

# Complete genomic sequence of an infectious pancreatic necrosis virus isolated from rainbow trout (*Oncorhynchus mykiss*) in China

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**Abstract** Infectious pancreatic necrosis (IPN) is a significant disease of farmed salmonids resulting in direct economic losses due to high mortality in China. However, no gene sequence of any Chinese infectious pancreatic necrosis virus (IPNV) isolates was available. In the study, moribund rainbow trout fry samples were collected during an outbreak of IPN in Yunnan province of southwest China in 2013. An IPNV was isolated and tentatively named ChRtm213. We determined the full genome sequence of the IPNV ChRtm213 and compared it with previously identified IPNV sequences worldwide. The sequences of

different structural and non-structural protein genes were compared to those of other aquatic birnaviruses sequenced to date. The results indicated that the complete genome sequence of ChRtm213 strain contains a segment A (3099 nucleotides) coding a polyprotein VP2–VP4–VP3, and a segment B (2789 nucleotides) coding a RNA-dependent RNA polymerase VP1. The phylogenetic analyses showed that ChRtm213 strain fell within genogroup 1, serotype A9 (Jasper), having similarities of 96.3% (segment A) and 97.3% (segment B) with the IPNV strain AM98 from Japan. The results suggest that the Chinese IPNV isolate has relative closer relationship with Japanese IPNV strains. The sequence of ChRtm213 was the first gene sequence of IPNV isolates in China. This study provided a robust reference for diagnosis and/or control of IPNV prevalent in China.

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**Keywords** Aquatic birnaviruses · Genogroups · Infectious pancreatic necrosis virus ChRtm213 · Molecular characterization · Phylogeny · Rainbow trout

## Introduction

The aquatic birnaviruses are the largest and most diverse group of viruses within the family *Birnaviridae*. They include a variety of viruses from numerous species of fish and marine invertebrates worldwide [1, 2]. Infectious pancreatic necrosis (IPN) was first described in a freshwater brook trout (*Salvelinus fontinalis*) facility in North America in the 1950s [3] with the subsequent isolation of the virus reported by Wolf et al. [4]. In Europe, the disease was initially reported in freshwater rainbow trout (*Oncorhynchus mykiss*) farms in France [5], Denmark [6], Scotland [7], and Norway [8]. By the mid-1970s, IPN had

**Table 1** Characteristics of the primers used to amplify genome cDNA of IPNV ChRtm213 isolate

Primer	Name	Nucleotide position	Product length (bp)
5' CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCA GAGT 3'	UPM-long		
5' CTAATACGACTCACTATAGGGC 3'	UPM-short		
5' AAGCAGTGGTATCAACGCAGAGT 3'	NUP		
5' GGT CGA ATT CTA GTG CCG T 3'	5'RACE-A	348–366	366
5' AAG AAG TGA AGG ACG CCG AAG TG 3'	3'RACE-A	2258–2281	595
5' CAG ACG GCA CTA GAA TTC GAC CAG TGG C 3'	AU	345–373	1998
5' CAT GGT GGC CCC TGA TGC AGT TGT 3'	AL	2319–2343	
5' GGAAACAGTGGGTCAACG 3'	B1U	1–18	1524
5' CCGTGGGTCTTCAGTGAAGATTC 3'	B1L	1500–1524	
5' CAAACGAGGATGGCTCTCCGCGGTACAATC 3'	B2U	1383–1413	1407
5' GGGGTCCCTGGCGGAAC 3'	B2L	2772–2789	

Composition and locations of the oligonucleotide primers used for cloning are shown. The UPM pairs and NUP were used in RACE as RNA joints. The primers of RACE and AU/L were used to amplify genome cDNA of complete segment A, and the primers of B1U/L and B2U/L were used to amplify genome cDNA of complete segment B. The positions where the primers bind (nucleotide numbers) are according to the published sequence [32]

been reported in North America, Europe, and Japan [9]. In 1986, the first outbreak of IPN was recorded in hatcheries for juvenile rainbow trout in Shanxi Province, northwest China [10]. This was followed by a series of IPN outbreaks in cultured juvenile rainbow trout in various districts of China [11–13].

IPN, a significant disease of farmed fish worldwide, is caused by infectious pancreatic necrosis virus (IPNV), a member of the family *Birnaviridae*, a family of non-enveloped, double-stranded RNA viruses with a bi-segmented (A and B) genome encoding five or six proteins [14]. The genome segment A (3.1 kb) contains two partially overlapping open reading frames (ORFs), and the large one encodes a 106 kDa polyprotein (pVP2–VP4–VP3), which will be co-translationally cleaved by the viral non-structural protease (VP4; 29 kDa) to generate pre-VP2 (pVP2, 63 kDa) and VP3 (29–31 kDa) proteins. There is a small ORF which overlaps with the amino-terminal end of the large ORF and generates a 15 kDa (VP5) non-structural polypeptide [15–17]. Both VP2 (449 aa) and VP3 (217 aa) are major components of the virion, while VP4 (252 aa) is present in lesser amounts. Major neutralizing epitopes are carried on VP2, suggesting that it is at least partly exposed in the outer surface of the capsid, and the VP2 polypeptide is glycosylated in the virion [16, 18, 19]. The nucleotide sequence of segment B encoding the protein VP1 is found in two forms: RNA-dependent RNA polymerase (RdRp), which occurs as a free polypeptide, and a protein VPg, which is linked to the 5' end of both genome segments [20]. The aquabirnaviruses can be divided into two distinct serogroups, A and B, based on results from cross-

neutralization assays [21]. Serogroup A contains the majority of IPNV isolates, including those associated with disease in salmonids, and within this group nine serotypes exist: A1 (reference strain WB)(West Buxton), A2 (Sp)(Spajarup), A3 (Ab)(Abild), A4 (He)(Hecht), A5 (Te)(Tellina), A6 (C1)(Canada 1), A7 (C2)(Canada 2), A8 (C3)(Canada 3), and A9 (Ja)(Jasper) [22]. Serotype A1 includes USA isolates, serotypes A6–A9 are mainly detected in Canada, and serotypes A2–A5 and B1 are found in Europe and Asia [23]. Blake et al. demonstrated that IPNV strains were clustered into six genogroups (1–6) based on amino acid sequences of the VP2 [1]. Many researchers suggested that all marine birnaviruses should form a new genogroup (genogroup 7) different from the six IPNV genogroups [24–26].

In China, an increase in outbreaks of IPN in rainbow trout has been reported in recent years [12, 27]. However, no gene sequences of any Chinese IPNV strains have been submitted to the GenBank database. There is only one complete gene sequence of a Chinese marine birnavirus strain, *Paralichthys olivaceus* birnavirus (POBV), that is available [28]. However, the marine birnavirus is of the different genogroups and serotypes with the IPNV isolated from salmonids. In this study, a novel IPNV strain was isolated from farmed rainbow trout in Yunnan Province, southeast China, in 2013. This strain was designated as IPNV ChRtm213. To facilitate the detailed comparison of the sequence characteristics of a Chinese IPNV strain with other phylogenetically related IPNV strains isolated from around the world, the complete IPNV ChRtm213 genomic sequences were described. The sequence of IPNV

**Table 2** Segment A of aquabirnaviruses isolates used in this study

No.	Isolate	Host	Origin	Accession no.
1	AM-98	Amago salmon	Japan	AY283780
2	Y-6	Yellowtail	Japan	AY283781
3	YT-01A	Yellowtail	Japan	AY283782
4	NC1	Japanese flounder	South Korea	AY283784
5	AY-98	<i>Plecoglossus altivelis</i>	Japan	AY283785
6	Mexican	Rainbow trout	Mexico	JX174178
7	Iran	Rainbow trout	Iran	KF279643
8	2310	Salmon	Spain	AJ489225
9	Buhl	Trout	USA	AF343573
10	Jasper	Unk	Canada	NC_001915
11	HL-1	Unk	Korea	D26526
12	Connecticut-1	Salmo salar	USA	JF440810
13	VR299	Trout	USA	AF343572
14	GC-1	Rockfish	Korea	AY064396
15	West Buxton (WB)	Trout	USA	AF342727
16	Sp	Trout	Denmark	AF342728
17	N1	Atlantic salmon	Norway	D00701
18	He	Pike	Germany	AF342730
19	Canada1 (C1)	Trout	Canada	AF342731
20	Canada2 (C2)	Trout	Canada	AF342733
21	Canada3 (C3)	<i>Arctic char</i>	Canada	AF342734
22	1146	Trout	Spain	AJ489222
23	2284	Trout	Spain	AJ489223
24	YTAV-06	Yellowtail	Japan	AB281673
25	POBV	<i>Paralichthys olivaceus</i>	China	EU161285
26	578	Turbot	Spain	AJ489228
27	2290	Salmon	Spain	AJ489224

ChRtm213 was the first sequence of IPNV isolates in China, and it will be used as a reference strain of IPNV in China in the future.

## Materials and methods

### Cell line and virus strain

Moribund rainbow trout fry samples were collected from Yunnan province of southeast China in 2013. The Chinook salmon Embryo cell line (CHSE-214) was used to isolate and propagate the IPNV isolate. The methods of virus stock, growth, and purifications refer to a previous study [17]. The IPNV isolate was passaged two times prior to sequencing.

### Primers design

To design the overlapping primers, available complete genome sequences of IPNV isolates from the National

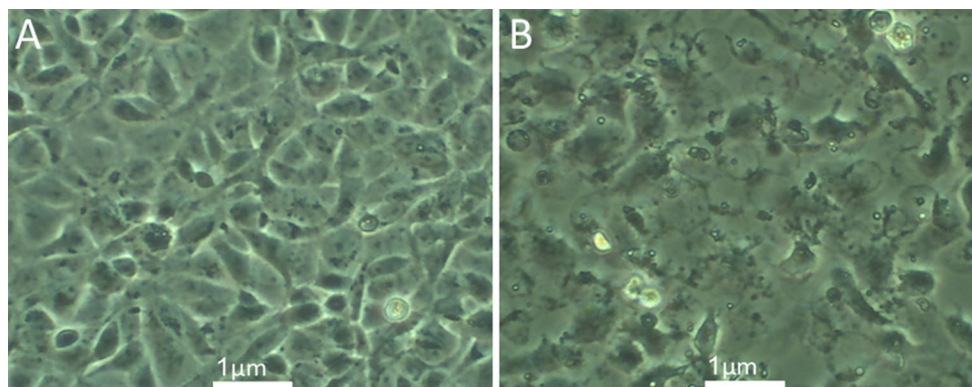
Center for Biotechnology Information were aligned. Five pairs of overlapping primers that cover the entire IPNV genome were designed using Oligo 6.0 and Primer Premier Version 5 software on the basis of the full-length genome sequences of IPNV isolates in GenBank database. The 5' rapid amplification of cDNA ends (5' RACE) [29] represents useful PCR-based approaches for the determination of 5' flanking cDNA sequence. The 3' RACE by lariat-dependent nested PCR (3' RACE LaNe) [30] is a simple and rapid method that allows "two-sided" gene-specific, fully nested PCR series for the elucidation of 3' flanking cDNA sequence without the requirement for ligation [31]. All primers were synthesized by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. Table 1 presents the primers used in this study.

### Amplification of the IPNV complete genome

Total RNA was extracted from the cell culture supernatant using the RNeasy RNA extraction kit (Qiagen, Germany)

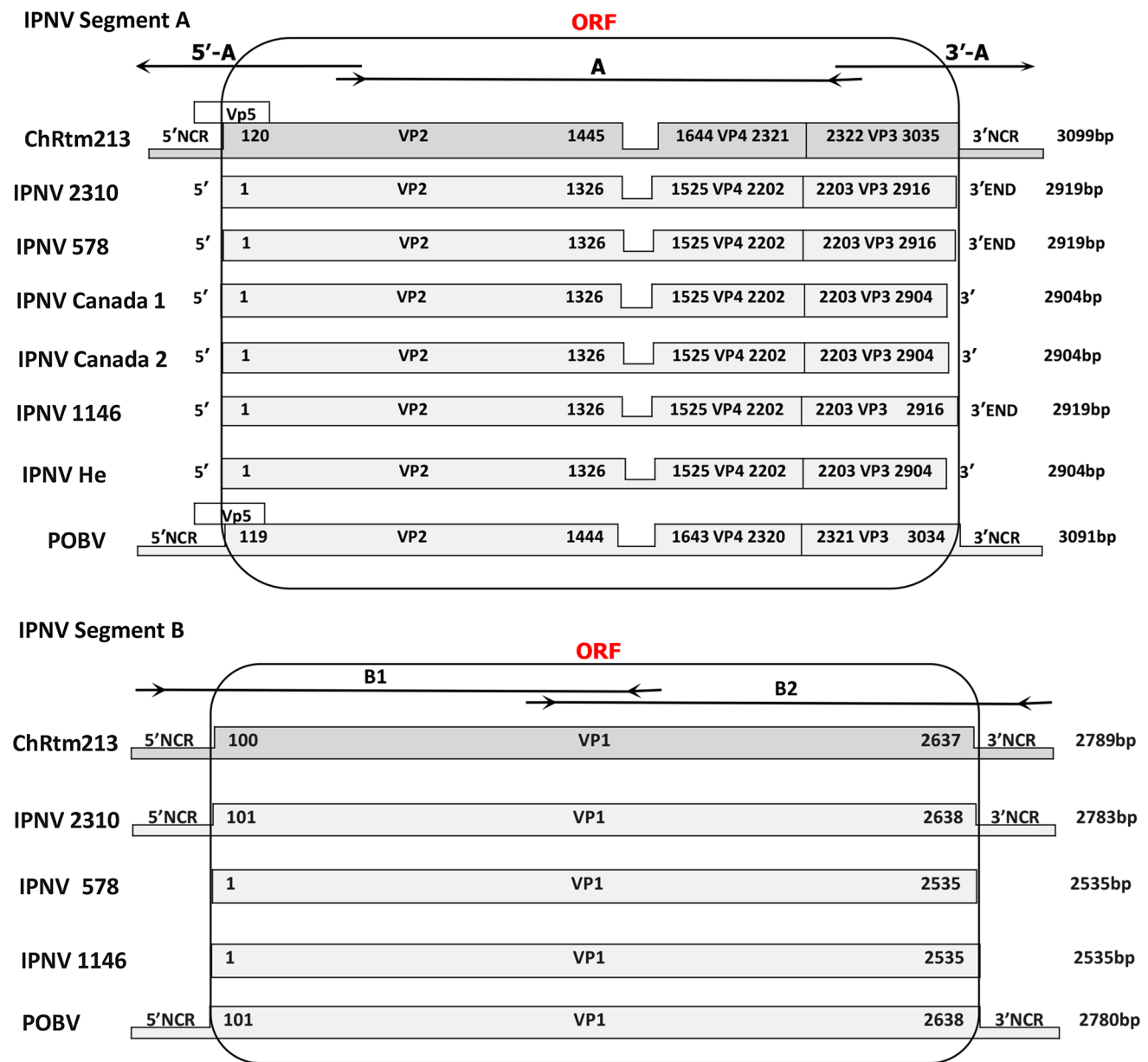
**Table 3** Segment B of aquabirnaviruses used in this study

No.	Isolate	Host	Origin	Accession no.
1	2284	Rainbow trout	Spain	AJ489239
2	31-75	Rainbow trout	France	AJ622823
3	IRIPNV	Fry rainbow trout	Iran	KC900161
4	YTAV-06	Yellowtail	Japan	AB281674
5	West Buxton	Unk	USA	AF078669
6	2310	Salmon	Spain	AJ489241
7	AY-98	<i>Plecoglossus altivelis</i>	Japan	AY123970
8	Y-6	Yellowtail	Japan	AY129662
9	YT-01A	Yellowtail	Japan	AY129663
10	AM-98	Amago salmon	Japan	AY129664
11	H1	Yellowtail	Japan	AY129665
12	NC1	Japanese flounder	Korea	AY129666
13	Sp103	Unk	Norway	AY354522
14	Sp116	Unk	Norway	AY354523
15	Sp122	Unk	Norway	AY354524
16	NVI-016	Unk	Norway	AY379743
17	17F2	Greenland halibut	Spain	AY780927
18	VP5-deficient Sp103	Unk	Norway	AY823633
19	HL-1	Unk	Korea	D26527
20	POBV	<i>Paralichthys olivaceus</i>	China	EU161286
21	Mexican	Rainbow trout	Mexico	EU665685
22	TAB 98	<i>Salmo salar</i>	Australia	EU672430
23	TABV 11-04755	Rainbow trout	Australia	KP268676
24	Jasper	Unk	Canada	NC_001916
25	1146	Trout	Spain	AJ489238
26	578	Turbot	Spain	AJ489244

**Fig. 1** Cytopathic effect (CPE) of CHSE-214 cells caused by IPNV. Healthy CHSE-214 cells were used as a negative control (a). Monolayers of CHSE-214 cultured in six-well plate were inoculated with IPNV ChRtm213 with a multiplicity of infection (MOI) of 0.1 (b)

following manufacturer's instructions and then stored at  $-80^{\circ}\text{C}$ . Reverse transcription polymerase chain reaction (RT-PCR) was performed in different tubes with use of the PrimeScript<sup>TM</sup> One-step RT-PCR kit (TaKaRa, Shiga, Japan). Viral RNA (1  $\mu\text{g}$  in 4  $\mu\text{L}$   $\text{H}_2\text{O}$ ) was mixed with 0.5  $\mu\text{g}$  of random hexamers in a final volume of 7.2  $\mu\text{L}$ . Concentration of primers used in PCR is 1 mmol/L.

Amplification reactions were carried out on polymerase chain reaction (PCR) instrument (Eppendorf AG, Germany) using the polymerase Ex Taq (TaKaRa, Shiga, Japan) with heated lid, programmed to perform 1 cycle of  $50^{\circ}\text{C}$  for 30 min, then, 1 cycle of  $94^{\circ}\text{C}$  for 2 min followed by 30 cycles of denaturing at  $94^{\circ}\text{C}$  for 30 s, annealing at  $55^{\circ}\text{C}$  for 30 s and extension at  $72^{\circ}\text{C}$  for



**Fig. 2** Schematic diagram of the deduced genomic organization of ChRtm213 and comparison with IPNV isolates from genogroup 1–7, including 2310, 578, Canada 1, Canada 2, 1146, He, and POBV, respectively. The straight lines with arrow show locations of the

cDNA clones used in determining the ChRtm213 complete genome. Shaded areas indicated the coding regions, and the unshaded areas represent non-coding regions (NCR). Numbers indicated the start and end of the respective ORF

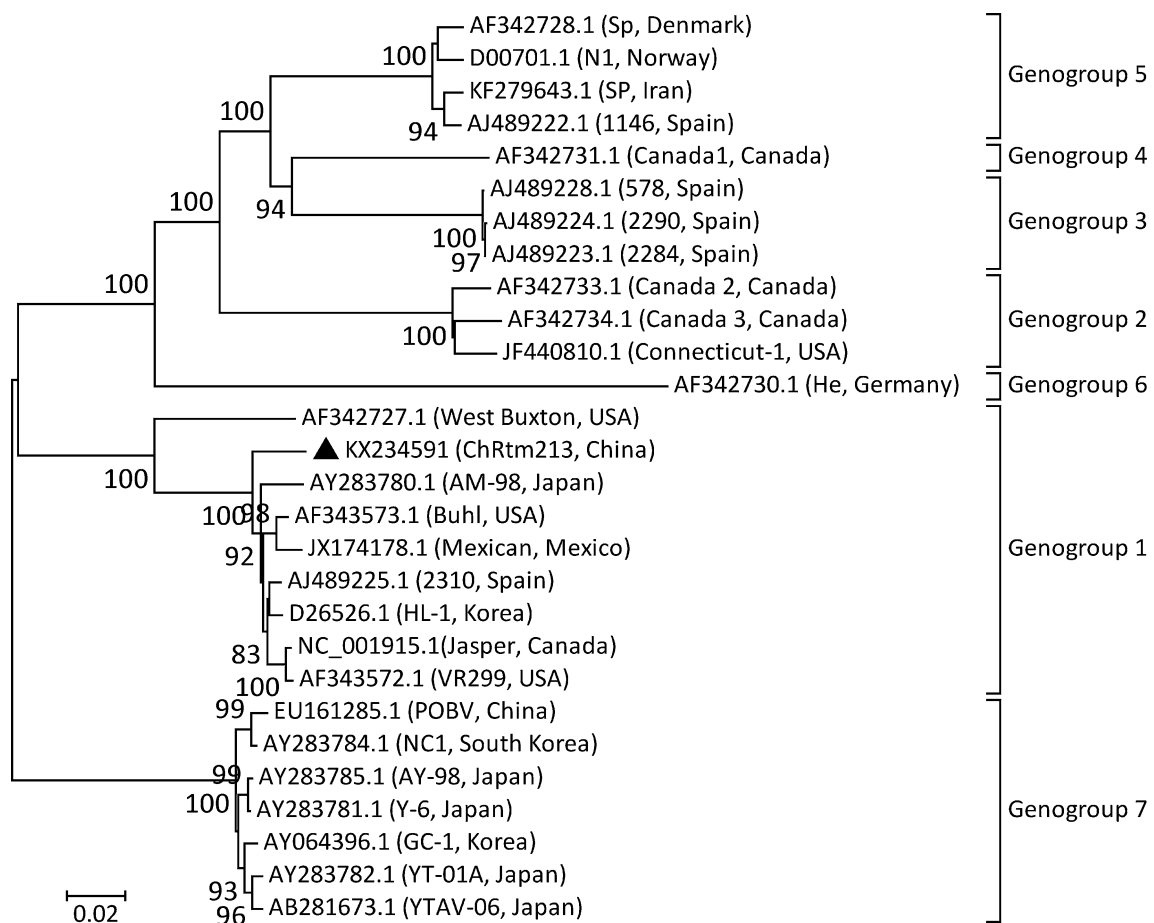
2 min followed by a single extension step of 5 min at 72 °C. The amplified products were analyzed by electrophoresis on a 1% agarose gel. The gel was stained with ethidium bromide and visualized under UV light.

The sequences of two ends of segment A were determined using the 5' RACE and 3' RACE systems for amplification of cDNA ends Version 2.0 (Invitrogen) using both dC-tailing and dA-tailing methods according to the manufacturer's instructions. This assay was done in duplicate to obtain the complete nucleotide sequence of segment A and segment B of the Chinese IPNV isolate

ChRtm213, and the sequences were deposited in GenBank database.

**Sequence alignment and analyses**

All PCR products with the expected size were separated from the agarose gel and purified using an AxyPrep DNA Gel Extraction Kit (Axygen, USA). The purified products were cloned using a pMD18-T simple TA vector (TaKaRa, Dalian). For each of the overlapping fragments, at least three positive clones were randomly selected and



**Fig. 3** Phylogenetic relationship of ChRtm213 and other reference aquabirnaviruses strains based on complete genome of segment A. Phylogenetic tree was constructed using the neighbor-joining method

and bootstrap analysis ( $n = 1000$ ) in MEGA 5.05. The ChRtm213 strain is highlighted in *black triangle*

sequenced by Shenzhen, China-BGI Tech Solutions Co., Ltd.

The contiguous region between the forward and reverse sequences was assembled and verified using DNASIS software (Hitachi Software Engineering Co., Ltd.). Laser-gene software (DNASStar, Inc., USA) was used to translate and conduct multiple alignments of the sequences, as well as to determine the percentage identities and similarity scores. To analyze the percentage identities and similarity scores, both nucleotide and amino acid sequences were aligned using the default options in Clustal W. Phylogenetic trees were constructed using the neighbor-joining method using MEGA Version 5.05 software [33]. The Kimura two-parameter distance measurements were used as the substitution model. The reliability of the phylogeny groupings was evaluated using bootstrapping with 1000 replicates. Aquabirnaviruses isolates used for analysis in this study are presented in Table 2 (segment A) and Table 3 (segment B), respectively. All these isolates had clear genogroups and were commonly used as reference strains in previous studies [14, 34].

## Results

### Genome organization of the IPNV strain ChRtm213

The IPNV isolate ChRtm213 was isolated and propagated in CHSE-214 cells, and was harvested when most of the cells showed obvious cytopathic effect (CPE) (Fig. 1). After two passages in CHSE-214 cells, IPNV was collected and subjected to genome sequencing. Segments A and B of IPNV isolate of ChRtm213 that covered all ORFs and flanking regions were assembled from RT-PCR amplifications. Segment A of the ChRtm213 is 3099 bp long and producing fragments of 1359, 918, 447, and 750 bp encoding VP2, VP4, VP5, and VP3 genes, respectively. Segment B of the ChRtm213 genome is 2789 bp long and encodes VP1 protein, a minor internal protein (93 kDa). The segment B of ChRtm213 begins with the conserved sequence GATTC and ends with the sequence GGCGAC, containing the ORF of VP1, which consists of 2535 nucleotides, beginning at the nucleotide 135 and ending with a TGA termination codon at nucleotide 2670. The full-length genomic nucleotide sequence of strain ChRtm213 was

**Table 4** Similarities of IPNV ChRtm213 with other aquabirnaviruses

No.	Isolate Segment A	Similarity (%)	Isolate Segment B	Similarity (%)
1	AM-98	96.3	AM-98	97.3
2	2310	97.3	2310	97.8
3	Mexican	96.7	Mexican	96.2
4	Jasper	96.8	Jasper	89.3
5	West Buxton (WB)	90.4	West Buxton	90.7
6	HL-1	96.5	HL-1	97.4
7	POBV	84.3	POBV	85.5
8	2284	78.4	2284	81.3
9	AY-98	84.7	AY-98	85.4
10	Y-6	84.6	Y-6	85.7
11	YTAV-06	84.4	YTAV-06	85.3
12	YT-01A	84.3	YT-01A	85.6
13	NC1	84.5	NC1	85.6
14	Buhl	97.0	17F2	90.7
15	VR299	96.9	H1	85.6
16	GC-1	84.3	TAB 98	82.0
17	Sp	78.9	TABV 11-04755	81.9
18	N1	77.2	Sp103	81.2
19	He	74.7	Sp116	81.2
20	Iran	78.9	Sp122	81.2
21	Connecticut-1	78.3	NVI-016	81.3
22	Canada1 (C1)	78.2	VP5-deficient Sp103	81.2
23	Canada2 (C2)	78.5	31-75	81.0
24	Canada3 (C3)	77.9	IRIPNV	81.3
25	1146	78.8	1146	81.6
26	578	78.5	578	81.3
27	2290	78.4		

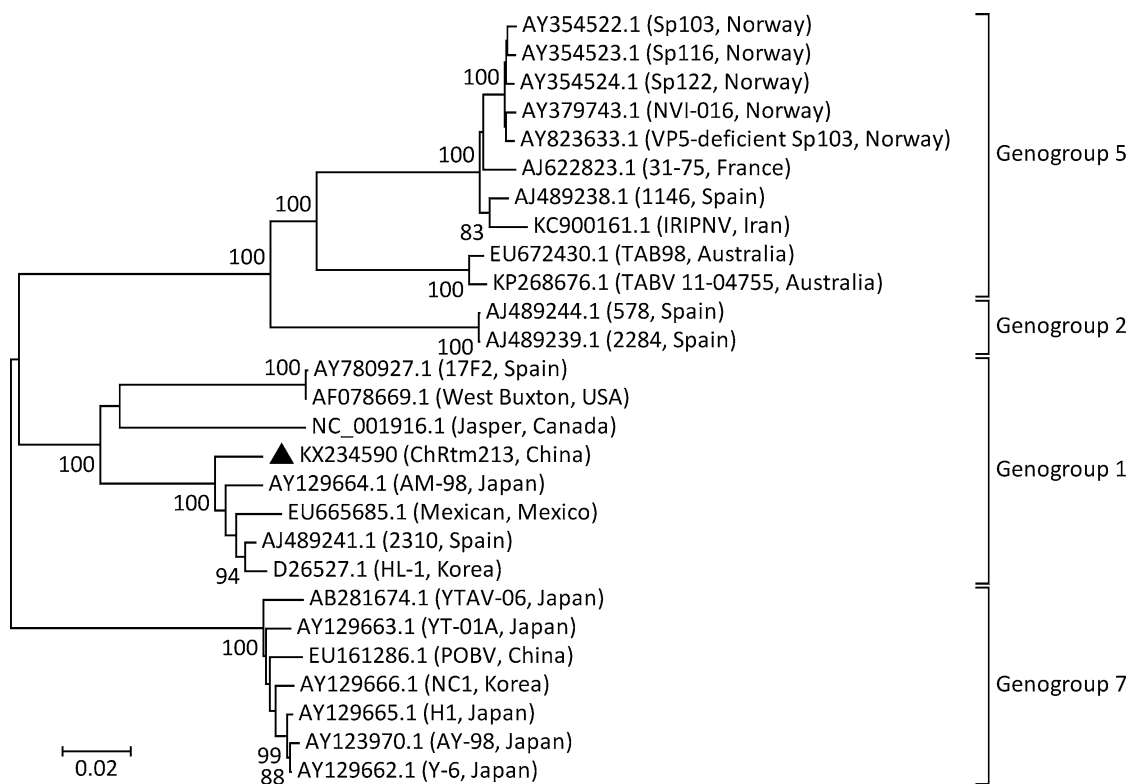
deposited in the GenBank database under Accession numbers KX234591 (segment A) and KX234590 (segment B). Schematic diagram of the deduced genomic organization of ChRtm213 and comparison with IPNV isolates from different genogroups are shown in Fig. 2.

### Homology and phylogenetic analyses

Unrooted phylogenetic trees for genomic segment A and B were built by the neighbor-joining method [35–37]. The phylogenetic tree analyses of the sequences of the segment A and B of various IPNV strains are shown in Figs. 2 and 3, respectively. Aquabirnavirus strains were clustered into seven genogroups in the phylogenetic tree based on segment A (Fig. 3). The results of phylogenetic analysis of the segment A showed that ChRtm213 clustered with IPNV strains including Spain 2310, Japanese AM98, American VR299, Buhl, WB, Korean HL-1, Canadian Jasper, and Mexican Mexican within the genogroup 1 (Fig. 3), and maintained 97.3, 96.3, 96.9, 97.0, 90.4, 96.5, 96.8, and 96.7% nucleotide

identity with the eight strains, respectively (Table 4). The succeeding nearest neighbor of ChRtm213 was Japanese aquabirnavirus Y-6 and had 84.6% nucleotide identity for the segment A. The results of phylogenetic analysis of the segment B were accordant with the results from the phylogenetic analysis of the segment A, and the ChRtm213 also clustered with these IPNV strains within the genogroup 1 (Fig. 4). The succeeding nearest neighbor of ChRtm213 also was Japanese aquabirnavirus Y-6 and had 85.7% nucleotide identities for the segment B. The ChRtm213 isolate had a relative close relationship with a Chinese marine birnavirus POBV isolated from *P. olivaceus*, and the identities of segment A and B were 84.3 and 84.5%, respectively (Table 4). The results indicated that the ChRtm213 isolate belonged to genogroup 1, and has closer relationship with reference strains 2310, Jasper, and AM-98.

Amino acid similarities of each ORF from ChRtm213 with other aquabirnaviruses from different genogroups were analyzed based on genomic sequences. Single IPNV isolates representing genogroups 1–6 and a marine birnavirus representing



**Fig. 4** Phylogenetic relationship of ChRtm213 and other reference aquabirnavirus strains based on complete genome of segment B. Phylogenetic tree was constructed using the neighbor-joining method

and bootstrap analysis ( $n = 1000$ ) in MEGA 5.05. The ChRtm213 strain is highlighted in *black triangle*

genogroup 7 were used in the analysis (Table 5). The results showed that the ChRtm213 had the highest identities with Spain 2310 strain of genogroup 1. The similarities of VP1, VP2, VP3, VP4, and VP5 between ChRtm213 and Spain 2310 were 99.4, 97.1, 98.2, 99.2, and 91.0%, respectively. The results showed accordance with that of the phylogenetic tree analyses, strongly supporting that the ChRtm213 belongs to genogroup 1. Among these isolates from different genogroups, an isolate which has the succeeding highest similarity with ChRtm213 was the Chinese marine birnavirus POBV belonging to genogroup 7. The similarity identities of VP1, VP2, VP3, VP4, and VP5 between ChRtm213 and POBV were 97.0, 89.0, 89.4, 88.1, and 80.6%, respectively (Table 5). Compared to other viral proteins of IPNV, VP5 is not conservative, and the similarities of VP5 between ChRtm213 and other strains were from 59.4 to 91.0%. In the ChRtm213, three extra termination codons were induced in the location of amino acid 43, 48, and 104 in the VP5, resulting in a truncated VP5 (Fig. 5).

## Discussion

The first report of an IPNV outbreak in northwest China involved the rainbow trout fry and occurred in 1986 [10] followed by outbreaks of IPN occurred in Heilongjiang,

Jilin, Shandong, Liaoning, and Gansu provinces of China at the late 1980s [10, 11, 13, 38]. In these previous reports, Chinese IPNV strains were isolated and characterized, and serological analyses showed that all the isolated IPNV strains more likely belonged to A2 (Spajarup, Sp) serotype [10, 11, 21]. After this event, no available information of IPN in China can be obtained until 2011 in which another IPNV strain was isolated from a rainbow trout farm in Liaoning province of China [12]. Although there are many reports on IPNV in China, no sequence of any Chinese IPNV isolates is available, and previous studies showed that IPN mainly occurred in the northern part of China. However, in this study, the IPNV ChRtm213 was isolated in Yunnan province, southwest China in 2013, and complete genomic sequence of the ChRtm213 was sequenced and deposited in the GenBank database.

Cutrín et al. proposed placing aquabirnaviruses into six genogroups, four of which can be subdivided into two genotypes based on a two-step restriction analysis, and the genotyping corresponds with serotyping as follows: genogroup I includes two genotypes corresponding to serotypes A9 (genotype I.1) and A1 (genotype I.2); genogroup II corresponds to serotype A3; genogroup III includes genotypes III.1 (serotype A2) and III.2 (serotype B1); genogroups IV and V include two genotypes, each



**Table 5** Similarities of ORFs between IPNV ChRtm213 and aquabirnaviruses from genogroups 1–7

Isolate	Genogroup	Host	Country	GenBank Accession (VP1)	VP1 (%) (844 aa)	GenBank Accession (polyprotein)	pVP2 (%) (449 aa)	pVP3 (%) (217 aa)	pVP4 (%) (252 aa)	GenBank Accession (VP5)	pVP5 (%) (133 aa)
IPNV 2310	1	Trout	USA	AAC71004.1	99.4	AAC71003.1	97.1	98.2	99.2	AAC71002.1	91.0
IPNV 578	2	Turbot	Spain	CAD32986.1	90.6	CAD32970.1	88.6	79.7	82.5	CAD32978.1	59.4
IPNV Canada 1	3	Trout	Canada	NA	–	AAK32158.1	87.5	80.6	81.3	NA	–
IPNV Canada 2	4	Trout	Canada	NA	–	AAK32159.1	89.1	82.0	81.0	AAA92627.1	63.9
IPNV 1146	5	Trout	Spain	CAD32980.1	90.5	CAD32964.1	88.9	80.6	82.5	CAD32972.1	62.4
IPNV He	6	Pike	Germany	NA	–	AAK32156.1	84.0	84.7	78.2	NA	–
POBV	7	<i>Paralichthys olivaceus</i>	China	ABW07822.1	97.0	ABW07821.1	89.0	89.4	88.1	ABW07820.1	80.6

NA not available

corresponding to serotypes A5, A6, A7, and A8 (genotypes IV.1, IV.2, V.1, and V.2, respectively); and genogroup VI corresponds to serotype A4 [39, 40]. In this study, the phylogenetic trees of aquabirnaviruses genomic nucleotide sequences of segment A showed that aquabirnaviruses were clustered into seven genogroups (genogroup 1–7), with six (genogroup 1–6) for IPNV and one (genogroup 7) for marine birnaviruses. The ChRtm213 belongs to the genogroup 1, which included the Spain 2310, Japanese AM98, American VR299, Buhl, WB, Korean HL-1, Canadian Jasper, and Mexican Mexican. The genogroup 1 corresponded to the genogroup I in Cutrin’s study [39]. The genogroup I consisted of genotype I.1 and genotype I.2, and the ChRtm213 was clustered into genotype I.1 along with the Japanese AM98, Canadian Jasper, and Korean HL-1. The ChRtm213 was supposed to share the same serotype with those isolates. Therefore, the ChRtm213 more likely belonged to serotype A9 (Ja). Due to the high similarities between the ChRtm213 with each strain within the genogroup 1, the source of the ChRtm213 was still uncertain. Although phylogenetic analysis alone is insufficient to conclude from which country IPNV was introduced into China, the historical record demonstrates that 4,000,000 rainbow trout eggs from Japan were imported to a fish farm in northeast China from 1986 to 1987, and IPNV and IHNV (Infectious hematopoietic necrosis virus) were isolated from rainbow trout fry in northeast China after the importation of eggs from Japan [11]. IHNV isolates in China have been proved to be imported from Japan after 1980s by comprehensive phylogenetic analyses based on a large number of Chinese IHNV isolates [11]. However, there is no gene sequence of Chinese IPNV isolates available; therefore, we were not able to perform extensive sequence analyses on Chinese IPNV isolates. Although the ChRtm213 had high similarities on genomic sequences with Japanese AM98 strain, there still were no data to support the source country of Chinese IPNV was Japan. Previous studies demonstrated that Chinese IPNV strains isolated in the late 1980s were of serotype A2 (Sp) with high virulence and mainly found in the northern part of China [10, 13]. The results suggest that the ChRtm213 is a novel strain that is different from the Chinese IPNV isolates described previously. For now, there is only one genomic sequence of Chinese aquabirnaviruses, and the marine birnavirus strain POBV submitted to the GenBank database [24]. The POBV was isolated from flounder *P. olivaceus*, and clustered to the genogroup 7 in this study. Although it is having relevant high similarities with ChRtm213, the marine birnavirus POBV still has many differences with the IPNV ChRtm213, such as the genogroup, serotype, and host. It is more meaningful to analyze ChRtm213 with other Chinese IPNV stains but the POBV. The results suggest that more

**Fig. 5** Alignment of pVP5 from ChRtm213 with other IPNV strains. *Red squares* indicate termination codons (Color figure online)

ChRtm213	MNHE HI QGNRNL LE I HY AS RE WAS KHS GRHNREAYTKTRDLV I . L	44
Canada 2	MQDE HKQG NRNL FE I HY AS RDWAS KHP GRHNGETHLKTRDLV I QL	45
West Buxton	MNHE HNKGNRNL LE I HY AS RE WTS KHS GRHNRETHTKTRDL I I QL	45
Y-6	MNHE HNKGNRNL LE I HY AS RE WSS KHS GRHNRETHTKTRDLV I QL	45
578	MQDE HKQS NRNI LE VHCAS RDWTF QHS GRHNGETHLKTRDFVL QL	45
1146	MQDE HKQG NRNL LE I HY AS RDWTS KHP GRHNGETHLKTRDLV I QL	45
ChRtm213	RGL . I RK WAS CL LP WGS WI QS RCP L QVE SE PDGT RI RP VARDVTG	88
Canada 2	RGL RL RK WAS SLL PGRTRL ACWCS LQVE CEPDGARI RPVAGDVAG	90
West Buxton	RGL RL RK WAS CL LP WGS RI QS RCP L QVE SE PDGT GI RPVVGNI TG	90
Y-6	RGL RL RK WAS CL LP WSS WI QS RCP L QVE SE PDGT GI RPVAGNI TG	90
578	RGL GL RK WCS CL FP WSTR I KGRCS LQME CEPDGT RVRPVAGDVTG	90
1146	RGL RL RK WNS CL FP WGT RLADRCTL QVE CEPDGAGVRPVAGDVAG	90
ChRtm213	PKEGI QL RETDLTEI . HPELNPPRWSVCTQWDPKRRHLRGKS	129
Canada 2	PEEGL QLWEAYLKEI R HSELHLTS RPLCTEWDPKCRHIRRQS	132
West Buxton	PEESI QLREVDLTEI R HPELDPSRWPLCTQRDPECSYLRRKS	132
Y-6	PEEGI QLREADLTEI RRPELNPTRWVCPEDWPERDLRRKS	132
578	PEESF QLREADIQEI RRSKLHAAGWALCPEWDHQRHLRRQS	132
1146	PEESL QVREADLKEI R HSKLHTTGRSLCSERDAQRCHLRRQP	132

sequences of Chinese IPNV isolates should be determined and submitted to GenBank database or some other databases, which will play an important role in the analyses of the source and evolution of IPNV in Chinese fish farming environment. The genomic sequence of the ChRtm213 characterized in this study was the first sequence of IPNV in China. The information of IPNV ChRtm213, such as genomic sequence, genogroup, and serotype, will provide an important reference to the research of Chinese IPNV isolates in the future.

The VP5 gene in aquabirnaviruses was not as conserved as the polyprotein or RdRp, which has been detected in virus-infected cells, but so far there is no evidence of the protein being found in purified virions. Some studies support the presence of VP5 [41], and a weak VP5 ORF-expressed product in CHSE-214 cells infected by the Jasper strain of IPNV has been detected [42]. Additionally, a previous study demonstrated that the VP5 is not essential for virulence or for the establishment of persistent IPNV infection. The role of VP5 in persistent infection was investigated by challenging Atlantic salmon fry with the recovered viruses, as well as with the low-virulence field strain Sp103 and a naturally occurring VP5-deficient mutant of Sp103. The results showed that VP5 is not required for viral replication in vivo, and its absence did not alter the virulence characteristics of the virus or the establishment of persistent IPNV infection [43–45]. In this study, a truncated VP5 was observed and confirmed in the ChRtm213 strain. However, it did not affect propagation of IPNV in CHSE-214 cells either. Thus, we conjectured that the VP5 is not essential for viral replication in cell line.

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**Author contributions** FJI conducted IPNV complete genome cloning and wrote the manuscript; JZ & ML identified and separated the IPNV ChRtm213 strain; TL & HL collected the diseased tissue samples of rainbow trout; JY & LMX designed the study and were responsible for the whole organization of the manuscript.

#### Compliance with ethical standards

**Conflict of interest** All authors declare that no conflicts of interest exist.

**Human and animal rights** This article does not contain any studies with human participants or animals performed by any of the authors.

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