

26 **Abstract**

27

28 Genetic diversity within *Trialeurodes vaporariorum* (Westwood, 1856) remains
 29 largely unexplored, particularly within regions of Sub-Saharan Africa. In this study, *T.*
 30 *vaporariorum* samples were obtained from three locations in Kenya: Katumani,
 31 Kiambu and Kajiado counties. DNA extraction, PCR and Sanger sequencing were
 32 carried out on ~750 bp fragment of the mitochondria cytochrome c oxidase I (COI)
 33 gene from individual whiteflies. In addition, global populations were assessed and 19
 34 haplotypes were identified, with three main haplotypes (Hp_19, Hp_10, Hp_011)
 35 circulating within Kenya. Measures of genetic diversity among *T. vaporariorum*
 36 populations resulted in haplotype diversity of 0.411, nucleotide diversity 0.00096,
 37 and Tajima's D -0.30315, ($P > 0.10$). Analysis of population structure across global
 38 sequences using Structurama indicated one population globally, with posterior
 39 probability of 0.72. Bayesian and maximum likelihood phylogenetic analysis gave
 40 support for two clades (Clade I = an admixed global population and Clade II = subset
 41 of Kenyan and 1 Greek sequence). Species delimitation between the two clades was
 42 assessed by four parameters; posterior probability, Kimura's two parameter (K2P),
 43 Rodrigo's P (Randomly distinct) and Rosenberg's reciprocal monophyly (P(AB). The
 44 two clades within the phylogenetic tree showed evidence of distinctness based on;
 45 Kimura two parameters (K2P) ($p = -1.21E-01$), Rodrigo's P (RD) ($p = 0.05$) and
 46 Rosenberg's P(AB) ($p = 2.3E-13$). Overall, low genetic diversity within the Kenyan
 47 samples is a likely indicator of recent population expansion and colonization with
 48 this region and plausible signs of species complex formation in Sub-Saharan Africa.

49 **Key Words:** *Trialeurodes vaporariorum*, Population Structure, Phylogenetics, Species
 50 Delimitation, Genetic Diversity, Kenya

51
52 Whiteflies are important agricultural pests with a global distribution (Anderson *et al.*,
53 2004; Lapidot *et al.*, 2014). Their agricultural importance is associated with damage
54 caused during feeding on the plant phloem and the production of honeydew (Colvin
55 *et al.*, 2006; Prijović *et al.*, 2013). Honeydew serves both to reduce transpiration and
56 as an inoculation point for saprophytic fungi which grows over the leaf surface
57 reducing photosynthesis (Colvin *et al.*, 2006). Among the most economically
58 important whiteflies is the greenhouse whitefly, *Trialeurodes vaporariorum*
59 (Westwood, 1856) which transmits a number of plant viruses of the genera *Crinivirus*
60 and *Torradovirus* (Navas-Castillo *et al.*, 2011; Navas-Castillo *et al.*, 2014). Of these,
61 the *Criniviruses*, Tomato chlorosis virus (ToCV) and Tomato infectious chlorosis virus
62 (TICV) are of major economic importance in tomato production globally (Wisler 1998,
63 Wintermantel *et al.*, 2009). In Africa, ToCV and TICV have been reported from
64 Morocco and South Africa (EPPO, 2005) and more recently from within greenhouses
65 in Sudan (Fiallo-Olivé, *et al.*, 2011). The viruses are able to infect a wide host range
66 beyond tomato (Fortes *et al.*, 2012). Production loss attributed to *T. vaporariorum*
67 transmitted viruses such as the sweet potato chlorotic stunt virus are a major
68 constraint on sweet potato production in Kenya, with uninfected sweet potatoes
69 yielding 50% more production than infected plants (Ateka *et al.*, 2004; Miano *et al.*,
70 2008).

71

72 **Phylogeographical structure of *T. vaporariorum* in Sub-Saharan Africa (SSA)**

73 Though *T. vaporariorum* is globally distributed, few studies have explored the
74 phylogeographical structuring and genetic diversity of *T. vaporariorum* populations

75 from Sub-Saharan Africa and in particular Kenya (Mound and Halsey 1978; Njaramb,
76 2000). The majority of phylogeographical studies of *T. vaporariorum* have been
77 confined to temperate regions (Roopa *et al.*, 2012; Kapantaidaki *et al.*, 2014; Prijović
78 *et al.*, 2014). In most of these studies there was lack of distinct phylogeographical
79 structuring that was further coupled with low genetic diversity.

80

81 Roopa *et al.*, (2012) used both mitochondrial COI (mtCOI) and nuclear markers to
82 assess the genetic diversity and phylogeographical structuring of *T. vaporariorum*
83 within populations in India. They reported no significant differences among *T.*
84 *vaporarioum* populations. Kapantaidaki *et al.* (2014) supported these findings using a
85 combination of mtCOI and secondary endosymbionts. In addition, Prijović *et al.*
86 (2013) reported a similar lack of phylogeographical structuring across populations in
87 Serbia and surrounding countries. However, some studies have reported population
88 structuring in different regions, with several drivers implicated as influencing
89 population structure. In particular, Ovčarenko *et al.* (2014) reported population
90 structuring in Finland and attributed this to habitats, field versus greenhouses
91 populations. Gao *et al.*, (2014) reported population structuring across the different
92 regions of China and suggested the primary driver was multiple introduction points
93 into China. There have been no similar studies in southern Africa.

94

95 On the basis of these studies we posed the following question: Do *T. vaporarioum*
96 samples from sub-Saharan Africa and in particular Kenya have distinct
97 phylogeographical structuring compared to global populations?

98

99 **How the hypothesis will be tested**

100 To help answer the question, we carried out field survey in 2014 across smallholder
 101 farms located in the Kenyan highlands, which produce primarily common bean
 102 (*Phaseolus vulgaris*, var *humilis* Alef). Using mtCOI we compared the genetic
 103 diversity found in Kenya with that previously observed for *T. vaporariorum* in other
 104 parts of the world using records stored in GenBank. We assessed the genetic
 105 diversity of the different *T. vaporariorum* global populations and explored signals of
 106 species complex formation; this was based on several species delimitation methods.
 107 We report for the first time the phylogeographical structuring of *T. vaporariorum*
 108 samples within Sub-Saharan Africa and in particular Kenya, compared to global *T.*
 109 *vaporariorum* samples.

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115 MATERIALS AND METHODS

116

117 Sample Collection

118 Sample collection of whiteflies was carried out on private property, following
 119 informed consent from the landowners. Adult whiteflies were collected on common
 120 beans (*Phaseolus vulgaris*) growing within smallholder farms in Kenya. Sampling
 121 was done in the short rain season of November 2014, across counties sub-counties
 122 in Katumani/Machakos, Kiambu and Kajiado (Fig 1). In each farm, common bean
 123 plants were examined for the presence of whiteflies across a 'z' transect. Whiteflies
 124 were collected using a mouth aspirator and aspirated directly into a falcon tube (BD,
 125 Biosciences USA) with RNAlater (Sigma, USA). Whiteflies were then shipped to the
 126 University of Western Australia and stored at room temperature.

127

128 Genomic DNA Isolation

129 Genomic DNA was extracted from individual whiteflies collected from each of the
 130 three sites using the Zymo Insect and Tissue extraction kit (Zymo, USA) as described
 131 by the manufacturer. Individual whiteflies were added into a ZR Bashing bead lysis
 132 tube containing 750 µl lysis solution. Bashing beads were then mounted on a Fast
 133 Prep®-24 MP bead beater and ground at 6 M/S for 40 seconds. Bashing beads were
 134 centrifuged at 10,000 x g for 1 minute. Subsequent steps were essentially as
 135 described by the manufacturer, with the only modification being the elution of
 136 genomic DNA with 30 µl of nuclease free water followed by a centrifugation step at
 137 10,000 x g for 30 seconds. Genomic DNA was stored at -20°C until analysis was
 138 carried out.

139

140 **Polymerase Chain Reaction and Sequencing**

141 Mitochondria COI *Trialeurodes vaporariorum* specific primers CO1-F:

142 5'-GCCTGGTTTTGGCATTA-3' and the reverse primer CO1-R:

143 5'-GCTTATTAGCACCCACTCTA-3' were used to amplify ~752 bp of COI gene (Gao, et

144 al., 2014). Polymerase chain reaction (PCR) was carried out in a total reaction mix of

145 20 µl with 5 ul of whitefly DNA as template, 10 ul Bulk AccuPower ^(R) PCR Premix

146 containing 1U of Top DNA polymerase, 250 µl of dNTP (dATP, dCTP, dGTP, dTTP),

147 10mM Tris-HCl (pH9.0), 30mM KCl, 1.5mM MgCl₂, 1 µl of forward and reverse

148 primers and 3 µl of nuclease free water. The PCR reaction was run on an eppendorf

149 thermocycler (Eppendorf, Hamburg Germany) under the following conditions; Initial

150 denaturation for 2 min at 94°C followed by 35 cycles of 94°C for 20 seconds,

151 annealing for 30 seconds at 52°C, elongation for 1 minute at 72°C, final elongation

152 for 30 min at 72°C. PCR amplicons were separated in a 2% agarose gel pre-stained

153 with gel red (Biotium, CA, USA) and run in 0.5 X Tris Boric EDTA (TBE) electrophoresis

154 buffer for 35 minutes at 100V and detected under UV light (Biorad, CA, USA). PCR

155 products were purified using Qiaquick PCR purification kit (Qiagen Inc.) as described

156 by the manufacturer. Purified PCR products were sent for bi-directional Sanger

157 sequencing at Macrogen Korea.

158

159 **Sequence Alignment and GenBank Data Retrieval**

160 A total of n=31 forward and reverse sequences of the 3' end of mtCOI were obtained.

161 Raw chromatograms were manually inspected using Geneious version 8

162 (<http://www.geneious.com>, Kearse et al., 2012) across all the bases, with

ambiguities corrected. Consensus sequences were generated for each whitefly, and used for subsequent analysis. Basic local alignment search (BLAST) was carried out to confirm that all sequences were *T. vaporariorum*. In addition, n = 228 *T. vaporariorum* COI sequences were retrieved from GenBank and combined with sequences from this study (Supplementary Table 1). The combined sequences were aligned using MAFFT in Geneious version 8 with default parameters (Kearse et al., 2012). Manual inspection, adjustment of gaps and trimming of overhangs was carried out.

171

172 ***Trialeurodes vaporariorum* Haplotype Distribution Across Geographical Locations**

To better understand the distribution of *T. vaporariorum* haplotypes across geographical locations and reduce redundancy within COI sequences deposited in GenBank. *T. vaporariorum* COI sequences were categorised based on geographical locations considered to have similar climatic properties as illustrated within global ecoregion maps (Olson, et al., 2002) Representative sequences from each of the haplotypes and across the five geographical locations were selected creating a subset of 90 sequences for subsequent analysis (Table 2). Classification was as follows: location 1, - n = 40 sequences from Europe Asia, and North Africa; location 2, - n = 31 sequences from Sub-Saharan (Kenya) (this study), location 3 - n = 11 sequences from South America, Central America, and the Caribbean; location 4, India, China - n=7 sequences from India and China; and location 5 - n=1 sample sequences from North America (USA). Location 5 was excluded from all population analysis, since only one sequence could be retrieved from the GenBank.

186

187 **Inter-Population Genetic Diversity**

188 The number of haplotypes within *T. vaporariorum* sequences was determined using
189 GenAlex (Peakall & Smouse, 2012). mtCOI variation across the sequences was
190 assessed using DNAsp v 5.0 (Librado & Rozas, 2009). Default program parameters
191 were used during both the analyses.

192

193 **Haplotypes Networks Based on MtCOI Sequences of *Trialeurodes vaporariorum***

194 Association between haplotypes across the four global geographical locations were
195 determined using median joining algorithms implemented in NETWORK version 5.0
196 (Bandelt *et al.*, 1999) with two outgroups *T. ricin* and *T. lauri* included under default
197 parameters.

198

199 **Phylogenetic Analysis**

200 Selection of the optimal model for phylogenetic analysis was determined using
201 jModeltest 2 (Darriba *et al.*, 2012), using 11 substitution schemes. The optimal
202 model selected based on the Akaike information criterion was (TPM2uf+G), while
203 the optimal model in Bayesian information criterion was (HKY+I). The model HKY+I
204 was selected since it considered variable base frequencies of one transition rate and
205 one transversion rate. MrBayes was run on the Magnus Supercomputer at the
206 Pawsey Supercomputer Centre at the University of Western Australia using 76
207 core/hours (274230s). Bayesian analysis was carried out for two independent runs
208 using the command “lset nst=2 rates=gamma” for 50 million generations, with trees
209 sampled every 1000 generations. In each of the runs, the first 25% (2,500) trees
210 were discarded as burn in. Convergence of the runs was determined using Tracer

211 v1.6.0 (Rambaut & Suchard, 2014). Maximum Likelihood (ML) phylogenetic trees
212 with bootstraps and nucleotide model set at $T_i \neq T_v$ rate (2 ST) and were generated
213 in PAUP* 4.0a147 (Swofford, 2003).

214

215 **Species Delimitation**

216 Clades identified within the *T. vaporariorum* phylogeny were assessed as potential
217 emerging species of *Trialeurodes vaporariorum* using the species delimitation plugin
218 (Masters, Fan, & Ross, 2011) in Geneious version 8 (<http://www.geneious.com>,
219 Kearse *et al.*, 2012). We used several species delimitation parameters on the mtCOI
220 sequences; Kimura two parameter (K2P), Rodrigo's (P(random distinct) posterior
221 probabilities (liberal and strict) and Rosenberg's P(AB) to evaluate either presence
222 species delimitation within *T. vaporariorum* COI sequences (Table 5).

223

224 **Evaluation of *T. vaporariorum* Population Structure using Bayesian Analysis**

225 To determine the population structure of *T. vaporariorum* mtCOI sequences across
226 different geographical locations we analysed using Structurama 2.0 (Huelsenbeck,
227 Andolfatto, & Huelsenbeck, 2011), which implements a Dirichlet process prior for
228 the number of populations. The default parameters of Gamma (0.100, 10.000) were
229 run, and the model without admixture was used. MCMC was run for one million
230 generations and samples were collected every 1000 generations. Two independent
231 runs were carried out to confirm that the MCMC algorithm converged to the same
232 values (Table 4)

233

234 **Results**

235

236 **Distribution of *T. vaporariorum* Haplotypes Across Global Populations**

237

238 In this study, 31 *T. vaporariorum* sequences were generated and deposited in
239 GenBank under accession numbers KX058201-KX058231 (Table 1) and compared
240 with 228 sequences obtained from GenBank (Supplementary Table 1). Analysis
241 across the combined global dataset based on representative 90 sequences revealed
242 19 haplotypes (Table 2).

243

244 **Inter-Population Genetic Diversity**

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246 Comparison of the genetic variation within *T. vaporariorum* was based on 443 bp
247 fragments of COI sequences across all the populations. A majority of the haplotypes
248 were from geographical location 1 (Europe Asia, and North Africa), while
249 geographical location 2 Sub Saharan (this study) had the fewest (n = 3) haplotypes
250 (Table 2). Assessment of population expansion based on neutrality test showed low
251 values for Tajima's D statistics across all four geographical locations indicative of
252 population contraction; location 1 (-1.58699, p > 0.10), location 2 (-0.30315, p >
253 0.10), location 3 (0.00351, p > 0.05) and location 4 (-1.23716, p>0.10) (Table 3),
254 however none of the values were found to be statistically significant (Table 3).

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260 Median Joining Networks Based on MtCOI Sequences of *Trialeurodes*

261 *vaporariorum*

262 Assessments of genetic relationships among the 19 haplotypes identified (Table 2)
263 was based on a median joining network of the COI sequences (Fig 2), showing two
264 ancestral haplotypes highlighted in red, red/black colour, while the rest of the
265 haplotypes diverged from them. Haplotypes 9 (HP_9) and 18 (HP_018) were the
266 dominant haplotypes across all geographical locations. Within the location 2 (Kenya),
267 haplotype 9, 10 and 11 (Hp_9, Hp_10, Hp_011) were present.

268

269 Phylogenetic analysis and species delimitation analysis of *Trialeurodes*

270 *vaporariorum*

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272 Phylogenetic relationships of *T. vaporariorum* across the geographical locations was
273 assessed using Bayesian phylogenetic inference as implemented in MrBayes 3.2.2
274 (Huelsenbeck *et al.*, 2011) and maximum likelihood methods. Phylogenetic trees
275 from both methods (Supplementary Fig. 1 and Supplementary Fig. 2) yielded two
276 main clades, that are supported with 50% bootstrap support and >0.9 posterior
277 probability respectively. Clade I was the largest clade, with a majority of *T.*
278 *vaporariorum* including a subset of samples from geographical location 2 (SSA-
279 Kenya). Clade II comprised of mainly *T. vaporariorum* individual sequences from
280 geographical location 2 (SSA-Kenya) and one sequence from Greece. *T. ricin* and *T.*
281 *lauri* were used as the outgroups for rooting the tree based on phylogenetic rooting
282 methods described in (Kinene *et al.*, 2016).

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284

285 **Bayesian analysis of *Trialeurodes vaporariorum* Population Structure**

286

287 Population structure of *T. vaporariorum* was evaluated using Structurama 2.0, with
288 default parameter settings and no admixture. All of the *T. vaporariorum* sequences
289 clustered as one population (K=1), supported by a posterior probability of 0.72
290 (Table 4), with posterior probability of 0.17 for K=2 populations. All other numbers of
291 populations received posterior probability of less than 0.10.

292

293 **Species Delimitation**

294

295 The two clades within the phylogenetic tree showed evidence of distinctness based
296 on Kimura two parameters (K2P) ($p = -1.21E-01$), Rodrigo's P (RD) ($p = 0.05$) and
297 Rosenberg's P(AB) ($p = 2.3E -13$) (Table 5). Across the two clades all the sequences
298 were monophyletic with genetic distance ranging between (0.2) and (0.7) (Table 5).
299 The posterior probabilities of correct assignment of unknown species based on strict
300 P ID (strict) and P ID (liberal) were ($p > 0.94$) and ($p > 0.99$) respectively (Table 5).

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304 **DISCUSSION**

305 In this study, we assessed the phylogeographical structuring and genetic diversity of
 306 *T. vaporariorum* within a previously underreported region (Sub-Saharan Africa).
 307 Phylogeographical structuring resulted in two main clades across representative
 308 global isolates with clade I (globally admixed) and II (Kenyan and one Greek sample).
 309 In contrast, genetic diversity across these global *T. vaporariorum* samples was low
 310 (Table 3). Species delimitation measures showed differences between *T.*
 311 *vaporariorum* isolates from Kenya compared to other geographical locations
 312 revealing plausible signs of species complex formation within a previously
 313 monophyletic group.

314

315 **Genetic Diversity of *T. vaporariorum* Across Global Samples**

316 Genetic diversity of *T. vaporariorum* and haplotypes counts were based on a 443bp
 317 fragment of mtCOI sequence. A total of 19 *T. vaporariorum* haplotypes were present
 318 among all global sequences, with 3 haplotypes (Hp-9, Hp-10, Hp-11) present within *T.*
 319 *vaporariorum* from Kenya geographical location 2 (Table 2). Haplotype diversity
 320 ranged between 0.411 in population 2 to 0.956 in geographical location 3 (Table 3).
 321 Neutrality tests based on Tajima's D statistic indicated low levels of genetic diversity
 322 across the global populations (Table 3). Our findings are comparable to previous
 323 genetic and population diversity studies of *T. vaporariorum*, indicating low genetic
 324 diversity (Roopa *et al.*, 2012, Shin *et al.*, 2013, Kapantaidaki *et al.*, 2014, Prijović *et*
 325 *al.*, 2014). Low genetic diversity within *T. vaporariorum* indicates recent population
 326 expansion and colonization into the regions. A possible explanation of the low

327 genetic diversity of *T. vaporariorum* across global locations and in specifically from
 328 this study could be two-fold. Firstly, there has been an increase in global trade on
 329 horticultural products; mainly cut flowers from Kenya (Ulrich, 2014). This has
 330 resulted in increased importation of flower cuttings as propagation material from
 331 temperate ecoregions. Flower cuttings are potential carriers of *T. vaporariorum*
 332 because the eggs are invisible but readily hatch under ideal climatic conditions. This
 333 mode of *T. vaporariorum* movement has been implicated as one of the main modes
 334 of distribution of invasive pests globally (Grapputo *et al.*, 2005). Secondly,
 335 proliferation of polyhouses for horticulture production potentially provide
 336 appropriate microclimatic conditions sufficient for the emergence of *T. vaporariorum*
 337 and subsequent release into the open field. The importance of the polyhouse in
 338 building up *T. vaporariorum* populations and controlling gene flow have previously
 339 been reported in India, Northern and Southern Europe (Roopa *et al.*, 2012;
 340 Ovčarenko *et al.*, 2014).

341

342 **Haplotype Networks**

343 Evaluation of the relationship among the 19 *T. vaporariorum* haplotypes (Table 2)
 344 across five global geographical location based on the median joining network (Fig. 2)
 345 showed two ancestral nodes (Hp_09, Hp_018) (Fig. 2) that were present across all
 346 geographical locations. Within the SSA population three main haplotypes were in
 347 circulation (Hp_09, Hp_10, Hp_11) (Table 1) while Haplotype_11 was found as a
 348 distinct haplotype within this population. Our findings concur with previous reports
 349 on the number of haplotypes within *T. vaporariorum* (Prijović *et al.*, 2014). An
 350 overlap in haplotypes across the geographical location could indicate a single source

351 of *T. vaporariorum* lineages that are easily adapted to different niche habitats
352 (Kapantaidaki *et al.*, 2014). However distinct haplotypes and clades within location 2
353 (SSA-Kenya) could be indications of local adaptations of *T. vaporariorum*.

354

355 **Phylogeographical Structuring and Species Delimitation Genotypes**

356 The evolutionary relationships among the *T. vaporariorum* sequences across
357 geographical locations were reconstructed using both maximum likelihood and
358 Bayesian phylogenetic analysis. Both phylogenetic trees resulted in two main clades
359 (Supplementary Fig.1 and Supplementary Fig. 2). Clade I was the largest clade
360 composed of *T. vaporariorum* samples from all the geographical locations while
361 clade II comprised of a subset of *T. vaporariorum* sequences from SSA (Kenya) and
362 one Greek samples. This is contrary to previous phylogenetic relationships of *T.*
363 *vaporariorum*, where a single clade was observed across *T. vaporariorum*
364 populations collected (Roopa *et al.*, 2012; Prijović *et al.*, 2014; Kapantaidaki *et al.*,
365 2014). In this study, we report existence of at least two main clades within *T.*
366 *vaporariorum* that are supported by bootstrap values of >60% and posterior
367 probabilities above 0.90 (Supplementary Fig. 1 and Supplementary Fig. 2). Evaluation
368 of the two clades as distinct species revealed signs of species complex within *T.*
369 *vaporariorum* based on species delimitation measures (Table 5). Clade distinctness
370 was supported by Kimura two parameters (K2P) ($p = -1.21E-01$), Rodrigo's P (RD) (p
371 $=0.05$) and Rosenberg's P(AB) ($p = 2.3E -13$) (Table 5).

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375 **Population structure based on Bayesian analysis**

376 Population structure analysis of *T. vaporariorum* using Structurama based on a
 377 variable number of populations showed the strongest support for one population
 378 (posterior probability 0.72; Table 4). There was lack of distinct clustering from *T.*
 379 *vaporariorum* based on global geographical location (Table 5). Population structure
 380 results of one population were contrary to the phylogenetic tree showing the
 381 existence of two main groups within *T. vaporariorum* (Supplementary Fig. 1 and
 382 Supplementary Fig. 2). However, our finding concur with (Gao *et al.*, 2014) where
 383 they report lack of geographical structuring within the *T. vaporariorum* populations
 384 though several populations were reported. This further supports our hypothesis of a
 385 single source of *T. vaporariorum* introduced into Sub Saharan Africa.

386
 387 Our findings highlight differences in the genetic diversity of the two whiteflies: *T.*
 388 *vaporariorum* and *B. tabaci*, which are both circulating in the regions sampled in
 389 Kenya. *B. tabaci* is recognized as a species complex of 34 species that are
 390 morphologically indistinguishable (Boykin *et al.*, 2007; De Barro & Ahmed,
 391 2011; Boykin & De Barro, 2014). *B. tabaci* putative species exhibit differences in host
 392 range, phylogeographical clustering and potential to transmit viruses (Abdullahi *et*
 393 *al.*, 2003; Sseruwagi *et al.*, 2006, De Barro & Ahmed, 2011; Ashfaq *et al.*, 2014; Legg
 394 *et al.*, 2014). These differences are yet to be reported within *T. vaporariorum* and
 395 may be present based on distinct clustering observed and plausible species complex
 396 formation revealed in this study and would warrant further investigation.

397

398 Future studies should include a multilocus approach to provide for a more robust
 399 and stringent species delimitation calculation to authenticate the possible existence
 400 of a species complex formation within *T. vaporariorum*. However, it is probable that
 401 the existence of two clades are early indicators of impending divergence within *T.*
 402 *vaporariorum* potentially driven by phylogeographical factors as observed within *B.*
 403 *tabaci* and *T. vaporariorum* in Europe (Boykin *et al.*, 2013; Ovčarenko *et al.*, 2014). In
 404 addition, differences in the bacterial endosymbionts among *T. vaporariorum*
 405 haplotypes might induce genetic sweeps within the mitochondria and thus drive the
 406 diversification of *T. vaporariorum* (Kapantaidaki *et al.*, 2014). In addition, an
 407 expanded sampling from outside of Europe and throughout Kenya will greatly
 408 improve the understanding of this highly invasive pest.

409

410

411 **Conclusion**

412 This study provides the evidence of phylogeographical structure of *T. vaporariorum*
 413 within Sub-Saharan Africa. In addition, the data presented here supports a putative
 414 species complex formation within *T. vaporariorum*. Our results provide preliminary
 415 findings for future research on the drivers for genetic population structuring and
 416 species complex formation within *T. vaporariorum* in nascent geographical regions.

417

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432

REFERENCES

- 433
- 434 **Abdullahi, I., Winter, S., Atiri, G. I. & Thottappilly, G. (2003)** Molecular
435 characterization of whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae)
436 populations infesting cassava. *Bulletin of Entomological Research*, 93, 97–106.
437 doi:10.1079/BER2003223
- 438 **Anderson, P. K., Cunningham, A. A., Patel, N. G., Morales, F. J., Epstein, P. R. &**
439 **Daszak, P. (2004)** Emerging infectious diseases of plants: Pathogen pollution,
440 climate change and agrotechnology drivers. *Trends in Ecology and Evolution*,
441 19(10), 535–544. doi:10.1016/j.tree.2004.07.021
- 442 **Ateka, E. M., Njeru, R. W., Kibaru, A. G., Kimenju, J. W., Barg, E., Gibsson, R. W. &**
443 **Vetten, H. J (2004)** Identification and distribution of viruses infecting sweet
444 potato in Kenya, 371–379.
- 445 **Bandelt, H. J., Forster, P. & Rohl, A. (1999)** Median-Joining Networks for Inferring
446 Intraspecific Phylogenies. *Molecular Biology*, 16(1), 37–48.
447 doi:10.1093/oxfordjournals.molbev.a026036
- 448 **Boykin, L. M., Bell, C. D., Evans, G., Small, I. & De Barro, P. J. (2013)** Is agriculture
449 driving the diversification of the *Bemisia tabaci* species complex (Hemiptera:
450 *Sternorrhyncha: Aleyrodidae*)?: Dating, diversification and biogeographic
451 evidence revealed. *BMC Evolutionary Biology*, 13(1), 228. doi:10.1186/1471-
452 2148-13-228
- 453 **Boykin, L. M. & De Barro, P. J. (2014)** A practical guide to identifying members of the
454 *Bemisia tabaci* species complex: and other morphologically identical species.
455 *Frontiers in Ecology and Evolution*, 2(August), 1–5.
456 doi:10.3389/fevo.2014.00045
- 457 **Boykin, L. M., Shatters, R. G., Rosell, R. C., McKenzie, C. L., Bagnall, R. A., De Barro,**
458 **P. & Frohlich, D. R. (2007)** Global relationships of *Bemisia tabaci* (Hemiptera:
459 *Aleyrodidae*) revealed using Bayesian analysis of mitochondrial COI DNA
460 sequences. *Molecular Phylogenetics and Evolution*, 44, 1306–1319.

doi:10.1016/j.ympev.2007.04.020

Colvin, J., Omongo, C. A., Govindappa, M. R., Stevenson, P. C., Maruthi, M. N., Gibson, G., Seal, S. E & Muniyappa, V. (2006) Host-Plant Viral Infection Effects on Arthropod-Vector Population Growth, Development and Behaviour: Management and Epidemiological Implications. In *Advances in Virus Research* (Vol. 67, pp. 419–452). doi:10.1016/S0065-3527(06)67011-5

Darriba, D., Taboada, G. L., Doallo, R. & Posada, D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, 9(8), 772–772. doi:10.1038/nmeth.2109

DeBarro, P. & Ahmed, M. (2011) Genetic networking of the *Bemisia tabaci* cryptic species complex reveals pattern of biological invasions. *PLoS ONE*, 6(10). doi:10.1371/journal.pone.0025579

EPPO (2005) Tomato chlorosis *crinivirus*. *OEPP/EPPO Bulletin*, (35), 439–441. doi:10.1111/j.1365-2338.2005.00888.x

Fiallo-Olivé, E., Hamed, A. A., Moriones, E., & Navas-Castillo, J. (2011) First Report of Tomato chlorosis virus Infecting Tomato in Sudan. *Plant Disease*, 95(12), 1592. doi:10.1094/PDIS-08-11-0631

Fortes, I. M., Moriones, E. & Navas-Castillo, J. (2012) Tomato chlorosis virus in pepper: Prevalence in commercial crops in southeastern Spain and symptomatology under experimental conditions. *Plant Pathology*, 61(5), 994–1001. doi:10.1111/j.1365-3059.2011.02584.x

Grapputo, A., Boman, S., Lindström, L., Lyytinen, A. & Mappes, J. (2005) The voyage of an invasive species across continents: Genetic diversity of North American and European Colorado potato beetle populations. *Molecular Ecology*, 14(14), 4207–4219. doi:10.1111/j.1365-294X.2005.02740.x

- 487 **Huelsenbeck, J. P., Andolfatto, P. & Huelsenbeck, E. T. (2011)** Structurama:
488 Bayesian inference of population structure. *Evolutionary Bioinformatics*,
489 2011(7), 55–59. doi:10.4137/EBO.S6761
- 490 **Kapantaidaki, D. E., Ovčarenko, I., Fytro, N., Knott, K. E., Bourtzis, K. &**
491 **Tsagkarakou, A. (2014)** Low Levels of Mitochondrial DNA and Symbiont
492 Diversity in the Worldwide Agricultural Pest, the Greenhouse Whitefly
493 *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae). *The Journal of Heredity*,
494 1–13. doi:10.1093/jhered/esu061
- 495 **Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Simon**
496 **Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B.,**
497 **Meintjes, P & Drummond, A. (2012)** Geneious Basic: An integrated and
498 extendable desktop software platform for the organization and analysis of
499 sequence data. *Bioinformatics*, 28(12), 1647–1649.
500 doi:10.1093/bioinformatics/bts199
- 501
502 **Kinene, T., Wainaina, J., Maina, S., & Boykin, L. M. (2016)** Rooting Trees, Methods
503 for. *Encyclopedia of Evolutionary Biology*, 3, 489–493. doi:10.1016/B978-0-12-
504 800049-6.00215-8
- 505
506 **Lapidot, M., Legg, J. P., Wintermantel, W. M. & Polston, J. E. (2014)** *Management of*
507 *Whitefly- Transmitted Viruses in Open-Field Production Systems. Control of Plant*
508 *Virus Diseases* (1st ed., Vol. 90). Elsevier Inc. doi:10.1016/B978-0-12-801246-
509 8.00003-2
- 510 **Legg, J. P., Sseruwagi, P., Boniface, S., Okao-Okuja, G., Shirima, R., Bigirimana, S.,**
511 **Gashakae, G., Herrmannf, H-W., Jeremiahg, S., Obieroh, H., Ndyetabulai, I.,**
512 **Tata-Hangyj, W., Masembe, C. & Brown, J. K (2014)** Spatio-temporal patterns
513 of genetic change amongst populations of cassava *Bemisia tabaci* whiteflies
514 driving virus pandemics in East and Central Africa. *Virus Research*, 186, 61–75.
515 doi:10.1016/j.virusres.2013.11.018

- 516 **Librado, P. & Rozas, J. (2009)** DnaSP v5: a software for comprehensive analysis of
517 DNA polymorphism data, 25(11), 1451–1452.
518 doi:10.1093/bioinformatics/btp187
- 519 **Masters, B. C., Fan, V. & Ross, H. A. (2011)** Species delimitation-a geneious plugin
520 for the exploration of species boundaries. *Molecular Ecology Resources*, 11(1),
521 154–157. doi:10.1111/j.1755-0998.2010.02896.x
- 522 **Mound L. A & Halsey S.H. (1978)** Whitefly of the World. A systematic catalogue of
523 the *Aleyrodidae (Homoptera)* with host plant and natural enemy data. British
524 Museum (Natural History) & John Wiley & Sons, Chichester (Great Britain)
- 525 **Miano, D. W., LaBonte, D. R. & Clark, C. A. (2008)** Identification of molecular
526 markers associated with sweet potato resistance to sweet potato virus disease
527 in Kenya. *Euphytica*, 160(1), 15–24. doi:10.1007/s10681-007-9495-2
- 528 **Navas-Castillo, J., Lopez, J. J. & Aranda, M. A (2014)** Whitefly-transmitted RNA
529 viruses that affect intensive vegetable production, 165, 155–171.
530 doi:10.1111/aab.12147
- 531 **Navas-Castillo, J., Fiallo-Olivé, E. & Sánchez-Campos, S. (2011)** *Emerging virus*
532 *diseases transmitted by whiteflies. Annual review of phytopathology* (Vol. 49).
533 doi:10.1146/annurev-phyto-072910-095235
- 534 **Njaramb, T. (2000)** Assessment of the Level of Resistance to Bifenthrin,
535 Cypermethrin and Methomyl Insecticides in *Bemisia tabaci (Gennadius)* and
536 *Trialeurodes vaporariorum (Westwood)* Whitefly Populations from Selected
537 Sites in Kenya (Msc.thesis).
- 538 **Olson, D. M., Dinerstein, E., Global, T. H. E., & David, P. (2002)** The Global 200:
539 Priority Ecoregions for Global Conservation. *Missour Botanical Garden*, 89(2),
540 199–224.
- 541 **Ovčarenko, I., Kapantaidaki, D., Lindström, L., Gauthier, N., Tsagkarakou, A., Knott,**
542 **K. & Vänninen, I. (2014)** Agroecosystems shape population genetic structure of

- 543 the greenhouse whitefly in Northern and Southern Europe. *BMC Evolutionary*
544 *Biology*, 14, 165. doi:10.1186/s12862-014-0165-4
- 545 **Peakall, R. & Smouse, P. E. (2012)** GenAlEx 6 . 5: genetic analysis in Excel .
546 Population genetic software for teaching and research — an update, 28(19),
547 2537–2539. doi:10.1093/bioinformatics/bts460
- 548 **Prijić, M., Marčić, D., Drobnjaković, T., Međo, I. & Perić, P. (2013)** Life History
549 Traits and Population Growth of Greenhouse Whitefly on Different Tomato
550 Genotypes. *Pesticid Phytomed*, 28(4), 239–245. doi:10.2298/PIF1304239P
- 551 **Prijić, M., Skaljic, M., Drobnjaković, T., Zanić, K., Perić, P., Marčić, D., & Puizina, J.**
552 **(2014)** Genetic variation of the greenhouse whitefly, *Trialeurodes vaporariorum*
553 (*Hemiptera: Aleyrodidae*), among populations from Serbia and neighbouring
554 countries, as inferred from COI sequence variability. *Bulletin of Entomological*
555 *Research*, 1, 1–10. doi:10.1017/S0007485314000169
- 556 **Swofford, D. L. (2003)** PAUP*. Phylogenetic Analysis Using Parsimony (*and Other
557 Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.
- 558 **Rambaut A, Suchard, M. A., Xie, D. & Drummond, A. J (2014)** Tracer v1.6,
559 Available from <http://beast.bio.ed.ac.uk/Tracer>
560
- 561 **Roopa, H. K., Kumar, N. K. K., Asokan, R., Rebijith, K. B., Mahmood, R. & Verghese,**
562 **A. (2012)** Phylogenetic Analysis of *Trialeurodes Spp.*(*Hemiptera: Aleyrodidae*)
563 from India Based on Differences in Mitochondrial and Nuclear Dna. *Florida*
564 *Entomologist*, 95(December), 1086–1094. doi:10.1653/024.095.0438
- 565 **Gao, R. R., Zhang, W.-P., Wu, H.-T., Zhang, R.-M., Zhou, H.-X., Pan, H.-P., Zhang, Y.-J.,**
566 **Brown, J. K. & Chu, D. (2014)** Population Structure of the Greenhouse Whitefly,
567 *Trialeurodes vaporariorum* (Westwood), an Invasive Species from the Americas,
568 60 Years after Invading China. *International Journal of Molecular Sciences*, 15(8),
569 13388–13400. doi:10.3390/ijms150813388

570

571 **Shin, D., Mo, H. H., Lee, S. E., Park, J. J. & Cho, K. (2013)** Elucidation of the genetic
572 differences in *Trialeurodes vaporariorum* populations under vegetable
573 greenhouse conditions by using the allozyme approach. *Entomological Research*,
574 43(5), 271–281. doi:10.1111/1748-5967.12032

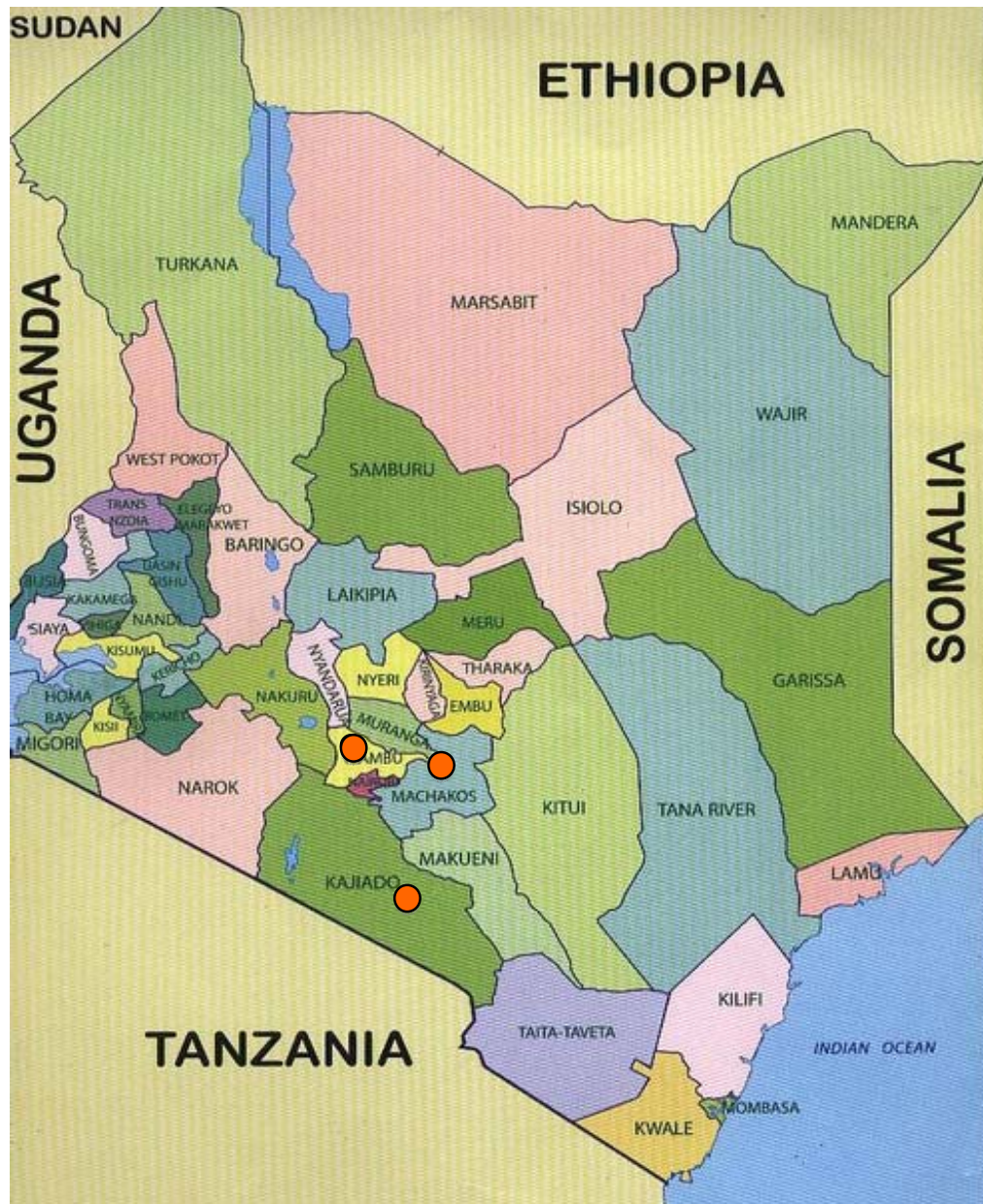
575 **Sseruwagi, P., Maruthi, M. N., Colvin, J., Rey, M. E. C., Brown, J. K. & Legg, J. P.**
576 **(2006)** Colonization of non-cassava plant species by cassava whiteflies (*Bemisia*
577 *tabaci*) in Uganda. *Entomologia Experimentalis et Applicata*, 119(Cmd), 145–153.
578 doi:10.1111/j.1570-7458.2006.00402.x

579 **Ulrich, A. (2014)** Export-Oriented Horticultural Production in Laikipia, Kenya:
580 Assessing the Implications for Rural Livelihoods. *Sustainability*, 6, 336–347.
581 doi:10.3390/su6010336

582 **Wintermantel, W. M., Hladky, L. L., Gulati-Sakhuja, A., Li, R., Liu, H. Y. & Tzanetakis,**
583 **I. E. (2009)** The complete nucleotide sequence and genome organization of
584 tomato infectious chlorosis virus: A distinct *crinivirus* most closely related to
585 lettuce infectious yellows virus. *Archives of Virology*, 154(8), 1335–1341.
586 doi:10.1007/s00705-009-0432-7

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591 **Figure 1:** Map of Kenya with Sampling Points included in this study are highlighted

592 **Source:** www.map1907.holes

593 **Key**

594  Sampling points for whitefly

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597 **Table 1:** *Trialeurodes vaporariorum* across geographical location and host plant

598 collected in this study.

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*Location	Coordinates	Host Plant	No of samples sequenced	GenBank Accession Numbers
Kajiado	-1.842073, 36.79186	<i>Phaseolus vulgaris</i> (Common Bean)	2	KX058204, KX058211
Katumani	-1.618715, 37.215651	<i>Phaseolus vulgaris</i> (Common Bean)	4	KX058202 KX058205 KX058207 KX058214
Kiambu	-1.280825, 36.652756	<i>Phaseolus vulgaris</i> (Common Bean)	25	KX058201- KX058203 KX058206 KX058208- KX058209 KX058210 KX058212 KX058213 KX058215- KX058231

602 *Location is the county or sub county in Kenya where whiteflies were collected in

603 2014.

604

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606

607 **Table 2:** *Trialeurodes vaporariorum* haplotypes from across geographical locations

608 determined using GenAlex 6.502

609

Haplotypes	Location 1	Location 2	Location 3	Location 4	Location 5	N	*GenBank Accession Number
Hp-1	-	-	1	-	-	1	JF682887
Hp-2	2	-	-	-	-	2	KC843068
Hp-3	6	-	-	-	-	6	KC843065
Hp-4	2	-	-	-	-	2	KC843067
Hp-5	-	-	1	-	-	1	JF682883
Hp-6	-	-	1	-	-	1	JF682882
Hp-7	-	-	1	-	-	1	JF682881
Hp-8	-	-	1	-	-	1	JF682885
Hp-9	4	23	2	-	-	29	LN614547
Hp-10	1	7	-	-	-	8	KX058224
Hp-11	-	1	-	-	-	1	KX058231
Hp-12	-	-	1	-	-	1	JF682888
Hp-13	1	-	-	-	-	1	KJ475455
Hp-14	1	-	-	-	-	1	KJ475454
Hp-15	2	-	-	-	-	2	KF921192
Hp-16	-	-	-	2	-	2	JX841223
Hp-17	1	-	-	-	-	1	HE863766
Hp-18	20	-	2	4	1	27	KC113579
Hp-19	-	-	1	1	-	2	JQ995233

610

611 **Key**

612 *Representative GenBank Accession number provided for each haplotype.

613 *N= Total Number of Haplotypes across the five world *Trialeurodes vaporariorum*
614 populations

615 **Geographical Location 1:** Europe Asia, and North Africa

616 **Geographical Location 2:** Sub Saharan (Kenya, this study)

617 **Geographical Location 3:** South America, Central America, and the Carribean

618 **Geographical Location 4:** India and China

619 **Geographical Location 5:** North America (USA)

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625 **Table 3:** Variation of mitochondria COI sequences from *Trialeurodes vaporariorum*

626 across four geographical locations calculated using DNAsp v 5.0.

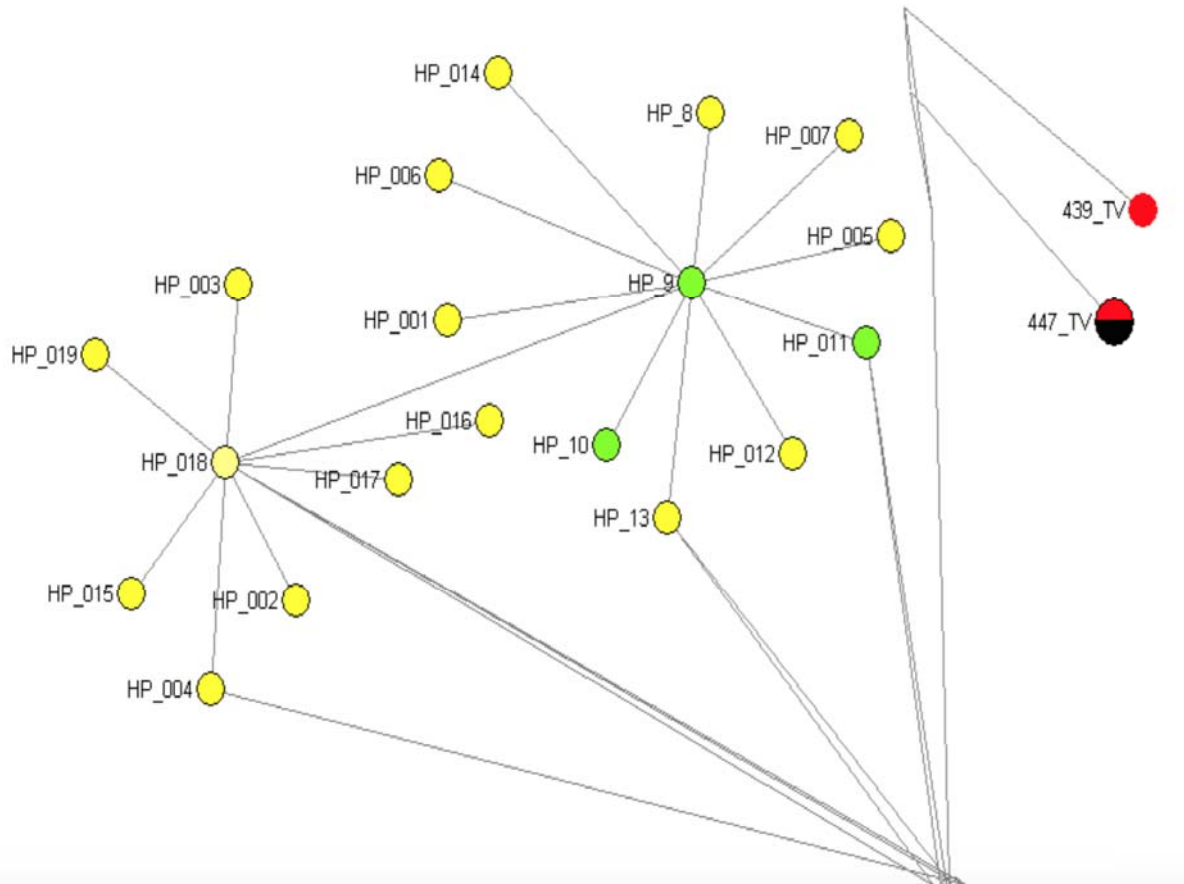
	Pop 1	Pop 2	Pop 3	Pop 4
Number of Individuals	40	31	10	7
Number of Haplotypes (h)	10	3	8	3
Haplotype (gene) diversity (Hd)	0.726	0.411	0.956	0.524
Nucleotide diversity (per site) (Pi)	0.00248	0.00096	0.00351	0.00129
Neutrality Tests				
Tajima's D	-1.58699	-0.30315	0.00351	-1.23716
	(P > 0.10)	(P > 0.10)	(P > 0.05)	(P > 0.10)

627 *Four of the five population were considered; Population 5 was dropped since only
628 one *Trialeurodes vaporariorum* sequence present from the GenBank.

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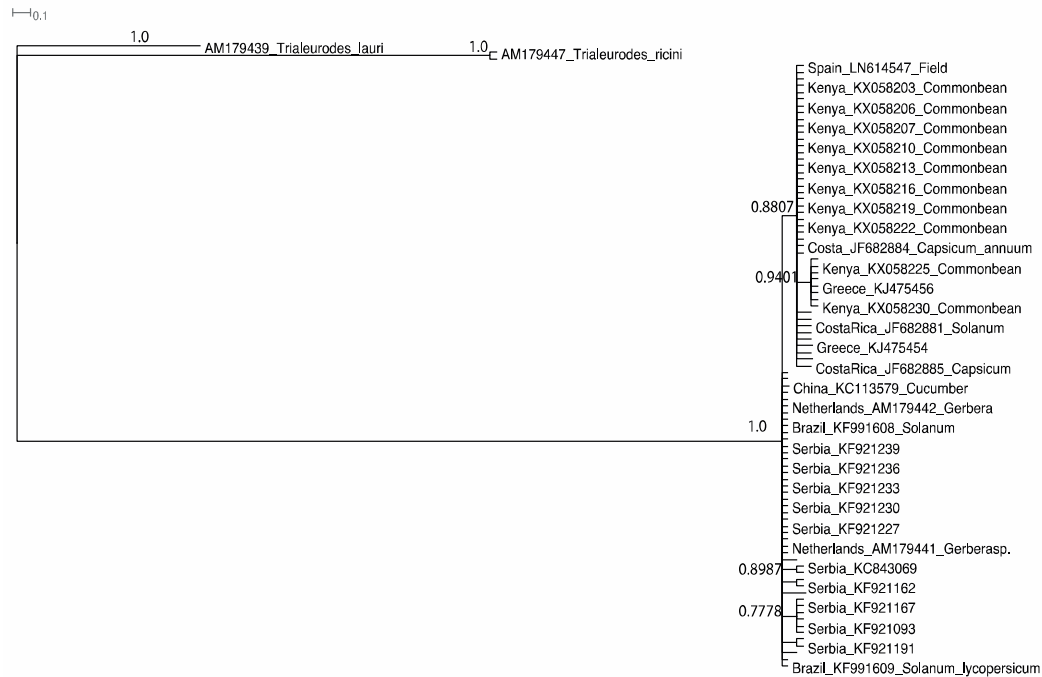
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Fig 2: Median joining network for 19 MtCO1 haplotypes of *Trialeurodes vaporariorum* and 3 outgroup sequences (*Trialeurodes ricin*, *Trialeurodes lauri*). Median joining networks were generated using the program NETWORK version 5.0. Haplotypes in circulation in Kenya are highlighted in green while the ancestral haplotypes are in red/black.

643
644 **Fig 3.** Bayesian phylogenetic relationships of representative *T. vaporariorum* and two
645 outgroups (*T. ricin*, *T. lauri*) generated with MrBayes 3.2.2. Branches in
646 supplemental figure 2 with zero branch length were collapsed.



647

648
 649 **Table 4:** Posterior probabilities on the number of populations (K) within *Trialeurodes*
 650 *vaporariorum* for the COI sequences across geographical locations using
 651 Structurama 2.0.

Number of Population (K)	Posterior Probability
1	0.72
2	0.17
3	0.07
4	0.02
5	0.01
6	0.0

Table 5: Species Delimitation estimation using the species delimitation plugin in Geneious 8.1.8 on representative *Trialeurodes vaporariorum* across five global geographical locations

Species	Closet Species	Monophyletic	Intra Dist	Inter Dist Closest (K2P(% difference))	Intra/Inter	P ID(Strict)	P ID(Liberal)	Av (MRCA-tips)	P (Randomly distinct)	Clade Support	Rosenberg's P (AB)
1	2	Yes	-2.44E-03	-1.21E-01	0.02	0.94 (0.83, 1.0)	1.00 (0.94, 1.0)	-1.22E-03	0.05	83.56	2.3E-13
2	1	Yes	-7.92E-03	-1.21E-01	0.07	0.97 (0.92, 1.0)	0.99 (0.97, 1.0)	-2.09E-02	0.05		2.3E-13

Supplementary Table 1: *Trialeurodes vaporariorum* COI sequence from GenBank and this study with respective haplotype and Geographical regions assigned

Country	Geographical Location Assigned	Haplotype (HP_)	GenBank Accession Numbers
Costa Rica	South America, Central America, and the Caribbean	1	JF682887
Serbia	Europe, Asia, and North Africa	2	KC843068
Serbia	Europe, Asia, and North Africa	2	KF921191
Serbia	Europe, Asia, and North Africa	3	KC843065
Serbia	Europe, Asia, and North Africa	3	KF921167
Serbia	Europe, Asia, and North Africa	3	KF921104
Serbia	Europe, Asia, and North Africa	3	KF921096
Serbia	Europe, Asia, and North Africa	3	KF921093
Serbia	Europe, Asia, and North Africa	3	KF921092
Serbia	Europe, Asia, and North Africa	4	KC843067
Serbia	Europe, Asia, and North Africa	4	KF921162
Costa	South America, Central America, and the Caribbean	5	JF682883
Costa Rica	South America, Central America, and the Caribbean	6	JF682882
Costa Rica	South America, Central America, and the Caribbean	7	JF682881
Costa Rica	South America, Central America, and the Caribbean	8	JF682885
Spain	Europe, Asia, and North Africa	9	LN614547
Kenya	Sub Saharan	9	KX058201
Kenya	Sub Saharan	9	KX058202
Kenya	Sub Saharan	9	KX058203
Kenya	Sub Saharan	9	KX058204
Kenya	Sub Saharan	9	KX058205
Kenya	Sub Saharan	9	KX058206
Netherlands	Europe, Asia, and North Africa	9	AM179446
Greece	Europe, Asia, and North Africa	9	KJ475453
Kenya	Sub Saharan	9	KX058207
Kenya	Sub Saharan	9	KX058208
Kenya	Sub Saharan	9	KX058209
Kenya	Sub Saharan	9	KX058210
Kenya	Sub Saharan	9	KX058211
Kenya	Sub Saharan	9	KX058212
Kenya	Sub Saharan	9	KX058213
Kenya	Sub Saharan	9	KX058214
Kenya	Sub Saharan	9	KX058215
Kenya	Sub Saharan	9	KX058216
Kenya	Sub Saharan	9	KX058217
Kenya	Sub Saharan	9	KX058218
Kenya	Sub Saharan	9	KX058219
Kenya	Sub Saharan	9	KX058220

Kenya	Sub Saharan	9	KX058221
Kenya	Sub Saharan	9	KX058222
Kenya	Sub Saharan	9	KX058223
Costa Rica	South America, Central America, and the Caribbean	9	JF682886
Costa Rica	South America, Central America, Caribbean	9	JF682884
Spain	Europe, Asia, and North Africa	9	AM179446
Kenya	Sub Saharan	10	KX058224
Kenya	Sub Saharan	10	KX058225
Kenya	Sub Saharan	10	KX058226
Kenya	Sub Saharan	10	KX058227
Greece	Europe, Asia, and North Africa	10	KJ475456
Kenya	Sub Saharan	10	KX058228
Kenya	Sub Saharan	10	KX058229
Kenya	Sub Saharan	10	KX058230
Kenya	Sub Saharan	11	KX058231
Costa Rica	South America, Central America, and the Caribbean	12	JF682888
Greece	Europe, Asia, and North Africa	13	KJ475455
Greece	Europe, Asia, and North Africa	14	KJ475454
Serbia	Europe, Asia, and North Africa	15	KC843069
Serbia	Europe, Asia, and North Africa	15	KF921192
India	Europe, Asia, and North Africa	16	JX841223
Morocco	Europe, Asia, and North Africa	17	HE863766
India	Europe, Asia, and North Africa	18	JQ995232
India	Europe, Asia, and North Africa	18	JQ995231
China	Europe, Asia, and North Africa	18	KC113579
United Kingdom	Europe, Asia, and North Africa	18	AM179444
Netherlands	Europe, Asia, and North Africa	18	AM179443
Netherlands	Europe, Asia, and North Africa	18	AM179442
Netherlands	Europe, Asia, and North Africa	18	AM179445
Greece	Europe, Asia, and North Africa	18	KJ475452
Brazil	South America, Central America, Caribbean	18	KF991608
Brazil	South America, Central America, Caribbean	18	KF991609
Serbia	Europe, Asia, and North Africa	18	KF921271
Serbia	Europe, Asia, and North Africa	18	KF921270
Serbia	Europe, Asia, and North Africa	18	KF921269
Serbia	Europe, Asia, and North Africa	18	KF921268
Serbia	Europe, Asia, and North Africa	18	KF921267
Serbia	Europe, Asia, and North Africa	18	KF921266
Serbia	Europe, Asia, and North Africa	18	KF921265
Serbia	Europe, Asia, and North Africa	18	KF921264
Serbia	Europe, Asia, and North Africa	18	KF921263
Serbia	Europe, Asia, and North Africa	18	KF921262
Serbia	Europe, Asia, and North Africa	18	KF921261

Serbia	Europe, Asia, and North Africa	18	KF921260
Serbia	Europe, Asia, and North Africa	18	KF921259
Serbia	Europe, Asia, and North Africa	18	KF921258
Serbia	Europe, Asia, and North Africa	18	KF921257
Serbia	Europe, Asia, and North Africa	18	KF921256
Serbia	Europe, Asia, and North Africa	18	KF921255
Serbia	Europe, Asia, and North Africa	18	KF921254
Serbia	Europe, Asia, and North Africa	18	KF921253
Serbia	Europe, Asia, and North Africa	18	KF921252
Serbia	Europe, Asia, and North Africa	18	KF921251
Serbia	Europe, Asia, and North Africa	18	KF921250
Serbia	Europe, Asia, and North Africa	18	KF921249
Serbia	Europe, Asia, and North Africa	18	KF921248
Serbia	Europe, Asia, and North Africa	18	KF921247
Serbia	Europe, Asia, and North Africa	18	KF921246
Serbia	Europe, Asia, and North Africa	18	KF921245
Serbia	Europe, Asia, and North Africa	18	KF921244
Serbia	Europe, Asia, and North Africa	18	KF921243
Serbia	Europe, Asia, and North Africa	18	KF921242
Serbia	Europe, Asia, and North Africa	18	KF921241
Serbia	Europe, Asia, and North Africa	18	KF921240
Serbia	Europe, Asia, and North Africa	18	KF921239
Serbia	Europe, Asia, and North Africa	18	KF921238
Serbia	Europe, Asia, and North Africa	18	KF921237
Serbia	Europe, Asia, and North Africa	18	KF921236
Serbia	Europe, Asia, and North Africa	18	KF921235
Serbia	Europe, Asia, and North Africa	18	KF921234
Serbia	Europe, Asia, and North Africa	18	KF921233
Serbia	Europe, Asia, and North Africa	18	KF921232
Serbia	Europe, Asia, and North Africa	18	KF921231
Serbia	Europe, Asia, and North Africa	18	KF921230
Serbia	Europe, Asia, and North Africa	18	KF921229
Serbia	Europe, Asia, and North Africa	18	KF921228
Serbia	Europe, Asia, and North Africa	18	KF921227
Serbia	Europe, Asia, and North Africa	18	KF921226
Serbia	Europe, Asia, and North Africa	18	KF921219
Serbia	Europe, Asia, and North Africa	18	KF921218
Serbia	Europe, Asia, and North Africa	18	KF921217
Serbia	Europe, Asia, and North Africa	18	KF921216
Serbia	Europe, Asia, and North Africa	18	KF921215
Serbia	Europe, Asia, and North Africa	18	KF921214
Serbia	Europe, Asia, and North Africa	18	KF921213
Serbia	Europe, Asia, and North Africa	18	KF921212
Serbia	Europe, Asia, and North Africa	18	KF921211

Serbia	Europe, Asia, and North Africa	18	KF921210
Serbia	Europe, Asia, and North Africa	18	KF921209
Serbia	Europe, Asia, and North Africa	18	KF921208
Serbia	Europe, Asia, and North Africa	18	KF921207
Serbia	Europe, Asia, and North Africa	18	KF921206
Serbia	Europe, Asia, and North Africa	18	KF921205
Serbia	Europe, Asia, and North Africa	18	KF921204
Serbia	Europe, Asia, and North Africa	18	KF921203
Serbia	Europe, Asia, and North Africa	18	KF921202
Serbia	Europe, Asia, and North Africa	18	KF921201
Serbia	Europe, Asia, and North Africa	18	KF921200
Serbia	Europe, Asia, and North Africa	18	KF921199
Serbia	Europe, Asia, and North Africa	18	KF921198
Serbia	Europe, Asia, and North Africa	18	KF921197
Serbia	Europe, Asia, and North Africa	18	KF921196
Serbia	Europe, Asia, and North Africa	18	KF921195
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Netherland	Europe, Asia, and North Africa	18	AM1794

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