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Phylogeny and colonization history of *Pringlea antiscorbutica* (Brassicaceae), an emblematic endemic from the South Indian Ocean Province

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ABSTRACT

The origins and evolution of sub-Antarctic island floras are not well understood. In particular there is uncertainty about the ages of the contemporary floras and the ultimate origins of the lineages they contain. *Pringlea* R. Br. (Brassicaceae) is a monotypic genus endemic to four sub-Antarctic island groups in the southern Indian Ocean. Here we used sequences from both the chloroplast and nuclear genomes to examine the phylogenetic position of this enigmatic genus. Our analyses confirm that *Pringlea* falls within the tribe Thelypodieae and provide a preliminary view of its relationships within the group. Divergence time estimates and ancestral area reconstructions imply *Pringlea* diverged from a South American ancestor ~5 Myr ago. It remains unclear whether the ancestor of *Pringlea* dispersed directly to the South Indian Ocean Province (SIOP) or used Antarctica as a stepping-stone; what is clear, however, is that following arrival in the SIOP several additional long-distance dispersal events must be inferred to explain the current distribution of this species. Our analyses also suggest that although *Pringlea* is likely to have inherited cold tolerance from its closest relatives, the distinctive morphology of this species evolved only after it split from the South American lineage. More generally, our results lend support to the hypothesis that angiosperms persisted on the sub-Antarctic islands throughout the Pliocene and Pleistocene. Taken together with evidence from other sub-Antarctic island plant groups, they suggest the extant flora of sub-Antarctic is likely to have been assembled over a broad time period and from lineages with distinctive biogeographic histories.

1. Introduction

The historical biogeography of Southern Hemisphere vascular plants has long fascinated biologists. First discussed by Hooker (1853) explanations for these now well-recognized patterns have been highly controversial. However, over the last 15 years molecular phylogenetic analyses (e.g., Lockhart et al., 2001; Meudt and Simpson, 2006; Schuettpelz and Hoot, 2004; Wagstaff et al., 2002; Winkworth et al., 2002a) have done much to improve our understanding of Southern Hemisphere biogeography. In particular, these studies have shown that for plants trans-oceanic dispersal has had much more important impacts on contemporary biogeographic patterns than Gondwanan vicariance (Sanmartín and Ronquist, 2004; Sanmartín et al., 2007; Winkworth et al., 1999, 2002b). To date such phylogenetic studies have focused predominantly on plant groups from Australia, New Caledonia, New Zealand and South America (e.g., Bartish et al., 2005; Knapp et al., 2005; Meudt and Simpson, 2006; Stöckler et al., 2002). In contrast, while sub-Antarctic island forms have been included in phylogenies for specific taxa (e.g., Gillespie et al., 2008; Tay et al., 2010; Wagstaff et al., 2002) the origins and evolution of sub-Antarctic island vascular plant floras have received more limited attention (but see Mitchell et al., 1999; Wagstaff and Hennion, 2007; Wagstaff et al., 2011).

Although small relative to their continental neighbors, the sub-Antarctic islands (Fig. 1) are critical to our understanding of Southern Hemisphere biogeography. It has previously been suggested that these islands host representatives of extinct Antarctic floras (e.g., Convey et al., 2009; Hooker, 1847; Werth, 1911) or have acted as dispersal stepping-stones to the lower latitude landmasses (e.g., Ali and Aitchison, 2009; Fleming, 1962; Kemp and Harris, 1975; Wardle, 1968). Most of the sub-Antarctic islands have relatively

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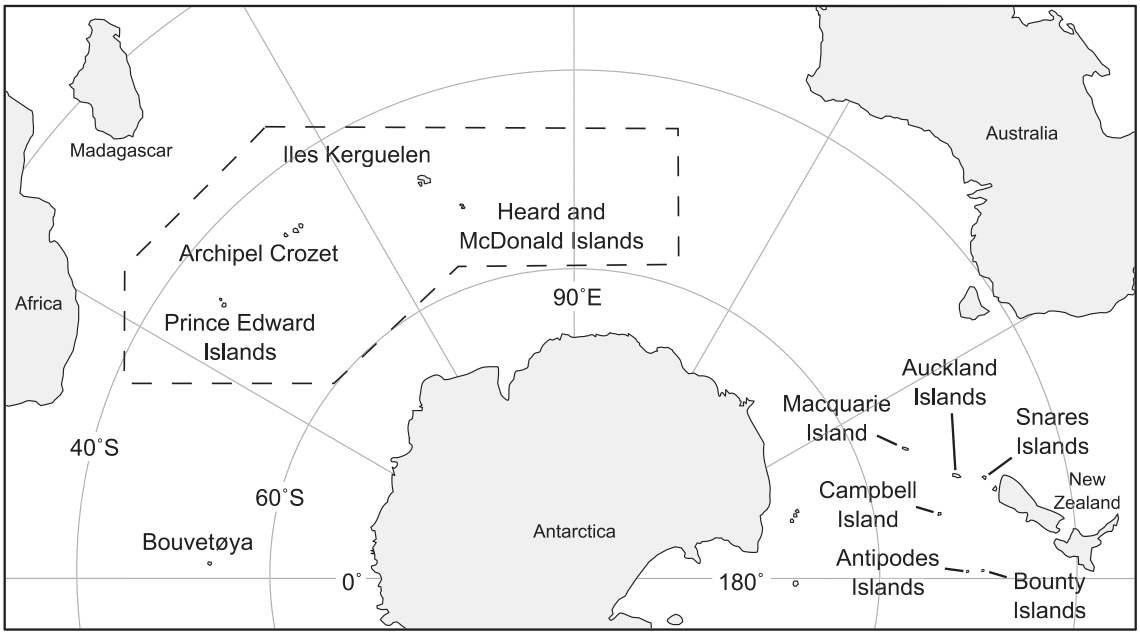

Fig. 1. Map showing eastern hemisphere in (southern) polar projection. Islands and island groups falling within the sub-Antarctic zone are labeled. The islands that compose the Southern Indian Ocean Province and, hence, distribution of *Pringlea* are indicated by the dotted line.

recent volcanic origins (Quilty, 2007) so uncertainty about the history of their floras is not a question of recent dispersal versus ancient Gondwanan vicariance. Instead the issue is whether the contemporary floras contain lineages that survived Pleistocene glaciation *in situ* or they were assembled *de novo* following the last glacial maximum (LGM). Post-LGM assembly of these floras is consistent with earlier studies suggesting complete glaciation of the sub-Antarctic islands (e.g., Colhoun and Goede, 1974; Hall, 1979; Sugden and Clapperton, 1977) and therefore the extinction of pre-Pleistocene assemblages (Smith, 1984). In contrast, recent data suggest that many of the islands supported ice-free areas (e.g., Bentley et al., 2007; Hall, 2002; Hall et al., 2011; Rosqvist et al., 1999). This, together with paleobotanical evidence (Van der Putten et al., 2010) implies that long-term survival *in situ* was possible. These alternatives reflect a more general debate concerning the origins and history of biotas in the Antarctic region as a whole (e.g., Chown, 1990; Convey et al., 2008, 2009; Gressitt, 1970; Stevens et al., 2006).

The origins of the plant lineages that occur in the sub-Antarctic have also been uncertain. One possibility is that these groups arrived from the lower latitude landmasses (e.g., Australia, New Zealand, and South America). Indeed, where sub-Antarctic island forms have been included in phylogenetic analyses they have often been shown to have close links to relatives on these landmasses (e.g., Tay et al., 2010; Wagstaff et al., 2002; Winkworth et al., 2002a). Alternatively the ancestors of these sub-Antarctic island forms may be derived from now extinct Antarctic floras. Evidence for the timing of complete glaciation on Antarctica remains uncertain (Convey et al., 2008; Siegert, 2008) but the continent may have hosted a tundra-like flora up until as recently as the mid-Pliocene (Ashworth and Cantrill, 2004). If so it is possible some of elements of these assemblages escaped extinction by dispersal to the sub-Antarctic islands. One potential example is the Iles Kerguelen endemic *Lyallia*. Molecular age estimates imply a long period of isolation and are interpreted as support for an Antarctic origin (Wagstaff and Hennion, 2007).

Pringlea antiscorbutica R. Br. (Brassicaceae), the "Kerguelen cabbage", is endemic to the four island groups of the South Indian Ocean Province (SIOP; *sensu* Smith, 1984). The province is composed of Iles Kerguelen, Archipel Crozet, Prince Edward Islands, and the Heard and McDonald Islands (Fig. 1). *Pringlea* is a long-lived perennial (usually living more than 7 years) with ramified, prostrate shoots that end in a rosette of large leaves (up to 80 cm in diameter). On plants more than 3–4 years old the shoots also bear 3–5 lateral inflorescences. There is high intra-population variability in plant size and the number of flowering stems (Hennion et al., 2006). *Pringlea* occurs in a wide array of habitat types but always on wet, yet free draining, soils. The monotypic *Pringlea* is distinctive within the Brassicaceae because although some of its morphological characteristics are found in other members of the family, they do not occur in similar combinations. Depending on the characters considered, links to at least six different tribes have been suggested (e.g., Delaveau et al., 1973; Hedge, 1976; Hooker, 1879; Werth, 1911) while von Hayek (1911) considered *Pringlea* distinctive enough to warrant its own tribe, Pringleeae.

Previous molecular analyses have gone some way toward resolving the tribal status of *Pringlea*. These studies place *Pringlea* within the otherwise exclusively New World tribe Thelypodieae (*sensu* Warwick et al., 2009) but do not convincingly resolve relationships within this large clade (Warwick et al., 2002, 2009). While suggestive these analyses are not sufficient to explicitly test hypotheses about the origins and evolution of *Pringlea*; instead they raise a further question. Most members of the Thelypodieae fall within one of two broad ecological categories, (i) those that occupy warm and dry habitats (e.g., species of *Caulanthus*, *Streptanthus*) and (ii) those that occupy cold and wet climate habitats (e.g., species of *Chilocardamum*, *Mostacillastrum*). Since cold and wet environments seem likely to have characterized the SIOP since the late Miocene there are two possible historical scenarios. First, if a group or groups of species from warm dry habitats are most closely related to *Pringlea* then the current ecological tolerances of this species evolved following arrival in the SIOP; perhaps in response to climatic cooling during the late Miocene and Pliocene. The alternative is that *Pringlea* is derived from a group that occupies cold and wet habitats. In this case *Pringlea* may have arrived in the SIOP with adaptations to the climatic conditions on these islands.

In this study we investigate both the phylogenetic position and historical biogeography of *Pringlea*. We reconstruct a phylogeny for *Pringlea* and a sample of species assigned to the Thelypodieae and

related tribes. We then use this tree to estimate divergence times and as a basis for reconstructing ancestral areas. More specifically we address the following questions. When did the ancestor of *Pringlea* arrive in the SIOP? Where did it arrive from? And to what extent did the unique biology of this species evolve in the SIOP? We also briefly discuss what our results imply in terms of the assembly processes of sub-Antarctic island floras.

2. Materials and methods

2.1. Sampling of Pringlea for molecular analyses

Seeds were sampled from three of the four archipelagos where *Pringlea* occurs: Iles Kerguelen, Archipel Crozet, and the Heard Island group (including Heard Island and the McDonald Islands). Seeds from Iles Kerguelen and Archipel Crozet were germinated and seedlings grown in a phytotron at UMR-CNRS Ecobio (University of Rennes 1, France). Seeds from two Heard Island populations were cultivated under similar conditions at the Royal Botanic Gardens Hobart (Tasmania, Australia). One individual from each population was randomly selected and its leaves used for DNA extraction. Leaves were collected from two populations on Marion Island (Prince Edward Islands). In each case sampled populations are geographically distinct; details of collection sites are provided in Appendix A.

2.2. DNA isolation, PCR amplification, and sequencing

Total genomic DNA was isolated from fresh or dried leaf tissue using either the DNAeasy plant DNA extraction kit (Qiagen) or a modified CTAB method (Doyle and Doyle, 1990). The nuclear ribosomal internal transcribed spacer (nrITS) and chloroplast *trn*L–*trn*F intergenic spacer (IGS) were PCR amplified using reaction volumes of 25 μl and a standard PCR mix; we used the primer pairs ITS-1/ITS-4 (White et al., 1990) and c/f (Taberlet et al., 1991) for amplification of nrITS and the *trn*L–*trn*F IGS, respectively. The thermal cycling profile for amplification of both loci was: 5 min at 95 °C, followed by 35 cycles of 30 s at 95 °C, 30 s at 50 °C, and 90 s at 72 °C, with a final incubation of 72 °C for 8 min. Amplification products were purified using the QIAquick PCR purification kit (Qiagen). We used the Big Dye Terminator Sequencing kit (PE Applied Biosystems,) and amplification primers for sequencing reactions. The resulting fragments were separated and analyzed on an ABI377 Prism Automated DNA Sequencer (Perkin Elmer).

2.3. Data matrices

Recent molecular phylogenetic (e.g., Al-Shehbaz et al., 2006; Beilstein et al., 2006, 2010; Couvreur et al., 2010; Warwick et al., 2009) and taxonomic studies (e.g., Al-Shehbaz, 2004, 2006) provided a framework for taxon selection. In addition to *Pringlea* our sampling included 21 species of Thelypodieae plus representatives of Arabideae, Brassiceae, Isatideae and Sisymbrieae. GenBank accession details for included taxa are provided in Appendix B.

Multiple sequence alignments were prepared using ClustalX (Larkin et al., 2007) with subsequent manual adjustments; for example, regions at either end of matrices were excluded if less than 50% of sequences were represented. To maintain positional similarity gaps were inferred in both the nrITS and *trn*L–*trn*F IGS matrices. For phylogenetic analyses gapped positions were excluded if less than 50% of sequences were represented or alignment was ambiguous.

We constructed a combined molecular data set by concatenating the nrITS and *trn*L–*trn*F IGS matrices. Prior to phylogenetic analysis we tested for incongruence between these data sets using the Incongruence Length Difference (ILD; Farris et al., 1994) test as implemented in PAUP*4.0b10 (Swofford, 2002). The test used 1000 replicates with default settings for heuristic searches; the exception being that MULTREES was turned off. We created a fourth matrix by adding an indel partition to the combined molecular data set. Gaps were coded as binary characters. Only simple (i.e., not those that appeared to result from independent overlapping events) and parsimony informative (i.e., present in two or more taxa) gaps were coded. Those at the ends of mononucleotide strings were omitted.

2.4. Phylogenetic analyses

We conducted maximum parsimony and Bayesian analyses on the nrITS and *trn*L–*trn*F IGS matrices. Maximum parsimony analyses were performed using PAUP version 4.0b10 (Swofford, 2002). Heuristic MP searches used TBR branch swapping, all characters equally weighted, and zero-length branches collapsed. Analyses were repeated 10,000 times with RANDOM ADDITION. Support for nodes was estimated using a full heuristic bootstrap with 1000 replicates. Bayesian searches were performed using MrBayes version 3.1.2 (Ronquist and Huelsenbeck, 2003). Best-fit substitution models were determined for each locus using the Bayesian Information Criterion (BIC; Schwartz, 1978) as implemented in JModelTest (Guindon and Gascuel, 2003; Posada, 2008). Searches used best-fit models and default settings for other run parameters. MCMC runs were initiated with a random starting tree and run for 2×10^7 generations, sampling from the posterior distribution every 500 generations (for a total of 40,000 samples each). Burn-in was determined using convergence diagnostics and trees sampled prior to stationarity eliminated.

MP and Bayesian analyses of the combined datasets were conducted as described for individual matrices. We applied a single best-fit substitution model (using JModelTest) to the combined sequence partition in analyses of both combined matrices; we used a standard discrete model (Lewis, 2001) for the indel partition in the combined molecular with indels analysis.

2.5. Divergence time analyses

We used BEAST (version 1.6.0; Drummond and Rambaut, 2007) to estimate divergence times. We used the majority-rule tree from our analysis of combined nucleotide sequence and indel matrices with a single model for sequence data as a start tree for these analyses. Several age estimates from Beilstein et al. (2010) were used to provide temporal calibrations for this tree; in each case the constraint was applied using normally distributed priors with means and standard deviations determined by the means and highest probability distributions of the corresponding nodes in the Beilstein et al. (2010) tree. The constraints are listed in Table 1 and their positions shown in Fig. 2.

We conducted a pair of BEAST analyses. For the first we fixed the entire tree topology (to match the MrBayes estimate) and for the second we enforced monophyly on the calibrated nodes as well as on the *Pringlea* crown group and the South American Thelypodieae clade. These two latter constraints are applied because indel information cannot be included in BEAST analyses but our analyses indicate indels provide additional support for these two clades. The constrained nodes in this second analysis are well supported in Bayesian analyses of our full combined data set (see Fig. 2). They are also consistent with the resolution provided by analyses of all but the nrITS matrix, as reported in Supplementary Figs. 1–3. For each analysis we conducted a pair of BEAST runs, each 2.5×10^7 generations in length and sampled every 500 generations. Both runs used an uncorrelated lognormal model of rate evolution (Drummond et al., 2006), the SYM + I model of sequence

Table 1
Age constraints used to time-calibrate the Thelypodieae phylogeny. Letters correspond to labeled nodes in Fig. 2.

Constrained node	Age (Ma, mega-annum) and standard deviation (Ma) used for calibration
A	38.40, 2.0
B	36.25, 2.0
C	30.76, 2.5
D	12.92, 2.0
E	26.69, 2.5
F	11.48, 3.0

evolution and a Yule speciation model (Yule, 1924). After removal of the first 1.25×10^6 generations as burn-in, the posterior distributions of divergence time estimates (as well as associated statistics) and trees for each run were combined using LogCombiner (version 1.6.0). Estimates and trees were examined using Tracer (version 1.5) and TreeAnnotator (version 1.6.0), respectively.

2.6. Ancestral area reconstruction

We conducted a pair of ancestral area reconstructions, each using a different character state assignment. For the first analysis taxa were assigned to one of four areas based on extant distributions. The areas were Old World, North America, South America, and the SIOP. The same areas were used for the second analysis with the exception of the SIOP. For this analysis we replaced this area with states corresponding to each of the four island archipelagos that make up the SIOP (Fig. 1).

Maximum-likelihood ancestral area reconstructions were performed using the Markov *k*-state one-parameter model as implemented in Mesquite version 2.74 (Maddison and Maddison, 2009). We performed optimizations on a random sample of 1000 trees from the BEAST runs in which we constrained a subset of nodes rather than the entire topology. Ancestral states were summarized on the maximum clade credibility tree for the corresponding BEAST runs.

3. Results

3.1. Sequences and data matrices

We sequenced the *trn*L–*trn*F IGS and nrITS from five and eight *Pringlea* populations, respectively; at least one accession from the Prince Edward Islands, Archipel Crozet, Iles Kerguelen and the Heard Island group were represented for both loci. GenBank accession details for these sequences are provided in Appendix A.

For both the *trn*L–*trn*F IGS and nrITS multiple sequence alignments were straightforward; however, several short regions in the nrITS alignment were excluded from further analysis as a result of alignment ambiguity introduced by inclusion of sequences from more distantly related groups. The ILD test indicated no significant incongruence between the two matrices ($P = 0.0864$). The final indel matrix consisted of 12 phylogenetically informative indels. Summary statistics for the individual and combined matrices are given in Appendix C.

3.2. Phylogenetic analyses

Majority rule trees from separate analyses of the *trn*L–*trn*F IGS (Supplementary Fig. 1) and nrITS (Supplementary Fig. 2) data sets are broadly consistent with one another. The differences between them reflect, for the most part, contrasting levels of resolution rather than conflicting phylogenetic signal. For example, the *trn*L–*trn*F IGS topology provides resolution of relationships within the Thelypodieae but not among the tribes of Brassicaceae. In contrast, the Thelypodieae form a large polytomy (together with the Brassiceae) in the nrITS tree but relationships among the remaining tribes are, at least, partially resolved. Individual datasets also provide differing levels of resolution with respect to *Pringlea*. In the nrITS tree *Pringlea* accessions are monophyletic but relationships among them are unresolved, whereas the *trn*L–*trn*F IGS topology provides some resolution of relationships among the accessions. Comparison of majority rule trees based on analyses of individual and combined datasets (Fig. 2, Supplementary Fig. 3) suggest the phylogenetic signal from the individual matrices is additive. There are minor differences between trees based on the two combined datasets; these involve resolution of relationships near the base of the tree, and the monophyly of the South American Thelypodieae. These differences reflect addition of the indel matrix. Support for the suggested relationships also varies between analyses with higher bootstrap (BS) and posterior probabilities (PP) associated with the combined data sets (Fig. 2; Supplementary Figs. 1–3). Summary statistics for our phylogenetic analyses are presented in Appendix C.

The topology resulting from analysis of the combined molecular and indel data set is the best resolved and supported (Fig. 2); this tree is the basis for our subsequent inferences. In this tree relationships among the included Brassicaceae tribes are fully resolved with PP of 1.0; support from MP bootstrapping is more variable. The suggested relationships are identical to those described by Beilstein et al. (2010). Also consistent with previous analyses we find strong support (BS = 86%, PP = 1.0) for the placement of *Pringlea* within Thelypodieae. Resolution and support for relationships within the tribe is variable. However, there is support for the sampled populations of *Pringlea* forming a monophyletic group (BS = 71%, PP = 1.0) that is nested within a clade (PP = 1.0) containing South American representatives of Thelypodieae. We recover two subclades within *Pringlea*; one that unites populations from the Iles Kerguelen and Heard Island group (PP = 0.93) and the other containing populations from the Prince Edward Archipelago (PP = 0.98).

3.3. Age and ancestral area reconstructions

Divergence time estimates and ancestral area reconstructions are reported in Fig. 3. Our analyses suggest initial diversification of the Thelypodieae crown group began in North America 11.8 Ma (mega-annum) ago (95% HPD 17.3–6.7 Ma ago) and was followed by colonization of and rapid diversification in South America from 7.5 Ma ago (95% HPD 11.3–4.1 Ma ago). The stem and crown ages of *Pringlea* are 5.0 Ma (95% HPD 7.7–2.6 Ma) and 2.4 Ma (95% HPD 4.7–0.6 Ma), respectively.

4. Discussion

4.1. The phylogenetic position of Pringlea: implications for understanding its taxonomy and morphological evolution

The taxonomic affinities of *Pringlea* have been uncertain since it was first collected more than 150 years ago. Only recently have molecular studies (e.g., Warwick et al., 2002, 2009) provided some resolution of this problem. These analyses have indicated that *Pringlea* is a member of the New World tribe Thelypodieae (*sensu* Warwick et al., 2009) but have been unable to determine its closest relatives due to a lack of resolution and support for relationships within the tribe (e.g., Warwick et al., 2009). We acknowledge that some of these limitations remain in our analyses. However, the combined sequence and indel matrix data supports additional phylogenetic structure and this provides some novel insights. Specifi-

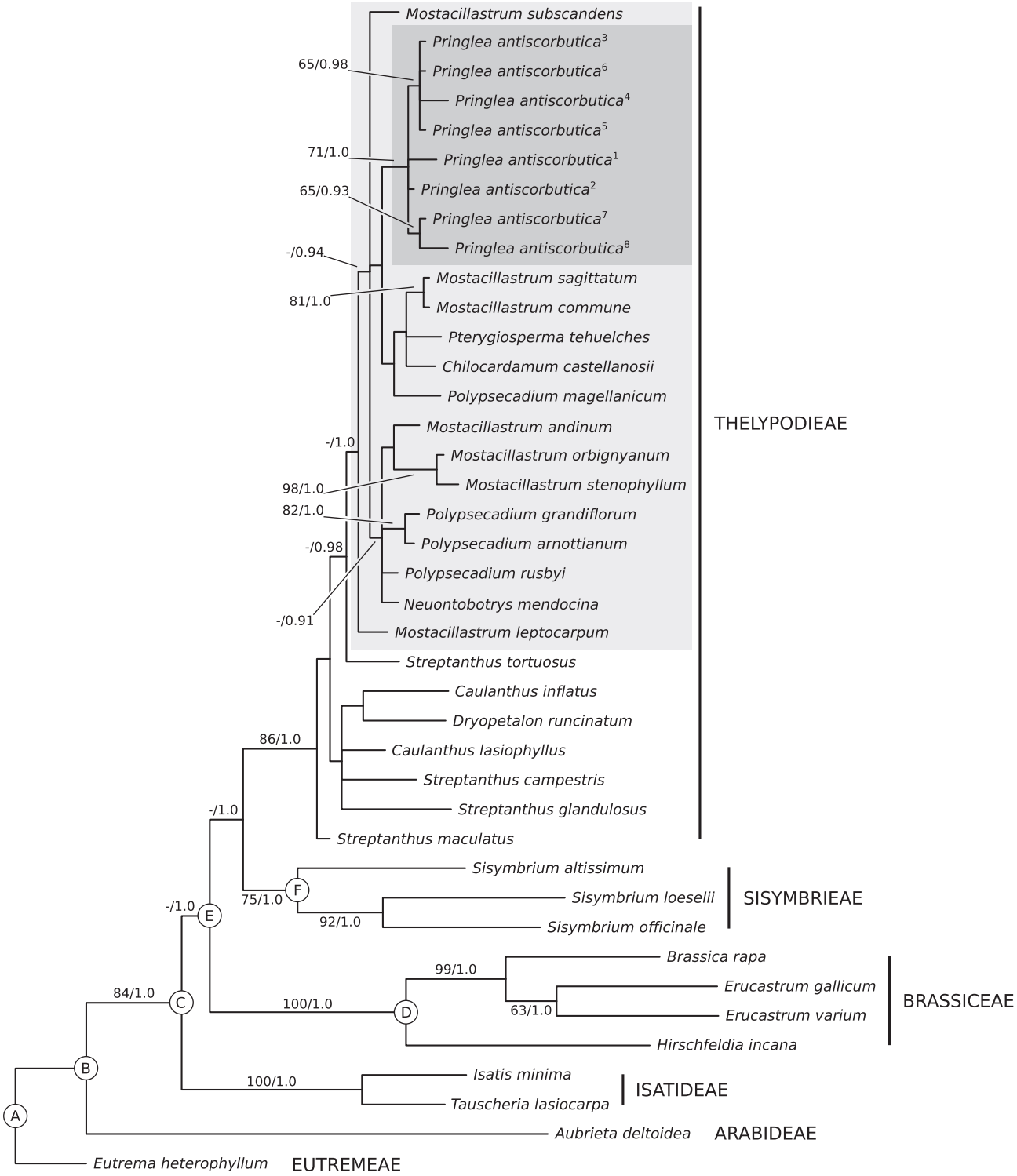

Fig. 2. Bayesian probability estimate of Thelypodieae phylogeny based on the combined molecular with indels dataset. Branch lengths are proportional to the mean of the corresponding posterior probability density. Bootstrap support values (from the corresponding MP analysis) and posterior probabilities are given for branches where posterior probabilities are >0.90; a dash indicates the bootstrap support value is <50%. Tribal affiliations are indicated on the right. The Thelypodieae occur, with the exception of *Pringlea* (dark grey box), in South (light grey box) and North (unshaded) America. Superscript numbers indicate accessions of *Pringlea* from different geographical locations; location details are provided in Appendix A and are also indicated in Fig. 3. Nodes labeled A–F are those constrained for divergence time estimation (see Table 1 for list of age constraints).

cally, we identify a clade (BS = 86%, PP = 1.0) containing the South American Thelypodieae plus *Pringlea* and show that the *Pringlea* accessions form a monophyletic group (BS = 71%, PP = 1.0) that is well nested within this clade (i.e., two nodes with PP > 0.90). So although we are reasonably confident *Pringlea* is most closely related to the South American Thelypodieae, the limited support for wider relationships within this group makes it difficult to identify its closest relatives. In the 50% majority rule trees from analyses of the *trn*L–*trn*F and both combined matrices (Fig. 2, Supplementary Figs. 1 and 3) the closest relatives include members

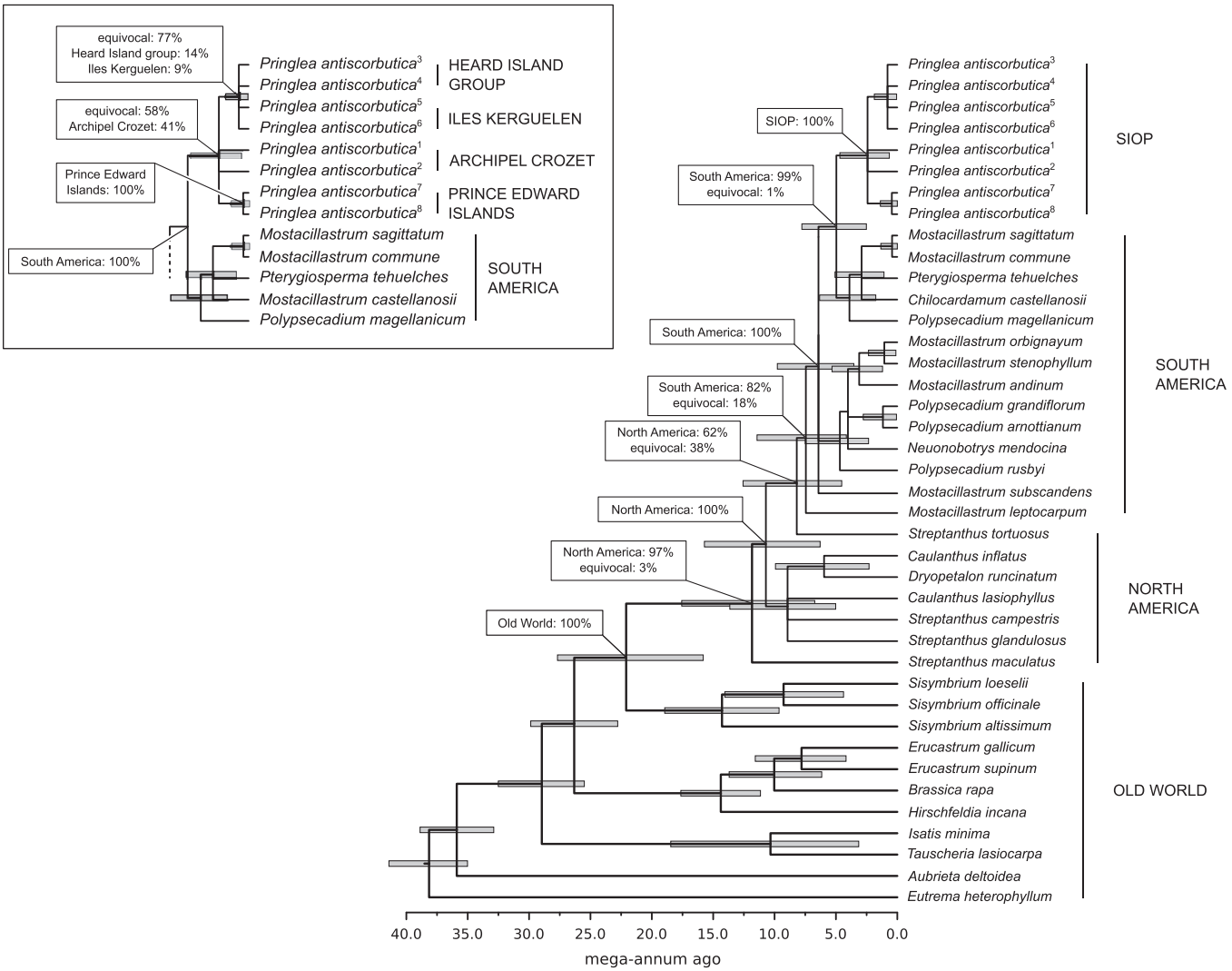

Fig. 3. Time-calibrated phylogeny for Thelypodieae. Branch lengths are proportional to time with gray bars indicating 95% highest probability densities. The relative likelihoods of biogeographic areas are given at internal nodes; these are calculated from only those sampled trees in which the node appears. For clarity we have omitted the likelihoods when these are the same as a more basal node (in all cases the omitted reconstructions are unambiguous). In the main tree the SIOP has been treated as a single area. In the inset the island archipelagos are coded separately, the remainder of the reconstruction is consistent with the main image.

of several genera (e.g., *Mostacillastrum* and *Polypsecadium*); this relationship receives a PP = 0.91 in the *trn*L–*trn*F tree.

In large part it is the distinctive morphology of *Pringlea* that has made identifying its tribal affinities so difficult. Indeed, there appear to be few morphological characters that clearly link *Pringlea* to Thelypodieae. Our analyses suggest the *Pringlea* lineage arose relatively recently (~5.0 Ma ago; Fig. 3) and therefore that its distinctiveness is the result of rapid morphological evolution. We suspect that rapid change in *Pringlea* may be the result of insularity. This is certainly consistent with evidence for the rapid evolution of morphological differences between insular and continental form in other plant groups (e.g., Baldwin et al., 1990; Böhle et al., 1996; Mitchell et al., 1999; Wagstaff et al., 2011). The morphology of *Pringlea* is also implies of a role for insularity. For example, the sublignose thickening of *Pringlea* stems (Hooker, 1847; Werth, 1911) is suggestive of insular woodiness; this characteristic has evolved independently in the insular representatives of many otherwise herbaceous angiosperm genera (Carlquist, 1974). Böhle et al. (1996) suggest that in *Echium* this characteristic may reflect selection pressures associated with a requirement for outbreeding, longevity and conspicuous inflorescences in island colonizers. A similar explanation may also apply in *Pringlea* where partial inbreeding appears to have only recently replaced insect-pollination and outcrossing (Schermann-Legionnet et al., 2007).

Our results provide important insights but a better understanding of the phylogenetic relationships within the South American Thelypodieae clade is needed if we are to examine the evolution of *Pringlea* in greater detail. In particular, we need to be able to confidently identify the closest relatives in order to explain the origins of this plants' unique set of morphological characteristics.

4.2. The biogeography of Pringlea

Our analyses suggest that the processes responsible for contemporary species diversity in Thelypodieae began in North America during the mid Miocene (Fig. 3). The North American species occur predominantly in the Madrean vegetation of western North America (Raven and Axelrod, 1978). Assuming Thelypodieae originated in this region (*c.f.*, Raven and Axelrod, 1978) their diversification may be associated with the expansion of summer-dry climates ~15 Ma ago (Axelrod, 1992; Flower and Kennett, 1994). Diversification in North America appears to have given rise to a number

of distinct lineages, one of which colonized South America during the late Miocene. Our reconstruction suggests a single event involving one or a few related colonists derived from the lineage represented by *Strepanthus tortuosus* (Fig. 3). Divergence time estimates suggest this colonization occurred well before the establishment of a land connection between North and South America (3.5–3.1 Ma ago; Coates and Obando, 1996). Similar results have been described for Valerianaceae (Bell and Donoghue, 2005) and Sapotaceae (J. Richardson, personal communication, 2012). Once established in South America the Thelypodieae radiated extensively. There are ~60 extant South American species distributed predominantly along the Andean mountain chain from the tropics to Tierra del Fuego (Warwick et al., 2009). That this radiation is largely cotemporal with the Andean orogeny in the late Miocene (Gregory-Wodzicki, 2000) suggests niche availability in newly uplifted areas may have been an important driver of diversification; similar inferences have been reported for *Halenia* (von Hagen and Kadereit, 2003), *Lupinus* (Hughes and Eastwood, 2006), and Valerianaceae (Bell and Donoghue, 2005).

Our analyses strongly suggest that *Pringlea* arose from a South American ancestor. The *Pringlea* clade is nested well within the South American radiation; several of the surrounding nodes receive PP > 0.90 (albeit with weaker BS support; Fig. 2) and are convincingly reconstructed as South American (Fig. 3). In the 50% majority rule trees from analyses of the *trn*L–*trn*F IGS and both combined matrices (Fig. 2; Supplementary Figs. 1 and 3) *Pringlea* is sister to a clade containing species that occur, like many South American taxa, in cold climate habitats (i.e., either high altitude or latitude). This relationship implies that cold tolerance had arisen within Thelypodieae prior to the divergence of *Pringlea*. Crisp et al. (2009) have shown that niche conservatism has strongly influenced the establishment of dispersing lineages in the Southern Hemisphere. Cool or cold climate habitats were likely to have dominated the SIOP by the Miocene (Bertler and Barrett, 2010). Given an assumption of niche conservatism, age estimates for the arrival of *Pringlea* in the SIOP suggest successful colonization would have required cold tolerance.

Although a South American origin for *Pringlea* is strongly supported we can be less certain about the dispersal route. Our results can certainly be interpreted in terms of direct long-distance dispersal. That is, arrival of *Pringlea* in the SIOP may have been the result of a single long-distance dispersal event from South America. On the other hand we cannot entirely rule out stepping-stone dispersal via Antarctica. Such a route is appealing since it reduces the distances that need to be covered by any single dispersal event. However, the availability a stepping-stone route depends on the timing of dispersal. Specifically, for stepping-stone dispersal to provide an explanation *Pringlea* must have arrived in the SIOP prior to the complete glaciation of Antarctica. The 95% HPD on the stem age for *Pringlea* suggests divergence from its South American ancestor 7.7–2.6 Ma ago. If complete Antarctic glaciation occurred during the mid Pliocene (e.g., Hambrey and McKelvey, 2000; Webb and Harwood, 1991), our stem age estimate is consistent with *Pringlea* having dispersed via Antarctica. In contrast, if complete glaciation occurred earlier (e.g., Denton et al., 1993; Flower and Kennett, 1994) then direct dispersal becomes an increasingly likely explanation. Improved estimates of lineage divergence times and for the timing of complete glaciation in Antarctica will be required if we are to differentiate between these two scenarios.

Our sampling of *Pringlea* from the individual archipelagos is limited and the pattern of colonization within the SIOP remains equivocal (Fig. 3). However, it is clear that following arrival in the SIOP additional long-distance dispersal events are needed to explain the current distribution of *Pringlea*. Assuming one archipelago was initially colonized, a minimum of three further events must be inferred. Point estimates for divergences within *Pringlea* suggest these dispersals occurred 0.8–0.4 Ma ago. Phylogeographic studies will be needed if we are to more fully examine dispersal patterns within the SIOP.

4.3. Mechanisms of long-distance dispersal in Pringlea

Pringlea seeds have outer cell layers that when exposed to water swell to form a mucilaginous coat that may function in dispersal (Hennion and Walton, 1997). This coating allows seeds to float for short periods; *Pringlea* seeds have been observed floating in running fresh water and along the Iles Kerguelen coastline (Chapuis et al., 2000; Hennion and Walton, 1997). However, floating time is limited so water-borne transport of seeds would seem to be unlikely for long-distance events. Alternatively the mucilaginous coating may provide a mechanism for the attachment of seeds to the feathers or feet of seabirds (Carlquist, 1974; Falla, 1960; Sorensen, 1986). If so, then the albatrosses and their relatives (Procellariiformes) would seem to be a likely dispersal vector (Moore, 1972; Winkworth et al., 2002b). These form colonies on many sub-Antarctic islands and are known to travel long distances across the Southern Ocean (Burg and Croxall, 2004; Croxall, 1984; Inchausti and Weimerskirch, 2002; Jouventin and Weimerskirch, 1990; Prince et al., 1992).

Similar mucilaginous coatings occur in other Brassicaceae (e.g., some *Streptanthus* and *Sisymbrium*) but this feature appears not to occur in the South American Thelypodieae (Al-Shehbaz, 2006). If a mucilaginous coat were absent from closely related South American species it would seem to imply that the feature evolved following arrival in the SIOP. In this case an alternative mechanism for long-distance dispersal would be needed to explain the arrival of *Pringlea* in the SIOP.

4.4. Implications for understanding the evolution of sub-Antarctic island floras

The origins of sub-Antarctic island biotas have been controversial especially in relation to the impacts of Pliocene–Pleistocene glaciation (Chown, 1990; Gressitt, 1970; Van der Putten et al., 2010). Divergence time estimates indicate *Pringlea* had arrived in and begun to spread across the SIOP well before the LGM. This strongly suggests that vascular plants were able to survive Pliocene–Pleistocene glacial cycles on one or more islands in the SIOP. Age estimates for *Lyallia* (Portulacaceae; Wagstaff and Hennion, 2007) and *Pleurophyllum* (Asteraceae; Wagstaff et al., 2011) also imply survival in the sub-Antarctic. Our results therefore further strengthen Van der Putten et al.'s (2010) argument that the assembly of contemporary sub-Antarctic island floras had begun prior to the Holocene. Similar patterns have been documented for other taxa (Mortimer et al., 2011; Stevens et al., 2006).

The origins of *Pringlea* appear to be in striking contrast to those of another SIOP endemic, *Lyallia*. For this species molecular analyses suggest a much longer period of isolation and potentially Antarctic ancestry (Wagstaff and Hennion, 2007). If so, then *Lyallia* and *Pringlea* would represent the two generalized hypotheses for the origin of sub-Antarctic island plants; that is, arrival from either Antarctica or a lower latitude landmass. To more fully describe the assembly of sub-Antarctic island floras we need molecular phylogenies for additional lineages as well as further studies of paleofloras. However, the emerging picture suggests that with a few possible exceptions contemporary sub-Antarctic island floras were not assembled *de novo* following the LGM. Lineages in the current floras likely arrived over an extended time period and from several source areas.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2012.07.023.

References

Ali, J.R., Aitchison, J.C., 2009. Kerguelen Plateau and the Late Cretaceous southern-continent bioconnection hypothesis: tales from a topographical ocean. J. Biogeogr. 36, 1778–1784.
Al-Shehbaz, I.A., 2004. A synopsis of the South American *Neuontobotrys* (Brassicaceae). Novon 14, 253–257.
Al-Shehbaz, I.A., 2006. The genus *Sisymbrium* in South America, with synopses of the genera *Chilocardamum, Mostacillastrum, Neuontobotrys,* and *Polypsecadium* (Brassicaceae). Darwiniana 44, 341–358.
Al-Shehbaz, I.A., Beilstein, M.A., Kellogg, E.A., 2006. Systematics and phylogeny of the Brassicaceae (Cruciferae): an overview. Plant. Syst. Evol. 259, 89–120.
Ashworth, A.C., Cantrill, D.J., 2004. Neogene vegetation of the Meyer Desert formation (Sirius Group) Transantarctic Mountains, Antarctica. Palaeogeogr. Palaeoclimatol. Palaeoecol. 213, 65–82.
Axelrod, D.I., 1992. Miocene floristic change at 15 Ma, Nevada to Washington, U.S.A. Palaeobotanist 41, 234–239.
Baldwin, B.G., Kyhos, D.W., Dvorak, J., 1990. Chloroplast DNA evolution and adaptive radiation in the Hawaiian silversword alliance (Asteraceae-Madiinae). Ann. Missouri Bot. Gard. 77, 96–109.
Bartish, I.V., Swenson, U., Munzinger, J., Anderberg, A.A., 2005. Phylogenetic relationships among New Caledonian Sapotaceae (Ericales): molecular evidence for generic polyphyly and repeated dispersal. Am. J. Bot. 92, 667–673.
Beilstein, M.A., Al-Shehbaz, I.A., Kellogg, E.A., 2006. Brassicaceae phylogeny and trichome evolution. Am. J. Bot. 94, 607–619.
Beilstein, M.A., Nagalingum, N.S., Clements, M.D., Manchester, S.R., Mathews, S., 2010. Dated molecular phylogenies indicate a Miocene origin for Arabidopsis thaliana. Proc. Natl. Acad. Sci. USA 107, 18724–18728.
Bell, C.D., Donoghue, M.J., 2005. Phylogeny and biogeography of Valerianaceae (Dipsacales) with special reference to the South American valerians. Org. Div. Evol. 5, 147–159.
Bentley, M.J., Evans, D.J.A., Fogwill, C.J., Hansom, J.D., Sugden, D.E., Kubik, P.W., 2007. Glacial geomorphology and chronology of deglaciation, South Georgia, sub-Antarctic. Q. Sci. Rev. 26, 644–677.
Bertler, N.A.N., Barrett, P.J., 2010. Vanishing polar ice sheets. In: Dodson, J. (Ed.), Changing Climates. Earth Systems and Society, Springer, Berlin, pp. 49–83.
Böhle, U.R., Hilger, H.H., Martin, W.F., 1996. Island colonization and evolution of the insular woody habit in *Echium* L. (Boraginaceae). Proc. Natl. Acad. Sci. USA 93, 11740–11745.
Burg, T.M., Croxall, J.P., 2004. Global population structure and taxonomy of the wandering albatross species complex. Mol. Ecol. 13, 2345–2355.
Carlquist, S., 1974. Island Biology. Columbia Univ. Press, New York.
Chapuis, J.L., Hennion, F., Le Roux, V., Le Cuziat, J., 2000. Growth and reproduction of the endemic cruciferous species *Pringlea antiscorbutica* in Kerguelen Islands. Pol. Biol. 23, 196–204.
Chown, S.L., 1990. Possible effects of Quaternary climatic change on the composition of insect communities of the South Indian Ocean Province Islands. S. Afr. J. Sci. 86, 386–391.
Coates, A.G., Obando, J.A., 1996. The geologic evolution of the central American Isthmus. In: Jackson, J.B.C., Budd, A.F., Coates, A.G. (Eds.), Evolution and Environment in Tropical America. Univ. Chicago Press, Chicago, pp. 21–56.
Colhoun, E.A., Goede, A., 1974. A reconnaissance survey of the glaciation of Macquarie Island. Pap. Proc. R. Soc. Tasmania 108, 1–19.
Convey, P., Gibson, J.A.E., Hillenbrand, C.-D., Hodgson, D.A., Pugh, P.J.A., Smellie, J.L., Stevens, M.I., 2008. Antarctic terrestrial life – challenging the history of the frozen continent? Biol. Rev. 83, 103–117.
Convey, P., Stevens, M.I., Hodgson, D.A., Hillenbrand, C.-D., Clarke, A., Pugh, P.J.A., Smellie, J.L., Cary, S.C., 2009. Antarctic terrestrial life – ancient evolutionary persistence or recent colonisation? Q. Sci. Rev. 28, 3035–3048.
Couvreur, T.L.P., Franzke, A., Al-Shehbaz, I.A., Bakker, F.T., Koch, M.A., Mummenhoff, K., 2010. Molecular phylogenetics, temporal diversification, and principles of evolution in the mustard family (Brassicaceae). Mol. Biol. Evol. 27, 55–71.
Crisp, M.D., Arroyo, M.T.K., Cook, L.G., Gandolfo, M.A., Jordan, G.J., McGlone, M.S., Weston, P.H., Westoby, M., Wilf, P., Linder, H.P., 2009. Phylogenetic biome conservatism on a global scale. Nature 458, 754–758.
Croxall, J.P., 1984. Seabirds and marine resources. In: Laws, E. (Ed.), Antarctic Ecology. Academic Press, London, pp. 533–619.
Delaveau, P., Hotellier, F., Guérin, H.P., Courcelle, A., 1973. Le Chou des Kerguelen (*Pringlea antiscorbutica* R.Br.). Plant. Méd. Phytothér. 7, 208–214.
Denton, G.H., Sugden, D.E., Marchant, D.R., Hall, B.L., Wilch, T.I., 1993. East Antarctic Ice Sheet sensitivity to Pliocene climate change from a Dry Valleys perspective. Geograf. Annal. 75, 155–204.
Doyle, J.J., Doyle, J.L., 1990. Isolation of plant DNA from fresh tissue. Focus 12, 13–15.
Drummond, A.J., Ho, S.Y.W., Phillips, M.J., Rambaut, A., 2006. Relaxed phylogenetics and dating with confidence. PLoS Biol. 4, 699–710.
Drummond, A.J., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7, 214.
Falla, R.A., 1960. Oceanic birds as dispersal agents. Proc. R. Soc. Lond. B 152, 655–659.
Farris, J.S., Kallersjo, M., Kluge, A.G., Bult, C., 1994. Testing significance of incongruence. Cladistics 10, 315–319.
Fleming, C., 1962. New Zealand biogeography – a paleontologists approach. Tuatara 10, 53–108.
Flower, B.P., Kennett, J.P., 1994. The middle Miocene climatic transition: East Antarctic ice sheet development, deep ocean circulation and global carbon cycling. Palaeogeogr. Palaeoclimatol. Palaeoecol. 108, 537–555.
Gillespie, L.J., Soreng, R.J., Bull, R.D., Jacobs, S.W.L., Refulio-Rodriguez, N.F., 2008. Phylogenetic relationships in subtribe Poinae (Poaceae, Poeae) based on nuclear ITS and plastid *trn*T-*trn*L–*trn*F sequences. Botany 86, 938–967.
Gregory-Wodzicki, K.M., 2000. Uplift history of the Central and Northern Andes: a review. Geol. Soc. Am. Bull. 112, 1091–1105.
Gressitt, J.L., 1970. Subantarctic entomology and biogeography. Pacif. Ins. Monogr. 23, 295–374.
Guindon, S., Gascuel, O., 2003. A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. Syst. Biol. 52, 696–704.
Hall, K., 1979. Late glacial ice cover and palaeotemperatures on sub-Antarctic Marion Island. Palaeogeogr. Palaeoclimatol. Palaeoecol. 29, 243–259.
Hall, K., 2002. Review of present and quaternary periglacial processes and landforms of the maritime and sub-Antarctic region. S. Afr. J. Sci 98, 71–81.
Hall, K., Meiklejohn, I., Bumby, A., 2011. Marion Island volcanism and glaciation. Ant. Sci. 23, 155–163.
Hambrey, M.J., McKelvey, B., 2000. Neogene fluctuations of the East Antarctic ice sheet: Stratigraphic evidence from the Lambert Glacier region. Geology 28, 887–890.
Hedge, I.C., 1976. A systematic and geographical survey of the Old World Cruciferae. In: Vaughan, J.G., MacLeod, A.J., Jones, M.B. (Eds.), The biology and chemistry of the Cruciferae. Academic Press, London, pp. 1–45.
Hennion, F., Frenot, Y., Martin-Tanguy, J., 2006. High flexibility in growth and polyamine composition of the crucifer *Pringlea antiscorbutica* in relation to environmental conditions. Physiol. Plant. 127, 212–224.
Hennion, F., Walton, D.W.H., 1997. Ecology and seed morphology of endemic species from Kerguelen phytogeographic zone. Pol. Biol. 18, 229–235.
Hooker, J.D., 1847. Botany [of] the Antarctic Voyage of the discovery Ships 'Erebus' and'Terror' in the Years 1831–1843 under the command of Captain Sir J.C. Ross, vol. 1. Flora Antarctica. Lovell Reeve, London.
Hooker, J.D., 1853. Botany [of] the Antarctic Voyage of the discovery Ships 'Erebus' and'Terror' in the Years 1831–1843 under the command of Captain Sir J.C. Ross, vol. 2. Flora Novae-Zelandiae. Lovell Reeve, London.
Hooker, J.D., 1879. Botany of Kerguelen Islands. Philos. Trans. R. Soc. Lond. 168, 1–14.
Hughes, C.E., Eastwood, R., 2006. Island radiation on a continental scale: exceptional rates of plant diversification after uplift of the Andes. Proc. Natl. Acad. Sci. USA 103, 10334–10339.
Inchausti, P., Weimerskirch, H., 2002. Dispersal and metapopulation dynamics of an oceanic seabird, the wandering albatross, and its consequences for its response to long-line fisheries. J. Anim. Ecol. 71, 765–770.
Jouventin, P., Weimerskirch, H., 1990. Satellite tracking of Wandering albatrosses. Nature 343, 746–748.
Kemp, E.M., Harris, W.K., 1975. The vegetation of Tertiary islands on the Ninetyeast Ridge. Nature 258, 303–307.
Knapp, M., Stöckler, K., Havell, D., Delsuc, F., Sebastiani, F., Lockhart, P.J., 2005. Relaxed molecular clock provides evidence for long-distance dispersal of *Nothofagus* (Southern Beech). PLoS Biol. 3, 38–43.
Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., Higgins, D.G., 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23, 2947–2948.
Lewis, P.O., 2001. A likelihood approach to estimating phylogeny from discrete morphological character data. Syst. Biol. 50, 913–925.
Lockhart, P.J., McLenachan, P.A., Havell, D., Glenny, D., Huson, D., Jensen, U., 2001. Phylogeny, dispersal and radiation of New Zealand alpine buttercups: molecular evidence under split decomposition. Ann. Missouri Bot. Gard. 88, 458–477.
Maddison, D.R., Maddison, W.P., 2009. Mesquite, version 2. Tucson, Arizona. <http://mesquiteproject.org>.
Meudt, H.M., Simpson, B.B., 2006. The biogeography of the austral, subalpine genus Ourisia (Plantaginaceae) based on molecular phylogenetic evidence. South

American origin and dispersal to New Zealand and Tasmania. Bot. J. Linn. Soc. 87, 479–513.
Mitchell, A.D., Meurk, C.D., Wagstaff, S.J., 1999. Evolution of *Stilbocarpa*, a megaherb from New Zealand's sub-Antarctic islands. N. Z. J. Bot. 37, 205–211.
Moore, D.M., 1972. Connections between cool temperate floras with particular reference to southern South America. In: Valentine, D.H. (Ed.), Taxonomy, Phytogeography, and Evolution. Academic Press, London, pp. 115–138.
Mortimer, E., Jansen van Vuuren, B., Lee, J.E., Marshall, D.J., Convey, P., Chown, S.L., 2011. Mite dispersal among the Southern Ocean Islands and Antarctica before the last glacial maximum. Proc. R. Soc. Lond. B 278, 1247–1255.
Posada, D., 2008. JModelTest: phylogenetic model averaging. Mol. Biol. Evol. 25, 1253–1256.
Prince, P.A., Wood, A.G., Barton, T., Croxall, J.P., 1992. Satellite tracking of wandering albatrosses (Diomedea *exulans)* in the South Atlantic. Ant. Sci. 4, 31–36.
Quilty, P., 2007. Origin and evolution of the sub-Antarctic islands: the foundation. Pap. Proc. R. Soc. Tasmania 141, 35–58.
Raven, P.H., Axelrod, D.I., 1978. Origin and relationships of the California flora. Univ. Calif. Publ. Bot. 72, 1–134.
Ronquist, F., Huelsenbeck, J.P., 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.
Rosqvist, G.C., Rietti-Shati, M., Shemesh, A., 1999. Late Glacial to middle Holocene climatic record of lacustrine biogenic silica oxygen isotopes from a Southern Ocean Island. Geology 27, 967–970.
Sanmartín, I., Ronquist, F., 2004. Southern Hemisphere biogeography inferred by event-based models: plant versus animal patterns. Syst. Biol. 53, 216–243.
Sanmartín, I., Wanntorp, L., Winkworth, R.C., 2007. West wind drift revisited: testing for directional dispersal in the Southern Hemisphere using event-based tree fitting. J. Biogeogr. 34, 398–416.
Schermann-Legionnet, A., Hennion, F., Vernon, P., Atlan, A., 2007. Breeding system of the subantarctic plant species *Pringlea antiscorbutica* R. Br. and search for potential insect pollinators in the Kerguelen Islands. Polar Biol. 30, 1183–1193.
Schuettpelz, E., Hoot, S.B., 2004. Phylogeny and biogeography of *Caltha* (Ranunculaceae) based on chloroplast and nuclear DNA sequences. Am. J. Bot. 91, 247–253.
Schwartz, G., 1978. Estimating the dimension of a model. Ann. Stat. 6, 461–464.
Siegert, M.J., 2008. Antarctic subglacial topography and ice-sheet evolution. Earth Surf. Process. Landforms 33, 646–660.
Smith, R.I., 1984. Terrestrial plant biology of the sub-Antarctic and Antarctic. In: Laws, R.M. (Ed.), Antarctic Ecology. Academic Press, London, pp. 61–162.
Sorensen, A.E., 1986. Seed dispersal by adhesion. Ann. Rev. Ecol. Syst. 17, 443–463.
Stöckler, K., Daniel, I.L., Lockhart, P.J., 2002. New Zealand Kauri (*Agathis australis* (D. Don) Lindl., Araucariaceae) survives Oligocene drowning. Syst. Biol. 51, 827–832.
Stevens, M.I., Greenslade, P., Hogg, I.D., Sunnucks, P., 2006. Southern Hemisphere springtails: could any have survived glaciation of Antarctica? Mol. Biol. Evol. 23, 874–882.
Sugden, D.E., Clapperton, C.M., 1977. The maximum ice extent on island groups in the Scotia Sea. Antarctica. Quat. Res. 7, 268–282.
Swofford, D.L., 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, MA.
Taberlet, P., Gielly, L., Pautou, G., Bouvet, J., 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Mol. Biol. 17, 1105–1109.
Tay, M.L., Meudt, H.M., Garnock-Jones, P.J., Ritchie, P.A., 2010. DNA sequences from three genomes reveal multiple long-distance dispersals and non-monophyly of sections in Australasian *Plantago* (Plantaginaceae). Aust. Syst. Bot. 23, 47–68.
Van der Putten, N., Verbruggen, C., Ochyra, R., Verleyen, E., Frenot, Y., 2010. Subantarctic flowering plants: pre-glacial survivors or post-glacial immigrants? J. Biogeogr. 37, 582–592.
von Hagen, K.B., Kadereit, J.W., 2003. The diversification of *Halenia* (Gentianaceae): ecological opportunity versus key innovation. Evolution 57, 2507–2518.
von Hayek, A., 1911. Entwurf eines Cruciferensystems auf phylogenetischer Grundlage. Beih. Bot. Centralbl. 27, 127–335.
Wagstaff, S.J., Bayly, M.J., Garnock-Jones, P.J., Albach, D.C., 2002. Classification, origin, and diversification of the New Zealand hebes (Scrophulariaceae). Ann. Missouri Bot. Gard. 89, 38–63.
Wagstaff, S.J., Breitwieser, I., Ito, M., 2011. Evolution and biogeography of *Pleurophyllum* (Astereae, Asteraceae), a small genus of megaherbs endemic to the subantarctic islands. Am. J. Bot. 98, 62–75.
Wagstaff, S.J., Hennion, F., 2007. Evolution and biogeography of *Lyallia* and *Hectorella* (Portulacaceae), geographically isolated sisters from the Southern Hemisphere. Ant. Sci. 19, 417–426.
Wardle, P., 1968. Evidence for an indigenous pre-Quaternary element in the mountain flora of New Zealand. N. Z. J. Ecol. 6, 120–125.
Warwick, S.I., Al-Shehbaz, I.A., Price, R.A., Sauder, C., 2002. Phylogeny of *Sisymbrium* (Brassicaceae) based on ITS sequences of nuclear ribosomal DNA. Can. J. Bot. 80, 1002–1017.
Warwick, S.I., Sauder, C., Mayer, M.S., Al-Shehbaz, I.A., 2009. Phylogenetic relationships in the tribes Schizopetaleae and Thelypodieae (Brassicaceae) based on nuclear ribosomal ITS region and plastid *ndhF* DNA sequences. Botany 87, 961–985.
Webb, P.-N., Harwood, D.M., 1991. Late Cenozoic glacial history of the Ross Sea embayment, Antarctica. Q. Sci. Rev. 10, 215–223.
Werth, E., 1911. Die vegetation der subantarktischen inseln kerguelen, possession- und heard-eiland. In: von Drygalski, E. (Ed.), Deutsche Südpolar-Expedition 1901–03, vol. 8. Würtzburg, pp. 125–371.
White, T.J., Bruns, Y., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal RNA genes for phylogenetics. In: Innis, M., Gelfand, D., Sninsky, J., White, T.J. (Eds.), PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, pp. 315–322.
Winkworth, R.C., Grau, J., Robertson, A.W., Lockhart, P.J., 2002a. The origins and evolution of the genus *Myosotis* L. (Boraginaceae). Mol. Phylogenet. Evol. 24, 180–193.
Winkworth, R.C., Wagstaff, S.J., Glenny, D.S., Lockhart, P.J., 2002b. Plant dispersal N.E.W.S from New Zealand. TREE 17, 514–520.
Winkworth, R.C., Robertson, A.W., Ehrendorfer, F., Lockhart, P.J., 1999. The importance of dispersal and recent speciation in the flora of New Zealand. J. Biogeogr. 26, 1323–1325.
Yule, G.U., 1924. A mathematical theory of evolution based on the conclusions of Dr. J.C. Willis. Philos. Trans. R. Soc. Lond. B. 213, 21–87.