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ANNOTATED SEQUENCE RECORD

Identification of a novel vitivirus from pineapple in Reunion Island

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Abstract

We report the complete genome sequence of a novel member of the genus *Vitivirus* (family *Betaflexiviridae*, subfamily *Trivirinae*) infecting pineapple. The complete genome sequence of this virus was obtained from total RNA extracted from pineapple leaf samples collected in Reunion Island, using a combination of high-throughput sequencing technologies. The viral genome is 6,757 nt long, excluding the poly(A) tail, and shares all the hallmarks of vitiviruses. Phylogenetic analysis performed on the replication-associated protein and capsid protein gene sequences unambiguously place this new virus, for which we propose the name "pineapple virus A", in the genus *Vitivirus*.

The genus *Vitivirus* (family *Betaflexiviridae*, subfamily *Trivirinae*) comprises viruses with filamentous particles 725-825 nm in length and 12 nm in diameter [1]. Each virion contains one copy of a 7- to 7.6-kb positive-sense RNA genome with five open reading frames (ORFs) encoding a replication-associated protein (RAP, ORF1) with an RNA-dependent RNA polymerase (RdRp) domain required for replication, a protein of unknown function (ORF2), a putative movement protein (MP, ORF3), a coat protein (CP, ORF4), and a putative nucleic acid binding protein (NABP, ORF5), respectively. The genus *Vitivirus* was initially created to accommodate grapevine viruses sharing similar genome features and organizations. It now also includes viruses infecting other hosts and currently has 17 species, whose members have mostly been identified in woody or perennial hosts [2]. Additional viruses that putatively belong to new species in the genus have also been reported but have yet to be recognized by the International Committee on Taxonomy of Viruses (ICTV) [2].

A leaf sample from a pineapple plant showing reddening and leaf tip dieback typical of pineapple mealybug wilt disease was collected in March 2016 in Saint Pierre (Reunion Island) and used to extract total RNA using an RNeasy Plant Mini Kit (QIAGEN, Courtabœuf, France). Illumina RNA sequencing was performed by Genewiz (Leipzig, Germany) after ribodepletion. A total of ~63M 2 x 150-nt paired-end reads were obtained. In parallel, Nanopore sequencing was performed using a MinION portable device and a cDNA-PCR Barcoding kit (Oxford Nanopore Technologies, Oxford, UK), and this generated ~1.1M reads with a mean quality of 9.8 (corresponding to an expected error rate of 10.47%) and with sizes ranging from 88 to 5,893 nt. Co-assembly of the reads generated by both techniques was then performed using SPAdes v3.13 [3]. Assembled contigs were used for BLASTn and BLASTx searches against a virus database derived from GenBank. A contig of ~7 kb showed similarity to vitivirus sequences. Other contigs with similarities to

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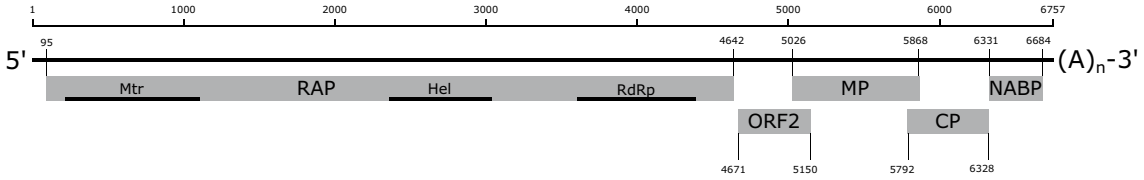

Fig. 1 Schematic representation of the genome organization of pineapple virus A. ORFs are represented as grey boxes. Mtr, methyl transferase domain; Hel, helicase domain; RdRp, RNA-dependent RNA polymerase domain; RAP, replication-associated protein; MP, movement protein; CP, coat protein; NABP, nucleic acid binding protein

ampeloviruses (genus *Ampelovirus*, family *Closteroviridae*) associated with pineapple mealybug wilt disease (PMWD) and described previously in Reunion Island [4] were also obtained.

The sequences of the 5' and 3' ends of the putative vitivirus genome were obtained from the MinION reads by searching for the strand-switching primer (SSP) incorporated during the MinION reaction and the poly(A) tail, respectively. All Illumina reads were mapped back onto the complete assembled viral genome sequence (mean position coverage of ~1,200), and the sequence was polished using Pilon V1.23 [5], resulting in a 6,757-nt genome sequence (excluding the poly(A) tail). The typical five ORFs of vitiviruses were predicted using DNAMAN software V5.2.2 (Lynnon Biosoft, San Ramon, USA). From 5' to 3', they encode a putative 1,515-aa replication-associated protein (RAP) with a methyltransferase domain located near the N-terminus, a helicase domain in its core, and an RdRp domain near the C-terminus, a 280-aa protein of unknown function, a 178-aa putative movement protein, a 159-aa coat protein, and a 117-aa nucleic acid binding protein most closely related to those of vitiviruses in the grapevine virus E (GVE) clade [2] (Fig. 1). The slightly shorter size of this genome compared to those of other vitiviruses could be attributed to the lack of the alkylation B (AlkB) domain usually present in ORF1 of vitiviruses. This domain is similarly absent in the genome of another vitivirus, Agave tequilina leaf virus (ALTV) [2].

Phylogenetic analysis of the RAP and CP sequences of the newly identified virus was performed on sequence alignments obtained using MAFFT [6]. Reference sequences of all members of the subfamily *Trivirinae* and those of yet unclassified putative vitiviruses were included in the analysis (Supplementary Table S1). The RAP and CP both sequences shared less than 72% nucleotide (nt) or 80% amino acid (aa) sequence identity with their counterparts from other members of the genus *Vitivirus* (Supplementary Table S2), which are the molecular demarcation criteria for new species in the family *Betaflexiviridae* [7]. Hence, the assembled genome sequence belongs to a virus for which the name "pineapple virus A" (PinVA) is proposed. PinVA RAP shares the highest similarity with mint virus 2 (59.1% nt and 36.3% aa sequence identity), whereas PinVA CP shares the highest similarity with blueberry green mosaic associated virus (55.3% nt sequence identity) and grapevine virus G (44.6% aa sequence). Maximum-likelihood phylogenetic trees corresponding to the aa sequences of RAP (Fig. 2A) and CP (Fig. 2B) were constructed using Fasttree V2.1.11 [8], with the LG substitution model with gamma-distributed rate among sites and the Shimodaira–Hasegawa-like test (SH-like) for branch support (1,000 replicates). The RAP and CP trees both show that PinVA is clearly a member of genus *Vitivirus*, branching at a basal position. However, phylogenetic analysis could not resolve whether PinVA is a closer relative to members of either the GVA or GVE clade defined by Maree *et al.* [2], even when complete genome sequences were used (data not shown). Our findings extend the known diversity and host range of vitiviruses. Additional work is now required to assess the role of PinVA, if any, in the etiology of PMWD. Interestingly, a previous study has suggested that grapevine-associated vitiviruses may not elicit discernible disease symptoms but may increase the severity of some grapevine diseases [9]. Understanding how PinVA interacts with pineapple mealybug wilt-associated ampeloviruses [10] may help unravel the etiology of pineapple mealybug-wilt disease.

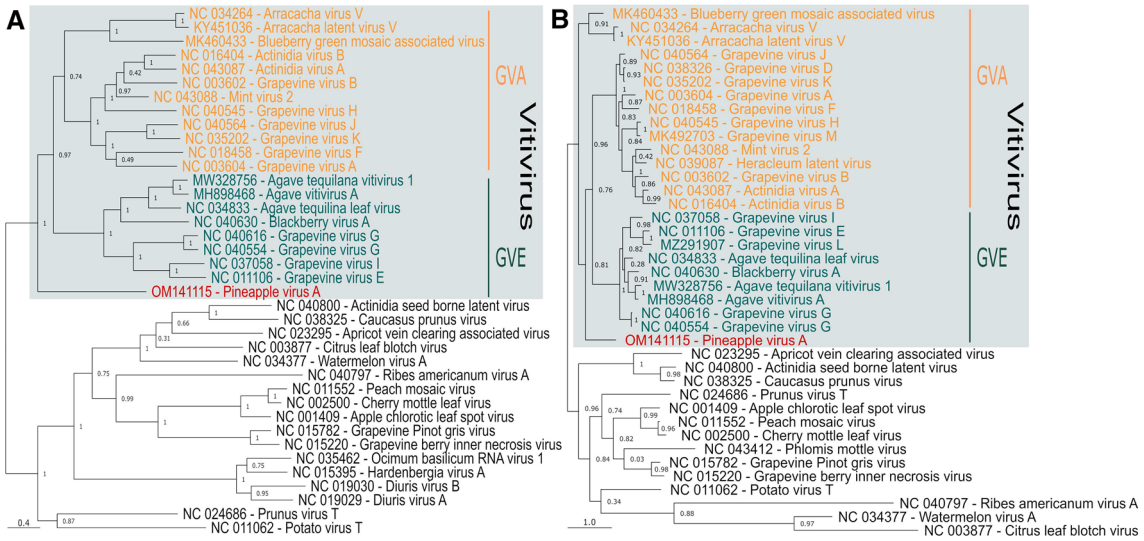

Fig. 2 Maximum-likelihood (ML) phylogenetic trees showing the placement of pineapple virus A (PinVA) among *Trivirinae* reference sequences and yet unclassified putative vitiviruses, based on the alignment of conceptually translated sequences of the replication-associated protein (A) and the capsid protein (B). The reference sequences of vitiviruses are shaded in grey, with the GVA clade indicated in orange, the GVE clade in green, and PinVA in red. Values associated with nodes indicate SH-like local support for the branches. The scale bar shows the number of substitutions per site. GenBank accession numbers are shown.

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Declarations

Conflict of interest The authors declare that they have 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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