



Complete Sequence of the Smallest Polyomavirus Genome, Giant Guitarfish (*Rhynchobatus djiddensis*) Polyomavirus 1

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Polyomaviruses are known to infect mammals and birds. Deep sequencing and metagenomic analysis identified the first polyomavirus from a cartilaginous fish, the giant guitarfish (*Rhynchobatus djiddensis*). Giant guitarfish polyomavirus 1 (GfPyV1) has typical polyomavirus genome organization, but is the smallest polyomavirus genome (3.96 kb) described to date.

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olyomaviruses have been found in a range of avian and mammalian species. Although some persist asymptomatically, other polyomavirus species cause diseases ranging from urinary tract hemorrhage to neoplasia (1-5). Historically, taxonomic classification included three genera, the Orthopolyomavirus and Wukipolyomavirus from mammals and Avipolyomavirus from birds (6, 7). However, a recent taxonomy proposal delineated four new genera, designated Alpha-, Beta-, Gamma-, and Deltapolyomavirus (8). Recently, black sea bass-associated polyomavirus 1 (BassPyV1, GenBank accession number KP071318), the first polyomavirus associated with a bony fish (Centropristis striata), was described (9). Here, a complete polyomavirus genome is reported from a cartilaginous fish, the giant guitarfish (Rhynchobatus djiddensis), a batoid elasmobranch (order Rajiformes). The presence of proliferative skin lesions, characterized microscopically by large intranuclear inclusions containing 75 nm icosahedral viral particles, initiated an investigation of the causative agent (10). To circumvent the lack of known viral genetic information in elasmobranchs, a sequenceindependent metagenomic approach was performed to identify viral sequences within the lesions (11, 12).

A complete, circular, double-stranded, 3,962 bp DNA genome was characterized. This virus, giant guitarfish polyomavirus 1 (GfPyV1), has characteristic polyomavirus arrangement of major open reading frames, including LT, VP1, and VP2. Although transmission electron microscopy failed to identify polyomavirus-like particles in tissue and virus isolation was not attempted due to lack of compatible cell lines, the presence of GfPyV1 LT and VP1 nucleic acids in skin lesions were confirmed using nested PCR and Sanger sequencing.

The genome size of 3.96 kb makes GfPyV1 the smallest described polyomavirus, compared with other genomes of 4.7 to 7.4 kb (2, 13, 14). While the GfPyV1 genome showed typical polyomavirus organization, its nucleotide sequence is highly divergent from other polyomaviruses. The predicted 1,794 bp large T (LT) protein is encoded by a single open reading frame, in contrast to the spliced LT genes of other polyomaviruses. BLAST searches revealed roughly 30% identity to a variety of mammalian and avian polyomavirus LT proteins. The LT from GfPyV1contains predicted DnaJ, Ori-binding, and helicase domains typical of polyomaviruses (15). A possible small T antigen-like open reading frame (ORF) encoding a 75 amino-acid-long protein was also predicted in the GfPyV1 early region, but a BLAST search revealed no sequence identity to any proteins in GenBank.

The predicted major capsid protein (VP1) contains 277 amino acids, smaller than all known VP1 proteins (2). It shares roughly 25% identity with various polyomavirus VP1 coat proteins by BLASTp search. At 500 amino acids, the predicted minor capsid protein (VP2) is longer than typical VP2 proteins (2). The VP2 encodes a possible N-terminal myristoylation signal.

Comparing the two fish polyomaviruses using Sequence Declamation Tool (SDT) v1.0 (16), BassPyV1 and GfPyV1 share 19.3%, 26%, 27.8%, and 22.9% protein identity in the viral genes LT, ST, VP1, and VP2, respectively. Although GfPyV1 DNA was present in associated tissues, preliminary data suggests that it was not the cause of the skin lesions.

Nucleotide sequence accession numbers. The complete genomic sequence of guitarfish polyomavirus 1 was deposited in GenBank under the GenBank accession numbers NC_026244 and KP264963.

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REFERENCES

- 1. Essbauer S, Ahne W. 2001. Viruses of lower vertebrates. J Vet Med B Infect Dis Vet Public Health 48:403–475. http://dx.doi.org/10.1046/ j.1439-0450.2001.00473.x.
- 2. DeCaprio JA, Imperiale MJ, Major EO. 2013. Polyomaviruses, p. 1633–1661. In Knipe DM, Howley PM (ed), Fields virology, 6th ed. Wolters Kluwer Health/Lippincott Williams & Wilkins, Philadelphia, PA.
- Baron HR, Howe L, Varsani A, Doneley RJ. 2014. Disease screening of three breeding populations of adult exhibition budgerigars (*Melopsittacus undulatus*) in New Zealand reveals a high prevalence of a novel polyomavirus and avian malaria infection. Avian Dis 58:111–117. http:// dx.doi.org/10.1637/10604-063013-REG.1.
- 4. Voyles BA. 1993. The biology of viruses. Mosby, St. Louis, MO.
- 5. Guerin JL, Gelfi J, Dubois L, Vuillaume A, Boucraut-Baralon C, Pingret JL. 2000. A novel polyomavirus (goose hemorrhagic polyomavirus) is the agent of hemorrhagic nephritis enteritis of geese. J Virol 74:4523–4529. http://dx.doi.org/10.1128/JVI.74.10.4523-4529.2000.
- 6. Flint SJ, Enquist LW, Racaniello VR, Skalka AM, Barnum DR, de Evaluación E. 2000. Principles of virology: molecular biology, pathogenesis and control. ASM Press, Washington, DC.
- Johne R, Buck CB, Allander T, Atwood WJ, Garcea RL, Imperiale MJ, Major EO, Ramqvist T, Norkin LC. 2011. Taxonomical developments in the family *Polyomaviridae*. Arch Virol 156:1627–1634. http://dx.doi.org/ 10.1007/s00705-011-1008-x.
- Calvignac-Spencer S, Feltkamp MCW, Daugherty MD, Moens U, Ramqvist T, Johne R, Ehlers B. 29 February 2016. A taxonomy update for the family *Polyomaviridae*. Arch Virol [Epub ahead of print]. http:// dx.doi.org/10.1007/s00705-016-2794-y.
- 9. Peretti A, FitzGerald PC, Bliskovsky V, Pastrana DV, Buck CB. 2015.

Genome sequence of a fish-associated polyomavirus, black sea bass (*Centropristis striata*) polyomavirus 1. Genome Announc 3(1):e01476-14. http://dx.doi.org/10.1128/genomeA.01476-14.

- Camus A, Dill J, McDermott A, Camus M, Ng TFF. 2016. Virusassociated papillomatous skin lesions in a giant guitarfish *Rhynchobatus djiddensis*: a case report. Dis Aquat Organ 117:253–258. http://dx.doi.org/ 10.3354/dao02956.
- Ng TF, Driscoll C, Carlos MP, Prioleau A, Schmieder R, Dwivedi B, Wong J, Cha Y, Head S, Breitbart M, Delwart E. 2013. Distinct lineage of vesiculovirus from big brown bats, United States. Emerg Infect Dis 19:1978–1980. http://dx.doi.org/10.3201/eid1912.121506.
- Ng TF, Wheeler E, Greig D, Waltzek TB, Gulland F, Breitbart M. 2011. Metagenomic identification of a novel anellovirus in Pacific harbor seal (*Phoca vitulina richardsii*) lung samples and its detection in samples from multiple years. J Gen Virol 92:1318–1323. http://dx.doi.org/10.1099/ vir.0.029678-0.
- Schuurman R, Sol C, Van Der Noordaa J. 1990. The complete nucleotide sequence of bovine polyomavirus. J Gen Virol 71:1723–1735. http:// dx.doi.org/10.1099/0022-1317-71-8-1723.
- Stevens H, Bertelsen MF, Sijmons S, Van Ranst M, Maes P. 2013. Characterization of a novel polyomavirus isolated from a fibroma on the trunk of an African elephant (*Loxodonta africana*). PLoS One 8:e77884. http://dx.doi.org/10.1371/journal.pone.0077884.
- An P, Sáenz Robles MT, Pipas JM. 2012. Large T antigens of polyomaviruses: amazing molecular machines. Annu Rev Microbiol 66: 213–236. http://dx.doi.org/10.1146/annurev-micro-092611-150154.
- Muhire BM, Varsani A, Martin DP. 2014. SDT: a virus classification tool based on pairwise sequence alignment and identity calculation. PLoS One 9:e108277. http://dx.doi.org/10.1371/journal.pone.0108277.