JEE ISSN: 2288-1220
https://doi.org/10.5141/jee.22.051
Journal of Ecology and Environment (2022) 46:27

SHORT COMMUNICATION

Comparison of detective ranavirus with major capsid protein gene from infected frogs (*Pelophylax nigromaculatus* and *Lithobates catesbeianus*) in South Korea

Jongsun Kim[1†], Nam-Ho Roh[2†], Jaejin Park[1] and Daesik Park[1*]

[1]Division of Science Education, Kangwon National University, Chuncheon 24341, Republic of Korea
[2]Department of Biological Sciences, Kangwon National University, Chuncheon 24341, Republic of Korea

ARTICLE INFO
Received July 19, 2022
Revised September 15, 2022
Accepted October 6, 2022
Published on October 24, 2022

***Corresponding author**
Daesik Park
E-mail parkda@kangwon.ac.kr

†These authors contributed equally to this work.

Ranaviruses are a primary cause of amphibian extinctions. More consistent ranavirus-infection reports and genetic characterizations of identified viruses are urgently needed, particularly from Asian countries. The objectives of this study were to obtain the partial major capsid protein (MCP) gene sequences (506 bp) of the ranavirus responsible for infecting frogs in South Korea, as our previous research had confirmed using qPCR, and to evaluate their genetic relationships with other previously reported ranavirus sequences. Three different ranavirus MCP sequences were obtained from *Pelophylax nigromaculatus* and *Lithobates catesbeianus*. All six different types of MCP sequence from the ranavirus identified in South Korea to date belonged to the Frog virus 3 (FV3)-like virus group in the genus *Ranavirus*. To better understand the origin and spread of ranaviruses in South Korea, further infection reports and full genome analyses of the identified ranaviruses are needed.

Keywords: Asia, frog virus 3, infectious disease, Iridoviridae

Introduction

Ranaviruses in the genus *Ranavirus*, Family Iridoviridae, can infect ectothermic organisms, including fishes, amphibians, and reptiles (Duffus et al. 2015; Jancovich et al. 2015). Ranavirus has extirpated local populations of amphibians in many countries and is known to be among the major causes of global amphibian declines (Brunner et al. 2021; Robert 2010). Currently, there are seven species belong to the genus *Ranavirus*, and Frog virus 3 (FV3), *Ambystoma tigrinum* virus (ATV), and Common midwife toad virus (CMTV) are representative species (Jancovich et al. 2015; Walker et al. 2021). FV3 was first discovered in leopard frogs (Granoff et al. 1965) and is known to infect a variety of amphibians, fishes, and reptiles (Duffus et al. 2015; Jancovich et al. 2015; Price et al. 2017; Waltzek et al. 2014). The most widely distributed virus FV3 is reported in almost all parts of the world, including the United States, Canada, Central and South America, Europe, Asia, and Africa (reviewed in Duffus et al. 2015). An elevated response level is needed to cope with increasing amphibian population declines due to ranavirus infections. This should include rigorous reporting of ranavirus infections and thorough genetic characterization of identified ranaviruses to allow global tracking of infection situations.

Recently, ranavirus-related research has increased in Asia, where research has been needed. Although most studies have been conducted in China, ranavirus-induced mass amphibian mortalities have also been reported in Japan, Taiwan, Korea, Malaysia, India, and Russia (Hazeri et al. 2017; Herath et al. 2021; Huang et al. 2011; Hsieh et al. 2021; Lisachov and Lisachova 2022; Mu et al. 2018; Sivasankar et al. 2017; Une et al. 2009; Xu et al. 2010). The first South Korean ranavirus-induced mass mortality was confirmed in 2009 involving larval gold-spotted pond frogs (*Pelophylax chosenicus*) (Kim et al. 2009). Since then, a total of seven cases have been reported, including infections of *Rana uenoi*, *R. huanrenensis*, *Kaloula borealis*, *P. nigromaculatus*, *Dryophytes japonicus*, and *Lithobates catesbeianus* (Kwon et al. 2017; Park et al. 2017; Park et al. 2021; Roh et al. 2022). Among the seven cases, three were confirmed by verification of a partial ranavirus major capsid protein (MCP) gene sequence (Kim et al. 2009; Kwon et al. 2017; Park et al. 2021). A recent report regarding the level of ranavirus prevalence using qPCR targeted three amphibian species (*P. nigromaculatus*, *D. japonicus*, and *L. catesbeianus*) inhabiting agricultural areas (Roh et al. 2022). In this study, between 16.1% and 50.0% of sampled

amphibians were infected, varying by species. Prior to conducting a comprehensive genome study of ranavirus in South Korea, it is necessary to analyze the MCP gene sequences of recently isolated ranaviruses to allow an evaluation of their genetic relationship with previously reported ranaviruses and of existence of multiple ranavirus strains across different amphibian species in South Korea.

The purposes of this study were to obtain the partial MCP sequences of the ranavirus that infects black-spotted pond frogs (*P. nigromaculatus*) and American bullfrogs (*L. catesbeianus*) inhabiting rice paddies, and to preliminarily evaluate their genetic relationship with ranaviruses previously reported in South Korea and abroad.

Materials and Methods

For this study, we selected larvae of *P. nigromaculatus* and *L. catesbeianus*, which were confirmed to be subjected to ranavirus infection through qPCR, with a low cycle threshold (CT) value of 32 or less in our previous study (Roh et al. 2022). For PCR, we used tadpole genomic DNA, which was extracted from the liver tissue, using a DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany), and stored at −80°C (Roh et al. 2022). Before PCR, we measured the concentration of extracted DNA using a Qubit3 Fluorometer (Invitrogen, Waltham, MA, USA) with a Qubit 1X dsDNA HS Assay Kit (Invitrogen) and adjusted it to a 1 ng/μL concentration with molecular biology grade water (Cytiva Korea, Incheon, Korea).

To amplify the partial MCP gene (500 bp), we used MCP4 and MCP5 primers, as used in our previous studies (Kwon et al. 2017; Mao et al. 1997; Park et al. 2021). We conducted PCR in a total volume of 20 μL, which contained 10 μL 2X TOPsimple PreMix nTaq (Enzynomics, Daejeon, Korea), 0.5 μL forward primer, 0.5 μL reverse primer, and 9 μL DNA template, using a SimpliAmp thermal cycler (Applied Biosystems, Waltham, MA, USA). All PCRs were performed with negative control of molecular biology grade water (Cytiva Korea). Targeted PCR products were confirmed on a 1.0% agarose gel, and the partial MCP sequences were obtained using an ABI 3730xl System (Applied Biosystems) at Macrogen, Inc. (Seoul, Korea).

We checked the resulting MCP sequences using Geneious Prime (Biomatters Ltd, Auckland, New Zealand). To examine the genetic relationship between the verified ranavirus types in this study and other previously known ranaviruses, we performed Basic Local Alignment Search Tool (BLAST) analysis and constructed a phylogenetic tree. For the custom nested BLAST analysis, we downloaded 39 partial MCP sequences of representative ranaviruses from the GenBank, such as the viruses previously reported from South Korea, including FV3-like viruses, CMTV-like viruses, and ATV-like viruses, and aligned the sequences using the default setting of MUSCLE (multiple sequence comparison by log-expectation) (Edgar 2004). We performed BLAST using Geneious Prime (Biomatters Ltd). For the lineage-based genetic relationship analysis, we constructed a Bayesian inference (BI) tree using the Markov chain Monte Carlo (MCMC) methods in MrBayes v3.2.4 (Ronquist et al. 2012). We ran 60 million tree generations, starting with a random tree, while saving every 1,000th tree into a file and discarding the first 5% of the sampled generations as burn-ins. For the analysis, we included 44 ranavirus MCP sequences, which were used for the BLAST, and the MCP sequence of short-finned eel ranavirus (FJ358612) was used as an outgroup.

Results and Discussion

Among the ten tadpoles that we previously analyzed to confirm ranavirus infection in a previous study (Roh et al. 2022), we successfully obtained partial sequences (506 bp) of ranavirus MCP gene from four of the *P. nigromaculatus* and two *L. catesbeianus* tadpoles. The MCP sequences of the ranavirus from three *P. nigromaculatus* (one collected from Pohang and two from Yeongam) and one *L. catesbeianus* (from Gimje) were identical (called Type A hereafter, accession number OP009373-009376). Ranavirus from one *P. nigromaculatus* (from Hongcheon, Type B, accession number OP009377) and from one *L. catesbeianus* (from Imja, Type C, accession number OP009378) had a unique MCP sequence (Table 1, Fig. 1).

In the BLAST analysis, Type A sequence showed 99.6% similarity with KRV-1 from South Korea (HM133594), Rana catesbeiana virus from Japan (AB474588), and Rana grylio iridovirus from China (JQ654586), and Type C sequence showed 100% similarity with these three virus types. Type B sequence showed 100% similarity with FV3 (MH332773, KF646249, MF360246, and MF411070) reported in Canada, the United States, the Netherlands, and the United Kingdom and with Lacerta monticola ranavirus (KM516719) reported in Portugal. The similarity between the three MCP sequence types in this study and the two types (KY264204-5), which were previously reported from two mountain frog species (*R. uenoi* and *R. huanrenensis*) in South Korea (Kwon et al. 2017; Park et al. 2021), ranged from 99.2% to 99.8% (Table 1). In the genetic relationship analysis, the three types of partial MCP gene sequences obtained in this study were all placed within the FV3-like ranavirus group in the genus *Ranavirus* (Fig. 1).

In this study, we successfully obtained the partial MCP gene sequence of the ranavirus from four *P. nigromaculatus* and two *L. catesbeianus*. As a result, in South Korea, ranavirus infection was confirmed by MCP gene sequence verification in a total of five anuran species (*P. chosenicus*, *R. uenoi*, *R. huanrenensis*, *P. nigromaculatus*, *L. catesbe-*

Table 1 Results of the custom nested BLAST using partial MCP DNA sequences (506 bp) of the three ranavirus types (Type A, B, and C) from *Pelophylax nigromaculatus* and *Lithobates catesbeianus* in this study

Sequence (accession number)	Host species	Country	Identical sites (%)		
			Type A	Type B	Type C
Type A (OP009373-009376)	*Pelophylax nigromaculatus*, *Lithobates catesbeianus*	Korea		99.4	99.6
Type B (OP009377)	*Pelophylax nigromaculatus*	Korea	99.4		99.8
Type C (OP009378)	*Lithobates catesbeianus*	Korea	99.6	99.8	
Rana catesbeiana virus (AB474588)	*Rana catesbeiana*	Japan	99.6	99.8	100.0
KRV-1 (HM133594)	*Pelophylax chosenicus*	Korea	99.6	99.8	100.0
Rana grylio iridovirus (JQ654586)	*Rana grylio*	China	99.6	99.8	100.0
Rana catesbeiana virus (KC756964)	*Rana catesbeiana*	Taiwan	99.4	99.6	99.8
Soft shelled turtle iridovirus (DQ335253)	*Trionyx sinensis*	China	99.4	99.6	99.8
Rana grylio iridovirus (KY264204)	*Rana huanrenensis*	Korea	99.4	99.6	99.8
Frog virus 3 (MH332773)	*Lithobates sp.*	Canada	99.4	100.0	99.8
Frog virus 3 (KF646249)	*Scaphirhynchus albus*	USA	99.4	100.0	99.8
Frog virus 3 (MF360246)	*Oophaga pumilio*	Netherlands	99.4	100.0	99.8
Frog virus 3 (MF411070)	*Rana temporaria*	UK	99.4	100.0	99.8
Lacerta monticola ranavirus (KM516719)	*Lacerta monticola*	Portugal	99.4	100.0	99.8
Rana grylio iridovirus (KY264205)	*Rana dybowskii* *Rana huanrenensis*	Korea	99.2	99.4	99.6
Frog virus 3 (DQ897669)	*Rana catesbeiana*	Brazil	99.0	99.6	99.4
Bohle iridovirus (FJ358613)	*Limnodynastes ornatus*	Australia	99.0	99.2	99.4
Zoo ranavirus (KF699143)	*Anaxyrus boreas boreas*	USA	98.8	99.0	99.2
German gecko ranavirus (KP266742)	*Uroplatus fimbriatus*	Germany	98.8	99.0	99.2
Common midwife toad virus (JQ231222)	*Alytes obstetricians*	Spain	98.6	98.8	99.0
Common midwife toad virus (MF125269)	*Pelophylax esculentus*	Netherlands	98.4	98.6	98.8
Tiger frog virus (MT512504)	*Hoplobatrachus tigerinus*	Thailand	98.4	98.6	98.8
Tortoise ranavirus (KM516713)	*Testudo hermanni*	Germany	98.2	98.4	98.6
Pike perch iridovirus (FJ358610)	*Stizostedion lucioperca*	Finland	98.2	98.4	98.6
Blood python ranavirus (KM516715)	*Python brongersmai*	Germany	98.0	98.2	98.4

BLAST: Basic Local Alignment Search Tool; MCP: major capsid protein.
Reference ranavirus sequences were downloaded from GenBank.

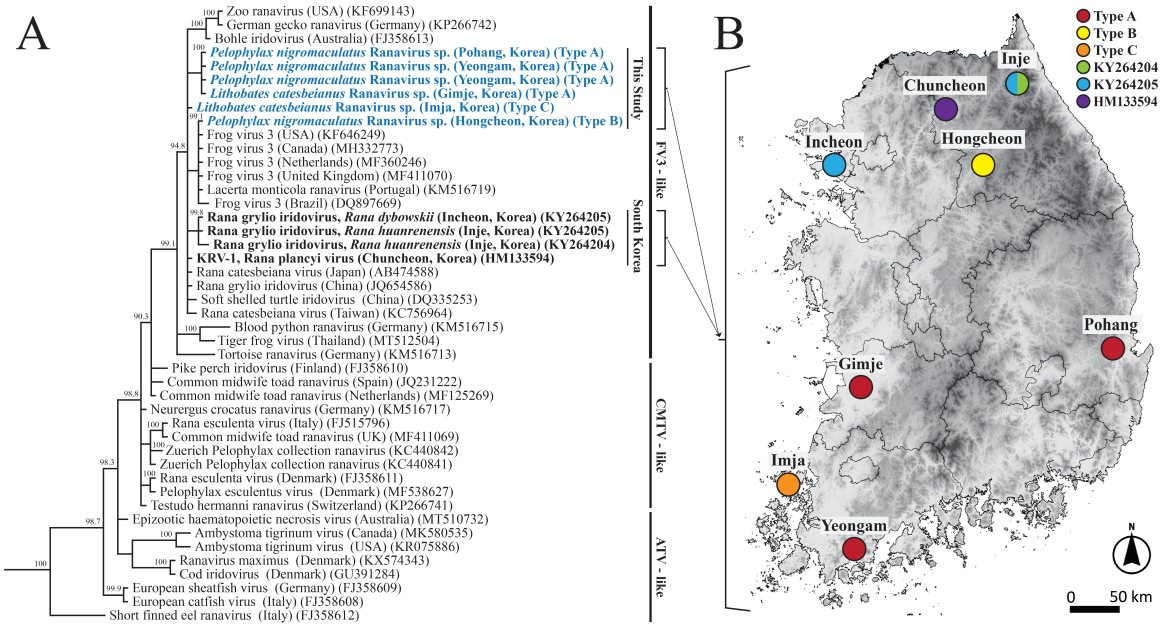

Fig. 1 Bayesian inference (BI) tree (**A**) based on the partial major capsid protein (MCP) DNA sequences (506 bp) of the ranaviruses from *Pelophylax nigromaculatus* and *Lithobates catesbeianus* in this study and the 39 sequences from GenBank (accession number) and the detected location (**B**) of six different FV3-like ranavirus types, which reported in South Korea to date. In BI tree, bold indicates the ranavirus previously reported in South Korea, and blue indicates the ranavirus identified in this study. Each different ranavirus type is indicated on the map with distinct colors.

ianus) (Kim et al. 2009; Kwon et al. 2017; Park et al. 2021; Roh et al. 2022). In particular, this study is the first report on the detection of FV3-like ranavirus in domestic wild American bullfrogs through MCP sequence confirmation in South Korea. In addition, we confirmed, through comparison of partial MCP sequences, that all six types of ranavirus MCP sequence identified in South Korea thus far belong to the FV3-like virus group in the genus *Ranavirus*.

Ranaviruses belonging to the FV3-like virus group in the genus *Ranavirus* are dominantly responsible for infecting Korean anuran amphibians. In the genetic relationship analysis, three newly discovered ranavirus MCP types and four previously reported ranavirus MCP types in South Korea were all placed within the FV3-like virus group. In the genus *Ranavirus*, Family Iridoviridae, FV3, ATV, CMTV, etc., are known to infect amphibians (Granoff et al. 1965; Jancovich et al. 2015; Speare and Smith 1992). In particular, FV3 infects not only amphibians but also fishes (Price et al. 2017) and reptiles (Waltzek et al. 2014), but there have been no such reports in fishes and reptiles in South Korea (Do et al. 2005; Kim et al. 2013; Lee et al. 2019). FV3 has been detected in various wild and cultured amphibian populations throughout the United States and Canada (Duffus et al. 2015; Price et al. 2017) and from introduced American bullfrogs in Brazil, the United Kingdom, Japan, and Taiwan (Ferreira et al. 2021; Gray et al. 2007; Hsieh et al. 2021; Une et al. 2009). Our results show that FV3-like ranaviruses in the genus *Ranavirus* could be the dominant ranavirus species infecting anuran amphibians in South Korea.

All six types of ranavirus MCP sequence reported in Korean amphibians to date belong to the FV3-like virus group with genetic variations. According to previous studies, genetic variations in FV3-like viruses occur frequently (Stöhr et al. 2015), caused by either genetic recombination between viruses of different strains or the process of adapting to different hosts and environments over time (Cronin et al. 2010; Grant et al. 2019; Vilaça et al. 2019). Regarding the possibility of interspecies genetic recombination, evaluation is not currently possible in South Korea. Unfortunately, studies on iridoviruses have not been conducted in other ectothermic organisms that cohabitate with amphibians. The genetic variations in FV3-like viruses due to the adaptation process may be applicable to our results. In this study, various types of ranavirus MCP sequence, within the FV3-like virus group, tended to differ according to host species and collection region. For example, virus MCP sequence types identified from *R. uenoi* and *R. huanrenensis* in the mountain valleys were more closely related to each other. These results suggest that the observed MCP sequence diversity of the ranaviruses in South Korea may be caused by differences in host species and their habitats.

In this study, we confirmed ranavirus infection in two more anuran species (*P. nigromaculatus* and *L. catesbeianus*) in South Korea through MCP sequencing. Additionally, we showed the genetic variety and phylogenetic positions of the ranavirus MCP sequences identified in this study and previous studies from South Korea. Considering increasing cases of ranavirus-induced mass mortalities of amphibians in South Korea (Kim et al. 2009; Kwon et al. 2017; Park et al. 2017; Park et al. 2021), further infection reports and full genome analyses of the identified ranaviruses are urgently needed to better understand the origin and spread of ranavirus across Korean amphibian populations.

Abbreviations

ATV: Ambystoma tigrinum virus
BI: Bayesian inference
BLAST: Basic Local Alignment Search Tool
CMTV: Common midwife toad virus
CT: Cycle threshold
FV3: Frog virus 3
MCMC: Markov chain Monte Carlo
MCP: Major Capsid Protein

Acknowledgements

We thank Woo-Jin Choi, Il-Kook Park, Ji-Ho Park for their help during field sampling and MinWoo Park, Ho-Jun Jung for their help during the dissection.

Authors' contributions

JK did data curation, formal analysis, investigation, and writing-original draft. NHR did data curation, investigation, formal analysis, and writing-review and editing. JP and DP did conceptualization, funding acquisition, supervision, writing-original draft, and writing-review and editing.

Funding

This study was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (No. 2020R1A6A3A1306094911).

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate

This study was reviewed and approved by the Institutional Animal Care and Use Committee of Kangwon National University (KW-200618-3).

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

References

Brunner JL, Olson DH, Gray MJ, Miller DL, Duffus ALJ. Global patterns of ranavirus detections. FACETS. 2021;6:912-24. https://doi.org/10.1139/facets-2020-0013.

Cronin JP, Welsh ME, Dekkers MG, Abercrombie ST, Mitchell CE. Host physiological phenotype explains pathogen reservoir potential. Ecol Lett. 2010;13(10):1221-32. https://doi.org/10.1111/j.1461-0248.2010.01513.x.

Do JW, Cha SJ, Kim JS, An EJ, Park MS, Kim JW, et al. Sequence variation in the gene encoding the major capsid protein of Korean fish iridoviruses. Arch Virol. 2005;150(2):351-9. https://doi.org/10.1007/s00705-004-0424-6.

Duffus ALJ, Waltzek TB, Stöhr AC, Allender MC, Gotesman M, Whittington RJ, et al. Distribution and host range of ranaviruses. In: Gray MJ, Chinchar VG, editors. Ranaviruses. Cham: Springer; 2015. p. 9-57.

Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 2004;32(5):1792-7. https://doi.org/10.1093/nar/gkh340.

Ferreira CM, Subramaniam K, de Sousa RLM, Tavares LS, Corrêa TC, Waltzek TB. Genomic sequencing of a frog virus 3 strain from cultured American bullfrogs (Lithobates catesbeianus) in Brazil. Arch Virol. 2021;166(7):1961-4. https://doi.org/10.1007/s00705-021-05094-y.

Granoff A, Came PE, Rafferty KA Jr. The isolation and properties of viruses from Rana pipiens: their possible relationship to the renal adenocarcinoma of the leopard frog. Ann N Y Acad Sci. 1965;126(1):237-55. https://doi.org/10.1111/j.1749-6632.1965.tb14278.x.

Grant SA, Bienentreu JF, Vilaça ST, Brunetti CR, Lesbarrères D, Murray DL, et al. Low intraspecific variation of Frog virus 3 with evidence for novel FV3-like isolates in central and northwestern Canada. Dis Aquat Organ. 2019;134(1):1-13. https://doi.org/10.3354/dao03354.

Gray MJ, Miller DL, Schmutzer AC, Baldwin CA. Frog virus 3 prevalence in tadpole populations inhabiting cattle-access and non-access wetlands in Tennessee, USA. Dis Aquat Organ. 2007;77(2):97-103. https://doi.org/10.3354/dao01837.

Hazeri M, Hassan MD, Abba Y, Omar AR, Allaudin ZN, Soltani M, et al. Molecular characterisation of grouper iridovirus isolates from Peninsular Malaysia. J Vet Malaysia. 2017;29(1):1-6.

Herath J, Ellepola G, Meegaskumbura M. Patterns of infection, origins, and transmission of ranaviruses among the ectothermic vertebrates of Asia. Ecol Evol. 2021;11(22):15498-519. https://doi.org/10.1002/ece3.8243.

Hsieh CY, Rairat T, Chou CC. Detection of ranavirus by PCR and in situ hybridization in the American bullfrog (Rana catesbeiana) in Taiwan. Aquaculture. 2021;543:736955. https://doi.org/10.1016/j.aquaculture.2021.736955.

Huang SM, Tu C, Tseng CH, Huang CC, Chou CC, Kuo HC, et al. Genetic analysis of fish iridoviruses isolated in Taiwan during 2001-2009. Arch Virol. 2011;156(9):1505-15. https://doi.org/10.1007/s00705-011-1017-9.

Jancovich JK, Steckler NK, Waltzek TB. Ranavirus taxonomy and phylogeny. In: Gray MJ, Chinchar VG, editors. Ranaviruses. Cham: Springer; 2015. p. 59-70.

Kim S, Sim MY, Eom AH, Park DS, Ra NY. PCR detection of ranavirus in gold-spotted pond frogs (Rana plancyi chosenica) from Korea. Korean J Environ Biol. 2009;27(1):110-3.

Kim WS, Choi SY, Kim DH, Oh MJ. A survey of fish viruses isolated from wild marine fishes from the coastal waters of southern Korea. J Vet Diagn Invest. 2013;25(6):750-5. https://doi.org/10.1177/1040638713504755.

Kwon S, Park J, Choi WJ, Koo KS, Lee JG, Park D. First case of ranavirus-associated mass mortality in a natural population of the Huanren frog (Rana huanrenensis) tadpoles in South Korea. Anim Cells Syst. 2017;21(5):358-64. https://doi.org/10.1080/19768354.2017.1376706.

Lee ES, Cho M, Min EY, Jung SH, Kim KI. Genetic relatedness of Megalocytivirus from diseased fishes in Korea. J Fish Pathol. 2019;32(2):49-57. https://doi.org/10.7847/jfp.2019.32.2.049.

Lisachov AP, Lisachova LS, Simonov E. First record of ranavirus (*Ranavirus* sp.) in Siberia, Russia. Herpetozoa. 2022;35:33-7. https://doi.org/10.3897/herpetozoa.35.e79490.

Mao J, Hedrick RP, Chinchar VG. Molecular characterization, sequence analysis, and taxonomic position of newly isolated fish iridoviruses. Virology. 1997;229(1):212-20. https://doi.org/10.1006/viro.1996.8435.

Mu WH, Geng Y, Yu ZH, Wang KY, Huang XL, Ou YP, et al. FV3-like ranavirus infection outbreak in black-spotted pond frogs (Rana nigromaculata) in China. Microb Pathog. 2018;123:111-4. https://doi.org/10.1016/j.micpath.2018.06.047.

Park IK, Koo KS, Moon KY, Lee JG, Park D. PCR detection of ranavirus from dead Kaloula borealis and sick Hyla japonica tadpoles in the wild. Korean J Herpetol. 2017;8(1):10-4.

Park J, Grajal-Puche A, Roh NH, Park IK, Ra NY, Park D. First detection of ranavirus in a wild population of Dybowski's brown frog (Rana dybowskii) in South Korea. J Ecol Environ. 2021;45:2. https://doi.org/10.1186/s41610-020-00179-2.

Price SJ, Ariel E, Maclaine A, Rosa GM, Gray MJ, Brunner JL, et al. From fish to frogs and beyond: impact and host range of emergent ranaviruses. Virology. 2017;511:272-9. https://doi.org/10.1016/j.virol.2017.08.001.

Robert J. Emerging ranaviral infectious diseases and amphibian decline. Diversity. 2010;2(3):314-30. https://doi.org/10.3390/d2030314.

Roh N, Park J, Kim J, Kwon H, Park D. Prevalence of ranavirus infection in three anuran species across South Korea. Viruses. 2022;14(5):1073. https://doi.org/10.3390/v14051073.

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, et al. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol. 2012;61(3):539-42. https://doi.org/10.1093/sysbio/sys029.

Sivasankar P, John KR, George MR, Mageshkumar P, Manzoor MM, Jeyaseelan MJP. Characterization of a virulent ranavirus isolated from marine ornamental fish in India. Virusdisease. 2017;28(4):373-82. https://doi.org/10.1007/s13337-017-0408-2.

Speare R, Smith JR. An iridovirus-like agent isolated from the ornate burrowing frog *Limnodynastes ornatus* in northern Australia. Dis Aquat Organ. 1992;14:51-7. https://doi.org/10.3354/dao014051.

Stöhr AC, López-Bueno A, Blahak S, Caeiro MF, Rosa GM, Alves de

Matos AP, et al. Phylogeny and differentiation of reptilian and amphibian ranaviruses detected in Europe. PLoS One. 2015;10(2): e0118633. https://doi.org/10.1371/journal.pone.0118633.

Une Y, Sakuma A, Matsueda H, Nakai K, Murakami M. Ranavirus outbreak in North American bullfrogs (Rana catesbeiana), Japan, 2008. Emerg Infect Dis. 2009;15(7):1146-7. https://doi.org/10.3201/eid1507.081636.

Vilaça ST, Bienentreu JF, Brunetti CR, Lesbarrères D, Murray DL, Kyle CJ. Frog virus 3 genomes reveal prevalent recombination between ranavirus lineages and their origins in Canada. J Virol. 2019;93(20): e00765-19. https://doi.org/10.1128/JVI.00765-19.

Walker PJ, Siddell SG, Lefkowitz EJ, Mushegian AR, Adriaenssens EM, Alfenas-Zerbini P, et al. Changes to virus taxonomy and to the International Code of Virus Classification and Nomenclature ratified by the International Committee on Taxonomy of Viruses (2021). Arch Virol. 2021;166(9):2633-48. https://doi.org/10.1007/s00705-021-05156-1.

Waltzek TB, Miller DL, Gray MJ, Drecktrah B, Briggler JT, MacConnell B, et al. New disease records for hatchery-reared sturgeon. I. Expansion of frog virus 3 host range into Scaphirhynchus albus. Dis Aquat Organ. 2014;111(3):219-27. https://doi.org/10.3354/dao02761.

Xu K, Zhu DZ, Wei Y, Schloegel LM, Chen XF, Wang XL. Broad distribution of Ranavirus in free-ranging Rana dybowskii in Heilongjiang, China. Ecohealth. 2010;7(1):18-23. https://doi.org/10.1007/s10393-010-0289-y.