AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Spectrum

The role of Molossidae and Vespertilionidae in shaping the diversity of alphacoronaviruses in the Americas

Diego Caraballo, Maria Sabio, Valeria Colombo, Maria Piccirilli, Lorena Vico, Stella Maris Hirmas Riade, Josefina Campos, Gustavo Martinez, Fernando Beltran, Elsa Baumeister, and Daniel Cisterna

Corresponding Author(s): Diego Caraballo, Instituto de Ecología, Genética y Evolución de Buenos Aires

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Reviewer(s): Disclosure of reviewer identity is with reference to reviewer comments included in decision letter(s). The following individuals involved in review of your submission have agreed to reveal their identity: Yousong Peng (Reviewer #2)

Transaction Report:

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September 6, 2022

Dr. Diego A. Caraballo
Instituto de Ecología, Genética y Evolución de Buenos Aires
Universidad de Buenos Aires, CONICET
Intendente Guiraldes 2160
Ciudad Universitaria, Pabellón 2, 4to piso
Buenos Aires 1428
Argentina

Re: Spectrum03143-22 (**The role of Molossidae and Vespertilionidae in shaping the diversity of alphacoronaviruses in the Americas**)

Dear Dr. Diego A. Caraballo:

Thank you for submitting your manuscript to Microbiology Spectrum.

Bats are among the natural hosts of coronaviruses (CoVs) and a large number of bat-CoVs have been reported worldwide. However, the diversity of bat-CoVs is currently underrepresented in the Americas, so your data contributes to knowledge of the genetic diversity and circulation of bat-CoVs in the area. The sequences in this study, as noted by the reviewer(s), are very short in length and have a small population size, which means they cannot adequately support some of the bioinformatic conclusions. I would suggest you emphasize the detection and genetic diversity of bat-CoVs and weaken the Bayesian inference, otherwise add more data to support the analysis.

When submitting the revised version of your paper, please provide (1) point-by-point responses to the issues raised by the reviewers as file type "Response to Reviewers," not in your cover letter, and (2) a PDF file that indicates the changes from the original submission (by highlighting or underlining the changes) as file type "Marked Up Manuscript - For Review Only". Please use this link to submit your revised manuscript - we strongly recommend that you submit your paper within the next 60 days or reach out to me. Detailed instructions on submitting your revised paper are below.

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Sincerely,

Biao He

Editor, Microbiology Spectrum

Journals Department
American Society for Microbiology
1752 N St., NW
Washington, DC 20036
E-mail: spectrum@asmusa.org

Reviewer comments:

Reviewer #1 (Comments for the Author):

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Staff Comments:

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Response:

We have a partial agreement with the Reviewer. The cross-species transmission of bat-CoVs in the Americas was not studied until a paper recently published by one of us (Caraballo 2022). In that paper, the clades that are the focus of this study were underrepresented, basically because there is a significant proportion of sequences generated in the present study. However, as we state below, we decided to narrow the CST analysis, in agreement with some weak points identified by the Reviewer.

We acknowledge that there is an evident gap in the geographical coverage of bat hosts and viruses. This was stated in the previous version of the manuscript. We agree to narrow the scope of our analysis and the conclusions drawn from it, but we would like to mention that our study contributes valuable results that enable gaining insight into the diversity of alphaCoVs in the Americas. To name some, we found independent lineages co-circulating in *Tadarida* and *Molossus*, as well as identified a host shift between these bat taxa. We also made a thorough analysis of the phylogenetic structure and diversity of the clades maintained by vespertilionids and molossids, showing that there is a strong host and geographic structure. Finally, we highlight *Myotis* as the main reservoir in terms of phylogenetic diversity. These conclusions were reached independently of the Bayesian inference of host shifts and the analysis of basal relationships among lineages (that could be influenced by uneven geographical sampling), which were excluded from the study after the Reviewer’s comments.

As, except for host shifts, all main sub-lineages are reciprocally monophyletic, we centered our analysis on shallow CSTs, and not on their basal relationships. Uneven geographic sampling may interfere with deep relationships between lineages, so we excluded the Bayes Factors inference (that accounts for both deep and shallow CSTs), and specifically discussed CSTs occurring at shallow levels of the phylogeny, between sympatric taxa which reflect traceable host shifts. In other words, it is likely that there would be missing links between two different bat genera occurring one in South America and the other one in North America. On the other hand, the host shift *Tadarida*->*Molossus* is likely, since the involved sequences were isolated from bats occurring in the same geographic region. The same applies to the remaining host shifts treated in the manuscript.

As to the number of sequences obtained, we would like to clarify that the aim of this work was not to perform a population study, but a phylogenetic one. We do not use sequences to infer demographic parameters, and even when molecular clocks are used, we do not treat time in absolute units. So, all our analysis derives from topology and branch lengths, which are essentially phylogenetic parameters, that would not require a denser population sampling.

We also acknowledge that there are limitations arising from the use of partial RNA-dependent RNA polymerase (RdRp) sequences. As occurs with the dataset produced in this study, the majority of bat-CoV sequences available in public databases were generated with the primers designed by Watanabe et al. (2010) which produce a fragment of 440 bp-800. However, it should be noted that the RdRp gene reflects vertical ancestry and is less prone to recombination than other regions of the CoV genome. In addition, it is worth noting that the general structure of the viral phylogenies inferred in this study showed high levels of resolution with highly supported nodes.

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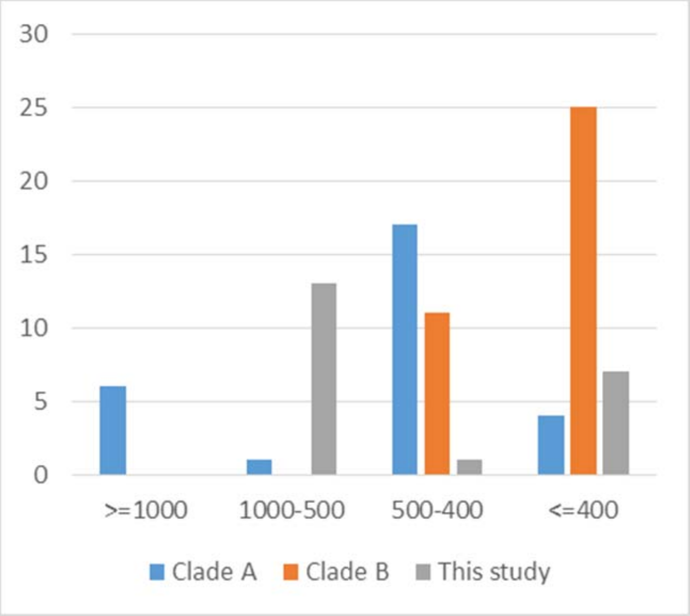

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We agree that this dataset has inherent limitations, and as such, the conclusions that follow this analysis must be taken with precaution (and this is reflected in the Discussion section). But if a strict criterion of sequence length is applied, the analysis cannot be done, and the opportunity to start gaining insight into batCoVs maintained by vespertilionds and molossids in the Americas would be lost. We are confident that there will be more batCoV sequences available in the next few years (we are personally working on generating full genome sequences in Argentina), but we are also convinced that these results could serve as good working hypotheses to guide future research and to point out precisely how important it would be to count with genomic analyses of virus and hosts diversity in the Americas.

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Response: We replaced these expressions with “$5x10^7$”, “$5x10^3$”, etc.

September 15, 2022

Dr. Diego A. Caraballo
Instituto de Ecología, Genética y Evolución de Buenos Aires
Universidad de Buenos Aires, CONICET
Intendente Guiraldes 2160
Ciudad Universitaria, Pabellón 2, 4to piso
Buenos Aires 1428
Argentina

Re: Spectrum03143-22R1 (**The role of Molossidae and Vespertilionidae in shaping the diversity of alphacoronaviruses in the Americas**)

Dear Dr. Diego A. Caraballo:

Please make sure these sequences reported in the study are publicly available, and you should provide a "Data Availability" paragraph at the end of the Materials and Methods section.

Thank you for submitting your manuscript to Microbiology Spectrum. As you will see your paper is very close to acceptance. Please modify the manuscript along the lines I have recommended. As these revisions are quite minor, I expect that you should be able to turn in the revised paper in less than 30 days, if not sooner. If your manuscript was reviewed, you will find the reviewers' comments below.

When submitting the revised version of your paper, please provide (1) point-by-point responses to the issues raised by the reviewers as file type "Response to Reviewers," not in your cover letter, and (2) a PDF file that indicates the changes from the original submission (by highlighting or underlining the changes) as file type "Marked Up Manuscript - For Review Only". Please use this link to submit your revised manuscript. Detailed instructions on submitting your revised paper are below.

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Editor, Microbiology Spectrum

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Corresponding authors may join or renew ASM membership to obtain discounts on publication fees. Need to upgrade your membership level? Please contact Customer Service at Service@asmusa.org.

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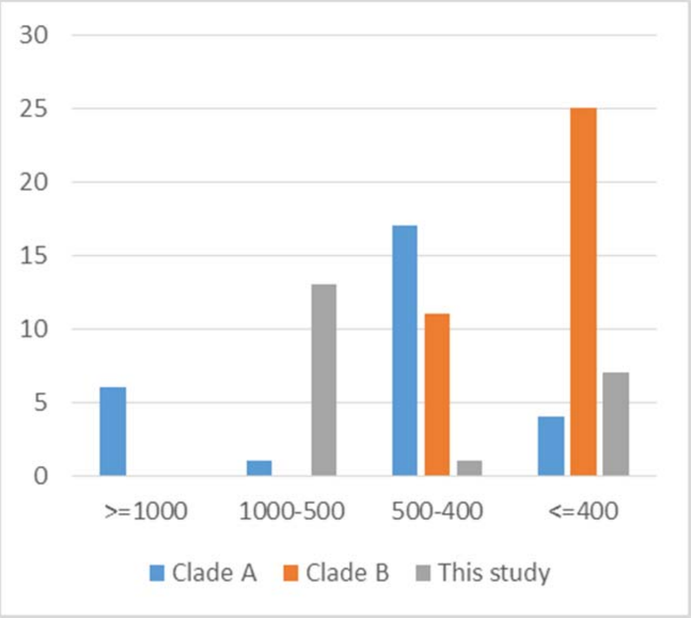

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Universidad de Buenos Aires, CONICET
Intendente Guiraldes 2160
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Buenos Aires 1428
Argentina

Re: Spectrum03143-22R2 (**The role of Molossidae and Vespertilionidae in shaping the diversity of alphacoronaviruses in the Americas**)

Dear Dr. Diego A. Caraballo:

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Sincerely,

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Editor, Microbiology Spectrum

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