Identification of Largemouth Bass Virus in the Introduced Northern Snakehead (*Channa argus)* Inhabiting the Chesapeake Bay Watershed

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Abstract

The Northern snakehead (*Channa argus*) is an introduced species that now inhabits the Chesapeake Bay. During a preliminary survey for introduced pathogens possibly harbored by these fish in Virginia waters, a filterable agent was isolated from five specimens that produced cytopathic effects in BF-2 cells. Based on PCR amplification and partial sequencing of the major capsid protein (MCP), DNA polymerase (DNApol) and DNA methyltransferase (Mtase) genes, the isolates were identified as largemouth bass virus (LMBV). Nucleotide sequences of the MCP (492 bp) and DNApol (419 pb) genes were 100% identical to those of LMBV. The nucleotide sequence of the Mtase (206 bp) gene was 99.5% identical with that of LMBV and the single nucleotide substitution did not lead to a predicted amino acid coding change. This is the first report of LMBV from the Northern snakehead, and evidence that non-Centrarchids may be susceptible to this virus.

Methods

Fish Collection

During September of 2006, Northern snakehead (n=15) were collected via electroshocking. Fish were collected from multiple sites within Dogue Creek and Little Hunting Creek. Both Dogue Creek and Little Hunting Creek are tidal tributaries located in Fairfax County, VA and are tributaries of the Potomac river. Liver, kidney and spleen were extracted and pooled from individual wild caught Northern snakehead and maintained on ice. All samples were processed within 12 h of arrival to the lab.

Cell Culture

- Tissues were homogenized and inoculated onto the BF-2, CHSE-214 and EPC cell lines.

Electron Microscopy

- Supernatant from cultures exhibiting cytopathic effect (CPE) were prepared for negative staining and imaging via electron microscopy (not shown).

Molecular Identification

- Based on electron microscopy results, it was suspected that the viral agent was a member of the Iridoviridae or a morphologically similar family of viruses.

- DNA was extracted from tissue culture supernatants or tissue culture cells exhibiting CPE were extracted with the Pure Viral nucleic acid (Roche) or DNeasy (Qiagen) extration kits. Extractions were performed as per manufacturer protocols.

- End point PCR was performed on all DNA preparations using a number of primer sets (Table 1)

- Direct sequencing of the products was conducted using a LMBV isolate as a positive control

Primer set (PS)	Primer Pair	Primer Sequence	Target	Expected Product size (bp)	Amplification *
PS1 [a]	MCP-F MCP-R	GACTTGGCCACTTATGAC GTCTCTGGAGAAGAAGAA	Major Capsid Protein	530	Yes
PS2 [b]	DNApolF DNAPolR	GTGTAYCAGTGGTTTTGCGAC TCGTCTCCGGGYCTGTCTTT	DNA polymerase	560	Yes
PS4 [d]	MtaseF MtaseR	TGTGGGCCAGTCCACCTTG GTAAGCGCAGTAATCCAACACG	DNA methyltransferase	244	Yes
PS5 [c]	LMBVscscF LMBVscscR	AAGCCTCTGGACCATGCAGAAAGA TTGAGATATGCGTCAGACACCGCA	DNA-dependent RNA polymerase, nucleoside triphosphate 1	251	Yes
PS6 [a]	NF-H1-F NF-H1-R	CCAAAGACCAAAGACCAG GTTGGTCTTTGGTCTCGCTC	Ranavirus Neurofilament-H1-like	639	No
PS7 [c]	LMBI_CY15F LMBI_CY15R	TGTCATCTGCACGTACACCCTGAA GTGCGTTTGCCAGATACGCTTCAT	CY15	635	No

Table 1. PCR conditions for all reactions included a pre-heating step (94 °C, 5 min) and a final extension (72 °C, 10 min). Other parameters differed by primer set.
[a] Denaturation (94 °C, 60 s), annealing (48 °C, 30 s), extension (72 °C, 60 s), cycles (30)
[b] Denaturation (94 °C, 60 s), annealing (50 °C, 30 s), extension (72 °C, 60 s), cycles (30)
[c] Denaturation (94 °C, 60 s), annealing (60 °C, 30 s), extension (72 °C, 60 s), cycles (30)
[d] Denaturation (55 °C, 30 s), extension (72 °C, 60 s), cycles (30)

Results

Molecular Identification

- Based on gross and hisological observations all 15 snakeheads were apparently-healthy

- CPE was observed in BF-2 cells from 5 of 15 fish

- End point PCR yielded product of expected size for LMBV for primer sets PS1, PS2, PS4 & PS5

- Direct sequencing of products demonstrated that nucleotide identity of MCP and DNA polymerase genes were identical to those of LMBV (AF080250 and DQ159940 and the positive LMBV (JQ178325 and JQ178326; Table 2).

- A single, synonymous mutation was noted in the DNA methyltransferase sequence from all snakehead isolates, but this nucleotide substitution is not predicted to affect protein coding.

Iridovirus	MCP		DNApol		Mtase	
	nucleotide	AA	nucleotide	AA	nucleotide	AA
LMBV (PB02-30, USA)	100	100	100	100	99.5	100
LMBV (USA)	100	100	100	100	99.5	100
LMBV (China)	97.9	98.1	NA	NA	95.6	98.5
DFV	98.1	98.1	100	100	97.5	98.5
GV6	98.1	98	100	100	NA	NA
EHNV	70.6	72.3	63.3	66.8	68.1	66.1
FV3	75.7	80.3	63.7	68.0	67.1	63.2
TFV	76.3	80.9	63.7	66.2	67.6	64.7
ECV	76.3	82.8	63.3	67.4	68.1	66.1
ATV	75.7	80.3	63.0	66.2	67.1	64.7
LCDV	51.2	49.4	44.4	26.9	53.6	54.4

Table 2. Identity of nucleotide (DNA) and protein (AA) between partial sequences the snakehead isolates and related iridoviruses. Viral genes included the 492 nucleotides of the major capsid protein (MCP; 492 bp), 519 nucleotides of DNA polymerase (DNApol; 519 bp), DNA methyltransferase (Mtase; 206 bp). Identity was denoted as not available (NA) if homologous sequence data were not present in the NCBI database. Nucleotide sequences for these comparisons (MCP, DNA polymerase and DNA methyltrasferase) included (1) Snakehead isolate; JQ178328, JQ178329 and JQ178330 2) Largemouth bass virus, LMBV (PB02-30, USA); JQ178325, JQ178326 and JQ178327 (3) LMBV (USA); AF080250, DQ159940 and AF100199 (4) LMBV (China); GU256635, NA and GU256634 (5) Doctor fish virus, DFV; FR677324, FJ374281, AF100202 6) Guppy virus 6, GV6; FR677325, FJ374282, and NA (7) Epizootic haematopoietic necrosis virus, EHNV; complete genome, FJ433873 (8) Frog virus 3, FV3; complete genome, AY548484 (9) Tiger frog virus, TFV; complete genome AF389451 (10) European catfish virus, ECV; complete genome, JQ724856 (11) *Ambystoma tigrinum stebbensi*, ATV; complete genome, AY150217 (12) Lymphocysis disease virus, LCDV, complete genome, AY380826.

Summary

- Originally it was suspected that the isolated snakehead virus was an invasive ‘hitch-hiker’ pathogen, based on the current sequence data it seems more likely that snakeheads were infected while in the US. This of course can only be confirmed via more rigorous, multi-gene molecular phylogeny studies.

- This is the first report of LMBV isolated from snakeheads, and one of a few instances of this pathogen isolated from a non-Centrarchids

- This isolate is not 100% identical to LMBV

- It differs from the largemouth bass iridovirus previously identified in Asia, and is most similar to the USA LMBV, DFV and GV6 (Figure 1)

- The significance of LMBV in snakeheads is unknown without subsequent pathogen challenge experiments

- Full genome sequencing of this isolate and others is necessary for phylogeographic, epidemiological studies, and may yield insights into molecular mechanisms of host-jumping

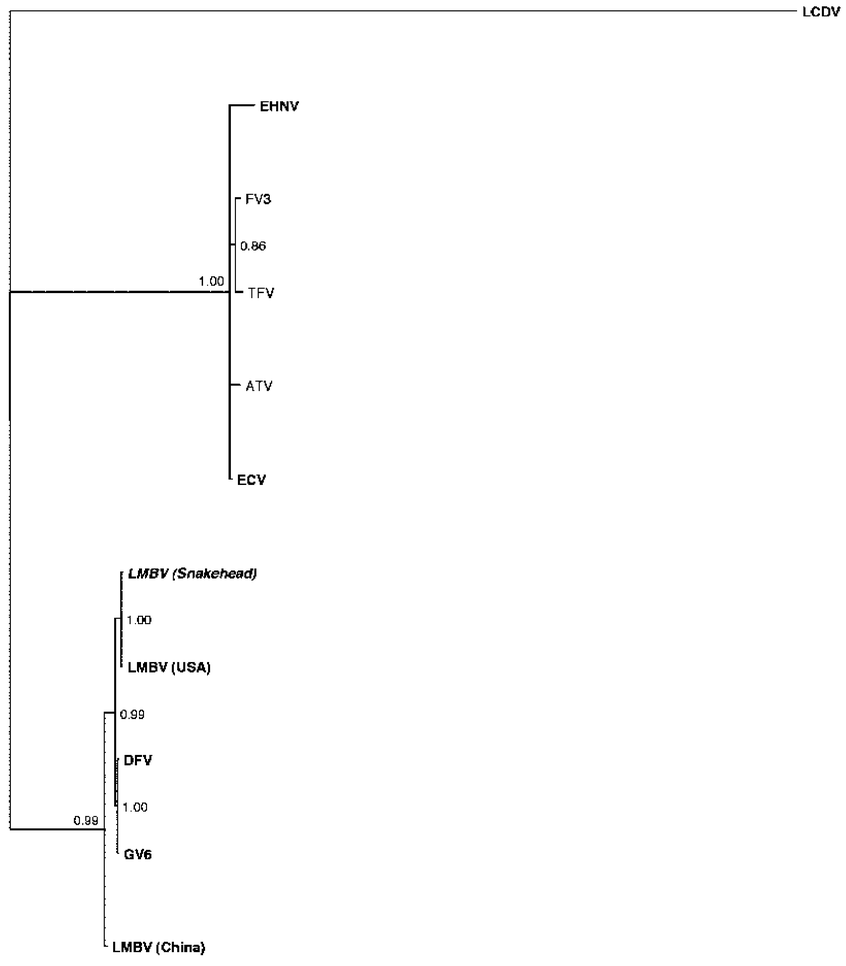

Figure 1. Phylogram of fish and amphibian Irodoviruses from concatenated sequences (1,217 bp) of the major capsid protein, DNA polymerase and DNA methyltransferase genes used in Table 2. Bayesian analysis was conducted with Mr. Bayes 3.1.1 software (Ronquist and Huelsenbeck 2003). Gaps were treated as informative data, and missing data was coded as such. Bayesian inference of phylogeny was conducted using the general time reversible (GTR+G) model. Four Markov chain Monte Carlo chains were run with default heating parameters until convergence. A consensus phylogram was constructed from 19,750 trees sampled from the posterior probability distribution. Iridoviruses that infect fish hosts are denoted with bold text. The isolate from snakehead is italicized and in bold text.

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