nature portfolio

Peer Review File

Ecology and evolutionary trajectories of morbilliviruses in Neotropical bats

Corresponding Author: Professor Jan Drexler

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The authors present work that represents a significant leap in our understanding of the standing diversity and origins of morbilliviruses. Limited genome samples previously existed for the family and with the authors' geographic and temporal sampling of bat and NHPs, coupled with data mining, the authors have identified a number of novel morbilliviruses. The methods in this manuscript are sound and the text and figures are of high quality. I have a number of minor points below that I believe would improve the quality of the manuscript, but overall I commend the authors on their engaging manuscript.

Minor points:
- Between figure 1 a and b, keep the species colours consistent.
- The authors have described how sampling bias may impact their results, as sampling was only performed in two Neotropical countries. On that point, sampling was also not performed on local rodent species. The rationale for this makes sense given the historical importance of bats for Morbilliviruses, however it would be good for the authors to comment more extensively on sampling bias.
- The authors use Vegan and Densitree rather than BEAST for host reconstruction, however they do use BEAST for tree estimation. BEAST can also estimate host jumps and it would be good to compare these methods and see if it concurs with the Densitree / Vegan output (possibly using the steps outlined in this tutorial: https://beast.community/markov_jumps_rewards).
- The authors limit their reconstruction analysis to the L gene and describe how this limits their analysis. Could the authors extract the coding sequences from each gene (or perhaps more than just L), align them and concatenate them together as a way of getting more data for the reconstruction?
- May be useful to include a schema of mammalian relatedness for the species sampled in this study for those less familiar with mammalian taxonomy and to help demonstrate the host-virus evolutionary histories (perhaps even a tanglegram).
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Reviewer #2

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The proposed article by Jo et al describes the identification and characterisation of a number of bat and primate born morbilliviruses from the Americas. The authors used extensive sample sets to identify the viruses, then sequenced them and examined their properties, mostly focusing on the genomic organisation and conclusions surrounding their phylogenetic relationship to each other, and extant sequences in the database. I found the manuscript easy to read and exciting, and I was impressed by the broad attempts at characterisation across in vitro biology and RNA virus evolution.
Major points:
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the high signals in the heart are more inconsistent with the known tissue tropism of 'classical' morbilliviruses like measles. Could the authors amplify Nectin-4 from their bat or NHP samples and confirm use of this receptor (I am unclear if these sequences are already known for these bat species and/or marmosets). This would go some way towards confirming that these bat morbilliviruses have the same biphasic life cycle in their mammalian hosts as measles, rinderpest, canine distemper etc. I understand live virus work is potentially challenging (due to sample degradation) but there are surrogate entry assays available, or recombinant protein binding experiments (ELISA, BLI, SPR) that could provide an answer (based on synthesis and construction of F and H expression constructs).
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This manuscript describes a very important effort to identify new morbillivirues in new world bats and their role in the evolution of this viral genus. The authors managed to identify and characterize the full or almost complete genome of five new morbillivirus species in New world bats, including one from existing sequencing read archives, and one new morbillivirus from marmosets collected in Brazil. One strain of Desmodus-associated morbillivirus (DesMV) could be isolated, allowing for assays to confirm bat-associated cell entry and frequent circulation in bats of Costa Rica. The new sequence data was combined with existing morbillivirus sequence data to einvestigate orgin of morbilliviruses and host shifts events. Cophylogenetic analyses showed evidence for host shifts from bats, carnivores and artiodactyls, notably for bat-associated spill-over at the origin of the Procine morbillivirus and Marmoset morbillivirus. Authors argue that their results also give evidence for an important role of bats in morbillivirus diversification. They call for further investigation into the risk of morbillivirus spill-over from bats to livestocks and primates.

The manuscript is well-written, and is based on a solid amont of data and diverse analyses, although there are some discrepencies between results described and data presented (see below). The major issue I found with this manuscript is that it appears that some results are over-interpreted to fit the narrative for the main role of New World bats in the origin of morbilliviruses. Some statements need to be tone down to represent better the results obtained and caveats of the data available. I think that some sections of the results and discussions described below would need to be revised before publication, or additional analyses may be needed to provide support for some statements.

One main issue concerns the evidence for a bat origin for morbilliviruses. This is mostly based on the posterior probability (PP) results presented for nodes A-D of a phylogenetic tree in Fig4c and d. PP values for these nodes are 30%, 42%, 66% and 82%, respectively. This is poor or, at best limited, statistical support for 3 out 4 nodes, even if PP value obtained for bats is higher than for other hosts. For the one strong support of 82%, it corresponds to node D which is itself poorly supported in the phylogeny (PP < 0.7). This limited statistical support should be made clear in results and discussion. Maybe the poor PP is due to the fact that the analysis is done on a partial L gene sequence (528nt). The authors went to a great extent to obtain complete or near-complete genome for 6 new morbilliviruses. I do not understand why they have not replicated the cophylogeny analysis on full genome data. Some virus and hosts for which only partial sequence is available would not be included, but some power might be gained on those deep nodes. The authors states in L293 that the poor power of short sequence was compensated by using different phylogenetic methods and tree topologies, but signal in the data is the limit, not the method. It would great if authors could do this extra analysis because the study would gain in strength. Cophylogeny results presented may be already good enough for publication in this journal, but the text must be amended to highlight their limitation.

Another issue concerns statements of bat-associated host shifts and evident reduction of the importance of host shifts from other orders. Their results show, as clearly stated in L211 that "bats, carnivores, and artiodactyls are main sources of cross-order host shifts in morbilliviruses", but the discussion, notably concerning MV's old world origin in cattle (L245-250), goes towards putting into doubts hosts shifts that are not of bat origin. In this section, host shifts from carnivors are totally omitted , apparently to fit with the narrative. The evidence fo bat-associated host shifts depends mostly on phylogenetic relationships between morbillivirus sequences, as for other hosts shifts. The discussion should be revised to represent better the diversity of host shifts in morbilliviruses, as shown in the results. Impact of bias in sampling should be better incorporated in the statements as well. The authors quickly mention sample bias (L267) and rightfully call for additional field investigation, but the possible impact of sample bias is not made as clear as it should. Any new morbillivirus sequence from old world bats or other hosts may change some of the results obtained concerning diversification of the virus and host shifts.

The authors state in the discussion (L290) that they account for sampling limitations with data mining from SRA, but their own results show that this method only provides poor results. Only one sequence of morbillivirus (which is already great!) was obtained out of thousands of SRA including from New World bats, so it can hardly be used to compare distribution of morbillivirus among different types of hosts. Interpretation of SRA mining results should be tone down accordingly.

There are multiple other statements that do not fit with the results presented and should be revised:
-L95 "was within the seroprevalence ranges detected for other morbilliviruses in wildlife populations". Only data from one paper on PMV is refered too so it is hardly all other morbillivirus in wildlife population.
- L112 "The number of morbilliviral species in bats exceeded those known in any other host order, i.e., six compared to four in artiodactyls (RPV, PPRV, CeMV, PoMV), three in carnivores (CDV, PDV, and FeMV) and one in primates (MV)". Is not not just a biais of sampling. Exceeding when it is 6 vs 4 is excessive
- L127 " most bat-associated morbilliviruses characterized on the full-genome level were monophyletic with the exception of MyoMV" This is based on genome data containing often only one of two sequences per morbillivirus. So how could it be otherwise. Their own data based on partial L gene could suggest that PhyMV may not be monophyletic. Such statement on limited data is not very useful
-L132 "...is consistent with low rates of recombination in negative-sense single- stranded RNA viruses" There is actually no evidence of recombination in any morbillivirus so far. Assessing recombination on genetic data only is not appropriate. See notably Han G.-Z. & Worobey M. (2011). – Homologous recombination in negative sense RNA viruses. Viruses, 3 (8), 1358-1373. doi: 10.3390/v3081358.)
-L161 "MarMV seperated by location not species"; looking at fig3b, there is clearly spearation by location and species. for example, all MarMG come from C penicillata. Again it is a statelent based on biased sample.
- L174 " confirming the marmoset host associations of morbillivirus-positive specimens. "- genetic results were exepcted to match with Callithrix expected as all 1400 sample, except 48, were marmoset. It only confirms that the sample was not contaminated by tissue from another animal. There is not enough samples from species other than marmoset to assess if their is a specific association with morbillivirus infection.

Finally, some results presented are not fully supported by the data made available:
- Table 1 and results mention 38 positive bat samples, but Supp Table 2 only list 24 positive samples. This list indicates the origin of 3 out 4 new bat morbillivirus. Notably sample CR209 at the origin of genome DesMV-1 is missing. Sample at the origin of the DesMV isolate is not indicated either.
-L71 "expanding the number of individual bats 4-fold". We don't know where this calculation come from.
- Fig1b and results describe RT-PCR results from multiple organs from 12 animals but the data associated is not in Supp Table 2 or anywhere else.
- Fig 1c: no information on what the circle in nodes stand for.
-Extended data 1a: Do the different colors in the labels of tree mean something?
- Fif 2e and f: Only Fig2e is mentioned in legend
- Fig3b The legend mentions the sue of PoMV as outgroup but it seems that PhyMV BOL is used as outgroup. From this analysis, it looks like PhyMV BOL is very different from PhyMV BRA (another species?). But it is maybe due to its positioning as an outgroup?
- Fig 3a what are the P hastatus and A planirostris samples indicated in fig 3a? New samples not mentioned in fig1a? From what I understand, fig 3a should show only samples collected during the study, but these samples are not mentioned in the text.
-Extended Data Table 4 not well described. What are AI and PS? where are marmoset- bats swine- sequences ? Are they grouped per location? It should be mentioned.

Decision Letter:

16th July 2024

*Please ensure you delete the link to your author homepage in this e-mail if you wish to forward it to your co-authors.

Dear Felix,

Thank you for your patience while your manuscript "Origins and evolutionary trajectories of morbilliviruses in Neotropical bats" was under peer-review at Nature Microbiology. It has now been seen by 3 referees, whose expertise and comments you will find at the end of this email. Although they find your work of some potential interest, they have raised a number of concerns that will need to be addressed before we can consider publication of the work in Nature Microbiology.

In particular, both reviewer #1 and #3 request to expand your phylogenetic analysis using additional viral genes beyond the L gene, and to extend the discussion on the limitations of your study. For example, but not limited to, discussing the limitations on the statistical support for some of the findings in the results and discussion sections. In addition, reviewer #1 asks to compare your host jump predictions using different tools. Reviewer #2 would like to see more results regarding the potential use of human CD150. We would be satisfied with sequence comparisons of CD150 homologues from relevant mammals and some careful speculations on whether interhost transmissions are likely.
This reviewer also suggests surrogate assays on Nectin-4 to inform on tissue tropism of the reported morbiliviruses or their according surrogate models.

Should further experimental data allow you to address these criticisms, we would be happy to look at a revised manuscript.

We are committed to providing a fair and constructive peer-review process. Please do not hesitate to contact us if there are specific requests from the reviewers that you believe are technically impossible or unlikely to yield a meaningful outcome.

We strongly support public availability of data. Please place the data used in your paper into a public data repository, if one exists, or alternatively, present the data as Source Data or Supplementary Information. If data can only be shared on request, please explain why in your Data Availability Statement, and also in the correspondence with your editor. For some data types, deposition in a public repository is mandatory - more information on our data deposition policies and available repositories can be found at https://www.nature.com/nature-research/editorial-policies/reporting-standards#availability-of-data.

Please include a data availability statement as a separate section after Methods but before references, under the heading "Data Availability". This section should inform readers about the availability of the data used to support the conclusions of your study. This information includes accession codes to public repositories (data banks for protein, DNA or RNA sequences, microarray, proteomics data etc…), references to source data published alongside the paper, unique identifiers such as URLs to data repository entries, or data set DOIs, and any other statement about data availability. At a minimum, you should include the following statement: "The data that support the findings of this study are available from the corresponding author upon request", mentioning any restrictions on availability. If DOIs are provided, we also strongly encourage including these in the Reference list (authors, title, publisher (repository name), identifier, year). For more guidance on how to write this section please see: http://www.nature.com/authors/policies/data/data-availability-statements-data-citations.pdf

If revising your manuscript:

* Include a "Response to referees" document detailing, point-by-point, how you addressed each referee comment. If no action was taken to address a point, you must provide a compelling argument. This response will be sent back to the referees along with the revised manuscript.

* If you have not done so already we suggest that you begin to revise your manuscript so that it conforms to our Article format instructions at http://www.nature.com/nmicrobiol/info/final-submission. Refer also to any guidelines provided in this letter.

* Include a revised version of any required reporting checklist. It will be available to referees (and, potentially, statisticians) to aid in their evaluation if the manuscript goes back for peer review. A revised checklist is essential for re-review of the paper.

When submitting the revised version of your manuscript, please pay close attention to our href="https://www.nature.com/nature-portfolio/editorial-policies/image-integrity">Digital Image Integrity Guidelines. and to the following points below:

-- that unprocessed scans are clearly labelled and match the gels and western blots presented in figures.
-- that control panels for gels and western blots are appropriately described as loading on sample processing controls
-- all images in the paper are checked for duplication of panels and for splicing of gel lanes.

Finally, please ensure that you retain unprocessed data and metadata files after publication, ideally archiving data in perpetuity, as these may be requested during the peer review and production process or after publication if any issues arise.

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Nature Microbiology is committed to improving transparency in authorship. As part of our efforts in this direction, we are now requesting that all authors identified as 'corresponding author' on published papers create and link their Open Researcher and Contributor Identifier (ORCID) with their account on the Manuscript Tracking System (MTS), prior to acceptance. This applies to primary research papers only. ORCID helps the scientific community achieve unambiguous attribution of all scholarly contributions. You can create and link your ORCID from the home page of the MTS by clicking on 'Modify my Springer Nature account'. For more information please visit please visit www.springernature.com/orcid.

If you wish to submit a suitably revised manuscript we would hope to receive it within 6 months. If you cannot send it within this time, please let us know. We will be happy to consider your revision, even if a similar study has been accepted for publication at Nature Microbiology or published elsewhere (up to a maximum of 6 months).

In the meantime we hope that you find our referees' comments helpful.

Yours sincerely,

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Reviewer Expertise:

Referee #1: Virology, phylogenetics, evolution
Referee #2: Virus infection, immunology
Referee #3: Zoonotic viruses, Virus diversity, Morbiliviruses

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The manuscript is well-written, and is based on a solid amont of data and diverse analyses, although there are some discrepencies between results described and data presented (see below). The major issue I found with this manuscript is that it appears that some results are over-interpreted to fit the narrative for the main role of New World bats in the origin of morbilliviruses. Some statements need to be tone down to represent better the results obtained and caveats of the data available. I think that some sections of the results and discussions described below would need to be revised before publication, or additional analyses may be needed to provide support for some statements.

One main issue concerns the evidence for a bat origin for morbilliviruses. This is mostly based on the posterior probability (PP) results presented for nodes A-D of a phylogenetic tree in Fig4c and d. PP values for these nodes are 30%, 42%, 66% and 82%, respectively. This is poor or, at best limited, statistical support for 3 out 4 nodes, even if PP value obtained for bats is higher than for other hosts. For the one strong support of 82%, it corresponds to node D which is itself poorly supported in the phylogeny (PP < 0.7). This limited statistical support should be made clear in results and discussion. Maybe the poor PP is due to the fact that the analysis is done on a partial L gene sequence (528nt). The authors went to a great extent to obtain complete or near-complete genome for 6 new morbilliviruses. I do not understand why they have not replicated the cophylogeny analysis on full genome data. Some virus and hosts for which only partial sequence is available would not be included, but some power might be gained on those deep nodes. The authors states in L293 that the poor power of short sequence was compensated by using different phylogenetic methods and tree topologies, but signal in the data is the limit, not the method. It would great if authors could do this extra analysis because the study would gain in strength. Cophylogeny results presented may be already good enough for publication in this journal, but the text must be amended to highlight their limitation.

Another issue concerns statements of bat-associated host shifts and evident reduction of the importance of host shifts from other orders. Their results show, as clearly stated in L211 that "bats, carnivores, and artiodactyls are main sources of cross-order host shifts in morbilliviruses", but the discussion, notably concerning MV's old world origin in cattle (L245-250), goes towards putting into doubts hosts shifts that are not of bat origin. In this section, host shifts from carnivors are totally omitted , apparently to fit with the narrative. The evidence fo bat-associated host shifts depends mostly on phylogenetic relationships between morbillivirus sequences, as for other hosts shifts. The discussion should be revised to represent better the diversity of host shifts in morbilliviruses, as shown in the results. Impact of bias in sampling should be better incorporated in the statements as well. The authors quickly mention sample bias (L267) and rightfully call for additional field investigation, but the possible impact of sample bias is not made as clear as it should. Any new morbillivirus sequence from old world bats or other hosts may change some of the results obtained concerning diversification of the virus and host shifts.

The authors state in the discussion (L290) that they account for sampling limitations with data mining from SRA, but their own results show that this method only provides poor results. Only one sequence of morbillivirus (which is already great!) was obtained out of thousands of SRA including from New World bats, so it can hardly be used to compare distribution of morbillivirus among different types of hosts. Interpretation of SRA mining results should be tone down accordingly.

There are multiple other statements that do not fit with the results presented and should be revised:
-L95 "was within the seroprevalence ranges detected for other morbilliviruses in wildlife populations". Only data from one paper on PMV is refered too so it is hardly all other morbillivirus in wildlife population.
- L112 "The number of morbilliviral species in bats exceeded those known in any other host order, i.e., six compared to four in artiodactyls (RPV, PPRV, CeMV, PoMV), three in carnivores (CDV, PDV, and FeMV) and one in primates (MV)". Is not not just a biais of sampling. Exceeding when it is 6 vs 4 is excessive
- L127 " most bat-associated morbilliviruses characterized on the full-genome level were monophyletic with the exception of MyoMV" This is based on genome data containing often only one of two sequences per morbillivirus. So how could it be otherwise. Their own data based on partial L gene could suggest that PhyMV may not be monophyletic. Such statement on limited data is not very useful
-L132 "...is consistent with low rates of recombination in negative-sense single- stranded RNA viruses" There is actually no evidence of recombination in any morbillivirus so far. Assessing recombination on genetic data only is not appropriate. See notably Han G.-Z. & Worobey M. (2011). – Homologous recombination in negative sense RNA viruses. Viruses, 3 (8), 1358-1373. doi: 10.3390/v3081358.)
-L161 "MarMV seperated by location not species"; looking at fig3b, there is clearly spearation by location and species. for example, all MarMG come from C penicillata. Again it is a statelent based on biased sample.
- L174 " confirming the marmoset host associations of morbillivirus-positive specimens. "- genetic results were exepcted to match with Callithrix expected as all 1400 sample, except 48, were marmoset. It only confirms that the sample was not contaminated by tissue from another animal. There is not enough samples from species other than marmoset to assess if their is a specific association with morbillivirus infection.

Finally, some results presented are not fully supported by the data made available:
- Table 1 and results mention 38 positive bat samples, but Supp Table 2 only list 24 positive samples. This list indicates the origin of 3 out 4 new bat morbillivirus. Notably sample CR209 at the origin of genome DesMV-1 is missing. Sample at the origin of the DesMV isolate is not indicated either.
-L71 "expanding the number of individual bats 4-fold". We don't know where this calculation come from.
- Fig1b and results describe RT-PCR results from multiple organs from 12 animals but the data associated is not in Supp Table 2 or anywhere else.
- Fig 1c: no information on what the circle in nodes stand for.
-Extended data 1a: Do the different colors in the labels of tree mean something?
- Fif 2e and f: Only Fig2e is mentioned in legend
- Fig3b The legend mentions the sue of PoMV as outgroup but it seems that PhyMV BOL is used as outgroup. From this analysis, it looks like PhyMV BOL is very different from PhyMV BRA (another species?). But it is maybe due to its positioning as an outgroup?
- Fig 3a what are the P hastatus and A planirostris samples indicated in fig 3a? New samples not mentioned in fig1a? From what

I understand, fig 3a should show only samples collected during the study, but these samples are not mentioned in the text.
-Extended Data Table 4 not well described. What are AI and PS? where are marmoset- bats swine- sequences ? Are they grouped per location? It should be mentioned.

****

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The authors have carried out a huge amount of work in their revisions and have addressed all my comments and recommendations. I have a couple of (very) minor points but am very satisfied with the revisions by the authors. I also particularly want to commend them on their high-quality figures.

Minor points:
Extended data fig 6e- can the authors include the host images in this panel too to make it clearer which colour is which.
Extended data fig 6f&g- can the authors describe the results in these panels in a little more detail for the reader's clarity.

Reviewer #2

(Remarks to the Author)
The authors have made significant effort to understand the receptor tropism of these viruses in vitro. This is important to understand the relative zoonotic risk of the different clades they have identified, e.g. a low risk for VamMV, and differential risk for MarMV and PBaMV dependent on whether you look at human CD150 or Nectin-4 usage. Indeed, predictions about 552 are prescient – I remember looking at that mutation before. A search uncovered this paper which the authors might want to reference: https://pmc.ncbi.nlm.nih.gov/articles/PMC6722581/. Also here: https://journals.asm.org/doi/10.1128/jvi.01248-18 authors aligned similar sequences. It's interesting to note that RPV sequences are F552Y at this position, (like the hCD150 using MarMVs) and RPV and MeV are thought to share a common ancestor. There is also species/host specific divergence at this residue so likely a key modulator of host-range. Beyond a few additions to the manuscript to reflect this prior discussion, I think it would be nice to see a phylogenetic tree added to compare the relative divergence of SLAMF1 and Nectin-4 sequences in morbillivirus hosts. I suspect one of these is more important than the other in driving receptor tropism/speciation in virus populations. I couldn't find this in the files, only the similarity matrices that I find a little difficult to read. Other than that, great work.

Reviewer #3

(Remarks to the Author)
I am fine with the revisions done to answer to my queries. I congratulate the authors for the immense job done in the revision.

However, statements on the macroevolutionary analyses remain too strong, based on PP of 0.40-0.73 for nodes B, C, D in Figure 6. The PPR value may be the highest of PP values obtained compared to other hosts, it still remains poor probability values.

Therefore, these two small changes are required before publication:

P6 L215-219: The probability of a bat origin was consistently highest in most major nodes in ASR (posterior probability [PP] 0.40-0.73 in nodes B-D, Fig. 6f), albeit the origin of morbilliviruses at the tree root remained unresolved (Fig. 6f, node A).

Please change to

The probability of a bat origin was consistently highest in most major nodes in ASR, although posterior pobability values remained small for most nodes (posterior probability [PP] 0.40-0.73 in nodes B-D, Fig. 6f). Notably, the origin of morbilliviruses at the tree root remained unresolved (Fig. 6f, node A).

P7 L257-258: Our macroevolutionary data project the origins of major morbilliviruses to non-recent host shifts, predominantly from bat hosts, in addition to artiodactyls and carnivores, discussed elsewhere20,28.

please change to:

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Decision Letter:

Our ref: NMICROBIOL-24061682A

14th February 2025

Dear Felix,

Thank you for submitting your revised manuscript "Origins and evolutionary trajectories of morbilliviruses in Neotropical bats" (NMICROBIOL-24061682A). It has now been seen by the original referees and their comments are below. The reviewers find that the paper has improved in revision, and therefore we'll be happy in principle to publish it in Nature Microbiology, pending minor revisions to satisfy the referees' final requests and to comply with our editorial and formatting guidelines.

If the current version of your manuscript is in a PDF format, please email us a copy of the file in an editable format (Microsoft Word or LaTex)-- we can not proceed with PDFs at this stage.

We are now performing detailed checks on your paper and will send you a checklist detailing our editorial and formatting requirements in about a week. Please do not upload the final materials and make any revisions until you receive this additional information from us.

Thank you again for your interest in Nature Microbiology Please do not hesitate to contact me if you have any questions.

Sincerely,

Reviewer #1 (Remarks to the Author):

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please change to:

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Version 2:

Decision Letter:

7th April 2025

Dear Felix,

I am delighted to accept your Article "Ecology and evolutionary trajectories of morbilliviruses in Neotropical bats" for publication in Nature Microbiology. Thank you for having chosen to submit your work to us and many congratulations.

Over the next few weeks, your paper will be copyedited to ensure that it conforms to Nature Microbiology style. We look particularly carefully at the titles of all papers to ensure that they are relatively brief and understandable.

Once your paper is typeset, you will receive an email with a link to choose the appropriate publishing options for your paper and our Author Services team will be in touch regarding any additional information that may be required. Once your paper has been scheduled for online publication, the Nature press office will be in touch to confirm the details.

You may wish to make your media relations office aware of your accepted publication, in case they consider it appropriate to organize some internal or external publicity. Once your paper has been scheduled you will receive an email confirming the publication details. This is normally 3-4 working days in advance of publication. If you need additional notice of the date and time of publication, please let the production team know when you receive the proof of your article to ensure there is sufficient time to coordinate. Further information on our embargo policies can be found here:
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After the grant of rights is completed, you will receive a link to your electronic proof via email with a request to make any corrections within 48 hours. If, when you receive your proof, you cannot meet this deadline, please inform us at rjsproduction@springernature.com immediately. You will not receive your proofs until the publishing agreement has been received through our system

Due to the importance of these deadlines, we ask you please us know now whether you will be difficult to contact over the next month. If this is the case, we ask you provide us with the contact information (email, phone and fax) of someone who will be able to check the proofs on your behalf, and who will be available to address any last-minute problems.

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As soon as your article is published, you will receive an automated email with your shareable link.

Congrats again to you and your co-authors! I am looking forward to seeing the paper published.

With kind regards,

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Rebuttal letter for

“Origins and evolutionary trajectories of morbilliviruses in Neotropical bats”

Tracking NMICROBIOL-24061682

Reviewer Comments

Reviewer #1 (Remarks to the Author):

The authors present work that represents a significant leap in our understanding of the standing diversity and origins of morbilliviruses. Limited genome samples previously existed for the family and with the authors’ geographic and temporal sampling of bat and NHPs, coupled with data mining, the authors have identified a number of novel morbilliviruses. The methods in this manuscript are sound and the text and figures are of high quality. I have a number of minor points below that I believe would improve the quality of the manuscript, but overall I commend the authors on their engaging manuscript.

Reply: We thank the reviewer for the critical comments and suggestions. We have addressed all comments and included the additional host reconstruction analyses using BEAST. In addition, we have changed all virus names according to new ICTV guidelines and consistent with the terminology used in a previously published paper[1]:

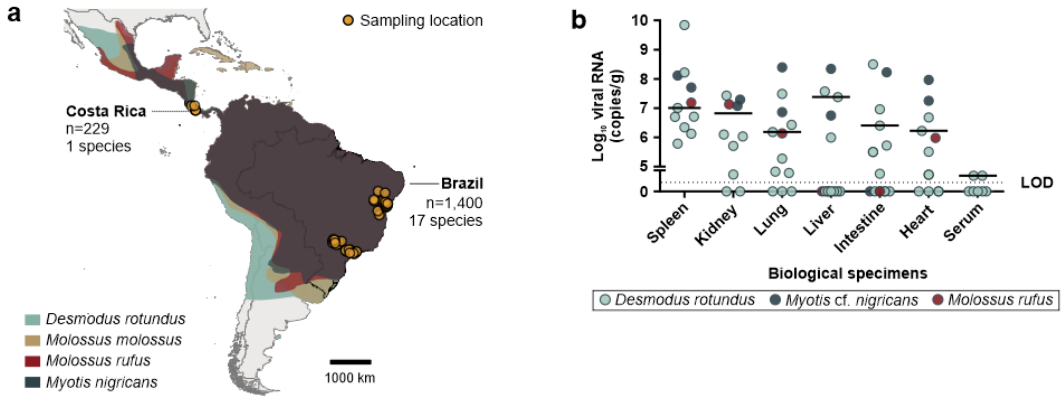
Virus abbreviations:

- MeV for measles virus and according to ICTV guidelines (previously MV)
- VamMV standing for vampire bat morbillivirus (previously DesMV)
- PBaMV standing for phyllostomid bat morbillivirus (previously PhyMV)
- MBaMV standing for myotis bat morbillivirus (previously MyoMV)

Minor points:

- Between figure 1 a and b, keep the species colours consistent.

Reply: Done as suggested.



(end of reply)

- The authors have described how sampling bias may impact their results, as sampling was only performed in two Neotropical countries. On that point, sampling was also not performed on local rodent species. The rationale for this makes sense given the historical importance of bats for Morbilliviruses, however it would be good for the authors to comment more extensively on sampling bias.

Reply: We agree with the reviewer. We have added our rationale for not sampling local rodent species in the methods under the Animal Sampling section and extended our rationale for not including rodent paramorbilliviruses in our analyses in our Dataset section. We also discuss how sampling bias of bats in the Neotropics may affect our evolutionary reconstructions and suggest targeted field work of shrews in the Neotropics and Old World bats.

Discussion

P7 L268-272: Whether the evolutionary origins of morbilliviruses date back to the dispersal histories of extant Neotropical bats (40 -13 million years ago)[2-4], to potential extinction events of morbilliviruses or their hosts in the Old World, or to macroevolutionary phenomena such as preferential host switching in sympatric animals or via intermediate hosts, or to whether those estimates are affected by sample bias remains to be investigated.

P7 L276-279: Targeted field work potentially elucidating the timing and hosts of morbilliviral macroevolution should include Neotropical shrews and Old World bat species genetically related to Neotropical bats hosting morbilliviruses, such as molossid and myotis bats.

Animal sampling

P9 L367-369: Local rodent species were not included as diverse rodent paramyxoviruses occur in phylogenetic sister relationship to morbilliviruses sensu stricto.

Dataset

P9 L446-451: We excluded diverse rodent paramyxoviruses that occur in phylogenetic sister relationship to viruses unambiguously classified as morbilliviruses by the ICTV, in part because these rodent paramyxoviruses had a different genome organization and lacked conserved key amino acids in the H glycoprotein for CD150 binding[5]. These rodent-borne viruses which are being classified as paramorbilliviruses by the ICTV may thus have had a different evolutionary pathway to classical (ortho)morbilliviruses.

(end of reply)

- The authors use Vegan and Densitree rather than BEAST for host reconstruction, however they do use BEAST for tree estimation. BEAST can also estimate host jumps and it would be good to compare these methods and see if it concurs with the Densitree / Vegan output (possibly using the steps outlined in this tutorial: https://beast.community/markov_jumps_rewards).

Reply: We thank the reviewer for this helpful suggestion. We conducted the continuous-time Markov chain (CTMC) analysis in a Bayesian framework (as suggested by the reviewer) and compared the results to our parsimony-based ancestral state reconstructions by Mesquite (updated **Figure 6d**). In general, the CTMC method generated higher host shift counts for all orders, as it accounts for uncertainties in the ancestral state reconstructions, whereas the parsimony-based approach aims to minimize the number of state changes in the reconstructions. Therefore, we highlighted only those host shifts whose values agreed in both methodological approaches, namely bats to artiodactyls and carnivores, from artiodactyls to primates, and from carnivores to bats, artiodactyls and shrews (updated **Fig. 6d**). No large difference was found in the main message. Host shifts during morbillivirus evolution predominantly originated from bats, artiodactyls and carnivores. In response to your query, we added the average Markov host shift count per host as an additional output (new **Fig. 6c** and updated **Fig. 6f**).

P6 L208-212: Total average counts of host shifts were highest from bats and artiodactyls to other host orders (**Fig. 6c**). Bayesian and parsimony-based ASR corroborated high average numbers (>0.5) of host shifts from bats to artiodactyls and carnivores, from artiodactyls to primates, and additionally from carnivores to bats, artiodactyls and shrews (**Fig. 6d**).

P7 L241-245: To investigate the sources of cross-order host shifts, we analysed morbilliviruses identified in a different host order than the putative reservoir host, including the MarMV described above. Average host shift counts in a Bayesian framework (**Fig. 6f***, right insert*) suggested a bat origin for MarMV and PoMV, as well as a carnivore origin for macaque-CDV and opossum-associated FeMV.

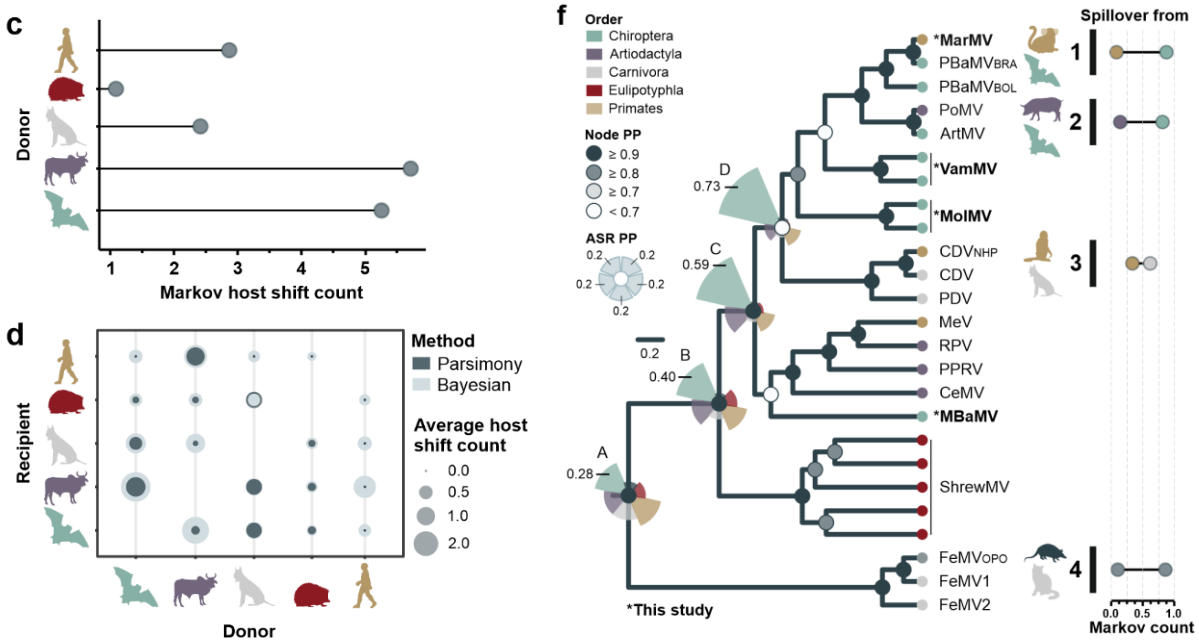


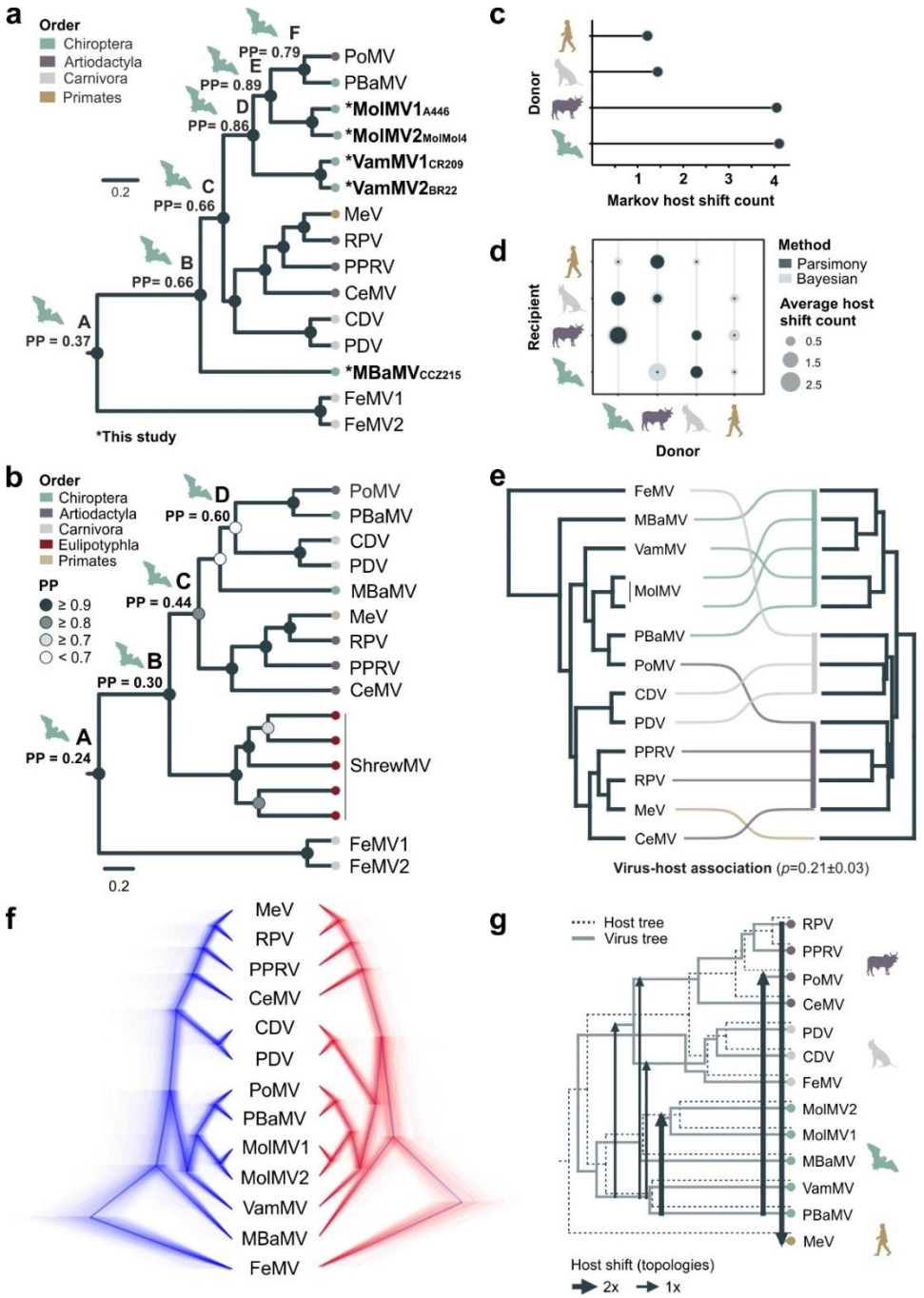
Fig. 6 Macroevolution of morbilliviruses
c) Average jump counts from each host order using continuous-time Markov chain in a Bayesian framework. d) Average number of morbillivirus host jumps from parsimony-based and Bayesian ancestral state reconstructions. f) f) *Left* insert, Bayesian phylogeny of representative morbillivirus species and sequences from suspected cross-order spillover events. Scale bar indicates genetic distance. BOL, Bolivia; BRA, Brazil; NHP, non-human primate; OPO, opossum. At nodes, circular bar plots denoting Bayesian ASR posterior probabilities (PP) for each evaluated node (A-D). *Right* insert, average Markov host shift counts for selected nodes. Node 1: bats 0.88 vs. marmosets 0.08, node 2: bats 0.81 vs. marmosets 0.14, node 3: carnivores 0.62 vs. primates 0.34, node 4: carnivores 0.86 vs. diprotodonts 0.10. See **Supplementary Tables S1-S2 and S6** for virus abbreviations and GenBank accession numbers.

(end of reply)

- The authors limit their reconstruction analysis to the L gene and describe how this limits their analysis. Could the authors extract the coding sequences from each gene (or perhaps more than just L), align them and concatenate them together as a way of getting more data for the reconstruction?

Reply: We thank the reviewer for the suggestion. We also agree that including more genetic information would help our reconstructions. However, our reconstruction was limited to the availability of morbillivirus sequences derived from shrews (GenBank accession numbers MT063254, MT063490, MT063273, MT157273, AB844351), and a few derived from bats (GenBank accession numbers KP963782.1, MN907425.1, KP963849.1), which we consider to be of substantial importance to include to exhaustively reconstruct the evolutionary history of morbilliviruses. Nevertheless, we performed the analyses using only complete concatenated translated ORFs (N,P,M,F,H, and L) and main results did not vary from results using only partial *L* gene sequences (updated **Extended Data Fig. 6**). In detail, for distance-based coevolutionary analyses, we found no signal for coevolution in both datasets (including one or two virus species of VamMV and FeMV) encompassing 1,000 randomly selected trees in ParaFit (p=0.058±0.011, p=0.21±0.03**; Extended Data Fig. 6e**), similar to what we reported originally based on the partial *L* gene sequences including those derived from shrews. For event-based coevolutionary analyses, we tested the two most likely topologies and consistently reconstructed an origin of PoMV, detected in pigs, from a bat-associated ancestor, an origin of MeV from a cattle-associated ancestor, and host switching among bat host species (**Extended Data Fig. 6g**), as we already reported including the partial *L* gene sequences from the shrews. Additionally, the importance of bats was more prominent as it led to increased statistical support for bat origins at the root (**Extended Data Fig. 6a**), which albeit supporting our conclusions, implies larger bias.

P6 L224-232: ASR in a Bayesian framework using full genome data generated high branch support values of >0.9 PP and led to increased statistical support for bat origins at the root and for all major nodes (0.37 – 0.86 in nodes A-D, **Extended Data Fig. 6a**) at the expense of excluding shrews as morbillivirus hosts, as only partial sequences were available. In contrast, removing the morbilliviral genomic sequences generated in this study led to decreased statistical support for bat origins (0.24 – 0.60 in nodes A-D, **Extended Data Fig. 6b**). Using full genome data, major findings in the host switch analyses and coevolutionary reconstructions were consistent with the results generated from partial *L* sequences (**Extended Data Fig. 6c-g**), suggesting robustness of our macroevolutionary analyses.



Extended Data Fig. 6. Macroevolution of morbilliviruses based on different datasets. a) Bayesian phylogeny of representative morbillivirus species. Circle at nodes indicate posterior probabilities >0.9 for branch support. Numbers at nodes A-F show the highest posterior probabilities (PP) for a host order by Bayesian ancestral state reconstructions (ASR). b) Bayesian phylogeny of previously published classical and divergent morbillivirus species. Tree was based on partial *L* gene (361 nucleotides). At nodes A-D, PP for a bat origin by Bayesian ASR. For a and b, scale bar indicates genetic distance. c) Average host shift counts from each host order using continuous-time Markov chain in a Bayesian framework d) Average number of morbillivirus host shifts from

Bayesian and parsimony-based ASR. e) Tanglegram of virus-host associations showing ParaFit *p*-value. f) DensiTree showing clustering morbillivirus sequences used to generate the two most likely topologies for event-based cophylogenies. g) Event-based coevolutionary analyses of the two most likely topologies. Arrow width represents the number of alternative topologies with the specified host shift. For a and c-g, analyses were performed using translated, concatenated ORFs. See **Supplementary Table S1-S2** for virus abbreviations and GenBank accession numbers.

(end of reply)

- May be useful to include a schema of mammalian relatedness for the species sampled in this study for those less familiar with mammalian taxonomy and to help demonstrate the host-virus evolutionary histories (perhaps even a tanglegram).

Reply: We agree and included a host tree based on the mammalian species tree[6] highlighting morbillivirus hosts (new **Fig. 6a**) and the representation of the ASR analyses evaluating the origins at the root of morbillivirus evolutionary tree. We also included a tanglegram to highlight the virus-host association (new **Fig. 6b**).

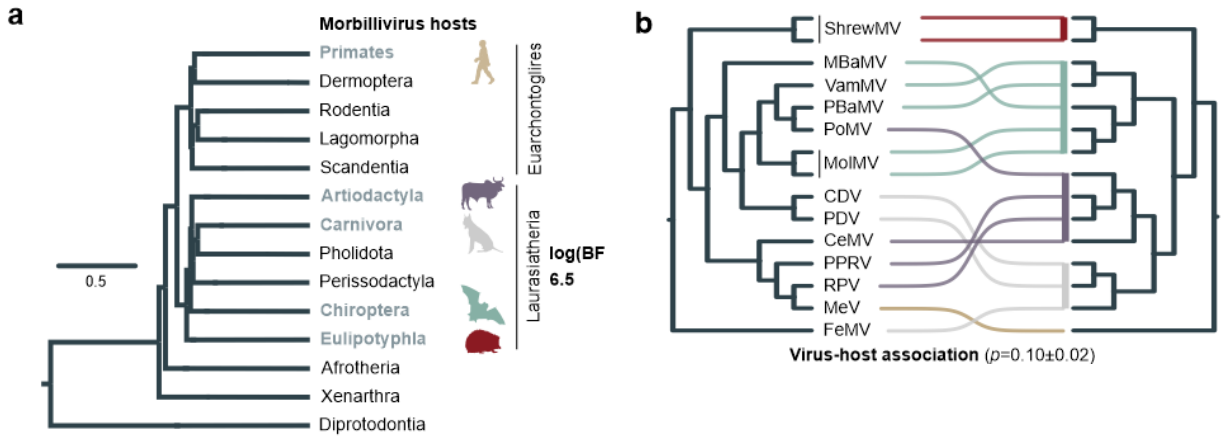


Fig. 6. a) Mammalian species tree[6] highlighting morbillivirus host orders/superorders. Outgroup Monotremata. Bayesian ancestral state reconstructions (ASR) comparing the superorders hosting morbilliviruses: Laurasiatheria (highest $\log_{10}$ Bayes factor [BF]) versus Euarchontoglires. b) Tanglegram of virus-host associations showing ParaFit *p*-value.

(end of reply)

- Figure 2 legend should read accession numbers, rather than accession nos.

Reply: Changed as suggested.

(end of reply)

- P7L232: Very briefly describe BaTS software for clarity as broad readership may not be familiar (Bayesian Tip-association Significance Testing may even be enough for clarity)

Reply: We have added a short description of the software BaTS in the main text as suggested.

P7 L245-249: Assigning country as a trait, Bayesian Tip-association Significance Testing (BaTS), tool that correlates trait values with phylogenies)[7], corroborated geographic association ($p<0.05$) for bat- and marmoset-associated morbilliviruses from Brazil as well as for bat- and pig-associated morbilliviruses from Mexico (**Fig. 6g, Extended Data Table 4**).

(end of reply)

- P10L373: Should the text read low quality reads were discarded rather than trimmed with Trimmomatic? Perhaps low quality ends were trimmed?

Reply: We have modified the text for better clarity.

P10 L387-388: Low quality reads of <Q30 were discarded, and low-quality ends were trimmed with Trimmomatic V.0.4.

(end of reply)

- Is it possible that there are morbilliviruses (or other viruses) that are more divergent in the data but lack the homology for finding hits? Could assembly and then matching help identify more.

Reply: We thank the reviewer for this comment. To identify highly divergent morbilliviruses, we used the Serratus Explorer tool for both nucleotide and amino acid (RdRP) sequence search available on the Serratus platform (https://serratus.io/toolkit). In addition, we used another tool that analyses the viral RdRP palmprint barcoding (palmID). In all instances we found classical morbilliviruses (e.g. MeV, PPRV, and CDV), as well as the reported morbillivirus sequence in a *M. molossus* in this study. We used a cutoff value of ≥82% identity to PBaMV (query), as sequences below that threshold belonged to henipa- and jeilongviruses, and a rodent paramorbillivirus in *Abrothrix olivaceus* (GenBank accession BK061229.1), that occur in phylogenetic sister relationship to morbilliviruses sensu stricto. This way we made sure to not miss any divergent unreported morbillivirus. We have now added a more detailed description in the Data mining section of Methods and added a supplementary table to enhance clarity of the methodology used:

P11 L423-434: We searched for unidentified divergent morbilliviruses by using the web tools Serratus explorer and palmID (analysis suite based on the viral RdRP palmprint barcoding sub-sequence) on the Serratus platform[8] (https://serratus.io/toolkit). The platform uses genomic sequencing data from the National Center for Biotechnology Information Short Read Archive (SRA) and screens for sequences that are similar to known viruses. For the identification of morbilliviruses, we used a cutoff value of ≥82% identity to PBaMV (GenBank accession MZ312422), which was used as query sequence for the morbillivirus polymerase barcode sequences retrieved from palmID. Sequences below that threshold belonged to a rodent paramorbillivirus (GenBank accession BK061229.1) and to viruses belonging to other paramyxovirus genera such as *Jeilongvirus* and *Henipavirus* (**Supplementary Table S13**). Sequences corresponding to MeV, PPRV, and CDV in prototypic hosts were excluded from downstream analyses. Once a divergent morbillivirus sequence was identified, the SRA sequence was downloaded and *de novo* assembly was performed.

(end of reply)

- Can the authors clarify if they de novo assembled all high-quality reads at any point for the novel samples (as done for the data mining)? If not, it would be good to compare results between the reference-based/ sequence homology approach and complete de novo assembly.

Reply: We thank the reviewer for this comment. For all morbillivirus-positive samples that were sequenced by HTS, we searched for potential morbillivirus reads that had >60% amino acid identity to any morbillivirus sequence in a morbillivirus protein reference database to get rid of host and other non-morbillivirus reads. The remaining "potential" morbillivirus reads were then *de novo* assembled, and only then we used a reference assembly with the newly generated morbillivirus contigs. We have now modified our methods section to enhance clarity.

P10 L387-394: Low quality reads of <Q30 were discarded, and low-quality ends were trimmed with Trimmomatic V.0.4. Remaining reads were aligned to a morbillivirus protein reference database (Ensemble) using sensitive default mode using DIAMOND (https://github.com/bbuchfink/diamond). Potential morbillivirus reads were extracted and *de novo* assembled using Geneious assembler within Geneious R.11.1.5 (https://www.geneious.com). A reference-based assembly using all high-quality

reads was then performed with the newly generated morbillivirus contigs using Bowtie2. Several iterations were performed until no more new reads were assembled.

(end of reply)

- The authors use one example sequence per virus species, however a quick search on NCBI virus shows there are ~1,600 complete or near-complete Morbillivirus genomes (taxid 11229). The phylogenetic analysis could benefit from greater numbers of samples. Is this something the authors considered?

Reply: In response to this query, we made a search for all near-complete morbillivirus sequences and obtained >1,600 morbillivirus sequences as the reviewer had already pointed out. Of these sequences, more than half of the sequences belonged to measles virus, and 1/4 to canine distemper virus, which would bias our macroevolutionary analyses. We believe inclusion of those data would be more important for dating or pressure analyses that are not covered in our study. We added this new data as supplementary **Table S14**.

Table S14. Near-complete morbillivirus sequences in NCBI Virus database

Virus	Complete sequences
Measles virus	984
Rinderpest virus	133
Peste des petits ruminants virus	231
Cetacean morbillivirus	35
Canine distemper virus	393
Phocine distemper virus	11
Porcine morbillivirus	1
Feline morbillivirus	29
Myotis bat morbillivirus	1
Phyllostomid bat morbillivirus	1
Total	1819

Note: sequences were accessed 27/12/2024

(end of reply)

Reviewer #2 (Remarks to the Author):

The proposed article by Jo et al describes the identification and characterisation of a number of bat and primate born morbilliviruses from the Americas. The authors used extensive sample sets to identify the viruses, then sequenced them and examined their properties, mostly focusing on the genomic organisation and conclusions surrounding their phylogenetic relationship to each other, and extant sequences in the database. I found the manuscript easy to read and exciting, and I was impressed by the broad attempts at characterisation across in vitro biology and RNA virus evolution.

Reply: We thank the reviewer for the critical comments and suggestions. We have addressed all comments and added functional experiments employing cell-to-cell fusion assays and investigation of nectin-4 usage. In addition, we have changed all virus names according to new ICTV guidelines and consistent with the terminology used in a previously published paper[1]:

Virus abbreviations:

- MeV for measles virus and according to ICTV guidelines (previously MV)
- VamMV standing for vampire bat morbillivirus (previously DesMV)
- PBaMV standing for phyllostomid bat morbillivirus (previously PhyMV)
- MBaMV standing for myotis bat morbillivirus (previously MyoMV)

Major points:

The tissue distribution of the virus in infected animals is interesting. The high signal in the spleen, would I agree, correlate with a lymphotropic virus – consistent with CD150 usage – which appears to be a conserved feature of morbillivirus entry. However, the high signals in the heart are more inconsistent with the known tissue tropism of 'classical' morbilliviruses like measles. Could the authors amplify Nectin-4 from their bat or NHP samples and confirm use of this receptor (I am unclear if these sequences are already known for these bat species and/or marmosets). This would go some way towards confirming that these bat morbilliviruses have the same biphasic life cycle in their mammalian hosts as measles, rinderpest, canine distemper etc. I understand live virus work is potentially challenging (due to sample degradation) but there are surrogate entry assays available, or recombinant protein binding experiments (ELISA, BLI, SPR) that could provide an answer (based on synthesis and construction of F and H expression constructs).

Reply: We agree. To address this comment, we added NCI-H358, cells expressing human nectin-4[9] (but not CD150), to our VamMV growth curve. No viral growth was detected by TCID50 assay nor with RT-qPCR, whereas MeV readily infected those cells and produced typical CPE (new **Supplementary Fig. S1**). We have updated **Fig. 2a** showing viral RNA (copies/mL) as instead of TCID50/mL (shown below) as all other cell lines, except Vero expressing vampire bat CD150, had value 0. Data from both RT-qPCR and TCID50 assays were added to the source data file associated to the figure panel. This result was incorporated into our results section.

P4 L93-105: No utilization of human CD150 (hCD150) nor nectin-4 (hnectin-4) by the VamMV isolate was shown (**Fig. 2a**), whereas MeV readily used those receptors (**Supplementary Fig. S1**). Lack of at least residual hnectin-4 usage was in contrast to another study showing that a myotis bat morbillivirus can use hnectin-4[1] to some extent, despite a pairwise amino acid sequence distance of 11-12% between human and myotis/vampire bat nectin-4 orthologues (**Fig. 2b, Supplementary Table S3**). It seems plausible that individual amino acid differences may limit VamMV usage of hnectin-4, or that different cell lines used may contribute to the observed difference. Lack of usage of the entry receptor CD150 and the egress receptor nectin-4 jointly corroborated limited zoonotic potential of VamMV.

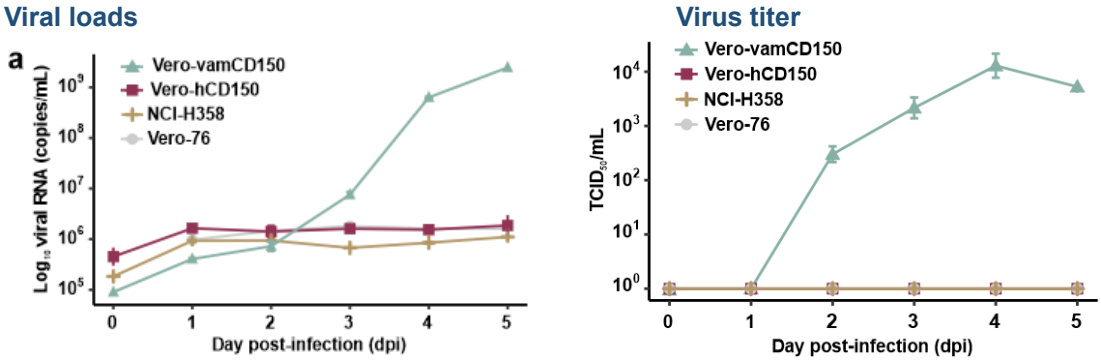


Fig. 2. a) Multistep growth curve analyses of VamMV1 (CR209) inoculated at a MOI of 0.01 for five days in Vero-vamCD150, Vero-hCD150, NCI-H358, and Vero-76 cells as control. Error bars are the standard error of the mean.

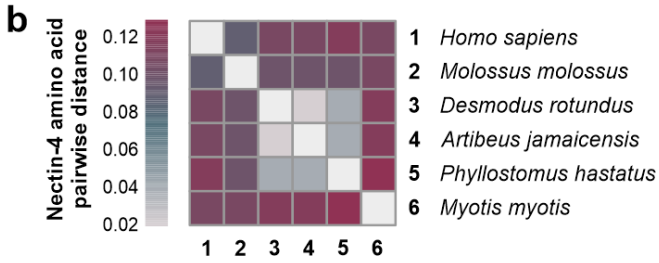
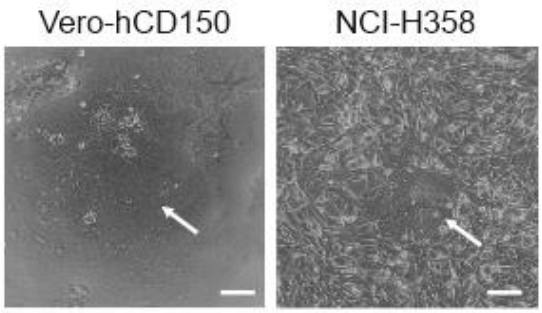


Fig. 2. b) Distance matrix comparing nectin-4 sequences between bat and human hosts of morbilliviruses. Cases in which sequence from the animal species was not available, a close genetically related species was used. See **Supplementary Table S3** for GenBank accession numbers.



Supplementary Fig. S1. Representative images of cytopathic effects (syncytia formation) produced by MeV. Scale bars, 100 µm.

Table S3. Pairwise amino acid sequence distances of nectin4 sequences from morbillivirus hosts

Sequences		A	B	C	D	E	F	G	H	I	J	K	L	M	N
AB755430.1 *Homo sapiens*	A														
XM_003735162.6 *Callithrix jacchus*	B	0.02													
XM_053915939.1 *Desmodus rotundus*	C	0.11	0.11												
XM_036343999.1 *Myotis myotis*	D	0.11	0.13	0.12											
XM_045838773.1 *Phyllostomus hastatus*	E	0.12	0.13	0.04	0.13										
XM_036272705.1 *Molossus molossus*	F	0.09	0.10	0.10	0.11	0.10									
XM_053659354.1 *Artibeus jamaicensis*	G	0.11	0.12	0.02	0.12	0.04	0.10								
NM_001313853.1 *Canis lupus familiaris*	H	0.06	0.07	0.09	0.10	0.11	0.08	0.10							
XM_032397196.1 *Phoca vitulina*	I	0.06	0.07	0.09	0.11	0.11	0.09	0.10	0.03						
XM_013996829.2 *Sus scrofa domesticus*	J	0.07	0.08	0.10	0.12	0.12	0.10	0.11	0.06	0.06					
XM_033856224.1 *Tursiops truncatus*	K	0.07	0.09	0.11	0.13	0.12	0.10	0.12	0.07	0.07	0.06				
XM_019822297.3 *Felis catus*	L	0.07	0.08	0.12	0.12	0.13	0.10	0.12	0.05	0.05	0.07	0.09			
XM_005677185.3 *Capra hircus*	M	0.07	0.09	0.10	0.12	0.11	0.09	0.11	0.06	0.06	0.04	0.06	0.08		
NM_001024494.1 *Bos taurus*	N	0.08	0.10	0.11	0.13	0.12	0.10	0.12	0.06	0.06	0.05	0.07	0.08	0.02	

To complement full virus-based infection experiments, we conducted cell-to-cell fusion assays using F and H glycoprotein expression constructs of marmoset and phyllostomid bat morbilliviruses on NCI-H358 cells. We chose the marmoset morbilliviruses as they were found in a primate host and therefore sequences of the nectin-4 receptor are closest to human with pairwise amino acid sequence distances of 2% (new **Supplementary Table S3**). We chose one marmoset morbillivirus strain per phylogenetic clade (see below), and phyllostomid bat morbillivirus, which is genetically the closest bat-associated morbillivirus. One marmoset morbillivirus strain from Minas Gerais (MG) showed fusion activity, corroborating usage of human nectin-4 by the newly found morbilliviruses is possible, albeit at low levels.

In addition, we performed cell-to-cell fusion assays on cells that express human CD150 (Vero-hCD150) and tamarin CD150 (B95a), as suggested by the reviewer. We showed that the two strains from Bahia (BA-1, BA-2) produced syncytia in Vero-hCD150 and B95a (**Fig. 5b**). The marmoset morbillivirus strain from MG and the phyllostomid bat morbillivirus, which grouped together, did not produce syncytia on those cells, again hinting at individual residues differentially restricting entry of those viruses. Our results suggest some level of zoonotic risk of MarMV at the entry stage. Results from the fusion assays were added to the new **figure 5**.

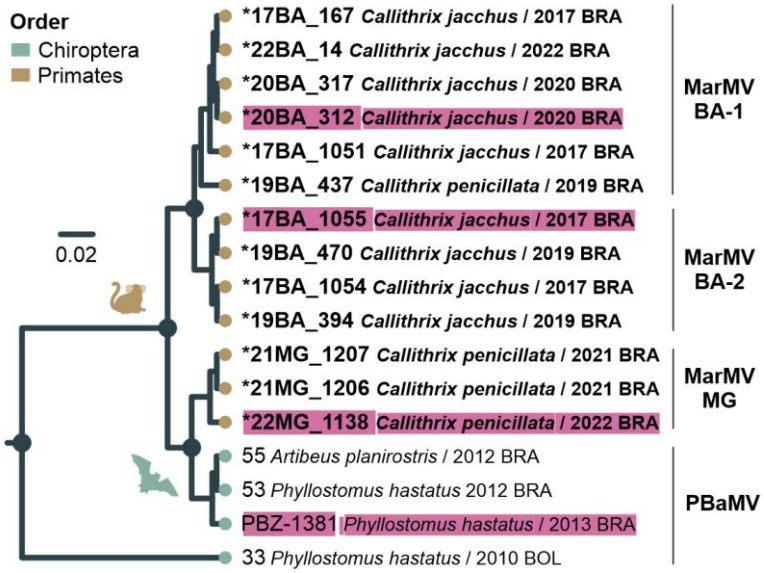


Figure. MarMV strains and PBaMV used for fusion experiments are highlighted

The following text block reports the data and methodology:

Results section

P5 L168-173: Pairwise amino acid sequence distances of tamarin and marmoset CD150 orthologues only show about 3% distance (**Fig. 5a, Supplementary Table S8**), and no differences at the MeV interaction sites[10] (**Fig. 5b**). Notably, MeV can use tamarin CD150[10] despite having a larger distance of 13% between tamarin and human CD150 amino acid sequences (**Fig. 5a, Supplementary Table S8**), and only one amino acid change in the interaction site 4 at position 119 (**Fig. 5b**).

P5 L175-190: We conducted cell-to-cell fusion assays to test whether MarMV can use tamarin and/or human CD150 as entry receptor despite the multiple amino acid changes in the interaction sites between H glycoprotein sequences (**Fig. 5c**). The two BA MarMV strains showed fusion activity in B95a and Vero-hCD150 cells, but not in hnectin-4 expressing NCI-H358 cells (**Fig. 5d**). Contrastingly, the MG MarMV strain that grouped with the previously sequenced PBaMV[11] showed fusion activity in NCI-H358 cells, corroborating functionality of viral glycoproteins, although fusion was neither observed in B95a, nor Vero-hCD150 cells. Notably, PBaMV showed no fusion activity in any of the tested cells, hinting at clade-associated properties within those genetically related bat- and marmoset-associated morbilliviruses. The only amino acid change between those strains inducing fusion and the ones that did not in B95a

and Vero-hCD150 cells was at position 552 (according to MeV), which participates in both CD150 interaction sites 2 and 4 (**Fig. 5c**). Position 552 may be a potential target for forward genetics investigating receptor use and host range. Notably, MeV glycoproteins induced fusion in all receptor-expressing cells (**Fig. 5d**), whereas none of the morbillivirus glycoproteins transfected individually or the empty vector induced fusion in tested cells or when cotransfected in Vero-E6 (**Fig. 5d, Extended Data Fig. 4**), which do not express morbillivirus receptors.

P6 L192-194: Thus, our data suggest some zoonotic risk of MarMV. However, whether a full replication cycle is possible and which amino acid residues potentially affect fusion activity or infection warrants further assessments, *e.g.* by reverse genetics[1].

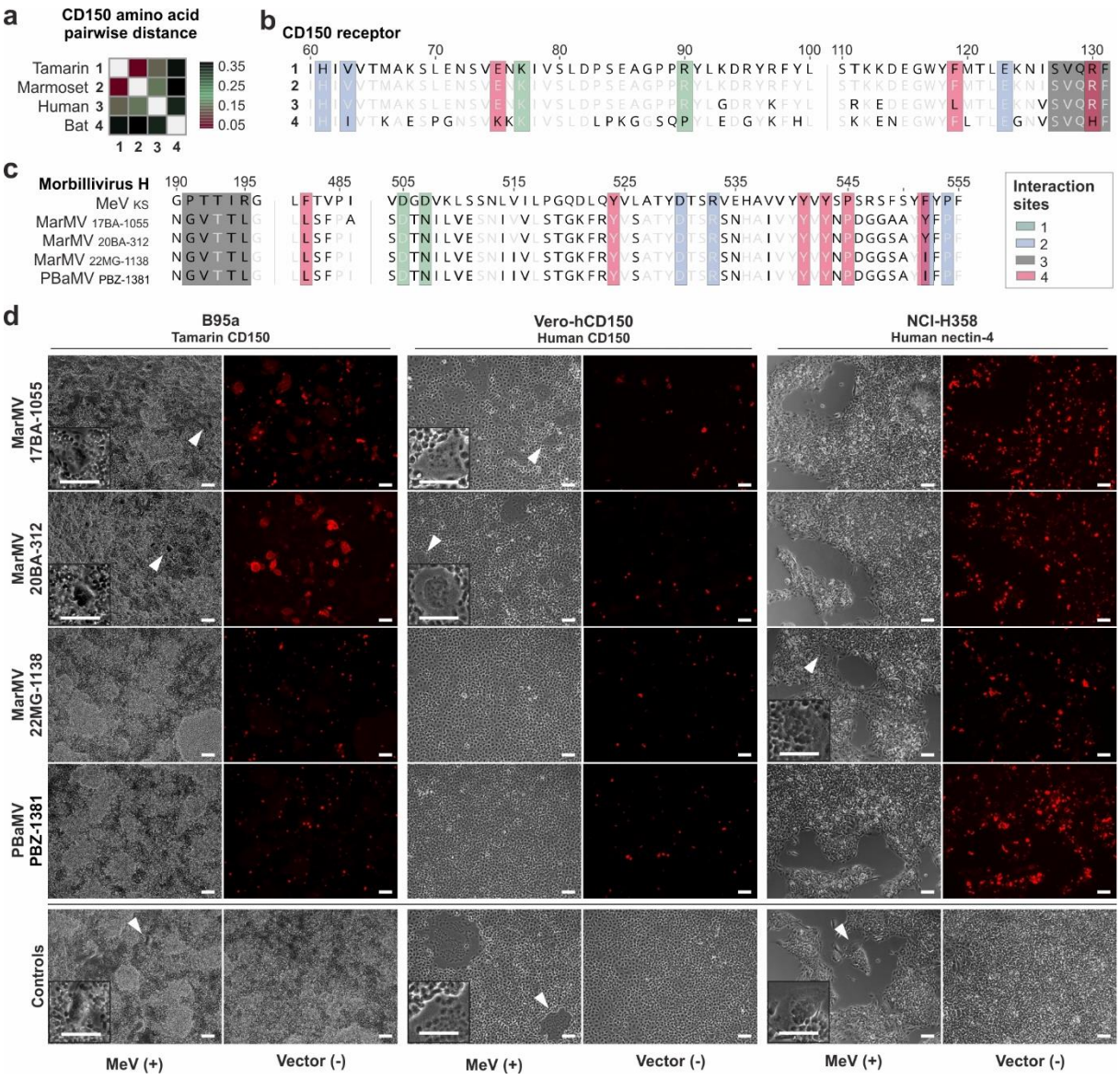
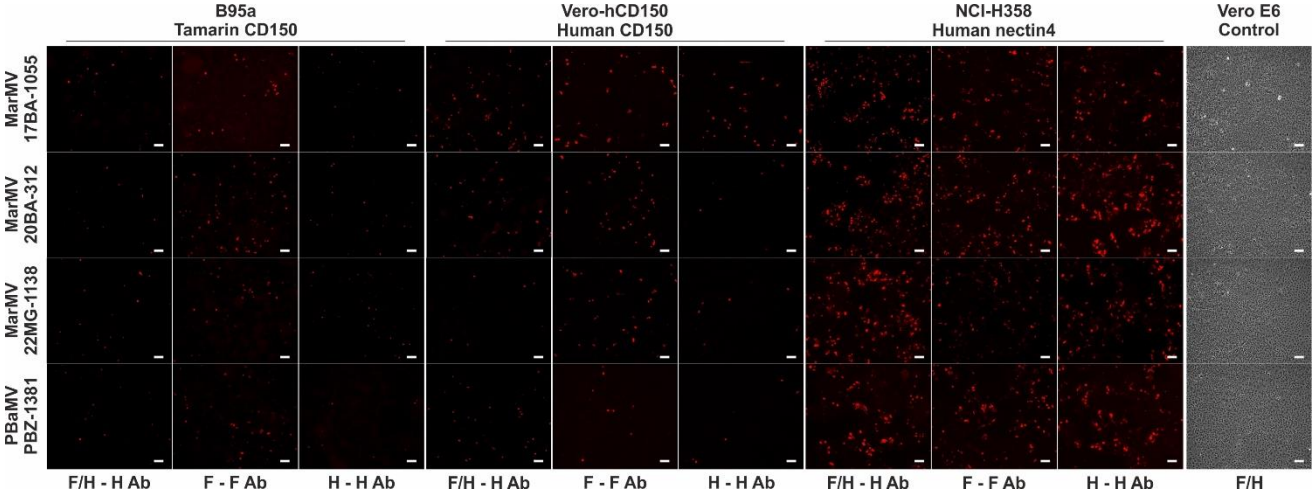


Fig. 5. Membrane fusion of marmoset-associated and conspecific bat-associated morbilliviruses
a) Distance matrix comparing relevant CD150 amino acid sequences to marmoset-associated morbilliviruses. See **Supplementary Table S8** for GenBank accession numbers. Bat refers to *Phyllostomus hastatus*, the host of PBaMV. b and c) Interaction sites between homologue morbillivirus H glycoproteins and CD150 receptors based on MeV H and tamarin CD150 crystal structure[10]. Numbers in panels a and b are linked and refer to the animal host. d) Cell-to-cell fusion assay of marmoset- and phyllostomid bat-associated morbilliviruses. Representative cotransfected B95a, Vero-hCD150, and NCI-H358 cells with F and H glycoproteins and stained with anti-F peptide-based primary antibody and anti-rabbit Cy3 secondary antibody. MeV F and H cotransfected cells as positive control and empty vector pCG1 transfected cells as negative control. Insert location is indicated by a white triangle. Scale 100µm. MarMV, marmoset-associated morbillivirus; PBaMV, phyllostomid bat morbillivirus; MeV, measles virus.



Extended Data Fig. 4. Controls of cell-to-cell fusion assay of marmoset-associated and conspecific bat-associated morbilliviruses

Cell-to-cell fusion assay of marmoset- and phyllostomid bat-associated morbilliviruses. Representative B95a, Vero-hCD150, and NCI-H358 cells transfected with fusion (F) and hemagglutinin (H) glycoproteins together (F/H) or individually (F or H) and stained with anti-H or anti-F peptide-based primary antibody and anti-rabbit Cy3 secondary antibody. Vero E6 cells were used to illustrate absence of fusion (negative control shown under light microscope). Scale 100 µm. MarMV, marmoset-associated morbillivirus; PBaMV, phyllostomid bat morbillivirus; MeV, measles virus; Ab, antibody.

Table S8. Pairwise amino acid sequence distances of CD150 sequences from morbillivirus hosts

Sequences		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
AF302038.1 *Saguinus oedipus*	A															
XM 002760176.6 *Callithrix jacchus*	B	0.03														
BC132792.1 *Homo sapiens*	C	0.13	0.15													
XM 045841470.1 *Phyllostomus hastatus*	D	0.35	0.37	0.33												
XM 024571326.1 *Desmodus rotundus*	E	0.35	0.36	0.31	0.07											
XM 037134224.2 *Artibeus jamaicensis*	F	0.36	0.38	0.33	0.08	0.10										
XM 036271887.1 *Molossus molossus*	G	0.37	0.37	0.34	0.29	0.28	0.30									
XM 014547315.1 *Myotis brandtii*	H	0.34	0.35	0.31	0.26	0.25	0.27	0.24								
AF329970.1 *Bos taurus*	I	0.35	0.37	0.34	0.27	0.25	0.29	0.32	0.28							
NM 001285726.1 *Capra hircus*	J	0.36	0.38	0.36	0.26	0.25	0.27	0.31	0.29	0.07						
NM 001243820.1 *Sus scrofa*	K	0.34	0.34	0.32	0.27	0.25	0.27	0.29	0.26	0.21	0.22					
XM 004327846.1 *Tursiops truncatus*	L	0.33	0.34	0.32	0.26	0.24	0.27	0.30	0.26	0.16	0.16	0.20				
MH430950.1 *Phoca vitulina*	M	0.33	0.35	0.31	0.25	0.22	0.25	0.27	0.25	0.26	0.25	0.22	0.23			
AF390108.1 *Canis lupus familiaris*	N	0.36	0.38	0.34	0.31	0.29	0.31	0.32	0.28	0.30	0.30	0.28	0.28	0.16		
NM 001278826.1 *Felis catus*	O	0.36	0.37	0.34	0.31	0.29	0.31	0.33	0.30	0.31	0.31	0.28	0.30	0.21	0.26	

Methods section (*P11 L464-487* and *P13 L539-553)*

Plasmids and antibodies

The plasmid pCAGGS-IgK-HA-vamCD150 was generated as previously published[12]. In brief, we subcloned a synthetic fragment containing the immunoglobulin IgK leader sequence, followed by HA epitope with a linker sequence to the vampire bat CD150 ORF (GenBank accession no. XM_024571326.1), for which we removed the signal sequence (amino acids 1-28) predicted by signal P 4.1 software (www.cbs.dtu.dk/services/SignalP/). Expression constructs of human codon-optimized F and H glycoprotein genes of morbilliviruses were commercially synthesized and cloned into the mammalian expression vector pCG1 (GenScript, Netherlands). The evaluated glycoproteins belong to the following morbilliviruses (strain, GenBank accession no.): MeV (KS, HM439386), PBaMV (PBZ-1381, MZ312422), and MarMVs (17BA-1055, PP059216; 20BA-312, PP850119; 22MG-1138, PP850121). The three strains of MarMV were selected based on their phylogenetic grouping: BA-1, BA-2, MG clades.

The antibodies used were anti-F rabbit polyclonal serum, anti-H rabbit polyclonal serum, and anti-rabbit IgG Cy3-conjugated antibody (Jackson ImmunoResearch). The custom polyclonal peptide-based

antibodies against the F and H glycoproteins of the selected MarMV strains and PBaMV (same as plasmids above) were commercially produced by Eurogentec (Seraing, Belgium) using their speedy 28-day program. The custom polyclonal antibodies were raised in peptide-immunized rabbits and purified by affinity chromatography on a matrix (AF-Amino TOYO). The anti-F peptide serum was raised against a peptide with sequence QDLTGTSRSYVKSL at the carboxyl-terminal. The anti-H peptide serum was raised against a peptide with sequence PA-S/P-ADKSNAFYKDS at the amino-terminal. Both peptides are positioned at the cytoplasmic tail of MarMV F and H glycoproteins and were selected as they were located in homologous regions to MeV peptides previously chosen to raise antibodies against MeV F and H[13].

Cell-to-cell fusion assay

Membrane fusion was evaluated in B95a, Vero-hCD150, NCI-H358, and Vero-E6 cells. All assays were performed using expression constructs of morbillivirus F and H glycoprotein genes of MarMV (strains 17BA-1055, 20BA-312, and 22MG-1138), PBaMV (strain PBZ-1381), and MeV (strain KS). We used human codon-optimized expression constructs to enhance gene expression in the human- and NHP-derived cell lines. Cells were transfected with 100ng of F and 100ng of H, only F, or only H using FuGENE® HD (Promega, Wisconsin, USA). Fusion experiments were conducted three times. To evaluate whether cells expressed the transfected proteins, cells were stained with the customized peptide-based antibodies against MarMV F and H glycoproteins. Briefly, cells were fixed with 4% paraformaldehyde at 48h after transfection. Cells were permeabilized with 0.2% Triton-X100 for 20 min, followed by a blocking step with 10% FBS for 30 min. F and H expression constructs were detected by addition of anti-F or anti-H peptide-based sera at 1:100 for 1 h followed by addition of anti-rabbit Cy3 at 1:400 for 1 h. Cell nuclei were stained with Hoechst solution (1 µg/ml) for 5 min. Three washes with 1x PBST were performed between each step. For MeV, 25ng of each plasmid construct were used due to its high fusion activity. Immunofluorescence was visualized in a Leica DMi8 microscope.

(end of reply)

Regarding the hypothesised extinction of old world morbilliviruses in bats. This is an interesting hypothesis that could explain how viruses like measles were introduced into the new world without having a clear (remaining) bat origin in the old world. However, I wonder is their precedence, or any other similarly hypothesised evidence, for any virus genus completely disappearing in this way? Given bat diversity in the old world, and the size of these continents, it seems that the likelihood of this sort of event is extremely low, even with millions of years of selection pressures. Maybe the original bat hosts have instead disappeared and therefore the reservoirs are gone, or alternatively the virus discovery efforts have just been unsuccessful and the viruses are still out there to be discovered?

Reply: We agree with the reviewer that the lack of bat-associated morbilliviruses in the Old World is very interesting and with the available data, it is difficult to point out if that is due to the extinction of the virus in Old World bats or the extinction of Old World bat hosts. Increased surveillance in Old World bats is therefore warranted. We have added the suggested points to our discussion.

P7 L266-272: However, no morbilliviruses have been detected in Old World bats despite intense surveillance[11,14,15], including the mining of sequence data in this study. Whether the evolutionary origins of morbilliviruses date back to the dispersal histories of extant Neotropical bats (40 -13 million years ago)[2-4], to potential extinction events of morbilliviruses or their hosts in the Old World, or to macroevolutionary phenomena such as preferential host switching in sympatric animals or via intermediate hosts, or to whether those estimates are affected by sample bias remains to be investigated.

P7 L276-279: Targeted field work potentially elucidating the timing and hosts of morbilliviral macroevolution should include Neotropical shrews and Old World bat species genetically related to Neotropical bats hosting morbilliviruses, such as molossid and myotis bats.

(end of reply)

I think I'm right that hematophagic bats are only found in the Americas and that these diversified c. 26 million years ago. There isn't much mention of this in the paper and how this unique interaction with mammals might change the dynamics of spillover, co-evolution etc for this particular group versus the other insectivorous bats where other morbilliviruses were found. I am not an expert in this area at all but I was surprised that the vampire bat diet wasn't discussed in more detail!

Reply: We agree and added the feeding behavior of morbillivirus bat hosts as a discussion point below. However, the diversification of hematophagic bats was not highlighted, as three other genera within the family of Phyllostomidae were also morbillivirus hosts as indicated in the new **Figure 1d**. We do however mention dates of Neotropical bat dispersal histories based on morbillivirus bat hosts, namely myotises (13 million years ago [mya]), molossids (29 mya), and bats from the superfamily Noctillionoidea (40-50 mya) which includes the family Phyllostomidae.

P8 L285-300: Most of the morbillivirus bat hosts are frugivorous and/or insectivorous. Hence, potential transmission to pigs may occur from the feeding of bat-contaminated fruits, as reported for Nipah virus, which can also be transmitted directly to humans via consumption of bat-contaminated date palm sap[16]. Similarly, marmoset feeding of bat-contaminated fruits may be a plausible transmission route, as proposed for the transmission of Marburg virus to apes[17]. In Latin America, spillovers of bat-associated viruses to humans and NHPs have been reported for rabies virus, which is generally transmitted by bites or scratches[18,19]. Thus, opposed to the transmission route by frugivorous and/or insectivorous bats, predator-prey interactions may be another potential transmission route driving morbillivirus cross-order host shift, as suggested for carnivore-associated morbilliviruses[20]. The feeding behavior of vampire bats suggests hematophagy as a possible transmission route to their prey, including animals reared as livestock, pets and humans[21]. Blood feeding is a unique trait displayed only by the common vampire bat and two other vampire bat species from the subfamily Desmodontinae not predominantly feeding on mammals[22], all of which are endemic exclusively to the Neotropics.

P7 L268-279: Whether the evolutionary origins of morbilliviruses date back to the dispersal histories of extant Neotropical bats (40 -13 million years ago)[2-4], to potential extinction events of morbilliviruses or their hosts in the Old World, or to macroevolutionary phenomena such as preferential host switching in sympatric animals or via intermediate hosts, or to whether those estimates are affected by sample bias remains to be investigated.

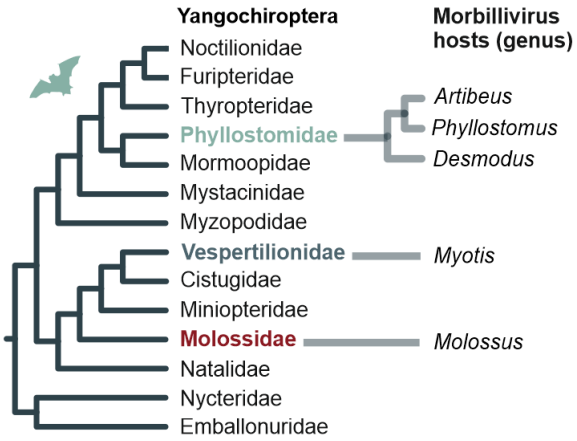


Fig. 1. d) Phylogeny of bat species[6] belonging to the suborder Yangochiroptera highlighting the genera and families positive for morbillivirus.

(end of reply)

Regarding the virus isolation in Veros, the authors state that degradation prevented any isolation of live virus. Could they be clearer in the text – was this actually attempted? If so other interpretations are also available: that the CD150 used was incompatible or that these viruses do not grow in Veros.

Reply: We agree. MarMV isolation tries were indeed performed, however, we were not successful potentially due to virus degradation or suboptimal receptor usage, consistent with data from our fusion

assay. To enhance clarity, we have slightly modified the text (see below) and included additional interpretations.

P5 L163-168: Isolation attempts of MarMV from 12 available RT-PCR-positive liver samples were neither successful in the cotton-top tamarin (*Saguinus oedipus*) lymphoblastoid cell line B95a traditionally used for morbillivirus isolation, nor in Vero cells expressing human CD150 (Vero-hCD150). Failed virus isolations may be potentially due to virus degradation during sampling and storage, or suboptimal receptor usage. The cotton-top tamarin CD150 was the source of the CD150 and MeV H crystal structure[10], and tamarins are genetically related to marmosets.

(end of reply)

Comparison with rodent viruses and their nomenclature. I wasn't too aware of these shrew sequences and their potential relationship to the other rodent morbilliviruses that have recently been characterised. However, it should be noted that the latter have been reclassified by ICTV as paramorbilliviruses. Are the shrew viruses distinct from these, and could the authors clarify the relationship between the viruses they have isolated and this new genus.

Reply: We agree this taxonomic aspect merits more attention. Feline morbillivirus is considered an orthomorbillivirus and clusters basal to all morbilliviruses sensu stricto, including the newly identified sequences in bats and in shrews. We characterized the newly identified bat-associated morbilliviruses, which had prototypic morbillivirus genomic properties including genome length and organization. The shrew-associated morbilliviruses formed a monophyletic clade and were included in our evolutionary reconstructions due to their importance during the orthomorbillivirus evolutionary history (shrew-associated morbilliviruses were highlighted in red in **Fig. 6f**). Unfortunately, only partial *L* gene sequences are available, and therefore genomic characterizations were not possible. Contrastingly, rodent morbilliviruses occur in phylogenetic sister relationship to morbilliviruses sensu stricto, have a different genome organization and lack conserved key amino acids in the H glycoprotein for CD150 binding. Therefore, rodent paramorbilliviruses were not included in our analyses, which rationale is included in our Dataset section in methods.

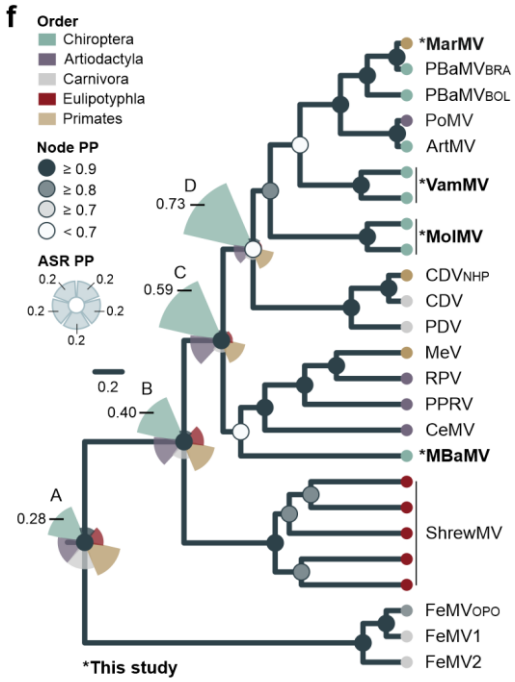


Fig. 6. f) Bayesian phylogeny of representative morbillivirus species and sequences from suspected cross-order spillover events. Asterisk (*) indicates sequences from this study and scale bar indicates genetic distance. BOL, Bolivia; BRA, Brazil; NHP, non-human primate; OPO, opossum. At nodes, circular bar plots denoting Bayesian ASR posterior probabilities (PP) for each evaluated node (A-D).

P11 L446-451: We excluded diverse rodent paramyxoviruses that occur in phylogenetic sister relationship to viruses unambiguously classified as morbilliviruses by the ICTV, in part because these rodent paramyxoviruses had a different genome organization and lacked conserved key amino acids in the H glycoprotein for CD150 binding[5]. These rodent-borne viruses which are being classified as paramorbilliviruses by the ICTV may thus have had a different evolutionary pathway to classical (ortho)morbilliviruses.

(end of reply)

The lack of virus in sera is, as the authors suggest, likely down to the absence of any lymphocytes – but was blood ever sampled and/or tested? Establishing whether these viruses cause a viraemia, transient or chronic, would be highly significant. As would sampling tissues from the upper respiratory tract to understand potential respiratory transmission routes.

Reply: We thank the reviewer for this comment. However, we did not obtain any blood samples from the bats nor from the non-human primates which were found dead during yellow fever surveillance. Tissues from the upper respiratory tract were also not available. This is now specified in the text.

P3 L52-54: Neither tissues from the upper respiratory tract, nor blood specimens were available.

(end of reply)

Availability or sequencing of host CD150 or Nectin-4 genes from bats/NHPs. From a quick search on Genbank it seems the Callithrix jacchus CD150 sequence is available. How close is this to humans, e.g. at the known interaction sites between CD150 and H. This seems an important thing to consider, especially given the potential for these viruses to jump directly into humans, which is only touched on at the very end of the discussion, when talking about the implications of cessation of measles vaccination post eradication. Ideally it would be nice to broaden the understanding of the CD150 tropism of these viruses to marmoset and human CD150, in light of the fact that restrictions have previously been reported (e.g. by using surrogate entry assays as above). The restriction of DesMV to hCD150 (fig. 1g) might not be universal and should be investigated. This wet lab work might be beyond the scope of this paper but analysis of the sequence conservation and predictions based on this are certainly warranted here. Since MarMV clearly uses marmoset CD150, and MV can use both marmoset and human CD150, what are the differences between these Hs that might help us to determine the zoonotic potential of the MarMV and PhyMV viruses?

Reply: To address this comment, we performed comparisons between relevant CD150 and H interaction sites as well as cell-to-cell fusion assays using F and H expression constructs of marmoset and phyllostomid bat morbilliviruses on cells that express human CD150 (Vero-hCD150), tamarin CD150 (B95a), and human nectin-4 (NCI-H358), as described above for the new **Fig. 5**.

(end of reply)

Minor points:

Figure 1g ang 1h: Which particular isolate of DesMV was used in these experiments?

Reply: We have now added the sample ID and the country in the Results section, and the Figure legend for 2a and 2c (previously 1g and 1h).

P3 L77-79: We successfully isolated VamMV (CR209, Costa Rica) from a vampire bat lung in Vero cells expressing the vampire bat CD150 ortholog, showing canonical CD150-mediated cell entry.

Fig. 2. a) Multistep growth curve analyses of VamMV-1 (CR209) inoculated at a MOI of 0.01 for five days in Vero-vamCD150, Vero-hCD150, NCI-H358, and Vero-76 cells as control. c) Neutralization titers against VamMV (CR209) in Costa Rican vampire bat sera. Experiment was performed in duplicate.

(end of reply)

Fig 2.d: The specific residues highlighted here are not mentioned in the text. It would be more useful to summarise how these vary between different morbilliviruses, and where the CD150 sequences might also vary.

Reply: We thank the reviewer for this suggestion. We have added comparisons between CD150 sequences of bat and human hosts in the extended data (updated **Extended Data Fig. 1e** and **Supplementary Table S8**), as well as between human and non-human primate hosts discussed above (**Fig. 5a-b**).

P4 L123-127: Homology modelling of the receptor binding H glycoprotein of bat-associated morbilliviruses revealed a typical β-sheet augmentation as in the published interaction site between MeV H glycoprotein and tamarin CD150[10] (**Fig. 3e; Extended Data Fig. 1c-d**), despite multiple amino acid changes at individual sites (**Extended Data Fig. 1e**).

Extended Data Fig. 1. Characterization of morbilliviruses

e) Interaction sites between bat homologue morbillivirus H glycoproteins and CD150 receptors based on MeV and tamarin CD150 crystal structure[10]. Cases in which sequence from the animal species was not available, a close genetically related species was used. See **Supplementary Table S8** for GenBank accession numbers.

(end of reply)

Fig .2e/f: please specify which DesMV (1 or 2) is used here? And add no-sera control data to establish the background neutralisation levels.

Reply: We specify now that we used VamMV1 (previously DesMV-1). We did have no-sera control data with 100% infection, which we had included in the source data. However, as the graph is made using a logarithmic scale, we couldn't plot a value at 0. The value for the no-sera control is now added to the legend.

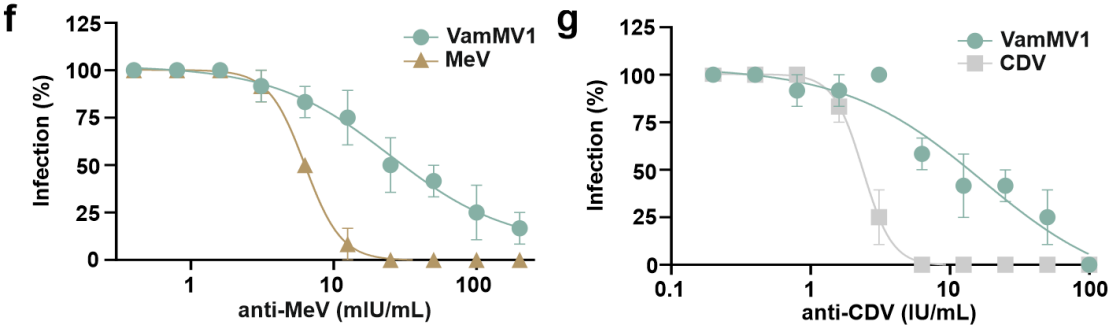


Fig. 3. f) Neutralization curves of VamMV1 (Vero-vamCD150) and MeV (Vero-hCD150) by anti-MeV human sera. g) Neutralization curves of VamMV1 (Vero-vamCD150) and CDV (Vero-76) by anti-CDV sera. In f and g, no-sera controls produced 100% infection; sera concentrations are measured in international units (IU); error bars are the standard error of the mean.

(end of reply)

Figure 3b: a minor point but I would have liked to see the phylogenetic relationship of the marmoset-associated viruses on the same tree as the bat-associated viruses detailed in Figs 1 and 2. I think this would give a more complete picture of the identifications. This collapsed tree with only PhyMV left me constantly comparing Fig. 3b and 2c. I realise this is essentially provided in Fig.4C but it would have been nice to see this earlier.

Reply: We agree and have expanded this phylogenetic tree with more bat-associated morbillivirus sequences to highlight where in the tree are those marmoset morbilliviruses (updated **Fig. 4b**).

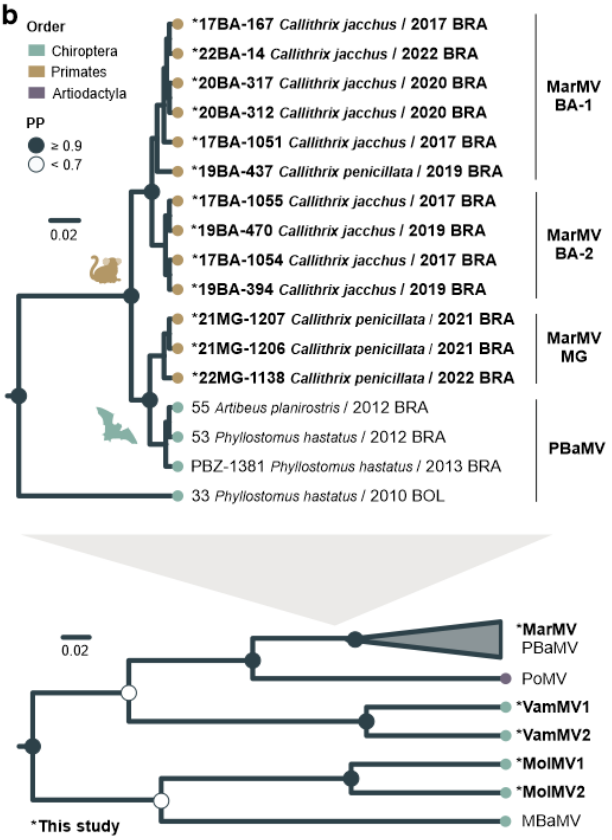


Fig. 4. b) Bayesian phylogeny of MarMV and related morbilliviruses using partial *L* gene (361 nucleotides), midpoint rooted. Expanded view of the collapsed clade of MarMV and PBaMV is displayed in the upper insert. Scale bar indicates genetic distance. Asterisk (*) indicates sequences from this study. See **Supplementary Tables S1** and **S6** for virus abbreviations and GenBank accession numbers.

(end of reply)

Reviewer #3 (Remarks to the Author):

This manuscript describes a very important effort to identify new morbillivirues in new world bats and their role in the evolution of this viral genus. The authors managed to identify and characterize the full or almost complete genome of five new morbillivirus species in New world bats, including one from existing sequencing read archives, and one new morbillivirus from marmosets collected in Brazil. One strain of Desmodus-associated morbillivirus (DesMV) could be isolated, allowing for assays to confirm bat-associated cell entry and frequent circulation in bats of Costa Rica. The new sequence data was combined with existing morbillivirus sequence data to einvestigate orgin of morbilliviruses and host shifts events. Cophylogenetic analyses showed evidence for host shifts from bats, carnivores and artiodactyls, notably for bat-associated spill-over at the origin of the Procine morbillivirus and Marmoset morbillivirus. Authors argue that their results also give evidence for an important role of bats in morbillivirus diversification. They call for further investigation into the risk of morbillivirus spill-over from bats to livestocks and primates.

The manuscript is well-written, and is based on a solid amont of data and diverse analyses, although there are some discrepencies between results described and data presented (see below). The major issue I found with this manuscript is that it appears that some results are over-interpreted to fit the narrative for the main role of New World bats in the origin of morbilliviruses. Some statements need to be tone down to represent better the results obtained and caveats of the data available. I think that some sections of the results and discussions described below would need to be revised before publication, or additional analyses may be needed to provide support for some statements.

Reply: We thank the reviewer for the critical comments. We have conducted additional analyses using full genomic sequences to address the reviewer's main criticism of using partial *L* gene for our macroevolutionary reconstructions. We have also toned down our statements regarding the role of bats in the evolutionary history of morbilliviruses. In addition, we have changed all virus names according to new ICTV guidelines and consistent with the terminology used in a previously published paper[1]:

Virus abbreviations:

- MeV for measles virus and according to ICTV guidelines (previously MV)
- VamMV standing for vampire bat morbillivirus (previously DesMV)
- PBaMV standing for phyllostomid bat morbillivirus (previously PhyMV)
- MBaMV standing for myotis bat morbillivirus (previously MyoMV)

One main issue concerns the evidence for a bat origin for morbilliviruses. This is mostly based on the posterior probability (PP) results presented for nodes A-D of a phylogenetic tree in Fig4c and d. PP values for these nodes are 30%, 42%, 66% and 82%, respectively. This is poor or, at best limited, statistical support for 3 out 4 nodes, even if PP value obtained for bats is higher than for other hosts. For the one strong support of 82%, it corresponds to node D which is itself poorly supported in the phylogeny (PP < 0.7). This limited statistical support should be made clear in results and discussion. Maybe the poor PP is due to the fact that the analysis is done on a partial L gene sequence (528nt). The authors went to a great extent to obtain complete or near-complete genome for 6 new morbilliviruses. I do not understand why they have not replicated the cophylogeny analysis on full genome data. Some virus and hosts for which only partial sequence is available would not be included, but some power might be gained on those deep nodes. The authors states in L293 that the poor power of short sequence was compensated by using different phylogenetic methods and tree topologies, but signal in the data is the limit, not the method. It would great if authors could do this extra analysis because the study would gain in strength. Cophylogeny results presented may be already good enough for publication in this journal, but the text must be amended to highlight their limitation.

Reply: We understand the concerns expressed by the reviewer, and we have therefore conducted the analyses with complete translated concatenated ORFs. All nodes were supported by a PP of >0.9 and we showed increased statistical support for bat origins at the root and for all major nodes with ASR PP values of 0.37, 0.66, 0.66, 0.86 in nodes A-D, respectively (**Extended Data Fig. 6a**).

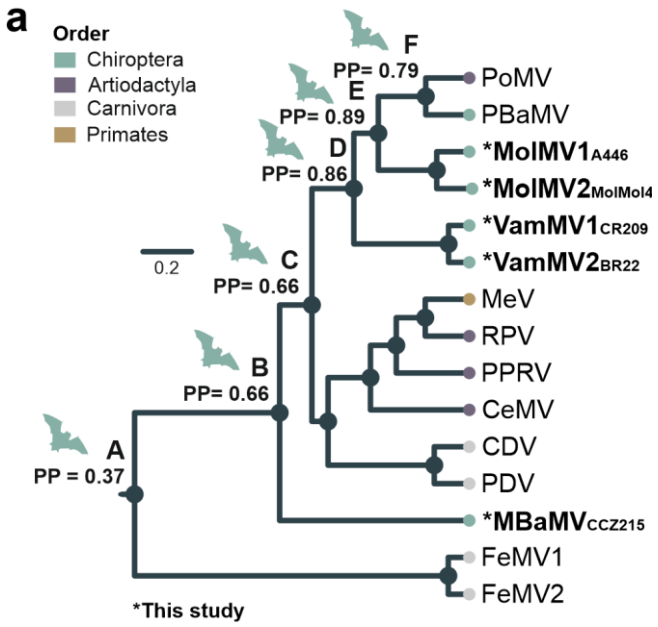
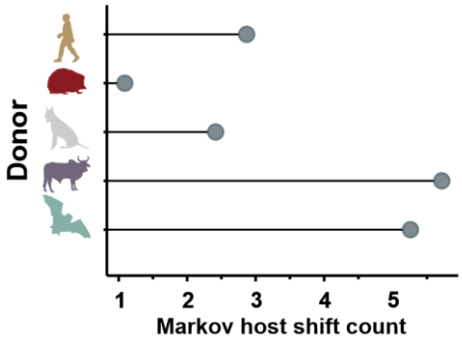


Fig. 6. a) Bayesian phylogeny of representative morbillivirus species. Circle at nodes indicate posterior probabilities >0.9. Numbers at nodes A-F show the highest posterior probabilities (PP) for a host order by Bayesian ancestral state reconstructions (ASR). Scale bar indicates genetic distance.

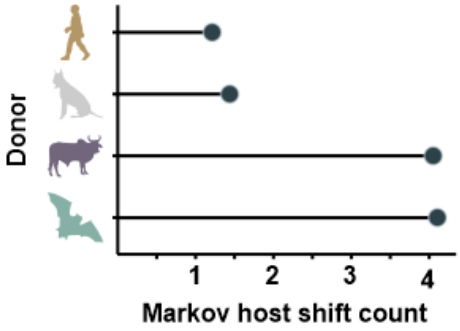
Similar to the results obtained from analyses of partial *L* gene sequences, host shift analyses indicated that most host shifts were from bats and artiodactyls (new analyses – **Figure 6c** and **Extended Data Fig. 6c**).

Data sets:

Partial *L* gene



Concatenated ORFs



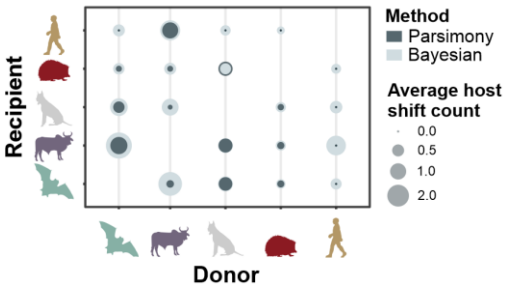
c) Average host shift counts from each host order using continuous-time Markov chain (CTMC) in a Bayesian framework

Bayesian and parsimony-based approaches also indicated that the highest host shifts were from bat to artiodactyl and carnivores, from artiodactyl to primate, and from carnivores to bats and artiodactyls. The additional Bayesian analyses were performed using a continuous-time Markov chain analysis to compare with parsimony-based method. We highlighted only those host shifts whose values agreed in both methodological approaches (updated host shift panels – **Figure 6d** and **Extended Data Fig. 6d**).

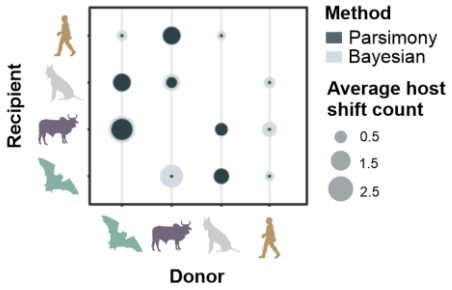
P6 L210-212: Bayesian and parsimony-based ASR corroborated high average numbers (>0.5) of host shifts from bats to artiodactyls and carnivores, from artiodactyls to primates, and additionally from carnivores to bats, artiodactyls and shrews (**Fig. 6d**).

Data sets:

Partial *L* gene



Concatenated ORFs

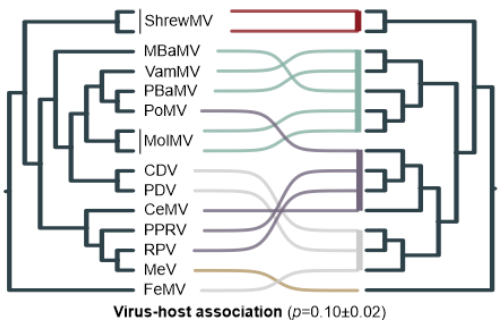


d) Average number of morbillivirus host shifts from Bayesian and parsimony-based ASR.

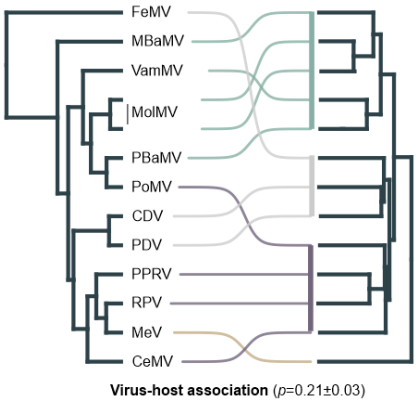
No signal for coevolution was detected using one virus per host species in both datasets. New tanglegram representations were added for better visualization of virus-host associations (new panels – **Figure 6b** and **Extended Data Fig. 6e**)

Data sets:

Partial *L* gene



Concatenated ORFs

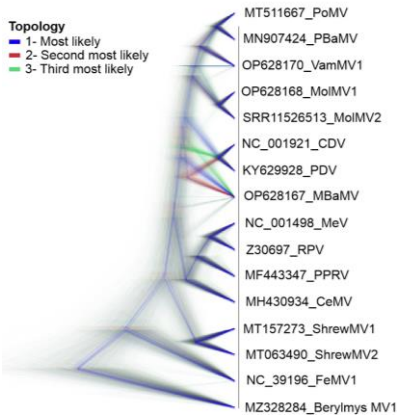


b) Tanglegram of virus-host associations showing ParaFit *p*-value.

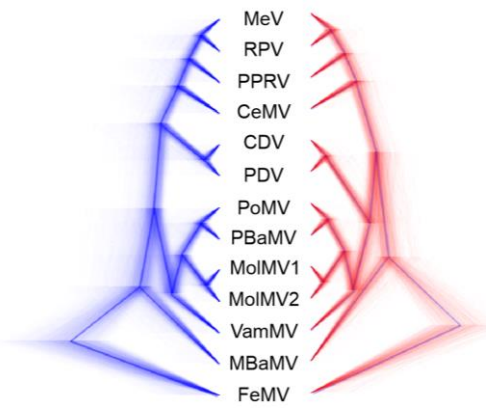
For event-based cophylogenetic reconstructions, only two topologies were predominant in comparison to the partial *L* gene tree (**Extended Data Fig. 4c**, and new **Extended Data Fig. 6f**).

Data sets:

Partial *L* gene



Concatenated ORFs

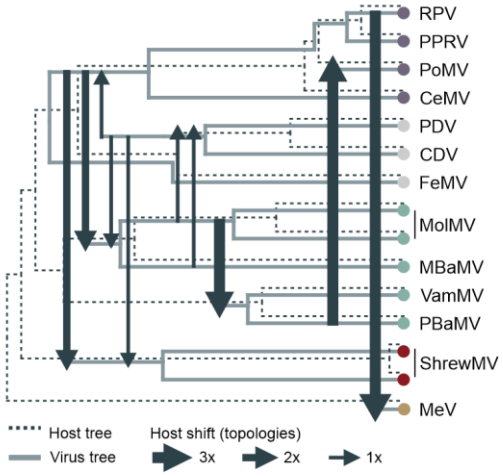


c) DensiTree showing clustering morbillivirus sequences used to generate the most likely topologies for event-based cophylogenies.

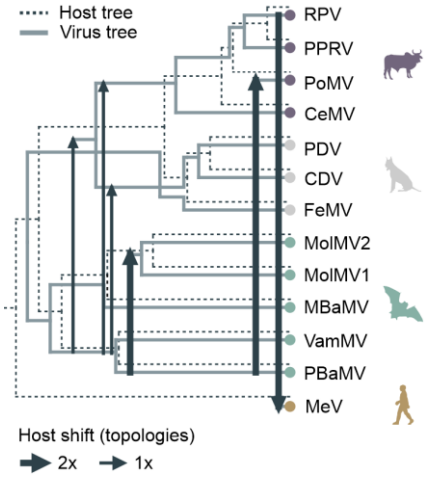
The most important host shifts were from bat to artiodactyl (PBaMV to PoMV), artiodactyl to primate (RPV to MeV), and between bat hosts in both datasets (**Fig. 6e**, and new **Extended Data Fig. 6g)**.

Data sets:

Partial *L* gene



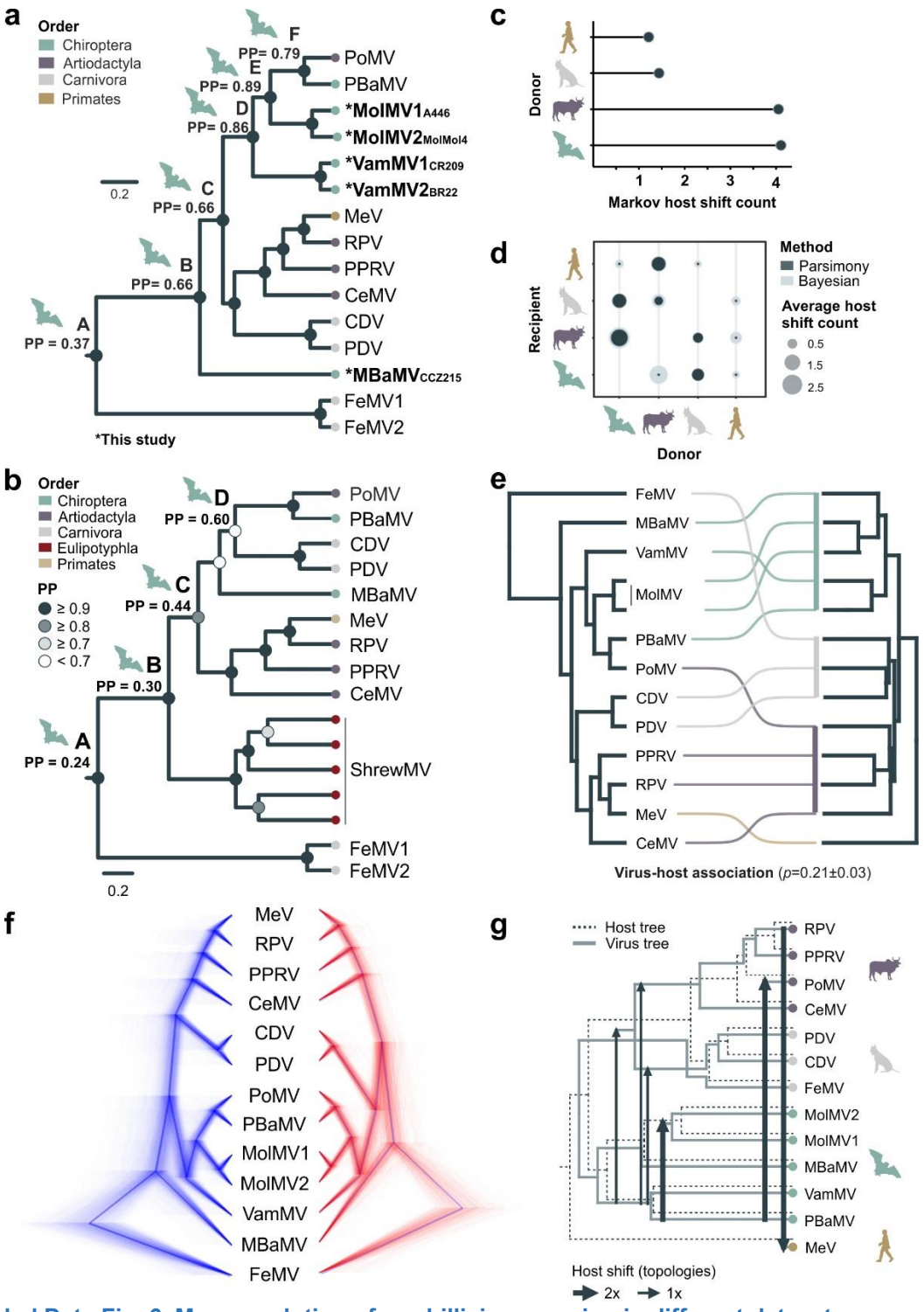
Concatenated ORFs



Event-based coevolutionary analyses of the three (partial *L* gene) and two (concatenated ORFs) most likely topologies. Arrow width represents the number of topologies with the specified host shift.

Although we obtained good support for all nodes, major findings were largely similar to those obtained with partial *L* gene suggesting host switches as a key process in the evolution of morbilliviruses, *e.g.* the origin of porcine morbillivirus from bat. However, due to the exclusion of morbilliviruses found in shrews (which were only partially sequenced in the studies reporting them), the importance of bats was more prominent, which albeit supporting our conclusions, implies a larger bias. We therefore kept the original dataset using partial *L* gene with additional suggested host reconstruction analyses for the main figures (**Fig. 6**), whereas the analyses relying on full genomes were added to the extended data (**Extended Data Fig. 6**). Text block related to updated **Extended Data Fig. 6**:

P6 L224-232: ASR in a Bayesian framework using full genome data generated high branch support values of >0.9 PP and led to increased statistical support for bat origins at the root and for all major nodes (0.37 – 0.86 in nodes A-D, **Extended Data Fig. 6a**) at the expense of excluding shrews as morbillivirus hosts, as only partial sequences were available. In contrast, removing the morbilliviral genomic sequences generated in this study led to decreased statistical support for bat origins (0.24 – 0.60 in nodes A-D, **Extended Data Fig. 6b**). Using full genome data, major findings in the host switch analyses and coevolutionary reconstructions were consistent with the results generated from partial *L* sequences (**Extended Data Fig. 6c-g**), suggesting robustness of our macroevolutionary analyses.



Extended Data Fig. 6. Macroevolution of morbilliviruses using in different datasets.

a) Bayesian phylogeny of representative morbillivirus species. Circle at nodes indicate posterior probabilities >0.9 for branch support. Numbers at nodes A-F show the highest posterior probabilities (PP) for a host order by Bayesian ancestral state reconstructions (ASR). b) Bayesian phylogeny of previously published classical and divergent morbillivirus species. Tree was based on partial *L* gene (361 nucleotides). At nodes A-D, PP for a bat origin by Bayesian ASR. For a and b, scale bar indicates genetic distance. c) Average host shift counts from each host order using continuous-time Markov chain in a Bayesian framework d) Average number of morbillivirus host shifts from Bayesian and parsimony-based ASR. e) Tanglegram of virus-host associations showing ParaFit *p*-value. f) DensiTree showing clustering morbillivirus sequences used to generate the two most likely topologies for event-based cophylogenies. g) Event-based coevolutionary analyses of the two most likely topologies. Arrow width represents the number of alternative topologies with the specified host shift. For a and c-g, analyses were performed using translated, concatenated ORFs. See **Supplementary Table S1-S2** for virus abbreviations and GenBank accession numbers.

Furthermore, we have proceeded to tone down our statements regarding morbillivirus origins in bat hosts.

P6 L215-219: Our results imply that bats, carnivores, and artiodactyls are main sources of cross-order host shifts in morbilliviruses. The probability of a bat origin was consistently highest in most major nodes in ASR (posterior probability [PP] 0.40-0.73 in nodes B-D, **Fig. 6f**), albeit the origin of morbilliviruses at the tree root remained unresolved (**Fig. 6f,** node A).

(end of reply)

Another issue concerns statements of bat-associated host shifts and evident reduction of the importance of host shifts from other orders. Their results show, as clearly stated in L211 that "bats, carnivores, and artiodactyls are main sources of cross-order host shifts in morbilliviruses", but the discussion, notably concerning MV's old world origin in cattle (L245-250), goes towards putting into doubts hosts shifts that are not of bat origin. In this section, host shifts from carnivors are totally omitted , apparently to fit with the narrative. The evidence fo bat-associated host shifts depends mostly on phylogenetic relationships between morbillivirus sequences, as for other hosts shifts. The discussion should be revised to represent better the diversity of host shifts in morbilliviruses, as shown in the results. Impact of bias in sampling should be better incorporated in the statements as well. The authors quickly mention sample bias (L267) and rightfully call for additional field investigation, but the possible impact of sample bias is not made as clear as it should. Any new morbillivirus sequence from old world bats or other hosts may change some of the results obtained concerning diversification of the virus and host shifts.

Reply: We thank the reviewer for this comment. We have added, changed, and removed statements accordingly. First, we removed our discussion on the origins of MeV from alternative reservoirs as this might be taken too speculatively. Second, host shifts from carnivores were highlighted in the last section of our results on evidence for cross-order spillover events and are also mentioned in our discussion on potential spillover events from bat-associated morbilliviruses. As morbillivirus host shifts from artiodactyls and carnivores have been discussed elsewhere, we focused mainly on the potential transmission routes from bats. This is now added to the text to make it clearer. Third, we have added the potential impact that new morbilliviruses may have on our analyses.

P7 L238-241: Cross-order transmissions have been reported for CDV, including to NHPs[23,24] and collared peccaries[25] (New World pigs, order Artiodactyla). Other reported cross-order transmissions include the detection of FeMV in opposums[26] (order Diprotodontia), and CeMV in seals[27] (order Carnivora).

P7 L257-258: Our macroevolutionary data project the origins of major morbilliviruses to non-recent host shifts, predominantly from bat hosts, in addition to artiodactyls and carnivores, discussed elsewhere[20,28].

P8 L291-294: Thus, opposed to the transmission route by frugivorous and/or insectivorous bats, predator-prey interactions may be another potential transmission route driving morbillivirus cross-order host shift, as suggested for carnivore-associated morbilliviruses[20].

P7 L274-276: Hypothetically existing divergent morbilliviruses in Old World hosts may impact macroevolutionary analyses, requiring increased surveillance and repetition of macroevolutionary analyses upon future morbillivirus findings.

(end of reply)

The authors state in the discussion (L290) that they account for sampling limitations with data mining from SRA, but their own results show that this method only provides poor results. Only one sequence of morbillivirus (which is already great!) was obtained out of thousands of SRA including from New World bats, so it can hardly be used to compare distribution of morbillivirus among different types of hosts. Interpretation of SRA mining results should be tone down accordingly.

Reply: We agree and have toned this down accordingly.

P8 L302-305: Limitations to our study include sampling in only two Neotropical countries and heterogeneous numbers of publicly available genomic sequences per host. Thus, the likelihood of identifying divergent morbilliviruses was low, but the combination of methods allowed us to yield a large genomic data set.

(end of reply)

There are multiple other statements that do not fit with the results presented and should be revised:
-L95 "was within the seroprevalence ranges detected for other morbilliviruses in wildlife populations". Only data from one paper on PMV is refered too so it is hardly all other morbillivirus in wildlife population.

Reply: We agree with the reviewer. As seroprevalence data of wildlife populations is limited due to the availability of the vaccines for CDV, PPRV, and MeV, we changed it to “unvaccinated populations” instead of “wildlife populations”. In addition to PDV seroprevalence ranges in different seal populations, we added a reference on a FeMV seroprevalence study that incorporates FeMV seroprevalence ranges obtained from previous studies.

P3 L91-92: The antibody detection rate in vampire bats was within the seroprevalence ranges detected for other morbilliviruses in unvaccinated populations[29,30].

Added reference:

Chaiyasak, Surangkanang, Chutchai Piewbang, Jadsada Ratthanophart, Navapon Techakriengkrai, Kittipong Rattanaporn, and Somporn Techangamsuwan. 2024. "Detection of Antibodies against Feline Morbillivirus by Recombinant Matrix Enzyme-Linked Immunosorbent Assay" *Viruses* 16, no. 8: 1339. https://doi.org/10.3390/v16081339

(end of reply)

- L112 "The number of morbilliviral species in bats exceeded those known in any other host order, i.e., six compared to four in artiodactyls (RPV, PPRV, CeMV, PoMV), three in carnivores (CDV, PDV, and FeMV) and one in primates (MV)". Is not not just a biais of sampling. Exceeding when it is 6 vs 4 is excessive

Reply: We agree and have deleted the sentence.

(end of reply)

- L127 " most bat-associated morbilliviruses characterized on the full-genome level were monophyletic with the exception of MyoMV" This is based on genome data containing often only one of two sequences per morbillivirus. So how could it be otherwise. Their own data based on partial L gene could suggest that PhyMV may not be monophyletic. Such statement on limited data is not very useful

Reply: Changed as suggested.

P4 L115-117: Bayesian phylogenetic analysis of translated, concatenated ORFs revealed that bat-associated morbilliviruses were not monophyletic, occurring in two different clades within the morbillivirus tree (**Fig. 3d**).

(end of reply)

-L132 "...is consistent with low rates of recombination in negative-sense single- stranded RNA viruses" There is actually no evidence of recombination in any morbillivirus so far. Assessing recombination on genetic data only is not appropriate. See notably Han G.-Z. & Worobey M. (2011). – Homologous recombination in negative sense RNA viruses. Viruses, 3 (8), 1358-1373. doi: 10.3390/v3081358.)

Reply: We thank the reviewer for providing this reference. As there is no evidence to disprove the reported recombination events in measles virus and canine distemper virus (listed also in Han & Worobey 2011), we adapted the sentence and added the suggested reference.

P4 L118-121: formal analyses (**Supplementary Table S5**) yielded no evidence for recombination involving bat-associated morbilliviruses, consistent with low rates or absence of recombination in negative-sense single-stranded RNA viruses[31,32].

Added reference:
Han GZ, Worobey M. Homologous recombination in negative sense RNA viruses. Viruses. 2011 Aug;3(8):1358-73. doi: 10.3390/v3081358. Epub 2011 Aug 4. PMID: 21994784; PMCID: PMC3185808.

(end of reply)

-L161 "MarMV seperated by location not species"; looking at fig3b, there is clearly spearation by location and species. for example, all MarMG come from C penicillata. Again it is a statelent based on biased sample.

Reply: To avoid statements based on potential sampling biases, we have deleted this statement.

(end of reply)

- L174 " confirming the marmoset host associations of morbillivirus-positive specimens. "- genetic results were exepcted to match with Callithrix expected as all 1400 sample, except 48, were marmoset. It only confirms that the sample was not contaminated by tissue from another animal. There is not enough samples from species other than marmoset to assess if their is a specific association with morbillivirus infection.

Reply: This is a very valid point and we have removed the sentence regarding marmoset host association and instead highlighted that the absence of host reads other than *Callithrix* spp. suggest instead that the sample was not contaminated by tissue from another animal, as suggested by the reviewer.

P10 L408-411: None of the marmoset samples for which near-complete morbillivirus genomes were generated by deep sequencing contained any read that mapped to COI of animal species other than *Callithrix* spp. with 99-100% mean identity (**Supplementary html files**), ruling out the possibility of laboratory contamination.

(end of reply)

Finally, some results presented are not fully supported by the data made available:

- Table 1 and results mention 38 positive bat samples, but Supp Table 2 only list 24 positive samples. This list indicates the origin of 3 out 4 new bat morbillivirus. Notably sample CR209 at the origin of genome DesMV-1 is missing. Sample at the origin of the DesMV isolate is not indicated either.

Reply: This was caused by the unintentional submission of filtered country and virus tabs showing only morbillivirus-positive samples from Brazil as instead of showing all investigated bat samples (morbillivirus positive and negative) from both countries. This has been corrected in the resubmission.

(end of reply)

- L71 "expanding the number of individual bats 4-fold". We don't know where this calculation come from.

Reply: This was calculated on the available number of morbilliviral sequences in bats. However, we have proceeded to delete “4-fold” to avoid potential biased statements.

(end of reply)

- Fig1b and results describe RT-PCR results from multiple organs from 12 animals but the data associated is not in Supp Table 2 or anywhere else.

Reply: The RT-qPCR results can be found in the source data file for Figure 1b. The number of animals was updated to 16 and we specify in the legend that those were the tested organs, since not all organs were available or tested for all animals (as shown in the source data file).

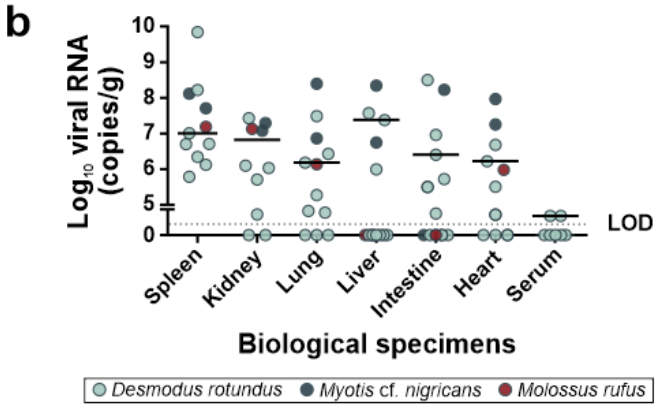


Fig. 1

b) Viral loads of bat morbilliviruses in tested organs from individual bats (n=16).

Source data for Fig. 1b:

	Log10 viral RNA (copies/g)						
Virus	**Spleen**	**Kidney**	**Lung**	**Liver**	**Intestine**	**Heart**	**Serum**
VamMV BR22	9.842609	5.708421	6.431364	7.572872	6.958564	6.682145	na
VamMV BR27	7.004321	6.09691	na	na	5.720159	6.225309	na
VamMV BR222	na	7.429752	6.184691	5.996074	6.401401	na	na
MBaMV CCZ213	7.705864	7.075547	6.863323	6.745855	8.227887	7.963788	na
MBaMV CCZ215	8.117271	7.290035	8.39794	8.342423	na	7.252853	na
MolMV A446	6.127105	7.133539	6.139879	na	na	5.983175	na
VamMV CR191	5.786041	na	4.694605	na	4.214844	na	neg
VamMV CR207	6.701568	6.033424	5.278754	na	na	4.0086	na
VamMV CR209	8.222716	na	7.485721	7.374748	8.501059	5.506505	na
VamMV CR217	6.342423	4.053078	na	na	5.499687	na	na
VamMV CR123	6.708421	na	4.390935	na	na	na	neg
VamMV CRC128	7.187521	na	na	na	na	na	neg
VamMV CR163	nt	nt	nt	nt	nt	nt	neg
VamMV CR183	nt	nt	nt	nt	nt	nt	neg
VamMV CR220	nt	nt	nt	nt	nt	nt	3.783892
VamMV CR221	nt	nt	nt	nt	nt	nt	3.783892

neg = negative
na = not available
nt = not tested

(end of reply)

- Fig 1c: no information on what the circle in nodes stand for.

Reply: The circles stand for posterior probability of >0.9. This information is now included in the figure legend.

(end of reply)

- Extended data 1a: Do the different colors in the labels of tree mean something?

Reply: Colored taxa indicate morbillivirus sequences found in bats. This was added to the legend.

(end of reply)

- Fif 2e and f: Only Fig2e is mentioned in legend

Reply: This is now modified.

(end of reply)

- Fig3b The legend mentions the sue of PoMV as outgroup but it seems that PhyMV BOL is used as outgroup. From this analysis, it looks like PhyMV BOL is very different from PhyMV BRA (another species?). But it is maybe due to its positioning as an outgroup?

Reply: PoMV was indeed used as an outgroup in the previous figure. We have re-done the phylogenetic tree and replaced the previous tree with an extended one including other bat-associated morbilliviruses. Regarding the genetic distances between the phyllostomid bat morbillivirus from Bolivia and the ones from Brazil, the virus from Bolivia was genetically more distant and grouped basal to the phyllostomid bat morbilliviruses found in Brazil, including the morbilliviruses found in marmosets. In comparison to other morbillivirus species, the genetic distance was similar to the distances found between the morbilliviruses from vampire bats in Brazil and Costa Rica, or the two morbilliviruses from molossid bats from Panama and Brazil. However, we remain cautious as only 361 nucleotides were available for the analyses.

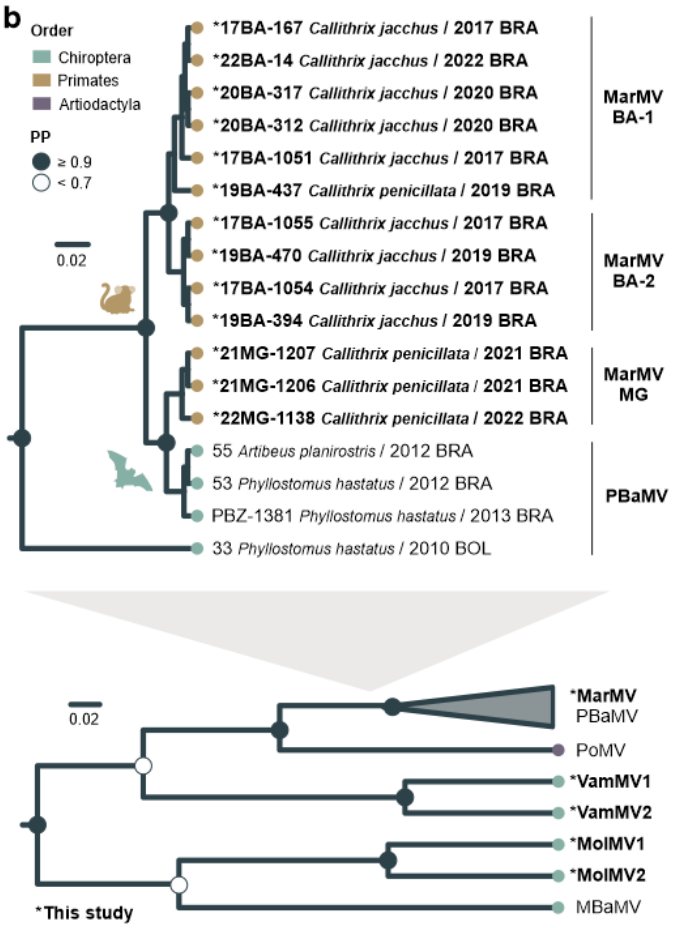


Fig. 4. b) Bayesian phylogeny of MarMV and related morbilliviruses using partial *L* gene (361 nucleotides), midpoint rooted. Expanded view of the collapsed clade of MarMV and PBaMV is displayed in the upper insert. Scale bar indicates genetic distance. Asterisk (*) indicates sequences from this study. See **Supplementary Tables S1** and **S6** for virus abbreviations and GenBank accession numbers.

(end of reply)

- Fig 3a what are the P hastatus and A planirostris samples indicated in fig 3a? New samples not mentioned in fig1a? From what I understand, fig 3a should show only samples collected during the study, but these samples are not mentioned in the text.

Reply: These samples were already in **Fig 1a** as they are not from our study. In **Fig. 4a** (previously Fig. 3a), we have now specified the animals sampled in this study.

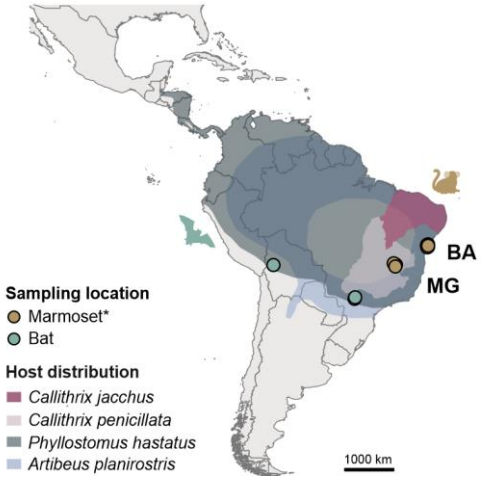


Fig. 4. a) Geographical distribution of marmoset and bat hosts of related morbilliviruses and sampling locations. Asterisk indicates samples from this study. BA, Bahia; MG, Minas Gerais.

(end of reply)

- Extended Data Table 4 not well described. What are AI and PS? where are marmoset- bats swine-sequences ? Are they grouped per location? It should be mentioned.

Reply: AI stands for “association index” and PS stands for “parsimony score”, abbreviations which have been added to the table notes. Country or location was used as trait (now added to the text) for the analyses and therefore the marmoset-associated morbillivirus sequences are in the Brazil clade, whereas the swine-associated morbillivirus sequences are in the Mexico clade.

P7 L245-249: Assigning country as a trait, Bayesian Tip-association Significance Testing (BaTS), tool that correlates trait values with phylogenies)[7], corroborated geographic association (p<0.05) for bat- and marmoset-associated morbilliviruses from Brazil as well as for bat- and pig-associated morbilliviruses from Mexico (**Fig. 6g, Extended Data Table 4**).

Extended Data Table 4. Geographic association of bat-, marmoset-, and swine-associated morbilliviruses by BaTS

Statistic / MC traits (location)	null mean (95% HDP CIs)	*p* value
AI	0.62 (0.3, 0.8)	**0.00**
PS	2.95 (2.8, 3.0)	**0.01**
MC (Brazil)	5.51 (3.1, 10.0)	**0.01**
MC (Bolivia)	1.00 (1.0, 1.0)	1.00
MC (Mexico)	1.05 (1.0, 1.2)	**0.02**

100 state randomizations; country (trait) as discrete state; AI, association index; PS, parsimony score; MC, monophyletic clade; HPD CIs = highest posterior density confidence intervals (credible sets)

(end of reply)

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Rebuttal letter for

"Origins and evolutionary trajectories of morbilliviruses in Neotropical bats"

Tracking NMICROBIOL-24061682A

Reviewer Comments

Reviewer #1 (Remarks to the Author):

The authors have carried out a huge amount of work in their revisions and have addressed all my comments and recommendations. I have a couple of (very) minor points but am very satisfied with the revisions by the authors. I also particularly want to commend them on their high-quality figures.

Minor points:

Extended data fig 6e- can the authors include the host images in this panel too to make it clearer which colour is which.

Reply: Done as suggested.

Extended data fig 6f&g- can the authors describe the results in these panels in a little more detail for the reader's clarity.

Reply: Done as suggested.

P6, L232-L236: Using full genome data, major findings in the host switch analyses and coevolutionary reconstructions were consistent with the results generated from partial *L* sequences (**Extended Data Fig. 6c-g**), in which main host shifts were from bat to artiodactyl, artiodactyl to primates, and between bat hosts (**Extended Data Fig. 6 f-g**), suggesting robustness of our macroevolutionary analyses.

Reviewer #2 (Remarks to the Author):

The authors have made significant effort to understand the receptor tropism of these viruses in vitro. This is important to understand the relative zoonotic risk of the different clades they have identified, e.g. a low risk for VamMV, and differential risk for MarMV and PBaMV dependent on whether you look at human CD150 or Nectin-4 usage. Indeed, predictions about 552 are prescient – I remember looking at that mutation before. A search uncovered this paper which the authors might want to reference: https://pmc.ncbi.nlm.nih.gov/articles/PMC6722581/. Also here: https://journals.asm.org/doi/10.1128/jvi.01248-18 authors aligned similar sequences. It's interesting to note that RPV sequences are F552Y at this position, (like the hCD150 using MarMVs) and RPV and MeV are thought to share a common ancestor. There is also species/host specific divergence at this residue so likely a key modulator of host-range.

Reply: Done as suggested. We have now added a few lines on the amino acid change at position 552.

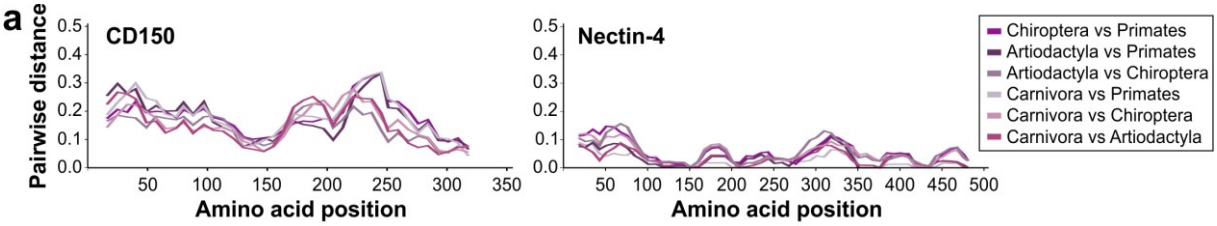
P5, L188-L190: Notably, RPV contains the same amino acid change F552Y as the MarMV strains producing fusion activity[1], and changes at that position have been previously found to reduce fusion activity[2].

Beyond a few additions to the manuscript to reflect this prior discussion, I think it would be nice to see a phylogenetic tree added to compare the relative divergence of SLAMF1 and Nectin-4 sequences in morbillivirus hosts. I suspect one of these is more important than the other in driving receptor tropism/speciation in virus populations. I couldn't find this in the files, only the similarity matrices that I find a little difficult to read. Other than that, great work.

Reply: We thank the reviewer for this suggestion. We added sequence distance plots (**Extended Data Fig. 1a**) to show the relative divergence between SLAMF1 and Nectin-4 sequences. We have, however, refrained from showing phylogenetic trees of the receptors as the generated trees using either maximum

likelihood method or in a Bayesian framework did not show monophyly among animal orders nor the clustering followed the mammalian species tree, which was reconstructed using concatenated conserved genes[3]. Therefore, we considered it misleading to show those unresolved trees.

P3, L85-L86: Notably, nectin-4 coding sequences are less diverse than CD150 coding sequences across animal orders (**Extended Data Fig. 1a**).



Extended Data Fig. 1a) Sequence distance plots between morbillivirus receptors of orthologues grouped by animal order.

Reviewer #3 (Remarks to the Author):

I am fine with the revisions done to answer to my queries. I congratulate the authors for the immense job done in the revision. However, statements on the macroevolutionary analyses remain too strong, based on PP of 0.40-0.73 for nodes B, C, D in Figure 6. The PPR value may be the highest of PP values obtained compared to other hosts, it still remains poor probability values.

Therefore, these two small changes are required before publication:

P6 L215-219: The probability of a bat origin was consistently highest in most major nodes in ASR (posterior probability [PP] 0.40-0.73 in nodes B-D, Fig. 6f), albeit the origin of morbilliviruses at the tree root remained unresolved (Fig. 6f, node A).

Please change to

The probability of a bat origin was consistently highest in most major nodes in ASR, although posterior pobability values remained small for most nodes (posterior probability [PP] 0.40-0.73 in nodes B-D, Fig. 6f). Notably, the origin of morbilliviruses at the tree root remained unresolved (Fig. 6f, node A).

P7 L257-258: Our macroevolutionary data project the origins of major morbilliviruses to non-recent host shifts, predominantly from bat hosts, in addition to artiodactyls and carnivores, discussed elsewhere20,28.

please change to:

Our macroevolutionary data project the origins of major morbilliviruses to non-recent host shifts, including from bat hosts in addition to artiodactyls and carnivores, discussed elsewhere20,28.

Reply: Done as suggested.

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