

Neolamarckia cadamba hosts a putative novel deltapartitivirus: a revelation by transcriptome data-mining

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Short Report

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Abstract

Neolamarckia cadamba (Roxb.) Bosser is a medicinally important, fast-growing, timber-yielding tree species. In the present study, the virome of *N. cadamba* was explored using the publicly available *N. cadamba* transcriptome datasets and a putative novel virus, tentatively named as Neolamarckia cadamba virus 1 (NcV1), was identified. NcV1 contained two genome segments, each coding for a single protein. NcV1 RNA1 (1564 nt) encoded for an RNA dependent RNA polymerase (RdRp) protein while NcV1 RNA2 (1492 nt) encoded for a coat protein (CP). Phylogenetic and sequence similarity analyses revealed the relatedness of NcV1 to pepper cryptic virus 1 and pittosporum cryptic virus 1. Based on the species demarcation criteria, genome organization and phylogeny, NcV1 can be regarded a new member of the genus *Deltapartitivirus*.

Full Text

Neolamarckia cadamba (Roxb.) Bosser (Rubiaceae), native to Southeast Asia, is an underexploited miracle medicinal tree species found globally in tropical and subtropical climates. The tree is fast-growing with a straight, cylindrical trunk and broad canopy (Dubey et al. 2011). *N. cadamba* has been used in traditional medicine for centuries (Pandey and Negi 2016). It has antimicrobial, anti-inflammatory, and antioxidant properties, and have shown therapeutic potential in the treatment of various diseases/disorders (Devgan et al. 2012) including diabetes, anaemia, stomatitis, leprosy, cancer and other infectious diseases (Pandey and Negi 2016). The timber of *N. cadamba*, highly valued for its strength and durability, is used for construction purposes, furniture, and boat making. Besides, the tree is used in paper production and its leaves are used as fodder for livestock. *N. cadamba* is one of the best landscape trees for urban greening, and suitable for woody forage produce (Ouyang et al. 2013). However, *N. cadamba* is susceptible to several insect pests such as defoliators, mealy bugs, scale insects, and diseases including die-back, damping-off and root rots (Chung et al. 2009; Yanti et al. 2018).

Among the biotic constraints, viral pathogens cause huge losses worldwide owing to the economically important diseases they incite in a wide range of plant species (Rubio et al. 2020). In the Next Generation Sequencing (NGS) era, the enormous transcriptomic and genomic data generated globally and available publicly provides an opportunity for the exploration of species-specific viromes through various computational tools. Alongside, novel viral sequences that may be present in the NGS data will also be unveiled, a process known as 'Data Driven Virus Discovery' (DDVD) (Lauber and Seitz, 2022). Our previous DDVD studies have identified several putative novel plant viral sequences associated with targeted plant species (Sidharthan and Baranwal 2021; Sidharthan et al. 2021a, b; Sidharthan et al. 2022; Sidharthan et al. 2023). In the present study, we explored the *N. cadamba* transcriptome datasets for novel viral sequences and identified a putative novel delatapartitivirus.

Four transcriptome libraries (SRR1107780, SRR1107781, SRR1107782, SRR7130587) of *N. cadamba* available in National Centre for Biotechnology Information's (NCBI) Sequence Read Archive (SRA) database were searched for putative novel plant viral reads using RNA-dependent RNA polymerase

(RdRp) search tool of Serratus explorer (Edgar et al. 2022) as described in Sidharthan et al. (2023). For genome recovery of identified novel virus, raw reads of virus-positive library were retrieved, trimmed using Trimmomatic v. 0.36 (Bolger et al. 2014) (quality score: 25), *de novo* assembled using rnaSPAdes v 3.15.4 (Bushmanova et al. 2019) and mapped against related viral sequence using blastx option of ncbi blast + v 2.10.1 (Cock et al. 2015) in Galaxy Australia server (<https://usegalaxy.org.au/>). Recovered genome segments were annotated based on the predicted motifs in the encoded proteins and BLAST analysis as described in Sidharthan et al. (2023). Phylogenetic analysis was carried out after MUSCLE alignment of protein sequences encoded by the recovered viral genome segments along with other related viral sequences using neighbourhood-joining method (NJ) and Poisson model with 1000 bootstrap replicates in MEGA 7 v 7.0.26 (Kumar et al. 2016). Sequence identity matrices were created using Sequence Demarcation Tool v 1.2 (Muhire et al. 2014). Trimmed reads were mapped onto recovered genome segments and mean coverage values were obtained as described in Sidharthan et al. (2023).

Reads of a putative novel deltapartitivirus was identified in the library SRR7130587 derived from the Bioproject PRJNA464271. The identified putative novel virus was tentatively named as Neolamarckia cadamba virus 1 (NcV1). After assembly and BLAST analysis against related viral sequences, two genome segments of NcV1 were recovered. NcV1 RNA1 (1564 nt) contains a single open reading frame (ORF) of 1440 nt encoding for a 54.3 kDa protein (479 aa) with viral RNA dependent RNA polymerase (RdRp) motif (PF00680). NcV1 RNA2 (1492 nt) contains a single ORF of 1230 nt encoding for a 47.1 kDa protein (409 aa) with no predicted motif (Fig. 1a). BLAST analysis showed that NcV1 proteins 1 and 2 shared a maximum of 74.74% and 51.08% sequence identities (at 100% query coverage), respectively with RdRp (AVL84362.1) and coat protein (CP) (AVV48359.1) sequences of pepper cryptic virus 1 (PCV-1). Phylogenetic analysis based on RdRp and CP sequences grouped NcV1 with PCV-1 and pittosporum cryptic virus 1 (PiCV1) (Fig. 1b, c). Sequence identity analysis revealed that NcV1 RdRp and CP sequences shared a maximum of 76.0% and 53.9% identities, respectively with the corresponding sequences of PiCV1 (Fig. 2, 3). Reads of NcV1 could not be detected in other three *N. cadamba* transcriptome libraries in MEGABLAST searches.

Deltapartitiviruses are plant-infecting double stranded (ds) RNA genome containing viruses with two or three genome segments (Vainio et al. 2018). Members of deltapartitiviruses are regarded as new species if they share $\leq 90\%$ and $\leq 80\%$ sequence similarities, respectively for RdRp and CP sequences with other known members (Vainio et al. 2018). Based on this species demarcation criteria, NcV1 can be regarded as a novel deltapartitivirus. A consensus statement report by Simmonds et al. (2017) stated that viruses that are known only by sequences from metagenomics data should be incorporated into the official International Committee on Taxonomy of Viruses (ICTV) taxonomy scheme. Thus, the identified virus can be regarded as a *bona fide* one. The current study paves way for further studies on understanding biological properties and distribution of the identified virus.

Declarations

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Author contributions

V. Kavi Sidharthan: conceptualization, methodology, formal analysis and investigation, writing- original draft preparation; Mushineni Ashajyothi: formal analysis and investigation, writing- review and editing.

Data availability

The viral genome sequences described in the study are submitted to NCBI Third Party Annotation database, and are provided in the ESM_1.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

Research involving human participants and/or animals

This work does not contain any animal or human participants.

Informed consent

This work does not contain any animal or human participants.

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Figures

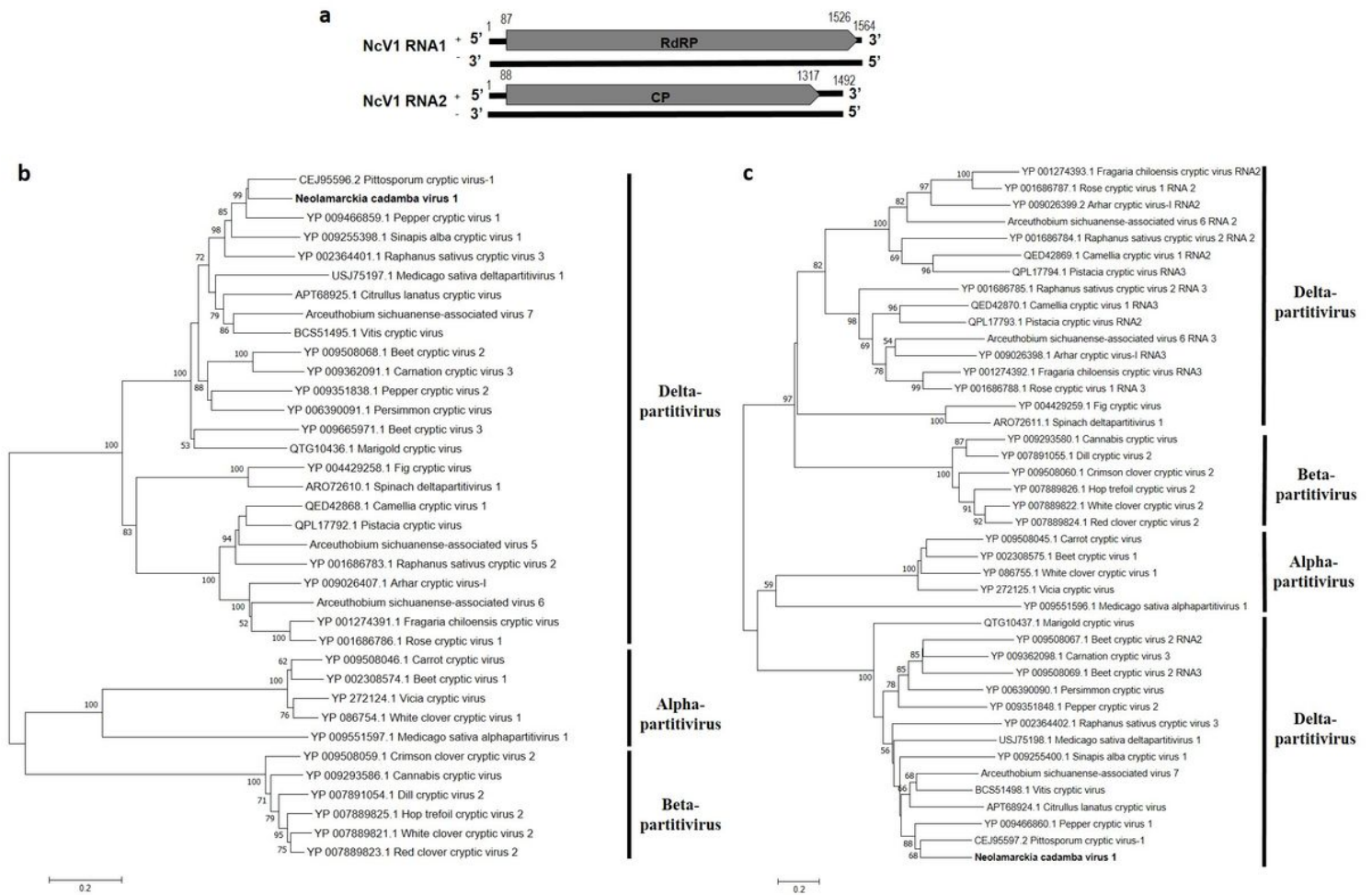


Figure 1

Genome organization of *Neolamarckia cadamba* virus 1 (NcV1) identified in the current study **(a)**. Phylogenetic relationship of NcV1 with other partitiviruses based on RdRp **(b)** and CP sequences **(c)**. Bootstrap values more than 50% are only indicated. NcV1 is shown in bold.

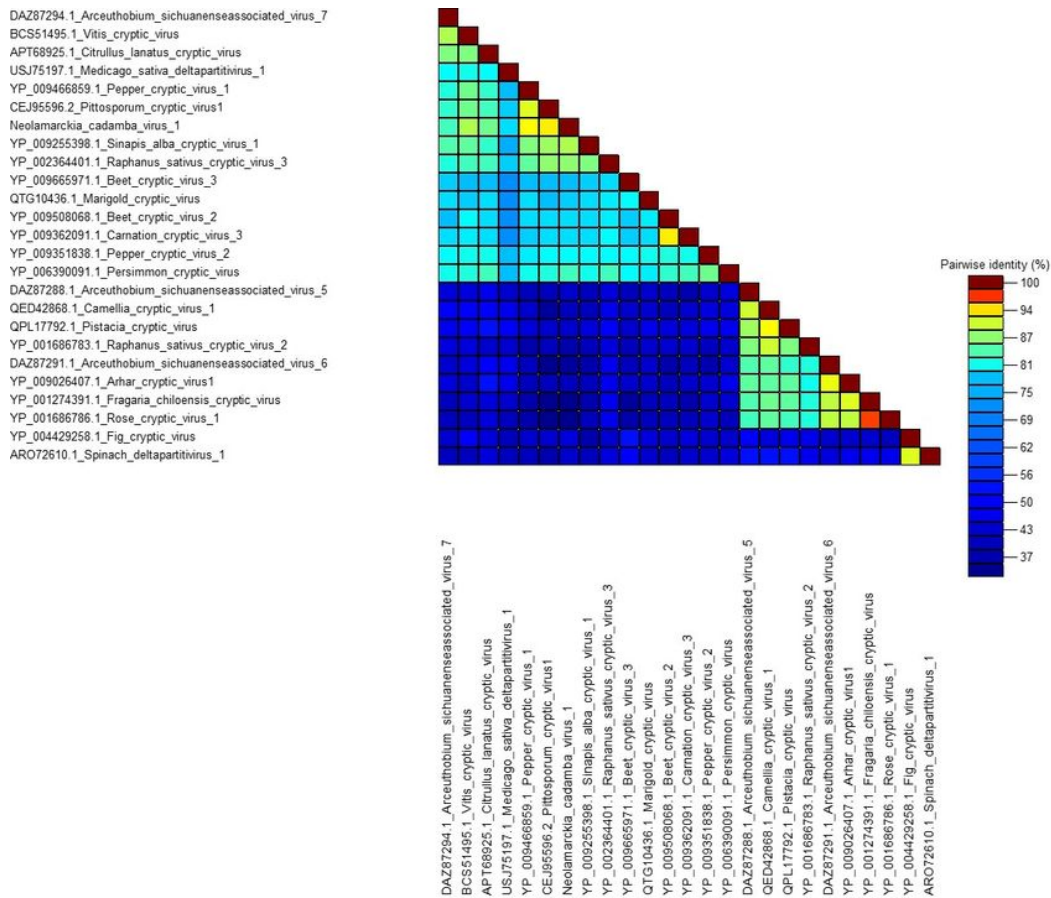


Figure 2

RNA-dependent RNA polymerase (RdRp) protein sequence identity matrix of Neolamarckia cadamba virus 1 (NcV1) and other deltapartitiviruses

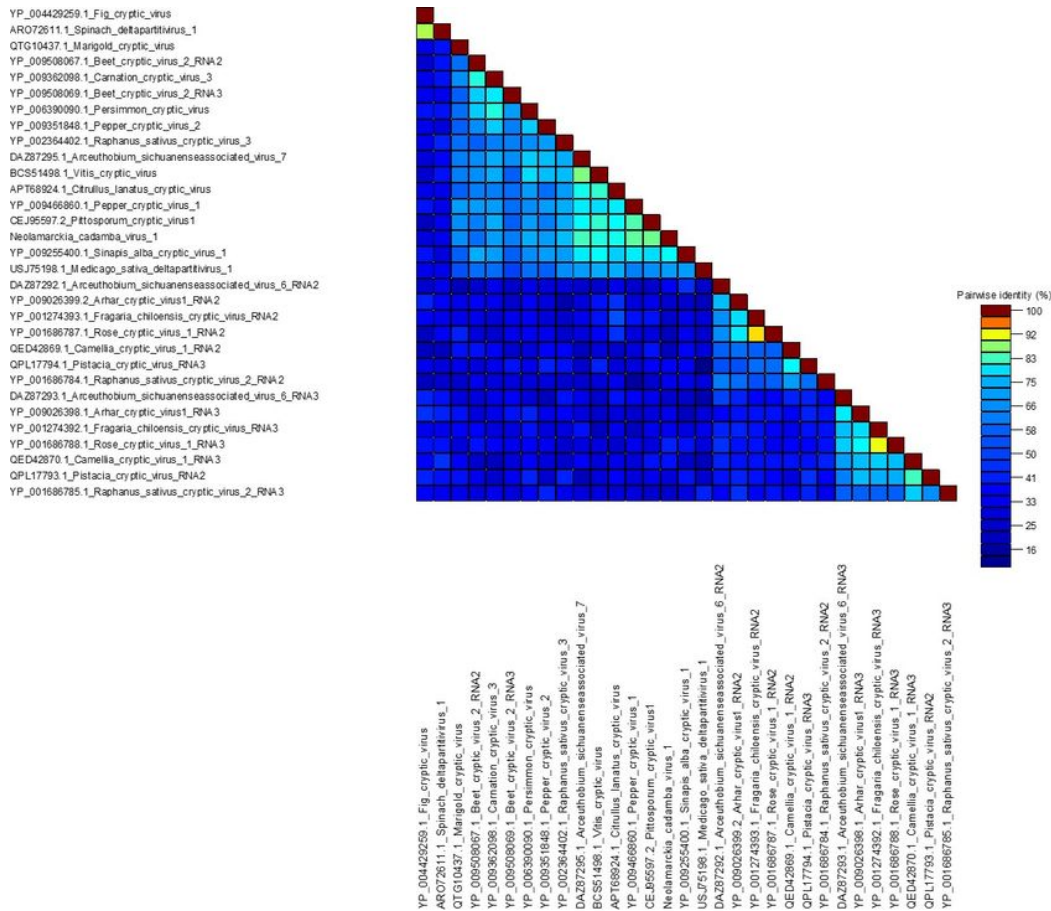


Figure 3

Coat protein (CP) sequence identity matrix of Neolamarckia cadamba virus 1 (NcV1) and other deltapartitiviruses