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Air potato (*Dioscorea bulbifera*) plants displaying virus-like symptoms are co-infected with a novel potyvirus and a novel ampelovirus

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Abstract

Air potato (*Dioscorea bulbifera*) plants being grown at the Florida Department of Agriculture and Consumer Services Division of Plant Industry Biological Control Laboratory II in Alachua County, Florida were observed exhibiting foliar mosaic symptoms characteristic of virus infection. A double-stranded RNA library generated from a symptomatic plant underwent high-throughput sequencing to determine if viral pathogens were present. Sequence data revealed the presence of two viral genomes, one with properties congruent with members of the genus *Potyvirus* (family *Potyviridae*), and the other with members of the genus *Ampelovirus* (family *Closteroviridae*). Sequence comparisons and phylogenetic placement indicate that both viruses represent novel species. The names "dioscorea mosaic virus" and "air potato virus 1" are proposed for the potyvirus and ampelovirus, respectively.

Keywords Air potato · Ampelovirus · Potyvirus · High-throughput sequencing · Genome characterization · Plant virus

The genus *Potyvirus* (family *Potyviridae*) harbors a large number of related positive-strand RNA viruses that infect a wide range of plant taxa. Some potyvirus species have a very narrow host range, whereas other may infect many host species [1, 2]. Many of the viruses produce severe disease symptoms on economically important crops, making this genus one of the most well-studied by plant virologists [3]. Potyviruses are typically transmitted in a non-persistent manner by several aphid species, as well as through mechanical inoculation [2]. Vertical transmission through seed has also been reported [2, 4]. Another taxon representing plant viruses of economic importance is the family *Closteroviridae*. Within this family is the genus *Ampelovirus*, which comprises related viruses that are transmitted in a semi-persistent manner by mealybugs [5]. Ampeloviruses can be further sub-grouped based on the content and organization of their positive-strand RNA genomes. Ampeloviruses in Subgroup I have larger, more complex genomes, whereas those in Subgroup II have reduced genome size and complexity [6]. The current members of the genus infect a wide range of host crops including grapevine, pineapple, stone fruit, and berries.

The air potato (*Dioscorea bulbifera*) is a widely cultivated member of the yam family. It has become naturalized in many regions outside of its native range, and is considered highly invasive in some locations such as Florida, USA. Across the Southeastern USA, widespread efforts are underway to reduce or eliminate incipient air potato populations using cultural, chemical, and biological control [7]. In March 2016, 10% of potted specimens of air potato plants being grown at the Florida Department of Agriculture and Consumer Services Division of Plant Industry (FDACS-DPI) Biological Control Laboratory II in Gainesville, FL were observed exhibiting symptomatic foliage consistent with virus infection. Leaf symptoms varied from a light green to chlorotic mosaic, at times associated with distinct green islands and mild leaf distortion (Fig. 1). Samples were submitted to the FDACS-DPI Plant Pathology Section, Gainesville, FL and subsequently the University of Hawaii for diagnosis. Approximately 5 g of symptomatic leaf tissue was

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Fig. 1 Air potato (*Dioscorea bulbifera*) leaves displaying mosaic symptoms

collected from a symptomatic plant and stored in RNAlater (ThermoFisher Scientific) at − 20 °C. Double-stranded (ds) RNAs were isolated from this tissue using CF-11 cellulose (Whatman) column chromatography based on the method of Morris and Dodds [8]. The dsRNAs were resolved by 1% agarose gel electrophoresis, revealing a prominent band approximately 15 kb in size (data not shown). A library was generated from the dsRNA template [9], and 1 ng of the resulting dsDNA was prepared for high-throughput sequencing (HTS) using a Nextera DNA Library Preparation Kit (Illumina) following the manufacturer's directions. HTS was performed at the University of Hawaii's Advanced Studies in Genomics, Proteomics, and Bioinformatics laboratory using MiSeq platform and a v3 kit with 600 cycles (Illumina). A total of 4,054,960 useable paired end reads were submitted to the VirFind pipeline [10]. Most of the contiguous sequences (contigs) generated were homologous to members of the genera *Ampelovirus* or *Potyvirus*, although the sequence homologies suggested both of these putative viruses were novel species in the respective taxa. To characterize their genomes, the paired end reads were merged using Geneious Pro 5.6.5 (BioMatters), and a VirFind-derived contig from each virus was used as a reference sequence in an iterative process to extend the length of the contig in both the 5′ and 3′ directions using Geneious Pro 5.6.5. To determine the terminal sequences of the ampleo-like virus genome and the 5′ terminus of the poty-like virus genome, the dsRNAs underwent RLM-RACE based on the procedure of Coutts et al. [11]. Amplicons from RLM-RACE were ligated into pGEM-T Easy (Promega) and at least five clones for each targeted terminus underwent Sanger-based sequencing. To determine the 3′ terminus of the poty-like virus genome, RT-PCR with an oligo-$dT_{(15)}$ primer targeting the poly(A) terminus was performed [12].

The genome of the poty-like virus from air potato was estimated to be 9,550 nt in length, excluding the 3′-terminal poly(A) tail (GenBank MH206616). The genome possessed a large ORF encoding a 3054 aa (351 kDa) polyprotein. This polyprotein is typical of members of the genus *Potyvirus* and is predicted to cleave into ten mature proteins at nine cleavage sites (Fig. 2a). A small overlapping ORF consistent with PIPO [13] was also predicted to initiate at $G_{(2)}A_{(6)}$ (nt 2920–2927) via a ribosomal frameshift. The polyprotein was most similar to the polyprotein of *Yam mild mosaic virus* (YMMV), with a 53% of the residues being identical. The mature coat protein (CP) was determined to be 58% identical to the YMMV ortholog. To determine the phylogenetic placement of this virus, the polyprotein was aligned using ClustalX2 [14] with the orthologs of current potyvirus species which shared the highest amino acid identity. Neighbor-joining (NJ), maximum parsimony (MP), and maximum likelihood (ML) algorithms were used with 1000 bootstrap repetitions to generate unrooted phylogenetic trees in MEGA 7.0.21 [15]. NJ, MP, and ML trees placed the poty-like virus from air potato in the genus *Potyvirus* clade, and indicated a close relationship to YMMV (Fig. 3a and data not shown). These results, in conjunction with CP amino acid sequence identity values to other potyviruses being less than < 80%, clearly indicate that this virus represents a distinct species within the genus *Potyvirus* [2]. The name "dioscorea mosaic virus" (DMV) is proposed for this new potyvirus species.

The genome of the ampelo-like virus was estimated to be 13,398 nt in length (GenBank Accession MH206615), and possessed seven open reading frames (ORFs), with an organization typical of Subgroup II members of the genus *Ampelovirus* (Fig. 2b). ORF1a was found to be a polyprotein 2191aa in length, possessing protease, methyltransferase, and helicase domains. Immediately downstream of ORF1a and putatively expressed via a + 1 ribosomal frameshift, ORF1b was found to encode an RNA-dependent RNA polymerase (RdRp) which shared the highest sequence identity (47%) with the RdRp of *Plum bark necrosis stem pitting-associated virus* (PBNSPaV). Downstream of the RdRp was a small ORF encoding a 9 kDa (53 aa) protein with no homologous sequences in GenBank as determined by blastp

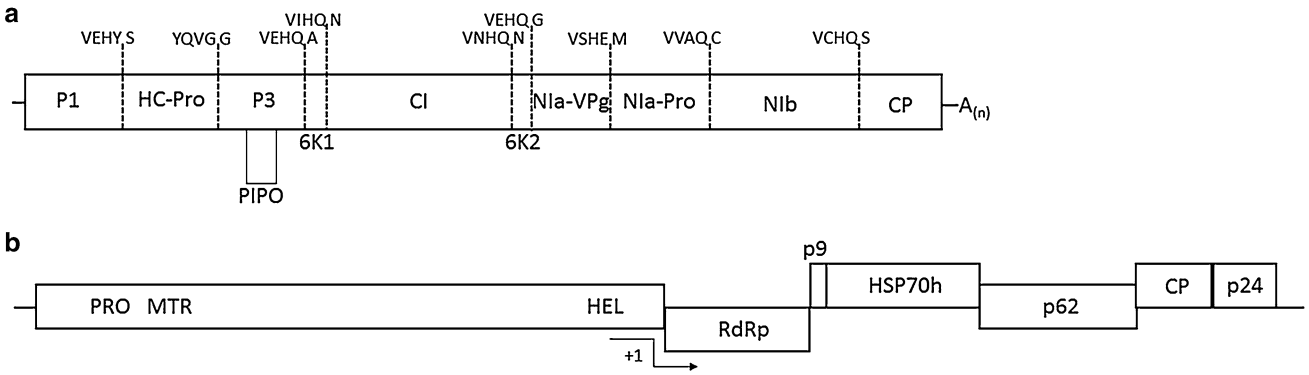

Fig. 2 Genome organization of the two viruses infecting air potato (*Dioscorea bulbifera*). Boxes represent open reading frames (ORFs) and are roughly to scale. **a** Dioscorea mosaic virus, a putative new species in the genus *Potyvirus* with a 9550 nt genome. Vertical dashed lines predict cleavage sites, with the predicted cleavage sequence provided above. A(n) is a polyadenylated 3′ terminus. **b** Air potato virus 1, a putative new species in the genus *Ampelovirus* with a 13,398 nt genome. The arrow represents expression of the RdRp via a +1 ribosomal frameshift. ORF abbreviations are provided in the text

analysis. This putative protein possessed a transmembrane domain from position 15–37, analogous to the putative protein products of similarly positioned ORFs in the genomes of members of the family *Closteroviridae*. Downstream was a 533 aa ORF determined to be the heat shock protein 70 homolog (HSP70h), a hallmark of the family Closteroviridae. This HSP70h shared highest sequence identity (50%) with the HSP70h of PBNSPaV. Downstream of the HSP70h was an ORF encoding a 547 aa protein with a predicted molecular weight of 62 kDa and homology to similarly positioned ORFs in members of the family *Closteroviridae*. Downstream was an ORF predicted to encode the 28 kDa CP of the virus, which shared the highest sequence identity (48%) with the CP of PBNSPaV. The 3′-terminal ORF encoded a 217 aa (24 kDa) protein which is similar in size to the proteins encoded by the 3′-terminal ORF in other Subgroup II ampeloviruses, but it shares no sequence homology with these or other protein sequences in GenBank. To determine the phylogenetic placement of this virus, the taxonomically relevant RdRp, HSP70h, and CP amino acid sequences of this virus were linked and aligned with orthologs from the current ampelovirus species using ClustalX2 [14]. Phylogenetic analyses were performed as described above. Both NJ and ML trees placed the putative ampelovirus from air potato in the Subgroup II clade of the genus on a branch with PBNSPaV (Fig. 3b and data not shown). These results, in conjunction with amino acid sequence identity values <75% for the RdRp, HSP70h, and CP, clearly indicate that this virus represents a distinct species within the genus *Ampelovirus*. The name "air potato virus 1" (AiPoV-1) is proposed for this new ampelovirus species.

This investigation into the cause of virus-like symptoms in air potato revealed the presence of two novel virus species by HTS; a potyvirus and an ampelovirus. Of the 4,054,960 useable paired end reads produced by HTS, 362,640 (8.9%) mapped to the potyvirus genome and 1,954,939 (48.2%) mapped to the ampelovirus genome, suggesting a higher viral titer of the latter in the infected tissue. This was also reflected in the dsRNA extraction, where only a prominent band at approximately 15 kb was observed. The higher ampelovirus titer is surprising, given the phloem-associated nature of their infection (versus infection of almost all cells types by potyviruses) [2, 16]. This disparity in titer may also be a result of the two viruses producing different amounts of dsRNAs or differing extraction efficiencies by CF-11 cellulose chromatography. Co-infecting viruses in symptomatic plants complicates the identification of the causal agent. One virus may be largely responsible for the observed symptoms, while the other may be cryptic. Since potyviruses are often responsible for mosaic-like symptoms and ampeloviruses are often cryptic or responsible for vascular diseases, it would seem the potyvirus may be largely responsible for the observed symptoms, despite its apparent lower titer [2, 16]. Viral co-infection may also result in synergistic effects, amplifying or creating additional symptomology. Sweet potato virus disease, caused by co-infection by a potyvirus and the crinivirus *Sweet potato chlorotic stunt virus* (the genus *Crinivirus* is a sister taxon to the genus *Ampelovirus* within the family *Closteroviridae*) is an example that may be relevant to this disease of air potato [17]. Additional studies are required to elucidate the etiology of this new disease of air potato.

Control efforts are currently underway in several locations where air potato has been deemed invasive. The air potato leaf beetle, *Lilioceris cheni* (Coleoptera: Chrysomelidae: Criocerinae), initially released as a biological control agent (BCA) targeting air potato in Florida [18] has also been released in Georgia, Louisiana, and Texas. The two viral pathogens of air potato described in this study are already present in Florida, and depending

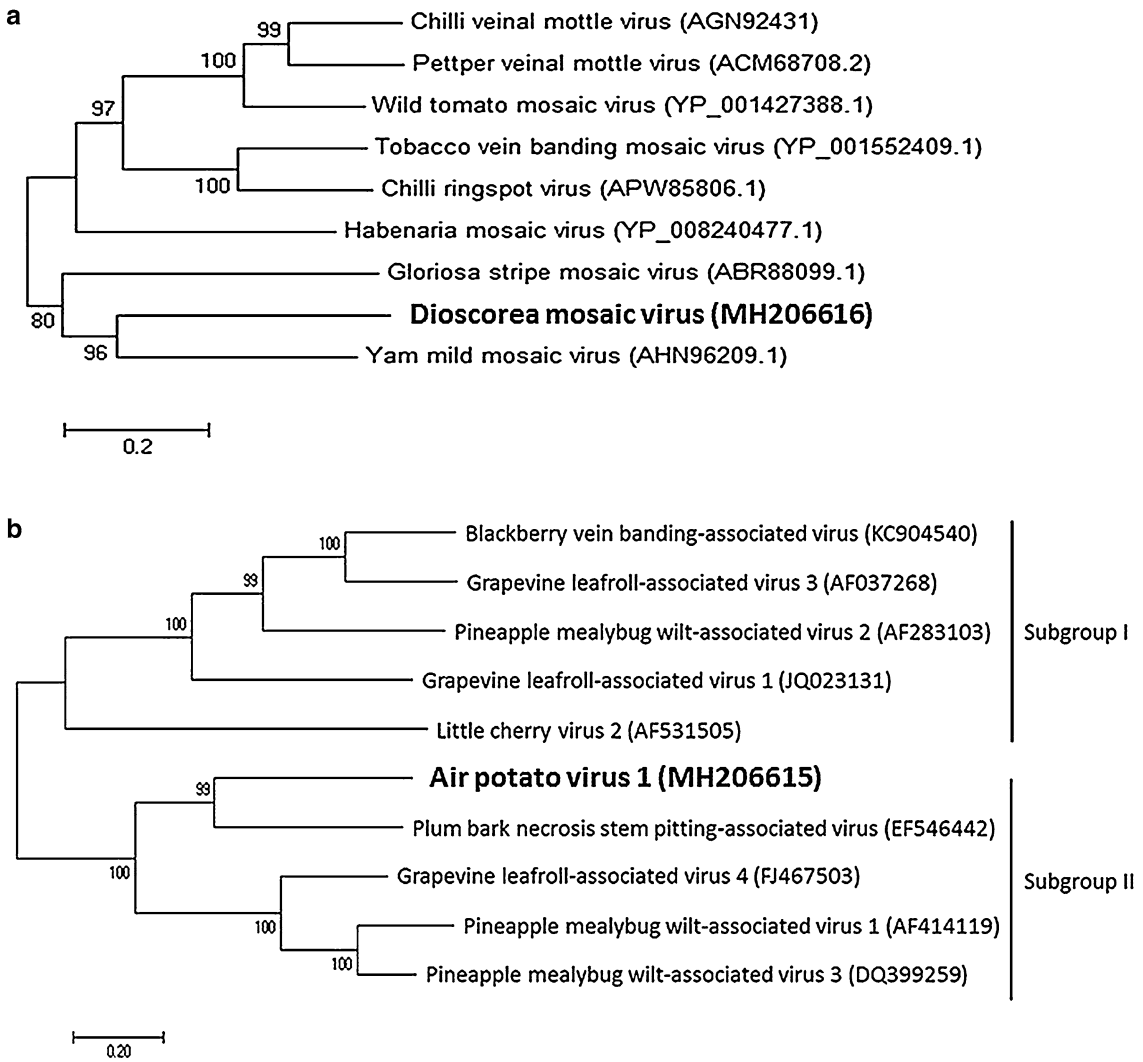

Fig. 3 Phylogenetic placement of two novel viruses infecting air potato (*Dioscorea bulbifera*) with their closest relatives as determined using amino acid sequences with a Maximum Likelihood algorithm. **a** The novel potyvirus, dioscorea mosaic virus, was placed on a branch with *Yam mild mosaic virus* based on the entire potyvirus polyprotein. **b** The novel ampelovirus, air potato virus 1, was placed within Subgroup II of the genus *Ampelovirus* on a branch with *Plum bark necrosis stem pitting-associated virus* based on the combined sequence of the RNA-dependent RNA polymerase, heat shock protein 70 homolog, and coat protein. The numbers below branches represent their percent support based on 1000 bootstrap repetitions. The scales represent the branch length for the given number of position substitutions. The GenBank accession numbers used in each phylogenetic analysis are provided next to the virus name

on their natural host range, may represent additional BCAs that can aid in these and future control efforts.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflicts of interest associated with this study.

Research involving human and animal participants This article does not contain any studies with human participants or animals performed by any of the authors.

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