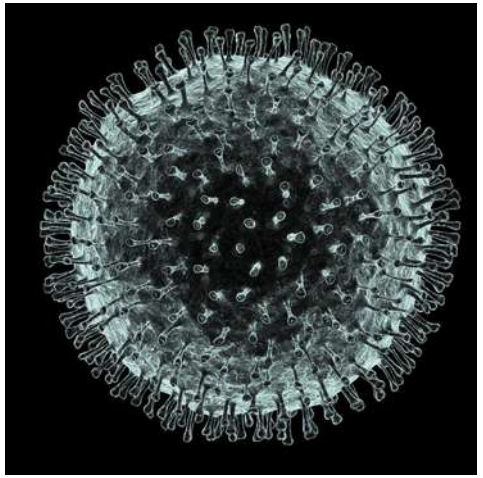


Yeni Koronavirus (2019-nCoV)'un Özellikleri ve Tanısı

Kenan Midilli

İ.Ü.-Cerrahpaşa

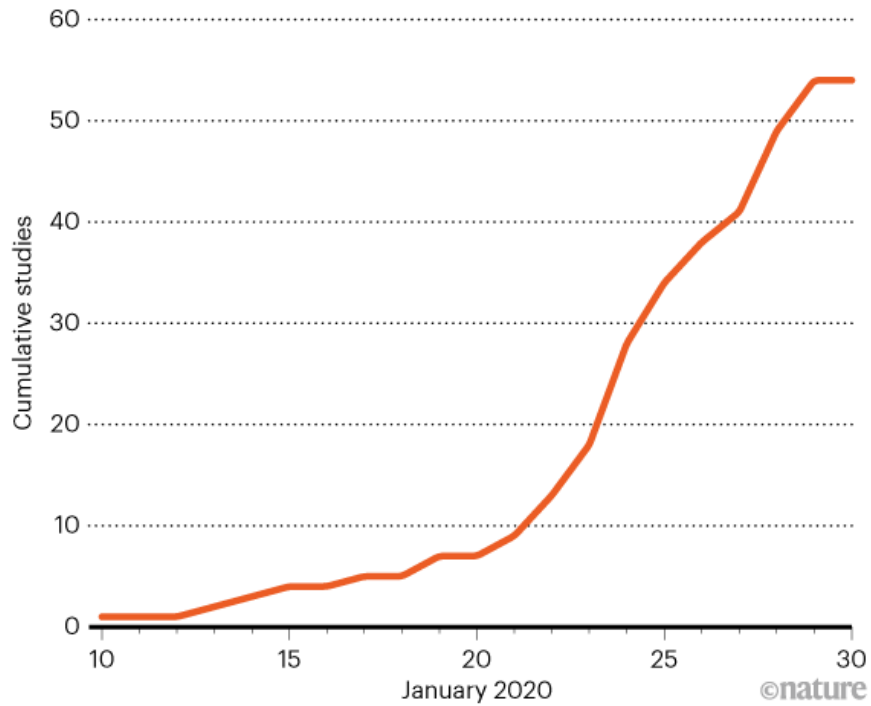
Cerrahpaşa Tıp Fakültesi

Tıbbi Mikrobiyoloji Anabilim Dalı

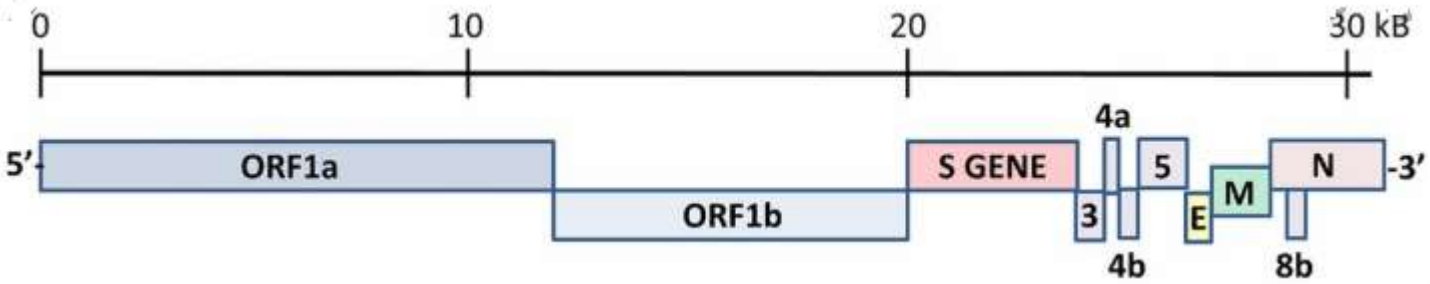
Tıbbi Viroloji Bilim Dalı

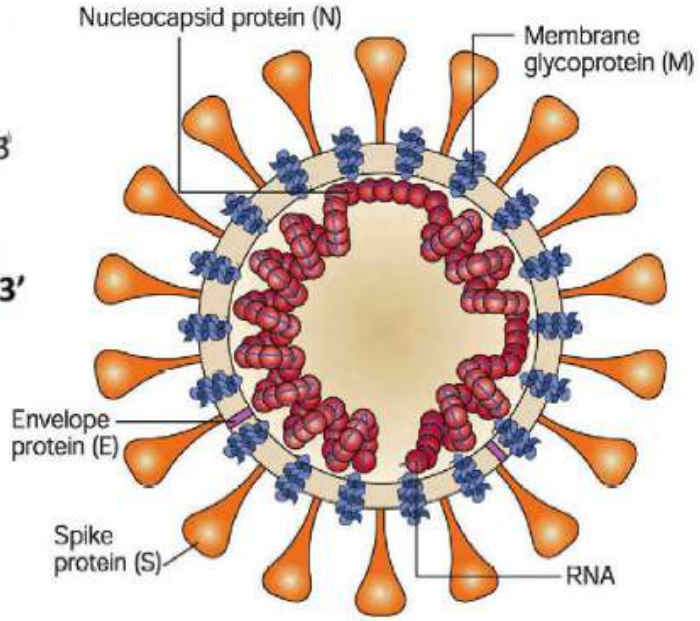
CORONAVIRUS RESEARCH

Dozens of studies about the virus have been published since the outbreak began.



ource: Analysis by Nature news team





Virionlar: Zarflı ve 15–20 nm uzunluğunda yüzey çıkıntıları

Nükleokapsid: Helikal, çok sayıda bazik fosfoproteinler (N) içerir

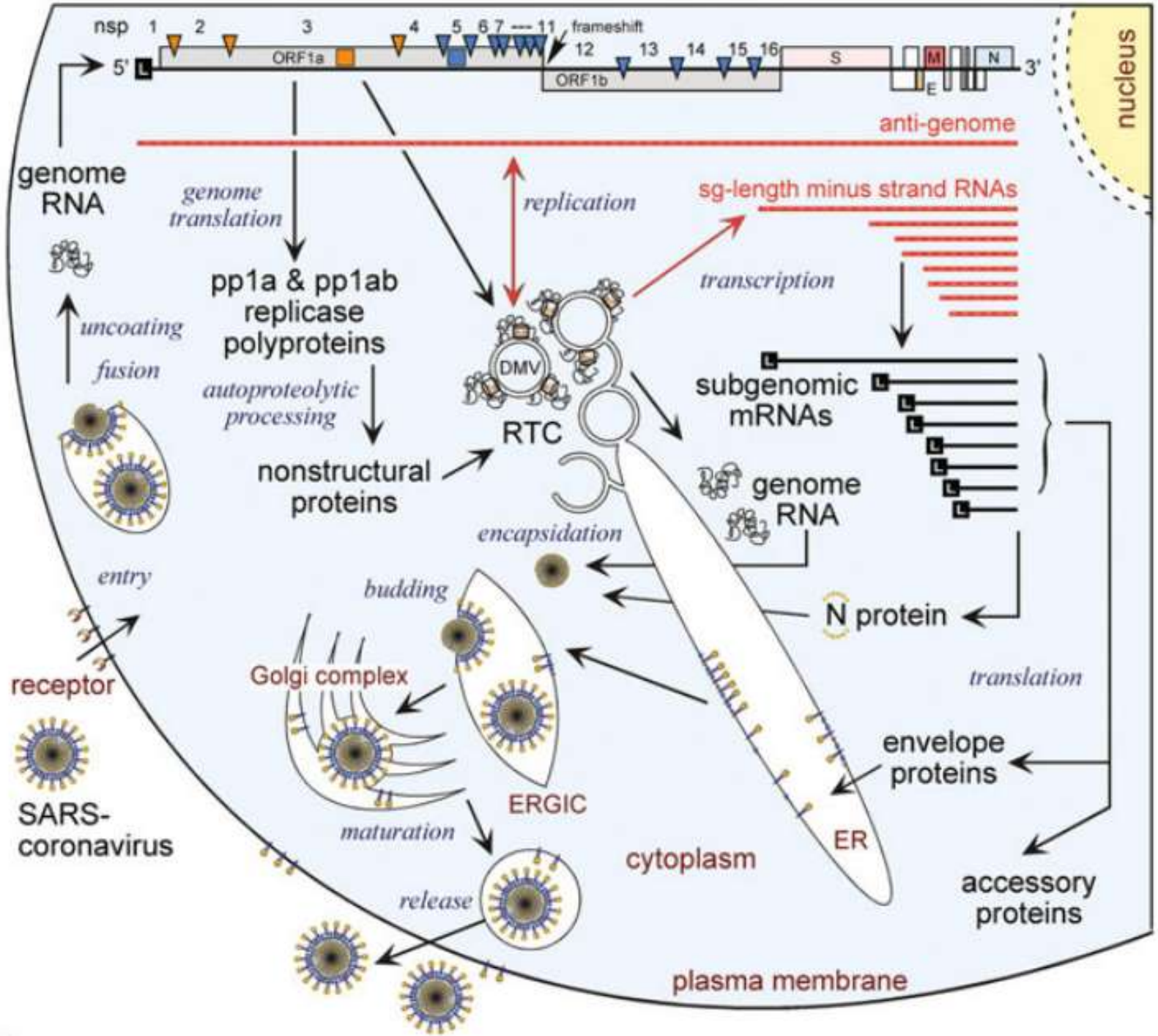
Zarf: Değişen sayılarda membran proteinleri içerir (ikisi korunmuştur): M proteini ve S proteini (tutunma ve füzyon, yüksek oranda glikozillenmiştir); ikisi mofogenez ve infektivite için gereklidirler

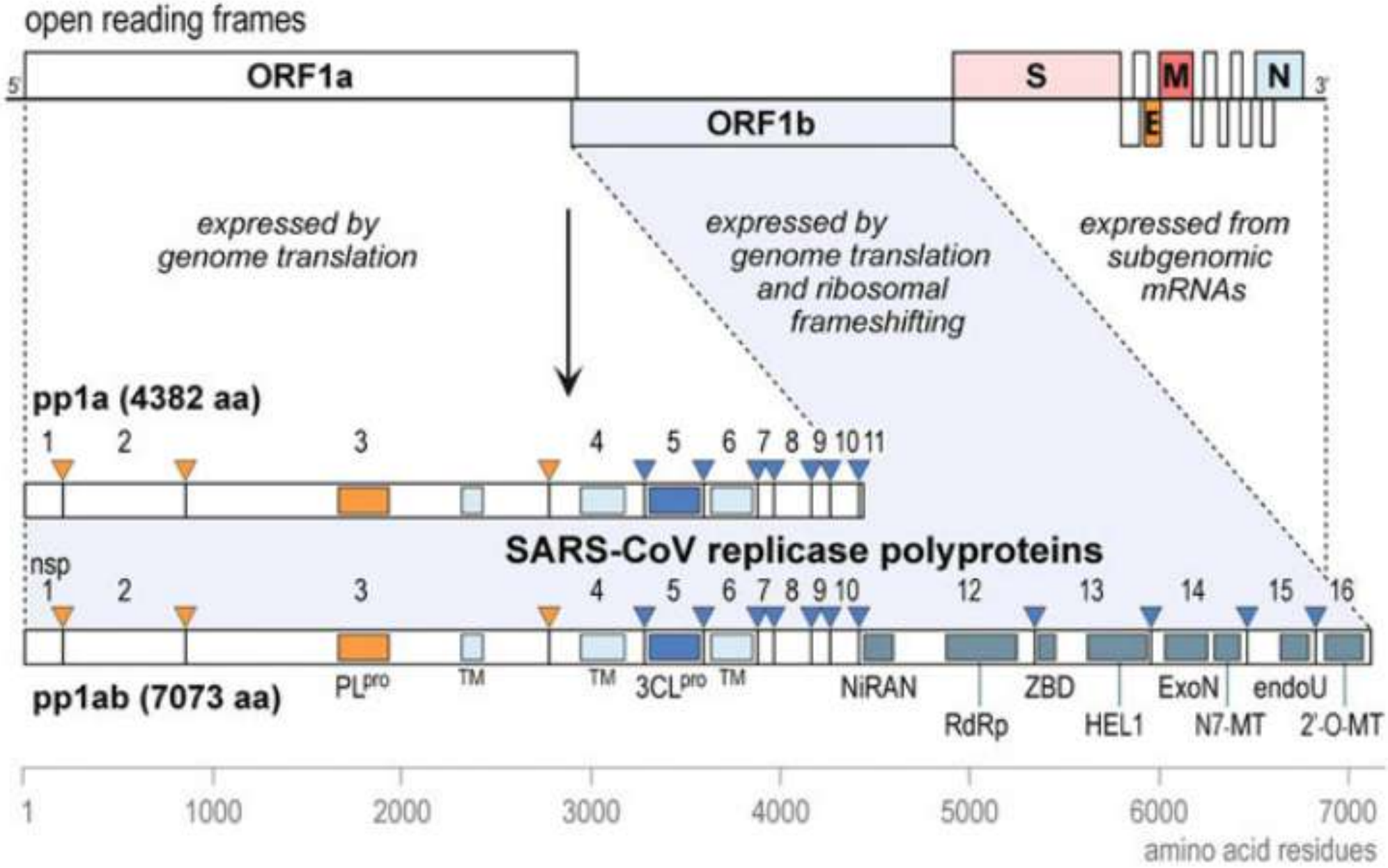
Genom: Pozitif anlamlı RNA, lineer, tek sarmallı, segmentsiz, infeksiyöz, 26–32 kb uzunluğunda, kapaklı, poliadenillenmiş ve yapısal olarak polisistronik

Genel genom organizasyonu: 5'-UTR-replikaz-S-M-N-UTR-3'; genome replikaz için mRNA işlevi görür

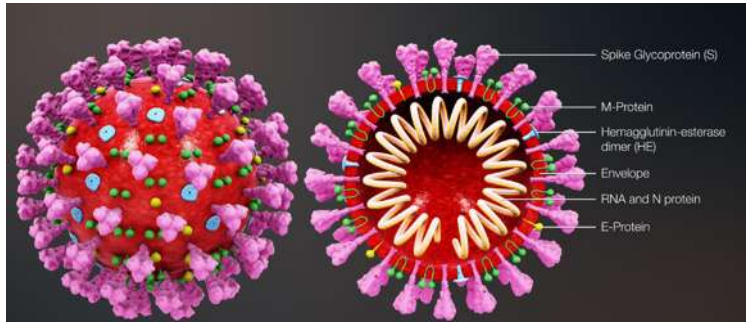
Replikaz geni: Örtüşen ORF 1a ve 1b'den oluşur → oldukça büyük pp1a and pp1ab poliproteinlerini kodlar; otokatalizle küçük parçalara bölünür

Replikazın ilerisinde yer alan ORF'ler: Poliadenillenmiş ve kapaklı subgenomik mRNA'lar kullanılarak ekspresse olurlar
Toparlanma: Nükleokapsid proteinleri pürtüksüz ER membranları/erken Golgi kompartmanlarında toparlanır ve tomurcuklanma ile serbestlenirler.


nsp 1 2 3 4 5 6 7 --- 11 frameshift 12 13 14 15 16
5'
ORF1a
ORF1b
S
M
E
N
3'
nucleus
anti-genome
genome RNA
genome translation
replication
sg-length minus strand RNAs
transcription
uncoating
fusion
pp1a & pp1ab replicase polyproteins
autoproteolytic processing
DMV
RTC
subgenomic mRNAs
nonstructural proteins
genome RNA
encapsidation
entry
budding
N protein
receptor
Golgi complex
translation
envelope proteins
SARS-coronavirus
ERGIC
maturation
ER
cytoplasm
accessory proteins
release
plasma membrane

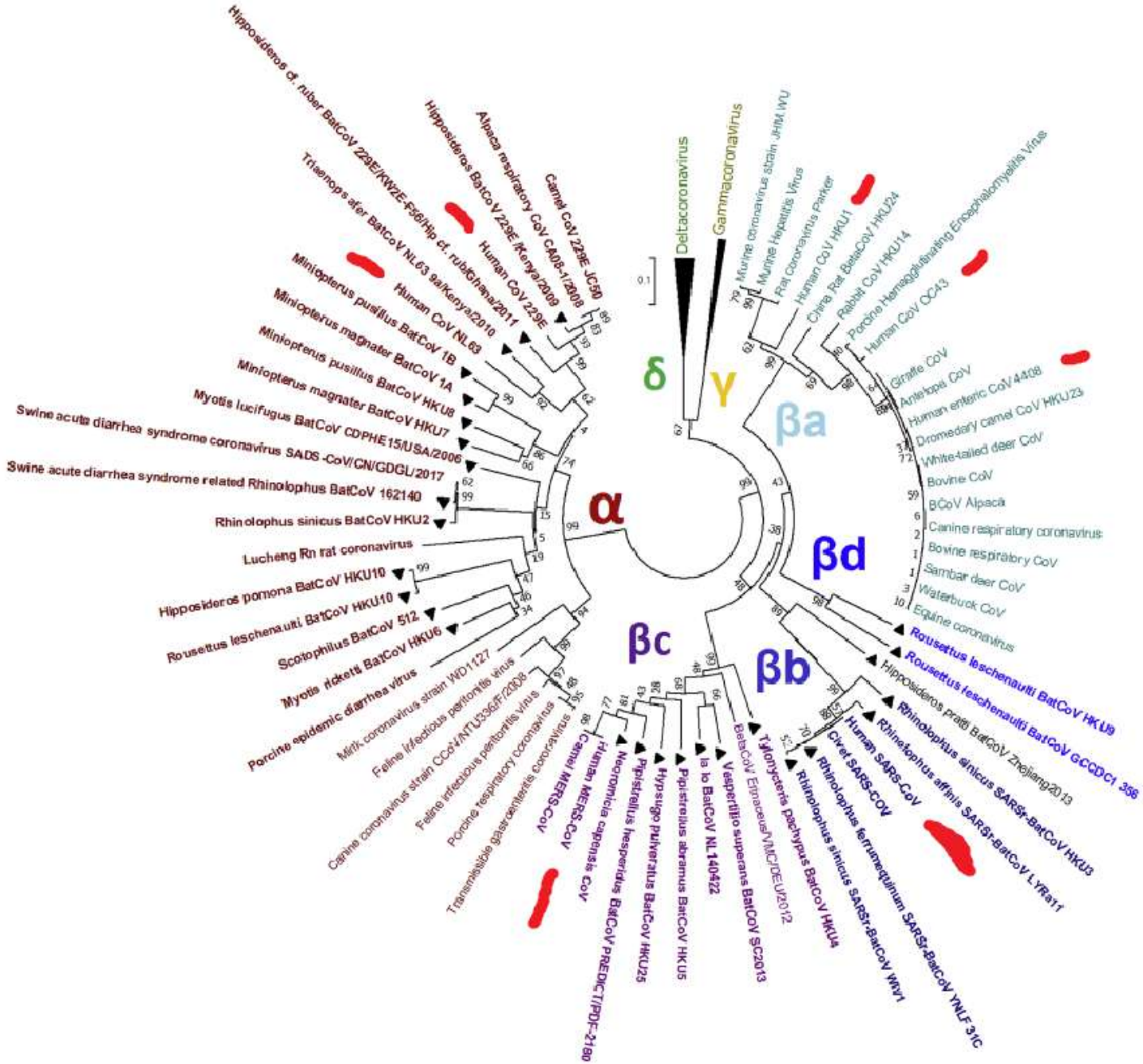

open reading frames
5'
ORF1a
ORF1b
S
M
E
N
3'
expressed by genome translation
expressed by genome translation and ribosomal frameshifting
expressed from subgenomic mRNAs
pp1a (4382 aa)
1 2 3 4 5 6 7 8 9 10 11
SARS-CoV replicase polyproteins
nsp
1 2 3 4 5 6 7 8 9 10 12 13 14 15 16
pp1ab (7073 aa)
PLpro
TM
TM
3CLpro
TM
NiRAN
RdRp
ZBD
HEL1
ExoN
N7-MT
endoU
2'-O-MT
1 1000 2000 3000 4000 5000 6000 7000
amino acid residues

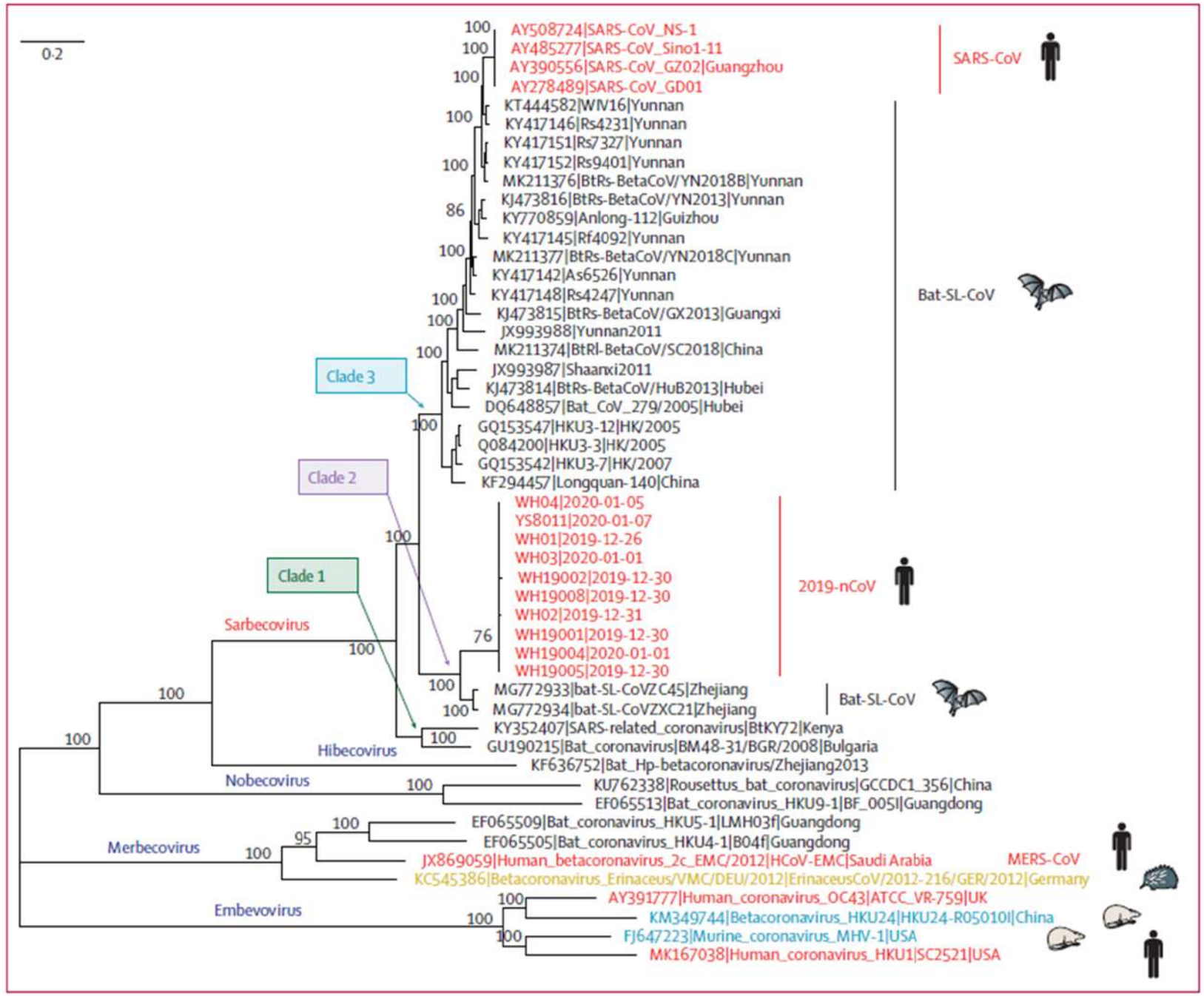
Aile: *Coronaviridae*
Altaile: *Letovirinae*
Altaile: *Orthocoronavirinae*

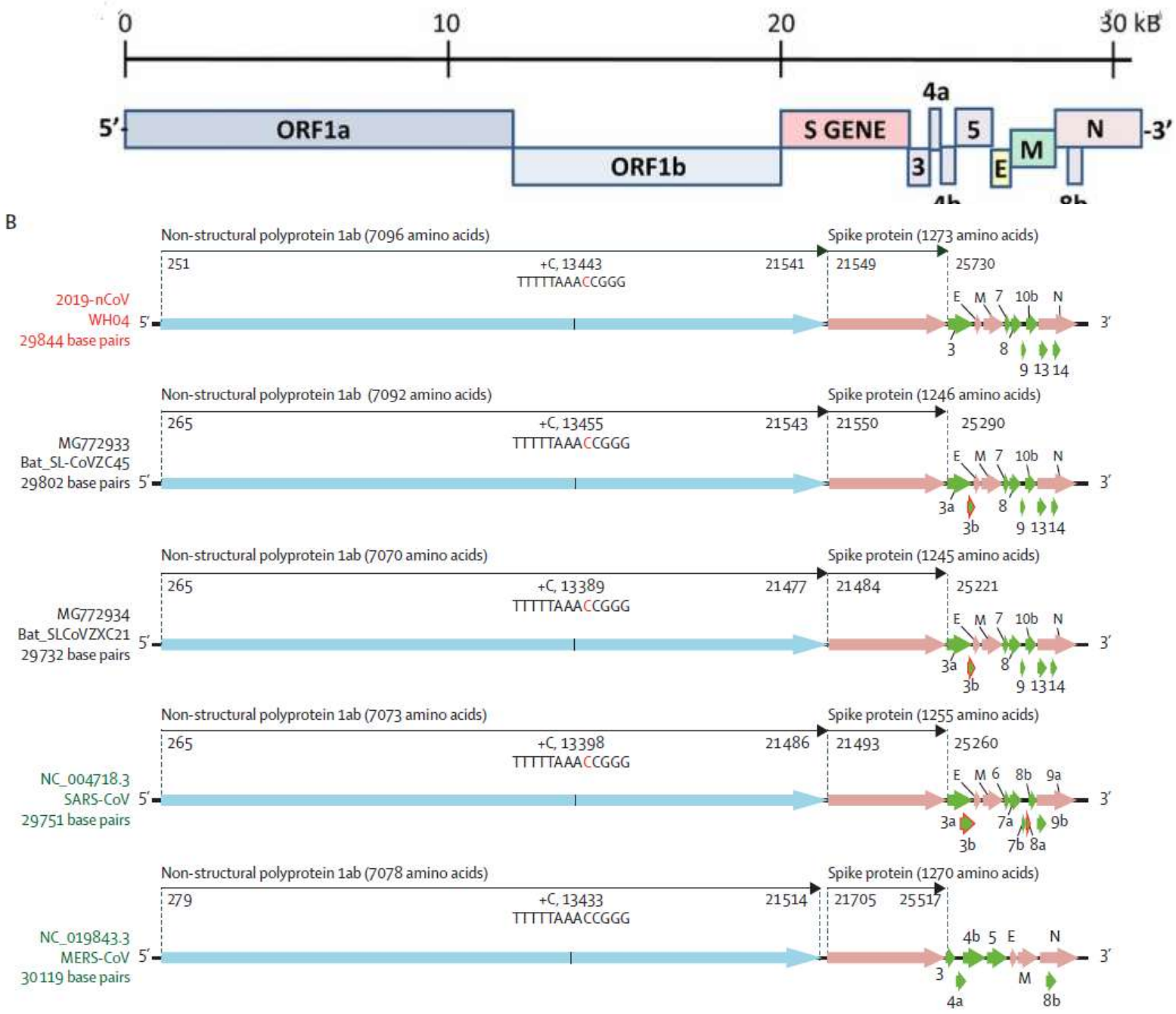


Orthocoronavirinae

- **Genus:** *Alphacoronavirus*
  - + **Subgenus:** *Colacovirus*
  - + **Subgenus:** *Decacovirus*
  - − **Subgenus:** *Duvinacovirus*
    - **Species:** *Human coronavirus 229E*
  - + **Subgenus:** *Luchacovirus*
  - + **Subgenus:** *Minacovirus*
  - + **Subgenus:** *Minunacovirus*
  - + **Subgenus:** *Myotacovirus*
  - + **Subgenus:** *Nyctacovirus*
  - + **Subgenus:** *Pedacovirus*
  - + **Subgenus:** *Rhinacovirus*
  - − **Subgenus:** *Setracovirus*
    - **Species:** *Human coronavirus NL63*
    - **Species:** *NL63-related bat coronavirus strain BtKYNL63-9b*
- − **Genus:** *Betacoronavirus*
  - − **Subgenus:** *Embecovirus*
    - **Species:** *Betacoronavirus 1*
    - **Species:** *China Rattus coronavirus HKU24*
    - **Species:** *Human coronavirus HKU1*
    - **Species:** *Murine coronavirus*
  - + **Subgenus:** *Hibecovirus*
  - − **Subgenus:** *Merbecovirus*
    - **Species:** *Hedgehog coronavirus 1*
    - **Species:** *Middle East respiratory syndrome-related coronavirus*
    - **Species:** *Pipistrellus bat coronavirus HKU5*
    - **Species:** *Tylonycteris bat coronavirus HKU4*
  - + **Subgenus:** *Nobecovirus*
  - − **Subgenus:** *Sarbecovirus*
    - **Species:** *Severe acute respiratory syndrome-related coronavirus*
- − **Genus:** *Deltacoronavirus*
  - + **Subgenus:** *Andecovirus*
  - + **Subgenus:** *Buldecovirus*
  - + **Subgenus:** *Herdecovirus*
  - + **Subgenus:** *Moordecovirus*
- − **Genus:** *Gammacoronavirus*
  - + **Subgenus:** *Cegacovirus*
  - + **Subgenus:** *Igacovirus*


Deltacoronavirus
Gammacoronavirus
δ
γ
α
βa
βb
βc
βd
Murine coronavirus strain JHM.WU
Murine Hepatitis Virus
Rat coronavirus Parker
Human CoV HKU1
China Rat BetaCoV HKU24
Rabbit CoV HKU14
Porcine Hemagglutinating Encephalomyelitis Virus
Human CoV OC43
Giraffe CoV
Antelope CoV
Human enteric CoV 4408
Dromedary camel CoV HKU23
White-tailed deer CoV
Bovine CoV
BCoV Alpaca
Canine respiratory coronavirus
Bovine respiratory CoV
Sambar deer CoV
Waterbuck CoV
Equine coronavirus
Rousettus leschenaulti BatCoV HKU9
Rousettus leschenaulti BatCoV GCCDC1 356
Hipposideros pratti BatCoV Zhejiang2013
Rhinolophus sinicus SARSr-BatCoV HKU3
Rhinolophus affinis SARSr-BatCoV LYRa11
Rhinolophus ferrumequinum SARSr-BatCoV YNLF 31C
Civet SARS-CoV
Human SARS-CoV
Rhinolophus sinicus SARSr-BatCoV WIV1
Rhinolophus pachypus BatCoV HKU4
Tylonycteris pachypus BatCoV HKU4
BetaCoV Eptesicus/NMC/DEU/2012
Vespertilio superans BatCoV SC2013
Ia io BatCoV NL140422
Pipistrellus abramus BatCoV HKU5
Hypsugo Pulveratus BatCoV HKU25
Pipistrellus hesperidus BatCoV HKU25
Neoromicia capensis CoV
Human MERS-CoV
Camel MERS-CoV
MERS-CoV PREDICT/PDF-2180
Transmissible gastroenteritis coronavirus
Porcine respiratory coronavirus
Feline infectious peritonitis virus
Canine coronavirus strain CCoV/NTU336/FF/2008
Feline infectious peritonitis virus
Mink coronavirus strain WD1127
Porcine epidemic diarrhea virus
Myotis ricketti BatCoV HKU6
Scotophilus BatCoV 512
Rousettus leschenaulti BatCoV HKU10
Hipposideros pomona BatCoV HKU10
Lucheng Rn rat coronavirus
Rhinolophus sinicus BatCoV HKU2
Swine acute diarrhea syndrome related Rhinolophus BatCoV 162140
Swine acute diarrhea syndrome coronavirus SADS-CoV/GN/GDGU/2017
Myotis lucifugus BatCoV CDPHE15/USA/2006
Miniopterus magnater BatCoV HKU7
Miniopterus pusillus BatCoV HKU8
Miniopterus magnater BatCoV 1A
Miniopterus pusillus BatCoV 1B
Human CoV NL63
Triaenops afer BatCoV NL63 gal/Kenya/2010
Hipposideros BatCoV 229E/Hip cf. ruber/Ghana/2011
Alpaca respiratory CoV C408-1/2008
Human CoV 229E
Camel CoV 229E JC50
Hipposideros cf. ruber BatCoV 229E/KWZE-F56/Hip cf. ruber/Ghana/2011


0·2
AY508724|SARS-CoV_NS-1
AY485277|SARS-CoV_Sino1-11
AY390556|SARS-CoV_GZ02|Guangzhou
AY278489|SARS-CoV_GD01
SARS-CoV
KT444582|WIV16|Yunnan
KY417146|Rs4231|Yunnan
KY417151|Rs7327|Yunnan
KY417152|Rs9401|Yunnan
MK211376|BtRs-BetaCoV/YN2018B|Yunnan
KJ473816|BtRs-BetaCoV/YN2013|Yunnan
KY770859|Anlong-112|Guizhou
KY417145|Rf4092|Yunnan
MK211377|BtRs-BetaCoV/YN2018C|Yunnan
KY417142|As6526|Yunnan
KY417148|Rs4247|Yunnan
KJ473815|BtRs-BetaCoV/GX2013|Guangxi
JX993988|Yunnan2011
MK211374|BtRl-BetaCoV/SC2018|China
JX993987|Shaanxi2011
KJ473814|BtRs-BetaCoV/HuB2013|Hubei
DQ648857|Bat_CoV_279/2005|Hubei
GQ153547|HKU3-12|HK/2005
Q084200|HKU3-3|HK/2005
GQ153542|HKU3-7|HK/2007
KF294457|Longquan-140|China
Bat-SL-CoV
Clade 3
Clade 2
Clade 1
WH04|2020-01-05
YS8011|2020-01-07
WH01|2019-12-26
WH03|2020-01-01
WH19002|2019-12-30
WH19008|2019-12-30
WH02|2019-12-31
WH19001|2019-12-30
WH19004|2020-01-01
WH19005|2019-12-30
2019-nCoV
Sarbecovirus
MG772933|bat-SL-CoVZC45|Zhejiang
MG772934|bat-SL-CoVZXC21|Zhejiang
Bat-SL-CoV
KY352407|SARS-related_coronavirus|BtKY72|Kenya
GU190215|Bat_coronavirus|BM48-31/BGR/2008|Bulgaria
Hibecovirus
KF636752|Bat_Hp-betacoronavirus/Zhejiang2013
Nobecovirus
KU762338|Rousettus_bat_coronavirus|GCCDC1_356|China
EF065513|Bat_coronavirus_HKU9-1|BF_005I|Guangdong
EF065509|Bat_coronavirus_HKU5-1|LMH03f|Guangdong
EF065505|Bat_coronavirus_HKU4-1|B04f|Guangdong
Merbecovirus
JX869059|Human_betacoronavirus_2c_EMC/2012|HCoV-EMC|Saudi Arabia
MERS-CoV
KC545386|Betacoronavirus_Erinaceus/VMC/DEU/2012|ErinaceusCoV/2012-216/GER/2012|Germany
AY391777|Human_coronavirus_OC43|ATCC_VR-759|UK
Embevovirus
KM349744|Betacoronavirus_HKU24|HKU24-R05010I|China
FJ647223|Murine_coronavirus_MHV-1|USA
MK167038|Human_coronavirus_HKU1|SC2521|USA


0
10
20
30 kB
5'
ORF1a
ORF1b
S GENE
4a
3
5
E
M
N
-3'
4b
8b
B
Non-structural polyprotein 1ab (7096 amino acids)
Spike protein (1273 amino acids)
251
+C, 13443
TTTTTAAACCGGG
21541
21549
25730
2019-nCoV
WH04
29844 base pairs
5'
3'
E M 7 10b N
3
8
9 13 14
Non-structural polyprotein 1ab (7092 amino acids)
Spike protein (1246 amino acids)
265
+C, 13455
TTTTTAAACCGGG
21543
21550
25290
MG772933
Bat_SL-CoVZC45
29802 base pairs
5'
3'
E M 7 10b N
3a
3b
8
9 13 14
Non-structural polyprotein 1ab (7070 amino acids)
Spike protein (1245 amino acids)
265
+C, 13389
TTTTTAAACCGGG
21477
21484
25221
MG772934
Bat_SLCoVZXC21
29732 base pairs
5'
3'
E M 7 10b N
3a
3b
8
9 13 14
Non-structural polyprotein 1ab (7073 amino acids)
Spike protein (1255 amino acids)
265
+C, 13398
TTTTTAAACCGGG
21486
21493
25260
NC_004718.3
SARS-CoV
29751 base pairs
5'
3'
E M 6 8b 9a
3a
3b
7a
7b 8a
9b
Non-structural polyprotein 1ab (7078 amino acids)
Spike protein (1270 amino acids)
279
+C, 13433
TTTTTAAACCGGG
21514
21705
25517
NC_019843.3
MERS-CoV
30119 base pairs
5'
3'
4b 5 E N
3
M
4a
8b

A

	Strain	Complete genome (%)	Gene region (%)											
			1ab	1a	1b	S	3	E	M	7	8	10b	N	13	14
Nucleotide sequences	Bat-SL-CoVZC45	87·6	88·9	90·7	86·0	75·2	87·8	98·7	93·4	95·2	88·8	88·5	91·1	89·1	96·7
	Bat-SL-CoVZXC21	87·5	88·7	90·3	86·1	74·7	88·9	98·7	93·4	95·2	89·1	88·5	91·2	89·5	96·7
	SARS-CoVGZ02	79·0	79·5	75·4	86·3	72·7	75·6	93·5	85·1	74·5	82·1	..	88·1	..	..
Amino acid sequences	Bat-SL-CoVZC45	..	95·6	95·6	95·8	80·2	90·9	100·0	98·6	93·4	87·6	94·2	94·3	73·2	92·9
	Bat-SL-CoVZXC21	..	95·2	95·1	95·5	79·6	92·0	100·0	98·6	93·4	88·4	94·2	94·3	73·2	92·9
	SARS-CoVGZ02	..	86·2	80·5	95·6	76·2	73·1	94·7	90·1	68·9	85·2	..	90·3	..	..

B

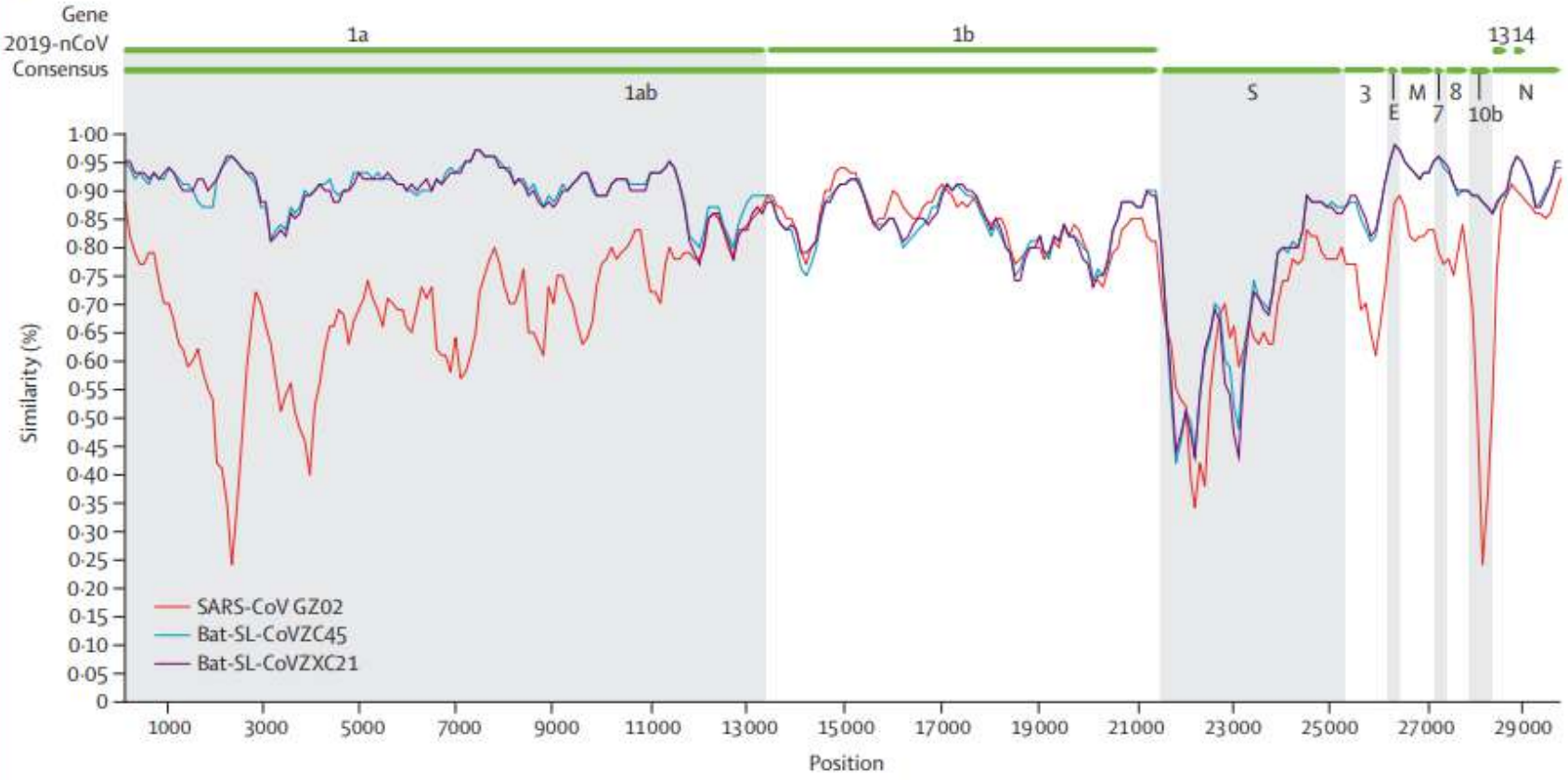
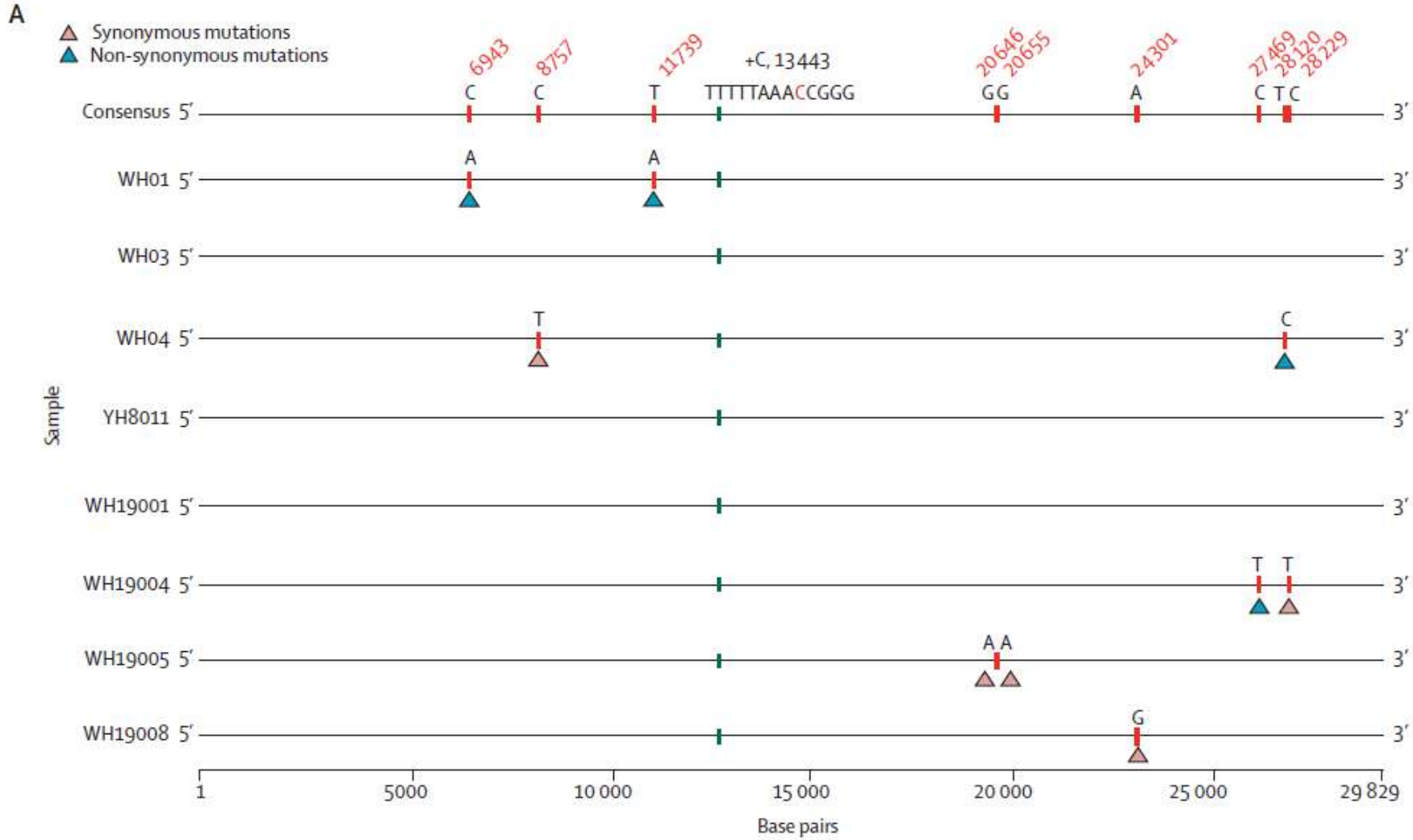
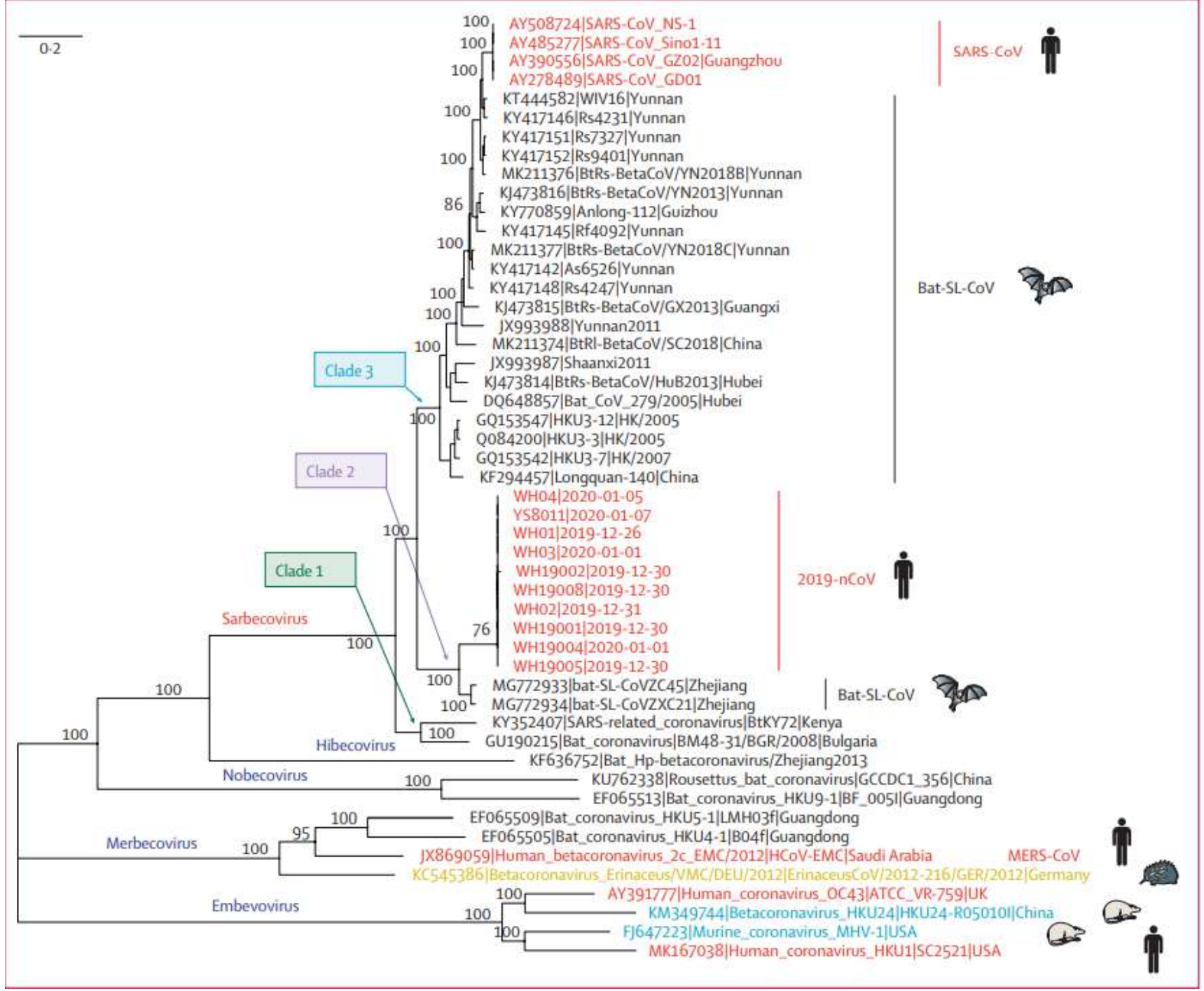


Figure 2: **Sequence identity between the consensus of 2019-nCoV and representative betacoronavirus genomes**


A
Synonymous mutations
Non-synonymous mutations
6943
8757
11739
+C, 13443
20646
20655
24301
27469
28120
28229
Consensus 5′ C C T TTTTTAAACCGGG GG A C T C 3′
WH01 5′ A A 3′
WH03 5′ 3′
WH04 5′ T C 3′
YH8011 5′ 3′
WH19001 5′ 3′
WH19004 5′ T T 3′
WH19005 5′ A A 3′
WH19008 5′ G 3′
Sample
1
5000
10 000
15 000
20 000
25 000
29 829
Base pairs


0·2
AY508724|SARS-CoV_NS-1
AY485277|SARS-CoV_Sino1-11
AY390556|SARS-CoV_GZ02|Guangzhou
AY278489|SARS-CoV_GD01
SARS-CoV
KT444582|WIV16|Yunnan
KY417146|Rs4231|Yunnan
KY417151|Rs7327|Yunnan
KY417152|Rs9401|Yunnan
MK211376|BtRs-BetaCoV/YN2018B|Yunnan
KJ473816|BtRs-BetaCoV/YN2013|Yunnan
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KY417145|Rf4092|Yunnan
MK211377|BtRs-BetaCoV/YN2018C|Yunnan
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KJ473815|BtRs-BetaCoV/GX2013|Guangxi
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GQ153547|HKU3-12|HK/2005
Q084200|HKU3-3|HK/2005
GQ153542|HKU3-7|HK/2007
KF294457|Longquan-140|China
Bat-SL-CoV
Clade 3
Clade 2
Clade 1
WH04|2020-01-05
YS8011|2020-01-07
WH01|2019-12-26
WH03|2020-01-01
WH19002|2019-12-30
WH19008|2019-12-30
WH02|2019-12-31
WH19001|2019-12-30
WH19004|2020-01-01
WH19005|2019-12-30
2019-nCoV
Sarbecovirus
MG772933|bat-SL-CoVZC45|Zhejiang
MG772934|bat-SL-CoVZXC21|Zhejiang
Bat-SL-CoV
KY352407|SARS-related_coronavirus|BtKY72|Kenya
GU190215|Bat_coronavirus|BM48-31/BGR/2008|Bulgaria
Hibecovirus
KF636752|Bat_Hp-betacoronavirus/Zhejiang2013
Nobecovirus
KU762338|Rousettus_bat_coronavirus|GCCDC1_356|China
EF065513|Bat_coronavirus_HKU9-1|BF_005I|Guangdong
EF065509|Bat_coronavirus_HKU5-1|LMH03f|Guangdong
EF065505|Bat_coronavirus_HKU4-1|B04f|Guangdong
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JX869059|Human_betacoronavirus_2c_EMC/2012|HCoV-EMC|Saudi Arabia
MERS-CoV
KC545386|Betacoronavirus_Erinaceus/VMC/DEU/2012|ErinaceusCoV/2012-216/GER/2012|Germany
AY391777|Human_coronavirus_OC43|ATCC_VR-759|UK
KM349744|Betacoronavirus_HKU24|HKU24-R05010I|China
FJ647223|Murine_coronavirus_MHV-1|USA
MK167038|Human_coronavirus_HKU1|SC2521|USA
Embevovirus

Virus species	Host	Attachment factor	Main receptor
Alphacoronavirus 1			
Canine coronavirus type I	Dog		?
Canine coronavirus type II	Dog		APN (aminopeptidaz N)
Feline coronavirus type I	Cat		?
Feline coronavirus type II	Cat		APN
Transmissible gastroenteritis virus	Pig	Sialic acid	APN
Human coronavirus 229E	Human		APN
Human coronavirus NL63	Human		ACE2
Betacoronavirus 1			
Bovine coronavirus	Cow		9-O-Ac Sia?
Equine coronavirus	Horse		9-O-Ac Sia?
Human coronavirus OC43	Human		9-O-Ac Sia? (9-O-acetyl-sialogycans)
Porcine hemagglutinating encephalomyelitis virus	Pig		9-O-Ac Sia?
*Murine coronavirus**	Mouse	4-O- or 9-O-Ac Sia	CEACAM1a
SARS-related coronavirus	Human		ACE2
MERS	Human		DPP4

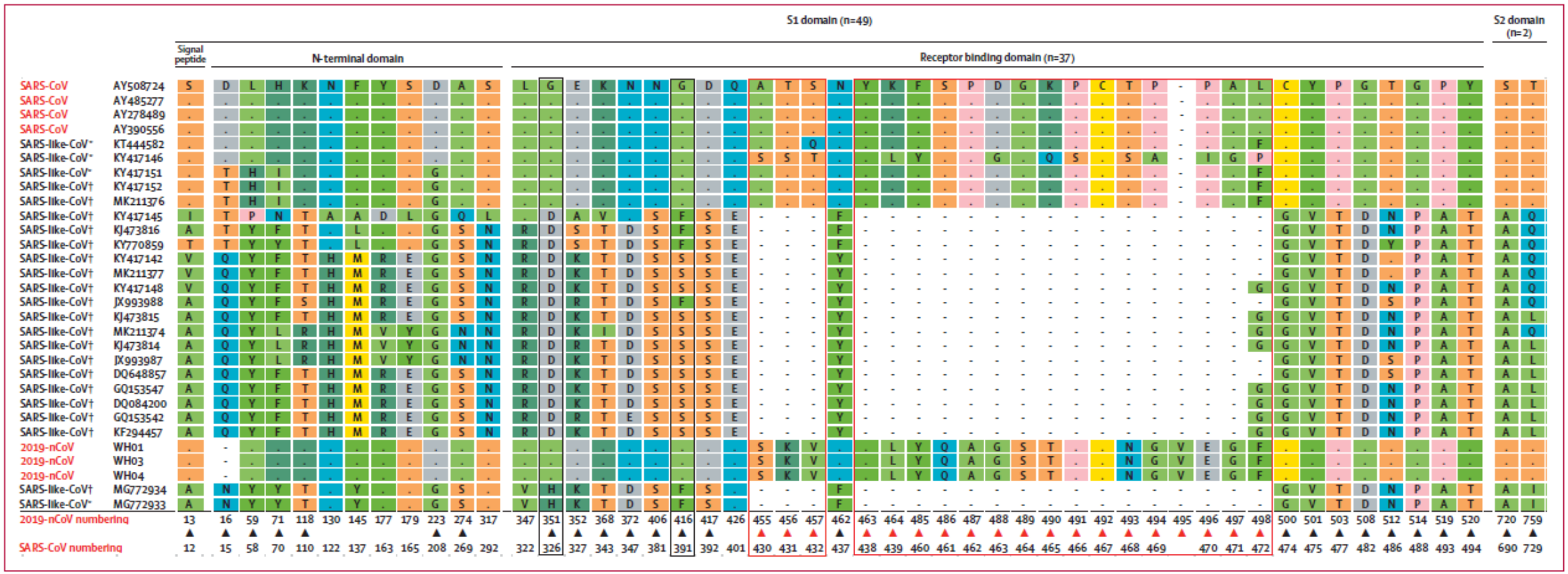


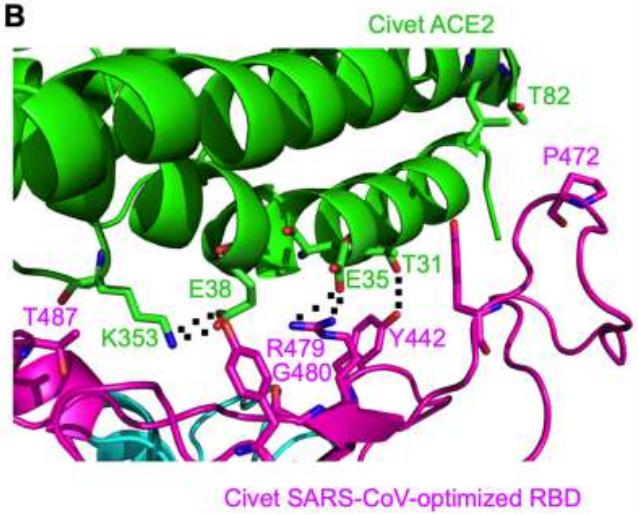
Figure 4: Specific amino acid variations among the spike proteins of the subgenus sarbecovirus

Viruses are ordered by the tree topology (as shown in figure 3) from top to bottom. One-letter codes are used for amino acids. CoV=coronavirus. 2019-nCoV=2019 novel coronavirus. SARS=severe acute respiratory syndrome. *Bat-derived SARS-like viruses that can grow in human cell lines or in mice. †Bat-derived SARS-like viruses without experimental data available.

A

ACE2	31	35	38	82	353
Human	K	E	D	M	K
Civet	T	E	E	T	K
Bat	K	K	D	N	K
Mouse	N	E	D	S	H
Rat	K	E	D	N	H
Pig	K	E	D	T	K
Ferret	K	E	E	T	K
Cat	K	E	E	T	K
Orangutan	K	E	D	M	K
Monkey	K	E	D	M	K

B



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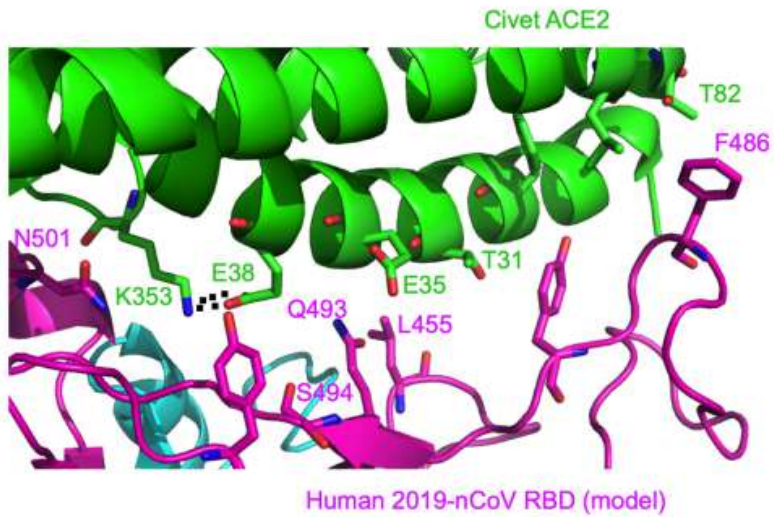

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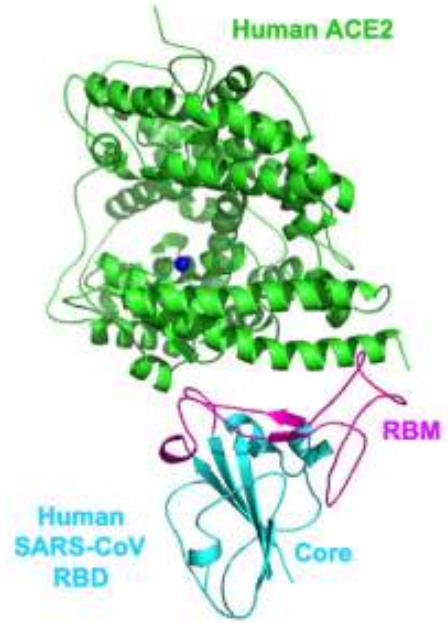
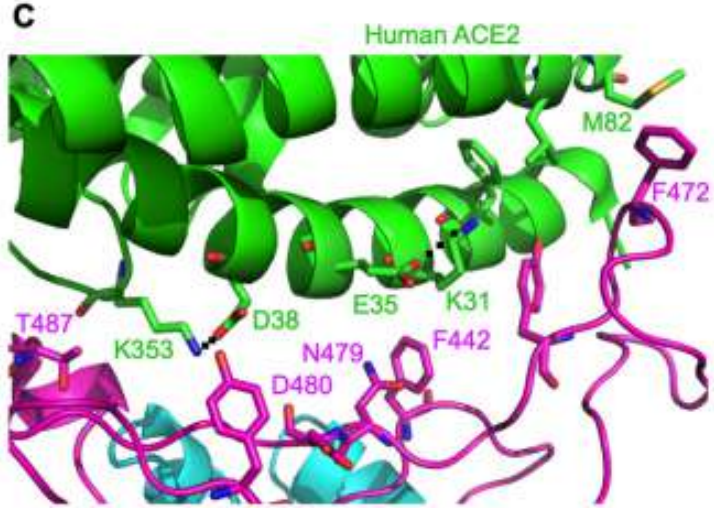


B

Virus	Year	442	472	479	480	487
SARS - human	2002	Y	L	N	D	T
SARS - civet	2002	Y	L	K	D	S
SARS - human/civet	2003	Y	P	N	G	S
SARS - civet	2005	Y	P	R	G	S
SARS - human	2008	F	F	N	D	S
Viral adaption to human ACE2		F > Y	F > L > P	N = R >>> K	D > G	T >>> S
Optimized - human	In vitro design	F	F	N	D	T
Viral adaptation to civet ACE2		Y > F	P = L > F	R > K = N	G > D	T > S
Optimized - civet	In vitro design	Y	P	R	G	T
SARS - bat	2013	S	F	N	D	N
2019-nCoV – human	2019	L (455)	F (486)	Q (493)	S (494)	N (501)

C



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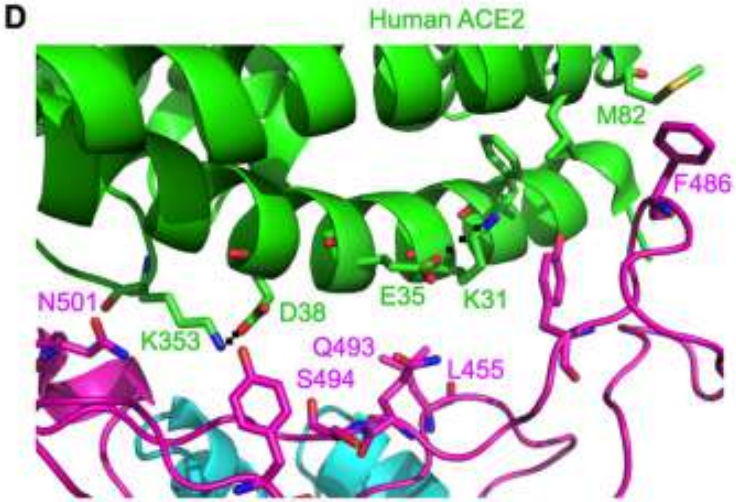


A

```
Human-SARS-2002    306 RVVPS GDVVRFPNIT NLCPFGEVFN ATKFPSVYAW ERKKISNCVA DYSVLYNSTF 360
Civet-SARS-2002    319 RVVPS GDVVRFPNIT NLCPFGEVFN ATKFPSVYAW ERKRISNCVA DYSVLYNSTS 373
Bat-SARS-2013      319 RVAPS KEVVRFPNIT NLCPFGEVFN ATTFPSVYAW ERKRISNCVA DYSVLYNSTS 373
2019-nCoV          319 RVQPT ESIVRFPNIT NLCPFGEVFN ATRFASVYAW NRKRISNCVA DYSVLYNSAS 373
                       ** *:  .:******* ********** ** * ***** :**:****** ********:

Human-SARS-2002    FSTFKCYGVS ATKLNDLCFS NVYADSFVVK GDDVRQIAPG QTGVIADYNY KLPDDFMGCV 420
Civet-SARS-2002    FSTFKCYGVS ATKLNDLCFS NVYADSFVVK GDDVRQIAPG QTGVIADYNY KLPDDFMGCV 433
Bat-SARS-2013      FSTFKCYGVS ATKLNDLCFS NVYADSFVVK GDDVRQIAPG QTGVIADYNY KLPDDFTGCV 433
2019-nCoV          FSTFKCYGVS PTKLNDLCFT NVYADSFVIR GDEVRQIAPG QTGKIADYNY KLPDDFTGCV 433
                   **********  ********: ********:: **:******* *** ****** ****** ***

Human-SARS-2002    LAWNTRNIDA TSTGNYNYKY RYLRHGKLRP FERDISNVPF SPDGKPCTP-P ALNCYWPLND 480
Civet-SARS-2002    LAWNTRNIDA TSTGNYNYKY RYLRHGKLRP FERDISNVPF SPDGKPCTP-P ALNCYWPLKD 493
Bat-SARS-2013      LAWNTRNIDA TQTGNYNYKY RSLRHGKLRP FERDISNVPF SPDGKPCTP-P AFNCYWPLND 493
2019-nCoV          IAWNSNNLDS KVGGNYNYLY RLFRKSNLKP FERDISTEIY QAGSTPCNGVE GFNCYFPLQS 494
                   :***:.*:*: .  ***** * * :*:.:*:* ******.  : . ...**.    .:***:**:.

Human-SARS-2002    YGFYTTTGIG YQPYRVVVLS FELLNAPATV CGPKL 515
Civet-SARS-2002    YGFYTTSGIG YQPYRVVVLS FELLNAPATV CGPKL 528
Bat-SARS-2013      YGFYITNGIG YQPYRVVVLS FELLNAPATV CGPKL 528
2019-nCoV          YGFQPTNGVG YQPYRVVVLS FELLHAPATV CGPKK 529
                   ***  *.*:* ********** ****:***** ****
```

B

Spike / RBD / RBM	SARS-human	SARS-civet	SARS-bat	2019-nCoV
SARS-human	100% / 100% / 100%			
SARS-civet	98.12% / 98.10% / 97.18%	100% / 100% / 100%		
SARS-bat	92.33% / 94.29% / 92.96%	92.75% / 94.76% / 91.55%	100% / 100% / 100%	
2019-nCoV	76.04% / 73.33% / 50.00%	76.78% / 74.29% / 50.00%	77.50% / 75.71% / 52.78%	100% / 100% / 100%

C

Spike /RBD /RBM	MERS-human
HKU4-bat	67.04% /57.69% /40.79%

Figure 3: Sequence comparison of 2019-nCoV and SARS-CoV. (A) Sequence alignment of SARS-CoV and 2019-nCoV RBDs. RBM residues are in magenta. The five critical residues in Fig. 1B are in blue. ACE2-contacting residues are shaded. Asterisks indicate positions that have a single, fully conserved residue. Colons indicate positions that have strongly conserved residues. Periods indicate positions that have weakly conserved residues. (B) Sequence similarities of SARS-CoV and 2019-nCoV in the spike protein, RBD and RBM, respectively. (C) Sequence similarities of MERS-CoV and HKU4 virus in the spike protein, RBD and RBM, respectively. GenBank accession numbers are: JX869059.2 for human MERS-CoV Spike; NC_009019.1 for bat HKU4-CoV Spike.

- 14 kritik amino asitten 8'i SARSv ile ortak

Table 1. Summary of the critical elements for ACE2 utilization present in the S protein of various SARSr-BatCoVs

SARS-CoV and SARSr-CoV	NTD genotype	5 a.a. deletion	442#	12 a.a. deletion	472#	479#	487#	491#
Human SARS-CoV TOR2	1	Retained	Y	Retained	L	N	T	Y
Civet SARSr-CoV SZ3	1	Retained	Y	Retained	L	K	S	Y
Civet SARSr-CoV civet007	1	Retained	Y	Retained	P	R	S	Y
SARSr-Rs-BatCoV WIV1	2	Retained	S	Retained	F	N	N	Y
SARSr-Rs-BatCoV WIV16	1	Retained	S	Retained	F	N	N	Y
SARSr-Rs-BatCoV Rs4874	1	Retained	S	Retained	F	N	N	Y
SARSr-Ra-BatCoV LYRa11	2	Retained	S	Retained	F	N	N	Y
SARSr-Rs-BatCoV Rs7327	2	Retained	S	Retained	F	N	N	Y
SARSr-Rs-BatCoV RsSHC014	2	Retained	W	Retained	P	R	A	H
SARSr-Rs-BatCoV Rs4231	1	Retained	W	Retained	P	R	A	H
SARSr-Rs-BatCoV Rs4084	2	Retained	W	Retained	P	R	A	H
SARSr-Rs-BatCoV Rs4081	3	Deleted	S	Deleted	Deleted	S	V	Y
SARSr-Rs-BatCoV Rs4075*	-	Deleted	S	Deleted	Deleted	S	P	Y
SARSr-Rs-BatCoV Rs4085*	-	Deleted	S	Deleted	G	S	N	Y
SARSr-Rf-BatCoV Rf4092	3	Deleted	S	Deleted	Deleted	S	V	Y
SARSr-Rf-BatCoV YNLF_31C	3	Deleted	S	Deleted	Deleted	S	V	Y

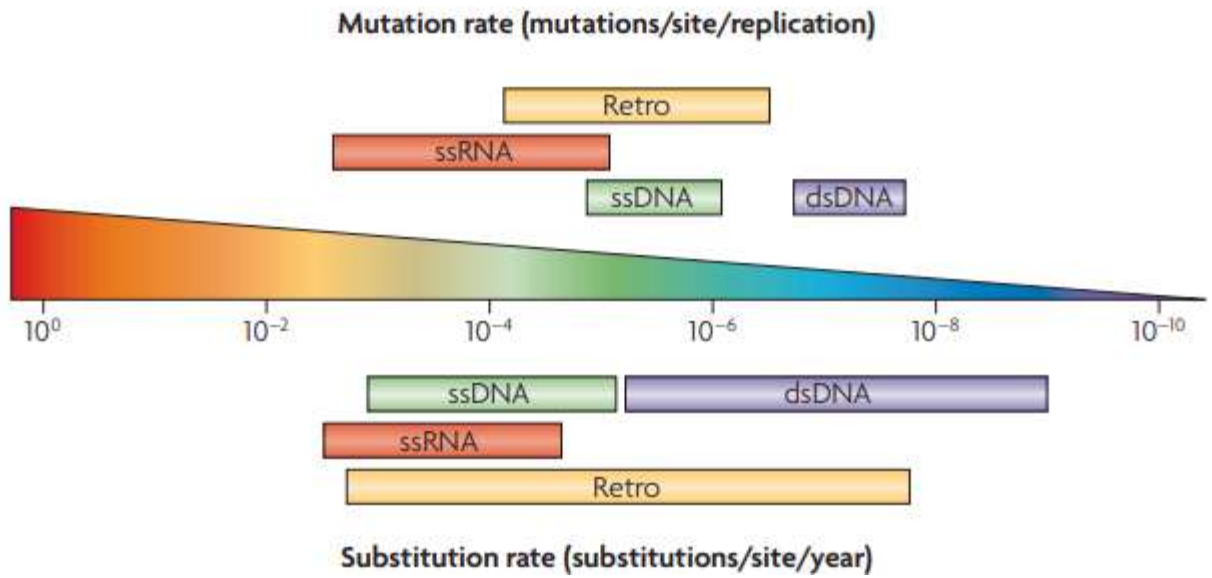
SARSr-BatCoVs that could replicate in cell lines ■

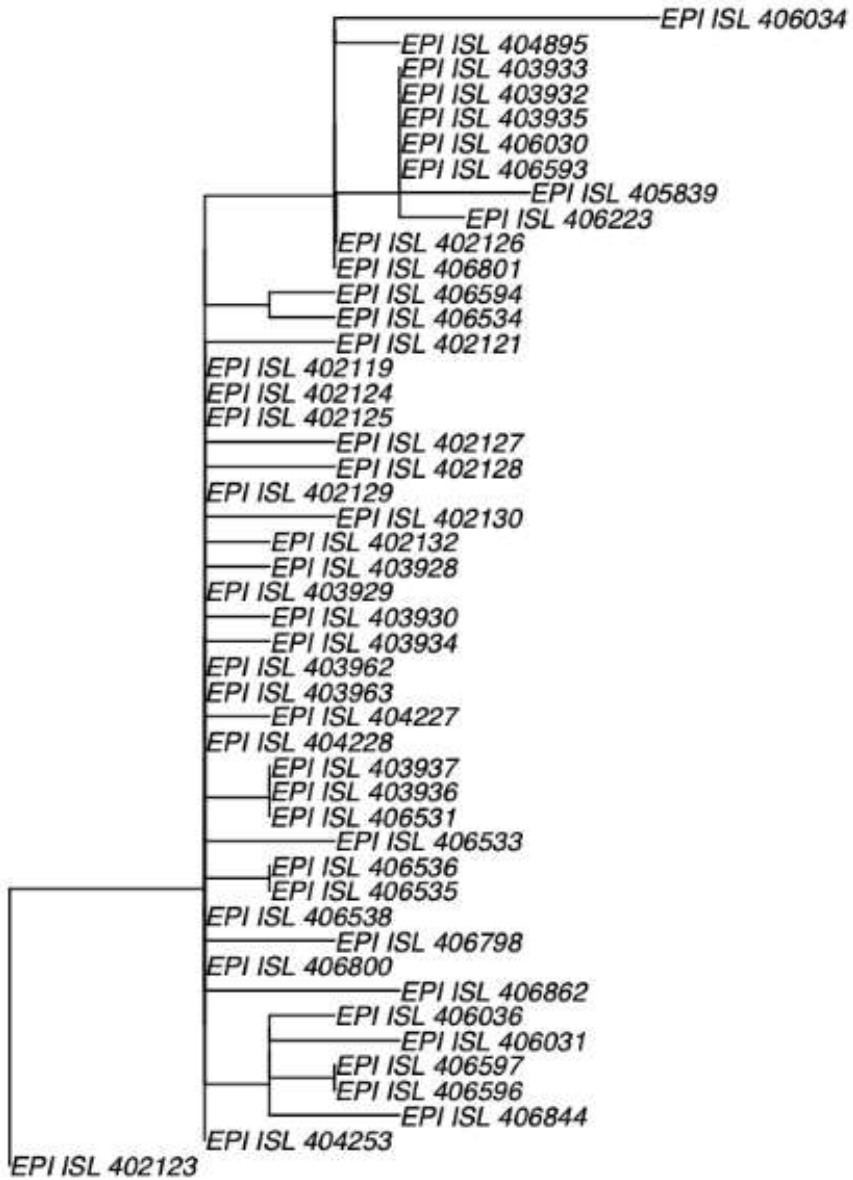
SARSr-BatCoVs that were reported to have slower replication kinetics (Menachery et al. 2015) ■

SARSr-BatCoVs that failed to replicate in cell lines ■

Critical a.a. residues on S protein determining interaction with ACE2

* Only RBD sequences are available in Genbank


Mutation rate (mutations/site/replication)
Retro
ssRNA
ssDNA
dsDNA
10^{0}
10^{-2}
10^{-4}
10^{-6}
10^{-8}
10^{-10}
ssDNA
dsDNA
ssRNA
Retro
Substitution rate (substitutions/site/year)



Model	Marginal likelihood	Evolutionary rate (subs/site/year)	TMRCA
SC heterochronous	-41028.38	1.23×10^{-3} (HPD: 5.63×10^{-4} – 1.98×10^{-3})	2019.89 (HPD: 2019.81 – 2019.95)
UCLN heterochronous	-41034.21	1.29×10^{-3} (HPD: 5.35×10^{-4} – 2.15×10^{-3})	2019.87 (HPD: 2019.74 – 2019.95)
UCLN isochronous	-41053.57	-	-
SC isochronous	-41053.70	-	-

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Leo Featherstone (leo.featherstone@unimelb.edu.au)
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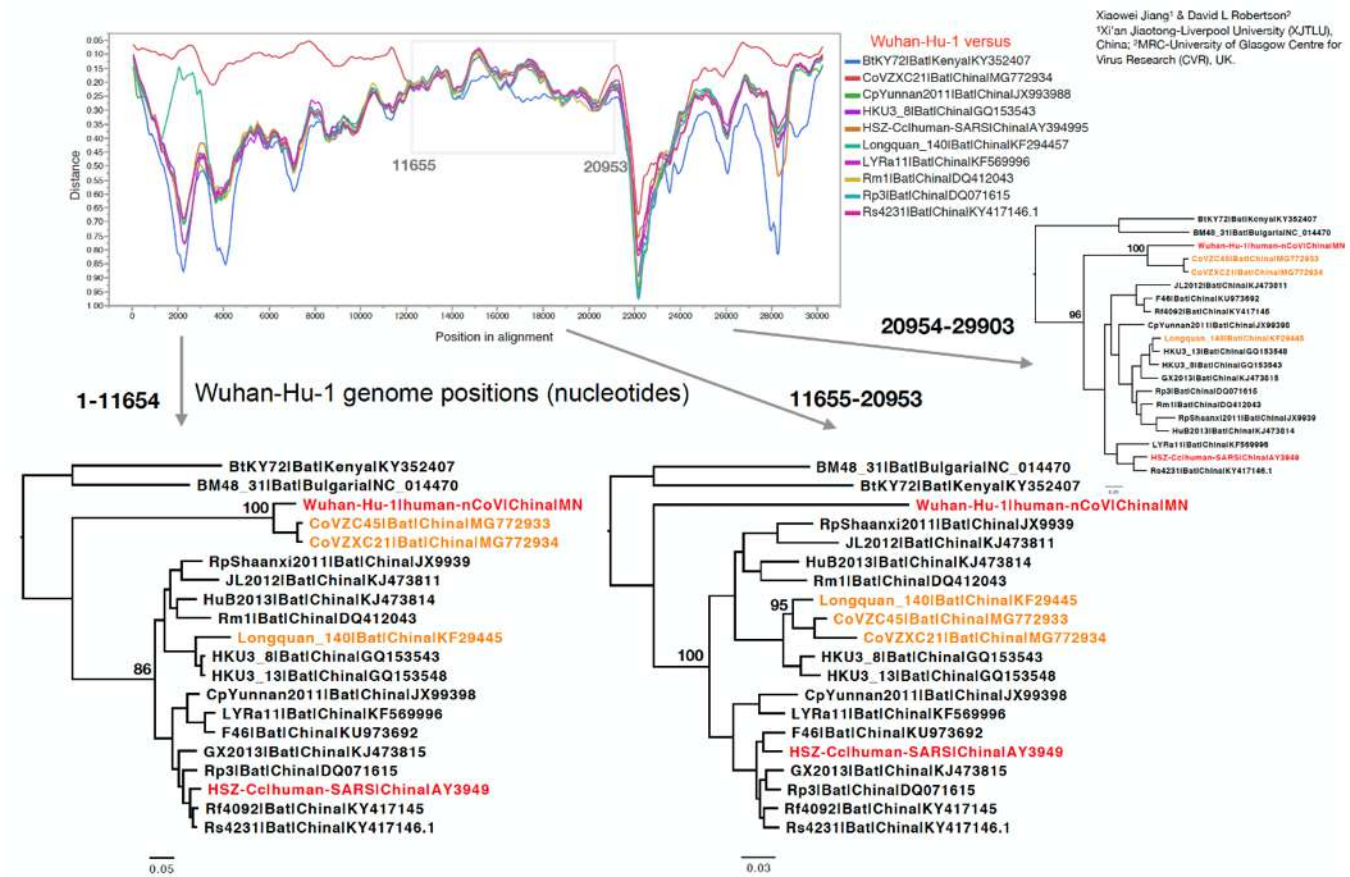
Peter Doherty Institute for Infection and Immunity

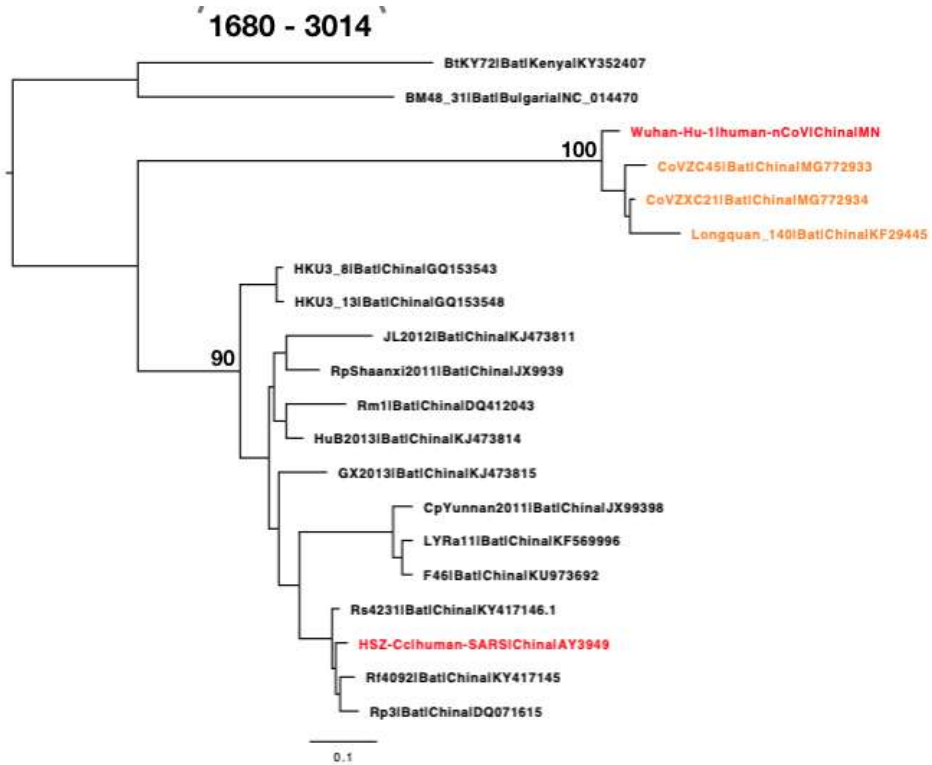
University of Melbourne, Melbourne, Australia

david.l.robertson 4 13d

With Xiaowei Jiang at XJTLU we've carried out a preliminary evolutionary analysis to characterise the evolutionary origins of the Wuhan virus, nCoV. Focus of our analysis is on the Wuhan-Hu-1 virus (accession no. MN908947, released on GenBank by Shanghai Public Health Clinical Center and School of Public Health, Fudan University, Shanghai, China) as all nCoV cluster together so will share the same evolutionary ancestry. It's clear from phylogenetic analysis the new human virus is most closely related to bat coronaviruses in the Betacoronaviruses genera. While this is apparent from both the previously reported BLAST and full-genome phylogenetic analysis the closest related bat viruses (CoVZC45 and CoVZXC21) are in fact recombinants with shared breakpoints either side of ORF1b:



Top, diversity plot across the Wuhan-Hu-1 genome alignment in 800 nucleotide windows, incremented 50 nts (generated using RDP4). Complete genomes were aligned with MAFFT using data from ViPR and GenBank. Phylogenetic trees from regions detected by the HyPhy software GARD to be between recombination locations are shown. Note, exact breakpoints locations are dependent on reference sets used. The trees were inferred using PhyML with a 'BEST' tree search and HKY substitution model. The same options were used to perform bootstrapping (1000 replicates). Trees were visualised with FigTree.



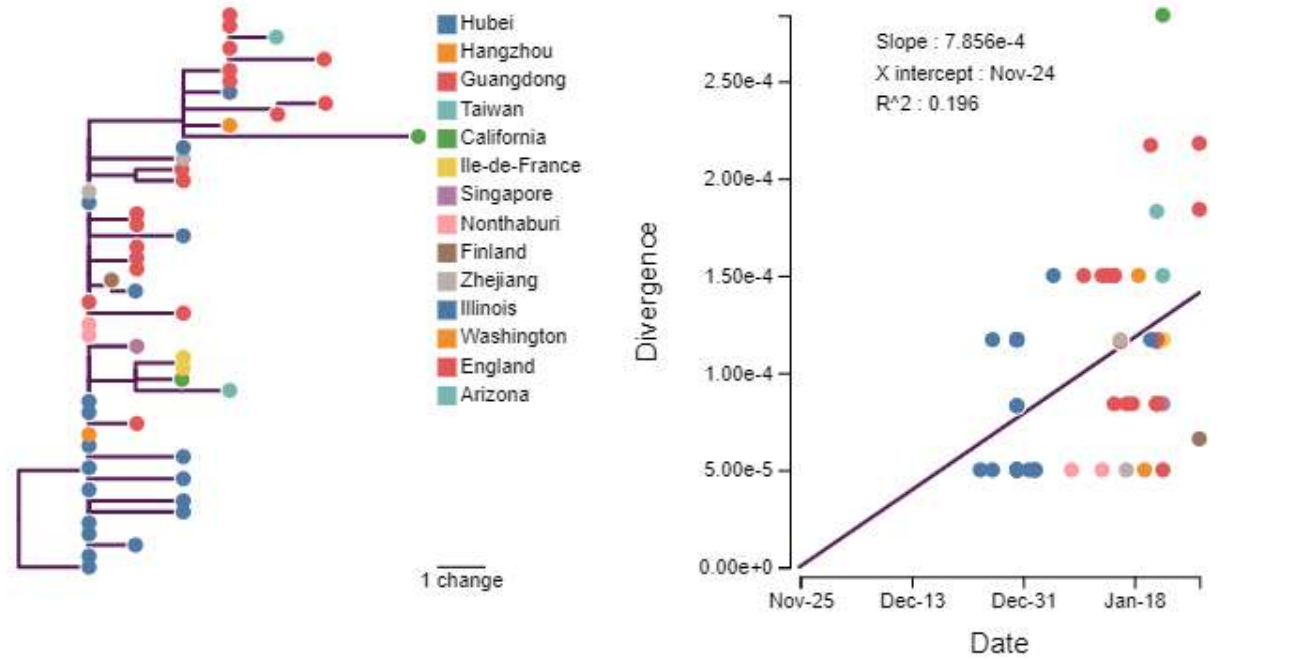
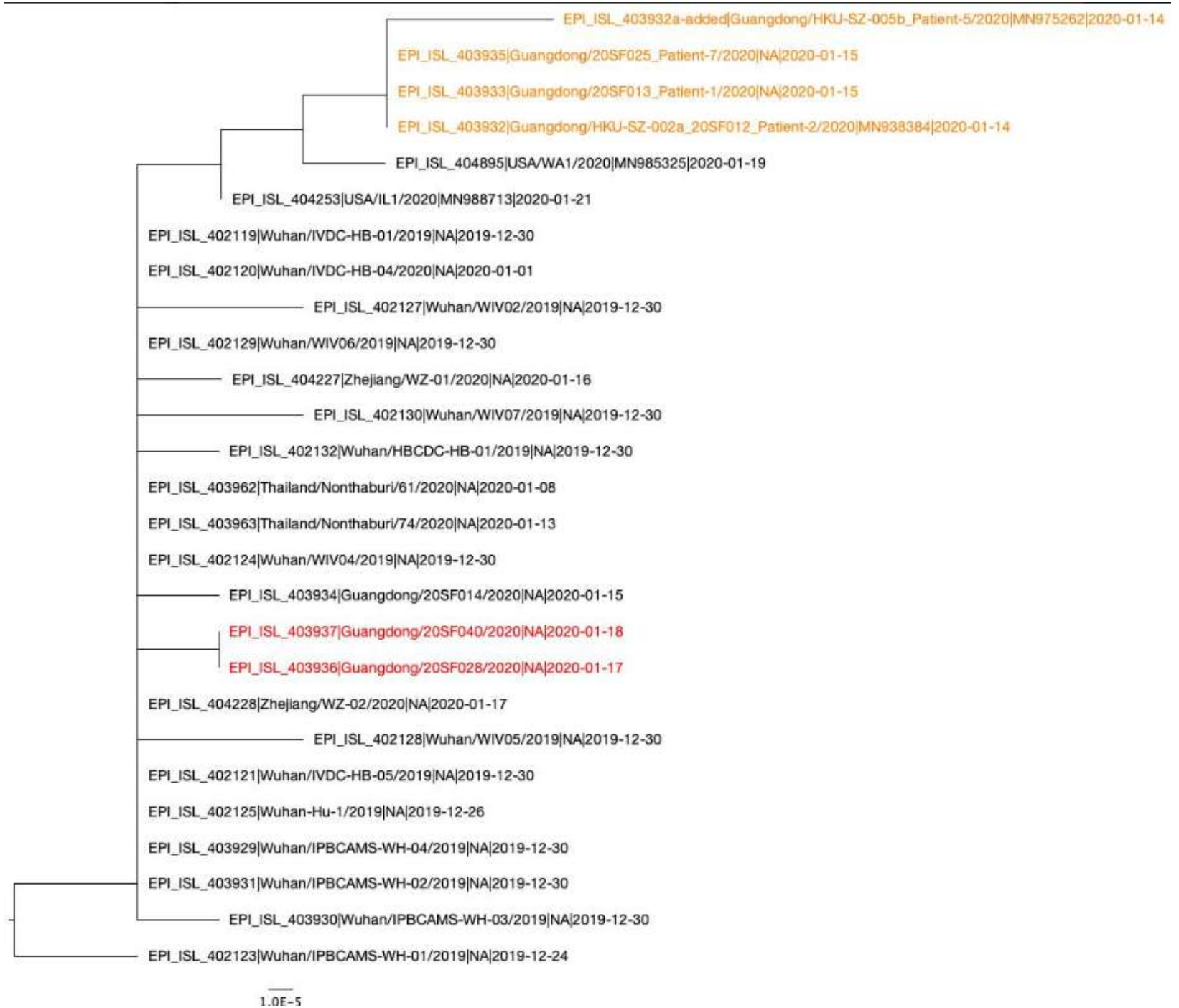


Figure 1 | Maximum likelihood tree of nCoV2019 genomes constructed using PhyML [1]. The tree is rooted using the oldest sequence but this is an arbitrary choice. Interactive tree figure by **@john.mccrone** using figtree.js 55 .

MRCA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EPI_ISL_403935|Guangdong/20SF025_Patient-7/2020|NA|2020-01-15
EPI_ISL_403933|Guangdong/20SF013_Patient-1/2020|NA|2020-01-15
EPI_ISL_403932|Guangdong/HKU-SZ-002a_20SF012_Patient-2/2020|MN938384|2020-01-14
EPI_ISL_404895|USA/WA1/2020|MN985325|2020-01-19
EPI_ISL_404253|USA/IL1/2020|MN988713|2020-01-21
EPI_ISL_402119|Wuhan/IVDC-HB-01/2019|NA|2019-12-30
EPI_ISL_402120|Wuhan/IVDC-HB-04/2020|NA|2020-01-01
EPI_ISL_402127|Wuhan/WIV02/2019|NA|2019-12-30
EPI_ISL_402129|Wuhan/WIV06/2019|NA|2019-12-30
EPI_ISL_404227|Zhejiang/WZ-01/2020|NA|2020-01-16
EPI_ISL_402130|Wuhan/WIV07/2019|NA|2019-12-30
EPI_ISL_402132|Wuhan/HBCDC-HB-01/2019|NA|2019-12-30
EPI_ISL_403962|Thailand/Nonthaburi/61/2020|NA|2020-01-08
EPI_ISL_403963|Thailand/Nonthaburi/74/2020|NA|2020-01-13
EPI_ISL_402124|Wuhan/WIV04/2019|NA|2019-12-30
EPI_ISL_403934|Guangdong/20SF014/2020|NA|2020-01-15
EPI_ISL_403937|Guangdong/20SF040/2020|NA|2020-01-18
EPI_ISL_403936|Guangdong/20SF028/2020|NA|2020-01-17
EPI_ISL_404228|Zhejiang/WZ-02/2020|NA|2020-01-17
EPI_ISL_402128|Wuhan/WIV05/2019|NA|2019-12-30
EPI_ISL_402121|Wuhan/IVDC-HB-05/2019|NA|2019-12-30
EPI_ISL_402125|Wuhan-Hu-1/2019|NA|2019-12-26
EPI_ISL_403929|Wuhan/IPBCAMS-WH-04/2019|NA|2019-12-30
EPI_ISL_403931|Wuhan/IPBCAMS-WH-02/2019|NA|2019-12-30
EPI_ISL_403930|Wuhan/IPBCAMS-WH-03/2019|NA|2019-12-30
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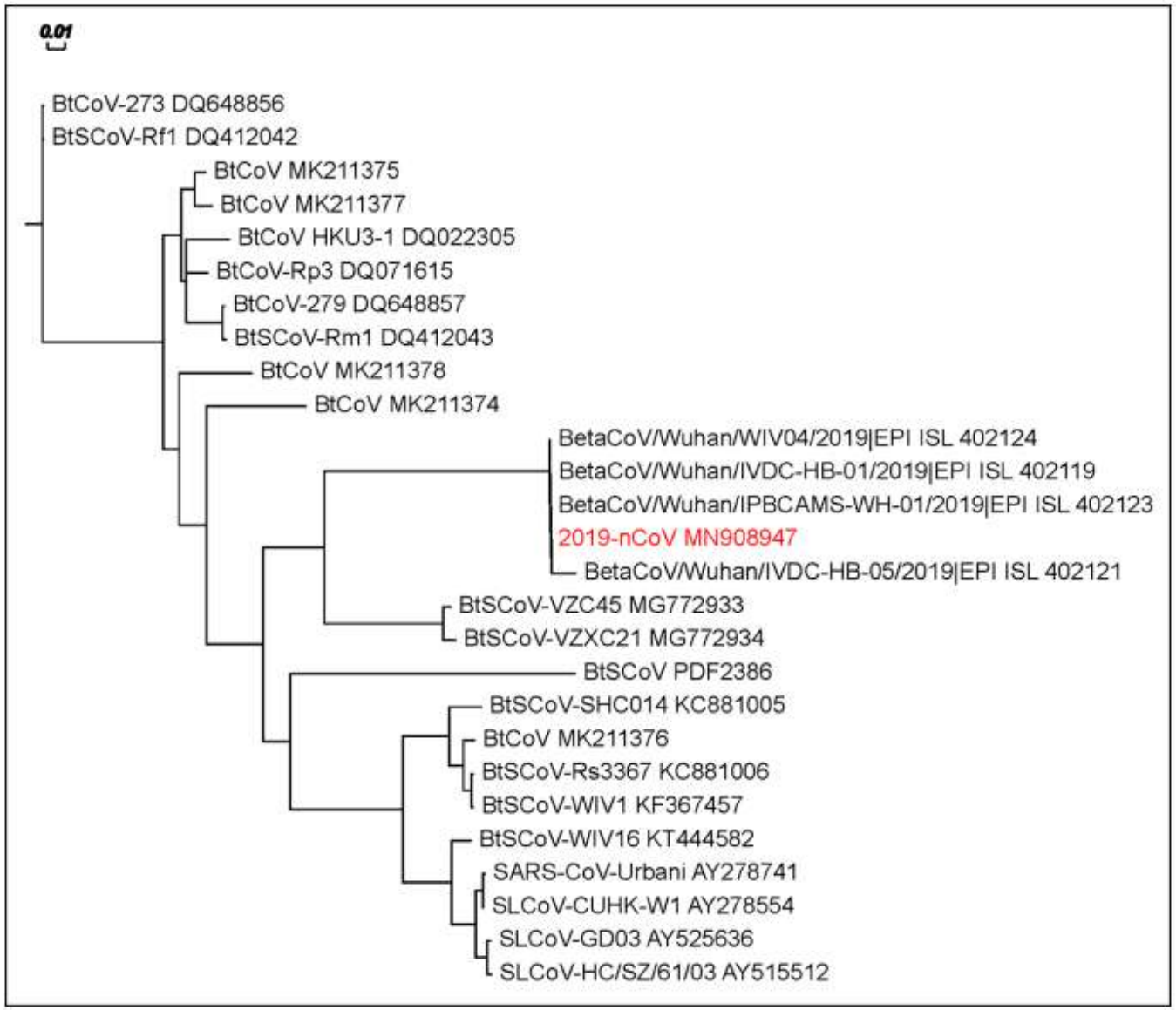
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BtSCoV-SHC014 KC881005
BtCoV MK211376
BtSCoV-Rs3367 KC881006
BtSCoV-WIV1 KF367457
BtSCoV-WIV16 KT444582
SARS-CoV-Urbani AY278741
SLCoV-CUHK-W1 AY278554
SLCoV-GD03 AY525636
SLCoV-HC/SZ/61/03 AY515512

TANI

- Reverse Transkripsiyon – Polimeraz zincir reaksiyonu (PCR)
  - Konvansiyonel (pan coronavirus → Dizileme)
  - Real time PCR

CDC protokolü

2019 nCoV_N1	2019 nCoV_N2	2019 nCoV_N3	RP	Result Interpretationa
+	+	+	±	2019-nCoV detected
If only one, or two, of three targets is positive			±	Inconclusive Result
–	–	–	+	2019-nCoV not detected
–	–	–	–	Invalid Result

2019-Novel Coronavirus (2019-nCoV) Real-time rRT-PCR Panel Primers and Probes				
Name	**Description**	**Oligonucleotide Sequence (5'>3')**	**Label[1]**	**Working Conc.**
2019-nCoV_N1-F	2019-nCoV_N1 Forward Primer	5'-GAC CCC AAA ATC AGC GAA AT-3'	None	20 µM
2019-nCoV_N1-R	2019-nCoV_N1 Reverse Primer	5'-TCT GGT TAC TGC CAG TTG AAT CTG-3'	None	20 µM
2019-nCoV_N1-P	2019-nCoV_N1 Probe	5'-FAM-ACC CCG CAT TAC GTT TGG TGG ACC-BHQ1-3'	FAM, BHQ-1	5 µM
2019-nCoV_N2-F	2019-nCoV_N2 Forward Primer	5'-TTA CAA ACA TTG GCC GCA AA-3'	None	20 µM
2019-nCoV_N2-R	2019-nCoV_N2 Reverse Primer	5'-GCG CGA CAT TCC GAA GAA-3'	None	20 µM
2019-nCoV_N2-P	2019-nCoV_N2 Probe	5'-FAM-ACA ATT TGC CCC CAG CGC TTC AG-BHQ1-3'	FAM, BHQ-1	5 µM
2019-nCoV_N3-F	2019-nCoV_N3 Forward Primer	5'-GGG AGC CTT GAA TAC ACC AAA A-3'	None	20 µM
2019-nCoV_N3-R	2019-nCoV_N3 Reverse Primer	5'-TGT AGC ACG ATT GCA GCA TTG-3'	None	20 µM
2019-nCoV_N3-P	2019-nCoV_N3 Probe	5'-FAM-AYC ACA TTG GCA CCC GCA ATC CTG-BHQ1-3'	FAM, BHQ-1	5 µM
RP-F	RNAse P Forward Primer	5'-AGA TTT GGA CCT GCG AGC G-3'	None	20 µM
RP-R	RNAse P Reverse Primer	5'-GAG CGG CTG TCT CCA CAA GT-3'	None	20 µM
RP-P	RNAse P Probe	5'-FAM – TTC TGA CCT GAA GGC TCT GCG CG – BHQ-1-3'	FAM, BHQ-1	5 µM

[1]TaqMan® probes are labeled at the 5'-end with the reporter molecule 6-carboxyfluorescein (FAM) and with the quencher, Black Hole Quencher 1 (BHQ-1) (Biosearch Technologies, Inc., Novato, CA) at the 3'-end.

Note: Oligonucleotide sequences are subject to future changes as the 2019-Novel Coronavirus evolves.

WHO protokolü: N tarama; nsp-14 doğrulama olarak

Primer and probe sequences

Assay 1 (Target: ORF1b-nsp14)

Forward primer (HKU-ORF1b-nsp14F): 5'-TGGGGYTTTACRGGTAACCT-3'

Reverse primer (HKU- ORF1b-nsp14R): 5'-AACRCGCTTAACAAAGCACTC-3'

Probe (HKU-ORF1b-nsp141P): 5'-FAM-TAGTTGTGATGCWATCATGACTAG-TAMRA-3'

Assay 2 (Target: N)

Forward primer (HKU-NF): 5'-TAATCAGACAAGGAACTGATTA-3'

Reverse primer (HKU-NR): 5'-CGAAGGTGTGACTTCCATG-3'

Probe (HKU-NP): 5'-FAM-GCAAATTGTGCAATTTGCGG-TAMRA-3'

Figure 1. Relative positions of amplicon targets on the SARS coronavirus and the 2019 novel coronavirus genome

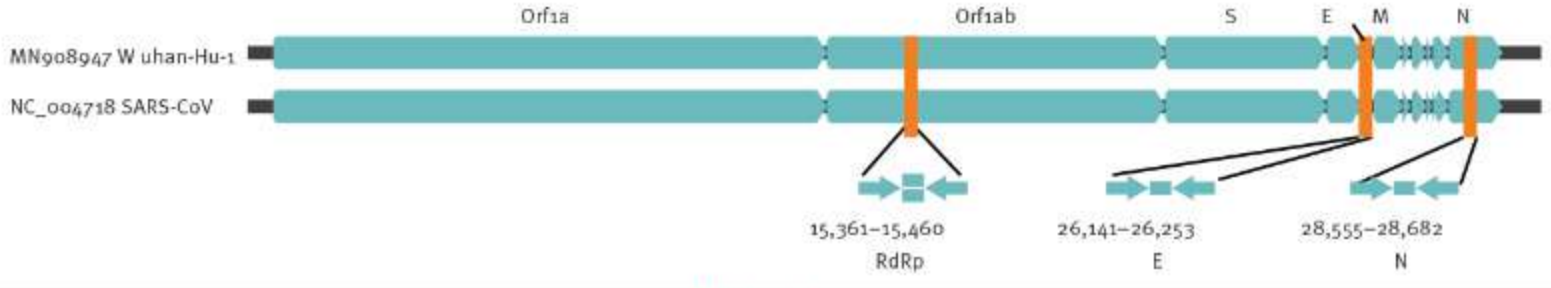


Table 1. Primers and probes, real-time RT-PCR for 2019 novel coronavirus

Toggle display: ▾ Open fullscreen

Assay/use	Oligonucleotide	Sequence[a]	Concentration[b]
RdRP gene	RdRp_SARSr-F	GTGARATGGTCATGTGTGGCGG	Use 600 nM per reaction
	RdRp_SARSr-P2	FAM-CAGGTGGAACCTCATCAGGAGATGC-BBQ	Specific for 2019-nCoV, will not detect SARS-CoV. Use 100 nM per reaction and mix with P1
	RdRP_SARSr-P1	FAM-CCAGGTGGWACRTCATCMGGTGATGC-BBQ	Pan Sarbeco-Probe will detect 2019-nCoV, SARS-CoV and bat-SARS-related CoVs. Use 100 nM per reaction and mix with P2
	RdRp_SARSr-R	CARATGTTAAASACACTATTAGCATA	Use 800 nM per reaction
E gene	E_Sarbeco_F	ACAGGTACGTTAATAGTTAATAGCGT	Use 400 nm per reaction
	E_Sarbeco_P1	FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ	Use 200 nm per reaction
	E_Sarbeco_R	ATATTGCAGCAGTACGCACACA	Use 400 nm per reaction
N gene	N_Sarbeco_F	CACATTGGCACCCGCAATC	Use 600 nm per reaction
	N_Sarbeco_P	FAM-ACTTCCTCAAGGAACAACATTGCCA-BBQ	Use 200 nm per reaction
	N_Sarbeco_R	GAGGAACGAGAAGAGGCTTG	Use 800 nm per reaction

[a] W is A/T; R is G/A; M is A/C; S is G/C. FAM: 6-carboxyfluorescein; BBQ: blackberry quencher.

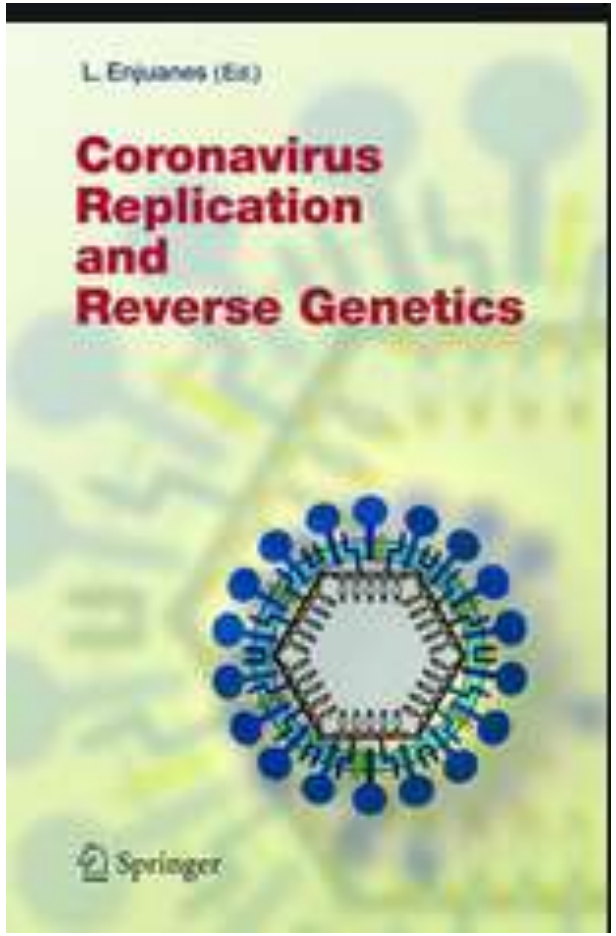
[b] Optimised concentrations are given in nanomol per litre (nM) based on the final reaction mix, e.g. 1.5 µL of a 10 µM primer stock solution per 25 µL total reaction volume yields a final concentration of 600 nM as indicated in the table.

Önce E gen bölgesi; sonra RdRp

- İlaçlar:

- Proteaz inhibitörleri: Litonavir/r
- Remdesevir
- Sofusbuvir
- IFN-beta
- Ribavirin?
- Oseltamivir?

Komplo teorileri



Reverse genetics with a full-length infectious cDNA of the Middle East respiratory syndrome coronavirus

Trevor Scobey, Boyd L. Yount, Amy C. Sims, Eric F. Donaldson, Sudhakar S. Agnihothram, Vineet D. Menachery, Rachel L. Graham, Jesica Swanstrom, Peter F. Bove, Jeeho D. Kim, Sonia Grego, Scott H. Randell, and Ralph S. Baric

PNAS October 1, 2013 110 (40) 16157-16162; https://doi.org/10.1073/pnas.1311542110

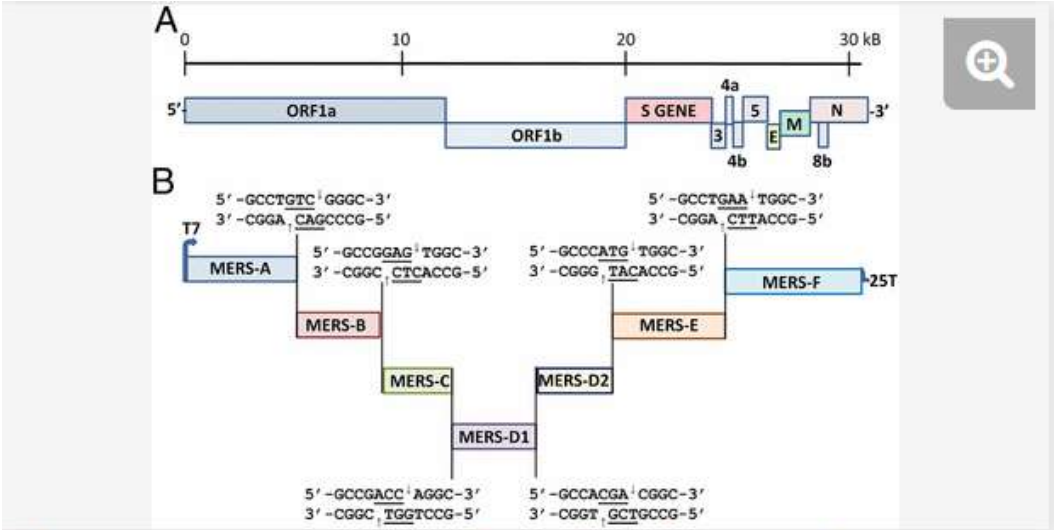


Fig. 1.

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Organization of the MERS-CoV molecular clone. (*A*) The organization of the MERS-CoV genome. (*B*) The full-length MERS-CoV genome was ultimately divided into seven contiguous cDNAs designated MERS A–F and flanked by unique BglI sites that allow for directed assembly of a full-length cDNA: MERS A (nucleotides 1–4692), MERS-B (4693–8811), MERS-C (8812–12258), MERS-D1 (12259–15470), MERS-D2 (15471–18806), MERS-E (18807–24397), and MERS-F (24398–30119).

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Questions Surround Canadian Shipment of Deadly Viruses to China

The same Winnipeg lab that sent Ebola and Henipah viruses to Beijing recently removed a number of researchers for an "administrative issue."

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NICOLETTA LANESE

1.8K

Sergei Kolesnikov