

Species Diversity and Geographical Distribution of Whiteflies (Hemiptera: Aleyrodidae) in Zimbabwe

Munyaradzi Kennedy Mutimbu

Manicaland State University of Applied Sciences

Friday Nguvayasvika Mudondo Kubiku

fridaykubiku@gmail.com

Manicaland State University of Applied Sciences

Nyasha Sakadzo

Manicaland State University of Applied Sciences

Kyeong-Yeoll Lee

Kyungpook National University

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Abstract

Whiteflies (Hemiptera: Aleyrodidae) cause significant agricultural losses through direct phloem feeding and as vectors of plant viruses. This study presents the first multi-site molecular survey of whitefly diversity in Zimbabwe, using the mitochondrial cytochrome oxidase subunit I (mtCO1) gene to identify 14 individuals from seven host plant species across 10 localities during the 2018–2019 growing season. Phylogenetic analysis assigned five individuals to *Bemisia tabaci* SSA1-SG3 and nine to *Trialeurodes vaporariorum*. All *B. tabaci* specimens were identical to SSA1-SG3 sequences from Tanzania (0.00% divergence), while showing substantial divergence from SSANS, MED Q2, and MEAM1 biotypes. All *T. vaporariorum* specimens were genetically uniform, showing only 0.16% divergence from the closest reference sequence. Endosymbiont screening revealed *Arsenophonus* in 100% of individuals, *Cardinium* and *Rickettsia* each in 57.1%, *Wolbachia* in 7.1% (*B. tabaci* only), and *Hamiltonella* was absent. Begomovirus screening identified Tomato yellow leaf curl virus (TYLCV) in one *B. tabaci* from Kadoma and Squash leaf curl China virus (SLCCV) in three *T. vaporariorum* individuals from Marondera and Harare, yielding an overall infection rate of 28.6%. These findings establish the first molecular baseline for whitefly species composition, endosymbiont communities, and begomovirus associations in Zimbabwe, and constitute the first record of SLCCV in *T. vaporariorum* in southern Africa. The presence of economically important viruses across multiple host plants and locations has direct implications for phytosanitary risk assessment, surveillance, and integrated pest management.

1 Introduction

Whiteflies (Hemiptera: Aleyrodidae) represent one of the most economically significant groups of agricultural insect pests globally, inflicting damage through two principal pathways. Direct damage results from the mass extraction of phloem sap by adult and nymphal feeding stages, causing yellowing, wilting, and premature senescence, while the copious honeydew produced during feeding supports the growth of sooty mould fungi that reduce photosynthetic capacity and render produce unmarketable (Colvin et al. 2006; Prijović et al. 2013). Indirect damage, often far more severe, arises from the transmission of plant viruses – particularly begomoviruses (Geminiviridae) – which can devastate susceptible crops even when population densities remain relatively low (Anderson et al. 2004; Lapidot et al. 2014).

The whitefly species of greatest global concern are *Bemisia tabaci* (Gennadius) and *Trialeurodes vaporariorum* (Westwood), both members of the family Aleyrodidae. *Bemisia tabaci* is now understood to constitute a cryptic species complex of at least 34 molecularly distinct entities that are morphologically indistinguishable but differ substantially in their biology, host range, pesticide resistance profiles, and vector competence (Dinsdale et al. 2010; Boykin and De Barro 2014). Molecular identification using the mitochondrial cytochrome oxidase subunit I (mtCO1) gene has become the standard approach for resolving cryptic species within this complex, with a threshold of 3.5% pairwise sequence divergence used to delimit species boundaries (Dinsdale et al. 2010). In sub-Saharan Africa, the predominantly cassava-colonising lineages are classified into five major groups (SSA1–SSA5), of which SSA1 is further subdivided into five subgroups (SSA1-SG1 to SSA1-SG5) distinguished by host association, geography, and epidemiological impact (Legg et al. 2014).

Trialeurodes vaporariorum, the greenhouse whitefly, is a cosmopolitan species with particular economic importance in temperate and subtropical horticultural systems. It transmits Crinivirus-type pathogens such as Sweet potato chlorotic stunt virus (SPCSV), which in combination with Sweet potato feathery mottle virus can cause total yield loss in sweet potato (Ateka et al. 2004; Miano et al. 2008). Although genetic surveys of *T. vaporariorum* have been conducted in Europe, India, and parts of East Africa, comprehensive molecular data from Zimbabwe and the broader southern African region remain scarce (Kapantaidaki et al. 2015).

Beyond their direct roles as pests and vectors, whitefly fitness and population dynamics are substantially modulated by secondary endosymbiotic bacteria harboured within their body tissues. These include *Arsenophonus*, *Hamiltonella*, *Cardinium*, *Rickettsia*, and *Wolbachia*, all of which can influence host survival, reproduction, immune function, pesticide tolerance, and interactions with viruses and parasitoids (Zchori-Fein and Brown 2002; Ghanim and Kontsedalov 2009; Kontsedalov et al. 2012). The composition and prevalence of endosymbiont communities can therefore have practical implications for pest management, particularly for the efficacy of biological control agents and insecticides.

Zimbabwe is an agriculturally important southern African nation where smallholder and commercial farmers grow a diverse range of vegetable, field, and horticultural crops – including tomato, bean, cotton, sweet potato, and Brassica – that are known hosts for both *B. tabaci* and *T. vaporariorum*. Crop losses attributable to whitefly feeding and whitefly-vectored viruses have been reported, yet no comprehensive molecular survey of whitefly genetic diversity and begomovirus associations across the country has been conducted to date. Existing studies were limited to two sites and focused on Mediterranean-type *B. tabaci* from cassava, providing insufficient coverage to characterise the broader whitefly community (Berry et al. 2004). The present study therefore aimed to: (i) characterise the species composition and mtCO1-based genetic diversity of whitefly populations collected from multiple host plants across geographically distinct regions of Zimbabwe; (ii) determine the prevalence and host associations of secondary endosymbionts in the identified whitefly species; and (iii) identify the begomoviruses carried by these whitefly populations and assess the potential risk they pose to vegetable and field crops. The results are intended to provide a molecular baseline to support surveillance, invasion monitoring, and the development of integrated pest management strategies in Zimbabwe and the broader southern African region.

2. Materials and methods

2.1 Study Area and Sample Collection

The study was conducted across 10 localities spanning six provinces of Zimbabwe during the 2018–2019 crop growing season (Fig. 1; Table 1). Zimbabwe is a landlocked country located in southern Africa (approximately 15–23°S, 26–33°E), characterised by a tropical climate with a rainy season from November to March and a dry season from April to October. The sampling design covered the major horticultural and crop-production zones of the country, with sites concentrated in the central provinces of Mashonaland East, Mashonaland West, Mashonaland Central, Harare, Midlands, and Masvingo, which collectively account for the majority of commercial and smallholder vegetable production (Fig. 1).

Adult whiteflies were collected from seven host plant species: bean (*Phaseolus vulgaris*), cotton (*Gossypium hirsutum*), tomato (*Solanum lycopersicum*), sweet potato (*Ipomoea batatas*), silk melon (*Luffa acutangula*), spider plant (*Cleome gynandra*), and Brassica (*Brassica oleracea*). Specimens were aspirated individually, preserving sex where possible, and stored in 90% ethanol in labelled micro-centrifuge tubes on a per-plant-per-site basis. Samples were maintained at – 20°C until DNA extraction. A total of 14 individual adults were selected for molecular analysis, representing the range of host plant and location combinations (Table 1).

Table 1

Collection sites, host plants, dates, identified whitefly species, and GenBank accession numbers for the 14 individuals analysed in this study.

Sample	Location	Region	Host plant	Identified species	GenBank Acc.
Nya_zw1	Nyahoni	Mashonaland East	<i>Phaseolus vulgaris</i>	<i>B. tabaci</i> SSA1-SG3	MN312148
Hama_zw2	Hamamavhaire	Masvingo	<i>Phaseolus vulgaris</i>	<i>B. tabaci</i> SSA1-SG3	MN312149
Mush_zw3	Mushandike	Masvingo	<i>Phaseolus vulgaris</i>	<i>B. tabaci</i> SSA1-SG3	MN312150
Chin_zw4	Chinamhora	Mashonaland East	<i>Ipomoea batatas</i>	<i>B. tabaci</i> SSA1-SG3	MN312151
Kado_zw9	Kadoma	Midlands	<i>Gossypium hirsutum</i>	<i>B. tabaci</i> SSA1-SG3	MN312152
Maro_zw5	Marondera	Mashonaland East	<i>Solanum lycopersicum</i>	<i>T. vaporariorum</i>	MN312153
Nyab_zw6a	Nyabira	Mashonaland West	<i>Solanum lycopersicum</i>	<i>T. vaporariorum</i>	MN312154
Nyab_zw6b	Nyabira	Mashonaland West	<i>Gossypium hirsutum</i>	<i>T. vaporariorum</i>	MN312155
Mazo_zw7	Mazowe	Mashonaland Central	<i>Solanum lycopersicum</i>	<i>T. vaporariorum</i>	MN312156
Marl_zw8	Marlborough	Harare	<i>Solanum lycopersicum</i>	<i>T. vaporariorum</i>	MN312157
Nyab_zw6c	Nyabira	Mashonaland West	<i>Luffa acutangula</i>	<i>T. vaporariorum</i>	MN312158
Maro2_zw10	Marondera	Mashonaland East	<i>Cleome gynandra</i>	<i>T. vaporariorum</i>	MN312159
Hre1_zw11	Harare	Harare	<i>Solanum lycopersicum</i>	<i>T. vaporariorum</i>	MN312160
Hre2_zw12	Harare	Harare	<i>Brassica oleracea</i>	<i>T. vaporariorum</i>	MN312161

2.2 DNA Extraction

Genomic DNA was extracted from individual whole adult whiteflies using the PureLink Genomic DNA Mini Kit (Invitrogen, Carlsbad, CA, USA). Each specimen was homogenised in a 1.5 mL microcentrifuge tube containing 180 µL genomic digestion buffer and 20 µL Proteinase K (50 µg/mL) using a disposable micro-pestle. Digestion was carried out at 55°C for 30 min, followed by standard spin-column purification as per the manufacturer's protocol. DNA concentration and purity were assessed using a NanoPhotometer™ (Implen, Munich, Germany), and samples were stored at – 20°C until use.

2.3 PCR Amplification

Four distinct PCR assays were performed for each individual: (i) amplification of the mtCO1 barcode region for species identification; (ii–vi) detection of five secondary endosymbionts; and (vii) detection of begomovirus DNA. All primer sequences, annealing temperatures, and expected amplicon sizes are listed in Table 2, and PCR thermal cycling conditions for each target are summarised in Table 3.

The mtCO1 fragment (658 bp) was amplified using the universal primers LCO1490 and HCO2198 (Folmer et al. 1994). Secondary endosymbionts were screened using gene-specific primers targeting 16S or 23S rDNA sequences of *Arsenophonus* (Thao and Baumann 2004), *Cardinium* (Weeks and Breeuwer 2003), *Hamiltonella* (Zchori-Fein and Brown 2002), 2002), *Rickettsia* (Gottlieb et al. 2006), and *Wolbachia* (Gottlieb et al. 2010). Begomovirus detection used degenerate primers (BegomoF1/BegomoR1) targeting a 1100 bp fragment of the DNA-A component (Sattar 2012). All reactions were performed in a total volume of 30 µL containing 15 µL Smart-Taq Pre-Mix (SolGent Co., Daejeon, Korea), 2 µL each of forward and reverse primer (10 pmol/µL), 4 µL template DNA (~ 40 ng), and 8 µL nuclease-free water. PCR products were resolved by electrophoresis on 1% agarose gels stained with ethidium bromide and visualised under UV illumination.

Table 2

Primer sequences used for PCR amplification of the whitefly mtCO1 barcode, secondary endosymbionts, and begomovirus DNA-A.

Primer	Sequence (5'-3')	Anneal °C / Size (bp)	Target gene	Reference
LC01490 / HCO2198	GGTCAACAAATCATAAAGATATTGG / TAAACTTCAGGGTGACCAAAAAATCA	52 / 658	mtCO1	(Folmer et al. 1994)
Ars23S1 / Ars23S2	CGTTTGATGAATTCATAGTCAAA / GGTCCTCCAGTTAGTGTTACCCAAC	60 / 600	Arsenophonus 23S rDNA	(Thao and Baumann 2004)
CFB-F / CFB-R	GCGGTGTAAAATGAGCGTG / ACCTMTTCTTAACTCAAGCCT	58 / 400	Cardinium 16S rDNA	(Weeks and Breeuwer 2003)
HbF / HbR	TGAGTAAAGTCTGGAATCTG / AGTTCAAGACCGCAACCTC	58 / 700	Hamiltonella 16S rDNA	(Zchori-Fein and Brown 2002)
Rickettsia F / R	GCTCAFAACGAACGCTATC / GAAGGAAAGCATCTCTGC	60 / 900	Rickettsia 16S rDNA	(Gottlieb et al. 2006)
Wolbachia F / R	CGGGGGAAAAATTTATTGCT / AGCTGTAATACAGAAAGTAAA	55 / 650	Wolbachia 16S rDNA	(Gottlieb et al. 2006)
BegomoF1 / BegomoR1	CGTGCTGCTGCCCCCATTGTCCGCGTCAC / CTGCCACAACCATGGATTCACGCACAGGG	65 / 1100	Begomovirus DNA A	(Bull et al. 2003)

Table 3

PCR thermal cycling conditions for whitefly species identification, secondary endosymbiont detection, and begomovirus screening.

Organism	Gene	Denaturation	Annealing °C	Extension	Cycles
<i>Whitefly</i> (<i>mtCO1</i>)	mtCO1	94°C 1 min	51°C 1 min	72°C 1 min	35
<i>Arsenophonus</i>	23S rDNA	95°C 30 sec	60°C 30 sec	72°C 45 sec	35
<i>Cardinium</i>	16S rDNA	94°C 1 min	58°C 1 min	72°C 1 min	35
<i>Hamiltonella</i>	16S rDNA	94°C 1 min	58°C 1 min	72°C 1 min	35
<i>Rickettsia</i>	16S rDNA	92°C 30 sec	60°C 30 sec	72°C 30 sec	35
<i>Wolbachia</i>	16S rDNA	94°C 30 sec	55°C 30 sec	72°C 1 min	35
<i>Begomovirus</i>	DNA A	95°C 2 min initial; 94°C 40 sec	65°C 1 min	72°C 30 sec; 72°C 7 min final	35

2.4 DNA Sequencing and BLAST Analysis

PCR products yielding clear single bands were either directly submitted for sequencing or first cloned into the T-Blunt™ Easy Plasmid Vector (Promega Co., Madison, WI, USA) to improve sequence quality. Sequencing was performed by SolGent Co. (Daejeon, Korea) using BigDye® Terminator Cycle Sequencing chemistry (Applied Biosystems, Foster City, CA, USA) and analysed on an ABI 3100 Capillary DNA Sequencer. Resulting sequences were subjected to BLAST searches against the NCBI GenBank nucleotide database (Schäffer et al. 2001) to identify the closest reference sequences. Confirmed mtCO1 sequences from the 14 Zimbabwe specimens were deposited in GenBank under accession numbers MN312148–MN312161 (COI) and MN305810–MN305823 (endosymbiont sequences).

2.5 Sequence Alignment and Phylogenetic Analysis

Nucleotide sequences were trimmed and aligned in Clustal Omega (Larkin et al. 2007) to produce 658 bp mtCO1 alignments. Reference sequences retrieved from GenBank were aligned together with the Zimbabwe specimens. Phylogenetic trees were reconstructed for each dataset (mtCO1, four endosymbiont loci, and begomovirus DNA-A) using the Maximum Likelihood method implemented in MEGA 11 (Tamura et al., 2021), based on the Tamura-3 parameter model with gamma-distributed rate variation among sites. Nodal support was assessed with 1000 bootstrap replicates (Felsenstein 1985). For *B. tabaci* mtCO1 analysis, *Bemisia afer* (NC024056) was used as the out-group. For *T. vaporariorum*, *Trialeurodes ricini* (AM179447) was used as the out-group. Species delimitation followed the > 3.5% pairwise divergence rule (Dinsdale et al., 2010). Pairwise genetic distances were calculated under the Kimura 2-parameter (K2P) model.

3 Results

3.1 Whitefly Species Identification and Geographical Distribution

Molecular analysis of 14 adult whiteflies collected from 10 localities and seven host plant species identified two distinct whitefly species: *Bemisia tabaci* SSA1-SG3 (n = 5) and *Trialeurodes vaporariorum* (n = 9) (Table 4). The geographical distribution of both species, as shown in Fig. 1, was largely restricted to the central corridor of Zimbabwe, reflecting the distribution of intensive crop production in the country. All 14 COI sequences were deposited in GenBank (MN312148–MN312161).

Table 4

The PCR detection on S-endosymbionts and begomovirus in whitefly species identified in Zimbabwe.

Genetic group	Location	Host	A	C	H	R	W	Begomovirus
<i>B. tabaci</i> SSA1-SG3	Nyahoni 1	<i>Phaseolus vulgaris</i>	+	+	-	+	-	
<i>B. tabaci</i> SSA1-SG3	Hamamavhaire2	<i>Phaseolus vulgaris</i>	+	+	-	-	+	
<i>B. tabaci</i> SSA1-SG3	Mushandike	<i>Phaseolus vulgaris</i>	+	-	-	-	-	
<i>B. tabaci</i> SSA1-SG3	Chinamhora	<i>Ipomoea batatas</i>	+	+	-	-	-	
<i>B. tabaci</i> SSA1-SG3	Kadoma	<i>Gossypium</i>	+	-	-	+	-	TYLCV
<i>T. vaporariorum</i>	Marondera	<i>Solanum lycopersicum</i>	+	-	-	+	-	
<i>T. vaporariorum</i>	Nyabira	<i>Solanum lycopersicum</i>	+	+	-	+	-	
<i>T. vaporariorum</i>	Nyabira	<i>Gossypium</i>	+	+	-	+	-	
<i>T. vaporariorum</i>	Mazowe	<i>Solanum lycopersicum</i>	+	-	-	+	-	
<i>T. vaporariorum</i>	Marlborough	<i>Solanum lycopersicum</i>	+	+	-	-	-	
<i>T. vaporariorum</i>	Nyabira	<i>Luffa acutangula</i>	+	+	-	-	-	
<i>T. vaporariorum</i>	Marondera	<i>Cleome gynandra</i>	+	-	-	+	-	SLCCV
<i>T. vaporariorum</i>	Harare	<i>Solanum lycopersicum</i>	+	+	-	-	-	SLCCV
<i>T. vaporariorum</i>	Harare	<i>Brassica</i>	+	-	-	+	-	SLCCV

(A: *Arsenophonus*, C: *Cardinium*, H: *Hamiltonella*, R: *Rickettsia*, W: *Wolbachia*)

3.1.1 *Bemisia tabaci*

Five *B. tabaci* individuals were collected from three host plants — bean (*Phaseolus vulgaris*) at Nyahoni and Hamamavhaire, sweet potato (*Ipomoea batatas*) at Chinamhora, and cotton (*Gossypium hirsutum*) at Kadoma (Table 4). Phylogenetic analysis of mtCO1 sequences placed all five Zimbabwe specimens in a single, well-supported clade (bootstrap value 99–100%) within the SSA1-SG3 sub-group (Fig. 2). The five Zimbabwe specimens were genetically identical to each other (0.00% pairwise divergence) and indistinguishable from *B. tabaci* SSA1-SG3 from Tanzania (LR535717; Table 5). Divergence values relative to other cryptic species were 9.4% compared to SSANS specimens from Spain (GQ867713 and KJ190492), 17.1% compared to MED Q2 from Israel (MH205753), and 15.5% compared to MEAM1 from Australia (KY951449) — all well above the 3.5% species delimitation threshold, confirming SSA1-SG3 as the sole *B. tabaci* cryptic species present in the surveyed localities (Table 4; Fig. 2).

Table 5

Pairwise mtCO1 sequence divergences (%) between *Bemisia tabaci* SSA1-SG3 specimens from Zimbabwe and selected reference sequences. Values < 3.5% indicate conspecificity; values in bold exceed the species delimitation threshold.

Sample	Genotype	Acc. No.	1–5 (ZW)	SSA1- SG3 Tz	SSANS Spain (ws9)	SSANS Spain (SPN1)	MED Q2 Israel	MEAM1 Aus.
Nya_zw1– Kado_zw9 (n = 5)	<i>B. tabaci</i> SSA1-SG3 (Zimbabwe)	MN312148– 52	0.00	0.00	9.40	9.40	17.10	15.50
SSA1-SG3 Tz	<i>B. tabaci</i> SSA1-SG3 (Tanzania)	LR535717	0.00	—	9.40	9.40	17.10	15.50
SSANS ws9	<i>B. tabaci</i> SSANS (Spain)	GQ867713	9.40	9.40	—	0.00	16.00	16.20
SSANS SPN1	<i>B. tabaci</i> SSANS (Spain)	KJ190492	9.40	9.40	0.00	—	16.00	16.20
MED Q2	<i>B. tabaci</i> MED Q2 (Israel)	MH205753	17.10	17.10	16.00	16.00	—	8.90
MEAM1	<i>B. tabaci</i> MEAM1 (Australia)	KY951449	15.50	15.50	16.20	16.20	8.90	—

3.1.2 *Trialeurodes vaporariorum*

Nine *T. vaporariorum* individuals were collected primarily from tomato (*Solanum lycopersicum*) at four sites (Marondera, Nyabira, Mazowe, Marlborough, Harare), from cotton at Nyabira, from silk melon (*Luffa*

acutangula) at Nyabira, from *Cleome gynandra* at Marondera, and from *Brassica* at Harare (Table 4). All nine Zimbabwe specimens were genetically identical to each other (0.00% inter-individual divergence) and clustered in a single clade with 99% bootstrap support alongside Indian *T. vaporariorum* sequences (HM153751, HM185763, JQ339322), with only 0.16% divergence from the closest Indian reference sequence (MK490851) (Table 6; Fig. 3). The Reunion island isolate (AJ550183) formed a clearly distinct outgroup lineage. The uniformity of the Zimbabwe *T. vaporariorum* population across geographically dispersed sites and diverse host plants suggests a single introduction event or a highly homogenous founder population.

Table 6

Pairwise mtCO1 sequence divergences (%) between *Trialeurodes vaporariorum* specimens from Zimbabwe and selected reference sequences. All Zimbabwe specimens are identical inter-se; values > 3.5% would indicate species-level separation.

Sample	Genotype	Acc. No.	ZW 1–9 (inter-se)	vs India (HM153751–JQ339322)	vs India MK490851
MN312153–MN312161 (n = 9)	<i>T. vaporariorum</i> (Zimbabwe)	9 sequences	0.00	0.00	0.16
HM153751	<i>T. vaporariorum</i> (India)	HM153751	—	0.00	0.16
HM185763	<i>T. vaporariorum</i> (India)	HM185763	—	0.00	0.16
JQ339322	<i>T. vaporariorum</i> (India)	JQ339322	—	0.00	0.16
MK490851	<i>T. vaporariorum</i> (India)	MK490851	0.16	0.16	—

3.2 Secondary Endosymbiont Detection and Phylogeny

Four of the five targeted secondary endosymbionts were detected across the 14 whitefly specimens. Hamiltonella was not detected in any individual. Infection rates varied among endosymbionts and between host species (Table 7; Fig. 4).

Arsenophonus was the most prevalent endosymbiont, detected in all 14 individuals regardless of whitefly species or collection site (100%; Table 7; Fig. 4). Phylogenetic analysis of Arsenophonus 23S rDNA sequences revealed two distinct clades: clade A1, containing sequences from both *B. tabaci* SSA1-SG3 and *T. vaporariorum* specimens from Zimbabwe alongside *B. tabaci* Asia II 7 from China; and clade A4, containing additional *T. vaporariorum* sequences from Zimbabwe clustered with diverse *B. tabaci* biotypes (MED from Burkina Faso, Asia I from India, Asia II 1 from China and Nepal, and SSA2 from Uganda) (Fig. 5). The co-clustering of *B. tabaci* and *T. vaporariorum* sequences within both Arsenophonus clades indicates that this endosymbiont is distributed horizontally across whitefly genera.

Cardinium was detected in 57.1% of all individuals (8/14; Table 7; Fig. 4): 60% of *B. tabaci* SSA1-SG3 (3/5) and 55.5% of *T. vaporariorum* (5/9). The Cardinium sequences from Zimbabwe – spanning both whitefly species – formed a single clade (C2) in the phylogenetic tree (Fig. 6), clustering alongside *B. tabaci* Asia II sequences from India, suggesting a shared strain lineage with Asian whitefly populations. Notably, *B. tabaci* SSA1-SG3 and *T. vaporariorum* sequences were interleaved within this clade, consistent with horizontal transmission of this Cardinium strain across whitefly genera.

Rickettsia was detected in 57.1% of all individuals (8/14): 40% of *B. tabaci* SSA1-SG3 (2/5) and 66.6% of *T. vaporariorum* (6/9). All Rickettsia sequences from Zimbabwe formed a single clade (R1) in the phylogenetic tree (Fig. 7), clustering with *B. tabaci* Asia II sequences from China and Nepal, again consistent with the presence of a single introduced Rickettsia strain that infects both whitefly species in Zimbabwe.

Wolbachia was detected at low prevalence: only 7.1% of all individuals (1/14), specifically one *B. tabaci* SSA1-SG3 specimen from Hamamavhaire. No *T. vaporariorum* individual tested positive for Wolbachia. The single Zimbabwe Wolbachia sequence fell within clade W2 (Fig. 8), clustering with Wolbachia from diverse *B. tabaci* biotypes including MEAM1 (Australia), Asia I (Bangladesh), and Asia II (India and Indonesia). Hamiltonella was entirely absent from all 14 specimens, consistent with the known competitive exclusion between Hamiltonella and Arsenophonus in whitefly populations.

Table 7

Infection rates of secondary endosymbionts in *Bemisia tabaci* SSA1-SG3 and *Trialeurodes vaporariorum* collected from Zimbabwe. Values in parentheses indicate the number of positive individuals.

Secondary endosymbiont	<i>B. tabaci</i> SSA1-SG3 (n = 5)	<i>T. vaporariorum</i> (n = 9)	Overall (n = 14)
Arsenophonus	100% (5/5)	100% (9/9)	100% (14/14)
Cardinium	60% (3/5)	55.5% (5/9)	57.1% (8/14)
Hamiltonella	Not detected	Not detected	Not detected
Rickettsia	40% (2/5)	66.6% (6/9)	57.1% (8/14)
Wolbachia	20% (1/5)	Not detected	7.1% (1/14)

3.3 Begomovirus Detection and Phylogeny

Begomoviruses were detected in 4 of the 14 whitefly individuals screened (overall infection rate: 28.6%; Table 8). Two distinct begomovirus species were identified by BLAST analysis and confirmed by phylogenetic reconstruction (Fig. 9; Fig. 10).

Tomato yellow leaf curl virus (TYLCV) was detected in a single *B. tabaci* SSA1-SG3 individual collected from cotton (*Gossypium hirsutum*) at Kadoma (Kado_zw9), representing 20% of the *B. tabaci* sample (1/5; Fig. 10). The TYLCV sequence from Zimbabwe clustered with 95% bootstrap support alongside *B. tabaci* MED sequences from South Korea and MEAM1 from Japan (Fig. 8), consistent with the predominant global TYLCV haplotype associated with MEAM1 and MED biotypes. The detection of TYLCV in an SSA1-

SG3 individual — a cassava-lineage biotype not previously well-characterised as a TYLCV vector — is a notable epidemiological finding requiring follow-up vector competence studies.

Squash leaf curl China virus (SLCCV) was detected in three *T. vaporariorum* individuals (33.3% of the *T. vaporariorum* sample; Fig. 10): one from Cleome gynandra at Marondera (Maro2_zw10), one from tomato at Harare (Hre1_zw11), and one from Brassica at Harare (Hre2_zw12). The SLCCV sequences from Zimbabwe formed a single clade with 100% bootstrap support alongside *T. vaporariorum*-associated SLCCV sequences from China, and were also related to *T. vaporariorum* sequences from India (Fig. 9). The detection of SLCCV in *T. vaporariorum* on three taxonomically diverse host plants across two geographically separated sites demonstrates that this virus is present across multiple hosts and locations in Zimbabwe.

Table 8

Begomovirus infection rates by whitefly species and overall across the Zimbabwe sample collection.

Virus	<i>B. tabaci</i> SSA1-SG3 (n = 5)	<i>T. vaporariorum</i> (n = 9)	Overall infection rate (n = 14)
Tomato yellow leaf curl virus (TYLCV)	20% (1/5)	0%	7.1% (1/14)
Squash leaf curl China virus (SLCCV)	0%	33.3% (3/9)	21.4% (3/14)
Overall begomovirus	20% (1/5)	33.3% (3/9)	28.6% (4/14)

4. Discussion

4.1 Species Composition and Whitefly Diversity in Zimbabwe

This study represents the first comprehensive multi-site molecular survey of whitefly species diversity in Zimbabwe, identifying *B. tabaci* SSA1-SG3 and *T. vaporariorum* as the two species present across the surveyed cropping systems. The exclusive detection of SSA1-SG3 — to the exclusion of MEAM1, MED, and other invasive biotypes documented in neighbouring countries — is a significant biogeographic finding. The SSA1 clade is considered indigenous to sub-Saharan Africa and was first characterised as the dominant cassava-colonising lineage in East and Central Africa (Legg et al. 2002; Berry et al. 2004). Our phylogenetic tree places all five Zimbabwe *B. tabaci* specimens within a single well-supported clade (bootstrap 99–100%) that is identical to SSA1-SG3 from Tanzania, reflecting the established regional distribution of this subgroup across East and southern Africa.

The ability of SSA1-SG3 to colonise diverse host plants beyond cassava — including bean, sweet potato, and cotton in the present study — has important implications for crop protection. This polyphagous behaviour has previously been documented in Uganda (Sseruwagi et al. 2006) and is consistent with the broad host range attributed to SSA1 lineages in coastal East Africa. The absence of MEAM1 and MED

biotypes, which dominate in North Africa and parts of southern Africa and are associated with more aggressive pesticide resistance and wider vector competence, is reassuring from a management perspective, but the situation warrants continuous surveillance given the documented northward and southward expansion of these invasive biotypes in recent decades (De Barro et al. 2011).

The *T. vaporariorum* population in Zimbabwe showed remarkable genetic homogeneity: all nine specimens were identical inter-se (0.00% divergence) and showed only 0.16% divergence from Indian reference sequences – well below the 3.5% species delimitation threshold. This extreme uniformity, observed across geographically dispersed sites and taxonomically diverse host plants, is consistent with a recent single-introduction event followed by rapid range expansion. The clustering of Zimbabwe *T. vaporariorum* with Indian sequences, at only 0.16% divergence, is consistent with trade-linked introduction through imported planting material – a pathway well-documented for *T. vaporariorum* dispersal globally (Gao et al. 2014). The previous detection of *T. vaporariorum* in only two Zimbabwean sites – where Mediterranean-type *B. tabaci* from cassava was also found – underscores the incomplete prior characterisation of the whitefly fauna and reinforces the importance of systematic national surveys.

4.2 Secondary Endosymbiont Prevalence and Implications

The universal detection of *Arsenophonus* across all 14 individuals (100%) makes it the most prevalent endosymbiont recorded in Zimbabwean whiteflies and is consistent with the high *Arsenophonus* rates reported in *B. tabaci* populations globally. The phylogenetic placement of Zimbabwe *Arsenophonus* sequences in two distinct clades (A1 and A4), with both *B. tabaci* and *T. vaporariorum* sequences co-clustering within each clade, provides phylogenetic evidence for horizontal transfer of this endosymbiont across whitefly genera. *Arsenophonus* has been shown to manipulate host reproduction and confer resistance to certain parasitoids, and its near-universal distribution in Zimbabwe's whitefly populations indicates that it is an established component of the endosymbiont community across both whitefly species in the surveyed localities (Thao and Baumann 2004; Moran et al. 2005).

The absence of *Hamiltonella* in all specimens is consistent with the known competitive exclusion between *Hamiltonella* and *Arsenophonus* – these two endosymbionts rarely co-occur within the same individual or population, and in communities where *Arsenophonus* is fixed at or near 100%, *Hamiltonella* is typically absent (Thao and Baumann 2004). This pattern strengthens confidence in the detection methodology.

Cardinium and *Rickettsia* showed intermediate and similar overall prevalence (both at 57.1%), but with different distributions across host whitefly species. *Cardinium* was slightly more prevalent in *B. tabaci* SSA1-SG3 (60%) than *T. vaporariorum* (55.5%), while *Rickettsia* showed the reverse pattern – significantly more prevalent in *T. vaporariorum* (66.6%) than *B. tabaci* (40%). *Cardinium* is known to induce cytoplasmic incompatibility and sex ratio distortion in arthropods (Hunter et al. 2003; Weeks and Breeuwer 2003), and the co-clustering of Zimbabwe *Cardinium* sequences from both whitefly species within clade C2 alongside *B. tabaci* Asia II sequences from India suggests the presence of a single shared *Cardinium* strain capable of infecting both whitefly genera. *Rickettsia*, similarly, placed all Zimbabwe sequences in clade R1, a lineage shared with Asian *B. tabaci* biotypes. The high *Rickettsia* prevalence in *T. vaporariorum* is noteworthy because *Rickettsia* has been shown to affect vector competence for certain plant pathogens

and to enhance fitness under specific environmental conditions (Gottlieb et al. 2006; Kontsedalov et al. 2012).

Wolbachia was detected in only one individual – a *B. tabaci* SSA1-SG3 specimen from Hamamavhaire (7.1%) – and was entirely absent from *T. vaporariorum*. While Wolbachia infections in *B. tabaci* have been reported globally, they are typically at low prevalence in African populations and absent from *T. vaporariorum*, consistent with our findings. The single Zimbabwe Wolbachia sequence clustered in clade W2 alongside diverse global *B. tabaci* biotypes (Fig. 7), consistent with horizontal acquisition from diverse global *B. tabaci* biotypes.

4.3 Begomovirus Detection and Epidemiological Significance

The detection of begomoviruses in 28.6% of the screened whitefly individuals (4/14) highlights the potential role of Zimbabwe's whitefly populations as active vectors of economically damaging plant viruses. Two distinct viruses were identified, each associated with a different whitefly species and occurring in different parts of the country, suggesting independent epidemiological cycles.

The detection of Tomato yellow leaf curl virus (TYLCV) in a *B. tabaci* SSA1-SG3 individual from a cotton field in Kadoma is notable for two reasons. First, TYLCV is primarily associated with the MEAM1 and MED biotypes globally (De Barro et al. 2011; Farres et al. 2012), and its detection in an SSA1-SG3 individual – a biotype not previously characterised as a primary TYLCV vector – documents a previously unrecorded vector-virus association in Zimbabwe that warrants vector competence investigation. Second, detection of TYLCV in a cotton field is epidemiologically significant: TYLCV has been reported on a range of hosts beyond tomato, and the detection in cotton-associated SSA1-SG3 indicates that this crop and its associated whitefly populations constitute a potential source of inoculum within the agricultural landscape. The TYLCV sequence from Zimbabwe clustered with MED and MEAM1-associated strains from South Korea and Japan (Fig. 8), consistent with a globally dispersed TYLCV haplotype; vector competence studies with SSA1-SG3 are required to determine the transmission capacity of this biotype.

Squash leaf curl China virus (SLCCV) was detected in three *T. vaporariorum* specimens across two geographical sites (Marondera and Harare) and three distinct host plants – *Cleome gynandra*, tomato, and Brassica. The detection of SLCCV in *T. vaporariorum* in Zimbabwe constitutes a new record for this host-vector-virus combination in southern Africa. SLCCV was originally described from cucurbit crops in China and was subsequently reported in association with *T. vaporariorum* in Asia. Its detection on three taxonomically diverse hosts – *Brassica*, *Cleome gynandra*, and tomato – across two geographically separated sites confirms a broad host range within Zimbabwean *T. vaporariorum* populations. The clustering of Zimbabwe SLCCV sequences with *T. vaporariorum*-associated sequences from China, with 100% bootstrap support, establishes that the Zimbabwean SLCCV lineage shares a common origin with East Asian strains. Given the wide cultivation of Brassica vegetables by smallholder farmers throughout Zimbabwe, SLCCV is present on a widely grown crop host and its epidemiological impact warrants urgent investigation.

4.4 Geographical Distribution Patterns

The spatial distribution of whitefly species across Zimbabwe's collection sites showed a clear pattern: *B. tabaci* SSA1-SG3 was collected from sites in the central and southern provinces, predominantly on leguminous (bean) and cash crops (cotton, sweet potato), while *T. vaporariorum* was predominantly collected from vegetable crops (tomato, *Brassica*) in the northern and peri-urban areas around Harare, Mazowe, and Nyabira. This distributional divergence reflects the different ecological optima and crop associations of the two species. *B. tabaci* SSA1-SG3 has a well-documented association with tropical and sub-tropical field crops and thrives in warmer lowland conditions, while *T. vaporariorum* is more commonly associated with temperate vegetable production and greenhouse crops, consistent with its predominance around the cooler highland areas of Harare and Mashonaland East (Kapantaidaki et al. 2015).

The restriction of all sampling sites to the central and northern provinces reflects both the distribution of Zimbabwe's major crop production zones and the constraints of the sampling design rather than the absolute southern limit of whitefly distribution. The southern and western regions of Zimbabwe – characterised by lower rainfall and more extensive ranching than horticulture – were not represented in this sample, and future surveys should extend coverage to these areas to determine whether both species occur on suitable host plants during the rainy season.

5. Conclusion

This study provides the first molecular characterisation of whitefly species diversity, endosymbiont communities, and begomovirus associations across multiple sites and host plants in Zimbabwe. Two whitefly species were identified from the 14 specimens collected: *Bemisia tabaci* SSA1-SG3 (n = 5) and *Trialeurodes vaporariorum* (n = 9). The exclusive presence of SSA1-SG3 within the *B. tabaci* species complex, to the exclusion of the invasive MEAM1 and MED biotypes, reflects the indigenous southern African composition of the *B. tabaci* fauna at the surveyed sites. All five *B. tabaci* SSA1-SG3 specimens were genetically identical and showed 0.00% divergence from Tanzanian SSA1-SG3, confirming the East African lineage of this population. Pairwise divergences of 9.4–17.1% from other biotypes (SSANS, MED Q2, MEAM1) confirmed that all specimens belong to a single indigenous cryptic species. All nine *T. vaporariorum* specimens were genetically identical (0.00% inter-individual divergence) and showed only 0.16% divergence from Indian reference sequences, consistent with a genetically uniform population sharing recent common ancestry with Indian lineages. *Arsenophonus* was universally present in both whitefly species (100%), with phylogenetic evidence of cross-species horizontal transmission. *Cardinium* and *Rickettsia* were each detected in 57.1% of specimens; *Wolbachia* in 7.1% (*B. tabaci* only); *Hamiltonella* was absent from all individuals, consistent with *Arsenophonus*-mediated competitive exclusion. Two begomoviruses were detected at an overall rate of 28.6%: TYLCV in one *B. tabaci* SSA1-SG3 individual from a cotton field in Kadoma, and SLCCV in three *T. vaporariorum* individuals from Marondera and Harare, representing the first record of SLCCV in *T. vaporariorum* in southern Africa. Collectively, these results establish a molecular baseline for whitefly-begomovirus interactions in Zimbabwe and demonstrate that both whitefly species present in the country already carry economically important plant viruses across

multiple host plants and locations. The findings have direct implications for phytosanitary risk assessment, the design of monitoring programmes, and the development of integrated pest management strategies targeting both whitefly vectors and the viruses they transmit.

6. Recommendations

Based on the key findings of this study, the following critical recommendations are made:

1. Establish a systematic national whitefly monitoring programme covering all five agro-ecological regions of Zimbabwe, including the southern and western provinces not represented in the present study. Standardised seasonal sampling at sentinel sites in major vegetable production zones should be conducted at least twice per year to detect biotype range shifts and new introductions.
2. Enhance border phytosanitary surveillance of imported planting material (tomato, sweet pepper, cucumber, squash seedlings). The near-identical genetic relationship of Zimbabwean *T. vaporariorum* to Indian sequences and the detection of SLCCV sequences of East Asian affinity demonstrate that imported propagating material is a documented introduction pathway for exotic whitefly lineages and begomovirus strains; PCR-based screening should be integrated into existing border inspection protocols.
3. Implement targeted virus management for TYLCV and SLCCV, including removal and destruction of infected plant material, isolation of tomato nurseries from cotton and other confirmed virus-harbouring hosts, and certification of virus-free transplant production. Farmers should be informed that *B. tabaci* SSA1-SG3 on cotton and bean, and *T. vaporariorum* on *Brassica* and *Cleome gynandra*, are confirmed carriers of these viruses and constitute direct inoculum sources for susceptible vegetable crops.

7. Directions for Future Research

The small sample size ($n = 14$) and geographic scope of this study identify three critical priorities directly arising from the key findings:

- Conduct nationwide surveys with substantially larger sample sizes (minimum $n = 200$ per season, stratified by province and crop type) to obtain statistically robust estimates of species frequencies, biotype composition, endosymbiont prevalence, and begomovirus infection rates across Zimbabwe's five agro-ecological regions. The current dataset ($n = 14$ from 10 localities) establishes a molecular baseline but is insufficient for population-level inference.
- Determine the vector competence of Zimbabwean *B. tabaci* SSA1-SG3 for TYLCV through controlled inoculation trials. Detection of TYLCV in an SSA1-SG3 individual from cotton in Kadoma documents a previously unrecorded vector-virus association; confirming or refuting transmission capacity of this biotype has direct implications for TYLCV disease management across southern Africa's tomato production systems.
- Investigate the vector competence and host range of *T. vaporariorum* for SLCCV, with particular focus on *Brassica*, tomato, and other vegetable crops. Detection of SLCCV in *T. vaporariorum* on three host

plants across two sites constitutes the first record of this virus in southern Africa, establishing an urgent need to determine the range of crops at risk and the transmission efficiency of *T. vaporariorum* under Zimbabwean field conditions.

Declarations

Conflicts of Interest

The authors declare no conflicts of interest.

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Author Contribution

All authors reviewed the manuscript

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Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request

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Figures

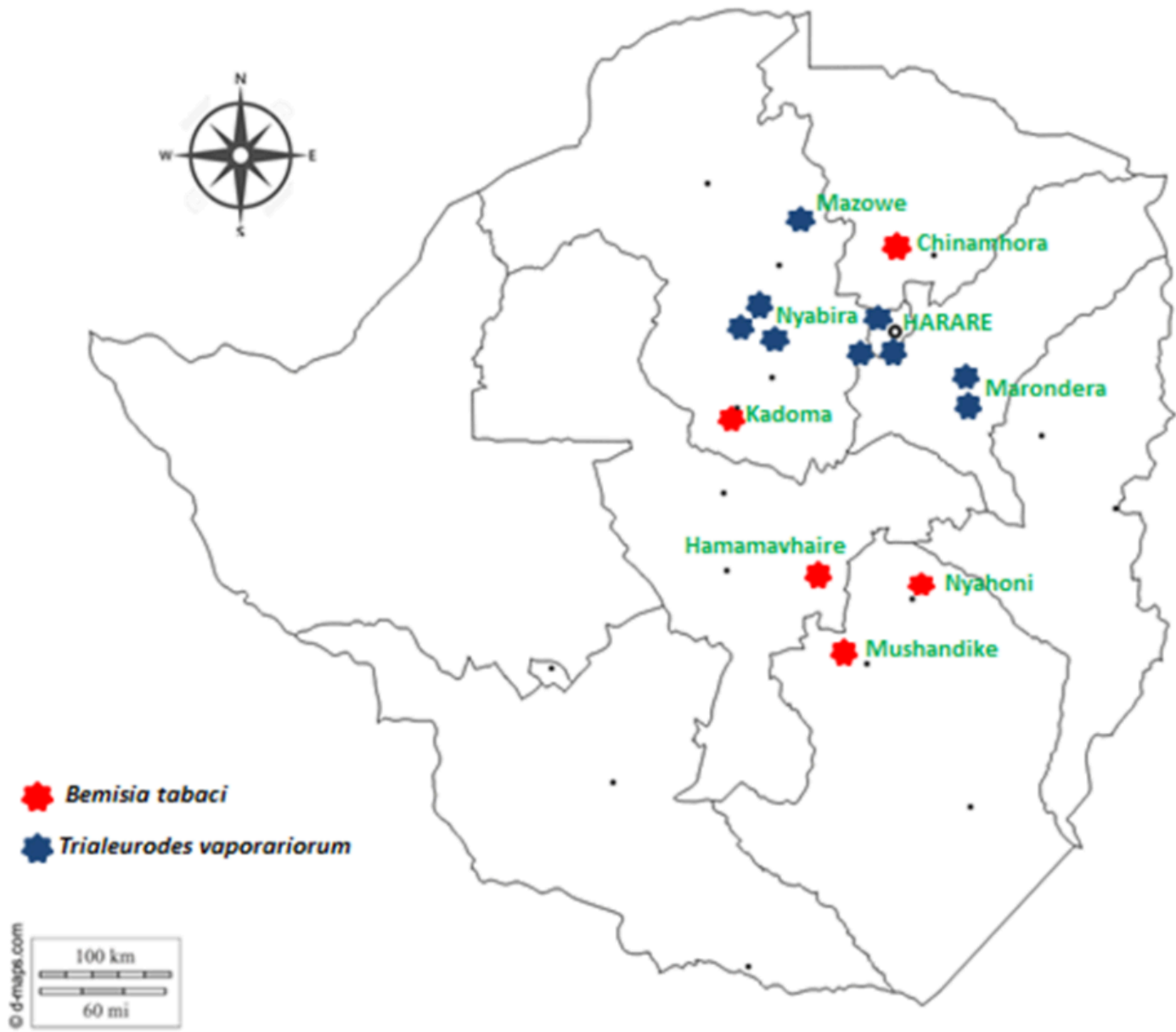


Figure 1

Map of Zimbabwe showing whitefly collection sites. Red markers indicate *Bemisia tabaci* SSA1-SG3 collection points; blue markers indicate *Trialeurodes vaporariorum* collection points. Samples were concentrated in the central agricultural provinces. Site names correspond to sample codes in Table 1.

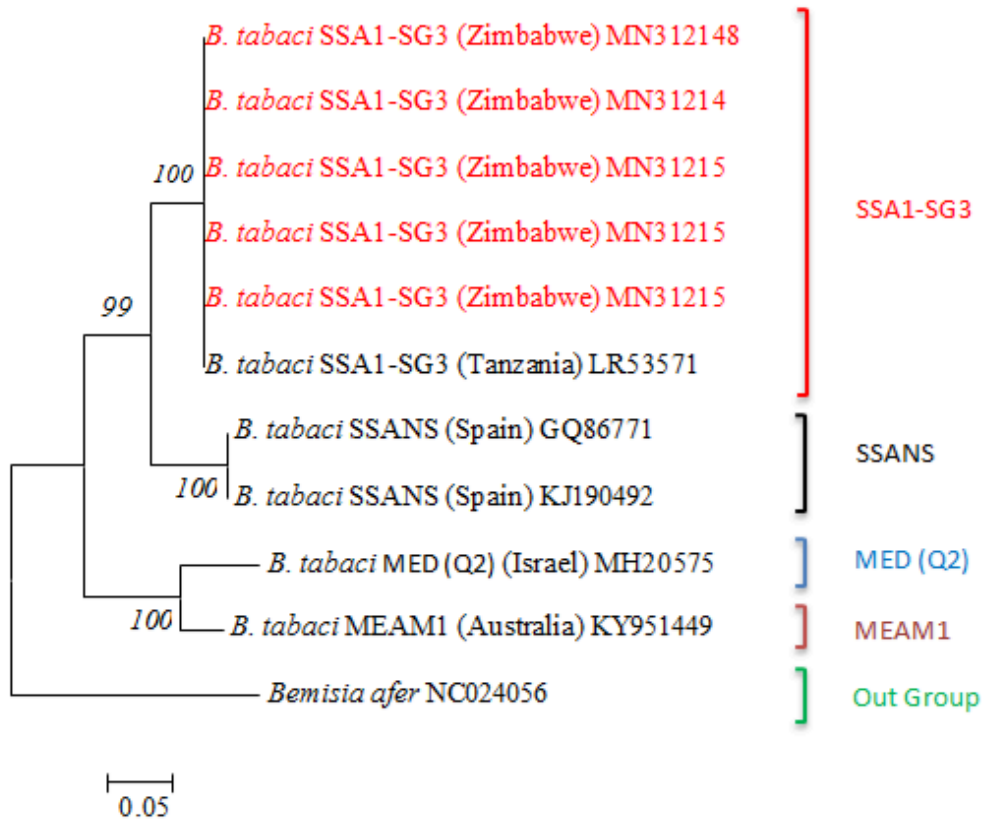


Figure 2

Maximum Likelihood phylogenetic tree based on mtCO1 sequences of *Bemisia tabaci* collected in Zimbabwe (highlighted in red), reference sequences from GenBank, and *Bemisia afer* (NC024056) as outgroup. Bootstrap support values (1000 replicates) are shown at nodes. Scale bar = 0.05 substitutions per site. All Zimbabwe specimens form a single clade within SSA1-SG3.

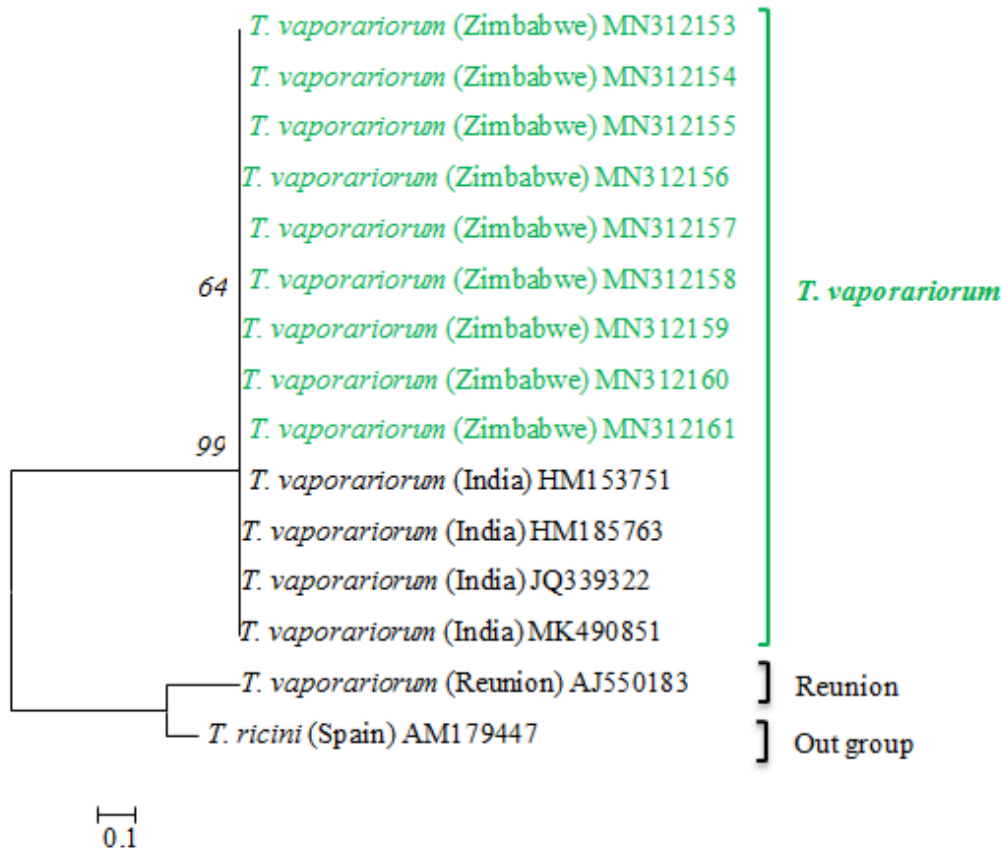


Figure 3

Maximum Likelihood phylogenetic tree based on mtCO1 sequences of *Trialeurodes vaporariorum* from Zimbabwe (highlighted in green) and reference sequences, with *Trialeurodes ricini* (AM179447) as the outgroup. Bootstrap support values (1000 replicates) are shown at nodes. Scale bar = 0.1 substitutions per site. All Zimbabwe specimens form a single clade with high identity to Indian sequences.

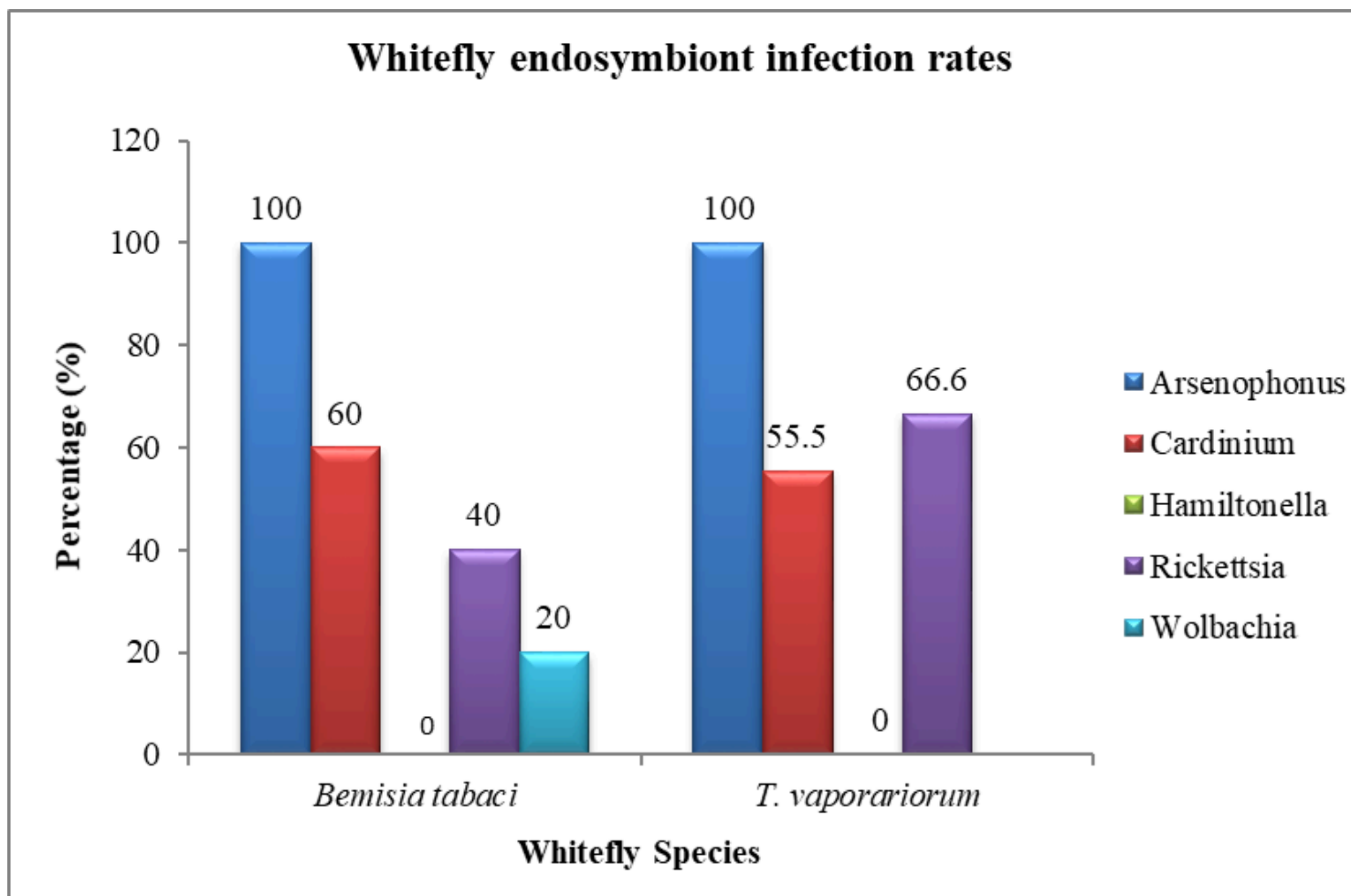


Figure 4

Percentage infection frequencies of secondary endosymbionts of whitefly species in Zimbabwe

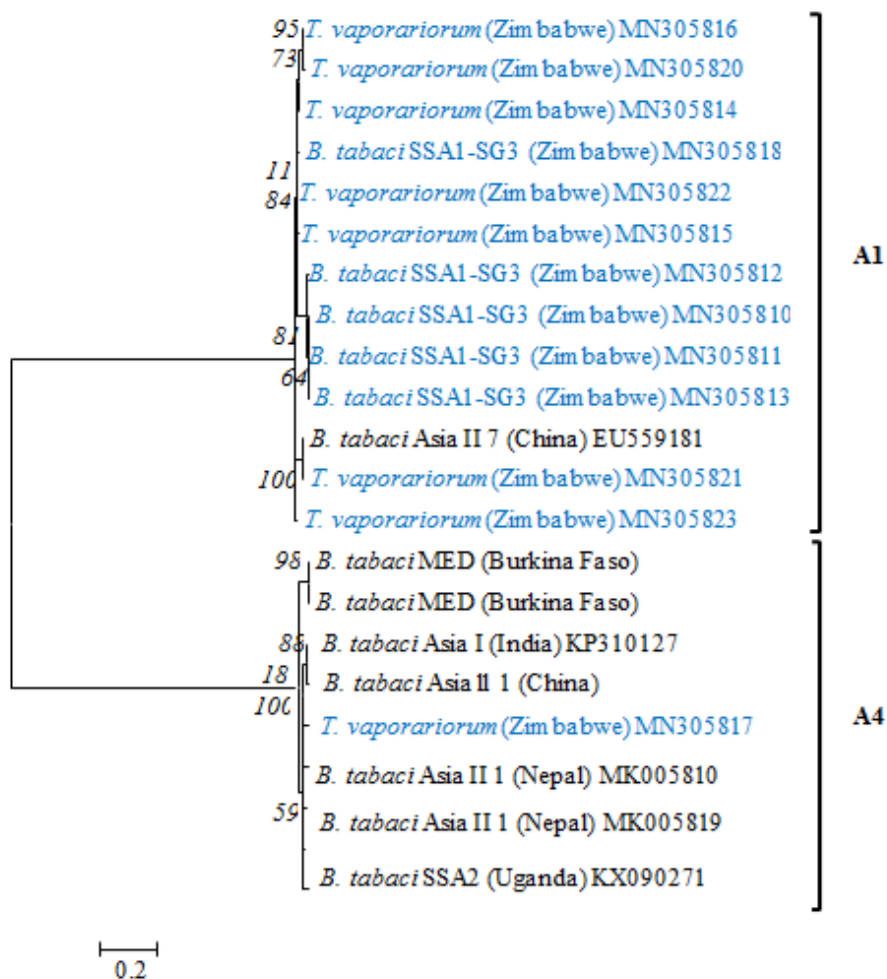


Figure 5

Maximum Likelihood phylogenetic tree of *Arsenophonus* 23S rDNA sequences from whitefly species in Zimbabwe. Zimbabwe *B. tabaci* SSA1-SG3 sequences are shown in red; Zimbabwe *T. vaporariorum* sequences are in green. Two clades (A1 and A4) are labelled. Scale bar = 0.2. Bootstrap values (1000 replicates) shown at key nodes.

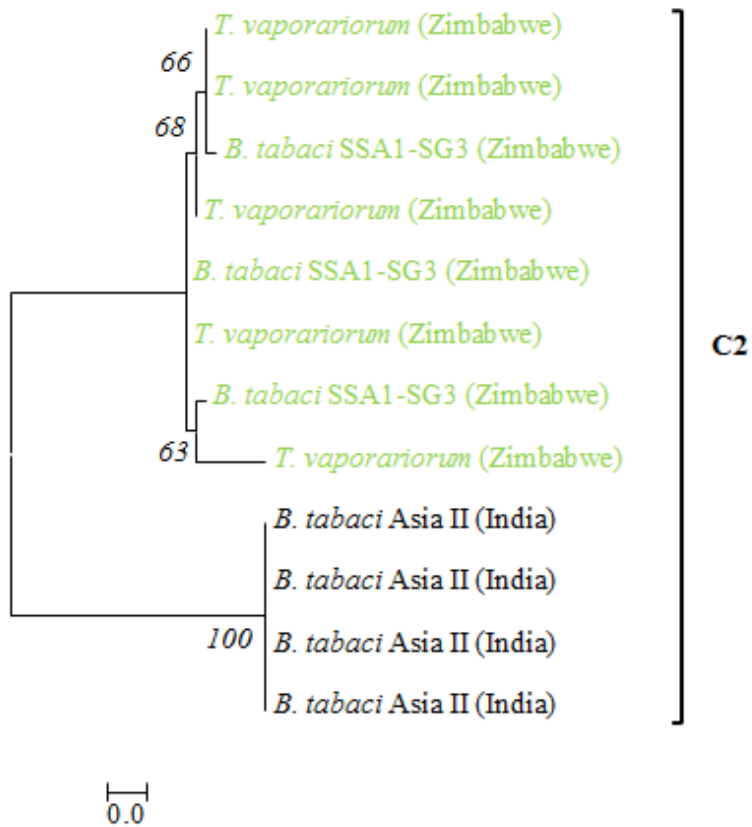


Figure 6

Maximum Likelihood phylogenetic tree of Cardinium 16S rDNA sequences from whitefly species in Zimbabwe. Zimbabwe sequences (both *B. tabaci* SSA1-SG3 and *T. vaporariorum*) cluster in clade C2 alongside *B. tabaci* Asia II from India. Scale bar = 0.0. Bootstrap values shown at nodes.

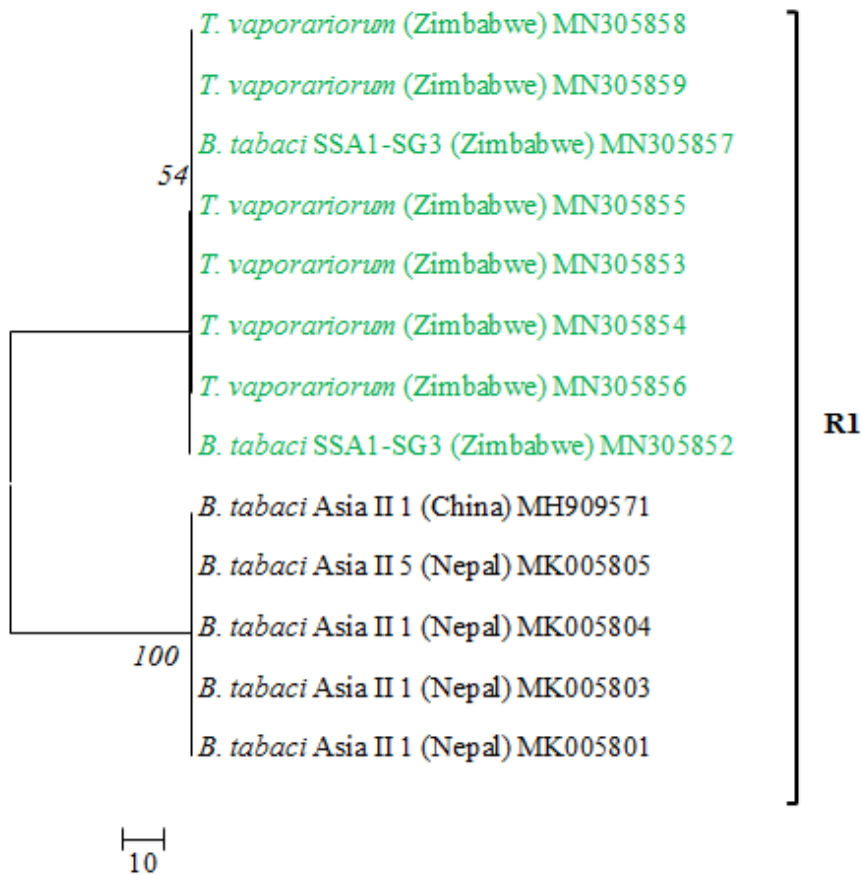


Figure 7

Maximum Likelihood phylogenetic tree of Rickettsia 16S rDNA sequences from whitefly species in Zimbabwe. All Zimbabwe sequences (*B. tabaci* SSA1-SG3 and *T. vaporariorum*) form a single clade (R1) with *B. tabaci* Asia II sequences from China and Nepal. Scale bar = 10. Bootstrap values shown at nodes.

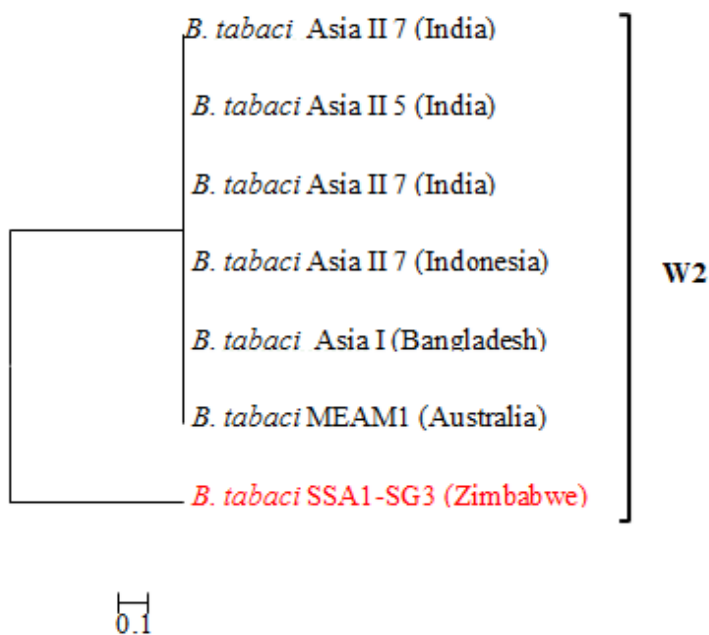


Figure 8

Maximum Likelihood phylogenetic tree of Wolbachia 16S rDNA sequences from *B. tabaci* SSA1-SG3 in Zimbabwe (red). The single Zimbabwe sequence clusters in clade W2 alongside diverse *B. tabaci* biotypes from Asia and Australia. Scale bar = 0.1. Bootstrap values shown at nodes.

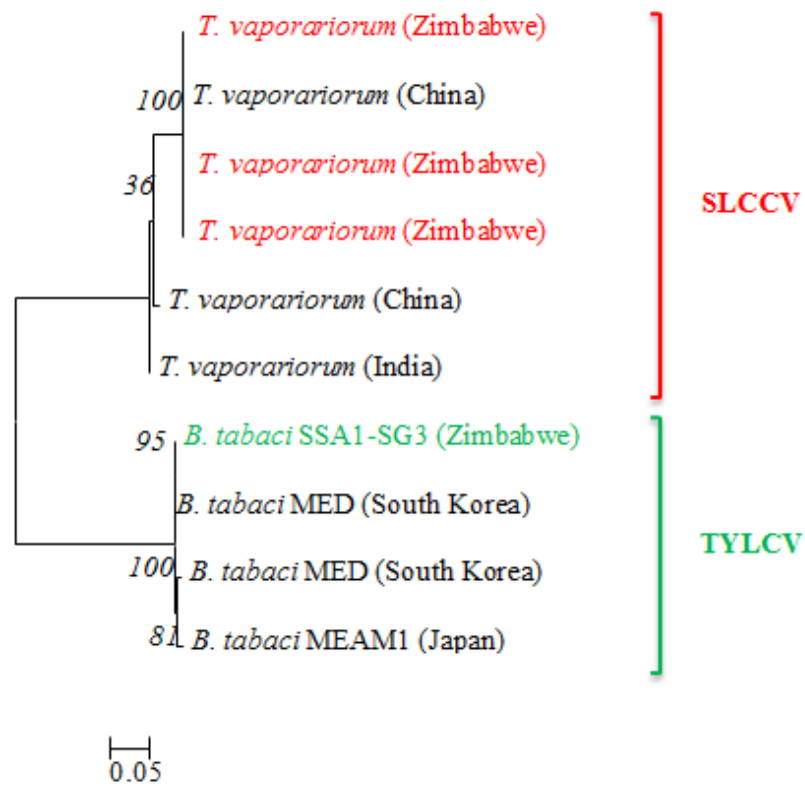


Figure 9

Maximum Likelihood phylogenetic tree of begomovirus sequences from whitefly species in Zimbabwe. Zimbabwe *B. tabaci* SSA1-SG3 (red) clusters within the TYLCV clade alongside *B. tabaci* MED (South Korea) and MEAM1 (Japan); Zimbabwe *T. vaporariorum* sequences (green) cluster within the SLCCV clade with *T. vaporariorum* from China. Scale bar = 0.05.

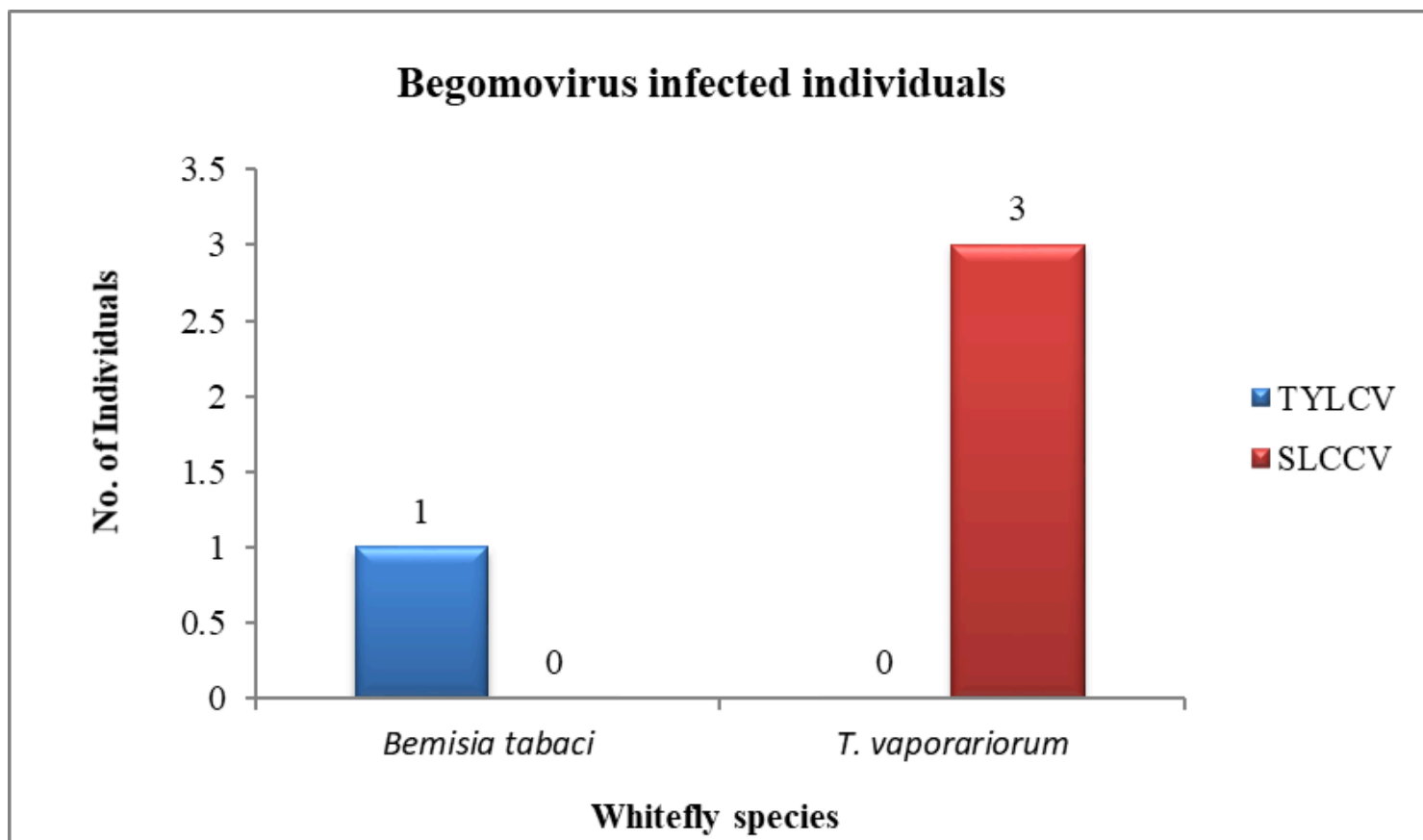


Figure 10

Number of whitefly individuals which acquired begomovirus amongst the samples collected from Zimbabwe