Research Article

Australian *Bryobia* mites (Trombidiformes: Tetranychidae) form a complex of cryptic taxa with unique climatic niches and insecticide responses

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Abstract

BACKGROUND: *Bryobia* (Koch) mites belong to the economically important spider mite family, the Tetranychidae, with >130 species described worldwide. Due to taxonomic difficulties and most species being asexual, species identification relies heavily on genetic markers. Multiple putative *Bryobia* mite species have been identified attacking pastures and grain crops in Australia. In this study, we collected 79 field populations of *Bryobia* mites and combined these with 134 populations that were collected previously. We characterised taxonomic variation of mites using 28S rDNA amplicon-based DNA metabarcoding using next-generation sequencing approaches and direct Sanger sequencing. We then undertook species distribution modelling of the main genetic lineages and examined the chemical responses of multiple field populations.

RESULTS: We identified 47 unique haplotypes across all mites sampled that grouped into four distinct genetic lineages. These lineages have different distributions, with three of the four putative lineages showing different climatic envelopes, as inferred from species distribution modelling. *Bryobia* mite populations also showed different responses to a widely used insecticide (the organophosphate, omethoate), but not to another chemical (the pyrethroid, bifenthrin) when examined using laboratory bioassays.

CONCLUSION: Our findings indicate that cryptic diversity is likely to complicate the formulation of management strategies for *Bryobia* mites. Although focussed on Australia, this study demonstrates the challenges of studying *Bryobia* and highlights the importance of further research into this complex group of mites across the world.
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Keywords: DNA barcoding; cryptic species; pest; species distribution modelling; insecticide responses

1 INTRODUCTION

Bryobia (Koch) mites belong to the economically important spider mite family, the Tetranychidae. They are widely distributed across North America, South America, Europe, Africa, New Zealand, Asia and Australia,[1–6] with >130 species described worldwide,[1] although these are likely to contain synonyms and species with overlapping descriptions.[2] Species have previously been described based on morphological characters and plant host associations.[4,6–9] However, suitable morphological characteristics are limited in these small mites, making identification difficult,[2,4] and species descriptions based primarily on plant-host associations are often problematic.[10]

To further compound difficulties with taxonomic resolution of *Bryobia* species, the majority of described species reproduce asexually by thelytokous parthenogenesis, with populations made up entirely of females; males have only been described from a few species.[2,11,12] *Bryobia* and other spider mites are ancestrally haplodiploid, where fertilised eggs give rise to diploid females and

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unfertilised eggs give rise to haploid males.[2,13,14] Many *Bryobia* species are also known to be infected with *Wolbachia* and *Cardinium*, maternally inherited intracellular bacteria known to alter the reproductive mode of infected hosts to enhance their spread.[11,15–17] *Wolbachia* infections in at least two *Bryobia* species induce parthenogenesis through a process known as gamete duplication, and it is likely that *Wolbachia* is responsible for parthenogenetic reproduction within this genus more generally.[11]

Bryobia mites are highly polyphagous, with numerous species being considered horticultural pests attacking a variety of fruits trees, while others feed on various grasses, herbaceous plants and grain crops.[1,2,18] Mites feed on plant material by puncturing plant cells with their mouth parts and sucking out the contents, leaving etiolated trails composed of whitish-grey spots.[4,13,19] In Australia, *Bryobia* mites are significant pests of winter grain crops and pastures, and their importance as pests in these agricultural commodities has increased over recent decades.[20–22] They are known to damage canola, wheat, oats, lucerne, clover and pulse crops, and tend to be most damaging at the establishment phase of plant development.[21,23] This is similar to other parts of the world, including South Africa,[24] India,[25] Europe[26–28] and North America.[29] In Australia, *Bryobia* consist of a species complex comprised of at least 10 putatively discrete taxa based on morphology and DNA sequence information.[3,30,31] Seven of these putative species (*B.* sp. I, *B.* sp. IV, *B.* sp. VII, *B.* sp. VIII, *B.* sp. IX, *B.* sp. X and *Bryobia praetiosa* Koch) are known to be present in Australian pastures and grain crops.[31] Of these, *B.* sp. VIII, *B.* sp. IX and *B.* sp. I are believed to be the most common, although this is based on limited sampling, mostly from the state of Victoria.[31] Little is known about the broader biogeographic distribution and abundances of different *Bryobia* taxa across Australia.

The presence of cryptic species can complicate pest management decisions, as separate control strategies may be needed for individual species. In Australia, *Bryobia* mites are largely controlled using insecticides through foliar sprays or insecticide seed treatments, with the organophosphate, omethoate, and the pyrethroid, bifenthrin, being the most commonly used.[32] However, there have been increasing concerns around chemical control difficulties in the field involving *Bryobia* mites.[32,33] Although *Bryobia* mites can possess inherent tolerances to chemicals,[32] it is unclear how these might differ between species, nor is it known whether different species have experienced varying levels of selection for increased insecticide tolerance. Such issues have previously arisen in cryptic mite species from the genus *Penthaleus*, which are also pests in Australian grain crops and pastures.[34–36] To date, only the chemical sensitivity of *Bryobia* sp. I has been investigated in Australia.[32]

In mites, DNA-based markers widely used to identify intraspecific and interspecific variation include mitochondrial DNA (mtDNA) and nuclear ribosomal DNA (rDNA); the mitochondrial cytochrome *c* oxidase subunit I gene (COI), and the internal transcribed spacers (ITS1 and ITS 2) are the most commonly used markers.[37,38] Sequences of the COI gene and ITS regions have been widely utilized to delineate and identify morphologically cryptic species, particularly within the Tetranychidae.[37,39–46] These genes along with the D1 region within the nuclear 28S rDNA gene have previously been used to determine the phylogenetic relationship among *Bryobia* mite species in Europe,[2] Australia[31] and Africa.[39]

In this paper, we investigated the species status and distribution of *Bryobia* mites in Australian pastures and winter grain crops. We undertook amplicon-based DNA barcoding with 28S rDNA using next-generation sequencing (NGS) approaches and Sanger

sequencing on *Bryobia* mites collected from winter grain crops, pastures and roadside vegetation throughout Western Australia (WA), South Australia (SA), Tasmania (Tas), New South Wales (NSW), Queensland (Qld) and Victoria (Vic). Sequence variation was analysed using Bayesian inference and hierarchical clustering of genetic distances, which led us to infer that Australian *Bryobia* mites form at least four distinct genetic lineages. Using presence-absence data and spatial climatic data, we then modelled the Australian distributions of these four lineages and identified climatic variables that were correlated with their distributions. We also explored the niche overlap of lineages commonly observed in this study and tested the responses of multiple mite populations to commonly used insecticides to further determine if there are phenotypic differences between *Bryobia* lineages.

2 MATERIALS AND METHODS

2.1 Mite collections

In this study, we utilized historical collections of *Bryobia* mites and undertook targeted field surveys in parts of Australia that had not been well sampled previously. One hundred and thirty-four populations of *Bryobia* mites collected from Vic, NSW, SA and the southern part of WA were utilised from Arthur *et al.*[22] Additionally, field collections of *Bryobia* mites were undertaken between April 2017 and October 2019, mostly targeting previously unsampled regions, including Tas and southern Qld (Fig. 1). Mites were collected along roadsides or within pasture fields and winter grain crops. Sites were targeted based on known plant hosts of *Bryobia* mites,[1,23,31] including cereals, lucerne (*Medicago sativa*), capeweed (*Arctotheca calendula*), clover (*Trifolium* spp.), barley grass (*Hordeum leporinum*), wild radish (*Raphanus raphanistrum*), wild oats (*Avena* spp.), vetch (*Vicia* spp.) and canola (*Brassica napus*). Mites were collected via suction using a Stihl SH55 blower vacuum with a fine gauze mesh placed over the end of the vacuum tube. Samples were placed into plastic containers and transferred to the laboratory. *Bryobia* mites were then distinguished from other mite species based on morphology (colour, size and arrangement of dorsal setae) using a stereo microscope at ×20 magnification and stored in 100% ethanol at −20 °C for DNA barcoding. Global Positioning System coordinates and plant host were recorded at each sampling location. Collections undertaken between 2017 and 2019 yielded an additional 79 field populations of *Bryobia* mites (Fig. 1).

2.2 DNA extraction, sequencing and bioinformatics

We used an amplicon NGS metabarcoding approach following Fadrosh *et al.*[47] to determine *Bryobia* haplotypes present within the 134 samples collected by Arthur *et al.*[22] and the 79 populations collected in this study. Approximately 20 mites per location were pooled into a single 2 mL tube, homogenised by hand and DNA extracted using DNeasy Blood & Tissue Kits (Animal Tissue Spin-Column Protocol; Qiagen, Victoria, Australia). Using the primers described in Ros *et al.*,[2] we amplified a region of the 28S rDNA nuclear region from each DNA extraction sample using a two-step PCR protocol. The first-round PCR primers contained a (5′–3′) universal adaptor sequence[47] and the forward and reverse primers were from Ros *et al.*[2]: nextera_28S_D1F-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG<u>ACCCSCTGAAYTTAAGCAT</u> and nextera_28S_D1R-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG<u>AACTCTCTCMTTCARAGTTC</u>. Reaction matrices contained 2 μL DNA, 1× PCR buffer, 3 mM $MgCl_2$, 0.3 μM each primer and 0.2 U of KAPA Plant DNA Polymerase (Kapa Biosystems,

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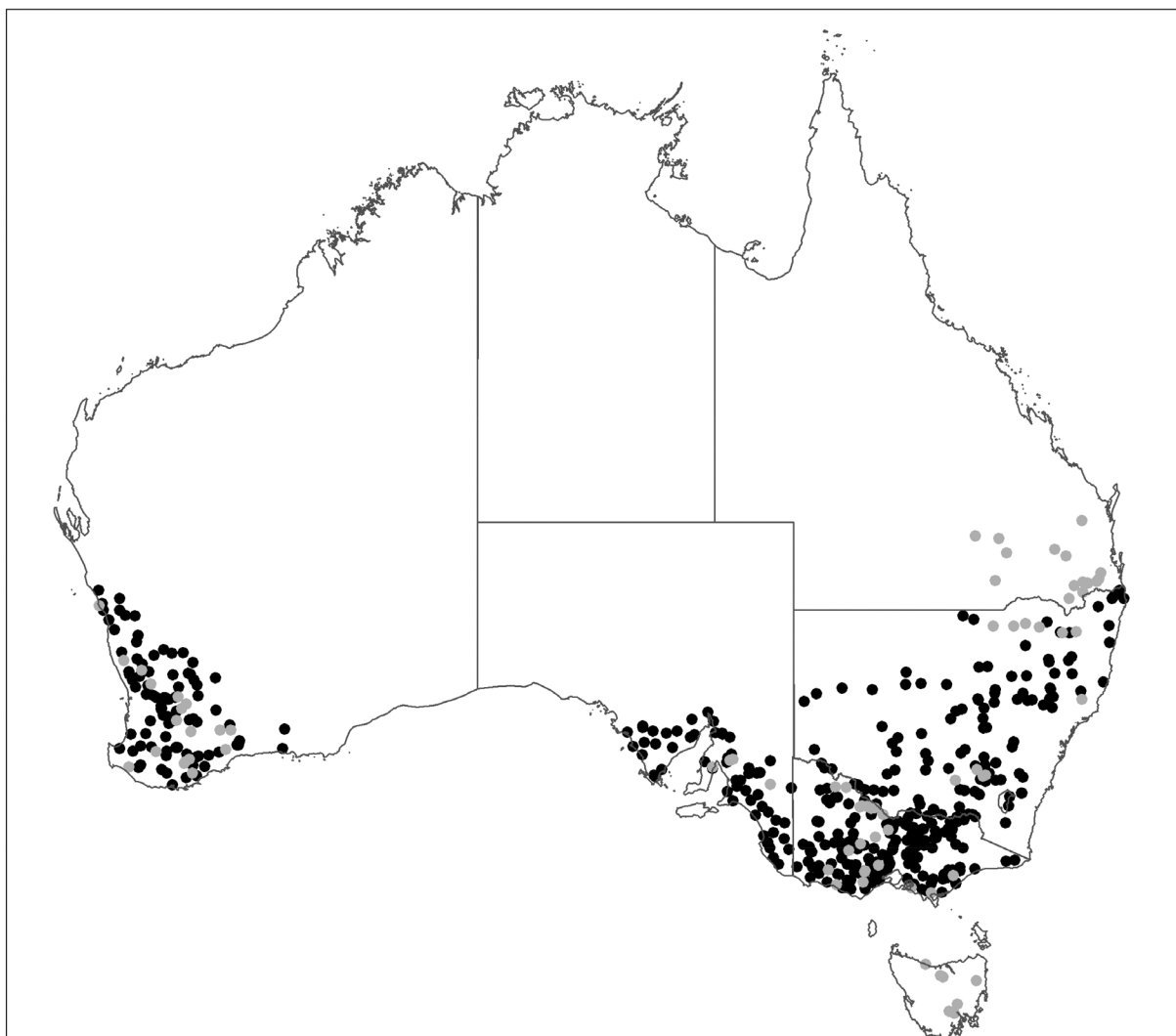
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Figure 1. Map of Australia showing the distribution of sample locations for *Bryobia* mites used in this study. Black circles indicate samples collected in Arthur *et al.*[22] Grey circles indicate locations where *Bryobia* mites were collected in this study.

Massachusetts, USA) in a final volume of 10 μL. Reaction conditions were as follows: one cycle at 95 °C for 3 min, 40 cycles at 95 °C for 20 s, 48 °C for 15 s, 72 °C for 30 s, and one cycle at 72 °C for 1 min. Two technical replicates were performed for each extraction sample. Following PCR, replicate reactions were pooled together in equal volumes. PCR products were then cleaned with ExoSAP-it (Applied Biosystems, Massachusetts, USA).

Second-round PCR primers contained the Illumina p5 or p7 binding region, an 8 bp index sequence[47] and a universal adaptor sequence. Unique forward and reverse index combinations were used for each population sample. Reactions contained 2 μL of cleaned PCR product, 0.5 μM of each primer and 1× Phusion Hot Start II High-Fidelity PCR Master Mix (Thermo Fisher Scientific, Massachusetts, USA) in a final volume of 20 μL. Reaction conditions were as follows: one cycle at 98 °C for 1 min, 15 cycles at 98 °C for 10 s and 72 °C for 45 s, and one cycle at 72 °C for 10 min. Samples were pooled and then subjected to size selection to remove nonspecific fragments outside of the desired size range using Sera-Mag SpeedBead Carboxylate-Modified Magnetic Particles (GE Healthcare Life Sciences, Massachusetts, USA). Sequencing

was performed on an Illumina MiSeq platform (Illumina, California, USA) using 300 bp PE chemistry at the Monash Health Translation Precinct (Victoria, Australia). Twelve controls (PCR negative containing water and no DNA) were included in the library.

For some population samples, only a small number of *Bryobia* mites (fewer than five) were available. For these samples, DNA extraction (DNeasy Blood & Tissue Kits, as above), PCR amplification and Sanger sequencing (ABI 3730xl, Macrogen, South Korea) were performed on individuals using the 28S_D1_F and 28S_D1_R primers as per Ros *et al.*[2]

Bioinformatic analyses were performed with a custom analysis pipeline that incorporated the software programs VSEARCH v2.9.0[48] and cutadapt.[49] Forward and reverse reads were first merged before trimming to remove primer sequences. Reads were then de-replicated and those with an abundance of less than 10 were excluded from further analyses. Reference sequences for phylogenetic reconstructions were obtained from data deposited by Ros *et al.*[2] and Arthur *et al.*,[31] which included 28S sequences for *Bryobia*, with the *Petrobia* genus as an outgroup (GenBank accessions: GU979863-72, EU487049-65).

2.3　Sequence analyses

Multiple sequence alignments and construction of a phylogenetic tree were performed using Seaview v4.7,[50] with neighbour-joining methods to obtain a distance-based tree and pairwise genetic distances between haplotypes. Preliminary analyses suggested that a small number of samples included traces of DNA from the *Petrobia* genus, grouping with *Petrobia* sequences used as outgroups. We therefore proceeded with only those haplotypes that fell within the *Bryobia* subtree. On this dataset, a further filtering step was conducted to retain only those haplotypes at a frequency ≥5% (representing ~1 mite in a sample of 20). Following this, sequences from the Sanger and NGS datasets were combined and filtered to retain only unique haplotypes. Phylogenetic reconstruction was undertaken using Bayesian inference (BI) in Mr Bayes v3.2.[51] We first determined the optimum nucleotide substitution model in Modeltest 3.7[52] using the Akaike's Information Criterion.[53] The same BI settings were then implemented as in Arthur *et al.*,[31] with five million generations (convergence indicated by average standard deviation of split frequency values <0.01). After discarding the burn-in, remaining trees were summarized in a consensus tree with posterior probabilities as nodal support.

Given the large number of unique haplotypes and the relatively low nodal support from the BI analysis, we also undertook a clustering analysis in R. The 28S rDNA gene alignment was imported into R using the APE package.[54] Only *Bryobia* species identified from previous analyses and unique operational taxonomic units (OTUs) identified in this study were analysed, excluding the outgroup *Petrobia*. A genetic distance matrix between pairwise sequences was constructed using the DIST.DNA function from APE, with a K80 mutational model and no-missing data (no pairwise deletion). The HCLUST function was used to visualise pairwise genetic distances.

The genetic distance matrix was then subjected to a principal coordinate analysis (PCoA), which decomposes the variance in the distance matrix to derive new orthogonal axes that summarise pairwise relationships among sequences. PCoA was performed using the PCoA function within the APE package. To identify those principal coordinate (PCo) axes that explained significant variation in the genetic distance matrix, relative to random expectations, we implemented a broken stick method,[55] using the BSDIMENSION function from the PCDIMENSION package.[56] We tested the total number of positive eigenvalues. Only the first two eigenvalues were identified as being significant. Therefore, the first two PCo axes (PCo1 and PCo2) were used as variables to identify putative taxonomic clusters. Four distinct clusters were observed, which we herein refer to as lineages 1, 2, 3 and 4. Visualisation of our clustering analyses was performed using the GGPLOT2[57] and GGTREE[58] packages.

2.4　Distribution models

We developed species distributions models utilising presence data for the *Bryobia* mite lineages identified in this study, in addition to absence data for 282 locations taken from Arthur *et al.*[22] Species distribution models were fit using the boosted-regression-tree (BRT) approach, which is a machine learning technique used for classification problems that produces a prediction from an ensemble (boosting) of many weaker models (decision trees).[59,60] We chose a bagging size of 0.5, which is appropriate for smaller data sets[59] and implemented the model in R using the code provided by Elith *et al.*[60] A *k*-fold cross-validation procedure was used to train (90%) and test (10%) each model, whereby the data was randomly divided into 10 subsets, with each subset sequentially omitted from the training process and used to test the model predictions. A learning rate of 0.001 was used, resulting in models with 1500–3050 trees depending on the *Bryobia* lineage. The relative contribution of each predictor variable to the model was estimated using standard practice.[59,60]

Predictor variables for our BRT models consisted of a subset of four standard BIOCLIM variables obtained from the WorldClim database,[61] which are derived from average monthly (1950–2000) temperature and precipitation data. The variables describe means, trends and seasonal variations of temperature and precipitation, which are more likely to represent physiological limits for species.[62] To enable comparison of estimated environmental responses, we used the same BIOCLIM variable subset across all models fitted to each *Bryobia* lineage. This subset was selected by examining pair-wise Pearson's correlation coefficients of predictor variables across the sampling locations in the study. BIOCLIM variables with the highest correlation were removed stepwise until no pairwise correlation coefficient exceeded 0.5 (preferencing lower BIOCLIM variables). This resulted in four BIOCLIM variables that were used across all lineages: BIO01 – Annual Mean Temperature; BIO3 – Isothermality (Mean Diurnal Range/Temperature Annual Range) (×100); BIO9 – Mean Temperature of Driest Quarter; and BIO12 – Annual Precipitation.

This study focused on the winter cropping regions of Australia, largely excluding the large interior regions of Australia's rangelands. Thus, to avoid model extrapolation, the analysis was restricted to areas outside of Australia's rangelands. This was achieved using Australian rangeland boundaries as defined by the Australian Government's Department of Agriculture, Water and the Environment, and the Australian Collaborative Rangelands Information System (ACRIS), reducing the total number of locations used in our climate suitability analysis from 495 to 454.

2.5　Niche overlap

Following Schoener,[63] we calculated the niche overlap $n_{x,y}$ between mite lineages *x* and *y* as the proportional overlap of presence observations across all sample sites where at least one lineage was recorded or $n_{x,y} = 1 - \frac{1}{2}\sum_i |p_{x,i} - p_{y,i}|$, where $p_{x,i}$ and $p_{y,i}$ are the relative frequencies of lineage *x* and *y*, respectively, across site *i*.

2.6　Chemical responses of mites

To examine the chemical responses of *Bryobia* mites, five populations were collected from fields and roadsides from Vic and NSW in July 2019 (Table 1). Mites were collected via suction as described above using a Stihl SH55 blower vacuum. Populations were placed into plastic containers with paper towelling and vegetation, and then transported back to the laboratory. Mites were kept cool prior to undertaking insecticide bioassays.

Two commonly used insecticides, the organophosphate omethoate (Le-Mat, Cheminova, NSW, Australia) and the pyrethroid bifenthrin (Talstar 250EC, FMC Australasia, NSW, Australia), were used to generate sensitivity data. Le-Mat contains 290 g L^{-1} of omethoate and the solution representing the recommended field rate for *Bryobia* mites was 348 mg a.i. L^{-1}. Talstar 250 EC contains 250 g L^{-1} of bifenthrin and the solution representing the recommended field rate for *Bryobia* mites was 200 mg a.i. L^{-1} of bifenthrin. Bioassays were performed following the methodology described in Arthur *et al.*[32] but adapted for plastic vials. Seven to

Table 1. Collection details of *Bryobia* populations collected and screened against omethoate and bifenthrin using laboratory bioassays

Population	Latitude	Longitude	Date collected	Plant host
Manangatang	−35.056	143.003	03 July 2019	*Medicago sativa*
Coolamon	−34.813	147.145	04 July 2019	Broadleaf grasses (Gramineae)
Cootamundra	−34.443	147.966	04 July 2019	*Urtica dioica*
Harden	−34.659	148.184	05 July 2019	*Urtica dioica*
Beggan	−34.636	148.294	05 July 2019	*Urtica dioica*

nine concentrations of omethoate ranging from 1×10^{-5} to the field rate and eight concentrations of bifenthrin ranging from 1×10^{-5} to the field rate, with the addition of the surfactant Tween at 0.01% (v/v) (Ecoteric 20; Thermo Fisher Scientific, Victoria, Australia), were serially diluted and tested against each population. For each insecticide concentration, approximately 10 mL of solution was poured into a 15-mL plastic vial and swirled to ensure complete coating, with excess liquid discarded. Between six and eight replicate vials were coated for each concentration and left to dry upside down overnight. The control vials were treated in the same manner, except water (with the addition of 0.01% Tween) was used. Eight *Bryobia* adults were then placed into each replicate vial along with two leaves of common vetch (*Vicia sativa*), which provided food and humidity. Vials were placed at 18 °C for 8 h. After this time, individuals were scored as alive (moving freely), incapacitated (inhibited movement) or dead (no movement over a 5-s period).[32] After scoring, mites from each population were separately placed into Eppendorf tubes containing 100% ethanol and frozen at −20 °C. Fourteen individual mites from each population were later Sanger sequenced to identify *Bryobia* haplotypes following the methods described above.

As incapacitated individuals invariably die and therefore do not contribute to the next generation, we pooled incapacitated individuals with dead individuals for data analysis across all bioassays. Mite mortality in each bioassay (Y) was modelled as a binomial response variable $Y \sim \text{Binomial}(p, n)$, where p is the probability of mortality and n is the number of mites in each replicate. The probability of mortality was related to linear covariates with a logistic link function $\text{logit}(p) = a_i + b_i \ln(x)$, where x is chemical dose (mg a.i. L^{-1}) and a_i and b_i are intercepts and slope coefficients, respectively, for each population i.[64,65] Variance was robustly estimated by allowing for overdispersion.[66]

Each chemical product was analysed separately, with differences in population effects (intercepts) assessed using an analysis of variance (ANOVA) and then examined in pairwise comparisons using Tukey's honest significant difference (HSD) method at the 0.05 significance level, which corrects for type I errors when performing multiple hypotheses tests.[67] LC$_{50}$ values (with 95% confidence intervals) and slope coefficient estimates were computed. All analyses were performed using the R language for statistical computing.[68]

3 RESULTS

3.1 Bryobia haplotypes

In total, mites from almost 200 locations across Australia, representing approximately 1388 individual *Bryobia*, were sequenced for the partial (~355 bp) 28S rDNA region using either the pooled NGS amplicon approach (1260 mites, 167 locations) and/or the individual mite Sanger sequencing approach (128 mites, 29

locations). Population samples sequenced included mites from 62 locations in NSW, 42 locations in WA, 41 locations in Vic, 23 locations in Qld, 14 locations in SA and seven locations in Tas. Phylogenetic analyses included 272 bp of the 28S rDNA region, allowing the inclusion of sequences from Ros *et al.*[2] and Arthur *et al.*[31] We obtained 47 unique haplotypes from these sequences after filtering. The phylogeny generated from these sequences and the BI node support are shown in Fig. 2. Of the 47 haplotypes, seven were identical to the putative species identified in Arthur *et al.*[31] (*B.* sp. I, *B.* sp. IV, *B.* sp. VII, *B.* sp. VIII, *B.* sp. IX, *B.* sp. X and *B. praetiosa*). Similar support to the topology found in Arthur *et al.*[31] was also found here, with the same two major clades identified: clade 1 contained putative species *B.* sp. I, *B.* sp. IV, *B.* sp. V, *B.* sp. VII, *B.* sp. VIII, *B.* sp. IX, and *B.* sp. X and clade 2 contained *B. praetiosa*, *B. kissophila*, *B. berlesei*, *B. sarothamni*, *B. rubioculus*, *B.* sp. III and *B.* sp. VI. Numerous new haplotypes (indicated here as different OTUs) were also found within or in-between each of the seven putative species from Arthur *et al.*[31] With the new OTUs identified in this study, there was less support for some of the putative species identified previously, as indicated by the BI posterior probabilities for nodes (Fig. 2).

The PCoA yielded two significant axes that explained the majority of the variation in pairwise genetic distances among haplotype sequences; PCo1 explained 59.6% of the variation and PCo2 explained 26.7% of the variation. Other PCo axes explained less than 5% of the variation in genetic distances and were not significantly different from random expectations. There were four distinct clusters identifiable in PCo space (Fig. 3), which is largely congruent with the BI tree, but some clusters include separate taxa with strong nodal support (Fig. 2). Cluster 1 (herein referred to as lineage 1) included *B.* sp. I, *B.* sp. V, *B.* sp. IX, *B.* sp. X and 28 OTUs, while cluster 2 (herein referred to as lineage 2) included *B.* sp. VII and four OTUs. Cluster 3 (herein referred to as lineage 3) is taxonomically diverse and included *B. praetiosa* and OTU43. The clade where this cluster is located also included *B. kissophila*, *B. sarothamni*, *B. cf. rubrioculus*, *B.* sp. III and *B.* sp. VI, but importantly showed deep nodes with bootstrap values that support the separation of these taxa (Fig. 2). Cluster 4 (herein referred to as lineage 4) included *B.* sp. IV, *B.* sp. VIII and seven OTUs.

Our analysis raises doubt over the differentiation of the putative *Bryobia* species identified in Arthur *et al.*[31] given some previously defined species clustered together in this study which incorporated many more sequences. Instead, *Bryobia* mites in Australia exhibit a hierarchically structured species complex of four major clades, with several morphologically identified species and putative species nested within one of these clades (lineage 3). This is further supported by the genetic distances within and between the previously defined species of *Bryobia* sequenced for the 28S rDNA gene (Table S1). The pairwise distances among *B.* sp. I, *B.* sp. V, *B.* sp. IX and *B.* sp. X (lineage 1) ranged from 0.7% to 2.6%,

Figure 2. Bayesian inference tree for 28S rDNA sequence showing relationships between unique haplotypes (OTUs) from this study and putative species identified in Ros *et al.*[2] and Arthur *et al.*[31] as well as other related taxa. The broad genetic lineages identified through clustering analysis in this study are shown. Numbers at nodes indicate posterior probabilities (nodal support). Genbank accession numbers are provided in parenthesis.

while the genetic divergence between other putative species were all >7.1%. Pairwise distances between *B.* sp. VII (lineage 2) and other putative species were substantial, ranging from 7.1% to 9.1%, while pairwise distances between *B. praetiosa* (lineage 3) and other putative species ranged from 7.9% to 11.2%. The genetic distances among *B.* sp. IV and *B.* sp. VII (lineage 4) were <0.6%, with divergence between other putative species in the range 9.9–16.1% (Table S1).

3.2 Distribution of mite lineages and environmental suitability

Distributions of the four *Bryobia* lineages from sampling locations across Australia are shown in Fig. 4. The most common genetic lineages detected across the 454 locations were lineage 1 (127 locations) and lineage 4 (88 locations). Lineage 2 was found at 15 locations. Mites comprising lineage 3 were only found at five locations. Lineage 1 was found across WA, SA, Vic, NSW, Tas and Qld, while the distribution of lineage 4 was slightly more restricted and found in WA, SA, Vic and NSW. *Bryobia* lineage 2 was restricted to south-eastern Australia (Vic, NSW and Tas), while

the *Bryobia* clade comprising lineage 3 was only found in Vic and the southern region of NSW.

Environmental suitability maps based on the species distribution models are also depicted in Fig. 4. With the exception of the *Bryobia* taxa falling into lineage 3, the species distribution models fit the data well, achieving a receiver operating characteristic (ROC) score of 0.88–0.96 for the training data and 0.73–0.82 for the cross-validation (cv) dataset depending on the lineage. A suitable distribution model could not be fitted to lineage 3, which is not surprising given the relatively few records detected here for *B. praetiosa* and related OTUs. BIO12 (annual precipitation) was generally the most influential variable in the models, contributing 11.5–46.9% to the models, while BIO03 (isothermality) contributed the least at 10.9–25.3% (Fig. S1). The low overlap in ecological niche observable in Fig. 4 is also demonstrated by the low values calculated for Schoener's index (Table S2), with the exception of lineages 1 and 4, which exhibited a moderate overlap of 0.35.

The estimated environmental responses varied for mite lineages (Fig. 5). The environmental response of *Bryobia* lineage 1 tended to increase with higher annual mean temperatures (BIO01) but decrease with higher mean temperature of the driest quarter
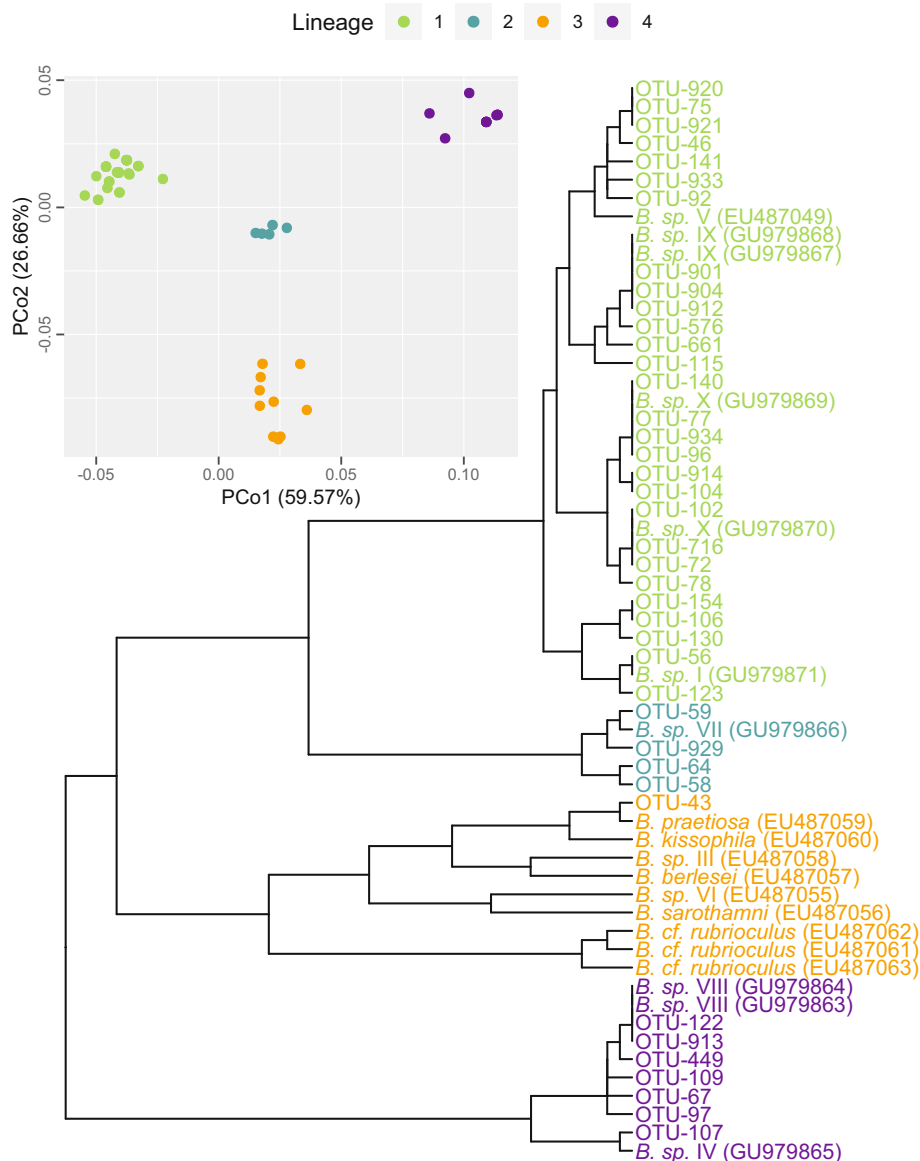
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2865

Figure 3. A hierarchical clustering tree of genetic distances between *Bryobia* haplotypes (OTUs) identified from Arthur *et al.*,[31] Ros *et al.*[2] and this study. Inset: scatter plot of sequences in PCo space. The labels of the tree and the points in the scatter plot are coloured with respect to $K = 4$ groups, as inferred from K-means analysis of PCo1 and PCo2. Genbank accession numbers are provided in parenthesis.

(BIO09) (Fig. 5). In contrast, the response of lineage 2 tended to increase with annual precipitation (BIO12) and decrease with high annual temperatures (BIO01), leading to a predicted distribution concentrated in cooler areas on the Australian mainland and most of Tas (Figs 4 and 5). Similar to lineage 1, *Bryobia* lineage 4 was found across a wide range, with the exception of eastern coastal regions, which appears to be driven by the strong negative relationship with annual precipitation (BIO12) (Figs 4 and 5).

3.3 Chemical responses of Bryobia mites

Control mortality of mites was less than 2% across all insecticide laboratory bioassays (Fig. 6). LC$_{50}$ values for bifenthrin ranged from 5.59 [95% confidence interval (CI) 0.37–84.68] to 17.12 (0.01–22 668.9) mg a.i. L^{-1} and regression coefficients from 0.475 ($\pm$0.061) to 2.668 ($\pm$3.538). LC$_{50}$ values for omethoate ranged from 0.12 (0.03–0.61) to 4.87 (1.65–14.31) mg a.i. L^{-1} and regression coefficients from 1.058 ($\pm$0.599) to 1.735 ($\pm$0.960) (Table 2).

There was no significant difference in the response of the five mite populations to bifenthrin ($\chi^2 = 1.75$, df = 4, $P = 0.78$) and there were also no differences when comparing LC$_{50}$ values which had overlapping 95% CIs (Table 2). This indicates similar bifenthrin sensitivity across all tested populations. Unique regression slopes for each population exposed to bifenthrin did not significantly improve the model ($\chi^2 = 1.2$, df = 4, $P = 0.88$). In contrast, differences in dose responses were detected between populations exposed to omethoate ($\chi^2 = 10.08$, df = 4, $P = 0.04$), although unique regression slopes did not significantly improve the model ($\chi^2 = 0.50$, df = 4, $P = 0.97$). Population differences are further supported by nonoverlapping 95% CIs of LC$_{50}$ values (Table 2). Manangatang exhibited the highest LC$_{50}$ value for omethoate, which was 40.5-fold greater than the LC$_{50}$ value of the most susceptible population from Cootamundra, while Coolamon had an LC$_{50}$ value approximately 9-fold greater than Cootamundra. Mites from Beggan,
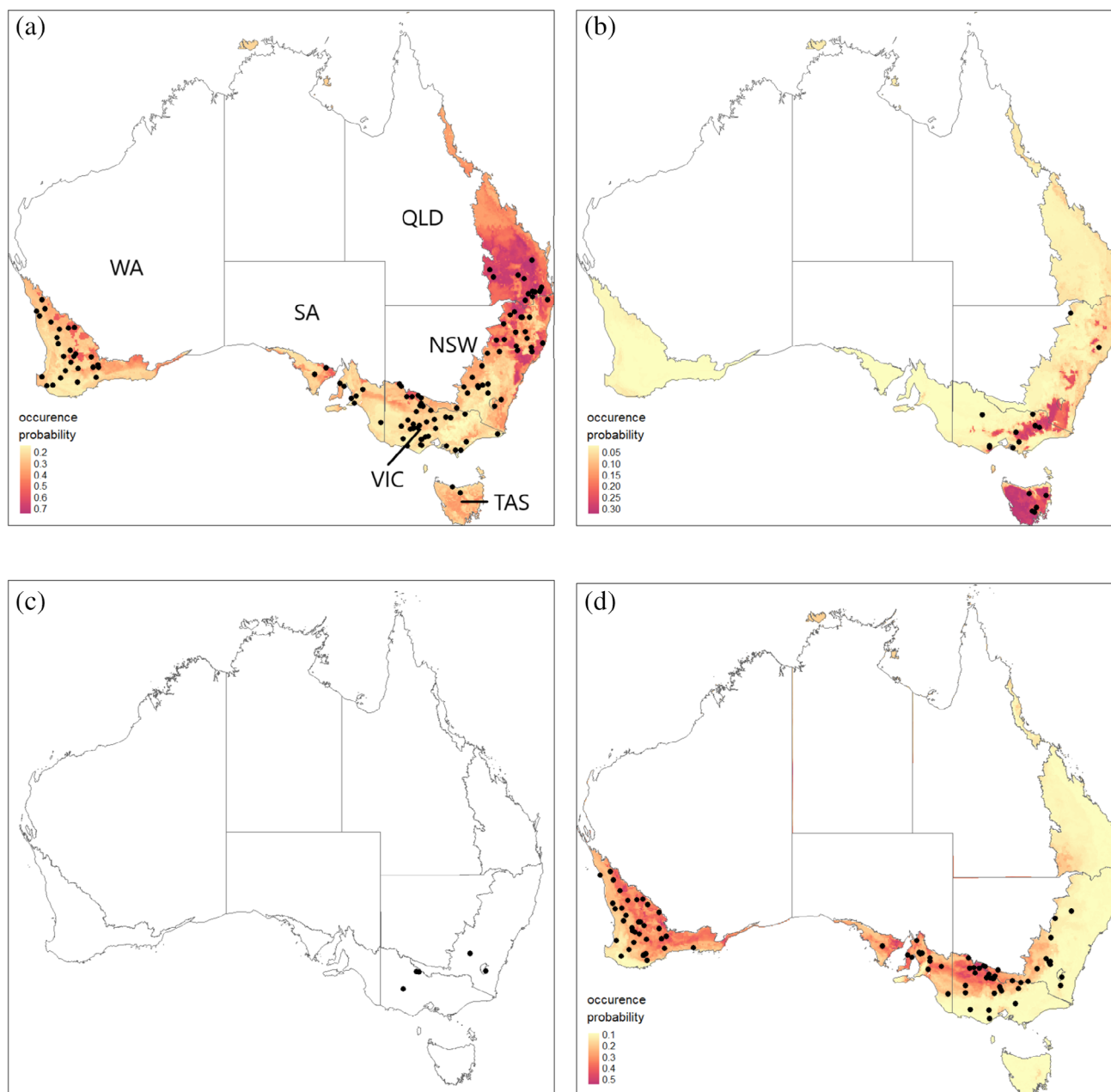
Figure 4. Distributions and boosted regression tree (BRT) model outputs of climate suitability for *Bryobia* mite lineages in Australia at a resolution of 0.05° (~5 km). (a) *Bryobia* lineage 1, (b) *Bryobia* lineage 2, (c) *Bryobia* lineage 3 and (d) 4 *Bryobia* lineage 4. The shading represents the predicted probability of presence based on BIOCLIM variables and fitted BRT models. Black circles represent locations where each lineage was detected.

Harden and Cootamundra had similar dose–response curves and LC_{50} values for omethoate (Fig. 6 and Table 2).

Sequencing of the 28S rDNA region revealed that Manangatang and Coolamon consisted of mites from putative species *B.* sp. IX (i. e. lineage 1), while Cootamundra, Harden and Beggan consisted of mites from putative species *B.* sp. VIII (i.e. lineage 4). These results indicate that mite lineages differ in chemical responses.

4 DISCUSSION

Bryobia mites are a significant pest of pastures and winter grain crops in Australia and elsewhere,[23–25,27,29] yet studies on this mite complex are limited. Arthur *et al.*[31] were the first to apply genetic markers to explore the biology of Australian *Bryobia* mites and suggested the presence of cryptic species. In this study, we explored the 28S rDNA gene region, which has been successfully used previously to infer species relationships among *Bryobia* species.[2] Not only did we find the same seven putative species (*B.* sp. I, *B.* sp. IV, *B.* sp. VII, *B.* sp. VIII, *B.* sp. IX, *B.* sp. X and *B. praetiosa*) identified in Arthur *et al.*,[31] but we also identified a further 40 unique haplotypes compared with only 10 identified by Arthur *et al.*[31] for the 28S rDNA region. Consequently, there is less support for the clear differentiation of these seven putative species. Rather, our more comprehensive dataset supports the existence of several broad genetic lineages of *Bryobia* mites within Australian grain crops and pastures. One of these, lineage 3, shows strong
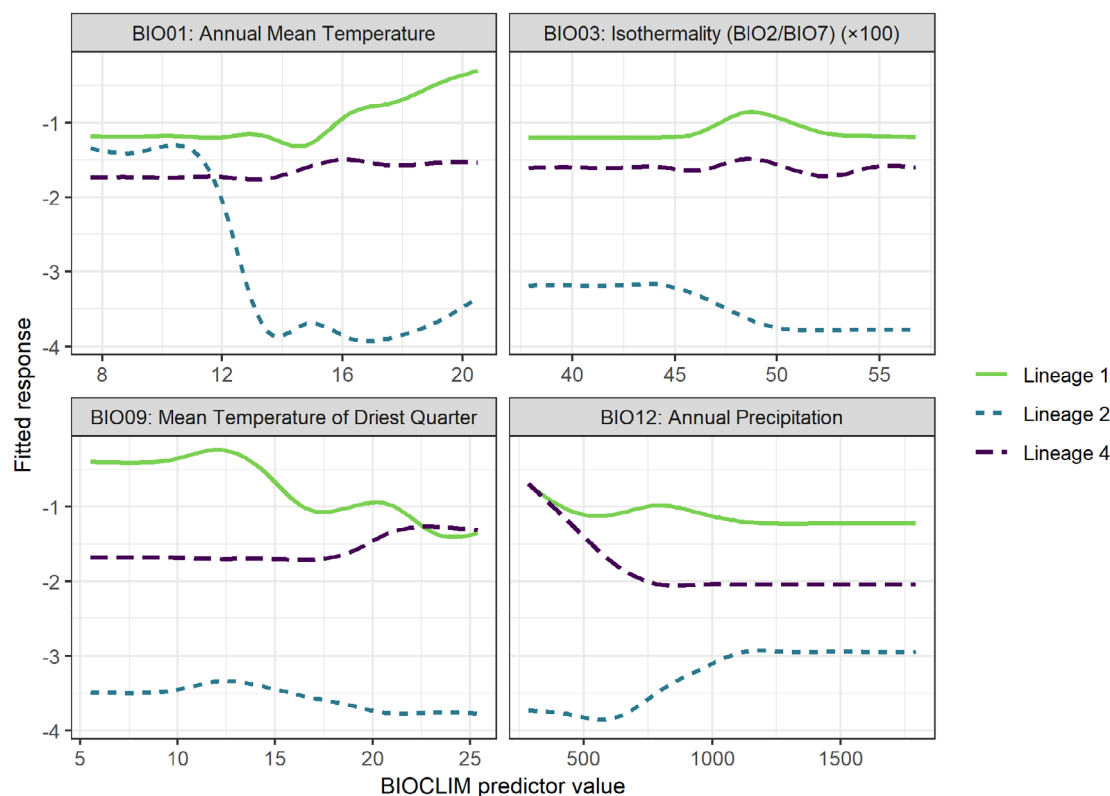
Figure 5. Partial dependency plots for four BIOCLIM predictors for *Bryobia* lineages 1, 2 and 4, as determined from a boosted regression tree model (other variables fixed at their average values). Lines are smoothed using a generalised additive model regression spline. The *y* axes are on a logit scale.

nodal support that indicates the presence of multiple species nested within this particular clade. Each of the remaining three lineages may very well represent three distinct species, although delineating species based on genetic data alone (even genome wide analysis) is challenging (see Galtier[69]), especially in taxa that undergo obligate or facultative asexual reproduction, like many *Bryobia*.

Bryobia mites are widespread in regions of Australia exhibiting Mediterranean-type climates (WA, SA, Vic, Tas and NSW), which are characterised by hot dry summers and cool moist winters. However, the *Bryobia* lineages characterised in this study had different climatic niches based on our species distribution models. *Bryobia* lineage 1 showed the broadest distribution, being found in all Australian states sampled; it was also the most abundant lineage sampled and had the highest haplotypic diversity (32 haplotypes, 127 locations). *Bryobia* lineage 2, consisting of five haplotypes, was only detected at 15 sites, and these were restricted to the south-eastern region of Australia. Mites comprising *Bryobia* lineage 3 were only found at five sites in Vic and southern NSW, and likely represent the cosmopolitan species *B. praetiosa*. Similar to lineage 1, *Bryobia* lineage 4 was abundant among our sample sites, with a widespread distribution across WA, SA, Vic and NSW, although it was absent from eastern coastal regions. The species distribution models highlight that *Bryobia* mite lineages respond to climatic factors in quite different ways. Lineages 1 and 4 generally expand into Australia's interior rangelands, where precipitation is low. Conversely, lineage 2 appears to be more limited by average temperatures and is predominantly found in cooler areas. The unique ecological niches of these lineages were also supported by the generally low values estimated for Schoener's index. These

different environmental responses are likely linked to inherent variation in traits like tolerance of thermal extremes and dry conditions. Such traits have been previously associated with distributions and adaptive shifts in other crop mites in Australia[70] and may be useful in generating hypotheses for future work around interspecific and interclonal variation.

Apart from lineage 3, which includes different putative species, the high degree of haplotype diversity detected in our study in some lineages likely reflects clonal diversity given the majority of *Bryobia* species reproduce asexually, with males in populations being rare.[2,31] Lineage 3 contains 10 haplotypes, most of which were not found in this study. However, the plant hosts for some of these putative species (or haplotypes) were not sampled here. To understand the extent of variation in this lineage and delineate species more accurately, further sampling should focus on other hosts, as well as other geographic locations. For instance, congruous with Arthur *et al.*,[31] we did not detect *B. rubrioculus* or *B. kissophila*, which have previously been morphologically identified from Australia.[3,30] This likely reflects our sampling approach, which focused on grain crops, pastures and roadside vegetation and did not cover host plants known to be important for these species, such as fruit trees (*B. rubrioculus*) and English ivy (*B. kissophila*).[1,2,18] Also similar to Arthur *et al.*,[31] we did not detect *B. graminum*, which is surprising given this species has been identified from Australia[3,30] and is reported to feed on pasture plants.[1,18] Interestingly, *Bryobia praetiosa* (including OTU-43) was identified in our study, but only at five locations, which is inconsistent with the notion that this species is a major pest of pasture plants and grain crops in southern Australia.[21,71]

The amplicon NGS metabarcoding approach adopted here for the 28S rDNA region was an efficient method for screening a large
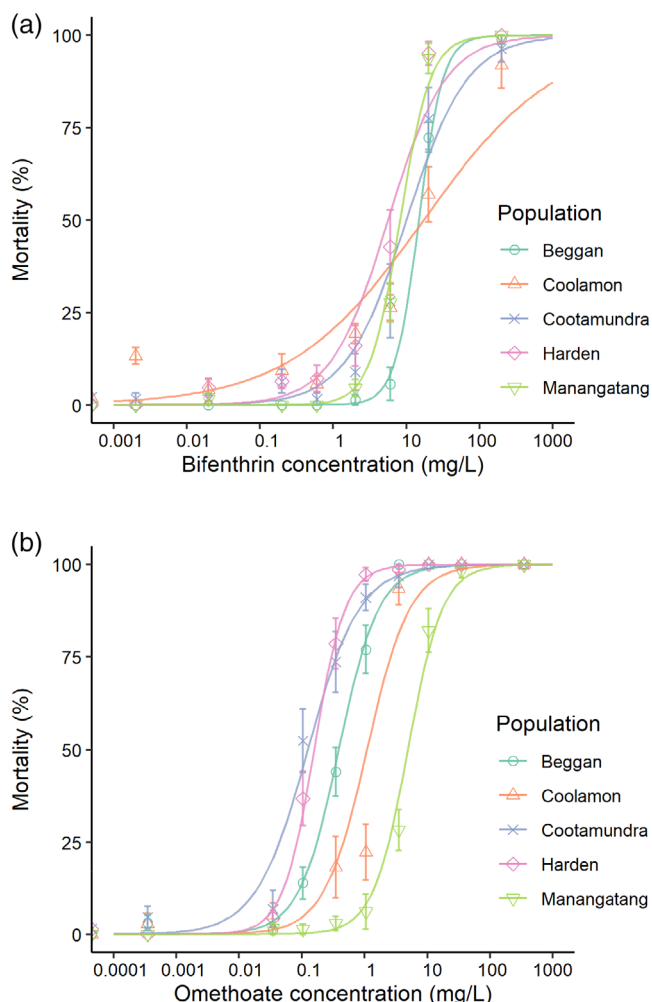
Figure 6. Dose–response curves for field populations of *Bryobia* mites after exposure to (a) bifenthrin and (b) omethoate for 8 h in laboratory bioassays. Error bars represent standard error of the mean. Manangatang and Coolamon are putative species *B.* sp. IX (i.e. lineage 1). Cootamundra, Harden and Beggan are putative species *B.* sp. VIII (i.e. lineage 4).

number of samples collected from over 167 populations. The method identified 47 haplotypes from >1200 mites, highlighting its efficiency compared with regular Sanger sequencing.

However, this approach may under-represent the true genetic diversity found in Australian *Bryobia* mites, as the pooled amplicon-based NGS method and conservative filtering approach adopted here to account for sequencing error will miss some low frequency haplotypes. Nevertheless, our results should reflect the dominant lineages and haplotypes of *Bryobia* found in Australian grain and pasture environments.

The discovery of multiple lineages of *Bryobia* mites inhabiting vast areas of agricultural landscapes in Australia signifies possible management challenges for farmers. A series of studies undertaken in the late 1990s and early 2000s showed considerable differences between cryptic *Penthaleus* mites, which share many life-history attributes with *Bryobia* species, including asexuality.[72] The three *Penthaleus* pest species present in Australia differ markedly in their distributions,[35,73] host plants[74] and summer diapause strategies.[75] These species also differ in their response to insecticides,[36,76] which is correlated with chemical control failures in the field.[36,72] The insecticide bioassays conducted in this study showed significant population differences also exist in *Bryobia*. Of the five *Bryobia* populations tested in our study, three were found to belong to lineage 4 and two to lineage 1. The lineage 1 populations had significantly higher LC_{50} values for omethoate (Manangatang 4.87 mg a.i. L^{-1}, Coolamon 1.09 mg a.i. L^{-1}) compared with all three populations belonging to lineage 4 (which ranged from 0.12 to 0.39 mg a.i. L^{-1}), indicating a higher level of tolerance to this chemical. These results suggest important insecticide tolerance differences between genetic lineages of *Bryobia* mites, potentially explaining inconsistencies experienced by Australian farmers when attempting to control *Bryobia* mites with insecticides registered in grain crops and pastures.[22,33] Differences in the climatic envelopes inhabited by *Bryobia* lineages may help inform management strategies in the absence of rapid field diagnostics. For example, lineage 1 is far more common in eastern Australia (NSW and Qld) compared with lineage 4; if the chemical tolerances observed in the present study hold across their observed range, it may be prudent for farmers in eastern NSW and Qld to minimise usage of organophosphates for *Bryobia* mite control.

In conclusion, this study supports the presence of a diverse species complex of *Bryobia* mites in Australia. However, contrary to an earlier delineation of seven putative species in Australia,[31] 28S rDNA barcoding of *Bryobia* populations collected across much of the Australian grain-growing region supports the differentiation

Table 2. LC_{50} values [and 95% confidence intervals (CIs)] and regression coefficients (and standard errors) for *Bryobia* mite populations when exposed to omethoate and bifenthrin for 8 h

Insecticide	Population	*Bryobia* lineage	LC_{50} value (95% CI) mg a.i. L^{-1}	Regression coefficient $b \pm SE$
Bifenthrin	Coolamon	1	17.12 (0.01–22 668.9)	0.475 ± 0.608
	Manangatang	1	8.15 (0.87–76.03)	2.118 ± 3.078
	Beggan	4	14.68 (2.96–72.80)	2.668 ± 3.538
	Cootamundra	4	9.94 (0.34–290.11)	1.048 ± 1.242
	Harden	4	5.59 (0.37–84.68)	1.115 ± 1.131
Omethoate	Coolamon	1	1.09 (0.20–5.79)	1.275 ± 0.840
	Manangatang	1	4.87 (1.65–14.31)	1.578 ± 0.816
	Beggan	4	0.39 (0.13–1.18)	1.370 ± 0.631
	Cootamundra	4	0.12 (0.03–0.61)	1.058 ± 0.599
	Harden	4	0.16 (0.05–0.45)	1.753 ± 0.960

The genetic lineage of each population, as determined by 28S rDNA sequencing, is also shown.

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2869

of four broad lineages, one of which (lineage 3) contains a number of distinct species. Additionally, our findings demonstrate that important ecological differences exist between these lineages, including their distribution, climatic suitability and response to insecticides. This highlights the importance of developing management strategies that consider genetic lineages separately, which necessitates the development of tools to readily distinguish *Bryobia* mites in the field. Currently, this requires considerable skill and experience, and is heavily reliant on genetic markers, with morphological identification by itself unlikely to be satisfactory for separating some lineages.[2,4,31,39] Although focused on Australia, this study demonstrates the challenges of studying *Bryobia* and highlights the importance of further research into this complex group of mites across the world.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

REFERENCES

1 Bolland HR, Gutierrez J and Flechtmann CHW, *World Catalogue of the Spider Mite Family (Acari: Tetranychidae)*. Brill, Leiden, Boston, Koln (1998).
2 Ros VID, Breeuwer JAJ and Menken SBJ, Origins of asexuality in *Bryobia* mites (Acari: Tetranychidae). *BMC Evol Biol* **8**:153 (2008).
3 Halliday RB, *Mites of Australia: A Checklist and Bibliography*. CSIRO Publishing, Melbourne, Victoria, Australia (1998).
4 Roosje GS and van Dinther JBM, The genus Bryobia and the species *Bryobia praetiosa* Koch. *Entomol Ber* **14**:327–336 (1953).
5 Pritchard AE and Keifer HH, Two new species of *Bryobia* with a revised key to the genus (Acarina: Tetranychidae). *Ann Entomol Soc Am* **51**:503–506 (1958).
6 Smith Meyer MK, Four new species of *Bryobia* Koch (Acari: Tetranychidae) from South Africa, with a revised key to the African species. *Phytophylactica* **24**:1–8 (1992).
7 van Eyndhoven GL and Vacante V, The Berlesei-group of the genus *Bryobia* Koch (Acari, Tetranychidae). *Redia* **68**:377–437 (1985).
8 Smith Meyer MKP, African Tetranychidae (Acari: Prostigmata) – with reference to the world genera. *Entomol Mem Dep Agric Water Supply Repub South Africa* **69**:1–175 (1987).
9 Hatzinikolis EN and Panou HN, Two new species of *Bryobia* (Acari: Tetranychidae) from forest trees in Greece. *Acarologia* **38**:51–56 (1997).
10 Menken SB, Pattern and process in the evolution of insect-plant associations: *Yponomeuta* as an example. *Entomol Exp Appl* **80**:297–305 (1996).
11 Weeks AR and Breeuwer JAJ, *Wolbachia*-induced parthenogenesis in a genus of phytophagous mites. *Proc R Soc London Ser B Biol Sci* **268**:2245–2251 (2001).
12 Norton RA, Kethley JB, Johnston DE and O'Connor BM, Phylogenetic perspectives on genetic systems and reproductive modes of mites, in *Evolution and Diversity of Sex Ratio in Insects and Mites*, ed. by Wrensch DL and Ebbert MA. Chapman and Hall, New York, pp. 8–99 (1993).
13 Helle W and Sabelis MW, *World Crop Pests 1A. Spider Mites. Their Biology, Natural Enemies and Control*. Elsevier Science Publishing, Amsterdam (1985).
14 Helle W and Bolland HR, Karyotypes and sex-determination in spider mites (Tetranychidae). *Genetica* **38**:43–53 (1967).
15 Ros VID and Breeuwer JAJ, The effects of, and interactions between, Cardinium and Wolbachia in the doubly infected spider mite *Bryobia sarothamni*. *Heredity (Edinb)* **102**:413–422 (2009).
16 Ros VID, Fleming VM, Feil EJ and Breeuwer JAJ, How diverse is the genus Wolbachia? Multiple-gene sequencing reveals a putatively new *Wolbachia* supergroup recovered from spider mites (Acari: Tetranychidae). *Appl Environ Microbiol* **75**:1036–1043 (2009).
17 Ros VID, Fleming VM, Feil EJ and Breeuwer JAJ, Diversity and recombination in *Wolbachia* and *Cardinium* from *Bryobia* spider mites. *BMC Microbiol* **12**:1–15 (2012).
18 Jeppson LR, Keifer HH and Baker EW, *Mites Injurious to Economic Plants*. University of California Press, Berkeley, USA (1975).
19 Anderson NH and Morgan CVG, Life-histories and habits of the clover mite, *Bryobia praetiosa* Koch, and the brown mite, *B. arborea* M. & A., in British Columbia (Acarina: Tetranychidae). *Can Entomol* **90**:23–42 (1958).
20 Hoffmann AA, Weeks AR, Nash MA, Mangano GP and Umina PA, The changing status of invertebrate pests and the future of pest management in the Australian grains industry. *Aust J Exp Agric* **48**:1481–1493 (2008).
21 Micic S, Hoffmann AA, Strickland G, Weeks AR, Bellati J, Henry K *et al.*, Pests of germinating grain crops in southern Australia: an overview of their biology and management options. *Aust J Exp Agric* **48**:1560–1573 (2008).
22 Arthur AL, Weeks AR, Hill MP and Hoffmann AA, The distribution, abundance and life cycle of the pest mites *Balaustium medicagoense* (Prostigmata: Erythraeidae) and *Bryobia* spp. (Prostigmata: Tetranychidae) in Australia, *Aust. Aust J Entomol* **50**:22–36 (2011).
23 Arthur AL, Weeks AR, Umina PA and Hoffmann AA, Survival and reproduction of the pest mites *Balaustium medicagoense* and *Bryobia* spp. on winter grain crops. *Exp Appl Acarol* **52**:141–153 (2010).
24 Smith Meyer MKP and Craemer C, Mites (Arachnida: Acari) as crop pests in southern Africa: an overview. *African Plant Prot* **5**:37–51 (1999).
25 Prasad V, Plant feeding mites of India (Acarina: Tetranychoidea): II. Genera *Bryobia*, *Bryobiella*, *Neopetrobia* and *Petrobia*. *Int J Acarol* **1**:12–20 (1975).
26 Hatzinikolis EN and Emmanouel NG, A revision of the genus *Bryobia* in Greece (Acari: Tetranychidae). *Entomol Hell* **9**:21–34 (1991).
27 Gratwick M, *Bryobia Mites, Crop Pests in the UK*. Springer, Dordrecht, pp. 329–333 (1992).
28 Skorupska A and Kozlowski J, Intensity of occurrence of eriophyoid mites (Acari, Eriophyoidea) and spider mites (Acari, Tetranychidae) on wild grasses. *J Plant Prot Res* **43**:415–420 (2003).
29 Beaulieu F and Knee W, Plant-feeding mites of the Canadian prairies. *Arthropods Can Grasslands* **3**:29–72 (2014).
30 Halliday RB, Additions and corrections to mites of Australia: a checklist and bibliography. *Aust J Entomol* **39**:233–235 (2000).
31 Arthur AL, Miller AD and Weeks AR, Genetic markers indicate a new species complex of emerging pest mites in Australian grains. *Ann Entomol Soc Am* **104**:402–415 (2011).
32 Arthur AL, Hoffmann AA, Umina PA and Weeks AR, Emerging pest mites of grains (*Balaustium medicagoense* and *Bryobia* sp.) show high levels of tolerance to currently registered pesticides. *Aust J Exp Agric* **48**:1126–1132 (2008).
33 PestFacts, A big year for *Bryobia* mites, 2015. http://www. cesaraustralia.com/sustainable-agriculture/pestfacts-south-eastern/past-issues/2015/pestfacts-issue-no-2-22nd-may-2015/bryobia-mites/.
34 Qin TK and Halliday RB, The Australian species of Chromotydaeus Berlese and Penthaleus C. L. Koch (Acarina: Penthaleidae). *J Nat Hist* **30**:1833–1848 (1996).
35 Weeks AR and Hoffmann AA, The biology of *Penthaleus* species in southeastern Australia. *Entomol Exp Appl* **92**:179–189 (1999).
36 Umina PA and Hoffmann AA, Tolerance of cryptic species of blue oat mites (*Penthaleus* spp.) and the redlegged earth mite (*Halotydeus destructor*) to pesticides. *Aust J Exp Agric* **39**:621 (1999).

37 Navajas M and Fenton B, The application of molecular markers in the study of diversity in acarology: a review. *Exp Appl Acarol* **24**:751–774 (2000).

38 Cruickshank RH, Molecular markers for the phylogenetics of mites and ticks. *Syst Appl Acarol* **7**:3–14 (2002).

39 Fashing NJ, Ueckermann EA, Fashing PJ, Nguyen N, Back AM and Allison LA, Bryobia abyssiniae (Prostigmata: Tetranychidae), a new species from the highlands of Ethiopia. *Int J Acarol* **42**:366–376 (2016).

40 Li G, Xue X-F, Zhang K-J and Hong X-Y, Identification and molecular phylogeny of agriculturally important spider mites (Acari: Tetranychidae) based on mitochondrial and nuclear ribosomal DNA sequences, with an emphasis on Tetranychus. *Zootaxa* **15**:1–15 (2010).

41 Hinomoto N, Tran DP, Pham AT, Ngoc Le TB, Tajima R, Ohashi K *et al.*, Identification of spider mites (Acari: Tetranychidae) by DNA sequences: a case study in northern Vietnam. *Int J Acarol* **33**:53–60 (2007).

42 Ros VID and Breeuwer JAJ, Spider mite (Acari: Tetranychidae) mitochondrial COI phylogeny reviewed: host plant relationships, phylogeography, reproductive parasites and barcoding. *Exp Appl Acarol* **42**:239–262 (2007).

43 Nishimura S, Hinomoto N and Takafuji A, The genetic variations among populations of *Tetranychus kanzawai* complex (Acari: Tetranychidae) indicated by using mitochondrial, ribosomal, and microsatellite DNA markers. *J Acarol Soc Japan* **16**:109–119 (2007).

44 Navajas M, Host plant associations in the spider mite *Tetranychus urticae* (Acari: Tetranychidae) insights from molecular phylogeography. *Exp Appl Acarol* **22**:201–214 (1998).

45 Hinomoto N and Takafuji A, Genetic diversity and phylogeny of the Kanzawa spider mite, *Tetranychus kanzawai* in Japan. *Exp Appl Acarol* **25**:355–370 (2001).

46 Hurtado MA, Ansaloni T, Cros-Arteil S, Jacas JA and Navajas M, Sequence analysis of the ribosomal internal transcribed spacers region in spider mites (Prostigmata: Tetranychidae) occurring in citrus orchards in eastern Spain: use for species discrimination. *Ann Appl Biol* **153**:167–174 (2008).

47 Fadrosh DW, Ma B, Gajet P, Sengamalay N, Ott S, Brotman RM *et al.*, An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the Illumina MiSeq platform. *Microbiome* **2**:6 (2014).

48 Rognes T, Flouri T, Nichols B, Quince C and Mahe F, VSEARCH: a versatile open source tool for metagenomics. *PeerJ* **4**:e2584 (2016).

49 Martin M, Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet J* **17**:10–12 (2011).

50 Gouy M, Guindon S and Gascuel O, SeaView version 4: a multiplatform graphical user interface for sequence alignmnet and phylogenetic tree building. *Mol Biol Evol* **27**:221–224 (2010).

51 Ronquist F, Teslenko M, van der Mark P, Ayres D, Darling A, Hohna S *et al.*, MrBayes 3.2.: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst Biol* **61**:539–542 (2012).

52 Posada D and Crandall KA, MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**:817–818 (1998).

53 Akaike H, *Information Theory as an Extension of the Maximum Likelihood Principle, Second International Symposium on Information Theory, 2–8 September 1971, Tsahkadsor, Armenia*. Akademiai Kiado, Budapest, Hungary, pp. 267–281 (1973).

54 Paradis E and Schliep K, ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* **35**:526–528 (2019).

55 Jackson DA, Stopping rules in principal components analysis: a comparison of heuristical and statistical approaches. *Ecology* **74**:2204–2214 (1993).

56 Coombes KR and Wang M, *PCDimension: Finding the Number of Significant Principal Components. R package version 1.1.11* (2019).

57 Wickham H, *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag, New York (2016).

58 Guangchuang Y, Using ggtree to visualize data on tree-like structures. *Curr Protoc Bioinforma* **69**:e96 (2020).

59 Friedman JH, Stochastic gradient boosting. *Comput Stat Data Anal* **38**:367–378 (2002).

60 Elith J, Leathwick JR and Hastie T, A working guide to boosted regression trees. *J Anim Ecol* **77**:802–813 (2008).

61 Hijmans RJ, Cameron SE, Parra JL, Jones PG and Jarvis A, Very high resolution interpolated climate surfaces for global land areas. *Int J Climatol* **25**:1965–1978 (2005).

62 Graham CH and Hijmans RJ, A comparison of methods for mapping species ranges and species richness. *Glob Ecol Biogeogr* **15**:578–587 (2006).

63 Schoener TW, Nonsynchronous spatial overlap of lizards in patchy habitats. *Ecology* **51**:408–418 (1970).

64 Robertson JL and Preisler HK, *Pesticide Bioassays with Arthropods*. CRC, Boca Raton (1992).

65 Venables WN and Ripley BD, *Modern Applied Statistics with S*. Springer, New York (2002).

66 McCullagh P and Nelder JA, *Generalized Linear Models*. Chapman and Hall, London, UK (1989).

67 Hsu JC, *Multiple Comparisons*. Chapman and Hall, London (1996).

68 R Core Team, *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria (2020).

69 Galtier N, Delineating species in the speciation continuum: a proposal. *Evol Appl* **12**:657–663 (2019).

70 Hill MP, Chown SL and Hoffmann AA, A predicted niche shift corresponds with increased thermal resistance in an invasive mite, Halotydeus destructor. *Glob Ecol Biogeogr* **22**:942–951 (2013).

71 Pavri C, Legume pastures, in *Pests of Field Crops and Pastures: Identification and Control*, ed. by Bailey PT. Landlinks Press, Melbourne, Victoria, Australia, pp. 412–424 (2007).

72 Umina PA, Hoffmann AA and Weeks AR, Biology, ecology and control of the Penthaleus species complex (Acari: Penthaleidae). *Exp Appl Acarol* **34**:211–237 (2004).

73 Hill MP, Hoffmann AA, MacFadyen S, Umina PA and Elith J, Understanding niche shifts: using current and historical data to model the invasive redlegged earth mite, *Halotydeus destructor*. *Divers Distrib* **18**:191–203 (2012).

74 Umina PA and Hoffmann AA, Plant host associations of *Penthaleus* species and *Halotydeus destructor* (Acari: Penthaleidae) and implications for integrated pest management. *Exp Appl Acarol* **33**:1–20 (2004).

75 Umina PA and Hoffmann AA, Diapause and implications for control of *Penthaleus* species and *Halotydeus destructor* (Acari: Penthaleidae) in southeastern Australia. *Exp Appl Acarol* **31**:209–223 (2003).

76 Robinson MT and Hoffmann AA, Additional tests on the effects of pesticides on cryptic species of blue oat mite (*Penthaleus* spp.) and the redlegged earth mite (*Halotydeus destructor*). *Aust J Exp Agric* **40**:671–678 (2000).