AUSTRALIAN VETERINARY JOURNAL
AUSTRALIA'S PREMIER VETERINARY SCIENCE TEXT
WILDLIFE & ZOOS

ORIGINAL ARTICLE

Evidence of a highly divergent novel parvovirus in Australia's critically endangered western ground parrot/kyloring (*Pezoporus flaviventris*)

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Detecting pathogens in endangered animal populations is vital for understanding and mitigating threats to their survival. The critically endangered western ground parrot (*Pezoporus flaviventris*, WGP), with a population as low as 150 individuals in Australia, faces an imminent risk of extinction. Despite this urgency, research on viral pathogens in this species remains limited. This study aimed to identify and characterise viruses present in faecal samples from seven individual WGP using a viral metagenomic approach. Analysis of the sequenced datasets revealed the presence of a novel virus belonging to the Parvoviridae family, named psittaciform chaphamaparvovirus 7 (PsChPV-7). The genome of PsChPV-7 contains typical structural and functional gene sequences found in Parvoviridae but is highly divergent, indicating its classification as a distinct species. Phylogenetic analysis placed PsChPV-7 within a unique sub-clade of the *Chaphamaparvovirus* genus, suggesting its evolutionary significance as an ancient lineage within this group. These findings may contribute to the development of strategic management and biosecurity plans aimed at conserving this endangered WGP.

Keywords evolution; next-generation sequencing; parvovirus; virome; western ground parrot

Aust Vet J 2024;102:570–575 doi: 10.1111/avj.13378

Viral pathogens can pose significant challenges at various levels, from individual animals to entire ecosystems. While our understanding of avian viruses is still limited, much of the existing knowledge focuses on zoonotic viruses like avian influenza,[1] economically important viruses such as Newcastle disease virus and those that cause significant mortality such as beak and feather disease virus (BFDV).[2–9] However, little is known about the viruses of many endangered Australian bird species, making it difficult to understand the potential risks surrounding viruses and the impact they may have on these species.

One of the critically endangered species of concern is the western ground parrot (WGP), also known as kyloring (*Pezoporus flaviventris*), which is currently found in only two locations in southwestern Australia. This species is one of the rarest parrots globally, with an estimated population of fewer than 150 birds. Conservation efforts face various challenges, including habitat loss and degradation, exacerbated by increased fire frequency, predation by introduced carnivores and reduced rainfall. While recovery efforts have prevented extinctions by protecting wild populations from these threats or vulnerability of its habitat pose ongoing challenges, making their future outlook precarious.[10] Additionally, our understanding of infectious agents affecting this species is limited. In addition, not much research has been conducted to understand the harbouring pathogens, specifically viruses in WGP.

Parvoviruses are small, nonenveloped DNA viruses with diameters typically ranging from 19 to 24 nm. Their genome is relatively compact, consisting of single-stranded DNA arranged linearly, spanning between 4 to 6 kilobases (kb).[11,12] This genome contains a gene that encodes non-structural protein (NS), including the enzymes necessary for replication such as NS1 (replicase), along with NS2 and NS3 produced through alternative splicing. It also encodes structural proteins; the capsid gene (VP1).[13] These viruses are significant pathogens capable of infecting a broad range of hosts, spanning both vertebrates and invertebrates.[14] Within the family Parvoviridae, there are three subfamilies: *Parvovirinae*, *Densovirinae* and *Hamaparvovirinae*, which are categorised based on phylogenetic analysis. *Parvovirinae*, for example, includes 10 genera with viruses infecting mammals, birds and reptiles. *Densovirinae* consists of eight genera affecting invertebrates like insects, crustaceans and echinoderms. Lastly, *Hamaparvovirinae* encompasses five genera containing viruses identified in both vertebrate and invertebrate hosts.[15]

Within the subfamily *Hamaparvovirinae*, parvoviruses of the genus *Chaphamaparvovirus* have been detected in faecal samples from various animals, including chickens, turkeys, peafowls, rats, pigs, red-crowned crane and straw-coloured fruit bat. These viruses have also been found in a variety of tissue samples such as serums, rectal swabs, nasal swabs and lung lavages from pigs.[14,16–20] Moreover, chaphamaparvoviruses have been identified in liver tissue samples from parrot species, including the rainbow lorikeet (*Trichoglossus moluccanus*) and little corella (*Cacatua sanguinea*).[21,22] To our

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knowledge, very little is known about circulating viruses that may be harboured by the western ground parrot. Despite this urgency, research on viral pathogens in this species remains limited. Furthermore, there is a limited information on parvoviruses infects Australian avifauna. This study aimed to identify and characterise viruses present in faecal samples from seven apparently healthy/asymptomatic individual captive WGP using a viral metagenomic approach.

Materials and methods

Sampling and ethical consideration

In 2023, seven fresh faecal samples, one from each bird were collected from individually housed captive western ground parrot/kyloring maintained as part of a captive insurance population at Perth Zoo, Western Australia. The samples were collected from food trays and the aviary floor during routine animal care and stored at −80°C until further processing. The sample collection process did not involve handling or manipulating the WGPs and was conducted with approval from the Zoo (ZA/3947-3).

Virus enrichment and virus nucleic acid extraction

Following the removal of potential impurities such as host cells, bacteria, food particles and free nucleic acids from the faecal samples, enrichment of virus particles was performed as per previously stated methods,[2,23] with minor variations. Briefly, the faecal material was centrifuged at 17,000 g for 3 min at room temperature (RT) after being aseptically resuspended and vigorously homogenised in sterile phosphate-buffered saline (PBS) (1:10). The supernatant was filtered using a 0.80 µm syringe filter and the filtrate was then processed downstream. The samples were then ultracentrifuged at 178,000 g for 1 h at 30 PSI at 4°C using the Hitachi Ultracentrifuge CP100NX. After discarding the supernatant, the pellet was suspended in 130 µL of sterile PBS. The filtrates were then nuclease-treated using 2 µL of benzonase nuclease (25–29 U/µL, purity >90%, Millipore) and 1 µL of micrococcal nuclease (2,000,000 gel units/mL, New England Biolabs) and incubated at 37°C for 2 h. The nuclease reaction was stopped by adding 3 µL of 500 mM ethylenediaminetetraacetic acid (EDTA). Using the QIAamp Viral RNA Mini Kit (Qiagen, Valencia, CA, USA), viral nucleic acids were extracted without adding any carrier RNA, enabling the simultaneous extraction of viral DNA and RNA. The quantity and quality of the isolated nucleic acids were determined using Nanodrop and an Agilent Tape Station (Agilent Technologies, Mulgrave, VIC, Australia) by the Genomic Platform, La Trobe University.

Next-generation sequencing

Before library construction, extracted nucleic acids (RNA) were subjected to cDNA synthesis, and amplification was carried out using the Whole Transcriptome Amplification Kit (WTA2, Sigma-Aldrich, Darmstadt, Germany) as per manufacturer's instructions. Amplified PCR products were then purified using the Wizard® SV Gel and PCR Clean-Up kit (Promega, Madison, WI, USA). The quantity and quality of the purified product were then checked using a Qubit dsDNA high sensitivity assay kit with Qubit Fluorometer v3.0 (Thermo Fisher Scientific, Waltham, MA, USA).

The library construction was performed individually for each of the seven samples using the Illumina DNA Prep (Illumina, San Diego, CA, USA) as per kit instructions, starting with 250 ng of DNA as measured by Qubit (Invitrogen). The quality and quantity of the prepared libraries were assessed by the Australian Genome Research Facility (AGRF), Melbourne, Australia. The prepared libraries were normalised and pooled in equimolar quantities. Prior to sequencing by the facility, the quality and quantity of the final pooled libraries were further assessed as described above. According to the manufacturer's instructions, cluster generation and sequencing of the libraries was performed with read lengths of 150-bp paired-end on Illumina® NovaSeq chemistry.

Bioinformatic analyses

All raw reads were subjected to a preliminary quality assessment, which involved pre-processing to eliminate ambiguous base calls and low-quality reads, followed by trimming to remove the Illumina adapter sequences. Trimmed sequence reads were mapped against the chicken genome (*Gallus gallus*, GenBank accession number NC_006088) in order to remove any possible host DNA contamination. In addition, reads were further mapped to the genomic sequence of the *Escherichia coli* bacteria (GenBank accession no. U00096) to remove any potential bacterial contamination. Unmapped cleaned reads were used as input data for *de novo* assembly using a SPAdes assembler (version 3.10.1)[24] under the 'careful' parameter in the LIMS-HPC system (a High-Performance Computer specialised for genomics research in La Trobe University). The resulting contigs were compared against the nonredundant nucleotide and protein databases on GenBank using BLASTN and BLASTX,[25] respectively, with an E-value threshold of 1×10^{-5} to remove potential false positives. Contigs that were significant BLAST hits with bacteria, eukaryotes or fungi were filtered out to remove non-viral reads. Non-phageous viral contigs of interest greater than 300 nucleotides (nt) were imported in Geneious Prime software (Biomatters Ltd., New Zealand, version 2023.1.1) for further functional analysis. Average coverage of the viral contigs was calculated using the clean raw reads in Geneious Prime software (version 2023.1.1).

Functional annotations

The assembled complete viral genome detected in this study was annotated as per stated protocol[2,20] using Geneious Prime (version 2023.1.1, Biomatters, Ltd., Auckland, New Zealand). The viral taxonomy was determined through comparative analysis via GenBank's BlastN, BlastX and BlastP, with the highest match being chosen against selection criteria (*e*-value = <0.0). Annotation of the open reading frames (ORFs) in the identities of viral genome was completed by comparison against specific criteria (>30 AA, methionine start codon, <50% overlap of a gene) via NCBI's database. They were then compared against conserved domain databases (NCBI, Bethesda, MA, USA).[25]

Comparative genomics and phylogenetic analyses

Genomic comparison of the newly sequenced complete viral genome was performed using Geneious Prime (version 2023.1.1). The sequence similarity between the selected viral sequences was identified against representative viral sequences by MAFFT alignment

L-INS-I in Geneious Prime (version 2023.1.1, Biomatters, Ltd., Auckland, New Zealand).

For phylogenetic analysis, representative viral genome or complete NS1 gene sequences were downloaded from GenBank, and a tree was constructed using Geneious Prime (version 2023.1.1, Biomatters, New Zealand). Amino acid sequences of protein-coding genes were aligned using the MAFTT L-INS-I algorithm implemented in Geneious Prime (version 7.388).[26] The phylogenetic tree was constructed using RAxML and Gamma Blosum62 protein model, with 1000 bootstrap support in Geneious Prime (version 2023.1.1).

Results

Seven faecal samples, one from each bird were gathered from seven different WGPs and underwent next-generation sequencing (NGS) technology. Among these samples, only one WGP (sample ID WGP01B, Kopin) was found to be infected with a parvovirus. No additional viral sequences were identified in any of the collected samples.

Genomic features of the detected PsChPV-7

The assembled genome of the novel psittaciform chaphamaparvovirus (PsChPV-7) detected was a linear single-stranded DNA (ssDNA) molecule spanning 4060 nucleotides (nt) in length, with a sequencing coverage of 58.0×. It exhibited a basic organisation akin to previously documented members of the Parvoviridae family. The PsChPV-7 genome showcased an A + T content of 45.9% and a C + G content of 54.1%, aligning with typical parvovirus genomic compositions. Structurally, PsChPV-7 resembled other parvoviruses, featuring two primary predicted open reading frames (ORFs). These ORFs usually encoded a replication initiator protein known as NS1, and a viral capsid protein (VP1), reflecting the fundamental genomic arrangement common among parvoviruses.

Comparative genomic shows a rich diversity in PsChPV-7

Comparative analysis of the protein sequences encoded by the predicted ORFs, utilising BLASTX and BLASTP, revealed significant protein sequence similarities (E value ≤ 10–5) for two major ORFs according to the BLAST database. The 5′ ORF1 spans 417 nt, and a BLAST search using the putative amino acid sequence did not yield any significant protein similarities.

The PsChPV-7 NS protein 1 (NS1) ORF measured 2058 nt in length and exhibited the highest amino acid identity with the porcine parvovirus, also known as ungulate protoparvovirus 1 (protein identity 25.12%, GenBank accession no. WNO15321.1), followed by porcine parvovirus 7 (PChPV-7) (protein identity 24.63%, GenBank accession no. AYC49708.1). Consistent with other parvoviruses, the complete NS1 gene of PsChPV-7 spanned 685 amino acids and encoded the helicase, featuring the conserved ATP- or GTP-binding Walker A loop (GPxNTGKT/S; $_{476}$**G**DTTI**GKS**$_{483}$), Walker B (xxxWEE; $_{517}$VIL**AEE**$_{522}$), Walker B′ (KQxxEGxxxxxPxK; $_{534}$**K**AVLR**G**NSVQVQI**K**$_{547}$) and Walker C (PxxxTxN; $_{558}$**P**VMGSL**N**$_{564}$) motifs. Moreover, the NS1 protein harboured two conserved replication initiator (endonuclease) motifs, xxHuHxxxx (IP$_{159}$**H**M**H**$_{161}$MLLE) and YxxK ($_{209}$**Y**LA**K**$_{212}$) (conserved amino acids are indicated in bold letters, and 'u' indicates a hydrophobic residue).

The major 3′ ORF, spanning 1275 nt, encoded a protein similar to the capsid protein VP1 of the Parvoviridae family. At the amino acid level, the VP1 protein of PsChPV-7 demonstrated the highest protein identity with a hypothetical protein of Parvoviridae sp. (protein identity 26.35%, GenBank accession no. QKE54999.1).

Evolutionary relationships of PsChPV-7

Phylogenetic reconstruction based on the sequences of the parvoviral replication initiator protein (NS1) supported the classification of the newly sequenced PsChPV-7 within the genus *Chaphamaparvovirus*. In the resulting maximum likelihood (ML) tree, PsChPV-7 formed a distinct sub-clade within the *Chaphamaparvovirus* genus (see Figure 1), indicating its probable position as an ancient evolutionary lineage within this group. Notably, analysis revealed a low amino acid sequence identity between PsChPV-7 and other parvovirus sequences available in GenBank, providing further evidence for its unique phylogenetic position. This finding is further supported with a low level of protein identity between PsChV-7 and ungulate protoparvovirus 1 (25.12%, 1), followed by PChPV-7 (24.63%). With no clear close relationship to other chaphamaparvoviruses, PsChPV-7's evolutionary relationship raises the possibility that it may be an intermediate evolutionary lineage with other chaphamaparvoviruses.

Discussion

This study presents the first genome sequence of a novel parvovirus, specifically psittaciform chaphamaparvovirus 7 (PsChPV-7), which was sequenced from the critically endangered Australian native WGP. The PsChPV-7 genome includes all major structural and functional gene sequences typical of the Parvoviridae family. However, it stands out as being highly divergent, clearly constituting a distinct species. This differentiation is evident in the low degree of amino acid sequence identity observed in the NS1 gene compared to the closest chaphamaparoviruses, such as the ungulate chaphamaparovirus 1.

The evolutionary relationship of PsChPV-7 is characterised by its lack of close association with other chaphamaparvoviruses. Nonetheless, the PsChPV-7 was positioned distinctively in a sub-clade within the genus *Chaphamaparvovirus*, suggests that it may represent an intermediate evolutionary lineage within the genus. This distinctiveness implies that PsChPV-7 has likely undergone significant evolutionary divergence from its closest relatives, which may be the result of unique adaptive pressures or evolutionary history. A similar scenario has been observed in several recent studies on other viruses infecting Australian bird species.[27–29] The authors of these studies highlighted that the evolutionary relationship between viruses in the genera *Atadenovirus* and *Siadenovirus* appears to be unfolding and that the viruses emerged as different lineages and sub-lineages that were not monophyletic, indicating that they originated with a host-switching event followed by virus-species co-evolution. Therefore, to resolve the exact order of phylogenetic position of PsChPV-7, further investigation into the parvoviral genomic sequences from a diverse avian host species across different geographic regions is essential.

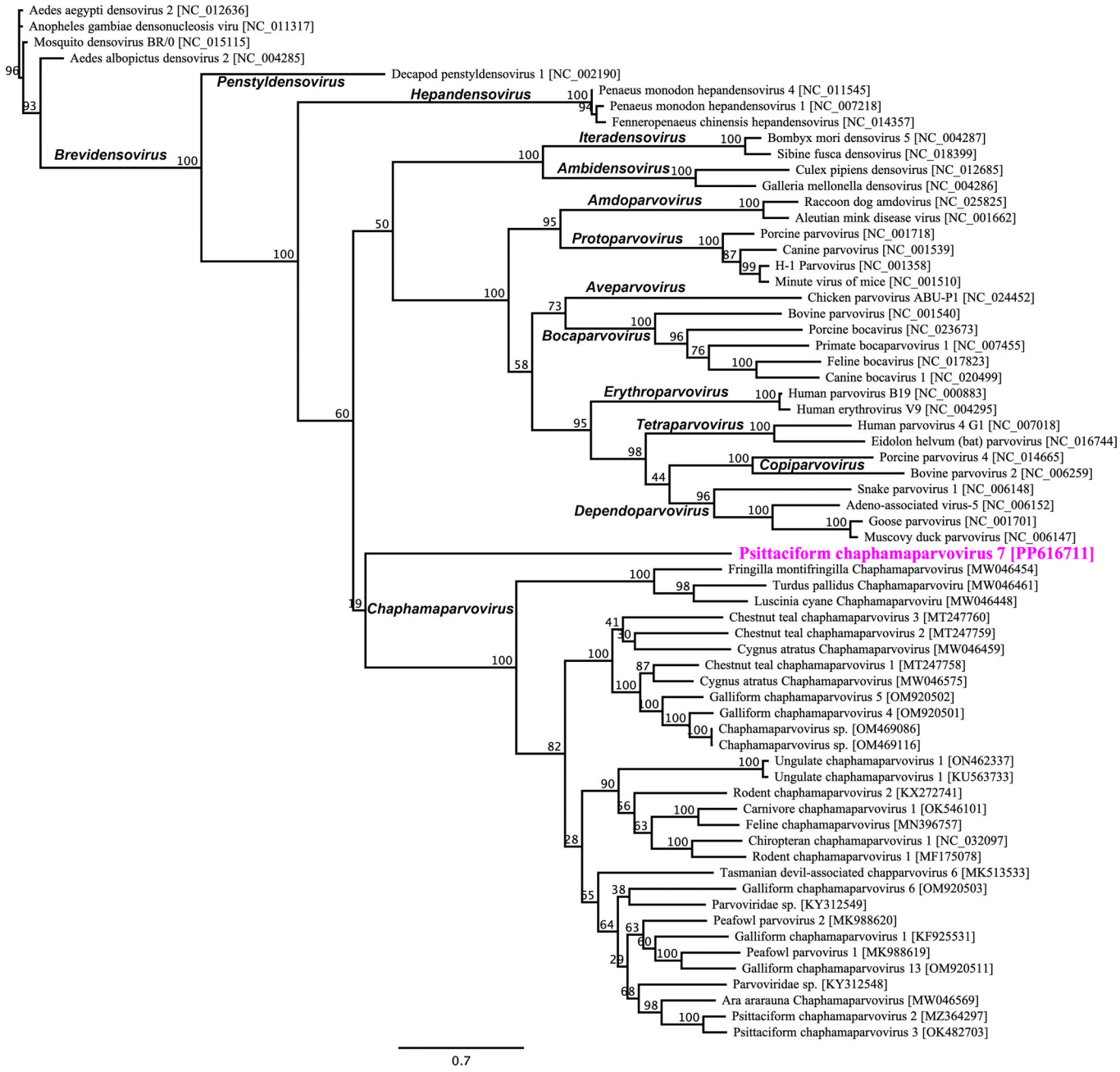

Figure 1. Unrooted phylogenetic tree for tracking the possible evolutionary relationship of psittaciform chaphamaparvovirus 7 with other selected parvoviruses. The maximum likelihood (ML) tree was constructed using the amino acid sequences of complete nonstructural protein (NS1). The numbers on the left show bootstrap values as percentages, and the labels at the branch tips refer to the original parvoviruses' species names, followed by their GenBank accession numbers in parentheses. The psittaciform chaphamaparvovirus 7, sequenced in this study, is distinctly marked in pink to highlight its position in the tree.

Metagenomics, a widely used technique, is crucial for screening and characterising microbes like viruses and bacteria.[30–32] It offers valuable insights into the diversity of microbiomes, particularly in poorly studied populations,[30–34] such as the WGP (*P. flaviventris*). These metagenomic studies are indispensable for developing strategies to prevent and mitigate threats to endangered species.[35] Amidst numerous recent viral outbreaks impacting avian hosts, including the H5N1 avian influenza,[36] there remains a substantial knowledge gap concerning the evolution and spread of viruses among captive populations of endangered Australian parrots, including potential unidentified strains and their impact on species decline.[35] The WGP is vulnerable to pathogenic novel diseases, yet little is known about their existing virome. This study aimed to bridge this gap by characterising the virome of a select group of captive WGPs using

high-throughput NGS technology, thereby enhancing our understanding of viral diversity within these birds. However, it is not known whether this novel virus is likely pathogenic or not, which warrant further investigation.

The genomic and phylogenetic analysis techniques used in this study have certain limitations, notably due to the lack of sufficient number of sequenced parvoviruses from Australian Psittaciformes in existing database. Clear evolutionary relationships are difficult to ascertain due to this data gap, as evidenced by the ML phylogenetic tree presented in Figure 1. Consequently, it becomes challenging to fully evaluate the significance of our findings. Additionally, these findings do not facilitate the identification of past viral infections or the prediction of the consequences of future infections. Therefore, it is imperative to conduct routine surveillance in order to trace the evolution and transmission of novel, distinct, or significant pathogens. Enriching the database with more sequenced viruses will improve the accuracy of evolutionary analysis, aiding in better understanding of transmission dynamics and their presence within populations. Ultimately, this knowledge is crucial for devising more effective conservation strategies for endangered parrot species.

Author contribution

Conceptualisation: SS; investigation: SS and NK; funding acquisition: SS; formal analysis: SS; wet-lab experiments: SS and NK; data analysis SS and ST, SDG; data curation: SS; supervision: SS; writing (original draft preparation): SS; writing (review and editing): NK, ST, SDG and RVH All authors have read and agreed to the published version of the manuscript.

Acknowledgments

The authors would like to acknowledge Arthur Ferguson, Matt Ricci, Harriet Mills, Peta Moore and the Department of Biodiversity, Conservation & Attractions, Perth Zoo for providing faecal samples of western ground parrot. Subir Sarker is the recipient of an Australian Research Council Discovery Early Career Researcher Award (grant number DE200100367) funded by the Australian Government. The authors would like to thank La Trobe University for providing the research opportunity, as well as the members of the Sarker Lab for the guidance and overview of this project.

Conflicts of interest and sources of funding

The authors declare that there are no conflicts of interests.

Ethical approval

The faecal sampling was conducted during routine animal husbandry practice without touching animals. The Animal Ethics Committee at La Trobe University was informed that findings from the material were to be used in a publication, and a formal waiver of ethics approval was granted.

Data availability statement

The data that support the findings of this study are openly available in GenBank at https://www.ncbi.nlm.nih.gov/, reference number PP616711.

References

1. Lupiani B, Reddy SM. The history of avian influenza. *Comp Immunol Microbiol Infect Dis* 2009;32(4):311–323.
2. Sarker S. Metagenomic detection and characterisation of multiple viruses in apparently healthy Australian Neophema birds. *Sci Rep* 2021;11(1):20915.
3. Sutherland M, Sarker S, Vaz PK et al. Disease surveillance in wild Victorian cacatuids reveals co-infection with multiple agents and detection of novel avian viruses. *Vet Microbiol* 2019;235:257–264.
4. Fogell DJ, Martin RO, Groombridge JJ. Beak and feather disease virus in wild and captive parrots: an analysis of geographic and taxonomic distribution and methodological trends. *Arch Virol* 2016;161:1–16.
5. Das S, Sarker S, Peters A et al. Evolution of circoviruses in lorikeets lags behind its hosts. *Mol Phylogenet Evol* 2016;100:281–291.
6. Sarker S, Forwood JK, Ghorashi SA et al. Beak and feather disease virus genotypes in Australian parrots reveal flexible host-switching. *Aust Vet J* 2015;93(12):471–475.
7. Raidal SR, Sarker S, Peters A. Review of psittacine beak and feather disease and its effect on Australian endangered species. *Aust Vet J* 2015;93(12):466–470.
8. Sarker S, Ghorashi SA, Forwood JK et al. Phylogeny of beak and feather disease virus in cockatoos demonstrates host generalism and multiple-variant infections within *Psittaciformes*. *Virology* 2014;460–461:72–82.
9. Peters A, Patterson EI, Baker BG et al. Evidence of psittacine *beak and feather disease virus* spillover into wild critically endangered orange-bellied parrots (*Neophema chrysogaster*). *J Wildl Dis* 2014;50(2):288–296.
10. Hub NESPTSR. National Environmental Science Program Threatened Species Research Hub (2019) Threatened Species Strategy Year 3 Scorecard – Western Ground Parrot. Australian Government, Canberra. Available from: http://www.environment.gov.au/biodiversity/threatened/species/20-birds-by-2020/western-ground-parrot 2019.
11. Day JM, Zsak L. Determination and analysis of the full-length chicken parvovirus genome. *Virology* 2010;399(1):59–64.
12. Kisary J, Nagy B, Bitay Z. Presence of parvoviruses in the intestine of chickens showing stunting syndrome. *Avian Pathol* 1984;13(2):339–343.
13. Kailasan S, Agbandje-McKenna M, Parrish CR. Parvovirus family conundrum: what makes a killer? *Annu Rev Virol* 2015;2(1):425–450.
14. Kapgate SS, Kumanan K, Vijayarani K et al. Avian parvovirus: classification, phylogeny, pathogenesis and diagnosis. *Avian Pathol* 2018;47(6):536–545.
15. Pénzes JJ, Söderlund-Venermo M, Canuti M et al. Reorganizing the family Parvoviridae: a revised taxonomy independent of the canonical approach based on host association. *Arch Virol* 2020;165(9):2133–2146.
16. Sarker S. Characterization of a novel complete-genome sequence of a galliform Chaphamaparvovirus from a free-range laying chicken clinically diagnosed with spotty liver disease. *Microbiol Resour Announc* 2022;11(11):e0101722.
17. Duarte M, Silva JM, Brito C et al. Faecal virome analysis of wild animals from Brazil. *Viruses* 2019;11(9):803.
18. Palinski RM, Mitra N, Hause BM. Discovery of a novel Parvovirinae virus, porcine parvovirus 7, by metagenomic sequencing of porcine rectal swabs. *Virus Genes* 2016;52(4):564–567.
19. Reuter G, Boros Á, Delwart E et al. Novel circular single-stranded DNA virus from Turkey faeces. *Arch Virol* 2014;159(8):2161–2164.
20. Sarker S, Talukder S, Anwar A et al. Unravelling bile Viromes of free-range laying chickens clinically diagnosed with spotty liver disease: emergence of many novel Chaphamaparvoviruses into multiple lineages. *Viruses* 2022;14(11):2543.
21. Chang W-S, Eden J-S, Hall J et al. Metatranscriptomic analysis of virus diversity in urban wild birds with paretic disease. *J Virol* 2020;94(18):e00606–20. https://doi.org/10.1128/jvi.00606-20.
22. Sutherland M, Sarker S. Liver virome of a little Corella (Cacatua sanguinea) reveals coinfection with a novel parvovirus and two beak and feather disease viruses. *Aust Vet J* 2023;101(9):366–372.
23. Vibin J, Chamings A, Collier F et al. Metagenomics detection and characterisation of viruses in faecal samples from Australian wild birds. *Sci Rep* 2018;8(1):8686.

24. Bankevich A, Nurk S, Antipov D et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 2012; 19(5):455–477.
25. Benson DA, Cavanaugh M, Clark K et al. GenBank. *Nucleic Acids Res* 2013;41-(Database issue):D36–D42.
26. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol* 2013;30(4): 772–780.
27. Athukorala A, Phalen DN, Das A et al. Genomic characterisation of a highly divergent Siadenovirus (psittacine Siadenovirus F) from the critically endangered Orange-bellied parrot (Neophema chrysogaster). *Viruses* 2021;13(9):1714.
28. Athukorala A, Forwood JK, Phalen DN et al. Molecular characterisation of a novel and highly divergent passerine adenovirus 1. *Viruses* 2020;12(9):1036.
29. Athukorala A, Helbig KJ, Mcsharry BP et al. Adenoviruses in avian hosts: recent discoveries shed New light on adenovirus diversity and evolution. *Viruses* 2022;14(8):1767.
30. Fernández-Correa I, Truchado DA, Gomez-Lucia E et al. A novel group of avian astroviruses from neotropical passerine birds broaden the diversity and host range of Astroviridae. *Sci Rep* 2019;9(1):9513.
31. New FN, Brito IL. What is metagenomics teaching us, and what is missed? *Annu Rev Microbiol* 2020;74:117–135.
32. Ko KK, Chng KR, Nagarajan N. Metagenomics-enabled microbial surveillance. *Nat Microbiol* 2022;7(4):486–496.
33. White RT, Taylor W, Klukowski N et al. A discovery down under: decoding the draft genome sequence of Pantoea stewartii from Australia's critically endangered western ground parrot/kyloring (Pezoporus flaviventris). *Microbial Genomics* 2023;9(9):001101.
34. White RT, Jelocnik M, Klukowski N et al. The first genomic insight into chlamydia psittaci sequence type (ST)24 from a healthy captive psittacine host in Australia demonstrates evolutionary proximity with strains from psittacine, human, and equine hosts. *Vet Microbiol* 2023;280:109704.
35. Smith KF, Acevedo-Whitehouse K, Pedersen AB. The role of infectious diseases in biological conservation. *Animal Conservation* 2009;12(1):1–12.
36. Feare CJ. Role of wild birds in the spread of highly pathogenic avian influenza virus H5N1 and implications for global surveillance. *Avian Dis* 2010; 54(s1):201–212.

(Accepted for publication 17 September 2024)