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# Virus genomes and virus-host interactions in aquaculture animals

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Over the last 30 years, aquaculture has become the fastest growing form of agriculture production in the world, but its development has been hampered by a diverse range of pathogenic viruses. During the last decade, a large number of viruses from aquatic animals have been identified, and more than 100 viral genomes have been sequenced and genetically characterized. These advances are leading to better understanding about antiviral mechanisms and the types of interaction occurring between aquatic viruses and their hosts. Here, based on our research experience of more than 20 years, we review the wealth of genetic and genomic information from studies on a diverse range of aquatic viruses, including iridoviruses, herpesviruses, reoviruses, and rhabdoviruses, and outline some major advances in our understanding of virus–host interactions in animals used in aquaculture.

**aquaculture, viral genome, antiviral defense, iridoviruses, reoviruses, rhabdoviruses, herpesviruses, host-virus interactions**

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Aquaculture has become the fastest and most efficient agricultural production industry in the world over the last three decades, and China is believed to be a major contributor to it [1–3]. According to official figures, the production of aquatic products has reached 61.72 million tons, with the 45.6 million tons from aquaculture accounting for 73.9% of the total produced [4]. Over the last 20 years, China's aquaculture output has accounted for about 2/3 of the total global aquaculture production [5]. However, viral diseases, which have been frequently reported in aquaculture animals, have hampered aquaculture development [6–8]. Concurrently, a natural decline in populations of aquatic vertebrates, especially the global decline or extinction events seen with some frogs and amphibians, have been reported by ecologists; hence, the question "why are all the frogs 'croaking'?" has been asked [9–11]. To help resolve these problems, researchers have looked for and identified a large number of diverse pathogenic viruses in aquaculture and natural aquatic animals including iridoviruses, herpesviruses, reoviruses and rhabdoviruses; these pathogenic iridoviruses have been found to be the cause of epizootic diseases in aquaculture animals and the global decline of amphibian populations [12–15]. In the last 10 years especially, more than 100 viral genomes have been genetically characterized via the rapid advances in genome sequencing technologies [16–19]. These advances have enabled great progress to be made in understanding the mechanisms underlying interactions between viruses and their aquatic host animals [19]. Here, we review recent progress in the genomic and genetic characterization of some important pathogenic viruses, such as iridoviruses, herpesviruses, reoviruses, and rhabdoviruses, and virus–host interactions in aquaculture animals.

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## 1 Iridoviruses and their genomes

Iridoviruses (family Iridoviridae) comprise the following five genera: *Ranavirus*, *Lymphocystivirus*, *Megalocytivirus*, *Iridovirus* and *Chloriridovirus*. The genomes of this family of viruses generally contain a single molecule of double-stranded DNA [19,20]. *Ranavirus*, *Lymphocystivirus* and *Megalocytivirus* infect more than 140 species of aquatic vertebrates including fish, amphibians and reptiles, and cause high mortality in aquaculture and problems with wildlife conservation [19–21]. For example, lymphocystis disease virus (LCDV), has been identified as the causative agent of lymphocystis disease in more than 100 different seawater and freshwater fish species [22,23]. In particular, diverse ranaviruses (genus *Ranavirus*) have been reported to infect about 70 amphibian species from at least 14 families, more than 100 fish species and dozens of reptiles; hence, some experts believe that ranaviruses infect not only frogs but also numerous different aquatic vertebrates, and are, therefore, promiscuous pathogens of cold-blooded vertebrates [24–33].

In total, 22 genomes from *Ranavirus*, *Lymphocystivirus* and *Megalocytivirus* have been completely sequenced; the smallest (105 kb) is that of the tiger frog virus (TFV), while the largest (186 kb) belongs to the Chinese strain of LCDV (LCDV-C) (Table 1).

**Table 1** Known iridoviruses of aquatic animals and their genomes

| No. | Genus and strain | Known host | Isolation region/time | Genome size (kb) | GC% | Potential ORFs | Accession No. | References |
|---|---|---|---|---|---|---|---|---|
| Ranavirus | | | | | | | | |
| 1 | FV3 (Frog virus 3) | Frog | America, 1966 | 105.903 | 55 | 98 | AY548484 | [32–35] |
| 2 | ESV (European sheatfish virus) | Fish | Europe, 1985 | 127.732 | 54 | 136 | JQ724856 | [36] |
| 3 | EHNV (Epizootic haematopoietic necrosis virus) | Fish | Australia, 1986 | 127.011 | 54 | 100 | FJ433873 | [37] |
| 4 | RGV (*Rana grylio* virus) | Frog | China, 1995 | 105.791 | 55 | 106 | JQ654586 | [38–43] |
| 5 | ATV (*Ambystoma tigrinum* virus) | Salamander | America, 2003 | 106.332 | 54 | 96 | AY150217 | [44,45] |
| 6 | SGIV (Singaporegrouper iridovirus) | Fish | Singapore,1998 | 140.131 | 48 | 162 | AY521625 | [46,47] |
| 7 | STIV (Soft-shelled turtle iridovirus) | Turtle | China, 1999 | 105.890 | 55 | 105 | EU627010 | [48] |
| 8 | GIV (Grouper iridovirus) | Fish | Taiwan, 2000 | 139.793 | 49 | 120 | AY666015 | [49] |
| 9 | TFV (Tiger frog virus) | Frog | China, 2002 | 105.057 | 55 | 105 | AF389451 | [50] |
| 10 | CMTV (Common midwife toad ranavirus) | Toad | Europe, 2007 | 106.878 | 55 | 104 | JQ231222 | [51] |
| 11 | ADRV (*Andrias davidianus*ranavirus) | Giant salamander | China, 2013 | 106.734 | 55 | 101 | KC865735 | [52] |
| 12 | ADRV-2 | Giant salamander | China, 2014 | 106.719 | 55 | 101 | KF033124 | [54] |
| 13 | CGSIV (Chinese giant salamander iridovirus) | Giant salamander | China, 2014 | 105.375 | 55 | 112 | KF512820 | [55] |
| Lymphocystivirus | | | | | | | | |
| 14 | LCDV-1 (lymphocystis disease virus-1) | Fish | Red Sea, 1962 | 102.653 | 29 | 110 | L63545 | [22,56] |
| 15 | LCDV-C (lymphocystis disease virus-China) | Fish | China, 2004 | 186.247 | 27 | 240 | AY380826 | [23,43,57] |
| Megalocytivirus | | | | | | | | |
| 16 | RSIV (Red seabream iridovirus) | Fish | Japan, 1992 | 112.415 | 53 | 116 | BD143114 | [61] |
| 17 | ISKNV (Infectious spleenand kidney necrosis virus) | Fish | China, 1998 | 111.362 | 55 | 124 | AF371960 | [60] |
| 18 | RBIV (Rock bream iridovirus) | Fish | Korea, 2000 | 112.080 | 53 | 118 | AY532606 | [62] |
| 19 | LYCIV (Large yellow croaker iridovirus) | Fish | China, 2001 | 111.767 | 54 | – | AY779031 | [63] |
| 20 | TRBIV (Turbot reddish body iridovirus) | Fish | China, 2004 | 110.104 | 55 | 115 | GQ273492 | [64] |
| 21 | OSGIV (Orange-spotted grouper iridovirus) | Fish | China, 2005 | 112.636 | 54 | 121 | AY894343 | [65] |
| 22 | RBIC-C1 (Rock bream iridovirus isolate from China) | Fish | China, 2012 | 112.333 | 55 | 119 | KC244182 | [66] |

### 1.1 Ranaviruses and their genomes

The following 13 ranavirus genomes have been completely sequenced: (i) Frog virus 3 is a species of the genus *Ranavirus*. Frog virus 3 infection results in considerable morbidity and mortality in a wide range of wild and cultivated amphibian species [32–35]. (ii) European sheatfish virus is a fish ranavirus isolated from moribund sheatfish (*Silurus glanis*) fry [36]. (iii) Epizootic hematopoietic necrosis virus is a fish ranavirus that causes serious hematopoietic necrosis in redfin perch and rainbow trout, resulting in serious economic losses in aquaculture and severe decline in wild populations of these fish [37]. (iv) *Rana grylio* virus (RGV). RGV, a ranavirus isolated from China, causes systemic hemorrhagic disease with a high mortality rate in frogs. It is also a model system for molecular characterization of ranaviruses [38–43]. (v) *Ambystoma tigrinum* virus is a lethal ranavirus originally isolated from Sonora tiger salamanders in southern Arizona, USA [44,45]. (vi) Singapore grouper iridovirus is a fish ranavirus isolated from a diseased grouper in Singapore [46,47]. (vii) Soft-shelled turtle iridovirus is a reptile ranavirus that causes viral disease in cultured soft-shelled turtles [48]. (viii) Grouper iridovirus is a fish ranavirus isolated from the spleen tissues of a diseased yellow grouper [49]. (ix) TFV is a frog ranavirus isolated from diseased tiger frogs [50]. (x) Common midwife toad ranavirus is a toad ranavirus responsible for an outbreak of a systemic hemorrhagic disease that caused high mortality in toads from northern Spain [51]. (xi) *Andrias davidianus* ranavirus (ADRV), the first sequenced ranavirus, is associated with high mortality in Chinese giant salamanders [52]. This ranavirus causes high mortality in wild and farmed Chinese giant salamanders [52,53]. (xii) ADRV-2, another ranavirus strain isolated from Chinese giant salamanders after ADRV, shares a high level genome identity with ADRV [54]. (xiii) Finally, the Chinese giant salamander iridovirus, which is another ADRV, is the third sequenced ADRV strain [55].

Based on their genome sizes, gene contents and phylogenetic analyses, the sequenced ranaviruses (Table 1) have been divided into two subgroups: the amphibian subgroup and the fish subgroup. The amphibian subgroup includes ADRV, Common midwife toad ranavirus, RGV, Frog virus 3, TFV, and *Ambystoma tigrinum* virus, while the fish subgroup comprises Epizootic hematopoietic necrosis virus, European sheatfish virus, Grouper iridovirus, and Singapore grouper iridovirus [52]. From extensive analysis of the genome architectures and major genes of this diverse array of ranaviruses (especially ADRV and RGV genomes), we have proposed a hypothetical evolutionary model for ADRV [52]. In this model, ADRV is proposed to emerge (with its current genome) from a common ancestor of the amphibian ranavirus subgroup through changes in its genome architecture and variations in some of its major virulence-related genes (Figure 1). This hypothesis is based on the architectural changes observed in current ranavirus genomes; these include segment inversion, fragment insertion and deletion, and several variations in major genes, such as high diversification in two duplicate genes encoding the US22 family-like proteins, truncated domains in the virulence-related gene encoding vIF2α, and the appearance of novel genes with nuclear localization signal and nuclear export signal motifs [52]. Therefore, our current model provides possible routes leading to evolutionary genetic change and cross-species transmission mechanisms in this diverse range of ranaviruses.

### 1.2 LCDV genomes

LCDVs (genus *Lymphocystivirus*) cause lymphocystis disease in marine and freshwater fish. The genomes of two LCDVs, LCDV-1 and LCDV-C have been completely sequenced. LCDV-1, which infects plaice and flounder, was isolated from the propagated cell lines of bluegill and centrarchid fish in 1966 [32–35] and had its genome completely sequenced in 1997 [22]. LCDV-C was originally iso-

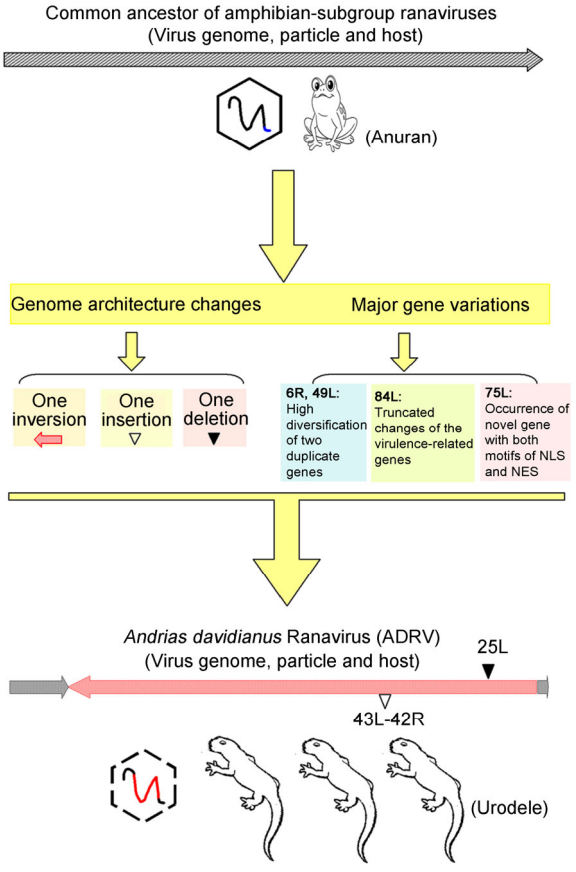


**Figure 1** A hypothetical model of ADRV evolutionary emergence from a common ancestor of the amphibian subgroup of ranaviruses. During evolutionary processes, several changes leading to the current genome architecture and major virulence-related gene variations are proposed to have happened. Red hatched arrow: genome segment inversion; black triangles: fragment insertion; blank triangles: fragment deletion; 6R, 49L, 84L, 75L and 25L: different open reading frames (ORFs) in ADRV genome [52].

lated from a flounder with lymphocystis disease by Chinese scientists in 2003, and its complete genome sequence was reported in 2004 [23,43,57]. The LCDV-C genome remains the largest among all known vertebrate iridoviruses sequenced thus far (Table 1).

It is worth mentioning that LCDV-C is listed as a typical *Iridoviridae* strain in the "Virus Taxonomy Ninth Report" by the International Committee on Taxonomy of Viruses [20]. LCDV-C genomic data can be used as a reference resource for identifying other LCDVs and iridoviruses and for performing gene function analyses. This resource will enable virologists to explore the genetic characteristics of these large DNA viruses. In addition, several novel emerging LCDVs, such as LCDV-PF from the paradise fish *Macropodus opercularis* [58], and GLCDV, which was isolated from cultured grouper [59], have been identified.

### 1.3 Megalocytivirus genomes

To date, seven megalocytiviruses have been subjected to complete genome sequencing: (i) Infectious spleen and kidney necrosis virus—a megalocytivirus causing high mortality in mandarin fish—is characterized by cell hypertrophy in the spleen, kidney, cranial connective tissue and endocardium of this fish [60]. (ii) Red sea bream iridovirus is a piscine iridovirus that causes an acute and highly contagious disease in Red sea bream from Japan and Korea [61]. (iii) Rock bream iridovirus is a megalocytivirus that occurs in cultured rock bream from Korea [62]. (iv) Large yellow croaker iridovirus is a megalocytivirus causing gill paleness, liver congestion, spleen and kidney hypertrophy in cultured large croaker [63]. (v) Turbot reddish body iridovirus is a megalocytivirus that causes serious systemic diseases with high mortality in cultured turbot [64]. (vi) Orange-spotted grouper iridovirus is the causative agent of serious systemic diseases with high mortality in the cultured orange-spotted grouper [65]. (vii) Lastly, rock bream iridovirus isolated from China, is a megalocytivirus that caused a severe disease epidemic in Chinese farmed rock bream [66].

Iridoviruses of the *Ranavirus* and *Lymphocystivirus* genera have broadly similar genome sizes and potential gene contents as iridoviruses of the *Megalocytivirus* genus; their genome sizes range from 110 to 113 kb, while their potential number of genes range from 115 to 124 (Table 1).

### 1.4 Important core genes and their functions in iridoviruses

Gene annotation and comparative genomic analysis have confirmed there are 26 core genes in iridoviruses [67]. Extensive comparisons of these important core genes has provided evidence for cross-species transmission in these iridoviruses, especially for the ranaviruses [68,69]. Moreover, some important genes encoding enzymes, structural proteins and immune-related proteins, such as the RGV 3β-hydroxysteroid dehydrogenase gene (*RGV 3β-HSD*) [70] and the RGV deoxyuridine triphosphatase gene (RGV *dUTPase*) [71], have been characterized and functionally analyzed. LCDV-C thymidylate synthase (LCDV-C TS) is able to promote cell cycle progression into S and G2/M phase. In comparison with control cells, TS-expressed cells have faster growth rates, and induce foci formation and anchorage-independent growth. These findings indicate that LCDV-C TS potentially exhibits the ability to transform cells (tumor formation) [72].

RGV *53R* is a core gene in iridoviruses, and encodes a viral envelope protein that plays an important role in virus assembly and infection [73,74]. Recently, we have chosen RGV *53R* as a target gene to construct a conditional lethal recombinant RGV (i53R-RGV-lacIO) containing the inducible lac repressor/operator system that can be regulated by IPTG, and have found that the *53R* expression level, plaque formation ability and viral titers in i53R-RGV-lacIO are significantly reduced in the absence of IPTG. These results indicate that RGV *53R* is not only essential for virus replication and assembly, but also contributes to virus infection and virion formation [75]. RGV *50L* contains a nuclear localization signal and helix-extension-helix motif, and is an immediate-early gene. Immuno-fluorescence assays indicate that *50L* expression occurs early during infection and persists in RGV-infected cells. RGV *50L* exhibits a cytoplasm-nucleus-viromatrix distribution pattern and viromatrix distribution pattern, indicating that it encodes a structural protein, and plays an important role in viral assembly and life cycle [76]. RGV *2L* is a core gene encoding an envelope protein. To investigate the role of *2L* in viral infections, we constructed a conditional lethal mutant virus containing the lac repressor/operator system and dual fluorescent labeling. Significantly, when *2L* expression is repressed, its plaque formation ability and virus titers were strongly reduced. Functional analysis indicates that the *2L* protein is essential for iridovirus infection and its study has provided new insights into iridovirus envelope proteins [77].

Besides the controllable recombinant virus technique mentioned above [77], gene knockout methodology has been also used to investigate gene function in iridoviruses [78]. Expression inhibition of a structural protein gene and RNA polymerase gene by morpholino knockdown or gene-specific silencing has been observed to cause a significant reduction in the yield of virus progeny [79,80]. Additionally, analysis of gene expression timing and infection pathways have been undertaken in iridovirus using transcriptomics [81]. For example, transcriptional analysis of TFV infection (along with other approaches) has revealed that TFV entry into HepG2 cells occurs via a pH-dependent, atypical, caveola-mediated endocytosis pathway [82]. Also, miRNAs have been recently found to have a significant impact on interactions between iridoviruses and their host aquaculture animals [83]; indeed, some miRNAs may me-

diate viral evasion [84].

## 2 Aquareovirus genomes

All members of the virus Reoviridae family are nonenveloped, and their genomes are composed of multiple (10, 11, or 12) segments of linear double-stranded RNAs housed within an icosahedral capsid. This family contains two subfamilies (Spinareovirinae and Sedoreovirinae) and 15 different genera (*Aquareovirus*, *Orthoreovirus*, *Orbivirus, Rotavirus*, *Coltivirus*, *Seadornavirus*, *Cardoreovirus*, *Cypovirus*, *Idnoreovirus*, *Phytoreovirus*, *Fijivirus*, *Oryzavirus*, *Mycoreovirus*, *Mimoreovirus*, and *Dinovernavirus*) [20].

Reoviruses that infect aquaculture fish belong to the *Aquareovirus* genus, and their genomes generally contain 11 segments of linear double-stranded RNA [85,86]. At least 15 reovirus genomes have been completely sequenced (Table 2). Of these, 11 reoviruses are different isolates from cultured grass carp obtained in various years and from different regions [85,87–92]; the other four reoviruses were isolated from cultured golden shiner, chum salmon, Atlantic salmon (Piscine reovirus), and turbot (*Scophthalmus maximus* reovirus), respectively [93–95].

Recently, our laboratory analyzed and compared the complete genome sequences and major core protein sequences of various grass carp reoviruses (GCRV), and this revealed significant genetic diversity among them [87,90]. GCRV can be divided into three groups. Most of them cluster into the first major group; these viruses are not cytopathic and contain a fiber-like protein. GCRV members of the second group are cytopathic and possess a fusion-associated small transmembrane (FAST) protein. GCRV 104, a lone member of the third group, is also cytopathic and has a fiber-like protein. However, the various genotypes are not associated with their regional distributions [90]. Therefore, more studies on the evolutionary and geographical relationships between genomic diversity and reovirus transmission should be performed on grass carp reoviruses.

*Scophthalmus maximus* reovirus, a novel reovirus isolated from marine fish, contains a FAST protein translated from a non-AUG start site that has been shown to partially contribute to the cytopathic effect caused by infection with this virus [94]. As a new reovirus equally related to members of the *Orthoreovirus* and *Aquareovirus* genera, Piscine reovirus, which is linked to heart and skeletal muscle inflammation in farmed Atlantic salmon (*Salmo salar* L.), has been suggested by whole genome comparisons to be more closely related to orthoreoviruses and, therefore, a new species of the *Orthoreovirus* genus [96]. Genome comparisons show that Piscine reovirus contains 10 genomic segments

**Table 2** Known aquareoviruses and their genomes

| No. | Virus strains | Host | Isolation region/time | Genome size (kb) | Segments/ ORFs | Accession No. | References |
|---|---|---|---|---|---|---|---|
| 1 | GCHV (Grass carp hemorrhage virus | Grass carp | China, 1980 | 21.366 | 11/11 | AF260511–3 AF251262 F239175 AF239174 F259053 AF284504 F236688 F234321 | [85] |
| 2 | GCRV-873 (Grass carp reovirus-873) | Grass carp | China, 1983 | 25.000 | 11/12 | AF260511–3 AF403390–7 | [85] |
| 3 | AGCRV (American grass carp reovirus) | Grass carp | America, 2001 | 23.576 | 11/12 | EF589098–EF589108 | [91] |
| 4 | GCRV-HuNan794 (Grass carp reovirus-HuNan794) | Grass carp | China, 2007 | 24.780 | 11/11 | KC238676–KC238686 | [92] |
| 5 | GCRV HZ08 (Grass carp reovirus HZ08) | Grass carp | China, 2008 | 24.707 | 11/11 | GQ896334–7 GU350742–8 | [87] |
| 6 | GCRV106 (Grass carp reovirus 106) | Grass carp | China, 2009 | 24.778 | 11/11 | KC201166–KC201176 | [97] |
| 7 | GCRV-HeNan988 (Grass carp reovirus-Henan988) | Grass carp | China, 2009 | 24.780 | 11/11 | KC847320–KC847330 | [97] |
| 8 | GCRV918 (Grass carp reovirus 918) | Grass carp | China, 2010 | 24.780 | 11/11 | KC201177–KC201187 | [97] |
| 9 | GCRV-109 (Grass carp reovirus-109) | Grass carp | China, 2014 | 24.625 | 11/11 | KC201177–KC201187 | [90] |
| 10 | GCRV-GD108 (Grass carp reovirus-GD108) | Grass carp | China, 2009 | 24.703 | 11/12 | HQ231198–HQ231208 | [88] |
| 11 | HGDRV (GCRV 104) (Hubei grass carp disease reovirus) | Grass carp | China, 2009 | 23.706 | 11/12 | JN967629–JN967639 | [89] |
| 12 | PRV(Piscine reovirus) | Atlantic salmon | Norway, 2012 | 23.320 | 10/11 | GU994013–GU994022 | [95] |
| 13 | GSRV (Golden shiner reovirus) | Golden shiner | America, 1979 | 23.695 | 11/12 | AF403398–AF403408 | [93] |
| 14 | CSRV (CHSRV) (chum salmon reovirus) | Salmon | Japan, 1981 | 23.015 | 11/12 | AF418294–AF418304 | [93] |
| 15 | SMReV (Turbot *Scophthalmus maximus* reovirus) | Turbot | China, 2012 | 24.042 | 11/12 | HM989930–HM989940 | [94] |

(not 11 as in all recognized aquareoviruses) and an outer-fiber protein that is present in most members of the *Orthoreovirus* genus [96,97]. Moreover, phylogenetic evidence of long distance dispersal and transmission has been revealed by comparing the protein coding sequences S1, S2 and S4 in Piscine reovirus genomic segments between farmed and wild Atlantic salmon [98].

As an ideal model system for studying the cell entry mechanism used by nonenveloped viruses, single-particle cryo-electron microscopy has been used to observe the 3.3 Å structure of the primed, infectious subvirion GCRV particle, thereby providing structural insight into the coupling of virion assembly [99,100]. Additionally, new insight into the mechanisms of viral factory formation and pathogenesis of aquareoviruses has been acquired from functional studies on aquareoviral genes where NS80, a nonstructural protein of fish reovirus, has been confirmed to be crucial for recruiting viral components to form aquareoviral factories [101].

## 3 Rhabdovirus genomes

Rhabdoviruses are a group of enveloped, single-stranded, negative-sense RNA viruses. The Rhabdoviridae family includes the following nine genera: *Cytorhabdovirus*, *Ephemerovirus*, *Lyssavirus*, *Novirhabdovirus*, *Nucleorhabdovirus*, *Perhabdovirus*, *Sigmavirus*, *Tibrovirus* and *Vesiculovirus* [20]. All known fish rhabdoviruses have been assigned to the following three genera: *Vesiculovirus*, *Novirhabdovirus* and *Perhabdovirus* [102,103].

Fish rhabdoviruses can cause severe hemorrhagic septicemia in freshwater and marine fish. In the last 10 years, virologists isolated and identified the following rhabdoviruses from aquaculture fish: *Siniperca chuatsi* rhabdovirus [104], *Scophthalmus maximus* rhabdovirus [105,106], *Paralichthys olivaceus* rhabdovirus [107], *Monopterus albus* rhabdovirus [108], snakehead rhabdovirus [109,110], Hirame rhabdovirus [111], and pike fry rhabdovirus [112]. Spring viremia of carp virus (SVCV), an earlier identified rhabdovirus, causes infectious hemorrhagic septicemia in common carp (*Cyprinus carpio*) [102,113]. Perch rhabdovirus causes lethal hemorrhagic disease in different farmed species of perch, bass, grayling and trout [114,115]. Viral hemorrhagic septicemia virus (VHSV) and infectious haematopoietic necrosis virus (IHNV) are two typical rhabdoviruses of the *Novirhabdovirus* genus. VHSV is a viral pathogen affecting both wild and cultured fish worldwide; infected species include salmon, trout, cod, herring, sole, catfish, pike, turbot, and flounder, among others [115]. IHNV causes severe losses to the salmon fish industry in the USA and Canada, and many other countries in Asia and Europe [18].

IHNV is the first fish rhabdovirus that has had its complete genome sequenced [116]. Currently, more than 100 fish rhabdovirus genomes have been completely sequenced, and over 80 of them are from different VHSV isolates or strains (Table 3). Fish rhabdovirus genomes are negative-sense, single-stranded RNA molecules, and their sizes range from 11 to 16 kb. In fish rhabdoviruses of the *Vesiculovirus* genus, such as SVCV, pike fry rhabdovirus, *Siniperca chuatsi* rhabdovirus, and *Scophthalmus maximus* rhabdovirus, their genomes encode the following five proteins: nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), and RNA-dependent RNA polymerase (L) in the order 3′-N-P-M-G-L-5′, whereas in fish rhabdoviruses of the *Novirhabdovirus* genus, such as VHSV, IHNV, Hirame rhabdovirus, snakehead rhabdovirus and *Paralichthys olivaceus* rhabdovirus, their genomes encode the following six proteins: N, P, M, G, non-virion protein (NV), and L, in the order 3′-N-P-M-G-NV-L-5′. In comparison with *Vesiculovirus* genus members, novirhabdoviruses possess an additional NV gene [107].

Because of their small genomes, short generation times and rapid mutation rates, fish rhabdoviruses, especially VHSV and IHNV, have been extensively used to analyze their evolutionary patterns, genetic diversity and biogeography of the numerous variants. Pierce and Stepien [117] evaluated the phylogenetic and biogeographic relationships of various VHSV isolates by comparing their corresponding genomic sequences, and depicted an evolutionary history of relatively rapid population diversifications in star-like patterns, following a quasispecies model. Furthermore, He et al. [118] applied the Bayesian coalescent method to the time-stamped entire coding sequences of each VHSV gene. Through age calculations on six genes, the first bifurcation event of the isolates they analyzed was estimated to have occurred within the last 300 years. Additionally, comprehensive phylogenetic analyses have been performed by comparing the corresponding gene sequences of worldwide VHSV or IHNV isolates [114,119,120].

In fish rhabdoviruses of the *Vesiculovirus* genus, SVCV genomes, which can be classified into different clades and genogroups, have been described as possessing high levels of diversity and plasticity [121,122]. Recently, Xiao et al. [123] performed recombination analysis of all known complete SVCV genomic sequences, and found evidence of homologous recombination in these genomes. This finding sheds light on recombination and the evolutionary process in various isolates of fish rhabdoviruses.

To allow functional studies to be conducted on fish rhabdovirus genes, several IHNV-VHSV chimeric recombinant viruses were constructed to allow the researchers to identify virulence genes through reverse genetics. Through comparative challenge experiments in rainbow trout fingerlings, recombinant IHNV gained higher virulence following substitution of the G gene with that of each individual VHSV strain [124]. Additionally, an *in vivo* superinfection assay has recently been developed to examine the role of virulence in IHNV of fish rhabdoviruses [125].

**Table 3** Known fish rhabdoviruses and their genomes

| No. | Virus strains | Date of isolation | Isolation region | Host | Genome size (knt) | Genotype (gene order) | Accession No. | References |
|---|---|---|---|---|---|---|---|---|
| Rhabdoviruses of different fish species | | | | | | | | |
| 1 | IHNV (Infectious haematopoietic necrosis virus) | 1979 | North America | Salmonids | 11.137 | 3′N-M1-M2-G-NV-L-5′ | X89213 | [116, 124] |
| | | | | | 11.131 | 3′N-M1-M (or M2)-G-NV-L-5′ | NC_001652.1 | |
| 2 | SCRV (*Siniperca chuatsi* rhabdovirus) | 1999 | China | Mandarin fish | 11.545 | 3′-N-P-M/Ms-G-L-5′ | DQ399789 | [104] |
| 3 | SMRV (*Scophthalmus maximus*rhabdovirus) | 2007 | China | Turbot | 11.492 | 3′N-Ps-P/C-M-G-L 5′ | HQ003891 | [105, 106] |
| 4 | PORV (*Paralichthys olivaceus* rhabdovirus) | 2005 | China | Flounder | 11.182 | 3′-N-P-M-G-NV-L-5′ | KC685626 | [107] |
| 5 | SVCV (Spring viraemia of carp virus) | 2007 | China | Common carp | 11.019 | 3′-N-P-M-G-L-5′ | DQ097384 | [102, 113,121–123] |
| | | | | | 11.019 | 3′-N-P-M-G-L-5′ | AJ318079 | |
| | | | | | 11.100 | 3′-N-P-M-G-L-5′ | DQ097384 | |
| | | | | | 11.020 | 3′-N-P-M-G-L-5′ | NC_002803.1 | |
| 6 | PFRV (Pike fry rhabdovirus) | 1973 | Netherlands | Pike fry | 11.097 | 3′ N-P-M-G-L-5′ | FJ872827 | [112] |
| 7 | EVEX (Eel virus European X) | 2013 | Europe | eel | 11.778 | 3′ N-P-M-G-L-5′ | JX827265 | |
| 8 | TenRV (Tench rhabdovirus) | 2013 | Europe | tench | 11.082 | 3′ N-P-M-G-L-5′ | KC113517 | |
| 9 | GrCRV (Grass carprhabdovirus) | 2013 | Europe | grass carp | 11.096 | 3′ N-P-M-G-L-5′ | KC113518 | |
| 10 | SVCV_Fijan (Spring viraemia of carp virus-Fijan) | 1971 | Yugoslavia | Common carp | 11.019 | 3′ N-P-M-G-L-5′ | AJ318079 | [102] |
| 11 | HIRRV CA-9703 (Hirame rhabdovirus-CA-9703) | 1986 | Korea | Japanese flounder | 11.034 | 3′ N-P-M-G-NV-L-5′ | AF104985 | [111] |
| 12–30 | PRV(Perch rhabdovirus) | 1980–2010 | France, Denmark, Italy, Finland Baltic Sea, Sweden, Netherlands | Pikeperch, Pike, Perch, Black bass, Grayling, Sea trout, Eel, Trout Brown | e.g. 11.487 | e.g. 3′ N-P-M-G-L-5′ | JX679246.1 JF502607, JF502603, JF502604 JF502609, JF502605, JF502596，JF502608, KC408701, KF146312 KC408697, KF146314, KC408700 KF146311, KC408699, KF146310 KC408698, KF146309, KF146308. KF146313, KF146315, AF434991 AF434992, FN557213, | [114] |
| Viral hemorrhagic septicemia virus (VHSV) from different fish species | | | | | | | | |
| 31–116 | VHSV (Viral haemorrhagic septicaemia virus) | 1962–2007 | Denmark, Norway France, Georgia English Baltic Sea, Kattegat Finland Archipelago Sea Scotland Ireland USA, Japan Italy, Sweden, Netherlands | Rainbow trout Cod, Sprat Herring Lamprey Turbot Pout, Eel Coho salmon Japanese flounder | e.g. 11.184 | e.g. 3′-N-P-M-G-NV-L-5′ | GQ385941 AF345857, AY546621, AY546616, Z93412, Z93414, AY546619, U28800, AY356632, AF345859, AF345858, AF143862, AY546575, AY546612, AY546623, AM086355,AM086356, AM086357, AM086358, AM086359, M086360, AM086361, AM086362, AM086363, AM086364, AM086365, AM086366, AM086367, AM086368,AM086369, AM086370, AM086371, AM086372, AM086373, AM086374, AM086375, AM086376, AM086377, AM086378, AM086379, AM086380, AM086381, AB179621 AM086382, AM086383, FJ384761, AY546576, AY546577, AY546578, GQ504013, HQ112198-HQ112200, HQ112234, HQ112201- HQ112203, HQ112235, HQ112204- HQ112206, HQ112236, HQ112207-HQ112209, HQ112237, HQ112210-HQ112212, HQ112238, HQ112213-HQ112215, HQ112239,HQ112216, HQ112217, HQ112240, HQ112218, HQ112241, HQ112248, HQ112219-HQ112221, HQ112243, HQ112222-HQ112224 HQ112244, HQ112225-HQ112227, HQ112245, HQ112228-HQ112229, HQ112246 HQ112242, HQ112233, HQ112232, HQ112230, HQ112231, HQ112247, AY546628, AY546582, AY546620, AY546632, EU547740, Y546618, U28747 | [117–119, 122] |

## 4 Herpesvirus genomes

Herpesviruses are enveloped viruses containing large, double-stranded, linear DNA genomes. They are host-specific pathogens, and are wide spread among vertebrates such as mammals, birds, amphibians and fish [126]. After two new virus families, namely, Alloherpesviridae (incorporating fish and frog herpesviruses) and Malacoherpesviridae (containing mollusks) were recognized in 2009, the Herpesvirales order includes the following three families: Herpesviridae (containing mammals and birds), Alloherpesviridae, and Malacoherpesviridae. The Alloherpesviridae family is divided into the following four genera: *Batrachovirus*, *Cyprinivirus*, *Ictalurivirus*, and *Salmonivirus* [20]. The *Cyprinivirus* genus contains four species: three of them, cyprinid herpesvirus 1 (CyHV-1), cyprinid herpesvirus 2 (CyHV-2), and cyprinid herpesvirus 3 (CyHV-3), are associated with common carp or goldfish, while one of them, anguillid herpesvirus 1 (AngHV1), is associated with freshwater eels [127,128].

Cyprinid herpesviruses have been reported to cause serious mortality in common carp and crucian carp [129]. CyHV-1 is the cause of carp pox, CyHV-3 is fatal in carp and koi fish, and CyHV-2 is the etiological agent of herpesviral hematopoietic necrosis disease in common carp, goldfish, crucian carp, and gibel carp [130]. Through use of bioluminescence imaging, the skin covering a fish's fins and body has been shown to be the major port of entry for cyprinid herpesviruses [131]. Tissue culture and RT-PCR testing results indicate that herpesviruses may become latent in leukocytes and other tissues following a primary infection, and that they can be reactivated from latency by temperature stress [132]. Recently, B cells have been identified as a major site where CyHV-3 can become latent [133].

CyHV-3 has been observed to cause significant morbidity and mortality in koi and common carp. The pathological signs include epidermal abrasions, excess mucus production, necrosis of gills and internal organs, and lethargy. CyHV-3 propagates well in the intestines and kidneys, and high numbers of infectious viruses can be observed in the droppings of infected fish [134]. Several primary culture cell lines can be used to propagate cyprinid herpesviruses and for isolating these etiologic agents of disease [135].

So far, the complete genomes of 11 herpesviruses have been sequenced from aquatic animals, nine of which are members of the Alloherpesviridae family, while two are members of the Malacoherpesviridae family. The genomes of these herpesviruses range in size from about 134.2 kb for the smallest (Ictalurid herpesvirus 1) to about 295.2 kb for the largest (Koi herpesvirus-J); their potential numbers of genes range from 77 to 163 (Table 4).

The complete genome sequences of five cyprinid herpesviruses, the diseases of which are fatal in common carp, koi carp, goldfish, crucian carp, and gibel carp [130] have been reported. These genomes are characterized by a unique region flanked at each terminus by a sizeable direct repeat. About 120 orthologous genes are shared by these cyprinid herpesviruses, and 55 of them also share sequence conser-

**Table 4** Known herpesviruses of aquatic animals and their genomes

| No. | Genus/strain | Host/symptom | Isolation region/time | Genome size (kb) | Potential ORFs | Accession No. | References |
|---|---|---|---|---|---|---|---|
| lloherpesviridae | | | | | | | |
| 1 | Ictalurivirus/Ictalurid herpesvirus 1 (IcHV-1) | Channel catfish | USA, 1971 | 134,226 | 77 | M75136 | [138,139] |
| 2 | Ictalurivirus/Anguillid herpesvirus 1 (AngHV-1) | Eels (Anguilla) | Netherlands, 1996 | 248.526 | 134 | FJ940765 | [140–142] |
| 3 | Cyprinivirus/Cyprinid herpesvirus1 (CyHV-1) | Common carp (*Cyprinus carpio*) and koi carp (a variety of *Cyprinus carpio*)/carp pox, papilloma | Japan, 1985 | 291,144 | 143 | JQ815363 | [91,128] |
| 4 | Cyprinivirus/Cyprinid herpesvirus 2 (CyHV-2) | Goldfish (*Carassius auratus*)/goldfish hematopoietic necrosis | Japan, 1992 | 290,304 | 154 | JQ815364 | |
| 5 | Cyprinivirus/Cyprinid herpesvirus 3 (CyHV-3) KHV-U | Koi and *Cyprinus carpio*/Bleeding gills, sunken eyes, pale patches or skin blistering | USA, 2003 | 295.146 | 163 | DQ657948 | [136] |
| 6 | KHV-J | | Japan, 2004 | 295,271 | 156 | AP008984 | |
| 7 | KHV-I | | Israel, 1998 | 295,138 | | DQ177346 | |
| 8 | Batrachoviru/Ranid herpesvirus 1 (RaHV-1) | Leopard frog, *Rana pipiens*/Renal carcinoma or Lucke tumor | North American, 1964 | 220,859 | 132 | DQ665917 | [138,139] |
| 9 | Batrachoviru/Ranid herpesvirus 2 (RaHV-2) Frog virus 4 | Leopard frog, *Rana pipiens*/Renal carcinoma or Lucke tumor | North American, 1964 | 231,801 | 147 | DQ665652 | |
| Malacoherpesviridae | | | | | | | |
| 10 | Ostreavirus/Oyster herpesvirus OsHV1 | Oyster | New Zealand, 1995 | 207,439 | 124 | AY509253 | [128] |
| 11 | Ostreavirus/Acute viral necrosis virus (AVNV) | Chinese scallop *Chlamys farreri* | China, 1998 | 210,993 | 123 | GQ153938 | [144] |

vation with AngHV1 of the *Cyprinivirus* genus. Significantly, only 12 genes were found to be conserved convincingly in all the sequenced alloherpesviruses [136–138].

Ictalurid herpesvirus 1 is the type species of the *Ictalurivirus* genus, and is the first fish herpesvirus for which the complete genome is known [139]. AngHV1 also frequently causes fatal disease in freshwater eels. After complete genome sequencing [140] and deep-sequencing of the AngHV1 transcriptome [141] were finalized, a genome-wide transcription analysis was performed using reverse transcription quantitative PCR, and a temporal regulation fashion similar to mammalian herpesviruses was observed in this fish herpesvirus [142].

Chelonid herpesvirus 5 is closely related to fibropapillomatosis, a neoplastic disease of marine turtles. Its genomic sequence has been shown to be largely collinear with the genomes of typical alphaherpesviruses [143]. In addition, the complete genome sequence of the acute viral necrosis virus, which belongs to the *Malacoherpesviridae* family, has also been reported recently [144].

In recent years, after cyprinid herpesvirus disease became widely reported in the world, (especially in China) [145–147], numerous studies on cyprinid herpesviruses and identification of immune-related genes have been conducted [148–150]. It is envisaged, therefore, that new insight and better understanding of these cyprinid herpesviruses will emerge and lead to efficient antiviral approaches being developed in the near future.

## 5 Virus-host interactions in aquatic animals

The diverse viruses discussed above are serious pathogens of aquatic animals, especially those used in aquaculture. To understand their pathogenetic mechanisms and thereby provide protective strategies again them, some significant experimental methods and high-throughput technologies such as transcriptomics and proteomics have been recently used to gain better knowledge of these viruses and their hosts. Such studies have greatly expanded our knowledge about the innate and acquired immune systems of aquatic animals [151–155]. In China especially, comparative immunological studies of aquaculture animals have flourished over the last 10 years, financial support for research in this area has increased, and progress in this field has been reported in several reviews [156–162]. Figure 2 is a schematic diagram outlining the interactions occurring between the diverse range of viruses discussed herein and their aquatic hosts. The diagram also shows how innate and acquired immunity, as well as related factors such as physical barriers, operate in aquaculture animals under attack by pathogenic viruses.

As shown in Figure 2, when viruses, such as iridoviruses, herpesviruses, reoviruses, or rhabdoviruses attempt to enter a host cell, they first meet physical barriers on the skin and interact with a continuous layer of mucus and the complex regulatory networks that control skin immunity [163–166]. After such viruses pass the first line of defense and enter the host cell, the infected cell immediately initiates a series of innate immune responses; these include an inflammatory

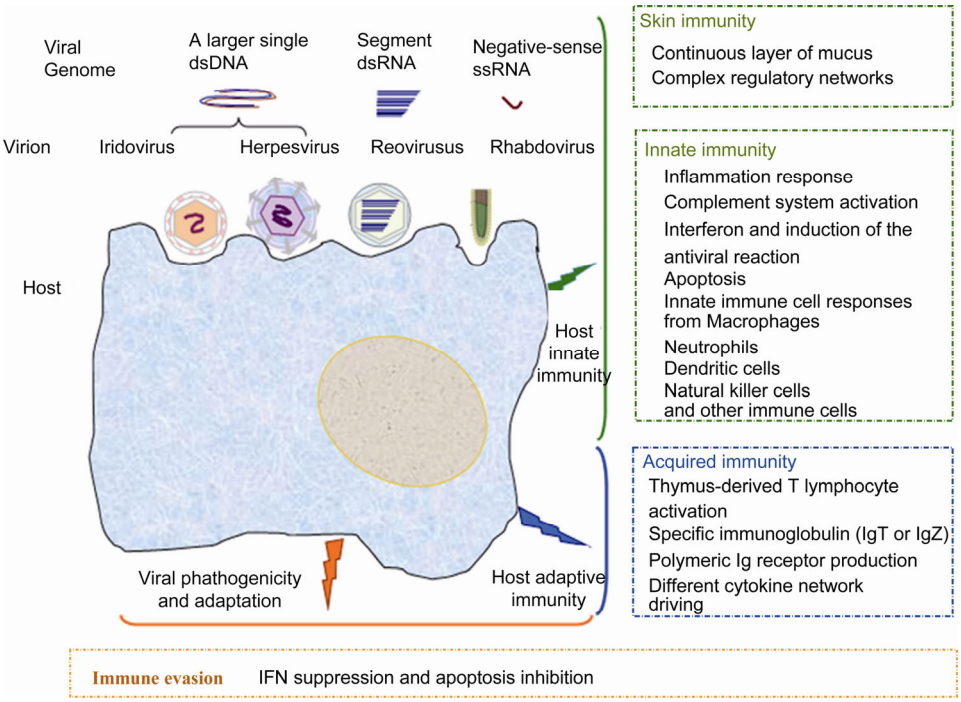


**Figure 2** Schematic diagram illustrating the types of interactions that can occur between viruses and a host cell from an aquatic animal.

response, complement system activation, interferon production, induction of antiviral reactions, cell apoptosis, and innate immune cell responses from macrophages, neutrophils, dendritic cells, natural killer and other immune cells that prevent viral replication and inhibit virus propagation [153,156,167]. Subsequently or concurrently, acquired (adaptive) immune responses, such as thymus-derived T lymphocyte activation, specific immunoglobulins (IgT or IgZ), polymeric Ig receptor production, and differential cytokine network activation, amongst others, enables collaboration between the cellular and humoral immune systems leading to destruction of the invading viruses [168–171]. Additionally, some aquatic viruses have developed immune evasion mechanisms and strategies to combat host immune systems through IFN suppression and apoptosis inhibition; such viruses have increased pathogenicity and have acquired the ability to cross species barriers in their transmission [153,167,172].

## 6 Conclusion and outlook

Over the last decade, a large number of diverse pathogenic viruses, such as iridoviruses, herpesviruses, reoviruses, and rhabdoviruses, have been identified from aquaculture and natural aquatic animals, and many of their genomes have been completely sequenced. Comparative genomic and phylogenetic analyses have provided new insight into the origins of these viruses, as well as the different routes that have led to genetic change and evolutionary processes, and cross-species transmission mechanisms. Additionally, functional studies on genes have yielded crucial information about viral factory formation and pathogenesis in these viruses. Along with their genome architectures and genetic characterization, the interactions between these viruses and their aquatic animal hosts have become an important focus in aquaculture. Finally, significant progress has been made in understanding the following: (i) the molecular mechanisms underlying virus-host interactions [173–176], (ii) innate antiviral immune responses in fish, and (iii) gene identification in the fish interferon system [177–179]. Armed with this knowledge, it is hoped that new drugs and strategies to protect aquaculture animals against pathogenic viruses will be developed in the near future.

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