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doi:10.1093/aob/mct057, available online at www.aob.oxfordjournals.org

ANNALS OF BOTANY
Founded 1887

Elucidating the native sources of an invasive tree species, *Acacia pycnantha*, reveals unexpected native range diversity and structure

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Received: 1 August 2012 Returned for revision: 6 November 2012 Accepted: 25 January 2013

• *Background and Aims* Understanding the introduction history of invasive plant species is important for their management and identifying effective host-specific biological control agents. However, uncertain taxonomy, intra- and interspecific hybridization, and cryptic speciation may obscure introduction histories, making it difficult to identify native regions to explore for host-specific agents. The overall aim of this study was to identify the native source populations of *Acacia pycnantha*, a tree native to south-eastern Australia and invasive in South Africa, Western Australia and Portugal. Using a phylogeographical approach also allowed an exploration of the historical processes that have shaped the genetic structure of *A. pycnantha* in its native range.
• *Methods* Nuclear (nDNA) and plastid DNA sequence data were used in network and tree-building analyses to reconstruct phylogeographical relationships between native and invasive *A. pycnantha* populations. In addition, mismatch distributions, relative rates and Bayesian analyses were used to infer recent demographic processes and timing of events in Australia that led to population structure and diversification.
• *Key Results* The plastid network indicated that Australian populations of *A. pycnantha* are geographically structured into two informally recognized lineages, the wetland and dryland forms, whereas the nuclear phylogeny showed little geographical structure between these two forms. Moreover, the dryland form of *A. pycnantha* showed close genetic similarity to the wetland form based on nDNA sequence data. Hybrid zones may explain these findings, supported here by incongruent phylogenetic placement of some of these taxa between nuclear and plastid genealogies.
• *Conclusions* It is hypothesized that habitat fragmentation due to cycles of aridity inter-dispersed with periods of abundant rainfall during the Pleistocene (approx. 100 kya) probably gave rise to native dryland and wetland forms of *A. pycnantha*. Although the different lineages were confined to different ecological regions, we also found evidence for intraspecific hybridization in Victoria. The invasive populations in Portugal and South Africa represent wetland forms, whereas some South African populations resemble the Victorian dryland form. The success of the biological control programme for *A. pycnantha* in South Africa may therefore be attributed to the fact that the gall-forming wasp *Trichilogaster signiventris* was sourced from South Australian populations, which closely match most of the invasive populations in South Africa.

Key words: *Acacia pycnantha*, biological control, biological invasions, genetic structure, hybridization, lineage divergence, native provenance, Pleistocene, systematics, taxonomy.

INTRODUCTION

Understanding the processes that shape species distributions and their evolutionary trajectories has long interested biogeographers, ecologists and phylogeographers. From a phylogeographical perspective, delineating genealogical relationships among taxa informs us about the historical processes that shaped patterns of gene flow, local adaptation and, ultimately, speciation. There is growing interest in better understanding processes that underpin the evolutionary trajectories of contemporary species movements, especially those that involve the transfer of species that become established and invasive (Richardson *et al.*, 2011). Historical and contemporary biogeographies are both important, with the former influencing the latter in several ways (e.g. Hui *et al.*, 2011; Rosenmeier *et al.*, 2013). Biological invasions are typically characterized by stochasticity, founder events and strong genetic drift, and introduced genotypes usually encompass only a small proportion of the total genetic diversity of the taxon (Bossdorf *et al.*, 2005; Kliber and Eckert, 2005). It is therefore important to understand the structure of natal populations when building a framework for testing hypotheses about the processes driving biological invasions, such as introduction histories, hybridization, gene diversity and, ultimately, evolutionary potential (Stepien *et al.*, 2002).

Relating historical biogeography to contemporary patterns of gene diversity associated with species introductions and invasions is, however, not always straightforward (Le Roux *et al.*, 2011). Invasive plant populations can arise from individuals introduced from several previously allopatric parts of the native range. This creates opportunities for admixture, hybridization and, consequently, genetic novelty (Prentis *et al.*, 2008). For example, *Acacia cyclops* sourced from different localities in Australia was introduced to a single location in

South Africa that led to intraspecific hybridization (Le Roux *et al.*, 2011). On the other hand, a congener, *A. saligna*, shows high intraspecific diversification in its native range (Millar *et al.*, 2011), whereas invasive populations in South Africa comprise genetic entities not found in the native range (Thompson *et al.*, 2012). Similarly, the most invasive *Tamarix* genotype in the United States is a hybrid of two species, which are allopatric in their native Eurasian range (Gaskin and Schaal, 2002). Such complex introduction scenarios are typical of species introduced for agroforestry in its widest sense, as species are often introduced from multiple sources on multiple occasions and are normally subjected to strong artificial selection following introduction.

Understanding introduction and invasion histories has important practical implications. The selection of effective host-specific biocontrol agents on invasive plants can depend on identifying which subspecific entities of the plant were introduced (Harris, 1998; Wardill *et al.*, 2005; Goolsby *et al.*, 2006). Identification of the native provenance of an invasive species can also improve the design of host-specificity lists and subsequent host testing under the assumption that historical biogeographical processes similarly influenced hosts and agents, and thus co-evolution (Wardill *et al.*, 2005; McLeish *et al.*, 2007). However, it should be noted that the identification of the native provenance of invasive species can be complicated by long histories of plantings and cultivation within the native range of the species (Thompson *et al.*, 2012).

Here we aim to place populations of *Acacia pycnantha* that are invasive in South Africa, Western Australia and Portugal in the context of historical biogeographical patterns in the native range of the species in south-eastern Australia. Specifically, we use plastid and nuclear DNA (nDNA) markers to: (1) reconstruct phylogenetic relationships among invasive (South Africa, Portugal, western Australia) and native (eastern Australia) populations of *A. pycnantha*; (2) compare genetic diversities in these invasive and native populations; and (3) infer the historical processes that may have shaped genetic structure in the natal regions of the species.

MATERIALS AND METHODS

Study system

Australia's national flower, *Acacia pycnantha* (the golden wattle), is native to New South Wales, Victoria and South Australia and is introduced and invasive in Western Australia. It is probably also naturalizing in some areas of New South Wales and South Australia (Eyre Peninsula) from cultivated plantings in revegetation projects and along roadsides. The species, like many Australian acacias, has visible phenotypic variation across its native range (Maslin, 2001; Maslin and McDonald, 2004). In particular, informal morphological classification recognizes two distinct ecotypes, the dryland and wetland forms (Fig. 1). These have distinct phyllodes, flower colours and distributional ranges. Despite this variation, the species has not been formally separated into subspecific entities.

Seeds of *A. pycnantha* were introduced to South Africa on at least two occasions (1865 and 1890) for tannin production and dune stabilization (Poynton, 2009), but the exact origin of the introduced seeds from the native range is unknown. Following introduction to South Africa, seeds of *A. pycnantha* were distributed extensively throughout the country (Poynton, 2009). Since then populations have become invasive in several locations with seven known localities of widespread invasions in the Eastern and Western Cape Provinces. *A. pycnantha* is now listed as a category-1 invasive plant meaning that all uses of the species are prohibited (Henderson, 2001; Nel *et al.*, 2004).

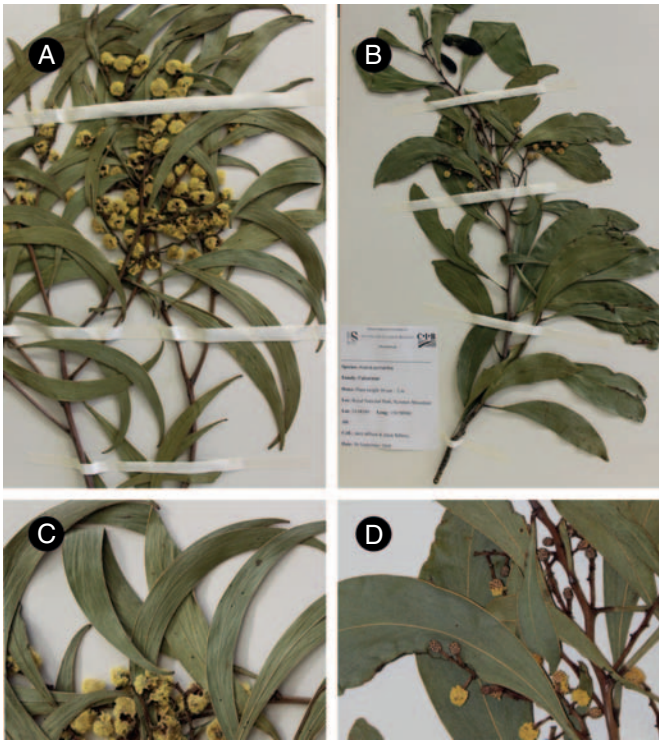

FIG. 1. Herbarium specimens of the extreme ecotypes of *Acacia pycnantha* in Australia for the dryland form (A) characterized by thin phyllodes (C) and the wetland form (B) characterized by broad phyllodes (D).

Following the success of other biological control agents against Australian acacias in South Africa, a gall-forming wasp, *Trichilogaster signiventris*, was introduced in 1987 (Hoffmann *et al.*, 2002; Impson *et al.*, 2011), and a seed-feeding weevil, *Melanterius maculates*, was introduced in 2003 (Impson *et al.*, 2011). The wasp dramatically reduced seed production, but the impact of the weevil is still to be determined (Impson *et al.*, 2011).

Elsewhere in the world, *A. pycnantha* is invasive in Portugal (Richardson and Rejmánek, 2011) and naturalized in the United States (California) (http://www.calflora.org). An initial assessment of the potential for biocontrol of Australian acacias based on the experience in South Africa is underway in Portugal (Marchante *et al.*, 2011), with *T. signiventris* identified as a potential candidate for release.

Plant collection

Phyllodes of *A. pycnantha* were collected from throughout its native range (from what appear to be natural populations

in south-eastern Australia) and invasive (Western Australia and South Africa) ranges during 2009 (Appendix). To prevent sampling individuals from reforestation projects, most native populations were sampled away from roadsides, e.g. in national parks. Between two and five trees were sampled from each population. A single population of *A. pycnantha* was also sampled in August 2010 in Portugal (see Appendix for locality data). Phyllode material was dried in silica gel and kept at room temperature until DNA extraction. Duplicate voucher specimens were collected from each sampled population and deposited at the State Herbarium in Adelaide, South Australia, and the Stellenbosch University Herbarium in Stellenbosch, South Africa.

DNA extraction, PCR amplification and sequencing

DNA was extracted from dried leaf samples using the CTAB method (Doyle and Doyle, 1987) modified by the addition of 5 M NaCl. DNA concentrations were measured using a Nanodrop spectrophotometer (Infinite 200 PRO NanoQuant, Tecan Group Ltd, Switzerland) and diluted to 100 ng μL^{-1} and stored at −80 °C until further use. The plastid *rpl32-trnL* region was amplified using the primers rpl32-F (5′-CAGTT CCAAAAAAACGTACTT-3′) and $trnL^{(UAG)}$ (5′-CTGCTT CCTAAGAGCAGCGT-3′) (Shaw *et al.*, 2007). In addition, two nuclear loci, the external transcribed spacer region (ETS) and internal transcribed spacer region (ITS) were amplified using primers described in Murphy *et al.* (2010). All PCR reactions were carried out in 50-μL reactions consisting of 5 μL (5 μM) of each primer, 5 μL of DNA template, 1 μL dNTP mix (20 mM), 2·0 mM $MgCl_2$, 5 μL of 10× buffer and 0·5 U Taq DNA polymerase (Super-Therm JMR-801; Southern Cross Biotechnologies, Cape Town, South Africa). For the plastid region the following thermocycler programme was used: an initial denaturation of 95 °C for 2 min followed by 30 cycles of 95 °C for 30 s, 60 °C for 30 s and 72 °C for 60 s, followed by a final extension at 72 °C for 10 min. For both nuclear loci the following programme was used: 95 °C for 3 min followed by 30 cycles of 94 °C for 1 min, 60 °C for 1 min and 72 °C for 2 min and a final extension of 72 °C for 10 min. Sequencing for all the three regions was carried out using Big dye Terminator cycle sequencing (Applied Biosystems, Foster City, CA, USA). Given the relative short lengths of all regions included here, sequencing was done in one direction only for all regions. All DNA sequences have been deposited in GenBank (http://www.ncbi.nlm.nih.gov/genbank/).

Phylogenetic analysis

DNA sequences were aligned using MAFFT (Katoh *et al.*, 2002) with manual adjustments in BIOEDIT version 7·0·5·3 (Hall, 1999) for all regions separately. The nuclear ETS and ITS datasets were later combined into a single nDNA dataset. A haplotype network was constructed for the plastid dataset using statistical parsimony as implemented in TCS version 1·21 (Clement *et al.*, 2000). Phylogeny reconstruction was conducted separately on the nuclear dataset. Bayesian inference of phylogenetic relationships was done for the nDNA datasets using Mr Bayes 3·1·2 (Ronquist and Huelsenbeck, 2003). The best fit models were first estimated using jModelTest (Guindon and Gascuel, 2003; Posada, 2008). The nDNA dataset was run for two million generations and trees sampled every 1000 generations. After discarding 25 % of the burn-in trees a consensus tree was generated. Posterior probabilities were calculated using the 50 % majority rule consensus method.

Genetic distances among *A. pycnantha* populations were estimated using the library seqinR in the R statistical environment (Charif and Lobry, 2007). To obtain an indication of the expected intraspecific variation for the *rpl32-*$trnL^{(UAG)}$ region, the genetic distances between subspecific entities of *A. pycnantha* and other species (*A. nilotica*, *Carex elata*, *Linaria viscosa*, *Linaria multicaulis*, *Limnanthes floccosa* and *Centaurea aeolica*) were calculated using data downloaded from GenBank.

To test for neutrality in the plastid and nuclear datasets we used Tajima's D test, Fu and Li's D* test and Fu and Li's F* test in dnaSP version 5·0 (Librado and Rozas, 2009). Mismatch distributions and Harpending's raggedness index for both datasets were also determined using dnaSP version 5·0 (Librado and Rozas, 2009).

An estimate of the divergence times of different native forms of *A. pycnantha* was based on previously published nucleotide substitution rates for acacias for the plastid genome of 0·1 % per million years (Byrne *et al.*, 2002). The value obtained from the nucleotide diversity estimate is very broad (Byrne *et al.*, 2002). Consequently, to validate this estimate, divergence times were also inferred using a relaxed molecular clock and a substitution rate of 0·1 per million years using a Bayesian Markov chain Monte Carlo (MCMC) procedure as implemented in BEAST 1·4·7 (Drummond and Rambaut, 2007). We used a relaxed molecular clock as our data did not meet the assumptions of a strict molecular clock, as inferred from a likelihood ratio test (Verbruggen *et al.*, 2009). The MCMC model was run under a general time-reversible model of nucleotide substitution with rate variation among sites modelled using a gamma-distribution implemented in jModelTest (Guindon and Gascuel, 2003; Posada, 2008). The Yule speciation process was selected as the tree prior. Three independent MCMC analyses were each run for ten million steps and parameter values were sampled every ten thousand steps.

RESULTS

The aligned plastid DNA matrix was 608 bp long, requiring 12 gaps (indels) with an average length of 2 bp. Twelve haplotypes were identified in *A. pycnantha* (Fig. 2). Five (A, D, F, H and I) unique haplotypes occurred in the distributional ranges of the wetland form in Australia, two haplotypes (B and C) encompassed both Victorian dryland and wetland taxa, and two haplotypes (M and L) occurred in the drier Southern Flinders Ranges, where the dryland form occurs. Three haplotypes (E, G and J) were found in South Africa only (Fig. 2). The haplotype most commonly recorded in the natural range (A) also occurred in four invasive populations in South Africa (Fig. 2). Two of the invasive Australian populations (Western Australia) and the population from Portugal also had haplotype A. The three remaining invasive populations

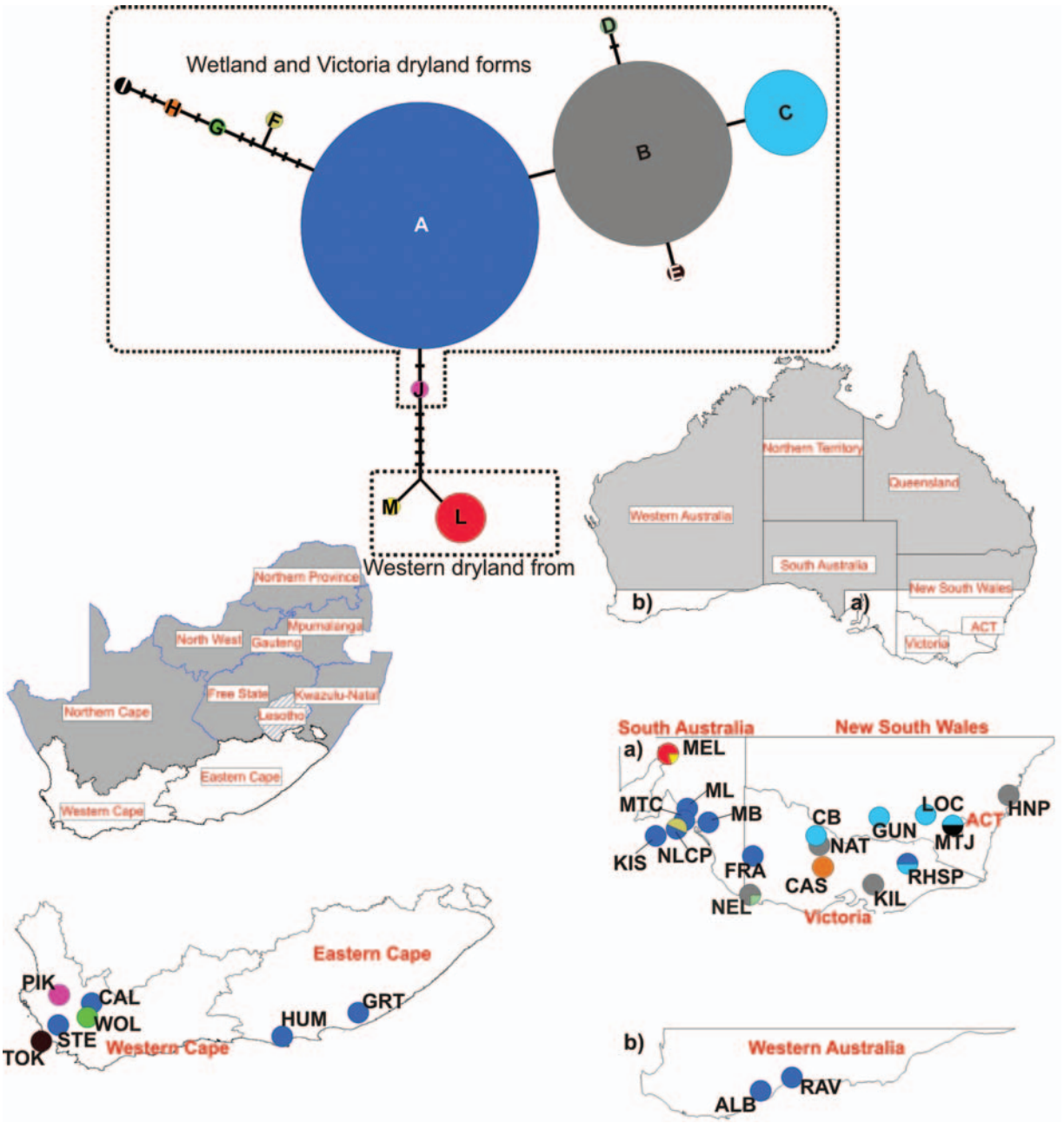

FIG. 2. Haplotype network of *Acacia pycnantha* (based on the *rpl32-trnL*$^{(UAG)}$ region) and geographical distribution of haplotypes in the native south-eastern Australia and invasive South Africa and western Australian ranges. Each unique haplotype is represented by a coloured circle and the size of the circle is proportional to the number of individuals possessing that haplotype. Pie charts represent the proportion and distribution of haplotypes across native (South Australia and New South Wales) and invasive (Western Australia and South Africa) ranges.

from South Africa were closely related to haplotypes A, B and G and haplotype H (Fig. 2). The Flinders Range haplotypes (L and M), corresponding to the dryland form of *A. pycnantha*, were not found in any of the invasive ranges.

Strong spatial clustering occurred throughout the range of *A. pycnantha* in Australia, with most haplotypes having restricted distributions. Moreover, these groupings correspond to the morphologically recognized lineages in *A. pycnantha*: Flinders Range dryland (haplotypes M and L; slender trees with narrow phyllodes at the species western natural range); wetland and Victoria dryland (haplotypes A, B, C, D, F, H and I) (Fig. 2). There was geographical overlap of haplotypes in Reef Hills State Park, Victoria (haplotype A and C). A distinct haplotype (haplotype I) was found in Mount Jeramborerra close to the Australian Capital Territory (Fig. 2).

The best-fit model of evolution used in Bayesian inference, according to the Akaike information criterion (AIC) for the combined nuclear regions, was also the GTR model with a gamma-distribution. The nuclear gene tree retrieved from the Bayesian analysis was incongruent with the plastid DNA

haplotype network in the placement of certain samples. Dryland samples from the Flinders Range clustered with the Natimuk samples from Victoria. In addition, South Australian populations from Mt Compass were shown to be closely related to the Flinders Range population. As in the plastid haplotype network, four South African populations clustered with South Australian populations. Two of the South African populations (Humansdorp and Wolseley) were closely related to the dryland form of *A. pycnantha* (Fig. 3). The Nelson population formed a distinct cluster which was closely related to nearby Victorian populations. Overall, there was no clear geographical structuring of *A. pycnantha* in the nuclear analysis.

Genetic distances ranged from 0 to 0·018 % between plastid DNA haplotypes (Table 1). Haplotypes L and M, which were collected from Melrose (MEL, Flinders Range), had larger distances of up to 0·018 % from the South Australia, Victoria and New South Wales haplotypes (mostly wetland haplotypes; Table S1).

Tests of neutrality for the plastid region using Tajima's D (−2·10088, $P < 0·05$), Fu and Li's D* (−3·54960, $P < 0·02$) and Fu and Li's F* (−3·62302, $P < 0·02$) all yielded negative values that were significantly different from zero. The population expansion hypothesis was investigated by computing the distribution of pairwise differences using dnaSP version 5. The mismatch distribution showed slight bimodality (Fig. 4A) with a Harpending's raggedness value resembling constant size population ($r = 0·3667$, see Zink *et al.*, 2000). Using the rate of nucleotide divergence previously published for the same region (*rpl32-trnL*) revealed that wetland and Flinders Range dryland forms of *A. pycnantha* diverged approx. 110 kya. The upper limit of the Bayesian estimate of the age of the most recent common ancestor (TMRCA) was in agreement with the nucleotide diversity divergence times, indicating that these lineages split approx. 66 kya (95 % confidence interval 58–87 kya).

Similar to plastid DNA, neutrality tests on the nDNA of Tajima's D (−2·84, $P < 0·001$), Fu and Li's D* (−5·88, $P < 0·02$) and Fu and Li's F* (−5·71, $P < 0·02$) yielded negatively significant values. However, pairwise mismatch distribution of the nuclear data yielded was unimodal with a raggedness index of 0·0265, indicative of recent population expansion (Fig. 4B).

DISCUSSION

Linking contemporary and historical biogeography remains a central theme in invasion biology (Stepien *et al.*, 2002). We were able to identify that invasive South African populations of *A. pycnantha* originated from the wetland form and Victorian dryland form of the species, whereas Portuguese and Western Australian invasive populations represent the wetland form only. Most invasive populations originated from the areas around Adelaide and the Mt Lofty ranges. This has important implications for the management of *A. pycnantha* in South Africa and Portugal as discussed below. We found evidence to support previous informal morphological classifications of *A. pycnantha* as two distinct ecotypes (wetland and dryland forms) based on plastid genealogies, providing a platform for guiding conservation efforts in the native range. However, the link between phylogenetic relatedness and morphological classification is not clear cut. The dryland forms of *A. pycnantha* found in Victoria, which had similar phyllode morphologies to the Flinders Range dryland variant, shared a close phylogenetic relationship with the wetland variants. However, the dryland variants found in the Flinders Range showed distinct genetic variation from the wetland/dryland Victoria group (Fig. 2).

Native range phylogeography

We suspect that the phylogeographic structure of *A. pycnantha* in eastern Australia is a result of relatively recent geological climatic shifts during the Pleistocene (Worth *et al.*, 2009, 2010). During the Pleistocene (approx. 16 kya – 1·8 Mya) eastern Australia experienced a series of cycles of pluviality and aridity (Toon *et al.*, 2007). Numerous and isolated Pleistocene refugia during the extreme dry periods have been previously identified (Fig. 5; Ford, 1987), separated by arid barriers, and climatic and edaphic factors (Ladiges *et al.*, 2011). This fragmented landscape resulted in allopatric speciation in many Australian plant groups (Byrne *et al.*, 2002, 2003; Millar *et al.*, 2008). Using an estimate of 0·1 % DNA sequence divergence per million years for plastid DNA as reported for other acacias (Byrne *et al.*, 2003), the divergence between the Flinders dryland and wetland/Victoria dryland form of *A. pycnantha* is estimated to have taken place around 100 kya. This coincides with the mid Pleistocene, a period with high sedimentation rates and aridity in eastern Australia (Prideaux *et al.*, 2007). This is supported by the *A. pycnantha* plastid structure, which indicated the presence of two main haplotype groups that is indicative of the two distinct ecological zones where the different *A. pycnantha* variants occur. Kangaroo Island was connected to mainland Australia during the late Pleistocene and the cut-off time of the island from mainland Australia is estimated at about 8800–13 500 years BP (Hope *et al.*, 1977). This timeframe is in agreement with the *A. pycnantha* collections from Kangaroo Island, which are genetically closely related to the wetland form (KIS 823 and KIS 825, Fig. 2).

For the dryland and wetland lineages, three putative refugia existed in the past: the Eyre Peninsula (Flinders Range dryland form), South East and Mt Lofty refugia (for the wetland and dryland Victorian lineage) (Fig. 5; Ford, 1987). These refugia were separated by the Eyrean and Mallee geographical barriers (Ford, 1987; Toon *et al.*, 2007) and represent areas that had low rainfall (Ford, 1987). Since this time, *A. pycnantha* has expanded its range eastwards and northwards. These expansions are supported by the presence of putative intraspecific hybrids identified by incongruent nDNA and plastid DNA phylogenetic placements (e.g. NAT18 Victoria, Natimuk) and by the patterns of nDNA mismatch distribution, which was unimodal, indicative of recent population expansion (Hwang *et al.*, 2003).

Morphologically, the wetland form of *A. pycnantha* has broader phyllodes and darker golden inflorescences than the dryland form (with narrower phyllodes and often pale yellow flowers, Fig. 1). However, slight morphological variants of *A. pycnantha* also exist in the wetland distributional range in South Australia, Victoria and New South Wales (Maslin and McDonald, 2004). The putative hybrid from Natimuk,

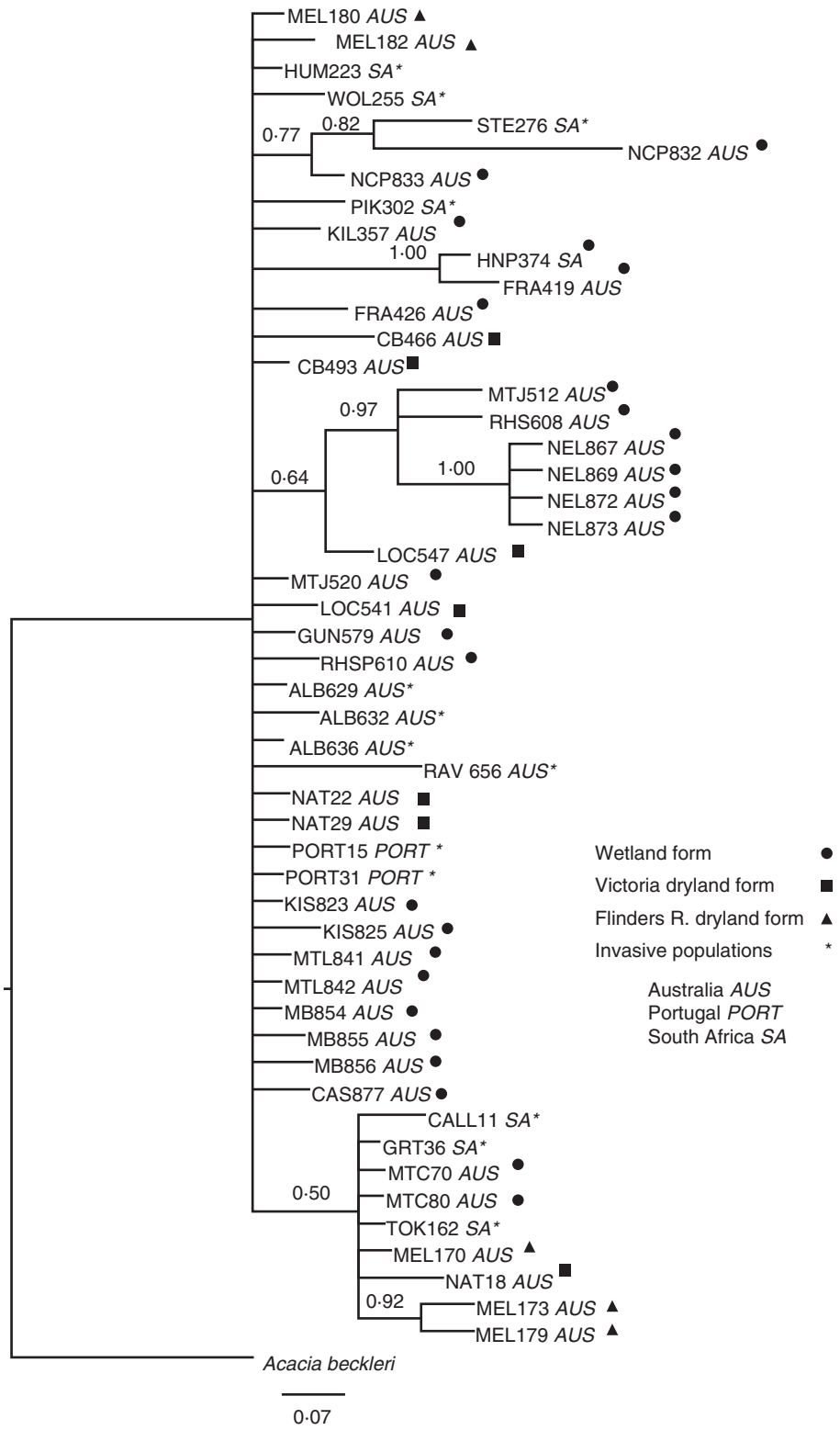

FIG. 3. Bayesian tree for *Acacia pycnantha* using combined ITS and ETS regions. Numbers above the branches represent posterior probabilities derived from the analysis. Invasive taxa are indicated by asterisks for Australian, Portuguese and South African collections, as indicated in the key; also indicated are wetland, Victoria dryland and Flinders Range dryland forms of *A. pycnantha*.

Victoria, showed intermediate morphological characters between the wetland haplotypes and the Flinders Range haplotype (J. Ndlovu and J. J. Le Roux, pers. obs.). The presence of these intermediates in Victoria is probably the result of hybridization through pollen exchange between the wetland and the dryland forms.

TABLE 1. *Genetic distances between* rpl32-trnL$^{(UAG)}$ *plastid haplotypes of* Acacia pycnantha*; A–H correspond to haplotypes as indicated in Fig. 1*

	A	B	C	D	E	F	G	H	I	J	L	M
B	0											
C	0	0										
D	0	0	0									
E	0	0	0·002	0								
F	0	0·004	0·007	0·004	0·004							
G	0·002	0·002	0·002	0·002	0·002	0·006						
H	0·004	0·004	0·002	0·004	0·004	0·004	0·002					
I	0·008	0·009	0·009	0·009	0·009	0·012	0·005	0·009				
J	0·002	0·002	0·002	0·002	0·002	0·007	0	0	0·005			
L	0·01	0·009	0·009	0·009	0·009	0·012	0·011	0·012	0·018	0·002		
M	0·01	0·011	0·012	0·011	0·011	0·015	0·011	0·015	0·016	0·01	0·002	

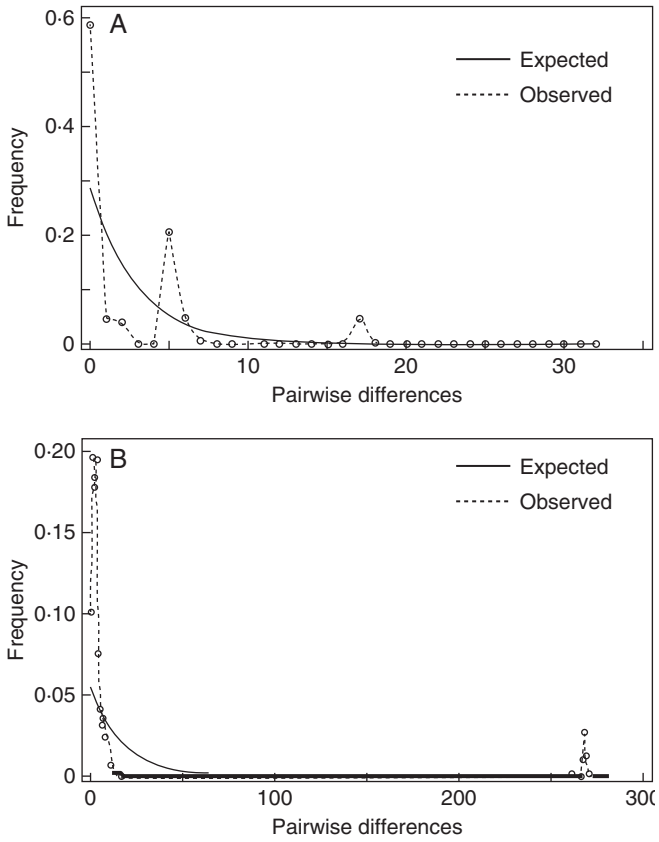

FIG. 4. Mismatch distributions for DNA sequence data of the plastid (A) and nuclear (B) genomes for *Acacia pycnantha* in Australia (native range). The solid line represents the expected mismatch distribution of a constant-size population and the dotted line represents the observed mismatch distribution based on *A. pycnantha* DNA sequence data.

To determine whether the genetic divergences between the extreme forms of *A. pycnantha* potentially represent different subspecies, we calculated genetic differences for known subspecies complexes and closely related species. At the interspecific level genetic divergence ranged from 0·04 % (*Acacia pulchella* and *A. koa*) to 0·15 % (*A. pulchella* and *A. longifolia*) and at the intraspecific level between 0 % (recognized subspecies of *A. nilotica*) and 0·005 % (recognized subspecies of *Linaria multicaulis*); the level of genetic divergence at the intraspecific level for *A. pycnantha* ranged between 0 and 0·018 %.

Overall, informal morphological classifications and our genetic results suggest that the *A. pycnantha* species complex requires taxonomic revision. The genetic divergence found between different forms, at least for plastid DNA, exceeds known divergences between some other taxa consisting of subspecies (Table 1). However, the combination of our genetic and morphological data is not sufficient to separate the species into a subspecies complex. These attempts are hampered by many (other) characters showing geographical variation between the distributional ranges of the dry and wetland forms. For example, the Victorian dryland form, despite its geographical disparity, resembles the variant from the Flinders Range. These problems are also exacerbated by the history of cultivation of *A. pycnantha* in its natural range, possibly obscuring the natural distribution of subspecific taxa in the species. We recommend a finer-scale population genetic study with a more extensive sampling scheme to resolve some of these issues.

Invasive range phylogeography and consequences for management

High-frequency genotypes in the invasive range in South Africa, Western Australia and Portugal occur predominantly in South Australia, with some evidence of wetland variants from Victoria and New South Wales having also been introduced. Notably, however, *A. pycnantha* in South Africa harbours only a fraction of the genetic diversity found in its Australian range (Fig. 2). Clearly there has been a genetic bottleneck during introduction and/or invasion (Fig. 2).

Assuming that historical genetic structure possibly determines co-evolutionary relationships between host plants and their antagonists and mutualists, our findings may explain why *Trichilogaster signiventris*, a gall-forming wasp initially released from Lake Natimuk in Victoria for biological control, did not establish successfully and did not achieve significant control of *A. pycnantha* in South Africa (Hoffmann *et al.*, 2002), as this region does not appear to be the source of invasive populations. However, the reintroduction of the same wasp species collected from Mt Compass in South Australia, a region identified here as a potential source of the

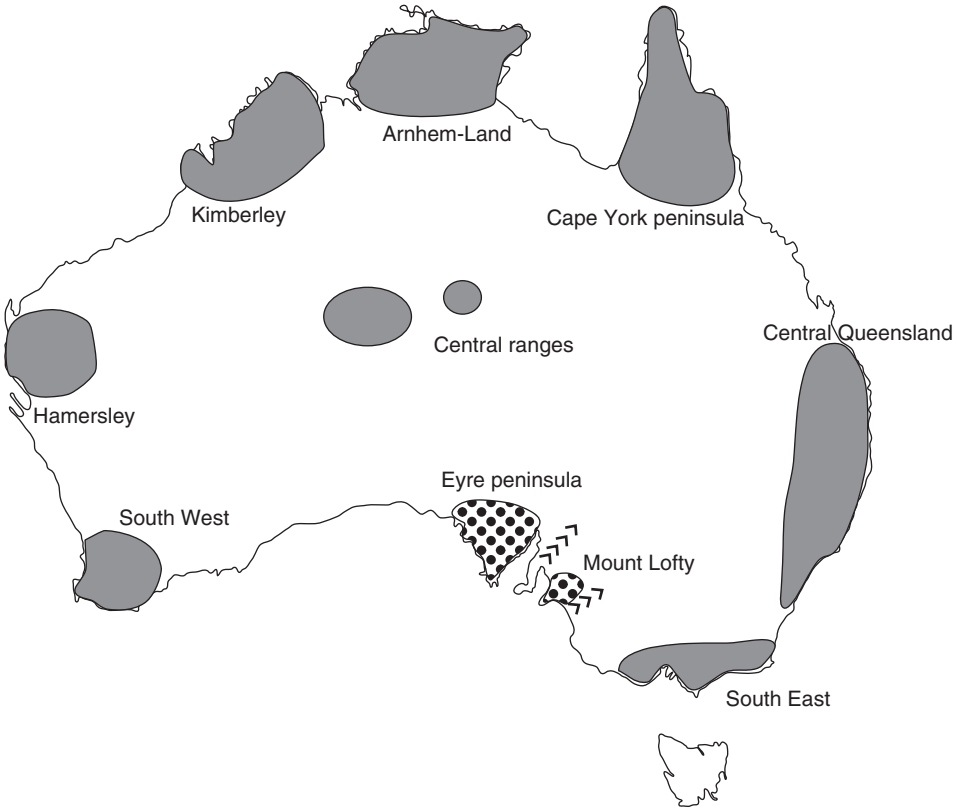

FIG. 5. Refuge areas and geographical barriers in Australia during the Pleistocene. Shaded areas represent refugia in Australia and those with black dots represent refugia for *Acacia pycnantha* (Eyre Peninsula and Mt Lofty). The arrows show the Eyrean and Mallee geographical barriers (Ford *et al.*, 1987).

invasive populations in South Africa, has led to successful establishment and substantial impacts and control of invasive populations in South Africa (Hoffmann *et al.*, 2002). We therefore have reason to conclude that host-specificity and compatibility may be linked to natal phylogeographical structure in this system.

These results have important implications. First, we would expect populations in the native range to be able to survive drier conditions. If the dryland form was introduced, there could potentially be a large expansion in the invasive range in South Africa. Second, the recommendations that biocontrol agents that have proved successful in South Africa should be considered for introduction to other countries where the species is invasive (e.g. Wilson *et al.*, 2011) might need to take subspecific identity and phylogenetic affinity into account (e.g. St. Quinton *et al.*, 2011). Initial results, however, are promising. *A. pycnantha* in Portugal appears to have a similar genetic origin to *A. pycnantha* in South Africa, suggesting the substantial reductions in seed production caused by *T. signiventris* in South Africa (Hoffmann *et al.*, 2002; Impson *et al.*, 2011) might be replicated in Portugal.

Concluding remarks

Identifying where invasive *Acacia* spp. originate from in their natal range is important for determining priorities for biological control. A comprehensive genetic diversity and phylogeographical study in the native range for all the known invasive acacias could potentially form a basis for recommendations of host-specific biological control organisms. In the case of *A. pycnantha*, the invasive genotype found in South Africa is similar to the invasive genotypes in Portugal and Western Australia and thus introduction of the same variant of *T. signiventris* for biological control released in South Africa is recommended. Such studies for all the *Acacia* spp. should be based on plastid genetic diversity as the plastid region has been shown to be more reliable for identifying source populations in this study and in other species in Australia. We therefore recommend that biological control efforts for Australian acacias must recognize the importance of genetic diversity by verifying the source of the invader before releasing biocontrol organisms.

ACKNOWLEDGEMENTS

This work was funded by the DST-NRF Centre of Excellence for Invasion Biology (CIB) and the Working for Water Programme through their collaborative research project on 'Research for Integrated Management of Invasive Alien Species' and grants from South Africa's National Research Foundation and the Hans Sigrist Foundation. We thank Kate Le Roux for assistance during field collections.

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APPENDIX

Localities in South Africa, Australia and Portugal where Acacia pycnantha *was collected.*

Sample ID	Latitude	Longitude	Country	Locality and abbreviation	Status	*rpl32-trnL*	ETS	ITS
CAL11	−33·10701	19·29755	RSA	Caledon (CAL), WC	invasive	JF276987	KC261682	KC261745
GRT36	−33·46032	26·15991	RSA	Grahamstown (GRT), EC	invasive	JF276999	KC261683	KC261746
MTC70	−35·40585	145·95586	AUS	Mt Compass (MTC), SA	native	JF276989	KC261684	KC261747
MTC80	−35·40585	145·95586	AUS	Mt Compass (MTC), SA	native	JF276990	KC261685	KC261748
TOK162	−33·84179	18·66602	RSA	Tokai (TOK), WC	invasive	JF276991	KC261686	KC261749
MEL170	−32·78187	138·1973	AUS	Melrose (MEL), SEA	native	KC261785	KC261687	KC261750
MEL173	−32·78187	138·1973	AUS	Melrose (MEL), SEA	native	KC261786	KC261688	KC261751
MEL179	−32·78187	138·1973	AUS	Melrose (MEL), SEA	native	KC261787	KC261689	KC261752
MEL180	−32·78187	138·1973	AUS	Melrose (MEL), SEA	native	JF276992	KC261690	KC261753
MEL182	−32·78187	138·1973	AUS	Melrose (MEL), SEA	native	KC261788	KC261691	KC261754
HUM223	−34·03989	24·78687	RSA	Humansdorp (HUM), EC	invasive	JF276993	KC261692	KC261755
WOL255	−33·34012	19·16109	RSA	Wolseley (HUM), WC	invasive	JF276994	KC261693	KC261756
STE276	−34·06024	18·41480	RSA	Stellenrust (HUM), WC	invasive	JF276995	KC261694	KC261757
PIK302	−32·80084	18·71501FC	RSA	Piketberg (PIK), WC	invasive	JF276996	KC261695	KC261758
KIL357	−37·22176	145·021	AUS	Kilmore (PIK), VIC	native	JF276997	KC261696	KC261759
HNP374	−34·08589	150·989	AUS	Heathcote NP (HNP), NSW	native	JF276988	KC261697	KC261760
FRA419	−36·77054	141·18135	AUS	Frances (FRA), VIC	native	JF277005	KC261698	KC261762
FRA426	−36·77054	141·18135	AUS	Frances (FRA), VIC	native	JF277008	KC261699	KC261763
CB466	−35·99273	143·76538	AUS	between Charlton & Boorte (CB)	native	JF277011	KC261700	KC261764
CB493	−35·99273	143·76538	AUS	between Charlton & Boorte (CB)	native	JF277022	KC261701	KC261765
MTJ512	−35·36866	149·20332	AUS	Mt Jeramborera (MTJ), ACT	native	JF276998	KC261702	KC261767
MTJ520	−35·36866	149·20332	AUS	Mt Jeramborera (MTJ), ACT	native	JF277009	KC261703	KC261768
LOC541	−35·36866	146·64549	AUS	Lockheart (LOC), NSW	native	JF277012	KC261704	KC261769
LOC547	−35·36866	146·64549	AUS	Lockheart (LOC), NSW	native	JF277021	KC261705	KC261771
GUN579	−35·21065	147·76425	AUS	Gundagai (GUN), NSW	native	JF277023	KC261706	KC261772
RHSP608	−36·59888	145·95586	AUS	Reef Hills SP (RHSP), VIC	native	JF277004	KC261707	KC261773
RHSP610	−36·59888	145·95586	AUS	Reef Hills SP (RHSP), VIC	native	JF277007	KC261708	KC261774
ALB629	−34·31586	118·79919	AUS	Albany (ALB), WA	invasive	JF277016	KC261709	KC261775
ALB632	−34·31586	118·79919	AUS	Albany (ALB), WA	invasive	JF277013	KC261710	KC261776
ALB636	−34·31586	118·79919	AUS	Albany (ALB), WA	invasive	JF277019	KC261711	KC261777
RAV656	−33·59650	120·17688	AUS	Ravernsthorpe (RAV), WA	invasive	JF277016	KC261712	KC261778
NAT18	−36·00409	143·76041	AUS	Natimuk (NAT), VIC	native	KC261791	KC261814	KC261779
NAT22	−36·00409	143·76041	AUS	Natimuk (NAT), VIC	native	KC261792	KC261815	KC261780
NAT29	−36·00409	143·76041	AUS	Natimuk (NAT), VIC	native	KC261793	KC261816	KC261781
PORT15	NA	NA	PORT		invasive	KC261794	KC261817	KC261783
PORT31	NA	NA	PORT		invasive	KC261795	KC261818	KC261784
KIS823	−35·75669	137·89486	AUS	Kangaroo Island (KIS), SA	native	KC261796	KC261713	KC261727
KIS825	−35·75669	137·89486	AUS	Kangaroo Island (KIS), SA	native	KC261797	KC261714	KC261728
NCP832	−35·61298	138·47950	AUS	Newlands C. Park (NLCP), SA	native	KC261798	KC261715	KC261729
NCP833	−35·61298	138·47950	AUS	Newlands C. Park (NLCP), SA	native	KC261799	KC261716	KC261730
MTL841	−34·97175	138·6653	AUS	Mt Lofty (ML), SA	native	KC261800	KC261717	KC261731
MTL842	−34·97175	138·6653	AUS	Mt Lofty (ML), SA	native	KC261801	KC261718	KC261732
MB854	−35·31895	139·51193	AUS	Murray Bridge (ML), SA	native	KC261803	KC261719	KC261734
MB855	−35·31895	139·51193	AUS	Murray Bridge (ML), SA	native	KC261804	KC261720	KC261735
MB856	−35·31895	139·51193	AUS	Murray Bridge (ML), SA	native	KC261805	KC261721	KC261736
NEL867	−38·05003	141·01510	AUS	Nelson (NEL), VIC	native	KC261807	KC261722	KC261738
NEL869	−38·05003	141·01510	AUS	Nelson (NEL), VIC	native	KC261808	KC261723	KC261739
NEL872	−38·05003	141·01510	AUS	Nelson (NEL), VIC	native	KC261810	KC261724	KC261741
NEL873	−38·05003	141·01510	AUS	Nelson (NEL), VIC	native	KC261811	KC261725	KC261742
CAS877	−37·10758	144·09283	AUS	Castlemaine (CAS), VIC	native	KC261813	KC261726	KC261744

AUS, Australia; RSA, Republic of South Africa; Port, Portugal. WC, Western Cape; EC, Eastern Cape; VIC, Victoria; WA, Western Australia; NSW, New South Wales; SA, South Australia; SEA, south-east Australia; ACT, Australian Capital Territory; NA, not available.