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NEW DISEASE REPORT

First report of the severe Uganda variant of *East African cassava mosaic virus* in Zambia

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The *East African cassava mosaic virus-Uganda* (EACMV-Ug) variant, which originated through recombination between an *African cassava mosaic virus* (ACMV) and an *East African cassava mosaic virus* (EACMV) (Legg & Winter, 2020), has been associated with a severe cassava mosaic disease pandemic affecting cassava (*Manihot esculenta*) in sub-Saharan Africa.

EACMV-Ug has not been found in Zambia despite repeated surveys, though it has been identified in surrounding countries (Legg & Winter, 2020). However, severe symptoms typical of EACMV-Ug have been observed in Zambian fields (Chikoti et al., 2015) (Figure. 1). In February 2013, 88 fields were visited in four provinces (Central, Eastern, Lusaka and Western) and 186 cassava leaf samples with moderate to severe cassava mosaic disease symptoms were collected (Tembo, 2016). All 186 samples tested positive by PCR (JSP001/002 primers for ACMV, EAB555F/R for EACMV, 30 cycles with a 55°C annealing temperature; Tembo, 2016): ACMV was detected in 34.7% of positive samples, EACMV in 4.5%, and the remainder (60.8%) were mixed infections of

FIGURE 1 Symptoms resembling those caused by *East African cassava mosaic virus-Uganda* observed in Rufunsa, Lusaka, Zambia in a field expressing extreme reduction of leaf area, leaf narrowing, drooping, and leaf yellowing in the crop, observed in February 2013.

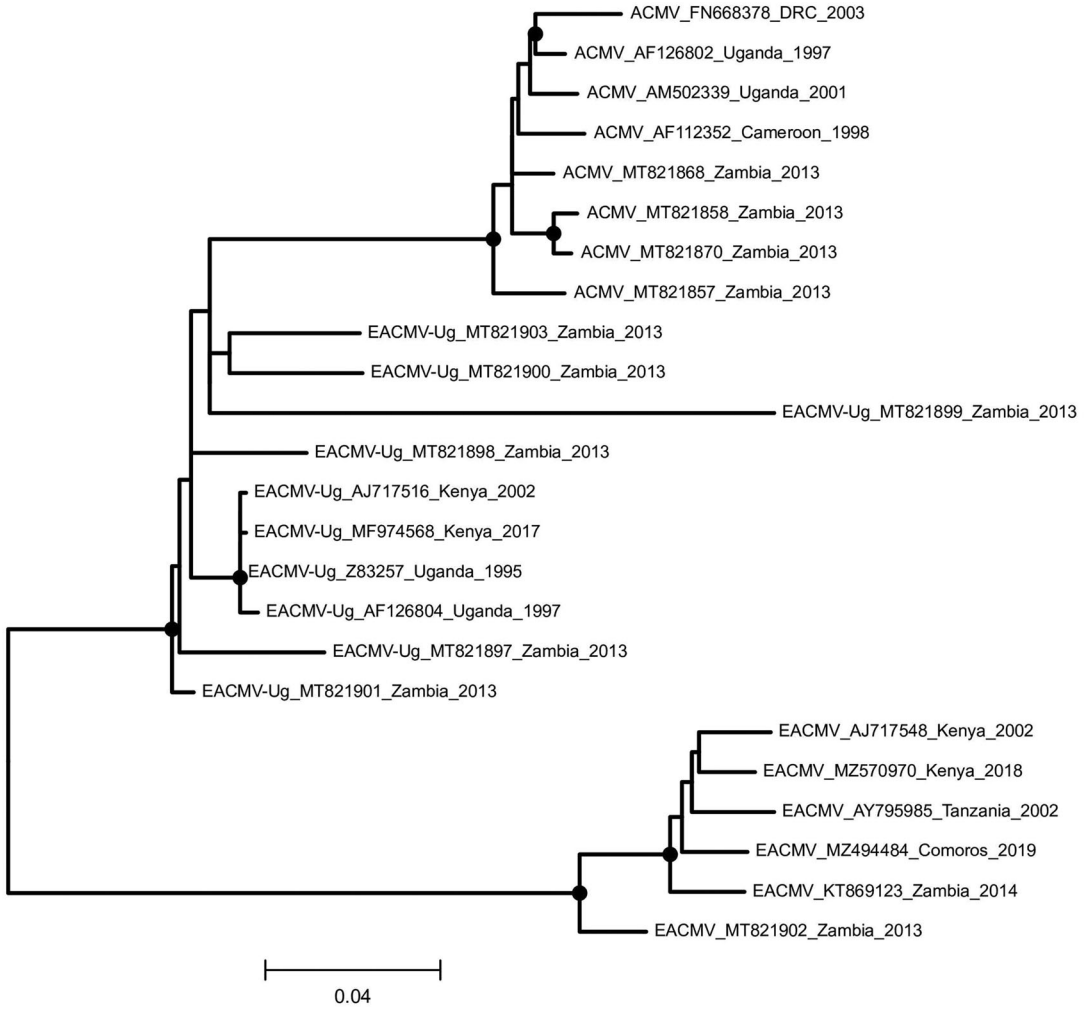

FIGURE 2 Midpoint-rooted maximum likelihood phylogeny for *African cassava mosaic virus*, *East African cassava mosaic virus* and *East African cassava mosaic virus-Uganda* coat protein nucleotide sequences. The maximum likelihood phylogenetic tree was constructed using IQ-Tree v2.07 with automatic selection of the best-fit substitution model (GTR+I+G4). Tree inference was performed with 3000 UFBoot replicates and a stopping rule of 500 iterations between unsuccessful improvements to the local optimum. UFboot branch support values ≥ 90% are indicated by filled circles.

ACMV and EACMV. The DNA-A segment of 25 of these samples were sequenced.

Phylogenetic analysis found that six of 25 sequences from PCR-positive samples (GenBank Accession Nos. MT821897-MT821901 and MT821903) were EACMV-Ug (Figure. 2), indicating that this variant has been present in Zambia for at least the last decade. The sequences were further confirmed to be EACMV-Ug recombinants by seven recombination detection methods (RDP5 v5.23; Martin et al., 2021) in an analysis of all ACMV and EACMV sequences deposited in GenBank prior to February 2023 (events 1 and 2 in Table 1, alignment available at https://github.com/acrespo-virevol/EACMV-UG-phylo-dating). Although isolate MT821899 was not grouped as a descendant of the recombinant event shared by all other 97 EACMV-Ug isolates collected to date (event 1, Table 1), it has similar predicted recombination breakpoints and the same putative parental species and can be considered a product of the same event. The six Zambian isolates were not immediately identified as EACMV-Ug because there have been additional recombination events spanning the coat protein (events 3–8), and overprinted recombination events in these Zambian strains may confound the identification of the clade-defining recombination event found in typical EAMCV-Ug isolates. Pairwise nucleotide identity analysis with SDTv1.2 (Muhire et al., 2014) confirmed that five of the six Zambian isolates are >94% identical to all other EACMV-Ug sequences over the whole DNA-A genomic segment, which is the threshold for being members of the same *Begomovirus* strain (Brown et al., 2015). The remaining isolate, MT821899, which may be the product of a different recombination event, is >94% identical to only 16/98 publicly available EACMV-Ug sequences but is 92.3-93.9% identical to all others.

In addition to this being the first report of EACMV-Ug in Zambia, this is also the first report of additional recombination events in the coat protein-coding region of EACMV-Ug.

TABLE 1 Recombination events involving MT821897-MT821901, MT821903 detected within a full DNA-A alignment of ACMV and EACMV sequences.

	Event number	EACMV-Ug recombinant sequence(s)	Recombination breakpoint		Parental sequence		Method[c]
			Start	End	Major	Minor	
Ancestral							
	1	MT821897, MT821898, MT821900, MT821901, MT821903 (plus 92 other sequences)	541[a]	1015[a]	AY795985 (EACMV)	AF112352 (ACMV)	RGBMCST
	2	MT821899	548	1014	AJ717545 (EACMV)	AF112352 (ACMV)	RGBMCST
Secondary							
	3	MT821897	1825	237	Unknown	AJ717524 (EACMV-Ug)	RGBMCST
	4	MT821898	1009[b]	1847	AF126804 (EACMV-Ug)	Unknown	RGBMCST
	5	MT821899	1817	176	Unknown	AJ717524 (EACMV-Ug)	RGBMCST
	6	MT821900	1821	317[b]	Unknown	AJ717517 (EACMV-Ug)	RGBMCST
	7	MT821901	1846	548[b]	Unknown	JN053452 (EACMV-Ug)	RGBMCST
	8	MT821903	1846	293[b]	Unknown	JN053452 (EACMV-Ug)	RGBMCST

[a]Breakpoint based on sequence accession MT821897.
[b]Actual breakpoint is undetermined; most likely overprinted by subsequent recombination event.
[c]R, RDP; G, GeneConv; B, Bootscan; M, MaxChi; C, Chimera; S, SisScan; T, 3SEQ.

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