



H5H8 suspicion

EUROPEAN UNION

Intra trade certificate

Part I : Details of dispatched consignment	I.1. Consignor			I.2. Certificate reference number		I.2.a Local reference number::																	
	Name TIS-VET KFT			INTRA.HU.2019.0005723		3862019																	
	Address KOSSUTH LAJOS U. 43																						
	Country Kömpöc Hungary (HU)			I.3. Central Competent Authority HU00000 Ministry of Agriculture																			
				I.4. Local Competent Authority HU00300 Kecskemét																			
	I.5. Consignee			I.6. No.(s) of related original certificates No.(s) of accompanying documents																			
	Name Rössig International SARL																						
	Address 20 AV. du General Azibert																						
	Country 11430 Gruissan France (FR)			I.7. Dealer Name Approval number																			
	I.8. Country of origin		ISO code	I.9. Region of origin		Code	I.10. Country of destination		ISO code	I.11. Region of destination		Code											
Hungary		HU				Bulgaria		BG															
I.12. Place of origin/Place of harvest												I.13. Place of destination											
Holding ☒ Assembly centre ☐ Dealer's premise ☐												Holding ☒ Assembly centre ☐ Dealer's premise ☐											
Approved body ☐ Semen centre ☐ Approved aquaculture holding ☐												Approved body ☐ Semen centre ☐ Approved aquaculture holding ☐											
Embryo team ☐ Establishment ☐ Other ☐												Embryo team ☐ Establishment ☐ Other ☐											
Name Zámbóné Maróti Erika												Name Donika 2011 EOOD											
Approval number 4553496												Approval number 5568-0328											
Address Alsópálos, tanya 408.												Address village Lisec											
Postal code / Region 6135 Csólyospálos												Postal code / Region 5568 Lisec - Jincen											
I.14. Place of loading												I.15. Date and time of departure											
Postal code / Region 6135 Csólyospálos												04/03/2019 16:00 (UTC +0100)											
I.16. Means of transport												I.17. Transporter											
Aeroplane ☐ Ship ☐ Railway wagon ☐												Name "Niki Trans-2006"Ltd											
Road vehicle ☒ Other ☐												Approval number 1772/22.10.2008											
Identification:: PB 5110 TT PB 4630 EH												Address Pyrvomaï, obl Plovdiv											
Number(s):												Postal code / Region 4270 Pyrvomaï - Member state BG											
I.21. Temperature of products												I.20. Number/Quantity				I.22. Number of packages							
Ambient ☐ Chilled ☐ Frozen ☐												3.200 unit				1							
I.23. Identification of container/Seal number																							
I.25. Animals certified for/products certified for:																							
Slaughter ☒																							

***Control measures,
sampling
(14.03.2019)***

- Local veterinary authority visit
- Farm of origin
 - 60 cloacal+60 tracheal swabs
 - 3 ducks (9 weeks old) from the same flock dispatched to Bulgaria
 - Diagnostic killing (carcass, blood)
- Four other contact holdings
 - Birds transported within the supposed latency period (03.03.2019-12.03.2019)
 - 2019.03.06., Bócsa holding 1 (1782 animals)
 - 2019.03.10. Bócsa holding 2 (2700 animals)
 - 2019.03.12. Móricgát (1220 animals)
 - 2019.03.12. Felsőmonostor (750 animals)
 - 4x (60 cloacal+60 tracheal swabs)
- Official surveillance and movement restriction in all holdings

- Original farm
 - 120 swabs, 3 carcasses M gene rRT-PCR negative
 - 3 blood **Influenza A antibody ELISA positive**, HI: H5, H7 negative, **H6 positive**
- Contact holdings
 - **2019.03.06. Bócsa holding 1 (1782 animals)**
 - **30 cloacal, 30 tracheal swabs M gene rRT-PCR positive (cT 17.85-37.44)**
 - 2019.03.10. Bócsa holding 2 (2700 animals)
 - **2019.03.12. Móricgát (1220 animals)**
 - **2 cloacal swabs M gene rRT-PCR positive (cT 29,8 és 33,72)**
 - 2019.03.12. Felsőmonostor (750 animals)
- H5, H7, N1, N5, N6 PCR negative, **N8 positive**, (Fereidouni et al., 2008)
- Sequencing HA (Hoffmann et al., 2001) and NA (Phipps et al., 2004): **H6N8**
- Full genome sequencing (Watson et al., 2015), Illumina
 - **A/duck/Hungary/11447/2019(H6N8)**

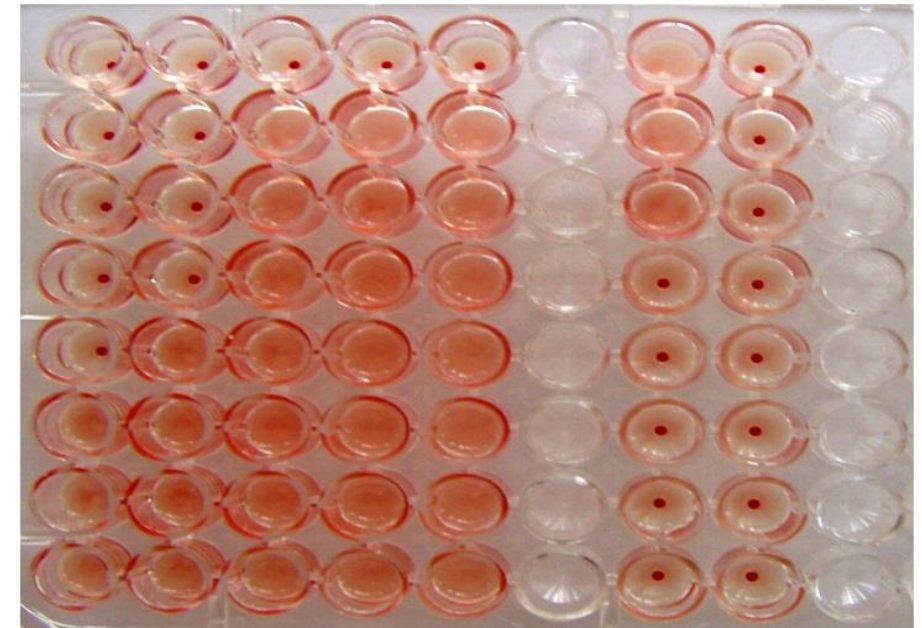
*Molecular biology,
serology*

Virology

- Virus isolation
 - Embryo death within 48 hours
 - HAU: 512
 - rRT-PCR: cT 12.5
- HAI
 - (H5, H7, NDV)
 - **H6**
- IVPI: 0
 - ELISA positive
- Oronasal challenge experiment
 - No clinical sign



H6N1 H6N3 H5N1 H7N7 NDV



HA gene phylogeny (ML tree)

Most related viruses

A/mallard duck/Georgia/7/2015
A/duck/Hubei/ZYSYG15/2015
A/teal/Toguchin/1157/2016

Subtype

H6N1
H6N2
H6N1

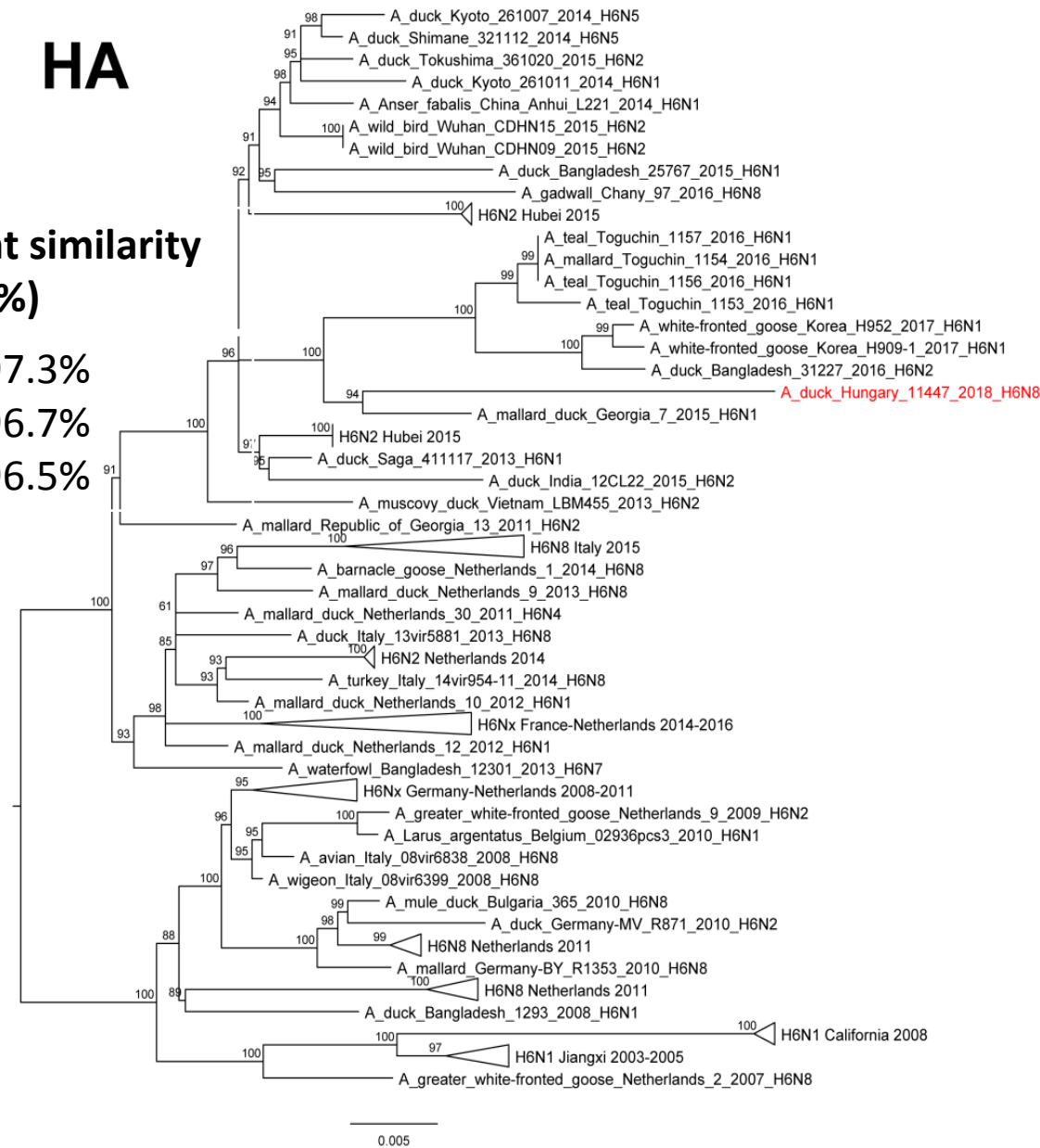
nt similarity (%)

97.3%
96.7%
96.5%

PQIETR↓GLF

No potential additional glycosylation sites

HA



**NA gene
phylogeny
(ML tree)**

Most related viruses

A/common shelduck/Mongolia/2076/2011
A/duck/Mongolia/53/2011
A/common shelduck/Mongolia/2185/2011

No stalk deletion
No potential additional glycosylation sites
No markers of antiviral drug resistance

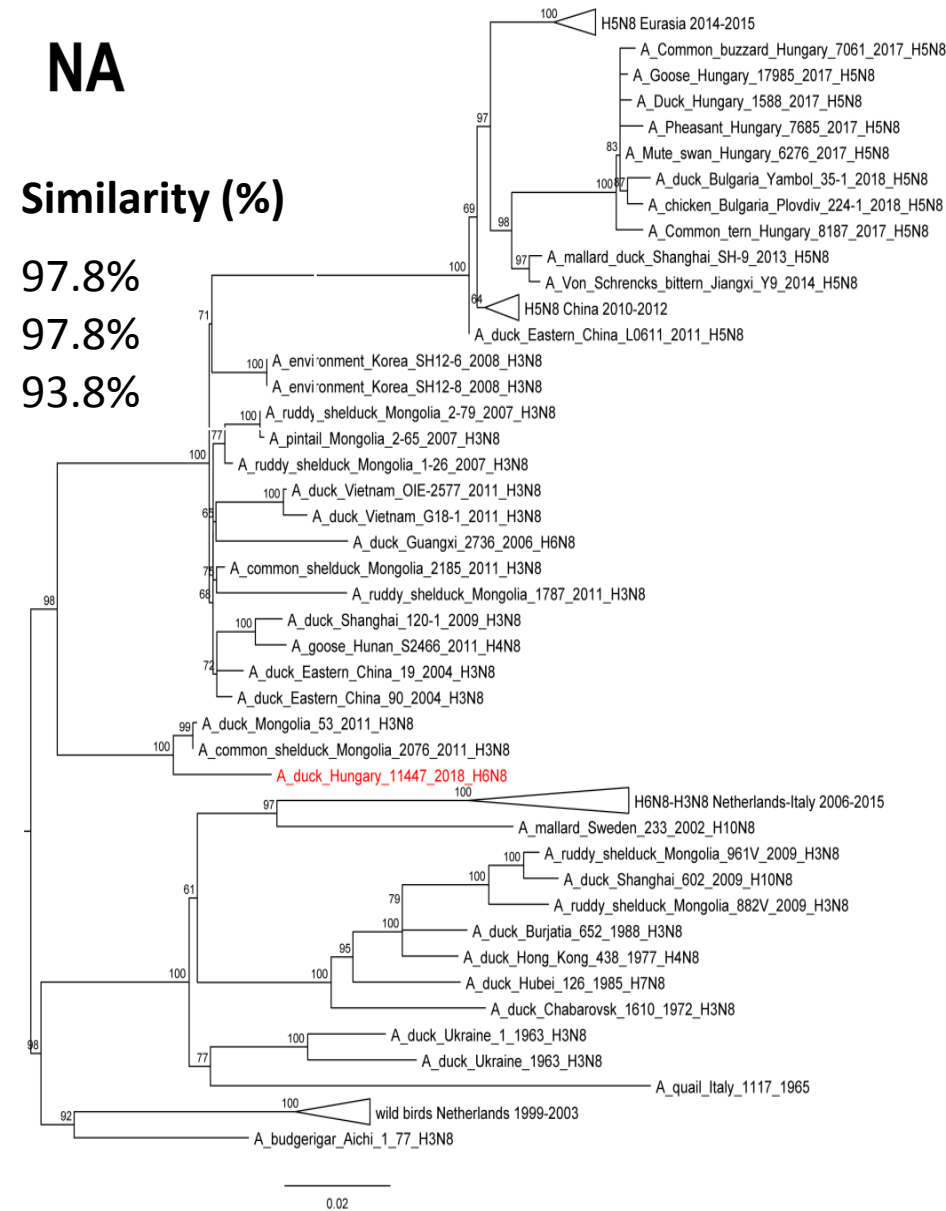
Subtype

H3N8

NA

Similarity (%)

97.8%
97.8%
93.8%



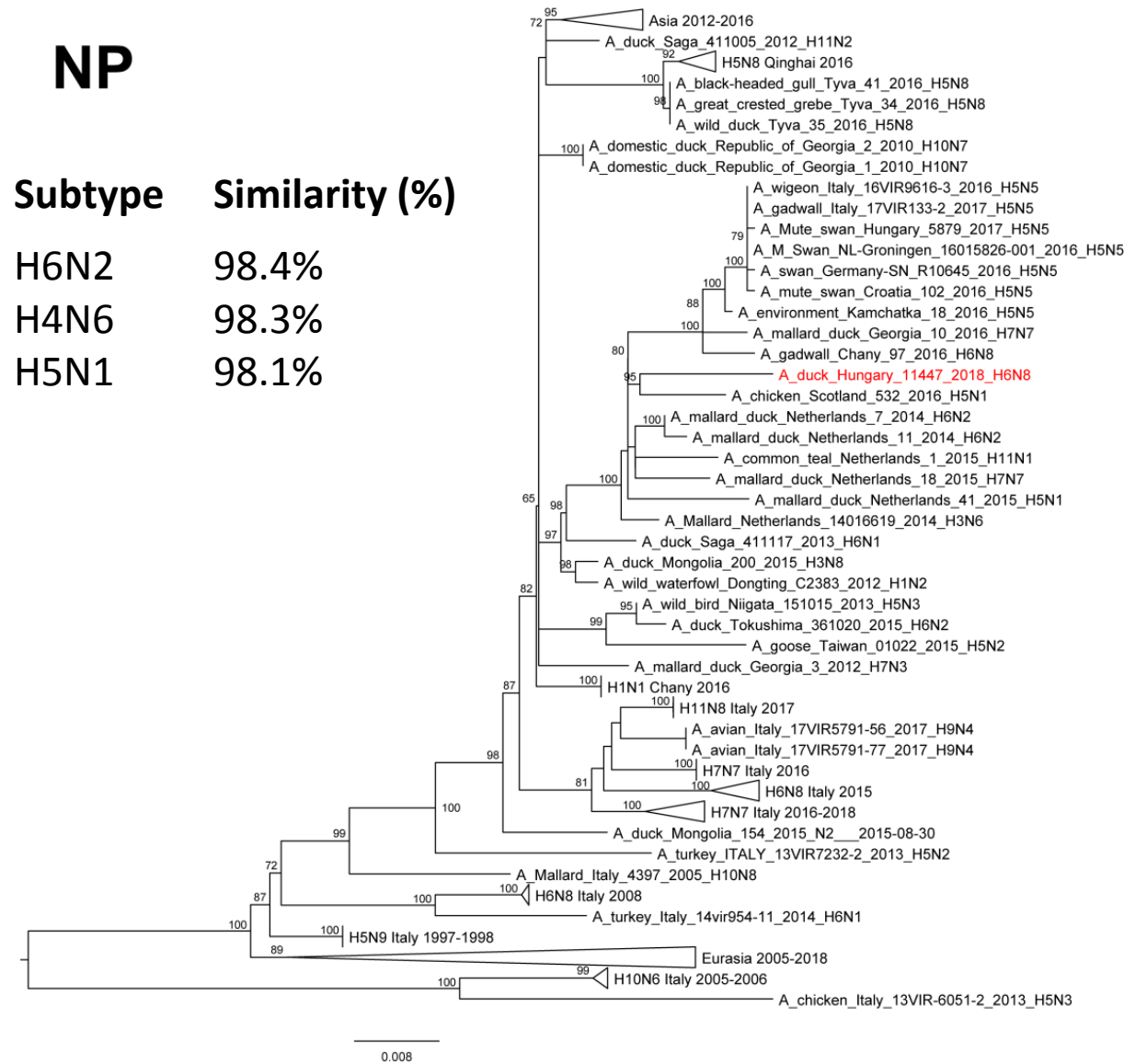
NP gene phylogeny (ML tree)

Most related viruses

A/mallard duck/Netherlands/7/2014
A/mallard/Italy/18VIR6264-78/2018
A/chicken/Scotland/532/2016

NP

Subtype	Similarity (%)
H6N2	98.4%
H4N6	98.3%
H5N1	98.1%



***M* gene phylogeny
(ML tree)**

Most related viruses

- A/mallard duck/Netherlands/19/2012
- A/mallard duck/Netherlands/6/2009
- A/duck/Moscow/4781/2012

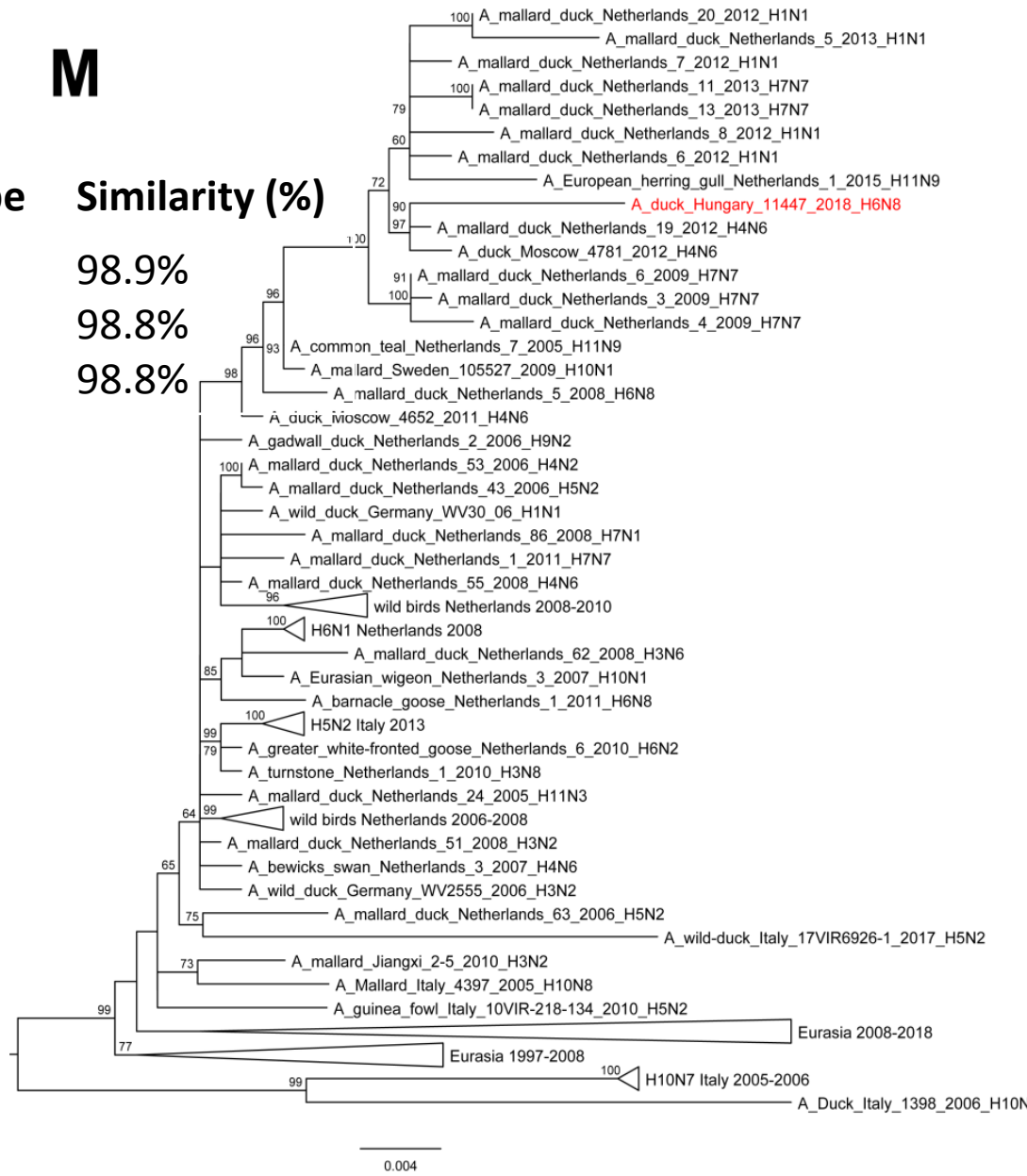
No markers of antiviral drug resistance

Subtype

- H4N6
- H7N7
- H4N6

Similarity (%)

- 98.9%
- 98.8%
- 98.8%



***NS gene phylogeny
(ML tree)***

Most related viruses

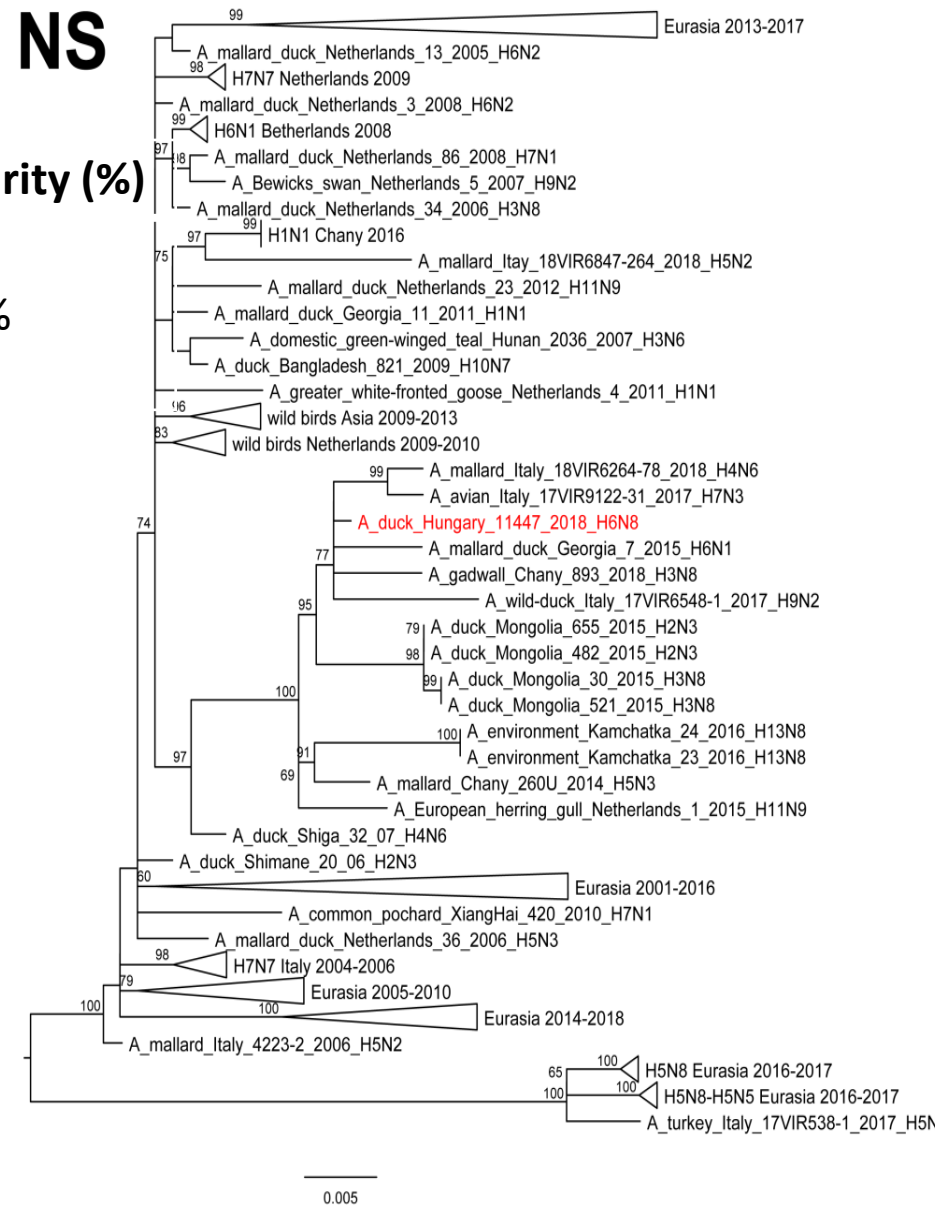
A/mallard duck/Georgia/7/2015
A/gadwall/Chany/893/2018
A/mallard/Italy/18VIR6264-78/2018

Subtype

H6N1
H3N8
H4N6

Similarity (%)

99.3%



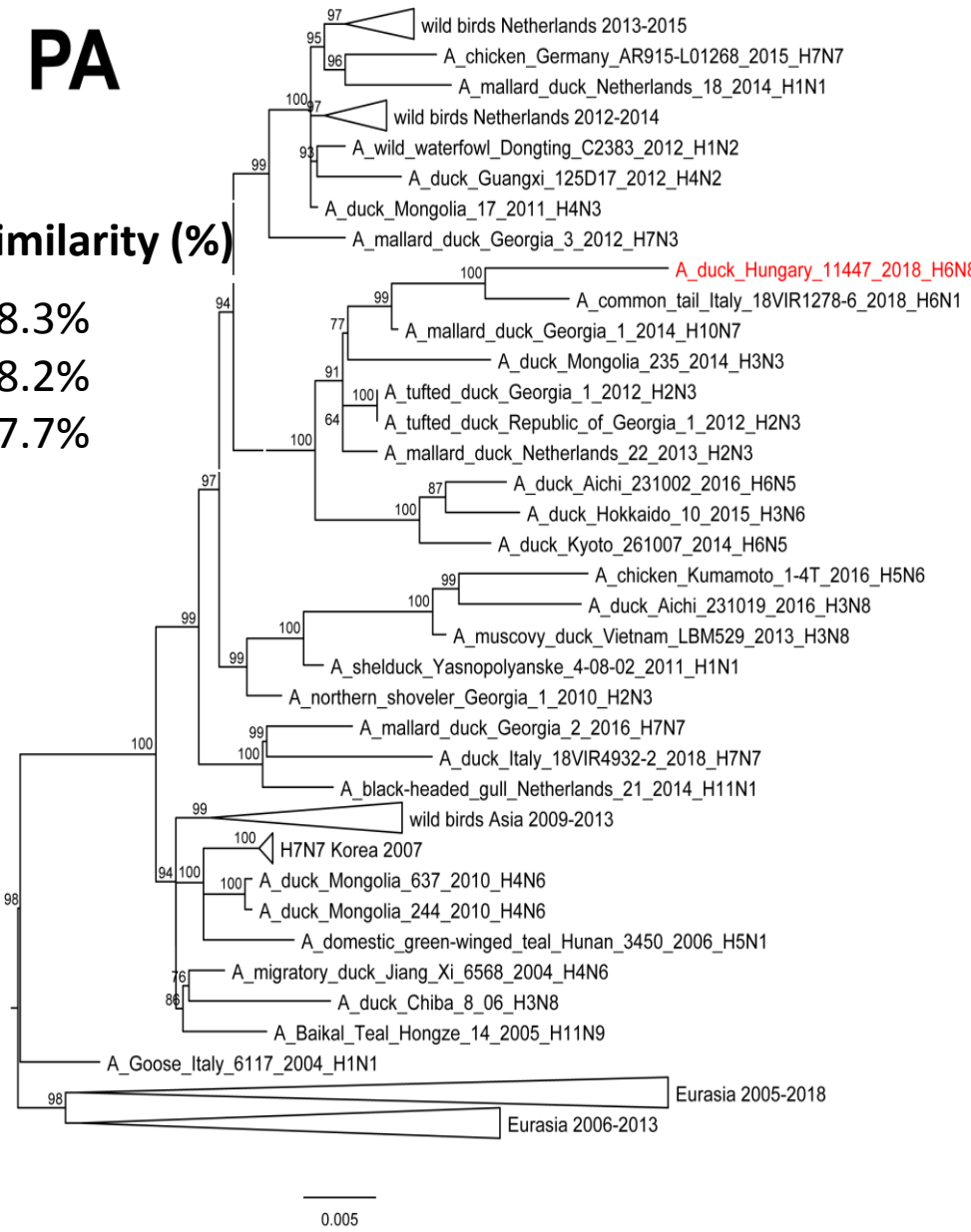
PA gene phylogeny
(ML tree)

Most related viruses

- A/common tail/Italy/18VIR1278-6/2018
- A/mallard duck/Georgia/1/2014
- A/tufted duck/Georgia/1/2012

Subtype	Similarity (%)
H6N1	98.3%
H10N7	98.2%
H2N3	97.7%

PA



***PB2 gene
phylogeny
(ML tree)***

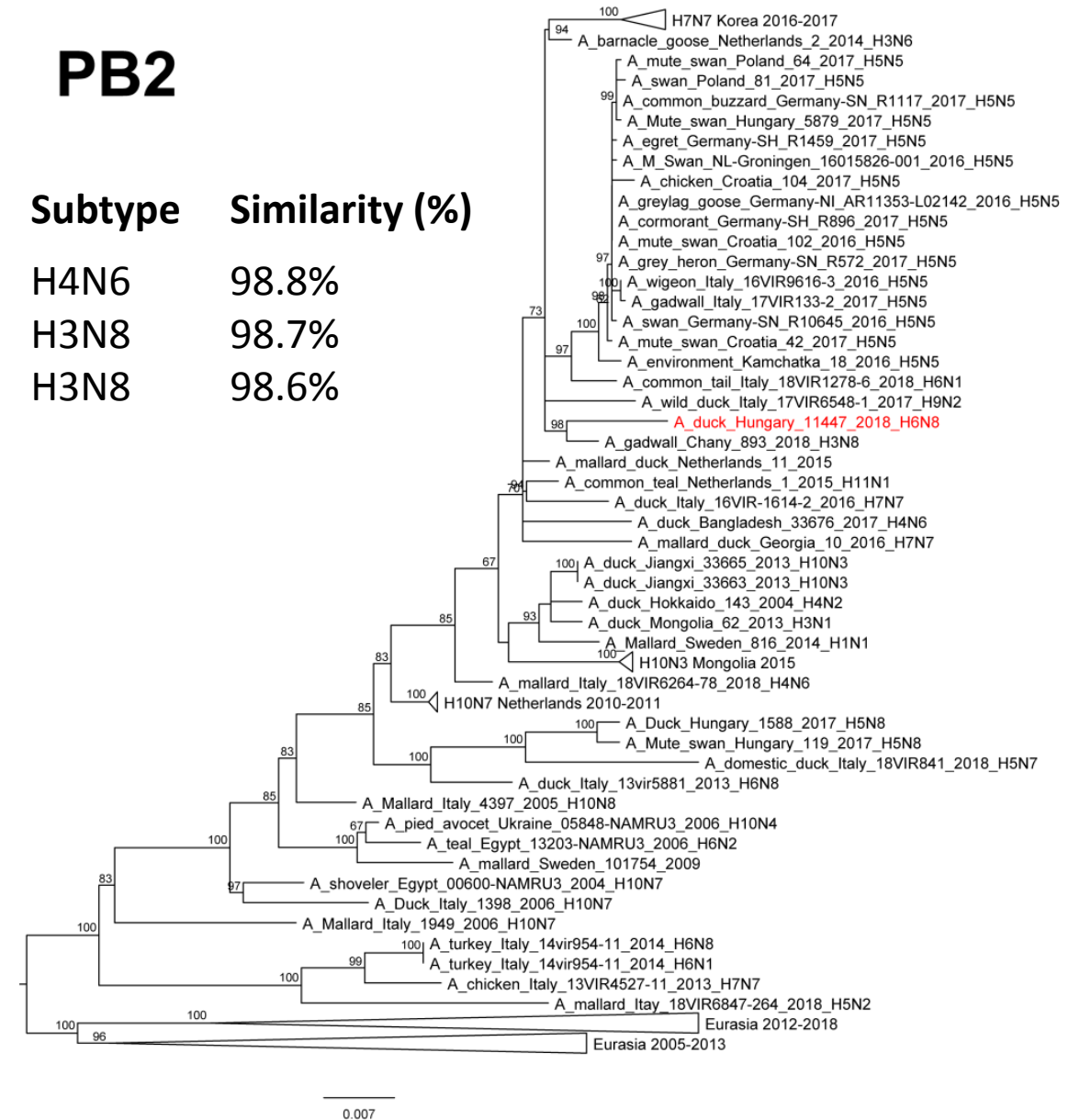
Most related viruses

A/mallard/Italy/18VIR6264-78/2018
A/gadwall/Chany/893/2018
A/barnacle_goose/Netherlands/2/2014

PB2

Subtype Similarity (%)

H4N6 98.8%
H3N8 98.7%
H3N8 98.6%



PB1

PB1 gene phylogeny (ML tree)

Most related viruses

A/Mute swan/Hungary/5879/2017
A/mute swan/Croatia/102/2016
A/swan/Poland/81/2017

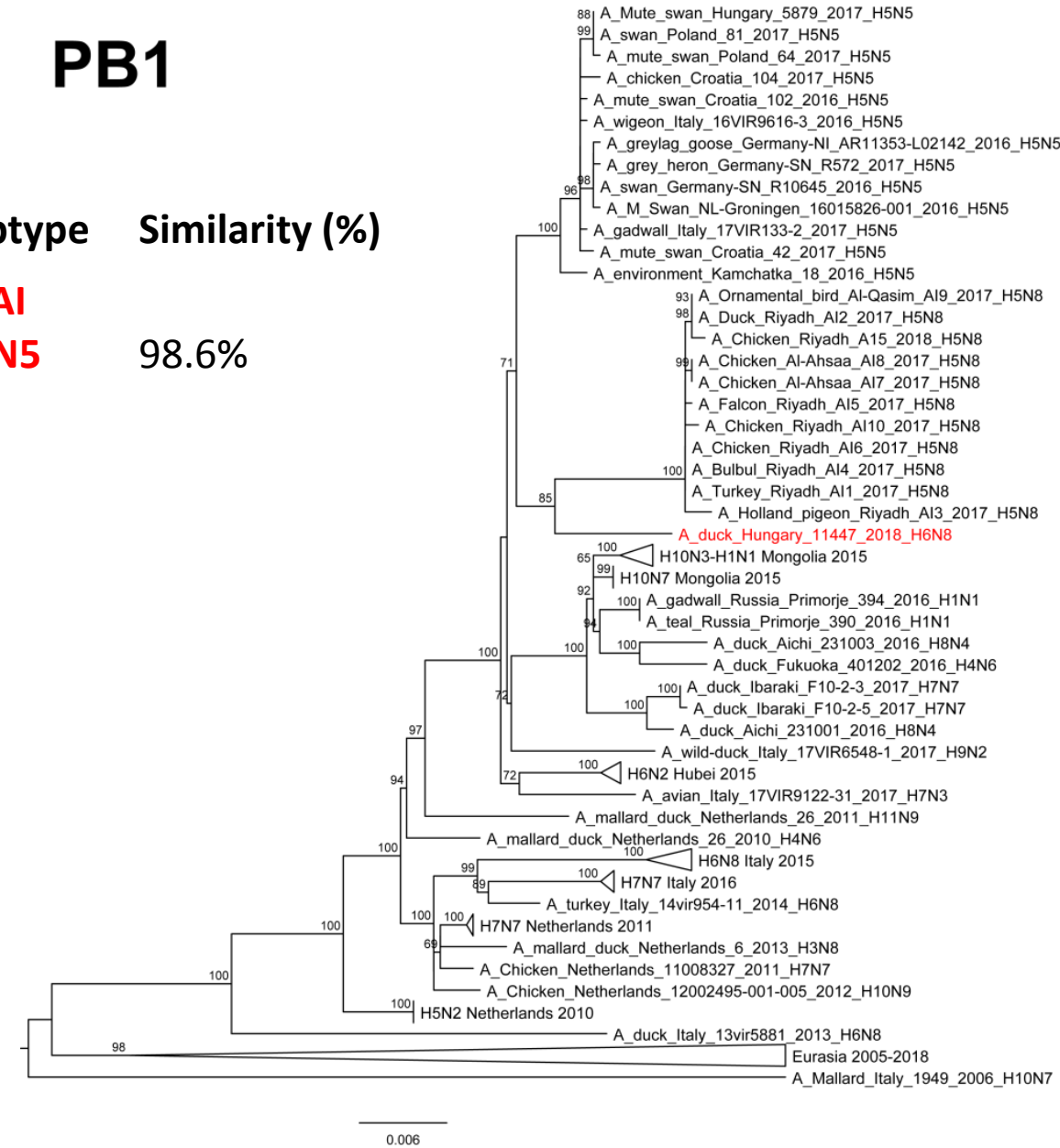
Subtype

HPAI

H5N5

Similarity (%)

98.6%



Phylogenetic tree showing the evolution of H5N8 and H5N1 viruses from 2007 to 2020. The tree is rooted on the left and branches out to the right. Nodes are labeled with bootstrap values. The x-axis represents time in years, with a scale bar of 2.0. The y-axis represents the genetic distance. The H5N8 clade is highlighted in red, and the H5N1 clade is highlighted in blue.

Key nodes and labels include:

- 2007, 91: A_mallard_duck_Netherlands_26_2010_H4N6_2010-09-13
- 2008, 09: A_mallard_duck_Netherlands_26_2011_H11N9_2011-10-10
- 2011, 58: A_duck_Mongolia_326_2015_H10N3_2015-08
- 2011, 77: A_duck_Hubei_ZYSYG8_2015_H6N2_2015-11-21
- 2011, 83: A_duck_Mongolia_245_2015_H10N3_2015-08
- 2012, 47: A_duck_Hubei_ZYSYG7_2015_H6N2_2015-11-21
- 2012, 49: A_duck_Hubei_ZYSYG3_2015_H6N2_2015-11-21
- 2012, 58: A_duck_Mongolia_520_2015_H1N1_2015-08-31
- 2012, 58: A_duck_Mongolia_141_2015_H2_2015-08-30
- 2012, 58: A_duck_Mongolia_626_2015_H10N7_2015-08-31
- 2012, 58: A_duck_Mongolia_709_2015_H10N7_2015-08-31
- 2012, 58: A_duck_Mongolia_499_2015_H10N7_2015-08-31
- 2013, 81: A_teal_Russia_Primorje_390_2016_H1N1_2016-04-11
- 2013, 81: A_gadwall_Russia_Primorje_394_2016_H1N1_2016-04-11
- 2013, 81: A_duck_Fukuoka_401202_2016_H4N6_2016-12-14
- 2013, 81: A_duck_Aichi_231003_2016_H8N4_2016-10-18
- 2013, 81: A_duck_Isaraki_F10-2-3_2017_H7N7_2016-02-21
- 2013, 81: A_duck_Isaraki_F10-2-5_2017_H7N7_2016-02-21
- 2013, 81: A_duck_Aichi_231001_2016_H8N4_2016-10-18
- 2013, 81: A_wild-duck_Italy_17VIR6548-1_2017_H9N2_2017
- 2013, 81: A_duck_Hubei_ZYSYG8_2015_H6N2_2015-11-21
- 2013, 81: A_duck_Hubei_ZYSYG7_2015_H6N2_2015-11-21
- 2013, 81: A_duck_Hubei_ZYSYG3_2015_H6N2_2015-11-21
- 2013, 81: A_avian_Italy_17VIR9122-31_2017_H7N3_2017
- 2013, 83: A_duck_Mongolia_326_2015_H10N3_2015-08
- 2013, 83: A_duck_Mongolia_245_2015_H10N3_2015-08
- 2013, 83: A_duck_Mongolia_520_2015_H1N1_2015-08-31
- 2013, 83: A_duck_Mongolia_141_2015_H2_2015-08-30
- 2013, 83: A_duck_Mongolia_626_2015_H10N7_2015-08-31
- 2013, 83: A_duck_Mongolia_709_2015_H10N7_2015-08-31
- 2013, 83: A_duck_Mongolia_499_2015_H10N7_2015-08-31
- 2013, 83: A_teal_Russia_Primorje_390_2016_H1N1_2016-04-11
- 2013, 83: A_gadwall_Russia_Primorje_394_2016_H1N1_2016-04-11
- 2013, 83: A_duck_Fukuoka_401202_2016_H4N6_2016-12-14
- 2013, 83: A_duck_Aichi_231003_2016_H8N4_2016-10-18
- 2013, 83: A_duck_Isaraki_F10-2-3_2017_H7N7_2016-02-21
- 2013, 83: A_duck_Isaraki_F10-2-5_2017_H7N7_2016-02-21
- 2013, 83: A_duck_Aichi_231001_2016_H8N4_2016-10-18
- 2013, 83: A_wild-duck_Italy_17VIR6548-1_2017_H9N2_2017
- 2013, 83: A_duck_Hubei_ZYSYG8_2015_H6N2_2015-11-21
- 2013, 83: A_duck_Hubei_ZYSYG7_2015_H6N2_2015-11-21
- 2013, 83: A_duck_Hubei_ZYSYG3_2015_H6N2_2015-11-21
- 2013, 83: A_avian_Italy_17VIR9122-31_2017_H7N3_2017
- 2013, 83: A_duck_Mongolia_326_2015_H10N3_2015-08
- 2013, 83: A_duck_Mongolia_245_2015_H10N3_2015-08
- 2013, 83: A_duck_Mongolia_520_2015_H1N1_2015-08-31
- 2013, 83: A_duck_Mongolia_141_2015_H2_2015-08-30
- 2013, 83: A_duck_Mongolia_626_2015_H10N7_2015-08-31
- 2013, 83: A_duck_Mongolia_709_2015_H10N7_2015-08-31
- 2013, 83: A_duck_Mongolia_499_2015_H10N7_2015-08-31
- 2013, 83: A_teal_Russia_Primorje_390_2016_H1N1_2016-04-11
- 2013, 83: A_gadwall_Russia_Primorje_394_2016_H1N1_2016-04-11
- 2013, 83: A_duck_Fukuoka_401202_2016_H4N6_2016-12-14
- 2013, 83: A_duck_Aichi_231003_2016_H8N4_2016-10-18
- 2013, 83: A_duck_Isaraki_F10-2-3_2017_H7N7_2016-02-21
- 2013, 83: A_duck_Isaraki_F10-2-5_2017_H7N7_2016-02-21
- 2013, 83: A_duck_Aichi_231001_2016_H8N4_2016-10-18
- 2013, 83: A_wild-duck_Italy_17VIR6548-1_2017_H9N2_2017
- 2013, 83: A_duck_Hubei_ZYSYG8_2015_H6N2_2015-11-21
- 2013, 83: A_duck_Hubei_ZYSYG7_2015_H6N2_2015-11-21
- 2013, 83: A_duck_Hubei_ZYSYG3_2015_H6N2_2015-11-21
- 2013, 83: A_avian_Italy_17VIR9122-31_2017_H7N3_2017
- 2013, 83: A_duck_Mongolia_326_2015_H10N3_2015-08
- 2013, 83: A_duck_Mongolia_245_2015_H10N3_2015-08
- 2013, 83: A_duck_Mongolia_520_2015_H1N1_2015-08-31
- 2013, 83: A_duck_Mongolia_141_2015_H2_2015-08-30
- 2013, 83: A_duck_Mongolia_626_2015_H10N7_2015-08-31
- 2013, 83: A_duck_Mongolia_709_2015_H10N7_2015-08-31
- 2013, 83: A_duck_Mongolia_499_2015_H10N7_2015-08-31
- 2013, 83: A_teal_Russia_Primorje_390_2016_H1N1_2016-04-11
- 2013, 83: A_gadwall_Russia_Primorje_394_2016_H1N1_2016-04-11
- 2013, 83: A_duck_Fukuoka_401202_2016_H4N6_2016-12-14
- 2013, 83: A_duck_Aichi_231003_2016_H8N4_2016-10-18
- 2013, 83: A_duck_Isaraki_F10-2-3_2017_H7N7_2016-02-21
- 2013, 83: A_duck_Isaraki_F10-2-5_2017_H7N7_2016-02-21
- 2013, 83: A_duck_Aichi_231001_2016_H8N4_2016-10-18
- 2013, 83: A_wild-duck_Italy_17VIR6548-1_2017_H9N2_2017
- 2013, 83: A_duck_Hubei_ZYSYG8_2015_H6N2_2015-11-21
- 2013, 83: A_duck_Hubei_ZYSYG7_2015_H6N2_2015-11-21
- 2013, 83: A_duck_Hubei_ZYSYG3_2015_H6N2_2015-11-21
- 2013, 83: A_avian_Italy_17VIR9122-31_2017_H7N3_2017
- 2013, 83: A_duck_Mongolia_326_2015_H10N3_2015-08
- 2013, 83: A_duck_Mongolia_245_2015_H10N3_2015-08
- 2013, 83: A_duck_M

Conclusions

- HA, NA, PB2, PA, NP, M, NS of A/duck/Hungary/11447/2019(H6N8)
 - LPAI viruses of different subtypes identified in Europe and Asia
- PB1 gene
 - HPAI H5N5 European viruses (2016-2017)
 - HPAI H5N8 viruses collected in Saudi Arabia (2017-2018)
 - (Donor for H5H5)
- Intense gene exchange between viruses circulating in the wild reservoir