Vol. 109: 9–14, 2014
doi: 10.3354/dao02724

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published April 23

# Genetic positioning of aquabirnavirus isolates from cultured Japanese eel *Anguilla japonica* in Korea

**Wi-Sik Kim, Myung-Joo Oh***

**Department of Aqualife Medicine, College of Fisheries and Ocean Science, Chonnam National University, Yeosu 550-749, Korea**

ABSTRACT: Aquabirnavirus is an epizootic virus in Japanese eel *Anguilla japonica* farms in Korea, although its origin is unclear. In the present study, nucleotide sequences of the VP2/NS junction region of 9 Korean aquabirnaviruses from cultured eel in various areas of Korea during 2000–2009 were analyzed to evaluate their genetic relatedness to worldwide isolates. The nucleotide sequences showed more than 94.2% identity among the 9 Korean eel isolates, 71.2% identity among 16 Korean isolates from freshwater and marine fish, and 71.1% identity among 25 worldwide isolates. All 9 isolates in this study were phylogenetically classified into genogroup II, including isolates from Denmark, Spain, Taiwan and Japan, and were discrete from salmonid and marine fish isolates (genogroup I and VII) in Korea. These results suggest that the Korean eel isolates have most likely been introduced from outside the country and not from coastal areas of Korea.

KEY WORDS: Aquabirnavirus · *Anguilla japonica* · VP2/NS junction region · Phylogeny



## INTRODUCTION

Viruses in the family *Birnaviridae* belong to 4 genera: *Aquabirnavirus*, *Avibirnavirus*, *Blosnavirus* and *Entomobirnavirus* (Delmas et al. 2012). *Aquabirnavirus* is the largest and most diverse of these genera, and aquabirnaviruses have been isolated from a variety of aquatic animals in freshwater, brackish and seawater environments throughout the world (Wolf 1988, Reno 1999). Representative examples of these agents are infectious pancreatic necrosis virus (IPNV) from trout and salmonids, marine birnavirus (MABV) from marine fish and shellfish, and eel virus European (EVE) from eel (Wolf 1988, Reno 1999). All of these viruses are a threat to the farming industry.

Aquabirnaviruses have a non-enveloped, icosahedral capsid approximately 65 nm in diameter containing the bisegmented, double-stranded RNA genome (Delmas et al. 2012). The smaller segment B (2.8 kb) encodes VP1, the RNA polymerase of the virus. Segment A (3.1 kb) contains 2 partially overlapping open reading frames (ORFs): a large ORF for the polyprotein (relative molecular weight [$M_r$] 106 kDa, $NH_2$-pVP2-NS-VP3-COOH) and a small ORF for VP5 ($M_r$ 17 kDa). The polyprotein is cleaved into 3 polypeptides: pVP2, the precursor of the major capsid protein, VP2; NS, a non-structural protein with protease activity associated with the polyprotein cleavage; 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, Dobos 1995, Delmas et al. 2012). A molecular phylogenetic analysis based on the VP2 coding or VP2/NS junction regions of aquabirnaviruses revealed the existence of 7 discrete genogroups that generally correlate with geographical origin and serological classification (Blake et al. 2001, Cutrín et al. 2004, Nishizawa et al. 2005): genogroup I (serotypes A1 and A9), genogroup II (A3), genogroup III (A2 and B1), genogroup IV (A5 and A6), genogroup V (A7 and A8), genogroup VI (A4) and genogroup VII (unclear).

IPNV was first isolated from chum salmon *Oncorhynchus keta* and goldfish *Carassius auratus* in

*Corresponding author: ohmj@chonnam.ac.kr

Korea in 1983 (Hah et al. 1984). Since then, IPNV infections have been reported in rainbow trout *O. mykiss* and chum salmon (Hedrick et al. 1985, Park et al. 1989, Lyoo et al. 1991, Jeon et al. 2011). In 1994, an aquabirnavirus was first isolated from the marine olive flounder *Paralichthys olivaceus* (Sohn et al. 1995). Thereafter, aquabirnaviruses have been detected not only from various cultured marine fish, but also from marine invertebrates and wild fish in Korean coastal waters (Oh et al. 1999, 2006, Azumi et al. 2007, Kitamura et al. 2007, Jung et al. 2008, Kim et al. 2013). The existence of 3 different genogroups (I, II and VII) was revealed by phylogenetic analyses of Korean aquabirnaviruses (Jung et al. 2008, Jeon et al. 2011, Kim et al. 2013). Genogroups I and II are composed of isolates from rainbow trout and chum salmon. Genogroup VII includes marine isolates from aquatic animals in the coastal waters of Korea, which has been suggested to include indigenous viruses from Korean and Japanese coastal areas (Nishizawa et al. 2005, Kim et al. 2013).

Eel is the most important freshwater fish cultured in Korea. Eel culture in Korea began in the 1960s, and about 10 000 t of Japanese eel *Anguilla japonica* were produced in 2007. However, due to high culture densities, infectious diseases caused by anguillid herpesvirus 1, *Pseudodactylogyrus* sp., *Edwardsiella tarda* and others have been implicated in high mortality (Kim et al. 2011, 2012). Among them, aquabirnaviruses have been commonly isolated from diseased eels in various areas of Korea (Kim et al. 2011). It is presumed that the aquabirnaviruses from eels may have been introduced by wild glass eels collected from Korean coastal waters or outside Korea because many wild glass eels were imported from several countries, such as Taiwan, Japan, China, Hong Kong and Denmark. However, there is no evidence to support this hypothesis. Thus, in the present study, we analyzed the nucleotide sequence of the VP2/NS junction region of 9 Korean aquabirnaviruses from cultured Japanese eel in various areas of Korea during 2000–2009 and compared the sequences with those of existing Korean and worldwide isolates to evaluate their genetic relatedness.

## MATERIALS AND METHODS

### Virus isolates

Nine aquabirnavirus Korean isolates (Kor-ESC00, Kor-EGS00, Kor-EGS01, Kor-ENJ01, Kor-EGC01, Kor-EAS02, Kor-EYG02, Kor-EYC02 and Kor-EGJ09) were obtained from eel farms in Korea during 2000–2009 (Kim et al. 2011) (Fig. 1). Kor-E of each isolate indicates the country of isolation and host fish as Korea and eel, respectively. The next 2 letters indicate the area of isolation: i.e. SC, GS, NJ, GC, AS, YG, YC and GJ denote Suncheon, Gokseong, Naju, Gochang, Asan, Yeonggwang, Yeocheon and Gangjin, respectively. The numbers at the end of each isolate indicate the year of isolation. For example, Kor-ESC00 denotes an eel isolate from Suncheon in 2000. The 9 isolates were obtained from diseased eels during an epidemic (Kim et al. 2011). These isolates were propagated in Chinook salmon embryo (CHSE-214) cells maintained at 20°C in Eagle's minimum essential medium (Gibco) supplemented with 10% fetal bovine serum (Gibco), 100 IU $ml^{-1}$ penicillin G, and 100 µg $ml^{-1}$ streptomycin sulfate. The isolates used for the nucleotide sequence analysis had undergone a maximum of 3 passages in culture.

### RT-PCR

Viral genomic RNA was extracted using TRIZOL reagent (Gibco) according to the manufacturer's instructions. RT-PCR was performed with the ABV-P1 (5′-AGA GAT CAC TGA CTT CAC AAG TGA C-3′) and ABV-P2 primers (5′-TGT GCA CCA CAG GAA AGA TGA CTC-3′) targeting the VP2/NS junction region between nucleotide positions 1403 and 1761 of segment A of the viral genome (Nishizawa et

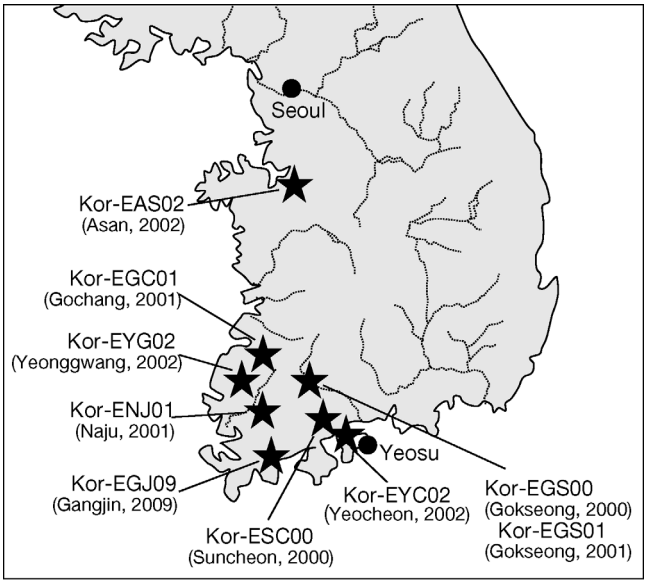


Fig. 1. Sampling locations and years of aquabirnavirus isolates from cultured eel. Stars indicate sites of origin for the 9 Korean aquabirnavirus isolates

al. 2005). Briefly, viral genomic RNA was denatured at 95°C for 5 min, and then incubated at 42°C for 30 min in 10 µl of RT buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl) containing 100 U M-MLV reverse transcriptase (Bioneer), 2.5 µM ABV-P2 primer, 1 mM dNTP and 5 mM $MgCl_2$ for reverse transcription. The synthesized cDNA was amplified in 50 µl PCR buffer containing 0.5 µM of each PCR primer, 1.25 U Ex-*Taq* DNA polymerase (Takara), 0.2 mM dNTP and 2 mM $MgCl_2$ by 30 cycles (95°C for 1 min, 52°C for 1 min and 72°C for 1 min) in a thermal cycler (MyGenie 96 thermal block, Bioneer).

### Nucleotide sequence analysis

PCR products were analyzed by 1.5% agarose gel electrophoresis and purified with a QIAquick gel extraction kit (Qiagen) for nucleotide sequence analysis using an ABI PRISM 3730 XL DNA Analyzer (Applied Biosystems), according to the manufacturer's instructions. The resulting sequences were assembled with Genetyx Win ver. 5.1 software. The sequence of the VP2/NS junction region was submitted to GenBank (accession nos. KF536953–KF536961) and compared with 16 worldwide isolates including 7 Korean isolates (Tables 1 & 2). In addition, a multiple alignment was constructed using Clustal X software (Thompson et al. 1997) to infer genetic relationship among 86 worldwide isolates with neighbor-joining criteria. GenBank accession numbers for these comparison sequences are shown in Table 1 and previous studies (Joh et al. 2000, Blake et al. 2001, Nishizawa et al. 2005, Jeon et al. 2011, Kim et al. 2013). The final phylogenetic tree was drawn with the MEGA4 program (Tamura et al. 2007).

## RESULTS AND DISCUSSION

PCR products of approximately 360 bp were obtained by RT-PCR with the ABV-P1 and ABV-P2 primers from the 9 Korean aquabirnavirus isolates (data not shown). The PCR products were sequenced and the nucleotide sequences of the VP2/NS junction region without the PCR primer sequences were com-

Table 1. Descriptions of representative aquabirnaviruses cited in this study. Asterisks indicate isolates found in the present study. (–) Host species only identified by common name in literature

| Isolate name | Host species | | Origin | Genogroup | GenBank no. |
|---|---|---|---|---|---|
| VR299 | Trout | – | USA | I | AF343572 |
| Jasper | Trout | – | Canada | I | M18049 |
| DRT | Rainbow trout | *Oncorhyncus mykiss* | Korea | I | D26526 |
| ChYy07VR | Chum salmon | *Oncorhyncus keta* | Korea | I | GQ866115 |
| Ab | Trout | – | Denmark | II | AF342729 |
| EEV | Eel | – | Japan | II | AY026486 |
| EIS | Eel | – | Taiwan | II | AY026487 |
| ChYy07AB | Chum salmon | *Oncorhyncus keta* | Korea | II | GQ866116 |
| Kor-EGS00* | Eel | *Anguilla japonica* | Korea | II | KF536956 |
| Kor-ESC00* | Eel | *Anguilla japonica* | Korea | II | KF536958 |
| Kor-EGS01* | Eel | *Anguilla japonica* | Korea | II | KF536957 |
| Kor-EGC01* | Eel | *Anguilla japonica* | Korea | II | KF536959 |
| Kor-ENJ01* | Eel | *Anguilla japonica* | Korea | II | KF536960 |
| Kor-EYG02* | Eel | *Anguilla japonica* | Korea | II | KF536954 |
| Kor-EAS02* | Eel | *Anguilla japonica* | Korea | II | KF536955 |
| Kor-EYC02* | Eel | *Anguilla japonica* | Korea | II | KF536961 |
| Kor-EGJ09* | Eel | *Anguilla japonica* | Korea | II | KF536953 |
| Sp | Trout | – | Denmark | III | AF342728 |
| N1 | Atlantic salmon | *Salmo salar* | Norway | III | D00701 |
| ASV | Atlantic salmon | *Salmo salar* | Canada | IV | AY026490 |
| Canada 1 | Trout | – | Canada | IV | AF342732 |
| Canada 2 | Trout | – | Canada | V | AF342733 |
| Canada 3 | Arctic char | *Salvelinus alpinus* | Canada | V | AF342734 |
| Hecht | Pike | *Esox lucius* | Germany | VI | L40583 |
| Y-6 | Yellowtail | *Seriola quinqueradiata* | Japan | VII | AY283781 |
| NC1 | Olive flounder | *Paralichthys olivaceus* | Korea | VII | AY283784 |
| DS | Olive flounder | *Paralichthys olivaceus* | Korea | VII | AY064395 |
| CGJJ11-1 | Chicken grunt | *Parapristipoma trilineatum* | Korea | VII | KC693770 |
| TSWD11 | Temminck's surfperch | *Ditrema temminckii* | Korea | VII | KC693773 |

Table 2. Pairwise comparisons (%) of nucleotide sequence identities of the VP2/NS junction region among 25 worldwide isolates (isolate details in Table 1). Identities >94% are in **bold**

| Isolate | Kor-ESC00 | Kor-EGS01 | Kor-EGC01 | Kor-ENJ01 | Kor-EYG02 | Kor-EAS02 | Kor-EYC02 | Kor-EGJ09 | ChYy07AB | NC1 | DS | CGJJ11-1 | TSWD11 | ChYy07VR | DRT | VR299 | Ab | EEV | EIS | Sp | ASV | Canada 2 | Hecht | Y-6 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Eel isolates found in this study** | | | | | | | | | | | | | | | | | | | | | | | | |
| Kor-EGS00 | **94.8** | **94.8** | **97.4** | **94.8** | **97.4** | **94.8** | **94.2** | **97.1** | **94.5** | 72.6 | 72.9 | 73.2 | 72.9 | 72.2 | 72.7 | 72.0 | **94.5** | **94.2** | **95.1** | 89.0 | 88.3 | 82.3 | 73.2 | 73.2 |
| Kor-ESC00 | | **100** | **96.1** | **100** | **96.1** | **100** | **95.5** | **95.8** | **99.7** | 75.5 | 75.8 | 76.5 | 75.8 | 74.8 | 75.2 | 74.6 | **99.7** | **96.8** | **97.7** | 88.4 | 89.6 | 82.6 | 72.6 | 76.1 |
| Kor-EGS01 | | | **96.1** | **100** | **96.1** | **100** | **95.5** | **95.8** | **99.7** | 75.5 | 75.8 | 76.5 | 75.8 | 74.8 | 75.2 | 74.6 | **99.7** | **96.8** | **97.7** | 88.4 | 89.6 | 82.6 | 72.6 | 76.1 |
| Kor-EGC01 | | | | **96.1** | **99.4** | **96.1** | **95.5** | **99.0** | **95.8** | 73.2 | 73.5 | 73.9 | 73.5 | 72.8 | 74.0 | 72.7 | **95.8** | **95.5** | **96.4** | 88.7 | 89.0 | 82.9 | 74.2 | 73.9 |
| Kor-ENJ01 | | | | | **96.1** | **100** | **95.5** | **95.8** | **99.7** | 75.5 | 75.8 | 76.5 | 75.8 | 74.8 | 75.2 | 74.6 | **99.7** | **96.8** | **97.7** | 88.4 | 89.6 | 82.6 | 72.6 | 76.1 |
| Kor-EYG02 | | | | | | **96.1** | **95.5** | **99.7** | **95.8** | 73.5 | 73.9 | 74.2 | 73.9 | 73.1 | 74.3 | 73.0 | **95.8** | **95.5** | **96.4** | 88.7 | 89.3 | 83.2 | 73.6 | 74.2 |
| Kor-EAS02 | | | | | | | **95.5** | **95.8** | **99.7** | 75.5 | 75.8 | 76.5 | 75.8 | 74.8 | 75.2 | 74.6 | **99.7** | **96.8** | **97.7** | 88.4 | 89.6 | 82.6 | 72.6 | 76.1 |
| Kor-EYC02 | | | | | | | | **95.8** | **95.1** | 73.9 | 73.9 | 74.8 | 74.2 | 71.2 | 72.3 | 71.1 | **95.1** | **94.8** | **95.8** | 86.8 | 86.7 | 81.6 | 73.2 | 74.5 |
| Kor-EGJ09 | | | | | | | | | **95.5** | 73.9 | 74.2 | 74.5 | 74.2 | 72.8 | 74.0 | 72.7 | **95.5** | **95.1** | **96.1** | 88.4 | 89.0 | 82.9 | 73.9 | 74.5 |
| **Korean isolates** | | | | | | | | | | | | | | | | | | | | | | | | |
| ChYy07AB | | | | | | | | | | 75.0 | 75.3 | 76.0 | 75.3 | 75.1 | 75.7 | 75.1 | **100** | **97.1** | **98.1** | 88.6 | 89.9 | 82.5 | 73.7 | 75.6 |
| NC1 | | | | | | | | | | | **99.0** | **94.5** | **99.0** | 78.6 | 79.0 | 78.4 | 75.2 | 76.5 | 77.3 | 73.2 | 75.0 | 76.5 | 72.9 | **99.0** |
| DS | | | | | | | | | | | | **94.8** | **98.7** | 79.2 | 79.7 | 79.0 | 75.5 | 76.1 | 76.3 | 73.5 | 75.3 | 76.5 | 73.5 | **99.4** |
| CGJJ11-1 | | | | | | | | | | | | | **94.5** | 78.9 | 79.4 | 78.7 | 76.1 | 77.6 | 78.9 | 74.2 | 74.4 | 75.2 | 72.6 | **95.5** |
| TSWD11 | | | | | | | | | | | | | | 78.9 | 79.4 | 78.7 | 75.5 | 76.8 | 77.6 | 73.5 | 75.3 | 76.5 | 73.2 | **98.7** |
| ChYy07VR | | | | | | | | | | | | | | | **98.4** | **100** | 75.1 | 73.5 | 74.4 | 74.8 | 75.1 | 71.8 | 71.6 | 78.9 |
| DRT | | | | | | | | | | | | | | | | **98.4** | 75.6 | 74.6 | 76.0 | 74.8 | 76.1 | 73.2 | 73.3 | 79.4 |
| **Worldwide isolates** | | | | | | | | | | | | | | | | | | | | | | | | |
| VR299 | | | | | | | | | | | | | | | | | 74.9 | 73.3 | 74.7 | 74.6 | 75.1 | 71.9 | 72.0 | 78.7 |
| Ab | | | | | | | | | | | | | | | | | | **97.1** | **98.1** | 88.7 | 89.9 | 82.3 | 72.9 | 75.8 |
| EEV | | | | | | | | | | | | | | | | | | | **99.0** | 88.3 | 88.6 | 82.5 | 73.8 | 77.2 |
| EIS | | | | | | | | | | | | | | | | | | | | 89.3 | 89.3 | 82.8 | 74.0 | 75.6 |
| Sp | | | | | | | | | | | | | | | | | | | | | 85.7 | 81.6 | 75.6 | 73.9 |
| ASV | | | | | | | | | | | | | | | | | | | | | | 82.8 | 72.2 | 75.0 |
| Canada 2 | | | | | | | | | | | | | | | | | | | | | | | 72.9 | 76.1 |
| Hecht | | | | | | | | | | | | | | | | | | | | | | | | 73.8 |

pared with those of 86 worldwide aquabirnavirus isolates. The nucleotide sequences showed more than 94.2% identity among the 9 Korean eel isolates, 71.2% identity among 16 Korean isolates and 71.1% identity among 25 worldwide isolates (Table 2). The Korean eel isolates showed lower nucleotide identity (72.6–76.5%) with marine isolates (NC1, DS, CGJJ11-1 and TSWD11) from aquatic animals in the coastal waters of Korea, but exhibited higher nucleotide identity (94.2–99.7%) with ChYy07AB (host: chum salmon; origin: Korea), Ab (trout, Denmark), EEV (eel, Japan) and EIS (eel, Taiwan) isolates.

A phylogenetic tree based on the VP2/NS gene sequences revealed 7 genogroups as follows (Fig. 2): genogroup I includes strains from Canada, the USA, Korea and Japan; genogroup II includes isolates from Denmark, Spain, Taiwan, Korea and Japan; genogroup III includes isolates from France, Norway, Thailand, England, Spain and Denmark; genogroup IV includes isolates from Canada and England; genogroup V includes Canadian isolates; genogroup VI includes a single German isolate; and genogroup VII is composed of Korean and Japanese isolates from marine fish and molluscan shellfish. All 9 isolates in this study were classified into genogroup II. Four isolates (Kor-ESC00, Kor-EGS01, Kor-ENJ01 and Kor-EAS02) were nearly identical to the Ab isolate, whereas the other 5 isolates (Kor-EGS00, Kor-EGC01, Kor-EYC02, Kor-EYG02 and Kor-EGJ09) were closely related to the EIS isolate (Fig. 2). From these results, it was confirmed that 3 different genogroups (I, II and VII) were present among Korean aquabirnaviruses, and that the Korean eel isolates were distinct from the marine isolates (genogroup VII) in Korean coastal areas. These results indicate that the aquabirnavirus infection in eel did not occur in a Korean seawater environment.

Aquabirnaviruses are thought to be spread from continent to continent by importation of salmonid fish or fish eggs,

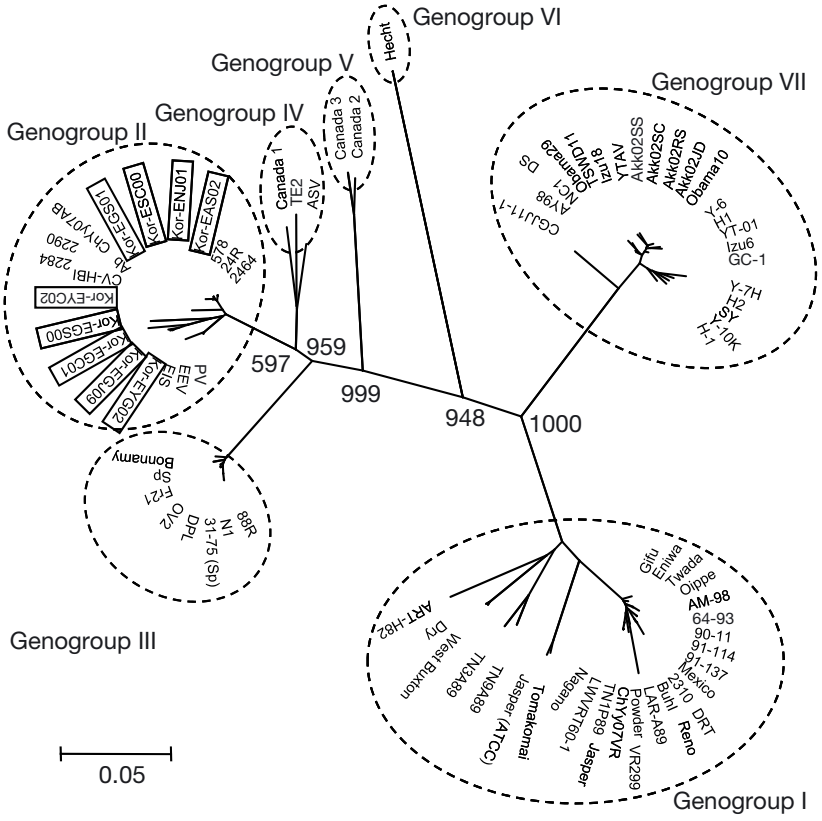


Fig. 2. Molecular phylogenetic tree of the genetic relationship among 86 aquabirnaviruses based on VP2/NS gene nucleotide sequences. Bootstrap values for 1000 replicates are shown at major nodes on the tree. Distance marker refers to the expected number of substitutions per site. Boxes indicate isolates found in this study

but it is likely that the virus had a global distribution prior to the widespread dissemination of salmonids in the 19th and early 20th centuries (Reno 1999). Aquabirnaviruses are generally separated by geographical origin. However, it is interesting that the Korean aquabirnaviruses were classified into 3 different genogroups: I, II and VII. In Korea, IPNV of the VR-299 serotype was first isolated from goldfish and chum salmon in 1983 (Hah et al. 1984). Since then, several VR-299 serotype IPNV isolates and isolates (ChYy07AB and DRT) belonging to the same genogroup I as VR-299 have been detected in chum salmon and rainbow trout (Hedrick et al. 1985, Chung et al. 1994, Jeon et al. 2011). Thus, IPNV of the VR-299 type (genogroup I) have been commonly found in Korean freshwater environments since 1983. During the 1960s–1990s, several species of salmon fry and eggs were imported from the USA and Japan to Korea, where the VR-299 type IPNV existed. Therefore, it is likely that VR-299 type IPNV was introduced to Korea during that time, although no supportive evidence is available.

Another isolate (ChYy07AB) belonging to genogroup II was also detected from chum salmon in 2007 in Korea (Jeon et al. 2011). In the present study, all Korean eel isolates from 2000 to 2009 belonged to genogroup II and were closely related to eel isolates from Taiwan and Japan and trout isolates from Denmark. During the 1990s–2000s, many glass eel were imported from Taiwan, Japan, China, Hong Kong, Denmark, Spain and France. Additionally, aquabirnaviruses belonging to genogroup II were not detected in Korea until 1999, even though isolates belonging to genogroup I were endemic viruses in Korean freshwater environments. Therefore, these records and results suggest that Korean aquabirnavirus isolates from eel have most likely been introduced by importation of glass eels.

As artificial reproduction of eel is not yet possible on a commercial scale, production of eel is based entirely on wild glass eel catches. Thus, the success of eel culture depends on the natural resource. The demand for glass eels (Japanese eel) is increasing in Korea, Japan and China, making their value very high. Therefore, several kinds of eel such as European eel *Anguilla anguilla*, shortfin eel *A. bicolor*, African longfin eel *A. mossambica* and American eel *A. rostrata* are currently imported to Korea from Denmark, Greece, France, Canada, China, the USA, the Philippines, Indonesia and Madagascar. This may lead to the potential introduction of eel disease agents into aquaculture production systems. Thus, the imported glass eels should undergo a period of quarantine and health testing to confirm a negative status for these dangerous pathogens.

*Acknowledgements*. This research was supported by the Technology Development Program for Fisheries, Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea.

LITERATURE CITED

Azumi K, Nakamura S, Kitamura SI, Jung SJ, and others (2007) Accumulation of organotin compounds and mar-

ine birnavirus detection in Korean ascidians. Fish Sci 73: 263–269

Blake S, Ma JY, Caporale DA, Jairath S, Nicholson BL (2001) Phylogenetic relationships of aquatic birnaviruses based on deduced amino acid sequences of genome segment A cDNA. Dis Aquat Org 45:89–102

Chung HK, Lee SH, Lee HH, Lee DS, Kim YS (1994) Nucleotide sequence analysis of the VP2-NS-VP3 genes of infectious pancreatic necrosis virus DRT strain. Mol Cells 4:349–354

Cutrín JM, Barja JL, Nicholson BL, Bandín I, Blake S, Dopazo CP (2004) Restriction fragment length polymorphisms and sequence analysis: an approach for genotyping infectious pancreatic necrosis virus reference strains and other aquabirnaviruses isolated from northwestern Spain. Appl Environ Microbiol 70:1059–1067

Delmas B, Mundt E, Vakharia VN, Wu JL (2012) Family *Birnaviridae*. In: King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ (eds) Virus taxonomy: Ninth report of the International Committee on Taxonomy of Viruses. Elsevier/Academic Press, London, p 499–507

Dobos P (1995) The molecular biology of infectious pancreatic necrosis virus (IPNV). Annu Rev Fish Dis 5: 25–54

Duncan R, Dobos P (1986) The nucleotide sequence of infectious pancreatic necrosis virus (IPNV) dsRNA segment A reveals one large ORF encoding a precursor polyprotein. Nucleic Acids Res 14:5934

Duncan R, Nagy E, Krell PJ, Dobos P (1987) Synthesis of the infectious pancreatic necrosis virus polyprotein, detection of a virus encoded protease, and fine structure mapping of genome segment A coding regions. J Virol 61: 3655–3664

Hah YC, Hong SW, Kim MH, Fryer JL, Winton JR (1984) Isolation of infectious pancreatic necrosis virus from goldfish (*Carassius auratus*) and chum salmon (*Oncorhynchus keta*) in Korea. Korean J Microbiol 22:85–90

Håvarstein LS, Kalland KH, Christie KE, Endresen C (1990) Sequence of the large double-stranded RNA segment of the N1 strain of infectious pancreatic necrosis virus: a comparison with other *Birnaviridae*. J Gen Virol 71: 299–308

Hedrick RP, Eaton WD, Fryer JL, Hah YC, Park JW, Hong SW (1985) Biochemical and serological properties of birnaviruses isolated from fish in Korea. Fish Pathol 20: 463–468

Jeon CH, Kim SR, Kim WS, Lee CH, and others (2011) Monitoring of viruses in chum salmon (*Oncorhynchus keta*) migrating to Korea. Arch Virol 156:1025–1030

Joh SJ, Kim JH, Heo GJ (2000) Genetic analysis of the VP2/NS junction region on segment A of marine birnavirus isolated from cultured flounder *Paralichthys olivaceus.* J Microbiol 38:44–47

Jung SJ, Kim SR, Joung IY, Kitamura SI, Ceong HT, Oh MJ (2008) Distribution of marine birnavirus in cultured olive flounder *Paralichthys olivaceous* in Korea. J Microbiol 46:265–273

Kim WS, Ok HN, Kim DH, Kim HY, Oh MJ (2011) Current status of pathogen infection in cultured eel *Anguilla japonica* between 2000 and 2010. J Fish Pathol 24: 237–245 (in Korean with English Abstract)

Kim HJ, Yu JH, Kim DW, Kwon SR, Park SW (2012) Molecular evidence of anguillid herpesvirus-1 (AngHV-1) in the farmed Japanese eel, *Anguilla japonica* Temminck & Schlegel, in Korea. J Fish Dis 35:315–319

Kim WS, Choi SY, Kim DH, Oh MJ (2013) A survey of fish viruses isolated from wild marine fishes from the coastal waters of southern Korea. J Vet Diagn Invest 25:750–755

Kitamura SI, Ko JY, Lee WL, Kim SR, and others (2007) Seasonal prevalence of lymphocystis disease virus and aquabirnavirus in Japanese flounder *Paralichthys olivaceous* and blue mussel *Mytilus galloprovincialis*. Aquaculture 266:26–31

Lyoo YS, Chang CH, Jean YH, Lee JO, Rhee JC (1991) Isolation of infectious pancreatic necrosis virus from rainbow trout in Korea. Korean J Vet Res 31:195–199 (in Korean with English Abstract)

Magyar G, Dobos P (1994) Evidence for the detection of the infectious pancreatic necrosis virus polyprotein and the 17-kDa polypeptide in infected cells and of the NS protease in purified virus. Virology 204:580–589

Manning DS, Leong JC (1990) Expression in *Escherichia coli* of the large genomic segment of infectious pancreatic necrosis virus. Virology 179:16–25

Manning DS, Mason CL, Leong JC (1990) Cell-free translational analysis of the processing of infectious pancreatic necrosis virus polyprotein. Virology 179:9–15

Nishizawa T, Kinoshita S, Yoshimizu M (2005) An approach for genogrouping of Japanese isolates of aquabirnaviruses in a new genogroup, VII, based on the VP2/NS junction region. J Gen Virol 86:1973–1978

Oh MJ, Jung SJ, Kim YJ (1999) Detection of birnavirus from cultured marine fish using polymerase chain reaction. J Fish Pathol 12:49–55 (in Korean with English Abstract)

Oh MJ, Jung SJ, Kitamura SI, Kim HR, Kang SY (2006) Viral diseases of olive flounder in Korean hatchery. J Ocean Univ China 5:45–48

Park JW, Lee JJ, Jeong GJ, Hah YC (1989) Characterization of the infectious pancreatic necrosis virus (IPNV) isolated from pancultured rainbow trout in Korea. Korean J Microbiol 27:225–230

Reno PW (1999) Infectious pancreatic necrosis and associated aquatic birnaviruses. In: Woo PTK, Bruno DW (eds) Fish diseases and disorders, Vol 3. CABI Publishing, New York, NY, p 1–55

Sohn SG, Park MA, Do JW, Choi JY, Park JW (1995) Birnavirus isolated from cultured flounder in Korea. Fish Pathol 30:279–280

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882

Wolf K (1988) Infectious pancreatic necrosis. In: Wolf K (ed) Fish viruses and fish viral diseases. Cornell University Press, New York, NY, p 115–157

*Editorial responsibility: Mark Crane, Geelong, Victoria, Australia*

*Submitted: October 17, 2013; Accepted: January 20, 2014*
*Proofs received from author(s): April 2, 2014*