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Chapter 20

Coronaviruses in Aquatic Organisms

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20.1 TAXONOMY

The *Coronaviridae* are a family of enveloped viruses with a single positive-strand RNA genome, and belong to the order *Nidovirales*. This order was first introduced by the International Committee on Taxonomy of Viruses (ICTV; Pringle, 1996) in 1996, and comprises the families *Arteriviridae* and *Coronaviridae*. The designation is derived from the Latin term *nidus* and reflects the large nested arrangements of subgenomic (sg) messenger RNA (mRNA) (Cavanagh, 1997). The morphological and molecular structures of nidoviruses differ widely, and so the viruses were assigned to the families *Arteriviridae, Coronaviridae, Roniviridae* and *Mesoniviridae*, according to their ultrastructural and genomic characteristics (de Groot et al., 2012a; Lauber et al. 2012; Nga et al., 2011; Zirkel et al., 2013). *Nidovirales* have a unique set of genetic markers: a putative multinuclear zinc-binding domain (ZBD) and a uridylate-specific endoribonuclease (NendoU) domain (Gorbalenya et al., 2006). The classification of *Nidovirales*, including the type species, is summarized in Table 20.1.

The family *Coronaviridae* comprises many genera and species, including important human and veterinary pathogens that cause respiratory, gastrointestinal, cardiovascular and neurological diseases. The main feature of coronaviruses is the coronal structure, which displays prominent projections from the surface of the envelope. Coronaviruses were originally classified according to their antigenic relationships and host species. During recent decades, numerous coronavirus isolates/genomes have been identified, and the classifications have been revised accordingly.

The family *Coronaviridae* is divided into two subfamilies: *Coronavirinae* and *Torovirinae* (González et al., 2003; de Groot et al., 2012b). Members of *Coronaviridae* infect three groups of vertebrates: mammals (genera *Alphacaronavirus*, *Betacoronavirus*, *Gammacoronavirus* and *Torovirus*), birds (genera *Gammacoronavirus* and *Deltacoronavirus*) and fish (genus *Bafinivirus*) (de Groot et al., 2012b). A nidovirus recently isolated from an Indian python (python nidovirus [PNV]) probably belongs to the subfamily *Torovirinae* (Bodewes et al., 2014), although it has not been formally classified.

Coronaviruses are classified according to prominent genetic features, which are localized within the replicase polyprotein pp1ab. Viruses showing more than 90% homology (at the amino acid level) within the conserved replicase domains belong to the same species (de Groot et al., 2012b). The following conserved domains and certain motifs of the polyprotein pp1ab are applied as demarcation criteria: 3C-like protease ($3CL^{pro}$), RNA-dependent RNA polymerase (RdRp), ADP-ribose1″-phosphatase (ADRP), 3′-to-5′ exoribonuclease (ExoN), superfamily 1 helicase with an N-terminal ZBD, NendoU, a putative ribose-2′-*O*-methyltransferase domain (O-MT) and a ribosomal frame shift signal (RFS) (de Groot et al., 2012b).

20.2 VIRUS STRUCTURE

The name, *Coronaviridae*, is derived from the Latin term *Corona*, and reflects the typical crownlike appearance conferred by the prominent surface projections. Coronaviruses are enveloped particles, which may be spherical (*Coronavirinae*), kidney-shaped (*Torovirus*) or bacilliform (*Bafinivirus*). The average diameter of coronaviruses ranges from 120 to 160 nm. Rod-shaped torovirus particles are 100–140 nm in length and 32–42 nm in width, whereas bafinivirus measures 150–185 × 57–70 nm (Fig. 20.1). The mean relative molecular mass (M_r) of coronavirus particles is 400×10^6. The buoyant density of coronavirus in sucrose is 1.15–1.20 g/cm^3, whereas that of torovirus and bafinivirus is 1.14–1.18 g/cm^3 and 1.17–1.19 g/cm^3, respectively (de Groot et al., 2012a).

Virions comprise an internal nucleocapsid structure, which is covered by an envelope derived from the Golgi apparatus or intermediate compartment of the infected cell. The envelope comprises a unit membrane, which bears club-shaped projections measuring 12–24 nm in length. These projections, which comprise spike (S) glycoprotein trimers, allow the virions to attach to specific receptors expressed on the plasma membrane of the host cell (Delmas and Laude, 1990). A typical

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TABLE 20.1 Classification of *Nidovirales* Accepted by the International Committee on Taxonomy of Viruses (ICTV)

Family	Subfamily	Genus	Type Species
Arteriviridae		*Arterivirus*	Equine arteritis virus (EAV)
Coronaviridae	*Coronavirinae*	*Alphacoronavirus*	Alphacoronavirus 1
		Betacoronavirus	Murine coronavirus
		Gammacoronavirus	Avian coronavirus
		Deltacoronavirus	Bulbul coronavirus HKU11
	Torovirinae	*Torovirus*	Equine torovirus
		Bafinivirus	White bream virus
Mesoniviridae		*Alphamesonivirus*	Alphamesonivirus 1
Roniviridae		*Okavirus*	Gill-associated virus

feature of coronaviruses is the formation of syncytia, which are induced by the S protein. Betacoronaviruses exhibit a second type of surface projection, which comprises a homodimeric hemaglutinin-esterase (HE) glycoprotein; this protein also mediates receptor binding. Viruses expressing the HE protein bind to and agglutinate erythrocytes (Modrow et al., 2010). The matrix (M) glycoprotein is located on the cytoplasmic side of the viral envelope and plays a central role in viral budding and contributes to the stability of the viral core structure (Escors et al., 2001a, b; Kuo and Masters, 2002).

Coronaviruses express a few copies of the small nonglycosylated envelope (E) protein, which is not expressed by *Torovirinae*. The E protein plays an important role in virus assembly and morphogenesis because of its ion channel and/or membrane-permeabilizing activity. Although the E protein is not essential for all coronaviruses, its loss results in a marked reduction in viral titer or inhibited virus maturation, release and spread (Ortego et al., 2002; Fischer et al., 1998; Kuo and Masters, 2003).

The internal ribonucleocapsid has a helical (*Coronavirinae*), tubular (*Torovirus*) or cylindrical (*Bafinivirus*) core structure and comprises a helical viral RNA genome complexed with multiple copies of the RNA-binding nucleocapsid (N) protein. The N protein is responsible for genome encapsidation and packaging. It is also essential for sg RNA synthesis and genome replication (Almazan et al., 2004; Schelle et al., 2005).

20.2.1 Viral Genome

Coronaviruses have the largest genome of all the RNA viruses. The viral genome is infectious and comprises a positive single-stranded RNA encompassing 25.4 kb (eg, Porcine Deltacoronavirus; PDCoV) to 31.8 kb (eg, bottlenose dolphin coronavirus HKU22). The as-yet-unassigned PNV genome comprises >33 kb and is probably the largest known RNA genome.

The capped and polyadenylated viral RNA has untranslated regions (UTR) at the 5′ and 3′ termini. The largest open reading frames (ORFs) are 1a and 1b, which are localized at the 5′ end and make up 60%–75% of the viral genome. These ORFs encode the nonstructural proteins and the viral replicase components. Both ORFs overlap within a small region containing a -1 RFS signal (Gorbalenya et al., 2006). ORFs encoding the smaller structural S, M, N, HE and E proteins are located downstream of ORF 1b. Both the number and the order of encoded structural genes may vary within and between different genera of *Coronaviridae*. In general, the viral genome is organized as follows: 5′-UTR-replicase-S-M-N-UTR-3′ (de Groot et al., 2012b).

20.2.2 Morphogenesis

Detailed information about the replication of nidoviruses isolated from aquatic organisms is lacking. Nevertheless, coronaviruses and toroviruses share a similar genome organization and replication strategy. The principle features of the viral infection cycle, as reviewed by de Vries et al. (1997) and Ruch and Machamer (2012), are described next.

The first step of the infection cycle is attachment of a virion to its cognate cellular receptor. This step is mediated by the S glycoprotein. The conformation of the S glycoprotein changes upon binding, thereby promoting fusion of the viral envelope with the cell membrane (Lewicki and Gallagher, 2002; Belouzard et al., 2012). Some coronaviruses require cleavage of the

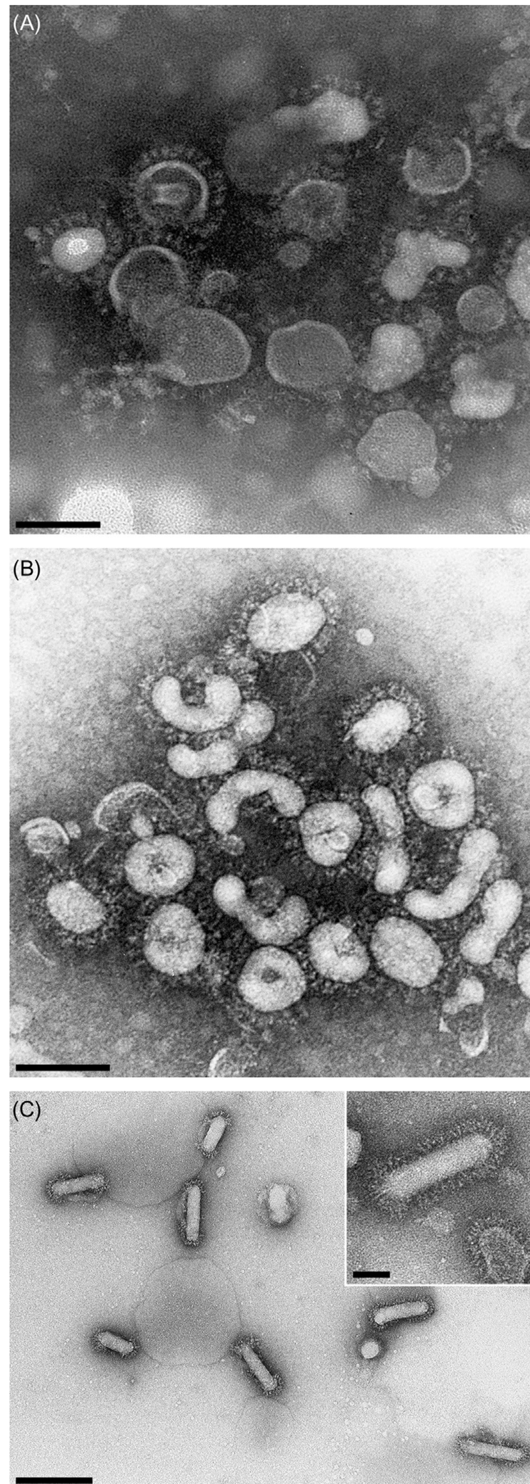

FIGURE 20.1 Electron micrograph showing representative *Coronaviridae* after negative contrast staining. (A) Transmissible gastroenteritis virus (TGEV) (family, *Coronaviridae*; genus, *Alphacoronavirus*). Bar, 100 nm. (B) Equine torovirus (EToV) (subfamily, *Torovirinae*; genus, *Torovirus*). Bar, 100 nm. (C) White bream virus (WBV) (subfamily, *Torovirinae*; genus, *Bafinivirus*). Bar, 200 nm (50 nm in inset). The photographs were kindly provided by Dr. H. Granzow (Federal Research Institute for Animal Health, Insel Riems, Germany).

S glycoprotein for optimal fusion; however, noncleavage does not prevent fusion (Cavanagh, 2005). After fusion of the viral envelope with the cell membrane, the viral genome is released into the cytoplasm. Virus replication takes place entirely within the cytoplasm of the infected cell. The first step involves attachment of the genomic RNA to ribosomes to facilitate synthesis of viral RdRp, which is encoded by ORF 1b. Structural proteins are produced from sg mRNAs. The viral replicase is encoded by two large ORFs, which overlap at the end of ORF 1a and the beginning of ORF 1b. A conserved "slippery" sequence comprising seven nucleotides is located upstream of a pseudoknot structure at the end of ORF 1a. This specific structure facilitates a ribosomal frame shift, resulting in the translation of two long polyproteins named pp1a and pp1ab. These large nonstructural proteins have never been observed in infected cells (de Groot et al., 2012a). Co- and post-translational processing by viral proteinases generates a range of mature nonstructural proteins, including the replicative proteins. These enzymes and proteins are conserved within coronaviruses and are typical features of nidoviruses. The replicase complex produces complementary copies of the genome. The resulting negative-stranded RNA intermediates serve as template for the generation of positive-stranded viral RNA (genome replication) and for the synthesis of a 3′ co-terminal nested set of sg mRNAs (transcription) (Sawicki, 2009). The latter are polyadenylated at the 3′ terminus. With the exception of toroviruses, each sg mRNA carries a short 5′ leader sequence, which is identical to the 5′ end of the viral genome. In coronaviruses and bafiniviruses, transcription of genome templates is regulated by a short AU-rich sequence (transcription regulating sequence; TRS); however, in toroviruses, the process is regulated by putative terminator/promoter elements (TPs). The TRS and TPs are not equivalent in terms of function (de Groot et al., 2012a). Coronaviruses and bafiniviruses generate mRNA via discontinuous synthesis, whereas toroviruses employ a mixed transcription procedure (Cavanagh, 2005; de Groot et al., 2012a,b).

Translation of viral glycoproteins takes place on the rough endoplasmic reticulum. The S, HE and M glycoproteins are then transported to the Golgi complex. The assembly process is initiated by the formation of M protein homomultimers, which accumulate at the membrane of the intermediate compartment between the endoplasmic reticulum and the Golgi (termed the ERGIC) (Neuman et al., 2011; De Haan et al., 1998, 2000). Envelope formation and recruitment of the M homomultimers is promoted by the integral membrane protein E (Ruch and Machamer, 2012; Ye and Hogue, 2007). The viral RNA combines with the N protein to form the RNP; meanwhile, the M and E proteins distort the membrane to form a sphere around the nucleocapsid. The M proteins then recruit the viral core and spike proteins. The newly formed virions bud into the lumen of the ERGIC. Virus containing Golgi vesicles move to the plasma membrane, where virions exit the cell via exocytosis (Siu et al., 2008; Krijnse-Locker et al., 1994).

20.2.3 Recombination

The diversity of nidoviruses is generated via mutation and recombination. The estimated mutation rate for nidoviruses is similar to that of other RNA viruses with smaller genomes; however, the replication rate is associated with a high rate of recombination. Both homologous and heterologous recombination have been demonstrated in tissue culture, in infected animals and in cases of natural infection (Lai, 1990; Cavanagh, 2005; Gorbalenya et al., 2006). It seems probable that the discovery of new nidoviruses will provide new insight into the mechanisms underlying recombination. Such discoveries will also further our understanding of viral evolution and improve taxonomic classification.

20.2.4 Antigenic Components

The S and HE glycoproteins are the major antigens expressed by coronaviruses and both induce the production of neutralizing antibodies and a cell-mediated immune response in the host (Siddell et al., 1983; Lai and Cavanagh, 1997). The N protein may also trigger a protective immune response (Du et al., 2009). In addition, closely related coronaviruses show serological cross-reactivity (Bradburne, 1970) [ie, Human coronavirus 229E (HCoV 229E), Transmissible gastroenteritis virus (TGEV), Porcine respiratory coronavirus ISU-1 (PRCV ISU-1) and Feline infectious peritonitis virus WSU79-1146 (FIPV 79-1146)].

20.3 CORONAVIRUSES IN AQUATIC ORGANISMS

Few reports describe the presence of coronaviruses in aquatic organisms. Coronaviruses have been isolated from marine mammals, including beluga whale (*Delphinapterus leucas*) and bottlenose dolphin (*Tursiops aduncus*), as well as from freshwater cyprinid species such as white bream (*Blicca bjoerkna*) and fathead minnow (*Pimephales promelas*). However, not all of these viruses are officially classified as coronaviruses by the ICTV. Table 20.2 provides an overview of coronaviruses detected in aquatic organisms, along with their classification status. At present, no information is available regarding the ecology, route of infection, cell tropism, mode of transmission and/or putative vectors of coronaviruses that infect aquatic organisms.

TABLE 20.2 Coronaviruses That Infect Aquatic Organisms

Genus	Virus	References	GenBank Accession Number
Alphacoronavirus	Harbor seal coronavirus	Bossart and Schwartz (1990)	FJ766501
Betacoronavirus	n.d.		
Gammacoronavirus	Beluga whale coronavirus SW1	Mihindukulasuriya et al. (2008)	EU111742
	Bottlenose dolphin coronavirus	Woo et al. (2014)	KF793824, KF793825, KF793826
Deltacoronavirus	n.d.		
Torovirus	n.d.		
Bafinivirus	White bream virus	Granzow et al. (2001), Schuetze et al. (2006)	DQ898157
	Fathead minnow virus	Iwanowicz and Goodwin (2002), Batts et al. (2012)	GU002364, GU002365
	Chinook salmon bafinivirus	Lord et al. (2014)	

Viruses officially listed by ICTV are Beluga whale coronavirus SW1 and the White bream virus. To date, no beta-/deltacoronaviruses or toroviruses have been detected.
n.d., not detected.

20.3.1 Alphacoronaviruses

20.3.1.1 Harbor Seal Coronavirus (Unassigned)

The only probable case of coronavirus infection of harbor seals was reported by Bossart and Schwartz (1990). In 1987 three seals (*Phoca vitulina*) housed at the Miami Seaquarium were affected by an acute necrotizing enteritis. Two of the seals died without showing any clinical signs; however, the third exhibited marked leukocytosis, dehydration, hypernatremia and hyperchloremia. Pathological sections from all three seals revealed extensive focal bronchoalveolar hemorrhage and edema, with severe diffuse pulmonary congestion. Moderate-to-severe lymphoid depletion was detected in the spleen and in visceral and peripheral lymphnodes. Coronavirus-specific antigens were detected in the intestinal mucosa. Immunofluorescence staining with antibodies against TGEV, FIPV and canine coronavirus (CCoV) (all alphacoronaviruses) yielded positive results, whereas immunofluorescence staining for bovine coronavirus (BCoV) (a betacoronavirus) was negative. Cross-reactivity among coronaviruses is limited to closely related species (de Groot et al., 2012b). Based on antigenic cross-reactivity, the coronavirus infecting the harbor seals most probably belonged to the genus *Alphacoronavirus*. However, because none of the ICTV demarcation criteria were met, the pathogen was not assigned to the genus.

A short sequence derived from a coronavirus infecting another harbor seal, which encoded the RNA-dependent RNA polymerase, has also been published (NCBI Genbank Acc. No. FJ766501). It is assumed that further studies will confirm the presence of related viruses in harbor seals.

20.3.2 Gammacoronaviruses

20.3.2.1 Beluga Whale Coronavirus SW1 (BWCoV SW1)

The first report about identification of the complete genome from a coronavirus isolated from a marine mammal was published in 2008 (Mihindukulasuriya et al., 2008). A captive-born beluga whale died from acute liver failure after suffering a short generalized pulmonary disease. Histological examination confirmed acute hepatic necrosis. Electron microscopic examination of liver tissue revealed a great number of round viral particles in the cytoplasm of hepatic cells. The estimated particle size (60–80 nm in diameter, with a core of 45–50 nm) differed markedly from that of known coronaviruses. Attempts to isolate the pathogen in noncongeneric cell lines failed, indicating that the virus had a specific host tropism. The viral genome was approximately 31.7 kb in size and encoded nonstructural (replicase; ORF 1a and 1b) and structural (S (ORF 2), E (ORF 3), M (ORF 4) and N (ORF 11)) proteins, which were flanked by UTRs. In addition, eight putative ORFs were localized between the genes coding the M and N proteins. The deduced amino acid sequences showed no similarity to proteins from known coronaviruses. Nevertheless, characteristic molecular features were identified: that is, transmembrane domains (ORFs 5 and 9), putative secretion signal sequences (ORFs 7 and 8) and a uridine kinase motif (ORF 10). Phylogenetic analyses of the structural and replicase proteins revealed that the virus was most closely related to

avian coronaviruses, such as infectious bronchitis virus and turkey coronavirus. Although the homology of the conserved replicase domain (<67%) was markedly lower than the species demarcation threshold (90%), BWCoV SW1 was characterized as a species belonging to the genus *Gammacoronavirus* (de Groot et al., 2012b). Woo et al. (2014) suggested that BWCoV SW1, along with bottlenose dolphin coronaviruses, be listed as a distinct species, *Cetacean coronavirus*, within the genus *Gammacoronavirus*.

20.3.2.2 Bottlenose Dolphin Coronavirus (BdCoV HKU22, Unassigned)

A marine surveillance study conducted in the Ocean Park in Hong Kong between 2008 and 2010 identified a coronavirus in Indo-Pacific bottlenose dolphins (Woo et al., 2014). The coronavirus genome was detected in fecal samples from three apparently healthy animals. In contrast to the isolate from beluga whale, which caused liver damage, the BdCoV did not induce any clinical signs/symptoms, indicating an asymptomatic or mild infection. The viral genome was detected during the infectious stage, whereas N-specific antibodies were detected in serum samples at 4–8 weeks postinfection. The nearly identical viral genomes isolated from the three bottlenose dolphins belonged to the genus *Gammacoronavirus*. Attempts to replicate the viruses in several nonhost-specific cell lines were unsuccessful and information about the ultrastructure of the pathogen is lacking. Therefore, the evidence for BdCoV is based on characterization of the viral genome. Genetic analyses suggested that BdCoV was closely related to BWCoV SW1. Both viruses possess a genome of approximately 31.7 kb and both genomes show similar organization. The N protein and conserved domains within replicase polyprotein pp1ab of BdCoV and BWCoV SW1 show 99.6–99.3% homology; however, they are less homologous with avian coronaviruses (35.4–74.9%). The major difference between the two viruses was detected in the spike protein. Phylogenetic analysis and pairwise amino acid alignments indicated that BWCoV and BdCoV clustered separately within the genus *Gammacoronavirus*. This finding is consistent with the close genetic relationship between the respective hosts, both of whom belong to the order *Cetacea*. Therefore, Woo et al. (2014) proposed a new viral species: *Cetacean coronavirus*.

20.3.3 Bafinivirus

20.3.3.1 White Bream Virus

White bream virus (WBV) is the type species of the genus *Bafinivirus* within the subfamily *Torovirinae*. The name bafinivirus was coined because the virus shows bacilliform morphology (ba-) and is a fish (fi-) nidovirus (ni-). The pathogen was isolated from a healthy white bream (*B. bjoerkna* L.) caught in the Federal State of Saxonia-Anhalt in Germany during a program designed to monitor pathogens in wild freshwater fish (Granzow et al., 2001). The virus replicates at 20–26°C in *Epithelioma papulosum cyprini* (EPC; CCLV Rie 173), Blue gill fry (BF-2; CCLV Rie 290), fathead minnow (FHM; CCLV Rie 057) and carp pituitary (CaPi; CCLV Rie 112) cells and induces cytopathic effects. The pathogen is sensitive to chloroform. Viral particles exhibit ultrastructural features similar to those described for *Roniviridae* [eg, gill-associated virus (GAV-AUS) and yellow head virus (YHV)] isolated from prawns (Chantanachookin et al., 1993; Spann et al., 1997). The mature enveloped virions measure 170–200 nm in length and 75–88 nm in diameter and are surrounded by fuzzy spikes approximately 20–25 nm long. The rigid rod-shaped nucleocapsid measures 120–150 × 19–22 nm and the buoyant density of the virus in sucrose is 1.17–1.19 g/cm^3. The viral genome is 26.6 kb in length and encodes five ORFs, representing the large replicase gene and the structural proteins S, M and N. The virus does not encode a hemagglutinin esterase protein or a homolog of the coronavirus E protein. WBV exhibits molecular features typical of nidoviruses, including a polycistronic genome, the production of sg RNAs during transcription, RFS to express the replicase core domains at the translational level, and post-translational proteolytic processing of viral proteins (Schuetze et al., 2006). Comparative sequence analyses showed that WBV is more closely related to toroviruses than to the other members of the order *Nidovirales*. Although WBV is related to toroviruses, it is different in several important aspects. First, WBV does not encode the structural gene HE. Second, all WBV sg RNAs possess a 5′ leader sequence, whereas three out of the four equine torovirus (strain Berne) sg RNAs lack a 5′ leader. Third, *Bafinivirus* does not encode cyclic nucleotide phosphodiesterase, which is conserved in bovine and equine toroviruses. Fourth, the nonstructural gene products and the structural proteins M, N and S of WBV and torovirus are poorly conserved (Schuetze et al., 2006). Finally, cyprinids are the natural hosts of WBV, whereas mammals are the natural hosts of toroviruses.

Pathogens with similar morphological, physicochemical and genetic features to the WBV have been isolated from other cyprinids, including goldfish (*Carassius a. auratus*), tench (*Tinca tinca*) and grass carp (*Ctenopharyngodon idella*) (Fichtner et al., 2005). Some fish suffer hemorrhage on the gills and skin, or ulceration of the skin. Cross-reactivity between anti-WBV antibodies and viral isolates from goldfish, tench and grass carp confirms a relationship between these

pathogens. Other WBV-like pathogens were isolated from tench and carp after clinical events and fatalities in Germany in 2007 (Fichtner et al., 2009). Infection of specific pathogen-free carp, goldfish and crucian carp (*Carassius carassius*) with a WBV-like pathogen isolated from a dead carp caused nonspecific clinical signs such as exhaustion, reduced food intake, increased mucus production, pseudofeces and petechiae. No mortalities were recorded (Fichtner et al., 2009). Thus coronaviruses appear to be prevalent in cyprinids. Genomic analyses of these pathogens are necessary to confirm a relationship with bafinivirus WBV and to support a clear taxonomic classification of cyprinid coronaviruses.

20.3.4 Unassigned Viruses

20.3.4.1 Fathead Minnow Virus (FHMNV, An Unassigned Member of the Nidovirales)

Fathead Minnow Virus (FHMNV) was first isolated from moribund fathead minnows (*P. promelas*) on a baitfish farm in central Arkansas in 1997 (Iwanowicz and Goodwin, 2002). Affected fish exhibited hemorrhage of the skin and eyes, as well as hepatic, splenic and renal lesions. The virus replicates in EPC, FHM and RTG (rainbow trout gonad) cells between 15°C and 25°C. A unique feature of this pathogen is the production of syncytia in both tissues and cell culture. The bacilliform particles measure 130–185×31–47 nm. The complete genome sequence suggests that the virus belongs to the genus *Torovirus* (Batts et al., 2012). The viral ssRNA is approximately 27 kb in length and displays the same genome organization as WBV, with molecular features typical of a nidovirus genome. Comparison of FHMNV helicase (pp1ab domain), S, M, N and ORF 1ab gene products with those of WBV reveals differing levels of homology, ranging from 15% (S protein) to 70% (helicase). The FHMNV pp1ab sequence is 11% homologous with that of equine arteritis virus, whereas the FHMNV helicase is 35% homologous with that of Equine torovirus (EToV). Phylogenetic analysis of the conserved helicase domain revealed that, although FHMNV is a distinct virus, it is the closest relative of WBV identified to date (Batts et al., 2012).

20.3.4.2 Chinook Salmon Bafinivirus (An Unassigned Member of the Nidovirales)

The first report of a bafinivirus isolated from Chinook salmon (family, *Salmonidae*; genus *Oncorhynchus*) was published in 2014 (Lord et al., 2014). The virus replicates and induces cytopathic effects in RTG-2 and EPC cells at 15°C, 20°C and 25°C. The enveloped particles have a bacilliform structure and are 45 nm in diameter and 120–130 nm in length. The virus possesses a genome of approximately 27 kb, with a gene organization consistent with that of bafiniviruses. Amino acid alignments suggest that this new virus is related to WBV and FHMV.

20.3.4.3 Carp Viruses

Several reports describe the isolation of potential coronaviruses from carp. Next is an overview of other putative coronaviruses reported in the literature. Their relationship to coronaviruses is unclear from an ultrastructural and morphogenetic perspective. Furthermore, it should be noted that these isolates do not fulfill the criteria for taxonomic classification (they are missing genomic and/or morphological data) and are therefore not officially listed by the ICTV.

20.3.4.4 Ctenopharyngodon idella Virus Hungary 33/86 (CIVH 33/86)

In 1986 an unknown virus was isolated from an apparently healthy grass carp (*C. idella*) imported into Germany from Hungary (Ahne et al., 1987). The isolate, CIVH 33/86, multiplies in CAR cells (goldfish [*Carassius auratus*] fin cells), CLC cells (common carp [*Cyprinus carpio*] leukocytes) and FHM cells between 20°C and 25°C. Infected cells fuse and then lyse. The virus is inactivated by chloroform, acid solutions (pH 3) and a temperature of 56°C. Treatment with 5-iododeoxyuridine (IUdR) has no effect on viral replication. Treatment with acridine orange confirmed the presence of a single-stranded viral RNA genome. Electron micrographs revealed enveloped rod-shaped particles measuring 170–220 nm in length and 50–55 nm in width. Finally, the isolate exhibits morphological features consistent with those of toroviruses (Ahne et al., 1987).

20.3.4.5 Carp Coronavirus

Sano et al. (1988) isolated a coronavirus from a common carp that died after suffering an acute infection; clinical signs included erythema on the abdomen and hepatic and renal necrosis. The virus is transmitted to carp fry via the water at a temperature of 20 °C. The pathogen was isolated from the kidney, liver and spleen, and replicates in BF-2, EPC, RTG-2 and FHM cells at 20°C. The enveloped particles measure 60–100 nm in diameter.

20.3.4.6 Carp Viremia-Associated Ana-Aki-Byo

A putative coronavirus, named carp viremia-associated ana-aki-byo, was responsible for high mortality rates in color carp (*C. carpio*) between 1997 and 1998 in Japan (Miyazaki et al., 2000). Necrotic lesions were observed in the visceral organs and virus particles were detected in hematopoietic tissue and spleen. The virus was resistant to IUdR, but sensitive to ether, suggesting that it was enveloped RNA virus. EPC cells were susceptible to infection at 25°C, but EK-1 (eel kidney), FHM, CHSE-214 (chinook salmon embryo) and RTG-2 cells were not. Infected EPC cells displayed karyopyknosis and intracytoplasmic vacuolization. The virus was round and displayed spike projections on the surface. The diameter of the virion ranged from 100 to 180 nm. Tubular structures (diameter, 60–70 nm) of various lengths and crystalline inclusions were observed in the cytoplasm of infected cells.

In summary, studies suggest that coronaviruses are prevalent in cyprinids and salmonids. It is hoped that new analytical tools, methods and technologies will enable the discovery and identification of other coronaviruses and facilitate further research into their molecular biology, phylogeny and evolution. Increased knowledge about the prevalence, distribution and transmission of these viruses, along with further identification of affected host species and ecological factors, will aid efforts to monitor and control infections and to assess the risk of transmission of coronaviruses in aquatic organisms.

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