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The diversity of aphid parasitoids in East Africa and implications for biological control

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Abstract

BACKGROUND: Hymenopteran parasitoids provide key natural pest regulation services and are reared commercially as biological control agents. Therefore, understanding parasitoid community composition in natural populations is important to enable better management for optimized natural pest regulation. We carried out a field study to understand the parasitoid community associated with *Aphis fabae* on East African smallholder farms. Either common bean (*Phaseolus vulgaris*) or lablab (*Lablab purpureus*) sentinel plants were infested with *Aphis fabae* and deployed in 96 fields across Kenya, Tanzania, and Malawi.

RESULTS: A total of 463 parasitoids emerged from sentinel plants of which 424 were identified by mitochondrial cytochrome oxidase I (COI) barcoding. *Aphidius colemani* was abundant in Kenya, Tanzania and Malawi, while *Lysiphlebus testaceipes* was only present in Malawi. The identity of *Aphidius colemani* specimens were confirmed by sequencing LWRh and 16S genes and was selected for further genetic and population analyses. A total of 12 *Aphidius colemani* haplotypes were identified. Of these, nine were from our East African specimens and three from the Barcode of Life Database (BOLD).

CONCLUSION: *Aphidius colemani* and *Lysiphlebus testaceipes* are potential targets for conservation biological control in tropical smallholder agro-ecosystems. We hypothesize that high genetic diversity in East African populations of *Aphidius colemani* suggests that this species originated in East Africa and has spread globally due to its use as a biological control agent. These East African populations could have potential for use as strains in commercial biological control or to improve existing *Aphidius colemani* strains by selective breeding.

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

Keywords: parasitoid; *Aphidius colemani*; biological control; *Aphis fabae*; DNA barcoding

1 INTRODUCTION

Parasitoids are important natural enemies which can regulate herbivores insect pests. Most parasitoids are within the order Hymenoptera. Parasitoid larvae typically develop on or in an arthropod host, killing it either directly or indirectly as a result of its development.[1] Parasitoids are a highly diverse group of insects, for example, the subfamily Aphidiinae (Hymenoptera; Braconidae) alone contains approximately 650 species of aphid parasitoids.[2] Many parasitoid species are yet to be identified,[3] in part due to the prevalence of cryptic species that are difficult to distinguish morphologically.[4] *Aphidius* spp. are economically and ecologically important parasitoids regulating insect herbivores naturally and as commercial biological control agents.[5] They are, therefore, a key group to study to better understand the biology and population structure of parasitoids.

Many *Aphidius* spp. have relatively low intraspecific genetic variation and in some cases also low interspecific diversity.[6–8] In some cases this makes it difficult to discriminate between these species by DNA barcoding. For example, *Aphidius ervi* Haliday and *Aphidius microlophii* Pennacchio & Tremblay have the same cytochrome oxidase I (COI) haplotype but are morphologically distinct.[9,10] In contrast, the closely related *Aphidius platensis*

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Brethes, *Aphidius transcaspicus* Telenga and *Aphidius colemani* Viereck which are part of the *Aphidius colemani* group have been distinguished by DNA barcoding.[8,10] Sequencing of the COI region of *Aphidius colemani* and *Aphidius platensis* found that genetic variation within these species shows no association between host species or geographic location.[8] Both species were found to have numerous haplotypes, but these were present across multiple countries and emerged from different aphid species.[8] In contrast, evidence supporting a relationship between spatial distribution and genetic diversity for *Aphidius transcaspicus* is mixed, with one study finding a relationship,[11] but a larger and more recent study seeing no association.[8] Counter to other *Aphidius* spp., *Aphidius rhopalosiphi* De Stefani Perez shows a high level of genetic variation with a genetic distance of up to 2.4% within this species; however, this variation shows no relationship to geographic distribution or host species.[7] *Aphidius colemani* is globally one of the most commercialised parasitoids in agriculture because it parasitizes economically damaging aphids including *Aphis craccivora* Koch and *Aphis fabae* Scop.,[12,13] which are highly destructive pests of legumes in East Africa.[14,15] Crop damage by aphids is caused by direct feeding, which reduces plant growth and yield,[16] and by vectoring plant virus diseases.[17] *Aphidius colemani* is such an effective natural pest regulator it has been reared commercially for use in biological control and, as a consequence of its escape from glasshouses or intentional release, now has a widespread distribution.[12,18] *Aphidius colemani* is most widely thought to originate from India or Pakistan.[12,19]

There has been relatively little research investigating the genetic diversity of parasitoid communities in tropical ecology and this is an important knowledge gap because genetic diversity in parasitoids may improve their efficacy as biological control agents. Furthermore, understanding the biology and community of parasitoids in an agro-ecosystem can inform conservation of these natural enemies or the introduction of appropriate commercial biological control agents.[20] Currently in East Africa, pest control is limited by a high cost, lack of farmer knowledge and limited research on pest control methods and how to incorporate them in the farming system. Where control techniques are used, there is often a reliance on synthetic chemical pesticides, which have a negative effect on human health and the environment.[21] Conservation biological control could represent a safer, more cost effective and accessible way for smallholders to control pests. Conservation methods include the use of floral resources to increase parasitoid efficacy, longevity and fecundity,[22–25] although these floral resource plants need to be chosen carefully to benefit important parasitoids and not pest species.[23,26,27]

The aim of the present study was to use DNA barcoding of the mitochondrial COI gene to identify parasitoids of *Aphis fabae* across three countries in East Africa, which could provide information about the genetic diversity of parasitoid species at a regional scale as well as inform future biological control. The crop systems used in the study were common bean (*Phaseolus vulgaris* L.) in Malawi and Tanzania and lablab (*Lablab purpureus* (L.) Sweet) in Kenya. Both legumes are important local subsistence crops that are significantly affected by *Aphis fabae*. As *Aphidius colemani* was identified as one of the most important aphid parasitoids in East African smallholder legume farming systems, the genetic and population diversity of this species was investigated in greater depth using COI sequence analysis and compared to *Aphidius colemani* sequences from the Barcode of Life Database (BOLD).

2 MATERIALS AND METHODS

2.1 Sample collection

A total of 96 sampling locations were selected across Malawi, Kenya and Tanzania (coordinates in Supporting Information, Table S1; Fig. S1). There were 32 sites in each country, each divided into two fields, one containing a monocrop and the other an intercrop. Orphan legume crops were cultivated in all fields as either a monocrop or intercrop, with common bean (*Phaseolus vulgaris* L.) being grown in Malawi and Tanzania and lablab (*Lablab purpureus* (L.) Sweet) grown in Kenya. Sentinel plants (common bean or lablab according to country, grown in pots in a screen-house) were infested with 50–60 apterous *Aphis fabae* and used to collect parasitoids as described by Mkenda *et al.*[28] After infestation of sentinel plants, *Aphis fabae* were left to settle before being placed into fields. Sentinel plants were placed in fields 4 and 7 weeks after seedling emergence for common bean or 8 and 12 weeks after emergence for lablab. These times were selected to coincide with the vegetative and flowering/podding stages of the legumes in the field to ensure that parasitoids were collected across the cropping season. Two sentinel plants were placed in each field, one in the centre of the crop and one in the field margin, because the abundance and diversity of aphid parasitoid species has been found to vary between crop and field margin habitat.[29] Sentinel plants were left in the field for 7–14 days to allow parasitism of *Aphis fabae*, before being returned to the laboratory where they were maintained in fine mesh cages for 28 days. All emerging parasitoids were collected and preserved in 95% ethanol for subsequent DNA barcoding.

2.2 DNA sequencing

DNA was extracted from parasitoids using the Chelex method. As *Aphidius colemani* were selected to be analysed in detail later, they were morphologically identified by forewing shape prior to DNA extraction.[8] Briefly, insects were ground using a micropestle in 1.5 mL Eppendorf tubes containing 50 µL of Chelex buffer (10% *w/v* Chelex resine (Bio-Rad, Hercules, CA, USA) in TE solution), the micropestle was then rinsed with a further 50 µL of Chelex buffer. Polymerase chain reaction (PCR) was performed using HCO2198 and LCO1490 primers[30] (10 µmol L^{-1}) and MyTaq DNA polymerase (Meridian Bioscience, Cincinnati, OH, USA) following manufacturer's instructions. The following PCR conditions were used: initial denaturation of 94 °C for 3 min, 37 cycles of 94 °C for 30 s, 52 °C for 1 min, 72 °C for 1 min 30 s and a final extension step of 72 °C for 10 min. Where this initial PCR was unsuccessful, the LepF1/C_ANTMRID[4,31] and MLepF1/LepR1[31,32] primer pairs were used to amplify shorter DNA fragments using the conditions described by Smith *et al.*[4] The PCR products were visualized using gel electrophoresis on a 1.2% agarose gel in 0.5 × TBE stained with GelRed (Biotium, Fremont, CA, USA) they were then purified using a GeneJET PCR purification kit (ThermoFisher Scientific, Waltham, MA, USA). The PCR product was then sequenced by GATC Biotech (Eurofins Scientific, Luxembourg) using the forward primer (5 µmol L^{-1}).

2.3 DNA barcoding

Full length COI barcodes produced with LCO/HCO primers were trimmed in Geneious Prime 2020.0.5 (https://geneious.com). Where sequencing using both LepF1/C_ANTMRID and MLepF1/LepR1 primer pairs was successful, this software was also used

to produce a consensus sequence (full length COI barcode) from these shorter sequences. Where only one of these shorter sequences was successfully produced, this short 'mini-barcode' was used for identification of the sample,[28] but not for further analysis. DNA barcodes were compared to published sequences (Table S2) in the BOLD[33] (http://www.boldsystems.org/) for identification. If the sequence yielded a close match (greater than 99% for species, greater than 95% for genus and greater than 90% for family) and the sequence clustered with a monophyletic group of this species/genus/family, then the unknown sequence was assigned to this taxon.[3] Sequences were uploaded to the National Centre for Biotechnology Information (NCBI) database. The quantity and identity of parasitoids emerged from *Aphis fabae* on sentinel plants were visualized using the ggplot2[34] package in R version 3.6.1.[35] Following barcoding, the diversity of all parasitoids was assessed by calculating family diversity using Shannon's (*H*) and Simpson's (*D*) diversity indices.

2.4 Analysis of *Aphidius colemani* sequences

As *Aphidius colemani* were the most abundant parasitoid, they were selected for further analysis. A total of 27 published *Aphidius colemani* reference sequences were obtained from BOLD (Table S2). All sequences were quality checked in MEGAX[36] and sequences that contained missing or ambiguous nucleotides were removed. Published sequences were aligned with DNA barcodes produced in Section 2.3 and trimmed in MEGAX to give 501 bp sequences. To confirm the identity of specimens whose DNA barcodes gave a 98–99% sequence match to *Aphidius colemani* sequences in BOLD, Automatic Barcode Gap Discovery (ABGD)[37] was performed using the ABGD web application (https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html) following calculation of pairwise distances in MEGAX using Kimura 2-Parameter (K2P) and Jukes–Cantor models. Default values for *P* between 0.001 and 0.1 were used and the number of steps was set to 20. Values of *X* between 0.1 and 1.5 were used. Published sequences of the closely related *Aphidius platensis* and *Aphidius transcaspicus* were included in this analysis (Table S2) to ensure that this method effectively distinguished between *Aphidius* species. Furthermore, a subset of these *Aphidius colemani* were selected for sequencing of the LWRh and 16S genes.[38] Amplification conditions were as described by Derocles *et al*.[38] These sequences were first trimmed to 323 and 282 bp, respectively, then compared to sequences in the NCBI database. They were then concatenated with the COI sequence (trimmed to 464 bp), which were aligned with sequences of *Aphidius* spp. and *Lysiphlebus fabarum* from Derocles *et al*.,[38] using MEGA X. A phylogenetic tree was constructed using the maximum likelihood method and a Tamura 3-parameter model[39] with Gamma distribution in MEGAX and visualized using Interactive Tree of Life[40] (iTOL).

A phylogenetic tree was constructed using *Aphidius colemani* sequences from BOLD and *Aphidius* specimens from our study using the maximum likelihood method and a Hasegawa–Kishino–Yano model[41] with a Gamma distribution in MEGAX and a bootstrap test was performed with 1000 replications. The phylogenetic tree was visualized using iTOL. Following this, a haplotype network was constructed using popART (v.1.7) software[42] and the minimum spanning networks method.[43] A haplotype accumulation curve was produced to determine whether sampling in this study and samples in BOLD was sufficient to describe *Aphidius colemani* haplotype diversity using the HACSim package[44] in R, with a total of 100 000 permutations and 95% confidence intervals. For population genetic analysis, it was necessary to group samples into populations. Due to the relatively low number of *Aphidius colemani* sequences from individual sampling locations available in sequence databases, they were grouped together into a subpopulation (Table S2).[45] Therefore the groups analysed were 'East Africa' (EA) and 'rest of the world' (ROTW). An AMOVA (analysis of molecular variance; 1000 permutations) was performed using Arlequin v. 5.3.2[46] to identify significant variation between and within these groups.

3 RESULTS

3.1 DNA barcoding of parasitoids emerged from sentinel plants

A total of 463 primary and secondary parasitoids emerged from *Aphis fabae* on sentinel plants across the sites in all three countries, 311 parasitoids from lablab plants in Kenya, 109 from bean plants in Tanzania and 43 from bean plants in Malawi (Fig. 1). Of these 424 were identified by DNA barcoding (Table S3), DNA extraction, PCR or sequencing was not successful for the remaining samples. Tanzania had the greatest species diversity of parasitoids ($D = 0.47$, $H = 0.88$), followed by Kenya ($D = 0.73$, $H = 0.47$) and Malawi ($D = 1$, $H = 0$). *Aphidius* spp. were the most abundant primary parasitoid in Tanzania and Kenya, while *Lysiphlebus testaceipes* (Cresson) was most abundant primary parasitoid in Malawi although *Aphidius* spp. were also present. Other primary parasitoids caught in Tanzania include *Diaeretiella rapae* McIntosh and unidentified species of Braconidae. The *Aphidius* spp. that emerged from sentinel plants showed the greatest similarity to *Aphidius colemani* DNA barcodes in the BOLD database. Those with greater than 99% similarity were initially designated as *Aphidius colemani*, however other *Aphidius* spp., particularly from Kenya, showed a 98–99% similarity to existing *Aphidius colemani* barcodes. These specimens clustered with *Aphidius colemani* in phylogenetic trees produced in BOLD, therefore they were most likely also *Aphidius colemani* and were included in later genetic and population analyses.

Tanzania had the greatest proportion (62.1%) of secondary parasitoids (Fig. 1), with the most abundant being in the family Pteromalidae. Other secondary parasitoids identified include *Asaphes* sp., *Pachyneuron* sp., *Phaenoglyphis* spp., Encyrtidae spp., Eulophidae spp. and Figitidae spp. No secondary parasitoids were identified in Malawi, while a low proportion were identified from Kenya (13.4%).

3.2 Genetic analysis of *Aphidius colemani*

After trimming and quality control 501 bp DNA barcodes were produced, 223 from our study and 36 from the BOLD database (Tables S2 and S3). Barcodes were aligned and it was determined that mutations observed in all samples except one were synonymous. ABGD with both Jukes–Cantor and K2P models with relative gap width of 1 or 1.5 and maximum intraspecific divergence of 0.8 to 1.3% identified 2–6 species with both initial and recursive partitions (Table S4). Of these partitions, only those that identified three species correctly grouped *Aphidius platensis* and *Aphidius transcaspicus*, hence these are the most likely to be correct. In these partitions all *Aphidius colemani* sequences from BOLD grouped with those thought to be *Aphidius colemani* in our study. This along with their morphological similarity, being caught in the same location and same host species support their identity as *Aphidius colemani*. The LWRh and 16S genes were sequenced for a subset of 16 *Aphidius colemani* from our study. The 16S

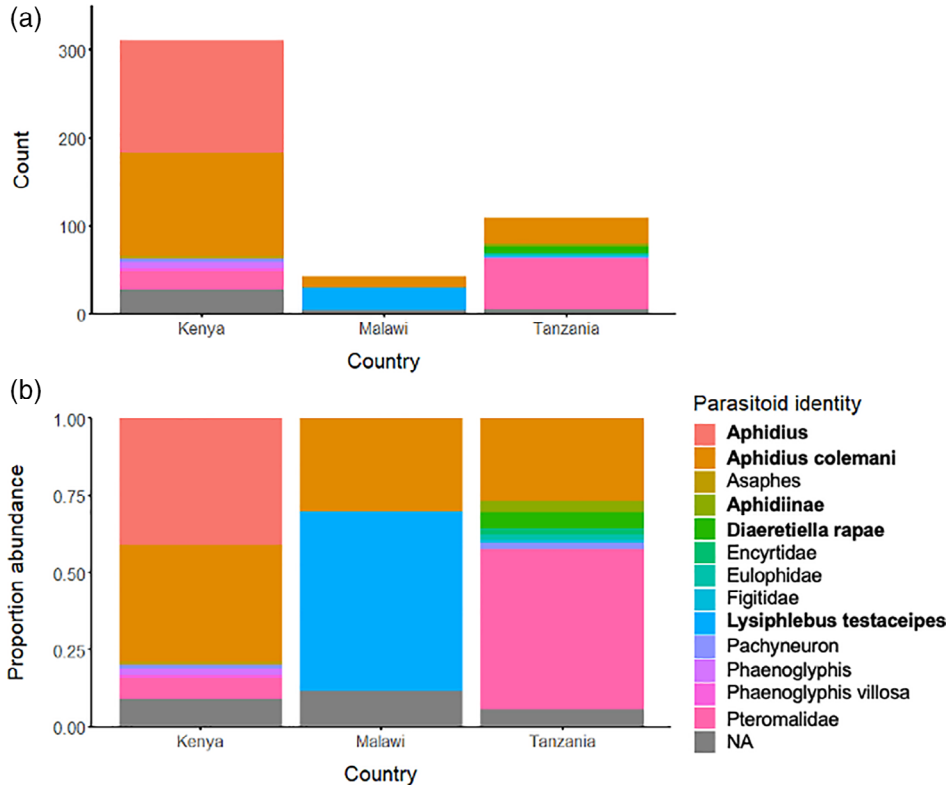

Figure 1. The number (a) or relative abundance (b) of primary and secondary parasitoids emerged from *Aphis fabae* on sentinel plants in Kenya, Tanzania and Malawi. NA represents where sequencing was unsuccessful or a sufficient match was not obtained from the BOLD database for specimen identification. Primary parasitoids are in bold and secondary parasitoids are not.

sequences show 98.94–99.65% similarity to *Aphidius colemani* sequences in the NCBI database (JQ240494) and LWRH sequences showed between 99.07–100% similarity to *Aphidius colemani* sequences (JN620704) in the NCBI database (Table S5). These high levels of similarity support the identification of *Aphidius colemani* in our study. Additionally, the COI-16S-LWRH concatenated sequences from our study group with known *Aphidius colemani* sequences in a phylogenetic tree (Fig. S2). Following this, a phylogenetic tree was constructed for all *Aphidius* COI sequences (Fig. 2). A large proportion of samples from our study form separate clades, as well as those from the online databases, giving four clades in total. These clades have bootstrap values of greater than 0.8, which suggests that there are significant differences between these clades and the other *Aphidius colemani* sequences.

Using the aligned 501 bp COI sequences, 12 *Aphidius colemani* haplotypes were identified (H1–H12; Fig. 3; sequences in

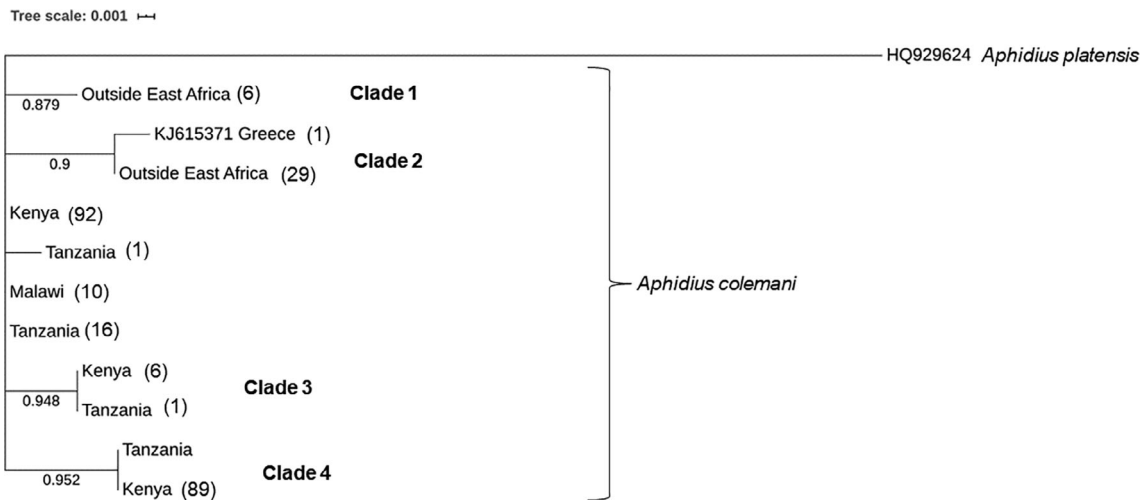

Figure 2. Phylogenetic tree constructed using the maximum likelihood method, a Hasegawa–Kishino–Yano model[41] with a Gamma distribution, showing the relationship between *Aphidius colemani* sequences from our study and BOLD. The tree has been condensed, with the number of samples from each country/region in brackets. Bootstrap values are shown next to branches, a bootstrap cut-off value of 0.7 was used.

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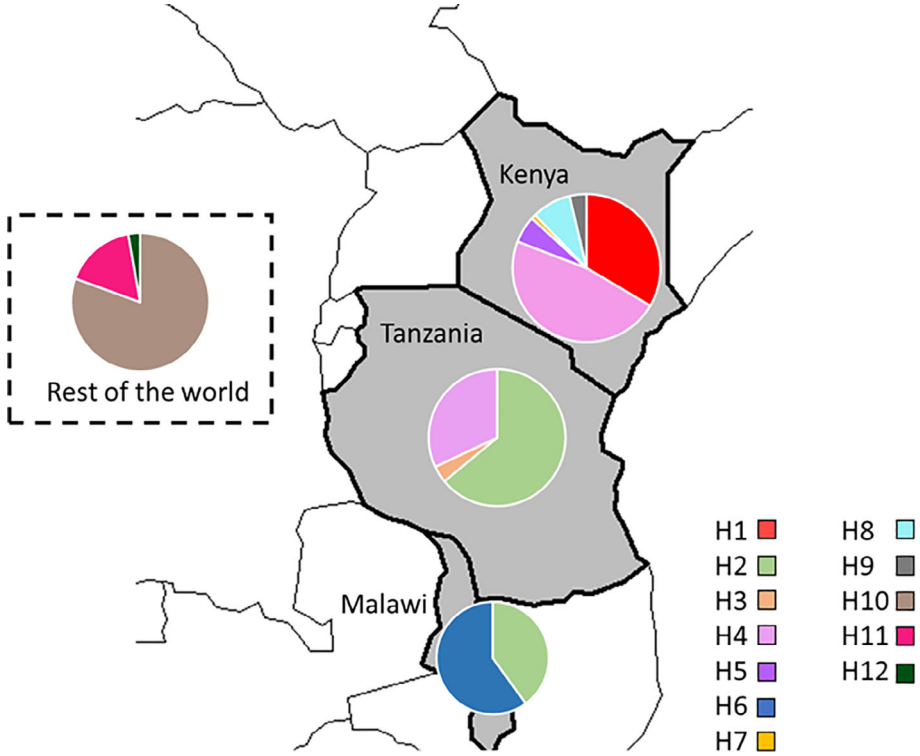

Figure 3. Haplotype map illustrating the proportion of *Aphidius colemani* haplotypes (H1–H12) in different areas. Haplotypes were identified from samples in this project (Kenya, Tanzania and Malawi) and sequences in BOLD (rest of the world). Haplotypes were identified using the minimum spanning network method.[41] Each colour represents a different unique haplotype.

Table S6) among the 259 specimens. Nine haplotypes were identified in the East African samples from our project, compared with only three haplotypes from BOLD sequences. There was no overlap between the haplotypes identified in East African and BOLD samples (Fig. 3), but within these groups some haplotypes (H2, H4, H10, H11) were found in multiple countries. Samples from Kenya showed the greatest diversity of haplotypes with six being identified, although this may be due to a greater sample size, followed by Tanzania where three haplotypes were identified (Fig. 3). The most common haplotype ($n = 89$) was H4, which comprises samples from Kenya and Tanzania. A haplotype accumulation curve was produced using specimens collected in this study and those in BOLD (Fig. 4). It was estimated that the 259 samples used for this analysis represent 92.8% of all observed haplotypes for this species. In order to identify at least 95% of all haplotype variation for *Aphidius colemani* it was estimated that a further 99 samples would need to be collected to give $N = 358$ (95% confidence interval: 355.862–360.138).

The AMOVA showed that the between groups variation explained 45.17% of the total genetic variation ($F_{CT} = 0.45$, $P = 0.016$), indicating that there were significant genetic differences between the East Africa and rest of the world groups. There was also significant variation within populations, which explained 48.38% of the total variation ($F_{ST} = 0.52$, $P < 0.001$). The remaining 6.45% of variation was explained by variation between populations within groups ($F_{SC} = 0.12$, $P < 0.001$). This supports the separation identified by haplotype analysis, whereby populations in East Africa have a high level of genetic differentiation from populations elsewhere, but there is little genetic differentiation between populations outside of East Africa, irrespective of their location.

4 DISCUSSION

This study characterized the aphid parasitoid diversity in Kenya, Malawi and Tanzania. *Aphidius colemani* was the most abundant primary parasitoid in Tanzania and Kenya, while *Lysiphlebus testaceipes* was the most abundant in Malawi. *Aphidius colemani* has been previously recorded in Tanzania[28] and Kenya[47] and has a widespread distribution.[8,12] In contrast *Lysiphlebus testaceipes* is a native Nearctic species that is likely a recent invasive species to Malawi. While *Lysiphlebus testaceipes* has been previously recorded in other parts of Africa, including Benin, Tunisia and Algeria,[48] it has not been recorded in Malawi and it is not known how it was introduced and established.[48] In the mid-1900s attempts were made to establish *Lysiphlebus testaceipes* for biological control in South Africa and Kenya,[47] however, it is not known whether these attempts were successful. Our study indicates that one of these source populations, or individuals from another introduction, may have established in Malawi. The two most abundant aphid primary parasitoids identified in this study, *Aphidius colemani* and *Lysiphlebus testaceipes*, parasitize several species of aphid,.[8,13,49,50] As these are the two most important primary parasitoid species in the East African smallholder agroecosystems we surveyed, conservation biological control of aphids should focus on providing resources, such as floral resource plants, to maintain populations of these parasitoids.[24,25] It is important to consider that as *Lysiphlebus testaceipes* appears to be a recent invasive species to Malawi, it may not be appropriate to promote or conserve this population as it may disrupt native parasitoid or host species. Due to the high numbers of *Lysiphlebus testacepies* seen in our study, it is likely already established in the area so it may be difficult to prevent the spread of this species. Therefore, conservation

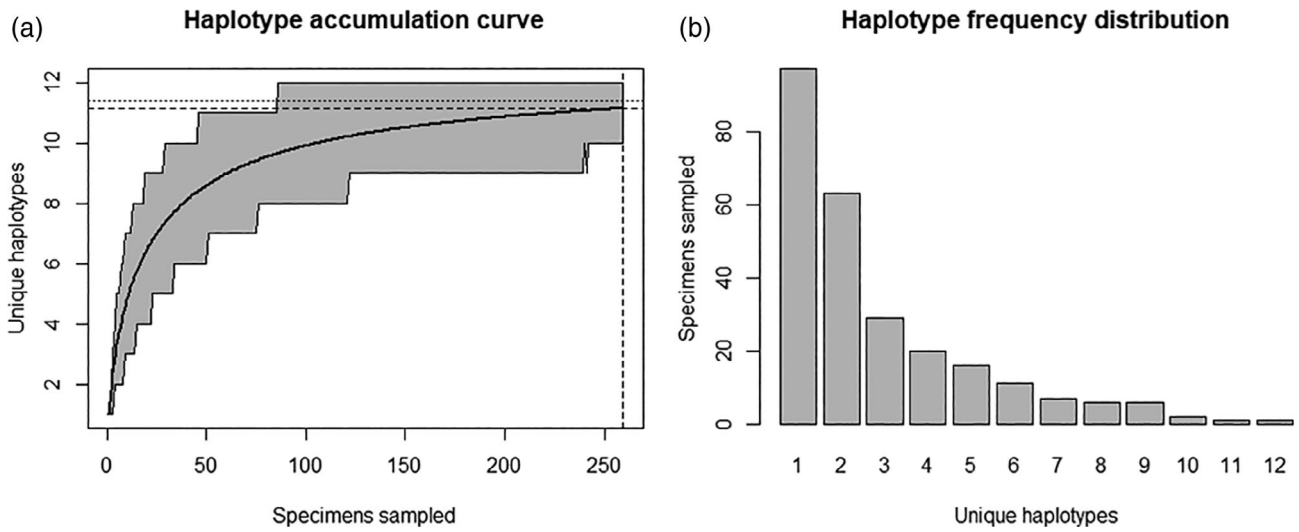

Figure 4. (a) Haplotype accumulation curve and (b) haplotype frequency bar plot for *Aphidius colemani* collected in this study and from the NCBI and BOLD databases. In (a) the grey shaded area represents 95% confidence interval. The dashed line represents the number of observed and the corresponding number of sampled individuals. The dotted line is the expected number of haplotypes at a 95% haplotype recovery level.

biological control could focus on harnessing native parasitoid species where identified.

This study also found significant genetic differences between East African populations of *Aphidius colemani* and those from other countries. We also identified high intraspecific diversity of populations in East Africa when compared to previously studied populations. Insects generally show a considerable reduction in genetic diversity in introduced populations relative to founder populations.[51] This supports our hypothesis that *Aphidius colemani* originates from East Africa, with populations elsewhere showing significantly less diversity due to small founding populations. Alternatively, the populations of *Aphidius colemani* in East Africa may represent very closely related cryptic species. It is unlikely that the high levels of variation in East African populations of *Aphidius colemani* are only due to the greater number of samples taken during this study in comparison to those available in BOLD. This is because the sequences in BOLD were from many different locations and all showed a low level of diversity that is reflected in this and previous population genetics studies.[8,52] To confirm our findings, further studies should be conducted with more *Aphidius colemani* specimens, particularly those from India and Pakistan, where this species was thought to originate.[12,19] A study by Tomanović *et al.*,[8] identified five *Aphidius colemani* haplotypes in Europe, of which none differed from another by more than one mutation. Our study only identified two of these haplotypes, which could be due to different lengths of DNA barcodes between these studies and ours. Understanding the biology of *Aphidius colemani* is important for its effective use in conservation biological control and as a commercial biological control agent.[5] This may have relevance for existing biological control programmes where East African *Aphidius colemani* populations could be used to increase diversity in commercial strains by selective breeding.[53] This could potentially improve qualities such as resilience to climate fluctuations, parasitism rate, host range and reduced detection by hyperparasitoids. Alternatively, these new populations could be investigated as novel biological control agents, although more information is required about their host preferences and tolerance to various environmental factors.

Whilst primary parasitoids provide biological control of pest aphids, it is important to consider the influence of secondary parasitoids on both the primary parasitoid and insect host species in an agro-ecosystem.[54,55] They are also an important consideration when developing conservation biological control strategies.[56] If the population of a secondary parasitoid is high compared to the primary parasitoid, for example it may disproportionately benefit from an introduced floral resource plant, it may result in populations of primary parasitoids being too low for effective pest control. In our study, the greatest proportion of secondary parasitoids emerged from sentinel plants in Tanzania, with none from those in Malawi. Secondary parasitoids are thought to be more sensitive to increasing landscape complexity than primary parasitoids[57] and are more abundant when non-crop habitat is present,[58] and in general the landscape in Tanzania was more heterogeneous. In our study it was only possible to identify most of these secondary parasitoids to family level using DNA barcoding, with the most common being Pteromalidae. This family is one of the most important groups of secondary aphid parasitoids.[59]

There were differences in the number and diversity of parasitoids identified from sentinel plants between Kenya, Malawi and Tanzania. There are a number of possible reasons for this: primarily, host–parasitoid food webs vary with altitude[60]; there may also have been effects of farm management practices, temperature and rainfall on the ability of parasitoids to parasitize sentinel aphids during the sampling period.[61] In some experimental systems, parasitism rate increases with greater parasitoid species richness[62]; however, this was not the case in our study, where Tanzania had the greatest parasitoid species richness but a lower parasitism rate than Kenya. Similar to our study, Macfadyen *et al.*,[63] found that on English farms there was no correlation between parasitoid species richness and parasitism rates, although farms with a greater parasitoid species richness had lower variability in parasitism rates over time. Whether a more genetically diverse population of a parasitoid species exhibits higher parasitism rates relative to a less genetically diverse population is not known. It has been postulated that where parasitoid species have a similar niche, i.e. use the same pest species,

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increasing parasitoid diversity would not have an effect on the pest abundance due to ecological redundancy of the parasitoids.[20] This would likely be particularly pronounced if parasitoid species target the same life stage of the insect. Finally, increasing landscape diversity or complexity can increase the abundance of parasitoids and parasitism rates,[57,64–68] although this is not the case for all parasitoid species[64,65,69] and likely depends on their differing resource requirements.

In conclusion, our study identified *Aphidius colemani* and *Lysiphlebus testaceipes* to be the most abundant aphid primary parasitoids in Kenya, Tanzania and Malawi, hence they should be the target of conservation biological control in these tropical smallholder agro-ecosystems. However, it would be important to take into account the effect of these measures on secondary parasitoid and pest species, so that they do not also benefit from these interventions. Furthermore, high intraspecific diversity of *Aphidius colemani* populations in East Africa when compared to previously studied populations, suggests that *Aphidius colemani* may have originated in East Africa and spread globally due to its use as a biological control agent. These populations have potential to be used in commercial biological control as a new strain or by selective breeding with existing strains.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article

SUPPORTING INFORMATION

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

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