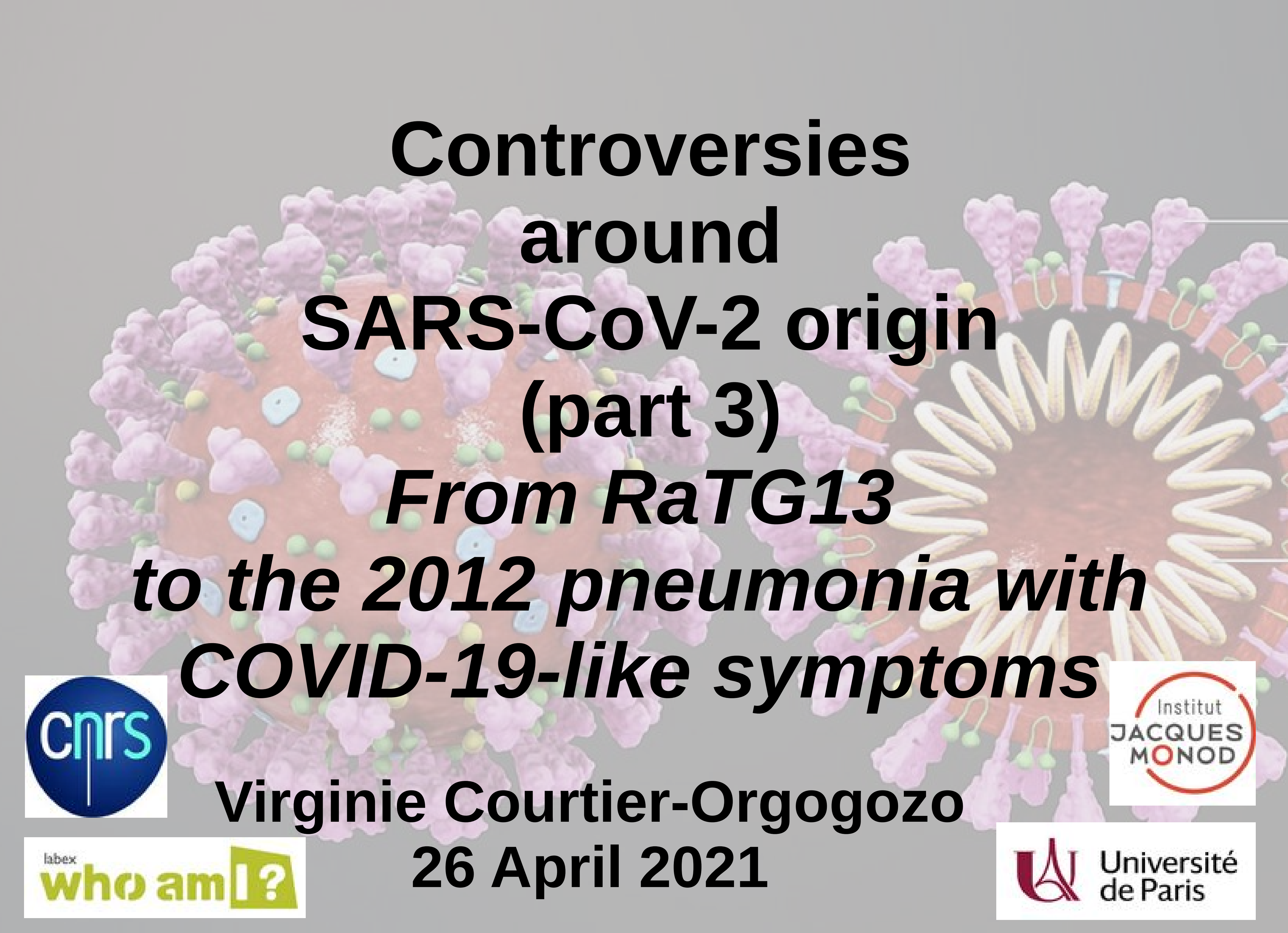


Controversies around SARS-CoV-2 origin

(part 3)

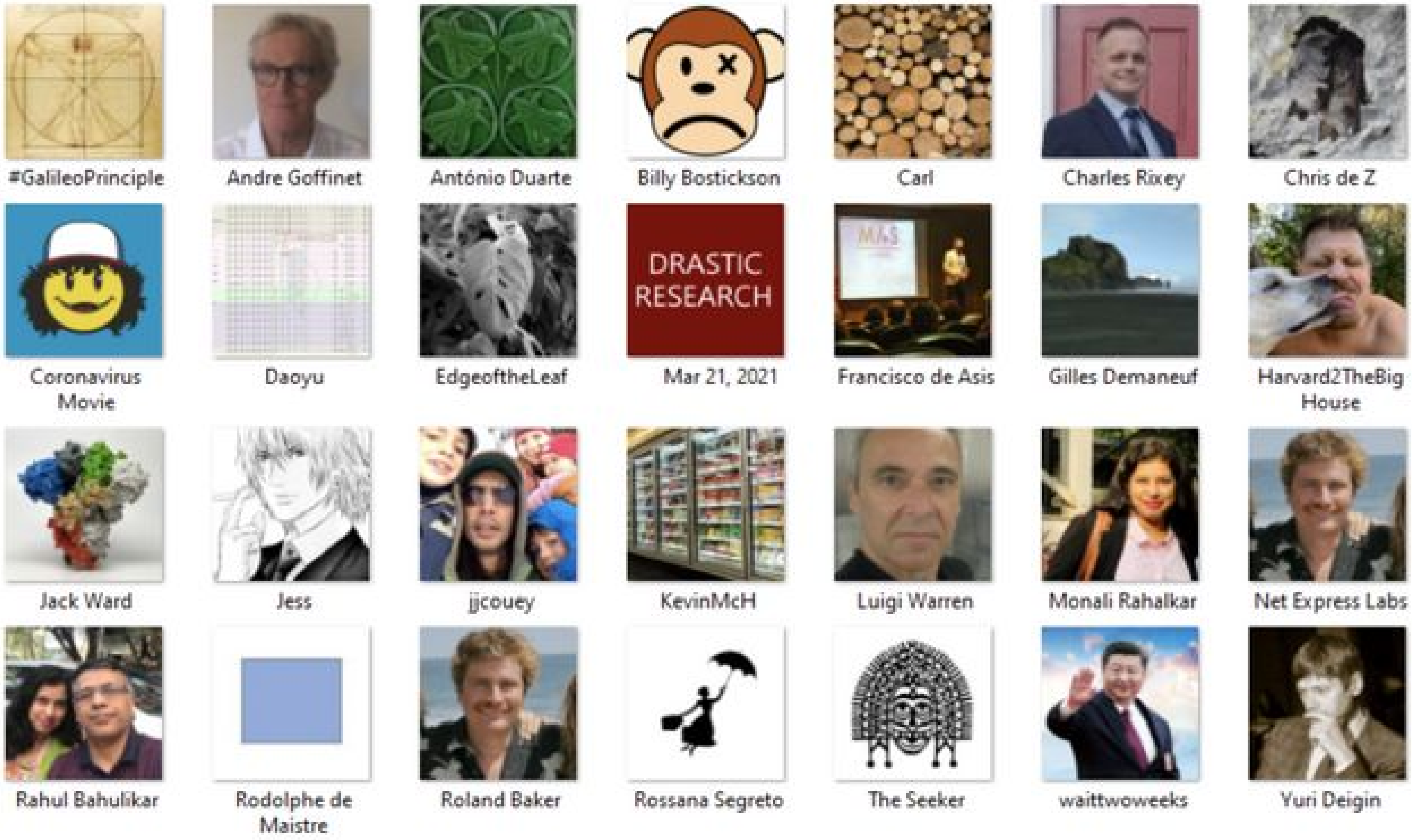
From RaTG13 to the 2012 pneumonia with COVID-19-like symptoms

Virginie Courtier-Orgogozo

26 April 2021

Thanks

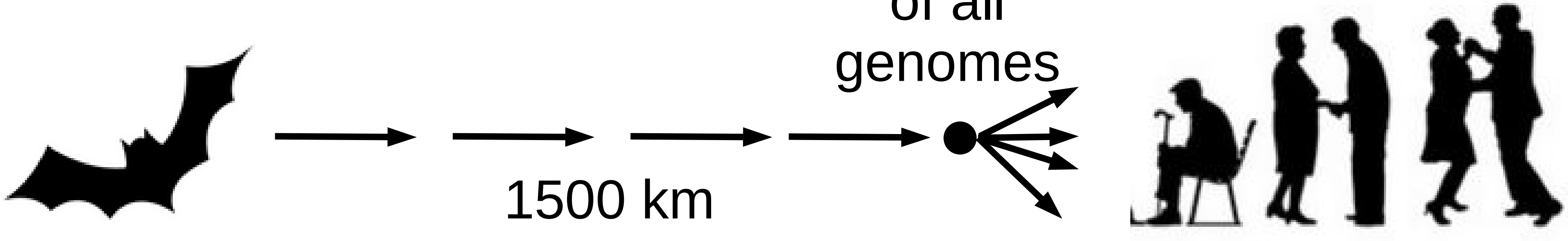
Monali
Francisco
Seeker



D.R.A.S.T.I.C. 2020: ORIGINS

(Decentralized Radical Autonomous Search Team Investigating COVID-19)

https://drasticresearch.org/the-team/



Bats

20-30 years
ago

South
of China

1500 km

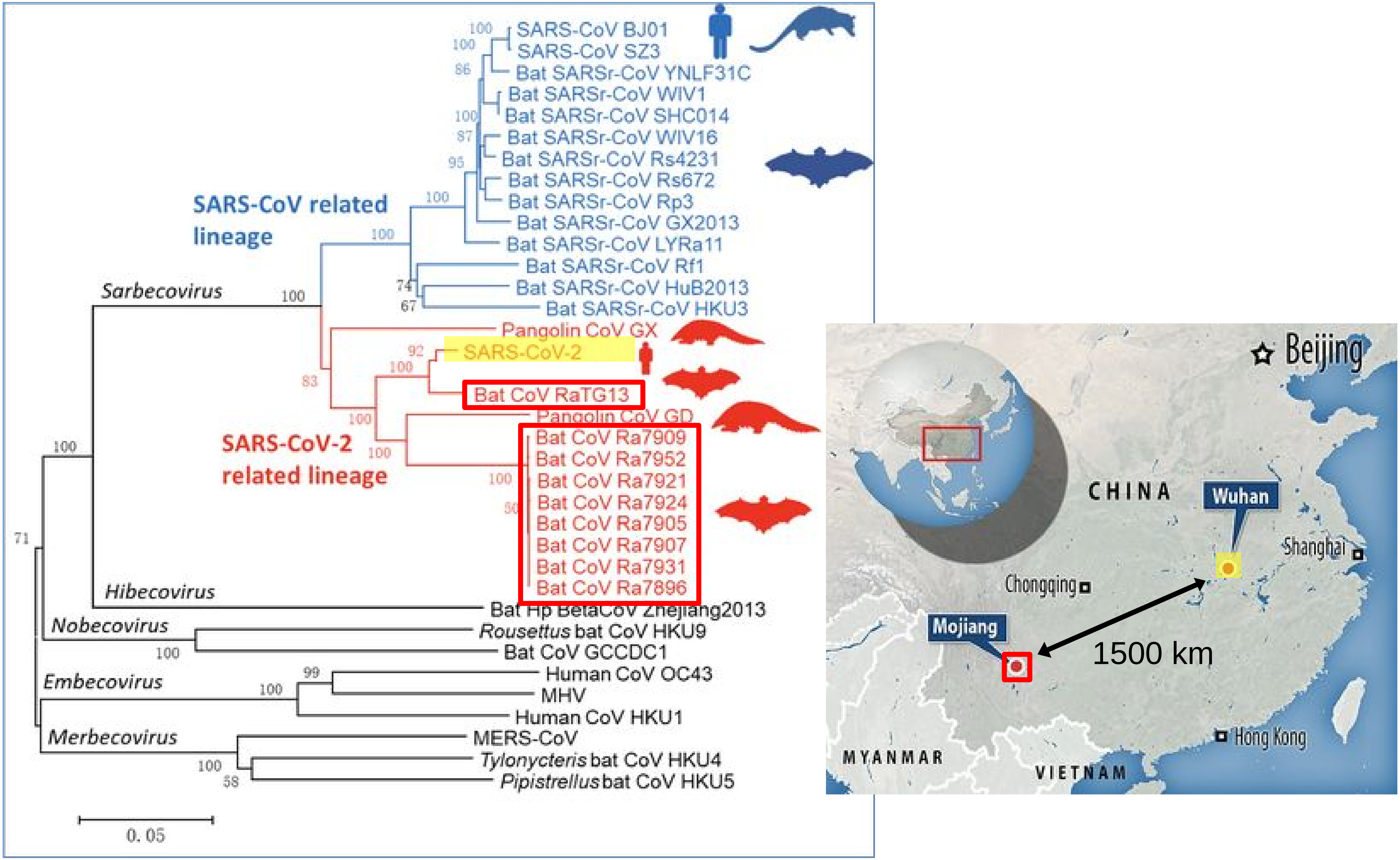
Mother
of all
genomes

Oct-Nov
2019

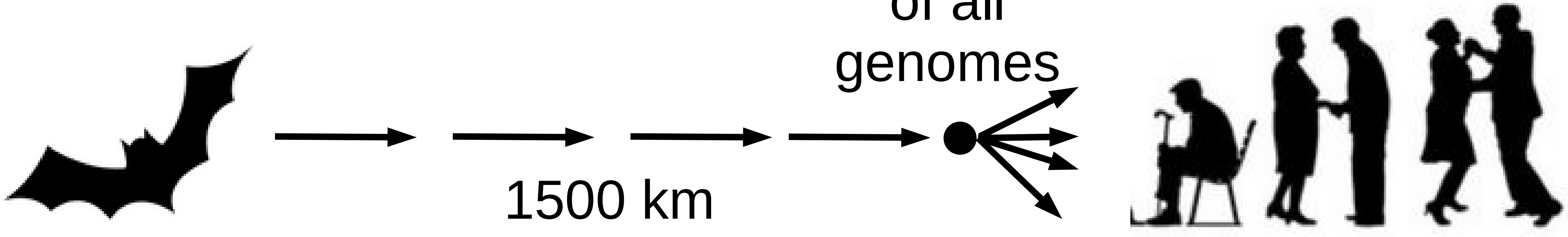
Wuhan

Outbreak in Wuhan

December 2019



Adapted from Zheng-Li Shi



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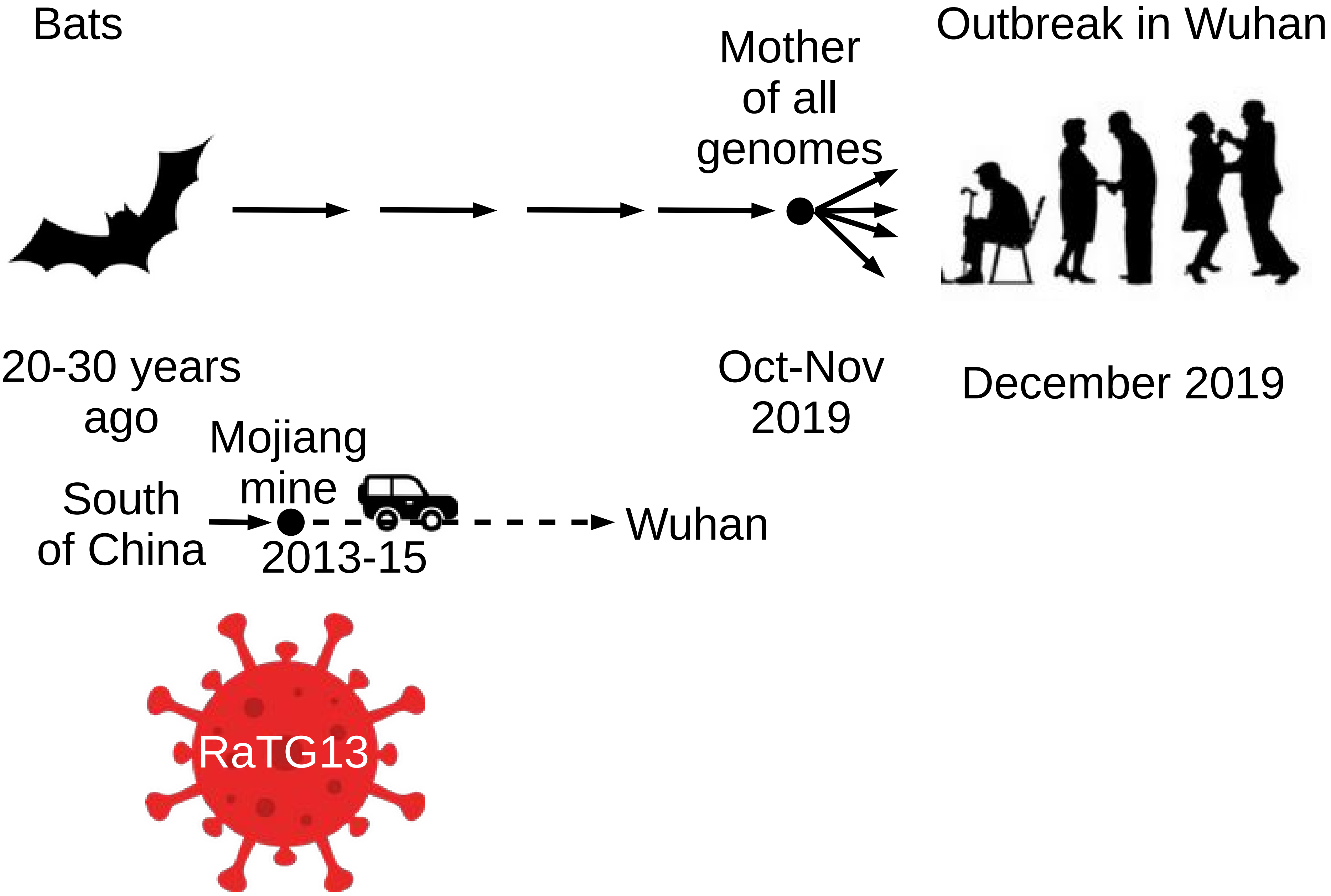


Bats

Mother
of all
genomes

Outbreak in Wuhan

20-30 years
ago

Oct-Nov
2019

December 2019

Mojiang
mine

South
of China

2013-15

Wuhan

RaTG13

Article

A new coronavirus associated with human respiratory disease in China

5,220 citations

https://doi.org/10.1038/s41586-020-2008-3

Received: 7 January 2020

Accepted: 28 January 2020

Fan Wu[1,7], Su Zhao[2,7], Bin Yu[3,7], Yan-Mei Chen[1,7], Wen Wang[4,7], Zhi-Gang S
Zhao-Wu Tao[2], Jun-Hua Tian[3], Yuan-Yuan Pei[1], Ming-Li Yuan[2], Yu-Ling Zh
Yi Liu[1], Qi-Min Wang[1], Jiao-Jiao Zheng[1], Lin Xu[1], Edward C. Holmes[1,5] & Yo

Emerging infectious diseases, such as severe acute respiratory syr

Article

A pneumonia outbreak associated with a new coronavirus of probable bat origin

10,840 citations

https://doi.org/10.1038/s41586-020-2012-7

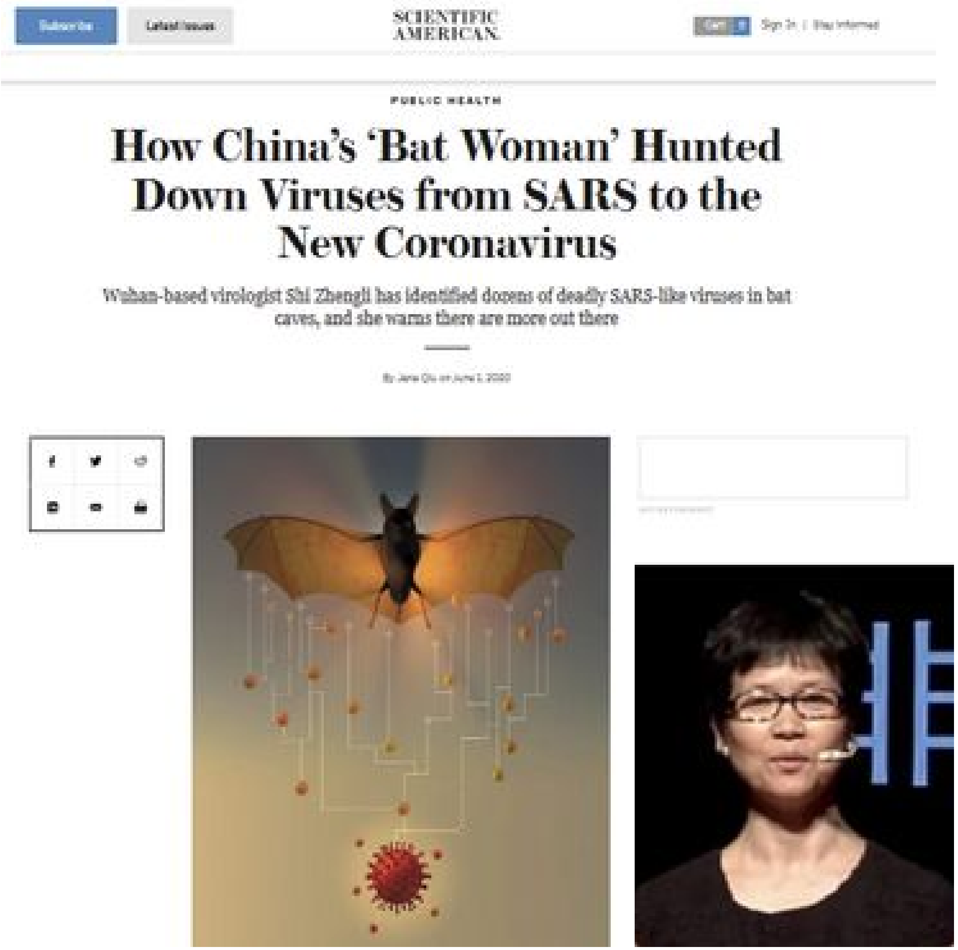
Received: 20 January 2020

Accepted: 29 January 2020

Peng Zhou[1,5], Xing-Lou Yang[1,5], Xian-Guang Wang[2,5], Ben Hu[1], Lei Zhang
Hao-Rui Si[1,3], Yan Zhu[1], Bei Li[1], Chao-Lin Huang[2], Hui-Dong Chen[2], Jing C
Hua Guo[1,3], Ren-Di Jiang[1,3], Mei-Qin Liu[1,3], Ying Chen[1,3], Xu-Rui Shen[1,3], Xi
Xiao-Shuang Zheng[1,3], Kai Zhao[1,3], Quan-Jiao Chen[1], Fei Deng[1], Lin-Lin Li
Fa-Xian Zhan[4], Yan-Yi Wang[1], Geng-Fu Xiao[1] & Zheng-Li Shi[1]✉

First thought: check lab data

“She frantically went through her own lab’s records from the past few years to check for any mishandling of experimental materials, especially during disposal. Shi breathed a sigh of relief when the results came back: **none of the sequences matched those of the viruses her team had sampled from bat caves**. “That really took a load off my mind,” she says. “I had not slept a wink for days.”



PUBLIC HEALTH

How China’s ‘Bat Woman’ Hunted Down Viruses from SARS to the New Coronavirus

Wuhan-based virologist Shi Zhengli has identified dozens of deadly SARS-like viruses in bat caves, and she warns there are more out there

By Jane Qiu on June 1, 2020

https://www.scientificamerican.com/article/how-chinas-bat-woman-hunted-down-viruses-from-sars-to-the-new-coronavirus1/

First thought: check lab data

The obvious question that is being asked is: is it really just a coincidence that an outbreak of a bat coronavirus could happen in the city of Wuhan, home to the world's leading bat coronavirus research laboratories?

"That's why we lost sleep the first few nights, but it is a very, very low chance," Linfa Wang said. For one thing, the SARS-CoV-2 sequence was not found in the records of the Wuhan Institute of Virology.

"We are scientists, we are professionals. How could I have a live virus in my freezer and not know about it?" he asked.

"Zero chance," he added.

Linfa Wang
Duke University
Singapore

https://www.rte.ie/news/primetime/2021/0318/1204794-covid-19-origins-china-wuhan-bats-lab-leak-frozen-food/

20 January, short commentary: no RaTG13

The phylogenetic analysis revealed that the gene sequence of 2016-nCoV is 89% identical to that of bat SARS-like coronavirus ZXC21 (bat-SL-CoVZXC21, accession no. MG772934.1) and ZC45 (MG772933.1), and 82% identical to that of SARS-CoV Tor2 (JX163927), suggesting that 2019-nCoV also belongs to betacoronavirus Lineage B, but has closer homology to bat-SL-CoVZC45 and bat-SL-CoVZXC21 than SARS-CoV [2]

Commentary

An emerging coronavirus causing pneumonia outbreak in Wuhan, China: calling for developing therapeutic and prophylactic strategies

Shibo Jiang, Lanying Du & Zhengli Shi

Pages 275-277 | Received 20 Jan 2020, Accepted 21 Jan 2020, Published online: 31 Jan 2020

https://doi.org/10.1080/22221751.2020.1723441

https://www.tandfonline.com/doi/full/10.1080/22221751.2020.1723441

20 January: paper submitted to Nature

23 January: preprint in bioRxiv

We then found a short RdRp region from a bat coronavirus termed BatCoV **RaTG13** which we previously detected in *Rhinolophus affinis* from Yunnan Province showed high sequence identity to nCoV-2019.

We did full-length sequencing to this RNA sample. Simplot analysis showed that nCoV-2019 was highly similar throughout the genome to RaTG13 (Fig. 1c), with 96.2% overall genome sequence identity. The phylogenetic analysis also showed that RaTG13 is the closest relative of the nCoV-2019 and form a distinct lineage from other SARSr-CoVs (Fig. 1d).

bioRxiv is receiving many new papers on coronavirus SARS-CoV-2. A reminder: these are preliminary reports that have not be
practice/health-related behavior, or be reported in news media as established information.

New Results

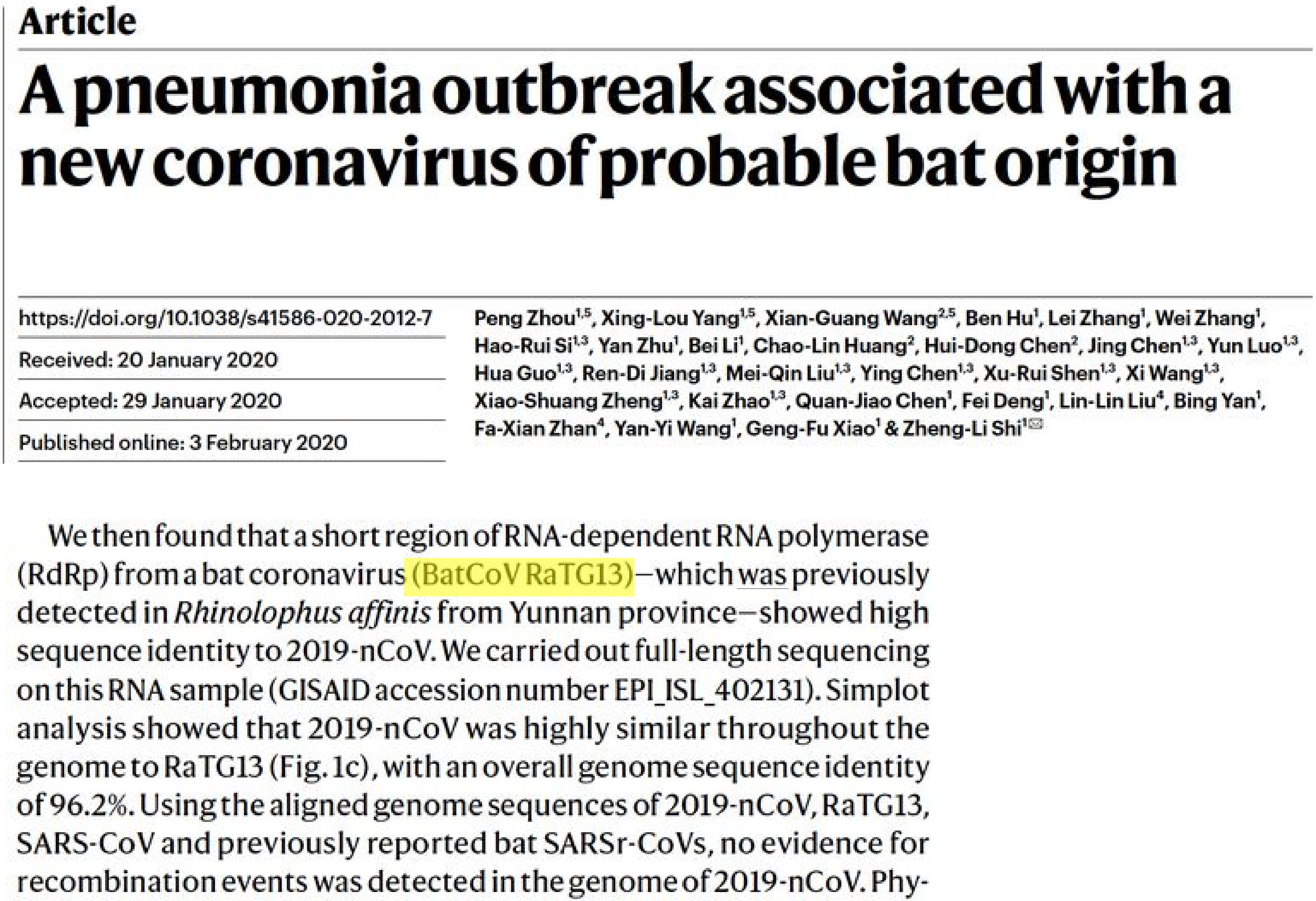
Discovery of a novel coronavirus associated with the recent pneumonia outbreak in humans and its potential bat origin

Peng Zhou, Xing-Lou Yang, Xian-Guang Wang, Ben Hu, Lei Zhang, Wei Zhang, Hao-Rui Si, Yan Zhu, Bei Li, Chao-Lin Huang, Hui-Dong Chen, Jing Chen, Yun Luo, Hua Guo, Ren-Di Jiang, Mei-Qin Liu, Ying Chen, Xu-Rui Shen, Xi Wang, Xiao-Shuang Zheng, Kai Zhao, Quan-Jiao Chen, Fei Deng, Lin-Lin Liu, Bing Yan, Fa-Xian Zhan, Yan-Yi Wang, Geng-Fu Xiao, Zheng-Li Shi

doi: https://doi.org/10.1101/2020.01.22.914952

Now published in *Nature* doi: 10.1038/s41586-020-2012-7

https://www.biorxiv.org/content/10.1101/2020.01.22.914952v1.article-info



Article

A pneumonia outbreak associated with a new coronavirus of probable bat origin

https://doi.org/10.1038/s41586-020-2012-7

Received: 20 January 2020

Accepted: 29 January 2020

Published online: 3 February 2020

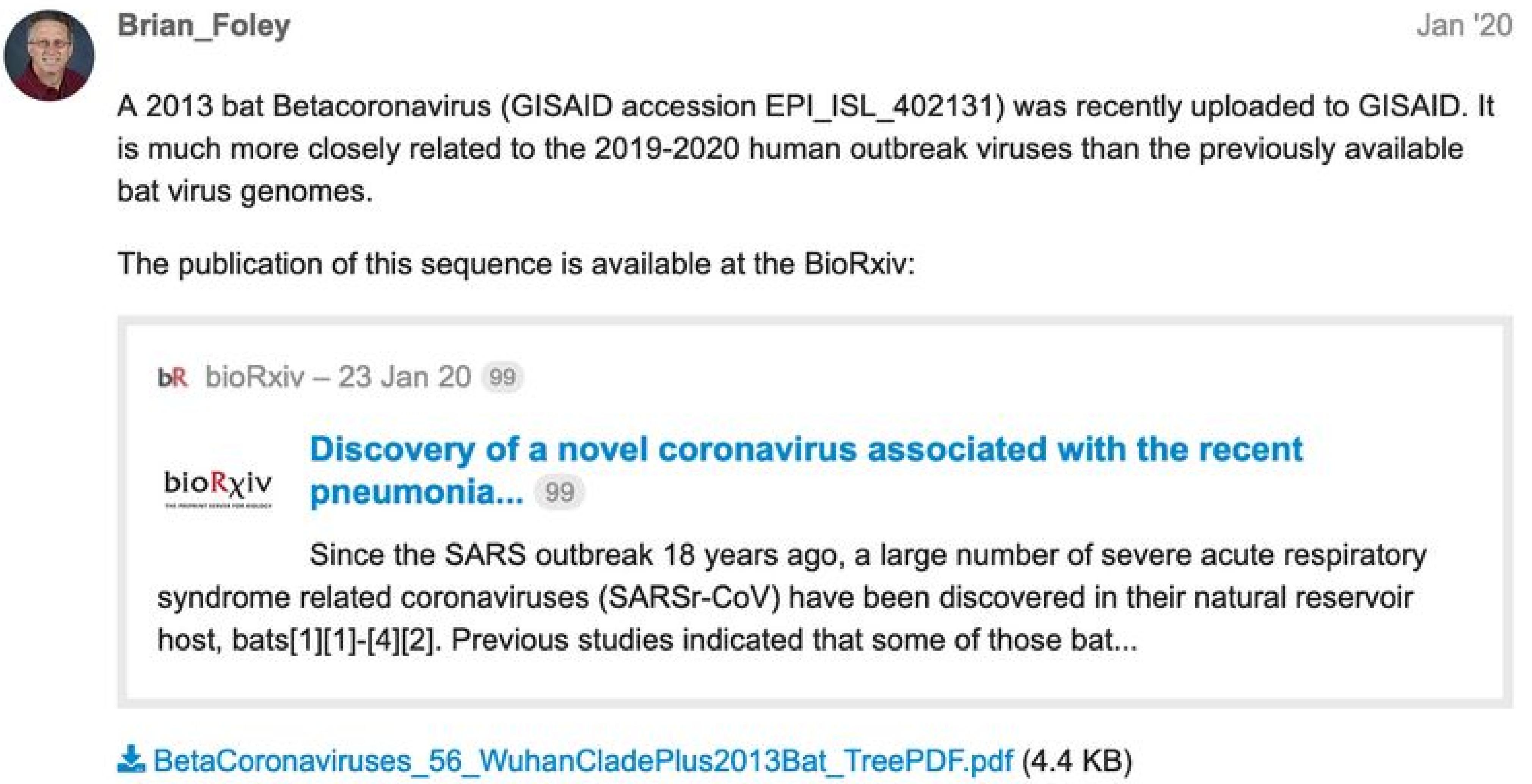
Peng Zhou[1,5], Xing-Lou Yang[1,5], Xian-Guang Wang[2,5], Ben Hu[1], Lei Zhang[1], Wei Zhang[1], Hao-Rui Si[1,3], Yan Zhu[1], Bei Li[1], Chao-Lin Huang[2], Hui-Dong Chen[2], Jing Chen[1,3], Yun Luo[1,3], Hua Guo[1,3], Ren-Di Jiang[1,3], Mei-Qin Liu[1,3], Ying Chen[1,3], Xu-Rui Shen[1,3], Xi Wang[1,3], Xiao-Shuang Zheng[1,3], Kai Zhao[1,3], Quan-Jiao Chen[1], Fei Deng[1], Lin-Lin Liu[4], Bing Yan[1], Fa-Xian Zhan[4], Yan-Yi Wang[1], Geng-Fu Xiao[1] & Zheng-Li Shi[1]✉

We then found that a short region of RNA-dependent RNA polymerase (RdRp) from a bat coronavirus (BatCoV RaTG13)—which was previously detected in *Rhinolophus affinis* from Yunnan province—showed high sequence identity to 2019-nCoV. We carried out full-length sequencing on this RNA sample (GISAID accession number EPI_ISL_402131). Simplot analysis showed that 2019-nCoV was highly similar throughout the genome to RaTG13 (Fig. 1c), with an overall genome sequence identity of 96.2%. Using the aligned genome sequences of 2019-nCoV, RaTG13, SARS-CoV and previously reported bat SARSr-CoVs, no evidence for recombination events was detected in the genome of 2019-nCoV. Phy-

https://www.nature.com/articles/s41586-020-2012-7

24 January:
RaTG13 sequence submitted to GISAID

29 January:



Brian_Foley Jan '20

A 2013 bat Betacoronavirus (GISAID accession EPI_ISL_402131) was recently uploaded to GISAID. It is much more closely related to the 2019-2020 human outbreak viruses than the previously available bat virus genomes.

The publication of this sequence is available at the BioRxiv:

bR bioRxiv – 23 Jan 20 99

bioRxiv

Discovery of a novel coronavirus associated with the recent pneumonia... 99

Since the SARS outbreak 18 years ago, a large number of severe acute respiratory syndrome related coronaviruses (SARSr-CoV) have been discovered in their natural reservoir host, bats[1][1]-[4][2]. Previous studies indicated that some of those bat...

BetaCoronaviruses_56_WuhanCladePlus2013Bat_TreePDF.pdf (4.4 KB)

https://virological.org/t/novel-2019-coronavirus-genome/319/26

When was RaTG13 sequenced?

Where does RaTG13 come from?

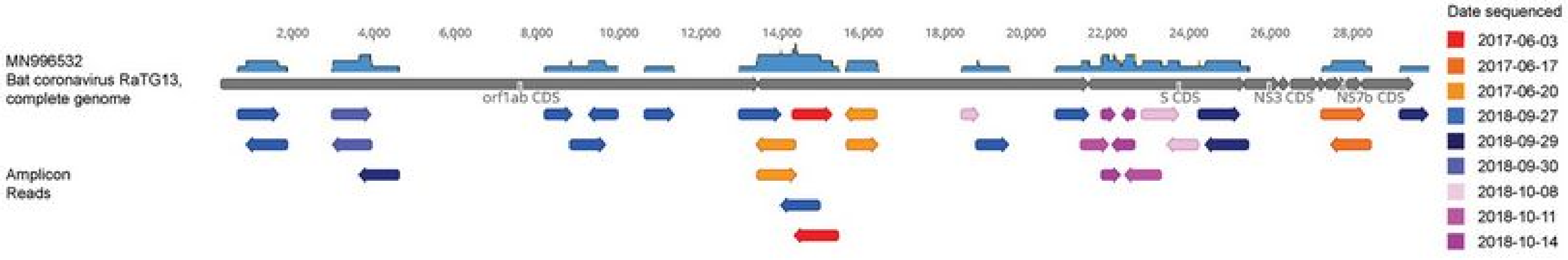
RaTG13 sequence data appeared sequentially

24 January 2020: full sequence submitted to GISAID (EPI_ISL_402131)

27 January: full sequence submitted to NCBI GenBank (MN996532)

13 February: Illumina reads become available at NCBI GenBank (SRX7724752)

19 May: raw amplicons become available at NCBI GeneBank (SRR11806578)



https://twitter.com/Ayjchan/status/1279761424919732224

https://www.ncbi.nlm.nih.gov/sra/SRX8357956

EPI_ISL_402131
additional location information: Pu’er City (not known when this information was added)
Illumina HiSeq 3000
CLC Genomics Workbench v12.0

Raw reads
1 ILLUMINA (Illumina HiSeq 3000) run: 11.6M spots, 3.3G bases, 1.7Gb downloads
Accession: SRX7724752 (published 2020-02-13),
SRP249482, PRJNA606165
RNA-Seq of Rhinolophus affinis:Fecal swab

Error during sequence submission

Total RNA was extracted from bronchoalveolar lavage fluid using the QIAamp Viral RNA Mini Kit following the manufacturers instructions. An RNA library was then constructed using the TruSeq Stranded mRNA Library Preparation Kit (Illumina, USA). Paired-end (150 bp) sequencing of the RNA library was performed on the HiSeq 3000 platform (Illumina).

`Sars_SL3_R1_171127.fastq.gz`
`Sars_SL3_R2_171127.fastq.gz`

sequenced in Nov 2017?

https://www.ncbi.nlm.nih.gov/sra/?term=SRX7724752
https://trace.ncbi.nlm.nih.gov/Traces/sra/?run=SRR11085797

https://www.ncbi.nlm.nih.gov//bioproject/PRJNA606165

When was RaTG13 full genome sequenced?

Its scientists “went back to that sample in 2020, in early January or maybe even at the end of last year, I don’t know." (The Times, 4 July 2020)

http://archive.is/7TCsc

“We found the closest relative to the current SARS-CoV-2 in a bat in China in 2013. We sequenced a bit of the genome, and then it went in the freezer; because it didn’t look like SARS, we thought it was at a lower risk of emerging. With the Global Virome Project, we could have sequenced the whole genome, discovered that it binds to human cells and upgraded the risk. And maybe then when we were designing vaccines for SARS, those could have targeted this one too, and we would have had something in the freezer ready to go if it emerged.” (9 May 2020)

https://www.theaustralian.com.au/weekend-australian-magazine/coronavirus-how-to-beat-the-next-pandemic/news-story/77989c4bfcb4fc7a120533e159dd35ff

Peter Daszak
EcoHealth Alliance
New-York City

16 March: 100% match with a 370-bp fragment submitted in 2016: BtCov/4991

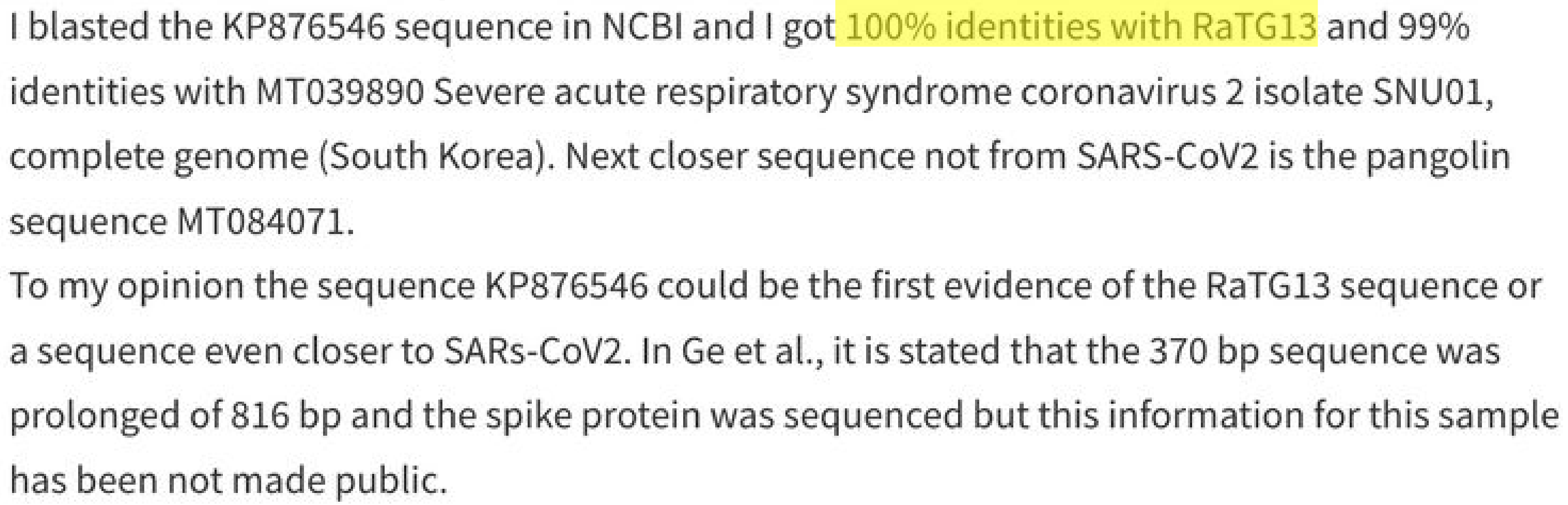

Rossana Segreto

@Rossana38510044 Vous suit

virology blog

About viruses and viral disease

=BtCov/4991

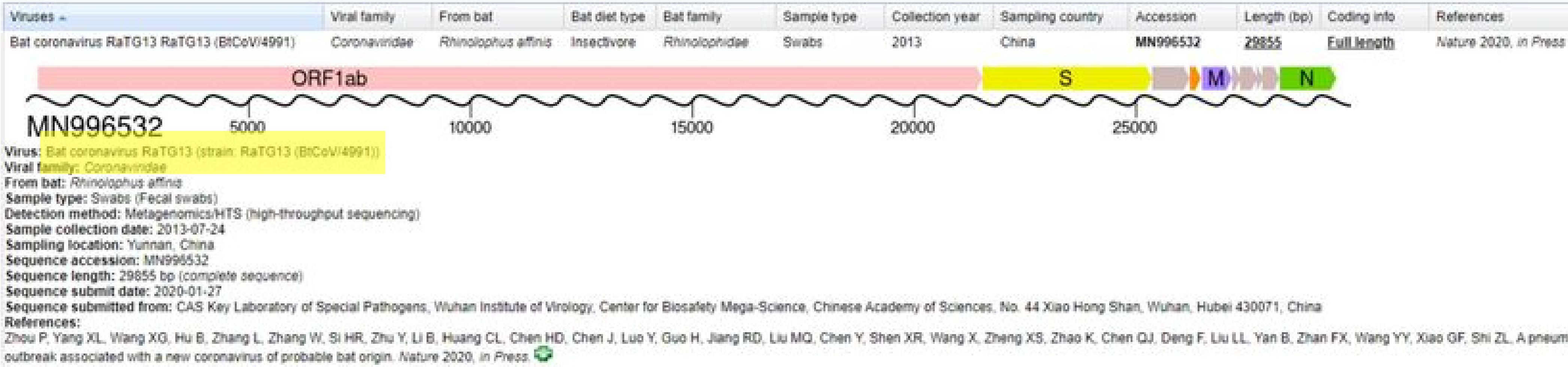

I blasted the KP876546 sequence in NCBI and I got 100% identities with RaTG13 and 99% identities with MT039890 Severe acute respiratory syndrome coronavirus 2 isolate SNU01, complete genome (South Korea). Next closer sequence not from SARS-CoV2 is the pangolin sequence MT084071.

To my opinion the sequence KP876546 could be the first evidence of the RaTG13 sequence or a sequence even closer to SARs-CoV2. In Ge et al., it is stated that the 370 bp sequence was prolonged of 816 bp and the spike protein was sequenced but this information for this sample has been not made public.

https://www.virology.ws/2020/02/20/pangolins-and-the-origin-of-sars-cov-2-coronavirus/

21 April: preprint
RaTG13 and BtCov/4991 in same entry

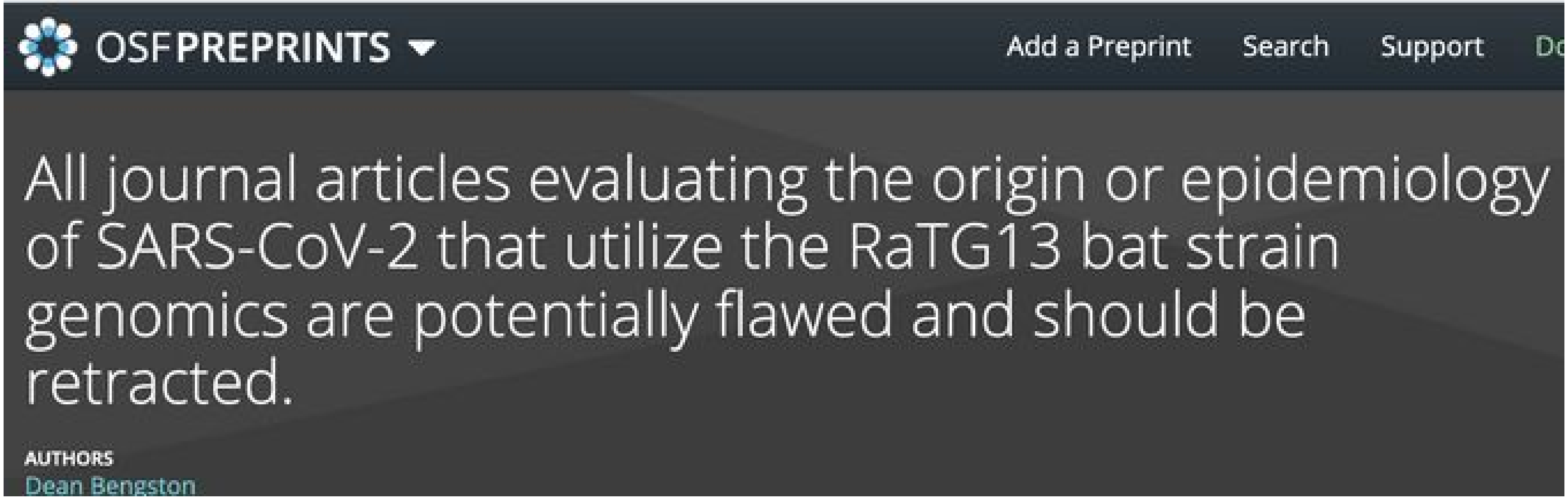
www.mgc.ac.cn/DBatVir database updated on 17 March 2020
to include both RaTG13 and 4991



Viruses	Viral family	From bat	Bat diet type	Bat family	Sample type	Collection year	Sampling country	Accession	Length (bp)	Coding info	References
Bat coronavirus RaTG13 RaTG13 (BtCoV/4991)	*Coronaviridae*	*Rhinolophus affinis*	Insectivore	*Rhinolophidae*	Swabs	2013	China	**MN996532**	29855	Full length	*Nature* 2020, *in Press*

Virus: Bat coronavirus RaTG13 (strain: RaTG13 (BtCoV/4991))
Viral family: Coronaviridae
From bat: *Rhinolophus affinis*
Sample type: Swabs (Fecal swabs)
Detection method: Metagenomics/HTS (high-throughput sequencing)
Sample collection date: 2013-07-24
Sampling location: Yunnan, China
Sequence accession: MN996532
Sequence length: 29855 bp (complete sequence)
Sequence submit date: 2020-01-27
Sequence submitted from: CAS Key Laboratory of Special Pathogens, Wuhan Institute of Virology, Center for Biosafety Mega-Science, Chinese Academy of Sciences, No. 44 Xiao Hong Shan, Wuhan, Hubei 430071, China
References:
Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL, Chen HD, Chen J, Luo Y, Guo H, Jiang RD, Liu MQ, Chen Y, Shen XR, Wang X, Zheng XS, Zhao K, Chen QJ, Deng F, Liu LL, Yan B, Zhan FX, Wang YY, Xiao GF, Shi ZL. A pneum
outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020, *in Press.*

Fig. 2 *Bat coronavirus RaTG13/BtCoV/4991information from the Institute of Pathogen Biology, CAMS&PUMC.*



All journal articles evaluating the origin or epidemiology of SARS-CoV-2 that utilize the RaTG13 bat strain genomics are potentially flawed and should be retracted.

AUTHORS

Dean Bengston

https://archive.is/K0f6L

http://www.mgc.ac.cn/DBatVir/update.xml

https://osf.io/wy89d/

Emerging Microbes & Infections >
Volume 9, 2020 - Issue 1

Articles

RNA based mNGS approach identifies a novel human coronavirus from two individual pneumonia cases in 2019 Wuhan outbreak

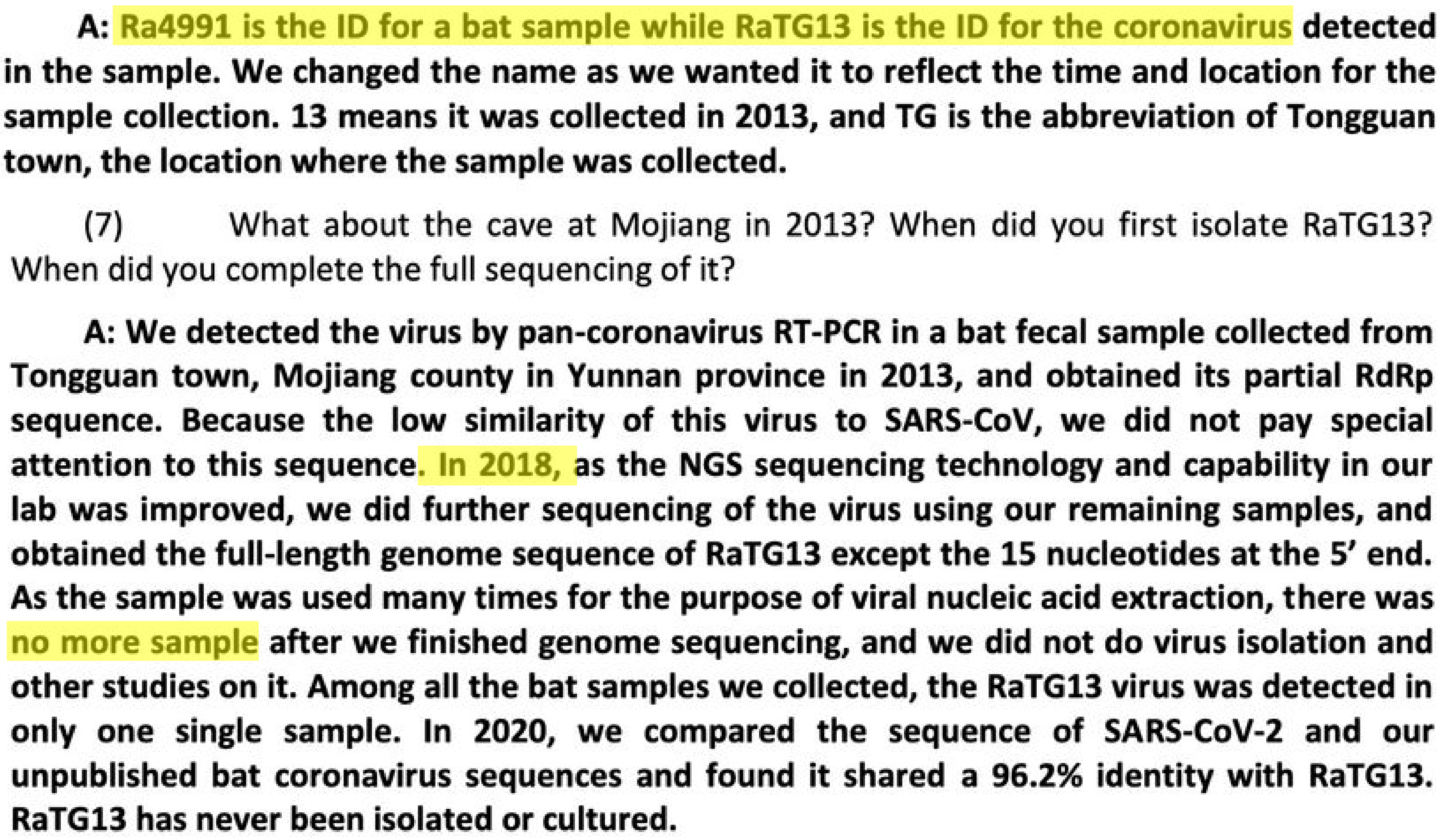
Liangjun Chen, Weiyong Liu, Qi Zhang, Ke Xu, Guangming Ye, Weichen Wu,

Pages 313-319 | Received 27 Jan 2020, Accepted 30 Jan 2020, Published online: 05 Feb 2020

“Phylogenetic analysis indicates that 2019-nCoV is close to coronaviruses (CoVs) circulating in Rhinolophus (Horseshoe bats), such as 98.7% nucleotide identity to partial RdRp gene of bat coronavirus strain **BtCoV/4991** (GenBank KP876546, 370 nt sequence of RdRp and lack of other genome sequence) and 87.9% nucleotide identity to bat coronavirus strain **bat-SL-CoVZC45** and **bat-SL-CoVZXC21**.”

https://www.tandfonline.com/doi/full/10.1080/22221751.2020.1725399

15 July: Dr Shi replied to Science editor questions



A: Ra4991 is the ID for a bat sample while RaTG13 is the ID for the coronavirus detected in the sample. We changed the name as we wanted it to reflect the time and location for the sample collection. 13 means it was collected in 2013, and TG is the abbreviation of Tongguan town, the location where the sample was collected.

(7) What about the cave at Mojiang in 2013? When did you first isolate RaTG13? When did you complete the full sequencing of it?

A: We detected the virus by pan-coronavirus RT-PCR in a bat fecal sample collected from Tongguan town, Mojiang county in Yunnan province in 2013, and obtained its partial RdRp sequence. Because the low similarity of this virus to SARS-CoV, we did not pay special attention to this sequence. In 2018, as the NGS sequencing technology and capability in our lab was improved, we did further sequencing of the virus using our remaining samples, and obtained the full-length genome sequence of RaTG13 except the 15 nucleotides at the 5' end. As the sample was used many times for the purpose of viral nucleic acid extraction, there was no more sample after we finished genome sequencing, and we did not do virus isolation and other studies on it. Among all the bat samples we collected, the RaTG13 virus was detected in only one single sample. In 2020, we compared the sequence of SARS-CoV-2 and our unpublished bat coronavirus sequences and found it shared a 96.2% identity with RaTG13. RaTG13 has never been isolated or cultured.

https://www.sciencemag.org/sites/default/files/Shi%20Zhengli%20Q%26A.pdf

When was RaTG13 full genome sequenced?

MISINFORMATION

Its scientists “went back to that sample in 2020, in early January or maybe even at the end of last year. I don’t know." (The Times, 4 July 2020)

http://archive.is/7TCsc

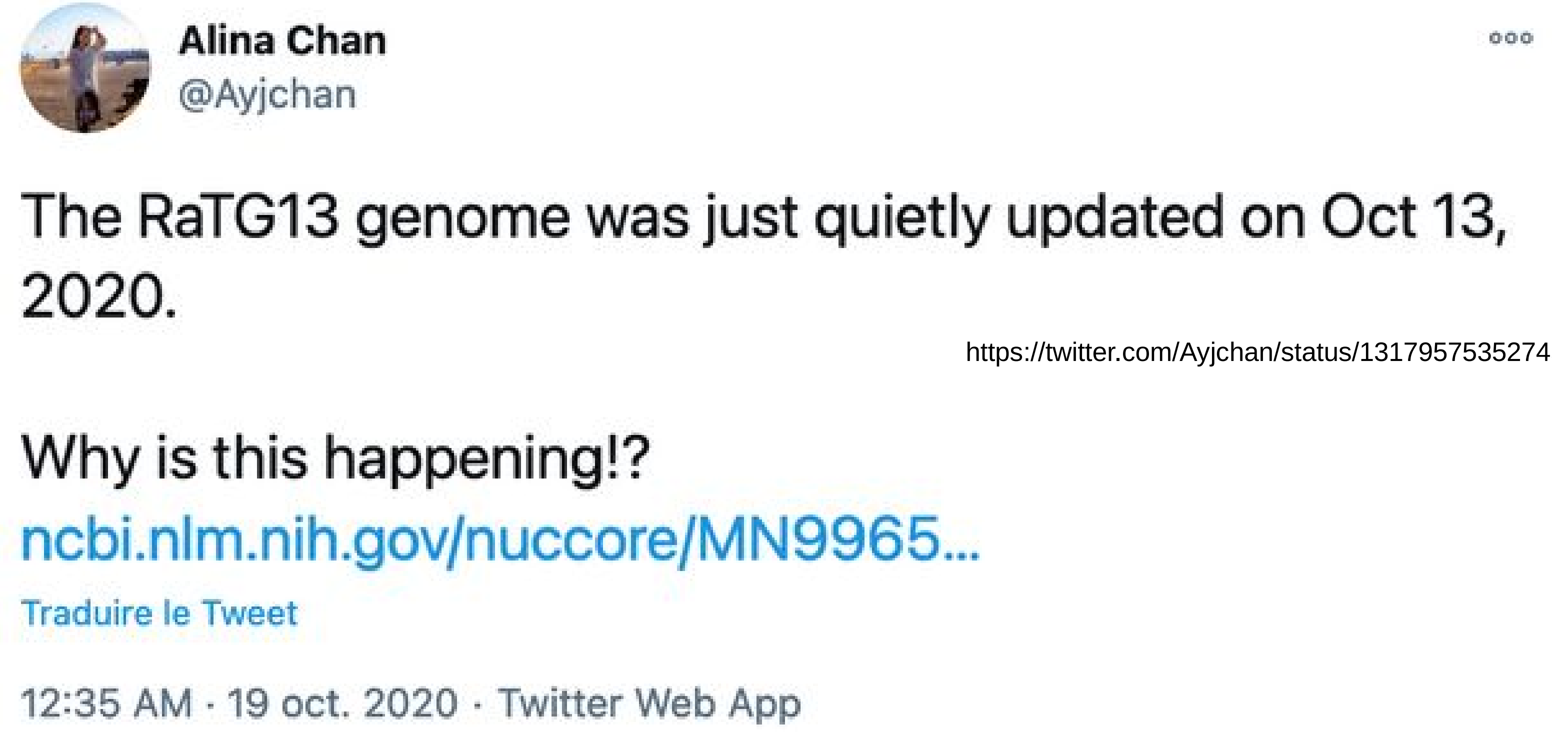
MISINFORMATION

“We found the closest relative to the current SARS-CoV-2 in a bat in China in 2013. We sequenced a bit of the genome, and then it went in the freezer; because it didn’t look like SARS, we thought it was at a lower risk of emerging. With the Global Virome Project, we could have sequenced the whole genome, discovered that it binds to human cells and upgraded the risk. And maybe then when we were designing vaccines for SARS, those could have targeted this one too, and we would have had something in the freezer ready to go if it emerged.” (9 May 2020)

https://www.theaustralian.com.au/weekend-australian-magazine/coronavirus-how-to-beat-the-next-pandemic/news-story/77989c4bfcb4fc7a120533e159dd35ff

Peter Daszak
EcoHealth Alliance
New-York City

Controversies around the RaTG13 sequence still ongoing



Alina Chan
@Ayjchan

The RaTG13 genome was just quietly updated on Oct 13, 2020.

Why is this happening!?
ncbi.nlm.nih.gov/nuccore/MN9965...

12:35 AM · 19 oct. 2020 · Twitter Web App

https://twitter.com/Ayjchan/status/1317957535274569728?s=20

in orf1ab: 4 single base changes and 1 double base change that researchers have already noticed (mismatch between amplicon and first published sequence).

15 nucleotides at 5’ end added.

https://www.ncbi.nlm.nih.gov/nuccore/MN996532

When was RaTG13 sequenced?

Where does RaTG13 come from?

bat RaTG13 (**genome assembly**)

```
LOCUS       MN996532            29855 bp    RNA     linear   VRL 24-MAR-2020
JOURNAL   Submitted (27-JAN-2020) CAS Key Laboratory of Special Pathogens,
          Wuhan Institute of Virology, Center for Biosafety Mega-Science,
          Chinese Academy of Sciences, No. 44 Xiao Hong Shan, Wuhan, Hubei
          430071, China

DT   30-JAN-2020 (Rel. 143, Created)
DT   26-MAR-2020 (Rel. 144, Last updated, Version 5)
RL   Submitted (27-JAN-2020) to the INSDC.
RL   CAS Key Laboratory of Special Pathogens, Wuhan Institute of Virology,
RL   Center for Biosafety Mega-Science, Chinese Academy of Sciences, No. 44 Xiao
RL   Hong Shan, Wuhan, Hubei 430071, China
CC   ##Assembly-Data-START##
CC   Assembly Method       :: CLC Genomics Workbench v. 12.0
CC   Sequencing Technology :: Illumina
CC   ##Assembly-Data-END##
FT   source          1..29855
FT                   /organism="Bat coronavirus RaTG13"
FT                   /host="Rhinolophus affinis"
FT                   /isolate="RaTG13"
FT                   /mol_type="genomic RNA"
FT                   /country="China"
FT                   /isolation_source="fecal swab"
FT                   /collection_date="24-Jul-2013"
FT                   /db_xref="taxon:2709072"
```

No precise location

https://www.ncbi.nlm.nih.gov/nuccore/MN996532.1

RaTG13/4991 comes from an abandoned mine

Research Article | Published: 18 February 2016

Coexistence of multiple coronaviruses in several bat colonies in an abandoned mineshaft

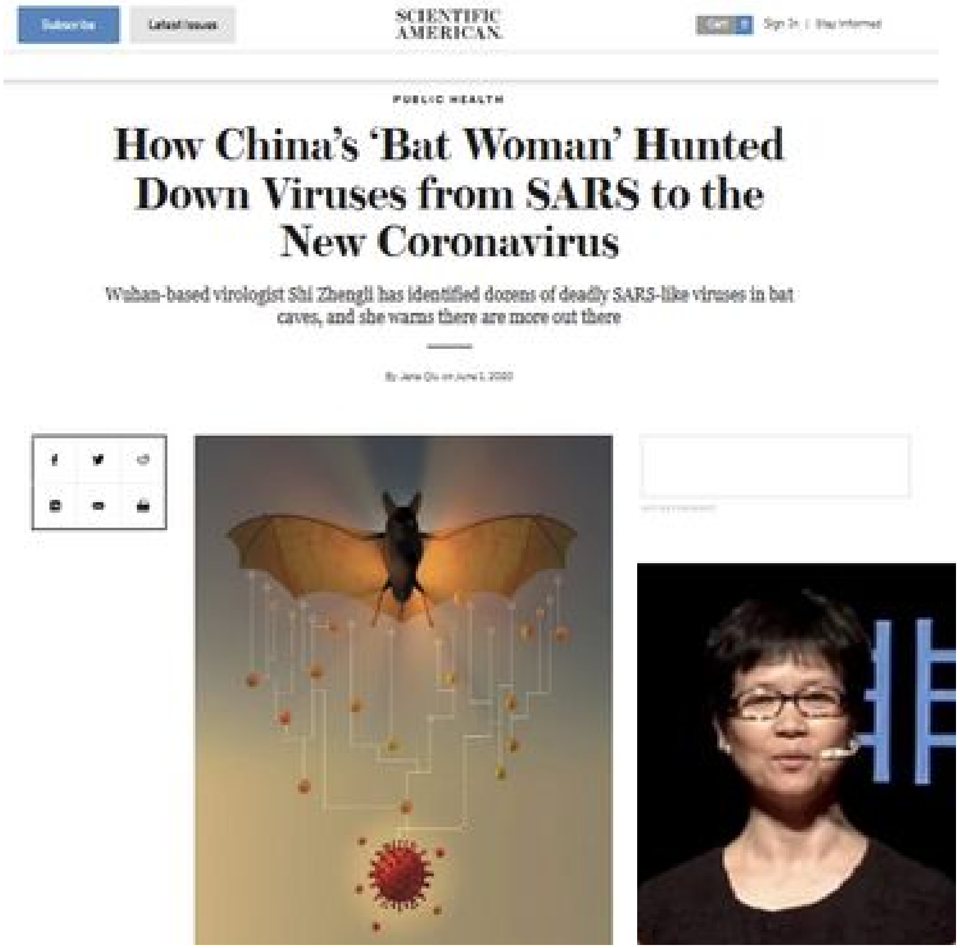
Xing-Yi Ge, Ning Wang, Wei Zhang, Ben Hu, Bei Li, Yun-Zhi Zhang, Ji-Hua Zhou, Chu-Ming Luo, Xing-Lou Yang, Li-Jun Wu, Bo Wang, Yun Zhang, Zong-Xiao Li & Zheng-Li Shi

Virologica Sinica **31**, 31–40(2016) | Cite this article

5788 Accesses | **31** Citations | **184** Altmetric | Metrics

Figure 1. Sample collection site in Mojiang, Yunnan Province. The mineshaft where bat samples were collected is indicated with a red dot, three county towns (Mojiang, Zhenyuan, and Ning'er) near the mineshaft are labeled with green dots.

Three years earlier Shi's team had been called in to investigate the virus profile of a mine shaft in Yunnan's mountainous Mojiang County—famous for its fermented Pu'er tea—where six miners suffered from pneumonialike diseases and two died. After sampling the cave for a year, the researchers discovered a diverse group of coronaviruses in six bat species. In many cases, multiple viral strains had infected a single animal, turning it into a flying factory for new viruses.



PUBLIC HEALTH

How China's 'Bat Woman' Hunted Down Viruses from SARS to the New Coronavirus

Wuhan-based virologist Shi Zhengli has identified dozens of deadly SARS-like viruses in bat caves, and she warns there are more out there

By Jane Qiu on June 1, 2020

https://www.scientificamerican.com/article/how-chinas-bat-woman-hunted-down-viruses-from-sars-to-the-new-coronavirus1/

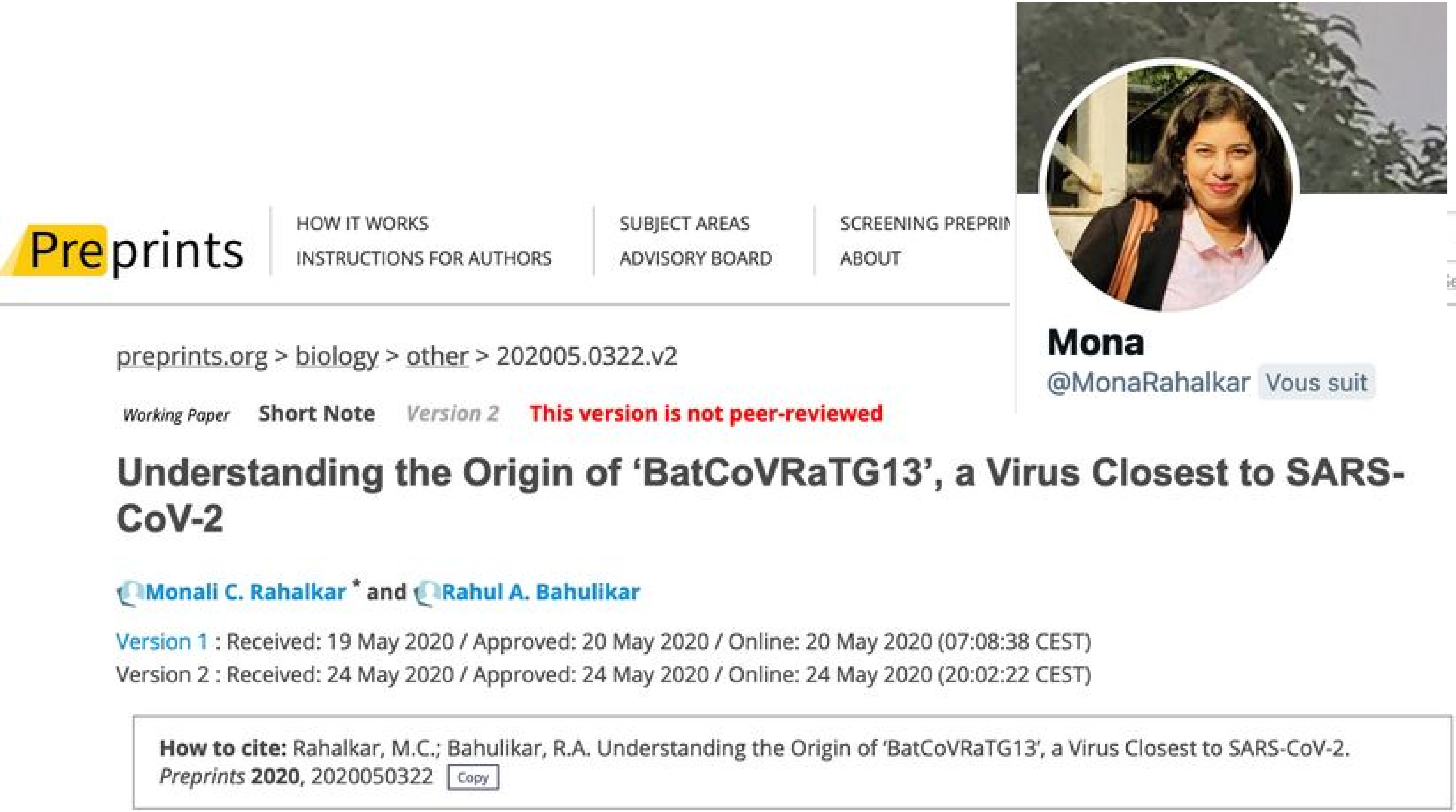
Hot on the trail. Chinese disease sleuths at work in the cave in Mojiang. COURTESY OF QI JIN

A New Killer Virus in China?

By **Richard Stone** | Mar. 20, 2014 , 3:45 PM

In June 2012, three men removing slag from a derelict copper mine in southwestern China fell ill with severe pneumonia and died. Six months later, researchers went spelunking in the mine—an artificial cave hewn from a hillside—in search of pathogens. After taking anal swabs from bats, rats, and musk shrews living in the cave, the team has discovered what it says is a new virus that may have felled the workers.

https://www.sciencemag.org/news/2014/03/new-killer-virus-china



Mona
@MonaRahalkar Vous suit

preprints.org > biology > other > 202005.0322.v2

Working Paper **Short Note** *Version 2* **This version is not peer-reviewed**

Understanding the Origin of 'BatCoVRaTG13', a Virus Closest to SARS-CoV-2

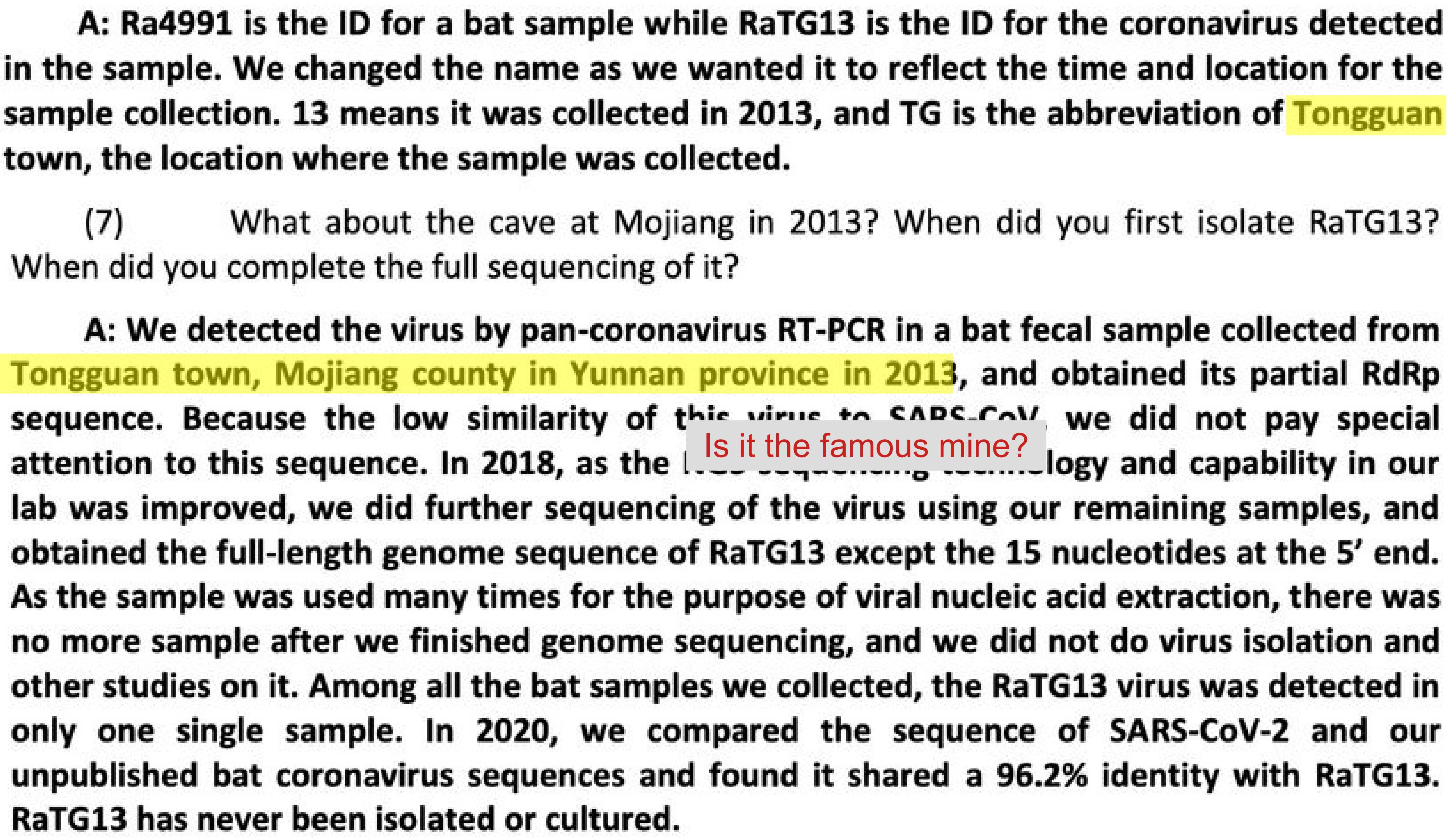
Monali C. Rahalkar * and **Rahul A. Bahulikar**

Version 1 : Received: 19 May 2020 / Approved: 20 May 2020 / Online: 20 May 2020 (07:08:38 CEST)
Version 2 : Received: 24 May 2020 / Approved: 24 May 2020 / Online: 24 May 2020 (20:02:22 CEST)

How to cite: Rahalkar, M.C.; Bahulikar, R.A. Understanding the Origin of 'BatCoVRaTG13', a Virus Closest to SARS-CoV-2. *Preprints* **2020**, 2020050322 Copy

https://www.preprints.org/manuscript/202005.0322/v2

15 July: Dr Shi replied to Science editor questions



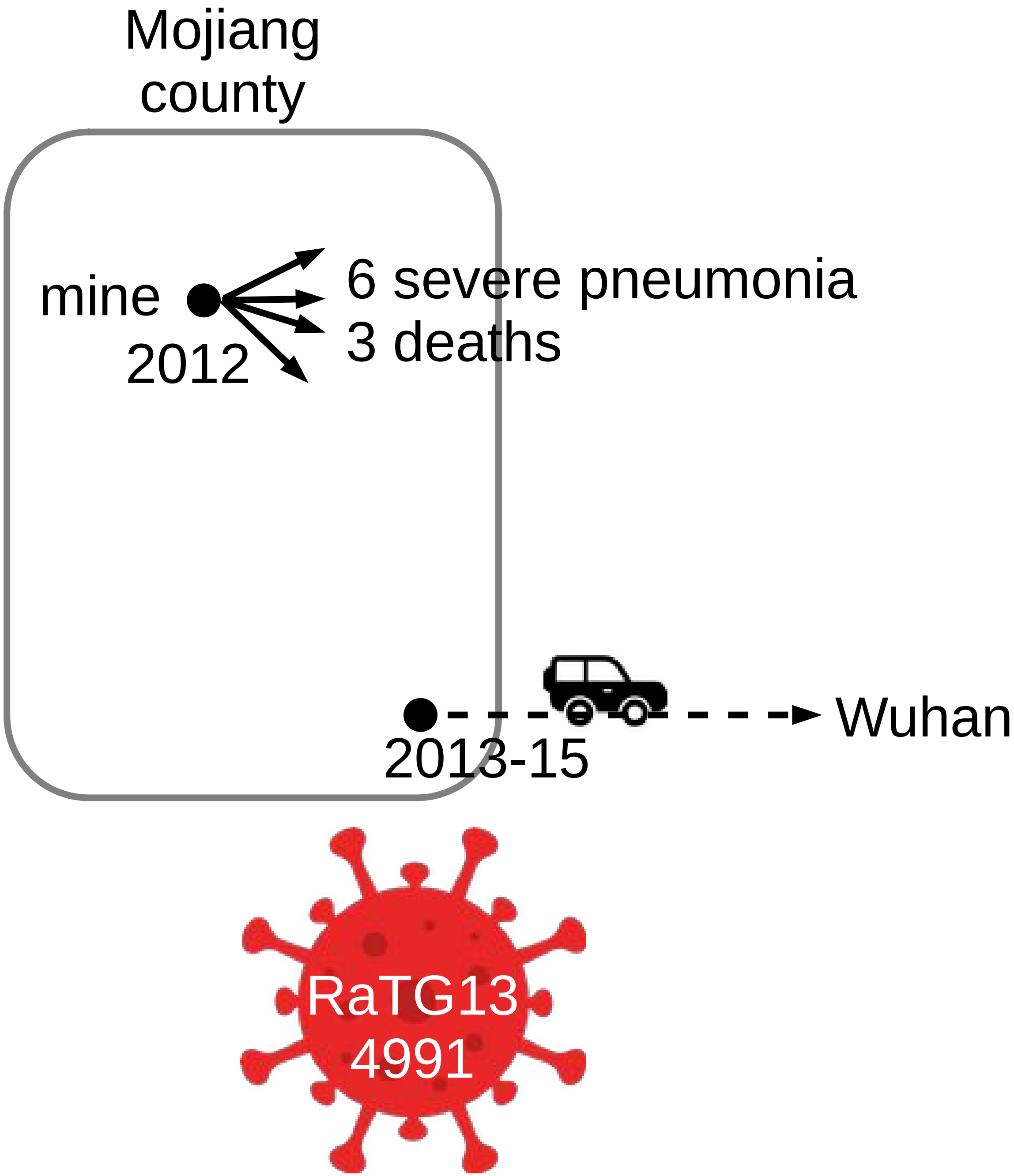
A: Ra4991 is the ID for a bat sample while RaTG13 is the ID for the coronavirus detected in the sample. We changed the name as we wanted it to reflect the time and location for the sample collection. 13 means it was collected in 2013, and TG is the abbreviation of Tongguan town, the location where the sample was collected.

(7) What about the cave at Mojiang in 2013? When did you first isolate RaTG13? When did you complete the full sequencing of it?

A: We detected the virus by pan-coronavirus RT-PCR in a bat fecal sample collected from Tongguan town, Mojiang county in Yunnan province in 2013, and obtained its partial RdRp sequence. Because the low similarity of this virus to SARS-CoV, we did not pay special attention to this sequence. In 2018, as the NGS sequencing technology and capability in our lab was improved, we did further sequencing of the virus using our remaining samples, and obtained the full-length genome sequence of RaTG13 except the 15 nucleotides at the 5′ end. As the sample was used many times for the purpose of viral nucleic acid extraction, there was no more sample after we finished genome sequencing, and we did not do virus isolation and other studies on it. Among all the bat samples we collected, the RaTG13 virus was detected in only one single sample. In 2020, we compared the sequence of SARS-CoV-2 and our unpublished bat coronavirus sequences and found it shared a 96.2% identity with RaTG13. RaTG13 has never been isolated or cultured.

Is it the famous mine?

https://www.sciencemag.org/sites/default/files/Shi%20Zhengli%20Q%26A.pdf



Mojiang
county

mine
2012

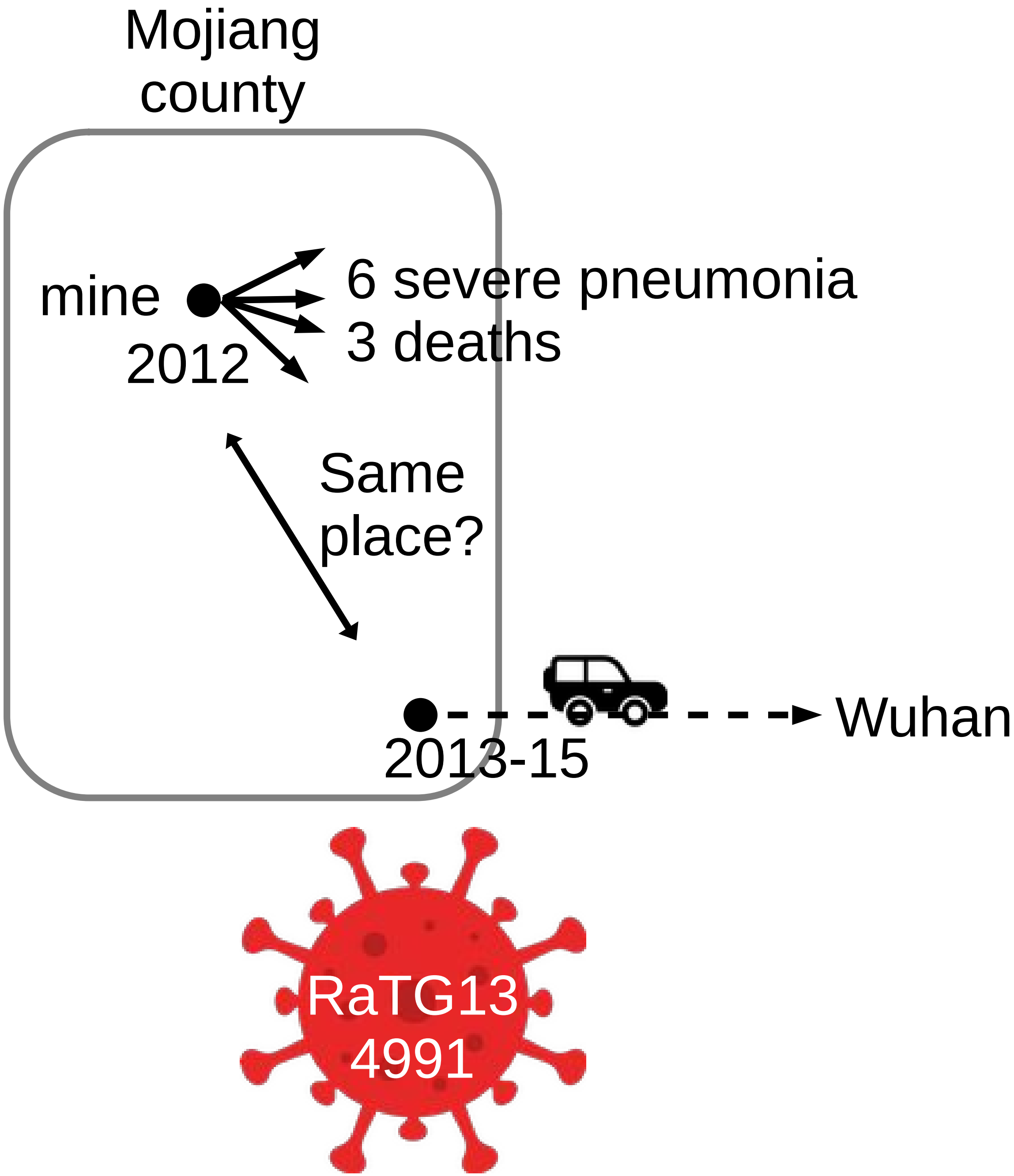
6 severe pneumonia
3 deaths

2013-15

Wuhan

RaTG13
4991

Was RaTG13 collected in the famous mine?



Mojiang county

mine

2012

6 severe pneumonia

3 deaths

Same place?

2013-15

Wuhan

RaTG13

4991

August 2020: NBC News' Janis Mackey Frayer interviews the Director of Wuhan National Biosafety Laboratory, Yuan Zhiming

"Was RaTG13 can you clarify, was it found in a mine where people had died of a covid-like illness?"

"No"

7:50

https://www.youtube.com/watch?v=P5EjqVkxms4&t=468s

17 Nov 2020

Addendum | Open Access | Published: 17 November 2020

Addendum: A pneumonia outbreak associated with a new coronavirus of probable bat origin

Peng Zhou, Xing-Lou Yang, [...] Zheng-Li Shi

Nature (2020) | Cite this article

5109 Accesses | **4** Citations | **213** Altmetric | Metrics

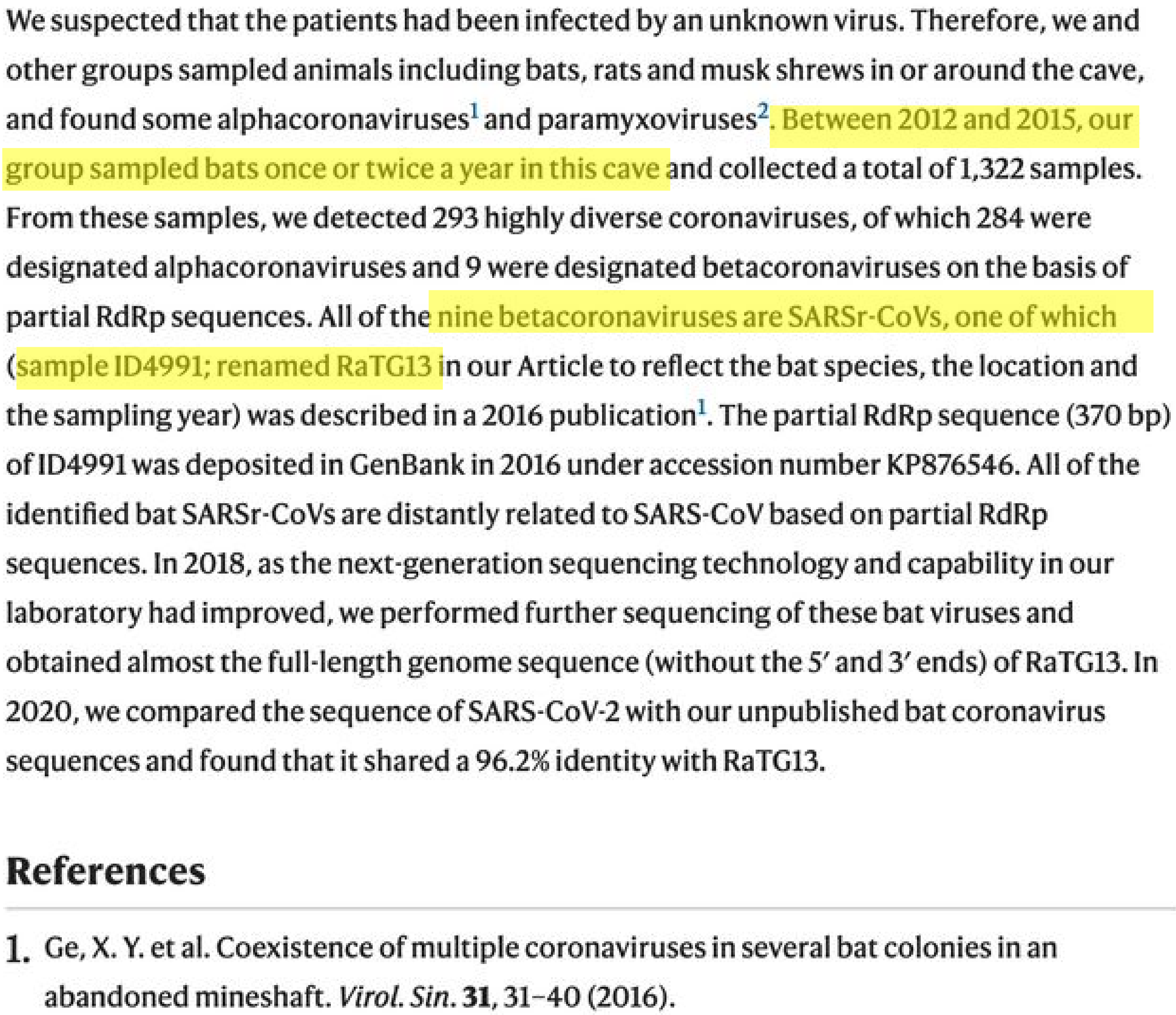
The Original Article was published on 03 February 2020

Addendum to: *Nature* https://doi.org/10.1038/s41586-020-2012-7 Published online 03 February 2020

Here we provide further information about the bat SARS-related coronavirus (SARSr-CoV) strain RaTG13 reported in our Article. Between 1 July and 1 October 2012, we received 13 serum samples collected from 4 patients (one of whom was deceased) who showed severe respiratory disease. These patients had visited a mine cave in Tongguan town, Mojiang County, Yunnan Province, China, to clean bat faeces in order to mine copper before being admitted to the First Affiliated Hospital of Kunming Medical University on 26–27 April 2012. The samples we received were collected by the hospital staff in June, July, August and September 2012. To investigate the cause of the respiratory disease, we tested the samples using PCR methods developed in our laboratory targeting the RNA-dependent RNA polymerases (RdRp) of Ebola virus, Nipah virus and bat SARSr-CoV Rp3, and all of the samples were negative for the presence of these viruses. We also tested the serum samples for the presence of antibodies against the nucleocapsid proteins of these three viruses, and none of the samples gave a positive result. Recently, we retested the samples with our validated enzyme-linked immunosorbent assay (ELISA) against the SARS coronavirus 2 (SARS-CoV-2) nucleocapsid protein—which has greater than 90% amino acid sequence identity with bat SARSr-CoV Rp3—and confirmed that these patients were not infected by SARS-CoV-2.

Same place!

https://www.nature.com/articles/s41586-020-2951-z



We suspected that the patients had been infected by an unknown virus. Therefore, we and other groups sampled animals including bats, rats and musk shrews in or around the cave, and found some alphacoronaviruses[1] and paramyxoviruses[2]. Between 2012 and 2015, our group sampled bats once or twice a year in this cave and collected a total of 1,322 samples. From these samples, we detected 293 highly diverse coronaviruses, of which 284 were designated alphacoronaviruses and 9 were designated betacoronaviruses on the basis of partial RdRp sequences. All of the nine betacoronaviruses are SARSr-CoVs, one of which (sample ID4991; renamed RaTG13 in our Article to reflect the bat species, the location and the sampling year) was described in a 2016 publication[1]. The partial RdRp sequence (370 bp) of ID4991 was deposited in GenBank in 2016 under accession number KP876546. All of the identified bat SARSr-CoVs are distantly related to SARS-CoV based on partial RdRp sequences. In 2018, as the next-generation sequencing technology and capability in our laboratory had improved, we performed further sequencing of these bat viruses and obtained almost the full-length genome sequence (without the 5′ and 3′ ends) of RaTG13. In 2020, we compared the sequence of SARS-CoV-2 with our unpublished bat coronavirus sequences and found that it shared a 96.2% identity with RaTG13.

References

1. Ge, X. Y. et al. Coexistence of multiple coronaviruses in several bat colonies in an abandoned mineshaft. *Virol. Sin.* **31**, 31–40 (2016).

https://www.nature.com/articles/s41586-020-2951-z

August 2020: NBC News' Janis Mackey Frayer interviews the Director of Wuhan National Biosafety Laboratory, Yuan Zhiming

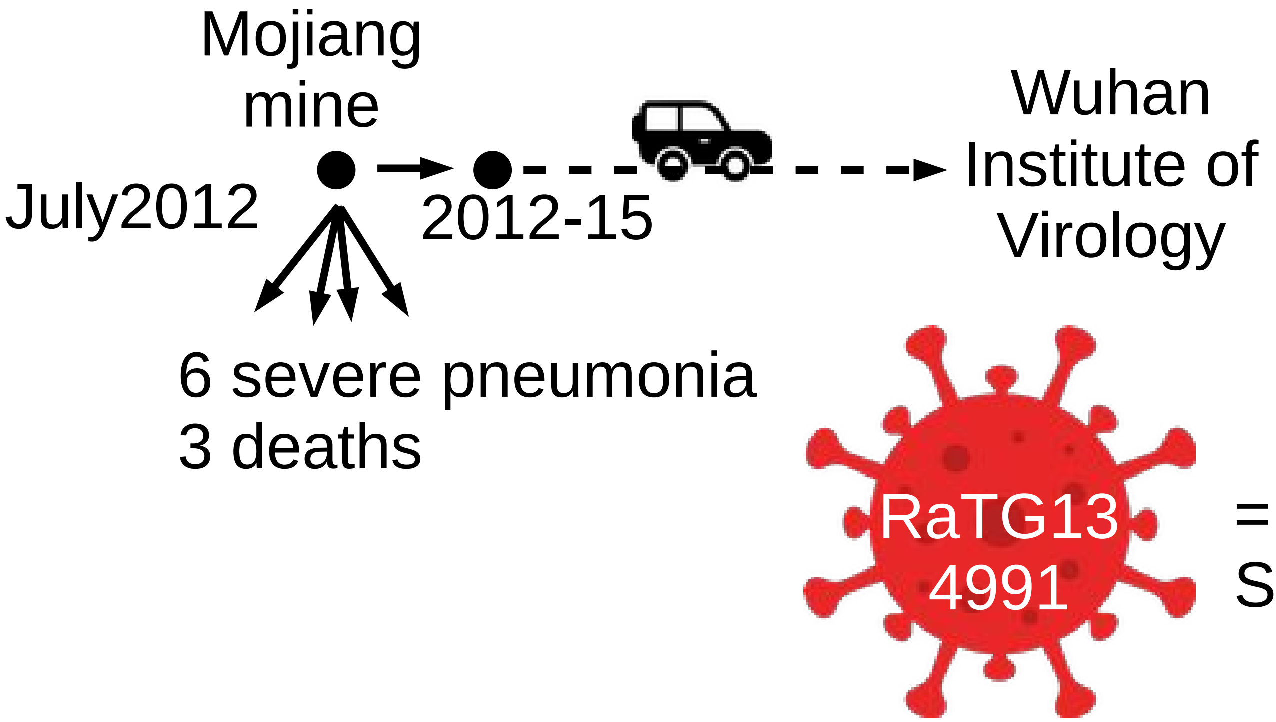
MISINFORMATION

"Was RaTG13 can you clarify, was it found in a mine where people had died of a covid-like illness?"

“No”

7:50

https://www.youtube.com/watch?v=P5EjqVkxms4&t=468s



Mojiang
mine

July2012

2012-15

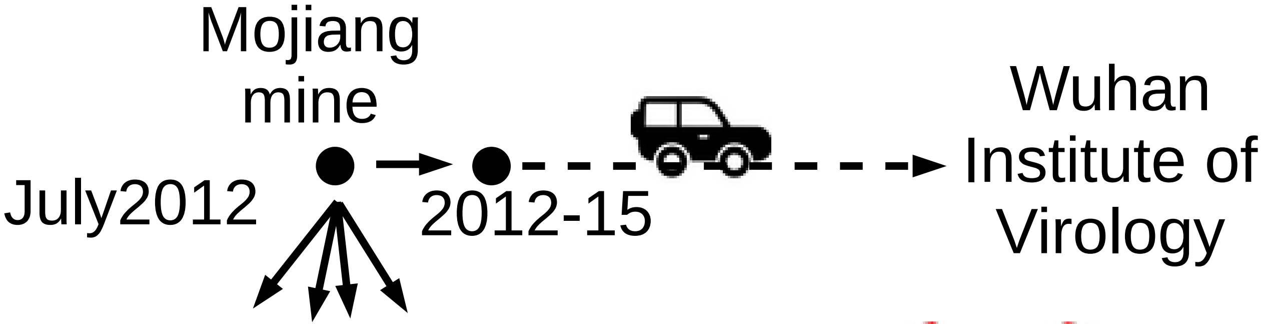
Wuhan
Institute of
Virology

6 severe pneumonia
3 deaths

RaTG13
4991

= most closely related to
SARS-CoV-2 (a cousin)

Was the 2012 pneumonia caused by a SARS-like virus?



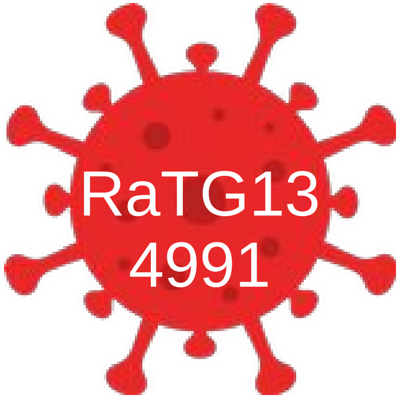
Mojiang mine

July2012

2012-15

Wuhan Institute of Virology

6 severe pneumonia
3 deaths



= most closely related to SARS-CoV-2 (a cousin)

Information not promptly disclosed

20 Jan 2020	**RaTG13 not mentioned**
23 Jan 2020	**RaTG13 mentioned**
Jul 2020	**sequenced in 2017-2018** **is 4991** **collected in Mojiang (mine?)**
Nov 2020	**collected in the Mojiang mine** **where severe pneumonia cases**
Ongoing controversy	**SARS-like pneumonia?**

15 May: @TheSeeker268 sends Master thesis to DRASTIC

The Seeker
@TheSeeker268 Vous suit

I am.

昆明医科大学
硕士学位论文

未知病毒引起重症肺炎6例分析

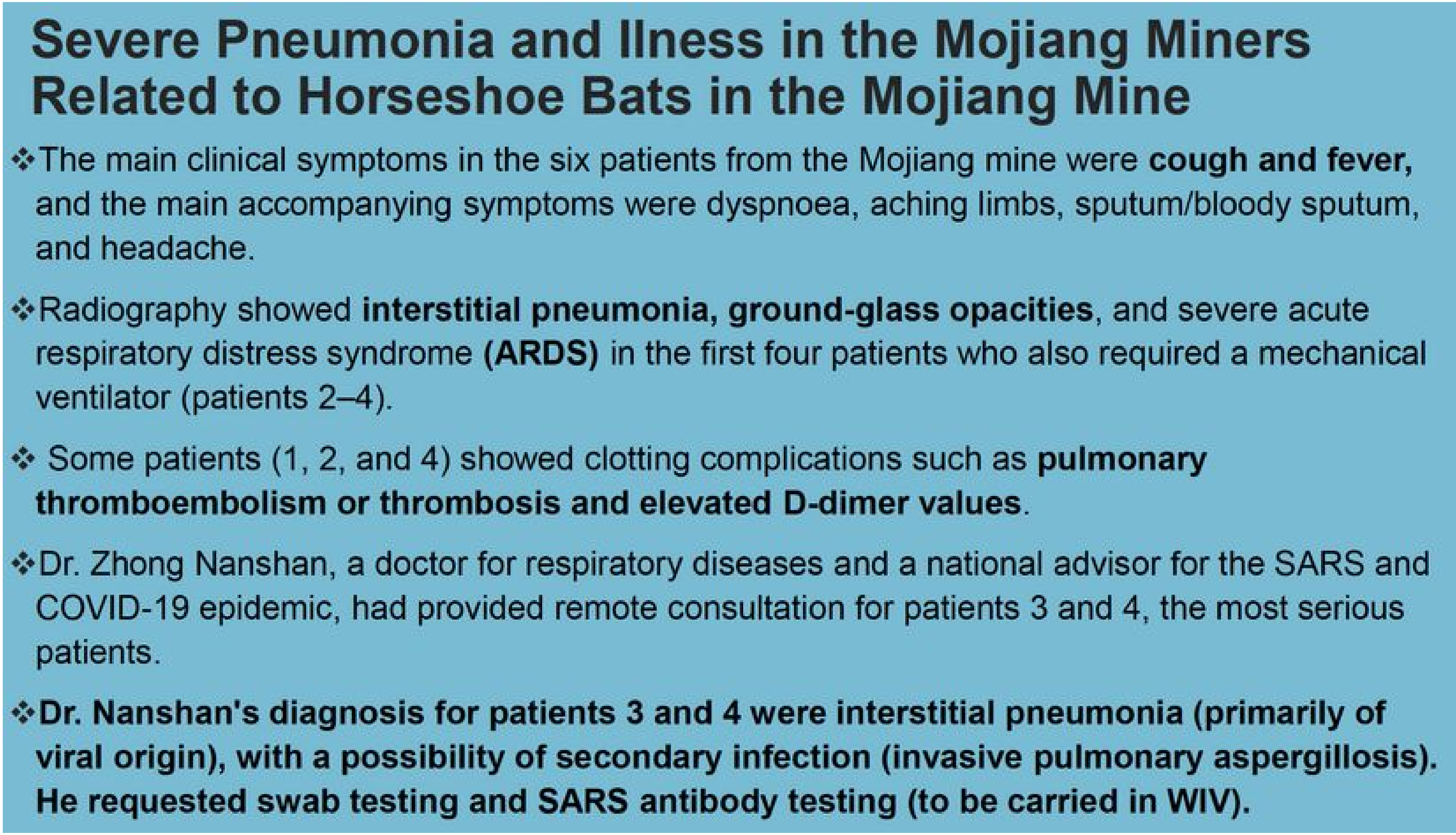
二〇一三年五月

There were 6 patients with severe pneumonia caused by unknown viruses sent to Dep. Emergency, the first affiliated hospital of Kunming medical university in April,May,2012. They were all workers at the same mine where had a lot of bats and bats feces. After the treatment, 3 patients died and 3 patients survived. According to the appraisal of the Kunming institute of zoology, Chinese academy of sciences, the type of the bat in mine where 6 patients worked is *Rhinolophus sinicus*, from which was extracted...

https://www.sutori.com/story/d-r-a-s-t-i-c-2020-origins--xCvdWonoJTx4TYVtAC4EhQ1b

http://eng.oversea.cnki.net/Kcms/detail/detail.aspx?filename=1013327523.nh&dbcode=CMFD&dbname=CMFD2014

https://www.documentcloud.org/documents/6981198-Analysis-of-Six-Patients-With-Unknown-Viruses.html



Severe Pneumonia and Ilness in the Mojiang Miners Related to Horseshoe Bats in the Mojiang Mine

- The main clinical symptoms in the six patients from the Mojiang mine were **cough and fever,** and the main accompanying symptoms were dyspnoea, aching limbs, sputum/bloody sputum, and headache.
- Radiography showed **interstitial pneumonia, ground-glass opacities**, and severe acute respiratory distress syndrome **(ARDS)** in the first four patients who also required a mechanical ventilator (patients 2–4).
- Some patients (1, 2, and 4) showed clotting complications such as **pulmonary thromboembolism or thrombosis and elevated D-dimer values**.
- Dr. Zhong Nanshan, a doctor for respiratory diseases and a national advisor for the SARS and COVID-19 epidemic, had provided remote consultation for patients 3 and 4, the most serious patients.
- **Dr. Nanshan's diagnosis for patients 3 and 4 were interstitial pneumonia (primarily of viral origin), with a possibility of secondary infection (invasive pulmonary aspergillosis). He requested swab testing and SARS antibody testing (to be carried in WIV).**

Slide from Monali Rahalkar

28 May: @TheSeeker268 finds PhD thesis

The Seeker
@TheSeeker268

En réponse à @ydeigin @luigi_warren et 20 autres personnes

Guess I found the mention of the exact location of the Tongguanzhen cave

Novel Virus Discovery in Bat and the Exploration of Receptor of Bat Coronavirus HKU9 (Canping Huang ; Supervisor: Gao Fu ; Shu Yuelong ; China CDC, 2018)

eng.oversea.cnki.net/kcms/detail/de...

Traduire le Tweet

中国疾病预防控制中心病毒病预防控制所博士论文

样本，并对其中可能携带的病原进行了检测。

材料和仪器，同第一章。

预防控制中心和当地疾控中心工作人员的协助下，我们在云南
县通关镇的一个废弃铜矿里（N 23°10′36″ E 101°21′28″）捕获
中 84 只为中华菊头蝠（*Rhinolophus sinicus*），3 只为亚洲长翼
liginosus），当天晚上在山洞里设置捕鼠器，第二天捕获一只野
后采集了肠道、肺脏、肝脏和脾脏组织，由于蝙蝠体型较小，
捕获的野鼠作同样处理，所有组织分别存放于 2 mL 细胞冻存
类包装在纱布袋中后立即存放于液氮，运送至云南省疾病预防
直至需要检测时取出。本研究通过了中国疾病预防控制中心病
云南省疾病预防控制中心的伦理和动物福利和使用委员会的

3.1. 2 Sample collection
With the assistance of the staff of Yunnan Provincial Center for Disease Control and Prevention and local CDC, we are in Yunnan Province of Mojiang Hani Autonomous County Tongguan Town One in an abandoned copper mine (N 2 3 °1 0 3 6 ″ E 1 0 1°2 r28 ″) Captured There were 87 bats, of which 84 were Zhonghuaju heads (and / sinicus) and 3 were Asian long wings Bats (MzWoptems flili gi nino sus). One day, a mousetrap was set in the cave at night, and the second day was captured. After all bats were sacrificed, intestinal, lung, liver and spleen tissues were collected. Due to the smaller size of the bat, Unable to collect anal swabs. The captured wild rats are treated

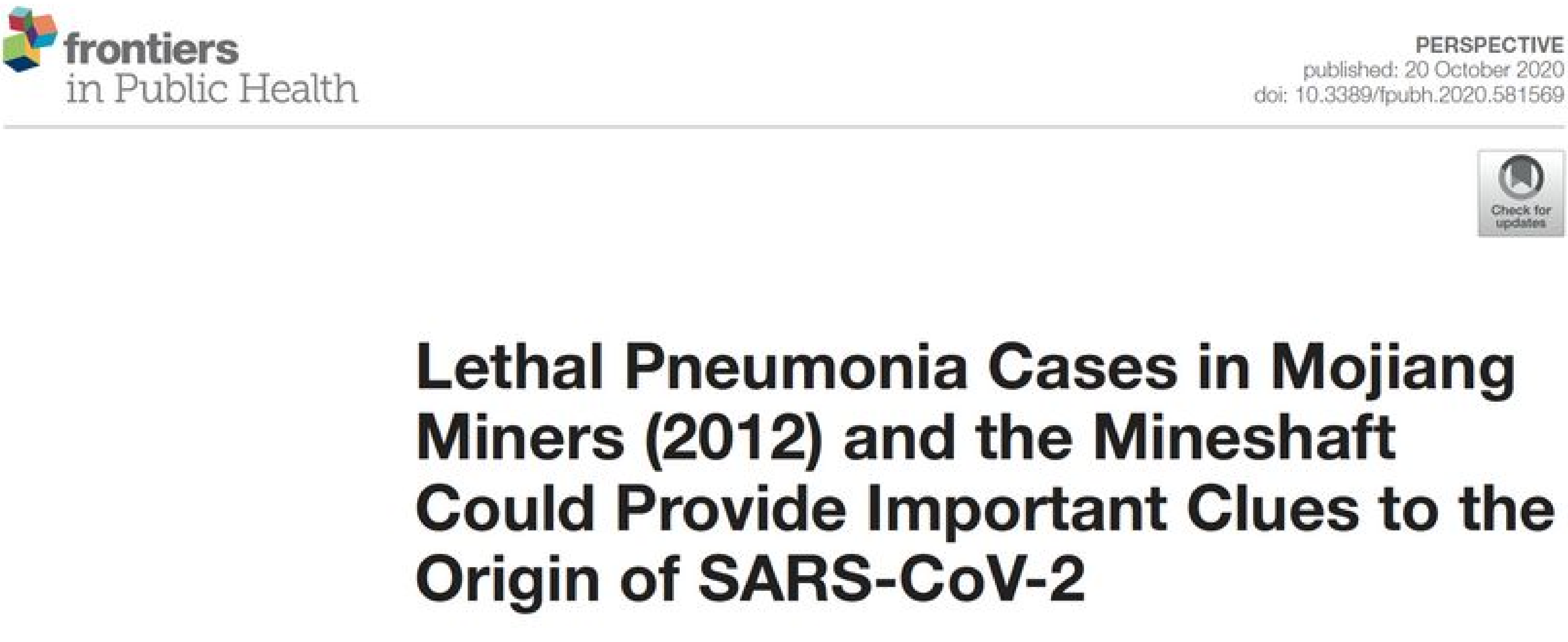
12:59 AM · 29 mai 2020 · Twitter Web App

https://twitter.com/TheSeeker268/status/1266142181208870912?s=20

Ph.D. thesis by Canping Huang (supervised by Dr. George Gao, present Director China CDCP)

https://twitter.com/TheSeeker268/status/1314258619144171520?s=20

http://eng.oversea.cnki.net/kcms/detail/detail.aspx?
CurRec=1&dbname=CDFDLAST2018&filename=1017118517.nh



frontiers
in Public Health

PERSPECTIVE
published: 20 October 2020
doi: 10.3389/fpubh.2020.581569

Lethal Pneumonia Cases in Mojiang Miners (2012) and the Mineshaft Could Provide Important Clues to the Origin of SARS-CoV-2

Monali C. Rahalkar[1*] *and Rahul A. Bahulikar*[2]

Mona
@MonaRahalkar Vous suit

https://www.frontiersin.org/articles/10.3389/fpubh.2020.581569/full

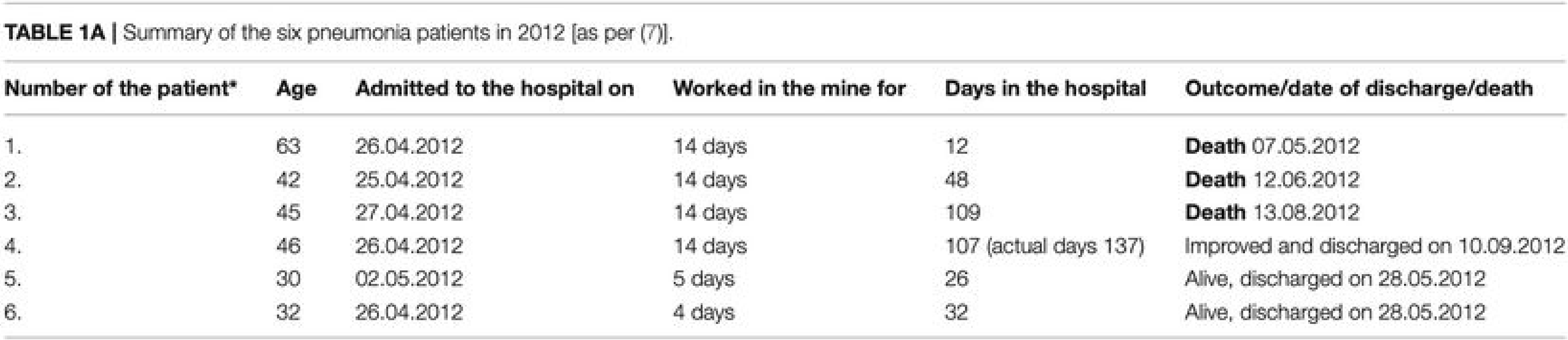


TABLE 1A | Summary of the six pneumonia patients in 2012 [as per (7)].

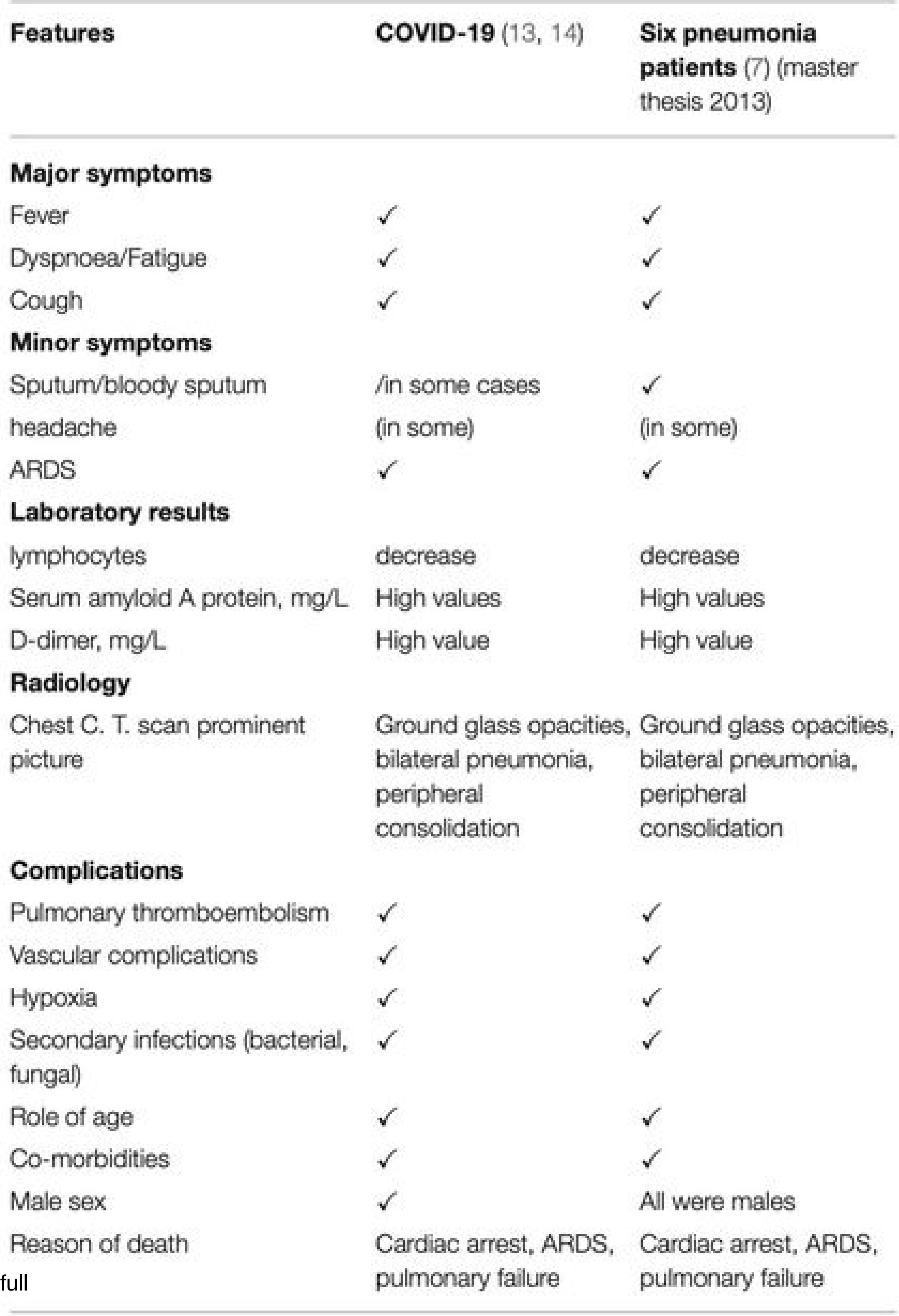
Number of the patient*	Age	Admitted to the hospital on	Worked in the mine for	Days in the hospital	Outcome/date of discharge/death
1.	63	26.04.2012	14 days	12	**Death** 07.05.2012
2.	42	25.04.2012	14 days	48	**Death** 12.06.2012
3.	45	27.04.2012	14 days	109	**Death** 13.08.2012
4.	46	26.04.2012	14 days	107 (actual days 137)	Improved and discharged on 10.09.2012
5.	30	02.05.2012	5 days	26	Alive, discharged on 28.05.2012
6.	32	26.04.2012	4 days	32	Alive, discharged on 28.05.2012

https://www.frontiersin.org/articles/10.3389/fpubh.2020.581569/full

Symptoms similar to COVID-19

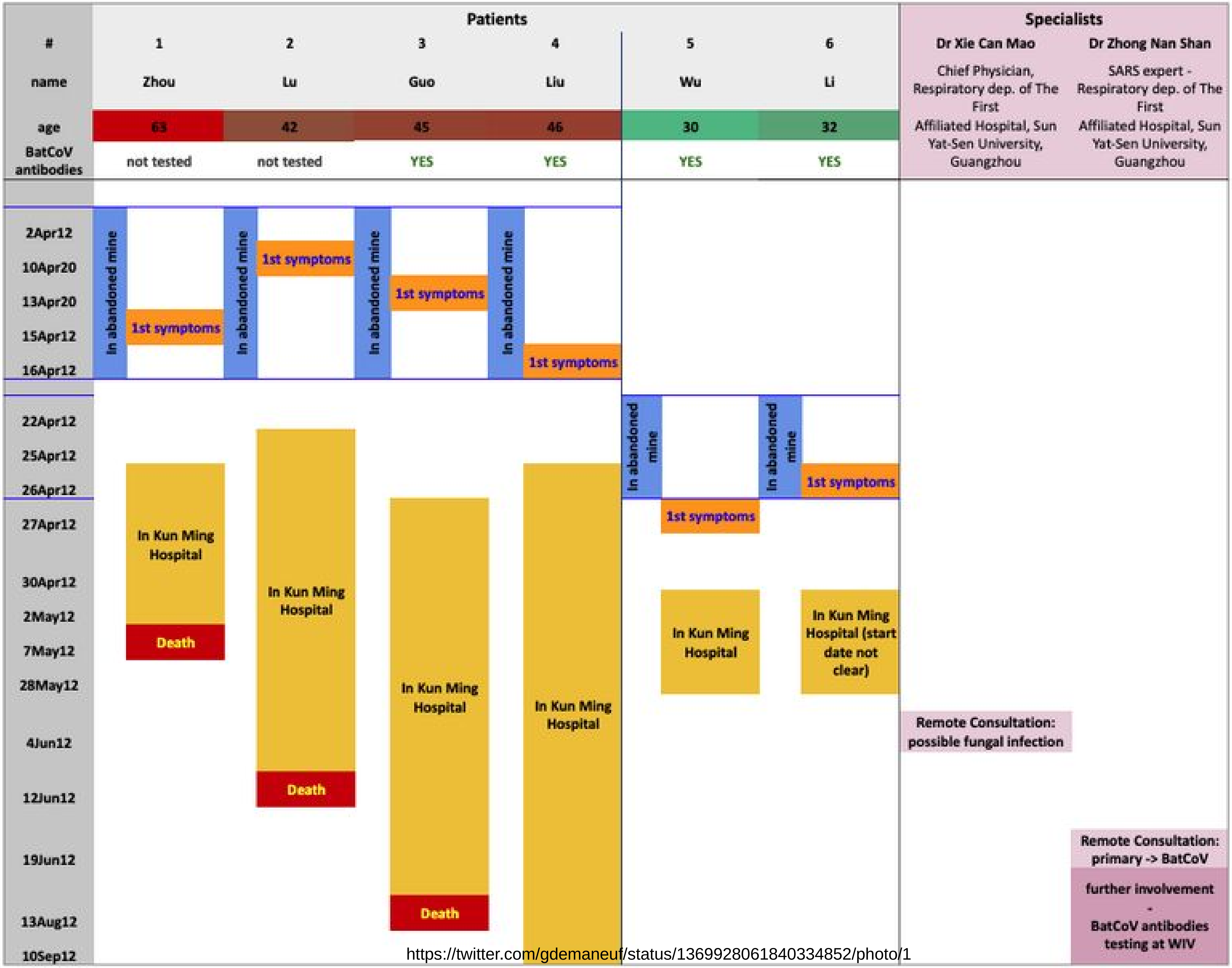
SARS-1:
unilateral pneumonia

https://www.ajronline.org/doi/full/10.2214/AJR.20.22969



Features	**COVID-19** (13, 14)	**Six pneumonia patients** (7) (master thesis 2013)
Major symptoms		
Fever	✓	✓
Dyspnoea/Fatigue	✓	✓
Cough	✓	✓
Minor symptoms		
Sputum/bloody sputum	/in some cases	✓
headache	(in some)	(in some)
ARDS	✓	✓
Laboratory results		
lymphocytes	decrease	decrease
Serum amyloid A protein, mg/L	High values	High values
D-dimer, mg/L	High value	High value
Radiology		
Chest C. T. scan prominent picture	Ground glass opacities, bilateral pneumonia, peripheral consolidation	Ground glass opacities, bilateral pneumonia, peripheral consolidation
Complications		
Pulmonary thromboembolism	✓	✓
Vascular complications	✓	✓
Hypoxia	✓	✓
Secondary infections (bacterial, fungal)	✓	✓
Role of age	✓	✓
Co-morbidities	✓	✓
Male sex	✓	All were males
Reason of death	Cardiac arrest, ARDS, pulmonary failure	Cardiac arrest, ARDS, pulmonary failure

https://www.frontiersin.org/articles/10.3389/fpubh.2020.581569/full



	Patients						Specialists	
#	1	2	3	4	5	6	Dr Xie Can Mao	Dr Zhong Nan Shan
name	Zhou	Lu	Guo	Liu	Wu	Li	Chief Physician, Respiratory dep. of The First Affiliated Hospital, Sun Yat-Sen University, Guangzhou	SARS expert - Respiratory dep. of The First Affiliated Hospital, Sun Yat-Sen University, Guangzhou
age	63	42	45	46	30	32		
BatCoV antibodies	not tested	not tested	YES	YES	YES	YES		

	1 Zhou	2 Lu	3 Guo	4 Liu	5 Wu	6 Li	Dr Xie Can Mao	Dr Zhong Nan Shan
2Apr12	In abandoned mine	In abandoned mine	In abandoned mine	In abandoned mine				
10Apr20		1st symptoms						
13Apr20			1st symptoms					
15Apr12	1st symptoms							
16Apr12				1st symptoms				
22Apr12		In Kun Ming Hospital			In abandoned mine	In abandoned mine		
25Apr12	In Kun Ming Hospital			In Kun Ming Hospital				
26Apr12			In Kun Ming Hospital			1st symptoms		
27Apr12					1st symptoms			
30Apr12					In Kun Ming Hospital	In Kun Ming Hospital (start date not clear)		
2May12								
7May12	Death							
28May12								
4Jun12							Remote Consultation: possible fungal infection	
12Jun12		Death						
19Jun12								Remote Consultation: primary -> BatCoV
13Aug12			Death					further involvement - BatCoV antibodies testing at WIV
10Sep12								

https://twitter.com/gdemaneuf/status/1369928061840334852/photo/1

17 Nov 2020

Addendum | Open Access | Published: 17 November 2020

Addendum: A pneumonia outbreak associated with a new coronavirus of probable bat origin

Peng Zhou, Xing-Lou Yang, […] Zheng-Li Shi

Nature (2020) | Cite this article

5109 Accesses | **4** Citations | **213** Altmetric | Metrics

The Original Article was published on 03 February 2020

Addendum to: *Nature* https://doi.org/10.1038/s41586-020-2012-7 Published online 03 February 2020

Here we provide further information about the bat SARS-related coronavirus (SARSr-CoV) strain RaTG13 reported in our Article. Between 1 July and 1 October 2012, we received 13 serum samples collected from 4 patients (one of whom was deceased) who showed severe respiratory disease. These patients had visited a mine cave in Tongguan town, Mojiang County, Yunnan Province, China, to clean bat faeces in order to mine copper before being admitted to the First Affiliated Hospital of Kunming Medical University on 26–27 April 2012. The samples we received were collected by the hospital staff in June, July, August and September 2012. To investigate the cause of the respiratory disease, we tested the samples using PCR methods developed in our laboratory targeting the RNA-dependent RNA polymerases (RdRp) of Ebola virus, Nipah virus and bat SARSr-CoV Rp3, and all of the samples were negative for the presence of these viruses. We also tested the serum samples for the presence of antibodies against the nucleocapsid proteins of these three viruses, a none of the samples gave a positive result. Recently, we retested the samples with our validated enzyme-linked immunosorbent assay (ELISA) against the SARS coronavirus 2 (SARS-CoV-2) nucleocapsid protein—which has greater than 90% amino acid sequence identity with bat SARSr-CoV Rp3—and confirmed that these patients were not infected by SARS-CoV-2.

Serum samples are not expected to contain viruses.

And 2 months after disease onset.

Contradiction with PhD thesis

https://monalirahalkar.wordpress.com/2020/12/11/critique-to-the-addendum-zhou-et-al-2020-and-other-contradictions-in-reporting-the-facts-about-ratg13-and-its-history/

https://www.nature.com/articles/s41586-020-2951-z

Swabs not mentioned in Addendum

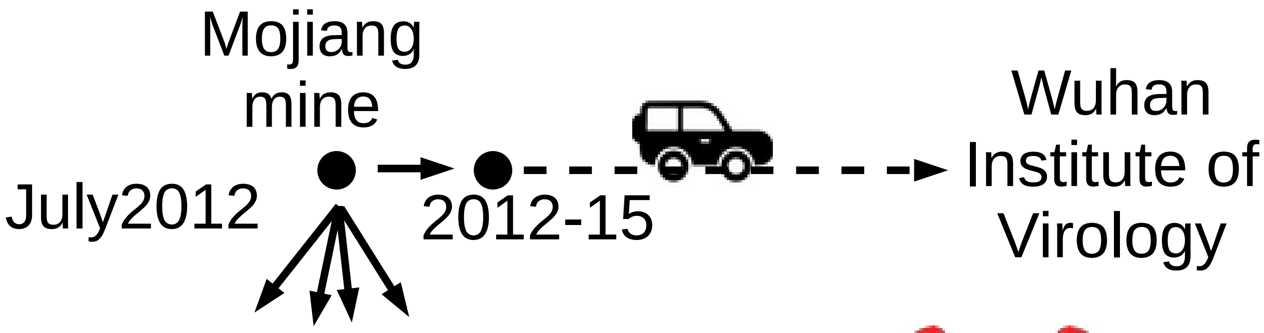
Master thesis: Dr. Zhong Nanshan had ordered to send swabs to WIV for testing for coronaviruses.

http://eng.oversea.cnki.net/Kcms/detail/detail.aspx?filename=1013327523.nh&dbcode=CMFD&dbname=CMFD2014

Contradiction with Addendum

PhD thesis, lines 283–285, page 9: the "blood test results of four cases showed that: **four people carried SARS virus IgG antibodies**, of which two were discharged with higher antibody levels (patients 5 and 6) and two which were hospitalized had lower antibody levels (patients 3 and 4) (Wuhan, Chinese Academy of Sciences) Virology Institute)"

http://eng.oversea.cnki.net/kcms/detail/detail.aspx?dbcode=CDFD&QueryID=11&CurRec=1&dbname=CDFDLAST2018&filename=1017118517.nh



Mojiang mine

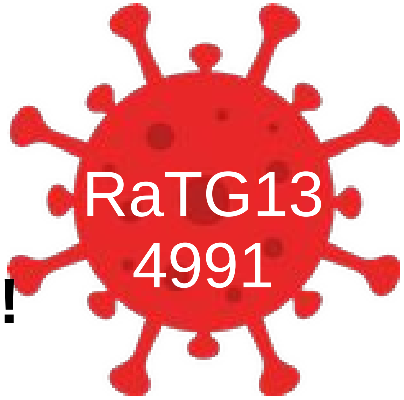
July2012

2012-15

Wuhan Institute of Virology

6 severe pneumonia
3 deaths

COVID-19-like
No intermediate host!



The Seeker
@TheSeeker268

En réponse à @TheSeeker268 @ydeigin et 21 autres personnes

The PhD thesis by Huang Canping is now deleted.

But it's archived:

Traduire le Tweet

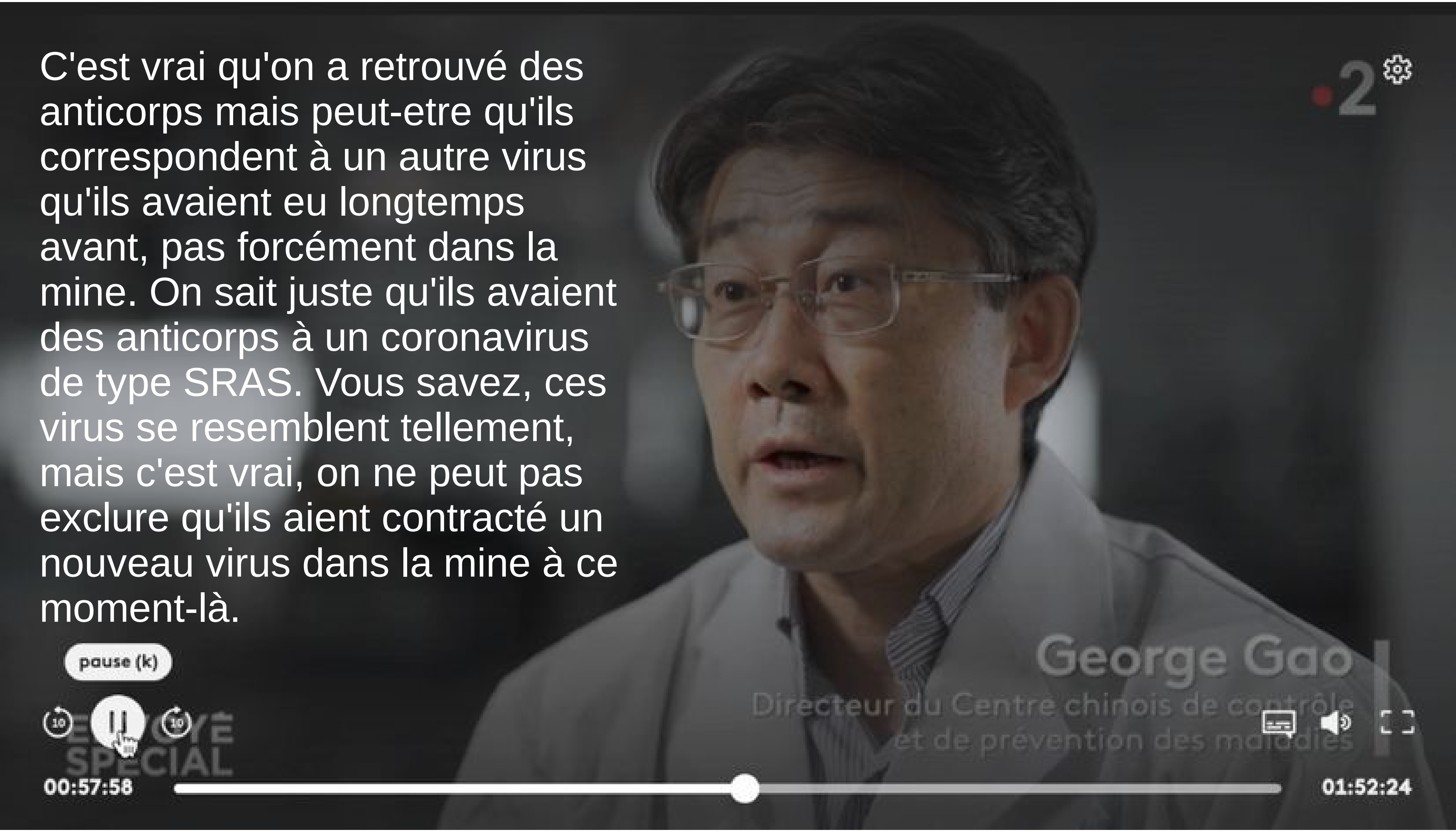
蝙蝠宿主中新病毒发现及蝙蝠冠状病毒HKU9受体的探索 - C...
archived 29 May 2020 12:33:22 UTC
archive.is

7:37 PM · 8 oct. 2020 · Twitter Web App

https://twitter.com/TheSeeker268/status/1314258619144171520?s=20

France 2 Elise Lucet interviews Georges Gao

Envoyé Spécial, 9 March 2021



C'est vrai qu'on a retrouvé des anticorps mais peut-etre qu'ils correspondent à un autre virus qu'ils avaient eu longtemps avant, pas forcément dans la mine. On sait juste qu'ils avaient des anticorps à un coronavirus de type SRAS. Vous savez, ces virus se resemblent tellement, mais c'est vrai, on ne peut pas exclure qu'ils aient contracté un nouveau virus dans la mine à ce moment-là.

0:57:00

https://www.francetvinfo.fr/replay-magazine/france-2/envoye-special/envoye-special-du-jeudi-11-mars-2021_4302957.html

1 June 2020: Scientific American

PUBLIC HEALTH

How China's 'Bat Woman' Hunted Down Viruses from SARS to the New Coronavirus

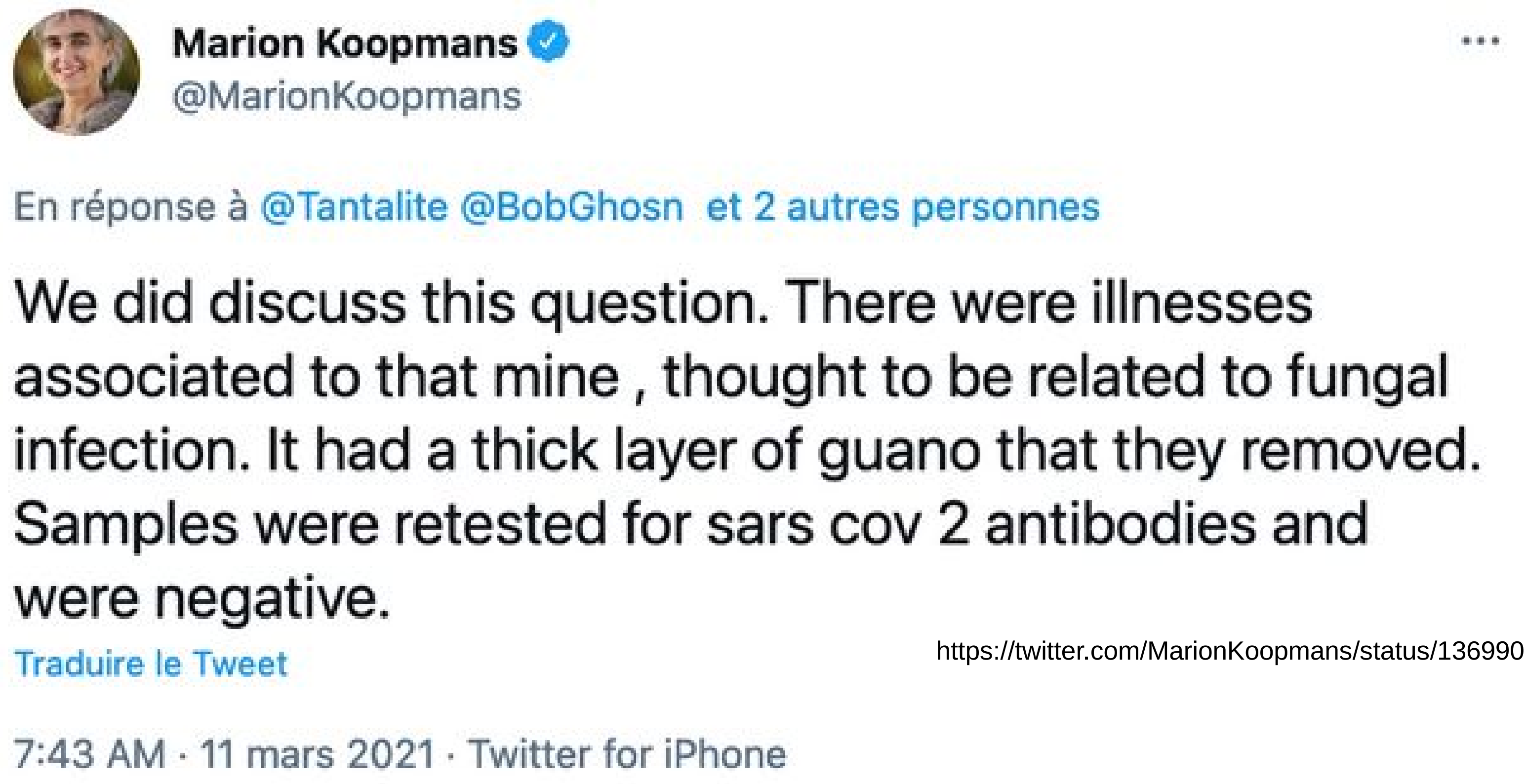
Wuhan-based virologist Shi Zhengli has identified dozens of deadly SARS-like viruses in bat caves, and she warns there are more out there

By Jane Qiu on June 1, 2020

Three years earlier Shi's team had been ca
profile of a mine shaft in Yunnan's mount
for its fermented Pu'er tea—where six min
pneumonialike diseases and two died. Aft
the researchers discovered a diverse grou
species. In many cases, multiple viral stra
turning it into a flying factory for new viru

"The mine shaft stunk like hell," says Shi, who, like her colleagues, went in wearing a protective mask and clothing. "Bat guano, covered in fungus, littered the cave." Although the fungus turned out to be the pathogen that had sickened the miners, she says it would have been only a matter of time before they caught the coronaviruses if the mine had not been promptly shut.

https://www.scientificamerican.com/article/how-chinas-bat-woman-hunted-down-viruses-from-sars-to-the-new-coronavirus1/



Marion Koopmans
@MarionKoopmans

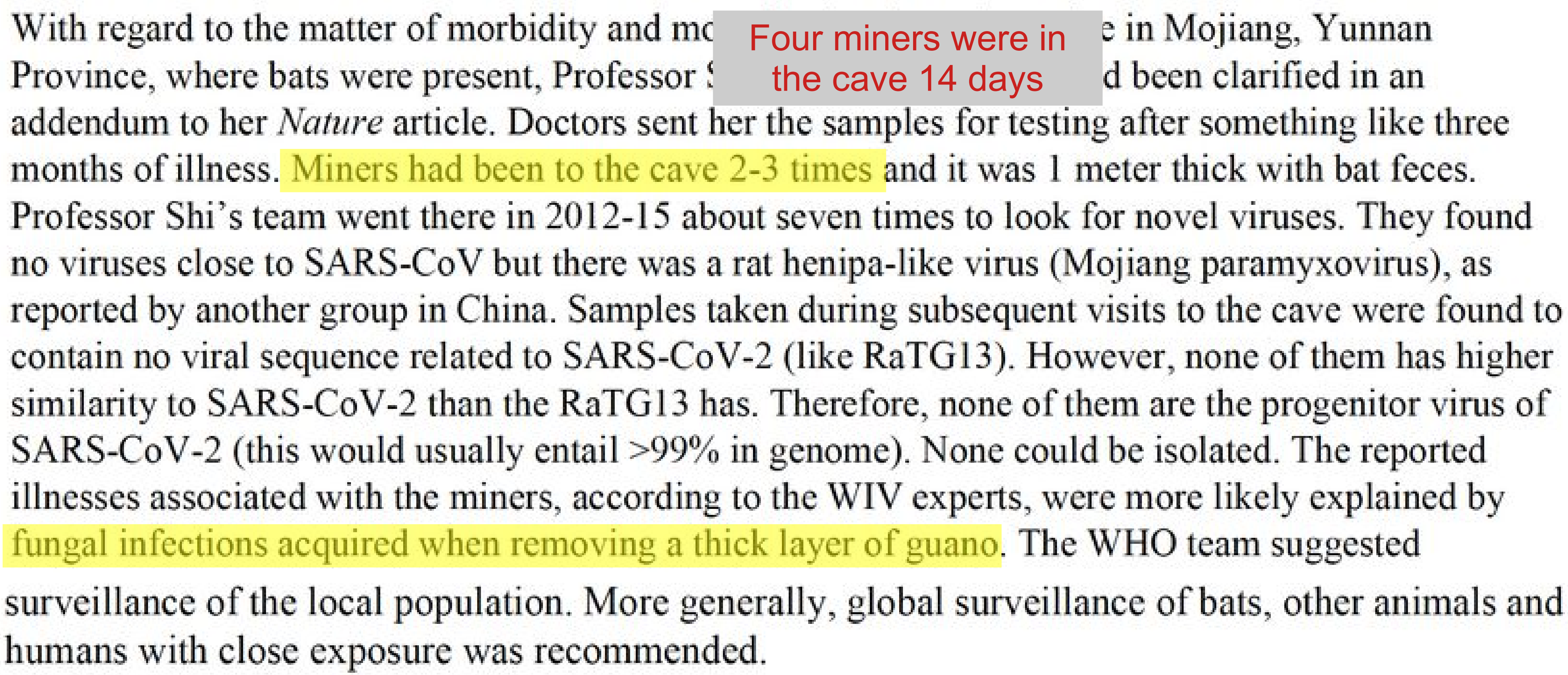
En réponse à @Tantalite @BobGhosn et 2 autres personnes

We did discuss this question. There were illnesses associated to that mine , thought to be related to fungal infection. It had a thick layer of guano that they removed. Samples were retested for sars cov 2 antibodies and were negative.

7:43 AM · 11 mars 2021 · Twitter for iPhone

https://twitter.com/MarionKoopmans/status/1369901874313330690?s=20

30 March 2021: WHO-China joint report



With regard to the matter of morbidity and mo e in Mojiang, Yunnan
Province, where bats were present, Professor S d been clarified in an
addendum to her *Nature* article. Doctors sent her the samples for testing after something like three months of illness. Miners had been to the cave 2-3 times and it was 1 meter thick with bat feces. Professor Shi's team went there in 2012-15 about seven times to look for novel viruses. They found no viruses close to SARS-CoV but there was a rat henipa-like virus (Mojiang paramyxovirus), as reported by another group in China. Samples taken during subsequent visits to the cave were found to contain no viral sequence related to SARS-CoV-2 (like RaTG13). However, none of them has higher similarity to SARS-CoV-2 than the RaTG13 has. Therefore, none of them are the progenitor virus of SARS-CoV-2 (this would usually entail >99% in genome). None could be isolated. The reported illnesses associated with the miners, according to the WIV experts, were more likely explained by fungal infections acquired when removing a thick layer of guano. The WHO team suggested surveillance of the local population. More generally, global surveillance of bats, other animals and humans with close exposure was recommended.

Four miners were in the cave 14 days

https://www.who.int/docs/default-source/coronaviruse/who-convened-global-study-of-origins-of-sars-cov-2-china-part-annexes.pdf

Issues with Zhou et al. Nature paper

Does not cite Zhang paper of the first SARS-CoV-2 genome sequence.

Text written as if RaTG13 had been sequenced in 2020.

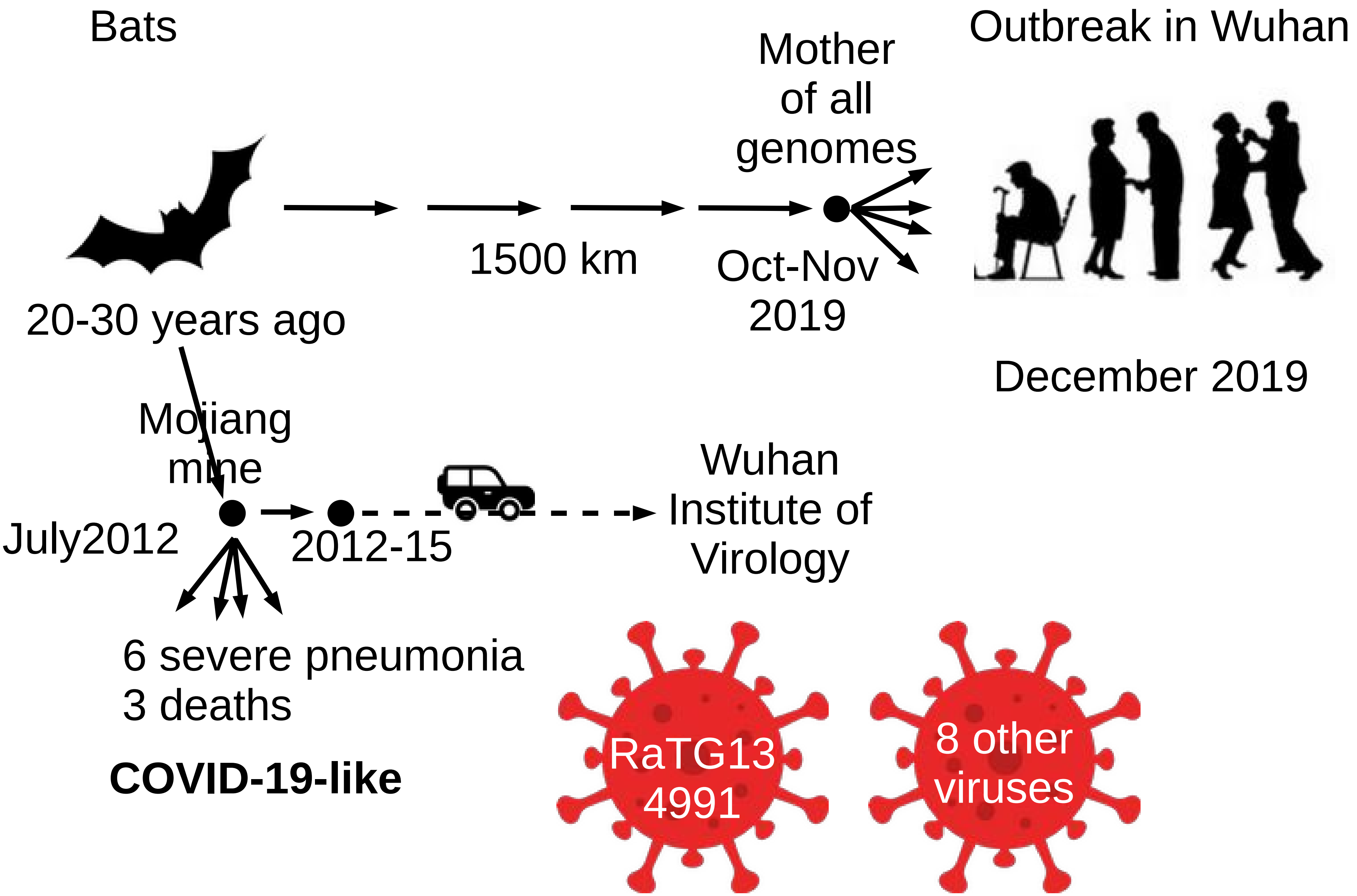
Does not cite her own Ge et al. 2016 paper about 4991.

Does not mention the 2012 pneumonia.

Does not provide method for RaTG13 genome assembly.

Mentions “no evidence for recombination events”.

Does not mention the furin cleavage site.



Bats

Outbreak in Wuhan

Mother
of all
genomes

1500 km

Oct-Nov
2019

20-30 years ago

December 2019

Mojiang
mine

July2012

2012-15

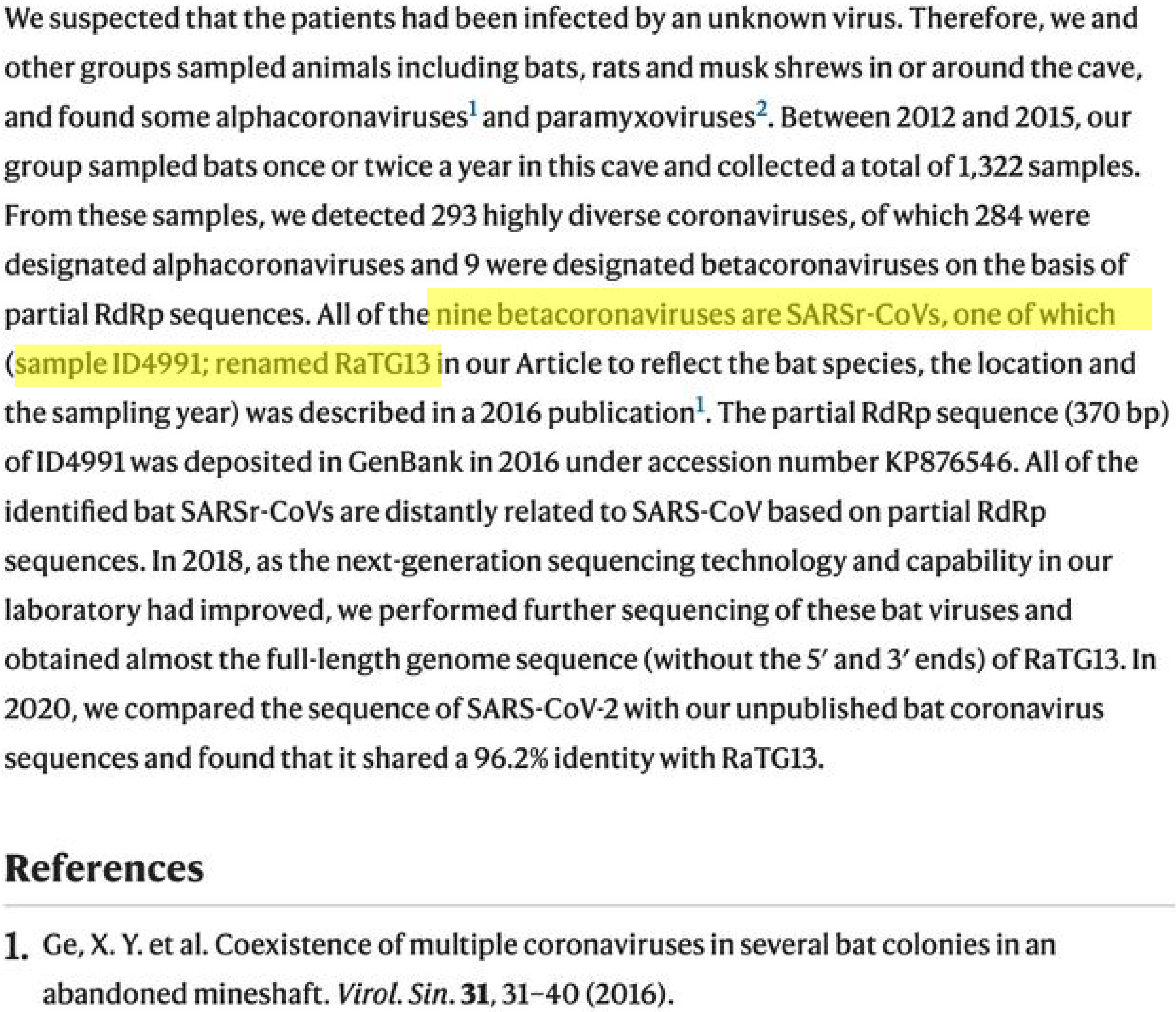
Wuhan
Institute of
Virology

6 severe pneumonia
3 deaths

COVID-19-like

RaTG13
4991

8 other
viruses



We suspected that the patients had been infected by an unknown virus. Therefore, we and other groups sampled animals including bats, rats and musk shrews in or around the cave, and found some alphacoronaviruses[1] and paramyxoviruses[2]. Between 2012 and 2015, our group sampled bats once or twice a year in this cave and collected a total of 1,322 samples. From these samples, we detected 293 highly diverse coronaviruses, of which 284 were designated alphacoronaviruses and 9 were designated betacoronaviruses on the basis of partial RdRp sequences. All of the nine betacoronaviruses are SARSr-CoVs, one of which (sample ID4991; renamed RaTG13 in our Article to reflect the bat species, the location and the sampling year) was described in a 2016 publication[1]. The partial RdRp sequence (370 bp) of ID4991 was deposited in GenBank in 2016 under accession number KP876546. All of the identified bat SARSr-CoVs are distantly related to SARS-CoV based on partial RdRp sequences. In 2018, as the next-generation sequencing technology and capability in our laboratory had improved, we performed further sequencing of these bat viruses and obtained almost the full-length genome sequence (without the 5′ and 3′ ends) of RaTG13. In 2020, we compared the sequence of SARS-CoV-2 with our unpublished bat coronavirus sequences and found that it shared a 96.2% identity with RaTG13.

References

1. Ge, X. Y. et al. Coexistence of multiple coronaviruses in several bat colonies in an abandoned mineshaft. *Virol. Sin.* **31**, 31–40 (2016).

https://www.nature.com/articles/s41586-020-2951-z

Conclusion

From RaTG13 to the 2012 pneumonia: information not promptly disclosed

Ongoing controversy about the cause of the pneumonia

8 other betacoronaviruses whose full genome sequence has not yet been disclosed

Difficult access to the mine