Complete genome sequence of a putative novel ilarvirus isolated from *Eleocharis dulcis*

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Abstract

The complete genomic sequence of a novel ilarvirus from *Eleocharis dulcis*, tentatively named "water chestnut virus A" (WCVA), was determined using next-generation sequencing (NGS) combined with reverse transcription polymerase chain reaction (RT-PCR) and rapid amplification of cDNA ends (RACE) PCR. The three genomic RNA components of WCVA were 3578 (RNA1), 2873 (RNA2), and 2073 (RNA3) nucleotides long, with four predicted open reading frames containing conserved domains and motifs typical of ilarviruses. Phylogenetic analysis of each predicted protein consistently placed WCVA in subgroup 4 of the genus *Ilarvirus*, together with prune dwarf virus, viola white distortion associated virus, Fragaria chiloensis latent virus, and potato yellowing virus. The genetic distances and lack of serological reaction to antisera against other ilarviruses suggest that WCVA is a novel member of the genus.

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of viruses belonging to the genus *Ilarvirus* (family *Bromoviridae*) with the highest nucleotide sequence identity of 57.5%, 74.0%, and 59.4% to different members of the genus, suggesting the presence of a distinct ilarvirus. Ilarviruses are a group of isometric and labile viruses that are distributed worldwide and infect many hosts, including vegetables, ornamentals, and fruit trees [5–7]. Their genomes encode four or five proteins on three RNA strands, and they have mostly been classified into four phylogenetic subgroups [8].

To determine the complete genomic RNA sequence of the water chestnut virus, specific primer sets were designed (Supplementary Table S1) to amplify each of the RNAs in two overlapping segments for cloning and splicing. Total RNA was extracted from the infected water chestnut sample using an EASYspin RNA Plant Mini Kit (Aidlab Biotechnologies Co., Ltd, China). The RNA was polyadenylated using poly(A) polymerase (TAKARA, Japan) and then reverse transcribed using the primer M4-T and a First Strand cDNA Synthesis Kit (Toyobo, Osaka, Japan) [9]. Rapid amplification of cDNA ends (RACE) (Tiosbio, Beijing, China) was used to ensure that the complete sequences were obtained. Each PCR product was inserted into the pEASY-T5 Zero Cloning Vector (TransGen Biotech, Beijing), and more than five clones per reaction were sequenced (Ykang, Hangzhou, China). The 5' and 3' RACE fragments amplified using the corresponding primers were then assembled using DNA-MAN 8.0 software (Lynnon Biosoft, Canada).

The complete genomic sequences of the three RNA segments were 3578 nt (RNA1), 2873 nt (RNA2) and 2073 nt (RNA3) in length (Fig. 1) and were deposited in the GenBank database (accession nos. MZ170696-MZ170698) with the provisional name "water chestnut virus A" (WCVA). The 5' untranslated regions (UTRs) of RNA2 and RNA3 of WCVA (49 and 101 nt in length, respectively) both start with TAAA, while the 3'-UTRs of RNA1 and RNA3 both end with GAUGGC. Sequence analysis suggested that WCVA has four ORFs (Fig. 1). The single ORF on RNA1 (P1: nt 43–3387; 125.11 kDa) had two conserved domains, identified by comparison to sequences in the protein families

![Fig. 1: Genome organization of water chestnut virus A (WCVA) GenBank accession nos. MZ170696-MZ170698. Conserved domains/motifs predicted by Pfam are shown.](image-url)
A putative novel ilarvirus isolated from *Eleocharis dulcis*
database (Pfam: http://pfam.xfam.org/ [10]): viral methyltransferase and helicase (Fig. 1). A BLASTp search showed that the P1 protein of WCVA had the highest amino acid sequence identity (98% query coverage and 57.3% identity) to prune dwarf virus (PDV) (ASJ26572). RNA2 also has a single predicted ORF (P2: nt 50–2710; 99.99 kDa) (Fig. 1) that encodes an RNA-dependent RNA polymerase. The P2 protein had the highest amino acid sequence identity to Gun-gahlin flea-associated ilarivirus (GFAIV) (QIJ70041, 89% query coverage and 58.1% identity). RNA3 is predicted to contain two non-overlapping ORFs, encoding a movement protein (MP: nt 102–912; 33.80 kDa) and a coat protein (CP: nt 1088–1765; 24.76 kDa) (Fig. 1). These had the highest amino acid sequence identity to isolates of potato yellow- ing virus (PYV) (85% query coverage and 61.7% identity to AZZ70614 and 98% query coverage and 40.6% identity to QBO24586).

To determine the relationship between WCVA and other known ilarviruses, phylogenetic trees were constructed for each of the ORFs using representative sequences of all previous reported ilarviruses (Supplementary Table S2), with CMV (genus Cucumovirus) included as an outgroup. Analysis was conducted in MEGA X [11] using the maximum-likelihood method with 1000 bootstrap replicates. In all ORFs, WCVA clustered in subgroup 4 with PDV, viola white distortion associated virus (VWDAV), Fragaria chiloensis latent virus (FCILV), and PYV, but the exact clustering pattern among these viruses differed slightly (Fig. 2). In addition, the P2 protein of WCVA showed the closest relationship to that of the GFAIV. However, the RNA2 is the only sequence of GFAIV that has been reported so far. Inspection of the trees suggests that three unclassified viruses — surrounding legume associated ilarivirus (SLAIV), grapevine associated ilarivirus (GAIV), and Solanum nigrum ilarivirus 1 (SNIV-1) — are very closely related and are probably members of a single (new) species.

Total protein extracted from leaves of infected water chestnut were tested in DAS-ELISA with antiserum to the potato leaf roll virus (PLRV), potato virus Y (PVY), tobacco mosaic virus (TMV), tobacco streak virus (TSV), PDV, and prunus necrotic ringspot virus (PNRSV) (Adgia), but there were no positive reactions. Sequence similarity criteria for demarcation of species within the genus Ilarivirus have not been defined [12], but the low amino acid sequence identity to known ilarviruses in all of the predicted proteins (< 62%) and the serological results suggest that WCVA is a distinct novel member of the genus. Further research will focus on the incidence of this virus in different water chestnut varieties and its host range and pathogenicity.

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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.

Additional information The nucleotide sequences reported in this manuscript have been deposited in the GenBank database under accession numbers MZI70696-MZ170698.

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