# *Distribution and genetic diversity of five invasive pests of* Eucalyptus *in sub-Saharan Africa*

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ORIGINAL PAPER

# Distribution and genetic diversity of five invasive pests of *Eucalyptus* in sub-Saharan Africa

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**Abstract** *Eucalyptus* is one of the most planted tree genera across the world, but is heavily challenged by invasive insect pests originating from the native range of these trees. The rate of introduction of non-native *Eucalyptus*-feeding insects has increased globally, including in sub-Saharan Africa where *Eucalyptus* trees have an important socio-economic role. In this study, we mapped the distribution and examined the genetic diversity of non-native *Eucalyptus* insect pests in 14 countries across sub-Saharan Africa. We focused on five foliage-feeding insect pests of *Eucalyptus* which are known to be present in the region, namely the bluegum chalcid wasp, *Leptocybe invasa*; the redgum lerp psyllid, *Glycaspis brimblecombei*; the bronze bug, *Thaumastocoris peregrinus*; the Eucalyptus weevil, *Gonipterus* sp.n.2; and the Eucalyptus gall wasp, *Ophelimus maskelli*. Insect samples were collected through structured surveys and small-scale sampling which were both combined with published literature to determine the distribution of these insect pests. Genetic diversity of each of these insect pests was estimated/assessed based on mitochondrial

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cytochrome oxidase I (COI) or cytochrome b (Cyt b) sequence data. Except *O. maskelli*, which is a relatively recent arrival, the other insect pests were found broadly distributed across the sampled countries, with first reports in many countries. Analysis of genetic diversity confirmed a common origin of geographically distant populations for *G. brimblecombei* and *O. maskelli*, moderate diversity for *T. peregrinus* and *Gonipterus* sp.n.2 and at least two distinct lineages for *L. invasa*. Two divergent haplogroups of *L. invasa*, with overlapping geographic range were confirmed in Ghana, Malawi, Sierra Leone, South Africa and Zimbabwe. Compared to published literature, new haplotypes were detected for *T. peregrinus*, *Gonipterus* sp.n.2 and *L. invasa*, suggesting multiple introduction of those pests in the region. Results of this study will have implications for quarantine, management and future research of *Eucalyptus* insect pests in the region and beyond.

**Keywords** Plantation forestry · COI · Cyt b · *Glycaspis brimblecombei* · *Gonipterus* · *Leptocybe invasa* · *Ophelimus maskelli* · *Thaumastocoris peregrinus*

## Introduction

*Eucalyptus* has been grown in sub-Saharan Africa since the nineteenth century when the bluegum, *Eucalyptus globulus*, was introduced into the Cape Province of South Africa (Bennett and Kruger 1983). Following this, many other *Eucalyptus* species have been introduced to South Africa and other countries in Southern Africa (Olivier 2009). In the second half of the 19th and early twentieth century, *Eucalyptus* plantings have dramatically expanded in East Africa in countries such as Ethiopia, Kenya, Rwanda, Uganda, Sudan and Burundi, to the extent that these trees have become a prominent part of the urban and rural landscapes (Louppe and Depommier 2010; FAO 2011). The recent increase in *Eucalyptus* plantings in sub-Saharan Africa is partly attributed to established plantations in Uganda, Rwanda, Tanzania, Zimbabwe (https://www.saazimbabwe.org, accessed September 2018), Ghana and Sierra Leone (https://www.miroforestry.com, accessed September 2018).

The huge gap between demand and supply of wood in sub-Saharan Africa makes the use of non-native tree species indispensable, with *Eucalyptus* currently being the most common species in planted forests in the region (Olivier 2009; Louppe and Depommier 2010; FAO 2011). *Eucalyptus* provides many socio-economic benefits including its use for construction materials, fuelwood, poles, timber, paper, pulp, oil, medicine, tannin and fibre, and is also a source of employment (Reynolds 1986; FAO 2011). *Eucalyptus* generates substantial household income and helps in buffering financial problems for many poor farmers in sub-Saharan Africa (Munishi 2007; Teshome 2009; FAO 2011). In rural communities, *Eucalyptus* stands are used as important guarantors of land tenure, access

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to credit, honey production and environmental conservation through their role in gully stabilization, soil conservation and strengthening road embankments (FAO 2011).

Insect pests and diseases challenge the production of *Eucalyptus* worldwide. This is due both to the accidental introduction of non-native insect pests and pathogens from the native range of *Eucalyptus* (Australia, Papua New Guinea and Indonesia), and the expansion of host range of native insects and pathogens to new *Eucalyptus* hosts used as plantation species (Wingfield et al. 2008, 2015). A recent review showed that the rate of introduction of non-native eucalypt-feeding insects globally has increased nearly five-fold between the 1980s and 2010s (Hurley et al. 2016). This includes many major eucalypt-feeding insects that have been introduced to sub-Saharan Africa, such as the bluegum chalcid wasp, *Leptocybe invasa* Fisher and La Salle (Hymenoptera: Eulophidae); the redgum lerp psyllid, *Glycaspis brimblecombei* Moore (Hemiptera: Psyllidae); the bronze bug, *Thaumastocoris peregrinus* Carpintero and Dellapé (Hemiptera: Thaumastocoridae); an undescribed species of the *Gonipterus scutellatus* species complex, *Gonipterus* sp.n.2 (Coleoptera: Curculionidae); and the Eucalyptus gall wasp, *Ophelimus maskelli* Ashmead (Hymenoptera: Eulophidae) (FAO 2009; Bush et al. 2016; Hurley et al. 2017). These invasive insects inevitably pose a serious threat to the sustainability of *Eucalyptus* forestry in the region.

*Leptocybe invasa* and *O. maskelli* are minute gall-forming wasps. *Leptocybe invasa* has become established in numerous countries, spanning the Mediterranean Basin, Europe, Asia, Africa, South America and North America (Nugnes et al. 2015; Le et al. 2018). Infestation by this wasp produces distinct galls on leaf petioles, midribs and stems of new foliage of both young and mature *Eucalyptus* trees and in severe cases this may lead to stunted growth and tree mortality (Dittrich-Schröder et al. 2018). In sub-Saharan Africa, it was first reported in Ethiopia in 2002 (Giliomea 2011), and subsequently spread to several countries in the continent (Mutitu et al. 2007; Zheng et al. 2014). *Ophelimus maskelli* has become established in Argentina, Ethiopia, France, Indonesia, Israel, Italy, Portugal, South Africa, Spain and Vietnam (Mendel et al. 2007; Branco et al. 2009; Burks et al. 2015; Asfaw 2018). It damages *Eucalyptus* through induction of numerous small pimple-like galls on both sides of the leaf surface (Branco et al. 2009). In sub-Saharan Africa, it was recently reported in Ethiopia (Asfaw 2018) and South Africa (Bush et al. 2016) and has a potential to spread to other countries in the region.

*Thaumastocoris peregrinus* and *G. brimblecombei* are sap-sucking insects. *Thaumastocoris peregrinus* has become established in New Zealand and several countries in Africa, Europe, and South America (Nadel et al. 2010; Laudonia and Sasso 2012; Sopow and George 2012; Garcia et al. 2013; Martins and Zarbin 2013). Both adults and nymphs feed on fully expanded leaves, initially leading to reddening of leaves. As the infestation progresses the whole canopy turns reddish-yellow to reddish-brown and eventually results in loss of canopy leaves (Nadel and Noack 2012). In sub-Saharan Africa, it was first reported from South Africa in 2003 (Jacobs and Neser 2005) and since then has spread to other countries in the region. *Glycaspis brimblecombei* has become established in Africa, Europe, North and South America and the Mediterranean (Reguia and Peris-Felipo 2013; Spodek et al. 2015). Both the adults and the nymphs feed on the sap, which eventually leads to leaf discoloration and in case of heavy infestations to severe leaf drop, twig dieback and occasional whole tree death (Bella and Rapisarda 2013). In sub-Saharan Africa, it was first reported from Mauritius in 2001 (Sookar et al. 2003) and has continued to spread to other countries in the region.

Cryptic species of the *Gonipterus scutellatus* complex have become significant pests of *Eucalyptus* in their origin in Australia and invasive ranges in Africa, the Americas, Europe and New Zealand (Mapondera et al. 2012). Both adults and larvae feed on leaves and cause severe damage to *Eucalyptus* trees (Tooke 1955). A taxonomic study of *G. scutellatus* populations across its native and invasive ranges proved that it is composed of eight cryptic species, the population in South Africa, France and Italy being an undescribed species *Gonipterus* sp.n.2 (Mapondera et al. 2012). In sub-Saharan Africa, the first report of this weevil dates back to 1916 in South Africa (Tooke 1955) and since then has spread to other countries in the region.

Other non-native insect pests of *Eucalyptus* such as the eucalyptus psyllid, *Blastopsylla occidentalis* Taylor (Hemiptera: Psyllidae); the bluegum psyllid,

*Ctenarytaina eucalypti* (Maskell) (Hemiptera: Psyllidae); the shell lerp psyllid, *Spondyliaspis* c.f. *plicatuloides* Froggatt (Hemiptera: Psyllidae); the eucalyptus tortoise beetle, *Trachymela tincticollis* (Blackburn) (Coleoptera: Chrysomelidae), the eucalyptus longhorned borers *Phoracantha recurva* Newman and *Phoracantha semipunctata* (Fabricius) (Coleoptera, Cerambycidae) have also been introduced to sub-Saharan Africa (FAO 2009; Bush et al. 2016; Hurley et al. 2017). However, most of these insects were not observed causing significant threats to *Eucalyptus* production in the region.

Knowledge of non-native insect pests of *Eucalyptus* in sub-Saharan Africa is limited. In their review, Hurley et al. (2017) presented a list of major non-native insect pests of plantation trees, including *Eucalyptus*, for the region. However, the list includes information for only six countries, and even for those countries it is often based on informal literature. The broader distribution of these insects within the region is poorly documented. In addition, their populations may contain multiple divergent lineages and cryptic species. For instance, at least two lineages of the invasive *L. invasa* have been confirmed to have spread across the globe (Nugnes et al. 2015; Dittrich-Schröder et al. 2018). The eucalyptus weevil, *G. scutellatus*, has been shown to be a species complex, with three known species introduced outside their native range (Mapondera et al. 2012). More generally, the distribution and the genetic diversity of invasive *Eucalyptus* insects in sub-Saharan Africa is largely unknown, bringing concerns about the correct identification of pest species and the implementation of effective management approaches.

We examined the distribution and genetic diversity of non-native *Eucalyptus* insect pests in sub-Saharan Africa. Insect specimens were collected from 14 countries in the region through structured surveys and small-scale sampling. Sequence data from COI or Cyt b genes were used to confirm species identification, determine presence of cryptic species, and examine genetic diversity. The collections focused on the main insect pests of *Eucalyptus* that feed on the foliage and are known to be present in the region, namely *L. invasa*, *G. brimblecombei*, *T. peregrinus*, *Gonipterus* sp.n.2, and *O. maskelli* (FAO 2009; Bush et al. 2016; Hurley et al. 2017).

## Materials and methods

### Collection of insect pests

The distribution and genetic diversity of *Eucalyptus* insect pests was investigated in 14 countries in sub-Saharan Africa, namely the Democratic Republic of Congo (DRC), Ethiopia, Ghana, Kenya, Madagascar, Malawi, Mauritius, Reunion, Rwanda, Sierra Leone, South Africa, Uganda, Zambia and Zimbabwe (Table 1). Information on the presence of *Eucalyptus* insect pests in these countries was collected through structured surveys, small-scale sampling, personal communications with collaborating experts across the region and from published literature.

Surveys were conducted in six countries, including Ethiopia, Ghana, Rwanda, Sierra Leone, Zimbabwe and South Africa in a range of 14 to 48 sites per country. A maximum of 100 trees per site were assessed for the presence of *Eucalyptus* insect pests. Trees were consecutively assessed and the direction of the transect line was changed at every fifth tree in a 'zig-zag' fashion. In sites where the infestation was high, the assessment was stopped as soon as the first 50 infested trees were obtained, even when it happened before assessing 100 trees. In each infested site, insect specimens were collected from multiple trees along the transect. In the other eight countries, small-scale sampling were conducted. Compared to the surveys, small-scale sampling were less structured collections obtained from one or two localities per country. Insect samples were collected from February 2017 to October 2018 (Table 1). For *T. peregrinus* and *Gonipterus* sp.n.2, most life stages, including eggs, larvae/nymphs and adults and for *G. brimblecombei* nymphs and adults were collected directly from infested trees. For *L. invasa* and *O. maskelli*, infested plant parts were collected, incubated in emergence cages and the emerging adults were captured. All insect specimens were preserved in absolute ethanol and kept frozen at − 20 °C until DNA extraction.

### DNA extraction, amplification and sequencing

DNA was extracted from insect samples that were collected from multiple trees per site to reduce the probability of including samples of related individuals, i.e. offspring from same parent. The preserved insect specimens were rinsed with sterilized distilled

**Table 1** Sub-Saharan Africa countries included in this study and insects collected

| No. | Country | Insect pests collected | Collection year | No. of locality | No. trees sampled | No. of insects sequenced |
|---|---|---|---|---|---|---|
| 1 | DRC | *Thaumastocoris peregrinus* | 2017 | 1 | 3 | 3 |
| 2 | Ethiopia | *Leptocybe invasa* | 2018 | 8 | 68 | 32 |
| | | *Glycaspis brimblecombei* | 2018 | 10 | 57 | 25 |
| | | *Ophelimus maskelli* | 2018 | 1 | 5 | 25 |
| 3 | Ghana | *Leptocybe invasa* | 2018 | 2 | 26 | 25 |
| 4 | Kenya | *Glycaspis brimblecombei* | 2018 | 11 | 10 | 25 |
| | | *Thaumastocoris peregrinus* | 2018 | 5 | 6 | 25 |
| | | *Gonipterus* sp.n.2 | 2018 | 2 | 2 | 21 |
| 5 | Madagascar | *Leptocybe invasa* | 2017–2018 | 2 | 15 | 23 |
| | | *Glycaspis brimblecombei* | 2017 | 1 | 2 | 2 |
| | | *Thaumastocoris peregrinus* | 2017 | 1 | 2 | 2 |
| | | *Ophelimus maskelli* | 2015 | 1 | 4 | 7 |
| 6 | Malawi | *Leptocybe invasa* | 2017 | 3 | 3 | 54 |
| | | *Glycaspis brimblecombei* | 2017 | 4 | 9 | 25 |
| | | *Thaumastocoris peregrinus* | 2017 | 2 | 2 | 20 |
| 7 | Mauritius | *Glycaspis brimblecombei* | 2017 | 1 | 4 | 30 |
| | | *Thaumastocoris peregrinus* | 2017 | 5 | 5 | 5 |
| 8 | Reunion | *Leptocybe invasa* | 2018 | 3 | 5 | 5 |
| | | *Glycaspis brimblecombei* | 2017 | 5 | 7 | 38 |
| | | *Thaumastocoris peregrinus* | 2017 | 3 | 7 | 9 |
| 9 | Rwanda | *Leptocybe invasa* | 2017 | 3 | 2 | 5 |
| | | *Glycaspis brimblecombei* | 2017 | 5 | 12 | 25 |
| | | *Thaumastocoris peregrinus* | 2017 | 5 | 30 | 48 |
| | | *Gonipterus* sp.n.2 | 2017 | 3 | 14 | 17 |
| | | *Ophelimus maskelli* | 2017 | 5 | 30 | 48 |
| 10 | Sierra Leone | *Leptocybe invasa* | 2018 | 2 | 25 | 25 |
| 11 | South Africa | *Glycaspis brimblecombei* | 2013 | 3 | 30 | 28 |
| | | *Thaumastocoris peregrinus* | 2018 | 2 | 2 | 20 |
| | | *Gonipterus* sp.n.2 | 2018–2019 | 4 | 33 | 25 |
| | | *Ophelimus maskelli* | 2014 | 2 | 2 | 5 |
| | | | 2018 | 1 | 3 | 20 |
| 12 | Uganda | *Glycaspis brimblecombei* | 2017 | 4 | 7 | 26 |
| | | *Thaumastocoris peregrinus* | 2017 | 2 | 3 | 27 |
| 13 | Zimbabwe | *Leptocybe invasa* | 2017 | 7 | 70 | 47 |
| | | *Glycaspis brimblecombei* | 2017 | 6 | 60 | 25 |
| | | *Thaumastocoris peregrinus* | 2017 | 3 | 7 | 13 |
| | | *Gonipterus* sp.n.2 | 2017 | 2 | 5 | 5 |
| 14 | Zambia | *Glycaspis brimblecombei* | 2017 | 2 | 3 | 21 |
| | | *Thaumastocoris peregrinus* | 2017 | 2 | 5 | 24 |

water and DNA was extracted from the whole insect, except for *Gonipterus* sp.n.2 where DNA was extracted from the thorax muscles. Total genomic DNA was extracted from 957 specimens (215 *L. invasa*, 348 *G. brimblecombei*, 221 T. *peregrinus*, 68 *Gonipterus* sp.n.2 and 105 *O. maskelli*) using

*prep*GEM™ Insect DNA extraction kit (ZyGEM) following the manufacturer's protocol (ZyGEM Quick-Start Guide). Quality of the extracted DNA was assessed through electrophoresis on 1% agarose gel. All extracted DNA was stored at − 20 °C.

*Leptocybe invasa*—A 530 bp fragment of the barcoding region of COI gene was amplified from 215 specimens (Online Resource 1) using the primers LiLCO1490: 5′-ATT TGA TCT GGA ATT TTA GG-3′ (Dittrich-Schröder et al. 2018) and HCO2198 (C1-N-2173): 5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′ (Folmer et al. 1994). Amplification reactions were performed following the procedures in Dittrich-Schröder et al. (2018).

*Glycaspis brimblecombei*—A 451 bp fragment of COI gene was amplified from 348 specimens (Online Resource 1) using the set of universal primers C1-N-2191: 5′-CCC GGT AAA ATT AAA ATA TAA ACT TC-3′ and C1-J-1718: 5′-GGA GGA TTT GGA AAT TGA TTA GTT CC-3′ (Simon et al. 1994). Amplification reactions were performed in a 25 µL reaction volume which included 15.5 µL distilled water, 5 µL 5 × MyTaq consisting of dNTPs, $MgCl_2$ and ready to use enhancers (Bioline), 1 µL of each primer (10 µM), 0.5 µL MyTaq™ DNA polymerase (5U $\mu L^{-1}$, Bioline) and 2 µL of diluted insect genomic DNA (50 ng $\mu L^{-1}$). The PCR reactions were run at 95 °C for 3 min, followed by 35 cycles at 95 °C for 20 s, 54 °C for 30 s and 72 °C for 30 s and a final extension at 72 °C for 7 min.

*Thaumastocoris peregrinus*—A 462 bp fragment of COI gene was amplified from 221 specimens (Online Resource 1) using the primers Tp2390F: 5′-ACC CGA GCA TAC TTT ACT TC-3′ (Nadel et al. 2010) and TL2-N-3014: 5′-TCC AAT GCA CTA ATC TGC CAT ATT A-3′ (Simon et al. 1994). Amplification reactions were performed using a slight modification of the procedures in Nadel et al. (2010) with 12.5 µL distilled water, 2.5 µL dNTP mix (10 mM each), and 1 µL of each primer (10 µM).

*Gonipterus* sp.n.2—A 652 bp fragment of COI gene was amplified from 68 specimens (Online Resource 1) using the primers GON-F: 5′-GGA GTA CTC GGG ATA ATT TAC G-3′ (Mapondera et al. 2012) and TL2-N-3014 (PAT): 5′-TCC AAT GCA CTA ATC TGC CAT ATT A-3′ (Simon et al. 1994). Amplification reactions were performed in a 25 µL reaction volume which included 13.5 µL distilled water, 2.5 µL 10 × PCR buffer (Roche, Roche Diagnostics GmbH, Mannheim, Germany), 3 µL $MgCl_2$ (25 mM) (Roche), 2.5 µL dNTP mix (10 mM each) (Roche), 1 µL of each primer (10 µM), 0.5 µL FastStart *Taq* DNA polymerase (5U $\mu L^{-1}$) (Roche) and 1 µL of diluted insect genomic DNA (50 ng $\mu L^{-1}$). The reactions were run at 96 °C for 5 min, followed by 35 cycles at 94 °C for 1 min, 58 °C for 1 min and 72 °C for 1 min and 30 s and final extension at 72 °C for 10 min.

*Ophelimus maskelli*—A 704 bp fragment of mitochondrial Cyt b gene was amplified from 105 specimens (Online Resource 1) using the primers CP1: 5′-GAT GAT GAA ATT GGA TC-3′ (Harry et al. 1998) and CB2: 5′-ATT ACA CCT CCT AAT TTA TTA GGA AT-3′ (Jermiin and Crozier 1994). Here, Cyt b gene was used as a marker in order to compare sequences of *O. maskelli* samples from sub-Saharan Africa with GenBank Cyt b sequences of *O. maskelli* from Israel and South Africa. Moreover, previously this gene region was reliably used for identification of wasps, including *O. maskelli* (Bush et al. 2016).

Amplification reactions were performed using a slight modification of the procedure for *Gonipterus* sp.n.2 with 12.8 µL distilled water, 0.2 µL FastStart *Taq* DNA polymerase and 2 µL diluted insect genomic DNA (50 ng $\mu L^{-1}$). The reactions were run at 95 °C for 7 min, followed by 35 cycles at 95 °C for 1 min, 49 °C for 1 min and 72 °C for 1 min and final extension at 72 °C for 10 min.

For all specimens, electrophoresis of a mixture of 4 µL PCR aliquots and 2 µL GelRed™ (Biotium, USA) was run along with a 100 bp DNA molecular weight marker (Thermo Scientific O'Gene Ruler™) on 2% agarose gel. The amplified DNA fragments were visualized under UV light and gel images were captured using BioRad Gel Doc™ EZ Imager. The PCR products were cleaned by adding 8 µL of ExoSAP-IT (USB Corporation, Cleveland, OH) and incubating the mixture at 37 °C and 80 °C; 15 min at each temperature point.

The forward and reverse sequencing reactions were performed in a 12 µL reaction volume which included 6.5 µL distilled water, 2 µL sequencing buffer, 0.5 µL BigDye 3.1, 1 µL primers (10 µM) and 2 µL purified PCR product (50 ng $\mu L^{-1}$). The reactions were then run as follows: *L. invasa*.; 96 °C for 2 min followed by 30 cycles at 96 °C for 30 s, 55 °C for 15 s and 60 °C for 4 min; *G. brimblecombei*; 96 °C for 2 min followed by 30 cycles at 96 °C for 10 s, 54 °C for

15 s and 60 °C for 4 min; *T. peregrinus*; 95 °C for 10 s, followed by 30 cycles at 95 °C for 10 s, 48 °C for 5 s and 60 °C for 4 min; *Gonipterus* sp.n.2; 96 °C for 2 min, followed by 30 cycles at 96 °C for 30 s, 57 °C for 15 s and 60 °C for 4 min; and *O. maskelli*; 96 °C for 2 min, followed by 30 cycles at 96 °C for 30 s, 55 °C for 15 s and 60 °C for 4 min. The sequencing products were cleaned and sequenced using an ABI Prism™ 3100 Genetic Analyser (Applied BioSystems, USA). For most specimens from Reunion, Madagascar, Mauritius and DRC, PCR products were purified and sequenced by a subcontractor (Macrogen, Seoul, Republic of Korea).

### Genetic diversity analysis using sequence data

The raw sequence data were edited using Biological Sequence Alignment Editor (BioEdit) (Hall 1999) version 7.0.9. For comparison purpose, insect sequences from the native (Australia) and invasive ranges of the pests were obtained from GenBank. Sequences were aligned using an online Multiple Sequence Alignment Program (MAFFT) version 7 (https://mafft.cbrc.jp/alignment/software/) (Katoh and Standley 2013). The aligned sequences were further edited in BioEdit by comparing against sequencing trace files. Genetic diversity parameters such as the number of segregating sites (S), number of haplotypes (h), haplotype diversity (Hd), the average number of nucleotide differences (K) and nucleotide diversity (Pi) were calculated for each of the species using DNA Sequence Polymorphism (DnaSP) version 5.10.01 software (Librado and Rozas 2009). Pairwise and overall mean sequence divergence among haplotypes (haplogroups in case of *L. invasa*) were computed for all the insect species (except *O. maskelli* which has only one haplotype) using the Kimura 2-parameter model in molecular evolutionary genetics analysis (MEGA) software (Tamura et al. 2011). Haplotype network was constructed for all the insect species using Network software (Bandelt et al. 1999).

### Mapping the distribution of insect pests

The distribution of *Eucalyptus* insect pests in sub-Saharan Africa was mapped based on a combination of information obtained from the survey, small-scale sampling, personal communications and published literature. This distribution map indicates the presence of the respective insect pests in the different countries included in this study. The collections in these countries, especially for the small-scale sampling, was limited in terms of area covered and time of sampling, and thus the data does not confirm the absence of the insect pests in those countries.

## Results

### Distribution of non-native insect pests of Eucalyptus in sub-Saharan Africa

The current study identified and confirmed the presence of five major non-native insect pests of *Eucalyptus*, namely, *L. invasa*, *G. brimblecombei*, *T. peregrinus*, *Gonipterus* sp.n.2 and *O. maskelli*, in different countries across sub-Saharan Africa. *Leptocybe invasa* was confirmed to be present in thirteen of the fourteen countries included in the study. It is widely spread in East Africa (Ethiopia, Kenya, Rwanda, Uganda), West Africa (Ghana, Sierra Leone), Southern Africa (Malawi, South Africa, Zambia, Zimbabwe) and in all the islands included in the study, namely Madagascar, Mauritius and Reunion (Fig. 1b). *Glycaspis brimblecombei* was confirmed to be present in 11 of the countries included in this study, namely Ethiopia, Kenya, Madagascar, Malawi, Mauritius, Reunion, Rwanda, South Africa, Uganda, Zambia and Zimbabwe (Fig. 1c, d). Similarly, *T. peregrinus* was confirmed to be present in 11 of the countries included in this study, namely DRC, Kenya, Madagascar, Malawi, Mauritius, Reunion, Rwanda, South Africa, Uganda, Zambia and Zimbabwe (Fig. 1d). *Gonipterus* sp.n.2 and *O. maskelli* were confirmed from a relatively smaller number of countries. *Gonipterus* sp.n.2 was confirmed in Kenya, Rwanda, South Africa and Zimbabwe and *O. maskelli* was confirmed in Ethiopia, Madagascar, Rwanda and South Africa (Fig. 1e, f). The current study confirmed that out of the eight cryptic species of *Gonipterus scutellatus*, it is only *Gonipterus* sp.n.2 that is present in surveyed/sampled countries in sub-Saharan Africa.

Many of the species occurrences confirmed in this study were the first report of those insects in the countries. This is the first published report of *L. invasa* in Madagascar, Mauritius, Reunion, Sierra Leone and Zambia; of *G. brimblecombei* in Kenya, Malawi, Reunion, Rwanda and Uganda; of *T. peregrinus* in

DRC, Madagascar, Mauritius, Rwanda and Zambia; of *Gonipterus* sp.n.2 in Rwanda; and of *O. maskelli* in Madagascar and Rwanda. Beside these countries where the presence of the pests were confirmed through sampling in the current study, literature indicates the presence of *L. invasa* in Mozambique, Tanzania and Zambia (Dittrich-Schröder et al. 2018); of *T. peregrinus* in Tanzania and Mozambique (CABI 2018a, b, c), of *G. brimblecombei* in Tanzania (Petro et al. 2017), of *Gonipterus scutellatus* complex in Lesotho, Madagascar, Malawi, Mauritius, Mozambique, Swaziland, Tanzania and Uganda (Williams et al. 1951; CABI 2018a, b, c; Jeger et al. 2018) (Fig. 1b–f).

### *Genetic diversity of non-native* Eucalyptus *insect pests in sub-Saharan Africa*

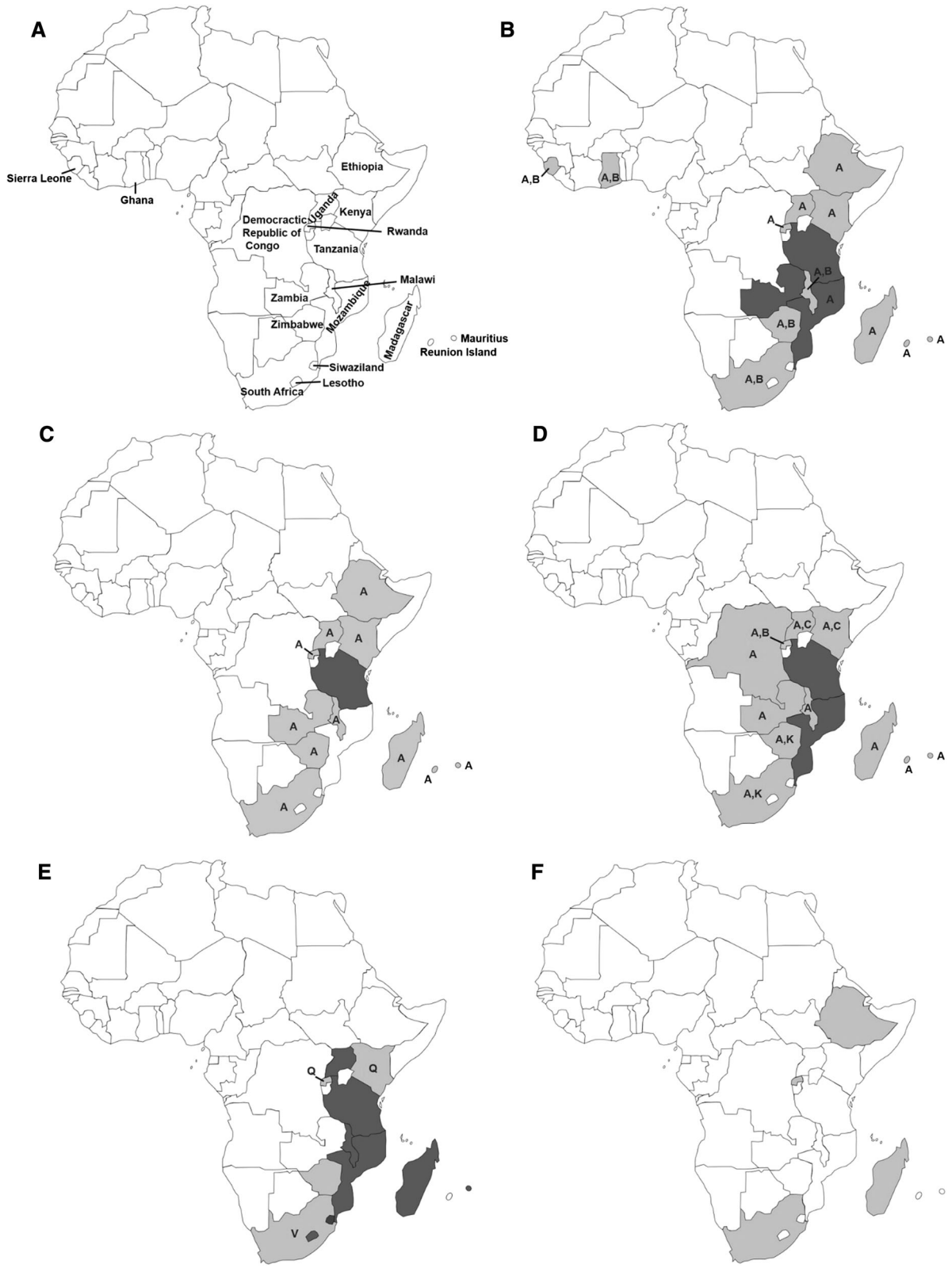
Analysis of sequences of COI gene of *L. invasa* revealed the presence of three haplotypes, namely haplotypes A, B and E across its range in sub-Saharan Africa (Table 2, Fig. 2). These haplotypes grouped with two of the three haplogroups (A, B and C) of the wasp which were previously identified across its geographic ranges, including in sub-Saharan Africa (Dittrich-Schröder et al. 2018; Le et al. 2018; Fig. 2). Haplotype A grouped with haplogroup A, and haplotype B and E with haplogroup B (Fig. 2). These two haplogroups are highly divergent from each other, with a mean sequence divergence of 3.7% (Table 3). In this study, haplogroup A was confirmed present in 12 sub-Saharan African countries (Ethiopia, Ghana, Kenya, Madagascar, Malawi, Mozambique, Reunion, Rwanda, Sierra Leone, South Africa, Uganda and Zimbabwe). Haplogroup B was found in Ghana, Malawi, Sierra Leone, South Africa and Zimbabwe (Fig. 2). It is now confirmed that haplogroup A and B are geographically co-occurring in at least five countries, including Ghana, Malawi, Sierra Leone, South Africa and Zimbabwe. An estimated overall nucleotide diversity (Pi) of 0.0140 (S = 18, h = 3, Hd = 0.415, K = 7.031) was obtained from the population in sub-Saharan Africa (Table 2). Haplogroup C, a lineage which was previously confirmed present in Australia (Dittrich-Schröder et al. 2018) was not found in our current survey in sub-Saharan Africa (Fig. 2).

Sequence analysis of COI gene of *G. brimblecombei* revealed the presence of only one haplotype, namely haplotype A throughout the invasive range of the psyllid in sub-Saharan Africa (Fig. 3). This is the same haplotype that was introduced in North America and South America. The other two haplotypes (B and C) in Fig. 3 were detected only in Australia. None of them were detected during our survey in sub-Saharan Africa. A low sequence divergence (0.22%) was detected between the haplotype in sub-Saharan Africa (Haplotype A) and the two other haplotypes in Australia (Haplotype B and C, Table 3).

Four closely related mitochondrial DNA haplotypes of *T. peregrinus*, namely A, B, C and K (overall mean sequence divergence = 0.04%) were detected across its range in sub-Saharan Africa (Table 3, Fig. 4). Haplotype A was found shared among Australia and several countries in sub-Saharan Africa, including DRC, Kenya, Madagascar, Malawi, Mauritius, Reunion, Rwanda, South Africa, Uganda, Zambia and Zimbabwe. Haplotype B and C were found exclusively in sub-Saharan Africa (haplotype B in Rwanda and haplotype C in Kenya and Uganda). Haplotype K was found shared among Australia, South Africa and Zimbabwe. Overall, the population in sub-Saharan Africa had an estimated nucleotide diversity of (Pi) of 0.0004 (S = 5, h = 4, Hd = 0.075, K = 0.167). The other seven haplotypes depicted in the network represent haplotypes previously reported from other regions, including Australia, South America and Europe (Nadel et al. 2010). None of these haplotypes were detected in sub-Saharan Africa.

Analysis of mitochondrial DNA diversity of *Gonipterus* sp.n.2 revealed the presence of two closely related haplotypes, namely haplotype Q and V (Overall mean sequence divergence = 0.7%) in sub-Saharan Africa (Table 3, Fig. 5). Haplotype Q was found exclusively in sub-Saharan Africa, shared between Kenya and Rwanda, and haplotype V was detected in South Africa and Australia. The other 22 haplotypes

**Fig. 1** Maps showing the distribution of *Eucalyptus* insect pests ▶ in sub-Saharan Africa including a blank map labelled with the names of countries included in this study and other countries where presence of pests were reported in literature (**a**), the distribution of *Leptocybe invasa* (**b**), *Glycaspis brimblecombei* (**c**), *Thaumastocoris peregrinus* (**d**), *Gonipterus* sp.n.2. (**e**), and *Ophelimus maskelli* (**f**). The letters in the maps represent the mitochondrial variants (haplogroups or haplotypes) of the respective insect species referred to in Figs. 2, 3, 4 and 5. The dark grey color indicates countries where information on the presence of the insects was obtained from literature or personal communications. In dark grey colored countries in map E, it is the presence of *Gonipterus scullatus* complex (not *Gonipterus* sp.n.2) that was reported in literature



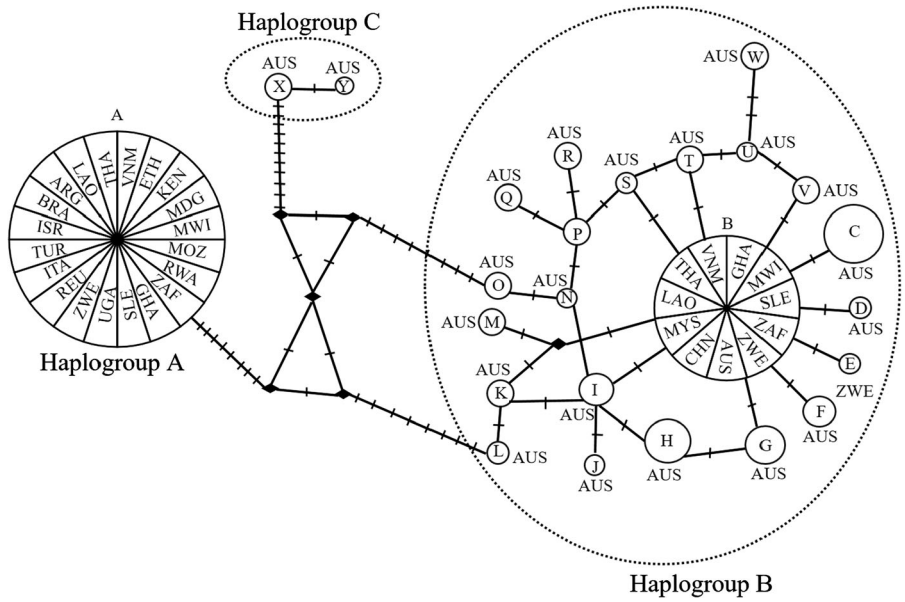



**Fig. 2** Haplotype network of *Leptocybe invasa*. Of the three mitochondrial haplogroups (A, B and C) previously detected across the entire range of the wasp (Dittrich-Schröder et al. 2018), two (A and B) were confirmed present in various countries in sub-Saharan Africa. Each circle represents a single haplotype and circle sizes are proportional to haplotype frequencies. The hyphen marks on the lines connecting the circles show the number of mutational steps. The country codes denote countries where haplotypes/haplogroups were detected. ETH—Ethiopia, GHA—Ghana, KEN—Kenya, MDG—Madagascar, MWI—Malawi, MOZ—Mozambique, RWA—Rwanda, REU—Reunion, ZAF—South Africa, SLE—Sierra Leone, UGA—Uganda, ZWE—Zimbabwe, AUS—Australia, ITA—Italy, TUR—Turkey, ISR—Israel, BRA—Brazil, CHN—China, MYS—Malaysia, LAO—Laos, THA—Thailand, VNM—Vietnam

illustrated in the network were previously reported from Australia (Mapondera et al. 2012). In the present study, none of these haplotypes were detected in sub-Saharan Africa.

Analysis of sequences of Cyt b gene of *O. maskelli* confirmed the presence of only a single haplotype in its invasive range in sub-Saharan Africa (Ethiopia, Madagascar, Rwanda and South Africa). This is the same haplotype which was previously reported from Israel and South Africa (Bush et al. 2016).

For some of the insect pests new haplotypes were detected; two for *T. peregrinus* (haplotype B and C, the former in Rwanda and the latter in Kenya and Uganda), one for *Gonipterus* sp.n.2 (haplotype Q) shared between Rwanda and Kenya and one for *L. invasa* (haplotype E) in Zimbabwe.

## Discussion

The results from our survey clearly show that five of the main global *Eucalyptus* insect pests have become broadly distributed within sub-Saharan Africa. Three of the major pests, namely *L. invasa*, *T. peregrinus* and *G. brimblecombei* were reported from sub-Saharan Africa in early 2000. *Leptocybe invasa* was first reported in Ethiopia in 2002 (Giliomee 2011), *T. peregrinus* in South Africa in 2003 (Jacobs and Neser 2005) and *G. brimblecombei* in Mauritius in 2001 (Sookar et al. 2003). In the intervening years, these insects have spread nearly in the entire region, except *T. peregrinus* and *G. brimblecombei* which are not yet detected in West Africa (Saavedra et al. 2015; Bush et al. 2016; Chungu et al. 2017; Petro et al. 2017; Le et al. 2018; Ndlela et al. 2018; Yirgu and Tanga 2019). *Ophelimus maskelli*, one of the recent arrivals in the region (Bush et al. 2016; Asfaw 2018) was confirmed to be present already in four countries. It is likely that this wasp will continue to spread in the region, signifying a need for an effective monitoring plan to enhance its early detection and timely management intervention. *Gonipterus* sp.n.2, one of the early arrivals in sub-Saharan Africa in 1916, was confirmed present in a relatively smaller number of countries

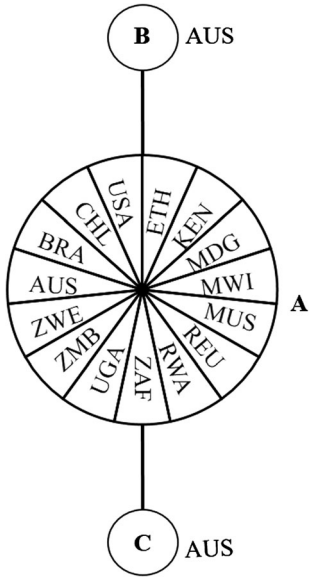



**Fig. 3** Mitochondrial haplotype network of *Glycaspis brimblecombei*. Three haplotypes were detected across the native and invasive range of the psyllid, of which only one (Haplotype A) was found in sub-Saharan Africa. Each circle represents a single haplotype and circle sizes are proportional to haplotype frequencies. The hyphen marks on the lines connecting the circles show the number of mutational steps. The country codes denote countries where haplotypes were detected. ETH—Ethiopia, KEN—Kenya, MDG—Madagascar, MWI—Malawi, MUS—Mauritius, REU—Reunion, RWA—Rwanda, ZAF—South Africa, UGA—Uganda, ZMB—Zambia, ZWE—Zimbabwe, AUS—Australia, BRA—Brazil, CHL—Chile and USA—United States of America

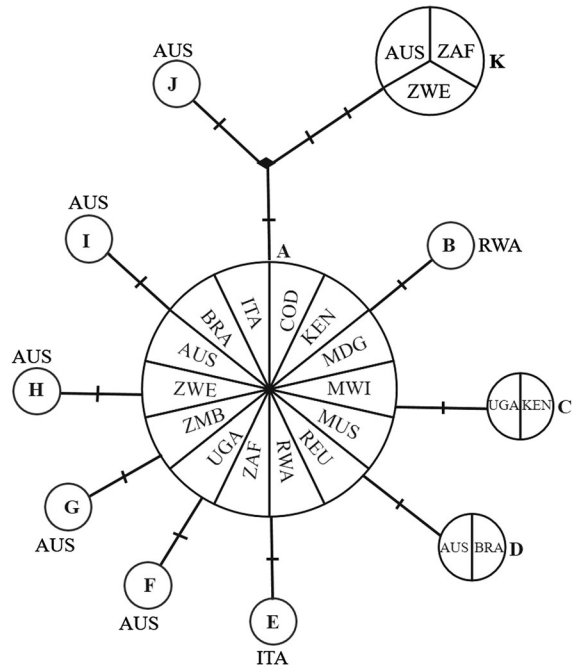



**Fig. 4** Mitochondrial haplotype network of *Thaumastocoris peregrinus*. Eleven haplotypes were detected across the native and invasive range of the insect, of which four (Haplotype A, B, C and K) were found in the sub-Saharan Africa. Each circle represents a single haplotype and circle sizes are proportional to haplotype frequencies. The hyphen marks on the lines connecting the circles show the number of mutational steps. The country codes denote countries where haplotypes were detected. COD—Democratic Republic of Congo, ITA—Italy, KEN—Kenya, MDG—Madagascar, MWI—Malawi, MUS—Mauritius, REU—Reunion, RWA—Rwanda, ZAF—South Africa, UGA—Uganda, ZMB—Zambia, ZWE—Zimbabwe, AUS—Australia and BRA—Brazil

compared to recent arrivals such as *L. invasa*, *T. peregrinus* and *G. brimblecombei*.

Two different haplogroups of *L. invasa* were found, with an overlapping geographic range in Ghana, Malawi, Sierra Leone, South Africa and Zimbabwe. The other four insect pests (*Gonipterus* sp.n.2, *T. peregrinus*, *G. brimblecomei* and *O. maskelli*) had a single or only a few closely related mtDNA haplotypes shared among countries across sub-Saharan Africa, suggesting common sources for these pests. These common sources may not necessarily be populations in the origin or native range of the pests, but could be from bridgehead populations. Bridgehead populations are successful invasive populations that serve as sources of colonists for remote new territories (Lombaert et al. 2010; Garnas et al. 2016). However, knowledge on the genetic diversity within the native areas and bridgehead populations would be needed to support or reject such a hypothesis. Hurley et al. (2016) showed that *Eucalyptus* insect pests were first detected outside their native range in only a small number of countries and these countries later served as bridgeheads to other regions. Compared to previous studies (Nadal et al. 2010; Mapondera et al. 2012; Dittrich-Schröder et al. 2018), new haplotypes were detected for *L. invasa*, *T. peregrinus* and *Gonipterus* sp.n.2. Most likely these have resulted from mutation as all the new haplotypes differ from the existing ones only by a few single nucleotide polymorphic sites. It is also possible that these are the results of multiple introduction events with different haplotypes or a single introduction event that contains different haplotypes of the pests. Further work should explore such scenarios with different (more variable) genetic

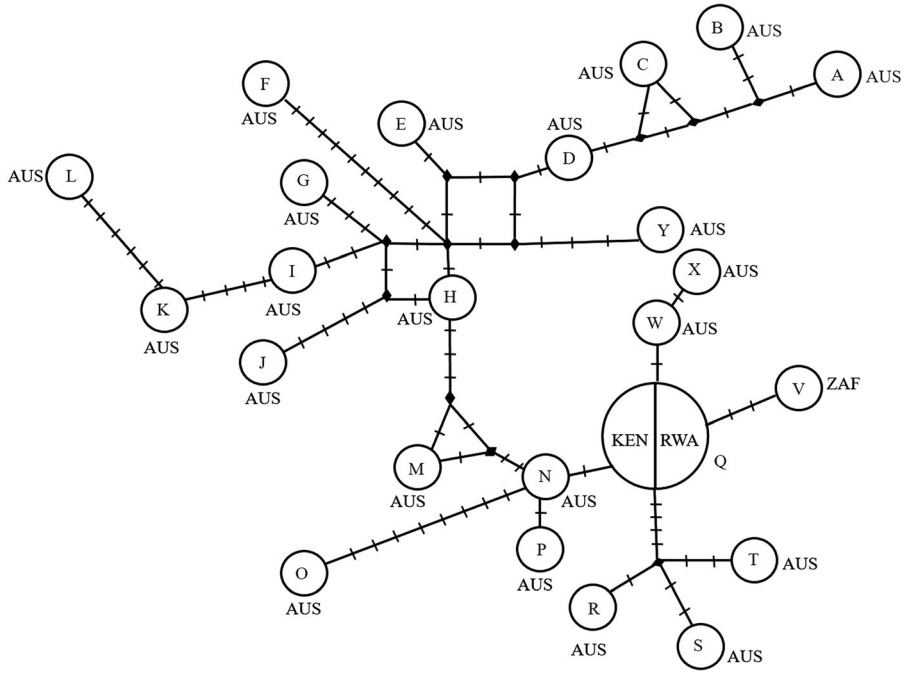



**Fig. 5** Mitochondrial haplotype network of *Gonipterus* sp.n.2. Twenty-four haplotypes of the beetle were detected across Australia and its invasive range in Africa, of which only two (Haplotype Q and V) were found in the sub-Saharan Africa. Each circle represents a single haplotype and circle sizes are proportional to haplotype frequencies. The hyphen marks on the lines connecting the circles show the number of mutational steps. The country codes denote countries where haplotypes were detected. KEN—Kenya, RWA—Rwanda, ZAF—South Africa and AUS—Australia

markers and statistics such as Approximate Bayesian Computation.

The present study confirmed the presence of two distinct mitochondrial haplogroups of *L. invasa*, namely Haplogroup A and B, across sub-Saharan Africa. This is in agreement with previous studies, which reported the presence of three mitochondrial haplogroups (Haplopgroup A, B and C), of which two (A and B) were confirmed present in sub-Saharan Africa (Dittrich-Schröder et al. 2018; Lee et al. 2018). The combined analysis of mitochondrial and nuclear sequence data, however, showed the presence of two distinct lineages of *L. invasa*, referred to as Lineage A, corresponding to Haplogroup A, and Lineage B, corresponding to Haplogroup B and C (Nugnes et al. 2015; Dittrich-Schröder et al. 2018). In our study, Lineage A was present in most of the countries sampled, with its first report in Ghana, Madagascar, Mauritius, Reunion, Rwanda and Sierra Leone. Lineage B, which was previously detected only in Ghana and South Africa, has now been detected in Malawi, Sierra Leone and Zimbabwe. The co-occurrence of the two *L. invasa* lineages in the region and within some countries, such as Ghana, Malawi, Sierra Leone, South Africa and Zimbabwe, raises concern for admixture (intra species hybridization) and the implications of the two lineages and potential admixture on management strategies. Admixtures between geographically overlapping distinct lineages were evident for the woodwasp, *Sirex noctilio* in some areas of its invasive range (Garnas et al. 2016). Control methods such as biological control agents and host resistance have been deployed against Lineage A and may not be effective against Lineage B. Moreover, the wasps may increase their adaptive fitness through admixture of the two lineages (Verhoeven et al. 2011). It is important to examine the ecology and management of the two lineages across sub-Saharan Africa, including host preference, response to available control methods and patterns of natural enemy recruitment.

Depending on the insect species, different or similar patterns of genetic diversity were observed

**Table 2** Genetic diversity parameters of five non-native *Eucalyptus* insect pests across sub-Saharan Africa

| Insect species | n | S | h | Hd | K | Pi |
|---|---|---|---|---|---|---|
| *Leptocybe invasa* | 215 | 18 | 3 | 0.415 | 7.032 | 0.0140 |
| *Thaumastocoris peregrinus* | 221 | 5 | 4 | 0.075 | 0.167 | 0.0004 |
| *Gonipterus* sp.n.2 | 68 | 2 | 2 | 0.074 | 0.148 | 0.0005 |
| *Glycaspis brimblecombei* | 348 | 0 | 1 | 0.000 | 0.000 | 0.0000 |
| *Ophelimus maskelli* | 105 | 0 | 1 | 0.000 | 0.000 | 0.0000 |

*n* sample size, *S* number of segregating sites, *h* number of haplotypes, *Hd* haplotype diversity, *K* mean number of nucleotide differences, *Pi* nucleotide diversity

**Table 3** Estimates of sequence divergence between haplotypes/haplogroups of *Eucalyptus* insect pests

| Haplogroups | **1** | **2** | **3** | |
|---|---|---|---|---|
| *Leptocybe invasa* | | | | |
| 1. Haplogroup A | | | | |
| 2. Haplogroup B | 0.037 | | | |
| 3. Haplogroup C | 0.039 | 0.046 | 0.000 | |
| Overall mean divergence = 0.0205 | | | | |
| **Haplotypes** | **1** | **2** | **3** | **4** |
| *Thaumastocoris peregrinus* | | | | |
| 1. Haplotype A | | | | |
| 2. Haplotype B | 0.001 | | | |
| 3. Haplotype C | 0.001 | 0.003 | | |
| 4. Haplotype K | 0.003 | 0.004 | 0.004 | 0.000 |
| Overall mean divergence = 0.0004 | | | | |
| **Haplotypes** | **1** | **2** | **3** | |
| *Glycaspis brimblecombei* | | | | |
| 1. Haplotype A | | | | |
| 2. Haplotype B | 0.002 | | | |
| 3. Haplotype C | 0.002 | 0.004 | 0.000 | |
| Overall mean divergence = 0.0003 | | | | |
| **Haplotypes** | **1** | **2** | | |
| *Gonipterus* sp.n.2 | | | | |
| 1. Haplotype Q | | | | |
| 2. Haplotype V | 0.006 | 0.000 | | |
| Overall mean divergence = 0.007 | | | | |

between sub-Saharan Africa and the rest of the world. For *G. brimblecombei* and *O. maskelli*, only single haplotypes of the respective species have become invasive in sub-Saharan Africa and the rest of the world. For *L. invasa* and *T. peregrinus*, the patterns differed from region to region. For *L. invasa*, the patterns in sub-Saharan Africa and Asia were similar, where two highly divergent haplogroups (A and B) have become invasive in both continents, whereas only one haplogroup (haplogroup A) was detected in South America and Europe. In general, haplogroup A was found throughout the invasive range of the wasp, making it the most invasive of all the three haplogroups. For *T. peregrinus*, relatively higher number of haplotypes (A, B, C and K) were detected in sub-Saharan Africa compared to one (A) and two (A and D) haplotypes in Europe and South America, respectively. Three haplotypes (B, C and K) were found

unique to sub-Saharan Africa compared to South America and Europe, while haplotype A was confirmed present in Africa, Europe, South America and its native range Australia. South America has one unique haplotype (D), compared to sub-Saharan Africa and Europe. A recent study on the population genetic of *T. peregrinus* in Australia has confirmed that haplotypes A and D are the most widespread and common haplotypes (Lo et al. 2019).

The pathways of introduction of *Eucalyptus* insect pests into and within sub-Saharan Africa are currently not well known, leaving the region vulnerable to further introductions. Trade in live plants such as infested eucalypt seedlings and propagation material are possible pathways of introduction for gall-forming insects such as *L. invasa* and *O. maskelli* (Csóka et al. 2017; Meurisse et al. 2018). Hitchhiking on vehicles, trailers and people's clothing and hair was reported for *T. peregrinus* (Meurisse et al. 2018). Dispersal through flight, wind and movement of firewood and other wood products are also likely to contribute to the introduction as well as range expansion of eucalypt insect pests (Todd and Horwitz 1990; Withers 2001; Csóka et al. 2017; Meurisse et al. 2018). Regular monitoring of insect pests, particularly around borders and ports of entry can assist in early detection of new introductions (Rabaglia et al. 2008). In addition, local capacity building in forest entomology is necessary to address current shortages in such expertise, and is essential for implementing effective monitoring systems and developing sustainable pest management strategies. Given the urgency of this need, it is necessary to evaluate the capacities of the research and training centres in the region to be leveraged for the monitoring and management of these pests. Shared and low genetic diversity of most *Eucalyptus* insect pests in sub-Saharan Africa should encourage countries in the region to collaborate and develop management practices that are effective against the shared pest problems. The need for such a collaborative effort for management through biological control, biosecurity and other tools has also been called for before (Garnas et al. 2012; Wingfield et al. 2015).

## Conclusions

To our awareness, this is the first comprehensive study that mapped the distribution and unravelled the genetic diversity of *Eucalyptus* insect pests across sub-Saharan Africa. Results of this study will have implications for quarantine, future research and management of *Eucalyptus* insect pests in the region and beyond. Implementing improved quarantine and regulatory measures that prevent/minimize new introductions and secondary spread is valuable for future management of *Eucalyptus* insect pests in sub-Saharan Africa. The relative low genetic diversity of *Eucalyptus* insect pests across sub-Saharan Africa suggests the possibility of using the same or similar management strategies across the invasive range of these pests and encourages collaborations between countries. Capacity building across sub-Saharan Africa and collaboration among experts in the region is essential in order to address these and similar pest problems.

Surveys of *Eucalyptus* insect pests in other *Eucalyptus* growing countries of sub-Saharan African region are needed in order to obtain a better understanding of their distribution and diversity. In this study we confirm the presence, but not the absence of the insect pests from the countries sampled. It is possible that the insect pests occur in some countries where they were not confirmed during our study, especially in countries where only small-scale sampling was conducted. It is also likely that the insect pests are present in other *Eucalyptus* growing countries such as Benin, Burundi, Mozambique, Sudan and Tanzania, which were neither surveyed nor sampled in the present study. Continuous monitoring of these pests and detailed analysis of their genetic diversity using nuclear DNA markers such as single sequence repeat (microsatellites) are required to better understand invasion pathways to the continent and spread within the region. Continuous monitoring would also enhance early detection and warning systems to assist management interventions. Studies on the level of damage and patterns of host utilization of these pests and enquiries on the diversity and prevalence of their natural enemies are equally important.

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## References

Asfaw BA (2018) First record of Eucalyptus Gall Wasp (EGW) (*Ophelimus maskelli* (Ashmead) on *Eucalyptus camaldulensis* in Ethiopia. Int J Agric For 8:112–117

Bandelt H-J, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. Mol Biol Evol 16:37–48

Bella S, Rapisarda C (2013) First Report from Greece of the invasive red gum lerp psyllid *Glycaspis brimblecombei* Moor (Hemiptera Psyllidae) and its associated parasitoid *Psyllaephagus bliteus* Riek (Hymenoptera Encyrtidae). Redia 96:33–35

Bennett BM, Kruger FJ (1983) Forestry and water conservation in South Africa: history, science and policy. World forest history series. The Australian National University (ANU) Press, Canberra, pp 1–265.

Branco M, Boavida C, Durand N, Franco JC, Mendel Z (2009) Presence of the eucalyptus gall wasp *Ophelimus maskelli* and its parasitoid *Closterocerus chamaeleon* in Portugal: First record, geographic distribution and host preference. Phytoparasitica 37:51–54

Burks RA, Mottern JL, Waterworth R, Paine TD (2015) First report of the eucalyptus gall wasp, *Ophelimus maskelli* (Hymenoptera: Eulophidae), an invasive pest on *Eucalyptus*, from the Western Hemisphere. Zootaxa 3926:448–450

Bush SJ, Slippers B, Neser S, Harney M, Dittrich-Schröder G, Hurley BP (2016) Six recently recorded Australian insects associated with *Eucalyptus* in South Africa. Afr Entomol 24:539–544

CABI (2018a) Invasive Species Compendium. *Gonipterus scutellatus* (Eucalyptus snout beetle). Accessed 26 Dec 2018. https://www.cabi.org/isc/datasheet/25719

CABI (2018b) Invasive Species Compendium. *Phoracantha recurva* (Eucalyptus longhorned borer). Accessed 26 Dec 2018. https://www.cabi.org/isc/datasheet/40371

CABI (2018c) Invasive Species Compendium. *Thaumastocoris peregrinus* (bronze bug). Accessed 26 Dec 2018. https://www.cabi.org/isc/datasheet/109741

Chungu D, Shakacite O, Chama H, Chungu BC, Mbindo K, Mulongwe L (2017) First record of the red gum lerp psyllid, *Glycaspis brimblecombei* Moore (Hemiptera: Psyllidae), in Zambia. Afr J Ecol 55:380–382

Csóka G, Stone GN, Melika G (2017) Non-native gall-inducing insects on forest trees: a global review. Biol Invasions 19:3161–3181

Dittrich-Schröder G, Hoareau TB, Hurley BP, Wingfield MJ, Lawson S, Nahrung HF, Slippers B (2018) Population genetic analyses of complex global insect invasions in managed landscapes: a *Leptocybe invasa* (Hymenoptera) case study. Biol Invasions 2018:1–26

FAO (2009) Global review of forest pests and diseases. A thematic study prepared in the framework of the Global Forest Resources Assessment 2005. FAO Forestry Paper 156, Rome, Italy

FAO (2011) *Eucalyptus* in East Africa, socio-economic and environmental issues, by Gessesse Dessie and Teklu Erkossa. Planted forests and trees working paper 46/E, Forest Management Team, Forest Management Division. FAO, Rome

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol 3:294–299

Garcia A, Figueiredo E, Valente C, Monserrat VJ, Branco M (2013) First record of *Thaumastocoris peregrinus* in Portugal and of the neotropical predator *Hemerobius bolivari* in Europe. Bull Insectol 66:251–256

Garnas JR, Auger-Rozenberg M-A, Roques A, Bertelsmeier C, Wingfield MJ, Saccaggi DL, Roy HE, Slippers B (2016) Complex patterns of global spread in invasive insects: eco-evolutionary and management consequences. Biol Invasions 18:935–952

Garnas JR, Hurley BP, Slippers B, Wingfield MJ (2012) Biological control of forest plantation pests in an interconnected world requires greater international focus. Int J Pest Manag 58:211–223

Giliomee JH (2011) Recent establishment of many alien insecs in south Africa-a cause of concern. Afr Entomol 19:151–155

Gomy Y (2000) Nouvelle liste chorologique des coléoptères de l'archipel des Mascareignes. Société réunionnaise des Amis du Muséum

Hall T (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 45:95–98

Harry M, Solignac M, Lachaise D (1998) Molecular evidence for parallel evolution of adaptive syndromes in Fig-Breeding Lissocephala (Drosophilidae). Mol Phylogenet Evol 9:542–551

Hurley BP, Garnas J, Wingfield MJ, Branco M, Richardson DM, Slippers B (2016) Increasing numbers and intercontinental spread of invasive insects on eucalypts. Biol Invasions 18:921–933

Hurley BP, Slippers B, Sathyapala S, Wingfield MJ (2017) Challenges to planted forest health in developing economies. Biol Invasions 19:3273–3285

Jacobs DH, Neser S (2005) *Thaumastocoris australicus* Kirkaldy (Heteroptera: Thaumastocoridae): a new insect arrival in South Africa, damaging to *Eucalyptus* trees: research in action. S Afr J Sci 101:233–236

Jeger M, Bragard C, Caffier D, Candresse T, Chatzivassiliou E, Dehnen-Schmutz K, Gilioli G, Miret JAJ, MacLeod A, Navarro MN, Niere B, Parnell S, Potting R, Rafoss T, Rossi

V, Urek G, Van Bruggen A, Van der Werf W, West J, Winter S, Santolamazza-Carbone S, Kertesz V, Aukhojee M, Gregoire JC (2018) Pest categorisation of the *Gonipterus scutellatus* species complex. EFSA Journal 16(1):5107

Jermiin LS, Crozier RH (1994) The cytochrome b region in the mitochondrial DNA of the ant *Tetraponera rufoniger*: sequence divergence in Hymenoptera may be associated with nucleotide content. J Mol Evol 38:282–294

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 30:772–780

Laudonia S, Sasso R (2012) The bronze bug *Thaumastocoris peregrinus*: a new insect recorded in Italy, damaging to *Eucalyptus* trees. Bull of Insectol 65:89–93

Le NH, Nahrung HF, Griffiths M, Lawson SA (2018) Invasive Leptocybe spp. and their natural enemies: Global movement of an insect fauna on eucalypts. Biol Control 125:7–14

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451–1452

Lo N, Montagu A, Noack A, Nahrung HF, Wei H, Eldridge M, Gray KA, Rose HA, Cassis G, Johnson RN, Lawson S (2019) Population genetics of the Australian eucalypt pest *Thaumastocoris peregrinus*: evidence for a recent invasion of Sydney. J Pest Sci 92:201–212

Lombaert E, Guillemaud T, Cornuet J-M, Malausa T, Facon B, Estoup A (2010) Bridgehead effect in the worldwide invasion of the biocontrol harlequin ladybird. PLoS ONE 5:e9743

Louppe D, Depommier D (2010) Expansion, research and development of the *Eucalyptus* in Africa. Wood production, livelihoods and environmental issues: an unlikely reconciliation? A communication given at the FAO/MEEATU Workshop "*Eucalyptus* in East Africa, the socio-economic and environmental issues", Bujumbura, 31 March–1 April, 2010

Mapondera TS, Burgess T, Matsuki M, Oberprieler RG (2012) Identification and molecular phylogenetics of the cryptic species of the *Gonipterus scutellatus* complex (Coleoptera: Curculionidae: Gonipterini). Austral Entomol 51:175–188

Martins CB, Zarbin PH (2013) Volatile organic compounds of conspecific-damaged *Eucalyptus benthamii* influence responses of mated females of *Thaumastocoris peregrinus*. J Chem Ecol 39:602–611

Mendel Z, Protasov A, Blumberg D, Brand D, Saphir N, Madar Z, La Salle J (2007) Note: Release and recovery of parasitoids of the eucalyptus gall wasp *Ophelimus maskelli* in Israel. Phytoparasitica 35:330–332

Meurisse N, Rassati D, Hurley BP, Brockerhoff EG, Haack RA (2018) Common pathways by which non-native forest insects move internationally and domestically. J Pest Sci 92:13–27

Munishi P (2007) The Eucalyptus controversy in Tanzania: TAF Annual General Meeting (AGM). Dodoma, Tanzania

Mutitu KE, Nyeko P, Otieno BO, Oeba V, Day RK (2007) Spatial distribution of the bluegum chalcid, *Leptocybe invasa* on *Eucalyptus* species in Kenya. Discov Innovat 19:369–374

Nadel RL, Noack AE (2012) Current understanding of the biology of *Thaumastocoris peregrinus* in the quest for a management strategy. Int J Pest Manage 58:257–266

Nadel RL, Slippers B, Scholes MC, Lawson SA, Noack A, Wilcken CF, Bouvet J, Wingfield MJ (2010) DNA barcoding reveals source and patterns of *Thaumastocoris peregrinus* invasions in South Africa and South America. Biol Invasions 12:1067–1077

Ndlela S, Manyangadze T, Sachisuko A, van der Lingen S, Makowe I (2018) The distribution and management of two invasive pests of *Eucalyptus*: the red gum lerp psyllid, *Glycaspis brimblecombei* (Hemiptera: Psylloidae), and the blue gum chalcid wasp, *Leptocybe invasa* (Hymenoptera: Eulophidae), in Zimbabwe. Afr Entomol 26:104–115

Nugnes F, Gebiola M, Monti MM, Gualtieri L, Giorgini M, Wang J, Bernardo U (2015) Genetic diversity of the invasive gall wasp *Leptocybe invasa* (Hymenoptera: Eulophidae) and of its *Rickettsia* endosymbiont, and associated sex-ratio differences. PLoS ONE 10:e0124660

Olivier W (2009) There is honey in the forest: the history of forestry in South Africa, 1st edn. Southern African Institute of Mining Forestry, Pretoria, South Africa

Petro R, Mpiri A, Mkude A (2017) Susceptibility of *Eucalyptus* species and clones to red gum lerp psyllid, G*lycaspis brimblecombei*, (Hemiptera: Psyllidae) in Mbizi Forest Plantation, Tanzania. Int J Environ Agric Biotech 2:417–420

Rabaglia R, Duerr D, Acciavatti R, Ragenovich I (2008) Early detection and rapid response for non-native bark and ambrosia beetles. Early Detection and Rapid Response Project Report, United States Department of Agriculture, Forest Service, Forest Health Protection, Washington, DC

Reguia K, Peris-Felipo FJ (2013) *Glycaspis brimblecombei* Moore, 1964 (Hemiptera: Psyllidae) invasion and new records in the Mediterranean area. Biodivers J 4:501–506

Reynolds E (1986) The ecological effects of *Eucalyptus*. FAO Forestry paper No. 59: JSTOR

Saavedra MC, Avila GA, Withers TM, Holwell GI (2015) The potential global distribution of the Bronze bug *Thaumastocoris peregrinus* Carpintero and Dellapé (Hemiptera: Thaumastocoridae). Agric For Entomol 17:375–388

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann Entomol Soc Am 87:651–701

Sopow S, George S (2012) Bronze bug, *Thaumastocoris peregrinus*: a new *Eucalyptus* pest in New Zealand. Surveillance (Wellington) 39(2):43–46

Sookar P, Seewooruthun S, Ramkhelawon D (2003) The redgum lerp psyllid, *Glycaspis brimblecombei*, a new pest of *Eucalyptus* sp. Mauritius. AMAS, Food and Agricultural Research Council, Réduit, Mauritius, pp 327–332

Spodek M, Burckhardt D, Protasov A, Mendel Z (2015) First record of two invasive eucalypt psyllids (Hemiptera: Psylloidea) in Israel. Phytoparasitica 43:401–406

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using likelihood, distance, and parsimony methods. Mol Biol Evol 10:2731–2739

Teshome T (2009) Is *Eucalyptus* ecologically hazardous tree species. Ethio e-j Res Innov For 1:128–134

Todd JJ, Horwitz PHJ (1990) Spreading insects through firewood collection in Tasmania. Aust For 53:154–159

Tooke FGC (1955) The Eucalyptus snout beetle, *Gonipterus scutellatus* Gyll. A Study of its ecology and control by biological means. Entomological Memoir, Department of Agriculture, South Africa 3:1–282

Verhoeven KJF, Macel M, Wolfe LM, Biere A (2011) Population admixture, biological invasions and the balance between local adaptation and inbreeding depression. Proc R Soc B 278:2–8

Williams RJ, Moutia LA, Hermelin PR (1951) The Biological Control of *Gonipterus scutellatus* Gyll. (Col. Curculionidae) in Mauritius. Bull Entomol Res 42:23–28

Wingfield MJ, Brockerhoff EG, Wingfield BD, Slippers B (2015) Planted forest health: The need for a global strategy. Science 349:832–836

Wingfield MJ, Slippers B, Hurley BP, Coutinho TA, Wingfield BD, Roux J (2008) Eucalypt pests and diseases: growing threats to plantation productivity. South For 70:139–144

Withers TM (2001) Colonization of eucalypts in New Zealand by Australian insects. Aust Ecol 26:467–476

Yirgu A, Tanga AA (2019) First record of *Glycaspis brimblecombei* Moore (hem.: Psyllidae) on *Eucalyptus camaldulensis* in Ethiopia. Phytoparasitica 47:67–70

Zheng X, Li J, Yang Z, Xian Z, Wei J, Lei C, Wang X, Lu W (2014) A review of invasive biology, prevalence and management of *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae: Tetrastichinae). Afr Entomol 22:68–79