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Complete genome sequence and architecture of crucian carp *Carassius auratus* herpesvirus (CaHV)

Xiao-Tao Zeng[1] · Zhong-Yuan Chen[1] · Yuan-Sheng Deng[1] · Jian-Fang Gui[1] · Qi-Ya Zhang[1]

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Abstract Crucian carp *Carassius auratus* herpesvirus (CaHV) was isolated from diseased crucian carp with acute gill hemorrhages and high mortality. The CaHV genome was sequenced and analyzed. The data showed that it consists of 275,348 bp and contains 150 predicted ORFs. The architecture of the CaHV genome differs from those of four cyprinid herpesviruses (CyHV1, CyHV2, SY-C1, CyHV3), with insertions, deletions and the absence of a terminal direct repeat. Phylogenetic analysis of the DNA polymerase sequences of 17 strains of *Herpesvirales* members, and the concatenated 12 core ORFs from 10 strains of alloherpesviruses showed that CaHV clustered together with members of the genus *Cyprinivirus*, family *Alloherpesviridae*.

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✉ Qi-Ya Zhang
zhangqy@ihb.ac.cn

Xiao-Tao Zeng
zengxt@ihb.ac.cn

Zhong-Yuan Chen
chenzy@ihb.ac.cn

Yuan-Sheng Deng
dengyuansheng@ihb.ac.cn

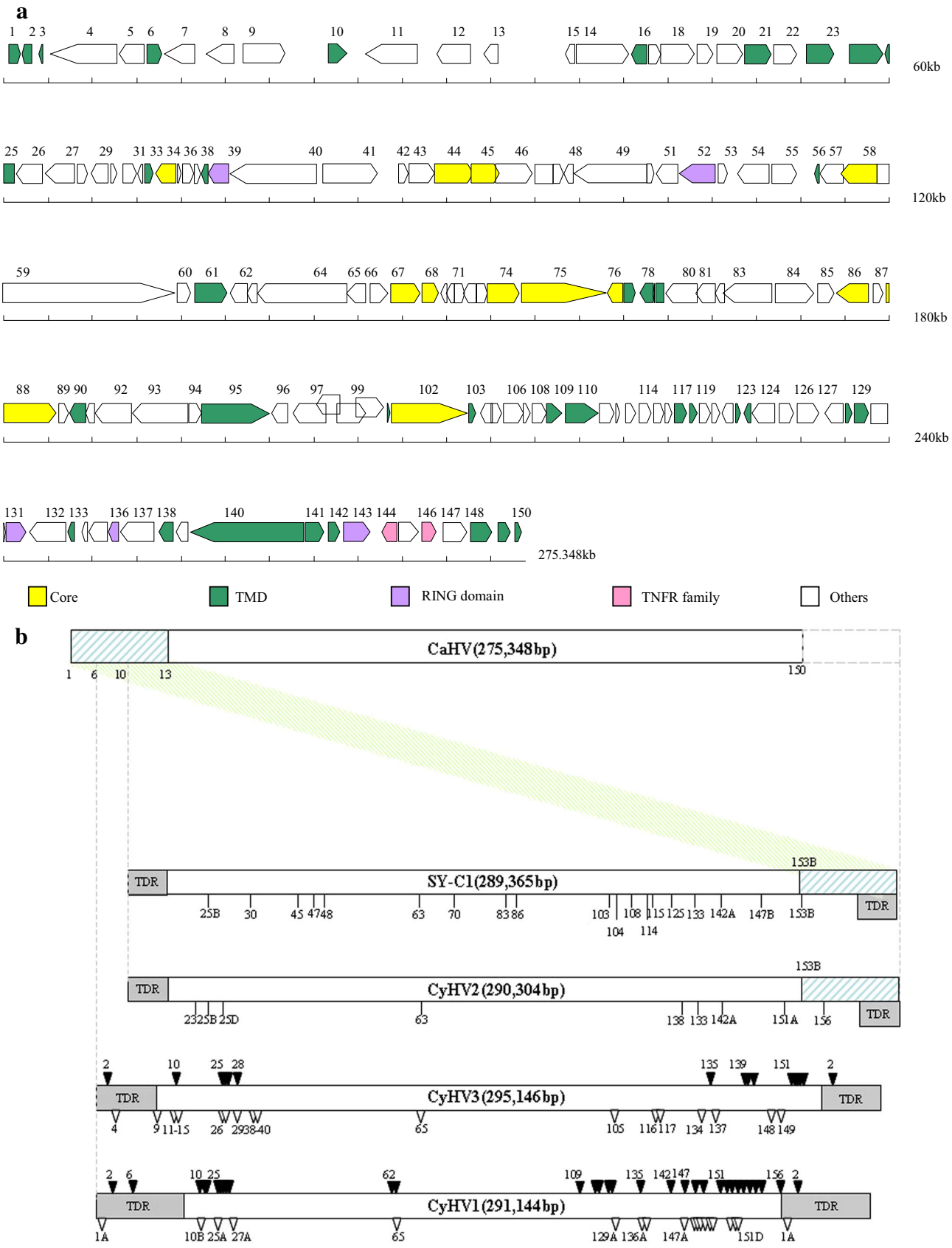
Jian-Fang Gui
jfgui@ihb.ac.cn

[1] State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, University of Chinese Academy of Sciences, Wuhan 430072, China

Emerging and re-emerging viral diseases have brought serious harms to aquaculture and the wild lower vertebrate population worldwide. The viruses causing these diseases, including iridoviruses, reoviruses, rhabdoviruses, nodaviruses and herpesviruses [1–5], are considered emerging threats to global aquaculture. For example, iridoviruses have been reported in fish, amphibians and reptiles [6–8].

Fish herpesviruses have been responsible for severe losses of cyprinid fishes in recent years [2, 9, 10]. The complete genomes of seven fish herpesviruses in the family *Alloherpesviridae* have been reported, five of which belong to cyprinid herpesviruses, including cyprinid herpesvirus 1 (CyHV1, 291,144 bp), cyprinid herpesvirus 2 (CyHV2, 290,304 bp) [11], a variant CyHV2 (SY-C1, 289,365 bp) [12], cyprinid herpesvirus 3 (CyHV3 295,146 bp) [5], and a Chinese CyHV3 isolate (KHV-GZ11, 295,119 bp) [13]. The complete genomes of five cyprinid herpesviruses consist of a unique sequence flanked by terminal direct repeats (TDRs). Twelve genes (termed core genes) are significantly conserved in all sequenced alloherpesviruses. Cyprinid herpesviruses have been reported to cause serious mortality in common carp and crucian carp (*Carassius auratus*). CyHV1 is the cause of carp pox, and CyHV2 causes herpesviral haematopioetic necrosis (HVHN) disease in common carp, goldfish, crucian carp and gibel carp [2]. CyHV3, the archetype of fish alloherpesviruses, infects common and koi carp [14].

Recently, a crucian carp herpesvirus (CaHV) was isolated from the tissues of diseased crucian carp with acute gill hemorrhages and high mortality. Crucian carps were infected experimentally by injection with tissue filtrate (CaHV viral suspension) from naturally infected fish, which caused similar symptoms to those observed in naturally infected fish, and high mortality. A PCR assay showed that CaHV was detected in various tissues of


a
60kb
120kb
180kb
240kb
275.348kb
Core
TMD
RING domain
TNFR family
Others
b
CaHV(275,348bp)
SY-C1(289,365bp)
CyHV2(290,304bp)
CyHV3(295,146bp)
CyHV1(291,144bp)
TDR

◀**Fig. 1** Diagram of the CaHV genome organization and comparisons of the genomes of the CaHV and CyHVs. **a** The CaHV genome is 275,348 bp in size and contains 150 potential open reading frames (ORFs). Arrows indicate the size, location and orientation of the ORFs. The CaHV genome contains 12 core ORFs that are significantly conserved among alloherpesviruses (core), 38 ORFs with one or more transmembrane domains (TMDs), five ORFs with a RING-finger domain (RING domain), two ORFs belonging to the TNFR family (TNFR family) and 93 other unclassed ORFs (others). **b** Genome comparisons between CaHV and CyHVs. Long frames indicate the sizes of genomes. Dashed frames in the CaHV genome indicates missing nucleotides. The light green long oblique line between CaHV and SY-C1 (KM200722) indicates that the CaHV 5'-terminal ORFs 1-13 (light blue hatched frame) correspond to the 3'-terminus of CyHVs. Gray frames indicate terminal direct repeats (TDRs). The short vertical lines in SY-C1 and CyHV2 (NC_019495) indicate ORFs with less than 95% identity to their homologues in CaHV. The black triangles in CyHV3 (NC_009127) and CyHV1 (NC_019491) indicate insertions relative to CaHV. The white triangles in CyHV3 and CyHV1 indicate deletions relative to CaHV

infected fish. Varying degrees of pathological change were observed in the various tissues [15]. Mutations in the C-terminal region affecting subcellular localization of G-protein coupled receptor (GPCR) encoded by CaHV have been identified [16]. However, the genome structure of CaHV and the genome architecture differences between CaHV and CyHVs (which include CyHV1, SY-C1, CyHV2 and CyHV3) have not been studied. To investigate the molecular mechanisms of CaHV pathogenesis, the complete genome of CaHV was sequenced and analyzed in this study.

Diseased crucian carps were collected from a fish farm of Jiangsu Provience of China during 2011 to 2012. CaHV was isolated from livers, spleens and kidneys from diseased fish according to a previously reported procedure [16]. The CaHV genomic DNA of purified viral particles was prepared by phenol-chloroform extraction as described [17]. CaHV genome sequencing was commercially performed by SinoGenoMax Co., Ltd (Beijing, China). The CaHV genome was annotated as described previously [11, 12]. The complete genome sequence of CaHV was deposited in the GenBank database under the accession number KU199244.

The CaHV genome is a linear double-stranded DNA, consisting of 275,348 bp with a G+C content of 51.73 %, and contains 150 predicted open reading frames (ORFs), ranging in size from 62 to 4113 amino acids (aa). It includes 12 core ORFs that are significantly conserved among alloherpesviruses, 38 ORFs with one or more transmembrane domains (TMDs), five ORFs with a RING-finger (Really Interesting New Gene) domain, two ORFs belonging to the tumor necrosis factor receptor (TNFR) family and 93 unclassed ORFs, as shown in Fig. 1a. The nucleotide positions, numbers of amino acids and molecular weights of the predicted proteins, as well as homologues of the predicted ORFs of CaHV in CyHVs are shown in a supplemental file (Table S1).

Comparisons of the genome structures of CaHV and CyHVs were carried out, and some interesting changes related to genome architecture, insertion or deletion of ORFs, and differences in the nucleotide sequence and number of ORFs were found. No terminal direct repeat (TDR) was found in the CaHV genome, and this was confirmed by PCR assay. Each of the CyHV genomes is flanked by TDRs of different length. Thirteen, ten and seven 5'-terminal ORFs of CaHV correspond to the 3'-terminal of SY-C1, CyHV2, CyHV3 and CyHV1, respectively. The initial alignment of homologues was uneven. CaHV *10R* is homologous to the first ORF of SY-C1 and CyHV2, and CaHV *6R* is homologous to the first ORF of CyHV3 and CyHV1. The presence of the 5'-terminal sequences (ORF9, etc.) was verified by PCR assay. Furthermore, various changes in genes were found, such as gene insertions, deletions, and nucleotide changes. Nineteen and nine ORFs in CaHV showed less than 95 % identity to their homologues in SY-C1 and CyHV2, respectively. Compared with CaHV, there are 15 inserted ORFs and 21 deleted ORFs in CyHV3 and 31 inserted ORFs and 18 deleted ORFs in CyHV1. The inserted and deleted ORFs located primarily in the 5' and 3' terminal regions of CyHV3 and CyHV1 (Fig. 1b). These data reveal that CaHV is a novel herpesvirus.

Based on the alignment of DNA polymerase protein sequences from 17 representative strains of herpesviruses (including CaHV), phylogenetic analysis was performed by the neighbor-joining method with 1000 bootstrap replications, using ClustX 1.83 and MEGA 5.1. Members of the order *Herpesvirales* were divided into three groups corresponding to the families *Alloherpesviridae*, *Herpesviridae* and *Malacoherpesviridae*. The data show that CaHV is most closely related to CyHV2 and clusters closely with CyHVs of the family *Alloherpesviridae* (Fig. 2a).

Further phylogenetic analysis was performed based on the concatenated protein sequences of 12 core ORFs from CaHV and nine known alloherpesviruses (Fig. 2b). They were divided into three groups corresponding to the genera *Cyprinivirus*, *Ictalurivirus* and *Batrachovirus*. CaHV clustered with the members of the genus *Cyprinivirus* and was closest to SY-C1, and most distant from AngHV1.The phylogenetic analysis confirmed that CaHV is a novel herpesvirus.

In conclusion, the complete genome sequence and architecture of CaHV were analyzed and compared with those of different herpesviruses. Phylogenetic analysis based on the DNA polymerase genes of 17 herpesviruses and 12 concatenated core genes from known alloherpesviruses showed that CaHV clustered together with members of the genus *Cyprinivirus*, and was most

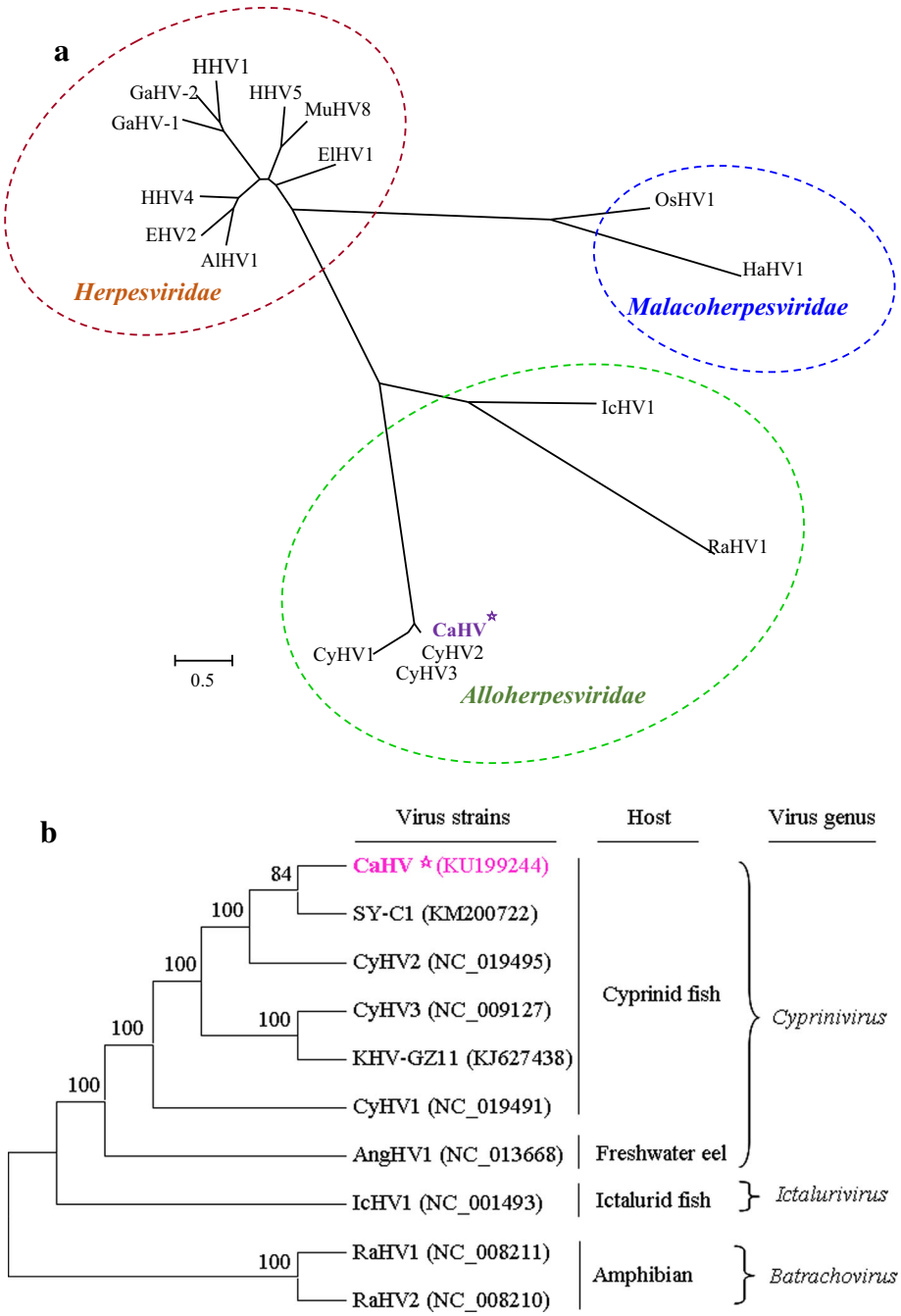


Fig. 2 Phylogenetic analysis. **a** Phylogenetic analysis based on the DNA polymerase genes of 17 members of the order *Herpesvirales*, which was divided into three families: *Herpesviridae*, *Alloherpesviridae* and *Malacoherpesviridae*. GenBank accession numbers are as follows: GaHV-1, JX646899; GaHV-2, AF243438; HHV1, NC_001806; HHV5, GU980198; MuHV8, NC_019559; ElHV1, NC_020474; HHV4, AP015016; AlHV1, NC_002531; EHV2, KM924294; CyHV2, NC_019495; CyHV3, NC_009127; CyHV1, NC_019491; IcHV1, NC_001493; RaHV1, NC_008211; OsHV1, KP412538; and HaHV1, HM631982. The scale bar represents 0.1 fixed mutation per amino acid position. **b** Phylogenetic analysis of CaHV with known alloherpesviruses based upon the concatenated amino acid sequences of 12 core ORFs (*34L, 44R, 45R, 58L, 67R, 68R, 74R, 75R, 76L, 86L, 88R, 102R*). Consensus bootstrap confidence values are indicated at the nodes of the branches. Viral hosts and genera are listed at the right

closely related to SY-C1. However, considerable genetic variations such as insertions and deletions were found, and the CaHV genome found to lack a direct repeat was revealed. These results indicate that CaHV is a novel herpesvirus, and the genetic variations that were identified here may contribute to virulence and host adaptation. This study extends our knowledge about members of the genus *Cyprinivirus* and provides important information for warning of epidemics of fish herpesviral disease.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Zhang QY, Gui JF (2012) Atlas of aquatic viruses and Viral diseases. Science Press, Beijing
2. Zhang QY, Gui JF (2015) Virus genomes and virus-host interactions in aquaculture animals. Sci China Life Sci 58:156–169
3. Murray AG (2013) Epidemiology of the spread of viral diseases under aquaculture. Curr Opin Virol 3(1):74–78
4. Lu MW, Lin CS (2003) Involvement of the terminus of grouper betanodavirus capsid protein in virus-like particle assembly. Arch. Virol. 148(2):345–355
5. Aoki T, Hirono I, Kurokawa K, Fukuda H, Nahary R, Eldar A, Davison AJ, Waltzek TB, Bercovier H, Hedrick RP (2007) Genome sequences of three koi herpesvirus isolates representing the expanding distribution of an emerging disease threatening koi and common carp worldwide. J Virol 81(10):5058–5065
6. Zhang QY, Xiao F, Xie J, Li ZQ, Gui JF (2004) Complete genome sequence of lymphocystis disease virus isolated from China. J Virol 78:6982–6994
7. Chinchar VG, Waltzek TB (2014) Ranaviruses: not just for frogs. PLoS Pathog 10(1):e1003850
8. Lei XY, Ou T, Zhu RL, Zhang QY (2012) Sequencing and analysis of the complete genome of *Rana grylio* virus (RGV). Arch Virol 157(8):1559–1564
9. Gotesman M, Kattlun J, Bergmann SM, El-Matbouli M (2013) CyHV-3: the third cyprinid herpesvirus. Dis Aquat Organ 105(2):163
10. Xu J, Zeng L, Zhang H, Zhou Y, Ma J (2013) Cyprinid herpesvirus 2 infection emerged in cultured gibel carp, *Carassius auratus* gibelio in China. Vet Microbiol 166(1):138–144
11. Davison AJ, Kurobe T, Gatherer D, Cunningham C, Korf I, Fukuda H, Hedrick RP, Waltzek TB (2013) Comparative genomics of carp herpesviruses. J Virol 87(5):2908–2922
12. Li LL, Luo YZ, Gao ZX, Huang J, Zheng XH, Nie HH, Zhang JM, Lin L, Yuan JF (2015) Molecular characterisation and prevalence of a new genotype of Cyprinid herpesvirus 2 in mainland China. Can J Microbiol 61(999):1–7
13. Li W, Lee XZ, Weng SP, He JG, Dong CF (2015) Whole-genome sequence of a novel Chinese cyprinid herpesvirus 3 isolate reveals the existence of a distinct European genotype in East Asia. Vet Microbiol 175(2):185–194
14. Boutier M, Ronsmans M, Rakus K, Jazowiecka-Rakus J, Vancsok C, Morvan L, Peñaranda MM, Stone DM, Way K, van Beurden SJ, Davison AJ, Vanderplasschen A (2015) Cyprinid herpesvirus 3: an archetype of fish alloherpesviruses. Adv Virus Res 93:161–256
15. Fang J, Deng YS, Wang J, Li T, Chen ZY, Zhou L, Zhang QY (2016) Pathological changes of acute viral hemorrhages in the gills of crucian carp. J Fish Sci China 23(2):336–343
16. Wang J, Gui L, Chen ZY, Zhang QY (2016) Mutations in the C-terminal region affect subcellular localization of crucian carp herpesvirus (CaHV) GPCR. Virus Genes 52(4):484–494
17. Chen ZY, Gui JF, Gao XC, Pei C, Hong YJ, Zhang QY (2013) Genome architecture changes and major gene variations of *Andrias davidianus* ranavirus (ADRV). Vet Res 44:101