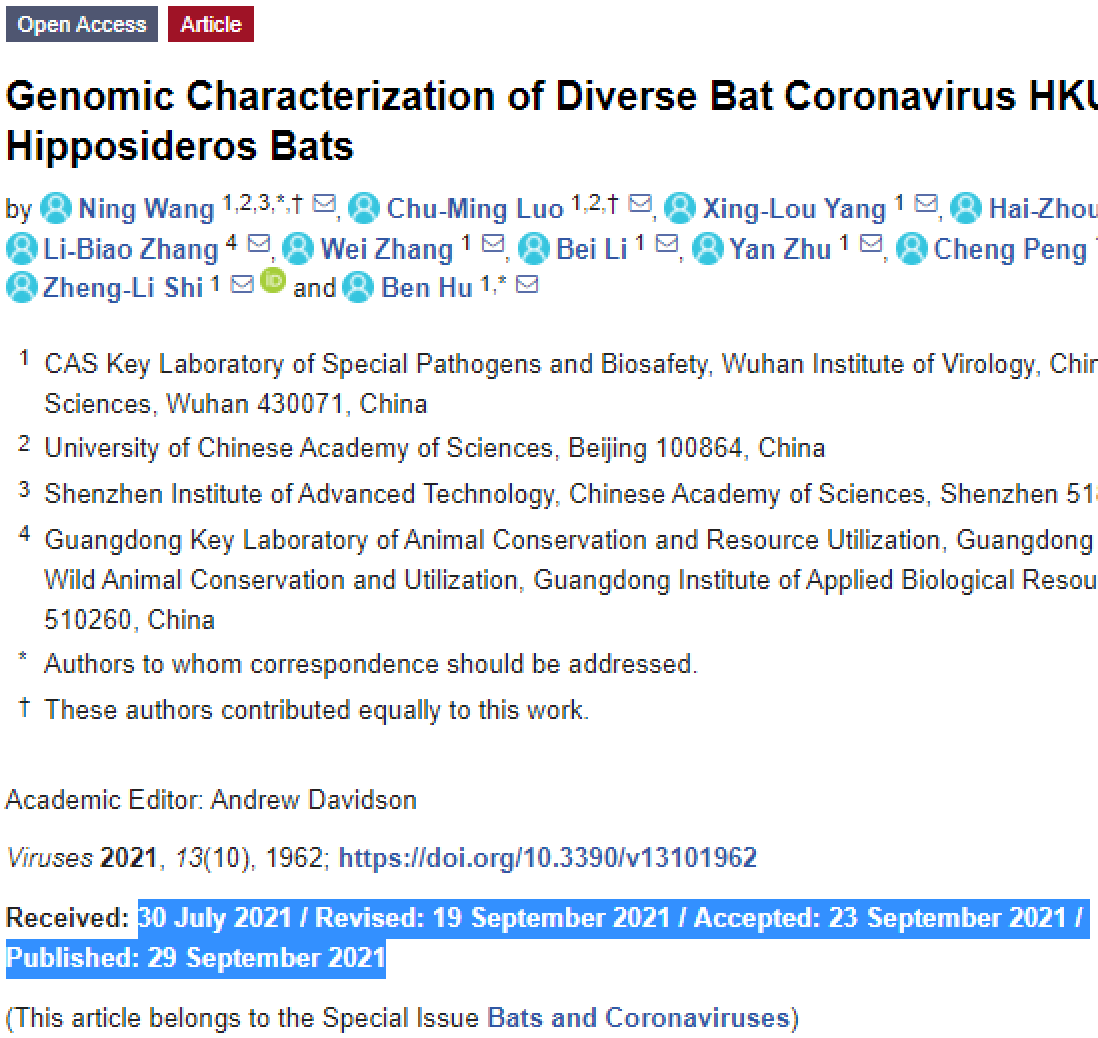
Twitter Thread by Francisco de Asis

Francisco de Asis
@franciscodeasis

[Thread] Wang et al. (2021):
Another article revealing trips to the Mojiang mine and Laos was kept in the limbo when the pandemic started.
Note: The article covers HKU10 viruses which are not related with SARS-like

It was "received" by the journal on 30 July 2021, but the sequences were accessioned in Genbank on 17-SEP-2019. So it seems that this article may have prior rejections or have been withdrawn from other journals.
https://t.co/laNmTabCuk



Open Access Article

Genomic Characterization of Diverse Bat Coronavirus HKU10 in Hipposideros Bats

by Ning Wang [1,2,3,*,†], Chu-Ming Luo [1,2,†], Xing-Lou Yang [1], Hai-Zhou Liu [1], Li-Biao Zhang [4], Wei Zhang [1], Bei Li [1], Yan Zhu [1], Cheng Peng [1], Zheng-Li Shi [1] and Ben Hu [1,*]

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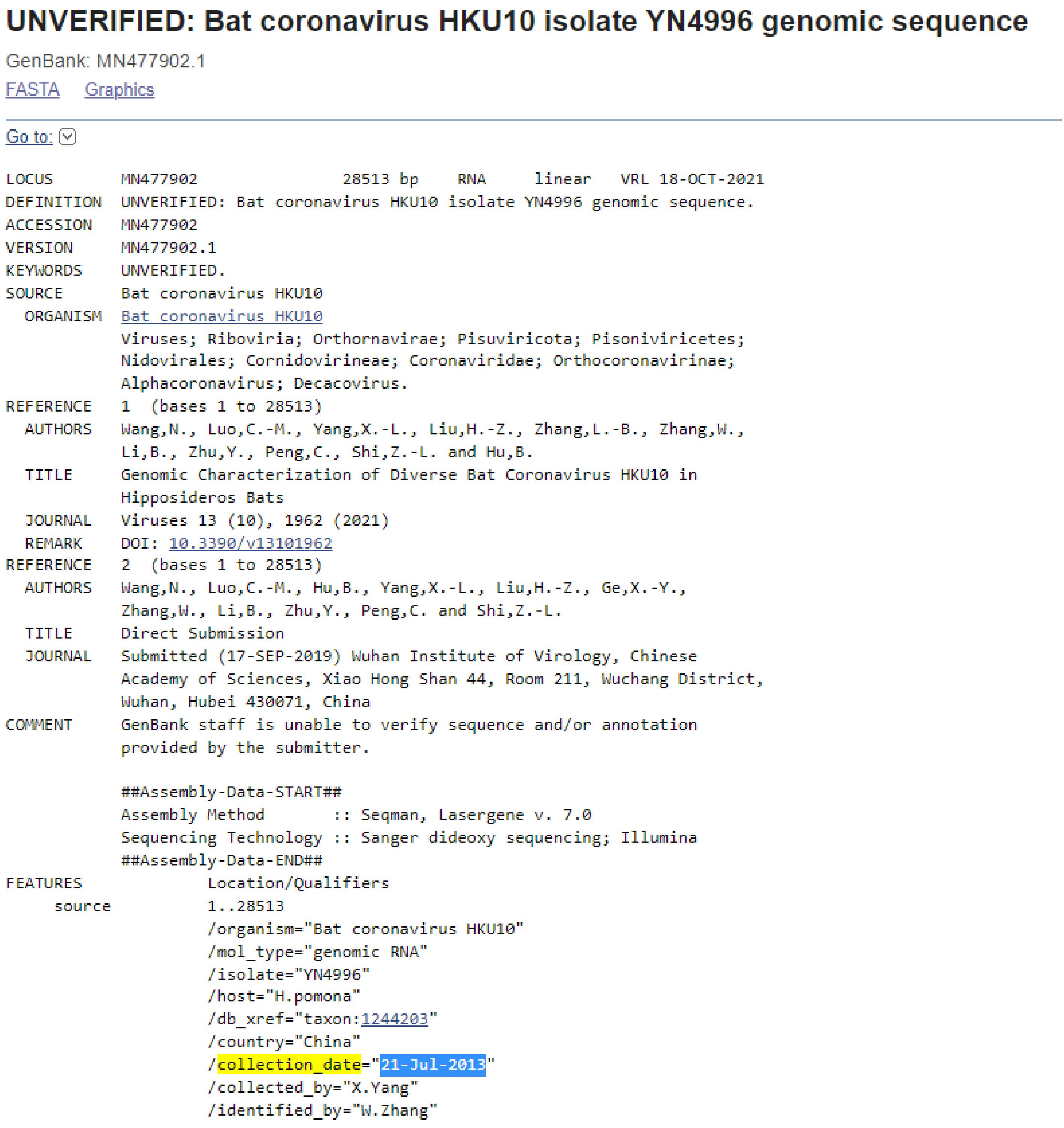
(This article belongs to the Special Issue Bats and Coronaviruses)

One of the samples is 4996 (note that RaTG13 = 4991).
There is a small discrepancy: 4991 was collected on 24-Jul-13 but this one on 21-Jul-13.
4996 is 100% identical to MJ_67C (from HKU team when they went to Mojiang) and clusters with 3723 (Mojiang).
https://t.co/XqMEyEi45T



UNVERIFIED: Bat coronavirus HKU10 isolate YN4996 genomic sequence

GenBank: MN477902.1

FASTA Graphics

Go to:

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DEFINITION  UNVERIFIED: Bat coronavirus HKU10 isolate YN4996 genomic sequence.
ACCESSION   MN477902
VERSION     MN477902.1
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SOURCE      Bat coronavirus HKU10
  ORGANISM  Bat coronavirus HKU10
            Viruses; Riboviria; Orthornavirae; Pisuviricota; Pisoniviricetes;
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            Alphacoronavirus; Decacovirus.
REFERENCE   1  (bases 1 to 28513)
  AUTHORS   Wang,N., Luo,C.-M., Yang,X.-L., Liu,H.-Z., Zhang,L.-B., Zhang,W.,
            Li,B., Zhu,Y., Peng,C., Shi,Z.-L. and Hu,B.
  TITLE     Genomic Characterization of Diverse Bat Coronavirus HKU10 in
            Hipposideros Bats
  JOURNAL   Viruses 13 (10), 1962 (2021)
  REMARK    DOI: 10.3390/v13101962
REFERENCE   2  (bases 1 to 28513)
  AUTHORS   Wang,N., Luo,C.-M., Hu,B., Yang,X.-L., Liu,H.-Z., Ge,X.-Y.,
            Zhang,W., Li,B., Zhu,Y., Peng,C. and Shi,Z.-L.
  TITLE     Direct Submission
  JOURNAL   Submitted (17-SEP-2019) Wuhan Institute of Virology, Chinese
            Academy of Sciences, Xiao Hong Shan 44, Room 211, Wuchang District,
            Wuhan, Hubei 430071, China
COMMENT     GenBank staff is unable to verify sequence and/or annotation
            provided by the submitter.

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            Sequencing Technology :: Sanger dideoxy sequencing; Illumina
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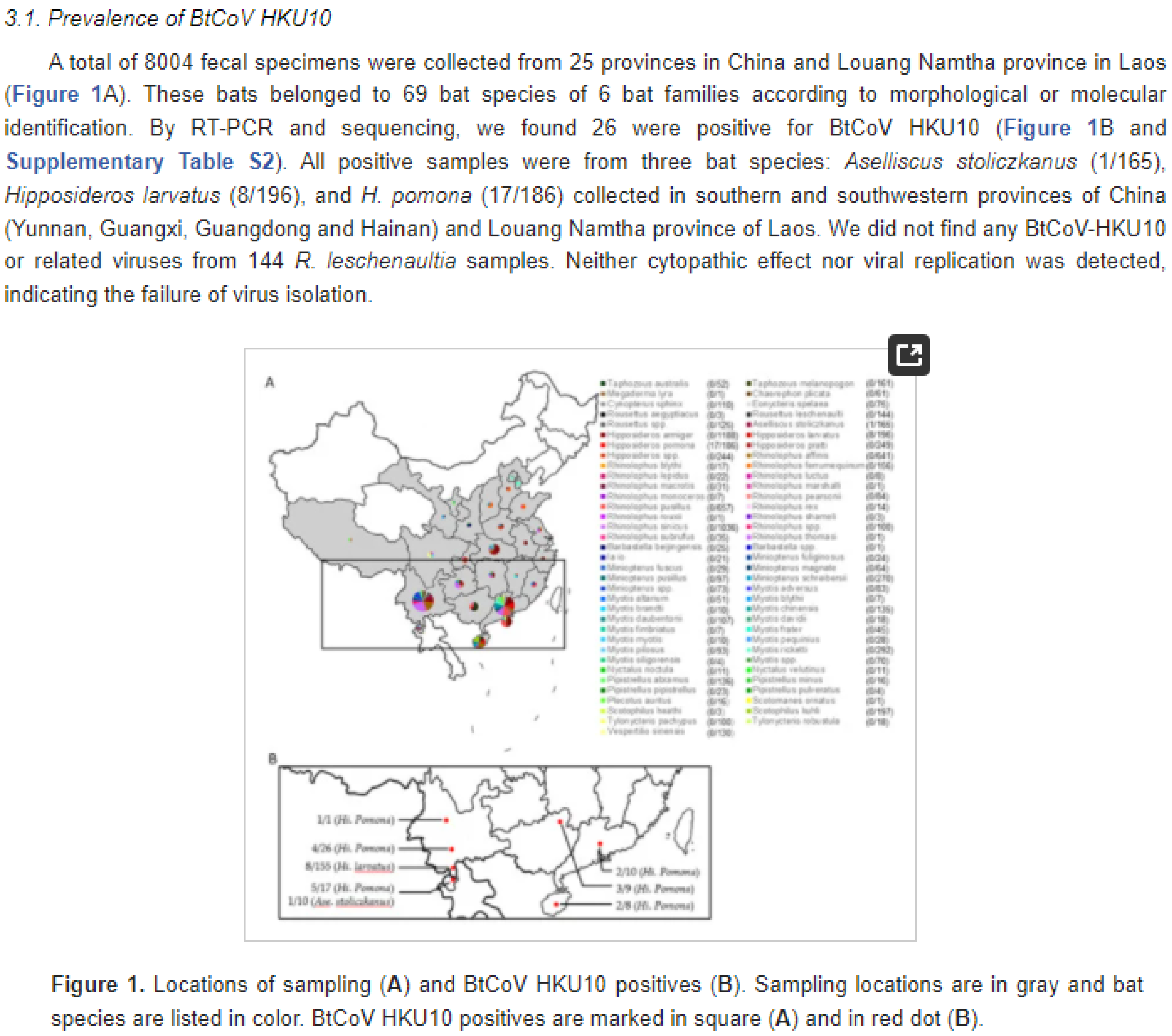
Btw, 3740 (Mojiang) and 7345 also cluster together, supporting that 7345 is from Tongguan/Mojiang as we already deduced a year ago.
https://t.co/gpwX4bhVcS

- Timeline of relevant visits to Yunnan for 2012-2015
- Probable samples from the mineshaft for 2014-2015 (detail and summary) pic.twitter.com/wNf3rWDCtQ

— Francisco de Asis (@franciscodeasis) November 28, 2020

The article does not explicitly say they collected samples from Mojiang, although the low-res map confirms it.



3.1. Prevalence of BtCoV HKU10

A total of 8004 fecal specimens were collected from 25 provinces in China and Louang Namtha province in Laos (**Figure 1**A). These bats belonged to 69 bat species of 6 bat families according to morphological or molecular identification. By RT-PCR and sequencing, we found 26 were positive for BtCoV HKU10 (**Figure** 1B and **Supplementary Table S2**). All positive samples were from three bat species: *Aselliscus stoliczkanus* (1/165), *Hipposideros larvatus* (8/196), and *H. pomona* (17/186) collected in southern and southwestern provinces of China (Yunnan, Guangxi, Guangdong and Hainan) and Louang Namtha province of Laos. We did not find any BtCoV-HKU10 or related viruses from 144 *R. leschenaultia* samples. Neither cytopathic effect nor viral replication was detected, indicating the failure of virus isolation.

Figure 1. Locations of sampling (**A**) and BtCoV HKU10 positives (**B**). Sampling locations are in gray and bat species are listed in color. BtCoV HKU10 positives are marked in square (**A**) and in red dot (**B**).

It is very surprising to see how they studied recombination events of 3 HKU10 viruses in the Mojiang mine (not mentioning the location) while showing great disregard for the SARS-like viruses also from there by not studying them at all.
Do you believe it?

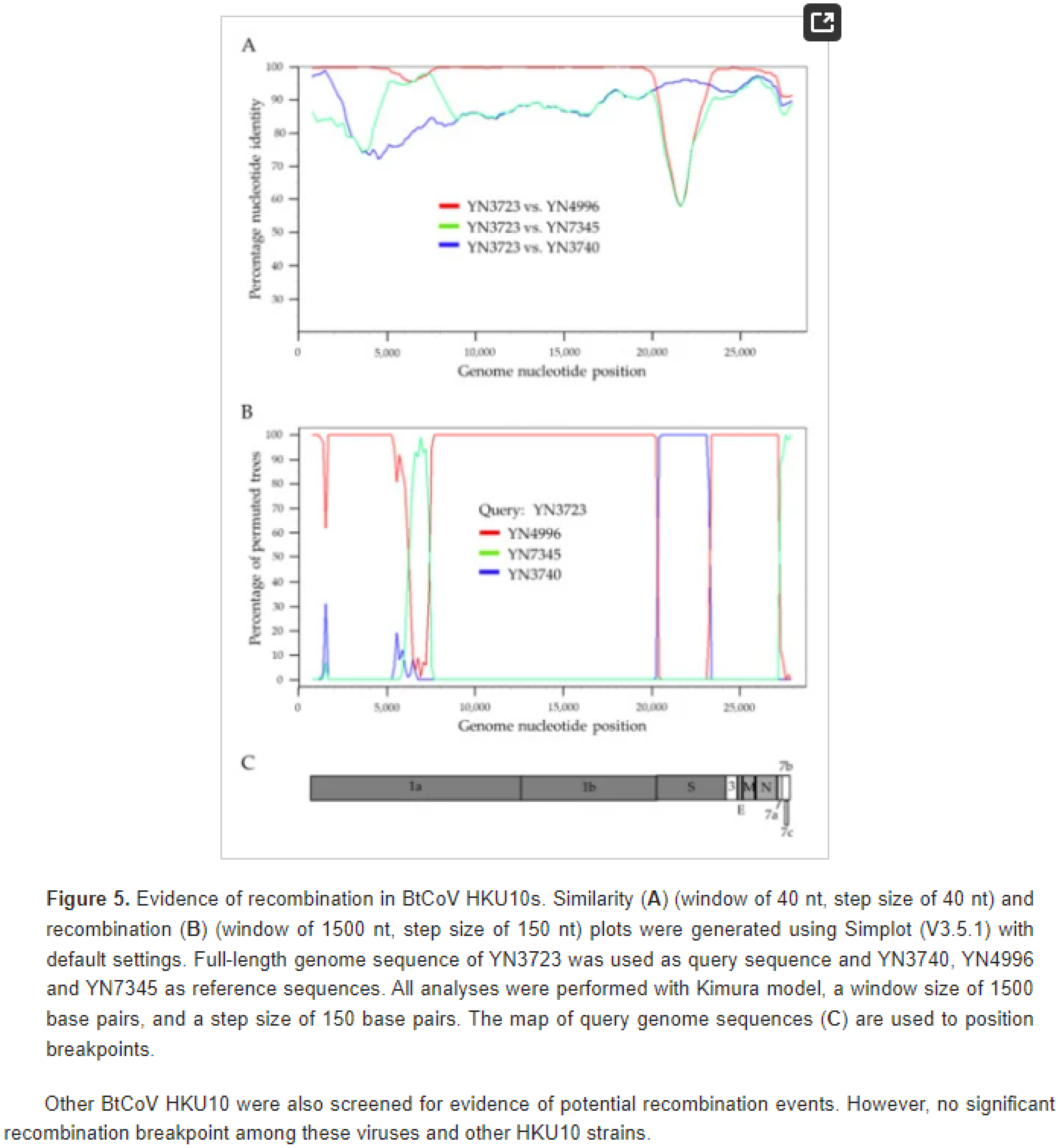


Figure 5. Evidence of recombination in BtCoV HKU10s. Similarity (**A**) (window of 40 nt, step size of 40 nt) and recombination (**B**) (window of 1500 nt, step size of 150 nt) plots were generated using Simplot (V3.5.1) with default settings. Full-length genome sequence of YN3723 was used as query sequence and YN3740, YN4996 and YN7345 as reference sequences. All analyses were performed with Kimura model, a window size of 1500 base pairs, and a step size of 150 base pairs. The map of query genome sequences (**C**) are used to position breakpoints.

Other BtCoV HKU10 were also screened for evidence of potential recombination events. However, no significant recombination breakpoint among these viruses and other HKU10 strains.

And we can now confirm Laos location for Latinne et al. (2020) https://t.co/CQqIY5xZAb

> Samples of Laos of Latinne et al. (2020) probably from Luang Namtha (same as what they said in a WIV thesis). Not far from BANAL location
>
> — Francisco de Asis (@franciscodeasis) September 23, 2021

This author Wang is the 2nd author of Ge et al. (2016), who published 4991. Her thesis is the precursor of this article and also of Wang et al. (2019).
https://t.co/0C4Lw1VkRN

第 3 章 结果

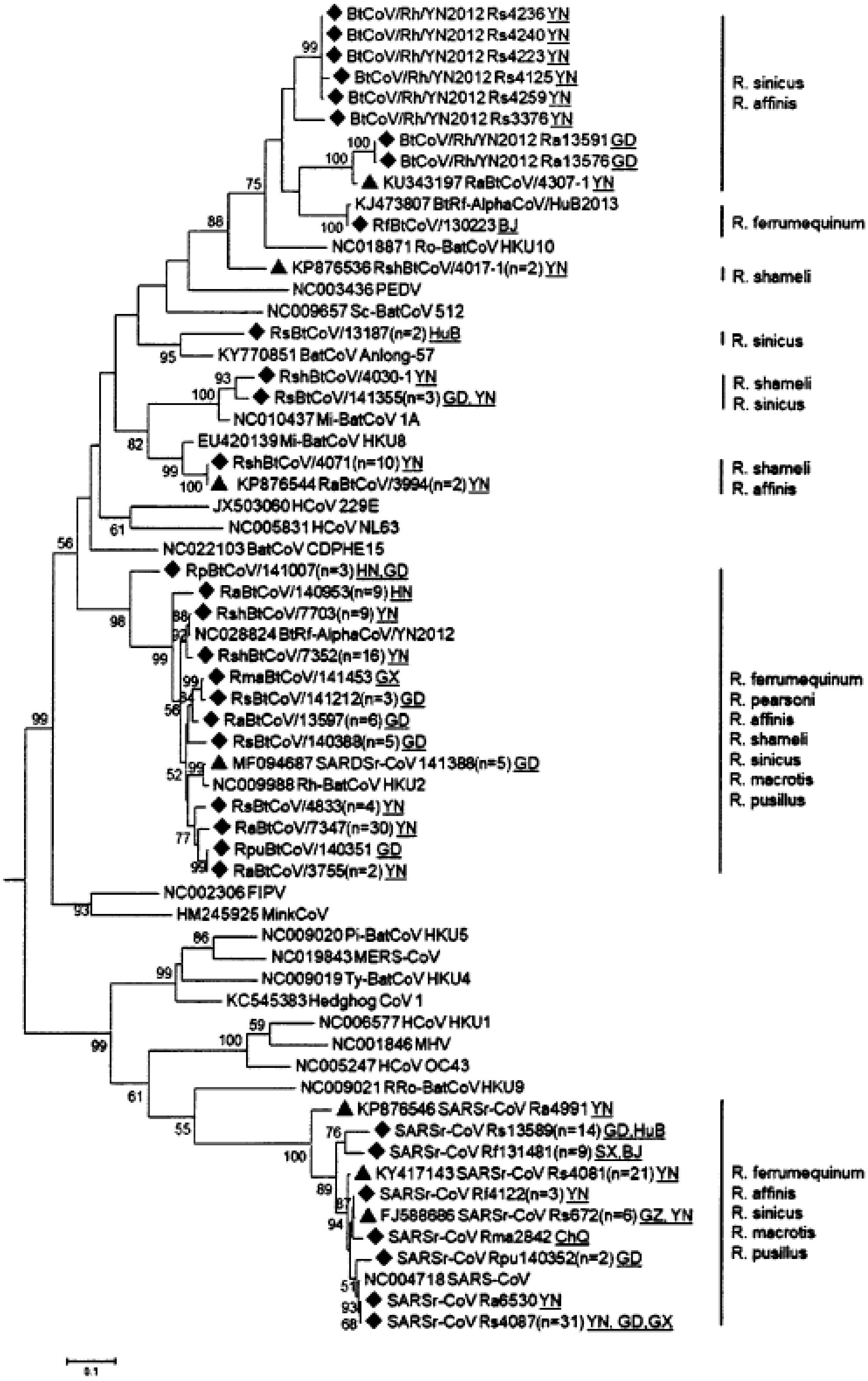


图 3.2 以菊头蝠中检测到的冠状病毒 RdRp 序列（327-bp）构建的系统进化树。

35

Wang (2018) \u8759\u8760\u65b0\u578b\u03b1\u51a0\u72b6\u75c5\u6bd2\u7684\u53d1\u73b0\u548cSARS\u76f8\u5173\u51a0\u72b6\u75c

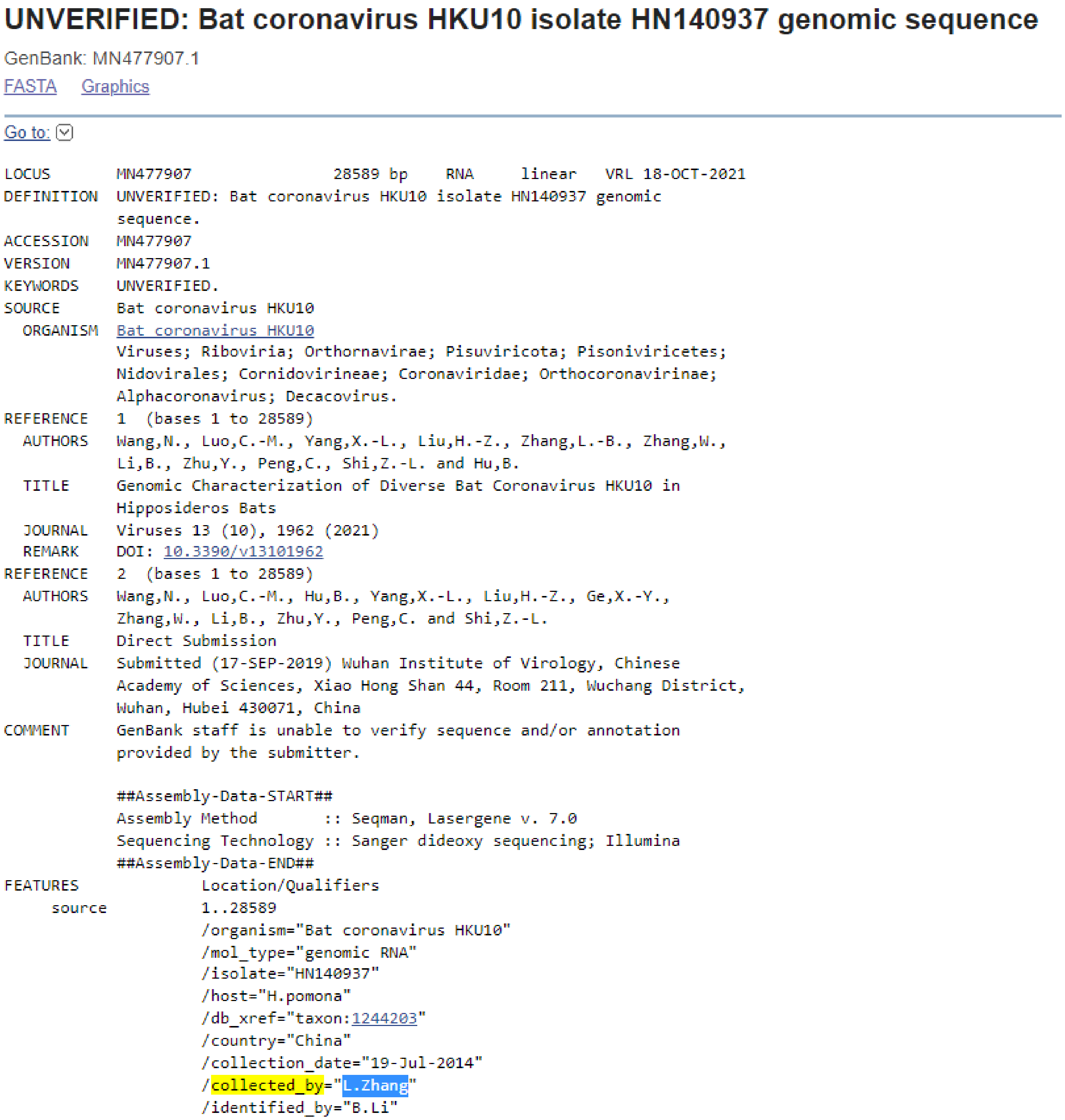
Discovery of novel bat alphacoronaviruses and SARS-related coronavirus serological detection in human.

cc @TheSeeker268 pic.twitter.com/bCWOPyv1J1

— Francisco de Asis (@franciscodeasis) November 21, 2021

Another interesting thing: Wang appears again as affiliated to WIV. Did she ended her academic stay with Shibo Jiang in NY? Or maybe it is because this is an "old" article?

LBZ is also a co-author. But only one accession acknowledge him as collector (HN140937 from Hainan) https://t.co/rJbP78MGvv



UNVERIFIED: Bat coronavirus HKU10 isolate HN140937 genomic sequence

GenBank: MN477907.1

FASTA Graphics

Go to:

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REFERENCE   1  (bases 1 to 28589)
  AUTHORS   Wang,N., Luo,C.-M., Yang,X.-L., Liu,H.-Z., Zhang,L.-B., Zhang,W.,
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  TITLE     Genomic Characterization of Diverse Bat Coronavirus HKU10 in
            Hipposideros Bats
  JOURNAL   Viruses 13 (10), 1962 (2021)
  REMARK    DOI: 10.3390/v13101962
REFERENCE   2  (bases 1 to 28589)
  AUTHORS   Wang,N., Luo,C.-M., Hu,B., Yang,X.-L., Liu,H.-Z., Ge,X.-Y.,
            Zhang,W., Li,B., Zhu,Y., Peng,C. and Shi,Z.-L.
  TITLE     Direct Submission
  JOURNAL   Submitted (17-SEP-2019) Wuhan Institute of Virology, Chinese
            Academy of Sciences, Xiao Hong Shan 44, Room 211, Wuchang District,
            Wuhan, Hubei 430071, China
COMMENT     GenBank staff is unable to verify sequence and/or annotation
            provided by the submitter.

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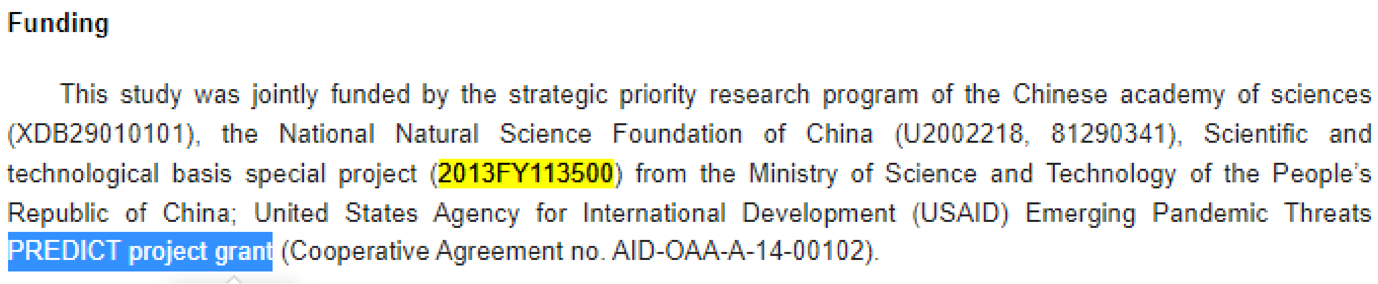
Famous 2013FY113500 grant and PREDICT grants again:



Funding

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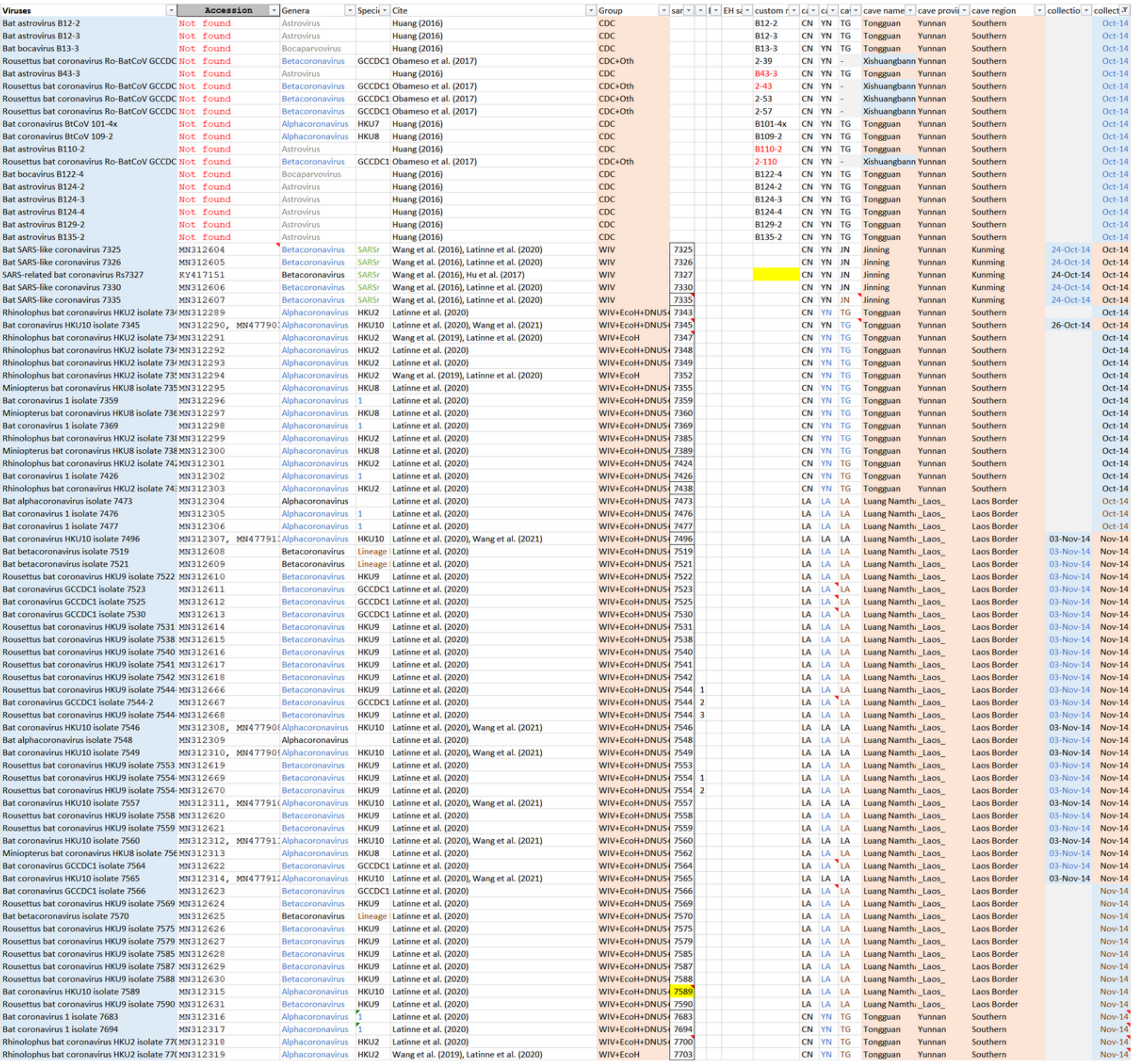
Apparently all accessions are now released because the accessions immediately before and after this range are published and used by other teams (MN477898 and MN477916).
But who knows, there could be interesting emails like those of Ben Hu regarding the delay of Latinne's seqs.

The records were not released when the article was published. I asked NCBI staff to release them since the article was finally published.
Are there more articles in the limbo or more sequences accessioned before the pandemic but not released yet?

What happened between 26-Oct-14 and 03-Nov-14? and after going to Laos? back to the mine? (6th trip)
There are some gaps of IDs, e.g. between last ID from Laos (7590) and 1st ID from the mine (7683). 92 samples is more than the average outcome for a day.
https://t.co/8J00bs8uwj



Viruses	Accession	Genera	Speci	Cite	Group	sar		E	EH s	custom r	ca	ca	ca	cave name	cave provi	cave region	collectio	collect
Bat astrovirus B12-2	Not found	Astrovirus		Huang (2016)	CDC					B12-2	CN	YN	TG	Tongguan	Yunnan	Southern		Oct-14
Bat astrovirus B12-3	Not found	Astrovirus		Huang (2016)	CDC					B12-3	CN	YN	TG	Tongguan	Yunnan	Southern		Oct-14
Bat bocavirus B13-3	Not found	Bocaparvovirus		Huang (2016)	CDC					B13-3	CN	YN	TG	Tongguan	Yunnan	Southern		Oct-14
Rousettus bat coronavirus Ro-BatCoV GCCDC	Not found	Betacoronavirus	GCCDC1	Obameso et al. (2017)	CDC+Oth					2-39	CN	YN	-	Xishuangbann	Yunnan	Southern		Oct-14
Bat astrovirus B43-3	Not found	Astrovirus		Huang (2016)	CDC					B43-3	CN	YN	TG	Tongguan	Yunnan	Southern		Oct-14
Rousettus bat coronavirus Ro-BatCoV GCCDC	Not found	Betacoronavirus	GCCDC1	Obameso et al. (2017)	CDC+Oth					2-43	CN	YN	-	Xishuangbann	Yunnan	Southern		Oct-14
Rousettus bat coronavirus Ro-BatCoV GCCDC	Not found	Betacoronavirus	GCCDC1	Obameso et al. (2017)	CDC+Oth					2-53	CN	YN	-	Xishuangbann	Yunnan	Southern		Oct-14
Rousettus bat coronavirus Ro-BatCoV GCCDC	Not found	Betacoronavirus	GCCDC1	Obameso et al. (2017)	CDC+Oth					2-57	CN	YN	-	Xishuangbann	Yunnan	Southern		Oct-14
Bat coronavirus BtCoV 101-4x	Not found	Alphacoronavirus	HKU7	Huang (2016)	CDC					B101-4x	CN	YN	TG	Tongguan	Yunnan	Southern		Oct-14
Bat coronavirus BtCoV 109-2	Not found	Alphacoronavirus	HKU8	Huang (2016)	CDC					B109-2	CN	YN	TG	Tongguan	Yunnan	Southern		Oct-14
Bat astrovirus B110-2	Not found	Astrovirus		Huang (2016)	CDC					B110-2	CN	YN	TG	Tongguan	Yunnan	Southern		Oct-14
Rousettus bat coronavirus Ro-BatCoV GCCDC	Not found	Betacoronavirus	GCCDC1	Obameso et al. (2017)	CDC+Oth					2-110	CN	YN	-	Xishuangbann	Yunnan	Southern		Oct-14
Bat bocavirus B122-4	Not found	Bocaparvovirus		Huang (2016)	CDC					B122-4	CN	YN	TG	Tongguan	Yunnan	Southern		Oct-14
Bat astrovirus B124-2	Not found	Astrovirus		Huang (2016)	CDC					B124-2	CN	YN	TG	Tongguan	Yunnan	Southern		Oct-14
Bat astrovirus B124-3	Not found	Astrovirus		Huang (2016)	CDC					B124-3	CN	YN	TG	Tongguan	Yunnan	Southern		Oct-14
Bat astrovirus B124-4	Not found	Astrovirus		Huang (2016)	CDC					B124-4	CN	YN	TG	Tongguan	Yunnan	Southern		Oct-14
Bat astrovirus B129-2	Not found	Astrovirus		Huang (2016)	CDC					B129-2	CN	YN	TG	Tongguan	Yunnan	Southern		Oct-14
Bat astrovirus B135-2	Not found	Astrovirus		Huang (2016)	CDC					B135-2	CN	YN	TG	Tongguan	Yunnan	Southern		Oct-14
Bat SARS-like coronavirus 7325	MN312604	Betacoronavirus	SARSr	Wang et al. (2016), Latinne et al. (2020)	WIV	7325					CN	YN	JN	Jinning	Yunnan	Kunming	24-Oct-14	Oct-14
Bat SARS-like coronavirus 7326	MN312605	Betacoronavirus	SARSr	Wang et al. (2016), Latinne et al. (2020)	WIV	7326					CN	YN	JN	Jinning	Yunnan	Kunming	24-Oct-14	Oct-14
SARS-related bat coronavirus Rs7327	KY417151	Betacoronavirus	SARSr	Wang et al. (2016), Hu et al. (2017)	WIV	7327					CN	YN	JN	Jinning	Yunnan	Kunming	24-Oct-14	Oct-14
Bat SARS-like coronavirus 7330	MN312606	Betacoronavirus	SARSr	Wang et al. (2016), Latinne et al. (2020)	WIV	7330					CN	YN	JN	Jinning	Yunnan	Kunming	24-Oct-14	Oct-14
Bat SARS-like coronavirus 7335	MN312607	Betacoronavirus	SARSr	Wang et al. (2016), Latinne et al. (2020)	WIV	7335					CN	YN	JN	Jinning	Yunnan	Kunming	24-Oct-14	Oct-14
Rhinolophus bat coronavirus HKU2 isolate 734	MN312289	Alphacoronavirus	HKU2	Latinne et al. (2020)	WIV+EcoH+DNUS	7343					CN	YN	TG	Tongguan	Yunnan	Southern		Oct-14
Bat coronavirus HKU10 isolate 7345	MN312290, MN47790	Alphacoronavirus	HKU10	Latinne et al. (2020), Wang et al. (2021)	WIV+EcoH+DNUS	7345					CN	YN	TG	Tongguan	Yunnan	Southern	26-Oct-14	Oct-14
Rhinolophus bat coronavirus HKU2 isolate 734	MN312291	Alphacoronavirus	HKU2	Wang et al. (2019), Latinne et al. (2020)	WIV+EcoH	7347					CN	YN	TG	Tongguan	Yunnan	Southern		Oct-14
Rhinolophus bat coronavirus HKU2 isolate 734	MN312292	Alphacoronavirus	HKU2	Latinne et al. (2020)	WIV+EcoH+DNUS	7348					CN	YN	TG	Tongguan	Yunnan	Southern		Oct-14
Rhinolophus bat coronavirus HKU2 isolate 734	MN312293	Alphacoronavirus	HKU2	Latinne et al. (2020)	WIV+EcoH+DNUS	7349					CN	YN	TG	Tongguan	Yunnan	Southern		Oct-14
Rhinolophus bat coronavirus HKU2 isolate 735	MN312294	Alphacoronavirus	HKU2	Wang et al. (2019), Latinne et al. (2020)	WIV+EcoH	7352					CN	YN	TG	Tongguan	Yunnan	Southern		Oct-14
Miniopterus bat coronavirus HKU8 isolate 735	MN312295	Alphacoronavirus	HKU8	Latinne et al. (2020)	WIV+EcoH+DNUS	7355					CN	YN	TG	Tongguan	Yunnan	Southern		Oct-14
Bat coronavirus 1 isolate 7359	MN312296	Alphacoronavirus	1	Latinne et al. (2020)	WIV+EcoH+DNUS	7359					CN	YN	TG	Tongguan	Yunnan	Southern		Oct-14
Miniopterus bat coronavirus HKU8 isolate 736	MN312297	Alphacoronavirus	HKU8	Latinne et al. (2020)	WIV+EcoH+DNUS	7360					CN	YN	TG	Tongguan	Yunnan	Southern		Oct-14
Bat coronavirus 1 isolate 7369	MN312298	Alphacoronavirus	1	Latinne et al. (2020)	WIV+EcoH+DNUS	7369					CN	YN	TG	Tongguan	Yunnan	Southern		Oct-14
Rhinolophus bat coronavirus HKU2 isolate 738	MN312299	Alphacoronavirus	HKU2	Latinne et al. (2020)	WIV+EcoH+DNUS	7385					CN	YN	TG	Tongguan	Yunnan	Southern		Oct-14
Miniopterus bat coronavirus HKU8 isolate 738	MN312300	Alphacoronavirus	HKU8	Latinne et al. (2020)	WIV+EcoH+DNUS	7389					CN	YN	TG	Tongguan	Yunnan	Southern		Oct-14
Rhinolophus bat coronavirus HKU2 isolate 742	MN312301	Alphacoronavirus	HKU2	Latinne et al. (2020)	WIV+EcoH+DNUS	7424					CN	YN	TG	Tongguan	Yunnan	Southern		Oct-14
Bat coronavirus 1 isolate 7426	MN312302	Alphacoronavirus	1	Latinne et al. (2020)	WIV+EcoH+DNUS	7426					CN	YN	TG	Tongguan	Yunnan	Southern		Oct-14
Rhinolophus bat coronavirus HKU2 isolate 743	MN312303	Alphacoronavirus	HKU2	Latinne et al. (2020)	WIV+EcoH+DNUS	7438					CN	YN	TG	Tongguan	Yunnan	Southern		Oct-14
Bat alphacoronavirus isolate 7473	MN312304	Alphacoronavirus		Latinne et al. (2020)	WIV+EcoH+DNUS	7473					LA	LA	LA	Luang Namth	_Laos_	Laos Border		Oct-14
Bat coronavirus 1 isolate 7476	MN312305	Alphacoronavirus	1	Latinne et al. (2020)	WIV+EcoH+DNUS	7476					LA	LA	LA	Luang Namth	_Laos_	Laos Border		Oct-14
Bat coronavirus 1 isolate 7477	MN312306	Alphacoronavirus	1	Latinne et al. (2020)	WIV+EcoH+DNUS	7477					LA	LA	LA	Luang Namth	_Laos_	Laos Border		Oct-14
Bat coronavirus HKU10 isolate 7496	MN312307, MN47791	Alphacoronavirus	HKU10	Latinne et al. (2020), Wang et al. (2021)	WIV+EcoH+DNUS	7496					LA	LA	LA	Luang Namth	_Laos_	Laos Border	03-Nov-14	Nov-14
Bat betacoronavirus isolate 7519	MN312608	Betacoronavirus	Lineage	Latinne et al. (2020)	WIV+EcoH+DNUS	7519					LA	LA	LA	Luang Namth	_Laos_	Laos Border	03-Nov-14	Nov-14
Bat betacoronavirus isolate 7521	MN312609	Betacoronavirus	Lineage	Latinne et al. (2020)	WIV+EcoH+DNUS	7521					LA	LA	LA	Luang Namth	_Laos_	Laos Border	03-Nov-14	Nov-14
Rousettus bat coronavirus HKU9 isolate 7522	MN312610	Betacoronavirus	HKU9	Latinne et al. (2020)	WIV+EcoH+DNUS	7522					LA	LA	LA	Luang Namth	_Laos_	Laos Border	03-Nov-14	Nov-14
Bat coronavirus GCCDC1 isolate 7523	MN312611	Betacoronavirus	GCCDC1	Latinne et al. (2020)	WIV+EcoH+DNUS	7523					LA	LA	LA	Luang Namth	_Laos_	Laos Border	03-Nov-14	Nov-14
Bat coronavirus GCCDC1 isolate 7525	MN312612	Betacoronavirus	GCCDC1	Latinne et al. (2020)	WIV+EcoH+DNUS	7525					LA	LA	LA	Luang Namth	_Laos_	Laos Border	03-Nov-14	Nov-14
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Rousettus bat coronavirus HKU9 isolate 7538	MN312615	Betacoronavirus	HKU9	Latinne et al. (2020)	WIV+EcoH+DNUS	7538					LA	LA	LA	Luang Namth	_Laos_	Laos Border	03-Nov-14	Nov-14
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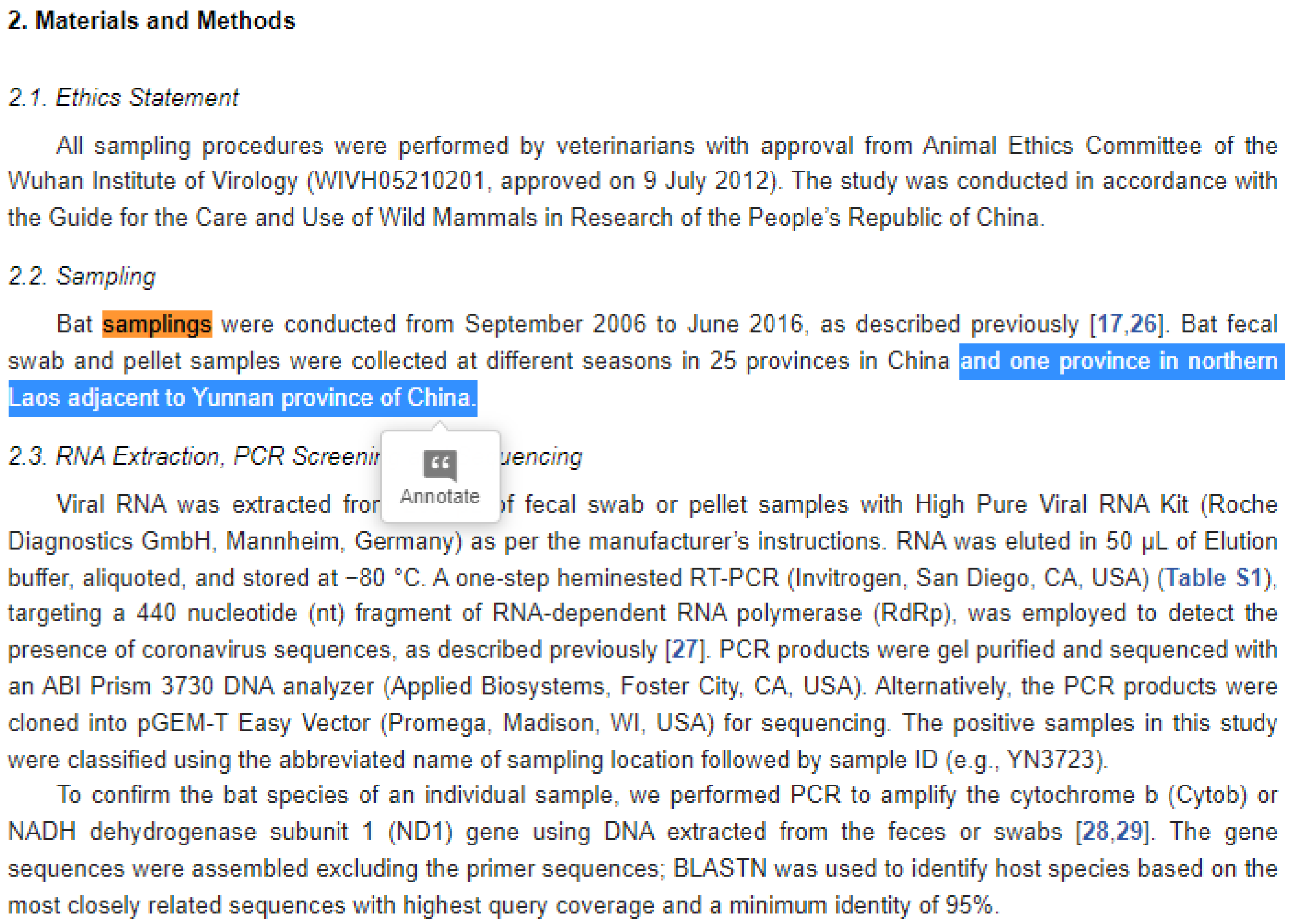
Rousettus bat coronavirus HKU9 isolate 7541	MN312617	Betacoronavirus	HKU9	Latinne et al. (2020)	WIV+EcoH+DNUS	7541					LA	LA	LA	Luang Namth	_Laos_	Laos Border	03-Nov-14	Nov-14
Rousettus bat coronavirus HKU9 isolate 7542	MN312618	Betacoronavirus	HKU9	Latinne et al. (2020)	WIV+EcoH+DNUS	7542					LA	LA	LA	Luang Namth	_Laos_	Laos Border	03-Nov-14	Nov-14
Rousettus bat coronavirus HKU9 isolate 7544-	MN312666	Betacoronavirus	HKU9	Latinne et al. (2020)	WIV+EcoH+DNUS	7544	1				LA	LA	LA	Luang Namth	_Laos_	Laos Border	03-Nov-14	Nov-14
Bat coronavirus GCCDC1 isolate 7544-2	MN312667	Betacoronavirus	GCCDC1	Latinne et al. (2020)	WIV+EcoH+DNUS	7544	2				LA	LA	LA	Luang Namth	_Laos_	Laos Border	03-Nov-14	Nov-14
Rousettus bat coronavirus HKU9 isolate 7544-	MN312668	Betacoronavirus	HKU9	Latinne et al. (2020)	WIV+EcoH+DNUS	7544	3				LA	LA	LA	Luang Namth	_Laos_	Laos Border	03-Nov-14	Nov-14
Bat coronavirus HKU10 isolate 7546	MN312308, MN47790	Alphacoronavirus	HKU10	Latinne et al. (2020), Wang et al. (2021)	WIV+EcoH+DNUS	7546					LA	LA	LA	Luang Namth	_Laos_	Laos Border	03-Nov-14	Nov-14
Bat alphacoronavirus isolate 7548	MN312309	Alphacoronavirus		Latinne et al. (2020)	WIV+EcoH+DNUS	7548					LA	LA	LA	Luang Namth	_Laos_	Laos Border	03-Nov-14	Nov-14
Bat coronavirus HKU10 isolate 7549	MN312310, MN47790	Alphacoronavirus	HKU10	Latinne et al. (2020), Wang et al. (2021)	WIV+EcoH+DNUS	7549					LA	LA	LA	Luang Namth	_Laos_	Laos Border	03-Nov-14	Nov-14
Rousettus bat coronavirus HKU9 isolate 7553	MN312619	Betacoronavirus	HKU9	Latinne et al. (2020)	WIV+EcoH+DNUS	7553					LA	LA	LA	Luang Namth	_Laos_	Laos Border	03-Nov-14	Nov-14
Rousettus bat coronavirus HKU9 isolate 7554-	MN312669	Betacoronavirus	HKU9	Latinne et al. (2020)	WIV+EcoH+DNUS	7554	1				LA	LA	LA	Luang Namth	_Laos_	Laos Border	03-Nov-14	Nov-14
Rousettus bat coronavirus HKU9 isolate 7554-	MN312670	Betacoronavirus	HKU9	Latinne et al. (2020)	WIV+EcoH+DNUS	7554	2				LA	LA	LA	Luang Namth	_Laos_	Laos Border	03-Nov-14	Nov-14
Bat coronavirus HKU10 isolate 7557	MN312311, MN47791	Alphacoronavirus	HKU10	Latinne et al. (2020), Wang et al. (2021)	WIV+EcoH+DNUS	7557					LA	LA	LA	Luang Namth	_Laos_	Laos Border	03-Nov-14	Nov-14
Rousettus bat coronavirus HKU9 isolate 7558	MN312620	Betacoronavirus	HKU9	Latinne et al. (2020)	WIV+EcoH+DNUS	7558					LA	LA	LA	Luang Namth	_Laos_	Laos Border	03-Nov-14	Nov-14
Rousettus bat coronavirus HKU9 isolate 7559	MN312621	Betacoronavirus	HKU9	Latinne et al. (2020)	WIV+EcoH+DNUS	7559					LA	LA	LA	Luang Namth	_Laos_	Laos Border	03-Nov-14	Nov-14
Bat coronavirus HKU10 isolate 7560	MN312312, MN47791	Alphacoronavirus	HKU10	Latinne et al. (2020), Wang et al. (2021)	WIV+EcoH+DNUS	7560					LA	LA	LA	Luang Namth	_Laos_	Laos Border	03-Nov-14	Nov-14
Miniopterus bat coronavirus HKU8 isolate 756	MN312313	Alphacoronavirus	HKU8	Latinne et al. (2020)	WIV+EcoH+DNUS	7562					LA	LA	LA	Luang Namth	_Laos_	Laos Border	03-Nov-14	Nov-14
Bat coronavirus GCCDC1 isolate 7564	MN312622	Betacoronavirus	GCCDC1	Latinne et al. (2020)	WIV+EcoH+DNUS	7564					LA	LA	LA	Luang Namth	_Laos_	Laos Border	03-Nov-14	Nov-14
Bat coronavirus HKU10 isolate 7565	MN312314, MN47791	Alphacoronavirus	HKU10	Latinne et al. (2020), Wang et al. (2021)	WIV+EcoH+DNUS	7565					LA	LA	LA	Luang Namth	_Laos_	Laos Border	03-Nov-14	Nov-14
Bat coronavirus GCCDC1 isolate 7566	MN312623	Betacoronavirus	GCCDC1	Latinne et al. (2020)	WIV+EcoH+DNUS	7566					LA	LA	LA	Luang Namth	_Laos_	Laos Border		Nov-14
Rousettus bat coronavirus HKU9 isolate 7569	MN312624	Betacoronavirus	HKU9	Latinne et al. (2020)	WIV+EcoH+DNUS	7569					LA	LA	LA	Luang Namth	_Laos_	Laos Border		Nov-14
Bat betacoronavirus isolate 7570	MN312625	Betacoronavirus	Lineage	Latinne et al. (2020)	WIV+EcoH+DNUS	7570					LA	LA	LA	Luang Namth	_Laos_	Laos Border		Nov-14
Rousettus bat coronavirus HKU9 isolate 7575	MN312626	Betacoronavirus	HKU9	Latinne et al. (2020)	WIV+EcoH+DNUS	7575					LA	LA	LA	Luang Namth	_Laos_	Laos Border		Nov-14
Rousettus bat coronavirus HKU9 isolate 7579	MN312627	Betacoronavirus	HKU9	Latinne et al. (2020)	WIV+EcoH+DNUS	7579					LA	LA	LA	Luang Namth	_Laos_	Laos Border		Nov-14
Rousettus bat coronavirus HKU9 isolate 7585	MN312628	Betacoronavirus	HKU9	Latinne et al. (2020)	WIV+EcoH+DNUS	7585					LA	LA	LA	Luang Namth	_Laos_	Laos Border		Nov-14
Rousettus bat coronavirus HKU9 isolate 7587	MN312629	Betacoronavirus	HKU9	Latinne et al. (2020)	WIV+EcoH+DNUS	7587					LA	LA	LA	Luang Namth	_Laos_	Laos Border		Nov-14
Rousettus bat coronavirus HKU9 isolate 7588	MN312630	Betacoronavirus	HKU9	Latinne et al. (2020)	WIV+EcoH+DNUS	7588					LA	LA	LA	Luang Namth	_Laos_	Laos Border		Nov-14
Bat coronavirus HKU10 isolate 7589	MN312315	Alphacoronavirus	HKU10	Latinne et al. (2020)	WIV+EcoH+DNUS	7589					LA	LA	LA	Luang Namth	_Laos_	Laos Border		Nov-14
Rousettus bat coronavirus HKU9 isolate 7590	MN312631	Betacoronavirus	HKU9	Latinne et al. (2020)	WIV+EcoH+DNUS	7590					LA	LA	LA	Luang Namth	_Laos_	Laos Border		Nov-14
Bat coronavirus 1 isolate 7683	MN312316	Alphacoronavirus	1	Latinne et al. (2020)	WIV+EcoH+DNUS	7683					CN	YN	TG	Tongguan	Yunnan	Southern		Nov-14
Bat coronavirus 1 isolate 7694	MN312317	Alphacoronavirus	1	Latinne et al. (2020)	WIV+EcoH+DNUS	7694					CN	YN	TG	Tongguan	Yunnan	Southern		Nov-14
Rhinolophus bat coronavirus HKU2 isolate 77	MN312318	Alphacoronavirus	HKU2	Latinne et al. (2020)	WIV+EcoH+DNUS	7700					CN	YN	TG	Tongguan	Yunnan	Southern		Nov-14
Rhinolophus bat coronavirus HKU2 isolate 77	MN312319	Alphacoronavirus	HKU2	Wang et al. (2019), Latinne et al. (2020)	WIV+EcoH	7703					CN	YN	TG	Tongguan	Yunnan	Southern		Nov-14

> the 589 samples of BIGD have some of the typical mistakes an intern would made when doing a manually, repetitive and boring work. But it is specially strange the mistake for the collection date of 7683, 7694, 7700 & 7703 (Jul-14 instead of somewhere between Oct-14 and May-15)
>
> — Francisco de Asis (@franciscodeasis) July 9, 2020

Sampling locations contradictions with Latinne et al. (2020): https://t.co/a0H8kublAV



2. Materials and Methods

2.1. Ethics Statement

All sampling procedures were performed by veterinarians with approval from Animal Ethics Committee of the Wuhan Institute of Virology (WIVH05210201, approved on 9 July 2012). The study was conducted in accordance with the Guide for the Care and Use of Wild Mammals in Research of the People's Republic of China.

2.2. Sampling

Bat samplings were conducted from September 2006 to June 2016, as described previously [17,26]. Bat fecal swab and pellet samples were collected at different seasons in 25 provinces in China and one province in northern Laos adjacent to Yunnan province of China.

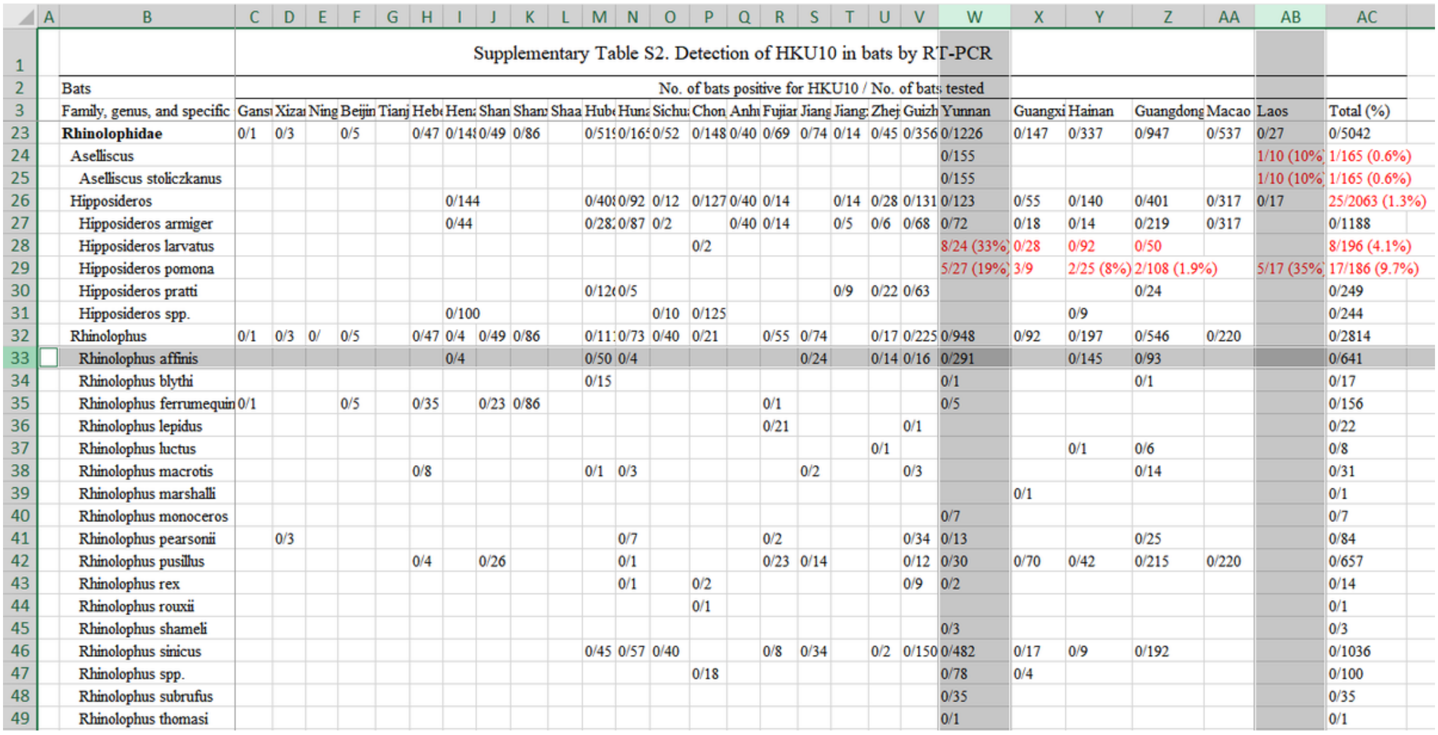
2.3. RNA Extraction, PCR Screenin uencing

Viral RNA was extracted fror f fecal swab or pellet samples with High Pure Viral RNA Kit (Roche Diagnostics GmbH, Mannheim, Germany) as per the manufacturer's instructions. RNA was eluted in 50 μL of Elution buffer, aliquoted, and stored at −80 °C. A one-step heminested RT-PCR (Invitrogen, San Diego, CA, USA) (Table S1), targeting a 440 nucleotide (nt) fragment of RNA-dependent RNA polymerase (RdRp), was employed to detect the presence of coronavirus sequences, as described previously [27]. PCR products were gel purified and sequenced with an ABI Prism 3730 DNA analyzer (Applied Biosystems, Foster City, CA, USA). Alternatively, the PCR products were cloned into pGEM-T Easy Vector (Promega, Madison, WI, USA) for sequencing. The positive samples in this study were classified using the abbreviated name of sampling location followed by sample ID (e.g., YN3723).

To confirm the bat species of an individual sample, we performed PCR to amplify the cytochrome b (Cytob) or NADH dehydrogenase subunit 1 (ND1) gene using DNA extracted from the feces or swabs [28,29]. The gene sequences were assembled excluding the primer sequences; BLASTN was used to identify host species based on the most closely related sequences with highest query coverage and a minimum identity of 95%.

> There was also a sub-trip to Laos border within the 5th visit (80km from TG). Those samples are presented as from Laos in Genbank, and are not present in Bigd.
> But Latinnet et al. (2020) was very clear: "solely from China"https://t.co/f45nzLS7DUhttps://t.co/wqyPJgUE4r pic.twitter.com/ydwZlDTyZS
>
> — Francisco de Asis (@franciscodeasis) November 28, 2020

Wang et al. (2021) acknowledge no Rhinolophus affinis from Laos contradicting PREDICT and EHA data: https://t.co/bDuSIQh0Qn



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC
1			Supplementary Table S2. Detection of HKU10 in bats by RT-PCR																										
2		Bats	No. of bats positive for HKU10 / No. of bats tested																										
3		Family, genus, and specific	Gans	Xiza	Ning	Beijin	Tianj	Hebe	Hena	Shan	Shan	Shaa	Hube	Huna	Sichu	Chon	Anhu	Fujiar	Jiang	Jiang	Zhej	Guizh	Yunnan	Guangxi	Hainan	Guangdong	Macao	Laos	Total (%)
23		**Rhinolophidae**	0/1	0/3		0/5		0/47	0/14	0/49	0/86		0/51	0/16	0/52	0/148	0/40	0/69	0/74	0/14	0/45	0/356	0/1226	0/147	0/337	0/947	0/537	0/27	0/5042
24		Aselliscus																					0/155					1/10 (10%	1/165 (0.6%)
25		Aselliscus stoliczkanus																					0/155					1/10 (10%	1/165 (0.6%)
26		Hipposideros							0/144				0/40	0/92	0/12	0/127	0/40	0/14		0/14	0/28	0/131	0/123	0/55	0/140	0/401	0/317	0/17	25/2063 (1.3%)
27		Hipposideros armiger							0/44				0/28	0/87	0/2		0/40	0/14		0/5	0/6	0/68	0/72	0/18	0/14	0/219	0/317		0/1188
28		Hipposideros larvatus														0/2							8/24 (33%	0/28	0/92	0/50			8/196 (4.1%)
29		Hipposideros pomona																					5/27 (19%	3/9	2/25 (8%)	2/108 (1.9%)		5/17 (35%	17/186 (9.7%)
30		Hipposideros pratti											0/12	0/5						0/9	0/22	0/63				0/24			0/249
31		Hipposideros spp.							0/100						0/10	0/125									0/9				0/244
32		Rhinolophus	0/1	0/3	0/	0/5		0/47	0/4	0/49	0/86		0/11	0/73	0/40	0/21		0/55	0/74		0/17	0/225	0/948	0/92	0/197	0/546	0/220		0/2814
33		Rhinolophus affinis							0/4				0/50	0/4					0/24		0/14	0/16	0/291		0/145	0/93			0/641
34		Rhinolophus blythi											0/15										0/1			0/1			0/17
35		Rhinolophus ferrumequin	0/1			0/5		0/35		0/23	0/86							0/1					0/5						0/156
36		Rhinolophus lepidus																0/21				0/1							0/22
37		Rhinolophus luctus																			0/1				0/1	0/6			0/8
38		Rhinolophus macrotis						0/8					0/1	0/3					0/2			0/3				0/14			0/31
39		Rhinolophus marshalli																						0/1					0/1
40		Rhinolophus monoceros																					0/7						0/7
41		Rhinolophus pearsonii		0/3										0/7				0/2				0/34	0/13			0/25			0/84
42		Rhinolophus pusillus						0/4		0/26				0/1				0/23	0/14			0/12	0/30	0/70	0/42	0/215	0/220		0/657
43		Rhinolophus rex												0/1		0/2						0/9	0/2						0/14
44		Rhinolophus rouxii														0/1													0/1
45		Rhinolophus shameli																					0/3						0/3
46		Rhinolophus sinicus											0/45	0/57	0/40			0/8	0/34		0/2	0/150	0/482	0/17	0/9	0/192			0/1036
47		Rhinolophus spp.														0/18							0/78	0/4					0/100
48		Rhinolophus subrufus																					0/35						0/35
49		Rhinolophus thomasi																					0/1						0/1

Other interesting things:

- 11 Ra samples <500km from the mine, in Laos in 2011
- sometimes they make mistakes mixing decimal and sexagesimal system for GPS coord.
- Some Nanling samples incorrectly geolocated in Kunming (e.g. NL13807) pic.twitter.com/9gavnAm90w

— Francisco de Asis (@franciscodeasis) February 16, 2021

Metadata from the Excel files for Table S1 and S5:
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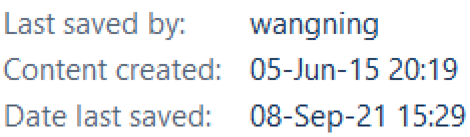
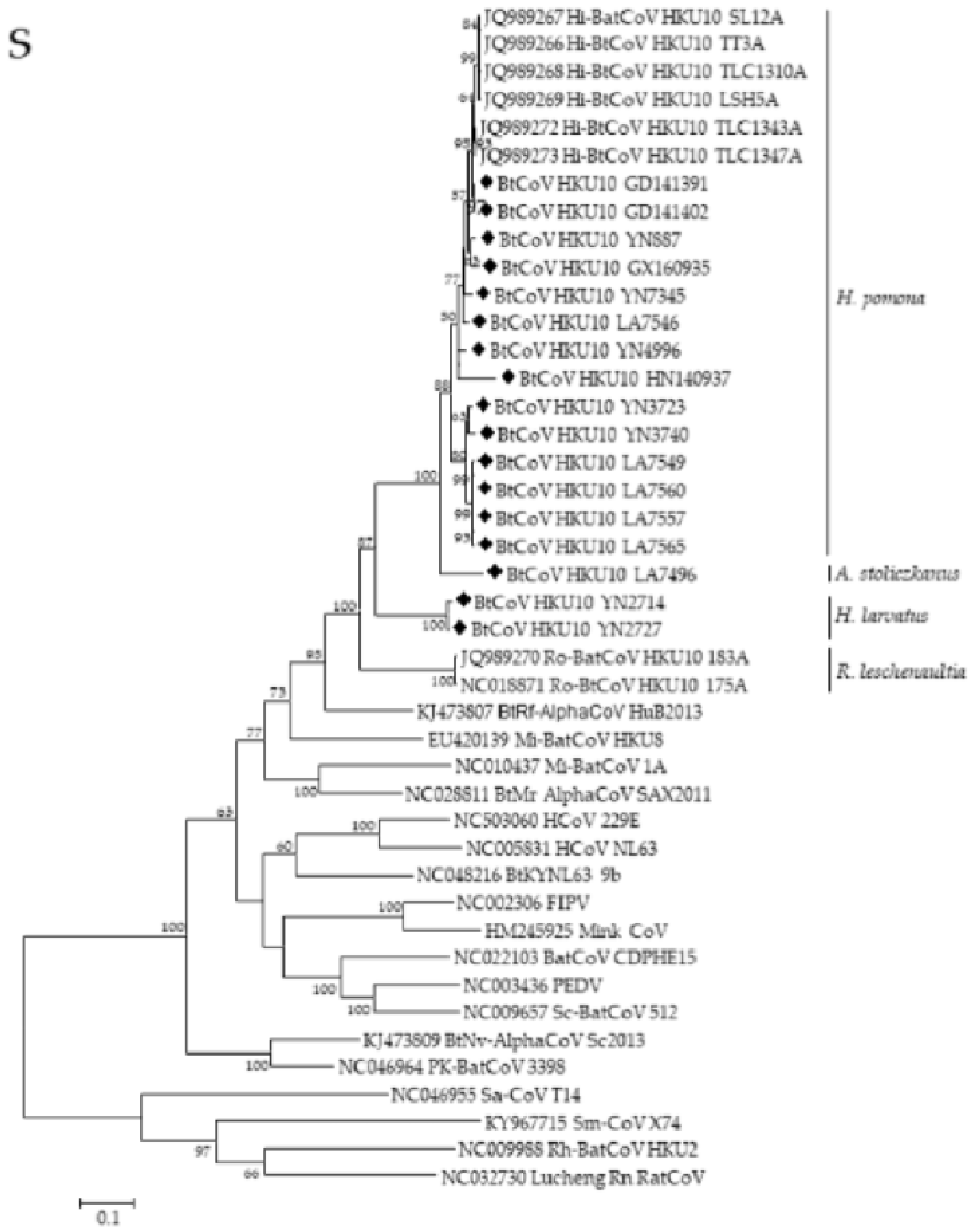
"Content created"="05-Jun-15"
(which is just after the 7th trip to Mojiang)



Last saved by:	wangning
Content created:	05-Jun-15 20:19
Date last saved:	08-Sep-21 15:29

Not easy to extrapolate HKU10 to SARS-like viruses, but see how viruses from Mojiang and Luang Namtha tend to cluster very close and even sandwiched.
e.g.: YN7345, LA7546 and YN4996

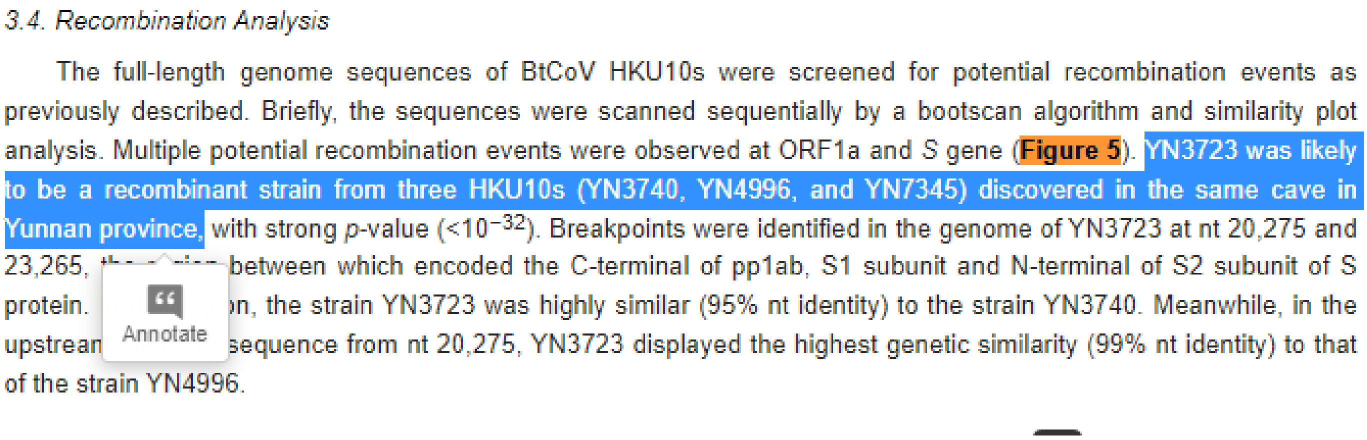


To end, just a reminder of the case of Latinne et al. (2020) https://t.co/F1XNupaGhl

[Thread] FOIA from @USRightToKnow regarding Latinne et al. (2020) and clade 7896
TLDR: No sequence was deleted/modified since Aug-2019, but it seems they wanted to buy time for not publishing the viruses very early in the pandemic.https://t.co/xEssWNv5TY

— Francisco de Asis (@franciscodeasis) October 12, 2021

I missed this one:
"cave"



3.4. Recombination Analysis

The full-length genome sequences of BtCoV HKU10s were screened for potential recombination events as previously described. Briefly, the sequences were scanned sequentially by a bootscan algorithm and similarity plot analysis. Multiple potential recombination events were observed at ORF1a and *S* gene (**Figure 5**). YN3723 was likely to be a recombinant strain from three HKU10s (YN3740, YN4996, and YN7345) discovered in the same cave in Yunnan province, with strong *p*-value ($<10^{-32}$). Breakpoints were identified in the genome of YN3723 at nt 20,275 and 23,265, [illegible] between which encoded the C-terminal of pp1ab, S1 subunit and N-terminal of S2 subunit of S protein. [illegible]on, the strain YN3723 was highly similar (95% nt identity) to the strain YN3740. Meanwhile, in the upstrea[illegible] sequence from nt 20,275, YN3723 displayed the highest genetic similarity (99% nt identity) to that of the strain YN4996.