

Molecular Characterization of Genome Sequences of Beak and Feather Disease Virus from the Australian Twenty-Eight Parrot (*Barnardius zonarius semitorquatus*)

Subir Sarker,^{a,c} Shubhagata Das,^{a,c} Seyed A. Ghorashi,^{a,c} Jade K. Forwood,^{b,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

Three complete genomes of beak and feather disease virus (BFDV) were recovered from wild twenty-eight parrots (*Polytelis antheoplus monarchoides*). The genomes consisted of 1,996 bp with 1,934 identical sites and a typically content stem-loop structure between ORF1 and ORF2. This is the first report of BFDV infection as well as the complete genome sequences for this host species globally.

Received 21 October 2014 Accepted 24 October 2014 Published 4 December 2014

Citation Sarker S, Das S, Ghorashi SA, Forwood JK, Raidal SR. 2014. Molecular characterization of genome sequences of beak and feather disease virus from the Australian twenty-eight parrot (*Barnardius zonarius semitorquatus*). *Genome Announc.* 2(6):e01255-14. doi: 10.1128/genomeA.01255-14.

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

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

Psittacine beak and feather disease (Pbfd) is a chronic immunosuppressive and often fatal viral infectious threat for both the wild and captive psittacine bird species globally (1–3). Its recent discovery in critically endangered orange-bellied parrots has raised concerns for the conservation of native parrots, all of which are threatened or endangered (4, 5). All *Psittaciformes* are considered susceptible to infection since it has been reported in more than 60 species of cockatoos and parrots (3, 6–9). The etiological agent of the disease—beak and feather disease virus (BFDV), a compact circular, ambisense single-stranded DNA (ssDNA) virus belonging to the genus *Circovirus* in the family *Circoviridae* (10, 11)—is perhaps the smallest and simplest pathogen known to infect vertebrates. Here, we report the molecular characterization of BFDV genome from three wild Australian ringneck parrots (commonly known as the twenty-eight parrot) in Western Australia.

The BFDV viral genomes were amplified from feather samples collected from three wild ringneck parrots in 1996 and stored at –20° C (sample numbers 96-B13, 96-B14, and 96-B15; GPS location: –32.533825°S, 115.5026522°E; –32.1318305°S, 116.029216°E; and –32.65904° S, 116.1297° E, respectively), and the genomic DNA was extracted using established protocols (12). Amplification of the complete genome sequences was carried out using established published protocols (13, 14). Briefly, the optimized reaction mixture contained 3 μl extracted genomic DNA, 2.5 μl of 10× High-Fidelity PCR buffer (Invitrogen), 1 μl of 25 μM of each primer, 1 μl of 50 mM MgSO₄, 4 μl of 1.25 mM deoxynucleoside triphosphates (dNTPs), 1 U of platinum *Taq* High-Fidelity DNA polymerase (Invitrogen), and distilled water added for a final volume of 25 μl. The optimized PCR conditions were as follows: 95°C for 3 min, followed by 40 cycles of 95°C for 30 s, 57°C for 45 s, 68°C for 2 min, and finally 68°C for 5 min. Amplified PCR products were TA-cloned into pGEM-T vector (Promega) and sequenced at AGRF Ltd. (Sydney, Australia). The sequenced contigs were assembled and the entire BFDV genome was constructed using Geneious software (version 6.1.8).

The three complete genomes of BFDV from the twenty-eight parrot consist of 1,996 bp with a G+C content of 53.2%. The genome has the same basic structure as other BFDV genomes, which includes two major bidirectional transcribed open reading frames (ORFs). A preliminary BLASTn (15) analysis of the assembled sequences showed a significant (>97%) pairwise nucleotide match to a BFDV genome from the red-tailed black cockatoo (KF385399). The newly amplified BFDV genomes shared >97.0% pairwise nucleotide identity with each other, and 84.3–97.4% nucleotide sequence homology with other BFDV genomes. Consequently, based on BLASTn (15) and BLASTp (16) analyses, ORF2 encoding the capsid protein was more diverse than the virion strand encoding a replication associated protein (ORF1). The identical sites were significantly lower in the ORF2 (693 sites; 92.4%) than the ORF1 (868 sites; 99.8%).

This study highlights the evidence of BFDV infection for the first time in Australian ringneck parrots, which may provide novel insights into the viral evolution and conservation of this host species.

Nucleotide sequence accession numbers. The three complete genomes of BFDV have been deposited at DDBJ/ENA/GenBank under the accession numbers [KF688548](https://www.ncbi.nlm.nih.gov/nuclseq/KF688548), [KF688549](https://www.ncbi.nlm.nih.gov/nuclseq/KF688549), and [KF688550](https://www.ncbi.nlm.nih.gov/nuclseq/KF688550). The versions described in this paper are the first versions.

ACKNOWLEDGMENT

We acknowledge the Australian Research Council's Discovery Projects funding scheme for financial support (grant DP1095408).

REFERENCES

1. 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>.
2. Ritchie PA, Anderson IL, Lambert DM. 2003. Evidence for specificity of

- psittacine beak and feather disease viruses among avian hosts. *Virology* 306:109–115. [http://dx.doi.org/10.1016/S0042-6822\(02\)00048-X](http://dx.doi.org/10.1016/S0042-6822(02)00048-X).
3. Sarker S, Ghorashi SA, Forwood JK, Bent SJ, Peters A, Raidal SR. 2014. Phylogeny of beak and feather disease virus in cockatoos demonstrates host generalism and multiple-variant infections within *Psittaciformes*. *Virology* 460–461:72–82. <http://dx.doi.org/10.1016/j.virol.2014.04.021>.
 4. 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>.
 5. Peters A, Patterson EI, Baker GB, Holdsworth M, Sarker S, Ghorashi SA, Raidal SR. 2014. Evidence of psittacine beak and feather disease virus spillover into wild critically endangered orange-bellied parrots (*Neophema chrysogaster*). *J. Wildl. Dis.* 50:288–296. <http://dx.doi.org/10.7589/2013-05-121>.
 6. Sarker S, Ghorashi SA, Forwood JK, Raidal SR. 2014. Rapid genotyping of beak and feather disease virus using high-resolution DNA melt curve analysis. *J. Virol. Methods* 208:47–55. <http://dx.doi.org/10.1016/j.jviro.2014.07.031>.
 7. 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 anthopeplus monarchoides*). *Genome Announc.* 2(1):e01243-13. <http://dx.doi.org/10.1128/genomeA.01243-13>.
 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. Das S, Sarker S, Forwood JK, Ghorashi SA, Raidal SR. 2014. Characterization of the whole-genome sequence of a beak and feather disease virus isolate from a Mallee Ringneck parrot (*Barnardius zonarius barnardi*). *Genome Announc.* 2(4):e00708-14. <http://dx.doi.org/10.1128/genomeA.00708-14>.
 10. Niagro FD, Forsthoefel AN, Lawther RP, Kamalanathan L, Ritchie BW, Latimer KS, Lukert PD. 1998. Beak and feather disease virus and porcine circovirus genomes: intermediates between the geminiviruses and plant circoviruses. *Arch. Virol.* 143:1723–1744. <http://dx.doi.org/10.1007/s007050050412>.
 11. Ritchie BW, Niagro FD, Latimer KS, Lukert PD, Steffens WL III, 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>.
 12. 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>.
 13. 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>.
 14. Sarker S, Ghorashi SA, Forwood JK, Metz S, Raidal SR. 2013. Characterization of the complete genome sequence of a beak and feather disease virus from a Moluccan red lory (*Eos bornea*). *Genome Announc.* 1(6):e00844-13. <http://dx.doi.org/10.1128/genomeA.00844-13>.
 15. 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).
 16. 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>.