



## RESEARCH ARTICLE

# Genome analysis of *Heliothis virescens* ascovirus 3h isolated from China

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No ascovirus isolated from China has been sequenced so far. Therefore, in this study, we aimed to sequence the genome of *Heliothis virescens* ascovirus 3h (HvAV-3h) using the 454 pyrosequencing technology. The genome was found to be 190,519-bp long with a G+C content of 45.5%. We also found that it encodes 185 hypothetical open reading frames (ORFs) along with at least 50 amino acids, including 181 ORFs found in other ascoviruses and 4 unique ORFs. Gene-parity plots and phylogenetic analysis revealed a close relationship between HvAV-3h and three other HvAV-3a strains and a distant relationship with *Spodoptera frugiperda* ascovirus 1a (SfAV-1a), *Trichoplusia ni* ascovirus 6a (TnAV-6a), and *Diadromus pulchellus* ascovirus 4a (DpAV-4a). Among the 185 potential genes encoded by the genome, 44 core genes were found in all the sequenced ascoviruses. In addition, 25 genes were found to be conserved in all ascoviruses except DpAV-4a. In the HvAV-3h genome, 24 baculovirus repeat ORFs (*bro*s) were present, and the typical homologous repeat regions (*hrs*) were absent. This study supplies information important for understanding the conservation and functions of ascovirus genes as well as the variety of ascoviral genomes.

**KEYWORDS** *Heliothis virescens* ascovirus 3h (HvAV-3h); genome organization; *bro* genes; phylogenetic relationship

## INTRODUCTION

*Ascoviridae* is an insect-specific family of viruses with double-stranded circular DNA genomes of 110–200 kb (Bigot et al., 2011; Wei et al., 2014). Ascoviruses can cause a chronic but ultimately fatal disease in the larvae of members of the *Noctuidae*, *Crambidae*, and *Plutellidae* families (Bigot et al., 2011). Based on virion morphology, DNA sequence information, host range, and tissue

tropism, two genera, *Ascovirus* and *Toursvirus*, have been recognized by the International Committee on Taxonomy of Viruses (ICTV) (Asgari et al., 2017). The genus *Ascovirus* contains three species—*Spodoptera frugiperda* ascovirus 1a (SfAV-1a), *Trichoplusia ni* ascovirus 2a (TnAV-2a), and *Heliothis virescens* ascovirus 3a (HvAV-3a) (Bigot et al., 2011)—while the newly assigned genus *Toursvirus* consists of only one species, *Diadromus pulchellus* ascovirus 4a (DpAV-4a), that was recently removed from the genus *Ascovirus* (Asgari et al., 2017).

A total of 18 ascoviral isolates or strains have been reported globally so far (Bigot et al., 2011; Huang et al., 2012a). Among these, the genomes of only 6 isolates have been sequenced: SfAV-1a (Bidishi et al., 2006), TnAV-6a (previously named as TnAV-2c, Wei et al.,

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2014) (Wang et al., 2006), HvAV-3e (Asgari et al., 2007), HvAV-3f (Wei et al., 2014), HvAV-3g (Huang et al., 2012b), and DpAV-4a (Bigot et al., 2009). Among the sequenced isolates, HvAV-3g contains the largest genome (199, 721 bp), while DpAV-4a has the smallest one (119, 343 bp) (Bigot et al., 2009; Huang et al., 2012b).

Based on the analysis of biological characteristics, the species HvAV-3a, which consists of 8 isolates, has been found to be the most diverse and widely distributed species from America to Asia and Australia (Hamm et al., 1998; Huang et al., 2012a). Among HvAV-3a species, the genome sequences of 3 isolates—HvAV-3e, HvAV-3g, and HvAV-3f—have been reported from Australia, Indonesia, and USA, respectively (Asgari et al., 2007; Huang et al., 2012b; Wei et al., 2014). However, no ascovirus isolated from China has been sequenced so far. To understand the diversity and phylogeny of HvAV-3a isolates, we aimed to sequence the complete genome of a HvAV-3h, which was isolated from China and transmitted by *Microplitis similis* (Li et al., 2016). We then went on to compare the sequence with the other 6 previously published ascovirus genome sequences. The results of our study will be helpful for providing insights into the origin and evolution of ascoviruses.

## MATERIALS AND METHODS

### Viral DNA extraction

For this study, HvAV-3h was propagated in *Spodoptera exigua* larvae as described previously (Huang et al., 2012a). The virions were then purified and viral DNA was extracted as previously described (Federici et al., 1990).

### Sequencing and bioinformatics analysis

The genome was sequenced with the Roche 454 GS FLX system by using shotgun strategy. The reads were assembled with Roche GS De Novo assembler software. A few regions that were not assembled into contigs were further amplified using PCR and sequenced.

Hypothetical ORFs were predicted using ORF finder (NCBI) with characteristics of containing a standard ATG start codon and a stop codon and potentially encoding at least 50 amino acids (aa). Gene annotation and comparisons were performed using the NCBI protein-protein BLAST algorithm (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Homologies among ascovirus genomes were investigated using Basic Local Alignment Search Tool (BLAST) (blastp) in NCBI (Altschul et al., 1997) and two sequences were aligned using blastp. MIROPEATS program (Parsons, 1995) was used to find repetitive regions. Restriction sites were predicted and the genome

map framework was drawn using the program Geneious version 8.04. The annotated genome sequence data were uploaded to GenBank under the accession number KU170628.

### Phylogenetic analysis

Phylogenetic analysis of the ascoviruses was performed using the amino acid sequences of DNA polymerase and Major capsid protein (MCP) from HvAV-3h and the 6 other sequenced *Ascovirus* and *Toursvirus* isolates, with homologs from *invertebrate iridescent virus 22* and *Wiseana iridescent virus* as the outgroups (Piégu et al., 2015). The sequences were then aligned using ClustalW (Larkin et al., 2007) with default parameters of MEGA 6.0 (Tamura et al., 2013). The maximum likelihood (ML) method was used with the program raxmlGUL 1.5 (<https://sourceforge.net/projects/raxmlgui/>) (Silvestro and Michalak, 2012), in which the analysis setting was chosen “ML+rapid bootstrap” and the “number of bootstrap replicates” was 1,000. The program MrBayes 3.1.2 (<http://morphbank.Ebc.uu.SE/mrbayes/>) was used to perform Bayesian inference (BI) analysis (Ronquist et al., 2003), with the MCMC analysis run for 300,000 generations and a burn-in series of 1,000.

## RESULTS

### General organization of the HvAV-3h genome

The HvAV-3h genome was sequenced using the Roche 454 GS FLX system with the shotgun strategy. A total of 43, 218 reads were obtained with the average length of 423 bp, and the genome was covered about 53 times. The HvAV-3h genome was assembled using Roche GS De Novo assembler software and Geneious version 8.04. A few regions that were not assembled into contigs were further amplified using PCR with the primers listed in [Supplementary Table S1](#), cloned, and sequenced. The final assembled contig representing the entire HvAV-3h genome sequence was confirmed using restriction digestion with 6 enzymes (*Bam*H I, *Hind* III, *Nde* I, *Pst* I, *Sac* I, and *Xho* I). The restriction profile ([Supplementary Figure S1](#)) matched well with the predicted fragments of the assembled genome ([Supplementary Table S2](#)). However, there were still some submolar bands ([Supplementary Figure S1](#)), indicating the presence of different genotypes in the isolate.

The HvAV-3h genome was assembled into a circular contiguous sequence of 190, 519 bp, which was significantly larger than the genome size (165 kbp) estimated previously (Huang et al., 2012a). This was possibly largely due to the missing small size fragments in the previously reported restriction enzyme digestion profiles (Huang et al., 2012a). A genomic map showing the or-

ganization of the ORFs with the predicted protein of over 50 aa in the HvAV-3h genome is presented in Figure 1. The genome has a G+C content of 45.5% and encodes 185 predicted ORFs, in which 104 are in the forward orientation and 81 are in the reverse orientation (Figure 1). The coding region accounts for 88.8% of the total sequences.

### Relationship with other ascoviruses

The size of the HvAV-3h genome was found to be smaller

than that of the HvAV-3g (199, 721 bp) and HvAV-3f genomes (198, 157 bp), but larger than that of the HvAV-3e genome (186, 262 bp). It is thus the third largest ascovirus genome described so far. Gene-parity plots of HvAV-3h against the 6 ascovirus isolates revealed high co-linearity of the gene order between HvAV-3h and HvAV-3g, HvAV-3f, or HvAV-3e, with only a few inversions and drifts, which may partially account for the difference in the genome size. Much lower co-linearity was found between HvAV-3h and SfAV-1a or TnAV-6a. No obvious

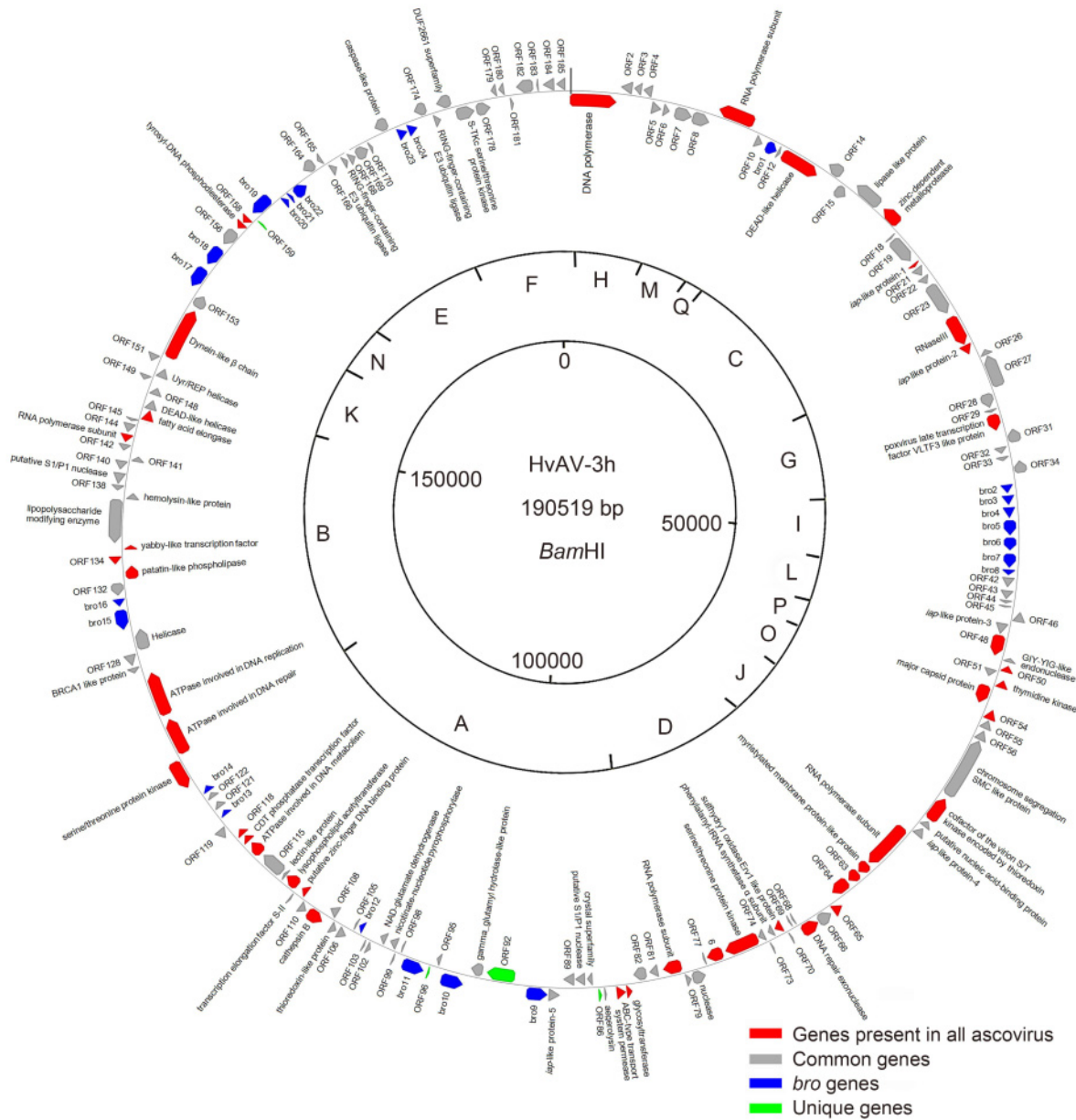


Figure 1. Circular map and gene organization of the HvAV-3h genome. The sites for the restriction enzyme *Bam*H I are presented; the fragments are indicated as A to Q according to the size from the largest to the smallest restriction fragment in Supplementary Figure S1. (Red color indicates core genes present in all ascovirus isolates, grey color indicates common genes, blue color indicates *bro* genes, and green color indicates unique genes).

collinear region could be found between HvAV-3h and DpAV-4a (Figure 2). The collinear regions between HvAV-3h and other ascovirus isolates, with the exception of DpAV-4a, indicated the presence of conserved regions among ascoviral genomes and their derivation from an ancestor ascovirus.

Phylogenetic analyses of the conserved genes of DNA polymerase and MCP from HvAV-3h and the 6 other Ascovirus and *Toursvirus* isolates also revealed a close relationship between HvAV-3h, HvAV-3g, HvAV-3f, and HvAV-3e (Figure 3). In fact, analysis using ML and Bayesian methods with a bootstrap value of 100% and a posterior probability of 1.00, respectively, revealed that these four HvAV-3a isolates form a monophyletic clade at high levels. The HvAV-3a isolates also appeared to have a distant relationship with SfAV-1a and TnAV-6a and an even more distant relationship with DpAV-4a (Figure 3).

### Comparison and classification of gene contents of the HvAV-3h genome with that of other ascoviruses

BLAST analysis revealed that, among the identified ORFs, 181 ORFs were related to genes reported from the other 6 ascovirus isolates, including HvAV-3g (174 ORFs, with average amino acid identity of 94.0%), HvAV-3f (172 ORFs, 92.9%), HvAV-3e (162 ORFs, 87.5%), SfAV-1a (116 ORFs, 62.7%), TnAV-6a (105 ORFs, 56.7%), and DpAV-4a (63 ORFs, 34.0%) (Supplementary Table S3).

Forty-four ascovirus ORFs were found to be conserved among HvAV-3h and the other 6 ascovirus and *toursvirus* genomes. Gene annotation indicated that these

genes are involved in DNA/RNA replication/transcription/metabolism, viral packaging and assembly, sugar and lipid metabolisms, etc. (Table 1). All the ascovirus isolates except DpAV-4a shared 25 ORFs with HvAV-3h (Table 2). Some of them appear to play roles in DNA/RNA replication/transcription/metabolism, but the functions of most of the shared genes are still unknown (Table 2). A total of 159 genes including the above-mentioned genes were found to be shared between HvAV-3h and the three closely related isolates of the species HvAV-3a. In addition, 19 genes were found to be shared between HvAV-3h and any one but not all three previously reported isolates of the species HvAV-3a. Six ORFs including ORF49 (GIY-YIG-like endonuclease), ORF51, ORF86, ORF92, ORF96, and ORF159 were not found in any of the other three isolates. ORF49 showed significant similarity to GIY-YIG-like endonuclease with E-value of  $6e-46$ . GIY-YIG-like endonuclease was found in SfAV-1a, TnAV-6a, and DpAV-4 as well as in viruses of the *Baculoviridae* and *Iridoviridae* families. Homologues of GIY-YIG-like endonuclease are known to be involved in viral DNA repair (Lindahl, 1982) and are required for efficient baculoviral virion production (Tang et al., 2013; Wu & Passarelli, 2012). ORF51 with an unknown function only showed significant similarity to ORF39 of SfAV-1a with E-value of  $1e-44$ . The other four ORFs, including ORF86, ORF92, ORF96, and ORF159, showed no significant levels of similarity to genes in the GenBank database; hence, they were considered unique genes of HvAV-3h.

### Bro genes

The baculovirus repeat ORF (*bro*) gene occurs as mul-

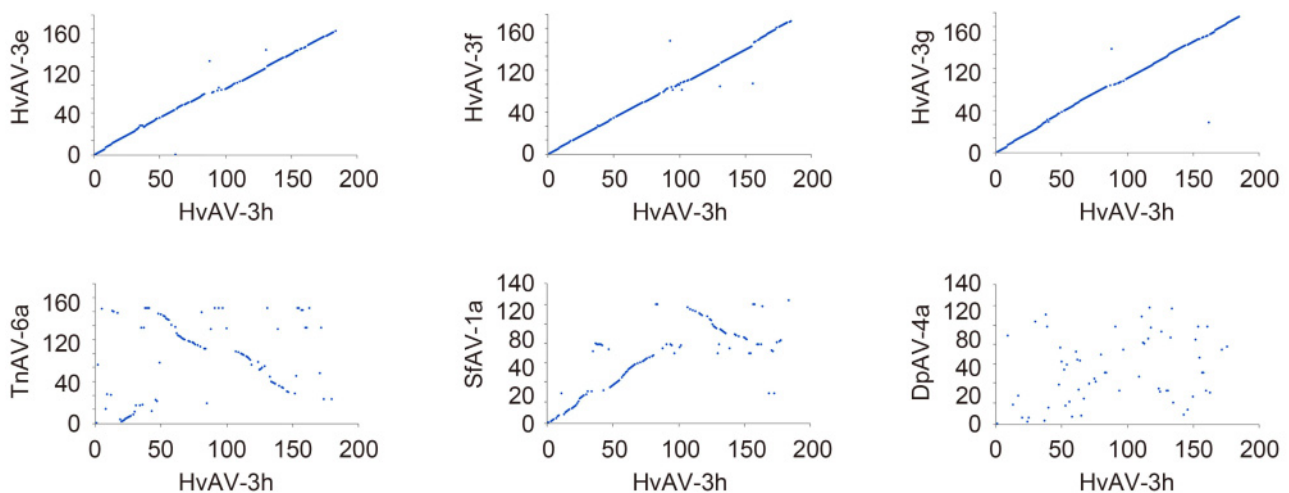


Figure 2. Gene Parity Plot analyses of HvAV-3h with the 6 other sequenced *Ascovirus* and *Toursvirus* isolates. Homologous genes were plotted based on their relative locations in the genomes. The x-axis represents ORFs from HvAV-3h and y-axis represents ORFs from each isolate indicated in the title of each panel.

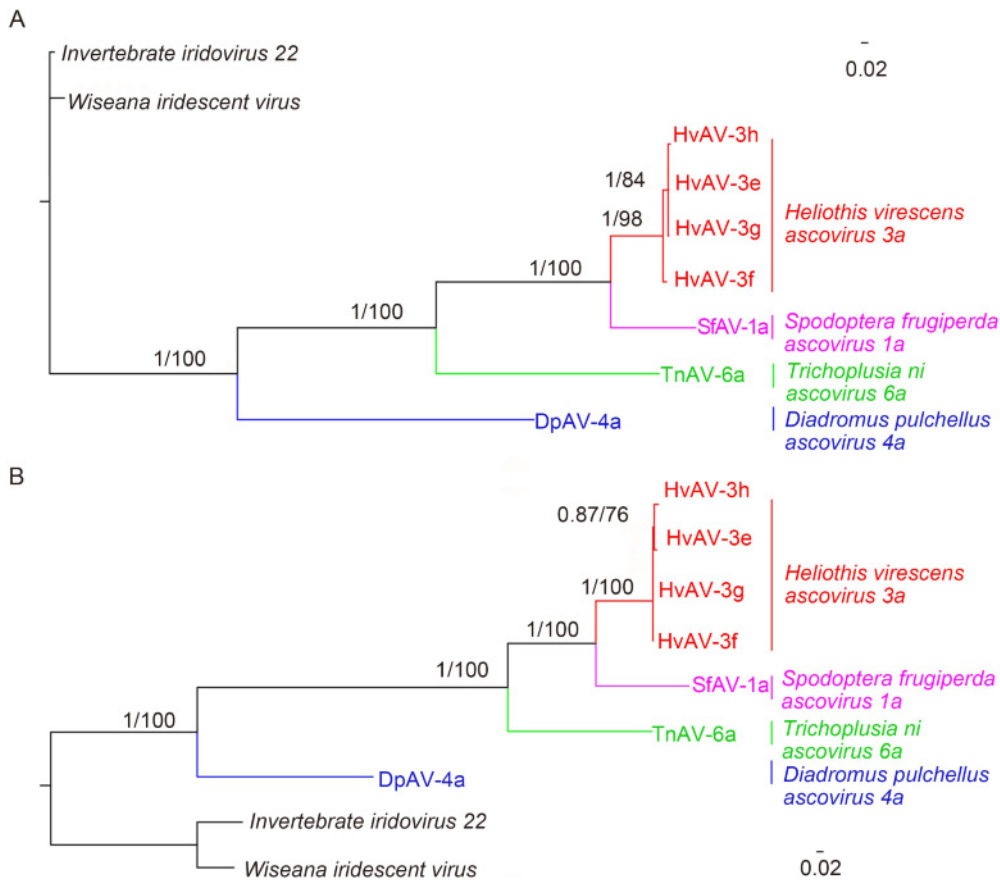


Figure 3. Phylogenetic tree based on amino acid sequences of MCP (A) and DNA polymerase (B) from the genomes of HvAV-3h and the 6 other ascovirus isolates, which were determined using Bayesian and Maximum likelihood (ML) analysis. The scale bar indicates the number of substitutions per spot. Numbers represent the node supports; the number on top indicate posterior probabilities for the Bayesian Inference and those below are based on 1000 bootstrap replicates for ML. The colors represent the haplogroups specific to each species.

Table 1. Classification of the 44 genes found in all Ascoviridae isolates

Classification	Genes
DNA/RNA/replication/transcription/metabolism (16)	DNA polymerase (orf1), RNA polymerase subunit (orf9), DEAD-like helicase (orf13), RNase III (orf24), poxvirus late transcription factor VLTf3 like protein (orf30), thymidine kinase (orf52), RNA polymerase subunit (orf61), DNA repair exonuclease (orf67), RNA polymerase subunit (orf80), ATPase involved in DNA metabolism (orf116), CDT phosphatase transcription factor (orf117), ATPase involved in DNA repair (orf125), ATPase involved in DNA replication (orf126), yabby-like transcription factor (orf135), RNA polymerase subunit (orf143), tyrosyl-DNA phosphodiesterase (orf157)
Packaging and assembly (2)	major capsid protein (orf53), sulfhydryl oxidase Erv1 like protein (orf71)
Sugar and lipid metabolism (4)	Glycosyl transferase (orf83), lysophospholipid acetyl transferase (orf113), patatin-like phospholipase (orf133), fatty acid elongase (orf146)
Others (12)	zinc-dependent metalloprotease (orf17), iap-like protein-1 (orf20), iap-like protein-2 (orf25), cofactor of the virion S/T kinase encoded by thioredoxin (orf58), myristylated membrane protein-like protein (orf62), serine/threonine protein kinase (orf75), ABC-type transport system permease (orf84), cathepsinB (orf109), putative zinc-finger DNA binding protein (orf111), serine/threonine protein kinase (orf124), dynein-like $\beta$ chain (orf152), bro*
Unknown (10)	orf48, orf50, orf54, orf63, orf64, orf65, orf76, orf118, orf134, orf158

Note: \* HvAV-3h encodes 24 bro: orf11, orf35, orf36, orf37, orf38, orf39, orf40, orf41, orf91, orf94, orf97, orf104, orf120, orf123, orf130, orf131, orf154, orf155, orf160, orf161, orf162, orf163, orf172, and orf173.

Table 2. Classification of the 25 genes found in all sequenced isolates of the genus *Ascovirus*

Classification	Genes
DNA/RNA/replication/transcription/metabolism (4)	<i>nuclease (orf78)</i> , <i>putative S1/P1 nuclease (orf88)</i> , <i>helicase (orf129)</i> , <i>putative S1/P1 nuclease (orf139)</i>
Others (5)	<i>iap-like protein-3 (orf47)</i> , <i>thioredoxin-like protein (orf107)</i> , <i>BRCA1 like protein (orf127)</i> , <i>lipopolysaccharide-modifying enzyme (orf136)</i> , <i>caspase-like protein (orf171)</i>
Unknown (16)	<i>orf5</i> , <i>orf8</i> , <i>orf14</i> , <i>orf19</i> , <i>orf21</i> , <i>orf22</i> , <i>orf23</i> , <i>orf26</i> , <i>orf27</i> , <i>orf31</i> , <i>orf34</i> , <i>orf43</i> , <i>orf55</i> , <i>orf66</i> , <i>orf74</i> , <i>orf81</i>

multiple copies per ascovirus genome (Asgari et al., 2007). For example, the genomes of SfAV-1a, TnAV-6a, HvAV-3e, HvAV-3f, HvAV-3g, and DpAV-4a show 7, 3, 23, 29, 25 and 12 *bro* genes, respectively (Asgari et al., 2007; Bideshi et al., 2006; Bigot et al., 2009; Huang et al., 2012b; Wang et al., 2006; Wei et al., 2014). In our study, we found that HvAV-3h contains 24 *bro* genes that encode from 99 to 521 residues (Figure 1, Supplementary Table S3). The *bro* genes in baculovirus have been believed to help the virus acquire a new functionality eventually (de Castro Oliveira et al., 2013), but their function in ascoviruses remains to be determined.

## DISCUSSION

HvAV-3h is the fourth sequenced isolate of the species HvAV-3a. HvAV-3h had a similar gene order and shared 159 ORFs with all the other isolates of the species HvAV-3a (Figure 2, Supplementary Table S3). However, there are still some gene reversions and drifts among the genomes. For example, 19 ORFs were found in any one but not all the four isolates, and 6 ORFs were only found in HvAV-3h (Figure 2, Supplementary Table S3). These findings show that although HvAV-3a isolates are closely related to each other, there are noticeable diversities in their genome organizations.

Comprehensive Geneparity Plots and phylogeny analyses of all the sequenced *Ascoviridae* isolates indicated that HvAV-3a isolates are distantly related to SfAV-1a and TnAV-6a and most distantly to DpAV-4a (Figure 2, Figure 3). All the *Ascoviridae* isolates shared 44 conserved genes (Table 1), which likely play important roles

during the life cycle of the viruses. Among the 44 conserved ORFs, 16–DNA polymerase (*orf1*), RNA polymerase subunits (*orf9*, *orf61*, *orf80*, and *orf143*), DEAD-like helicase (*orf13*), RNase III (*orf24*), poxvirus late transcription factor VLTF3 like protein (*orf30*), thymidine kinase (*orf52*), DNA repair exonuclease (*orf67*), ATPase involved in DNA metabolism (*orf116*), CDT phosphatase transcription factor (*orf117*), ATPase involved in DNA repair (*orf125*), ATPase involved in DNA replication (*orf126*), yabby-like transcription factor (*orf135*), and tyrosyl-DNA phosphodiesterase (*orf157*)—are predicted to be involved in DNA/RNA replication/transcription and metabolism (Table 1). All the ascoviruses appear to encode many genes involved in nucleotide metabolism and transcription/replication. For example, TnAV-6a encodes 16 related ORFs (Wang et al., 2006) and SfAV-1a encodes 9 (Bideshi et al., 2006). Serine/threonine protein kinase and AV-like serine/threonine protein kinase are also conserved in ascoviruses. These kinases likely participate in virus or virus-host regulation of signaling networks (Jacob et al., 2011); however, the roles of the kinases in ascovirus infection are not clear so far. Efforts also need to be made to reveal the functions of other unknown conserved ORFs (Table 1). Among the 44 core genes, only 2 were annotated to encode the structural proteins MCP (*orf53*) and sulfhydryl oxidase Erv1-like protein (*orf71*) (Long et al., 2009; Wu and Passarelli, 2010). As ascoviruses have complicated morphologies, it is tempting to assume that more structural genes may be identified from the remaining unknown core genes.

Ascovirus genomes often contain large interspersed repeats of 1–3 kbp (Bigot et al., 2000), but no repeat regions (*hrs*) were found in the HvAV-3h genome (Figure 1). Similarly, no repeat regions have been found in the DpAV-4a genome (Bigot et al., 2009). In contrast, 5 copies of repeats have been found in the HvAV-3e genome (*orf38* <*hr1*>, *orf84* <*hr2*>, *orf124* <*hr3*>, *orf156* <*hr4*> and *orf178* <*hr5*>) (Asgari et al., 2007) and the HvAV-3g genome (*orf35*, *orf68*, *orf104*, *orf126*, and *orf173*) (Huang et al., 2012b). Two repeat regions have been found in the HvAV-3f genome (*orf95* and *orf179*) (Wei et al., 2014) and the SfAV-1a (*orf34* and *orf77*) (Bideshi et al., 2006) and TnAV-6a (*Hr1* and *Hr2*) genomes (Wang et al., 2006). Additionally, 24 *bros* were found in the HvAV-3h genome in our study, and 12, 7, 3, 23, 26 and 29 *bros* have been found previously in DpAV-4a (Bigot et al., 2009), SfAV-1a (Bideshi et al., 2006), TnAV-6a (Wang et al., 2006), HvAV-3e (Asgari et al., 2007), HvAV-3g (Huang et al., 2012b), and HvAV-3f (Wei et al., 2014), respectively. The function of repeat regions, *bros*, and *hrs* in the evolution and variety of ascovirus genomes needs to be investigated in future studies.

In summary, we sequenced the genome of a HvAV-3h isolate from China and compared the sequence to those of 6 other previously published ascovirus genome sequences to establish the evolutionary relationship between different ascovirus species. Our findings indicate a close relationship between different HvAV-3a isolates and distant relationship between HvAV-3h and some other species of the *Ascoviridae* family. Future studies need to be focused on elucidating the diversities in the genome organizations of different HvAV-3h isolates and on understanding the role of different genes in the ascoviral genomes as well as the conservation of these genes.

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## COMPLIANCE WITH ETHICS GUIDELINES

The authors declare that they have no conflict of interest. This article does not contain any studies with human or animal subjects performed by any of the authors.

## AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: HGH, DHH, MW, XWC, ZH. Performed the experiments: HGH, DHH. Analyzed the data: HGH, DHH, ZH. Contributed reagents/materials/analysis tools: HGH, DHH. Wrote the paper: HGH, DHH, ZH.

Supplementary figure/tables are available on the websites of *Virologica Sinica*: [www.virosin.org](http://www.virosin.org); [link.springer.com/journal/12250](http://link.springer.com/journal/12250).

## REFERENCES

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller

- W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res*, 25: 3389–3402.
- Asgari S, Bideshi D, Bigot Y, Federici BA, Cheng XW, ICTV Report Consortium. 2017. ICTV Virus Taxonomy Profile: *Ascoviridae*. *J Gen Virol*, 98: 4–5.
- Asgari S, Davis J, Wood D, Wilson P, McGrath A. 2007. Sequence and organization of the *Heliothis virescens* ascovirus genome. *J Gen Virol*, 88: 1120–1132.
- Bideshi DK, Demattei MV, Rouleux-Bonnin F, Stasiak K, Tan Y, Bigot S, Bigot Y, Federici BA. 2006. Genomic sequence of *Spodoptera frugiperda* ascovirus 1a, an enveloped, double-stranded DNA insect virus that manipulates apoptosis for viral reproduction. *J Virol*, 80: 11791–11805.
- Bigot Y, Asgari S, Bideshi DK, Cheng X, Federici BA, Renault S. 2011. Family *Ascoviridae*. In: *Virus Taxonomy: Ninth Report of the International Committee on the Taxonomy of Viruses*, third ed. King, AMQ., Adams, MJ, Carstens, EB, Lefkowitz, EJ (Eds.). London: Elsevier Inc. pp. 147–152.
- Bigot Y, Renault S, Nicolas J, Moundras C, Demattei MV, Samain S, Bideshi DK, Federici BA. 2009. Symbiotic virus at the evolutionary intersection of three types of large DNA viruses; iridoviruses, ascoviruses, and ichnoviruses. *PLoS ONE*, 4: e6397.
- Bigot Y, Stasiak K, Rouleux-Bonnin F, Federici BA. 2000. Characterization of repetitive DNA regions and methylated DNA in ascovirus genomes. *J Gen Virol*, 81: 3073–3082.
- de Castro Oliveira JV, de Brito AF, Braconi CT, de Melo Freire CC, Iamarino A, de Andrade Zanotto PM. 2013. Modularity and evolutionary constraints in a baculovirus gene regulatory network. *BMC Syst Biol*, 7: 87.
- Federici BA, Vlcek JM, Hamm JJ. 1990. Comparative study of virion structure, protein composition and genomic DNA of three ascovirus isolates. *J Gen Virol*, 71: 1661–1668.
- Hamm JJ, Styer EL, Federici BA. 1998. Comparison of field-collected ascovirus isolates by DNA hybridization, host range, and histopathology. *J Invertebr Pathol*, 72: 138–146.
- Huang GH, Garretson TA, Cheng XH, Holztrager MS, Li SJ, Wang X, Cheng XW. 2012a. Phylogenetic position and replication kinetics of *Heliothis virescens* ascovirus 3h (HvAV-3h) isolated from *Spodoptera exigua*. *PLoS ONE*, 7: e40225.
- Huang GH, Wang YS, Wang X, Garretson TA, Dai LY, Zhang CX, Cheng XW. 2012b. Genomic sequence of *Heliothis virescens* ascovirus 3g isolated from *Spodoptera exigua*. *J Virol*, 86: 12467–12468.
- Jacob T, Broeke CVD, Favoreel HW. 2011. Viral Serine/Threonine Protein Kinases. *J Virol*, 85: 1158–1173.
- Larkin M A, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG. 2007. Clustal W and Clustal X version 2.0. *Bioinformatics*, 23: 2947–2948.
- Li SJ, Hopkins RJ, Zhao YP, Zhang YX, Hu J, Chen XY, Xu Z, Huang GH. 2016. Imperfection works: survival, transmission and persistence in the system of *Heliothis virescens* ascovirus 3h (HvAV-3h), *Microplitis similis* and *Spodoptera exigua*. *Sci Rep*, 6: 21296.
- Lindahl T. 1982. DNA repair enzymes. *Annu Rev Biochem*, 51: 61–87.
- Long CM, Rohrmann GF, Merrill GF. 2009. The conserved baculovirus protein p33 (Ac92) is a flavin adenine dinucleotide-linked sulfhydryl oxidase. *Virology*, 388: 231–235.
- Parsons J. 1995. Miropoints: graphical DNA sequence comparisons. *Comput Appl Biosci*, 11: 615–619.
- Piégu B, Asgari S, Bideshi D, Federici BA, Bigot Y. 2015. Evolutionary relationships of iridoviruses and divergence of ascovir-

- uses from invertebrate iridoviruses in the superfamily Megavirales. *Mol Phylogenet Evol*, 84: 44–52.
- Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19: 1572–1574.
- Silvestro D, Michalak I. 2012. Raxml GUI: a graphical front-end for RAxML. *Org Divers Evol*, 12: 335–337.
- Tamura K, Stecher G, Peterson D, Filipowski A, Kumar S. 2013. MEGA6: Molecular evolutionary genetics analysis version 6. 0. *Mol Biol Evol*, 30: 2725–2729.
- Tang Q, Li G, Yao Q, Chen L, Feng F, Yuan Y, Chen K. 2013. Bm65 is essential for the propagation of *Bombyx mori* nucleopolyhedrovirus. *Curr Microbiol*, 66: 22–29.
- Wang LH, Xue JL, Seaborn CP, Arif BM, Cheng XW. 2006. Sequence and organization of the *Trichoplusiani* ascovirus 2c (*Ascoviridae*) genome. *Virology*, 354: 167–177.
- Wei YL, Hu J, Li SJ, Chen ZS, Cheng XW, Huang GH. 2014. Genome sequence and organization analysis of *Heliothis virescens* ascovirus 3f isolated from a *Helicoverpa zea* larva. *J Invertebr Pathol*, 122: 40–43.
- Wu W, Passarelli AL. 2012. The *Autographa californica* M nucleopolyhedrovirus ac79 gene encodes an early gene product with structural similarities to UvrC and intron-encoded endonucleases that is required for efficient budded virus production. *J Virol*, 86: 5614–5625.
- Wu W, Passarelli AL. 2010. *Autographa californica* multiple nucleopolyhedrovirus Ac92 (ORF92, P33) is required for budded virus production and multiply enveloped occlusion-derived virus formation. *J Virol*, 84: 12351–12361.