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Research

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Genetic insights into the first detection of *Paracoccus marginatus* (Hemiptera: Pseudococcidae) in Australia

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Species spread in a new environment is often associated with founders' effect, and reduced effective population size and genetic diversity. However, reduced genetic diversity does not necessarily translate to low establishment and spread potential. *Paracoccus marginatus* Williams and Granara de Willink is a polyphagous pest that has invaded 4 continents in around 34 years. It was first detected in the Northern Territory, Australia in July 2023. Following this, we collected 45 specimens from 20 suburbs across 3 regions. Using mitochondrial cytochrome oxidase I and nuclear ribosomal RNA genes (18S and 28S), we examined molecular diagnostics robustness, genetic diversity, haplotype network, and demographics (Tajima D) of the recently detected Australian population. We compared our samples with publicly available sequences deposited on GenBank. All 3 genes were suitable for molecular diagnosis with a 100% identity score. For all 3 genes, we found low nucleotide diversity, haplotype diversity, and negative Tajima D in the newly detected *P. marginatus* population, suggesting a recent single founder event by a few individuals. Comparing our study's sequences with global sequences showed low haplotype diversity, nucleotide diversity, and Tajima's D, suggesting that despite low genetic diversity at the 3 genes, *P. marginatus* has successfully invaded South America, Africa, Asia, and Oceania. Our study highlights the role of demographic and life history traits in the species' invasion success. We provide a baseline dataset from the first detection of *P. marginatus* in Australia. Further analysis of the spread can provide insights into invasive species' adaptation in a novel environment.

Keywords: recent invasion, biosecurity, diagnostics, papaya mealybug, genetics

Introduction

Increasing rates of insect pest invasion pose significant threats to agriculture, forestry, and global biodiversity. For efficient and cost-effective management, it is more beneficial to prevent the introduction and control the spread of the invasive species during the early phases of invasion than attempting eradication once invasive species are established (Allendorf and Lundquist 2003, Simberloff et al. 2013). After arrival, the survival, establishment, and spread of a species are determined by its population biology, which includes life history traits, demography, ecological factors, propagule pressure (number of introductions and number of founding individuals), interspecific competition, and genetic effects (Sakai et al. 2001, Allendorf and Lundquist 2003). Colonization of introduced populations into a novel environment is often associated with a high genetic bottleneck due to a small number of founding individuals, leading to inbreeding depression, low allelic diversity, low effective population size, low population fitness, and a high impact of genetic drift (Allendorf and Lundquist 2003). Subsequent founder effects can further slow down population range expansion in a new area (Hagan et al. 2024). However, several studies have reported that the loss of genetic diversity might not be as prevalent as previously thought (Roman and Darling 2007). Invasive populations, despite experiencing founder effects and reduced genetic diversity, may still evolve rapidly in new environments, sometimes even outperforming native populations (Roman and Darling 2007, Dlugosch and Parker 2008). Understanding invasion routes, the number of introduction events, genetic diversity, and factors contributing to species invasion success can provide insights into invasion mechanisms and advance our knowledge of effective control and management measures.

Genetic diversity does not always predict invasiveness success (Allendorf and Lundquist 2003, Dlugosch and Parker 2008). For example, the pine wood nematode has spread to Europe and Asia despite low genetic polymorphism (Mallez et al. 2015). Similarly, the pest gall midge *Asynapta groverae* has established in new

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regions with low genetic diversity (Kang et al. 2023), and invasive populations of the wasp *Leptocybe invasa* have expanded globally with limited genetic differentiation and small introduced populations (Dittrich-Schröder et al. 2018). Invasive populations can utilize other mechanisms to survive and spread, such as asexual reproduction and maintenance of clonal invasive genotypes, ecological plasticity (Roman and Darling 2007), a greater number of introduced individuals and release events (strong propagule pressure) (Jaspers et al. 2021), long lag phases (Gaither et al. 2012), and advantageous life history traits. The availability of hosts and vectors, and the absence of predators, can further assist newly invaded populations in establishing themselves in novel environments. In addition, some species are intrinsically better competitors (Allendorf and Lundquist 2003). Therefore, the interaction of life history traits, genetic factors, and demographic factors in contributing to invasion success is still not well understood.

The papaya mealybug *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae) is an excellent example of a rapidly spreading polyphagous pest. The first outbreak of *P. marginatus* in Australia was recorded in Darwin, Northern Territory in July 2023. The initial detection was on a papaya plant (*Carica papaya*) but infestations were also found in the same area on frangipani (*Plumeria* sp.) and hibiscus (*Hibiscus* spp.) (Papaya mealybug | NT.GOV.AU). *Paracoccus marginatus* is native to the Americas and was originally described from a specimen from Mexico (Williams and Granara de Willink 1992). Since then, it has been recorded in 14 Caribbean countries (1994), the United States (1998), and the Pacific islands (2002 and 2003) (Krishnan et al. 2016). Between 2008 and 2016, *P. marginatus* spread to southern Asia and Indo-Pacific islands, West and East Africa, and Israel (Krishnan et al. 2016, Health et al. 2023). It has a wide host range, affecting over 200 plant genera, including tropical fruits, vegetables, and ornamental plants (Finch et al. 2021, Health et al. 2023). *Paracoccus marginatus* causes severe damage to the host plant including yellowing, crinkling, and curling of leaves, reduced plant growth, low yield, and sometimes destruction of the entire plant (Suganthy et al. 2012). *Paracoccus marginatus* is a small (2–3.5 mm) hemipteran (Fig. 1A) and reproduces sexually with a female usually laying 150–600 eggs. Females lack wings and crawl short distance during early instars, while adult males have a single pair of wings. Species dispersal can be assisted by wind, irrigation channels, and other organisms such as crows, bats, and ants. Long-distance transport can be achieved by human-assisted movement of infested plant material (Finch et al. 2021). It is most active in warm and dry weather, with an optimal temperature range of 25 ± 5 °C (Krishnan et al. 2016).

Mitochondrial and nuclear markers including cytochrome oxidase 1 (COI), 18s, and 28s are widely used to examine geographic

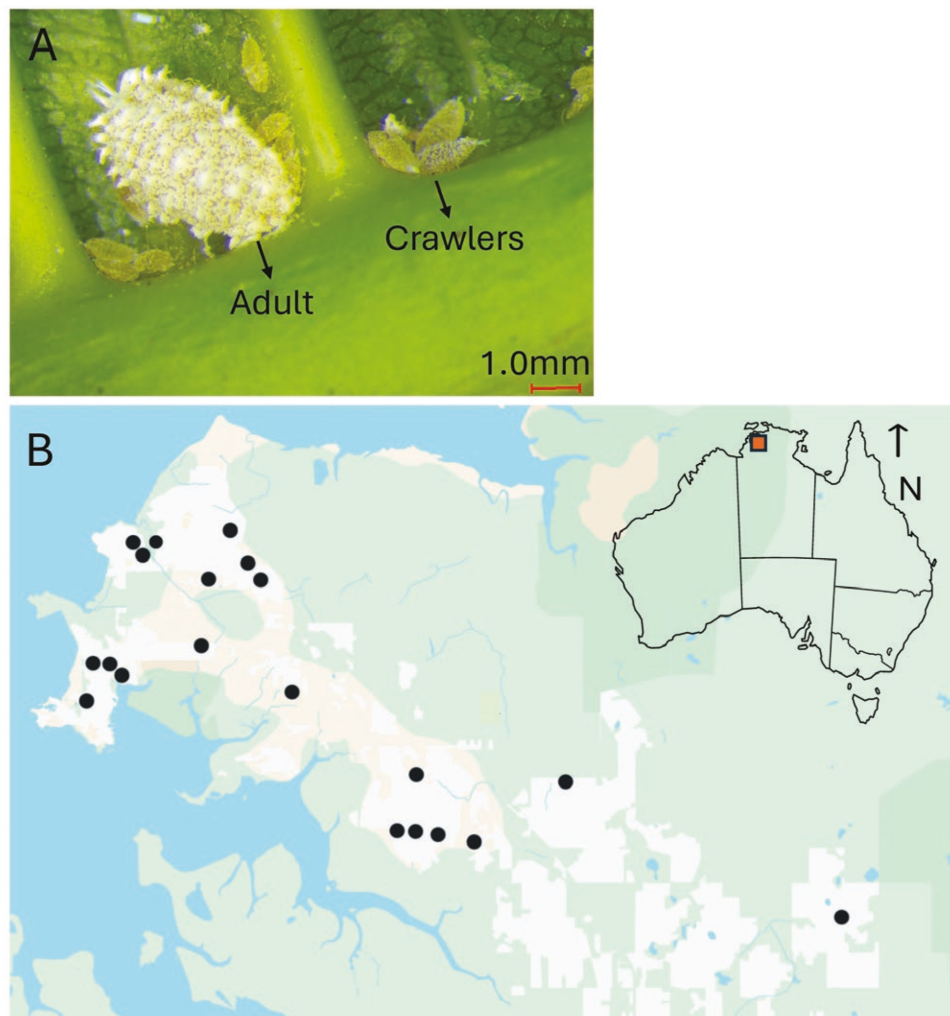

Fig. 1. A) Image of *Paracoccus marginatus* adult female and crawlers. B) A map showing the sampling locations in the Northern Territory, Australia.

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origin, haplotype diversity, and genetic heterogeneity in mealybugs (Malausa et al. 2011, Wu et al. 2015, Daane et al. 2018) and in *P. marginatus*. Genetic studies on *P. marginatus* have reported a single haplotype across Asia and Africa, including Cambodia, China, India, Indonesia, Malaysia, Thailand, and Mozambique (Ahmed et al. 2015), using the partial mitochondrial COI gene region. The species has a low 28S gene divergence in samples collected from 3 regions in Kenya, Africa (Heya et al. 2020), and a higher likelihood of spreading into new regions as predicted by climatic modeling (Heya et al. 2020).

In this study, we collected 45 adult individuals from 20 suburbs across the Darwin, Palmerston, and Litchfield regional areas in the Northern Territory between August 2023 and March 2024, following its first detection. Our sample collection spanned 5 seasons of the Gulumoerrgin seasonal calendar (Larrakia Indigenous people calendar): Gurrulwa guligi (season of "big wind"—August and September); Dalirrgang (build-up time—September and October); Balnba (rainy season—November and December); Dalay (monsoon season—January, February, and March); and Mayilema season (March and April). Using partial mitochondrial COI gene, and 2 nuclear ribosomal RNA genes—18S and 28S, we examined (i) the robustness of *P. marginatus* molecular diagnosis using 3 genes and (ii) the genetic diversity, haplotype diversity, and demographics (a) within the newly detected population of *P. marginatus* in Australia and (b) by comparing the newly invaded population in Australia with global populations. By examining genetic factors from the start of the invasion, our study provides insights into the introduction frequency, spread of the species into new areas, its adaptive and spread capacity.

Materials and Methods

Sample Collection

Samples were examined under the stereo microscope and if deemed necessary, specimens were slide-mounted for further morphological identification. Scale insects and mealybug morphological identification key (Mealybugs key | Scale Insects (idtools.org)) and redescription of *P. marginatus* Williams and Granara de Willink (Hemiptera: Coccoidea: Pseudococcidae) (Miller and Miller 2002) were used for morphological identification. Once morphologically determined to be *P. marginatus* the samples were stored in 90% ethanol for DNA extraction. A total of 1–4 individuals were included per suburb (Table 1; Fig. 1B).

DNA Extraction and PCR Amplification

Samples were washed with distilled water twice to remove the traces of ethanol and air-dried prior to DNA extraction. Genomic DNA was extracted from individual adult mealybugs using QIAGEN Blood and Tissue kit (Cat No. 69506) following the manufacturer's instructions with slight modifications in the final elution step. DNA was eluted in 60 µl DEPC-treated water (Thermofisher Scientific, AM9906). Concentration and quality of DNA was measured using NanoDrop Spectrophotometer (Thermofisher Scientific).

COI gene PCR was performed using primer pairs C1J2195: TGATTYTTTGGTCATCCAGAAGTandTL2N3014:TCCAATGCA CTAATCTGCCATATTA (Simon et al. 1994), 18S gene PCR was performed using primers 2880: CTGGTTGATCCTGCCAGTAG (Tautz et al. 1988) and B: CCGCGGCTGCTGGCACCAGA (von Dohlen and Moran 1995) and 28S PCR was performed using primers S3660: GAGAGTTMAASAGTACGTGAAAC (Dowton and Austin 1998) and A335: TCGGARGGAACCAGCTACTA (Whiting et al. 1997). The PCR reaction mixture of 25 µl contained 12.5 µl of 2 × MyTaq mix (BIOLINE BIO-25041), 1 µl of each primer (10 µM), 0.5 µl of BSA (20 mg/ml), and 2 µl of template DNA and water. The PCR cycling parameters used for COI gene amplification were 1 denaturation cycle of 95 °C for 4 min, followed by 35 cycles of 95 °C for 30 s, 48 °C for 45 s, and 72 °C for 1 min, followed by a final extension of 72 °C for 5 min (Wu et al. 2014). PCR conditions for amplification of 18S and 28S genes were 94 °C for 4 min, followed by 35 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 90 s, followed by a final extension of 72 °C for 4 min (Whiting et al. 1997). For amplification confirmation, PCR products were loaded on a 1.5% agarose gel, run at 80 V for 40 min, and visualized under the GelDoc Go gel Imaging system (BIORAD PTY Ltd). The amplicon sizes obtained by each PCR were around 746 bp for COI, 650 bp for 18S, and 700 bp for 28S.

Table 1. Sample collection site information. Samples of *Paracoccus marginatus* were collected from the 3 regions in the Northern Territory, Darwin, Australia

Suburb	No. of individual sequenced	Region
Alawa	3	Darwin
Bayview	1	Darwin
Berrimah	2	Darwin
Driver	2	Palmerston
Gardens	2	Darwin
Herbert	2	Litchfield
Howard Springs	1	Litchfield
Karama	3	Darwin
Leanyer	3	Darwin
Malak	3	Darwin
Marrara	3	Darwin
Millner	1	Darwin
Moulden	1	Palmerston
Parap	4	Darwin
Rapid Creek	1	Darwin
Roseberry	3	Palmerston
Winnellie	2	Darwin
Woodroffe	3	Palmerston
Woolner	2	Darwin
Zuccoli	3	Palmerston

Amplicon Sequencing

We sequenced COI and 18S gene amplicons on the Oxford Nanopore MinION sequencer (MinION Mk1C). PCR amplicons were sequenced using Flongle flow cells (FLO-FLG114). Library preparation for amplicon sequencing was completed using the Rapid barcoding kit v14 (SQK-RBK114.96) following the manufacturer's instructions (Oxford Nanopore Technologies, Oxford, UK). In 4 separate runs, sequencing libraries were loaded on to flow cell using Flongle sequencing expansion kit (EXP-FSE002) following the manufacturer's instructions. A quality control of sequencing pores was done before each run.

We performed sequencing runs using MinKNOW v23.07.12 (Oxford Nanopore Technologies, Oxford, U.K) for 24–48 h with high-accuracy basecalling tuned to "ON" mode. Minion basecalling was set to "high-accuracy model." To access the quality of run, we inspected run reports generated by the MinION sequencer. Generated fastq files were trimmed using the Nanofilt programme (De Coster et al. 2018). Reads were filtered for a minimum sequence length of 200 bp, a maximum sequence length of 1,000 bp, and a quality score of 15. Consensus was searched for forward and

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Table 2. NCBI GenBank accessions of 3 genes: COI, 18S, and 28S of *Paracoccus marginatus* from different countries used in the present study

NCBI Accession number	Publication/NCBI submission date	Country/Region	Amplicon size (bp)
COI gene			
KP745312	Ahmed et al. (2015)	China/Asia	712
PP291863.1	Unpublished/2024	India/Asia	764
MT309692.1	Unpublished/2020	Malaysia/Asia	303
EU267201.1	Unpublished/2016	Florida/USA	746
MW881818.1	Choi and Lee (2022)	Myanmar/Asia	410
OP757501	Unpublished/2022	Tanzania/Africa	725
OP757502.1	Unpublished/2022	Tanzania/Africa	755
18S gene			
EU188580	Unpublished/2008	USA	555
MW541966	Choi and Lee (2022)	South Korea/Asia	555
MN967101	Unpublished/2022	Malaysia/Asia	630
28S gene			
EU188474.1	Unpublished/2008	USA	289
MN447458.1	Unpublished/2020	Kenya/Africa	268
MN447462.1	Unpublished/2020	Kenya/Africa	241
MN447467.1	Unpublished/2020	Kenya/Africa	296
MW227324.1	Unpublished/2020	Malaysia/Asia	297
MW542033	Choi and Lee (2022)	South Korea/Asia	756
MZ407948.1	Unpublished/2021	China/Asia	428
MZ407949.1	Unpublished/2021	China/Asia	425
KY211345.1	Unpublished/2019	China/Asia	720
KP692333	Unpublished/2016	China/Asia	731
MK873100.1	Unpublished/2020	China/Asia	697

reverse primers and their reverse complements. Trimmed and filtered reads were de novo assembled using Geneious Prime software (https://www.geneious.com/; Biomatters, New Zealand). Contigs were inspected manually and consensus sequences with the highest number of supported reads were extracted as a final consensus. 28S amplicons were Sanger sequenced bidirectionally at the Australia Genomics Research Facility (www.agrf.org.au), Brisbane. Forward and reverse reads were de novo assembled in Geneious Prime and read ends were trimmed for low-quality bases.

The consensus sequences of all 3 genes generated in the present study were compared with the sequences deposited on the NCBI GenBank nucleotide database (https://www.ncbi.nlm.nih.gov/nucleotide/). For COI, a total of 191 *P. marginatus* sequences were downloaded from NCBI GenBank. Of these, 161 sequences (24 unique sequences) were excluded because they covered a different section of the COI gene. Among the remaining 30 sequences, only unique sequences were retained for further analysis, as some accessions were 100% identical, leaving a final set of 7 sequences. Accessions were available from Asia, Africa, and the United States (Table 2). Sequences were aligned using the MAFFT multiple alignment tool with default settings in Geneious Prime. Aligned sequences were used to obtain pairwise percentage identity scores between present sequences and other sequences. When several sequences from the same location were available on GenBank, only unique sequences were included in the analysis.

Genetic Analysis

Genetic and demographic parameters including nucleotide diversity (π), haplotype diversity (Hd), number of segregating sites, and deviations from neutrality test—Tajima's D statistic (Tajima 1989) were calculated for (i) all sequences from the present study and (ii) between aligned present sequences and sequences obtained from GenBank using DnaSP (Rozas et al. 2017). Tajima's D provides inference on population demographic expansion. Hd value < 0.5 and π value < 0.005 suggest a recent population bottleneck or founder event by a single or few lineages (Grant and Bowen 1998).

Haplotype Network

We examined the biogeographic pattern of genetic diversity and genetic structure at an intraspecific level between present study sequences and GenBank deposited sequences by constructing a haplotype network using the Templeton, Crandall, and Sing (TCS) method (Clement et al. 2002) in PopART (Leigh et al. 2015). TCS network method can estimate population-level genealogies when divergences are expected to be low (Clement et al. 2000). To assess haplotype accumulation, i.e., an evaluation of sample size required to obtain an accurate estimation of haplotype diversity within a species (Lindblom 2009, Phillips et al. 2019), haplotype rarefaction curves were generated in R using "HaploAccum()" function in the SpideR package (Brown et al. 2012) with 1,000 permutations. Nonparametric Chao 1 estimator of total haplotype diversity was calculated using the "chaoHaplo()" function.

Evolutionary Divergence

The evolutionary divergence between sequences from the present study and sequences from GenBank was calculated in MEGAX v11 (Tamura et al. 2021) using the Kimura-2 parameter distance mode. As the sequences in the present study were similar, only 1 sequence was randomly selected to calculate the evolutionary divergence.

Results

All samples successfully produced COI sequences ranging from 752 bp to 824 bp. All 45 sequences showed a 100% similarity with *P. marginatus*. For nuclear genes, 18S gene sequences ranged from 611 bp to 618 bp. All 45 sequences showed a 100% similarity with *P. marginatus*. For 28S, sequences ranged from 708 bp to 757 bp. One sample was removed due to low-quality base call (60%). All

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sequences showed a 100% similarity with *P. marginatus*. All 3 genes were able to identify the species with a 100% identity match score.

Genetic Analysis within Current Samples

For COI, nucleotide diversity (π) was 0.0002, haplotype diversity (Hd) was 0.1, and Tajima's D was −0.38 (nonsignificant) with only 1 segregating site. All samples had Hd < 0.5 and π < 0.005, suggesting a recent founder event by a single or few lineages (Grant and Bowen 1998, Huang et al. 2024). Negative Tajima's D suggested deviations from neutrality and a recent population bottleneck or population expansion. For 18S and 28S genes, no polymorphic sites were found.

Genetic Analysis between Samples from Present Study and Global Sequences

For COI, we compared 45 samples from the present study with 7 sequences from Asia, Africa, and the United States. The nucleotide diversity between sequences was 0.005, haplotype diversity was 0.1, and Tajima's D was −2.15 ($P < 0.05$) with 24 segregating sites (Table 3). For 18S, we compared 45 current sequences with 3 sequences from Asia and the United States. The nucleotide diversity between sequences was 0.00008, haplotype diversity was 0.04, and Tajima's D was −1.1 (nonsignificant $P > 0.05$) with 1 segregating site. For 28S, we compared 44 current samples with 11 sequences from Asia, Africa, and the United States. The nucleotide diversity between sequences was 0.001, haplotype diversity was 0.07, and Tajima's D was −1.76 (nonsignificant) with 3 segregating sites. Overall low nucleotide and haplotype diversity was observed between populations worldwide for all 3 genes. A negative Tajima's D, although nonsignificant for 18S and 28S genes, implies the global populations have gone through a demographic expansion.

Haplotype Network

For the COI gene, all sequences from the present study and sequences originating from Asia formed 1 group. Two sequences from Tanzania with 23 mutations formed the second group and 1 sequence from the United States with 1 mutation formed the third group (Fig. 2). For 18S gene, all current 45 samples and sequences originating from Asia formed 1 group. One sample originating from the United States differed by a single mutation (Fig. 2). For 28S gene, all current sequences and 9 sequences from the United States, Asia, and Africa formed 1 group. Two NCBI GenBank accessions originating from China (MZ407948.1 and MZ407949.1) with 2 mutations each formed a second group.

Haplotype accumulation plots for all 3 genes did not reach asymptote suggesting further global sampling efforts are likely to increase the haplotype diversity between populations across countries (Fig. 3). For COI gene, Chao 1 richness estimated 3.5 haplotypes potentially exist for this marker. For 18S gene, Chao 1 richness estimated 2 haplotypes. The low estimation is likely due to limited sequences ($n = 3$) available from other countries. For 28S gene, Chao 1 richness estimated 7 haplotypes, however, gaps in 1 of the NCBI sequences (MW542033.1) could have contributed to the slight overestimation of haplotype number.

Evolutionary Divergence

Low to no genetic divergence was observed in mitochondrial and nuclear genes. Consistent with haplotype analysis, for COI gene samples from Australia showed slight divergence from NCBI GenBank accessions OP757501, OP757502 (0.082), and EU267201 (0.003). For 18S gene, sample from the United States showed slight

Table 3. Population genetics and demographic parameters for samples from *Paracoccus marginatus* present study and global NCBI GenBank sequences. SD: standard deviation

Genetic marker	Number of sequences analyzed	Nucleotide diversity (π)	Haplotype diversity (Hd)	Tajima's D	No. of segregating sites
COI	45 (Current study)	0.0002	0.1 (SD 0.07)	−0.38 ($P > 0.1$)	1
	45 Australia, 4 Asia, 2 Africa, 1 USA	0.005	0.1 (SD 0.06)	−2.15 ($P < 0.05$)	24
18S	45 Australia, 1 USA, 2 Asia	0.00008	0.04 (SD 0.04)	−1.1 ($P = > 0.1$)	1
28S	44 Australia, 1 USA, 3 Africa, 7 Asia	0.0015	0.07 (SD 0.04)	−1.76 ($P > 0.1$)	3

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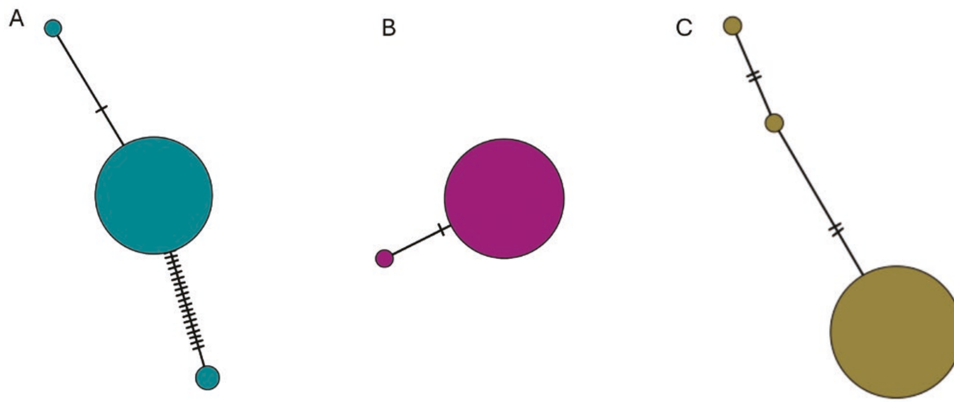

Fig. 2. TCS haplotype network between samples collected from the first detection of *Paracoccus marginatus* in Australia and other global samples submitted on NCBI GenBank; A: COI, B: 18S and C: 28S. Sizes of the circles represent the number of individuals in each group.

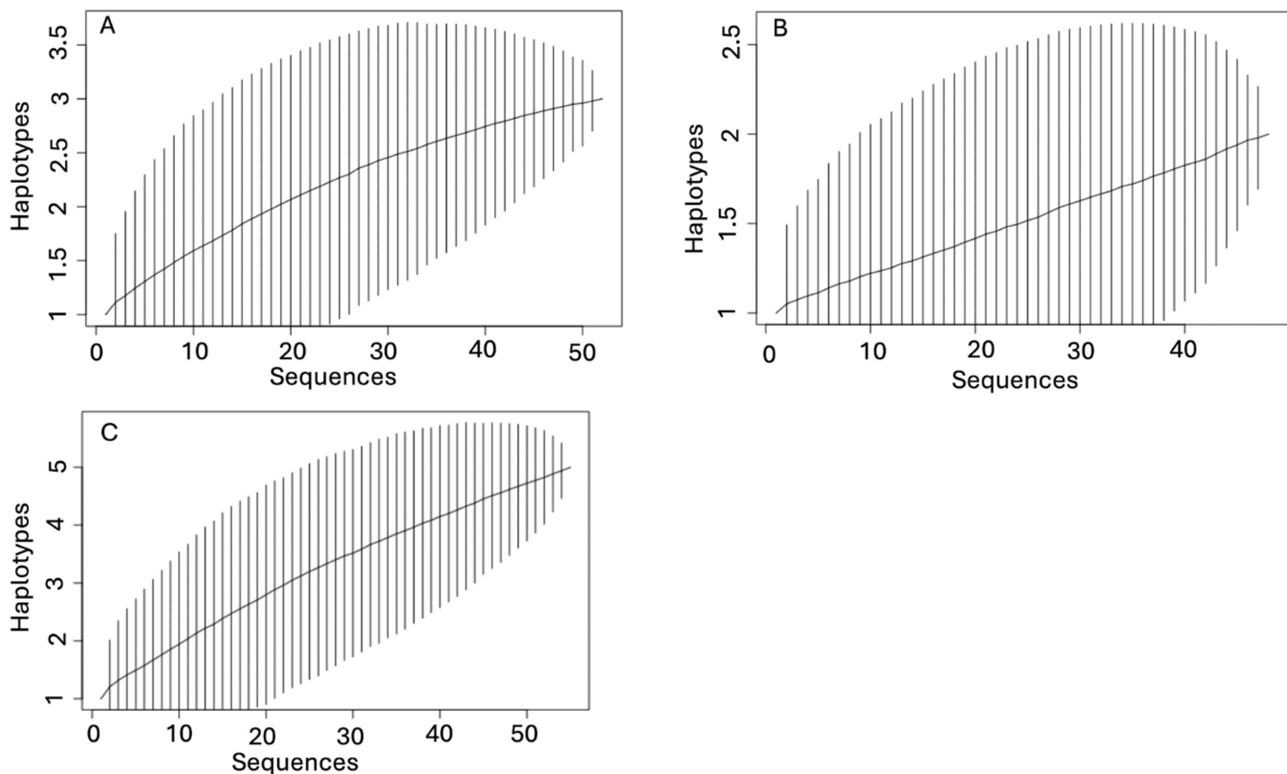

Fig. 3. Haplotype accumulation curves between sequences from the present study and NCBI GenBank sequences for A) COI, B) 18S, and C) 28S genes.

divergence from other sequences (0.001) and for 28S gene, evolutionary divergence ranged from 0.0 to 0.026 (Table 4).

Discussion

Increased global trade, live plant imports, and human-assisted movement have facilitated the introduction of non-native insect pests into novel environments (Liebhold et al. 2012). *Paracoccus marginatus* is a polyphagous pest first detected in Australia in Darwin, Northern Territory in July 2023. In this study, we present population diversity indices and demographics of the recently detected population in Australia. We found all 3 genes can identify *P. marginatus* with a high confidence of 100%. We detected very low to no nucleotide and haplotype diversity in the mitochondrial COI and nuclear 18S and 28S genes in the recently invaded population, suggesting a recent founder event by a single or few lineages. Significant negative Tajima's D for COI suggested deviations from neutrality and a recent population bottleneck or population expansion. The comparative analysis of sequences from the present study with publicly available sequences from Asia, Africa, and the United States indicated overall low genetic diversity, evolutionary divergence, and demographic expansion in the global populations. Haplotype networking placed samples from our study in a single group with sequences from Asia, suggesting an expansion of a single or a few haplotypes. However, further global sampling is likely to increase the estimates of genetic diversity. By collecting genetic data just after the first detection, we provide valuable insights into the early invasion genetics of this rapidly spreading pest.

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Table 4. Evolutionary divergence estimates between sequences from present study and global NCBI GenBank sequences for COI, 18S, and 28S genes

COI

	EU267201	KP745312	MT309692	MW881818	OP757501	OP757502	PP291863
KP745312	0.003						
MT309692	0.003	0.000					
MW881818	0.003	0.000	0.000				
OP757501	0.086	0.082	0.082	0.082			
OP757502	0.086	0.082	0.082	0.082	0.000		
PP291863	0.003	0.000	0.000	0.000	0.082	0.082	
PQ182143 (Current study)	0.003	0.000	0.000	0.000	0.082	0.082	0.000

18S gene

	EU188580	MN967101	MW541966
MN967101	0.001		
MW541966	0.001	0.000	
PQ182145 (Current study)	0.001	0.000	0.000

28S gene

	EU188474	KP692333	KY211345	MK873100	MW227324	MW542033	MZ407948	MZ407949	MN447458	MN447462	MN447467
KP692333	0.000										
KY211345	0.000	0.000									
MK873100	0.000	0.000	0.000								
MW227324	0.000	0.000	0.000	0.000							
MW542033	0.000	0.000	0.000	0.000	0.000						
MZ407948	0.016	0.016	0.016	0.016	0.016	0.017					
MZ407949	0.024	0.024	0.024	0.024	0.024	0.026	0.016				
MN447458	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.025			
MN447462	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.025	0.000		
MN447467	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.025	0.000	0.000	
PQ182830 (Current study)	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.024	0.000	0.000	0.000

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Early, rapid, and accurate diagnostics are crucial for biosecurity responses and incursion management. COI gene is the preferred barcode for insect species identification due to its high sequence variability at the interspecific level and availability of a comprehensive reference database (Hebert et al. 2003, Hajibabaei et al. 2006). In our study, COI provided a 100% identity score with *P. marginatus* sequences submitted on GenBank. However, COI may not always provide promising and accurate species identification for Hemiptera, especially in the case of closely related species and cryptic diversity (Park et al. 2011, Wang et al. 2016). A multilocus approach can improve the identification accuracy and provide reliable estimates of species evolutionary divergence (Wang et al. 2016). For mealybugs, the nuclear ribosomal RNA 18S and 28S genes have been frequently used in phylogenetic studies (Hardy et al. 2008, Kaydan et al. 2015, Choi and Lee 2022). The 2 genes have successfully diagnosed *P. marginatus* with a 100% identity score. A multigene diagnostics approach combined with a smaller and accessible MinION sequencer can provide results quickly and more robustly.

It is generally believed that newly invaded non-native populations experience genetic diversity loss due to the founder's effect, leading to a smaller effective population size and reduced adaptability (Sax and Brown 2000). We found very low genetic diversity and the presence of a single COI haplotype. Our results are consistent with another study suggesting the presence of a single haplotype in *P. marginatus* across Asian countries (Cambodia, China, India, Indonesia, Malaysia, and Thailand) (Ahmed et al. 2015). Furthermore, Heya et al. (2020) found low genetic divergence in the 28S gene across 3 regions of Kenya. The low genetic diversity and spread of a single haplotype have been noted in other insect pest species. For example, 2 invasive members of the *Bemisia tabaci* species complex, spread globally with low available genetic diversity (De Barro and Ahmed 2011). Similarly, in the invasive harlequin ladybird *Harmonia axyridis*, while 2 mitochondrial haplotypes dominate the native population, only 1 haplotype is dominant in the non-native range (Li et al. 2023) suggesting that the degree of invasiveness may differ between genotypes. It is possible that an "invasive" genotype of *P. marginatus* with a higher spread potential has been successful in invading tropical and subtropical regions with suitable climatic conditions.

Invasive populations with low genetic diversity have other ways to survive, spread, and establish. Arrival at the right time of the year with optimal environmental conditions can facilitate range expansion, regardless of genetic processes (Roman and Darling 2007). *Paracoccus marginatus* was first detected in Darwin during the dry season, which is described as a heavy dew time followed by big wind time in the Larrakia seasonal calendar. The cooler dry climatic conditions between 18 and 30 °C with no rain are optimal for this species (Krishnan et al. 2016). In addition, the presence of several host plants, especially Frangipani (*Plumeria* spp.), and a potential absence or low abundance of co-evolved, predators may have facilitated a rapid spread of the species (Sax and Brown 2000). The species is not a pest in its native distribution in Mexico and Central America due to the presence of natural enemies and predators (Tanwar et al. 2010). *Paracoccus marginatus* also has a high fecundity, with females laying around 300 eggs in optimal climatic conditions, which could facilitate its rapid spread. Overall, the *r*-selected life history trait and absence of predators and competitors may have assisted in the successful establishment and rapid spread of the species in northern Australia.

Significant negative values of Tajima's D test for COI gene suggest deviation from neutrality and population expansion. This aligns with the recent expansion of *P. marginatus* in Asia and 9 counties in Kenya (Ahmed et al. 2015). A rapid spread in Kenya is attributed to the trade of infested plant material between neighboring countries (Macharia et al. 2017). Within around 34 years, the species has spread across 4 continents: South America, Africa, Asia, and Oceania, mainly to the tropical and subtropical regions. It has been suggested that a further expansion of *P. marginatus* into Africa, Central America, and Asia is possible due to suitable climatic conditions and host plants (Finch et al. 2021). Environmental variables such as isothermality, temperature, and precipitation are substantially associated with its distribution range (Heya et al. 2020). A high adaptability potential, broad host range, and thermal tolerance are potential mechanisms that enable this species' rapid spread, despite low genetic diversity and founder effect.

While low genetic divergence has been observed between sequences from the present study and global sequences, our haplotype accumulation analysis suggests the likelihood of an increase in genetic diversity estimates with a larger global sample size. Native populations generally have more genetic diversity and standing genetic variation maintained through balancing selection than the invasive population (Stern and Lee 2020). For *P. marginatus*, additional sampling efforts from native range and invaded countries will refine the genetic diversity estimates for this species. Further studies incorporating genomic approaches can provide valuable insights into the spread, establishment, and subsequent range expansion of *P. marginatus* in novel environment, especially when freed from natural enemies, and supported by favorable environmental conditions and suitable hosts.

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Author contributions

Sonu Yadav (Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Visualization, Writing—original draft, Writing—review & editing [lead]), Shreya Patel (Formal analysis, Investigation, Writing—review & editing [supporting]), Sachinthi Kithulgoda (Methodology, Writing—review & editing [supporting]), Haidee Brown (Methodology, Writing—review & editing [supporting]), and Brian Thistleton (Methodology, Writing—review & editing [supporting])

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Conflicts of interest

None declared.

Data Availability

Selected data generated as a part of the study have been submitted to NCBI GenBank and accession numbers have been included in the manuscript.

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