Dear Dr. Carlson,   
  
Your manuscript entitled "Applying ecological network theory to re-estimate global viral diversity: host sharing matters" has now been seen by 3 reviewers, whose comments are attached. The reviewers have raised a number of concerns which will need to be addressed before we can offer publication in Nature Ecology & Evolution. We will therefore need to see your responses to the criticisms raised and to some editorial concerns, along with a revised manuscript, before we can reach a final decision regarding publication.   
  
In particular, Referee #2 feels that the presentation requires more clarity to distinguish it as a stand-alone piece of work, rather than a response to Carroll et al in Science. Referee #3 also feels that there are some issues with clarity of presentation, so we suggest re-formatting into our longer Article format (up to 3000 words for Intro+Results+Discussion), to give you room to address these comments.

We appreciate the opportunity to revise our manuscript, and have expanded to a longer format, aiming to produce a piece of broader relevance rather than simply a response to the Carroll *et al.* paper.

Please note that we also do not permit punctuation in titles, so you may wish to revise this now. Titles should be short and informative (ideally no more than 100 characters, including spaces). For example: "Re-estimating global viral diversity based on host sharing" or "Global viral diversity estimates incorporating shared hosts", something like that.

We have updated the title of the manuscript to “XXXXX”

Reviewers' comments:   
  
Reviewer #1 (Remarks to the Author):  
  
Review of C. J. Carlson et al. Nature Ecology and Evolution  
  
This manuscript presents a new estimate of global viral diversity in mammals that reduces current estimates based on linear scaling between host and virus diversity by two orders of magnitude. This re-estimation of viral diversity is based on host sharing, applying a power law scaling relationship for host-virus species interaction networks.  
  
I think this is an excellent paper. In the absence of clear theoretical expectations, the authors use a simulation method to re-estimate viral richness, based on iterative resampling, curve-fitting, and extrapolation on bipartite networks. They apply the method to an excellent database that covers 10% of mammal diversity and their 511 catalogued viruses. Their approximation works remarkably well: they slightly overestimate virus diversity (568). Then, they extrapolate to the global host-virus networks, considering both RNA and DNA viruses. The upper bound found is only 2-3 % of previous estimates.   
  
My only (relatively) major concern with this paper relies on the effects of sampling effort within individual host species on the estimation. The question is simple, but it is not clear in the manuscript: do the 511 catalogued viruses in the database represent the maximal sampling effort where diversity saturates for these hosts? In other words, can the authors provide rarefaction curves where viral diversity saturates, for at least some host species? In Table S1 they provide the degree for different orders, but it is not clear whether the degree depicted corresponds to the saturation phase of the rarefaction curve. If these curves do not saturate, hence, there is a “hidden” diversity not accounted for. If, on the contrary, sampling more individual hosts does not increase viral diversity, then, estimations are correct. In the methods, the authors state “…rarefaction curves were constructed for one or two individual species as a function of sampling effort”. But, do these curves saturate? Usually, in the study of other consumer-resource interaction networks, the number of individuals sampled should be large enough to provide a good estimation of the true degree of a species (see e.g. P. Jordano 2016, Functional Ecology <https://doi.org/10.1111/1365-2435.12763>).

This is tied up in the 6.7% shit.

The writing is clear and appealing for a general audience. Also, the figures are very nice, simple, and greatly illustrate and complement the message of the paper.  
  
  
Reviewer #2 (Remarks to the Author):  
  
In this study the authors estimate global virus diversity for mammals using a non-linear equation fitted as a power law to the number of hosts. The authors show that this method, in comparison with linear estimation used in a previous study earlier this year, provides order of magnitude lower estimation of mammal virus richness.   
  
Overall I find this paper timely, relevant and interesting. I agree with the authors that estimating richness of affiliate species linearly does not make much sense and I also find the estimation by Carroll et al way too high. So, I like the paper (and the fact that the code is already available!), but I do have comments (mostly since things were not explained well).  
  
Good luck!!  
  
MAJOR COMMENTS  
  
1. The manuscript reads as a response to the recent study published in Science earlier this year by Carroll et al (ref 1). The language of the current manuscript is brief (too brief), the tone sometimes harsh.

The writing style assumes that the reader is familiar with ref 1: it is impossible to understand the results and the introduction before reading the methods, to really understand what ref 1 did. In fact, I needed to read the Supplement of ref 1 to really get a really good idea of what they did. On the other hand, the authors make some broader analysis across network types in Fig 1 and show that estimating affiliate species typically produces a power law. They even give a package to perform this analysis making it broadly accessible. So, I think the editors and authors need to work together to decide if this is a brief communication as a response, or if it stand by its own and uses the Carroll paper as a mere example in which linear estimation has applied consequences.

This would require some rewriting and re-formulation of the problem. Does not seem too difficult to do.  
  
2. I know this is a brief communication but I think the results and the intro are just too brief. I really did not understand from reading this what was the problem and how the analysis was done. I had to read this a few times. What I suggest is to move some description from methods to the intro. For example, the explanation on how Carroll et al calculated virus diversity could easily go to the intro in line 24. And some of the methods for estimation should be explained somehow before the authors “throw” all these results at the reader.

3. I was missing an explanation on why the analysis was done that way. Specifically, why was there a need to resample? Why don’t just fit the number of viruses to a power law? Why, and in which way is this analysis different than the classical approach of fitting a degree distribution to an exponential/power law/truncated power law (sensu Bascompte et al 2003 Ecol Lett)? Was resampling done just to produce CI? or is there some other logic behind it?

4. Why did the authors choose to fit a power law (that this was previously done by ref 7 is not a good-enough motivation)? A power law has a special property of scale-invariance. Why is scale-invariance important for affiliate diversity? what does it mean biologically for the viral diversity? Also, if we try to fit a different function would that have a worse fit (e.g., a lower AIC in model selection)? Worth trying no?

5. It is tricky to use the taxonomy we typically use for species on viruses. Designation of viruses to different ‘species’ (L.22) does not make much sense, and this is well known. The influenza virus itself has so many strains each with its own epidemiological properties. What is the taxonomical units of the data set of Olival? How does using ‘species’ as a unit limit our ability to really know what is out there? We should use whole genomes, or OTUs, etc.

6. It was not clear to me if the viral diversity was estimated for host orders and not species (L. 245)? I guess species because it does not make sense to me to fit to orders. But if that was the case then why?

MINOR:  
  
7. L.28: change ‘severely’ with the exact quantity, or in terms of orders of magnitude. Also try to avoid harsh tone towards Carroll et al in general.

We have made this change:

8. L.24-25. Why? Why not considering host sharing a fundamental gap?

We are unsure how to interpret this review, as the sentence in question reads: “A fundamental gap in such projections is the lack of attention to host sharing patterns.”

9. L32—33. Is this independent estimate a mean for the host species?

Yes; we have now clarified this.  
  
10. L37-39. And? Is this way of correcting the estimates helpful?

We have somewhat reorganized this text to better clarify the problem with the approach (which also addresses Reviewer 3’s concern about why we refer to this as a “lower bound” approach):

“However, this approach omits any species that are shared between hosts, and can only provide a lower bound on the number of host-specific species (May 1990).”

11. L 43-44. Any ref for that? or is it just based on the Fig 1? If only based on the figure then I don’t think it is conclusive evidence.

We have rephrased this sentence to avoid this concern:

“This non-linear scaling between host and affiliate richness observed in host-helminth associations can be reproduced for several types of species interactions (Figure1A-D).”  
  
12. L49: change “that the pattern would” to “to”.

We have made this change.  
  
13. L50-51 Data and computational tools for that kind of analysis exit at least 10 or more years. I don’t agree about that “recently”.

With the viral data presented in the Olival study only made accessible this year, it is hard to argue the data to find these types of patterns—at least, for viral diversity—has not recently increased; however, we have removed reference to computational tools:

“….the pattern may be subtle enough at smaller scales to not have been evident without the kind of large network data that is increasingly available in community ecology.”  
  
14. L.51-52: Why? Also see my major comment #4.  
  
15. L66-68. Using the 6.7% is also linear extrapolation (as in Carroll et al). Just saying...  
  
16. I Would include humans in Fig 2d,e. It will be cool to show with which orders humans share more viruses. In that regard, authors are missing an important reference for parasite sharing: Gómez JM, Nunn CL, Verdú M. Centrality in primate-parasite networks reveals the potential for the transmission of emerging infectious diseases to humans. Proc Natl Acad Sci U S A. 2013;110: 7738–7741.

This one is a Casey one  
  
17. Why were edge weights proportional to number of species shared? How would another choice change the results? For example, Pilosof et al. (2015 PLOS One) used the jacquard index because two hosts that share similar parasite communities will be more prone to share a new parasite. Is it because you need the weights to fit the curves?

This one is a Casey one  
  
Reviewer #3 (Remarks to the Author):  
  
This manuscript addresses the question of estimating the global diversity of viruses that use mammals as hosts, and the number that potentially use humans as hosts (i.e. zoonoses). This is highly topical, given the recent launch of the Global Virome Project and similar schemes, which has involved much fanfare about the huge number of undiscovered viruses and the threats they might pose. The manuscript work points out a flawed assumption underpinning those estimates, and then develops and applies a rational approach rooted in ecological principles to make better estimates. The new numbers turn out to be drastically lower, and the authors make arguments for how their approach might be used to target on-going sampling efforts.   
  
I was startled to learn from this manuscript that the existing, well-publicized estimates of global viral diversity were based on the simplistic (and obviously wrong) assumption that host sharing could be ignored. The authors have done a major service to the field in uncovering this error and proposing a reasonable fix. They have also put the virus-host literature in an appropriate ecological context, by comparing the ‘affiliate-host’ relationships for viruses to those observed for four other classes of close species interactions (Fig 1 A-D). For these reasons I am enthusiastic about this study, and believe it merits placement in a high-ranked journal where it will get the attention it deserves.   
  
With that said, I do believe there are aspects that could be improved, either in substance or presentation. I detail these below.  
  
- The scaling exponents z are reported without uncertainties. This seems odd given that the power-law relationships are at the heart of the study. I recognize that the authors used a sub-sampling approach to estimate the uncertainty in total viral richness directly, but still the underlying claims about power-law behaviour should be supported statistically.

- In most or all of the power-law fits to data, the fitted line seems to run high at the upper limit of host diversity (i.e. the right-most part of the curve fit). Is this simply a manifestation of the overestimation problem described in Fig 1E? Or is there something else going on here? Did the authors try any other functional forms to fit the data?

- Uncertainties are reported (as 95% CIs) for many key quantities throughout the study, but they don’t seem to capture all the uncertainty at hand. In particular, there is a major leap in assuming that the viral database they are using (from the Olival et al paper) captures 6.7% of the viral richness for all host groups and all virus groups considered in the paper. This number is reached by comparing the relevant numbers from the viral database to numbers reached by intensive metagenomic screening of two host species. To their credit, the authors acknowledge in the Methods that this number is a ‘back-of-the-envelope estimate’ and is ‘tenuous’. However they also go on to apply this factor to re-scale every richness estimate reported in the manuscript, and it seems to be treated as having no uncertainty at all. While I am sympathetic to the predicament (some number is needed, and this seems to be the best one, and there is no obvious way to accurately capture the uncertainty on it), this approach undermines all the 95% CIs presented for richness estimates. Why bother propagating the small uncertainties from other steps in the process, while ignoring uncertainty in this factor? This only serves to make the uncertainty look smaller than it really is. It would be much better to take a swing at estimating CIs around each estimate that encompass all sources of uncertainty. Or at minimum, there needs to be explicit language about how the stated uncertainty ranges should be interpreted.

- Also, when the estimates of total zoonotic virus richness are presented on lines 88-89, the authors abandon the CIs and just state the numbers with a preceding ~ (‘approximately’). To me, this read as conceding the point that the uncertainty estimates wouldn’t be reliable anymore, since more factors with unknown uncertainties were used to reach these values. This is not very satisfying, though I am open to arguments about why it’s reasonable. However this approach is unevenly applied, since it seems it should also apply to the estimates relying on the 6.7% number, which also lacks an uncertainty.

- Why is the 6.7% rate applied to both DNA and RNA viruses? Shouldn’t there be different scaling factors for the two groups?

And while I like the idea of performing the analysis separately for DNA and RNA viruses, why stop at DNA vs RNA? Why not break the viruses down further, e.g. to ss vs ds, or viral families?

- Some details in the results confuse me. The authors use the ‘zoonotic rates’ from Olival et al (14.1% and 41.7%) to go from estimated total DNA and RNA virus richness to estimated numbers of zoonoses in each class. And they report them with ~ as discussed above. But then when using the 50% estimation method, they give 95% CIs for the zoonotic estimates, and the zoonotic rate for DNA viruses is clearly higher than 14%. At first these two differences made me think they had somehow extrapolated the zoonotic numbers directly. But then I calculated the zoonotic rates from the reported numbers, and found that both the DNA and RNA groups used the 41.7% rate. This appears to be an error. If so, then the later statement on lines 103-109 (about more zoonotic DNA viruses than zoonotic RNA viruses) does not hold. This would be fine, especially since the rationale provided in lines 107-109 seems tenuous.

- I didn’t get much out of Figure 2D-E. It’s a nice illustration, but there seems to be little information in the degree distribution (all the important nodes are roughly the same size, surprisingly). Nothing about the specific edges jumped out. So really it’s only the ‘node strength’ which differs significantly, and it’s not obvious to me why I should care about that. The text in lines 123-131 seems to imply that this mapping of diversity patterns will somehow help to guide future sampling, but I couldn’t follow the logic (or at least, I couldn’t see how the network in Fig 2 informed it). Can the authors please make their thinking more explicit here?

This is gonna be a Casey one

- Similarly I could not understand how the authors’ methods can standardize viral richness efforts for sampling effort (line 116), especially since current sampling is likely to be biased in unknown ways, and probably in different ways for different virus and host groups. (See the News & Views commentary about the Olival et al paper for more discussion of these possible biases.)  
  
  
Minor points  
  
Line 34: I was initially confused about the lower bound argument. It later became clear that this is contingent on assuming that every host species has at least one host-specific affiliate species, which is not obviously true. So I would argue that this isn’t really a lower bound, since the estimate could easily be lower if this assumption doesn’t hold.   
  
Lines 63-73 – This passage was a bit hard to understand, before reading the methods to learn about the different fitting approaches.   
  
It is sub-optimal to go through all the main results on viral species without alluding to the results shown in Fig 2A-C.   
  
Lines 167-179 – Please give the numbers of host and affiiliate species for each example.  
  
Line 249 – Is this statement restricted to non-human hosts? Must be, or else the point about zoonotic viruses makes no sense. Please make this explicit.

Caption to Fig 2 – first sentence of this caption is a bit technical, as it describes quantities that aren’t explained except in the Methods. Also how does node size scale with degree? (by area? Radius?)

This is a Casey one