Molecular characterization of a novel macluravirus from Curcuma alismatifolia identified by high-throughput sequencing

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Abstract

The complete sequence of a *Curcuma alismatifolia* bract virus (CurABV) from Zhangzhou City of Fujian Province was determined by high-throughput sequencing. The viral genome consists of 8273 nucleotides (nt) and encodes a large polyprotein of 2634 amino acids (aa). The polyprotein was predicted to be cleaved into nine mature proteins by two viral proteases. Sequence analyses indicated CurABV shares 48.60%~74.40% nt and 48.71%~71.49% aa sequence identity with other macluraviruses in the coat protein (CP) gene. Phylogenetic analysis indicates that CurABV is clustered with high confidence among representative members from the genus *Macluravirus*. These data above suggest that CurABV represents a putative new member within the genus *Macluravirus*. To our knowledge, this represents the first complete genome sequence reported for a novel macluravirus infecting *C. alismatifolia*.

Full Text

The genus *Curcuma* in the *Zingiberaceae* family has been extensively cultivated as a medicinal and ornamental plant in tropical and subtropical regions in recently years. As a member of the genus *Curcuma*, *Curcuma alismatifolia* has great potential for use as cut flowers, flowering pot plants, and garden plants in tropical landscaping due to its bright bract color, distinctive inflorescence, and long blooming period [2,12,14,15]. Since *C. alismatifolia* is still a relatively new blooming flower in China, researches on this plant has primarily focuses on growth habits, cultivation techniques, rapid tissue propagation, breeding of new varieties and cut flow preservation [7,13,16,18]. Up to now, reported disease affecting *C. alismatifolia* have mainly been caused by bacteria and fungi pathogens [17], leading to significant yield losses and quality deterioration. However, viral pathogen infecting this plant has not been documented or reported yet. Here, we reported for the first time a novel macluravirus identified in *C. alismatifolia* 'Siam Sitrone' using high-throughput sequencing.

The spike of *C. alismatifolia* consists of the upper and the lower bracts, with the large and colorful upper oblate sterile bracts serving as the main ornamental parts (Figure 1A). While the base portion of the upper bracts is uniform in color, the top portion often exhibits color variations or mottle spots (Figure 1B). To investigate the mechanism behind these color variations, samples of the base and the top portions of the upper bract from *C. alismatifolia* 'Siam Sitrone' were collected from Changtai County, Zhangzhou City of Fujian Province. Total RNA was extracted from both sections using Trizol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocol. RNA concentrations and integrity were assessed using a spectrophotometer (Nanodrop2000, Thermo Fisher Scientific, Wilmington, DE, USA) and a BioAnalyser 2100 (Agilent, Palo Alto, CA, USA), respectively. RNA libraries (mRNA and viral RNA) were constructed and sequenced on an Illumina NovaSeq™ X Plus platform [6]. Briefly, RNAs (mRNA and viral RNA) were purified using an oligo-dT column, followed by ribosomal RNA depletion, cDNA synthesis, adapters ligation, and PCR amplification for 10 cycles to generate sequencing libraries. After adapter trimming and filtration of low-quality bases using the trimmomatic tool, a total of 19,656,138 and 21,344,192 clean reads (35-76 bp in length) were obtained from the top and the base portion of the upper bract, respectively. *De novo* assembly using Trinity 2.1 generated 352,478 and 367,187 contigs from the top and the base portion of the upper bract, respectively. These contigs were annotated against the viral genome/protein database using NCBI-BLAST. One contig with a length of 8214 nucleotides (nts) exhibited expression level 30 times higher in the top portion compared to the base portion of the upper bract (data no shown). BLAST analysis revealed 73% nts and 78% amino acids (aas) sequence identity with cardamom mosaic virus (CdMV, accession no. NC_039088), a member of the genus *Macluravirus* in the family *Potyviridae*. Based on the species demarcation criteria of International Committee on Taxonomy of Viruses (ICTV), these sequence divergence values suggested this represent a novel virus species, which we provisionally named *Curcuma alismatifolia* bract virus (CurABV). The potential viral agents were observed under transmission electron microscope as previously described [8,9,10,11] . Viral particles with flexuous filaments (Figure 1C), a size of ca. 15×700 nm and wind wheel-shaped body structures (Figure 1D) when viewed in cross-section were found , indicating that the diseased *C. alismatifolia* plant was indeed infected by a filamentous virus. To obtain the complete genome sequence of this virus, 5' and 3' rapid amplification of cDNA ends (RACEs) was performed using SMARTer RACE (ClonTech). The resulting amplicons were cloned and sequenced (data no shown). Combining the contig sequence with RACE results, the complete genome sequence was assembled and deposited in GenBank with the Submission No. PX361094.

The complete genome sequence of CurABV is 8273 nts in length, including a 29-nt poly(A) tail at the 3' terminus (Figure 1E, supplementary Table 1). The genome organization includes a 116-nt 5'-untranslated region (UTR) and a 226-nt 3'-UTR (Figure 1E, supplementary Table 1). As expected, CurABV genome contains a large open reading frame (ORF, nt 117-8018) encoding a polyprotein of 2,634 aa (Figure 1E, supplementary Table 1), which is consistent with what has been observed for other members of the genus *potyvirus* [1]. The polyprotein is predicted to be cleaved into nine functional proteins: HC-Pro, P3, 7K, CI, 9K, VPg, NIa, NIb and CP (Figure 1E, supplementary Table 1). Unlike most *Potyviridae* members, this virus lacks a P1 proteinase (Figure 1E, supplementary Table 1) [5]. The predicted eight cleavage sites (GYYVG/SAAGEN, LQ/GGVKTSEKQ, LQ/MHLFDLAL, MQLQ/QDGKL, EVLE/MKGKRLN, PEQRLE/IDTRLS, CLQ/MNDTTIL, QQLQ/MNLDGSTQ) were identified by comparison of CurABV polyprotein aa sequence with those of other potyviruses, with the first cleavage mediated by HC-Pro cysteine proteinase and the remaining part by the NIa-Pro cysteine proteinase (Figure 1E). Similar to other viruses classified in the family *Potyviridae* [3,4], conserved motifs in polyprotein of CurABV, including the GSGKSXXXP and DEXH motifs in CI, H-X_{31}- D-X_{61}-T-X_4-C-X_{15}-H motif in NIa, GDD motif in NIb, VPg-attachment tyrosine, and RNA binding motifs (NGTS, WCANNGTSSE, AFDF and S-R-D) were also present. However, the typical potyvirus aphid-transmission motif DAG in the CP, motifs PTK and KITC in the HC-Pro are also absent [3,4]. Details of genome positions, nucleotide and protein sizes are presented in Figure 1E and supplementary Table 1.

Global alignment of the polyprotein, all nine mature proteins sequences and 5'/3'-UTRs of the virus were performed. For the polyprotein-coding region, CurABV shared 65.99%~72.16 % nt sequence and 49.58%~78.10 % aa sequence identity with other macluraviruses, with the highest identity to CdMV (Table 1). Similarly, most mature proteins showed less than 74.40% nt identity and 79.60% aa identity, except HC-Pro (81.60%), CI (84.20%), 9K (82.50%), VPg (81.50%) and NIa (83.80%) at the aa level (Table 1). The CurABV CP gene exhibited the highest (74.4 %) nt identity with CdMV and the lowest (48.6%) with broad-leafed dock virus A (BLDVA), while at the aa level, it shared the highest (71.49%) identity with alpinia mosaic virus (AlpMV) and the lowest (48.71%) with maclura mosaic virus (MacMV) (Table 1). Based on the nucleotide sequences of the polyprotein, a maximum-likelihood phylogenetic tree was constructed using IQ-Tree implemented in PhyloSuite, with the GTR+G4+I substitution model. Branch support was assessed using 10, 000 ultrafast bootstrap replicates. The analysis included eight reported macluraviruses and forty-five representative *Potyviridae* members as reference. The results showed that

CurABV was clustered into a well-supported monophyletic clade (bootstrap value =100%), together with the eight known macluraviruses (Figure 2), confirming its classification within the *Macluravirus* genus.

According to the *Potyviridae* species demarcation criteria, macluraviruses exhibiting < 76% nucleotide and < 82% amino acid identity across the complete ORF—or < 76% nucleotide and <80% amino acid identity for the CP gene—are considered distinct species. Our findings demonstrate that the virus identified in this study satisfies these criteria as a novel *Macluravirus* species. To our knowledge, this represents the first complete genome sequence reported for a novel macluravirus infecting *Curcuma alismatifolia*.

Declarations

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Contributions

Investigation, Huiwen Yu and Luanmei Lu; sequence analysis, Fangluan Gao; writing–review and editing, Hanhong Lan.

Compliance with ethical standards
Conflict of interest The authors declare that they have no conflicts of interest.
Ethical approval Not required

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Table 1

Table 1 Pairwise nucleotide and amino acid (in parenthesis) identities of the genome of Curcuma alismatifolia virus (CaV)with those of members of ten species in the genus *Macluravirus*

Name	CdMV (NC_039088)	AloMV (NC_055493)	CYNMV (NC_018455)	YCMV (NC_038561)	YCNV (NC_055491)	BLDVA (NC_038560)	NLV (KX979913)	ArLV (NC_026759)	AlpMV (NC_043
Polyprotein	72.16/78.10	68.98/67.44	66.07/54.67	65.99/53.55	69.48/53.17	66.40/49.58	67.71/50.08	68.01/49.79	-
5´-UTR	32.10/-	37.30/-	36.30/-	30.30/-	28.20/-	35.80/-	10.70/-	29.70/-	-
HC-Pro	72.10/81.60	62.40/60.30	55.70/49.60	53.00/44.60	56.20/50.00	54.90/48.00	55.70/51.10	53.30/48.80	-
P3	67.40/68.70	57.40/52.40	49.10/38.60	49.00/36.00	45.70/32.30	44.30/29.80	46.40/30.80	44.40/31.80	-
7K	74.40/79.60	70.80/76.50	68.20/68.70	66.60/70.30	68.70/71.80	60.90/57.80	70.30/75.00	69.70/71.80	-
CI	72.90/84.20	66.00/71.10	56.00/53.60	54.50/53.50	54.40/53.60	55.80/53.50	54.10/50.20	54.40/51.20	-
9K	69.10/82.50	64.50/65.00	54.10/48.70	54.10/47.50	57.90/51.20	50.00/40.00	51.60/43.70	49.10/42.50	-
VPg	72.90/81.50	68.10/73.70	57.20/54.60	57.60/54.90	54.80/51.30	50.00/43.90	53.00/45.20	50.80/44.10	-
NIa	73.10/83.80	72.10/77.80	64.20/62.60	63.70/63.10	62.80/61.70	59.70/58.00	58.60/52.90	58.80/54.80	-
NIb	72.44/78.83	68.30/72.80	68.65/63.64	68.62/66.22	69.48/63.06	66.40/60.00	67.71/60.07	68.01/63.03	72.71/7
CP	74.40/68.49	70.85/70.30	67.71/57.33	65.87/58.91	67.52/61.67	48.60/51.45	-/53.94	66.79/50.62	72.13/7
3´-UTR	53.60/-	450.20/	45.40/-	45.30/-	52.80/-	40.30/-	/37.60-	34.00/-	-

AlpMV, alpinia mosaic virus; AloMV, alpinia oxyphylla mosaic virus; ArLV, artichoke latent virus; BLDVA, broad-leafed dock virus A; CdMV, cardamom mosaic virus; CYNMV, Chinese yam necrotic mosaic virus; MacMV, maclura mosaic virus; NLV, narcissus latent virus; YCMV, yam chlorotic mosaic virus; YCNV, yam chlorotic necrosis virus; -, no data available.

Figures

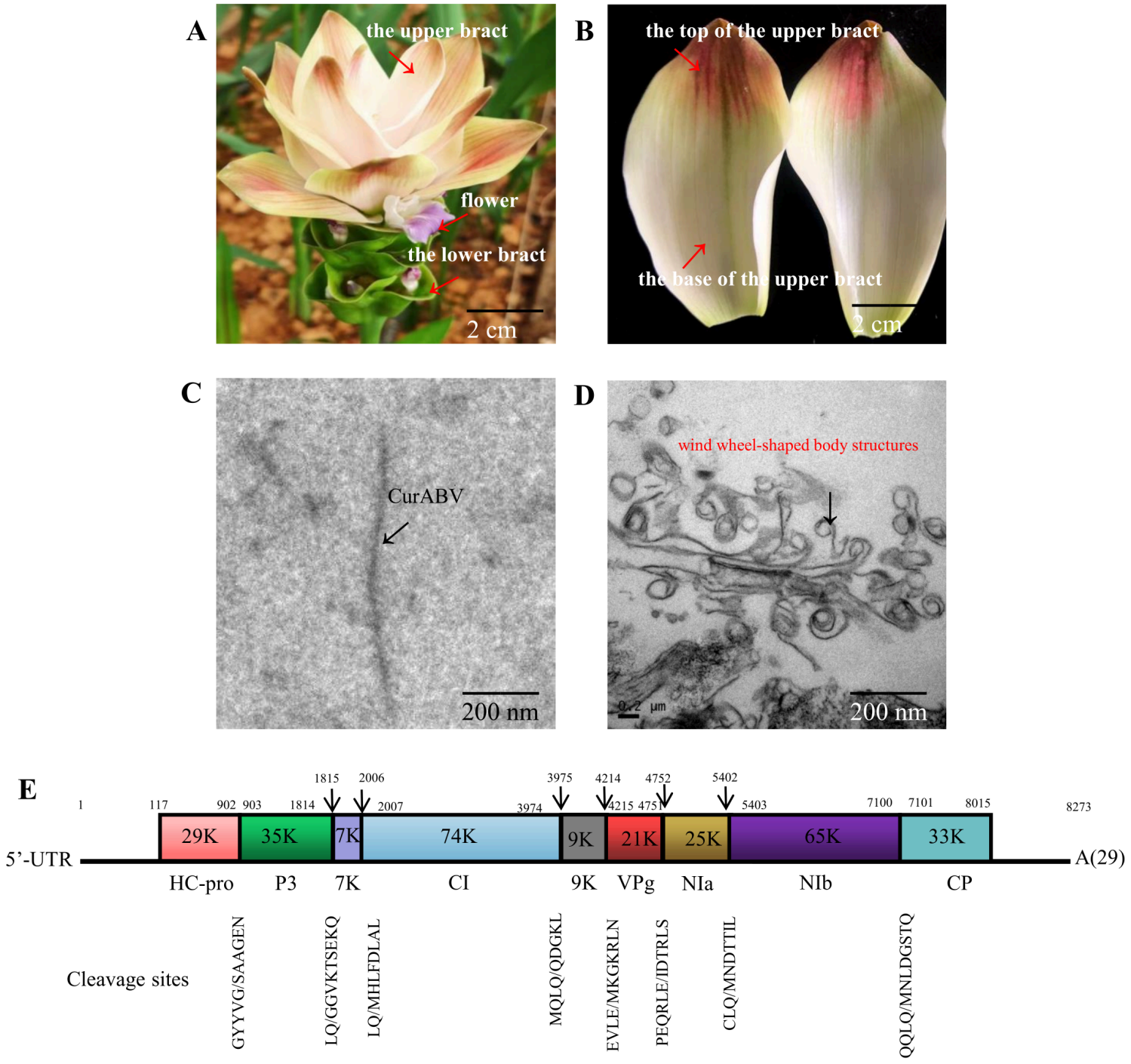

Figure 1

Detection of *Curcuma alismatifolia* bract virus (CurABV) infection in *Curcuma alismatifolia* 'Siam Sitrone' *sampled* from Changtai County, Zhangzhou City of Fujian Province. **A,** The spikes of *C. alismatifolia* 'Siam Sitrone'. Bars, 2 cm. **B**, The top and the base portions of the upper bracts. Bars, 2 cm. **C and D,** Electron microscopy of *C. alismatifolia* 'Siam Sitrone' *leaves* infected with CurABVshowing virus particle flexuous filaments morphology (C) and wind wheel-shaped body structures (D) through thin sections. Bars, 200 nm. **E,** Genome organization of CurABV. Numbers represent the nucleotide position of coding regions.

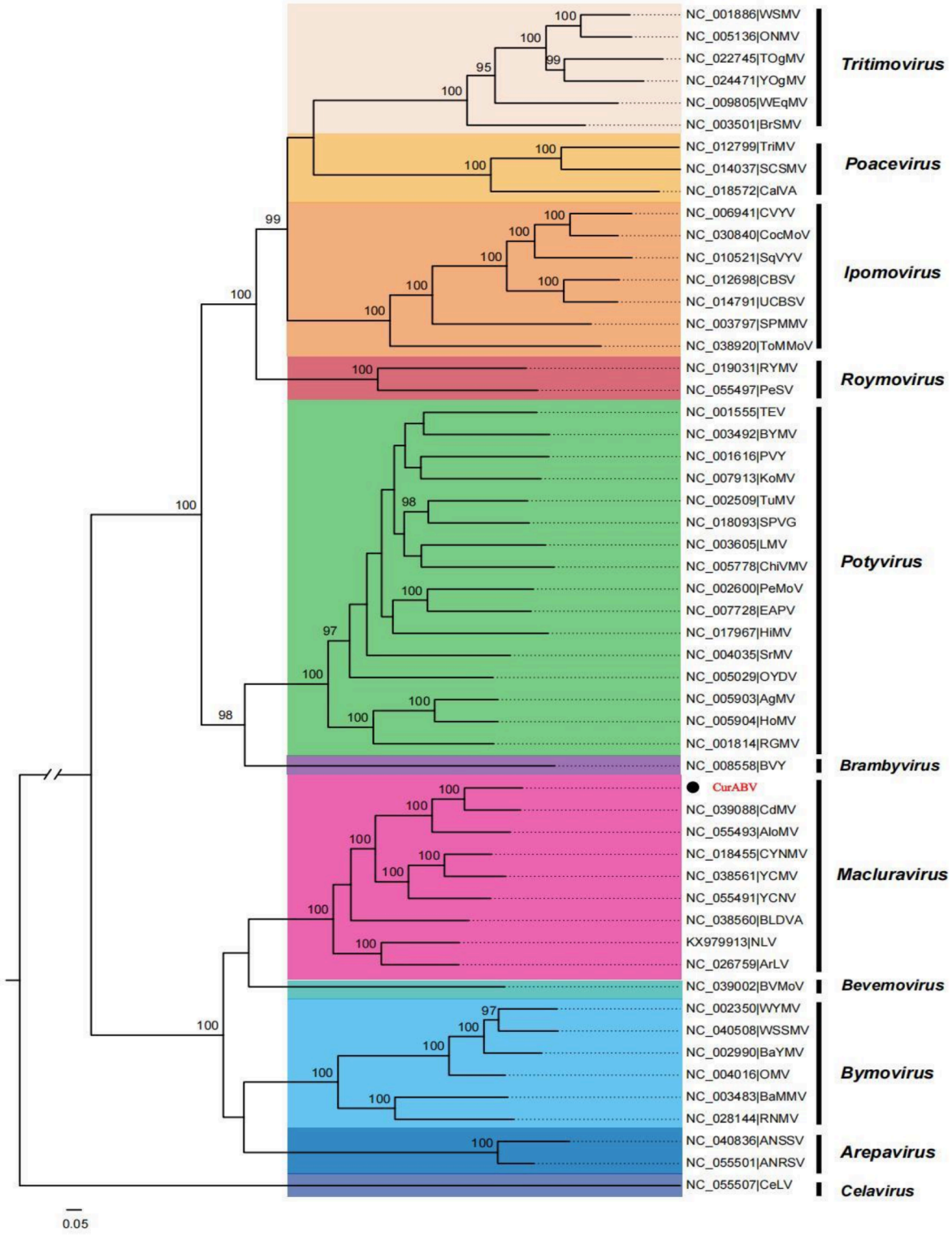

Figure 2

Phylogenetic analysis based on the nucleotide sequences of CurABV polyprotein and

those of all known members of the genus *Macluravirus*, as well as representative members

from other genera of the family *Potyviridae*. Phylogenetic tree was constructed using IQ-Tree implemented in PhyloSuite, with the GTR+G4+I substitution model. Branch support was assessed using 10, 000 ultrafast bootstrap replicates. Numbers at the nodes represent bootstrap values in percentage.

Supplementary Files

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- Supplementarytables20250913.doc