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RESEARCH ARTICLE

Diversity of symbiotic bacteria associated with *Bemisia tabaci* (Homoptera: Aleyrodidae) in cassava mosaic disease pandemic areas of Tanzania

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Abstract

All *Bemisia tabaci* individuals harbour an obligate bacterial symbiont (*Portiera aleyrodidarum*), and many also harbour non-essential facultative symbionts. The association of symbiotic bacteria with the various genetic groups of *B. tabaci* remains unknown for East Africa. This study aimed to assess any association between the various whitefly genetic groups and the endosymbionts they harbour; to investigate if a unique endosymbiont is associated with super-abundant whiteflies, and to provide baseline information on endosymbionts of whiteflies for a part of East Africa. Whiteflies collected during surveys in Tanzania were genotyped and screened for the presence of the obligate and six secondary symbionts (SS): *Rickettsia* (R), *Hamiltonella* (H), *Arsenophonus* (A), *Wolbachia* (W), *Cardinium* (C) and *Fritschea* (F). The results revealed the presence of Mediterranean (MED), East Africa 1 (EA1), Indian Ocean (IO) and Sub-Saharan Africa 1 (SSA1) genetic groups of *Bemisia tabaci*, with SSA1 further clustered into four sub-groups: SSA1-SG1, SSA1-SG2, SSA1-SG1/2 and SSA1-SG3. F was completely absent from all of the whiteflies tested while R was always found in double or multiple infections. In general, no particular symbiont appeared to be associated with the super-abundant SSA1-SG1 *B. tabaci,* although A or AC infections were common among infected individuals. The most striking feature of these super-abundant whiteflies, dominating cassava mosaic disease pandemic areas, was the high prevalence of individuals uninfected by any of the six SS tested. This study of the endosymbionts of *B. tabaci* in East Africa showed contrasting patterns of infection in crop and weed hosts.

Introduction

The whitefly species *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) was known for most of the 20th century as a secondary pest of many crops and ornamental plants in many countries (Patti & Rapisarda, 1981). During the last two decades, however, global movement of invasive forms of *B. tabaci* has dramatically increased its pest status all over the world, causing significant damage to agriculture both in protected environments and in open field cultivations (Brown *et al.,* 1995a; De Barro *et al.,* 2000). This whitefly has been found to be a cryptic species complex that comprises morphologically indistinguishable but genetically and biologically distinct species (De Barro *et al.,* 2011; Liu *et al.,* 2012). These species vary considerably in host range, fecundity, insecticide resistance and virus transmission ability (Bird, 1957; Costa *et al.,* 1993; Bedford *et al.,* 1994; Liu *et al.,* 2010; Xu *et al.,* 2011). At present, the *B. tabaci* species complex is thought to comprise at least 35 morphologically indistinguishable species (Dinsdale *et al.,* 2010; Hu *et al.,*

2011; Alemandri *et al.*, 2012; Chowda-Reddy *et al.*, 2012; Parrella *et al.*, 2012; Lee *et al.*, 2013; Legg *et al.*, 2013). Among the 35 morphologically indistinguishable species, sub-Saharan Africa 1 (SSA1) has recently been further divided into five sub-groups which include SSA1-SG1, SSA1-SG2, SSA1-SG3, SSA1-SG4 and an intermediate group between SG1 and SG2 termed SSA1-SG1/2 (Legg *et al.*, 2013).

In the tropics, cassava (*Manihot esculenta* Crantz) is an important source of calories, third in importance after rice and maize (FAO, 2008). However, for more than two decades cassava production in East and Central Africa has been limited by the outbreak and spread of a severe cassava mosaic disease (CMD) pandemic starting in Uganda in the late 1980s (Otim-Nape *et al.*, 1997). The unusually severe pandemic is mainly characterised by high CMD severity and incidence and greatly increased populations of the whitefly vector (Gibson *et al.*, 1996; Otim-Nape *et al.*, 1997; Legg & Ogwal, 1998). Hundred-fold increases were observed in whitefly abundance in pandemic-affected areas (Legg *et al.*, 2006). *Bemisia tabaci* whiteflies occurring at these unusually high densities have been termed 'super-abundant' (Legg, 2010). For the first time, an association between an invasive super-abundant *B. tabaci* group (SSA2) and a severe CMD epidemic was demonstrated in Uganda in the early 2000s (Legg *et al.*, 2002). Nearly a decade later, further evidence for a similar association of high densities of the *B. tabaci* group SSA1-SG1 with CMD pandemics in the wider region of East and Central Africa was provided through analyses of 14 years of data (Legg *et al.*, 2013).

All whiteflies harbour an obligate (primary) endosymbiotic bacterium essential for the host's survival and development – *Portiera aleyrodidarum* (Baumann *et al.*, 2004; Sloan & Moran, 2012). In addition, many harbour facultative (secondary) symbionts (Buchner, 1965). Different genetically distinct species or groups of *B. tabaci* host different complexes of several secondary symbionts (SS) that may include *Rickettsia, Hamiltonella, Arsenophonus, Cardinium, Wolbachia*, *Fritschea* and *Hemipteriphilus* (Zchori-Fein & Brown, 2002; Weeks *et al.*, 2003; Thao & Baumann, 2004; Everett *et al.*, 2005; Moran *et al.*, 2005; Gottlieb *et al.*, 2006; Bing *et al.*, 2013). The effect of many of these SS in whiteflies is not well known, although *Rickettsia* has been shown to increase the tolerance to heat shock in one population (Brumin *et al.*, 2011) and to induce fitness benefits and female bias in another (Himler *et al.*, 2011). In other hosts, SS have been shown to affect their hosts in several ways, such as: manipulation of reproduction, (Zchori-Fein *et al.*, 2001; Hunter *et al.*, 2003; Duron *et al.*, 2008; Werren *et al.*, 2008), conferring resistance to parasitism, fungal attack or virus (Oliver *et al.*, 2003; Hedges *et al.*, 2008; Lukasik *et al.*, 2013) or increasing their tolerance to temperature (Russell & Moran, 2006; Henry *et al.*, 2013).

Within the cryptic species complex of *B. tabaci*, major genetic groups are generally reproductively isolated (Xu *et al.*, 2010; Sun *et al.*, 2011) and can vary in both biological traits as well as the endosymbiotic community they harbour (Brown *et al.*, 1995a; Costa *et al.*, 1995; Brown, 2000; Gueguen *et al.*, 2010). For example, Chiel *et al.* (2007) demonstrated that *Arsenophonus* and *Wolbachia* were found only in the Mediterranean (MED) genetic group (Q biotype) and *Hamiltonella* was found only in the Middle-East Asia Mionr 1 (MEAM 1) genetic group (B biotype) in Israel where these whiteflies are sympatric.

Recently, the association of symbiotic bacteria with the various genetic groups of *B. tabaci* was published for some West African countries (Gnankiné *et al.*, 2012). The study revealed an association between symbiotic bacterial communities and *B. tabaci* genetic groups. However, such associations of *B. tabaci* genetic groups and their symbionts remain unknown for East Africa. Understanding the symbiotic bacterial community hosted by *B. tabaci* may be important given that symbiotic bacteria may influence virus transmission capabilities of whiteflies (Gottlieb *et al.*, 2010). These associations may be very problematic if they are influencing the rapid distribution and progression of the virus disease pandemics in cassava in East and Central Africa that are associated with unusually high populations of *B. tabaci* (Legg *et al.*, 2006, 2011).

This study was initiated in order to (a) assess associations between the various whitefly genetic groups on cassava and nearby plant hosts and the endosymbionts they harbour, (b) determine whether there is a unique endosymbiont that is associated with super-abundant whitefly populations and (c) provide baseline information on endosymbionts of whiteflies for a part of East Africa (Tanzania). Here we report the first description of the diversity of the symbiotic bacterial community hosted by various genetic groups of *Bemisia tabaci* in Tanzania, with a special focus on the CMD pandemic affected areas.

Materials and methods

Study area and whitefly collection

In a survey conducted during 2012 and 2013 in Tanzania, covering parts of six regions (Mwanza, Shinyanga, Tabora, Singida, Dodoma and Pwani) (Fig. 1), 148 *B. tabaci* adult samples were collected from 155 farmers' cassava fields (more than 500 individuals total). Some of the regions sampled include the area of cassava cultivation through which the pandemic of severe CMD is currently expanding, from northwest to central Tanzania. Previous studies have indicated the likely direction of the severe CMD pandemic (Legg, 2010).

Figure 1 The six sampling regions of the current study – shaded in grey.

Sampling was done in farmers' cassava fields and in the immediate area surrounding cassava, including non-cassava crops and common whitefly-bearing weeds. Fields were sampled every 10–15 km along main and secondary roads. Samples collected were immediately preserved in 1.5 ml tubes containing 95% ethanol and stored at −20°C until DNA extraction. Host plants included cassava (*Manihot esculenta* Crantz), pumpkin (*Cucurbita pepo* L.), sweet potato [*Ipomoea batatas* (L.) Lam.], a Lamiaceae weed – klip dagga or lion's ear [*Leonotis nepetifolia* (L.) R. Br.], okra [*Abelmoschus esculentus* (L.) Moench], tomato (*Solanum lycopersicum* L.) and cotton (*Gossypium hirsutum* L.).

DNA extraction and PCR of *Bemisia tabaci*

From 77 randomly selected whitefly samples, 210 individuals were subjected to molecular analyses to identify their genetic group and to assess their symbiotic bacterial communities (Table 1). DNA was extracted using a standard Chelex extraction method. Individual insects were first washed with distilled water to remove ethanol, homogenised under a microscope in a 3 µl drop of proteinase-K (20 mg/ml dH_2O) on a piece of parafilm, and then placed in a 0.5 ml microfuge tube containing 50 µl of 10% (w/v) Chelex solution kept on ice. The tubes were vortexed and centrifuged briefly before, during and at the end of a 1 h incubation period at 37°C. The tubes were then incubated at 96°C for 8 min to deactivate the proteinase-K, vortexed and centrifuged briefly before being stored in the freezer at −20°C until further use (White *et al.*, 2009).

Tubes were vortexed again and centrifuged briefly before using the supernatant as template DNA in PCR. Mitochondrial DNA fragments (*mtCOI*) from whitefly DNA were amplified using the forward primer C1-J-2195 and the reverse primer L2-N-3014 (Table 2, Simon *et al.*, 1994). PCR was performed using 2 µl aliquots of the DNA extract in a total reaction volume of 10.5 µl containing 0.8× FailSafe premix, 0.48 µM of each primer and 1.25 units *Taq* DNA polymerase (Invitrogen™, Thermo Fisher Scientific Inc. MA, USA). Initial DNA denaturation was conducted for 3 min at 94°C followed by 35 cycles of 1 min denaturation at 94°C, 1 min annealing at 52°C and 1:20 min amplification at 72°C. Final extension was done at 72°C for 10 min. PCR products (aliquots of 4 µl) were

Table 1 Localities, host plants and genetic groups of the whitefly individuals used for molecular analyses with the most frequent secondary symbiont they harboured[a]

Localities				
Region	District	Host Plants	Genetic group	Most frequent secondary symbiont infection
Pwani	Mkuranga			
Dodoma	Dodoma Urban			
Singida	Manyoni, Ikungi, Singida Urban	Cassava (n = 148)	SSA1 (n = 145)	A, AC, None
Tabora	Igunga, Nzega, Tabora, Urambo, Uyui		IO (n = 3)	AC
Shinyanga	Bukombe, Kahama, Maswa, Shinyanga, Shinyanga Rural, Ushirombo			
Mwanza	Misungwi, Nyang'hwale, Geita			
Tabora	Nzega, Urambo	Pumpkin (n = 23)	MED (n = 14)	RHCW, no A
Shinyanga	Bukombe, Kahama			
Mwanza	Kwimba		EA1 (n = 9)	RCW, A
Shinyanga	Bukombe, Kahama	Sweet potato (n = 19)	MED (n = 18)	RHW
Mwanza	Geita		SSA1 (n = 1)	A
Tabora	Nzega, Uyui, Igunga	Klip dagga (n = 14)	EA1 (n = 7)	HCW
			MED (n = 5)	only H
Shinyanga	Bukombe, Shinyanga Rural		IO (n = 2)	RHC
Shinyanga	Kahama	Okra (n = 3)	EA1 (n = 3)	RACW
Mwanza	Geita			
Shinyanga	Kahama	Tomato (n = 1)	SSA1 (n = 1)	A
Shinyanga	Bukombe	Cotton (n = 2)	EA1 (n = 2)	RACW
		Total n = 210		

R: *Rickettsia*, H: *Hamiltonella*, A: *Arsenophonus*, C: *Cardinium*, W: *Wolbachia*, F: *Fritschea*, None: no infection by secondary symbionts, SSA1, Sub-Saharan Africa 1, MED: Mediterranean; EA1: East Africa 1, IO: Indian ocean genetic group.

[a]The numbers in brackets represent the number of individuals on each host plant and belonging to the various genetic groups

separated on a 1% agarose gel and visualised with Gel Red under UV light. For a subset of individuals, PCR products of the expected size (~850 bp) were sent to be sequenced either at Macrogen Inc. (Macrogen MD, 20850, USA) or BMR Genomics (Via Redipuglia 21A, 35131 Padova, Italy).

Detection and identification of endosymbionts

The quality of DNA extraction was first checked by detecting the primary endosymbiont *Portiera aleyrodidarum* using the forward and reverse primers PortF and PortR, respectively (Thierry *et al.,* 2011). Secondary endosymbiont infection was then assessed using specific PCR primers targeting the *16S* rDNA genes for *Rickettsia, Hamiltonella, Arsenophonus, Wolbachia, Cardinium* and *Fritschea.* Specific endosymbiont primers and annealing temperatures are given in Table 2. PCR was performed using 1–2 µl of the DNA extract in a total reaction volume of 10.5 µl containing 0.8× FailSafe premix, 0.48–0.76 µM of each primer and 1.25 units *Taq* DNA polymerase (Invitrogen™). PCR products were separated and visualised under UV light. Selected bands of expected sizes were sequenced either at Macrogen Inc. (Macrogen MD, 20850, USA) or BMR Genomics (Via Redipuglia 21A, 35131 Padova, Italy).

Phylogenetic analysis of *Bemisia tabaci* and endosymbionts

Out of the 210 individuals, 96 were sequenced for a portion of the *mitochondrial COI* gene. Sequences were cleaned manually and ends trimmed using Chromas Lite version 2.1 (Technelysium Pty Ltd, 2012) and aligned using the clustalO option of SeaView (version 4.4.2) (Galtier *et al.,* 1996; Gouy *et al.,* 2010). DNA sequence identities were confirmed using NCBI-BLAST. After alignment, a standard 657 bp *mtCOI* fragment was used to generate the phylogenetic tree. Selected sequences were used from the global *Bemisia* dataset (release version 31_Dec_2012) (De Barro & Boykin, 2013) and from GenBank® for comparison with sequences from this study. Maximum likelihood phylogenetic trees were constructed with 1000 bootstrap replicates using MEGA 6 (Tamura *et al.,* 2013). A jModel test (jModelTest version 8 2.1.3) was performed and TrN + I + G was used as the best fit nucleotide substitution model. The outgroup used for *B. tabaci* analysis was *Trialeurodes vaporariorum* (Westwood), GenBank code: JF693935. The trees were summarised using Fig Tree (version 1.4.0) (Rambaut, 2009).

Phylogenetic analysis for representative endosymbiont sequences was performed in a similar manner, sequences were compared to known sequences in the GenBank®

Table 2 List of primers and PCR conditions used in the study

Targeted organism and/or gene	Name of Primer	Primer sequences (5′–3′)	Annealing temp. (°C)/Product size (bp)	Reference
B. tabaci	C1-J-2195	TTGATTTTTTGGTCATCCAGAAGT	52°/~850	Simon *et al.*, 1994
mtCOI	L2-N-3014	TCCAATGCACTAATCTGCCATATTA		
Portiera	Port-F	GGAAACGTACGCTAATAC	59°/~900	Thierry *et al.*, 2011
16S rDNA	Port-R	TGACGACAGCCATGCAGCAC		
Rickettsia	Rick 16S 528F	ACTAATCTAGAGTGTAGTAGGGGATGATGG	60°/~900	Chiel *et al.*, 2009
16S rDNA	Rick 16S 1044R	GTTTTCTTATAGTTCCTGGCATTACCC		
Hamiltonella	Ham-F	TGAGTAAAGTCTGGAATCTGG	60°/~700	Zchori-Fein & Brown, 2002
16S rDNA	Ham-R	AGTTCAAGACCGCAACCTC		
Arsenophonus	CAIf	GCCTGATGCAGCCATGCCGCGTGTATG	65°/~500	Dale *et al.*, 2006
16S rDNA	CAIr	GTCATCCCCACCTTCC		
Wolbachia	V1	TTGTAGCCTGCTATGGTATAACT	52°/~900	O'Neill *et al.*, 1992
16S rRNA	V6	GAATAGGTATGATTTTCATGT		
Cardinium	CLO F	GCGGTGTAAAATGAGCGTG	57°/~500	Weeks & Breeuwer, 2003
16S rDNA	CLO R	ACCTMTTCTTAACTCAAGCCT		
Fritschea	U23F	GATGCCTTGGCATTGATAGGCGATGAAGGA	55°/~600	Everett *et al.*, 2005
16S rDNA	23SIGR	TGGCTCATCATGCAAAAGGCA		

database to verify their identity and phylogenetic trees were constructed (Supporting Information).

Statistical analysis of association of SS in whitefly genetic groups and host plants

To assess whether the apparent differences in secondary symbionts infection status of our samples are randomly occurring by chance or whether there is some relation between SS and genetic group as well as host plants, a Chi-square test was performed. As we assume there could be sampling bias towards cassava, we tested secondary symbiont infected and uninfected whitefly individuals collected only from cassava and belonging to SSA1-SG1 on one hand and belonging to other groups on the other hand. This was done to see whether the striking feature of high numbers of uninfected SSA1-SG1 *B. tabaci* individuals could be expected by chance.

Results

Phylogenetic analysis of *Bemisia tabaci*

The phylogenetic analysis of *mtCOI* sequences revealed four putative species of the *B. tabaci* species complex, which included: SSA1, Mediterranean (MED), Indian Ocean (IO) and East Africa 1 (EA1) (Fig. 2). Four sub-groups were also detected under the SSA1 putative species, namely, SSA1-SG1, SSA1-SG2, SSA1-SG3 and SSA1-SG1/2.

Bemisia tabaci infection by SS and geographic distribution of the SS

Detection of the primary symbiont *P. aleyrodidarum* in all individuals confirmed the DNA extract quality. Different patterns of double and multiple infections (infection with three or more SS) were observed in the different genetic groups (Fig. 3). *Rickettsia* (R) was always found in double or multiple infections and *Fritschea* (F) was completely absent from all of the whitefly individuals examined in this study (Fig. 3, Table S1, Supporting Information). We did not test for *Hemipteriphilus*, which was documented in China for the first time in 2013 (Bing *et al.*, 2013).

The majority (81%) of MED whiteflies were infected by two or more SS. Whiteflies from this group had either single or multiple combinations of one to four symbionts, with *Hamiltonella* (H) and *Wolbachia* (W) appearing as single infections, but *Rickettsia, Cardinium* (C) and *Arsenophonus* (A) present only in multiply-infected hosts (Fig. 3, Supplemental Information: Table S1). Double and multiple infections were also common in whiteflies belonging to the IO and EA1 putative species. The majority of EA1 individuals harboured *Cardinium* and *Wolbachia* with several additional combinations of *Rickettsia*, *Hamiltonella* and *Arsenophonus*.

Generally, *Arsenophonus* was the most commonly found secondary symbiont in all the four sub-groups of SSA1. More than one-third of SSA1-SG1 individuals were free of SS (36%), but infected individuals frequently harboured *Arsenophonus*. AC was the most common double infection type in individuals in SSA1-SG1, while HA and HAC infections were infrequent. In this group, multiple infection was very rare, and only observed in 8% of the examined individuals. SSA1-SG1 was the only sub-group where individuals with no secondary symbiont infection were recorded (Fig. 3, Table S1). SSA1-SG1 is principally found in the north-west of Tanzania surrounding Lake Victoria, corresponding to the area affected by the severe

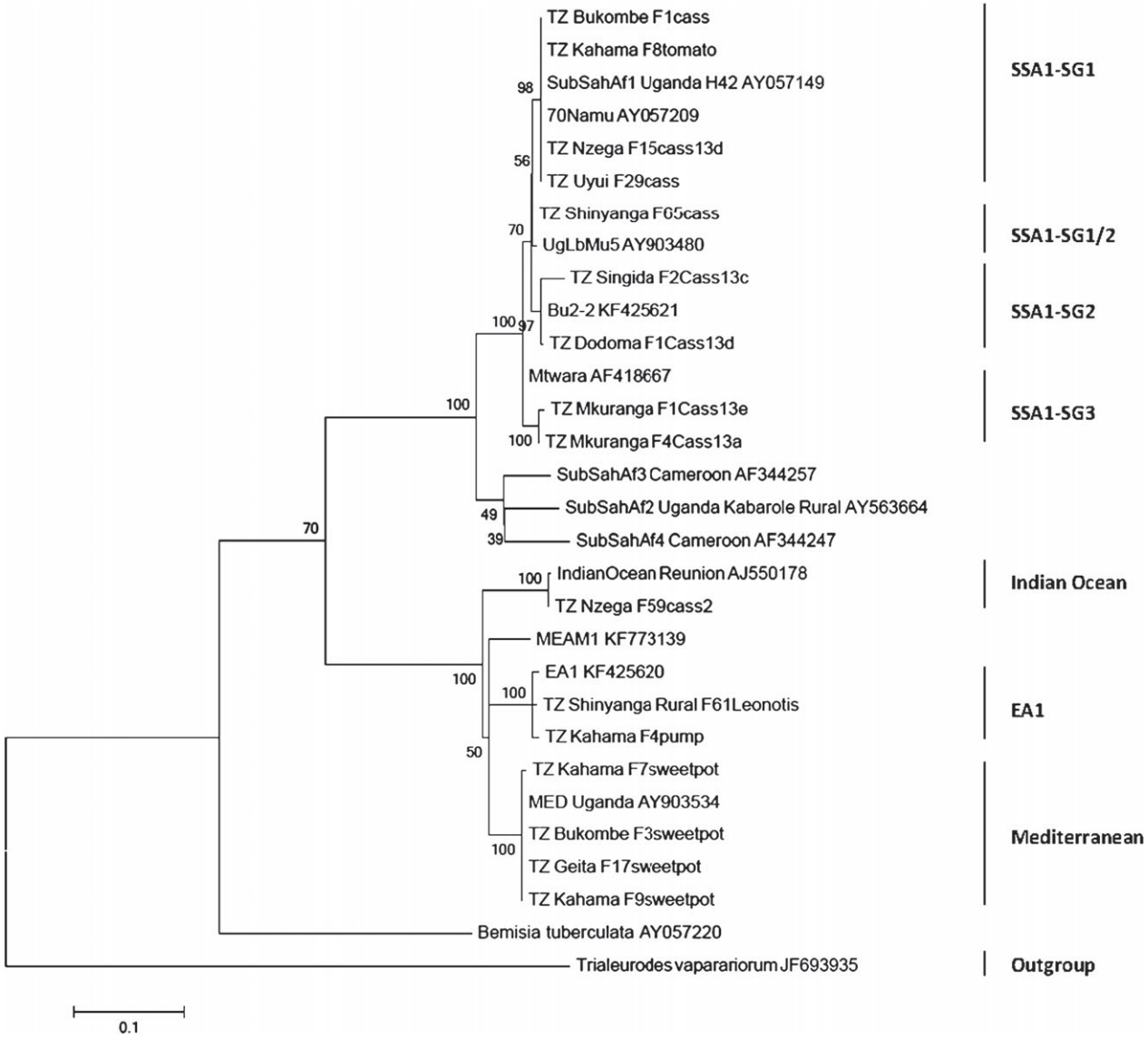

Figure 2 Maximum likelihood phylogeny of *mtCOI* sequences of *Bemisia tabaci* collected during 2012–2013 surveys with selected reference sequences from GenBank. All sequences starting with 'TZ_' are sequences produced in this study whereas all other sequences are reference sequences from GenBank. The tree is drawn to scale and the scale bar represents the number of substitutions per site. SSA1 represents Sub-Saharan Africa 1 genetic group; SG, sub-groups; and EA1, East Africa 1 genetic group.

CMD pandemic (Fig. 4). Conversely, all SSA1-SG1/2 and SSA1-SG2 individuals, which occurred primarily in the central part of Tanzania, harboured either one or more of the SS. Infections by AC and HAC were common in these two sub-groups. SSA1-SG3, which only occurred in coastal areas, frequently harboured R in combination with HA or HAC (Figs 3 and 4). Taking into consideration all 210 *B. tabaci* individuals screened for SS, multiple infection was the most common infection type followed by double infection, no infection and single infection (Fig. 5).

Host plants, *Bemisia tabaci* and SS

Whiteflies collected on different host plants belonged to different genetic groups and harboured different secondary endosymbionts. Whiteflies on okra, tomato and cotton were very few, so we were not able to make strong conclusions on these host plants. However, it was still possible to compare their symbiont profile with whiteflies of the same genetic group collected on other host plants. Most whiteflies collected on cassava and the one individual collected on tomato were SSA1, whereas whiteflies

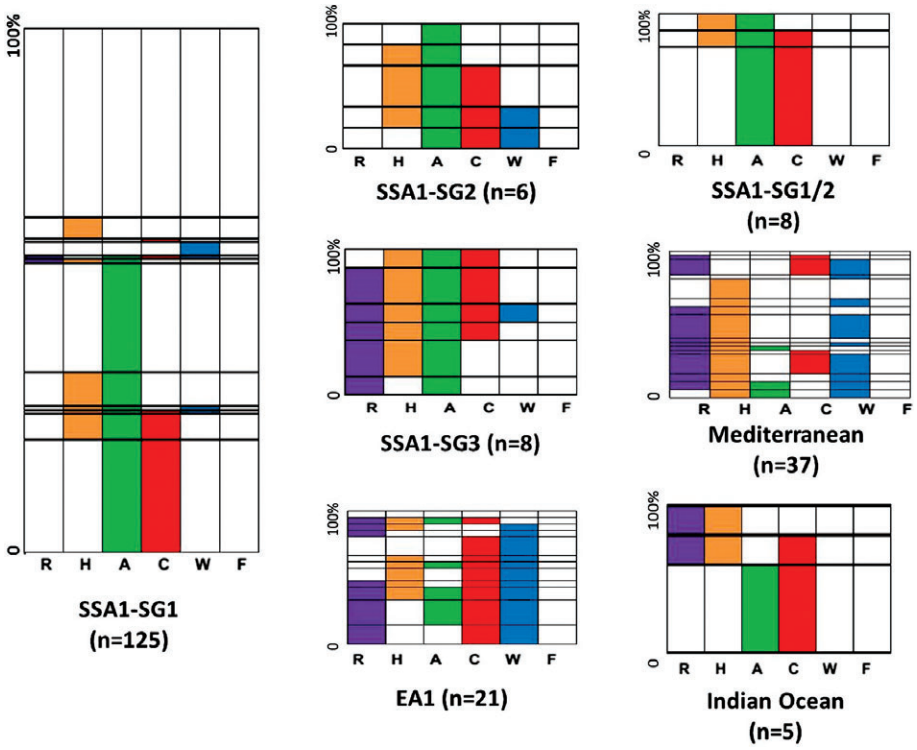

Figure 3 Secondary symbiont infection in *Bemisia tabaci* individuals belonging to the different genetic groups and sub-groups found in this study. The number of individuals tested and the genetic groups they belong to are indicated below each graph. The various colours represent the different secondary symbionts tested where R is *Rickettsia*; H, *Hamiltonella*; A, *Arsenophonus*; C, *Cardinium*; W, *Wolbachia*; and F, *Fritschea*. The combination of colours in rows represents whiteflies that shared that particular complex of symbionts. The width of the row indicates the percentage of individuals that have that combination of symbionts. SSA1 represents Sub-Saharan Africa 1 genetic group; SG, sub-groups; and EA1, East Africa 1 genetic group. The numbers in brackets indicate the number of individuals tested in each of the genetic groups and sub-groups.

collected on the remaining host plants mainly belonged to MED, EA1 and IO (Table 1). The whiteflies on cassava and on tomato harboured A, AC or no secondary endosymbionts at all. Almost all whiteflies on sweet potato were MED (except one SSA1 individual with only A infection) and harboured mainly RHW. In addition, some MED whiteflies had A and a few had C (A and C not shown in Table 1 which presents only the most frequent secondary symbiont infection) while those on okra and cotton were all EA1 genetic group, all with four SS (RACW). On the other hand, whiteflies on pumpkin and on the weed called klip dagga or lion's ear mainly belonged to both MED and EA1 with few on klip dagga belonging to IO (Table 1). MED whiteflies on pumpkin were infected by a different subset of four SS: RHCW. EA1 individuals from the same host plant, besides harbouring RHCW, also had A but fewer H. On the other hand, MED whiteflies on klip dagga only had single infection by H while EA1 individuals collected from the same host plant exhibited multiple infections: mainly HCW and some also with R and A (R and A not shown in Table 1 which presents only the most frequent secondary symbiont infection). Whiteflies belonging to IO genetic group were collected on cassava (n = 3) and klip dagga (n = 2). Although the number of individuals was very few, those on cassava harboured AC while those on klip dagga had RH and RHC (Table 1).

Almost all the uninfected whiteflies were collected on cassava (95.8%). Two individual whiteflies, one from okra and one from klip dagga were the only other whiteflies without SS in the study. All other individuals harboured either one or more of the SS in various combinations and frequencies.

Association of SS in whitefly genetic groups and host plants

A χ^2 analysis of secondary symbiont infection status of whiteflies collected on cassava revealed statistically significant differences [χ^2 (1, N = 148) = 13.14, $P < 0.001$] between SSA1-SG1 whiteflies and all others indicating that this group was less likely to be infected with SS than others (Table S2). Similar analyses revealed that SSA-SG1 whiteflies were more likely to be free of SS than were any

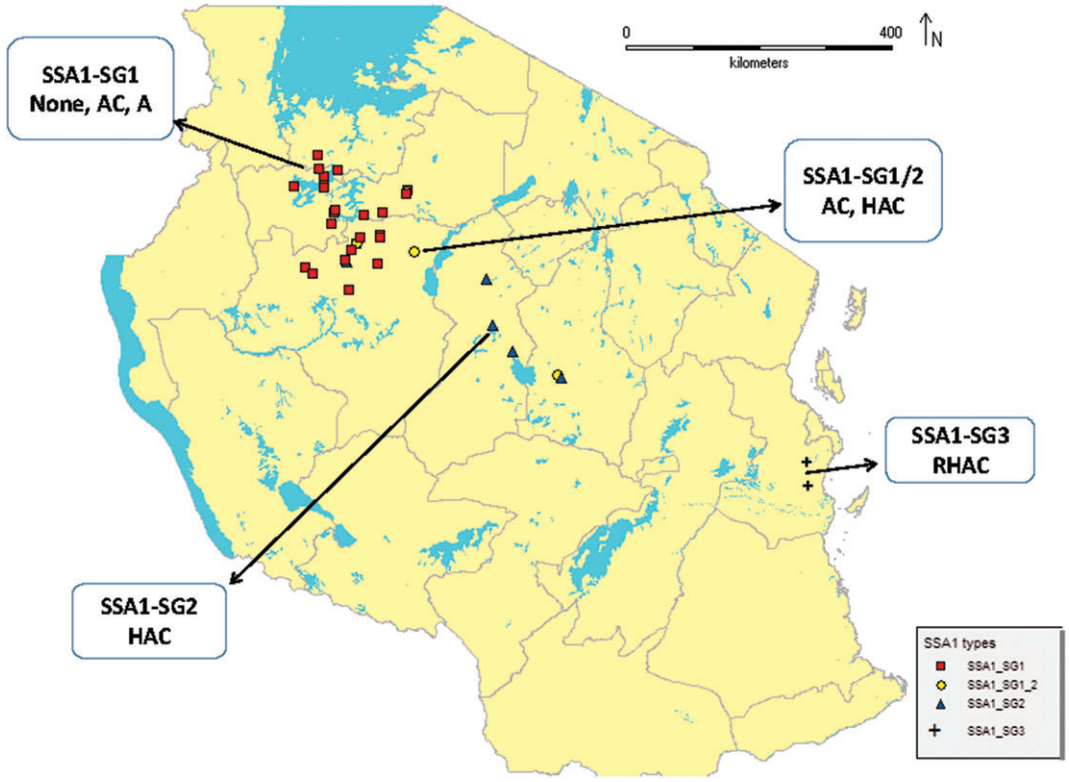

Figure 4 Geographic distribution of *Bemisia tabaci* genetic groups on cassava and their most frequent secondary symbionts. R, *Rickettsia*; H, *Hamiltonella*; A, *Arsenophonus*; C, *Cardinium*; W, *Wolbachia;* F, *Fritschea*; None, no infection by secondary symbionts; SSA1, Sub-Saharan Africa 1; SG1, Sub-group 1; SG2, Sub-group 2; SG1/2, intermediate Sub-group between 1 and 2; SG3, Sub-group 3.

other genetic group and sub-group collected in the CMD pandemic-affected area of the Lake Zone [χ^2 (1, N = 190) = 18.83, $P < 0.001$] (Table S3).

Phylogenetic analysis of endosymbionts

A total of 2–5 partial sequences were obtained for the *16S* rDNA gene of each of the endosymbionts found to infect the whiteflies tested in the study. The amplified sequences (between 388 and 666 bp, depending on the endosymbiont) were aligned together with selected reference sequences from GenBank® and the alignment was used to reconstruct the respective phylogenetic trees (Figure S1–S6). The phylogenetic trees revealed that the endosymbionts detected grouped with the reference sequences, confirming the identity of the sequences produced.

Discussion

SS and Bemisia tabaci *genetic groups*

About one quarter of the more than 1200 species of whitefly that have been described worldwide occur in Africa (Mound & Halsey, 1978). In the past years, molecular markers have been widely used to discriminate genetic variation in morphologically indistinguishable populations of *B. tabaci* whiteflies (Costa & Brown, 1991; Brown *et al.,* 1995b; Frohlich *et al.,* 1999; Delatte *et al.,* 2005; Dinsdale *et al.,* 2010). In our study we used the *mtCOI* gene to detect the various genetic groups of *B. tabaci*. The whiteflies studied clustered into four putative species; three of which are designated in Dinsdale *et al.* (2010) as SSA1, MED and IO. The fourth was the recently designated putative species EA1 (Legg *et al.,* 2013). SSA1 further clustered into four sub-groups: SG1, SG2, SG1/2 and SG3. The extent of gene flow within subgroups of SSA1 has yet to be studied, although successful matings have been demonstrated between sympatric populations of the SSA1 and SSA2 putative species (Maruthi *et al.,* 2002).

The current study assessed associations between SS and several genetic groups of *B. tabaci.* Generally, whiteflies belonging to SSA1 were shown to frequently harbour *Arsenophonus* but almost never *Rickettsia,* similar to the results reported in West Africa (Gnankiné *et al.,* 2012) for the sub-Saharan African non-Silver Leafing 1 (AnSL 1) 'biotype', which is equivalent to SSA1 (Dinsdale *et al.,*

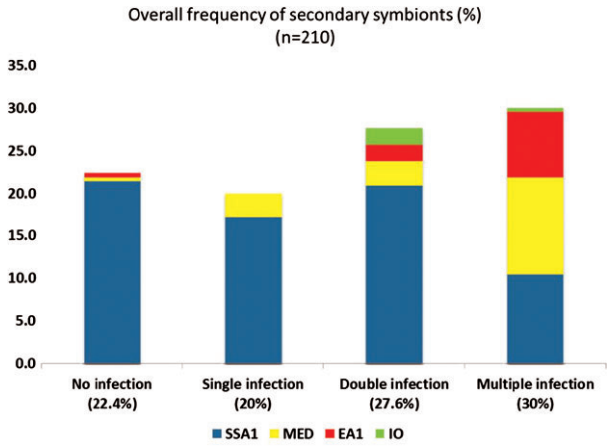

Figure 5 Overall frequency of secondary symbionts harboured by *Bemisia tabaci* individuals sampled during 2012 and 2013 in Tanzania. The various colours indicate the different genetic groups whereas SSA1 represents Sub-Saharan Africa 1; MED, Mediterranean; EA1, East Africa 1; IO, Indian Ocean genetic group. The numbers in brackets indicate the percentage of each secondary symbiont infection type among the total 210 whitefly individuals tested.

2010). However, while in this study a high frequency of uninfected individuals was detected from SSA1-SG1, in West Africa (Gnankiné *et al.,* 2012) it was from SSA1-SG3. Contrary to the findings in West Africa (Gnankiné *et al.,* 2012), the SSA1-SG3 individuals in our study frequently harboured *Rickettsia*. *Fritschea* was never detected in any of the samples in both studies.

Two previous studies (Gottlieb *et al.,* 2008; Gueguen *et al.,* 2010) indicated that *Hamiltonella* and *Arsenophonus* did not occur in double infections in whiteflies in the MED genetic group. However, in our study, double infection by *Hamiltonella* and *Arsenophonus* was not uncommon in MED individuals, similar to Parrella *et al.* (2014), as well as among EA1 individuals or SSA1 group whiteflies. From the previously identified four clades under the MED genetic group (Boykin *et al.,* 2007; Chu *et al.,* 2008; Gueguen *et al.,* 2010), the individuals in our study belonged to Q1 clade. Earlier studies reported *Rickettsia* to be absent in Q1 individuals while *Hamiltonella* was found near fixation (Gueguen *et al.,* 2010; Parrella *et al.,* 2014). Contrary to these previous studies, the MED (Q1) individuals, we tested were frequently infected by *Rickettsia*. According to Parrella *et al.* (2014), *Arsenophonus* had low prevalence among the southern Italy Q1 individuals which was similar to our findings for the Q1 individuals from Tanzania. However, contrary to their findings, our Q1 individuals harboured *Wolbachia* frequently. These findings suggest that the occurrence and frequency of double and multiple infections could vary according to location, whitefly population and time.

Our results indicated a clear association between *B. tabaci* putative species and host plant groupings, as has been widely reported elsewhere (Abdullahi *et al.,* 2003; Gnankiné *et al.,* 2012). SSA1 was largely confined to cassava whilst MED, IO and EA1 occurred on a diverse group of annual crop and weed hosts.

SS and 'super-abundance' of *Bemisia tabaci*

In Tanzania and other neighbouring countries in East and Central Africa, super-abundant whitefly populations on cassava crops have been reported from CMD pandemic affected areas (Legg & Ogwal, 1998; Legg, 2010). Our sampling covered CMD pandemic affected areas in north-west Tanzania, where a super-abundant SSA1-SG1 *B. tabaci* seems to be associated with the pandemic (Legg *et al.,* 2013). Survey collections were also made in central and eastern parts of the country not yet affected by the severe CMD pandemic. This study was initiated partly to assess the factors behind the 'super-abundance' of these whiteflies. *Rickettsia* is known to confer fitness to *B. tabaci* through increased fecundity and female bias (Himler *et al.,* 2011). In our study, however, the SSA1-SG1 known to occur in increased numbers in CMD affected areas almost never harboured *Rickettsia* (only 2 out of 125 individuals). Moreover, about 38% of the whiteflies collected on cassava and belonging to SSA1-SG1 showed no infection by secondary endosymbionts. Multiple infection was also very rare in this group.

Why super-abundant whiteflies are associated with the lack of a secondary symbiont infection is puzzling. In general, for strictly vertically transmitted symbionts, theory predicts that they will not be maintained or spread in populations unless they confer fitness benefits to their hosts, or manipulate host reproduction in such a way (e.g. female-biasing) as to confer a transmission advantage to the symbiont (O'Neill *et al.,* 1997). However, symbiont benefits may not occur if there is regular horizontal transmission, in which case symbionts can persist even if pathogenic. The possibility of horizontal transmission being involved in the patterns of symbionts in whiteflies observed in the current study (Table 1) cannot be determined from these data. However, previous studies have presented evidence that certain clades or species of aphids that acquired particular SS through horizontal transmission were able to utilise new host plants that were formerly unsuitable; clearly indicating that host plant utilisation is influenced by SS and that SS are important for adaptation and utilisation of new host plants (Tsuchida *et al.,* 2004; Tsuchida *et al.,* 2011; Henry *et al.,* 2013). Therefore, determining the possibility and the frequency of horizontal transmission in *Bemisia tabaci* through detailed phylogenetic and co-evolutionary

study of the whiteflies and their SS in relation to the different host plants they utilise in different geographic locations would be very useful in helping predict the role of host plants and the multiple SS observed in whitefly fitness. It would also be useful to improve understanding of the relationship between SS in cassava whiteflies and vector capacity. While symbionts have been implicated in increasing transmission of plant viruses of whiteflies (Gottlieb *et al.,* 2010), symbionts in *Drosophila* and mosquitoes have conferred resistance to human and insect viruses (Hedges *et al.,* 2008; Moreira *et al.,* 2009). Future study could address the question of whether SS of whiteflies on cassava reduce CMD vector competence.

This study reports for the first time the variable and complex symbiotic communities of the genetic groups of *B. tabaci* occurring on crop and weed hosts in a part of East Africa where whiteflies and viruses vectored by whiteflies threaten the viability of the cassava crop. In our study, no particular symbiont appeared to be associated with the super-abundant SSA1-SG1 *B. tabaci*. In contrast, a sizeable proportion of SSA1-SG1 individuals exhibited no infection by SS. The exact association between a higher frequency of individuals with no SS in the pandemic associated SSA1-SG1 *B. tabaci* and its apparent super-abundant nature remains to be determined and further studies are thus recommended. While future studies should be directed towards understanding the role of these symbionts in the biology and vector competence of the various *B. tabaci* genetic groups, it is important to note that similarly high levels of uninfected individuals as in SSA1-SG1 in our study were detected from SSA1-SG3 in West Africa. Hence it seems unlikely that endosymbionts are the causal factors for the super-abundance phenomenon of SSA1-SG1 in the Lake Zone in Tanzania.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Percentage of whitefly single, double and multiple infections by secondary endosymbionts. SSA1 represents Sub-Saharan Africa 1; SG, Sub-group; EA1, East Africa 1; R = *Rickettsia*; H = *Hamiltonella*; A = *Arsenophonus*; C = *Cardinium*; W = *Wolbachia;* F = *Fritschea*; and '-'= 0%

Table S2. Observed and expected (in brackets) secondary symbiont frequencies of whiteflies collected on cassava. SSA1-SG1 represents Sub-Saharan Africa 1 Sub-group 1 whereas Others represents all individuals other than SSA-SG1

Table S3. Observed and expected (in brackets) secondary symbiont frequencies of whiteflies collected in the CMD pandemic area in the Lake Zone. SSA1-SG1 represents Sub-Saharan Africa 1 Sub-group 1 whereas Others represents all individuals other than SSA-SG1

Figure S1. Maximum likelihood phylogeny of *Portiera aleyrodidarium* partial *16S* rDNA sequences and selected reference sequences from Genebank. Sequences starting with 'TZ_' are sequences produced in this study whereas all other sequences are reference sequences from GenBank. The tree is drawn to scale and the scale bar represents the number of substitutions per site.

Figure S2. Maximum likelihood phylogeny of *Rickettsia* partial *16S* rDNA sequences and selected reference sequences from Genebank. Sequences starting with 'TZ_' are sequences produced in this study whereas all other sequences are reference sequences from GenBank. The tree is drawn to scale and the scale bar represents the number of substitutions per site.

Figure S3. Maximum likelihood phylogeny of *Hamiltonella* partial *16S* rDNA sequences and selected reference sequences from Genebank. Sequences starting with 'TZ_' are sequences produced in this study whereas all other sequences are reference sequences from GenBank. The tree is drawn to scale and the scale bar represents the number of substitutions per site.

Figure S4. Maximum likelihood phylogeny of *Arsenophonus* partial *16S* rDNA sequences and selected reference sequences from Genebank. Sequences starting with 'TZ_' are sequences produced in this study whereas all other sequences are reference sequences from GenBank. The tree is drawn to scale and the scale bar represents the number of substitutions per site.

Figure S5. Maximum likelihood phylogeny of *Cardinium* partial *16S* rDNA sequences and selected reference sequences from Genebank. Sequences starting with 'TZ_' are sequences produced in this study whereas all other sequences are reference sequences from GenBank. The tree is drawn to scale and the scale bar represents the number of substitutions per site.

Figure S6. Maximum likelihood phylogeny of *Wolbachia* partial *16S* rDNA sequences and selected reference sequences from Genebank. Sequences starting with 'TZ_' are sequences produced in this study whereas all other sequences are reference sequences from GenBank. The tree is drawn to scale and the scale bar represents the number of substitutions per site.

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