Short Communication

An approach for genogrouping of Japanese isolates of aquabirnaviruses in a new genogroup, VII, based on the VP2/NS junction region

Toyohiko Nishizawa, Shinichi Kinoshita and Mamoru Yoshimizu

Graduate School of Fisheries Sciences, Hokkaido University, Hakodate, 041-8611, Japan

Correspondence
Toyohiko Nishizawa
jjnishi@fish.hokudai.ac.jp

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Aquabirnaviruses, represented by *Infectious pancreatic necrosis virus* (IPNV), have been isolated from epizootics in salmonids and a variety of aquatic animals in the world; six genogroups of aquabirnaviruses have been identified. In comparisons of nucleotide sequences of the VP2/NS junction region, maximum nucleotide diversities of 30·8 % were observed among 93 worldwide aquabirnavirus isolates. A phylogenetic tree revealed the existence of a new genogroup, VII, for Japanese aquabirnavirus isolates from marine fish and molluscan shellfish. Nucleotide diversities between genogroups VII and I–VI were 18·7 % or greater. At the nucleotide level, Japanese IPNV isolates from epizootics in salmonids were nearly identical to a genogroup I strain from the USA or Canada. It is suggested that Japanese IPNV isolates belonging to genogroup I were originally introduced from North American sources, whereas Japanese aquabirnavirus isolates of genogroup VII were from marine aquatic animals indigenous to Japan.

Infectious pancreatic necrosis virus (IPNV), the type species of the genus *Aquabirnavirus* in the family *Birnaviridae*, is the aetiological agent of infectious pancreatic necrosis. IPNV has been isolated from epizootics in cultured salmonids and a variety of aquatic animals in freshwater and sea-water environments in the world (Reno, 1999). In some cases, aquabirnavirus isolates lack any association with disease, although they are identified serologically as IPNV. Thus, aquabirnavirus isolates with no pathogenicity to salmonids are currently named as aquabirnaviruses, to be distinguished from IPNV (Reno, 1999; Leong *et al.*, 2000). The vast majority of IPNV and other aquabirnavirus isolates are classified into serogroups A and B, but no cross-relation by reciprocal-neutralization tests was observed between these serogroups. Most aquabirnavirus isolates belong to serotypes A1–A9 of the major serogroup A, whilst the minor serogroup B consists of a single serotype, B1 (Hill & Way, 1995; Reno, 1999). Serotype A1 contains most of the USA isolates, serotypes A6–A9 are found mainly in Canada and serotypes A2–A5 and B1 are found in European countries. *Yellowtail ascites virus* (YTAV), another member of the genus *Aquabirnavirus*, was originally isolated in Japan from an epizootic in yellowtail (*Seriola quinqueradiata*) with ascites (Sorimachi & Hara, 1985) and in Japanese flounder (*Paralichthys olivaceus*) with clinical haemorrhage (Kusuda *et al.*, 1989). YTAV isolates have also been detected from a variety of marine fish and molluscan shellfish in Japan (Suzuki & Nojima, 1999; Takano *et al.*, 2001; Watanabe *et al.*, 2002; Isshiki *et al.*, 2004). These YTAV isolates are serologically cross-reactive with IPNV of serogroup A, but genetically distinguishable from IPNV (Kusuda *et al.*, 1993; Hosono *et al.*, 1994, 1996; Takano *et al.*, 2001; Zhang & Suzuki, 2003).

Aquabirnaviruses have a non-enveloped, icosahedral capsid approximately 60 nm in diameter containing the bisegmented, double-stranded RNA genome (segments A and B; Leong *et al.*, 2000). Segment B (2·8 kb) encodes a minor internal polypeptide VP1 (M_r 94 kDa), the putative RNA-dependent RNA polymerase, whilst segment A (3·1 kb) contains two partially overlapping open reading frames (ORFs), a large ORF for the polyprotein (M_r 106 kDa, NH2-pVP2-NS-VP3-COOH) and a small ORF for VP5 (M_r 17 kDa). The polyprotein is cleaved into three polypeptides: pVP2, the precursor of the major capsid protein VP2; NS, a non-structural protein with protease activity associated with the cleavage of the polyprotein; and VP3, a minor capsid protein (Duncan & Dobos, 1986; Duncan *et al.*, 1987; Håvarstein *et al.*, 1990; Manning & Leong, 1990; Manning *et al.*, 1990; Magyar & Dobos, 1994; Leong *et al.*, 2000). Recently, molecular phylogenetic analysis, based on the VP2-coding region of IPNV and other aquabirnaviruses, has revealed the existence of six discrete genogroups that are correlated to the previously established serotypes, A1–A9 and B1 (Blake *et al.*, 2001; Cutrín *et al.*, 2004). The previous studies unfortunately included only a limited number of Japanese isolates; thus, in this study, a total of 14 Japanese isolates of IPNV and other aquabirnaviruses, differing in the year of isolation and geographical and host origins, together

The GenBank/EMBL/DDBJ accession numbers for the nucleotide sequences determined in this study are AB179699–AB179712.

with worldwide isolates, were subjected to molecular phylogenetic analysis based on the nucleotide sequence of the VP2/NS junction region in order to evaluate their genetic relatedness.

Six Japanese isolates of IPNV from affected salmonids (Eniwa, Tomakomai, Oippe, Towada, Gifu and Nagano) and eight Japanese aquabirnavirus isolates from apparently healthy marine fish (Obama10, Obama29, Izu6, Izu18, Akk02SS, Akk02RS, Akk02SC and Akk02JD) were used in this study. Eniwa and Tomakomai were isolated from rainbow trout (*Oncorhynchus mykiss*) at Hokkaido in the 1970s, whilst Oippe was also from masu salmon (*Oncorhynchus masou*) at Aomori Prefecture in the 1980s. Towada, Gifu and Nagano isolates were from rainbow trout on the mainland of Japan in 1987, the 1980s and 2000, respectively (Kimura *et al.*, 1984; Yoshimizu *et al.*, 1993). Obama10, Obama29, Izu6 and Izu18 were isolated from free-living Japanese flounder at Obama and Minami-Izu, Japan, in 1999 (Takano *et al.*, 2001). Isolates Akk02SS, Akk02RS, Akk02SC and Akk02JD were isolated respectively from the wild marine fish snowy sculpin (*Myoxocephalus blandti*), saffron cod (*Eleginus gracilis*), rainbow smelt (*Osmerus eperlanus mordax*) and Japanese dace (*Tribolodon hakonensi*) captured in the Akkeshi coastal area of Hokkaido, Japan, in 2002 (Kobayashi *et al.*, 2005).

Viral genomic RNA, prepared with an RNA-extraction kit (Isogen-LS; Nippon Gene) according to the manufacturer's instructions, was used as the template for RT-PCR with an annealing temperature of 52 °C. PCR primers ABV-P1 (5′-AGAGATCACTGACTTCACAAGTGAC-3′) and ABV-P2 (5′-TGTGCACCACAGGAAAGATGACTC-3′) were used for amplification of the VP2/NS junction region between nucleotide positions 1403 and 1761 of segment A of the viral genome (Heppell *et al.*, 1992). The PCR products were purified with a PCR-purification kit (Stratagene) and subjected to nucleotide sequence analysis. The resulting sequences were assembled with DNASIS (Hitachi) and multiple alignments of the sequences were constructed by using CLUSTAL W (Thompson *et al.*, 1994) to search for an optimal phylogenetic tree with neighbour-joining criteria. The final phylogenetic tree was drawn with the NJplot program (Perrière & Gouy, 1996). The determined nucleotide sequences were registered with GenBank/DDBJ under accession nos AB179699–AB179712. Deposited nucleotide sequences of 79 isolates of IPNV and aquabirnavirus in GenBank/DDBJ were used for comparative purpose (Fig. 1). Although 93 isolates were employed in total in the present study, the corresponding countries, host species and serotypes of 39 representative isolates are provided in Table 1.

In the comparison of nucleotide sequences of the VP2/NS junction region, a maximum nucleotide diversity of 30·8 % was observed among the 93 isolates of IPNV and aquabirnaviruses (Table 2). The present phylogenetic tree revealed seven major clusters as follows: genogroup I included the Jasper strain (Canada) and USA strains Reno, VR299, Buhl, Dry Mills and West Buxton; genogroup II included Abild (Denmark) and Asian isolates PV, E1S, EEV and CV-HB1; genogroup III included Bonnamy (France), N1 (Norway), DPL (Thailand), d'Honnincthum, OV2 (England) and Spajarup (Denmark); genogroup IV was for ASV (Canada), Canada 1 and Tellina (England); genogroup V was for Canada 2 and Canada 3; genogroup VI was for Hecht (Germany); and genogroup VII was composed of YTAV and aquabirnaviruses from marine fish and molluscan shellfish (Fig. 1; Table 1). Heppell *et al.* (1992, 1993) identified the existence of only three major genogroups for IPNV by comparison of amino acid sequences and restriction-fragment length-polymorphism patterns of the VP2/NS region. The present tree was also based on analysis of the VP2/NS region, but phylogenetic analysis enabled a more detailed genogrouping of the aquabirnaviruses. Although the VP2/NS region of 310 bp is a little shorter than the VP2 coding region (about 490 bp), no significant difference in topographic structures was observed between the phylogenetic trees based on the VP2/NS region and VP2 regions, with the exception of a clade of genogroup VII of the present tree. This could be caused by the VP2/NS and VP2 regions partially sharing one of the highly variable domains of the viral genome (Heppell *et al.*, 1993; Blake *et al.*, 2001). The new genogroup, VII, was composed of 25 isolates of YTAV and other aquabirnaviruses, all of which were isolated from marine fish and molluscan shellfish in Japan with the exception of one Korean isolate, NC1 (Fig. 1). The submitted aquabirnavirus isolates, Obama10, Obama29, Izu6, Izu18, Akk02SS, Akk02RS, Akk02SC and Akk02JD, were nearly identical to the Y6 isolate (GenBank/DDBJ accession no. AY283781), the type strain of YTAV in genogroup VII. The degree of nucleotide diversity within genogroup VII was 3·5 %, which was the same level as that within genogroups II and V (Table 2). Moreover, a high degree of nucleotide diversity ($\geqslant$18·7 %) between isolates of genogroup VII and the remaining genogroups, I–VI, is apparent (Table 2). These findings strongly suggest the existence of a new genogroup in the genus *Aquabirnavirus*, VII, for Japanese isolates of YTAV and other aquabirnaviruses from marine fish and molluscan shellfish.

Japanese isolates of YTAV and other aquabirnaviruses share common antigenic epitopes with IPNV strains VR299 (serotype A1 of genogroup I), Ab (A3 of genogroup II) and Sp (A2 of genogroup III), in reciprocal-neutralization tests (Kusuda *et al.*, 1993; Nakajima & Sorimachi, 1994; Takano *et al.*, 2001), but detailed serotyping of these Japanese isolates has not been clear. In the present results, these Japanese isolates were classified into the newly defined genogroup, VII, which was completely discrete from the remaining genogroups, I–VI (Fig. 1). Moreover, genogroups I–VI correlate with the serotypes A1–A9 and B1 as follows: genogroup I (A9 and A1), genogroup II (A3), genogroup III (A2 and B1), genogroup IV (A5 and A6), genogroup V (A7 and A8) and genogroup VI (A4) (Blake *et al.*, 2001; Cutrín *et al.*, 2004). It is thus suggested that the

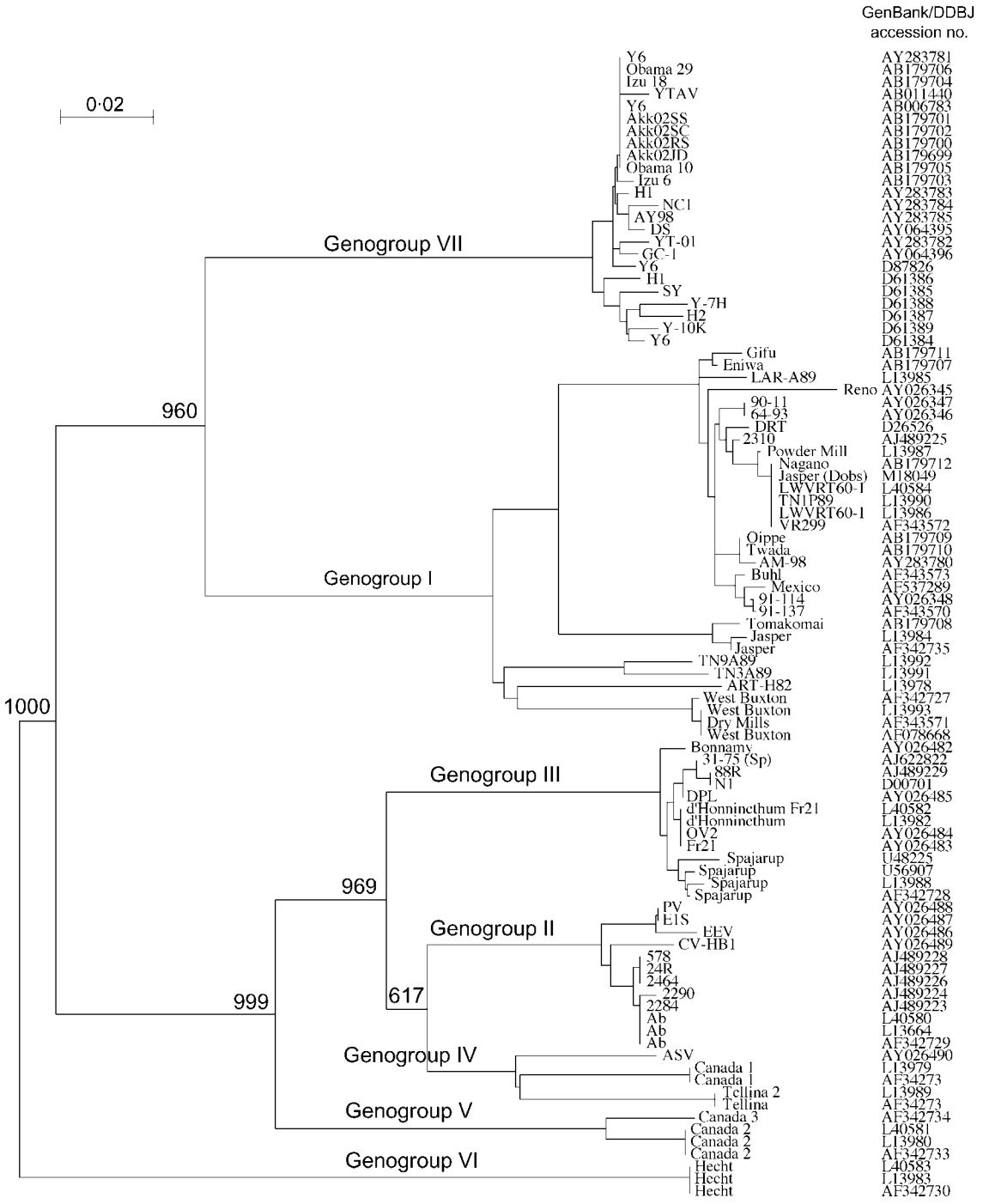

Fig. 1. Molecular phylogenetic tree based on nucleotide sequences of the VP2/NS junction region among 93 worldwide isolates of IPNV and other aquabirnaviruses, and corresponding GenBank/DDBJ accession numbers. Bootstrap values from 1000 replicates are shown at major nodes. Bar, 0·02 replacement nucleotides per site.

isolates of genogroup VII are classified into serogroup A, but may be subtly different from the others.

The Japanese isolates of IPNV, Eniwa, Tomakomai, Oippe, Towada, Gifu and Nagano, were all classified into genogroup I, but were divided into two of the three minor clusters of this genogroup (Fig. 1). The Tomakomai isolate was nearly identical to the Jasper strain, whereas the other five isolates were related closely to VR299 or the Buhl strain (Fig. 1; Table 1). It is thus indicated that the Tomakomai isolate is serotyped as A9, the same as the Jasper strain, whereas the other five isolates are classified as serotype A1,

Table 1. Nucleotide diversities based on the VP2/NS junction region among 39 representatives of the 93 isolates of IPNV and other aquabirnaviruses

Strain/isolate	Geographical origin	Host of origin	Type*	Nucleotide diversity (%)†																																						
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
Genogroup I																																										
North American																																										
1. Reno	Nevada, USA	Trout	A1	–	3·9	3·6	10·4	12·0	12·0	3·9	4·2	3·6	3·9	3·6	3·6	11·4	28·2	28·2	29·2	28·9	27·6	27·9	28·6	27·9	27·6	27·6	27·6	27·9	29·2	30·2	30·2	30·5	29·9	22·4	22·4	22·4	22·4	22·4	22·4	22·4	22·4	22·1
2. VR299	West Virginia, USA	Trout	A1		–	1·9	8·1	9·7	9·7	1·9	1·6	1·6	0·0	1·6	1·6	8·4	27·3	27·3	28·2	27·9	26·8	26·9	27·7	26·9	26·8	26·6	26·8	26·3	27·7	28·7	29·0	29·0	28·7	21·3	21·3	21·3	21·3	21·3	21·3	21·3	21·3	21·0
3. Buhl	Idaho, USA	Trout	A1			–	7·7	9·7	9·7	1·3	1·6	1·0	1·9	1·0	1·0	8·7	26·0	26·0	26·9	26·6	25·5	25·6	26·5	25·6	25·5	25·3	25·8	25·3	26·8	27·7	27·7	28·1	28·1	20·6	20·6	20·6	20·6	20·6	20·6	20·6	20·6	20·3
4. Jasper	Canada	Trout	A9				–	10·0	10·0	7·4	6·8	6·8	8·1	7·1	7·1	1·0	28·2	28·2	29·2	28·6	28·4	28·6	29·0	28·6	28·7	28·6	28·7	27·9	29·4	29·4	29·0	27·7	30·3	20·3	20·3	20·3	20·3	20·3	20·3	20·3	20·3	20·0
5. Dry Mills	Maine, USA	Trout	A1					–	0	9·4	8·1	8·7	9·7	9·0	9·0	9·7	28·6	28·6	29·5	28·9	27·7	28·2	28·7	28·2	28·1	28·2	28·1	28·2	28·7	28·4	27·7	27·4	29·0	19·4	19·4	19·4	19·4	19·4	19·4	19·4	19·4	19·0
6. West Buxton	Maine, USA	Trout	A1						–	9·4	8·1	8·7	9·7	9·0	9·0	9·7	28·6	28·6	29·5	28·9	27·7	28·2	28·7	28·2	28·1	28·2	28·1	28·2	28·7	28·4	27·7	27·4	29·0	19·4	19·4	19·4	19·4	19·4	19·4	19·4	19·4	19·0
Japanese																																										
7. AM98	Kochi, Japan	Land-locked trout	NT							–	1·6	1·0	1·9	0·3	0·3	8·4	26·3	26·3	27·3	26·3	25·8	25·6	26·5	25·6	25·5	25·3	25·8	25·6	26·8	28·1	27·1	27·4	27·7	21·0	21·0	21·0	21·0	21·0	21·0	21·0	21·0	20·6
8. Gifu	Gifu, Japan	Rainbow trout	NT								–	0·6	1·6	1·3	1·3	7·1	26·6	26·6	27·6	27·3	26·1	26·6	27·4	26·6	26·5	26·3	26·8	25·6	27·1	28·1	28·7	29·0	28·1	20·6	20·6	20·6	20·6	20·6	20·6	20·6	20·6	20·3
9. Eniwa	Hokkaido, Japan	Rainbow trout	NT									–	1·6	0·6	0·6	7·7	26·3	26·3	27·3	26·9	25·8	26·0	26·8	26·0	25·8	25·6	26·1	25·6	27·1	28·1	28·1	28·4	27·4	20·0	20·0	20·0	20·0	20·0	20·0	20·0	20·0	19·7
10. Nagano	Nagano, Japan	Rainbow trout	NT										–	1·6	1·6	8·4	27·3	27·3	28·2	27·9	26·8	26·9	27·7	26·9	26·8	26·6	26·8	26·3	27·7	28·7	29·0	29·0	28·7	21·3	21·3	21·3	21·3	21·3	21·3	21·3	21·3	21·0
11. Oippe	Aomori, Japan	Masu salmon	NT											–	0	8·1	26·3	26·3	27·3	26·3	25·8	26·0	26·8	26·0	25·8	25·6	26·1	25·6	26·8	28·1	27·4	27·7	28·1	20·6	20·6	20·6	20·6	20·6	20·6	20·6	20·6	20·3
12. Twada	Aomori, Japan	Rainbow trout	NT												–	8·1	26·3	26·3	27·3	26·3	25·8	26·0	26·8	26·0	25·8	25·6	26·1	25·6	26·8	28·1	27·4	27·7	28·1	20·6	20·6	20·6	20·6	20·6	20·6	20·6	20·6	20·3
13. Tomakomai	Hokkaido, Japan	Rainbow trout	NT													–	28·6	28·6	29·5	28·9	28·7	28·9	29·4	28·9	29·0	28·9	29·0	27·9	29·4	29·4	29·0	27·7	30·3	20·0	20·0	20·0	20·0	20·0	20·0	20·0	20·0	19·7
Genogroup II																																										
14. PV	Taiwan	Perch	A3														–	0	1·0	2·6	1·9	10·1	10·7	10·1	10·1	10·1	10·7	10·7	11·4	10·7	17·2	17·5	28·2	24·4	24·4	24·4	24·4	24·4	24·4	24·4	24·4	24·4
15. E1S	Taiwan	Eel	A3															–	1·0	2·6	1·9	10·1	10·7	10·1	10·1	10·1	10·7	10·7	11·4	10·7	17·2	17·5	28·2	24·4	24·4	24·4	24·4	24·4	24·4	24·4	24·4	24·4
16. EEV	Japan	Eel	A3																–	3·2	2·9	11·0	11·7	11·0	11·0	11·0	11·7	11·4	12·0	11·4	17·2	17·5	28·2	25·0	25·0	25·0	25·0	25·0	25·0	25·0	25·0	25·0
17. CV-HB1	Taiwan	Clam	A3																	–	1·9	10·7	10·7	10·7	10·7	10·7	11·4	9·7	10·1	9·7	15·6	15·9	29·5	24·7	24·7	24·7	24·7	24·7	24·7	24·7	24·7	24·7
18. Abild	Denmark	Trout	A3																		–	10·7	11·3	10·7	10·6	10·7	11·3	10·1	9·4	9·4	17·4	17·7	28·7	24·4	24·4	24·4	24·4	24·4	24·4	24·4	24·4	24·2
Genogroup III																																										
19. Bonnamy	France	Trout	A2																			–	1·3	0·6	1·0	1·0	1·3	14·0	15·3	13·6	17·5	17·5	26·0	26·3	26·3	26·3	26·3	26·3	26·3	26·3	26·3	26·3
20. N1	Norway	Atlantic salmon	A2																				–	0·6	1·0	1·0	1·3	14·3	14·8	13·2	17·1	17·7	26·5	26·8	26·8	26·8	26·8	26·8	26·8	26·8	26·8	26·8
21. DPL	Thailand	Snakehead	NT																					–	0·3	0·3	0·6	13·6	14·9	13·3	17·2	17·9	25·6	26·3	26·3	26·3	26·3	26·3	26·3	26·3	26·3	26·3
22. d'Honnincthum			A2																						–	0	1·0	14·0	15·2	13·5	16·8	17·4	26·1	26·1	26·1	26·1	26·1	26·1	26·1	26·1	26·1
23. OV2	England	Oyster	A2																							–	1·0	14·0	15·3	13·6	16·9	17·5	26·0	26·3	26·3	26·3	26·3	26·3	26·3	26·3	26·3	26·3
24. Spajarup	Denmark	Trout	A2																								–	14·3	15·5	13·9	17·7	18·4	26·1	26·1	26·1	26·1	26·1	26·1	26·1	26·1	26·1	26·1
Genogroup IV																																										
25. ASV	Canada	Atlantic salmon	A6																									–	6·5	7·5	16·6	17·2	28·6	25·0	25·0	25·0	25·0	25·0	25·0	25·0	25·0	25·0
26. Canada 1	Canada	Trout	A6																										–	7·7	18·1	19·4	30·0	25·2	25·2	25·2	25·2	25·2	25·2	25·2	25·2	25·2
27. Tellina	England	Tellina	A5																											–	18·4	18·7	30·0	25·2	25·2	25·2	25·2	25·2	25·2	25·2	25·2	25·2
Genogroup V																																										
28. Canada 3	Canada	Arctic char	A8																												–	3·5	30·0	25·2	25·2	25·2	25·2	25·2	25·2	25·2	25·2	25·2
29. Canada 2	Canada	Trout	A7																													–	29·4	23·9	23·9	23·9	23·9	23·9	23·9	23·9	23·9	24·2
Genogroup VI																																										
30. Hecht	Germany	Pike	A4																														–	27·7	27·7	27·7	27·7	27·7	27·7	27·7	27·7	27·7	27·7
Genogroup VII																																										
31. Y6	Kochi, Japan	Yellowtail	NT																															–	0	0	0	0	0	0	0	0·3
32. Akk02SS	Hokkaido, Japan	Snowy sculpin	NT																																–	0	0	0	0	0	0	0·3
33. Akk02RS	Hokkaido, Japan	Rainbow smelt	NT																																	–	0	0	0	0	0	0·3
34. Izu18	Shizuoka, Japan	Japanese flounder	NT																																		–	0	0	0	0	0·3
35. Akk02SC	Hokkaido, Japan	Saffron cod	NT																																			–	0	0	0	0·3
36. Akk02JD	Hokkaido, Japan	Japanese dace	NT																																				–	0	0	0·3
37. Obama29	Fukui, Japan	Japanese flounder	NT																																					–	0	0·3
38. Obama10	Fukui, Japan	Japanese flounder	NT																																						–	0·3
39. Izu6	Shizuoka, Japan	Japanese flounder	NT																																							–

*NT, Not typed.

†(No. replacement nucleotides per site) × 100.

Table 2. Nucleotide diversities among genogroups I–VII, based on the VP2/NS junction region, from 93 isolates of IPNV and other aquabirnaviruses

Genogroup	Nucleotide diversity (%)* between genogroups:							
	I (NA†)	I (J‡)	II	III	IV	V	VI	VII
I:								
NA†	<13·3	<11·4	25·3–30·8	25·0–31·0	25·0–30·3	26·8–30·5	27·4–30·6	18·7–24·0
NJ‡		<8·4	25·5–29·5	25·3–29·4	25·6–29·4	27·1–29·0	27·4–30·3	19·7–22·6
II			<3·2	10·1–12·7	9·4–12·0	15·6–18·1	28·2–29·5	23·9–26·3
III				<2·3	12·9–15·8	16·8–19·4	25·6–26·5	25·8–28·7
IV					<7·7	16·6–19·4	28·6–30·3	24·7–27·1
V						<3·5	29·4–30·0	23·5–26·8
VI							–	27·7–29·0
VII								<3·5

*(No. replacement nucleotides per site) × 100.

†NA, North American isolates.

‡J, Japanese isolates.

the same as the VR299 and Buhl strains. Additionally, genogroup II contained a Japanese strain, EEV, which was isolated from an imported European eel (Sano *et al.*, 1981) and serotyped as A3 (Blake *et al.*, 2001). Therefore, it is shown that aquabirnaviruses belonging to at least three different genogroups (including four serotypes) have been present in Japan and that genogroups I (serotype A1) and VII of aquabirnaviruses are predominant in Japan.

IPNV is thought to be spread from continent to continent with the importation of salmonid fish or fish eggs, but it is likely that the virus had a global distribution prior to the widespread dissemination of salmonids in the 19th and the early 20th centuries (Reno, 1999). Aquabirnaviruses from a variety of aquatic animal species are generally infected with the same genogroup as particular IPNV isolate(s) in the same area. However, it is interesting that Japanese IPNV and other aquabirnaviruses are classified into two different genogroups, I and VII, which are segregated completely by the environment in which they are distributed, i.e. the former is isolated from salmonids in freshwater environments, whereas the latter is detected in marine aquatic animals. Against this unique situation in Japan, we consider the following. Aquabirnaviruses of genogroup VII may have existed as indigenous viruses in Japanese marine environments prior to the widespread dissemination of salmonids, because genogroup VII isolates have been found to be limited to Japan and Korea; moreover, these isolates are not currently associated with any disease in their host. This assumption is further supported by the nucleotide diversity within genogroup VII (3·5 %), the same degree as that within other discrete clades, genogroups II and V (Table 2). In contrast, Japanese IPNV isolates belonging to genogroup I could have been introduced from North American sources by transportation of infected fish or fish eggs in the 1950s, because Japan was an important client for importation of salmonid fish and fish eggs from North America at that time (Kimura & Yoshimizu, 1991; Yoshimizu, 1996). As a consequence, the introduced viruses may have become established in Japanese freshwater environments. This is supported by the degree of nucleotide diversity (8·4 %) exhibited by Japanese IPNV isolates; however, the Tomakomai isolate was nearly identical to the Canadian Jasper strain (1·0 %), whereas the other remaining Japanese isolates were nearly identical to USA strains VR299 and Buhl ($\leqslant$1·9 %) (Tables 1 and 2). Therefore, we speculate that the segregated distribution of IPNV and aquabirnaviruses in Japan is an interesting situation that suggests that IPNV may originate in marine environments, as well as a theory for a marine origin of *Viral hemorrhagic septicemia virus*, a fish-pathogenic rhabdovirus (Smail, 1999; Nishizawa *et al.*, 2002; Einer-Jensen *et al.*, 2004).

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