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GENOME REPORT

Genome sequence of Ceratobium mosaic virus (species *Potyvirus ceratobii*)

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Significance

Ceratobium mosaic virus (CerMV; species *Potyvirus ceratobii*) has only been recorded from the east coast of Australia, where it infects native *Dendrobium* and allied orchid genera. Very little is known about this virus, although based on its classification as a potyvirus, it likely is transmitted in a non-persistent manner by aphids. When first discovered, only a RT-PCR amplicon covering the NIb and coat protein coding regions and 3' untranslated region of the viral genome was sequenced, which at the time was sufficient for official recognition of this virus as a novel species. However, the standard of evidence now required for taxonomic proposals is a complete or near complete genome sequence. In this report, we present the complete genome sequence of an isolate of CerMV from New South Wales, allowing a more comprehensive analysis of its classification and phylogeny.

Keywords Orchid virus · *Dendrobium speciosum* var. *pendunculatum* · *Thelychiton speciosus* · High throughput sequencing · Native plant · Environmental threat

Introduction

There are approximately 25,000–35,000 species of orchid (Orchidaceae) in the world, many threatened by processes such as habitat clearance, illegal collecting, and climate change (Besi et al. 2023; Wraith and Pickering 2019). While usually not posing an existential threat, plant viruses can interfere with orchid conservation efforts, and they are serious concerns for orchid growers because of their effects on plant growth and flowering. There is no recent estimate of the number of plant viruses infecting orchids, but the current tally probably numbers in the hundreds (Pérez et al. 2025).

In Australia, 21 different virus species from 11 different genera have been reported infecting orchids, many indigenous to this country, but others cosmopolitan in distribution such as Cymbidium mosaic virus and Odontoglossum ringspot virus (Geering and Thomas 2022; Chao et al. 2022). One virus that is thought to be indigenous is Ceratobium mosaic virus (CerMV; species *Potyvirus ceratobii*), a potyvirus that was discovered in the orchid collection of the Australian National Botanic Gardens in Canberra, ACT, and in a private orchid collection at Toowoomba, Queensland (Mackenzie et al. 1998; Gibbs et al. 2008). Potyviruses in general are transmitted by aphids in the non-persistent manner (Inoue-Nagata et al 2022). Natural hosts of CerMV include *Grastidium* spp., *Dendrobium discolor*, and two synthetic hybrids of Australian *Dendrobium* spp. Only six partial genome sequences of CerMV are available covering the NIb and coat protein coding domains of the polyprotein, and the 3' untranslated region.

In 2020, leaves of a diseased orchid plant were submitted to our laboratory for diagnosis, and potyvirus-like virions were observed under the transmission electron microscope. Following high-throughput sequencing, the virus was identified as CerMV, and the first complete genome sequence of this virus is presented here.

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Materials and methods

To visualise virions, a crude leaf extract was adsorbed to a carbon-coated copper grid, stained with 1% ammonium molybdate, pH 5.8, and examined under a JEOL JEM-1400 transmission electron microscope.

RNA extraction, high-throughput sequencing and sequence assembly were done as described by Chao et al. (2022). Briefly, total RNA was extracted from fresh leaves using a TRIzol™ Plus RNA Purification Kit (Thermo Fisher Scientific, Waltham, MA, USA) kit. Sequencing was outsourced to the Australian Genome Research Facility, where libraries were prepared using a TruSeq® Stranded Total RNA Library Preparation Kit with Ribo-Zero™ Plant (Illumina, San Diego CA, USA). Sequencing was done using a NovaSeq 6000 System with NovaSeq 6000 SP Reagent Kit v1 (200 cycles, Illumina) to generate 100 bp pair-end reads. De novo sequence assembly was done using SPAdes v3.11.1. To map the terminus of the genome, 5' RACE was done as described by Tran et al. (2024).

For phylogenetic analyses, entire polyprotein sequences were first aligned using the MUSCLE algorithm and then back-translated to give the nucleotide alignment. The Maximum likelihood method of phylogenetic inference was used, as implemented using W-IQ-TREE with default parameter settings (Trifinopoulos et al. 2016). Each of the ten different protein coding domains in the polyprotein ORF was partitioned and models of evolution for each region of sequence automatically selected. Support for the branch nodes was assessed using 1000 bootstrap alignment replicates. The role of RNA recombination in viral evolution was analysed using RDP5 (Martin et al. 2020).

Results and discussion

An orchid grower from Casula, Sydney, reported a *Dendrobium speciosum* var. *pedunculatum* 'Herberton Gold' × 'Herberton' plant with abnormal purple pigmentation on the flowers (Supplementary Fig. 1) in September 2020. Irregular, small, purple spots were present on the lateral and dorsal sepals, and to a lesser extent on the petals. The central veins of these floral structures were also darkly pigmented purple on the underside. In comparison, the flowers of healthy plants are typically uniform off-white to light yellow, except for purple pigmentation in the labellum (https://therocklilyman.com/dendrobium-speciosum/variety-pedunculatum/). Leaf samples from this plant were submitted for virus testing, and flexuous, filamentous particles c. 680 nm length observed in the sap extract when viewed under a transmission electron microscope (Supplementary Fig. 2). RNA was extracted from a portion of the leaves and submitted for high throughput sequencing, while remaining tissue was lyophilised and deposited in the Queensland Department of Primary Industries plant virus collection under isolate number 7014.

After de novo sequencing assembly, a single contig was obtained that matched the five existing partial genome sequences of CerMV (AF022442, AF022443, AF022444, AF022445 and AF022446) with 92.3–99.5% nucleotide identity across aligned regions when a BLASTN search of GenBank was done. This contig was assembled from 4,150,294 reads and had an average sequence depth of 52,422.1 reads. After 5' RACE and removal of the poly(A) tail, the complete genome sequence of the virus isolate was determined to be 9622 nt in length. Genome organisation was typical of a potyvirus, with a single long ORF (nt 117–9380) identified that conceptually encodes a precursor polyprotein with a molecular mass of 352.3 kDa and contains 3087 amino acid residues. Putative cleavage sites for the P1, HC-Pro, and NIa-Pro protease enzymes were identified within the polyprotein sequence (Goh and Hahn 2021), marking the sites of processing of the polyprotein into the ten mature proteins (Supplementary Table 1). A conserved polymerase slippage motif (GA6) was present at nt 2892–2898, which typically leads to the addition of an A in this part of the sequence in a small proportion of transcripts, thus generating the frameshift protein, P3N-PIPO (Olspert et al. 2015).

When a BLASTX search of GenBank was done using the polyprotein ORF sequence, the highest scoring matches were to paris mosaic necrosis virus (PMNV), wisteria vein mosaic virus (WVMV) and kudzu chlorotic ring blotch virus (KCRBV), suggesting that CerMV belongs to the bean common mosaic virus (BCMV) subgroup of potyviruses, as previously suggested by Gibbs et al. (2008) using coat protein sequences for the analysis. To further explore evolutionary relationships and to place the virus within previously determined geographic groupings (Hajizadeh et al. 2024), a phylogenetic analysis was done using complete polyprotein coding sequences (Supplementary Fig. 3). CerMV isolate 7014 (CerMV-7014) branched within the subclade of viruses that is thought to have had a Southeast or East Asian origin and shared a most recent common ancestor with PMNV, a virus that thus far has only been found in Yunnan, China, where it infects plant species in both monocot and eudicot families, including *Paris polyphylla* (Melanthiaceae), *Panax notoginseng* (Araliaceae), and *Polygonatum kingianum* (Asparagaceae) (Hu et al. 2022; Lan et al. 2018). In pairwise sequence comparisons, the polyproteins of CerMV-7014 and PMNV had 73.8% amino acid identity, confirming the status of CerMV as a distinct potyvirus

species. No evidence for RNA recombination in the CerMV genome was found.

Based on our phylogenetic analysis, we conclude that CerMV is an introduced pathogen in Australia, and likely originated from Southeast Asia, which is the primary centre of diversity for *Dendrobium* sensu lato (Xiang et al. 2016). Supporting this conclusion of non-indigeneity, CerMV has only ever been found in cultivated orchids in urban environments in Australia, and not in wild orchid populations. While there have not been any dedicated surveys for this virus, there have been numerous surveys of native orchids for other scientific purposes, and one would assume that CerMV would have come to the attention of plant pathologists if it was already widespread in the field given the distinctiveness of the disease symptoms (Mackenzie et al. 1998). There is still much to be learnt about the biology of CerMV such as its host range, which is likely to include other plant species in the Orchidaceae and possibly even in other botanical families.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13313-026-01124-y.

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Authors' contributions H-YC prepared the RNA samples for high-throughput sequencing and compiled the genome sequence. MW and KC did the 5' RACE and electron microscopy, respectively. ADWG did the phylogenetic analyses, identified the protease cleavage sites, and wrote the initial draft of the manuscript. All authors reviewed the manuscript and agreed with the publication of the paper.

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Data availability The data that support the findings of this study are available from the corresponding author upon request. The genome sequence of CerMV-7014 has been deposited in GenBank and provided with the accession code PX392580.1.

Declarations

Ethical approval No ethics approvals were required for this study.

Competing interests The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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