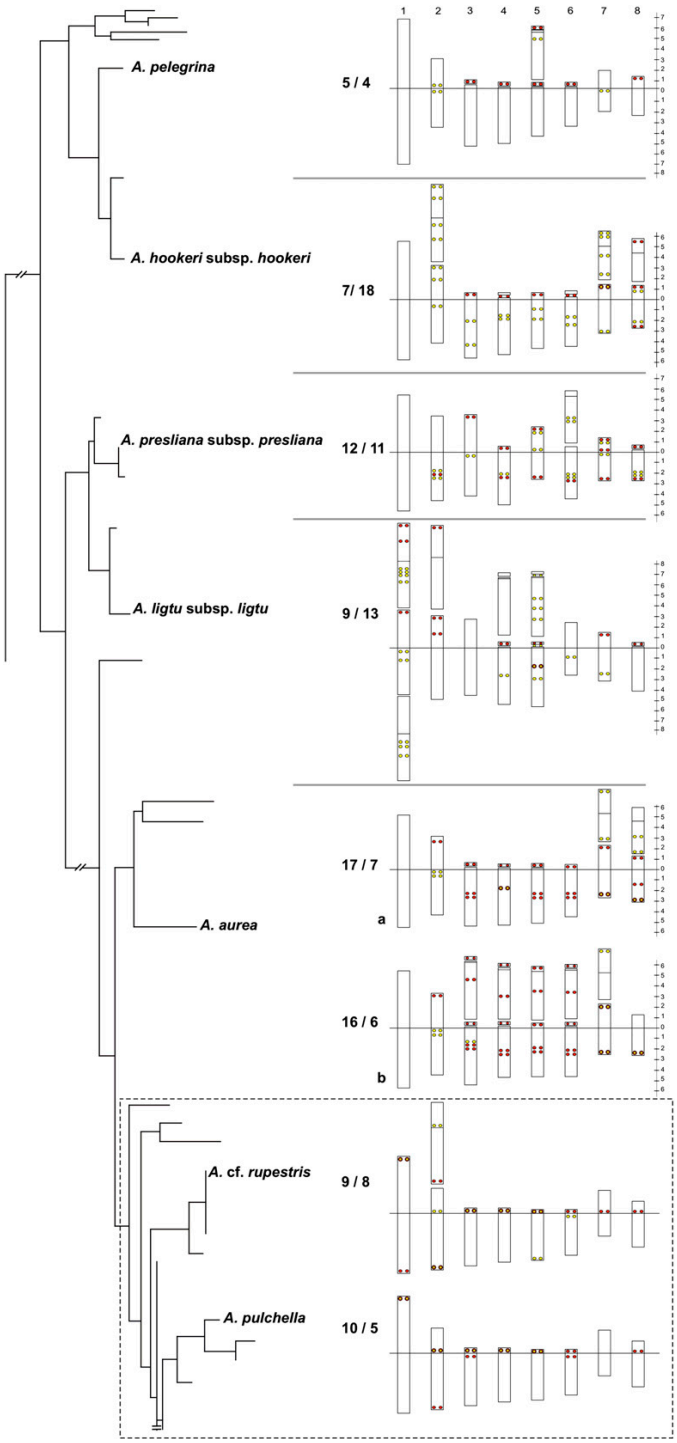

Fig. 3. Simplified ML tree showing the phylogenetic relationships of the *Alstroemeria* species included in the chromosome analyses, with a portion of the Brazilian clade highlighted in the dotted box. The idiograms next to each species show the localization of the 18S-25S rDNA (red) and the 5S rDNA (yellow) probes on the chromosomes. The numbers correspond to the total of number of 18S-25S rDNA sites/total number of 5S rDNA sites. Scales to the right of ideograms indicate the relative length of chromosome arms (%) according to Baeza et al. (2007). The 5S and 45S rDNA signals of *A.* cf. *rupestris* and *A. pulchella* were placed according to karyogram observations. (a) *A. aurea* accession 1090, (b) *A. aurea* accessions 4193, 4201, and 4202.

cf. *rupestris* and *A. pulchella* belong. The geographic distribution of these clades is shown on the inset map in Fig. 2.

Measurement and classification of karyotypes— Karyotypes of the Chilean species, *Alstroemeria aurea*, *A. hookeri*, *A. ligtu*, *A. pelegrina* and *A. presliana*, were investigated for structural differentiation by measuring 10 metaphases for each species (Table 1 in Baeza et al., 2007). The results, including the standard deviation of arm lengths, are provided in the karyograms of Fig. 3. When karyotypes are classified by arm ratio (Levan et al., 1964; Table 2 in Baeza et al., 2007), three species have four metacentric to submetacentric chromosomes and four acrocentric chromosomes, while two (*A. presliana* and *A. ligtu*) have five metacentric to submetacentric and three acrocentric chromosomes. The Brazilian species *Alstroemeria* cf. *rupestris* and *A. pulchella* have $2n = 16$ chromosomes, with four metacentric, four submetacentric and eight acrocentric chromosomes (karyotype formula = 4M + 4SM + 8A; Fig. 4).

Distribution of the 18S-25S and 5S rDNA signals—*Alstroemeria hookeri*, *A. pelegrina*, *A. presliana*, *A. ligtu*, and *A. aurea*, respectively, had 7, 5, 12, 9, and sixteen to seventeen 18S-25S rDNA sites and 18, 4, 11, 13, and six to seven 5S rDNA sites (Fig. 3 and online Appendix S3). *Alstroemeria* cf. *rupestris* displayed nine 18S-25S rDNA sites, two in the terminal regions of the first metacentric chromosome pair, one in the terminal region of the first submetacentric chromosome pair, four in the terminal region of its four acrocentric chromosome pairs, one in the centromeric region (interstitial) of the second metacentric chromosome pair, and another interstitial site in the second submetacentric chromosome pair (Figs. 3, 4, 5c). The same species had eight 5S rDNA sites, one localized in the terminal region of one metacentric chromosome pair, three terminal sites on the short arms of three of its four acrocentric pairs (one of them very weak), an additional terminal site on the long arm of the third acrocentric pair, and one interstitial site on the fourth acrocentric chromosome pair. One of the submetacentric chromosome pairs of the same species had a 5S site in its centromeric region and an additional terminal 5S signal (Figs. 3, 4, 5b). *Alstroemeria pulchella* had ten 18S-25S rDNA sites, one in the terminal region of the first metacentric chromosome pair, two in the first submetacentric chromosome pair (one in the centromeric region and the other in the terminal region), four in the terminal region of its four acrocentric chromosome pairs, two interstitial sites in two of the four acrocentric chromosome pairs, and one interstitial site in the second submetacentric pair (Figs. 3, 4, 5f). The same species also had five 5S rDNA sites, one localized in the terminal region of one metacentric chromosome pair, three in the terminal regions of three acrocentric chromosome pairs, and one in the centromeric region of one submetacentric pair (Figs. 3, 4, 5e). A summary of the distribution of the rDNA sites is provided in the Table 2.

The two Brazilian *Alstroemeria* showed a high variation of detectable signals. In the case of *A.* cf. *rupestris* (Fig. 3), the 5S rDNA site located on the short arm of the chromosome pair number 5 was only seen in one cell (see Appendix S4), while six 5S rDNA sites were seen in all cells (Figs. 3, 4, 5b), and a small site located on the long arm of an acrocentric chromosome pair was only observed in few cells (Appendix S4). In *A. pulchella*, four 5S rDNA sites were always detected. An additional small site (indicated with red arrowheads in Fig. 5e) was not always seen (see Appendix S4, and Fig. 3). Of the 18S-25S rDNA sites, seven were always observed in *A.* cf. *rupestris* and *A. pulchella* (Figs. 3, 4, 5c, 5f), while weak signals close to the centromeric region of the smallest submetacentric and metacentric chromosome pairs were seen only twice in *A.* cf. *rupestris* (Fig. 3; Appendix S4). In *A. pulchella*, small terminal sites on the largest metacentric chromosome pair and on the long arm of the largest submetacentric pair were also seen only rarely. Interstitial 18S-25S rDNA sites on the acrocentric chromosomes pairs 3 and 6, and on the second submetacentric chromosome pair were also observed in only a few cells (Appendix S4).

Overall, most 18S-25S rDNA signals were located terminally, while most 5S rDNA signals were interstitial (Table 2). Only in *A. aurea* were 18S-25S rDNA signals largely interstitial (chromosomes 3 to 6, and 8 of accession 1090, and 3 to 6 in accessions 4193, 4201, and 4202), but 5S rDNA signals terminal (see chromosomes 7 and 8 in Fig. 3). Four interstitial 18S-25S rDNA sites were also present on chromosomes 2, 3, 6, and 8 of *A. pulchella* (Table 2, Fig. 4, 5f), and interstitial 5S rDNA was seen on chromosome 2 of this species and chromosomes 2 and 6 of *A.* cf. *rupestris* (Table 2, Fig. 5b, 5e). Satellites with 18/25S rDNA signals were observed in *A. aurea* (chromosomes 3–5 of accession 1090, and 3, 4, and 6 of accessions 4193, 4201, and 4202), *A. ligtu* (chromosomes 4, 5, and 8), and *A. presliana* (chromosome 8), and *Alstroemeria hookeri* was the only species with 18/25S rDNA signals on the secondary constriction of chromosomes 4 and 6 (Fig. 3, Table 2).

Some of the 18S-25S and 5S rDNA sites were located very close to each other or adjacent (Garcia et al., 2007; Mazzella et al., 2010). Such was the case in four plants of *A. aurea* on

TABLE 2. Summary of the results obtained in the FISH experiments for number of rDNA sites (No.) and their location on the chromosomes of *Alstroemeria*.

Species	18S–25S		5S		No. adjacent sites (Chromosome pair)
	No.	Location	No.	Location	
A. aurea 1090	17	6 T, 8 I, 3 Sat	7	3 T, 3 I, 1 P	1 (4), 1 (7), 1 (8)
A. aurea 4193, 4201, 4202	16	5 T, 8 I, 3 Sat	6	3 T, 2 I, 1 P	2 (7), 1 (8)
A. cf. *rupestris*	9	7 T, 2 I	8	6 T, 2 I	1 (1), 1 (2), 1 (3), 1 (4), 1 (5)
A. hookeri subsp. *hookeri*	7	5 T, 2 SC	18	7 T, 11 I	1 (7)
A. ligtu subsp. *ligtu*	9	3 T, 3 I, 3 Sat	13	1 T, 10 I, 1 P, 1 SC	1 (5)
A. pelegrina	5	1 T, 4 Sat	4	1 I, 3 P	0
A. presliana subsp. *presliana*	12	7 T, 3 I, 1 P, 1 Sat	11	2 T, 6 I, 3 P	0
A. pulchella	10	6 T, 4 I	5	4 T, 1 I	1 (1), 1 (2), 1 (3), 1 (4), 1 (5)

Notes: Locations are abbreviated as follows: T, terminal/subterminal; I, interstitial; P, pericentromeric; Sat, satellite; SC, secondary constriction. The number of adjacent rDNA signals in homologous chromosome is also shown.

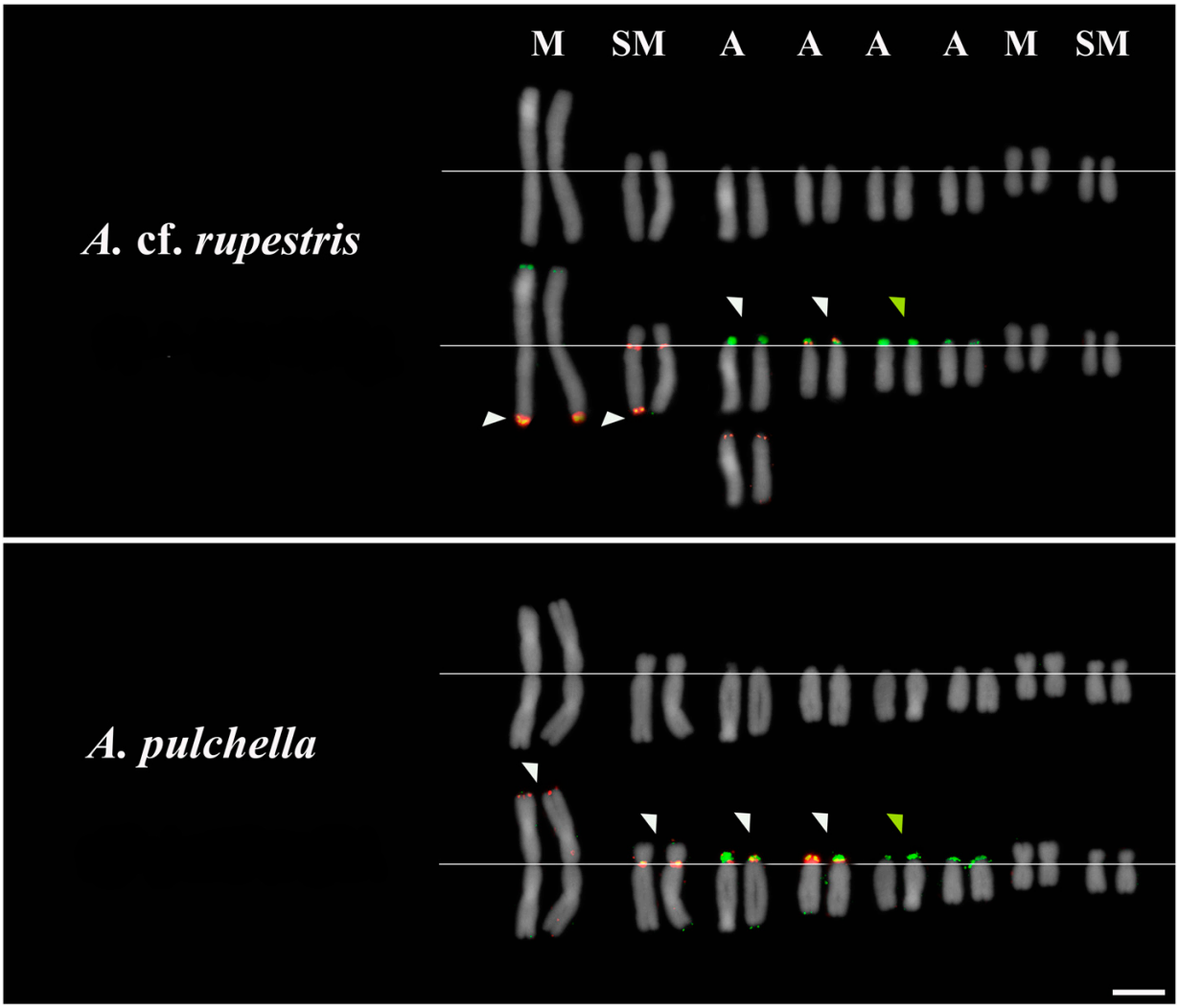

Fig. 4. Karyograms of *Alstroemeria* cf. *rupestris* and *A. pulchella* with $2n = 16$ showing the overlapping of 5S rDNA (red) and 18/25S rDNA (green) probes. White arrowheads indicate chromosome pairs with adjacent sites; yellow arrowheads indicate chromosome pairs in which adjacent sites were also observed although not in this particular metaphase. M, metacentric; SM, submetacentric; A, acrocentric. Scale bar = 10 μm.

chromosomes 7 and 8 and also in plant 1090 on chromosome 4 (Fig. 3; Appendix S3). In *A. hookeri*, adjacent 18S-25S and 5S rDNA sites were present on chromosome 7 and in *A. ligtu* on the long arm of chromosome 5 (Fig. 3). The highest number of adjacent 18S-25S and 5S rDNA sites was observed for the two Brazilian species (Fig. 4). Intraspecific differences found among the four population samples of *A. aurea* are discussed later.

***Insterstitial telomeric sites*—**In *Alstroemeria* cf. *rupestris*, our telomeric probe revealed an interstitial (centromeric) telomeric site on one chromosome and in a few additional metaphases of two or three homologous chromosomes. No interstitial telomeric sites were observed in *A. pulchella* (Figs. 5A, D).

***Intraspecific polymorphism in FISH signals*—**In the Chilean species *A. aurea*, plant 1090 (Fig. 3A) differed from plants 4193, 4201, and 4202 (Fig. 3B) in chromosomes 3–8 (Fig. 3; see Table 1 for their geographic origin). Furthermore, chromosomes 3–6 were polymorphic in plants 4193 and 4202 (Appendix S3). Polymorphism was also found in *A. ligtu* plants from different populations (Fig. 3 shows the three "versions" of chromosome 1 and the two "versions" of chromosomes 2, 4, and 5, one above the other) as well as in *A. pelegrina* and *A. presliana* (Fig. 3 shows the homologous versions of chromosomes above each other).

DISCUSSION

***Revised interpretation of* Alstroemeria *cytogenetic changes resulting from the phylogenetic context*—**The main clades of *Alstroemeria* (labeled in Fig. 2) are well supported, while species

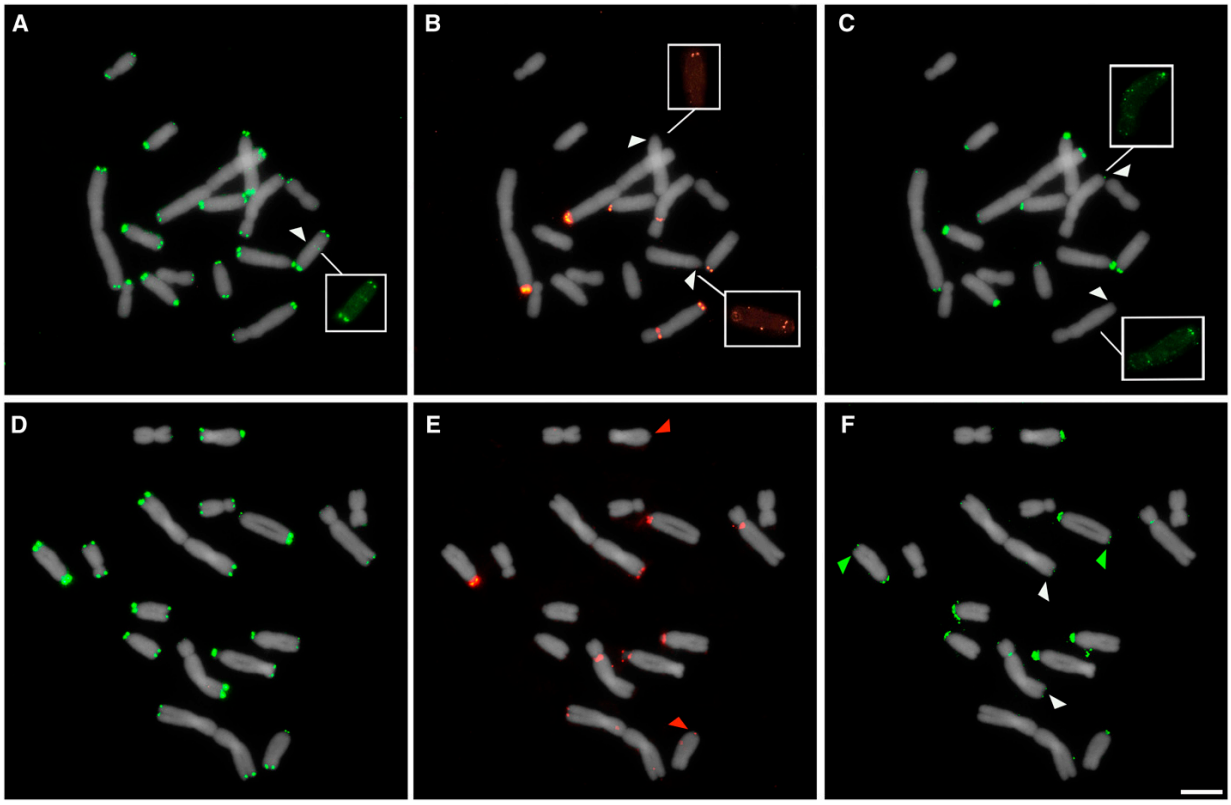

Fig. 5. Fluorescence in situ hybridization (FISH) on mitotic metaphase chromosomes of (A-C) *Alstroemeria* cf. *rupestris* and (D-F) *A. pulchella*. Distribution of (A, D) telomeric sequences, (B, E) 5S rDNA sites, and (C, F) 18S-25S rDNA sites. Insert in (A) shows an interstitial telomeric site, and in (B) and (C) chromosome pairs with weak sites not visible after the overlap with DAPI. Arrowheads in (E) indicate sites that were difficult to detect, and in (F) the telomeric probe signal in the terminal region of some chromosomes (green arrowheads), including weak signals (white arrowheads). Scale bar = 10 μm.

groups from central-south Chile/Argentina and Brazil lack statistical support. In a study of Alstroemeriaceae biogeography that applied a molecular clock, the stem lineage of the Brazilian clade (clade c in Fig. 2) dates to about 9.2 million years ago (Ma) (Chacón et al., 2012), which provides a rough temporal context for the documented cytogenetic changes. Notably, the Brazilian clade is evolutionarily derived from Chilean/Argentinean ancestors (Fig. 2), meaning that one cannot construct a contrast between all Chilean species on the one hand and all Brazilian ones on the other.

The Chilean alstroemerias in clade a (Fig. 2) grow in regions with long periods of drought (Muñoz-Schick and Moreira-Muñoz, 2003; Moreira-Muñoz, 2011). The Brazilian species in general grow in more humid, less drought-stressed habitats. These ecological differences between the species may have led Buitendijk et al. (1997) to contrast Chilean and Brazilian "karyotype groups" that supposedly differ in PI/DAPI ratios and 2C values: group 1 comprised *A. magnifica**, *A. pelegrina**, *A. philippii**, and *A. pulchra*; group 2 *A. angustifolia*, *A. aurea**, and *A. hookeri**; group 3 *A. ligtu* subsp. *ligtu* and *A. ligtu* subsp. *simsii**; and group 4 *A. brasiliensis**, *A. caryophyllaea**, *A. inodora**, and *A. psittacina** (species shown in our Fig. 2 are marked by an asterisk). Groups 1 and 4 are recovered in our molecular tree (Fig. 2), while group 2 is unnatural (the monophyly of group 3 is not addressed by our data since we only included one of the two subspecies of *A. ligtu*).

***Localization and inter- and intraspecific variability in the number of rDNA sites*—**The number of 18S-25S rDNA sites can vary from 5–7 sites in the *A. hookeri*/*A. pelegrina* clade, to 16–17 in *A. aurea* (Fig. 3), with closely related species, such as *A. hookeri* and *A. pelegrina*, having 18 or just four 5S rDNA sites (Table 2), implying a rapid increase or decrease of these sites (Cajas et al., 2009 for a study focusing on *A. hookeri*). The only Brazilian species so far studied have nine (*Alstroemeria* cf. *rupestris*) and 10 (*A. pulchella*) 18S-25S rDNA signals (Figs. 3, 4). Variation in rDNA sites among closely related species often characterizes diploids and their polyploid relatives (Hasterok et al., 2006; Malinska et al., 2010). A recent study on *Paphiopedilum*, an orchid genus with no known polyploids (Lan and Albert, 2011), however, also found high variation in the number and distribution of the 5S and 25S rDNA sites among close relatives, which the authors explained by chromosomal rearrangements and dynamic double-strand break repair processes that characterize hotspots in pericentromeric and telomeric regions (Schubert and Lysak, 2011). This could also be the case in *Alstroemeria* in which no polyploids are known either and which presents telomeric sequences near most 18/25S and 5S rDNA terminal sites (Fig. 5).

Adjacent rDNA signals (with 18S-25S and 5S probes close to each other) as found in *A. aurea*, *A. hookeri*, *A. ligtu*, *A. pulchella*, and *A.* cf. *rupestris* are rare, but have been reported from the monocots *Iris* (Martínez et al., 2010), *Lilium* (Lim et al., 2001), *Lycoris* (Chang et al., 2009), and *Maxillaria* (Cabral et al., 2006), the dicots *Artemisia* (Garcia et al., 2007) and *Linum* (Muravenko et al., 2004) and the conifer *Picea* (Siljak-Yakovlev et al., 2002). While the function of adjacent rDNA sites is not known, it has been suggested that adjacent 45S and 5S sites at telomeric ends may relate to the stabilization of centromeric fission products (Chang et al., 2009; also Dobigny et al., 2003).

If all 78 species of *Alstroemeria* turn out to have $2n = 16$ chromosomes (as found in the 29 species so far counted: Appendix S1), genome evolution in this genus would exclusively have involved reorganizations of chromosome structure, rather than polyploidy as in many other species-rich monocot genera (e.g., Taketa et al., 1999: *Hordeum*; Adams et al., 2000: *Aloe*; Martínez et al., 2010: *Iris* subgenus *Xiphium*). Alstroemeriaceae in general exhibit little variation in chromosome numbers, especially compared to their sister family, Colchicaceae. Among the three genera studied (there are no data yet for the fourth genus, *Drymophila*), *Alstroemeria* has the largest chromosomes and highest karyotype asymmetry. The extensive genome reorganization in this genus inferred here from the dynamic rDNA sites (within species and among close relatives) could have involved DNA insertions, inversions, or translocations. Indeed, pericentric inversions have been invoked to explain the patterns of heterochromatin location in the eight *Alstroemeria* karyotypes analyzed by Buitendijk and Ramanna (1996). Alternatively, or in addition, interchromosomal symmetric reciprocal translocations as described by Schubert and Lysak (2011; see their Fig. 2d) could have led to the equilocal position of the rDNA sites in some *Alstroemeria* (Fig. 3).

Besides such primary rearrangements, mobility in rDNA sites can also result from transposon-mediated transpositions (Datson and Murray, 2006; Raskina et al., 2008) which can be activated by abiotic stresses, for example, drought (Kalendar et al., 2000; Aprile et al., 2009). Drought stress-related transposon activity in *Alstroemeria* might have increased during the fluctuating dry/wet climatic conditions in Miocene South America when the plant clade studied here evolved (Chacón et al., 2012). Attributing cytogenetic features to this or other factors, such as the Andean uplift (e.g., Buitendijk and Ramanna, 1996), however, remains speculative until more in-depth studies.

***Genome size variation in* Alstroemeria—**The molecular phylogeny of *Alstroemeria* also provides a basis for interpreting genome size changes in this genus (Buitendijk et al., 1997; 1998; our Fig. 6), which appear unrelated to rDNA dynamics. For example, *A. hookeri* with very few 18S-25S rDNA sites (7 sites, Table 2) and *A. aurea* with many (17 and 16, Table 2) have almost the same genome size (C-values of 26 pg vs. 26.8 pg, respectively, Fig. 6). Likewise, *A. hookeri* and *A. pelegrina* (Fig. 2) with divergent numbers of 5S rDNA sites (18 vs. 4, respectively, Table 2) have similar genome sizes (1C-values of 26 pg and 22.1 pg, respectively, Fig. 6). The underlying explanation for the different genome sizes likely is the differential accumulation of transposable elements (Hawkins et al., 2006; Piegu et al., 2006; Piednoel et al., 2012).

***Implications of the observation of interstitial telomeric sites in* Alstroemeria *cf.* rupestris—**We detected interstitial telomeric sites near the centromeres in *A.* cf. *rupestris* (Fig. 5A), which hints at a Robertsonian fusion of chromosomes (Leitch and Leitch, 2012). Such fusions have been invoked to explain bimodal karyotype organization in Asparagaceae (McKain et al., 2012) and may also underlay the bimodal karyotypes in *Alstroemeria*. A hypothesis of end-to-end fusion (resulting in a reduction in chromosome number) would provide an explanation for *Bomarea* having $2n = 18$ (Appendix S1), while *Alstroemeria* has $2n = 16$. Further cytogenetic studies using telomeric probes are required to test this hypothesis.

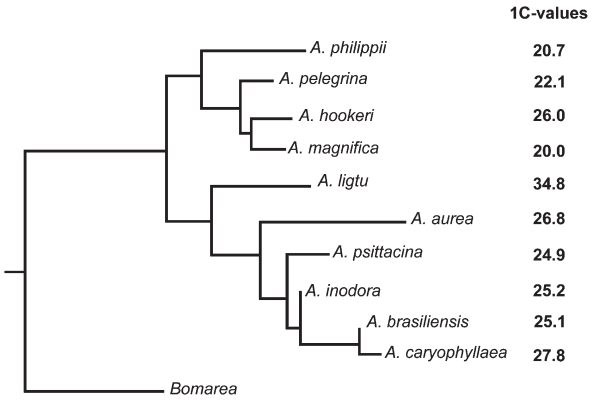

Fig. 6. Phylogram showing the relationships among the *Alstroemeria* species for which the genome size is known. The 1C-values (pg DNA) were obtained from Buitendijk et al. (1997, 1998). The topology is based on the ML tree shown in Fig. 2.

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Appendix S1. Species of Alstroemeriaceae studied cytogenetically, with geographic species distribution, technique used, chromosome numbers reported, and reference (CA: Conventional analysis; CB: C-Banding; FISH: Fluorescens *in situ* Hybridization).

Species	**Geographic distribution**	**Technique**	**2*n***	**Reference**
Alstroemeria andina subsp. *venustula* Ehr. Bayer	Argentina, Chile	CA	16	Sanso (2002)
Alstroemeria angustifolia Herb. subsp. *angustifolia*	Chile	CB	16	Buitendijk and Ramanna (1996)
Alstroemeria aurantiaca D. Don (synonym of *A. aurea* Graham)	Argentina, Chile	CA	16; 25	Tsuchiya and Hang (1989); Chen et al. (2003)
Alstroemeria aurea Graham	Argentina, Chile	CB, FISH	16	Buitendijk and Ramanna (1996); Kamstra et al. (1997); Buitendijk et al. (1998); Baeza et al. (2007a)
Alstroemeria caryophyllaea Jacq.	Brazil	CA	16	Tsuchiya and Hang (1989)
Alstroemeria chilensis Cree	Chile	CA	16	Strasburger (1882); Tsuchiya and Hang (1989)
Alstroemeria graminea Phil.	Chile	CB	16	Jara-Seguel et al. (2004)
Alstroemeria haemantha Ruiz and Pav.	Chile	CA	16	Sanz de Cortazar (1948); De Nordenflycht (1981); Tsuchiya and Hang (1989)
Alstroemeria hookeri Lodd.	Chile	CA	16	Tsuchiya and Hang (1989)
Alstroemeria hookeri Lodd. subsp. *cummingiana* Ehr. Bayer	Chile	CA	16	Sanso (2002); Baeza et al. (2010)
Alstroemeria hookeri Lodd. subsp. *hookeri*	Chile	CA, FISH	16	Baeza et al. (2007a; 2010); Cajas et al. (2009)
Alstroemeria hookeri Lodd. subsp. *maculata* Ehr. Bayer	Chile	CA	16	Baeza et al. (2010)
Alstroemeria hookeri Lodd. subsp. *recumbens* Ehr. Bayer	Chile	CA	16	Sanso (2002); Baeza et al. (2010)
Alstroemeria hygrophila Meerow, Tombolato and F. K. Mey.	Brazil	CA	16	Meerow et al. (1999)
Alstroemeria inodora Herb.	Brazil	CB	16	Buitendijk and Ramanna (1996); Kamstra et al. (1997);

				Kuipers et al. (2002)
Alstroemeria isabelleana Herb.	Argentina, Brazil	CA	16	Sanso and Hunziker (1998)
Alstroemeria japonica L.*	?	CA	16, 13	Venkateswarlu and Lakshmi (1975); Lakshmi (1976, 1980)
Alstroemeria ligtu L. subsp. *ligtu*	Chile	CB; CA; FISH	16	Buitendijk and Ramanna (1996); Buitendijk et al. (1998); Baeza et al. (2006; 2007a)
Alstroemeria ligtu L. subsp. *incarnata* Ehr. Bayer	Chile	CB	16	Buitendijk et al. (1998)
Alstroemeria ligtu L. subsp. *simsii* Ehr. Bayer	Chile	CA, CB	16	Buitendijk et al. (1998); Baeza et al. (2006)
Alstroemeria magnifica Herb. subsp. *magnifica*	Chile	CB	16	Buitendijk and Ramanna (1996); Buitendijk et al. (1998)
Alstroemeria orchidioides Meerow, Tombolato and F. K. Mey.	Brazil	CA	16	Meerow et al. (1999)
Alstroemeria pallida Graham	Chile	CA	16	Sanso (2002)
Alstroemeria patagonica Phil.	Argentina, Chile	CA	16	Moore (1981); Sanso (2002)
Alstroemeria pelegrina L.	Chile	CA, CB, FISH	16	Tsuchiya and Hang (1989); Stephens et al. (1993); Buitendijk and Ramanna (1996); Baeza et al. (2007a)
Alstroemeria philippii Baker	Chile	CB	16	Buitendijk and Ramanna (1996)
Alstroemeria presliana Herb. subsp. *australis* Ehr. Bayer.	Chile	CA	16	Baeza et al. (2001; 2008a)
Alstroemeria presliana Herb. subsp. *presliana*	Chile	CA, FISH	16	Baeza et al. (2007a, b; 2008a)
Alstroemeria pseudospathulata Ehr. Bayer	Argentina	CA	16	Sanso (2002)
Alstroemeria psittacina Lehm.	Argentina, Brazil	CA, CB	16	Tsuchiya and Hang (1989); Buitendijk and Ramanna (1996); Sanso and Hunziker (1998); Kuipers et al. (2002)
Alstroemeria pulchella L. f.	Brazil	CA, FISH	16	Venkateswarlu and Lakshmi (1975); Lakshmi (1976); Tsuchiya and Hang (1989); this study
Alstroemeria pulchra Sims	Chile	CA	16	De Nordenflycht (1981); Tsuchiya and Hang (1989)
Alstroemeria pygmaea Herb.	Argentina	CA	16	Sanso (2002)
Alstroemeris cf. *rupestris* M. C. Assis	Brazil	FISH	16	This study

Alstroemeria versicolor Ruiz and Pav.	Chile	CA	16	Tsuchiya and Hang (1989)
Alstroemeria violacea Phil.	Chile	CA	16	Tsuchiya and Hang (1989)
Bomarea boliviensis Baker	Argentina, Bolivia	CA	18	Sanso and Hunziker (1998)
Bomarea edulis Herb.	From Mexico to Argentina and Brazil	CA	18	Hunziker and Xifreda (1990); Sanso and Hunziker (1998)
Bomarea involucrosa Baker	Bolivia, Chile, Peru	CA	18	Palma-Rojas et al. (2007)
Bomarea macrocephala Pax	Argentina, Bolivia	CA	18	Sanso and Seo (2005)
Bomarea ovallei Ravenna	Chile	CA, CB	18	Palma-Rojas et al. (2007); Jara-Seguel et al. (2005)
Bomarea patinii Baker subsp. *patinii*	Colombia, Ecuador	CA	18	Baeza et al. (2008b)
Bomarea salsilla Mirb.	Chile	CA	18	Palma-Rojas et al. (2007)
Bomarea salsilloides M. Roem. (synonym of *B. edulis* Herb.)	Brazil	CA	18	Guerra (1986)
Bomarea setacea Herb.	Colombia, Ecuador, Peru	CA	18	Hunziker and Xifreda (1990)
Bomarea stans Kraenzl.	Argentina, Bolivia	CA	18	Sanso and Seo (2005)
Drymophila cyanocarpa R. Br.	Tasmania	CA	24	Menadue and Orchard (1985)
Drymophila cyanocarpa R. Br.	Tasmania	CA	20	Wiltshire and Jackson (2003)
Drymophila moorei Baker	Australia	CA	20	Conran (1985)
Luzuriaga marginata Benth. & Hook.	Chile, Falkland islands	CA	20	Moore (1967)
Luzuriaga parviflora Kunth	New Zealand	CA	20	Bfuzenberg and Hair (1963)
Luzuriaga polyphylla J. F. Macbr.	Chile	CA	20	Jara-Seguel and Zúñiga (2005)
Luzuriaga radicans Ruiz & Pav.	Chile, Argentina	CA	20	Jara-Seguel and Zúñiga (2005); Jara-Seguel et al. (2010)

* Unresolved name

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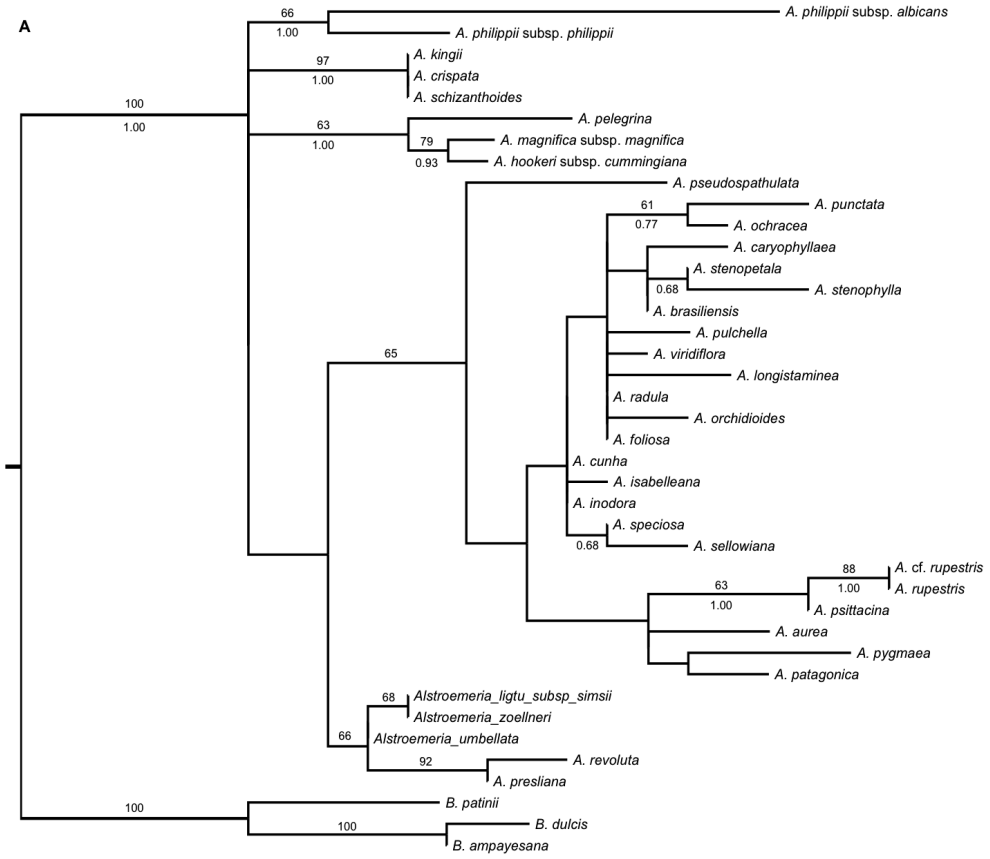
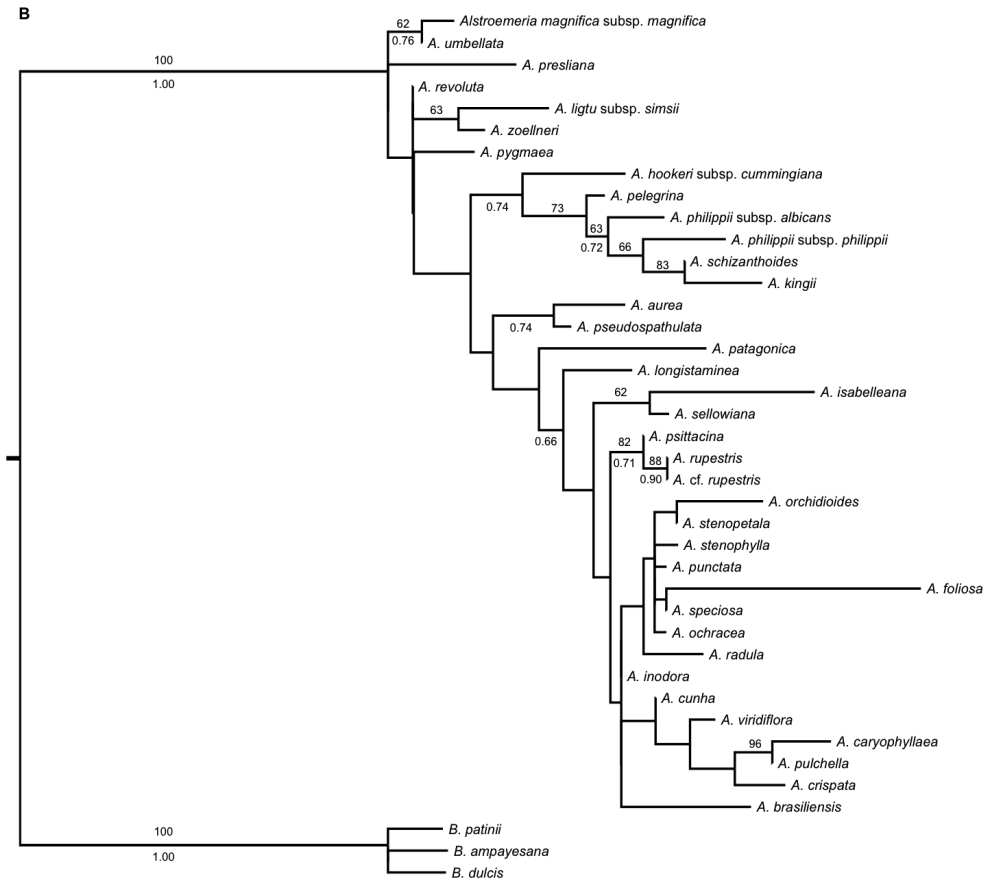
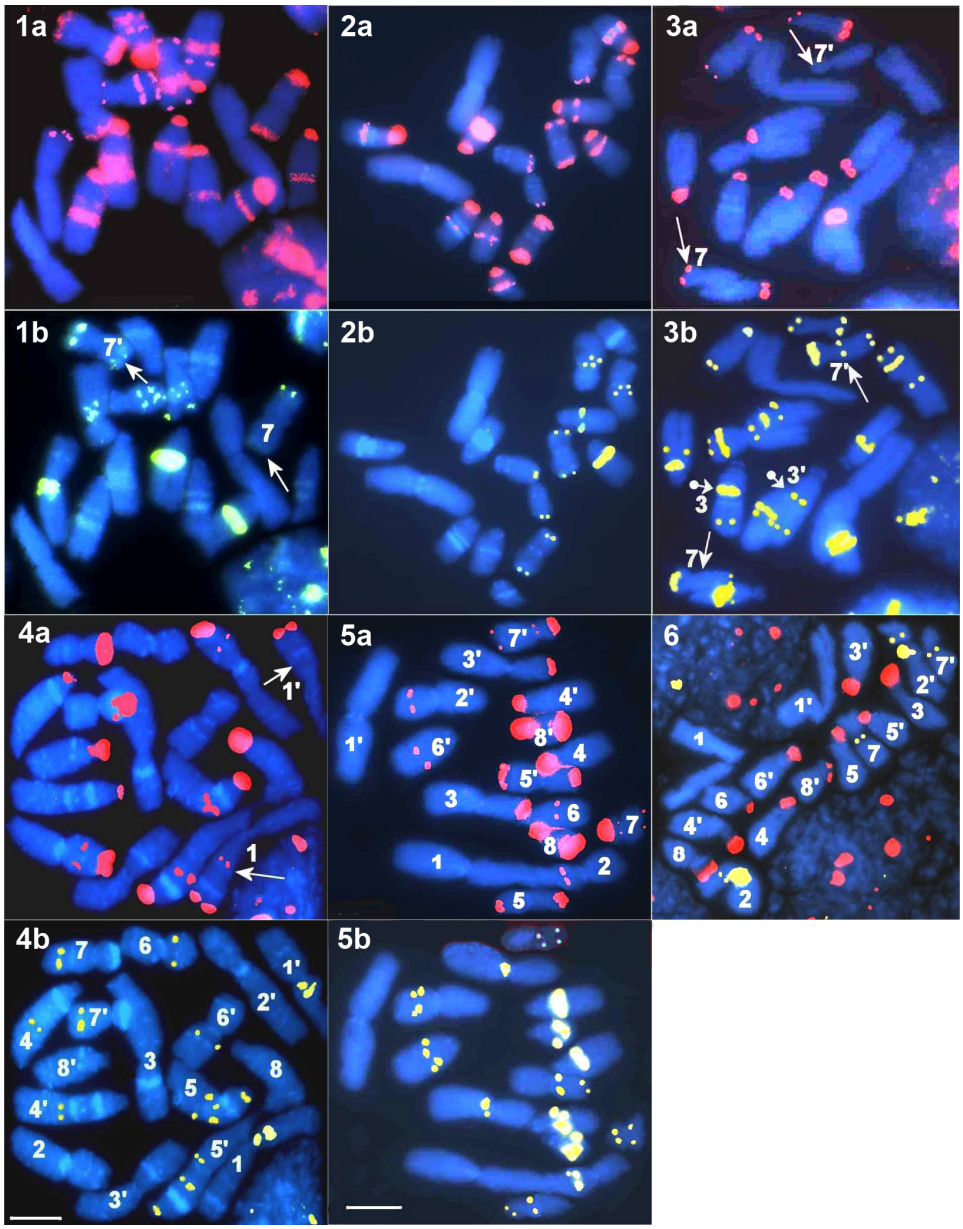
Appendix S2. Maximum likelihood phylograms of the genus *Alstroemeria* based on the individual analyses of the organellar sequences (A) and the nuclear ITS sequences (B). The trees are rooted on the *Alstroemeria* sister clade, *Bomarea*. Maximum likelihood bootstrap support from 100 replicates is shown above branches, and posterior probability from a Bayesian analysis of the same data below branches.

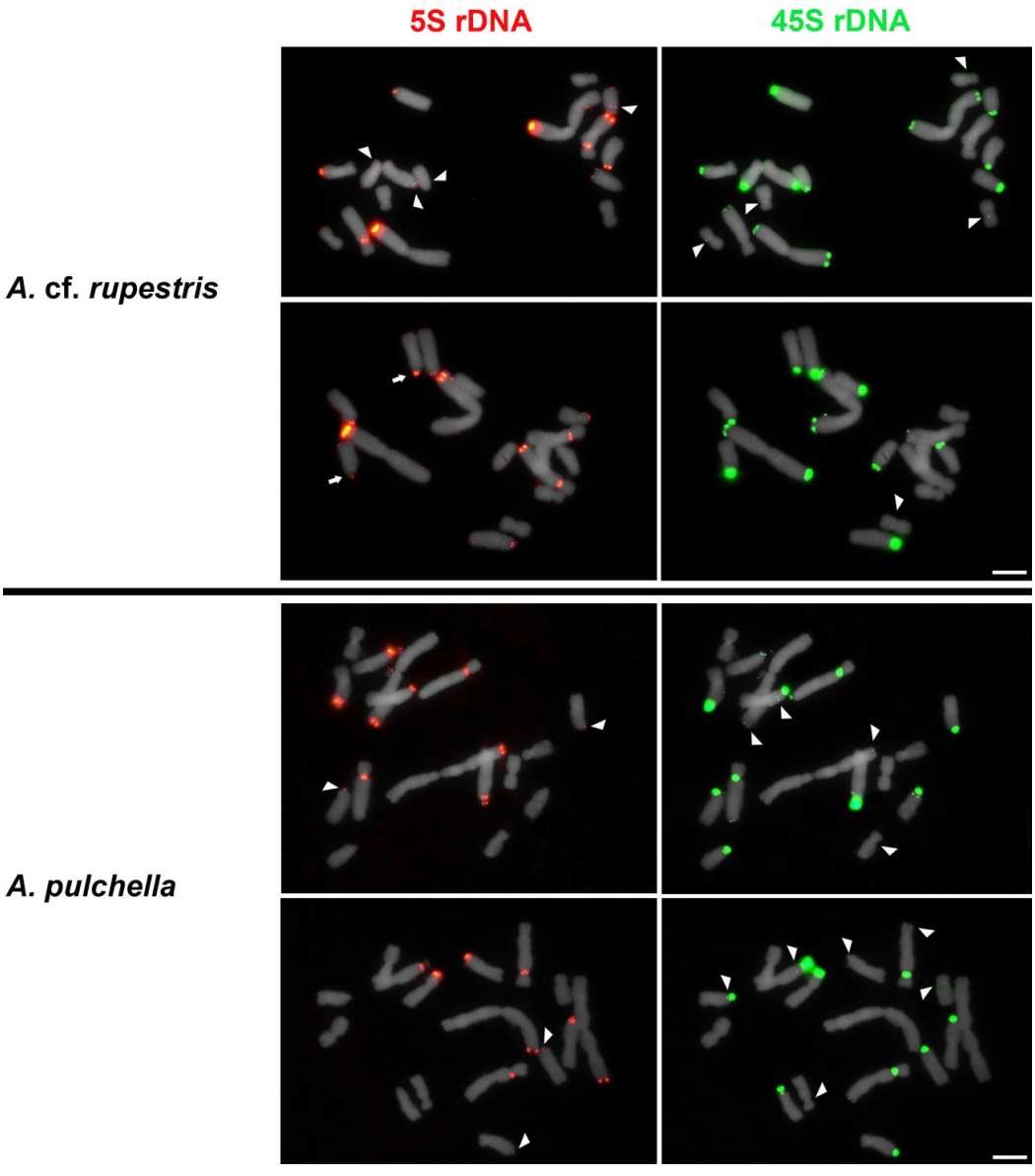



B
62
0.76
Alstroemeria magnifica subsp. magnifica
A. umbellata
100
1.00
A. presliana
A. revoluta
63
A. ligtu subsp. simsii
A. zoellneri
A. pygmaea
A. hookeri subsp. cummingiana
0.74
73
A. pelegrina
63
A. philippii subsp. albicans
0.72
66
A. philippii subsp. philippii
83
A. schizanthoides
A. kingii
A. aurea
0.74
A. pseudospathulata
A. patagonica
A. longistaminea
62
A. isabelleana
A. sellowiana
0.66
82
A. psittacina
0.71
88
A. rupestris
0.90
A. cf. rupestris
A. orchidioides
A. stenopetala
A. stenophylla
A. punctata
A. foliosa
A. speciosa
A. ochracea
A. radula
A. inodora
A. cunha
A. viridiflora
96
A. caryophyllaea
A. pulchella
A. crispata
A. brasiliensis
100
1.00
B. patinii
B. ampayesana
B. dulcis

Appendix S3. Results of the FISH analysis of the five Chilean species using 18/25S (a, red) and 5S (b, yellow) rDNA probes in **1**. *Alstroemeria aurea* 1090; **2.** *A. aurea* 4193, 4201, 4202; **3.** *A. hookeri* 4181; **4.** *A. ligtu*, and **5.** *A. presliana*. Double FISH of 18/25S (red) and 5S rDNA (yellow) is shown in **6.** *A. pelegrina*. Counterstaining (blue) in DAPI polymorphic hybridization sites are marked by arrows. Chromosomes were designated according the measurements shown in Baeza et al. (2007). Scale bars correspond to 5 µm.



Appendix S4. Additional metaphases of *Alstroemeria* cf. *rupestris* and *A. pulchella* showing the distribution of 5S and 45S rDNA (i.e. 18/25S rDNA). The small rDNA sites that were not observed in all cells are indicated by arrowheads. In the case of *A.* cf. *rupestris,* the small arrows indicate 5S rDNA sites that were only seen in this metaphase. Bars correspond to 10 µm.



Chapter 6

THE EVOLUTION OF COLCHICACEAE, WITH A FOCUS ON CHANGES IN CHROMOSOME NUMBERS

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Abstract

The lily family Colchicaceae consists of geophytic herbs distributed on all continents except the Neotropics. It is particularly diverse in southern Africa, where 80 of the 270 species occur. Colchicaceae exhibit a wide range of ploidy levels, from *2n* = 14 to *2n* = 216. To understand where and how this cytogenetic diversity arose, we generated multilocus phylogenies of the Colchicaceae and the *Colchicum* clade that respectively included 82 or 137 species plus relevant outgroups. To infer the number of polyploidization events and single chromosome changes (dysploidy) that could explain the observed numbers in the living species, we compared a series of likelihood models on phylograms and ultrametric trees containing the 52 or 122 species with published chromosome counts. Models were optimized under maximum likelihood. The results show that the main mechanism of chromosome number evolution in most Colchicaceae clades was the gain or loss of single chromosomes, with the exception of *Colchicum* in which polyploidization events are concentrated, presumably as the result of hybridization and allopolyploidization.

Keywords: African Colchicaceae, ancestral chromosome number, maximum likelihood inference, polyploidy.

Introduction

The Colchicaceae are the third largest family of the Liliales (after the Liliaceae and Smilacaceae) and have some 270 species in 15 genera, distributed in Africa, Asia, Australasia, North America and Europe. No species occur in South and Central America (Vinnersten and Manning, 2007). Their closest relatives are the Alstroemeriaceae, which have most of their species in South America and which were the subject of recent phylogenetic and biogeographic work (Chacón et al., 2012a). Together, the two form the sister clade to the Petermanniaceae, a monospecific family restricted to tropical Australia (Vinnersten and Reeves, 2003; Fay et al., 2006; APG III, 2009). All Colchicaceae contain colchicine, an alkaloid traditionally used in the treatment of gout, and also in cytogenetics due to its properties as a microtubule polymerization inhibitor (Vinnersten and Larsson, 2010). Colchicaceae are long-lived

cormose or rhizomatous geophytes with rather large flowers with six, usually free tepals (Fig. 1), each more or less enveloping a stamen, and nectaries on the base of filaments or tepals (Nordenstam, 1998). African Colchicaceae in the Namaqualand desert often have leaves with helical shapes and hairy margins that serve to harvest water from dew and fog, which then dripps to the soil and reaches the root zone where it is ultimately stored in the corms (Vogel and Müller-Doblies, 2011).

Figure 1. Morphological diversity and floral variability in Colchicaceae. A, *Gloriosa modesta*; B, *Wurmbea marginata*; C, *Colchicum bulbocodium*; D, *Colchicum cuspidatum*. Photographs C. Bräuchler (A), A. Fleischmann (B), J. Chacón (C, D), used with permission.

Colchicaceae have been the subject of several molecular-phylogenetic studies that clarified generic relationships and the circumscriptions of the Australian/African

genus *Wurmbea*, the Mediterranean/Irano-Turanian genus *Colchicum* (the latter extending east to Afganistan and Kirgiztan; Persson, 2007), and the small genus *Gloriosa*, with 10 species in Africa, India, and Southeastern Asia (Vinnersten and Reeves, 2003; Vinnersten and Manning, 2007). A redefinition of *Colchicum* to include all c. 60 species of *Androcymbium* was proposed by Manning et al. (2007) and Persson (2007), while del Hoyo and Pedrola-Monfort (2008) and del Hoyo et al. (2009) preferred to maintain *Androcymbium* and *Colchicum* as separate genera.

A striking feature of the Colchicaceae is their high karyological variation (Table 1), with chromosome numbers ranging from *2n* = 14 (e.g., *Uvularia grandiflora*; Therman and Denniston, 1984) to *2n* = 216 (in *Colchicum corsicum*; Persson, 2009). Such variation contrasts with the sister family, Alstroemeriaceae, in which the chromosome numbers vary between *2n* = 16 and *2n* = 20 (Chacón et al., 2012b). The cytogenetics of the genus *Colchicum* is especially complex, with different species having variable chromosome numbers as well as ploidy levels (from tetra- to 24-ploid; Persson et al., 2011), perhaps related with the presence of colchicine (Nordenstam, 1998). The effect of colchicine on the separation of chromosomes after the anaphase of mitosis was discovered by B. Pernice in 1889 and described more fully by Eigsti et al. (1945); it revolutionized cytogenetics because it permitted experimental doubling of the entire complement of a cell's chromosome set.

Table 1. Chromosome numbers available for the Colchicaceae genera (see details of the species and references in Appendix 2).

Genus	No. of species	No. of species counted	Chromosome number	
			n	*2n*
Baeometra Salisb. ex Endl.	1	1		22
Burchardia R. Br.	6	5	48	24
Camptorrhiza Hutch.	2	1		22
Colchicum L.	ca. 157	97		14, 18, 20, 21, 22, 24, 27, 32, 40, 42–44, 36, 38, 46, 48, 50, 52, 54, 58, 90, 92, 94, 96, 102, 106, 108, ca. 110, ca. 120, 140, 146, 182, ca. 216
Disporum Salisb. ex G.Don	20	11		14, 16, 18, 30, 32

Continued

Table 1 *Continued*

Genus	No. of species	No. of species counted	Chromosome number	
			n	*2n*
Gloriosa L.	10	7		20, 21, 22, 44, 66, 88
Hexacyrtis Dinter	1	1		22
Iphigenia Kunth	12	6	11	22
Kuntheria Conran & Clifford	1	1		14
Ornithoglossum Salisb.	8	4		24
Sandersonia Hook.	1	1		24
Shelhammera R. Br.	2	2		14, 36
Tripladenia D. Don	1	1		14
Uvularia L.	5	3	7	14
Wurmbea Thunb.	ca. 50	3		14, 20, 40

Besides through polyploidy, chromosome numbers can change through chromosome fission (ascending dysploidy) or chromosome fusion (descending dysploidy; Schubert and Lysak, 2011). While polyploidy is thought to promote ecological diversification by facilitating the adaptation to new environments through novel biochemical, physiological, and developmental traits not found in the progenitors (Levin, 1983; Abbott et al., 2013), dysploidy is thought to arise accidentally, and we know of no adaptive reason for its spread. Knowing the distribution of polyploidy or instead dysploidy in a particular clade or geographic region can help set up testable hypotheses about evolutionary pathways, for example about the frequency of past hybridizations.

Here we investigate chromosome number evolution in the Colchicaceae using the likelihood approach of Mayrose et al. (2010), which models the frequency of past events that could explain the observed chromosome numbers in a group. The approach requires either a phylogram or an ultrametric tree and parameterizes four types of changes, duplication of the entire chromosome complement, fusion (loss) of chromosomes, fission (gain) of chromosomes, and triploidization (called demi-duplication by Mayrose et al.). The method was tested using artificial and empirical datasets in the original work by Mayrose and colleagues, and has so far been used in five studies (Ness et al., 2011: Pontederiaceae; Cusimano et al., 2012: Araceae;

Ocampo and Columbus, 2012: *Portulaca*; Harpke et al., 2013: *Crocus*; Metzgar et al., 2013: fern genus *Cryptogramma*). Based on these (still few) studies, it does not appear to be biased towards inferring either polyploidy or chromosome losses or gains. For the Pontederiaceae, for example, it inferred four full genome duplications and one demi-duplication within the crown clade, while in the Araceae, chromosome fusion (loss) was the predominant inferred event and polyploidization appeared infrequent.

We here use almost 150 available chromosome counts for the Colchicaceae, a modified recent phylogeny of the family (Chacón and Renner, in review), and a newly compiled phylogeny of *Colchicum* to infer the chromosomal history of the family. Our main questions were (i) Are there predominant modes of chromosome number change in the family's different clades? And (ii) can changes in chromosome number plausibly be related to conincidental arrival in a new region or habitat type where a single polyploid or dysploid ancestor might then have radiated?

Materials and Methods

Taxon sampling and phylogenetic analyses

We used two data sets. The first consisted of a phylogram and ultrametric tree (chronogram) for 82 species of Colchicaceae (representing all genera) plus nine outgroups (representing the Alstroemeriaceae, Petermanniaceae, Ripogonaceae, and Philesiaceae) obtained from five chloroplast regions (*matK*, *ndhF*, *rbcL*, *rps16,* and *trnL-F*), one mitochondrial gene (*matR*), and the internal transcribed spacer of nuclear ribosomal DNA (Chacón and Renner, in review). Species authors, geographic origin, herbarium voucher specimen, and GenBank accession numbers are listed in Appendix 1. The second data set included 137 species of *Colchicum* (including 41 of the 57 species of *Androcymbium* transferred to *Colchicum* by Manning et al. [2007] plus 96 *Colchicum* species) some with multiple accessions (the total tree includes 185 *Colchicum* accessions), plus *Hexacyrtis dickiana* and *Ornithoglossum vulgare* as outgroups based on Vinnersten and Reeves (2003). This second matrix included sequences of the *trn*L intron, *trn*L-*trn*F intergenic spacer (IGS), *trn*Y-*trn*D IGS, *trnH-psbA* IGS, *atp*B-*rbc*L IGS, and *rps*16 intron from the studies of del Hoyo et al. (2009), Vinnersten and Reeves (2003), and Persson et al. (2011). Sequences were

aligned with MAFFT v. 6 (Katoh and Toh, 2008) for an alignment of 5042 nucleotides, which was then analyzed under maximum likelihood (ML) using RAxML v. 7.0.4 (Stamatakis, 2006) through the CIPRES Science Gateway (Miller et al., 2010). The substitution model used was the GTR + G model, this being the best-fitting model using the Akaike information criterion (AIC) in FindModel (http://hcv.lanl.gov/content/sequence/findmodel/ findmodel.html; Posada and Crandall, 1998). Statistical support for nodes was assessed by 1000 ML bootstrap replicates under the same model. An ultrametric tree was obtained in R with the function "chronopl" of the APE package v. 3.0-6 (Paradis et al., 2004), which implements the penalized likelihood method of Sanderson (2002) including appropriate cross-validation to find the best smoothing parameter.

Inference of chromosome number change

The chromosome numbers for 144 species of Colchicaceae and eight outgroup taxa were obtained from the Index to Plant Chromosome Numbers (http://www.tropicos.org/Project/IPCN; October 2012) and other literature (Appendix 2; this includes all species with published chromosome numbers). Chromosome numbers were available for 48 of the 82 species included in the family trees (phylogram and ultrametric) and for 120 of the species included in the *Colchium* trees. In a few cases, multiple GenBank sequences labeled as the same species do not group together; however, since all sequences used here have herbarium vouchers, doubtful identifications can in principle be verified later.

For maximum likelihood and Bayesian phylogenetic inferences of ancestral haploid chromosome numbers we relied on ChromEvol v. 1.3 (Jan. 2012; Mayrose et al., 2010; http://www.tau.ac.il/~itaymay/cp/chromEvol/index.html) with an extension provided by I. Mayrose (Tel Aviv University; personal communication, 29 January, 2013) that allows fixing the root node number. ChromEvol implements eight models of chromosome number change, which include the following six parameters: polyploidization (chromosome number duplication with rate ρ, "demi-duplication" or triploidization with rate μ) and dysploidization (ascending: chromosome gain rate λ; descending: chromosome loss rate δ) as well as two linear rate parameters, λ_1 and δ_1, for the dysploidization rates λ and δ, allowing them to depend on the current number

of chromosomes. Four of the models have constant rates, whereas the other four include two linear rate parameters. Both model sets also have a null model that assumes no duplication events. We first fit all models to the data with 1000 simulations per model in order to determine which one performed best. We then re-ran the analysis for the best-fit model using 10,000 simulations to compute the expected number of changes along each branch as well as the ancestral haploid chromosome numbers at nodes. The null hypothesis (no polyploidy) was tested using AIC.

Past haploid chromosome numbers were inferred on ML phylograms as well as ultrametric trees. Species for which no chromosome number information was available were cut from the trees, resulting in 52 species in the Colchicaceae tree (instead of 91) and 122 species (143 accessions) instead of 139 (187 accessions) in the *Colchicum* tree. To run ChromEvol on the Colchicaceae ultrametric tree we had to adjust the branch lengths of the tree because the root-to-tip distance was large, which can cause ChromEvol to overestimate the number of transitions. Using artifical data, Mayrose et al. (2010) showed that reliable reconstructions are obtained with root-to-tip distances ranging from 0.1 to 0.8. We therefore adjusted the branch length of the ultrametric family tree by a factor of 0.002 (resulting in a length of 0.234) to give it a similar root-tip height as that in the phylogram (with 0.248). The branch length of the phylogram of the *Colchicum* clade was adjusted by a factor of 2 (Table 2). We ran additional analyses with double or half these tree lengths to test if the results would differ substantially; this was not the case.

The maximum haploid number of chromosomes was set to 10 more than the highest empirical number (i.e., 108 + 10 = 118), the minimum number to 1. In some runs, we fixed the haploid chromosome number at the *Colchicum*+outgroups root node, once to $a = 11$ with a probability of 1 (because this was the number inferred for this node in the family-wide analysis using the phylogram) and once to $a = 8$ or 9, both with a probability of 0.5 (this being the numbers inferred for this node using the ultrametric tree for the family).

Table 2. Results of the analyses carried out in chromEvol to infer chromosome number changes in the Colchicaceae and the *Colchicum* clade using a phylogram (P) or an ultrametric tree (U).

Analysis	**Tree**							**Number of expected events along branches inferred by simulations**					**Rates**				**Chromosome no. at root node inferred by**		
		Factor	Root-tip length	Total tree length	Best model	Log-lik	AIC	Gains	Losses	Duplications	Demi-duplications	Total	λ	δ	ρ	μ	Bayes: best n - PP	Bayes: 2nd best n - PP	ML
Colchicaceae	P	1	0.183	1.139	cr	-131.4	268.8	17.4	14.6	8.1	7.3	47.4	21.1	19.5	8.9	= ρ	6-0.21	7-0.21	7
Colchicaceae	U	0.002	0.178	2.423	cr	-123.6	253.3	33.7	0	5.7	7.3	46.7	15.7	0.0	4.0	= ρ	6-0.34	5-0.33	7
***Colchicum* clade**	P	2	0.197	1.503	crde	-435	878	18.5	20.6	15.8	27.7	82.6	23.3	23.3	14.3	27.8		Fixed to 11	
***Colchicum* clade**	U	1	0.10	4.92	crde	-356.7	739.4	14.4	20.4	19.6	31.5	85.9	5.8	6.4	4.2	8.3		Fixed to 11	

The factor with which the branch of the tree have been adjusted for the analysis and the resulting tree lengths are given. Logarithmic likelihood (Log-lik) and AIC scores; best model: cr = constant rate model with duplication rate equal to the demiduplication rate; crde = constant rate model with duplication rate different to the demi-duplication rate; rate parameters: λ = chromosome gain rate, δ = chromosome loss rate, ρ = duplication rate, μ = demi-duplication rate; frequency of the four possible event types with an expectation > 0.5; haploid chromosome number (*a*) inferred at the root node under Bayesian optimization with the respective PP, and under maximum likelihood optimization (ML).

Results

Molecular phylogeny of Colchicaceae

The combined plastid, mitochondrial and nuclear data (6451 aligned nucleotides) yielded a robust phylogeny for the 82 Colchicaceae with most clades having >80% bootstrap support (Fig. 2). The monotypic genus *Kuntheria* forms a clade with *Schelhammera undulata* and *Tripladenia cunninghamii*. *Burchardia*, a genus of six species of which three are included in the phylogeny including the type species *B. umbellata* R. Brown, forms a grade at the base of the tree. All other genera with more than one species are monophyletic.

Ancestral chromosome numbers in Colchicaceae

The best model on either the phylogram or the ultrametric tree for the 48 Colchicaceae with chromosome counts assumes a duplication rate equal to the demi-duplication rate ($\rho = \mu = 8.9$ for the phylogram, and $\rho = \mu = 4$ on the ultrametric tree, Table 2). Despite the best model being the same for both trees, the inferred parameter values differ (see the rates and some of the numbers inferred along the backbone; Table 2), especially the number of inferred dysploidy events. The gain rate inferred on the phylogram was $\lambda = 21.1$, the loss rate $\delta = 19.5$. The majority of inferred events were gains (17.4) and losses (14.6). There were 8.1 duplications and 7.3 demi-duplications (Table 2). On the ultrametric tree, the inferred gain rate was $\lambda = 15.7$ and the loss rate $\delta = 0$. Of the inferred events 33.7 were gains, 0 losses, 5.7 duplications, and 7.3 demi-duplications (Table 2).

On the phylogram, the root node of the Colchicaceae was inferred to have had haploid numbers of $a = 6$, 7 or 8, with $a = 7$ having the highest posterior probability (PP; Fig. 3). Changes from $a = 7$ to 11 and back down to 10 and 9 were inferred along the backbone (Fig. 3). On the ultrametric tree, the inferred number at the root was $a = 6$, with a gradual increase along the backbone via individual chromosome gains ($a = 6$ to 7 to 8 to 9; Appendix 3). In both trees, all other first-diverging taxa have numbers based on $a = 7$ (*Uvularia*, *Disporum cantoniense*, *Kuntheria*, *Schelhammera*, and *Tripladenia*), with the exception of some *Disporum* species with higher numbers (Fig. 3 and Appendix 2).

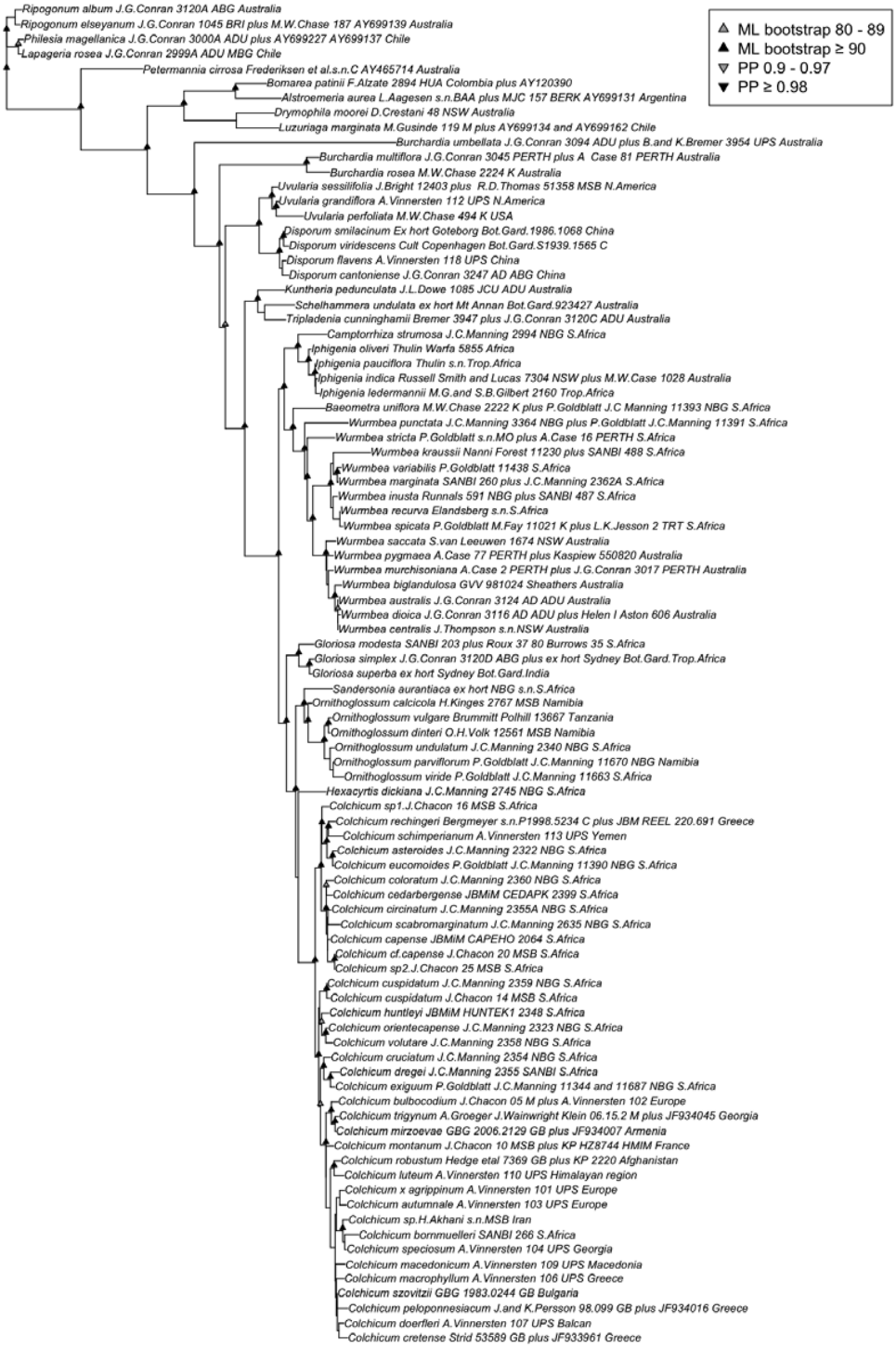

Figure 2. Maximum Likelihood phylogeny for Colchicaceae based on the combined analysis of plastid, mitochondrial, and nuclear markers. The tree is rooted on the sister clade, Alstroemeriaceae, plus species of Petermanniaceae, Ripogonaceae, and Philesiaceae. Bootstrap support and posterior probabilities for each clade are indicated with the triangles according to the values explained in the inset.

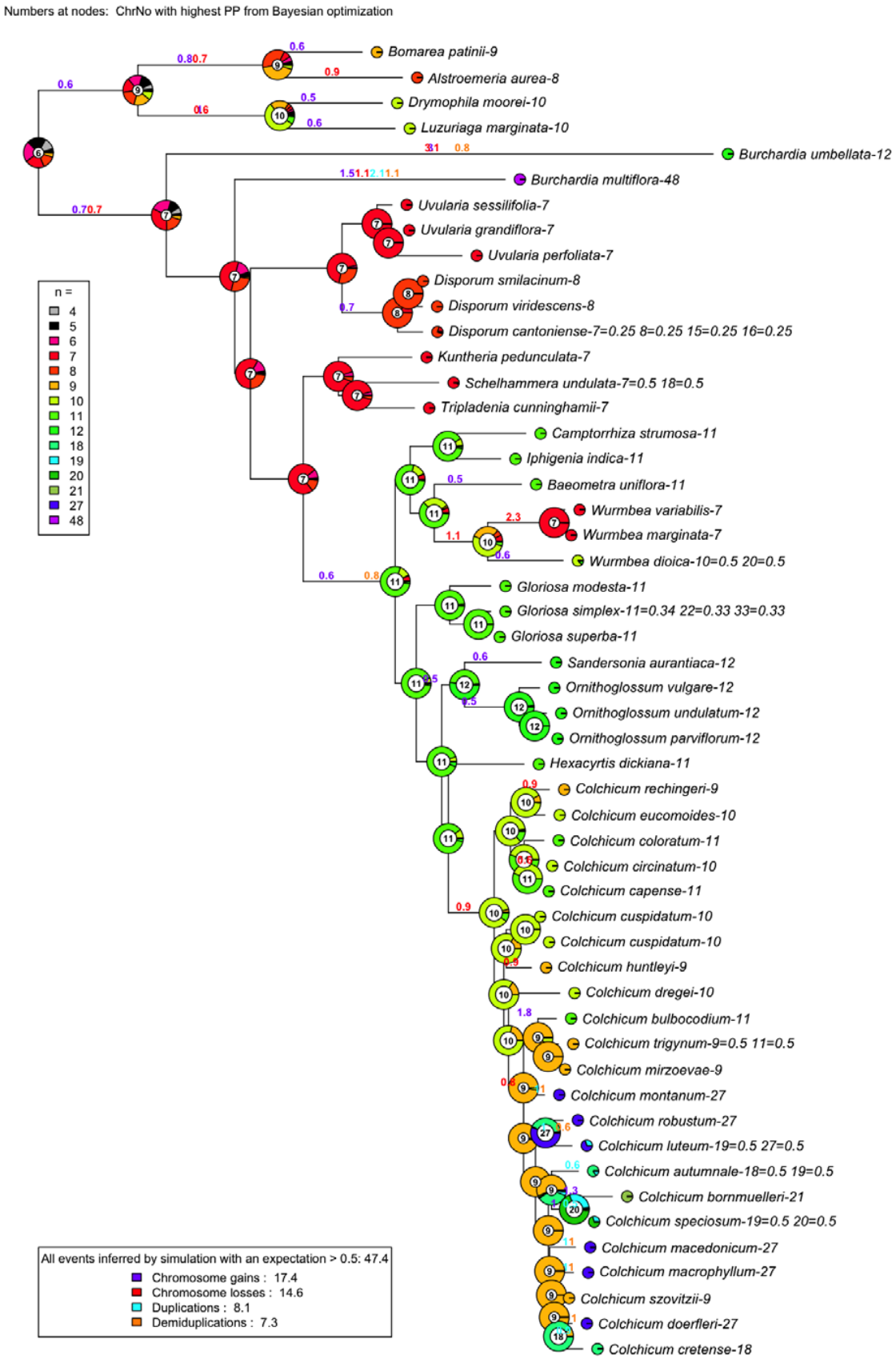

Figure 3. *Continued*

(Figure 3. *Continued)* Chromosome number reconstruction for the Colchicaceae family inferred on the maximum likelihood phylogeny, with outgroups included. Numbers at the tips are the haploid chromosome numbers of species. Pie charts at nodes and tips represent the probabilities of the inferred haploid chromosome numbers; the color-coding of the chromosome numbers is explained in the inset. Numbers inside the pie charts are the chromosome numbers with the highest probability. Numbers above branches represent the expected number of the four possible events, i.e. gains, losses, duplications, and demi-duplications occuring along that branch inferred with an expectation > 0.5. The color-coding of events is explained in the insets, the sum of the single events and the total number of events are also indicated there.

Another difference between inferences on the phylogram vs. the ultrametric tree concerns the haploid chromosome number of the crown node of *Wurmbea*, which in the phylogram is $a = 10$, while in the ultrametric tree it is $a = 7$ (Fig. 3 and Appendix 3, respectively). In both trees, the ancestral chromosome number for the *W. marginata*/*W. variabilis* clade is $a = 7$, while for *W. dioica* it is $a = 10$. Therefore, 2.3 chromosome losses were inferred on the phylogram for the branch leading to *W. marginata*/*W. variabilis* (change from $a = 10$ to 7; Fig. 3), and instead 1.3 chromosome gains and 0.6 demi-duplications for the branch leading to *W. dioica* on the ultrametric tree (change from $a = 7$ to 10; Appendix 3). In the phylogram, a chromosome loss on the stem of *Colchicum* led to $a = 10$, decreasing further to $a = 9$ through another loss (Fig. 3; see next section about *Colchicum*). In the ultrametric tree instead, single chromosome gains are inferred to have led from $a = 8$ to $a = 9$ (Appendix 3).

Molecular phylogeny of *Colchicum*

Figure 4 shows a phylogeny for 187 accessions representing 137 species of *Colchicum*, rooted on the two outgroup taxa and with maximum likelihood bootstrap values. Nine major clades with interesting chromosome number changes are labeled along the backbone (A to I; Fig. 4). A large clade of species previously placed in *Androcymbium* (clade A in Fig. 4; see Appendices 1 and 2 with the *Androcymbium* species names) with two subclades, one with 17 species (21 accessions) and one with 14 species (22 accessions), is sister to a clade containing the remaining *Colchicum*

species (B). While some of the clades A – I lack statistical support, the distribution of chromosome numbers (next section) matches the topology (Figs. 4, 5).

Figure 4. *Continued*

(**Figure 4.** *Continued***)** Maximum likelihood phylogeny of *Colchicum* based on chloroplast sequences from the studies of Persson et al. (2011), del Hoyo et al. (2009), and Vinnersten and Reeves (2003). The tree is rooted on *Hexacyrtis dickiana* and *Ornithoglossum vulgare.* Bootstrap support for each clade are indicated with the triangles according to the values explained in the inset.

Reconstruction of ancestral chromosome numbers in *Colchicum*

The best-fitting model of chromosome number evolution on the phylogram and ultrametric tree for *Colchicum* (141 accessions representing 120 species) inferred a = 10 as ancestral in the clade (PP = 0.7; see Fig. 5 and Appendix 4; note that we allowed a maximal root node number of a = 11, based on the results from the family-level analysis). There follows an inferred reduction to a = 9 and three increases to a = 11 in *C. coloratum* (unknown subsp.), *C. capense* and *C. coloratum* subsp. *burchelli* (see tip labels in Fig. 5). A duplication and a demi-duplication (from a = 9 to 18 and to 27, from a = 9 to 27, and from a = 9 to 12 and to 27) could explain the six *Colchicum* clades composed exclusively of species with n = 27 (clades 1, 2, 5, 6, 10 and within clade 8). Lower numbers, such as a = 9, appear mostly in clade 3 (Fig. 5). Along the backbone, a reduction in ancestral chromosome number to a = 8 is inferred on the branch leading to clade G, followed by an increase in chromosome number to a = 12 by a demi-duplication on the branch leading to clade H.

Discussion

Chromosome number evolution in early-diverging Colchicaceae

Maximum likelihood phylogenies for 82 species of Colchicaceae or 137 species of *Colchicum* (Figs. 2 and 4) were here used to infer probable events that could explain the observed chromosome number range in this family (Figs. 3 and 5). A genus first sequenced in the present study is the monospecific Australian *Kuntheria*, which forms a clade with *Schelhammera undulata*, the type species of an Australian genus that has two other species, and the monospecific Australian *Tripladenia*, all three with a chromosome number of $2n$ = 14 (Fig. 3 and Appendix 2) and an inferred haploid ancestral number of a = 7 (Fig. 3).

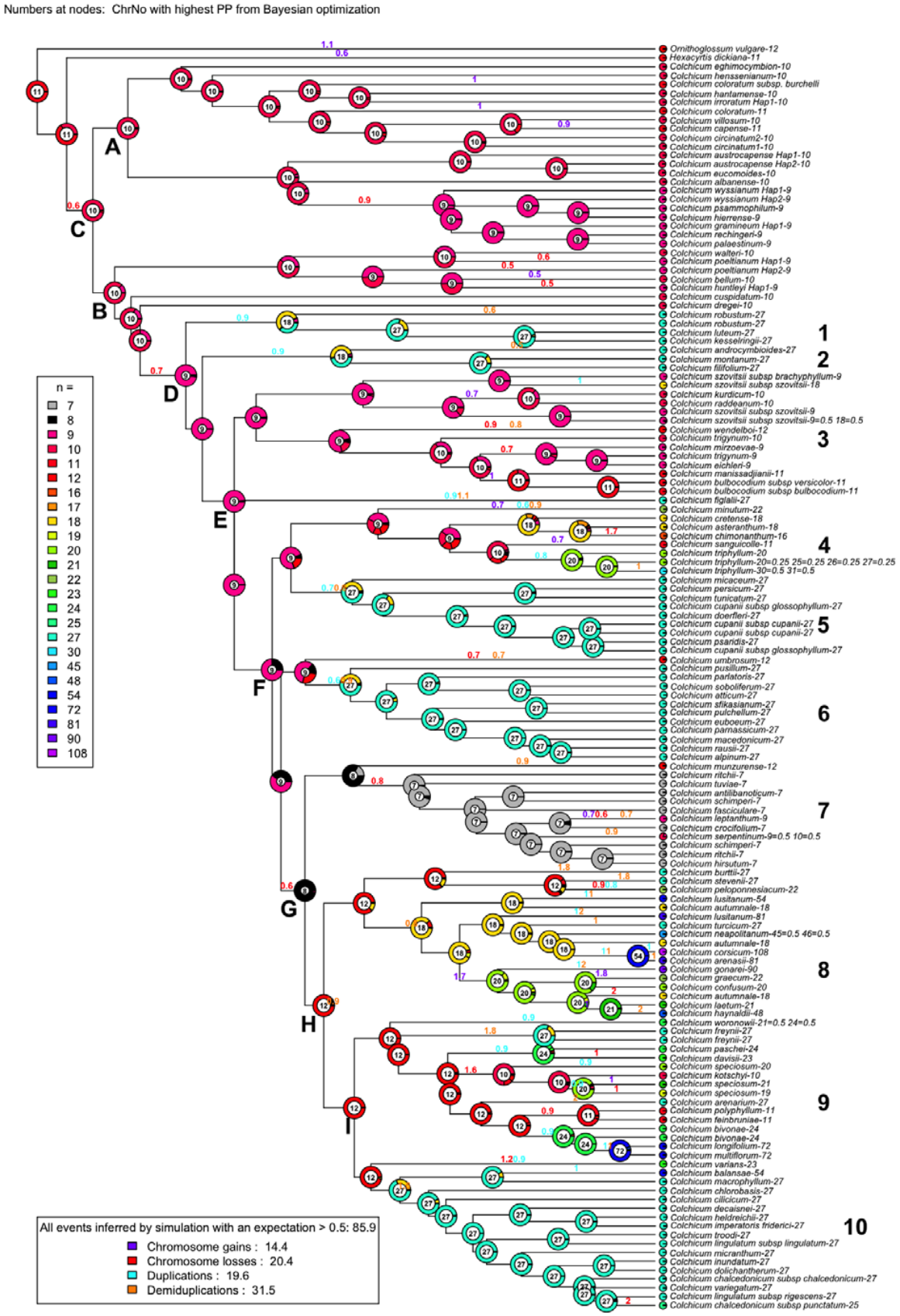

Figure 5. *Continued*

(**Figure 5.** *Continued*) Chromosome number reconstruction in *Colchicum* inferred on the ultrametric tree, with outgroups included. In this analysis the root node number has been fixed to $a = 11$. Numbers at the tips are the haploid chromosome numbers of species. Pie charts at nodes and tips represent the probabilities of the inferred haploid chromosome numbers; the color-coding of the chromosome numbers is explained in the inset. Numbers inside the pie charts are the chromosome numbers with the highest probability. Numbers above branches represent the expected number of the four possible events, i.e. gains, losses, duplications, and demi-duplications occuring along that branch inferred with an expectation >0.5. The color-coding of events is explained in the insets, the sum of the single events and the total number of events are also indicated there.

The ancestral chromosome number of the Colchicaceae may have been $a = 6$, 7 or 8, and $a = 7$ apparently was maintained in the non-African groups, such as the Asian/North American *Disporum*-*Uvularia* clade (Fig. 3), which began diversifying around 16 million years ago (Ma; Chacón and Renner, in review). Among the early-diverging Colchicaceae is the Australian *Burchardia umbellata*, the type species of the genus *Burchardia*, which groups far from the remaining five species traditionally placed in this genus (Fig. 2; Keighery and Muir 2005). Solving this problem will require transfer of the names *B. bairdiae*, *B. congesta*, *B. monantha*, *B. multiflora*, and *B. rosea* to a new genus. The chromosome numbers in this unnatural assembly are $2n = 24$ in *B. congesta* and *B. umbellata*, $2n = 48$ in *B. monantha* and *B. bairdiae*, and $2n = 96$ in *B. multiflora* (see Appendix 2).

The early-diverging branches of Colchicaceae are distributed in Australia (*Burchardia umbellata*, the ex-*Burchardia* clade, *Tripladenia*, *Kuntheria, Schelhammera*), Asia (*Disporum*), and North America (*Uvularia*) and have $a = 7$, while the younger, mainly African taxa (see geographic distributions in Appendix 1) share $a = 11$, apparently as the result of chromosome fissions and demi-duplications (Fig. 3). The initial diversification of the African clade began during the Eocene, apparently after a single long-distance dispersal event from Australia about 46 Ma (Chacón and Renner, in review) and involved expansion into arid-adapted vegetation; all the African species (including the African *Colchicum*; next section) are perennial herbs with an underground corm, adapted to high seasonality and aridity (Nordenstam, 1998; Vinnersten 2003).

Wurmbea, a genus of c. 50 species distributed in Africa and Australia, likely as the result of a "return" dispersal event from Africa eastwards across the Indian Ocean (Chacón and Renner, in review), has three published chromosome numbers, two from South African species (*W. variabilis* and *W. marginata*, both *2n* = 14) and one from Australia for *W. dioica* with *2n* = 20 and 40 (Fig. 3 and Appendices 2 and 3). Different from all other Colchicaceae, the 30 Australian species of *Wurmbea* usually have unisexual flowers in addition to, or instead of, bisexual flowers. Species can be dioecious or gynodioecious (Barrett and Case 2006; Case et al. 2008). In *W. dioica*, which is gynodioecious, the individuals with bisexual flowers suffer high levels of selfing (Vaughton and Ramsey, 2003). It would be interesting to test the possibility of widespread polyploidy in the Australian clade of *Wurmbea*, with an accompanying loss of self-incompatibility and selection for unisexual flowers to reduce selfing and inbreeding depression.

Chromosome number evolution in *Colchicum*

Previous less-densely sampled phylogenies already suggested that *Colchicum* and *Androcymbium* were not mutually monophyletic (Vinnersten and Reeves, 2003 and Manning et al., 2007: both with the same 18 species of *Androcymbium* and 9 species of *Colchicum*; del Hoyo and Pedrola-Monfort, 2008: 29 species of *Androcymbium* and 5 species of *Colchicum*; del Hoyo et al., 2009: 41 species of *Androcymbium* and 6 species of *Colchicum*; Persson et al., 2011: 3 species of *Androcymbium* and 96 species of *Colchicum*). The phylogeny presented here with 41 species previously placed in *Androcymbium* and 96 of *Colchicum* (Appendix 1) shows beyond doubt that the type species of *Androcymbium, A. melanthoides* (*C. melanthiodes*), is more closely related to species of *Colchicum* than it is to many species placed in *Androcymbium*, supporting Manning et al.'s (2007) sinking of *Androcymbium* into *Colchicum.*

The Mediterranean and northern African species of *Colchicum* (*C. gramineum, C. rechingeri*, *C. palaestinum, C. wyssianum*, *C. hierrense*, and *C. psammophilum*) apparently descend from South African ancestors that dispersed northward from the Namib Desert sometime during the Pliocene (ca. 3.5 Ma; del Hoyo et al., 2009). The North African species have asymmetrical karyotypes and *2n* = 18, while the South African species have symmetrical karyotypes and *2n* = 20 or 22 (Caujapé-Castells et

al., 2001). Caujapé-Castells et al. proposed that descending dysploidy (from 22 or 20 to 18) might explain these numbers, similar to the best-fitting model found with ChromEvol (Fig. 5).

The ancestral haploid chromosome number of *Colchicum* inferred here is *a* = 10, while Persson et al. (2011) using parsimony-based trait reconstruction with the chromosome numbers coded as seven states: 0 = 9; 1 = 8; 2 = 7; 3 = 10; 4 = 11; 5 = 12; ? = unknown (aneuploid?) inferred a base number of x = 9. They also inferred reductions from 9 to 8 and from 9 to 7 as well as increases to 10 or 11, just as inferred here (Fig. 5). However, there are also discrepancies. For instance, the clade formed by *C. szovitsii* / *C. raddeanum* / *C. kurdicum* is inferred to have *a* = 9 in our study, but 10 by Persson et al. These may be minor differences, but they illustrate the uncertainty in any reconstructions of karyological evolution. The approach proposed by Mayrose et al. (2010), however has the advantage of quantifying the uncertainty, which is not possible under parsimony-based chromosome number reconstruction.

Contrasting with the stable chromosome numbers found in much of the family, *Colchicum* (including *Androcymbium*) shows high levels of variation in ploidy levels (Fig. 5). The frequent polyploidization has been attributed to the presence of colchicine in this genus (Nordenstam, 1998), but since the entire family contains this alkaloid (Vinnersten and Larsson, 2011) its presence is unlikely by itself to explain the polyploidy in *Colchicum*. Instead, there is also dysploidy, with reductions to *a* = 7, increases to *a* = 10, 11, 12, 18, 20, 21, 24, 27, in addition to the ploidy changes to 54 and 72 (see Fig. 5). Interspecific hybridization seems likely based on observations of intermediate morphologies, sterility in some cultivars, and mathematical addition of haploid chromosome numbers (Persson, 1999; Persson et al., 2011). For instance, the cultivated species *C. laeutum* (*2n* = 44–45) could be a hybrid between *C. autumnale* (*2n* = 36) and *C. cilicicum* (*2n* = 54) since 18 + 27 = 45 (Persson et al., 2011). Unfortunately, no experimental crosses or other studies addressing hybridization appear to have been published, and the extent to which past hybridization explains the ploidy lability in *Colchicum* therefore remains an open question.

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Supporting information

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

Appendix 1. Species sampled in this study, voucher information, geographic origin, and GenBank accession numbers.

Appendix 2. Chromosome numbers available for the Colchicaceae and the outgroup species

Appendix 3. Chromosome number reconstruction for the Colchicaceae family inferred on the ultrametric tree.

Appendix 4. Chromosome number reconstruction in *Colchicum* inferred on the maximum likelihood phylogeny.

Appendix 1. Species sampled in this study, voucher information, geographical origin and GenBank accession numbers. Species for which sequences from different plants were combined are marked by an asterisk. Previously accepted *Androcymbium* names are writen in parenthesis.

Species name	Voucher	Geographical origin	*ndh*F	*rbc*L	*atp*B-*rbc*L	*mat*K	*rps*16	*trn*L-*trn*F	*mat*R	ITS
COLCHICACEAE										
Baeometra uniflora (Jacq.) G. J. Lewis*	M.W. Chase 2222 (K)	South Africa	JQ404638	JQ404747		JQ404867			JQ404979	JQ405061
Baeometra uniflora (Jacq.) G. J. Lewis*	P. Goldblatt & J.C. Manning 11393 (NBG)	South Africa			AJ554246		AJ551201			
Burchardia multiflora Lindl.*	J.G. Conran 3045 (PERTH, ADU)	Western Australia, c. 12 km W of Walpole along SW Hwy	KC899383	KC899449		KC899619			KC899512	KC899567
Burchardia multiflora Lindl.*	A. Case 81 (PERTH)	Australia			EU044616			EU044684		
Burchardia rosea Keighery	M.W. Chase 2224 (K)	Australia			AJ554248		AJ551203	AJ560295		
Burchardia umbellata R. Br.*	K. Bremer 3954 (UPS)	Australia					AJ551204	AJ551349		
Burchardia umbellata R. Br.*	J.G. Conran 3094 (AD, ADU)	South Australia, Muddy Flat NFS 5.6 km N of Nangwarry off Riddock Hwy	KC899384	KC899450		KC899620			KC899513	
Camptorrhiza strumosa (Baker f.) Oberm.	J.C. Manning 2994 (NBG)	South Africa, Mpumalanga	JQ404641	AJ554249	AJ554249	JQ404870	AJ551205	AJ551350		
Colchicum asteroides (J.C. Manning & Goldblatt) J.C. Manning & Vinn. (*Androcymbium asteroides* Manning & Goldblatt)	J.C. Manning 2322 (NBG)	South Africa, Northern Cape Province	KC899369	KC899435	AJ554228	KC899605	AJ551183	AJ551333	KC899499	KC899555
Colchicum autumnale L.	A. Vinnersten 103 (UPS)	Bulgaria	KC899385	KC899451	AJ554251	KC899621	AJ551207	AJ551352	KC899514	KC899568
Colchicum bornmuelleri Freyn	SANBI LHMS #266	South Africa	JQ404640	JQ404749		JQ404869			JQ404981	JQ405062
Colchicum bulbocodium Ker Gawl.*	J. Chacón 05 (M)	Europe	JQ404639	JQ404748		JQ404868			JQ404980	
Colchicum bulbocodium Ker Gawl.*	A. Vinnersten 102 (UPS)	Spain			AJ554247		AJ551202	AJ551348		
Colchicum capense (L.) J.C. Manning & Vinn. (*Androcymbium capense* (L.) K. Krause)	JBM CAPEHO 2064 (HMiM)	South Africa, Cape Town, Hopefield			EU236983		EU237064			
Colchicum cedarbergense (U. Müll.-Doblies, Hähnl., U.U. Müll.-Doblies & D. Müll.-Doblies) J.C.	JBM CLANPK 2399 (HMiM)	South Africa, Wuppertal, Clanwilliam			EU236984		EU237065			

Manning & Vinn. (*Androcymbium cedarbergense* U. Müll.-Doblies, Hähnl., U.U. Müll.-Doblies & D. Müll.-Doblies)										
Colchicum cf. *capense* (L.) J.C. Manning & Vinn. (*Androcymbium* cf. *capense* (L.) K. Krause)	J. Chacón 20 (MSB, BOL)	South Africa, Western Cape, Katbakkies	KC899370	KC899436		KC899606			KC899500	KC899556
Colchicum circinatum (Baker) J.C. Manning & Vinn. (*Androcymbium circinatum* Baker)	J.C. Manning 2355A (NBG)	South Africa, Northern Cape, Springbok	KC899371	KC899437	AJ554232	KC899607	AJ551187	AJ551335	KC899501	KC899557
Colchicum coloratum J.C. Manning & Vinn. (*Androcymbium latifolium* Schinz)	J.C. Manning 2360 (NBG)	South Africa, Nieuwoudtville	KC899376	KC899442	AJ554238	KC899612	AJ551193	AJ551341	KC899506	KC899561
Colchicum cretense Greuter	A. Strid 53589 (GB)	Greece, Crete	KC899386	KC899452	JF933961	KC899622	JF934213	JF934464		
Colchicum cruciatum (U. Müll.-Doblies & D. Müll.-Doblies) J.C. Manning & Vinn. (*Androcymbium cruciatum* U. Müll.-Doblies & D. Müll.-Doblies)	J.C. Manning 2354 (NBG)	South Africa, Cape Province	KC899372	KC899438	AJ554233	KC899608	AJ551188	AJ551336	KC899502	KC899558
Colchicum cuspidatum (Baker) J.C. Manning & Vinn. (*Androcymbium cuspidatum* Baker) 1	J. Chacón 14 (MSB, BOL)	South Africa, Western Cape, Cederberg Wilderness Area	KC899373	KC899439		KC899609			KC899503	KC899559
Colchicum cuspidatum (Baker) J.C. Manning & Vinn. (*Androcymbium cuspidatum* Baker) 2	J.C. Manning 2359 (NBG)	South Africa	KC899374	KC899440	AJ554234	KC899610	AJ551189		KC899504	KC899560
Colchicum doerfleri Haláksy	A. Vinnersten 107 (UPS)	Macedonia	KC899387	KC899453	AJ554252	KC899623	AJ551208	AJ551353		KC899570
Colchicum dregei (C. Presl.) J.C. Manning & Vinn. (*Androcymbium dregei* C. Presl.)	J.C. Manning 2355 (NBG)	South Africa, Northern Cape, Springbok	JQ404520	JQ404669	AJ554235	JQ404777	AJ551190	AJ551338	JQ404901	JQ405011
Colchicum eucomoides (Jacq.) J.C. Manning & Vinn. (*Androcymbium eucomoides* (Jacq.) Willd.)	P. Goldblatt & J.C. Manning 11390 (NBG)	South Africa, Western Cape, Caledon, Hermanus			AJ554236		AJ551191	AJ551339		
Colchicum exiguum subsp. *vogelii* (U. Müll.-Doblies & D. Müll.-Doblies) J.C. Manning & Vinn.* (*Androcymbium exiguum* subsp. vogelii (U.Müll.-Doblies & D.Müll.-Doblies)	P. Goldblatt & J.C. Manning 11344 (NBG)	Namibia, Spitskop	KC899375	KC899441		KC899611			KC899505	

U.Müll.-Doblies & D.Müll.-Doblies)										
Colchicum exiguum subsp. *vogelii* (U. Müll.-Doblies & D. Müll.-Doblies) J.C. Manning & Vinn.* (*Androcymbium exiguum* subsp. *vogelii* (U.Müll.-Doblies & D.Müll.-Doblies) U.Müll.-Doblies & D.Müll.-Doblies)	P. Goldblatt & J.C. Manning 11687 (NBG)	South Africa			AJ554243		AJ551198	AJ551345		
Colchicum huntleyi (Pedrola, Membrives, J.M. Monts. & Caujape) J.C. Manning & Vinn. (*Androcymbium huntleyi* Pedrola, Membrives, J.M. Monts. & Caujape)	JBM HUNTEK1 2348 (HMiM)	South Africa, Vioolsdrif, Eksteenfontein			EU236998		EU237075	AY622713		
Colchicum luteum Baker	A. Vinnersten 110 (UPS)	Himalayan region	KC899388	KC899454	AJ554253	KC899624	AJ551209	AJ551354	KC899515	KC899569
Colchicum macedonicum Kosanin	A. Vinnersten 109 (UPS)	Macedonia	KC899389	KC899455	AJ554254	KC899625	AJ551210	AJ551355	KC899516	KC899571
Colchicum macrophyllum B. L. Burtt	A. Vinnersten 106 (UPS)	Greece, Crete	KC899390	KC899456	AJ554255	KC899626	AJ551211	AJ551356	KC899517	KC899572
Colchicum mirzoevae (Gabrieljan) K. Perss.	B. Zhirair GBG 2006-2129 (GB)	Armenia, Ijevan	KC899391	KC899457	JF934007	KC899627	JF934259	JF934510		
Colchicum montanum L.*	J. Chacon 10 (MSB)	France, Nantes	KC899392	KC899458		KC899628			KC899518	KC899573
Colchicum montanum L.*	K. Persson HZ8744 (HMiM)	Spain, Huesca			EU237023		EU237101			
Colchicum orienticapense (U. Müll.-Doblies & D. Müll.-Doblies) J.C. Manning & Vinn. (*Androcymbium orientecapense* U. Müll.-Doblies & D. Müll.-Doblies)	J.C. Manning 2323 (NBG)	South Africa, Northern Cape, Hanover	KC899377	KC899443	AJ554240	KC899613	AJ551195	AJ551342	KC899507	KC899562
Colchicum peloponnesiacum Rech. f. & P.H. Davis	J. & K. Persson 98-099 (GB)	Greece, Achaea	KC899393	KC899459	JF934016	KC899629	JF934267	JF934519	KC899519	KC899574
Colchicum rechingeri (Greuter) J.C. Manning & Vinn.* (*Androcymbium rechingeri* Greuter)	E. Bergmeier s.n. P1998.5234(C)	Greece, Crete	KC899378	KC899444		KC899614				
Colchicum rechingeri (Greuter) J.C. Manning & Vinn.* (*Androcymbium rechingeri* Greuter)	JBM REEL 220.691 (HMiM)	Greece, Crete, Elafonisos			EU237012		EU237089	AY608525		
Colchicum robustum (Bunge) Stef. (s.str.)*	I.C. Hedge et al. 7369 (GB)	Greece	KC899394	KC899460		KC899630				

Colchicum robustum (Bunge) Stef. (s.str.)*	K. Persson 2220	Afghanistan, Parwan			EU237024		EU237102	EU237048		
Colchicum scabromarginatum (Schltr. & K. Krause) J.C. Manning & Vinn. (*Androcymbium scabromarginatum* Schltr. & K.Krause)	J.C. Manning 2635 (NBG)	South Africa, Little Namaqualand	KC899379	KC899445	AJ554241	KC899615	AJ551196	AJ551343	KC899508	KC899563
Colchicum schimperianum (Hochst.) C.Archer (*Androcymbium schimperianum* (Hochst.) K.Perss.)	A. Vinnersten 113 (UPS)	Yemen			AJ554272		AJ551228	AJ551367		
Colchicum sp.	H. Akhani s.n. (MSB)	Iran	KC899395	KC899461		KC899631			KC899520	KC899575
Colchicum sp.2	J. Chacón 25 (MSB, BOL)	South Africa, Western Cape, Katbakkies	KC899381	KC899447		KC899617			KC899510	KC899565
Colchicum sp1.	J. Chacón 16 (MSB, BOL)	South Africa, Western Cape, Hondsverbrand	KC899380	KC899446		KC899616			KC899509	KC899564
Colchicum speciosum Steven	A. Vinnersten 104 (UPS)	Georgia	KC899396	KC899462	AJ554256	KC899632	AJ551212		KC899521	KC899576
Colchicum szovitzii Fisch. & C.A. Mey. subsp. *szovitsii*	GBG 1983-0244 (GB)	Bulgaria, Stara Zagora	KC899397	KC899463	JF934043	KC899633	JF934291	JF934418		
Colchicum trigynum (Steven ex Adams) Stearn.*	A. Groeger & J. Wainwright Klein 06.15.2 (MSB)	Georgia	KC899398	KC899464		KC899634			KC899522	KC899577
Colchicum trigynum (Steven ex Adams) Stearn.*	J. Cuba 90-088 (GB)	Georgia, Tbilisi			JF934045		JF934045	JF934548		
Colchicum volutare (Burch.) J.C. Manning & Vinn. (*Androcymbium volutare* Burch.)	J.C. Manning 2358 (NBG)	South Africa	KC899382	KC899448	AJ554244	KC899618	AJ551199	AJ551346	KC899511	KC899566
Colchicum x *agrippinum* Baker	A. Vinnersten 101 (UPS)	Europe	KC899399	KC899465	AJ554250	KC899635	AJ551206	AJ551351	KC899523	KC899578
Disporum cantoniense (Lour.) Merr.	J.G. Conran 3247 (AD, ABG)	Australia, cultivated Adelaide Bot. Gard.	KC899400	KC899466		KC899636			KC899524	KC899579
Disporum flavens Kitag.	A. Vinnersten 118 (UPS)	China	KC899401	KC899467	AJ554257	KC899637	AJ551213	AJ551357	KC899525	KC899580
Disporum smilacinum A. Gray	Ex hortus Göteborg Bot. Gard. 1986-1068	China	KC899402	KC899468	AJ554258	KC899638	AJ551214	AJ551358	KC899526	KC899581
Disporum viridescens (Maxim.) Nakai	Cultivated S1939-1565 (C)	China	JQ404647	JQ404755		JQ404876			JQ404986	JQ405064
Gloriosa modesta (Hook.) J.C.Manning & Vinn.*	SANBI LHMS #203	South Africa	KC899403	KC899469		KC899639			KC899527	KC899582
Gloriosa modesta (Hook.) J.C.Manning & Vinn.*	J.E. Roux 37/80 & J.P. Burrows 35 (NBG)	South Africa			AJ554269		AJ551225	AJ551365		

Gloriosa simplex L.*	J.G. Conran 3120D (ABG)	Australia, cultivated Adelaide Bot. Gard.	KC899404	KC899470		KC899640			KC899528	KC899583
Gloriosa simplex L.*	Ex hortus Sydney Bot. Gard.	Tropical Africa			AJ554262		AJ551216	AJ551360		
Gloriosa superba L.	Ex hortus Sydney Bot. Gard.	India	KC899405	KC899471	AJ554261	KC899641	AJ551218	AJ551362	KC899529	KC899584
Hexacyrtis dickiana Dinter	J.C. Manning 2745 (NBG)	South Africa	KC899406	KC899472		KC899642			KC899530	KC899585
Iphigenia indica (L.) A. Gray ex Kunth*	M.W. Chase 1028 (K)	India	AY224999	AJ417893	EU044593		AJ551223	AJ551392		
Iphigenia indica (L.) A. Gray ex Kunth*	J. Russell-Smith & D. Lucas 7304 (NSW)	Australia, Northern territory	KC899407	KC899473						
Iphigenia ledermannii Engl. & K.Krause	M.G. & S.B. Gilbert 2160 (UPS)	Tropical Africa			AJ554265		AJ551221	AJ551394		
Iphigenia oliveri Engl.	M. Thulin & Warfa 5855 (UPS)	Africa			AJ554266		AJ551222	AJ551364		
Iphigenia pauciflora Martelli	M. Thulin et al. 3504 (UPS)	Tropical Africa			AJ554268		AJ551224	AJ551393		
Kuntheria pedunculata (F. Muell.) Conran & Clifford	J.L. Dowe 1085 (JCU, ADU)	Australia, Queensland, Josephine Creek Falls	KC899408	KC899474		KC899643			KC899531	KC899586
Ornithoglossum calcicola Krause & Dinter	H. Kinges 2767 (MSB)	Namibia, Omuramba	KC899409	KC899475		KC899644			KC899532	KC899587
Ornithoglossum dinteri Krause	O.H. Volk 12561 (MSB)	Namibia, Maltahöhe	KC899410	KC899476		KC899645			KC899533	
Ornithoglossum parviflorum B. Nord.	P. Goldblatt & J.C. Manning 11670 (NBG)	South Africa, Cape Province	KC899411	KC899477	AJ554276	KC899646	AJ551232	AJ551370	KC899534	KC899588
Ornithoglossum undulatum Sweet	J.C. Manning 2340 (NBG)	South Africa	KC899412	KC899478	AJ554277	KC899647	AJ551233	AJ551371	KC899535	KC899589
Ornithoglossum viride Aiton	P. Goldblatt & J.C. Manning 11663	South Africa, Cape Province			AJ554278		AJ551234	AJ551372		
Ornithoglossum vulgare B. Nord.*	R.K. Brummitt & R.M. Polhill 13667 (UPS)	Tanzania			AJ554279		AJ551235	AJ551373		
Ornithoglossum vulgare B. Nord.*	W. Giess et al. 11089 (MSB)	Namibia, Tsumkwe	KC899413	KC899479		KC899648			KC899536	KC899590
Sandersonia aurantiaca Hook.	Ex hortus NBG s.n.	South Africa	KC899418	KC899484	AJ554280	KC899652	AJ551236	AJ560299	KC899541	KC899592
Schelhammera undulata R. Br.	Ex hortus Mt. Annan Bot. Gard. 923427	Australia	KC899419	KC899485	AJ554281	KC899653	AJ551237	AJ551374	KC899542	KC899593
Tripladenia cunninghamii D. Don*	K. Bremer 3947 (UPS)	Australia			AJ554282		AJ551238	AJ551375		
Tripladenia cunninghamii D. Don*	J.G. Conran 3120C (AD, ABG)	Australia, cultivated Adelaide Bot. Gard.	KC899420	KC899486		KC899654			KC899543	KC899594
Uvularia grandiflora Sm.	A. Vinnersten 112 (UPS)	North America	KC899421	KC899487	AJ554284	KC899655	AJ551240	AJ551377	KC899544	KC899595
Uvularia perfoliata L.	M.W. Chase 494	USA	JQ404662	JQ404767		JQ404891		AJ560300	JQ405001	KC899596

	(K)									
Uvularia sessilifolia L.*	J. Bright 12403 (UPS)	North America			AJ554286		AJ551242	AJ551378		
Uvularia sessilifolia L.*	R.D. Thomas 51358 (MSB)	USA, Louisiana	KC899422	KC899488		KC899656			KC899545	
Wurmbea australis (R.J.Bates) R.J.Bates	J.G. Conran 3124 (AD, ADU)	South Australia, Battery Track, Mt Remarkable NP at N edge of park	KC899423	KC899488		KC899657			KC899546	
Wurmbea biglandulosa (R. Br.) T. D. Macfarl.	Sheathers GVV 981024	Australia	KC899424	KC899489	AJ554288	KC899658	AJ551244	AJ551380	KC899547	KC899597
Wurmbea centralis T. D. Macfarlane	J. Thompson s.n. (NSW)	South Australia	KC899425							
Wurmbea dioica (R. Br.) F. Muell.*	J.G. Conran 3116 (AD, ADU)	South Australia, Anstey Hill Recreation Reserve above gate #3	KC899426	KC899490		KC899659			KC899548	KC899598
Wurmbea dioica (R. Br.) F. Muell.*	H.I. Aston 606 (UPS)	Australia			AJ554289		AJ551245	AJ551381		
Wurmbea glassii (C.H.Wright) J.C.Manning & Vinn.	J.C. Manning 2168 (NBG)	South Africa, Cape Province			AJ554273		AJ551229	AJ551368		
Wurmbea inusta (Baker) B. Nord	P. Runnalls 591 (NBG)	South Africa, Western Cape, Somerset West	KC899427	KC899491	AJ554291	KC899660	AJ551247	AJ551383	KC899549	KC899599
Wurmbea kraussii Baker	I. Nänni & F. Forest 11230 (NBG)	South Africa	JQ404663	JQ404768	AJ554292	JQ404892	AJ551248	AJ551384	JQ405002	JQ405070
Wurmbea marginata (Desr.) B. Nord.	J.C. Manning 2362A (NBG)	South Africa, Western Cape, Caledon	KC899428	KC899492	AJ554293	KC899661	AJ551249	AJ551385	KC899550	KC899600
Wurmbea murchisoniana T.D.Macfarl.*	A. Case 2 (PERTH)	Australia			EU044604			EU044703		
Wurmbea murchisoniana T.D.Macfarl.*	J.G. Conran 3017 (PERTH)	Western Australia, 200 m S of Murchison R xing	KC899429	KC899493		KC899662			KC899551	KC899601
Wurmbea punctata (L.) J.C. Manning & Vinn.*	J.C. Manning 3364 (NBG)	South Africa, Western Cape, Elandsberg Farm	KC899430	KC899494		KC899663			KC899552	KC899602
Wurmbea punctata (L.) J.C. Manning & Vinn.*	P. Goldblatt & J.C. Manning 11391 (NBG)	South Africa			AJ554274		AJ551230	AJ551369		
Wurmbea pygmaea (Endl.) Benth.*	A. Case 77 (PERTH)	Australia	AF547012							
Wurmbea pygmaea (Endl.) Benth.*	B. Kaspiew 550820	Australia			AJ554297		AJ551253	AJ551389		
Wurmbea recurva B. Nord.	A. Vinnersten s.n. (UPS)	South Africa	KC899431	KC899495		KC899664			KC899553	KC899603
Wurmbea saccata T. D. Macfarl. & S. J. van Leeuwen	S. van Leeuwen 1674 (NSW)	Western Australia	KC899432	KC899496	AJ554299		AJ551255	AJ551391		

Wurmbea spicata (Burm.f.) T. Durand & Schinz*	P. Goldblatt M. Fay 11021 (K)	South Africa, Western Cape, Piketberg	KC899433							
Wurmbea spicata (Burm.f.) T. Durand & Schinz*	L.K. Jesson 2 (TRT)	South Africa			EU044613			EU044691		
Wurmbea stricta (Burm.f.) J.C.Manning & Vinn.	P. Goldblatt s. n. (MO)	South Africa	JQ404659	JQ404765		JQ404888		AJ560298	JQ404998	JQ405068
Wurmbea stricta (Burm.f.) J.C.Manning & Vinn.*	A. Case 16 (PERTH)	South Africa, Canberra			EU044615					
Wurmbea variabilis B. Nord.	P. Goldblatt 11438	South Africa, Cape Province	KC899434	KC899498	AJ554295		AJ551251	AJ551387	KC899554	KC899604
OUTGROUPS										
ALSTROEMERIACEAE										
Alstroemeria aurea Graham*	L. Aagesen s. n. (BAA)	Argentina, Chubut	JQ404511	AY120359		JQ404771	AY120373		JQ404895	JQ405005
Alstroemeria aurea Graham*	M.C. Sheahan s.n. (K)	Chile			AY699131			AY699225		
Bomarea patinii Baker*	J. Chacón 02 (ANDES)	Colombia, Valle del Cauca	JQ404585	JQ404711		JQ404823			JQ404944	EU159951
Bomarea patinii Baker*	F. Alzate 2894 (HUA)	Colombia, Cundinamarca								EU159951
Bomarea edulis (Tussac) Herb.*	L. Aagesen w/n (SI)	Argentina					AY120390			
Drymophila moorei Baker	D. Crestani 48 (NSW)	Australia, New South Wales	JQ404645	JQ404753		JQ404874			JQ404984	JQ405063
Luzuriaga marginata (Gaertn.) Benth. & Hook.f.*	M. Gusinde 119 (M)	Chile, Magallanes	JQ404650	JQ404757		JQ404878	AY120393		JQ404988	JQ405066
Luzuriaga radicans Ruiz & Pav.*	M.W. Chase 499 (K)				AY699134			AY699162		
PETERMANNIACEAE										
Petermannia cirrosa F. Muell.	S. Frederiksen et al. s.n. (C)	Australia	AY465662	AY465714	AY699144					
PHILESIACEAE										
Lapageria rosea Ruiz & Pav.	J.G. Conran 2999A (ADU)	Cultivated Mt. Lofty Bot. Gard.	KC899414	KC899480		KC899649			KC899537	
Philesia magellanica J.F.Gmel.*	J.G. Conran 3000A (ADU)	Cultivated Mt. Lofty Bot. Gard.	KC899415	KC899481		KC899650			KC899538	
Philesia magellanica J.F.Gmel.*	M.W. Chase 545 (K)	Chile			AY699137			AY699227		
RIPOGONACEAE										
Ripogonum album R. Br.	J.G. Conran 3120A (AD)	Australia, Cultivated Adelaide Bot. Gard.	KC899416	KC899482		KC899651			KC899539	KC899591
Rhipogonum elseyanum F. Muell.*	M.W. Chase 187 (NCU)	Australia, Sydney	AF276016	Z77309	AY699139			AY699164		
Rhipogonum elseyanum F. Muell.*	J.G. Conran 1045 (ADU, BRI)	Australia, SE Queensland, Lyrebird Ridge, Springbrook Plateau	KC899417	KC899483					KC899540	

Appendix 2. Chromosome numbers available for the Colchicaceae and the outgroup species. See the literature cited below the table. The *Androcymbium* species names cited in the corresponding reference is writen in parenthesis.

Species	*2n*	References
Colchicaceae		
Baeometra uniflora (Jacq.) G.J.Lewis	22	Nordenstam 1998
Burchardia bairdiae Keighery	24	Keighery 1984; Macfarlane 1987
Burchardia congesta Lindl.	24	Keighery and Muir 2005
Burchardia monantha Domin	24	Keighery 1984
Burchardia multiflora Lindl.	48	Keighery 1984;
Burchardia umbellata R. Br.	24	Keighery and Muir 2005
Camptorrhiza strumosa (Baker) Oberm.	22	Nordenstam 1998
Colchicum alpinum DC.	54, ca 120*	Feinbrun 1958; Cecchi and Fiorini 2002; Camarada 1979*
Colchicum arenarium Waldst. & Kit.	38	Feinbrun 1958
Colchicum asteranthum Vassil. & K. Perss.	36	Vassiliades and Persson 2002
Colchicum atticum Spruner ex. Tomm.	54	Phitos et al. 1989
Colchicum austrocapense (U.Müll.-Doblies & D.Müll.-Doblies) J.C.Manning & Vinn. (*Androcymbium austrocapense* U. Müll.-Doblies & D. Müll.-Doblies)	20	Montserrat Martí et al. 2002
Colchicum autumnale L.	36, 38*	Feinbrun 1958*; Sveshnikova and Krichfalushij 1985; Krichphalushi 1989; Dobea and Hahn 1997; Persson 1999
Colchicum autumnale subsp. *autumnal*e L.	38	Murin and Majovsky 1979
Colchicum autumnale subsp. *pannonicum* (Griseb. & Schenk.) Nyman	38	Murin and Majovsky 1987
Colchicum balansae Planch.	108	Persson 1999
Colchicum baytopiorum C.D. Brickell	46	Conran 1985

Colchicum bellum (Schltr. & K.Krause) J.C.Manning & Vinn. (*Androcymbium bellum* Schltr. & Krause)	20	Montserrat Martí et al. 2002
Colchicum bivonae Guss.	32, 36*, 52*, 54*	Feinbrun 1958*; Papanicolaou 1984; Sik and Küçüker 1998; Persson 1998; Peruzzi and Cesca 2002
Colchicum boissieri Orph.	36	Sik and Küçüker 1998
Colchicum bulbocodium Ker Gawl.	22	Wetschnig 1992
Colchicum capense (L.) J.C. Manning & Vinn. (*Androcymbium capense* (L.) Krause)	22	Montserrat Martí et al. 2002
Colchicum cf. *stevenii* Kunth	14, 38	Garbari and Crisman 1988
Colchicum chalcedonicum Azn.	50	Küçüker 1984
Colchicum chalcedonicum subsp. *chalcedonicum* K. Perss.	54	Persson 1998
Colchicum chalcedonicum subsp. *punctatum* K. Perss.	50	Persson 1998
Colchicum chimonanthum K. Perss.	32	Persson 1999
Colchicum chlorobasis K. Perss.	54	Persson 2005
Colchicum cilicicum (Boiss.) Dammer	54	Persson 1999
Colchicum circinatum (Baker) J.C. Manning & Vinn. (*Androcymbium circinatum* Baker)	20	Montserrat Martí et al. 2002
Colchicum clanwilliamense (Pedrola, Membrives & J.M.Monts.) J.C.Manning & Vinn. (*Androcymbium albanense* subsp. *clanwilliamense* Pedrola, Membrives & G. Monts.)	20	Montserrat Martí et al. 2002
Colchicum coloratum J.C. Manning & Vinn. (*Androcymbium latifolium* Schinz)	22	Montserrat Martí et al. 2002
Colchicum coloratum J.C.Manning & Vinn. subsp. burchellii (Baker) J.C.Manning & Vinn. (*Androcymbium burchellii* Baker)	22	Montserrat Martí et al. 2002
Colchicum confusum K. Perss.	40	Persson 1999
Colchicum corsicum Baker	ca. 216	Persson 1993
Colchicum cretense Greuter	36	Persson et al. 2011
Colchicum cupanii Guss.	54	Feinbrun 1958; Camarada 1979; Arrigoni and Mori 1980;

		Colombo et al. 1982
Colchicum cupanii var. *latifolium* Guss.	54	Bartolo et al. 1981
Colchicum cuspidatum (Baker) J.C. Manning & Vinn. (*Androcymbium cuspidatum* Baker)	20	Montserrat Martí et al. 2002
Colchicum davisii C.D. Brickell	46	Persson 1999
Colchicum decaisnei Boiss.	54	Feinbrun 1958; Persson 1999
Colchicum doerfleri Halácsy	54	Persson et al. 2011
Colchicum dolichantherum K. Perss.	54	Persson 1999
Colchicum dregei (C. Presl.) J.C. Manning & Vinn. (*Androcymbium dregei* Presl.)	20	Montserrat Martí et al. 2002
Colchicum eghimocymbion (U.Müll.-Doblies & D.Müll.-Doblies) J.C.Manning & Vinn. (*Androcymbium eghimocymbion* U. Müll.-Doblies & D. Müll.-Doblies)	20	Montserrat Martí et al. 2002
Colchicum eichleri (Regel) K. Perss.	18	Bokeriya 1988
Colchicum euboeum (Boiss.) K. Perss.	54	Persson 1998
Colchicum eucomoides (Jacq.) J.C. Manning & Vinn. (*Androcymbium eucomoides* (Jacq.) Willd.)	20	Margeli et al. 1999
Colchicum feinbruniae K. Perss.	22	Persson 1992
Colchicum gonarei Camarada	182	Camarada 1979
Colchicum graecum K. Perss.	42-44	Persson 1988
Colchicum gramineum (Cav.) J.C. Manning & Vinn. (*Androcymbium gramineum* (Cav.) J.F. Macbr.)	18	Margeli et al. 1995, 1999
Colchicum hantamense (Engl.) J.C.Manning & Vinn. (*Androcymbium hantamense* Engl.)	20	Montserrat Martí et al. 2002
Colchicum haynaldii Heuff.	96	Persson 1999
Colchicum heldreichii K. Perss.	54	Persson 1999
Colchicum henssenianum (U.Müll.-Doblies & D.Müll.-Doblies) J.C.Manning & Vinn. (*Androcymbium henssenianum* U. Müll.-Doblies & D. Müll.-Doblies)	20	Montserrat Martí et al. 2002
Colchicum hiemale Freyn	54	Feinbrun 1958
Colchicum hierosolymitanum Feinbr.	18	Feinbrun 1958
Colchicum hierrense (A.Santos) J.C.Manning & Vinn. (*Androcymbium hierrense* A. Santos)	18	Margeli et al. 1995, 1999; Pedrola-Monfort

		and Caujapé-Castells 1998
Colchicum huntleyi (Pedrola, Membrives, J.M. Monts. & Caujape) J.C. Manning & Vinn. (*Androcymbium huntleyi* Pedrola, Membrives, J.M. Monts. & Caujapé)	18	Montserrat Martí et al. 2002
Colchicum imperatoris-friderici Siehe ex K. Perss.	54	Persson 1999
Colchicum inundatum K. Perss.	54	Persson 1999
Colchicum irroratum (Schltr. & K.Krause) J.C.Manning & Vinn. (*Androcymbium irroratum* Schltr. & Krause)	20	Montserrat Martí et al. 2002
Colchicum kotschyi Boiss.	20	Persson 1999
Colchicum laetum Steven	42	Magulaev 1992
Colchicum leptanthum K. Perss.	18	Persson 2001
Colchicum lingulatum Boiss. & Spruner	48	Conran 1985
Colchicum lingulatum Boiss. & Spruner subsp. *lingulatum*	54	Persson 1998
Colchicum lingulatum subsp. *rigescens* K. Perss.	54	Persson 1998
Colchicum liparochiadys Woronow	42, 48	Bokeriya 1988
Colchicum lusitanicum Brot.	90/92†, 94/96†106, ca 110*	Camarada 1979*; Baldini 1997; Fridlender et al. 2002†
Colchicum lusitanum Brot.	102, 106	Feinbrun 1958
Colchicum luteum Baker	38, 54*	Feinbrun 1958; Persson et al. 2011*
Colchicum macedonicum Kosanin	54	Persson et al. 2011
Colchicum macrophyllum B.L. Burtt	54	Persson 1999
Colchicum micaceum K. Perss.	54	Persson 1999
Colchicum micranthum Boiss.	54	Küçüker 1984
Colchicum minutum K. Perss.	44	Persson 1999
Colchicum mirzoevae (Gabr.) K. Perss.	18	Pogosian 1997
Colchicum montanum L.	54	Persson et al. 2011
Colchicum munzurense K. Perss.	24	Persson 1999
Colchicum neapolitanum Ten.	38, 140, 146*	Feinbrun 1958; Camarada 1979*
Colchicum palaestinum (Baker) Boulos	18	Margeli et al. 1995, 1999

(*Androcymbium palaestinum* Baker)		
Colchicum parnassicum Sart., Orph. & Heldr.	54	Persson 1988
Colchicum paschei K. Perss.	48	Persson 1999
Colchicum poeltianum (U.Müll.-Doblies & D.Müll.-Doblies) J.C.Manning & Vinn. (*Androcymbium poeltianum* U. Müll.-Doblies & D. Müll.-Doblies)	18+1-2B	Montserrat Martí et al. 2002
Colchicum psammophilum (Svent.) J.C.Manning & Vinn. (*Androcymbium psammophilum* Svent.)	18	Margeli et al. 1995, 1999; Pedrola-Monfort and Caujapé-Castells 1998
Colchicum pulchellum K. Perss.	54	Persson 1988
Colchicum pusillum Sieber	27, 54, 58	Kamari and Matthas 1986
Colchicum rausii K. Perss.	54	Persson 1999
Colchicum rechingeri (Greuter) J.C. Manning & Vinn. (*Androcymbium rechingeri* Greuter)	18+0-2B	Margeli et al. 1995, 1999
Colchicum ritchii R. Br.	14	Feinbrun 1958
Colchicum robustum (Bunge) Stef.	54	Persson et al. 2011
Colchicum sanguicolle K. Perss.	22	Persson 1999
Colchicum schimperi Janka ex Stef	14	Feinbrun 1958
Colchicum sfikasianum Kit Tan & Iatroú	54	Persson 1998
Colchicum speciosum Steven	38, 40, 42	Feinbrun 1958, Bokeriya 1988; Persson 1999
Colchicum stevenii Kunth	54	Feinbrun 1958
Colchicum szovitsii Fisch. & C.A. Mey.	18	Bokeriya 1988
Colchicum trigynum (Steven ex Adam) Stearn	18 22*, 24†	Bojeryia 1988; Magulaev 1992†; Johnson and Brandham 1997*
Colchicum triphyllum Kunze	20, 21, 42*, 54†	Feinbrun 1958; Lentini et al. 1988*; Sik and Küçüker 1998†
Colchicum tunicatum Feinbr.	54	Feinbrun 1958
Colchicum turcicum Janka	52	Küçüker 1984
Colchicum tuviae Feinbr.	14	Feinbrun 1958
Colchicum umbrosum Steven	24	Bokeriya 1988
Colchicum variegatum L.	42, 44	Feinbrun 1958; Sik and Küçüker 1998
Colchicum villosum (U.Müll.-Doblies & D.Müll.-Doblies) J.C.Manning & Vinn.	20	Montserrat Martí et al. 2002

(*Androcymbium villosum* U. Müll.-Doblies & D. Müll.-Doblies)			
Colchicum walteri (Pedrola, Membrives & J.M.Monts.) J.C.Manning & Vinn. (*Androcymbium walteri* Pedrola, Membrives & G. Monts.)		20	Montserrat Martí et al. 2002
Colchicum woronowii M.R. Bokeriya		42, 48	Bokeria 1990
Colchicum wyssianum (Beauverd & Turrett.) J.C.Manning & Vinn. (*Androcymbium wyssianum* Beauverd & Turrett.)		18 18+0-1B	Margeli et al. 1995, 1999
Colchicum zangezurum Grossh.		18	Bokeriya 1988
Disporum calcaratum D. Don		16,18? (16+2B?)	Hara 1988
Disporum cantoniense (Lour.) Merrill var. *cantoniense*		14,16, 30	Hara 1988
Disporum cantoniense var. *kawakamii* (Hayata) Hara		16, 32	Hara 1988
Disporum cantoniense var. *multiflorum* (Blume) Hara		16	Hara 1988
Disporum kawakamii Hayata		16	Saito et al. 2009
Disporum leucanthum Hara		16	Hara 1988
Disporum longistylum (Lèv. et Van.) Hara		16	Hara 1988
Disporum lutescens (Maxim.) Koidzumi		16	Hara 1988
Disporum ovale Ohwi		16	Hara 1988
Disporum sessile (Thunb.) D. Don ex Schult. & Schult.f.		16 (24)	Therman 1956; Hara 1988
Disporum smilacinum A. Gray		16	Hara 1988
Disporum uniflorum Baker		16	Hara 1988
Disporum viridescens (Maxim.) Nakai		16	Therman 1956; Hara 1988
Gloriosa carsonii Baker x *G. richmondensis*		44	Narain 1979
Gloriosa lutea auct. x *G. plantii* (Planch.) Loudon		22	Narain 1979
Gloriosa modesta (Hook.) J.C. Manning & Vinn.		22	Amano et al. 2008
Gloriosa simplex L.		22, 44, 88	Karihaloo 1985
Gloriosa superba L.	irr.	20*, 21*, 22, 66†	Narain 1981; Tarar et al. 1985; Vishwakarma and

			Tarar 1989*; Lugade and Hegde 1992; Vijayavalli and Mathew 1990a†,b, 1992†; Amano et al. 2008
Gloriosa virescens Lindl. x *G. richmondensis* x *G. superba* L.		44	Narain 1979
Hexacyrtis dickiana Dinter		22	Nordenstam 1998
Iphigenia indica (L.) A. Gray ex Kunth	11*	22	Sarkar and Datta 1978*; Rama et al. 1987
Iphigenia magnifica Ansari & R. Rao	11*	22	Rama et al. 1983, 1987; Lugade and Hegde 1994; Sarkar and Datta 1978*
Iphigenia mysorensis Arekal & Swamy	11*	22	Rama et al. 1983, 1987; Sarkar and Datta 1978*
Iphigenia novae-zelandiae (Hook.f.) Baker		20	Hair and Beuzenberg 1966
Iphigenia pallida Baker	11*	22	Rama et al. 1983, 1987; Sarkar and Datta 1978*
Iphigenia stellata Blatt.	11*	22	Rama et al. 1983, 1987; Sarkar and Datta 1978*
Kuntheria pedunculata (F.Muell.) Conran & Clifford		14	Conran 1985
Ornithoglossum parviflorum B. Nord.		24	Nordenstam 1982
Ornithoglossum undulatum Sweet		24	Nordenstam 1982
Ornithoglossum vulgare B. Nord.		24	Nordenstam 1982
Ornithoglossum zeyheri (Baker) B. Nord.		24	Nordenstam 1982
Sandersonia aurantiaca Hook.		24	Pandey and Pal 1980
Schelhammera multiflora R. Br.		14	Conran 1985
Schelhammera undulata R. Br.		14, 36*	Conran 1985; Briggs et al. 2002*
Tripladenia cunninghamii D. Don		14	Nordenstam 1998
Uvularia grandiflora Sm.	7		Therman and Denniston 1984
Uvularia perfoliata L.		14	Utech 1980
Uvularia sessilifolia L.		14	Utech 1980; Love and Love 1981; Plante 1995
Wurmbea dioica (R. Br.) F. Muell.		20, 40	Wiltshire and Jackson 2003
Wurmbea marginata (Desr.) B. Nord.		14	Nordenstam 1986

Wurmbea variabilis B. Nord.		14	Nordenstam 1986
Outgroups			
Alstroemeriaceae			
Alstroemeria aurea Graham		16	Buitendijk and Ramanna 1996; Buitendijk et al. 1998
Bomarea patinii Baker		18	Baeza et al. 2008
Drymophila moorei Baker	10		Conran 1985
Luzuriaga marginata (Gaertn.) Benth. & Hook.f.		20	Moore 1967
Petermanniaceae			
Petermannia cirrosa F. Muell.		10	Conran 1985
Philesiaceae			
Lapageria rosea Ruiz & Pav.		30+1B	Hanson et al. 2003
Philesia magellanica J.F.Gmel.		12	Moore 1981
Ripogonaceae			
Ripogonum album R.Br.		30	Hanson et al. 2003

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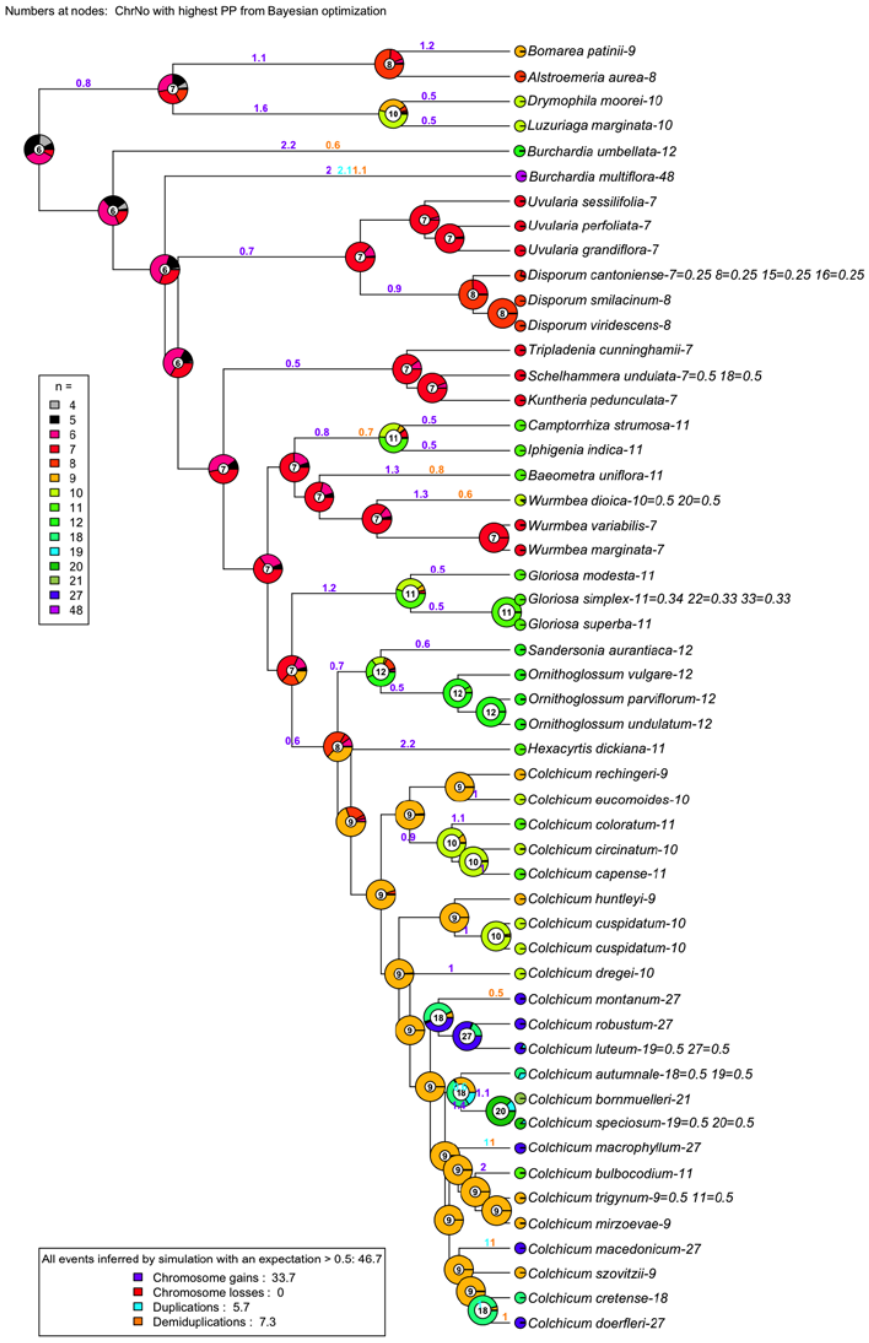
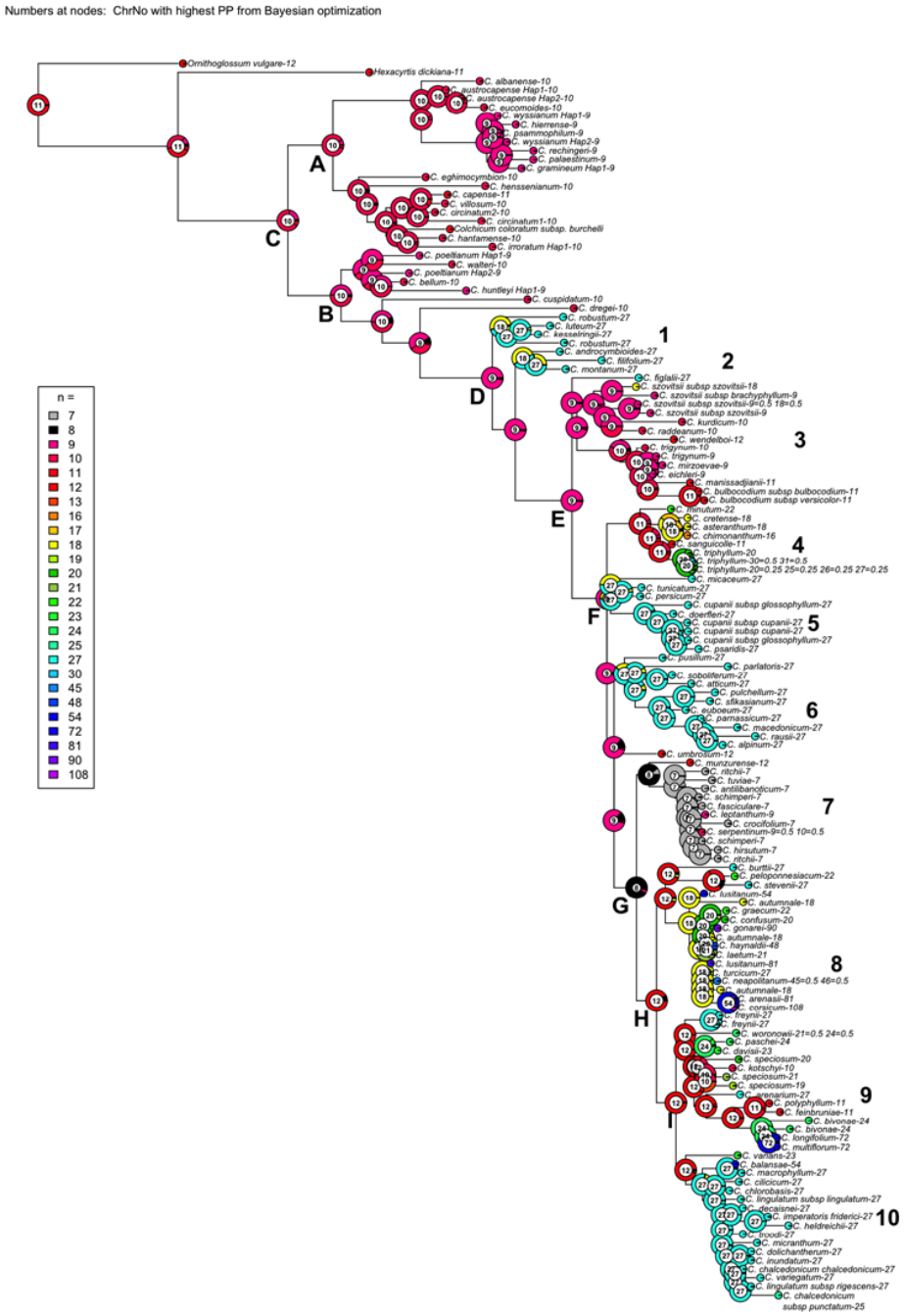
Appendix 3. Chromosome number reconstruction for the Colchicaceae family inferred on the ultrametric tree. Numbers at the tips are the haploid chromosome numbers of species. Pie charts at nodes and tips represent the probabilities of the inferred haploid chromosome numbers; the color-coding of the chromosome numbers is explained in the inset. Numbers inside the pie charts are the chromosome numbers with the highest probability. Numbers above branches represent the expected number of the four possible events, i.e. gains, losses, duplications, and demi-duplications occuring along that branch inferred with an expectation >0.5. The color-coding of events is explained in the insets, the sum of the single events and the total number of events are also indicated there.



Appendix 4. Chromosome number reconstruction in *Colchicum* inferred on the maximum likelihood phylogeny. Numbers at the tips are the haploid chromosome numbers of species. Pie charts at nodes and tips represent the probabilities of the inferred haploid chromosome numbers; the color-coding of the chromosome numbers is explained in the inset. Numbers inside the pie charts are the chromosome numbers with the highest probability. Numbers above branches represent the expected number of the four possible events, i.e. gains, losses, duplications, and demi-duplications occuring along that branch inferred with an expectation >0.5. The color-coding of events is explained in the insets, the sum of the single events and the total number of events are also indicated there.



General Discussion

Phylogenetics and evolution of Alstroemeriaceae and Colchicaceae

Relationships in the order Liliales

The results presented in Chapters 2, 4, and 6 contribute significantly to the knowledge of the evolution of the Liliales, a Linnean order and natural clade formed by ten families among which the Alstroemeriaceae and the Colchicaceae are the third and fourth most species-rich (after Liliaceae and Smilacaceae; Stevens, 2001 onwards). The molecular phylogeny of Alstroemeriaceae (Chapter 2) represents the first comprehensive phylogeny for the family, with 125 out of 204 species from all four genera sampled for both nuclear and plastid DNA sequences. The molecular phylogeny of Colchicaceae presented in Chapter 4 builds on the work of Vinnersten and Bremer (2001) by including a larger sampling of genes and species, with DNA sequences from the three plant genomes (the nucleus, mitochondria, and chloroplast) analyzed for 83 out of 270 species from all genera. The phylogeny of *Colchicum* obtained in Chapter 6 is also the first comprehensive phylogeny for this genus and includes 137 of the 157 species from the group's distribution range.

The addition of the Australian species *Petermannia cirrosa* plus supplementary outgroups from the Liliales provided maximal support (100% bootstrap) for the sister-group relationship between Petermanniaceae and the Alstroemeriaceae-Colchicaceae clade, a point that had remained unclear in the last molecular phylogenies of the Liliales (<65% bootstrap in both Fay et al., 2006 and Petersen et al., 2012). The clade formed by the three families shares the presence of a well developed primary root (Stevens, 2001 onwards).

Biogeography of Alstroemeriaceae

Ancestral area reconstruction and molecular dating in combination support Vinnersten and Bremer's (2001) hypothesis that the Alstroemeriaceae-Colchicaceae lineage dates back to a time when Australia, Antarctica, and South America were still connected, about 93.4 Ma (Fig. 1 in Iglesias et al., 2011; Fig. 3 in Chapter 2). The *Alstroemeria/Bomarea* clade diverged from the Australasian/Chilean *Luzuriaga*/*Drymophila* clade at 57.5 Ma ago (Fig. 3 in Chapter 2) during the

Paleocene-Eocene Thermal Maximum, when subtropical climates extended as far as 30ºS latitude (Zachos et al., 2001; Hinojosa and Villagrán, 2005; Iglesias et al., 2011).

The uplift of the Patagonian Andes and the establishment of the South American Arid Diagonal, less than 16 Ma (Blisniuk et al., 2005), provided the setting for the radiation of *Alstroemeria* at c. 18.4 Ma (Figs. 1 and 3 in Chapter 2). The complete absence of *Alstroemeria* along this dry belt could be the result of population extinction with the increasing aridity. The creation of arid conditions apparently resulted in the replacement of a subtropical vegetation by a xerophytic and shrubby-herbaceous vegetation, as shown by the Miocene palynological record of Patagonia (Quattrocchio et al., 2011). The stem group age of the Brazilian *Alstroemeria* clade (9.2 Ma, Fig. 3 in Chapter 2) falls towards the end of a phase of global cooling (Zachos et al., 2001: 10–14 Ma) and predates the expansion of C4 grasslands in northwest Argentina (Blisniuk et al., 2005: 7–8 Ma). A monocot group with a similar distribution range in Brazil, the Laeliinae orchids, radiated 11–14 Ma (Antonelli et al., 2010), at about the same time as the Patagonian/Brazilian *Alstroemeria* (13.5 Ma, Fig. 3 in Chapter 2).

The inferred diversification of the Andean *Bomarea* clade at c. 14.3 Ma matches the Miocene radiation of extant hummingbird lineages, which occurred between 17 and 12 Ma ago (Bleiweiss, 1998). Judging from the morphology, color, shape, nectar rewards, diurnal anthesis, and orientation of the flowers, most species of *Bomarea* are hummingbird pollinated, and this is supported by field observations for a few species (del Hoyo et al., 1999; Dziedzioch et al., 2003; Fogden and Fogden, 2006; Hofreiter and Rodriguez, 2006; Gutiérrez-Zamora, 2008; Paulsch et al., 2012). Hummingbirds are reliable pollinators in tropical forests and at mid- and high altitudes in the Andes, and adaptation to these pollinators may have contributed to range expansion, establishment and maintainance of isolated populations, and thus species formation and diversification of *Bomarea*. This could also have been the case for the Brazilian Alstroemerias, a clade that started diversifying around 9.2 Ma (Fig. 3 in Chapter 2) with numerous endemic species (44 of the c. 78 *Alstroemeria* species are endemic in Brazil). The adaptations to hummingbird pollination are evident (Buzato et al., 2000; see also Appendix 2), and the inflorescences of the Brazilian species resemble those of the Andean Bomareas. The fastest episodes of Andean mountain building occurred in the Huancabamba region (Garzione et al., 2008; Capitanio et al., 2011), which

harbors some 33 species of *Bomarea*, including 13 endemic species (Hofreiter, 2007). This deflection, located at 6 °S, is the deepest and widest depression in the high Andes and a dispersal barrier for plants and animals (Weigend, 2002). Few *Bomarea* species occur on both sides of this depression (Hofreiter, 2007), but my sampling does not permit inferring population-level divergence times. Between 5 and 2 Ma, *Bomarea* reached Central America, my species and gene sampling are, however, insufficient to infer the precise divergence times of the four endemic Central American species.

The Alstroemeriaceae are one of only five Austral-Antarctic flowering plant families that entered South America from Antarctica and expanded northwards into tropical latitudes. The other four families are Calceolariaceae, Cunoniaceae, Escalloniaceae, and Proteaceae (Table 3 in Chapter 2). Together, they comprise 670 species or <1% of Neotropical plant diversity (assuming a total of 90,000 seed plant species for the Neotropics; Gentry, 1982), and they are thus a very small floristic component compared to northern migrants into South America. Comparison of the five "southern immigrants" reveals a few similarities (Table 3 in Chapter 2): Five entered South America well before the uplift of the Patagonian Andes. Besides the Alstroemeriaceae, these are the Calceolariaceae (ca. 260 species in South America), Cunoniaceae (ca. 83 species in South America), Escalloniaceae (41 species in South America), and Proteaceae (85 species in South America). All five families expanded their geographic ranges by adapting to montane habitats and migrating northwards along the raising Andean chain. All also adapted to subtropical climates in southeastern Brazil (the cerrado shrub land and/or Atlantic coastal forests), an area they may have reached before the development of extremely dry conditions in the South American Arid Diagonal (Figs. 1 and 3 in Chapter 2). This ecological barrier may indeed be a major factor in explaining the rarity of south-to-north migration in the Neotropics.

Discovery of the first Alstroemeriaceae fossil

A fossil discovered by John G. Conran, Jennifer M. Bannister, Dallas C. Mildenhall, and Daphne E. Lee in mining pits near Otago, New Zealand, could be placed in the genus *Luzuriaga* on the basis of anatomical and morphological characteristics of the

leaves that ressemble the living species *L. parviflora* (Chapter 3). Some of these characteristics are the presence of isodiametric adaxial epidermal cells with straight to rounded walls and slightly sunken stomata (Fig. 5 in Chapter 3). As a result, the new fossil species *L. peterbanisteri* Conran, Bannister, Mildenh. & D.E.Lee sp. nov. was described. The discovery of this fossil is of great importance for studies of the biogeography of Alstroemeriaceae and related Liliales because (as mentioned in the *Introduction* of this thesis) no other fossils for the Alstroemeriaceae/Colchicaceae clade are known.

When I explored two different placements for the *Luzuriaga* fossil in different calibration nodes of the Alstroemeriaceae tree (Fig. 3 in Chapter 3), I found that the estimated times were congruent with the ages obtained in the analyses where this fossil was not included. The age of the fossil implies that Luzuriagoideae existed in New Zealand around 23 Ma ago. Like so many other New Zealand clades (Pole, 1994; Landis et al., 2008; Jordan et al., 2010) they then must have gone extinct, perhaps during times of submergence, and reached New Zealand again by long-distance dispersal from southern Chile (Fig. 3 in Chapter 2). A similar situation has been reported for the New Zealand Richeeae (Ericaceae), which date to <7 Ma, yet have New Zealand fossils that are 25–20 Ma old (Jordan et al., 2010).

Biogeography of Colchicaceae

What can be learn from the LAGRANGE experiments?

The Colchicaceae have an almost worldwide range (Appendix 1), and the chronogram showed that their history spans from the Upper Cretaceous to the Holocene (Fig. 4 in Chapter 4). These two factors made the family a suitable study system to explore certain capabilities of the LAGRANGE biogeographic software, such as the option to subdivide time into slices for which different geographic scenarios can be assigned different propabilities. To better assess the program's sensitivity to modified input trees (for example, with different numbers of nodes per time slice), I also created artificial data by modifying the empirical Colchicaceae tree.

The results illustrated how the two user-defined matrices, the adjacency matrix and the area-dispersal matrix, alone and in combination influence the outcome of my experiments (Table 2 and Appendix S4 in Chapter 4). Obviously, it is desirable that

these matrices determine the estimations made by LAGRANGE (this being their entire point). However, before my study nobody appears to have analyzed to what extent one matrix affects the other. On the other hand, as a result of my experiments, a bug in the program's likelihood calculations was revealed, which has since been fixed (R. Ree, email of May 27th 2013). In the empirical Colchicaceae data and in the artificial data I created, the simplest biogeographic models without time slices had the highest likelihoods (Fig. 5 in Chapter 4), but different results have been obtained in other study systems. For instance, in a study of the Hawaiian genus *Psychotria* the likelihood scores were better for the more constrained models (Ree and Smith, 2008). In the case of the Colchicaceae, however, the use of a constrained model would imply the *a priori* rejection of long-distance dispersal, which is implausible given the geographic disjunctions of genera such as *Wurmbea*, which has about the same number of species in South Africa and Australia.

Adding too many parameters or constraints to a model is undesirable because it can result in over-fitting, which occurs when the number of parameters is high relative to the number of observations (data). A model that has been over-fit will generally have poor predictive performance. In my experiments, I observed that the models with constrained adjacency matrices and more time slices were the most ambiguous, i.e., they inferred (postdicted) a higher number of alternative ancestral areas than did the less constrained models (Table 4 in Chapter 4). Some constrained models inferred ancestral ranges comprising Central and South America, where no Colchicaceae species occur today (Fig. 4 in Chapter 4). Although the possibility of an ancient migration through these landmasses cannot be dismissed, the likelihood scores of these models were worse than of models with fewer constraints, indicating poor fit to the data (Fig. 5 in Chapter 4). As pointed out by Ree and Sanmartín (2009), an important challenge for model-based biogeographic methods is to achieve a balance between the complexity and the realism of models against computational feasibility and inferential power (predictive performance).

Biogeographic history of Colchicaceae

According to the chronogram and ancestral area reconstruction obtained with the best-fit model (using an unconstrained adjacency matrix, 2 time slices, and 5 categories of dispersal probabilities, that is, model MC2, see Fig. 2 in Chapter 4) the initial

radiation of the Colchicaceae took place about 75 Ma in Australia (Fig. 4 in Chapter 4). Based on the MC2 biogeographic model, further range expansion into Asia could have taken place during the Palaeogene, some 62.8 Ma. However, the LAGRANGE reconstruction for the range of the relevant node in the phylogeny was uncertain, with an alternative expansion to Africa, instead of Asia, having a slighlty higher likelihood (Fig. 4 and Appendix S3-B in Chapter 4). The further diversification of the Colchicaceae in Southern-Middle Africa started about 54.2 Ma (Table 1 in Chapter 4). As Africa moved north and the Tethys Sea was closing, the ancestor of the *Disporum*/*Uvularia* clade dispersed to Southeast Asia probably via Arabia and from there to North America via the Bering land bridge (28.3–16.1 Ma, Table 1 and Fig. 4 in Chapter 4).

The main radiation of the Colchicaceae took place in Southern-Middle Africa during the Oligocene and Miocene, and several long-distance dispersal events occurred in genera such as *Wurmbea*, *Iphigenia,* and *Androcymbium* (Fig. 4 in Chapter 4). The dispersal of *Wurmbea* eastwards across the Indian Ocean from southern Africa to Australia was inferred as having taken place c. 25.2 Ma (Table 1 in Chapter 4). It could have involved oceanic rafting facilitated by the West Wind Drift (Berg and Linder, 2009). The dispersal of *Androcymbium* from southern Africa to the Mediterranean region in Europe and Northern Africa may have taken place about 19 Ma, with subsequent diversification of species in eastern Europe and the Arabian Peninsula. These species form today's *Colchicum* clade. These results contradict the findings of del Hoyo et al. (2009), who inferred three long-distance dispersal events starting at the end of the Miocene, c. 7 Ma, as a result of the formation of the late Miocene-Pliocene arid track in the east of Africa. By contrast, I inferred that the diversification of *Androcymbium* started between 30–24.4 Ma, during the Oligocene (Table 1 in Chapter 4), a date closer to the estimated diversification times for other plant lineages of the South African Cape Region, where *Androcymbium* is most diverse.

Chromosome evolution in Alstroemeriaceae and Colchicaceae

Distribution patterns of rDNA in *Alstroemeria*

The molecular phylogeny of *Alstroemeria* provided the basis for an evolutionary interpretation of cytogenetic features, such as the distribution of the FISH rDNA signals on the chromosomes and the genome size. The maximum likelihood phylogram of *Alstroemeria* (Fig. 2 in Chapter 5) revealed two monophyletic groups: A clade of species distributed in north-central Chile, and a clade occurring in south-central Chile, Argentina, and Brazil. The Brazilian clade is nested among Chilean/ Argentinean ancestors, meaning that one cannot construct a contrast between all Chilean species on the one hand and all Brazilian ones on the other as done in the study by Buitendijk and Ramanna (1996).

The Chilean alstroemerias in "clade a" (Fig. 2 in Chapter 5) grow in regions with long periods of drought (Muñoz-Schick and Moreira-Muñoz, 2003; Moreira-Muñoz, 2007), while the Brazilian species in general grow in more humid, less drought-stressed habitats. The ecological differences between the species may have led Buitendijk et al. (1997) to contrast Chilean and Brazilian "karyotype groups" that supposedly differ in PI/DAPI ratios and 2C values: Group 1 comprised *A. magnifica*, *A. pelegrina*, *A. philippii* and *A. pulchra*; group 2 *A. angustifolia*, *A. aurea* and *A. hookeri*; group 3 *A. ligtu* ssp. *ligtu* and *A. ligtu* ssp. *simsii*; and group 4 *A. brasiliensis*, *A. caryophyllaea*, *A. inodora* and *A. psittacina*. Groups 1 and 4 are recovered in my molecular tree (Fig. 2 in Chapter 5), while group 2 is unnatural (the monophyly of group 3 is not addressed since I only included one of the two subspecies of *A. ligtu*).

Regarding the localization and variability in the number of rDNA sites, the number of 18/25S rDNA sites can vary from 5–7 sites in the *A. hookeri*/*A. pelegrina* clade, to 16–17 in *A. aurea* (Fig. 3 in Chapter 5), with closely related species, such as *A. hookeri* and *A. pelegrina*, having 18 or just 4 5S rDNA sites (Table 2 in Chapter 5), implying rapid increase or decrease of these sites (Cajas et al., 2009 for a study focusing on *A. hookeri*). The only Brazilian species studied so far have nine (*Alstroemeria* cf. *rupestris*) and ten (*A. pulchella*) 18/25S rDNA signals (Figs 3 and 4 in Chapter 5). Such relatively drastic changes in rDNA sites usually indicate chromosomal rearrangements, such as typically occur in pericentromeric and telomeric regions (Schubert and Lysak, 2011). This could also be the case in

Alstroemeria, which presents telomeric sequences near most 18/25S and 5S rDNA terminal sites (Fig. 5 in Chapter 5). If all 78 species of *Alstroemeria* turn out to have $2n = 16$ chromosomes (Appendix S1 in Chapter 5), genome evolution in this genus would exclusively have involved reorganizations of chromosome structure, rather than polyploidy as in many other species-rich monocot genera (e.g., Taketa et al., 1999: *Hordeum*; Adams et al., 2000: *Aloe*; Martínez et al., 2010: *Iris* subgenus *Xiphium*). An earlier study also invoked pericentric inversions to explain the patterns of heterochromatin location in eight *Alstroemeria* karyotypes (Buitendijk and Ramanna, 1996).

Besides such primary rearrangements of chromosome structure, mobility in rDNA sites can also result from transposon-mediated transpositions (Datson and Murray, 2006; Raskina et al., 2008) that can be activated by abiotic stresses, for example, drought (Kalendar et al., 2000; Aprile et al., 2009). Drought stress-related transposon activity in *Alstroemeria* might have increased during the fluctuating dry/wet climatic conditions in Miocene South America when the plant clade studied here diversified (see Chapter 2). Attributing cytogenetic features to this or other factors, such as the Andean uplift (e.g., Buitendijk and Ramanna, 1996), however remains speculative until more in-depth studies.

The discovery of interstitial telomeric sites near the centromeres in *A.* cf. *rupestris* (Fig. 5a in Chapter 5) hints at a Robertsonian fusion of chromosomes (Leitch and Leitch, 2012). Such fusions have been invoked to explain bimodal karyotype organization in Asparagaceae (McKain et al., 2012) and may also underlay the bimodal karyotypes in *Alstroemeria*. The hypothesis of end-to-end fusion (resulting in a reduction in chromosome number) would provide an explanation for *Bomarea* having $2n = 18$ (Appendix S1 in Chapter 5), while *Alstroemeria* has $2n = 16$. Further cytogenetic studies using telomeric probes are required to test this hypothesis.

Chromosome number evolution in the Colchicaceae

One of the main contributions of the family-level phylogeny that I generated for the Colchicaceae was the placement of the monospecific Australian genus *Kuntheria* (never sequenced before) in a clade with *Schelhammera* and *Tripladenia* (Fig. 2 in

Chapter 6). These three Australian genera share a chromosome number of *2n* = 14 (Appendix 2 in Chapter 6) and an inferred haploid ancestral number of *a* = 7 (Fig. 3 in Chapter 6). Based on morphological similarities, Vinnersten and Manning (2007) ranked them as a tribe, Tripladeniae.

The analyses conducted in the ChromEvol software program suggested that the most plausible haploid ancestral chromosome numbers of the Colchicaceae were *a* = 6, 7 or 8, and that *a* = 7 was maintained in the Asian/North American *Disporum-Uvularia* clade (Fig. 3 in Chapter 6).

The gain or loss of single chromosomes, either by dysploidy or by aneuploidy was the main event responsible for changes in chromosome number in the Colchicaceae (Table 2 in Chapter 6). We found that the early-diverging branches of Colchicaceae, which are distributed in Australia (*Burchardia*, *Tripladenia*, *Kuntheria, Schelhammera*), Asia (*Disporum*), and North America (*Uvularia*) have *a* = 7, while the younger, mainly African taxa share *a* = 11 (Fig. 3 in Chapter 6). The inferred changes could have taken place during the initial diversification of the African clade, which involved expansion into arid-adapted vegetation (Chapter 4).

In *Wurmbea,* a genus with species in South Africa and Australia (see Chapter 4), changes in chromosome number may relate to changes in sexual systems. Different from all other Colchicaceae, which are hermaphrodites, the 30 Australian species of *Wurmbea* usually have unisexual and/or bisexual flowers, and the species can be dioecious (i.e. having male and female flowers on separate plants) or gynodioecious (i.e. species in which individual plants bear only female flowers or only bisexual flowers; Barrett and Case, 2006; Case et al., 2008). Best studied is the Australian *W. dioica*, a gynodioecious species for which polyploidy has been reported (Appendix 2 in Chapter 6) and in which individuals with bisexual flowers suffer high levels of selfing (Vaughton and Ramsey, 2003). It would be interesting to test the possibility of widespread polyploidy in the Australian clade of *Wurmbea*, with an accompanying loss of self-incompatibility and selection for unisexual flowers to reduce selfing and inbreeding depression.

Chromosome number evolution in *Colchicum*

Previous less-densely sampled phylogenies already suggested that *Colchicum* and *Androcymbium* were not mutually monophyletic (Vinnersten and Reeves, 2003 and Manning et al., 2007: both with the same 18 species of *Androcymbium* and 10 species of *Colchicum*; del Hoyo and Pedrola-Monfort, 2008: 29 species of *Androcymbium* and 5 species of *Colchicum*; del Hoyo et al., 2009: 41 species of *Androcymbium* and 6 species of *Colchicum*; Persson et al., 2011: 3 species of *Androcymbium* and 96 species of *Colchicum*; Nguyen et al., 2013: 11 species of *Androcymbium* and 6 species of *Colchicum*). The phylogeny of *Colchicum* that I obtained with help of Dr. Cusimano with 41 species previously placed in *Androcymbium* and 96 of *Colchicum* (Appendix 1 in Chapter 6) shows beyond doubt that the type species of *Androcymbium, A. melanthoides* (*C. melanthiodes*), is more closely related to species of *Colchicum* than it is to many species placed in *Androcymbium*, supporting Manning et al.'s (2007) sinking of *Androcymbium* into *Colchicum.* In order to uphold the principle of monophyly I therefore decided to accept the last taxonomic treatment and refer only to *Colchicum sensu lato*.

The ancestral haploid chromosome number of *Colchicum* (including *Androcymbium*) inferred in our analyses was $a = 10$ (Fig. 5 in Chapter 6), while Persson et al. (2011) inferred a base number of x = 9, using parsimony-based trait reconstruction with the chromosome numbers coded as seven states: 0 = 9; 1 = 8; 2 = 7; 3 = 10; 4 = 11; 5 = 12; ? = unknown (aneuploid?). They also inferred reductions from 9 to 8 and from 9 to 7 as well as increases to 10 or 11, just as inferred in our study (Fig. 5 in Chapter 6). However, for some *Colchicum* species Persson et al. (2011) obtained different ancestral numbers and this could be related with the uncertainty associated with any reconstruction of karyological evolution. The ChromEvol modeling approach developed by Mayrose et al. (2010), which can be carried out in a Bayesian framework, at least has the advantage of quantifying the uncertainty (as posterior probabilities), which is not possible under parsimony-based chromosome number reconstruction.

Compared to the remaining Colchicaceae, *Colchicum* showed a striking variation in ploidy levels (Fig. 5 in Chapter 6). The frequent polyploidization has been attributed to the presence of colchicine (Nordenstam, 1998), but since the entire family contains this alkaloid (Vinnersten and Larsson, 2011) its presence is unlikely

by itself to explain the polyploidy in *Colchicum*. Another explanation could be hybridization, judging from intermediate morphologies, sterility in some cultivars, and mathematical addition of haploid chromosome numbers (Persson, 1999; Persson et al., 2011). For instance, the cultivated species *C. laeutum* (*2n* = 44–45) could be a hybrid between *C. autumnale* (*2n* = 36) and *C. cilicicum* (*2n* = 54) since 18 + 27 = 45 (Persson et al., 2011). Unfortunately, no experimental crosses, sequencing of nuclear genes (allowing the detection of paralogs), or other studies addressing hybridization (such as FISH experiments) appear to have been published, and the extent to which past hybridization explains the ploidy lability in *Colchicum* therefore remains an open question.

General Conclusions

My research on the Alstroemeriaceae and Colchicaceae has contributed to the knowledge and understanding of two aspects of the evolution of plants; first, the untangling of the biogeogeographic patterns of the southern hemisphere and the strengths and risks of a parametric method of ancestral area reconstruction, and second, the understanding of chromosome evolution in a clade of Liliales from a phylogenetic perspective.

The biogeographic studies revealed that the most recent common ancestor of the Alstroemeriaceae/Colchicaceae lived during the Cretaceous in East Gondwana. The Alstroemeriaceae is one of only five southern hemisphere plant families that entered South America before the main Andean uplift and diversified in the Neotropics northwards until reaching Central America and eastern Brazil. The evolution of the Alstroemeriaceae during the Miocene was strongly influenced by abiotic factors, such as the Andean orogenesis and the establishment of the South American Arid Diagonal. The adaptations to hummingbird pollination in *Bomarea* and the Brazilian *Alstroemeria* probably played a role in the diversification of these clades. The discovery of fossil leaves of the extint species *Luzuriaga peterbannisteri* Conran, Bannister, Mildenh., & D.E.Lee sp. nov. in mining pits in New Zealand indicates a long paleogeographic history of *Luzuriaga*, and evidences the biogeographic connections between South America and Australasia during the Oligo–Miocene.

After the split from the Alstroemeriaceae, the Colchicaceae continued diversifying in Australia during the Cretaceous. The main species radiation occured in southern Africa between the Palaoecene and Miocene, forming a clade of plants adapted to arid conditions. Long-distance dispersal played an important role in the evolution of the family. For instance, during the Miocene-Oligocene *Wurmbea* dispersed back to Australia while *Colchicum* (*sensu lato*) migrated to the Mediterranean region in Europe and northern Africa. Another lineage diversified in Asia (*Disporum*) and then reached North America likely through the Bering Land Bridge (*Uvularia*). My experiments with a recently developed biogeographic software program underlined the inherent difficulties of the model-based methods of ancestral area reconstruction. As perhaps expected, the models are very sensitive to user's *ad hoc* specification of the non-default parameters that are supposed to incorporate

information on plausible or impossible past range expansion pathways. Although this option constitutes the main advantage of LAGRANGE a thorough design of the adjacency matrix and time slices is necessary to avoid model over-parameterization. It is also advisable to compare results obtained with constrained versus unconstrained matrices before trusting in the results.

My FISH study on *Alstroemeria* chromosomes revealed an extremely high variation in the 5S and 18/25S rDNA sites of closely related species, indicating a rapid increase, decrease, or translocations of these ribosomal genes. The observation of telomeric sites near the centromeres of the chromosomes of *Alstroemeria* cf. *rupestris* probably resulted from a Robertsonian fusion, a mechanism that could also explain $n = 8$ chromosomes in *Alstroemeria* compared to $n = 9$ in the sister genus *Bomarea*.

Different mechanisms of chromosomal evolution were inferred for the Colchicaceae, a clade with a high variation of chromosome numbers and ploidy levels, especially in *Colchicum*. The maximum-likelihood method implemented in ChromEvol suggested that the main events behind the changes in chromosome number in the Colchicaceae probably were gains or losses of single chromosomes. To achieve a deeper understanding of the ploidy variation in *Colchicum*, it will be necessary to sequence single-copy nuclear genes to better resolve species relationships and then to elucidate the possible role of hybridization in the polyploidization.

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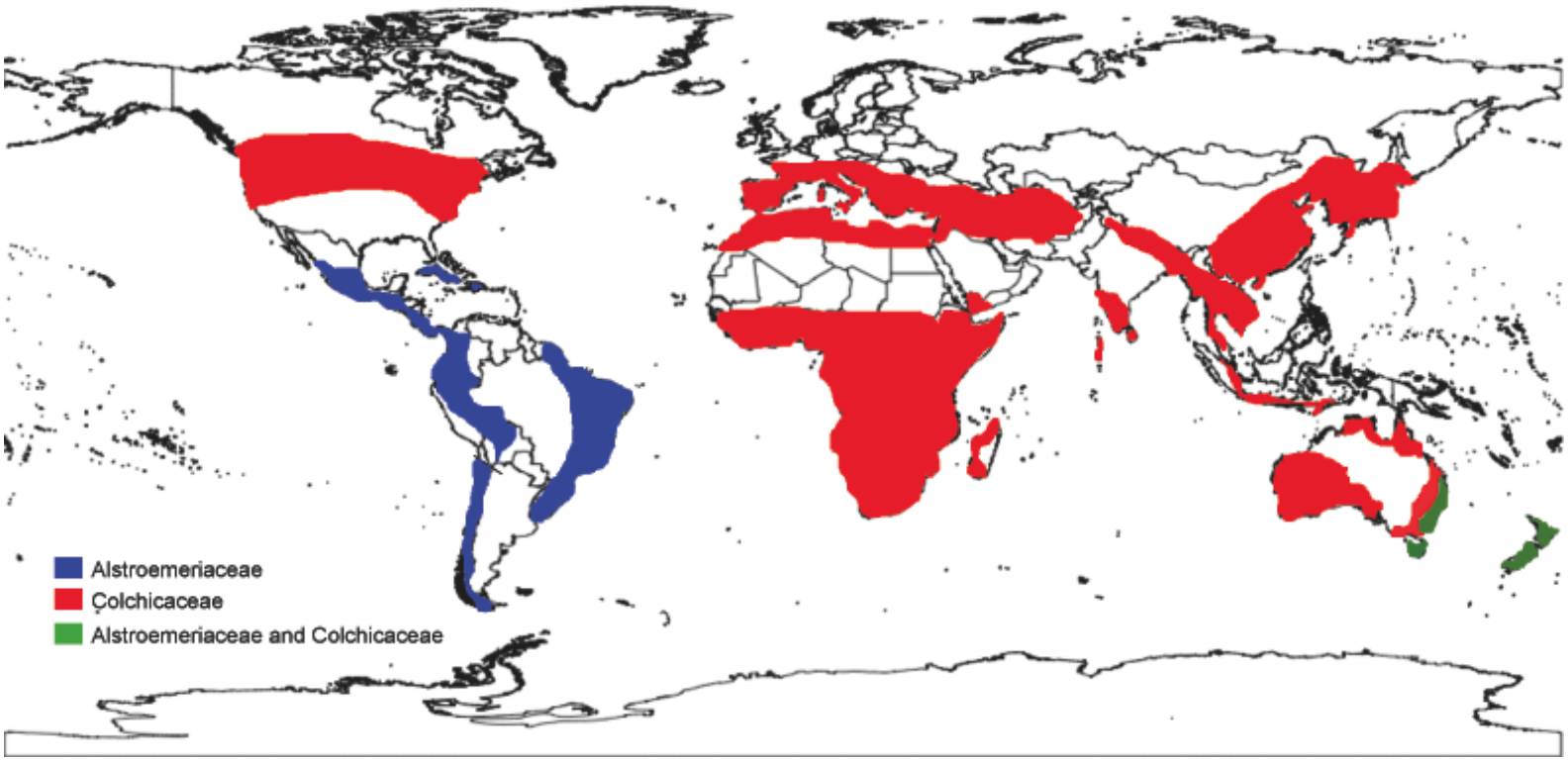
Appendices

Appendix 1. Geographic distribution of the Alstroemeriaceae-Colchicaceae clade. The Alstroemeriaceae is shown in blue, the Colchicaceae in red, and the only area where species of both families are found is shown in green.



Appendix 2. *Alstroemeria isabellana* (left) and *A. inodora* (right) are part of the hummingbird-pollinated floras of the Atlantic rain forests of southeastern Brazil (photo from I. Sazima used with permission).

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PUBLICATIONS

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GRANTS AND PRICES

2012 EES Young Researcher Prize for PhD students, EES^{LMU} Conference 2012, Munich Graduate School for Evolution, Ecology and Systematics, Ludwig-Maximilans University, Munich, Germany, October 4 – 5 2012.

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Ginés - Mera Fellowship for Postgraduate Studies in Agrobiodiversity, October 2003 – March 2005, from the International Center for Tropical Agriculture – CIAT, Cali, Colombia

ORAL AND POSTER PRESENTATIONS

Chacón, J. and S. S. Renner. When do models that account for changing continental connectivities make a difference? An example from the Colchicaceae. 66th International Conference of the International Biogeography Society. North Miami, Florida, USA, January 8–13, 2013

Chacón, J. History of a pair of clades that parted ways in East Gondwana and then followed non-overlapping trajectories: corms and fire-adaptations in South Africa, the Mediterranean, and Australia vs. hummingbird pollination in South America. EES Conference 2012, Ludwig-Maximilans University, Munich, Germany, October 4–5, 2012.

Chacón, J. and S. S. Renner. Alstroemeriaceaeae, a plant family with an Austral-Antarctic distribution that expanded into tropical latitudes – inferring the when and how.

14th Nordic Meeting on Tropical Botany, Gothenburg, Sweden, 6–8 August 2012

Chacón, J., A. Vinnersten, and S. S. Renner. Nuclear and mitochondrial data tell a new story about genus boundaries and biogeography of the Colchicaceae.

Southern African Society for Systematic Biology 10th Meeting, SASSB X, Arniston, South Africa, July 16 – 20, 2012

Chacón, J., M. Camargo de Assis, A. W. Meerow, and S. S. Renner. New insights into the biogeography of the Austral floristic realm from a complete phylogeny for the Alstroemeriaceae.

XVIII International Botanical Congress IBC 2011, Melbourne, Australia, July 23 – 30 2011

Chacón, J. and S. S. Renner. Phylogeny and biogeography of the Alstroemeriaceae, an important clade of the Austral floristic realm.

BioSystematics Berlin 2011, Berlin, Germany, February 21 – 27, 2011

Chacón, J. and S. S. Renner. Molecular phylogenetics and biogeography of Alstroemeriaceae, an important clade of the Austral floristic realm (Poster).

VW Status Symposium in Evolutionary Biology, Frauenchiemsee, Germany, May 9 – 12, 2010

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Chacón, J., S. Madriñán, M. W. Chase and J. J. Bruhl. Molecular Phylogenetics and Historical Biogeography of *Oreobolus* (Cyperaceae).

XVII International Botanical Congress IBC 2005, Vienna. Abstracts Book, XVII International Botanical Congress, Vienna, Austria: Austria Center Vienna, 2005. Pp: 214.

Chacón, J., S. Madriñán and J. Tohme. Phylogenetic patterns in the genus *Manihot* Mill. (Euphorbiaceae): biogeography and comparative ecology of Central American and South American species.

III Congreso Colombiano de Botánica, 2004, Popayán. Abstracts Book, III Congreso Colombiano de Botánica, Popayán: Universidad del Cauca, 2004. Pp: 359 – 360.

Chacón, J., and S. Madriñán (Poster). VIII Congreso Latinoamericano de Botánica y II Congreso Colombiano de Botánica 2002, Cartagena de Indias, Colombia.

ATTENDED WORKSHOPS/MEETINGS

2nd meeting of the Network for Neotropical Biogeography, January 14, 2013, Montgomery Botanical Center, Coral Gables, Miami, Florida, USA

TreeBOL Meeting, November 2008, New York Botanical Garden, New York, USA

III Congreso Colombiano de Biotecnología, II Seminario Internacional de Bionegocios, August 2008, Bogotá, Colombia

MolConnect Workshop "Bridging Genomics and Biodiversity" September 2006, Bogotá, Colombia

Workshop on Molecular Evolution 2004, July – August, 2004, Marine Biological Laboratory, Woods Hole, MA, USA

INTERNSHIPS

Visiting Researcher, May – August 2002, Supervisor: Dr. Mark W. Chase, Jodrell Laboratory, Royal Botanic Gardens, Kew, UK

MEMBERSHIPS

International Biogeography Society

LANGUAGES

Spanish (native language), English (fluent), and German (basic knowledge)

BIOINFORMATICS

BEAST, DnaSP, FigTree, Geneious, LAGRANGE, MacClade, MAFFT, Mesquite, Modeltest, MrBayes, PAUP*, RASP, RAxML, S-DIVA, Sequencher, Sequin, R BioGeomancer, Dendroscope, Google Earth, GPS Visualizer, Quantum GIS, TreeEdit