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INVASION NOTE

Population structure of the invasive ambrosia beetle, *Euwallacea fornicatus*, indicates multiple introductions into South Africa

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Abstract The ambrosia beetle *Euwallacea fornicatus* (Polyphagous Shot Hole Borer; PSHB), native to Asia, was documented in South Africa for the first time in 2012. Death of susceptible host trees is caused by blocking of xylem tissues by the mutualistic plant-pathogenic fungus, *Fusarium euwallaceae* and extensive tunnelling by the beetles into the sapwood. Within a few years, PSHB has spread from its putative entrance point in the coastal province of KwaZulu-Natal to nearly every other province in South Africa. This study serves as a preliminary assessment of dispersal pathways and population genetic relationships of PSHB in South Africa. PSHB individuals were collected from five provinces across South Africa. In addition, data on PSHB from three provinces in its native range in China and invasive PSHB from California were also generated here and supplemented by sequence data of PSHB available from GenBank. Comparisons of Cytochrome Oxidase Subunit I (COI) sequences of PSHB in South Africa revealed a nearly homogenous population. The majority of individuals have the same haplotype as is present in California, Israel and Vietnam (H33). A second haplotype was present in only two localities in KwaZulu-Natal and the Western Cape. This haplotype is also present in Vietnam and China (H38). The placement of the two haplotypes identified within South Africa, into different haplogroups suggests more than one invasion event. This pilot project justifies the use of more comprehensive genomic tools to finely map the relationships, global invasion pathways and within-country dispersal patterns of PSHB to better inform management of this invasive species.

Keywords Invasive species · Ambrosia beetle · Cytochrome oxidase · *Euwallacea fornicatus* · South Africa · Polyphagous shot hole borer

Introduction

Euwallaceae fornicatus (*Coleoptera: Curculionidae: Scolytinae, Xyleborini*; Polyphagous Shot Hole Borer; PSHB) has become an established pest in Israel (Mendel et al. 2012), Southern California (Eskalen et al. 2012), South Africa (Paap et al. 2018), Hawaii (Rugman-Jones et al. 2020) and Australia (CABI2020; Government of Western Australia 2022) and Europe (Schuler et al. 2021, in press). These beetles cause damage and ultimately, death of

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highly susceptible trees by cultivating a symbiotic ambrosia fungus (*Fusarium euwallaceae*) that causes Fusarium dieback disease in combination with beetle mass attack (Eskalen et al. 2013; Paap et al. 2018). PSHB poses a significant threat to agricultural, urban and natural forests (Eskalen et al. 2012) and has been recorded on more than 247 hosts according to studies in California (Lynch et al. 2021) and 130 in South Africa (van Rooyen et al. 2021). In urban areas (e.g., Johannesburg, South Africa), conservative estimates of tree losses are estimated at 25% (de Wit et al. 2021) as the majority of the urban forests (> 50%) consist of *E. fornicatus* reproductive hosts (de Wit et al. 2021). The importance of healthy urban environments is highlighted by the fact that by 2050 Africa will have 1.26 billion people living in cities, representing the fastest rate of urbanisation of any continent. These beetles are now considered high-risk quarantine pests of international concern (CABI 2015).

Stouthamer et al. (2017) identified 21 haplotypes of *E. fornicatus* based on Cytochrome Oxidase C Subunit I (COI) gene sequence data. The dominant invasive haplotype found in California, Israel and South Africa (Stouthamer et al. 2017) was designated as Haplotype 33 (H33) and has a putative origin in Vietnam. The range of localities where H33 has invaded (Israel, California, South Africa) could allude to a bridgehead effect as the mechanism of spread for this haplotype (Lantschner et al. 2020). California also harbours a second haplotype (H35) which may point toward multiple introductions into this region (Stouthamer et al. 2017). Surveys of genetic diversity of *E. fornicatus* at a global scale are still scant and the recent confirmation of *E. fornicatus* in Hawaii (Rugman-Jones et al. 2020) and Australia (CABI 2020; Government of Western Australia 2022) highlights the constantly growing global spread and shifting genetic landscape of this invasive beetle. In South Africa, *E. fornicatus* has been present since at least 2012 (Stouthamer et al. 2017) and became a more prominent pest, identified in KwaZulu Natal as of 2017 (Paap et al. 2018). It has now spread to every province except Limpopo, representing the largest geographical outbreak of this invasive pest, but a population genetic survey of the growing invasion has yet to be conducted. In this study, we provide an urgently needed, rapid assessment of the genetic structure of *E. fornicatus* in South Africa based on mitochondrial Cytochrome Oxidase C Subunit I (COI) gene data to understand its genetic diversity and pathways of dispersal.

Materials and methods

Euwallacea fornicatus sampling

Individuals were collected between June 2020 and April 2021 from five provinces in South Africa where *E. fornicatus* has been observed (Van Rooyen et al. 2021) (Table 1). Sub-locations within provinces were defined as sampling sites separated by some geographic barrier, e.g., a large break in green space separated by major highways. This amounted to the sampling of five provinces and 25 sub-locations. Individuals were collected from 23 different tree species, both indigenous and alien.

To collect fresh individuals, a method by Paap et al. (2018) was followed wherein an infested branch section was removed from the tree and placed in sealed containers (10L buckets) for transport to the laboratory. The branch was then split to verify gallery formation, and the presence of adult beetles, eggs, larvae and pupae. Beetles were collected from exposed galleries with a needle or paintbrush and placed individually into 1.5 mL Eppendorf tubes with absolute Ethanol. Tubes were labelled, sealed with parafilm and stored at − 20 °C until use.

For each host tree, GPS coordinates and tree species identity were recorded, and photographs of infestation sites or entry holes were taken. For each individual beetle, GPS coordinates and host tree species were recorded. Each beetle was digitally imaged using a Leica MZ16A microscope. Representative individuals and photographic images of beetles are stored at Stellenbosch University, Conservation Ecology & Entomology.

DNA extraction, polymerase chain reaction (PCR) and sequence data

Beetle heads and thorax were dissected from the abdomen to be used for DNA extraction. Extractions were carried out using the Qiagen DNEasy Blood & Tissue kit with adapted protocol [Purification of total DNA from insects (DY14 Aug-06; p 2–3; Qiagen (cat. no. 69504 or 69506)]. The head and thorax were ground in liquid Nitrogen using a plastic pestle in

Table 1 Locations and host trees sampled in South Africa

Province	Location	GPS coordinates (in decimal degrees (S;E))	Tree species	Number of individuals from various galleries in a single infested branch	Haplotypes	GenBank accession
Free State	Bloemfontein Golf Course	− 29.116945; 26.257500	*Quercus robur*	3	H33	
Free State	Bloemfontein Golf Course	− 29.117037; 26.257553	*Fraxinus* sp.	1	H33	
Free State	Bloemfontein Golf Course	− 29.117027; 26.257408	*Unknown*	3	H33	
Free State	Bloemfontein Golf Course	− 29.117041; 26.257509	*Ulmus parvifolia*	1	H33	
Free State	Bloemfontein Golf Course	− 29.117031; 26.257458	*Platanus* sp.	1	H33	
Free State	Bloemfontein, Langenhoven Park	− 29.100525; 26.157888	*Acer negundo*	1	H33	
Free State	Fitchard Park Suburbs, Bloemfontein	− 29.137278; 26.185417	*Acer pseudoplatanus*	1	H33	
Free State	Fitchard Park Suburbs, Bloemfontein	− 29.146328; 26.185402	*Acer buergerianum*	3	H33	
Free State	Fitchard Park Suburbs, Bloemfontein	− 29.138988; 26.175032	*Quercus robur*	4	H33	
Gauteng	Houghton Estate	− 26.164143; 28.058382	*Acer buergerianum*	5	H33	
Gauteng	Houghton Estate	− 26.165053; 28.060573	*Acer negundo*	3	H33	
Gauteng	Houghton Estate	− 26.163333; 28.064167	*Koelreuteria paniculata*	2	H33	
Gauteng	Houghton Estate	− 26.163618; 28.064328	*Platanus* sp.	2	H33	
Gauteng	Houghton Golf Club	− 26.165718; 28.067459	*Combretum erythrophyllum*	1	H33	
Gauteng	Johannesburg, Melrose	− 26.144144, 28.048351	*Robinia pseudoacacia*	4	H33	
Gauteng	Kempton Park CoffeeShop	− 26.100526; 28.225426	*Acer negundo*	3	H33	
Gauteng	Kempton Park, Dries Niemandt Park	− 26.104660; 28.217413	*Quercus robur*	2	H33	
Gauteng	Kempton Park, Dries Niemandt Park	− 26.104858; 28.217199	*Platanus* sp.	3	H33	
Gauteng	Kenneth Stainbank Nature Reserve	− 29.916389; 30.940001	*Acalypha glabrata*	1	H33	
Gauteng	Krugersdorp Gardenworld	− 26.042803; 27.885929	*Platanus* sp.	5	H33	

Table 1 (continued)

Province	Location	GPS coordinates (in decimal degrees (S;E))	Tree species	Number of individuals from various galleries in a single infested branch	Haplotypes	GenBank accession
Gauteng	Pretoria Rietondale	− 25.732548; 28.220006	*Acer negundo*	6	H33	
Gauteng	Pretoria Zoo	− 25.736082; 28.190388	*Acer negundo*	1	H33	
Gauteng	Pretoria Zoo	− 25.736082; 28.190388	*Quercus agrifolia*	1	H33	
Gauteng	Sandton	− 26.111646; 28.065979	*Quercus robur*	3	H33	
Gauteng	Walter Sisulu National Botanical Garden	− 26.086741, 27.844260	*Heteromorpha arborescens*	1	H33	
Kwa-Zulu Natal	Durban Botanical Garden	− 29.847288; 31.007526	*Afzelia quanzensis*	38	H33	OK598063
Kwa-Zulu Natal	Durban Botanical Garden	− 29.847288; 31.007526	*Afzelia quanzensis*	5	H38	OK598062
Kwa-Zulu Natal	Simbithi Eco Estate	− 29.508165; 31.216709	*Erythrina lysistemon*	2	H38	
Kwa-Zulu Natal	Simbithi Eco Estate	− 29.521257; 31.221115	*Indigofera jucunda*	21	H33	
Kwa-Zulu Natal	Simbithi Eco Estate	− 29.521284; 31.22118	*Albizia adianthifolia*	11	H33	
Northern Cape	Jan Kemp Dorp, Pecan Farms	− 27,920,200; 24.831107	*Carya illinoensis*	1	H33	
Western Cape	George Botanical Gardens	− 33.954098; 22.455410	*Psoralea* sp.	7	H33	
Western Cape	George Botanical Gardens	− 33.954098; 22.455410	*Psoralea* sp.	1	H38	
Western Cape	George Golf Course	− 33.955097; 22.441805	*Widdringtonia nodiflora*	1	H33	
Western Cape	George Golf Course	− 33.956802; 22.440058	*Fraxinus* sp.	1	H33	
Western Cape	Sedgefield Market Gas Station	− 34.009842; 22.778262	*Acer negundo*	6	H33	
Western Cape	Sedgefield Market Gas Station	− 34.009842; 22.778262	*Acer negundo*	4	H38	
Western Cape	Somerset West Buitengracht St	− 34.071430; 18.856550	*Acer negundo*	9	H33	
Western Cape	Somerset West Harewood Ave	− 34.051556; 18.836365	*Populus* sp.	15	H33	
Western Cape	Somerset West Lourensford Montessori school	− 34.063477; 18.885941	*Acer negundo*	2	H33	
Western Cape	Somerset West Lourensford Montessori school	− 34.063477; 18.885941	*Erythrina lysistemon*	1	H33	

Table 1 (continued)

Province	Location	GPS coordinates (in decimal degrees (S;E))	Tree species	Number of individuals from various galleries in a single infested branch	Haplotypes	GenBank accession
Western Cape	Somerset West: Vergelegen	− 34.077373; 18.891624	*Pinus* sp.	1	H33	
Western Cape	Somerset West: Vergelegen	− 34.077373; 18.891624	*Acer negundo*	3	H33	

1.5 mL Eppendorf tubes. Ground samples were lysed at 65 °C for 2 h and eluted into 50 µL of Buffer AE. DNA concentration in samples were quantified using a Nanodrop (ThermoFisher).

PCR amplification of the 711 bp Cytochrome Oxidase C Subunit I partial CDS was carried out using universal primers LCO1490 and HCO2198 (Leray et al. 2013). DNA was diluted 1:20 for quantities measured between 0.7 and 135 ng/µL, and 5 µL of the diluted DNA was used for PCR. The PCR reaction volume contained 12.5 µL AccuStart II PCR Master Mix (QuantBio), 0.75 µL Forward Primer (10 µM); 0.75 µL Reverse Primer (10 µM), and 6 µL water to a total reaction volume of 25 µL. PCR reaction conditions followed: 3 min 94 °C; 40 cycles of 30 s 94 °C, 30 s 50 °C, 1 min 72 °C. Agarose gel electrophoresis (1.5% agarose and SmartGlow Loading dye (Accuris Reagents)) was used to verify the amplification of single fragments. Samples showing single amplification were submitted for Sanger sequencing in both directions to the Central Analytical Facility at Stellenbosch University. Forward and reverse sequences were aligned using MAFFT (https://mafft.cbrc.jp/alignment/server/) to obtain consensus sequences. Anomalies in the alignments were verified for sequencing errors using TEAL Trace Analysis Viewer (https://www.gear-genomics.com/teal/). Consensus sequences were aligned to the *E. fornicatus* sequence from Paap et al. (2018) (GenBank accession MG642816) to confirm sample identities as that of *E. fornicatus*. This sequence was selected based on the morphological and genetic characterization of the specimen in addition to its confirmed association with the *F. euwallacea* fungal symbiont. Stouthamer et al. (2017) was the first to delineate the cryptic species complex of *Euwallacea* into four distinct clades and seperate haplotypes. The sequence generated by Paap et al. (2018) corresponds to haplotype 33 (Stouthamer et al. 2017) and is described as *E. fornicatus*. Though an older sequence from South Africa has been available since 2012, it was only classified as *E. fornicatus* in the study by Stouthamer et al. (2017) where it also corresponded to haplotype 33.

In addition to individuals collected and sequenced from South Africa, individuals from three provinces in China (Guizhou, Guiyang; Fujian, Fuzhou and Hainan, Haikou) and an individual from the USA (Los Angeles County, California) was sequenced and sequences submitted to GenBank (Table 2). Additional *E. fornicatus* Cytochrome C Oxidase Subunit 1 (COI) gene (partial cds; mitochondrial) sequences were obtained from NCBI GenBank (https://www.ncbi.nlm.nih.gov/) based on analyses performed by O'Donnell et al. (2015), Stouthamer et al. (2017), Gomez et al. (2018), Paap et al. (2018) and Rugman-Jones et al. (2020). Table 2 lists these sequences, their accession numbers and localities along with GPS coordinates. A total of 40 additional sequences were added including accession MG642816, the sequence from the *E. fornicatus* individual originating from the first report in South Africa (Paap et al. 2018) and corresponding to haplotype H33 (KU727021) as described by Stouthamer et al. (2017).

Statistical analyses

All sequences were aligned using MAFFT (multiple alignment using fast Fourier transform) version 7.471 (http://mafft.cbrc.jp/alignment/server/) and trimmed to an equal length of 458 bp. Sequences were collapsed into haplotypes using R (version 3.6.3) packages *ape* and *pegas* (Paradis and Schliep 2019). Sample completeness was estimated using R *iNEXT* (Hsieh et al. 2016) to plot extrapolated sample

Table 2 Sequences generated from non-South African samples and sequences obtained from GenBank

Country	Region	Assigned GPS coordinates (in decimal degrees, S and W are negative)	Haplotypes	GenBank Accession
China	Guizhou	23.196233; 113.286929	H44	MH276936
China		28.726854; 111.163393	H25	KU727013
China		28.726854; 111.163393	H29	KU727017
China	Guizhou, Guiyang	23.196233; 113.286929	H44	OK598064
China	Fujian, Fuzhou	25.856025; 119.402126	H54	OK598066
China	Hainan, Haikou	19.979365; 110.22285	H51	OK595067
China	Hainan, Haikou	19.979365; 110.22285	H52	OK598068
China	Guizhou	23.196233; 113.286929	H44	MH276937
China	Hong Kong	22.348853; 114.166830	H38	MH276941
China	Hong Kong	22.348853; 114.166830	H38	MN266860
Hawaii		19.599681; − 155.412709	H43	KX818247
Israel		13.066336; 108.42431	H33	KM406722
Japan	Okinawa	26.756046; 128.248521	H39	KU727027
Japan	Okinawa	26.756046; 128.248521	H39	MH276939
South Africa		− 29.847288; 31.007526	H33	MG642816
Taiwan		23.723134; 121.105754	H22	KU727010
Taiwan		23.723134; 121.105754	H30	KU727018
Taiwan		23.723134; 121.105754	H36	KU727024
Taiwan		23.723134; 121.105754	H37	KU727025
Taiwan		23.723134; 121.105754	H40	KU727028
Taiwan		23.723134; 121.105754	H41	KU727029
Taiwan		23.723134; 121.105754	H42	KU727030
Thailand	Chiang Mai	18.712459; 98.877048	H28	KU727016
Thailand	Phetchabun	16.255357; 101.098660	H28	MH276938
Thailand	Chiang Mai	18.712459; 98.877048	H46	MH276943
USA	Los Angeles County; California	34.087289; − 118.528494	H35	KM406726
USA	Los Angeles County; California	34.087289; − 118.528494	H33	JX912724
USA	Los Angeles County; California	34.087289; − 118.528494	H35	OK598065
USA	Los Angeles County; California	34.087289; − 118.528494	H35	KM406727
USA	Los Angeles County; California	34.087289; − 118.528494	H35	KU727023
USA	Los Angeles County; California	34.087289; − 118.528494	H35	MH276942
Vietnam		13.066336; 108.42431	H23	KU727011
Vietnam	Gai Lai	10.967924; 106.679761	H26	KU727014
Vietnam		13.066336; 108.42431	H27	KU727015
Vietnam		13.066336; 108.42431	H32	KU727020
Vietnam	Yen Bai	21.68373; 104.847182	H38	KU727026
Vietnam	Phu Yen	13.149835; 109.221556	H24	KU727012
Vietnam	Yen Bai	21.68373; 104.847182	H31	KU727019
Vietnam		13.066336; 108.42431	H33	KU727021
Vietnam		13.066336; 108.42431	H34	KU727022
Vietnam	Hoan Kiem	21.032153; 105.857712	H45	MH276940
Vietnam	Cao Bang	22.643500; 106.244756	H47	MN266858
Vietnam	Thua Thien-Hue	16.379175; 107.587390	H48	MN266859
Vietnam	Ninh Binh	20.241406; 105.957448	H49	MN266861

coverage within individuals from native and invasive regions based on the number of haplotypes sampled per country. Native regions comprised Vietnam, China, Thailand, Taiwan, and Japan while invasive regions comprised South Africa, California, Israel and Hawaii.

Maximum likelihood (ML) analyses were conducted to determine genealogical relationships among sequences using R packages *ape* and *phangorn* (Paradis and Schliep 2019) based on a distance matrix of sequence alignment. A range of substitution models were tested using the function *modelTest* and the optimum model was selected for constructing the final tree. Non-parametric bootstrap analysis was conducted with 100 bootstrap samples. The tree was unrooted as only *E. fornicatus* was used in the analysis and a Neighbor Joining tree was constructed.

Sequence divergence between individuals was quantified as Kimura two-parameter distances (K2P) using R package *ape* and the *dist.dna* function. Percentage sequence divergence was calculated as the percentage of point mutations within the 458 bp COI sequences. Tajima's *D* uses information on segregating sites (mutation frequency) to detect deviation from neutrality due to population bottleneck or expansion, directional selection or introgression (Tajima 1989) and was calculated using R packages *ape* and *pegas*.

Results

A total of 194 *E. fornicatus* individuals were collected from five provinces and 23 tree species in South Africa. Most of these individuals were collected in KwaZulu-Natal (Table 1), where the beetle was first detected in the country (Stouthamer et al. 2017; Paap et al. 2018). Tree species *Acer pseudoplatanus*, *Koelreuteria paniculata*, *Robinia pseudoacacia*, *Acalypha glabrata* and *Indigofera jucunda* are here reported as reproductive hosts for the first time in South Africa. This excludes new species where the beetles were collected on the external surface and gallery formation or reproduction could not be confirmed (*Widdringtonia nodiflora*, *Heteromorpha arborescens*, *Quercus agrifolia* and *Pinus* sp.).

A sample completeness curve (Fig. 1) depicting sample coverage per interpolated and extrapolated number of sampled individuals from native and invasive ranges showed sufficient sample coverage (> 0.9)

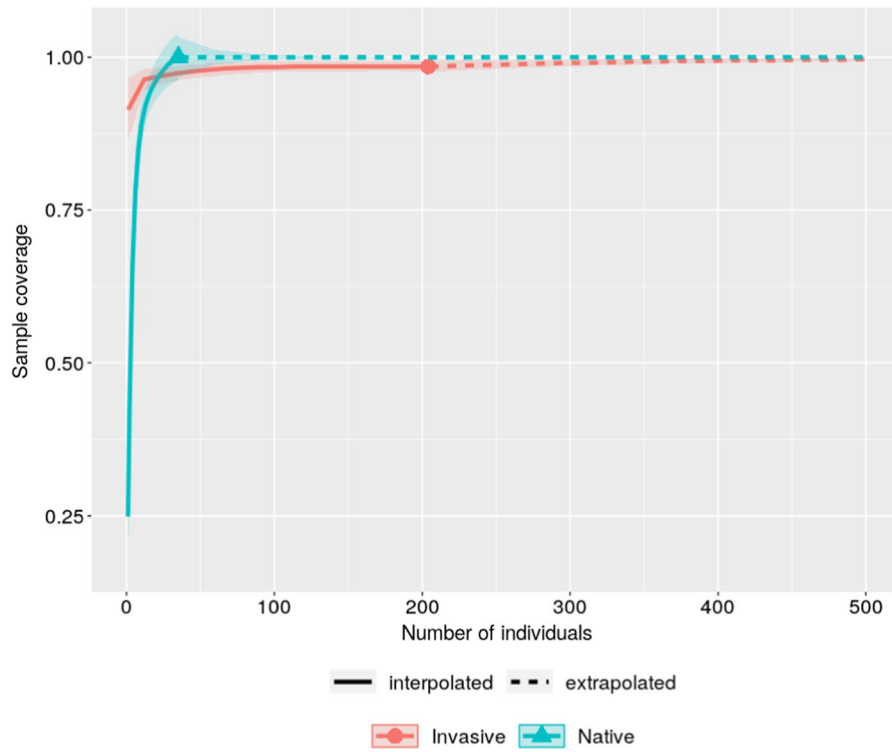

Fig. 1 Sample completeness curve depicting sample coverage per interpolated and extrapolated number of sampled individuals from native and invasive ranges

for invasive samples collected, mostly, from South Africa. Sample coverage for native samples from Asia could be improved (> 0.6) however it is not unusually low and was limited by the availability of sequences on public databases.

Sequence alignment of all individuals produced an alignment of 458 bp of the COI gene region. COI sequences of most individuals from South Africa matched the H33 haplotype as described by Stouthamer et al. (2017) for *Euwallacea fornicatus* and the sequence generated by Paap et al. (2018) (GenBank Accession: KU727021 and MG642816 respectively) with 100% sequence similarity. The exception was twelve individuals from four locations and four tree species in KwaZulu Natal and the Western Cape, South Africa (Table 1). These samples showed three different point mutations when compared to the sequence from Paap et al. (2018) (MG642816). The first two mutations substitute cytosine for thymine and the third substituting thymine for cytosine.

Haplotype analyses of the dataset produced 30 distinct haplotypes (Tables 1, 2). Samples from South Africa were divided into two distinct haplogroups (Fig. 2). Haplotype 33 was assigned to South African individuals that matched the H33 haplotype sequence as generated by Stouthamer et al. (2017) and the sequence described from KwaZulu Natal by Paap et al. (2018) (GenBank Accession: KU727021 and MG642816 respectively).South African individuals that did not match the H33 haplotype were assigned to haplotype 38 (Stouthamer et al. 2017; GenBank Accession: KU727026) (Table 1). Haplotype 33 is also found in California, Israel and Vietnam, likely originating from the latter (Fig. 2). Haplotype 38 is also found in Vietnam, Yen Bai and China, Hong Kong (Fig. 2). In addition to haplotype H33, California also contained a unique, invasive haplotype (H35). Another unique, invasive haplotype was identified from Hawaii (H43). The greatest haplotype diversity was found in Vietnam (thirteen haplotypes), followed by China (eight haplotypes).

Phylogenetic relationships among individuals were examined using maximum likelihood (ML) analysis. The best substitution model selected was the GTR + G + I which represented the base frequencies of the dataset (AT rich) best and had the lowest Akaike information criterion (AIC) and Bayesian information criterion (BIC) values (2455.829 and 2711.695 respectively compared to 2734.582 and 2949.18 respectively for the Jukes and Cantor model (Jukes and Cantor 1969)) with a log likelihood of − 1165.914. The 30 haplotypes clustered into three main clades (Fig. 2). Haplotypes from South Africa clustered into two unique haplogroups within the largest clade.

Genetic differentiation

Sequence divergence between haplotypes was quantified as Kimura two-parameter distances (K2P) (Supplementary Table 1). K2P values of 0 indicate no sequence divergence between haplotypes while greater divergence is indicated by numbers closer to 1. South African individuals corresponding to haplotype 33 show low sequence divergence from South African samples corresponding to haplotype 38 (0,006608026). This agrees with the maximum likelihood analysis depicted in Fig. 2 though despite the low sequence divergence, these two haplotypes are placed in differing haplogroups (Fig. 2). Percentage sequence divergence (Supplementary Table 1) shows the number of mutations (sequence differences from MG642816) within the 458 bp COI sequences as a percentage. The highest percentage (7.74%), or greatest difference from MG642816, is seen for haplotype 51 followed by 52 and 22. This agrees with the ML analysis which places these sequences as a separate clade (Fig. 2). In the current study, Tajima's D was negative (− 0.76; p value normal 0.44). This would suggest recent directional selection or population growth within the *E. fornicatus* species after a recent bottleneck event (Avtzis et al. 2019).

Discussion

In this study we show that *E. fornicatus* is genetically diverse in its native regions, as expected, and that the South African invasive populations are genetically more homogenous. As with other studies we confirm that most invasive populations and haplotypes originate from Vietnam (Stouthamer et al. 2017). This included one of the two haplotypes from California (H33), two from South Africa (H33, H38) and one from Israel (H33). The newly identified haplotype in South African haplotype, H38, is also found in China, Hong Kong and Vietnam, Yen-Bai making its exact

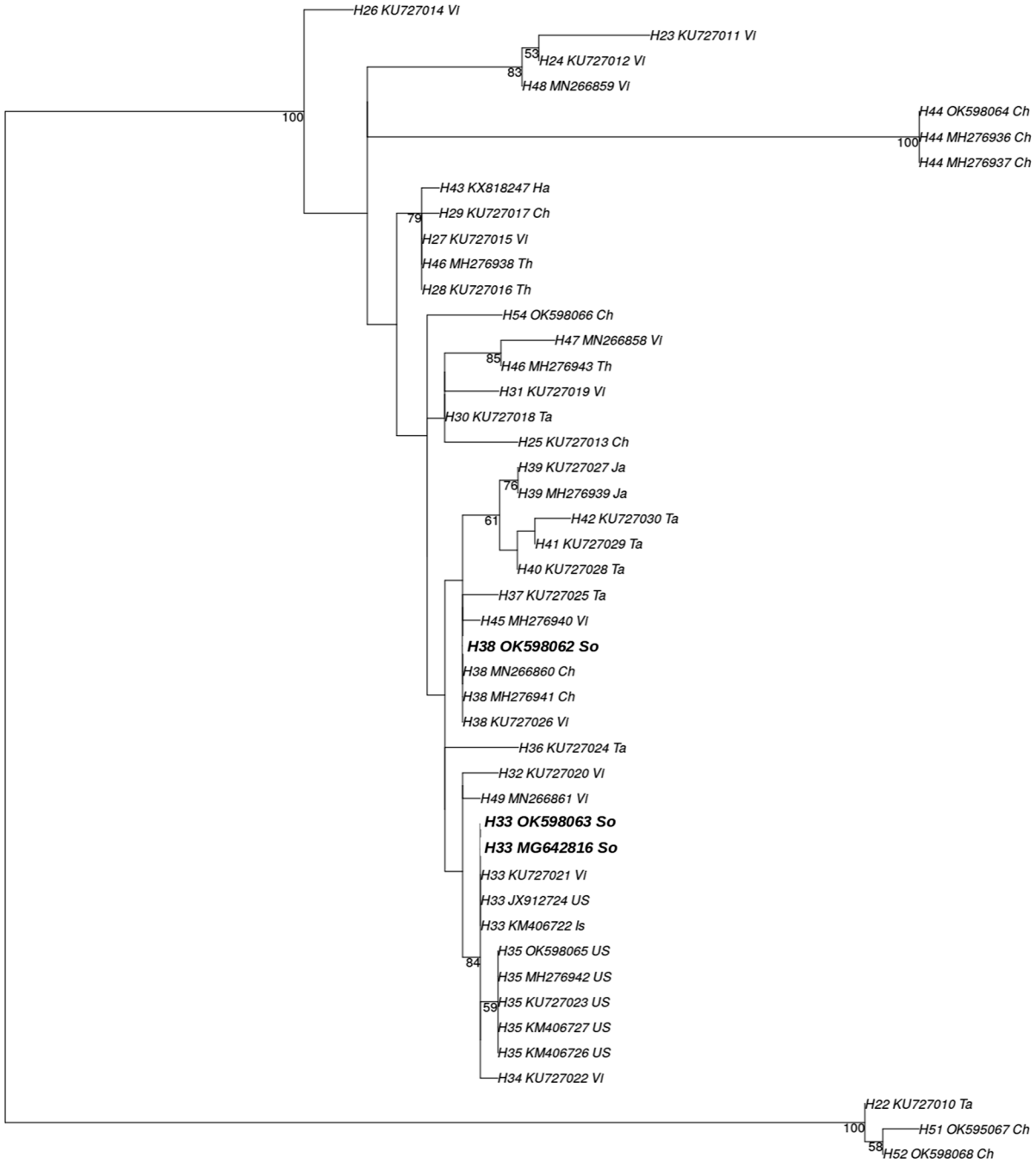

Fig. 2 Neighbor Joining, unrooted phylogenetic tree based on Maximum Likelihood analysis under the GTR + G + I substitution model. Nodes are named by Haplotype number (H); GenBank accession number; Country of origin for accession (abbreviations as follows: Vi: Vietnam; Ch: China; Ha: Hawaii; Th: Thailand; Ta: Taiwan; Ja: Japan; So: South Africa; US: California; Is: Israel)

origin, unclear. Two additional invasive haplotypes, one from California (H35) (O'Donnell et al. 2015; Stouthamer et al. 2017) and one from Hawaii (H43) (Rugman-Jones et al. 2020) are not represented in any

of the sequences available on GenBank and therefore have no clear origin as yet. However, sequence divergence is smallest between H35 and H33, which originates from Vietnam. And in the case of H43, sequence divergence is smallest between this haplotype and H27 from Vietnam and H28, H46 from Thailand.

The ease with which these invasive taxa can move around the globe and establish in non-native environments is likely due to their cryptic habits and ecologies, wide host ranges and specialised haplodiploid breeding systems (van Rooyen et al. 2021). The haplodiploid mating system in particular can promote establishment of new populations as these can be initiated from extremely low population numbers. It is therefore not surprising that all invasive populations of PSHB seem to have restricted genetic diversity. Despite this, they have spread over large areas and genetic variability does not seem to be a critical factor in their success (Peer and Taborsky 2005; Andersen et al. 2012).

In South Africa, PSHB has spread from KwaZulu-Natal to most major provinces in only a few years (Stouthamer et al. 2017; Paap et al. 2018). The exact date of entry into South Africa and the number of invasion events cannot be confirmed, however first accounts date back to 2012 (Stouthamer et al. 2017). The invasion is therefore relatively recent and the low genetic variability that was observed within the country is expected under the assumption that the invasion originated from a small initial population(s).

Here we identified the two PSHB haplotypes present in South Africa, for the first time. Haplotype 33 is much more common and widespread than haplotype 38 and the latter may represent a second, more recent colonization event. Alternatively, the more widespread haplotype (H33) may be more successful as an invader if both haplotypes had been introduced simultaneously. Haplotype 33 is also observed in California and Israel, supporting its claim as a very successful invader in comparison to haplotypes 35, 38 and 43 that have all only been found in single locations (California, South Africa and Hawaii respectively).

The presence of haplotype 33 across three continents could be attributed to invasive bridgehead effects. *Euwallacea fornicatus* was detected in California for the first time in 2003 with initial damage in Long Beach observed in 2010. However, it did not become a serious concern until 2012 (Eskalen et al. 2012). In India, severe damage to avocado caused by *E. fornicatus* has been known since 2005 (Mendel 2012). Research of native specimens of *E. fornicatus* haplotype 33 is needed to determine whether this invasive success can be attributed to the environment it occupies in Vietnam (e.g. surrounding an international port) or whether the haplotype holds some genetic superiority.

Stouthamer et al. (2017), divided the *E. fornicatus* clade in two. In their study, one branch is depicted by a single haplogroup (H22) diverging with a K2P value of 0.079 and a p-distance (pairwise distances per site) of 0.074 from the other samples forming the second clade. This is reflected in the current study where haplogroup 22 from Taiwan together with haplogroups 51 and 52 (originating from China, Hainan, Haikou and not represented in the Stouthamer et al. 2017 study) diverge from most of the samples with a K2P value of ± 0.08 and a percentage sequence divergence of 7.64% (Fig. 2; Supplementary Table 1). In this study, H26 appears as a separate clade under ML analysis (Fig. 2), diverging from the remaining haplotypes with a K2P value of ± 0.02 and a percentage sequence divergence of 1.97% (Supplementary Table 1). This is similar to the within-clade values Stouthamer et al. (2017) reported for *E. fornicatus* and is therefore likely not a separate clade. Haplotype 44 also shows strong divergence from the remaining haplogroups (Fig. 2) with a K2P value of ± 0.08 and percentage sequence divergence of 4.8% (Supplementary Table 1). The K2P value for haplotype 44 is like that of the two separate *E. fornicatus* clades as presented by Stouthamer et al. (2017) and it therefore more likely that haplotype 44, could be considered a separate clade. An argument could be made that these separate clades represent cryptic species as mentioned by Stouthamer et al. (2017). However, based only on COI sequence data such conclusions cannot be drawn as in the *Xyleborini*, high intraspecific divergence has been reported in the COI gene (Dole et al. 2010; Andersen et al. 2012; Jordal & Kambestad, 2014) though nuclear divergence at the 28S Ribosomal gene is low. Gauthier (2010), similarly, uses both mitochondrial (COI gene) and nuclear (microsatellite) markers to show the delineation of a species complex. Whole-genome applications such as genome-wide Single Nucleotide Polymorphism (SNP) markers

would provide the clearest insight as to the state of cryptic species (Marchán et al. 2020).

Tajima's *D* detects selective neutrality of the observed nucleotide polymorphisms (Tajima 1989). In the current study, Tajima's D was calculated at − 0.76 (*p* value normal 0.44). This would imply that the population is not selectively neutral (D ≈ 0) and the negative value suggests recent directional selection or population growth after a recent bottleneck with an abundance of rare alleles within *E. fornicatus*. Even though, the p value in this instance is not significant ($p > 0.02$) and this parameter would be better investigated in genome-wide SNP studies where genetic signatures of selection can be investigated, this result does provide impetus for such studies in *E. fornicatus.*

In conclusion, this study provides evidence for novel haplotypes of *E. fornicatus* in South Africa and internationally. The most abundant *E. fornicatus* haplotype present in South Africa, originated from Vietnam. This haplotype (H33) is also invasive in California and Israel. However, the native populations in Vietnam are extremely genetically diverse and severely under sampled. Inferences on population of origin should therefore be interpreted with caution. The origin of the newly reported haplotype in South Africa, is less clear as this haplotype (H38) is found in Vietnam and China. In South Africa is it only found in two coastal provinces, KwaZulu-Natal and the Western Cape and it can be assumed that this represents a second introduction, likely after 2012 when the first specimen, corresponding to haplotype 33 (Stouthamer et al. 2017), was collected as part of the Barcode of Life Project (BOLD: ETKC270-13). This raises concern for further introductions of more haplotypes of *E. fornicatus* or other *Scolytinae* beetles into South Africa with unknown potential for agricultural, urban and environmental damage. For this reason, increased efforts to monitor points of entry into the country for living *Scolytinae* is urgently needed.

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Data availability The datasets generated during and/or analysed during the current study are available in the GenBank repository, https://www.ncbi.nlm.nih.gov/ under accession numbers: OK598062 – 598070. The datasets and images generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors have not disclosed any competing interests.

References

Andersen HF, Jordal BH, Kambestad M, Kirkendall LR (2012) Improbable but true: the invasive inbreeding ambrosia beetle *Xylosandrus morigerus* has generalist genotypes. Ecol Evol 2:247–257. https://doi.org/10.1007/s10340-021-01443-7

Avtzis DN, Lakatos F, Gallego D et al (2019) Shallow genetic structure among the European populations of the six-toothed bark beetle *Ips sexdentatus* (Coleoptera, Curculionidae, Scolytinae). Forests 10:1–10. https://doi.org/10.3390/f10020136

CABI (2015) *Euwallacea fornicatus* (tea shot-hole borer). In: Invasive species Compend. http://www.cabi.org/cpc/search/?q=Euwallacea+fornicates

CABI (2020) *Euwallacea fornicatus*. In: Invasive species Compend. https://www.cabi.org/isc/datasheet/57163#REF-DDB-165911

De Wit MP, Crookes DJ, Blignaut JN et al (2021) Invasion of the polyphagous shot hole borer beetle in South Africa: A preliminary assessment of the economic impacts [document on the Internet]. c2021 [cited 2021 Sept]. https://doi.org/10.21203/rs.3.rs-220132/v1

Dole SA, Jordal BH, Cognato AI (2010) Polyphyly of *Xylosandrus reitter* inferred from nuclear and mitochondrial genes (Coleoptera: Curculionidae: Scolytinae). Mol Phylogenet Evol 54:773–782. https://doi.org/10.1016/j.ympev.2009.11.011

Eskalen A, Gonzalez A, Wang D et al (2012) First report of a *Fusarium* sp. and its vector tea shot hole borer (*Euwallacea fornicatus*) causing Fusarium dieback on avocado in California. Plant Dis 96:1070

Eskalen A, Stouthamer R, Lynch S et al (2013) Host range of Fusarium dieback and its ambrosia beetle (Coleoptera: Scolytinae) vector in southern California. Plant Dis 97:938–951. https://doi.org/10.1094/PDIS-11-12-1026-RE

Gauthier N (2010) Multiple cryptic genetic units in Hypothenemus hampei (Coleoptera: Scolytinae): evidence from microsatellite and mitochondrial DNA sequence data. Biol J Linn Soc 101:113–129. https://doi.org/10.1111/j.1095-8312.2010.01483.x

Gomez DF, Skelton J, Steininger MS et al (2018) Species delineation within the *Euwallacea fornicatus* (Coleoptera: Curculionidae) complex revealed by morphometric and phylogenetic analyses. Insect Syst Divers. https://doi.org/10.1093/isd/ixy018

Government of Western Australia (2022) Polyphagous shot-hole borer. https://www.agric.wa.gov.au/borer. Accessed 2 Mar 2022

Hsieh T, Ma K, Chao A (2016) iNEXT: an R package for rarefaction and extrapolation of species diversity (H ill numbers). Methods Ecol Evol 7:1451–1456. https://doi.org/10.1111/2041-210X.12613

Jordal BH, Kambestad M (2014) DNA barcoding of bark and ambrosia beetles reveals excessive NUMTs and consistent east–west divergence across Palearctic forests. Mol Ecol Resour 14:7–17. https://doi.org/10.1111/1755-0998.12150

Jukes T, Cantor C (1969) Evolution of protein molecules. In: Mammalian protein metabolism, pp 21–132

Lantschner MV, Corley JC, Liebhold AM (2020) Drivers of global Scolytinae invasion patterns. Ecol Appl 30:1–12. https://doi.org/10.1002/eap.2103

Leray M, Yang JY, Meyer CP et al (2013) A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: application for characterizing coral reef fish gut contents. Front Zool. https://doi.org/10.1186/1742-9994-10-34

Lynch SC, Eskalen A, Gilbert GS (2021) Host evolutionary relationships explain tree mortality caused by a generalist pest-pathogen complex. Evol Appl 14:1083–1094. https://doi.org/10.1111/eva.13182

Marchán DF, Fernández R, Domínguez J et al (2020) Genome-informed integrative taxonomic description of three cryptic species in the earthworm genus Carpetania (Oligochaeta, Hormogastridae). Syst Biodivers 18:203–215. https://doi.org/10.1080/14772000.2020.1730474

Mendel Z, Protasov A, Sharon M et al (2012) An Asian ambrosia beetle *Euwallacea fornicatus* and its novel symbiotic fungus *Fusarium* sp. pose a serious threat to the Israeli avocado industry. Phytoparasitica 40:235–238. https://doi.org/10.1007/s12600-012-0223-7

O'Donnell K, Sink S, Libeskind-Hadas R et al (2015) Discordant phylogenies suggest repeated host shifts in the *Fusarium–Euwallacea* ambrosia beetle mutualism. Fungal Genet Biol 82:277–290. https://doi.org/10.1016/j.fgb.2014.10.014

Paap T, de Beer Z, Migliorini D et al (2018) The polyphagous shot hole borer (PSHB) and its fungal symbiont *Fusarium euwallaceae*: a new invasion in South Africa. Australas Plant Pathol 47:231–237. https://doi.org/10.1007/s13313-018-0545-0

Paradis E, Schliep K (2019) ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics 35:526–528. https://doi.org/10.1093/bioinformatics/bty633

Peer K, Taborsky M (2005) Outbreeding depression, but no inbreeding depression in haplodiploid ambrosia beetles with regular sibling mating. Evolution (N Y) 59:317–323. https://doi.org/10.1111/j.0014-3820.2005.tb00992.x

Rugman-Jones PF, Au M, Ebrahimi V et al (2020) One becomes two: second species of the *Euwallacea fornicatus* (Coleoptera: Curculionidae: Scolytinae) species complex is established on two Hawaiian Islands. PeerJ. https://doi.org/10.7717/peerj.9987

Schuler H, Witkowski R, van de Vossenberg B et al (2021) Recent invasion and eradication of two members of the *Euwallacea fornicatus* species complex (Coleoptera: Curculionidae: Scolytinae) from tropical greenhouses in Europe. Research Square Preprint [cited Sept 2021]. https://doi.org/10.21203/rs.3.rs-640781/v2

Stouthamer R, Rugman-Jones P, Thu PQ et al (2017) Tracing the origin of a cryptic invader: phylogeography of the *Euwallacea fornicatus* (Coleoptera: Curculionidae: Scolytinae) species complex. Agric Entomol 19:366–375. https://doi.org/10.1111/afe.12215

Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585–595

Van Rooyen E, Paap T, de Beer ZW et al (2021) The Polyphagous shot hole borer (PSHB) beetle: current status of a perfect invader in South Africa. S Afr J Sci 117:1–10