

1 First report of banana bunchy top virus in banana and plantain (*Musa* spp.) in 2 Tanzania

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15 Banana (including plantain; *Musa* spp.) is an important vegetatively propagated food staple
16 grown as a semi-perennial crop in fields and backyard gardens in Tanzania. Banana
17 bunchy top disease (BBTD), caused by the banana bunchy top virus (BBTV, genus
18 *Babuvirus*), is the most economically important viral disease of banana, infection of which
19 results in severe stunting and reduction in fruit production by 90-100% within two seasons
20 (Kumar et al., 2015). The virus is spread by the banana aphid, *Pentalonia*
21 *nigronervosa*, and through vegetative propagation of infected sources. BBTV is an
22 introduced virus first reported in sub-Saharan Africa (SSA) in the 1960s in the Democratic
23 Republic of Congo (Kumar et al., 2011). Since then, BBTV spread was confirmed in 15
24 countries in Central, Southern, and Western African regions but was not detected in any
25 previous surveys in the East African sub-region (Kumar et al., 2011). During banana pests

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and disease surveys conducted in December 2020 – January 2021 in Buhigwe District in the Kigoma Region of Tanzania revealed banana plants with typical BBTv symptoms (severe stunting, leaves with shortened petioles, chlorotic streaks, and yellow leaf margins) in several banana fields in Muhinda (lon. 29.78662, lat. -4.53672) and Mwayaya (lon. 29.8218, lat. -4.49203) villages. Most of the affected plantations were 5 to 15 years old. Leaf samples (N=21) from symptomatic (N=6) and asymptomatic (N=15) banana plants were collected and used for total DNA extraction and BBTv detection by polymerase chain reaction (PCR) using the primer pair BBTv-1 and BBTv-2 to amplify ~240 bp sequence of DNA-R encoding for core master replication initiator protein gene (Mansoor et al. 2005). All samples from symptomatic plants tested positive and asymptomatic plants were negative. To further confirm the virus identity, four samples, each from symptomatic (PCR positive) and asymptomatic (PCR negative) plants from Muhinda and Mwayaya villages, were tested by Triple Antibody Sandwich-Enzyme-linked Immunosorbent Assay (TAS-ELISA) using BBTv ELISA reagent set (Cat. # SRA24700-1000, Agdia, France) following the manufacturer's protocol. Samples from symptomatic plants reacted positively in TAS-ELISA, and asymptomatic plants were negative. The 240-bp PCR product of two isolates was purified, and both strands were sequenced. A BLAST search of the nucleotide sequences (NCBI GenBank Acc.# MW711671 and MW711672) revealed 99% identity with DNA-R sequences of several other BBTv isolates from Africa (Acc. No# JF755994). Further analysis of the 240-bp nucleotide sequences with Maximum-likelihood phylogenetic analysis using MEGA-X software has grouped the two BBTv sequence isolates with the SSA sub-clade of the South-Pacific group (Kumar et al., 2015). To our knowledge, this is the first report of BBTv infecting bananas in Tanzania, and East Africa endowed with rich banana diversity and popular East African Highland banana clone. BBTv presents a new threat to banana production in this sub-region due to the high risk of

further spread through vegetative propagation, traditional planting material exchange practices, and the ubiquitous ~~insect-~~ banana aphid vector. This study warrants delimitation surveys to assess the extent of spread, with simultaneous efforts to raise awareness about BBTD recognition and control measures among banana growers, including eradicating infected mat~~s~~ and replanting with healthy planting material to recover banana production.

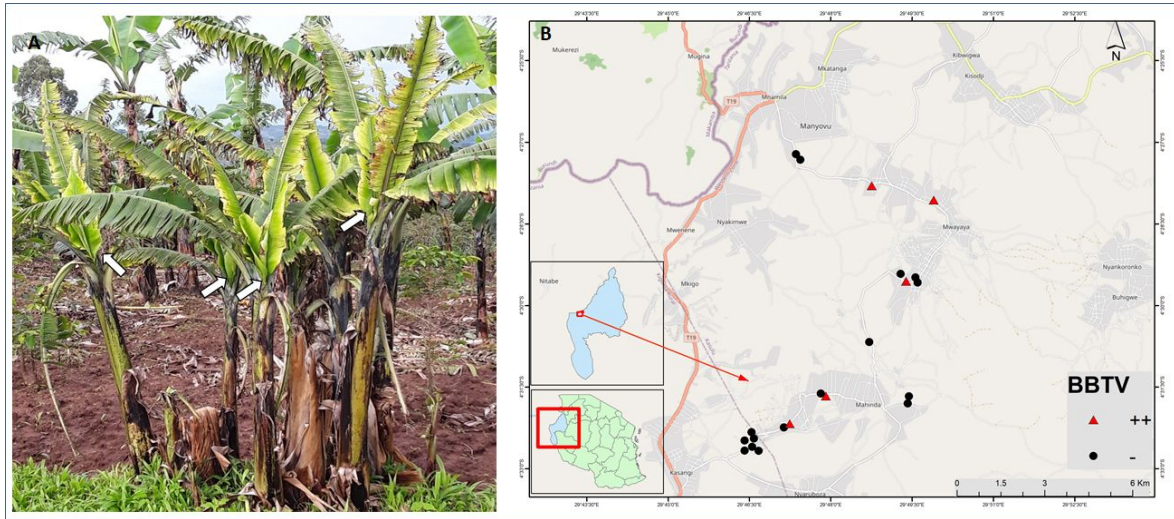
Acknowledgements

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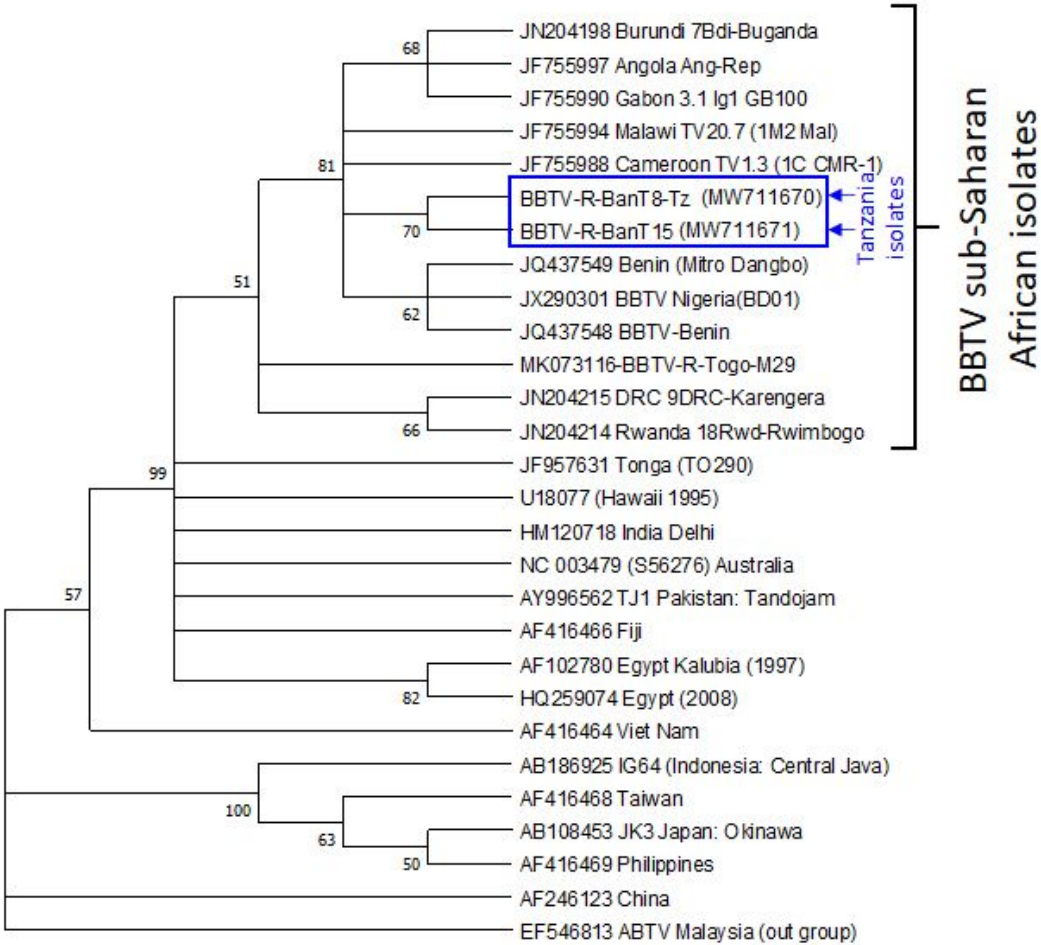
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Supplementary Figures



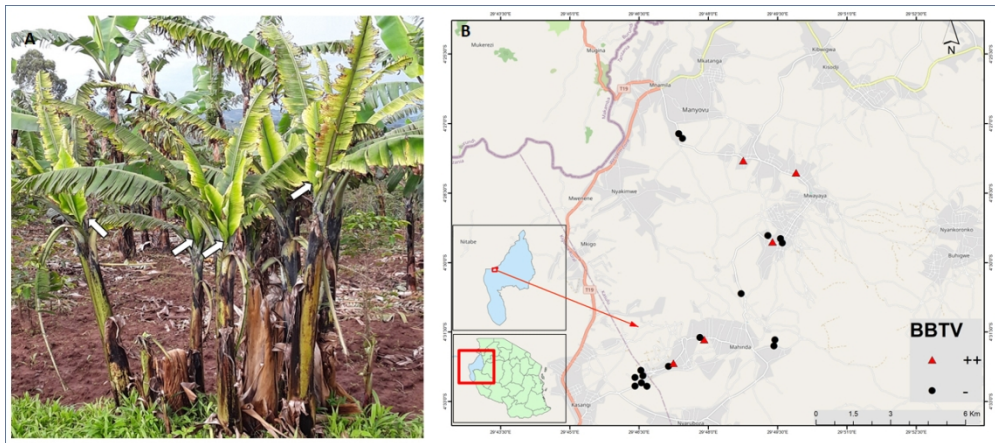
Supplementary Figure 1. (A) East African highland banana plants showing typical BBTv symptoms in Muhinda village in Buhigwe District, Kigoma Region in Tanzania; (B) Geographic map of Tanzania showing survey sites (●) and locations of banana fields with confirmed BBTv- infected plants (▲).

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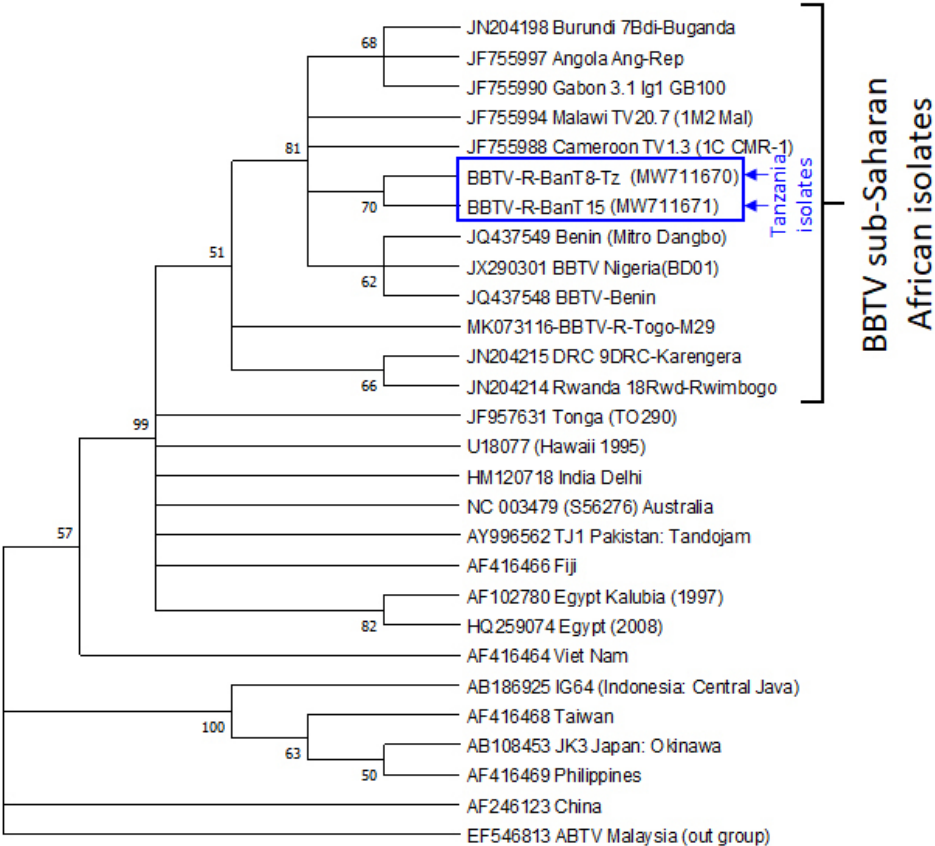
Supplementary Figure 2. Phylogenetic position of some BBTv isolates in GenBank and the BBTv Tanzanian isolates (blue border). The evolutionary history was inferred by using the Maximum-Likelihood method based on the Tamura-Nei model. The tree with the highest log likelihood (-874.59) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 26 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 244 positions in the final dataset. Evolutionary analyses were conducted in MEGA X.

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(A) East African highland banana plants showing typical BBTV symptoms in Muhinda village in Buhigwe District, Kigoma Region in Tanzania; (B) Geographic map of Tanzania showing survey sites (□) and locations of banana fields with confirmed BBTV- infected plants (▲).

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Phylogenetic position of some BBTv isolates in GenBank and the BBTv Tanzanian isolates (blue border).

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