Supplementary material Tables

Table S1. Primers used in this study.

virus	Primer	Nucleotide information
PDCoV	PDCoV-F	5'-TCCTCCAAGGAGGCTATGC-3'
	PDCoV-R	5'-GCGAATTCTGGATCGTTGT-3'
PEDV	PEDV-F	5'-CCCACCAACTTTAGTATGAGT-3'
	PEDV-R	5'-GTACCATGACACCAGAGTAAT-3'
PTV	PTV-F	5'-GGCGACAGGGTACAGAAGAG-3'
	PTV-R	5'- GGCCAGCCGCGACCCTGTC-3'
PKV	PKV-F	5'-TGTGCCTTCCGCATTGTT-3'
	PKV-R	5'- TGTACCTGTCGTAAAACT-3'
TGEV	TGEV-F	5'-ACTCGCTATCGCATGGTGAA-3'
	TGEV-R	5'-GATGGACGAGCATAGGCATT-3'
PRRSV	PRRSV-F	5'-GCCTCGTGTTGGGTGGCAGAA-3'
	PRRSV-R	5'-CGCCCTAATTGAATAGGTGACTT-3'
PRV	PRV-F	5'-CACGGAGGACGAGCTGGGGCT-3'
	PRV-R	5'-GTCCACGCCCCGCTTGAAGCT-3'
PCV2	PCV2-F	5'-AGAAGCTCTCTATCGGAG-3'
	PCV2-R	5'-AAGGTTGAATTCTGGCCC-3'
CSFV	CSFV-F	5'-TAGGGTGGACGGGTGTCATAGAGT-3'
	CSFV-R	5'-AAGCATATATTGCTGGAAGTAGCT-3'

Table S2. PDCoV sequences information.

	GenBank accession	**Strains**	**Hosts**	**Country**	**Collection Date**
Genome	MN520193※	AH2018-81	pig	China: Anhui	2018
	MN520199※	AH2018-93	pig	China: Anhui	2018
	MN520200※	AH2018-94	pig	China: Anhui	2018
	MN520190※	AH2018-322	pig	China: Anhui	2018-03
	MN520198※	AH2019-H	pig	China: Anhui	2019
	MN520202※	GD2018-1	pig	China: Guangdong	2018-05
	MN520201※	GD2018-186	pig	China: Guangdong	2018
	MN520203※	GX2018-1	pig	China: Guangxi	2018-11
	MN520204※	HN2018-LH2	pig	China: Henan	2018-05

	MN520205※	HN2019-C115	pig	China: Henan	2019-03
	MN520206※	HN2019-C132	pig	China: Henan	2019-03
	MN520197※	SD2018-4	pig	China: Shandong	2018-04
	MN520194※	SD2018-10	pig	China: Shandong	2018-10
	MN200481※	SD2018-300	pig	China: Shandong	2018-03
	MN520195※	SD2018-304	pig	China: Shandong	2018-03
	MN520196※	SD2018-306	pig	China: Shandong	2018-03
	MN520191※	SD2019-426	pig	China: Shandong	2019
	MN520207※	JS2018-QF49	pig	China: Jiangsu	2018-10
	MN520208※	JS2018-YC15	pig	China: Jiangsu	2018-07
	MN520209※	JS2019-A1414	pig	China: Jiangsu	2019-01
	MN520192※	ZJ2018-D	pig	China: Zhejiang	2018-08
	NC_039208	HKU15-155	pig	China: Hong Kong	2010
	MH708124	HNZK-04	pig	China	2018-03-20
	MH708123	HNZK-02	pig	China	2018-03-20
	MH708125	HNZK-06	pig	China	2018-03-20
	KR131621	PDCoV/CHJXNI2/2015	pig	China	2015-03
	KY293677	CH/JXJGS01/2016	pig	China	2016-05-23
	KY293678	CH/JXJGS02/2016	pig	China	2016-05-23
	KJ769231	OhioCVM1/2014	pig	USA	2014-03-01
	JQ065042	HKU15-44	pig	China: Hong Kong	2009
	MF948005	HB-BD	pig	China	2017-08-10
	KJ567050	8734/USA-IA/2014	pig	USA: Iowa	2014-02-20
	KJ462462	OH1987	pig	USA: Ohio	2014-01-31
	KJ569769	IN2847	pig	USA: Ohio	2014-02-13
	KJ584355	IL2768	pig	USA: Ohio	2014-02-12
	KJ584356	SD3424	pig	USA: South: Dakota	2014-02-20
	KJ584357	KY4813	pig	USA: Kentucky	2014-03-07
	KJ584358	PA3148	pig	USA: Pennsylvania	2014-02-18
	KJ584359	NE3579	pig	USA: Nebraska	2014-02-21
	KJ620016	MI6148	pig	USA: Michigan	2014-03-18
	KM820765	KNU14-04	pig	South Korea	2014-04
	KP981395	USA/IL/2014/026PDV_P11	unknown	USA: Illinois	2014
	KT381613	OH11846	pig	USA: Ohio	2014-05-07
	KY354363	DH1	pig	South Korea: Kyunggido	2016-04-01
	KY354364	DH2	pig	South Korea: Kyunggido	2016-04-01
	KY364365	KNU16-07	pig	South Korea	2014-07
	LC216914	S579N	pig	China: Hong Kong	2014-02-13

KY513725	CH/Jiangsu/2014	pig	China: Jiangsu	2014
KP757890	CHN-AH-2004	pig	China	2004-05-24
KP757891	CHN-HB-2014	pig	China	2014-12-26
KP757892	CHN-JS-2014	pig	China	2014-12-20
KT336560	CHN-HN-2014	pig	China	2014-11-24
KU981059	NH	pig	China	2015-02-16
MF431742	GD	pig	China	2015
MF642322	CHN/GS/2016/1	pig	China	2016-08
MF642323	CHN/GS/2016/2	pig	China	2016-08
MF642324	CHN/GS/2017/1	pig	China	2017-04
MF642325	CHN/QH/2017/1	pig	China	2017-03
KT021234	CH/SXD1/2015	pig	China	2015-03-20
KY926512	KNU16-11	pig	South Korea	2016-11
MG832584	CHN-HN-1601	pig	China	2016-07
MH715491	PDCoV/CHGD/2016	pig	China	2016
MF431743	SD	pig	China	2014
MF041982	SHJS/SL/2016	pig	China	2016-12-23
MG242062	CHN-HeB1-2017	pig	China	2017
KY065120	CHN/Tianjin/2016	pig	China: Tianjin	2016
KY513724	CH/Hunan/2014	pig	China: Hunan	2014
KM012168	Michigan/8977/2014	pig	USA: Michigan	2014-03-17
KJ601777	PDCoV/USA/Illinois133/2014_from_USA	pig	USA: Illinois	2014-01-08
KJ481931	PDCoV/USA/Illinois121/2014_from_USA	pig	USA	2014-01-04
KX834351	PDCoV/Swine/Vietnam/HaNoi6/2015	pig	Viet Nam	2015-10-10
KX834352	PDCoV/Swine/Vietnam/Binh21/2015	pig	Viet Nam	2015-12-08
LC216915	S582N	pig	China: Hong Kong	2014-02-13
KU051641	PDCoV/Swine/Thailand/S5011/2015	pig	Thailand	2015-06-10
KU051649	PDCoV/Swine/Thailand/S5015L/2015	pig	Thailand	2015-06-30
KX118627	P1_16_BTL_0115/PDCoV/2016/Lao	pig	Laos	2016-01-20
KJ601778	PDCoV/USA/Illinois134/2014_from_USA	pig	USA: Illinois	2014-01-08

	KJ601779	PDCoV/USA/Illinois136/2014_from_USA	pig	USA: Illinois	2014-01-11
	KJ601780	PDCoV/USA/Ohio137/2014_from_USA	pig	USA: Ohio	2014-01-26
	KT266822	CH/Sichuan/S27/2012	pig	China: Sichuan	2012
	KR265861	USA/Nebraska210/2014	pig	USA: Nebraska	2014-02-05
	KX443143	CH-01	unknown	China	2016
	KU984334	TT_1115	pig	Thailand	2015-11
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	MF280390	CHN-GD-2016	pig	China	2016
	KR265859	USA/Minnesota159/2014	pig	USA: Minnesota	2014-02-11
	KR265856	USA/Illinois272/2014	pig	USA: Illinois	2014-02-23
	MF095123	CHN-HG-2017	pig	China	2017-02-15
	KR150443	USA/Arkansas61/2015	pig	USA: Arkansas	2015-03-24
	KR265848	USA/Minnesota214/2014	pig	USA: Minnesota	2014-03-14
	KR265860	USA/Nebraska209/2014	pig	USA: Nebraska	2014-02-05
	KY363867	CHN-GD16-03	pig	China	2016-03-18
	KR265864	USA/Minnesota292/2014	pig	USA: Minnesota	2014-03-14
	KR265847	USA/Minnesota442/2014	pig	USA: Minnesota	2014-03-06
	KR265850	USA/Michigan448/2014	pig	USA: Michigan	2014-04-02
	KR265851	USA/Indiana453/2014	pig	USA: Indiana	2014-05-13
	KR265852	USA/Illinois449/2014	pig	USA: Illinois	2014-04-21
	KR265853	USA/Minnesota/2013	pig	USA: Minnesota	2013-10-14
	KR265854	USA/Minnesota454/2014	pig	USA: Minnesota	2014-05-21
	KR265855	USA/Minnesota455/2014	pig	USA: Minnesota	2014-05-21

	KR265857	USA/Illinois273/2014	pig	USA: Illinois	2014-02-23
	KR265858	USA/NorthCarolina452/2014	pig	USA: North: Carolina	2014-05-06
	KR265862	USA/Ohio444/2014	pig	USA: Ohio	2014-03-26
	KR265863	USA/Ohio445/2014	pig	USA: Ohio	2014-03-27
	KR265865	USA/Iowa459/2014	pig	USA: Iowa	2014-06-05
	KX022603	PDCoV/USA/Minnesota140/2015	pig	USA: Minnesota	2015-12-18
	KR265849	USA/Michigan447/2014	pig	USA: Michigan	2014-04-02
	KX022602	PDCoV/USA/Iowa136/2015	pig	USA: Iowa	2015-10-15
	KX022604	PDCoV/USA/Nebraska137/2015	pig	USA: Nebraska	2015-11-27
	KU665558	CHN-LYG-2014	unknown	China	2014-06-26
	LC260038	AKT/JPN/2014	pig	Japan: Akita	2014-05
	LC260039	GNM-1/JPN/2014	pig	Japan: Gunma	2014-05
	LC260040	GNM-2/JPN/2014	pig	Japan: Gunma	2014-05
	LC260041	IWT/JPN/2014	pig	Japan: Iwate	2014-05
	LC260042	MYZ/JPN/2014	pig	Japan: Miyazaki	2014-03
	LC260043	OKN/JPN/2014	pig	Japan: Okinawa	2014-08
	LC260044	YMG/JPN/2014	pig	Japan: Yamagata	2014-12
	LC260045	HKD/JPN/2016	pig	Japan: Hokkaido	2016-09
	KX022605	PDCoV/USA/Nebraska145/2015	pig	USA: Nebraska	2015-12-21

※: Newly sequenced strains in this study.

Table S3. Sequence identity between this study and reference sequences.

Isolated strains	Sequence homology among isolated strains	Sequence homology between isolated strains and reference strain
S gene (nt)	96.1%-100%	95.6%-99.9%
S gene (aa)	96.7%-100%	95.6%-100%
Full-length	98.9%-99.8%	91.2%-99.9%

Table S4. Positively selected amino acid polymorphisms among different lineages.

site	Lineage			
	Early China	Thailand	China	USA
107	Q	Q/L	Q/L	L

149	D	A/N/R	H/Y/R/Q	H/Y
183	T	T	T/I	T/I
630	A	A	A/L/V/T	A/V/T
698	S	S	S/A/T	S

Supplementary material Figures

Fig S1. Identification of PDCoV strains SD2018 and AH2019/H in LLC-PK1 cells. A) LLC-PK1 cells were infected with virus isolates from pigs and subsequently tested by rtPCR or PCR for various porcine viruses including Porcine Epidemic Diarrhea (PEDV), Porcine Kobuvirus (PKV), Porcine teschovirus (PTV), Porcine Transmissible Gastroenteritis Virus (TGEV), Classical Swine Fever Virus (CSFV), Porcine reproductive and respiratory syndrome virus (PRRSV), and Pseudorabies virus (PRV). B) Detection of the two PDCoV strains in LLC-PK1 cells by indirect immunofluorescence assay (IFA) at 18 hpi at 0.1 MOI. A monoclonal antibody (MAb) for PDCoV N protein and fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse immunoglobulin G (IgG) were used as primary and secondary antibodies, followed by staining with 4,6-diamidino-2-phenylindole (DAPI). Magnification: 200x.

Fig S2. ML tree based on non-recombination complete genomes. The ML tree was reconstructed using RAxML (v8.4.10) using the general time-reversible substitution model with gamma-distributed rates across sites. 1,000 bootstraps were evaluated to assess support values. The Thailand lineage is represented in blue, the Early China lineage in red, the USA lineage in orange, and the China lineage in green.

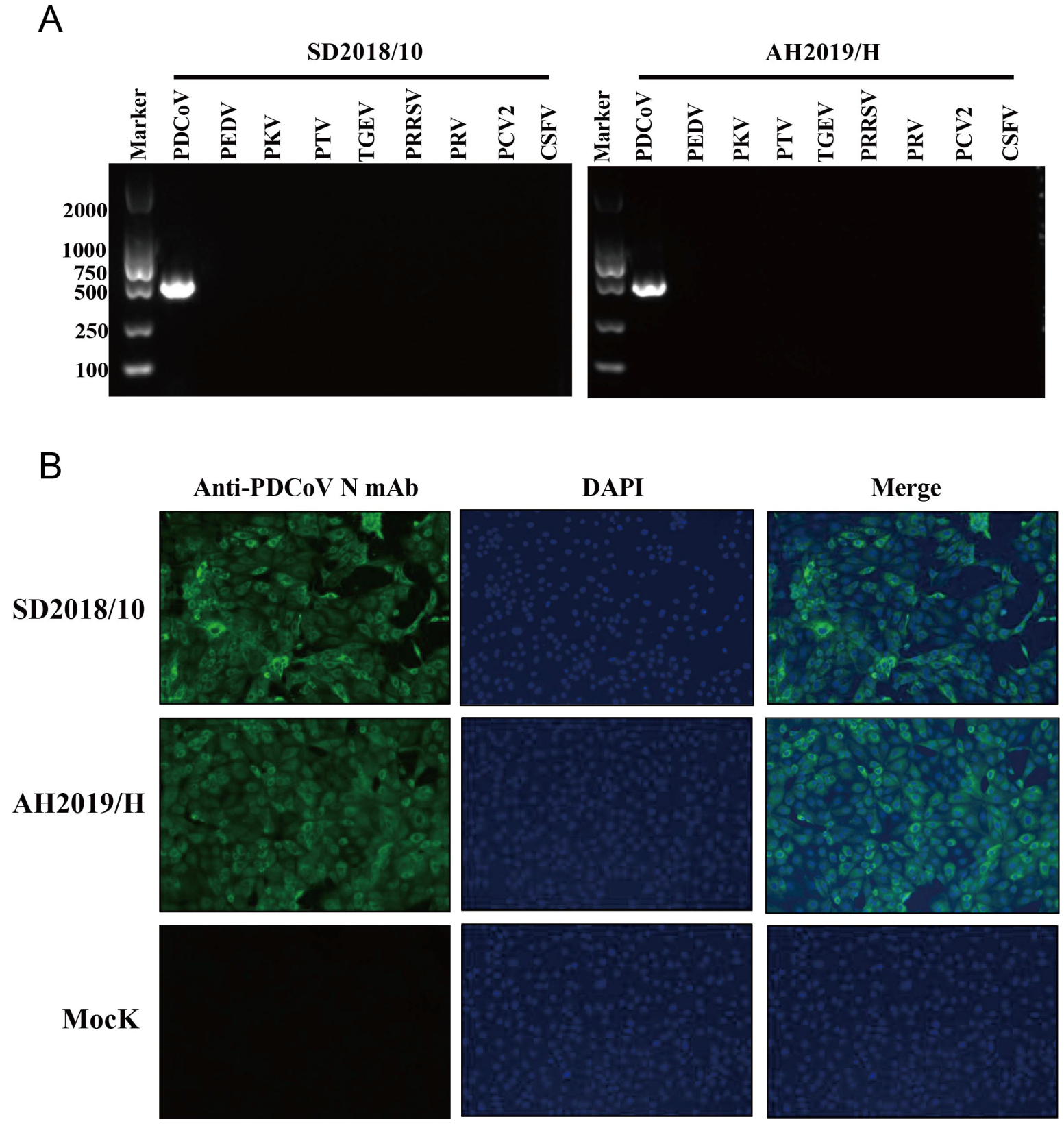
Fig S3. A) ML phylogenetic trees inferred for 194 PDCoV S gene under the general time-reversible substitution model with gamma-distributed rates across sites. 1,000 bootstraps were evaluated to assess support values. B) Temporal signal testing based on an ML tree using TempEst (version 1.5.3). C) tMRCA and evolutionary rate of PDCoV. The tMRCA and evolutionary rate of PDCoV as well as each lineage separately were estimated using BEAST (version 1.10.5).

Fig S4. Location of selected amino acids in the structure of S. Surface representation of the

tip of a S monomer. The amino acids under selection are shown as red spheres. Note that His 149 and Ser 98 are exposed at the surface of the molecule.

Fig S5. Linear regression between patristic and geographic distances between sampling locations considered in the discrete phylogeographic inference. Patristic distances were computed on the MCC tree obtained from the discrete phylogeographic analysis performed with the generalized linear model (GLM) extension.

Fig S6. Comparison between the continuous phylogeographic reconstructions obtained under the relaxed random walk (RRW) and the directional random walk (RDW) models. The plots show the mapped MCC tree and 95% HPD regions based on trees subsampled from the post burn-in posterior distribution of trees. The tree nodes are colored according to a color scale ranging from brown (the time to the most recent common ancestor, TMRCA) to green (most recent sampling time). 95% HPD regions were computed for successive time layers and superimposed using the same color scale reflecting time. On the maps, subnational Chinese province borders are represented by white lines.


A
SD2018/10
AH2019/H
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PDCoV
PEDV
PKV
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TGEV
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PRV
PCV2
CSFV
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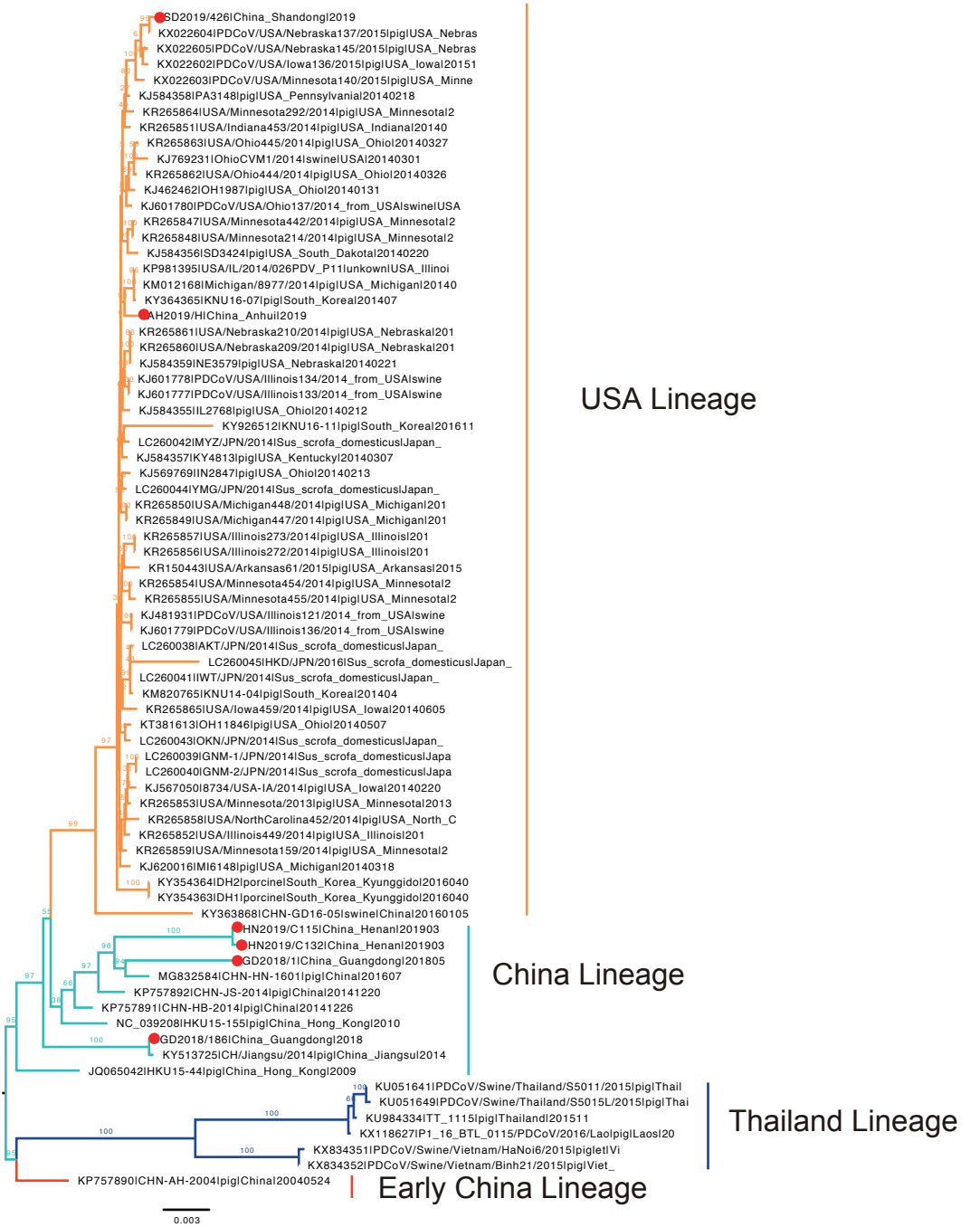
750
500
250
100
B
Anti-PDCoV N mAb
DAPI
Merge
SD2018/10
AH2019/H
MocK

Figure S1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KJ584358|PA3148|pig|USA_Pennsylvania|20140218
KR265864|USA/Minnesota292/2014|pig|USA_Minnesota|2
KR265851|USA/Indiana453/2014|pig|USA_Indiana|20140
KR265863|USA/Ohio445/2014|pig|USA_Ohio|20140327
KJ769231|OhioCVM1/2014|swine|USA|20140301
KR265862|USA/Ohio444/2014|pig|USA_Ohio|20140326
KJ462462|OH1987|pig|USA_Ohio|20140131
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KR265847|USA/Minnesota442/2014|pig|USA_Minnesota|2
KR265848|USA/Minnesota214/2014|pig|USA_Minnesota|2
KJ584356|SD3424|pig|USA_South_Dakota|20140220
KP981395|USA/IL/2014/026PDV_P11|unknown|USA_Illinoi
KM012168|Michigan/8977/2014|pig|USA_Michigan|20140
KY364365|KNU16-07|pig|South_Korea|201407
AH2019/H|China_Anhui|2019
KR265861|USA/Nebraska210/2014|pig|USA_Nebraska|201
KR265860|USA/Nebraska209/2014|pig|USA_Nebraska|201
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KJ569769|IN2847|pig|USA_Ohio|20140213
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KR265850|USA/Michigan448/2014|pig|USA_Michigan|201
KR265849|USA/Michigan447/2014|pig|USA_Michigan|201
KR265857|USA/Illinois273/2014|pig|USA_Illinois|201
KR265856|USA/Illinois272/2014|pig|USA_Illinois|201
KR150443|USA/Arkansas61/2015|pig|USA_Arkansas|2015
KR265854|USA/Minnesota454/2014|pig|USA_Minnesota|2
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KJ601779|PDCoV/USA/Illinois136/2014_from_USA|swine
LC260038|AKT/JPN/2014|Sus_scrofa_domesticus|Japan_
LC260045|HKD/JPN/2016|Sus_scrofa_domesticus|Japan_
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LC260040|GNM-2/JPN/2014|Sus_scrofa_domesticus|Japa
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KR265853|USA/Minnesota/2013|pig|USA_Minnesota|2013
KR265858|USA/NorthCarolina452/2014|pig|USA_North_C
KR265852|USA/Illinois449/2014|pig|USA_Illinois|201
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KJ620016|IMI6148|pig|USA_Michigan|20140318
KY354364|IDH2|porcine|South_Korea_Kyunggido|2016040
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HN2019/C132|China_Henan|201903
GD2018/1|China_Guangdong|201805
MG832584|CHN-HN-1601|pig|China|201607
KP757892|CHN-JS-2014|pig|China|20141220
KP757891|CHN-HB-2014|pig|China|20141226
NC_039208|HKU15-155|pig|China_Hong_Kong|2010
GD2018/186|China_Guangdong|2018
KY513725|CH/Jiangsu/2014|pig|China_Jiangsu|2014
JQ065042|HKU15-44|pig|China_Hong_Kong|2009
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KX118627|P1_16_BTL_0115/PDCoV/2016/Lao|pig|Laos|20
KX834351|PDCoV/Swine/Vietnam/HaNoi6/2015|pig|et|Vi
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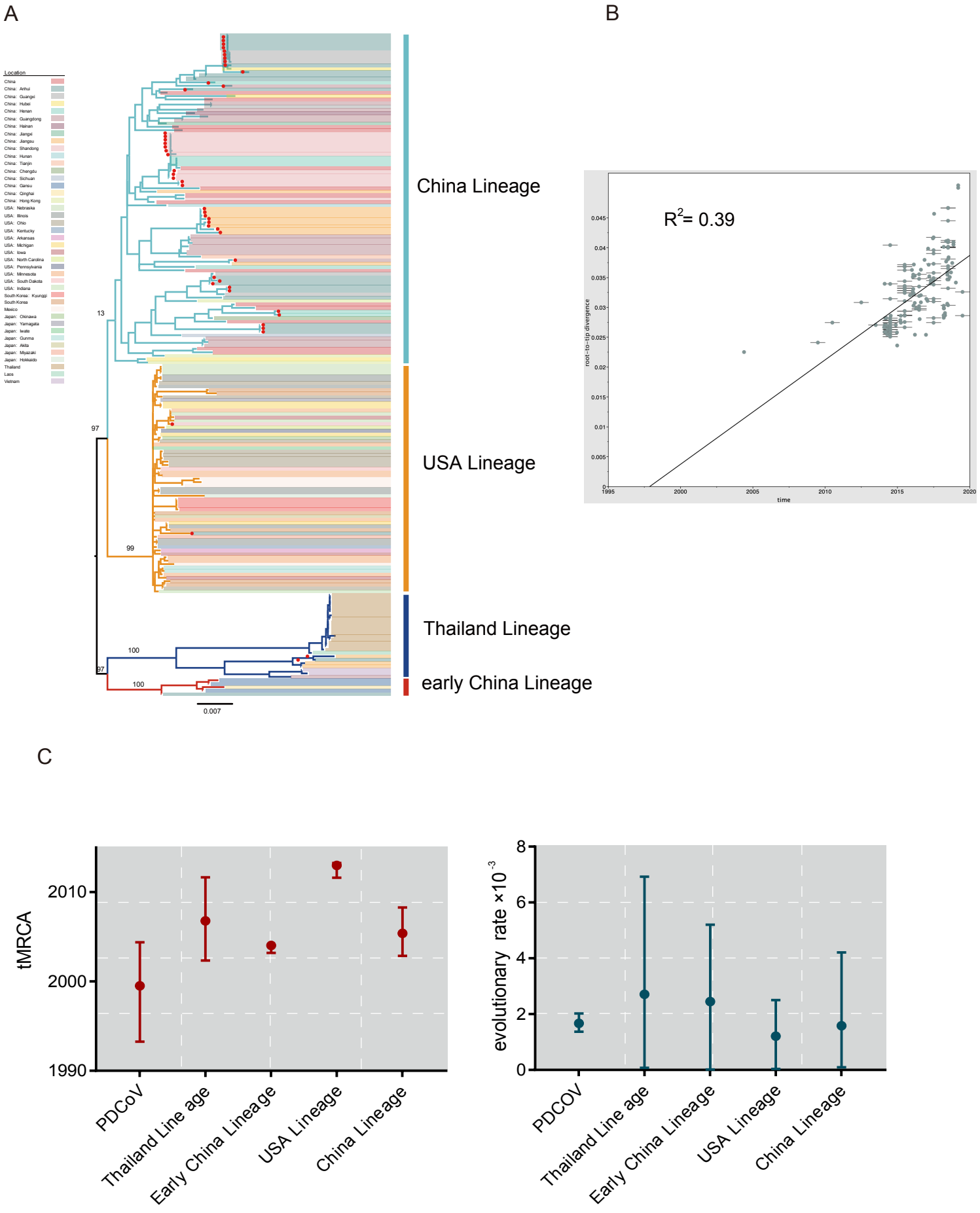
0.003
USA Lineage
China Lineage
Thailand Lineage
Early China Lineage

Figure S2


A
B
C
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China: Shandong
China: Hunan
China: Tianjin
China: Chengdu
China: Sichuan
China: Gansu
China: Qinghai
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USA: Kentucky
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Thailand
Laos
Vietnam
13
97
99
100
97
100
0.007
China Lineage
USA Lineage
Thailand Lineage
early China Lineage
R2= 0.39
root-to-tip divergence
time
tMRCA
2010
2000
1990
evolutionary rate ×10-3
8
6
4
2
0
PDCoV
Thailand Line age
Early China Lineage
USA Lineage
China Lineage

Figure S3

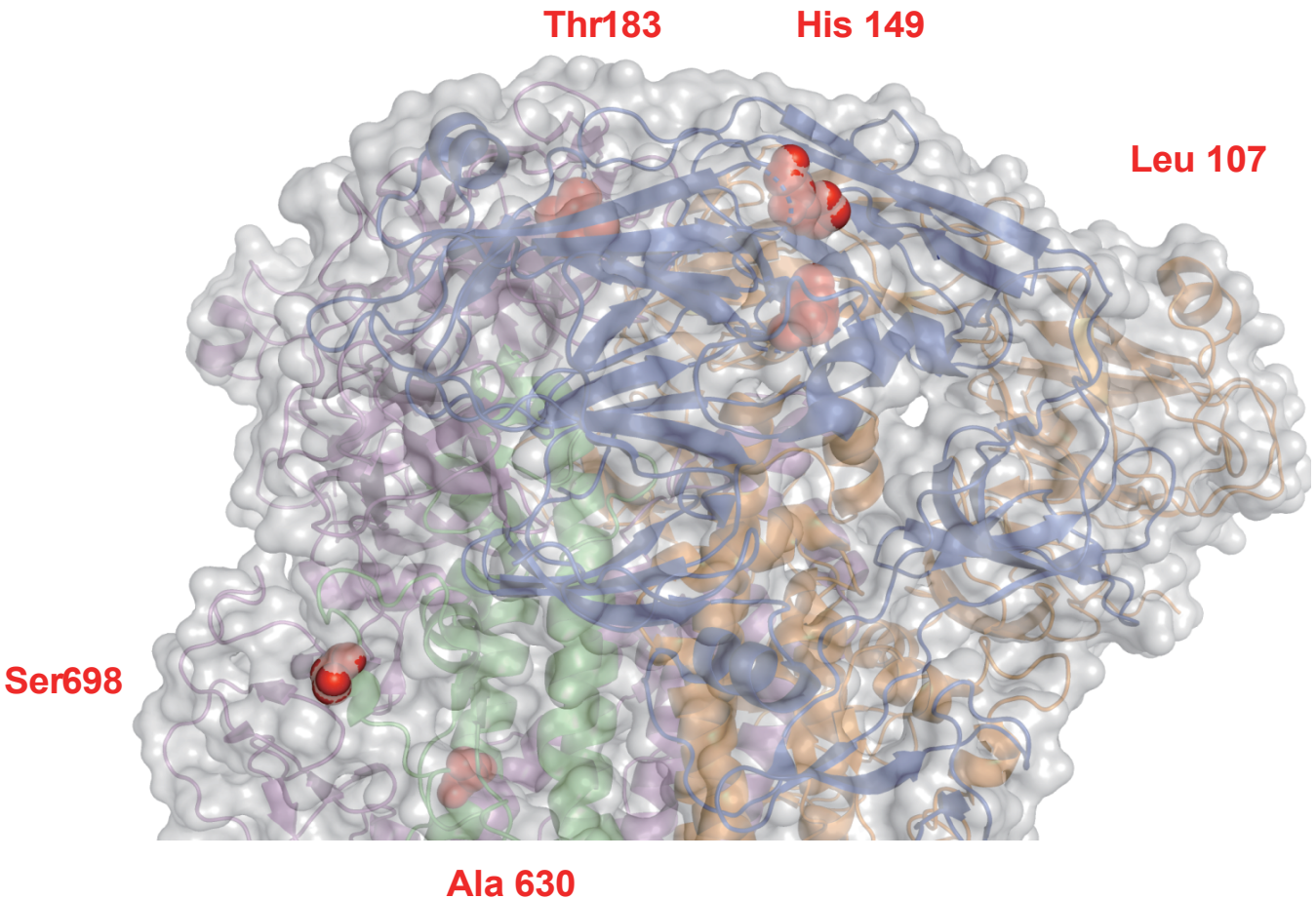
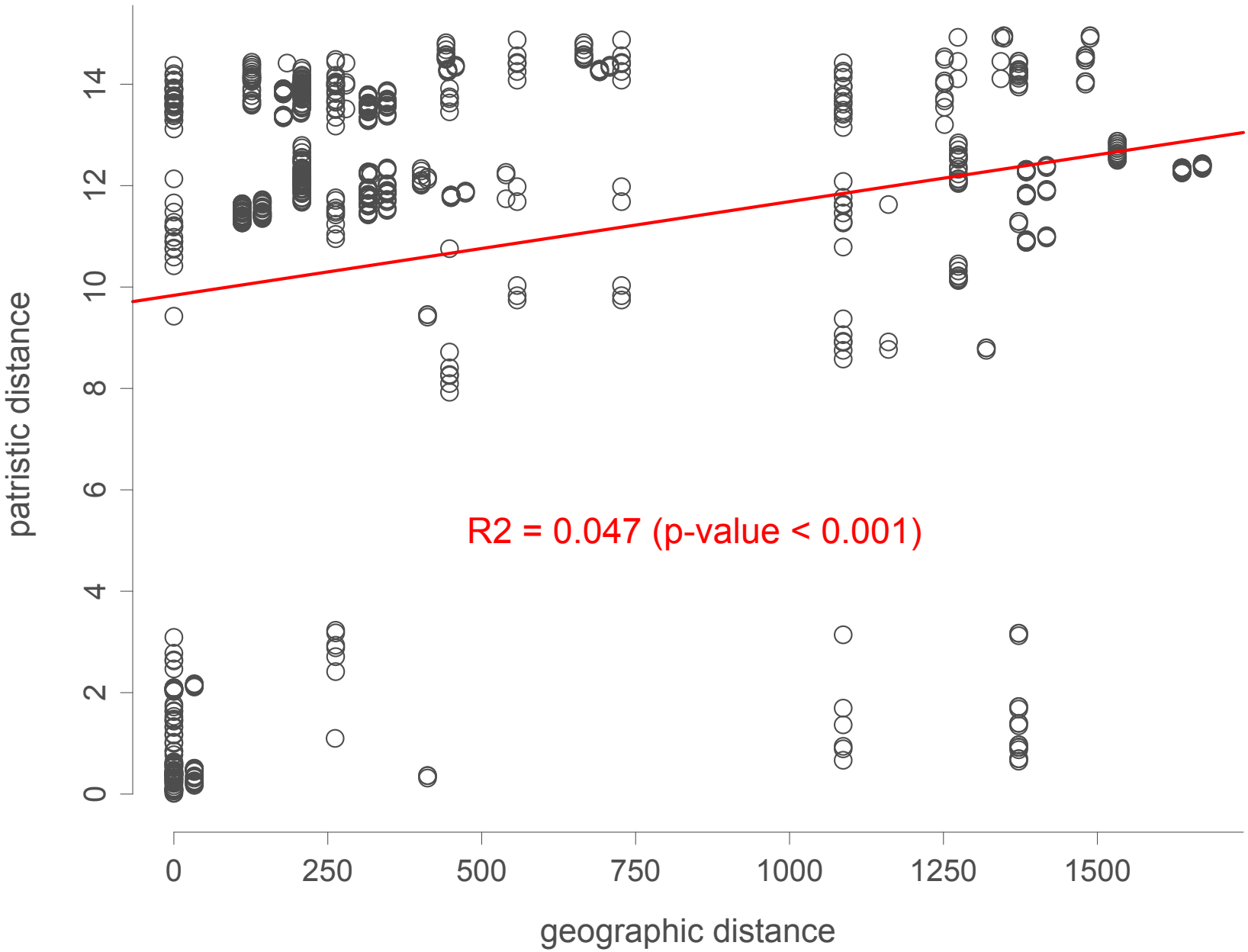


Figure S4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4
6
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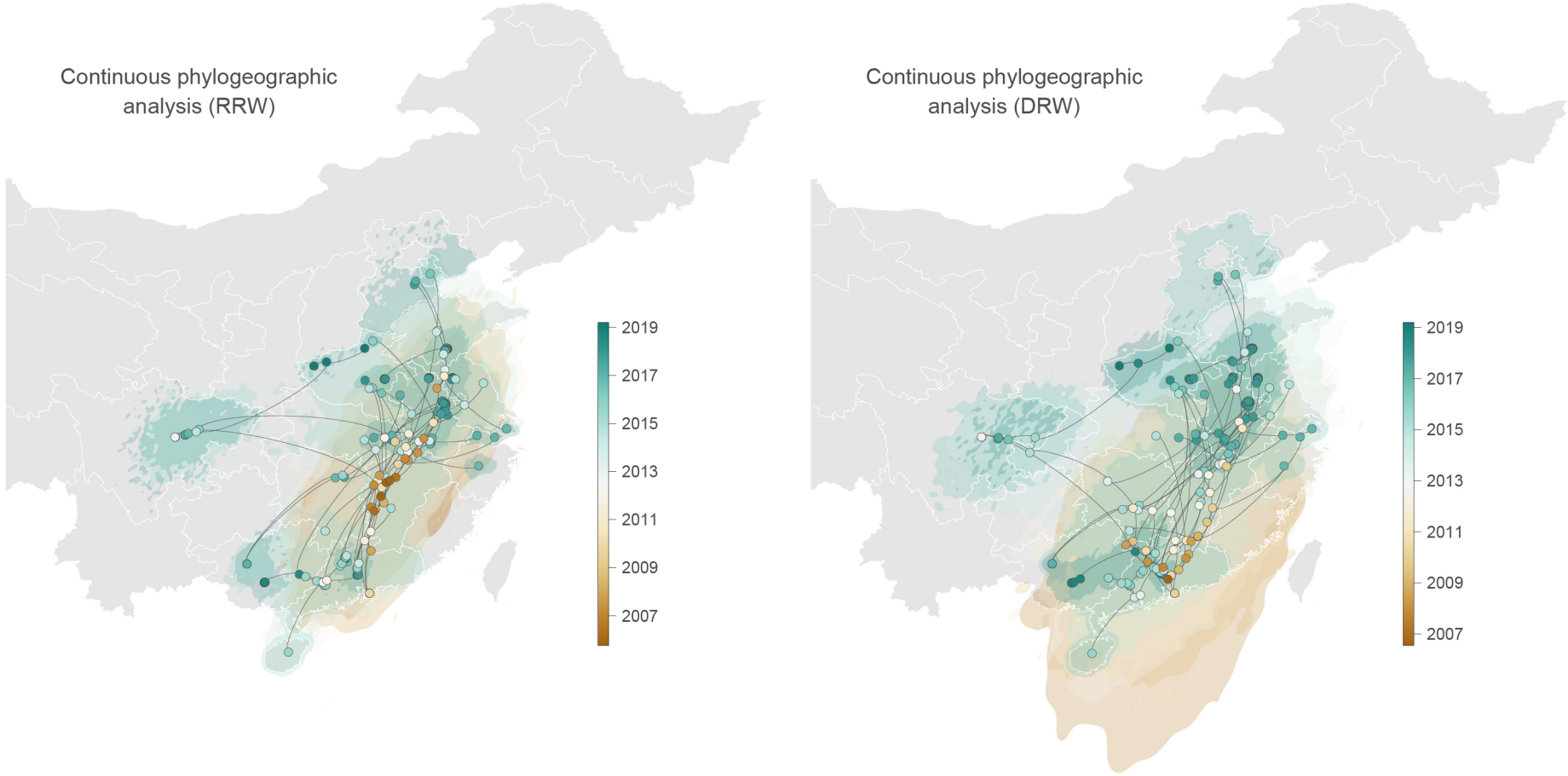
500
750
1000
1250
1500
geographic distance

Figure S5


Continuous phylogeographic analysis (RRW)
2019
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2011
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2007
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2017
2015
2013
2011
2009
2007

Figure S6