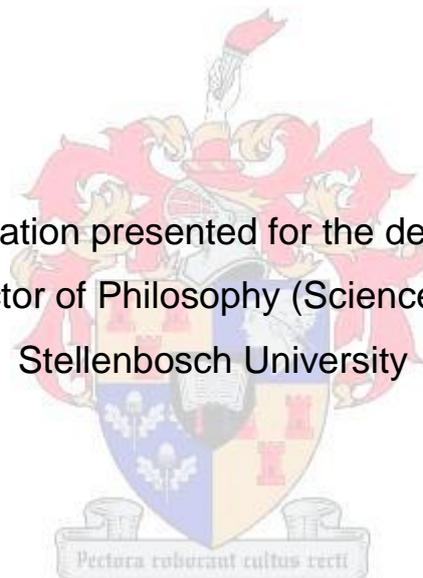


**Molecular ecology of two invasive legumes  
(*Acacia saligna* and *Paraserianthes lophantha*)**

by

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Dissertation presented for the degree of  
Doctor of Philosophy (Science) at  
Stellenbosch University



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December 2012

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## DECLARATION

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Genevieve D. Thompson

Date: 26 July 2012 .....

## ABSTRACT

Large-scale human-mediated movements of organisms promote the establishment of species outside their native ranges and a very small proportion of these species become invasive. Invasive species management typically assumes that introduced species are single, static evolutionary units that are genetically analogous to their native counterparts. However, studies have shown that native and introduced populations of a number of introduced plants differ vastly in their genetic composition. These differences may negatively affect the overall success of control and management programmes, particularly for species that are intra-specifically diverse. The influence of intra-specific diversity on the invasion process was tested in two widely exported tree species that are native to Western Australia, *Acacia saligna* (three subspecies) and *Paraserianthes lophantha* (two subspecies).

Climate matching between the native and introduced range (using species distribution models, SDM) is widely used to forecast future invasion risks, however, it is unknown if SDMs can detect intra-specific niche differences in invasive plants. The SDMs I developed for the subspecies of *A. saligna* detected intra-specific differences within the native range, but did not predict the full invasive distribution in South Africa. Unsurprisingly, SDMs agreed with genetic analyses (based on nuclear microsatellites, nuclear DNA, and chloroplast DNA) and did not assign South African populations to any subspecies of *A. saligna*. South African populations were assigned to a novel genetic entity likely produced by human cultivation practices. A global phylogeny identified this cultivated genotype in introduced populations in eastern Australia and Portugal, while the remaining introduced populations differed markedly in their genetic composition. Overall, *A. saligna*'s high intra-specific diversity and complex introduction history generated a variety of genetic patterns across the current global distribution of the taxon.

Global populations of *P. lophantha* were processed using a similar approach to that used for *A. saligna*, and aimed to determine if the same pathways and modes of introduction produced analogous genetic patterns in a closely related species. Diverse arrays of genotypes were identified in introduced populations of *P. lophantha*, suggesting inconsistent sampling of a variety

of native sources. Further work is however needed to clarify the morphological and genetic differences (if any) between the intra-specific entities, and identify exactly which *P. lophantha* subspecies were introduced outside of their native range,

The variation in the global distribution of genetic diversity observed in *A. saligna* and *P. lophantha* demonstrated that intra-specific genetic variation, human usage, and the pathway and manner of introduction interact during several phases of the invasion process and collectively determine the introduced genetic patterns. The dissimilarity in the distribution of genotypes in both species suggests that they might not behave the same way throughout their introduced range. Consequently, management insights might not be transferrable between regions. More generally, my findings provide an important contribution to the debate whether (and how quickly) introduced and native populations should be treated as fundamentally different entities.

## OPSOMMING

Grootskaalse menslike verskuiwing van organismes bevorder die vestiging van spesies buite hul natuurlike voorkomsareas en 'n klein hoeveelheid van hierdie spesies word indringers. Tydens die bestuur van indringerspesies word dit tipies aanvaar dat ingevoerde indringerspesies enkele, statiese evolusionêre eenhede is wat analoog is aan hul inheemse eweknieë. Studies het egter getoon dat inheemse en uitheemse populasies van 'n aantal ingevoerde plante aansienlik verskil in hul genetiese samestelling. Hierdie verskille kan 'n negatiewe invloed op die algehele sukses van beheer- en bestuursprojekte hê, veral vir die spesies wat intra-spesifiek divers is. Die invloed van intra-spesifieke diversiteit op die indringingsproses is getoets aan twee boomspesies, inheems aan Wes-Australië, wat wyd uitgevoer word: *Acacia saligna* (drie subspesies) en *Paraserianthes lophantha* (twee subspesies).

Vergelyking van klimaatstoestande tussen 'n spesie se in- en uitheemse voorkomsareas word wyd gebruik om toekomstige indringingsrisiko te voorspel. Dit was voor hierdie navorsing onduidelik of spesie verspreiding modelle (SVMs) intra-spesifieke nis-verskille in indringerplante kan uitwys. SVMs wat vir die subspesies van *A. saligna* ontwikkel is, kon intra-spesifieke verskille in Wes-Australië uitwys, maar het nie die volle verspreiding van die spesies in Suid-Afrika voorspel nie. Onverbasend, is geen Suid-Afrikaanse populasies deur genetiese analise (gebaseer op die kern mikrosatelliete, kern-DNS, en chloroplas-DNS) toegewys aan 'n subspesie van *A. saligna* nie. Suid-Afrikaanse populasies het 'n nuwe genetiese entiteit wat waarskynlik gekweek is deur menslike verbouingspraktyke. 'n Globale filogenie het hierdie verboude genotipe in addisionele ingevoerde populasies in die ooste van Australië en Portugal geïdentifiseer. Mikrosatelliet genotipes van uitheemse populasies wêreldwyd in Oos-Australië, Israel, Italië, Nieu-Seeland, Portugal, Suid-Afrika, Spanje en die VSA verskil merkbaar in hul genetiese samestelling. *A. saligna* se hoë intra-spesifieke diversiteit en komplekse geskiedenis van invoer (wat verbouing, wye verspreiding en hoë "propagule" druk betrek), het 'n verskeidenheid van genetiese patrone oor die huidige globale verspreiding van die takson gegenereer.

Om te bepaal of 'n globale uiteenlopende genetiese patroon binne nouerwante spesies bestaan, is globale bevolkings van *Paraserianthes lophantha* verwerk deur gebruik te maak van 'n soortgelyke benadering as wat vir *A. saligna* gebruik is. Globale populasies van beide studietoepesies bestaan uit 'n diverse verskeidenheid van genotipes. Resultate dui daarop dat *P. lophantha* van 'n verskeidenheid inheemse bronne ingevoer is. Om te identifiseer watter *P. lophantha* subspesies buite hul natuurlike voorkomsarea versprei is, word verdere werk benodig om die morfologiese en genetiese verskille (indien enige) tussen die intra-spesifieke entiteite vas te stel.

In hierdie tesis het ek gewys dat intra-spesifieke genetiese variasie, menslike gebruik en invoering-geskiedenis saam werk om genetiese patrone in uitheemse populasies te vorm. Verder het ek die waarde van die gebruik van verskillende molekulêre benaderings om indringing geskiedenis te verstaan, gedemonstreer. Die verskil in die verspreiding van die genotipes van *A. saligna* en *P. lophantha* dui daarop dat hulle moontlik nie op dieselfde manier dwarsdeur hul uitheemse verspreidingsarea mag optree nie. Bestuursinsigte mag gevolglik nie oordraagbaar wees tussen streke nie. Meer algemeen, bied my bevindings 'n belangrike bydrae tot die debat of (en hoe vinnig) inheemse en ingevoerde populasies behandel moet word as fundamenteel verskillende entiteite.

## ACKNOWLEDGEMENTS

This thesis would certainly never have been possible without the support, in many ways and forms, from the following people and institutions:

- This work was funded by DST-NRF Centre of Excellence for Invasion Biology (C•I•B) and the Working for Water Programme through their collaborative research project on “Integrated Management of Invasive Alien Species”. Thanks also go to Stellenbosch University and the European Weed Research Society for financial support during my time at Stellenbosch University
- Ben-Erik Van Wyk, for instilling within me a passion for plants and for science.
- My four supervisors, Dave Richardson, Jaco Le Roux, John Wilson and Dirk Bellstedt for providing guidance and expertise through-out my time at the CIB. To Dave, thank-you for always maintaining the ‘bigger picture’, having an open door and for making it look easy. To Jaco, thank-you for many hours of help on all aspects of the project - in the field, lab and office. To Dirk, thank-you for always being patient and always willing to help in any way you can. To John, thank-you for ‘sticking to your guns’, being objective and thorough.
- Co-author Mark Robertson, for being patient and teaching me the in’s and outs of MAXENT and species distribution modelling.
- Distant co-author Bruce Webber, for being thorough and always readily available for Q and A Skype sessions.
- Christy Momberg, for hours of technical and emotional support.
- Dane Paijmans, Megan Koordom and Leri Koegelenberg for lab assistance.
- Friends, fellow students and researchers at the Centre for Invasion Biology and the Department of Botany and Zoology for support, assistance and discussion.
- A special thanks to Alana Den Breeÿen and Mirijam Gaertner, your support, advice, guidance and friendship is immeasurable.
- Words cannot express my thanks and gratitude to my family for their support and encouragement during my PhD. To Sam, for being enthusiastic about ‘my plants’ and several years of study, for having boundless faith in me and for always providing constructive criticism.

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# CHAPTER 1 General Introduction

- This chapter has been adapted from published conference proceedings, citation: Thompson, G.D., Le Roux, J.J., Bellstedt, D.U., Richardson, D.M. & Wilson, J.R.U. (2011). Molecular research as tool for managing biological invasions: *Acacia saligna* as a case study. In: *Proceedings of the 2nd International Workshop on Invasive Plants in Mediterranean Type Regions of the World*. 2-6 August 2010 (Ed. Brunel S). pp. 107-117. Council of Europe Publishing, Mèze, France.

## 1.1 Literature review

Expanding human populations have radically increased the magnitude of global transport and trade (Richardson *et al.*, 2000), and increased the ease and speed with which humans have moved non-native species to new environments. On rare occasions, the introduction of non-native propagules result in the formation of invasive populations. Invasive populations pose a threat to native biodiversity, are major drivers of ecosystem change and cause substantial economic losses (Powell *et al.*, 2011).

In order to reduce the impacts of biological invasions, management strategies are developed to manage current invasions and help prevent future invasions. Ideally, management aims to eradicate invasive populations but this approach rarely succeeds. An alternative and more effective approach is to prevent the spread of species outside of their native range (Simberloff, 2003). However, management plans often implicitly or explicitly assume that invasive species are single inviolate entities that are indistinguishable from their native counterparts. Such assumptions may substantially affect the overall success of management programmes designed to be effective before (e.g. predictive methods such as risk assessment) or after the establishment of introduced populations (e.g. reducing spread through biological control).

Invasive species risk assessments are capable of predicting which species are most likely to become invasive and are a valuable tool in managing biological invasions. Risk assessment

protocols may be developed using prominent invasive species as models to determine how and why successful invasion occurred. For instance, the attributes of model invasive species can be used to compile a list of typical 'invasive' characteristics against which currently non-invasive species can be compared (e.g. Rejmánek & Richardson, 1996; Goodwin *et al.*, 1999, Daehler *et al.*, 2000; Peterson & Vieglais, 2001; Moles, 2008; Whitney & Gabler, 2008). If the introduction of a non-native species is proposed, and that species possesses a number of typically invasive characteristics, the proposed introduction should be managed with caution as the species may become invasive in the new range.

Many studies have already attempted to predict which species are likely to become invasive by associating a range of biotic and abiotic characteristics with invasive success (Kolar & Lodge, 2001). High genetic diversity has often been associated with invasive success (Ellstrand & Schierenbeck 2000, Mack *et al.*, 2000; Lavergne & Molofsky, 2007) as it assists environmental adaptation to the new range (Sakai *et al.*, 2001). Successful invasion is also constrained by the characteristics of the invader, by the properties of the ecosystem that is being invaded (Richardson & Pyšek, 2006), and the manner in which the species was introduced to the new range (Wilson *et al.*, 2009). Overall, the majority of studies have concluded that no single set of characteristics or features is common amongst all invaders (Thuiller *et al.*, 2006; Le Roux & Wiczorek, 2009). Nonetheless, spatial and temporal patterns in genetic diversity continue to be studied in introduced populations (Sakai *et al.*, 2001), but are increasingly being studied together with other ecological or biological parameters (e.g. biomass and leaf surface area in different genotypes, or high propagule pressure and human usage, Ross & Auge, 2008).

Recent studies have highlighted propagule pressure as a common contributor to invasive success (Kolar & Lodge, 2001; Lockwood *et al.*, 2005; Simberloff, 2009) but obtaining information on the number of propagules introduced to a new environment is difficult as detailed introduction records are often limited. However, High propagule pressure or multiple introductions frequently results in high genetic diversity in the introduced range because the repeated introduction of propagules may also mean the repeated introduction of genetic material. Thus, if the introduced range is as diverse as the native range, one could speculate that many propagules were

introduced to the region (i.e. high propagule pressure). In all cases, one would need to be mindful of the fact that high genetic diversity may be caused by other factors, including a single introduction from multiple diverse sources, or hybridisation that produces novel invasive genotypes. In any event, high propagule pressure (through sheer weight of numbers) and high genetic diversity (by increasing the chance of introducing a suitable genotype) are likely to be correlated causative factors in an invasion.

Insight drawn from the application of molecular tools to introduced populations provides two potential outputs: 1) insight into the genetic patterns associated with invasive success, and 2) information that can be used to allocate resources to control or prevent further introductions. However, the dynamics of species invasions can be extremely complex, where a number of species attributes and human mediated processes interact to determine the genetic patterns in the introduced range. Such complexities make it challenging to distinguish between a range of factors and their influence on the genetic diversity in the introduced range. This challenge may be further exacerbated by complexities in the native range. For example the introduction dynamics of intra-specifically diverse species significantly affects their genetic diversity in the new range, the opportunity for intra-specific hybridization (e.g. *Tamarix* spp., Gaskin & Schall, 2002) and the possibility of novel genotypes or hybrids (e.g. *Schinus terebinthifolius*, Williams *et al.*, 2005); all features that have been linked to highly successful invaders.

Australian acacias are an important model genus in invasion ecology (Richardson *et al.*, 2011), and possess many of the aforementioned features that are of interest to invasion biologists, molecular ecologists and invasive species managers, viz.

- About a third of the Australian acacia group (1012 recognised species in subgenus *Phyllodineae*) have been introduced to countries outside of Australia (Richardson *et al.*, 2011).
- The exports of acacias have been well documented, providing records of success or failure in post introduction establishment. Success or failure can be related to

particular introduction histories, life history traits, genetic and/or ecosystem characteristics (of both the source and receiving ecosystem).

- Several Australian acacias are successful invaders, especially in Mediterranean-type regions of the world where they displace native biodiversity and considerably alter ecosystem structure and function (Macdonald & Jarman, 1984; Richardson & Rejmánek, 2011).
- Microsatellite markers have been developed for acacias (*A. mangium*, *A. saligna*, Butcher *et al.*, 2000; Millar & Byrne, 2007) and their relatives (*Paraserianthes lophantha*; Brown *et al.*, 2011) and may be transferable to other species within the genus. Microsatellite markers enable fine-scale genetic processes to be quantified and compared at a range of spatial scales. This provides opportunities to compare the native and introduced genetic differences of introduced acacias to their introduction dynamics, invasive intra-specific diversity and population genetic structure.

Australian acacias in South Africa represent an excellent system to study many concepts pertinent to invasion biologists, and were selected as the focus of this thesis. There are fourteen invasive acacias in South Africa that have considerable negative effects on native biodiversity (Le Maitre *et al.*, 2011; van Wilgen *et al.*, 2011; Wilson *et al.*, 2011). These fourteen species have been present in South Africa for periods ranging between 107 years (*A. elata*, van Wilgen *et al.*, 2011) and 184 years (*A. longifolia*). In addition, the manner, rate and mode in which they were introduced varies (see Roux, 1961; Shaughnessy, 1980; Poynton, 2009; Le Roux *et al.*, 2011; van Wilgen *et al.*, 2011) and influences the size of their invasive ranges.

In order to select an Australian acacia for a population genetic study, information on the introduction history and anthropogenic use of invasive acacias in South Africa was collated (Table 1.1). Table 1.1 lists the fourteen major invaders, their purpose and date of introduction (Poynton, 2009), the size of their invasive ranges in quarter-degree grid cells (QDGCs, Henderson, 2001;

Wilson *et al.*, 2007) and whether they were introduced on multiple or single occasions (Poynton, 2009). To obtain a global view of the same fourteen acacias, records from the Global Biodiversity Information Facility (GBIF, 2010, <http://www.gbif.org>) were collated and their distributions mapped globally (Fig. 1.1a). The same approach was used for South Africa (Fig. 1.1b). The global distributions show that the Australian acacias that occur in South Africa also occur in other Mediterranean-type regions around the world, although they are not necessarily invasive (Fig. 1.1a). This provides the opportunity to compare patterns in genetic diversity in a number of regions simultaneously to identify commonalities or differences that may be associated with their invasive success (or lack thereof).

From the 14 invasive acacias in South Africa, *Acacia saligna* and *Paraserianthes lophantha* were selected as study species because:

- They are invasive in South Africa and have substantial negative impacts on the environment; the South African government consequently invests large sums of money in their control (van Wilgen *et al.*, 2012).
- They occur in regions outside of South Africa as introduced or invasive species providing the opportunity to test genetic patterns in more than one introduced region.
- They are intra-specifically diverse and biogeographically structured in their native range providing the opportunity to test the influence that strong genetic structure, or intra-specific variation has on invasiveness.
- They were introduced to South Africa on more than one occasion (i.e. multiple introductions), although records indicate that *A. saligna* was introduced in much higher volumes (high propagule pressure, Poynton, 2009) on at least double the number of occasions as *P. lophantha* (i.e. low propagule pressure). This allows the introduced genetic signature to be compared to propagule pressure.
- Molecular markers (microsatellites) have been developed for both species and additional microsatellites developed for *A. mangium* may be transferrable to both species.

- Prior to this study, there were no known assessments of the introduced genetic diversity of the two study species in the main study region (South Africa) or, to my knowledge, in any other introduced populations.
- They are native to the same biogeographical areas in Western Australia, but their usage by humans and introduction histories differ.

## **1.2 Motivation, aims and thesis structure**

South Africa is home to one of the world's most biodiverse regions, the Cape Floristic Region (CFR). This biodiversity hotspot encompasses the greatest non-tropical concentration of higher plant species in the world (Goldblatt & Manning, 2002). Climate change, urbanization and invasive species pose serious threats to the native biodiversity and natural water resources contained within the CFR (Rouget *et al.*, 2003). Invasive Australian acacias are the dominant woody invaders over much of the CFR (Macdonald & Jarman, 1984) as they are widely distributed (Henderson, 2001) and planted for their economic and aesthetic value (Carruthers *et al.*, 2011; Griffin *et al.*, 2011). The mode, timing, site and rate at which acacias were introduced to South Africa varies (Poynton, 2009), as do their biological characteristics and intraspecific diversity (see Le Roux *et al.*, 2011). Little is known about the population genetic structure, intraspecific diversity and ecological niche preferences of invasive acacias in South Africa (see the special issue [Volume 17(3)] of *Diversity and Distributions* on “Human-mediated introductions of Australian acacias – a global experiment in biogeography”). This provides the opportunity to test the effect of particular genetic or climatic characteristics on the invasive success of a number of closely related species (broadly) under the same environmental conditions.

In this thesis I explore the introduction dynamics, invasive intra-specific diversity, population genetic structure and ecological niche preferences of two invasive Australian legumes (*A. saligna* and *P. lophantha*) in South Africa and other Mediterranean-type climates. Specifically, I aim to test mechanisms and processes such as hybridization, multiple introductions (propagule pressure) and increased genetic diversity as stimuli for invasive success. The results are placed in the context of national management strategies.

This thesis has been structured into 6 chapters, with the following aims:

- Chapter 1 is the general introduction and covered relevant background literature.

- Chapter 2 entitled “Predicting the subspecific identity of invasive species using distribution models: *Acacia saligna* as an example”, was published in *Diversity and Distributions* (Thompson *et al.*, 2011). I tested the ability of correlative species distribution models to predict the distributions of the different subspecies of *A. saligna*.
- Chapter 3 entitled “Cultivation shapes genetic novelty in a globally important invader”, was published in *Molecular Ecology* (Thompson *et al.*, 2012). I assessed the population genetic and phylogeographic structure of native (Western Australia) and introduced populations (South Africa) of *A. saligna*.
- Chapter 4 entitled “A tree well-travelled: Global phylogeography of the invasive *Acacia saligna*” has been submitted to the *Journal of Biogeography* for review. Here I built on the findings of Chapter 3, and determined the native provenance(s), subspecies identity, and spatial patterns of genetic diversity within and among global populations of *A. saligna*.
- Chapter 5 is entitled “Microsatellite markers trace the introduction history of the invasive legume, *Paraserianthes lophantha*”. I determined the native provenance(s) and spatial patterns of genetic diversity within and among globally introduced populations of *P. lophantha* using nuclear microsatellites and one nuclear gene region. This chapter built on the findings of a study I conducted in conjunction with my supervisors during the course of the PhD (see Le Roux *et al.*, 2011).
- Chapter 6 summarises the findings of the thesis and provides general conclusions.

For the relative contributions of myself, my supervisors and co-authors to all aspects of this PhD thesis, please see the “*Author contributions*” section at the end of each published chapter.

### 1.3 Study species

#### (a) *Acacia saligna* (Labill.) H. L. Wendl

<i>Synonyms:</i>	<i>Acacia cyanophylla</i> Lindley, <i>Racosperma saligna</i> (Labill.) Pedley, <i>A. falcata</i> (Roux, 1961), <i>A. foliata</i> (Roux, 1961).
<i>Common names</i>	Port Jackson Willow, Golden Wreath Wattle, Orange Wattle, Blue-leafed Wattle, Western Australian Golden Wattle (ILDIS, 2011).
<i>Native range</i>	Southern regions of Western Australia (Fig. 1.2, grey circles).
<i>Introduced range</i>	Chile, Cyprus, Ethiopia, Israel, Italy, Kenya, Morocco, New Zealand, Portugal, South Africa and Spain (Maslin & McDonald, 2004; Richardson & Rejmánek, 2011; Wilson <i>et al.</i> , 2011, Fig. 1.2, grey squares).
<i>Human use</i>	Dune reclamation, shelter, leather tanning, livestock fodder, firewood (Henderson, 2001; Maslin & McDonald, 2004; Orwa, 2009).
<i>Control methods</i>	Biological control by a rust fungus and seed feeding insects (Morris, 1991, 1997, Old <i>et al.</i> , 2002; Wood & Morris., 2007; Impson <i>et al.</i> , 2011); mechanical extraction, herbicide application (Henderson, 2001), seed bank control via soil solarisation (Cohen <i>et al.</i> , 2008).

#### *Species description*

Bushy shrub or tree, ranging from 3–7 m in height (George *et al.*, 2006). Bark is grey, branchlets pendulous and glabrous. Phyllodes vary in shape, size and colour (Maslin & McDonald, 2004) but possess a prominent midrib. A gland is present 0–3 mm above pulvinus, and is 1–2 mm wide and coarsely wrinkled. The inflorescences are mostly 2–10-headed racemes of globular flowers. The seed pods are linear, flat, shallowly constricted between seeds, 8–12 cm long, 4–6 mm wide, dark brown to black. The *A. saligna* species complex currently contains four informal subspecies that have been characterized based on their morphology (worldwidewattle.com, George *et al.*, 2006; Millar *et al.*, 2008b). This has been recently revised based on microsatellite markers to 3 major genetic groups or subspecies (Millar *et al.*, 2011).

### *Invasive characteristics*

*Acacia saligna* is a hardy, drought-resistant species that survives in a wide range of environments, and grows on all substrates where sufficient water is available (Milton & Hall, 1981). It is well adapted to dominate disturbed areas, such as road sides, but is a poor invader of undisturbed areas (Poynton, 2009). In South Africa it invades coastal sand dunes, woodlands and the fynbos (Macdonald & Jarman, 1984; Henderson, 2001; Yelenik *et al.*, 2004). It is a “category 1” invader in South Africa (invasive species requiring a compulsory control, removal and destruction approach, Nel *et al.*, 2004), and outcompetes native species (Witkowski, 1991). *Acacia saligna*'s ability to readily fix nitrogen through symbiosis with root nodulating bacteria and mycorrhizal fungi (Richardson *et al.*, 2000) may contribute to its invasive success; especially in environments with nutrient-poor soils characteristic of Mediterranean-type climates (Kruger *et al.*, 1989). Reproductive characteristics of invasive importance include its ability to reproduce vegetatively through root suckering and coppicing, its production of voluminous seed banks (Milton & Hall, 1981; Strydom *et al.*, 2012) and highly outcrossing mode of pollen dispersal (Millar *et al.*, 2008a). Anthropogenic uses that have facilitated its introduction include its wide use as an ornamental or forestry species (Maslin & McDonald, 2004), or as a fodder for livestock (Midgley & Turnbull, 2003). Long-distance dispersal of the species occurs via sand used in road construction and agriculture. Local seed dispersal agents are predominantly by ants and baboons (Stirton, 1978), while pollination is predominantly by bees in South Africa (Gibson *et al.*, 2011).

**(b) *Paraserianthes lophantha* (Willd.) I.C. Nielsen**

<i>Synonyms</i>	<i>Acacia lophantha</i> Willd., <i>Albizia distachya</i> (Vent.) J.F. Macbr., <i>Albizia lophantha</i> (Willd.) Benth. <i>Feuillea distachya</i> (Vent.), <i>Mimosa distachya</i> Vent., <i>Mimosa lophantha</i> (Willd.) Pers.
<i>Common names</i>	Brush Wattle, Cape Leeuwin Wattle, Stinkbean
<i>Native range</i>	<i>P. lophantha</i> sub-species <i>lophantha</i> is native to south Western Australia; <i>P. lophantha</i> sub-species <i>montana</i> is native to Indonesia (Fig. 1.3, grey circles).
<i>Introduced range</i>	Temperate Australia (New South Wales, South Australia, Victoria), Hawaii, New Zealand, South Africa ( <a href="http://www.hear.org">www.hear.org</a> ), southern California (Stirton, 1978), the Canary Islands and Chile (Randall, 2002), see Fig. 1.3, grey squares.
<i>Human use</i>	Ornamental and for land rehabilitation (Henderson, 2001)
<i>Control methods</i>	Biocontrol by seed feeding insects (Henderson, 2001; Impson <i>et al.</i> , 2011), rust fungus (Old <i>et al.</i> , 2002)

***Species description***

Evergreen shrub or tree, 4–15 metres in height, possessing dark green, hairy branchlets, leaf-rachi and peduncles. The leaf pinnae occur in pairs of 8-10, with 20-40 pairs of oblong leaflets 6-8 mm long, about 2 mm wide ([www.hear.org](http://www.hear.org)). The petioles possess a gland near the base. The flowers are auxiliary spikes, shortly peduncled, 3-10 cm long and more than 3 cm thick ([www.hear.org](http://www.hear.org)), flowering from June to August. The brown seed pods are 6-10 cm long, 12-18 mm broad, and compressed between seeds, creating a corrugated affect (Stirton, 1987). The seeds produce a distinct odour when damaged, giving it its common name in South Africa- the stink bean (Henderson, 2001).

***Invasive characteristics***

*Paraserianthes lophantha* occurs predominantly as localised, small stands in South Africa (pers. obs.). It was introduced to South Africa for ornamental and agricultural purposes (Henderson, 2001). It invades forest margins, riverbanks and the wetter areas of the Fynbos (Stirton, 1987). Like *A. saligna*, *P. lophantha* also forms symbiotic relationships with rhizobia for nitrogen fixation and accumulates very large seed banks in the soil (Henderson, 2001). The use of *P. lophantha* as an ornamental plant appears to be the chief reason for its introduction to South Africa (Henderson, 2001). Long-distance dispersal occurs by human transport, while local dispersal agents are mainly birds (Stirton, 1987). *Paraserianthes lophantha* appears to be less aggressive than *A. saligna* as an invader, the reasons for which are currently unclear.

## 1.4 Tables and Figures

**Table 1.1** Fourteen major invasive *Acacia* species occurring in South Africa. Details of the introduction histories are given as the purpose and year of introduction, as well as their invasive range size in South Africa.

Species <sup>φ</sup>	Reason for introduction	Date	Multiple introductions †	Invasive range size*
<i>Acacia baileyana</i>	ornamental	1919	yes	87
<i>Acacia cyclops</i>	dune stabilisation	1835	yes	167
<i>Acacia dealbata</i>	silviculture	1858	yes	256
<i>Acacia decurrens</i>	silviculture	1880	yes	101
<i>Acacia elata</i>	ornamental	1904	yes	38
<i>Acacia implexa</i>	unknown	c. 1880	unknown	3
<i>Acacia longifolia</i>	dune stabilisation	1827	yes	95
<i>Acacia mearnsii</i>	silviculture	1858	yes	432
<i>Acacia melanoxylon</i>	silviculture	1848	yes	138
<i>Acacia paradoxa</i>	unknown	c. 1850	unknown	1
<i>Acacia podalyriifolia</i>	ornamental	1894	yes	56
<i>Acacia pycnantha</i>	dune stabilisation, tanbark	1865	yes	35
<i>Acacia saligna</i>	dune stabilisation, tanbark	1833	yes	160
<i>Acacia stricta</i>	unknown	?	unknown	2

\* Invasive range size is a crude estimate, and is based on the number of quarter-degree grid cells occupied by each species (Henderson *et al.*, 2001; Wilson *et al.*, 2007). One quarter-degree grid cell is equal to approximately 25 km<sup>2</sup>. <sup>φ</sup> van Wilgen *et al.*, 2011; † Poynton, 2009.

## Figure Legends

**Figure 1.1** Global distribution of fourteen *Acacia* species classified as major invaders in South Africa (van Wilgen *et al.*, 2011) based on records from the Global Biodiversity Information Facility (GBIF, 2010, <http://www.gbif.org>). Their distributions are represented (a) at a global scale in their native (green circles) and introduced ranges (red circles), and (b) in their introduced range in South Africa. Occurrences for *A. saligna* in South Africa are represented by red crosses.

**Figure 1.2** Global distribution of *Acacia saligna* based on native records from the Australian Virtual Herbarium and introduced records from the Global Biodiversity Information Facility (GBIF, 2010, <http://www.gbif.org>) and the South African Plant Invaders Atlas (SAPIA).

**Figure 1.3** *Acacia saligna* in flower during September in South Africa (a), morphology and the species' native and naturalised geographical distribution in Australia (b, [worldwidewattle.com](http://worldwidewattle.com)).

**Figure 1.4** Global distribution of *Paraserianthes lophantha* based on native records from the Australian Virtual Herbarium and introduced records from the Global Biodiversity Information Facility (GBIF, 2010, <http://www.gbif.org>) and the South African Plant Invaders Atlas (SAPIA).

**Figure 1.5** *Paraserianthes lophantha* along a roadside in South Africa (a, b), floral morphology (c).

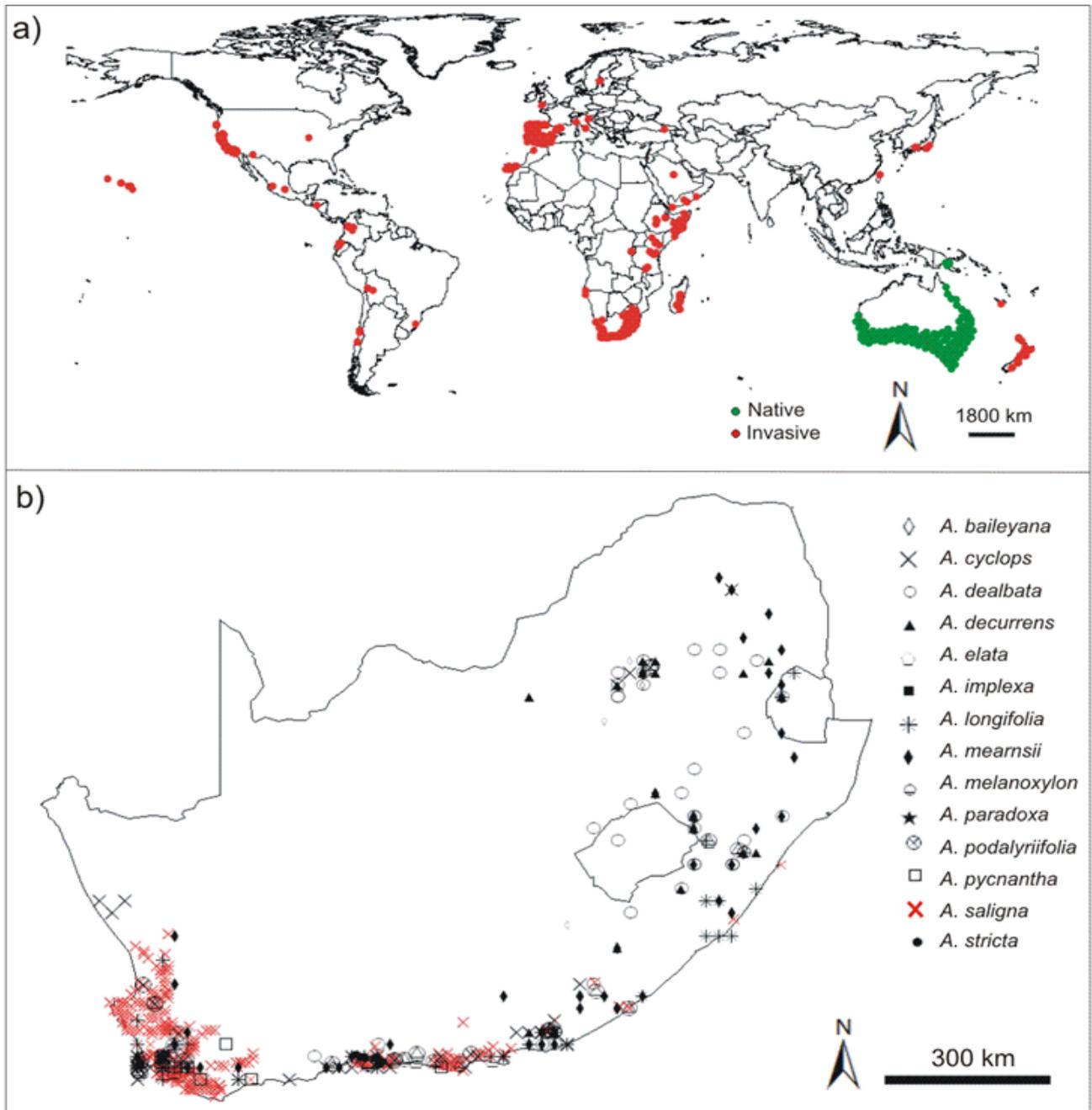


Figure 1.1



Figure 1.2

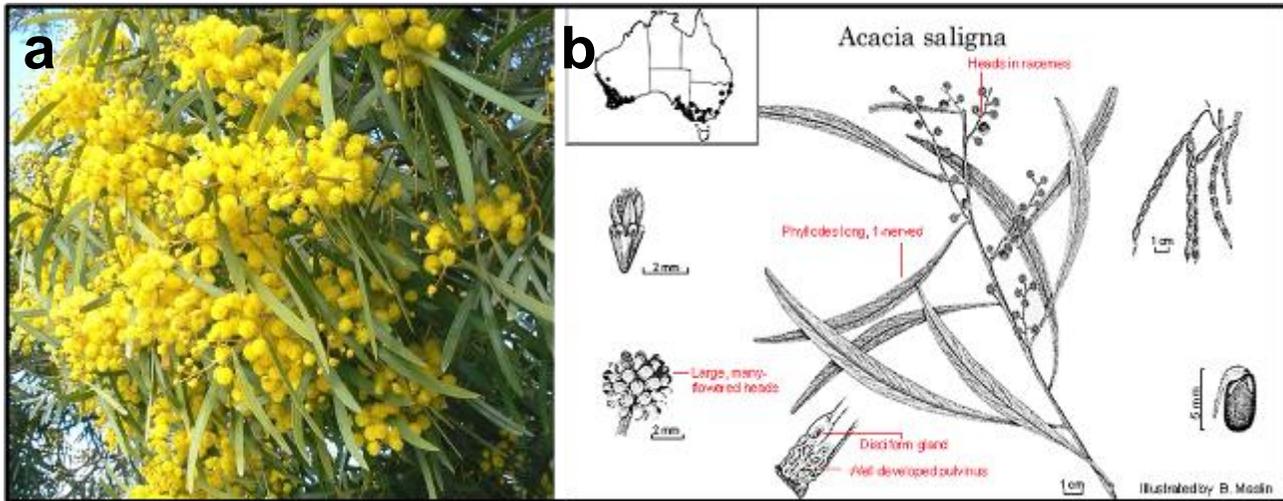


Figure 1.3



Figure 1.4



Figure 1.5

## CHAPTER 2 Predicting the subspecific identity of invasive species using distribution models: *Acacia saligna* as an example

- This chapter has been published, citation: Thompson, G.D., Robertson, M.P., Webber, B.L., Richardson, D.M., Le Roux, J.J. & Wilson, J.R.U. (2011) Predicting the subspecific identity of invasive species using distribution models: *Acacia saligna* as an example. *Diversity and Distributions*, 17, 1001-1014.

### Abstract

We aimed to explore whether the subspecific genetic entities of *Acacia saligna* occupy different bioclimatic niches in their native and introduced ranges, and whether these niches are predictable using species distribution models (SDMs). SDMs were developed in MAXENT using six climatic variables to calculate the climatic suitability of the ranges of *A. saligna* in Australia, South Africa and the Mediterranean Basin. We assessed: 1) the subspecific niche differences identified by SDMs using measures of niche overlap and model performance; 2) the ability of SDMs to predict the most likely subspecific genetic entities present in South Africa based on comparisons to genetic data; and 3) the ability of SDMs to predict the most likely subspecific genetic entities present in the Mediterranean Basin. All model projections were assessed for sensitivity and modelled prevalence as indicators of model fit or predictability. The SDMs identified different subspecific bioclimatic niches in the native range. Sensitivity and modelled prevalence show that none of the models correctly predicted the full range of *A. saligna* in South Africa or the Mediterranean Basin. Models also show that the South African niche is different to that in the native range. We concluded that the subspecies of *A. saligna* occupy quantifiably distinct bioclimatic niches in their native ranges, implying that they should occupy distinct niches in their invasive ranges. However, projections to the introduced range did not correspond with known occurrences. Our SDMs are unable to predict the full introduced niche of *A. saligna* at a species or subspecies level in either South Africa or the Mediterranean Basin. Range

limits in the native and introduced ranges may be determined by additional factors not used in the SDMs developed in this study.

## **2.1 Introduction**

Human activities are changing the geographical ranges of species in many ways at faster rates and at broader scales than ever before (Vitousek *et al.*, 1997; Walther *et al.*, 2009). Many types of changes to the environment caused by anthropogenic factors affect the capacity of organisms to persist at a given locality. Such changes, together with a re-shuffling of associated biotic interactions, have radically altered the distribution of species worldwide. Rapidly growing human populations with increasing mobility, diversified needs and technological advances have created new pathways for the movement of species to areas far removed from their native ranges (Wilson *et al.*, 2009). A proportion of introduced species become invasive (i.e. spread from introduction sites), in some cases displacing native species, altering ecosystem functioning, and causing environmental and economic damage (Pyšek & Richardson, 2010).

Species' distributions are constrained by biotic and abiotic factors that define the space or the 'niche' that a species can occupy (Elith & Leathwick, 2009; Alexander & Edwards, 2010). Species distribution models (SDMs), also termed bioclimatic models and ecological niche models, are used to understand the distribution of species (Elith & Leathwick, 2009). They attempt to incorporate a number of meaningful biological and environmental factors that influence a species' range. For invasive plant species, there is often a marked similarity between the climate in the native range and other areas where the species is most invasive (Thuiller *et al.*, 2005). Consequently, climate matching between the native and introduced range of a species using SDMs is widely used to forecast future invasion risks (Tucker & Richardson, 1995; Peterson *et al.*, 2003; Mau-Crimmins *et al.*, 2006; Richardson & Thuiller, 2007; Gordon *et al.*, 2010).

Most SDMs assume the subject taxon (usually a species) comprises a uniform entity; that is, that the subject taxon has similar environmental adaptations throughout its range. This is rarely the case. SDMs also assume that a species' niche is conserved between the native and introduced range (niche conservatism *sensu* Peterson *et al.*, 1999). However, realised niches (the niche actually occupied by a species) are unlikely to be the same once an invader is released from its natural enemies and competitors in the introduced range (Lee, 2002), or if genetic drift occurs. Genetic drift may result in the introduced species being represented by only a small part of the total genetic diversity present in the native range (i.e. a genetic bottleneck; Mooney & Cleland, 2001).

The amount and structure of genetic diversity in the introduced range will likely affect a species' ability to withstand competition or environmental pressures in its new range. Any positive effects, such as faster growth rates or resistance to herbivory, may allow an introduced species to expand its range or move beyond its native realised niche. Whether the differences between a species' native and introduced genetic structure will consistently enable an invader to alter its niche (lack of niche conservatism; see Peterson *et al.*, 1999; Wiens & Graham, 2005) is currently unknown (Rödder & Lötters, 2009).

Previous research has shown that the genetic structure of a species interacts with aspects of the introduction history to determine the genetic diversity and structure in the introduced range (e.g. Le Roux *et al.*, 2011). Several studies have reported admixture (mating between two genetically distinct groups) resulting in highly invasive novel genotypes as a consequence of multiple introductions from a highly structured and diverse native range (Gaskin & Schall, 2002; Genton *et al.*, 2005; Lavergne & Molofsky, 2007; Facon *et al.*, 2008; Prentis *et al.*, 2008).

The capacity of SDMs to accurately project potential distributions may be substantially affected by a number of parameters including: whether subspecific entities have adapted to different climatic niches; if the processes defining niches differ in the introduced and native ranges, or if an invader undergoes substantial genetic change upon introduction. Given the significant implications for

obtaining meaningful output from SDMs, it is surprising that very few studies have tested or incorporated known subspecific information into SDMs, even for conservation-focused models of native species (see Scoble & Lowe, 2010). A small number of studies have used molecular data and SDMs to: delimit the range of a number of closely related species (Leaché *et al.*, 2009); assess changes in spatial genetic structure of a tree species with climate change (Sork *et al.*, 2010); assess the change in niche occupancy of sister species with change in climate (Pearman *et al.*, 2010); assess the biogeographic history of two congeneric species (Jakob *et al.*, 2007) and possible speciation mechanisms (Graham *et al.*, 2004; Peterson & Nyári, 2007). However, to our knowledge SDMs have not been developed at a subspecies level for an invasive alien plant.

Australian acacias (species in *Acacia* subgenus *Phyllodineae* native to Australia; Miller *et al.*, 2011; Richardson *et al.*, 2011) are an excellent system for exploring these ideas as many invasive acacias have geographically structured intra-specific variation in their native range and have different introduction histories (see Le Roux *et al.*, 2011). We test the potential for using subspecific information on the *Acacia saligna* (Labill.) H. L. Wendl. species complex. *Acacia saligna* is native to Western Australia and has been widely introduced around the world, becoming an aggressive invader in many regions (Henderson, 2001; Nel *et al.*, 2004; Richardson & Rejmánek, 2011). It has been well studied from both an ecological and molecular perspective in its native (Marsudi *et al.*, 1999; George *et al.*, 2006; Maslin *et al.*, 2004; Millar *et al.*, 2008; Millar *et al.*, 2011) and introduced range (Milton & Hall, 1981; Witkowski, 1991; Holmes & Cowling, 1997; Yelenik *et al.*, 2004; Wood & Morris, 2007, Le Roux *et al.*, 2011), providing a substantial source of verifiable distribution records from which a SDM can be built.

Previous genetic research has shown that a number of subspecific entities of *A. saligna* exist in Western Australia (George *et al.*, 2006; Millar *et al.*, 2008), but their “morphological taxonomic classification is problematic” (Millar *et al.*, 2011) making field identification challenging. Millar *et al.* (2008) identified four genetic lineages or subspecies, consistent with the morphological groupings of

the species complex (Maslin *et al.*, 2006), each geographically associated with a particular ecological habitat: subspecies *lindleyi* (water courses, sand dunes, coastal plains), subspecies *pruinescens* (deep soil in swamp-like areas), subspecies *saligna* (coastal plains) and subspecies *stolonifera* (watercourses and forest-like areas). More recently, following extensive population genetic characterization in the native range, the *A. saligna* species complex has been revised to comprise only three lineages. Millar *et al.* (2011) identified these three groups as: 1) subspecies *lindleyi*, 2) subspecies *stolonifera*, 3) subspecies *saligna* and *pruinescens*. For simplicity throughout this manuscript, we use the term subspecies to refer both to the original four taxa based on morphological traits (Maslin *et al.*, 2006) and the more recent three derived from molecular research (Millar *et al.*, 2011), but recognise that neither scheme has been formalised.

*Acacia saligna* was introduced to South Africa on at least five separate occasions between 1845 and 1922, with over 200 million seeds introduced during this period (Poynton, 2009). A comparative phylogeographic study of native and introduced *A. saligna* populations showed that only a very small proportion of *A. saligna*'s native genetic diversity is present in South Africa (Le Roux *et al.*, 2011). This is despite the fact that introductions into South Africa were from multiple sources, including the native range in Australia, France, and other unknown sources (Poynton, 2009). The species has a long residence time (*ca.* 170 years) in South Africa and has been very widely dispersed. It has likely reached its bioclimatic limits at the broad scale in the region (Rouget *et al.*, 2004).

This study draws on available ecological and genetic research on the *A. saligna* species complex to: 1) assess whether the different subspecies occupy areas in their native range that can be distinguished by correlative SDMs; 2) explore the predictive ability of subspecific SDMs for the introduced range in South Africa considering known occurrences and current genetic data (Le Roux *et al.*, 2011); and 3) use SDMs to predict which subspecies are present in other biogeographical regions where *A. saligna* has been introduced (*i.e.* in the Mediterranean Basin).

## 2.2 *Methods*

### (a) *Modelling approach*

Our approach incorporated the most recent recommendations and approaches in the literature associated with correlative modelling of introduced species (see Webber et al., 2011), with each modelling approach tailored to the ecological questions being asked. Two data source regions were used to calibrate models representing the native range (Western Australia, Fig. 2.1a) and an introduced range (South Africa, Fig. 2.1b) of *A. saligna*. While native and introduced records are often combined in the same model to improve projections of the potential invasive range (e.g. Broennimann & Guisan, 2008), this would obscure any pattern attributable to the species' subspecific bioclimatic distribution and we therefore chose not to use this approach.

To determine whether SDMs can detect subspecific niche differences (aim one) we first built models using all native records to test the predictability of the full native niche of the *A. saligna* species complex. Second, we built models using native records per subspecies to test the predictability of the niche for each subspecies. In both cases models were projected to the model training domain in the south-western part of Western Australia. We then used several methods to compare the identified climatic niches occupied by the subspecies.

To explore the predictive power of SDMs in the introduced range in South Africa, relative to known occurrences and genetic data (aim two), we used several approaches. First, models were built using all native records and projected to South Africa, to assess the niche that the entire *A. saligna* species complex would occupy in the introduced range. Second, models were built using records per subspecies and projected to South Africa to assess subspecific niche differences in the introduced range. Third, models were built using various combinations of subspecies records and projected to South Africa. These combinations were selected to incorporate the most recent molecular groupings within the species complex (Millar et al., 2011), and molecular evidence (Le Roux et al., 2011) suggesting only a small proportion of the native genetic diversity is present in South Africa. The

combinations tested were: (*lindleyi* + *stolonifera*; *pruinescens* + *saligna*; *lindleyi* + *pruinescens* + *saligna*; *pruinescens* + *saligna* + *stolonifera*). Fourth, models were built using all introduced South African records and projected to the whole of South Africa. Fifth, models were built using records from the introduced range in South Africa and projected back to the native range in Western Australia. The fifth component compares the native and introduced niche within the same environmental space, allowing for any changes in the occupied range between countries to be assessed.

To determine whether SDMs can predict subspecies present in other biogeographical regions where *A. saligna* has been introduced (aim three) we followed three approaches. Each approach projected to an area with a Mediterranean-type climate similar to that in the south-western parts of Western Australia. For this aim, projections to the Mediterranean Basin enabled us to further explore the practicality of predicting subspecific identities of introduced *A. saligna* populations. First, models were built using all native records to assess the niche occupied by the entire *A. saligna* species complex. Second, models were built using native records per subspecies to assess subspecific niche differences. Third, models were built using South African *A. saligna* records to assess whether the invasive type present in South Africa is conserved in the Mediterranean Basin.

### *(b) Distribution records*

Native distribution records for each subspecies of *A. saligna* in Western Australia were obtained from herbarium records from Australia's Virtual Herbarium online database (<http://avh.rbg.vic.gov.au>, accessed 1 October, 2010). We only considered records that were assigned morphologically to one of the four subspecies groups by the taxonomic authority on *A. saligna* (Bruce Maslin, Department of Environment and Conservation, Western Australia). Records that appeared to be outliers (i.e. located on the periphery of the known distributions of each subspecies) were verified by Bruce Maslin using the original specimen sheets. To ensure that presence records only reflected the natural climate suitability at a site, we omitted records that (i) were identified as cultivated or growing in managed environments, (ii) occurred in microclimates not detectable at a 5' grid scale (e.g.

along rivers in arid areas), or (iii) had locality information at a resolution coarser than 5'. After quality control, and restricting records to one per 5' grid cell per subspecies, for each subspecies (i.e. regularisation to minimise sampling bias), a total of 442 occurrence records were used: 249 records for subspecies *lindleyi*, 44 records for subspecies *pruinescens*, 108 records for subspecies *saligna*, and 41 records for subspecies *stolonifera* (Fig 1.1a).

Distribution records for the introduced range in South Africa were compiled from the South African Plant Invaders Atlas (SAPIA; Richardson *et al.*, 2005; Henderson *et al.*, 2007), as well as field observations and collections by the authors. These records were collected at a spatial precision of at least 5', and subjected to the same quality control methods as the native range records. A total of 210 regularised occurrence records were used (Fig. 1.1b). These records contain no information on subspecific identity.

Distribution records for the introduced range in eastern Australia and the Mediterranean Basin were sourced from Australia's Virtual Herbarium online database and the Global Biodiversity Information Facility (GBIF, 2010), respectively. These records were subjected to the same degree of scrutiny as those records used to build the models. Only occurrences collected at a spatial precision of a 5' grid cell were used. A total of 24 regularised occurrence records were used. These records contain no information on subspecific identity.

### (c) *Bioclimatic variables*

We wanted to build the models using bioclimatic variables that represent ecologically relevant climatic factors for *Acacia* distributions in Mediterranean-type environments (Maslin *et al.*, 2006; Droppelmann & Berliner, 2000; Degen *et al.*, 1995; Witkowski, 1991; Jeffery *et al.*, 1988). During variable selection we placed a priority on choosing a set of variables that minimise multi-collinearity between variables. Multi-collinearity was assessed using a Pearson correlation coefficient analysis (see supporting information, Table S1) using ENMTools version 1.0 (Warren *et al.*, 2010). We

downloaded global gridded bioclimatic data at 5' resolution from the WorldClim database (<http://www.worldclim.org>; Hijmans *et al.*, 2005) for the six selected bioclimatic ('BioClim') variables: temperature seasonality (Bio4), mean temperature of the hottest quarter (Bio10), mean temperature of the coldest quarter (Bio11), precipitation seasonality (Bio15), precipitation of the hottest quarter (Bio18), and precipitation of the coldest quarter (Bio19).

#### (d) *Species distribution modelling*

MAXENT version 3.3.3e (Phillips *et al.*, 2006) was used throughout as it is a widely used and accepted SDM method that can produce robust results (Elith *et al.*, 2006; Phillips & Dudík, 2008; Elith *et al.*, 2011). The software builds a model using environmental layers, occurrence records (presence points) and a geographically defined background area for taking pseudo-absence points for a particular species, to define a set of constraints under which that species is likely to persist. We applied default parameters: "logistic output", "create response curves", "jackknife measures of variable importance", "do clamping", and a regularization value of 1. We restricted the feature type to "hinge features"; selecting only hinge features means that the MAXENT model produces smoother response curves where the models are more focussed on the "strongest trends" in the data (Elith *et al.*, 2010). This approach is recommended for introduced species and produces models that are likely to be more ecologically realistic (e.g. Elith *et al.*, 2010).

MAXENT uses pseudo-absence data drawn randomly from a geographically defined background in lieu of actual absence records to define environmental conditions for where the species has not been recorded. The background from which pseudo-absences are drawn can however, significantly influence the model results (Phillips *et al.*, 2009, VanDerWal *et al.*, 2009), and so it is recommended that the background be restricted to the region in which the species would reasonably be expected to occur (Elith *et al.*, 2011). Moreover, it is necessary to achieve a balance between a background that gives good regional performance driven by relevant climate variables, and one that can perform reasonably at a continental scale by not being overly constrained by a reduced set of

variables largely unrelated to the species in question (Elith *et al.*, 2010; Webber *et al.*, 2011). Following the methods of Webber *et al.* (2011), we used the Köppen-Geiger climate classification (or vegetation classes) to define our model backgrounds. Köppen-Geiger classifications, following the rules defined in Kriticos *et al.* (2011), were applied to the 5' resolution WorldClim global climatology, which is the same source for the BioClim variables used in the models. Ten thousand pseudo-absences (Phillips & Dudík, 2008) were then drawn from an area defined by the Köppen-Geiger polygons within which one or more distribution records were located. For models based on native range subspecies records, we used a single background that corresponded to the combined distribution records of *A. saligna*. For all other models we used the rules outlined above to define a background based on the distribution records used in that model.

Model projections to new areas, particularly to other continents, are likely to include regions where the model is extrapolating beyond the climate space encompassed by the training domain (i.e. the background). Thus it is imperative that novel areas should be identified in projections so that the model output in these regions can be carefully interpreted against the response curves and assessed for plausibility (Elith *et al.*, 2010). Projections to novel climates (model extrapolation) were assessed using multivariate environmental similarity surface (MESS) maps (Elith *et al.*, 2010). MESS maps provide an indication of the similarity of the bioclimatic data in the projected region compared to the training region. Areas of dissimilar bioclimatic data (novel environments) are given negative values (MESS-, extrapolation), while areas of similar bioclimatic data are given positive values (MESS+, interpolation). MESS- areas were carefully interpreted by visually inspecting the response curves (Fig. S2.2) and limiting bioclimatic variables (Fig. S2.3) to provide an indication of the variables driving the models in different regions. We used the minimum training presence, or lowest presence threshold (LPT; Pearson *et al.*, 2007) to define climatically suitable areas. The LPT is the lowest generated suitability value from model projections that intersects with a distribution record, and therefore represents a non-arbitrary threshold particularly suited for modelling invasive species (Liu *et al.*, 2005;

Webber *et al.*, 2011). Colour raster displays were separated into 20 classes ranging from the LPT value (green) to moderately suitable (yellow, probability value of 0.5) and highly suitable (red, probability value of 1). All values below the LPT were designated unsuitable (white).

*(e) Niche differences*

To test whether the climatic niches derived from the four subspecies differed, we conducted niche similarity tests using ENMTools version 1.0 (Warren *et al.*, 2010) in the native range of *A. saligna*. ENMTools calculates Schoener's D index (1968) and the Hellinger-based similarity statistic (I) (van der Vaart, 1998) for each grid cell of the model projection. This approach, suggested by Warren *et al.* (2008), provides an ecologically meaningful measure (D) that is combined with a statistically robust measure (I). In ENMTools both measures range from 0 (no overlap) to 1 (complete overlap).

*(f) Model assessment*

Correlative model fits were calculated by assessing the ability of the model to correctly assign presence or absence (more often expected than at random) in relation to an actual presence or pseudo-absence. We used the LPT to define "presence" or "absence" and based calculations on 5' grid cells using regularised distribution record data. We calculated: 1) model sensitivity, which is the proportion of correctly predicted observed presences (omission errors), and its statistical significance using an exact one-tailed binomial test (following the methods of Anderson *et al.*, 2002); 2) modelled prevalence, which is the proportion of the complete projection region estimated to be climatically suitable (for more details see Webber *et al.*, 2011). In all cases, we assessed modelled prevalence in relation to model sensitivity, novel regions, and extrapolation within these regions by examining the response of the bioclimatic variables in the model (response curves, Fig. S2.2).

Models that display high sensitivity (as close to 1 as possible), and are statistically significant (according to the exact one-tailed binomial test), are important for invasive species as they are at least able to correctly project occurrences in the introduced range. Models that display low sensitivity

suggest models that are unable to project climatic suitability in regions where there are known occurrences. Further, models that display statistically non-significant outputs do not warrant further investigations as projections are unlikely to be robust. We did not use a commonly applied method (Area Under Curve values of the Receiver Operating Characteristic) to measure model performance because its usefulness for model interpretation is questionable (Lobo *et al.*, 2008), particularly when assessing models developed for invasive species (Webber *et al.*, 2011).

## 2.3 Results

### (a) Models projected to the native range

Niche similarity tests (Schroener's D index and Hellinger based distance) based on the projected climatic suitability for each subspecies indicated that the subspecies occupy different bioclimatic niches in their native range with respect to the six variables used in our models (Table 2.1). In addition, niche overlap for subspecies projections indicate that subspecies *lindleyi* and *pruinescens* have the most similar niches; while subspecies *lindleyi* and *saligna* have the most dissimilar niche (Table 2.1). These differences were mirrored by variation in the sensitivity and modelled prevalence for models calibrated using records for each subspecies (Table 2.1, aim one).

All models trained and tested with native range occurrences displayed statistically significant results according to the exact binomial test ( $P < 0.0001$ , Table 2). Highly sensitive (Table 2.1) subspecies level projections to the native range (Fig. 2.1) indicate that the four subspecies of *A. saligna* occupy different climatic niches. Further, results showed that the climatic niche occupied by the species complex as a whole is broader than the niche occupied by each subspecies (Fig. 2.2a versus Fig. 2.2b-e). The projections for *A. saligna* subspecies *pruinescens*, *saligna* and *stolonifera* were subsets of the broad projected region of climatic suitability for *A. saligna* subspecies *lindleyi* (Table 2.1 and Fig. 2.2).

Overall, native models trained using all *A. saligna* records, or records per subspecies, displayed perfect sensitivity but variable modelled prevalence (Table 2.1, aim one). Models for pairwise comparisons between the subspecies displayed the highest levels of sensitivity and modelled prevalence for models trained with subspecies *lindleyi* and *pruinescens*; while pairwise comparisons for models trained with subspecies *saligna* and *stolonifera* produced much lower sensitivity and modelled prevalence (Table 2.1).

### *(b) Models projected to South Africa*

All models projecting to the introduced range in South Africa displayed statistically significant results according to the exact binomial test ( $P < 0.0001$ , Table 2.2). Within full native model projections (MESS+ and MESS- areas; Fig. 2.3b-j) no single model was able to predict the full current distribution of *A. saligna* in South Africa (i.e. no model obtained perfect sensitivity; Table 2.2, aim two). Sensitivity was highest for the models trained using South African records (0.99, Table 2.2) and displayed almost no MESS – regions (Fig. 2.3a). Sensitivity of models trained using native records was highest for the pairwise model for subspecies *lindleyi* and *stolonifera*, and the individual model for subspecies *lindleyi* (Table 2.2). All these models displayed low modelled prevalence (Table 2.2). The largest areas of modelled prevalence (Table S2.2) were for subspecies *saligna* (Fig. 2.3e) and *pruinescens* (Fig. 2.3d), however much of this area fell within MESS- areas (model extrapolation).

Models trained with native records and projected to South Africa indicate that the Western and Northern Cape had climates similar to those used to construct the model in the native range in Australia (i.e. MESS+ areas Fig. 2.3b-j) and were not limited by any single bioclimatic variable (Fig. S2.3). Within these MESS+ areas and across all native models, regions that were projected to be climatically suitable were consistent with at least some of the current introduced distribution of *A. saligna* (Fig. 2.3b-j, Table 2.2, aim two)

Within MESS- areas, models varied in their ability to correctly predict areas of climatic suitability within the distribution of *A. saligna*. Models that were built on combinations of subspecies occurrences did not project climatically suitable areas in the northern parts of South Africa in areas far beyond the known distribution of *A. saligna* (Fig. 2.3g-j). However individual models for subspecies *saligna* (Fig. 2.3d) and *pruinescens* (Fig. 2.3d) projected climatic suitability along the east coast of South Africa, consistent with known occurrences of *A. saligna*. For these models, the dominant climatic variable (limiting factors, *sensu* Elith *et al.*, 2010) influencing model projections in the north eastern regions of South Africa was precipitation in the hottest quarter (Bio18, Fig. S2.2 b and c). In these cases, Bio18 displayed open-ended response curves that maintained high suitability values (Fig. S2.2 b and c).

*(c) South African models projected to Australia*

Models constructed using introduced South African records and back projected to Australia displayed statistically significant results according to the exact binomial test ( $P < 0.0001$ , Table 2.2, aim two). Regions of high projected suitability occur along the coastal regions of south-western Western Australia, consistent with the native distribution of *A. saligna* (Fig. 2.4, blue circles). These models suggest that the entities present in South Africa occupy at least the full native niche of *A. saligna*, i.e. perfect sensitivity (Table 2.2). Moreover, projected climatic suitability extended in a north-easterly direction beyond the native range of *A. saligna*, into the inland areas of south-western Western Australia (Fig. 2.4). However, areas of projected suitability did not include the full introduced range of *A. saligna* in eastern Australia (Fig. 2.4, black circles), despite projected suitability intersecting with the majority of naturalised occurrences of *A. saligna* in this region. Taken together, these models suggest that there may be additional regions of climatic suitability for (South African) *A. saligna* in south-western Western Australia and eastern Australia (MESS+ space) that are not currently occupied.

#### (d) *Models projected to the Mediterranean Basin*

Only models for all subspecies combined, and subspecies *pruinescens* projecting to the introduced range in the Mediterranean Basin displayed statistically significant results according to the exact binomial test ( $P < 0.0001$ , Table 2.2, aim three). Areas of projected climatic suitability in the Mediterranean Basin (Fig. 2.5) and modelled prevalence and sensitivity (Table 2.2, aim three) varied substantially between subspecies. Models displayed the highest sensitivity for projections trained using all native records, followed by models trained with South African records, followed by models trained with subspecific records (Table 2.2). Overall a large proportion of the Mediterranean Basin represented MESS- climates relative to the native range of *A. saligna* (Fig. 2.5a, and 2.5c-f). Models trained using introduced South African records produced the smallest MESS- area (Fig. 2.5b). Within MESS- and MESS+ space, no model projected climatic suitability intersecting with all known occurrences in the region (i.e. perfect sensitivity was not achieved).

## 2.4 *Discussion*

Our models indicate that all subspecies of *A. saligna* occupy different climatic niches within their native and introduced ranges. This variation in climatic space is confirmed by multiple lines of evidence: model projections, quantification of climate occupancy, and the sensitivity and modelled prevalence of projections. The degree of dissimilarity between model projections for the four subspecies, and their combination projections (i.e. models calibrated with groups of subspecies) to South Africa indicate that the realised niches will likely differ irrespective of the morphological (Maslin *et al.*, 2006) or genetic groupings (Millar *et al.*, 2011).

Our models show that in Western Australia *A. saligna* subspecies *stolonifera* occupies the most spatially and climatically narrow niche; while *A. saligna* subspecies *lindleyi* occupies the widest niche. Assuming that subspecies distributions are primarily defined by climatic limitations, this suggests that subspecies *lindleyi*, followed closely by subspecies *pruinescens* have the widest

environmental tolerance. These subspecies should be considered a slightly higher risk of becoming naturalised elsewhere relative to the other subspecies.

Models projecting to the introduced range of *A. saligna* in South Africa indicate that South African populations currently occur outside the range of climates occupied by all native subspecies, as represented by the climatic variables used in our modelling. Moreover, models for *A. saligna* subspecies *lindleyi*, and the combination model of subspecies *lindleyi* + *stolonifera* most closely reflect *A. saligna*'s current introduced distribution in South Africa. Phylogeographic data from Western Australian and South African *A. saligna* populations suggest a substantial genetic bottleneck and the presence of only a subset of the native subspecies in South Africa (Le Roux *et al.*, 2011). Based on the assumption that the native geographical distribution of these subspecies is partially explained by the climatic variables used to build the models in this study, it is most likely that either subspecies *lindleyi* or *stolonifera* are present in South Africa. However, back projections to Australia are inconsistent with native data projections to South Africa, as they suggest that the South African entities occupy a wider climatic space than the currently occupied native range of *A. saligna*. That is, that the drier inland regions of Western Australia and eastern Australia would be occupied by South African entities.

Models projecting to the Mediterranean Basin do not provide evidence linking a particular *A. saligna* subspecies to the introduced range as no model intersected with all known occurrences in the region. However, projections for subspecies *pruinescens* intersected with known occurrences of *A. saligna*, but these were within novel climate space. This suggests these subspecies would be most suited to the climates in the Mediterranean Basin; but do caution that it would be imprudent to interpret these results as meaning that the other subspecies pose a lower risk of becoming invasive in areas with Mediterranean-type climates.

In light of these findings, it is important to consider the influence that a species' introduction history can have on genetic structure in the introduced range (see Le Roux *et al.*, 2011). *Acacia*

*saligna* was introduced on a number of occasions, and has been widely and actively distributed in South Africa (Shaughnessy, 1980; Poynton, 2009). Despite the multiple introductions, our modelling suggests that not all native genetic entities are present in South Africa. This is in agreement with the amount of genetic diversity found in Australia, compared to South Africa (Le Roux *et al.*, 2011). In addition, our models were unable to confirm the presence of only one particular subspecies or genetic group in South Africa. It may also be that a niche shift has taken place; this would explain the inability of all models built with native data to predict the introduced distribution in South Africa. A shift may also be due to novel genetic entities that have arisen in the invasive range, or the impact of human mediated dispersal (e.g. Theoharides & Dukes, 2007). These uncertainties highlight the need for further research on the link between genetic variation and niche partitioning in the native and introduced range of invasive species, and the use of common garden experiments to elucidate links between genetic variation and quantifiable differences in plant fitness.

In summary, although the models displayed high levels of subspecific predictability (i.e. high sensitivity and relatively low prevalence) in their native range, they displayed poor predictability when applied to their introduced ranges. This may be due to a niche shift upon introduction (e.g. genetic drift) or that the SDMs developed for *A. saligna* do not incorporate climatic variables that are restricting the species' current distribution. The very gradual climatic gradients in the south-west of Western Australia mean that the absolute differences in climate space between the subspecies may be far smaller relative to the range experienced in other Mediterranean-type regions to which the species has been introduced, and that factors other than climate may also be important for explaining range limits in Australia. We recognise, for example, that non-climatic variables such as edaphic features may also influence the niche occupied by *A. saligna*. However, we were unable to account for such influences due to a lack of appropriate edaphic data for all the regions we investigated.

This study represents the first SDM to be developed for an invasive plant species complex. Models showed that subspecies of *A. saligna* vary substantially in their climatic and spatial extent in

Western Australia, providing evidence that SDMs can detect bioclimatic niche differences below the species level. Further, we found that models based on data from the native range did not adequately predict the distribution of *A. saligna* in South Africa or the Mediterranean Basin. These findings provide putative support for the observations that genetic diversity and structure in the South African range differ considerably from the native range, and is consistent with the molecular data of Le Roux *et al.* (2011). Furthermore, we provide evidence for a lack of niche conservatism between the native and introduced range of *A. saligna*. Further research is required to test whether niche conservatism is violated between the native and introduced range of other species.

### *Acknowledgements*

We acknowledge financial support from the DST-NRF Centre of Excellence for Invasion Biology and the Working for Water Programme through the collaborative research project on “Research for Integrated Management of Invasive Alien Species” and the CSIRO Climate Adaptation Flagship. The Oppenheimer Memorial Trust and Stellenbosch University funded the October 2010 workshop in Stellenbosch at which a preliminary version of this paper was presented. We thank Bruce Maslin (Department of Environment and Conservation, Western Australia) for identifying herbarium specimens, Noboru Ota (CSIRO, Australia) for creating the 5' Köppen-Geiger layer, Zahn Munch for assistance with ArcGIS, Darren Kriticos (CSIRO, Australia) for an alternative statistical approach to AUC, and three anonymous reviewers for useful comments on earlier drafts of the manuscript.

### *Author contributions*

M.P.R., G.D.T., J.R.U.W., J.J. Le Roux and D.M.R conceived the research ideas, G.D.T., M.P.R., J.J. Le Roux, J.R.U.W., and D.M.R designed research methods, G.D.T. and B.L.W. processed the distribution data, G.D.T. ran the models, G.D.T., M.P.R. and B.L.W. analysed the model output, B.L.W. and M.P.R. provided SDM expertise and G.D.T. led the writing.

## 2.5 Tables and Figures

**Table 2.1** Variation in the bioclimatic niche similarity of the subspecies of *Acacia saligna* in Western Australia. Pairwise similarities were calculated based on model (ten replicates) projections to the native range. Two measures describe the climatic similarity: niche overlap using Schoener's D index, and niche similarity using the Hellinger-based similarity statistic. Both measures range from 0 (no overlap/similarity) to 1 (complete overlap/similarity).

<i>Acacia saligna</i> subspecies	Schoener's index (D)	Hellinger similarity statistic (I)
<i>lindleyi</i> - <i>stolonifera</i>	0.381	0.620
<i>pruinescens</i> - <i>stolonifera</i>	0.487	0.531
<i>lindleyi</i> - <i>saligna</i>	0.295	0.552
<i>pruinescens</i> - <i>saligna</i>	0.315	0.552
<i>saligna</i> - <i>stolonifera</i>	0.356	0.556
<i>lindleyi</i> - <i>pruinescens</i>	0.445	0.647

**Table 2.2** Correlative model fit based on the sensitivity and modelled prevalence of distribution models developed for *Acacia saligna* relative to each of the three aims. The training and testing datasets and the region of projection varied between models and were grouped based on the aims.

Aim	Subspecies training data	Testing dataset	Region of projection	Sensitivity	Modelled prevalence
1) Assess whether the subspecies occupy areas in their native range that can be distinguished by correlative models	All subspecies	All subspecies	Western Australia	1.00	0.53
	<i>A.s. ssp. lindleyi</i>	<i>A.s. ssp. lindleyi</i>	Western Australia	1.00	0.54
	<i>A.s. ssp. pruinescens</i>	<i>A.s. ssp. pruinescens</i>	Western Australia	1.00	0.34
	<i>A.s. ssp. saligna</i>	<i>A.s. ssp. saligna</i>	Western Australia	1.00	0.17
	<i>A.s. ssp. stolonifera</i>	<i>A.s. ssp. stolonifera</i>	Western Australia	1.00	0.09
	<i>A.s. ssp. lindleyi</i>	<i>A.s. ssp. saligna</i>	Western Australia	1.00	0.55
	<i>A.s. ssp. lindleyi</i>	<i>A.s. ssp. pruinescens</i>	Western Australia	0.98	0.55
	<i>A.s. ssp. lindleyi</i>	<i>A.s. ssp. stolonifera</i>	Western Australia	1.00	0.55
	<i>A.s. ssp. pruinescens</i>	<i>A.s. ssp. lindleyi</i>	Western Australia	0.76	0.35
	<i>A.s. ssp. pruinescens</i>	<i>A.s. ssp. saligna</i>	Western Australia	1.00	0.36
	<i>A.s. ssp. pruinescens</i>	<i>A.s. ssp. stolonifera</i>	Western Australia	1.00	0.36
	<i>A.s. ssp. saligna</i>	<i>A.s. ssp. lindleyi</i>	Western Australia	0.37	0.15
	<i>A.s. ssp. saligna</i>	<i>A.s. ssp. pruinescens</i>	Western Australia	0.24 <sup>†</sup>	0.15
	<i>A.s. ssp. saligna</i>	<i>A.s. ssp. stolonifera</i>	Western Australia	0.61	0.15
<i>A.s. ssp. stolonifera</i>	<i>A.s. ssp. lindleyi</i>	Western Australia	0.09 <sup>†</sup>	0.07	
<i>A.s. ssp. stolonifera</i>	<i>A.s. ssp. pruinescens</i>	Western Australia	0.71	0.07	
<i>A.s. ssp. stolonifera</i>	<i>A.s. ssp. saligna</i>	Western Australia	0.52	0.07	
2) Explore predictive power of models in South Africa relative to current molecular information	All subspecies	South African <i>A. saligna</i>	South Africa	0.73	0.03
	<i>A.s. ssp. lindleyi</i>	South African <i>A. saligna</i>	South Africa	0.90	0.04
	<i>A.s. ssp. pruinescens</i>	South African <i>A. saligna</i>	South Africa	0.41	0.18
	<i>A.s. ssp. saligna</i>	South African <i>A. saligna</i>	South Africa	0.29	0.49
	<i>A.s. ssp. stolonifera</i>	South African <i>A. saligna</i>	South Africa	0.17	0.00
	<i>pruinescens+saligna+stolonifera</i>	South African <i>A. saligna</i>	South Africa	0.44	0.02
	<i>lindleyi+pruinescens+saligna</i>	South African <i>A. saligna</i>	South Africa	0.69	0.03
	<i>stolonifera+lindleyi</i>	South African <i>A. saligna</i>	South Africa	0.91	0.04
	<i>pruinescens+saligna</i>	South African <i>A. saligna</i>	South Africa	0.41	0.01
	South African <i>A. saligna</i>	South African <i>A. saligna</i>	South Africa	0.99	0.12
	South African <i>A. saligna</i>	All native subspecies	Australia	1.00	0.23
3) Predict which subspecies are present in other countries	All subspecies	Mediterranean <i>A. saligna</i>	Mediterranean Basin	0.61	0.24
	<i>A.s. ssp. lindleyi</i>	Mediterranean <i>A. saligna</i>	Mediterranean Basin	0.22 <sup>†</sup>	0.29
	<i>A.s. ssp. pruinescens</i>	Mediterranean <i>A. saligna</i>	Mediterranean Basin	0.04	0.42
	<i>A.s. ssp. saligna</i>	Mediterranean <i>A. saligna</i>	Mediterranean Basin	0.04 <sup>†</sup>	0.02
	<i>A.s. ssp. stolonifera</i>	Mediterranean <i>A. saligna</i>	Mediterranean Basin	0.00 <sup>†</sup>	0.00
	South African <i>A. saligna</i>	Mediterranean <i>A. saligna</i>	Mediterranean Basin	0.30 <sup>†</sup>	0.19

† - statistically non-significant results based on the exact binomial test (P < 0.0001)

Note: Model sensitivity was defined as the proportion of correctly predicted observed presences, where presence was defined by the lowest presence threshold (LPT) at which there was an actual presence in the projected range. Modelled prevalence was defined as the proportion of complete projection region estimated to be climatically suitable.

## Figure Legends

**Figure 2.1** Distribution records (black circles) of *Acacia saligna* overlaid with an environmentally informative background (Köppen-Geiger region; grey shading) from which pseudo-absence data were drawn for a) the four proposed subspecies in the native range in Western Australia, and b) the introduced range in South Africa and Lesotho.

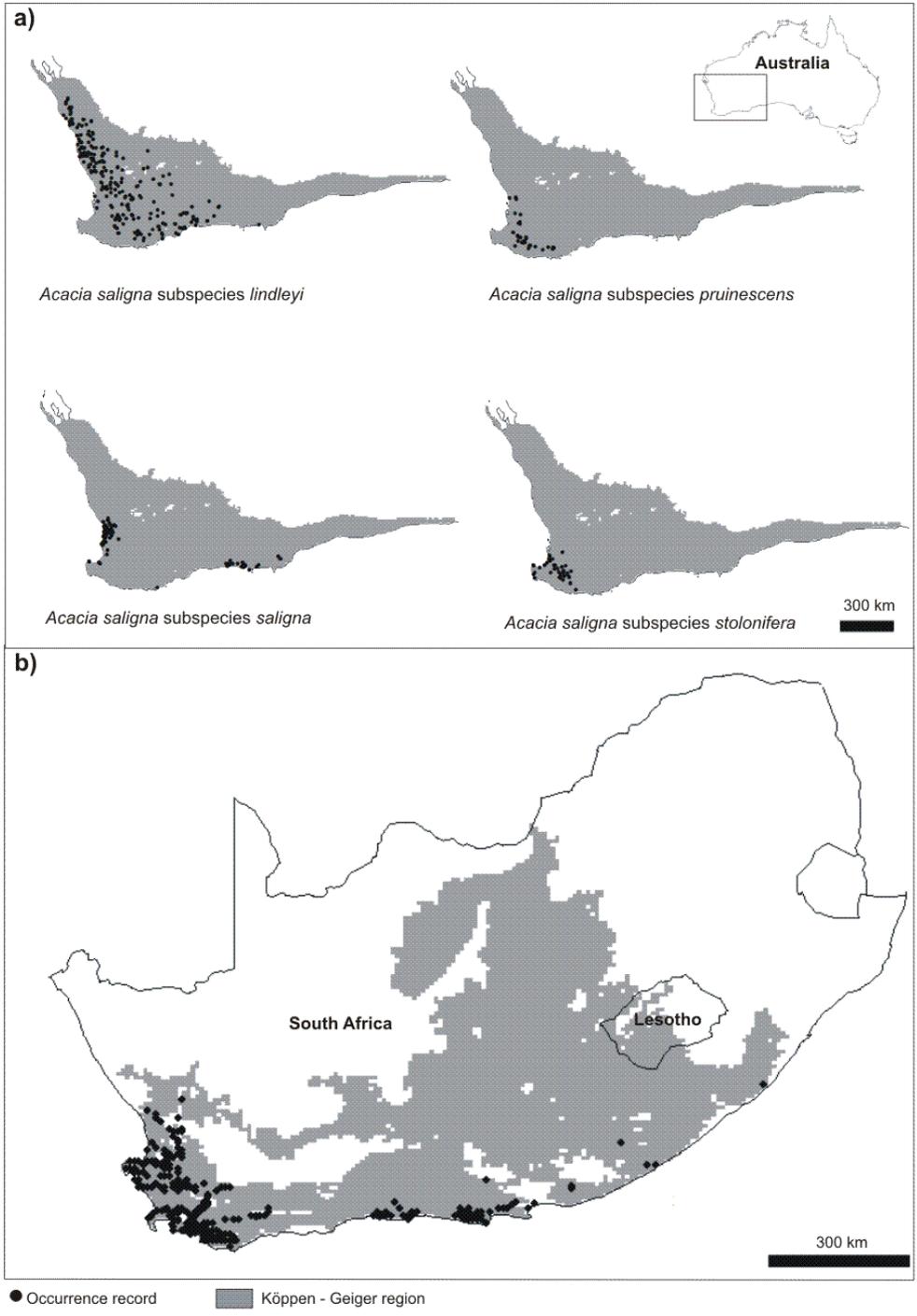
**Figure 2.2** Projected bioclimatic niches identified by correlative distribution models for each subspecies of *Acacia saligna* in Western Australia. Projections were based on the mean of ten replicate models. The colour scale depicts areas of projected climatic suitability, ( $\geq$  lowest presence threshold, LPT), ranging from highly suitable (red) to suitable (yellow) to marginally suitable (green) and unsuitable (white). Models were calibrated with native occurrence records from Western Australia (blue circles) and pseudo-absence data drawn from a single, environmentally informative background. The size of the projected range is indicated by the number of five minute grid cells (bottom right corner) projected as climatically suitable by the model. Hatched overlays indicate areas of model extrapolation (MESS-; i.e. at least one climatic variable has a value outside the range of the variables in the training region).

**Figure 2.3** Variation in the bioclimatic niches identified by correlative distribution models in the introduced range of *Acacia saligna* in South Africa. Potential distributions were constructed considering current molecular data from Le Roux *et al.*, (2011) and aimed to predict which subspecies are likely to be present in South Africa. Projections were based on the mean of ten replicate models, and were calibrated with: (a) introduced South African occurrences, (b) all native occurrences, (c-f) native occurrences per subspecies, and (g-j) various combinations of subspecies. Pseudo-absence data were drawn from a single, environmentally informative background. The colour scale depicts areas of projected climatic suitability, ( $\geq$  lowest presence threshold, LPT), ranging from highly suitable (red) to suitable (yellow) to marginally suitable (green) and unsuitable (white). The size of the potential introduced range is indicated by the number of five minute grid cells (bottom right corner) projected as climatically suitable by the model. Hatched overlays indicate areas of model extrapolation (MESS-; i.e. at least one climatic variable has a value outside the range of the variables in the training region).

**Figure 2.4** Potential distributions of introduced South African populations of *Acacia saligna* in Australia. Projections were based on the mean of ten replicate models, and aimed to assess niche differences between the Western Australian niche and the South African niche within the same bioclimatic space. South African pseudo-absence data were drawn from a single, environmentally informative background. The colour scale depicts areas of projected climatic suitability, ( $\geq$  lowest

presence threshold), ranging from highly suitable (red) to suitable (yellow) to marginally suitable (green) and unsuitable (white). Hatched overlays indicate areas of model extrapolation (MESS-; i.e. at least one climatic variable has a value outside the range of the variables in the training region).

**Figure 2.5** Projected bioclimatic niches of *Acacia saligna* in the Mediterranean Basin. Projections were based on the mean of ten replicate models and aimed to predict which subspecies are likely to be present in a biogeographical region similar to the native range in Western Australia, and the introduced range in South Africa. Models were calibrated with: (a) all native occurrences, (b) introduced South African occurrences, (c-f) native occurrences per subspecies. Pseudo-absence data were drawn from a single, environmentally informative background. Hatched overlays indicate areas of model extrapolation (MESS-; i.e. at least one climatic variable has a value outside the range of the variables in the training region). The colour scale depicts areas of projected climatic suitability, ( $\geq$  lowest presence threshold, LPT), ranging from highly suitable (red) to suitable (yellow) to marginally suitable (green) and unsuitable (white). The size of the potential introduced range is indicated by the number of five minute grid cells (bottom right corner) projected as climatically suitable by the model. Hatched overlays indicate areas of model extrapolation (MESS-; i.e. at least one climatic variable has a value outside the range of the variables in the training region).



**Figure 2.1**

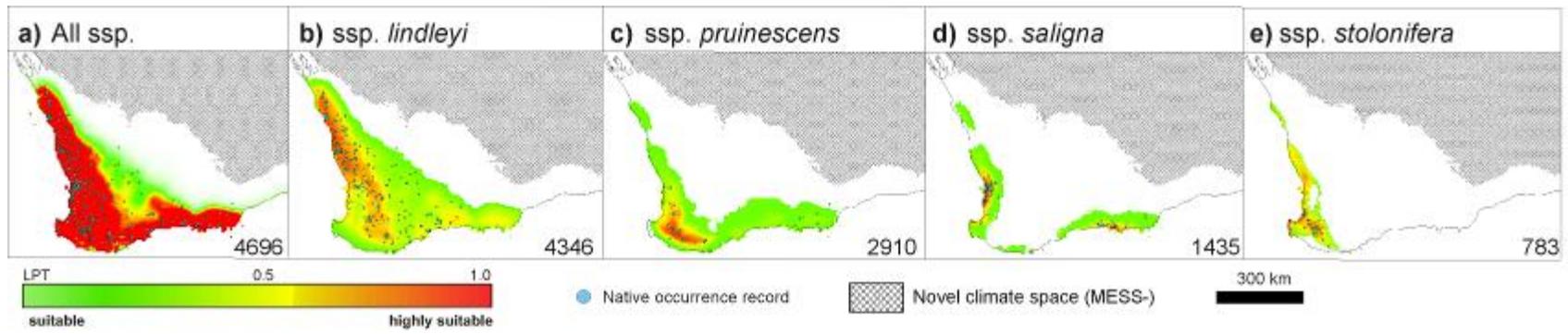


Figure 2.2

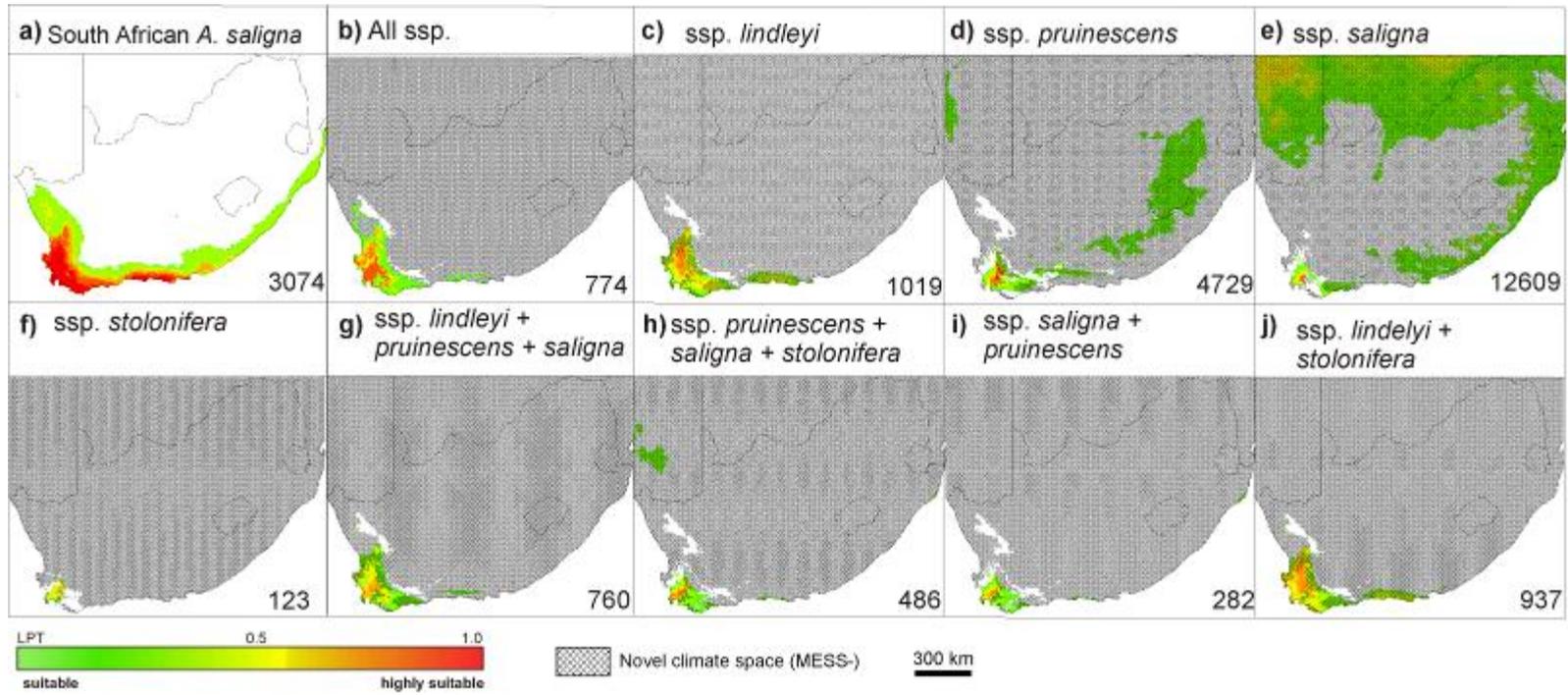
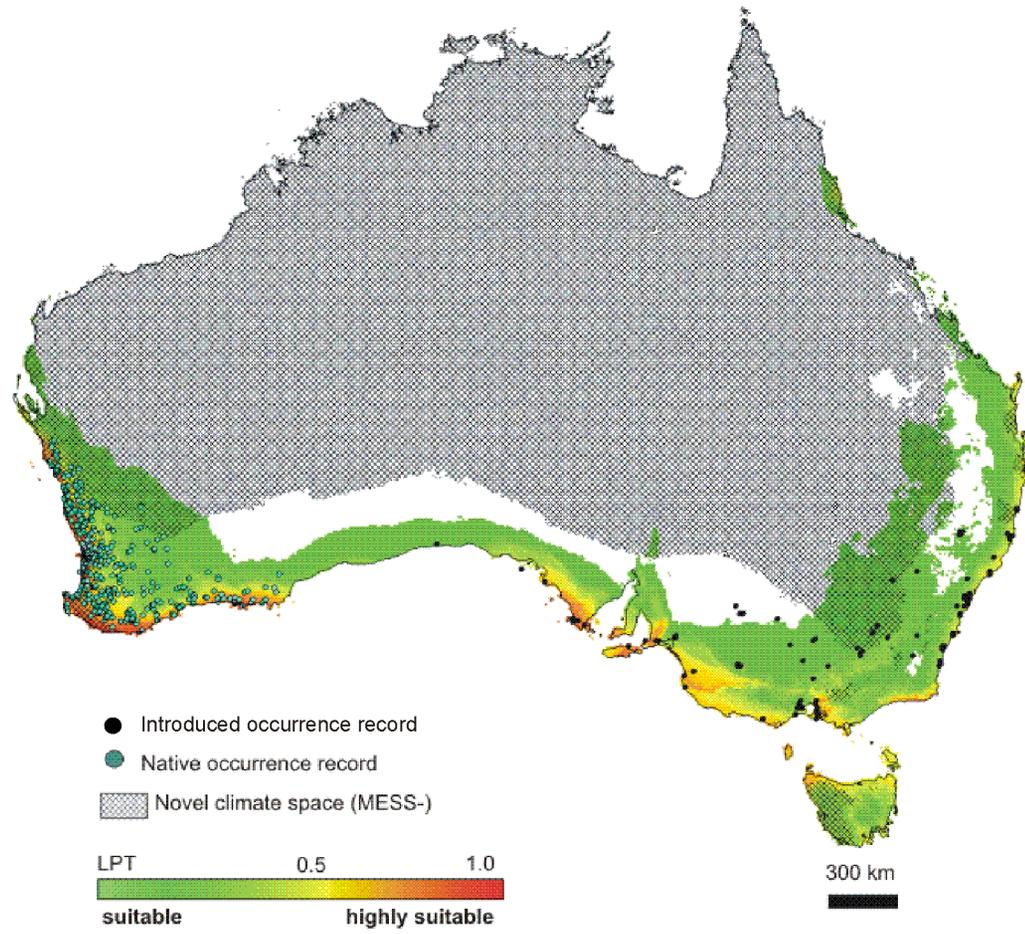


Figure 2.3



**Figure 2.4**

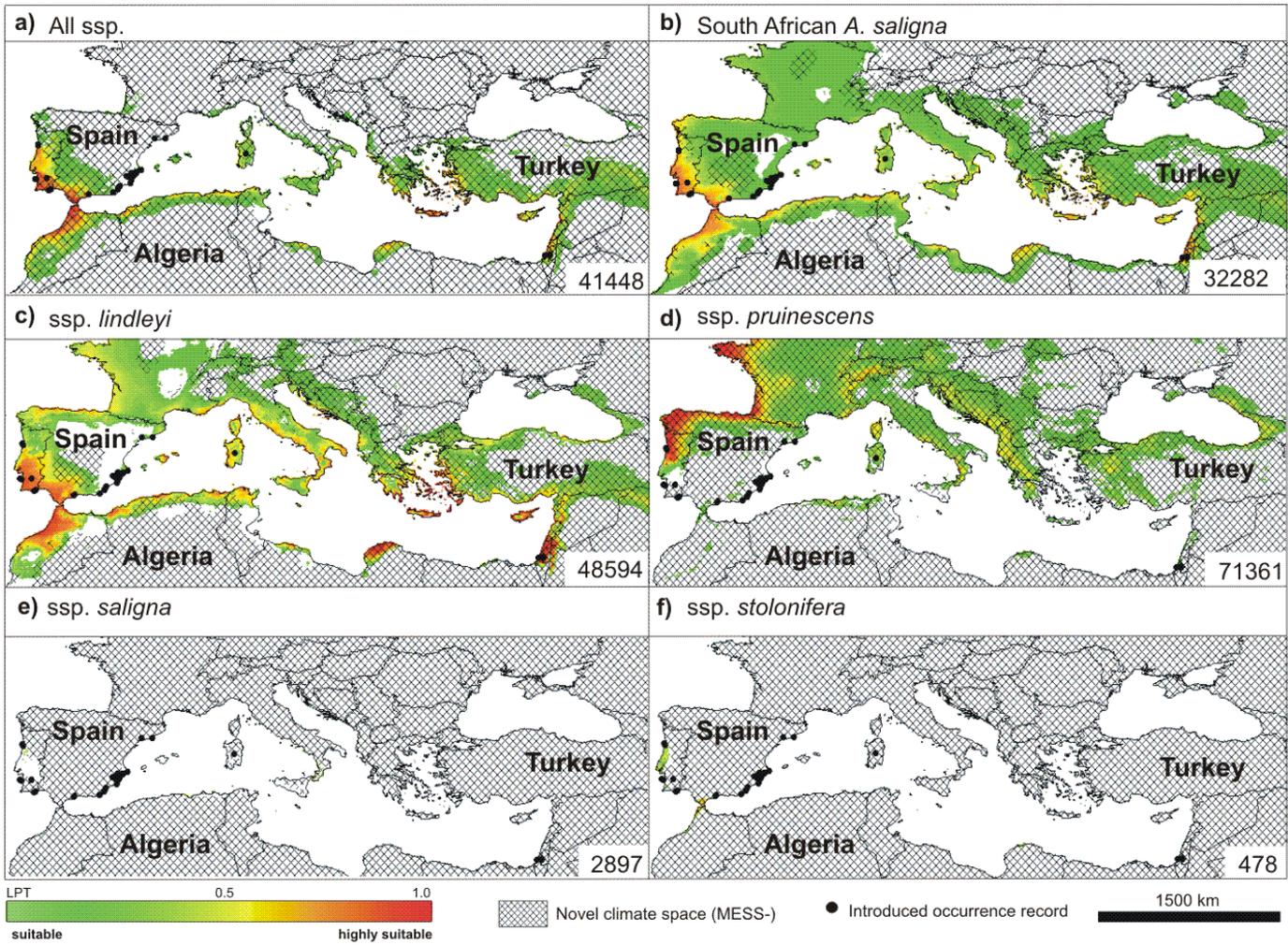
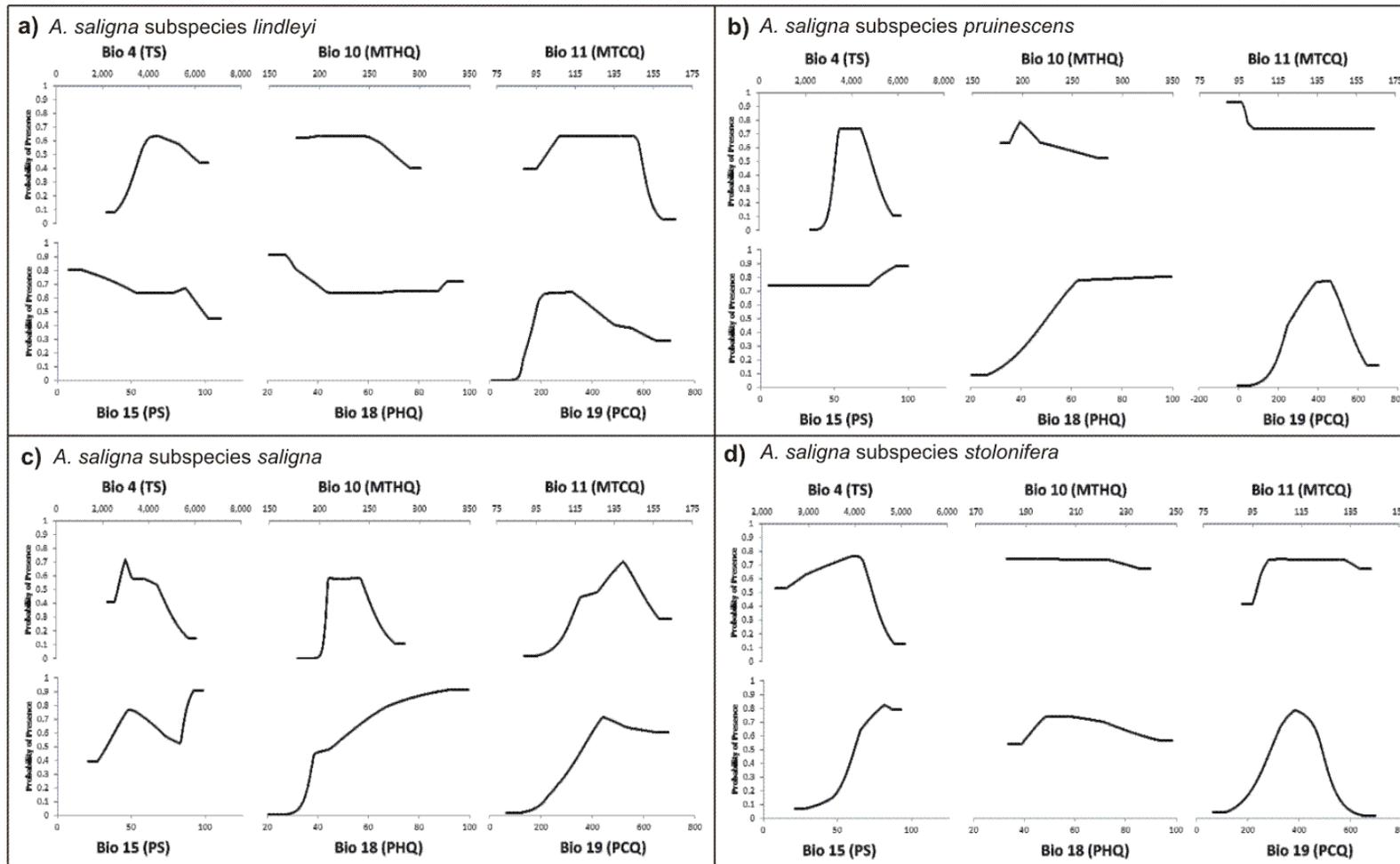


Figure 2.5

## 2.6 Appendices

### Appendix S2 Bioclimatic response curves



**Figure S2.1** Bioclimatic response curves for the 4 proposed subspecies of *Acacia saligna*, generated by ten replicate MAXENT models trained and projected to the native range (Western Australia). Bioclimatic variables used: temperature seasonality (TS; Bio4), mean temperature of the hottest quarter (MTHQ; Bio10), mean temperature of the coldest quarter (MTCQ; Bio11), precipitation seasonality (PS; Bio15), precipitation of the hottest quarter (PHQ; Bio18), and precipitation of the coldest quarter (PCQ; Bio19).

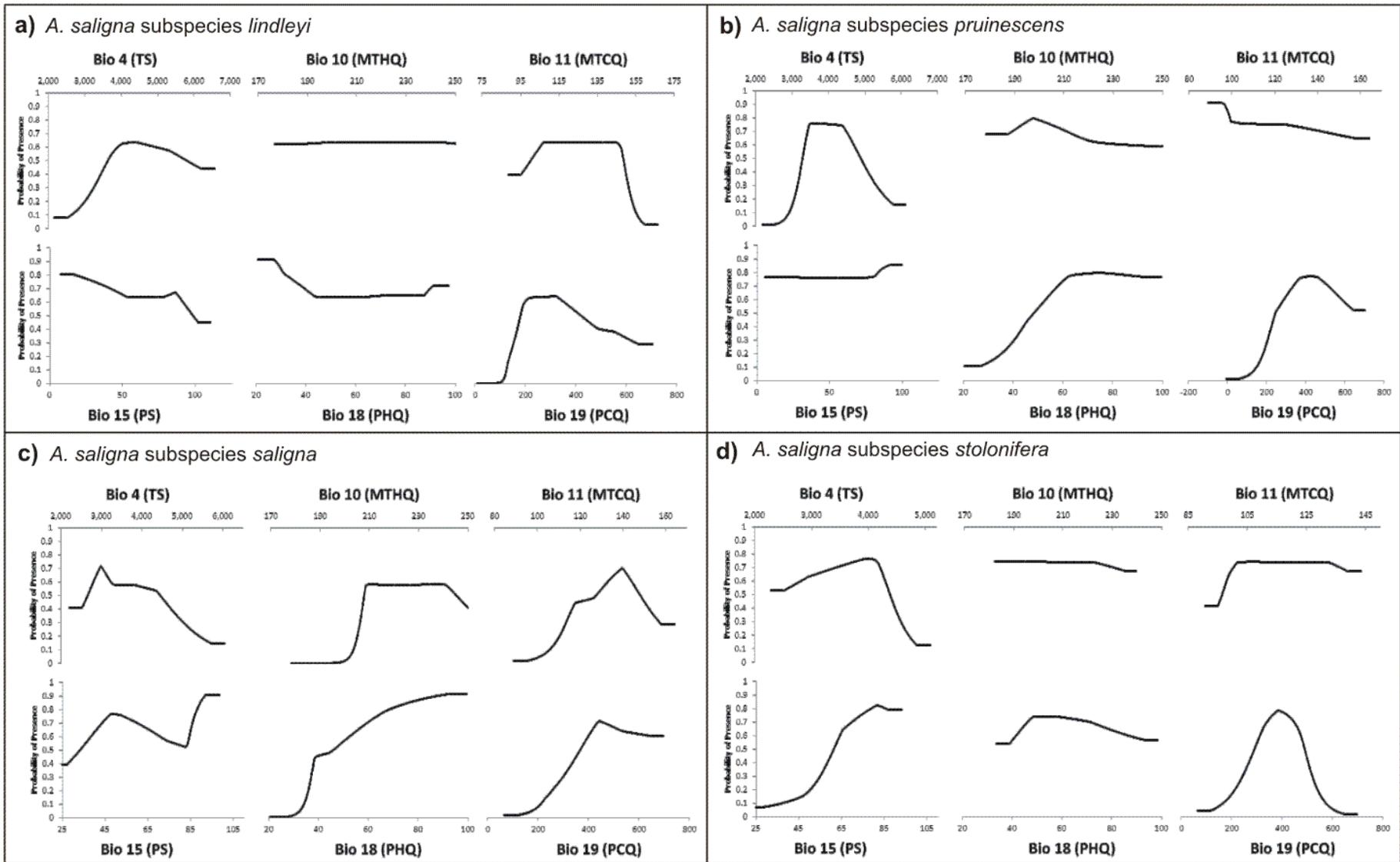
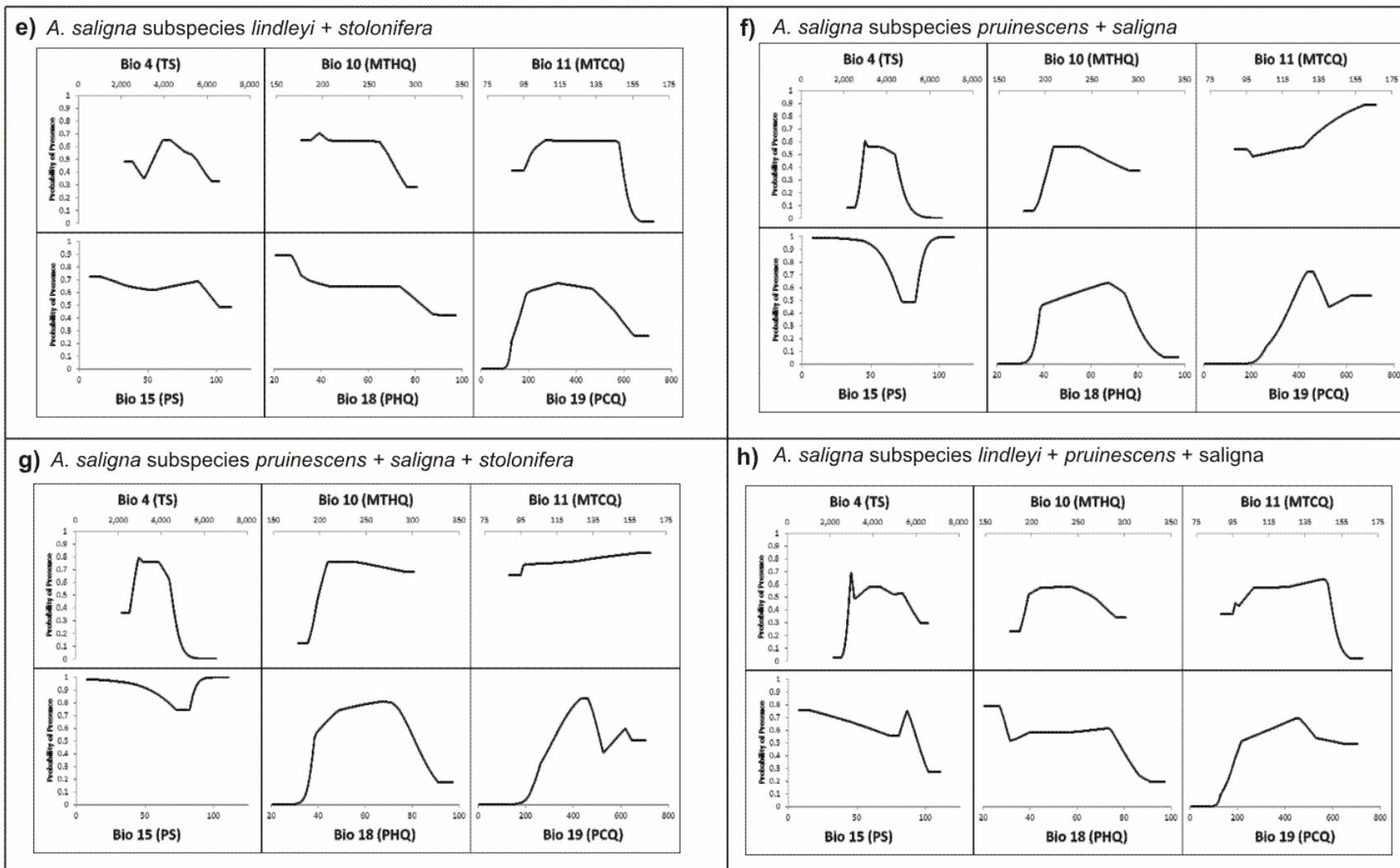
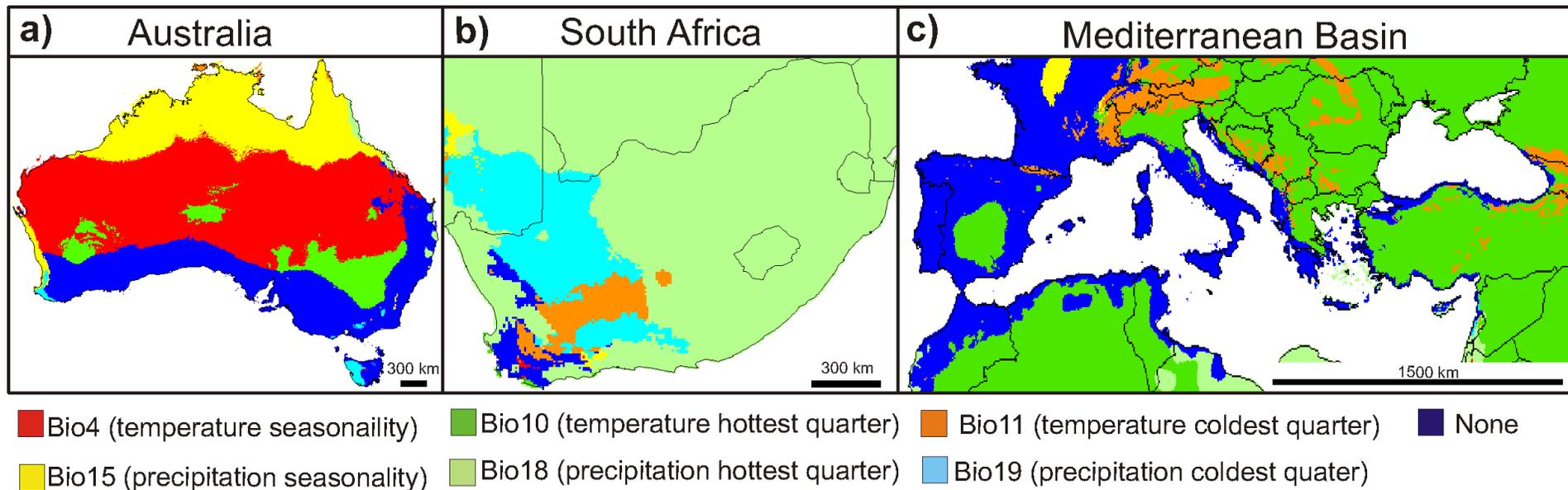


Figure S2.2 legend on following page



**Figure S2.2** Bioclimatic response curves generated by ten replicate MAXENT models trained on native range data (Western Australia) for *Acacia saligna*, and projected to South Africa. Models were constructed using occurrences per subspecies (a- d); and various combinations of subspecies (e-h). Bioclimatic variables used: temperature seasonality (TS; Bio4), mean temperature of the hottest quarter (MTHQ; Bio10), mean temperature of the coldest quarter (MTCQ; Bio11), precipitation seasonality (PS; Bio15), precipitation of the hottest quarter (PHQ; Bio18), and precipitation of the coldest quarter (PCQ; Bio19).



**Figure S2.3** Regional distribution of the 6 bioclimatic variables that spatially limit the MAXENT models for the *Acacia saligna* species complex, based on models trained in a) South Africa and projected to Australia; b) Western Australia (native range) and projected to South Africa; and c) Western Australia and projected to the Mediterranean Basin.

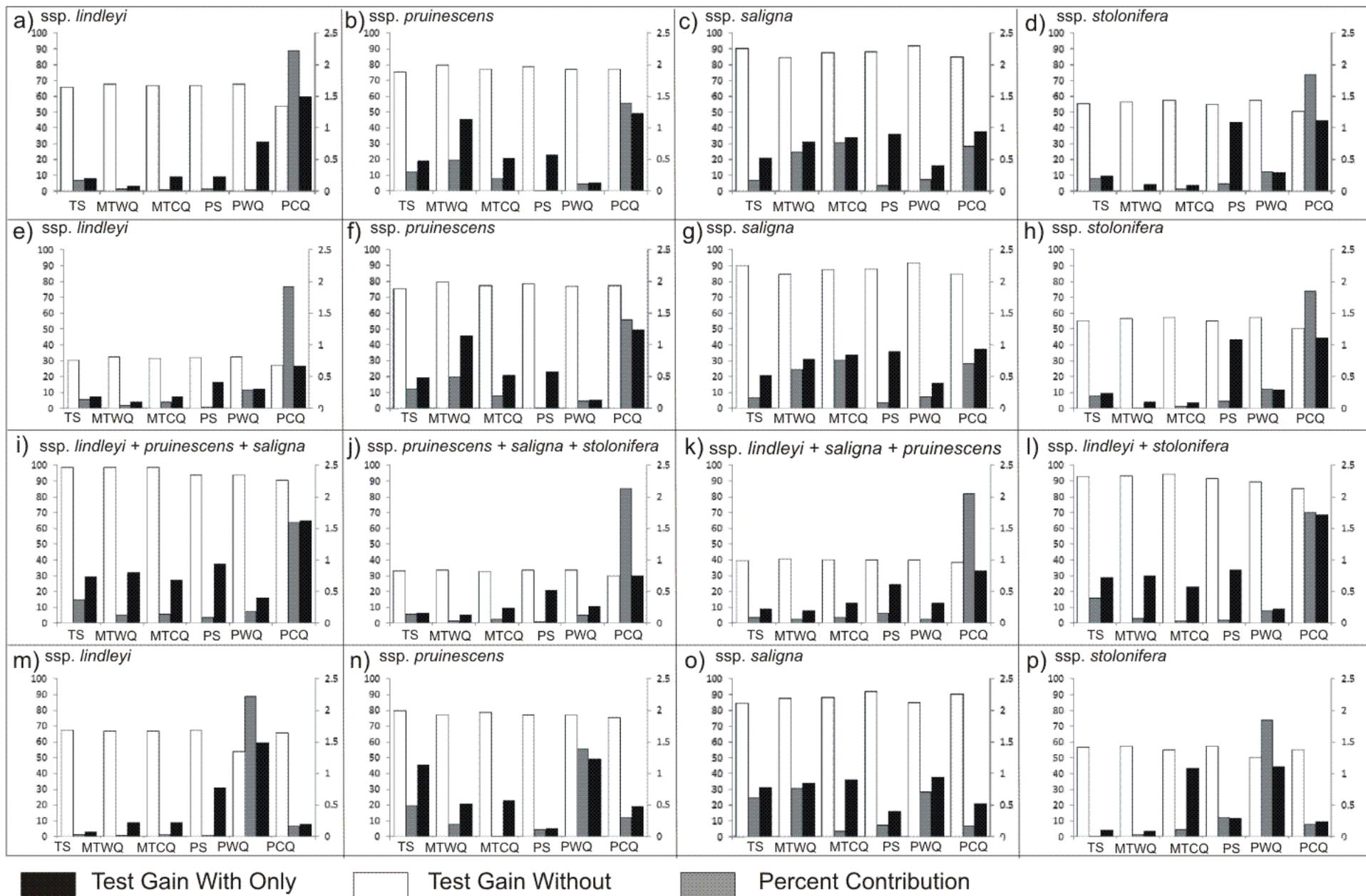


Figure S2.4 Legend on following page

**Figure S2.4** Bioclimatic variable contributions (primary  $y$ -axis) and testing gains with and without each variable (secondary  $y$ -axis), for the mean of ten replicate MAXENT models projected to the native range in Western Australia (a-d), introduced range in South Africa (e-l) and the introduced range in the Mediterranean Basin (m-p). Six bioclimatic variables represented are: temperature seasonality (TS; Bio4), mean temperature of the hottest quarter (MTHQ; Bio10), mean temperature of the coldest quarter (MTCQ; Bio11), precipitation seasonality (PS; Bio15), precipitation of the hottest quarter (PHQ; Bio18), and precipitation of the coldest quarter (PCQ; Bio19).

**Table S2.1** Multi-collinearity assessment based on a Pearson correlation coefficient of the bioclimatic variables from the species' native climatic space using ENMTools.

	Bio18	Bio15	Bio11	Bio10	Bio4
Bio15	0.558				
Bio11	0.563	0.907			
Bio10	0.102	0.627	0.733		
Bio4	0.678	0.491	0.497	0.226	
Bio19	0.017	0.388	0.527	0.747	0.194

**Table S2.2** Data used to calculate sensitivity and modelled prevalence of distribution models developed for *Acacia saligna* relative to each of the three aims. The training and testing datasets and the region of projection varied between models and were grouped based on the aims.

Subspecies training dataset	Source of testing dataset	Testing dataset	Region of projection	LPT Value	SC in region	UC in region	SC with presence	UC with presence
<b>Aim 1 analyses</b>								
All subspecies	AVH	All subspecies	Western Australia	0.0212	4696	4092	614	2
<i>A.s. ssp. lindleyi</i>	AVH	<i>A.s. ssp. lindleyi</i>	Western Australia	0.0417	4346	3747	332	0
<i>A.s. ssp. pruinescens</i>	AVH	<i>A.s. ssp. pruinescens</i>	Western Australia	0.0163	2910	5533	66	0
<i>A.s. ssp. saligna</i>	AVH	<i>A.s. ssp. saligna</i>	Western Australia	0.0080	1435	7008	162	0
<i>A.s. ssp. stolonifera</i>	AVH	<i>A.s. ssp. stolonifera</i>	Western Australia	0.0954	783	7660	56	0
<i>A.s. ssp. lindleyi</i>	AVH	<i>A.s. ssp. saligna</i>	Western Australia	0.0268	4662	3781	162	0
<i>A.s. ssp. lindleyi</i>	AVH	<i>A.s. ssp. pruinescens</i>	Western Australia	0.0268	4662	3781	65	1
<i>A.s. ssp. lindleyi</i>	AVH	<i>A.s. ssp. stolonifera</i>	Western Australia	0.0268	4662	3781	56	0
<i>A.s. ssp. pruinescens</i>	AVH	<i>A.s. ssp. lindleyi</i>	Western Australia	0.0128	2973	5470	251	81
<i>A.s. ssp. pruinescens</i>	AVH	<i>A.s. ssp. saligna</i>	Western Australia	0.0159	3007	5436	162	0
<i>A.s. ssp. pruinescens</i>	AVH	<i>A.s. ssp. stolonifera</i>	Western Australia	0.0159	3007	5436	56	0
<i>A.s. ssp. saligna</i>	AVH	<i>A.s. ssp. lindleyi</i>	Western Australia	0.0082	1273	7170	124	208
<i>A.s. ssp. saligna</i>	AVH	<i>A.s. ssp. pruinescens</i>	Western Australia	0.0082	1273	7170	16	50
<i>A.s. ssp. saligna</i>	AVH	<i>A.s. ssp. stolonifera</i>	Western Australia	0.0082	1273	7170	34	22
<i>A.s. ssp. stolonifera</i>	AVH	<i>A.s. ssp. lindleyi</i>	Western Australia	0.0977	581	7862	31	301
<i>A.s. ssp. stolonifera</i>	AVH	<i>A.s. ssp. pruinescens</i>	Western Australia	0.0977	581	7862	47	19
<i>A.s. ssp. stolonifera</i>	AVH	<i>A.s. ssp. saligna</i>	Western Australia	0.0977	581	7862	84	78
<b>Aim 2 analyses</b>								
All subspecies	AVH	South African <i>A. saligna</i>	South Africa	0.0212	774	24868	188	70
<i>A.s. ssp. lindleyi</i>	SAPIA + CIB	South African <i>A. saligna</i>	South Africa	0.0417	1019	24623	232	26
<i>A.s. ssp. pruinescens</i>	SAPIA + CIB	South African <i>A. saligna</i>	South Africa	0.0163	4729	20913	107	151
<i>A.s. ssp. saligna</i>	SAPIA + CIB	South African <i>A. saligna</i>	South Africa	0.0080	12609	13033	75	183
<i>A.s. ssp. stolonifera</i>	SAPIA + CIB	South African <i>A. saligna</i>	South Africa	0.0954	123	25519	43	215
<i>pruinescens+saligna+stolonifera</i>	SAPIA + CIB	South African <i>A. saligna</i>	South Africa	0.0080	486	25156	113	145
<i>lindleyi+pruinescens+saligna</i>	SAPIA + CIB	South African <i>A. saligna</i>	South Africa	0.0182	760	24882	179	79
<i>stolonifera+lindleyi</i>	SAPIA + CIB	South African <i>A. saligna</i>	South Africa	0.0628	937	24705	234	24
<i>pruinescens+saligna</i>	SAPIA + CIB	South African <i>A. saligna</i>	South Africa	0.0115	282	25360	105	153
South African <i>A. saligna</i>	SAPIA + CIB	South African <i>A. saligna</i>	South Africa	0.0078	3074	22568	256	2
South African <i>A. saligna</i>	SAPIA + CIB	All subspecies	Australia	0.0163	23954	78731	442	0
<b>Aim 3 analyses</b>								
All subspecies	AVH	Mediterranean <i>A. saligna</i>	Mediterranean Basin	0.0212	41448	127764	14	9
<i>A.s. ssp. lindleyi</i>	GBIF	Mediterranean <i>A. saligna</i>	Mediterranean Basin	0.0417	48594	120618	5	18
<i>A.s. ssp. pruinescens</i>	GBIF	Mediterranean <i>A. saligna</i>	Mediterranean Basin	0.0163	71361	97851	1	22
<i>A.s. ssp. saligna</i>	GBIF	Mediterranean <i>A. saligna</i>	Mediterranean Basin	0.0080	2897	166315	1	22
<i>A.s. ssp. stolonifera</i>	GBIF	Mediterranean <i>A. saligna</i>	Mediterranean Basin	0.0954	478	168734	0	23
South African <i>A. saligna</i>	SAPIA + CIB	Mediterranean <i>A. saligna</i>	Mediterranean Basin	0.0078	32282	136930	7	16

SC – climatically suitable 5' grid cell

US – climatically unsuitable 5' grid cell

## CHAPTER 3 Cultivation shapes genetic novelty in a globally important invader

- This chapter has been published, citation: Thompson, G.D., Bellstedt, D.U., Byrne, M., Millar, M.A., Richardson, D.M., Wilson, J.R.U & Le Roux, J.J. (2012) Cultivation shapes genetic novelty in a globally important invader. *Molecular Ecology*, **21**, 3187-3199.

### Abstract

*Acacia saligna* is a species complex that has become invasive in a number of countries worldwide where it has caused substantial environmental and economic impacts. Understanding genetic and other factors contributing to its success may allow managers to limit future invasions of closely related species. We used three molecular markers (nDNA, cpDNA and nuclear microsatellites) to compare the introduced range (South Africa) to the native range (Western Australia). Nuclear markers showed that invasive populations are divergent from native populations, and most closely related to a cultivated population in Western Australia. We also found incongruence between nuclear and chloroplast data that, together with the long history of cultivation of the species, suggest that introgressive hybridization may have occurred within *A. saligna*. While we could not definitively prove introgression, the genetic distance between cultivated and native *A. saligna* populations was comparable to known interspecific divergences among other *Acacia* species. Therefore, cultivation, multiple large-scale introductions, and possibly introgressive hybridization, have rapidly given rise to the divergent genetic entity present in South Africa. This may explain the known global variation in invasiveness and inaccuracy of native bioclimatic models in predicting potential distributions.

### 3.1 Introduction

Biological invasions tend to promote rapid evolution due to the introduction process itself and the novel selection pressures that arise in the introduced range (Prentis *et al.* 2008). Indeed, post-introduction establishment has been associated with a number of genetic characteristics, including high genetic diversity (e.g. reed canarygrass, Lavergne & Molofsky 2007; European paper wasp, Johnson & Stark 2004, European starling, Rollins *et al.* 2009), increased phenotypic plasticity (e.g. Chinese tallow tree, Zou *et al.* 2009; anolis lizards, Kolbe *et al.*, 2009) and novel genotypes arising from hybridisation (e.g. *Casuarina* spp., Gaskin *et al.* 2009; freshwater sculpin, Nolte *et al.*, 2005).

Cultivation plays an important role in determining the influence of such processes for two main reasons. First, species used in horticulture and silviculture are typically introduced on multiple occasions, in large quantities, and are planted widely with resources to facilitate establishment, i.e. there is likely to be high propagule pressure, high genetic diversity, and opportunities for novel genetic combinations to arise (Ellstrand & Schierenbeck 2000; Wilson *et al.* 2009). Second, breeding and selection can favour traits associated with invasiveness, e.g. fast growth rates and robustness to adverse environmental conditions (e.g. Richardson 1998; Paynter *et al.* 2003; Richardson & Rejmánek 2011).

A species' native phylogeographic structure or evolutionary history can similarly influence introduced genetic diversity as it defines the genetic pool from which the invader is drawn (Taylor & Keller 2007; Le Roux *et al.* 2011), and so to some extent affects the degree to which cultivation and introduction dynamics can create new genetic entities. Such patterns and process that occur prior, during, or post introduction, are likely to have a substantial effect on the evolutionary trajectories of species complexes that are introduced outside of their native range. That is, their introduction dynamics and native phylogeographic structure can act in concert to determine their introduced intra-specific diversity, the opportunity for intra-specific hybridization (admixture), or the development of novel genotypes. Indeed, sympatric introductions of different genotypes have

produced entities that are more phenotypically plastic, with greater fitness than their native counterparts (e.g. Thompson 1991; Durka *et al.* 2005; Sun *et al.* 2005).

Woody plants used in silviculture and agriculture have been widely distributed and cultivated for centuries, in many instances resulting in invasive populations (Richardson 1998; Thuiller *et al.* 2006; Richardson & Rejmánek 2011). Australian acacias (1012 recognized species native to Australia, previously grouped in *Acacia* subgenus *Phyllodineae*) are a model group for the study of woody plant invasions (Richardson *et al.* 2011). More than a third of taxa in the group (386 species) have been introduced outside their natural range, and many of them have been repeatedly introduced to the same region, or to multiple regions (Richardson *et al.* 2011). A number of species display very high levels of intraspecific genetic diversity and structure in their native ranges (Le Roux *et al.* 2011), allowing repeated tests of the influence of the invasion dynamics of a species on the genetic signature in the introduced range. Their introduction histories are relatively well documented (Griffin *et al.* 2011; Le Roux *et al.* 2011; Richardson *et al.* 2011), providing definitive records on introduction mode and date. Furthermore, many species were selected for introduction because of fast growth rates, their ability to survive in adverse conditions, and incidentally their weediness (Griffin *et al.* 2011).

*Acacia saligna* (Labill.) H. L. Wendl., a species complex native to Western Australia, is one of the most frequently exported Australian acacia taxa (Griffin *et al.* 2011) and now occurs in at least 20 countries worldwide (Richardson *et al.* 2011; Richardson & Rejmánek 2011). It has been used for timber, as an ornamental plant, as a source of fodder, fuel, fibre and tannin, and for erosion control (Orwa *et al.* 2009; Kull *et al.* 2011). *Acacia saligna* is an allogamous, diploid ( $2n = 26$ , Ghimpu 1929), insect-pollinated shrub or tree (Atchison 1948; Millar *et al.* 2008, Gibson *et al.* 2011) that bears hermaphroditic, globular inflorescences (Maslin & McDonald 2004). The species displays high levels of ecological, phenotypic, and genetic variation throughout its native range (Maslin 1974; Maslin & McDonald 2004). This variation is not easily ascribed to sub-specific entities, and the number and categorisation of taxonomically distinct entities has been a matter of

debate (Maslin 1974; Maslin & McDonald 2004; George *et al.* 2006; Millar *et al.* 2008; Millar *et al.* 2011a). As no classification has been formalised, throughout this manuscript we refer to both the most recent morphological treatment: 1) subspecies '*lindleyi*' ('typical' variant), 2) subspecies '*stolonifera*' ('forest' variant), 3) subspecies '*saligna*' ('cyanophylla' variant), and 4) subspecies '*pruinescens*' ('Tweed River' variant, Maslin & McDonald 2004, [worldwidewattle.com](http://worldwidewattle.com)); and the three main genetic groups identified by Millar *et al.* (2011a). The only difference between the two treatments is that the morphological treatment combines subspecies '*saligna*' and '*pruinescens*', Each informal subspecies has differing ecological traits (seed set, reproductive success and biomass production) and a preference for particular environmental conditions e.g. the 'cyanophylla' variant prefers deep sandy soils while the 'typical' variant is common on seasonally dry water courses and around granite rocks (Maslin & MacDonald 2004). Such ecological characteristics might be expected to persist in the introduced range and aid or impede invasive success.

*Acacia saligna* was introduced to South Africa in about 1833 for dune stabilisation and ornamental purposes (Roux 1961; Shaughnessy 1980), and was later used as a wood and tannin source. From 1833 to 1890, over fifty million seeds were distributed and several thousand seedlings planted (Roux 1961). These populations have since expanded considerably, and *A. saligna* now extends over some 1.8 million ha of natural and semi-natural land in South Africa (Le Maitre 2000). Despite relatively detailed records of the time, number, and locations of introductions of *A. saligna* to South Africa, the source of seeds and the subspecific identity of invasive populations remain unknown. Various control measures have been used including mechanical, chemical and biological control (Wood & Morris, 2007). While the introduction of classical biological control agents has substantially reduced the density of infestations (Impson *et al.* 2011), *A. saligna* remains one of the most costly invasive plants in South Africa (van Wilgen *et al.* 2012).

Our overall goal in this study was to improve our understanding of *A. saligna* invasions by examining which subspecific entities are present in South Africa using pure native lineages (i.e. referred to as reference populations from here on) from Western Australia (Millar *et al.* 2011a).

Ultimately we hope this will guide the use of future biological control agents, and provide insight into the invasion dynamics of other invasive acacia species already present in South Africa and other regions. Specifically, we use DNA sequence and microsatellite variation to: 1) place the invasive populations within a framework of spatial genetic structure among different subspecies/genetic lineages of *A. saligna* in their native range; 2) compare levels of genetic diversity in invasive populations of *A. saligna* to those in the native range; 3) relate the population genetic structure of invasive *A. saligna* to its known invasion dynamics; and 4) discuss the implications of our findings for the management of *A. saligna* in South Africa.

## **3.2 Methods**

### ***(a) Sampling design and DNA isolation***

Phyllode material of *A. saligna* was collected from 163 individuals from the introduced range in South Africa. We also included a single native individual from Wilbinga, as well as individuals introduced to New South Wales and South Australia (five individuals), Israel (two individuals) and Spain (one individual, Table 1). Due to low sample sizes these (non-South African) accessions were only included in our phylogeographic datasets (see below). For comparisons between South Africa and the native range, we used DNA from eight reference populations included in Millar *et al.* (2011a) to identify intra-specific variants (Table 3.1); these are considered to represent pure native lineages of *A. saligna*. Collections were also made from seven additional populations from Western Australia that did not have definitive subspecies identifications. In addition, a collection was made at the original locality (Busselton) from which the fungal biological control agent, *Uromycladium tepperianum*, was collected for release in South Africa (Morris 1991). We also downloaded one ETS sequence from GenBank for a cultivated specimen of *A. saligna* that originated from a glasshouse specimen in Canberra, Australian Capital Territory, Australia (GenBank number: FJ868448.1; herbarium specimen number CANB 634053.1). Phyllode material was dried and stored on silica gel until DNA extraction. Genomic DNA was extracted using a

modified cetyltrimethyl ammonium bromide (CTAB) method (Doyle & Doyle 1990) with the addition of 0.2 M sodium sulphite to the extraction and wash buffers following Byrne *et al.* (2001).

*(b) DNA sequencing and data analysis*

One nuclear (external transcribed spacer, Brown *et al.* 2008) and one chloroplast (trnQ - 5'rps16, Shaw *et al.* 2007) gene were amplified for all accessions where possible. See Appendix A in the supporting information for amplification conditions.

Sequence data were aligned and edited using BIOEDIT v 7.0.5.3 (Hall 1999). DnaSP v.5 (Librado & Rozas 2009) was used to identify different ETS sequences, and calculate the average number of haplotypes ( $N_H$ ), haplotype diversity ( $h$ ) and nucleotide diversity ( $p$ ) for the native and introduced ranges. For nDNA we used MODELTEST v.3.7 to determine the best-fit nucleotide substitution model (Posada & Crandall 2001) under the Akaike Information Criterion (AIC). Maximum-Likelihood analysis was conducted in PAUP\* v.4b10 (Swofford 1999), using the TPM1uf model selected by MODELTEST (Kimura 1981), and the heuristic search option. Support for internal branches was evaluated using 10 000 bootstrap replicates (Felsenstein 1985). nDNA phylogenetic reconstructions were rooted using two closely related species (*A. cupularis* and *A. rostelifera*, GenBank numbers: JF420247 and JF420272, respectively) known to be sister to *A. saligna* (see Miller *et al.* 2011). Population pairwise  $\Phi_{ST}$  was calculated with 10 000 permutations in ARLEQUIN v.3.5 (Excoffier *et al.* 2005). To assess genetic differentiation among sampling sites, we conducted a hierarchical analysis of molecular variance (AMOVA; Excoffier *et al.* 1992) in ARLEQUIN using 10 000 permutations. Relationships among the trnQ - 5'rps16 haplotypes were examined using statistical parsimony to reconstruct haplotype networks generated at the 95% connection limit with TCS v.1.21 (Clement *et al.* 2000). Due to the low resolution present within the trnQ - 5'rps16 region for *A. saligna*, we did not conduct further analyses to assess population structure (i.e. population pairwise  $\Phi_{ST}$ ), or the distribution of genetic variation (i.e. AMOVA).

(c) *Microsatellite genotyping and data analysis*

Ten nuclear microsatellite loci previously developed and characterised for *A. saligna* (Millar & Byrne 2007) were PCR-amplified in two separate multiplex reactions (5 loci per multiplex) for each sample. Populations that had less than 5 individuals were not genotyped (populations from Wilbinga, South Australia, New South Wales, Spain and Israel). Each 10  $\mu$ L reaction contained 0.25 U Taq polymerase (KapaBiosystems, Cape Town, South Africa), 1.5 mM  $MgCl_2$ , 10 mM Tris-HCl (pH 8.4), 50 mM KCl, 0.2 mM of each dNTP, 5  $\mu$ M of each primer and  $\sim$ 10 ng/ $\mu$ L genomic DNA. Thermocycling consisted of initial denaturation at 95  $^{\circ}C$  for 2 min, followed by 35 cycles of 95  $^{\circ}C$  for 15 s, 56  $^{\circ}C$  for 30 s, 72  $^{\circ}C$  for 10 s; no final extension was required. PCR fragments were separated on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, USA), using GENESCAN<sup>TM</sup>-500 (-250) as an internal size standard (Applied Biosystems). Allele sizes were visualized and scored using GENEMARKER<sup>®</sup> v1.95 (SoftGenetics LLC<sup>®</sup>, Pennsylvania, USA).

(d) *Isolation by distance*

Recent studies have suggested that the presence of strong isolation by distance (IBD) in microsatellite data can lead to incorrect deductions on the history of populations (Guillot *et al.* 2009). Consequently, we chose to test for IBD prior to further tests of genetic diversity and population structure. IBD analyses were computed for South Africa and Western Australia separately using Mantel tests and the online resource IBDWS v 3.16 (Jensen *et al.* 2005). For these, matrices of pairwise genetic distances ( $F_{ST}$  values calculated in ARLEQUIN) were plotted against geographical distances (Euclidian distances calculated in GENALEX v 6.4). The upper and lower 95 % confidence limits were set using 10 000 permutations.

(e) *Genetic diversity*

For the combined native and introduced dataset, microsatellite data were tested for departures from Hardy-Weinberg Equilibrium (HWE) using 1 000 000 steps in the Markov chain in

ARLEQUIN (Excoffier & Lischer 2010). We also tested for linkage disequilibrium for all pairs of loci in ARLEQUIN. For a broad overview of within population genetic diversity parameters, we compared the native (Western Australia) and introduced range (South Africa) by calculating the total number of alleles ( $N_A$ ), allelic richness ( $R_S$ ), mean observed and expected heterozygosities ( $H_E$  and  $H_O$ ), the fixation index ( $F_{ST}$ ), and the inbreeding coefficient ( $F_{IS}$ ) in FSTAT v. 2.9.3.2 (Goudet 2001). FSTAT was used as it compensates for unequal sample sizes between populations. For a finer scale analysis of diversity within individual populations, the mean of the following parameters were computed for polymorphic loci in ARLEQUIN (Excoffier & Lischer 2010): number of alleles ( $N_A$ ), observed and expected heterozygosity ( $H_O$  and  $H_E$ ) and inbreeding coefficients ( $F_{IS}$ ). We also calculated the mean number of private alleles per population ( $P_A$ ) in GENALEX (Peakall & Smouse 2006).

*(f) Population genetic structure*

Several Bayesian clustering algorithms are available to determine the most likely number of biological populations or genetic demes ( $K$ , see Guillot *et al.* 2009 for a review of methods and software), each with advantages and drawbacks (e.g. Rowe & Beebee 2007). We used three different, spatially explicit Bayesian clustering algorithms. We chose spatially explicit models that incorporate admixture in all cases as these models are more robust than models that do not incorporate admixture, and are better able to identify the optimal number of genetic clusters (François & Durand 2010). The three models employed were implemented in STRUCTURE v 2.3.2 (Falush *et al.* 2007), GENELAND v 3.1.4 (Guillot *et al.* 2005) and TESS v 2.3.1 (Chen *et al.* 2007) respectively. For more details on model parameters and settings refer to Appendix B in the supporting information. To assess the effect allelic associations might have on genetic clustering of populations and regions (e.g. see Rosenthal *et al.* 2008) we compared both the number of private alleles in native and introduced ranges, and identified differences in allelic frequencies that exceeded ten percent between ranges in GENALEX.

(g) *Comparative genetic distances between species and subspecies*

In order to compare the divergence present within species and subspecies in the genus *Acacia* (subgenus *Phyllodineae*) to the divergence present within *A. saligna*, we downloaded available ETS DNA sequence data from GenBank. For species comparisons we selected one of the closest relatives of *A. saligna*, *A. rostellifera* (Miller *et al.* 2011). For subspecies comparisons we selected *A. longifolia* (subspecies *sophorae* and subspecies *longifolia*). A matrix of pairwise genetic distances was calculated using DNADist in BIOEDIT (Hall 1999).

(h) *Visualisation of genetic distance*

To provide further support for the genetic groups inferred by phylogenetic reconstructions we plotted pairwise genetic distances for the nuclear and chloroplast DNA sequences using a non-metric multidimensional scaling analysis. Pairwise genetic distances between all individuals for the ETS and trnQ-5'rps16 genes were calculated in BIOEDIT (Hall 1999). Accessions were clustered using a non-metric multidimensional scaling analysis (NMDS), and the 'ratio + bounds' setting in PERMAP v 11.8a (Heady & Lucas 2007), and a highly accurate convergence value of 0.000005. Ten iterations were conducted, where each new iteration was initiated manually when the objective function moved towards a minimum value. Proximity co-ordinates for each individual were obtained from the solution with the lowest objective function value and plotted in R using the 'car' package (Fox & Weisberg 2011).

(i) *Spatial distribution of genetic diversity*

To determine the distribution of genetic variation (in the nuclear microsatellites and nDNA sequence data) between groups of individuals or populations at different scales (e.g. country or continental scale) we conducted an analysis of molecular variance (AMOVA) using ARLEQUIN (Excoffier & Lischer 2010). We partitioned total genetic variance at three hierarchical levels—among invasive and native regions, among populations within regions, and within populations. The

degree of population differentiation and spatial variation were also estimated by computing population pairwise  $F_{ST}$  values for all populations in Western Australia and South Africa independently. This analysis was conducted in ARLEQUIN where the  $F_{ST}$  significance levels were assessed using a Bonferroni adjustment for multiple comparisons (Weir 1996). Lastly, the distribution of genotypes in the native and invasive range was further assessed by combining genetic and geographic distance for all sampled individuals of *A. saligna* using a co-variance standardized Principal Coordinate Analysis (PC<sub>O</sub>A) in GENALEX (Peakall & Smouse 2006) and 1 000 permutations.

### 3.3 **Results**

#### (a) *Genetic diversity*

There was no evidence of significant IBD in either the native ( $r^2 = 0.0255$ ,  $p = 0.9245$ ) or introduced ( $r^2 = 0.0009$ ,  $p = 0.5510$ ) ranges of *A. saligna* (Appendix 3C - Fig. S3.1, Supporting Information). Consequently, the effects of IBD were not considered in further analyses. Only two pairs of loci displayed significant levels of linkage disequilibrium ( $p = 0.001$ , data not presented).

All ten microsatellite loci were found to be polymorphic. At these loci there was a larger number of alleles ( $N_A$ ), higher levels of allelic richness ( $R_S$ ), and more unbiased gene diversity ( $H_S$ ) in native populations compared to introduced populations; whereas introduced populations were more inbred and had less differentiation than native populations (Table 3.2, Appendix 3C - Table S3.1, Supporting Information).

#### (b) *Native genetic structure*

The clustering algorithms varied in the optimal number of native genetic clusters:  $K_{\text{native}} = 3$  for STRUCTURE (Fig. 3.1, Appendix 3C – Fig. S3.2, Supporting Information),  $K_{\text{native}} = 5$  for TESS (Appendix C - Fig. S3.3, Supporting Information) and  $K_{\text{native}} = 6$  for GENELAND (Appendix 3C - Fig. S3.4, Supporting Information). Despite this incongruence, the most frequently retrieved genetic

cluster (i.e. most dominant) in all analyses was consistent with the findings of Millar *et al.* (2011a) and morphological identification of herbarium specimens by Bruce Maslin (*Acacia* expert, Dept. of Environment and Conservation, Western Australia). This cluster included individuals of *A. saligna* subspecies '*saligna*' and subspecies '*pruinescens*' (Figs. 3.1, Appendix 3C – Fig. S3.2, S3.5, Supporting Information), and is consistent with the findings of Millar *et al.* (2011a) i.e. subspecies '*saligna*' and '*pruinescens*' are indistinguishable based on microsatellite data.

Assignments of native populations by STRUCTURE (Fig. 3.1, Appendix 3C – Fig. S3.2, Supporting Information) and TESS (Appendix 3C - Fig. S3.3, Supporting Information) were congruent for the majority of sites. The clusters retrieved were similar to those identified by Millar *et al.* (2011a). We used reference populations from Millar *et al.* (2011a) to assign subspecies names to each cluster: cluster 1 - *A. saligna* subspecies '*saligna*'; cluster 2 - *A. saligna* subspecies '*lindleyi*', and cluster 3 - *A. saligna* subspecies '*stolonifera*'. Overall, these groups were consistent with the relationships resolved by maximum likelihood based on ETS data (Fig. 3.2). GENELAND (Appendix 3C – Fig. S3.4, Supporting Information) gave somewhat different results – it identified a greater degree of genetic structure and did not identify any substantially mixed populations. It assigned the majority of populations (8 of 14) to a single cluster (different populations compared to STRUCTURE and TESS), and identified a number of geographically localised populations (Muntagin, Leschnault Inlet, Wanneroo and Ravensthorpe) that were assigned to unique genetic clusters.

All ten microsatellite loci yielded allelic frequencies that differed by more than ten percent between the native and introduced ranges (Appendix 3C - Fig. S3.6, Supporting Information). We identified a total of 32 alleles, seven of which represented alleles only found in native populations (i.e. they were unique to Western Australia).

(c) *DNA sequence variation and phylogeography*

The 485 bp ETS alignment contained a total of 109 polymorphic sites, 49 of which were parsimony-informative. Within the 50 individuals, we identified 27 distinct sequences, with 12 sequences unique to Western Australian, 12 sequences unique to South Africa and two sequences shared between regions. The remaining sequence was unique to South Australia (see 2310, Appendix 3C - Fig. S3.7C, Supporting Information). A number of native gene sequences were restricted to single populations in the native range (Appendix 3C - Fig. S3.7A, Supporting Information), which was not the case for introduced populations (Appendix 3C - Fig. S3.7B, Supporting Information). The most common DNA sequence in Western Australia was restricted to two geographically adjacent populations (Muntagin and Wickepin) in the north-eastern part of the native range of *A. saligna* (Appendix 3C - Fig. S3.7A, Supporting Information). These populations were identified as *A. saligna* subspecies '*lindleyi*' during Bayesian clustering (Fig. 3.1).

Sequence variation identified a number of features that were congruent with the nuclear microsatellite clustering of native populations. Individuals collected in populations that displayed mixed affinities in nuclear microsatellite clustering also lacked phylogenetic affinity in well supported clades (notably the Busselton population, Fig. 3.2). The ETS phylogeny identified two main clades in the native range of the *A. saligna* species complex, while evidence for a third cluster containing all individuals representative of *A. saligna* subspecies '*lindleyi*' was present, but did not have significant support (nodal support < 70, Fig. 3.2). These results were not in complete agreement with the microsatellite structure identified by the STRUCTURE and TESS assignment analyses. The first clade represented *A. saligna* subspecies '*saligna*', while the second well supported clade represented *A. saligna* subspecies '*stolonifera*'. The remaining accessions of *A. saligna* subspecies '*lindleyi*' were identified as sister taxa to the *A. saligna* subspecies '*saligna*' clade. The clustering of pairwise genetic distances for nDNA accessions (Appendix 3C - Fig. S3.8B, Supporting Information) supported the three clades identified by microsatellite clustering in STRUCTURE.

Statistical parsimony of cpDNA also identified two very divergent lineages within *A. saligna*. However, these divergent lineages were incongruent with the ETS phylogeny in the placement of taxa (Fig.3. 2). The 722 bp trnQ-5'rps16 alignment contained only four parsimony-informative sites and eight distinct haplotypes were identified (Fig.3. 2B). The first lineage included native reference individuals of *A. saligna* subspecies '*lindleyi*' and *A. saligna* subspecies '*saligna*', as well as introduced individuals from South Africa, South Australia, New South Wales, Israel and Spain (Fig. 3.2B). The second lineage included native reference individuals of *A. saligna* subspecies '*stolonifera*', additional individuals from the Busselton population in Western Australia, and two individuals from South Australia (Fig. 3.2B).

NMDS of pairwise genetic distances for native and introduced populations further illustrated incongruences between the cpDNA and nDNA (Appendix 3C - Fig. S3.8, Supporting Information). However, the same incongruences were identified by phylogenetic reconstructions and Bayesian clustering. The two major groups retrieved for cpDNA data clearly differentiated *A. saligna* subspecies '*stolonifera*' from all other native and introduced accessions (Appendix 3C - Fig. S3.8A, Supporting Information). Three major native groups were retrieved from the nDNA data representing the subspecies of *A. saligna*; and one additional group (all South African accessions, and accessions from Busselton, Dinninup and Wanneroo in Western Australia, Appendix 3C - Fig. S3.8B, Supporting Information).

The PCoA identified similar results to the NMDS analysis (Appendix 3C - Fig. S3.5 and S3.8 respectively, Supporting Information). Specifically, the PCoA identified three groups: Group one included native populations of *A. saligna* subspecies '*lindleyi*'. Group two included populations of *A. saligna* subspecies '*saligna*' and *A. saligna* subspecies '*stolonifera*'. Group three included all South African populations, and Tuart Forest and Busselton populations from Western Australia.

Assessment of genetic variance between the native and introduced ranges showed a moderate level of microsatellite diversity was partitioned among the native and introduced range (14.5 %), while the majority of microsatellite diversity was partitioned within populations (Table

3.3). A similar pattern was found in the nuclear sequence data, with moderate but lower diversity among populations (7.7 %) and the majority of diversity was partitioned within populations (61.2 %, Table 3.3). Overall, population pairwise  $F_{ST}$  values indicate moderate to high differentiation in native populations, and low to moderate population genetic differentiation in the introduced range (Appendix 3C - Table S3.1, Supporting Information).

The Bayesian clustering algorithms all broadly separated native and introduced populations, although they identified different numbers of optimal K-clusters (Fig. 3.1, Appendix 3C – Fig. S3.2, S3.3, S3.4, Supporting Information). STRUCTURE identified two genetic clusters, broadly corresponding to native and introduced populations (Appendix 3C - Fig. S3.9, Supporting Information). Overall the STRUCTURE analysis showed that all introduced South African populations displayed the closest genetic affinity to populations Busselton and Tuart Forest from the native range (Fig. 3.3A, Appendix 3C – Fig. S3.5, S3.7, S3.9 Supporting Information). GENELAND identified seven clusters, and assigned individuals to a genetic cluster with membership coefficients ( $q_i$ ) of greater than 0.95. GENELAND was also the only algorithm that identified more than one genetic cluster in the introduced populations (Fig. 3.3). TESS identified five clusters (Appendix 3C - Fig. S3.3B, Supporting Information). Similarly to the STRUCTURE results, the TESS analysis showed that introduced populations displayed the strongest genetic affinity to populations at Busselton (assigned with a  $q_i$  of greater than 0.7).

Overall, the divergence present within the native subspecies of *A. saligna*, and native and introduced clades was substantially greater than divergences observed within other subspecies or species of *Acacia* (Appendix 3C - Table S3.2, Supporting Information). The genetic distance between the native and introduced clade of *A. saligna* (see Fig. 3.2) was within the same order of magnitude as the genetic distance between native *A. saligna* subspecies '*lindleyi*', and its closest relative, *A. rostellifera* (Appendix 3C - Table S3.2, Supporting Information). In addition, the genetic distance between the native subspecies of *A. saligna* was an order of magnitude greater than the genetic distance between the different subspecies of *A. longifolia*.

### 3.4 Discussion

Our results indicate that the introduction efforts of *A. saligna* into South Africa have led to an invasion that is characterized by unstructured, high genetic diversity that is divergent from that found in pure native lineages in Western Australia. Genetic divergence and novelty of this magnitude can arise through numerous processes, including strong drift (e.g. Roy & Buronfosse 2011), post-introduction selection (e.g. Lavergne & Molofsky 2007), admixture (e.g. Kolbe 2007) and inter-specific hybridization (e.g. Prentis *et al.* 2009).

Both sets of nuclear data (microsatellite and DNA sequence) suggest that admixture between different subspecies has not occurred in South Africa. Indeed, South African populations shared no close relationship with any of the known informal subspecies of *A. saligna* (i.e. reference populations, ETS data). Furthermore, we rule out the possibility that paralogous gene regions may explain the observed patterns as we sequenced multiple cloned gene copies for the ETS region for a number of taxa, and never retrieved multiple copies from the same individual from both major clades. It is also unlikely that the invasive lineage represents an un-sampled native lineage since: a) we extensively sampled the *A. saligna* complex throughout its distribution in Western Australia; and b) we included populations representative of the three known genetic lineages in our analyses (Millar *et al.* 2011a). The South African populations shared limited ETS genetic information with additionally sampled populations from the native range, one of which (the Busselton population) appears to be cultivated or planted.

*Acacia saligna* has been widely planted for agroforestry and as a roadside species throughout Western Australia (Maslin & MacDonald 2004). Unfortunately, identification of planted stands in the field is very difficult, even for experts (W. O'Sullivan, pers. comm.). Field inspection of the Western Australian population (Busselton, south of Perth) most closely related to South Africa populations, confirmed that this site was indeed planted (B. Maslin and W. O'Sullivan, pers. comm.). Microsatellite divergence between the same population (Busselton) and pure native lineages of *A. saligna* was too large to assign an existing subspecies identity to this population.

Field inspection of other Western Australian populations closely related to South African populations based on nuclear sequence data (i.e. Wanneroo), suggested that these populations may be natural.

Unfortunately, there are no detailed historical records of the location of *A. saligna* plantings in Western Australia, and no information on the source of seed used in these plantings, nor is the source of seeds exported from Australia known. Our genetic results suggest that the origin of South African propagules is the same as the source of Western Australian plantings. The earliest herbarium record of a cultivated *A. saligna* tree is from Western Australia in 1838 in the Swan River region (Royal Botanic Gardens Melbourne, MELISR database, accessed 18 August 2011). Interestingly, the earliest records of seeds imported to South Africa was at a similar time (in 1833, Poynton 2009). The number of introductions and scale of seeds introduced to South Africa (several thousand to several million; Roux 1961, Poynton 2009) suggest that collections of seeds must have come from large mature stands likely only present in the native range. In addition, the presence of a central seed distributor in South Africa (Cape Seed Store, see Poynton 2009) may explain the lack of genetic structure throughout South Africa.

While cultivation could give rise to the genetic differences observed between planted, invasive and native *A. saligna* populations, the incongruence between cpDNA and nDNA phylogenies is currently unexplained. There were no genetic similarities between introduced South African populations and native reference populations of *A. saligna* subspecies '*stolonifera*' at the nDNA or cpDNA gene regions examined (Fig. 3.2, Appendix 3C – Fig. S3.3, S3.8, Supporting Information). However, South African individuals and native individuals of *A. saligna* subspecies '*lindleyi*', '*pruinescens*' and '*saligna*' (Millar *et al.* 2011a) appeared to be related (cpDNA, Fig. 3.3, 3.4A). It is thus unlikely that South African populations originated from populations of *A. saligna* subspecies '*stolonifera*' in Western Australia, but may have originated from hybridisation between a number of parental lineages of *A. saligna* subspecies '*lindleyi*', '*pruinescens*' and '*saligna*'. Although discrepancies between nuclear and chloroplast phylogenies can be caused by a variety

of factors [e.g. lineage sorting of ancestral polymorphisms or non-homologous sampling of duplicated genes (unlikely as we included multiple clones accessions of the nuclear gene)], we suggest that our results most likely represent introgressive hybridization (hybridization followed by backcrossing) and led to chloroplast capture within *A. saligna*.

If introgression had occurred between parental lineages of *A. saligna* (subspecies '*lindleyi*', '*pruinescens*' and '*saligna*') and a closely related, but currently unknown species, then we would expect that the genetic distance between the South African clade and different subspecies of *A. saligna* would be substantial. This is precisely what we observed in the nuclear DNA. The genetic distance between the South African clade and the native clade was approximately the same order of magnitude to the distance between native *A. saligna* and its sister taxa, *A. rostellifera* (Table S3.2). Indeed, the distance between the native and introduced clade far exceeds our observations (based on available data retrieved from GenBank) of divergences between subspecies of Australian acacias (but see Wardill *et al.* 2003), and is on a level with divergences at the species level. Our microsatellite analyses also support this hypothesis. It appears that the divergence between native and introduced ranges is largely driven by private alleles present within each range despite a thorough sampling of native populations. This is further supported when considering differences in allele frequencies, with one fifth of all alleles that differed by more than 10 % in their frequencies between ranges, being private alleles restricted to the native range.

Many species that are now invasive were introduced to new regions for their economic value. Such species have been subject to cultivation and breeding practices to artificially select advantageous traits to promote faster growth rates or higher biomass production. Consequently, cultivated genotypes present in the introduced range may be fitter than their native counterparts (e.g. Lavergne & Molofsky 2007), and these may pose a greater threat as an invasive species (e.g. *Mahonia aquifolium*; Ross & Auge 2008). A number of successful invaders have been subject to some form of cultivation or breeding that may have facilitated persistence in a new environment

(Ross 2009). Thus, the selection pressures imposed on a species by cultivation may play a substantial role in invasive success.

We recommend that future research should focus on comparing quantitative and qualitative traits of native and invasive genotypes of *A. saligna* under common garden conditions. Such experiments would allow the testing of native genetic variation in concert with heritable phenotypic variation. Furthermore, the genetic dissimilarity of native and introduced populations may be related to possible increased fitness effects of cultivation in the native range.

In agreement with our genetic data, previous work has shown that the subspecies of *A. saligna* differ dramatically in the bioclimatic niches they occupy in Western Australia, and their potential range in South Africa (Thompson *et al.* 2011). The novel genetic entity identified here means that predictions of potential range size using environmental tolerances of genetic entities in the native range will be inaccurate. In such cases, where the taxon has had sufficient residence time to sample potential invulnerable sites, predictions based on introduced environmental distribution correlates, are likely to offer better results (Rouget *et al.* 2004). Clearly, the assumption that introduced taxa lumped under the name of "*A. saligna*" will perform similarly throughout their introduced range is problematic.

The dissimilarity in genetic composition between the native and introduced range and habit of biological control agents could significantly affect the overall success of control programmes. Assuming that genetic similarity will translate into host-specificity, our findings suggest that the biocontrol agent (*U. tepperianum*) was, perhaps fortuitously, collected from a suitable Western Australian source (Busselton) of *A. saligna*. The suggested common garden experiments, including pathogenicity and host-specificity tests on various sources of *U. tepperianum* from Western Australia, may further enhance control in South Africa.

In summary, our results show how cultivation, the number and size of introduction events, human-mediated transport, genetic drift, and possibly introgressive hybridization, can act swiftly and concurrently to create genotypic novelty. Such genotypic novelty has important implications for

management, e.g. in predicting potential range and assessing options for classical biological control. In the absence of such a holistic approach, we have demonstrated that taxonomic identity and biogeographic provenance(s) alone, aspects crucial for the initial implementation of successful management, can easily lead to erroneous deductions. Our study not only shows the value of using different molecular approaches to understand invasion histories, but also raises the fundamental question of whether (and how quickly) introduced species can be regarded as fundamentally different entities to their native counter-parts (Müller-Schärer *et al.* 2004).

### *Acknowledgements*

This research was funded by the DST-NRF Centre of Excellence for Invasion Biology and the Working for Water Programme through the collaborative research project on “Research for Integrated Management of Invasive Alien Species”. DMR and JJLR acknowledge additional funding from the National Research Foundation. We thank Bruce Maslin and Wayne O’ Sullivan for providing valuable support and advice throughout the project, and Australian herbaria for providing detailed records for *A. saligna*. We also thank Jean-Marc Dufour and Oscar Godoy for providing samples from Israel and Spain respectively.

### *Author contributions*

J.R.U.W, D.M.R, M.B and M.A.M initiated the collaboration. G.D.T, J.J. Le Roux, J.R.U.W and D.M.R conceived the research ideas. G.D.T and J.J. Le Roux designed the research methods. M.B and M.A.M contributed genomic DNA. G.D.T. generated and analysed all molecular data and led the writing.

### 3.5 Tables and Figures

**Table 3.1** Microsatellite genetic diversity indices for native and introduced populations of *Acacia saligna*.

Locality	ID	N	$N_A$	$N_{PA}^{**}$	$H_o$	$H_e$	Latitude	Longitude
<b>Native</b>								
<i>Western Australia</i>								
Parkeyerring <sup>‡</sup>	PAR	5	3.6	4	0.439	0.683	-33.362	117.356
Ravensthorpe <sup>‡</sup>	RAV	5	3	2	0.439	0.567	-33.258	119.751
Wellesley	WEL	21	3.1	4	0.443	0.475	-33.148	115.742
Busselton	BUS	21	4.6	1	0.451	0.522	-33.661	115.358
Tuart Forest	TUA	11	3.7	2	0.394	0.498	-33.54	115.508
Dinninup <sup>‡</sup>	DIN	28	5.2	7	0.415	0.603	-33.813	116.534
Wilbinga <sup>‡</sup>	WIL	1	-	-	-	-	-31.438	115.663
Wanneroo <sup>†</sup>	WAN	13	3.1	1	0.338	0.428	-31.438	115.663
Leshnault Inlet <sup>†</sup>	WEI	14	3.3	2	0.38	0.391	-33.218	115.693
Mount Ney <sup>†</sup>	MTN	14	3.5	7	0.462	0.52	-33.398	122.466
Preston <sup>†</sup>	PRE	14	3.6	3	0.365	0.529	-33.529	115.97
Muntagin <sup>†</sup>	MUN	15	3.7	6	0.482	0.484	-31.758	118.583
Tweed River <sup>†</sup>	TWR	14	2.9	2	0.36	0.386	-34.58	116.492
Wickepin <sup>†</sup>	WIC	15	4.4	5	0.468	0.544	-32.63	117.384
Boyatup Hill <sup>†</sup>	BOY	12	3.2	4	0.408	0.444	-33.738	123.044
<b>Introduced</b>								
<i>South Africa</i>								
Cinsta <sup>‡</sup>	CIN	31	3.8	3	0.29	0.392	-32.845	28.113
Ebenhaezer <sup>‡</sup>	EBE	24	3.3	5	0.334	0.434	-31.586	18.242
Breede River <sup>‡</sup>	BRE	14	3.5	3	0.412	0.485	-34.12	20.034
Port Alfred	PA	28	2.8	1	0.305	0.363	-33.554	26.893
Sedgefield	SED	15	3.3	2	0.393	0.496	-34.011	22.779
Jeffrey's Bay	JBAY	33	4	5	0.288	0.499	-34.052	24.922
Albertinia <sup>*</sup>	ALB	18	3.2	1	0.299	0.375	-34.137	21.699
<i>Australia</i>								
Tintinara, South Australia <sup>*</sup>	TIN	2	-	-	-	-	-35.921	140.101
Sydney, New South Wales <sup>*</sup>	SYD	3	-	-	-	-	-33.765	151.233
<i>Eurasia</i>								
Israel	ISR	2	-	-	-	-	31.736	34.617
Spain <sup>*</sup>	SPA	1	-	-	-	-	36.72	-4.42

\*\* Calculated in GENALEX

<sup>†</sup> reference populations of *A. saligna* from Millar *et al.* (2011).

<sup>\*</sup>Populations without ETS data

<sup>‡</sup>Populations without trnQ-5'rps16 data

Note:  $N$ , number of individuals genotyped / sequenced at site,  $N_A$ , mean number of alleles;  $N_{PA}$ , number of private alleles;  $H_o$ , mean observed heterozygosity;  $H_e$ , mean expected heterozygosity

**Table 3.2** Overall microsatellite genetic diversity indices for the native and introduced range of *Acacia saligna*.

	$R_S$	$H_S$	$H_O^*$	$F_{IS}^*$	$F_{ST}^*$
Native	1.490	0.506	0.414	0.181	0.330
Invasive	1.452	0.457	0.310	0.322	0.132

\*  $P < 0.05$

Note: Allelic richness ( $R_S$ ), unbiased gene diversity ( $H_S$ ), observed heterozygosity ( $H_O$ ), inbreeding coefficient ( $F_{IS}$ ) and among population differentiation ( $F_{ST}$ ).

**Table 3.3** A hierarchical AMOVA partitioning of genetic variation in *Acacia saligna* for nuclear microsatellite and sequence variation (nDNA) at various spatial scales: among native and invasive regions; among populations and within populations within native and invasive regions.

Source of variation	d.f.	Sum of squares	Variance	Percent variation (%)	Fixation index*
<b>nDNA</b>					
Among native and invasive range	1	800.15	33.546	31.1	0.11118
Among populations	15	1308.29	8.248	7.7	0.38795
Within populations	31	2044.06	65.937	61.2	0.31139
<b>Microsatellite</b>					
Among native and invasive range	1	159.88	0.37722	14.5	0.34044
Among populations	19	362.26	0.51057	19.6	0.22890
Within populations	709	1219.48	1.72000	66.0	0.14465

\* All values significant, significance was tested using 10 000 random permutations.

## Figure Legends

**Figure 3.1** Bayesian assignment of native genetic groups within the *Acacia saligna* complex, overlaid with known native distribution records for each of the four subspecies. Distribution records are based on morphological identification and were obtained from Australia's Virtual Herbarium online database ([avh.rbg.vic.gov.au](http://avh.rbg.vic.gov.au), accessed 1 October 2010). Membership of each individual's genome ( $q_i$ ) to the three identified genetic clusters is indicated by vertical bars. Pie charts show overall genotype assignment for each population to particular genetic clusters. Reference populations of known informal subspecies were labelled according to Millar *et al.* (2011): lin (subspecies '*lindleyi*'), sto (subspecies '*stolonifera*'), pru+sal (subspecies '*pruinescens*' and subspecies '*saligna*').

**Figure 3.2** Phylogenetic relationships within and among native and introduced populations of *Acacia saligna* based on (A) nDNA (maximum likelihood [CI = 0.950, RI = 0.981]) and (B) cpDNA (parsimony haplotype network, inset). In (A) all individuals are labelled by population name and symbols indicate the subspecies identified in Millar *et al.* (2011). Tree branch lengths are scaled according to genetic distance and bold branches represent strongly supported relationships (nodal support > 70). In (B) the shading differentiates between native and introduced populations. For both analyses native subspecies were identified in Millar *et al.* (2011), while introduced populations are labelled by country or state of origin.

**Figure 3.3** Identification of the number of distinct genetic groups of *Acacia saligna* in the native (Western Australia) and introduced (South Africa) range using three Bayesian clustering algorithms. The data sets contain a total of 365 individuals genotyped at 10 nuclear microsatellite loci. Membership of each individual's genome ( $q_i$ ) to the inferred number of genetic clusters is indicated by vertical bars. Pie charts show overall genotype assignment for each population to particular genetic clusters. Reference populations of known informal subspecies were labelled according to Millar *et al.* (2011): lin (subspecies '*lindleyi*'), sto (subspecies '*stolonifera*'), pru (subspecies '*pruinescens*') and sal (subspecies '*saligna*').

## 3.6 Appendices

**Appendix 3A** METHODS FOR AMPLIFICATION OF GENE REGIONS.

**Appendix 3B** BAYESIAN CLUSTERING METHODS.

**Appendix 3C** ADDITIONAL TABLES AND FIGURES FROM RESULTS

**Figure S3.1** Isolation by distance analysis for native and introduced populations of *A. saligna*.

**Figure S3.2** Principal Component analysis of microsatellite data

**Figure S3.3** Bayesian clustering of native populations using GENELAND and TESS.

**Figure S3.4** Identification of the number of clusters in the native and introduced range using TESS.

**Figure S3.5** Hierarchical clustering in the native range using STRUCTURE.

**Figure S3.6** Native and introduced allelic frequencies.

**Figure S3.7** nDNA statistical parsimony network of native and introduced *A. saligna* accessions.

**Figure S3.8** Multi-dimensional scaling of ETS and trnQ data.

**Figure S3.9** Hierarchical clustering in the native and introduced range using STRUCTURE.

**Table S3.1** Population genetic structure in the native and introduced range.

**Table S3.2** Genetic distances between species and subspecies of acacias.

### Appendix 3A AMPLIFICATION OF NUCLEAR AND CHLOROPLAST GENE REGIONS

The nuclear ETS region was amplified using the primers described in Brown *et al.* (2008), and the PCR setup and conditions described in Le Roux *et al.* (2011). Accessions that did not produce clean sequences were cloned using the pGEM® -T Easy Vector System (Promega, Anatech, Johannesburg, South Africa), and had a number of inserts sequenced. The chloroplast region (trnQ - 5'rps16) was amplified using the primers described in Shaw *et al.* (2007), and the following PCR conditions: each 50 µL reaction contained approximately 30 ng of genomic DNA, 200 µM of each dNTP (AB gene; Southern Cross Biotechnologies, Cape Town, South Africa), 25 µmol of each primer, 0.5 U Taq DNA polymerase (Super-Therm JMR-801; Southern Cross Biotechnologies), 10 X PCR reaction buffer, 3 mM MgCl<sub>2</sub>. The reaction was held at 95 °C for 5 minutes prior to the addition of Taq. Thermocycling consisted of initial denaturation at 95 °C for 2 min, 35 cycles of 95 °C for 15 s, 56 °C for 30 s, 72 °C for 10 s; and a final extension of 72 °C for 10 min. Amplified DNA fragments were purified using the QIAquick PCR Purification kit (Qiagen, Cape Town, South Africa, Southern Cross Biotechnologies), sequenced using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction kit (forward only) and an automated ABI PRISM 377XL DNA sequencer (PE Applied Biosystems, Foster City, CA, USA).

## Appendix 3B BAYESIAN CLUSTERING METHODS

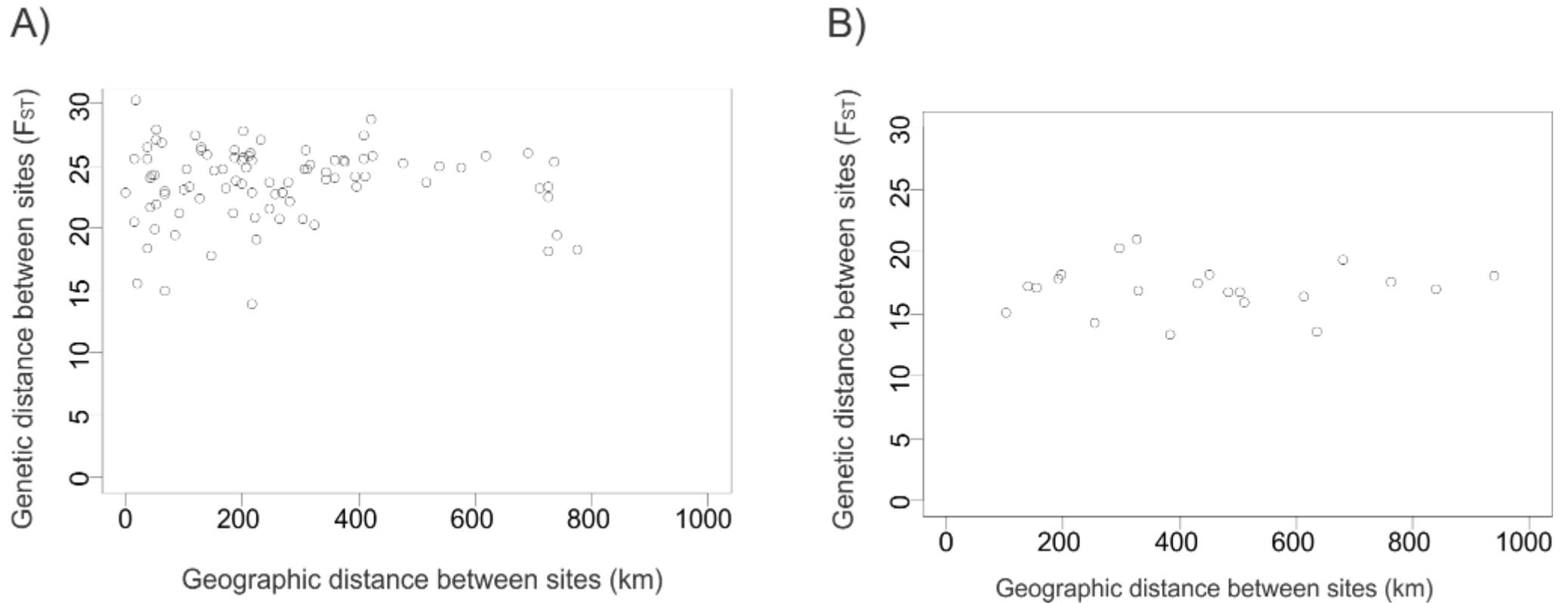
First we used a hierarchical clustering approach (Le Roux *et al.* 2010) implemented in STRUCTURE (Falush *et al.* 2007) and the  $\Delta K$  method of Evanno *et al.* (2005) to determine all levels of population structure in the native range ( $K_{\text{native}}$ ). This approach was repeated for the native and invasive range ( $K_{\text{combined}}$ ). We followed the methods of Rollins *et al.* (2009), simulating K values from one upwards until either K exceeded the total number of populations, or the number of individuals per population was insufficient to allow for further analyses of population structure. Individuals that could not be assigned with more than 60% of their scored loci to a particular group were not included in subsequent analyses. Furthermore, individuals within a single population that were assigned to a genetic group outside of their population of origin were separated into their respective genetic groups for the next level of the analysis.

Second, we conducted an analysis using the spatial model in GENELAND (Guillot *et al.* 2005), implemented in R (Ihaka & Gentleman 1996, R Development Core Team 2004). GENELAND produces results that are robust when fine scale population structure is present (see Guillot 2008). GENELAND uses spatial co-ordinates as artificial centres around which the genetic groups are clustered. In this way it is able to incorporate spatial information without actually using the physical location of the sampled populations. We conducted ten independent runs using correlated allele frequencies, and 1 million iterations of the Markov chain Monte Carlo procedure, saving every 1 000<sup>th</sup> iteration.

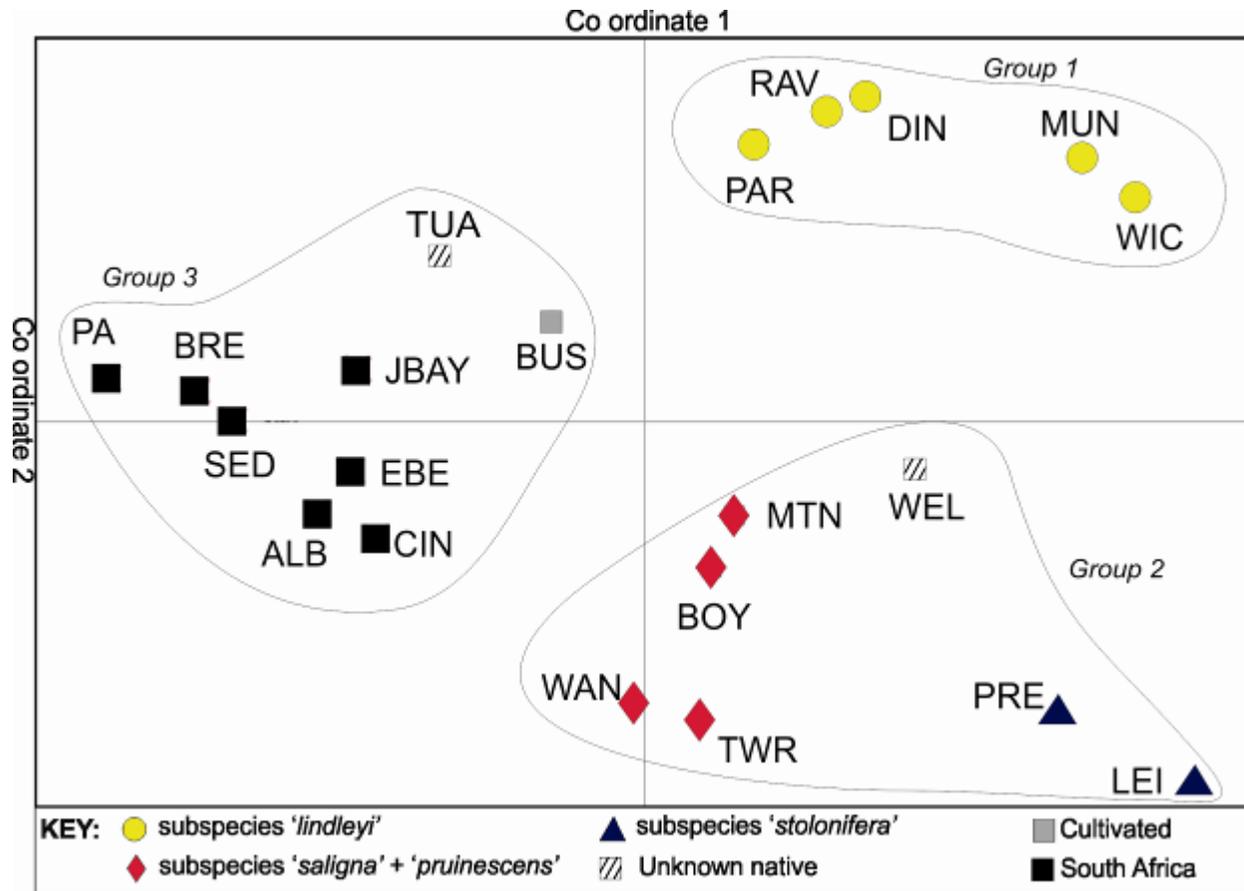
Third, we determined the optimal number of K clusters for *A. saligna* using TESS (Chen *et al.* 2007) so we could compare our results to Millar *et al.* (2011). We used spatial information when determining  $K_{\text{native}}$ , but not for  $K_{\text{combined}}$ . We followed the methods of Millar *et al.* (2011) for all parameters, except that we used an admixture model for the reason outlined in François and Durand (2010). For each value of  $K_{\text{max}}$ , we computed the Deviance Information Criterion (DIC), and averaged the estimated admixture coefficients over 20 % of the runs with the lowest DIC values plotted against K for all runs. We then selected the minimum value of K for which  $\text{DIC}_K$  was not

significantly different from the mean of all values of DIC greater than K based on a one-sample t-test.

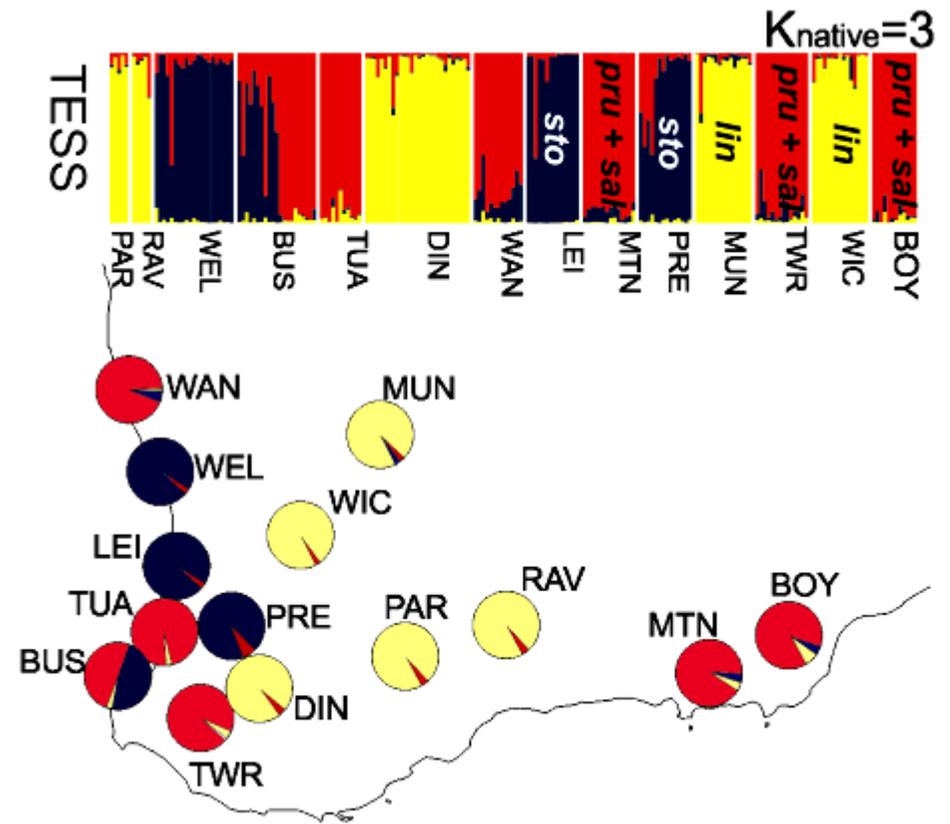
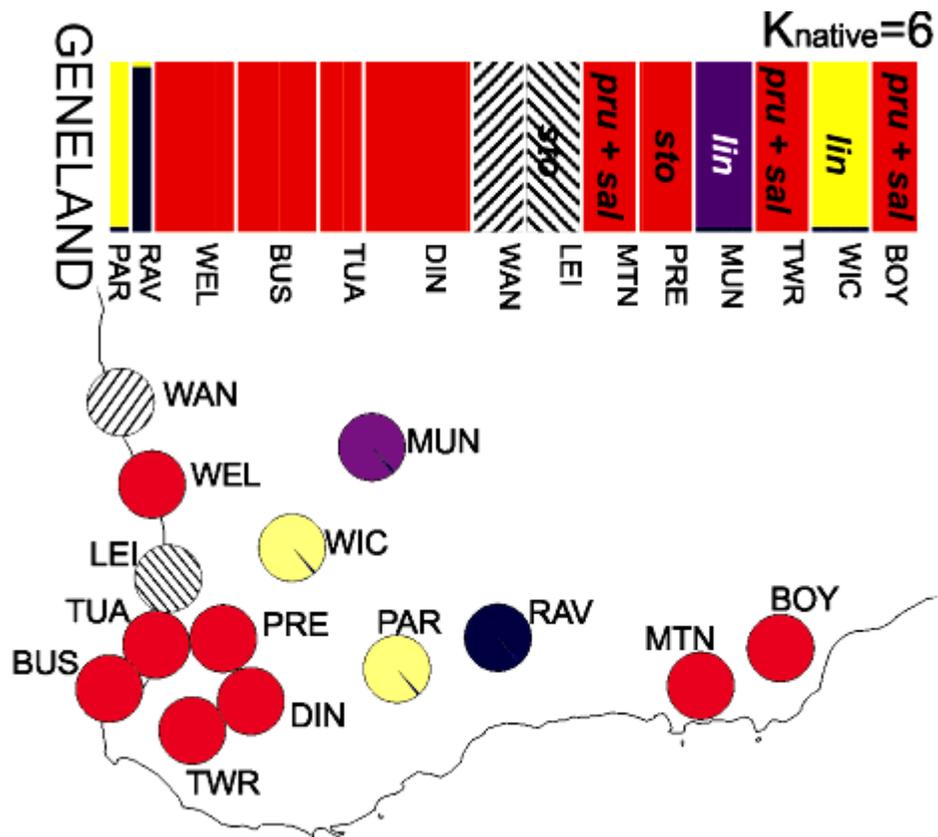
Appendix 3C ADDITIONAL RESULTS (TABLES AND FIGURES)



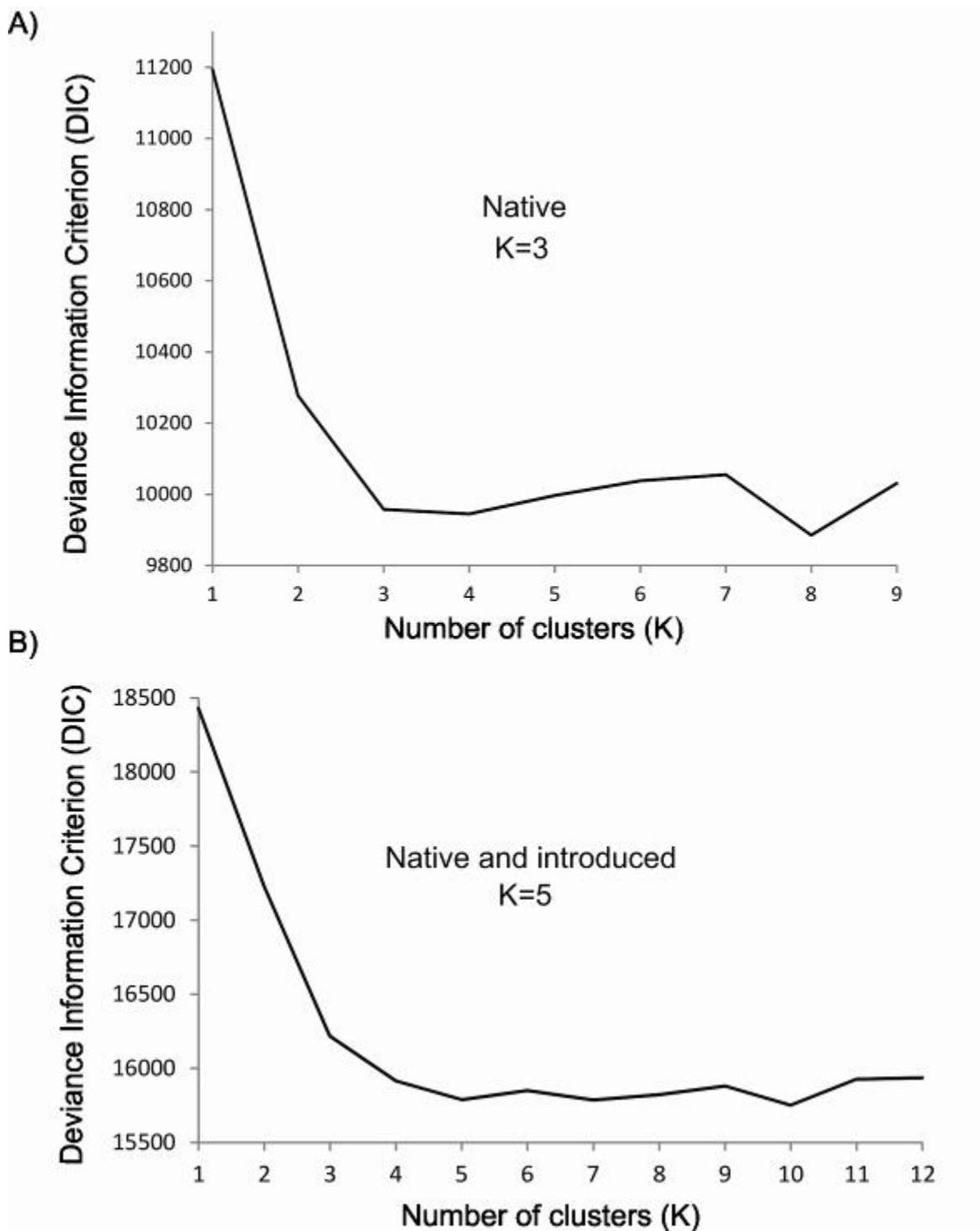
**Figure S3.1** Relationship between genetic and geographic distance for *Acacia saligna* populations in the native range in Western Australia (A) and the introduced range in South Africa (B) based on 10 nuclear microsatellite loci. Population pairwise genetic ( $F_{ST}$  calculated in ARLEQUIN) and geographic (Euclidean) distance was correlated using a Mantel test and the online “isolation by distance” service (<http://ibdws.sdsu.edu/~ibdws>).



**Figure S3.2** Genetic correlation between native lineages of *Acacia saligna* (circles, diamonds, triangles) and introduced populations from South Africa (crosses) using a principle co-ordinate analysis of microsatellite data. Native lineages include *A. saligna* subspecies 'lindleyi' (Group 1), and subspecies 'stolonifera' and subspecies 'saligna' (Group 2). Group 3 comprised invasive South African populations and two native populations (Tua and Bus), and clustered separately from the two major native Groups. Co-ordinate 1 explained 34.6% of the variation, and Co-ordinate 2 explained 22.6% of the variation.



**Figure S3.3** Bayesian clustering of native populations of *Acacia saligna* based on 10 nuclear microsatellite loci in the software GENELAND and TESS



**Figure S3.4** Identification of the optimal number of clusters in the native range (A), as well as the native and introduced range (B) of *Acacia saligna*. The data sets contain a total of A) 202 and B) 365 individuals for 10 diploid, nuclear microsatellite loci. For each region (native and introduced), we performed 1000 independent runs of 10 000 sweeps using an admixture parameter  $\alpha = 0.6$  in TESS. We kept 20% of runs that had the lowest DIC or  $\ln P(D|K)$  values, averaged their outputs per K value, and plotted these averages against K.

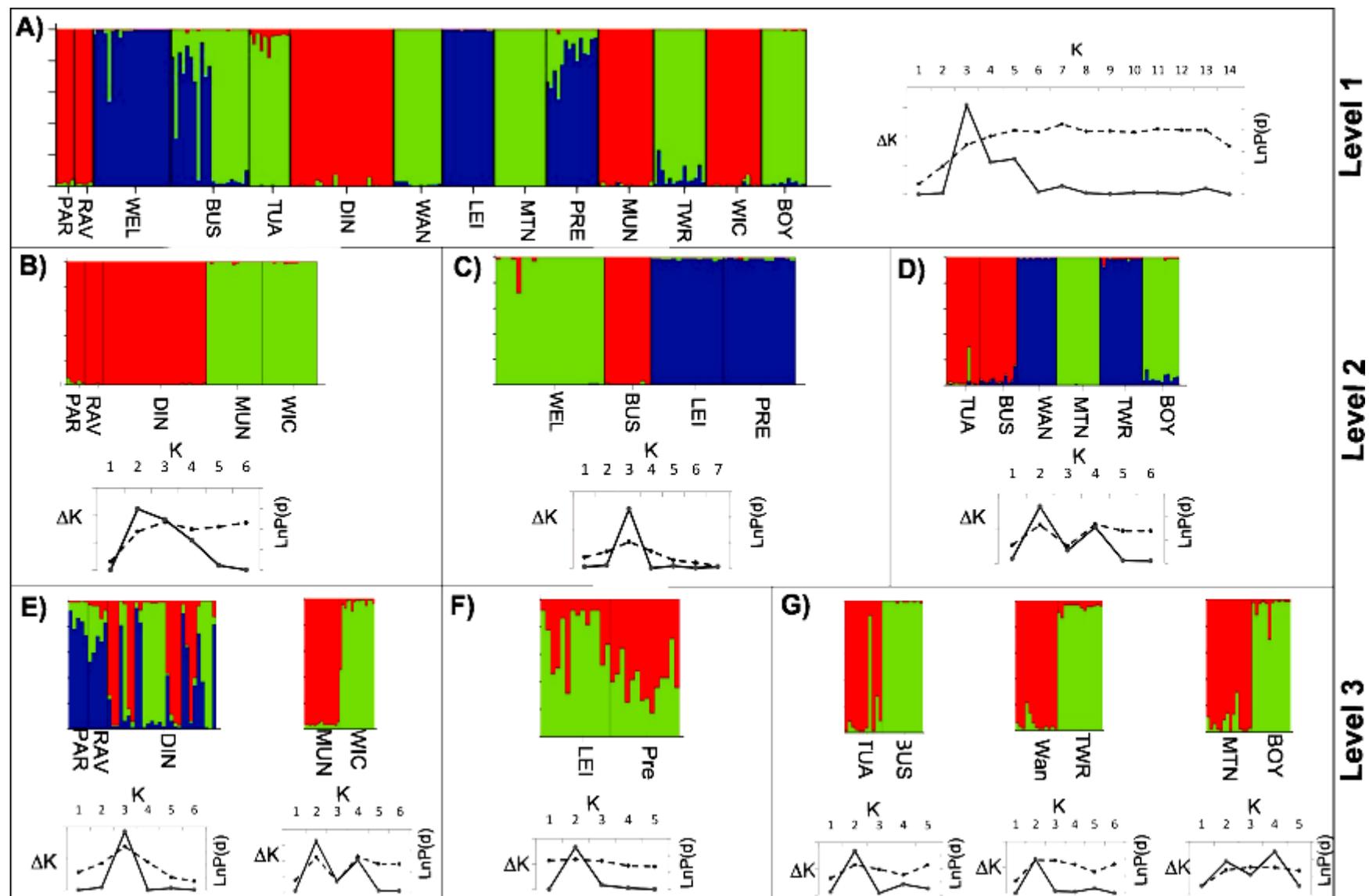
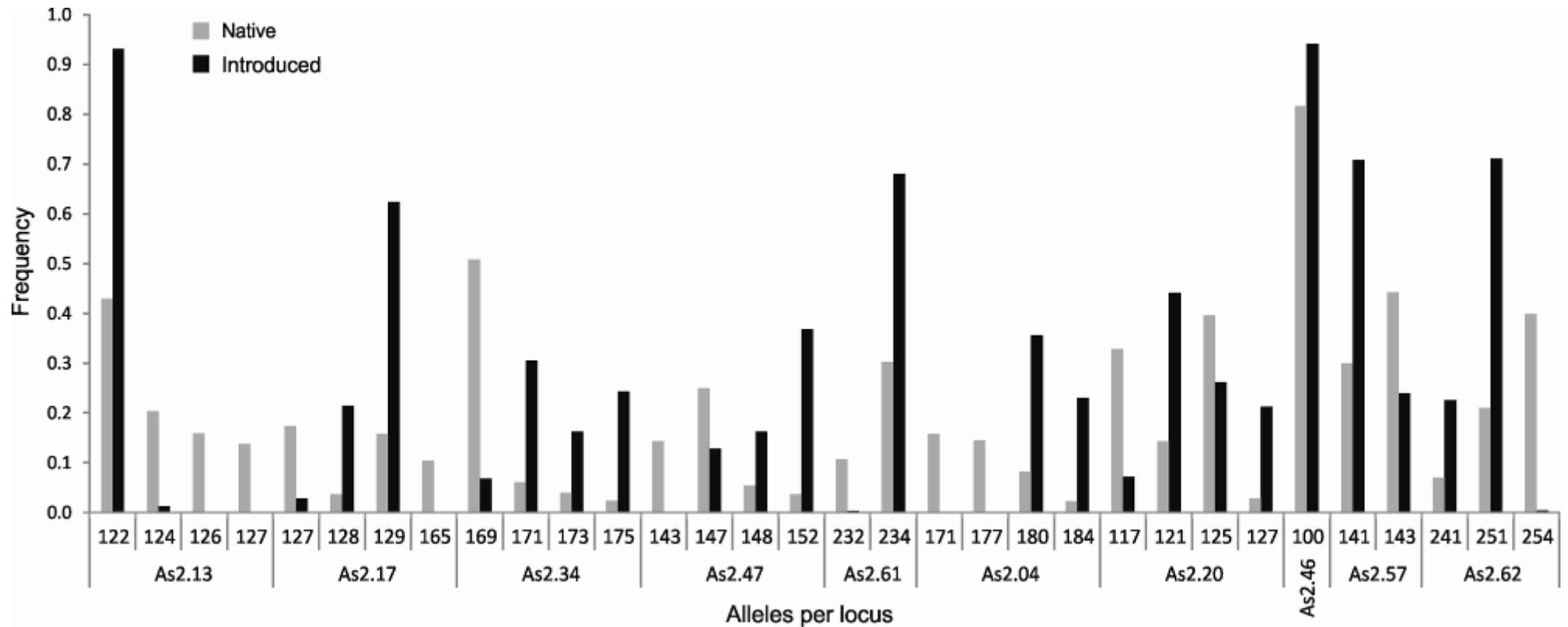


Figure S3.5 – see legend on next page.

**Figure S3.5** Identification of the optimal number of clusters ( $K_{\text{native}}$ ) for *Acacia saligna* in the native range in Western Australia using hierarchical Bayesian clustering in the software STRUCTURE. The data set contains a total of 14 populations containing 202 individuals genotyped at 10 nuclear microsatellite loci that were clustered at 3 hierarchical levels: Level 1 (A), Level 2 (B, C, D) and Level 3 (E, F, G). The estimated proportional membership is represented by bar plots, where each bar is an individual that is divided into K-coloured segments representing the proportional membership of each individual's genome ( $q_i$ ) to a particular K cluster. The optimal K for each level of clustering was identified using the  $\Delta K$  method (Evanno *et al.* 2005) and is graphed with each plot. Sampling site labels are indicated below each plot.



**Figure S3.6** Distribution of microsatellite allelic frequencies that differed by at least 10 %between the native and introduced ranges of *Acacia saligna*.

Loci and alleles presented were selected to display the maximum variation between the native and introduced range.

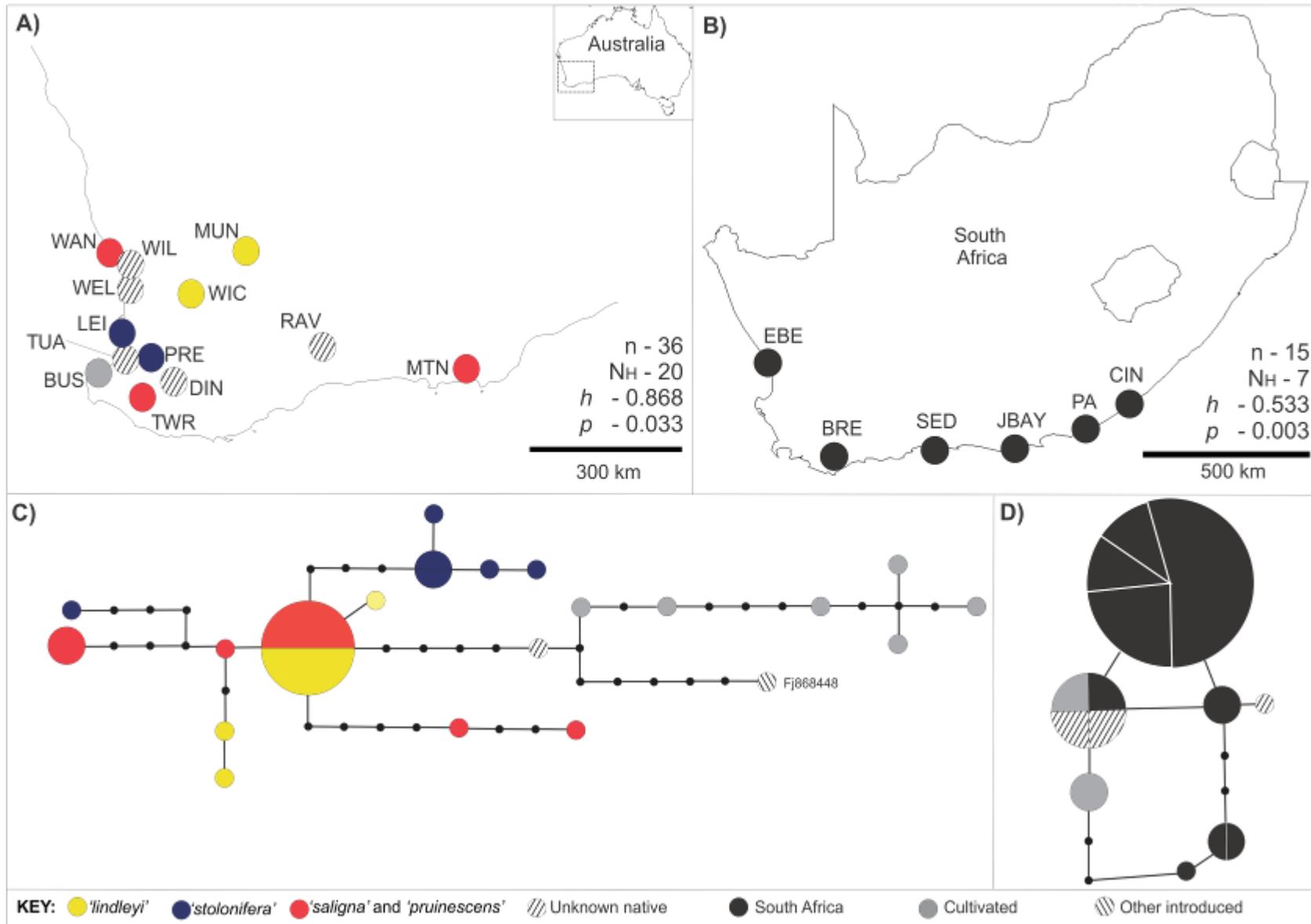
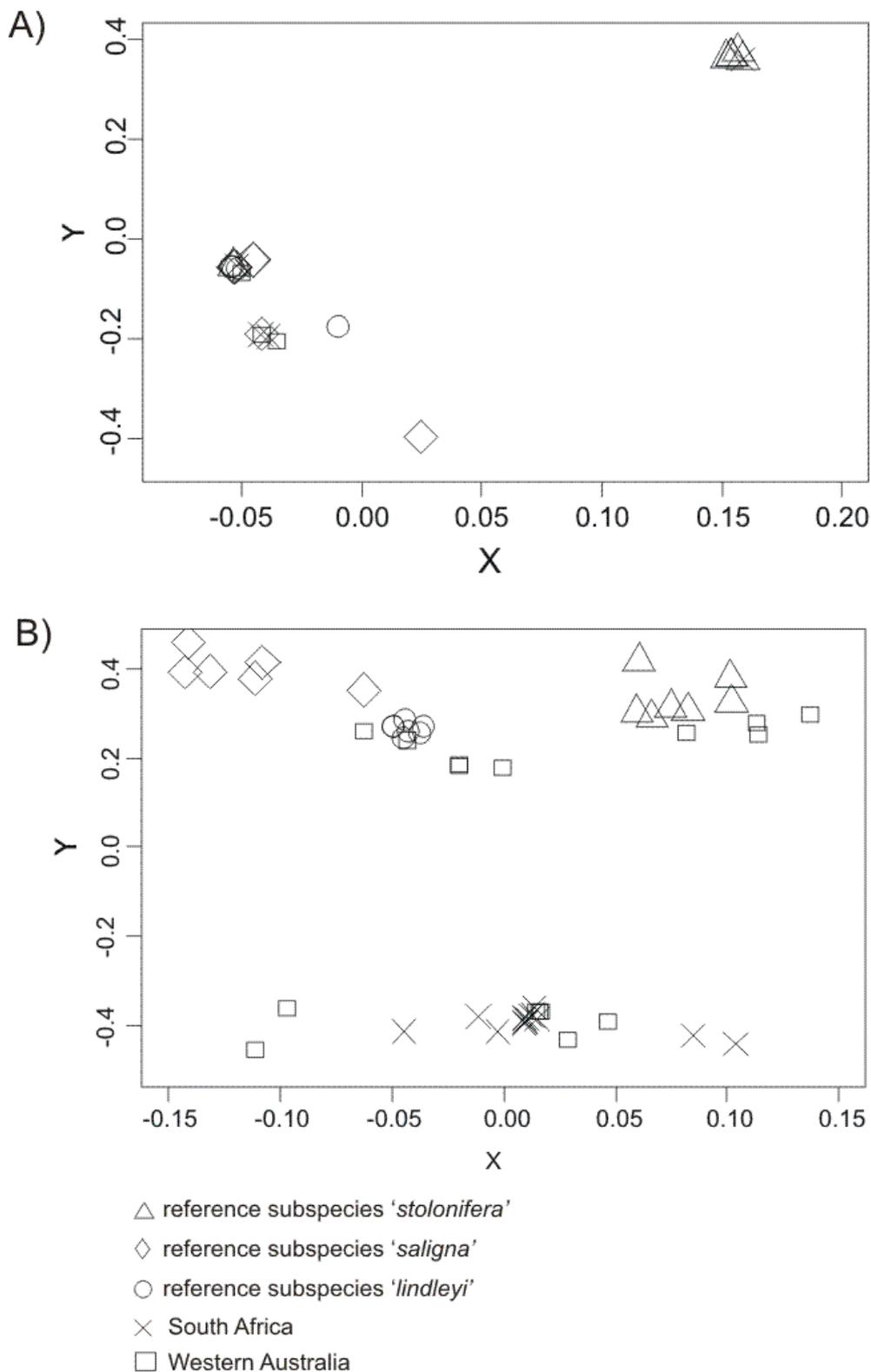


Figure S3.7- see legend on next page.

**Figure S3.7** Spatial distribution of sequence variation (external transcribed spacer region) for *Acacia saligna* accessions in (A) native Western Australia and (B) introduced South African populations. Statistical parsimony networks were constructed in TCS for *Acacia saligna* accessions in (C and D), where each circle represents a sampled haplotype (size proportional to frequency) and each link between haplotypes indicates one mutational event. The pie slices of a circle indicate the proportion of localities at which that haplotype was collected. Angle of bifurcation and length of link between haplotypes have no significance. Number of haplotypes ( $N_H$ ), haplotype diversity ( $h$ ) and nucleotide diversity ( $p$ ) are presented in bottom left corner for Western Australia (A) and South Africa (B).



**Figure S3.8** Separation of native and introduced individuals of *Acacia saligna* based on pairwise genetic distances for (A) cpDNA, the trnQ-5'rps16 region, and (B) nDNA, the ETS region. Genetic distances were translated into proximity co-ordinates using a nonmetric multidimensional scaling analysis.

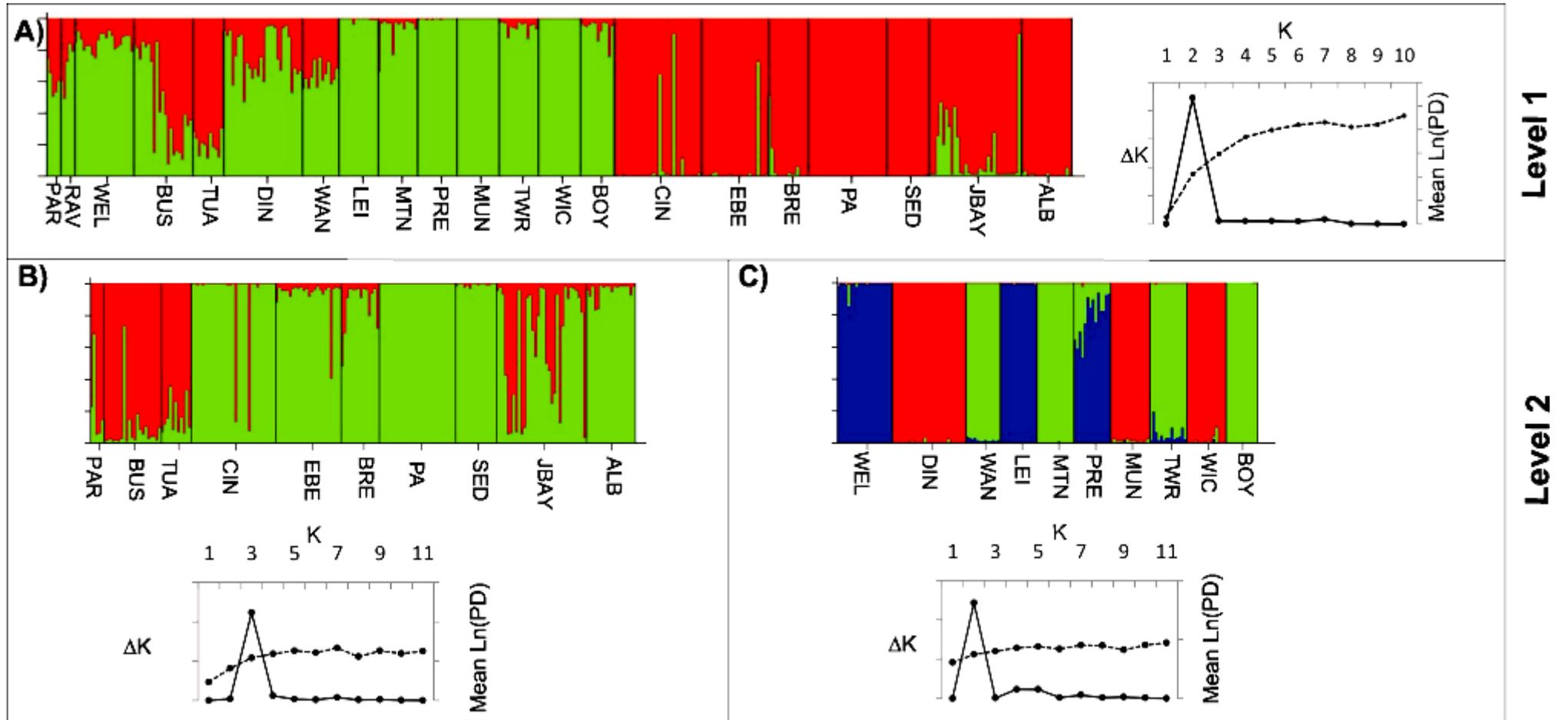


Figure S3.9- see legend on next page.

**Figure S3.9** Identification of the optimal number of clusters (K) for *Acacia saligna* in the native (Western Australia) and introduced (South Africa) range using hierarchical Bayesian clustering in the software STRUCTURE. The data sets contain a total of 21 populations containing 365 individuals genotyped at 10 nuclear microsatellite loci and clustered at 2 hierarchical levels: Level 1 (A) and Level 2 (B, C). The estimated proportional membership is represented by bar plots, where each bar is an individual that is divided into K-coloured segments representing the proportional membership of each individual's genome ( $q_i$ ) to a particular K cluster. The optimal K for each level of clustering was identified using the  $\Delta K$  method (Evanno *et al.* 2005) and is graphed with each plot. Sampling sites are indicated below each plot.

**Table S3.1** Population pairwise genetic structure for the native (Western Australia) and introduced (South Africa) range of *Acacia saligna*. Population pairwise  $F_{ST}$  values were calculated in ARLEQUIN using genotypic data generated from ten nuclear microsatellite loci.

<b>Western Australia</b>	PAR	RAV	WEL	BUS	TUA	DIN	WAN	LEI	MTN	PRE	MUN	TWR	WIC
Parkeyerring													
Ravensthorpe	<b>0.004</b>												
Wellesley	0.149	0.226											
Busselton	0.138	0.253	0.167										
Tuart Forest	0.193	0.343	0.273	0.007									
Dinninup	0.005	0.026	0.150	0.197	0.224								
Wanneroo	0.281	0.347	0.348	0.244	0.231	0.333							
Leshnault Inlet	0.355	0.433	0.258	0.392	0.504	0.288	0.386						
Mount Ney	0.296	0.370	0.432	0.310	0.275	0.283	0.307	<b>0.506</b>					
Preston	0.239	0.310	0.199	0.275	0.360	0.213	0.258	0.085	0.420				
Muntagin	0.180	0.182	0.216	0.304	0.374	0.130	0.419	0.335	0.388	0.219			
Tweed River	0.323	0.374	0.362	0.219	0.172	0.340	0.164	0.418	0.296	0.302	0.426		
Wickepin	0.174	0.190	0.221	0.298	0.342	0.119	0.412	0.318	0.359	0.202	0.020	0.423	
Boyatup Hill	0.253	0.319	0.336	0.212	0.246	0.256	0.189	0.399	0.144	0.248	0.355	0.219	0.335
<b>South Africa</b>	CIN	EBE	BRE	PA	SED	JBAY							
Cinsta													
Ebenhaezer	0.130												
Breede River	0.172	0.036											
Port Alfred	0.237	0.130	0.083										
Sedgefield	0.086	<b>0.034</b>	0.035	0.115									
Jeffrey's Bay	0.195	0.063	0.045	0.132	0.073								
Albertinia	0.106	0.122	0.174	<b>0.268</b>	0.086	0.204							

Note: Bold values indicate highest and lowest  $F_{ST}$  values

**Table S3.2** Pairwise comparisons of nuclear genetic distances (external transcribed spacer) between species and subspecies within the *Acacia* genus.

Pairwise comparisons	Genetic distance
<b>Species - species</b>	
<i>A. rostellifera</i> <sup>1</sup> vs. <i>A. saligna</i> subspecies ' <i>lindleyi</i> '	0.091
<i>A. rostellifera</i> <sup>1</sup> vs. introduced <i>A. saligna</i> (South African)	0.161
<b>Subspecies - subspecies</b>	
<i>A. longifolia</i> subspecies <i>longifolia</i> <sup>2</sup> vs. subspecies <i>sophorae</i> <sup>3</sup>	0.003
<i>A. saligna</i> subspecies ' <i>lindleyi</i> ' vs. subspecies ' <i>saligna</i> '	0.016
<i>A. saligna</i> subspecies ' <i>lindleyi</i> ' vs. subspecies ' <i>stolonifera</i> '	0.012
<i>A. saligna</i> subspecies ' <i>saligna</i> ' vs. subspecies ' <i>stolonifera</i> '	0.021
<b>Native clade - Introduced clade</b>	
<i>A. saligna</i> subspecies ' <i>stolonifera</i> ' vs. introduced <i>A. saligna</i> (South Africa)	0.085
<i>A. saligna</i> subspecies ' <i>lindleyi</i> ' vs. introduced <i>A. saligna</i> (South Africa)	0.061
<i>A. saligna</i> subspecies ' <i>saligna</i> ' vs. introduced <i>A. saligna</i> (South Africa)	0.089

GenBank numbers:

<sup>1</sup>JF420272

<sup>2</sup>HM007639.1

<sup>3</sup>HM007647

## CHAPTER 4 A tree well-travelled: Global phylogeography of the invasive *Acacia saligna*

- This chapter has been submitted to Journal of Biogeography for review: Thompson, G.D., Bellstedt, D.U., Richardson, D.M., Wilson, J.R.U & Le Roux, J.J. A tree well-travelled: Global phylogeography of the invasive *Acacia saligna*. *Journal of Biogeography* (submitted).

### Abstract

Invasiveness in one region is often used to predict and manage introductions in another region, however there can be substantial intra-specific genetic differences in introduced populations. Here, we conducted a global phylogenetic assessment of a widely introduced and invasive wattle species, *Acacia saligna*. Our overall aim was to determine the native provenance(s), subspecies identity, and spatial patterns of genetic diversity within and among global populations. Sites of *A. saligna* were sampled in the native (Western Australia) and introduced (eastern Australia, Israel, Italy, New Zealand, Portugal, South Africa, Spain and the USA) ranges. Phylogenetic trees and statistical parsimony networks were used to determine the phylogenetic relationships of introduced populations from different parts of the world to potential native source(s), representing three proposed subspecies of *A. saligna*. Sequence data were generated from 13 native populations and 18 globally introduced localities for one nuclear gene (ETS) and one chloroplast gene (trnQ-5'rps16). Our results showed that introduced populations of *A. saligna* differ markedly in their genetic composition. All the known subspecies of *A. saligna* have been moved around the world, and have been recorded as naturalising and spreading. A uniquely invasive genotype, previously identified only in South Africa, is also identified in eastern Australia and Portugal. With different intra-specific genetic lineages present in different countries, it is unclear whether lessons learned managing *A. saligna* invasions in one region can be generalised for all introduced regions. Further work is needed to conclusively link

the relative extent of invasions to genetic differences (in particular whether genetic novelty can explain the widespread invasions observed in South Africa).

## **4.1 Introduction**

Determining the patterns and processes that lead to successful plant invasions may help to reduce their extent and impact (Byers *et al.*, 2002). The invasive success of introduced plants has often been linked with the genetic features of introduced populations (Sakai *et al.*, 2001; Lee, 2002; Le Roux & Wieczorek, 2009), including high genetic diversity and novel genotypes (e.g. Lavergne & Molofsky, 2007; Thompson *et al.*, 2012). The amount and distribution of gene diversity in invasive populations is shaped by stochastic processes (such as founder events and drift) and human-mediated processes (such as cultivation and artificial selection) that occur prior, during, and after introduction (e.g. Le Roux *et al.*, 2011). A better understanding of these processes and how they affect the evolutionary trajectories of species in their introduced ranges is important for understanding the factors that mediate invasive success.

Many successful plant invaders have substantial economic value. Indeed, pathways created by enterprises such as agroforestry, biofuel production, commercial forestry and ornamental horticulture, are important drivers of invasions in many parts of the world (Richardson & Rejmánek, 2011). Such introduction and dissemination pathways often facilitate establishment and survival, promote large scale introductions and dispersal, and involve the selection of favourable traits (e.g. high reproductive output, resistance to harsh environmental conditions, rapid growth rate; Richardson, 1998; Ross, 2009; Wilson *et al.*, 2009, Richardson & Blanchard, 2011).

Australian acacias (1012 recognized species in *Acacia* subgenus *Phyllodineae*) have been planted in many parts of the world over the past two centuries for forestry and other purposes (Griffin *et al.*, 2011). Many Australian acacias have been subject to selective breeding programmes (Richardson *et al.*, 2011) and unsurprisingly, many acacias are widely invasive (Richardson & Rejmánek, 2011). *Acacia saligna* (native to Western Australia) is the most widely planted non-timber Australian *Acacia* species (Griffin *et al.*, 2011) and has been cultivated in and outside of its native range in Australia (see Fig. S1) and in many other parts of the world (Maslin & McDonald, 2004; Griffin *et al.*, 2011). The species is currently invasive in at least the following countries: Algeria, Chile, Cyprus, Israel, Italy, Kenya, Morocco, Portugal, South Africa and Spain (Maslin & McDonald, 2004; Richardson & Rejmánek, 2011; Wilson *et al.*, 2011). It is not clear why *A. saligna* has not (yet) become invasive in all regions to which it has been introduced (Wilson *et al.*, 2011).

The introduction pathway of a species can substantially affect the genetic signature in the introduced range (Lee, 2002; Le Roux *et al.*, 2011). It is thus important to consider the number and timing of species introductions in conjunction with the genetic characteristics of native and introduced populations when attempting to reconstruct invasion histories (Le Roux *et al.*, 2011). The introduction history of *A. saligna* has been well documented in some cases (e.g. South Africa, Poynton, 2009) but less so in others (e.g. Portugal, E. Marchante, pers. comm.). *Acacia saligna* was exported on a few occasions in the 1800s, but dissemination around the world increased rapidly with modern transport and trade, particularly with the formation of the Australian Tree Seed Centre in 1962 (Griffin *et al.*, 2011). The earliest recorded exports were to South Africa in 1833 (Poynton, 2009), Portugal in 1869 (António Gouveia, pers. comm., grey literature), Libya and Ethiopia in 1870 (Griffin *et al.*, 2011), and Israel in approximately 1920 (Kull *et al.*, 2011). The species also occurs, but is not known to be invasive in Egypt, France, Iran (Derbel *et al.*, 2009), Kenya (Droppelman *et al.*, 2000), New Zealand ([www.gbif.org](http://www.gbif.org); ILDIS,

2011), Tunisia (Degen *et al.*, 1995) and the USA (ILDIS, 2011), and dates of introduction to these regions are unknown. *Acacia saligna* has become naturalized outside of its native ranges within temperate Australia (South Australia, Victoria, Tasmania, New South Wales and southeast Queensland) where it was widely planted to control dry-land salinity and provide woody biomass (Maslin & McDonald, 2004).

*Acacia saligna* comprises four informal subspecies that are grouped into three major genetic lineages: *A. saligna* subspecies '*lindleyi*', *A. saligna* subspecies '*saligna*' + '*pruinescens*' and *A. saligna* subspecies '*stolonifera*' (Millar *et al.*, 2011). Previous work has shown that these subspecies occupy bioclimatically distinct niches in Western Australia (Thompson *et al.*, 2011); and that invasive populations in South African are genetically more different from native range populations than expected as a result of purely stochastic processes, admixture or multiple introductions (Thompson *et al.*, 2012). Thompson *et al.* (2012) argued that the novel South African genotype may have arisen through introgressive hybridization (during cultivation) prior to introduction to South Africa.

Many introduced acacias were sourced from central seed stores and forestry stations directly in Australia or secondarily from other countries (e.g. France, Poynton, 2009) before being distributed globally (Griffin *et al.*, 2011, Kull *et al.*, 2011). It is therefore conceivable that genotypes similar to those found in South Africa may have been introduced to other localities. While analyses of a limited number of European and non-native Australian individuals showed that the South African genotype was genetically unique (Thompson *et al.*, 2012), further sampling was needed to confirm the initial results.

Our study expands on the population genetic and phylogenetic study conducted by Thompson *et al.* (2012) by using the same molecular markers (nDNA and cpDNA) in order to determine the native provenance(s), subspecies identity, and spatial patterns of genetic

diversity in seven countries to which *A. saligna* has been introduced. Through an assessment of the global genetic patterns in *A. saligna* we aim to: 1) determine whether particular genotypes or subspecies are always invasive; 2) determine whether novel genotypes previously identified in South Africa (Thompson *et al.*, 2012) have been introduced elsewhere; and 3) improve our understanding of the history of introduction and invasion of the species worldwide. We also consider the implications of results for the management of the species.

## **4.1 Methods**

### **(a) Field collections and DNA extraction**

Mature phyllodes of *Acacia saligna* were collected from the native range in Western Australia (13 native populations) and from invasive ranges in eastern Australia, New Zealand, Israel, Italy, Portugal, South Africa, Spain and the United States (18 introduced populations, Table 1). Western Australian localities were identified for sampling based on their overlap with the known distributions of the intra-specific variants of *A. saligna* ([worldwidewattle.com](http://worldwidewattle.com)), and included populations used by Millar *et al.* (2011) to delimit the genetic relationships between subspecies of *A. saligna*. Material was dried and stored on silica gel until DNA extraction. Genomic DNA was extracted using a modified cetyltrimethyl ammonium bromide (CTAB) method (Doyle & Doyle, 1990) with the addition of 0.2 M sodium sulphite to the extraction and wash buffers (Byrne *et al.*, 2001). DNA quality and quantity was measured using a Nanodrop spectrophotometer (Infinite 200 PRO NanoQuant, Tecan Group Ltd, Switzerland) and all DNA diluted to a final concentration of *ca.* 30 ng/ $\mu$ L.

(b) *Sequencing and data analysis*

The nuclear (nDNA) external transcribed spacer (ETS) and chloroplast (cpDNA) trnQ - 5'rps16 regions were amplified using the methods described in Thompson *et al.* (2012). The final dataset comprised 50 sequences generated for previous work (Le Roux *et al.*, 2011; Thompson *et al.*, 2012), and 59 additional sequences generated for this study.

Sequence data were aligned and edited using BIOEDIT version 7.0.5.3 (Hall, 1999) followed by manual editing. For nDNA a Maximum-Likelihood analysis was conducted in PAUP\* v.4b10 (Swofford, 1999), using the TVM+G model selected by MODELTEST v.3.7 (Kimura, 1981) under the Akaike Information Criterion (AIC). A heuristic search was conducted and the bootstrap support for branches evaluated using 10 000 replicates (Felsenstein, 1985). Phylogenetic trees were rooted using *A. cupularis* and *A. rostellifera*, (GenBank numbers: JF420247 and JF420272 respectively), sister species to *A. saligna* (Miller *et al.*, 2011). For both nDNA and cpDNA data we reconstructed networks using statistical parsimony as implemented in TCS v.1.21 (Clement *et al.*, 2000), and a 95 % connection limit to examine relationships among the sampled individuals.

To support the placement of global populations in the nDNA phylogenetic tree, we used a non-metric multidimensional scaling (NMDS) analysis to plot pairwise genetic distances between all ETS sequences. Pairwise genetic distances were calculated in BIOEDIT version 7.0.5.3 (Hall, 1999). Sequences were clustered using ten iterations of a 'ratio + bounds' analysis, and a highly accurate convergence value of 0.000005 in the software PERMAP (Heady & Lucas, 2007). Proximity co-ordinates for each individual were obtained from the solution with the lowest objective function value and plotted in R using the 'car' package (Fox & Weisberg, 2011).

### *(c) Genetic diversity*

Chloroplast sequence data was not variable enough to allow for the assessment of the distribution of genetic variation (i.e. AMOVA) or the measurement of haplotype diversity. Therefore, only the distribution of nuclear genetic diversity between the native and introduced range was assessed in ARLEQUIN v.3.5 (Excoffier & Lischer, 2010). We used two approaches, firstly we calculated the sequence and nucleotide diversity between ranges (native vs. invasive), and secondly we conducted a hierarchical analysis of molecular variance between ranges (AMOVA; Excoffier & Lischer, 2010) using 10 000 permutations.

## **4.3 Results**

### *(a) Sequence variation and genetic diversity*

Both nDNA and cpDNA parsimony analyses identified two major networks (Fig. 1, 2, Table S1). However the placement of native and introduced populations was in some instances incongruent for the two gene regions. The 439 bp ETS alignment contained a total of 124 polymorphic sites, 70 of which were parsimony-informative. Across all native and introduced populations (107 individuals) we identified 62 distinct sequences that were partitioned into two major clades (Fig. 2) that were separated by a 70 bp indel. Every country sampled except New Zealand possessed at least one unique nDNA sequence, with the most unique sequences coming from Israel (Fig. 1). The trnQ-5'rps16 cpDNA alignment contained a total of 722 base pairs, four parsimony informative sites and retrieved eight haplotypes in two networks (Network A and Network B, Table S1). Overall, populations in the native range had marginally higher sequence and nucleotide diversity, and a greater number of polymorphic sites than those in

introduced ranges (Table 2, S2). The majority of genetic diversity was partitioned among populations, and the minority of genetic diversity was partitioned among ranges (Table S2).

*(b) Subspecies identity of introduced populations*

An analysis of the variation in nDNA showed that native populations and introduced populations were clustered into two major groups (Fig. 1, 2, S2 and Table S1). The majority of South African populations (Fig1B, black fill) were divergent from native and introduced populations (i.e. they were contained within a separate network), however genotypes were shared between South Africa, Portugal and eastern Australia. More specifically, *A. saligna* subspecies '*stolonifera*' shared sequences with Spain, Portugal and New Zealand, while *A. saligna* subspecies '*saligna*' + '*pruinescens*' shared sequences with Italy and Portugal, and *A. saligna* subspecies '*lindleyi*' shared sequences with other Western Australian populations, as well as populations found in Portugal and the USA (Fig. 1). The remaining sequences were unique to their country of origin.

NMDS of pairwise genetic distances for native and introduced populations mirrored the results of the nDNA statistical parsimony networks, and phylogenetic reconstructions in all aspects (Fig. S2). In contrast, cpDNA identified a number of shared haplotypes between two of the three native subspecies of *A. saligna* (subspecies '*lindleyi*' and '*saligna*') and Israel, South Africa and Spain (Table S1). Populations of *A. saligna* subspecies '*stolonifera*' grouped separately from all populations, excluding one individual collected in eastern Australia.

### (c) *Phylogenetic relationships*

Topologies retrieved in the phylogenetic tree were similar to the genetic relationships retrieved by the network analyses, with two well supported clades identified (Fig. 2). The first clade included eight individuals from Western Australia known to be planted/cultivated (see Thompson *et al.*, 2012), the majority of South African individuals, and individuals from Portugal and eastern Australia (Fig 2, accession FJ868448). The second well supported clade contained the remaining native and introduced individuals (Fig. 2).

## **4.4 Discussion**

The impressive extent of dissemination, high propagule pressure, cultivation, and known high native genetic structure of *A. saligna* has generated interesting and in some instances, surprising genetic signatures across the current global distribution of the taxon. Our phylogenetic assessment of the genetic patterns within and among native and introduced populations of *A. saligna* suggests two main conclusions. First, populations of *A. saligna* around the world comprise a variety of different genetic entities, where no single genetic entity is present in all introduced populations. Given the native range distribution of these entities, this implies wide geographical sampling of diverse *A. saligna* propagules prior to their global introductions. Second, the invasive South African genotype (with no genetically closely related native lineages), is also present in cultivated populations in Western Australia, naturalised populations in eastern Australia and some introduced populations in Portugal.

In some cases only a single subspecies appears to have been introduced to a region (e.g. only *A. saligna* subspecies '*stolonifera*' occurs in Spain), whereas other regions (e.g. Portugal) appear to have been sourced from a variety of subspecific populations in Western

Australia. This genetic pattern does not appear to be associated with sampling effort in Portugal and Spain; however more extensive sampling is required for Italy, New Zealand and the USA. On the basis of shared sequences, we conclude that subspecies are represented in the respective countries as follows: *A. saligna* subspecies '*lindleyi*' in Portugal and the USA; *A. saligna* subspecies '*saligna*' + '*pruinescens*' in Italy and Portugal; *A. saligna* subspecies '*stolonifera*' in New Zealand, Portugal and Spain.

The large number of unique genotypes in Israel and a lack of shared sequences between Israel and any other region sampled, suggest that either the source of Israeli populations has not been sampled in Australia, or that the genotypes arose post-introduction to Israel. While the former is unlikely given our widespread sampling across *A. saligna*'s native range (Millar *et al.*, 2011; Thompson *et al.*, 2012), it is possible that these unique genotypes were not sampled in Western Australia. The *in situ* evolution of introduced genotypes would require a substantial amount of time. While *A. saligna* has been present in Israel since the early 19<sup>th</sup> century (Dufour-Dror, 2012; Kull *et al.*, 2011), this allows for only ca. 46 generations since its introduction (assuming a sexual maturity of 2 years, see Gibson *et al.*, 2012). It is therefore highly unlikely that post-introduction evolution would have given rise to the large number of unique genotypes identified in the region (see Fitzpatrick *et al.*, 2012).

The introduction histories of many invasive species are poorly documented (Jeschke & Strayer, 2005; Puth & Post, 2005). Clearly, *A. saligna* introductions represent a mixed case of detailed (e.g. South Africa, Poynton, 2009) and virtually unrecorded (e.g. Cyprus) introductions. The limited global records that are available for *A. saligna* indicate that: 1) introductions to Portugal and South Africa occurred within the same period, and 2) South African introductions were sourced from Australia, as well as from Europe (Poynton, 2009). Indeed, the novel genotype identified by Thompson *et al.* (2012) was only shared between populations in Portugal, cultivated populations in Western Australia and a naturalized population in eastern

Australia (Sydney and Canberra sites). The transfer of the same genotype between the aforementioned regions is further supported by the origin of *A. saligna*'s common name in South Africa – the Port Jackson Willow (Henderson, 2001). Port Jackson is in fact a natural bay outside *A. saligna*'s native range that encompasses Sydney harbour in Australia. Thus, considering the genetic and historical evidence, a likely source of South African propagules are naturalised populations in eastern Australia.

Despite the absence of the novel genotype from the remaining invasive (and not naturalized) populations we sampled in Israel and Spain, both these regions possessed several divergent genotypes. This initially suggests that invasiveness in *A. saligna* can be associated with high genetic diversity, however wider sampling of naturalized populations is needed to support this (e.g. in Italy and the USA). Overall, wider sampling across the introduced distribution, coupled with a better understanding of the history of use of the species is needed for further deductions on the processes (natural or human-mediated) that may have shaped all introduced genetic patterns.

#### *Implications for management*

The presence of different subspecies and/or divergent genotypes of *A. saligna* in the regions sampled have several implications for management. Ideally management recommendations developed in one region should be extrapolated to other regions (Wilson *et al.*, 2011). But, the differing biological and ecological attributes associated with each *A. saligna* subspecies (Table S3, also see Thompson *et al.*, 2011; Thompson *et al.*, 2012) and the diversity of entities across the different regions sampled, suggest that management experiences may not be transferable between regions for *A. saligna*. This also means that predictions based on differences in habitat (Table S3) or bioclimatic niches occupied by each subspecies in the native range, might not provide much predictive value for the introduced ranges (Thompson *et al.*,

2011). Moreover, novel or genetically divergent genotypes may respond differently to biological control agents that are host-specific to native genotypes of *A. saligna* (cf. Goolsby *et al.*, 2006). This means that the agents that provide substantial levels of control in South Africa might not achieve similar levels of control in other regions, and so rather than exporting agents from South Africa (Wilson *et al.*, 2011), successful biological control might require additional surveys in the native range.

In conclusion, our study shows that *A. saligna* is a genetically diverse taxon that has been extensively moved around the world, displays high levels of genetic diversity within introduced populations, and no single or dominant genotype(s) is consistently associated with invasions. These genetic patterns suggest that substantial geographic variation in sampling efforts prior to introductions (which may often occur during agroforestry introductions), have important ecological, evolutionary and management consequences for subsequent invasive populations.

### *Acknowledgements*

We acknowledge financial support from the DST-NRF Centre of Excellence for Invasion Biology and the Working for Water Programme through the collaborative research project on “Research for Integrated Management of Invasive Alien Species”. We thank Bruce Maslin (Department of Environment and Conservation, Western Australia) for identifying herbarium specimens and for answering diverse questions over several years. Collectors from around the world are thanked for the collection of genetic material: Margaret Byrne and Melissa Millar (DNA from Western Australia), Colin Ogle (New Zealand), Jean-Marc Dufour-Dror (Israel), Giuseppe Brundu (Italy), Elizabete Marchante, Hélia Marchante (Portugal), Oscar Godoy (Spain), John Brock and Michelle Gibson (USA).

### *Author contributions*

All authors conceived the research ideas and designed the research methods. G.D.T. generated and analysed all molecular data and led the writing.

## 4.5 Tables and figures

**Table 4.1** Locality information and population identification for native and introduced populations of *Acacia saligna*.

Country	Locality	Region	ID	n (ETS)	Latitude	Longitude
<b>Native</b>						
Australia	Wellesley	Western Australia	Wel	2	-33.148	115.742
Australia	Busselton	Western Australia	Bus	3	-33.661	115.358
Australia	Tuart Forest	Western Australia	Tua	5	-33.258	119.751
Australia	Wannerroo	Western Australia	Wan	2	-31.247	115.276
Australia	Leshnault Inlet	Western Australia	LeI	5	-33.784	115.250
Australia	Mount Ney	Western Australia	MtN	2	-33.239	115.202
Australia	Preston	Western Australia	Pre	2	-33.190	115.349
Australia	Muntagin	Western Australia	Mun	4	-31.273	118.210
Australia	Tweed River	Western Australia	TwR	2	-34.210	116.177
Australia	Wickepin	Western Australia	Wic	3	-32.227	117.138
Australia	Willibinga	Western Australia	Wil	2	-31.438	115.663
Australia	Dinninup	Western Australia	Din	3	-33.813	116.534
Australia	Parkeyerrin	Western Australia	Par	2	-33.362	117.356
<b>Introduced</b>						
Portugal	Sines	Setubal	Sin	5	37.979	-8.876
Portugal	Dunas Mira	Cantanhede	Dua	4	40.466	-8.799
Portugal	Costa Caparica	Almada	Cos	3	38.654	-9.229
Portugal	Carrapateira	Aljezur	Car	3	37.206	-8.892
Israel	Nitzanim Nature Reserve	Israeli Coastal Plain	Isr	13	31.736	34.617
Italy	Sorgono	Sardinia	Ita	2	40.030	9.070
New Zealand	Wanganui	North Island	NZ	1	-39.924	175.043
USA	East Chandler Heights	Arizona	Ech	2	33.897	-111.815
South Africa	Cintsa	Eastern Cape	Cin	2	-32.845	28.113
South Africa	Ebenhaezer	Western Cape	Ebe	4	-31.586	18.242
South Africa	Breede River	Western Cape	Bre	2	-34.120	20.034
South Africa	Port Alfred	Eastern Cape	PA	1	-33.554	26.893
South Africa	Sedgefield	Western Cape	Sed	3	-34.011	22.779
South Africa	Jeffreys Bay	Eastern Cape	JBay	9	-34.052	24.922
Spain	Malaga city	Malaga	Mal	11	36.720	-4.420
Australia	Sydney	Victoria	Syd	3	-33.765	151.233
Australia	Adelaide	New South Wales	Ade	1	-35.921	140.101
Australia	ca. Stirling Range National Park	Western Australia (cultivated)	Cul	1	-34.350	118.017

**Table 4.2** Nuclear DNA genetic diversity indices of *Acacia saligna* populations in the native range in Western Australia and the introduced range (eastern Australia, New Zealand, Israel, Italy, Portugal, Spain, South Africa and the USA).

	n	Sequence diversity	Nucleotide diversity	Polymorphic sites	Average pairwise differences
Western Australia	38	0.929 (0.029)	0.02477 (0.00424)	35	32.0 (14.3)
Introduced	71	0.703 (0.051)	0.02376 (0.00283)	32	38.6 (17.0)

Note: Standard deviation in parenthesis.

## Figure Legends

**Figure 4.1** Phylogenetic relationships within and among native and introduced populations of *Acacia saligna* based on statistical parsimony of nuclear DNA sequences. Relationships between sequences of the external transcribed spacer (ETS) region were assessed using TCS and a 95% connection limit. Native reference populations of the three genetic lineages of *A. saligna* (defined by Millar *et al.*, 2011) are represented by colours, while introduced populations are represented by shading, stripes or a hatching pattern.

**Figure 4.2** Phylogenetic relationships within and among native and introduced populations of *Acacia saligna* based on maximum likelihood of nDNA. Tree branch lengths are scaled according to genetic distance. Values above or left of branches represent bootstrap support values. Native subspecies were identified based on Millar *et al.* (2011) and are represented by symbols (not all accessions are depicted). Introduced populations are labelled by country of origin. Western Australian populations (of unknown subspecies origin) are labelled by an acronym for their source population.

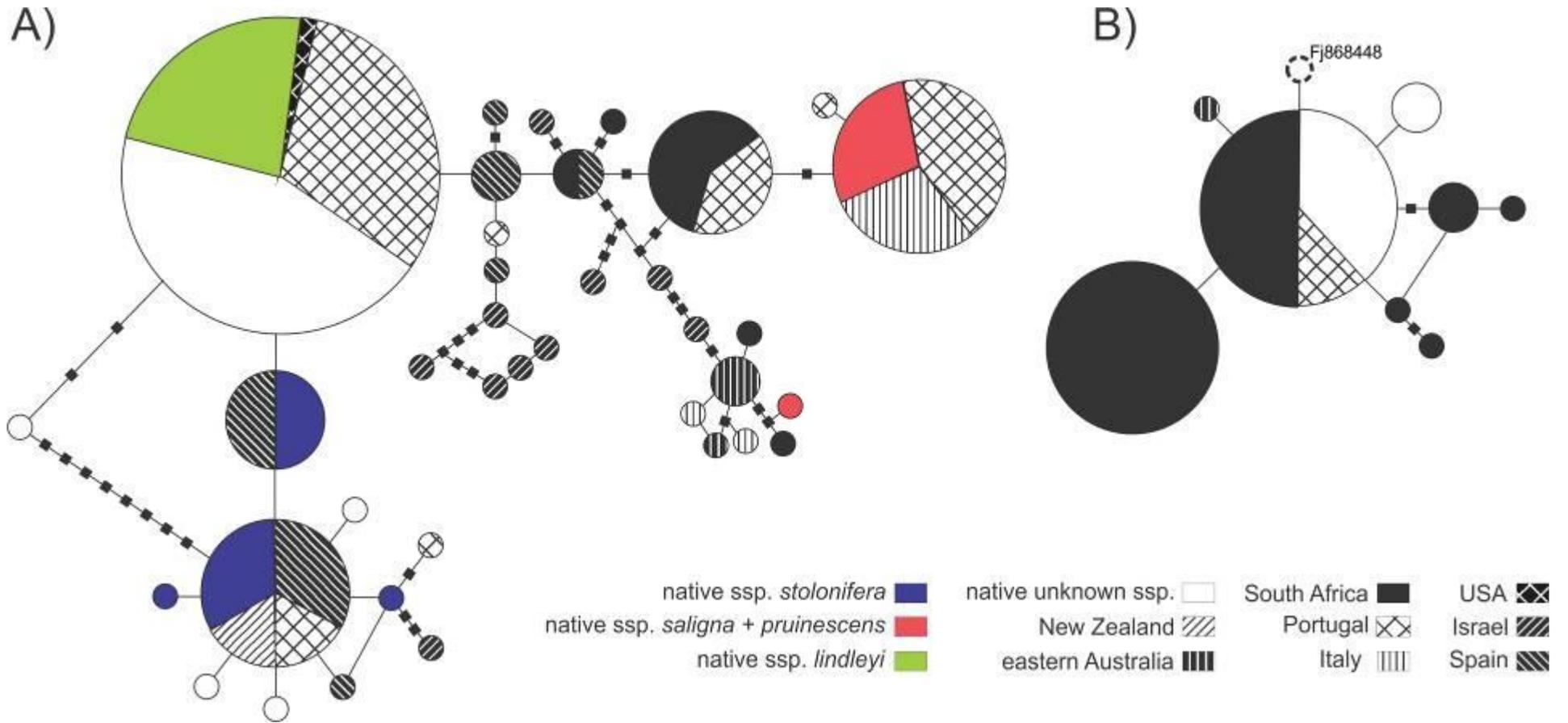
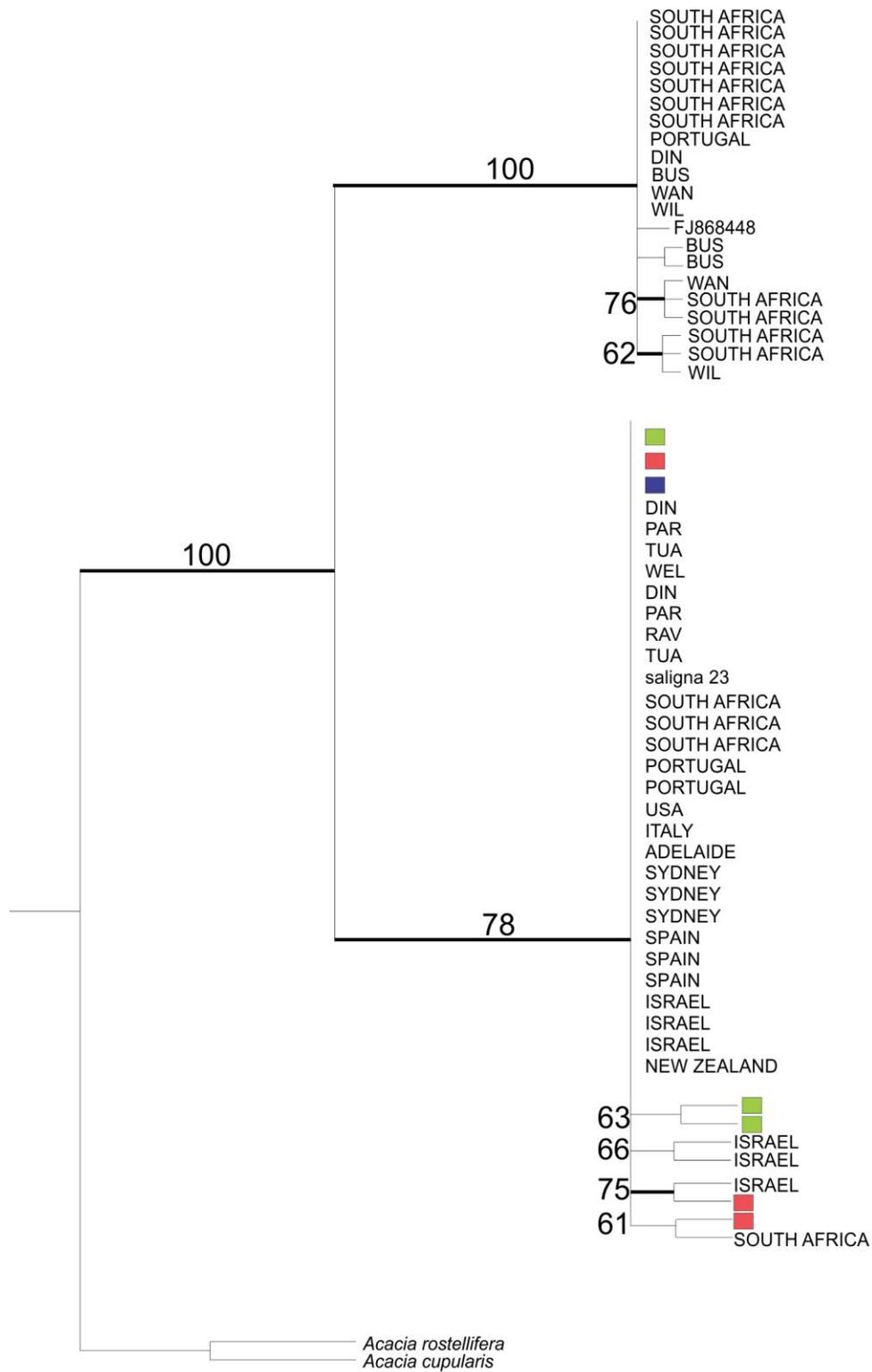


Figure 4.1



— 20 mutational changes    ■ native ssp. *lindleyi*    ■ native ssp. *saligna + pruinescens*    ■ native ssp. *stolonifera*  
**Figure 4.2**

## 4.6 Appendices

### Appendix S4.6.1 Additional tables and figures from results

**Table S4.1** Chloroplast DNA (trnQ-5'rps16) haplotypes retrieved using TCS.

Haplotype number	Regions containing haplotype	<i>Acacia 'saligna'</i> subspecies
<b>Network A</b>		
I	Western Australia (native and cultivated), Israel	'lindleyi' and 'saligna'
II	Western Australia (native only), Spain, Israel, South Australia, South Africa	'lindleyi' and 'saligna'
III	Western Australia (cultivated), South Australia, South Africa	NA
IV	Western Australia (native)	NA
V	South Australia	NA
<b>Network B</b>		
VI	Western Australia (native)	'stolonifera'
VII	Western Australia (native and cultivated)	'stolonifera'
VIII	South Australia	NA

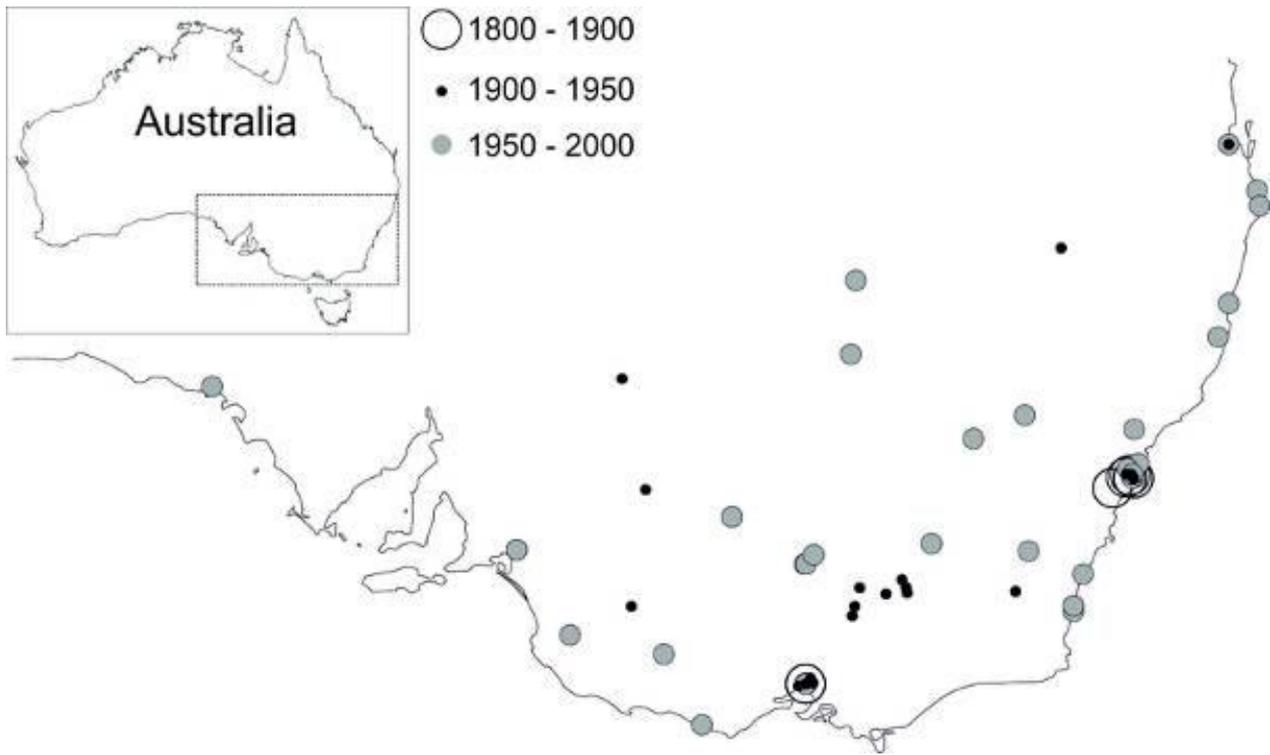
**Table S4.2** Distribution of molecular variance in the native range in Western Australia, and introduced range of *Acacia saligna* in eastern Australia, Italy, Israel, New Zealand, Portugal, South Africa, Spain and the USA.

Source of variation	d.f.	Sum of squares	Fixation index	Percent variation (%)
Among native and introduced ranges	1	23.714	$\Phi_{RT} = -0.06153$	-6.15*
Among populations	24	1385.606	$\Phi_{PR} = 0.65637$	71.79*
Within populations	80	506.653	$\Phi_{PT} = 0.67629$	34.36

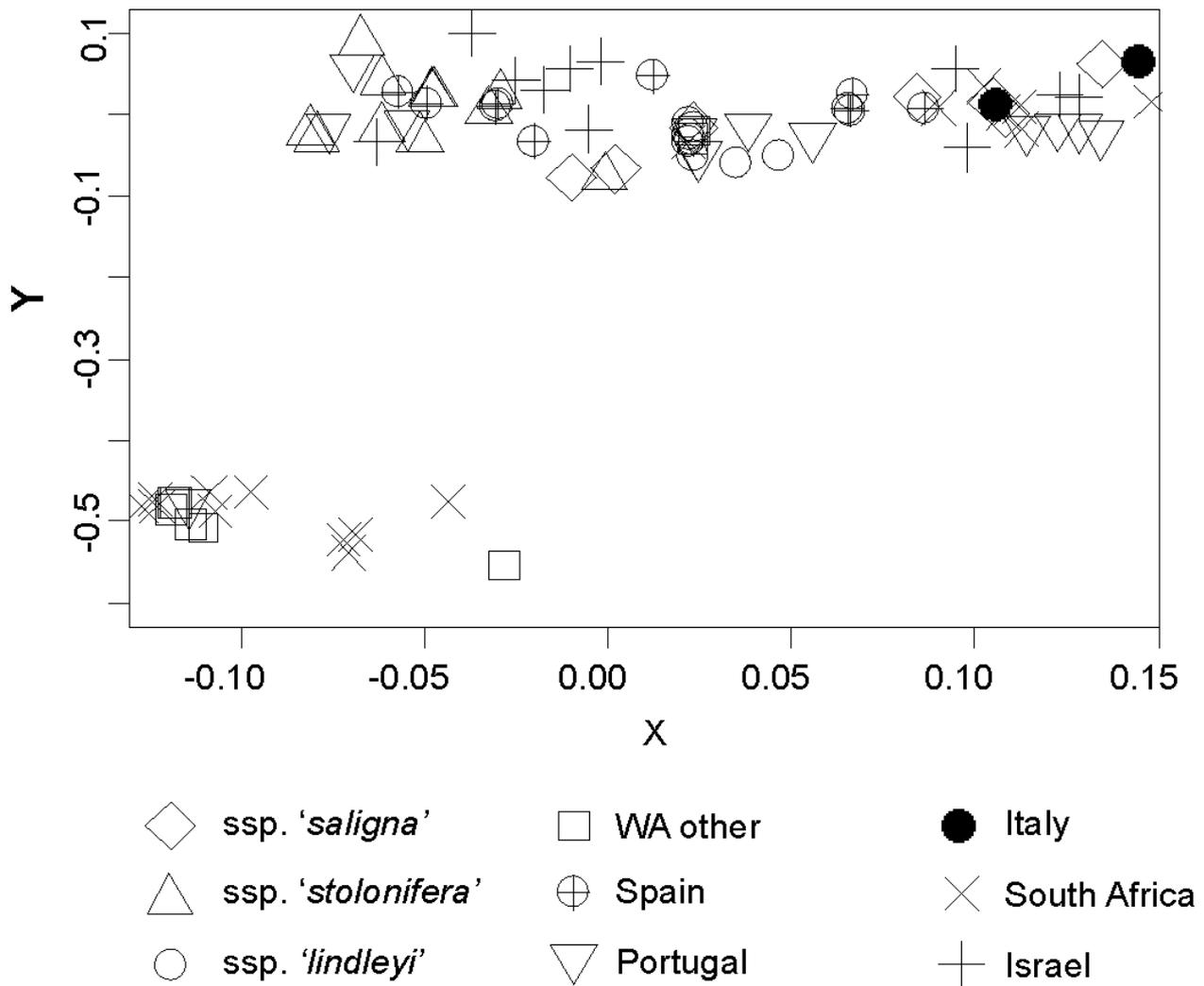
\* significant

**Table S4.3** Biological and ecological attributes of the four informal subspecies of *Acacia saligna* in the native range in Western Australia (adapted from worldwidewattle.com).

<i>A. saligna</i>		Inflorescence			
subspecies	Environment occupied	Phyllode	bud shape	Suckering ability	Bark description
<i>saligna</i>	coastal plains	narrow, dull	obtuse	yes	smooth, red
<i>stolonifera</i>	forest	dull	conical	yes, strongly	friable
<i>lindleyi</i>	wheat belt	shiny	conical	no	smooth
<i>pruinescens</i>	inland coastal region	glaucous, dull	conical	yes, strongly	white, friable



**Figure S4.1** Non-native herbarium records of *Acacia saligna* collated from eastern Australian herbaria and classed according to their year of collection.



**Figure S4.2** Plot of pairwise genetic distances between native and introduced individuals of *Acacia saligna* based on nDNA (external transcribed spacer) sequences. Nonmetric multidimensional scaling was used to translate genetic distances into 2D proximity co-ordinates.

## CHAPTER 5    **Microsatellite markers trace the introduction history of the invasive legume, *Paraserianthes lophantha***

- This chapter is in draft format.
- Authors: Thompson, G.D., Bellstedt, D.U., Richardson, D.M., Wilson, J.R.U & Le Roux, J.J.

### **Abstract**

The interplay between intra-specific genetic variation, human usage and the manner and pathway of introduction can substantially influence the distribution of invasiveness in Australian acacias (*Acacia* subgenus *Phyllodineae*). I further explore this using an intra-specifically diverse relative of the acacias, *Paraserianthes lophantha* (subspecies *lophantha* and subspecies *montana*). Introduced populations in eastern Australia, Portugal, New Zealand, South Africa and the USA exhibited a diverse array of genotypes, not all of which are present in Western Australia. This genetic pattern was supported by Bayesian clustering of nuclear microsatellites and phylogenetic reconstructions of nDNA. The distribution and number of genotypes present in introduced populations suggest a diverse array of native sources were sampled, and each source was sampled unequally. Unfortunately, lack of data for the native range of *P. lophantha* subspecies *montana* makes it impossible to conclude that only subspecies *lophantha* has been introduced outside of its native range.

### **5.1 Introduction**

Large-scale human-mediated movements of non-native species have in many instances led to the establishment of invasive plant populations (Wilson *et al.*, 2009). A species' genetic composition and the manner, site and rate at which it is introduced can affect the genetic

signature in its introduced range (Taylor & Keller, 2007) and its ability to become invasive (Lee, 2002). The relationship between genetic composition and introduction history is further influenced when native range populations are intra-specifically diverse and biogeographically structured (see Le Roux *et al.*, 2011). Indeed, high intra specific diversity in invasive plants could translate into ecological differences (e.g. *Acacia saligna* species complex; Thompson *et al.*, 2011). Identifying these differences is not only important for understanding the dynamics of biological invasions but also for their management, especially biological control (e.g. Scott *et al.*, 1998; Wardill *et al.*, 2005).

Multiple introductions of non-native species may allow introduced populations to overcome the genetic bottlenecks that are often experienced during introduction (Barret & Husband, 1990). The introduction of previously disjunct (or genetically diverse) native sources has led to new genetic combinations, increased genetic diversity and higher adaptive potential in introduced populations (Sakai *et al.*, 2001; Lee, 2002). Mating between diverse native entities in conjunction with novel selection pressures in the introduced range may contribute to rapid evolution and increased invasiveness (Lavergne & Molofsky, 2007). The number of recombination events may be increased if introduced populations have been present in the new range for a long time, or if non-native species are selectively bred for human-use.

Woody plant species used in silviculture and agriculture have been widely distributed and cultivated for centuries, in many instances resulting in invasive populations (Thuiller *et al.*, 2006; Richardson & Rejmánek, 2011). Such species tend to have complex introduction histories (Le Roux *et al.*, 2011) and are often introduced repeatedly to a range of different ecosystems over an extended period of time (e.g. *A. mearnsii*; Poynton, 2009). Many silvicultural species also possess a number of 'invasive' traits, including an ability to grow rapidly in a range of different environments. Australian acacias (more than 1000 species) are a large group of species that

have been widely disseminated for economic gain and are considered a model group in invasion biology (Richardson *et al.*, 2011).

*Paraserianthes* is the sister genus to the Australian acacias (Brown *et al.*, 2011, Miller *et al.*, 2011). This woody tree species shares similar ecological attributes (i.e. preference for Mediterranean-climates) and invasive distributions with a number of acacias and it is also subject to similar management strategies. For example, many introduced acacias and *P. lophantha* are subject to biological control by seed-feeding insects in South Africa (Impson *et al.*, 2011). *Paraserianthes lophantha* (Willd.) I.C. Nielsen, one of four species in the genus, displays high levels of morphological variation (Brown *et al.*, 2011). Two sub-species are currently recognised: subspecies *lophantha* in Western Australia and subspecies *montana* in Indonesia, New Guinea and the Solomon Islands (Nielsen *et al.*, 1983, see Fig. 1).

Overall, global introductions of *P. lophantha* are poorly recorded and the subspecific identity of introduced populations is unknown. Similar to many wattles, *P. lophantha* was exported for its wood, as an ornamental and for forestry (Henderson, 2001). The species is considered invasive in Hawaii, New Zealand, South Africa ([www.hear.org](http://www.hear.org)), southern California (Stirton, 1978), the Canary Islands and Chile (Randall, 2002), and is naturalized in the temperate parts of Australia (New South Wales, South Australia, Victoria; Nielsen *et al.*, 1983; Nielsen, 1992; Barneby & Grimes, 1996; Entwisle *et al.*, 1996). In South Africa, *P. lophantha* invades forest margins and riverbanks (Henderson, 2001) and is considered an environmental transformer that alters the ecosystems it invades (Stirton, 1978). The species produces large seed banks that are viable for several years making control of established infestations as challenging as wattle invasions (Wilson *et al.*, 2011).

The unusual native distribution of *P. lophantha* (spanning two major land masses) could have substantial effects on the ecological and genetic attributes of introduced populations. Such

geographically disjunct distributions may suggest that the two subspecies are genetically divergent and have a preference for differing environmental conditions. The sympatric introduction of two very divergent entities can substantially affect the evolutionary trajectory of introduced populations (through novel genetic combinations), or increase the ability of the species to tolerate a much wider range of climatic conditions (compared to native populations).

In this study I aimed to improve our understanding of *P. lophantha* invasions by assessing the genetic diversity, intra-specific identity, and population genetic structure of native (Australia) and introduced populations (eastern Australia, South Africa, Hawaii and Portugal) using nuclear DNA and nuclear microsatellites. In doing so, I hope to identify the genetic patterns in introduced populations of the species, and provide potential management recommendations for biological control.

## **5.2 Methods**

### **(a) Sampling design and DNA isolation**

*Paraserianthes lophantha* subspecies *lophantha* was sampled from eleven localities across the native range in Western Australia (Table S1); eleven sites elsewhere in Australia (New South Wales, 2; South Australia, 4; Victoria, 5); 13 sites in South Africa; and one site each in Portugal and the USA. I was unable to obtain suitable material of *P. lophantha* subspecies *montana* from its native range and supplemented with material from its introduced range in the Hawaiian Archipelago. Leaf material was dried and stored on silica gel until DNA extraction. Whole genomic DNA was extracted using the cetyltrimethyl ammonium bromide (CTAB) method as described by Doyle and Doyle (1990).

(b) *Amplification and data analysis of microsatellite markers*

Forty-three nuclear microsatellites (Butcher *et al.*, 2000, Ng *et al.*, 2005, Millar & Byrne, 2008) were tested for cross-amplification to *P. lophantha* using the methods described in the supplementary information (S1). Eleven highly polymorphic nuclear microsatellites were selected for a population genetic study. All markers were amplified using the conditions described in the Supplementary Information (S1).

Microsatellite data was tested for linkage disequilibrium and departures from Hardy-Weinberg Equilibrium (HWE) using 10 000 steps in the Markov chain in ARLEQUIN v 3. 5 (Excoffier & Lischer, 2010). Isolation by distance (IBD) analyses may provide insights into the reproductive and dispersal limits of invasive species. IBD was tested in native (Western Australia) and introduced populations (South Africa) via Mantel tests using the online resource IBDWS v 3.16 (Jensen *et al.*, 2005). Mantel tests were conducted using a matrix of pairwise genetic distances ( $F_{ST}$  values) was plotted against geographical distances (Euclidian distance).

Genetic diversity between the native and introduced range was compared by calculating the observed and expected heterozygosities ( $H_E$  and  $H_O$ ), fixation indexes ( $F_{ST}$ ), and inbreeding coefficients ( $F_{IS}$ ) in FSTAT v. 2.9.3.2 (Goudet, 2001) using 10 000 permutations. Genetic diversity at the population level was assessed by calculating the mean number of alleles ( $N_A$ ), observed and expected heterozygosity ( $H_O$  and  $H_E$ ) and inbreeding levels ( $F_{IS}$ ) in ARLEQUIN; lastly the mean number of private alleles per population ( $P_A$ ) was calculated in GENALEX v 6.4 (Peakall & Smouse, 2006). The statistical significance of genetic diversity at the population level was assessed using a two sided T-test.

The distribution of genetic variation between native and introduced populations was assessed using an analysis of molecular variance (AMOVA) based on microsatellite allele frequencies ( $R_{ST}$  like) in ARLEQUIN (Excoffier & Lischer, 2010). Genetic variation was assessed

at three hierarchical levels using 10 000 permutations. I partitioned total genetic variance among invasive and native regions, among populations within regions and within populations. Genetic and spatial population differentiation in Western Australia and South Africa was independently assessed by computing population pairwise  $F_{ST}$  values in ARLEQUIN. 10 000 permutations, and the significance of  $F_{ST}$  values were assessed using a Bonferroni adjustment for multiple comparisons (Weir, 1996).

I used a spatially explicit Bayesian clustering algorithm that incorporates admixture and a hierarchical clustering approach (Le Roux *et al.*, 2009) implemented in STRUCTURE v 2.3.2 (Falush *et al.*, 2007). A spatially explicit, admixed model was selected as it is better able to identify the optimal number of genetic clusters (François & Durand, 2010). STRUCTURE HARVESTER (Dent & vonHoldt, 2012) and the  $\Delta K$  method of Evanno *et al.* (2005) were used to determine all levels of population structure. K values were simulated from one upwards until the number of individuals per population was insufficient to allow for further analyses, or until K exceeded the total number of populations. Individuals unassigned to a particular group with more than 60% of their genome were not included in the next level of the analysis. Two datasets were analysed using the aforementioned parameters: the first dataset included all native populations and aimed to determine the number of genetic demes present in Australia; the second dataset included all native and introduced populations and aimed to determine the number of genetic demes in the native and introduced.

### (c) *DNA sequencing and data analysis*

The nuclear external transcribed spacer (ETS) was amplified using the primers described in Brown *et al.* (2008), and the PCR setup and conditions described in Le Roux *et al.* (2011). Sequence data was visualized, edited manually in BIOEDIT version 7.0.5.3 (Hall, 1999). I also downloaded ETS sequences from GenBank representative of *P. lophantha* subspecies

*lophantha* (GenBank numbers HM800432, HM800431). The final dataset comprised 41 sequences generated for previous work (Le Roux *et al.*, 2011), and 21 sequences generated for this study.

To assess genetic differentiation between the native and introduced range I calculated gene diversity and nucleotide diversity in DnaSP v.5 (Librado & Rozas, 2009). I also reconstructed networks using statistical parsimony with a 95 % connection limit in TCS v.1.21 (Clement *et al.*, 2000). I assessed the distribution of genetic variation between native and introduced populations using an analysis of molecular variance (AMOVA) using 10 000 permutations in ARLEQUIN (Excoffier & Lischer, 2010), and the same hierarchical setup as the microsatellite data.

To support the placement of native and introduced populations identified by statistical parsimony, I conducted a non-metric multidimensional scaling analysis (NMDS). Here, pairwise genetic distances between ETS sequences (calculated in BIOEDIT; Hall, 1999) were clustered using a 'ratio + bounds' analysis in the software PERMAP (Heady & Lucas, 2007). Ten iterations and a convergence value of 0.000005 were used to find the solution with the lowest objective function value. Proximity co-ordinates for each individual were plotted in R using the 'car' package (Fox & Weisberg, 2011).

## **5.2 Results**

### **(a) Microsatellite diversity and population genetic structure**

There was no evidence of significant isolation by distance in either the Western Australian ( $r^2 = 0.009$ ,  $p = 0.2960$ ) or South African ( $r^2 = 0.0144$ ,  $p = 0.2200$ ) ranges of *P. lophantha* (Fig. S5.1). When genetic diversity in the native and introduced range was compared at the

population level ( $N_A$ ,  $H_O$ ,  $H_E$ ,  $P_A$ ,  $F_{IS}$ ; Table 5.1) or at the regional level (Table 5.1) there were no significant differences in genetic diversity between ranges. The hierarchical AMOVA indicated that the majority of microsatellite diversity resided within populations (Table 5.3).

In the native range, the Bayesian clustering algorithm identified five genetic demes in the seven native populations ( $K=5$ , Fig. S5.2). On average, individuals that were collected within populations in the field also clustered within genetic demes during Bayesian clustering. That is, each genetic deme represented a population in the field, except the Pemberton individuals that were assigned to two genetic groups (pink and blue demes, Fig. S5.2). Native populations and South African populations displayed moderate to high population genetic differentiation (Table S5.2).

Overall, hierarchical Bayesian clustering identified six genetic demes in the native and introduced populations ( $K=6$ , Fig. S5.3). All six genetic demes were identified in the native populations, while only five of these were present in the introduced range (Fig. 5.1, S5.3). These five genetic demes were differentially distributed across the sampled range (Fig. 5.1). Only a single genetic deme was present in each introduced population in Portugal (POR) and the USA (HAW), while five genetic demes were spread across the South African range (BOT, CIT, GEO, HAN, KIR, KUR, PIK, SCA, STE, TSI, VAN), and four genetic demes were present in the eastern Australian range (ADE, CCP, MTJ, PEI; Fig. 5.1, S5.3).

### *(b) DNA sequence variation and phylogeography*

Overall, nuclear DNA (423 bp. ETS region) identified low intra-specific divergence within the 62 individuals of *P. lophantha*. Statistical parsimony retrieved a total of 10 different sequences that were contained within a single network (Fig. 5.2). As with the microsatellite data, genetic diversity was similar between the native and introduced range (Table 5.3, S5.3), where the majority of diversity was partitioned within populations (Table 5.2).

Two high frequency sequences were shared between individuals collected in Western Australia, eastern Australia and South Africa, as well as sequences representative of *P. lophantha* ssp. *lophantha* (data downloaded from GenBank, cf. Brown *et al.*, 2011). A total of five geographically unique sequences were identified. Four were present in introduced populations - three in the USA and one in South Africa. The remaining unique sequences were representative of *P. lophantha* ssp. *lophantha* (data downloaded from GenBank, cf. Brown *et al.*, 2011). Statistical parsimony and NMDS identified similar patterns within native and introduced populations (Fig. 5.2, 5.3). Overall, native and introduced sequences displayed close genetic relationships, and did not cluster into ranges (i.e. native vs. introduced) or geographically localised groups (i.e. per sampled population or country, Fig. 5.3).

### **5.3 Discussion**

High genetic diversity in the introduced range has been repeatedly linked with both invasive success and multiple introductions (e.g. Lavergne & Molofsky, 2007; Rollins *et al.*, 2009; Tang *et al.*, 2009; Gaudeul *et al.*, 2011). The presence of high genetic diversity in the introduced range is however dependant on the genetic structure present within the native range, and the species' history of introduction (number, site and time of introduction). Nuclear DNA and microsatellite data showed substantial intra-specific diversity in the native range of *P. lophantha*. Furthermore, results show that almost all native genotypes of *P. lophantha* subspecies *lophantha* have been introduced outside of Western Australia. Similar native genetic patterns have been identified in acacias that are native to Western Australia (e.g. *A. cyclops*, Le Roux *et al.*, 2011; *A. saligna*, Millar *et al.*, 2011). Similar introduced genetic patterns have been identified in invasive acacias that have been introduced to South Africa and many of the other regions sampled for *P. lophantha* (e.g. *A. saligna* in Spain and Portugal, see Chapter 3 and 4).

On the basis of genetic data we showed that introduced populations were most likely introduced from the following areas or regions with similar allele frequencies as those listed: naturalised populations in eastern Australia were sourced from northern and southern populations in Western Australia (sites Canning Hills, Pemberton, Porongurup); South African invasions were sourced from across Western Australia and/or from eastern Australia (but excluding the Margaret River site); Portuguese invasions were sourced from the south-eastern parts of Western Australia and/or secondarily from eastern Australia; American/Hawaiian invasions were sourced from a limited number of populations in the south-western parts of Western Australia (sites Pemberton, Porongurup and Margaret River).

The introduction of *P. lophantha* to regions outside of its native range has been poorly documented for all the introduced regions I sampled, except for South Africa. The species was introduced to the Cape on two separate occasions in 1833 and 1835, where introductions were most likely in small numbers and from Australia (Stirton, 1978). Our study showed that South African populations are almost as diverse and structured as native Western Australian populations of *P. lophantha* subspecies *lophantha*, and were sourced from a range of geographically disjunct sites. Well documented introduction histories for other Australian acacias provide evidence for similarly broad-scale collection efforts prior to introductions to South Africa (e.g. *A. mearnsii* was collected from more than 20 sites; Poynton, 2009).

In contrast to the introduced range in South Africa, Portuguese and American/Hawaiian populations were much less diverse and comprised substantially fewer genotypes (Hawaii only) than invasive South African populations. While it is possible that the observed patterns (reduced genetic diversity) are artefacts of limited sampling effort, it is also possible that the introduced populations in Portugal and Hawaii underwent a genetic bottleneck during introduction. These populations may consequently be subject to inbreeding depression in the introduced range, and a reduced ability to expand their range.

The presence of unique ETS genotypes in the USA for Hawaiian populations of *P. lophantha* subspecies *montana* (but closely related to subspecies *lophantha*) has more than one possible explanation. The most parsimonious is that the low levels of phylogenetic divergence between Hawaiian sequences and those representative of *P. lophantha* subspecies *lophantha* (only one mutational step); as well as shared microsatellite genotypes between Hawaii and Western Australia, indicate that Hawaiian populations were incorrectly identified and do not represent *P. lophantha* subspecies *montana*. Alternatively, our data may support the view of Barneby and Grimes (1996) who proposed an alternative classification reducing *P. lophantha* to a single species that is morphologically variable but displays low level of genetic divergence.

Unfortunately, lack of data for the native range of *P. lophantha* subspecies *montana* makes it impossible to conclude that only subspecies *lophantha* has been introduced outside of its native range. However, even if *P. lophantha* is revised to a single species, the high levels of intra-specific variation present in Western Australia are still likely to substantially influence the introduced genetic diversity and the opportunity for novel genetic combinations. On a broader scale, such divergent genetic variation will affect the evolutionary trajectories of introduced populations of *P. lophantha*.

Overall, this approach was able to identify contrasting genetic signatures in introduced populations of *P. lophantha* and reinforces the need to consider native intra-specific diversity and a species history of introduction when attempting to reconstruct invasion pathways. Further, the study shows that introduced genetic signatures cannot always be attributed to a particular manner of introduction, particularly for economically valuable species (Le Roux *et al.*, 2011).

## 5.4 Tables and Figures

**Table 5.1** Genetic diversity indices for native and introduced populations of *Paraserianthes lophantha* genotyped at 11 nuclear microsatellite loci.

Country	Label	Location	N	$N_A$	$H_O$	$H_E$	$F_{IS}$	$P_A$
Native								
Australia	CAN	Canning Hills, Western Australia	4	2.09	0.36	0.35	0.39	0.55
Australia	HAM	Hamel, Western Australia	4	2.09	0.23	0.38	0.61	0.27
Australia	JAR	Jarrahdale, Western Australia	5	1.45	0.31	0.15	0.29	0.27
Australia	KRD	Kordabup, Western Australia	25	5.36	0.12	0.57	0.79	1.64
Australia	MAR	Margaret River, Western Australia	4	2.18	0.88	0.38	-0.31	0.64
Australia	PEM	Pemberton, Western Australia	16	6.18	0.15	0.66	0.77	2.00
Australia	POG	Porongurup, Western Australia	12	5.09	0.15	0.63	0.77	1.18
<i>Average</i>				3.49	0.31	0.45	0.47	0.94
Introduced								
Australia	ADE	Adelaide, South Australia	4	2.09	0.09	0.36	0.80	0.00
Australia	CCP	Cleland Park, South Australia	9	1.64	0.11	0.22	0.80	0.09
Australia	MTJ	Mount Jagged, South Australia	4	0.91	0.02	0.09	0.53	0.00
Australia	PEI	Port Elliot, South Australia	7	2.45	0.10	0.32	0.72	0.09
Portugal	HT	Portugal	10	1.45	0.05	0.20	0.79	0.09
South Africa	BOT	Bot River, Western Cape	5	0.82	0.00	0.07	1.00	0.00
South Africa	CIT	Citrusdal, Western Cape	6	3.09	0.22	0.46	0.59	0.12
South Africa	GEO	George, Western Cape	21	3.27	0.25	0.45	0.47	0.12
South Africa	HAN	Hankey, Eastern Cape	4	1.36	0.11	0.14	0.35	0.00
South Africa	KIR	Kirstenbosch, Western Cape	5	2.82	0.17	0.48	0.71	0.36
South Africa	KUR	Kurland, Western Cape	5	2.36	0.05	0.35	0.88	0.09
South Africa	PIK	Piketberg, Western Cape	6	1.73	0.09	0.21	0.63	0.12
South Africa	SCA	Scarborough, Western Cape	6	3.00	0.15	0.45	0.71	0.14
South Africa	STE	Stellenbosch, Western Cape	23	4.00	0.11	0.39	0.73	0.09
South Africa	TSI	Tsitsikamma, Eastern Cape	19	3.45	0.16	0.42	0.63	0.09
South Africa	VAN	Vanrhynsdorp, Western Cape	14	2.55	0.15	0.38	0.63	0.12
USA	HAW	Maui, Hawaii	20	6.09	0.30	0.59	0.51	0.67
<i>Average</i>				2.53	0.13	0.33	0.67	0.13

Note:  $N$  – number of individuals,  $N_A$  – mean number of alleles,  $H_O$  – mean observed heterozygosity,  $H_E$  – mean expected heterozygosity,  $F_{IS}$  – mean inbreeding levels,  $P_A$  – mean number of private alleles per population

**Table 5.2** Genetic diversity indices for native and introduced populations of *Paraserianthes lophantha*, genotyped at 11 nuclear microsatellite loci.

	$H_S$	$H_O$	$F_{IS}$	$F_{ST}$
Native	0.564	0.164	0.709	0.269
Introduced	0.442	0.174	0.607	0.360

Note:  $H_S$  - unbiased gene diversity,  $H_O$  - observed heterozygosity,  $F_{IS}$  – inbreeding coefficient,  $F_{ST}$  – among population differentiation.

**Table 5.3** A hierarchical AMOVA partitioning genetic variation in *Paraserianthes lophantha* at three spatial scales: among native and invasive ranges; among populations and within populations within native and invasive ranges.

Source of variation	d.f.	Sum of squares	Fixation index	Percent variation (%)
<b>Microsatellite data</b>				
Among native and invasive range	1	65.34	0.403*	10.18
Among populations	22	65.34	0.376*	34.40
Within populations	452	524.98	0.043	55.42
<b>nDNA</b>				
Among native and invasive range	1	0.68	0.445*	2.31
Among populations	14	6.98	0.431*	42.15
Within populations	38	5.38	0.023	55.54

\* denotes  $P < 0.0001$

## Figure Legends

**Figure 5.1** Global distribution of the six genetic demes identified by Bayesian clustering in native and introduced populations of *Paraserianthes lophantha*. The native distribution of the two subspecies of *P. lophantha* (ssp. *lophantha* and ssp. *montana*) are encircled. The dataset comprised 238 individuals genotyped at 11 nuclear microsatellite loci. Pie charts show overall genotype assignment for each population to particular genetic clusters.

**Figure 5.2** Genetic relationships between native and introduced populations of *Paraserianthes lophantha* determined by statistical parsimony of nuclear DNA (external transcribed spacer). Populations are coded according to their geographic population of origin.

**Figure 5.3** Pairwise genetic distances for *Paraserianthes lophantha* populations collected in the native range in Western Australia and introduced range in eastern Australia (New South Wales, South Australia, Victoria), South Africa and the USA. Nonmetric multidimensional scaling was used to translate nDNA pairwise genetic distances into proximity co-ordinates.

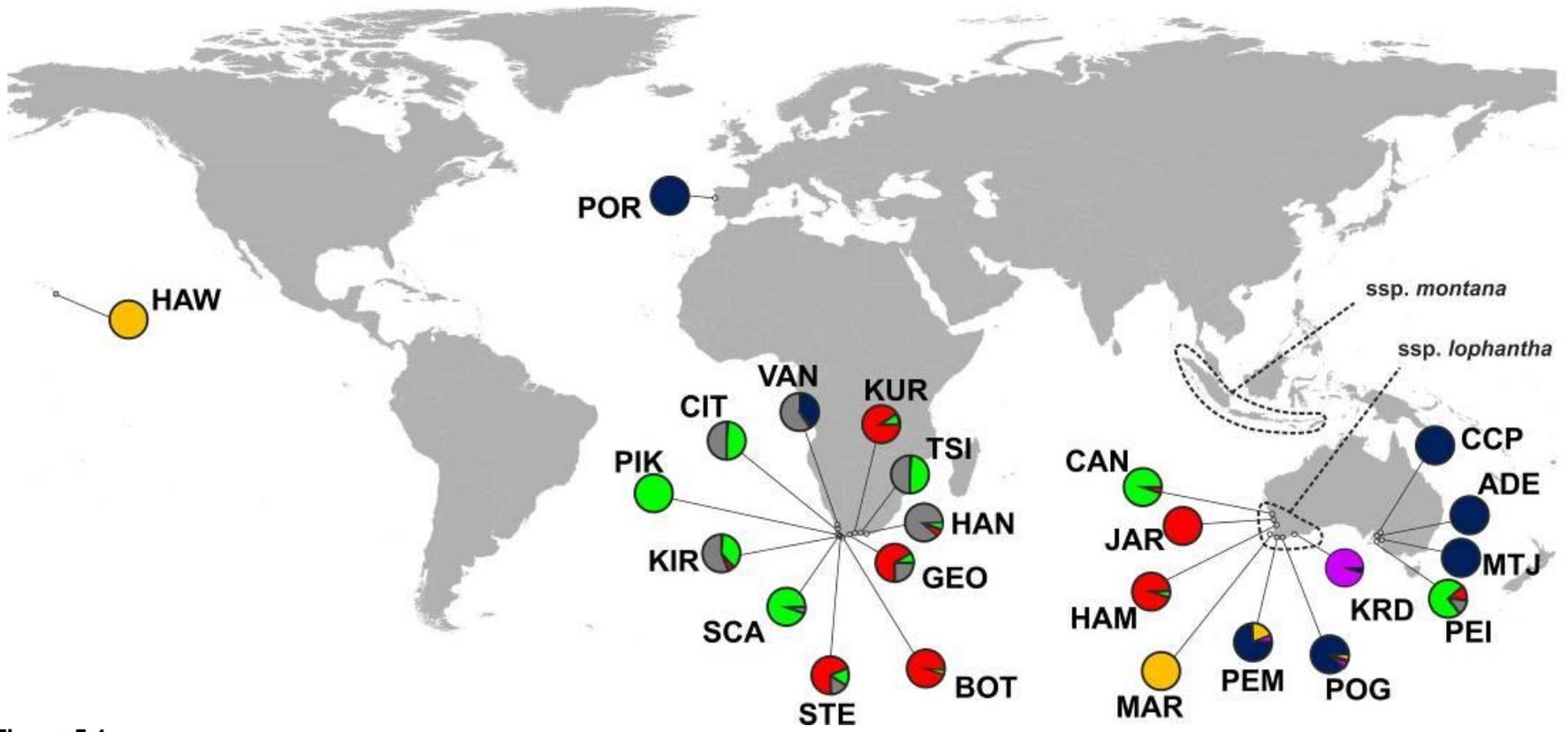
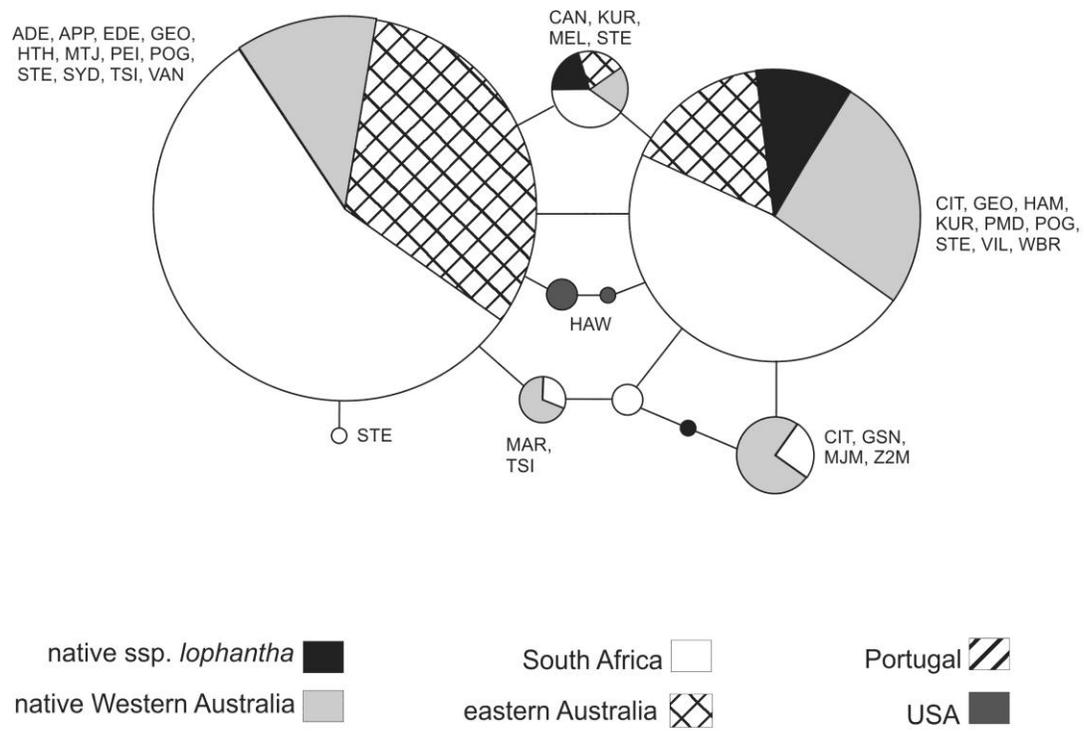
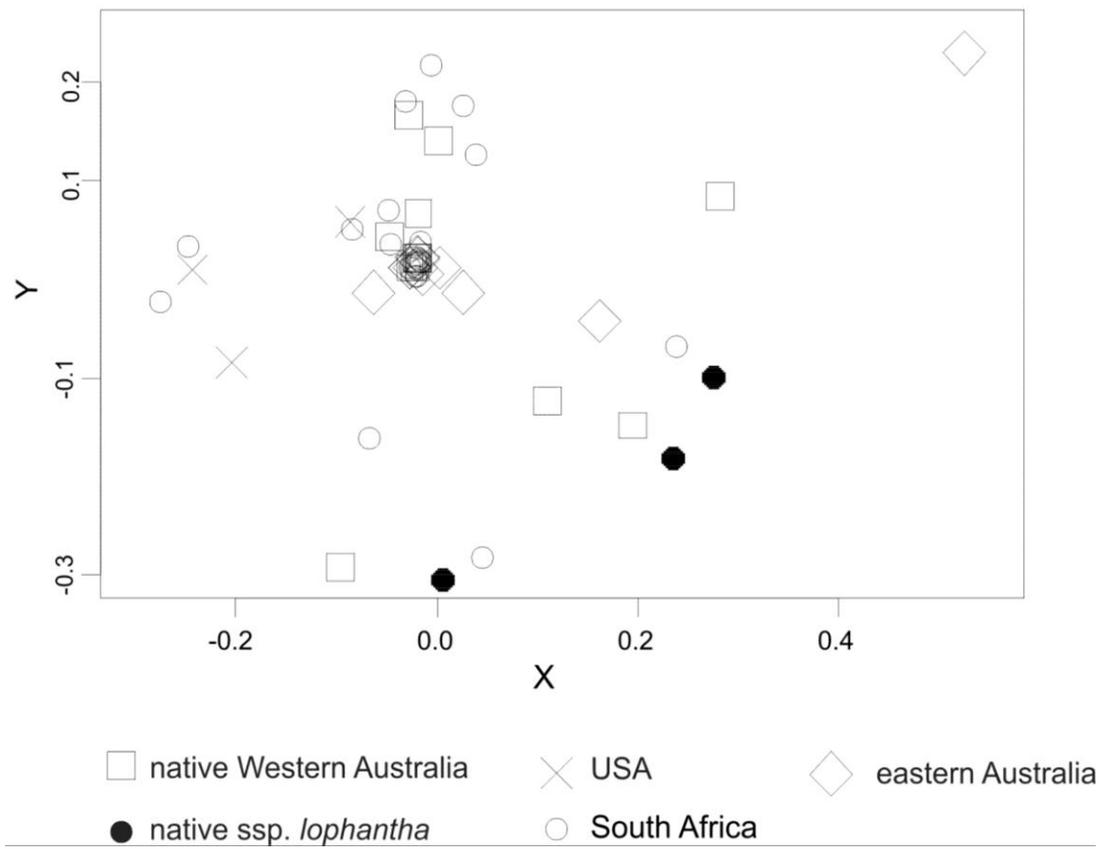


Figure 5.1



**Figure 5.2**



**Figure 5.3**

## 5.5 Appendices

### Appendix S5.1 Transfer of nuclear microsatellites to *Paraserianthes lophantha*

Forty-three nuclear microsatellites previously developed for *Acacia mangium* (Butcher *et al.*, 2000), *Acacia saligna* (Millar & Byrne, 2008), and an *Acacia* hybrid (*Acacia mangium* X *Acacia auriculiformis*, Ng *et al.*, 2005) were tested for amplification using conditions described for their development. All PCRs were conducted on a Multigene Cycler (Labnet International, Inc.). Fluorescence was visualised using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, USA) and GENEMARKER® version 1.95 (SoftGenetics LLC®, Pennsylvania, USA). PCR products were sized relative to a molecular size standard (LIZ500 (-250), PE Applied Biosystems).

Loci that did not amplify using their developmental conditions were PCRd with a temperature gradient PCR at 48–60°C, and at three different MgCl<sub>2</sub> concentrations (1.0 mM, 1.5 mM and 2.0 mM). Each 10 µL reaction contained 0.25 U HotStart Taq polymerase (KapaBiosystems, Cape Town, South Africa), 1.5 mM MgCl<sub>2</sub>, 10 mM Tris-HCl (pH 8.4), 50 mM KCl, 0.2 mM of each dNTP, 5 µM of each primer and ~25 ng/µL genomic DNA. The PCR cycle in the KapaBiosystems HotStart Kit. The PCR cycle and reaction setup was optimized for all loci until single bands were clearly distinguishable on a 2% Agarose gel, run at 80 V for 120 min. The forward primer from each pair was fluorescently end-labelled with 6-FAM, HEX, VIC, NED or PET.

All successfully transferred microsatellites (15 nuclear microsatellites), as well as six additional microsatellites developed for *P. lophantha* (Brown & Gardner, 2011) were tested for polymorphism in 24 native individuals (collected in Western Australia) and 24 introduced individuals (collected in Hawaii, USA). Only highly polymorphic nuclear microsatellites (> 6 alleles) were selected for a population genetic study on *P. lophantha*.

**Table S5.1** Sample locality information for all native and introduced sampling localities of *Paraserianthes lophantha*.

Country	Label	Location	Latitude	Longitude
Australia	CAN	Canning Hills, Western Australia	-32.055	116.106
Australia	GSN	Gingilup Swamps Nature Reserve, Western Australia	-34.315	115.414
Australia	HAM	Hamel, Western Australia	-32.866	115.918
Australia	JAR	Jarrahdale, Western Australia	-32.326	116.099
Australia	KRD	Kordabup, Western Australia	-35.017	117.095
Australia	MJM	Manjimup, Western Australia	-34.216	115.940
Australia	MOR	Morangup, Western Australia	-31.684	116.325
Australia	PEM	Pemberton, Western Australia	-34.415	116.096
Australia	POG	Porongurup Range, Western Australia	-34.673	117.904
Australia	UNK	Western Australia	-34.673	117.904
Australia	Z2M	William Bay, Western Australia	-34.994	117.247
Australia	ADE	Adelaide, South Australia	-35.251	138.550
Australia	CCP	Cleland Conservation Park, South Australia	-34.957	138.676
Australia	MTJ	Mount Jagged, South Australia	-35.390	138.633
Australia	PEI	Port Elliot, South Australia	-35.593	138.363
Australia	APP	Apex Park, Victoria	-37.881	147.972
Australia	MEL	Melbourne, Victoria	-37.634	144.490
Australia	PMD	Port Macdonnell, Victoria	-38.058	141.093
Australia	WBR	Woolamai Beach Rd, Phillip Island, Victoria	-38.530	145.335
Australia	WYR	Wye River, Victoria	-38.641	143.890
Australia	EDE	Eden, New South Wales	-37.069	149.910
Australia	SYD	Sydney, New South Wales	-33.779	151.238
Portugal	HT	Portugal	40.771	-8.695
South Africa	BOT	Bot River, Western Cape	-34.221	19.174
South Africa	CIT	Citrusdal, Western Cape	-32.620	18.957
South Africa	GEO	George, Western Cape	-34.023	22.332
South Africa	HAN	Hankey, Eastern Cape	-33.901	24.830
South Africa	HTH	Hottentots Holland, Western Cape	-33.938	19.162
South Africa	KIR	Kirstenbosch, Western Cape	-33.993	18.436
South Africa	KUR	Kurland, Western Cape	-33.966	23.453
South Africa	PIK	Piketberg, Western Cape	-32.800	18.695
South Africa	SCA	Scarborough, Western Cape	-34.205	18.397
South Africa	STE	Stellenbosch, Western Cape	-33.944	18.877
South Africa	TSI	Tsitsikamma, Eastern Cape	-33.998	24.228
South Africa	VAN	Vanrhynsdorp, Western Cape	-31.615	18.742
South Africa	VIL	Viljoenshof, Western Cape	-34.670	19.692
USA	HAW	Maui, Hawaii	20.677	-156.332

**Table S5.2** Population pair-wise  $F_{ST}$  values calculated in ARLEQUIN using 10 000 permutations for native and introduced populations of *Paraserianthes lophantha*. The dataset comprised 184 individuals from 18 populations genotyped at 11 nuclear microsatellite loci.

<b>Western Australia</b>	<b>CAN</b>	<b>HAM</b>	<b>JAR</b>	<b>KRD</b>	<b>MAR</b>	<b>PEM</b>	<b>POG</b>				
Hamel (HAM)	0.564										
Jarrahdale (JAR)	0.689	0.502									
Kordabup (KRD)	0.409	0.357	0.446								
Margaret River (MAR)	0.578	0.406	0.593	0.369							
Pemberton (PEM)	0.325	0.165	0.335	0.203	0.231						
Porongurup Range (POG)	0.343	0.242	0.375	0.233	0.285	0.130					

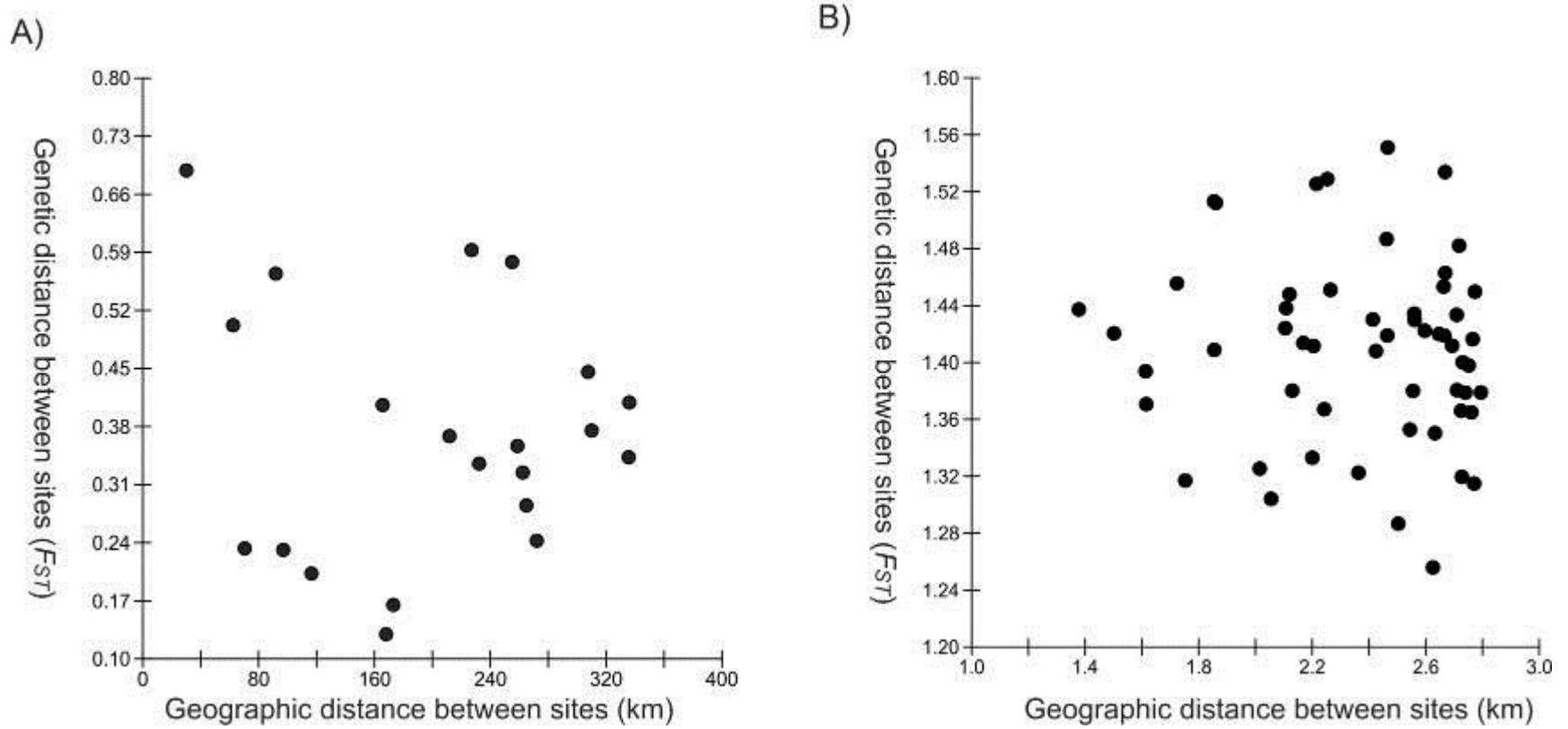
  

<b>South Africa</b>	<b>BOT</b>	<b>CIT</b>	<b>GEO</b>	<b>HAN</b>	<b>KIR</b>	<b>KUR</b>	<b>PIK</b>	<b>SCA</b>	<b>STE</b>	<b>TSI</b>
Citrusdal (CIT)	0.384									
George (GEO)	0.048	0.261								
Hankey (HAN)	0.686	0.467	0.335							
Kirstenbosch (KIR)	0.246	0.273	0.246	0.327						
Kurland (KUR)	-0.033	0.299	0.237	0.528	0.235					
Piketberg (PIK)	0.596	0.451	0.439	0.670	0.335	0.505				
Scarborough (SCA)	0.276	0.345	0.344	0.509	0.257	0.373	0.288			
Stellenbosch (STE)	-0.288	0.354	0.209	0.439	0.268	0.088	0.455	0.389		
Tsitsikamma (TSI)	0.319	0.298	0.307	0.369	0.175	0.352	0.375	0.307	0.378	
Vanrhynsdorp (VAN)	0.360	0.184	0.293	0.450	0.302	0.374	0.465	0.421	0.398	0.289

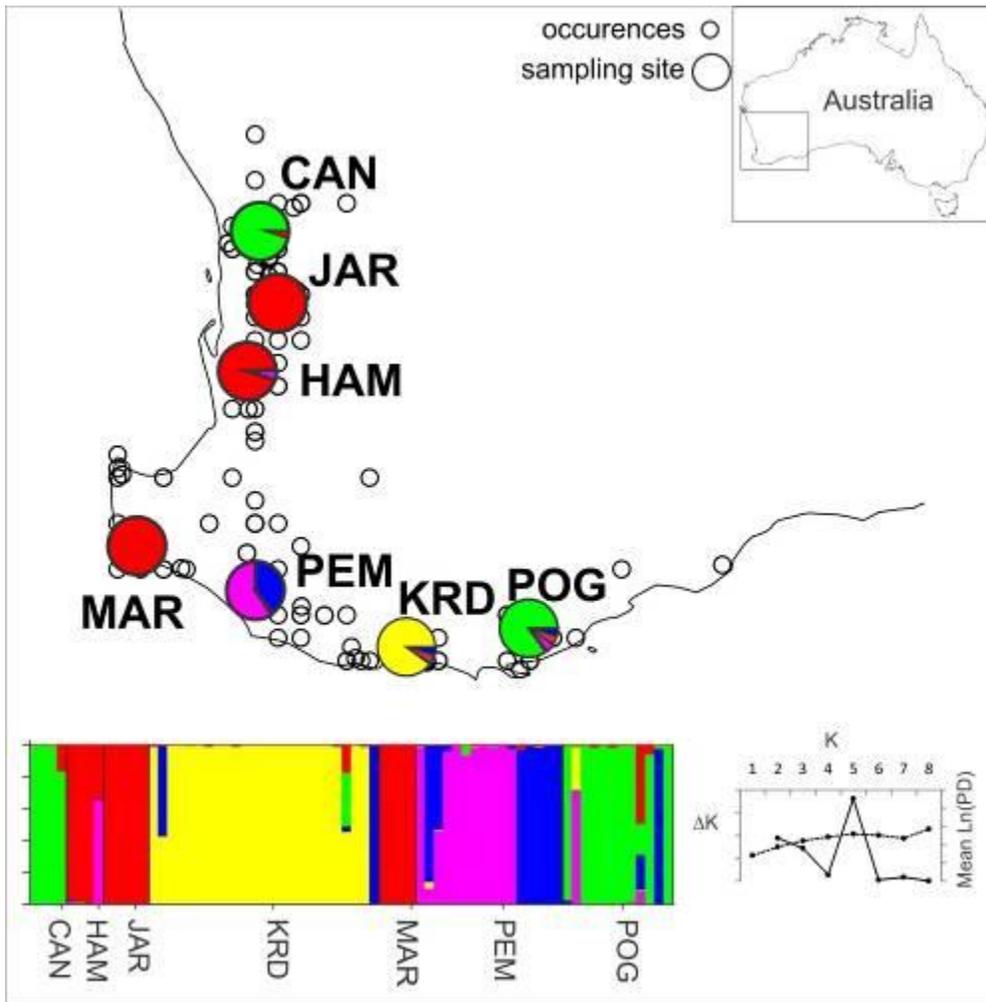
**Table S5.3** Genetic diversity indices for populations of *Paraserianthes lophantha* in the native (Australia) and introduced range (Portugal, South Africa and the USA).

	N	Gene diversity	Nucleotide diversity
Native	26	0.452 (0.109)	0.00141 (0.00039)
Introduced	32	0.387 (0.105)	0.00108 (0.00033)

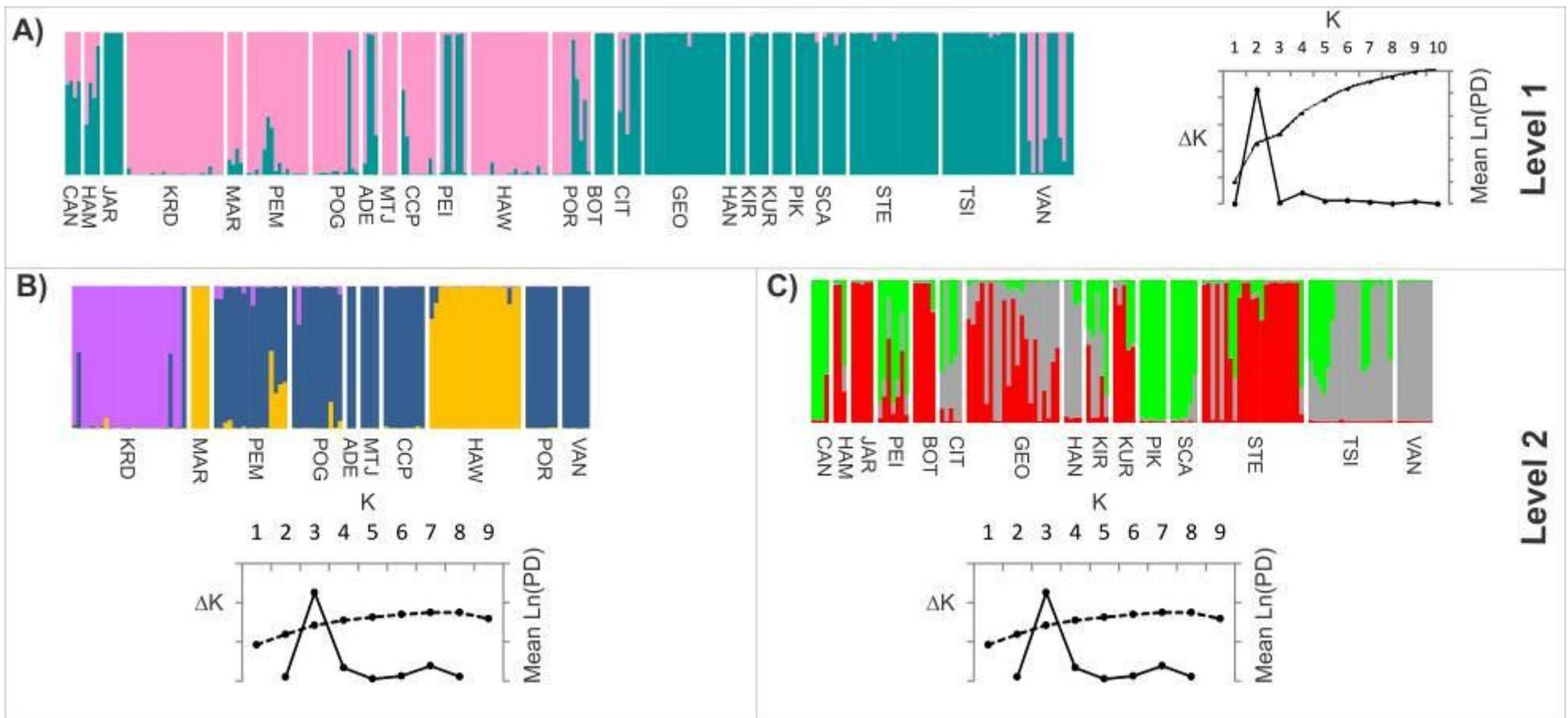
Note: N – number of ETS sequences / individuals, standard deviation in parenthesis.



**Figure S5.1** Population pairwise relationships between genetic (microsatellite  $F_{ST}$ ) and geographic distance for populations of *Paraserianthes lophantha* in the native range in Western Australia (A) and the introduced range in South Africa (B). Population pairwise genetic and geographic (Euclidean) distances were correlated using a Mantel test and the online “isolation by distance” service (<http://ibdws.sdsu.edu/~ibdws>).



**Figure S5.2** Bayesian assignment of native populations of *Paraserianthes lophantha* based on 11 nuclear microsatellite loci. Pie charts show overall genotype assignment for each population to particular genetic clusters. Membership of each individual's genome ( $q_i$ ) to the three identified genetic clusters is indicated by vertical bars.



**Figure S5.3** Hierarchical clustering of native and introduced populations of *Paraserianthes lophantha* from Australia (ADE, CAN, MTJ, CCP, HAM, JAR, KRD, MAR, PEI, PEM, POG), South Africa (BOT, CIT, GEO, HAN, KIR, KUR, PIK, SCA, STE, TSI, VAN), Portugal (POR) and the USA (HAW). 238 individuals (24 populations) were genotyped using 11 nuclear microsatellites and clustered at two hierarchical levels using a Bayesian clustering algorithm in STRUCTURE. Membership of each individual's genome (qi) to the three identified genetic clusters is indicated by vertical bars.

## CHAPTER 6 Conclusion

*Acacia saligna* and its close relative *Paraserianthes lophantha*, are intra-specifically diverse invaders that have significant negative effects on native biodiversity in South Africa and other parts of the world. Prior to this thesis, species distribution models had not been developed for an invasive plant at the intra-specific level, nor had the influence of high intra-specific (i.e. subspecies) variation on introduced genetic patterns been tested. Population genetic studies have been conducted on *A. saligna* in its native range, but there were no known assessments of the patterns of genetic diversity within introduced populations of *A. saligna*, or native and introduced populations of *P. lophantha*.

The work presented in this thesis showed that high intra-specific diversity can affect the ecological niche preferences and distribution of genetic diversity in introduced populations of *A. saligna* and *P. lophantha*. The first publication (Chapter 2) showed that correlative species distribution models are able to detect quantifiable differences between the native niche preferences of the subspecies of *A. saligna*. However models were unable to accurately predict the introduced distributions of the subspecies. A possible reason for the inaccuracy of correlative models was identified in the second publication (Chapter 3), when molecular markers showed that introduced populations in South Africa were genetically distinct from all native subspecies of *A. saligna*. The observed genetic patterns and the history of use of the species suggest introgressive hybridisation occurred during cultivation and may have facilitated the development of a novel nuclear genotype of *A. saligna* in South Africa. This study showed that human-mediated processes (such as selective breeding and cultivation) substantially affected the evolutionary trajectory of *A. saligna* in South Africa and other parts of the world.

Chapter 4 built on the findings of Chapter 3, and the hypothesis that the wide use of *A. saligna* in agroforestry (Griffin *et al.*, 2011) may have led to the occurrence of the cultivated genotype in other globally invasive populations. A global phylogenetic assessment of introduced localities showed that the novel genotype was indeed present in other invasive populations (in

eastern Australia and Portugal). Interestingly, this genetic pattern was in agreement with the history of introduction (multiple introductions) of the species to these two regions. The pattern was however inconsistent for all the regions sampled. I concluded that a wider breadth of sampling is needed to fully understand the genetic relationships between all invasive *A. saligna* populations.

Overall, the molecular work on *A. saligna* showed that introduced genetic signatures are influenced by high intra-specific diversity, but also by the introduction history (manner, site and rate at which a species is introduced to the new range) and human use of a species (i.e. cultivation). Although these patterns were consistent for the studies on *A. saligna*, it was unclear whether these patterns could be generalised for closely related species such as *P. lophantha*.

In Chapter 5, I explored this possibility by conducting a similar analysis on *P. lophantha*, to that conducted on *A. saligna*. The introduction history of *P. lophantha* shows that the species was introduced on fewer occasions and in fewer numbers than *A. saligna*. Surprisingly, the molecular results showed that introduced populations of *P. lophantha* possess similar levels of genetic diversity compared to native populations. Furthermore, despite the low number of introduction events of *P. lophantha* to South Africa, introduced populations of the species comprised a range of native genotypes sourced from across the native range of the species in Western Australia. This genetic pattern is in contrast to the single genetic entity of *A. saligna* that was introduced to South Africa, despite *A. saligna*'s history of multiple introductions. Clearly, the two study species were not sampled in the same manner prior to their introduction to South Africa.

The introduction dynamics of non-native species clearly has substantial effects on the genetic patterns in the new range, particularly for species that are commercially important. Both *A. saligna* and *P. lophantha* have been widely exported for ornamental and land rehabilitation purposes (Maslin & McDonald, 2004; Dufour-Dror, 2012; Wilson *et al.*, 2011, Griffin *et al.*, 2011; Degen *et al.*, 1995; Randall, 2002, Kull *et al.*, 2011), however the distribution of genetic diversity varies between species, across their introduced ranges. A global variation in the distribution of genotypes has been shown for invasive species that were introduced in high numbers or on

multiple occasions. For example, Le Roux and colleagues (2011) demonstrated this for several closely related acacias (see Le Roux *et al.*, 2011), while Prentis and colleagues (2009) demonstrated this for invasive plants that have overlapping native ranges (Prentis *et al.*, 2009). This suggests that reduced genetic diversity in the new range is not as common place as previously thought. In fact there is mounting evidence to support the hypothesis that high propagule pressure and multiple introductions is able to overcome the effects of founder events that would traditionally result in genetic bottlenecks. This thesis clearly demonstrated that the genetic patterns within introduced populations of *A. saligna* and *P. lophantha* vary in different parts of the world. The contrasting introduction histories, levels of genetic diversity and number of haplotypes in both species suggest caution should be taken when extrapolating introduced genetic patterns to the manner and rate at which a species was introduced to the new range.

Understanding the processes that shape the genetic makeup of invasive plant populations has important theoretical and applied implications. In this thesis I explored the interplay of introduction histories, intra-specific diversity and biogeographic processes (drift, selection, climate) that influence the introduced genetic patterns of two prominent invasive tree species. Overall, the research presented herein shows that: 1) intra-specific genetic variation can influence the predictive accuracy of species distribution models and hence their usefulness in invasive risk analyses; 2) high native intra-specific diversity, multiple introductions and human use (i.e. cultivation) can affect the evolutionary trajectories of introduced populations; 3) different molecular approaches are valuable tools for improving our understanding of invasion histories and patterns of invasiveness; 4) subspecific information should be considered when native provenances are being selected as potential sources of biological control agents (assuming that genetic similarity will translate into host-specificity); 5) subspecies and indeed native and introduced populations should not necessarily be treated as the same entities when developing management programmes.

The dissimilarity in the introduced distribution of genotypes and discordance with the known introduction histories of *A. saligna* and *P. lophantha* suggest that the use of introduced genetic patterns alone may not accurately reflect the true invasion history of a species. Providing that

genetic variation translates into heritable phenotypic variation (and possibly variation in fitness between regions), the dissimilarity in the global distribution of genotypes also suggests that no introduced populations can be successfully managed or controlled in the same way. Clearly, invasive taxa lumped under generic taxon names such as “*A. saligna*” or “*P. lophantha*” are unlikely to perform similarly throughout their introduced range, and may not respond the same way to management. Management approaches may be rendered completely ineffective if introduced populations are markedly different from their native counterparts (e.g. South African populations of *A. saligna* and native subspecies, see Chapter 3). Further research is needed on invasive and intra-specifically diverse species to see whether similar patterns exist in introduced populations. Such research should also test whether the observed variation in genotypes translates into phenotypic variation, and if this ultimately affects invasiveness.

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