

Tracing the distribution of natural enemies of non-native invasive *Eucalyptus* insect pests in sub-Saharan Africa

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Abstract

Eucalyptus forestry in sub-Saharan Africa is challenged by non-native *Eucalyptus*-feeding insects. In recent studies, six invasive *Eucalyptus* insect pests, namely *Blastopsylla occidentalis*, *Glycaspis brimblecombei*, *Gonipterus* sp.n.2, *Leptocybe invasa*, *Thaumastocoris peregrinus* and *Ophelimus maskelli* were confirmed present in sub-Saharan Africa. We investigated the diversity and distribution of natural enemies of these pests in six sub-Saharan

African countries. Plant parts (leaves, petioles, stem) infested by the insect pests were sampled from multiple sites in each country. The emerged natural enemies were identified using morphological characteristics and DNA sequence data. Nine species of natural enemies were confirmed present in the surveyed countries, namely *Anaphes nitens*, *Closterocerus chamaeleon*, *Megastigmus* sp., *M. pretorianensis*, *Psyllaephagus blastopsyllae*, *P. bliteus*, *Quadrastichus mendeli*, *Selitrichodes kryceri* and *S. neseri*. No natural enemies were found in Ghana and Sierra Leone despite the presence of *L. invasa* in both of those countries. Interestingly, most of these natural enemies were unintentionally introduced into the surveyed countries. Results of this study showed that both insect pests and natural enemies introduced into one country are likely to affect many other countries in the region. These findings call for a more coordinated approach to the management of plantation pests in the region.

Keywords: biological control, plantation forestry, *Anaphes nitens*, *Closterocerus chamaeleon*, *Psyllaephagus*, *Quadrastichus mendeli*

Introduction

Natural enemies are an important regulating factor of many insect populations, including invasive insect pests (Vacante and Bonsignore 2017). According to the enemy release hypothesis, non-native pests remain fairly free of natural enemies at the early stage of introduction (Keane and Crawley 2002; Roy et al. 2011). However, natural enemies accumulate gradually through the formation of novel associations between invasive insects and native natural enemies (Cornell and Hawkins 1993; Wondafrash et al. 2018) and/or accidental introduction of natural enemies from other regions (Bush et al. 2016; 2018; Kenis et al. 2017; Wondafrash et al 2018). Recruitment of natural enemies native to the invaded region is a very slow process and often characterized by low prevalence of natural enemies. It requires behavioural, biological and phenological adjustments for the native natural enemies to successfully exploit the new/introduced host (Cornell and Hawkins 1993; Grabenweger et al. 2010; Zappala et al. 2013). On the other hand, co-evolved natural enemies which accidentally

arrive in regions where their hosts have become invasive are likely to establish and become prevalent.

Besides the accidental arrival of co-evolved natural enemies in regions where their hosts have become invasive, these natural enemies can also be intentionally introduced as part of a Classical Biological Control (CBC) programme. CBC involves the introduction of natural enemies of non-native origin with the aim to permanently control pests, usually also non-native. It has been used for the control of a wide variety of organisms, including against insect pests by using parasitoids, predators and pathogens (Kenis et al. 2017). CBC is one of the most common pest management options in forestry (Garnas et al. 2012; Kenis et al. 2017), and has been successfully used in *Eucalyptus* plantations (Garnas et al. 2012).

Eucalyptus production is heavily challenged by insect pests globally. These insect pests are often non-native, originating from the native range of the trees in Australia, Papua New Guinea and Indonesia (Wingfield et al. 2008; 2015). Similar to other parts of the world, the rate of introduction of non-native *Eucalyptus*-feeding insects has been increasing in sub-Saharan Africa (Hurley et al. 2016; 2017). In recent studies, six non-native invasive *Eucalyptus* insect pests, namely the bluegum chalcid wasp, *Leptocybe invasa* Fisher (Hymenoptera: Eulophidae); the redgum lerp psyllid, *Glycaspis brimblecombei* Moore (Hemiptera Psyllidae); the bronze bug, *Thaumastocoris peregrinus* Carpintero & Dellapé (Hemiptera: Thaumastocoridae); an undescribed species of the *Gonipterus scutellatus* species complex, *Gonipterus* sp.n.2 (Coleoptera: Curculionidae); the eucalyptus gall wasp, *Ophelimus maskelli* Ashmead (Hymenoptera: Eulophidae); and the Eucalyptus psyllid, *Blastopsylla occidentalis* Taylor (Hemiptera: Psyllidae) were confirmed to be present and broadly distributed across sub-Saharan Africa (Tamesse et al. 2014; Bush et al. 2016; Wondafrash et al. 2020).

These insect pests pose a huge threat to the production of *Eucalyptus*, the commonly grown and socio-economically important tree genus in the region (FAO 2011). Both the adults

and the nymphs of *B. occidentalis*, *G. brimblecombei* and *T. peregrinus* feed on the sap of the leaves. In the case of *G. brimblecombei*, this leads to leaf discoloration, leaf drop, twig dieback and occasional whole tree death (Bella and Rapisarda 2013). The lerps also cause sooty mould when they get damp. Infestation by *T. peregrinus* starts with reddening of the leaves and gradually the whole canopy turns reddish- yellow to reddish-brown. This may lead to the loss of canopy leaves as the infestation progresses (Nadel and Noack 2012). For *B. occidentalis*, no severe feeding damage was reported in its invasive range, except minor damage to young shoots (Bouvet et al. 2005). However, the nymphs of this species produce honeydew and copious white flocculence, which enhances growth of sooty mould on the leaves (Santana and Burckhardt 2007). *Leptocybe invasa* and *O. maskelli* are gall inducing pests. Infestation by *L. invasa* leads to the formation of galls on various plant parts, including leaf petioles, midribs and stems of new foliage of both young and mature trees. This may lead to stunted growth and tree mortality in severe cases (Dittrich-Schroder et al. 2018). *Ophelimus maskelli* causes damage by inducing numerous small pimple-like galls on both sides of the leaf surface (Branco et al. 2009). Both the larvae and the adults of *Goniopteris* sp.n.2 feed on *Eucalyptus* leaves and cause severe damage to the trees (Took 1955).

A number of co-evolved natural enemies of the above-mentioned insect pests have been intentionally (CBC) or unintentionally introduced in sub-Saharan Africa. For most of these, the country of release or first report was South Africa. Examples of these include the intentional introduction of the egg parasitoid *Anaphes nitens* (Girault) (Hymenoptera: Mymaridae) against the *G. scutellatus* species complex (Tooke 1955; Schröder et al. 2020) and *Cleruchoides noackae* Lin & Huber against *T. peregrinus* in South Africa (Mutitu et al. 2013). *Selitrichodes neseri* Kelly & La Salle (Hymenoptera: Eulophidae) was intentionally introduced in Mauritius, Malawi, South Africa and Zimbabwe for the control of *L. invasa* (Kim et al. 2008; Dittrich-Schröder et al. 2014; Bush SJ et al., unpublished data). *Closterocerus chamaeleon* (Girault)

(Hymenoptera: Eulophidae), the parasitoid of *O. maskelli*, and *Quadrastichus mendeli* Kim & La Salle (Hymenoptera: Eulophidae), the parasitoid of *L. invasa*, were recently reported from South Africa as unintentional introductions (Bush et al. 2016; 2018). Similarly, *Psyllaephagus bliteus* Riek (Hymenoptera: Encyrtidae), the parasitoid of *G. brimblecombei*, was recently reported from South Africa and Zimbabwe (Bush et al. 2016; Ndlela et al. 2018). *Psyllaephagus blastopsyllae* Tamesse, Soufo, Tchanatame, Dzokou, Gumovsky & Coninck (Hymenoptera: Encyrtidae), a parasitoid of *Blastopsylla occidentalis* Taylor (Hemiptera: Psyllidae), was also detected in Cameroon and South Africa (Tamesse et al. 2014; Bush et al. 2016). However, knowledge on the presence and distribution of natural enemies of *Eucalyptus* insect pests is lacking in most parts of sub-Saharan Africa.

In this study, we investigated the diversity and distribution of natural enemies of these six invasive *Eucalyptus* insect pests, five of which were recorded in sub-Saharan Africa in the recent study by Wondafrash et al. (2020). We collected plant material infested with these insect pests, including *B. occidentalis*, *G. brimblecombei*, *Gonipterus* sp.n.2, *T. peregrinus*, *L. invasa*, and *O. maskelli* from six countries across sub-Saharan Africa. Our collections focused on the insect growth stages associated with the natural enemies that were known to have been introduced to the region. We used taxonomic characteristics and DNA sequence data to identify the natural enemies.

Materials and methods

Natural enemy surveys

Surveys were conducted in six countries, namely Ethiopia, Ghana, Malawi, Rwanda, Sierra Leone and Zimbabwe in 2017 and 2018. A total of 125 sites (36 in Ethiopia, 19 in Ghana, 16 in Malawi, 18 in Rwanda, 14 in Sierra Leone and 22 in Zimbabwe) were assessed for the presence of *Eucalyptus* insect pests and their natural enemies (Table 1). Site selection was

Table 1. Site information for collection of natural enemies of *Eucalyptus* insect pests in six sub-Saharan African countries.

Province/Zone/Region	District	No. sites	<i>Eucalyptus</i> species	Tree age (yr.)	Elevation (m. a. s. l.)
Ethiopia					
East Showa	Dire Gerbicha	1	<i>E. camaldulensis</i> , coppice	8	1969
	Gelan	1	<i>E. camaldulensis</i> , coppice	5	2261
West Arsi	Shashemene	3	<i>E. globulus</i> , <i>E. saligna</i> , <i>E. grandis</i> , coppice	0.5 – 4	2629-2654
Sidama	Shebedino	1	<i>E. camaldulensis</i> , coppice	1	1838
	Yirgalem	1	<i>E. camaldulensis</i> , coppice	3	1779
Welayta	Damot Weyde	1	<i>E. camaldulensis</i> , coppice	1	1759
Jimma	Kersa	3	<i>E. camaldulensis</i> , coppice	0.2 - 2	1693-1769
	Seka Chekorsa	1	<i>E. camaldulensis</i>	2	1819
Bonga	Gimbo	2	<i>E. saligna</i> , <i>E. camaldulensis</i>	1 - 2	1508
Gurage	Kebena	1	<i>E. camaldulensis</i> , coppice	4	1815
Southwest Sowa	Weliso	4	<i>E. camaldulensis</i> , <i>E. camaldulensis</i> , <i>E. camaldulensis</i> , coppice	1 - 5	1944 - 2316
North Showa	Debre Libanos	1	<i>E. globulus</i> , coppice	2	2505
East Gojam	Machakel	2	<i>E. camaldulensis</i> , <i>E. grandis</i> , coppice	1 – 3	2230-2390
Awi	Enjibara	2	<i>E. camaldulensis</i> , <i>E. globulus</i> , coppice	0.7	2499
	Fagta Lokoma	2	<i>E. camaldulensis</i> , coppice	0.5 - 0.7	2379-2393
	Dangila	1	<i>E. camaldulensis</i> , coppice	0.5	2134
West Gojam	North Achefer	1	<i>E. camaldulensis</i>	1.5	1971
	Machakel	1	<i>E. camaldulensis</i>	1.5	2033
North Gonder	Gonder Zurya	1	<i>E. camaldulensis</i> , coppice	2	1909
	Gonder City	1	<i>E. camaldulensis</i> , coppice	2	2070
North Wollo	Meket	1	<i>E. globulus</i> , coppice	2	2735
	Gazo	1	<i>E. globulus</i> , coppice	2	3222
	Guba Lafto	1	<i>E. camaldulensis</i> , coppice	3	2086
	Habru	1	<i>E. camaldulensis</i>	2	1898
South Wollo	Kalu	1	<i>E. camaldulensis</i> , coppice	0.3	1964
Ghana					
Ashanti	Agogo	12	<i>E. urophylla</i> , GU clones, <i>E. pellita</i>	0.33 – 2	184-353
	Drabonso	7	<i>E. camaldulensis</i> , <i>E. pellita</i> , <i>E. urophylla</i> , GU clones	0.66 – 1	275-305
Malawi					
Central Region	Lilongwe	5	<i>E. camaldulensis</i>	-	1195
	Dowa	1	<i>E. grandis</i>	-	1072
	Ntcheu	1	<i>E. camaldulensis</i>	-	1132
Northern Region	Rumphi	3	<i>E. camaldulensis</i>	-	1152
Eastern Region	Zomba	4	<i>E. camaldulensis</i> , <i>E. grandis</i>	-	765 - 1019
Southern Region	Mulanje	1	<i>E. grandis</i>	-	751

Province/Zone/Region	District	No. sites	<i>Eucalyptus</i> species	Tree age (yr.)	Elevation (m. a. s. l.)
	Mwanza	1	<i>E. grandis</i>	-	673
Rwanda					
Kigali	Gasabo	1	Mixed, coppice	Mixed	1987
North	Rulindo	1	Mixed, coppice	3	1839
North	Gicumbi	2	<i>E. maidenii</i>	4	1847-1979
East	Rwamagana	1	Mixed, coppice	Mixed	1598
East	Gatsibo	1	Mixed, coppice	Mixed	1537
East	Nyagatare	1	Mixed, coppice	Mixed	1496
East	Kayonza	1	Mixed, coppice	Mixed	1599
East	Kirehe	1	Mixed, coppice	Mixed	1633
South	Kamonyi	1	Mixed, coppice	Mixed	1446
South	Nyanza	1	Mixed, coppice	Mixed	1624
South	Huye	1	Mixed, coppice	Mixed	1678
South	Gisagara	1	Mixed, coppice	Mixed	1709
South	Nyaruguru	1	Mixed, coppice	Mixed	1898
West	Rubavu	1	<i>E. maidenii</i>	2	1567
West	Rutsiro	2	<i>E. maidenii</i> , Mixed coppice	4	1876-2016
West	Karongi	1	Mixed, coppice	Mixed	2207
Sierra Leone					
Northern Province	Tonkolili	14	<i>E. camaldulensis</i> , <i>E. pellita</i> , <i>E. urophylla</i> , <i>E. teriticornis</i> , GU clones	1.4-2	65-96
Zimbabwe					
Mashonaland West	Hurungwe	1	<i>E. camaldulensis</i>	1.4	1075
Mashonaland West	Lion's Den	1	<i>E. camaldulensis</i>	2	1182
Mashonaland West	Highbury	1	<i>E. camaldulensis</i>	2	1175
Mashonaland Central	Nyabiria	1	<i>E. grandis</i>	1	1424
Mashonaland Central	Concession	2	<i>E. camaldulensis</i>	2-3	1432
Harare	Darwendale	1	<i>E. grandis</i>	3	1332
Harare	Chivhu	1	<i>E. camaldulensis</i>	1	1410
Harare	Chikomba	2	<i>E. camaldulensis</i> , <i>E. grandis</i>	1-2	1407-1432
Mashonaland East	Macheke	2	<i>E. grandis</i> , <i>E. camaldulensis</i>	2	1532-1544
Manicaland	Headlands	1	<i>E. grandis</i>	2	1524
Manicaland	Vumba Estate	3	<i>E. cloeziana</i> , <i>E. grandis</i>	1-2	1226, 1334-1504
Manicaland	Mutasa	2	<i>E. grandis</i> , <i>E. cloeziana</i> , coppice	1	1434-1511
Manicaland	Chimanimani	4	<i>E. cloeziana</i> , coppice	0.4-1	960-1063

made in such a way as to obtain a fair representation of the major *Eucalyptus* growing provinces/zones and the common *Eucalyptus* species grown in the surveyed countries. Eight *Eucalyptus* species (*E. camaldulensis*, *E. cloeziana*, *E. globulus*, *E. grandis*, *E. maidenii*, *E. pellita*, *E. tereticornis* and *E. urophylla*) and a *E. grandis*×*urophylla* (GU) clone were assessed during the survey. Stand age was used as a criterion for site selection. Young stands often less than three years old and coppices of less than five years old were selected for the assessment in order to access the canopy easily (Table 1).

Sampling strategy

A maximum of 100 consecutive trees per site were assessed for the presence of *Eucalyptus* insect pests. The assessment was made along a ‘zig-zag’ transect where the direction of the transect was changed at every fifth tree to attain increased area coverage compared to a straight line transect. Trees were checked for the presence of life stages and/or any damage/infestation symptoms of the respective insect pests. Plant parts (leaves, petioles, stem) infested with any life stages (eggs, larvae, nymphs and adults) of the insect pests, and galls were collected from ten trees per site along the transect. The infested plant parts were kept in a plastic container, with separate containers for each site. In sites with a high infestation level, the transect was stopped after infested material was collected from 50 trees. A total of 10 876 trees (2 739 in Ethiopia, 1 826 in Ghana, 1 600 in Malawi, 1 712 in Rwanda, 1 359 in Sierra Leone and 1 640 in Zimbabwe) were assessed for the presence of *Eucalyptus* insect pests.

The samples were transported to the quarantine facility at the Forestry and Agricultural Biotechnology Institute (FABI) Biocontrol Centre, the University of Pretoria, South Africa following standard phytosanitary procedures (FAO 2015). In the quarantine facility, the samples were kept in plastic boxes with lids at 23 °C for 3-6 weeks, depending on how fast the materials were colonized with mould. Pieces of paper towel were placed in the plastic boxes to

balance the moisture level. The emerged pests and natural enemies were collected by using a camel brush and an insect aspirator, preserved in absolute ethanol and used to confirm species identification.

Morphological identification of the pests and natural enemies

Specimens of pests and natural enemies were identified using morphological characteristics and mitochondrial DNA sequence data. Morphologically, all the collected specimens were investigated under a SMZ745/745T (Nikon, Japan) stereomicroscope at a $\times 40$ magnification and grouped into different morphospecies. External morphological characteristics such as body size and colour, shape and structure of wings, antennae, legs and ovipositors were used for the initial grouping (Fig. 1). Representative specimens from these groups were then identified by comparing them with photographs and specimens of previously identified natural enemies of *Eucalyptus* insect pests.

Molecular identification of the pests and natural enemies

The morphological identification was confirmed by mitochondrial DNA sequence data analysis. For the pest species, identification was confirmed by sequencing a segment of cytochrome oxidase I (COI) and cytochrome b (Cyt b) gene (see Wondafrash et al. 2020). For the natural enemies, a segment of the cytochrome b (Cyt b) gene of the representative specimens of each morphospecies was sequenced. The specimens were rinsed with sterilized distilled water and total genomic DNA was extracted from the whole insect using prepGEM™ Insect DNA extraction kit (ZyGEM) following the manufacturer's protocol (ZyGEM Quick-Start Guide). The quantity and quality of the DNA was determined through electrophoresis using 1% agarose gel and a NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, U.S.A.). DNA was stored at -20°C until further use.

DNA was amplified 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' (Jermin and Crozier 1994). All amplification reactions were performed in a 25 μL reaction solution made of 12.6 μL of ultrapure (SABAX) water, 2.5 μL of 10 \times concentrated PCR reaction buffer (Roche, Roche Diagnostics GmbH, Mannheim, Germany), 3 μL of MgCl_2 (25 mM), 2.5 μL of deoxynucleotide triphosphate (dNTP) mix (10 mM; 2.5 mM each), 1 μL of each primer (10 mM), 0.4 μL of FastStart *Taq* DNA polymerase (5 U μL^{-1}) (Roche) and 2 μL of diluted insect genomic DNA (50 ng μL^{-1}). The reactions were run at 95 $^\circ\text{C}$ for 7 min, followed by 35 cycles at 95 $^\circ\text{C}$ for 1 min, 49 $^\circ\text{C}$ for 1 min and 72 $^\circ\text{C}$ for 1 min and final extension at 72 $^\circ\text{C}$ for 10 min.

For all specimens, electrophoresis of a mixture of 4 μL PCR aliquots and 2 μL GelRedTM (Biotium, USA) was run along with a 100 bp DNA molecular weight marker (Thermo Scientific O'Gene RulerTM) on 2% agarose gel. The amplified DNA fragments were visualized under UV light and gel images were captured using BioRad Gel DocTM EZ Imager. The PCR products were cleaned by adding 8 μL of ExoSAP-IT (USB Corporation, Cleveland, OH) and incubating the mixture at 37 $^\circ\text{C}$ and 80 $^\circ\text{C}$ for 15 min at each temperature point. The forward and reverse sequencing reactions were performed in a 10 μL reaction volume which included 6 μL distilled water, 2 μL sequencing buffer, 0.5 μL BigDye 3.1, 0.5 μL primers (10 μM) and 1 μL purified PCR product (50 ng μL^{-1}). The reactions were run at 96 $^\circ\text{C}$ for 2 min, followed by 30 cycles at 96 $^\circ\text{C}$ for 30 s, 55 $^\circ\text{C}$ for 15 s and 60 $^\circ\text{C}$ for 4 min. The sequencing products were cleaned and sequenced using an ABI PrismTM 3100 Genetic Analyser (Applied BioSystems, USA). The raw sequence data were edited using Biological Sequence Alignment Editor (BioEdit) (Hall 1999) version 7.0.9. Specimen identification was made by comparing sequence data from the present study with sequences in GenBank and from previously

identified specimens. Specimens with a sequence identity of 95% and above were considered to be conspecific.

Results

Identification and distribution of the pest species

Six non-native *Eucalyptus* insect pests, namely *B. occidentalis*, *G. brimblecombei*, *Gonipterus* sp.n.2, *L. invasa*, *O. maskelli* and *T. peregrinus* were detected and identified across the surveyed countries. *Blastopsylla occidentalis* was confirmed present in Ethiopia and Rwanda; *G. brimblecombei* in Ethiopia, Malawi, Rwanda and Zimbabwe; *Gonipterus* sp.n.2 in Rwanda and Zimbabwe; *L. invasa* in all the surveyed countries; *O. maskelli* in Ethiopia and Rwanda; and *T. peregrinus* in Malawi, Rwanda and Zimbabwe (see Wondafrash et al. 2020).

Identification of natural enemies

Nine species of natural enemies of *Eucalyptus* insect pests were confirmed to be present across the surveyed countries. These included *A. nitens*, *C. chamaeleon*, *M. pretorianensis*, an unknown *Megastigmus* species (herein referred to as *Megastigmus* sp.), *P. blastopsylla*, *P. bliteus*, *Q. mendeli*, *S. kryceri*, and *S. neseri* (Table 2, Fig. 1). All of these species are native to Australia and the majority of them have been released as biological control agents of different *Eucalyptus* insect pests elsewhere in the world. The initial morphological identification matched the molecular identification using DNA sequence data, except *Megastigmus* sp. which was morphologically misidentified as *M. zebrinus*.

Table 2. The presence of natural enemies of *Eucalyptus* insect pests in the surveyed six countries. No natural enemies were collected from Ghana and Sierra Leone.

	<i>Anaphes nitens</i>	<i>Closterocerus chamaeleon</i>	<i>Megastigmus</i> sp.	<i>Megastigmus pretorianensis</i>	<i>Psyllaephagus blastopsyllae</i>	<i>Psyllaephagus bliteus</i>	<i>Quadrastichus mendeli</i>	<i>Selitrichodes kryceri</i>	<i>Selitrichodes neseri</i>
Ethiopia	NC	P*	P*	NC	P*	P*	NC	NC	NC
Ghana	NC	NC	NC	NC	NC	NC	NC	NC	NC
Malawi	NC	NC	P*	NC	NC	P*	NC	NC	P
Rwanda	P*	NC	NC	P*	P*	P*	NC	P*	NC
Sierra Leon	NC	NC	NC	NC	NC	NC	NC	NC	NC
Zimbabwe	NC	NC	P*	P*	NC	P	P*	NC	P

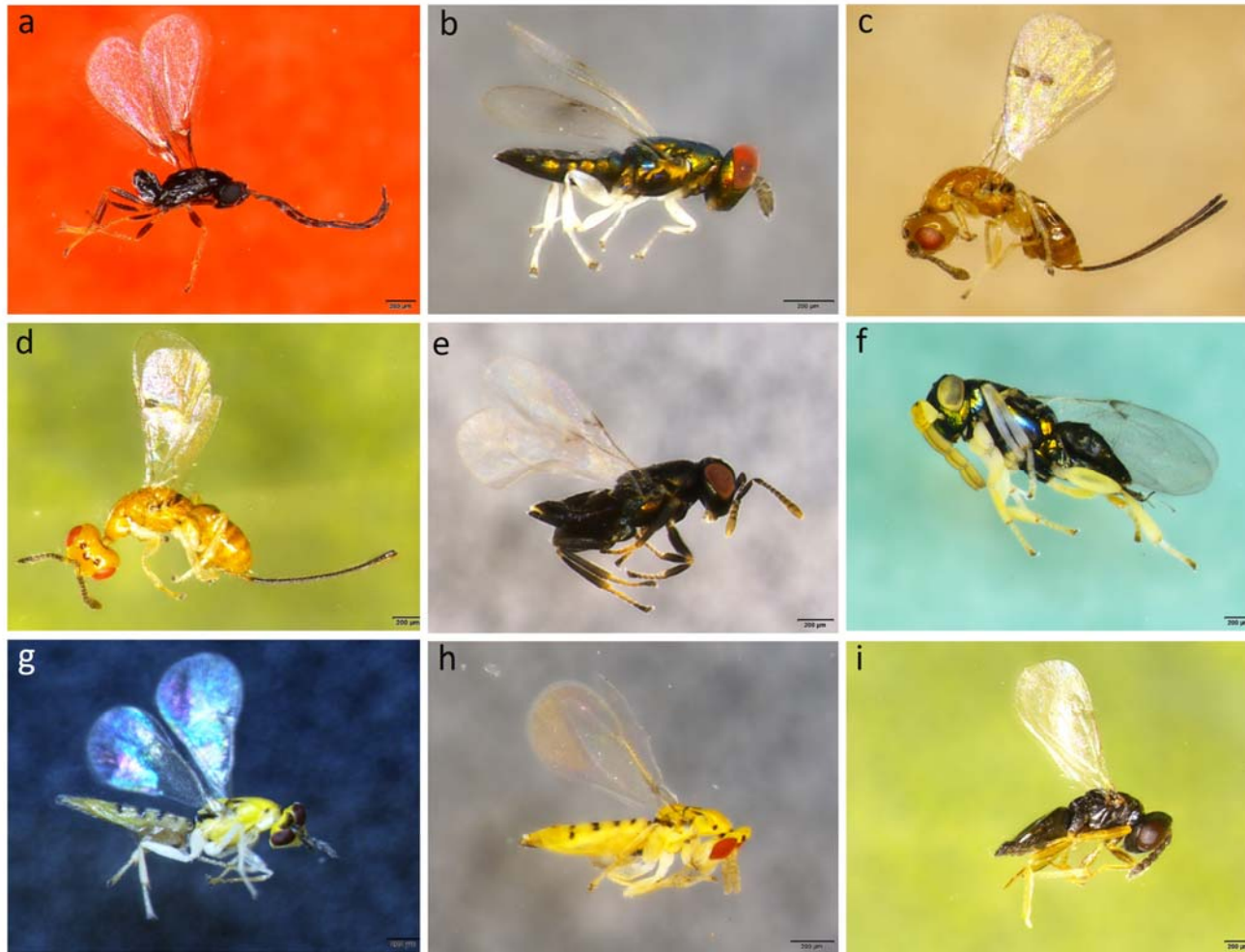


Figure 1: Natural enemies of eucalypt insect pests detected in sub-Saharan Africa. (a) *Anaphes nitens*, (b) *Closterocerus chamaeleon*, (c) *Megastigmus pretorianensis*, (d) *Megastigmus* sp., (e) *Psyllaephagus blastopsyllae*, (f) *Psyllaephagus bliteus*, (g) *Quadrastichus mendeli*, (h) *Selitrichodes kryceri*, and (i) *Selitrichodes neseri*

Distribution of natural enemies

The natural enemies were found in four of the six countries surveyed (Table 2, Fig. 2). *Anaphes nitens* was confirmed to be present in Rwanda; *C. chamaeleon* in Ethiopia; *M. pretorianensis* in Rwanda and Zimbabwe; *Megastigmus* sp. in Ethiopia, Malawi and Zimbabwe; *P. bliteus* in Ethiopia, Malawi, Rwanda and Zimbabwe; *P. blastosyllae* in Ethiopia and Rwanda; *S. kryceri* in Rwanda; *S. neseri* in Malawi and Zimbabwe; and *Q. mendeli* in Zimbabwe. No natural enemies were found in Ghana and Sierra Leone despite the presence of *L. invasa* in both of those countries. Most of these natural enemies were the first reports for the surveyed countries. This is the first published report of *A. nitens* and *S. kryceri* in Rwanda; of *C. chamaeleon* in Ethiopia; of *M. pretorianensis* in Rwanda and Zimbabwe; of *Megastigmus* sp. in Ethiopia, Malawi and Zimbabwe; of *P. bliteus* in Ethiopia, Malawi and Rwanda; of *P. blastosyllae* in Ethiopia and Rwanda, and of *Q. mendeli* in Zimbabwe.

In most cases, the natural enemies were found in a number of sites in the surveyed countries (Fig. 2, Supplementary Material 1). For example, in Zimbabwe, *Megastigmus* spp. (*M. pretorianensis* and *Megastigmus* sp.), *P. bliteus* and *S. neseri* were found in 13, 7 and 8 sites, respectively, of the 22 surveyed sites. Similarly, in Ethiopia, *Megastigmus* sp. and *P. bliteus* were present in 6 and 8 sites, respectively, of the 36 surveyed sites. In Malawi, *Megastigmus* sp. were found in 4 of the 10 surveyed sites. In Rwanda, *A. nitens* was confirmed present in 4 of the 18 surveyed sites. The other natural enemies such as *P. blastosyllae*, *Q. mendeli* and *S. kryceri* were found only in a single or a few sites (Fig. 2, Supplementary Material 1).

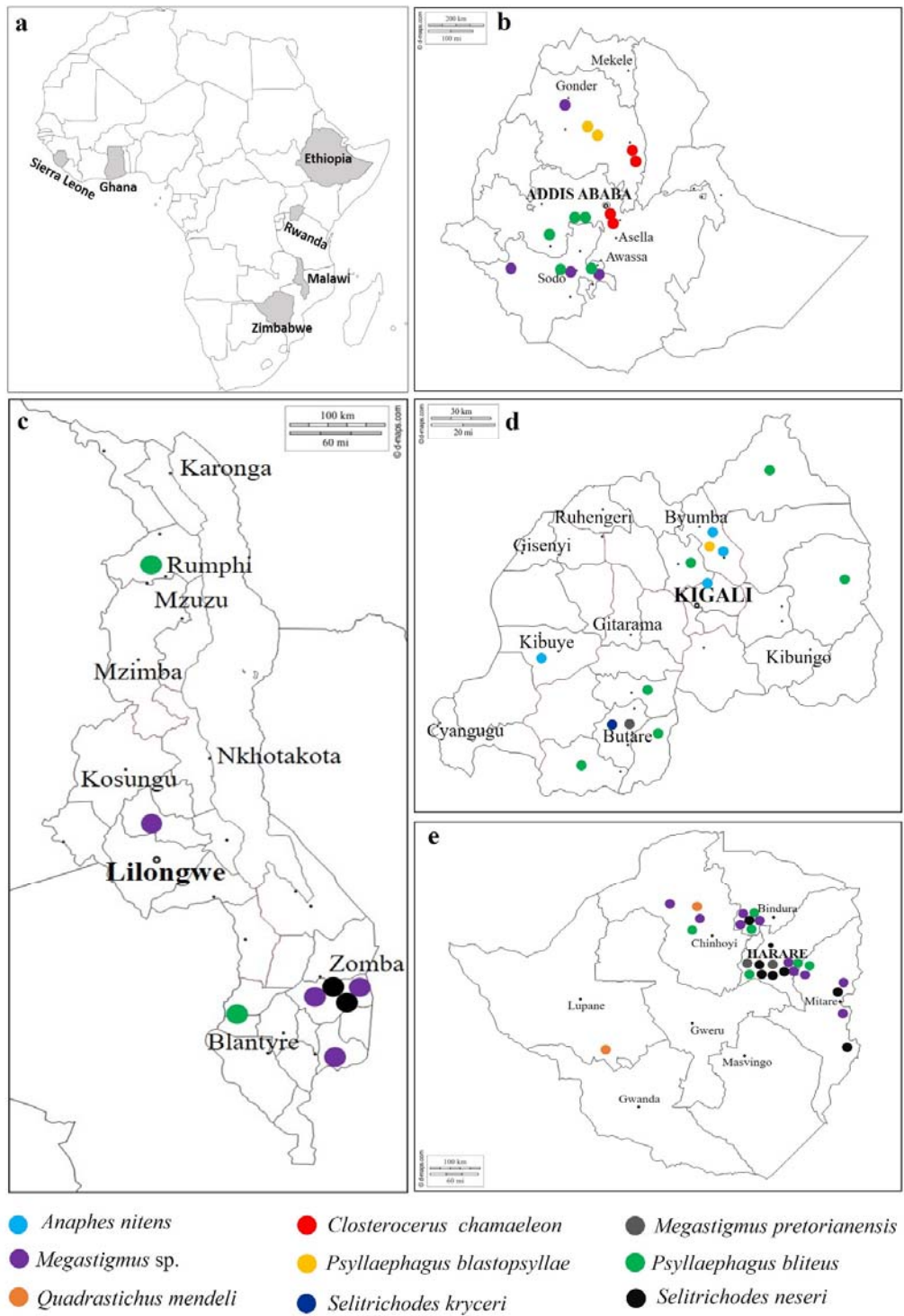


Figure 2: (a) Sub-Saharan African countries surveyed for natural enemies of eucalypt insect pests, and the distribution of the different natural enemies in (b) Ethiopia, (c) Malawi, (d) Zimbabwe. No natural enemies were found in Ghana and Sierra Leone, The different symbols on each map indicate the presence and distribution of natural enemies.

Discussion

This is the first study to identify and map the distribution of natural enemies of *Eucalyptus* insect pests in sub-Saharan Africa. Nine species of natural enemies of various *Eucalyptus* insect pests were recorded, with many first reports in the surveyed countries. All of these natural enemies are native to Australia. In most cases, these natural enemies were confirmed present in multiple sites in the surveyed countries.

Most of the natural enemies reported in this study were biological control agents previously known to be introduced in Southern Africa either intentionally or unintentionally. Results of the current study showed that these natural enemies are present in other countries in sub-Saharan Africa where there were no records of their intentional introduction. For example, *A. nitens* which was intentionally introduced to South Africa for the control of *G. scutellatus* (now *Gonipterus* sp.n.2) in 1926 (Tooke 1955; Schröder et al. 2020) was detected in Rwanda. *Closterocerus chamaeleon*, a recent arrival in South Africa (Bush et al. 2016), was confirmed present in Ethiopia. Similarly, *P. bliteus* which was recently reported from South Africa and Zimbabwe (Bush et al. 2016) has now been detected in Ethiopia, Malawi and Rwanda; and *Quadrastichus mendeli* which was recently reported from South Africa (Bush et al. 2018) was detected in the neighbouring Zimbabwe. It is possible that these natural enemies arrived accidentally together with their hosts via human-mediated pathways such as transport of live plant material, tourism and hitchhiking on vehicles/baggage, or through natural dispersal by flight or the aid of wind currents (Meurisse et al. 2019).

Some natural enemies which were not previously known to be present in sub-Saharan Africa were also detected in this study. These include *Megastigmus* sp. which was detected in Ethiopia, Malawi and Zimbabwe; and *S. kryceri* in Rwanda. *Megastigmus* sp. was recently detected in Australia associated with *L. invasa* (Dittrich-Schroder G., unpublished sequence

data), whereas *S. kryceri* was intentionally introduced in Israel in 2007 for the control of *L. invasa* (Kim et al. 2008).

Unintentional spread of some of the natural enemies recorded in this study was previously reported from other *Eucalyptus* growing regions of the world. For example, *C. chamaeleon*, since its intentional release in Israel in 2005 (Protasov et al. 2007), has spread to different *Eucalyptus*-growing regions, including the Mediterranean region (Doganlar and Mendel 2007; Borrajo et al. 2008; Branco et al. 2009), Chile and Argentina (Aquino et al. 2014; Bush et al. 2016). *Psyllaephagus bliteus* was intentionally introduced in California, Mexico and Chile in 2000, 2002 and 2005, respectively (Daane et al. 2005; Plascencia-González et al. 2005; Ide et al. 2006) and subsequently spread to Brazil, Colombia, a number of Mediterranean countries (Caleca et al. 2011; Pérez-Otero et al. 2011; Bella and Rapisarda 2013; Reguia and Peris-Filipo 2013; Karaca et al. 2015) and Portugal (Dhari et al. 2014). Following its deliberate introduction in Israel in 2007 (Kim et al. 2008), *Q. mendeli* has accidentally spread to several countries, including Argentina (Aquino et al. 2017), China (Zheng et al. 2014); Cambodia, Laos, Thailand, Vietnam (ACIAR 2016) and Italy (Nugnes et al. 2016), and South Africa (Bush et al. 2018).

Some of the natural enemies reported in the current study have not been intentionally introduced elsewhere in the world as biological control agents. These include *Megastigmus* sp., *M. pretorianensis*, and *P. blastopsyllae*. Interestingly, *M. pretorianensis* and *P. blastopsyllae* were previously reported from sub-Saharan Africa and beyond. *Megastigmus pretorianensis*, a potential natural enemy of *L. invasa* was recently described from South Africa (Doganlar 2015) and detected in Argentina and Thailand (Huang et al. 2018). It was more recently reported from Australia (Le et al. 2020), where it is assumed to be native. In this study, it was found in Rwanda and Zimbabwe. *Psyllaephagus blastopsyllae*, a congener of *P. bliteus* and a parasitoid of *B. occidentalis*, was recently described from Cameroon (Tamesse et al. 2014) and

subsequently reported from South Africa (Bush et al. 2016). In the current study, this parasitoid was confirmed as present in Ethiopia and Rwanda.

The unintentional spread of these natural enemies into countries where they were not intentionally introduced is of benefit for *Eucalyptus* growers in the region as these parasitoids could play an important role in regulating the populations of the pest species. For example, *A. nitens* has been the main biological control agent for *Gonipterus* populations for many decades in South Africa, South America and Europe (Tooke 1955; Schröder et al. 2020). Similarly, *C. chamaeleon*, *P. bliteus*, *Q. mendeli* and *S. kryceri* have been reported to control the populations of their respective host species (Dahlsten et al. 2005; Plascencia-González et al. 2005; Ide et al. 2006). However, there is a risk associated with unintentional movement of natural enemies between countries. Unintentionally introduced natural enemies could compete with/displace other natural enemies or biocontrol agents. They may also attack non-target insects.

Acquiring knowledge on the presence and distribution of natural enemies of non-native pests in the invaded regions is a crucial step in developing a CBC program (Kenis et al. 2019). For example, such information can inform decisions as to which, if any, biological control agents should be introduced, to which areas they should be introduced and if there is an opportunity to collect natural enemies from one area of a country and release in another area where the natural enemy is not present. In our study, none of the known natural enemies of *L. invasa* were detected in Ghana, Ethiopia and Sierra Leone despite the significant level of damage it causes on *Eucalyptus* trees. This suggests the need for introduction of biological control agents such as *S. neseri*, *S. kryceri* and *Q. mendeli*. Some of the natural enemies detected in this study, for example, *P. blastosyllae*, *Q. mendeli* and *S. kryceri* were found only in a single or a few sites. These natural enemies can be collected, reared and released in other areas of those countries where their host has become a problem.

Conclusions

The current study developed baseline information on the presence and distribution of natural enemies of *Eucalyptus* insect pests in sub-Saharan Africa. It showed that populations of insect pests and natural enemies are very connected across *Eucalyptus* plantations in the region. These connected and shared pest and natural enemy populations call for a coordinated and concerted management approach among the affected countries. Garnas et al. (2012) and Wingfield et al. (2015) made a similar call for a coordinated and globally connected management strategy of plantation pests. It is important to monitor the parasitism rate and distribution of these natural enemies and their impact on the pest population over time. The interaction between the different natural enemies and its potential consequence on pest management also needs to be investigated. In addition, it would be beneficial to conduct similar surveys in sub-Saharan African countries not included in this study, but where *Eucalyptus* is an important socio-economic crop.

References

- ACIAR. 2016. Annual report- project FST2012/091- Biological control of galling insect pests of eucalypt plantations in the Mekong Region. Annual report- project FST2012/091, Issue 07/2016.
- Aquino DA, Andorno AV, Pathauer PS, Botto EN, López SN. 2017. Centro de Estudios ParasitPrimer a cita de *Quadrastichus mendeli* (Hymenoptera: Eulophidae: Tetrastichinae) de Argentina, asociado a agallas de *Leptocybe invasa* (Hymenoptera: Eulophidae: Tetrastichinae). *Acta Zoológica Lilloana* 62 (Suplemento), 2018.
- Aquino DA, Hernández CM, Cuello EM, Andorno AV, Botto EN. 2014. Primera cita de la Argentina de *Ophelimus maskelli* (Ashmead) (Hymenoptera: Eulophidae) y su

- parasitoide, *Closterocerus chamaeleon* (Girault) (Hymenoptera: Eulophidae). *Revista de la Sociedad Entomológica Argentina* 73: 79-182.
- Bella S, Rapisarda C. 2013. First record from Greece of the invasive red gum lerp psyllid *Glycaspis brimblecombei* Moore (Hemiptera: Psyllidae) and its associated parasitoid *Psyllaephagus bliteus* Riek (Hymenoptera Encyrtidae). *Redia* 96: 33-35.
- Borrajo P, López MA, Ocete R, López G, Ruiz F. 2008. Comunicación primera cita de *Closterocerus chamaeleon* Girault (Hymenoptera, Eulophidae), parasitoide de *Ophelimus maskelli* Ashmead (Hymenoptera, Eulophidae) en la provincial de Huelva (SO España). *Boletín de Sanidad Vegetal - Plagas* 34: 383-385.
- Bouvet JPR, Harrand L, Buckhardt D. 2005. Primera cita de *Blastopsylla occidentalis* y *Glycaspis brimblecombei* (Hemiptera: Psyllidae) para la república argentina. *Revista de la Sociedad Entomológica Argentina* 64: 99–102.
- 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.
- Bush SJ, Slippers B, Nesar S, Harney M, Dittrich-Schroder G, Hurley BP. 2016. Six recently recorded Australian insects associated with *Eucalyptus* in South Africa. *African Entomology* 24: 539-544.
- Bush SJ, Dittrich-Schröder G, Nesar S, Gevers C, Baffoe KO, Slippers B, Hurley BP. 2018. First record of *Quadrastichus mendeli*, a parasitoid of *Leptocybe invasa*, in South Africa. *Southern Forests: a Journal of Forest Science* 80: 275-277.
- Caleca V, Lo Verde G, Maltese M. 2011. First record in Italy of *Psyllaephagus bliteus* Riek (Hymenoptera: Encyrtidae) parasitoid of *Glycaspis brimblecombei* Moore (Hemiptera: Psyllidae). *IL Naturalista Siciliano* 4: 435-444.

- Cornell HV, Hawkins BA. 1993. Accumulation of native parasitoid species on introduced herbivores: a comparison of hosts as natives and hosts as invaders. *American Naturalist* 141: 847-865.
- Daane KM, Sime KR, Dahlsten DL, Andrews Jr JW, Zuparko RL. 2005. The biology of *Psyllaephagus bliteus* Riek (Hymenoptera: Encyrtidae), a parasitoid of the red gum lerp psyllid (Hemiptera: Psylloidea). *Biological Control* 32: 228-235.
- Dahlsten DL, Daane KM, Paine TD, Sime KR, Lawson AB, Rowney DL, Roltsch WJ, Andrews Jr JW, Kabashima JN, Shaw DA, Robb KL, Downer JA, Geisel PM, Chaney WE, Ingels CA, Varela LG, Bianchi ML, Taylo G. 2005. Imported parasitic wasp helps control red gum lerp psyllid. *California Agriculture* 59: 229-234.
- Dahri S, Jamaa MLB, Boavida C, Branco M. 2014. Presence of *Glycaspis brimblecombei* and its parasitoid *Psyllaephagus bliteus* in Tunisia and Portugal. *Silva Lusitana* 22: 99-115.
- Dittrich-Schröder G, Harney M, Naser S, Joffe T, Bush S, Hurley BP, Wingfield MJ, Slippers B. 2014. Biology and host preference of *Selitrichodes neseri*: a potential biological control agent of the Eucalyptus gall wasp, *Leptocybe invasa*. *Biological Control* 78: 33-41.
- Dittrich-Schroder 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.
- Doganlar M, Mendel Z. 2007. First record of the Eucalyptus gall wasp *Ophelimus maskelli* and its parasitoid, *Closterocerus chamaeleon*, in Turkey. *Phytoparasitica* 35: 333-335.
- Doganlar M. 2015. Diagnosis of *Megastigmus* spp. (Hymenoptera: Torymidae) reared from galls of *Leptocybe invasa* FISHER & LASALLE, 2004, (Hymenoptera: Eulophidae) on *Eucalyptus* spp. (Myrtaceae), with description of a new species from South Africa. *Entomofauna* 43: 561-580.

- 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, Italy.
- FAO. 2015. Global phytosanitary manuals, standard operating procedures and training kits. Terminal report prepared for the Standard and Trade Development Facility (STDF). AGP: MTF/GLO/368/STF. Rome.
- Garnas JR, Hurley BP, Slippers B, Wingfield MJ. 2012. Biological control of forest plantation pests in an interconnected world requires greater international focus. *International Journal of Pest Management* 58: 211-223.
- Grabeweger G, Kehrl P, Zweimuller I, Augustin S, Avtzi N, Bacher S, Freise J, Girardoz S, Guichard S, Heitland W, Lethmayer C, Stolz M, Tomov R, Volter L, Kenis M. 2010. Temporal and spatial variations in the parasitoid complex of the horse chestnut leafminer during its invasion of Europe. *Biological Invasions* 12: 2797-2813.
- Hall T. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/ NT. *Nucleic Acids Symposium Series* 45: 95-98.
- Harry M, Solignac M, Lachaise D. 1998. Molecular evidence for parallel evolution of adaptive syndromes in Fig-Breeding *Lissocephala* (Drosophilidae). *Molecular Phylogenetics and Evolution* 9: 542-551.
- Huang Z-Y, Jun Li J, Lu W, Zheng X-L, Yang Z-D. 2018. Parasitoids of the eucalyptus gall wasp *Leptocybe* spp.: a global review. *Environmental Science and Pollution Research* 25: 29983-29995.
- Hurley BP, Slippers B, Sathyapala S, Wingfield MJ. 2017. Challenges to planted forest health in developing economies. *Biol Invasions* 19: 3273-3285.

- Hurley BP, Garnas J, Wingfield MJ, Branco M, Richardson DM, Slippers B. 2016. Increasing numbers and intercontinental spread of invasive insects on eucalypts. *Biological Invasions* 18: 921-933.
- Ide SM, Muñoz CA, Beéche MC, Mondaca JE, Jaques LR, González PE, Goycoolea CP. 2006. Detección y control biológico de *Glycaspis brimblecombei* Moore (Hemiptera: Psyllidae). Servicio Agrícola y Ganadero, Division de Proteccion Agrícola, Santiago, Chile. 32 pp.
- Jermin 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. *Journal of Molecular Evolution* 38: 282-294.
- Karaca I, Kayahan A, Simsek B, Çelikpençe Y. 2015. First record of *Glycaspis brimblecombei* Moore (Hemiptera: Aphalaridae), in Turkey. *Phytoparasitica* 43: 171-175.
- Keane RM, Crawley MJ. 2002. Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution* 17: 164-170.
- Kenis M, Hurley BP, Colombari F, Lawson S, Sun J, Wilcken C, Weeks R, Sathyapala S. 2019. Guide to the classical biological control of insect pests in planted and natural forests, FAO Forestry Paper No. 182. FAO, Rome, Italy.
- Kenis M, Hurley BP, Hajek AE, Matthew JW. 2017. Classical biological control of insect pests of trees: facts and figures. *Biological Invasions* 19: 3401-3417.
- Kim I-K, Mendel Z, Protasov A, Blumberg D, La Salle J. 2008. Taxonomy, biology, and efficacy of two Australian parasitoids of the eucalyptus gall wasp, *Leptocybe invasa* Fisher and La Salle (Hymenoptera: Eulophidae: Tetrastichinae). *Zootaxa* 1910: 1-20.
- Le NH, Nahrung HF, Morgan JAT, Lawson SA. 2020. Multivariate ratio analysis and DNA markers reveal a new Australian species and three synonymies in eucalypt-ga II-

- associated *Megastigmus* (Hymenoptera: Megastigmidae). *Bulletin of Entomological Research* 110: 709-724.
- Meurisse N, Rassati D, Hurley BP, Brockerhof EG, Haack RA. 2019. Common pathways by which non-native forest insects move internationally and domestically. *Journal of Pest Science* 92: 13-27.
- Mutitu EK, Garnas JR, Hurley BP, Wingfield MJ, Harney M, Bush SJ, Slippers B. 2013. Biology and rearing of *Cleruchoides noackae* (Hymenoptera: Mymaridae), an egg parasitoid for the biological control of *Thaumastocoris peregrinus* (Hemiptera: Thaumastocoridae). *Journal of Economic Entomology* 106: 1980-1985.
- Nadel RL, Noack AE. 2012. Current understanding of the biology of *Thaumastocoris peregrinus* in the quest for a management strategy. *International Journal of Pest Management* 58:257–266.
- 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: Psylloidea), and the blue gum chalcid wasp, *Leptocybe invasa* (Hymenoptera: Eulophidae), in Zimbabwe. *African Entomology* 26: 104-115.
- Nugnes F, Gebiola M, Gualtieri L, Russo E, Sasso R, Bernardo U. 2016. When exotic biocontrol agents travel without passport: first record of *Quadrastichus mendeli*, parasitoid of the blue-gum chalcid *Leptocybe invasa*, in Italy. *Bulletin of Insectology* 69: 85-91.
- Pérez-Otero R, Borrajo P, Mansilla JP, Ruiz F. 2011. Primera cita en España de *Psyllaephagus bliteus* Riek (Hymenoptera, Encyrtidae), parasitoide de *Glycaspis brimblecombe* (Hemiptera, Psyllidae). *Boletín de Sanidad Vegetal Plagas* 37: 37-44.

- Plascencia-González A, Cibrián-Tovar D, Llanderal-Cázares C, López-Pérez I, Arriola-Padilla V. 2005. Biología del parasitoide *Psyllaephagus bliteus* (Hymenoptera: Encyrtidae). *Revista Chapingo Serie Ciencias Forestales y del Ambiente* 11: 11-17.
- Protasov A, Blumberg D, Brand D, Lasalle J, Mendel Z. 2007. Biological control of the *Eucalyptus* gall wasp *Ophelimus maskelli* (Ashmead): Taxonomy and biology of the parasitoid species *Closterocerus chamaeleon* (Girault), with information on its establishment in Israel. *Biological Control* 42: 196-206.
- Reguia K, Peris-Felipo FJ. 2013. *Glycaspis brimblecombei* Moore, 1964 (Hemiptera Psyllidae) invasion and new records in the Mediterranean area. *Biodiversity Journal* 4: 501-506.
- Roy HE, Handley L-JI, Schönrogg K, Poland RL, Purse BV. 2011. Can the enemy release hypothesis explain the success of invasive alien predators and parasitoids? *Biocontrol* 56: 451- 468.
- Santana DLT, Burckhardt D. 2007. Introduced *Eucalyptus* psyllids in Brazil. *Journal of Forestry Research* 12:337–344.
- Schröder ML, Slippers B, Wingfeld MJ, Hurley BP. 2020. Invasion history and management of *Eucalyptus* snout beetles in the *Gonipteris scutellatus* species complex. *Journal of Pest Science* 93: 11-25.
- Tamesse JL, Soufo L, Tchanatame EC, Dzokou VJ, Gumovsky A, De Coninck E. 2014. Description of *Psyllaephagus blastopsyllae* sp.n. (Encyrtidae), new species, endoparasitoid of *Blastopsylla occidentalis* Taylor (Psyllidae, Spondyliaspidae) in Cameroon. *Journal of Biodiversity and Environmental Sciences* 5: 228-236.
- Tooke FGC. 1955. The *Eucalyptus* Snout Beetle: a study of its ecology and control by biological means. Entomology Memoirs Vol. 3, Department of Agriculture, Pretoria, South Africa, pp. 1-282.

- Vacante V, Bonsignore CP. 2017. Natural enemies and pest control. In: Handbook of Pest Management in Organic Farming (ed. by V Vacante, S Kreiter), pp 60-77, CAB International, Wallingford, UK.
- 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, Coutinh TA, Wingfield BD, Roux J. 2008. Eucalypt pests and diseases: growing threats to plantation productivity. *Southern Forests* 70: 139-144.
- Wondafrash M, Slippers B, Garnas J, Hurley BP. 2018. Parasitoid assemblage associated with a North American pine weevil in South Africa. *Agricultural and Forest Entomology* 20: 208-216.
- Wondafrash M, Slippers B, Zambazimana A, Kayumba I, Nibouche S, Van Der Lingen S, Asfaw BA, Jenya H, Mutitu EK, Makowe IA, Chungu D, Kiwuso P, Kulimushi E, Razafindrakotomamonjy A, Bosu PP, Sooka P, Hurley BP. 2020. Distribution and genetic diversity of five invasive pests of *Eucalyptus* in sub-Saharan Africa. *Biological Invasions* 22: 2205-2221.
- Zappala L, Biondi A, Alma A, Al-Jboory IJ, Arno J, Bayram A, Chailleux A, El-Arnaouty A, Gerling D, Guenaoui Y, Shaltiel-Harpaz L, Siscaro G, Stavrinides M, Tavella L, Aznar RV, Urbaneja A, Desneux N. 2013. Natural enemies of the South American moth, *Tuta absoluta*, in Europe, North Africa and Middle East, and their potential use in pest control strategies. *Journal of Pest Science* 86: 635-647.
- Zheng XL, Li J, Yang ZD, Xian ZH, Wei JG. 2014. A review of invasive biology, prevalence and management of *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae: Tetrastichinae). *African Entomology* 22: 68-79.

ZyGEM. n.d. *DNA extraction using prepGEM® Insect*. ZyGEM Quick-Start Guide.

<https://www.genetargetsolutions.com.au/wp-content/uploads/2014/03/prepGEM-Insect-Quick-Start-Guide.pdf>