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ANNOTATED SEQUENCE RECORD

Arachis ampelovirus 1, a novel ampelovirus infecting forage peanut

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Abstract

A novel ampelovirus, designated "arachis ampelovirus 1" (ArAV1), was identified through high-throughput sequencing in forage peanut (*Arachis pintoi*). The ArAV1 genome sequence, confirmed by Sanger sequencing, comprises 12,940 nucleotides and contains seven open reading frames (ORFs), indicating a typical ampelovirus genome structure. The HSP70 homolog, RdRp, and CP proteins were found to exhibit less than 75% amino acid sequence identity to those of other ampeloviruses. Phylogenetic analysis based on amino acid sequences of the HSP70 homolog and the RdRp showed that pineapple mealybug wilt-associated virus 1 is the most closely related to ArAV1, whereas analysis based on CP amino acid sequences revealed that the pineapple mealybug wilt-associated virus 3 is the closest relative of ArAV1. These analyses suggest that ArAV1 is a member of a new species within the genus *Ampelovirus*, for which we propose the binomial name "*Ampelovirus arachii*".

Forage peanut (*Arachis pintoi*) is a perennial stoloniferous tropical legume that is widely planted for grazing purposes in tropical regions, including Brazil [1]. In this study, 22 symptomatic and asymptomatic *Arachis pintoi* accessions were obtained from the Active Germplasm Bank (BGA) of forage peanuts at the Embrapa Acre Institute (Rio Branco, Acre, Brazil). Total nucleic acids were extracted using a Purelink Viral RNA/DNA Mini Kit (Thermo Fisher Scientific, Waltham, USA), followed by DNase treatment using a DNA-free DNA Removal Kit (Thermo Fisher Scientific). RNA extracted from the 22 *A. pintoi* plants was pooled to construct a complementary DNA library, using a Complete ScriptSeq Kit (Epicenter, Illumina, Inc. San Diego, USA), followed by transcriptome sequencing using an Illumina HiSeq2500 platform at the Functional Genomics Facility of the Esalq, University of São Paulo (Piracicaba, Brazil). The raw reads were trimmed to remove adapter sequences and low-quality reads, and the resulting sequences were assembled using CLC Main Workbench v. 7.0.3 (QIAGEN, Germantown, USA). The assembled contigs were compared using tBLASTx to sequences in the virus genome database, using Geneious software v.9.1.5 (Biomatters Ltd, Auckland, New Zealand) [2]. Finally, the extended sequences were reassembled into selected contigs using the 'map-to-reference' function in Geneious. The longest contig assembled contained 12,984 nucleotides (nt) aligned with 15,084 reads (out of 195,850,656 total reads), with a mean coverage of 115.9×. The specific primers AmpF and AmpR (Supplementary Table S1), were designed to amplify the RNA-dependent RNA polymerase (RdRp) gene to detect ampelovirus sequences in the individual *A. pintoi* plants.

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The ampelovirus was detected in three of the 22 accessions. High-throughput sequencing (HTS) also revealed the presence of arachis virus Y, cucumber mosaic virus subgroup IB, arachis mottle-associated virus, peanut mottle virus (PeMoV), and cowpea chlorotic mottle virus (CCMV), which were subsequently confirmed by RT-PCR, using the primers shown in Supplementary Table S1. Accession A116, which showed mild mosaic symptoms and was coinfected with PeMoV and CCMV, was selected to determine the complete genome sequence by Sanger sequencing. The cDNA fragments were amplified by RT-PCR, using RNA extracted from a single plant of A116 and specific primers (Supplementary Table S1) designed based on the HTS results. 5'- and 3'-RACE were performed as described previously [3]. The resulting amplicons were sequenced by the Sanger method, and the virus was partially purified from leaves from accession A116 as described previously, with modifications [4]. A semi-purified preparation was examined by transmission electron microscopy (JEM 1011, JEOL, Tokyo, Japan) after negative staining with uranyl acetate [5], revealing the presence of flexuous particles ca. 1440–2200 nm in length (Fig. 1a).

The genome of arachis ampelovirus 1 (ArAV1, accession number LC851044) is 12,940 nt in length including 5' and 3' untranslated regions of 212 and 127 nt, respectively. It contains seven putative open reading frames (ORFs), which are analogous to those found in other viruses of the genus *Ampelovirus* (Fig. 1b). ORF1a encodes a putative 229.2-kDa protein with a conserved viral methyltransferase domain at amino acid positions 538–851. The stop codon of ORF1a is UAG and is preceded by two uracil nucleotides (UUUAG, nt positions 6403–6407), suggesting that a+1 ribosomal frameshift, as observed in other *Closteroviridae* members [6], may allow the product of ORF1b to be fused to the ORF1a product, resulting in a 286.2-kDa protein. This putative fusion protein contains a motif that is conserved in RdRps. ORF2 encodes a putative 5.77-kDa protein (P6). ORF3 is predicted to encode a 59.27-kDa protein that is homologous to the heat shock protein 70 homolog (HSP70h). ORF4 encodes a putative 62.8-kDa protein with a conserved domain belonging to the viral HSP90h superfamily located between amino acid (aa) residues 40 and 524. ORF5 encodes the capsid protein (CP), which has a molecular weight of 29.6 kDa. This CP sequence contains a closterovirus CP superfamily domain at aa 105–206. ORF6, the final ORF in the genome, encodes a putative 23.4-kDa protein (P23). The complete genome sequence of ArAV1 and the HSP70h, RdRp, and CP sequences were compared to those of members of the genus *Ampelovirus*, using ClustalW [8], which showed that pineapple mealybug wilt-associated virus 1 (PMWaV1) had the most sequence similarity to ArAV1 in both the HSP70h (71.3% identity) and the RdRp (72.7% identity), whereas the most sequence similarity in the CP (69.1% identity) was observed with pineapple mealybug wilt-associated virus 3 (PMWaV3). The species demarcation criteria for the genus *Ampelovirus* are at least

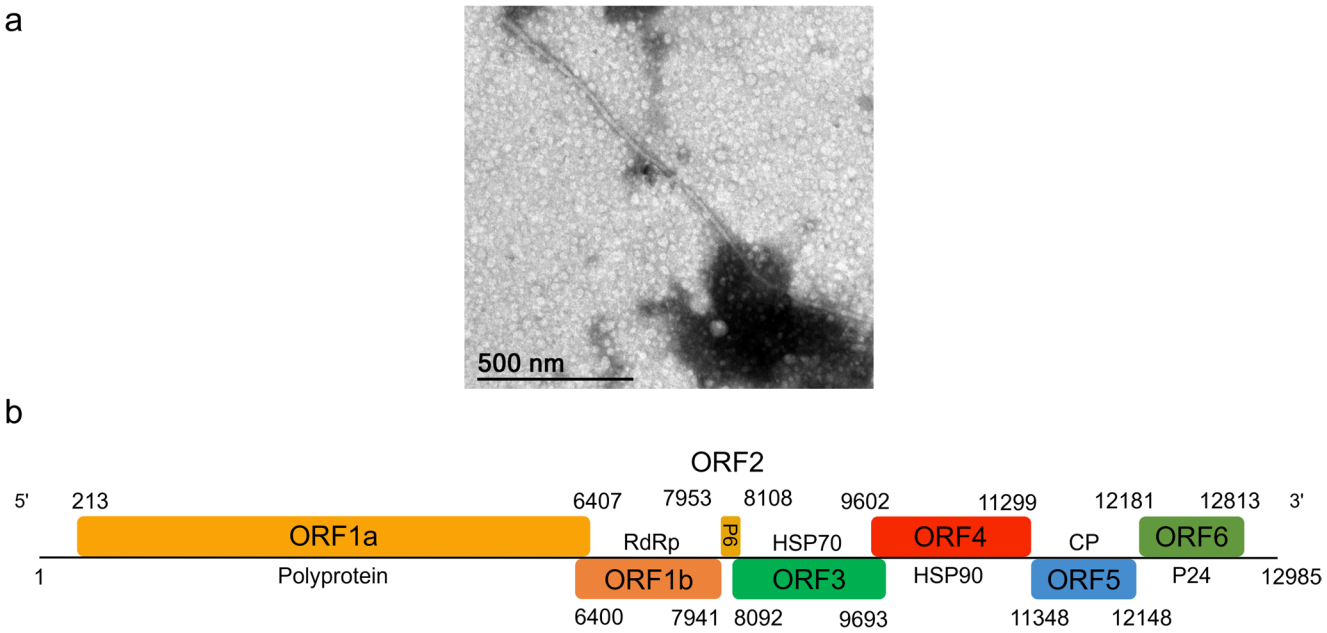

Fig. 1 (**a**) Transmission electron microscopy image of a semi-purified preparation of arachis ampelovirus 1 (ArAV1) negatively stained with uranyl acetate, showing a flexuous virion. (**b**) Genome organization of ArAV1. The nucleotide positions of the regions encoding the mature proteins are shown. Rectangles represent the relative positions of the open reading frames (ORFs). ORF1, polyprotein; ORF1b, RNA-dependent RNA polymerase (RdRp); ORF2, 6-kDa protein (P6); ORF3, heat shock protein 70 homolog (HSP70h); ORF4, heat shock protein 90 homolog (HSP90h); ORF5, coat protein (CP); ORF6, 23-kDa protein (P23)

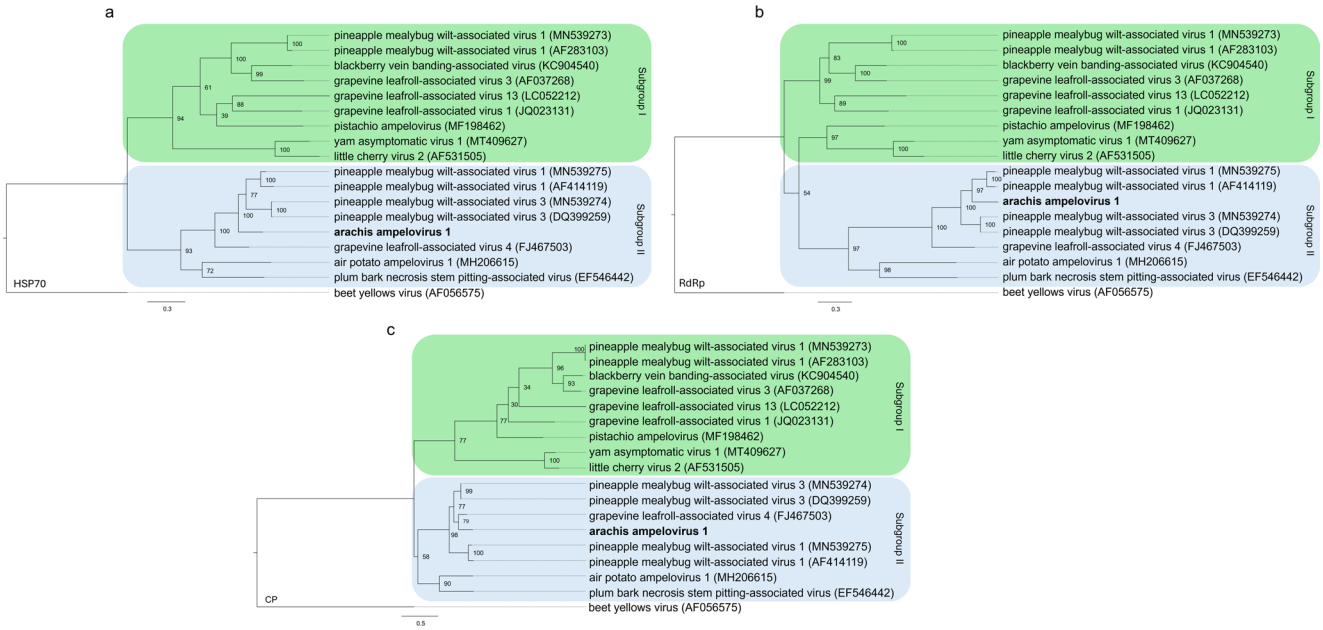

Fig. 2 Phylogenetic analysis based on amino acid sequences of the (**a**) heat shock protein 70 (HSP70), (**b**) RNA-dependent RNA polymerase (RdRp), and (**c**) coat protein (CP) amino acid sequences of ArAV1 and members of the genus *Ampelovirus*. The analysis was performed using the maximum-likelihood method with 1000 bootstrap replicates. Beet yellows virus (AF056575) was used as an outgroup

25% amino acid sequence divergence in the HSP70h, RdRp, and CP [7]. Therefore, ArAV1 appears to represent a new species in this genus. Phylogenetic analysis was performed using IQ-TREE [9], with the optimal model determined by ModelFinder [10]. The WAG+F+R3 model was applied to the HSP70h aa sequence dataset, the LG+F+I+G4 model was used for the RdRp aa sequences, and the LG+I+G4 model was applied to the CP sequence dataset. The resulting phylogenetic trees (Fig. 2) showed that ArAV1 clustered with other members of the genus *Ampelovirus* and was most closely related to PMWaV1 based on HSP70h and RdRp sequences, and to PMWaV3 based on CP sequences. These data suggest that ArAV1 should be classified as a member of a new species in the genus *Ampelovirus*, family *Closteroviridae*, for which we propose the binomial species name "*Ampelovirus arachii*".

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00705-025-06444-w.

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Declarations

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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