



Complete Genome Sequence of Frog virus 3, Isolated from a Strawberry Poison Frog (*Oophaga pumilio*) Imported from Nicaragua into the Netherlands

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ABSTRACT *Frog virus 3* was isolated from a strawberry poison frog (*Oophaga pumilio*) imported from Nicaragua via Germany to the Netherlands, and its complete genome sequence was determined. *Frog virus 3* isolate Op/2015/Netherlands/UU3150324001 is 107,183 bp long and has a nucleotide similarity of 98.26% to the reference *Frog virus 3* isolate.

Ranaviruses (family *Iridoviridae*, subfamily *Alphairidovirinae*) have caused declines of poikilotherm populations worldwide (1–3). These viruses are commonly spread through international trade (4–8), sometimes resulting in the introduction of virulent strains into wild populations (9). In the Netherlands, there are two documented cases of ranaviruses in imported specimens; the first involved frog virus 3 (FV3) in red-tailed knobby newts (*Tylototriton kweichowensis*) from China (10), and the second involved common midwife toad virus (CMTV) in poison dart frogs (*Dendrobates auratus*, *Phyllobates bicolor*) from an undetermined location (11). Poison dart frogs imported from the Netherlands also experienced a frog virus 3-associated die-off upon arrival to Japan (12).

In 2015, two strawberry poison frogs (*Oophaga pumilio*), imported from Nicaragua via Germany, died upon arrival to the Netherlands. No gross lesions were observed, but histopathology revealed mild liver necrosis and intracytoplasmic basophilic inclusions in hepatocytes and bone marrow hematopoietic cells.

Conventional PCR and sequencing of the major capsid protein from liver samples of both animals revealed FV3, a clade of ranaviruses that, unlike CMTV, has not been reported to occur in Dutch nature (3, 13, 14). Subsequently, a 10% organ suspension from one animal was mixed with 1% antibiotics and inoculated on epithelioma papulosum cyprini cells. Once full cytopathic effect was observed, the virus was purified by high-speed ultracentrifugation on a 36% sucrose cushion as described previously (14). After resuspension in ice-cold phosphate-buffered saline (PBS), the DNA was extracted with the QIAamp DNA blood minikit (Qiagen) according to the manufacturer's protocol. The DNA was sheared by sonication, and a library was prepared using the KAPA library preparation kit. A MiSeq system running a V3 chemistry platform (Illumina) was used to generate 2 × 300-nucleotide paired-end sequence reads. After quality control of the sequence reads using Trim Galore (<http://github.com/FelixKrueger/TrimGalore>), a *de novo* assembly using SPAdes (15) produced a contig of 107,183 bp with a total G+C content of 54.95%. Annotations were performed manually with ORF finder at the NCBI website to highlight putative protein products using the genome of the American FV3 isolate (FV3-reference; GenBank accession no. AY548484) as a reference (16).

The genomic structure of the FV3 *Oophaga pumilio* isolate (FV3-Op) showed a nucleotide similarity of 98.26% to the genome of FV3-reference and a 97.92% nucle-

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otide similarity to FV3-SSME (KJ175144) (17). All 98 putative open reading frames present in other FV3-like virus counterparts were identified, with only a few features distinct from FV3-reference, including the lack of truncation in the eukaryotic initiation factor 2 alpha protein. It has been suggested that strains with the capacity to express the full version of this immunomodulatory protein are more pathogenic than those with a truncated version (18). Phylogenetic characterization using 45 ranavirus genes positioned FV3-Op in the FV3 clade, in a separate cluster from soft-shelled turtle iridovirus (EU627010.1) (19), *Rana grylio* iridovirus (JQ6545861.1) (20), and tiger frog virus (AF389451) (21). The isolation of FV3 from imported amphibians from Nicaragua highlights the constant risk of trade-associated introduction of foreign *Ranavirus* spp.

Accession number(s). This whole-genome shotgun project has been deposited in DDBJ/ENA/GenBank under the accession no. [MF360246](https://doi.org/10.1016/j.cub.2014.09.028). The version described in this paper is the first version, MF360246.1.

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REFERENCES

- Price SJ, Garner TW, Nichols RA, Ballou F, Ayres C, Mora-Cabello de Alba A, Bosch J. 2014. Collapse of amphibian communities due to an introduced *Ranavirus*. *Curr Biol* 24:2586–2591. <https://doi.org/10.1016/j.cub.2014.09.028>.
- Teacher AGF, Cunningham AA, Garner TWJ. 2010. Assessing the long-term impact of *Ranavirus* infection on wild common frog populations. *Anim Conserv* 13:514–522. <https://doi.org/10.1111/j.1469-1795.2010.00373.x>.
- Rijks JM, Saucedo B, Spitzen-van der Sluijs A, Wilkie GS, van Asten AJ, van den Broek J, Boonyarittichaij R, Stege M, van der Sterren F, Martel A, Pasmans F, Hughes J, Gröne A, van Beurden SJ, Kik MJL. 2016. Investigation of amphibian mortality events in wildlife reveals an on-going ranavirus epidemic in the north of the Netherlands. *PLoS One* 11: e0157473. <https://doi.org/10.1371/journal.pone.0157473>.
- Picco AM, Collins JP. 2008. Amphibian commerce as a likely source of pathogen pollution. *Conserv Biol* 22:1582–1589. <https://doi.org/10.1111/j.1523-1739.2008.01025.x>.
- Schloegel LM, Picco AM, Kilpatrick AM, Davies AJ, Hyatt AD, Daszak P. 2009. Magnitude of the US trade in amphibians and presence of *Batrachochytrium dendrobatidis* and *Ranavirus* infection in imported North American bullfrogs (*Rana catesbeiana*). *Biol Conserv* 142:1420–1426. <https://doi.org/10.1016/j.biocon.2009.02.007>.
- Stöhr AC, Blahak S, Heckers KO, Wiechert J, Behncke H, Mathes K, Günther P, Zwart P, Ball I, Rüschoff B, Marschang RE. 2013. *Ranavirus* infections associated with skin lesions in lizards. *Vet Res* 44:84. <https://doi.org/10.1186/1297-9716-44-84>.
- Kolby JE, Smith KM, Berger L, Karesh WB, Preston A, Pessier AP, Skerratt LF. 2014. First evidence of amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) and ranavirus in Hong Kong amphibian trade. *PLoS One* 9:1–6. <https://doi.org/10.1371/journal.pone.0090750>.
- Stöhr AC, López-Bueno A, Blahak S, Caeiro MF, Rosa GM, Alves de Matos AP, Martel A, Alejo A, Marschang RE. 2015. Phylogeny and differentiation of reptilian and amphibian ranaviruses detected in Europe. *PLoS One* 10:e0118633. <https://doi.org/10.1371/journal.pone.0118633>.
- Epstein B, Storfer A. 2015. Comparative genomics of an emerging amphibian virus. *G3* 6:15–27. <https://doi.org/10.1534/g3.115.023762>.
- Pasmans F, Blahak S, Martel A, Pantchev N, Zwart P. 2008. *Ranavirus*-associated mass mortality in imported red tailed knobby newts (*Tylotriton kweichowensis*): a case report. *Vet J* 176:257–259. <https://doi.org/10.1016/j.tvjl.2007.02.028>.
- Kik M, Stege M, Boonyarittichaij R, van Asten A. 2012. Concurrent ranavirus and *Batrachochytrium dendrobatidis* infection in captive frogs (*Phyllobates* and *Dendrobates* species), The Netherlands, 2012: a first report. *Vet J* 194:247–249. <https://doi.org/10.1016/j.tvjl.2012.09.016>.
- Une Y, Kudo T, Tamukai K, Murakami M. 2014. Epidemic ranaviral disease in imported captive frogs (*Dendrobates* and *Phyllobates* spp.), Japan, 2012: a first report. *JMM Case Rep*. <https://doi.org/10.1099/jmmcr.0.001198>.
- Kik M, Martel A, Sluijs AS, Pasmans F, Wohlsein P, Gröne A, Rijks JM. 2011. *Ranavirus*-associated mass mortality in wild amphibians, The Netherlands, 2010: a first report. *Vet J* 190:284–286. <https://doi.org/10.1016/j.tvjl.2011.08.031>.
- van Beurden SJ, Hughes J, Saucedo B, Rijks J, Kik M, Haenen OLM, Engelsma MY, Gröne A, Verheije HM, Wilkie G. 2014. Complete genome sequence of a common midwife toad-like ranavirus associated with mass mortalities in wild amphibians in the Netherlands. *Genome Announc* 2(6):e01293-14. <https://doi.org/10.1128/genomeA.01293-14>.
- Nurk S, Bankevich A, Antipov D, Gurevich AA, Korobeynikov A, Lapidus A, Pribelski AD, Pyshkin A, Sirotkin A, Sirotkin Y, Stepanauskas R, Clin-genpeel SR, Woyke T, McLean JS, Lasken R, Tesler G, Alekseyev MA, Pevzner PA. 2013. Assembling single-cell genomes and mini-metagenomes from chimeric MDA products. *J Comput Biol* 20:714–737. <https://doi.org/10.1089/cmb.2013.0084>.
- Tan WGH, Barkman TJ, Gregory Chinchar VG, Essani K. 2004. Comparative genomic analyses of frog virus 3, type species of the genus *Ranavirus* (family *Iridoviridae*). *Virology* 323:70–84. <https://doi.org/10.1016/j.virol.2004.02.019>.
- Morrison EA, Garner S, Echaubard P, Lesbarrères D, Kyle CJ, Brunetti CR. 2014. Complete genome analysis of a frog virus 3 (FV3) isolate and sequence comparison with isolates of differing levels of virulence. *Virology* 466:11–46. <https://doi.org/10.1186/1743-422X-11-46>.
- Rothenburg S, Chinchar VG, Dever TE. 2011. Characterization of a ranavirus inhibitor of the antiviral protein kinase PKR. *BMC Microbiol* 11:56. <https://doi.org/10.1186/1471-2180-11-56>.
- Huang Y, Huang X, Liu H, Gong J, Ouyang Z, Cui H, Cao J, Zhao Y, Wang X, Jiang Y, Qin Q. 2009. Complete sequence determination of a novel reptile iridovirus isolated from soft-shelled turtle and evolutionary analysis of *Iridoviridae*. *BMC Genomics* 10:224. <https://doi.org/10.1186/1471-2164-10-224>.
- Lei XY, Ou T, Zhu RL, Zhang QY. 2012. Sequencing and analysis of the complete genome of *Rana grylio* virus (RGV). *Arch Virol* 157:1559–1564. <https://doi.org/10.1007/s00705-012-1316-9>.
- He JG, Lü L, Deng M, He HH, Weng SP, Wang XH, Zhou SY, Long QX, Wang XZ, Chan SM. 2002. Sequence analysis of the complete genome of an iridovirus isolated from the tiger frog. *Virology* 292:185–197. <https://doi.org/10.1006/viro.2001.1245>.