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ANNOTATED SEQUENCE RECORD

Complete genome sequence of a new bipartite begomovirus infecting *Boehmeria leiophylla* in China

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Abstract

A bipartite begomovirus was identified from a *Boehmeria leiophylla* plant sample exhibiting yellow mosaic symptoms collected in Nabanhe National Nature Reserve, Xishuangbanna, Yunnan, China. Five complete DNA-A and four DNA-B genome sequences were obtained by rolling-circle amplification (RCA), cloned, and sequenced. All DNA-A sequences were determined to be 2759 nucleotides in size, sharing 99.9%–100% nucleotide sequence identity with each other. The DNA-B sequences were comprised of 2673 nucleotides, sharing 98.6–100% nucleotide sequence identity with each other. Genomic organization of the begomovirus was typical of Old World bipartite begomoviruses. Sequence analysis revealed 81.84% nucleotide sequence identity to tomato leaf curl Hsinchu virus (ToLCHsV) from China for the DNA A component and 67.23% identity to the squash leaf curl China virus (SLCCNV) from India for the DNA B component. The sequence comparisons suggest that this bipartite begomovirus represents a novel species for which we propose the name "*Ramie yellow mosaic virus*".

Begomoviruses (taxonomy: genus *Begomovirus*, family *Geminiviridae*) have circular single-stranded (ss) DNA genomes, which are encapsidated in twinned icosahedral particles and transmitted by the whitefly *Bemisia tabaci* [1]. The majority of begomoviruses originating from the New World (NW) have bipartite genomes; the genome of many begomoviruses from the Old World (OW) are monopartite, although there are a few with bipartite genomes [1, 2]. The genomic components of the bipartite viruses, known as DNA-A and DNA-B, are approximately 2.5–2.8 kb in size, whereas the monopartite genome is homologous to the DNA-A component of bipartite begomoviruses [3]. DNA-A encodes one or two proteins (CP, V2/AV2) in the virion sense, and four in the complementary sense (Rep, REn, TrAP, and AC4/C4), while DNA-B encodes two proteins (NSP and MP) [3–5]. The DNA-A and DNA-B components share a ~ 200 nucleotide sequence with high identity known as the common region (CR). The CR contains the nonanucleotide sequence (TAATATTAC) that marks the origin of virion-strand DNA replication, the TATA box and repeated sequences (known as "iterons") which are sequence specific binding sites for Rep [6]. The TATA-box with typical sequence, TATA, is a regulatory element involved in the formation of a transcription initiation complex [7].

Begomoviruses have emerged as damaging pathogens that infect a broad range of dicotyledonous plants including many economically important crops [8, 9]. *Boehmeria leiophylla* plants are protected plants across China, and are often found in rainforests. Begomovirus infections, e.g. tomato leaf curl Hsinchu virus (ToLCHsV), of plants of this genus were reported in Jiangsu and Zhejiang provinces in 2010 [10]. ToLCHsV is one of the few bipartite begomoviruses in the OW and has mostly been identified from ramie samples (*Boehmeria niv*ea), and rarely identified in tobacco and tomato.

In January 2015, leaves with yellow mosaic symptoms were observed on *B. leiophylla* plants in Nabanhe National Nature Reserve in Xishuangbanna, a southern prefecture of Yunnan province, China (Fig. 1). Leaf samples (YN4818,

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Fig. 1 Yellow mosaicking of leaves observed in *Boehmeria leiophylla* plants in the Nabanhe National Nature Reserve in Xishuangbanna, Yunnan Province during 2015

YN4819, and YN4820) were collected from three symptomatic *B. leiophylla* plants respectively, and total DNA was extracted with the CTAB method [11]. Total DNA was used for PCR-based detection of begomovirus DNA-A, DNA-B, betasatellites and alphasatellites using four previously reported primer sets to amplify DNA-A, DNA-B, betasatellites, and alphasatellites [12–15]. All samples tested positive with universal degenerate primer pairs PA/PB (for DNA-A), and PCRc1/PBL1v2040 (for DNA-B), but were negative with betasatellite and alphasatellite primer sets. Amplification products of the sample YN4819 were cloned, and four clones with the expected insert size were selected for sequencing at Life Technologies, Shanghai, China. Sequence comparisons using the BLASTn algorithm revealed that the sequences of the amplicons generated with the DNA-A primer set shared 82% identity with the *CP* gene of ToLCHsV-FJSm04, while DNA-B amplicons shared 72% identity with a partial DNA-B sequence of tomato leaf curl New Delhi virus (ToLCNDV).

Rolling circle amplification (RCA) was performed using a TempliPhiTM kit (GE Healthcare, Bucks, UK) to obtain enriched circular viral DNA from the YN4819 leaf sample [16]. RCA fragments of approximately 2.8 kb were obtained from this sample by cleaving the circular DNA with *Hin*dIII or *Sma*I restriction endonucleases and these were cloned into the pGEM®-7Zf (+) plasmid vector (Promega, Madison, WI, USA), which was cleaved previously with the same enzymes. Four DNA sequences of the cloned inserts (*Hin*d-1, *Hin*d-3, *Sma*I-1 and *Sma*I-7) were selected and sequenced using the primer walking strategy. Further, specific primer pairs 4819DNA-A-F (5′-ACCTCT ATAAAGAACTTG-3′), 4819DNA-A-R (5′-GTACACGCA CTAAATAAG-3′) and 4819DNA-B-F (5′- CAACCACTC ACGCATTAAGT-3′), 4819DNA-B-R (5′-CCAGGTGTC CCACTAACAGT-3′) were designed for amplification of the full-length genome of DNA-A and DNA-B, respectively, from the RCA product. The amplified fragments (approximately 2.8 kb) were cloned into the pGEM-T easy vector (Promega). Five clones (4819-5, 4819-10, 4819-12, 4819-1 and 4819-4) were selected and completely sequenced using the primer walking strategy. Sequence comparisons using the BLASTn algorithm revealed that five DNA-A and four DNA-B sequences were obtained. The sequences of the five DNA-A clones (KU522485, KU522486, KU522487, MG825412, and MG825413) and the four DNA-B clones (MG825411, MF319552, MF319553, and MG825414) were determined to be 2759 and 2673 nucleotides, respectively. All sequences had a genome organization typical of bipartite begomoviruses: two virus-sense genes encoding the capsid protein (CP/AV1) and AV2 protein (AV2) and four complementary-sense genes encoding the replication-associated protein (Rep/AC1), a transcriptional activator protein (TrAP/AC2), a replication enhancer protein (REn/AC3), and the AC4 protein (AC4) in the DNA-A, and one virus-sense gene encoding the nuclear shuttle protein (BV1) and a complementary-sense gene (BC1) in the DNA-B segment (Table S1). The two components had a common region (CR) of 180 nt (96.1% identity), which included the highly conserved nonanucleotide sequence (5′-TAATATT/AC-3′), TATA box, and iteron sequences (GGTGT and GGGGT).

Following the recommendations of the *Geminiviridae* Study Group of the ICTV [17], the pairwise nucleotide identities for the viral components were calculated using the MUSCLE option in SDT v1.2 [18] using sequences obtained from the initial BLASTn analysis. The results demonstrated that complete DNA-A sequences identities ranged from 99.92 to 100% among these five isolates, while the complete DNA-B sequences shared 98.62 to 100% sequence identity with each other. These results indicate that they are isolates representing a single species, based on presently applicable species demarcation criteria for begomoviruses [17]. With regards to other begomoviruses, the DNA-A component has the highest (81.84%) nucleotide (nt) sequence identity with ToLCHsV (KC171652) from China, while the DNA-B component was most closely related to the DNA-B of SLCCNV from India, with 67.23% nt sequence identity. According to current recommendations for begomovirus classification [17], the begomovirus identified from *B. leiophylla* is a new begomovirus isolate, for which the name ramie yellow mosaic virus (RaYMV) is proposed. The proposed species name is therefore accordingly "*Ramie yellow mosaic virus*".

Phylogenetic trees were constructed using the neighbor-joining method algorithm with 1000 bootstrap replications available in MEGA6 version 6.0 [19]. Phylogenetic analysis revealed that RaYMV DNA-A clustered with DNA-A of ToLCHsV from China and within a subclade containing monopartite begomoviruses from Philippines and Thailand (Fig. 2A). The RaYMV DNA-B was most closely related

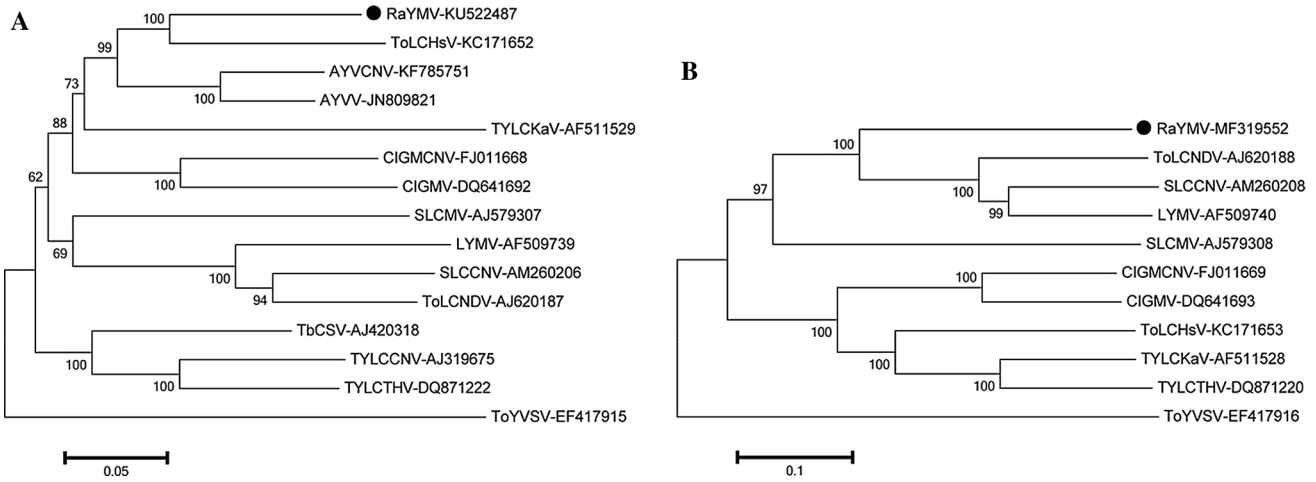

Fig. 2 Phylogenetic trees based on full-length sequences of begomovirus DNA-A components (A) and DNA-B components (B) selected from the database. The phylogenetic trees were constructed using the neighbor-joining method with MEGA6, using 1,000 bootstrap replicates. The sequences generated in this work are labeled with black circles. The begomovirus acronyms are as follows: tomato leaf curl Hsinchu virus (ToLCHsV), ageratum yellow vein China virus (AYVCNV), ageratum yellow vein virus (AYVV), tomato yellow leaf curl Kanchanaburi virus (TYLCKaV), clerodendron golden mosaic China virus (ClGMCNV), clerodendron golden mosaic virus (ClGMV), Sri Lankan cassava mosaic virus (SLCMV), luffa yellow mosaic virus (LYMV), squash leaf curl China virus (SLCCNV), tomato leaf curl New Delhi virus (ToLCNDV), tobacco curly shoot virus (TbCSV), tomato yellow leaf curl China virus (TYLCCNV), tomato yellow leaf curl Thailand virus (TYLCTHV) and tomato yellow vein streak virus (ToYVSV)

to ToLCNDV, LYMV, and SLCCNV isolates (Fig. 2B). These results showed that the DNA-A and DNA-B components of RaYMV grouped into different branches, with different species closely related to begomoviruses identified in Asia.

At present, only a few bipartite begomoviruses have been reported in China including Kudzu mosaic virus, clerodendrum golden mosaic China virus, and ToLCHsV [10, 20, 21]. The majority of these viruses are isolated from wild/non-cultivated plants. In our study, we also identified a novel bipartite begomovirus from wild/non-cultivated plants.

Nabanhe National Nature Reserve in Xishuangbanna was established nearly 60 years ago. Plants have been allowed to grow completely undisturbed by agricultural practices within the nature reserve. Since *B. leiophylla* is a perennial shrub, it is likely that these protected plants have been coexisting with this distinct virus for a very long time. Thus, further studies are required to determine the prevalence of this virus in the national nature reserve and its potential to cause loss of these protected plants, as well as other plant species in the area.

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Compliance with ethical standards

The authors have read and have abided by the statement of ethical standards for manuscripts submitted to Archives of Virology.

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

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

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