Complete Genome Analysis of a Novel Iflavivirus From a Spotted Lanternfly, *Lycorma Delicatula*

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Abstract

The spotted lanternfly, *Lycorma delicatula*, is an invasive pest that causes serious economic losses to fruit and wood. Here, we identified a novel iflavirus from the spotted lanternfly, temporarily named *Lycorma delicatula* iflavirus 1 (LDIV1). The full genome sequence of LDIV1 was 10,222 nt in length, which encoded a polyprotein that contained a picornavirus capsid-protein-domain-like domain, a cricket paralysis virus capsid superfamily domain, a RNA helicase domain, a peptidase C3 superfamily domain, and a RNA-dependent RNA polymerase (RdRp) domain. LDIV1 efficiently replicated in host insect and activated the small interfering RNA (siRNA)-based host antiviral immunity. Phylogenetic analysis demonstrated that LDIV1 was the closest to Picornavirales sp, with 61.7% sequence identity in the RdRp region, and 57.6% sequence identity in the coat protein region, which met the demarcation criteria of new species in the genus *Iflavirus*. To the best of our knowledge, LDIV1 is the first virus discovered in *L. delicatula*.

Full Text

The spotted lanternfly, *Lycorma delicatula* (Hemiptera: Fulgoridae) is a sap-feeding insect native to China. It is highly polyphagous and feeds on more than 70 plant species, including apples and grapes [1]. In recent years, owing to the increasing frequency of international trade, the distribution range of *L. delicatula* has been expanding from China to Japan, South Korea and the United States, causing serious damage to the global fruit and wood industry [2, 3]. The current management strategies for *L. delicatula* mainly rely on pesticide application, which is often incompatible with the natural enemies and may result in pest resurgence. In 2019, a high-quality reference genome of *L. delicatula* became available [4], which provided a valuable resource for investigating this invasive pest. However, as far as we know, there is little or even no information about the viruses infecting *L. delicatula*.

Iflaviridae is a family of positive-stranded, non-enveloped RNA viruses with a small linear genome (approximately 9-11 kilobases in length) [5]. As shown by the latest ICTV Online Report (<http://ictv.global/report/>), the family *Iflaviridae* contains only 15 species in one genus, *Iflavirus*. Also, genome architecture analysis demonstrates that iflavirus contains a single open reading frame (ORF), that is directly translated into a polyprotein, followed by posttranslational processing to generate the non-structural and structural proteins [5]. All viruses in the family *Iflaviridae* identified so far are restricted to infecting invertebrates, usually the insects [6-8]. It is reported that some iflaviruses are the insect pathogens, which cause premature mortality, behavioural changes, and developmental abnormalities to their hosts, thus become the promising agents for the biological control of specific insects [9, 10].

The *L. delicatula* analyzed in this study was collected from the tree of heaven, *Ailanthus altissima*, in Xiangshan, Zhejiang, China in 2020. The insect was subsequently sent to our laboratory for RNA extraction. The whole single insect was used to extract the total RNA using the TRIzol Total RNA Isolation Kit (Takara, Dalian, China). The RNA sample was then sent to Novogene (Beijing, China) for transcriptomic sequencing as previously described [11]. Later, the output raw data were filtered, and the

clean data were assembled using Trinity (version 2.8.5) [12] with default parameters. To confirm the insect species, the assembled contigs were compared with cytochrome oxidase subunit 1 (COI) from the Barcode of Life Data (BOLD) Systems (<https://www.boldsystems.org/>). As a result, the lanternfly analyzed in this study was *L. delicatula*, which had a COI sequence identical to the reported COI sequence (accession number MN607209).

To further identify the potential iflavirus, the assembled contigs were compared with a locally generated viral database containing sequences from all members of the genus *Iflavirus*. As a result, an ifla-like viral contig, which represented an almost complete viral genome, was detected. Thereafter, the viral contig was validated by reverse transcription PCR (RT-PCR), and the full genome sequence of the ifla-like virus was successfully obtained by rapid amplification of cDNA ends (RACE) using the SMARTer® RACE 5'/3' kit (Takara). The primers are listed in Supplementary Table S1. The new virus discovered in *Lycorma delicatula* was temporarily named *Lycorma delicatula* iflavirus 1 (LDIV1), and its complete genome sequence was submitted to the GenBank (accession number OM728531).

Excluding the polyA tail, the whole genome sequence of LDIV1 was 10,222nt in length, which contained a large ORF (573 to 9992 nt), a 5'-UTR (572 nt) and a 3'-UTR (230 nt). The large ORF encoded a 3,140 aa polyprotein. Later, the typical domains of iflaviruses were predicted by InterPro Scan (<https://www.ebi.ac.uk/interpro>), which included a picornavirus capsid-protein-domain-like (rhv-like), a cricket paralysis virus capsid superfamily domain (CRPV), a RNA helicase domain (Hel), a peptidase C3 superfamily domain (peptidase) and a RNA-dependent RNA polymerase domain (RdRp) (Fig. 1A). Moreover, the abundance and coverage of LDIV1 were assessed by rearranging RNA-seq reads into a reconstructed complete genome sequence of LDIV1. The results showed that RdRp was relatively abundant in the whole virus genome, suggesting that LDIV1 might replicate efficiently in the host insects (Fig. 1B). The amino acid sequence identity between the polyprotein of LDIV1 and Picornavirales sp (MT138389) was also determined. Notably, the identity value of CP is one of the criteria to define new iflaviruses. The highest percentage identity was 57.6% for CP and 61.7% for RdRp when compared with Picornavirales sp, which met the demarcation criteria of new species in the genus *Iflavirus*, with the sequence identity of CP being less than 90% at amino acid level [13]. These results indicated that LDIV1 was a new iflavirus. To determine the taxonomic status of LDIV1, the RdRp amino acid sequence was aligned to those of the previously reported iflaviruses using MAFFT (version 7.450) [14]. The substitution model was evaluated with ModelTest-NG, and a maximum likelihood phylogenetic tree was constructed in RAxML-NG (version 0.9.0), with 1000 bootstrap replicates [15]. As revealed by phylogenetic analysis, LDIV1 was the closest to Picornavirales sp and Nilaparvata lugens honeydew virus 3 (Fig. 2).

To explore the small interfering RNA (siRNA)-based host antiviral immunity, the small RNA (sRNA) library of *L. delicatula* was also sequenced, and the virus-derived siRNA (vsiRNA) was comprehensively characterized as previously described [16]. The sRNA library was prepared by the Illumina TruSeq small RNA sample preparation kit (Illumina, USA), and sequenced on the Illumina HiSeq 2500 platform. Besides, the sRNA reads were processed by removing adapters, low-quality sequences and garbage sequences, while sRNAs with the length of 18-30 nt were extracted. As a result, the majority of LDIV1-derived vsiRNAs

were 22-23 nt in length (Fig. 3A), and were almost equally derived from the sense and antisense chains of the virus genome (Fig. 3B). These LDIV1-derived vsiRNAs strongly suggested that the RNAi antiviral pathway of *L. delicatula* was activated in response to LDIV1 infection.

In conclusion, our study reveals the full genome of LDIV1 from an invasive pest, *L. delicatula*. LDIV1, a member of iflavirus, is evolutionary related to Picornavirales sp. LDIV1 can efficiently replicate in host insects and activate the RNAi antiviral pathway. In the future, it will be interesting to determine the influence of LDIV1 on the host insects, and investigate the potential value of this virus in the biological control of agricultural pests.

Declarations

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Conflict of interest

The authors declare no conflicts 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

Schematic diagram of genome structure (A) and transcriptome raw reads coverage (B) of *Lycorma delicatula* iflavirus 1 (LDIV1). rhv-like, picornavirus capsid-protein-domain-like; CRPV, cricket paralysis virus capsid superfamily domain; Hel, RNA helicase domain; peptidase, peptidase C3 superfamily domain; RdRp, RNA-dependent RNA polymerase domain.

Figure 2

Phylogenetic relationships of *Lycorma delicatula* iflavirus 1 (LDIV1) with other iflaviruses. Maximum-likelihood phylogenetic tree based on the RNA-dependent RNA polymerase domain was constructed with a bootstrap of 1000. Scale bars represent percent divergence. *Drosophila immigrans* Nora virus and *Spodoptera exigua* virus were used as an outgroup. Nodes with bootstrap values >50% are marked with solid blue circles, and the larger circles indicate higher bootstrap values.

Figure 3

Analysis of *Lycorma delicatula* iflavirus 1 (LDIV1)-derived small interfering RNAs (siRNA). (A) The size distribution of LDIV1-derived siRNAs with 18-30 nt in length. (B) Distribution of LDIV1-derived siRNA on the viral genome. Black represents sRNAs derived from the antisense strand of genome (Minus), and black represents sRNAs derived from the sense strand of genome (Plus)

Supplementary Files

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