

DNA sequences from three genomes reveal multiple long-distance dispersals and non-monophyly of sections in Australasian *Plantago* (Plantaginaceae)

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In our recent paper (Tay *et al.* 2010), several errors arose in Figs 5 and 6, mostly at the drafting stage: we neglected a polytomy in both figures; the tree topology in Fig. 6 was incorrect; the names of seven terminal taxa were associated with the wrong branches in Fig. 6; and *P. euryphylla* was spelled incorrectly in Fig. 5. The figures presented here correct these errors.

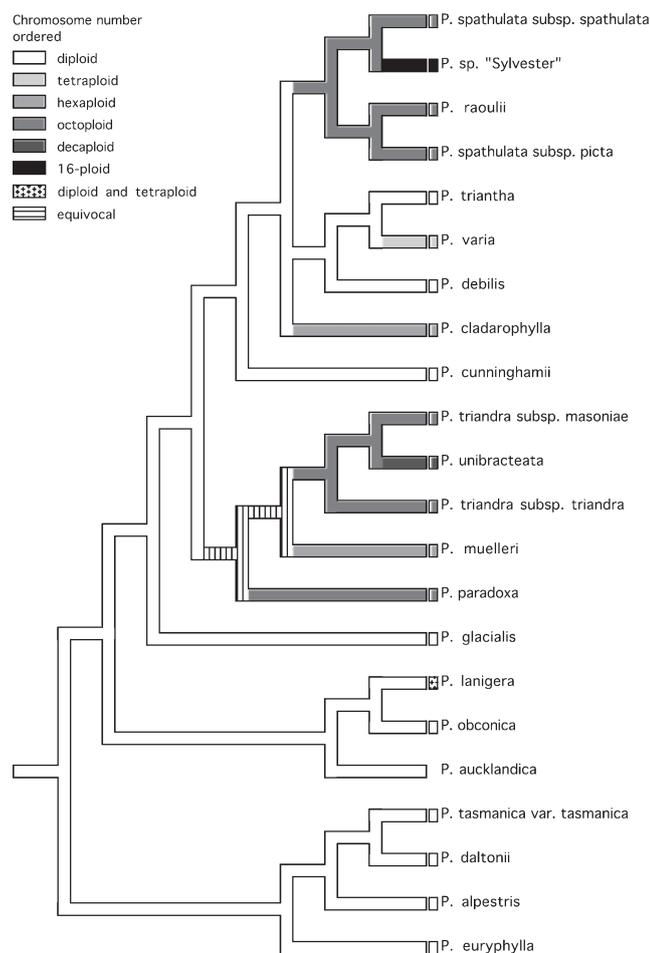


Fig. 5. Evolution of the chromosome number in Australasian *Plantago*. The following chromosome numbers were plotted with MacClade ver. 4.08 onto a pruned tree from Fig. 2, showing only Australasian species: diploid ($2n=12$), tetraploid ($2n=24$), hexaploid ($2n=36$), octoploid ($2n=48$), decaploid ($2n=60$) and 16-ploid ($2n=96$) character states.

Additionally, we have updated the data presented by including the diploid chromosome number of *P. daltonii* (Brown 1981).

Fortunately, the discussion and conclusions of the original paper are still consistent with the revised figures.

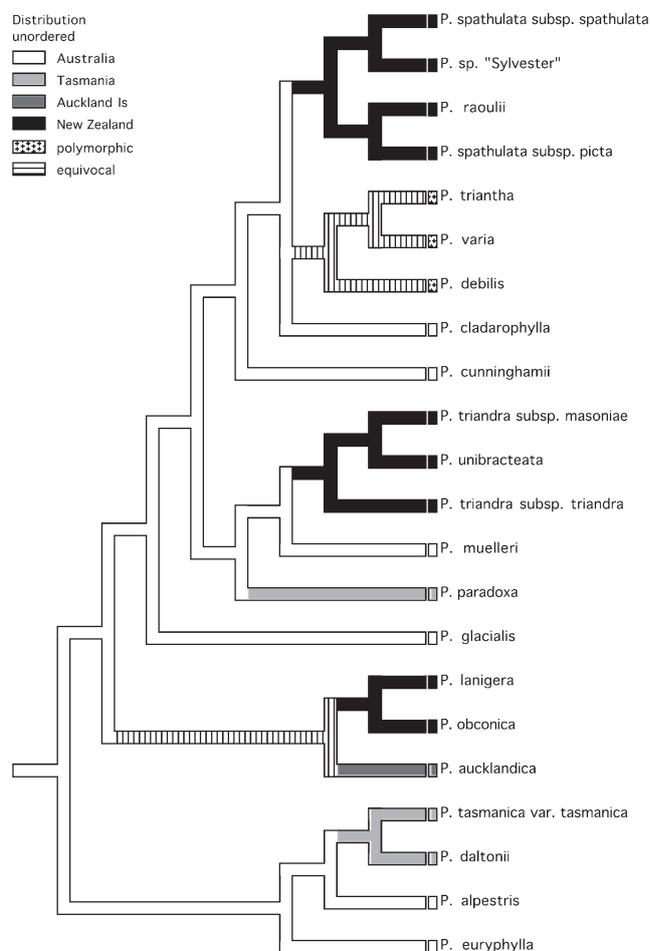


Fig. 6. Biogeographic patterns in Australasian *Plantago*. The following four biogeographic areas were plotted with MacClade ver. 4.08 onto a pruned tree from Fig. 2, showing only Australasian species: mainland Australia, Tasmania, Auckland Islands and New Zealand.

Acknowledgement

We thank Jennifer Tate for drawing our attention to the errors corrected here.

References

Brown MJ (1981) An experimental taxonomic study of *Plantago tasmanica* Hook. f. and *P. daltonii* Dene. *Australian Journal of Botany* **29**, 441–452. doi:[10.1071/BT9810441](https://doi.org/10.1071/BT9810441)

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DNA sequences from three genomes reveal multiple long-distance dispersals and non-monophyly of sections in Australasian *Plantago* (Plantaginaceae)

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Abstract. We examined the geographic origins and taxonomic placements of New Zealand and Australian *Plantago* (Plantaginaceae) by using molecular phylogenetic data. *Plantago* comprises over 200 species distributed worldwide. Analyses of three markers from the nuclear (ITS), chloroplast (*ndhF-rpl32*) and mitochondrial (*coxI*) genomes showed that the New Zealand species form three distinct, well supported clades that are not each others' closest relatives, and were each derived relative to the sampled Australian species. Therefore, at least three long-distance directional dispersal events into New Zealand can be inferred for *Plantago*, likely from Australian ancestors. This result differs from the biogeographic pattern often reported for New Zealand plant genera of a single dispersal event followed by rapid radiation, and may be attributed to ready biotic dispersal of mucilaginous seeds and habitat similarities of the Australasian species. Molecular dating placed the arrival time and diversification of the New Zealand species between 2.291 and 0.5 million years ago, which coincides with the geological dates for the uplift of mountain ranges in New Zealand. The mitochondrial DNA substitution rate of the Australasian clade relative to the rest of the genus is discussed, as well as implications of the non-monophyly of sections *Oliganthos*, *Mesembrynia* and *Plantago* within subgenus *Plantago*.

Additional keywords: biogeography, cpDNA, dispersal, molecular dating, molecular phylogeny, mtDNA, nrDNA.

Introduction

Plant biogeography in the southern hemisphere has garnered much attention since early studies by Hooker (1853), Darwin (1859) and others. In particular, increased use of molecular phylogenetics since the 1990s has allowed for large-scale evolutionary studies in the region (e.g. Sanmartín and Ronquist 2004; Sytsma *et al.* 2004; Sanmartín *et al.* 2007). Recent studies have revealed that long-distance dispersal in the southern hemisphere is far more common than previously thought (Winkworth *et al.* 1999, 2002b) and that directional dispersal from west to east is common (west wind drift; Winkworth *et al.* 2002b; Muñoz *et al.* 2004; Sanmartín *et al.* 2007).

As an important part of the southern hemisphere, Australasia in particular has been shown to have been strongly influenced by biotic dispersal. Many phylogenetic studies have found that several Australian and New Zealand groups have closely related species or species complexes, suggesting recent or frequent dispersal or both (Breitwieser *et al.* 1999; von Hagen and Kadereit 2001; Wagstaff *et al.* 2002; Smissen *et al.* 2003; Meudt and Simpson 2006; Ford *et al.* 2007; Perrie and Brownsey 2007; Perrie *et al.* 2007). Groups with dispersible propagules might have repeatedly crossed the Tasman Sea (e.g. *Drosera*, Rivadavia *et al.* 2003). Consistent with the west wind drift, long-distance dispersal appears to be more common from Australia to

New Zealand (Wagstaff *et al.* 1999; Wagstaff and Wege 2002; Ford *et al.* 2007), although there have been a few well supported cases of dispersals in the other direction (Wagstaff and Garnock-Jones 2000; Lockhart *et al.* 2001; Wagstaff *et al.* 2002; Wanntorp and Wanntorp 2003; Meudt and Bayly 2008).

New Zealand especially poses interesting questions in biogeography, taxonomy and evolution because of its isolation (2000 km from the nearest major landmass), its continental geology and mostly Mesozoic age of its rocks, the relationships of its biota with the animals and plants of other regions (Darlington 1965; Nelson 1975; Trewick *et al.* 2007; McDowall 2008), and geological evidence of extensive submergence of the landmass during the Oligocene (Pole 1994; Cooper and Cooper 1995; Trewick *et al.* 2007). Recent literature (e.g. Winkworth *et al.* 1999; Stöckler *et al.* 2002; Wagstaff *et al.* 2002; Knapp *et al.* 2005, 2007) suggests that the origins of New Zealand flora may be the result of a mixture of older vicariance and more recent long-distance dispersal events. For the latter, a common pattern observed is a single dispersal event followed by rapid radiation (Wagstaff and Garnock-Jones 1998; Winkworth *et al.* 1999; Perrie *et al.* 2003; Albach *et al.* 2005; Meudt and Simpson 2006; Ford *et al.* 2007). Despite increasing numbers of molecular studies involving New Zealand plants, general evolutionary and biogeographic

patterns of the New Zealand flora are still not well known, particularly those regarding species in the alpine region (Lockhart *et al.* 2001).

Plantago is a large worldwide genus in the family Plantaginaceae, with more than 200 species. They are found on most continents and many oceanic islands and range from widespread, weedy species to specialised island endemics. Within Australasia (Fig. 1), there are 32 described species, native to New Zealand (8 species and 2 subspecies; Sykes 1988, 1998; Rahn 1996) and Australia (24 spp.; Briggs 1992). There is also a 16-ploid entity from New Zealand that probably represents an undescribed species, tag-named *P. sp.* 'Sylvester' (Groves and Hair 1971). In New Zealand, species of *Plantago* are distributed widely across both the North and South Islands; whereas in Australia, they are found on the mainland south of 25°S, and in Tasmania. Australasian species of *Plantago* also occur on offshore islands; e.g. *P. aucklandica* is endemic to the Auckland Islands, and *P. triantha* is native to the Auckland Islands and Tasmania. Plants are mostly small rosette herbs and are usually found in damp areas (such as seepage areas and near bogs, tarns and river edges) from alpine regions to lowland and coastal herbfields. Flowers are wind-pollinated and are either solitary or clustered into spicate inflorescences.

Rahn (1996) grouped all species of *Plantago* into six subgenera and several sections on the basis of phylogenetic analysis of morphological data. According to his study, all 32 Australasian species belong to the large subgenus *Plantago* (comprising 133 species in total); however, they were placed in three different sections within it (Rahn 1996). Thus, section *Mesembrynia* has 32 species, 20 of which are Australasian (3 from New Zealand, 17 from Australia), and 12 others are from Tonga (1), Amsterdam & St Paul Islands in the Indian Ocean (2), New Guinea (3) and Eurasia (6) (although the inclusion of these six Eurasian species in this section 'may be incorrect', K. Rahn, pers. comm., cited in Rønsted *et al.* 2002: 333). Section *Oliganthos* has 23 species and is further subdivided into three series, namely series *Oliganthos* (8 spp., 7 South American and 1 Tasmanian), series *Carpophorae* (2 Andean spp.) and series *Microcalyx* (13 spp., 4 each from New Zealand and New Guinea, and 5

from Australia). Only 2 of the 42 species in section *Plantago* are Australasian (*P. aucklandica* from the Auckland Islands and *P. hedleyi* from Lord Howe Island), with the rest of the species from Eurasia, Oceania and the Americas. The remaining two sections *Micropsyllium* and *Virginica* (6 and 28 spp., respectively) are largely American sections, and contain no Australasian species.

According to Rahn's morphological phylogenetic analysis, within a monophyletic subgenus *Plantago*, sections *Micropsyllium*, *Oliganthos* and *Virginica* were monophyletic, whereas section *Mesembrynia* was paraphyletic (with sect. *Virginica* nested within it) and section *Plantago* was paraphyletic relative to all the other sections (Rahn 1996). None of the sections that comprise Australasian species has a unique synapomorphy. Section *Oliganthos* was differentiated by few flowers (usually 1–3) which also occurs in subgenera *Littorella* and *Psyllium*. Sections *Mesembrynia* and *Virginica* are linked by the presence of an apical fifth seed (or at least its upper compartment), although neither section has a synapomorphy. Section *Plantago* is differentiated by many crowded flowers on the spike, although Rahn (1996) considered this character pleisiomorphic. Rahn (1996) noted that species relationships and infrageneric classification within subgenus *Plantago* were especially difficult to resolve and uncertain because of a lack of morphological characters, although he did note the likely close affinity of species from the largely southern hemisphere sections *Mesembrynia* and *Oliganthos*. With respect to the biogeography of these southern hemisphere species, Rahn (1996) suggested that the current distribution was achieved through vicariance and subsequent extinctions.

A molecular phylogenetic analysis of ITS and *trnL-F* sequences from 57 *Plantago* species representing all subgenera and most sections (see Rønsted *et al.* 2002) tested Rahn's morphological groups and biogeographic hypotheses. In the study of Rønsted *et al.*, subgenus *Plantago* was monophyletic (as were three other subgenera as defined by Rahn) although relationships among the 19 species of subgenus *Plantago* sampled were not well supported. The four species of section *Mesembrynia* sampled (*P. debilis* from Australia, *P. raoulii* and *P. spatulata* from New Zealand, and *P. stauntonii* from the Amsterdam & St Paul Islands) formed a highly supported monophyletic group in this clade, whereas the two sampled species from section *Oliganthos* (*P. uniglumis* and *P. rigida* from South America) were not monophyletic and were in unresolved positions near the section *Virginica* clade. A more recent study using a nuclear-encoding single-copy gene, *SUC1* (Ishikawa *et al.* 2009), sampled 18 of the 19 individuals of subgenus *Plantago* included in Rønsted *et al.* (2002), plus four additional species including *P. depressa* and *P. camtschatica*, two Asian species of section *Mesembrynia*. The topology of their phylogeny was similar to that in Rønsted *et al.* (2002) although with higher resolution and support values (Ishikawa *et al.* 2009). With respect to biogeography, both Rønsted *et al.* (2002) and Ishikawa *et al.* (2009) inferred that the distribution of these southern hemisphere species, and indeed of the genus as a whole, was largely influenced by long-distance dispersal.

Another study included seven different species of *Plantago* from sections *Mesembrynia* and *Oliganthos* in their molecular phylogenetic analyses of ITS sequences of 23 species of *Plantago*

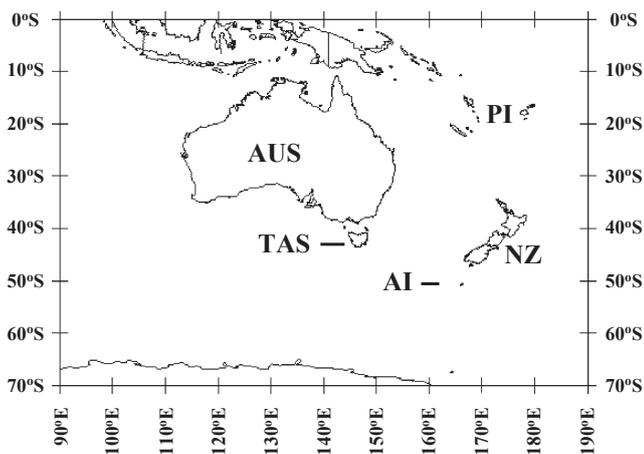


Fig. 1. The Australasian region. AI = Auckland Islands, AUS = Australia, NZ = New Zealand, PI = Pacific Islands and TAS = Tasmania.

and three species of the closely related genus *Littorella* (Hoggard *et al.* 2003). In that study, subgenus *Plantago* (14 sampled species) was highly supported as monophyletic, although relationships within subgenus *Plantago* were not well resolved or supported by high bootstrap values. The six Australasian species from sections *Mesembrynia* (*P. tasmanica*, *P. daltonii*, *P. euryphylla* and *P. hispida*, all from Australia) and *Oliganthos* (*P. paradoxa*, Australia, and *P. triandra*, New Zealand) formed a monophyletic group within that clade, although the two sections were not monophyletic (Hoggard *et al.* 2003). In fact, section *Mesembrynia* was polyphyletic because the Asian species, *P. camtschatica*, was more closely related to *P. sparsiflora* from the USA (sect. *Plantago*) than to the Australian species of section *Mesembrynia*.

Thus, 14 of 56 species (25%) of sections *Mesembrynia* and *Oliganthos*, including only 9 of 32 (28%) of Australasian species of *Plantago*, have been included in three different molecular phylogenies (two with non-overlapping sampling), and there has been little resolution or support for their evolutionary relationships. We obtained molecular sequence data from 21 Australasian species (including 12 species that had not been included in previous studies) to investigate biogeographic patterns of *Plantago* within Australasia, with a particular focus on resolving relationships among all the New Zealand species. In increasing taxonomic sampling and adding novel molecular data from all three genomes, we will also make a significant contribution towards the bigger goal of achieving a molecular phylogeny for the southern hemisphere species and indeed all species of the large, worldwide genus *Plantago*.

Materials and methods

Molecular techniques

Tissue samples were either preserved in silica gel from field collections, or were obtained from existing herbarium specimens. After manual disruption of dried tissue with a pestle and mortar, DNA extractions were performed with either the DNEasy Plant Mini kit (Qiagen, Hilden, Germany) or by a cetyltrimethylammonium bromide (CTAB) protocol modified from Doyle and Doyle (1990).

The use of different markers from several genomes may provide insights into events such as hybridisation, introgression, reticulation and incomplete lineage sorting if these have occurred in the past (Vriesendorp and Bakker 2005). We chose the internal transcribed spacers (ITS) from nuclear rDNA (nrDNA), *ndhF-rpl32* spacer from chloroplast DNA (cpDNA) and *coxI* from mitochondrial DNA (mtDNA) from a primer assay to elucidate the evolutionary history of the Australasian species of *Plantago*. The mtDNA region is particularly interesting for *Plantago* because unprecedented elevated rates of mtDNA evolution have been reported in the genus (Cho *et al.* 2004), whereas the mitochondrial genome is normally characterised by slow rates of evolution in plants (Wolfe *et al.* 1987).

The primers used to amplify the ITS region (ITS1, 5.8s nrDNA and ITS2), *ndhF-rpl32* spacer region, and *coxI* mitochondrial gene, respectively, were as follows: *ITS28CC*–CGCCGTTAC TAGGGGAATCCTTGTAAG (Wagstaff and Garnock-Jones 1998) and *ITS5*–GGAAGTAAAAGTCGTAACAAGG (White

et al. 1990); *ndhF*–AAAGGTATKATCCAYGMATATT and *rpl32R*–AATATCCCTTYTTTCCAA from (Shaw *et al.* 2007); and *coxIF4*–GGATATCTAGGYATGGTTTATGC and *coxIR3*–AAGCTGGAGGACTTTGTAC (Cho *et al.* 2004). PCR amplification was performed by using a final volume of 25 μ L of the following: 16.35 μ L water, 1 \times ThermoPol reaction buffer (10 mM KCl, 10 mM (NH₄)₂SO₄, 20 mM TRIS–HCl (pH 8.8), 2 mM MgSO₄, 0.1% Triton X-100) (New England BioLabs, Ipswich, MA), 0.4 mg mL⁻¹ BSA, 250 μ mol dNTPs, 10 pmol each primer, 0.75 U of *Taq* DNA polymerase (New England BioLabs), and 0.4 μ L DNA template. The amplification was carried out with a thermocycling profile of an initial 2 min at 94°C, followed by 30 cycles of 1 min at 94°C, 1.5 min at 50°C, 1 min at 72°C, and ending with a final extension time of 5 min at 72°C. This PCR protocol was used to successfully amplify regions for all primer pairs. PCR products were visualised on 1.5% agarose gels before being purified with the High Pure PCR Purification Kit (Roche Diagnostics, Mannheim, Germany). The purified PCR products were sequenced in both directions with an ABI3730 Genetic Analyzer (Applied Biosystems, Melbourne) by the Allan Wilson Centre Genome Service (Massey University, Palmerston North, New Zealand).

Study group and taxonomic sampling

Plantago samples for the study were collected from wild populations across New Zealand and Australia. Location of samples, along with voucher information and GenBank accession numbers are presented in Table 1. DNA sequences were obtained for all nine native New Zealand species (including the tag-named *P. sp.* ‘Sylvester’), and 12 of the 24 native Australian species from subgenus *Plantago* sections *Oliganthos* (9 spp.), *Mesembrynia* (11 spp.) and *Plantago* (1 sp.). Sequences were also obtained for five introduced *Plantago* species that have established in New Zealand, including two from subgenus *Plantago* (*P. major*, section *Plantago*, and *P. australis*, section *Virginica*), two from the sister subgenera of subgenus *Plantago* (*P. coronopus*, subgenus *Coronopus*, and *P. lanceolata*, subgenus *Albicans*; Rønsted *et al.* 2002), and *P. sp.*, an unidentified and possibly newly introduced species in New Zealand. New Zealand species *Veronica hookeriana* and *V. salicornioides* (Garnock-Jones *et al.* 2007) were used as distant outgroups because *Veronica* is a close relative of *Plantago* (Rønsted *et al.* 2002; Cho *et al.* 2004). This sampling strategy resulted in datasets comprising 36 individuals, representing 27 species of *Plantago* and two species of *Veronica* (only 35 individuals were included in the *ndhF-rpl32* dataset because *V. salicornioides* was not able to be sequenced for this marker). All sequences in these datasets have been deposited in GenBank (Table 1). A concatenated dataset of the 35 individuals common to all three datasets was compiled by combining sequences from all three regions.

In addition, an expanded ITS dataset was also assembled, comprising 252 sequences, including 239 from *Plantago*, 10 from *Littorella*, one from *Aragoa* and two from *Veronica*. Of these, 100 sequences were downloaded from GenBank (see Appendix 1), 36 were generated for the previous dataset (Table 1) and 116 were generated for a separate study on evolutionary patterns and species boundaries of the

Table 1. Collection details and GenBank accession numbers for species included in the present study

Locations: AI = Auckland Islands, AUS = Australia, NSW = New Zealand and TAS = Tasmania. Collectors: *BGB* = Barbara Briggs, *MLT* = Mei Lin Tay, *PBH* = Peter Heenan, *PGJ* = Phil Garnock-Jones, *PB* = Peter Beveridge, *PJL* = Peter Lockhart, *RL* = Rodney Lewington and *VT* = Vanessa Thorne. Herbarium vouchers: *CHR* = Allan Herbarium, Landcare Research, Christchurch, New Zealand; *NSW* = National Herbarium of New South Wales, Australia; and *WELTU* = H. D. Gordon Herbarium in Victoria University of Wellington, New Zealand; n.a., not available

Species	Indigenous distribution	Collected from	Collection number	ITS	GenBank accession number	Herbarium voucher
					<i>ndhF-rp32</i>	<i>cox1</i>
Section <i>Mesembrynia</i>						
<i>Plantago alpestris</i>	AUS	Kosciuszko National Park, NSW, AUS	<i>BGB9748</i>	FJ024611	FJ024647	NSW742962
<i>P. cladarophylla</i>	AUS	Barrington Tops National Park, NSW, AUS	<i>JRHosking 2682</i>	FJ024612	FJ024648	WELTU20189
<i>P. cunninghamii</i>	AUS	Brigalow Park Nature Reserve, NSW, AUS	<i>JRHosking 2752</i>	FJ024613	FJ024649	WELTU20186
<i>P. daltonii</i>	TAS	St Clair National Park, TAS, AUS	<i>BGB9782</i>	FJ024617	FJ024653	NSW743874
<i>P. debilis</i>	AUS/TAS	Barrenjoey Headland, NSW, AUS	<i>BGB9738</i>	FJ024608	FJ024644	NSW742894
<i>P. eurypphylla</i>	AUS	Kosciuszko National Park, NSW, AUS	<i>BGB9743</i>	FJ024607	FJ024643	NSW742956
<i>P. spathulata</i> subsp. <i>picta</i>	NZ	East Cape, Gisborne, NZ	n.a.	FJ024590	FJ024626	CHR439486
<i>P. spathulata</i> subsp. <i>spathulata</i>	NZ	Marfells Beach, Marlborough, NZ	<i>PGJ2629 & MLT</i>	FJ024589	FJ024625	WELTU20117
<i>P. sp.</i> 'Sylvester'	NZ	Lake Sylvester, Nelson, NZ	<i>MLT022 & PGJ</i>	FJ024600	FJ024636	WELTU20150
<i>P. raoulitii</i> (1)	NZ	Lake Sarah, Cass, NZ	<i>PGJ2259</i>	FJ024592	FJ024628	WELTU20153
<i>P. raoulitii</i> (2)	NZ	Waiuomata Valley, Wellington, NZ	<i>PB & RL s.n.</i>	FJ024591	FJ024627	WELTU20155
<i>P. tasmanica</i> var. <i>tasmanica</i>	TAS	St Clair National Park, TAS, AUS	<i>BGB9780</i>	FJ024615	FJ024651	WELTU20188
<i>P. varia</i>	AUS/TAS	Kosciuszko National Park, NSW, AUS	<i>BGB9767</i>	FJ024609	FJ024645	NSW743869
Section <i>Oliganthos</i>, series <i>Microcalyx</i>						
<i>P. glactalis</i>	AUS	Kosciuszko National Park, NSW, AUS	<i>BGB9753</i>	FJ024610	FJ024646	NSW743813
<i>P. lanigera</i> (1)	NZ	Sugar Loaf Pass, Mt Aspiring National Park, NZ	<i>Mike Thorsen s.n.</i>	FJ024596	FJ024632	WELTU20133
<i>P. lanigera</i> (2)	NZ	Sugar Loaf Pass, Mt Aspiring National Park, NZ	<i>Mike Thorsen s.n.</i>	FJ024597	FJ024633	WELTU20133
<i>P. lanigera</i> (3)	NZ	Hall Range, Canterbury, NZ	<i>PBH s.n.</i>	FJ024593	FJ024629	WELTU20143
<i>P. lanigera</i> (4)	NZ	Wilberg Range, Westland, NZ	<i>MLT027 et al.</i>	FJ024594	FJ024630	WELTU20147
<i>P. lanigera</i> (5)	NZ	Wilberg Range, Westland, NZ	<i>MLT027 et al.</i>	FJ024595	FJ024631	WELTU20147
<i>P. muelleri</i>	AUS	Kosciuszko National Park, NSW, AUS	<i>BGB9752</i>	FJ024606	FJ024642	NSW743812
<i>P. obconica</i> (1)	NZ	Hector Mountains, Otago, NZ	n.a.	FJ024603	FJ024639	CHR573261
<i>P. obconica</i> (2)	NZ	Cardrona Ski Field, Wanaka, NZ	<i>PGJ2600 et al.</i>	FJ024604	FJ024640	WELTU20121
<i>P. paradoxo</i>	TAS	St Clair National Park, TAS, AUS	<i>BGB9781</i>	FJ024616	FJ024652	WELTU20187
<i>P. triandra</i> subsp. <i>masoniae</i>	NZ	Paturau Coast, Nelson, NZ	<i>PJL s.n.</i>	FJ024598	FJ024634	WELTU20168
<i>P. triandra</i> subsp. <i>triandra</i>	NZ	Lake Sylvester, Nelson, NZ	<i>MLT021 & PGJ</i>	FJ024599	FJ024635	WELTU20163
<i>P. unibracteata</i> (1)	NZ	Mt Ruapehu, NZ	<i>MLT053 et al.</i>	FJ024602	FJ024638	WELTU20173
<i>P. unibracteata</i> (2)	NZ	Lake Sylvester, Nelson, NZ	<i>MLT024 & PGJ</i>	FJ024601	FJ024637	WELTU20175
Section <i>Oliganthos</i>, series <i>Oliganthos</i>						
<i>P. triantha</i>	TAS/AI	Enderby Island, AI, NZ	<i>VT55</i>	FJ024614	FJ024650	WELTU20177
Section <i>Plantago</i>						
Section <i>Plantago</i>						
Section <i>Plantago</i>						
Outgroups						
<i>P. australis</i>	AI	AI, NZ (cult. Otari-Wilton Bush)	n.a.	FJ024605	FJ024641	WELTU20185
<i>P. coronopus</i>	America	Hunua Ranges, Auckland, NZ	<i>MLT019 & PGJ</i>	FJ024618	FJ024654	WELTU20181
<i>P. lanceolata</i>	Mediterranean, Europe	Island Bay, Wellington, NZ	<i>PGJ2549</i>	FJ024621	FJ024657	WELTU20183
<i>P. major</i>	Cosmopolitan	Karori, Wellington, NZ	<i>PGJ2551</i>	FJ024622	FJ024658	WELTU20184
<i>P. sp.</i> ^A	Cosmopolitan	Ben Burn Park, Karori, NZ	<i>PGJ2550</i>	FJ024619	FJ024655	WELTU20180
<i>Veronica hookeriana</i>	?	Pukerua Bay, Wellington, NZ	<i>PGJ2566 & MLT</i>	FJ024620	FJ024656	WELTU20178
<i>V. salicornioides</i>	NZ	Whanahuia Range, Ruahine Mountains, NZ	<i>PGJ2458</i>	FJ024623	FJ024659	WELTU20200
	NZ	Jacks Pass, Hammer, Canterbury, NZ	n.a.	FJ024624	n.a.	CHR512475

^AThe taxon referred to as *P. sp.* in this study is an introduced species that has never been reported in New Zealand. The ITS sequence for this species was identical to a *P. asiatica* (subg. *Plantago*, sect. *Plantago*) sequence downloaded from GenBank and further morphological work is needed to clarify the identity of this sample.

New Zealand *Plantago* species from multiple individuals and populations of each of the New Zealand species (Tay 2008; M. L. Tay, H. M. Meudt, P. J. Garnock-Jones and P. Ritchie, unpubl. data). After removing identical sequences to improve the efficiency of the analyses, the expanded ITS dataset had 177 sequences. Compilation of this dataset allowed for the first time simultaneous analysis of all the sequences used in Rønsted *et al.* (2002) and Hoggard *et al.* (2003), plus our newly generated sequences. This resulted in the best available sampling for phylogenetic analysis of subgenus *Plantago* (32% of species included in the present study) and sections *Mesembrynia* and *Oliganthos* (45%). Specifically, within subgenus *Plantago*, the following were included: sections *Mesembrynia* (47%), *Oliganthos* (42%), *Plantago* (26%), *Micropsyllium* (50%) and *Virginica* (18%).

Dataset alignment and analyses

MEGA ver. 3.1 (Kumar *et al.* 2004) was used to assemble forward and reverse sequences for a given DNA marker for each individual, and then to align individual sequences with one another. Characteristics of the individual sequence datasets were examined with MEGA ver. 3.1 and DAMBE ver. 4.5.9 (Xia and Xie 2001).

Analyses were performed individually on each of the five datasets (ITS, *coxI*, *ndhF-rpl32*, concatenated and expanded ITS). Preliminary analyses of our datasets suggested that gaps are informative and should be included in the dataset. Gaps were coded by a modified complex indel coding (MCIC) method using SeqState ver. 1.32 (Müller 2005) because MCIC has been found to outperform other gap-coding methods (Simmons *et al.* 2007). The ITS dataset had 26 indel characters, the *ndhF-rpl32* region had 36 indel characters, and there were no indels in the *coxI* dataset. The concatenated dataset included 61 indel characters because an indel was present in the ITS sequence of *Veronica salicornioides* but the sequence was not included in the concatenated dataset. Ambiguous characters in the datasets were excluded from phylogenetic analyses. The final concatenated dataset comprised 2194 bp (2255 bp when indel characters were included as coded characters). The expanded ITS dataset comprised 656 bp (733 bp when indel characters were included as coded characters).

The substitution model for each dataset was selected with Modeltest ver. 3.7 (Posada and Crandall 1998), using the Akaike information weights criterion (AIC) because it has advantages over the hierarchical likelihood ratio test (Posada and Buckley 2004). Maximum parsimony (MP) and maximum likelihood (ML) analyses were conducted on all datasets (ML analyses were not conducted for the expanded ITS dataset) using PAUP* ver. 4.0b10 (Swofford 2002). Heuristic searches were conducted under an MP criterion by using 10 000 replicates of random sequence addition and tree-bisection–reconnection (TBR) branch swapping, with a maximum of 10 000 trees. For ML analyses, 100 replicates of random sequence addition and TBR branch swapping were implemented. Non-parametric bootstrap support was assessed with 200 replicates for all of the analyses, with random sequence addition and TBR branch swapping (or subtree pruning and regrafting, SPR, for the expanded ITS dataset only).

Heuristic searches were conducted on all five datasets with MrBayes ver. 3.1.1 (Huelsenbeck and Ronquist 2001), with each dataset analysed initially with four chains and 500 000 generations (1 000 000 for the expanded ITS dataset). Additional generations were added as needed, to reach a standard deviation of split frequencies of <0.01, which ensures that the runs have converged on a stationary distribution. For the concatenated dataset, two separate analyses were conducted to check the suitability of the models applied to data with potentially very different evolutionary patterns. First, one model was used across the whole dataset, and then a partitioned dataset was used such that separate models were used for each dataset as recommended by Modeltest. For each run, 25% of the trees were excluded as burn-in.

Topological congruence was examined first by visually comparing trees generated from the ITS, *coxI* and *ndhF-rpl32* datasets for each analysis. A partition–homogeneity (ILD) test was also run in PAUP* (1000 replicates, TBR branch swapping, 100 random replicates of random taxa addition, and maximum of 10 000 trees) for all combinations of datasets. Additionally, a supertree network approach (McBreen and Lockhart 2006) was used to identify the strongest phylogenetic signal within each of the individual datasets. Supertree networks were constructed from ML trees from the three datasets with SplitsTree ver. 4.8 (Huson and Bryant 2006).

Characters such as geographic distributions, ploidy levels and key features of the sections of subgenus *Plantago* were mapped onto the concatenated tree (Fig. 2) with MacClade ver. 4.08 (Maddison and Maddison 2005), to investigate evolutionary patterns and taxonomic significance. For geographic distributions, we used the following four areas: mainland Australia (we did not attempt a finer resolution into biogeographic areas within this region because our sample did not include all the species present and because our question of interest was dispersal to New Zealand), Tasmania, Auckland Islands and New Zealand.

Molecular dating

An ITS dataset consisting of 38 individuals, including representatives from each *Plantago* and *Veronica* species collected for the present study and several species each of *Aragoa* and *Littorella* obtained from GenBank, was used to date nodes for the Australasian species (see Appendix 2). ITS sequences were used only because ITS sequences (but not the other markers) for *Littorella* and *Aragoa* are readily available on GenBank. A likelihood ratio test showed that the ITS sequences did not evolve in a clock-like manner (Felsenstein 1981); thus, BEAST ver. 1.4.6 (Drummond and Rambaut 2007) was used to implement a relaxed-clock model (Drummond *et al.* 2006) to estimate divergence times for the lineages in the dataset. Monophyletic groups with high support in the ML tree were set up as groups for dating nodes with BEAUti ver. 1.4.6 (Drummond and Rambaut 2007).

Analyses in BEAST were conducted with 10 000 000–20 000 000 runs, until estimated sample sizes (ESS) for each parameter were large enough. A GTR+I+G model was implemented in all cases as recommended by Modeltest. TRACER ver. 1.4 (Drummond and Rambaut 2007) was used to

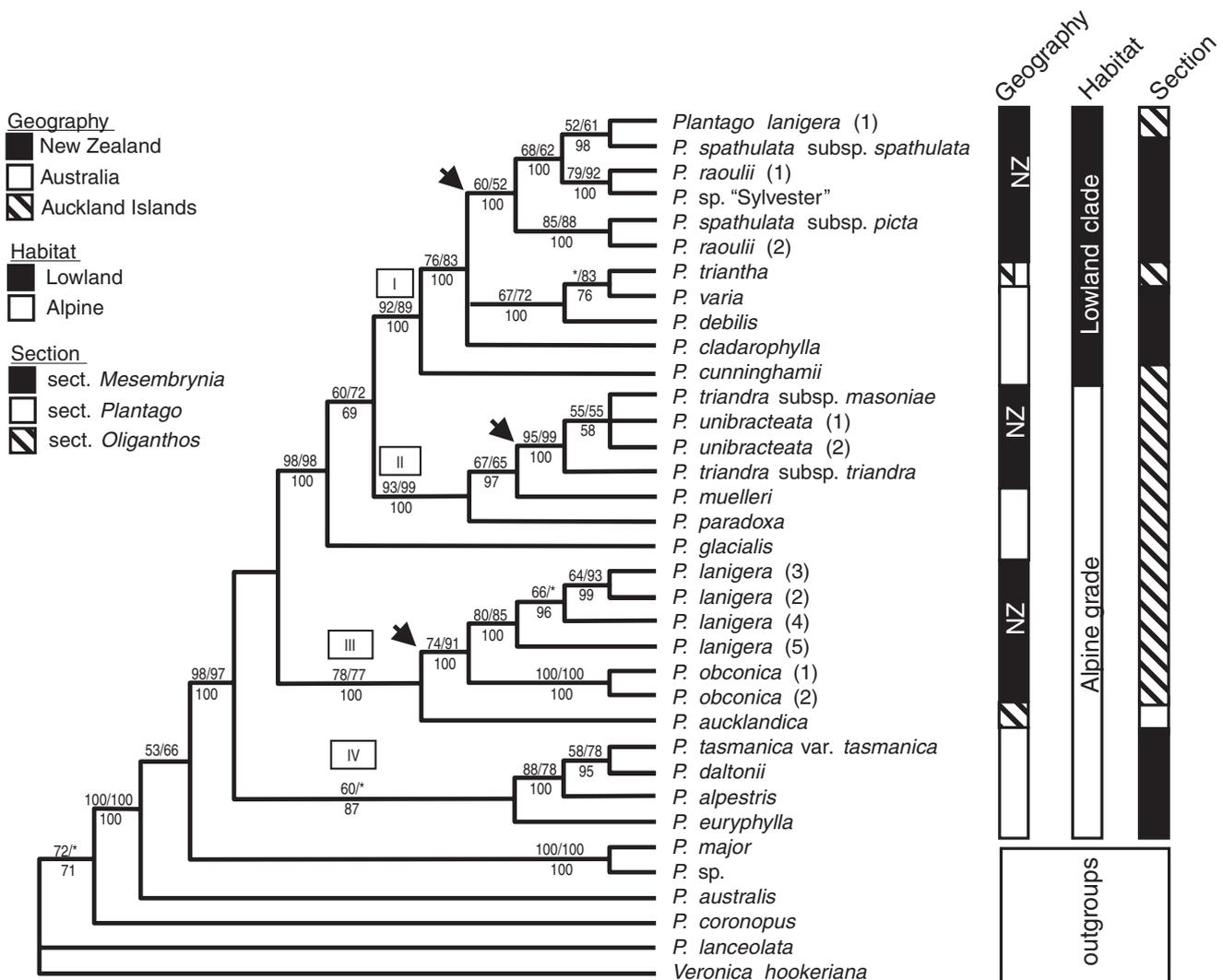


Fig. 2. Maximum likelihood (ML) tree reconstructed using concatenated data (ITS, *ndhF-rp132* and *cox1*) of native and introduced Australasian *Plantago* species (likelihood score = -7738.89). Values displayed above branches are from maximum parsimony/ML bootstrap analyses, whereas values presented below branches are posterior probabilities from MrBayes analyses (* indicates less than 50% support). Numbers in parentheses following epithets represent individuals sampled from the same species. Arrows indicate a trans-Tasman dispersal event to New Zealand from an ancestral Australian population. Clades I-IV are mentioned in the text. Bars to the right of the tree indicate geography, habitat and sectional classification following Rahn (1996).

analyse results. Visual comparison of the trees reconstructed by BEAST showed identical topology of the ITS tree reconstructed by using ML analysis in all cases.

To obtain estimates for the divergence dates of Australasian species, two different (secondary) calibration points were used. First, the dates 5.47 million years ago for the radiation of the *Plantago-Littorella* group and 7.1 million years ago since the divergence of this group and *Aragoa* (see Rønsted *et al.* 2002) were used. These dates had been estimated previously by using a non-parametric rate smoothing approach on an ITS molecular phylogeny calibrated with the age of New Amsterdam, an oceanic island that arose 0.5–0.7 million years ago and to which *P. stauntonii* subsequently dispersed (Rønsted *et al.* 2002). (Although a more direct dating approach would be ideal, unfortunately we were unable to directly calibrate

the tree based on this particular node as the placement of *P. stauntonii* was not resolved in our ITS phylogeny.)

Second, we used the date estimate of 2.766 (± 0.08) million years ago for the split of the *Plantago-Littorella* group from *Aragoa*. We estimated this date by downloading the dataset used by Rønsted *et al.* (2002) and running BEAST with their tree calibrated to the age of New Amsterdam for *P. stauntonii*. It is important to note that the non-parametric rate smoothing method used to estimate rates of evolution in Rønsted *et al.* (2002) may produce results different from those with BEAST analyses, and indeed this is the case here for the age of the *Plantago-Littorella* and *Aragoa* split (7.1 million years ago v. 2.8 million years ago). Thus, we re-ran the BEAST analysis on our dataset with this newly estimated divergence date (2.766 (± 0.08) million years ago) and compared the date of

divergence recovered for Australasian *Plantago* to those estimated previously.

Results

Dataset statistics

Inspection of the three datasets revealed that the *ndhF-rpl32* dataset had more variable sites than did the ITS and *coxI* datasets, and a lower percentage of parsimony-informative sites than did the ITS dataset (see Table 2 for a summary of statistics for all datasets). All individuals sequenced had unique ITS ($n=36$) and *ndhF-rpl32* ($n=35$) sequences, whereas only 15 of 36 individuals had unique sequences for *coxI*. Some Australian species had *coxI* sequences identical to those of some New Zealand species (*P. muelleri* was identical to *P. triandra* and *P. unibracteata*; *P. debilis* and *P. triantha* were identical to *P. raoulii* and *P. spathulata* subsp. *picta*; and *P. euryphylla* and *P. glacialis* were identical to *P. lanigera*, *P. novae-zelandiae*, *P. obconica* and *P. aucklandica*). Chi-square tests of all base frequencies showed a significant base heterogeneity for only the *ndhF-rpl32* dataset and among variable sites for the *coxI* dataset. In the ITS dataset, some sequences had a few ambiguous sites that may have resulted from multiple copies of the rDNA array in the genome.

Partition-homogeneity (ILD) tests indicated that the datasets were not congruent for ITS+*ndhF-rpl32*+*coxI* (P -value=0.001), ITS+*coxI* ($P=0.002$), ITS+*ndhF-rpl32* ($P=0.001$) and *ndhF-rpl32*+*coxI* ($P=0.016$). Out of these, the organellar DNA datasets (*ndhF-rpl32* and *coxI*) showed the least conflict. Visual analysis of the topology and the SplitsTree graph (refer to Appendix 3) indicated that four species (*Plantago muelleri*, *P. paradoxa*, *P. triandra* and *P. unibracteata*) were providing conflicting signals among the regions. This may be the cause of the low P -values of the ILD test. However, we found that the three datasets were still significantly incongruent when ILD tests were run excluding these four species. Although traditionally used to check congruence of

phylogenies from different regions, the ILD test has been shown to be highly inaccurate even when the topologies of trees are congruent (Reeves *et al.* 2001; Yoder *et al.* 2001) and may continue to indicate conflict even after the incongruent sequences are removed (Manos *et al.* 1999). Thus, analyses were carried out using the concatenated dataset because the individual tree topologies were not vastly different, there were no highly supported clades that were incongruent with each other, and there was low support for the placement of the conflicting species in the ITS and *coxI* trees.

Phylogenetic analyses

Topologies of trees reconstructed by using different tree-construction methods were very similar for each of the datasets. Additionally, using one model *v.* individual models for each molecular marker in MrBayes resulted in trees with similar topology (data not shown). The posterior probabilities (PP) for trees reconstructed using a Bayesian inference of phylogeny were found to be similar to bootstrap (BP) support values obtained with ML, and higher than those obtained with MP. Trees reconstructed with ML are presented here for the concatenated dataset (Fig. 2) and the individual ITS, *coxI* and *ndhF-rpl32* datasets (Fig. 3), and a Bayesian 50% majority rule tree is presented for the expanded ITS dataset (Fig. 4). Support values <50% were considered low support and are not shown in the figures. Support values are considered high if they are >70% for the MP and ML bootstrap analyses, and >95% for MrBayes analyses.

Concatenated dataset

Multiple clades were resolved with good support in the trees reconstructed from the concatenated dataset (Fig. 2). The Australasian species form a highly supported clade (98/97/100 MP BP/ML BP/Bayesian PP support values), comprising four highly supported monophyletic groups.

Table 2. Summary of statistics for the datasets used in the study

	ITS (nuclear)	<i>ndhF-rpl32</i> (chloroplast)	<i>coxI</i> (mitochondrial)	Concatenated
Base frequencies of all sites (%)	T-23.0 C-26.4 A-22.2 G-28.4	T-37.0 C-12.5 A-37.3 G-13.2	T-32.8 C-23.2 A-23.3 G-20.6	T-31.2 C-20.4 A-28.0 G-20.4
Base frequencies of variable sites (%)	T-31.6 C-26.2 A-22.5 G-19.6	T-33.0 C-19.0 A-25.4 G-22.5	T-26.1 C-34.1 A-25.5 G-14.3	T-30.5 C-25.1 A-24.7 G-19.7
Range of sequence length (aligned length) in bp including outgroups	577–622 (639)	548–737 (942)	613 (613)	1774–1972 (2194)
No. of recoded gaps appended (aligned length with recoded gaps)	26 (665)	36 (978)	0 (613)	61 (2255)
No. of variable sites and parsimony-informative sites including outgroups (%)	211 (20%)	286 (10%)	150 (19%)	638 (13%)
No. of variable sites and parsimony-informative sites for ingroup sequences (%)	78 (6%)	95 (6%)	39 (2%)	212 (5%)
Modeltest model (AIC)	GTR+G	TVM+G	TVM+G	TIM+I+G
Gamma shape estimate	0.4645	2.0817	0.2834	0.9143
Pinvar	0	0	0	0.2309
No. of transitions (all sites included)	17	14	10	38
No. of transversions (all sites included)	10	17	11	38
Transition/transversion ratio (all sites included)	1.6	0.8	0.9	1.0
% missing data	0.43	0.64	0.44	0.52

Clade IV (60/<50/87) comprises four Australian species from section *Mesembrynia* and is sister to the rest of the clades. Clade III (78/77/100) comprises two New Zealand species from section *Oliganthos* series *Microcalyx* (*P. lanigera* and *P. obconica*), which together are sister to *P. aucklandica* (section *Plantago*) from the Auckland Islands. This clade is sister to the rest of the Australasian species. Australian species *P. glacialis* (section

Oliganthos series *Microcalyx*) is sister to Clades I and II (99/98/100). Clade I (92/89/100) comprises New Zealand and Australian species from section *Mesembrynia* plus *P. triantha* (section *Oliganthos* series *Oliganthos*), whereas Clade II (93/99/100) comprises solely New Zealand and Australian species from section *Oliganthos* series *Microcalyx*. Clades I and II each contain a grade of Australian species at the base of a New Zealand clade.



Fig. 3. Maximum likelihood phylogenies with branch lengths and bootstrap values for each of the following three datasets: (A) ITS, (B) *ndhF-rpl32* and (C) *cox1*. Note branch-length difference among the trees with respect to the outgroup species *Plantago coronopus* and *P. lanceolata*, *Veronica* species, and the Australasian species. Likelihood scores for these trees are -2513.31 , -3045.43 and -8393.42 , respectively.

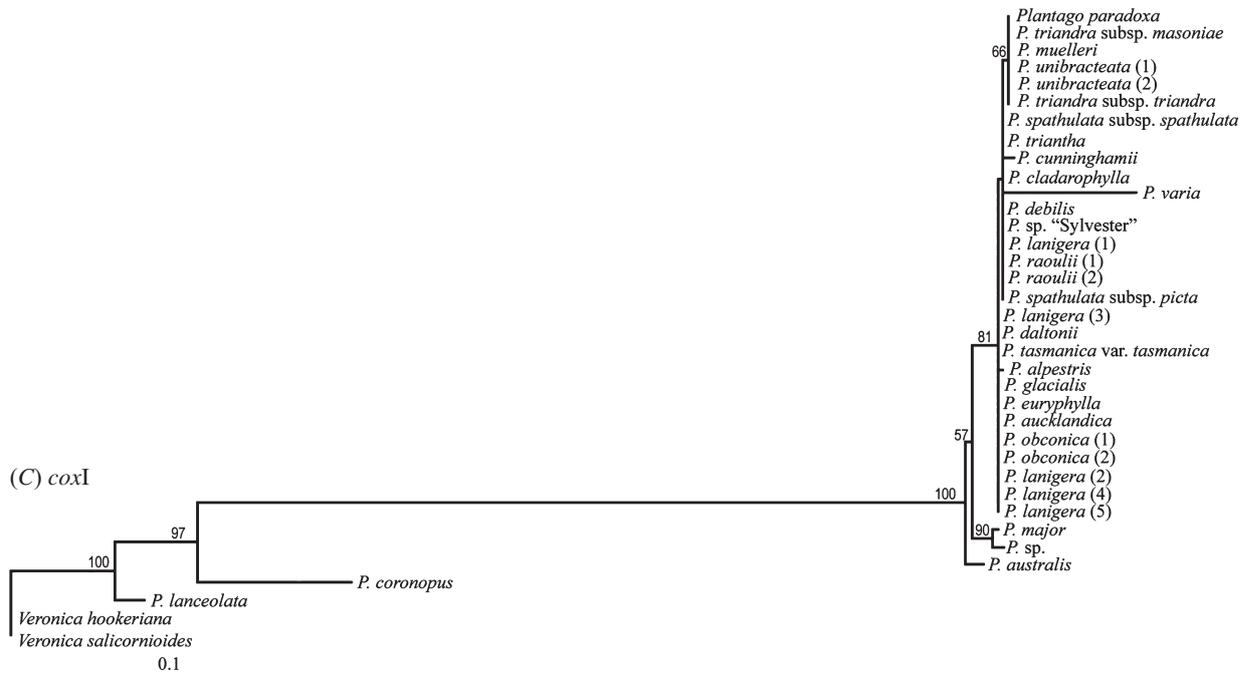


Fig. 3. (continued)

In addition, Clade I is a group of Australasian *Plantago* found in lowland areas, whereas Clades II, III and IV, along with *P. glacialis*, are found in alpine and subalpine areas.

Sequences from multiple individuals of *Plantago raoulii* and *P. lanigera*, and of subspecies of *P. spathulata* and *P. triandra*, do not form monophyletic groups matching the circumscriptions of those species (Fig. 2). This is being investigated in a separate study focusing on taxonomic issues and relationships of individuals of New Zealand species at the population level (M. L. Tay, H. M. Meudt, P. J. Garnock-Jones and P. Ritchie, unpubl. data). The supernetwork constructed using SplitsTree has a topology similar to the concatenated tree (see Appendix 3), with the same four distinct groups visible, and some uncertainties regarding the placement of the species within Clade II, which was expected owing to conflict between the phylogenetic trees reconstructed from the three datasets.

Individual datasets

Overall, the individual ML trees and the concatenated ML tree revealed similar species groupings. However, some differences were evident and are noted below. The placement of close outgroups in the ITS ML tree (Fig. 3A) differs from the concatenated tree (*Plantago lanceolata* and *P. coronopus* have switched positions). *P. major*, *P. sp.* and *P. australis* form a clade that is nested within the Australasian species, as opposed to forming a grade at the base of the Australasian clade in the concatenated phylogeny. The ingroup clades are similar except that *P. muelleri* and *P. paradoxa* do not form a clade with *P. unibracteata* and *P. triandra*. Instead, *P. unibracteata* and *P. triandra* are placed at the base of the tree, whereas *P. muelleri* and *P. paradoxa* are placed as sister species to other Australian

species further up in the tree. Furthermore, *P. debilis*, *P. varia* and *P. triantha* form a clade with *P. spathulata* subspecies *spathulata*. The placements of *P. muelleri*, *P. paradoxa*, *P. debilis* and *P. varia* in the ITS tree are incongruent with the concatenated tree and both organellar DNA trees.

The close outgroups (*P. lanceolata* and *P. coronopus*) are sister species in the *ndhF-rpl32* ML tree (Fig. 3B). This differs from the ITS, *coxI* and concatenated ML trees. It is worth noting that the topology of the trees reconstructed using the concatenated dataset most closely resembled that of the *ndhF-rpl32* trees. This is likely to be because there is more phylogenetic signal in the *ndhF-rpl32* dataset.

There was very little resolution in the ML analysis of the *coxI* dataset (Fig. 3C), with only two Australasian nodes resolved and only one with good bootstrap support (81 BP). Interestingly, this moderately supported clade (*P. paradoxa*, *P. triandra*, *P. muelleri* and *P. unibracteata*) is also present in the *ndhF-rpl32* dataset and in the concatenated dataset, and is the clade that causes conflict between the ITS tree and the organellar DNA trees. The other nodes within the Australasian groups are poorly resolved and may suggest either a recent separation of Australian and New Zealand taxa, or the unsuitability of this marker for reconstructing the phylogeny of this group of plants. Finally, a peculiar pattern found in the *coxI* ML tree is a long branch separating the ingroup *Plantago* species from the outgroup *Plantago* species and an unexpectedly short branch length between *Plantago* as a whole and the *Veronica* outgroup. In contrast, the ITS and *ndhF-rpl32* datasets have short branches between the ingroup species and a long branch between *Plantago* as a whole and *Veronica*. There is also a long branch leading to *P. varia* in the *coxI* tree that is not evident in the other two phylogenies.

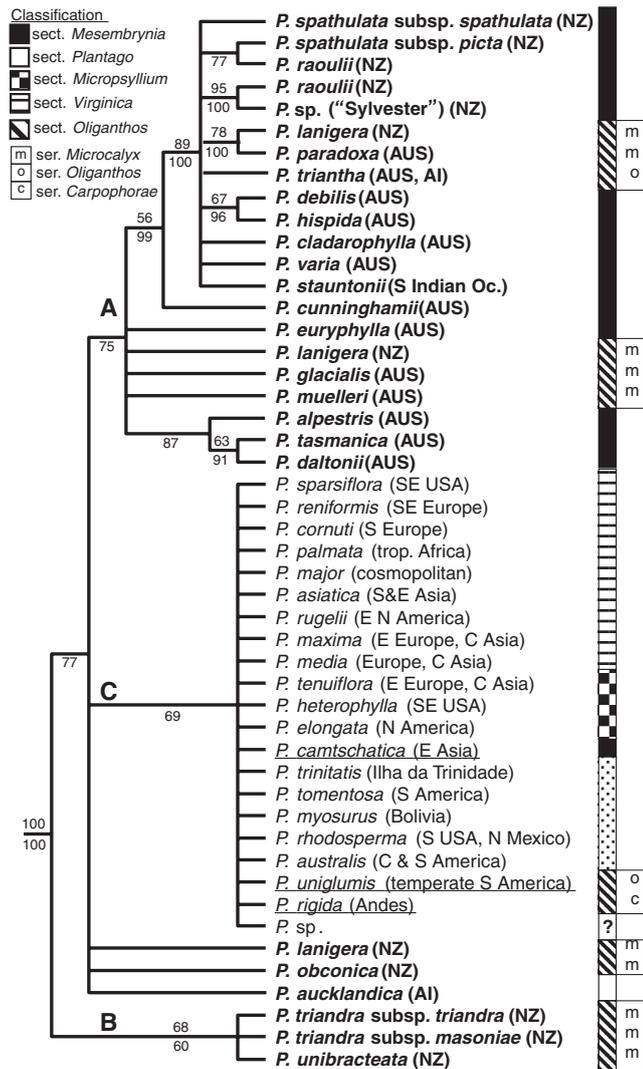


Fig. 4. Simplified Bayesian 50% majority rule tree for the expanded ITS dataset for *Plantago* subgenus *Plantago* (average tree likelihood score = -7531.24). Numbers above branches represent MP bootstrap values (BP), whereas numbers below branches represent posterior probability values (PP). Geographic location is indicated after species names, and sectional and series classification following (Rahn 1996) are also indicated by the bars to the right (see legend for details). Note that each Australasian species in the tree (shown in **boldface type**) represents 1–25 individual sequences (NZ = New Zealand, AUS = Australia, AI = Auckland Islands). The three non-Australasian species of sections *Mesembrynia* and *Oliganthos* are underlined. The fully resolved tree including all individuals sampled can be found in Tay (2008); see text for details.

Expanded ITS dataset

A simplified tree showing the main topological features of the Bayesian 50% majority rule consensus tree of the expanded ITS dataset is shown in Fig. 4, with MP bootstrap support values and posterior probabilities (the tree in its entirety can be found in Appendix 2 (O) of Tay, 2008). Subgenus *Plantago* was monophyletic with high support (98/100 MP BP/Bayesian PP support values) and can be further subdivided into three clades that were not highly supported, and, interestingly, do not

correspond to sections as currently circumscribed. The relationships of these clades relative to one another, and of species within each clade, were not well resolved. Clade A (<50/75) comprised species of sections *Mesembrynia* and *Oliganthos*, including most of the Australasian species from Clades I, II and IV in the concatenated ML tree (Fig. 2) (plus *P. stantonii*). Clade B (51/60) contained *P. triandra* and *P. unibracteata* (which were also part of Clade II in the concatenated ML tree). The three remaining Australasian species (*P. obconica*, *P. lanigera* and *P. aucklandica*), which comprised Clade III from the concatenated ML tree, were unresolved within subgenus *Plantago*. Finally, Clade C (<50/69) comprised a mixture of species from sections *Plantago*, *Virginica*, *Micropsyllum*, as well as the two sampled South American species of section *Oliganthos* (*P. uniglumis* and *P. rigida*) and one Asian species of section *Mesembrynia* (*P. camtschatica*).

Molecular dating

Monophyletic groups with strong support were used to derive dates for molecular dating. These were (1) a clade containing all of the *Plantago*, *Littorella* and *Aragoa* species, (2) a clade of all the *Plantago* and *Littorella* species, (3) a clade with all ingroup *Plantago* but excluding *P. coronopus* and *P. lanceolata*, and (4) Clade I from the ML concatenated tree, excluding *P. cunninghamii*. Molecular dating of the ITS dataset with BEAST, using dates previously estimated by Rønsted *et al.* (2002) provided a date of 2.291 (± 0.0039) million years ago for the divergence of the clade comprising the Australasian *Plantago* species, *P. major*, and *P. sp.*, whereas the node of Clade I (excluding *P. cunninghamii*) was dated to 1.520 (± 0.030) million years ago. Re-estimating rates with BEAST instead of rate smoothing (as in Rønsted *et al.* 2002) provided an estimate of 0.943 (± 0.02) million years ago for the divergence of the clade with all Australasian *Plantago*, *P. major* and *P. sp.* and 0.5 million years ago (± 0.01) for the node of Clade I (excluding *P. cunninghamii*). These analyses thus provide a range of possible dates of origin for the Australasian species.

Discussion

The aim of the present study was to investigate biogeographic patterns and species relationships for the Australasian species of *Plantago*. The concatenated dataset of regions from the three genomes used in the study was able to resolve most of the relationships among the Australasian species, and will be discussed further below together with the findings of the expanded ITS dataset.

Biogeography of Australasian *Plantago*

Evidence for multiple trans-Tasman dispersals was found. In the concatenated tree (Figs 2, 6), three New Zealand clades are nested within the Australasian group, and the nodes of the New Zealand clades are placed in a more recently derived position than are the nodes of the Australian species. This pattern suggests at least three independent long-distance dispersal events to New Zealand, each of which has given rise to a small species radiation there. On the basis of the current sampling, all three dispersals appear to be from Australia to New Zealand. Two additional dispersals involving the Auckland Islands' species are likely. First, the location of the

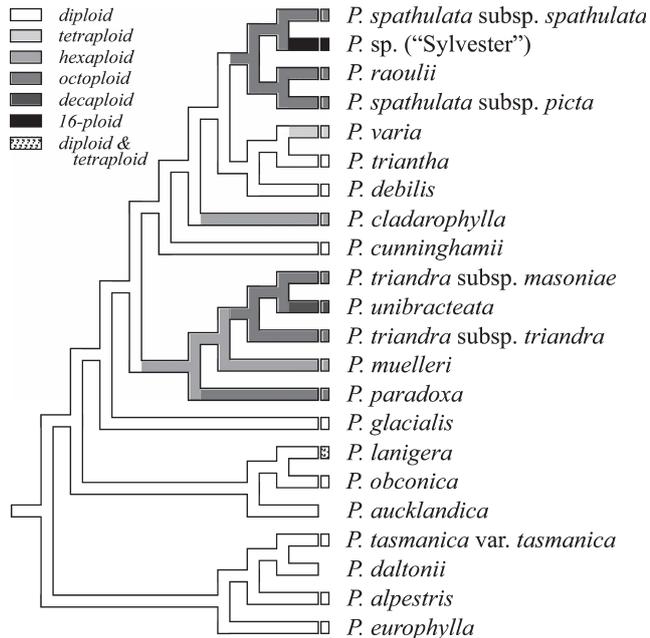


Fig. 5. Evolution of the chromosome number in Australasian *Plantago*. The following chromosome numbers were plotted with MacClade ver. 4.08 onto a pruned tree from Fig. 2, showing only Australasian species: diploid ($2n=12$), tetraploid ($2n=24$), hexaploid ($2n=36$), octoploid ($2n=48$), decaploid ($2n=60$) and 16-ploid ($2n=96$) character states.

ancestor of *P. aucklandica* is equivocal in Fig. 6 (*P. aucklandica* and the *P. lanigera/obconica* clade share a common ancestor that originated in Australia; however, the following three explanations, each requiring two dispersals, are possible: Australia–New Zealand–Auckland Is; Australia–Auckland Is–New Zealand; or Australia–Auckland Is and Australia–New Zealand). Second, it seems likely that *P. triantha* dispersed to the Auckland Islands from Tasmania. Such west–east dispersal is congruent with expectations because of prevailing westerly winds (west wind drift; Raven 1973; Winkworth *et al.* 2002b; Sanmartín *et al.* 2007). Although material of South American, Pacific and Asian species of sections *Oliganthos* and *Mesembrynia* was unavailable for the current study, the expanded ITS analysis (Fig. 4) included *P. uniglumis* (AY101875, section *Oliganthos*, South America), *P. rigida* (AY101876, AF313037; section *Oliganthos*, South America) and *P. camtschatica* (AJ548971; section *Mesembrynia*, Asia). These three species were more closely related to species from section *Plantago*, *Virginica* and *Micropsyllium* than to the Australasian species from sections *Mesembrynia* and *Oliganthos* (albeit with PP support values <95%). The topology of Ishikawa *et al.* (2009) also agrees with this result. Although sampling of additional DNA markers and species of subgenus *Plantago* – particularly from southern hemisphere sections *Oliganthos* and *Mesembrynia* – is needed to confirm the origin and direction of these dispersal events, the present results are nevertheless unambiguous in supporting multiple dispersals of *Plantago* into New Zealand.

The finding of multiple dispersals across the Tasman Sea differs from the common pattern of a single dispersal event followed by rapid radiation that is found in many other

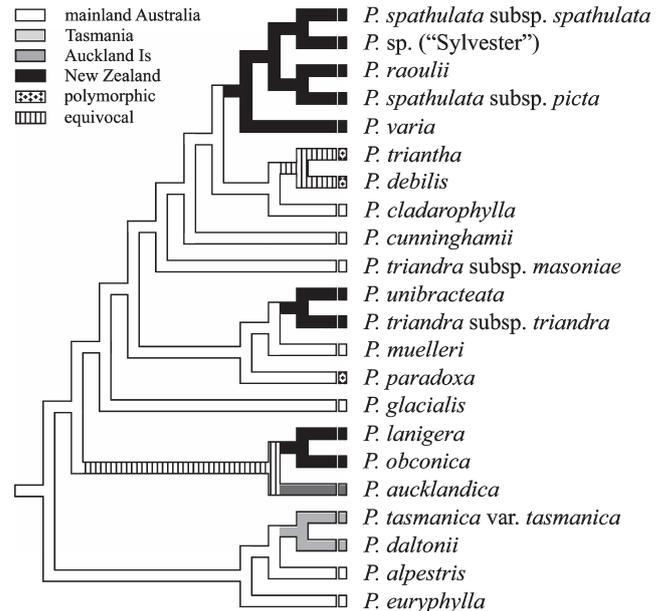


Fig. 6. Biogeographic patterns in Australasian *Plantago*. The following four biogeographic areas were plotted with MacClade ver. 4.08 onto a pruned tree from Fig. 2, showing only Australasian species: mainland Australia, Tasmania, Auckland Islands and New Zealand.

New Zealand plant genera (Wagstaff and Garnock-Jones 1998; Winkworth *et al.* 1999; Perrie *et al.* 2003; Albach *et al.* 2005; Meudt and Simpson 2006). If the inference of dispersal from Australia to New Zealand is correct, the three dispersal events reflect two independent dispersals to the alpine and/or subalpine region in New Zealand (including the subantarctic islands) and one to the lowlands, where in each case the closest Australian relative is also from similar habitats. One explanation for this pattern is that the New Zealand species may have evolved from a common ancestor that had already established in that bioclimatic zone (i.e. alpine to alpine, lowland to lowland), which may have increased the success of establishment in New Zealand. Such ‘biome conservatism’ has been recently shown to be very common in southern hemisphere plants, particularly those dispersing from Australia to New Zealand (Crisp *et al.* 2009). The lowland Australasian species form a clade that is nested within an alpine clade, which suggests that following arrival in Australasia, alpine *Plantago* groups have subsequently dispersed to lowland and coastal regions (Fig. 2).

The unusual finding of multiple dispersals in Australasian *Plantago* fits in with the evolutionary history of the genus. The fact that there are many cosmopolitan species in *Plantago*, and that even small oceanic islands have a mixture of native and introduced species from the genus indicates that the plants are capable of dispersal over long distances and establishment. Within the Australasian taxa, species are commonly distributed across geographic barriers that often separate other biota, such as the Cook Strait in New Zealand, which separates the North and South Islands, and Bass Strait, which separates Tasmania from Australia. Although dispersal has been postulated for many species of *Plantago* (Rønsted *et al.* 2002; Dunbar-Co *et al.* 2008), the dispersal mechanisms of the Australasian species are largely

unknown. It has been hypothesised that the seeds, which exude adhesive mucilage in many *Plantago* species when wetted, could be dispersed by sticking to a vector such as oceanic birds (Rønsted *et al.* 2002). We have noted extremely powerful adhesive exudates for the wetted seeds of several New Zealand species of *Plantago* (P. J. Garnock-Jones, pers. obs.). Other *Plantago* species, such as *P. lagopus*, have hairs on their capsules, which have been shown to facilitate overland dispersal by sticking to the coat of migrating ungulates (Manzano and Malo 2006).

There was a noticeable lack of sequence divergence between the Australian and New Zealand species, as has been found in previous studies (Rønsted *et al.* 2002; Hoggard *et al.* 2003). This is especially evident in analysis of the *coxI* dataset, where several Australian and New Zealand species even share identical sequences, suggesting recent diversification of the Australasian species. Molecular dating suggests that the timing of arrival and diversification of the New Zealand species (estimated here to be between 2.291 (± 0.0039) and 0.5 (± 0.01) million years ago) coincides with the geological dates of the uplift of the New Zealand mountains during the last 5 million years (Winkworth *et al.* 2002a).

Polyploid evolution

Chromosome numbers are diverse in *Plantago* and have an assumed base chromosome number of $x=6$ (although $x=4$ and $x=5$ are also present in the genus; Briggs 1973; Rahn 1996). Australasian *Plantago* are a prime example of this diversity, with six levels of ploidy recorded ($2n=12, 24, 36, 48, 60$ and 96 ; Groves and Hair 1971; Briggs 1973; Dawson 1989). Within Australasia, the alpine grade is mostly diploid, although hexa-, octo- and decaploids occur in Clade II (Fig. 5). Most species of the lowland clade are polyploids, including tetra-, hexa-, octo- and 16-ploids, although diploidy is presumed ancestral in the clade (Fig. 5). Thus, polyploid series appear to have evolved at least four times in Australasian *Plantago*. Interestingly, polyploidy does not appear to have played a major role in the evolutionary history outside of subgenus *Plantago*, so its importance within sections *Oliganthos* and *Mesembrynia* from the southern hemisphere (Rahn 1984) is of great interest. Ishikawa *et al.* (2009) were able to show that, of the species they sampled within subgenus *Plantago*, one is of autopolyploid origin and 10 are of allopolyploid origin, including *P. spathulata* and *P. raoulii*. We are currently counting chromosomes to resolve several variable and unknown species in New Zealand (B. Murray, H. M. Meudt, P. J. Garnock-Jones and M. L. Tay, unpubl. data), and further studies to determine the nature of the New Zealand polyploids are also planned (B. Murray, pers. comm.).

Taxonomic implications

The phylogeny reconstructed from the concatenated dataset revealed that within subgenus *Plantago*, sections *Plantago*, *Mesembrynia* and *Oliganthos*, and series *Microcalyx* are probably not monophyletic (Fig. 2). Our highly supported phylogeny builds on results from previous studies (Rønsted *et al.* 2002; Hoggard *et al.* 2003) to refute the morphological sectional classifications within subgenus *Plantago* (Rahn 1996). This was accomplished by significantly increasing sampling within sections *Mesembrynia* (12/32 sampled) and *Oliganthos* (9/24, including 7 individuals from series *Microcalyx* that are

included for the first time) and improving resolution and support values on the tree via the use of three different DNA markers from all three genomes.

Flower number was an important character in Rahn's (1996) sectional classification within subgenus *Plantago*, in which section *Oliganthos* was distinguished by its few-flowered inflorescences, whereas section *Plantago* has many crowded flowers (Rahn 1996). Our results showed that a reduction in the flower number is likely to have evolved on several occasions and is thus the result of convergence (see species labelled section *Oliganthos* in Fig. 2). In addition, Rahn (1996) observed that although New Zealand members of section *Mesembrynia* sometimes can have a reduced number of flowers (not shown in Fig. 2), the flowers are densely crowded, unlike in section *Oliganthos*. This character incongruence also indicates the homoplastic nature of these floral characters. The presence of an apical third compartment in the fruit, which was thought to be synapomorphic for sections *Mesembrynia* + *Virginica*, also appears to have evolved multiple times within the subgenus (see species labelled section *Mesembrynia* in Fig. 2) and elsewhere in the genus. Thus, none of the morphological characters used to define these sections appears to be useful from a taxonomic point of view. However, while we recommend rejection of the current sectional classification within subgenus *Plantago* (Rahn 1996), we do not propose a replacement system here. A much more detailed molecular phylogenetic study of subgenus *Plantago*, including many more of its 133 species and additional DNA markers, is warranted.

For the New Zealand species, many of which had not been sequenced before the present study, some discrepancies between current taxonomic classifications and the reconstructed phylogeny, and other interesting species relationships, deserve mention here. First, the two subspecies of *P. spathulata* do not form a monophyletic group. Sykes (1988) noted that the main difference between the two is a persistent taproot in subsp. *picta*, although they may also be differentiated by hairs either on the keels or margins of bracts and sepals (Moore 1961). Additional samples of both subspecies should be sequenced and added to the phylogeny to determine whether subsp. *picta* should be recognised at species rank, as it was originally described. Second, *P. obconica* is placed next to *P. lanigera* in the phylogeny, and does not appear to be closely related to *P. triandra* as hypothesised by Sykes (1988) when he described the species. This grouping is evident in all three sequenced regions, thus providing strong support for the relationship. Third, the undescribed polyploid *P. sp.* 'Sylvester' appears to be closely related to *P. raoulii*, with which it may share many morphological similarities (M. L. Tay, H. M. Meudt and P. J. Garnock-Jones, pers. obs.); however, sequences from more samples are needed to determine placement of this species within the phylogeny. Finally, in the present study, we could not differentiate between *P. lanigera* and *P. novae-zelandiae* as described by Sykes (1988) and Spence and Sykes (1989) by using either morphological characters or molecular data, and hence we have identified the five individuals sampled in the present study as *P. lanigera*. Because of character similarities, and the fact that these two entities have highly overlapping distributions and comprise both diploid and tetraploid individuals (Spence and Sykes 1989), we are conducting further studies to delimit species in this complex.

Incongruence of phylogenies from nuclear DNA and organellar DNA

In the present study, trees reconstructed using regions from the three different genomes yielded similar topologies, although there were a few discrepancies. The Australian species *P. muelleri* and *P. paradoxa*, and the New Zealand species *P. triandra* and *P. unibracteata* appear to have conflicting evolutionary histories of nuclear and organellar genomes. The four species form a clade in the *ndhF-rpl32* tree and the tree of the concatenated dataset, whereas they are interspersed among other Australasian species in the ITS tree. One explanation for this conflict may be hybridisation, which is not surprising because the group is relatively young. Hybridisation is not uncommon in *Plantago* (Dunbar-Co *et al.* 2008), although we consider it unlikely to occur across the Tasman Sea which separates these Australian and New Zealand species. Other processes such as incomplete lineage sorting cannot be discounted. However, additional analyses using our expanded ITS dataset (M. L. Tay, H. M. Meudt, P. J. Garnock-Jones and P. Ritchie, unpubl. data) suggested that the lack of resolution in phylogenetic analyses of this region, which do not resolve the placement of *P. triandra* and *P. unibracteata*, may be the most likely explanation for the conflict. Sequencing of additional nuclear regions may be able to further separate patterns formed from incomplete lineage sorting from reticulate evolution (e.g. Morgan-Richards *et al.* 2009).

Elevated substitution rates of mtDNA in Australasian Plantago?

The elevated rates of mtDNA substitutions in *Plantago* that were reported by Cho *et al.* (2004) are not evident within the Australasian clade. There was a long branch between the close *Plantago* outgroups and the ingroup *Plantago*, with a short branch between *Plantago* and *Veronica* in the *coxI* marker (Fig. 3). The opposite was found in the ITS and *ndhF-rpl32* phylogenies. Branch lengths are very short within the clades containing the Australasian species for all three regions, with the sole exception of a long branch leading to *Plantago varia* in the *coxI* tree. This finding of long branch lengths between the close *Plantago* outgroups and the ingroup clade may be a result of recent rapid radiations, a process common in endemic New Zealand groups (Wagstaff and Garnock-Jones 1998; Heenan *et al.* 2002; Murray *et al.* 2004); or an increased substitution rate in *coxI* along the stem of the Australasian clade that has subsequently decreased within the Australasian clade. Elevated rates of mtDNA evolution have been previously reported in some lineages of *Plantago* (Cho *et al.* 2004), and offer a more plausible explanation for this finding than an unusually rapid radiation, because there is no evidence of reciprocal lowered substitution rates in ITS or *ndhF-rpl32* sequences in the Australasian species as would be expected for such a radiation. *P. varia* seems to represent a reversal to rapid *coxI* substitution rates in an Australasian species, and deserves further investigation.

Conclusions

In summary, the present study has shown evidence for an uncommon pattern of at least three independent long-distance dispersal events of *Plantago* to New Zealand, which, on the

basis of current sampling, are hypothesised to have originated in Australia. In all cases, the ancestors of these species in New Zealand appear to have dispersed to habitats similar to those their relatives occupy in Australia. We speculate that multiple dispersals might have been facilitated by sticky seed exudates. By using molecular dating, the node of the Australasian clade was estimated to be between 2.291 and 0.5 million years ago, coinciding with the uplift of the mountain ranges in New Zealand. Following establishment in Australasia, the alpine species appear to have subsequently shifted to lowland habitats. The phylogeny was also able to provide insights into species relationships, highlighting the need for taxonomic revisions in several cases.

While we have obtained sequences for most of the Australasian species, sequences for the remaining Australian species, and in particular others from South America and New Guinea, will be invaluable for elucidating a more complete picture of the biogeography of *Plantago* in the southern hemisphere. Additional nuclear regions (e.g. *SUC1*, Ishikawa *et al.* 2009) may help further resolve the phylogeny and determine whether the incongruence found in the present study could be explained by hybridisation or other processes such as incomplete lineage sorting. Further, sequencing of the *coxI* region from other *Plantago* species would verify whether the high rate of mtDNA evolution has slowed down in other lineages that are outside of Australasia. Last, we have shown that several taxonomic revisions are needed at the level of species in New Zealand as well as at section and subgenus levels in the genus as a whole. Integration of molecular data with the large morphological dataset from Rahn (1996) will be a powerful tool in investigating species relationships, especially those among closely related species with short branch lengths (e.g. Hardy *et al.* 2008).

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References

- Albach DC, Utteridge T, Wagstaff SJ (2005) Origin of Veroniceae (Plantaginaceae, formerly Scrophulariaceae) on New Guinea. *Systematic Botany* 30, 412–423. doi:10.1600/0363644054223666
- Breitwieser I, Glenny D, Thorne A, Wagstaff SJ (1999) Phylogenetic relationships in Australasian Gnaphalieae (Compositae) inferred from ITS sequences. *New Zealand Journal of Botany* 37, 399–412.
- Briggs BG (1973) Chromosomal studies on *Plantago* in Australia. *Contributions from the NSW National Herbarium* 4, 399–405.

- Briggs BG (1992) *Plantago*. In 'Flora of New South Wales, Vol. 3'. (Ed. G Harden) pp. 592–598. (Royal Botanic Gardens & Domain Trust: Sydney)
- Cho Y, Mower JP, Qiu YL, Palmer JD (2004) Mitochondrial substitution rates are extraordinarily elevated and variable in a genus of flowering plants. *Proceedings of the National Academy of Sciences, USA* **101**, 17741–17746. doi:10.1073/pnas.0408302101
- Cooper A, Cooper RA (1995) The Oligocene bottleneck and New-Zealand biota: genetic record of a past environmental crisis. *Proceedings of the Royal Society of London. Series B. Biological Sciences* **261**, 293–302. doi:10.1098/rspb.1995.0150
- Crisp MD, Arroyo MTK, Cook LG, Gandolfo MA, Jordan GJ, McGlone MS, Weston PH, Westoby M, Wilf P, Linder HP (2009) Phylogenetic biome conservatism on a global scale. *Nature* **458**, 754–756. doi:10.1038/nature07764
- Darlington PJ (1965) 'Biogeography of the southern end of the world.' (Harvard University Press: Cambridge, MA)
- Darwin C (1859) 'On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life.' (Murray: London)
- Dawson MI (1989) Contributions to a chromosome atlas of the New Zealand flora – 30 miscellaneous species. *New Zealand Journal of Botany* **27**, 163–165.
- Doyle JJ, Doyle JD (1990) Isolation of plant DNA from fresh tissue. *Focus* **12**, 13–15.
- Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **7**, 214. doi:10.1186/1471-2148-7-214
- Drummond AJ, Ho SYW, Phillips MJ, Rambaut A (2006) Relaxed phylogenetics and dating with confidence. *PLoS Biology* **4**, 699–710. doi:10.1371/journal.pbio.0040088
- Dunbar-Co S, Wiczeorek AM, Morden CW (2008) Molecular phylogeny and adaptive radiation of the endemic Hawaiian *Plantago* species (Plantaginaceae). *American Journal of Botany* **95**, 1177–1188. doi:10.3732/ajb.0800132
- Felsenstein J (1981) Evolutionary trees from DNA-sequences – a maximum-likelihood approach. *Journal of Molecular Evolution* **17**, 368–376. doi:10.1007/BF01734359
- Ford KA, Ward JM, Smissen RD, Wagstaff SJ, Breitwieser I (2007) Phylogeny and biogeography of *Craspedia* (Asteraceae: Gnaphalioideae) based on ITS, ETS and *psbA-trnH* sequence data. *Taxon* **56**, 783–794.
- Garnock-Jones PJ, Albach DC, Briggs BG (2007) Botanical names in southern hemisphere *Veronica* (Plantaginaceae): sect. *Detneria*, sect. *Hebe*, and sect. *Labiatoideae*. *Taxon* **56**, 571–582.
- Groves BE, Hair JB (1971) Contributions to a chromosome atlas of the New Zealand flora – 15 miscellaneous families. *New Zealand Journal of Botany* **9**, 569–575.
- Hardy CR, Moline P, Linder HP (2008) A phylogeny for the African Restionaceae and new perspectives on morphology's role in generating complete species phylogenies for large clades. *International Journal of Plant Sciences* **169**, 377–390. doi:10.1086/526467
- Heenan PB, Mitchell AD, Koch M (2002) Molecular systematics of the New Zealand *Pachycladon* (Brassicaceae) complex: generic circumscription and relationship to *Arabidopsis* sens. lat. and *Arabis* sens. lat. *New Zealand Journal of Botany* **40**, 543–562.
- Hoggard RK, Kores PJ, Molvray M, Hoggard GD, Broughton DA (2003) Molecular systematics and biogeography of the amphibious genus *Littorella* (Plantaginaceae). *American Journal of Botany* **90**, 429–435. doi:10.3732/ajb.90.3.429
- Hooker JD (1853) 'Flora Novae-Zelandiae.' (Lovell Reeve: London)
- Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**, 754–755. doi:10.1093/bioinformatics/17.8.754
- Huson DH, Bryant D (2006) Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution* **23**, 254–267. doi:10.1093/molbev/msj030
- Ishikawa N, Yokoyama J, Tsukaya H (2009) Molecular evidence of reticulate evolution in the subgenus *Plantago* (Plantaginaceae). *American Journal of Botany* **96**, 1627–1635. doi:10.3732/ajb.0800400
- Knapp M, Stöckler K, Havell D, Delsuc F, Sebastiani F, Lockhart PJ (2005) Relaxed molecular clock provides evidence for long-distance dispersal of *Nothofagus* (southern beech). *PLoS Biology* **3**, 38–43. doi:10.1371/journal.pbio.0030014
- Knapp M, Mudaliar R, Havell D, Wagstaff SJ, Lockhart PJ (2007) The drowning of New Zealand and the problem of *Agathis*. *Systematic Biology* **56**, 862–870. doi:10.1080/10635150701636412
- Kumar S, Tamura K, Nei M (2004) MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. *Briefings in Bioinformatics* **5**, 150–163. doi:10.1093/bib/5.2.150
- Lockhart PJ, McLenachan PA, Havell D, Glenny D, Huson D, Jensen U (2001) Phylogeny, radiation, and transoceanic dispersal of New Zealand alpine buttercups: molecular evidence under split decomposition. *Annals of the Missouri Botanical Garden* **88**, 458–477. doi:10.2307/3298586
- Maddison DR, Maddison WP (2005) 'MacClade 4.08: analysis of phylogeny and character evolution.' (Sinauer Associates: Sunderland, MA)
- Manos PS, Doyle JJ, Nixon KC (1999) Phylogeny, biogeography, and processes of molecular differentiation in *Quercus* subgenus *Quercus* (Fagaceae). *Molecular Phylogenetics and Evolution* **12**, 333–349. doi:10.1006/mpev.1999.0614
- Manzano P, Malo JE (2006) Extreme long-distance seed dispersal via sheep. *Frontiers in Ecology and the Environment* **4**, 244–248. doi:10.1890/1540-9295(2006)004[0244:ELSDVS]2.0.CO;2
- McBreen K, Lockhart PJ (2006) Reconstructing reticulate evolutionary histories of plants. *Trends in Plant Science* **11**, 398–404. doi:10.1016/j.tplants.2006.06.004
- McDowall RM (2008) Process and pattern in the biogeography of New Zealand – a global microcosm? *Journal of Biogeography* **35**, 197–212.
- Meudt HM, Bayly MJ (2008) Phylogeographic patterns in the Australasian genus *Chionohebe* (*Veronica* s.l., Plantaginaceae) based on AFLP and chloroplast DNA sequences. *Molecular Phylogenetics and Evolution* **47**, 319–338. doi:10.1016/j.ympev.2007.12.019
- Meudt HM, Simpson BB (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. *Biological Journal of the Linnean Society. Linnean Society of London* **87**, 479–513. doi:10.1111/j.1095-8312.2006.00584.x
- Moore LB (1961) Plantaginaceae. In 'Flora of New Zealand'. (Ed. HH Allan) pp. 780–786. (P. D. Hasselberg, Government printer: Wellington, New Zealand)
- Morgan-Richards M, Smissen RD, Shepherd LD, Wallis GP, Hayward JJ, Chan C-h, Chambers GK, Chapman HM (2009) A review of genetic analyses of hybridisation in New Zealand. *Journal of the Royal Society of New Zealand* **39**, 15–34.
- Müller K (2005) SeqState: primer design and sequence statistics for phylogenetic DNA datasets. *Applied Bioinformatics* **4**, 65–69.
- Muñoz J, Felicísimo AM, Cabezas F, Burgaz AR, Martínez I (2004) Wind as a long-distance dispersal vehicle in the southern hemisphere. *Science* **304**, 1144–1147. doi:10.1126/science.1095210
- Murray BG, Datson PM, Lai EL, Sheath KM, Cameron EK (2004) Polyploidy, hybridization and evolution in *Pratia* (Campanulaceae). *New Zealand Journal of Botany* **42**, 905–920.
- Nelson GJ (1975) Review: Biogeography, the vicariance paradigm, and continental drift. *Systematic Zoology* **24**, 490–504. doi:10.2307/2412908
- Perrie LR, Brownsey PJ (2007) Molecular evidence for long-distance dispersal in the New Zealand pteridophyte flora. *Journal of Biogeography* **34**, 2028–2038. doi:10.1111/j.1365-2699.2007.01748.x

- Perrie LR, Brownsey P, Lockhart PJ, Brown EA, Large MF (2003) Biogeography of temperate Australasian *Polystichum* ferns as inferred from chloroplast sequence and AFLP. *Journal of Biogeography* **30**, 1729–1736. doi:10.1046/j.1365-2699.2003.00938.x
- Perrie LR, Bayly MJ, Lehnebach CA, Brownsey PJ (2007) Molecular phylogenetics and molecular dating of the New Zealand Gleicheniaceae. *Brittonia* **59**, 129–141. doi:10.1663/0007-196X(2007)59[129:MPAMDO]2.0.CO;2
- Pole M (1994) The New-Zealand flora – entirely long-distance dispersal. *Journal of Biogeography* **21**, 625–635. doi:10.2307/2846036
- Posada D, Buckley TR (2004) Model selection and model averaging in phylogenetics: advantages of akaike information criterion and Bayesian approaches over likelihood ratio tests. *Systematic Biology* **53**, 793–808. doi:10.1080/10635150490522304
- Posada D, Crandall KA (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics (Oxford, England)* **14**, 817–818. doi:10.1093/bioinformatics/14.9.817
- Rahn K (1984) *Plantago* sect. *Oliganthos* in southern South America, a taxonomic revision. *Nordic Journal of Botany* **4**, 601–627. doi:10.1111/j.1756-1051.1984.tb01986.x
- Rahn K (1996) A phylogenetic study of the Plantaginaceae. *Botanical Journal of the Linnean Society* **120**, 145–198.
- Raven PH (1973) Evolution of subalpine and alpine plant groups in New Zealand. *New Zealand Journal of Botany* **11**, 177–200.
- Reeves G, Chase MW, Goldblatt P, Rudall P, Fay MF, Cox AV, Lejeune B, Souza-Chies T (2001) Molecular systematics of Iridaceae: evidence from four plastid DNA regions. *American Journal of Botany* **88**, 2074–2087. doi:10.2307/3558433
- Rivadavia F, Kondo K, Kato M, Hasebe M (2003) Phylogeny of the sundews, *Drosera* (Droseraceae), based on chloroplast *rbcL* and nuclear 18S ribosomal DNA sequences. *American Journal of Botany* **90**, 123–130. doi:10.3732/ajb.90.1.123
- Rønsted N, Chase MW, Albach DC, Bello MA (2002) Phylogenetic relationships within *Plantago* (Plantaginaceae): evidence from nuclear ribosomal ITS and plastid *trnL-F* sequence data. *Botanical Journal of the Linnean Society* **139**, 323–338. doi:10.1046/j.1095-8339.2002.00070.x
- Sanmartín I, Ronquist F (2004) Southern hemisphere biogeography inferred by event-based models: plant versus animal patterns. *Systematic Biology* **53**, 216–243. doi:10.1080/10635150490423430
- Sanmartín I, Wanntorp L, Winkworth RC (2007) West wind drift revisited: testing for directional dispersal in the Southern Hemisphere using event-based tree fitting. *Journal of Biogeography* **34**, 398–416. doi:10.1111/j.1365-2699.2006.01655.x
- Shaw J, Lickey EB, Schilling EE, Small RL (2007) Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. *American Journal of Botany* **94**, 275–288. doi:10.3732/ajb.94.3.275
- Simmons MP, Müller K, Norton AP (2007) The relative performance of indel-coding methods in simulations. *Molecular Phylogenetics and Evolution* **44**, 724–740. doi:10.1016/j.ympev.2007.04.001
- Smitsen RD, Garnock-Jones PJ, Chambers GK (2003) Phylogenetic analysis of ITS sequences suggests a Pliocene origin for the bipolar distribution of *Scleranthus* (Caryophyllaceae). *Australian Systematic Botany* **16**, 301–315. doi:10.1071/SB01032
- Spence JR, Sykes WR (1989) Are *Plantago novae-zelandiae* L. Moore and *P. lanigera* Hook. f. (Plantaginaceae) different? *New Zealand Journal of Botany* **27**, 499–502.
- Stöckler K, Daniel IL, Lockhart PJ (2002) New Zealand Kauri (*Agathis australis* (D. Don) Lindl., Araucariaceae) survives Oligocene drowning. *Systematic Biology* **51**, 827–832. doi:10.1080/10635150290102474
- Swofford DL (2002) 'PAUP*. Phylogenetic analysis using parsimony (*and other methods).' (Sinauer Associates: Sunderland, MA)
- Sykes WR (1988) Notes on New Zealand *Plantago* Species. *New Zealand Journal of Botany* **26**, 321–323.
- Sykes WR (1998) Plantaginaceae. In 'Flora of New Zealand, Vol. IV. Naturalised pteridophytes, gymnosperms, dicotyledons'. (Eds CJ Webb, WR Sykes, PJ Garnock-Jones) pp. 942–955. (Botany Division DSIR: Christchurch, New Zealand)
- Sytsma KJ, Litt A, Zijhara ML, Pires JC, Nepokroeff M, Conti E, Walker J, Wilson PG (2004) Clades, clocks, and continents: historical and biogeographical analysis of Myrtaceae, Vochysiaceae, and relatives in the southern hemisphere. *International Journal of Plant Sciences* **165**, S85–S105. doi:10.1086/421066
- Tay ML (2008) Evolution of Australasian *Plantago* (Plantaginaceae). MSc Thesis, Victoria University, Wellington, New Zealand.
- Trewick SA, Paterson AM, Campbell HJ (2007) Hello New Zealand. *Journal of Biogeography* **34**, 1–6. doi:10.1111/j.1365-2699.2006.01643.x
- von Hagen KB, Kadereit JW (2001) The phylogeny of *Gentianaella* (Gentianaceae) and its colonization of the southern hemisphere as revealed by nuclear and chloroplast DNA sequence variation. *Organisms, Diversity & Evolution* **1**, 61–79. doi:10.1078/1439-6092-00005
- Vriesendorp B, Bakker FT (2005) Reconstructing patterns of reticulate evolution in angiosperms: what can we do? *Taxon* **54**, 593–604.
- Wagstaff SJ, Garnock-Jones PJ (1998) Evolution and biogeography of the *Hebe* complex (Scrophulariaceae) inferred from ITS sequences. *New Zealand Journal of Botany* **36**, 425–437.
- Wagstaff SJ, Garnock-Jones PJ (2000) Patterns of diversification in *Chionohebe* and *Parahebe* (Scrophulariaceae) inferred from ITS sequences. *New Zealand Journal of Botany* **38**, 389–407.
- Wagstaff SJ, Wege J (2002) Patterns of diversification in New Zealand Stylidiaceae. *American Journal of Botany* **89**, 865–874. doi:10.3732/ajb.89.5.865
- Wagstaff SJ, Heenan PB, Sanderson MJ (1999) Classification, origins, and patterns of diversification in New Zealand Carmichaelinae (Fabaceae). *American Journal of Botany* **86**, 1346–1356. doi:10.2307/2656781
- Wagstaff SJ, Bayly MJ, Garnock-Jones PJ, Albach DC (2002) Classification, origin, and diversification of the New Zealand hebes (Scrophulariaceae). *Annals of the Missouri Botanical Garden* **89**, 38–63. doi:10.2307/3298656
- Wanntorp L, Wanntorp H-E (2003) The biogeography of *Gunnera* L.: vicariance and dispersal. *Journal of Biogeography* **30**, 979–987.
- White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In 'PCR protocols: a guide to methods and applications'. (Eds M Innis, C Gelfand, J Sninsky, T White) pp. 315–322. (Academic Press: San Diego, CA)
- Winkworth RC, Robertson AW, Ehrendorfer F, Lockhart PJ (1999) The importance of dispersal and recent speciation in the flora of New Zealand. *Journal of Biogeography* **26**, 1323–1325. doi:10.1046/j.1365-2699.1999.00392.x
- Winkworth RC, Grau J, Robertson AW, Lockhart PJ (2002a) The origins and evolution of the genus *Myosotis* L. (Boraginaceae). *Molecular Phylogenetics and Evolution* **24**, 180–193. doi:10.1016/S1055-7903(02)00210-5
- Winkworth RC, Wagstaff SJ, Glenny D, Lockhart PJ (2002b) Plant dispersal NEWS from New Zealand. *Trends in Ecology & Evolution* **17**, 514–520. doi:10.1016/S0169-5347(02)02590-9
- Wolfe KH, Li WH, Sharp PM (1987) Rates of nucleotide substitution vary greatly among plant mitochondrial, chloroplast, and nuclear DNAs. *Proceedings of the National Academy of Sciences, USA* **84**, 9054–9058. doi:10.1073/pnas.84.24.9054
- Xia X, Xie Z (2001) DAMBE: software package for data analysis in molecular biology and evolution. *Journal of Heredity* **92**, 371–373. doi:10.1093/jhered/92.4.371
- Yoder AD, Irwin JA, Payseur BA (2001) Failure of the ILD to determine data combinability for slow loris phylogeny. *Systematic Biology* **50**, 408–424. doi:10.1080/106351501300318003

Appendix 1. Details of *Plantago* samples that were included in the ITS phylogeny

Samples that were collected and sequenced (DNA) for this study are indicated in bold, with details of collection location. n.a. = not available

Ref. in Rahn (1996)	Species	Indigenous distribution	Collection location	GenBank accession number	Reference or voucher ^A
155	<i>Plantago afra</i>	S Africa		AY101892	Rønsted <i>et al.</i> (2002)
184	<i>P. albicans</i>	Mediterranean		AY101905	Rønsted <i>et al.</i> (2002)
75	<i>P. alpestris</i>	Australia	Kosciuszko National Park, NSW, AUS	FJ024611 n.a.	NSW742962 (BGB9748)/ NSW742963 (BGB9749)
132	<i>P. alpine</i>	Europe		AY101877	Jensen <i>et al.</i> (1996)
175	<i>P. amplexicaulis</i>	Mediterranean		AY101900	Rønsted <i>et al.</i> (2002)
146	<i>P. arborescens</i>	Macaronesia		AY101886/ AJ548954	Rønsted <i>et al.</i> (2000)/ Hoggard <i>et al.</i> (2003)
154	<i>P. arenaria</i>	Mediterranean		AY101891/ AY692082	Rønsted <i>et al.</i> (2002)/ Dhar <i>et al.</i> (2006)
210	<i>P. aristata</i>	E USA		AY101911/ AJ548983	Rønsted <i>et al.</i> (2002)/ Hoggard <i>et al.</i> (2003)
29	<i>P. asiatica</i>	S & E Asia		AY101862/ AJ548977	Rønsted <i>et al.</i> (2002)/ Hoggard <i>et al.</i> (2003)
166	<i>P. atrata</i>	Europe, W Asia		AY101895	Rønsted <i>et al.</i> (2002)
15	<i>P. aucklandica</i>	Auckland Islands	Auckland Islands (cult. in Otari-Wilton Bush, Wellington, NZ)	FJ024605	Otari cult. (2003.2037)
108	<i>P. australis</i>	America	Hunua Ranges, Auckland, NZ Waverly, NZ (cult. population)	FJ02416 n.a.	WELTU20181 (MLT019 & PGJ) WELTU20182 (Colin Ogle s. n.)
178	<i>P. bellardii</i>	Mediterranean		AY101874/ AF313038	Rønsted <i>et al.</i> (2000)/ Albach and Chase (2001)
52	<i>P. camtschatica</i>	E Asia		AY101902	Rønsted <i>et al.</i> (2000)
190	<i>P. ciliata</i>	Mediterranean		AJ548971	Hoggard <i>et al.</i> (2003)
67	<i>P. cladarophylla</i>	Australia	Barrington Tops National Park, NSW, AUS	AY101906 FJ024612	Rønsted <i>et al.</i> (2002) NSW744803 (J. R. Hosking 2682)
23	<i>P. cornuti</i>	S Europe		AY101859	Rønsted <i>et al.</i> (2002)
140	<i>P. coronopus</i>	Mediterranean, Europe	Island Bay, Wellington, NZ	FJ024621	WELTU20183 (PGJ2549)
140	<i>P. coronopus</i>	Mediterranean, Europe		AY101882/ AJ548987	Rønsted <i>et al.</i> (2002)/ Hoggard <i>et al.</i> (2003)
137	<i>P. crassifolia</i>	Mediterranean, S Africa		AY101881	Rønsted <i>et al.</i> (2002)
176	<i>P. cretica</i>	E Mediterranean		AY101901	Rønsted <i>et al.</i> (2002)
57	<i>P. cunninghamii</i>	Australia	Brigalow Park Nature Reserve, NSW, AUS	FJ024613	NSW744804 (J. R. Hosking 2752)
74	<i>P. daltonii</i>	Tasmania	St. Clair National Park, AUS, Tasmania	FJ024617	NSW743874 (BGB9782)
60	<i>P. debilis</i>	Australia	Barrenjoey Headland, NSW, AUS	AJ548968 FJ024608	Hoggard <i>et al.</i> (2003) NSW742894 (BGB9738)
46	<i>P. elongata</i>	W USA		AY101868	Rønsted <i>et al.</i> (2002)
207	<i>P. erecta</i>	W USA		AJ548974 AY101909/ AJ548982	Hoggard <i>et al.</i> (2003) Rønsted <i>et al.</i> (2002)/ Hoggard <i>et al.</i> (2003)
72	<i>P. euryphylla</i>	Australia	Kosciuszko National Park, NSW, AUS	n.a.	NSW743824 (BGB9760)/ NSW743822 (BGB9758)/

Appendix 1. (continued)

Ref. in Rahn (1996)	Species	Indigenous distribution	Collection location	GenBank accession number	Reference or voucher ^A
				FJ024607	NSW742956 (BGB9743)/ NSW (BGB9741)
148	<i>P. famarae</i>	Macaronesia		AJ548966	Hoggard <i>et al.</i> (2003)
129	<i>P. glacialis</i>	Australia	Kosciuszko National Park, NSW, AUS	AY101888 n.a.	Ronsted <i>et al.</i> (2002) NSW742960 (BGB9746)/ NSW743813 (BGB9753)
45	<i>P. heterophylla</i>	SE USA		AJ548975	Hoggard <i>et al.</i> (2003)
77	<i>P. hispida</i>	Tasmania, E Australia		AJ548967	Hoggard <i>et al.</i> (2003)
212	<i>P. hookeriana</i>	S USA		AY101913	Ronsted <i>et al.</i> (2002)
169	<i>P. lagopus</i>	Mediterranean		AY101897/ AY692078	Ronsted <i>et al.</i> (2002)/ Dhar <i>et al.</i> (2006)
170	<i>P. lanceolata</i>	cosmopolitan	Karori, Wellington, NZ	FJ024622 AY101898/ AF313036/ AJ548984/ AY692077 n.a.	WELTU20184 (PGJ2551) Albach and Chase (2001)/ Ronsted <i>et al.</i> (2002)/ Hoggard <i>et al.</i> (2003)/ Dhar <i>et al.</i> (2006) WELTU20131 (PBH s. n.)/ WELTU20143 (PBH s. n.) WELTU20125 (PBH s. n.)/ WELTU20124 (PBH s. n.) WELTU20133 (Mike Thorsen s. n.) WELTU20142 (PGJ2611 et al.)/ WELTU20141 (PGJ2607 et al.)/ WELTU20128 (MLT040 et al.)/ WELTU20140 (MLT036 et al.)/ WELTU20136 (MLT039 & PGJ) WELTU20134 (Mike Thorsen s. n.) WELTU20147 (MLT027 et al.) WELTU20132 (MLT034 & PGJ) WELTU20138 (MLT031 et al.) WELTU20130 (HMM273/2 & BS) WELTU20145 (MLT051 et al.)/ WELTU20139 (MLT052 et al.) WELTU20123 [Otari cult. (2005.0122)] WELTU20148 [Otari cult. (2005.0112)]
120, 122	<i>P. lanigera</i>	New Zealand	Hall Range, Canterbury, NZ Sealy Tarns, Mt. Cook, NZ Sugarloaf Pass, Otago, NZ The Remarkables, Queenstown, NZ Mt. Hikurangi, East Cape, NZ Wilberg Range, Westland, NZ Shotover Saddle, Otago, NZ Cardrona Skifield, Wanaka, NZ Rock and Pillar Range, Otago, NZ Ruahine Ranges, North Island, NZ Tararua Ranges, Wellington, NZ Thomson Mountains, Otago, NZ	n.a. n.a. FJ024596/7 n.a. n.a. FJ024594/5 n.a. n.a. n.a. n.a. n.a. n.a. n.a.	

Appendix 1. (continued)

Ref. in Rahn (1996)	Species	Indigenous distribution	Collection location	GenBank accession number	Reference or voucher ^A
171	<i>P. leiopetala</i>	Madeira	Eyre Mountains, Otago, NZ	n.a.	CHR580877 (Kerry Ford s. n.)
194	<i>P. lundborgii</i>	San Ambrosio Is.		AY101899/ AJ548985	Rønsted <i>et al.</i> (2002)/ Hoggard <i>et al.</i> (2003)
141	<i>P. macrorrhiza</i>	Mediterranean		AY101907	Rønsted <i>et al.</i> (2002)
26	<i>P. major</i>	cosmopolitan	Kingsland, Auckland, NZ	n.a.	WELTU20179 (MLT018 & LT)
			Karori, Wellington, NZ	FJ024619	WELTU20180 (PGJ2550)
				AY101861/ AY692079	Rønsted <i>et al.</i> (2003)/ Dhar <i>et al.</i> (2006)
135	<i>P. maritima</i>	cosmopolitan		AY101879/ AJ548986	Rønsted <i>et al.</i> (2002)/ Hoggard <i>et al.</i> (2003)
151	<i>P. mauritanica</i>	NW Africa		AY101890	Rønsted <i>et al.</i> (2002)
40	<i>P. maxima</i>	E Europe, C Asia		AY101864	Rønsted <i>et al.</i> (2002)
41	<i>P. media</i>	Europe, C Asia		AY101865/ AJ548964	Rønsted <i>et al.</i> (2002)/ Hoggard <i>et al.</i> (2003)
131	<i>P. muelleri</i>	Australia	Kosciuszko National Park, NSW, AUS	FJ024606	NSW743812 (BGB9752)/
				n.a.	NSW742951 (BGB9742)
91	<i>P. myosuroides</i>	S America		AY101873	Rønsted <i>et al.</i> (2002)
168	<i>P. nivalis</i>	S Spain		AY101896	Rønsted <i>et al.</i> (2000)
162	<i>P. nubicolata</i>	Peru, Bolivia, NW Argentina		AJ548972	Hoggard <i>et al.</i> (2003)
–	<i>P. obconica</i>	New Zealand	Hector Mountains, Otago, NZ	FJ024603	CHR573261
			Cardrona Skifield, Wanaka, NZ	n.a.	WELTU20122 (MLT030 et al.)/
				FJ024604	WELTU20121 (PGJ2600 et al.)
179	<i>P. ovata</i>	Mediterranean, W USA		AY101903/ AJ548973/ AY692076	Rønsted <i>et al.</i> (2002)/ Hoggard <i>et al.</i> (2003)/ Dhar <i>et al.</i> (2006)
24	<i>P. palmata</i>	trop. Africa		AY101860	Rønsted <i>et al.</i> (2002)
121	<i>P. paradoxa</i>	Tasmania	St. Clair National Park, NSW, AUS	FJ024616	WELTU20187 (BGB9781)
				AJ548969	Hoggard <i>et al.</i> (2003)
211	<i>P. patagonica</i>	W USA, Argentina		AY101912	Rønsted <i>et al.</i> (2000)
68	<i>P. raoulii</i>	New Zealand	Manaia, Taranaki, NZ	n.a.	WELTU20156 (MLT054 et al.)
			George Creek, Wainuiomata, Wellington, NZ	FJ024591	WELTU20152 (PB & RL s. n.)
			Owhiro Bay, Wellington, NZ	n.a.	WELTU20151 (MLT016 et al.)/
					WELTU20157 (MLT017 et al.)
			Maungatawharau, Waiouru, NZ	n.a.	Cult. in Victoria University (Colin Ogle s. n.)
			Maungaharuru, Hawkes Bay, NZ	n.a.	WELT (LP4448 & LS)
			Tararua Ranges, Wellington, NZ	n.a.	WELT (LP4539 & LS)
			Cape Palliser, Wairarapa, Wellington, NZ	n.a.	WELT (LP4996 & LS)

Appendix 1. (continued)

Ref. in Rahn (1996)	Species	Indigenous distribution	Collection location	GenBank accession number	Reference or voucher ^A
			Lake Sarah, Cass, NZ	FJ024592	WELTU20153 (PGJ2559)
22	<i>P. reniformis</i>	SE Europe		AY101858/ AJ548978	Rønsted <i>et al.</i> (2002)/ Hoggard <i>et al.</i> (2003)
94	<i>P. rhodosperma</i>	S USA, N Mexico		AJ548976/ AY692081	Hoggard <i>et al.</i> (2003)/ Dhar <i>et al.</i> (2006)
118	<i>P. rigida</i>	Andes		AY101876/ AF313037	Rønsted <i>et al.</i> (2002)/ Albach and Chase (2001)/
35	<i>P. rugelii</i>	E N America		DQ006026 AY101863/ AY692080	Kress <i>et al.</i> (2005) Rønsted <i>et al.</i> (2002)/ Dhar <i>et al.</i> (2006)
160	<i>P. sarcophylla</i>	E Mediterranean		AY101893	Rønsted <i>et al.</i> (2002)
149	<i>P. sempervirens</i>	SW Europe		AY101889	Rønsted <i>et al.</i> (2002)
200	<i>P. sericea</i>	Andes		AY101910	Rønsted <i>et al.</i> (2003)
136	<i>P. serraria</i>	Mediterranean		AY101880	Rønsted <i>et al.</i> (2002)
	<i>P. sp.</i>	?	Pukerua Bay, Wellington, NZ	FJ024620	WELTU20178 (PGJ2566 & MLT)
20	<i>P. sparsiflora</i>	SE USA		AJ548979	Hoggard <i>et al.</i> (2003)
56	<i>P. spathulata</i> subsp. <i>picta</i>	New Zealand	East Cape, Gisborne, NZ	FJ024590	CHR439486
76	<i>P. spathulata</i> subsp. <i>spathulata</i>	New Zealand	Cass, NZ	n.a.	WELTU20118 (PGJ2557)
			Cape Palliser, Wairarapa, Wellington, NZ	n.a.	WELTU20120 (PGJ2567 & MLT)/ WELTU20119 (PGJ2568 & MLT)
			Marfells Beach, Marlborough, NZ	FJ024589	WELTU20117 (PGJ2629 & MLT)
161	<i>P. squarrosa</i>	E Mediterranean		AY101894	Rønsted <i>et al.</i> (2002)
78	<i>P. stauntoni</i>	Amsterdam & St. Paul Is.		AY101870	Rønsted <i>et al.</i> (2000)
182	<i>P. stocksii</i>	W Asia		AY101904	Rønsted <i>et al.</i> (2002)
142	<i>P. subspathulata</i>	Madeira		AY101884	Rønsted <i>et al.</i> (2002)
133	<i>P. subulata</i>	Mediterranean		AY101878	Rønsted <i>et al.</i> (2000)
–	<i>P. sp.</i> “<i>sylvester</i>”	New Zealand	Lake Sylvester, Nelson, NZ	FJ024600	WELTU20150 (MLT022)
				n.a.	WELTU20149 (MLT023)
205	<i>P. tandilensis</i>	E Argentina		AY101908	Rønsted <i>et al.</i> (2002)
73	<i>P. tasmanica</i> var. <i>tasmanica</i>	Tasmania	St. Clair National Park, NSW, AUS	FJ024615	NSW743928 (BGB9780)
43	<i>P. tenuiflora</i>	E Europe, C Asia		AJ548970 AY101866	Hoggard <i>et al.</i> (2003) Rønsted <i>et al.</i> (2002)
84	<i>P. tomentosa</i>	S America		AY101872	Rønsted <i>et al.</i> (2002)
124	<i>P. triandra</i> subsp. <i>triandra</i>	New Zealand	Kettlehole Tarn, Cass, NZ	n.a.	WELTU20158 (PGJ2558)
			St. Arnaud, Nelson, NZ	n.a.	WELTU20162 (MLT025 & PGJ)
			Lake Sylvester, Nelson, NZ	FJ024599	WELTU20163 (MLT021 <i>et al.</i>)
			Harihari, Westland, NZ	n.a.	WELTU20164 (MLT029 <i>et al.</i>)
			Waipapa River, Northland, NZ (cult. in Otari- Wilton Bush, Wellington, NZ)	n.a.	WELTU20165 [Otari cult. (1005.0115)]
				AJ548965	Hoggard <i>et al.</i> (2003)

Appendix 1. (continued)

Ref. in Rahn (1996)	Species	Indigenous distribution	Collection location	GenBank accession number	Reference or voucher ^A
–	<i>P. triandra</i> subsp. <i>masoniae</i>	New Zealand	Paturau Coast, Nelson, NZ Manaia, Taranaki, NZ	FJ024598 n.a.	WELTU20160 (<i>PJL s. n.</i>) WELTU20167 (<i>MLT055 et al.</i>)
109	<i>P. triantha</i>	Tasmania, Subantarctic Islands	Enderby Island, Auckland Islands	FJ024614	WELTU20177 (<i>VT55</i>)
81	<i>P. trinitatis</i>	Ilha Trindade		AY101871	Rønsted <i>et al.</i> (2002)
119	<i>P. unibracteata</i>	New Zealand	Cardrona Skifield, Wanaka, NZ Wilberg Range, Westland, NZ Lake Sylvester, Nelson, NZ Rainbow Skifield, Nelson, NZ The Remarkables, Queenstown, NZ Ruapehu Skifield, Mt. Ruapehu, NZ	n.a. n.a. FJ021601 n.a. n.a. FJ024602	WELTU20171 (<i>PGJ2603 & MLT</i>) WELTU20160 (<i>PGJ2599 et al.</i>) WELTU20172 (<i>MLT028 et al.</i>) WELTU20175 (<i>MLT024 & PGJ</i>) WELTU20174 (<i>MLT026 & PGJ</i>) WELTU20176 (<i>MLT038 & PGJ</i>) WELTU20173 (<i>MLT053 & PGJ</i>)
114	<i>P. uniglumis</i>	S America		AY101875	Rønsted <i>et al.</i> (2002)
65	<i>P. varia</i>	Australia	Kosciuszko National Park, Australia	n.a. FJ024609	NSW 743869 (<i>BGB9766</i>) NSW743869 (<i>BGB9767</i>)
147	<i>P. webbii</i>	Macaronesia		AY101887	Rønsted <i>et al.</i> (2002)
143	<i>Litorea uniflora</i>	Europe		AJ548962/ AJ548960/ AJ548963/ AJ548961/ AF515218	Hoggard <i>et al.</i> (2003)/ Hoggard <i>et al.</i> (2003)/ Hoggard <i>et al.</i> (2003)/ Hoggard <i>et al.</i> (2003)/ Albach <i>et al.</i> (2004)/
144	<i>Litorea americana</i>	North America		AJ548956/ AJ548958/ AJ548957/ AJ548955	Hoggard <i>et al.</i> (2003)/ Hoggard <i>et al.</i> (2003)/ Hoggard <i>et al.</i> (2003)/ Hoggard <i>et al.</i> (2003)
145	<i>Litorea australis</i>	South America		AJ548959	Hoggard <i>et al.</i> (2003)
–	<i>Aragoa corrugatifolia</i>	Colombia		AJ548980	Hoggard <i>et al.</i> (2004)
–	<i>Aragoa cupressina</i>	Northern Andes		AJ459402	Bello <i>et al.</i> (2002)
–	<i>Veronica hookeriana</i>	New Zealand	Whanahuia Range, Ruahine Mts, NZ	FJ024623	WELTU (<i>PGJ2458</i>)
–	<i>Veronica salicornioides</i>	New Zealand	Jacks Pass, Hanmer, Canterbury, NZ	FJ024624	CHR512475

^ACollectors: *BGB*=Barbara G. Briggs, *BS*=Barry Sneddon, *HMM*=Heidi Meudt, *LP*=Leon Perrie, *LS*=Lara Shepherd, *LT*=Leah Tooman, *MLT*=Mei-Lin Tay, *PBH*=Peter Heenan, *PGJ*=Phil Garnock-Jones, *PB*=Peter Beveridge, *PJL*=Peter J. Lockhart, *RL*=Rodney Lewington and *VT*=Vanessa Thom. Herbarium vouchers: CHR = Allan Herbarium, Landcare Research, Christchurch, New Zealand; NSW = National Herbarium of New South Wales, Australia; WELT = Herbarium of Museum of New Zealand, Te Papa Tongarewa, Wellington, New Zealand; and WELTU = H. D. Gordon Herbarium in Victoria University of Wellington, New Zealand.

Appendix 2. A list of voucher information and GenBank accession numbers of ITS sequences for individuals of *Plantago* used for molecular dating in this study

Species	Locality ^A	Reference or herbarium voucher ^B
<i>Plantago</i> sp. "Sylvester" (FJ024600)	Lake Sylvester, Nelson, NZ	WELTU20150
<i>P. alpestris</i> (FJ024611)	Kosciuszko National Park, NSW, AUS	NSW742962
<i>P. aucklandica</i> (FJ024605)	Auckland Islands, NZ	In cult. Otari (2003.2037)
<i>P. australis</i> (FJ024618)	Hunua Ranges, Auckland, NZ	WELTU20181
<i>P. cladarophylla</i> (FJ024612)	Barrington Tops National Park, NSW, AUS	NSW744803
<i>P. coronopus</i> (FJ024621)	Island Bay, Wellington, NZ	WELTU20183
<i>P. cunninghamii</i> (FJ024613)	Brigalow Park Nature Reserve, NSW, AUS	NSW744804
<i>P. daltonii</i> (FJ024617)	St. Clair National Park, AUS, Tasmania	NSW743874
<i>P. debilis</i> (FJ024608)	Barrenjoey Headland, NSW, AUS	NSW 742894
<i>P. euryphylla</i> (FJ024607)	Kosciuszko National Park, NSW, AUS	NSW742956
<i>P. glacialis</i> (FJ024610)	Kosciuszko National Park, NSW, AUS	NSW743813
<i>P. lanceolata</i> (FJ024622)	Karori, Wellington, NZ	WELTU20184
<i>P. lanigera</i> (3) (FJ024593)	Hall Range, Canterbury, NZ	WELTU20143
<i>P. major</i> (FJ024619)	Ben Burn Park, Karori, NZ	WELTU20180
<i>P. muelleri</i> (FJ026064)	Kosciuszko National Park, NSW, AUS	NSW743812
<i>P. obconica</i> (2) (FJ024604)	Cardrona Skifield, Wanaka, NZ	WELTU20121
<i>P. paradoxa</i> (FJ024616)	St. Clair National Park, AUS, Tasmania	WELTU20187
<i>P. raoulii</i> (1) (FJ024592)	Lake Sarah, Cass, NZ	WELTU20153
<i>P. sp.</i> (FJ024620)	Pukerua Bay, Wellington, NZ	WELTU20178
<i>P. spathulata</i> subsp. <i>picta</i> (FJ024590)	East Cape, Gisborne, NZ	CHR439486
<i>P. spathulata</i> subsp. <i>spathulata</i> (FJ024589)	Marfells Beach, Marlborough, NZ	WELTU20117
<i>P. tasmanica</i> var. <i>tasmanica</i> (FJ024615)	St. Clair National Park, AUS, Tasmania	WELTU20188
<i>P. triandra</i> subsp. <i>masoniae</i> (FJ024598)	Paturau Coast, Nelson, NZ	WELTU20168
<i>P. triandra</i> subsp. <i>triantra</i> (FJ024599)	Lake Sylvester, Nelson, NZ	WELTU20163
<i>P. triantha</i> (FJ024614)	Enderby Island, AI, NZ	WELTU20177
<i>P. unibracteata</i> (2) (FJ024601)	Lake Sylvester, Nelson, NZ	WELTU20175
<i>P. varia</i> (FJ024609)	Kosciuszko National Park, NSW, AUS	NSW743869
<i>P. stauntonii</i> (AY101870)	Amsterdam & St. Paul Islands	Rønsted <i>et al.</i> (2002)
<i>Aragoa corrugatifolia</i> (AJ548980)	Colombia	Hoggard <i>et al.</i> (2003)
<i>A. cupressina</i> (AJ459402)	northern Andes	Bello <i>et al.</i> (2002)
<i>L. americana</i> (AJ548958)	New Brunswick, Canada	Hoggard <i>et al.</i> (2007)
<i>L. americana</i> (AJ548955)	Michigan, USA	Hoggard <i>et al.</i> (2007)
<i>L. australis</i> (AJ548959)	Falkland Islands	Hoggard <i>et al.</i> (2007)
<i>L. uniflora</i> (AJ548960)	Denmark	Hoggard <i>et al.</i> (2007)
<i>L. uniflora</i> (AJ548963)	Iceland	Hoggard <i>et al.</i> (2007)
<i>L. uniflora</i> (AJ548961)	Sweden	Hoggard <i>et al.</i> (2007)
<i>Veronica hookeriana</i> (FJ024623)	Whanahuaia Range, Ruahine Mts, NZ	WELTU20200
<i>V. salicornioides</i> (FJ024624)	Jacks Pass, Hanmer, Canterbury, NZ	CHR512475

^ALocations: AI = Auckland Islands, AUS = Australia, NSW = New South Wales, NZ = New Zealand.

^BHerbarium vouchers: WELTU = H. D. Gordon Herbarium in Victoria University of Wellington, New Zealand; NSW = National Herbarium of New South Wales, Australia; CHR = Allan Herbarium, Landcare Research, Christchurch, New Zealand.

Appendix 3. Neighbour-net analyses of the concatenated regions ITS, *ndhF-*rpl32** and *cox1* of the Australasian species of *Plantago* using SplitsTree ver. 4.8 (using samples listed in Table 1)

