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Genetic analyses reveal a complex introduction history of the globally invasive tree *Acacia longifolia*

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Abstract

Acacia longifolia (Sydney golden wattle) is considered one of the most problematic plant invaders in Mediterranean-type ecosystems. In this study, we investigate the species' invasion history by comparing the genetic diversity and structure of native (Australia) and several invasive range (Brazil, Portugal, South Africa, Spain, and Uruguay) populations and by modelling different introduction scenarios using these data. We sampled 272 *A. longifolia* individuals – 126 from different invasive ranges and 146 from the native range – from 41 populations. We genotyped all individuals at four chloroplast and 12 nuclear microsatellite markers. From these data we calculated diversity metrics, identified chloroplast haplotypes, and estimated population genetic structure based on Bayesian assignment tests. We used Approximate Bayesian Computation (ABC) models to infer the likely introduction history into each invaded country. In Australia, population genetic structure of *A. longifolia* appears to be strongly shaped by the Bass Strait and we identified two genetic clusters largely corresponding to mainland Australian and Tasmanian populations. We found invasive populations to represent a mixture of these clusters. Similar levels of genetic diversity were present in native and invasive ranges, indicating that invasive populations did not go through a genetic bottleneck. Bayesian assignment tests and chloroplast haplotype frequencies further suggested a secondary introduction event between South Africa and Portugal. However, ABC analyses could not confidently identify the native source(s) of invasive populations in these two countries, probably due to the known high propagule pressure that accompanied these introductions. ABC analyses identified Tasmania as the likely source of invasive populations in Brazil and Uruguay. A definitive native source for Spanish populations could also not be identified. This study shows that tracing the introduction history of

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A. longifolia is difficult, most likely because of the complexity associated with the extensive movement of the species around the world. Our findings should be considered when planning management and control efforts, such as biological control, in some invaded regions.

Keywords
Australian acacias, genetic diversity, haplotypes, introduction history, microsatellite markers, multiple introductions, population structure, propagule pressure

Introduction

Australian acacias (genus *Acacia* Mill.) are considered some of the world's most problematic plant invaders (Richardson et al. 2011). At least 25 species are known to be invasive (Richardson et al. 2015; Magona et al. 2018) and are amongst some of the best studied taxa in invasion biology (Richardson et al. 2011). *Acacia* species have been introduced worldwide for several purposes, such as land reclamation, tannin production and as ornamental plants (Griffin et al. 2011). These introductions frequently involved high propagule pressure (i.e., the number and size of introduction events), exemplified by invasive populations that often have high levels of genetic diversity and that experience no molecular inbreeding (Vicente et al. 2021).

Knowledge of the introduction history of invasive species may aid their management, such as biological control (Jourdan et al. 2019). For example, the earleaf acacia, *Acacia auriculiformis*, is considered highly invasive in Florida, USA (Kull et al. 2008; Richardson and Rejmánek 2011; McCulloch et al. 2021). In its native range, the species shows deep phylogenomic divergence between northern Queensland and the Northern Territory in Australia, and Papua New Guinea (McCulloch et al. 2021). This deep genetic divergence is mirrored in populations of some herbivorous insects feeding on *A. auriculiformis*, such as the specialist leaf-feeding beetle, *Calomela intemerata* (Nawaz et al. 2021). This holds important implications for the utility of this insect as a potential biocontrol agent for *A. auriculiformis*. For example, genomic analyses identified the Northern Territory as the source of invasive populations of the wattle in Florida (McCulloch et al. 2021) and, therefore, *Calomela intemerata* from this part of Australia may be particularly well-suited for biocontrol release in this invaded region.

Molecular studies have been instrumental in disentangling the introduction histories of invasive species (Le Roux 2021). For example, genetic studies allow researchers to trace the routes of, and estimate the propagule pressure that founded, invasions (Wardill et al. 2005; Pyšek et al. 2013). Invasive Australian acacias have been particularly well-studied in this regard (e.g., Thompson et al. 2012, 2015; Le Roux et al. 2013; Ndlovu et al. 2013; Vicente et al. 2018; Hirsch et al. 2019, 2021). For instance, the *Acacia saligna* species complex (also known as Coojong) comprises three distinct genetic lineages (ssp. *lindleyi*, ssp. *stolonifera*, and ssp. *salinga* and *pruinescens*; Millar et al. 2011) with different distributions in Australia (Thompson et al. 2011). This species was introduced into South Africa in 1833 (Poynton 2009), Portugal in 1869

(Thompson et al. 2015), Libya and Ethiopia in 1870 (Griffin et al. 2011), and Israel in 1920 (Kull et al. 2011). It was also introduced into countries such as Italy and the USA (California), where introduction dates are unknown (Thompson et al. 2015). Using genetic data, Thompson et al. (2012, 2015) found that all lineages have been introduced outside Australia and that extensive admixture is occurring in some invaded ranges. However, in South Africa a novel genetic lineage (which likely originated from an introgressive hybridisation event) was identified (Thompson et al. 2012). This lineage was subsequently identified in Italy and Portugal (Thompson et al. 2015). *Acacia pycnantha*, also known as the Golden wattle, is a species native to eastern and southern Australian that has two recognised ecotypes, the so-called wetland and dryland forms (Ndlovu et al. 2013). This species was introduced into South Africa in 1865 and again in 1890 and is now considered highly invasive in the country (Poynton 2009). The species also has invasive populations in Portugal and Western Australia (Richardson and Rejmánek 2011; Ndlovu et al. 2013). In Australia, Le Roux et al. (2013) identified admixture between the two ecotypes, probably because of the extensive movement of seeds/plants for restoration. Invasive populations in South Africa have similar levels of genetic diversity and admixture to native range populations (Le Roux et al. 2013).

Acacia longifolia (Andrews) Willd. is native to south-eastern Australia and Tasmania, with two formally described subspecies: *A. l.* ssp. *longifolia* and *A. l.* ssp. *sophorae* (Flora of Australia Volume 11B, Mimosaceae, *Acacia* part 2 2001). These subspecies are distinguished by phyllode shape, size and colour, and seed pod shape. They also have slightly different, but mostly overlapping, distributions in Australia (Flora of Australia Volume 11B, Mimosaceae, *Acacia* part 2 2001). This species has been introduced into several countries as an ornamental tree and for coastal dune stabilisation and is now considered one of the worst plant invaders in many Mediterranean regions. *Acacia longifolia* was initially introduced into South Africa in 1827. Subsequent introductions occurred in 1845 (from Australia) and between 1895 and 1908 (secondarily from Paris and California, and the Botanical Gardens of Adelaide; Poynton 2009). In 1984, *A. longifolia* was considered the second most impactful invader of the country's hyperdiverse fynbos biome (Macdonald and Jarman 1984; Dennill 1987). Between 1982 and 1983, the Australian gall-forming wasp, *Trichilogaster acaciaelongifoliae*, was introduced from Australia to control the species (Dennill 1985, 1987; Neser 1985). This biocontrol agent is considered highly successful (Janion-Scheepers and Griffiths 2020).

In Portugal, specimens of *A. longifolia* were recorded in the catalogue of the University of Coimbra's Botanical Gardens in 1878 (Henriques 1879). However, the first official introduction record of the species into Portugal likely dates to 1897 (Fernandes 2008; Carruthers et al. 2011; Kull et al. 2011). Irrespective of when the species was introduced, the origins of these introduction(s) remain unknown. The species was used by forestry services in several coastal afforestation projects over the years. A few examples are the dunes in São Jacinto (1888–1929; Marchante 2011), Costa da Caparica (1906; Lourenço 2009), Quiaios-Mira (1924 and 1948; Rei 1925; Marchante 2001, 2011), and Vila Nova de Milfontes [late 1960s/early 1970s; Miguel Prado, personal communication, but see Vicente (2016); Vicente et al. (2018)]. A molecular study

including three invasive populations from Portugal found similar genetic diversity and very low differentiation among them, which led to the hypothesis that the introduction of the species into Portugal involved a single seed source that was used to establish nursery stock, and these plants were then disseminated for afforestation projects (Vicente et al. 2018). However, the introduction records mentioned above suggest a more complex introduction history of the species into Portugal. The invasion success of *A. longifolia* in Portugal has led to the biological control release of *Trichilogaster acaciaelongifoliae*, imported from South Africa (Marchante et al. 2011). The wasp has successfully established along the Portuguese coast (López-Núñez et al. 2021).

In South America the introduction of *A. longifolia* occurred much more recently, in mid-20th century, into southern Brazil (Rico-Arce 2007; Zenni and Ziller 2011), Uruguay (Boelcke 1946; Rico-Arce 2007) and Argentina (Boelcke 1946; Celsi 2016). In Uruguay the first record for this species dates to 1946 (Boelcke 1946), and in Brazil the first official record of the species is from 1979 in Santa Catarina (Burkart 1979). There are also several observational records for the species in other states of Brazil, Argentina, and Uruguay (e.g., Base de dados de espécies exóticas invasoras do Brasil, http://bd.institutohorus.org.br; Tropicos.org, http://www.tropicos.org/Name/13024183). No biological control agents have been released into South America and no information is available on the origins of *A. longifolia* introductions to the continent.

Here we compare the population genetic diversity and structure of native and globally invasive populations of *A. longifolia*. We also aim to infer the introduction histories of the species into Brazil, Portugal, South Africa, Spain, and Uruguay using Approximate Bayesian Computation (ABC) modelling. Based on available historical records and the results from molecular studies of invasive acacias mentioned above, we hypothesised that the genetic diversity in invasive populations will be comparable to that of Australian populations, but with low population genetic structure. Regarding introduction scenarios, we hypothesised that Portuguese *A. longifolia* populations originated from a single introduction event from Australia, whereas in South Africa, we expected our modelling results to support the known multiple and independent introductions of the species into the country (Poynton 2009). We thus hypothesised that this invasion would be characterised by high levels of genetic admixture. We had no clear prediction for the introduction history of *A. longifolia* in South America given the scarcity of historical information for the region.

Methods

Collection of Plant Material and DNA Extraction

Phyllodes of *Acacia longifolia* were collected from individual plants in several invasive [Portugal, POR; Spain – Galicia, ESP; South Africa, RSA; Brazil, BRA (Vicente et al. 2020); and Uruguay, URU] and native (mainland southeast Australia, AUS; and Tasmania, TAS) range populations. Within each country we sampled more than one popula-

tion where possible (see Table 1). In Portugal, individuals from Vila Nova de Milfontes (VNMF), Pinheiro da Cruz (PC) and two individuals from Osso da Baleia (included in the Mira population) were previously sampled by Vicente et al. (2018). In Australia, additional material was obtained from seedlings grown from seeds acquired from local nurseries. In total, 272 individuals were included in our analyses (Table 1, Fig. 1A), 126 from invaded ranges (Table 1A, Fig. 1B–D) and 146 from Australia (Table 1B, Fig. 2).

DNA was extracted using the method of Doyle and Doyle (1987), as modified by Weising et al. (1994) and adapted for *A. longifolia* by Vicente et al. (2018). DNA was quantified using spectrophotometry.

Microsatellite amplification and genotyping

Ten chloroplast microsatellite loci (cpSSRs; ccmp1-10) described by Weising and Gardner (1999) were tested for cross-amplification in *A. longifolia* (Suppl. material 1: table S1). For these cross-amplification tests, we first used the PCR conditions described by Weising and Gardner (1999) and, when needed, adjusted $MgCl_2$ concentrations between 1.5 mM to 2.5 mM and annealing temperatures between 50 °C to 52 °C to optimise amplifications of individual loci. Loci were selected based on visualisation of PCR products on 2% agarose gels. Four loci (ccmp3, 4, 5 and 7) consistently yielded high quality amplicons and were selected for amplification in all samples. Details of PCR primers can be found in Suppl. material 1: table S2. For cpSSRs each 15 µL reaction contained 20ng DNA, 2.5 mM $MgCl_2$, 0.2 mM dNTP mix (Promega, USA), 0.5 µM fluorescently labelled (with either 6-FAM or HEX) forward primer and 0.5 µM reverse primer (STAB Vida, Portugal), 1 U of GoTaq Flexi DNA polymerase and 1× colourless GoTaq Flexi buffer (Promega, USA). The following thermal cycle was used: initial denaturation at 94 °C for 5 min, followed by 40 cycles of 1 min at 94 °C, 1 min at 52 °C and 1 min at 72 °C, with a final extension period of 8 min at 72 °C. As a routine procedure, each reaction included a negative control that did not include DNA and at least 10% of repeated samples that were run in a 2% agarose gel to confirm amplification before fragment analysis.

We searched the literature for studies that have developed nuclear microsatellites (nSSRs) in *Acacia* species. We also tested primers that we designed for *A. cyclops* (unpublished data). This led to a total of 32 primer pairs that were screened for cross-amplification in *A. longifolia* (Suppl. material 1: table S1). For optimisation, $MgCl_2$ concentrations were varied between 1.5 mM and 2.5 mM, annealing temperature between 52 °C and 60 °C, extension times between 15s and 30s, and primer concentrations between 0.1 µM and 0.2 µM. Loci were selected based on visualisation of PCR amplification products on 2% agarose gels. Eleven nSSR loci were retained for amplification in our samples. Primers for locus DCLOC (Roberts et al. 2013) were previously optimised for analysis in *A. longifolia* by Vicente et al. (2018) and were also included in this study. Genotyping data for this locus for samples from Vila Nova de Milfontes (VNMF), Pinheiro da Cruz (PC) and two samples from Osso da Baleia (included in the Mira population) were obtained from Vicente et al. (2018) and incorporated into

Table 1. Number of samples (n), population code, latitude, and longitude of the collection sites. **A** invasive range (Brazil, Portugal, South Africa, Spain, and Uruguay) **B** native range (mainland Australia and Tasmania). Collection sites in mainland Australia in **bold** represent seedling samples obtained from nurseries.

		1A		
Collection Site	**Code**	**N**	**Latitude / Longitude**	**Sampling year**
Portugal	***POR***	***38***		
Vila Nova de Milfontes	VNMF	5	37.685292, -8.791508	2015
Vila Nova de Milfontes			37.675608, -8.766397	2015
Vila Nova de Milfontes			37.511822, -8.440267	2015
Pinheiro da Cruz	PC	6	38.250377, -8.752181	2015
Osso da Baleia	Mira	7	40.000884, -8.901132	2015
Mira			40.527170, -8.673730	2018
Mira			40.450170, -8.768960	2018
Mira			40.461380, -8.708500	2018
Foz do Arelho	FA	6	39.429354, -9.223632	2019
Monte Gordo	MG	6	37.183880, -7.448606	2019
Moledo	Mol	8	41.866359, -8.855380	2017
Spain (Galicia)	***ESP***	***12***		
Muros	Muros	6	42.820272, -9.065278	2019
San Vicente	SanVic	6	42.464995, -8.908974	2019
South Africa	***RSA***	***36***		
Stellenbosch	Stell	5	-33.947222, 18.834920	2017
Grahamstown	Graham	6	-33.327920, 26.499520	2018
Grahamstown			-33.321640, -33.32164	2018
Clarkson	Clark	6	-34.071800, 24.404570	2018
R102			-33.981450, 24.043820	2018
Sedgefield	Sedge	8	-34.068380, 22.948030	2018
Hermanus	Herm	5	-34.395970, 19.218410	2018
Lasikisiki	Lasiki	6	-31.413750, 29.712980	2018
Brazil	***BRA***	***25***		
Tramandaí	Tram	5	-29.890618, -50.097749	2019
Cassino Beach	Cass	5	-32.188509, -52.169312	2019
Hermenegildo	Hmng	5	-33.639901, -53.420143	2019
Lagoa do Peixe National Park	Peixe	5	-31.250075, -51.026285	2019
Moçambique Beach	Moca	5	-27.486697, -48.393998	2019
Uruguay	***URU***	***15***		
Cabo Polonio	Polonio	5	-34.407141, -53.878037	2019
Hotel Paque Oceánico Beach	Hotel	5	-33.908477, -53.512534	2019
Brazil/Uruguay Frontier	Front	5	-33.728768, -53.468794	2019
		1B		
Mainland Australia	***AUS***	***76***		
Clovelly, NSW	Clov	8	-33.914732, 151.263171	2017
Green Point, NSW	Green	8	-32.250278, 152.536667	2020
Bilpin, NSW	Bilpin	6	-33.491667, 150.533333	2020
Ulladulla, NSW	Ulladulla	8	-35.350000, 150.483333	2020
Vaucluse, NSW	Vaucluse	8	-33.852778, 151.263889	2020
Torrington, NSW	Torrington	6	-29.207500, 151.686389	2020
Marulan, NSW	Marulan	8	-34.683333, 150.066667	2020
Beachport, SA	Beachport	8	-37.516667, 140.083333	2020
Curdievale, VIC	Curdievale	7	-38.508123, 142.899504	2020
Bermagui, NSW	Bermagui	9	-36.443373, 150.061346	2020

1B				
Collection Site	**Code**	**N**	**Latitude / Longitude**	**Sampling year**
Tasmania	***TAS***	***70***		
Bridport	Bridport	8	-40.999805, 147.393570	2020
St. Helens Conservation Area (Private property)	Helens	8	-41.328235, 148.294909	2020
Seven Mile Beach	SMile	8	-42.850198, 147.522249	2020
Southwest National Park	SouthW	8	-43.606146, 146.817540	2020
Three Sisters National Park	ThreeS	8	-41.129030, 146.125828	2020
Freycinet National Park	Freycinet	8	-42.173890, 148.279790	2020
Whale Bone Point	Whale	8	-43.439267, 147.235150	2020
Stanley	Stanley	8	-40.780950, 145.277317	2020
Arthur River	Arthur	6	-41.033333, 144.666667	2020

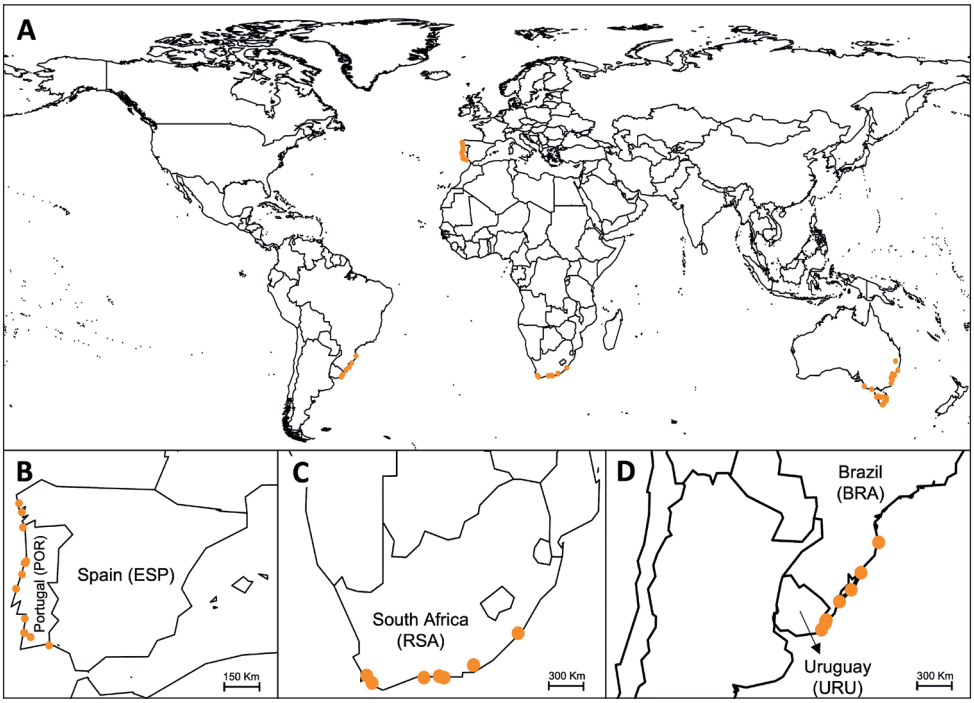

Figure 1. Map of the collection sites of *Acacia longifolia*. Each dot represents the exact location of each collection site (see coordinates in Table 1) **A** all sampled countries/locations **B** Iberian Peninsula **C** South Africa **D** Brazil and Uruguay. Map was drawn using the *maptools* R package (Lewin-Koh et al. 2011; R Core Team 2016).

the dataset generated in this study. Thus, in total, 12 nSSR loci were analysed in this study (see Suppl. material 1: table S2 for further details).

PCRs of nSSR loci were performed in 15 μL reaction volumes, each containing 10 ng DNA, 1.5–2.5 mM of $MgCl_2$ depending on primers used (Suppl. material 1: table S2), 0.2 mM dNTP mix (Promega, USA), 0.2 μM primer forward labelled with

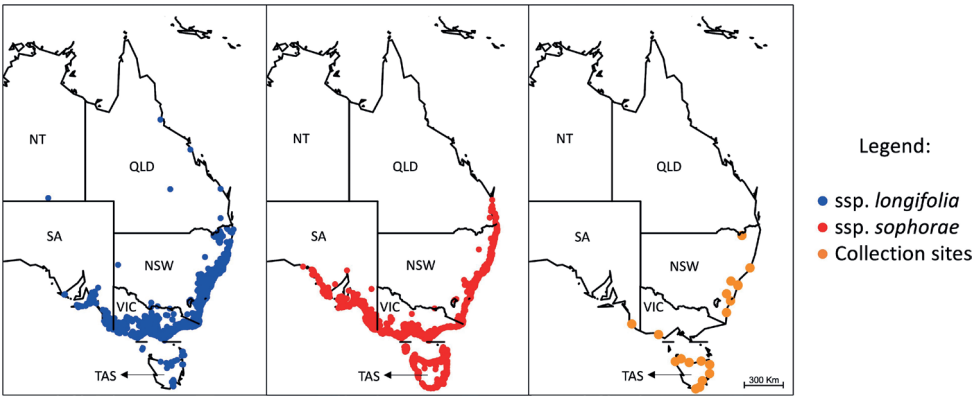

Figure 2. Maps of the native range distribution of *Acacia longifolia*. Distributions of *A. l.* ssp. *longifolia* and *A. l.* ssp. *sophorae* are presented in blue and red, respectively, and the sample collection sites of this study are presented in orange. Each orange dot represents the exact location of each collection site (see Table 1). Maps were drawn using the *maptools* R package (Lewin-Koh et al. 2011; R Core Team 2016). Geo-referenced occurrence records for each subspecies were obtained from the Atlas of Living Australia database (ALA 2021a, b) and the Global Biodiversity Information Facility (GBIF 2021a,b), with duplicates and erroneous records (i.e., records with coordinates that fell in the ocean or with no registered coordinates) manually removed in R statistical environment (R Core Team 2016) with the *maptools*, *raster* (Hijmans and van Etten 2010) and *rgdal* (Keitt et al. 2010) R packages. NSW: New South Wales. NT: Northern Territory. QLD: Queensland. SA: South Australia. TAS: Tasmania. VIC: Victoria.

a fluorescent dye (either 6-FAM, HEX or ATTO-550), 0.2 μM reverse primer (STAB Vida, Portugal), 1 U of GoTaq Flexi DNA polymerase and 1× colourless GoTaq Flexi buffer (Promega, USA). The PCR cycle consisted of an initial denaturation at 95 °C for 5 min, followed by 40 cycles of 30 sec at 94 °C, 1 min at the annealing temperature optimised for each primer pair (Suppl. material 1: table S2), and 30 sec at 72 °C, with a final extension period of 7 min at 72 °C. For the two primer pairs, Division/Bell and The/Wall, extension time was reduced to 15 s. Positive and negative controls were included in each reaction, as explained above. Fragments for all loci were separated on an Applied Biosystems 3100 Series System with the Dye Set DS-30 (internal standard ROX) by STAB Vida (Portugal) and scored using Peak Scanner in the ThermoFisher AppConnect online software portal (ThermoFisher Scientific, USA).

Population genetic diversity and structure

Haplotypes were identified based on the combination of alleles across all cpSSR loci/individual (see Results section). The number of different alleles (N_a), number of effective alleles (N_e), Shannon's Information Index (I), and haplotype diversity (h) were calculated using GenAlEx v6.5 (Peakall and Smouse 2006, 2012). The Network v10.2 software (www.fluxus-engineering.com) was used to draw a median-joining (MJ; Bandelt et al. 1999) haplotype network.

Micro-Checker v2.2 (Van Oosterhout et al. 2004) was used to check for scoring errors and large allele dropouts in our nSSR genotype dataset. This software was also used to generate a dataset that has been corrected for null alleles, using the Oosterhout algorithm with 95% confidence intervals (Van Oosterhout et al. 2004). Expected heterozygosity (H_E), observed heterozygosity (H_O), allelic richness (A_r), and inbreeding coefficients (F_{IS}) were calculated for the corrected and uncorrected genotype datasets using the *diveRsity* R package (Keenan et al. 2013). These metrics were compared between datasets using the independent 2-group Mann-Whitney U test in R environment (R Core Team 2016) to check if the presence of null alleles affected our results. The mean frequency of null alleles across all loci and populations was calculated with the FreeNA software (Chapuis and Estoup 2007), as well as the ENA-corrected (i.e., excluding null alleles; ENA method, Chapuis and Estoup 2007) and uncorrected global (i.e., at each locus and population) and pairwise population fixation indices (F_{ST}). To further check for the effects of null alleles on our analyses, statistical differences between the ENA-corrected and uncorrected global and pairwise F_{ST} values were tested using the independent 2-group Mann-Whitney U test in R environment (R Core Team 2016). Since null alleles had an overall low mean frequency and did not significantly affect any of the calculated metrics (see Results), no correction for null alleles was applied and all further analyses were performed using the uncorrected nSSR genotype dataset. Allele frequency departures from Hardy-Weinberg equilibrium (HWE) for all loci were tested using GenAlEx v6.5 (Peakall and Smouse 2006, 2012).

The four descriptive population diversity metrics mentioned above (H_E, H_O, A_r, and F_{IS}) were compared among countries using a Kruskal-Wallis rank sum test in R environment (R Core Team 2016). In this analysis, individuals from Spain were excluded as only two populations were sampled in this country. Instead, the analysis was repeated by grouping the samples from Spain with the ones from Portugal (i.e., Iberian Peninsula; IBP).

Bayesian assignment tests were performed using the STRUCTURE v2.3.4 program (Pritchard et al. 2000) to investigate the genetic structure of the native and invasive *A. longifolia* populations. An admixture model with correlated allele frequencies was used to test for the number of genetic clusters (K), ranging from 1 to 15, with 100,000 burn-in iterations, 250,000 Markov Chain Monte Carlo repetitions (MCMC), and 15 iterations per K value. We then performed a hierarchical analysis by removing samples belonging to the smallest genetic cluster identified by this analysis (13 samples belonging to Portugal and South Africa; see Results) to infer structure among the remaining populations. We repeated this analysis with the same conditions as above, but with K ranging from 1 to 10. For all STRUCTURE analyses the optimum number of genetic clusters was evaluated in STRUCTURE HARVESTER web v0.6.94 (Earl and vonHoldt 2012) by applying the delta K method of Evanno et al. (2005) and also analysing the likelihood distributions [LnP(K)]. Graphical visualisations of these results were obtained using the CLUMPAK server main pipeline (Kopelman et al. 2015).

To better understand population structure within the native range, we also ran a separate STRUCTURE analysis that included data from native range individuals only, with *K* ranging from 1 to 10, implementing an admixture model with correlated allele frequencies using 10,000 burn-in iterations, 50,000 MCMC repetitions, and 10 iterations per *K* value. The optimum number of genetic clusters and the graphical visualisation of the results were obtained as described above. This analysis identified $K = 2$ as the optimum number of genetic clusters, with one cluster predominantly corresponding to mainland Australia and the other predominantly to Tasmanian populations (see Results). To check for population genetic substructure within these two identified clusters, ancestry coefficients for each individual were averaged by cluster over the 10 iterations for $K = 2$, and individuals having a minimum ancestry coefficient cut-off value of 0.7 for their corresponding cluster were selected for further cluster-specific (i.e., mainland Australia or Tasmania) analyses. We used the same parameters described above to identify the optimum number of genetic clusters and to graphically visualise these. We also tested for Isolation by Distance (IBD) by performing a Mantel test with 9,999 permutations using the *ade4* R package (Dray et al. 2022) by comparing the linearised pairwise F_{ST} values matrix (i.e., $F_{ST}/1\text{-}F_{ST}$) with the geographical distance matrix obtained with the Geographic Distance Matrix Generator v1.2.3 software (Ersts 2022). We compared the mean pairwise F_{ST} values among populations within mainland Australia, populations within Tasmania, and populations from mainland Australia *vs* Tasmania using a Kruskal-Wallis rank sum test followed by a post-hoc independent 2-group Mann-Whitney U test in R environment (R Core Team 2016).

Inference of invasion sources

Approximate Bayesian Computations (ABC) were performed in the DIYABC v2.1.0 software (Cornuet et al. 2014) to test different invasion scenarios for each invaded country separately. In these analyses, we took the two major genetic clusters in the native range identified by the STRUCTURE analysis (see Results) into consideration, i.e., populations from mainland Australia (AUS) and Tasmania (TAS), while invaded populations that were pooled by country. All tested scenarios assumed that the AUS and TAS populations diverged from a native ancestral unsampled population, with the following variations: 1) the invasive population originated from the AUS populations; 2) the invasive population originated from the TAS populations; 3) the invasive population originated from an admixed unsampled population resulting from multiple introductions which originated from both AUS and TAS populations; 4) the invasive population originated from multiple introductions ("ghost" unsampled populations) originating from AUS populations; 5) the invasive population originated from multiple introductions ("ghost" unsampled populations) originating from TAS populations; and 6) the invasive population originated from an unknown native population which is related to the AUS and TAS genetic lineages (see Fig. 3). We ran 3×10^6 simulations for each scenario for each invaded country. We did not consider any genetic bottlenecks in these scenarios, as we found no evidence for their existence (see Results section).

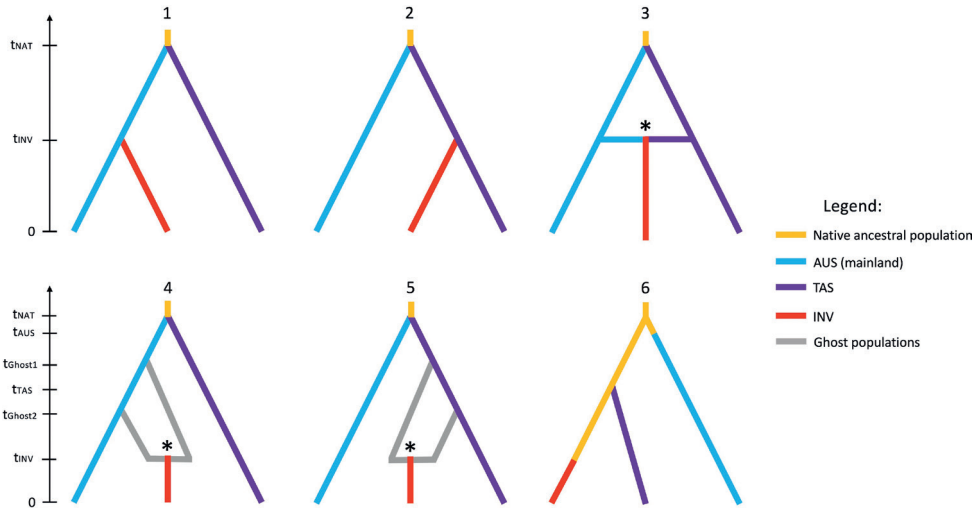

Figure 3. Diagrams of the six tested scenarios for each invaded country via DIYABC analyses. Asterisks represent merging and admixture of two populations. The "native ancestral population" is an unsampled population genetically related to both the mainland south-eastern Australia (AUS) and Tasmania (TAS) clusters identified via STRUCTURE analyses. "Ghost populations": unsampled populations. INV: Invasive population. See Materials and Methods for detailed descriptions of each scenario. Parameter descriptions, prior and posterior values are provided in Suppl. material 1: tables S3–S5.

The summary statistics selected for the models were the following: mean number of alleles, mean genetic diversity, mean allele size variance, mean M index, fixation index (F_{ST}), mean index of classification, shared allele distance and $\delta\mu^2$ genetic distance. The description of the parameters, their prior distributions, and parameter rules are provided in Suppl. material 1: tables S3–S5. The priors for time of invasion (t_{INV}) varied between countries according to the latest year of sample collection and the year of first introduction reported in historical records and literature (see Introduction for details), assuming a generation time of 2–3 years (Maslin and McDonald 2004; Vicente et al. 2021). For the purposes of this analysis, since there is no available information on a specific introduction year into Spain (Galicia), the year of introduction into Portugal was assumed to coincide with the introduction of *A. longifolia* into Spain (see Introduction for details). This allowed an estimation of the maximum possible number of generations that *A. longifolia* has been present in each invaded country. The posterior probabilities of each scenario were calculated using a logistic approach on the 1% simulated datasets that were closest to the observed dataset. For the scenario(s) with the highest probability for each country, the posterior distribution of parameters was estimated using a logit transformation on the 1% simulated datasets that were closest to the observed dataset. The precision of these parameters was assessed using the "bias and precision" option on 500 test datasets to estimate the relative median absolute deviation (RMedAD) and means of the median relative bias (MedRB). The adequacy of the chosen scenario(s) was assessed using the "model checking" option by simulat-

ing 1000 datasets using a logit transformation of parameters, with a different set of summary statistics to avoid overestimating the fit of the model. The chosen statistics were the mean number of alleles, mean genetic diversity, mean allele size variance, and $\delta\mu^2$ genetic distance. Type I and Type II errors were also computed for the scenarios(s) with the highest posterior probability using the "confidence in scenario choice" option using a logistic regression and simulating 100 test datasets.

Due to the identification of a cluster connecting South Africa and Portugal (see Results section), we also tested 6 scenarios with data from these two countries, that correspond to scenarios 1, 2 and 6 described above with and without a bridgehead between South Africa and Portugal. We ran simulations and "model checking" analyses as described above for non-bridgehead scenarios.

Results

Dataset characteristics

We identified between two and three alleles per cpSSR locus (mean 2.5), ranging between 93–154 bp in size. We identified between two and nine alleles per nSSR locus (mean 5.08), ranging between 81 bp to 328 bp in size. Scoring errors due to stuttering were found for locus C51M0 (Mira population, POR; Table 1A) and locus AH3-1 (Seven Mile beach population, TAS; Table 1B). All loci showed departures from Hardy-Weinberg equilibrium, and within populations some were monomorphic. However, mean null allele frequency was low in our dataset (mean = 0.027; SD = 0.073) and ENA-corrected and uncorrected F_{ST} values were not significantly different between null allele-corrected and uncorrected datasets for both global and pairwise estimates (Mann-Whitney U test $p > 0.5$; Suppl. material 1: fig. S1). Similarly, population diversity metrics (H_E, H_O, A_r, and F_{IS}) were not significantly different between the corrected and uncorrected datasets (Mann-Whitney U test $p > 0.5$; Suppl. material 1: fig. S2). Therefore, null allele corrections were not considered in all further nSSR analyses.

Population genetic diversity and structure

Our cpSSR data identified eight unique haplotypes (hereafter haplotypes A–H; Fig. 4A). Haplotype E was the most dominant in invasive ranges, while haplotype D was the most frequent one in the native range. Haplotypes B, C and G only occurred in the native range. Portugal and South Africa shared one haplotype (A), and also had a unique haplotype each (F and H, respectively). Our network analysis found that haplotypes B, D and E were closely related to most other haplotypes (Fig. 4B). The number of different alleles (N_a) ranged from 1 (BRA and ESP) to 2.25 (RSA; Table 2), while the number of effective alleles (N_e) ranged from 1 to 1.659 (similar to *Na*; Table 2). In invaded countries, South African populations had the highest haplotype diversity (h = 0.407, Table 2), while Spanish and Brazilian populations had a single

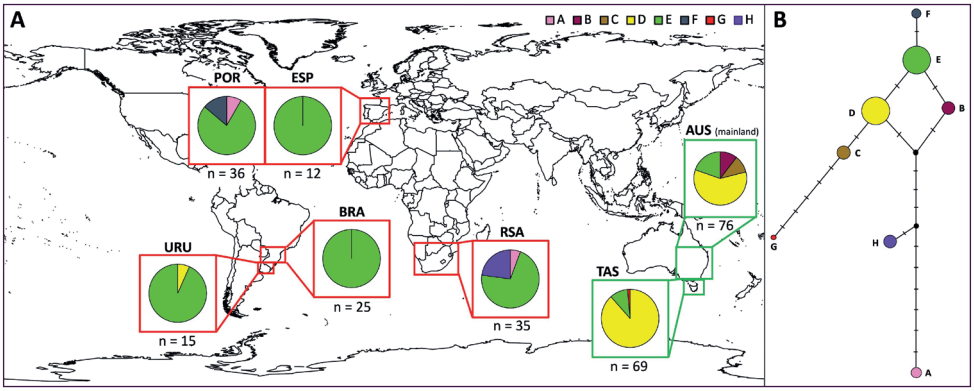

Figure 4. Haplotypes based on chloroplast microsatellites **A** geographical distribution of the eight identified haplotypes (A–H). The number of samples (n) is shown below pie charts **B** median-joining network analysis of eight chloroplast haplotypes. The size of each circle is proportional to the frequency of each haplotype, and branch markings indicate mutational steps between haplotypes.

Table 2. Diversity metrics of all native and invasive *Acacia longifolia* populations for chloroplast microsatellites. N_a – Number of different alleles; N_e – Number of effective alleles; h – Haplotype diversity. Standard error (SE) is shown in parenthesis.

Range	Country	N_a	N_e	H
Invasive	POR	2.000 (0.408)	1.229 (0.119)	0.165 (0.074)
Invasive	ESP	1.000 (0.000)	1.000 (0.000)	0.000 (0.000)
Invasive	RSA	2.250 (0.250)	1.659 (0.049)	0.396 (0.018)
Invasive	BRA	1.000 (0.000)	1.000 (0.000)	0.000 (0.000)
Invasive	URU	1.250 (0.250)	1.036 (0.036)	0.031 (0.031)
Native	AUS	1.750 (0.479)	1.360 (0.287)	0.184 (0.129)
Native	TAS	1.750 (0.479)	1.073 (0.054)	0.059 (0.050)

haplotype (Table 2). Haplotype diversity in Australian populations ranged between 0.06 (TAS) to 0.186 (AUS; Table 2). Haplotype B was only found in South Australia (SA; Beachport population, Table 1B) while haplotype G occurred only in Tasmania (TAS; St. Helens Conservation Area population, Table 1B). Haplotype E was found in New South Wales (NSW; Clovelly and Torrington populations, Table 1B) and in one population in eastern Tasmania (St. Helens Conservation Area population). Haplotype C was predominantly restricted to mainland Australia, while haplotype D was widespread across the native range.

For invasive populations, and for nSSRs, mean expected heterozygosity (H_E) ranged from 0.29 (ESP) to 0.33 (POR), while mean observed heterozygosity (H_O) ranged from 0.33 (RSA) to 0.40 (BRA), mean allelic richness (A_r) ranged from 1.74 (ESP) to 1.97 (POR), and mean inbreeding coefficient (F_{IS}) ranged from -0.146 (RSA) to -0.317 (BRA; Table 3). In the native range, mean H_E ranged from 0.25 (AUS) to 0.27 (TAS), while mean H_O ranged from 0.31 (TAS) to 0.32 (AUS), mean A_r ranged from 1.73 (AUS) to 1.77 (TAS), and mean F_{IS} ranged from -0.196 (TAS) to -0.303

Table 3. Diversity metrics of all native and invasive populations of *Acacia longifolia* for nuclear microsatellites. H_E – Expected heterozygosity; H_O – Observed heterozygosity; A_r – Allelic richness; F_{IS} – Inbreeding coefficient; SD – Standard deviation.

Range	Country	Population	H_E	H_O	A_r	F_{IS}
Invasive	POR	VNMF	0.29	0.35	1.84	-0.222
Invasive	POR	PC	0.29	0.42	1.75	-0.458
Invasive	POR	Mira	0.53	0.32	2.70	0.397
Invasive	POR	FA	0.28	0.36	1.75	-0.268
Invasive	POR	MG	0.26	0.33	1.75	-0.292
Invasive	POR	Mol	0.31	0.39	2.01	-0.247
		Mean	***0.33***	***0.36***	***1.97***	***-0.181***
		SD	***0.10***	***0.04***	***0.37***	***0.294***
Invasive	ESP	Muros	0.31	0.37	1.76	-0.176
Invasive	ESP	SanVic	0.27	0.32	1.72	-0.195
		Mean	***0.29***	***0.35***	***1.74***	***-0.185***
		SD	***0.03***	***0.04***	***0.03***	***0.014***
Invasive	RSA	Stell	0.24	0.37	1.59	-0.529
Invasive	RSA	Graham	0.31	0.38	1.86	-0.209
Invasive	RSA	Clark	0.45	0.40	2.37	0.112
Invasive	RSA	Sedge	0.22	0.31	1.51	-0.436
Invasive	RSA	Herm	0.23	0.20	1.55	0.126
Invasive	RSA	Lasiki	0.32	0.31	1.91	0.059
		Mean	***0.30***	***0.33***	***1.80***	***-0.146***
		SD	***0.09***	***0.07***	***0.33***	***0.288***
Invasive	BRA	Tram	0.33	0.35	1.95	-0.050
Invasive	BRA	Cass	0.32	0.42	2.01	-0.289
Invasive	BRA	Hmng	0.32	0.47	2.03	-0.443
Invasive	BRA	Peixe	0.31	0.40	1.83	-0.283
Invasive	BRA	Moca	0.24	0.37	1.60	-0.517
		Mean	***0.30***	***0.40***	***1.88***	***-0.317***
		SD	***0.04***	***0.05***	***0.18***	***0.180***
Invasive	URU	Polonio	0.33	0.38	1.98	-0.162
Invasive	URU	Hotel	0.29	0.35	1.73	-0.221
Invasive	URU	Front	0.28	0.38	1.76	-0.386
		Mean	***0.30***	***0.37***	***1.82***	***-0.256***
		SD	***0.03***	***0.02***	***0.14***	***0.116***
Native	AUS	Clov	0.31	0.38	1.85	-0.216
Native	AUS	Green	0.26	0.31	1.73	-0.179
Native	AUS	Bilpin	0.27	0.31	1.70	-0.133
Native	AUS	Ulladulla	0.21	0.33	1.60	-0.558
Native	AUS	Vaucluse	0.27	0.39	1.79	-0.441
Native	AUS	Torrington	0.12	0.18	1.29	-0.472
Native	AUS	Marulan	0.34	0.43	2.18	-0.247
Native	AUS	Beachport	0.21	0.25	1.57	-0.189
Native	AUS	Curdievale	0.30	0.37	1.82	-0.226
Native	AUS	Bermagui	0.26	0.33	1.88	-0.283
		Mean	***0.25***	***0.32***	***1.73***	***-0.303***
		SD	***0.06***	***0.07***	***0.23***	***0.144***
Native	TAS	Bridport	0.20	0.26	1.56	-0.342
Native	TAS	Helens	0.35	0.43	2.05	-0.256
Native	TAS	SMile	0.38	0.40	2.23	-0.068

Range	Country	Population	H_E	H_O	A_r	F_{IS}
Native	TAS	SouthW	0.27	0.36	1.76	-0.346
Native	TAS	ThreeS	0.28	0.34	1.80	-0.218
Native	TAS	Freycinet	0.25	0.20	1.70	0.196
Native	TAS	Whale	0.26	0.30	1.68	-0.190
Native	TAS	Stanley	0.19	0.18	1.57	0.086
Native	TAS	Arthur	0.22	0.36	1.62	-0.625
		Mean	***0.27***	***0.31***	***1.77***	***-0.196***
		SD	***0.06***	***0.09***	***0.23***	***0.245***

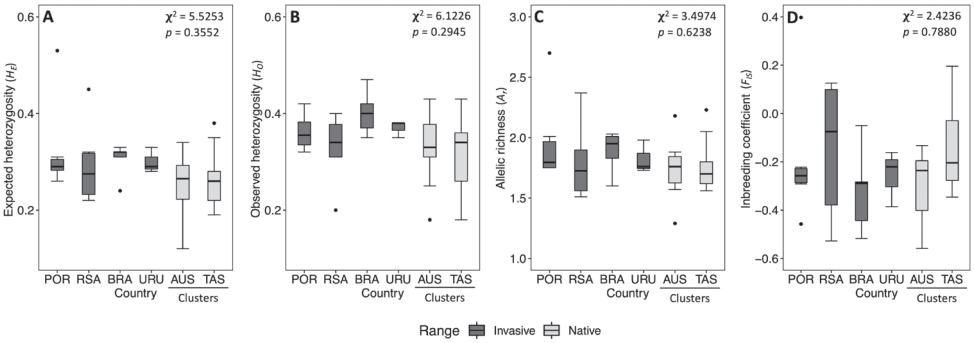

Figure 5. Comparisons of diversity metrics of *Acacia longifolia* between invaded countries and two native range genetic clusters **A** expected heterozygosity **B** observed heterozygosity **C** allelic richness **D** inbreeding coefficient. The two native range clusters were identified via STRUCTURE analysis. Kruskal-Wallis test results are shown on the upper right corner, with 5 degrees of freedom. Spain was excluded from the analysis due to the low number of populations sampled.

(AUS; Table 3). Diversity metric comparisons (excluding Spanish populations due to low population numbers) found similar levels of genetic diversity and lack of inbreeding between native and different invasive ranges (Kruskal-Wallis test $p > 0.05$ for all diversity metrics; Fig. 5). Similar results were obtained when these analyses were repeated with individuals from Spain and Portugal lumped together as Iberian Peninsula (IBP; Suppl. material 1: fig. S3).

Our initial STRUCTURE analysis based on all nSSR data identified two genetic clusters (Suppl. material 1: fig. S4A, B), one that included individuals from Portugal (Mira population) and South Africa (Clarkson and Sedgefield populations) and another that included all other populations (Fig. 6A). STRUCTURE analysis of the latter cluster (i.e., hierarchical analysis) revealed two further genetic subclusters (Suppl. material 1: fig. S4C, D). In this second analysis, native range populations separated in two genetic clusters roughly corresponding to those from mainland Australian (AUS) and Tasmania (TAS), while populations from the invaded ranges were admixtures of these two genetic clusters (Fig. 6B, and see Suppl. material 1: fig. S5 for bar plots of $K = 3 – 6$).

Analysis of the native range-only data also identified two genetic clusters (Suppl. material 1: fig. S6), corresponding to mainland Australia and Tasmania (Fig. 7A). We

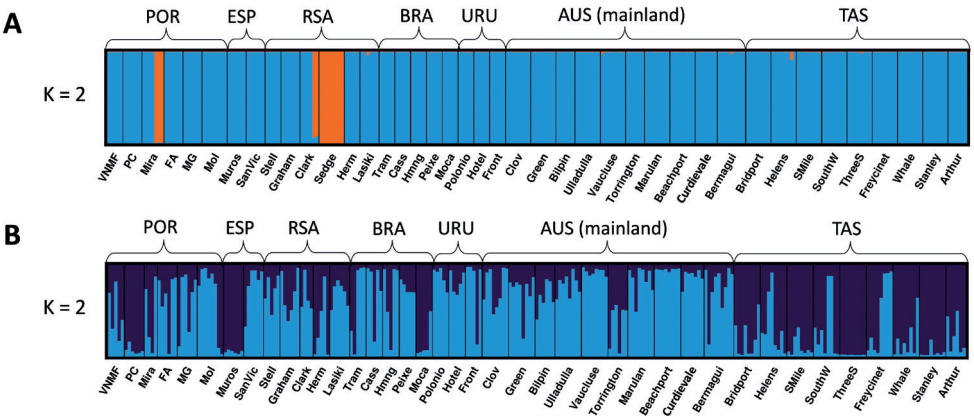

Figure 6. STRUCTURE bar plots ($K = 2$) in the invasive and native ranges of *Acacia longifolia* **A** bar plot for the complete dataset **B** bar plot for the hierarchical analysis of the blue cluster in A. Population names underneath the plots correspond to the codes provided in Table 1.

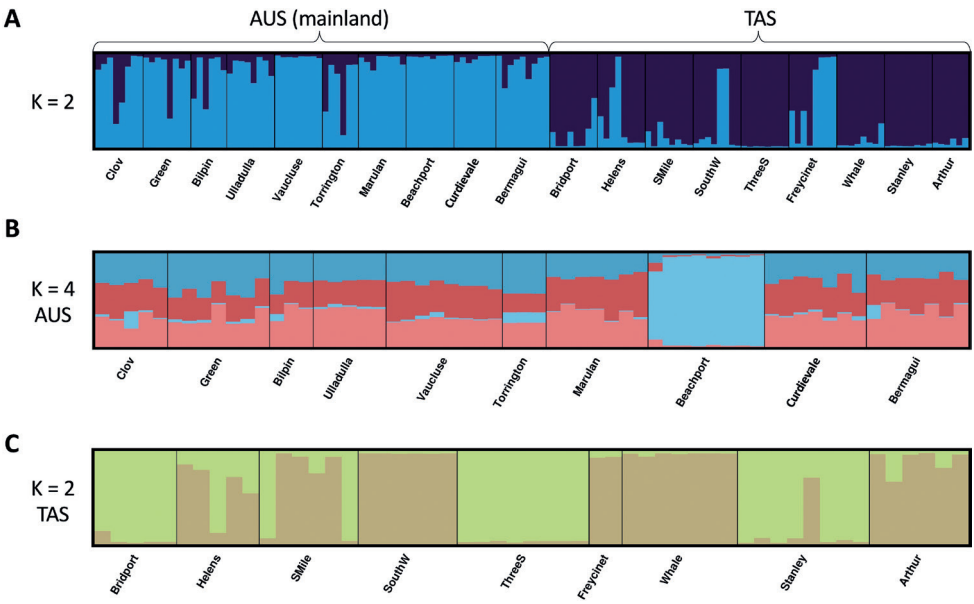

Figure 7. STRUCTURE bar plots for the identified optimum number of clusters in *Acacia longifolia*'s native range **A** bar plot for the overall native range ($K = 2$) **B** bar plot for the mainland Australia cluster ($K = 4$) **C** bar plot for the Tasmania cluster ($K = 2$). Population names underneath the plots correspond to the codes provided in Table 1.

identified significant IBD (Mantel test r = 0.159, $p = 0.04$) and found pairwise fixation indices among populations within mainland Australia and within Tasmania to be significantly lower than the fixation indices among populations from mainland Australia and Tasmania (Fig. 8, Kruskal-Wallis test $\chi^2 = 12.848$, $p = 0.002$, followed by a

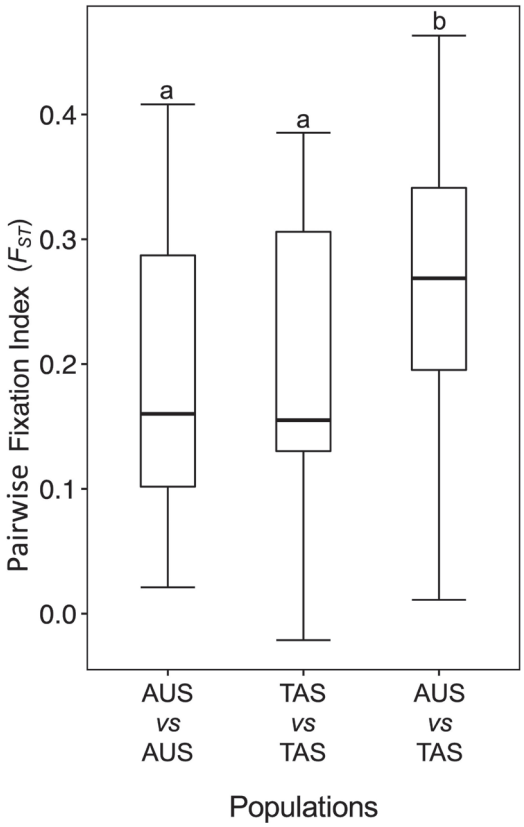

Figure 8. Comparison of pairwise fixation indices (F_{ST}) among Australian *Acacia longifolia* populations. Comparisons were made among populations within mainland Australia (AUS vs AUS), populations within Tasmania (TAS vs TAS), and among population of mainland Australia vs Tasmania (AUS vs TAS). Kruskal-Wallis test $p < 0.05$, and letters show the result of the post-hoc Mann-Whitney U test.

post-hoc Mann-Whitney U test), further supporting the STRUCTURE assignment of individuals to two genetic clusters corresponding to mainland Australia and Tasmania. However, higher values of *K* suggested that substructure existed within these two clusters (Suppl. material 1: fig. S7). Hierarchical STRUCTURE analyses revealed four clusters within mainland Australia and two clusters within Tasmania (Fig. 7B, C, respectively; Suppl. material 1: fig. S8A, B and C, D, respectively). Within mainland Australia, the population from Beachport appears to be distinct from all other populations (Fig. 7B; it also has a unique cpSSR haplotype B, Fig. 4), while all other populations show equal and symmetrical levels of admixture (i.e., being panmictic; also visible for other higher *K* values, Suppl. material 1: fig. S9). Within Tasmania, the Bridport, Three Sisters National Park, and Stanley populations seem to be genetically distinct from all other populations (Fig. 7C), with the Three Sisters National Park population being the most distinct (Suppl. material 1: fig. S10, $K = 6$). Bar plots for higher values of *K* are presented in the Suppl. material 1 (Suppl. material 1: figs S9, S10).

Inference of invasion sources

A similar introduction scenario was inferred for South Africa and Portugal, with the origin of invasive populations being an unknown population related to both the AUS and TAS genetic lineages (i.e., scenario 6, Fig. 3). This scenario had very high probability for both countries (p = 0.997, 95% CI = 0.995–0.998, and p = 0.928, 95% CI = 0.906–0.95, respectively; Suppl. material 1: fig. S11). Similar introduction scenarios were identified for Brazil and Uruguay: single or multiple introductions from Tasmania, i.e., scenarios 2 and 5, respectively (Fig. 3). Statistical support for these scenarios was lower for Brazil (scenario 2: p = 0.417, 95% CI = 0.391–0.443; scenario 5: p = 0.425, 95% CI = 0.399–0.451; Suppl. material 1: fig. S11) and Uruguay (scenario 2: p = 0.403, 95% CI = 0.378–0.429; scenario 5: p = 0.418, 95% CI = 0.392–0.444; Suppl. material 1: fig. S11) compared with the support for the most likely scenarios for Portugal and South Africa. Our ABC analysis for Spain was inconclusive, thus any discussion (see below) regarding these populations does not rely on this result.

Based on the scenarios with highest posterior probability for each invaded country, several parameters such as effective population size, time of events (e.g., introductions), admixture, and mutation rates were computed (Suppl. material 1: table S3). The mean time since first introduction of *A. longifolia* into South Africa and Portugal was 53.4 and 34.1 generations, respectively (Suppl. material 1: table S4). The mean time since first introduction ranged from 18.6 to 29.6 generations for Uruguay and from 13.8 to 16.3 generations for Brazil, depending on the scenario (2 or 5; Suppl. material 1: table S5). Bias values (i.e., RMedAD and MedRB) indicated that our model estimates are plausible (Suppl. material 1: tables S4, S5). Low Type I and Type II errors were inferred for both the Portuguese and South African scenarios (ranging from 0.02 to 0.12; Suppl. material 1: table S6). Conversely, the scenarios for Brazil and Uruguay showed higher Type I and Type II errors (ranging from 0.45 to 0.60; Suppl. material 1: table S6), but their posterior probabilities were low (compared with the scenario with highest probability for Portugal and South Africa). Results for the adequacy of the scenarios (i.e., "model checking", Suppl. material 1: table S7) showed that all high posterior probability scenarios had a slight deviation between the observed and simulated mean allelic size variance statistic, and in some cases also the mean genetic distance, but overall indicate that these scenarios sufficiently explained the observed data.

Results from the bridgehead scenario analysis (Suppl. material 1: fig. S12) showed that scenario 5 had the highest posterior probability (p = 0.716, 95% CI = 0.617–0.818). This scenario (Suppl. material 1: fig. S13) represents independent introductions of *A. longifolia* into South Africa and Portugal from an unknown native source that is related to both the AUS and TAS genetic lineages, thus not supporting a bridgehead introduction event between these two countries. The "model checking" analysis (Suppl. material 1: table S8) showed many deviations between the observed and simulated values of several summary statistics, indicating that this scenario does not explain our data better than those tested above.

Discussion

Our results suggest a complex introduction history of *A. longifolia* around the world during the 19th and 20th centuries. We found support for our initial hypothesis that invasive populations have high genetic diversity and low population structure. This agrees with the known history of multiple introductions, often of large propagule sizes, of the species into many parts of the world (Kull et al. 2007, 2008, 2011; Carruthers et al. 2011). Multiple introductions, especially when originating from multiple sources, hamper inferences of the native sources of invasive populations, even when historical records are available. For example, for Portugal, we were unable to identify the native source(s) of introduction and found evidence of multiple introductions, thus refuting the single introduction hypothesis from a previous study (Vicente et al. 2018). Similarly, we were unable to draw any conclusions on the sources of invasive *A. longifolia* in Galicia (Spain), likely due to the low number of populations and individuals we sampled in the country. Regarding South Africa, as expected, we were not able to identify the native source of the invasion. On the other hand, we were able to identify Tasmania as the likely native source of invasive populations in South America. Below we discuss how our limited sampling in Victoria and South Australia (one population in each state) and lack of samples from Queensland may have impacted our inferences of the introduction histories of *A. longifolia* to different parts of the world.

We identified population structure in Australian *A. longifolia*, with populations from mainland Australia and Tasmania corresponding to two distinct genetic clusters (Figs 6, 7A). Most invasive populations appeared to be admixtures between these two clusters, providing support for multiple introductions. One possible explanation for the existence of the two genetic clusters in Australia is the Bass Strait that separates Tasmania from the mainland, and thus acts as a strong barrier to gene flow as has been observed for *A. dealbata* (Hirsch et al. 2017, 2018) and other Australian natives such as *Eucalyptus regnans* (Nevill et al. 2010) and *Tasmannia lanceolata* (Worth et al. 2010). We also identified substructure within the mainland Australia and Tasmania clusters. Within mainland Australia, the Beachport population is genetically distinct from all others (Fig. 7B), and this result is also corroborated by the cpSSR data. This population is from South Australia and corresponds to the most western sampling site in our analyses, and thus these results might be due to disjunct sampling. Future work should include populations sampled throughout this state to fully understand the species' population structure in Australia. Regarding the Tasmanian cluster, the Bridport, Three Sisters National Park, and Stanley populations were found to be genetically distinct from all others (Fig. 7C). Interestingly, these are the only three sampled Tasmanian populations in close proximity to the Bass Strait, and these results can possibly suggest some level of gene flow among these populations and those from mainland Australia close to the Bass Strait. However, only one sampled population in mainland Australia is situated within this region (Curdievale, Victoria), thus further sampling is required to clarify these genetic relationships.

While we identified substructure within mainland Australia and Tasmania, we used the two overall genetic clusters as putative source areas in our ABC modelling for several reasons. First, the overall clustering of Australian populations into two genetic clusters was well supported by our isolation by distance (IBD) analyses and regional comparisons of fixation indices (Fig. 8). Second, fine-scale source inferences using hierarchical clusters would be more affected by unsampled "ghost" populations (which we included in our modelling scenarios, Fig. 3) than inferences based on broadscale regional genetic clusters. Our limited sampling in several Australian states makes the issues associated with modelling fine-scale invasion sources even more problematic. Lastly, co-evolutionary diversification between the host plants and prospective biocontrol agents is generally evident at broader, rather than narrow, geographic scales (e.g., Goolsby et al. 2006). The earleaf wattle example we discussed earlier provides a case in point (see Introduction). Co-diversification between this wattle and its specialist leaf-feeding beetle, *Calomela intemerata*, has been identified over broad geographic scales (Nawaz et al. 2021). Therefore, knowledge of the broadscale native range source(s) of invasive populations can play an important role in matching potential biological control agent biotypes with invasive host plant lineages.

We also found evidence for a unique genetic cluster shared between Portugal and South Africa. Invasive populations in these two countries also shared one cpSSR haplotype (Fig. 4). These findings suggest a possible secondary introduction event or bridgehead, likely from South Africa into Portugal based on historical occurrence records of the species in both countries (Rei 1925; Lourenço 2009; Poynton 2009; Marchante 2011; Vicente 2016; Vicente et al. 2018). Our ABC modelling did not support this scenario and this aspect of the introduction history of *A. longifolia* warrants further investigation. Overall, globally invasive populations do not appear to have gone through genetic bottlenecks upon introduction, a common feature of invasive acacia populations (Vicente et al. 2021).

While we could not infer introduction histories for all invaded regions with high levels of confidence, we do provide evidence that these likely differed among different parts of the world. For instance, we found higher cpSSR haplotype diversity in South Africa and Portugal compared with South America, probably because these two countries have been invaded for longer or, more likely, their introductions had much higher propagule pressure than to those into South America. South Africa had the highest haplotype diversity (higher than in the native range populations we sampled), while Spain and Brazil had the lowest. These findings agree with historical records indicating that wattle seeds, including those of *A. longifolia*, were often imported into South Africa from several locations, both from within and outside Australia (Poynton 2009). Our finding that South African populations harbour genetic diversity levels similar to those in native range populations is therefore unsurprising. Taken together, this corroborates our Approximate Bayesian Computation (ABC) modelling inferences, which failed to identify the native source of South African populations based on the scenarios we analysed. Similar findings have been made for other invasive acacias with complicated introduction histories characterised by multiple introductions of high propagule sizes

from diverse sources (e.g., *A. dealbata*; Hirsch et al. 2019, 2021). In our case, it is also possible that the "ghost" sources of South African invasions are areas in Victoria, South Australia, or Queensland, from where we had limited or no samples, and this limitation may also be the reason for the identification of divergent chloroplast haplotypes in invaded areas (e.g., haplotypes A and H in South Africa, Fig. 4).

The introduction history of *A. longifolia* in Portugal, as for South Africa, is likely characterised by multiple and genetically diverse introduction events. Historical records indicate that various *Acacia* species were planted along the Portuguese coast at different times (e.g., Rei 1925; Lourenço 2009; Marchante 2011; Marchante et al. 2011; Vicente 2016; Vicente et al. 2018). A previous genetic study hypothesised that the same seed allotment was used to establish nursery stock from which plants were sourced for plantings along the Portuguese coast (Vicente et al. 2018). Yet, genetic diversity of Portuguese populations is similar to that in South African and Australian populations, suggesting that multiple introductions from different origins likely established invasive populations in Portugal. A similar introduction history was recently described for *A. dealbata* into Portugal (Hirsch et al. 2021). Again, the sources of Portuguese *A. longifolia* invasions may include native range areas in Victoria, South Australia, or Queensland, from where we had limited or no samples.

For Brazil and Uruguay our ABC analyses indicated that the most likely origin of introduction is Tasmania, either as single or multiple introduction events. Historical records from these countries are scarce but considering that the genetic diversity of these populations is similar to that found in South African, Portuguese and Australian populations, multiple introductions seem likely. We could not conclusively infer the source(s) of Spanish *A. longifolia* populations, mostly likely because of the low number of plants we sampled in this country. However, we speculate that these plants were introduced in similar fashion to that of Portugal.

Conclusion

Our work shows that the origins of *A. longifolia* introductions around the world are hard to trace, likely because of the extensive historical efforts to introduce the species for dune 'restoration' and as an ornamental plant (Kull et al. 2011). The similar levels of genetic diversity among native and invasive ranges are illustrative of this. Multiple introduction events of large size (i.e., high propagule pressure) will not only help introduced population to overcome demographic and stochastic impacts associated with small populations sizes but will also provide high adaptive capacity, afforded by high genetic diversity, to these populations. Our results showing extensive admixture between native range genetic clusters also suggest that exploration for new biocontrol agents can be done throughout the native range of *A. longifolia* as no 'pure' genetic lineages are invasive. Moreover, the success of existing biocontrol agents such as *Trichilogaster acaciaelongifoliae* in places such as South Africa is likely to be replicated in other invaded regions. 'Piggy-backing' on the biocontrol programs of countries such

as South Africa may substantially shorten the amount of time needed to implement programs in other parts of the world, as was the case for the introduction of *T. acaciae-longifoliae* into Portugal (e.g., Marchante et al. 2011; López-Núñez et al. 2021).

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Supplementary material 1

Supplementary methodology details (primers, PCR conditions) and data analyses.

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Data type: Figures and tables

Explanation note: Comparison of the A) global fixation indices over all loci and populations of *Acacia longifolia*, and B) pairwise fixation indices, with and without the ENA correction (Chapuis and Estoup 2007). Comparisons of A) expected heterozygosity, B) observed heterozygosity, C) allelic richness, and D) inbreeding coefficent, using uncorrected and null allele-corrected datasets (Micro-Checker v2.2; Van Oosterhout et al. 2004). Box plots showing comparisons of expected heterozygosity (A), observed heterozygosity (B), allelic richness (C) and inbreeding coefficient (D) among *Acacia longifolia* populations from invaded countries (with populations from Portugal and Spain combined as Iberian Peninsula, IBP) and the two native range clusters identified by STRUCTURE analysis. Likelihood distribution [LnP(*K*)] and Delta *K* plots (Evanno method; Evanno et al. 2005) from STRUCTURE HARVESTER analysis for A-B) the complete dataset, and C-D) further analysis of the largest cluster (indicated in blue in Fig. 6A in the main text) identified in the analysis of the complete dataset (hierarchical analysis), respectively. STRUCTURE bar plots for $K = 3 – 6$ from the hierarchical analysis of the largest cluster from analysis of the complete dataset (indicated in blue in Fig. 6A in the main text). Results from the STRUCTURE HARVESTER analysis of the native range dataset. STRUCTURE bar plots for $K = 3 – 6$ from the analysis of the native range data only. Likelihood distribution [LnP(*K*)] and Delta *K* plots (Evanno method; Evanno et al. 2005) from STRUCTURE HARVESTER analysis for A-B) mainland Australia data, and C-D) Tasmania data, respectively. STRUCTURE bar plots for $K = 2 – 6$ (except $K = 4$) from the analysis of the mainland Australia data only. STRUCTURE bar plots for $K = 3 – 6$ from the analysis of the Tasmania data

only. Posterior probabilities of the six tested scenarios by invaded country. Details of microsatellite primers tested for cross-amplification in *Acacia longifolia*. Primers selected for cpSSRs and SSRs analyses and their corresponding repeat motifs, identified alleles, fluorescent dye labels used, PCR conditions (annealing temperature and concentration of magnesium chloride). Descriptions of parameters included in the ABC analyses. Prior and posterior values of parameters for Scenario 6 of ABC analyses, selected as the best scenario for Portugal (POR) and South Africa (RSA). Prior and posterior values of parameters for A) Scenario 2 and B) Scenario 5 of ABC analyses, selected as the best scenarios for Brazil (BRA) and Uruguay (URU). Type I and Type II errors for the chosen scenarios of each invaded country in ABC analyses. Results of the "model checking" analysis for the best scenarios selected by country in ABC analyses. Posterior probabilities of the six bridgehead tested scenarios between RSA and POR (600 000 simulations in total). DIYABC drawing of scenario 5, which had the higher posterior probability of the six bridgehead tested scenarios between RSA and POR (p = 0.7161, 95% CI = 0.6165-0.8157). Results of the "model checking" analysis for the best bridgehead scenarios selected (scenario 5) in ABC analyses.

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Link: https://doi.org/10.3897/neobiota.82.87455.suppl1

Supplementary material 2

Genotype data used in this study in GenAlEx format.

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Data type: Genotype data

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