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Presence and distribution of banana bunchy top virus in Laos

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Abstract
Banana bunchy top virus is reported for the first time in Laos. Infected plants showed typical disease symptoms and the two complete genome sequences reported place the isolates in the Southeast Asian subgroup.

Keywords Nanovirus · Genome sequence · Lao PDR · *Musa* spp. · Viral disease

Banana bunchy top virus (BBTV) is the most damaging viral pathogen of bananas (Kumar et al. 2015). It has a multi-component, circular, single-stranded DNA genome and is horizontally transmitted by its primary vector, the banana aphid (*Pentalonia nigronervosa*), as well as vertically transmitted between plant generations by vegetative propagation practices (Thomas 2019). BBTV likely originated in Southeast Asia (Stainton et al. 2015) but a notable gap in knowledge of the distribution of the virus within this region is Laos (Thomas 2019). Plants with symptoms of banana bunchy top disease (Fig. 1) were observed in Bolikhamxay, Luang Namtha and Vientiane Provinces, and Vientiane Prefecture in Laos (Fig. 2) during surveys conducted between 2015 and 2017 (Table 1). Occurrences of this disease were mostly in scattered plants in household gardens and smallholder plantings and never at high incidence in any particular region. In this disease note, we present the complete genome sequences of two virus isolates from Laos and provide positive identifications for several other isolates from a range of cultivars and locations.

Leaf samples of banana plants displaying symptoms typical of banana bunchy top disease were dried over silica gel for later laboratory analysis, and a subset (n = 39) imported into Australia under licence and archived in the Queensland Department of Agriculture and Fisheries Plant Virus Collection (Ecosciences Precinct, Dutton Park, Queensland). Diagnoses of imported samples were done by either immunocapture (IC) PCR using DNA-R specific primers BBT1 and BBT2 (Sharman et al. 2000) or PCR using DNA-R fwd and rev primers (Stainton et al. 2012). For the latter, total plant DNA was extracted using an ISOLATE II Plant DNA kit (Bioline) according to the manufacturer's instructions. PCR amplicons from six isolates were gel-purified using an ISOLATE II PCR and Gel kit (Bioline) and used for direct sequencing or sequenced after cloning using an Invitrogen TOPO TA cloning kit (Thermo Fisher Scientific). Sanger sequencing was outsourced to the Australian Genome Research Facility. As a limited confirmatory test, two samples were also checked by triple antibody sandwich ELISA (Geering and Thomas 1996). All samples with banana bunchy top disease symptoms tested were positive by PCR

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Fig. 1 Typical symptoms caused by banana bunchy top virus in field samples from Laos. Isolates Q6400 (A) and Q6653 (B) from Bolikxamay Province, Q6644 (C, D) from Luang Namtha Province. Typical whole plant symptoms of stunting, narrow, erect bunched leaves with chlorotic margins in panels A, B, D; Characteristic dark green streaks on the petiole and minor leaf veins in panel C

and/or IC-PCR and the two samples tested by ELISA were also positive (Table 1).

The two virus isolates selected for complete genome sequencing were DAF accession Q6624, collected from near the village of Ban Nakhorng (N18.303606, E102.565024), and DAF accession Q6634, collected from near the village of Patsum (N18.427711, E103.71643), both on 29 June 2016. Total plant DNA was extracted using a DNeasy Plant Mini Kit (QIAGEN), and circular DNA molecules selectively enriched by rolling circle amplification using an Illustra TempliPhi 100 Amplification Kit (GE Biosciences), as per the manufacturer's instructions. The TempliPhi products were then submitted to the Beijing Genomics Institute (BGI; Shenzen, China) for sequencing on an Illumina Hiseq 4000 platform, with a library construction size of 300–500 bp and pair-ended reads of 150 bp. Sequence trimming was done by BGI and all other analyses done in house using CLC Genomics Workbench 10.1.1.

Following merging of the pair-ended reads, *de novo* genome assemblies were generated and six contigs obtained, each *c*. 1 kb in length and representing a large majority of the sequence reads (26,072–219,980 and 10,811–144,941 reads per DNA component for Q6624 and Q6634, respectively). BLASTN searches of GenBank suggested that each contig represented one component of the BBTV genome, with closest matches to isolates from Thailand. No evidence for the presence of satellite components or other viruses was obtained. Final genome assemblies were done by mapping reads to a previously sequenced genome of BBTV from Nong Khai Province in Thailand (GenBank Accessions KY427060.1- KY427065.1), which was among the highest matching hits (97–99% nucleotide identity) in the previous BLASTN analyses. Isolates Q6624 and Q6634 were 99.2 to 99.9% identical across the six genome components (Table 2).

Other BBTV isolates from Laos were partially characterised by sequencing of PCR amplicons and pairwise nucleotide identities of 97.3–100% were calculated among partial and complete DNA-R component sequences. The Laos isolates fell within the Southeast Asian subgroup of BBTV and were most closely related to isolates from neighbouring countries (Fig. 3).

In conclusion, we report the presence of BBTV in Laos for the first time and confirm its presence in Vientiane Prefecture and the provinces of Bolikhamxay, Luang Namtha and Vientiane (Fig. 3), in both commercial Cavendish and local cultivars. There are no coordinated efforts to control BBTV in Laos, yet the incidence of BBTV has not reached a level that poses an existential threat to the local banana industry as it did when the virus was introduced into Australia in the early 1900s (Thomas 2019). It is possible that the local cultivars of banana have a degree of resistance to BBTV that prevents major epidemics from occurring.

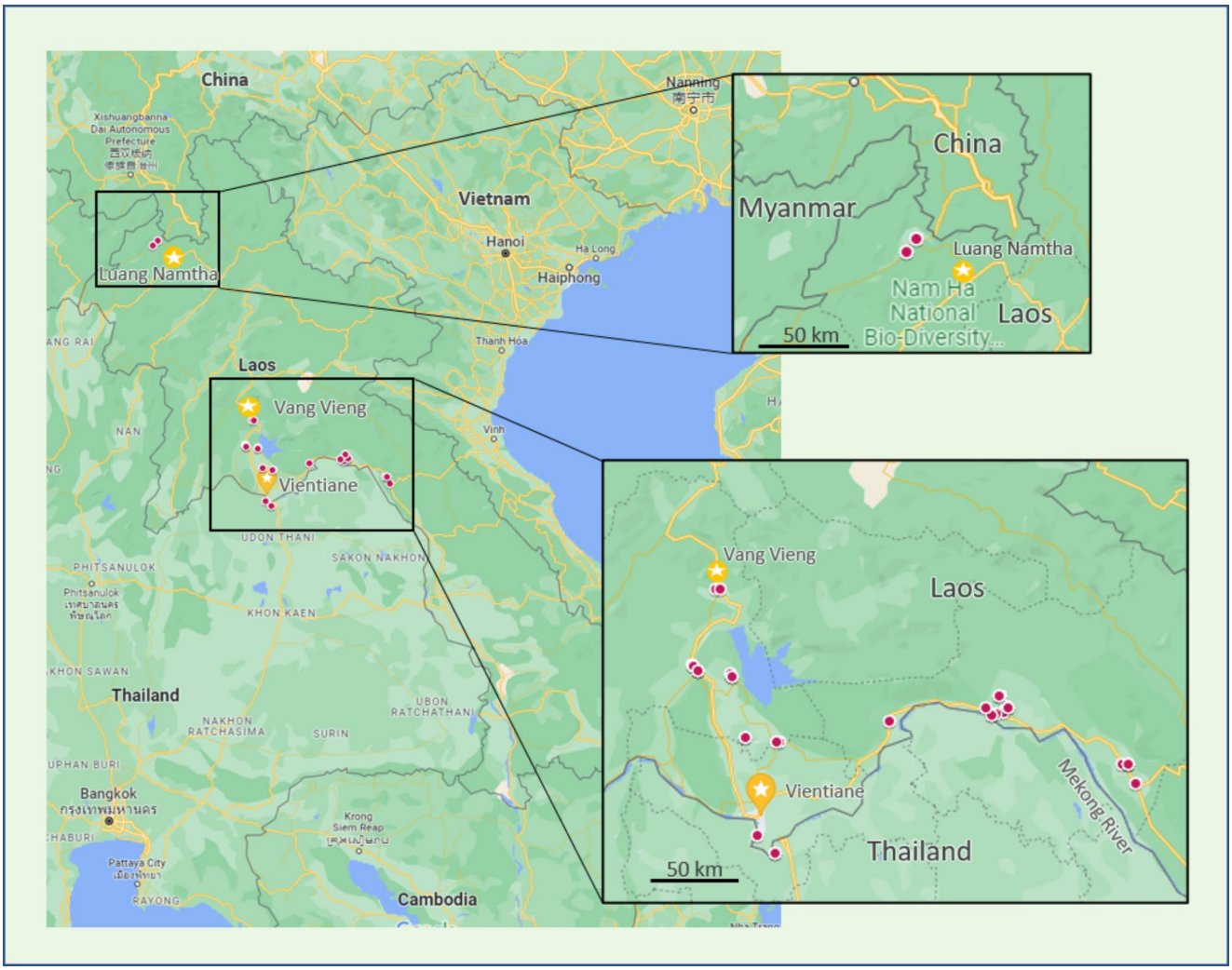

Fig. 2 Distribution of confirmed banana bunchy top virus isolates from Laos described in this work indicated by red dots

Table 1 Confirmed BBTV isolates from Laos. "+" signifies, as appropriate, positive PCR with BBT1/BBT2 or DNA-R fwd/rev primers obtained for each isolate

Isolate	Local cultivar names	GPS latitude	GPS longitude	Province	BBTV BBT1,2 PCR [b]	BBTV DNA-R PCR[b]	GenBank code
Q6400[c]	Hom	17.81853	102.69254	Vientiane[a]	nt	+	
Q6401	Hom	17.81955	102.69254	Vientiane[a]	+	nt	
Q6405[c]	unknown	17.88909	102.61162	Vientiane[a]	+	+	
Q6429	Knao	18.07235	102.53390	Vientiane[a]	nt	+	
Q6617	Jeang	18.39402	103.64869	Bolikhamxay	+	nt	
Q6618	Klai	18.59825	102.33205	Vientiane	nt	+	
Q6619	Jeang	18.30361	102.56503	Vientiane	+	+	
Q6620	Klai	18.10906	104.27908	Bolikhamxay	nt	+	
Q6621	Hom	18.39446	103.64793	Bolikhamxay	+	nt	
Q6622	Jeang	18.39908	103.66482	Bolikhamxay	nt	+	
Q6623	Hom	18.60573	102.32871	Vientiane	nt	+	
Q6624	Jeang	18.30361	102.56503	Vientiane	+	+	OP293737, OP297849-53
Q6625	Nam	18.40741	103.70114	Bolikhamxay	+	nt	
Q6626	Cavendish	21.18041	101.15705	Luang Namtha	+	+	OP485239
Q6627	Klai	18.28380	102.71323	Vientiane	+	nt	

Table 1 Continued

Q6628	Hom	18.36763	103.19582	Bolikhamxay	+	+	OP538684
Q6629	Klai	18.91992	102.42963	Vientiane	+	+	OP538685
Q6630	Jeang	18.55261	102.50401	Vientiane	+	+	OP538686
Q6631	Hom	18.42771	103.71643	Bolikhamxay	+	nt	
Q6632	Klai	18.91874	102.45049	Vientiane	nt	+	
Q6633	Kung	18.55255	102.50410	Vientiane	+	+	
Q6634	Figle	18.42771	103.71643	Bolikhamxay	+	nt	OP297854-59
Q6635	Musi	18.56477	102.49913	Vientiane	+	+	OP538687
Q6636	Hom	18.28490	102.69923	Vientiane	+	nt	
Q6637	Klai	18.56387	102.49869	Vientiane	nt	+	
Q6638	Jeang	18.42829	103.71414	Bolikhamxay	nt	+	
Q6639	Hom	18.19197	104.22105	Bolikhamxay	+	nt	
Q6644	Cavendish	21.11860	101.09366	Luang Namtha	+	+	OP538688
Q6650	Hom	18.47652	103.68278	Bolikhamxay	+	+	
Q6651	Cavendish	18.47652	103.68278	Bolikhamxay	+	nt	
Q6652	Hom	18.47791	103.67943	Bolikhamxay	+	nt	
Q6653	Hom	18.47763	103.67988	Bolikhamxay	+	nt	
Q6654	Klai	18.19101	104.24592	Bolikhamxay	+	nt	
Q6655	Klai	18.19100	104.24587	Bolikhamxay	+	+	
Q6656	Hom	18.19104	104.24551	Bolikhamxay	+	nt	
Q6658	Hom	18.42494	103.61960	Bolikhamxay	+	nt	
Q6659	Klai	18.57740	102.35112	Vientiane	+	nt	
Q6660	Klai	18.57728	102.35129	Vientiane	+	+	
Q6661	Klai	18.57723	102.35122	Vientiane	+	nt	

[a]Vientiane Prefecture

[b] nt – not tested

[c] Also positive for BBTV by ELISA

Table 2 Nearest identities read numbers for the six genome components of banana bunchy top virus isolates Q6624 and Q6634

Virus isolate	DNA component	GenBank code	Reads[1]	Size (nt)	Nearest BLAST match BBTV isolate[2]	Nearest match		% Identity Q6624 vs. Q6634
						GenBank code	% Identity	
Q6624	R	OP293737	26,072	1104	Nakhon Ratchasima-TH16, DNA-R	MF039879.1	99.6	99.8
	S	OP297850	47,474	1060	Chaiyaphum-TH16 DNA-S	MF039874.1	98.1	99.7
	M	OP297852	195,794	1047	NongKhai-TH16, DNA-M	KY427061.1	99.3	99.7
	C	OP297851	76,170	1015	NongKhai-TH16, DNA-C	KY427060.1	99.5	99.9
	N	OP297853	247,470	1077	Chaiyaphum-TH16, DNA-N	MF039872.1	99.9	99.9
	U3	OP297849	219,980	1053	NongKhai-TH16 DNA-U3	KY427065.1	99.0	99.2
	Total BBTV reads		812,960					
	Total sample reads		2,365,796					
Q6634	R	OP297854	27,151	1104	Nakhon Ratchasima-TH16, DNA-R	MF039879.1	99.6	
	S	OP297858	10,811	1059	Chaiyaphum-TH16, DNA-S	MF039874.1	97.9	
	M	OP297856	73,755	1047	NongKhai-TH16, DNA-M	KY427061.1	99.6	
	C	OP297855	108,366	1015	NongKhai-TH16, DNA-C	KY427060.1	99.6	
	N	OP297857	139,895	1077	Chaiyaphum-TH16, DNA-N	MF039872.1	99.8	
	U3	OP297859	144,941	1053	NongKhai-TH16, DNA-U3	KY427065.1	98.8	
	Total BBTV reads		504,919					
	Total sample reads		1,124,838					

[1] Number of DNA component-specific reads

[2] All isolates are from Thailand

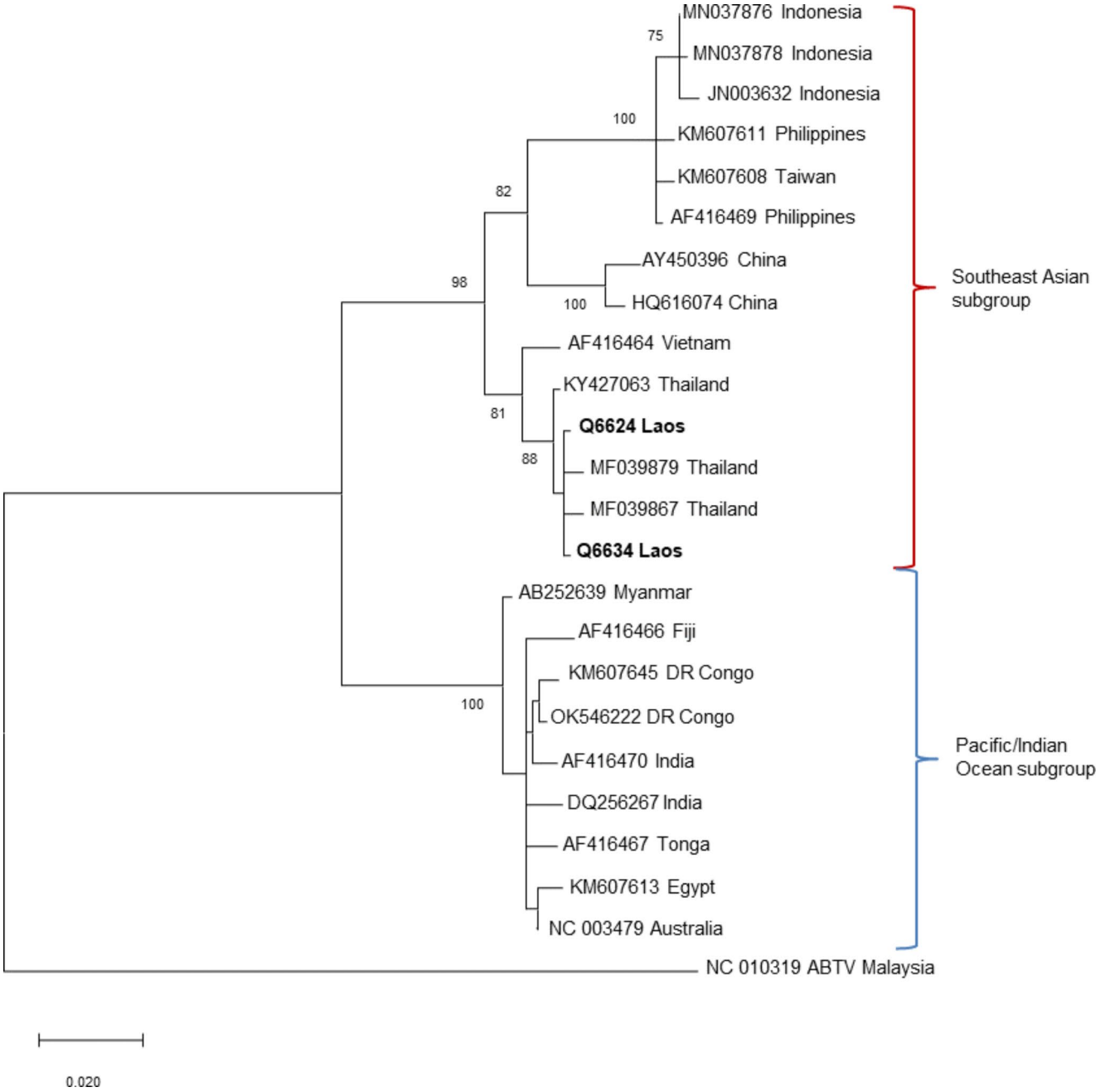

Fig. 3 Maximum likelihood phylogenetic tree showing the relationships between banana bunchy top virus isolates from Laos (bold) and representatives of the two virus subgroups, based on the DNA-R nucleotide coding sequence. The related nanovirus abaca bunchy top virus (ABTV) serves as an outgroup. Bootstrap values >70% are shown at the nodes of the branches. The scale bar represents the number of substitutions per site

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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