

Incursion of Novel Eurasian Low Pathogenicity Avian Influenza H5 Virus, Australia, 2023

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Australia is a sink for low pathogenicity avian influenza viruses, with isolated circulation occurring on the continent. We report the incursion of a Eurasian low pathogenicity avian influenza H5 virus into Australia. This report benefits surveillance and diagnostic work because of the risk and current absence of highly pathogenic avian influenza A(H5N1).

Australia is a sink for the diversity of avian influenza viruses, wherein viruses circulating in Eurasia and North America are occasionally introduced to the continent, followed by long-term, isolated circulation (1). Whereas a diversity of low pathogenicity avian influenza (LPAI) viruses exist, Oceania is the only continent where the goose/Guangdong lineage of highly pathogenic avian influenza (HPAI) A(H5N1) is absent (2). An HPAI incursion has never been detected in Oceania, although several HPAI outbreaks have occurred in poultry, all following evolution of endemic Australian lineage LPAI H7 viruses from the wild bird reservoir (3). Because of the risk to poultry and wild birds in Australia, a key aim of the National Avian Influenza in Wild Birds (NAIWB) surveillance program is the detection and characterization of circulating H5 and H7 viruses (4).

The Study

In 2023, through both the NAIWB targeted surveillance (4) and passive surveillance, we detected 17 LPAI H5 viruses by quantitative PCR (qPCR) and recovered 8 genomes. The genomes comprised 7 complete or near-complete genomes and 1 partial genome, which included hemagglutinin (HA), nucleoprotein, neuraminidase (NA), matrix, and nonstructural protein (NS) segments. We recovered viral HA fragments from 2 additional detections that identified lineage only (Table). We collected, screened, and sequenced samples as described in previous publications (1). We deposited the sequences into GenBank (GenBank accession nos. PP947529–52 and PP922943–79).

The HA segments of LPAI H5 viruses we sequenced were unrelated to LPAI H5 viruses previously characterized in Australia. The HA sequences had a PQRETR/GLF cleavage site, which contrasts with the PQKATR/GLF cleavage site found in all LPAI H5 viruses reported in Australia since 2005 (1). The detections we found all belonged to a single Eurasian-lineage LPAI H5 that was detected in 4 Australia jurisdictions over a 7-month period. We detected the first occurrence of the LPAI H5 in April 2023 (Figure

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Table. Genome constellations for each virus sequenced in the study of an incursion of novel Eurasian low pathogenicity avian influenza H5 virus, Australia, 2023*

Virus designation	Date	PB2	PB1	PA	HA	NP	NA	M	NS
A/chestnut teal/Victoria/23-01686-0034/2023 (H5N3)	2023 Apr 26	Aus	Aus (B)	Aus	H5-EUR-novel	Aus	N3-Aus	Aus (A)	A-Aus
A/Pacific black duck/Victoria/23-01686-0039/2023 (H5N3)	2023 Apr 26	Aus	Aus (A)	Aus	H5-EUR-novel	Aus	N3-Aus	Aus (A)	B-Aus
A/gray teal/Victoria/23-01688-0045/2023 (mixed)†	2023 Apr 26	Aus	Aus (A)	Aus	H5-EUR-novel	Aus	N3-Aus/ N7-Aus	Aus (A)	B-Aus
A/Pacific Black Duck/Victoria/17363/2023 (H5N3)	2023 Jun 22	Aus	Aus (A)	Aus	H5-EUR-novel	Aus‡	N3-Aus	Aus (A)	A-Aus
A/wild waterbird/Queensland/ P23-02457-48/2023 (H5N3)	2023 Jun 23	Aus	Aus (C)	Aus	H5-EUR-novel	Aus	N3-Aus	Aus (B)	A-Aus
A/wild waterbird/Queensland/ P23-02457-51/2023 (H5N3)	2023 Jun 23	Aus	Aus (C)	Aus	H5-EUR-novel	Aus	N3-Aus	Aus (B)	A-Aus
A/wild waterbird/South Australia/23-80999145-13/2023 (H5N9)	2023 Jul 31	Aus	Aus (C)	Aus	H5-EUR-novel	Aus‡	N9-Aus	Aus (A)	B-Aus
A/Radjah Shelduck/Northern Territory/ 20231282-03/2023 (H5N1)	2023 Oct 30	ND	ND	ND	H5-EUR-novel	Aus‡	N1-Aus	Aus (A)	A-Aus

*In cases where viruses fell into different Australian lineages we added an indicatory letter in parentheses to show the different lineages. In the case of NS, whether the virus fell into the A or B allele is indicated before the lineage name. (e.g., B-Aus for an Australian lineage in the B allele). Similarly, for the neuraminidase subtype information is incorporated (e.g., N1-Aus for an Australian lineage in the N1). Aus, established Australian clade; EUR-novel, novel incursion from Eurasian lineage; HA, hemagglutinin; M, matrix protein; ND, no sequence data available; NA, neuraminidase; NP, nucleoprotein; NS, nonstructural protein; PA, polymerase acidic; PB, polymerase basic.

†A/gray teal/Victoria/23-01688-0045/2023 comprised H5 and H10, and N3 and N7.

‡Cases where viruses fell into the same Australian lineage but were not sister to each other.

1) in Victoria from 3 hunter shot waterfowl: 1 Pacific black duck (*Anas superciliosa*), 1 gray teal (*Anas gracilis*), and 1 chestnut teal (*Anas castanea*). In June 2023, we detected another LPAI H5 occurrence in Victoria in a live-captured Pacific black duck and 2 LPAI H5 occurrences in Queensland from wild waterbird fecal environmental samples. We detected another LPAI H5 occurrence in July 2023 from wild waterbird fecal samples in South Australia. Finally, we detected 1 LPAI H5 virus in a wild duck in October 2023 through passive surveillance in the Northern Territory.

Samples collected from live birds were done in accordance with Deakin University Animal Ethics (ethics approval no. B39-2019). Ethics approvals were not required for fecal environmental samples, samples collected from hunter-shot birds, or samples collected for disease investigations.

We conducted BLASTn analysis (<https://blast.ncbi.nlm.nih.gov>) by using the National Center for Biotechnology Information nucleotide database. We conducted phylogenetic analysis of the HA sequences and found the sequenced viruses

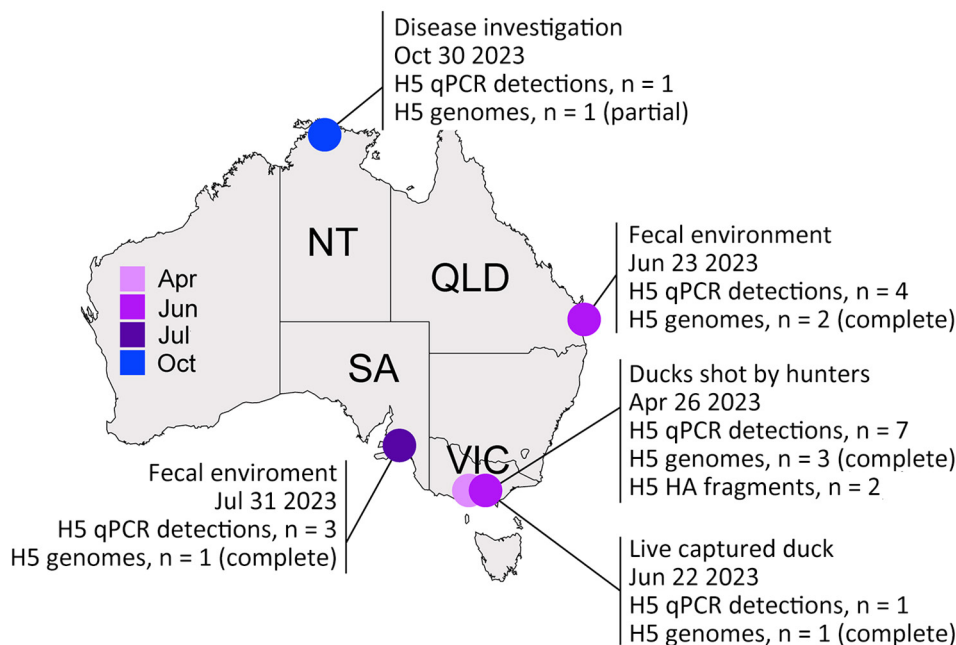


Figure 1. Locations of Eurasian lineage low pathogenic avian influenza H5 virus detections in Australia, 2023. Information is provided about the location, sample type, and date of H5 detections. NT, Northern Territory; QLD, Queensland; SA, South Australia; VIC, Victoria.

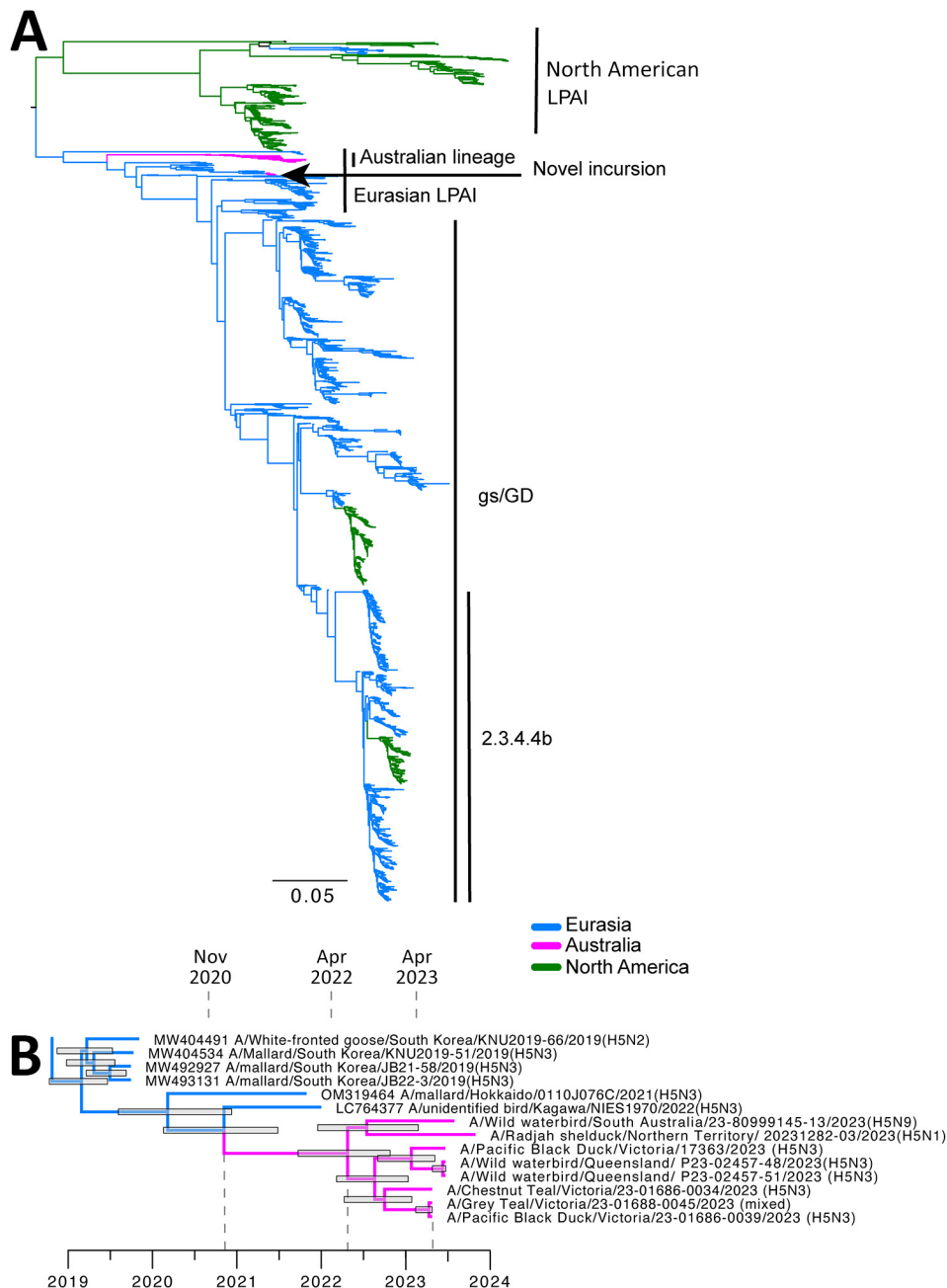


Figure 2. Phylogenetic analysis of Eurasian lineage LPAI H5 virus detected in Australia, 2023. A) Maximum-likelihood phylogenetic tree of all H5 sequences from Asia, North America, and Oceania since 2010. Major clades have been highlighted. The arrow indicates the novel lineage detection in Australia. The tree has been rooted between the North American and Eurasian lineages. Scale bar indicates number of substitutions per site. B) Cropped time-structured phylogenetic tree. Dashed lines indicate the date of divergence from GenBank reference sequences, the most recent common ancestor of all Australian sequences, and the date of first detection. Node bars comprise the 95% highest posterior density. Scale bar is time in years. gs/GD, goose/Guangdong lineage of highly pathogenic avian influenza A(H5N1); LPAI, low pathogenicity avian influenza.

were most closely related ($\leq 98\%$) to LPAI H5 viruses detected during 2019–2022 in Japan and South Korea (Figures 1, 2). Those countries contribute most influenza genomes recovered from the East Asian–Australasian databases. Alignments and .xml files for all trees are available at <https://github.com/michellewille2/Eurasian-LPAI-H5-incursion-to-Australia>. We investigated the potential temporal incursion window in Australia by using time-structured phylogenetic analysis, as outlined in previous publications (1). The most recent common

ancestor of the 8 complete HA sequences was from April 2022 (95% highest posterior density [HPD] September 2021–October 2022), almost a year before the first detection, but with a large HPD (Figure 1). To examine H5 activity in the previous year, we queried all H5 detections for 2022 reported to the NAIWB surveillance program and found 12 LPAI H5 viruses were detected by qPCR in wild bird samples (New South Wales, $n = 3$; Tasmania, $n = 1$; Victoria, $n = 5$; Western Australia, $n = 3$) (5). Of the viruses sequenced, all HA sequences fell into the previously described

Australian LPAI H5 lineage that has been circulating since 2007, although it was likely present since the 1990s (1). Hence, cryptic circulation of the novel Eurasian lineage LPAI H5 in Australia may have occurred in locations or in avian hosts that are not included in NAIWB surveillance and therefore were not detected in 2022. Alternatively, incursion might have only occurred in early 2023, but rather than a single viral introduction, multiple viruses with closely related HA sequences were separately introduced. Unfortunately, the scarcity of available HA reference sequences from 2022 and 2023 from Asia because of limitations in wild bird surveillance or sequence deposition into GenBank hinders understanding of introduction dynamics.

We investigated the reassortment patterns in the segments to gain additional insight. All other gene segments fall into viral lineages already present in Australia (Table; Appendix Figure, Tables 1, 2, <http://wwwnc.cdc.gov/EID/article/30/12/24-0919-App1.pdf>). Genome constellations were partially conserved, and all viruses shared the same polymerase basic (PB) 1, polymerase acidic, nucleoprotein, and matrix segment lineages, although within-clade segments were not always 100% identical (Appendix Figure). We detected 3 different PB1 lineages and 2 different NS lineages (both the A and B alleles). We obtained full genomes of 4 viruses from wild birds in Victoria, 3 from the same sampling event. Of the full genomes obtained, 2 had identical genome constellations, although A/gray teal/Victoria/23-01688-0045/2023 was a mixed infection with H5, H10, N3 and N7 segments recovered. Co-sampled A/chestnutTeal/Victoria/23-01686-0034/2023(H5N3) was different in both the PB1 and NS sequence compared with the other 2 viruses. A/Pacific black duck/Victoria/17363/2023(H5N3), which was detected 2 months after the initial detections in Victoria, had a similar genome constellation to the other sequences from Victoria, despite having an HA sequence sister to those from Queensland (detected June 2023), rather than those from Victoria. A/wild waterbird/South Australia/23-80999145-13/2023(H5N9) had a different PB1 and NA sequence, and A/radjah shelduck/Northern Territory/20231282-03/2023(H5N1) also had a different NA subtype. The 2 genomes from Queensland shared the same PB1 sequence as A/wild waterbird/South Australia/23-80999145-13/2023(H5N9) but had unique M segments. A reasonable explanation for those results is a single introduction followed by a reassortment event incorporating 7 Australian-lineage segments. As the virus spread across Australia, additional reassortment events contributed to the genome constellation diversity (i.e., diversity in the PB1, NA, and NS segments).

Conclusions

There are 3 implications of this viral incursion. First, the delayed detection highlights limitations in the national targeted surveillance program that, in combination with investigation of consequential wild bird illness and death events, is necessary to rapidly detect and respond to an incursion of goose/Guangdong HPAI H5N1 into Australia. Second, while the KATR cleavage site found in Australian-lineage viruses has not yet been associated with evolution of LPAI to HPAI, the RETR cleavage site in the novel Eurasian lineage incursion has been identified as a precursor to HPAI H5 viruses (6). This incursion may increase the risk for evolution of HPAI H5 in Australia. Finally, as part of the surveillance and diagnostic processes, H5 qPCRs are designed to rapidly differentiate LPAI H5 of Australian lineage from Eurasian lineage LPAI and HPAI. The presence of a novel LPAI lineage requires a diagnostic update. Understanding viral incursions is a global concern, as demonstrated by the numerous incursions and intercontinental spread of HPAI H5N1 clade 2.3.4.4b. Determining the patterns of viral incursion and spread and identifying the hosts involved is necessary for effective disease risk management and communication that supports disease preparedness and response.

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About the author

Dr. Wille is a senior research fellow at the Centre for Pathogen Genomics, University of Melbourne. Her interests include viruses of birds, ecology and evolution of avian influenza, and ecology of bird viral communities.

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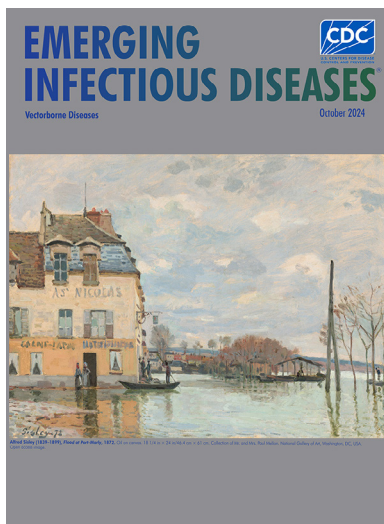
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Appendix.

Appendix Table 1. Top 5 Blast hits for neuraminidase segments against the NCBI nt database (September 2024)

NA Subtype	Designation	Top blast hit	% identity
N1	A/Radjah Shelduck/Northern Territory/20231282-03/2023(H5N1)	OL370361 A/wild waterbird/South Australia/19-9013889-61/2019(mixed)	98.01%
		OL370353 A/wild waterbird/South Australia/19-9013889-37/2019(N1)	98.01%
		OL371577 A/wild waterbird/South Australia/19-9013889-35/2019(H1N1)	97.94%
		OL371593 A/wild waterbird/South Australia/19-9013889-43/2019(H7N1)	97.94%
		OL370347 A/wild waterbird/South Australia/19-9013889-13/2019(N1)	97.87%
N3	A/Chestnut Teal/Victoria/23-01686-0034/2023 (H5N3)	PP549216 A/emu/Victoria/21-03712/2021(H10N3)	98.23%
		PP549221 A/wild waterfowl/Western Australia/AS-22-0673-0006/2022(H10N3)	98.16%
		OL370601 A/grey teal/South Australia/18-2812337-47/2018(H1N3)	97.87%
		OL370585 A/grey teal/South Australia/17-3278836-7/2017(H9N3)	97.59%
		OL371081 A/wild duck/Queensland/P20-03375-0084-01/2020(H1N3)	97.45%
	A/Pacific Black Duck/Victoria/23-01686-0039/2023 (H5N3)	PP549216 A/emu/Victoria/21-03712/2021(H10N3)	98.58%
		PP549221 A/wild waterfowl/Western Australia/AS-22-0673-0006/2022(H10N3)	98.51%
		OL370601 A/grey teal/South Australia/18-2812337-47/2018(H1N3)	98.23%
		OL370585 A/grey teal/South Australia/17-3278836-7/2017(H9N3)	97.94%
		OL371081 A/wild duck/Queensland/P20-03375-0084-01/2020(H1N3)	97.80%
	A/Grey Teal/Victoria/23-01688-0045/2023 (mixed)	PP549216 A/emu/Victoria/21-03712/2021(H10N3)	98.23%
		PP549221 A/wild waterfowl/Western Australia/AS-22-0673-0006/2022(H10N3)	98.16%
		OL370601 A/grey teal/South Australia/18-2812337-47/2018(H1N3)	97.87%
		OL370585 A/grey teal/South Australia/17-3278836-7/2017(H9N3)	97.59%
		OL371081 A/wild duck/Queensland/P20-03375-0084-01/2020(H1N3)	97.45%
	A/Pacific Black Duck/Victoria/17363/2023 (H5N3)	PP549216 A/emu/Victoria/21-03712/2021(H10N3)	98.23%
		PP549221 A/wild waterfowl/Western Australia/AS-22-0673-0006/2022(H10N3)	98.16%
		OL370601 A/grey teal/South Australia/18-2812337-47/2018(H1N3)	97.87%
		OL370585 A/grey teal/South Australia/17-3278836-7/2017(H9N3)	97.59%
		OL371081 A/wild duck/Queensland/P20-03375-0084-01/2020(H1N3)	97.45%

NA Subtype	Designation	Top blast hit	% identity
	A/wild waterbird/Queensland/ P23-02457-48/2023(H5N3)	PP549216 A/emu/Victoria/21-03712/2021(H10N3)	98.30%
		PP549221 A/wild waterfowl/Western Australia/AS-22-0673-0006/2022(H10N3)	98.23%
		OL370601 A/grey teal/South Australia/18-2812337-47/2018(H1N3)	97.94%
		OL370585 A/grey teal/South Australia/17-3278836-7/2017(H9N3)	97.66%
		OL371081 A/wild duck/Queensland/P20-03375-0084-01/2020(H1N3)	97.52%
	A/wild waterbird/Queensland/ P23-02457-51/2023 (H5N3)	PP549216 A/emu/Victoria/21-03712/2021(H10N3)	98.30%
		PP549221 A/wild waterfowl/Western Australia/AS-22-0673-0006/2022(H10N3)	98.23%
		OL370601 A/grey teal/South Australia/18-2812337-47/2018(H1N3)	97.94%
		OL370585 A/grey teal/South Australia/17-3278836-7/2017(H9N3)	97.66%
		OL371081 A/wild duck/Queensland/P20-03375-0084-01/2020(H1N3)	97.52%
N7 A/Grey Teal/Victoria/23-01688-0045/2023 (mixed)	OL370323 A/wild duck/New South Wales/M19-11593-66/2019(H7N7)	97.88%	
	PP549421 A/chicken/New South Wales/M21-10080-0006/2021(H10N7)	97.53%	
	OL370529 A/Pacific black duck/Victoria/18-01589-232/2018(H7N7)	97.53%	
	PP549261 A/Grey teal/South Australia/22-64233564-17/2022(H10N7)	96.96%	
	PP549293 A/Grey teal/Victoria/15848/2022(H10N7)	96.82%	
N9 A/wild waterbird/South Australia/23-80999145-13/2023(H5N9)	OR681022 A/Wild waterfowl/Australia/22-04155-0048/2022(H11N9)	99.79%	
	OL370420 A/wild waterbird/Queensland/JCU-272/2019(H5N9)	97.81%	
	OL370617 A/grey teal/Victoria/10657/2017(H11N9)	97.17%	
	OL371681 A/wild waterbird/Victoria/19-00581-009/2019(H11N9)	97.10%	
	OL370448 A/Pacific black duck/Victoria/11889/2019(H11N9)	97.03%	

Appendix Table 2. Top 5 Blast hits for all internal segments against the NCBI nt database (September 2024)

Segment	Designation	Top blast hit	% identity
PB2	A/Chestnut Teal/Victoria/23-01686-0034/2023 (H5N3)	PP549211 A/emu/Victoria/21-03712/2021(H10N3)	98.77%
		PP549416 A/chicken/New South Wales/M21-10080-0006/2021(H10N7)	98.73%
		PP549264 A/Grey teal/South Australia/22-64233564-20/2022(H4N6)	98.64%
		OQ201826 A/Wild waterfowl/Australia/22-02927-0052/2022(H4N6)	98.46%
		PP549400 A/Tawny frogmouth/Western Australia/AS-21-1823-0007/2021(H10N7)	98.38%
	A/Pacific Black Duck/Victoria/23-01686-0039/2023 (H5N3)	PP549264 A/Grey teal/South Australia/22-64233564-20/2022(H4N6)	99.12%
		PP549416 A/chicken/New South Wales/M21-10080-0006/2021(H10N7)	98.33%
		PP549211 A/emu/Victoria/21-03712/2021(H10N3)	98.29%
		OQ201826 A/Wild waterfowl/Australia/22-02927-0052/2022(H4N6)	98.07%
		PP549400 A/Tawny frogmouth/Western Australia/AS-21-1823-0007/2021(H10N7)	97.98%
A/Grey Teal/Victoria/23-01688-0045/2023 (mixed)	PP549264 A/Grey teal/South Australia/22-64233564-20/2022(H4N6)	99.21%	
	PP549416 A/chicken/New South Wales/M21-10080-0006/2021(H10N7)	98.42%	
	PP549211 A/emu/Victoria/21-03712/2021(H10N3)	98.38%	
	OQ201826 A/Wild waterfowl/Australia/22-02927-0052/2022(H4N6)	98.16%	

Segment	Designation	Top blast hit	% identity
		PP549400 A/Tawny frogmouth/Western Australia/AS-21-1823-0007/2021(H10N7)	98.07%
	A/Pacific Black Duck/Victoria/17363/2023 (H5N3)	PP549416 A/chicken/New South Wales/M21-10080-0006/2021(H10N7)	98.55%
		PP549211 A/emu/Victoria/21-03712/2021(H10N3)	98.51%
		PP549264 A/Grey teal/South Australia/22-64233564-20/2022(H4N6)	98.46%
		OQ201826 A/Wild waterfowl/Australia/22-02927-0052/2022(H4N6)	98.29%
		ON505898 A/Mallard(Anas platyrhynchos)/South Korea/KNU2021-49/2021(H7N7)	98.25%
	A/wild waterbird/Queensland/ P23-02457-48/2023(H5N3)	PP549240 A/Chestnut teal/Victoria/16148/2022(H10N7)	98.90%
		OR681027 A/Wild waterfowl/Australia/22-04155-0048/2022(H11N9)	98.77%
		PP549211 A/emu/Victoria/21-03712/2021(H10N3)	98.64%
		PP549416 A/chicken/New South Wales/M21-10080-0006/2021(H10N7)	98.55%
		OQ201826 A/Wild waterfowl/Australia/22-02927-0052/2022(H4N6)	98.33%
	A/wild waterbird/Queensland/ P23-02457-51/2023 (H5N3)	PP549240 A/Chestnut teal/Victoria/16148/2022(H10N7)	98.90%
		OR681027 A/Wild waterfowl/Australia/22-04155-0048/2022(H11N9)	98.77%
		PP549211 A/emu/Victoria/21-03712/2021(H10N3)	98.64%
		PP549416 A/chicken/New South Wales/M21-10080-0006/2021(H10N7)	98.55%
		OQ201826 A/Wild waterfowl/Australia/22-02927-0052/2022(H4N6)	98.33%
	A/wild waterbird/South Australia/23-80999145-13/2023(H5N9)	OR681027 A/Wild waterfowl/Australia/22-04155-0048/2022(H11N9)	99.47%
		PP549240 A/Chestnut teal/Victoria/16148/2022(H10N7)	98.99%
		PP549416 A/chicken/New South Wales/M21-10080-0006/2021(H10N7)	98.68%
		PP549211 A/emu/Victoria/21-03712/2021(H10N3)	98.64%
		OQ201826 A/Wild waterfowl/Australia/22-02927-0052/2022(H4N6)	98.51%
PB1	A/Chestnut Teal/Victoria/23-01686-0034/2023 (H5N3)	OR681026 A/Wild waterfowl/Australia/22-04155-0048/2022(H11N9)	98.11%
		OL370533 A/Pacific black duck/Victoria/19-01749-033/2019(H10N5)	97.93%
		OL371677 A/wild waterbird/Victoria/19-00581-009/2019(H11N9)	97.93%
		OL371661 A/wild waterbird/Tasmania/20-2052-11/2020(H1N6)	97.89%
		OL371093 A/wild duck/Queensland/P20-03375-0183-01/2020(H6N1)	97.89%
	A/Pacific Black Duck/Victoria/23-01686-0039/2023 (H5N3)	OL370356 A/wild waterbird/South Australia/19-9013889-61/2019(mixed)	98.77%
		OL370343 A/wild waterbird/South Australia/19-9013889-13/2019(mixed)	98.77%
		OL371565 A/wild waterbird/South Australia/19-9013889-31/2019(H7N1)	98.68%
		OL371589 A/wild waterbird/South Australia/19-9013889-43/2019(H7N1)	98.68%
		OL371485 A/wild waterbird/South Australia/17-6661506-14/2017(H7N5)	97.98%
	A/Grey Teal/Victoria/23-01688-0045/2023 (mixed)	OL370356 A/wild waterbird/South Australia/19-9013889-61/2019(mixed)	98.55%
		OL370343 A/wild waterbird/South Australia/19-9013889-13/2019(mixed)	98.55%
		OL371565 A/wild waterbird/South Australia/19-9013889-31/2019(H7N1)	98.46%
		OL371589 A/wild waterbird/South Australia/19-9013889-43/2019(H7N1)	98.46%
		OL371485 A/wild waterbird/South Australia/17-6661506-14/2017(H7N5)	97.85%
	A/Pacific Black Duck/Victoria/17363/2023 (H5N3)	OL370356 A/wild waterbird/South Australia/19-9013889-61/2019(mixed)	98.55%

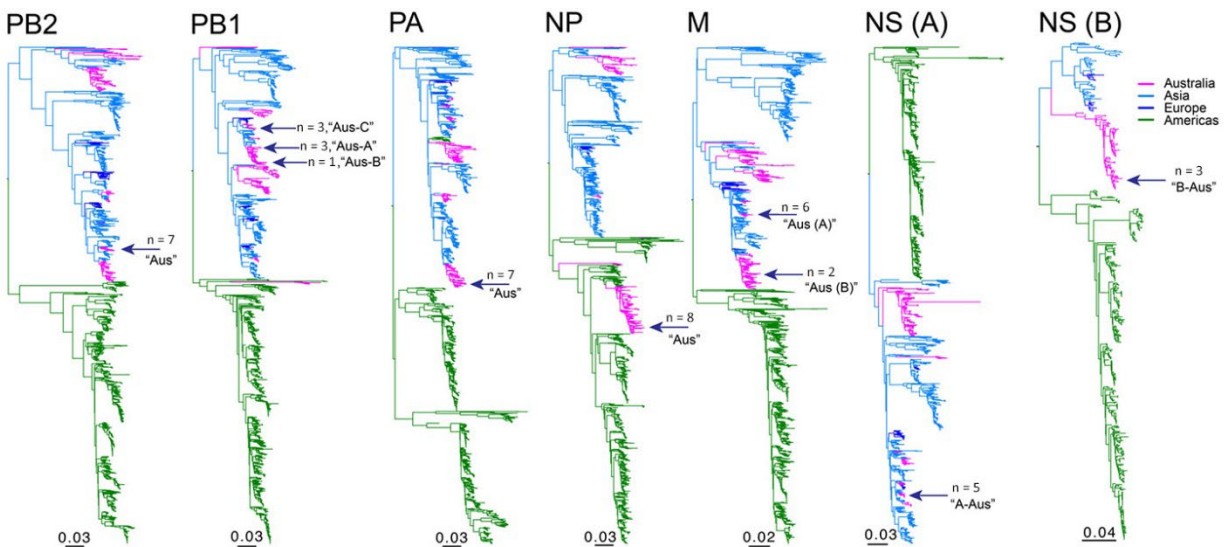
Segment	Designation	Top blast hit	% identity
		OL370343 A/wild waterbird/South Australia/19-9013889-13/2019(mixed)	98.55%
		OL371565 A/wild waterbird/South Australia/19-9013889-31/2019(H7N1)	98.46%
		OL371589 A/wild waterbird/South Australia/19-9013889-43/2019(H7N1)	98.46%
		OL371485 A/wild waterbird/South Australia/17-6661506-14/2017(H7N5)	97.85%
	A/wild waterbird/Queensland/ P23-02457-48/2023(H5N3)	PP549241 A/Chestnut teal/Victoria/16148/2022(H10N7)	99.08%
		OQ201827 A/Wild waterfowl/Australia/22-02927-0052/2022(H4N6)	98.90%
		PP549417 A/chicken/New South Wales/M21-10080-0006/2021(H10N7)	98.72%
		ON495895 A/Bean Goose(Anser fabalis)/South Korea/KNU2021-42/2021(H1N1)	98.42%
		OK217082 A/Wild Duck/South Korea/KNU2020-11/2020(H4N6)	98.28%
	A/wild waterbird/Queensland/ P23-02457-51/2023 (H5N3)	PP549241 A/Chestnut teal/Victoria/16148/2022(H10N7)	99.08%
		OQ201827 A/Wild waterfowl/Australia/22-02927-0052/2022(H4N6)	98.90%
		PP549417 A/chicken/New South Wales/M21-10080-0006/2021(H10N7)	98.72%
		ON495895 A/Bean Goose(Anser fabalis)/South Korea/KNU2021-42/2021(H1N1)	98.42%
		OK217082 A/Wild Duck/South Korea/KNU2020-11/2020(H4N6)	98.28%
	A/wild waterbird/South Australia/23-80999145-13/2023(H5N9)	PP549241 A/Chestnut teal/Victoria/16148/2022(H10N7)	99.08%
		OQ201827 A/Wild waterfowl/Australia/22-02927-0052/2022(H4N6)	98.90%
		PP549417 A/chicken/New South Wales/M21-10080-0006/2021(H10N7)	98.72%
		ON495895 A/Bean Goose(Anser fabalis)/South Korea/KNU2021-42/2021(H1N1)	98.42%
		OK217082 A/Wild Duck/South Korea/KNU2020-11/2020(H4N6)	98.28%
PA	A/Chestnut Teal/Victoria/23-01686-0034/2023 (H5N3)	PP549274 A/Grey teal/South Australia/22-64233564-37/2022(H10N2)	99.12%
		PP549258 A/Grey teal/South Australia/22-64233564-17/2022(H10N7)	99.02%
		OL371678 A/wild waterbird/Victoria/19-00581-009/2019(H11N9)	98.51%
		PP549250 A/Grey teal/New South Wales/14340X/2020(H4N6)	98.42%
	A/Pacific Black Duck/Victoria/23-01686-0039/2023 (H5N3)	OL370510 A/Pacific black duck/Victoria/11840/2019(H2N1)	98.33%
		PP549274 A/Grey teal/South Australia/22-64233564-37/2022(H10N2)	98.65%
		PP549258 A/Grey teal/South Australia/22-64233564-17/2022(H10N7)	98.56%
		OL371678 A/wild waterbird/Victoria/19-00581-009/2019(H11N9)	98.33%
		OL370510 A/Pacific black duck/Victoria/11840/2019(H2N1)	98.14%
		PP549250 A/Grey teal/New South Wales/14340X/2020(H4N6)	98.09%
	A/Grey Teal/Victoria/23-01688-0045/2023 (mixed)	PP549274 A/Grey teal/South Australia/22-64233564-37/2022(H10N2)	98.65%
		PP549258 A/Grey teal/South Australia/22-64233564-17/2022(H10N7)	98.56%
		OL371678 A/wild waterbird/Victoria/19-00581-009/2019(H11N9)	98.33%
		OL370510 A/Pacific black duck/Victoria/11840/2019(H2N1)	98.14%
		PP549250 A/Grey teal/New South Wales/14340X/2020(H4N6)	98.09%
	A/Pacific Black Duck/Victoria/17363/2023 (H5N3)	PP549274 A/Grey teal/South Australia/22-64233564-37/2022(H10N2)	98.61%
		PP549258 A/Grey teal/South Australia/22-64233564-17/2022(H10N7)	98.51%
		OL371678 A/wild waterbird/Victoria/19-00581-009/2019(H11N9)	98.19%

Segment	Designation	Top blast hit	% identity
		OL370510 A/Pacific black duck/Victoria/11840/2019(H2N1)	98.00%
		PP549250 A/Grey teal/New South Wales/14340X/2020(H4N6)	98.00%
A/wild waterbird/Queensland/ P23-02457-48/2023(H5N3)		PP549274 A/Grey teal/South Australia/22-64233564-37/2022(H10N2)	98.79%
		PP549258 A/Grey teal/South Australia/22-64233564-17/2022(H10N7)	98.70%
		OL371678 A/wild waterbird/Victoria/19-00581-009/2019(H11N9)	98.09%
		PP549250 A/Grey teal/New South Wales/14340X/2020(H4N6)	98.09%
A/wild waterbird/Queensland/ P23-02457-51/2023 (H5N3)		OL370445 A/Pacific black duck/Victoria/11889/2019(H11N9)	97.95%
		PP549274 A/Grey teal/South Australia/22-64233564-37/2022(H10N2)	98.79%
		PP549258 A/Grey teal/South Australia/22-64233564-17/2022(H10N7)	98.70%
		OL371678 A/wild waterbird/Victoria/19-00581-009/2019(H11N9)	98.09%
		PP549250 A/Grey teal/New South Wales/14340X/2020(H4N6)	98.09%
A/wild waterbird/South Australia/23-80999145-13/2023(H5N9)		OL370445 A/Pacific black duck/Victoria/11889/2019(H11N9)	97.95%
		PP549274 A/Grey teal/South Australia/22-64233564-37/2022(H10N2)	98.84%
		PP549258 A/Grey teal/South Australia/22-64233564-17/2022(H10N7)	98.74%
		OL371678 A/wild waterbird/Victoria/19-00581-009/2019(H11N9)	98.14%
		PP549250 A/Grey teal/New South Wales/14340X/2020(H4N6)	98.14%
NP	A/Radjah Shelduck/Northern Territory/ 20231282-03/2023(H5N1)	OL370445 A/Pacific black duck/Victoria/11889/2019(H11N9)	98.09%
		OL371920 A/wild waterbird/Western Australia/AS19-3999-1/2019(H8N4)	98.20%
		OL372192 A/wild waterfowl/Western Australia/AS18-1331-10/2018(H11N3)	97.66%
		OL370274 A/wild waterfowl/Western Australia/AS18-1331-84/2018(H11N9)	97.60%
		OL370267 A/wild waterfowl/Western Australia/AS18-1331-79/2018(H11N9)	97.53%
A/Chestnut Teal/Victoria/23-01686-0034/2023 (H5N3)		OL370512 A/Pacific black duck/Victoria/11840/2019(H2N1)	97.46%
		OL370413 A/wild duck/Queensland/P20-00339-68/2020(mixed)	98.40%
		PP549236 A/Chestnut teal/Victoria/16099/2022(H10N7)	98.20%
		OL371680 A/wild waterbird/Victoria/19-00581-009/2019(H11N9)	98.20%
		OL370600 A/grey teal/South Australia/18-2812337-47/2018(H1N3)	98.20%
A/Pacific Black Duck/Victoria/23-01686-0039/2023 (H5N3)		PP549228 A/Chestnut teal/Victoria/16047/2022(H10N7)	98.13%
		PP549236 A/Chestnut teal/Victoria/16099/2022(H10N7)	98.73%
		PP549228 A/Chestnut teal/Victoria/16047/2022(H10N7)	98.66%
		OL370413 A/wild duck/Queensland/P20-00339-68/2020(mixed)	98.60%
		OL371680 A/wild waterbird/Victoria/19-00581-009/2019(H11N9)	98.53%
		OL370600 A/grey teal/South Australia/18-2812337-47/2018(H1N3)	98.53%
A/Grey Teal/Victoria/23-01688-0045/2023 (mixed)		PP549236 A/Chestnut teal/Victoria/16099/2022(H10N7)	98.73%
		PP549228 A/Chestnut teal/Victoria/16047/2022(H10N7)	98.66%
		OL370413 A/wild duck/Queensland/P20-00339-68/2020(mixed)	98.60%
		OL371680 A/wild waterbird/Victoria/19-00581-009/2019(H11N9)	98.53%
		OL370600 A/grey teal/South Australia/18-2812337-47/2018(H1N3)	98.53%
A/Pacific Black Duck/Victoria/17363/2023 (H5N3)		PP549260 A/Grey teal/South Australia/22-64233564-17/2022(H10N7)	98.60%
		PP549348 A/Pacific black duck/Tasmania/22-1184-5/2022(H4N1)	98.20%

Segment	Designation	Top blast hit	% identity
		MN826602 A/Chestnut teal/Australia/CT08.18-12952/2018(H9N2)	98.06%
		OL370447 A/Pacific black duck/Victoria/11889/2019(H11N9)	98.00%
		OL370600 A/grey teal/South Australia/18-2812337-47/2018(H1N3)	98.00%
	A/wild waterbird/Queensland/ P23-02457-48/2023(H5N3)	OR681023 A/Wild waterfowl/Australia/22-04155-0048/2022(H11N9)	99.00%
		OL370413 A/wild duck/Queensland/P20-00339-68/2020(mixed)	98.26%
		OL371680 A/wild waterbird/Victoria/19-00581-009/2019(H11N9)	98.20%
		OL370600 A/grey teal/South Australia/18-2812337-47/2018(H1N3)	98.20%
		OL370328 A/wild waterbird/South Australia/19-6528228-82/2019(H11)	98.13%
	A/wild waterbird/Queensland/ P23-02457-51/2023 (H5N3)	OR681023 A/Wild waterfowl/Australia/22-04155-0048/2022(H11N9)	99.00%
		OL370413 A/wild duck/Queensland/P20-00339-68/2020(mixed)	98.26%
		OL371680 A/wild waterbird/Victoria/19-00581-009/2019(H11N9)	98.20%
		OL370600 A/grey teal/South Australia/18-2812337-47/2018(H1N3)	98.20%
		OL370328 A/wild waterbird/South Australia/19-6528228-82/2019(H11)	98.13%
	A/wild waterbird/South Australia/23-80999145-13/2023(H5N9)	OL370944 A/wild bird/Victoria/20-03245-0029/2020(H9N2)	98.46%
		OL371080 A/wild duck/Queensland/P20-03375-0084-01/2020(H1N3)	98.46%
		OL371104 A/wild duck/Queensland/P20-03558-0029-01/2020(H1N3)	98.40%
		OL370367 A/wild waterbird/South Australia/19-9013889-76/2019(H7N1)	98.20%
		OL371600 A/wild waterbird/South Australia/19-9013889-45/2019(H7N1)	98.13%
M	A/Radjah Shelduck/Northern Territory/ 20231282-03/2023(H5N1)	OQ201832 A/Wild waterfowl/Australia/22-02927-0052/2022(H4N6)	99.80%
		MW466354 A/duck/Bangladesh/41797/2019(H3N8)	99.80%
		OR949087 A/duck/Chiayi/19WB0021F/2018(H4N6)	99.80%
		ON505895 A/Mallard(Anas platyrhynchos)/South Korea/KNU2021-41/2021(H9N2)	99.80%
		OK235643 A/Wild Duck/South Korea/KNU2020-74/2020(H3N8)	99.80%
	A/Chestnut Teal/Victoria/23-01686-0034/2023 (H5N3)	OQ201832 A/Wild waterfowl/Australia/22-02927-0052/2022(H4N6)	99.80%
		MW466354 A/duck/Bangladesh/41797/2019(H3N8)	99.49%
		OR758553 A/chicken/Dhaka/BAIV-1050_M/2021(H9N2)	99.49%
		OR949087 A/duck/Chiayi/19WB0021F/2018(H4N6)	99.49%
		ON505895 A/Mallard(Anas platyrhynchos)/South Korea/KNU2021-41/2021(H9N2)	99.49%
	A/Pacific Black Duck/Victoria/23-01686-0039/2023 (H5N3)	PP549238 A/Chestnut teal/Victoria/16099/2022(H10N7)	99.29%
		MW466354 A/duck/Bangladesh/41797/2019(H3N8)	99.19%
		PP549230 A/Chestnut teal/Victoria/16047/2022(H10N7)	99.19%
		PP549310 A/Grey teal/Victoria/16156/2022(H10N7)	99.19%
		OR949087 A/duck/Chiayi/19WB0021F/2018(H4N6)	99.19%
	A/Grey Teal/Victoria/23-01688-0045/2023 (mixed)	PP549238 A/Chestnut teal/Victoria/16099/2022(H10N7)	99.29%
		MW466354 A/duck/Bangladesh/41797/2019(H3N8)	99.19%
		PP549230 A/Chestnut teal/Victoria/16047/2022(H10N7)	99.19%
		PP549310 A/Grey teal/Victoria/16156/2022(H10N7)	99.19%
		OR949087 A/duck/Chiayi/19WB0021F/2018(H4N6)	99.19%
	A/Pacific Black Duck/Victoria/17363/2023 (H5N3)	PP549238 A/Chestnut teal/Victoria/16099/2022(H10N7)	98.98%
		MW466354 A/duck/Bangladesh/41797/2019(H3N8)	98.88%
		PP549230 A/Chestnut teal/Victoria/16047/2022(H10N7)	98.88%
		PP549310 A/Grey teal/Victoria/16156/2022(H10N7)	98.88%
		OR949087 A/duck/Chiayi/19WB0021F/2018(H4N6)	98.88%
	A/wild waterbird/Queensland/ P23-02457-48/2023(H5N3)	OL370602 A/grey teal/South Australia/18-2812337-47/2018(H1N3)	99.08%
		OL370421 A/wild waterbird/Queensland/JCU-272/2019(H5N9)	98.88%

Segment	Designation	Top blast hit	% identity
		MN826604 A/Chestnut teal/Australia/CT08.18-12952/2018(H9N2)	98.88%
		OL371538 A/wild waterbird/South Australia/19-6529700-80/2019(H8N4)	98.88%
		OL371530 A/wild waterbird/South Australia/19-6529700-27/2019(H6N8)	98.78%
	A/wild waterbird/Queensland/ P23-02457-51/2023 (H5N3)	OL370602 A/grey teal/South Australia/18-2812337-47/2018(H1N3)	99.08%
		OL370421 A/wild waterbird/Queensland/JCU-272/2019(H5N9)	98.88%
		MN826604 A/Chestnut teal/Australia/CT08.18-12952/2018(H9N2)	98.88%
		OL371538 A/wild waterbird/South Australia/19-6529700-80/2019(H8N4)	98.88%
		OL371530 A/wild waterbird/South Australia/19-6529700-27/2019(H6N8)	98.78%
	A/wild waterbird/South Australia/23-80999145-13/2023(H5N9)	OR950095 A/duck/Tainan/16WB3443-16-20/2016(H7N7)	99.49%
		OR675273 A/Spot-billed duck(Anas poecilohyncha)/Korea/KNU27/2023(H11N15)	99.39%
		MW466354 A/duck/Bangladesh/41797/2019(H3N8)	99.39%
		OR949087 A/duck/Chiayi/19WB0021F/2018(H4N6)	99.39%
		OQ201832 A/Wild waterfowl/Australia/22-02927-0052/2022(H4N6)	99.39%
NS	A/Radjah Shelduck/Northern Territory/ 20231282-03/2023(H5N1)	PP549407 A/Tawny frogmouth/Western Australia/AS-21-1823-0007/2021(H10N7)	99.40%
		ON495909 A/Mallard(Anas platyrhynchos)/South Korea/KNU2021-44/2021(H1N1)	99.28%
		MF694254 A/domestic duck/Georgia/11/2016(H4N6)	99.28%
		MN208025 A/teal/Egypt/MB-D-125OP/2015(H7N3)	99.28%
		MF694195 A/domestic duck/Georgia/4/2016(H4N6)	99.28%
	A/Chestnut Teal/Victoria/23-01686-0034/2023 (H5N3)	PP549407 A/Tawny frogmouth/Western Australia/AS-21-1823-0007/2021(H10N7)	99.52%
		ON495909 A/Mallard(Anas platyrhynchos)/South Korea/KNU2021-44/2021(H1N1)	99.40%
		MF694254 A/domestic duck/Georgia/11/2016(H4N6)	99.40%
		MN208025 A/teal/Egypt/MB-D-125OP/2015(H7N3)	99.40%
		MF694195 A/domestic duck/Georgia/4/2016(H4N6)	99.40%
	A/Pacific Black Duck/Victoria/23-01686-0039/2023 (H5N3)	PP549210 A/wild waterbird/South Australia/22-68204541-55/2022(H4N6)	99.52%
		PP549231 A/Chestnut teal/Victoria/16047/2022(H10N7)	99.40%
		OL370450 A/Pacific black duck/Victoria/11889/2019(H11N9)	99.40%
		PP549311 A/Grey teal/Victoria/16156/2022(H10N7)	99.28%
		OR681024 A/Wild waterfowl/Australia/22-04155-0048/2022(H11N9)	99.16%
	A/Grey Teal/Victoria/23-01688-0045/2023 (mixed)	PP549210 A/wild waterbird/South Australia/22-68204541-55/2022(H4N6)	99.52%
		PP549231 A/Chestnut teal/Victoria/16047/2022(H10N7)	99.40%
		OL370450 A/Pacific black duck/Victoria/11889/2019(H11N9)	99.40%
		PP549311 A/Grey teal/Victoria/16156/2022(H10N7)	99.28%
		OR681024 A/Wild waterfowl/Australia/22-04155-0048/2022(H11N9)	99.16%
	A/Pacific Black Duck/Victoria/17363/2023 (H5N3)	PP549407 A/Tawny frogmouth/Western Australia/AS-21-1823-0007/2021(H10N7)	99.16%
		ON495909 A/Mallard(Anas platyrhynchos)/South Korea/KNU2021-44/2021(H1N1)	99.05%
		MF694254 A/domestic duck/Georgia/11/2016(H4N6)	99.05%
		MN208025 A/teal/Egypt/MB-D-125OP/2015(H7N3)	99.05%
		MF694195 A/domestic duck/Georgia/4/2016(H4N6)	99.05%
	A/wild waterbird/Queensland/ P23-02457-48/2023(H5N3)	PP549407 A/Tawny frogmouth/Western Australia/AS-21-1823-0007/2021(H10N7)	99.76%
		ON495909 A/Mallard(Anas platyrhynchos)/South Korea/KNU2021-44/2021(H1N1)	99.64%
		MF694254 A/domestic duck/Georgia/11/2016(H4N6)	99.64%
		MN208025 A/teal/Egypt/MB-D-125OP/2015(H7N3)	99.64%
		MF694195 A/domestic duck/Georgia/4/2016(H4N6)	99.52%
	A/wild waterbird/Queensland/ P23-02457-51/2023 (H5N3)	PP549407 A/Tawny frogmouth/Western Australia/AS-21-1823-0007/2021(H10N7)	99.76%

Segment	Designation	Top blast hit	% identity
		ON495909 A/Mallard(Anas platyrhynchos)/South Korea/KNU2021-44/2021(H1N1)	99.64%
		MF694254 A/domestic duck/Georgia/11/2016(H4N6)	99.64%
		MN208025 A/teal/Egypt/MB-D-125OP/2015(H7N3)	99.64%
		MF694195 A/domestic duck/Georgia/4/2016(H4N6)	99.52%
	A/wild waterbird/South Australia/23-80999145-13/2023(H5N9)	OR681024 A/Wild waterfowl/Australia/22-04155-0048/2022(H11N9))	99.76%
		PP549210 A/wild waterbird/South Australia/22-68204541-55/2022(H4N6)	99.64%
		PP549231 A/Chestnut teal/Victoria/16047/2022(H10N7)	99.52%
		OL370450 A/Pacific black duck/Victoria/11889/2019(H11N9)	99.52%
		PP549311 A/Grey teal/Victoria/16156/2022(H10N7)	99.40%



Appendix Figure. Maximum likelihood trees for internal segments. In cases where all viruses are similar only a single arrow is shown. In cases where viruses fall into different Australian lineages (i.e. a result of different virus incursion events). Lineage names presented in Table 1 and the number of sequences in each of those lineages have been included adjacent to arrows. Lineage names are for clarity only. No internal segments are available for either virus from Queensland, and PB1, PB2 and PA segments were unavailable for A/Radjah shelduck/Northern Territory/20231282-03/2023(H5N1). Alignments for all trees are available on <https://github.com/michellewille2/Eurasian-LPAI-H5-incursion-to-Australia>