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Cassava green mite genetic diversity from three geographical sites in Kenya

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Abstract

Cassava green mite (CGM) of the *Mononychellus* genus is an invasive species in Africa introduced from South America. Its genetic diversity over geographical localities has never been assessed in Kenya. We extracted DNA on internal transcribed spacer 2 (ITS2) and cytochrome oxidase subunit I (COI) and compared phylogenetic variations of CGM from the three sites in Kenya. We searched for species identify from the NCBI Genebank and found identical species nucleotide from Congo and Benin. Sequences from the three sites in Kenya were found to be 100% similar to CGM nucleotide from the Cong-Benin accessions (X79902.1) on ITS2 gene region. On COI, a 98-99% site sequences similarity was observed on *M. progresivus* accession X79901.1. The CGM race sequence from coastal Kenya was found to have the highest phylogenetic divergence from the Congo-Benin sequences. A small biogeographic genetic divergence was evident from the analyses among the sites. While the results confirm *M. progresivus* species identity in Kenya it also indicates intra-species phylogenetic variations on the COI gene region.

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Introduction

Cassava, *Manihot esculenta* Crantz is an important staple food for over 800 million people (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.*, 1989). Reports indicate that the CGM was accidentally introduced in Africa when cassava was imported from South America and reported first in Uganda during the 1970s from there it spread everywhere inclusive of East Africa (Nyiira, 1972; Megevand *et al.*, 1987; Yaninek and Herren, 1988). Yaninek and Hanna(2003) reported that success of biological control of *Mononychellus tanajoa* Bondar (= *M. progresivus* Doreste) in warm-humid regions in Africa was after sourcing for the predatory mite *Typhlodromalus aripo* De Leon of family Phytoseiidae from South America. Little success on CGM population has been reported in the hot-dry regions of the continent (Malindagabo and Birandano, 1984; Kariuki *et al.*, 2002; Jones, 2002).

Earlier, Gutiérrez (1987) had reviewed *Mononychellus* species complex citing eight species found on cassava in South Ameica; *Mononychellus tanajoa* Bondar, *M. progresivus* Doreste, *M. manihoti* Doreste, *M. bondari* Paschoal, *M. caribbeanae* McGregor, *M. mcgregori* Fletchman & Baker and *M. estradai* Baker & Pritchard. Cassava is highly susceptible to the CGM and high levels of infestation have been reported in several localities particularly the dry lowlands of Sub-Sahara Africa (Yaninek and Herren, 1987). Navajas *et al* (1994) showed complete identical similarity of the genomic characteristics of different African populations of CGM to those of Columbia, whereas the populations from Brazil (South America) were found to be different. Tetranychid species diversity has been reported since the last century to present (Boudreaux, 1963; Navajas *et al.*, 1994). The possible effect of geographical localities to genetic diversity of CGM aroused our interest to determine how the species *Mononychellus progresivus* Doreste differ from one place to another on two gene regions, the ribosomal and mitochondrial.

The present study was therefore carried out to determine CGM species comparative genetic diversity in Kenya from three sites of distinct geographical localities.

Materials and methods

Mite collection sites

A cassava green mite *Mononychellus* species specimens recovered from cassava plants in three geographical zones; low midlands at Katumani (LM4), Kiboko (LM5) and the coastal lowlands at Mtwapa (CL3) (Table 1) of Kenya. In each field, 15 plants were sampled at random. The phytophagous specimens were collected by beating the selected plants with a stick (60 cm long) over a circular plastic blue board of 32 cm diameter. With the aid of a head loop lens (Mag.X4) the green phytophagous mites were picked with a camel hair brush (size ooo) from the plastic board with a hair camel brush (size ooo). The phytoseiid mites were preserved in 70% alcohol after separation from any other arthropods.

Table 1. Cassava production sampling zones and the respective yearly climatic conditions.

Zone	No. plants	locality	GPS coordinates	altitude (m)	temp range (°C)	Rainfall range (mm)
LM 4	15	Katumani	01° 20'58"S 037° 8'39"E	1602	20–24	600–1200
LM 5	15	Kiboko	02° 7'43"S, 037° 8'41"E,	934	24–30	400–600
CL 3	15	Mtwapa	03° 9'37" S, 039° 33'44"E	18	26–28	1500–2000

LM=Lower midlands, UM= upper midlands and CL= coastal lowlands.

DNA extraction

Genomic Deoxyribonucleic acid (DNA) was extracted from individual mite specimens of collected green mite specimens using tissue kit (Qiagen, GHBB, German) according to the manufacturer's instructions. The DNA samples of four specimens were first eluted in30µl of buffer AE and stored at 4 °C. Polymerase chain reaction (PCR) was performed in a total volume of 20µl containing 1X PCR buffer, 1.5 mM MgCl2, 10mM of dNTP mix, 10 pM of each primer, 1.5 units Taq Expand TM high fidelity PCR reagents (Roche Diagnostics, Mannheim, Germany) and2 µl (approx 5ng) and the mixture was incubated in thermal cycler (Applied Biosystems 9700). The PCR products were amplified by an initial denaturing at 95 °C for 4 min, followed by 35 cycles of 92 °C for 1min, 51 0C 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'-TGATTTTTTGGTCACCCAGAAG-3'and 5'-ACAGCTCCTATAGATAAAAC-3' (Navajas *et al.*, 1994). The amplified PCR products of 20ul were stained with ethidium bromide and visualized by using 1% agar rose gel electrophoresis , then later purified using the QIAquick® PCR purification kit (QIAGEN, Germany) according to the manufacturer's instructions. The purified products (4-5ul) were directly sequenced by using an ABI 3100 series automated sequencer (Applied Biosystems Inc).

Site mite nucleotide match (%)

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 by use of MEGA 5.2 on COI and ITS2 regions of CGM sequences from the three sites. Bootstraps replications of 1000 were applied for signification measure of nucleotide divergence.

Results

Species molecular identity match

The sites' mite nucleotide on ITS2 were 100% similar to NCBI Genebank accession X79902.1 of species *Mononychellus progresivus* Doreste (Table 2). No other species taxa from NCBI were found related to *M. progresivus* from the sites of Kiboko, Katumani and Mtwapa.

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.

Site /zone	bp (letters)	% match	NCBI accession	Gene Region	Species
Kiboko (LM5)	796	100	emb/X79902.1	18SrRNA	*Mononychellus progresivus* Doreste
Katumani (LM4)	845	100	emb/X79902.1	18SrRNA	*Mononychellus progresivus* Doreste
Mtwapa (CL3)	805	100	emb/X79902.1	18SrRNA	*Mononychellus progresivus* Doreste

On the COI the sequences blast (NCBI) results from Kiboko were found closely similar to *M. progresivus* (X79901.1) at 99 and 90% to *Tetranychus urticae* (Koch) accessions DQ017588.1 and KF544952.1, respectively (Table3). The sequences from Katumani were 99 and 91% similar to the same accessions of *M. progresivus* and *T. urticae* respectively. The least similarity match of 98 and 89% on the same accessions were observed from sequences from Mtwapa.

Table 3. Comparative cytochrome oxidase subunit I (mtCO1) BLAST genetic match results of *Mononychellus progresivus* from different sites in Kenya to species nucleotides from NCBI date base.

Site /zone	bp (letters)	% match	NCBI accession	Gene region	Species
Kiboko (LM5)	371	99	X79901.1	mtCOI	*Mononychellus progresivus* Doreste
		90	DQ017588.1	mtCOI	*Tetranychus urticae* Koch
		90	KF544952.1	mtCOI	*Tetranychus urticae* Koch
Katumani (LM4)	368	99	X9901.1	mtCOI	*Mononychellus progresivus* Doreste
		91	DQ0175588.1	mtCOI	*Tetranychus urticae* Koch
		91	KF544952.1	mtCOI	*Tetranychus urticae* Koch
Mtwapa (CL3)	370	98	X79901.1	mtCOI	*Mononychellus progresivus* Doreste
		89	DQ0175588.1	mtCOI	*Tetranychus urticae* Koch
		89	KF544952.1	mtCOI	*Tetranychus urticae* Koch

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/X79902.1of *M. progresivus* (Fig.1). 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.

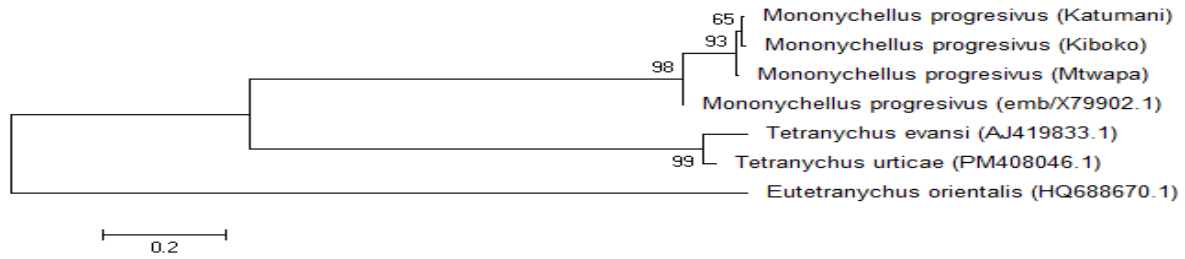

Fig. 1. Neighbour-Joining phylogenetic tree based on internal transcribed spacer 2 (ITS2) nucleotide divergences of *Mononychellus progresivus* showing phylogeny positions among related taxa from NCBI. Bootstrap values (>50%) based on 1000 replications are shown at branch nodes.

The COI phylogeny tree showed that Kiboko and Mtwapa had the highest genetic divergence from NCBI accession X79901.1 (Fig.2). 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*.

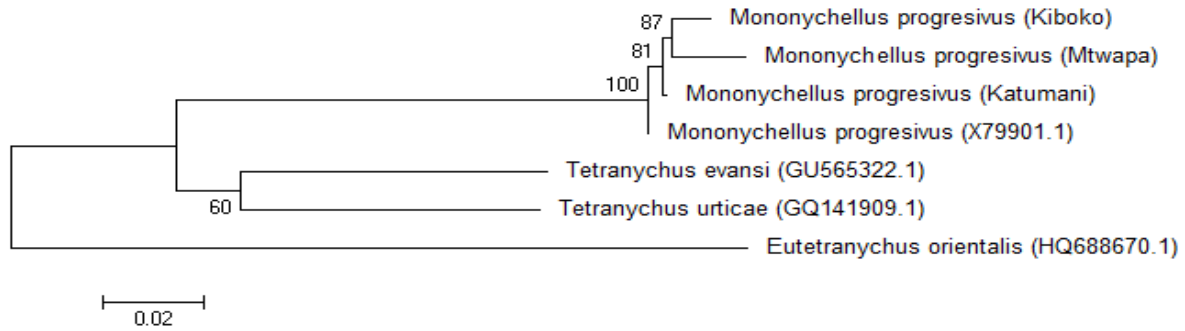

Fig. 2. Neighbour-Joining phylogenetic tree based on cytochrome oxidase subunit I (COI) nucleotide divergence of *Mononychellus progresivus* showing phylogeny positions of related taxa from NCBI. Bootstrap values (>50%) based on 1000 replicates are shown at branch nodes.

Discussion

The NCBI Blast search showed that the cassava species in Kenya was *M. progresivus*, similar to the Congo-Benin CGM sequences carried out by Navajas *et al* (1994). A close look of the percentage match of the three site sequences to the NCBI Genebank showed that there was no nucleotide variation between Kenya *M. progresivus* and Congo-Benin (X79902.1) race on the ITS2 region, while on the COI genetic divergence was observed of 1-2%. The highest divergence of 2% was realized on the sequence from Mtwapa to accession X79901.1. The same genetic divergence was reported between sequences of Benin and Congo by Navajas *et al* (1994). This indicates that some geographical genetic variations are inherent on *M. progresivus.*

The ITS2 phylogeny tree showed the *M. progresivus* sequences from Kiboko and Katumani were the most distant to Congo-Benin sequences from NCBI. On the other hand the COI phylogeny tree showed that both Kiboko and Mtwapa sequences were the most distant from the same accession. Mtwapa is warmest and humid, than the hot dry Kiboko and the cooler Katumani site.

Some workers allude that the ITS(2) is the most stable for molecular systematics at the species level, a fact confirmed on *M. progresivus* sequences in Kenya being more similar to the species in central and west Africa (Morrison, 2006; Knowles and Carstens, 2007). Murega *et al* (1989) demonstrated that Kenya and Uganda CGM populations were compatible after a crossing experimental study. The Kenya sites' *M. progresivus* sequence variation on the COI region showed geographical race genetic divergence manifestation. While the COI region is acclaimed as the DNA region for species barcoding 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) has reported some intra-species biogeographic variation of predator genus *Phytoseiulus*. Where possible a study on how different *M. progresivus* races responds to different cassava cultivars would lead to more insight on suitable cassava varieties for enhanced phytoseiid *T. aripo* presence on cassava as recently reported (Onzo *et al.* 2012). Such study would lead to enhanced information on predator-plant relationship leading to non- economic injury level density-models of CGM on cassava (Bakker *et al.*, 1993).

In conclusion it can be noted that since the report on accidental introduction of CGM in East Africa in 1970s, the present study can only point that the COI gene region could be reliably analyzed for geographical race variation and nucleotide substitutions difference. The present work can be a baseline for further evaluations on geographical site mite resistance to acaricide use in comparison to what was reported recently on two-spotted mite resistance to abamectin on tomatoes in Iran (Memarizadeh *et al.*, 2013).

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References

Bakker FM, Klein ME, Mesa NC, Braun AR. 1993. Saturation deficit tolerance spectra of phytophagous mites and their phytoseiid predators on cassava. Experimental an Applied Acarology, **17,** 97-113.

Boudreaux HB. 1963. Biological aspects of some phytophagous mites. Annual Review of Entomology, **8,** 137-154.

Food and Agriculture Organisation (FAO). 2007. Cassava production. www.fao.org/2007/y5548e/5548e.htm

Gutierrez J. 1987. The cassava green mite in Africa: one or two species (Acari: Tetranychidae). Experimental and Applied Acarology **3**, 163-168.

Jones A. 2002. Implementing cassava green mite control in Mozambique. In: R. Hanna and M. Toko (eds).The proceedings of the 3rd international meeting of Africa-wide cassava Green Mite Biocontrol Project, 20 -22 February, Cotonou, Benin. 12-23.

Kanouh M, Tixier MS, Okassa M, Kreiter S. 2010. Phylogenetic and biogeographic analysis of the genus *Phytoseiulus* (Acari: Phytoseiidae). *Zoologica Scripta,* **39,** 450–461.

Kariuki CW, Toko M, Hanna R, Ngari BMP. 2002. The progress of biological control of cassava green mite using *Typhlodromalus aripo* in Kenya. In: Proceedings of the Regional Meeting of the cassava Green mite Bio-control Project, 15-17 Nov. Dar es Salaam, Tanzania 27-41 (Eds. R. Hanna and M. Toko).

Knowles LL, Carstens BC. 2007. Delimiting species without monophyletic gene trees, Systematics Biology, **56(6),** 887-895.

Malindagabo J, Birandano B. 1984. Some problems of cassava production in Rwanda. In: Integrated pest management of cassava green mite. Proceedings of a Regional Training Workshop in East Africa 30th April- May 4th 1984. 95-100

Megevand B, Yaninek JS, Friese DD. 1987. Classical biological control of cassava green mite. Symposium XI of the International Conference of Tropical Entomology. Africa–Wide Biological Programme of Cassava Pests. Insect science and its application, **18**, 871-874.

Memarizadeh N, Ghadamyari M, Zamani P, Sajedi RH. 2013. Resistance mechanism to abamectin in Iranian populations of the two-spotted mite, *Tetranychus urticae* Koch. Acarologia, **53(3)**, 235-246.

Morrison DA. 2006. Multiple sequence alignment for phylogenetic purposes, Australian Systematic Botany, **19,** 479-559.

Murega T N. 1989. Cross-breeding studies on the Cassava green mite *Mononychellus sp* (Acari: Tetranychidae) in East Africa. Experimental and Applied Acarology, **6,** 85-90.

Navajas M, Gutierrez J, Bonato O, Bolland HR, Mapangou–Devassa S. 1994. Intraspecific diversity of the cassava green mite *Mononychellus progresivus* (Acari:Tetranychidae) using comparisons of mitochondria and nuclear ribosomal DNA sequences and cross breeding. Experimental and Applied Acarology, **18,** 351-360.

Navajas M, Gutierrez J, Lagnel., J. 1996. Mitochondrial cytochrome oxidase I in tetranychid mites: a comparison between molecular phylogeny and changes of morphological and life history traits. Bull of Entomol Res, **86,** 407-417.

Nyiira ZM. 1972. Report on investigation on cassava mite, *Mononychellus tanajoa* (Bondar). Department of Agriculture, Kawanda Research Station. Unpublished annual report,14.

Nweke FI. 1996. Cassava: A cash crop in Africa. COSCA Working Paper No. 14. Collaborative study of cassava in Africa. International Institute of tropic Agriculture, Ibadan, Nigeria, 11.

Onzo A, Hanna R, Sabelis MW. 2012. The predatory *Typhlodromalus aripo* prefers green-mite induced plants oduors from pubescent cassava varieties. Experimental Applied Acarology, **58 (4),** 359-370.

Yaninek JS, Hanna R. 2003. Cassava green mite in Africa: a unique example of successful classical

biological control of a mite pest on a continental scale. Borgemeister, P, Borgemeister, C, Langewald, J (eds.), Biological control in IPM systems in Africa, CABI, 61-75.

Yaninek JS, Herren HR. 1988. Introduction and spread of the cassava green mite, *Mononychellus tanajoa* Bondar (Acari: Tetranychidae). A exotic pest in Africa and search for appropriate control mrethods. A review: Bulletin of Entomology Research, **79,** 1-13.

Yaninek JS, Herren HR, Gutierrez HP. 1987. The biological basis for the seasonal out breaks of cassava green mites in Africa. Insect Science Application **8,** (6) 861-865.