

Avian Influenza Molecular Diagnostics in ARRIAH (2010-2013)

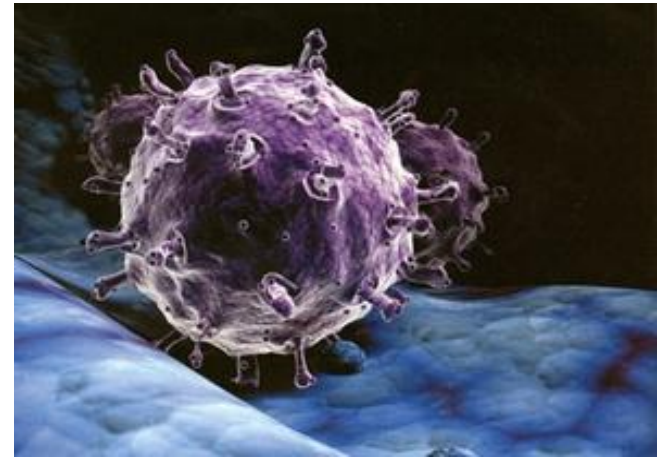
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Avian Influenza Molecular Diagnostics Tasks

- **Molecular-biological characteristics of avian influenza virus isolates** (typing, subtyping, determination of potential pathogenicity degree of virus isolates, nucleotide sequences genetic analysis, phylogenetic analysis).
- **Development of methods for detection and subtyping of AI viruses on the basis of RT-PCR and RRT-PCR** (development of methods for genome indication and subtype identification of AI virus, primers selection).



Avian Influenza Molecular Diagnostics

RNA extraction
Samples-any
(tissue, swabs, etc.)



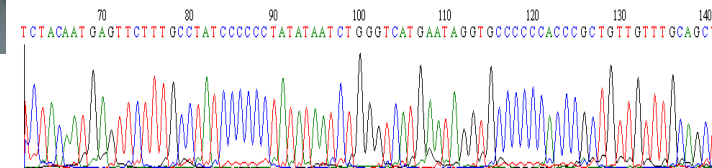
Conventional
RT-PCR



Real-time
RT-PCR;
Visualization



Visualization; purification;
sequencing;
interpreting the results



Molecular-diagnostic Methods

**Universal RNA/DNA
extraction kit**



**Tecan Robotic extraction
station Freedom Evo-100**



**R-Time PCR amplifier,
Corbett Life Science**



BIO RAD gel doc video system



**ABI Prism Genetic analyzers,
Applied Biosystems**



**Pyrosequencing technology,
Roche**



Avian influenza PCR results 2005 - 2013

Analyzed	Poultry	Wild birds	Total
Samples	1912	6830	8742
Type A	227	257*	484 (5,53%)
A/H5N1	224	110	334 (69,00%)

* - include H3N6, H3N8, H4N2, H4N6 and H9N2 subtypes

Avian influenza PCR results (2006-2013)

2

Period	Positive type A results	HPAI/H5N1	Total
2006	145	107	1014
2007	62	55	833
2008	15 (1-H3, 4-H4, 4-H5 -LP)	2	1577
2009	23 (1-H4 -LP)	22	475
2010	29 (6-H3, 4-H4 -LP)	7	587
2011	11 (1-H3, 3-H4, 1-H3+H4) -LP	-	762
2012	19 (3-H9N2, 9-H9) -LP	-	921
2013	6 (1-H3N6) - LP	-	2002

Avian influenza PCR results (2010)

Russian Federal district / Country	Poultry		Wild birds		Total
	Positive samples A/H5N1	Negative samples	Positive samples type A/	Negative samples	
1. Central	-	434	3 (1-H4N6)	37	474
2. Siberian	-	5	7- H5N1, 19 (6-H3N8, 3-H4N6)	65	96
3. North-Caucasian	-	4	-	2	6
4. Volga	-	3	-	-	3
5. Ural	-	-	-	1	1
6. North-Western	-	-	-	3	3
7. Rep. of Kazakhstan	-	1	-	-	1
8. Rep. of Belarus	-	3	-	-	3
Total:	-	450	29	108	587

Avian influenza PCR results (2011)

Russian Federal district / Country	Poultry		Wild birds		Total
	Positive samples A/H5N1	Negative samples	Positive samples type A/	Negative samples	
1. Central	-	33	-	291	324
2. Siberian	-	6	11 (1- H3N8, 3 - H4N6, 1- H3+H4)	162	179
3. North-Caucasian	-	17	-	174	191
4. Southern	-	-	-	47	47
5. Far-Eastern	-	-	-	7	7
6. Volga	-	1	-	-	1
7. Rep. of Uzbekistan	-	12	-	-	12
8. Rep. of Tajikistan	-	1	-	-	1
Total:	-	70	11	681	762

Avian influenza virus subtypes identified in ⁵ the Russian Federation in 2010-2011

Avian Species	PCR positive results type A	AIV subtype	HPAI/H5N1	Virus isolation
1. teal	15	4-H3N8, 2-H4N6, 1-H3+H4	-	7
2. mallard	8	2-H3N8, 2-H4N6	-	4
3. gadwall	4	1-H3N8	-	1
4. wild duck	2	1-H4N6	-	1
5. pintail	2	1-H4N6	-	1
6. pochard	1	1-H4N6	-	1
7. shoveler	1	-	-	-
8. great crested grebe	7	-	7	3
Total:	40	7-H3N8 7-H4N6 1-H3+H4	7-H5N1	18

Avian influenza PCR results (2012)

Russian Federal district	Poultry		Wild birds		Total
	Positive samples type A/	Negative samples	Positive samples type A/	Negative samples	
1. Central	-	6	9 (H9)	89	104
2. Siberian	-	30	7 (Excluded H5,H7,H9)	211	248
3. North-Caucasian	-	4	-	214	218
4. South	-	5	-	127	132
5. Volga	-	-	-	62	62
6. North-Western	-	6	-	60	66
7. Far-Eastern	2 (H9N2)	21	1(H9N2)	67	91
Total:	2 (H9N2)	72	17 (1-H9N2, 9-H9)	830	921

Avian influenza PCR results (2013)

Russian Federal district /Country	Poultry		Wild birds		Total
	Positive samples type A/	Negative samples	Positive samples type A/	Negative samples	
1. Central	-	35	1 (Excluded H5,H7,H9)	262	298
2. Siberian	-	-	3 (1 – H3N6)	406	409
3. North-Caucasian + South	-	378	-	402	780
4. Volga	-	1	-	207	208
5. North-Western	-	17	2 (Excluded H5,H7,H9)	98	117
6. Far-Eastern	-	175	-	-	175
7. Rep. of Kazakhstan	-	15	-	-	15
Total:	-	621	6 (1 – H3N6)	1375	2002

Avian influenza positive PCR results (2013)

Region	Date	Specie	PCR	Notice
Arkhangelsk region	24.07	common gull	M-gene +/ H5, H7, H9 -	not isolated
Arkhangelsk region	25.07	common gull	M-gene +/ H5, H7, H9 -	not isolated
Vladimir region	27.09	wild duck	M-gene +/ H5, H7, H9 -	not isolated
Krasnoyarsk krai	17.10	mallard	M-gene +/ H5, H7, H9 -	not isolated
Altai krai	20.11	wild duck	M-gene +/ H5, H7, H9 -	not isolated
Altai krai	21.11	wild duck	M-gene +/ H3N6	Isolated/ KQTR_GLF

Wild birds samples investigation (2010-2013)

Avian Species	PCR positive results type A	H5N1	Virus isolation	Total
1. pigeon	1	-	1	705
2. synanthropic birds	-	-	-	479
3. wild birds	-	-	-	239
4. wild ducks	21	-	2	224
5. sparrow	-	-	-	179
6. crow	-	-	-	149
7. wild geese	-	-	-	148
8. mallard	9	-	4	136
9. great crested grebe	7	7	3	123
10. teal	15	-	7	95
11. bald-coot	-	-	-	39
12. gadwall	4	-	1	37
13. pochard	1	-	1	15
14. shoveler	1	-	-	10
15. pintail	2	-	1	2
16. other birds (quail, gull..)	2	-	-	502
Total:	63	7	20	3082

Wild birds samples investigation (2010-2013)

Avian group	PCR positive results type A	HPAI/ H5N1	Virus isolation	Total
1. Synanthropic birds	1	-	1	1512 - 49%
2. Wild ducks	53	-	16	519 - 17%
3. Dif. species (gull, quail...)	2	-	-	502 - 16%
4. Other waterfowl (w. goose, g.c.grebe, bald-coot)	7	7	3	310 - 10%
5. Wild birds (unknown species)	-	-	-	239 - 8%
Total:	63	7	20	3082

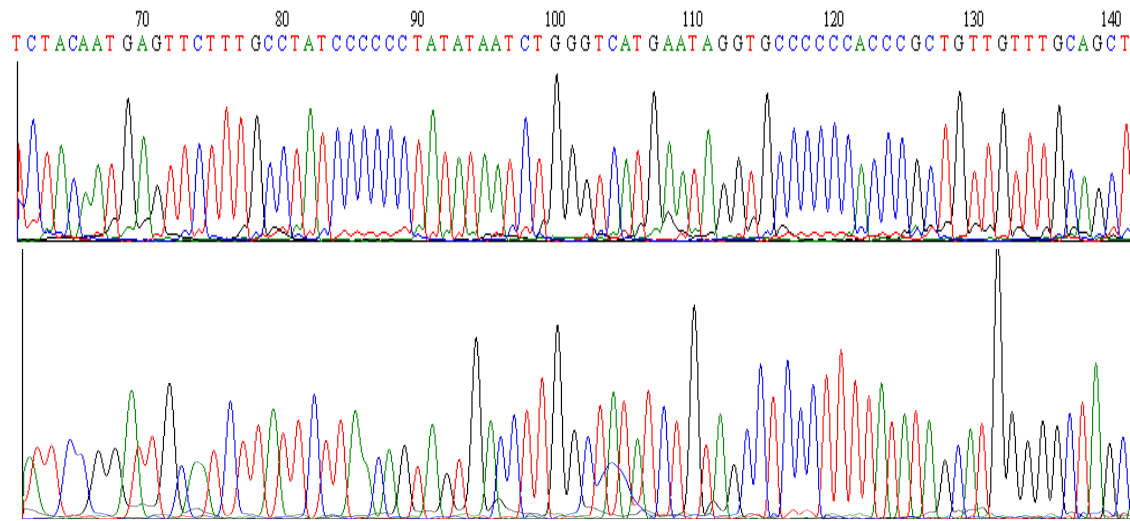
Avian influenza PCR results (2014...)

Russian Federal district	Poultry		Wild birds		Total
	Positive samples type A/	Negative samples	Positive samples type A/	Negative samples	
1. Central	-	5	-	76	81
2. Volga	-	195	-	-	195
3. Siberian	-	-	3 (Excluded H5,H7,H9)	47	50
Total:	-	200	3	123	326

AIV H5N1 full genome:

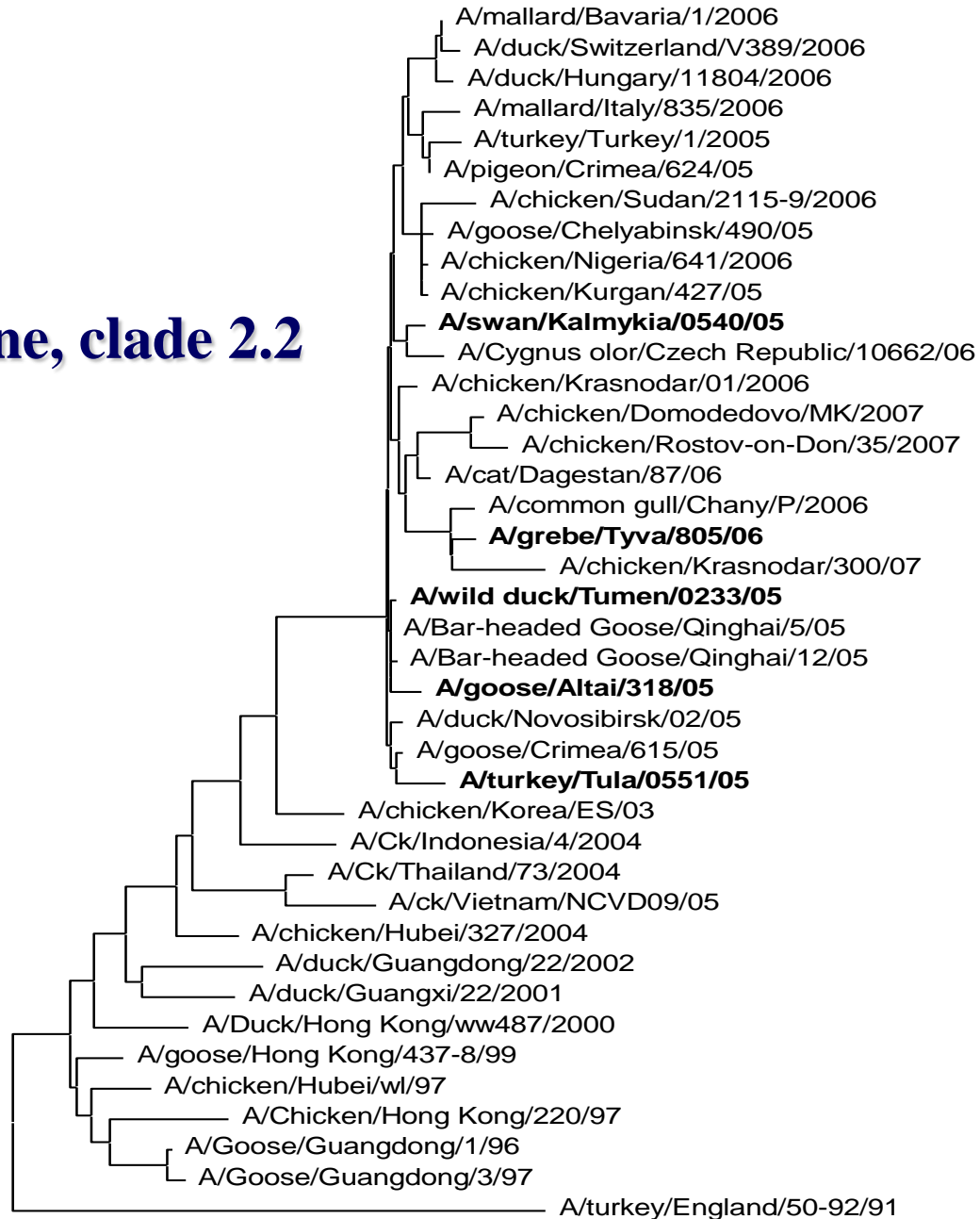
- A/duck/Novosibirsk/02/05*
- A/goose/Altai/318/05
- A/w_duck/Tumen/233/05
- A/goose/Chelyabinsk/490/05
- A/chicken/Kurgan/427/05
- A/turkey/Tula/551/05
- A/swan/Kalmykia/540/05
- A/pigeon/Crimea/624/05
- A/cat/Dagestan/87/06
- A/grebe/Tyva/805/06
- A/chicken/Krasnodar/19/06
- A/swan/Stavropol/0035/06
- A/turkey/KBR/42/06
- A/chicken/Dagestan/79/06
- A/chicken/Volgograd/236/06
- A/chicken/Krasnodar/776/07
- A/chicken/Primorskii/85/08*
- A/gull/Tyva/96/09
- A/shelduck/Tyva/99/09
- A/grebe/Tyva/100/09
- A/grebe/Tyva/433/10

* - vaccine strain



Genetic line A/Gs/Gd/96

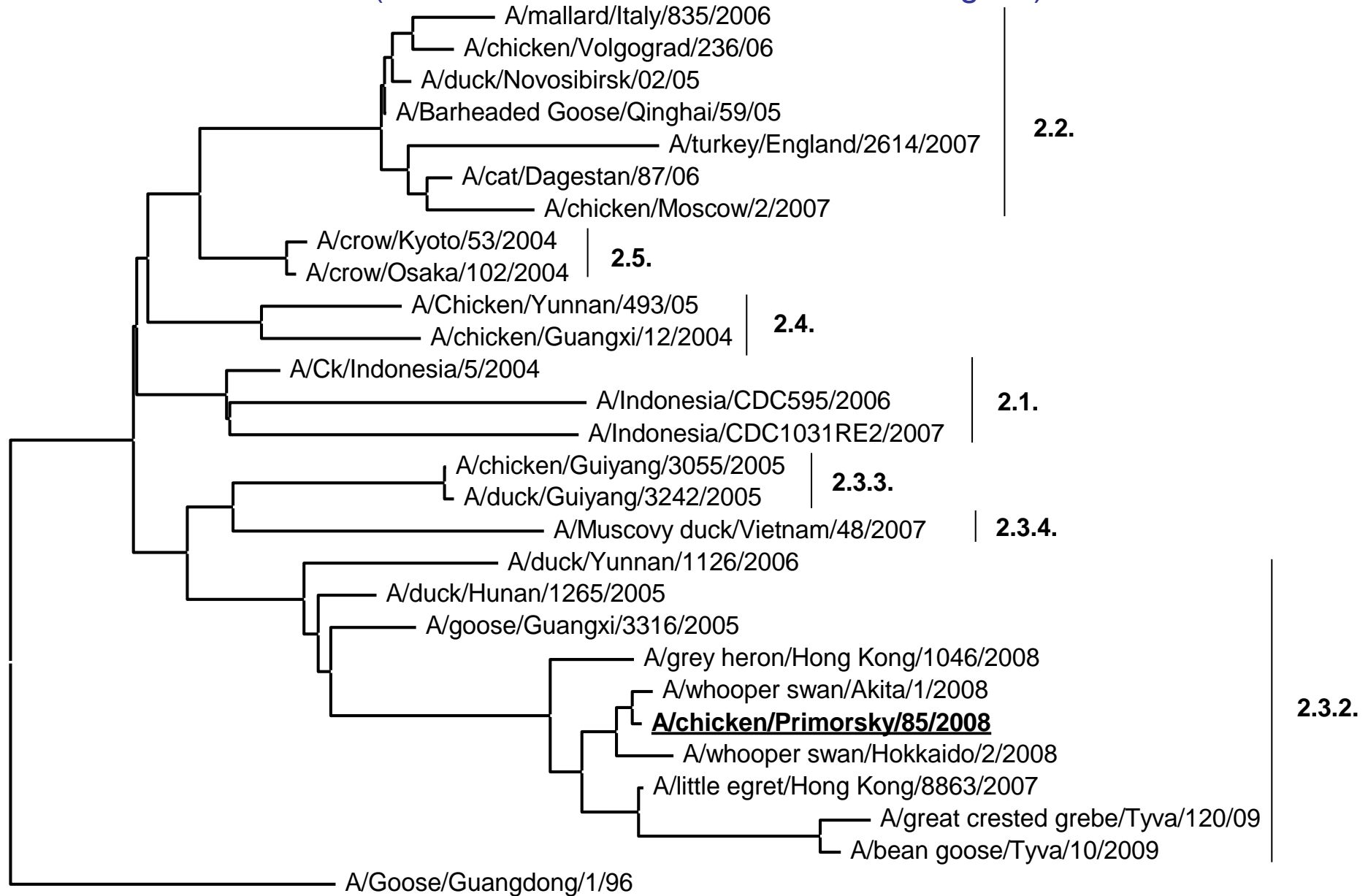
Qinghai subline, clade 2.2



1

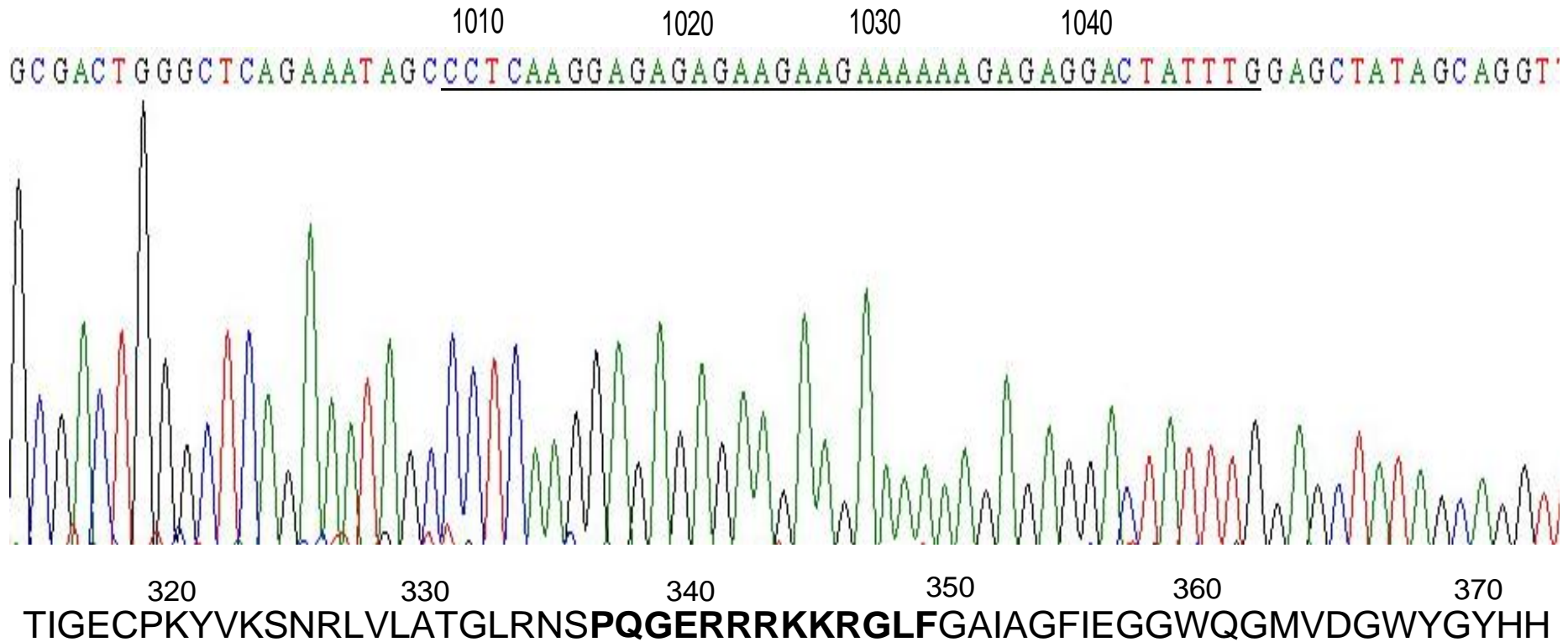
0.01

Only one outbreak in wild migrating birds in Tyva Republic in 2009
(in lakes common for Russia and Mongolia)



0.005

HAo cleavage site structure of HPAI virus isolates H5N1

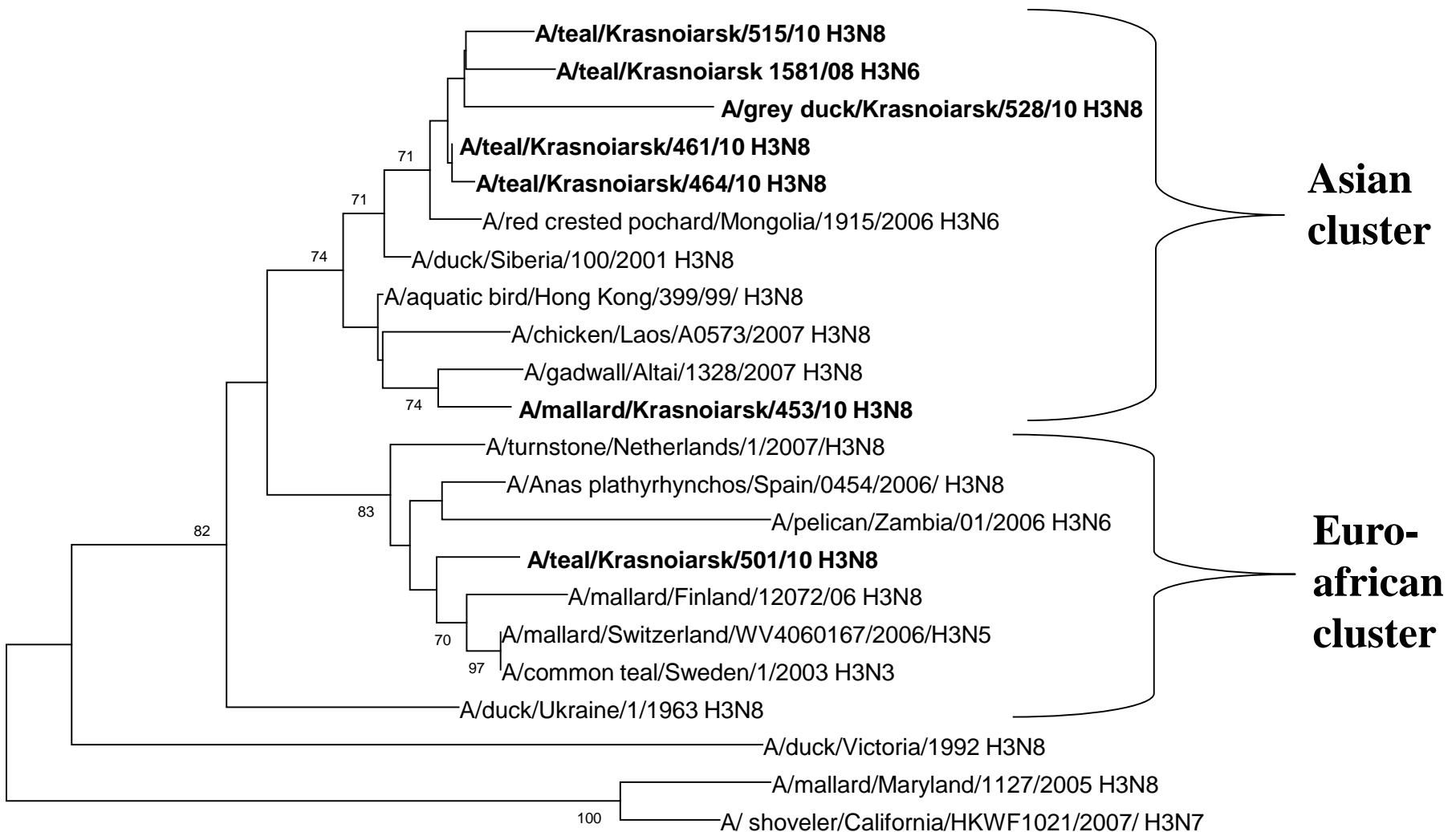


Clade 2.2

PQRERRRKK-RGLF

Clade 2.3.2

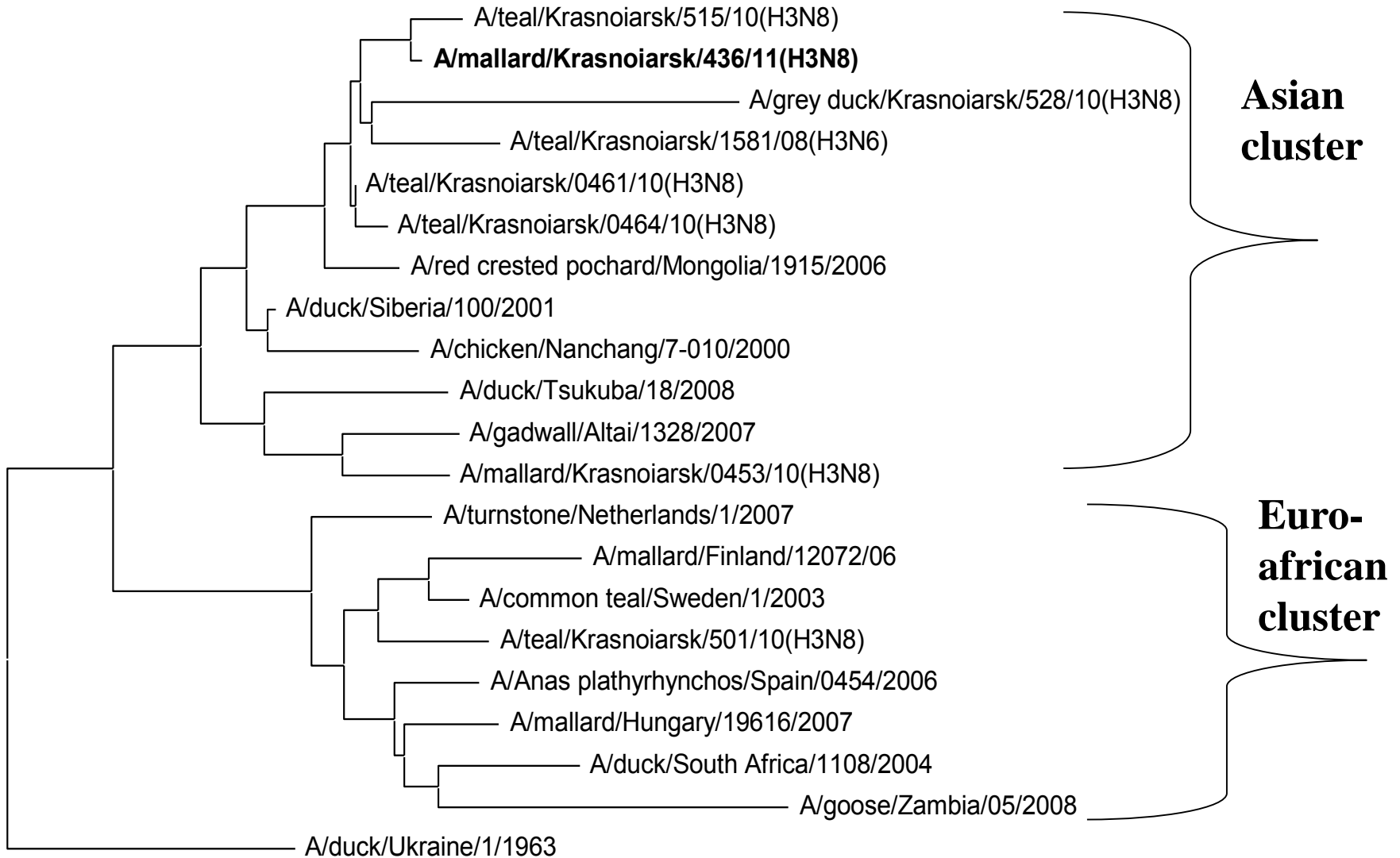
LP AI H3 isolates (gene H)_2010



HA cleavage site: PEKQTRGLF

Nucleotide sequences phylogenetic analysis results of H-gene (H3) fragment (721-1084 b.p.)

LP AI H3 isolates (gene H)_2011

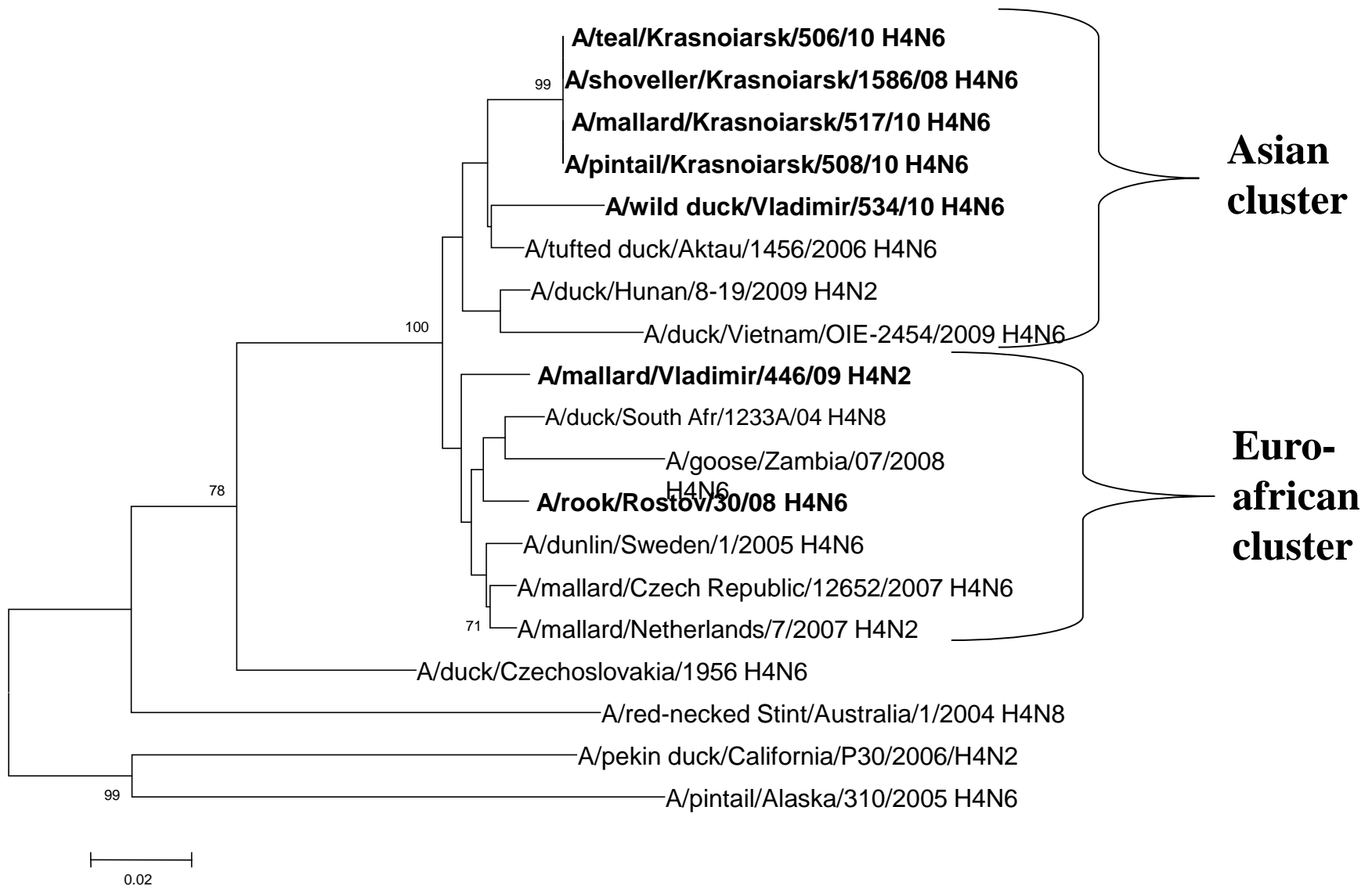


0.005

HA cleavage site: PEKQTRGLF

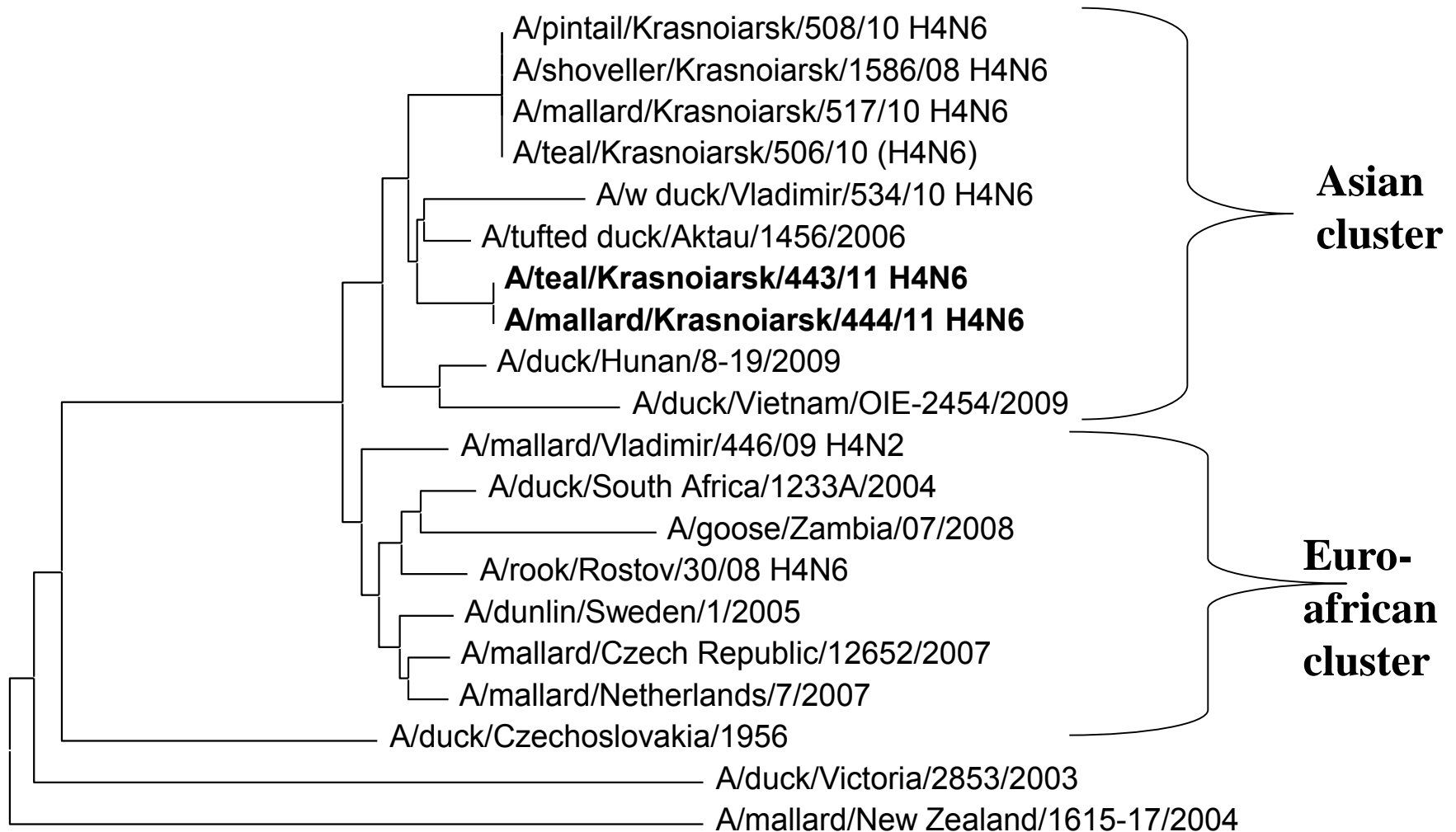
Nucleotide sequences phylogenetic analysis results of H-gene (H3) fragment (721-1084 b.p.)

LP AI H4 isolates (gene H)_2010



Nucleotide sequences phylogenetic analysis results of H-gene (H4) fragment (745-1143 b.p.)

LPAl H4 isolates (gene H)_2011



Nucleotide sequences phylogenetic analysis results of H-gene (H4) fragment (745-1143 b.p.)

HAo cleavage site structure of H4 LPAI virus isolates

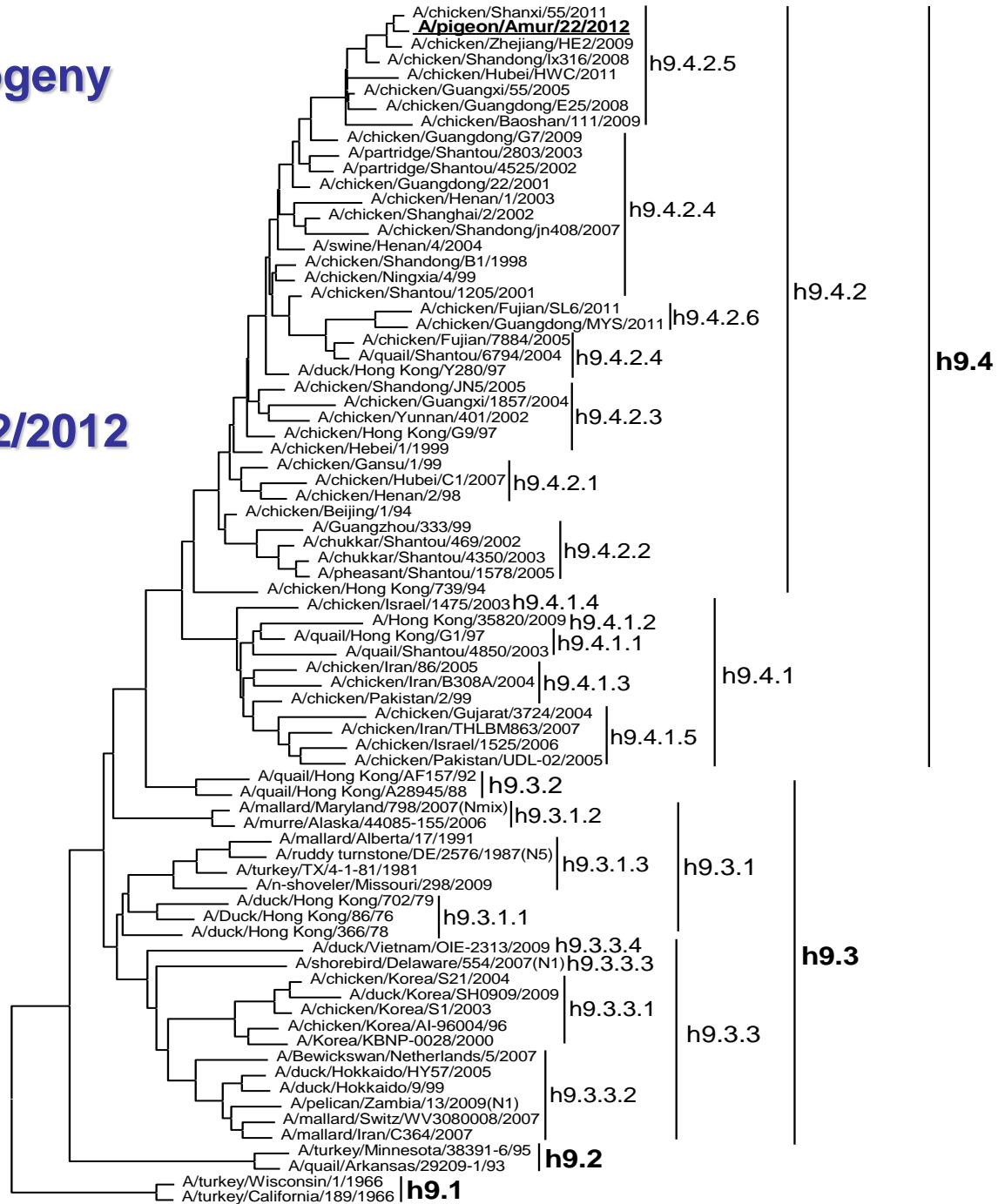
Isolate	HA cleavage site sequence
A/swine/Ontario/01911-2/99 (H4N6)	KATRGLF
A/mallard/Maryland/1241/2005 (H4N6)	KATRGLF
A/pochard/Buryatiya/1903/2000 (H4N6)	KAPRGLF
A/muskrat/Buryatiya/1944/2000 (H4N6)	KAPRGLF
A/duck/Czechoslovakia/1956 (H4N6)	KASRGLF
A/dunlin/Sweden/1/2005(H4N6)	RASRGLF
A/duck/Mongolia/583/02 2002 (H4N7)	KASRGLF
A/mallard/Netherlands/7/2007 (H4N2)	KASRGLF
A/rook/Rostov/30/08 (H4N6)	RASRGLF
A/shoveller/Krasnoiarsk/1586/08 (H4N6)	KESRGLF
A/mallard/Vladimir/446/09 (H4N2)	KASRGLF
A/teal/Krasnoiarsk/506/10 (H4N6)	KESRGLF
A/pintail/Krasnoiarsk/508/10 (H4N6)	KESRGLF
A/mallard/Krasnoiarsk/517/10 (H4N6)	KESRGLF
A/wild duck/Vladimir/534/10 (H4N6)	KASRGLF
A/teal/Krasnoiarsk/443/11 (H4N6)	KASRGLF
A/mallard/Krasnoiarsk/444/11 (H4N6)	KASRGLF

H9N2 Avian influenza virus subtype identified in the Russian Federation in 2012

Isolate	Region	Date	Cleavage site	HI test	IVPI
A/chicken/Amur/3/12 (H9N2)	Amur oblast	07.02.12	PSRSSR_GLF	1:256	0.0
A/pigeon/Amur/22/12 (H9N2)	Amur oblast	27.02.12	PSRSSR_GLF	1:64	-
A/duck/Moscow/cons/12	Moscow oblast	03.12.12	PAASDR_GLF	-	-

LPAI H9N2 phylogeny (gene H)

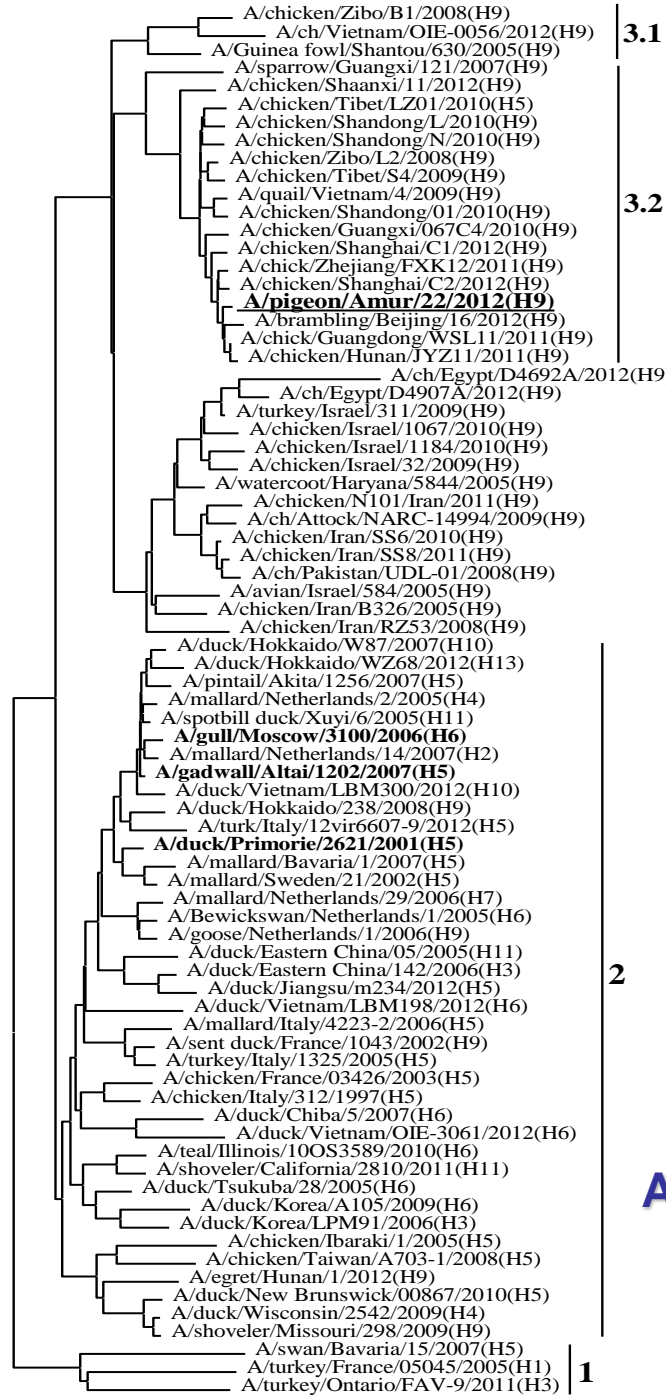
A/pigeon/Amur/22/2012 (H9N2)



0.02

N2

N2 phylogenetic analysis (gene N)



3

3.3

2

1

0.02

A/pigeon/Amur/22/2012 (H9N2)

Thank you for attention!

