

Whole-Genome Sequence Characterization of a Beak and Feather Disease Virus in a Wild Regent Parrot (*Polytelis anthoepplus monarchoides*)

Subir Sarker,^{a,c} Jade K. Forwood,^{b,c} Seyed A. Ghorashi,^{a,c} David McLelland,^d Andrew Peters,^{a,c} Shane R. Raidal^{a,c}

School of Animal and Veterinary Sciences, Charles Sturt University, Wagga Wagga, New South Wales, Australia^a; School of Biomedical Sciences, Charles Sturt University, Wagga Wagga, New South Wales, Australia^b; Graham Centre for Agricultural Innovation, Wagga Wagga, New South Wales, Australia^c; Zoos South Australia, Adelaide, South Australia, Australia^d

The whole-genome sequence of beak and feather disease virus (BFDV) from a wild Australian regent parrot (*Polytelis anthoepplus monarchoides*) was characterized. The genome consists of 1,993 bp and has a typical stem-loop structure between open reading frame 1 (ORF1) and ORF2. This is the first evidence of BFDV infection as well as the complete genome sequence for this host species, globally.

Received 20 December 2013 Accepted 31 December 2013 Published 30 January 2014

Citation Sarker S, Forwood JK, Ghorashi SA, McLelland D, Peters A, Raidal SR. 2014. Whole-genome sequence characterization of a beak and feather disease virus in a wild regent parrot (*Polytelis anthoepplus monarchoides*). *Genome Announc*. 2(1):e01243-13. doi:10.1128/genomeA.01243-13.

Copyright © 2014 Sarker et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 3.0 Unported license](http://creativecommons.org/licenses/by/3.0/).

Address correspondence to Shane R. Raidal, shraidal@csu.edu.au, or Subir Sarker, ssarker@csu.edu.au.

Beak and feather disease virus (BFDV) is one of the smallest and simplest pathogens, in terms of both genome and particle size, of any vertebrate, and it causes psittacine beak and feather disease (Pbfd). It belongs to the family *Circoviridae* (1–3), with the circular single-stranded DNA (ssDNA) genome of BFDV surrounded by a nonenveloped icosahedral capsid 19 to 22 nm in diameter, and it must use host cell machinery to replicate (4, 5). The emergence of the disease in critically endangered species has been recently highlighted as a key threat to the orange-bellied parrot (6). It is estimated that no more than 2,900 adult regent parrots (*Polytelis anthoepplus monarchoides*) exist across the range of the Murray-Darling river catchment of southeastern Australia; as a result, the regent parrot is also currently listed as vulnerable under the Australian Government *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act) and endangered under the NSW *Threatened Species Conservation Act 1995* (7). Pbfd presents with chronic symmetrical feather loss, as well as beak and claw deformities eventually leading to death or acutely infected juvenile birds, causing sudden death even in the absence of clinical signs (5, 8–10). Here, BFDV is reported for the first time in a wild regent parrot in Australia.

Dried blood spots collected from a regent parrot (sample ID, 13-1683; year of sampling, 2013; global positioning system [GPS] location, 34.100°S 140.4440°E) was used as a source of genomic DNA, and extraction of DNA was performed using established protocols (11–13). The whole-genome sequence was amplified using the primers and PCR conditions described by Sarker et al. (14, 15). Briefly, the optimized reaction mixture contained 3 μ l extracted genomic DNA, 2.5 μ l of 10 \times High Fidelity PCR buffer (Invitrogen), 1 μ l of 25 μ M each primer, 1 μ l of 50 mM MgSO₄, 4 μ l of 1.25 mM dinucleoside triphosphates (dNTPs), 1 U platinum *Taq* DNA Polymerase High Fidelity (Invitrogen), and dH₂O added for a final volume of 25 μ l. The optimized PCR conditions were 95°C for 3 min, followed by 40 cycles of 95°C for 30 s, 57°C

for 45 s, and 68°C for 2 min, and finally 68°C for 5 min. Amplified PCR products were TA cloned into pGEM-T vector (Promega, USA) and sequenced at the Australian Genome Research Facility (AGRF), Ltd. (Sydney, Australia). The sequenced contigs were assembled, and the entire BFDV genome was constructed using Geneious software (version 6.1.6).

The complete genome consists of 1,993 bp, with a G+C content of 53.5%. The genome has the same basic structure as other BFDV genomes, which includes two major open reading frames (ORFs), ORF1 on the virion strand, encoding a replication-associated protein (Rep), and ORF2, encoding the capsid protein (Cap). The overall nucleotide (nt) identity of this new isolate compared with the BFDV genome available on GenBank (16) ranges from >82 to 95%. Consequently, based on BLASTn (17) and BLASTp (18), ORF2 is more diverse than ORF1. The nucleotide identity of ORF2 of the regent parrot BFDV genome varies from 88 to 95% compared with other BFDV genomes, while the identity of the amino acid sequence ranges from 77 to 96%.

This study highlights the evidence of BFDV infection in an endangered regent parrot, which may provide novel insights into the management and recovery of this host species.

Nucleotide sequence accession number. This complete genome of BFDV has been deposited at DDBJ/EMBL/GenBank under the accession no. [KF850537](https://www.ncbi.nlm.nih.gov/nuccore/KF850537). The version described in this paper is the first version.

ACKNOWLEDGMENT

We acknowledge the Australian Research Council's Discovery Projects funding scheme for their financial support (grant no. DP1095408).

REFERENCES

1. Bassami MR, Berryman D, Wilcox GE, Raidal SR. 1998. Psittacine beak and feather disease virus nucleotide sequence analysis and its relationship to porcine circovirus, plant circoviruses, and chicken anaemia virus. *Virology* 249:453–459. <http://dx.doi.org/10.1006/viro.1998.9324>.

2. Ritchie BW, Niagro FD, Latimer KS, Lukert PD, Steffens WL, Rakich PM, Pritchard N. 1990. Ultrastructural, protein composition, and antigenic comparison of psittacine beak and feather disease virus purified from four genera of psittacine birds. *J. Wildl. Dis.* 26:196–203. <http://dx.doi.org/10.7589/0090-3558-26.2.196>.
3. Ritchie BW, Niagro FD, Lukert PD, Steffens WL III, Latimer KS. 1989. Characterization of a new virus from cockatoos with psittacine beak and feather disease. *Virology* 171:83–88. [http://dx.doi.org/10.1016/0042-6822\(89\)90513-8](http://dx.doi.org/10.1016/0042-6822(89)90513-8).
4. Bassami MR, Ypelaar I, Berryman D, Wilcox GE, Raidal SR. 2001. Genetic diversity of beak and feather disease virus detected in psittacine species in Australia. *Virology* 279:392–400. <http://dx.doi.org/10.1006/viro.2000.0847>.
5. Todd D. 2000. Circoviruses: immunosuppressive threats to avian species: a review. *Avian Pathol.* 29:373–394. <http://dx.doi.org/10.1080/030794500750047126>.
6. Sarker S, Patterson EI, Peters A, Baker GB, Forwood JK, Ghorashi SA, Holdsworth M, Baker R, Murray N, Raidal SR. 2014. Mutability dynamics of an emergent single stranded DNA virus in a naïve host. *PLoS One* 9:e85370. <http://dx.doi.org/10.1371/journal.pone.0085370>.
7. Baker-Gabb D, Hurley VG. 2011. National recovery plan for the regent parrot (eastern subspecies) *Polytelis anthopeplus monarchoides*. Department of Sustainability and Environment, Melbourne, Australia.
8. Raidal SR, McElnea CL, Cross GM. 1993. Seroprevalence of psittacine beak and feather disease in wild psittacine birds in New South Wales. *Aust. Vet. J.* 70:137–139. <http://dx.doi.org/10.1111/j.1751-0813.1993.tb06105.x>.
9. Pass DA, Perry RA. 1984. The pathology of psittacine beak and feather disease. *Aust. Vet. J.* 61:69–74. <http://dx.doi.org/10.1111/j.1751-0813.1984.tb15520.x>.
10. Doneley RJ. 2003. Acute beak and feather disease in juvenile African grey parrots—an uncommon presentation of a common disease. *Aust. Vet. J.* 81:206–207. <http://dx.doi.org/10.1111/j.1751-0813.2003.tb11472.x>.
11. Ypelaar I, Bassami MR, Wilcox GE, Raidal SR. 1999. A universal polymerase chain reaction for the detection of psittacine beak and feather disease virus. *Vet. Microbiol.* 68:141–148. [http://dx.doi.org/10.1016/S0378-1135\(99\)00070-X](http://dx.doi.org/10.1016/S0378-1135(99)00070-X).
12. Khalesi B, Bonne N, Stewart M, Sharp M, Raidal S. 2005. A comparison of haemagglutination, haemagglutination inhibition and PCR for the detection of psittacine beak and feather disease virus infection and a comparison of isolates obtained from loriiids. *J. Gen. Virol.* 86:3039–3046. <http://dx.doi.org/10.1099/vir.0.81275-0>.
13. Bonne N, Clark P, Shearer P, Raidal S. 2008. Elimination of false-positive polymerase chain reaction results resulting from hole punch carryover contamination. *J. Vet. Diagn. Invest.* 20:60–63. <http://dx.doi.org/10.1177/104063870802000111>.
14. Sarker S, Ghorashi SA, Forwood JK, Raidal SR. 2013. Whole-genome sequences of two beak and feather disease viruses in the endangered swift parrot (*Lathamus discolor*). *Genome Announc.* 1(6):e00842-13. <http://dx.doi.org/10.1128/genomeA.00842-13>.
15. Sarker S, Ghorashi SA, Forwood JK, Metz S, Raidal SR. 2013. Characterization of a beak and feather disease virus complete genome sequence in a Moluccan red lory (*Eos bornea*). *Genome Announc.* 1(6):e00844-13. <http://dx.doi.org/10.1128/genomeA.00844-13>.
16. Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW. 2013. GenBank. *Nucleic Acids Res.* 41:D36–D42. <http://dx.doi.org/10.1093/nar/gks1195>.
17. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *J. Mol. Biol.* 215:403–410. [http://dx.doi.org/10.1016/S0022-2836\(05\)80360-2](http://dx.doi.org/10.1016/S0022-2836(05)80360-2).
18. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* 25:3389–3402. <http://dx.doi.org/10.1093/nar/25.17.3389>.