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

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Eucalypt forestry in sub-Saharan Africa is challenged by non-native eucalypt-feeding insects. In recent studies, six invasive eucalypt 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 countries in the region. Plant parts (leaves, petioles and stem) infested with 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: *Anaphes nitens*, biological control, *Closterocerus chamaeleon*, plantation forestry, *Psyllaephagus*, *Quadrastichus mendeli*

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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; Kenis et al. 2017; Bush et al. 2018; Wondafrash et al. 2018). Recruitment of natural enemies native to the invaded region is a very slow process and often characterised 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 of permanently controlling pests, usually also non-native. It has been used to control 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 used successfully in eucalypt plantations (Garnas et al. 2012).

Eucalypt 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; Wingfield et al. 2015). Similar to other parts of the world, the rate of introduction of non-native eucalypt-feeding insects has been increasing in sub-Saharan Africa (Hurley et al. 2016; Hurley et al. 2017). In recent studies, six non-native invasive eucalypt insect pests, namely the blue gum chalcid wasp, *Leptocybe invasa* Fisher (Hymenoptera: Eulophidae); the red gum 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 eucalypt gall wasp, *Ophelimus maskelli* Ashmead (Hymenoptera: Eulophidae); and the eucalypt 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 eucalypt, 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, when they get damp, also cause sooty mould on the tree parts. 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 *Gonipterus* sp.n.2 feed on eucalypt leaves and cause severe damage to the trees (Tooke 1955).

Several 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). *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; Bush et al. 2018). Similarly, *Psyllaephagus bliteus* Riek (Hymenoptera: Encyrtidae), the parasitoid of *G. brimblecombei,* was recently reported from South Africa (Bush et al. 2016) and Zimbabwe (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 (Tamesse et al. 2014) and South Africa (Bush et al. 2016). However, knowledge on the presence and distribution of natural enemies of eucalypt 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 eucalypt 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 eucalypt insect pests and their natural enemies (Table 1). Sites were selected to ensure a fair representation of the major eucalypt growing provinces/zones and the common eucalypt species grown in the surveyed countries. Eight eucalypt species (*E. camaldulensis* Dehnh., *E. cloeziana* F.Muell., *E. globulus* Labill/, *E. grandis* W.Hill, *E. maidenii (*F.Muell.) J.B.Kirkp., *E. pellita* F.Muell., *E. tereticornis* Sm. and *E. urophylla* S.T.Blake) 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 eucalypt 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 and 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,

Table 1: Site information for collection of natural enemies of eucalypt insect pests in six sub-Saharan African countries

Province/ zone/ region	District	No. sites	*Eucalyptus* species	Tree age (years)	Elevation a.s.l. (metres)
Ethiopia					
East Showa	Dire Gerbicha	1	*E. camaldulensis*, coppice	8	1 969
	Gelan	1	*E. camaldulensis*, coppice	5	2 261
West Arsi	Shashemene	3	*E. globulus*, *E. saligna*, *E. grandis*, coppice	0.5–4	2 629–2 654
Sidama	Shebedino	1	*E. camaldulensis*, coppice	1	1 838
	Yirgalem	1	*E. camaldulensis*, coppice	3	1 779
Welayta	Damot Weyde	1	*E. camaldulensis*, coppice	1	1 759
Jimma	Kersa	3	*E. camaldulensis*, coppice	0.2–2	1 693–1 769
	Seka Chekorsa	1	E. camaldulensis	2	1 819
Bonga	Gimbo	2	*E. saligna*, *E. camaldulensis*	1–2	1 508
Gurage	Kebena	1	*E. camaldulensis*, coppice	4	1 815
South West Sowa	Weliso	4	*E. camaldulensis*, *E. camaldulensis*, coppice	1–5	1 944–2 316
North Sowa	Debre Libanos	1	*E. globulus,* coppice	2	2 505
East Gojam	Machakel	2	*E. camaldulensis*, *E. grandis*, coppice	1–3	2 230–2 390
Awi	Enjibara	2	*E. camaldulensis*, *E. globulus*, coppice	0.7	2 499
	Fagta Lokoma	2	*E. camaldulensis*, coppice	0.5–0.7	2 379–2 393
	Dangila	1	*E. camaldulensis*, coppice	0.5	2 134
West Gojam	North Achefer	1	*E. camaldulensis*	1.5	1 971
	North Machakel	1	*E. camaldulensis*	1.5	2 033
North Gonder	Gonder Zurya	1	*E. camaldulensis*, coppice	2	1 909
	Gonder City	1	*E. camaldulensis*, coppice	2	2 070
North Wollo	Meket	1	*E. globulus*, coppice	2	2 735
	Gazo	1	*E. globulus*, coppice	2	3 222
	Guba Lafto	1	*E. camaldulensis*, coppice	3	2 086
	Habru	1	*E. camaldulensis*	2	1 898
South Wollo	Kalu	1	*E. camaldulensis*, coppice	0.3	1 964
Ghana					
Ashanti	Agogo	12	*E. urophylla*, GU clones, *E. pellita*	0.33–2	184–353
	Drabonso	7	*E. camaldulensis, E. pellita, E. urophylla*, GU clones	0.66–1	275–305
Malawi					
Central Region	Lilongwe	5	*E. camaldulensis*	–	1 195
	Dowa	1	*E. grandis*	–	1 072
	Ntcheu	1	*E. camaldulensis*	–	1 132
Northern Region	Rumphi	3	*E. camaldulensis*	–	1 152
Eastern Region	Zomba	4	*E. camaldulensis*, *E. grandis*	–	765–1 019
Southern Region	Mulanje	1	*E. grandis*	–	751
	Mwanza	1	*E. grandis*	–	673
Rwanda					
Kigali	Gasabo	1	Mixed, coppice	Mixed	1 987
North	Rulindo	1	Mixed, coppice	3	1 839
North	Gicumbi	2	*E. maidenii*	4	1 847–1 979
East	Rwamagana	1	Mixed, coppice	Mixed	1 598
East	Gatsibo	1	Mixed, coppice	Mixed	1 537
East	Nyagatare	1	Mixed, coppice	Mixed	1 496
East	Kayonza	1	Mixed, coppice	Mixed	1 599
East	Kirehe	1	Mixed, coppice	Mixed	1 633
South	Kamonyi	1	Mixed, coppice	Mixed	1 446
South	Nyanza	1	Mixed, coppice	Mixed	1 624
South	Huye	1	Mixed, coppice	Mixed	1 678
South	Gisagara	1	Mixed, coppice	Mixed	1 709
South	Nyaruguru	1	Mixed, coppice	Mixed	1 898
West	Rubavu	1	*E. maidenii*	2	1 567
West	Rutsiro	2	*E. maidenii*, Mixed coppice	4	1 876–2 016
West	Karongi	1	Mixed, coppice	Mixed	2 207
Sierra Leone					
Northern Province	Tonkolili	14	*E. camaldulensis*, *E. pellita*, *E. urophylla*, *E. teriticornis*, GU clones	1.4–2	65–96
Zimbabwe					
Mashonaland West	Hurungwe	1	*E. camaldulensis*	1.4	1 075
Mashonaland West	Lion's Den	1	*E. camaldulensis*	2	1 182

Table 1: (cont.)

Province/ zone/ region	District	No. sites	*Eucalyptus* species	Tree age (years)	Elevation a.s.l. (metres)
(Zimbabwe, contd)					
Mashonaland West	Highbury	1	*E. camaldulensis*	2	1 175
Mashonaland Central	Nyabiria	1	*E. grandis*	1	1 424
Mashonaland Central	Concession	2	*E. camaldulensis*	2–3	1 432
Harare	Darwendale	1	*E. grandis*	3	1 332
Harare	Chivhu	1	*E. camaldulensis*	1	1 410
Harare	Chikomba West	2	*E. camaldulensis, E. grandis*	1–2	1 407–1 432
Mashonaland East	Macheke	2	*E. grandis, E. camaldulensis*	2	1 532–1 544
Manicaland	Headlands	1	*E. grandis*	2	1 524
Manicaland	VumbaEstate	3	*E. cloeziana, E. grandis*	1–2	1 226, 1 334–1 504
Manicaland	Mutasa	2	*E. grandis, E. cloeziana, coppice*	1	1 434–1 511
Manicaland	Chimanimani	4	*E. cloeziana,* coppice	0.4–1	960–1 063

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 eucalypt insect pests.

The samples were transported to the quarantine facility at the Forestry and Agricultural Biotechnology Institute (FABI) Biocontrol Centre at 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 colonised 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 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 ×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 (Figure 1). Representative specimens from these groups were then identified by comparing them with photographs and specimens of previously identified natural enemies of eucalypt 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 sterilised 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, USA). 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× concentrated polymerase chain reaction (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 °C for 7 minutes, followed by 35 cycles at 95 °C for 1 minute, 49 °C for 1 minute and 72 °C for 1 minute and final extension at 72 °C for 10 minutes.

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 visualised 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 °C and 80 °C for 15 minutes 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 °C for two minutes, followed by 30 cycles at 96 °C for 30 seconds, 55 °C for 15 seconds and 60 °C for four minutes. 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.

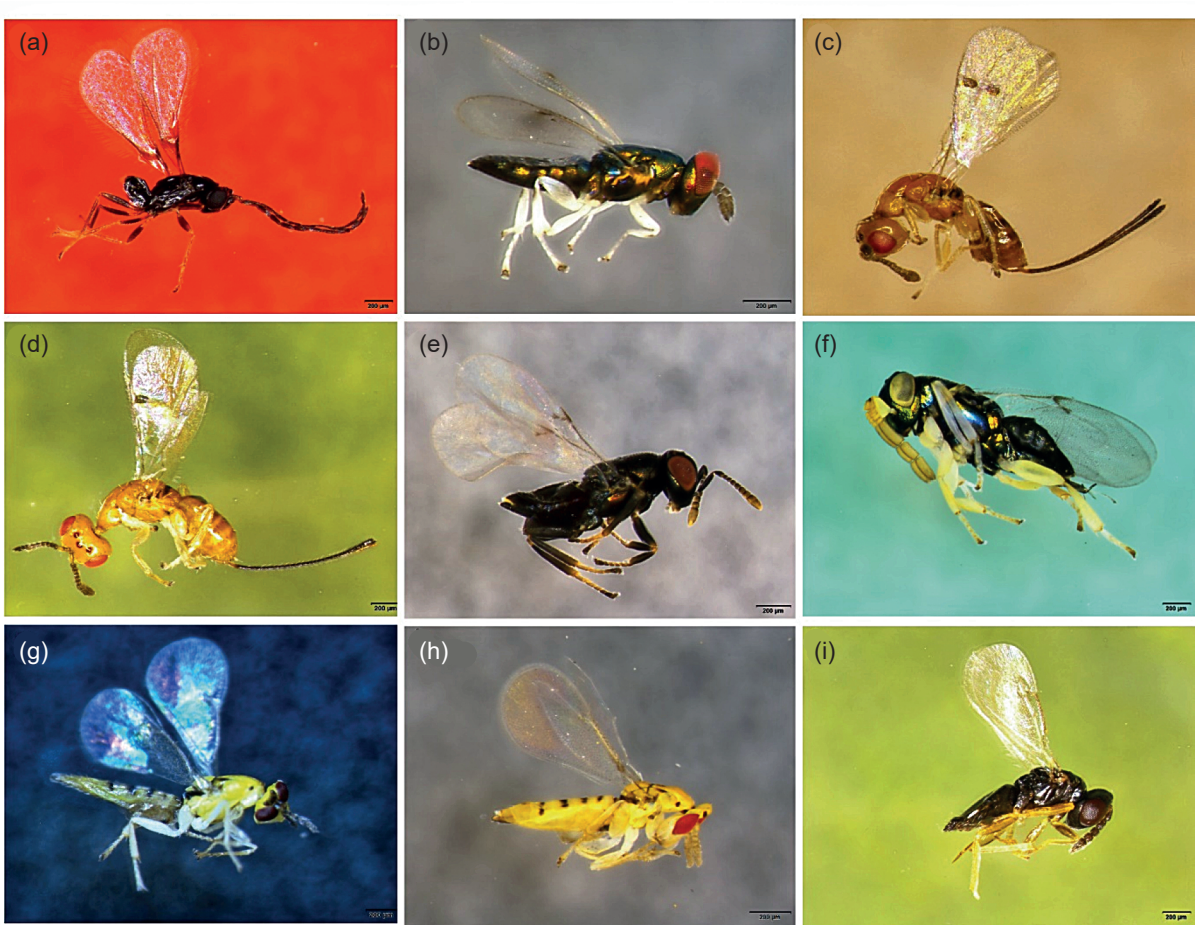

Figure 1: Natural enemies of eucalypt insect pests detected in sub-Saharan Africa. (a) *Anaphes nitens*, (b) *Closterocerus chamaeleon*, (c) *Megastigmus pretorianensis*, (d) *Megastigmus sp.*, (e) *Psyllaephagus blastopsyllae*, (f) *Psyllaephagus bliteus*, (g) *Quadrastichus mendeli*, (h) *Selitrichodes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

Results

Identification and distribution of the pest species

Six non-native eucalypt 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 eucalypt 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, Figure 1). All of these species are native to Australia and most of them have been released as biological control agents of different eucalypt insect pests elsewhere in the world. The initial morphological identification matched the molecular identification using DNA sequence data, except *Megastigmus* sp. was morphologically misidentified as *M. zebrinus*.

Distribution of natural enemies

The natural enemies were found in four of the six countries surveyed (Table 2; Figure. 2). *Anaphes nitens* was confirmed to be present in Rwanda; *C. chamaeleon* in Ethiopia; *M. pretorianesis* in Rwanda and Zimbabwe; *Megastigmus* sp. in Ethiopia, Malawi and Zimbabwe; *P. bliteus* in Ethiopia, Malawi, Rwanda and Zimbabwe; *P. blastopsyllae* 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 Rwanada and Zimbabwe; of *Megastigmus* sp. in Ethiopia, Malawi and Zimbabwe; of *P. bliteus* in Ethiopia, Malawi and Rwanda; of *P. blastopsyllae* in Ethiopia and Rwanda, and of *Q. mendeli* in Zimbabwe.

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

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

NC = not collected P = present, but not the first report P* = present, and the first report

In most cases, the natural enemies were found in several sites in the surveyed countries (Figure 2; see also Supplementary information, Table S1). 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. blastopsyllae*, *Q. mendeli* and *S. kryceri* were found only in a single or a few sites (Figure 2; Supplementary information, Table S1).

Discussion

This is the first study to identify and map the distribution of natural enemies of eucalypt insect pests in sub-Saharan Africa. Nine species of natural enemies of various eucalypt 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 our 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. scutelatus* (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 eucalypt 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 eucalypt-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, several 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 our 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 our study, this parasitoid was confirmed as present in Ethiopia and Rwanda.

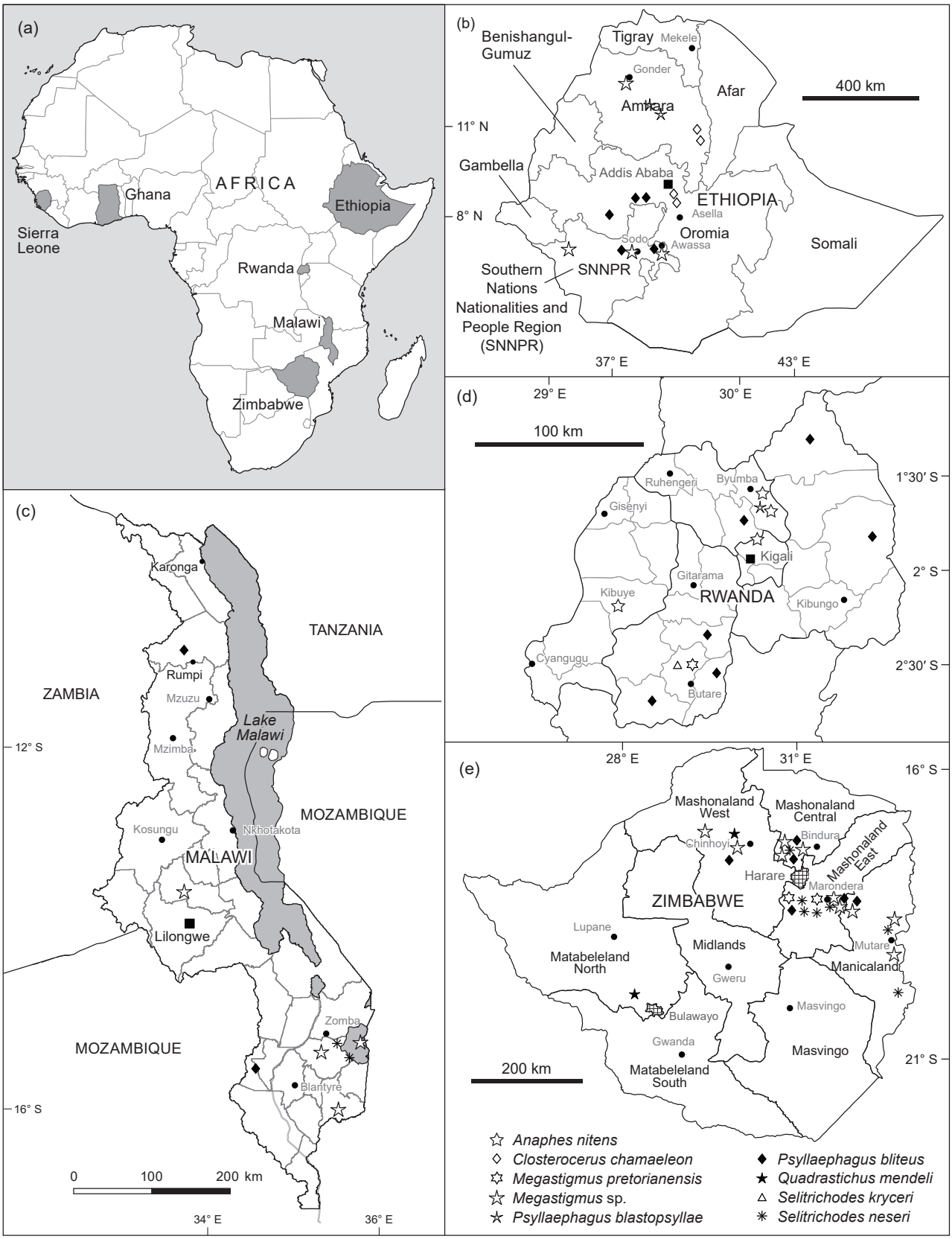

Figure 2: (a) Sub-Saharan African countries surveyed for natural enemies of eucalypt insect pests, and the distribution of the different natural enemies in (b) Ethiopia, (c) Malawi, (d) Rwanda and (e) Zimbabwe. No natural enemies were found in Ghana and Sierra Leone. The different symbols on each map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

The unintentional spread of these natural enemies into countries where they were not intentionally introduced is of benefit for eucalypt 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 programme (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 eucalypt 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. blastopsyllae*, *Q. mendeli* and *S. kryceril*, 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

Our study developed baseline information on the presence and distribution of natural enemies of eucalypt insect pests in sub-Saharan Africa. It showed that populations of insect pests and natural enemies are very connected across eucalypt 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 eucalypt is an important socio-economic crop.

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