Mitogenomic analysis of diversity of key whitefly pests in Kenya and its implication to their sustainable management

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Whiteflies (Hemiptera: Aleyrodidae) are devastating agricultural pests of economic importance vectoring pathogenic plant viruses. Knowledge on their diversity and distribution in Kenya is scanty, limiting development of effective sustainable management strategies. The present study is aimed at identifying whitefly pest species present in Kenya across different agroecological zones and establish predictive models for the most abundant species in Africa. Whiteflies were sampled in Kenya from key crops known to be severely infested and identified using 16S rRNA markers and complete mitochondrial genomes. Four whitefly species were identified: Aleyrodes proletella, Aleurodicus dispersus, Bemisia afer and Trialeurodes vaporariorum, the latter being the most dominant species across all the agroecology. The assembly of complete mitogenomes and comparative analysis of all 13 protein coding genes confirmed the identities of the four species. Furthermore, prediction spatial models indicated high climatic suitability of T. vaporariorum in Africa, Europe, Central America, parts of Southern America, parts of Australia, New Zealand and Asia. Consequently, our findings provide information to guide biosecurity agencies on protocols to be adopted for precise identification of pest whitefly species in Kenya to serve as an early warning tool against T. vaporariorum invasion into unaffected areas and guide appropriate decision-making on their management.
The complete mitogenome sequences of whiteflies from Kenya. In the present study, $1.7 \times 10^7$, $1.9 \times 10^7$, $1.9 \times 10^7$ and $1.8 \times 10^7$ reads were generated for \textit{A. dispersus}, \textit{B. afer}, \textit{T. vaporariorum} and \textit{A. proletella} samples, respectively, from MiSeq sequencing. A de novo assembly of the \textit{A. proletella} sample generated 812,145 contigs ($\geq 1000$ bp). One contig of 14,563 bp was identified by BLASTn. For the reference-based assembly of the whitefly samples, 39,900, 145,385 and 164,997 reads were generated for \textit{A. dispersus}, \textit{B. afer} and \textit{T. vaporariorum}, respectively. Referenced to KR063274, NC_024056 and KY426015, contigs of 17,327 bp, 17,664 bp and 16,580 bp were identified for \textit{A. dispersus}, \textit{B. afer} and \textit{T. vaporariorum}, respectively.

Sequence annotation showed that all the mitogenomes comprised of the entire set of 37 genes typical of metazoan mitochondrial genomes, including 13 protein-coding genes (PCGs), 22 transfer RNA (tRNA) genes and two ribosomal RNA (rRNA) genes. All the three complete mitogenomes had the same gene order, gene direction and start/stop codons of each PCGs as that of the reference sequences (Supplementary Table 6a to 6c).

The pairwise similarity between the samples from this study and the GenBank reference samples showed 94.01% between \textit{A. dispersus} and the reference mitogenome, 99.56% between \textit{B. afer} and this study and the reference mitogenome and 94.52% between \textit{T. vaporariorum} from this study and the reference mitogenome (Supplementary Table 7). The maximum likelihood tree of the 13 PCGs from the samples in this study combined with representative sequences, showed a paraphyletic relationship between the \textit{Aleurodicus} genus and the other whitefly genera (Fig. 4).

The combined length of all tRNA genes was 1309 bp, 1459 bp and 1422 bp for \textit{A. dispersus}, \textit{B. afer} and \textit{T. vaporariorum}, respectively; with individual genes ranging from 57 to 80 bp for \textit{A. dispersus}, 57 to 83 bp for \textit{B. afer} and 55 to 70 bp for \textit{T. vaporariorum}. Analysis of the tRNA secondary structure showed that all the tRNAs in \textit{A. dispersus} (Supplementary Fig. 1) and \textit{T. vaporariorum} (Supplementary Fig. 2) had the typical clover leaf structure except for trnS1 and trnS2, which lack the dihydrouridine (DHU) arm (Fig. 5). Nineteen of the tRNAs in \textit{B. afer} (Supplementary Fig. 3) showed the typical clover leaf structure, except for trnA, trnS1 and trnS2 which lacked the DHU arm (Fig. 5).

Potential distribution of \textit{Triaeleurodes vaporariorum} under current and future scenarios

The predicted current global distribution of \textit{T. vaporariorum} obtained from the MaxEnt model showed the highest likelihood of establishment in most European countries, the USA, southern Canada, Brazil, Uruguay, Argentina, Australia, New Zealand, Northern Iran, South eastern China and Japan (Fig. 6) with an establishment risk index (ERI) of $>0.6$. The predicted future global distribution showed the risk of establishment of T.
**T. vaporariorum** showed an increase under future climate scenario, with ERI ranging between >0.4–0.6 (Fig. 7). The predicted current distribution of *T. vaporariorum* in Africa ranged between 0.101 and 0.78. The countries considered to be highly suitable for the pest establishment with an ERI of >0.4, included Algeria, Cameroon, Ethiopia, Gabon, Kenya, Morocco, South Africa, Tanzania, and Uganda (Fig. 8a). Under the future climate scenario, the predicted distribution in Africa ranged between 0.0981 and 0.754 (Fig. 8b).

**Discussion and conclusion**

In this study, we identified the various whitefly species present on a range of vegetable crops in Kenya and their species diversity. Subsequently we identified the most abundant whitefly species (*T. vaporariorum*) then modelled the potential current and future risk of establishment of *T. vaporariorum* to provide critical information on the global distribution and establishment of the species.
Frohlich demonstrated the importance of the mitochondrial cytochrome oxidase subunit I (COI) as a region for analysing nucleotide divergence between and within populations. However, we experienced low amplification efficiencies with the primers used in the study described by Folmer, which is in tandem with the findings reported by Shatters who also observed low quality and suboptimal product quantities when using mtCOI primers; C1-J-2195 and L2-N-3014. Considering this, our study focused on using the whitefly 16S ribosomal subunit.

**Figure 2.** Maximum-likelihood tree based on a 650 bp alignment of 188 sequences of the 16S ribosomal RNA (rrna) gene using the 4119/4118 markers from whitefly samples collected from Kenya and reference whitefly sequences available in GenBank (n = 5), with *Aphis gossypii* as an outgroup. The number of sequences obtained in this study are indicated in square brackets. Branchsupport was based on 1000 bootstrap replicates.

**Figure 3.** Maximum-likelihood tree based on a 650 bp alignment of 132 sequences of the 16S ribosomal RNA (rrna) gene using the whitefly specific from whitefly samples collected from Kenya and reference whitefly sequences available in GenBank (n = 5), with *Aphis gossypii* as an outgroup. The number of sequences obtained in this study is indicated in square brackets. Branchsupport was based on 1000 bootstrap replicates.

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In nature, insects are known to co-evolve depending on the prevailing biotic factors and most often they develop morphologically indistinct sibling species as observed in black flies, mosquitoes among others. Whiteflies have shown similar attributes hence the rise to the numerous biotypes distributed in the world. However, according to the review done by De Barro, the use of biotype in describing *B. tabaci* and other whitefly species was thought to be erroneous and misleading. We report on the characterization of four distinct whitefly species found in various agro-ecological zones of Kenya with two of the described species co-existing on the same crop. To date, *B. tabaci* has at least 24 known morphologically indistinguishable species. This possesses a real challenge in developing sustainable effective control approaches since these species differ in terms of their susceptibility to natural enemies, host range, insecticide resistance, fecundity and vector competence for begomoviruses.

From our study, we were able to detect four different species of whiteflies from the various host plants assayed in the country. Among these species, *T. vaporarium* is the most abundant in Kenya. This was mainly found on

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**Figure 4.** Maximum-likelihood tree of full mitogenomes (13 protein coding genes) based on complete mitogenome sequences excluding tRNA, rRNA and control regions of *Aleurodicus dispersus*, *Aleyrodes proletella*, *Bemisia afer* and *Trialeurodes vaporariorum* from Kenya including *Bemisia tabaci* mitogenome available in GenBank, with *Aphis gossypii* mitogenome as an outgroup. Branch support was based on 1000 bootstrap replicates. Numbers in the brackets represent the GenBank accession numbers.

**Figure 5.** Transfer RNA secondary structure of trnA and trnS1 in *Aleurodicus dispersus* and *Bemisia afer* as predicted by MITOS showing presence and absence of dihydrouridine arm.
Figure 6. Maxent and BIOCLIM average model plots depicting current establishment of *Trialeurodes vaporariorum* with an establishment risk index (ERI) > 0.6 in Europe, Central America, parts of Southern America (Brazil, Uruguay and Argentina), parts of Australia, New Zealand and Asia (China and Japan).

Figure 7. Maxent and BIOCLIM average model plots depicting future changes in the establishment of *Trialeurodes vaporariorum*. Prediction is up to year 2050 showing increase in risk of *Trialeurodes vaporariorum* establishment (ERI > 0.4–0.6) probably due to the rising temperatures attributed to global warming.
both tomato and French bean crops while the traditionally known whitefly, *B. tabaci* was not detected, which is contrary to earlier findings in tomato agroecology of Kenya where it was reported as the dominant species collected\(^2\). Our sequences matched at ≥98% with *Trialeurodes vaporarium* available at GenBank. This level of homology was confirmed via use of 3 different markers. The analysis of the complete mitochondrial genomes of the four species also confirmed the homology of each species to its reference mitogenome. When the reference sequences from GenBank were included in the phylogenetic assays, it was noticed that the Kenyan whitefly samples completely diverged from the *B. tabaci* complex. It was also observed that none of the samples mapped to *B. tabaci* in the assembly of the NGS sequences. We can therefore infer that though *B. tabaci* is a devastating pest, it could either have been displaced by *T. vaporarium* or out-competed from the Kenyan agroecological zones, or its current biotype population is low, and thus we may be faced with threats from other whiteflies species which were previously not considered to be economically important. Effects of indiscriminate chemical use over time cannot be understated, and hence we cannot rule out the possibility of near 'almost complete' wipe-out of *B. tabaci* in the Kenyan agro-ecological zones. In this study, *B. afer* (which is an out-group in the *B. tabaci* complex) and *A. dispersus* were found co-existing on the same cassava plant, a known host plant of *B. tabaci* whereas *A. proletella* which was identified on kales has been documented to prefer cabbage\(^4\,\^5\) as a host plant.

The maximum likelihood analysis supported the separation of the Aleyrodidae into subfamilies Aleurodicinae and Aleyrodinae with *A. dugessii* and *A. dispersus* belonging to Aleurodicinae. This is consistent with the reports of Chen\(^4\), who also demonstrated that *A. dugessii* belonged to the subfamily Aleurodicinae. In the Aleyrodinae subfamily, *A. spiniferus* formed a clade with *T. acacia* while *B. tabaci* species grouped together with *B. afer*. These results are consistent with the morphological classification and phylogenetic studies of whiteflies\(^4\,\^5\). The analysis showed a paraphyletic relationship among the Aleyroididae family. Monophyly was observed between *Bemisia* clade and *Aleochara spiniferus* and *Tetraleurodes acaciae* clades.

Gamarra\(^4\) used life table parameters to map out the distribution of *T. vaporarium* and constructed the first risk atlas for Africa. The study predicted that agroecological zones with ERI ≥0.6 would have permanent/life-long establishment of this pest. This is in congruence with our findings whereby our models (Fig. 6) projected ERI >0.6 in Europe, Central America, parts of Southern America (Brazil, Uruguay, and Argentina), parts of Australia, New Zealand, and Asia (China and Japan). In areas where open field farming is practiced, as evidence of the greater Asian and African continents, the ERI lessened to 0.2–0.4 (Fig. 6). Global temperature changes and climatic conditions especially rising temperatures attributed to global warming, the difference will probably not affect the long-term establishment of *T. vaporarium* as the average ERI falls within the range of permanent establishment of the pest\(^4\). Here in this study, the detection of different whitefly species clearly indicates that the biological control agents must be well screened for host specificity, if meaningful control options are anticipated. The most abundant species collected was *T. vaporarium*, and if the dispersal trajectory is maintained, the life-long establishment of *T. vaporarium* would be global. Hence, *T. vaporarium* will remain a potential risk and threat for food production in the high-risk regions if sustainable effective measures are not developed to suppress the pest dynamics.
dispersus, reference sequences were retrieved from GenBank and included in the analyses. These included
A. based on the Kimura 2-parameter model as implemented in MEGA X. The reliability of the trees was assessed
ary relationships between the various samples were inferred using the maximum likelihood algorithm method
(5 mM dNTPs, 15 mM MgCl2, stabilizers and enhancers) (Bioline, London, UK), 0.5 pmol µl −1 of each primer,
Table 3). The PCR was carried out in a total reaction volume of 20 µL containing 5X My Taq Reaction Buffer
(5 mM dNTPs, 15 mM MgCl2, stabilizers and enhancers) (Bioline, London, UK), 0.5 pmol µl −1 of each primer, 0.5 mM MgCl2, 0.0625 U µl −1 My Taq DNA polymerase and 15 ng µl −1 of DNA template. This reaction was set up in the Mastercycler gradient Nexus thermal cycler (Eppendorf, Hamburg, Germany). The following cycling conditions were used: initial denaturation for 2 min at 95 °C, followed by 40 cycles of 30 s at 95 °C, 40 s annealing and 1 min at 72 °C, then a final elongation step of 10 min at 72 °C. The target gene region was 650–700 base
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pairs. The amplified PCR products were resolved through a 1.2% agarose gel. The DNA bands on the gel were
The successful sequences were assembled and edited using Geneious Version 8 (http://www.geneious.com) followed by multiple alignments done in Clustal X version 2.1. For conclusive identification of the species, both similarity and phylogenetic analyses were carried out. Similarity searches were conducted by querying the consensus sequences via the Basic Local Alignment Search Tool (BLAST). The (BLAST) algorithm was used to identify regions of similarity between sequences from this study and publicly available sequences in the GenBank database (Supplementary Table 4). Using the clean assembled sequences, phylogenetic and molecular evolutionary analyses were conducted in MEGA X. In order to infer the biotypes of the Kenyan whitefly collected, reference sequences were retrieved from GenBank and included in the analyses. These included A. dispersus (KR063274), A. proletella (GQ867758), B. afer (GQ677747), B. tabaci (K778614) and T. vaporariorum (GQ867761). Pairwise and multiple alignment of the sequences was done using Clustal W in MEGA. Evolutionary relationships between the various samples were inferred using the maximum likelihood algorithm method based on the Kimura 2-parameter model as implemented in MEGA X. The reliability of the trees was assessed using 1000 bootstrap replications.

Complete mitogenome and phylogenetic analyses. Reference based assembly of the mitogenome sequences were carried out in Geneious Version 8 using the partial mitochondrial genome sequence of A. dispersus (KR063274) and complete mitochondrial genome sequence of B. afer (K7734608) and T. vaporariorum (NC_006280) as reference. De-novo assembly of mitogenome sequence for A. proletella, which did not have a partial or complete record in GenBank, was performed with SPAdes v.3.13.0. Contigs were identified by BLASTn
v.2.2.3057. The mitogenome sequence was annotated by MITOS58 (the complete mitogenomes of A. dispersus, B. afer and T. vaporarorum have been uploaded to GenBank (Table 1).

Reference mitogenomes of A. dispersus (KR063274), B. afer (KF734668), B. tabaci (KJ778614) and T. vaporarorum (NC_006280) (Supplementary Table 5) were downloaded from the GenBank and assembled mitogenomes from this study were aligned using Clustal X. Thirteen Protein coding genes (PCGs) excluding the tRNAs, rRNAs and putative control regions were extracted and aligned with the reference mitogenomes using Clustal X. Maximum likelihood method was implemented in PhyML for the construction of the phylogenetic tree. The mitogenome of Aphis gossypii (NC_024581) was used as outgroup. Bootstrap analysis of 1000 replicates were used for nodal support.

**Trialeurodes vaporariorum spatial distribution modelling.** Global occurrence data were obtained from CABI Invasive Species Compendium (https://www.cabi.org/ISC/datasheet/54660)59. Environmental predictors included 19 bioclimatic variables of 2.5 km spatial resolution for the current scenario using the baseline average [1950–2000]60, and for the future scenario using the Coupled Model Intercomparison Project, Phase 5 (CMIP) 2055 average [2041–2070]61. The environmental variables were obtained from the WorldClim database (http://www.worldclim.org/). The bioclimatic variables represent annual trends (e.g., mean annual temperature, annual precipitation), seasonality (e.g., annual range in temperature and precipitation) and extreme or limiting environmental factors (e.g., temperature of the coldest and warmest month, and precipitation of the wet and dry quarters)62. All these variables were created using the 'bioclims' function in the R package 'dismo' version 3.4.1 and Maxent version 3.4.1 (R studio). The strongly correlated variables among all the bioclimatic variables were removed using the “findCorrelation” function in the 'Care' package63 in R v3.4.1 and only the uncorrelated predictor variables [Annual mean temperature (Bio 1), Temperature annual range (Bio 2), Mean diurnal range (Bio 7), Mean temperature of warmest quarter (Bio 10), Precipitation of wettest month (Bio 13), Precipitation of driest month (Bio 14), Precipitation seasonality (Bio 15), Precipitation of warmest quarter (Bio 18) and Precipitation of coldest quarter (Bio 19)] were subsequently used. Presence locations for T. vaporariorum (occurrence data) were compared against all the pseudo-absence points that are available in the study area to avoid model overfitting of spatially clustered presence points and inability to predict spatially independent data. The model was trained using 75% of the presence data and validated using 25%. The model was run with 5000 iterations and >10,000 background points for both current and future climate scenarios.

**Ethics approval.** All handling and experiments were performed as per the procedures at the ICIPE Animal Rearing and Quarantine Unit as approved by the National Commission of Science, Technology and Innovations, Kenya.

**Data availability**

The datasets generated and/or analysed during the current study are available from the corresponding author upon request and shall also be made available through open source platforms.

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Author contributions
F.M.K. and S.E. conceptualised the research. F.M.K. and F.L.O.O. generated the data. F.M.K., F.L.O.O., I.J.A., K.S.A. and C.M.T. analysed the data. F.M.K., K.S.A., S.S. and S.E. provided the funds. F.M.K., F.L.O.O., I.J.A. and K.S.A. wrote the original draft of the manuscript. F.M.K., F.L.O.O., I.J.A., K.S.A., S.S., S.A.M., T.D., C.M.T. and K.S.A. and C.M.T. conceptualised the research. F.M.K. and F.L.O.O. generated the data. F.M.K., F.L.O.O., I.J.A., K.S.A. and C.M.T. contributed to the writing—reviewing, editing and final approval of the manuscript.

Competing interests
The authors declare no competing interests.

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