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Diversity and Introduction History of *Glycaspis brimblecombei* Reflects a History of Bridgeheads and Distinct Invasions

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Glycaspis brimblecombei is an invasive insect pest of *Eucalyptus* that has spread rapidly around the world since its first report in California in 1998. The pest now occurs on at least four continents where *Eucalyptus* is grown as a non-native plantation species. To characterize global routes of invasion for this insect, we characterized the sequences of a portion of the Cytochrome Oxidase 1 (COI) gene from 105 individuals from the invasive and native range, including from Australia, Brazil, Chile, La Réunion, Mauritius, South Africa and the United States. In addition, we developed 13 polymorphic microsatellite markers, of which we used 11 to characterize the diversity in the same 105 specimens. Our results suggest that there have been two independent introduction events from Australia, which is assumed to be the origin, to distinct parts of the adventive range. The first introduction was into the United States, from where it appears to have spread to South America and eventually to South Africa. This finding highlights the threat of bridgehead populations to accelerate pest invasions in *Eucalyptus*, even if those populations are on widespread non-commercial populations of *Eucalyptus* (as in California). A second introduction appears to have occurred on the islands of Mauritius and La Réunion and provides another example of the establishment of independent lineages of invasive global insect pests. This complex invasion pattern mirrors that found in other *Eucalyptus* pests.

Keywords: invasion pathways, invasive insect pest, *Eucalyptus* spp., population genetics, forest health

INTRODUCTION

Invasive insects threaten biodiversity and ecosystem function in both natural and highly managed ecosystems such as forestry and agriculture (Hoffmann et al., 2008; Wingfield et al., 2015; Hurley et al., 2016). Increasingly, such threats are global in scope. Together with climate change and increasing volumes of global trade, the homogenization of ecosystems, in part due to the widespread

planting of common species for agriculture or forestry, have increased the rate of insect spread and establishment worldwide (Ali et al., 2014; Garnas et al., 2016). Although the invasion mechanisms and patterns of movement vary among organisms, the increase in accidental insect spread is strongly linked to anthropogenic pathways (Estoup and Guillemaud, 2010; Foucaud et al., 2010; Garnas et al., 2016). Understanding these patterns of movement is important for managing existing invasive populations, as well as avoiding future introductions.

Eucalyptus species are native to Australasia and have become one of the most widely planted hardwood species worldwide, now occurring in more than 100 countries and on all continents with the exception of Antarctica and covering over 20 million hectares (Brockerhoff et al., 2013). Since the first report of invasive *Eucalyptus* pests reported outside their native range, the number of invasive insect pest outbreaks has been continuously rising, with a sharp increase in the number of emergences since the 1980's (Wingfield et al., 2015; Hurley et al., 2016). This number is expected to continue to increase rapidly in future given the consistent pattern over the last 20 years.

Glycaspis brimblecombei Moore, 1964 (Hemiptera, Psylloidea, Aphalaridae), commonly known as the red gum lerp psyllid, has become an important threat to *Eucalyptus* plantations worldwide (de Queiroz et al., 2013). It was first reported outside its native range in 1998 in California, United States (Brennan et al., 2001) and has since rapidly spread to several countries (Garnas et al., 2016; Makunde et al., 2020). Within 10 years after its first report from California, it was noted in at least seven countries in the Americas (de Queiroz et al., 2013; Tsagkarakis et al., 2014), Mauritius and Madagascar (Laudonia and Garonna, 2010). Subsequently, *G. brimblecombei* has also been observed from several countries in Europe and Africa (Laudonia and Garonna, 2010; Bella and Rapisarda, 2013; Frasconi et al., 2013; Ben Attia and Rapisarda, 2014; Laudonia et al., 2014; Spodek et al., 2015; Ndlela et al., 2018; Makunde et al., 2020).

Heavy infestations of *G. brimblecombei* on *Eucalyptus* trees have the potential to cause premature leaf fall (including total defoliation in severe cases), dieback and reduction of the tree growth (de Queiroz et al., 2013). Physiological stresses caused by *G. brimblecombei* on the tree leads to increased susceptibility of the tree to other pests and diseases and can result in the death of susceptible varieties (Frasconi et al., 2013). Although *G. brimblecombei* mainly attack species such as *E. camaldulensis* Dehnh and *E. tereticornis* Smith, it also affects hybrids such as *E. grandis× E. tereticornis,* and *E. grandis × E. camaldulensis* (Brennan et al., 2001; de Queiroz et al., 2013; Ben Attia and Rapisarda, 2014; Lucia et al., 2016; Bush et al., 2020). Classic biological control, pesticides and the planting of tolerant tree species and/or hybrid clones are currently being used as management strategies for *G. brimblecombei* (Gonçalves et al., 2013; Garnas et al., 2016; Ndlela et al., 2018; Kojwang, 2019). Biological control using the psyllid-specific parasitic wasp *Psyllaephagus bliteus* Riek (Hymenoptera: Encyrtidae) has been attempted in a few countries (Dahlsten et al., 2005; Huerta et al., 2011). However, the effectiveness of *P. bliteus* varies geographically and studies have concluded that these differences are linked to habitat and temperature (Dahlsten et al., 2005;

Gonçalves et al., 2013; Margiotta et al., 2017). Similarly, studies have shown the dependence on environmental factors also of development and invasion capacity of *Glycaspis brimblecombei* (Laudonia et al., 2014; Caleca et al., 2018; Junk et al., 2020).

The impact of *G. brimblecombei* as an invasive pest, the need to increase the efficiency of biological control management options, as well as the history of complex invasions (including cryptic species and establishment of multiple independent lineages) in other *Eucalyptus* pests, inspired this study to characterize the diversity of and invasion history of global populations of *G. brimblecombei*. We use reports of the emergence of *G. brimblecombei,* together with nuclear and mitochondrial DNA markers to characterize the genetic diversity within and between populations from its native (Australia) and invasive (including the United States, various countries in South America, South Africa, La Réunion and Mauritius) range to suggest the historical pattern of the invasion.

MATERIALS AND METHODS

Glycaspis brimblecombei Sample Collection and DNA Extraction

Whole specimens of *Glycaspis brimblecombei* were obtained from across the invasive range from Brazil ($N = 11$), Chile (Valparaiso Region; Santiago) ($N = 16$), La Réunion ($N = 15$), Mauritius ($N = 13$), South Africa (Gauteng; KwaZulu-Natal; Limpopo; Mpumalanga) ($N = 30$), the United States (California—Laguna Grande; Riverside) ($N = 11$), and Australia (Queensland) ($N = 9$) and were stored in 95% ethanol at $-20°C$ until used for DNA extraction. The sampling was carried out from 2012 to 2014. A modified phenol-chloroform DNA extraction protocol was used to obtain genomic DNA from individual insects (Nishiguchi et al., 2002). Prior to DNA extraction, specimens were preserved in 95% ethanol.

Glycaspis brimblecombei Mitochondrial Diversity

A portion of the Cytochrome Oxidase subunit I (COI) of the mitochondrial gene from a total of 105 individuals was amplified using the universal primers C1-N-2191 (5'-CCC GGT AAA ATT AAA ATA TAA ACT TC-3') (alias Nancy) and C1-J-1718 (5'-GGA GGA TTT GGA AAT TGAT TAG TTC C-3') (alias Ron) (Simon et al., 1994). PCR amplifications were performed using the Veriti Thermal Cycler (Applied Biosystems, Foster City, California, Unites States) in a total volume of 25 µl which contained 5 µl 5X MyTaq Reaction Buffer consisting of dNTPs, $MgCl_2$, and ready to use enhancers (Bioline, London, United Kingdom), 2.5 U MyTaq[TM] DNA Polymerase (Bioline, London, United Kingdom), 10 µM of each C1-N-2191 and C1-J-1718 primer, 16.5 µl SABAX pure water (Adcock Ingram, Midrand, South Africa) and a minimum of 10 ng/µl of DNA. The PCR cycling parameters included an initial denaturation step of 95°C for 3 min., followed by 35 cycles of denaturation at 95°C for 20 s, annealing at 54°C for 30 s, and a final extension step of at 72°C for 7 min. The PCR product was visualized on a 2% agarose

gel using a BioRad Gel Doc™ Ez Imager and the software Image Lab v4.0 build 16 (Hercules, California, United States).

The PCR products were purified using Sephadex G-50 (Sigma, St. Louis, Missouri, United States) columns for 3 min. at 800 rcf in a desktop centrifuge (Eppendorf, Hamburg, Germany). Subsequently the PCR products were visualized on a 2% agarose gel using a BioRad Gel Doc™ Ez Imager and the software Image Lab v4.0 build 16 (Hercules, California, United States). Purified PCR amplicons were used for cycle sequencing incorporating 6.4 µl sterile SABAX water (Adcock Ingram, Midrand, South Africa), 2.1 µl of 5 X sequencing buffer (Thermo Fisher Scientific, Waltham, Massachusetts, United States), 0.5 µl of BigDye terminator 3.1, 10 µM of each primer. The cycle sequencing conditions included 96°C for 2 min., followed by 30 cycles of 96°C for 30 s, 54°C for 15 min. and 60°C for 4 min. Subsequently, the cycle sequencing product was cleaned using Ethanol/NaAc precipitation protocol, following guidelines from the ABI manual (2010).[1] Samples were sequenced using the ABI PRISM™ 3100 Genetic Analyzer (Applied Biosystems, Foster City, California, Unites States).

MEGA version 7 (Kumar et al., 2016) was used to edit individual sequences and create contigs. AliView Version 1.17.1 (Larsson, 2014) was used to translate nucleotide sequences to amino acid sequences to check for the presence of stop codons. Nucelotide diversity and haplotype diversity (Nei, 1987) were computed using DnaSP version 6.12.03 (Rozas et al., 2003). A multiple sequence alignment was conducted using ClustalΩ (Sievers and Higgins, 2002) with default parameters. A COI haplotype network was generated using Network Version 10.1.0.0 package (Polzin and Daneschmand, 2003).[2] A neighbor Joining analysis, using the Kimura-2 parameter substitution model, was conducted using MEGA version 7 (Kumar et al., 2016) with 1000 bootstrap replicates. *Blastopsylla occidentalis* (GenBank number MN272142.1) was used as an outgroup. Uncorrected percentage pairwise sequence divergence was also calculated using MEGA version 7 (Kumar et al., 2016).

Glycaspis brimblecombei Microsatellite Development, Amplification, and Genotyping

Low-coverage sequencing of the *G. brimblecombei* genome was performed using the Illumina MiSeq platform. Sequence reads for *G. brimblecombei* were assembled using VelvetOptimiser v.2.2.5 (Zerbino and Birney, 2008). All contigs larger than 1000 base pairs (bp) were used to search for microsatellites using MsatCommander 1.0.8 (Faircloth, 2008). Selection criteria included a minimum of seven and a maximum of 23 repeats. Microsatellites from di-, tri-, tetra-, and pentanucleotide classes were selected with product sizes ranging from 100 to 377 bp. Subsequently, Primer3 version 2.5.0 (Untergrasser et al., 2012) was used to design twenty primers which were evaluated for suitability.

Polymorphism were confirmed by PCR using three individuals per country (Australia, Brazil, Chile, La Réunion, Mauritius, South Africa and United States). Thirteen of the 20 microsatellite primers showed polymorphisms. The forward primers of these 13 primer pairs were fluorescently labeled (Thermo Fischer Scientific, Waltham, Massachusetts, United States) and subsequently used for PCR. The same 105 specimens used for the mitochondrial analysis were used for the microsatellite analysis. Microsatellite amplification was conducted in a 96-well plate with a total reaction volume of 11 µl comprising 1 µl PCR product, 0.15 µl GeneScan 500 LIZ dye Size Standard (Applied Biosystems, Foster City, California, Unites States) and 10.27 µl Formamide. Scoring was conducted using GeneMapper software (Version 4.1, Applied Biosystems, Foster City, California, Unites States).

Genetic Diversity Analysis

For each of the seven populations (Australia, Brazil, Chile, La Réunion, Mauritius, South Africa, and United States) genetic diversity indices were generated using Arlequin version 3.5.1.3 (Excoffier et al., 2005). An analysis of molecular variance (AMOVA) was conducted, with 100 000 permutations using Arlequin version 3.5.1.3 (Excoffier et al., 2005), to compare the genetic variance among regions (native and invasive), among populations within regions, and within populations. Pairwise F_{st} values were calculated for specimens in each of the seven populations using Arlequin version 3.5.1.3 (Excoffier et al., 2005).

Genetic Relatedness, Population Structure, and Scenario Testing

A Principle Coordinate Analysis (PCoA) was conducted using GenAlEx version 6.503 (Peakall and Smouse, 2012) to quantify and visualize genetic groupings in our data. In addition, a one-way PERMANOVA was conducted using PAST (PAleontological STatistics Version 3.20) with the Bray-Curtis similarity index and 9,999 permutations (Hammer et al., 2001). STRUCTURE v2.3.4 (Pritchard et al., 2000) was used to group specimens into populations based on genetic similarity. Default parameters were used, with the burn-in length set to 100,000 iterations and 100,000 simulations. The K-values tested included K = 1 to K = 10, with 20 repetitions. To define the most likely partition of the number of clusters ΔK was calculated (Evanno et al., 2005) using Structure Harvester (Earl and von Holdt, 2012). Cluster Markov Packager Across K (CLUMPAK) was used to visualize the results (Kopelman et al., 2015).

Approximate Bayesian Computation (ABC) using DIYABC software version 2.0.4 beta (Cornuet et al., 2008) was used to test different scenarios which hypotheses of the global movement of *G. brimblecombei*. The genetic diversity data from the microsatellites and historical records were used to infer the most likely dispersal route of *G. brimblecombei* between Australia, Africa and America. Eight scenarios were tested using 1,000,000 simulated data sets for each scenario. One and two sample summary statistics included *Mean Genic Diversity* and *Mean Size Variance*, and *Mean Number of Alleles*, *Mean Size Variance*, *Classification Index,* and *Distance between two samples*,
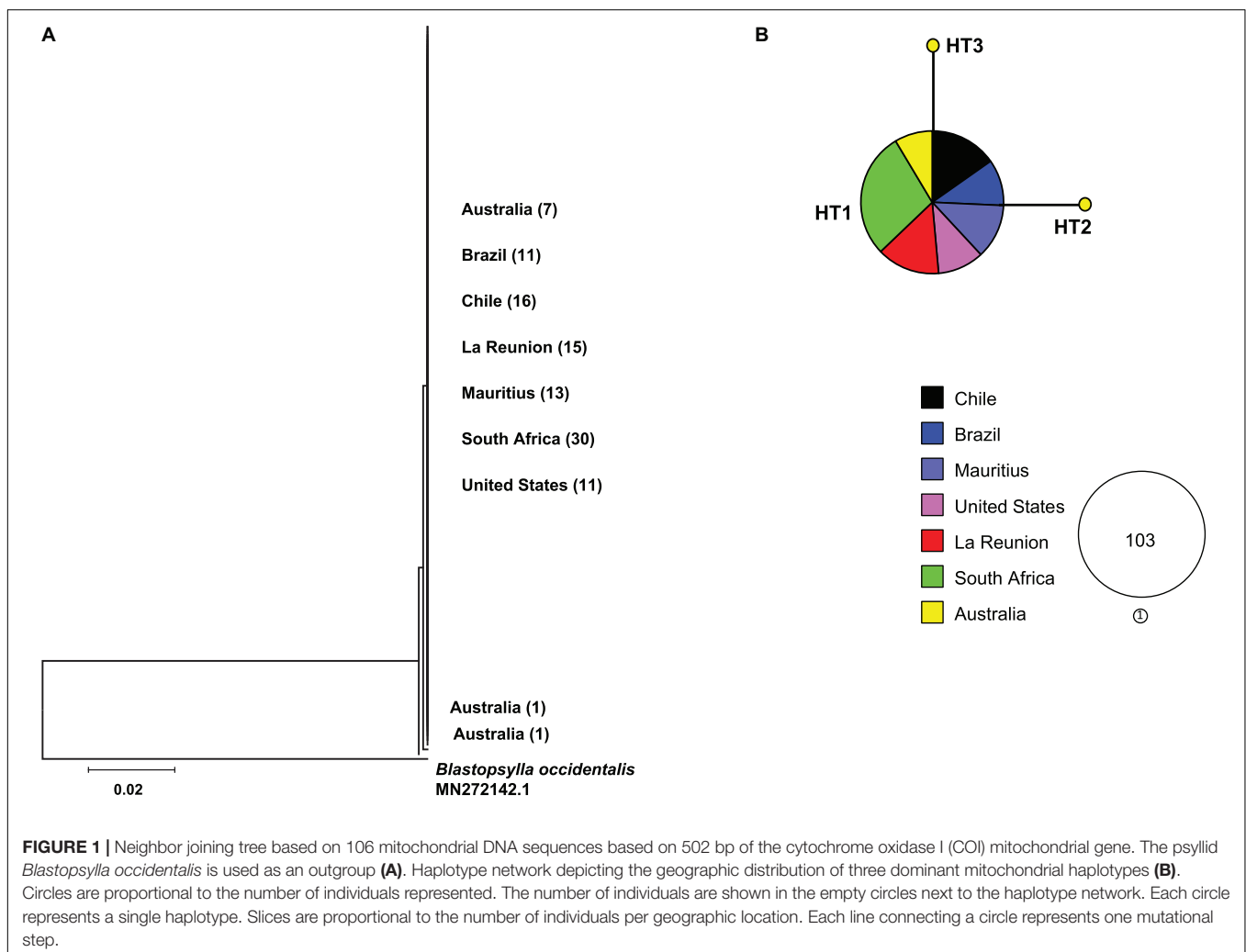
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[1] https://assets.thermofisher.com/TFS- Assets/LSG/manuals/cms_081527.pdf
[2] fluxus-engineering.com

respectively. The most likely scenario was chosen based on two criteria, namely that the simulated scenario was amongst the 1% comprising the most likely scenarios, and that the scenario had the highest posterior probability, calculated by using a logistic regression, of all the scenarios tested. Several assumptions were made in the construction of tested scenarios: Australia (AUS) was defined as the native range, South America (SAM) represented by Brazil and Chile, and Mauritius and La Réunion (ISL: islands) were combined into one population due to their genetic similarity at the COI mtDNA locus. Finally, the United States and South Africa (RSA) were regarded as separate populations. All scenarios have been included, in a visual format in the **Supplementary Material**.

Scenario 1 tested the hypothesis that three independent introductions of *G. brimblecombei* occurred from the native range—an introduction from Australia to the United States with the South American population establishing as a result of a bottleneck occurring during the movement from the United States to South America, a separate introduction from Australia to the ISL (Mauritius and La Réunion), and from Australia to South Africa. Scenario 2 tested the hypothesis that a direct introduction from Australia to the United States occurred, with a subsequent bottleneck event and three independent radiation events (South America, ISL and South Africa) from the United States. Scenario 3 tested the hypothesis that all four populations, the United States, South America, South Africa and the ISL, originated directly but independently from the origin. Scenario 4 tested the hypothesis that two introductions occurred, one from the origin to the ISL (Mauritius and La Réunion) and another from the origin to the Unites States, followed by movement from the United States to South America and the emergence of the South African population from the United States population. Scenario 5 tested the hypothesis of a step-wise introduction from the origin to the United States and the ISL (i.e., from the United States to South America and then to South Africa). Scenario 6 tested the hypothesis of an introduction from Australia to the United States, with a subsequent introduction from the United States into South America and then from South America to South Africa. Subsequently an introduction from the United States to both Mauritius and La Réunion occurred. Scenario 7 tested the hypothesis of a direct introduction from Australia to the

FIGURE 1 | Neighbor joining tree based on 106 mitochondrial DNA sequences based on 502 bp of the cytochrome oxidase I (COI) mitochondrial gene. The psyllid *Blastopsylla occidentalis* is used as an outgroup **(A)**. Haplotype network depicting the geographic distribution of three dominant mitochondrial haplotypes **(B)**. Circles are proportional to the number of individuals represented. The number of individuals are shown in the empty circles next to the haplotype network. Each circle represents a single haplotype. Slices are proportional to the number of individuals per geographic location. Each line connecting a circle represents one mutational step.

United States, then from the United States to South America, and from South America to the ISL and from the ISL to South Africa. Scenario 8 tested the hypothesis that a direct introduction of *G. brimblecombei* occurred from the origin to the United States and to the ISL followed by secondary introductions from ISL to South Africa and from the United States to South America.

RESULTS

Mitochondrial DNA Sequence Diversity

A 502 bp portion of the cytochrome oxidase I region (COI) of the mitochondrial DNA was amplified for 105 specimens (GenBank accession OK335882-OK335986) and used to generate a neighbor joining tree and a haplotype network (**Figure 1**). The sequences contained no stop codons. Three haplotypes were present. A single haplotype was present in all introduced populations, as well as in seven specimens (77%) from the native range. The remaining two haplotypes were present only in the native range of Australia (**Table 1**). The percent sequence divergence between the invasive haplotype and specimens in the Australian only group ranged from 0 to 0.2%. These divergent haplotypes differed by a single base pair each and were represented by a total of two individuals.

Microsatellite Diversity

The genome of *G. brimblecombei* as assembled using Illumina MiSeq reads indicated a genome size of 138 Mb, an N_{50} of 584 bp, contained 278,167 contigs, and a GC content of 31.7%. Of the initial twenty primers, 13 produced polymorphic fragments/alleles using a PCR analysis of three individuals; these were fluorescently labeled. Two of these primers were discarded due to scoring difficulties. The eleven remaining primers were used to produce length polymorphism data for the 105 specimens on which SSR diversity estimates are based.

Of the invaded geographical areas, the United States had the highest neutral genetic (SSR) diversity (calculated using allele frequency per locus) (0.66 ± 0.36), followed by Chile (0.57 ± 0.32), Brazil (0.56 ± 0.32), La Réunion (0.54 ± 0.30), Mauritius (0.49 ± 0.29), and South Africa (0.20 ± 0.15). The total number of alleles (A), mean number of alleles and average gene diversity was highest in the native range (Australia), as expected

(**Table 2**). Pairwise F_{st} values for the seven sampled populations ranged from 0.53 ± 0.02 to 1.000 (where $F_{st} = 1$ indicates no shared alleles) and did not differ significantly from zero for any population pairs. The AMOVA analysis indicated that 30.8% variation occurred among populations within regions (df = 5; $P < 0.001$) while within population variation was 67.6% (df = 203; $P < 0.001$; **Table 3**). Only 1.6% of variation was attributed to region (native v. invasive; df = 1; $P = 0.57$).

The Principal Component Analysis (PCoA) suggested two clusters, one for the island group (La Réunion and Mauritius) and the another for the rest of the samples, with South Africa comprising a mini-cluster within this second cloud (**Figure 2**). PC 1 and PC 2 explained 22.23% and 8.99% of variation, respectively. The PERMANOVA indicated that there were significant differences ($P < 0.05$) between all clusters except Mauritius and La Réunion, and Brazil and Chile (**Table 4**).

Analysis of population structure using STRUCTURE and STRUCTURE harvester, indicated the highest likelihood for two clusters ($K = 2$; **Figure 3**). At $K = 2$, one group consisted of samples from South America (Brazil and Chile), South Africa and the United States and the rest Australia and the ISL.

Hypothesis testing of eight scenarios intended to identify the most likely global pattern of movement of *G. brimblecombei,* showed highest support for Scenario 5 representing one independent introduction from Australia to the ISL (Mauritius and La Réunion) and another from Australia to the United States, followed by a secondary introduction from the United States to the southern hemisphere adventive range (i.e., South America and South Africa; Bayesian probability value [95% confidence interval] = 0.76 [0.64; 0.87]; **Figure 4** and **Supplementary Material**).

DISCUSSION

The current study is the first to characterize the genetic diversity of the red gum lerp psyllid, *Glycaspis brimblecombei,* across multiple regions and to evaluate among hypothesized invasion routes. We inferred the invasion routes using neutral genetic diversity from across the genome of the insect, using mitochondrial (CO1) diversity and historical records of introduction to guide which hypotheses to be tested. The study

TABLE 1 | Haplotype and nucleotide diversity of *Glycaspis brimblecombei* based on mitochondrial DNA data.
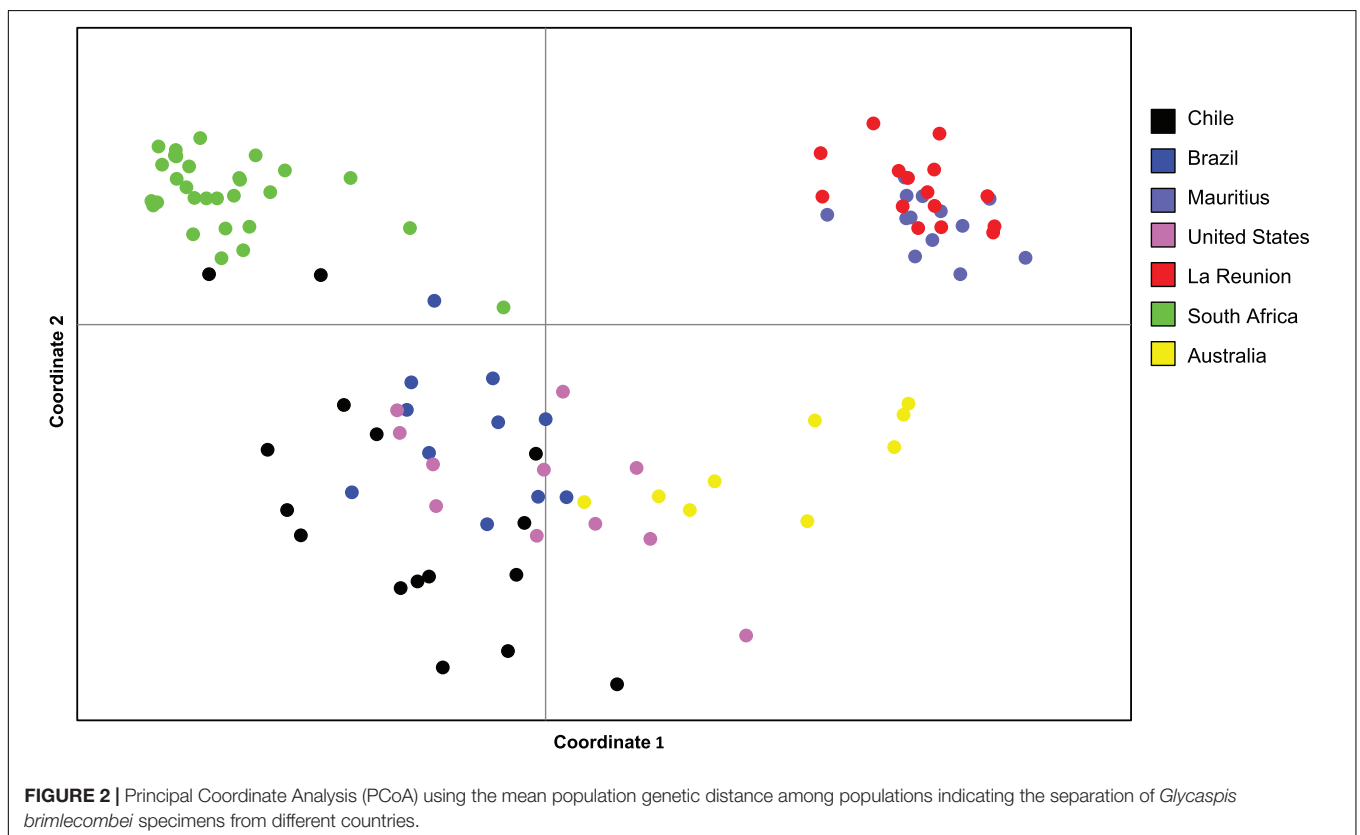
Geographical region	No. of individuals	No. of localities/countries	No. of haplotypes	Haplotype diversity (Hd)	Nucelotide diversity (Pi)
Native					
Australia	9	1	3	0.4167	0.00089
Invasive					
Mauritius	96	6	1	0.000	0.000
Reunion					
South Africa					
United States					
Chile					
Brazil					
	105				

TABLE 2 | Genetic diversity indices for *Glycaspis brimblecombei* based on SSR data.

Regions	Number of individuals	Total number of alleles (A)	Mean number of alleles (± SD) by locus	Number of polymorphic loci	Mean expected heterozygosity (± SD)	Mean observed heterozygosity (± SD) for polymorphic loci	Average gene diversity over loci (± SD)
Native							
Australia	9	84	10.81 ± 3.43	10	0.83 ± 0.10	0.60 ± 0.21	0.83 ± 0.45
Invasive							
Brazil	11	44	4.40 ± 1.77	7	0.62 ± 0.13	0.55 ± 0.23	0.55 ± 0.31
Chile	16	38	3.45 ± 1.21	8	0.59 ± 0.20	0.52 ± 0.25	0.56 ± 0.31
Mauritius	13	46	4.60 ± 2.27	7	0.61 ± 0.23	0.52 ± 0.23	0.49 ± 0.28
La Reunion	15	41	4.55 ± 1.81	9	0.66 ± 0.11	0.548 ± 0.26	0.54 ± 0.29
South Africa	30	34	4.85 ± 1.77	3	0.55 ± 0.20	0.44 ± 0.21	0.20 ± 0.14
United States	11	57	5.18 ± 2.13	10	0.68 ± 0.18	0.57 ± 0.28	0.66 ± 0.36

TABLE 3 | Analysis of the molecular variance (AMOVA) of the native (Australia) and introduced (Brazil, Chile, Mauritius, Reunion, South Africa, United States) populations of *Glycaspis brimblecombei* using SSR data.

Source of variation	df	Sum of squares	Variance components	Percentage of variation	*P*-value
Among regions (native vs. invasive)	1	20.39	0.046	1.64	0.57
Among populations within regions	5	143.06	0.86	30.78	<0.00001
Within populations	203	384.85	1.89	67.57	<0.00001
Total	209	548.30	2.81	100	

FIGURE 2 | Principal Coordinate Analysis (PCoA) using the mean population genetic distance among populations indicating the separation of *Glycaspis brimlecombei* specimens from different countries.
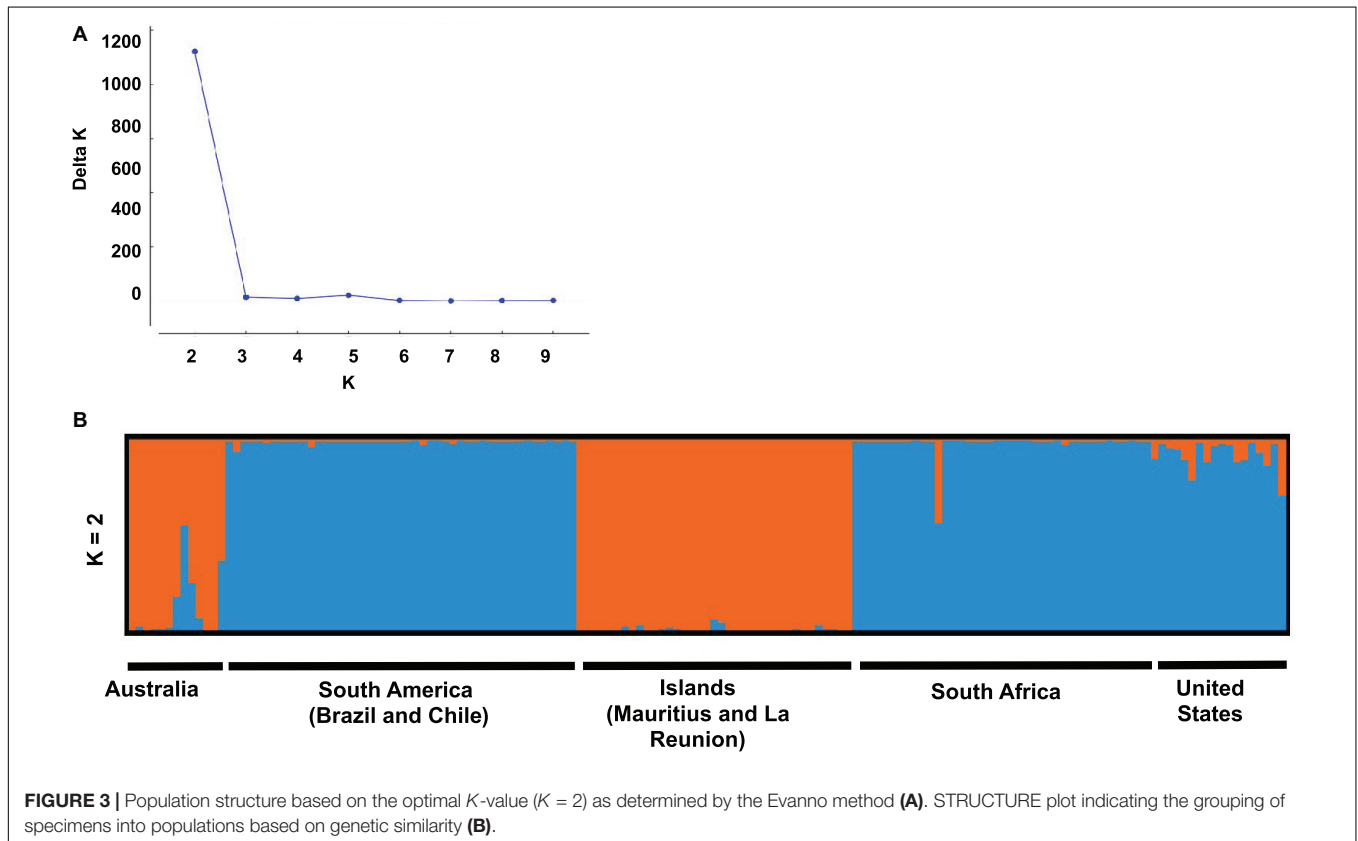
shows an invasion history outside the native range, as reported in other invasive insect pests associated with forestry (Nadel et al., 2009; Boissin et al., 2012), starting from a population established in the United States, and then spreading to South America and Africa. It also indicates that there has been a second, independent introduction into Mauritius and La Réunion. The small sample size used in this study was adequate to analyze the genetic structure of this insect population. There was only

TABLE 4 | One-way PERMANOVA, conducted using the Bray-Curtis similarity index, indicating *p*-values (uncorrected significance) for *Glycaspis brimblecombei* using SSR data.

	Australia	Brazil	Chile	Mauritius	Reunion	South Africa	United States
Australia		0.0008	0.0002	0.0005	0.0001	0.0006	0.0001
Brazil	0.0008		0.3523	0.0021	0.0001	0.0386	0.0011
Chile	0.0002	0.3523		0.0001	0.0001	0.0025	0.0001
Mauritius	0.0005	0.0021	0.0001		0.0861	0.0003	0.0003
Reunion	0.0001	0.0001	0.0001	0.0861		0.0002	0.0001
South Africa	0.0006	0.0386	0.0025	0.0003	0.0002		0.0003
United States	0.0001	0.0011	0.0001	0.0003	0.0001	0.0003	

FIGURE 3 | Population structure based on the optimal *K*-value (*K* = 2) as determined by the Evanno method **(A)**. STRUCTURE plot indicating the grouping of specimens into populations based on genetic similarity **(B)**.

a single haplotype present in the invaded range, which would indicate that all the diversity in the invaded range had been captured in these samples. A similar study, using specimens from a number of different regions indicated no genetic diversity in the invasive range, even though the sample size was substantially larger (Dittrich-Schröder et al., 2018).

Glycaspis brimblecombei is native to Australia and this region is also confirmed as the likely source population of the invasions using both mitochondrial and nuclear data. In addition, the mitochondrial and nuclear data suggest the occurrence of a bottleneck in the invasive populations. Evidence for a bottleneck event include (i) the absence of diversity at the CO1 mtDNA locus in the introduced range, (ii) a decrease in heterozygosity in the invaded range, and (iii) a decrease in the number of alleles in the invaded range (Roderick and Navajas, 2003; Arca et al., 2015). Comparison of the genetic diversity between and within

populations is a method regularly used to identify the source population (Zepeda-Paulo et al., 2010). However, these methods do not consider specific invasion scenarios, and hence additional methods are required.

We found three COI haplotypes across the native and introduced range. Haplotype 1 was observed throughout the invasive range as a whole as well as in the origin. Two haplotypes (Haplotype 2 and Haplotype 3) were each represented by a single individual from Australia. The low sequence divergence observed between the three haplotypes (0–0.2%) is typical of specimens of the same species, as is expected in this case with all collections identified as *G. brimblecombei*. Previous studies on psyllid species delimitation have indicated that a 3% uncorrected p-distance threshold for cytochrome oxidase I (COI) divergence is a likely guideline for psyllid species identification (Taylor et al., 2016; Percy, 2017; Wonglersak et al., 2017; Martoni et al., 2018).

FIGURE 4 | Global pathway of spread (Scenario 5) has been identified as the most highly supported invasive pathway of *G. brimblecombei*. The "Islands" refers to individuals from Mauritius and La Réunion and "South America" refers to individuals from Brazil and Chile.
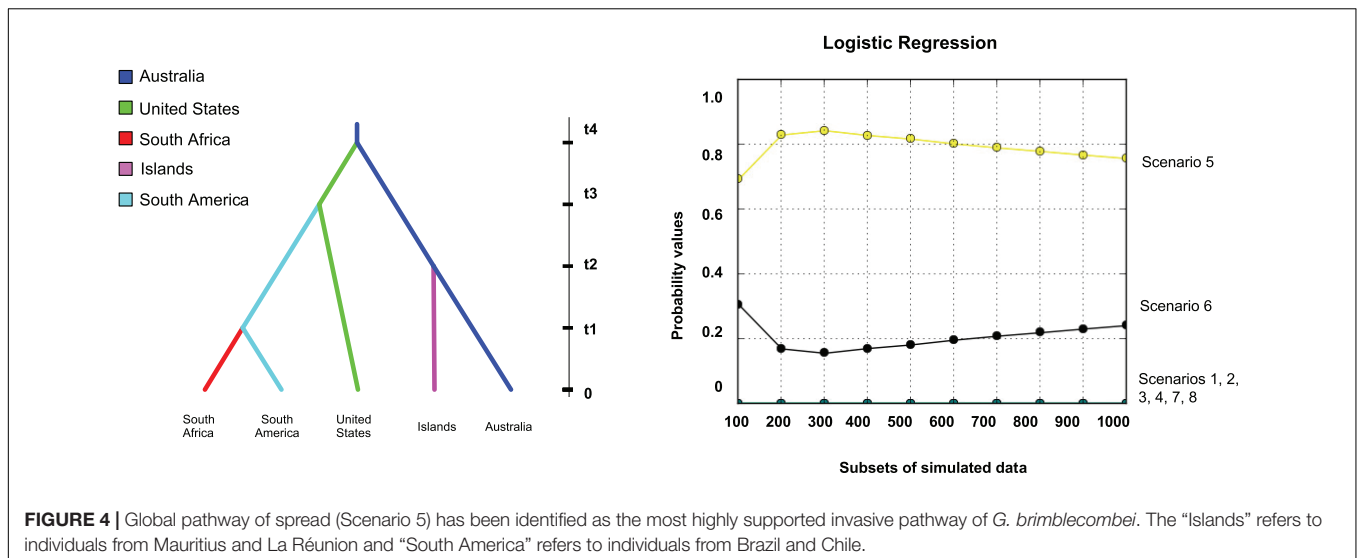
The Australian population of *G. brimblecombei* contained the highest genetic diversity, consistent with other studies that have shown more diversity in populations collected from the native range (Cifuentes et al., 2011). Of the invaded geographical areas, the United States had the highest genetic diversity followed by Chile, Brazil, La Réunion, Mauritius, and South Africa, respectively. The South African group had the lowest genetic diversity among the invaded populations, which could be due to a founder effect or genetic drift in this population following introduction. This pattern could reflect a genetic bottleneck if the number of colonizing individuals was low or genetic drift occurred during establishment. Reduced genetic variation is frequently observed within invasive ranges, though this pattern can be ameliorated by multiple introductions, admixture, and rapid population growth (Rius and Darling, 2014; Garnas et al., 2016). Similarly, reduced genetic variation has been observed in a number of other recently introduced invasive species in their invaded range [e.g., *Thaumastocoris peregrinus* (Nadel et al., 2009; Mutitu et al., 2020); *Anoplophora glabripennis* (Carter et al., 2010); *Halyomorpha halys* (Cesari et al., 2018); *Vespula germanica* (Eloff et al., 2020); amongst many others]. There is little evidence, however, that low genetic diversity represents a major constraint on adaptive evolution (Garnas, 2018).

Approximate Bayesian Computation (ABC) scenario testing supported two independent introduction events from the native Australian range; one into the United States with subsequent, secondary introductions into South America and South Africa, and another from Australia into Mauritius and La Réunion. The STRUCTURE analysis also provides support for two independent groups with the Australian, Mauritian and La Réunion populations grouping together, and the South American (Brazil and Chile), South African and United States populations grouping together. Similar patterns of multiple independent introductions from species' native ranges have been shown for various other plantation insect pests, such as *Thaumastocoris peregrinus* (Nadel et al., 2009), *Sirex noctilio* (Boissin et al., 2012), three species of *Gonipterus* weevils (Mapondera et al., 2012),

and *Leptocybe invasa* (Dittrich-Schröder et al., 2018). In some studies, multiple introductions were speculated to result from increases in the population of the introduced organism within the native range likely to have acted as a proximal source of invasive propagules. This suggests that monitoring of insect pests in native ranges of major crops, such as *Eucalyptus* in this case, could help predict potential future invasions. Little is known about *G. brimblecombei* in its native range (Santos et al., 2020).

The invasive United States population was the first to be detected outside of Australia and has served as a source of a number of subsequent introductions. Numerous examples exist where invasive species have become a source for further introductions and this process is referred to as the bridgehead effect (Lombaert et al., 2010; Ascunce et al., 2011; Yang et al., 2012). If this population emerges as a disproportionate driver of global spread, it could plausibly be considered a "bridgehead" population (Lombaert et al., 2010; Ascunce et al., 2011; Yang et al., 2012; Santos et al., 2020). One explanation of this phenomenon is that individuals in these bridgehead populations acquire traits associated with successful establishment and spread (Bertelsmeier and Keller, 2018). Such populations are important to monitor for the emergence of new genotypes and/or evidence of adaptive evolution. Alternatively, this effect could simply reflect larger population sizes or greater connectivity via global trade and travel (Garnas et al., 2012, 2016).

Scenario testing and the STRUCTURE results suggest that the *G. brimblecombei* invasion occurred from Australia to La Réunion and Mauritius (both islands), and not between these islands and South Africa, which could have been predictable due to their geographic proximity. Projections based on historical invasion rates of insect pests in Mauritius suggest that one new insect pest is expected to be recorded each year, perhaps increasing with time (Russel et al., 2017; Mauremootoo et al., 2019). Islands have been shown to be more susceptible to invasion by non-native species (Sol, 2000; Simberloff, 2003; Gimeno et al., 2006), including forest pests and pathogens (Brockerhoff and Liebhold, 2017). The high

invasibility of islands makes them a potential hub for future invasion elsewhere globally.

Understanding invasion pathways of insect pests is important in order to control spread as well as to prevent future escapes from the native range (Beggs et al., 2011; Masciocchi and Corley, 2013; Eloff et al., 2020). This study demonstrates how detailed knowledge of spread pathways can be obtained using neutral molecular markers and surveillance data (Eloff et al., 2020). Our results provide an additional example of multiple independent introductions followed by spread within the adventive range that would be difficult to predict without the use of molecular tools and associated analytical approaches. Further elucidation of these pathways is crucial to prevent additional introductions in future (Eloff et al., 2020). While genetic diversity of *G. brimblecombei* and other pests of forests and forestry is moderately reduced relative to the native range, this does not appear to impact population fitness across a range of environments nor the potential for future spread.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: NCBI (accessions: OK335882–OK335986).

AUTHOR CONTRIBUTIONS

GD-S, JG, DA-C, RA, BH, SL, and BS designed the research. GD-S and DA-C performed the research. GD-S, JG, DA-C, and BS analyzed the data. JG, RA, BH, SL, and BS provided material and contributed to interpretation of the results. All authors contributed to the writing of the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/ffgc.2021. 783603/full#supplementary-material

Supplementary Figure 1 | The eight scenarios tested to determine the most likely route of introduction of *Glycaspis brimblecombei*. The "Islands" refers to individuals from Mauritius and La Réunion and "South America" refers to individuals from Brazil and Chile.

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