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Characterisation of a novel aviadenovirus associated with disease in tawny frogmouths (*Podargus strigoides*)

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ABSTRACT

Aviadenoviruses are widespread in wild birds but rarely cause disease in nature. However, when naïve species are exposed to poultry or aviaries, aviadenoviruses can lead to disease outbreaks. This study characterised a novel aviadenovirus infection in a native Australian bird, the tawny frogmouth (*Podargus strigoides*) during an outbreak investigation. The identified complete genome of aviadenovirus, named tawny frogmouth aviadenovirus A (TwAviAdV-A) was 41,175 bp in length containing 52 putative genes. TwAviAdV-A exhibits the common aviadenovirus genomic organisation but with a notable monophyletic subclade in the phylogeny. The TwAviAdV-A virus was hepatotrophic and the six frogmouths presented to the wildlife hospitals in South Eastern Queensland most commonly exhibited regurgitation (in four frogmouths). Three were died or euthanized, two recovered, and one showed no signs. The detection of TwAviAdV-A in frogmouths coming into care re-emphasizes the need for strict biosecurity protocols in wildlife hospitals and care facilities.

1. Introduction

The genus *Aviadenovirus* is one of the three adenovirus genera whose species almost exclusively (*Aviadenovirus* and *Siadenovirus*) (Benko et al., 2021; Vaz et al., 2020) or commonly (*Atadenovirus*) (Vaz et al., 2020; Athukorala et al., 2022) infect birds. Aviadenovirus infections appear to be widespread in wild birds and infections have been detected in a diverse range of avian families during routine screening of apparently healthy wild birds in Europe (Rinder et al., 2020), Australia (Vaz et al., 2020), South America (Cortes-Hinojosa et al., 2021), and Japan (Kobayashi et al., 2022) and these viruses appear to be host-adapted (Vaz et al., 2020). Reports of adenoviruses causing disease in free-ranging birds are rare (Bodewes et al., 2013; Karamendin et al., 2021; Kumar et al., 2010; Reece et al., 1985) and with three exceptions these adenoviruses have not been characterised (Bodewes et al., 2013; Kumar et al., 2010). One of these viruses was fowl aviadenovirus which was isolated from black kites (*Milmus migrans)* (Kumar et al., 2010) in India that were believed to have been infected by feeding on domestic fowl carcasses (Kumar et al., 2010). Another was identified in dead nestling herring gull (*Larus argentatus*) and lesser black-backed gull (*Larus fuscus*) in the Netherlands (Bodewes et al., 2013), and a similar virus was detected in dead great black-headed gull (*Larus ichthyaetus*) and Caspian terns (*Hydroprogne caspia*), in a breeding colony on the shore of the Black Sea (Karamendin et al., 2021). Whether this virus is enzootic to these species or originated in another species is not known.

In contrast to free-ranging wild birds, where aviadenovirus associated-disease is rare, multiple outbreaks of aviadenovirus-associated disease have been documented in mixed collections of wild birds held for captive breeding (Kobayashi et al., 2022; Das et al., 2017) and aviadenoviruses are important causes of morbidity and mortality in

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Table 1
Origin, clinical presentations, Adenovirus status, and outcomes (survival and release, euthanasia, or died) of the tawny frogmouths included in this study.

ID	Where found	Weight (g)	BCS*	Reason for admission	Date admitted to Hospital	Date transferred to the aviary	Findings consistent with an adenovirus infection (date of onset)	Outcome	Date of outcome	Histopathological results	Adenovirus PCR results
Case 1	Noosa Heads, QLD	270	3/5	Right eye Injury Concussion	11/06/21	24/06/21	Started regurgitating in the aviary. Returned to the hospital 1/07/21)	Died	2/07/21	Severe hepatocellular necrosis with biliary trematodiasis, microfilariasis and intranuclear basophilic inclusion bodies	Positive - liver collected at necropsy
Case 2	Cooran, QLD	360	4/5	Fractured scapula	26/06/21	Never	Depressed and regurgitating 8/07/21	Euthanized	12/07/21	Severe hepatocellular necrosis with biliary trematodiasis, microfilariasis and intranuclear basophilic inclusion bodies	Positive - liver collected at necropsy
Case 3	Cooroy, QLD	260	2/5	Unable to fly; Significant elevations in GLDH, cholesterol, bile acids, AST & uric acid**	15/07/21	13/08/21	Signs resolved prior to transfer to the aviary	Released	19/10/21	NA	Positive – cloacal swab taken on admission; Positive – cloacal swab taken subsequently (3/9/21)
Case 4	Munruben, QLD	290	3/5	Fracture of the right ulna	July 22, 2021 (originally at Wacol since 24/06)	23/07/21	Started regurgitating in the aviary – returned to hospital16/08/21	Released	19/10/21	NA	Positive – cloacal swab, day after admission; July 23, 2021 Negative – cloacal swab (3/9/21); Negative – cloacal swab (24/09/21)
Case 5	Doonan, QLD	310	3/5	Unable to stand	11/07/21	30/07/21	Injured leg in aviary - returned to hospital 18/08/21. Started regurgitating 26/08/21	Euthanized as a result of a leg injury)	30/08/21	NA	Positive – Cloacal swab (30/08/21)
Case 6	Ringtail Creek, QLD	224	3/5	Mild concussion	22/09/21	Never	No signs	Released	29/09/21	NA	Positive – Cloacal Swab 22/09/21 on admission

Note: BCS; Body Condition Score, GLHD; glutamate dehydrogenase, AST; Aspartate Amino Transferase, NA; Not Applicable.

intensively raised poultry (Gallinaceous and Anseriformes spp.) (Fu et al., 2013; Schachner et al., 2018) and pigeons (*Columbia livia*) (Vereecken et al., 1998). Aviadenovirus outbreaks appear to occur in mixed collections of wild birds because naïve species are exposed to a host-adapted virus shed by other species in the same aviary (Das et al., 2017). Aviadenoviruses are enzootic in poultry (Schachner et al., 2018) and domestic pigeon flocks (Vereecken et al., 1998). In these species, disease outbreaks appear to occur as the result of exposure to particularly pathogenic strains of the viruses, concurrent infection with immunosuppressive viruses (Niczyporuk et al., 2020), and stress and other management-related issues. Transmission can occur both horizontally and vertically. When vertical transmission occurs, disease, if it occurs, may not develop until birds are sexually mature (Schachner et al., 2018). Pathogenic aviadenoviruses target one or more organ systems, including the liver (Tomaszewski and Phalen, 2007), ventricular mucosa (Goodwin, 1993), intestinal mucosa (Zhang et al., 2018), pancreas (Smith, 2018), Bursa of Fabricius (Bodewes et al., 2013), spleen (Tomaszewski and Phalen, 2007), respiratory mucosa of the trachea (Zhang et al., 2018) and the female reproductive tract (Fu et al., 2013).

Genomes of the aviadenoviruses are relatively large as compared to those of the other adenovirus families, ranging in size from 38,694 bp (*Psittacine aviadenovirus C*) (Milani et al., 2018) to 45,781 bp (*Fowl aviadenovirus B*) (Marek et al., 2013). Aviadenovirus genomes possess a common genomic architecture found in other adenoviruses that is composed of a casset of core open reading frames (ORFs) shared by all adenoviruses. The core genes are located in the central part of the adenovirus genome, encode structural proteins, DNA replication proteins, host protein binding sites that moderate DNA replication, and proteins that modulate the host immune response (Kulanayake and

Tikoo, 2021). Variations in virulence are usually influenced by the hexon protein, which contains specific antigenic determinants, and fiber proteins due to their involvement of in receptor binding. The presence of two fiber projections is commonly observed in aviadenoviruses. However, in some aviadenovirus species, such as *Fowl aviadenovirus C*, two fiber genes are present, while some others, like *Fowl aviadenovirus B* and *Fowl aviadenovirus D*, possess only one fiber gene (Zhang et al., 2018; Pallister et al., 1996; Pénzes et al., 2014; Schachner et al., 2014; To et al., 2014; Wang and Zhao, 2019).

As with all adenovirus families, the right hand or terminal portion of the aviadenovirus genome contains variable numbers of open reading frames that code for proteins whose function is largely unknown. The aviadenoviruses genomes also contain genus-specific dUTP pyrophosphate (dUTPase), NS1 gene and the three consecutive hypothetical protein-coding genes next to NS in the left genomic end. In addition, the right genomic end of the aviadenoviruses contain several genus-common hypothetical ORFs and some species carry a GAM-1 protein-coding gene (Athukorala et al., 2022). The roles that these genes play in the life cycle and pathogenicity of aviadenoviruses remains to be determined. Also, each genome end is capped by the inverted terminal repeat sequence that varying in length between 39 bp (*Goose aviadenovirus A*, serotype 4) (Kajan et al., 2012) and 721 bp (*Duck aviadenovirus B*, serotype 2) (Marek et al., 2014).

Tawny frogmouths (*Podargus strigoides*) are members of the globally distributed order caprimulgiformes (Grzimek et al., 2003). The tawny frogmouth is a solitary and nocturnal species that is distributed across the majority of the Australian mainland and Tasmania (BirdLife-Australia BirdLife Australia). They are common but appear to be declining across their range (BirdLife-Australia BirdLife Australia). They are predominately insectivorous but also feed on other invertebrates such as molluscs (e.g., snails and slugs), mice (*Mus musculus*) and small species of birds (AnimalSpot Tawny Frogmouth). There is a single report of a disease caused by an uncharacterised adenovirus in a nestling tawny frogmouth that presented to a zoo in Victoria, Australia. The bird did not eat for several days and died. Gross examination reveals an enlarged, friable liver with miliary white foci. Histologically, lesions consist of acute hepatic necrosis with eosinophilic intranuclear inclusion bodies. It did not eat in care and died. No specific signs that might be attributed to the adenovirus infection were reported. Histologically, it was found to have a multifocal necrotizing hepatitis and haemorrhagic typhlitis with intralesional inclusion bodies consistent with those caused by adenoviruses and adenovirus virions were imaged by electron microscopy in the diseased tissue (Reece et al., 1985). In the current manuscript, we describe the signs, clinicopathological manifestations and temporal and spatial distribution of an outbreak (A sudden increase in the number of affected species/individuals within a specie that is above the level considered normal for the given population.) of an aviadenovirus-associated disease in wild tawny frogmouths in Southeast Queensland, Australia. We also report the entire genome of this virus and its evolutionary relationship to representative adenoviruses.

2. Materials and methods

2.1. Source of sample and histopathological diagnosis

Livers from Case 1 and Case 2 (Table 1) were formalin-fixed, sectioned at 4 μm, and routinely stained with hematoxylin and eosin. Fresh liver from Case 1 was routinely cultured for aerobic bacteria.

2.2. Extraction of DNA, and confirmation of the presence of TwAviAdV-A DNA

Genomic DNA was extracted from liver and cloacal swabs using the commercially available Purelink™ Genomic DNA Mini Kit (Invitrogen, CA, USA). Adenovirus was detected in the extracted DNA using a nested-PCR reaction using primers that are designed to detect all known adenovirus infections (Wellehan et al., 2004; Yang et al., 2019). Amplicons were purified using Amicon Ultra- 0.5 centrifugal filter unit (Millipore, Sigma, St. Louis, MO, USA) according to the manufacturer's instructions and sequenced in both directions using the forward and reverse primers through the Australian Genome Research Facility (AGRF), Westmead, NSW, Australia.

2.3. Library construction and sequencing

The library construction was adapted using the Nextera DNA Flex Prep (Illumina, San Diego, CA, USA) as per kit instructions. The quality and quantity of the prepared library was assessed, and the prepared library was normalised and pooled in equimolar quantities. The quality and quantity of the final library was further assessed before sequencing by the AGRF facility. Cluster generation and sequencing of the library was performed with the read length of 150-bp paired-end on Illumina® NovaSeq chemistry according to the manufacturer's instructions.

2.4. Genome assembly

Sequencing data were analysed using Geneious Prime (version 21.1.1, Biomatters, Ltd., Auckland, New Zealand) and CLC Genomics Workbench (version 9.0.1, CLC bio, a QIAGEN company, Prismet, Aarhus C, Denmark) according to the previously established analysing pipeline (Sarker et al., 2017a, 2017b; Sutherland et al., 2019; Athukorala et al., 2020, 2021). The complete genome of TwAviAdV-A was obtained from a total number of 36.37 million paired-end reads. Initial quality evaluation for all raw reads was performed and pre-processed to remove ambiguous base calls and poor-quality reads. In addition, to remove the host DNA contamination, the trimmed reads were mapped against the chicken genome (*Gallus*, GenBank accession number NC_006088). Reads were further mapped to *Escherichia coli* bacterial genome (GenBank accession no. U00096) to remove possible bacterial contamination. Unmapped reads were then subjected to de novo assembly, using SPAdes assembler (version 3.10.1) (Bankevich et al., 2012), under the "careful" parameter in LIMS-HPC cluster (La Trobe Institute for Molecular Science—High Performance Computing cluster, specialised for genomics research in La Trobe University) (Altschu et al., 1990; Boratyn et al., 2013). The resulting contigs were compared against the nonredundant nucleotide and protein databases on GenBank using BLASTN and BLASTX (Benson et al., 2013), respectively, with an e-value threshold of 1×10^{-5} to remove potential false positives. BLASTn searches yielded a single contig of 41,175 bp corresponding to the TwAviAdV-A sequence.

2.5. Genome annotation and bioinformatics

The assembled TwAviAdV-A genome was annotated using the Geneious software (version 21.1.1, Biomatters, Ltd., Auckland, New Zealand). Several reference aviadenoviruses genomes were used for the annotation process to compare the predicted ORFs and to evaluate the consequences of potential truncations or extensions that can occur at the N- and C-termini. ORFs over 30 amino acids along with minimal overlapping (not exceeding 25% overlaps in one of the genes) to other open reading frames were selected and annotated. The predicted ORFs were extracted into FASTA files subsequently, and similarity searches were performed on annotated ORFs as potential genes to determine whether they shared significant sequence similarities to established viral or cellular genes (BLAST E value $\leq 10^{-5}$) or contained a putative conserved domain as predicted by protein searches (BLASTX and BLASTP) (Benson et al., 2014).

Multiple applications were used to predict the function of identified hypothetical ORFs and to identify their conserved domains or motifs. TMHMM package v.2.0 (DTU Health Tech, Lyngby, Denmark) (Krogh et al., 2001; Tusnady and Simon, 2001), SOSUI (Hirokawa. et al., 1998), and DAS (Cserzö. et al., 1997) were used to search for transmembrane

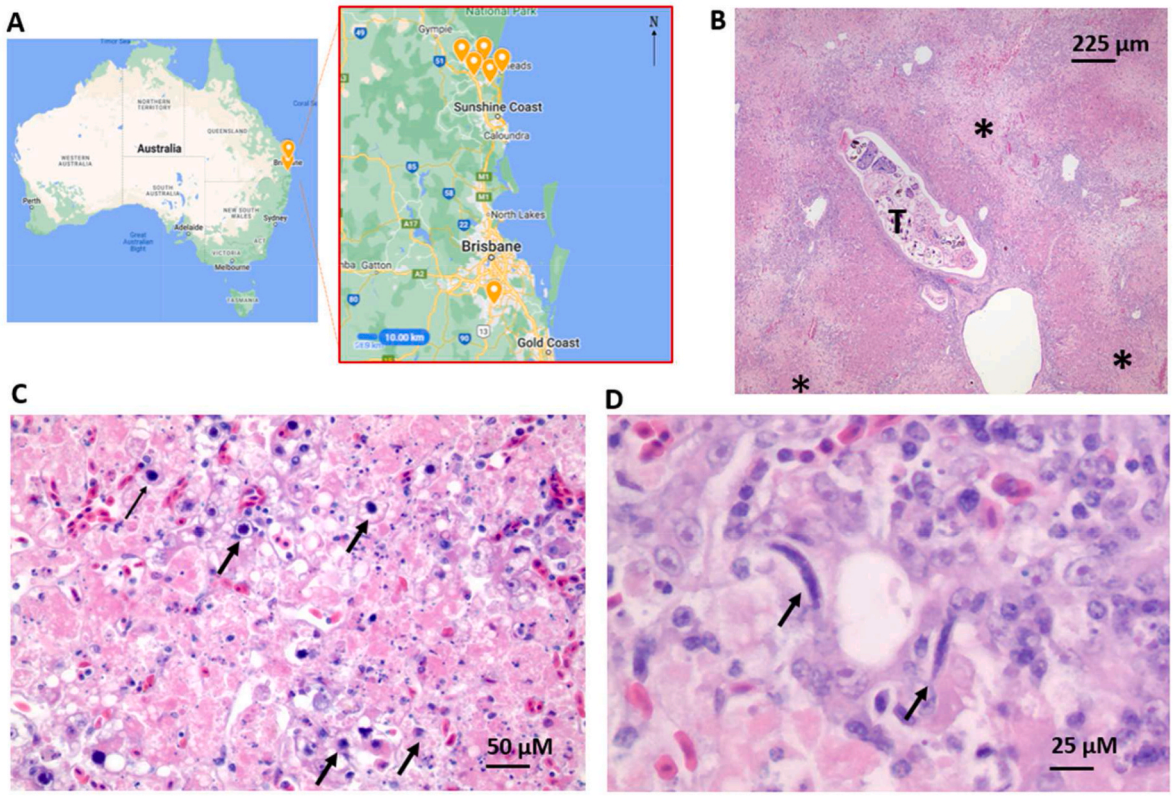

Fig. 1. **1A**: Locations where tawny frogmouths included in this study were found (Generated using Google Maps); **1B**: Hematoxylin and eosin-stained section of liver from a tawny frogmouth that died with an adenovirus hepatitis and had a concurrent trematode infection. A cross section of the trematode is marked with a T. There is extensive centrilobular to massive necrosis. Representative sections of necrosis are highlighted (*); **1C**: Higher magnification of the hematoxylin and eosin-stained section of the liver of from the tawny frogmouth shown in 1B. The liver is undergoing extensive peracute necrosis. Numerous hepatocytes (arrows) contain deeply basophilic intranuclear inclusions which are characteristic of those caused by adenovirus infections; **1D**: Higher magnification of the hematoxylin and eosin-stained section of the liver of from the tawny frogmouth shown in 1B. Microfilaria (arrows) are present within the lumens of capillaries.

(TM) helices. Protein homologs and conserved secondary structures were searched using Phyre2 (Kelley et al., 2015) and (HHpred) (Zimmermann et al., 2018), respectively to help predict the function of proposed hypothetical ORFs predicted in this study.

2.6. Comparative genomics

The organisation of the newly assembled tawny frogmouth aviadenovirus A (TwAviAdV-A) genome with other selected aviadenoviruses was visualized and compared using CLC Genomic Workbench (version 9.0.1, CLC bio, a QIAGEN Company, Prismet, Aarhus C, Denmark). Comparative G + C content (%) and pairwise identity of representative aviadenovirus species against TwAviAdV-A were obtained using Geneious software (version 21.1.1, Biomatters, Ltd., Auckland, New Zealand), based on the nucleotide sequences of the complete genome and the similarity percentage of selected aviadenoviruses core proteins sequences. Selected proteins were aligned using the MAFFT alignment (version 7.450) [51]in Geneious (version 21.1.1, Biomatters, Ltd., Auckland, New Zealand) and the similarity percentage of protein sequences was calculated following scoring matrix BLOSUM62 and Gap open penalty = 1.53. Blosum62 with threshold 1 (percentage of residues which have score $\geq$ 1 in the Blosum62 matrix) parameters.

2.7. Phylogenetic analyses

Phylogenetic analysis was performed to determine the evolutionary relationship of the assembled TwAviAdV-A using the sequence characterised in this study with other 53 representative adenoviruses, publicly available in GenBank. Initially, amino acid sequences of four conserved genes; DNA polymerase, pTP, hexon, and penton were extracted individually from the selected AdV genomes. Individual and concatenated sequences of the selected genes were then separately aligned with MAFTT (version 7.450), using G–INS–I (scoring matrix BLOSUM62; gap open penalty 1.53; offset value 0.123) in Geneious (version 21.1.1, Biomatters, Ltd., Auckland, New Zealand) (Katoh and Standley, 2013). Sequences were annotated with the virus species and GenBank accession number in parentheses. Using the individual and concatenated amino acids sequence alignments, maximum likelihood (ML)-based phylogenetic analyses were performed with 1000 non-parametric bootstrap replicates implemented in Geneious (version 21.1.1, Biomatters, Ltd., Auckland, New Zealand) (Guindon et al., 2008).

2.8. Recombination analyses

Recombination analyses were performed within the genus *Aviadenovirus*. Because of the limitation of recombination detection softwares, highly diverse sequences are not recommended to use in recombination detection and may lead false recombination events. Therefore, only the full-length aviadenovirus genome and selected gene sequences of aviadenoviruses were assessed for a detection of recombination signals using the RDP, Bootscan, MaxChi, GENECONV, Siscan, Chimaera, LARD, and 3Seq methods contained in the RDP4 program (Martin et al., 2010). Events detected with significant p-values from at least two above-mentioned methods were considered as possible events of recombination.

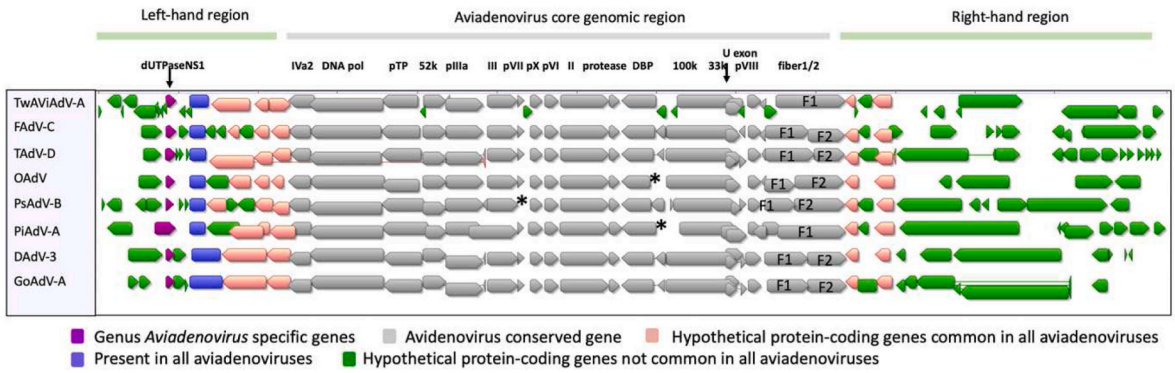

Fig. 2. Schematic illustration of aviadenovirus genome comparison. Complete aviadenovirus genomes were extracted from NCBI: tawny frogmouth aviadenovirus A (TwAviAdv-A), GenBank Ac No. ON642334; *Fowl aviadenovirus C* (FAdV-C), GenBank Ac No. KU569296; *Turkey aviadenovirus D* (TAdV-D), GenBank Ac No. KF477313; Owl adenovirus (OAdV), GenBank Ac No. LC638697; *Psittacine aviadenovirus B* (PsAdV-B), GenBank Ac No. KX577802; *Pigeon aviadenovirus A* (PiAdV-A), GenBank Ac No. FN824512; *Duck adenovirus 3* (DAdV-3), GenBank Ac No. MW677606, and *Goose aviadenovirus A* (GoAdV-A), GenBank Ac No. JF510462 were aligned with MAFTT (version 7.388) (Katoh and Standley, 2013) in Geneious (version 21.1.1). Conserved adenovirus genes, genus-common and species-specific hypothetical ORFs, and genes present in all avidenoviruses were presented in different colors as per the given color guide. Conserved genes that are not present in genomes are marked with X and F1 and F2 denotes fiber 1 and fiber 2, respectively.

3. Results

3.1. Clinical presentations and outcomes

The six tawny frogmouths in this study presented to the Royal Society for the Prevention of Cruelty to Animals (RSPCA) Wildlife Hospitals, at the Wacol Animal Care Campus, Wacol, QLD (−27° 34′ 59.99″ S.52° 55′ 59.99″ E) (1 bird) and the RSPCA Eumundi Wildlife Centre, Eumundi, QLD (−26° 28′ 59.99″ S: 152° 56′ 59.99″ E) (5 birds) between June 11, 2021 and September 22, 2021. (Fig. 1A and Table 1). The bird that originally presented at the Wacol campus was transferred to the Eumundi Centre after 28 days in care. Five were presented because of traumatic injuries. One presented ill and emaciated with biochemical changes consistent with severe diffuse liver disease and severe diffuse kidney disease, findings consistent with an adenovirus hepatitis and nephritis. Four of the tawny frogmouths that presented for traumatic injuries subsequently developed a sign (vomiting) that was likely the result of aviadenovirus disease (Table 1). Of these, one died spontaneously, one was euthanized because of a poor prognosis based on adenovirus-associated signs and an injury that occurred while in care, one had transient signs, recovered, and was released. The remaining tawny frogmouth never showed signs of adenovirus infection, recovered from its traumatic injury and was released. Signs that developed in injured tawny frogmouths in care occurred at 12, 19, 50, and 52 days after presentation. Repeat PCR testing of cloacal swabs was done on two tawny frogmouths. One was positive at presentation and was still shedding the adenovirus 49 days later, despite showing no signs of regurgitation for 21 days. This bird was released after a total of 96 days in care as it was free of clinical signs for roughly 2 months. One was positive when first tested in care and then was negative when retested 41 and 62 days later (Table 1).

3.2. Microbiological and histopathologic findings

A mixed population of *Pseudomonas* sp., *Enterococcus* sp., and *Escherichia coli* were isolated from the liver sample of Case 1. These were considered to represent a bacterial overgrowth of the liver after the bird died. Lesions identified in hematoxylin and eosin-stained liver sections from both Case 1 and Case 2 were characterised by extensive acute centrilobular to massive necrosis throughout the liver with collapse of some centrilobular lumens (Fig. 1B). Scattered among, and sometimes bordering necrotic regions, were individual hepatocytes with swollen nuclei and cytoplasmic vacuolation, some with peripheralized chromatin and one to multiple large central darkly basophilic inclusions (Fig. 1C). These nuclear changes were consistent with intranuclear inclusions causing an adenovirus infection.

In Case 1 and 2, superimposed on this acute lesion was a chronic active lesion characterised by dilated bile ducts that contained multiple cross-sections of trematodes, presumptively *Brachylecithum podargi* (Angel and Pearson, 1977) (Fig. 1B). Associated with the biliary trematode sections was an extensive mixed inflammation in the surrounding portal areas composed of lymphocytes, plasma cells, and macrophages. Many portal areas had moderate ductular hyperplasia composed of tortuous epithelial cords sometimes associated with poorly recognisable lumens (ductal reaction). Cross sections of trematodes were also observed in the liver section from Case 2, but they were uncommon and associated inflammation was relatively mild. An incidental finding was the presence of rare 4-micron-in-diameter microfilaria within necrotic regions of both cases (Fig. 1D).

3.3. Initial characterisation of the adenovirus detected in the samples from the tawny frogmouths

Adenovirus DNA sequences ranging from 218 to 483 nucleotides were obtained from the PCR amplicons. These sequences were identical (or nearly identical 99%) to each other and have been submitted to GenBank (GenBank aceession numbers OR637863-66). Based on the percentage nucleotide similarity of the members in aviadenovirus genus, e.g., *Turkey adenovirus B* (83.9%; GenBank Accession no GU936707) and *Pigeon adenovirus A* (83.9%; GenBank Accession no FN824512), this virus appeared to a be a novel aviadenovirus.

3.4. Genomic organisation and comparative analysis of TwAviAdV-A

The assembled tawny frogmouth aviadenovirus A (TwAviAdV-A) complete genome (GenBank accession no ON642334) was a double-stranded linear DNA molecule of 41,175 bp in length. The TwAviAdV-A genome encoded 52 predicted methionine-initiated ORFs that were annotated as putative genes and numbered from left to right (Fig. 2 and Table 2). The TwAviAdV-A complete genome was found to contain all expected conserved genes found in other species in the genus *Aviadenovirus*. TwAviAdV-A genome had a G + C content of 59.7% and based on the pairwise genome analysis, its genome is most closely related to *Pigeon aviadenovirus A* with 47.01% genome identity (Supplementary Table S1).

Table 2
Predicted protein-coding genes of TwAviAdV-A.

TFMAdVA gene synteny	genome coordinates	Length (nt)	Length (AA)	Description	Per. ident/query cover/ accession No	Note
TFMAdVA – 01	21–167	147	48	Hypothetical protein		unique to TFMAdVA
TFMAdVA – 02	1181–417	765	254	protein ORF52 [Pigeon adenovirus 2]	40.68/91%/YP_009310421.1	
TFMAdVA – 03	1332–1123	210	69	Hypothetical protein		unique to TFMAdVA
TFMAdVA – 04	2095–1307	789	262	protein ORF52 [Pigeon adenovirus 2]	39.04/94%/YP_009310421.1	
TFMAdVA – 05	3056–1944	1113	370	Hypothetical protein		unique to TFMAdVA
TFMAdVA – 06	3226–2975	252	83	Hypothetical protein		unique to TFMAdVA
TFMAdVA – 07	3244–3351	108	35	Hypothetical protein		unique to TFMAdVA
TFMAdVA – 08	3540–3385	156	51	Hypothetical protein		unique to TFMAdVA
TFMAdVA – 09	3736–3479	258	85	Hypothetical protein		unique to TFMAdVA
TFMAdVA – 10	3735–4295	561	186	dUTP pyrophosphatase [Fowl aviadenovirus A]	65.56/79%/APP94048.1	
TFMAdVA – 11	4801–4478	324	107	Hypothetical protein		unique to TFMAdVA
TFMAdVA – 12	5063–4842	222	73	Hypothetical protein		unique to TFMAdVA
TFMAdVA – 13	4974–5798	825	274	Rep protein [Fowl aviadenovirus A]	46.32/97%/NP_043871.1	
TFMAdVA – 14	6397–5837	561	186	ORF14A [Pigeon adenovirus 2a]	33.15/95%/APO40939.1	
TFMAdVA – 15	7414–6620	795	264	hypothetical protein [Psittacine adenovirus 1]	52.34/96%/AXB73035.1	
TFMAdVA – 16	7413–8543	1131	376	ORF12 [Fowl aviadenovirus 10]	34.65/79%/ABB18353.1	
TFMAdVA – 17	9777–8512	1266	421	IVa2 [Turkey aviadenovirus 5]	73.95/90%/YP_008719851.1	
TFMAdVA – 18	13,552–9689	3864	1287	DNA polymerase [Fowl aviadenovirus B]	63.10/96%/QCC26476.1	
TFMAdVA – 19	15,597–13522	2076	691	terminal protein precursor [Fowl aviadenovirus 5]	50.00/98%/YP_007985647.1	
TFMAdVA – 20	15,932–15732	201	66	Hypothetical protein		unique to TFMAdVA
TFMAdVA – 21	15,858–17087	1230	409	encapsidation protein 52K [Fowl aviadenovirus 7]	68.60/97%/ANJ02486.1	
TFMAdVA – 22	17,335–17081	255	84	capsid protein precursor pIIIa [Fowl aviadenovirus D]	74.03/90%/CCE04067.1	
TFMAdVA – 23	17,103–18821	1719	572	pIIIa [Duck adenovirus 4]	67.58/100%/QJC19250.1	
TFMAdVA – 24	18,928–20589	1662	553	penton [Adenoviridae sp.]	75.34/92%/QLI47685.1	
TFMAdVA – 25	20,595–20840	246	81	pVII [Fowl aviadenovirus E]	55.84/90%/QGQ62663.1	
TFMAdVA – 26	20,837–21046	210	69	Hypothetical protein		unique to TFMAdVA
TFMAdVA – 27	20,994–21566	573	190	major core protein [Fowl aviadenovirus 10]	57.67/97%/AAA92581.1	
TFMAdVA – 28	21,661–22362	702	233	capsid protein precursor pVI [Fowl aviadenovirus 3]	63.14/100%/ANJ02417.1	
TFMAdVA – 29	22,443–25277	2835	944	L3 hexon [Fowl aviadenovirus A]	73.66/100%/QGQ62264.1	
TFMAdVA – 30	25,302–25931	630	209	protease [Fowl aviadenovirus 5]	74.76/98%/QJP03680.1	
TFMAdVA – 31	27,851–26073	1779	592	DNA-binding protein [Fowl aviadenovirus D]	54.65/71%/NP_050289.1	
TFMAdVA – 32	28,412–27894	519	172	Hypothetical protein		unique to TFMAdVA
TFMAdVA – 33	28,927–28718	210	69	Hypothetical protein		unique to TFMAdVA
TFMAdVA – 34	28,926–31976	3051	1016	100K [Fowl aviadenovirus 5]	62.72/61%/YP_007985657.1	
TFMAdVA – 35	32,283–31642	642	213	encapsidation protein 22K [Pigeon adenovirus 2]	46.95/76%/YP_009310443.1	
TFMAdVA – 36	31,684–32202	519	172	22K [Fowl aviadenovirus A]	48.11/98%/AP_000423.1	
TFMAdVA – 37	32,338–32105	234	77	Hypothetical protein		unique to TFMAdVA
TFMAdVA – 38	32,509–33279	771	256	L4 pVIII [Fowl aviadenovirus A]	63.64/98%/ASU56024.1	
TFMAdVA – 39	33,532–33215	318	105	U exon [Fowl adenovirus 8b]	73.24/67%/AWT08537.1	
TFMAdVA – 40	33,489–33770	282	93	Hypothetical protein		unique to TFMAdVA
TFMAdVA – 41	33,796–35898	2103	700	fiber-2 [Pigeon adenovirus 2a]	35.95/83%/APO40960.1	
TFMAdVA – 42	36,402–35929	474	157	ORF22 [Turkey adenovirus 1]	36.36/98%/YP_003933598.1	
TFMAdVA – 43	36,401–36541	141	46	Hypothetical protein		unique to TFMAdVA
TFMAdVA – 44	37,024–36548	477	158	Hypothetical protein		unique to TFMAdVA
TFMAdVA – 45	37,998–37078	921	306	ORF20 [Duck adenovirus 4]	41.04/85%/QJC19264.1	
TFMAdVA – 46	38,440–38339	102	33	Hypothetical protein		unique to TFMAdVA
TFMAdVA – 47	38,752–38516	237	78	Hypothetical protein		unique to TFMAdVA

(*continued on next page*)

Table 2 (*continued*)

TFMAdVA gene synteny	genome coordinates	Length (nt)	Length (AA)	Description	Per. ident/query cover/ accession No	Note
TFMAdVA – 48	39,064–38927	138	45	Hypothetical protein		unique to TFMAdVA
TFMAdVA – 49	39,003–39875	873	290	GAM-1 [Pigeon adenovirus 2]	31.54/95%/YP_009310452.1	
TFMAdVA – 50	40,712–40047	666	221	Hypothetical protein		unique to TFMAdVA
TFMAdVA – 51	40,869–40705	165	54	Hypothetical protein		unique to TFMAdVA
TFMAdVA – 52	40,985–41125	141	46	Hypothetical protein		unique to TFMAdVA

Notes: TFMAdVA; tawny frogmouth aviadenovirus A, AA, amino acid; nt, nucleotide; %, percentage.

3.5. Genome annotation and comparative analyses of TwAviAdV-A

Comparative genomic analysis using BLASTX and BLASTP identified the homologs of the protein sequences encoded by the predicted ORFs with significant protein sequence similarity (E value: 10^{-5}) (Table 2). Among the predicted protein-coding genes in TwAviAdV-A genome, 23 ORFs encode species-specific hypothetical proteins (ORF-1, 3, 5–9, 11, 12, 20, 26, 32, 33, 37, 40,43, 44, 46–48 and 50–52) and 29 are homologous to other aviadenovirus gene products. BLASTP and BLASTX did not find any similarity with known functional proteins in the non-redundant protein database. These hypothetical ORFs encode proteins ranging from 35 to 370 amino acids in length (Table 2). Of the ORFs designated as hypothetical proteins, nine were identified in the left-hand region of the genome and eight in the right-hand region, including one next to the fiber gene. Whereas another six hypothetical proteins unique to TwAviAdV-A were discovered among core genes (Table 2 and Fig. 2). From the hypothetical ORFs, four (ORF-05, -11, −44 and −51) were predicted to be transmembrane proteins, each containing a single transmembrane helix (TMH) by TMHMM-2.0, HMMTOP, SOSUI, and DAS. Nevertheless, no evidence was found for conserved secondary structures or protein homologs by HHpred and Phyre2.

Conserved genes in TwAviAdV-A exhibited a similar orientation as those found in the complete genomes of *Pigeon aviadenovirus A* and *Goose aviadenovirus A* which have the highest and lowest genome identities to TwAviAdV-A respectively (Supplementary Table 1). The left-hand region of the TwAviAdV-A genome contained the *Aviadenovirus* genus-specific dUTP pyrophosphatase gene homolog followed by Rep protein and three other hypothetical protein-coding genes that are common to all aviadenoviruses along with a range of hypothetical proteins in the right-hand region (Fig. 2).

3.6. Evolutionary relationships of TwAviAdV-A

Phylogenetic reconstruction using concatenated amino acid sequences of two structural; hexon and penton and two non-structural; DNA polymerase and pTP proteins, confirmed that TwAviAdV-A is a member of the genus *Aviadenovirus* and formed a distinct subclade. TwAviAdV-A appeared to be evolved in between pigeon aviadenoviruses and psittacine aviadenoviruses in the aviadenovirus evolution (Fig. 3). Individual ML trees using the DNA polymerase and penton genes were demonstrated a similar tree topology for the TwAviAdV-A species (Supplementary Figures S1 and S2) while all four individual ML trees mapped TwAviAdV-A into the Genus *Aviadenovirus* (Supplementary Figures S3 and S4).

3.7. Indication of a rare recombination event

A single occurrence of a recombination event was detected in the hexon gene of TwAviAdV-A using RDP4 program analysis. Recombination was detected in the region of 394–1318 of the TwAviAdV-A and *Turkey aviadinovirus B* (GenBank accession no. GU936707) is the minor parental sequence where source of major parental sequence is unknown. Significantly higher p-values; RDP- 5.34×10^{-6}, Bootscan – 3.99×10^{-4}, Maxchi – 2.68×10^{-9}, and SiSscan – 2.98×10^{-15} were assured the recombinant event.

4. Discussion

Adenovirus infections in free ranging wild birds are common (Vaz et al., 2020; Cortes-Hinojosa et al., 2021; Kobayashi et al., 2022) but are rarely associated with disease (Bodewes et al., 2013; Karamendin et al., 2021). In this study we genetically characterised a novel aviadenovirus (tawny frogmouth aviadenovirus A) that caused an outbreak of disease in a free ranging Australian species, the tawny frogmouth.

Genomic and phylogenetic analysis uncovered the genus-specific characteristics and confirmed the classification of TwAviAdV-A in the genus *Aviadenovirus* as well as the distinctness of TwAviAdV-A from other aviadenoviruses. The central part of the TwAviAdV-A genome has the characteristic conserved genes and arrangement of every other adenovirus and in addition to 23 unique hypothetical proteins where there is no homology to known protein sequences. Four of them were predicted to encode transmembrane proteins but extensive analysis is recommended to better understand their structural and functional importance in host-virus interactions and pathogenicity. Unlike other aviadenoviruses, which typically have ORFs that code for two fiber proteins, it appears to only have a single ORF. Presence of ORF, coding for fiber 2 protein has been reported to associated with pathogenicity (Schachner et al., 2014) however a recent study also highlighted the significant roles of fiber 1 in pathogenic *Fowl aviadenovirus C* (Wang et al., 2020). Over the course of its evolution, it also appears to have undergone a recombination event with another aviadenovirus. Recombination events are well documented in human adenoviruses (Lukashev et al., 2008) and have been suggested to play an important role in their evolution. Multiple recombination events have also been documented in another aviadenovirus; Poicephalus aviadenovirus that caused disease in an African species of parrot (Red-bellied parrot -*Poicephalus rufiventris*) that was held in a mixed species collection of parrots that also contained a sub clinically infected Australian species, the Purple-crowned lorikeet (*Parvipsitta porphyrocephala*) (Das et al., 2017).

Phylogenetic reconstructions demonstrated a clear subclade for TwAviAdV-A, suggesting pigeon adenoviruses to be the closest relative but yet to be determined. The origin of TwAviAdV-A is unknown, and it could be a pathogenic strain of an aviadenovirus that was originally adapted to the tawny frogmouth. However, this would seem unlikely as multiple animals, over a wide geographic area, were infected in this outbreak and tawny frogmouths are not a social species and have a small home range, so how it would spread between them is not clear. It is also likely possible one of the tawny frogmouths in hospital infected the others as they were all in close proximity in the hospital and some shared the aviary at the same time. An alternate hypothesis about the origin of this outbreak is that the TwAviAdV-A is a host-adapted aviadenovirus of another small bird species, one that might be more gregarious and more likely to have a larger home range or to migrate and could be a prey species of the tawny frogmouth. Many adenoviruses have been found to

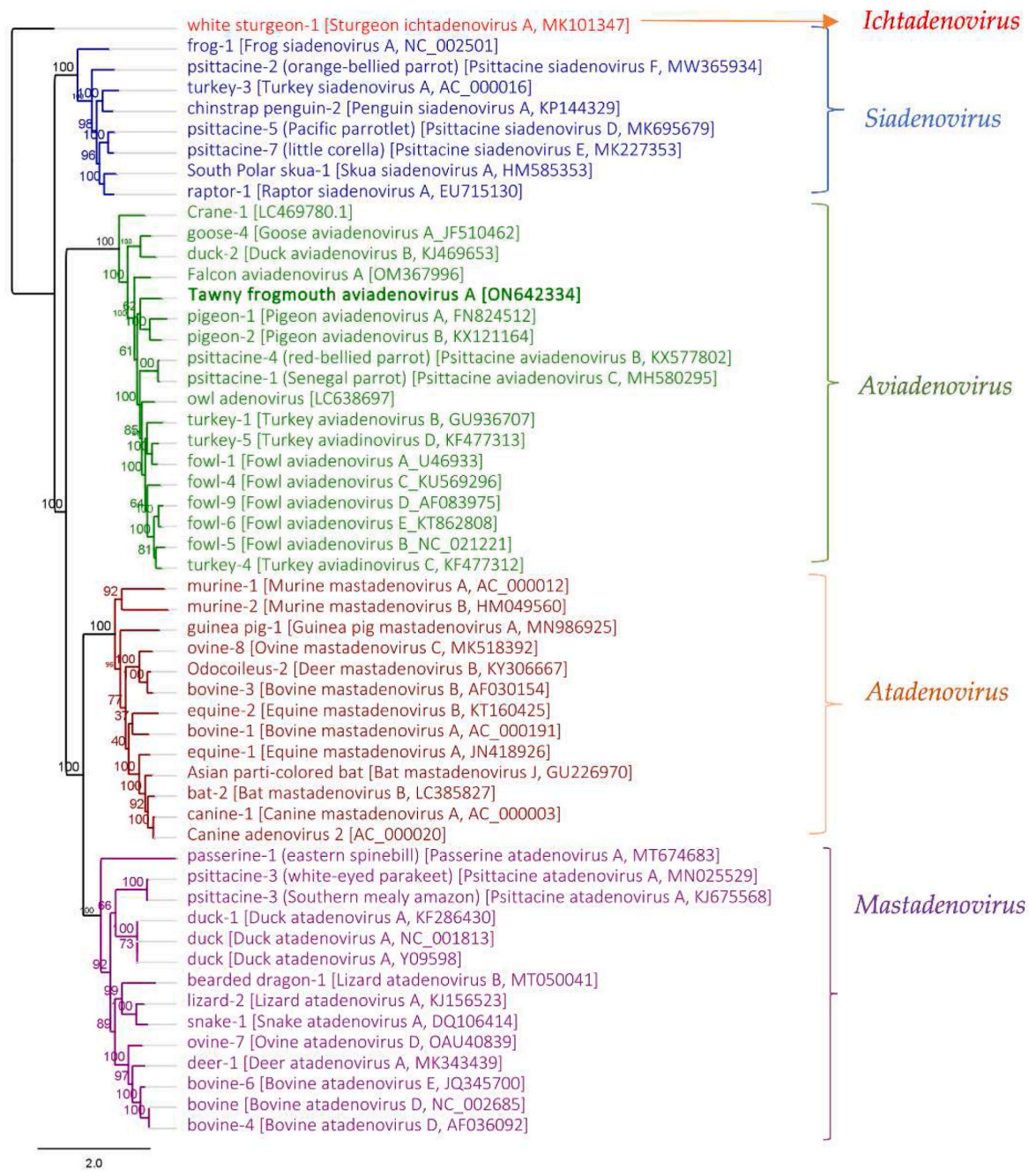

Fig. 3. The phylogenetic tree of selected AdVs, presented the possible evolutionary relationship of novel TwAviAdV-A. Concatenated amino acid sequences of the complete DNA-dependent DNA polymerase, penton, pTP, and hexon genes were used to construct Maximum likelihood (ML) tree. The unrooted ML tree was constructed under the WAG substitution model, and 500 bootstrap replicates using tools available in Geneious. Bootstrap values as percentages were shown at the beginning of the node and the branch tips were labelled with the original AdVs species name followed by GenBank accession number in parentheses. The novel TwAviAdV-A is highlighted in bold letters.

sub-clinically infect Australian passerine species (Vaz et al., 2020), however, none of these were closely related to TwAviAdV-A, so additional testing of tawny frogmouths and their prey species will be required to test these hypotheses.

One of the target organs for TwAviAdV-A appears to be the liver, based on the presence of intranuclear inclusion bodies consistent with those caused by adenoviruses in hepatocytes in cases one and two and elevations in serum liver enzymes and bile acids in Case 3. Whether other organs are also targeted by TwAviAdV-A remains unknown as only limited tissue sets were submitted for histopathology in this study. Therefore, a more comprehensive set of tissues should be collected from tawny frogmouths suspected of infection with TwAviAdV-A and disease in the future. Specifically, ventriculus, and multiple sections of the intestines, bursa, kidney, spleen and the lungs should be collected as these have been target organs for other adenovirus infections in birds (Bodewes et al., 2013; Fu et al., 2013; Tomaszewski and Phalen, 2007; Goodwin, 1993; Zhang et al., 2018; Smith, 2018).

A range of outcomes appear to be possible when a tawny frogmouth is infected with TwAviAdV-A. The TwAviAdV-A infection appeared to have killed Cases 1 and 2. However, three birds that tested positive, including one that appeared to have had an adenovirus-associated hepatitis on presentation, survived and were released, demonstrating

that not all infections are lethal and not all infections may result in signs. Ideally, birds shedding adenovirus would be isolated while in care and not released until they stopped shedding virus. Based on the findings of Case 3, virus shedding can occur for several weeks or more (49 days), but based on the findings in Case 4, virus shedding at detectable levels may eventually cease. Repeat screening of infected tawny frogmouths in future studies will be required to confirm this.

Viral infections pose a major, if not insurmountable, biosecurity challenge for wildlife hospitals and wildlife recovery and rehabilitation centres that care for birds. These facilities typically care for many species of birds, and they may be housed and treated in close quarters. As with the TwAviAdV-A, many viruses can sub-clinically infect wildlife (Martens et al., 2020; Zlabravec et al., 2021) and thus the potential for infection of uninfected birds of the same species and *trans*-species infection is high. Preventing bird to bird transmission of viruses requires scrupulous attention to cleanliness and sanitation. Also, isolation of birds suspected to have infectious diseases and housing birds by species rather than a mixed collection is indicated. Failure to prevent virus transmission to a naïve host could have significant ramifications in the treatment and rehabilitation facilities and in wild bird populations if sub-clinically infected birds were released to the wild.

At least two of the tawny frogmouth cases in this study had current infectious diseases. The liver fluke (*Brachylecithum podargi*) (Angel and Pearson, 1977), likely contributed to the chronic active lesions seen in the liver of these two birds. *Brachylecithum podargi* was first described in tawny frogmouths from South Eastern Queensland and its intermediate host is likely to be a snail. Given the potential pathogenicity of *B. podargi*, faeces should be examined from all tawny frogmouths coming into care and birds shedding trematodes eggs should be treated for this infection. Mircofilaria were also seen in the hepatic lesions of the two birds that died but were considered to be incidental findings. Nematodes producing microfilaria are found regularly in many species of birds and are generally not considered to be pathogenic (Villalva--Pasillas et al., 2020).

In conclusion, this study reports a novel aviadenovirus, tentatively named as tawny frogmouth aviadenovirus A causing an outbreak among tawny frogmouths in a wildlife facility. Clinical disease investigation evidenced the characteristics of adenovirus infection and ability of TwAviAdV-A to result in severe clinical signs and death. TwAviAdV-A occupied with all core genes that are common to adenoviruses as well as some unique ORFs and represents a distinct subclade phylogenetically within the aviadenoviruses. Additional studies screening aviadenoviruses among the wild avian population and generating whole genome sequences is recommended to enhance the awareness of aviadenoviruses diversity and evolution.

Institutional review board statement

The material used in this study was submitted for diagnostic purposes. The Animal Ethics Committee at the University of Sydney was informed that findings from the diagnostic material were to be used in a publication and a formal waiver of ethics approval has been granted.

Informed consent statement

Not applicable.

Data availability statement

The sequences and associated data analysed in this have been deposited in NCBI GenBank under the accession number ON642334.

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CRediT authorship contribution statement

Ajani Athukorala: Conceptualization, Methodology, Software, Formal analysis, Investigation, Writing – original draft, preparation, Writing – review & editing, Visualization. **Claude Lacasse:** Investigation, Writing – review & editing. **Jeffrey B. Curtiss:** Investigation, Writing – review & editing. **David N. Phalen:** Conceptualization, Methodology, Software, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, preparation, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition. **Subir Sarker:** Conceptualization, Methodology, Software, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, preparation, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References

Altschu, S., Gish, W., Miller, W., Myers, E., Lipman, D., 1990. Basic local alignment search tool. J. Mol. Biol. 215, 403–410.
Angel, L., Pearson, J., 1977. DICROCOELIID trematodes from AUSTRALIAN hosts. Trans. Roy. Soc. S. Aust. 101, 115–132.
AnimalSpot Tawny Frogmouth. https://www.animalspot.net/tawny-frogmouth.html (accessed 17/04/2023).
Athukorala, A., Forwood, J.K., Phalen, D.N., Sarker, S., 2020. Molecular characterisation of a novel and highly divergent passerine adenovirus 1. Viruses 12 (9).
Athukorala, A., Phalen, D.N., Das, A., Helbig, K.J., Forwood, J.K., Sarker, S., 2021. Genomic characterisation of a highly divergent Siadenovirus (psittacine Siadenovirus F) from the critically endangered orange-bellied parrot (neophema chrysogaster). Viruses 13 (9), 1714.
Athukorala, A., Helbig, K.J., McSharry, B.P., Forwood, J.K., Sarker, S., 2022. Adenoviruses in avian hosts: recent discoveries shed new light on adenovirus diversity and evolution. Viruses 14 (8).
Bankevich, A., Nurk, S., Antipov, D., Gurevich, A.A., Dvorkin, M., Kulikov, A.S., Lesin, V. M., Nikolenko, S.I., Pham, S., Prjibelski, A.D., Pyshkin, A.V., Sirotkin, A.V., Vyahhi, N., Tesler, G., Alekseyev, M.A., Pevzner, P.A., 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J. Comput. Biol. 19 (5), 455–477.
Benkő, M., Aoki, K., Arnberg, N., Davison, A.J., Echavarría, M., Hess, M., Jones, M.S., Kaján, G.L., Kajon, A.E., Mittal, S.K., Podgorski II, Martín, C.S., Wadell, G., Watanabe, H., B, H., 2021. ICTV virus taxonomy profile: adenoviridae 2021. J. Gen. Virol. 103 (3).
Benson, D.A., Cavanaugh, M., Clark, K., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J., Sayers, E.W., 2013. GenBank. Nucleic Acids Research 41, D36–D42. Database issue.
Benson, D.A., Clark, K., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J., Sayers, E.W., 2014. GenBank. Nucleic Acids Res 42, D32–D37. Database issue.
BirdLife-Australia BirdLife Australia - Birds on focus. https://www.calameo.com/read/0041078957045ff95451f?page=68 (accessed 17/04/2023).
Bodewes, R., van de Bildt, M.W., Schapendonk, C.M., van Leeuwen, M., van Boheemen, S., de Jong, A.A., Osterhaus, A.D., Smits, S.L., Kuiken, T., 2013. Identification and characterization of a novel adenovirus in the cloacal bursa of gulls. Virology 440 (1), 84–88.

Boratyn, G.M., Camacho, C., Cooper, P.S., Coulouris, G., Fong, A., Ma, N., Madden, T.L., Matten, W.T., McGinnis, S.D., Merezhuk, Y., Raytselis, Y., Sayers, E.W., Tao, T., Ye, J., Zaretskaya, I., 2013. BLAST: a more efficient report with usability improvements. Nucleic Acids Res. 41, W29–W33. Web Server issue.

Cortes-Hinojosa, G., Adkesson, M.J., Cardenas-Alayza, S., Seguel, M., Paves, H., Wellehan, J.F.X., 2021. Adenovirus diversity in Fur seal and penguin colonies of South America. J. Wildl. Dis. 57 (4), 964–969.

Cserzö, M., Wallin, E., Simon, I., von Heijne, G., A., E., 1997. Prediction of transmembrane alpha-helices in prokaryotic membrane proteins: the dense alignment surface method. Protein Eng. 10 (6), 673–676.

Das, S., Fearnside, K., Sarker, S., Forwood, J.K., Raidal, S.R., 2017. A novel pathogenic aviadenovirus from red-bellied parrots (Poicephalus rufiventris) unveils deep recombination events among avian host lineages. Virology 502, 188–197.

Fu, G., Chen, H., Huang, Y., Cheng, L., Fu, Q., Shi, S., Wan, C., Chen, C., Lin, J., 2013. Full genome sequence of egg drop syndrome virus strain FJ12025 isolated from muscovy duckling. Genome Announc. 1 (4).

Goodwin, M.A., 1993. Adenovirus inclusion body ventriculitis in chickens and captive bobwhite quail (Colinus virginianus). Avian disesases 37, 568–571.

Grzimek, B., 2003. Grzimek's animal life encyclopedia. In: Schlager, N., Grzimek, B., Jeff, T., Hutchins, M., Olendorf, D. (Eds.), Zoology - Encyclopedias. Gale Group, USA.

Guindon, S., Dufayard, J., Lefort, V., Anisimova, M., Hordijk, W., Gascuel, O., 2008. New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0.

Hirokawa, T., Boon-Cheng, S., S, M., 1998. SOSUI: classification and secondary structure prediction system for membrane proteins. Bioinformatics 14 (4), 378–379.

Kajan, G.L., Davison, A.J., Palya, V., Harrach, B., Benko, M., 2012. Genome sequence of a waterfowl aviadenovirus, goose adenovirus 4. J. Gen. Virol. 93 (Pt 11), 2457–2465.

Karamendin, K., Kydyrmanov, A., Fereidouni, S., 2021. High mortality in terns and gulls associated with infection with the novel gull adenovirus. J. Wildl. Dis. 57 (3), 662–666.

Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 30 (4), 772–780.

Kelley, L.A., Mezulis, S., Yates, C.M., Wass, M.N., Sternberg, M.J., 2015. The Phyre2 web portal for protein modeling, prediction and analysis. Nat. Protoc. 10 (6), 845–858.

Kobayashi, H., Uchida, Y., Fujino, K., Horie, M., Umezawa, E., Aihara, N., Kamiie, J., Shimoda, H., Maeda, K., Une, Y., Taharaguchi, S., 2022. Isolation and whole-genome sequencing of a novel aviadenovirus from owls in Japan. Arch. Virol. 167 (3), 829–838.

Krogh, A., Larsson, B., von Heijne, G., Sonnhammer, E.L., 2001. Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. J. Mol. Biol. 305 (3), 567–580.

Kulanayake, S., Tikoo, S.K., 2021. Adenovirus core proteins: structure and function. Viruses 13 (3).

Kumar, R., Kumar, V., Asthana, M., Shukla, S., Chandra, R., 2010. Isolation and identification of a fowl adenovirus from wild black kites (Milvus migrans). J. Wildl. Dis. 46 (1), 272–276.

Lukashev, A.N., Ivanova, O.E., Eremeeva, T.P., Iggo, R.D., 2008. Evidence of frequent recombination among human adenoviruses. J. Gen. Virol. 89 (Pt 2), 380–388.

Marek, A., Kosiol, C., Harrach, B., Kajan, G.L., Schlotterer, C., Hess, M., 2013. The first whole genome sequence of a Fowl adenovirus B strain enables interspecies comparisons within the genus Aviadenovirus. Vet. Microbiol. 166 (1–2), 250–256.

Marek, A., Kaján, G.L., Kosiol, C., Harrach, B., Schlötterer, C., Hess, M., 2014. Complete genome sequences of pigeon adenovirus 1 and duck adenovirus 2 extend the number of species within the genus Aviadenovirus. Virology 462–463, 107–114.

Martens, J.M., Stokes, H.S., Berg, M.L., Walder, K., Raidal, S.R., Magrath, M.J.L., Bennett, A.T.D., 2020. Beak and feather disease virus (BFDV) prevalence, load and excretion in seven species of wild caught common Australian parrots. PLoS One 15 (7), e0235406.

Martin, D.P., Lemey, P., Lott, M., Moulton, V., Posada, D., Lefeuvre, P., 2010. RDP3: a flexible and fast computer program for analyzing recombination. Bioinformatics 26 (19), 2462–2463.

Milani, A., Zamperin, G., Fusaro, A., Salviato, A., Bano, L., Zandonà, L., Brunetta, R., Monne, I., 2018. Complete genome sequence of psittacine adenovirus 1, identified from Poicephalus senegalus in Italy. Microbiol Resour Announc 7 (11).

Niczyporuk, J.S., Kozdrun, W., Czekaj, H., Stys-Fijol, N., Piekarska, K., 2020. Detection of fowl adenovirus D strains in wild birds in Poland by Loop-Mediated Isothermal Amplification (LAMP). BMC Vet. Res. 16 (1), 58.

Pallister, J., Wright, P.J., Sheppard, A., 1996. A single gene encoding the fiber is responsible forVariations in virulence in the fowl adenoviruses. J. Virol. 70 (8), 5115–5122.

Pénzes, J.J., Menéndez-Conejero, R., Condezo, G.N., Ball, I., Papp, T., Doszpoly, A., Paradela, A., Pérez-Berná, A.J., López-Sanz, M., Nguyen, T.H., van Raaij, M.J., Marschang, R.E., Harrach, B., Benkő, M., San Martín, C., 2014. Molecular characterization of a lizard adenovirus reveals the first atadenovirus with two fiber genes and the first adenovirus with either one short or three long fibers per penton. J. Virol. 88 (19), 11304–11314.

Reece, R.L., Pass, D.A., Butler, R., 1985. Inclusion body hepatitis in a tawny frogmouth (Podargus strigoides: caprimulgiformes). Aust. Vet. J. 62 (12), 426.

Rinder, M., Schmitz, A., Baas, N., Korbel, R., 2020. Molecular identification of novel and genetically diverse adenoviruses in Passeriform birds. Virus Gene. 56 (3), 316–324.

Sarker, S., Das, S., Lavers, J.L., Hutton, I., Helbig, K., Imbery, J., Upton, C., Raidal, S.R., 2017a. Genomic characterization of two novel pathogenic avipoxviruses isolated from pacific shearwaters (Ardenna spp.). BMC Genom. 18 (1), 298.

Sarker, S., Roberts, H.K., Tidd, N., Ault, S., Ladmore, G., Peters, A., Forwood, J.K., Helbig, K., Raidal, S.R., 2017b. Molecular and microscopic characterization of a novel Eastern grey kangaroopox virus genome directly from a clinical sample. Sci. Rep. 7 (1), 16472.

Schachner, A., Marek, A., Jaskulska, B., Bilic, I., Hess, M., 2014. Recombinant FAdV-4 fiber-2 protein protects chickens against hepatitis-hydropericardium syndrome (HHS). Vaccine 32 (9), 1086–1092.

Schachner, A., Matos, M., Grafl, B., Hess, M., 2018. Fowl adenovirus-induced diseases and strategies for their control - a review on the current global situation. Avian Pathol. 47 (2), 111–126.

Smith, D.A., 2018. Palaeognathae: apterygiformes, casuariiformes, rheiformes, struthioniformes; tinamiformes. In: Pathology of Wildlife and Zoo Animals, pp. 635–651.

Sutherland, M., Sarker, S., Vaz, P.K., Legione, A.R., Devlin, J.M., Macwhirter, P.L., Whiteley, P.L., Raidal, S.R., 2019. Disease surveillance in wild Victorian cacatuids reveals co-infection with multiple agents and detection of novel avian viruses. Vet. Microbiol. 235, 257–264.

To, K.K., Tse, H., Chan, W.M., Choi, G.K., Zhang, A.J., Sridhar, S., Wong, S.C., Chan, J.F., Chan, A.S., Woo, P.C., Lau, S.K., Lo, J.Y., Chan, K.H., Cheng, V.C., Yuen, K.Y., 2014. A novel psittacine adenovirus identified during an outbreak of avian chlamydiosis and human psittacosis: zoonosis associated with virus-bacterium coinfection in birds. PLoS Neglected Trop. Dis. 8 (12), e3318.

Tomaszewski, E.K., Phalen, D.N., 2007. Falcon adenovirus in an American kestrel (Falco sparverius). J. Avian Med. Surg. 21 (2), 135–139.

Tusnady, G.E., Simon, I., 2001. The HMMTOP transmembrane topology prediction server. Bioinformatics 17 (9), 849–850.

Vaz, F.F., Raso, T.F., Agius, J.E., Hunt, T., Leishman, A., Eden, J.S., Phalen, D.N., 2020. Opportunistic sampling of wild native and invasive birds reveals a rich diversity of adenoviruses in Australia. Virus Evol 6 (1) veaa024.

Vereecken, M., de Herdt, P., Ducatelle, R., 1998. Adenovirus infections in pigeons: a review. Avian Pathol. 27 (4), 333–338.

Villalva-Pasillas, D., Medina, J.P., Soriano-Vargas, E., Martinez-Hernandez, D.A., Garcia-Conejo, M., Galindo-Sanchez, K.P., Sanchez-Jasso, J.M., Talavera-Rojas, M., Salgado-Miranda, C., 2020. Haemoparasites in endemic and non-endemic passerine birds from central Mexico highlands. Int J Parasitol Parasites Wildl 11, 88–92.

Wang, Z., Zhao, J., 2019. Pathogenesis of hypervirulent fowl adenovirus serotype 4: the Contributions of viral and host factors. Viruses 11 (8).

Wang, W., Liu, Q., Li, T., Geng, T., Chen, H., Xie, Q., Shao, H., Wan, Z., Qin, A., Ye, J., 2020. Fiber-1, not fiber-2, directly mediates the infection of the pathogenic serotype 4 fowl adenovirus via its shaft and knob domains. J. Virol. 94 (17).

Wellehan, J.F., Johnson, A.J., Harrach, B., Benko, M., Pessier, A.P., Johnson, C.M., Garner, M.M., Childress, A., Jacobson, E.R., 2004. Detection and analysis of six lizard adenoviruses by consensus primer PCR provides further evidence of a reptilian origin for the atadenoviruses. J. Virol. 78 (23), 13366–13369.

Yang, N., McLelland, J., McLelland, D.J., Clarke, J., Woolford, L., Eden, P., Phalen, D.N., 2019. Psittacid Adenovirus-2 infection in the critically endangered orange-bellied parrot (Neophema chrysogastor): a key threatening process or an example of a host-adapted virus? PLoS One 14 (2), e0208674.

Zhang, Y., Liu, R., Tian, K., Wang, Z., Yang, X., Gao, D., Zhang, Y., Fu, J., Wang, H., Zhao, J., 2018. Fiber2 and hexon genes are closely associated with the virulence of the emerging and highly pathogenic fowl adenovirus 4. Emerg. Microb. Infect. 7 (1), 199.

Zimmermann, L., Stephens, A., Nam, S.Z., Rau, D., Kubler, J., Lozajic, M., Gabler, F., Soding, J., Lupas, A.N., Alva, V., 2018. A completely reimplemented MPI bioinformatics toolkit with a new HHpred server at its core. J. Mol. Biol. 430 (15), 2237–2243.

Zlabravec, Z., Trilar, T., Slavec, B., Krapez, U., Vrezec, A., Rojs, O.Z., Racnik, J., 2021. Detection of herpesviruses in passerine birds captured during autumn migration in Slovenia. J. Wildl. Dis. 57 (2), 368–375.