Identification of a novel vitivirus from pineapple in Reunion Island

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Abstract

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

Annotated Sequence Record

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 7-7.6 kb positive-sense RNA genome with five open reading frames (ORFs) encoding a replication-associated protein (RAP) with an RNA-dependent RNA polymerase (RdRp) domain required for replication (ORF1), 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 organization. It now also includes viruses infecting other hosts and today counts 15 species mostly identified from woody or perennial hosts [2]. Additional species in the genus have also been reported that 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 the pineapple mealybug wilt disease, was collected in March 2016 in Saint Pierre (Reunion Island), and used to extract total RNAs using the RNeasy Plant Mini Kit (Qiagen, Courtabœuf, France). Illumina RNA sequencing was performed by Genewiz (Leipzig, Germany) following ribodepletion. A total of ~126.1M 150 nt paired reads was obtained. In parallel, Nanopore sequencing was performed using a MinION portable device and the cDNA-PCR Barcoding kit (Oxford Nanopore Technologies, Oxford, UK) and generated ~1.1M reads with sizes ranging from 88 to 5,893 nt. Coassembly 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 ~7kb showed similarity with vitivirus sequences. Other contigs with similarities to ampeloviruses (genus *Ampelovirus*, family *Closteroviridae*), associated with pineapple mealybug wilt disease (PMWD) and described 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 looking respectively for strand-switching primer (SSP) incorporated during the MinION reaction and poly(A) tail. All Illumina reads were mapped back on 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 the 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] (Figure 1).

Phylogenetic analyses of the RAP and CP sequences of the newly identified virus were 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 (Supp. Table 1). 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* (Supp. Table 2), which are the molecular demarcation criteria for new species in the *Betaflexiviridae* family [7]. Hence, the assembled genome belongs to a virus for which the name pineapple vitivirus 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 the tentative species 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 nucleotide sequences of RAP (Figure 2A) and CP (Figure 2B) were reconstructed with Fasttree V2.1.10 [8]. Figure 2B suggests that PinVA belongs to the GVE clade defined by Maree *et al.* ([2]) whereas figure 2A places PinVA in a basal position compared to other vitiviruses. Our findings extend the known diversity and host range of vitiviruses. Additional work is now required to assess the role of PinVA in the etiology of PMWD. Interestingly, a previous study suggests 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.

Declarations

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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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Figures

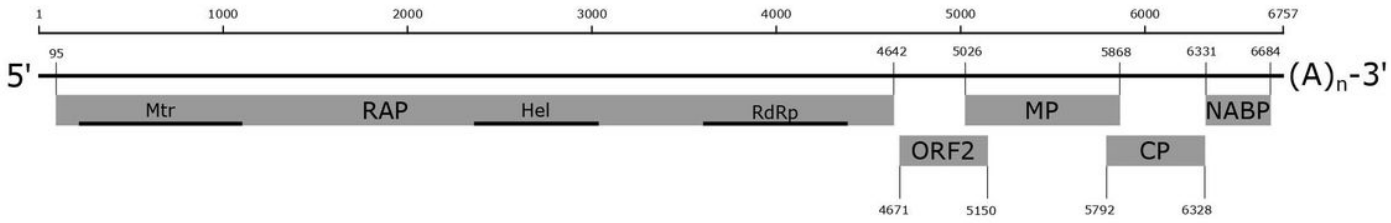

Figure 1

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.

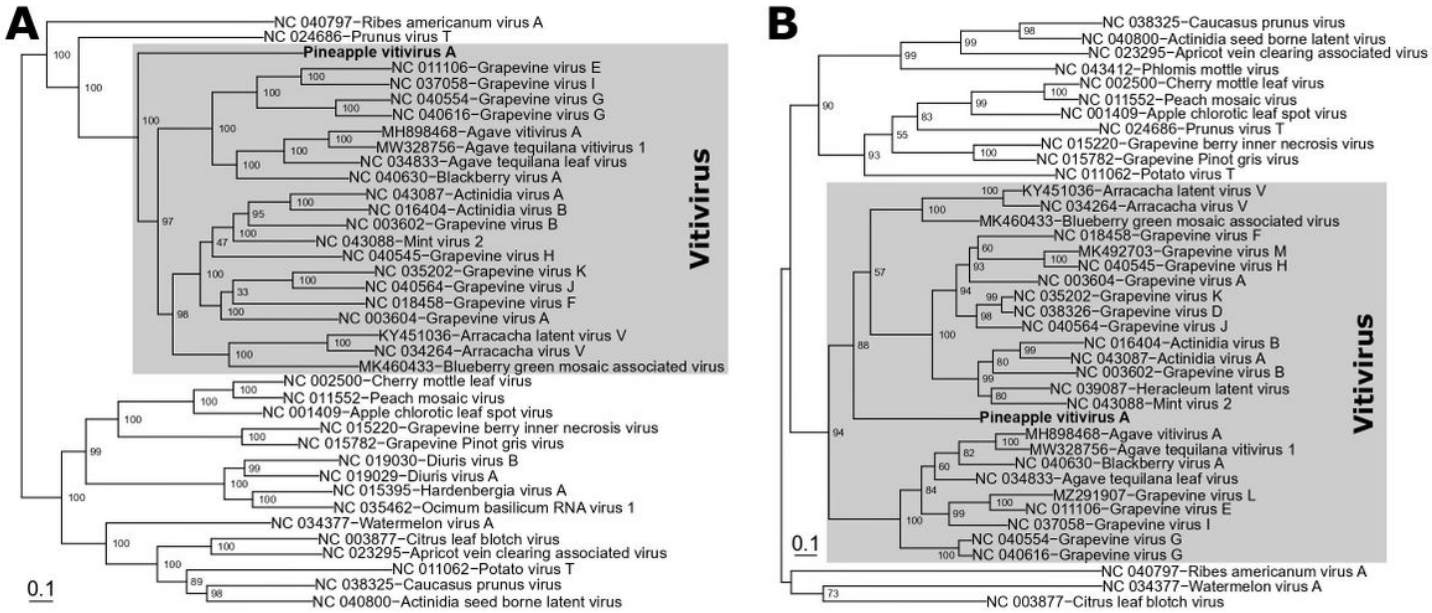

Figure 2

Maximum likelihood phylogenetic trees showing the placement of pineapple vitivirus A (PinVA) along *Trivirinae* reference sequences and yet unclassified putative vitiviruses based on the comparison of nucleotide sequences corresponding to the RAP gene (A) and the CP gene (B). The reference sequences of vitiviruses are squared in grey and PinVA is indicated in bold. Values associated with nodes indicate SH-like local support for the branches to their left. The scale bar shows the number of substitutions per site.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SuppTable1.xlsx
- SuppTable2.xlsx