Genetic diversity of aphid (Hemiptera: Aphididae) species attacking amaranth and nightshades in different agro-ecological zones of Kenya and Tanzania

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Aphids are major pests of African indigenous vegetables. Information on the genetic diversity and the role of host crop and environmental differentiation in their diversity in East Africa is scanty. The knowledge on genetic diversity is a critical component in the development of sound and sustainable integrated pest management strategy, from detection to control. A portion of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene was used to characterise the species of aphids on amaranth and nightshades at different agro-ecological zones of Kenya and Tanzania. Aphid samples were collected in localities growing the vegetables in low, mid and high altitude agro-ecological zones. Total DNA was isolated and amplified using universal barcoding primers targeting the 5′ end of the COI barcode region. Nucleotide sequences of the COI barcode, using the Basic Local Alignment Search Tool model, found high homology to four species of aphids: *Myzus persicae*, *Aphis fabae*, *Aphis craccivora* and *Macrosiphum euphorbiae.* Three subspecies of the *A. fabae* were also detected. Intraspecific diversity depicted *M. euphorbiae* having the lowest value, while *A. fabae* showed the highest diversity. Interspecific diversity between *A. fabae* and *A. craccivora* was the lowest while between *A. craccivora* and *M. persicae* it was the highest. The phylogenetic tree showed each species clustering together irrespective of the host crop or site where collected. Principal component analysis and haplotype network analyses confirmed these results. Low genetic diversity revealed by COI suggests that the environment or host crop contribute less to the genetic diversity of aphids in both countries.

Key words: cytochrome *c* oxidase, *Aphis fabae, Myzus persicae, Macrosiphum euphorbiae, Aphis craccivora*, agro-ecological zones, African indigenous vegetables.

INTRODUCTION

Amaranth and nightshades are widely grown African indigenous vegetables in many parts of East Africa where they play an important role in providing vital nutritional and economic benefits to the smallholder farmers (Seeiso & Materechera 2014). Unfortunately, they have received insufficient attention in mainstream food policies and were until recent times regarded as a poor man's food (Bosch *et al*. 2009). Amongst other challenges, pests continue to be the predominant impediments to their production. In Tanzania for example, arthropods and nematodes contribute to a loss of between 36 % and 42 % of amaranth and nightshade production in various agro-ecological zones (Keller 2004). Among these notorious arthropods are the aphids.

Aphids (Hemiptera: Aphididae) are among the most successful insects invading both food and non-food crops, including amaranth and nightshades worldwide. With documented species numbers of about 5012 (Favret 2014), aphids have the capacity to cause significant yield loss. Universally, aphids have been reported to cause over 70 % loss in the production of crops (Aslam *et al*. 2007). Directly, aphids pierce the stems and leaves of the plant using sharp mouthparts called stylets to obtain sap from the phloem tissues, eventually destroying the crop by depriving it of nutrients (Blackman & Eastop 2000). In the event, aphids indirectly transmit up to 50 % of plant potyviruses (Gray & Banerjee 1999). Moreover, aphids secrete honeydew, a sugary substance, which not only exposes the leaves to sunburn but also act as an attractant for secondary infections such as the sooty moulds that prevent the physiological functions of the plant and reduce the market appeal of

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the vegetables (Blackman & Eastop 2000; Lee *et al*. 2010).

Aphid species diversity is influenced by host-crop and environmental association (Miller & Foottit 2009; Peccoud *et al*. 2010). Since the availability and suitability of crops change seasonally, aphids are likely to become polyphagous and mobile. Thus, aphids tend to develop high morphological plasticity (Figueroa *et al*. 2005; Brévault *et al.* 2008). For example, species of the black bean aphids (*Aphis fabae*) are reported to exist in a complex of several unresolved subspecies (Béji *et al*. 2013). This makes both identification and understanding of their genetic makeup difficult. Furthermore, aphids depict sexual dimorphism in which some reproduce parthenogenetically where female aphids produce other females (anholocycly); others are holocylcic where a single sexual phase is included in the reproductive cycle (Orantes *et al.* 2012). In relation to host range, aphids can be either moneoecious, completing their life cycle in one host or heteroecious, spending some time in a primary host and move to a secondary host depending on the weather conditions and the availability of either primary or secondary host crop at a particular time of the year (Blackman & Eastop 2000).

The liberalisation of trade among the East African countries requires the development of an anticipatory model with the capacity to identify taxa that are deemed invasive in a country. Characterisations of aphid species on world crops have primarily relied on the morphological features and host crop association (Dixon 1998; Foottit *et al*. 2008; Chen *et al*. 2012). However, morphological features cannot be used to reliably discriminate aphid species that bear cryptic allometric features in many body parts like members of the genus *Aphis*. Because of these shortcomings, advancement of molecular biology techniques has since helped in fingerprinting the taxonomy of otherwise cryptic species, screening of germplasms, genetic diversity studies as well as testing accession stability and integrity (Kameswara 2004). Molecular markers are known to be versatile tools that give objective analysis of data, give results that are reproducible, are not amenable to environmental influence and demonstrate polymorphism. Besides, molecular markers occur frequently within the genome (Jonah *et al*. 2011) hence necessitating studies on a wide variety of functions such as generation of molecular maps. Some of the modern molecular markers include: Restriction fragment length polymorphism (RFLP), Random amplified polymorphic DNA (RAPD) which reveal molecular polymorphism instantly but have the disadvantage of being non-reproducible and therefore rendering the results unreliable, Amplified fragment length polymorphism (AFLP), Microsatellites or Simple sequence repeats (SSRs), Single nucleotide polymorphisms (SNPs), diversity arrays technology (DArT) and Mitochondrial DNA portions (Liu & Cordes, 2004; Semagn *et al*. 2006). The mitochondrial DNA portions like the cytochrome *c* oxidase (COI) gene has been used to genetically identify and characterise animal species with up to 100 % precision (Herbert *et al*. 2003a).

The COI gene is a eukaryotic marker whose portion (barcode region) has been used as a standard marker for bio-identification and genetic characterisation of more than 80 % of animal species (Coeur d'Acier *et al*. 2014), including aphids. Because of this, it has been referred to as the standard barcode (Herbert *et al*. 2003a; Herbert *et al*. 2003b; Hajibabaei *et al*. 2007; Wang & Qiao 2009; Rebijith *et al*. 2013). Unlike morphological identification, the barcode region allows for reliable identification, to species and subspecies level, at any developmental stage of the pest life cycle, including the eggs and degraded samples of animal specimens (Arif & Khan 2009). Furthermore, it lacks recombination events and possesses a robust resistance to molecular erosion (Castellana *et al*. Saccone 2011; Chen *et al*. 2012; Béji *et al.* 2015).

There is little information on the types of aphids infesting African indigenous vegetables in East Africa such as amaranth and nightshades. The current study aims at characterising the aphid species attacking amaranth and nightshades in various agro-ecological zones of Kenya and Tanzania using DNA barcoding. In particular, the study was undertaken to first identify the adult alate aphid species on these two vegetables and describe the role of three agro-ecological zones (high, mid and low) based on altitude, political boundaries and host crop in aphid species diversity in the two countries. This will form an important diagnostic component for management strategies to be put in place, for early detection systems of newly evolving biotypes, deployment of resistant crop cultivars and containment of invasive species.

MATERIAL AND METHODS

Study sites and sample collection technique

Adult and late instar pupae of aphids on cultivated amaranth and nightshades were collected from two localities in each of the six randomly selected counties in Kenya in February 2015 and from five Provinces in Tanzania in May 2015 (Tables 1 and 2, and Fig. 1). Sampling was done on crops growing at least 2 km apart to avoid collection of progeny from a single parthenogenetic female (Lokeshwari *et al*. 2015). At least 20 aphids were collected from a single crop, preserved in 95 % ethanol and transported to the International Centre of Insect Physiology and Ecology (ICIPE) Molecular Pathology Laboratories, in Arthropod Pathology Unit. The dorsal, ventral and lateral images of the insects were taken using the Leica LAS EZ4D stereo microscope at ×25 magnification prior to DNA extraction.

DNA isolation, quantification and qualification

Five insect samples from each locality were surface-sterilised in 3 % bleach (sodium hypochlorite) for a few seconds and rinsed twice with distilled water. Genomic DNA was extracted from individual aphid using Isolate II Genomic DNA Kit (Bioline, U.K.) according to the manufacturer's protocol. The quantity and quality of DNA was determined using Nanodrop 2000/2000c Spectrophotometer (Thermo Fisher Scientific, U.S.A.). Voucher DNA specimen and insect samples are deposited under the first author's name at the Arthropod Pathology Unit of the International Centre of Insect Physiology and Ecology (ICIPE), Duduvile Campus, Nairobi, Kenya.

DNA amplification, purification and sequencing

The barcode region was amplified using LCO1490 Forward (5'-GGTCAACAAATCATAAAG ATATTGG-3') and reverse primer – HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATA-3) (Folmer *et al*. 1994). Polymerase chain reaction (PCR) involved a 10 μl reaction volume containing: DNase/RNase free PCR water (Bioline, U.K.), 5X MyTaq reaction buffer, 10 pmol/μl of each primer, 25 mM $MgCl_2$, 0.625U of MyTaq™ DNA polymerase (Bioline, U.K.) and 15–50 ng/μl of crude DNA extract. Amplification was done in an Arktik thermocycler (Thermo Fisher Scientific, U.S.A.) under the following optimised conditions: initial denaturation of 95 °C for 2 min, followed by 40 cycles of 30 s denaturation at 95 °C, 45 s annealing at 50.6 °C and elongation for 1 min at 72 °C. The resultant PCR products were loaded with a 6X loading dye into preformed wells in a 1.5 % agarose gel. PCR products were separated *via* electrophoresis at 80 volts for 40 min and a well with a 1 kb DNA ladder (Fermentas, Thermo Fisher Scientific, U.S.A.) for base pair scoring. In addition, a negative control was included in every set of reaction round. The gel was viewed under UV light and images of all gels documented using KETA GL imaging system (Wealtec Corp., U.S.A.).

Successfully amplified samples were excised and purified using Isolate II PCR and Gel Kit (Bioline, U.K.) according to the manufacturer's protocol then sequenced bi-directionally at Macrogen Inc. Europe Laboratories (Amsterdam, Netherlands).

Analyses of the barcode region

Nucleotide chromatograms of the sense and antisense sequences were subjected to quality assessment through manual editing using Chromas Lite 2.0 (Technelysium Pty Ltd, Queensland, Australia). The homology searches were performed using the basic local alignment search tool for nucleotides (BLASTn) algorithm (Zhang *et al*. 2000) hosted at the National Centre for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/). Clustal X 2.1 (Thompson *et al*. 1997) generated multiple sequence alignments; while Jalview (Waterhouse *et al*. 2009) helped to perform sequence trimming. The software jModelTest 2 (Darriba *et al*. 2012) was used to determine the best fitting model for the phylogenetic analysis. TrN+G model was chosen based on the Akaike information criterion corrected for small sample (AICc) and the Bayesian information criterion (BIC) as the best fitting model. The maximum likelihood estimations were obtained from TrN+G model under general time reversible (GTR) substitution model with gamma-distributed rate variation across sites in RAxML 8.0 (Stamakis 2014). In order to assess robustness of the clades, bootstrap values for node support were calculated for 1000 pseudo-replicates. Molecular divergence summary for family, genus and species were calculated using distance summary tool using pairwise distance model in the Barcode of Life Data systems (BOLD).

The matrix of interspecific genetic distances and the maximum likelihood (ML) estimation of tran-

Table 1. Summary of Kenyan aphid samples collected in various agro-ecological zones.

Species name	Analysis code	Host crop	Site collected	Altitude (m a.s.l.)	GPRS coordinates	Accession numbers
M. persicae	MpBo1, MpBo2, MpBo3, MpBo4, MpBo5	Amaranth	Borabu (Kisii)	1881	00°79.929′S 035°01.373′E	KY323047, KY323046, KY323045, KY323044, KY323043
M. persicae	Mpkt1, Mpkt2, Mpkt3, Mpkt4	Amaranth	Kitutu (Kisii)	1869	00°68.989′S 034°86.338′E	KY323037, KY323036, KY323048, KY323035.
M. persicae	Mpka1, Mpka2, Mpka3, Mpka6, Mpka7	Nightshade	Kangaru (Embu)	1493	01°12.179′S 036°43.063′E	KY323042, KY323041, KY323040, KY323039, KY323038.
A. fabae	AfBo2	Amaranth	Borabu (Kisii)	1861	00°79.929′S 035°01.373′E	KY323029
A. fabae	AfLa1, AfLa3, AfLa7	Amaranth	Lari (Kiambu)	2252	00°59.185′S 036°37.146′E	KY322948, KY322947, KY322946.
A. fabae	AfKr3, AfKr5, AfKr6	Nightshade	Karura (Kiambu)	1844	01°12.188′S 036°43.059′E	KY323026, KY323025, KY323024.
A. craccivora	AcBo1, AcBo3, AcBo4, AcBo5	Amaranth	Borabu (Kisii)	1862	00°79.929′S 035°01.373′E	KY322905, KY322904, KY322903, KY322902
A. craccivora	AcKi2, AcKi3, AcK7, AcKi8, AcKi10, AcKis4, AcKis19, AcKis20.	Amaranth	Kisauni (Mombasa)	20	03°59.835′S 039°43.496′E	KY323013, KY323012, KY323011, KY323010, KY323009
A. craccivora	AcNy1, AcNy3, AcNy4, AcNy6, AcNy7	Amaranth	Nyali (Mombasa)	21	04°03.292′S 039°41.098′E	KY322994, KY322993, KY322992, KY322991, KY322990
A. craccivora	AcLi1, AcLi2, AcLi4, AcLi5, AcLi6	Amaranth	Likoni (Mombasa)	19	04°05.641′S 039°38.926′E	KY322989, KY322988, KY322987, KY322986, KY322985

Continued on p. 411

Table 1 (continued)

Species name	Analysis code	Host crop	Site collected	Altitude (m a.s.l.)	GPRS coordinates	Accession numbers
A. craccivora	AcMt2, AcMt3, AcMt5, AcMt6, AcMt7	Amaranth	Mtongwe (Mombasa)	22	04°04.974′S 039°38.429′E	KY322978, KY322977, KY322976, KY322975, KY322974.
A. craccivora	AcMa1, AcMa2, AcMa4, AcMa5, AcMa6	Amaranth	Magarini (Kilifi)	10	03°08.598′S 040°06.431′E	KY322973, KY322972, KY322971, KY322970, KY322969.
A. craccivora	AcMl1, AcMl2, AcMl3, AcMl4, AcMl5	Amaranth	Malindi (Kilifi)	3	03°09.858′S 040°05.248′E	KY322958, KY322957, KY322956, KY322955, KY322954.
A. craccivora	AcLa1, AcLa2, AcLa3, AcLa4, AcLa6	Amaranth	Lari (Kiambu)	2248	00°59.182′S 036°37.152′E	KY322953, KY322952, KY322951, KY322950, KY322949.
A. craccivora	AcKr1, AcKr2, AcKr3, AcKr4, AcKr5	Amaranth	Karura (Kiambu)	1884	01°12.179′S 036°43.063′E	KY322999, KY322998, KY322997, KY322996, KY322995.
A. craccivora	AcKa1, AcKa2, AcKa3, AcKa4, AcKa7	Amaranth	Kangaru (Embu)	1510	00°37.441′S 037°27.082′E	KY322891, KY322890, KY322889, KY322888, KY322887.
A. craccivora	AcKm1, AcKm3, AcKm4, AcKm5, AcKm6.	Nightshade	Kamiu (Embu)	1390	00°37.437′S 037°21.552′E	KY323004, KY323003, KY323002, KY323001, KY323000.
A. craccivora	AcKb3, AcKb4, AcKb6, AcKb7, AcKb8	Amaranth	Kimbimbi (Kirinyaga)	1203	00°37.135′S 037°21.553′E	KY322886, KY322885, KY323016, KY323015, KY323014.
M. euphorbiae	MeKm3, MeKm5	Amaranth	Kamiu (Embu)	1393	00°37.437′S 037°21.552′E	KY323034, KY323033

Table 2. Summary of Tanzanian aphid samples collected in various agro-ecological zones.

Species name	Analysis code	Host crop	Site collected	Altitude (m a.s.l.)	GPRS coordinates	Accession numbers
A. craccivora	AcMg1, AcMg2, AcMg3, AcMg5, AcMg7.	Amaranth	Morogoro Rural (Morogoro)	435	06°49.627′S 037°48.151′E	KY322945, KY322944, KY322943, KY322942, KY322941.
A. craccivora	AcBg1, AcBg2, AcBg3, AcBg4, AcBg5.	Amaranth	Bagamoyo (Pwani)	54	06°26.849′S 038°54.434′E	KY322910, KY322909, KY322908, KY322907, KY322906.
A. craccivora	AcMu1, AcMu2, AcMu3, AcMu5.	Amaranth	Muheza (Tanga)	216	05°10.600′S 038°48.002′E	KY322923, KY322922, KY322921, KY322920.
A.craccivora	AcLt1, AcLt2, AcLt3, AcLt4, AcLt5, AcLt6.	Amaranth	Lushoto (Tanga)	1124	04°50.879′S 038°20.129′E	KY322983, KY322982, KY322981, KY322980, KY322979.
A. craccivora	AcMs1, AcMs2, AcMs3, AcMs4, AcMs5, AcMs9, AcMs10, AcMs11, AcMs13.	Nightshade	Moshi (Kilimanjaro)	858	03°23.288′S 037°1.981′E	KY322932, KY322931, KY322930, KY322929, KY322928, KY322927, KY322926, KY322925, KY322924.
A. craccivora	AcHa3, AcHa4, AcHa5, AcHa6, AcHa7	Amaranth	Hai (Kilimanjaro)	1028	03°17.464′S 037°0.310′E	KY322896, KY322895, KY322894, KY322893, KY322892.
A. craccivora	AcH1, AcH2, AcH3, AcH4, AcH5.	Nightshade	Hai (Kilimanjaro)	1036	03°17.464′S 037°0.310′E	KY322933, KY322932, KY322931, KY322930, KY322929.
A. craccivora	AcMe1, AcMe2, AcMe3, AcMe4	Amaranth	Meru (Arusha)	1054	03°19.667′S 036°9.685′E	KY322968, KY322967, KY322966, KY322965
A. craccivora	AcMer2, AcMer3,	Amaranth	Meru (Arusha)	1053	03°19.667′S 036°9.685′E	KY322964, KY322963,

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Table 2 (continued)

Species name	Analysis code	Host crop	Site collected	Altitude (m a.s.l.)	GPRS coordinates	Accession numbers
	AcMer4, AcMer5, AcMer6, AcMer7.					KY322962, KY322961.
A. craccivora	AcAs1, AcAs2, AcAs3, AcAs5, AcAs8, AcAs9, AcAs10.	Nightshade	Arusha (Arusha)	1791	03°18.333′S 036°1.677′E	KY322884, KY322916, KY322915, KY322914, KY322913, KY322912, KY322911.
A. craccivora	AcMmr2, AcMmr3, AcMmr4, AcMmr5	Nightshade	Mt. Meru (Arusha)	1814	03°17.787′S 036°1.740′E	KY322936, KY322935, KY322934, KY322933.
A. craccivora	AcMm1, AcMm2, AcMm5, AcMm6	Amaranth	Mt. Meru (Arusha)	1854	03°18.122′S 036°1.957′E	KY322908, KY32290, KY322906, KY322905.
A. craccivora	AcTe3, AcTe6, AcTe7.	Nightshade	Tengeru (Arusha)	1222	03°23.123′S 036°8.463′E	KY322919, KY322918, KY322917.
A. fabae	AfMg1, AfMg6, AfMg8	Nightshade	Morogoro Rural (Morogoro)	445	06°50.088′S 037°47.213′E	KY323021, Y323020, KY323019.
A. fabae	AfLt4, AfLt6.	Nightshade	Lushoto (Tanga)	216	04°50.831′S 038°20.178′E	KY323023, KY323022.
A. fabae	AfMm1, AfMm3, AfMm5, AfMm6, AfMm7.	Nightshade	Mvomero (Morogoro)	585	06°57.609′S 037°31.936′E	KY323018, KY323031, KY323017, KY323030, KY323032.

sition/transversion bias were estimated using Kimura-2-parameter (K-2-P) model in MEGA 6.0 (Tamura *et al*. 2013). DNA sequence polymorphism (DnaSP v. 5.10.01) (Librado & Rozas, 2009) was used to generate the haplotypes, polymorphic, parsimony informative sites and haplotype diversity (*h*). Network 5.0.0.0 (Fluxus Technology Ltd) generated the haplotype network under the median-joining algorithm. Principal component analysis (PCoA) plot was constructed by GenAlEx 6.502 (Peakall & Smouse 2012) using the genetic distance matrix generated by MEGA 6.0. Sequences were submitted to the Barcode of Life Database (BOLD) and deposited in GenBank with accession numbers KY322884–KY323048 assigned to the samples as shown in Tables 1 and 2.

RESULTS

Species discrimination and sequences analyses

The data set consisted of 165 samples collected on amaranth and nightshades in both countries. Homology search positively identified four main

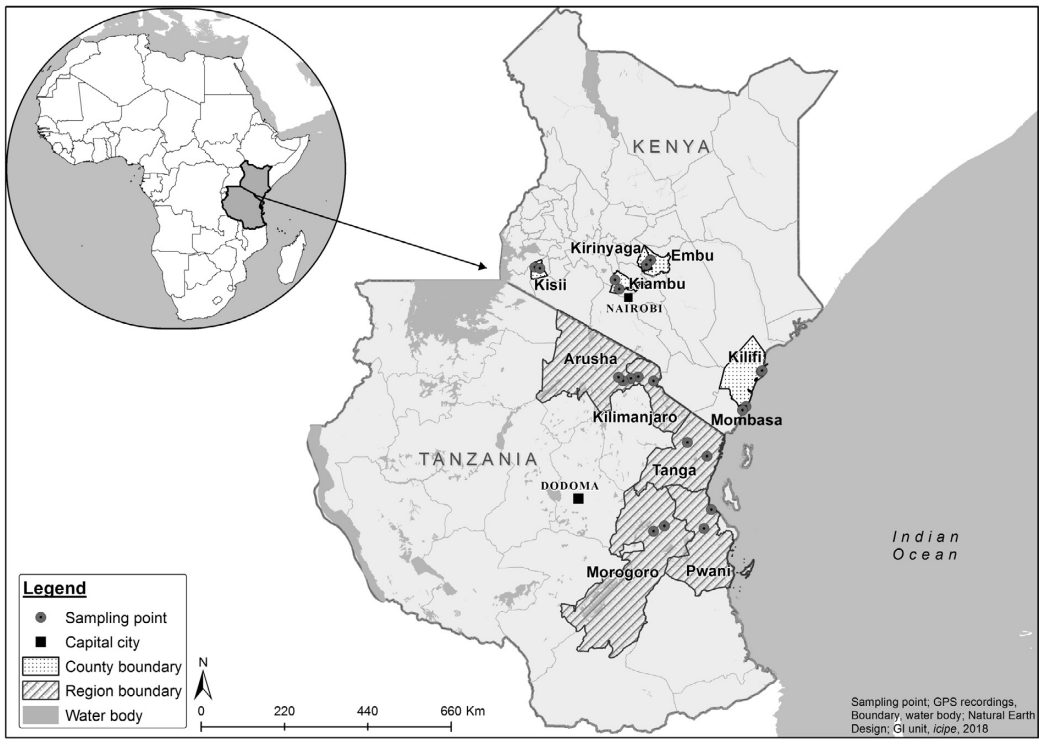

Fig. 1. Map of Kenya and Tanzania showing aphid sampling sites.

types of aphid species on the two host crops with ≥98 % hits. They are: the cowpea aphid, *Aphis craccivora* Koch, the black bean aphid, *Aphis fabae* Scopoli, green peach aphid, *Myzus persicae* Sulzer and the potato aphid, *Macrosiphum euphorbiae* Thomas. *Aphis fabae* and *M. euphorbiae* were found only on nightshades while *A. craccivora* and *M. persicae* were found on both crops.

The barcodes generated for all the samples were 662 bp in size. Out of this, 552 sites were invariable, 110 sites were polymorphic while 99 were parsimony informative sites. The average nucleotide composition (T = 40.8 %, C = 13.7 %, A = 35.0 % and G = 10.6 %) showed a bias towards thymine-adenine nucleotides. No stop codons were detected in any of the sequences analysed.

Intraspecific sequence divergence ranged from 0.0 (*M. euphorbiae*) to 0.07 (*A. fabae*). *Aphis craccivora* generated a 0.01 intraspecific divergence. The interspecific divergence (Table 3) showed that the genetic distance among the aphids sampled for *A. fabae* and *A. craccivora* was the lowest; whereas between *A. craccivora* and *M. persicae* was the highest. The average interspecific divergence for all the species collected was 0.0861. It also showed that *A. fabae* and *A. craccivora* are closely related as compared to *M. persicae* and *A. craccivora* which depicted the greatest interspecific divergence value of 0.106. The haplotype diversity (Hd) value was 0.464; whereas the nucleotide diversity (Pi) obtained was 0.0279.

The distance summary (Table 4) shows pairwise

Table 3. Estimates of evolutionary distances between four species of aphids sampled in Kenya and Tanzania obtained using the Kimura-2-Parameter model.

	A. fabae	*A. craccivora*	*M. persicae*	*M. euphorbiae*
A. fabae	0			
A. craccivora	0.063	0		
M. persicae	0.093	0.106	0	
M. euphorbiae	0.087	0.083	0.085	0

Table 4. Distribution of sequence divergence of different taxonomic levels of the four aphid haplotypes. The minimum, maximum, mean and standard error (S.E.) distance values were determined using the pairwise distance model in the distance summary tool hosted at the Barcode of Life Sciences.

	n	Taxa	Comparisons	Min dist (%)	Mean dist (%)	Max dist (%)	S.E. (%)
Within species	165	4	8990	0	0.29	6.8	0
Within genera	141	1	2128	0.3	5.89	7.4	0
Within family	165	1	2412	7.85	9.45	10.88	0

mean distance divergences between specimens of different genera belonging to the same family as 0.0589 (range 0.3–7.4) and mean pairwise divergence between samples of different families as 0.0945 (range 0.0785–0.1088) under a standard error (S.E.) of zero.

The phylogram (Fig. 2) shows samples of the same species clustering together strongly (≥99 %) in distinct clades independent of the locality or the host crop. The phylogenetic tree shows four main clusters representing each of the major species in the data set. Sub-clustering is seen on the members of the *A. craccivora* and *A. fabae.* For example, members of the *A. craccivora* collected on amaranth in Karura area (Kiambu County, Kenya) further formed a subcluster with a boostrap support value of 63%. Similarly, the *A. fabae* depicted the subclustering of the three subspecies of *A. fabae, i.e. A. fabae fabae, A. fabae solanella* and *A. fabae circiiacanthoidis* with an intraspecific divergence of 0.01.

The principal component analysis (PCoA) plot generated from the distance divergence matrix (Table 3) is shown in Fig. 3. The results showed that aphids of the same species clustered distinctly confirming results from the phylogenetic analysis. Clusters belonging to *A. fabae* and *A. craccivora* were found to be closer than those of *Myzus persicae* and *Macrosiphum euphorbiae* which were also closer to one another.

Furthermore, haplotype studies generated four haplotypes with an overall haplotype diversity (Hd) value of 0.464 (S.D. ± 0.048). The haplotype network (data not shown here) generated using median-joining algorithm revealed a clear separation of the various species of aphids in to their clusters per the number of substitutions between each of the four haplotypes (Hap1–Hap4). Hap1 composed of members of *A. craccivora.* This was the most dominant haplotype with 78.8 % (130 of the 165) samples in the study. Hap2 had 19 samples of *A. fabae*, Hap3 composed of 13 samples of *M. persicae* and Hap4 consisted of two samples of *M. euphorbiae*. The molecular diversity indices are as indicated in Table 5. The network shows how close or far away the collected aphid species evolved from each other. Fig. 3 shows *A. craccivora* and *A. fabae* to be closely related. Furthermore, the haplotype network also showed a close relationship between *M. persicae* and *M. euphorbiae*.

DISCUSSION

Knowledge on the types of aphids infesting amaranth and nightshades and their genetic diversity base is important in deployment of aphid resistant cultivars, understanding the susceptibility of species to environmental changes and selection pressures as well as development of pest management strategies (Xu *et al.* 2011). After

Table 5. Summary of molecular diversity indices of the mitochondrial cytochrome *c* oxidase I (COI) gene of aphids haplotypes collected in Kenya and Tanzania based on the Jukes and Cantor method.

Haplotype	Aphid type	No.	Hd (± S.D.)	Pi (± S.D.)	S	k
Hap1	*A. craccivora*	130	0.061 (0.029)	0.00021(0.00014)	8	0.138
Hap2	*A. fabae*	19	0.789 (0.099)	0.00567 (0.00150)	19	3.75
Hap3	*M. persicae*	14	0.0 (0.00)	0.0 (0.00)	0	0.0
Hap4	*M. euphorbiae*	2	0.0 (0.00)	0.0 (0.00)	0	0.0
All samples	All	165	0.464 (0.048)	0.0279 (0.00362)	107	18.481

Number of samples (No.), haplotype diversity (hd), nucleotide diversity (Pi) with standard deviations (S.D.), number of polymorphic (segregating) sites (S) and average number of nucleotide differences (k).

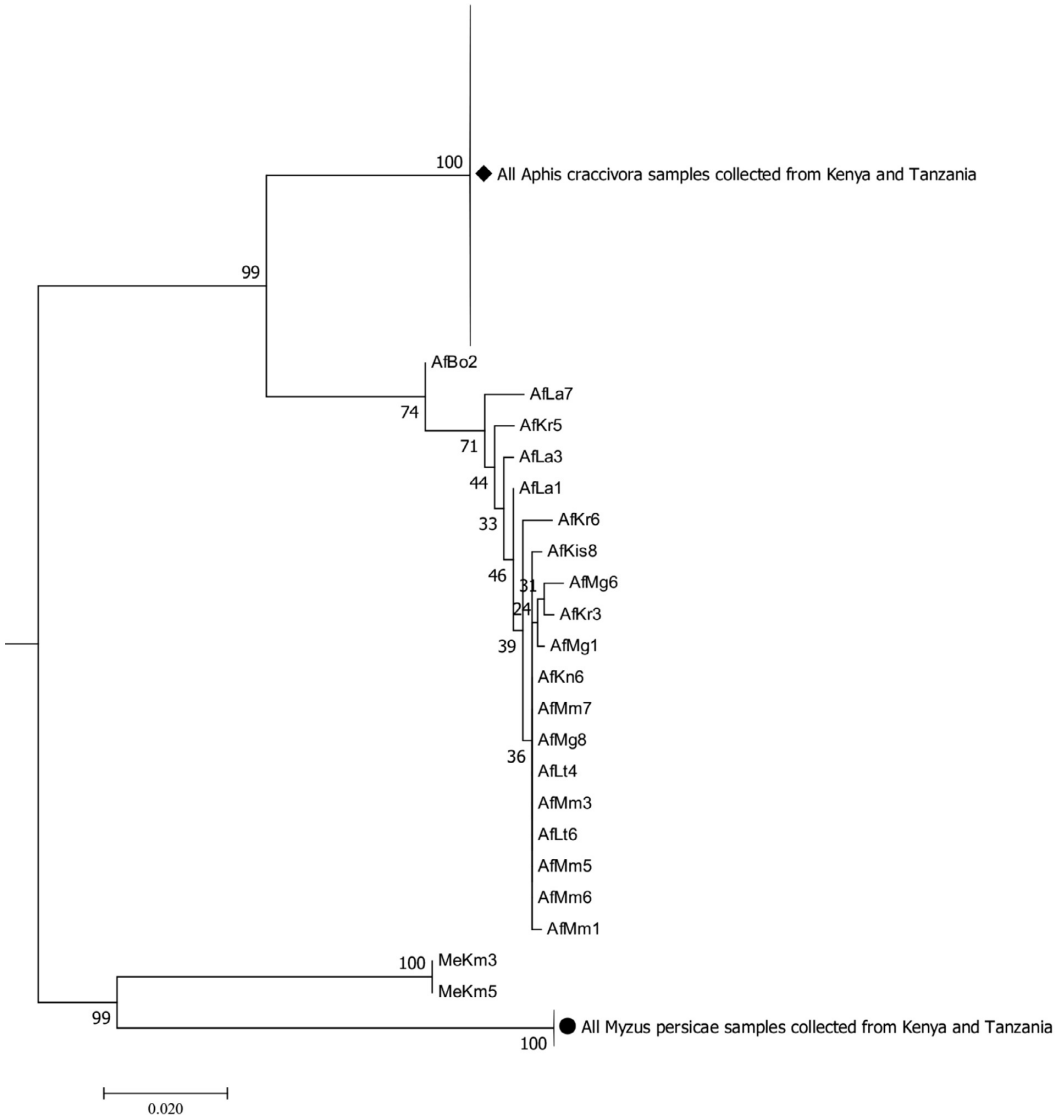

Fig. 2. The Maximum likelihood phylogenetic tree of the four aphid species collected in Kenya and Tanzania generated using TrN+G model under GTRγ in RAxMl. Values indicated at the nodes represent percentage bootstrap values after 1000 pseudoreplicates. The tree was drawn to scale and the branch lengths denote the rate of substitution per nucleotide position.

removal of primer sequences used in PCR, the effective sequences consisted of around 662 bp for all the 165 samples based on COI gene. Additionally, the sequences generated linked to four aphid species that attack the amaranth and nightshades in Kenya and Tanzania.

Among the identified species, the potato aphid, *Macrosiphum euphorbiae*, is reported here for the first time on amaranth and nightshades in the two countries. This was, however, a single observation on amaranth in Kamiu area of Kenya and this may suggest that amaranth may have been the preferred secondary host crop for the potato aphid during the dry season as this was the case at the sampling period. *Aphis fabae, A. craccivora* and *M. persicae* did not show host specificity, a feature that has largely been linked to these aphid species as reported by Blackman & Eastop (2000). This phenomenon could be as a result of unavailability of the primary host during certain times of the

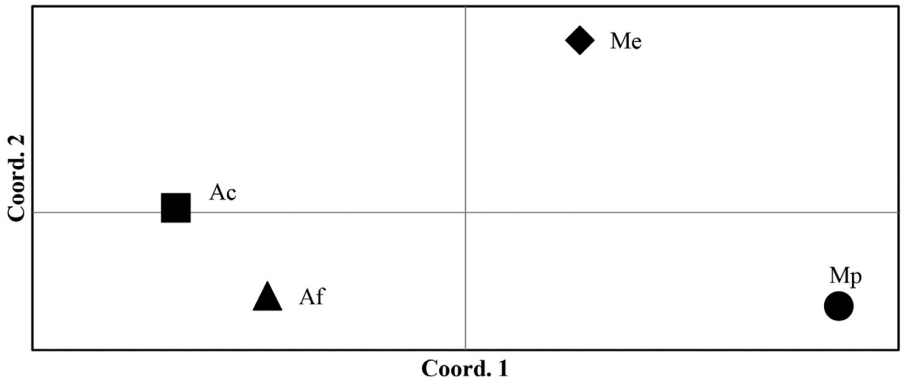

Fig. 3. PCoA plot generated in GenAlEx 6.502 for the interspecific distance matrix for the four aphid species collected in Kenya and Tanzania. The first and the second principal coordinates account for 60.97 % and 39.03 % of the variation, respectively.

year, a factor that forces aphids to be either oligophagous or polyphagous and therefore give aphids selective advantage (Raboudi *et al.* 2012). Living organisms increase their genetic diversity in order to successfully colonise and establish novel habitats; thus aphids tend to develop certain features that aid in adapting to their new habitats (Margaritopoulos *et al.* 1998). For instance, Margaritopoulos *et al*. (1998), using a morphological identification method, reported that the ultimate rostral segment in aphid species is known to vary with the host plant and the physiological status of the plant. Similarly, under restricted conditions, the body size and pigmentation of *M. persicae* were largely affected by the host plant (Clements *et al*. 2000).

The nucleotide bias towards adenine-thymine constituting 76.1 % depicted here is typical of other genetic characteristics of aphid species reported by other related studies that utilised the COI gene region (Kim *et al.* 2010; Wang *et al.* 2011; Chen *et al*. 2012; Papasotiropoulos *et al*. 2013; Rebijith *et al*. 2013; Kinyanjui *et al*. 2015). This is important since the two hydrogen bonds binding adenine and thymine requires lower amount of energy to break the DNA double helix during replication and subsequent transcription of this gene (Mitchel 1997). This region has also been linked to sequences responsible for control of replication and transcription of the mitochondrial genome (Krzywinskia *et al*. 2011). The higher intraspecific genetic divergence value as expressed by *A. fabae* in comparison to other species in this study can be explained by the existence of different morphotypes of this species (Béji *et al*. 2013). This result, however, is similar to a relatively lower genetic divergence in the population of *A. fabae* complex collected in Tunisia from different geographical zones (Béji *et al*. 2015). The mean family, genus and species distances as shown in Table 4 generated a normal divergence as expected at various taxonomic levels.

Furthermore, the current study showed various species clustering largely together irrespective of the host crop, country or the locality of collection. The clusters clearly separated one species from its nearest neighbour with a fairly strong bootstrap values indicating that species identification using homology search in the GenBank was precise. However, the cowpea aphids collected from Karura (Kiambu County, Kenya) formed a subcluster with a support value of 63 %. Such support was due to a single nucleotide transition (T/C) located in position 495 of the sequences analysed.

The overall mean interspecific divergence of 8.61 % generated is substantive to warrant identification using COI gene region between the various species of aphids collected from Kenya and Tanzania. The lowest interspecific divergence generated between the samples of *A. fabae* and *A. craccivora* is because the two belong to the same genus *Aphis*, and with almost indistinguishable morphological characters. *Aphis fabae* complex depict black or bluish-green colour and dorsal sclerites on the abdominal segment as the distinct morphometric traits found in its members while *Aphis craccivora*, has a shiny blackish-grey back colour with a shiny dorsal shield (Blackman & Eastop 2000). The greater sequence divergence between *M. persicae* and *A. craccivora* is in congruence with the study by Kinyanjui *et al*. (2016). Their morphological characteristics are quite distinct. Usually, the

adults of *M. persicae* are wingless, ranging in colour from pale greenish-yellow to various shades of green, pink and red. Its distinct frontal tubercles point inwards, cornicles are longer than cauda and of the same colour as the body, three longitudinal dark green stripes on the pear-shaped body (Liu & Sparks 2001). Besides, the two aphids belong to different genera. On the other hand, the intra-specific divergence values were least in *M. euphorbiae* which can be attributed to the low sample numbers rather than the differences in their genetic diversity. Studies on genetic diversity using microsatellite markers have revealed a general moderate to low diversity among the populations of *Aphis gossypii* in China (Wang *et al*. 2017); as well as among the populations of *Sitobion avenae* in China (Xin *et al.* 2014). On the contrary, Raboudi *et al*. (2011) using RAPD markers demonstrated a relatively higher genetic variability among 200 samples of *M. euphorbiae* sampled in Tunisia.

Samples of *Aphis fabae* showed paraphyletic distribution in the phylogenetic tree, consisting of three sister species, *Aphis fabae fabae* Scopoli, *A. fabae cirsiiacanthoidis* Scopoli and *Aphis fabae solanella* Theobald. However, these sister species are separated from one another by a very low genetic distance of 0.01. These members of the black bean aphid are quite similar in morphology. They coexist in their natural hosts and identification process using classical taxonomy is nearly impossible since the original classification relied on the ability of each subspecies to colonise their unique secondary host (Stroyan 1984). There is paucity of information on the genetic diversity of the black bean aphid subspecies based on COI gene region. Restriction of the mitochondrial ND5 region for the three subspecies with inclusion of *A. fabae mordwilkoi* by 13 restriction enzymes has previously yielded low variation in size of the restriction fragments (Raymond *et al.* 2001). Nuclear marker studies on the other hand suggest that two subspecies of the black bean aphid (*A. fabae fabae* and *Aphis fabae solanella*) are separated pre-zygotically by the differences in sex pheromones which prevent mating. Females of *A. fabae solanella*, when both subspecies are placed under a light controlled environment, release pheromones quite fast as compared to *A. fabae fabae* females that release the mating pheromone about an hour later than the former (Thieme & Dickson 1996). This assortative mating may infer the sympatric speciation between the two subspecies; hence making mating a cumbersome process. Besides, *A. f. fabae* was previously reported to possess a more pronounced phenotypic plasticity than *A. f. solanella and A. f. cirsiiacanthoidis* (Béji *et al*. 2015). The phylogenetic reconstruction revealed that aphid species clustered together distinctly independent of the geography or host crop. This confirmed the lack of geographical or host crop effect on the genetic diversity of aphids attacking amaranth and nightshade in Kenya and Tanzania. The lack of relations between the genetic diversity and host crop and environmental variation as shown in the phylogenetic tree suggests that such factors cannot infer the cause of a speciation event; rather they play an important role in the survival of the aphids. This agrees with previous reports showing near genetic homogeneity irrespective of the host crop or differences in geographical distribution of the aphids (Raboudi *et al*. 2011; Nibouche *et al*. 2014; Cocuzza *et al*. 2015).

The pattern of haplotype clustering conferred with that of the phylogenetic tree and the principal component analysis where samples of the genus *Aphis* were closely related than those of *Macrosiphum* and *Myzus*, further elaborating the single species cluster results. The results of the haplotype showed lower nucleotide diversity (Pi = 0.0279) as compared to haplotype diversity (hd = 0.464) in populations of aphids collected in Kenya and Tanzania. Similar results have been reported in *A. craccivora* populations in Thailand (Wongsa *et al*. 2017). This scenario could be indicative that there are bottleneck events (Wei *et al*. 2013) amongst the aphids in Kenya and Tanzania infesting amaranth and nightshades. Hap2 consisting of *A. fabae* demonstrated the highest haplotype diversity than any other haplotype and this could be attributed to presence of subspecies in these samples. Hap3 and Hap4 (*M. persicae* and *M. euphorbiae*, respectively) generated no haplotype diversity and nucleotide diversity. This could be due to low sampling sites in the study. The network analyses showed Hap1 and Hap2 having closer relatedness as compared with the other two which depicted a relatively close association.

This study underscores the utility of the DNA barcode region in fingerprinting of aphid species. The relatively low genetic diversity indices demonstrated here may mean that the adaptability to harsh environmental conditions of the sampled aphids is relatively weak, and the limited host crops and survival environment may lead to

relatively low genetic diversity amongst these aphids (Ran *et al*. 2015). However, there is need to undertake similar studies using nuclear markers and increase the number of samples to corroborate these results. In addition, COI proved to be an indispensable tool that can be used in confirming the results of classical taxonomy in identification of invasive aphid species. Identification process is a critical element since it avails quick and accurate identification tool for monitoring and detection of potentially invasive species, facilitating successful integrated pest management strategies and contributing to effective phytosanitary management systems, understanding and management of potyviruses and downstream biological studies, especially of aphid groups that do have different subspecies. Studies have shown that different insect species respond to control methods differently. For instance, in a study conducted over a period of one year, *M. persicae* demonstrated resistance to 71 synthetic chemical insecticides more than any other insect in the study (Vasquez 1995). Besides, it is the most effective vector of many viruses that infect the crops that they feed on (Vasquez 1995). Therefore, it is paramount to barcode the various insect species. This way, various control methods can be administered independently to these species and results portrayed case by case. This creates a specific control profile for individual group of insects when subjected to a certain control mechanism.

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