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Authors: Wagstaff, Steven J., and Tate, Jennifer A.

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Phylogeny and Character Evolution in the New Zealand Endemic Genus *Plagianthus* (Malveae, Malvaceae)

Steven J. Wagstaff[1,3] and Jennifer A. Tate[2]

[1]Allan Herbarium, Landcare Research, PO Box 40, Lincoln 7640, New Zealand
[2]Massey University, Institute of Molecular Biosciences, Private Bag 11222, Palmerston North, New Zealand
[3]Author for correspondence (wagstaffs@landcareresearch.co.nz)

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Abstract—As presently circumscribed, *Plagianthus* includes two morphologically distinct species that are endemic to New Zealand. *Plagianthus divaricatus*, a divaricate shrub, is a dominant species in coastal saline shrub communities, whereas *P. regius* is a tree of lowland and montane forests. Results from independent analyses of ITS and 5′ *trnK/matK* sequences are congruent, and when combined provide a robust framework to study character evolution. Our findings suggest the ancestor of *Plagianthus* originated in Australia where the sister genera *Asterotrichion* and *Gynatrix* are presently distributed. The stem age of *Plagianthus* was estimated at 7.3 (4.0–14.0) million years ago (Ma) and the crown radiation at 3.9 (1.9–8.2) Ma. Most of the characters optimized onto the molecular phylogeny were shared with source lineages from Australia and shown to be plesiomorphic. Only the divaricate branching pattern characteristic of *Plagianthus divaricatus* was acquired after the lineage became established in New Zealand and shown to be apomorphic. The initial *Plagianthus* founders were shrubs or small trees with deciduous leaves and small inconspicuous dioecious flowers. Juvenile vegetative morphology and sexual maturation are decoupled in *Plagianthus*; heteroblastic vegetative development is well documented in *Plagianthus* and close relatives.

Keywords—Bayesian analyses, divergence time estimates, long-distance dispersal, Malveae, New Zealand, *Plagianthus.*

Since the early days of botanical exploration, plants found on oceanic islands have been appreciated for their unique characteristics as compared to their continental relatives. The New Zealand flora is an excellent example of this situation. It is well known for its high level of endemism, where 82% of the species found in the archipelago are endemic (one endemic family and 65 endemic genera; de Lange et al. 2006), and high levels of morphological diversity. Lloyd (1985) suggested that the interplay between long-distance dispersal, establishment, and diversification were critical for the evolution of distinctive features of the New Zealand flora; in particular, the evolution of a relatively large proportion of gender-dimorphic flowers (Godley 1979; Webb et al. 1999), and the prevalence of plants with small, simple, nonshowy, white flowers that are pollinated by unspecialized insects (Godley 1979; Lloyd 1985; Newstrom and Robertson 2005). The woody plants of New Zealand are also overwhelmingly evergreen, but interestingly the few plants with deciduous leaves often share an unusual divaricate growth habit characterized by closely interlacing short wiry twigs that branch at a wide angle and bear small leaves (Kelly 1994; McGlone et al. 2004). Abrupt changes in leaf morphology during development (leaf heteroblasty) represent yet another distinctive feature of the New Zealand flora (Cockayne 1901, 1912; Burns and Dawson 2009). Here we use a phylogenetic framework to study the evolution of some of these distinctive traits in the New Zealand endemic genus *Plagianthus* J. R. Forst & G. Forst. (Malveae, Malvaceae).

Recent studies of Wagstaff et al. (2010) and Tate et al. (2005) suggest that *Plagianthus regius* (Poit.) Hochr. and *P. divaricatus* J. R. Forst & G. Forst. along with the monotypic Australian taxa *Gynatrix pulchella* (Willd.) Alef. and *Asterotrichion discolor* (Hook.) Melville form a well-supported monophyletic group, sister to the endemic New Zealand genus *Hoheria* A. Cunn. Bates (1968) included these genera in the *Plagianthus* alliance along with *Lawrencia* Hook. and *Selenothamnus* Melville (= *Lawrencia* sect. *Selenothamnus*, fide Lander 1984). Members of the *Plagianthus* alliance are characterized by the lack of involucral bracts, a reduction or loss of stipules, leaf venation that is often pinnate rather than palmate, and the presence of a solitary, pendulous ovule in each carpel. With the exception of *Hoheria*, members of the *Plagianthus* alliance have small unisexual flowers with stigma lobes that are decurrent on filiform or clavate style branches, a reduced number of carpels (varying from one to six), and partial seed abortion. *Hoheria*, with showy bisexual flowers, capitate stigmas and five–15 carpels, stands out and more closely resembles genera in the *Abutilon* alliance (Bates 1968). Both of the endemic New Zealand genera exhibit heteroblastic leaf development in which the juvenile leaves are markedly different from the adults, except for *P. regius* ssp. *chathamicus* (Cockayne) de Lange. *Plagianthus* and *Hoheria* also share a haploid chromosome number of $n = 21$ (Groves and Hair 1971; Dawson and Beuzenberg 2000), but chromosome numbers of other members of the *Plagianthus* alliance remain unknown (Tate et al. 2005).

The type of the genus, *Plagianthus divaricatus,* is readily distinguished from *P. regius* by its divaricate growth habit, linear entire leaves, and flowers that are solitary or found in small axillary cymes (Figs. 1A–E). *Plagianthus divaricatus* is a deciduous shrub with an unusual filiramulate divaricating growth habit (Cockayne 1958; Allan 1961). Its outward appearance may be wiry and close, or twiggy and open. The main stem is generally stout, with numerous lateral branches. The laterals pass upwards and outwards ultimately forming short wiry twigs that diverge at a wide angle and are closely interlacing. The interior branches are naked. The small, linear to linear-ovate leaves are slightly coriaceous and emerge from much reduced branchlets. The flowers are dioecious with pale yellow or whitish petals edged with purple. Only the male flowers are sweetly scented. The flowers are produced in early spring from September to October. *Plagianthus divaricatus* is commonly found near the coast in salt marshes (Fig. 2), but extends inland along tidal rivers where it occasionally hybridizes with *P. regius*. *Plagianthus regius* is a deciduous tree up to 15 m that is common in lowland forests particularly on nutrient-rich soils (Figs. 1F–K). The leaves are ovate to ovate-lanceolate, 2.5–7 cm long, coarsely toothed, and acuminate. The small yellowish flowers mature in the early spring and form conspicuous, compound cymes. The male flowers are sweetly scented, but the female flowers are unscented or only weakly scented (Cockayne 1958; Allan 1961). Two subspecies of *P. regius* are known: ssp. *regius* is widely

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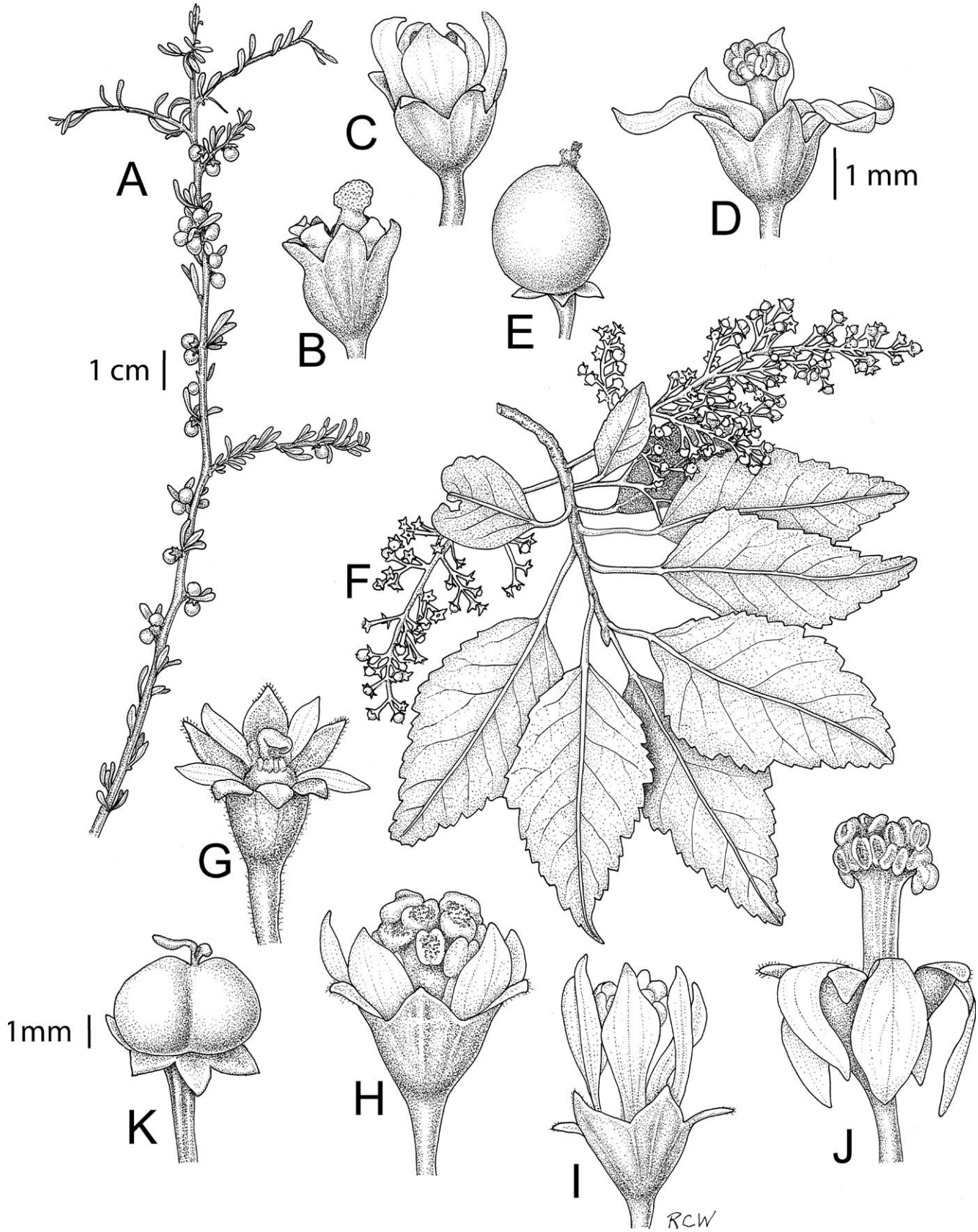

Fig. 1. Comparison of the morphological characteristics of *Plagianthus divaricatus* and *P. regius*. A-E *P. divaricatus*: A. Vegetative shoot with attached fruits. B. Pistillate flower. C. Immature staminate flower. D. Staminate flower at anthesis with reflexed petals. E. Mature fruit. F-K *P. regius*: F. Adult vegetative shoot with attached fruits. G. Immature pistillate flower showing sterile anthers. H. Mature pistillate flower. I. Immature staminate flower with stamens surrounded by petals. J. Staminate flower at anthesis with reflexed petals. K. Mature fruit. The scale bar = 1 cm for habit illustrations A, F; 1 mm for the flowers B, C, D, G, H, I, J and 1 mm for the fruits E, K.

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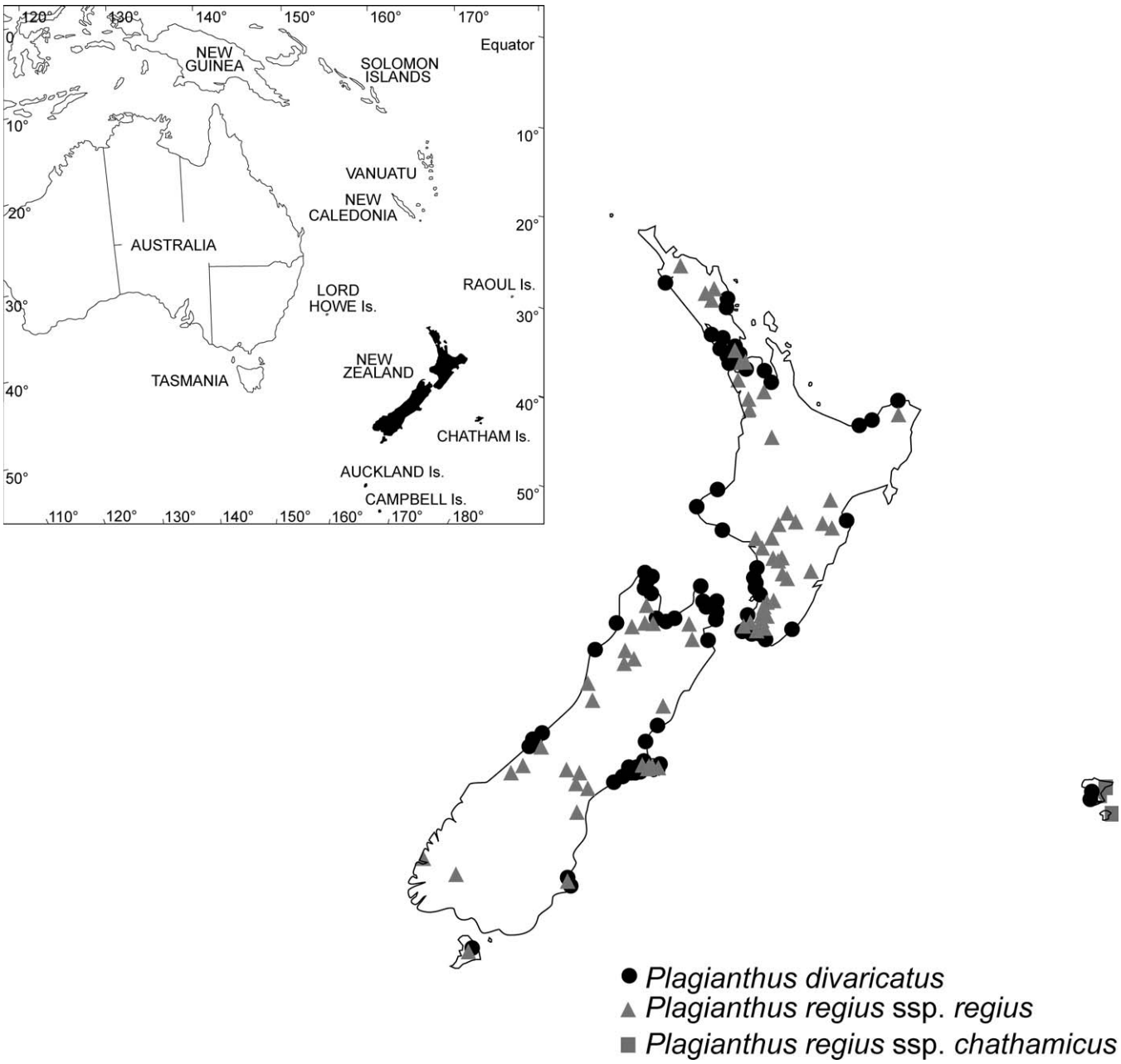

Fig. 2. Generalized distribution map of *Plagianthus divaricatus* and *P. regius*. Box shows the position of New Zealand in relationship to Australia. Symbols represent specimens held in the Allan Herbarium (CHR).

distributed on the North and South Islands of New Zealand (Fig. 2), whereas ssp. *chathamicus* is restricted to the Chatham Islands, a small isolated archipelago approximately 800 km east of the South Island (Mueller 1864; de Lange 2008). *Plagianthus regius* ssp. *chathamicus* differs from ssp. *regius* by the lack of a divaricating juvenile phase (de Lange 2008; Burns and Dawson 2009).

Lloyd (1985) proposed that nonrandom dispersal and establishment of plants with different characters was a form of species selection brought about by differential rates of migration, persistence, and speciation in island plants. He suggested that a phylogenetic framework could be used to test this hypothesis. Our research aims were threefold: first to infer phylogenetic relationships within *Plagianthus* and among closely related genera, second to estimate the age of the lineage in New Zealand, and third to infer the ancestral characteristics of the initial founding population of *Plagianthus*, distinguishing traits characteristic of the source from those that evolved after *Plagianthus* became established in New Zealand.

Materials and Methods

Taxon Sampling—This study builds upon our earlier phylogenetic studies of New Zealand Malveae (Heenan et al. 2005; Tate et al. 2005; Wagstaff et al. 2010) with the addition of chloroplast-encoded sequences to complement the ITS sequences. Our sample included 28 sequences with representatives of all six genera comprising the *Plagianthus* alliance of Bates (1968). To assess levels of intraspecific variation in *Plagianthus*, we included five samples of *P. divaricatus* and nine samples of *P. regius* collected throughout New Zealand (including two samples of *P. regius* ssp. *chathamicus* from the Chatham Islands). Outgroups included *Asterotrichion discolor*, *Gynatrix pulchella* (Tate et al. 2005), and one representative from the seven

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species currently included in *Hoheria* (Wagstaff et al. 2010). We included two herbaceous representatives of *Lawrencia* sect. *Lawrencia*, and two shrubs included in *Lawrencia* sect. *Selenothamnus* (Tate et al. 2005); we rooted our analysis along the long-branch leading to these taxa. Voucher information, along with GenBank accession numbers are presented in Appendix 1. The complete data sets are available on request from the first author and from TreeBASE (study number S10541).

DNA Extraction, Amplification and Sequencing—Total DNA was extracted from fresh leaves, leaves dried with silica gel, or from herbarium specimens, using a Qiagen DNeasy extraction kit (QIAGEN Pty Inc., Clifton Hill, Victoria, Australia) following the manufacturer's recommended protocols. Amplification and sequencing procedures for ITS and 5′ *trnK/matK* follow Wagstaff et al. (2010). Excess primers and unincorporated nucleotides were removed from PCR products by a Shrimp Alkaline Phosphatase (GE Healthcare, Global Headquarters, Cahlfont St. Giles, United Kingdom)/Exonuclease I (Fermentase International Inc, Burlington, Ontario Canada) treatment. Sequencing reactions were run on an ABI3730 sequencer (Applied Biosystems, Foster City, California) by the Allan Wilson Centre Genome Service at Massey University, Palmerston North, New Zealand. In all instances, both forward and reverse DNA strands were sequenced and edited using Sequencher 4.8 (Gene Codes Corporation, Ann Arbor, Michigan).

Sequence Alignment—Sequences were initially aligned using ClustalX (Thompson et al. 1997). The resulting alignments were visually inspected and gaps inserted manually to ensure positional homology prior to the phylogenetic analyses. Gaps in the same position were treated as homologous binary characters following Simmons and Ochoterena (2000). Gaps that differed in length, sequence, or position were treated as different characters. The presence and absence of indels were coded separately and included in the parsimony and Bayesian analyses, but excluded from the divergence time estimates. We assumed that the mutation rate for insertions or deletions differs from the nucleotide substitution rate and felt that it would be difficult to model this difference in substitution rate adequately.

Phylogenetic Analyses—We conducted independent maximum parsimony (MP) analyses of the two sequence data sets using PAUP* 4.0b10 (Swofford 2002). Searches used TBR branch swapping, MULPARS in effect, and RANDOM ADDITION with 1,000 replicates. Duplicate trees were eliminated using the condense trees option and collapsing branches with a maximum length of zero. Characters were unordered and equally weighted. Congruence of the molecular data matrices was assessed by the ILD test (Farris et al. 1994, 1995) with 100 data partition replicates excluding uninformative sites as was suggested by Hipp et al. (2004) and Ramirez (2006). In the absence of significant conflict as indicated by the ILD test results, we combined the sequence and gap data partitions. Support for clades was estimated by bootstrap (Felsenstein 1985) with 1,000 replicates excluding uninformative sites; starting trees were obtained by RANDOM ADDITION with one replication for each bootstrap replicate, TBR branch swapping, and MULPARS in effect.

We defined five data partitions that corresponded to the two DNA regions (ITS and 5′ *trnK/matK*), gap data sets for each DNA region, and a character data set comprised of some of the ecologically important attributes of *Plagianthus,* such as geographic distribution, habitat, and life form. Character states and their distribution for this latter data partition are described in Appendix 2. Since the five data partitions were unlinked, we applied different evolutionary models and parameters to each partition (Nylander et al. 2004). Appropriate maximum likelihood model and parameter estimates were determined by the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC), which are implemented in Modeltest 3.06 (Posada and Crandall 1998; Posada and Buckley 2004). The general time reversible (GTR) was identified as the most appropriate model of sequence evolution for the two sequence data partitions with flat priors for the rate matrix and nucleotide frequency and uniform priors for the proportion of invariable sites with a gamma shape parameter. The binary model with variable coding bias was used for the gap data partitions, and the standard discrete model was applied to the character data.

Using MrBayes 3.1.2, we ran two independent Markov Chain Monte Carlo (MCMC) analyses for 10 million generations, with each search starting from a different random tree (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). Metropolis coupling was used to improve the MCMC sampling of the target distribution. Each analysis consisted of four chains: three heated and one cold. We monitored the likelihood values to assure stationarity, and then discarded 25% of the samples from the cold chain discarded as burn-in. The temperature was reduced from the default value of 0.2 to 0.05 to improve the acceptance rate between chain swaps. Tree diagnostics and the chain sample frequency were calculated every 1,000 generations. The average standard deviation of the split frequencies was < 0.01, and the potential scale reduction factor approached 1.0 for all parameters.

Ancestral State Reconstructions—We chose 10 ecologically important attributes of *Plagianthus* that described current geographic distribution patterns, habitat, breeding systems and dispersal and scored these traits for each taxon included in the phylogeny. Character state assignments were based upon field studies, examination of herbarium specimens, and literature. All characters and their respective character state assignments are listed in Appendix 2. Based upon the preliminary molecular results, we selected three clades with 100% posterior probability support (Nodes A, B, and C shown in Fig. 4). In subsequent analyses, we first constrained each of these nodes to monophyly then estimated the PP of the ancestral character states at each node. This approach accounts for both phylogenetic and mapping uncertainty when inferring character evolution (Ronquist 2004). We also mapped characters using MacClade 4.08 (Maddison and Maddison 2004).

Divergence Time Estimates—We used a likelihood ratio test to determine whether the data satisfied the assumptions of a molecular clock using LR = −2 log LR; where LR is the difference between the –ln likelihood of the tree, with and without enforcing a molecular clock and the χ^2 distribution, with n–2 degrees of freedom, where n is the number of taxa (Felsenstein 1988). In the absence of a molecular clock, we used Bayesian (Drummond and Rambaut 2007) and penalized likelihood (Sanderson 1997, 2002a) approaches to accommodate rate heterogeneity across lineages.

Because of the paucity of the fossil record of Malveae, we based our divergence time estimates on three calibration points (see Ho and Phillips 2009). We placed an exponential prior distribution of 3 with a zero offset of 44.7 million years ago (Ma) with a probability distribution that tails off to 60 Ma for the most recent common ancestor (mrca) of *Lawrencia spicata* and *Hoheria angustifolia.* In our analyses this prior corresponds to the Eocene appearance of fossils attributed to the Malveae in Australia, New Zealand, and South America (Mildenhall 1980; Pocknall 1982; Zamaloa and Romero 1990; Barreda 1993; Macphail 1997; Dettmann and Clifford 2000; Mautino et al. 2004; Wilf et al. 2005; Barreda et al. 2007; Iglesias et al. 2007). A second prior, with a log-normal distribution of 13.5 Ma with 95% confidence intervals of 12.4–14.6, was placed on the node separating the western Australian endemic *Lawrencia helmsii* from *L. squamata,* which is widely distributed across southern Australia. Diversification of many Western Australian endemics accompanied increased aridity and the expansion of the Nullarbor Plains during the Miocene (Crisp et al. 2004; Crisp and Cook 2007; Byrne et al. 2008). A third uniform prior, with a lower bound of 1.0 and an upper bound of 3.0, was placed on the node separating the Chatham Island endemic *Plagianthus regius* ssp. *chathamicus* 1.52 from the mainland *Plagianthus regius* ssp. *regius* 8.42. This prior corresponds to the emergence of the Chatham Islands between 1 and 3 million years ago (Campbell et al. 1994; Trewick et al. 2007; Landis et al. 2008; Wallis and Trewick 2009). We applied Bayesian and maximum likelihood approaches to estimate divergence times at three well-supported nodes. The results are presented as means with confidence limits surrounding the mean values.

Two independent MCMC searches were undertaken using BEAST 1.4.8 (Drummond and Rambaut 2007) with a relaxed, uncorrelated, log-normal molecular clock model. We used the GTR substitution model with gamma and invariant sites, four gamma categories, and the base frequencies estimated. The tree prior was set to a Yule speciation process. The MCMC chain was run for three million generations, logging parameters every 1,000 generations. Log files were examined using Tracer 1.4 (Rambaut and Drummond 2007) to optimize priors and assess effective samples sizes (files are available upon request from the first author). LogCombiner and TreeAnnotator (Drummond and Rambaut 2007) were used to combine and summarize the information in the tree output files; a summary tree with 95% highest posterior density (HPD) confidence intervals on the branch divergence estimates was drawn using FigTree (Rambaut 2006–2008).

We used a likelihood approach to estimate a smoothing parameter by cross-validation using r8s (Sanderson 2002b). Confidence limits associated with the divergence dates were calculated using bootstrapping (Sanderson 2003). The initial ML tree was used as a constraint during a bootstrap search, and 100 rooted bootstrap trees with ML branch lengths were saved in an ALNEXUS format (without a translation table). This option saves trees with branch lengths and taxon labels as an integral part of the tree description. We then used the profile command to summarize confidence intervals to the divergence estimates at designated nodes in the ML tree.

Results

Analyses of the ITS and 5′ trnK/matK Data Partitions—Results from the ITS and 5′ *trnK/matK* data partitions were

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largely congruent (Fig. 3). The aligned ITS data set included 762 characters of which 683 were constant, 35 were parsimony-uninformative, and 44 were parsimony-informative (Table 1). None of the data were coded as missing, but gap symbols were placed in 279 character positions (1.2% of the ITS data matrix). Eight gaps with sizes varying from one to three bp were inferred. A heuristic search with 1,000 random addition replicates recovered a single island of 27 MP trees of 90 steps, each with a consistency index (CI) of 0.909 and a retention index (RI) of 0.980. The strict consensus of the 27 trees is shown in Fig. 3.

The aligned 5′ *trnK/matK* data set included 899 characters of which 848 were constant, 16 were parsimony-uninformative, and 25 were parsimony informative (Table 1). Ten gaps were inferred in the 5′ *trnK/matK* data set varying in size from one to 13 bp. However, we could not confidently align the gap in a polyA region spanning nucleotide positions 139–156, which was subsequently excluded from the analyses. Eighty-eight data cells were coded as missing, and gap symbols were placed in 472 positions, constituting about 2.1% of the data matrix. A heuristic search with 1,000 random addition replicates recovered 15 MP trees in a single island of 45 steps, each with a CI of 0.893 and a RI of 0.963. There was little homoplasy in the molecular data sets, as indicated by the

TABLE 1. Summary statistics for the ITS and 5′ *trnK/matK* data partitions including gaps. Parsimony-uninformative characters were excluded from the consistency index calculation. MPTs = maximum parsimony trees; CI = consistency index; RI = retention index.

Data partition	Total characters	Informative characters	MPTs	Tree length	CI	RI
ITS	762	44	27	90	0.909	0.980
ITS gaps	8	3	1	8	1.00	1.00
5′ *trnK/matK*	889	25	15	45	0.893	0.963
5′ *trnK/matK* gaps	10	7	1	10	1.00	1.00
Combined sequences and gaps	1,669	79	70	158	0.876	0.955

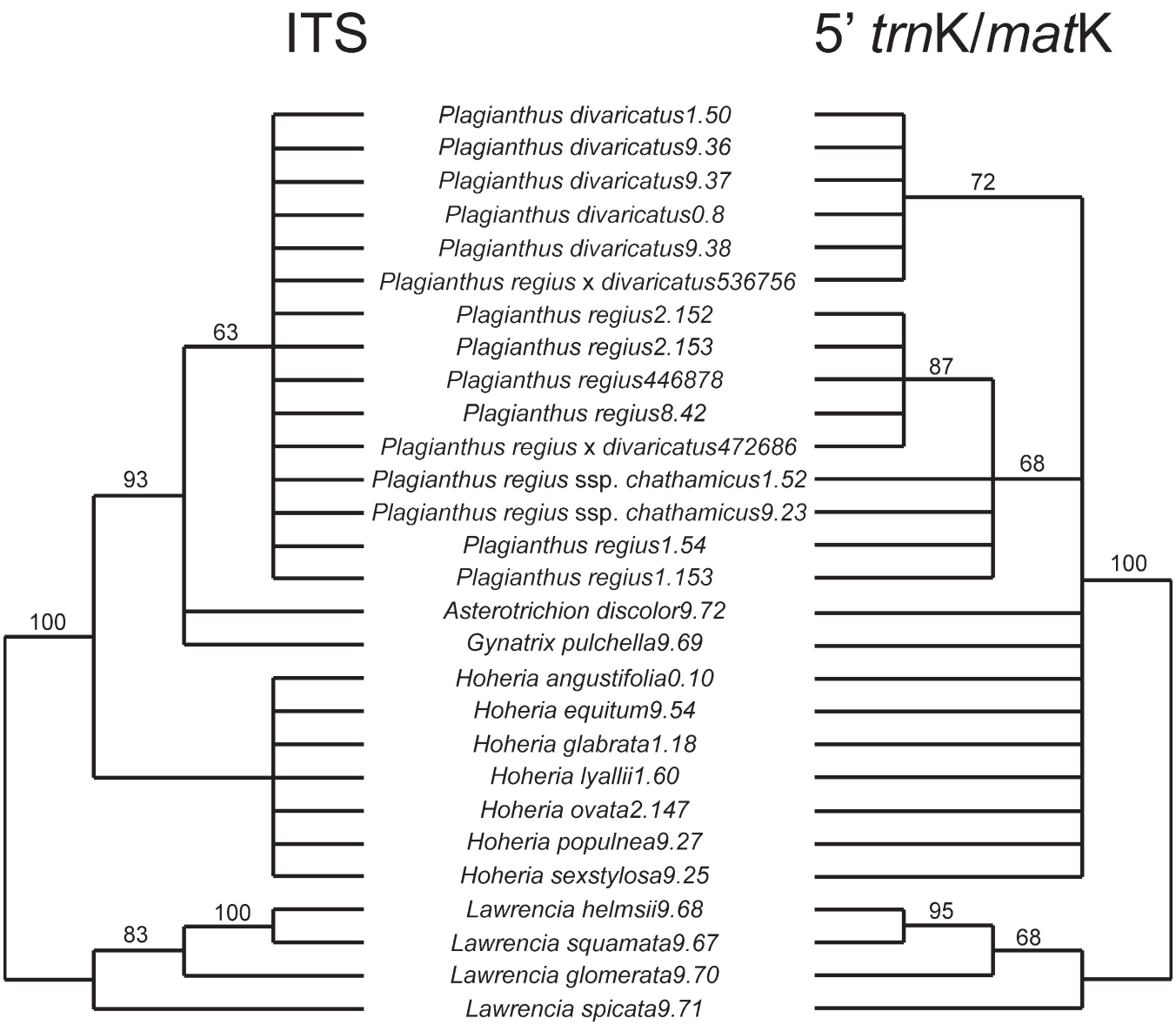

FIG. 3. Comparison of the strict consensus trees recovered from the ITS and 5′ *trnK/matK* data partitions. The tree topologies are largely congruent. Bootstrap values are provided above nodes.

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high consistency and retention indices (Table 1). An ILD test failed to reveal significant conflict in the data ($p = 0.24$) hence, the sequence and gap data sets were combined.

Within the two *Plagianthus* species, the cpDNA and ITS sequences were similar, suggesting either a recent radiation and/or a slow nucleotide substitution rate. Even after combining the two data sets, 11 *Plagianthus* sequences were considered redundant by the merge taxa option in MacClade. Even though their sequence character states were not identical, the sequences were considered redundant as long as a resolution of the missing or ambiguous data could make them identical. The redundant sequences fell into three groups: group 1 included *Plagianthus divaricatus* 1.50, *P. divaricatus* 9.37, *P. divaricatus* 0.8, *P. divaricatus* 9.38, *P. regius* × *divaricatus* 472686; group 2 included *P. regius* ssp. *chathamicus* 9.23 and *P. regius* ssp. *chathamicus* 1.52; and group 3 included *P. regius* 2.153, *P. regius* 446878, *P. regius* 2.152, and *P. regius* 8.42. To avoid including taxa with zero branch lengths in our divergence time estimates, we removed nine redundant sequences, including only one exemplar from each of these groups in subsequent analyses. Notably all outgroups had unique sequences in the combined data set.

The trees recovered from the Bayesian analysis are presented as a majority-rule consensus in Fig. 4. Four well-supported groups were recovered. The most prominent split in our data is the branch separating *Lawrencia* (100% posterior

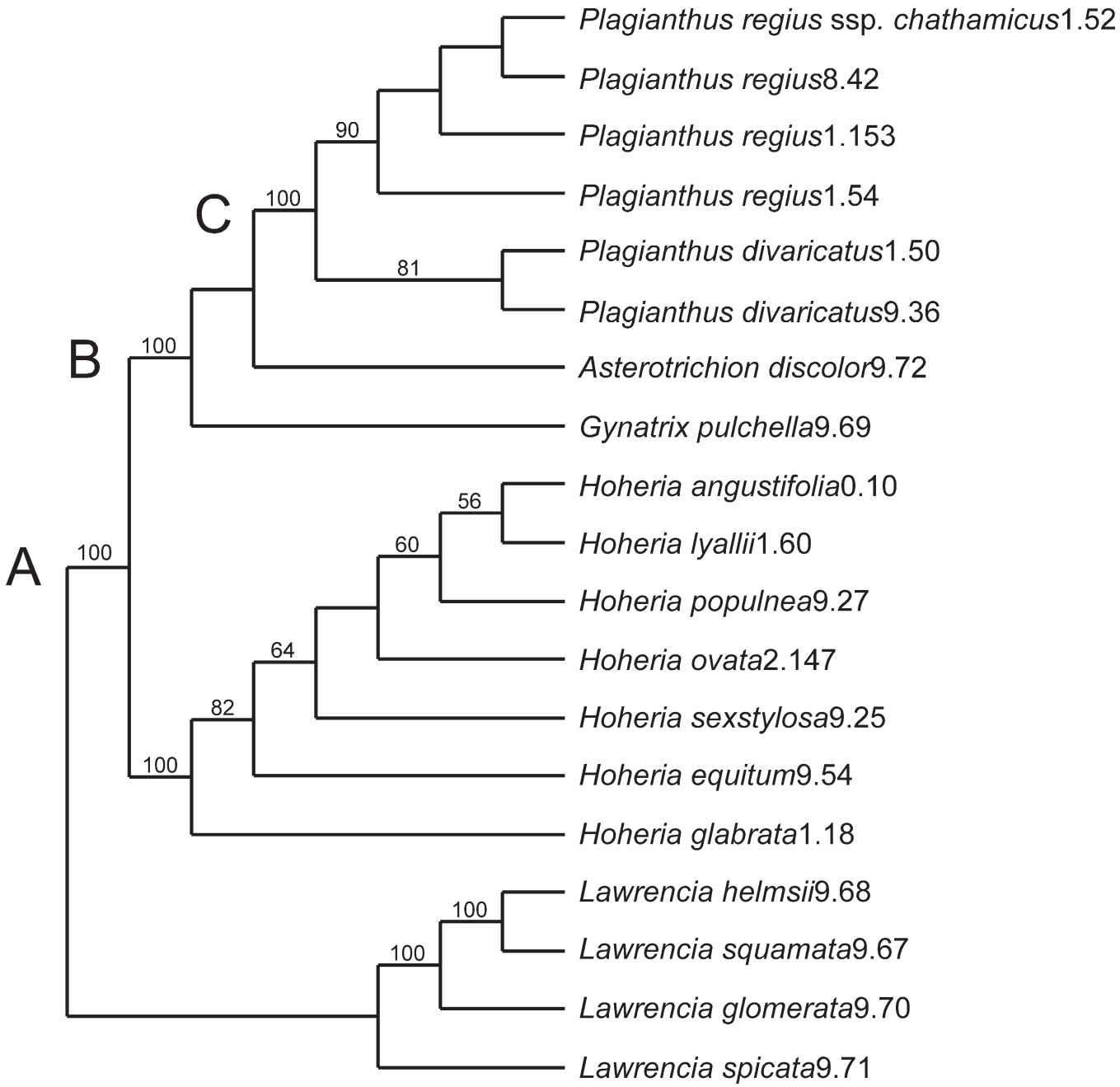

FIG. 4. Majority rule consensus tree from Bayesian analysis of the combined ITS and 5′ *trnK/matK* data partitions. Posterior probability values are provided above each node. Divergence time estimates and posterior probabilities of ancestral states were estimated at three well-supported nodes labelled A–C.

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probability, hereafter PP) from *Asterotrichion*, *Gynatrix*, *Hoheria*, and *Plagianthus*. Similarly, the seven species of *Hoheria* also form a well-supported clade, but there is little support for relationships within this group. *Asterotrichion*, *Gynatrix*, and *Plagianthus* form a third well-supported clade (100% PP) with either *Asterotrichion* or *Gynatrix* or a clade comprised by *Asterotrichion* and *Gynatrix* emerging as sister to *Plagianthus* (100% PP). Monophyly of *Plagianthus regius* (90% PP) and *P. divaricatus* (81% PP) is only moderately supported. Even though there were no exceptionally long or short branches, the data failed the assumptions of a molecular clock (Likelihood ratio test = 2 (3,254.54–3267.94) = 26.794, d.f. = 17, $p \leq 0.05$).

***Estimates of Divergence Times*—**The Bayesian estimates of divergence time are consistently younger than the ML estimates (Table 2), but the confidence intervals overlap. This difference may reflect the different methods of calibration and estimating uncertainty implemented in r8s and BEAST. Only one fixed calibration point was designated in r8s with maximum and minimum age constraints. The uncertainty surrounding the divergence estimates was calculated using a bootstrapping procedure. In contrast, we designated three calibration points in BEAST; these were assigned probability distributions, and the uncertainty was given as a 95% PP. Only the Bayesian estimates are presented in Fig. 5. The uncertainty surrounding the divergence estimates was greater in the Bayesian estimates, indicating that Bayesian values represent more conservative estimates than ML estimates.

Table 2. Comparison of divergence estimates derived from Bayesian and maximum likelihood approaches. The values given as million years (Ma) are presented as means ± SD for the maximum likelihood estimates using r8s vers. 1.71 (Sanderson 2002b), and the means ± 95% posterior probability for the Bayesian estimates using BEAST 1.4.8 (Drummond and Rambaut 2007).

Lineage	Bayesian	Maximum likelihood
Stem age of *Plagianthus*, *Asterotrichion* and *Gynatrix*	17.4 (8.0–23.0) Ma	12.5 ± 2.7 Ma (value calculated from 97 bootstrap trees; node collapsed in 3 trees)
Stem age of *Plagianthus*	7.3 (4.0–14.0) Ma	9.0 ± 2.4 Ma (value calculated from 85 bootstrap trees)
Crown age of *Plagianthus*	3.9 (1.9–8.2) Ma	5.4 ± 2.2 Ma

The Bayesian estimate for the stem age of the *Plagianthus*, *Asterotrichion*, and *Gynatrix* lineage is 17.4 (8.0–23.0) Ma, whereas the stem age of *Plagianthus* is 7.3 (4.0–14.0) Ma. The crown radiations occurred at about the same time in the New Zealand endemic genera, *Plagianthus* [3.9 (1.9–8.2) Ma] and *Hoheria* [4.7 (2.7–10.0) Ma] (Fig. 5; Table 2).

Discussion

Plagianthus is a small genus that represents an ecologically important element of the New Zealand flora. *Plagianthus divaricatus* is dominant in estuarine shrub communities,

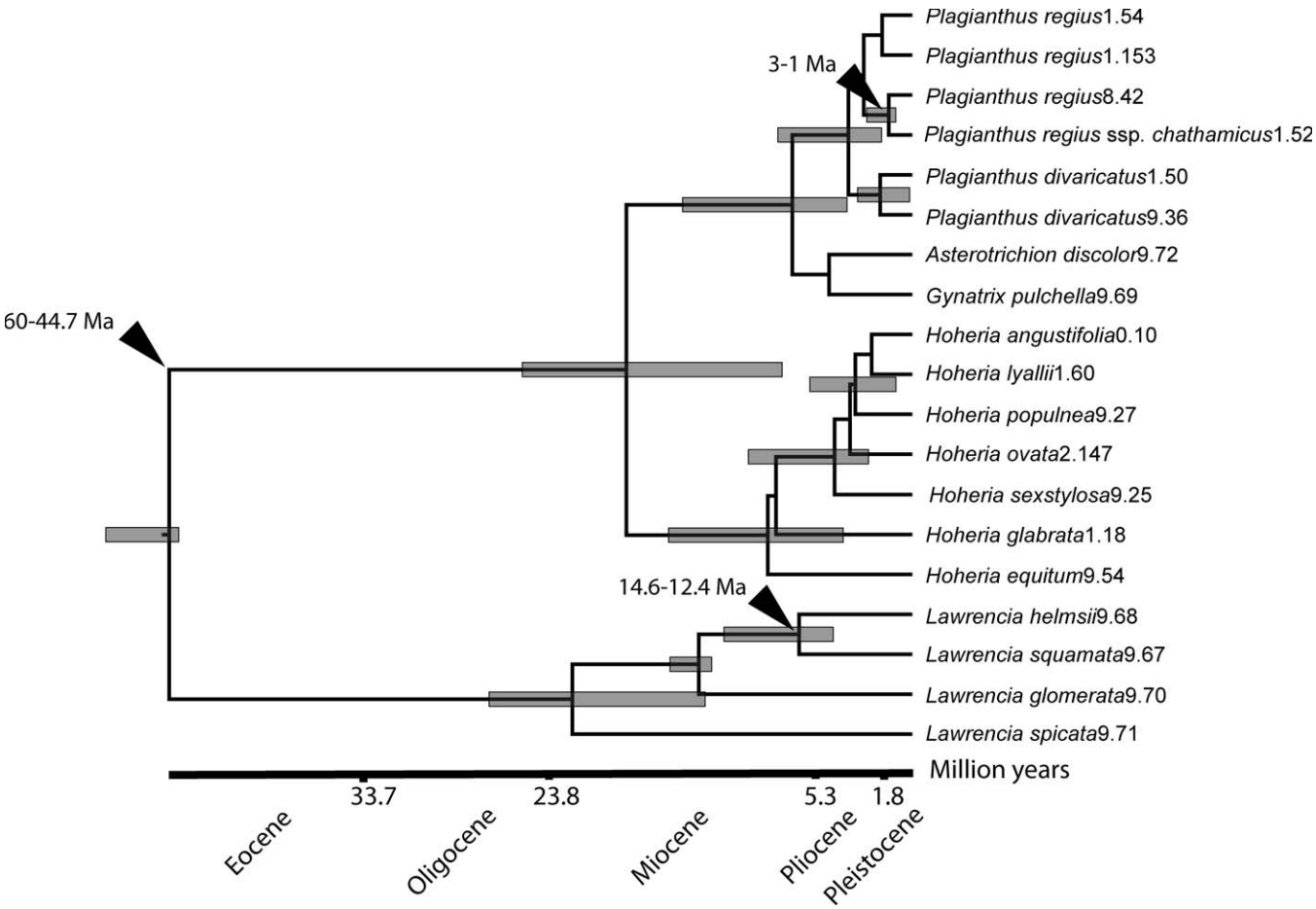

Fig. 5. A chronogram depicting divergence estimates in the *Plagianthus* alliance. Divergence estimates are summarized for the stem age of *Plagianthus*, *Asterotrichion* and *Gynatrix* (Node A), the stem age of *Plagianthus* (Node B), and the crown age of *Plagianthus* (Node C) in Table 2.

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whereas *P. regius* is a conspicuous component of lowland forests. We trace their ancestry to Australia and suggest that their progenitor dispersed to New Zealand recently. Most of the ancestral morphological characteristics were retained and few apomorphic characters were acquired after the founders became established in New Zealand.

Out of Australia—*Plagianthus* shares ancestry with the Australian endemic genera *Asterotrichion* and *Gynatrix* (Fig. 6A). However, the sister group of *Plagianthus* remains unresolved. *Asterotrichion*, *Gynatrix*, or a clade composed of the two genera were equally likely sister to *Plagianthus* (Figs. 4–6). *Asterotrichion* is endemic to Tasmania, while *Gynatrix* is found both in Tasmania and South Australia. The PP for an Australian origin at node A (Fig. 4) is 0.80 (Table 3). We obtained a single MP resolution of the character 'current distribution' within the *Plagianthus* alliance (Fig. 6A), suggesting independent Australian origins for the two New Zealand endemic genera, *Plagianthus* and *Hoheria,* with subsequent dispersal of *Plagianthus regius* ssp. *chathamicus* to one of the offshore island archipelagos, the Chatham Islands (see map in Fig. 1).

While fossils attributed to Malveae date back at least to the Eocene and possibly earlier, our results suggest that diversification within the *Plagianthus* alliance was more recent (Fig. 5; Table 2). The earliest fossils of Malvaceae extend back to the upper Cretaceous (Campanian and Maastrichtian) (Manchester 1992, 1994; Muller 1984); however, fossil pollen allied to Malveae first appears in Eocene deposits in Australia, New Zealand, and South America (Mildenhall 1980; Pocknall 1982; Zamaloa and Romero 1990; Barreda 1993; Macphail 1997; Dettmann and Clifford 2000; Mautino et al. 2004; Iglesias et al. 2007). Fossil fruits and leaves of Malvaceae are also reported from the mid-Eocene of Argentina (Wilf et al. 2005; Barreda et al. 2007). However, fossil leaves closely allied to the extant species *Plagianthus regius* first appear in the New Zealand Wanganui series, which only dates to Pliocene–Pleistocene (McQueen 1954). Bayesian and maximum likelihood estimates led to overlapping ages for *Plagianthus*: 7.3 (4.0–14.0) Ma and 9.0 ± 2.4 Ma for the stem age, respectively, and 3.9 (1.9–8.2) Ma and 5.4 ± 2.2 Ma for the crown age. It is interesting that *Hoheria* [4.7 (2.7–10.0) Ma] appears to have diversified around the same time as *Plagianthus*, but with different consequences for species richness and the evolution of morphological traits.

Ancestral vs. Newly Acquired Characteristics—Crisp et al. (2009) suggested that immigrants seldom shifted into a new biome following transoceanic dispersal successfully. This appears to be the case with *Plagianthus*, as most of the functional traits that we mapped are shared with the source lineages in Australia. Only the divaricate branching pattern characteristic of *Plagianthus divaricatus* was acquired after the genus became established in New Zealand.

Species of *Plagianthus* are widely distributed in New Zealand (Fig. 2), but are ecologically isolated; interspecific hybridization occurs only in rare instances. The ancestral habitat of *Plagianthus* is equivocal (Fig. 6B); with a PP for a coastal saline environment of 0.59 or a forest environment of 0.41 at Node A (Table 3). *Plagianthus divaricatus* is dominant in coastal saline shrub communities (Cockayne 1958; Wardle 1991), whereas *P. regius* is a conspicuous component of lowland forests, often coexisting with species of *Hoheria*.

Our results suggest that adaptation to a saline environment has occurred at least twice in the evolutionary history of the *Plagianthus* alliance (Fig. 6B). Lander (1984) suggested that the Australian genus *Lawrencia* originated in strand habitats, but with the onset of aridification dispersed along margins of inland saline lakes. Diversification within the genus may have been driven by the expansion and contraction of the inland lakes systems in Central and Western Australia. *Lawrencia spicata* (sect. *Lawrencia*) is widely distributed in southern Australia where it inhabits coastal salt marshes, estuaries, and stream banks (Lander 1984). *Lawrencia glomerata* (sect. *Lawrencia*) is also widely distributed and polymorphic, occurring in and around coastal inlets and estuaries, but spreading inland near playa lake basins and saline depressions. *Lawrencia squamata* and *L. helmsii* (sect. *Selenothamnus*) are shrubs with xerophytic tendencies. *Lawrencia squamata* is a widespread species comprising many fragmented allopatric populations found on the margins of inland playa lake basins, saline flats, and depressions, whereas *L. helmsii* is restricted to gypsum ridges and large margins in the Eremaean Botanical Province of Western Australia.

The most recent common ancestor of *Plagianthus* was a shrub or small tree with moderately sized (mesophyll) deciduous leaves (Fig. 6B–D). Adaptation to a forest environment was accompanied by the evolution of a fully arborescent growth form in *P. regius* in addition to evergreen leaves in some species of *Hoheria*. A reduction in leaf size has evolved independently in *P. divaricatus*, *L. squamata*, and *L. helmsii* (Fig. 6E). In *L. squamata* and *L. helmsii* the reduction in leaf size was likely an adaptation to arid environments. The branching patterns, leaf shape and size, and pubescence are highly variable in *L. squamata*, and it is the only species in the *Plagianthus* alliance to possess thorns.

In many New Zealand woody plants, vegetative growth often differs markedly between the juvenile and adult reproductive phases of development. In some instances a distinct divaricate juvenile form is retained in a reproductively mature plant for a number of years (Hooker 1852; Cockayne 1912). This phenomenon, known as heteroblasty (Jones 1999), is well documented in the *Plagianthus* alliance, appearing in *Plagianthus*, *Hoheria,* and *Lawrencia* (Fig. 6F). It may also occur, but has not been reported, in *Asterotrichion* or *Gynatrix*. The PP for the presence of heteroblasty at node A is 0.71 (Table 3). The juveniles of *Plagianthus regius* ssp. *regius* exhibit a filiramulate juvenile stage with slender interlacing shoots and minute apical buds. The leaves are typically small and distantly spaced. In some cases, the filiramulate growth phase is retained in shoots emerging at the base of a reproductively mature tree (Wardle 1991). However, a prolonged filiramulate growth phase is absent or only weakly expressed in the Chatham Island endemic *P. regius* ssp. *chathamicus* (Burns and Dawson 2009).

A divaricate growth form evolved shortly after *Plagianthus* became established in New Zealand (Fig. 6G). The PP for the presence of a divaricate habit at node A is only 0.01, and the probability only increases to 0.11 at node C (Table 3). A divaricate branching habit is an autapomorphy of *P. divaricatus* (Fig. 6G), but also characteristic of the juvenile phase of *P. regius* ssp. *regius* (Wardle 1991) and some species of *Hoheria*. It has apparently evolved in several independent lineages in New Zealand and was facilitated by changes in the timing of development.

Divaricate plants are small-leaved shrubs or juvenile trees that characteristically have a wide branching-angle (Greenwood and Atkinson 1977; McGlone and Webb 1981;

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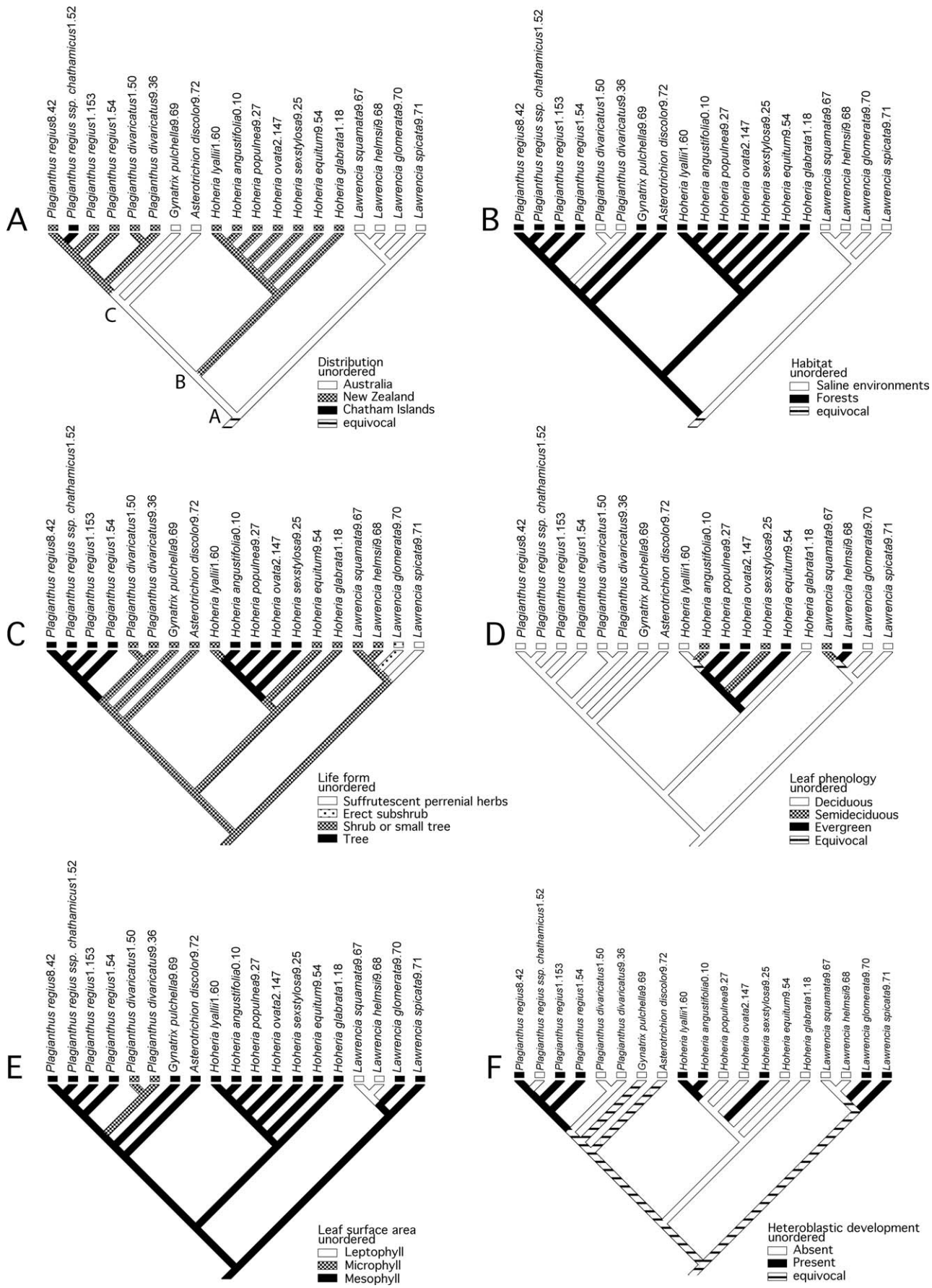

FIG. 6. Extant distributions, habitat and functional traits mapped onto the Bayesian tree shown in Fig. 5. A. Distribution. B. Habitat. C. Life form. D. Leaf phenology. E. Leaf surface area. F. Heteroblastic development. Well-supported nodes (shown in Fig. 5) are labelled A–C.

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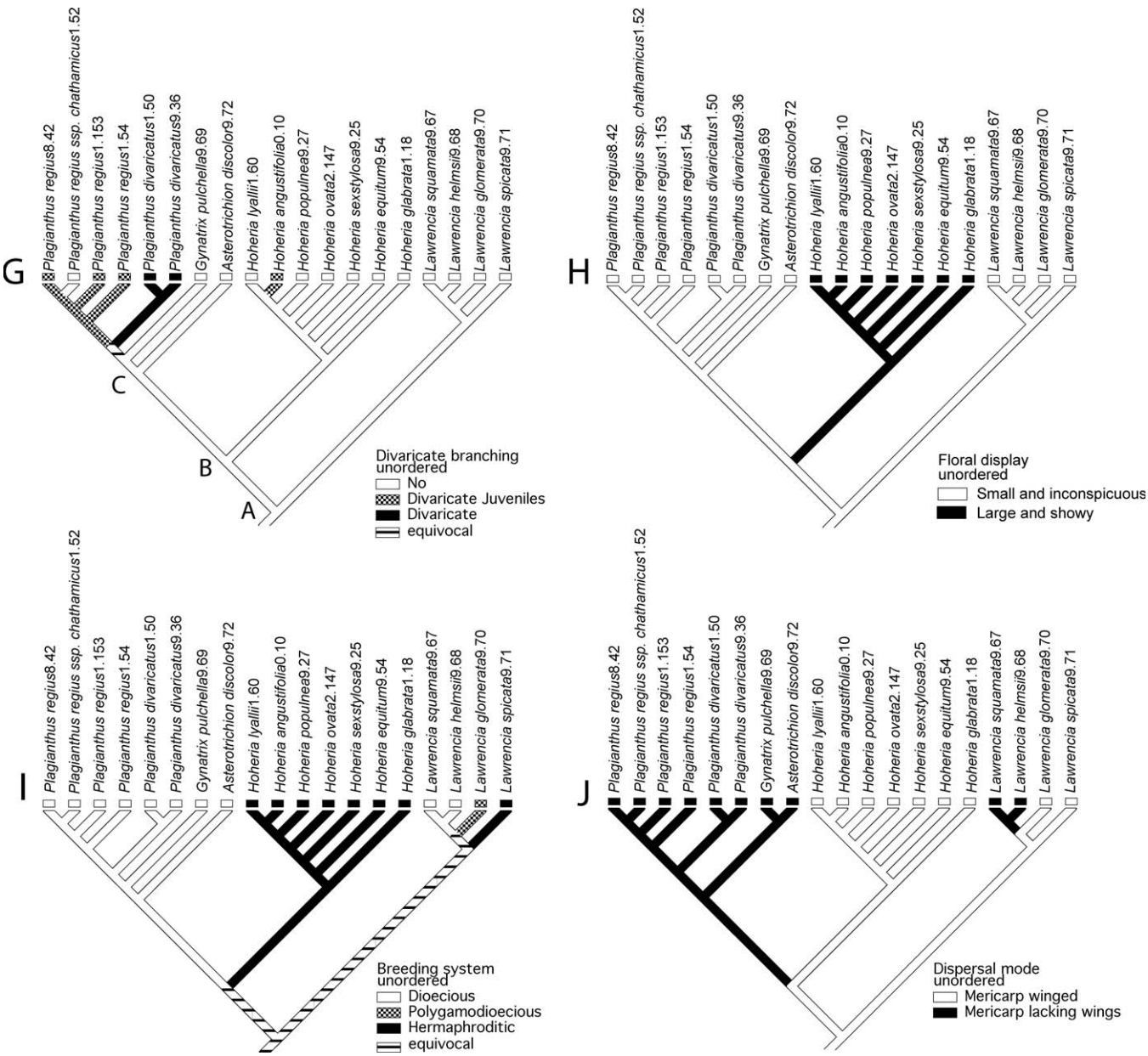

FIG. 6. Extant distributions, habitat and functional traits mapped onto the Bayesian tree shown in Fig. 5. G. Divaricate branching. H. Floral display. I. Breeding system. J. Dispersal mode. Well-supported nodes (shown in Fig. 5) are labelled A–C.

Kelly 1994). Furthermore, the branches have an interlaced structure with a relatively leafless exterior. Climatic factors have been offered as one possible explanation for the evolution of the divaricate habit. McGlone and Clarkson (1993) suggested the divaricate habit enhanced resistance to water stress either during the last ice age when water was scarce or in response to short droughts in the current forest environment. It might also provide resistance to frost or wind damage, as well as might optimize light capture in environments with high light intensity (Day 1998). Alternatively, the divaricate habit might represent a resistance to moa browsing (Greenwood and Atkinson 1977; Bond et al. 2004). Indeed, empirical ecological evidence suggests that *P. regius* was eaten by Dinorthid moas (Burrows 1980). A divaricate habit has evolved independently in several unrelated New Zealand genera, and at least 10% of the woody endemic species in New Zealand exhibit this growth form, yet it is quite rare elsewhere in the world (Greenwood and Atkinson 1977; McGlone and Webb 1981; Bond et al. 2004).

Both species of *Plagianthus* have inconspicuous (Fig. 6H), predominantly dioecious flowers (Fig. 6I). Rare instances of polygamodioecy and monoecy have been described (Allan 1961; Melville 1966). Small inconspicuous flowers are ancestral, but the breeding system present in the New Zealand founders is equivocal (Fig. 6I), with posterior probabilities of 0.59 for a dioecious system and 0.29 for a hermaphroditic system at node A (Table 3). Dioecy is rare in the Malvaceae, but common throughout the New Zealand flora (Webb et al. 1999). The other New Zealand endemic, *Hoheria,* is distinct from the other members of the *Plagianthus* alliance in having large, showy, hermaphroditic flowers (Fig. 6H–I). Godley (1979) identified five types of dioecious flowers in New Zealand plants. The functional flowers of *Plagianthus* retain a rudimentary remnant of the opposite sex (Fig. 1D), correspoding to the 'unisexual by

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TABLE 3. Posterior probabilities for character state distributions at three well-supported nodes. A. *Hoheria*, *Asterotrichion*, *Gynatrix* and *Plagianthus* clade, B. *Asterotrichion*, *Gynatrix* and *Plagianthus* clade, C. *Plagianthus* clade. See Fig. 6A–J for character state maps.

Character	States	Node A	Node B	Node C
1. Distribution	Australia	0.80	0.38	0.14
	New Zealand	0.18	0.59	0.84
	Chatham Islands	0.01	0.03	0.02
2. Habitat	Saline	0.59	0.09	0.13
	Forests	0.41	0.91	0.87
3. Life form	Suffrutescent perennial herbs	0.02	0.02	0.01
	Erect subshrub	0.41	0.02	0.01
	Shrub or small tree	0.55	0.92	0.91
	Tree	0.17	0.05	0.07
4. Leaf phenology	Deciduous	0.98	0.90	0.99
	Semideciduous	0.01	0.03	0.00
	Evergreen	0.01	0.07	0.00
5. Leaf surface area	Leptophyll (< 2.5 cm^3)	0.03	0.03	0.00
	Microphyll (2.5–20 cm^3)	0.22	0.04	0.10
	Mesophyll (20–180 cm^3)	0.75	0.93	0.90
6. Heteroblastic development	Absent	0.29	0.24	0.20
	Present	0.71	0.76	0.80
7. Divaricate branching	Absent	0.98	0.93	0.75
	Divaricate juveniles	0.01	0.04	0.14
	Divaricate	0.01	0.03	0.11
8. Floral display	Small and inconspicuous	0.85	0.65	0.97
	Large and showy	0.15	0.34	0.03
9. Breeding system	Dioecious	0.59	0.71	0.99
	Polygamodioecious	0.11	0.05	0.01
	Hermaphroditic	0.29	0.24	0.01
10. Dispersal mode	Fruit winged	0.55	0.56	0.11
	Fruit lacking wings	0.45	0.44	0.89

abortion' type (Godley 1979; Mitchell and Diggle 2005). In this morphological type, the initiation of androecial and gynoecial organs occurs in all flowers, but is followed by the termination of development in one or the other organ set.

The flowers of *Asterotrichion*, *Gynatrix*, and *Lawrencia* are inconspicuous, resembling those of *Plagianthus*. They are white, pale yellow, or green, ranging in size from two to 10 mm. However, the breeding systems are variable, with hermaphroditism, polygamodioecy, and complete dioecy expressed in *Lawrencia* (Lander 1984). The bisexual flowers of *Lawrencia* are protandrous, and the style branches reflex into the staminal cluster as the flowers mature, which increases the chances of self-pollination. The flowers of *L. spicata* are bisexual, whereas those of *L. glomerata* are either unisexual or bisexual. Both *L. squamata* and *L. helmsii* are dioecious as are *Asterotrichion* and *Gynatrix* (see Sakai and Weller 1999 for a review of ecological correlates of gender and sexual dimorphism in flowering plants).

Mechanisms of seed dispersal may have facilitated the spread of *Plagianthus* in New Zealand, but probably had little to do with long-distance dispersal between Australia and New Zealand. Transoceanic long-distance dispersal is most likely an infrequent random event with the probability of successful establishment being exceedingly small (Nathan et al. 2008). The evolution of dispersal mechanisms is equivocal in our character reconstructions. *Plagianthus* founders either lacked winged fruits or lost them soon after they were established in New Zealand (Fig. 6J); the PP of winged fruits at node A is 0.55 (Table 3).

Fruit morphology in members of the *Plagianthus* alliance involves basic modifications of the typical schizocarp of Tribe Malveae with trends towards a reduction in the number of carpels, a reduction in fertility, and abortion of sterile mericarps (Melville 1966; Lander 1984). The fruits of *L. spicata* and *L. glomerata* are indehiscent with five fertile mericarps in each fruit (Lander 1984). The pericarp becomes light, dry, and stiff at maturity, while the margins form narrow wings. There is substantial air space between the seed and the pericarp, and the bladder-like fruit could conceivably be dispersed by water or wind (Fig. 6J). While the mericarps of *Hoheria* are also winged (obscurely so in *H. lyallii* and *H. glabrata*), the fruit structure is different, and probably not homologous to the winged structure in *Lawrencia* (Lander 1984). *Lawrencia squamata* and *L. helmsii* are irregularly dehiscent with two to five mericarps in each fruit; in some cases only one or two of the mericarps are fertile. The pericarps lack wings or any other special aids for dispersal (Lander 1984). The naked seeds are released in the neighbourhood of the parent plant following the disintegration of the pericarp. The fruits of *P. divaricatus*, *P. regius*, and *Asterotrichion* are similar, but usually only one (sometimes two in *P. divaricatus*) of the mericarps are fertile; each with a single seed. *Gynatrix* differs from all other members of the *Plagianthus* alliance in possessing a crustaceous endocarp, which is responsible for the dehiscence of the mericarps (Melville 1966).

Australia was the source for many New Zealand plants groups such as *Plagianthus* (Cockayne 1958; Wardle 1991), yet the environmental conditions driving evolution in both regions are different. Our findings show that unravelling the evolution of New Zealand plants cannot be achieved in isolation, but requires an appreciation of allied floras and traits that have evolved elsewhere and subsequently persisted in their new home. Consistent with our previous phylogenetic analyses (Tate et al. 2005; Wagstaff et al. 2010), *Plagianthus* emerged as monophyletic. However, no geographic structure

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on the genetic variation of the two species across the North and South Islands was found. The exact sister group of *Plagianthus* is also still unresolved, as either *Asterotrichion* or *Gynatrix* or a clade composed of the two genera were shown to be equally likely sister to *Plagianthus*. Additional data and faster evolving markers (e.g. microsatellite loci) are needed to clarify the relationships of these intriguing endemics.

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Appendix 1. Taxa (and collection details) of plant material from which DNA was extracted for sequencing are listed alphabetically along with their DNA, Herbarium, and GenBank accession numbers (ITS, 5′ *trnK/matK*)

Asterotrichion discolor* (Hook. f.) Melville** (Australia, Tasmania, Bluff River, *A. Moscal 8224*, 1 June 1984), 9.72, CHR491243, AY591811, GU045813; ***Gynatrix pulchella* (Willd.) Alef.** (Australia, Canberra, CSIRO grounds, lower E. slopes of Black Mountain, *R. Pullen 8529*, 14 Oct. 1973), 9.69, CHR410595A, AY591826, GU045814; ***Hoheria angustifolia* Raoul** (New Zealand, Otago, Otago Peninsula, Taiaroa Bush, *A. Markey*, 11 Jan. 2000), 0.10, CHR534905A, AY944585, AY944608; ***Hoheria equitum* Heads** (New Zealand, cultivated, Christchurch, *P. B. Heenan*, 17 Sept. 1999, ex Aorangi Island, Poor Knights Islands), 9.54, CHR529193, AY944586, GU045804; ***Hoheria glabrata* Sprague & Summerh.** (New Zealand, Canterbury, Arthur's Pass National Park, Otira Valley, Windy Point lookout, *S. J. Wagstaff*, 9 Mar. 2001), 1.18, CHR541761, AY944588, AY944611; ***Hoheria lyallii* Hook. f.** (New Zealand, South Canterbury, South Branch Ashburton River, *S. J. Wagstaff*, 12 March 2002), 1.60, CHR559101, AY944601, AY944624; ***Hoheria ovata* G. Simpson & J. S. Thomson** (New Zealand, Nelson, Mt Burnett, *P. B. Heenan & P. J. de Lange*, 8.iv.1997), 2.147, CHR 512522A, AY944603, AY944626. ***Hoheria populnea* A. Cunn.** (New Zealand, cultivated, Lincoln, Canterbury Agriculture and Science Centre grounds, *S. J. Wagstaff*, 15 Feb. 1999), 9.27, CHR529983, AY944604, AY944627; ***Hoheria sexstylosa* Colenso** (New Zealand, cultivated, Lincoln, Canterbury Agriculture and Science Centre grounds, *S. J. Wagstaff*, 15 Feb. 1999, ex Banks Peninsula, Kaituna Reserve), 9.25, CHR529984, GU045785, GU045807; ***Lawrencia glomerata* Hook.** (Australia, South Australia, Lake Torrens Basin, Carrapateena Arm, Salt Creek, south of Archie Beavis Dam, *J. Z. Weber 1321*, 5 Sept. 1968), 9.70, CHR380446A, AY591836, GU045815; ***Lawrencia helmsii* (F. Muell. & Tate) Lander** (Australia, Western Australia, Lake Austin, *L. A. Craven 5033*, 22 Apr. 1978), 9.68, CHR380678, AY5911852, GU045816; ***Lawrencia spicata* Hook.** (Australia, Tasmania, Dorans Road, near Lauderdale, *A. M. Buchanan 4540*, 28 Nov. 1984), 9.71, CHR423878, AY591835, GU045817; ***Lawrencia squamata* Miq.** (Australia, South Australia, Nullabor Region, *N. N. Donner 7196*, 16 Aug. 1980), 9.67, CHR411138, AY591853, GU045818; ***Plagianthus divaricatus* J. R. Forst. & G. Forst.** (New Zealand, Westland, Karamea-Heaphy Road adjacent to Oparara Road lagoon. *D. K. Manning*, 8 Jan. 1985), 1.50, CHR419115, AY944606, AY944629; ***Plagianthus divaricatus* J. R. Forst. & G. Forst.** (New Zealand, Auckland, Lucas Creek Scenic Reserve, *R. O. Gardner 2083*, 1 Nov. 1978), 9.36, CHR397684, HM348800, HM348789; ***Plagianthus divaricatus* J. R. Forst. & G. Forst.** (New Zealand, Wellington, Wairarapa coast, NE of Oterei River mouth, *A. P. Druce*, Dec. 1978), 9.37, CHR312124, HM348801, HM348790; ***Plagianthus divaricatus* J. R. Forst. & G. Forst.** (New Zealand, Canterbury, Banks Peninsula, Teddington, *D. Banks 90/33 & P. Douglass*, 16 Nov. 1990), 9.38, CHR474070, GU045791, GU045819; ***Plagianthus divaricatus* J. R. Forst. & G. Forst.** (New Zealand, Otago, Otago Peninsula, Hoopers Inlet, *A. Markey*, 11 Jan. 2000), 0.8, CHR53488, HM348802, HM348791; ***Plagianthus regius* (Poit.) Hochr. ssp. *chathamicus

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(Cockayne) de Lange (New Zealand, Chatham Island, Te Matarae, *W. R. Sykes 426/93*, 2 Dec. 1993), 1.52, CHR496754, HM348803, HM348792; ***Plagianthus regius* (Poit.) Hochr. ssp.** *chathamicus* **(Cockayne) de Lange** (New Zealand, cultivated, Lincoln, Canterbury Agriculture and Science Centre grounds, *S. J. Wagstaff*, 15 Feb 1999, ex Chatham Island, Smith's Bush), 9.23, CHR529985, AY944607, AY944630; ***Plagianthus regius* (Poit.) Hochr.** (New Zealand, Canterbury, Summit Road, Sign of the Bellbird, *R. Elder & J. Thompson*, 27 Nov. 1988), 2.153, CHR465352, GU045792, GU045820; ***Plagianthus regius* (Poit.) Hochr.** (New Zealand, NW Nelson, Fyfe Ra., 360 m. *A. P. Druce*, Feb. 1989), 1.54, CHR395673, HM348793, HM348804; ***Plagianthus regius* (Poit.) Hochr.** (New Zealand, Tongariro, Turakina River, Rangiwaea, Turakina Valley Road, *C. C. Ogle 2564*, 18 Apr. 1993), 2.152, CHR 481818, HM348808, HM348797; ***Plagianthus regius* (Poit.) Hochr.** (New Zealand, near Geraldine, Waihi Gorge, F. J. Breteler 13.11.89), CHR 446878, HM348805, HM348794; ***Plagianthus regius* × *divaricatus*** (New Zealand, Lake Forsythe, P. Heenan & P. J. de Lange 8/2/00), CHR 472686, HM348806, HM348795; ***Plagianthus regius* × *divaricatus*** (New Zealand, Rakanui Rakaukeke Creek), CHR 536756, HM348807, HM348796; ***Plagianthus regius* (Poit.) Hochr.** (New Zealand, Clevedon, Wairoa River, *P. J. de Lange 6992*, Feb. 17, 2008), 8.42, AK300202, HM348810, HM348799; ***Plagianthus regius* (Poit.) Hochr.** (New Zealand, Westland Land District, Teremakau River, Harrington Creek, *B. H. Macmillan 96/9*. 16 Jan. 1996), 1.153, CHR 510106, HM348809, HM348798.

Appendix 2. Character state distributions. **1.** Distribution (0: Australia, 1: New Zealand, 2: Chatham Islands); **2.** Habitat (0: Saline environments, 1: Forests); **3.** Life form (0: Suffrutescent perrenial herbs, 1: Erect subshrub, 2: Shrub or small tree, 3: Tree); **4.** Leaf phenology (0: Deciduous, 1: Semi-deciduous, 2: Evergreen); **5.** Leaf surface area (0: Microphyll (2.5–20 cm^3), 1: Leptophyll (< 2.5 cm^3), 2: Mesophyll (20–180 cm^3); **6.** Heteroblastic leaf development (0: Absent, 1: Present); **7.** Divaricate branching (0: No, 1: Divaricate juveniles, 2: Divaricate); **8.** Floral display (0: Small and inconspicuous, 1: Large and showy); **9.** Breeding system (0: Dioecious, 1: Polygamodioecious, 2: Hermaphroditic); **10.** Dispersal mode (0: Mericarps winged, 1: Mericarps lacking wings).

Asterotrichion discolor 9.72: 0 1 2 ? 0 0 2 0 0 1; *Gynatrix pulchella* 9.69: 0 1 2 ? 0 0 2 0 0 1; *Hoheria angustifolia* 0.10: 1 1 3 1 1 1 2 1 2 0; *Hoheria equitum* 9.54: 1 1 2 0 0 2 2 1 2 0; *Hoheria glabrate* 1.18: 1 1 2 0 0 0 2 1 2 0; *Hoheria lyallii* 1.60: 1 1 2 1 0 0 2 1 2 0; *Hoheria ovata* 2.147: 1 1 3 0 0 2 2 1 2 0; *Hoheria populnea* 9.27: 1 1 3 0 0 2 2 1 2 0; *Hoheria sexstylosa* 9.25: 1 1 3 1 0 1 2 1 2 0; *Lawrencia glomerate* 9.70: 0 0 1 1 0 0 2 0 1 0; *Lawrencia helmsii* 9.68: 0 0 2 0 0 2 0 0 0 1; *Lawrencia spicata* 9.71: 0 0 0 1 0 0 2 0 2 0; *Lawrencia squamate* 9.67: 0 0 2 0 0 1 0 0 0 1; *Plagianthus divaricatus* 1.50: 1 0 2 0 2 0 1 0 0 1; *Plagianthus divaricatus* 9.36: 1 0 2 0 2 0 1 0 0 1; *P. regius* ssp. *chathamicus* 1.52: 2 1 3 0 0 0 2 0 0 1; *Plagianthus regius* 1.54: 1 1 3 1 1 0 2 0 0 1; *Plagianthus regius* 1.153: 1 1 3 1 1 0 2 0 0 1; *Plagianthus regius* 8.42: 1 1 3 1 1 0 2 0 0 1;

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