Transcriptional responses of Daphnis nerii larval midgut to oral infection by Daphnis nerii cypovirus-23

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Abstract

Background: Daphnis nerii cypovirus-23 (DnCPV-23) is a new type of cypovirus and has a lethal effect on the oleander hawk moth, Daphnis nerii which feeds on leaves of Oleander and Catharanthus et al. After DnCPV-23 infection, the change of Daphnis nerii responses has not been reported.

Methods: To better understand the pathogenic mechanism of DnCPV-23 infection, 3rd-instar Daphnis nerii larvae were orally infected with DnCPV-23 occlusion bodies and the transcriptional responses of the Daphnis nerii midgut were analyzed 72 h post-infection using RNA-seq.

Results: The results showed that 1979 differentially expressed Daphnis nerii transcripts in the infected midgut had been identified. KEGG analysis showed that protein digestion and absorption, Toll and Imd signaling pathway were down-regulated. Based on the result, we speculated that food digestion and absorption in insect midgut might be impaired after virus infection. In addition, the down-regulation of the immune response may make D. nerii more susceptible to bacterial infections. Glycerophospholipid metabolism and xenobiotics metabolism were up-regulated. These two types of pathways may affect the viral replication and xenobiotic detoxification of insect, respectively.

Conclusion: These results may facilitate a better understanding of the changes in Daphnis nerii metabolism during cypovirus infection and serve as a basis for future research on the molecular mechanism of DnCPV-23 invasion.

Keywords: Daphnis nerii cypovirus-23, Midgut, Transcriptome analysis

Introduction

The oleander hawk moth, Daphnis nerii (D. nerii), belongs to Lepidoptera, Sphingidae family, is a worldwide pest [1]. D. nerii larvae damages leave of Oleander, Catharanthus, Vinca, Adenium, Vitis, Tabernaemontana, Gardenia, Trachelospermum, Amsonia, Asclepias, Carissa, Rhazya, Thevetia, Jasminum and Ipomoea [2, 3], which affect the landscape and the medicinal value of these plants. At present, the chemical pesticide decamethrin is used to control D. nerii [2].

Cypovirus is a member of the Reoviridae family, and is characterized by its single layered capsid [4]. DnCPV-23 was isolated from naturally diseased D. nerii larvae. This was a new type of cypovirus based on different electrophoretic migration patterns and conserved terminal sequences [1, 5, 6]. In addition to Daphnis nerii, it has been found that DnCPV-23 can also induce infection and death in many species of Sphingidae insects, such as...
as *Cephalonomes hylas* Linnaeus, *Ampelophaga rubiginosa* Bremer & Grey, and *Agatha lycaenaria* Kollar. The genome of DnCPV-23 consists of ten segments of linear double-stranded RNA, referred to as genomic segments 1 (*SI*) to 10 (*S10*), in accordance with the fragments from longest to shortest [7]. Our previous research and unpublished data demonstrated that the virus could successfully replicate on the SF9 [8] and Manduca sexta cell lines QB-MS 2-2 [9]. However, the molecular mechanism of the interactions between the new type cypovirus and its hosts remains unclear. It is necessary to identify the interactions between the virus and its hosts to achieve an in-depth understanding and reveal the exploitation potential of the virus for future insecticide development.

Recently, many studies in the field have generated large amounts of data using the aforementioned high-throughput approaches, from the silkworms or BmN cells infected with BmCPV, including (1) The possible host’s RNAi response against BmCPV challenge in persistent and pathogenic Bombyx mori model was compared. During the pathogenic infection, it was found that higher level RNAi responses against BmCPV were observed, which further demonstrated the importance of RNAi as an antiviral mechanism [10]. (2) Gene expression profiles [11–19], DNA methylation [20], and lipidomic profile [21] of silkworm midgut or BmN cells after BmCPV infection were analyzed. These results suggested that many genes (for example, genes expressing Calreticulin, FK506-binding protein, and protein kinase c inhibitor gene, microRNAs, and activated protein kinase C) may play important roles in BmCPV replication. In addition, epigenetic regulation may influence silkworm-virus interaction, and BmCPV may modulate the lipid metabolism of cells for their self-interest.

Until now, the molecular mechanism underlying the midgut infection of DnCPV-23 is not clearly understood. Furthermore, since transcriptome analyses regarding *D. nerii* or DnCPV-23 have not yet been performed, this study aims to fill this gap about the new type cypovirus. The data and analysis presented here provide insights into the possible mechanism of DnCPV-23 infection and host defense and a basis for future DnCPV-23 relevant studies.

**Materials and methods**

**Daphnis nerii** larval midgut and virus stock

Newly wild-caught second instar larvae with a similar mass were used in this research investigation for the virus infection. Before infection, the *D. nerii* were supplied with 12-h day/night cycles under 50±5% relative humidity conditions and were nurtured on oleander leaves at 27±1 °C for three days. The midgut tissues were collected from four pathogenically infected larvae at 72 h [13, 15] after feeding with DnCPV-23. The same tissues were also collected from three uninfected control larvae at the same time point. DnCPV was originally isolated from the larvae of *D. nerii* and propagated in *D. nerii* larvae [1]. The polyhedra suspension of DnCPV-23 utilized for infecting the *D. nerii* was stored at 4 °C in the dark.

**Virus inoculation**

In this study, the DnCPV-23 viral stock was suspended in distilled water at a concentration of 2×10⁷ polyhedra/mL. Then, 100 μL of the viral suspension was spread evenly on one piece of oleander leaf measuring approximately 4 cm × 1.5 cm each in size. The leaf was then fed to four *D. nerii* larvae. The dose of infection was calculated as 2×10⁶ polyhedra per larva. In addition, three control larvae were fed the same quantity of leaves treated with only distilled water. After approximately 12 h, fresh oleander leaves were used to feed the inoculated larvae after the DnCPV-23-inoculated leaves had been completely consumed.

**Sample preparation**

The midguts of both DnCPV-23-infected and control larvae were collected at 72 h post-inoculation by dissecting the larvae on ice. The isolated midgut was then quickly washed in 0.8% diethylpyrocarbonate (DEPC)-treated physiologic saline solution to remove the attached leaf pieces, and then frozen in liquid nitrogen [13, 22].

**RNA sequencing**

All of the RNA-seq procedures were conducted by the Oebiotech Company (Shanghai, China). The total RNA was extracted from the *D. nerii* midgut tissue using TRIzol reagent (Invitrogen, USA) according to the manufacturer’s protocols. The RNA integrity and concentrations were checked using an Agilent 2100 Bioanalyzer (Agilent Technologies, USA). In addition, seven RNA samples (including three uninfected samples and four infected samples) with RNA integrity were used to construct the libraries. The cDNA libraries were prepared using a TruSeq RNA Sample Preparation Kit (Illumina, USA) according to the manufacturer’s protocols. Thereafter, the obtained cDNA libraries were sequenced on the Illumina HiSeq2500 platform, which generated paired-end raw reads of 125 bp.

**De novo assembly and functional annotation**

The raw data was pretreated by discarding reads with adaptors and low quality (quality scores <30). Then, the raw data was assembled using Trinity software with default parameters for de novo transcriptome assembly. Transcripts that were not shorter than 300 bp were used for subsequent analysis. To obtain the functional
Differential gene expression analysis

RNA sequencing results from the two groups were mapped to the assembled transcriptome using bow-tie2 [23] and express [24]. The FPKM (fragments per kb per million reads) method [25] was utilized to calculate the expression levels of the unigenes, which eliminated the influencing effects of the different gene lengths and sequencing levels. The differences in the unigene expressions between the two groups were calculated with DESeq [26] and any significant differences were determined with \( P < 0.05 \) and an absolute value of log2 fold change \( > 1 \).

Real-time quantitative reverse transcription PCR (Real-Time qRT-PCR)

This study utilized qRT-PCR to analyze the expression level of DnCPV-23 \( S1, S10 \) genes of transcriptome samples, and verify the DEGs recognized by the RNA-seq. The total RNA was isolated from the samples of the transcriptomic analysis using TRIzol reagent (Life Technologies) and was then treated with DNase I (Fermentas, Glen Burnie, MD, USA). We reversely transcribed 1 \( \mu \)g of the total RNA per sample into complementary DNA (cDNA) using a PrimeScript RT Reagent Kit (Takara). Then, qRT-PCR was performed using Talent qPCR Pre-Mix SYBR Green (Tiangen, China) on a QuantStudio™ 7 Flex Real-Time PCR System (Applied Biosystems™). One cycle was added for melting curve analysis for all the reactions to verify the product specificity. The expression level of each gene relative to that of the \( RPL13 \) gene was calculated using the \( 2^{-\Delta\Delta CT} \) method [27]. All of the primers for the aforementioned target genes are listed in Table 1. Results are representative of two to three independent experiments.

Results

Virus infection of the samples

Prior to the transcriptome analysis, qRT-PCR was used to detect the mRNA levels of the DnCPV-23 \( S1, S10 \) genes in the infected and uninfected samples. The results showed that the infected group had been successfully infected based on the high relative expression of the viral gene mRNA compared with uninfected group (Fig. 1).

Transcriptome sequencing and assembly

The RNA-Seq data from the DnCPV-23-infected and control groups contained 346.39 million reads, and 334.60 million clean reads after trimming, among which 96.17 to 97.39% per sample were determined to be useful. The acquired clean reads were assembled into 31,696 unigenes (>300 bp). The average length of these unigenes was 1347.61 bp, and the N50 length was 2348 bp; other information about these unigenes were shown in Table 2. This study then assembled 31,696 unigenes ranging from 301 bp to 32,420 bp. The total unigene length was 42,713,980.

Transcriptome annotation

A total of 31,696 assembled unigenes were searched against the public databases, including the NR, Swiss-prot, KOG, GO, and KEGG databases, among which 16,820 (53.1%) (Fig. 2) unigenes were annotated. The distribution patterns of the unigenes in the different databases were as follows: 16,615 unigenes in the NR database, 11,152 unigenes in the Swissprot database, 10,374 unigenes in the KOG, 10,468 unigenes in the GO, and 5501 unigenes in the KEGG databases (Table 3). Figure 2 shows the degree of overlap between the unigenes annotated in the different databases. It was found that 4353 (13.7%) unigenes overlapped in all five databases, while 12,390 (73.7%) unigenes overlapped in two or more databases.

Significant impacts of the viral infection on the hosts’ transcriptome expressions

As shown in Fig. 3, the main component PCA1 had reached 41.56%, and the main component PCA2 had reached 27.23%. Therefore, the percentage total of the two was 68.79%, which accounted for a high proportion and represented the overall population to a large extent. This study’s principal component analysis manifested a clear separation of the samples with the two treatments (Fig. 3A), which indicated that the samples had good repeatability. The heat map of the gene expressions is presented in Fig. 3B. The results suggested that these DEGs could distinguish the samples. The results revealed that the viral infection could exert apparent influences on the midgut gene expressions. In addition, the transcriptome results showed that 1166 genes were down-regulated (accounting for 3.68% of the total assembled unigenes) and 812 genes (accounting for 2.56% of the total assembled unigenes) were up-regulated as a response to the DnCPV-23 infection (Fig. 3C).

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Analysis of the differently expressed genes

In this study, KEGG function enrichment analysis was performed on the differential genes expressed in the DnCPV-23-infected and uninfected control groups to clarify the relevant biological pathways involved in the differential genes. Among all of the DEGs, 298 DEGs had KEGG annotations, of which 118 were up-regulated genes and 180 were down-regulated genes. According to the pValue of KEGG analysis of up-regulated and down-regulated signal pathways, we identified 20 most significant signal pathways each. These pathways play an important role in insect reproduction, immunity, digestion and absorption and xenobiotic metabolism and so on (Fig. 4).

### Table 1
Primer sequences used in the qRT-PCR for the viral RNA detection of transcriptome samples and validation of the RNA-seq

| No | Primer name | Primer sequence (5’ to 3’) | Tm (°C) | Gene id | Target gene |
|----|-------------|----------------------------|--------|---------|-------------|
| 1  | S1-RTPCR-F  | GTGCTGATGCTGCTGCTA        | 49.6   | N/A     | DnCPV S1   |
| 2  | S1-RTPCR-R  | TGATTGATGACGCATTAGAG      | 51.5   |         |             |
| 3  | S10-RTPCR-F | GTCCGATGCTGCTGCTGCTA     | 52.6   | N/A     | DnCPV S10  |
| 4  | S10-RTPCR-R | CGTATGCTGCTGCTGCTA       | 51.3   |         |             |
| 5  | CASP8-F     | ACTGAGGAGAATGAGGTTA       | 51.5   | TRINITY_DN10280_c0_g1_i1_3 | CASP8   |
| 6  | CASP8-R     | AGCGAGAGAATGAGGTTA        | 53.7   |         |             |
| 7  | CYP6A13-F   | GATCCATGCTGCTGCTGCTA     | 51.0   | TRINITY_DN11437_c0_g1_i1_6 | CYP6A13 |
| 8  | CYP6A13-R   | CAGTGTGCTGCTGCTGCTA      | 50.5   |         |             |
| 9  | CYP6B45-F   | GCGATGCTGCTGCTGCTA       | 53.4   | TRINITY_DN12532_c0_g7_i1_1 | CYP6B45 |
| 10 | CYP6B45-R   | ATGCGAGAATGAGGTTA        | 51.0   |         |             |
| 11 | DHR54-F     | TCTGAGGAGAATGAGGTTA      | 52.8   | TRINITY_DN12896_c1_g2_i3_3 | DHR54   |
| 12 | DHR54-R     | CGCGAGAATGAGGTTA         | 53.5   |         |             |
| 13 | PNLIP-F     | GACCTGTGCTGCTGCTGCTA     | 53.5   | TRINITY_DN12381_c0_g2_i1_6 | PNLIP   |
| 14 | PNLIP-R     | GATGAGAATGAGGTTA        | 53.2   |         |             |
| 15 | PRS1_2_3-F  | GATGGAGAATGAGGTTA       | 55.4   | TRINITY_DN10836_c0_g5_i1_6 | PRS1_2_3|
| 16 | PRS1_2_3-R  | TCGGAGAATGAGGTTA       | 53.5   |         |             |
| 17 | RDH12-F     | GTGCAATGCTGCTGCTA        | 52.5   | TRINITY_DN14445_c0_g1_i1_3 | RDH12   |
| 18 | RDH12-R     | GCTGAGAATGAGGTTA        | 52.2   |         |             |
| 19 | SCARB1-F    | AACAGAGAATGAGGTTA       | 53.0   | TRINITY_DN14140_c0_g1_i1_6 | SCARB1  |
| 20 | SCARB1-R    | GTGGGAGAATGAGGTTA       | 51.7   |         |             |
| 21 | SLC46A1-F   | GTCGAGAATGAGGTTA       | 53.7   | TRINITY_DN8071_c0_g1_i2_5 | SLC46A1 |
| 22 | SLC46A1-R   | GAGCAGAATGAGGTTA       | 51.7   |         |             |
| 23 | SLC52A3-F   | GGCTGAGAATGAGGTTA      | 52.5   | TRINITY_DN11521_c0_g1_i2_4 | SLC52A3 |
| 24 | SLC52A3-R   | GGCTGAGAATGAGGTTA      | 54.4   |         |             |
| 25 | ABCA3-F     | GGATGAGAATGAGGTTA       | 53.3   | TRINITY_DN12365_c0_g1_i6_2 | ABCA3   |
| 26 | ABCA3-R     | GAGCTGAGAATGAGGTTA      | 51.8   |         |             |
| 27 | ABCC4-F     | GAGCTGAGAATGAGGTTA      | 53.3   | TRINITY_DN11997_c1_g1_i2_4 | ABCC4   |
| 28 | ABCC4-R     | GAGCTGAGAATGAGGTTA      | 51.9   |         |             |
| 29 | CYP6B6-F    | GACCTGTGCTGCTGCTA       | 50.7   | TRINITY_DN13898_c0_g1_i1_4 | CYP6B6  |
| 30 | CYP6B6-R    | GTGGGAGAATGAGGTTA       | 50.5   |         |             |
| 31 | GAPDH-F     | TATGGGAGAATGAGGTTA      | 50.1   | TRINITY_DN5984_c0_g1_i2_2 | GAPDH   |
| 32 | GAPDH-R     | TATGGGAGAATGAGGTTA      | 52.4   |         |             |
| 33 | LYPLA3-F    | ACACTGCACTGCACTGCACTGCA | 52.8   | TRINITY_DN10250_c0_g1_i1_1 | LYPLA3  |
| 34 | LYPLA3-R    | GACCTGAGAATGAGGTTA      | 51.5   |         |             |
| 35 | NTE-F       | GAGCTGAGAATGAGGTTA      | 53.6   | TRINITY_DN14343_c0_g2_i1_4 | NTE     |
| 36 | NTE-R       | GAGCTGAGAATGAGGTTA      | 53.8   |         |             |
| 37 | UGT-F       | GACCTGAGAATGAGGTTA      | 51.3   | TRINITY_DN14215_c0_g5_i7_5 | UGT     |
| 38 | UGT-R       | GACCTGAGAATGAGGTTA      | 52.2   |         |             |
| 39 | DnRPL13-F   | GACCTGAGAATGAGGTTA      | 52     | TRINITY_DN4717_c0_g1_i2_3 | DnRPL13 |
| 40 | DnRPL13-R   | TATGGGAGAATGAGGTTA      | 54.5   |         |             |
To verify the reliability of the transcriptome data and the DEG results obtained by RNA-seq, seventeen DEGs were selected for qPCR analysis. As shown in Fig. 5, the fold-change values of DnCPV_1 sample vs Mock_1 sample obtained in the qPCR analysis results were consistent with the values obtained by the RNA-seq for all of the selected genes.

**Discussion**

This study analyzed the transcriptome of the uninfected *D. nerii* midgut and the DnCPV-23- infected *D. nerii* midgut presented unique gene expression profiles induced by DnCPV-23 infection for the first time. In addition, KEGG function enrichment analysis was performed on the differential genes expressed after DnCPV-23 infection. Compared with uninfected *D. nerii* midgut, the transcriptome profiles of the infected samples displayed universally changed transcript abundances for many pathways.
Table 3  Annotation statistics for each database

| Anno_Database | Annotated_Number | 300 < = length < 1000 | Length > = 1000 |
|---------------|-----------------|------------------------|-----------------|
| NR            | 16,615 (52.42%) | 6217 (19.61%)          | 10,398 (32.81%) |
| Swissprot     | 11,152 (35.18%) | 2921 (9.22%)           | 8231 (25.97%)   |
| KEGG          | 5501 (17.36%)   | 1694 (5.34%)           | 3807 (12.01%)   |
| KOG           | 10,374 (32.73%) | 2758 (8.70%)           | 7616 (24.03%)   |
| eggNOG        | 15,249 (48.11%) | 5239 (16.53%)          | 10,010 (31.58%) |
| GO            | 10,468 (33.03%) | 2670 (8.42%)           | 7798 (24.60%)   |
| Pfam          | 10,594 (33.42%) | 2505 (7.90%)           | 8089 (25.52%)   |

Fig. 3  Influence of DnCPV-23 infection on D. neri transcriptome:  

A  Plot of the 1st and 2nd principal component of the sample variations using the principal component analysis, in which the red dots represent samples without DnCPV-23 infection, and the green dots denote infected samples.  
B  Heat map of 1,978 differently expressed genes (DEGs) in the infected samples and controls.  
C  After infection, 812 genes were up-regulated (red bars) and 1166 genes were down-regulated (blue bars)
Fig. 4 KEGG classifications of DEGs after DnCPV-23 infection (Top 20): A. Down-regulated pathways; B. Up-regulated pathways. Horizontal axis of the figure is the enrichment score. The larger the bubble, the more the number of DEGs. The bubble color changes from purple to blue to green to red, indicating that the smaller the enrichment p-value and the greater the significance.
tein and amino acid metabolism in D. nerii [13, 28].

The down-regulation of midguts, which causes the disturbance of pro-

digestion and the absorption pathway way was down-regulated in the non-infected midgut [13]. In addition, protein diges-
tion and absorption pathway were down-regulated, consistent with the

transcriptome study about BmCPV infected midgut vs

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lism pathway, vitamin digestion, and absorption sig-

Fig. 5 Validation of RNA-seq profiles by real-time qPCR. To validate the RNA-seq data, the relative mRNA levels of 17 selected DEGs in the DnCPV_1 sample were examined by qPCR. The mRNA levels by qPCR are presented as the fold change compared with the Mock_1 sample after normalization against RPL13A. The relative expression levels from the RNA-seq analysis were calculated as RPKM values. Error bars show mean ± SEM

Based on the pValue of KEGG analysis regarding up-regulated and down-regulated signal pathways, we

identified 20 most significant signal pathways each. Among these signal pathways, the retinol metabo-
lism pathway, vitamin digestion, and absorption signal pathway were down-regulated, consistent with the transcriptome study about BmCPV infected midgut vs non-infected midgut [13]. In addition, protein digestion and absorption pathway was down-regulated in accord with previous research [10]. DnCPV infection may destroy the functions of digestion and the absorption of midguts, which causes the disturbance of protein and amino acid metabolism in D. nerii [13, 28]. Peptidoglycan recognition proteins (PGRPs) are pattern recognition molecules that are conserved from insects to mammals. PGRPs are the first receptors known to recognize, bind, or catalytically cleave the pathogenic microorganisms [29], PGRPs recognize bacteria and their unique cell wall component, peptidoglycan [30, 31]. This study observed nine transcripts of D. nerii isoforms of PGRP genes. Six transcripts were found to be down-regulated in the infected D. nerii midgut. The most highly expressed and most dramatically down-regulated was TRINITY_DN13195_c0_g1_i3_3, which was down-regulated by as much as 51-fold. The down-regulation of PGRP expression can lead to a decrease in the ability of the D. nerii's innate immune system to recognize bacterial peptidoglycans (PGN), which may lead to D. nerii more susceptible to bacterial infections. In addition, BmpPGRP-S2 was up-regulated upon BmCPV infection, overexpression of which can activate the Imd pathway and induce increased AMPs to enhance the antiviral capacity of transgenic silkworm against BmCPV [32]. Moreover, previous study demonstrates [33] that PGRPS2-1 and PGRPS2-2 can prevent BmCPV replication. Based on this work, was speculated that the down-regulation of PGRP was conducive to the replication of DnCPV-23. The gene CASP8 (KEGG gene name: caspase-8, Gene id: TRINITY_DN10280_c0_g1_i1_3) (Dredd in Drosophila) was down-regulated more than two folds, and other caspase genes changed non-
significantly. It is predicted to be involved in the cleavage of Relish, the Drosophila homolog of mammalian NF-kB, resulting in activating the immune-deficient pathway (IMD)-induced expression of antimicrobial peptides in response to Gram-negative bacteria [34–36], fungi and viruses [37]. Research performed by Li et al. proved BmDredd interacts with BmSTING to enhance antiviral signaling [38]. The down-regulation of this gene may be very important for DnCPV-23 to escape from the host innate immune system and replicate in the midgut. Our result conflicted with the work by Guo et al. [11]. We speculated the contradiction might be related to the different stages of virus-host interaction or the heterogeneity of different species against viruses. The pathways and the genes mentioned above are listed in Table 4 (The expression of genes in each sample is shown in Additional file 1).

In this study, the up-regulation of glycrophospho-
lipid metabolism was consistent with Zhang’s research [21]. The up-regulation of this pathway may be related to the viral replication [39, 40]. In addition, Glycine, serine and threonine metabolism were up-regulated in this transcriptome analysis. In the study by Wu et al., two genes related to this signaling pathway were up-regulated and the other down-regulated. In our study, the expression levels of the phosphoserine phosphatase genes were significantly higher in DnCPV-23-infected midgut than in the non-infected group, suggesting that serine metabolism disorders were induced after DnCPV-23 infection. Expression of many UGT genes was up-regulated; UDP-glucuronosyltransferase (UGT) isozymes take endogenic and exogenic toxic substances as substrates, catalyze detoxification of many chemical toxins in our daily diet and environment by conjugation to glucuronic acid or glucose [41, 42]. After DnCPV-23 infection, it was speculated that the D. nerii tended to strengthen the elimination of lipophilic endobiotics such as hormones and xenobiotics including phytoalexins and drugs conjugated by invertebrates and plants mainly with glucose [42] through promoting the transcription of UGTs by regulating the activi-
ties of nuclear-receptor family (CAR, PXR, FXR, LXR, and PPAR), the arylhydrocarbon receptor [43] or ubiquituous transcription factors (FOXA1, Sp1, and Cdx2)
### Table 4 The down-regulated pathways focused in the discussion section

| id          | Term                                      | pValue      | Enrichment score | gene_id                        | BaseMean_control_mock | BaseMean_case_DnPVP | FoldChange | pValue     | qValue      | Regulation | NR annotation | KEGG gene name | KEGG gene name |
|-------------|--------------------------------------------|-------------|------------------|--------------------------------|-----------------------|--------------------|------------|------------|-------------|------------|----------------|----------------|----------------|
| ko04974     | Protein digestion and absorption          | 1.33E–17    | 6845688889       | TRINITY_DNI2884_c1_g5_i1_1     | 2843.901036           | 2821.111777       | 0.099198662 | 1.72E–06   | 0.0007668  | Down       | LOW QUALITY PROTEIN carboxypeptidase B [Bombyx mori] | CPA2            |               |
|             |                                            |             |                  | TRINITY_DNI3745_c3_g2_i2_4     | 37,228.13358          | 6283.238748        | 0.168776625 | 0.04653249 | 0.7667028  | Down       | putative chymotrypsin, partial [Samia ricini] | CELA2           |               |
|             |                                            |             |                  | TRINITY_DNI3546_c1_g2_i2_1     | 33,763.67895          | 1798629852         | 0.005327115 | 1.26E–10   | 2.06E–07   | Down       | RecName: Full = Trypsin, alkaline C; Flags: Precursor PRSS1_2_3 | PRSS1_2_3               |               |
|             |                                            |             |                  | TRINITY_DNI1163_c0_g1_i2_3     | 576.0371804           | 2838155477         | 0.049270352 | 1.62E–08   | 1.12E–05   | Down       | trypsin, alkaline C-like [Spodoptera litura] PRSS1_2_3 | PRSS1_2_3               |               |
|             |                                            |             |                  | TRINITY_DNI3619_c0_g2_i1_3     | 701.0350489           | 1995322761         | 0.28462525  | 0.00871419 | 0.3479086  | Down       | sodium/potassium-transporting ATPase subunit alpha isoform X6 [Bombyx mori] ATP1A |               |               |
|             |                                            |             |                  | TRINITY_DNI13597_c0_g2_i2_4    | 683.142229            | 3696948382         | 0.054116803 | 0.00373058 | 0.7023823  | Down       | serine protease 62 [Mamestra configurata] PRSS1_2_3 | PRSS1_2_3               |               |
|             |                                            |             |                  | TRINITY_DNI0836_c0_g7_i1_6     | 407.2114124           | 52.04490715        | 0.127808076 | 0.00142694 | 0.1232753  | Down       | trypsin, partial [Manduca sexta] PRSS1_2_3               | PRSS1_2_3               |               |
|             |                                            |             |                  | TRINITY_DNI7116_c0_g1_i1_5     | 140.0397486           | 41.7398528         | 0.298057182 | 0.002906   | 0.6296715  | Down       | Prolylcarboxypeptidase [Danaus plexippus plexippus] PRCP PRSS1_2_3 | PRSS1_2_3               |               |
|             |                                            |             |                  | TRINITY_DNI5681_c0_g1_i1_6     | 1202.562374           | 41.63057875        | 0.034618228 | 0.00013096 | 0.0240473  | Down       | chymotrypsinogen-like protein 3 [Manduca sexta] PRSS1_2_3 | PRSS1_2_3               |               |
|             |                                            |             |                  | TRINITY_DNI0836_c0_g5_i1_6     | 3539.814633           | 11.52410727        | 0.003255568 | 0.00393418 | 0.2234223  | Down       | trypsin, alkaline C [Bombyx mori] PRSS1_2_3               | PRSS1_2_3               |               |
| id | Term | pValue | Enrichment_score | gene_id | BaseMean_control_mock | BaseMean_case_DnCPV | FoldChange | pValue | qValue | Regulation | NR annotation | KEGG gene name |
|----|------|--------|------------------|---------|------------------------|--------------------|------------|--------|--------|------------|---------------|----------------|
| TRINITY_DN14237_c1_g1_i3_3 | 2139.270665 126.8457966 | 0.059293945 | 0.00362815 | 0.2142243 | Down | hypotheti-cal protein BSV51_4161 [Heliothis virescens] | PRSS1_2_3 |
| TRINITY_DN18044_c0_g1_i1_4 | 61.209503460 | 0 | 0.00262208 | 0.1786659 | Down | RecName: Full = Trypsin, alkaline C; Flags: Precursor | PRSS1_2_3 |
| TRINITY_DN13619_c0_g2_i1_3 | 1098.504638 384.681457 | 0.350186466 | 0.02344348 | 0.5771173 | Down | Sodium/potassium-transporting ATPase subunit alpha [Papilio xuthus] | ATP1A |
| TRINITY_DN12770_c1_g2_i2_6 | 33,988.65545 24720.57397 | 0.072731838 | 0.03984473 | 0.7217322 | Down | serine protease 62 [Mamestra configurata] | PRSS1_2_3 |
| TRINITY_DN14161_c2_g2_i3_3 | 150,880.9838 10,699.40148 | 0.070912856 | 0.027439 | 0.6151748 | Down | trypsin, alkaline C-like [Spodoptera litura] | PRSS1_2_3 |
| TRINITY_DN12929_c2_g1_i1_6 | 9158.048497 41.0899202 | 0.004486755 | 0.00143302 | 0.1232753 | Down | trypsin [Manduca sexta] | PRSS1_2_3 |
| TRINITY_DN10836_c0_g1_i3_6 | 157,887.1736 53,448.64932 | 0.338524328 | 0.02226533 | 0.5641697 | Down | trypsin, alkaline C-like [Bombyx mori] | PRSS1_2_3 |
| TRINITY_DN12646_c0_g1_i3_6 | 18,799.76899 34,300.33323 | 0.018245082 | 0.00588226 | 0.271197 | Down | trypsin, alkaline C-like [Spodoptera litura] | PRSS1_2_3 |
| TRINITY_DN3826_c0_g1_i1_3 | 4708.243184 116,815.754 | 0.024810901 | 0.00083177 | 0.8534744 | Down | serine protease 5 [Mamestra configurata] | PRSS1_2_3 |
| TRINITY_DN12903_c0_g1_i1_6 | 588.272948 19,698.37276 | 0.03348509 | 7.39 E−10 | 8.85 E−07 | Down | silk gland derived serine protease [Bombyx mori] | PRSS1_2_3 |
| TRINITY_DN14269_c4_g1_i5_4 | 15,015.14212 207.7199418 | 0.013834031 | 0.00010037 | 0.019782 | Down | trypsin [Manduca sexta] | PRSS1_2_3 |
| id        | Term                             | pValue  | Enrichment score | gene_id                      | BaseMean_control_mock | BaseMean_case_DnCPV | FoldChange | pValue | qValue | Regulation | NR annotation | KEGG gene name |
|-----------|----------------------------------|---------|------------------|------------------------------|-----------------------|---------------------|------------|--------|--------|------------|----------------|----------------|
| 4501.305649 355.7861617 | 0.007904066 0.00033725 0.0475214 | Down    | trypsinogen-like protein 3 [Man- duca sexta] |
| 229.0935699 8006529495 | 0.349487308 0.03584292 0.6919927 | Down    | proton-coupled amino acid transport- |
| 1070.195299 2435172941 | 0.022754472 0.00153816 0.1284174 | Down    | carboxypeptidase B [Bom- byx mori] |
| 3577.8804260 | 0 | 0.00308839 0.197654 | Down    | trypsin CFT-1-like [Trichoplu- sia ni] |
| 2166.100895 6776280065 | 0.031283308 7.81 E−11 1.52 E−07 | Down    | trypsin precursor A2D2, partial [Agrotis ipsilon] |
| 166.8439433 24.32627007 | 0.145802536 0.01850363 0.5173478 | Down    | hypothetical protein B5V51_4161 | |
| 14,636.6041 1623956772 | 0.110951745 6.30 E−06 0.0020452 | Down    | trypsin, alkaline C-like [Spodoptera litura] |
| 159.1367963 7.893169411 | 0.049599901 235 E−05 0.0062453 | Down    | proton-coupled folate transporter isoform X2 [Bombyx mori] |
| 15,115.15447 69.82405532 | 0.004619473 1.51 E−05 0.0042801 | Down    | pancreatic triacylglycerol lipase E−like [Spodoptera litura] |
| id     | Term                                  | pValue | Enrichment score | gene_id                           | BaseMean_control mock | BaseMean_case_DnCPV | FoldChange | pValue  | qValue  | Regulation | NR annotation            | KEGG gene name |
|--------|---------------------------------------|--------|-----------------|-----------------------------------|------------------------|---------------------|------------|---------|---------|------------|--------------------------|----------------|
|        | scavenger receptor class B type 1    |        |                 | TRINITY_DN9781_c0_g1_i1_3         | 75.2460337 2063729453 | 0.274264217         | 0.03100164 | 0.6531949 | Down     | scavenger             | SCARB1          |
|        | protein 12 [Bombyx mori]             |        |                 | TRINITY_DN1521_c0_g1_i2_4         | 1196.401288 2800407483 | 0.234069247         | 0.00250876 | 0.1751687 | Down     | solute-carrier family S2, riboflavin transporter, member 3-B isoform X3 [Trichoplusia ni] | SLC52A3, RFT2 |
|        | pancreatic triacylglycerol lipase    |        |                 | TRINITY_DN14080_c0_g1_i4_5        | 13,236.92182 9084409062 | 0.068629317         | 0.03090689 | 0.6516395 | Down     | pancreatic triacylglycerol lipase [Bombyx mori] | PNLIP, PL       |
|        | [Hemolymph]                          |        |                 | TRINITY_DN17108_c0_g1_i1_5        | 6399.436654 1095005085 | 0.171109606         | 0.00016917 | 0.0286312 | Down     | sensory neuron membrane protein 2 [Bombyx mori] | SCARB1          |
| ko04624 | Toll and Imd signaling pathway       | 0.00016 | 3.943369176     | TRINITY_DN14140_c0_g1_i1_6        | 48,818.06612 7401059156 | 0.015160492         | 2.79 E−06  | 0.0010722 | Down     | peptidoglycan recognition protein 2 [Manduca sexta] | PGRP            |
|        |                                      |        |                 | TRINITY_DN13195_c0_g1_i3_3        | 74.85725235 0         | 0                   | 0.002832612 | 0.6208957 | Down     | Bacteriophage T7 lysozym E−like protein 1 (BTL-LP1) [Bombyx mori] | PGRP            |
|        |                                      |        |                 | TRINITY_DN1052_c0_g1_i2_5         | 1415.480197 5368363029 | 0.379260907         | 0.04150037 | 0.7315422 | Down     | caspas E−6 [Manduca sexta] | CASP8           |
|        |                                      |        |                 | TRINITY_DN10280_c0_g1_i3_3        | 16,714.28346 3187737419 | 0.019071936         | 3.82 E−10  | 5.17 E−07 | Down     | peptidoglycan recognition protein 2 [Manduca sexta] | PGRP            |
Table 4 (continued)

| id     | Term                        | pValue | Enrichment_score | gene_id                      | BaseMean_control_mock | BaseMean_case_DnCPV | FoldChange | pValue | qValue | Regulation | NR annotation | KEGG gene name |
|--------|-----------------------------|--------|------------------|------------------------------|-----------------------|---------------------|------------|--------|--------|------------|---------------|----------------|
| ko00830 | Retinol metabolism         | 0.000409 | 3.492698413      | TRINITY_DN14190_c1_g2_i2_4  | 4018.349256 72063212  | 0.179335362 0.04158394 | 0.7316189  | Down   | UDP-gluco-syltransferase isoform X1 [Bombyx mori] | UGT            |                |
|        |                             |        |                  | TRINITY_DN12319_c0_g2_i1_4  | 4465.699274 170972127  | 0.038285634 2.79E-06 | 0.0010722  | Down   | UDP-glyco-syltransferase UGT340C2 [Bombyx mori] | UGT            |                |
|        |                             |        |                  | TRINITY_DN12896_c1_g2_i3_3  | 4251.17249 1508008769  | 0.35472773 0.02274633 | 0.5685456  | Down   | PREDICTED: RNA-directed DNA polymerase from mobile element jockey-like [Papilio machaon] | DHR54          |                |
|        |                             |        |                  | TRINITY_DN13518_c1_g1_i6_6  | 745.6825793 1192237632 | 0.159885408 0.0001628 | 0.0278557  | Down   | UDP-glucosyltransferase UGT340C1 precursor [Bombyx mori] | UGT            |                |
|        |                             |        |                  | TRINITY_DN14445_c0_g1_i3_3  | 151.0781746 54.27468401 | 0.359249006 0.04671659 | 0.7685163  | Down   | hypothetical protein 85X24_HaOG201493 [Helicoverpa armigera] | RDH12          |                |
|        |                             |        |                  | TRINITY_DN9738_c0_g1_i1_6   | 438.41149 83.17535239  | 0.189719828 0.04256225 | 0.7412925  | Down   | uncharacterized protein LOC1 12052352 [Bicyclus anynana] | UGT            |                |
|        |                             |        |                  | TRINITY_DN8673_c0_g1_i3_3   | 839.7824168 1672848772  | 0.199200262 0.00073535 | 0.0803495  | Down   | PREDICTED: UDP-glucuronosyltransferase 2B19-like isoform X6 [Amyelois transitella] | UGT            |                |
| id          | Term                  | pValue | Enrichment score | gene_id     | BaseMean_control_mock | BaseMean_case_DnCPV | FoldChange | pValue   | qValue   | Regulation | NR annotation                      | KEGG gene name |
|------------|-----------------------|--------|------------------|-------------|------------------------|---------------------|------------|----------|----------|------------|-------------------------------------|----------------|
| TRINITY_DN17220_c0_g1_i1_4 | UDP-glycosyltransferase UGT340C1 precursor [Bombyx mori] | 3639.593263 39259259199 | 0.000615911 0.00570705 0.2744919 | Down | | | | | | | | |
| id     | Term                          | pValue | Enrichment_score | Gene_id                  | BaseMean_control_mock | BaseMean_case_DnCPV | FoldChange | pValue | qValue | Regulation | NR annotation | KEGG gene name          |
|--------|-------------------------------|--------|------------------|--------------------------|-----------------------|----------------------|------------|--------|--------|------------|---------------|-------------------------|
| ko00564 | Glycerophospholipid metabolism | 0.00046 | 3.794540796      | TRINITY_DN14020_c0_ gl1_l1_0 | 1066.209777           | 311.867512          | 3.1.06206287 | 0.027085 | 0.610813421 | Up          | phosphatidate phosphatase LPIN2 isoform X2 [Trichoplusia ni] | LPIN                     |
|        |                               |        |                  | TRINITY_DN14343_c0_ gl2_l1_4 | 25.87214477           | 100.8782352         | 3.899106012    | 0.02501  | 0.591372647 | Up          | hypothetical protein B5V51_748 [Heliothis virescens] | NTE, NRE                 |
|        |                               |        |                  | TRINITY_DN14343_c2_ gl1_l1_5 | 1212.926019           | 3816.360113         | 3.146407986    | 0.018214 | 0.514696311 | Up          | phosphatidate phosphatase LPIN3 isoform X1 [Bombyx mori] | LYPLA3                   |
|        |                               |        |                  | TRINITY_DN2180_c0_ gl1_l1_3 | 5.852454693           | 49.9148246          | 8.528298822    | 0.005837 | 0.276535057 | Up          | group XV phospholipase A2-like [Trichoplusia ni] | Lypla3                   |
|        |                               |        |                  | TRINITY_DN10230_c0_ gl1_l1_1 | 73.45139125           | 221.6966763         | 3.018277429    | 0.037654 | 0.337015221 | Up          | phosphatidylyserine decarboxylase [Opeophthera brumata] | PISD, PISD               |
|        |                               |        |                  | TRINITY_DN11518_c6_ gl1_l1_2 | 5.298336083           | 709.749683          | 13.39570899    | 0.008188 | 0.337015221 | Up          | neuropathy target esterase sws [Papilio xuthus] | NTE, NRE                 |
| ko00260 | Glycine, serine and threonine metabolism | 0.00232 | 4.238058552      | TRINITY_DN9933_c0_ gl1_l2_6 | 782.5009178         | 4185.641092      | 5.349055824    | 0.025238 | 0.593696583 | Up          | phosphoserine phosphatase isoform X3 [Trichoplusia ni] | betA, CHDH               |
Table 5 (continued)

| id  | Term                      | pValue | Enrichment_ score | Gene_id                      | BaseMean_ control | BaseMean_ case_DnCPV | FoldChange | pValue | qValue | Regulation | NR annotation | KEGG gene name          |
|-----|---------------------------|--------|-------------------|------------------------------|-------------------|----------------------|------------|--------|--------|------------|-----------------|--------------------------|
| ko00982 | Drug metabolism—cytochrome P450 | 0.0002 | 4.29382248 | TRINITY_DN12220_c1_g1_i9_4 | 107649078         | 517.8794976         | 4.8108122  | 0.00313 | 0.19793455 | Up               | PREDICTED: phosphoserine phosphatase [Amyelois transitella] | serB, PSPH               |
|     |                           |        |                   | TRINITY_DN10934_c0_g2_i2_1 | 2279078944        | 10414.52625        | 4.569620669 | 0.002617 | 0.17866588 | Up               | phosphoserine phosphatase isoform X1 [Bombyx mori] | serB, PSPH               |
|     |                           | 0.0005 |                   | TRINITY_DN11538_c1_g1_i3_2 | 2286892992        | 2097.050761        | 9.169868326  | 0.023489 | 0.577337157 | Up               | hypothetical protein BSV51_11710 [Heliothis virescens] | UGT                      |
|     |                           |        |                   | TRINITY_DN14215_c0_g5_i7_5 | 1270025656        | 13180.97268        | 103.7850898   | 0.020886 | 0.5511394 | Up               | UDP-glucuronyltransferase 1-7C-like [Trichoplusia ni] | UGT                      |
|     |                           |        |                   | TRINITY_DN7938_c0_g2_i1_2 | 393470221          | 637.5929402        | 162.0435058   | 6.29 E−05 | 0.013882511 | Up               | PREDICTED: uncharacterized protein LOC106102769 [Papilio polytes] | GST, gst                  |
|     |                           |        |                   | TRINITY_DN13727_c0_g2_i1_5 | 1859951388         | 4205.660038        | 22.61166644   | 0.002653 | 0.18004196 | Up               | UDP-glycosyltransferase UGT340C2 [Bombyx mori] | UGT                      |
|     |                           |        |                   | TRINITY_DN13616_c0_g3_i6_5 | 2595433532         | 8843.383538        | 340.7285692   | 0.014481 | 0.44908968 | Up               | UDP-glucuronosyltransferase 1-7C-like [Trichoplusia ni] | UGT                      |
|     |                           |        |                   | TRINITY_DN11622_c2_g4_i1_2 | 0                  | 23.44067936 Inf    | 0.026206     | 0.601380717 | Up               | UDP-glucuronosyltransferase 2B15-like isoform X1 [Helicoverpa armigera] | UGT                      |
| id       | Term                              | pValue | Enrichment_score | Gene_id                       | BaseMean_control | BaseMean_case_DnCPV | FoldChange | pValue  | qValue         | Regulation | NR annotation | KEGG gene name |
|----------|-----------------------------------|--------|------------------|-------------------------------|------------------|---------------------|------------|---------|----------------|------------|----------------|---------------|
| ko00980  | Metabolism of xenobiotics by cytochrome P450 | 0.00043 | 3.839182453      | TRINITY_DN11402_c0_g2_i13_2 | 663.9094166      | 6959.047076        | 0.000074  | 0.080554531 | Up            | UDP-glucuronosyltransferase 1-7C-like [Trichoplusia ni] | UGT            |
|          |                                   |        |                  | TRINITY_DN11538_c1_g1_i3_2   | 2.286892992      | 2097.050761        | 0.023489  | 0.577337157 | Up            | hypothetical protein B5V51_11710 [Heliothis virescens] | UGT            |
|          |                                   |        |                  | TRINITY_DN14215_c0_g6_i7_5   | 1.270025656      | 13,180.97268       | 0.020886  | 0.5511394 | Up            | UDP-glucuronosyltransferase 1-7C-like [Trichoplusia ni] | UGT            |
|          |                                   |        |                  | TRINITY_DN7938_c0_g2_i1_2    | 3.93470221       | 637.5929402        | 6.29E−05  | 0.013882511 | Up            | PREDICTED: uncharacterized protein LOC106102769 [Papilio polytes] | GST, gst       |
|          |                                   |        |                  | TRINITY_DN13727_c0_g2_i1_5   | 185.9951388      | 4205.660038        | 0.002663  | 0.180041496 | Up            | UDP-glycosyltransferase UGT340C2 [Bombyx mori] | UGT            |
|          |                                   |        |                  | TRINITY_DN13616_c0_g3_i6_5   | 25.95433532      | 8843.383538        | 0.014481  | 0.449089688 | Up            | UDP-glucuronosyltransferase 1-7C-like [Trichoplusia ni] | UGT            |
|          |                                   |        |                  | TRINITY_DN11622_c2_g4_i1_2   | 0                | 23.44067936 Inf   | 0.026206  | 0.601380717 | Up            | UDP-glucuronosyltransferase 2B15-like isoform X1 [Helicoverpa armigera] | UGT            |
|          |                                   |        |                  | TRINITY_DN11402_c0_g2_i13_2  | 663.9094166      | 6959.047076        | 0.000074  | 0.080554531 | Up            | UDP-glucuronosyltransferase 1-7C-like [Trichoplusia ni] | UGT            |
| id         | Term                                      | pValue | Enrichment_score | Gene_id                                      | BaseMean_control_mock | BaseMean_case_DnCPV | FoldChange | pValue  | qValue       | Regulation | NR annotation | KEGG gene name |
|------------|-------------------------------------------|--------|------------------|----------------------------------------------|------------------------|---------------------|------------|---------|--------------|------------|----------------|----------------|
| ko00983    | Drug metabolism—other enzymes            | 0.00101| 3.107909605      | TRINITY_DN11538_c1_g1_i3_2                  | 2.286892992            | 2097.050761         | 9.169868326 | 0.023489| 0.577337157 | Up         | hypotheti-cal protein BSV51_11710 [Heliothis virescens] | UGT             |
|            |                                           |        |                  | TRINITY_DN14215_c0_g5_i7_5                 | 1.270025656            | 131,800.97268       | 103.7850898 | 0.020886| 0.3511394   | Up         | UDP-glucuronosyltransferase-1-7C-like [Trichoplusia ni] | UGT             |
|            |                                           |        |                  | TRINITY_DN7938_c0_g2_i1_2                  | 3.93470221             | 637.5929402         | 162.0435058 | 6.29 E−05| 0.013882511 | Up         | PREDICTED: uncharacterized protein LOC106102769 [Papilio polytes] | GST, gst        |
|            |                                           |        |                  | TRINITY_DN13727_c0_g1_i1_5                 | 1.859951388            | 4205.660038         | 22.61166644 | 0.002663| 0.18004196 | Up         | UDP-glycosyltransferase UGT340C2 [Bombyx mori] | UGT             |
|            |                                           |        |                  | TRINITY_DN11728_c0_g1_i4_2                 | 1.306667581            | 6467.7549766        | 4949812684  | 0.001549| 0.128973078 | Up         | uridine phosphorylase 1 isoform X2 [Bombyx mori] | udp, UPP        |
|            |                                           |        |                  | TRINITY_DN13616_c0_g3_i6_5                 | 25.95433532            | 8843.383538         | 340.7285692 | 0.014481| 0.449089688 | Up         | UDP-glucuronosyltransferase-1-7C-like [Trichoplusia ni] | UGT             |
|            |                                           |        |                  | TRINITY_DN11622_c2_g4_i1_2                 | 0                     | 23.44067936         | Inf         | 0.026206| 0.601380717 | Up         | UDP-glucuronosyltransferase 2B15-like isoform X1 [Helicoverpa armigera] | UGT             |
|            |                                           |        |                  | TRINITY_DN11402_c0_g2_i13_2                | 663.9094166            | 6959.047076         | 10.48192254 | 0.000742| 0.080554531 | Up         | UDP-glucuronosyltransferase-1-7C-like [Trichoplusia ni] | UGT             |
However, the interactions between UGT and cypovirus still remain unclear. In Table 5, there were the pathways and genes mentioned above and genes expression of each sample is shown in Additional file 1.

Conclusion
This study revealed substantial differences in the transcriptions of the *D. nerii* genes related to digestion, immunity, glycerophospholipid metabolism and toxic substances metabolism induced by DnCPV-23 replication. Findings obtained in this research further enriched the understanding of cypovirus-*Spodoptera* insect interactions in midgut and provided additional basic information for the future exploitation of DnCPV-23.

### Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s12985-021-01721-x.

Additional file 1. All the different expression genes in the midgut after DnCPV-23 infection.

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### Authors' contributions
KW, YC, designed and performed the experiments andanalysed the data. ZZ, GL and CJ collected *Daphnis nerii* larval. WJ and LJ provided suggestions. JW, YC and JL wrote the manuscript. All Authors have read and approved the final version of the manuscript.

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### Availability of data and materials
The original data of the transcriptome will be released on 2021-10-05 or upon deposition in a public database. The online version contains supplementary material available at https://doi.org/10.1186/s12985-021-01721-x.

### Declarations

#### Ethics approval and consent to participate
Not applicable.

#### Consent to publication
Not applicable.

#### Competing interests
The authors declare that they have no competing interests.

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