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Complete genome sequence of mimosa mosaic virus, a new sobemovirus infecting Mimosa sensitiva L.

Caterynne Melo Kauffmann (caterynne.kauffmann@hotmail.com)
Universidade de Brasilia https://orcid.org/0000-0001-9366-2027
Alessandra de Jesus Boari
Embrapa Amazônia Oriental: Embrapa Amazonia Oriental
João Marcos Fagundes Silva
Universidade de Brasilia
Rosana Blawid
Universidade Federal Rural de Pernambuco
Tatsuya Nagata
Universidade de Brasilia

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Abstract

A sobemovirus, tentatively named “mimosa mosaic virus” (MimMV), was found by high-throughput sequencing and isolated from the mimosa (*Mimosa sensitiva* L.) plant. The genome sequence was also confirmed by Sanger sequencing and comprises 4595 nucleotides. Phylogenetic analysis of the amino acid (aa) sequences of the P2b (from ORF2b) and the coat protein showed 52.7% and 31.8% aa sequence identities with those of blueberry shoestring virus, respectively. The complete genome sequence identity to MimMV with other sobemoviruses was less than 47%. These analyses suggest that MimMV is considered a new species of the genus *Sobemovirus*.

Full Text

Mimosa (*Mimosa sensitiva* L.) is a spontaneous species in the *Fabaceae* family, subfamily *Mimosoideae*. Previously, an isometric virus was reported to cause mosaic symptoms in mimosa plants in the State of Pará, north region of Brazil [1]. To identify the unknown isometric virus, we collected mimosa leaf samples showing mosaic symptoms (Fig.1B) in July 2015, in Santo Antônio do Tauá, State of Pará. The sample collected was analyzed by high-throughput sequencing (HTS).

Initially, sample of mimosa leaves were processed for viral semi-purification procedure [2]. Total RNA was extracted from the semi-purified preparation using ZR Plant RNA MiniPrep Kit (Zymo Research, Irvine, USA). The extracted RNA was added to a pooled sample (total RNA from semi-purified samples of cowpea, black pepper, and patchouli to reduce the sequencing cost), then rRNA was removed from the pooled sample using RiboZero kit for plant (Illumina, San Diego, USA), and cDNA library was sequenced using Novaseq (Illumina) at Macrogen with 5 G scale (Macrogen Inc. Seoul, Korea).

The HTS reads was trimmed and the contigs were obtained by *de novo* assembly using SPAdes 4.2. [3]. The resulting contigs were analyzed with tBlastx against virus genome database using Geneious program v.8.1.9 (Biomatters Ltd, Auckland, New Zealand). Based on the contigs related to sobemovirus sequences, the longer contigs were assembled by reads mapping in Geneious program. The specific primers, SobemoR and SobemoF (Supplementary Table S1), were designed to the region of the RNA-dependent RNA polymerase gene to detect sobemovirus sequences in individual mimosa samples. To sequence the whole genome of the sobemovirus by Sanger method, cDNA fragments were amplified by RT-PCR using the extracted RNA of the single mimosa plant using specific primers (Supplementary Table S1) designed based on HTS analysis. The 5'- and 3'-RACE protocols were performed as described by [4]. These cDNA fragments were sequenced by Sanger methods.

The complete genome of the sobemovirus, tentatively named as mimosa mosaic virus (MimMV), has 4595 nt (accession no. OP456085) determined by Sanger sequencing. The genomic organization of MimMV is typical of the genus *Sobemovirus* [5] with five open reading frames (ORFs) (Fig. 1B). The 5' end sequence is ACAAAA, present in sobemoviruses [5] and the 3' non-coding region is 399 nt long, slightly longer than other sobemoviruses (44 to 283 nt) [6]. P1 is a movement protein (encoded by ORF1)

and has the conserved [C64-X_5-C70-X_{45}-H115-X_3-C119] putative zinc-finger motif, which is related to the suppression of RNA silencing and cell-to-cell movement ability [5;7]. The putative ORFx (174 aa) starts with a CTG codon [8]. *P2a* (ORFP2a) encodes the polyprotein with putative transmembrane domain, serine protease, and genome-linked viral protein (VPg). In the region of overlap with the putative ORFx, two transmembrane alpha-helix (TMH) were predicted at aa positions 7-26 and 31-53, using TMHMM (https://services.healthtech.dtu.dk). The protease motif in P2a TxxGxsG was identified as TAQGFSG in MimMV, and the VPg motif (WAD/WGD/WNK) followed by a D/E-rich region [5,9] was modified in MimMV as "WHEAEDEMDDAD" (Fig. 1A).

P2ab, as in other viruses of the genus, appears to be expressed through a (-1) frameshift in a slippery sequence (Fig. 1A), UUUAAAC, followed by a secondary stem-loop structure located seven nucleotides downstream [10]. The frameshift site of MimMV assumed starting at position 1909 of its genome. A stem-loop was predicted with RNAfold [11] 4 nt downstream of the frameshift site (Fig. 1A).

P2b encodes the RNA-dependent RNA polymerase (RdRp) and has the conserved GDD motif (SGSYCTSSTNX_{19-35}GDD). The ORF3 encodes the coat protein (CP). The CP motif (ACAAA) is in position 3397 nt in the genome and may constitute the beginning of a subgenomic RNA [5]. For genome recombination analysis, the RDP4 program was used [12], which did not show any signals of recombination for this isolate.

The MimMV genome sequence was compared to those of other sobemoviruses, using RefSeq accessions from the genus *Sobemovirus*. The P2b and CP regions were extracted based on alignments, obtained MAFFT version 7 (https://mafft.cbrc.jp/alignment/server), and used for further phylogenetic analysis. The phylogenetic tree was inferred using IQ-TREE2 [13] with the best substitution model estimated by ModelFinder [14]. Blueberry shoestring virus (BBSSV) and velvet tobacco mottle virus (VTMoV) were closely related to MimMV (Fig. 2A) showing 52.7% and 43.3% aa sequence identities in P2b, respectively. The higher aa sequence identities with MimMV CP were 31.8% and 26.3% for BBSSV and rubus chlorotic mottle virus (RuCMV), respectively (Fig. 2B). Pairwise sequence identity was calculated using SDT v1.2. and RefSeq accessions from the members of the genus *Sobemovirus.* The demarcation criterion for establishing a new species in the genus *Sobemovirus* is that the complete genome sequence below 75% nucleotide identity [15]. BBSSV was the closest relative to the MimMV, with 46.8% nt sequence identity at the complete genome (Fig. 2C). In conclusion, the analyses suggest that MimMV is classified as a new species in the genus *Sobemovirus*, family *Solemoviridae.*

Declarations

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Conflict of Interest The authors declare that they have no conflicts of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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Figures

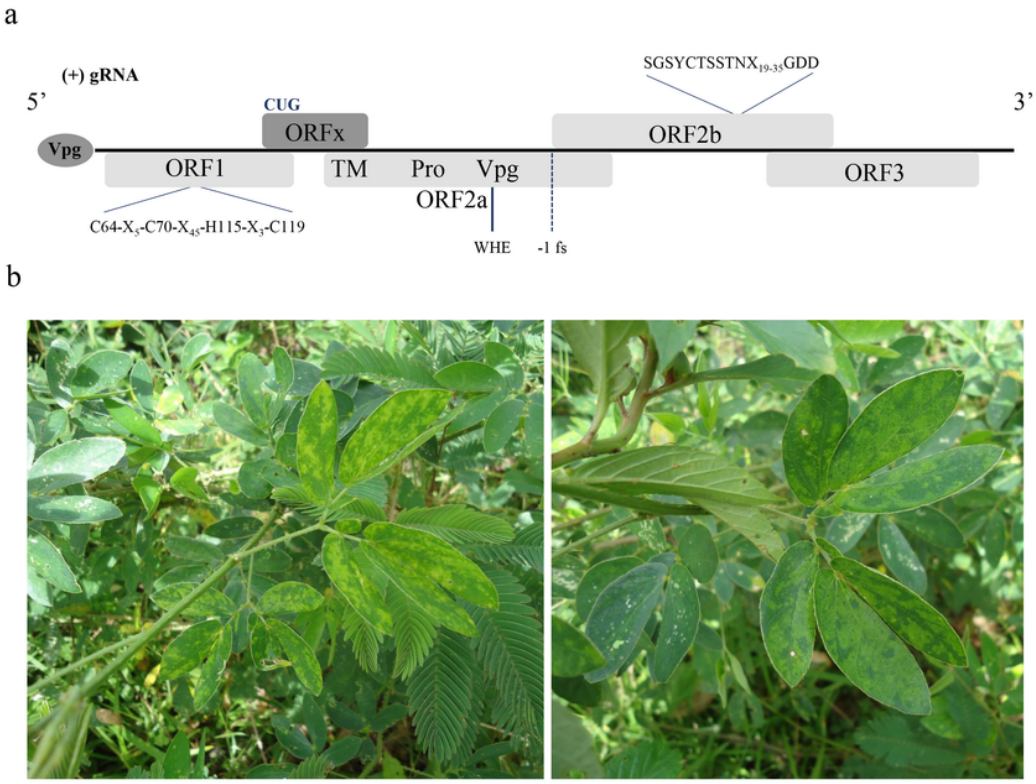


Figure 1

(a) Genome organization of mimosa mosaic virus (MimMV). Rectangles represent the relative position of the open reading frames (ORFs). ORF1, movement protein; ORFx, protein X; TM, transmembrane domain; Pro, serine protease domain; VPg, virus protein genome-linked; ORF2b, RNA-dependent RNA polymerase (RdRp); ORF3, coat protein. [C64-X_5-C70-X_{45}-H115-X_3-C119] putative zinc-finger motif in ORF1. WHE is VPg motif. (SGSYCTSSTNX_{19-35}GDD) is the GDD motif of the RdRp. (b) Mosaic symptoms in leaves of mimosa.

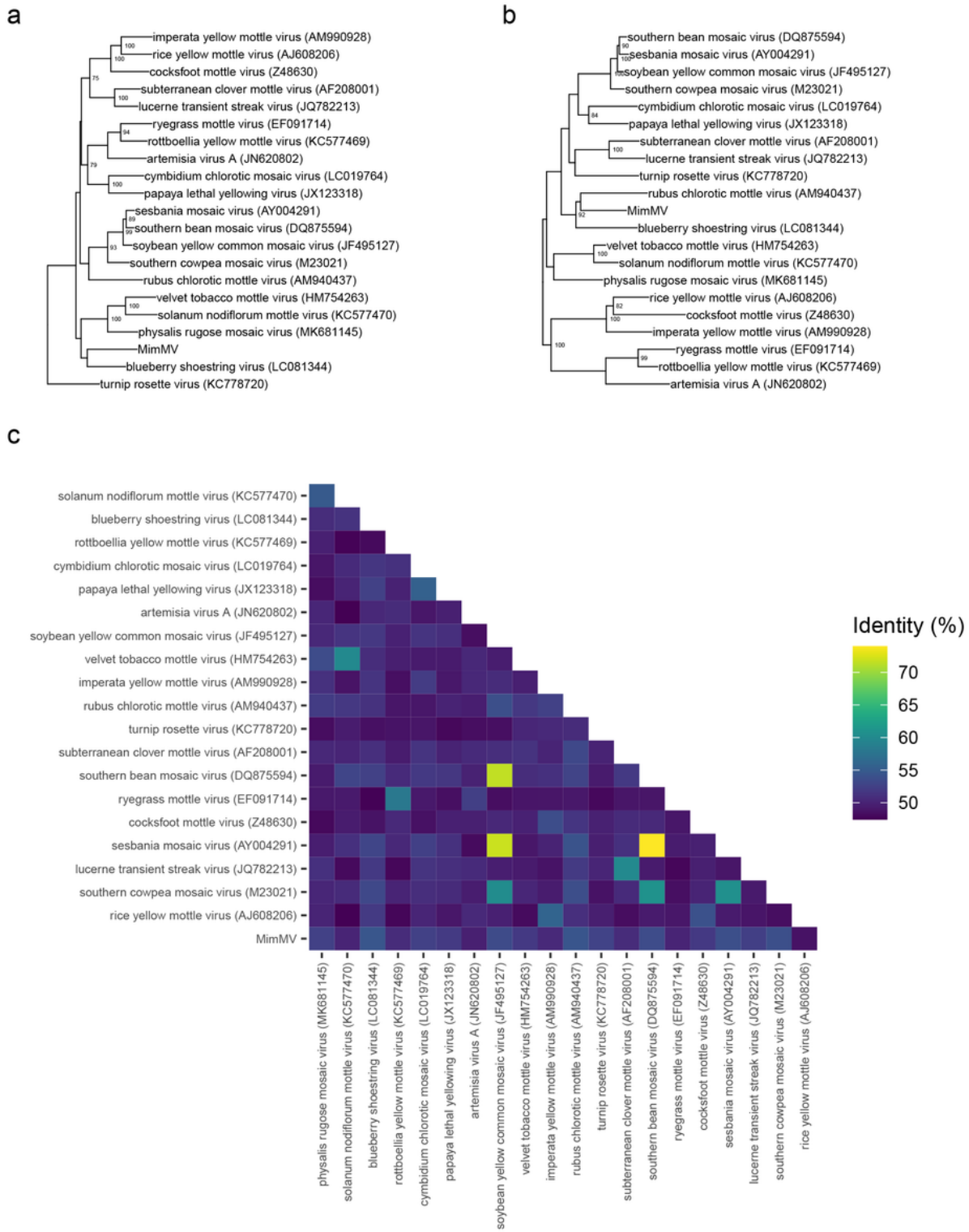


Figure 2

(a) Maximum-likelihood tree based on P2b amino acid sequences of mimosa mosaic virus (MimMV) and members of the genus *Sobemovirus* (b) Maximum-likelihood tree based on CP amino acid sequences of MimMV and members of the genus *Sobemovirus*. (c) Heatmap of pairwise based on complete genome nucleotide sequence identity (%) between MimMV and members of the genus *Sobemovirus*.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Mimosamosaicviruscompletegenome.txt
- TabeladeprimerMimMV.docx