

Discovery of a New Virus of the Family *Tymoviridae* Isolated from Mosquitoes of the Genus *Mansonia* in Brazil

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Abstract

A new virus, named Mutum virus, of the Family *Tymoviridae* was isolated from mosquitoes (*Mansonia* spp.). This virus was isolated in clone C6/36 cells. After complete sequencing, it presented a genome with 6,494 nt. The isolated virus is phylogenetically more related to two viruses isolated from *Culex* spp. mosquitoes: Ek Balam virus, isolated from Mexico, and Culex-originated Tymoviridae-like virus, isolated from China. Owing to the similarity of the protein coding domains, genomic organization, and phylogenetic analysis, we suggest that this virus belongs to a new genus, family *Tymoviridae*

Main Text

The family *Tymoviridae* is divided into three genera: *Maculavirus*, *Marafivirus*, *Tymovirus*. Virions contain single-stranded positive RNA [1]. Initially, the family *Tymoviridae* comprised only plant viruses, but in recent years, virus of this family has been isolated from mosquitoes: the insect-specific virus (ISV) Culex-originated Tymoviridae-like virus (CuTLV) and the Ek Balam virus (EkBV) [1-3].

Our study reports a third Tymo-like virus, called Mutum virus (MUTV), owing to the name of the mosquito collection area. MUTV was detected in female *Mansonia* sp. mosquitoes collected in 2018 in the vicinity of the Jirau hydroelectric dams in Nova Mutum Paraná, a rural village in the municipality of Porto Velho, State of Rondônia, Brazil (Online Resource 1). MUTV was isolated from four pools of female *Mansonia* mosquitoes in *Ae. albopictus* cells (C6/36) [4]. The viruses were registered as follows: BE AR 855909, BE AR 855928, BE AR 855911, and BE AR 855922.

MUTV was fully sequenced on an Ion torrent PGM platform (Thermo Fisher Scientific, MA, USA) according to the manufacturer's recommendations. A phylogenetic tree was constructed using the Maximum likelihood (ML) method with bootstrap test fixed 1000 replicates [5], in the RaxML v.8.0 program [6].

The sequences of MUTV have been deposited in GenBank with the following accession numbers: BE AR 855909 (MT656586), BE AR 855928 (MT656589), BE AR 855911 (MT656587), and BE AR 855922 (MT656588). The four MUTV samples showed nucleotide identity ranging from 99.9% to 100% and amino acid identity of 100%, indicating that is the same virus. MUTV had a genome of approximately 6,494 nt. The 5' and 3' non-coding regions presented 37 nt and 53 nt, respectively. MUTV has three ORFs: ORF 1 (RdRp) has 5,331 nt, 1,776 aa, and 201,061 kDa protein; ORF 2 (Coating protein) has 732 nt, 243 aa, and 26,134 kDa protein; and ORF 3 has 285 nt, 94 aa, and 10,729 kDa protein. The total readings and the coverage of the genome reads ranged from 55,312 to 139,811 and from 16.10x to 48.47x, respectively.

Analysis in the Interproscan databases, revealed conserved protein domains in the ORF 1 for Vmethyltransf, peptidase, helicase 1, and RdRp (Figure 1A), and for Tymo coat in the ORF 2 (Figure 1B). For ORF 3, there is no function described; therefore, it is characterized as a hypothetical ORF.

The MUTV is phylogenetically similar to two viruses isolated from mosquitoes: EkBV and CuTLV (bootstrap of 100%); this ISV group is apparently related to plant viruses, although genetically distant and also showed the grouping of the Bat Tymo-Like virus in this cluster (bootstrap of 74%) (Figure 2). These viruses isolated from mosquitoes are currently located in a group of unclassified viruses within the family *Tymoviridae*, out of the other genera already described. The data show amino acid identity of MUTV with EkBV, CuTLV, and Bat Tymo-like virus as 70.6%, 51.0%, and 39.0% and nucleotide identity as 67.0%, 56.3%, and 50.9%, respectively. In summary, we describe a new ISV, Mutum virus, related with ISVs in the family *Tymoviridae* until future definitions of classification.

Declarations

Acknowledgements

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Ethics Declaration

The samples were made available by project "Development of a methodology for monitoring behavioral dynamics for *Mansonia* Spp. and its relevance in hydroelectric use in the Amazon" (PD - 06631-0005/2017) with approval of Chico Mendes Institute for Biodiversity, protocol No. 37174–1.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Figures

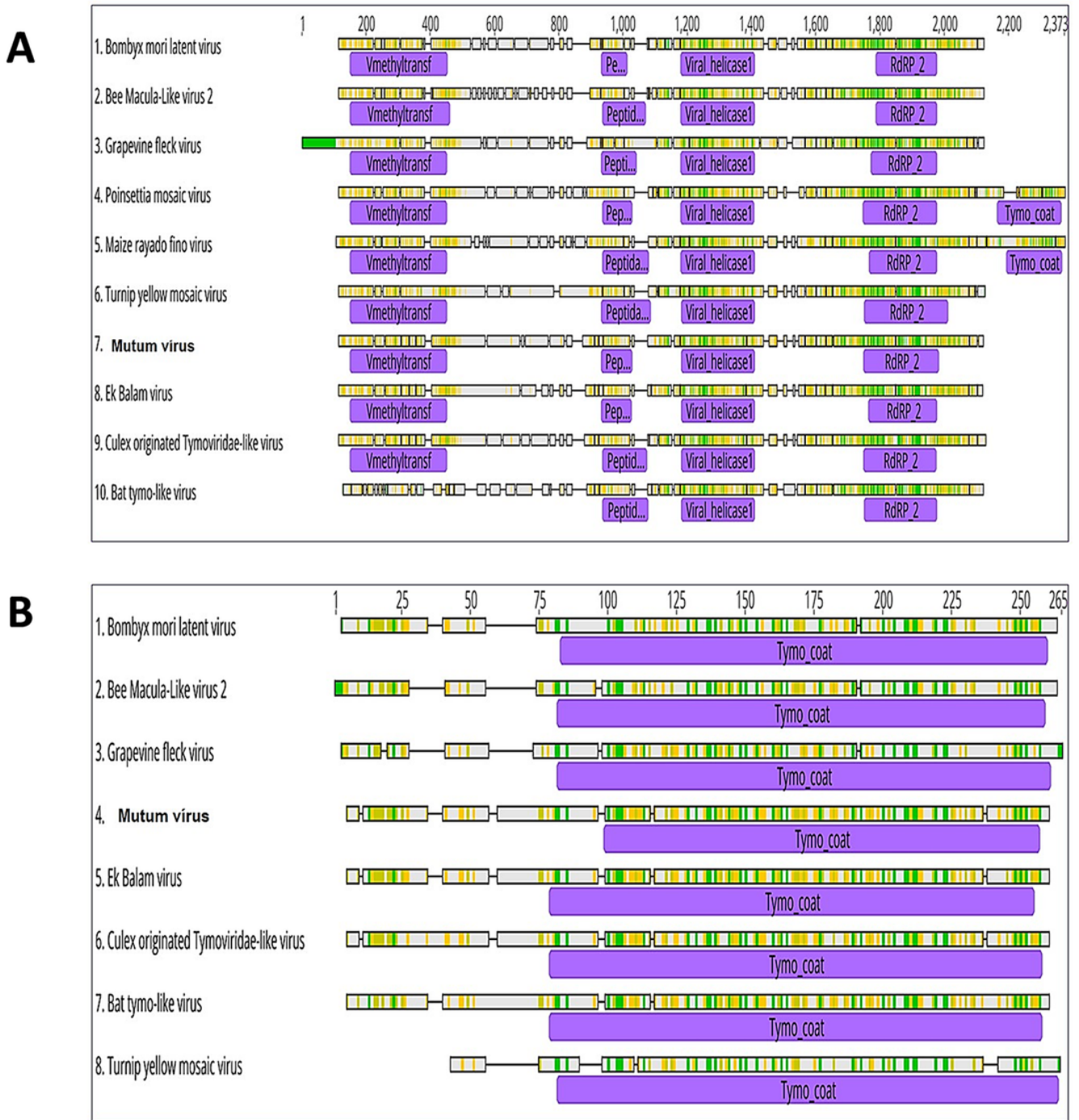


Figure 1

Conserved motifs predicted for the Mutum virus, EkBV, CuTLV, Bat tymo-like virus and reference viruses for each genus together with ungrouped viruses. A) ORF 1. B) ORF 2.

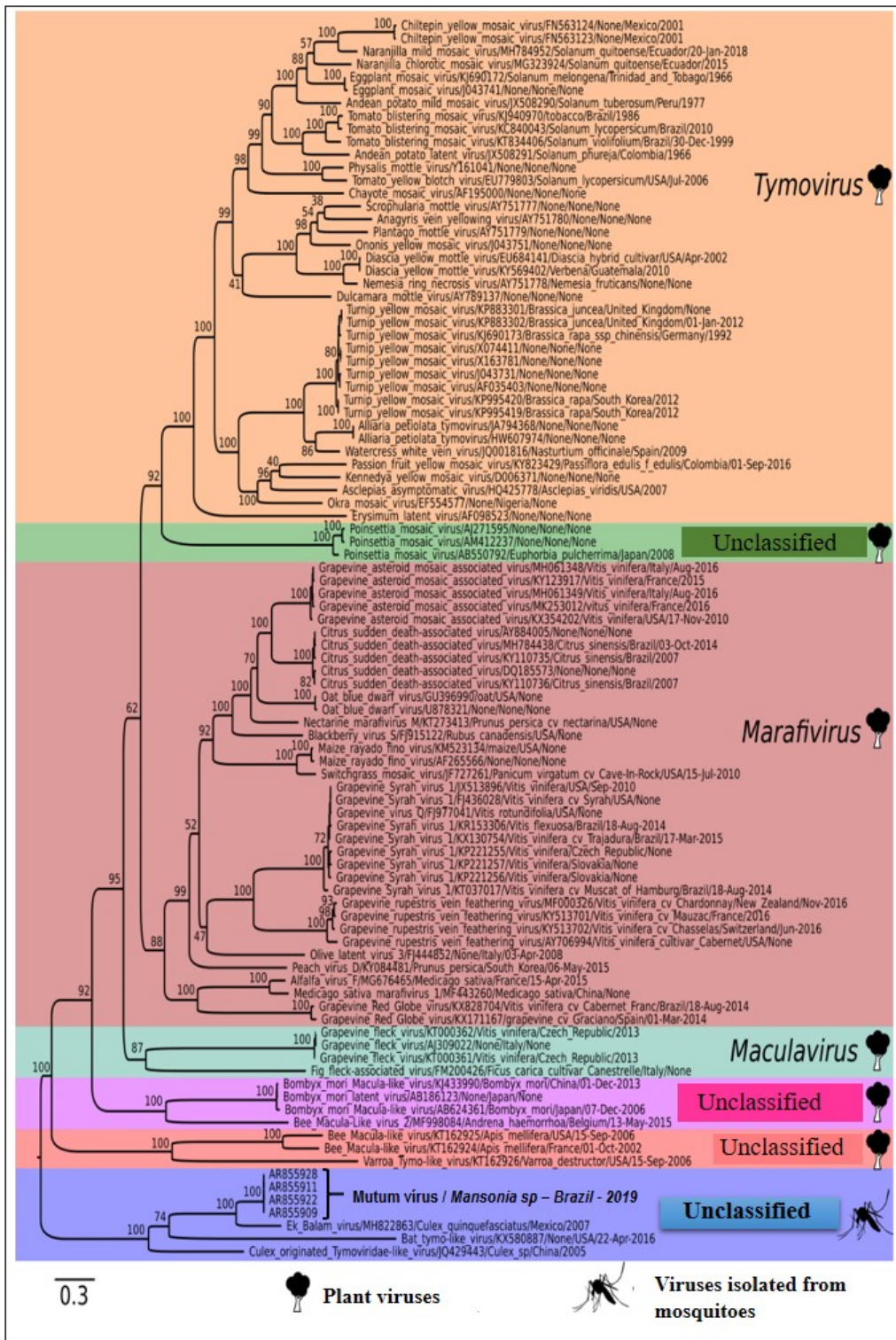


Figure 2

Phylogenetic tree of the family Tymoviridae using the ML method based on the complete amino acid sequences of the RNA-dependent RNA polymerase protein. The clusters are labeled in colors. The nodes of the tree correspond to bootstrap values in percent. The scale bar corresponds to genetic divergence.

Supplementary Files

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- [OnlineResource1.pdf](#)