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Muntingia yellow spot virus: a novel New World begomovirus infecting *Muntingia calabura* L.

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Abstract

The whole genome sequence of a begomovirus (family *Geminiviridae*) infecting *Muntingia calabura* L. (family Muntingiaceae) from the province of Guayas in Ecuador was determined in this work. The major symptom observed on this plant species was yellow spots on leaves. The nucleotide sequences of three DNA-A clones and one DNA-B clone were compared to those of other begomoviruses. The DNA-A clones displayed the highest similarity to isolates of pepper leafroll virus (PepLRV), with 87.4 to 88.1% sequence identity. Likewise, the DNA-B clone showed the highest similarity (79.3-79.6% sequence identity) to PepLRV isolates. According to the demarcation criteria for begomovirus species, the begomovirus described in this work, for which we propose the name "muntingia yellow spot virus", represents a novel species. To our best knowledge, this is the first report of a begomovirus infecting a plant of the family Muntingiaceae.

The genus *Begomovirus* (family *Geminiviridae*) is the largest plant virus genus according to reports of the International Committee on Taxonomy of Viruses (ICTV). The begomovirus genome comprises one or two circular single-stranded DNA components, referred to as DNA-A and DNA-B [1]. Begomoviruses are transmitted in a persistent circulative manner by the whitefly *Bemisia tabaci*. In recent decades, the widespread dissemination of *B. tabaci* has been associated with a dramatic upsurge of begomovirus infections throughout the world [2]. *Muntingia calabura* L., belonging to the family Muntingiaceae [3, 4], is a shrub that grows in tropical and subtropical regions worldwide and is native to southern Mexico, the Caribbean, and Central and South America [5]. This plant is a potential source of nutraceutical products [5]. In Ecuador, *M. calabura* (commonly known as "niguito" or "cerezo") is mainly found in the provinces of El Oro, Loja, Los Ríos, Manabí, Azuay, Guayas, and Galápagos, growing between 0 and 1100 meters above sea level [6]. During a survey carried out in the town of Sabanilla, Guayas province (Ecuador), in January 2017, a tissue leaf sample was collected from a *M. calabura* plant showing virus-like symptoms consisting of leaf deformation, interveinal chlorosis and yellow spots on leaves (Fig. 1A). This plant was found at the border of a bean field.

Total DNA was isolated using a FastDNA Kit (MP, Biomedicals, France) according to the manufacturer's instructions. The begomovirus infection was confirmed by PCR using the degenerate primers PAL1v1978 and PAR1c946 for begomoviruses [7]. The viral DNA components were amplified by rolling-circle amplification (RCA) using a TempliPhi Kit (GE Healthcare). The RCA products were digested with *Pst*I, and *Sac*I endonucleases. The linearized DNA-A and DNA-B components (*ca*. 2.6 kb) were purified and inserted into the appropriated restriction sites of pBluescript II SK+ (Stratagene, USA). The ligation products were used to transform NEB 10-beta chemically competent *Escherichia coli* cells. Three full-length DNA clones were obtained using *Pst*I and *Sac*I and sequenced by primer walking at Macrogen Inc. (Amsterdam, The Netherlands). Contigs of each DNA component were assembled using Geneious R11 (www.geneious.com). RCA products digested with *Sac*I generated three identical DNA-A clones, while RCA products digested with *Pst*I generated one DNA-B clone and two variants of DNA-A. Using BLASTn, the three variants of

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Fig. 1 *Muntingia calabura* L plant exhibiting yellow spot symptoms reminiscent of a begomovirus infection

DNA-A (E56S1A, E56P8A and E56P11A) and the DNA-B clone (E56P13B) were found to be related to New World (NW) begomoviruses. Multiple sequence alignments with genetically related begomoviruses were performed using MUSCLE [8], and pairwise sequence identity scores were determined using the software SDT v1.2 [9]. The phylogeny of DNA-A and DNA-B was reconstructed based on maximum-likelihood trees (500 bootstrap replicates) using MEGA 6 [10]. Recombination analysis was carried out using RDP4 [11] and Simplot [12].

The DNA-A clones E56S1A (2588 nt, GenBank no. MW032662), E56P8A (2591 nt, MW032663), and E56P11A (2590 nt, MW032664) have the typical genome organization of DNA-A from NW begomoviruses consisting of five ORFs: AV1, AC1, AC2, AC3, and AC4. Pairwise sequence comparisons showed that the three DNA-A sequences were 93.7-96.5% identical to each other and displayed the highest similarity to isolates of pepper leafroll virus (PepLRV), with nt sequence identity ranging from 87.4 to 88.1% (Supplementary Fig. S1A). PepLRV was first described in Peru infecting bean, tomato, pepper, and the weed species *Nicandra physaloides* (L.) Gaertn [13]. More recently, a PepLRV isolate (MH481901) was found infecting bean plants in Ecuador, where few begomoviruses have been described so far [14, 15]. The DNA-B clone E56P13B (2551 nt, MW032665) showed the typical genome organization of DNA-B components of begomoviruses, consisting of two ORFs, BV1 and BC1, and displayed the highest nucleotide sequence similarity (79.3-79.6% identity) to PepLRV isolates (Supplementary Fig. S1B). The common region (CR), found in both DNA-A and DNA-B, is about 158 nt long, and the components shared 85 to 93.9% nucleotide sequence identity in the CR. Two copies of the iteron GAGCAC were identified within the CR of each DNA component. Iterons are essential repeated sequence elements through which the replication-associated protein is bound to the viral DNA [16]. According to ICTV guidelines, begomoviruses showing less than 91% identity in the complete DNA-A sequence are considered members of different species [17]. As the DNA-A components identified in this study were > 93% identical to each other and < 91% identical to those of previously described begomoviruses, these isolates should be considered members of a new species, and we propose the name "muntingia yellow spot virus" (MuYSV) for this novel virus.

Phylogenetic analysis confirmed that the DNA-A and DNA-B components of MuYSV are closely related to the corresponding genome components of PepLRV isolates (Fig. 2A–B). Previous studies showed that both genome components of PepLRV grouped with different NW begomoviruses, suggesting their separate evolutionary origins [13]. Likewise, the DNA-A and DNA-B of MuYSV clustered with different NW begomoviruses (Fig. 1B-C), with the exception of PepLRV, indicating their separate evolutionary histories, as has been observed previously for other begomoviruses [18, 19]. Furthermore, recombination events were detected in both genome components of MuYSV by at least six recombination methods implemented in RDP4 software [11] (Table S1). Interestingly, the recombination event detected in one DNA-A clone of MuYSV, E56S1A, involved the AC1 genes and intergenic regions of the other two DNA-A clones, E56P11A and E56P8A, as putative parents. According to pairwise sequence analysis, the AC1 gene was the most variable gene in the DNA-A component of MuYSV (93.2-98.3% nucleotide sequence diversity) (data not shown). The perennial nature of *M. calabura* might contribute to the genetic diversity of MuYSV by allowing frequent mutations and potential recombination events to occur within a single host plant. Recombination events between strains of the same virus have been observed in other begomoviruses such as cotton leaf curl Multan virus [20] and tomato yellow leaf curl Sardinia virus [21]. Furthermore, a recombination event in the DNA-B of MuYSV was predicted to have involved the begomoviruses cleome leaf crumple virus (CleLCrV) and soybean blistering mosaic virus (SbBMV) (Table S1). Both viruses have been reported in South America. CleLCrV was first described in a weed, *Cleome affinis* (Blume) Spreng (family Cleomaceae), growing in the state Mato Grosso do Sul, Brazil [22], while the SbBMV isolate identified as a potential parent of MuYSV was found infecting pepper (*Capsicum annuum* L) in northwestern Argentina [23]. Finally, to our best knowledge, this is the first description of a begomovirus infecting a member of the family Muntingiaceae. This underscores the need to search for new and yet undiscovered begomoviruses in Ecuador to better understand the ecology of such viruses in cultivated and non-cultivated plant species.

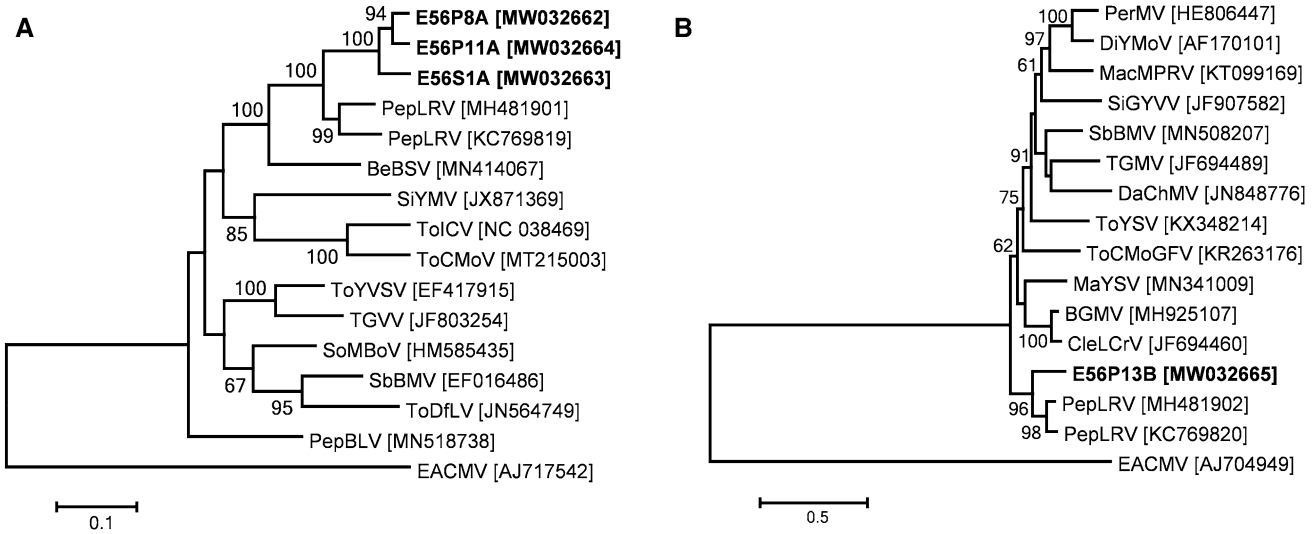

Fig. 2 Phylogenetic analysis of the MuYSV genome. (A) Phylogenetic tree based on an alignment of the DNA-A components of MuYSV (E56S1A, E56P8A and E56P11A) and related begomoviruses. (B) Phylogenetic tree based on an alignment of the DNA-B component of MuYSV (E56P13B) and related begomoviruses. Phylogenetic relationships were determined based on the maximum-likelihood method, using GTR+G as a nucleotide substitution model. Bootstrap values (500 iterations) above 60% are indicated for each node. MuYSV isolates are highlighted in bold. East African cassava mosaic virus (EACMV) sequences were used as outgroups in both trees. Nucleotide sequences are identified with their GenBank accession number in brackets. Sequences of the following begomoviruses were included in the analysis: bean bushy stunt virus (BeBSV), bean golden mosaic virus (BGMV), cleome leaf crumple virus (CleLCrV), dalechampia chlorotic mosaic virus (DaChMV), dicliptera yellow mottle virus (DiYMoV), macroptilium mosaic Puerto Rico virus (MacMPRV), macroptilium yellow spot virus (MaYSV), pepper blistering leaf virus (PepBLV), pepper leafroll virus (PepLRV), peristrophe mosaic virus (PerMV), sida golden yellow vein virus (SiGYVV), sida yellow mosaic virus (SiYMV), solanum mosaic Bolivia virus (SoMBoV), soybean blistering mosaic virus (SbBMV), tomato chlorotic mottle virus (ToCMoV), tomato chlorotic mottle Guyane virus (ToCMoGFV), tomato dwarf leaf virus (ToDfLV), tomato golden mosaic virus (TGMV), tomato golden vein virus (TGVV), tomato interveinal chlorosis virus (ToICV), tomato yellow spot virus (ToYSV), and tomato yellow vein streak virus (ToYVSV).

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Declarations

Conflict of interest The authors declare no conflict 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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