

**Genetic and molecular characterization of H9N2 and H5 avian influenza viruses
from live poultry markets in Zhejiang Province, eastern China**

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Supplementary Material:

Figure legends:

Figure S1. Percentage of avian influenza virus positive poultry swabs by month. Cloacal swabs
samples were collected from live poultry markets in Zhejiang Province, eastern China from
2013–2014. The percentages of the swabs that tested positive for avian influenza virus each month
during the collection period are shown.

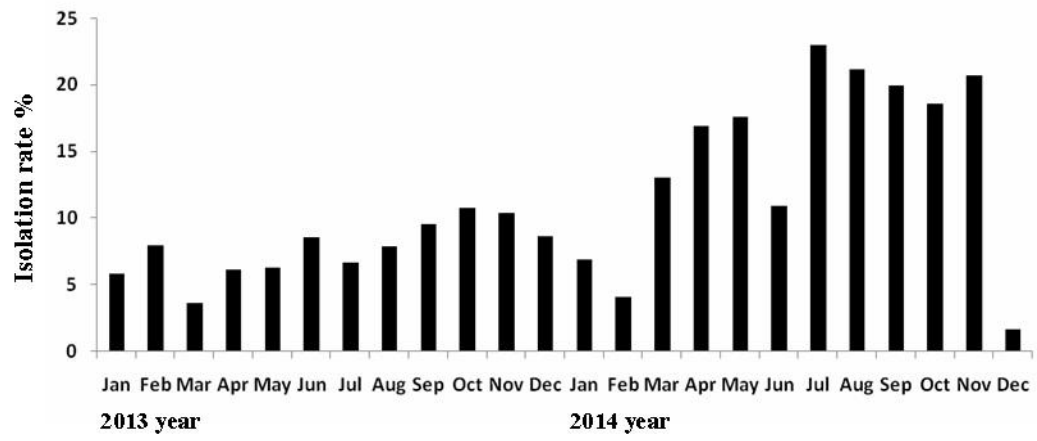
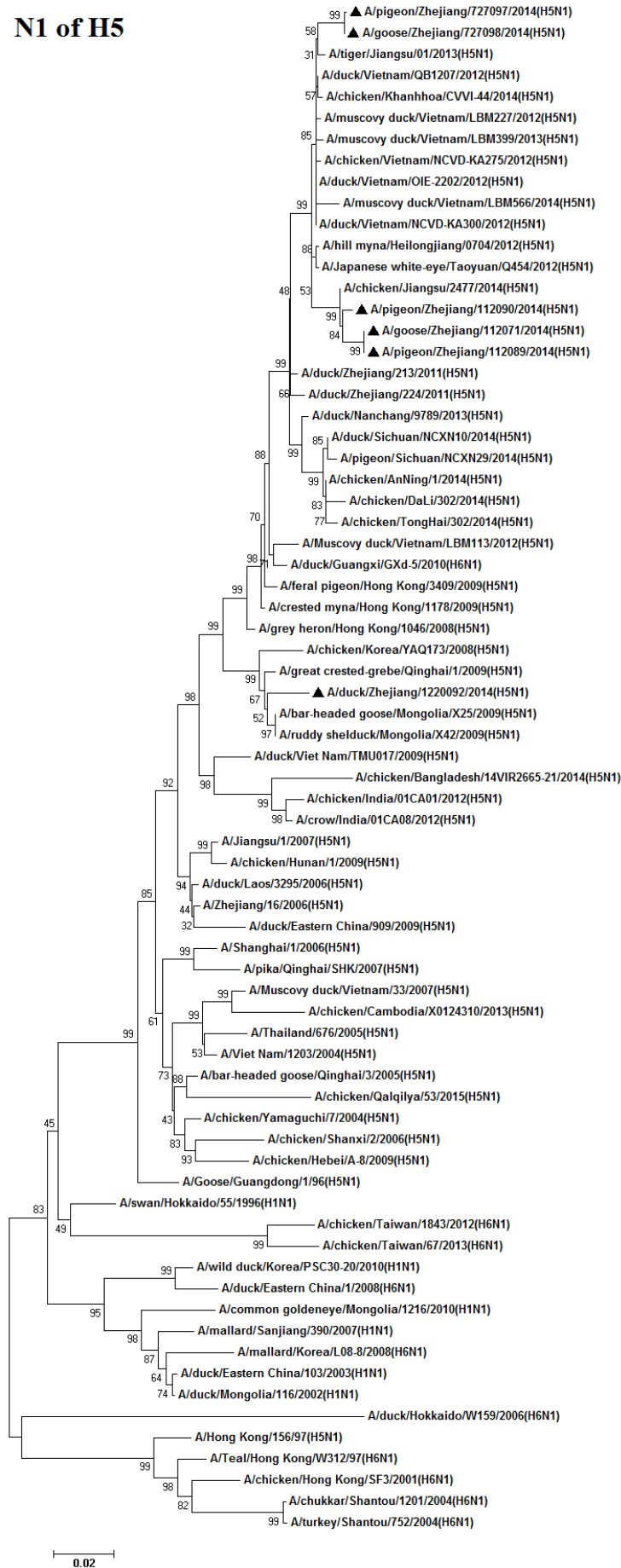
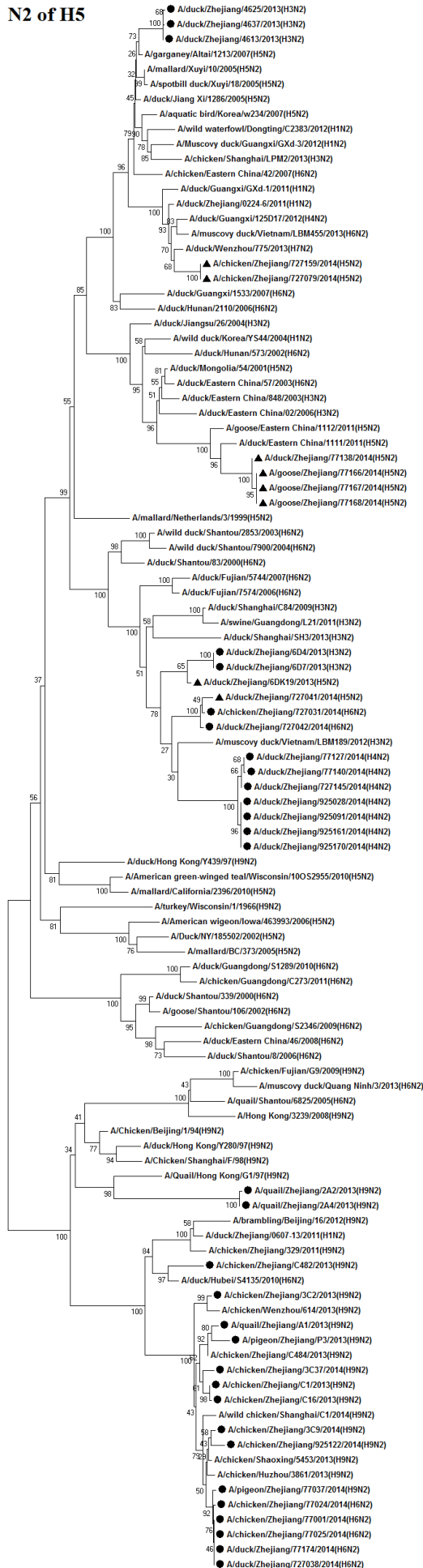


Figure S2. Phylogenetic analysis of the NA (N1, positions 1–1410; N2, 40–1340; N6, 40–1352; N8, 44–1352), PB2 (positions 1–2280), PB1 (positions 16–2268), PA (positions 1–2151), NP (positions: 1–1436), M (positions 29–923), and NS (positions 28–768) segments of the H5 avian influenza viruses compared to reference influenza viruses. The tree was created by the maximum likelihood method and bootstrapped with 1,000 replicates using the MEGA6 package. The H5 viruses from poultry in China identified in this study are highlighted by triangles. The non-H5 viruses from poultry in China identified in this study are highlighted by dots. Scale bar represents the distance unit between sequence pairs.

N1 of H5

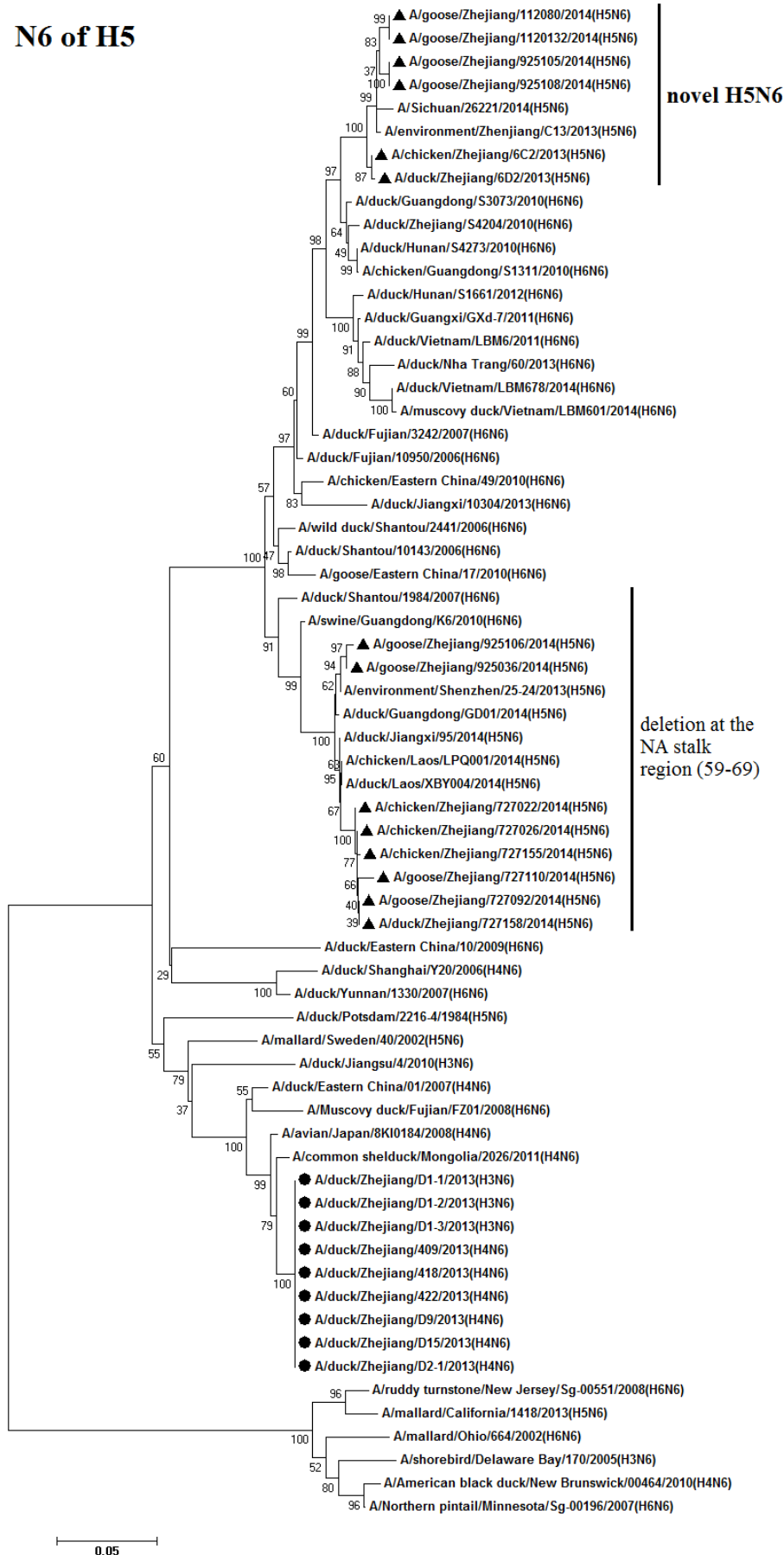


N2 of H5

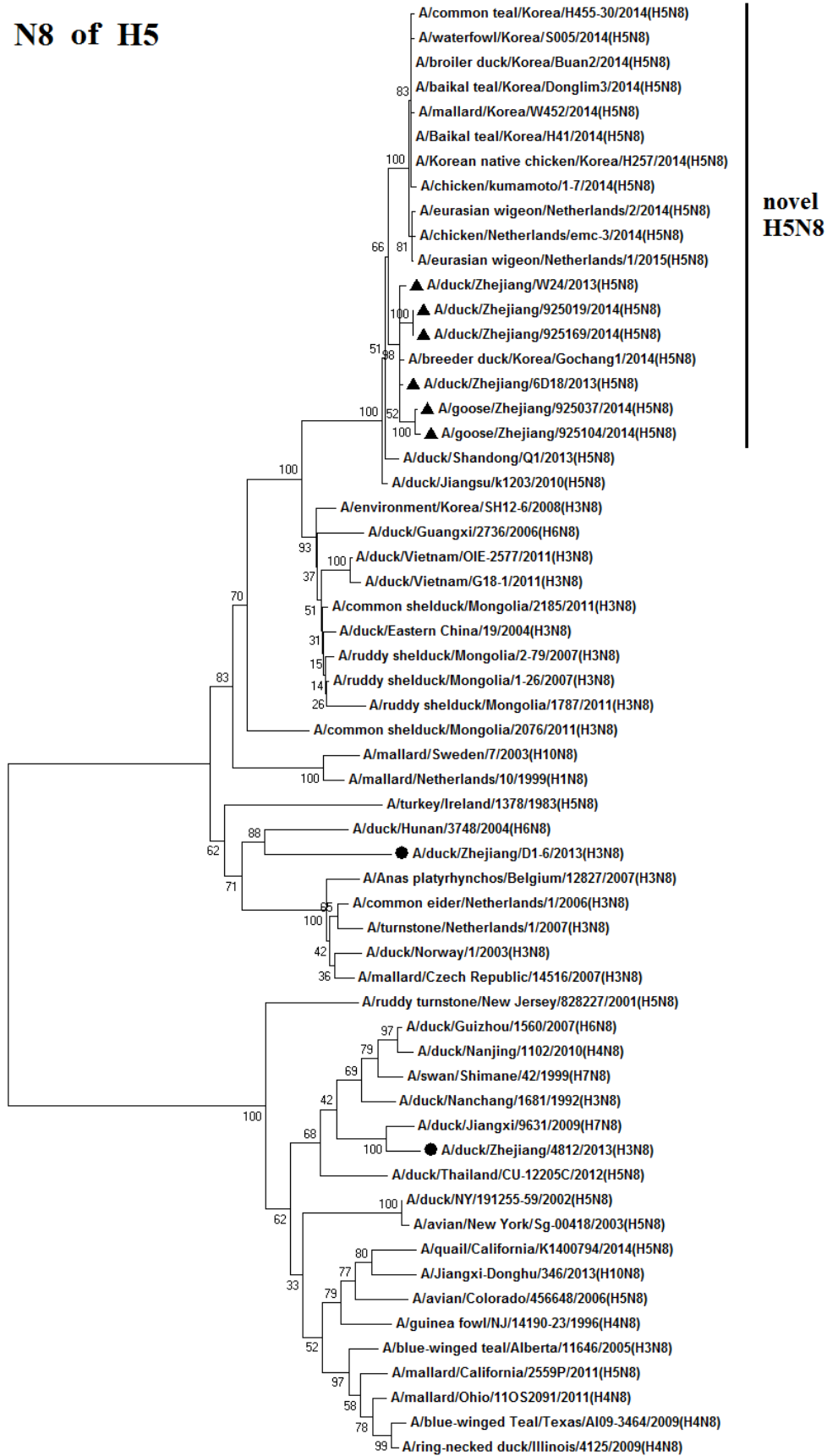


H9N2-like

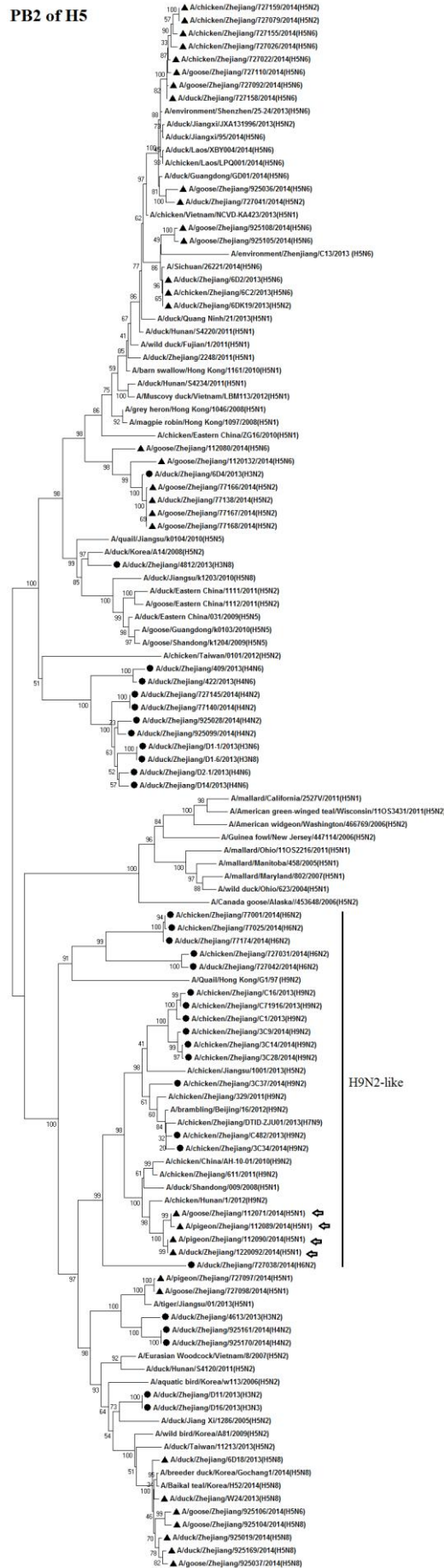
N6 of H5



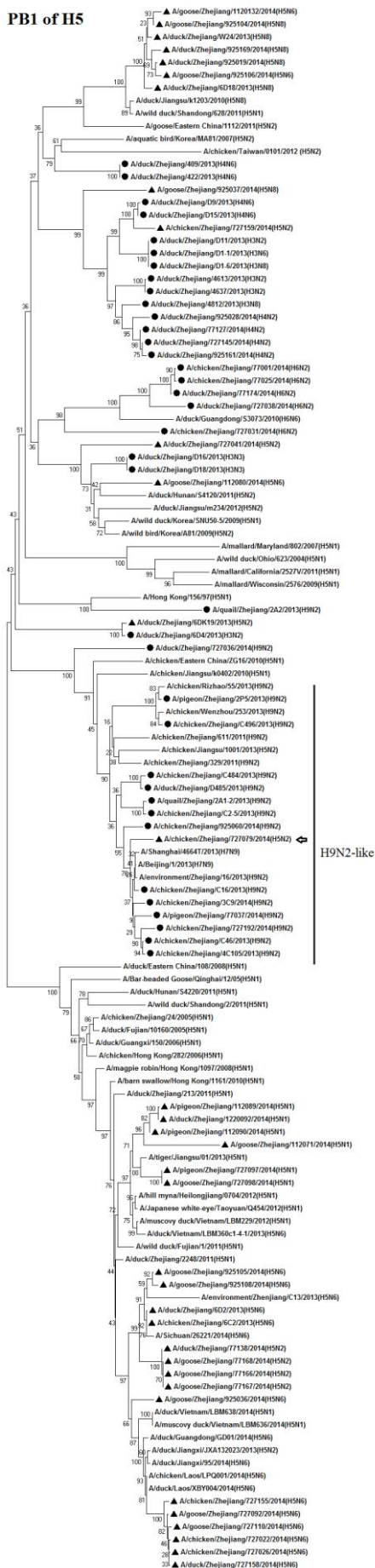
N8 of H5



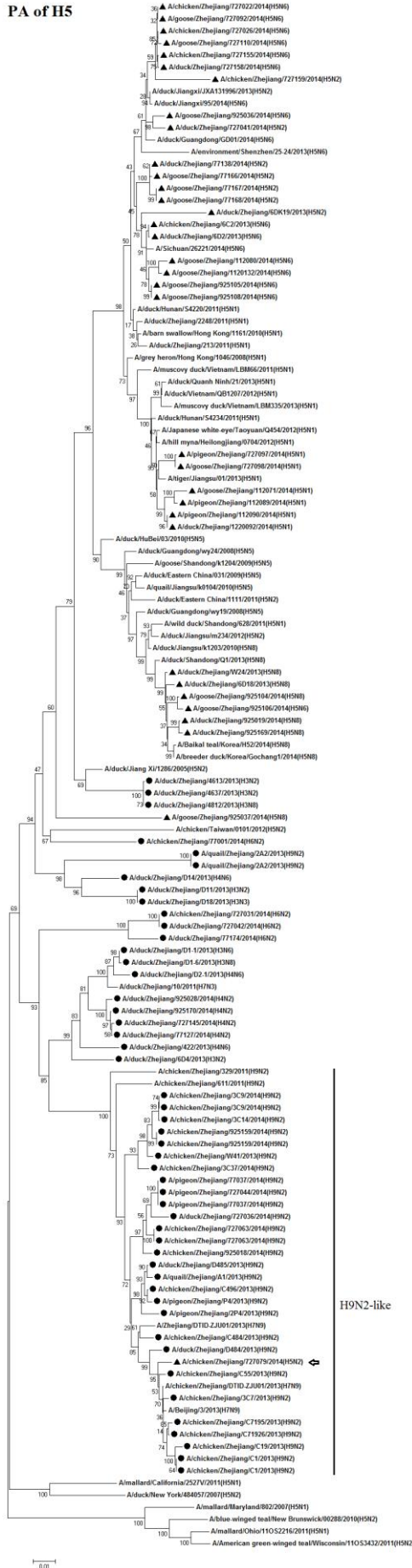
PB2 of H5



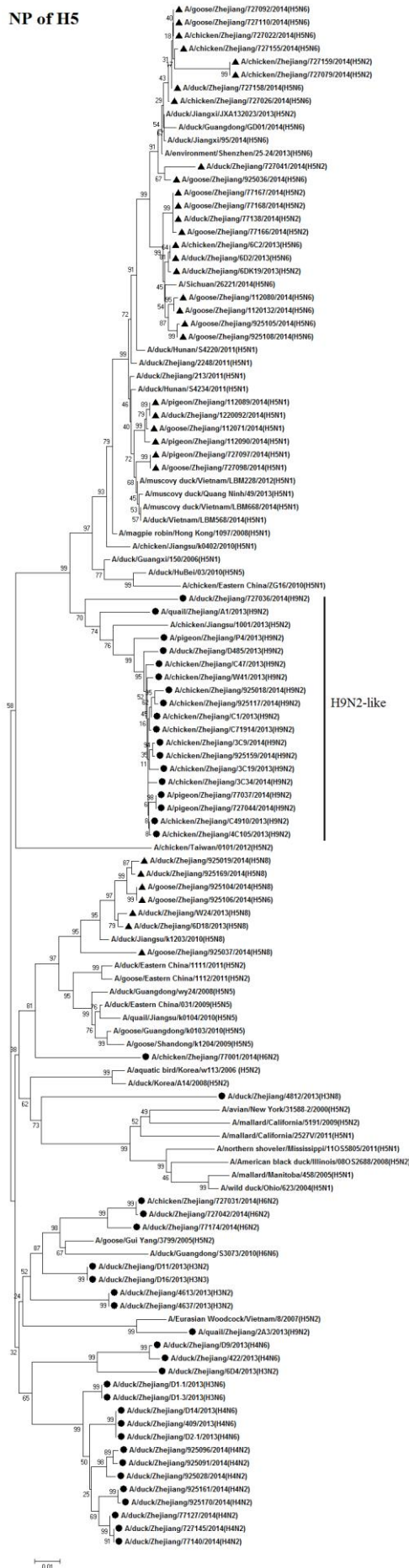
PB1 of H5



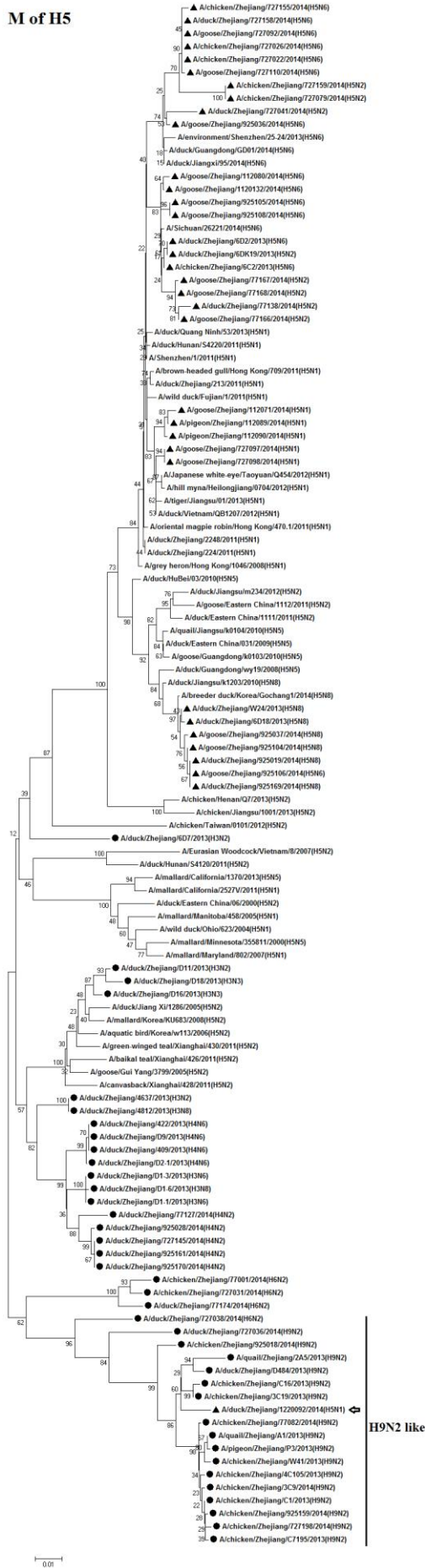
PA of H5



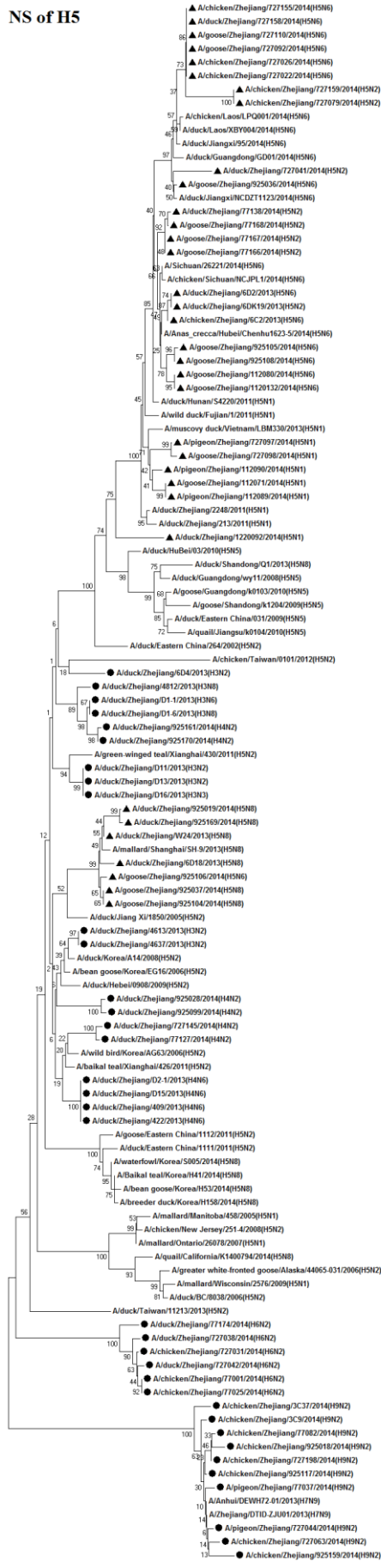
NP of H5



M of H5



NS of H5



deletion of position
80-84 in NS1

without
deletion of position
80-84 in NS1

H9N2-like

Table S1. Genotype descriptions of the classical reference viruses and H9N2 avian influenza virus field isolates

Representative isolates ^a	Lineage ^b at the following gene segment								Genotypes
	PB2	PB1	PA	HA	NP	NA	M	NS	
CK/BJ/1/94	BJ94	BJ94	BJ94	BJ94	BJ94	BJ94	BJ94	BJ94	A
CK/GD/6/97	G1	G1	BJ94	BJ94	BJ94	G9	BJ94	BJ94	B
CK/GX/10/99	G1	G1	G1	BJ94	BJ94	G9	BJ94	G1	C
CK/GX/9/99	G1	G1	G1	BJ94	BJ94	BJ94	BJ94	G1	D
CK/HLJ35//00	G1	G1	G1	Ws66	Ws66	G9	BJ94	BJ94	E
CK/GD/10/00	BJ94	BJ94	BJ94	BJ94	BJ94	G9	BJ94	BJ94	F
CK/HN/26/00	G1	BJ94	BJ94	BJ94	BJ94	BJ94	BJ94	BJ94	G
CK/SH/F/98	SH98	SH98	SH98	BJ94	SH98	BJ94	BJ94	BJ94	H
CK/GD/56/01	SH98	SH98	SH98	BJ94	SH98	G9	BJ94	BJ94	I
CK/JS/1/99	SH98	SH98	SH98	BJ94	SH98	BJ94	BJ94	HK76	J
CK/SD/1/03	BJ94	BJ94	Kor96	BJ94	BJ94	BJ94	BJ94	BJ94	K
DK/Js/3/05	SH98	SH98	SH98	BJ94	SH98	BJ94	BJ94	Kor96	L
CK/SD/B1/98	BJ94	BJ94	SH98	BJ94	BJ94	BJ94	BJ94	BJ94	M
CK/SD/B2/07	BJ94	SH98	SH98	BJ94	SH98	BJ94	BJ94	BJ94	N
CK/SD/B4/07	SH98	SH98	SH98	BJ94	SH98	BJ94	G1	BJ94	O
CK/ZB/B1/08	SH98	SH98	SH98	BJ94	SH98	G9	G1	BJ94	P
CK/HN/L1/08	SH98	BJ94	Y439	BJ94	SH98	G9	G1	BJ94	Q
A/chicken/Zhejiang/C1/2013	G1	SH98	SH98	BJ94	SH98	BJ94	G1	BJ94	S

A/quail/Zhejiang/2A2/2013 G1 G1 Y439 BJ94 G1 G1 G1 G1 Novel

Note: Viruses were harvested during surveillance of live poultry markets in Zhejiang, China from January 2013 through December 2014.

^a Genotypes of representative isolates were assigned as described previously ^{7, 26, 27, 49}.

^b The lineage for each viral gene segment is coded as: BJ94= Ck/BJ/1/94-like genes; SH98= Ck/SH/F/98-like genes; G1= Qa/HK/G1/97-like genes; Y439= Dk/HK/Y439/97-like genes; HK76= DK/HK/d73/76-like genes; Kor96= Ck/Kor/323/96-like genes.

Table S2. Comparison of amino acid sequences of HA and NA genes of H9N2 avian influenza viruses isolated from poultry in Zhejiang, China.

Representative isolates	Amino acids at the receptor-binding sites		Cleavage site aa 333–340	NA deletion (aa)
	Selected locations ^a	Left edge aa 232–237		
A/chicken/Zhejiang/C1/2013	YWTN AL Y	NGLMGR	PSR SS R/GL	62–64
A/chicken/Zhejiang/C19/2013	YWTN AL Y	NGLMGR	PSR SS R/GL	62–64
A/chicken/Zhejiang/C71916/2013	YWTN AL Y	NGLMGR	PSR SS R/GL	62–64
A/chicken/Zhejiang/C16/2013	YWTN T LY	NGLMGR	PS K SSR/GL	62–64
A/chicken/Zhejiang/727198/2014	YWTN T LY	NGLMGR	PSR SS R/GL	62–64
A/chicken/Zhejiang/925117/2014	YWTN T LY	NGLMGR	PSR SS R/GL	62–64
A/quail/Zhejiang/2A5/2013	YWTN V LY	NGLMGR	PSR SS R/GL	62–64
A/quail/Zhejiang/2A2/2013	YWTN T LY	NGLMGR	PSR SS R/GL	38–39
A/quail/Zhejiang/2A3/2013	YWTN T LY	NGLMGR	PSR SS R/GL	38–39
A/quail/Zhejiang/2A4/2013	YWTN T LY	NGLMGR	PSR SS R/GL	38–39
Ck/BJ/1/94	YWTN V LY	NG QQ GR	PAR SS R/GL	NO
Ck/SH/F/98	YWTN AL Y	NG QQ GR	PAR SS R/GL	62–64
Qa/HK/ G1/97	YWT HE LY	NDL Q GR	PAR SS R/GL	38–39
Dk/HK/Y439/97	YWT HE LY	ND QQ GR	PAAS N R/GL	NO
Dk/HK/Y280/97	YWTN T LY	NGL Q GR	PAR SS R/GL	62–64
A/turkey/Wisconsin/1/1966	YWT HE LY	NG QQ GR	PA V SSR/GL	NO

Note: ^a From left to right, ^{aa} 109, 161, 163, 191, 198, 202, and 203.

Residues in ***bold italics*** indicate changes from the consensus alignment.

Table S3. Novel H5 avian influenza virus strains isolated from poultry.

No.	Strain	Subtype	HA clade	District, city	Date of sample collection	Species	H5 virus isolation rate (%)
1	A/pigeon/Zhejiang/727097/2014(H5N1)	H5N1	2.3.2	Yuhang, Hangzhou	Jul 27, 2014	pigeon	1.17% (3/255)*
2	A/pigeon/Zhejiang/112089/2014(H5N1)	H5N1	2.3.2	Haining, Jiaxing	Nov 20, 2014		
3	A/pigeon/Zhejiang/112090/2014(H5N1)	H5N1	2.3.2	Haining, Jiaxing	Nov 20, 2014		
4	A/chicken/Zhejiang/727079/2014(H5N2)	H5N2	2.3.4.6	Yuhang, Hangzhou	Jul 27, 2014	chicken	0.29% (6/2031)
5	A/chicken/Zhejiang/727159/2014(H5N2)	H5N2	2.3.4.6	Yuhang, Hangzhou	Jul 27, 2014		
6	A/chicken/Zhejiang/6C2/2013(H5N6)	H5N6	2.3.4.6	Anji, Huzhou	Dec 14, 2013		
7	A/chicken/Zhejiang/727022/2014(H5N6)	H5N6	2.3.4.6	Yuhang, Hangzhou	Jul 27, 2014		
8	A/chicken/Zhejiang/727026/2014(H5N6)	H5N6	2.3.4.6	Yuhang, Hangzhou	Jul 27, 2014		
9	A/chicken/Zhejiang/727155/2014(H5N6)	H5N6	2.3.4.6	Yuhang, Hangzhou	Jul 27, 2014		
10	A/duck/Zhejiang/1220092/2014(H5N1)	H5N1	2.3.2	Tongxiang, Jiaxing	Dec 25, 2014	duck	1.07% (10/929)
11	A/duck/Zhejiang/6DK19/2013(H5N2)	H5N2	2.3.4.6	Yuhang, Hangzhou	Dec 14, 2013		
12	A/duck/Zhejiang/77138/2014(H5N2)	H5N2	2.3.4.6	Tongxiang, Jiaxing	Jul 7, 2014		
13	A/duck/Zhejiang/727041/2014(H5N2)	H5N2	2.3.4.6	Yuhang, Hangzhou	Jul 27, 2014		
14	A/duck/Zhejiang/6D2/2013(H5N6)	H5N6	2.3.4.6	Anji, Huzhou	Dec 14, 2013		
15	A/duck/Zhejiang/727158/2014(H5N6)	H5N6	2.3.4.6	Yuhang, Hangzhou	Jul 27, 2014		
16	A/duck/Zhejiang/W24/2013(H5N8)	H5N8	2.3.4.6	Tongxiang, Jiaxing	Nov 14, 2013		
17	A/duck/Zhejiang/6D18/2013(H5N8)	H5N8	2.3.4.6	Yuhang, Hangzhou	Dec 14, 2013		
18	A/duck/Zhejiang/925169/2014(H5N8)	H5N8	2.3.4.6	Deqing, Huzhou	Sep 25, 2014		
19	A/duck/Zhejiang/925019/2014(H5N8)	H5N8	2.3.4.6	Deqing, Huzhou	Sep 25, 2014		

20	A/goose/Zhejiang/727098/2014(H5N1)	H5N1	2.3.2	Yuhang, Hangzhou	Jul 27,2014	goose	30% (15/50)
21	A/goose/Zhejiang/112071/2014(H5N1)	H5N1	2.3.2	Haining, Jiaxing	Nov 20,2014		
22	A/goose/Zhejiang/77166/2014(H5N2)	H5N2	2.3.4.6	Tongxiang, Jiaxing	Jul 7, 2014		
23	A/goose/Zhejiang/77167/2014(H5N2)	H5N2	2.3.4.6	Tongxiang, Jiaxing	Jul 7, 2014		
24	A/goose/Zhejiang/77168/2014(H5N2)	H5N2	2.3.4.6	Tongxiang, Jiaxing	Jul 7, 2014		
25	A/goose/Zhejiang/727092/2014(H5N6)	H5N6	2.3.4.6	Yuhang, Hangzhou	Jul 27, 2014		
26	A/goose/Zhejiang/727110/2014(H5N6)	H5N6	2.3.4.6	Yuhang, Hangzhou	Jul 27, 2014		
27	A/goose/Zhejiang/925105/2014(H5N6)	H5N6	2.3.4.6	Yuhang, Hangzhou	Sep 25, 2014		
28	A/goose/Zhejiang/925108/2014(H5N6)	H5N6	2.3.4.6	Yuhang, Hangzhou	Sep 25, 2014		
29	A/goose/Zhejiang/925106/2014(H5N6)	H5N6	2.3.4.6	Yuhang, Hangzhou	Sep 25, 2014		
30	A/goose/Zhejiang/925036/2014(H5N6)	H5N6	2.3.4.6	Yuhang, Hangzhou	Sep 25, 2014		
31	A/goose/Zhejiang/112080/2014(H5N6)	H5N6	2.3.4.6	Haining, Jiaxing	Nov 20, 2014		
32	A/goose/Zhejiang/1120132/2014(H5N6)	H5N6	2.3.4.6	Haining, Jiaxing	Nov 20, 2014		
33	A/goose/Zhejiang/925037/2014(H5N8)	H5N8	2.3.4.6	Yuhang, Hangzhou	Sep 25, 2014		
34	A/goose/Zhejiang/925104/2014(H5N8)	H5N8	2.3.4.6	Yuhang, Hangzhou	Sep 25, 2014		

Note: Viruses were harvested during surveillance of live poultry markets in Zhejiang, China from January 2013 through December 2014