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This contribution is dedicated to the memory of Prof. Dan Gerling, a scientist, a colleague and a friend

Sub-Saharan Africa 1 is the dominant cryptic species of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) associated with cassava in Madagascar

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ABSTRACT

Cassava's productivity in Madagascar is affected by pests and diseases, among which the whitefly *Bemisia tabaci* (Gennadius) transmitting cassava mosaic begomoviruses (CMBs) is most important. The present study sought to establish the abundance and diversity of *B. tabaci* cryptic species on cassava and other host plants in Madagascar. In addition, cassava mosaic disease (CMD) incidence and symptom severity were assessed. The identity and genetic diversity of *B. tabaci* samples collected on cassava and other plant species in Central Highlands and Tsaratanana Massif in northwestern region were studied using the partial mitochondrial cytochrome oxidase I (mtCOI) gene. The analyses of the mtCOI sequences revealed three *B. tabaci* cryptic species—Sub-Saharan Africa 1 (SSA1), Mediterranean (MED) and the Indian Ocean (IO)—in the sampled areas. SSA1 was the dominant cryptic species, with 100% occurrence on cassava crops. For the first time, we report the occurrence of MED in Madagascar. Both MED and IO, the indigenous species in the South West Indian Ocean islands of Anajouan Mayotte, Grande Comore, Mauritius, Reunion, Seychelles and Madagascar, occurred on non-cassava hosts. As opposed to previous reports, we recorded no *B. tabaci* Middle East Asia Minor 1 – MEAM1 cryptic species (formerly also known as the B-biotype) on any of the sampled plants and locations. Generally, the abundance of adult whiteflies was low (<1 specimen per plant) on cassava in all the sampled locations, except in Analavory (3.93) and Ampitolova (3.00) in Central Highlands. Similarly, the whitefly abundance was low on the non-cassava plant species, likely hosts for *B. tabaci* IO and MED. Cassava mosaic disease was observed in 100% of the surveyed cassava fields. The disease symptoms were generally mild, with severities of 2.00–3.13 (average, 2.62). Locations differed significantly ($P<0.001$, LSD=5.00) in CMD incidences. The CMD incidence ranged between 30–100% (averaged *ca* 59%). Our findings provide current knowledge of the economically important *B. tabaci* species, which is vital to the development of sustainable management practices for the vector and cassava viral diseases in Madagascar.

KEYWORDS: Agricultural pests, cassava, *B. tabaci*, whitefly, genetic diversity, cryptic species, SSA1, MED, Afrotropical, Madagascar.

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INTRODUCTION

Bemisia tabaci (Gennadius, 1889) (Hemiptera: Aleyrodidae) is a pest of global importance due to its ability to transmit many plant viruses, particularly begomoviruses (Geminiviridae), to agricultural crops and cause considerable yield losses (Brown *et al.* 1995) in the tropical (Brown 2000) and subtropical (Brown & Bird 1992; Fishpool & Burban 1994; Jones 2003) regions of the world. There are at least 34 putative morphologically indistinguishable cryptic species that comprise this complex agricultural pest (Boykin *et al.* 2012, 2018). In high abundance, *B. tabaci* causes physical damage to plants through extraction of large quantities of phloem sap, induction of phytotoxic disorders, irregular fruit ripening, chlorosis of leaves and honey dew excretes, which encourage growth and development of sooty mold fungi that darkens foliage and fruit (Byrne & Bellows 1991; Costa & Brown 1991). In cassava (*Manihot esculenta* Crantz), physical damage by *B. tabaci* was reported to cause between 40–50% yield loss (Thresh *et al.* 1997; Legg *et al.* 2011).

In Africa and Asia, *B. tabaci* is the key vector of cassava mosaic begomoviruses (CMBs) (Bock & Woods 1983; Dubern 1994; Fishpool & Burban 1994), the causative agents of cassava mosaic disease (CMD). *Bemisia tabaci* was also reported to transmit cassava brown streak ipomoviruses (CBSIs) causing cassava brown streak disease (CBSD), albeit at low rates (Maruthi *et al.* 2005, 2017; Mware *et al.* 2009). Cassava mosaic disease seriously limits the production of cassava, a major staple crop for more than 300 million households in Sub-Saharan Africa (SSA) (Horton 1988; IITA 1990; Dahniya 1994). Annual economic losses attributed to CMD alone in East and Central Africa have been estimated at US$ 1.9–2.7 billion (Legg *et al.* 2006). The two viral diseases continue to devastate cassava crops in eastern, central and southern Africa, where they threaten food security of the mainly rural households (Alicai *et al.* 2007, 2016; Bigirimana *et al.* 2011; Legg *et al.* 2011, 2014, 2015; Mulimbi *et al.* 2012; Ndunguru *et al.* 2015; Patil *et al.* 2015; Ateka *et al.* 2017; Maruthi *et al.* 2017).

In Madagascar, CMD was first reported in 1932, but was of minor importance at that time (Francois 1937). In 1934, a CMD outbreak was reported west of Lake Alatroa on the central plateau, which spread in many cassava-growing areas of the island (Cours 1951) and resulted in the abandonment of cassava cultivation (Frappa 1938). The government intervened by initiating the first major cassava germplasm improvement program, which aimed at development of CMD-resistant varieties (Cours 1951). The first resistant varieties were widely distributed in the 1940s and drastically reduced the CMD frequency. In 1998, a country-wide survey was carried in key cassava producing regions of Madagascar to establish the status of whiteflies and whitefly-transmitted viruses (WTVs) on cassava and sweet potato by the System-wide Tropical Whitefly IPM project (Ranomenjanahary *et al.* 2005). The study found adult whitefly populations on cassava to be highest in Antananarivo (7.1) and lowest in Mahajanga (2.5), while whiteflies

were very scarce on sweet potato. The CMD incidence, mainly due to use of virus-infected cuttings, was responsible for 86% of all cases and was highest (71%) in Fianarantsoa and lowest (31%) in Antananarivo. The CMD symptoms were relatively mild in Antananarivo, but severe elsewhere (Ranomenjanahary *et al.* 2005).

Until recently, genetic methods targeting the diversity of the mitochondrial cytochrome oxidase I (mtCOI) gene were considered most reliable for distinguishing whitefly populations and cryptic species occurring on different crops and plant species (Brown 2000; De Barro *et al.* 2000, 2011; Bosco *et al.* 2006; Dinsdale *et al.* 2010). At least five *B. tabaci* cryptic species—SSA1, SSA2, SSA3, SSA4 and SSA5—have been reported on cassava in the Afrotropics (Legg *et al.* 2002, 2014; Sseruwagi *et al.* 2005, 2006; Boykin *et al.* 2007, 2018; Dinsdale *et al.* 2010; Mugerwa *et al.* 2012; Esterhuizen *et al.* 2013; Tocko-Marabena *et al.* 2017). In Madagascar and the SWIO Islands, previous studies identified the IO/MS and Middle East Asia Minor 1 (MEAM 1)/B biotype as the main *B. tabaci* cryptic species on cassava and several other hosts (Delatte *et al.* 2005, 2006, 2012). Recently, Wosula *et al*. (2017) used NextRAD sequencing of whitefly genomic DNA and identification of single nucleotide polymorphisms (SNPs), as well as comparisons of mtCOI sequences from the same whiteflies, to demonstrate that whiteflies collected during their surveys on cassava in eight African countries (including Madagascar) belonged to the SSA group; however, only limited whitefly collections were made in Madagascar. The identified nuclear SNPs will complement the ongoing efforts to obtain a species tree for the *B. tabaci* species complex. The development of other markers, such as the nuclear gene markers, to distinguish *B. tabaci* species has been elusive for a long time, due to the requirement for large amounts of insect RNA for transcriptome studies. However, the recent successful identification of SNPs that can differentiate cryptic species and reveal some level of hybridization between cryptic species along their distribution range (Wosula *et al.* 2017), and the method of extraction of the RNA and transcriptomes of individual field-collected *B. tabaci* specimens (Sseruwagi *et al.* 2017) provide a great breakthrough. These methods may not only resolve the species identity issues, but also identify a gene flow to track hybrids of different *B. tabaci* cryptic species.

Madagascar's economy relies heavily on agriculture, which depends on small-scale subsistence farmers yet accounts for almost 30% of the gross domestic product and employs about 75% of the work force (Raveloharison 2017). Rice is the key staple of the Malagasy diet and its paddy production was 2,550,000 tons in 1999 (FAO 1999). However, following declining yields and severe damage to rice crops by cyclones in the 1980–1990s, cassava's untapped potential as a food security and industrial crop in Madagascar was recognized. A drive to oversee the development of the crop led to the introduction and screening of new germplasm (Abass *et al.* 2012). Currently, however, like elsewhere in SSA, cassava's productivity is limited by pests and diseases, among which CMD is most important. To address threat to food security caused by CMD, collaborative efforts were established in

2017 between Mikocheni Agricultural Research Institute based in Tanzania and authorities in Madagascar, to share experience and expertise in diagnostics and management of cassava viral diseases and their insect vectors. The activity was carried out under the 'Disease diagnostics for sustainable cassava productivity in Africa' project, a regional effort that sought to build human and infrastructure capacity of National Agricultural Research Systems in seven countries in east and southern Africa, including Kenya, Malawi, Mozambique, Rwanda, Uganda, Zambia and Tanzania. Therefore, the main objective of this study was to establish diversity and abundance of *B. tabaci* species on cassava and several other host plant species in Madagascar. In addition, we also assessed the incidence and symptom severity of CMD and CBSD in small-holder farmers' fields.

MATERIALS AND METHODS

Whitefly and disease assessments

Adult whitefly abundance, CMD incidence and symptom severity were assessed as described by Sseruwagi *et al*. (2004) on 15 plants per field, in 3–5 months old cassava fields selected randomly at equal intervals along motorable roads in 11 locations, viz. Amboatany, Ambodahy, Ambohitravao, Amborovy, Ambovona, Ampitolova, Analavory, Itasy, Tsararano (Central Highlands region), Antsanitia and Betangirika (Tsaratanana Massif region in North-west), in January 2017. In each field, adult whitefly populations were counted on the top five leaves per plant. Cassava mosaic disease infection and symptom severity were assessed per plant using a scale of increasing severity from 1 (no symptoms) to 5 (very severe symptoms) (Hahn *et al.* 1980). Disease incidence was calculated as the proportion or percentage of plants with CMD symptoms (Fargette 1987). Geo-coordinates (latitude, longitude and altitude) were recorded for each location using a global positioning system (GPS) (etrex, HC Sumit) and the data (Table 1) used to construct a map showing the geographic distribution of sampled areas in Madagascar.

Whitefly sample collection and DNA extraction

Adult whiteflies were collected on cassava and non-cassava weed and shrub species growing within and near the sampled cassava fields in each location, using an aspirator and placed in Eppendorf tubes with 90% ethanol for laboratory analyses at Mikocheni Agricultural Research Institute, Dar es Salaam, Tanzania. At least three single female adult whiteflies were selected per plant species per location and genomic DNA was extracted according to Frohlich *et al*. (1999). Each insect was placed in a small well with 10 µl of extraction buffer (5 mM Tris-HCl, pH 8.0, 0.5 mM EDTA, 0.5% Nonidet P-40, 1 mg/mL proteinase K) on an inverted petri dish covered with parafilm and was gently ground using the tips of 0.2 µl polymerase chain reaction (PCR) tubes to release total DNA. Additional 30 µl of extraction buffer were added to each spot and the mix was transferred to new Eppendorf tubes. The extracts were incubated in a water bath (Gesellschaft für

Table 1. Locations and host plant species sampled for *Bemisia tabaci* in Central Highlands (CH) and Tsaratanana Massif/northwestern regions (TM) of Madagascar, January 2017. In Antananarivo, only one plant, a wild cassava relative was sampled. It was not considered as a farmer's field and therefore the location is not included in the disease assessments.

No.	Location	Region	Longitude	Latitude	Alt., m	Host plant	Sample ID
1	Antananarivo	CH	47.47805	-18.8700	1268.80	Wild Cassava	-
2	Ambohitravao	CH	47.55472	-18.8036	1277.95	Unknown	2A, 2B
3	Amboatany	CH	47.53750	-18.7622	1415.20	Cassava	-
4	Ambovona	CH	47.72030	-18.8111	1442.65	Cassava	-
5	Itasy	CH	47.14550	-19.0142	1274.90	Cassava	-
6	Analavory	CH	47.04083	-18.9866	1186.45	Unknown	6A
						Cassava	6B, 6C
7	Itasy	CH	46.72694	-18.0367	1241.35	Cassava	7B, 7C
8	Tsararano	CH	46.89098	-17.2123	308.00	Cassava	-
9	Ambodahy	CH	46.89556	-16.3501	92.30	Cassava	-
10	Amborovy	CH	46.35214	-15.6712	-16.17	Unknown	10A–C
11	Antsanitia	TM	46.42177	-15.5733	1.78	Cassava	11A–C
12	Ampitolova	CH	46.37484	-15.6604	11.89	Cassava	-
13	Betangirika	TM	46.40674	-15.6361	-15.86	Unknown	13A
						Cassava	13B, 13C

Labortechnik mbH, Germany) at 65 °C for 15 min and at 95 °C for 10 min using a block heater (Grant QBD2, England). The DNA was then centrifuged at 15,493× g for 5 min to pellet the debris. The supernatant was transferred to new Eppendorf tubes and the contents stored at -20 °C until use.

Mitochondrial DNA amplification and sequencing

Amplification of the partial mtCOI gene (850 bp) was obtained with the primer set: MT10/C1-J-2195 (5′-TTGATTTTTTGGTCATCCAGAAGT-3′) and MT12/TL2-N-3014 (5′-TCCAATGCACTAATCTGCCATATTA-3′) as per Simon *et al*. (1994). Polymerase chain reaction amplification was performed using a 2720 Thermal Cycler (Applied Biosystem, USA) as follows: first cycle at 95 °C for 2 min, 35 cycles at 94 °C for 30 sec, 54 °C for 30 sec, 72 °C for 1 min, and final extension at 72 °C for 10 min. A total reaction mixture of 25 µl was made up of 16.3 µl distilled water, 5 µl of 5X One Taq PCR reaction buffer, 0.5 µl of 10 mM dNTPs, 0.5 µl 10 µM of each primer, 0.2 µl of One Taq DNA polymerase (New England Biolabs, Ipswich, MA, USA) and 2.0 µl DNA template. PCR products were electrophoresed using Midicell Primo electrophoretic gel system in 1 % agarose gel stained in ethidium bromide at 100 V for 30 min in 1X TAE buffer. The gel was visualized and photographed using the UVP, BioDoc-It 201 Imaging System m-20V Trans illuminator, USA. For sequencing, the PCR products were shipped on dry ice to Fasteries SA, Plan-les-Ouates, Switzerland. The DNA sequences in this study are deposited in GenBank with accession numbers MG457760–MG457775.

Table 2. Result of blast algorithm of GenBank when comparing the most frequent *Bemisia tabaci* species from Madagascar to the worldwide diversity.

Query	Closest match	Verified nucleotide identity (%)	Accession no. of closest match	Country
6B	SubSahAf1	99.85	1251_32_SubSahAf1_JQ286457	Tanzania
6C	SubSahAf1	100	1251_32_SubSahAf1_JQ286457	Tanzania
7B	SubSahAf1	100	1251_32_SubSahAf1_JQ286457	Tanzania
7C	SubSahAf1	100	1251_32_SubSahAf1_JQ286457	Tanzania
11A	SubSahAf1	100	1251_32_SubSahAf1_JQ286457	Tanzania
11B	SubSahAf1	99.85	1251_32_SubSahAf1_JQ286457	Tanzania
11C	SubSahAf1	100	1251_32_SubSahAf1_JQ286457	Tanzania
13B	SubSahAf1	100	1251_32_SubSahAf1_JQ286457	Tanzania
13C	SubSahAf1	100	1251_32_SubSahAf1_JQ286457	Tanzania
13A	Indian Ocean	99.85	52_28_IndianOcean_AJ550171	Madagascar
2A	Indian Ocean	100	52_28_IndianOcean_AJ550171	Madagascar
2B	Indian Ocean	100	52_28_IndianOcean_AJ550171	Madagascar
10A	Mediterranean	99.71	358_29_Mediterranean_FJ766391	Burkina Faso
10B	Mediterranean	99.71	358_29_Mediterranean_FJ766391	Burkina Faso
10C	Mediterranean	96.01	117_29_Mediterranean_AY827606	Nigeria

Phylogenetic analysis

Initial species identification

The obtained sequences were loaded into the *Whiteflybase* (www.whiteflybase.org; Boykin *et al.* 2017), which allowed us to narrow down the reference data used in the downstream phylogenetic analyses. Thus, all sequences generated in this study matched *B. tabaci* SSA1, MED or IO with over 99% identical base pairs (Table 2), which meant that reference data only included unique haplotypes from SSA1, MED and IO in the MrBayes analyses (described below). The Uganda *B. tabaci* species was also included in the analyses as an outgroup. In addition, the New World and MEAM1 species were also included because they had been found elsewhere (Manani *et al.* 2017) to be in the same clade as the target species identified in our study. This contrasts with previous studies that recommended utilising all unique haplotypes in the phylogenetic analyses, which dramatically increases time to convergence for the MrBayes runs.

MrBayes phylogenetic analyses

The final alignment contained 491 of 687 nucleotides. We used MrBayes version 3.2.1 (Ronquist *et al.* 2012) that employs Markov Chain Monte Carlo (MCMC) sampling to approximate the posterior probabilities of phylogenies (Green 1995), the posterior probabilities are shown above the branches (Fig. 1). MrBayes 3.2.1 was run on the Magnus supercomputer (Pawsey Supercomputer Centre, Perth, Western Australia) utilising the BEAGLE library (Ayers 2012). MrBayes 3.2.1 was run with a GTR+I+G model of molecular evolution, utilising four chains for 30 million generations and trees were sampled every 1000 generations. All

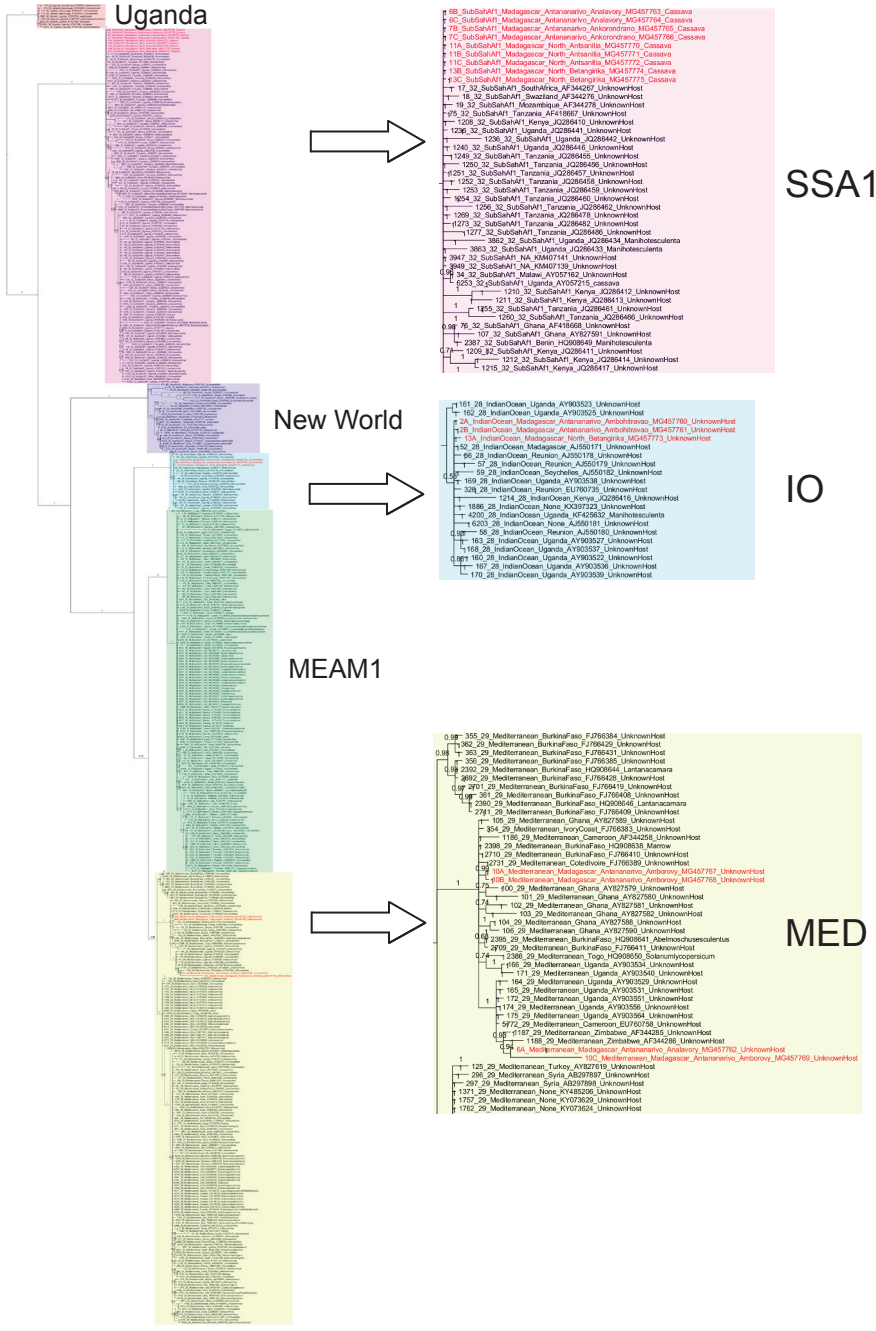

Fig. 1: MrBayes consensus tree for *Bemisia tabaci*. Madagascan samples are highlighted in red.

runs reached a plateau in likelihood score, which was indicated by the standard deviation of split frequencies (0.0015), and the potential scale reduction factor (PSRF) close to one, showing that the MCMC chains converged.

Statistical analysis of data

The whitefly population, CMD symptom severity and disease incidence data were tested for homogeneity of variance before analyses. Adult whitefly numbers (n) were logarithmically transformed (log(n+10)) to stabilize the variances due to zero counts in some of the cassava fields. The transformed data were subjected to a One-way analysis of variance (ANOVA) using Genstat statistical package version 12.1 (PC/Windows Vista), copyright 2009, VSN International Ltd.

RESULTS

Status of cassava fields and cultivars grown

A total of thirteen small-scale cassava fields were sampled in eleven locations with diverse agro-ecologies in the Central Highlands and Tsaratanana Massif/ Northwestern regions of Madagascar (Table 1). In all the locations visited, it was observed that the small-holder farmers were faced with multiple challenges concerning cassava production that included: (1) inferior cultivars of mainly low yielding landraces, (2) pests and diseases, and (3) poor husbandry practices. The main cultivars grown by the farmers across the two regions included *Orgaya*, *Madaras*, *Mahogo Pamba* and *Mahogo mena helika*.

Whitefly abundance, symptom severity and CMD incidence

Generally, very low numbers (mean = 0.85) of adult whiteflies occurred on cassava in all the surveyed locations. There were significantly lesser variations in adult whitefly abundance between cassava fields (P=1.000) than between sites (P<0.001, LSD=1.54). The whitefly abundance was highest in Analavory (3.93) followed by Ampitolova (3.00) in Central Highlands and was less than 1 (0–0.80) in the rest of the locations (Table 3). Similarly, whitefly abundance was low (<1) on non-cassava plants sampled for *B. tabaci*. However, a few farmers in Antsanitia, Tsaratanana Massif in the northwestern region, reportedly observed symptoms of sooty mold on cassava leaves in June, when populations are likely to be more abundant.

Cassava mosaic disease affected plants had typical mild green and yellow mosaic symptoms. There were no significant differences (P=0.43, LSD=0.78) in mean CMD symptom severity between locations. Disease symptom severity ranged between 2.00–3.13 and averaged only 2.62 (Table 3). Based on visual assessments of symptoms, CMD was observed in all surveyed fields. Locations differed significantly (P<0.001, LSD=5.00) in CMD occurrence. The incidence ranged between 30–100%, with an average of 59.29% (Table 3), and was highest in Betangirika (100%), Tsaratanana Massif/northwestern region, and Ampitolova (86.6%) in Central Highlands, and 60% and above in Amborovy (60%), Itasy

Table 3. Mean adult whitefly abundance and cassava mosaic disease incidence (%) and symptom severity (1–5 scale) in small-holder cassava fields in Central Highlands (CH) and Tsaratanana Massif/ northwestern (TM) regions of Madagascar, January 2017.

Location	Region	Mean abundance	S.E.	CMD incidence	S.E.	CMD severity	S.E.
Amboatany	CH	0.13	0.56	46.16	1.82	2.29	0.31
Ambodahy	CH	0.40	0.56	66.60	1.82	2.82	0.25
Ambohitravao	CH	0.40	0.40	36.65	1.28	2.91	0.25
Amborovy	CH	0.80	0.56	60.00	1.82	2.38	0.29
Ambovona	CH	0.00	0.56	40.00	1.82	2.67	0.33
Ampitolova	CH	3.00	0.56	86.60	1.82	2.62	0.23
Analavory	CH	3.93	0.56	33.30	1.82	2.00	0.36
Ankorondrano/Itasy	CH	0.57	0.40	63.30	1.28	2.74	0.19
Tsararano	CH	0.00	0.56	46.60	1.82	2.57	0.31
Antsanitia	TM	0.13	0.56	73.00	1.82	2.73	0.25
Betangirika	TM	0.00	0.77	100.00	2.49	3.13	0.29
Means		**0.85**		**59.29**		**2.62**	
LSD (5%)		**1.54**		**5.00**		**0.78**	
P (5%)		**<0.001**		**<0.001**		**0.433**	

(63.3%), Ambodahy (66.6%) Antsanitia (73%). Incidence was 30–50% in other sampled locations. The main source of disease infection was cutting-borne, which means that farmers were recycling the CMD-affected planting materials from previous seasons' crops. None of the disease-affected cassava plants had clear CBSD symptoms in the fields and locations sampled in our study.

Phylogenetic analysis of adult *Bemisia tabaci* mtCOI sequences

Bayesian analyses of the 15 sequences obtained from Madagascar (this study) and the 476 unique haplotypes in the *Whiteflybase* reference data for species identification (www.whiteflybase.org) revealed three cryptic species present in the sampled areas. Nine of the 15 *B. tabaci* samples were SSA1, three were IO and four turned to be MED (Fig. 1). The SSA1 sequences grouped together with those from southern Africa, i.e. South Africa, Swaziland and Mozambique. On the other hand, the IO sequences grouped with those obtained in Madagascar, Reunion, Seychelles and Uganda. The MED sequences grouped with similar haplotypes from Uganda, Zimbabwe and West Africa (Burkina Faso, Ghana and Ivory Coast). None of the mtCOI sequences grouped with the MEAM1 (B-biotype) genetic group in our study.

Geographical distribution of *B. tabaci* cryptic species

Three *B. tabaci* cryptic species were identified on the sampled plants in Madagascar in our study, viz. SSA1, IO and MED. *Bemisia tabaci* SSA1 occurred mainly on cassava in all the sampled locations in the Central Highlands and Tsaratanana Massif/northwestern regions. It was the only cryptic species identified on cassava. *Bemisia tabaci* IO occurred on non-cassava plant species in Betangirika,

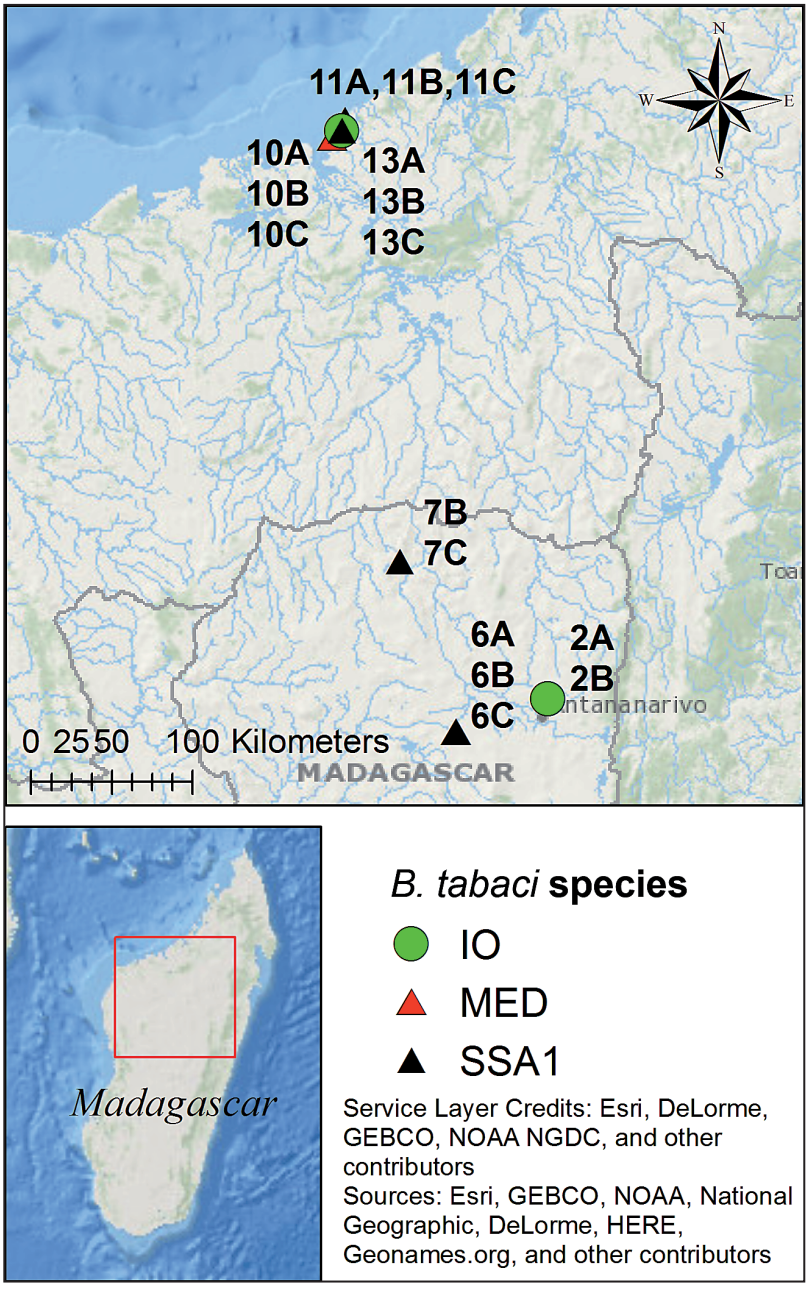

Fig. 2: The geographical distribution of *Bemisia tabaci* species in Central Highlands and Tsaratanana Massif/northwestern regions of Madagascar, January 2017.

Tsaratanana Massif/northwestern region and in two locations in Ambohitravao, west of Antananarivo in Central Highlands, while *B. tabaci* MED similarly occurred on the non-cassava plants in only Ambohitravao, Central Highlands region. In general, however, there was no correlation between the cryptic species and their geographic distribution in Madagascar (Fig. 2).

DISCUSSION

Our study established SSA1 to be the dominant cryptic species of *B. tabaci* on cassava in Central Highlands and Tsaratanana Massif/northwestern regions of Madagascar, occurring in 100% of the sampled cassava fields. We report for the first time the occurrence of the *B. tabaci* MED cryptic species in Madagascar, which, together with the IO, occurred on non-cassava plants in the sampled areas. Previous studies reported the IO and MEAM 1 as the main *B. tabaci* cryptic species on cassava and several other hosts in Madagascar and the SWIO Islands of Anjouan, Mayotte, Grande Comore, Mauritius, Reunion and Seychelles (Delatte *et al.* 2005, 2006, 2012). However, in the current study none of the cassava fields hosted IO or MEAM1. The occurrence of SSA1 in Madagascar is important, because it is associated with the transmission and spread of CMBs (Dubern 1994) and cassava brown streak viruses (CBSVs) (Maruthi *et al.* 2005, 2017; Mware *et al.* 2009) in Sub-Saharan Africa. In addition, SSA1 is the most widely distributed cryptic species currently associated with the upsurge in whitefly populations on cassava in East and Central Africa (Legg *et al.* 2011, 2014, 2015; Mugerwa *et al.* 2012, 2013; Tajebe *et al.* 2014) and rapid spread of a severe CMD outburst (Legg *et al.* 2002, 2014; Tajebe *et al.* 2014) that threatens food supply for millions of rural households dependent on cassava.

We confirm and expand (by using a larger number of samples) on the findings by Wosula *et al*. (2017), who studied a few samples using NextRAD sequencing of whitefly genomic DNA and identification of SNPs, as well as comparisons of mtCOI sequences from the same whiteflies, to demonstrate that SSA1 is the primary cryptic species occurring on cassava in Madagascar. The low abundance of adult whitefly (<1 specimen per plant) on cassava in the sampled fields and locations in our study may be attributed to a characteristic feature of the SSA1 *B. tabaci* populations in southern Africa that were reported to have low density on cassava (Legg *et al.* 2002; Berry *et al.* 2004). The SSA1 haplotypes from Madagascar (our study) grouped with the southern Africa clade including South Africa, Swaziland and Mozambique. However, the low whitefly populations recorded in our study may also reflect sampling in the 'low season' of the year, when whiteflies are least abundant as reported by some farmers in Antsanitia, Tsaratanana Massif in northwestern region, who observed symptoms of sooty mold on cassava plants in June. The development of sooty mold fungi that darkens foliage and fruit is due to excretion of honeydew usually by a large population of *B. tabaci* colonizing crops (Byrne & Bellows 1991). Cassava disease surveys in

Madagascar (Ranomenjanahary *et al.* 2005) and elsewhere in Sub-Saharan Africa (Sseruwagi *et al.* 2004; Jeremiah *et al.* 2007, 2015; Ndunguru & Tairo 2009, 2010; Ndunguru *et al.* 2011) have been carried when whitefly populations are lowest (July–September). Furthermore, since surveys are based on one-time assessments, they tend to underestimate the populations that occur on the cassava crops in an area in the cropping season (P. Sseruwagi, unpubl. data). Therefore, January may not have been an optimum period to sample for whitefly in Madagascar. Other factors including temperature, altitude, cultivar, have also been reported to influence whitefly abundance (Macfadyen *et al.* 2018). More studies should be conducted to understand the factors for the differences in abundance of the SSA1 populations in east and southern Africa.

The level of CMD was moderate to high (30–100%), with typical cutting-borne symptoms, i.e., damage occurring on the lowermost first-formed leaves of the affected plants (Sseruwagi *et al.* 2004), thus confirming that the disease was caused by propagation of virus infected cuttings. This is consistent with earlier findings, that reported 86.3% of total infection due to the same reason in 1998 (Ranomenjanahary *et al.* 2005). After these two decades since the last major survey in Madagascar, CMD remains a major problem in small-holder cassava fields. The situation is worsened by the evident lack of improved disease- and whitefly-resistant cassava cultivars in Madagascar, a testimony to the fact that more research is still needed to develop cassava germplasm for control of *B. tabaci* and WTVs, which was featured as a priority area and focus of the National Center for Applied Research and Rural Development (FOFIFA), the official agricultural research agency in Madagascar (Tairo *et al.* 2017).

CONCLUSIONS

This work highlights current knowledge of the *B. tabaci* cryptic species of economic importance to cassava and other plant species in Madagascar. It is through understanding their biology and implications for disease epidemiology that the development of sustainable management strategies for the vector and cassava viral diseases can be achieved. We have provided a novel bioinformatics routine for identifying *B. tabaci* cryptic species using the newly developed species identification tool – the *Whiteflybase* (www.whiteflybase.org). In the past, members of the *B. tabaci* cryptic species complex were identified using a large mtCOI dataset with all of the haplotypes, which required a supercomputer to analyze the data. We have shown that utilising the species tool in *Whiteflybase* allows the researcher to narrow down the reference data for the phylogenetic analyses therefore making species identification easier and faster.

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Field collection of *B. tabaci* samples: PS, LB, FT, JN, JU, RH.
Laboratory analysis: PS, TF, DK.
Sequences analysis and phylogenetic tree construction: LB, AS, PS.
Writing: PS, LB, FT, JN, JU, DK, AS.