First report of a newly detected potyvirus, *Commelina* mild mosaic virus, infecting *Commelina* spp. in Australia

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Abstract In 2007, a sample of scurvy weed (*Commelina cyanea*) showing mild mosaic leaf symptoms was collected from Rosedale, New South Wales, Australia. From this plant we obtained the NIb/CP gene sequence of a previously unrecognised potyvirus, for which we have suggested the name *Commelina* mild mosaic virus (CMMV). Sequence analysis indicated that CMMV has no close relationship to any potyvirus that has been sequenced previously. The relationship of this species to other previously identified, but not sequenced potyviruses isolated from *Commelina* spp. in other countries is also not known due to a lack of nucleotide sequence data. This is the first time a potyvirus has been reported to infect a native *Commelina* species in Australia.

Keywords New and emerging · Degenerate primers · Plant virus detection · Plant virus diagnostics · Universal primers

In 2007, a sample of native scurvy weed (*Commelina cyanea*) showing mild mosaic leaf symptoms was collected from

coastal woodland at Rosedale, a settlement near Batemans Bay on the south coast of New South Wales, Australia. *Commelina cyanea*, ‘scurvy weed’, was used as an anti-scorbutic by the first European settlers of these regions two centuries ago and it is widespread throughout the eastern coastline of Australia, mainly in forests and woodlands of Queensland, New South Wales and Northern Territory.

Nucleic acids were extracted from the leaf tissue of the sample and tested for the presence of potyviruses using reverse transcription PCR (RT-PCR). The sample tested positive for potyvirus infection using two sets of universal potyvirus primers “NIb2F-3R” (Zheng et al. 2010) and “Potyvird 1-2” (Gibbs and Mackenzie 1997a, b). The “NIb2F-3R” primers were used due to their wide range of detection and generated a 350 bp PCR product from the RNA replicase (NIb) gene of the potyvirus genome. The “Potyvird” primers amplified a larger PCR product of 1.62 kb in length, spanning part of the NIb gene, the entire coat protein (CP) gene and the 3′ untranslated region (UTR) from the potyvirus genome, which is often used for potyvirus species demarcation. Consequently, this larger PCR product was cloned into the vector pGEM-T using the manufacturer’s protocols (Easy II Vector System, Promega Corporation, Madison, WI, USA) and three clones containing the inserts were sequenced in both directions using the Big dye 3.1 kit (Applied Biosystems, California, USA). A consensus sequence of 1.62 kb (GenBank accession HQ225836) was created from the six sequences obtained and used to search GenBank using BLASTN (Altschul et al. 1990).

An alignment 1,166 bp long between a part of the 1.62 kb sequence and a nucleotide sequence from the Christmas bell potyvirus (GenBank accession: EF427894.1), with 67% (781/1166) sequence identity, suggested that the 1.62 kb sequence was of viral origin. No other significant similarity to sequences was found using BLASTN. The Christmas bell potyvirus is a species that has not yet been recognised by the

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Fig. 1 Consensus Minimum-Evolution tree of 57 potyviruses inferred from an alignment of their nucleotide sequences from the Nib, CP and 3'-UTR region targeted by the "Potyvirus" primers and rooted using the homologous ryegrass mosaic rymovirus sequence

International Committee on Taxonomy of Viruses but is placed with potyviruses in GenBank's unofficial taxonomy.

A translation of the nucleotide sequence showed it contained a long open reading frame, as would be expected from a potyvirus genome, which encoded a 540 amino acid

(aa) residue protein sequence. The deduced protein sequence was used to search the GenBank protein database using BLASTX. Almost all of the protein sequence, 490 aa, aligned with a region of the polyprotein of the potyvirus *Iris mild mosaic virus* (IMMV; ABD98799.2) with a

Fig. 2 Consensus Minimum-Evolution tree inferred from 57 amino acid sequences translated from the NIb and CP region of the potyvirus genome targeted by the “Potyvirus” primers and rooted with the homologous ryegrass mosaic rymovirus sequence

sequence identity score of 61% (302/490) and a similarity score, based on amino acid residues with similar properties, of 75% (369/490).

To further investigate the relationship of the unrecognised virus with other species within the *Potyvirus* genus, phylogenetic analyses were done using the nucleotide and translated amino acid sequences of Christmas bell potyvirus, IMMV and 54 distinct species representing all of the major clusters of potyvirus. Two trees were calculated from the nucleotide and amino acid alignments using the Minimum-Evolution method (Rzhetsky and Nei 1992) and MEGA version 4 (Tamura et al. 2007). Bootstrap analyses were done using 1,000 replicate samples from the alignments and bootstrap consensus trees were found (Felsenstein 1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates in both trees were collapsed. For the nucleotide tree, sequences from the NIb, CP and 3'-UTR regions targeted by the "Potyvrid" primers were used. For the amino acid tree, only the sequences from the NIb and CP region of the potyvirus genomes were used because the 3'-UTR does not encode a protein. The reference sequences from *Ryegrass mosaic virus* (type species of genus *Rymovirus*; NC_001814) were used as outgroups to root both trees (Figs. 1 and 2).

In the nucleotide tree, the sequence from the unrecognised virus was placed on a long branch in the same clade as Christmas bell potyvirus (Fig. 1). In the amino acid tree, the sequence from the unrecognised virus was placed in a cluster, but not the same clade, with Christmas bell potyvirus and IMMV (Fig. 2).

Both trees suggest that the virus is a distinct potyvirus species, as do our results from database searches and alignments. The nucleotide and amino acid sequences of the unrecognised virus were only 38.7–55.5% and 42.4–59.6% identical, respectively, to those of other potyvirus species included in the alignment. These identity scores are significantly lower than those recommended to distinguish potyvirus species (Adams et al. 2005), being 76–77% for nucleotides and 82% for amino acids, based on alignments of coat protein sequences.

Six RNA virus species have been reported to naturally infect *Commelina spp.*, four probably belonging to the genus *Potyvirus*, one belonging to the genus *Bromovirus* and one belonging to the genus *Cucumovirus* (Table 1). Of the potyviruses reported to infect *Commelina* species, none is known to occur in Australia and sequence from not one is available in GenBank, the latest one isolated remains uncharacterised (Grisoni et al. 2006). The relationship of this virus to other previously identified, but not sequenced potyviruses isolated from *Commelina spp.* is therefore difficult to determine without sequence information.

Table 1 The name and genus of virus species naturally infecting *Commelina spp.* plants

Virus species name	Synonyms	Genus	Geographical distributions	Sequence availability	References
<i>Ancilema mosaic virus</i>	AneMV	Potyvirus (tentative)	Not known	None	(Baker and Zettler 1988)
<i>Brome mosaic virus</i>	BMV	Bromovirus	Finland, South Africa, the central United States, Canada, Russia and the former USSR, and the former Yugoslavia	33 in total. (NC_002026, NC_002027 and NC_002028 for RNA 1, RNA 2 and RNA 3 respectively)	(Hamilton 1961)
<i>Commelina mosaic virus</i>	ComMV	Potyvirus	USA	None	(Morales and Zettler 1977; Baker and Zettler 1988)
<i>Commelina yellow mottle virus</i> ^a	ComYMV	Badnavirus	Gudeloupe and Sudan	1 in total. (NC_001343)	(Medberry et al. 1990)
<i>Cucumber mosaic virus</i>	CMV	Cucumovirus	World-wide distribution	1,125 in total. (NC_002034, NC_002035 and NC_001440 for RNA 1, RNA 2 and RNA 3 respectively)	(Baker and Zettler 1988)
Unknown potyvirus	Not available	Potyvirus	Madagascar	None	(Grisoni et al. 2006)
<i>Tradescantia mosaic virus</i>	TraMV	Potyvirus	USA	None	(Baker and Zettler 1988)

^a DNA virus

Commelina cyanea, ‘scurvy weed’, is native to Australia and at the time of writing, this is the first report of a potyvirus infecting a *Commelina* sp. in Australia. It seems likely, given the geographic location and novel host, that the virus differs from all the potyviruses previously isolated from *Commelina* spp. and it is a newly recognised species. We suggest the name ‘Commelina mild mosaic virus’ (CMMV) for this virus.

Interestingly, CMMV does not belong to the bean common mosaic virus lineage like most other potyviruses isolated from Australian native plants (Gibbs et al. 2008). Its closest relatives are Christmas bell potyvirus and IMMV, both of which have not been reported in Australia. IMMV was originally isolated from *Iris* ssp. in Netherlands, but it is now present in most commercially important cultivars and probably distributed worldwide (<http://www.ictvdb.org/ICTVdB/00.057.0.01.033.htm>). There are currently four IMMV sequences available in GenBank and the translated amino acid of one of the two New Zealand isolates (DQ436918) matched closest to CMMV. No other significant relationship can be elucidated from these two species.

Similarly, little insight can be gained from the relationship between CMMV and Christmas bell potyvirus due to the lack of host range, serological and transmission data for both species. The only isolate of Christmas bell potyvirus was isolated from *Sandersonia aurantiaca*, ‘Christmas bell’, from Taiwan and other than its nucleotide sequences deposited in GenBank, no other biological data exist for this tentative potyvirus species.

Further sampling of the CMMV population and biological experiments will be required to determine whether the virus is found in any other plant species and how the virus relates to other Australian potyviruses.

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