

Questions regarding Liu et al. (2020) in PLoS Pathogens

Sainath Suryanarayanan <sainath@usrtk.org> To: chenjp@giabr.gd.cn Bcc: Gary Ruskin <gary@usrtk.org> Wed, Oct 28, 2020 at 12:17 PM

Dear Dr. Chen,

I am working with the public interest non-profit U.S. Right to Know to help increase public awareness about what is known and not known about the origins of SARS-CoV-2. I am writing to seek your advice about Liu et al.'s (2020) *PLoS Pathogens* paper titled, "Are pangolins the intermediate host of the 2019 novel coronavirus (SARS-CoV-2)?", on which you are listed as the corresponding author.

In raising questions about Liu et al. (2020), it is not my intention to question your integrity. However, I believe that serious questions concerning the scientific accuracy and reliability of Liu et al. (2020) need to be addressed in a transparent manner, especially because SARS-CoV-2 continues to cause staggering harm worldwide and efforts to understand and resolve the current pandemic and future such pandemics turn on sound scientific evidence.

As you know, Liu et al. (2020) discovered coronaviruses in pangolins that were intercepted by Guangdong Customs in March and July of 2019. The authors found in these diseased pangolins coronaviruses with a level of whole genomic sequence similarity to SARS-CoV-2, at the time, second only to the bat coronavirus RaTG13 — the closest known relative of SARS-CoV-2. Further, there was a striking 98.6% sequence match between the discovered pangolin coronavirus and SARS-CoV-2 in the receptor binding motif, a critical region of the S protein essential for binding to host cell receptors. Liu et al.'s finding has been crucial for substantiating claims that there are coronaviruses circulating in the wild that have very high similarity to SARS-CoV-2 and that therefore, SARS-CoV-2 is likely the result of animal-to-human spillover potentially involving multiple animal species as intermediaries.

You may also know that serious issues have been raised about the veracity of the 3 pangolin individuals upon which Liu et al.'s entire analysis was based. Here are some of the key issues:

1. Liu et al.'s (2020) analysis was built primarily using the same metagenomic dataset published by Liu et al. *Viruses* in 2019 titled, "Viral Metagenomics Revealed Sendai Virus and Coronavirus Infection of Malayan Pangolins (*Manis javanica*)." Originally, the raw dataset of Liu et al. (2019) was published in NCBI's sequence read archive (SRA) on September 23, 2019. However, Liu et al. (2019) re-published their SRA on January 22, 2020. What was your reason for changing or re-publishing the original SRA? Did you inform PLoS Pathogens about your reason?

2. Two of the three sampled pangolins were obtained from a smuggled batch intercepted in March 2019, whereas the third one was apparently obtained from a different smuggled batch intercepted in July 2019. The authors claimed 99.54% nucleotide-sequence identity between the contigs of the three sampled individuals, while noting that the sequence reads from the July-caught pangolin were "relatively less abundant." The sequence data shared by the authors in GenBank only lists reads from the March 2019 pangolin sequences. Can you explain why you did not provide the sequence data for the July pangolin sample? How quickly can you correct this issue and deposit the sequence data for the July 2019 pangolin sample?

3. Two days after *PLoS Pathogens* was reportedly received Liu et al. (2020) *Nature* received an independent study by Xiao et al. (2020) titled "Isolation of SARS-CoV-2-related coronavirus from Malayan pangolins." Significantly, Liu et al. (2020) and Xiao et al. (2020) shared authors, and Xiao et al. "renamed pangolin samples first published by Liu et al. [2019] *Viruses* without citing their study as the original article that described these samples, and used the metagenomic data from these samples in their analysis" (Chan and Zhan, biorXiv). **Did you inform PLoS Pathogens about your close communications and data duplication with the authors of the** *Nature* **paper?**

USRTK Mail - Questions regarding Liu et al. (2020) in PLoS Pathogens

4. Did you share amplicon data with PLoS Pathogens? In the absence of such amplicon data, could you explain how the reviewers verified the pangolin coronavirus genome that you published? On this point, what do you think about the finding that Xiao et al.'s full pangolin CoV genome is a 99.95% match to Liu et al. PLoS Pathogens full pangolin CoV genome? How quickly do you think this needs to be publicly clarified by Liu et al. and PLoS Pathogens? How can Liu et al. and PLoS Pathogens quickly clarify the reasons behind the high level of shared identity between these two separately published genomes?

I hope you will consider shedding more light on these issues, which I believe are simply too important to be kept outside of public purview. Thanks so much for considering.

Sincerely,

Sainath

Sainath Suryanarayanan, PhD Staff Scientist, U.S. Right to Know usrtk.org/about/



陈金平 <chenjp@giabr.gd.cn> To: Sainath Suryanarayanan <sainath@usrtk.org> Wed, Oct 28, 2020 at 12:47 PM

Dear Sainath,

Many thanks for your interested with our Publication in Plos Pathogens, I suggest that you read our two papers carefully,only after you read, then you know the two papers are different content even together with parts of the same samples,and all these questions you raised we have answered to the Journal. there is not any relationship between my team and **the authors of the** *Nature* **paper** you said, Please know that our paper submitted before the Nature paper.We have supplied all the analysis data to the Journal, of course, if you are intersted, I also can send.

Jinping Chen

-----原始邮件-----发件人:"Sainath Suryanarayanan" <sainath@usrtk.org> 发送时间:2020-10-28 19:17:12 (星期三) 收件人: chenjp@giabr.gd.cn 抄送: 主题: Questions regarding Liu et al. (2020) in PLoS Pathogens [Quoted text hidden]



Questions regarding Liu et al. (2020) in PLoS Pathogens

陈金平 <chenjp@giabr.gd.cn> To: Sainath Suryanarayanan <sainath@usrtk.org> Wed, Oct 28, 2020 at 1:11 PM

Dear Sainath,

As your questions below, I can answer as follows:

1. Parts of the raw data of PLOS Pathogens were overlapped with the data used in the Viruses articles (published on Oct 24,2019, which firstly reported the sendai Virus and Coronavirus Infection of Malayan Pangolins, but we did not assemble the whole coronavirus genome), The sample IDs of the individuals with coronavirus infection was lung07, lung08, which had reported in our Viruses paper. We also detected reads belong to coronavirus in the sample lung09, which was listed in Table S1 in our PLOS Pathogens paper. The raw data of these three samples could be found under NCBI accession number PRJNA573298, and the BioSample ID were SAMN12809952, SAMN12809953, and SAMN12809954, moreover, other individual (F9) from different batch was also positive, the raw data can be seen in NCBI SRA SUB 7661929, which will be released soon for we have another MS (under review) and RT-PCR was amplified for all gaps in our PLOS pathogens paper.

2. When we published our 2019 viruses paper, we just found the coronavirus sequence in pangolin with short reads, and we haven't got its complete genome because we haven't SARS-Cov-2 reference sequence, after we got SARS-Cov-2 complete sequence, then we can re-analysis our data again and get the complete quences with the reference, that's the reason of our Plos pathogens paper. We haven't corrected our raw data, just with the SARS-Cov-2 as reference sequence, we can compose the whole pangolin Cov tother with RT-PCR amplication.

3.We submitted our PLOS Pathogens paper on Feb.14, 2020 before the Nature paper (the Reference 12 in our PLOS pathogens paper, they submitted on Feb.16, 2020 from their submit date in Nature), our PLOS pathogens paper explain that SARS-Cov-2 is not from pangolin coronavirus directly and pangolin not as intermediate host. We knew their work after their news briefing on Feb. 7,2020, and we have different opinions with them, the other two papers (Viruses and Nature) have been listed in the PLOS Pathogen paper as reference papers (reference number 10 and 12), we are different research groups from Nature paper authors, and there is no relationship with each other, and we took samples with detail sample information from the Guangdong wildlife rescue center with helps from Jiejian Zou and Fanghui Hou as our co-authors and we don't know where the samples of the Nature paper from. We got the pangolin CoV genome based on our Viruses paper published and RT-PCR sequence results for filling gaps, and our PLOS Pathogens paper have different opinions with the Nature paper (reference 12).

10/29/2020

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4. Some of the questions I have answered above, I am responsible for our data, and we have all the raw data, and also with the the pangolin coronavirus RNA, we can repeat our results again and again.

Welcome to any questions about our published paper.

Have a good day

Jinping

Institute of Zoology, Guangdong Academy of Sciences

-----原始邮件-----发件人:"Sainath Suryanarayanan" <<u>sainath@usrtk.org</u>> 发送时间:2020-10-28 19:17:12 (星期三) 收件人: chenjp@giabr.gd.cn 抄送: 主题: Questions regarding Liu et al. (2020) in PLoS Pathogens

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I hope you will consider shedding more light on these issues, which I believe are simply too important to be kept outside of public purview. Thanks so much for considering.

Sincerely,

Sainath

Sainath Suryanarayanan, PhD Staff Scientist, U.S. Right to Know usrtk.org/about/



Sainath Suryanarayanan <sainath@usrtk.org>

Wed, Oct 28, 2020 at 8:14 PM

To: 陈金平 <chenjp@giabr.gd.cn> Bcc: Gary Ruskin <gary@usrtk.org>

Dear Jinping,

Thank you so much for taking the time to respond. As a quick follow up, seeing as you used the July 2019 (F9) sample to assemble a genome that you published in Liu et al. (2020), would it not be appropriate to release the SRA of F9 immediately, and attribute it correctly to your PLoS Pathogens paper? Is PLoS Pathogens aware about where your new manuscript (with the F9 SRA data) is under review?

Also, could you explain why you have not published the amplicon sequencing data utilized by Liu et al. (2020)?

I appreciate your willingness and patience in answering my questions.

Thank you, Sainath

Sainath Suryanarayanan, PhD Staff Scientist, U.S. Right to Know usrtk.org/about/



陈金平 <chenjp@giabr.gd.cn>

Wed, Oct 28, 2020 at 11:58 PM

To: Sainath Suryanarayanan <sainath@usrtk.org>

Dear Sainath,

We have explained this to the Journal, and all our intermidiate analysis data you can track in genebank, which shown in the BioRvix paper you sent to me, you can know our firstly composed sequence, whole genome with filling gaps after RT-PCR, you can find our each step results .we can compose the pangoline coV genome without this sample F9, which can't affect our results in Plos pathogens for compose whole genome, we used RT—PCR to fill all the gaps, also we

have submitted F9 to genebank waiting for release. all RT—PCR primers used has sent to the Journal and I can send you all the RT- PCR sequence raw data if you prefer.

Best regards

Jinping Chen

在 2020-10-29 03:14:22, "Sainath Suryanarayanan" <sainath@usrtk.org> 写道:

>Dear Jinping,

> >

>Thank you so much for taking the time to respond. As a quick follow up, seeing as you used the July 2019 (F9) sample to assemble a genome that you published in Liu et al. (2020), would it not be appropriate to release the SRA of F9 immediately, and attribute it correctly to your PLoS Pathogens paper? Is PLoS Pathogens aware about where your new manuscript (with the F9 SRA data) is under review?

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> >

>Thank you, >Sainath

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>Sainath Suryanarayanan, PhD

>Staff Scientist, U.S. Right to Know >usrtk.org/about/

> usitk

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>On Wed, Oct 28, 2020 at 1:11 PM 陈金平 <chenjp@giabr.gd.cn> wrote:

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Sainath Suryanarayanan <sainath@usrtk.org> To: 陈金平 <chenjp@giabr.gd.cn> Bcc: Gary Ruskin <gary@usrtk.org> Thu, Oct 29, 2020 at 1:01 PM

Dear Jinping,

Thank you for your friendly reply and willingness to share data. Yes, please do send me at your earliest convenience all the RT- PCR sequence raw data utilized by Liu et al.'s PLoS Pathogens paper.

Thanks again, Sainath

Sainath Suryanarayanan, PhD Staff Scientist, U.S. Right to Know usrtk.org/about/



陈金平 <chenjp@giabr.gd.cn> To: Sainath Suryanarayanan <sainath@usrtk.org> Fri, Oct 30, 2020 at 6:03 AM

Dear Sainath,

Please see the attachment for RT-PCR primers and RT-PCR prodct sequence results, and please reference our Plos Pathogens paper if you will use these data, many thanks.

Best regards

Jinping Chen

Institute of Zoology, Guangdong Academy of Sciences

-----原始邮件-----发件人:"Sainath Suryanarayanan" <sainath@usrtk.org> 发送时间:2020-10-29 20:01:44 (星期四) 收件人: "陈金平" <chenjp@giabr.gd.cn> 抄送: 主题: Re: Re: Questions regarding Liu et al. (2020) in PLoS Pathogens [Quoted text hidden]

2 attachments

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RT-PCR Sequences for pangolin Cov2020.fas
18K

	primes	used fo	r pangolin	Cov -	llm-2020.xls	X
	17K					



Questions regarding Liu et al. (2020) in PLoS Pathogens

Sainath Suryanarayanan <sainath@usrtk.org>

Fri, Oct 30, 2020 at 3:09 PM

To: 陈金平 <chenjp@giabr.gd.cn>

Dear Jinping,

Thank you for the files. Would you be willing to also share the raw chromatogram files from the amplicon sequencing (extension is .ab1 or .scf)? We will cite Liu et al. if we use any of these.

Many Thanks, Sainath

Sainath Suryanarayanan, PhD Staff Scientist, U.S. Right to Know usrtk.org/about/



Questions regarding Liu et al. (2020) in PLoS Pathogens

陈金平 <chenjp@giabr.gd.cn> To: Sainath Suryanarayanan <sainath@usrtk.org> Sat, Oct 31, 2020 at 3:09 AM

Dear Sainath,

The files with extension .fas I sent to you is the amplicon sequencing without any changes and the same with the raw chromatogram files, I hope this can help you.

Best regards

Jinping Chen

-----原始邮件-----发件人:"Sainath Suryanarayanan" <sainath@usrtk.org> 发送时间:2020-10-30 22:09:20 (星期五) 收件人: "陈金平" <chenjp@giabr.gd.cn> 抄送: 主题: Re: Re: Re: Questions regarding Liu et al. (2020) in PLoS Pathogens [Quoted text hidden]