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PHYLOGENETIC DIVERSITY OF CASSAVA GREEN MITE, *Mononychellus progresivus* FROM DIFFERENT GEOGRAPHICAL SITES IN EAST AFRICA

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ABSTRACT

Cassava green mite (CGM) of the *Mononychellus* genus is an invasive species in Africa, introduced from South America. Its phylogenetic diversity over geographical localities has never been assessed in East Africa, where mite density dynamics oscillate from few individuals to a peak of hundreds. The objective of this study was to determine CGM species comparative phylogenetic diversity from seven distinct geographical sites in East Africa. Six sites were sampled for CGM races, two samples from each country. DNA was extracted on internal transcribed spacer 2 (ITS2) and cytochrome oxidase subunit I (COI), and compared for phylogenetic variations of CGM from different locations of East African region. A comparative search from the NCBI Gene bank resulted into identical species nucleotides from Congo and Benin. Sequences from the two sites in Kenya were 99-100% similar to CGM nucleotide from the Congo-Benin accessions (X79902.1) on ITS2 gene region. On COI, a 98-99% site sequences similarity was observed on *M. progresivus* accession X79901.1. A closely related divergence of specimens collected from Tanzania and Uganda was determined. Both Uganda and Tanzania had 99% match to X79901.1 on COI region. Similarly, the Uganda and Tanzania samples had 99% match to emb/X79902.1 on the 18Sr RNA region. The CGM sequence from coastal Kenya had the highest phylogenetic divergence from the Congo-Benin sequences. A small biogeographic phylogenetic divergence (0-1%) was evident from the analyses among the six collection sites. The results confirm *M. progresivus* identity in East Africa it also indicates intra-species phylogenetic variations on the COI gene region of interest.

Key Words: Benin, Congo, Kenya, *Mononychellus progresivus*

RÉSUMÉ

L'acarien vert du manioc (CGM) du genre *Mononychellus* est espece invasive Introduite de l'Amerique du Sud en Afrique. Sa diversite phyllogenetique au sein et entre les localites n'a jamais ete evaluee en Afrique de l'Est, ou la densite d'acarien varie de quelques individus a plusieurs centaines. L'objectif de cette etude etait de determiner la diversite phyllogenetiques des especes CGM et de comparer sept sites geographiquement differents en Afrique de l'Est. six sites ont ete echantillonnes pour des races de CGM, a raison de deux echantillons par pays. De l'AND a ete extrait sur l'espaceur interne transcrit 2 (ITS2) et sous-unite I de cytochrome oxidase (COI), ces echantillons ont ete compares pour les variations phyllogenetiques de CGM provenant de localites differentes de la region Est Africaine. Une etude comparee a la banque de gene de NCBI a revele des especes identiques en provenance de Congo et du Benin, de point de vue de leur nucleotides. Des sequences provenant des deux sites au Kenya ont exhibe 99-100% de similarite avec les nucleotides de CGM provenant des accessions Congo-Benin (X79902.1) sur la region genetique ITS2. Sur COI, une similarite de 98-99% a ete observee sur l'accession *M. progresivus* X79901.1. Une divergence de sujets genetiquement proches a ete observe chez des specimens

collectes en Tanzannie et en Ouganda. Ouganda et Tanzanie ont exhibe 99% de similitude a X79901.1 sur la region COI. De meme, les echantillons provenant de Ouganda et de Tanzannie presentaient 99% de similitude avec emb/X79902.1 sur la region 18Sr RNA. La sequence de CGM provenant de cote kenyane avait la divergence phyllogenique la plus elevee d'avec les sequences Congo-Benin. Une legere divergence phyllogenetique geographiaque (0-1%) a ete note des analyses dans les sites de collection. Les resultats conferment l'identite de *M. progresivus* identity en Afrique de l'Est. l'etude a aussi revele des variations phyllogenetiques sur la region de gene d'interet COI.

Mots Cles: Benin, Congo, Kenya, *Mononychellus progresivus*

INTRODUCTION

Cassava, *Manihot esculenta* Crantz, is an important staple food for over 800 million people world-wide (Nweke, 1996; FAO, 2007). The cassava green mite (CGM) pest of *Mononychellus* species, constrains the production of this important crop due to direct leaf damage leading to reduction of photosynthetic leaf area (Yaninek *et al.*, 1987). Early reports indicate that the CGM was accidentally introduced in Africa when cassava was imported from South America. It was first reported in Uganda during the 1970s, from where it spread everywhere in Africa (Megevand *et al.*, 1987; Yaninek and Herren, 1988). Previous studies have reported success in biological control of *Mononychellus tanajoa* Bondar (*M. progresivus* Doreste) in warm-humid regions in Africa, when a predatory mite, *Typhlodromalus aripo* De Leon, of family Phytoseiidae was released from South America (Kariuki *et al.,* 2002; Yaninek and Hanna, 2003). Pest mite density threshold was determined at >27 mites per leaf on various varieties (Mutisya *et al.,* 2014). Mutisya *et al.* (2015) explored effective management of CGM in different agro-ecological zones of Kenya, and found that it is only in the dry low midlands that the use of abamectin acaricide safeguarded the crop from leaf drop, during 3-5 months of drought. In the coastal and cool upper midland zones, predacious mites suppressed the pest mite density to below injury levels (Mutisya *et al.,* 2015).

Gutiérrez (1987) reviewed the *Mononychellus* species complex, citing eight species found on cassava in South America; namely *Mononychellus tanajoa* Bondar, *M. progresivus* Doreste, *M. manihoti* Doreste, *M. bondari* Paschoal, *M. caribbeanae* McGregor, *M. mcgregori* Fletchman and Baker and *M. estradai* Baker & Pritchard. Navajas *et al.* (1994) showed complete similarity of the genomic characteristics of different African populations of CGM to those of Colombia; whereas the populations from Brazil (South America) were found to be different. Tetranychid species diversity has been reported since the last century (Boudreaux, 1963; Navajas *et al.*, 1994). Memarizadeh *et al.* (2013) reported acaricide abamectin resistance by the red tomato mite, *Tetranychus evansi* Pritchard & Baker in Asian countries, where species intra-geographical variations were evident among the populations on ITS and COI regions. The possible effect of geographical localities on genetic diversity of CGM aroused our interest in determining how the species, *M. progresivus,* differ from one country to another in East Africa. The information is important in pest control, especially where use of acaricide is advocated for in advanced drought conditions of the marginal lands in Kenya with varied agro-ecological zones (Mutisya *et al.,* 2015). The objective of this study was to determine CGM species comparative phylogenetic diversity from seven distinct geographical sites in East Africa.

MATERIALS AND METHODS

Mite sampling sites. Specimens of cassava green mite, *Mononychellus* species, were recovered from cassava plants in six different geographical zones of East Africa (Table 1). These were low midlands at Kisumu (LM3) and Katumani (LM4) of Kenya; upper midlands at Namanga (UM3) and Sirare (UM2) of Tanzania sites. In Uganda, the sites were Kawanda and Namulonge of Lake Basin and Mbale Farm Lands. In each field, three leaves from different plants were sampled at random and hundreds of CGM actives mixed in a vial. The Geographical Positioning Service (GPS)

points were taken for positional recording. The phytophagous specimens were brushed with a 4-inch paint brush from the underside of the leaves onto A-4-paper and further picked with a camel hair (size 000) and inserted in 99-100% alcohol vial.

Mean mite density dynamics during dry and wet periods were scored for each country site, to predict times of peak (Fig. 1). Kenya had the highest population fluctuations of <10 to > 300 mites per leaf; followed by Tanzania with <10 to < 200 mites per leaf, respectively. The lowest range of mite density was Uganda, where the wet season had <5 to < 70 mites per leaf.

DNA extraction. Genomic deoxyribonucleic acid (DNA) was extracted from individual mite specimens using tissue kit (Qiagen, GHBB, Germany), according to the manufacturer's instructions. The DNA samples of four specimens were first eluted in 30 µl of buffer AE and stored at 4 °C. Polymerase chain reaction (PCR) was performed in a total volume of 20 µl containing 1X PCR buffer, 1.5 mM $MgCl_2$, 10mM of dNTP mix, 10 pM of each primer, 1.5 units Taq Expand TM high fidelity PCR reagents (Roche Diagnostics, Mannheim, Germany) and 2 µl (approx 5 ng), and the mixture was incubated in thermal cycler (Applied Biosystems 9700). The

TABLE 1. Sampled sites of cassava green mite (CGM) in East Africa where cassava is grown at specific agro-ecological zones (AEZs) of east Africa

Country	Locality	AEZ	Coordinates	Altitude (m)	AEZ description
Kenya	Kiboko	Low midlands (LM3)	02°5' 52" S37°25 57" E	1186	Hot-wet
	Katumani	Low midlands (LM4)	01 20' 51" S37 08' 24" E	1609	Warm-dry
Uganda	Kawanda	Upper midlands (LV-MF)	0° 24'25"N32° 32'07"E	1147	Warm-wet
	Namulonge	Upper midlands (LV-MF)	02° 38' 88" N36° 47' 02" E	1139	Warm-wet
Tanzania	Namanga	Upper midlands (UM3)	02° 3' 00" S36° 46' 00" E	1311	Warm-dry
	Sirare	Upper midlands (UM2)	01° 38' 00" S34° 10' 00" E	1658	Cool-wet

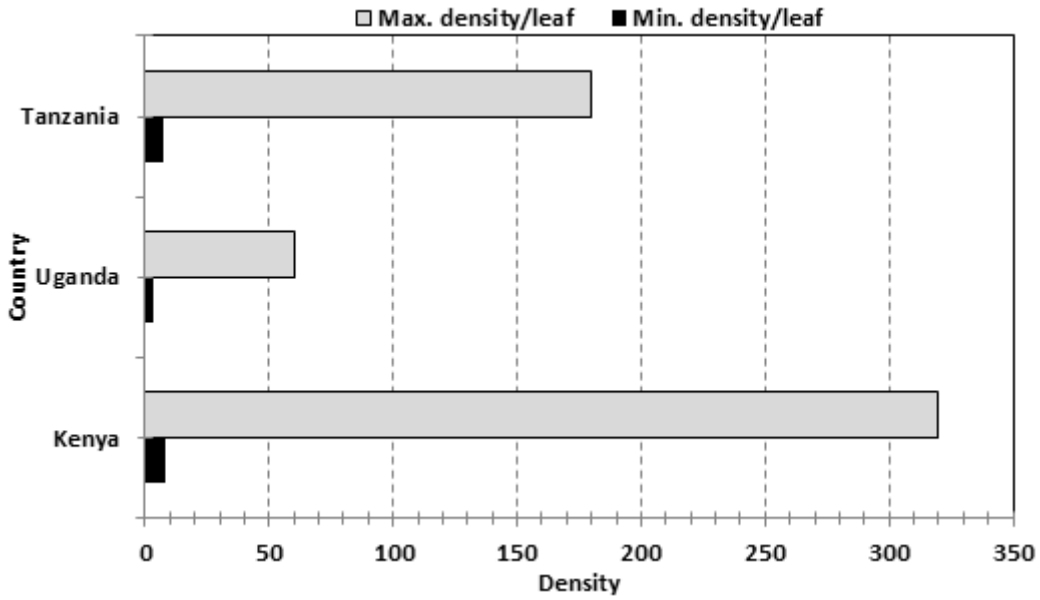

Figure 1. Distribution range (minimum and maximum peaks) of the cassava green mite *Mononychellus* species in different sampled sites of East Africa.

PCR products were amplified by an initial denaturing at 95 °C for 4 min; followed by 35 cycles of 92 °C for 1min, 51 °C for 2 min, 72 °C for 1min, and a final extension at 72 °C for 9 minutes. The ITS2 regions were amplified using the primers 5'AGAGGAAGTAAAAGTCGTAACAAG-3' for the 3' end of the 18SrDNA and 5'-ATATGCTTAAATTCAGGGGG-3' for the 5' end of the 28S. The mitochondrial COI primers used were 5'-TGATTTTTTGGT CACCCAGAAG-3'and 5'-ACAGCTCCTA TAGATAAAAC-3' (Navajas *et al.,* 1994). The amplified PCR products of 20 µl were stained with ethidium bromide and visualised using 1% agar rose gel electrophoresis at 80 voltage for 50 minutes; then later purified using the QIAquick® PCR purification kit (QIAGEN, Germany), according to the manufacturer's instructions. The purified products (4-5 µl) were directly sequenced by using an ABI 3100 series automated sequencer (Applied Bio-systems Inc).

Mite nucleotide analyses. The nucleotide sequences of mites from the three sites were Blast on NCBI database to determine phylogeny match (%) with other species of similar gene regions. The nucleotide sequences were aligned using BioEdit 5.0. ClustalX was used for the multiple alignments before construction of Neighbour-Joining phylogeny trees using MEGA 5.2.2 on COI and ITS2 gene regions of CGM sequences from the six sites. The Juke-Cantor Model of Maximum Composite Likelihood was used for analysis, where Bootstraps replications of 1000 were applied for significant measure of nucleotide divergence.

RESULTS

Species nucleotide identity match. The Kiboko CGM nucleotide had 100% similarity to NCBI Gene bank accession X79902.1 of species *Mononychellus progresivus* Doreste on ITS2 region (Table 2). Further, a 99% similarity to the same NCBI accession was observed for all other CGM nucleotides from the sites. This was 0-1% intra-divergence range of CGM races from the different sites. No other species taxa from NCBI were found related to *M. progresivus* from the sites in Kenya, Uganda and Tanzania.

TABLE 2. Comparative internal transcribed spacer 2 (ITS2) BLAST genetic match results of *Mononychellus progresivus* from different sites in Kenya to species nucleotides from NCB data base

Country	Site	Base pairs(letters)	Match (%)	NCBIAccession	Gene region	Species identity
Kenya	Kiboko	796	100	emb/X79902.1	18SrRNA	*Mononychellus progresivus* Doreste
	Katumani	838	99			
Uganda	Kawanda	919	99	emb/X79902.1	18SrRNA	*Mononychellus progresivus* Doreste
	Namulonge	801	99			
Tanzania	Namanga	863	99	emb/X79902.1	18SrRNA	*Mononychellus progresivus* Doreste
	Sirare	823	99			

On the COI, the sequences blast (NCBI) resulted into 99% similarity to *M. progresivus* (X79901.1) from Congo-Benin races; while a 90-91% similarity with *Tetranychus urticae* (Koch) accessions DQ017588.1 and KF544952.1, respectively, were noted (Table 3). The sequences from Katumani (Kenya) were 99 and 91% similar to the same accessions of *M. progresivus* and *T. urticae,* respectively. The nucleotide blast from Tanzanian sites of Namanga and Sirare were 99% similar to accession X79901.1 (*M. progresivus*) and 91% to accession KF544952.1 (*T. urticae*). On the other hand, the Uganda nucleotide sequence from Kawanda and Namulonge were 99% similar to X79901.1 (*M. progresivus*) and 90% to accessions CUC02469.1 and KFDQ017588.1.

Mite phylogeny diversity. The ITS2 phylogeny tree showed that the highest sequence divergence was from Katumani and Kiboko, in relation to NCBI similar species accession emb/X79902.1 of *M. progresivus* (Fig. 2). The sequence from Mtwapa was the closest to the *M. progresivus* (emb/X79902.1). The out-groups of *T. urticae* (PM408046.1), *T. evansi* (AJ419833.1) and *E. orientalis* (HQ688670.1) were clearly different genera.

The COI phylogeny tree showed that Kiboko and Mtwapa had the highest genetic divergence from NCBI accession X79901.1 (Fig. 3). Katumani sequence was genetically closest to the NCBI accession (X79901.1). The out-groups, *T. evansi* (GU565322.1), *T. urticae* (GQ141909.1) and *E. orientalis* (HQ688670.1) were clearly different taxa from *M. progresivus.*

Removing the outlier taxa of genera *Tetranychus* and *Eutetranychus,* and basing nucleotide divergence on the Congo-Benin accessions, enabled closer examination of CGM nucleotide at geographical intra-divergence level. Figure 4 shows that Kiboko, Namanga and Sirare

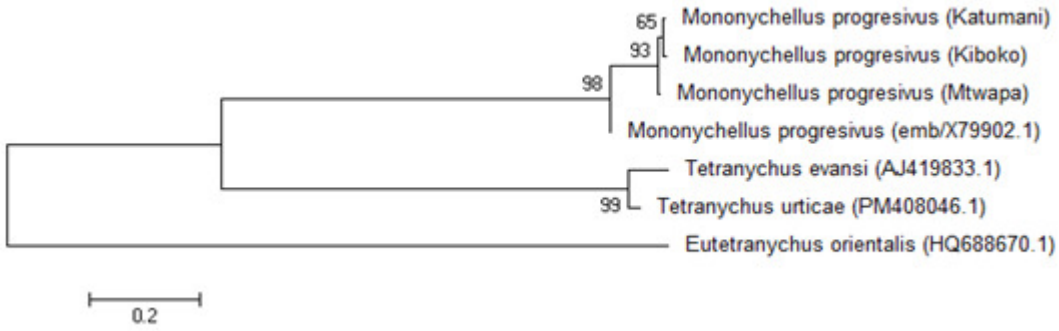

Figure 2. Neighbour-Joining phylogenetic tree based on internal transcribed spacer 2 (ITS2) nucleotide divergences of *Mononychellus progresivus* showing phylogeny positions among related taxa from NCBI. Bootstrap values (>50%) based on 1000 replications are shown at branch nodes.

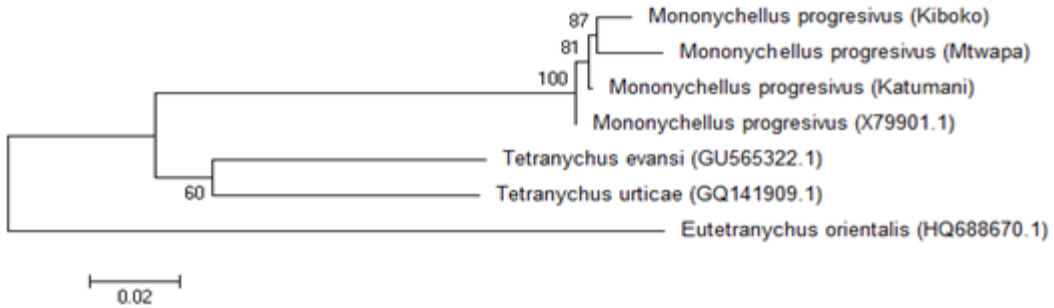

Figure 3. Neighbour-Joining phylogenetic tree based on cytochrome oxidase subunit I (COI) nucleotide divergence of *Mononychellus progresivus* showing phylogeny positions of related taxa from NCBI. Bootstrap values (>50%) based on 1000 replicates are shown at branch nodes.

TABLE 3. Comparative cytochrome oxidase subunit I (mtCO1) of two closest genetic match (%) results of cassava green mite species from different sites in East Africa to species nucleotides from NCBI data base

Country	Site	Base pairs (letters)	Match (%)	NCBI accession	Gene region	Species identity
Kenya	Kiboko	371	99	X79901.1	mtCOI	*Mononychellus progresivus* Doreste
			91	DQ017588.1	mtCOI	*Tetranychus urticae* Koch
	Katumani	371	99	X79901.1	mtCOI	*Mononychellus progresivus* Doreste
			90	DQ017588.1	mtCOI	*Tetranychus urticae* Koch
Uganda	Kawanda	441	99	X79901.1	mtCOI	*Mononychellus progresivus* Doreste
			90	CUC02469.1	mtCOI	*Tetranychus urticae* Koch
	Namulonge	481	99	X79901.1	mtCOI	*Mononychellus progresivus* Doreste
			90	DQ017588.1	mtCOI	*Tetranychus urticae* Koch
Tanzania	Namanga	450	99	X79901.1	mtCOI	*Mononychellus progresivus* Doreste
			91	KF544952.1	mtCOI	*Tetranychus urticae* Koch
	Sirare	483	99	X79901.1	mtCOI	*Mononychellus progresivus* Doreste
			91	KF544952.1	mtCOI	*Tetranychus urticae* Koch

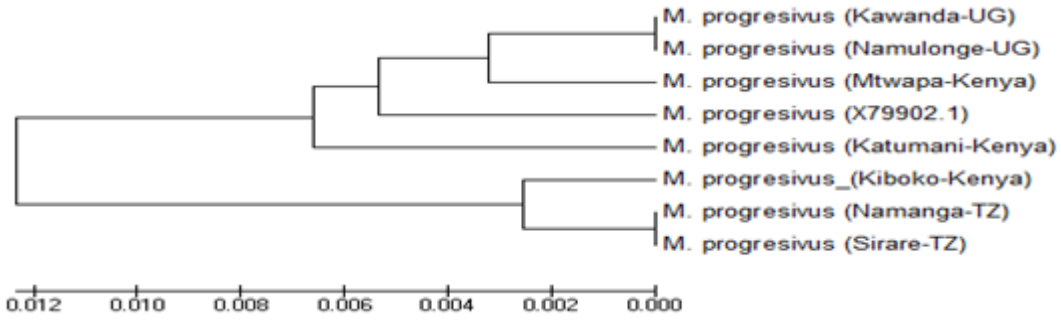

Figure 4. Neighbour-Joining phylogenetic tree based on internal transcribed spacer 2 (ITS2) nucleotide divergences of *Mononychellus progresivus* from different sites of East Africa in comparison to Cong-Benin accession X79902.1). Bootstrap values (>50%) based on 1000 replications are shown at branch nodes.

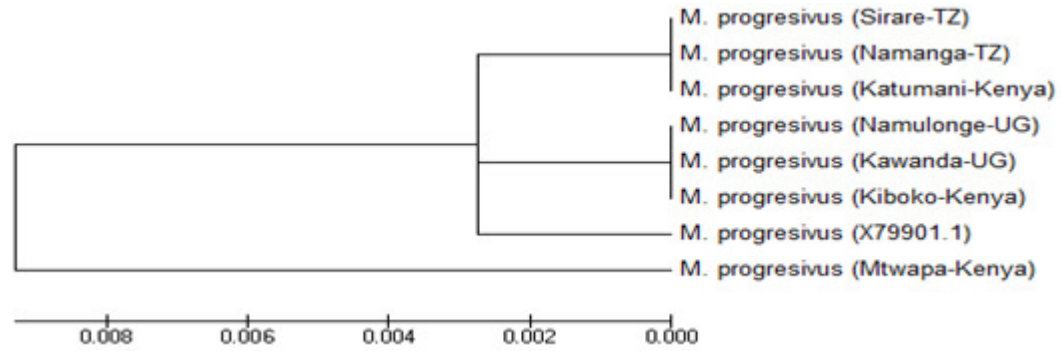

Figure 5. Neighbour-Joining phylogenetic tree based on cytochrome oxidase subunit I (COI) nucleotide divergence of *Mononychellus progresivus* showing species nucleotide from different sites from East Africa in comparison to Cong-Benin accession X79901.1). Bootstrap values (>50%) based on 1000 replicates are shown at branch nodes.

had the highest intra-divergence from NCBI accession X79902.1on ITS2 region.

On the other hand, Mtwapa CGM nucleotide indicated the highest intra-geographical difference from the rest of site nucleotides on COI region (Fig. 5).

DISCUSSION

Species nucleotide identity. The NCBI Blast search showed that the cassava species in Kenya and the East African region in general, was *M. progresivus*, similar to the Congo-Benin CGM sequences carried out by Navajas *et al.* (1994). A close look at the percentage match of the three site sequences to the NCBI Gene bank, showed no nucleotide variation between Kenya *M. progresivus* and Congo-Benin (X79902.1) race on the ITS2 region; where 0-1% intra-divergence was observed, while on the COI, a divergence of 1% was observed. Similar intra-geographical divergence was reported between sequences of Benin and Congo (Navajas *et al.*, 1994) where 0-2.1 and 0-0.4% intra-specific divergence on COI and ITS2, respectively. This indicates that some bio-geographical genetic variations are inherent on *M. progresivus* species in Africa. Similarly, the Uganda and Tanzania samples were indicative of 99% similarity to accession X79901.1 (*M. progresivus*), and in agreement to the earlier work by Murega (1989) on the race compatibility within the larger region of East Africa.

Mite phylogeny diversity. The ITS2 phylogeny tree showed that the *M. progresivus* sequences from Kiboko and Katumani were the most distant to Congo-Benin sequences from NCBI Gene bank. On the other hand, the COI phylogeny tree showed that both Kiboko and Mtwapa sequences were the most distant from the same

accession. Mtwapa site was both the warm and humid, compared to the hot dry Kiboko and the cooler Katumani sites of Kenya.

Some studies alluded to ITS(2) being the most stable for molecular systematics at the species level (Morrison, 2006; Knowles and Carstens, 2007). The allusion contrasts the results of the pioneer work by Navajas *et al.* (1994) on CGM races, where ITS2 region had 0-4%; while COI showed 0-2.1% nucleotide intra-divergence. Our study showed a 0-1% divergence on the ITS2; while COI region had 1%. Murega (1989) demonstrated that Kenya and Uganda CGM populations were compatible after a crossing study where a successful progeny was achieved in 100% of the test mature cohorts. The Kenya *M. progresivus* sequence variations on the COI region, showed geographical race genetic divergence manifestation. While the COI region is acclaimed as the DNA region for species bar coding within the range of 600 codon base pairs, its utility continue to be tested both in phylogeny and genetic studies in arthropods and vertebrates (Navajas *et al.*, 1996; Morrison, 2006).

Kanouh *et al.* (2010) reported some intra-species biogeographic variation of the predator genus *Phytoseiulus,* showing intra-geographic nucleotide divergence. Spider mite acaricide toxicity on red tomato spotted mite, *Tetranychus urticae* Koch, in Iran has shown reduced efficacious control as populations develop more chemical resistance (Memarizadeh *et al.,* 2013). Mutisya *et al*. (2015) have recommended abamectin spray of CGM in the hot-dry marginal lands where cassava is threatened by high densities of the pest mite. How such diverse intra-specific CGM races would respond to seasonal spray regimes of abamectin is worth a study in different regions in East Africa, where cassava suffers long drought period.

CONCLUSION

It is clear from this study that the COI gene region can be reliably analysed for bio-geographical race variation and nucleotide substitution difference. The CGM races from the sites of Kiboko (LM5), Namanga (UM3) and Sirare (UM3) have demonstrated the highest bio-geographic nucleotide divergence difference from the other three sites. This work can be a baseline for further evaluations on geographical site mite resistance to acaricide use. A future study on how different *M. progresivus* races respond to different cassava cultivars will lead to greater clarification on suitable cassava variety development and enhanced phytoseiid *T. aripo* presence on cassava plants. Such a study would lead to enhanced information on predator-plant relationship leading to non- economic injury level density-models of CGM on cassava in Africa.

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