



Genetic diversity of *Glycaspis brimblecombei* (Hemiptera: Aphalaridae) and its parasitoid *Psyllaephagus bliteus* (Hymenoptera: Encyrtidae) in Brazil

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Eucalyptus plantations are attacked by exotic pests that decrease their productivity and burden the production chain. Global trade has contributed to the introduction of exotic pests in many countries and these pests are now a main concern of the forestry sector (Brockhoff et al., 2006; Liebhold et al., 2012). Studies of the distribution patterns, genetic diversity, gene flow, and geographic origin of these pests are important to develop and implement pest-management programs in *Eucalyptus* plantations. Understanding the pests' origins and invasion patterns is essential to prevent future invasions (Wingfield et al., 2008; Nadel et al., 2010).

The red gum lerp psyllid, *Glycaspis brimblecombei* (Moore) (Hemiptera: Aphalaridae), is one of the main exotic pests of *Eucalyptus* in Brazil. This sap-sucking insect causes loss of photosynthetic area and dryness of the upper part of the crown, retarding trees growth. Severe attacks may even kill the trees (Wilcken et al., 2003). *Glycaspis brimblecombei*, originally from Australia, was first reported in California, USA in 1998 and in Mexico in 2000 (Brennan et al., 1999; Paine et al., 2006). Subsequently, *G. brimblecombei* was detected in Africa (Mauritius) and South America (Chile) in 2001, Brazil in 2003, and Europe in 2007, in the Iberian Peninsula (Wilcken et al., 2003; Olivares et al., 2004; Valente and Hodkinson, 2009). The most recent reports of *G. brimblecombei* invasions are in Ethiopia, New Zealand and Zambia (Chungu et al., 2017; Yirgu and Anjulo, 2019).

In Brazil, *G. brimblecombei* has been parasitized by the micro-wasp initially believed to be *Psyllaephagus bliteus* Riek (Hymenoptera: Encyrtidae). However, outbreaks of *G. brimblecombei* have coincided with low incidences of parasitism by *P. bliteus* in some parts of the country in recent years (Tuller et al., 2017). Understanding the diversity and possible genetic lineages of the pest and its parasitoid in different Brazilian regions can generate essential information to improve management of *G. brimblecombei* in Brazil. Our objectives were: (i) to evaluate the genetic diversity of *G. brimblecombei* and *P. bliteus* collected in different regions of Brazil; and (ii) to compare the mitochondrial

genetic lineages from Brazil of *G. brimblecombei* and *P. bliteus* with previously identified genetic lineages from other regions of the world.

At least 20 specimens of *G. brimblecombei* were collected in Grajaú, Maranhão (05°52'29"S 46°03'42"W), Três Lagoas, Mato Grosso do Sul (20°35'26"S 51°37'08"W), Itamarandiba, Minas Gerais (17°53'18"S 42°51'22"W), Telemaco Borba, Paraná (24°17'47"S 50°37'10"W), Guaíba, Rio Grande do Sul (30°08'57"S 51°21'12"W) and Botucatu, São Paulo (22°50'44"S 48°26'04"W), Brazil. *Psyllaephagus bliteus* specimens were collected from Três Lagoas, Mato Grosso do Sul (20°35'26"S 51°37'08"W), Itamarandiba, Minas Gerais (17°53'18"S 42°51'22"W) and Botucatu, São Paulo (22°50'44"S 48°26'04"W), Brazil. All specimens were collected from commercial *Eucalyptus* plantations and stored in 100% ethanol at –20 °C. Total genomic DNA was extracted from adult insects, using the CTAB (cetyltrimethylammonium bromide) protocol, adapted from Doyle and Doyle (1990).

For *G. brimblecombei*, a fragment of the Cytochrome c Oxidase Subunit I (COI) gene fragment was amplified using the C1-J-2441 and TL2-N-3014 primers (Simon et al., 1994). For *P. bliteus*, a fragment of the Cytochrome B gene fragment was amplified using the CP1 and CB2 primers described by Harry et al. (1998) and Jermin and Crozier (1994), respectively. The samples were sent for Sanger bidirectional sequencing at the Center for Agricultural Biotechnology (CEBETC/ESALQ), University of São Paulo.

A consensus dataset of 517-bp COI fragments was generated for 34 *G. brimblecombei* individuals (4-7 individuals per collection site). We included in our dataset 13 sequences of individuals from *G. brimblecombei* from Portugal (n = 5) and Australia (n = 8) (see Morrow et al., 2017). For *P. bliteus*, a 645-bp CytB fragment consensus dataset was generated for 12 individuals. Twenty-five sequences from different specimens of *Psyllaephagus* spp. (see Figure 1) were added to our dataset. The two datasets were individually aligned in MEGA X, using the ClustalW algorithm (Kumar et al., 2018). The sequences from Brazil were deposited at the NCBI

GenBank under access numbers MN615705 and MN615706 - MN615707 for *G. brimblecombei* and *Psyllaephagus*, respectively. Inference of the number of haplotypes for each species was performed using the software DnaSP 5.10 (Librado and Rozas, 2009). For *G. brimblecombei*, we constructed a haplotype network using the PopArt software (Leigh and Bryant, 2015). For *Psyllaephagus* spp., we constructed a Bayesian phylogenetic tree using Mr. Bayes (Huelsenbeck and Ronquist, 2001), following the steps described by Oliveira et al. (2013).

A single *G. brimblecombei* haplotype (H1) was found in Brazil and is shared with individuals from Portugal (Figure 2). The three other haplotypes, H2, H3 and H4, are originated from Australia. Our results suggest that one *G. brimblecombei* invasion events occurred in Brazil and it was probably originated from locals previously invaded as USA, Mexico or Chile and not from Australia, origin geographic region, as already reported for other pests in Brazil (Nadel et al., 2010; Dittich-Schröder et al., 2018; Bortolotto et al., 2019; Cordeiro et al., 2019; Ferronato et al., 2019).

The large genetic distance from the H4 haplotype to the other haplotypes suggests the presence of different *G. brimblecombei* genetic lineages in Australia, its region of origin (Figure 2). This result emphasizes the importance of phytosanitation planning to prevent the invasion of new *G. brimblecombei* genetic lineages into Brazil, since hybridization between different lineages could increase the adaptive potential of a pest and complicate efforts

to suppress populations (Bertelsmeier and Keller, 2018; Corrêa et al., 2019).

We found two mitochondrial haplotypes/lineages among the individuals that were initially identified morphologically as *P. bliteus* in Brazil. When submitted to BLAST-NCBI (NCBI, 2019), the P1 haplotype showed high similarity to *P. bliteus* (homology > 99%). However, the P2 haplotype showed 100% similarity to another species of *Psyllaephagus* from Australia (KU525267), which was not previously identified. The P1 haplotype identified as *P. bliteus* was found in four individuals collected from Botucatu and one from Itamarandiba. The P2 haplotype was found in four individuals from Três Lagoas and one individual from Botucatu. The Bayesian phylogenetic tree confirmed the presence of a high diversity of *Psyllaephagus* specimens in Australia, suggesting the occurrence of cryptic species that have not yet been taxonomically distinguished (Figure 1). The high diversity of the genus *Psyllaephagus* encourages prospecting for new natural enemies in Australia, to improve biological control of *G. brimblecombei* worldwide.

In conclusion, the presence of one haplotype in Brazil suggests that a single invasion event of *G. brimblecombei* originating from geographic areas previously invaded for this pest in the American continent. Furthermore, Brazil and Portugal share the same haplotype of *G. brimblecombei*, which shows a connection among invasion routes in the world. Finally, two distinct parasitoids species of the genus *Psyllaephagus* were found in Brazil, one them *P. bliteus* and the other one without taxonomic identification.

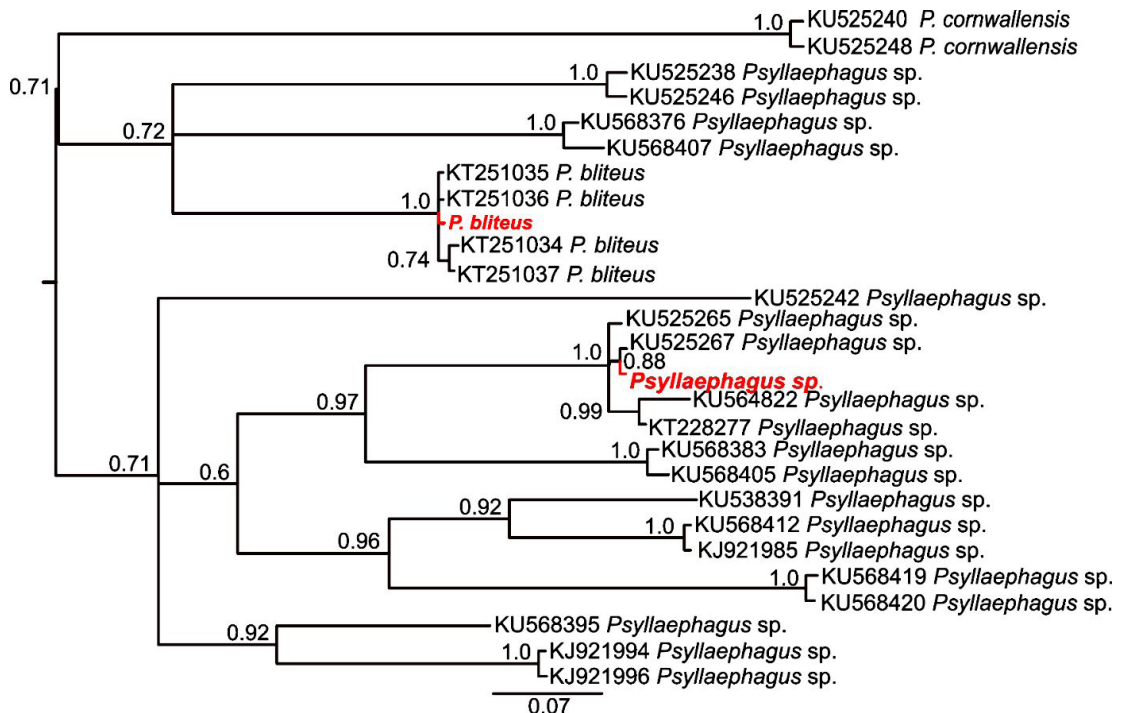


Figure 1. Bayesian phylogenetic tree of *Psyllaephagus* spp. based on the Cytochrome B gene fragment.

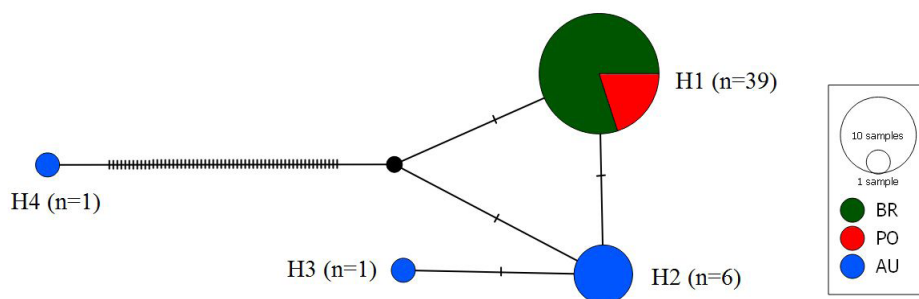


Figure 2. Haplotype network based on the Cytochrome c Oxidase Subunit I gene fragment for *Glycaspis brimblecombei* (Hemiptera: Aphalaridae) from Brazil (BR), Portugal (PO) and Australia (AU).

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