SS2:背景介绍

- 二型猪链球菌（SS2）是一种重要的动物病原，主要在猪群中传播并导致猪的死亡
- 人传染SS2能够引起脑炎、败血病、心内膜炎等疾病，并会导致死亡
- 至今为止，已记载的人感染病例已超过200例
- 1998和2005年在我国暴发了人群中的SS2流行

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SS2:背景介绍

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Streptococcal Toxic Shock Syndrome Caused by *Streptococcus suis* Serotype 2

Jiaqi Tang[1*☯], Changjun Wang[1☯], Youjun Feng[2,10☯], Weizhong Yang[3☯], Huaidong Song[4,9☯], Zhihai Chen[5☯], Hongjie Yu[3☯], Xiuzhen Pan[1], Xiaojun Zhou[6], Huaru Wang[1], Bo Wu[6], Haili Wang[1], Huamei Zhao[1], Ying Lin[7], Jianhua Yue[1], Zhenqiang Wu[7], Xiaowei He[7], Feng Gao[2], Abdul Hamid Khan[2,10], Jian Wang[8], Guo-Ping Zhao[9], Yu Wang[3*], Xiaoning Wang[7*], Zhu Chen[4,9], George F. Gao[2*]

RESEARCH

Human *Streptococcus suis* Outbreak, Sichuan, China

Hongjie Yu,*[1] Huaiqi Jing,†[1] Zhihai Chen,‡[1] Han Zheng,† Xiaoping Zhu,§ Hua Wang,¶ Shiwen Wang,* Lunguang Liu,§ Rongqiang Zu,* Longze Luo,§ Nijuan Xiang,* Honglu Liu,§ Xuecheng Liu,§ Yuelong Shu,* Shui Shan Lee,# Shuk Kwan Chuang,** Yu Wang,* Jianguo Xu,† Weizhong Yang,* and the *Streptococcus suis* study groups[2]

SS2:背景介绍

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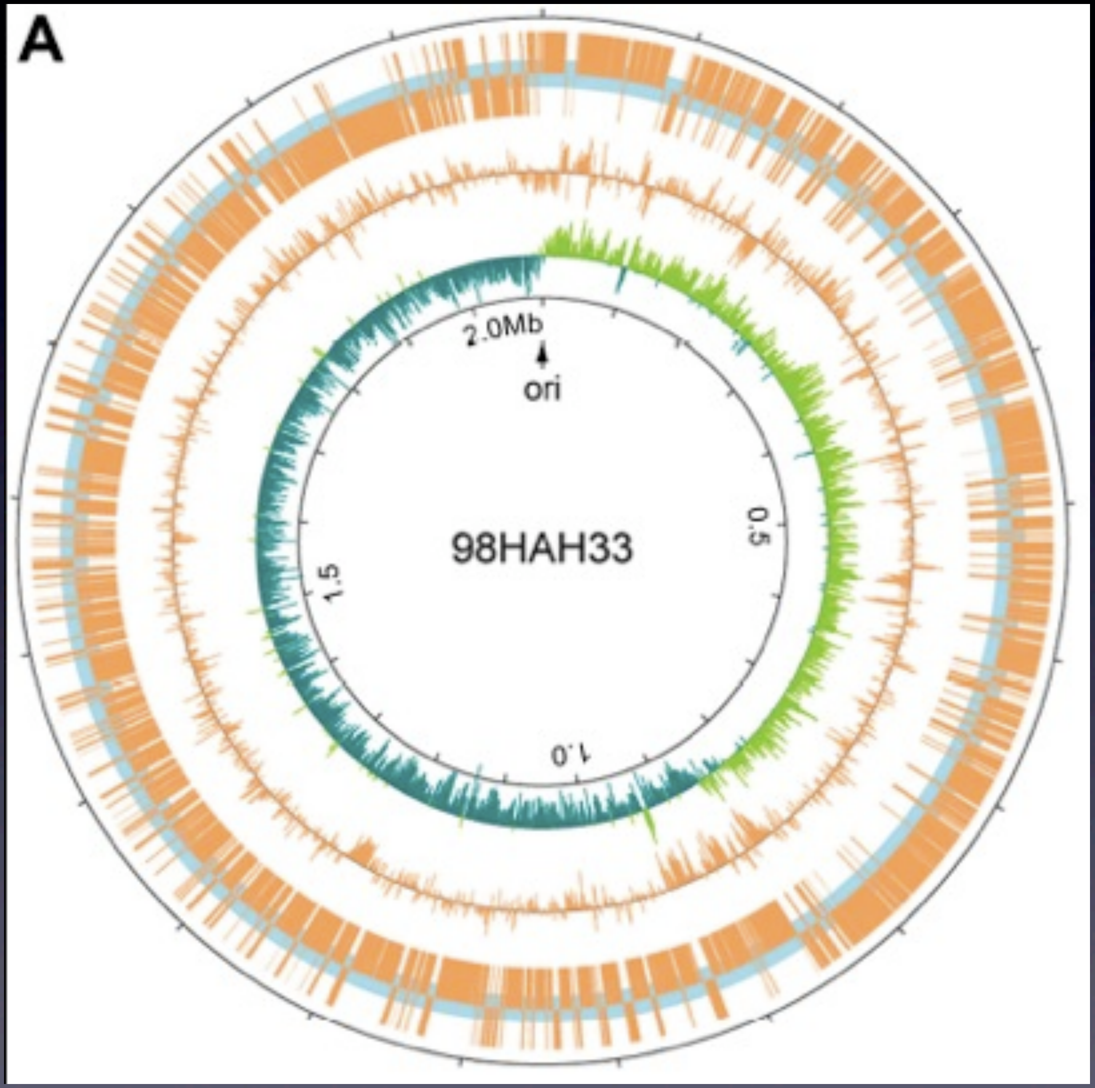

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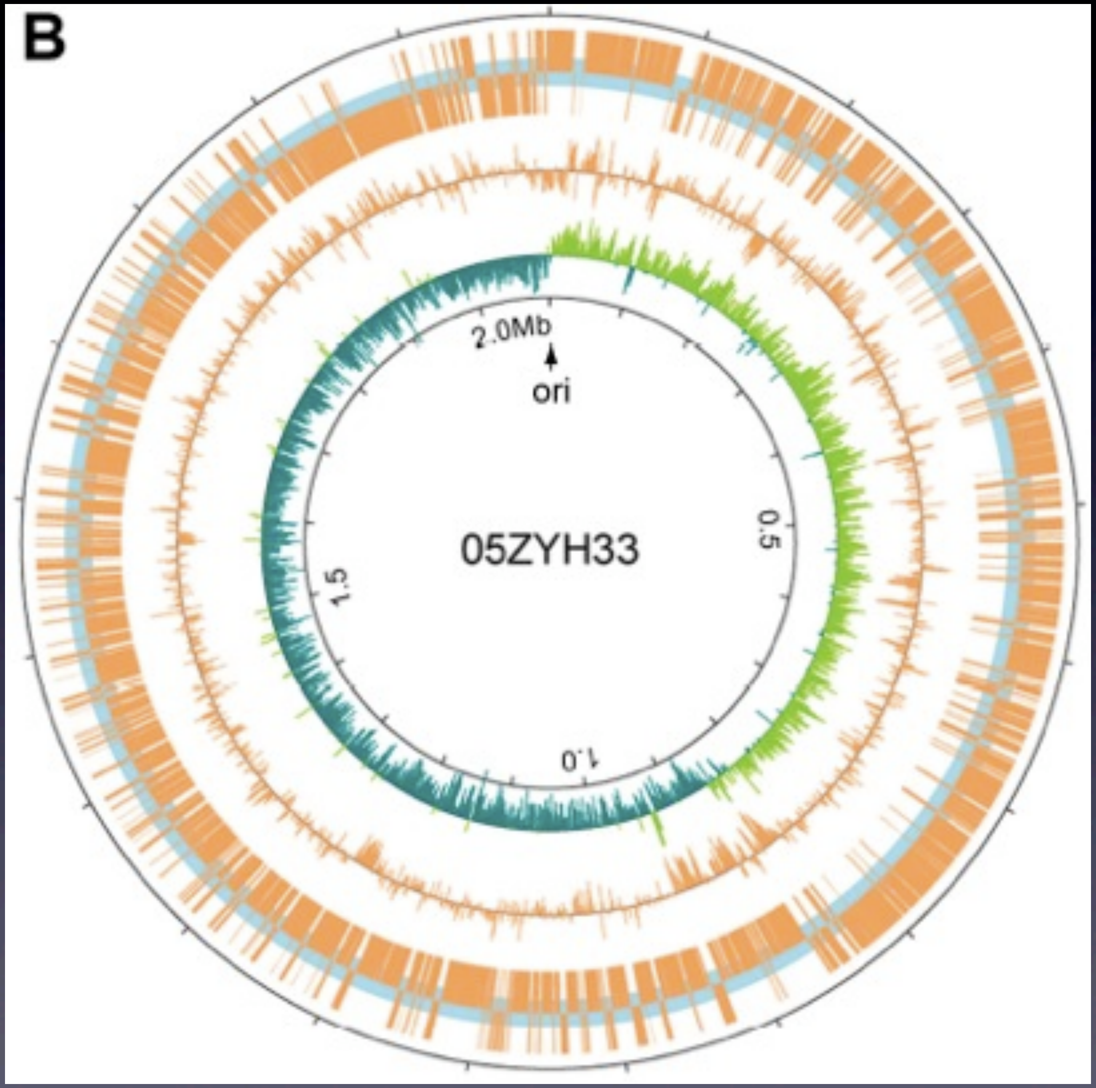

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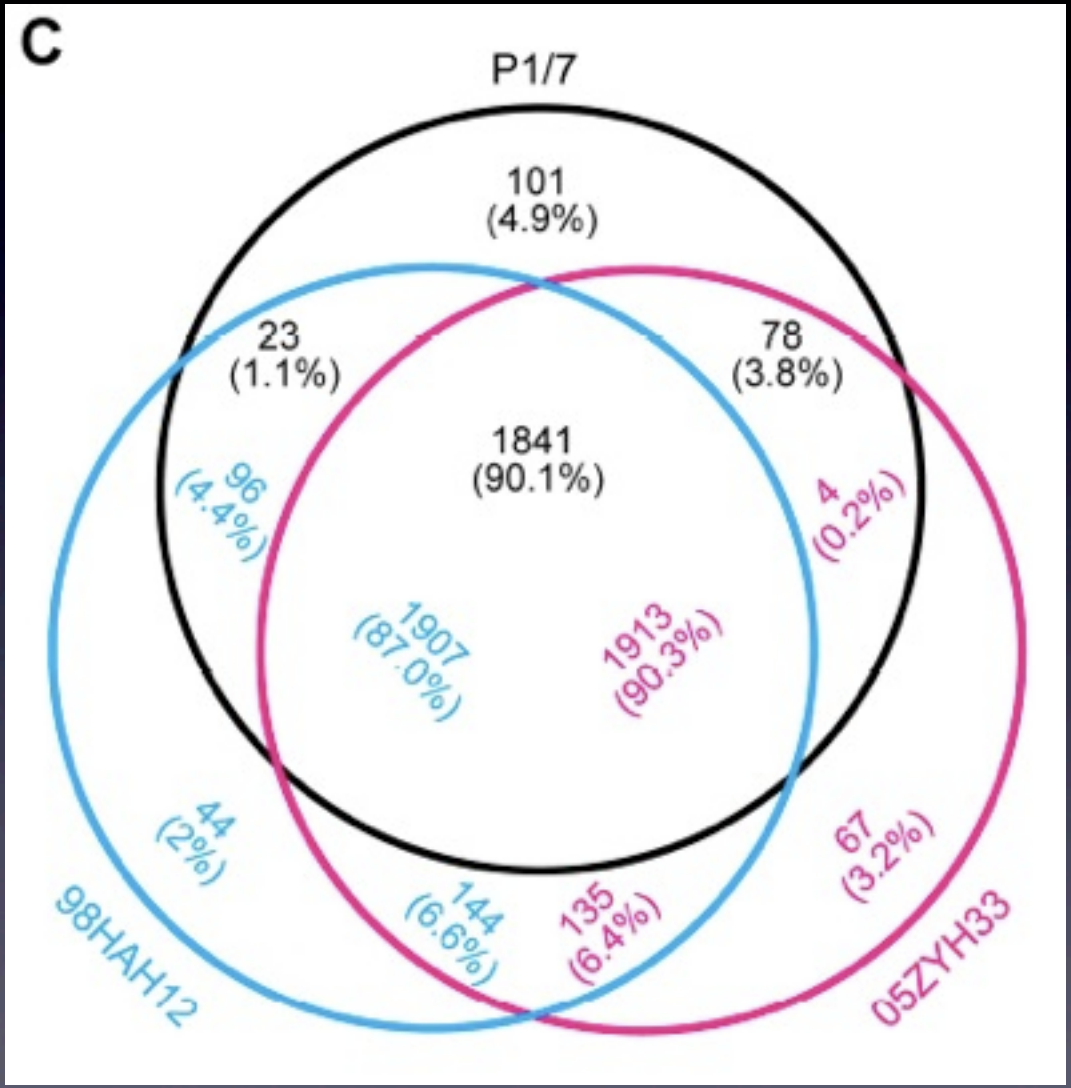
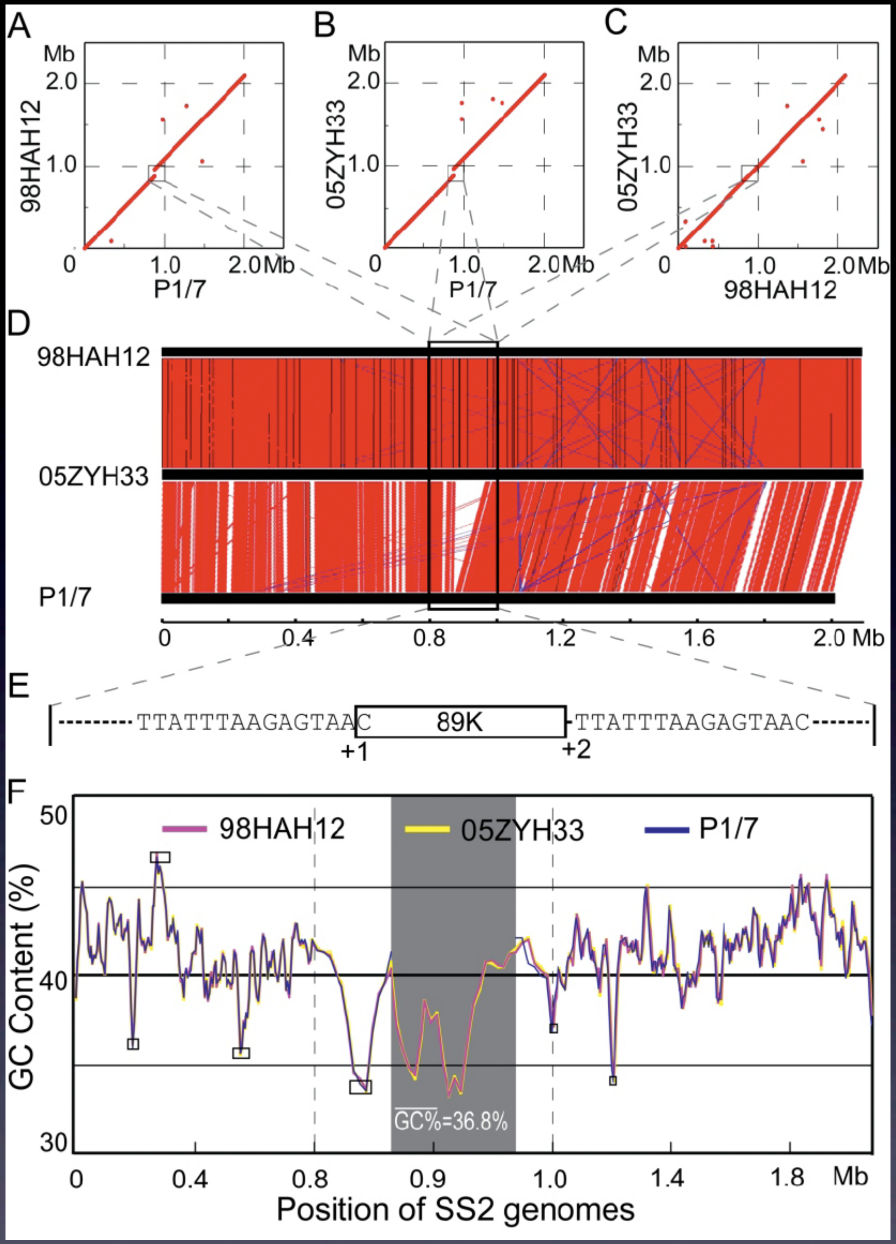
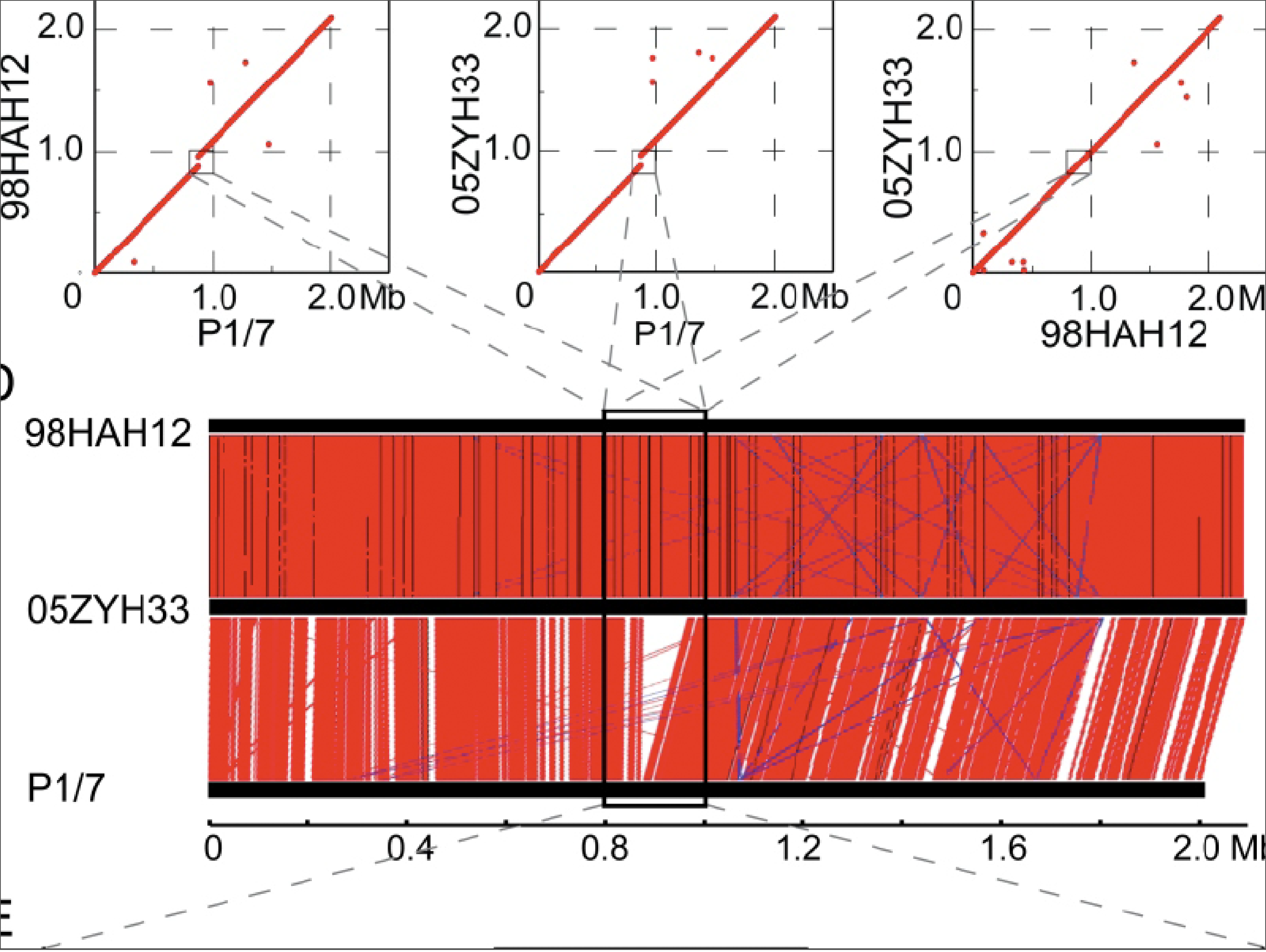

C
P1/7
101
(4.9%)
23
(1.1%)
78
(3.8%)
1841
(90.1%)
96
(4.4%)
4
(0.2%)
1907
(87.0%)
1913
(90.3%)
44
(2%)
67
(3.2%)
144
(6.6%)
135
(6.4%)
98HAH12
05ZYH33



SS2基因组比对和GC含量分析


98HAH12
2.0
1.0
0
1.0
2.0Mb
P1/7
05ZYH33
2.0
1.0
0
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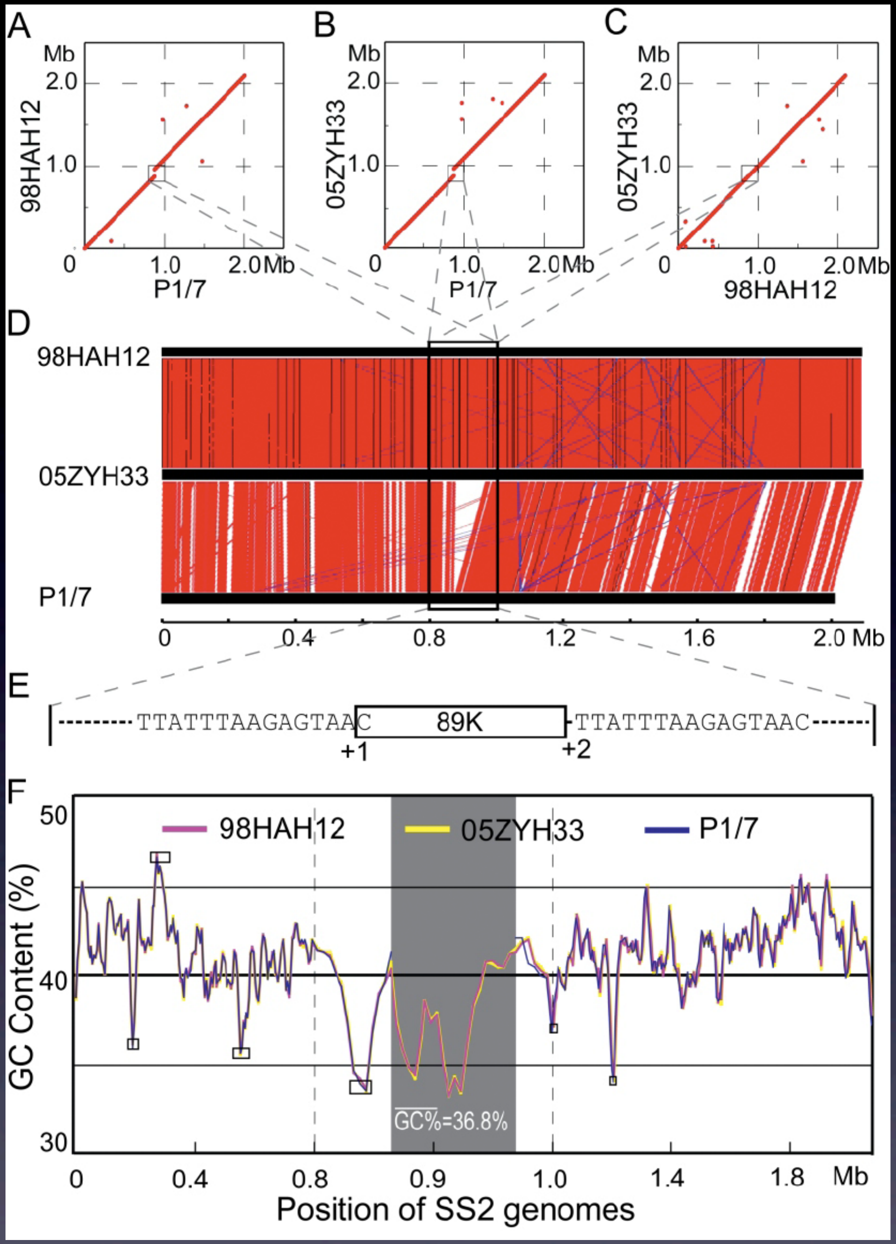
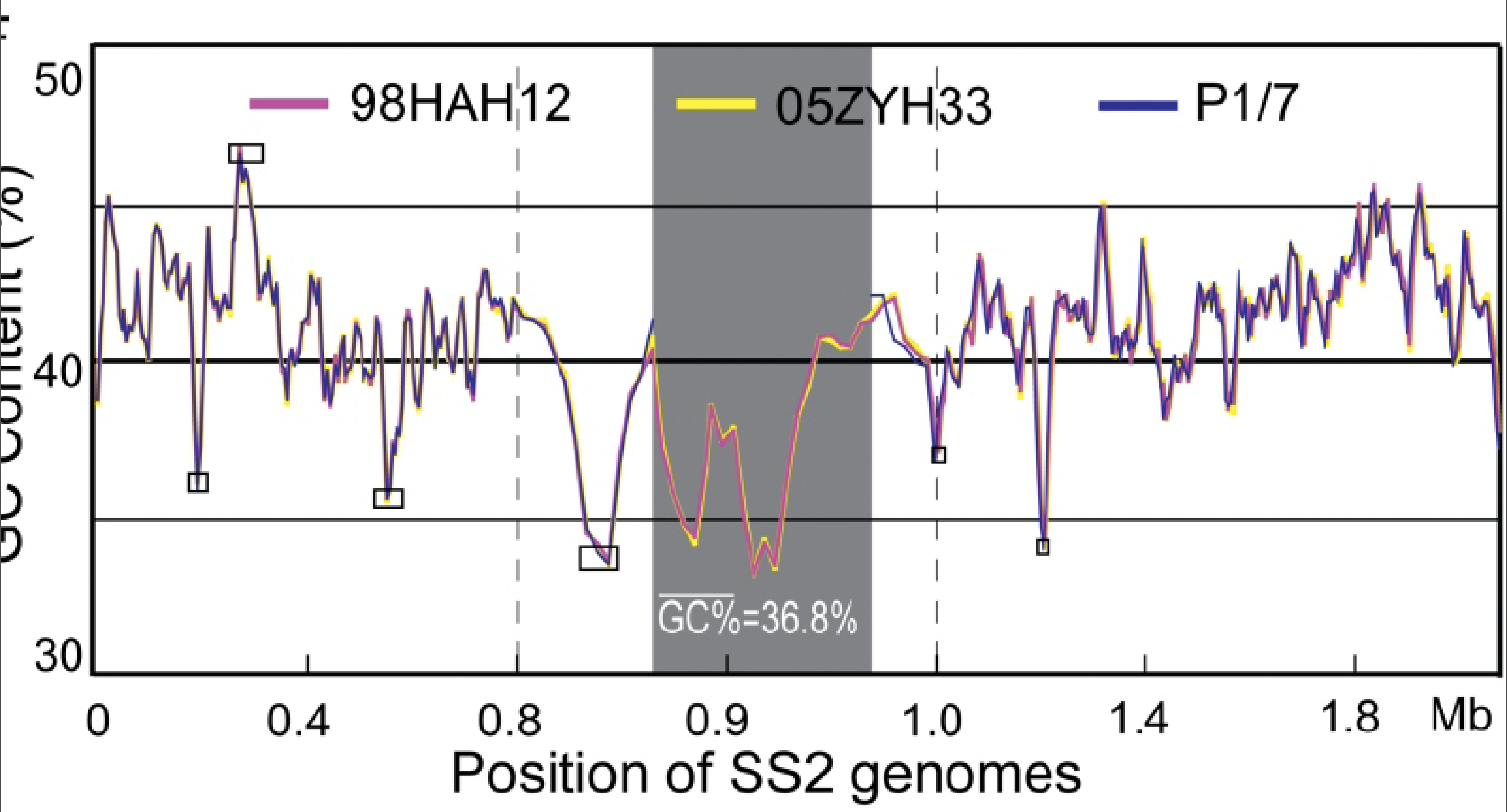
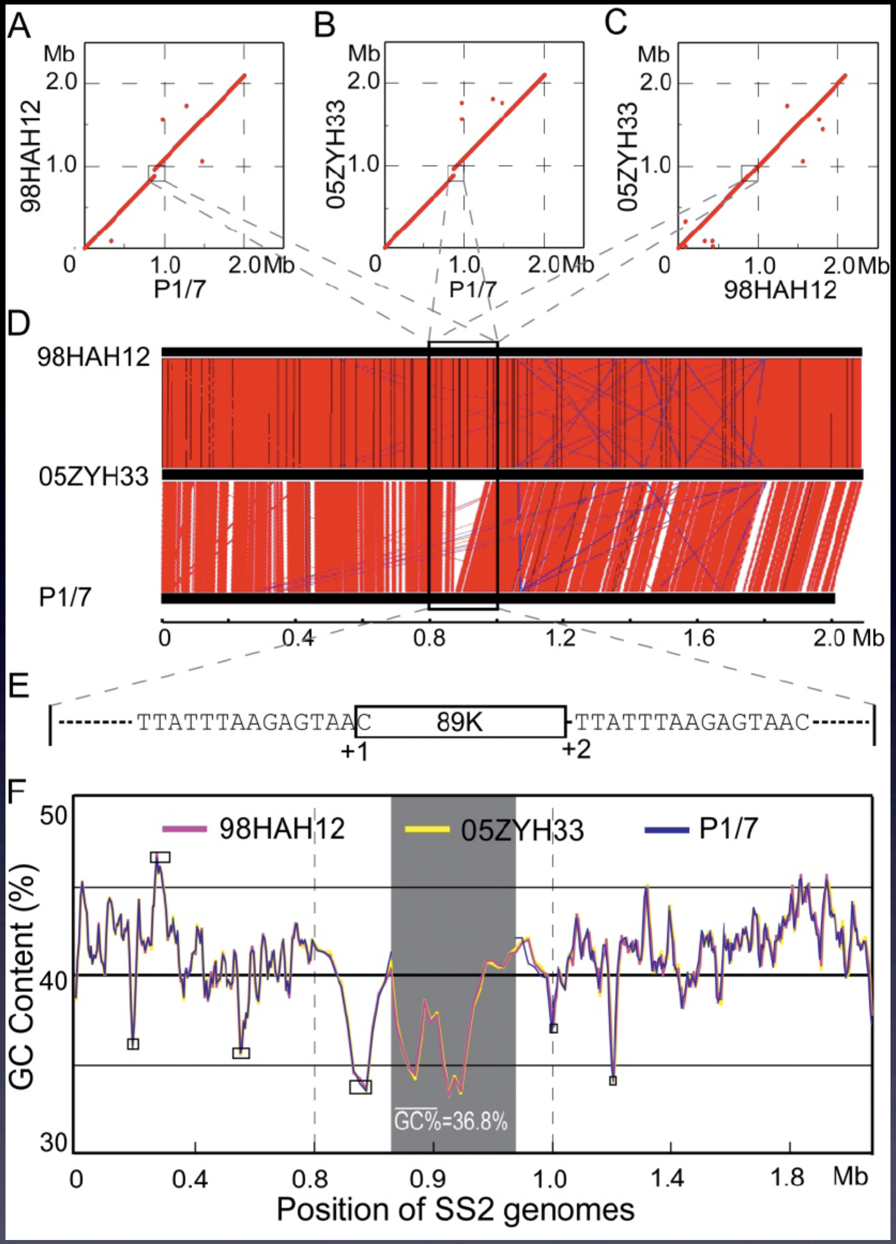
2.0Mb
P1/7
05ZYH33
2.0
1.0
0
1.0
2.0M
98HAH12
98HAH12
05ZYH33
P1/7
0
0.4
0.8
1.2
1.6
2.0 Mb



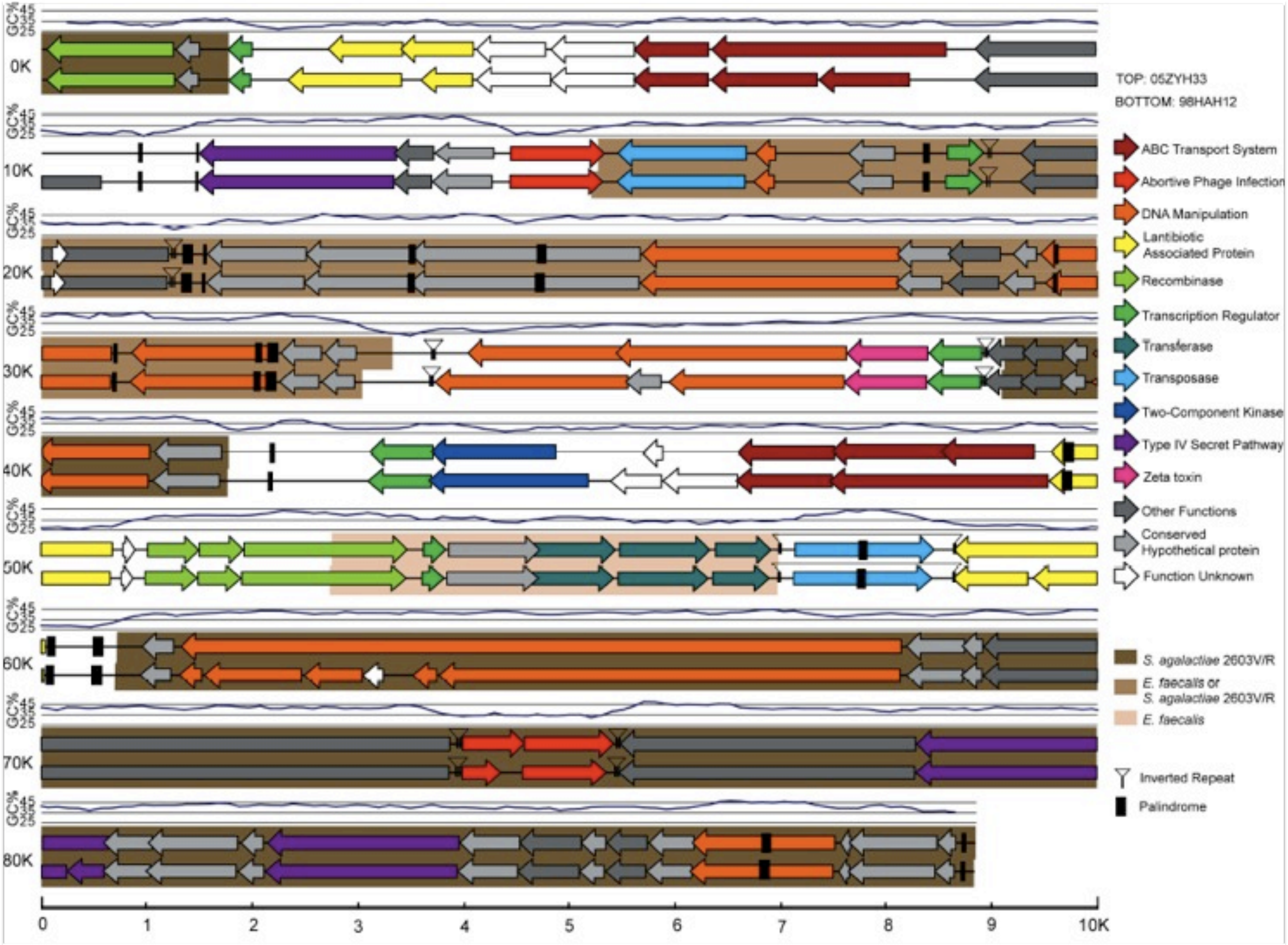
SS2基因组比对和GC含量分析



SS2基因组比对和GC含量分析



SS2基因组比对和GC含量分析


GC%
45
35
25
0K
10K
20K
30K
40K
50K
60K
70K
80K
TOP: 05ZYH33
BOTTOM: 98HAH12
ABC Transport System
Abortive Phage Infection
DNA Manipulation
Lantibiotic Associated Protein
Recombinase
Transcription Regulator
Transferase
Transposase
Two-Component Kinase
Type IV Secret Pathway
Zeta toxin
Other Functions
Conserved Hypothetical protein
Function Unknown
S. agalactiae 2603V/R
E. faecalis or S. agalactiae 2603V/R
E. faecalis
Inverted Repeat
Palindrome
0
1
2
3
4
5
6
7
8
9
10K

89K普遍存在于

98与05暴发的致病菌中

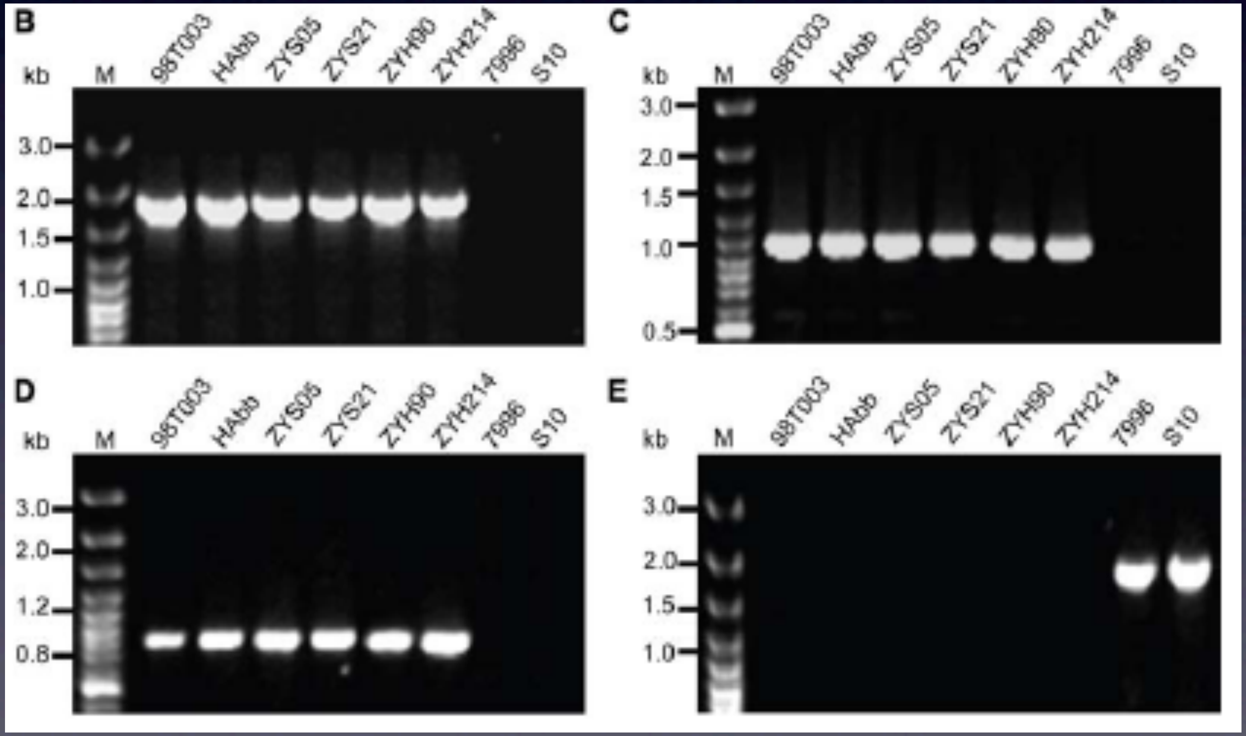
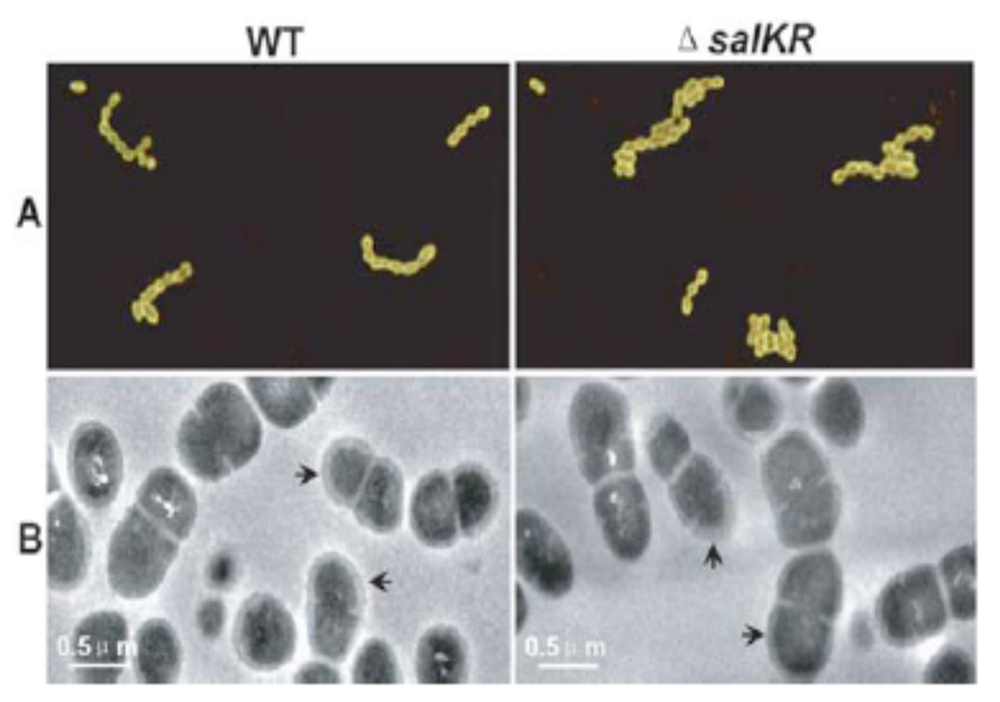
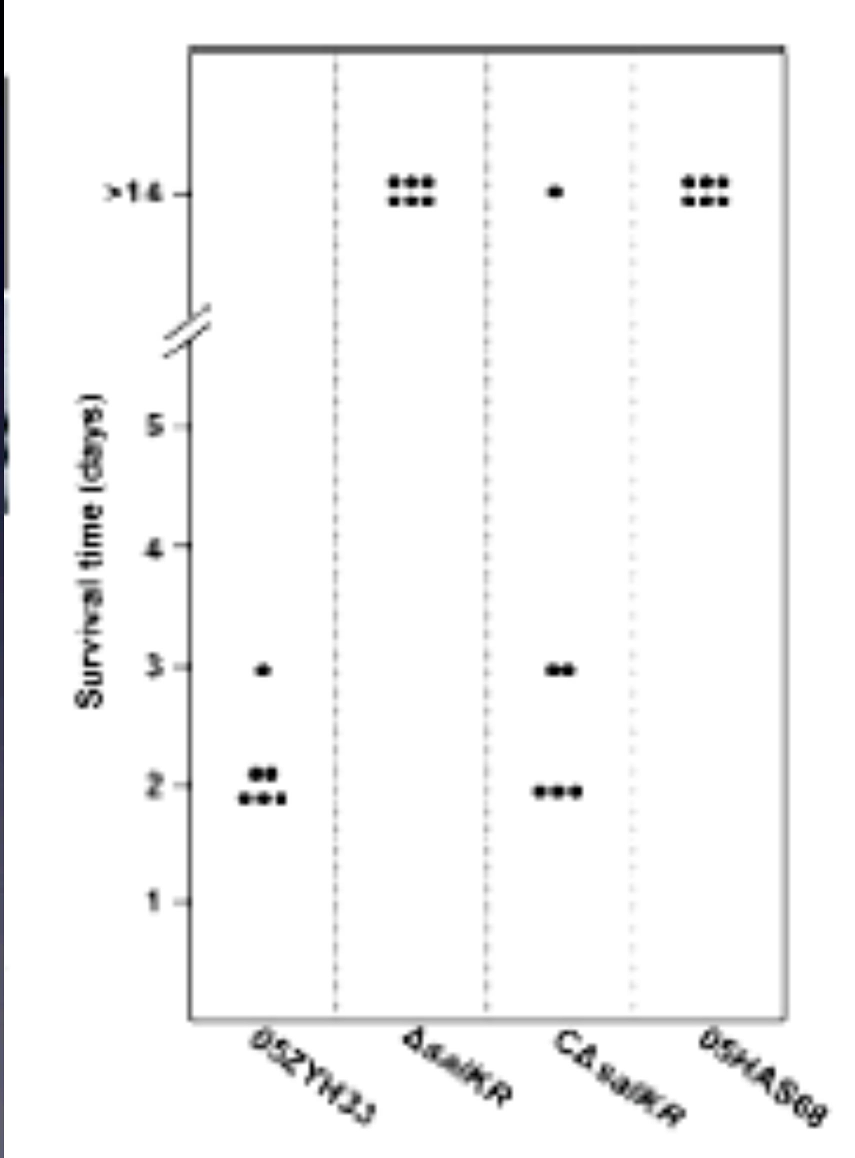


Table 2. Statistics of PCR detection for 89K in SS2

Strains	Origins (Years)	Locations	Virulence	89K
Chinese strains of SS2 (30 in total)				
17-19	Healthy swine, 2006	Jiangsu, China	Avirulent	−
05ZYH33*	STSS patient, 2005	Sichuan, China	Highly virulent	+
05ZYH36	STSS patient, 2005	Sichuan, China	Highly virulent	+
ZYH38	STSS patient, 2005	Sichuan, China	Highly virulent	+
ZYH45	STSS patient, 2005	Sichuan, China	Highly virulent	+
ZYH55	STSS patient, 2005	Sichuan, China	Highly virulent	+
ZYH87	STSS patient, 2005	Sichuan, China	Highly virulent	+
ZYH90	STSS patient, 2005	Sichuan, China	Highly virulent	+
ZYH214	STSS patient, 2005	Sichuan, China	Highly virulent	+
ZYH354-1	STSS patient, 2005	Sichuan, China	Highly virulent	+
ZYS05	Died swine, 2005	Sichuan, China	Highly virulent	+
ZYS19	Died swine, 2005	Sichuan, China	Highly virulent	+
ZYS21	Died swine, 2005	Sichuan, China	Highly virulent	+
ZYS22	Died swine, 2005	Sichuan, China	Highly virulent	+
05HAS68*	Healthy swine, 2005	Jiangsu, China	Avirulent	−
05-J2a	Healthy swine, 2005	Jiangsu, China	Avirulent	−
05-J2d	Healthy swine, 2005	Jiangsu, China	Avirulent	−
05-14e	Healthy swine, 2005	Jiangsu, China	Avirulent	−
98HAH12*	STSS patient, 1998	Jiangsu, China	Highly virulent	+
98T003	STSS patient, 1998	Jiangsu, China	Highly virulent	+
HAbb	STSS patient, 1998	Jiangsu, China	Highly virulent	+
98002	STSS patient, 1998	Jiangsu, China	Highly virulent	+
98147#	STSS patient, 1998	Jiangsu, China	Highly virulent	+
98150#	STSS patient, 1998	Jiangsu, China	Highly virulent	+
98145#	Died swine, 1998	Jiangsu, China	Highly virulent	+
98146#	Died swine, 1998	Jiangsu, China	Highly virulent	+
98148#	Died swine, 1998	Jiangsu, China	Highly virulent	+
98151#	Died swine, 1998	Jiangsu, China	Highly virulent	+
S006#	Swine, before 1998	China	Less virulent	−
S008#	Swine, before 1998	China	Less virulent	−
International strains of SS2 (10 in total)				
7996	Swine	Holland	Avirulent	−
S10	Swine	Holland	Highly virulent	−
T15	Swine	Holland	Avirulent	−
8004	Swine	Holland	Highly virulent	−
8011	Swine	Holland	Highly virulent	−
8012	Swine	Holland	Highly virulent	−
8014	Swine	Holland	Highly virulent	−
8019	Swine	Holland	Highly virulent	−
5735	Swine	Canada	Highly virulent	−
SS2-N	Swine	Germany	Highly virulent	−

89K与致病力的关系

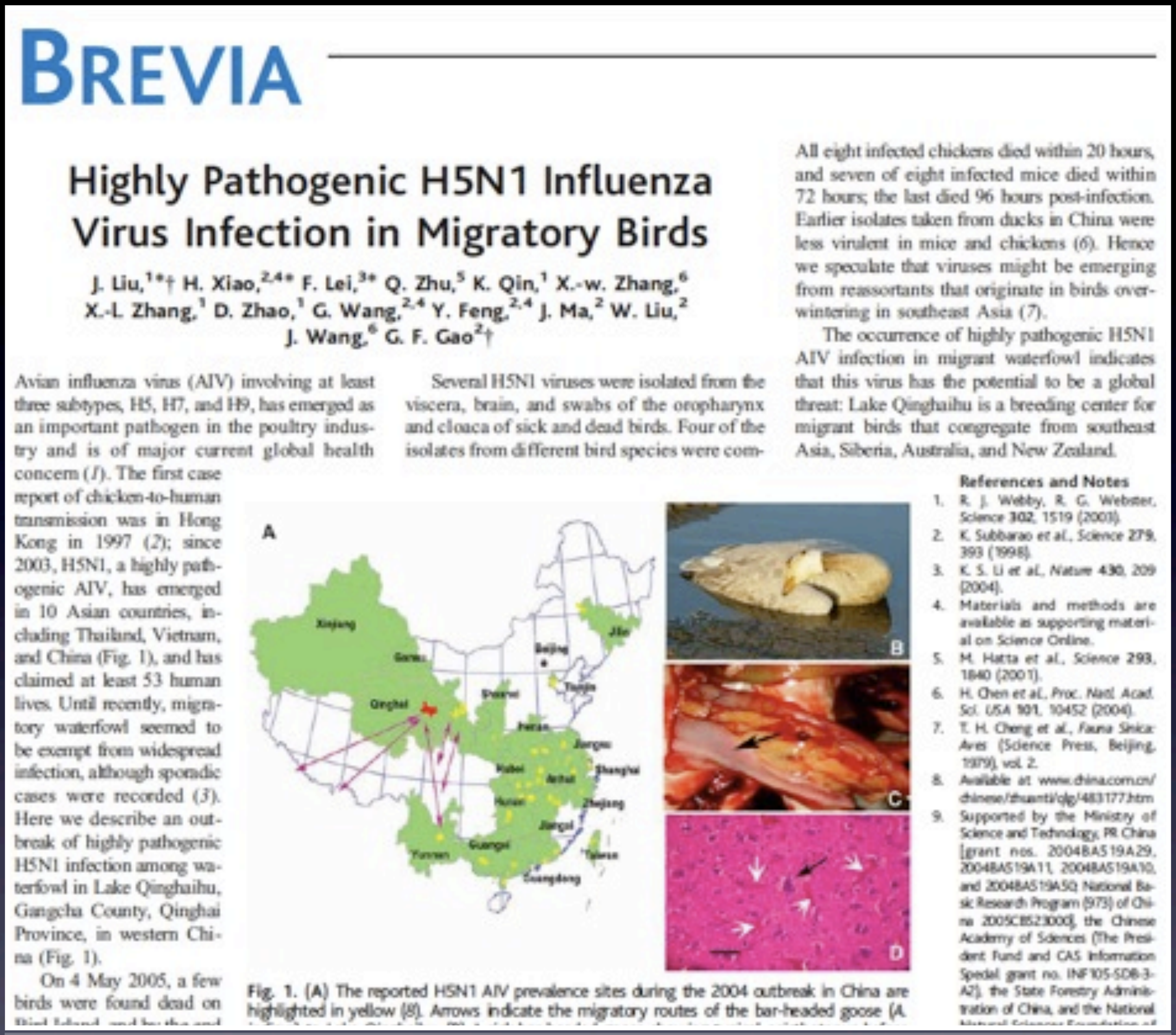

WT
Δ salKR
A
B
0.5 μm
0.5 μm


Survival time (days)
>14
5
4
3
2
1
05ZYH33
ΔsalKR
CΔsalKR
05HAS68

Phylogenetics

Qinghai Lake, and Influenza Virus

Start from 2005



BREVIA

Highly Pathogenic H5N1 Influenza Virus Infection in Migratory Birds

J. Liu,[1*†] H. Xiao,[2,4*] F. Lei,[3*] Q. Zhu,[5] K. Qin,[1] X.-w. Zhang,[6]
X.-L. Zhang,[1] D. Zhao,[1] G. Wang,[2,4] Y. Feng,[2,4] J. Ma,[2] W. Liu,[2]
J. Wang,[6] G. F. Gao[2]†

Avian influenza virus (AIV) involving at least three subtypes, H5, H7, and H9, has emerged as an important pathogen in the poultry industry and is of major current global health concern (*1*). The first case report of chicken-to-human transmission was in Hong Kong in 1997 (*2*); since 2003, H5N1, a highly pathogenic AIV, has emerged in 10 Asian countries, including Thailand, Vietnam, and China (Fig. 1), and has claimed at least 53 human lives. Until recently, migratory waterfowl seemed to be exempt from widespread infection, although sporadic cases were recorded (*3*). Here we describe an outbreak of highly pathogenic H5N1 infection among waterfowl in Lake Qinghaihu, Gangcha County, Qinghai Province, in western China (Fig. 1).

On 4 May 2005, a few birds were found dead on

Several H5N1 viruses were isolated from the viscera, brain, and swabs of the oropharynx and cloaca of sick and dead birds. Four of the isolates from different bird species were com-

All eight infected chickens died within 20 hours, and seven of eight infected mice died within 72 hours; the last died 96 hours post-infection. Earlier isolates taken from ducks in China were less virulent in mice and chickens (*6*). Hence we speculate that viruses might be emerging from reassortants that originate in birds over-wintering in southeast Asia (*7*).

The occurrence of highly pathogenic H5N1 AIV infection in migrant waterfowl indicates that this virus has the potential to be a global threat: Lake Qinghaihu is a breeding center for migrant birds that congregate from southeast Asia, Siberia, Australia, and New Zealand.

Fig. 1. (**A**) The reported H5N1 AIV prevalence sites during the 2004 outbreak in China are highlighted in yellow (*8*). Arrows indicate the migratory routes of the bar-headed goose (*A.*

References and Notes

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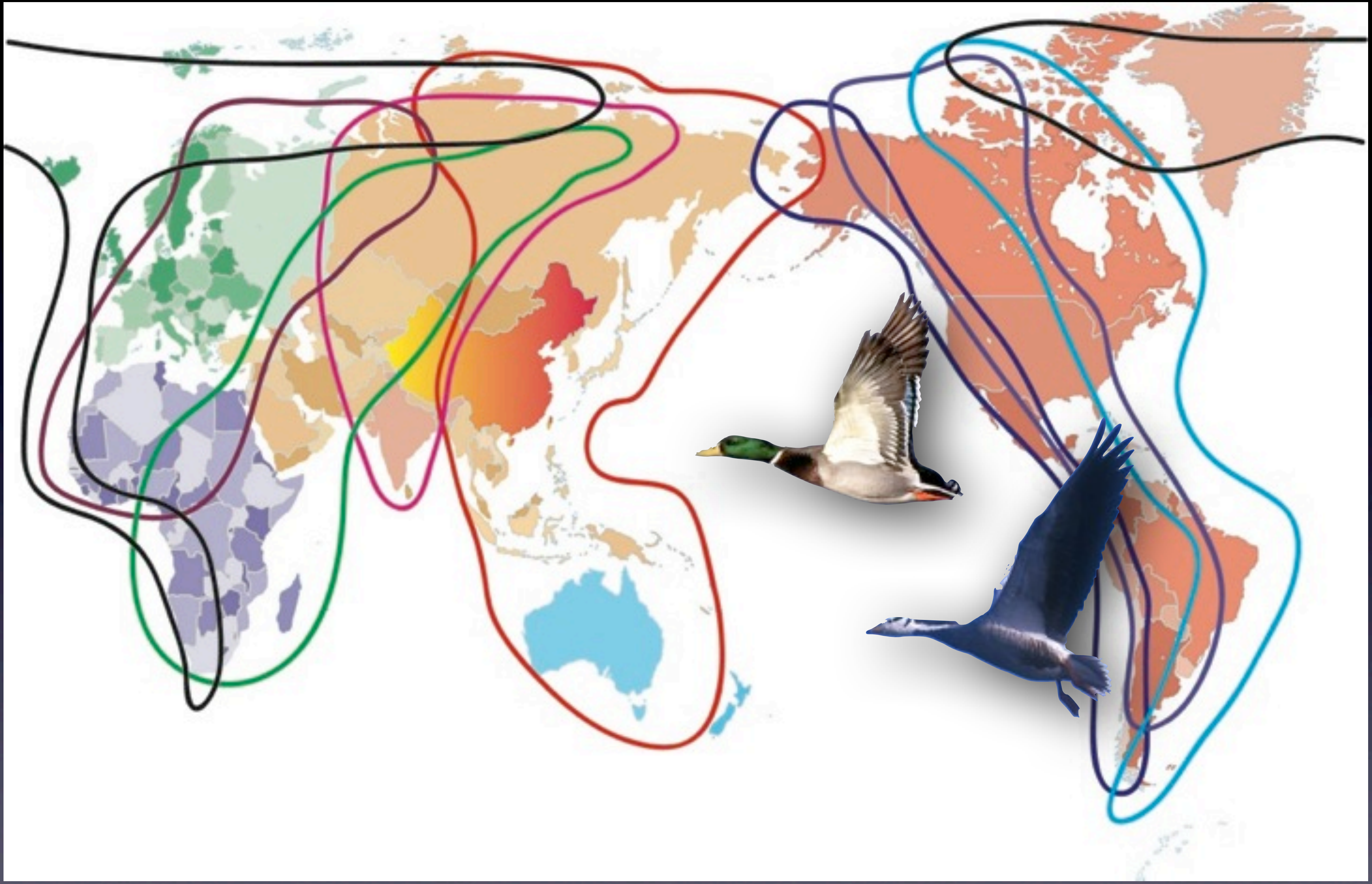
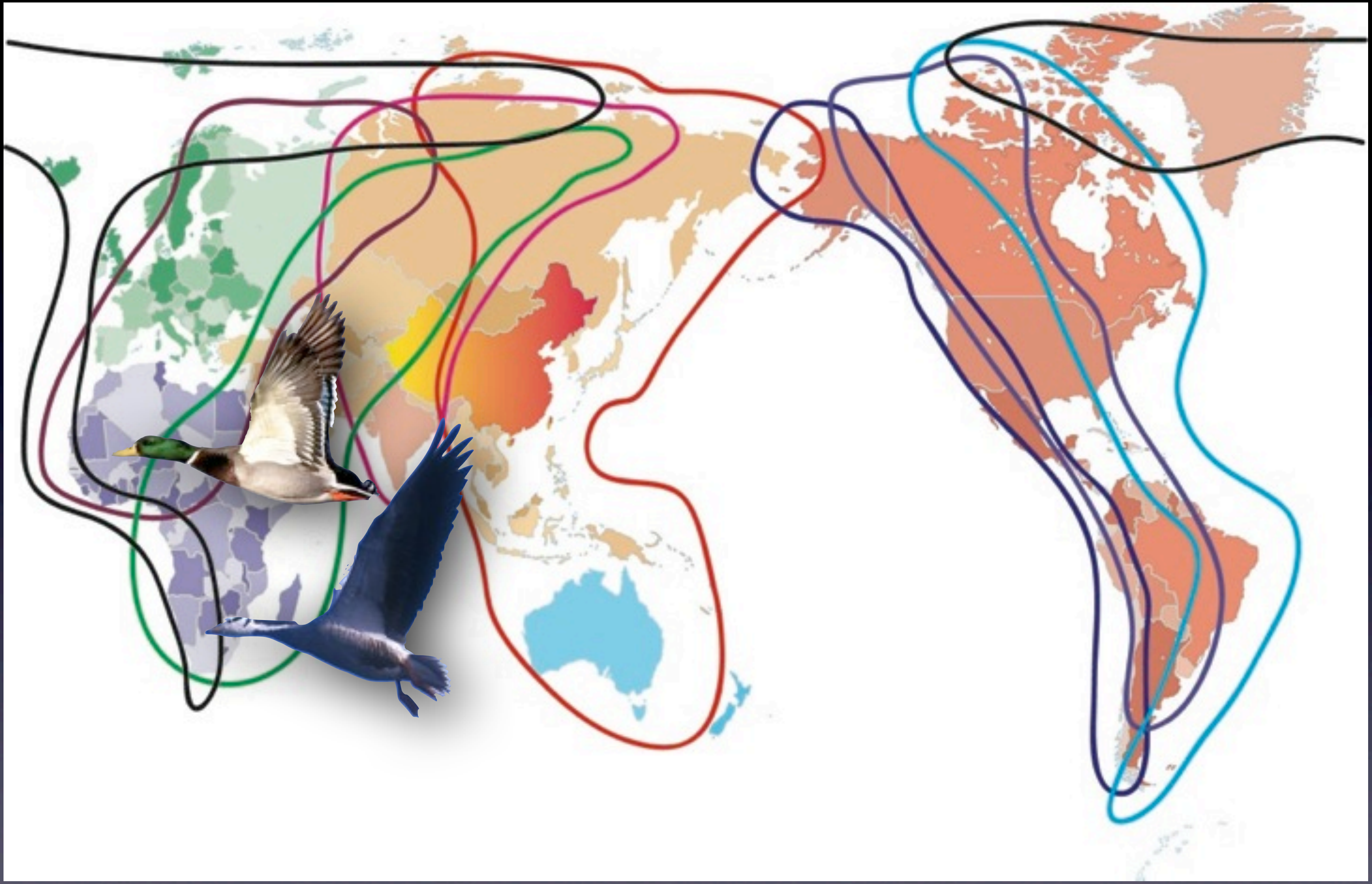
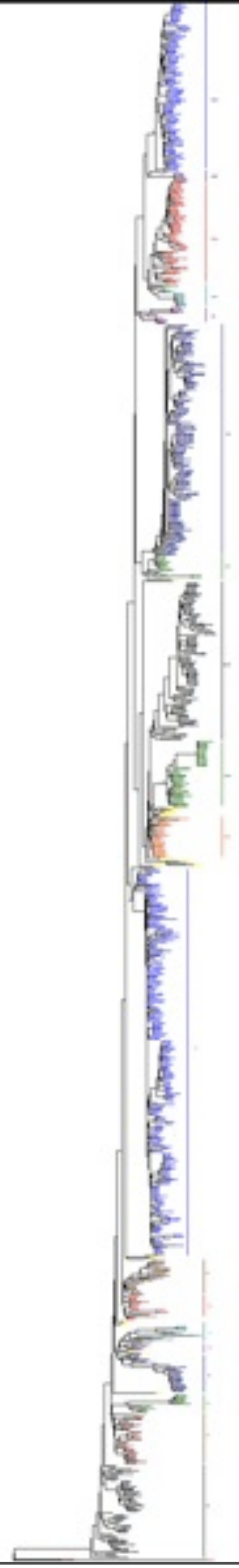
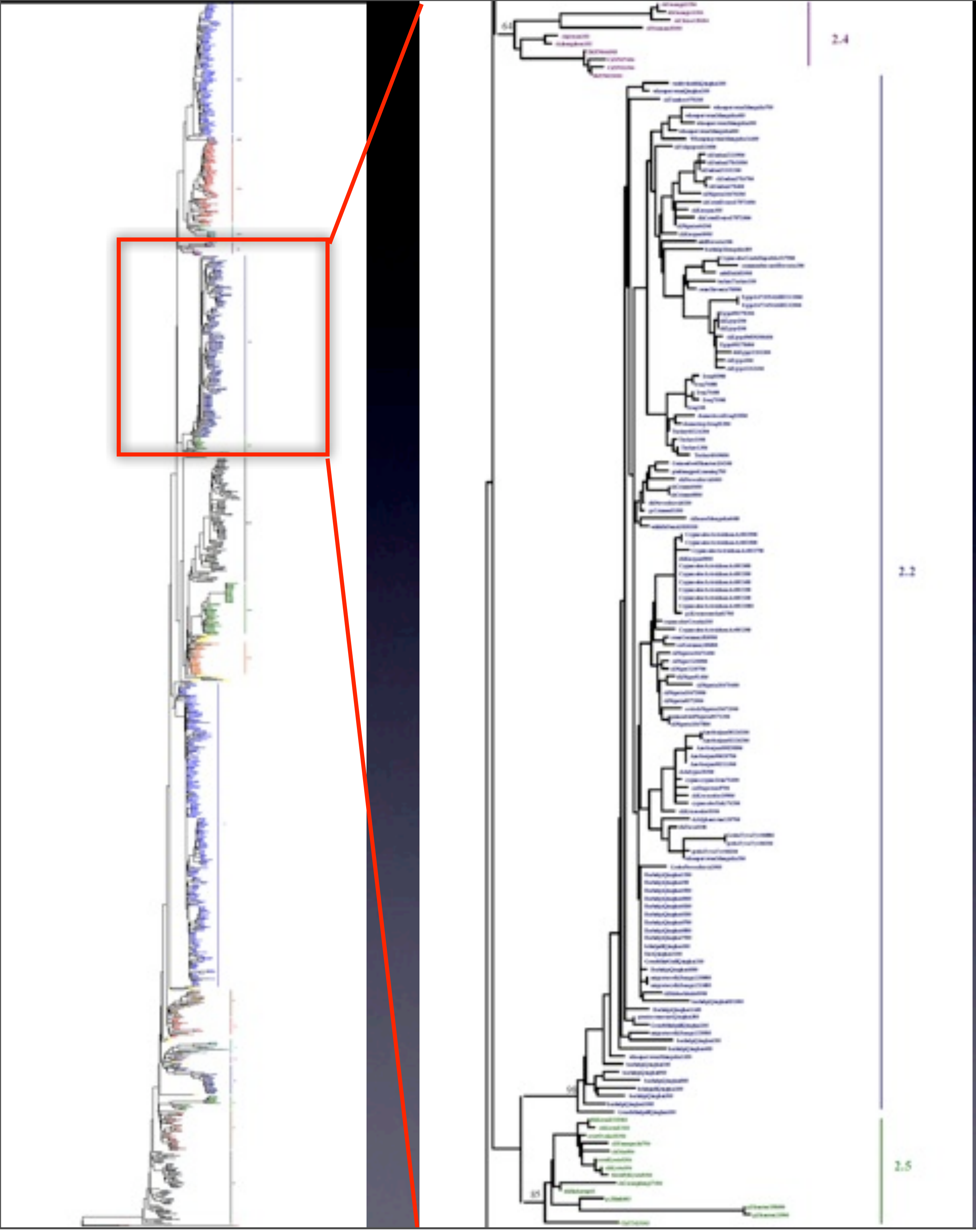
9. Supported by the Ministry of Science and Technology, PR China [grant nos. 2004BA519A29, 2004BA519A11, 2004BA519A10, and 2004BA519A50; National Basic Research Program (973) of China 2005CB523000], the Chinese Academy of Sciences (The President Fund and CAS Information Special grant no. INF105-SDB-3-A2), the State Forestry Administration of China, and the National

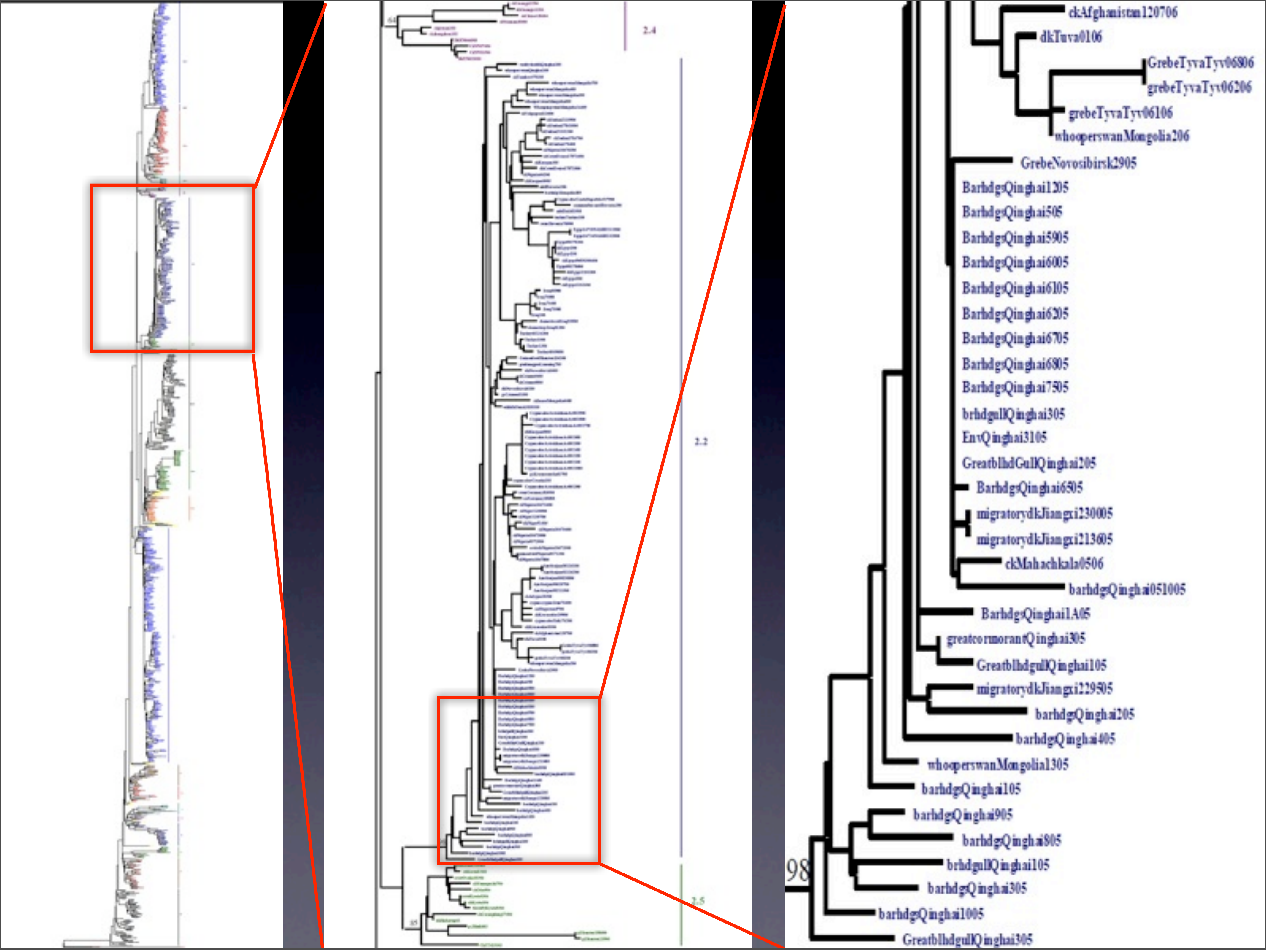
May, 2005
H5N1 Outbreak Qinghai Lake








64
2.4
2.2
96
85
2.5


64
2.4
2.2
2.5
85
ckAfghanistan120706
dkTuva0106
GrebeTyvaTyv06806
grebeTyvaTyv06206
grebeTyvaTyv06106
whooperswanMongolia206
GrebeNovosibirsk2905
BarhdgsQinghai1205
BarhdgsQinghai505
BarhdgsQinghai5905
BarhdgsQinghai6005
BarhdgsQinghai6105
BarhdgsQinghai6205
BarhdgsQinghai6705
BarhdgsQinghai6805
BarhdgsQinghai7505
brhdgullQinghai305
EnvQinghai3105
GreatblhdGullQinghai205
BarhdgsQinghai6505
migratorydkJiangxi230005
migratorydkJiangxi213605
ckMahachkala0506
barhdgsQinghai051005
BarhdgsQinghai1A05
greatcormorantQinghai305
GreatblhdgullQinghai105
migratorydkJiangxi229505
barhdgsQinghai205
barhdgsQinghai405
whooperswanMongolia1305
barhdgsQinghai105
barhdgsQinghai905
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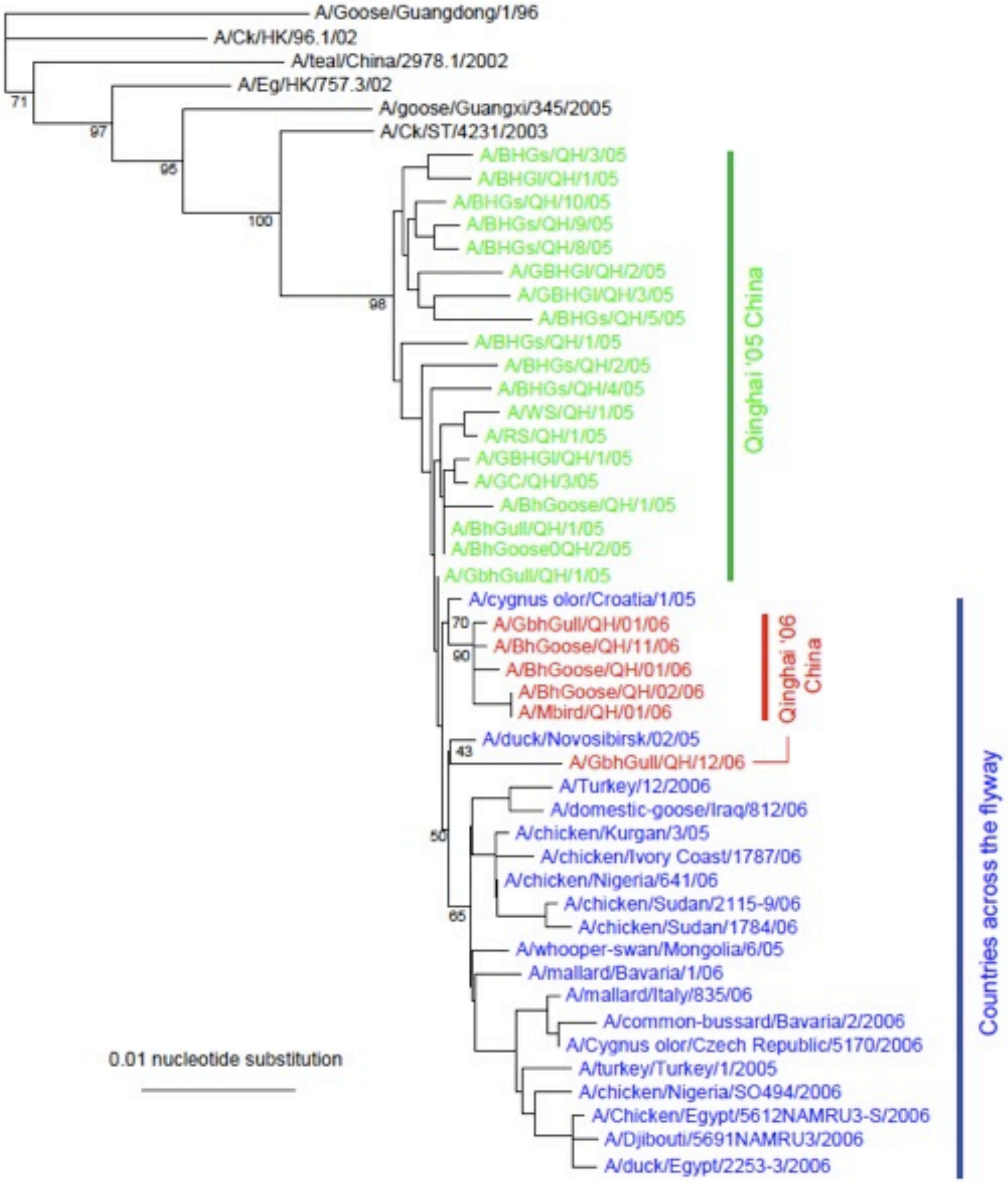
GreatblhdgullQinghai305
98

2006


A/Goose/Guangdong/1/96
A/Ck/HK/96.1/02
A/teal/China/2978.1/2002
A/Eg/HK/757.3/02
A/goose/Guangxi/345/2005
A/Ck/ST/4231/2003
71
97
95
100
98
A/BHGs/QH/3/05
A/BHGl/QH/1/05
A/BHGs/QH/10/05
A/BHGs/QH/9/05
A/BHGs/QH/8/05
A/GBHGl/QH/2/05
A/GBHGl/QH/3/05
A/BHGs/QH/5/05
A/BHGs/QH/1/05
A/BHGs/QH/2/05
A/BHGs/QH/4/05
A/WS/QH/1/05
A/RS/QH/1/05
A/GBHGl/QH/1/05
A/GC/QH/3/05
A/BhGoose/QH/1/05
A/BhGull/QH/1/05
A/BhGoose0QH/2/05
A/GbhGull/QH/1/05
Qinghai '05 China
A/cygnus olor/Croatia/1/05
70
90
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A/BhGoose/QH/11/06
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A/BhGoose/QH/02/06
A/Mbird/QH/01/06
Qinghai '06 China
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50
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A/Turkey/12/2006
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A/mallard/Bavaria/1/06
A/mallard/Italy/835/06
A/common-bussard/Bavaria/2/2006
A/Cygnus olor/Czech Republic/5170/2006
A/turkey/Turkey/1/2005
A/chicken/Nigeria/SO494/2006
A/Chicken/Egypt/5612NAMRU3-S/2006
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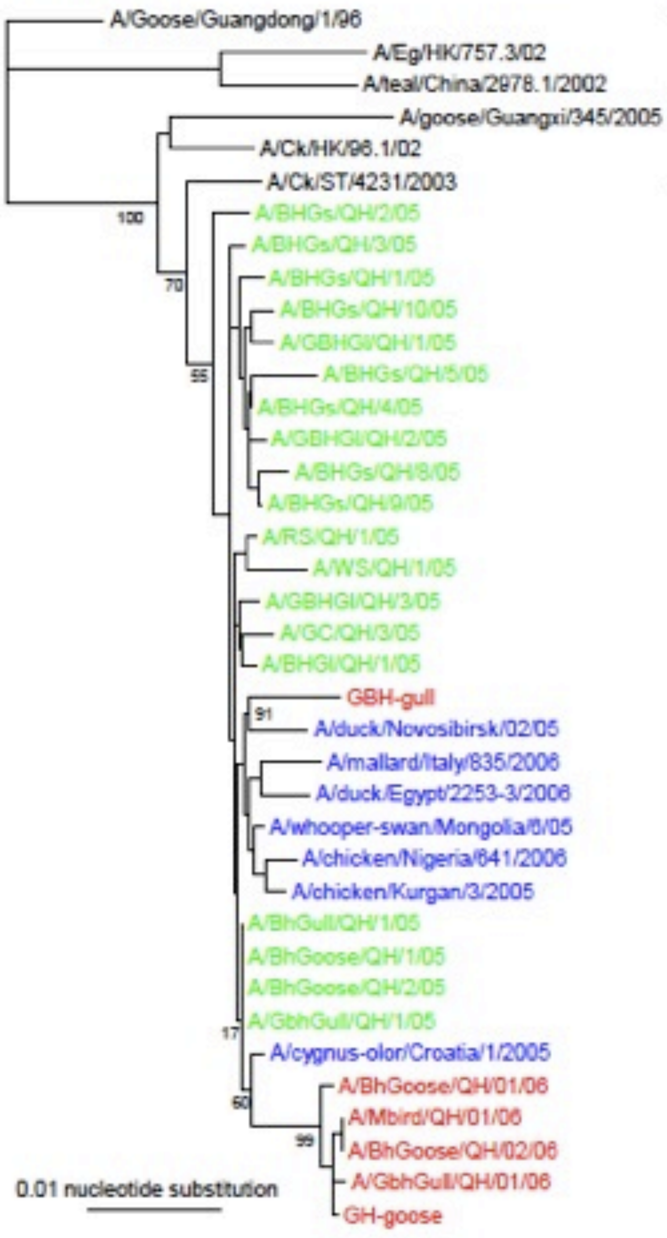
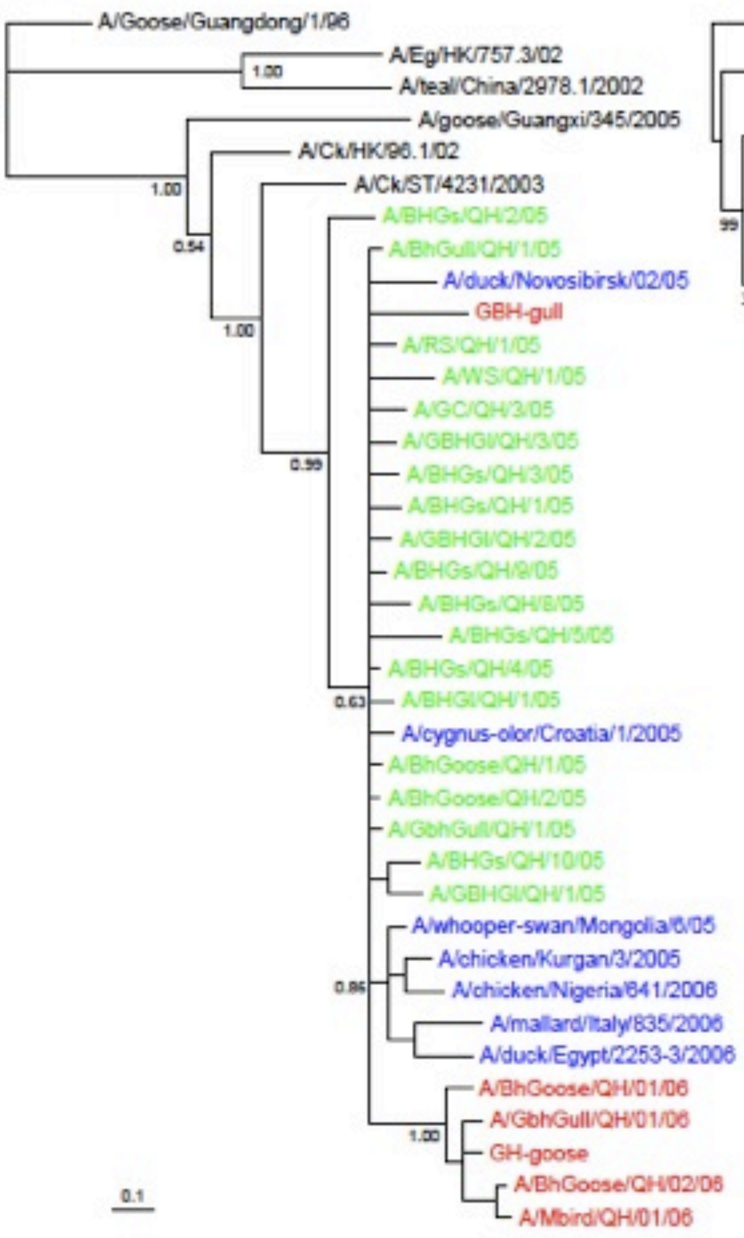
A/duck/Egypt/2253-3/2006
Countries across the flyway
0.01 nucleotide substitution

NA: NJ tree



NA: Bayesian tree



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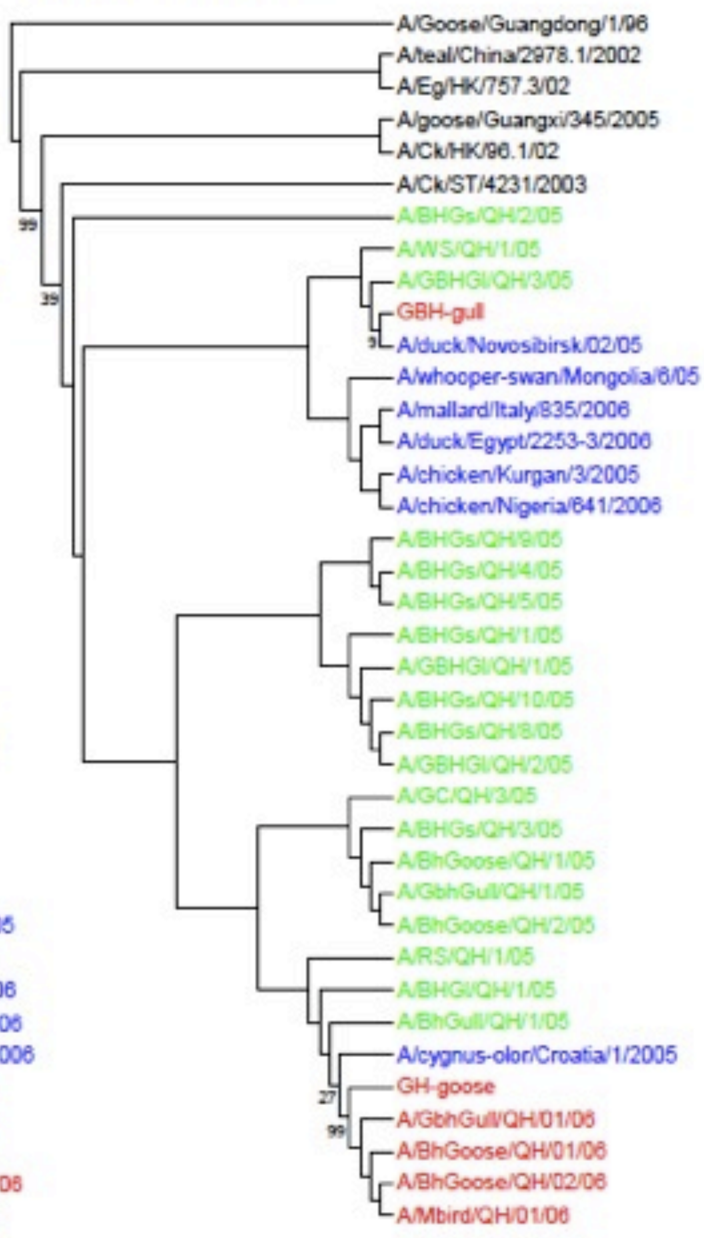

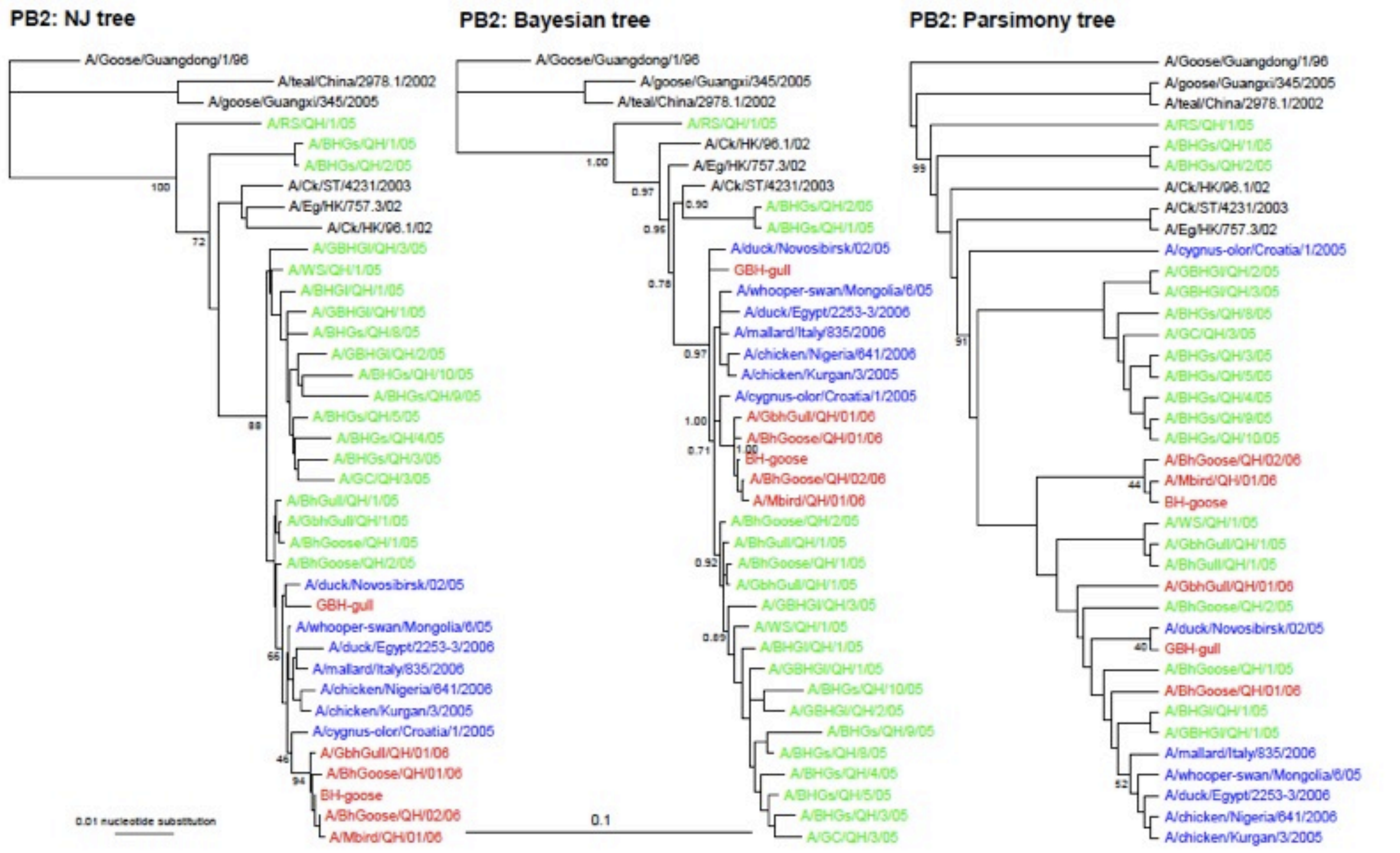
PB2: NJ tree
A/Goose/Guangdong/1/96
A/teal/China/2978.1/2002
A/goose/Guangxi/345/2005
A/RS/QH/1/05
A/BHGs/QH/1/05
A/BHGs/QH/2/05
A/Ck/ST/4231/2003
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100
72
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GBH-gull
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46
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BH-goose
A/BhGoose/QH/02/06
A/Mbird/QH/01/06
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PB2: Bayesian tree
A/Goose/Guangdong/1/96
A/goose/Guangxi/345/2005
A/teal/China/2978.1/2002
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A/BHGs/QH/4/05
A/BHGs/QH/5/05
A/BHGs/QH/3/05
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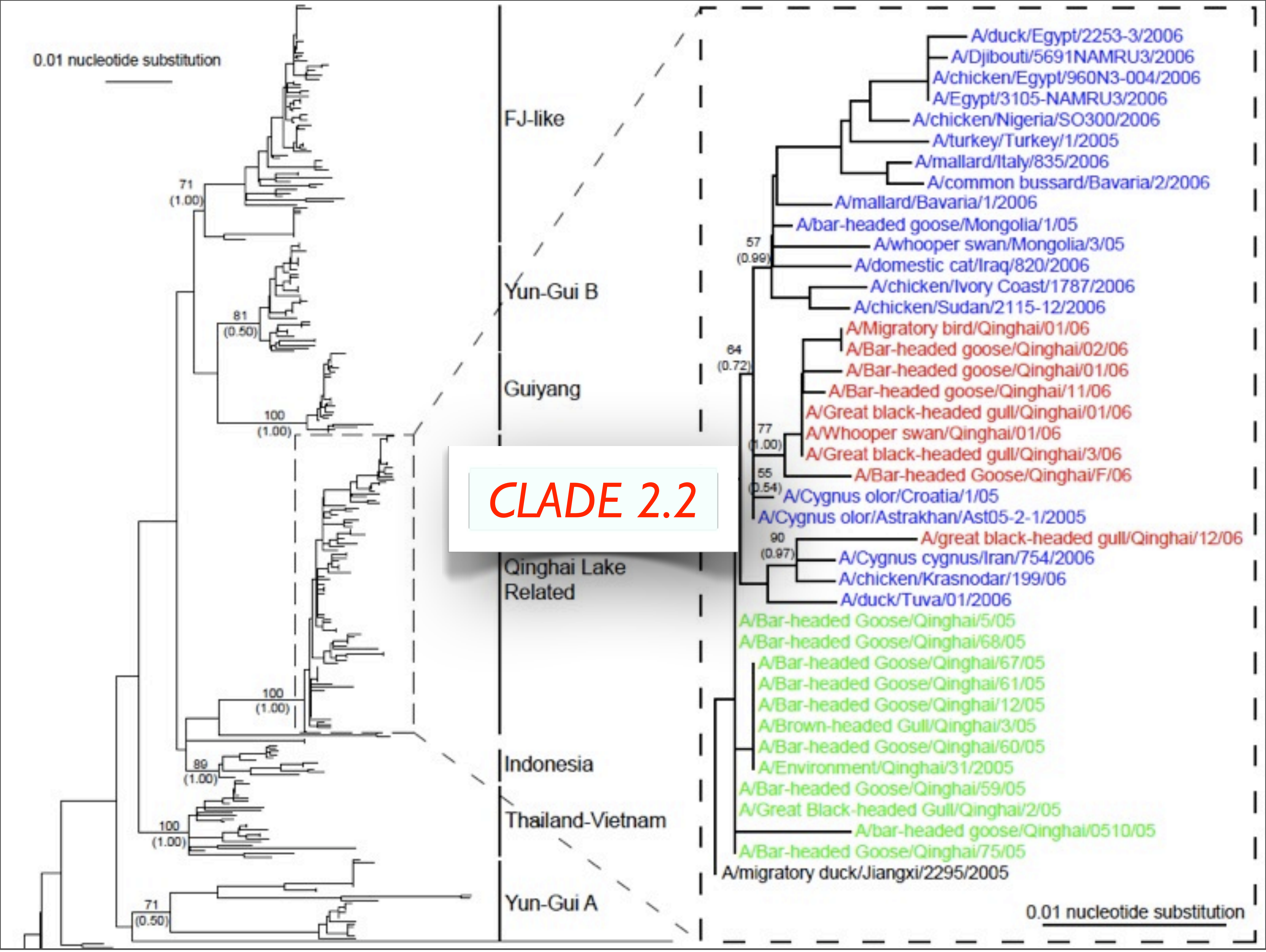
A/GC/QH/3/05
PB2: Parsimony tree
A/Goose/Guangdong/1/96
A/goose/Guangxi/345/2005
A/teal/China/2978.1/2002
A/RS/QH/1/05
A/BHGs/QH/1/05
99
A/BHGs/QH/2/05
A/Ck/HK/96.1/02
A/Ck/ST/4231/2003
A/Eg/HK/757.3/02
A/cygnus-olor/Croatia/1/2005
A/GBHGl/QH/2/05
A/GBHGl/QH/3/05
A/BHGs/QH/8/05
A/GC/QH/3/05
91
A/BHGs/QH/3/05
A/BHGs/QH/5/05
A/BHGs/QH/4/05
A/BHGs/QH/9/05
A/BHGs/QH/10/05
A/BhGoose/QH/02/06
44
A/Mbird/QH/01/06
BH-goose
A/WS/QH/1/05
A/GbhGull/QH/1/05
A/BhGull/QH/1/05
A/GbhGull/QH/01/06
A/BhGoose/QH/2/05
A/duck/Novosibirsk/02/05
40
GBH-gull
A/BhGoose/QH/1/05
A/BhGoose/QH/01/06
A/BHGl/QH/1/05
A/GBHGl/QH/1/05
A/mallard/Italy/835/2006
52
A/whooper-swan/Mongolia/6/05
A/duck/Egypt/2253-3/2006
A/chicken/Nigeria/641/2006
A/chicken/Kurgan/3/2005

J Gen Virol, 2008


0.01 nucleotide substitution
71
(1.00)
81
(0.50)
100
(1.00)
100
(1.00)
89
(1.00)
100
(1.00)
71
(0.50)
FJ-like
Yun-Gui B
Guiyang
Qinghai Lake
Related
Indonesia
Thailand-Vietnam
Yun-Gui A
A/duck/Egypt/2253-3/2006
A/Djibouti/5691NAMRU3/2006
A/chicken/Egypt/960N3-004/2006
A/Egypt/3105-NAMRU3/2006
A/chicken/Nigeria/SO300/2006
A/turkey/Turkey/1/2005
A/mallard/Italy/835/2006
A/common bussard/Bavaria/2/2006
A/mallard/Bavaria/1/2006
A/bar-headed goose/Mongolia/1/05
A/whooper swan/Mongolia/3/05
A/domestic cat/Iraq/820/2006
A/chicken/Ivory Coast/1787/2006
A/chicken/Sudan/2115-12/2006
57
(0.99)
64
(0.72)
A/Migratory bird/Qinghai/01/06
A/Bar-headed goose/Qinghai/02/06
A/Bar-headed goose/Qinghai/01/06
A/Bar-headed goose/Qinghai/11/06
A/Great black-headed gull/Qinghai/01/06
A/Whooper swan/Qinghai/01/06
A/Great black-headed gull/Qinghai/3/06
A/Bar-headed Goose/Qinghai/F/06
77
(1.00)
55
(0.54)
A/Cygnus olor/Croatia/1/05
A/Cygnus olor/Astrakhan/Ast05-2-1/2005
90
(0.97)
A/great black-headed gull/Qinghai/12/06
A/Cygnus cygnus/Iran/754/2006
A/chicken/Krasnodar/199/06
A/duck/Tuva/01/2006
A/Bar-headed Goose/Qinghai/5/05
A/Bar-headed Goose/Qinghai/68/05
A/Bar-headed Goose/Qinghai/67/05
A/Bar-headed Goose/Qinghai/61/05
A/Bar-headed Goose/Qinghai/12/05
A/Brown-headed Gull/Qinghai/3/05
A/Bar-headed Goose/Qinghai/60/05
A/Environment/Qinghai/31/2005
A/Bar-headed Goose/Qinghai/59/05
A/Great Black-headed Gull/Qinghai/2/05
A/bar-headed goose/Qinghai/0510/05
A/Bar-headed Goose/Qinghai/75/05
A/migratory duck/Jiangxi/2295/2005
0.01 nucleotide substitution

CLADE 2.2

2009

Specimen Collection and Virus Isolation of Qinghai Lake in 2009

- **Specimen**
  - **6 Great Black-headed Gulls**
  - **2 Great Cormorants**
  - **2 Brown-headed Gulls**
- **Virus**
  - **9 isolates**

A/great black-headed gull/Qinghai/1/2009
A/great black-headed gull/Qinghai/2/2009
A/great black-headed gull/Qinghai/3/2009
A/great black-headed gull/Qinghai/4/2009
A/great black-headed gull/Qinghai/5/2009
A/great black-headed gull/Qinghai/6/2009
A/great cormorant/Qinghai/1/2009
A/brown-headed gull/Qinghai/1/2009
A/brown-headed gull/Qinghai/2/2009

EID50

Virus	$\log EID_{50}$
GBHGull/QH/1/09	-9
GBHGull/QH/2/09	-7.5
GBHGull/QH/3/09	-8.3
GBHGull/QH/4/09	-8.1
GBHGull/QH/5/09	-7.9
GBHGull/QH/6/09	-7
GC/QH/1/09	-8.3
BHGull/QH/1/09	-8.1
BHGull/QH/2/09	-8.5

INTRAVENOUS PATHOGENICITY INDEX (IVPI)

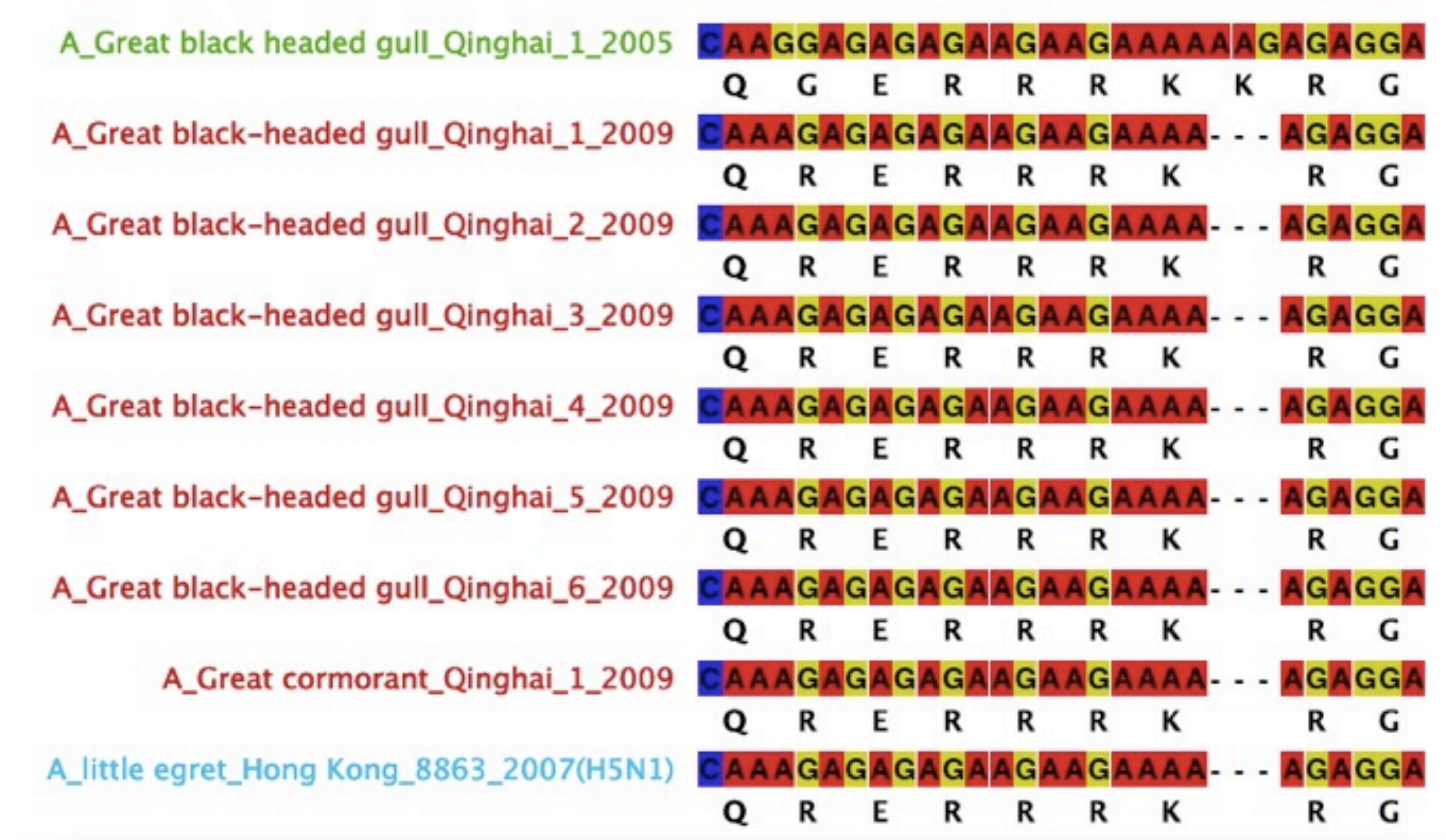
Virus	IVPI
GBHGull/QH/1/09	3
GBHGull/QH/6/09	2.94

GBHGull/QH/1/09: highest EID50
GBHGull/QH/6/09: lowest EID50

Antigenicity

	Antisera （chicken） of		
Virus	QH05	MH08	QH09
QH05 (2.2)	64	64	32
MH08 (2.3.2)	16	128	128
QH09 (2.3.2)	32	128	256

	Antisera （mouse） of	
Virus	QH05	QH09
QH05	512	128
MH08	128	256
QH09	128	512

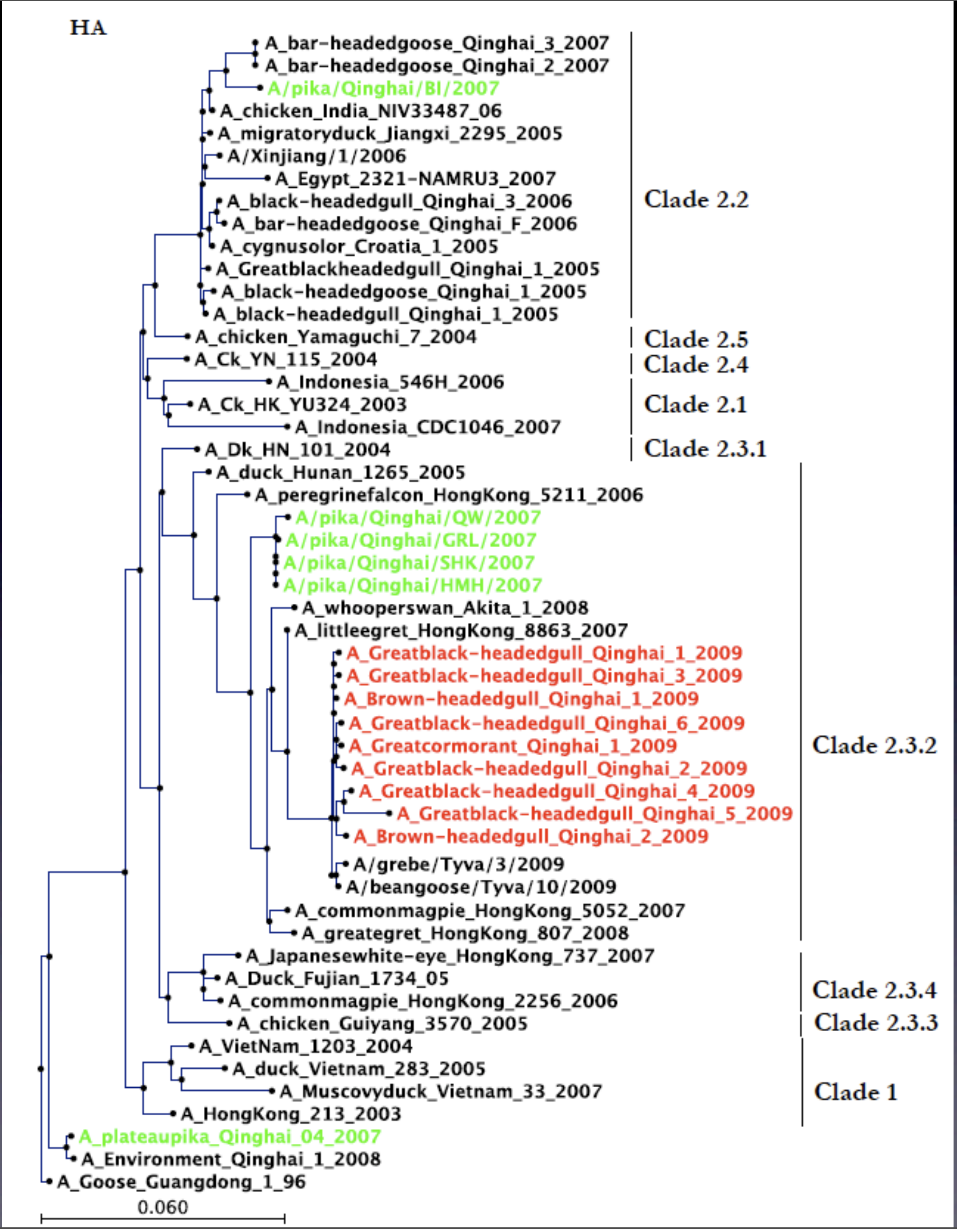


A_Great black headed gull_Qinghai_1_2005	CAAGGAGAGAGAAGAAGAAAAAAGAGAGGA	Q G E R R R K K R G
A_Great black-headed gull_Qinghai_1_2009	CAAAGAGAGAGAAGAAGAAAA- - - AGAGGA	Q R E R R R K R G
A_Great black-headed gull_Qinghai_2_2009	CAAAGAGAGAGAAGAAGAAAA- - - AGAGGA	Q R E R R R K R G
A_Great black-headed gull_Qinghai_3_2009	CAAAGAGAGAGAAGAAGAAAA- - - AGAGGA	Q R E R R R K R G
A_Great black-headed gull_Qinghai_4_2009	CAAAGAGAGAGAAGAAGAAAA- - - AGAGGA	Q R E R R R K R G
A_Great black-headed gull_Qinghai_5_2009	CAAAGAGAGAGAAGAAGAAAA- - - AGAGGA	Q R E R R R K R G
A_Great black-headed gull_Qinghai_6_2009	CAAAGAGAGAGAAGAAGAAAA- - - AGAGGA	Q R E R R R K R G
A_Great cormorant_Qinghai_1_2009	CAAAGAGAGAGAAGAAGAAAA- - - AGAGGA	Q R E R R R K R G
A_little egret_Hong Kong_8863_2007(H5N1)	CAAAGAGAGAGAAGAAGAAAA- - - AGAGGA	Q R E R R R K R G

PB2	CLADE 2.2	627K
	CLADE 2.3.2	627E
	QH09	627E

Phylogenetics

- Qinghai Lake 2009
- Qinghai Lake 2007, Pika (Zhou et al. 2009 JVI)
- Qinghai Lake 2007-2008 (He et al. submitted to GenBank)
- Reference Viruses (Clade 1 & 2, from WHO)
- ML tree with Bootstrap (RAxML)


HA
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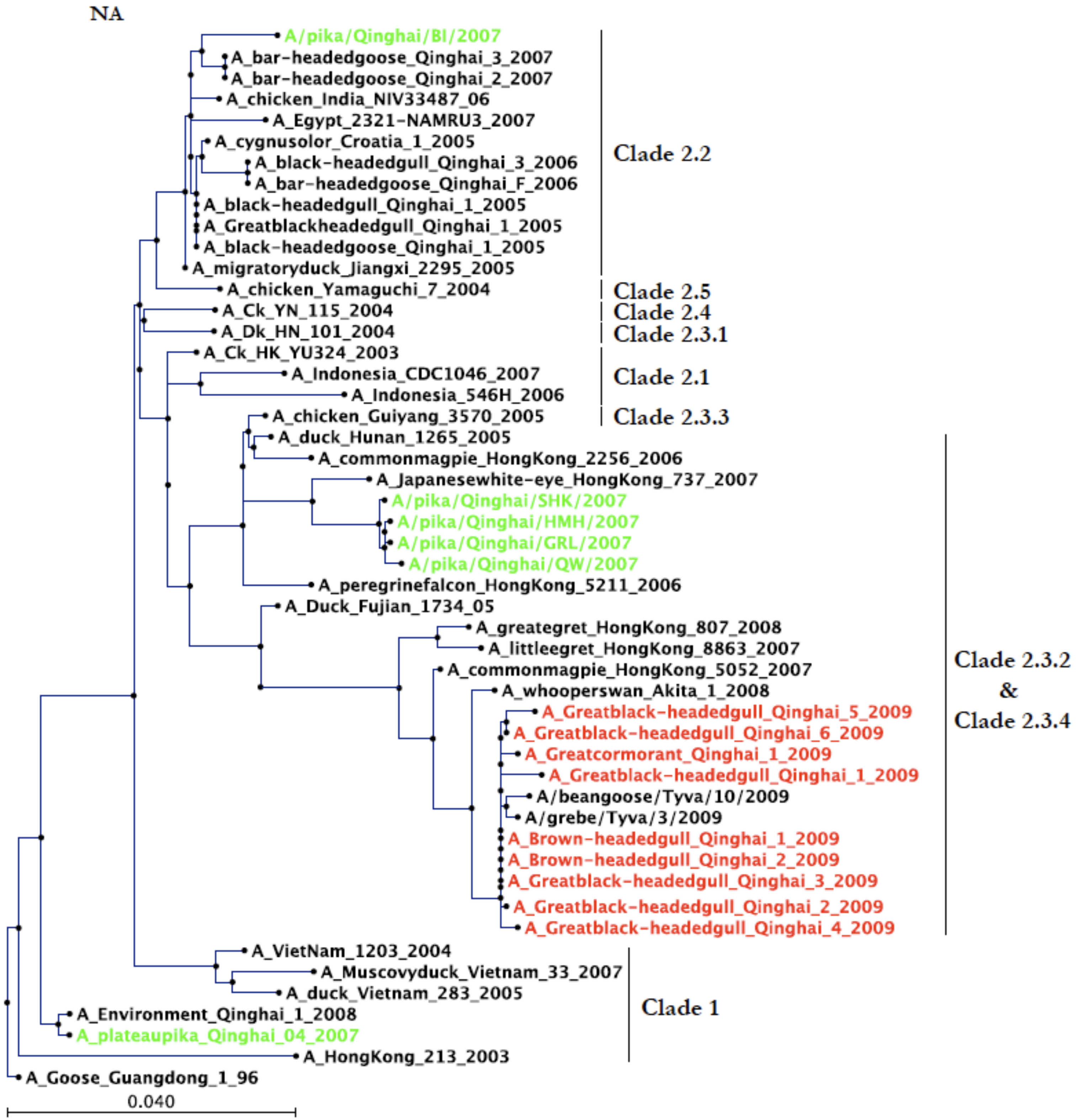
A_bar-headedgoose_Qinghai_2_2007
A/pika/Qinghai/BI/2007
A_chicken_India_NIV33487_06
A_migratoryduck_Jiangxi_2295_2005
A/Xinjiang/1/2006
A_Egypt_2321-NAMRU3_2007
A_black-headedgull_Qinghai_3_2006
A_bar-headedgoose_Qinghai_F_2006
A_cygnusolor_Croatia_1_2005
A_Greatblackheadedgull_Qinghai_1_2005
A_black-headedgoose_Qinghai_1_2005
A_black-headedgull_Qinghai_1_2005
Clade 2.2
A_chicken_Yamaguchi_7_2004
Clade 2.5
A_Ck_YN_115_2004
Clade 2.4
A_Indonesia_546H_2006
A_Ck_HK_YU324_2003
A_Indonesia_CDC1046_2007
Clade 2.1
A_Dk_HN_101_2004
Clade 2.3.1
A_duck_Hunan_1265_2005
A_peregrinefalcon_HongKong_5211_2006
A/pika/Qinghai/QW/2007
A/pika/Qinghai/GRL/2007
A/pika/Qinghai/SHK/2007
A/pika/Qinghai/HMH/2007
A_whooperswan_Akita_1_2008
A_littleegret_HongKong_8863_2007
A_Greatblack-headedgull_Qinghai_1_2009
A_Greatblack-headedgull_Qinghai_3_2009
A_Brown-headedgull_Qinghai_1_2009
A_Greatblack-headedgull_Qinghai_6_2009
A_Greatcormorant_Qinghai_1_2009
A_Greatblack-headedgull_Qinghai_2_2009
A_Greatblack-headedgull_Qinghai_4_2009
A_Greatblack-headedgull_Qinghai_5_2009
A_Brown-headedgull_Qinghai_2_2009
A/grebe/Tyva/3/2009
A/beangoose/Tyva/10/2009
A_commonmagpie_HongKong_5052_2007
A_greategret_HongKong_807_2008
Clade 2.3.2
A_Japanesewhite-eye_HongKong_737_2007
A_Duck_Fujian_1734_05
A_commonmagpie_HongKong_2256_2006
Clade 2.3.4
A_chicken_Guiyang_3570_2005
Clade 2.3.3
A_VietNam_1203_2004
A_duck_Vietnam_283_2005
A_Muscovyduck_Vietnam_33_2007
A_HongKong_213_2003
Clade 1
A_plateaupika_Qinghai_04_2007
A_Environment_Qinghai_1_2008
A_Goose_Guangdong_1_96
0.060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A_chicken_Yamaguchi_7_2004
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A_Ck_HK_YU324_2003
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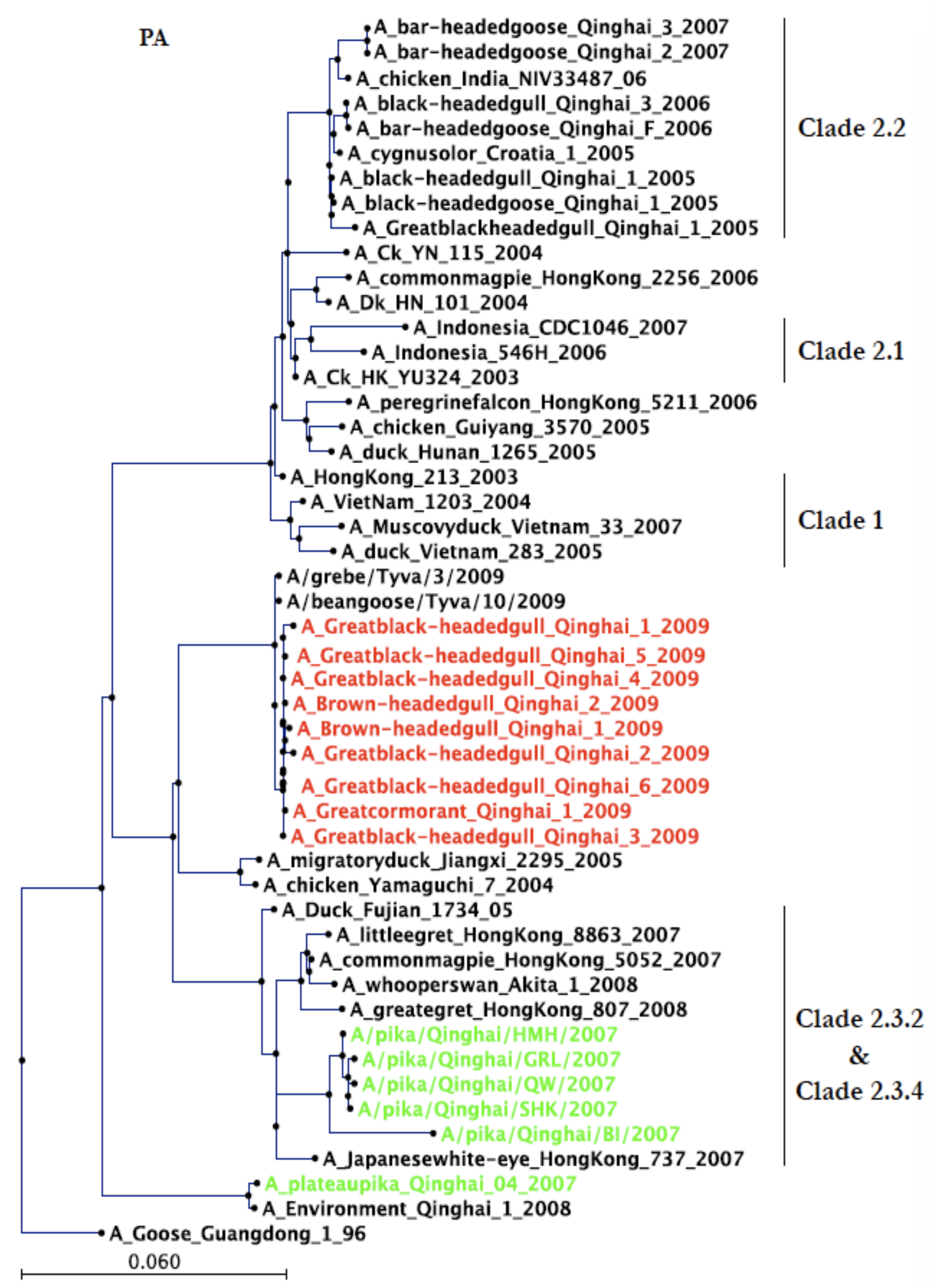
A_chicken_Guiyang_3570_2005
A_duck_Hunan_1265_2005
A_commonmagpie_HongKong_2256_2006
A_Japanesewhite-eye_HongKong_737_2007
A/pika/Qinghai/SHK/2007
A/pika/Qinghai/HMH/2007
A/pika/Qinghai/GRL/2007
A/pika/Qinghai/QW/2007
A_peregrinefalcon_HongKong_5211_2006
A_Duck_Fujian_1734_05
A_greategret_HongKong_807_2008
A_littleegret_HongKong_8863_2007
A_commonmagpie_HongKong_5052_2007
A_whooperswan_Akita_1_2008
A_Greatblack-headedgull_Qinghai_5_2009
A_Greatblack-headedgull_Qinghai_6_2009
A_Greatcormorant_Qinghai_1_2009
A_Greatblack-headedgull_Qinghai_1_2009
A/beangoose/Tyva/10/2009
A/grebe/Tyva/3/2009
A_Brown-headedgull_Qinghai_1_2009
A_Brown-headedgull_Qinghai_2_2009
A_Greatblack-headedgull_Qinghai_3_2009
A_Greatblack-headedgull_Qinghai_2_2009
A_Greatblack-headedgull_Qinghai_4_2009
A_VietNam_1203_2004
A_Muscovyduck_Vietnam_33_2007
A_duck_Vietnam_283_2005
A_Environment_Qinghai_1_2008
A_plateaupika_Qinghai_04_2007
A_HongKong_213_2003
A_Goose_Guangdong_1_96
0.040
Clade 2.2
Clade 2.5
Clade 2.4
Clade 2.3.1
Clade 2.1
Clade 2.3.3
Clade 2.3.2
&
Clade 2.3.4
Clade 1

Similar to results from Russia

Avian Influenza (H5N1) Outbreak among Wild Birds, Russia, 2009

To the Editor: Highly pathogenic avian influenza (HPAI) virus (H5N1) has been endemic in poultry in Southeast Asia since 2003 (*1*). In April 2005, an outbreak of influenza virus (H5N1) infection was detected in wild birds on Qinghai Lake in western China (*2*). Subsequently, the Qinghai-like (clade 2.2) HPAI virus (H5N1) lineage was detected in wild birds and poultry in many countries (*1*,*3*,*4*). The source of these introduc-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A_black-headedgoose_Qinghai_1_2005
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A_Ck_YN_115_2004
A_commonmagpie_HongKong_2256_2006
A_Dk_HN_101_2004
A_Indonesia_CDC1046_2007
A_Indonesia_546H_2006
A_Ck_HK_YU324_2003
Clade 2.1
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A_chicken_Guiyang_3570_2005
A_duck_Hunan_1265_2005
A_HongKong_213_2003
A_VietNam_1203_2004
A_Muscovyduck_Vietnam_33_2007
A_duck_Vietnam_283_2005
Clade 1
A/grebe/Tyva/3/2009
A/beangoose/Tyva/10/2009
A_Greatblack-headedgull_Qinghai_1_2009
A_Greatblack-headedgull_Qinghai_5_2009
A_Greatblack-headedgull_Qinghai_4_2009
A_Brown-headedgull_Qinghai_2_2009
A_Brown-headedgull_Qinghai_1_2009
A_Greatblack-headedgull_Qinghai_2_2009
A_Greatblack-headedgull_Qinghai_6_2009
A_Greatcormorant_Qinghai_1_2009
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A_migratoryduck_Jiangxi_2295_2005
A_chicken_Yamaguchi_7_2004
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A_littleegret_HongKong_8863_2007
A_commonmagpie_HongKong_5052_2007
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A_greategret_HongKong_807_2008
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A/pika/Qinghai/QW/2007
A/pika/Qinghai/SHK/2007
A/pika/Qinghai/BI/2007
A_Japanesewhite-eye_HongKong_737_2007
Clade 2.3.2
&
Clade 2.3.4
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A_Environment_Qinghai_1_2008
A_Goose_Guangdong_1_96
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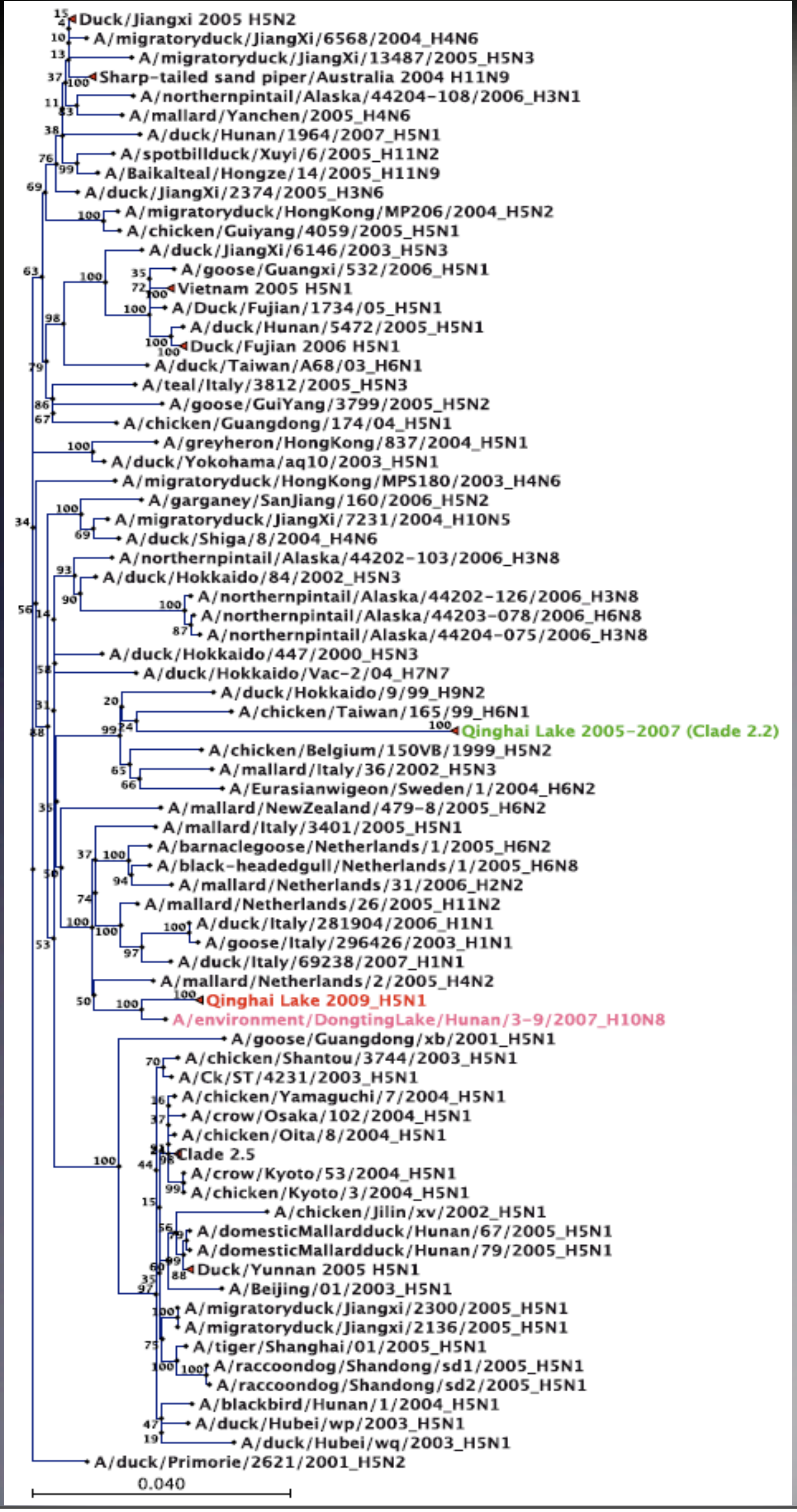
Duck/Jiangxi 2005 H5N2
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Sharp-tailed sand piper/Australia 2004 H11N9
A/northernpintail/Alaska/44204-108/2006_H3N1
A/mallard/Yanchen/2005_H4N6
A/duck/Hunan/1964/2007_H5N1
A/spotbillduck/Xuyi/6/2005_H11N2
A/Baikalteal/Hongze/14/2005_H11N9
A/duck/JiangXi/2374/2005_H3N6
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A/chicken/Guiyang/4059/2005_H5N1
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A/goose/Guangxi/532/2006_H5N1
Vietnam 2005 H5N1
A/Duck/Fujian/1734/05_H5N1
A/duck/Hunan/5472/2005_H5N1
Duck/Fujian 2006 H5N1
A/duck/Taiwan/A68/03_H6N1
A/teal/Italy/3812/2005_H5N3
A/goose/GuiYang/3799/2005_H5N2
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A/duck/Yokohama/aq10/2003_H5N1
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A/northernpintail/Alaska/44203-078/2006_H6N8
A/northernpintail/Alaska/44204-075/2006_H3N8
A/duck/Hokkaido/447/2000_H5N3
A/duck/Hokkaido/Vac-2/04_H7N7
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A/chicken/Belgium/150VB/1999_H5N2
A/mallard/Italy/36/2002_H5N3
A/Eurasianwigeon/Sweden/1/2004_H6N2
A/mallard/NewZealand/479-8/2005_H6N2
A/mallard/Italy/3401/2005_H5N1
A/barnaclegoose/Netherlands/1/2005_H6N2
A/black-headedgull/Netherlands/1/2005_H6N8
A/mallard/Netherlands/31/2006_H2N2
A/mallard/Netherlands/26/2005_H11N2
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A/mallard/Netherlands/2/2005_H4N2
Qinghai Lake 2009_H5N1
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A/Ck/ST/4231/2003_H5N1
A/chicken/Yamaguchi/7/2004_H5N1
A/crow/Osaka/102/2004_H5N1
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Clade 2.5
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A/chicken/Jilin/xv/2002_H5N1
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Duck/Yunnan 2005 H5N1
A/Beijing/01/2003_H5N1
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A/tiger/Shanghai/01/2005_H5N1
A/raccoondog/Shandong/sd1/2005_H5N1
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A/blackbird/Hunan/1/2004_H5N1
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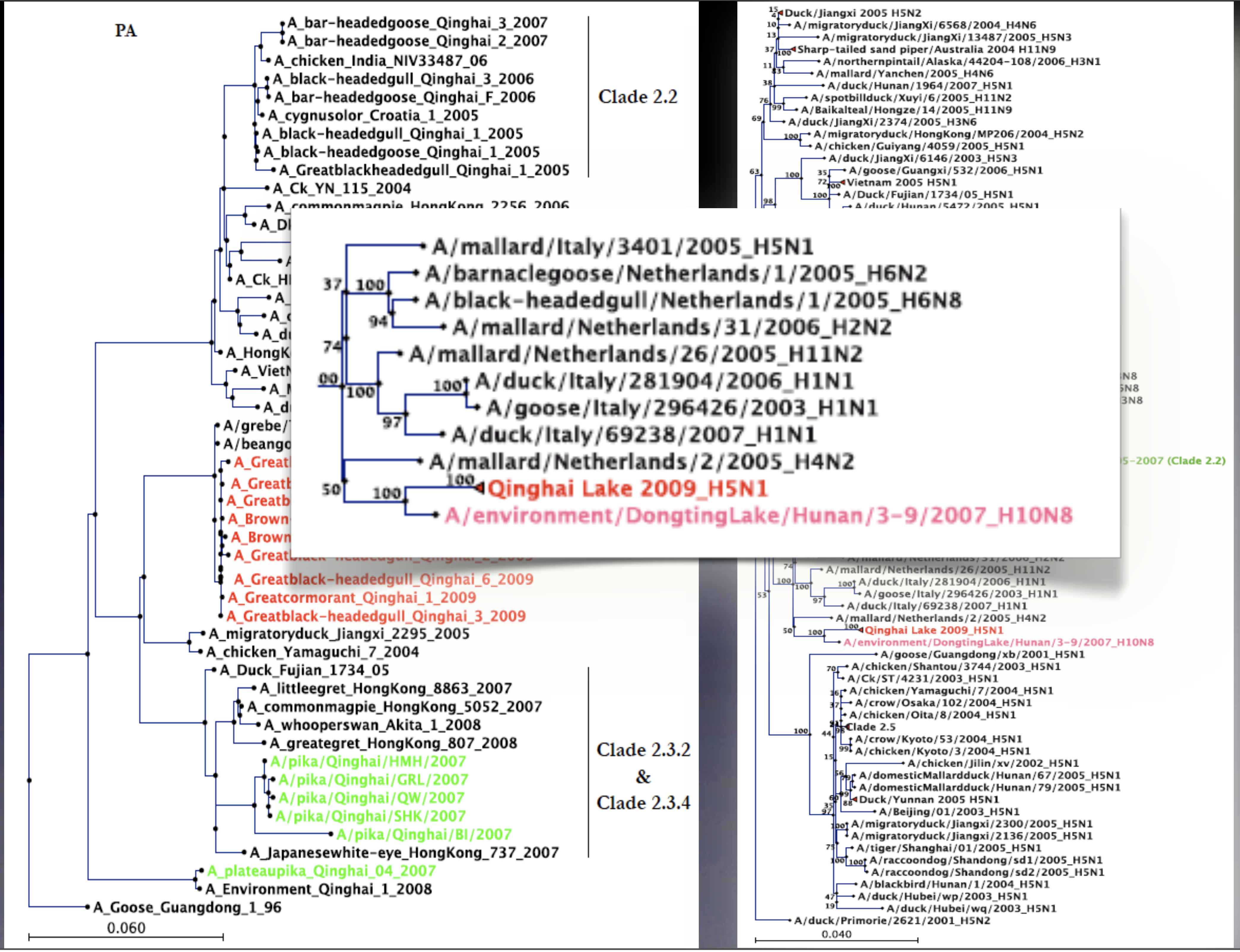
A/duck/Primorie/2621/2001_H5N2
0.040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A_bar-headedgoose_Qinghai_2_2007
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A_black-headedgull_Qinghai_3_2006
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A_cygnusolor_Croatia_1_2005
A_black-headedgull_Qinghai_1_2005
A_black-headedgoose_Qinghai_1_2005
A_Greatblackheadedgull_Qinghai_1_2005
Clade 2.2
A_Ck_YN_115_2004
A_Greatblack-headedgull_Qinghai_6_2009
A_Greatcormorant_Qinghai_1_2009
A_Greatblack-headedgull_Qinghai_3_2009
A_migratoryduck_Jiangxi_2295_2005
A_chicken_Yamaguchi_7_2004
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A_whooperswan_Akita_1_2008
A_greategret_HongKong_807_2008
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A/pika/Qinghai/GRL/2007
A/pika/Qinghai/QW/2007
A/pika/Qinghai/SHK/2007
A/pika/Qinghai/BI/2007
A_Japanesewhite-eye_HongKong_737_2007
Clade 2.3.2
&
Clade 2.3.4
A_plateaupika_Qinghai_04_2007
A_Environment_Qinghai_1_2008
A_Goose_Guangdong_1_96
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A/mallard/Netherlands/26/2005_H11N2
A/duck/Italy/281904/2006_H1N1
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A/duck/Italy/69238/2007_H1N1
A/mallard/Netherlands/2/2005_H4N2
Qinghai Lake 2009_H5N1
A/environment/DongtingLake/Hunan/3-9/2007_H10N8
37
100
94
74
100
100
97
50
100
100
Duck/Jiangxi 2005 H5N2
A/migratoryduck/JiangXi/6568/2004_H4N6
A/migratoryduck/JiangXi/13487/2005_H5N3
Sharp-tailed sand piper/Australia 2004 H11N9
A/northernpintail/Alaska/44204-108/2006_H3N1
A/mallard/Yanchen/2005_H4N6
A/duck/Hunan/1964/2007_H5N1
A/spotbillduck/Xuyi/6/2005_H11N2
A/Baikalteal/Hongze/14/2005_H11N9
A/duck/JiangXi/2374/2005_H3N6
A/migratoryduck/HongKong/MP206/2004_H5N2
A/chicken/Guiyang/4059/2005_H5N1
A/duck/JiangXi/6146/2003_H5N3
A/goose/Guangxi/532/2006_H5N1
Vietnam 2005 H5N1
A/Duck/Fujian/1734/05_H5N1
5-2007 (Clade 2.2)
A/mallard/Netherlands/26/2005_H11N2
A/duck/Italy/281904/2006_H1N1
A/goose/Italy/296426/2003_H1N1
A/duck/Italy/69238/2007_H1N1
A/mallard/Netherlands/2/2005_H4N2
Qinghai Lake 2009_H5N1
A/environment/DongtingLake/Hunan/3-9/2007_H10N8
A/goose/Guangdong/xb/2001_H5N1
A/chicken/Shantou/3744/2003_H5N1
A/Ck/ST/4231/2003_H5N1
A/chicken/Yamaguchi/7/2004_H5N1
A/crow/Osaka/102/2004_H5N1
A/chicken/Oita/8/2004_H5N1
Clade 2.5
A/crow/Kyoto/53/2004_H5N1
A/chicken/Kyoto/3/2004_H5N1
A/chicken/Jilin/xv/2002_H5N1
A/domesticMallardduck/Hunan/67/2005_H5N1
A/domesticMallardduck/Hunan/79/2005_H5N1
Duck/Yunnan 2005 H5N1
A/Beijing/01/2003_H5N1
A/migratoryduck/Jiangxi/2300/2005_H5N1
A/migratoryduck/Jiangxi/2136/2005_H5N1
A/tiger/Shanghai/01/2005_H5N1
A/raccoondog/Shandong/sd1/2005_H5N1
A/raccoondog/Shandong/sd2/2005_H5N1
A/blackbird/Hunan/1/2004_H5N1
A/duck/Hubei/wp/2003_H5N1
A/duck/Hubei/wq/2003_H5N1
A/duck/Primorie/2621/2001_H5N2
0.040

2010

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Clade 2.3.2 Avian Influenza Virus (H5N1), Qinghai Lake Region, China, 2009–2010

To the Editor: In 2005, a large population of wild migratory birds was infected with highly pathogenic avian influenza (HPAI) virus (H5N1) in the Qinghai Lake region of western People's Republic of China, resulting in the death of ≈10,000 birds (*1*,*2*). On the basis of phylogenetic analysis of the hemagglutinin (HA) gene, the virus was classified as clade 2.2 according to the World Health Organization guidelines. Subsequently, viruses from this clade were found in Mongolia, Russia, Europe, and Africa along the migratory flyways of birds (*3*,*4*). This unique distribution of the same clade of HPAI virus (H5N1) through different migratory routes indicates that migratory birds might play a global role in virus dissemination (*3*,*4*).

In 2006, viruses from the same clade were isolated in the Qinghai Lake region (*3*). Analysis of viral outbreaks along migratory flyways demonstrated a similar outbreak pattern for the past 4 years (2006–2009) (*5*). During that period, clade 2.2 avian influenza virus (H5N1) was isolated in China, Mongolia, Russia, Germany, Egypt, and Nigeria; all viruses were closely related to the Qinghai Lake virus. Despite the broad distribution of clade 2.2 viruses in migratory flyways, few isolates of clade 2.2 viruses in local domestic poultry were reported, especially in China (*6*). Outbreaks of these viruses were reported in poultry in Africa (*7*). The reason these viruses rarely cause outbreaks in poultry is unknown.

During May–June 2009 and 2010, several dead migratory birds were found in the Qinghai Lake region. Nine HPAI viruses (H5N1) were isolated in 2009 and 2 were isolated in 2010 from great cormorants (*Phalacrocorax carbo*), brown-headed gulls (*Chroicocephalus brunnicephalus*), great black-headed gulls (*Ichthyaetus ichthyaetus*), great-crested grebes (*Podiceps cristatus*), and bar-headed geese (*Anser indicus*) and serotyped as described (*3*). HA genes from all 11 isolates were subsequently amplified by using reverse transcription–PCR and sequenced.

Phylogenetic analysis of HA sequences and an additional HA gene sequence from the 2009 Qinghai Lake subtype H5N1 virus isolate from a great crested grebe (from the National Avian Influenza Virus Reference Laboratory, Harbin, China) (GenBank accession no. CY063318) showed that HA genes from all 12 viruses clustered as clade 2.3.2 (Figure); none clustered with clade 2.2 viruses. Additionally, the HA cleavage site in the new isolates is PQRERRRKRG, which is identical to that of clade 2.3.2 viruses. In clade 2.2, the cleavage site is PQRERRRKKRG.

A bootstrap (1,000×) maximum likelihood tree (*8*) also demonstrated that Qinghai 2009 and 2010 virus isolates are closely related to those isolated in Mongolia and Uvs Nuur Lake in 2009, as reported by Sharshov et al. (*5*). Qinghai Lake and Uvs Nuur Lake, which are found along the migratory flyway in central Asia, are major lakes for bird migration and breeding. Many birds fly from Qinghai Lake to Uvs Nuur Lake in the spring.

If one considers isolation date and bird species infected, viruses isolated in Mongolia and Russia and our isolates were likely transmitted between the 2 lake regions by bird migration. Moreover, HA sequences are closely related to viruses isolated from wild birds in Hong Kong and Japan during 2007–2008, which are the most recent isolates of clade 2.3.2 viruses before isolation of 2009 Qinghai Lake viruses. These results indicate that viruses in the Qinghai Lake region may be transmitted by wild birds along the migratory flyway in eastern

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[1] These authors contributed equally to this article.

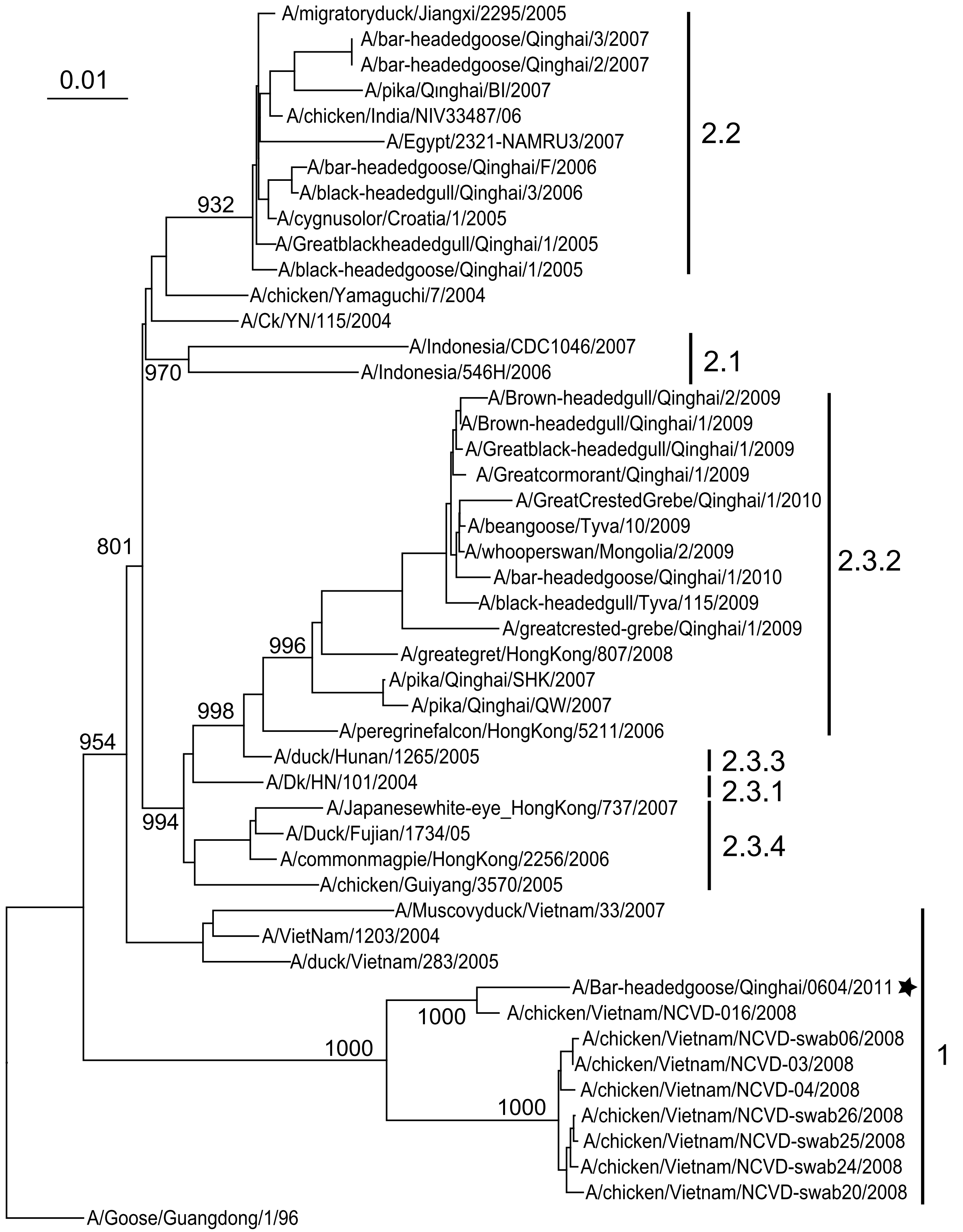
istent productive cough, who had adiographic abnormalities suggestive f TB, or who received TB treatment ither currently or in the 2 years receding the survey. Persons had n in-depth interview that included uestions on where they were treated or TB. Assessment of socioeconomic tatus was based on 9 household haracteristics (*7*).

Missing data were imputed by sing multiple imputation methods, ssuming that these data were nissing at random to adjust for onparticipation and missing data n facility of TB treatment (*8*). We sed the ice and mi commands in tata version 11 software (StataCorp P, College Station, TX, USA), vhich included age, area, zone, and ocioeconomic status.

Of the 103,924 eligible persons n selected districts, 94,179 (91%) vere screened, 7,498 were identified s having suspected TB, and 407 eported having been recently treated or TB: 316 (77.6%) in public ealth facilities (PHFs) reporting ases to the NTP, 8 (2.0%) in PHFs ot reporting cases to the NTP, and 9 (7.1%) in private health care acilities not reporting to the NTP. ifty-four (13.3%) did not provide nformation about where they were reated. Multiple imputation led to djusted proportions of 88.9%, 2.9%, nd 8.2%, respectively. Sensitivity nalyses, which assigned 54 persons vith missing data for location of TB reatment to PHFs or private clinics, esulted in a range of 7.1%–20.3% or private sector treatment.

Characteristics of participants by ype of facility where they received B treatment are shown in the able. Women, younger persons, and esidents of southern Vietnam were nore likely to seek treatment in the rivate sector. Urban populations and hose with the highest socioeconomic tatus were most likely to seek private are, but these differences were not ignificant (Table).

2011


0.01
A/migratoryduck/Jiangxi/2295/2005
A/bar-headedgoose/Qinghai/3/2007
A/bar-headedgoose/Qinghai/2/2007
A/pika/Qinghai/BI/2007
A/chicken/India/NIV33487/06
A/Egypt/2321-NAMRU3/2007
A/bar-headedgoose/Qinghai/F/2006
A/black-headedgull/Qinghai/3/2006
A/cygnusolor/Croatia/1/2005
A/Greatblackheadedgull/Qinghai/1/2005
A/black-headedgoose/Qinghai/1/2005
2.2
932
A/chicken/Yamaguchi/7/2004
A/Ck/YN/115/2004
A/Indonesia/CDC1046/2007
A/Indonesia/546H/2006
970
2.1
A/Brown-headedgull/Qinghai/2/2009
A/Brown-headedgull/Qinghai/1/2009
A/Greatblack-headedgull/Qinghai/1/2009
A/Greatcormorant/Qinghai/1/2009
A/GreatCrestedGrebe/Qinghai/1/2010
A/beangoose/Tyva/10/2009
A/whooperswan/Mongolia/2/2009
A/bar-headedgoose/Qinghai/1/2010
A/black-headedgull/Tyva/115/2009
A/greatcrested-grebe/Qinghai/1/2009
A/greategret/HongKong/807/2008
A/pika/Qinghai/SHK/2007
A/pika/Qinghai/QW/2007
A/peregrinefalcon/HongKong/5211/2006
2.3.2
801
996
998
A/duck/Hunan/1265/2005
2.3.3
954
A/Dk/HN/101/2004
2.3.1
A/Japanesewhite-eye_HongKong/737/2007
A/Duck/Fujian/1734/05
A/commonmagpie/HongKong/2256/2006
A/chicken/Guiyang/3570/2005
994
2.3.4
A/Muscovyduck/Vietnam/33/2007
A/VietNam/1203/2004
A/duck/Vietnam/283/2005
A/Bar-headedgoose/Qinghai/0604/2011 ★
A/chicken/Vietnam/NCVD-016/2008
1000
1000
A/chicken/Vietnam/NCVD-swab06/2008
A/chicken/Vietnam/NCVD-03/2008
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1000
A/chicken/Vietnam/NCVD-swab26/2008
A/chicken/Vietnam/NCVD-swab25/2008
A/chicken/Vietnam/NCVD-swab24/2008
A/chicken/Vietnam/NCVD-swab20/2008
1
A/Goose/Guangdong/1/96

About Phylogenetics

- Sequences => Alignment => Phylogeny
  - Sequence set is the MOST important
  - Align your sequence, codon by codon
  - Neighbor Joining? Bayesian? Maximum Likelihood?

Tree Presentation

- TreeView
- Tree-Illustrator
- CLC Sequence Viewer
- Tree => Vector Graphic

THE END!

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