



Phylogeny of 2019-nCoV and SARS-like CoVs of Human, Bat and Pangolin Origin

Abdellatif, MM

¹Department of Biology (Microbiology), Faculty of Science and Arts, Northern Border University, Rafha, Saudi Arabia²Department of Microbiology, Faculty of Vet. Science. University of Nyala, Sudan

*Corresponding author: muazm20@gmail.com

Article History: 20-120 Received: May 17, 2020 Revised: June 24, 2020 Accepted: July 20, 2020

ABSTRACT

A novel coronavirus first broke out in Wuhan, China in December, 2019 has been declared a pandemic by WHO on March, 2020. This work aimed to search for probable ancestor of the virus, phylogeny of 2019-nCoV and similar SL-CoVs based on the whole genome, *M*, *N*, *ORF1ab*, *orf3a*, and *S* gene sequences (n=84) obtained from GenBank using BLASTn software in the NCBI was done. Nucleotides of *ORF3a* and *S*-genes among 2019-nCoV are identical, whereas its similar on the whole genome (99.9-100%), *M*-gene (99.7-100%), *N*-gene (99.9-100%) and *ORF1ab*-gene (99.7-100%). nCoV are similar to *bat CoV/RaTG13* on the whole genome (96.2%), *M*-gene (95.0%), *N*-gene (97%), *ORF1ab*-gene (95.3%), *ORF3a*-gene (99.1%) and *S*-gene (90.7%). Likewise, nCoV exhibited homology to *bat-CoVZXC21* on *M*-gene (93.2%), *N*-gene (91.5%), *ORF1ab*-gene (93.1%) and *ORF3a*-gene (94.4%). The emergent viruses shared identity to *bat-CoVZC45* on *N*-gene (91.3%), *ORF1ab*-gene (92.8%) and *ORF3a*-gene (94.0%). In addition, *pangolin-CoV/MP789* exhibited common sequences on *M*-gene (91.0%), *N*-gene (96.3%) and *ORF3a*-gene (93.3%) to nCoV. Furthermore, *pangolin-CoV/MP789* is analogous to *bat CoV/RaTG13* (91.3%) and *bat-SL-CoVZXC21* (92.2%) on *M*-gene and to *bat CoV/RaTG13* (94.8%) on *N*-gene. Nevertheless, nCoV are distinct from the previously identified SL-CoVs of human origin. The present analysis indicates that nCoV may have transmitted from bats, pangolin and/or unidentified hosts.

Key words: Phylogeny - 2019-nCoV - SL-CoVs - Bats - Pangolin – Human

INTRODUCTION

Coronaviruses are enveloped viruses with a positive sense, single stranded RNA genome that belong to *Coronaviridae* (Tijssen *et al.*, 2011). It codes for spike (S), envelope (E), membrane (M), and nucleocapsid (N), as well as functional proteins (Perlman and Netland, 2009; Woo *et al.*, 2009b). CoVs are widespread in animals causing respiratory, enteric, hepatic and neurological signs. The unique viral multiplication and genetic instability allow the virus to adapt to new hosts and geographic areas (Lai and Cavanagh, 1997). Six different CoVs have been reported, HCoV-OC43 and -229E reported in 1960s (Hamre and Procknow, 1966; McIntosh *et al.*, 1967). HCoV-NL63 and -HKU1 occurred in 2004 and 2005, respectively (van der Hoek *et al.*, 2004; Woo *et al.*, 2005). Severe acute respiratory syndrome (SARS)-CoV in 2003 and the Middle East respiratory syndrome (MERS)-CoV in 2012, and recently a novel virus was described in China (Birmingham *et al.*, 2012; Drosten *et al.*, 2003; Ksiazek *et al.*, 2003; Peiris *et al.*, 2003; Zaki *et al.*, 2012; Zhou *et al.*, 2020). Literature provide evidence that all HCoVs have

evolved in bats and rodents involving intermediate hosts in contact with humans (de Groot *et al.*, 2013; Drexler *et al.*, 2014; Vijaykrishna *et al.*, 2007; Vijgen *et al.*, 2006; Vijgen *et al.*, 2005; Woo *et al.*, 2009a; Woo *et al.*, 2009b).

The present search was intended to find probable ancestor of 2019-nCoV based on nucleotide sequence homology of the whole genome, *M*, *N*, *ORF1ab*, *orf3a* and *S* genes among 2019-nCoV and SL-CoVs of bat, pangolin and human origins.

MATERIALS AND METHODS

Nucleotide sequences (n=84) of the 2019 n-CoVs and SL-CoVs of bat, pangolin and human origins were retrieved on 27 April 2020 from GenBank using BLASTn software on NCBI (<https://blast.ncbi.nlm.nih.gov/Blast>) (Table 1). Analysis was based on the whole genome, *membrane glycoprotein (M)*, *nucleocapsid phosphoprotein (N)*, *ORF1ab polyprotein*, *ORF3a* and *surface glycoprotein (S)* genes.

Phylogenetic trees were constructed using Neighbor-Joining method (Saitou and Nei, 1987) by means of *ClustalW* via *Lasergene 7* (DNASTAR, Inc, Madison, WI, USA).

Table 1: Description of 2019 n-CoVs and SL-CoVs sequences downloaded from GenBank for phylogenetic analysis

No	Isolate	Host	Country	GenBank	Reference
1.	SARS-CoV-2/Hu/DP/Kng/19-027	Human	Japan	LC528233	Unpublished
2.	SARS-CoV-2/USA-TX1/2020	Human	USA	MT106054	Unpublished
3.	SARS-CoV-2/USA-CA7/2020	Human	USA	MT106052	Unpublished
4.	SARS-CoV-2/01/human/2020/SWE	Human	Sweden	MT093571	Unpublished
5.	SARS-CoV-2/01/human/2020/CHN	Human	China	MT049951	Unpublished
6.	SARS-CoV-2/IPBCAMS-WH-05/2020	Human	China	MT019533	(Ren <i>et al.</i> , 2020)
7.	SARS-CoV-2/IPBCAMS-WH-03/2019	Human	China	MT019531	(Ren <i>et al.</i> , 2020)
8.	SARS-CoV-2 Wuhan seafood market	Human	China	LR757998	(Chen <i>et al.</i> , 2020)
9.	SARS-CoV-2 Wuhan seafood market	Human	China	LR757996	(Chen <i>et al.</i> , 2020)
10.	SARS-CoV-2/VIC01/2020	Human	Australia	MT007544	(Caly <i>et al.</i>)
11.	SARS-CoV-2/2019-nCoV WHU01	Human	China	MN988668	(Chen <i>et al.</i> , 2020)
12.	SARS-CoV-2/2019-nCoV_HKU-SZ-002a_2020	Human	China	MN938384	(Chan <i>et al.</i> , 2020)
13.	SARS-CoV-2 Wuhan-Hu-1	Human	China	NC_045512	(Robertson <i>et al.</i> , 2004)
14.	SARS-CoV-2/USA-CA3/2020	Human	USA	MT027062	Unpublished
15.	Bat coronavirus/RaTG13	Rhinolophus affinis	China	MN996532	(Zhou <i>et al.</i> , 2020)
16.	SARS-CoV-2/USA-CA6/2020	Human	USA	MT044258	Unpublished
17.	Bat SARS-like coronavirus/bat-SL-CoVZXC21	Rhinolophus affinis	China	MG772934	(Hu <i>et al.</i> , 2018)
18.	Bat SARS-like coronavirus/bat-SL-CoVZC45	Rhinolophus affinis	China	MG772933	(Hu <i>et al.</i> , 2018)
19.	Pangolin-CoV/MP789	Manis javanica	China	MT084071	(Liu <i>et al.</i> , 2020)
20.	Bat SARS-like coronavirus/Rs4255	Rhinolophus sinicus	China	KY417149	(Hu <i>et al.</i> , 2017)
21.	Civet SARS CoV/Civet SARS CoV SZ16/2003	A civet	China	AY304488	(Hu <i>et al.</i> , 2017)
22.	Bat SARS-like coronavirus/As6526	Aselliscus stoliczkanus	China	KY417142	(Hu <i>et al.</i> , 2017)
23.	Bat coronavirus/BtCoV/279/2005	Bat	China	DQ648857	(Tang <i>et al.</i> , 2006)
24.	SARS coronavirus ShanghaiQXC1	Human	China	AY463059	Unpublished
25.	SARS coronavirus Taiwan TC2	Human	Taiwan	AY338175	(Shih <i>et al.</i> , 2005)
26.	SARS coronavirus isolate HC/GZ/81/03	Paguma larvata	China	AY545917	(Guan <i>et al.</i> , 2003)
27.	SARS coronavirus Sino1-11	Human	China	AY485277	(Zhang <i>et al.</i> , 2005)
28.	SARS coronavirus TW9	Human	Taiwan	AY502932	(Yeh <i>et al.</i> , 2004)
29.	Bat/ Rs7327	Rhinolophus sinicus	China	KY417151	(Hu <i>et al.</i> , 2017)
30.	Bat/ Rs4231	Rhinolophus sinicus	China	KY417146	(Hu <i>et al.</i> , 2017)
31.	SARS-CoV-2/IQTC01/human/2020/CHN	Human	China	MT123290	Unpublished
32.	2019-nCoV/USA-CA9/2020	Human	USA	MT118835	Unpublished
33.	BetaCoV/Korea/SNU01/2020	Human	China	MT039890	(Kim <i>et al.</i> , 2020)
34.	2019 nCoV/Italy-INMII	Human	Italy	MT008022	Unpublished
35.	Coronavirus 2 WIV05/WIV05	Human	China	MN996529	(Zhou <i>et al.</i> , 2020)
36.	SARS-CoV-2/wuhan/ WIV02	Human	China	MN996527	(Zhou <i>et al.</i> , 2020)
37.	2019-nCoV_HKU-SZ-005b_2020	Human	China	MN975262	(Chan <i>et al.</i> , 2020)
38.	2019-nCoV/USA-IL2/2020	Human	USA	MT044257	Unpublished
39.	Cp/Yunnan2011	Chaerephon plicata	China	JX993988	(Wu <i>et al.</i> , 2016b)
40.	Bat SARS CoV Rp3/2004	Bat	China	DQ071615	(Li <i>et al.</i> , 2005)
41.	Bat SARS CoV Rp3/2004	Rhinolophus pusillus	China	JX993987	(Wu <i>et al.</i> , 2016b)
42.	Bat SARS coronavirus HKU3-7	Bat	China	GQ153542	(Lau <i>et al.</i> , 2010)
43.	Rhinolophus affinis coronavirus isolate LYRa11	Rhinolophus affinis	China	KF569996	(He <i>et al.</i> , 2014)
44.	icSARS-C3	Human	USA	MK062181	(Graham <i>et al.</i> , 2018)
45.	Bat SARS-like coronavirus/Rs4247	Rhinolophus sinicus	China	KY417148	(Hu <i>et al.</i> , 2017)
46.	SARS-like coronavirus WIV16	Rhinolophus sinicus	China	KT444582	(Yang <i>et al.</i> , 2016)
47.	Bat SARS-like coronavirus Rs3367	Rhinolophus sinicus	China	KC881006	(Ge <i>et al.</i> , 2013)
48.	SARS coronavirus MA15	Mus musculus	USA	JF292915	Unpublished
49.	SARS-CoV-2/HS_46/human/2020/CHN	Human	China	MT081063	(Ai <i>et al.</i> , 2020)
50.	SARS-CoV-2/HS_18/human/2020/CHN	Human	China	MT081061	(Ai <i>et al.</i> , 2020)
51.	SARSCoV-2/61-TW/human/2020/ NP	Human	Nepal	MT072688	(Bastola <i>et al.</i> , 2020)
52.	BetaCoV/Wuhan/IPBCAMS-WH-02/2019	Human	China	MT019530	(Ren <i>et al.</i> , 2020)
53.	SARS-CoV-2/HS_84/human/2020/CHN	Human	China	MT081065	(Ai <i>et al.</i> , 2020)
54.	Wuhan seafood market pneumonia virus	Human	China	LR757995	unpublished
55.	2019-nCoV/USA-IL1/2020	Human	USA	MN988713	Unpublished
56.	Wuhan seafood market pneumonia virus	Human	China	LR757997	unpublished
57.	BtRs-BetaCoV/YN2018D	Rhinolophus affinis	China	MK211378	(Han <i>et al.</i> , 2019)
58.	Bat SARS-like coronavirus Rs4237	Rhinolophus sinicus	China	KY417147	(Hu <i>et al.</i> , 2017)
59.	Bat SARS coronavirus Rp1	Bat	China	DQ071613	(Li <i>et al.</i> , 2005)
60.	SARS coronavirus Urbani icSARS-C7	Human	USA	MK062183	(Graham <i>et al.</i> , 2018)
61.	Bat SARS-like coronavirus isolate Rs9401	Rhinolophus sinicus	China	KY417152	(Hu <i>et al.</i> , 2017)
62.	SARS-CoV-2/NTU02/2020/TWN	Human	Taiwan	MT066176	unpublished
63.	BetaCoV/Wuhan/IPBCAMS-WH-01/2019	Human	China	MT019529	(Ren <i>et al.</i> , 2020)
64.	SARS coronavirus Urbani icSARS-C7-MA	Human	USA	MK062184	(Graham <i>et al.</i> , 2018)
65.	2019-nCoV/USA-WI1/2020	Human	USA	MT039887	Unpublished
66.	2019-nCoV/USA-WA1/2020	Human	USA	MN985325	(Harcourt <i>et al.</i> , 2020)
67.	Bat SARS-like coronavirus isolate Rs4874	Rhinolophus sinicus	China	KY417150	(Hu <i>et al.</i> , 2017)

68.	Bat SARS-like coronavirus	Rhinolophus monoceros	China	KF294457	(Lin <i>et al.</i> , 2017)
69.	Bat SARS coronavirus HKU3-13	Rhinolophus	China	GQ153548	(Lau <i>et al.</i> , 2010)
70.	Bat SARS coronavirus HKU3-11	Rhinolophus	China	GQ153546	(Lau <i>et al.</i> , 2010)
71.	Bat SARS coronavirus HKU3-1	Bat	China	DQ022305	(Lau <i>et al.</i> , 2005)
72.	SARS coronavirus Taiwan TC3	Human	Taiwan	AY348314	(Shih <i>et al.</i> , 2005)
73.	BetaCoV/Wuhan/IPBCAMS-WH-02/2019	Human	China	MT019532	(Ren <i>et al.</i> , 2020)
74.	2019-nCoV_HKU-SZ-001_2020	Human	China	MN938387	(Chan <i>et al.</i> , 2020)
75.	Bat coronavirus Rc-CoV-3	Rhinolophus cornutus	Japan	LC469301	unpublished
76.	Civet SARS CoV SZ3/2003	A civet	China	AY304486	(Guan <i>et al.</i> , 2003)
77.	SARS coronavirus Urbani icSARS-C3-MA	Human	USA	MK062182	(Graham <i>et al.</i> , 2018)
78.	SARS coronavirus Sin_WNV	Human	Singapore	AH013709	(Lim <i>et al.</i> , 2004)
79.	SARS coronavirus Shanhgai LY	Human	China	AH012999	unpublished
80.	PCoV_GX-P2V	Pangolin	China	MT072864	unpublished
81.	PCoV_GX-P5E	Manis javanica	China	MT040336	unpublished
82.	PCoV_GX-P5L	Manis javanica	China	MT040335	unpublished
83.	PCoV_GX-P1E	Manis javanica	China	MT040334	unpublished
84.	PCoV_GX-P4L	Manis javanica	China	MT040333	unpublished

RESULTS

Whole genome

All 2019-nCoVs cluster in single clade with *bat coronavirus/RaTG13*, other SL-CoVs arranged distinctly (Fig. 1). Identity among 2019-nCoVs is 99.9-100%, 79.4-96.2% compared to bat viruses, 84.1-85.5% to pangolin viruses and 79.4-79.6% to human CoVs. 2019-nCoVs nucleotides is 96.2% matching to *bat coronavirus/RaTG13* (Fig. 2).

M-gene

Phylogeny of *M-gene* sequences arranged nCoVs with *bat coronavirus/RaTG13* and *bat-SL-CoVZXC21* (Fig. 1). *M-gene* is 99.7-100% homologous between 2019-nCoVs, 84.6-95.0% to bat viruses, 89.1-91.0% to Pangolin-CoVs and 84.6-84.9% to human CoVs. nCoVs are closely related to *bat CoV/RaTG13* (95.0%), *bat CoV/SL-CoVZXC21* (93.2%) and *pangolin-CoV/MP789* (91.0%). Furthermore, *pangolin-CoV/MP789* is similar to *bat coronavirus/RaTG13* (91.3%) and *bat-SL-CoVZXC21* (92.2%) (Fig. 2).

N-gene

Evolutionary analysis constructed a tree that placed nCoVs close to *bat coronavirus/RaTG13* and *pangolin-CoV/MP789*, SL-CoVs are grouped in separate branches (Fig. 1). Similarity among nCoVs is 99.9-100%, 88.6-97% to bat viruses, 92.0-94.6% to pangolin-CoVs, 88.7-88.9% to human CoVs. 2019-nCoVs sequences are similar to *bat coronavirus/RaTG13* (97%), *bat-SL-CoVZXC21* (91.5%) and *pangolin-CoV/MP789* (96.3%). Moreover, *bat CoVs/RaTG13* shared 94.8% nucleotides when aligned to *pangolin-CoV/MP789*.

ORF1ab-gene

Alignment of *ORF1ab gene* sequences built a tree that classified nCoVs adjacent to *bat CoVs/RaTG13*, *bat-SL-CoVZXC21* and *bat-SL-CoVZXC45*, other SL-CoVs appeared in two groups (Fig. 1). Analysis showed 99.7-100% nt homology among nCoVs, 83.5-95.5% correlated to bat CoVs, 84.5-89.4% to pangolin-CoVs and 83.5-83.9% to human CoVs. nCoVs is similar to *bat CoV/RaTG13* (95.3%), *bat-SL-CoVZXC45* (92.8%) and *bat-SL-CoVZXC21* (93.1%) (Fig. 2).

ORF3a-gene

Analysis of *ORF3a* nt classified 2019-nCoVs together with *CoVs/RaTG13*, *bat-SL-CoVZXC21* and *bat-SL-CoVZXC45* and *pangolin-CoV/MP789*, other SL-CoVs accumulated distinctly (Fig. 1). nCoVs sequences are identical, 82.2-99.1% related to bat viruses, 27.4-93.3% to pangolin-CoVs and 83.5-84.2% to human CoVs. Sequences are close to *bat coronavirus/RaTG13* (99.1%), *bat-SL-CoVZXC21* (94.4%), *bat-SL-CoVZXC45* (94.0%) and *pangolin-CoV/MP789* (93.3%) (Fig. 2).

S-gene

Phylogeny of *S-gene* showed that nCoVs are close to *CoVs/RaTG13* and *pangolin-CoV/MP789*, other SL-CoVs appeared in scattered branches (Fig. 1). Sequences among nCoVs is identical, 86.0-90.7% to bat viruses, 81.3-87.9% to Pangolin-CoVs, 85.0-86.0% to human CoVs. 2019-nCoVs is similar to *Bat CoVs/RaTG13* (90.7%) (Fig. 2).

DISCUSSION

The study examined nucleotide homology among 2019-nCoVs and SL-CoVs of bat, pangolin and human origin retrieved from GenBank. Sequence Analysis revealed that nCoVs shared intragroup similarity on the whole genome, *M*, *N*, *ORF1ab-genes* and identical on *ORF3a* and *S-genes*. Results are in line with Ren *et al.* (2020), who reported nucleotide identities of 99.8% to 99.9%. Novel viruses are closely related to coronavirus/RaTG13, bat-SL-CoVZXC45 and bat-SL-CoVZXC21 identified from *Rhinolophus affinis* (Hu *et al.*, 2018; Ren *et al.*, 2020). Diversity of *S-gene* among CoVs may due to recombination of viruses within *Rhinolophus sinicus* or with CoVs from other hosts (Wu *et al.*, 2016a). Its anticipated that if the natural host is infected simultaneously with diverse viruses, recombination may yield new progeny variants, the error correction mechanisms may support the selection of recombinant virions (Eckerle *et al.*, 2010; Hanada *et al.*, 2004; Minskaia *et al.*, 2006). Previous reports indicate that all HCoVs may have been acquired from small mammals including bats (Drexler *et al.*, 2014; Vijaykrishna *et al.*, 2007; Woo *et al.*, 2009b). Bats are considered as a natural host because its natural and continuously infected with diverse CoVs outside the habitat of the present community (Drexler *et al.*, 2012; Greger, 2007; Haydon *et al.*, 2002).

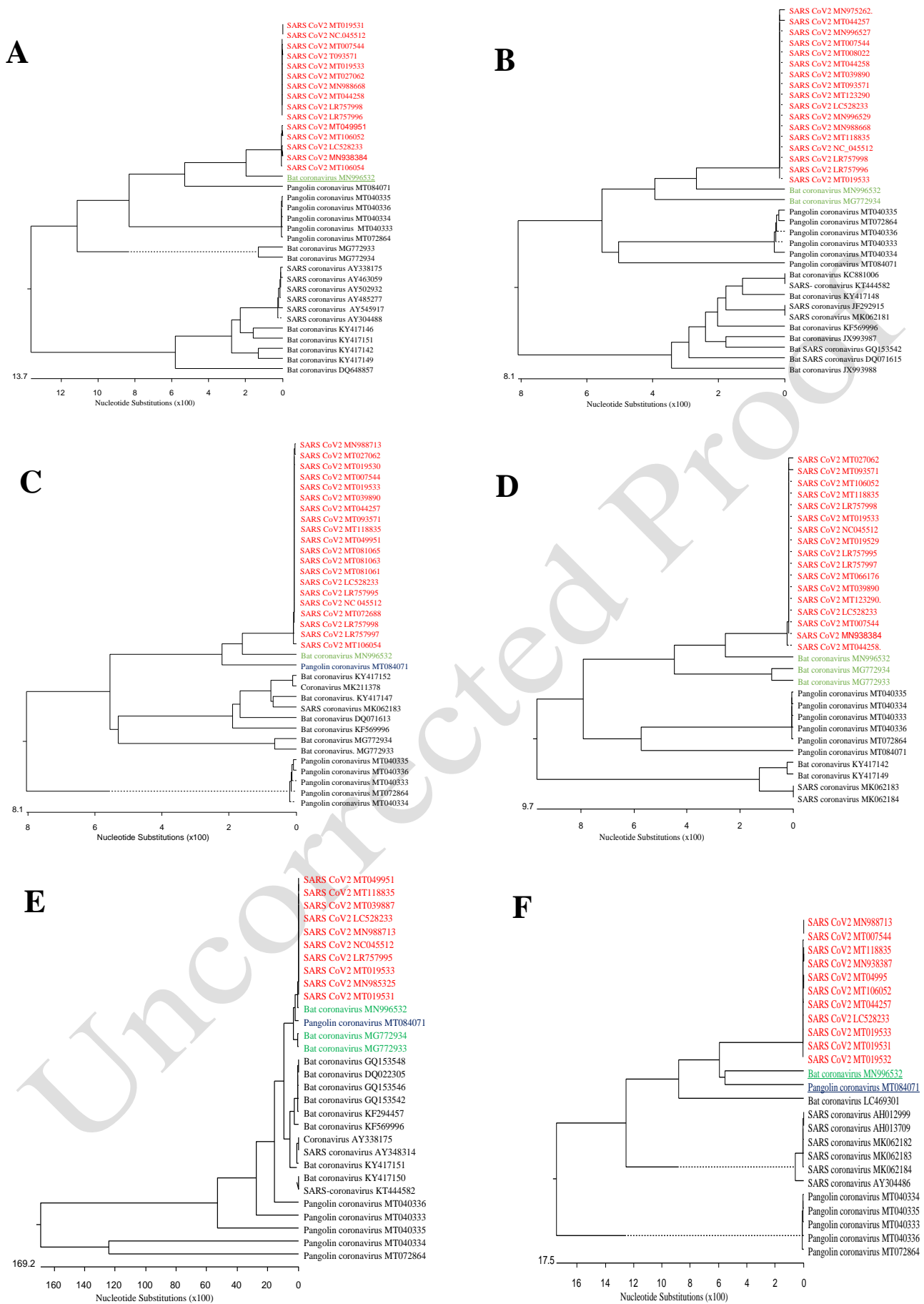


Fig. 1: Phylogeny of CoVs based on the whole genome (A), *M* (B), *N* (C), *ORF1ab* (D) and *orf3a* (E) and *S* gene sequences. Trees were constructed using the Neighbor-Joining method in *Lasergene 7* (Kumar *et al.*, 2016).

As previously published for SARS-CoV and MERS-CoV (Perlman and Netland, 2009; Zhong *et al.*, 2003), the bat may be the source for SARS-CoV-2 since it displays whole-genome similarity with batCoV RaTG13. However, previous coronavirus outbreaks occurred through intermediate hosts, such as civets or camels, before jumping to humans (Corman *et al.*, 2018; Zaki *et al.*, 2012). This may also apply to SARS-CoV-2, since the first COVID-19 infection described no contact at the seafood market (Ren *et al.*, 2020).

Apart from RaTG13, the Pangolin-CoV is the most closely interrelated to SARS-CoV-2. It exhibited 91.02% nucleotide identity with the SARS-CoV-2 genome. Bearing in mind that the earliest COVID-19 patient defined no close contact (Ren *et al.*, 2020), it is vital to discover the intermediate SARS-CoV-2 host to stop interspecies transmission. The present analysis indicates that 2019-nCoVs may have jumped to human from bats, pangolin and/or other unidentified hosts.

Conclusion

The present study showed that novel coronaviruses are similar to *CoV/RaTG13*, *bat-CoVZXC21*, *bat-CoVZC45* and *pangolin-CoV/MP789*, indicating that 2019-nCoVs may have evolved in bats, pangolin and/or other unidentified hosts.

REFERENCES

- Ai JW, Zhang HC, Xu T, *et al.*, 2020. Optimizing diagnostic strategy for novel coronavirus pneumonia, a multi-center study in eastern China. Medrxiv. DOI: <https://doi.org/10.1101/2020.02.13.20022673>.
- Bastola A, Sah R, Rodriguez-Morales AJ, *et al.*, 2020. The first 2019 novel coronavirus case in Nepal. *Lancet Infect Dis*, 20: 279-280.
- Birmingham A, Chand M, Brown C, *et al.*, 2012. Severe respiratory illness caused by a novel coronavirus, in a patient transferred to the United Kingdom from the Middle East. *Eurosurveillance*, 17: 20290.
- Caly L, Druce J, Roberts J, *et al.*, 2019. Isolation and rapid sharing of the 2019 novel coronavirus (SAR-Cov-2) from the first diagnosis of Covid-19 in Australia. *Med J Australia*, 212: 459-462.
- Chan JFW, Yuan S, Kok KH, *et al.*, 2020. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *The Lancet*, 395: 514-523.
- Chen L, Liu W, Zhang Q, Xu K, *et al.*, 2019. RNA based Mngs approach identifies a novel human coronavirus from two individual pneumonia cases in 2019 Wuhan outbreak. *Emerg Microb Infect*, 9: 313-319.
- Corman VM, Muth D, Niemeyer D and Drosten C., 2018. Hosts and sources of endemic human coronaviruses. *Adv Virus Res*, 100: 163-188.
- De Groot R. J., Baker SC, Baric RS, *et al.*, 2013. Commentary: Middle East Respiratory Syndrome Coronavirus (MERS-Cov): announcement of the coronavirus study group. *J Virol*, 87: 7790-7792.
- Drexler JF, Corman VM and Drosten C, 2014. Ecology, evolution and classification of bat coronaviruses in the aftermath of SARS. *Antivir Res*, 101: 45-56.
- Drexler JF, Corman VM, Müller MA, *et al.*, 2012. Bats host major mammalian paramyxoviruses. *Nat Commun*, 3: 1-13.
- Drosten C, Günther S, Preiser W, *et al.*, 2003. Identification of a novel coronavirus in patients with Severe Acute respiratory syndrome. *N Engl J Med*, 348: 1967-1976.
- Eckerle LD, Becker MM, Halpin RA, *et al.*, 2010. Infidelity Of SARS-Cov Nsp14-Exonuclease Mutant Virus Replication Is Revealed By Complete Genome Sequencing. *Plos Pathog*, 6: e1000896.
- Ge XY, Li JL, Yang XL, *et al.*, 2013. Isolation and characterization of a bat SARS-Like coronavirus that uses the ACE2 Receptor. *Nature*, 503: 535-538.
- Graham RL, Deming DJ, Deming ME, *et al.*, 2018. Evaluation of a recombination-resistant coronavirus as a broadly applicable, rapidly implementable vaccine platform. *Commun Biol*, 1: 1-10.
- Greger, M, 2007. The human/animal interface: emergence and resurgence of zoonotic infectious diseases. *Crit Rev Microbiol Crit*, 33: 243-299.
- Guan Y, Zheng B, He Y, *et al.*, 2003. Isolation and characterization of viruses related to the SARS coronavirus from animals in Southern China. *Science*, 302: 276-278.
- Hamre D and Procknow JJ, 1966. A new virus isolated from the human respiratory tract. *Proceedings Of The Society For Experimental Biology And Medicine*, 121: 190-193.
- Han Y, Du J, Su H, *et al.*, 2019. Identification of diverse bat alphacoronaviruses and betacoronaviruses in China provides new insights into the evolution and origin of coronavirus-related diseases. *Front Microbiol*, 10: 1900.
- Hanada K, Suzuki Y and Gojobori T, 2004. A large variation in the rates of synonymous substitution for RNA viruses and its relationship to a diversity of viral infection and transmission modes. *Mol Biol Evol*, 21: 1074-1080.
- Harcourt J, Tamin A, Lu X, *et al.*, 2020. Severe acute Respiratory Syndrome Coronavirus 2 from patient with 2019 novel coronavirus disease, United States. *Emerg Infect Dis*, 26: 1266.
- Haydon DT, Cleavel S, Taylor LH and Laurenson MK, 2002. Identifying reservoirs of infection: a Conceptual and practical challenge. *Emerg Infect Dis*, 8: 1468-1473.
- He B, Zhang Y, Xu L, *et al.*, 2014. Identification of diverse alphacoronaviruses and genomic characterization of a novel Severe Acute Respiratory Syndrome-Like Coronavirus from bats in China. *J Virol*, 88: 7070-7082.
- Hu B, Zeng LP, Yang XL, *et al.*, 2017. Discovery of a rich gene pool of bat SARS-Related Coronaviruses provides new insights into the origin of SARS Coronavirus. *Plos Pathog*, 13:e1006698.
- Hu D, Zhu C, Ai L, *et al.*, 2018. Genomic characterization and infectivity of a novel SARS-Like Coronavirus in Chinese bats. *Emerg Microbes. Infect*, 7: 1-10.
- Kim JY, Choe PG, Oh Y, *et al.*, 2020. The first case of 2019 novel Coronavirus pneumonia imported into Korea from Wuhan, China: implication for infection prevention and control measures. *J Korean Med Sci*, 35: e61.
- Ksiazek TG, Erdman D, Goldsmith C S, *et al.*, 2003. A novel Coronavirus associated with Severe Acute Respiratory Syndrome. *N Engl J Med*, 348: 1953-1966.
- Lai MM, And Cavanagh D, 1997. The molecular biology of Coronaviruses. *Adv Virus Res*, Elsevier, 48: 1-100.
- Lau SK, Li KS, Huang Y, *et al.*, 2010. Ecoepidemiology and complete genome comparison of different strains of Severe Acute Respiratory Syndrome-Related Rhinolophus Bat Coronavirus in China reveal bats as a reservoir for acute, self-limiting infection that allows recombination events. *J Virol*, 84: 2808-2819.
- Lau SK, Woo PC, Li KS, *et al.*, 2005. Severe Acute Respiratory Syndrome Coronavirus-Like Virus in Chinese horseshoe bats. *Proceedings Of The National Academy of Sciences*, 102: 14040-14045.
- Li W, Shi Z, Yu M, *et al.*, 2005. Bats are natural reservoirs of SARS-Like Coronaviruses. *Science*, 310: 676-679.
- Lim PL, Kurup A, Gopalakrishna G, *et al.*, 2004. Laboratory-acquired Severe Acute Respiratory Syndrome. *N Engl J Med*, 350: 1740-1745.

- Lin XD, Wang W, Hao ZY, *et al.*, 2017. Extensive diversity of Coronaviruses in bats from China. *Virology*, 507: 1-10.
- Liu P, Jiang JZ, Hua Y, *et al.*, 2020. Are Pangolins the intermediate host of the 2019 novel Coronavirus (2019-Ncov). *Biorxiv*. DOI: <https://doi.org/10.1101/2020.02.18.954628>.
- Mcintosh K, Dees JH, Becker WB, *et al.*, 1967. Recovery in tracheal organ cultures of novel viruses from patients with respiratory disease. *PNAS*, 57: 933.
- Minskaia E, Hertzog T, Gorbalenya A E, *et al.*, 2006. Discovery of an RNA virus 3'→ 5' exoribonuclease that is critically involved in Coronavirus RNA synthesis. *PNAS*, 103: 5108-5113.
- Peiris J, Lai S, Poon L, *et al.*, 2003. Coronavirus as a possible cause of Severe Acute Respiratory Syndrome. *Lancet*, 361: 1319-1325.
- Perlman S, and Netland J. 2009. Coronaviruses post-SARS: update on replication and pathogenesis. *Nat. Rev. Microbiol*, 7: 439-450.
- Ren LL, Wang YM, Wu ZQ, *et al.*, 2020. Identification of a novel Coronavirus causing severe pneumonia in human: a descriptive study. *Chin Med J*, 133: 1015-1024.
- Robertson MP, Igel H, Baertsch R, *et al.*, 2004. The structure of a rigorously conserved RNA element within the SARS virus genome. *Plos Biol*, 3: E5.
- Saitou N and Nei M. 1987. The Neighbor-Joining Method: A new method for reconstructing phylogenetic trees. *Mol Biol Evol*, 4: 406-425.
- Shih MC, Peck K, Chan WL, *et al.*, 2005. SARS-Cov infection was from at least two origins in the Taiwan area. *Intervirology*, 48: 124-132.
- Tang X, Zhang J, Zhang S, *et al.*, 2006. Prevalence and genetic diversity of Coronaviruses in bats from China. *J Virol*, 80: 7481-7490.
- Tijssen P, Agbandje-Mckenna M, Almendral J, *et al.*, 2011. Virus Taxonomy 9th Report Of The International Committee On Taxonomy Of Viruses.
- Van Der Hoek L, Pyrc K, Jebbink MF, *et al.*, 2004. Identification of a new human Coronavirus. *Nat. Med*, 10: 368-373.
- Vijaykrishna D, Smith GJ, Zhang JX, *et al.*, 2007. Evolutionary insights into the ecology of Coronaviruses. *J Virol*, 81: 4012-4020.
- Vijgen L, Keyaerts E, Lemey P, *et al.*, 2006. Evolutionary history of the closely related group 2 Coronaviruses: Porcine hemagglutinating encephalomyelitis virus, bovine Coronavirus, and human Coronavirus OC43. *J Virol*, 80: 7270-7274.
- Vijgen L, Keyaerts E, Moës E, *et al.*, 2005. Complete genomic sequence of human Coronavirus OC43: molecular clock analysis suggests a relatively recent zoonotic Coronavirus transmission event. *J Virol*, 79: 1595-1604.
- Woo P, Lau S, and Yuen K. 2009a. Clinical features and molecular epidemiology of Coronavirus-HKU1-associated community-acquired pneumonia. *Hong Kong Med J*, 15: 46-47.
- Woo PC, Lau SK, Chu CM, *et al.*, 2005. Characterization and complete genome sequence of a novel Coronavirus, Coronavirus HKU1, From Patients With Pneumonia. *J Virol*, 79: 884-895.
- Woo PC, Lau SK, Huang Y, *et al.*, 2009b. Coronavirus diversity, phylogeny and interspecies jumping. *Exp Biol Med*, 234: 1117-1127.
- Wu Z, Yang L, Ren X, *et al.*, 2016a. Deciphering the bat virome catalog to better understand the ecological diversity of bat viruses and the bat origin of emerging infectious diseases. *ISME J*, 10: 609-620.
- Wu Z, Yang L, Ren X, *et al.*, 2016b. ORF8-Related genetic evidence for Chinese horseshoe bats as the source of Human Severe Acute Respiratory Syndrome Coronavirus. *J Infect Dis*, 213: 579-583.
- Yang XL, Hu B, Wang B, *et al.*, 2016. Isolation and characterization of a novel bat Coronavirus closely related to the direct progenitor of Severe Acute Respiratory Syndrome Coronavirus. *J Virol*, 90: 3253-3256.
- Yeh SH, Wang HY, Tsai CY, *et al.*, 2004. Characterization of Severe Acute Respiratory Syndrome Coronavirus genomes in Taiwan: Molecular epidemiology and genome evolution. *PNAS*, 101: 2542-2547.
- Zaki A M, Van Boheemen S, Bestebroer TM, *et al.*, 2012. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. *N Engl J Med*, 367: 1814-1820.
- Zhang J, Liu Y, Hu L, *et al.*, 2005. Preparation and characterization of SARS in-house reference antiserum. *Vaccine*, 23: 5666-5669.
- Zhong N, Zheng B, Li Y, *et al.*, 2003. Epidemiology and cause of Severe Acute Respiratory Syndrome (SARS) in Guangdong, people's Republic Of China. *Lancet*, 362: 1353-1358.
- Zhou P, Yang XL, Wang XG, *et al.*, 2020. A pneumonia outbreak associated with a new Coronavirus of probable bat origin. *Nature*, 579: 270-273.