

Phylogeny, Pathogenicity, Transmission and Host Immune Responses of New H5N6 Avian Influenza Viruses in Chickens and Mice

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Supplemental material: Table S1-S4 and Fig. S1.

Table S1. Sequence comparisons of the four H5N6 avian influenza viruses with their Closest genetic relatives

Virus	Gene	Closest related virus strain	Homology (%)
A/duck/Guangdong/13087/2013 (GD13087)	PB2	A/duck/Hunan/S4220/2011(H5N1)	98.6
	PB1	A/duck/Zhejiang/224/2011(H5N1)	98.3
	PA	A/duck/Hunan/S4220/2011(H5N1)	99.1
	HA	A/wild/duck/Shandong/628/2011(H5N1)	97.4
	NP	A/duck/Hunan/S4220/2011(H5N1)	98.5
	NA	A/duck/Fujian/3242/2007(H6N6)	92.4
	M	A/duck/Hunan/S4220/2011(H5N1)	99.2
	NS	A/duck/Hunan/S4220/2011(H5N1)	98.9
A/goose/Guangdong/14016/2014 (GD14016)	PB2	A/duck/Hunan/S4220/2011(H5N1)	98.6
	PB1	A/duck/Zhejiang/224/2011(H5N1)	98.2
	PA	A/duck/Hunan/S4220/2011(H5N1)	99.0
	HA	A/wild duck/Shandong/628/2011(H5N1)	97.3
	NP	A/duck/Hunan/S4220/2011(H5N1)	98.5
	NA	A/duck/Fujian/3242/2007(H6N6)	92.6
	M	A/duck/Hunan/S4220/2011(H5N1)	99.2
	NS	A/duck/Hunan/S4220/2011(H5N1)	99.0
A/duck/Guangdong/14017/2014 (GD14017)	PB2	A/duck/Hunan/S4220/2011(H5N1)	98.6
	PB1	A/duck/Zhejiang/224/2011(H5N1)	98.2
	PA	A/duck/Hunan/S4220/2011(H5N1)	99.0
	HA	A/wild duck/Shandong/628/2011(H5N1)	97.4
	NP	A/duck/Hunan/S4220/2011(H5N1)	98.5
	NA	A/duck/Fujian/3242/2007(H6N6)	92.6
	M	A/duck/Hunan/S4220/2011(H5N1)	99.1
	NS	A/duck/Hunan/S4220/2011(H5N1)	99.0
A/duck/Guangdong/14085/2014 (GD14085)	PB2	A/duck/Hunan/S4220/2011(H5N1)	98.3
	PB1	A/duck/Zhejiang/224/2011(H5N1)	98.1
	PA	A/duck/Hunan/S4220/2011(H5N1)	98.9
	HA	A/wild duck/Shandong/628/2011(H5N1)	97.1
	NP	A/duck/Hunan/S4220/2011(H5N1)	98.3
	NA	A/duck/Guangdong/s3073/2010(H6N6)	97.9
	M	A/duck/Hunan/S4220/2011(H5N1)	98.9
	NS	A/duck/Hunan/S4220/2011(H5N1)	99.3

Table S2. Summary of Signature Amino Acid Mutation in GD13087, GD14016, GD14017, and GD14085 Viruses.

Gene	Amino Acid position	Virus			
		GD13087	GD14016	GD14017	GD14085
HA ^a	190	E	E	E	E
	225	G	G	G	G
	226	Q	Q	Q	Q
	227	R	R	R	R
	228	G	G	G	G
	325-336	PLRERRRRKRGL	PLRERRRRKRGL	PLRERRRRKRGL	PLREKRRKRGL
	(Cleavage site)	F	F	F	F
NA ^b	58-68 (Stalk region)	deletion	deletion	deletion	TIINNHPQNNF
	119	E	E	E	E
	275	H	H	H	H
	293	R	R	R	R
	295	N	N	N	N
PB2 ^c	627	E	E	E	E
	701	D	D	D	D
M2 ^c	26	L	L	L	L
	27	V	V	V	V
	30	A	A	A	A
	31	S	S	S	S
	34	G	G	G	G
NS1 ^c	42	S	S	S	S
	92	E	E	E	E

^a According to H3 numbering.

^b According to N6 numbering.

^c According to H5 numbering.

Table S3. Summary of the Potential glycosylation site in HA and NA genes of the GD13087, GD14016, GD14017, and GD14085 viruses.

Segments	Amino position ^a	Acid	Virus			
			GD13087	GD14016	GD14017	GD14085
HA1	26		NNS	NNS	NNS	NNS
	27		NST	NST	NST	NST
	39		NVT	NVT	NVT	NVT
	181		NNT	NNT	NNT	NNT
	302		NSS	NSS	NSS	NSS
HA2	499		NGT	NGT	NGT	NGT
	558		NGS	NGS	NGS	NGS
NA	51		NET	NET	NET	NET
	54		NPT	NPT	NPT	.. ^b
	70		NIT	NIT	NIT	NIT
	86		NLT	NLT	NLT	NLT
	146		NGT	NGT	NGT	NGT
	201		NAS	NAS	NAS	NAS

^a Amino acids sites (HA segment and NA segment) were located by taking A/goose/Guangdong/1/1996 (H5N1), A/duck/Guangdong/s3073/2010(H6N6) and A/duck/Fujian/3242/2007(H6N6).

^b The potential N-glycosylation sites at position is absent.

Table S4. Amino-acid Difference among the GD13087, GD14016, GD14017, and GD14085 viruses.

Gene	Amino Acid position	Virus			
		GD13087	GD14016	GD14017	GD14085
HA ^a	10	V	V	V	I
	16	S	S	S	G
	46	Q	Q	Q	R
	56	R	R	R	K
	74	G	G	G	G
	110	N	S	S	N
	130	T	T	T	I
	139	P	P	P	T
	142	E	E	E	D
	156	M	M	M	T
	167	T	T	T	I
	177	E	K	K	K
	178	M	M	M	I
	185	R	R	R	Q
	214	V	V	V	I
	261	G	E	G	G
	263	F	Y	F	F
	285	M	M	M	V
	341	R	R	R	K
	383	R	R	R	K
	393	I	V	V	V
	516	K	E	E	E
NA ^b	15	V	V	V	M
	22	E	E	E	E
	39	L	L	L	M
	44	T	T	T	N
	54	N	N	N	T
	58-68 (Stalk region)	deletion	deletion	deletion	TIINNHPQNNF
	77	N	N	N	T
	82	R	R	R	G
	83	T	T	T	H
	115	L	L	L	I
	143	R	R	R	K
	159	I	I	I	V
	209	R	R	R	G
	220	V	A	A	A
	235	K	K	K	G

Table S4. Amino-acid Difference among the GD13087, GD14016, GD14017, and GD14085 viruses. Continued

Gene	Amino position	Acid	Virus			
			GD13087	GD14016	GD14017	GD14085
NA ^b	250	K	K	K	K	R
	252	A	A	A	A	E
	264	Q	Q	Q	Q	K
	270	Q	Q	Q	Q	K
	272	N	N	N	N	D
	285	A	A	A	A	S
	286	G	G	G	G	E
	291	V	V	V	V	I
	303	I	I	I	I	V
	316	K	K	K	K	R
	338	D	D	D	D	E
	391	P	P	P	P	A
	392	T	T	T	T	I
	394	Y	Y	Y	Y	H
	396	L	L	L	L	I
	416	N	T	T	T	N
	436	I	S	S	S	S
	437	D	D	D	D	S
	445	M	M	M	M	I
	452	R	R	R	R	K
PB2 ^a	144	R	R	R	R	Q
	299	K	K	K	K	R
	451	I	I	I	I	L
	637	I	I	I	I	T
	754	I	I	I	I	N
PB1 ^a	386	K	K	K	K	R
	387	K	K	K	K	E
	430	R	R	R	R	K
	621	Q	Q	Q	Q	K
	635	R	R	R	R	K
	648	A	A	A	A	T
	654	G	G	G	G	S
PA ^a	254	N	N	N	N	D
	261	M	M	M	M	I
	262	K	K	K	K	R
	272	D	D	D	D	E
	300	E	E	E	E	K
	405	S	S	S	S	G
	677	E	G	G	G	E
	688	D	D	D	D	E

Table S4. Amino-acid Difference among the GD13087, GD14016, GD14017, and GD14085 viruses. Continued

Gene	Amino Acid position	Virus			
		GD13087	GD14016	GD14017	GD14085
NP ^a	27	A	T	T	A
	33	V	V	V	I
	105	I	I	I	M
	186	V	V	V	I
	450	N	N	N	S
M1 ^a	37	A	T	A	T
	75	Q	L	Q	Q
	116	A	S	A	S
M2 ^a	13	N	N	N	K
	50	Y	C	Y	C
	97	E	E	E	K
NS1 ^a	18	I	V	V	V
	122	S	S	S	T
	192	I	I	I	V

^a According to H5 numbering.

^b According to N6 numbering.

Fig S1. Phylogenetic analysis of PB2, PB1, PA, NP, M1, NS1. The trees were constructed by using the neighbor joining method with the Maximum Composite Likelihood model and MEGA version 4.0 (<http://www.megasoftware.net>) with 1,000 bootstrap replicates based on the following sequences: PB2 (S1A): nt 28 to 2307; PB1 (S1B): nt 25 to 2298; PA (S1C): nt 25 to 2175; NP (S1D): nt 46 to 1542; M1 (S1E): nt 26 to 784; NS1 (S1F): nt 27 to 704. Abbreviation: IDN, Indonesia; QH, Qinghai; VN, Vietnam; GY, Guiyang. The virus marked with black triangle '▲' is the H5N6 virus isolated and sequenced in this study.

Fig. S1A.

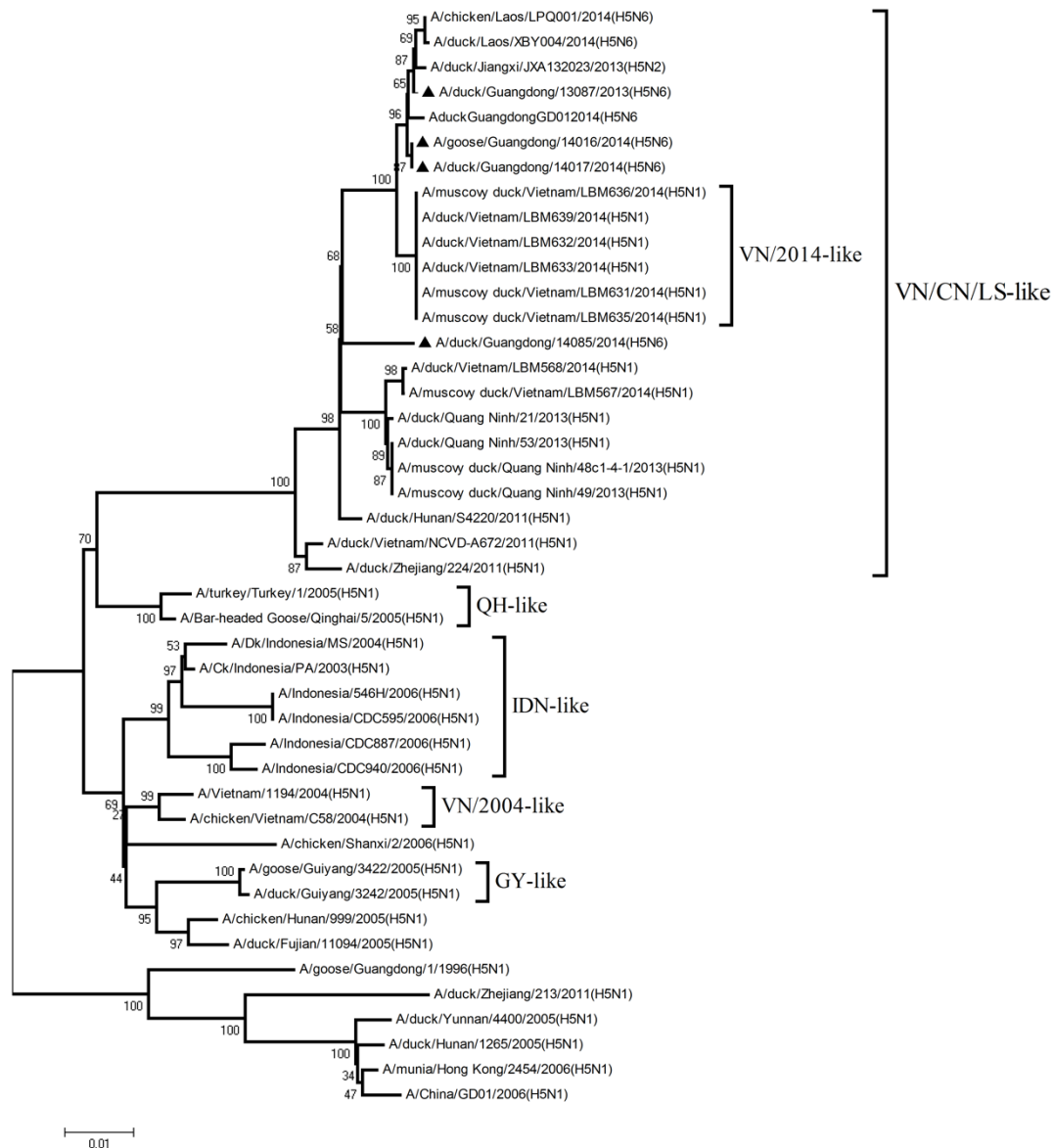


Fig. S1B.

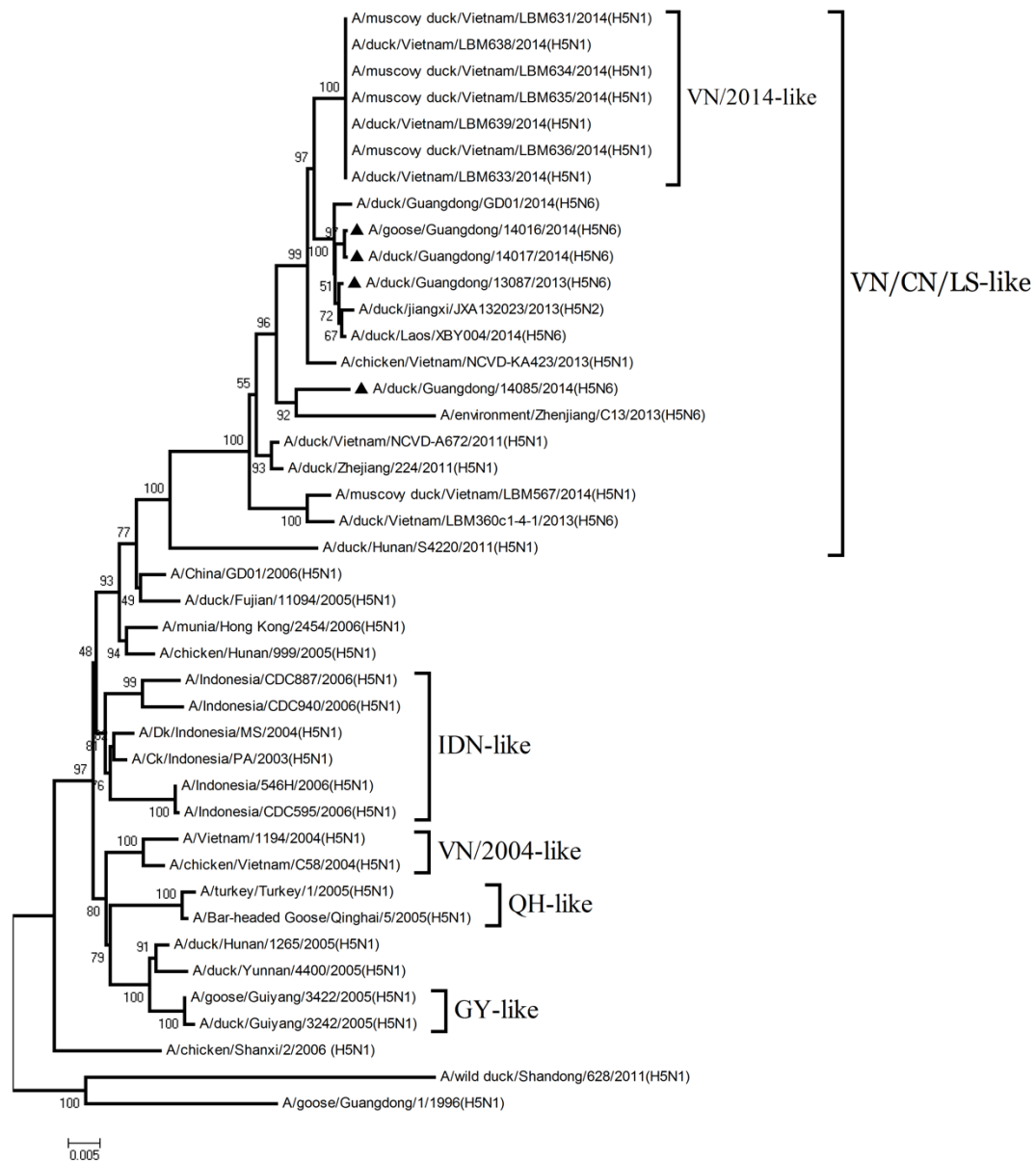


Fig. S1C.

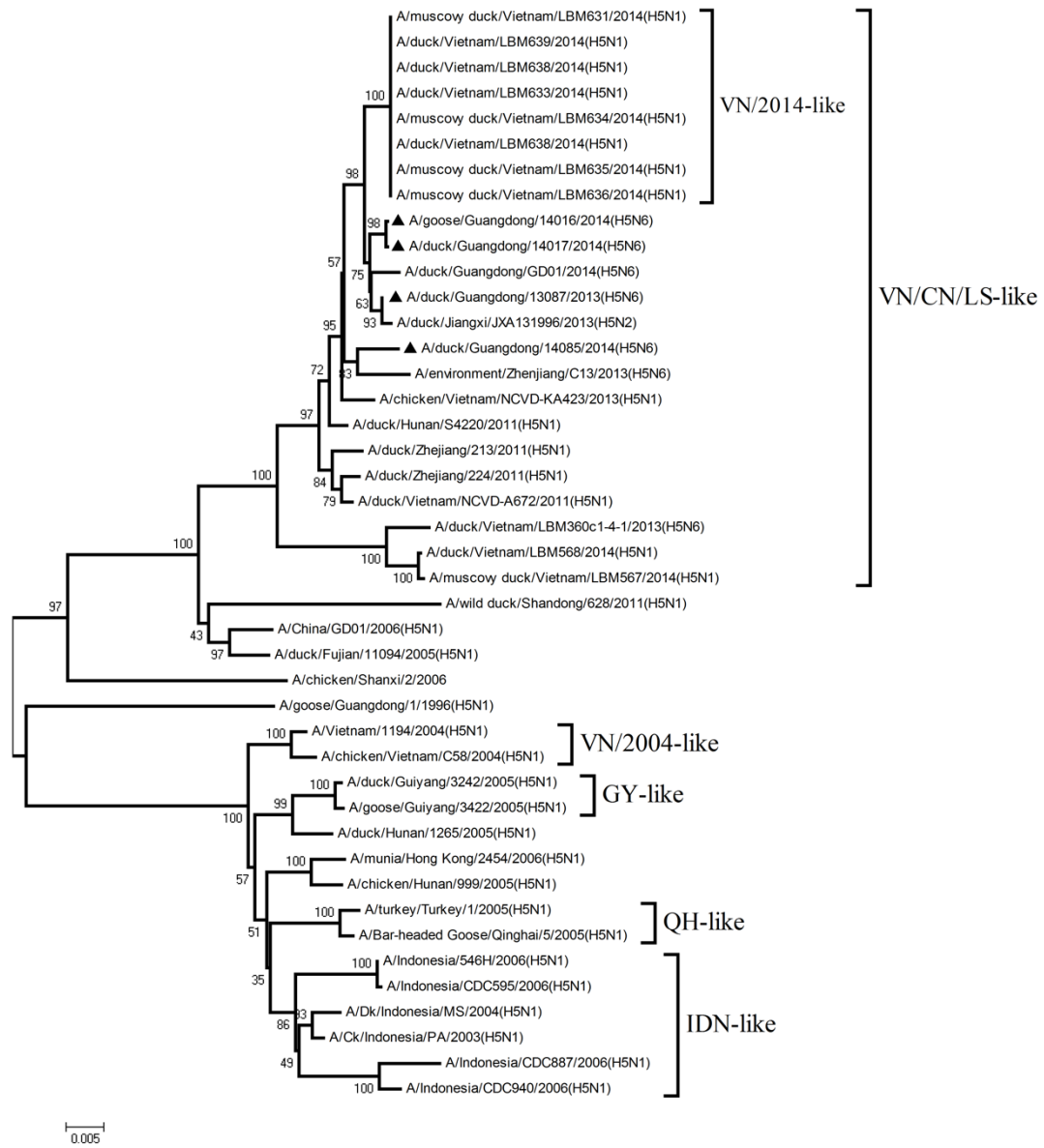


Fig. S1D.

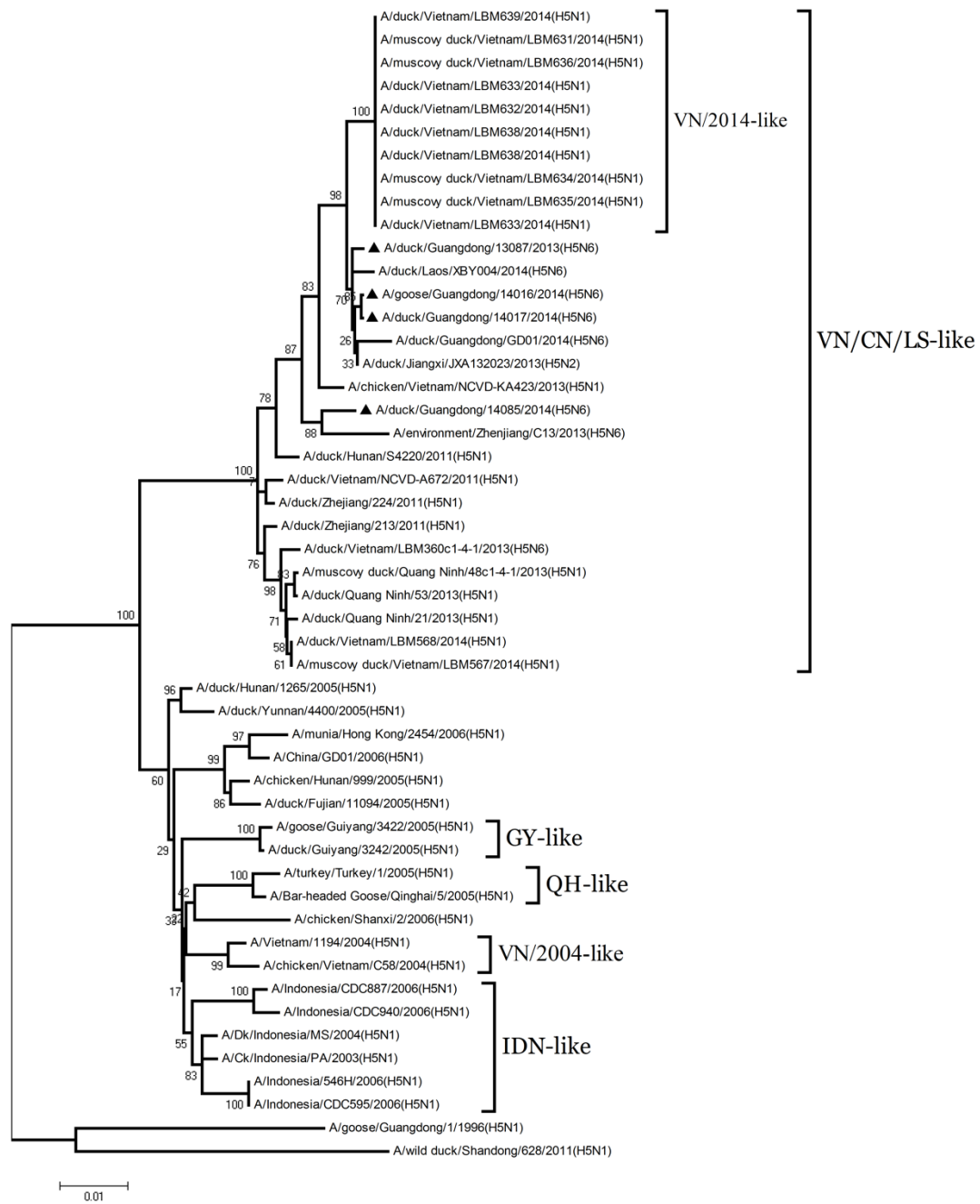


Fig. S1E.

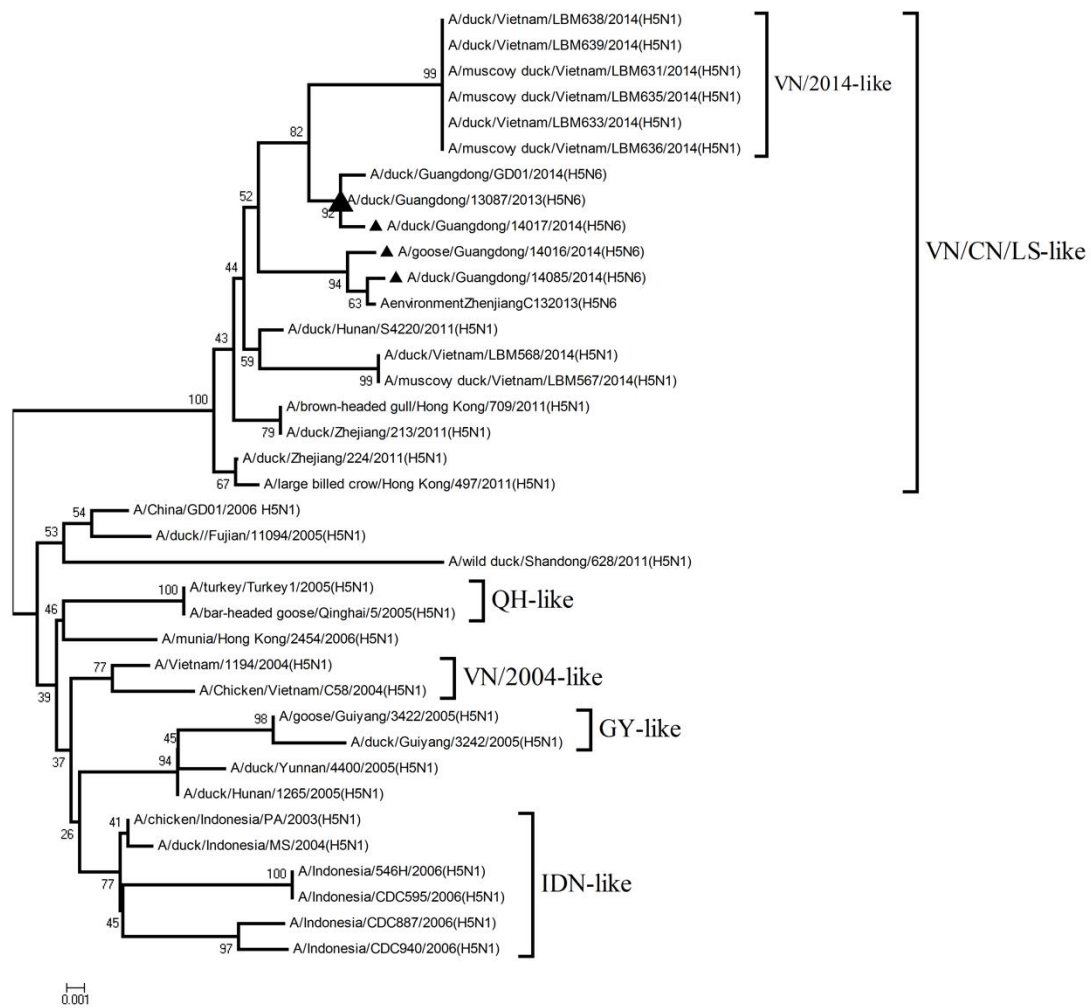


Fig. S1F.

