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From: Sent: To:	Joshua Hayward <u>\$22</u> Wednesday, 12 February 2020 3:15 PM mary.tachedjian; Kohl, Claudia; Adam Johnson; Dearnley, Megan (AAHL, Geelong AAHL); Brianna Jesaveluk; Christine Langer; Solymosi, Philip; Hille, Georg; Andreas; Cecilia Sanchez; Adam Werner; dimitri.kontos <u>\$22</u> Gary Crameri; Heidi Drummer; Andy Poumbourios; Glenn.Marsh; Michelle Baker; Edward Holmes; Wang Linfa: <u>\$22</u> Gilda Tachedijan
Subject: Attachments:	Re: HPG Paper PNAS Submission Response to reviewers and revised manuscript Response to Reviewers.pdf; HPG paper - Manuscript Revised V2.pdf; HPG paper - SI Appendix Revised V2 (Marked up).pdf
Dear All, Thank you for all of the edits and to PNAS. Marked-up versions of the subm Best, Josh	improvements! Please be advised that the revised manuscript has been submitted itted manuscript, SI, and response to reviewers are attached.

# Joshua Hayward PhD

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# 7<sup>th</sup> February 2020

Dear Editor,

Thank you for considering our manuscript entitled "Infectious KoRV-related retroviruses circulating in Australian bats" by Joshua A. Hayward, Mary Tachedjian, Claudia Kohl, Adam Johnson, Megan Dearnley, Brianna Jesaveluk, Christine Langer, Philip D. Solymosi, Georg Hille, Andreas Nitsche, Cecilia A. Sánchez, Adam Werner, Dimitri Kontos, Gary Crameri, Glenn A. Marsh, Michelle L. Baker, Pantelis Poumbourios, Heidi E. Drummer, Edward C. Holmes, Lin-Fa Wang, Ina Smith, Gilda Tachedjian for publication in *Proceedings of the National Academy of Sciences*.

We thank the editor and reviewers for their comments and suggestions for strengthening the paper. Please find our responses to the reviewer's questions below. Revisions within the manuscript are highlighted in yellow. We have changed the authorship positions for P. Poumbouros and H.E. Drummer due their contributions to additional experimental studies on bat serology.

Yours Sincerely,

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Prof. Gilda Tachedjian NHMRC, Senior Research Fellow Head, Life Sciences Discipline Head, Retroviral Biology and Antivirals Laboratory Burnet Institute



# Editor's comments:

Our reviewers were overall positive about the paper, but had specific suggestions for improvement. Both made very good points. Some weakening of claims (about possible relation to endogenous viruses, for example) might be in order. Reviewer #2 had several requests for additional experiments, and many were not difficult. Adding as many as feasible would strengthen the paper.

# Reviewer #1:

## **General comments:**

Here Hayward et al. identify and characterize the a full-length, replication-competent gammaretrovirus genome isolated from a bat in Australia, called HPG. In addition, four other related, partial retroviral genomes were isolated from other bat species in Australia and China. These retroviruses are closely related to koala retrovirus (KoRV) and gibbon ape leukemia virus (GALV) with similar sequence, tropism, and structure. HPG envelope antibodies were detected in multiple bat serum samples and HPG-like nucleic acids were detected in multiple bat serum samples and HPG-like nucleic acids were detected in multiple bat serum samples and HPG-like nucleic acids were detected in multiple bat feces samples. The authors propose that this novel gammaretrovirus is actively replicating in bats and that bats having overlapping habitats with koalas and gibbons may have led to interspecies transmission. The work is solid and novel, but a few key questions were not fully addressed in the study that could strengthen the manuscript.

## **Major Comments:**

**Comment 1:** It appears that KoRV-related retroviruses were only detected in bat mucosal excretions and not in blood. Is it known where these viruses may replicate in koalas (or bats based on receptor expression) and if this tropism would explain this finding? Related to this, how do the authors envision that interspecies transmission occurred? This would be particularly useful to include in the Discussion, as it is not clear when and how transmission(s) between the 2 species occurred.

**Response:** Although appropriate blood samples were not available for analysis, we were able to detect KoRVrelated sequences in feces and urine. While KoRV has been detected in the blood of infected koalas (Tarlinton, 2006, Nature, 442:7098; Simmons, 2012, Aus. Vet. J. 90:10; Waugh, 2017, Sci. Rep. 7:1), KoRV nucleic acids and/or proteins have also been identified in various tissues, including sperm (Tarlinton, 2006, Nature, 442:7098), breast milk (Xu, 2013, PNAS, 110:28; Morris, 2016, Sci. Rep. 6) as well as feces (Wedrowicz, 2016,

Conserv. Genet. Resour. 8:4). The closely related gibbon ape leukemia virus (GALV) has additionally been identified in the feces and urine of gibbons (Kawakami, 1977, J. Natl. Cancer. Inst. 268:5619).

KoRV-A and GALV utilize the PiT-1 (SLC20A1) receptor (reviewed in Denner, 2016, Viruses, 8:12), which is a phosphate transport protein ubiquitously expressed at variable levels throughout the mammalian body (Kavanaugh, 1994, PNAS, 91:15; Johann, 1992, J. Virol. 66(3); also see expression database entries:

Expression Atlas (Petryszak, 2015, Nucleic acids research, D746-D752) https://www.ebi.ac.uk/gxa/genes/ensg00000144136?bs=%7B%22homo%20sapiens%22%3A%5B%22ORGANIS M PART%22%5D%7D#baseline

<u>Baee</u> (Bastian, 2008, In International Workshop on Data Integration in the Life Sciences, Springer, Berlin, Heidelberg)

https://bgee.org/?page=gene&gene id=ENSG00000144136)

The highest expression levels of PiT-1 are in locations including the colon, testes, breast, bladder, placenta, and brain. Our experimental results (Fig 5A), including new data from infection interference assays (Fig 5B) suggests that HPG utilizes the same cell receptor, PiT-1, similar to KoRV-A and GALV. Taken together, these observations are consistent with detecting KoRV-related retroviruses (i.e. HPG) in the feces of bats as well as other pooled tissue samples.

Regarding potential routes for interspecies transmission we would like to clarify that we do not propose that a specific species-to species transmission from bats to koalas/gibbon apes occurred for the KoRV-related retroviruses identified in our study. Rather, our phylogenetic analysis suggests that there are likely retroviruses **more closely** related to KoRV/GaLV yet to be discovered. This was stated in the discussion section Page 11, Line 12 as follows:

"Hence, bat communities could in theory provide a route of transmission for KoRV-related viruses between Asia and Australia, although the immediate ancestor of KoRV remains uncertain and it is clear that additional animal species need to be sampled. Indeed, there are likely to be other currently unidentified species infected with KoRV-related viruses linking the habitats of R. hipposideros and Australian bats. The long phylogenetic branch length linking the KoRV clade to its closest known relatives in the GALV/WMV clade indicates that the phylogenetic picture remains incomplete, with additional as yet unknown viruses and host species existing between the KoRV and GALV/WMV lineages of gammaretroviruses."

Further regarding interspecies transmission, in general, given the diversity of body fluids within which KoRV and GALV (and by extension, KoRV-related viruses) might be found, a number of possible scenarios may be reasonably speculated. These include transmission via blood during fighting/predation, and contamination of food sources by feces and urine.

To address the comments raised by the reviewer, we have included the following paragraph in the Discussion at Page 11, Line 32:

"KoRV and GALV utilize the PiT-1 receptor for cell entry (19, 27, 28). This receptor is almost ubiquitously expressed throughout the mammalian body at variable levels (41-44), and is highly expressed in many tissues including the colon, breast, testes, bladder, placenta, and brain (41, 42). KoRV and GALV have been detected in numerous tissues and body fluids including blood, sperm, breast milk, feces, and urine (5, 27, 45-50). Given the wide distribution of PiT-1 expression and the detection of KoRV and GALV in body fluids including blood, urine, and feces, it is possible that interspecies transmission might occur along routes including blood during fighting/predation, and contamination of food sources by feces and urine."

**Comment 2:** On page 9, line 15 and 17, the authors state, " contain endogenous HPG-related sequences" and "...suggesting evidence of endogenization or latent infection with HPG-related viruses." However, on page 7, they report that HPG is unlikely to be an endogenous virus. First, these statements are contradictory and should be reconciled. Second, in the BLAST analysis, presumably bat ERVs were identified. What sequence identity do they have with HPG? The authors state that "sequences with high percent nucleotide similarity" were not observed. However, "high" is not defined.

**Response:** We thank the reviewer for raising this point and can appreciate why our statements appear contradictory. We agree that we cannot rule out the possibility that HPG is not endogenous in some proportion of the bat gene pool since we have only sampled a small number of bats. Accordingly, we have modified the manuscript as follows to soften our claims:

## We have modified our result heading from

"HPG is not an endogenous retrovirus"

to

"HPG sequences were not detected in the genomes of pteropid bats" on Page 7, Line 3.

Furthermore, within the discussion section, we have modified the text to read as follows on Page 12, Line 3:

"We searched carefully for the presence of HPG in the genomes of P. alecto and P. vampyrus using molecular analyses, and more broadly for KoRV related viruses in the SRA: we were unable to detect these viral sequences in any currently available bat genome sequence. While these data suggest that bat KoRV related viruses are not endogenous, we cannot exclude the possibility as we have only sampled a small proportion of bats within each species. In this regard, KoRV endogenization in koalas is relatively recent, and accordingly is not represented across the entire koala gene pool (45); existing in both endogenous and exogenous forms (5, 27, 51). Thus, given that HPG specific sequences have been identified across several bat species, either HPG is an exogenous virus or it is undergoing endogenization in real time. A possible example of the latter is FFRV1 (14), which was recently discovered in the brain tissue of a P. alecto bat, but which we were not able to identify within the genome of P. alecto or other bats. Serological and nucleic acid analyses revealed that numerous individual bats across several species have been exposed to HPG and HPG-related viruses (Supplementary Figure 12 and Supplementary Table 4), and that 6.7% (25/373) of the bat scat samples analyzed contained HPG specific RNA, suggesting that these bats are actively infected with HPG and, more generally, that HPG related viruses are currently circulating among the communities of multiple species of Australian pteropid bats. The close evolutionary relationship between the bat KoRV related viruses from several species of Australasian pteropid bats, Asian microbats, and the gibbon, koala, and rodent viruses suggest that bat populations in Australia and Asia play an important role in the transmission of KoRV-related viruses between bats and possibly other mammals."

Regarding our BLAST search within pteropid genomes for HPG sequences, when we analysed the genome of *P. alecto* for sequences similar to HPG, our BLAST analysis revealed that HPG and closely related retroviral sequences were not present. The closest identified hit against the HPG sequence in this analysis was a 546 nt sequence aligning to the *pol* gene of HPG, with a nucleotide identity of 69%.

To address this matter, we have replaced the sentence on Page 7, Line 5:

"No sequences with high percent nucleotide similarity to HPG were identified" with

"No sequences matching HPG were identified. The closest identified hit against the HPG sequence in this analysis was a 546 nt sequence within the genome of P. alecto, aligning to the pol gene of HPG, with an e-value of 5.0x10<sup>46</sup> and a nucleotide identity of 69%."

**Comment 3**: Regarding phylogenetic analysis with related gammaretroviruses, how much does time impact relatedness? For example, HPG was isolated from a bat obtained in 2011, but it is unclear when the other viruses were obtained and how this could affect evolution, particularly in new host species (i.e. species adaptation after transmission from bats to koalas).

**Response**: We apologize that the sampling period was not clear. The sampling period for these viruses was only over seven years, as stated on Page 4, Line 38, and accordingly is highly unlikely to have an impact on relatedness in the context of the evolutionary time scale depicted in our phylogeny which is almost certainly on the scale of thousands to millions of years (Holmes EC, (2009), The Evolution and Emergence of RNA Viruses. Oxford Series in Ecology and Evolution, Oxford University Press, Oxford).

**Comment 4**: On page 9, the authors describe 12 samples that were positive only for HPG DNA and not HPG RNA and state that they represent animals that are "latently infected with other HPG-related virus(es) or contain endogenous HPG-related sequences." An alternative explanation would be low quantity and/or low quality RNA present in these samples. It is unclear that RNA and DNA quantity or quality were controlled in the analysis.

**Response:** To clarify, the samples referenced in this sentence tested positive for HPG *specific* RNA, but not HPG*-related* RNA indicating that the quality of the RNA in these samples was adequate for this analysis. While we did not detect HPG-related RNA in these samples, we did detect HPG-related DNA.

Regarding quantitation of our samples and results, nucleic acids extracted from bat samples were quantitated using the QuantiFluor RNA system without DNase treatment, and all samples were found to have a total nucleic acid concentration of greater than 50 pg/µL. During the qRT-PCR analysis, bat samples were classified as positive or negative based on their fluorescence signal compared a standard curve generated using  $1 \times 10^{0} - 1 \times 10^{7}$  copies of the HPG-proviral plasmid. In the standard curve, signal was only generated down to a threshold of  $1 \times 10^{1}$  copies, the signal for which appeared at cycle 36 (CT 36), and this CT value served as the cut-off for determining a positive result.

While we cannot exclude that some amount of HPG-related RNA was present in the samples and below the limits of detection, we did utilize a highly sensitive kit (Thermo Power SYBR Green RNA-to-CT Kit) that is capable of detecting specific targets from sub-picogram levels of total RNA.

To address the reviewer's alternative explanation, we have modified this sentence to now read as Page 10, Line 26:

"While we cannot rule out that some or all of these samples may have contained HPG-related RNA below the limit of detection of this assay, these data suggest that 12 bat samples were actively infected with HPG and were either latently infected with other HPG-related virus(es) or contain endogenous HPG-related sequences."

Within the supplementary methods section "*RT-qPCR for detecting the presence of gammaretroviral nucleic acids in bat samples*" SI Page 8, Line 41 we have added the following details:

"Samples were quantitated using the QuantiFluor RNA system (Promega, Madison, USA) in 96-well plates on a Clariostar microplate reader (Isogen Life Science, De Meern, Netherlands). All samples contained a total nucleic acid concentration  $\geq$  50 pg/µL."

And on SI Page 9, Line 3:

"Bat samples were classified as positive or negative based on their fluorescence signal compared against a standard curve generated using  $1 \times 10^{9} - 1 \times 10^{7}$  copies of the HPG-proviral plasmid. The cut-off for determining a positive result was a cycle threshold of 36, representing  $1 \times 10^{1}$  copies of the HPG provirus."

## **Minor Comments:**

**Comment 5**: The Introduction (page 5, line 1) and the Discussion (page 10, line 3) state "the Daintree rainforest," which implies that the Daintree rainforest is part of the Australian east coast. However, the Results section (page 6, lines 6 7) state "373 bats along the east coast of Australia and 106 bats from the Daintree Rainforest (Queensland)" that seems to imply that the Daintree rainforest is separate from the east coast. The text should be consistent throughout the manuscript.

Response: To clarify this statement we have modified the text at various locations to read as follows:

Page 4, Line 38: "we collected bat samples (feces, blood, urine, and oral swabs) from towns and the Daintree rainforest along the east coast of Australia"

Page 6, Line 5: "To identify KoRV related viruses in bats, samples were collected from the east coast of Australia, including feces, oral swabs, blood, and urine. 373 samples were collected from towns in New South Wales and Queensland and 106 from the Daintree Rainforest (Queensland)."

Page 11, Line 2: "To determine whether KoRV related viruses are present in Australian bats, we collected samples from bats along the east coast of Australia"

**Comment 6**: Supplementary Table 2: it is assumed that Genbank accession numbers for the bat retroviruses will be forthcoming and included in the final version of the manuscript.

**Response:** The Genbank accession numbers for the bat retroviruses have been included in the updated version of Supplementary Table 2.

**Comment 7**: It is recommended that data presented in Supplementary Figure 7 be described in the Results section after Figure 5 (page 8, 2nd paragraph).

**Response:** We thank the reviewer for this suggestion and have included this description as follows in the results section on Page 8, Line 26:

"Alignment of the binding motif within mammalian PiT-1 genes supports this result as the binding sites within P. alecto and P. vampyrus PiT-1 share the permissive amino acid residues, which are distinct from the non permissive motif within mouse PiT-1 (29) (Supplementary Figure 9)."

**Comment 8**: Supplementary Figure 9 is not discussed anywhere in the text. It is suggested that it be removed or appropriately described in the text.

**Response:** A mislabeled reference to this Supplementary Figure (which has since been renumbered) within the text of the Supplementary Methods section has been corrected, and on SI Page 9, Line 27 now reads:

"SDS PAGE in the presence and absence of  $\beta$ -mercaptoethanol revealed a single diffuse band with a molecular weight range of ~80 90 kDa (Supplementary Figure 13), consistent with the molecular weight predicted from the amino acid sequence (62,805 Da) with 6 N-linked glycans (~ 18 kDa)."

# Reviewer #2:

#### **General comments:**

This is an interesting paper submitted by Hayward and colleagues, describing the discovery and biological characterization of KoRV-related gammaretrovirus sequences in samples from different Australian bat species. The bulk of the results focuses on sequences obtained from scat of P. Alecto, and referred to here as Hervey pteropid gammaretrovirus (HPG). A consensus is used to reconstruct a full HPG viral genome, and a variety of biochemical methods and EM is used to confirm production of gamma-like virions. If true, this may be the first description of an exogenous gammaretrovirus of bats. While the study is likely to be of broad interest, there are several caveats to interpretation that should be addressed, as well as some minor points.

**Comment 1**: Page 7, results first paragraph - An important caveat is that this could also be a recent, rare and unfixed ERV insertion, similar to many KoRV loci in Koalas, especially in southern koalas - that is to say, a similar approach in southern koalas might "miss" detecting a rare enKoRV sequence. The intact nature of the HPG is also consistent with something that could be present in both exogenous and endogenous forms. Effectively, this doesn't change the impact of the manuscript - either its an exogenous gammaretrovirus of bats, a very recently endogenized gammaretrovirus of bats, or both. My suggestion is to stay open to all possibilities - present it as an exogenous virus, but acknowledge that the actual samples might have detected a germline insertion (ERV).

**Response:** We agree with the comment made by the reviewer, and apologize that this was not clear in the manuscript. We have addressed this comment in our response to Reviewer 1's Major Comment 2.

**Comment 2**: Page 7, results - phylogenetic analysis. While a tree based on the full genomes is potentially robust, the authors should also analyze RT and env separately. Do they give the same/similar results as one another, and are they consistent with the tree based on the entire genomes? Recombination can obscure phylogenetic relationships, especially when one part of the genome is more divergent or has had a very different evolutionary trajectory. For example, it could be one gene, such as env, that separates one branch from the others, but is the result of a single recombination event and not of divergence over time. Gene-specific phylogenies could be added to supplemental data, and wouldn't be necessary in the main text (unless they reveal a more complex phylogenetic history, in which case the authors will want to make it part of the story).

**Response:** As suggested by the reviewer, we have now provided individual phylogenies for the *env*, *pol* and *gag* genes as Supplementary Figure 4. As can be seen, the tree topologies for *env* and *pol* genes are the same as that for the complete viral genomes (Figure 2). A slightly different topology was observed in the *gag* gene phylogeny, however, as all the relevant bootstrap values were very low (35%, 41%, 48%), a history of genomic recombination cannot be safely inferred since the difference in tree topology in the *gag* gene lacks phylogenetic resolution.

We have included the corresponding text within the Results section on Page 7, Line 22:

"This finding is supported by phylogenetic analyses of the individual pol and env genes, which reveal the same branching pattern (Supplementary Figure 4). While analysis of the gag gene resulted in a slightly different branching pattern, this is likely as a result of low phylogenetic resolution as indicated by low bootstrap support for key nodes on this tree (Supplementary Figure 4)."

We have also updated the Supplementary methods section to include description of the phylogenetic analysis as follows on SI Page 5, Line 6:

"To determine the evolutionary relationships among KoRV-related gammaretroviruses we performed phylogenetic analyses using aligned complete genome nucleotide sequences (Supplementary Table 2) and individual gene sequences. Accordingly, a multiple sequence alignment of 19 complete genomes was performed using a combination of MAFFT (8) and MUSCLE algorithms (9). Following alignment, regions of ambiguous and uncertain alignment were removed using Gblocks (10). For the complete genomes, this resulted in final alignment of 6,925 nt that was used to infer evolutionary relationships. Subsets of this alignment covering the gag, pol, and env gene regions were used for the individual gene analyses. Phylogenetic trees of these data were estimated using the maximum likelihood (ML) method available in the PhyML program (11), assuming a GTR model of nucleotide substitution with a proportion of invariant sites (I) and a gamma distribution of among site rate variation (I). To determine the robustness of each node a bootstrap resampling analysis (1,000 replications) was performed using the same nucleotide substitution model. For the complete genome tree (Figure 2), a Shimodaira-Hasegawa (SH) test was also conducted, providing additional nodal support. The Mus caroli ERV, McERV (Supplementary Table 2), sequence was used as an outgroup to root the tree."

**Comment 3**: Page 8, serological analysis the negative control (HIV Env) rules out general background, but does not rule out cross reactivity with other gamma-type retroviruses or ERV expression. How specific is this assay? Since the claim is "HPG seropositivity", it should include Env proteins from a distant relative (GaLV, KoRV) and even a different gamma lineage altogether (e.g., MLV Env). The conclusion could then be "HPG-seropositivity" or "KoRV-related retrovirus seropositivity" depending either result fits the story being described in the manuscript. But as is, it's not clear they can claim specificity for HPG.

**Response:** The reviewer has raised an important issue with regards to HPG-specific seropositivity across the tested bat samples. To address this issue we have undertaken a peptide binding analysis in a solid phase enzyme immunoassay to assess the seroreactivity of bat samples against short peptide sequences from the VRA region of Env specific to HPG, KoRV-A, GALV, and the more distantly related MLV, in addition to the HPG Env trimer. The assay was validated using high titre immune serum raised to HPG Env in rabbits, which showed specific binding to HPG VRA peptide, but not KoRV A, GALV or MLV VRA peptides. The rabbit immune serum was also not reactive against an unrelated HCV peptide sequence encoding the antigenic region of glycoprotein E2 residues 409-422 (data not included in manuscript). Additionally, a macaque immune serum raised to MLV only showed reactivity to MLV VRA peptide. While we cannot exclude that antibodies that develop in bats infected with HPG can cross-react with peptides from KoRV and GALV, the data strongly suggest that 32% of bats have been infected with HPG or other KoRV-related viruses.

These new serology results have been included as Supplementary Figure 12 and supersede the luminex data within the Results section, which now reads as follows, on Page 9, Line 21:

"To assess Australian bats for exposure to HPG or KoRV related viruses, we tested bat sera for the presence of antibodies reactive against the HPG Env protein. We also tested for the presence of HPG-specific nucleic acid in bat fecal samples.

Bat sera (87 samples collected from 9 bat species) were screened for the presence of antibodies reactive to the HPG Env trimer ectodomain (Glu<sup>38</sup> Ser<sup>603</sup>) and a synthetic peptide of the HPG VRA region of Env in a solid phase enzyme immunoassay. For sera reactive to HPG VRA, additional analysis was conducted against VRA peptides from KoRV-A, GALV and ecotropic MLV (Supplementary Figure 12).

A rabbit immune serum raised to the HPG Env trimer was used as a positive control and to determine crossreactivity to KoRV, GALV and MLV peptides. The immune sera reacted strongly to HPG Env trimer and the HPG VRA peptide sequence but did not show reactivity to KoRV, GALV or MLV peptides. In addition, immune serum raised to MLV reacted to the MLV peptides sequence but not to HPG, KoRV or GALV VRA peptides sequences (Supplementary Figure 12).

Of the 87 bat samples, 27 (31%) showed reactivity to the HPG Env trimer and of these 19 (22%) were reactive to the HPG VRA peptide [P. alecto (n = 17), P. conspicillatus (n = 1), Rhinolopus megaphyllus (n = 1)]. Of the 19 HPG VRA positive sera, 8 showed additional reactivity to KoRV A and 4 were additionally reactive to both KoRV A and GALV peptides. One serum, #20 P. alecto, was more strongly reactive towards the GALV VRA peptide than the HPG or KoRV A VRA peptide, or the HPG Env protein. Two samples, #7 P. alecto and #8 P. alecto, were reactive against the KoRV A and GALV VRA peptide, respectively, but were not reactive against the HPG VRA peptide. No bats demonstrated reactivity to MLV (Supplementary Figure 12).

These results reveal that 32% of bat samples were seropositive to HPG or other KoRV related protein sequences. Within the species P. alecto, 83% were seropositive to HPG and/or other KoRV related protein sequences, and 27% were only seropositive to HPG protein sequences."

The methodology in the supplementary methods section SI Page 9, Line 41, "Serological assay for the presence of anti HPG antibodies in bats" now reads:

"Bat sera were screened for the presence of antibodies reactive to the HPG Env trimer and the VRA region of Env using synthetic peptides in a solid phase enzyme immunoassay. N terminal biotinylated synthetic peptide encoding the HPG VRA region (LETWDIPDSDVSASTRVRPADSD, Genscript, USA) was added to Avidin coated plates (Nunc, Maxisorb) at 5  $\mu$ g/ml followed by the addition of serially diluted bat serum in PBS containing 2.5 mg/ml bovine serum albumin and Tween 20 (0.05%). Following the addition of bat sera, plates were incubated overnight at 4°C. Bound antibodies were detected with horseradish peroxidase labelled Protein A/G (Thermo Scientific, Rockford) followed by 3,3',5,5'-Tetramethylbenzidine (TMB) substrate (Sigma, USA). Antibody titers were calculated from curves fitted with the Hill slope equation and interpolation to achieve 5x background absorbance (Prism v8.3.1). In the absence of a bat serum verified as seronegative for gammaretroviral infection, conservative titer thresholds were used for the delineation of positive seroreactivity. For the -VRA peptides a seropositive titer of ≥ 280 was selected, and for HPG Env a seropositive titer of ≥ 50 was selected. Sera that displayed above 5x background levels of binding to HPG VRA were further screened for reactivity to biotinylated synthetic peptides of the equivalent regions of KoRV-A (LESWDIPELTASASQQARPPDSN), GALV (LESWDIPGTDVSSSKRVRPPDSD), and MLV (PSYWGLEYQSPFSSPPGPPCCS) in the same way. MLV-positive macaque serum was a kind gift from Damian Purcell (University of Melbourne)."

**Comment 4**: page 17, figure 2 - Need to explain the ratios at the nodes (e.g., 1/100, 1/97, etc). Are these bootstrap values, and if so, why are they presented this way (usually they are given as percentages)? This info should be in the figure legend.

**Response:** We apologize that these values were not clearly explained. The values are not fractions, but the depiction of two different measures of nodal support: SH-like branch support to the left and bootstrap support to the right

i.e. 'SH-value/Bootstrap-value'

We have updated the figure legend to clarify this, and it now reads as follows:

"...All branches are scaled according to the number of nucleotide substitutions per site, and branches representing bat retroviruses are shown in red. Support for key nodes on the phylogeny are shown in the form SH-like branch support/bootstrap support. Silhouettes represent the host species..."

**Comment 5**: Page 18, Figure 3 and related results section - The negative control (which is shown in Supp Fig 8) to confirm that the particles are produced by the transfected plasmid is not mentioned here or referred to in the main text. Authors also need to include some indication of how many images or fields were needed to detect the particles or, more specifically, to explain with what certainty the negative controls can be said to have less/no particles compared to the composites in figure 3. For example, were sufficient fields analyzed or similar numbers of cells visualized/ is this the result of comparing similar numbers of images/cells for transfected and control cells?

**Response**: In thin-section electron microscopy, the analysis is taking place on a single plane (70-90 nm) section of the cell, the height of which can be up to 20  $\mu$ m in a cell monolayer and larger in suspension depending on the cell's orientation and morphology. As such, we feel that detection of viral particles measured by EM

analysis alone is not the most efficient approach to measuring % infection and/or providing 100% confidence of finding (or not finding) viral particles in a test or control sample. For this reason, as described in the Supplementary Methods sections "Transfection of 293T cells for generation of HPG & M-MLV viral particles" and "Electron Microscopy and viral particle morphology", we complimented our EM analysis with a virionassociated reverse transcriptase activity (RT) assay on supernatant from each of the cell suspension samples that was to be analyzed by electron microscopy. A positive reading was obtained for MLV and HPG but not for the negative controls (cells- with no transfection and the empty plasmid) indicating virus budding and activity in the test samples only. In sample blocks containing MLV 293T cells and HPG 293T cells, viral particles were readily observed budding from the cell membrane or in inclusion bodies within the cells, indicating a relative abundance of virus in the cells. For the negative controls, which were untransfected 293T cells and 293T cells mock transfected with the pcDNA3.1 plasmid, no virus was observed in cells following extensive examination across numerous fields of view, multiple sections and on two separate grids. Furthermore, cell morphology and ultrastructure of control samples was consistent with healthy cells in tissue culture. In contrast, cells in the population that had transfected with MLV and HPG showed morphological indictors of infection such as fragmented cell and organelle membranes, extracellular debris (membrane) and in some instances, cytoplasmic or nuclear condensation. Together these data increase our confidence that control samples were truly negative for virus, whilst the MLV 293T cells and HPG 293T cells did contain replicating virus particles.

We have included the quality control data from the virion-associated RT assay alongside the EM negative controls in Supplementary Figure 5 as panel D. The legend for this figure now includes:

"D) The graph displays the result of a virion-associated RT assay, supporting the result that cells transfected with proviral Moloney murine leukemia virus (M-MLV) and Hervey pteropid gammaretrovirus (HPG) expression plasmids generate retroviral particles in contrast to untransfected and mock pcDNA3.1 transfected cells. Error bars represent the standard deviation of n 2 technical replicates"

We appreciate the reviewer's suggestion that we make appropriate mention of the negative EM controls in the main text and have modified our reference to these data in the results section Page 7, Line 35, which now reads: "Transfection of human 293T cells with a plasmid construct carrying the HPG provirus resulted in the generation and release of viral particles morphologically similar to ecotropic Moloney murine leukemia virus (M MLV), as determined by electron microscopy (Figure 3). In contrast, no virus was observed in un-transfected 293T cells and mock control 293T cells that were transfected with pcDNA3.1 (Supplementary Figure 5). These data are supported by virion associated reverse transcriptase analysis of each sample analyzed in concert with electron microscopy analysis (Supplementary Figure 5)."

The following sentence has been appended to the end of the legend of Figure 3 on Page 22:

"Negative controls were untransfected cells and cells mock transfected with the empty vector pcDNA3.1. These controls were not observed to contain or produce viral particles (Supplementary Figure 5)."

**Comment 6**: Page 20, figure 4: the HPG result is distinctive the MLV infection results in the expected plateau consistent with ongoing replication, whereas HPG replication peaks and drops quickly to background. This raises the possibility that the data don't represent ongoing replication cycles, but rather a burst of production from initially infected cells. Another possibility is that the HPG retrovirus is replicating, but is toxic to cells, similar to lentivirus replication in cell culture. An experiment to examine these possibilities and to definitively establish successive rounds of replication is important (e.g., passaging filtered supe to a second plate/flask followed by RT assay, or replication with and without inhibiting RT, etc).

**Response**: To address this possibility, and as suggested by the reviewer, we conducted a 'secondary infection assay', in which we established successive rounds of replication, and is included as Supplementary Figure 8. In brief, 293T cells were transfected with the HPG proviral plasmid; cell culture supernatant was later harvested and clarified. This clarified supernatant was used to establish a primary infection in 293T cells in the same manner as our original infectivity assay. We then collected the clarified supernatant of these cells and repeated the process, and successfully established a secondary infection in 293T cells, as determined by a virion associated PERT assay. The data from this experiment confirms that successive rounds of replication can be established by HPG in 293T cells. In contrast to our original experiments we used a PERT assay (RT qPCR) to confirm the presence of HPG for these new experiments, as we have ceased using the radiolabeled virion-associated reverse transcriptase assay due to increased and prohibitive costs of radiolabeled nucleotides in our region.

We have added this analysis to the Results section, Page 8, Line 16:

"HPG was confirmed to be capable of establishing successive rounds of replication through a secondary infection assay (Supplementary Figure 8)"

The methodology for this assay has been added to the Supplementary Methods as "Secondary infection assay" SI Page 7, Line 26, and reads:

"To confirm that HPG was capable of establishing successive rounds of infection, the HPG provirus was transfected into 293T cell culture as described in "Transfection of 293T cells for generation of HPG & M-MLV viral particles". To establish a primary infection from HPG virions, 293T cells were infected as described in "Replication kinetics assay". To establish a secondary infection, clarified supernatant containing HPG viral particles generated from the primary infection was collected and used to establish a second infection in 293T cells as described in "Replication kinetics assay". 5  $\mu$ L samples were collected at inoculation, 6 h following inoculation and washing (t = 0), and at 48 h following washing (t = 48). Collected samples were analyzed for the presence of virion-associated reverse transcriptase activity by PERT assay, as described in "Generation of HeLa cells persistently infected with HPG". Values derived from the PERT assay represent arbitrary units of RT activity in comparison to a dilution series (10-fold dilution series down to 1.0x10<sup>7</sup>) of HPG virions which were generated in-house, as described in "Transfection of 293T cells for generation of HPG & M-MLV viral particles."

## Other comments

**Comment 7**: The manuscript proposes that HPG uses the same receptor as KoRV-A and GaLV, and even includes a supplemental figure depicting the conserved binding site motif in PiT-1 of the relevant host species. This is presented as part of the argument in referring to this as a "KoRV related retrovirus", as in the title of the manuscript and elsewhere in the text. Given how easy it is to do, why not formally prove this? It should be straightforward, and there is plenty of precedent in the literature - either by adding PiT-1 expression to null cells (such as the NIH3T3 cells used in figure 5), or by means of a standard superinfection cross-interference assay. Either experiment can be done with existing reagents in a relatively short period, and would strengthen the manuscript.

**Response**: We thank the reviewer for this suggestion and have undertaken a superinfection interference assay, included as an additional panel (B) in Figure 5. Briefly, in this experiment we generated persistently HPG infected HeLa cells (Supplementary Figure 11), then challenged these cells with infection by Envelope-pseudotyped reporter retroviruses representing HPG, KoRV-A, GALV, Amphotropic MLV, Dualtropic MLV, and the vesicular stomatitis virus (VSV). Compared against uninfected HeLa cells, HPG-infected HeLa cells were strongly resistant to superinfection from HPG, KoRV-A, and GALV Env pseudotyped viral particles; they were moderately resistant to infection by amphotropic and dualtropic MLV (which respectively use the PiT 2 and PiT-1 & PiT-2 cell receptors [Feldman, 2004, J. Virol. 78:2; Miller, 1996, J. Virol. 70:8]) Env pseudotyped particles; almost no impact was observed on susceptibility to infection by VSV. The ecotropic MLV used in our infection kinetics assay was not utilized in this assay as it is incapable of infecting human cells. These results suggest that HPG utilizes the PiT-1 and PiT-2 receptors for cell entry.

We have added the following description of this analysis to the Results section, Page 9, Line 6:

"To further investigate receptor usage by HPG, we performed a superinfection interference assay (Figure 5B). In this assay, human HeLa cells persistently infected with HPG (Supplementary Figure 11) became strongly resistant to superinfection with a reporter virus pseudotyped with the envelope proteins of KoRV-A, GALV, or HPG (97.8 - 98.6% reduction in infectivity). Infections with viral particles pseudotyped with dualtropic or amphotropic MLV Env were also moderately inhibited (34.5% and 47.1% reduction in infectivity, respectively). Dualtropic MLV uses both PiT-1 & Pit-2 (SLC20A2) cell receptors (36), while amphotropic MLV exclusively uses PiT-2 (37). In contrast, superinfection by particles pseudotyped with the unrelated vesicular stomatitis virus (VSV) envelope G-protein was not restricted. These data are consistent with HPG utilizing the PiT-1 and possibly the PiT-2 receptors for cell entry. Taken together, these results indicate that HPG may share a similar host range as KoRV-A and GALV, with the caveat that the specific determinants of receptor usage and cell tropism for PiT-1 and PiT-2 are complex (30, 31, 38), and further investigation will be required to more accurately delineate the host range and cell tropism of HPG."

Given that this analysis revealed inhibition of superinfection by amphotropic and dualtropic MLV, both of which utilize the PiT-2 receptor, we have included these viruses in an updated receptor binding domain alignment (Supplementary Figure 10). This analysis revealed that as with HPG, amphotropic and dualtropic MLV also contained a significant insertion in the VRB domain relative to KoRV, GALV, and ecotropic MLV. We have updated our description of the results of the RBD alignment to read as follows, Page 8, Line 33:

"An alignment of the receptor binding domain (RBD) (32) within the Env sequence of HPG against other KoRV related viruses reveals numerous differences in the variable regions (VRA and VRB) within the RBD (Supplementary Figure 10). Within this region, the pathologically important CETTG motif (33), that is conserved in all other bat KoRV-related viruses, contains a threonine to serine mutation in HPG, resulting in a CETSG motif. HPG is more similar to GALV than to KoRV across both the VRA and VRB, where the RBD amino acid identities for HPG compared to GALV and KoRV are 66% and 62%, respectively. However, all of the KoRV related bat gammaretroviruses analyzed contain a large insertion within the VRB of 10 and 16 amino acids, respectively relative to GALV and KoRV. Amphotropic and dualtropic MLV similarly contain a large insertion within the VRB relative to KoRV, GALV, and ecotropic MLV. The VRB region of amphotropic MLV is essential for interaction with the PiT-2 cell receptor (59), and the large insertion within the VRB of HPG may be involved in its possible use of the PiT-2 receptor, demonstrated by the superinfection assay."

The following paragraph has been added to the discussion section, Page 13, Line 9:

"Infection of cells with a retrovirus can restrict the subsequent superinfection by viruses that use the same receptor by various mechanisms including downregulation of the receptor, and blocking the binding site on the cell receptor, preventing penetration or adsorption of the virus (54, 55). This method has been used to demonstrate the shared use of the PiT-1 receptor between KoRV-A and GALV (56). We undertook a superinfection interference assay which demonstrated that infection with HPG restricts superinfection by a reporter virus pseudotyped with the envelope protein of KoRV-A, GALV, amphotropic MLV, and dualtropic MLV. KoRV-A and GALV utilize the PiT-1 receptor (19, 27, 28), while amphotropic MLV utilizes PiT-2 (57), and dualtropic MLV utilizes both PiT-1 and PiT-2 (36). These results are consistent with HPG utilizing the PiT-1 receptor for cell entry."

The following section has been added to the Supplementary Methods , SI Page 6, Line 12, as "Generation of HeLa cells persistently infected with HPG":

"HPG virion-containing supernatants were generated as previously described in, "Transfection of 293T cells for generation of HPG & M-MLV viral particles", and used to infect HeLa cells. Cells were seeded at a density of 7x10<sup>5</sup> cells per T25 tissue culture flask (BD Biosciences, Bedford MA). Once cells reached 50% confluency, media was replaced with a mix of 4 ml DMEM, 1 ml HPG virion-containing supernatant and DEAE-Dextran (Sigma-Aldrich) at a final concentration of 10 µg/mL. Cells were incubated for 16 h at which point the supernatant was removed, cells were washed twice in PBS and 5 ml of fresh DMEM was added. At 48 hours post infection, cells were passaged at a concentration of 1:5 into a new T25 flask. Cells were routinely passaged 1:5 twice weekly for three weeks and supernatants were tested for the presence of virion-associated RT activity by a product-enhanced reverse transcriptase (PERT) Assay, as previously described (13), except using a PrecisionPLUS qPCR SYBR Master Mix (Primer Design, Chandler's Ford, UK) and analysed on a QuantStudio 7 Flex Real-Time PCR machine (Thermo Fischer Scientific)."

**Comment 8**: Page 32, line 21 supplemental methods refers to "Supp Figure 8" but probably is supposed to refer to Supp Figure 9.

**Response:** We thank the reviewer for noticing this. All of the supplementary figure labels have been updated in the revised manuscript.

**Comment 9**: Page 43, Supp figure 9 legend could use some additional info Are there control lanes (nontransfected or mock transfected) in the image? If so, are the controls the basis for establishing that the indicated bands are HPG Env? The lanes should be labeled or mentioned in the legend. Alternatively, If there are no control lanes, how can the authors claim that this isn't an unfortunate background band?

**Response**: We thank the reviewer for picking this up. The lane preceding HPG in both the reducing and nonreducing conditions is a control lane containing expressed supernatant before binding/column purification of the polyhistidine tagged (His<sub>6</sub> tag) HPG Env ectodomain protein. We have modified the figure and legend (Supplementary Figure 13) to include this information on SI Page 25.

\*\*\*\*\*\*

From:	Wang Linfa s22
Sent:	Wednesday, 12 February 2020 3:19 PM
То:	Joshua Hayward; mary.tachedjian; Kohl, Claudia; Adam Johnson; Dearnley, Megar (AAHL, Geelong AAHL); Brianna Jesaveluk; Christine Langer; Solymosi, Philip; Hille Georg; Andreas; Cecilia Sanchez; Adam Werner; dimitri.kontos <b>\$22</b> Gary Crameri; Heidi Drummer; Andy Poumbourios; Glenn.Marsh; Michelle Baker; Edwa Holmes; <ina.smith <b="">\$22 Gilda Tachedjian</ina.smith>
Subject:	RE: HPG Paper PNAS Submission Response to reviewers and revised manuscri
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- External Email

Dear All,

Thank you for all of the edits and improvements! Please be advised that the revised manuscript has been submitted to PNAS.

Marked up versions of the submitted manuscript, SI, and response to reviewers are attached. Best,

Josh

## Joshua Hayward PhD

Research Officer Retroviral Biology and Antivirals Research Laboratory

Burnet Institute Disease Elimination and Maternal & Child Health Programs

# GPO Box 2284, Melbourne, Victoria, Australia 3001 s22

The Macfarlane Burnet Institute for Medical Research and Public Health Ltd ABN 49 007 349 984

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	JLL
From:	Gilda Tachedjian s22
Sent:	Saturday, 15 February 2020 9:15 AM
To:	Joshua Hayward; Muzza Work; Kohl, Claudia; Adam Johnson; Dearnley, Megan (AAHL, Geelong AAHL); Brianna Jesaveluk; Christine Langer; Solymosi, Philip; Hille, Georg; Andreas; Cecilia Sanchez; Adam Werner; dimitri.kontos <b>\$22</b> Gary Crameri; Heidi Drummer; Andy Poumbourios; Glenn.Marsh; Michelle Baker; Edward Holmes; Wang Linfa; <ina.smit< td=""></ina.smit<>
Subject:	Re: HPG Paper - PNAS Submission Response to reviewers and revised manuscript

Dear All,

You all should have received an email from PNAS regarding submission of the revised manuscript.

This email may also have requested that you ensure your name, institutional affiliation and ORCID identifier (if you have one) are correct in the PNAS database.

Can you please action this request as soon as possible.

~??

FYI the manuscript has proceeded through, "editorial board review", "editor review" and is now "pending recommendation".

Cheers

Gilda

# Professor Gilda Tachedjian BSc (Hons) PhD

Head, Life Sciences Discipline Group Head, Retroviral Biology and Antivirals Lab NHMRC Senior Research Fellow

## **Burnet Institute**



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Cunningham"	s22	"James LN Wood	s22 "Li	n-Fa Wang" s22
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# Manuscript number: JGV-D-20-00049

Title: Achimota pararubulavirus 3: a new bat-derived paramyxovirus of the genus Pararubulavirus Authors: Kate S Baker, BVSc PhD; Mary Tachedjian; Jennifer Barr; Glenn A Marsh; Shawn Todd; Gary Crameri; Sandra Crameri; Ina Smith; Clare EG Holmes; Richard Suu-Ire; Andres Fernandez-Loras; Andrew A Cunningham; James LN Wood; Lin-Fa Wang

# Dear Dr Baker,

Your submission entitled 'Achimota pararubulavirus 3: a new bat-derived paramyxovirus of the genus Pararubulavirus' has been received by *Journal of General Virology*.

You will be able to check on the progress of your manuscript by logging on to Editorial Manager as an author.

The URL is https://www.editorialmanager.com/jgv/.

Your manuscript reference number is JGV-D-20-00049. Please use this in all correspondence relating to this manuscript.

Thank you for submitting your work to Journal of General Virology, a Microbiology Society journal.

Kind regards, Editorial Office Journal of General Virology Microbiology Society | microbiologyresearch.org

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Cc:	Brendan Crabb; Professor James Beeson; David Anderson; Rob Daly; Angus Morgan;
Culting	Tracy Parish; Margaret Hellard; Ben Coghlan; Tachedjian Lab Lab; David Harrison
Subject:	RE: MNAS INS# 2019 15400R Decision Notification

Well done everyone and pleasure to contribute!

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From: Gilda Tachedjian	s22				
Sent: Wednesday, 4 Mar	rch 2020 8:57 AM				
To: Joshua Haywar	s22	; Tachedjiar	n, Mary (H&B, G	ieelong AAHL)	
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Subject: Fwd: PNAS MS# 2019-15400R Decision Notification

# Dear All,

am thrilled to advise that our manuscript "Infectious KoRV related retroviruses circulating in Australian bats" has been accepted for publication in PNAS.

Many thanks to all for your contributions. It was truly a team effort reflecting around 8 years of work- or maybe more - as a "side project" with a terrific group of virologists based at Burnet, AAHL/CSIRO and beyond!

Mary, this long journey all began with your serendipitous discovery of unexpected "retroviral" sequences in a metagenomics analysis of bat scat from Queensland bats during a Hendra virus outbreak. Kudos goes to your exceptional skills in molecular biology and NGS and "hunting virus sequences in bats" as well as recognising that this odd snippet of a retroviral sequence was worth pursuing. Ina many thanks goes to you as well for your top notch skills in "isolating" viruses from bat scat for the metagenomics analysis.

Many thanks Linfa for giving my lab the amazing opportunity to work on this virus. I think I am correct that you said to Mary - "go work this out with your sister, she is a retrovirologist"

From there, with the help of our terrific team of coauthors, we have discovered the first ever reproduction competent retrovirus "Hervey Pteropid Gammaretrovirus, HPG" in bats and have serological and nucleic acid evidence that HPG and similar viruses are circulating in fruit bats in Australia.

Of note HPG is similar to Koala retroviruses currently endogenising Koalas in real time suggesting that bats may act as a reservoir for transmission of KoRV-related retroviruses to other species. Also thanks goes to Claudia and her team for agreeing to combine the "Daintree" viruses with our story to increase impact and for the marvellous Eddie Holmes working his magic with the phylogenetics analyses. Heidi and Andy, you put the icing on the cake with developing serological assays to distinguish Ab to HPG from related gammaretroviruses (KoRV and GaLV) to address one of the reviewers comments which has elevated the quality of the study.

Last but not least, I would like to thank Josh for writing the first draft, a huge undertaking pulling together all of the data. The supplementary section is around 20 pages. But I knew I was playing to Josh's strengths, he likes to write and he is passionate about viruses in bats!

The identification of a new virus in bats is quite relevant to the current situation with COVID 19/SARS CoV 2 i.e. the potential for spillover of viruses in bats to other species.

This paper is under embargo. I will notify you of the publication date so that any media can be coordinated with the Burnet Institute's comms team members - Tracy Parish and Angus Morgan cc'd on this email.

Once again many thanks for your contributions and looking forward to FINALLY seeing this work in print!

Best

Gilda

Professor Gilda Tachedjian BSc (Hons) PhD Head, Life Sciences Discipline Group Head, Retroviral Biology and Antivirals Lab NHMRC Senior Research Fellow

s22

Burnet Institute⊤	s22
	s22

The Macfarlane Burnet Institute for Medical Research and Public Health Ltd,

burnet.edu.au

Equity through better health

Begin forwarded message:

From: \$22 pnascentral.org Subject: PNAS MS# 2019-15400R Decision Notification Date: 4 March 2020 at 7:51:09 am AEDT To: gildat \$22 Cc: \$22 Reply-To: pnas \$22

March 3, 2020

Title: "Infectious KoRV-related retroviruses circulating in Australian bats " Tracking #: 2019-15400R Authors: Hayward et al.

#### Dear Prof. Tachedjian,

We are pleased to inform you that the PNAS Editorial Board has given final approval of your article for publication. Stephen Goff, the Editor who conducted the initial review of your manuscript [MS# 2019-15400R], will also be informed of the decision.

The editorial staff may contact you shortly if final publication ready files are needed. Please note you may be asked to shorten your manuscript upon receipt of the article proof if the work does not adhere to the <u>stated length requirements</u> and <u>additional fees</u> may apply.

Within 48 hours of receipt of your proofs, you will receive an email from <u>PNAS</u> with a link to your publication charge estimate in our online billing platform. Please see the <u>PNAS Author Center</u> for information about publication fees. Authors of research articles may pay a surcharge of \$1,700 to make their paper freely available through the PNAS Open Access option. If your institution has a current Site License, the open access surcharge is \$1,300. Authors of Brief Reports will be assessed a charge of \$2,200, which includes Open Access. Proofs should be returned within 48 hours.

Papers "in press" at PNAS are under embargo and not for public release before 3:00 PM Eastern Time, the Monday before publication. Authors may talk with the press about their work prior to the embargo but should coordinate this with the PNAS News Office or their institution's press office so that reporters are aware of PNAS policy and understand that papers are embargoed until the week of publication. If you plan to present your embargoed paper at a conference prior to publication, please contact the PNAS News Office immediately at 202-334 1310, or <u>PNASnews@nas.edu</u>.

Authors are invited to submit scientifically interesting and visually arresting cover illustrations. To view accepted cover art, please visit the <u>PNAS cover archive</u>. Please note that images must be original and that exclusive rights to publish will convey to PNAS. If selected for the cover, the image also may be used further in promotional materials, including but not limited to brochures, advertisements, and posters. To submit cover art candidates, please send files to <u>PNASCovers@nas.edu</u>

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\*\*\*You can now track your manuscript through the production process by clicking on the link below.\*\*\*

https://www.pnascentral.org/cgibin/main.plex?el=A4B5DVBw2B5BbFN6F3A9ftdlqmwpLKZJ2sexfplLZGgZ

Sincerely yours, May R. Berenbaum Editor in-Chief

\*\*\*\*\*

s22					
From:       Smith, Ina (H&B, Black Mountain)       S22         Sent:       Wednesday, 4 March 2020 9:34 AM         To:       'Gilda Tachedjian'; Joshua Hayward; Tachedjian, Mary (H&B, Geelong AAHL); Kohl, Claudia; Adam Johnson; Dearnley, Megan (AAHL, Geelong AAHL); Brianna Jesaveluk; Christine Langer; Solymosi, Philip; Hille, Georg; Andreas; Cecilia Sanchez; Adam Werner; dimitri.kontos@mh.org.au; Gary Crameri; Heidi Drummer; Andy Poumbourios; Marsh, Glenn (H&B, Geelong AAHL); Baker, Michelle (H&B, Geelong AAHL); Edward Holmos; Wong Linfa					
Cc:	Brendan Crabb; Professor James Beeson; David Anderson; Rob Daly; Angus Morgan; Tracy Parish; Margaret Hellard; Ben Coghlan; Tachedjian Lab Lab; David Harrison				
Subject:	RE: PNAS MS# 2019 15400R Decision Notification				
Wonderful news Gilda I too would like to thank everyor comments and especially thank l	ne for their contributions to get this great work published. I would like to echo Gilda her, Mary and Josh who have been the driving force behind publishing this work.				
Cheers Ina					
Ina Smith, PhD Senior Research Scientist   Risk Evaluation and Preparedness Program   Health and Biosecurity   s22					
	Duplicate Email - Removed				

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		Document 42				
	s22					
	From: Sent: To: Cc:	Joshua Hayward Wednesday, 4 March 2020 9:48 AM Brendan Crabb Edward Holmes; Smith, Ina (H&B, Black Mountain); Gilda Tachedjian; Tachedjian, Mary (H&B, Geelong AAHL); Kohl, Claudia; Adam Johnson; Dearnley, Megan (AAHL, Geelong AAHL); Brianna Jesaveluk; Christine Langer; Solymosi, Philip; Hille, Georg; Andreas; Cecilia Sanchez; Adam Werner; dimitri.kontos S <sup>22</sup> Gary Crameri; Hoidi Drummer; Andy Poumbouries; March, Clong (H&B, Geolong, AAHL); Baker				
	Subject:	Michelle (H&B, Geelong AAHL); Wang Linfa; Professor James Beeson; David Anderson; Rob Daly; Angus Morgan; Tracy Parish; Margaret Hellard; Ben Coghlan; Tachedjian Lab Lab; David Harrison Re: PNAS MS# 2019 15400R Decision Notification				
	Thanks, Brendan! It's a great out	come for us all!				
Joshua Hayward PhD						
Research Officer Retroviral Biology and Antivirals Research Laboratory						
	Burnet Institute Disease Elimination and Maternal &	& Child Health Programs				
	GPO Box 2284, Melbourne, Victo s22	oria. Australia 3001				
	The Macfarlane Burnet Institute fo	r Medical Research and Public Health Ltd ABN 49 007 349 984				
(	On Wed, 4 Mar 2020 at 09:46, B and special congrats to you Jo	rendan Crabb s22 wrote: sh, should have said that earlier. What a triumph.				
	Brendan					
	Professor Brendan Crabb AC Director & CEO	2				
Burnet Institute						
	s22					
	The Macfarlane Burnet Institute for N 85 Commercial Road, Melbourne, VIC <u>burnet.edu.au</u>	ledical Research and Public Health Ltd, 2 3004, Australia				
	Equity through better health					

On 4 Ma	ar 2020, at 9:41 am, Joshua Hayward	s22	wrote:
Fantasti	ic news and a big congratulations to all involved!!		
Joshua	Hayward PhD		
Resear Retrovi	ch Officer iral Biology and Antivirals Research Laborato	ry	
Burnet Disease	Institute Elimination and Maternal & Child Health Programs		
GPO Box	s22 x 2284, Melbourne, Victoria, Australia 3001 s22	-	
The Mac	farlane Burnet Institute for Medical Research and Pul	olic Health Ltd ABN 49	007 349 984
On Wed Brillian	, 4 Mar 2020 at 09:40, Edward Holmes t work Gilda!	s22	wrote:
Delight	ted to be involved.		
Cheers	,		
Eddie			
PROFE ARC A	ESSOR EDWARD C. HOLMES FAA FRS ustralian Laureate Fellow		
THE UN Marie E School The Un	IVERSITY OF SYDNEY Bashir Institute for Infectious Diseases & Biosecurity of Life & Environmental Sciences and School of Me iversity of Sydney   Sydney   NSW   2006   Australia s22	/, edical Sciences, a	
Υψ.	On 3 Mar 2020, at 2:33 pm, Smith, Ina (H&B, Blac s22 wrote:	k Mountain)	÷
	Wonderful news Gilda		
	I too would like to thank everyone for their contripublished. I would like to echo Gilda comments and Josh who have been the driving force behind	ibutions to get this gr nd especially thank h publishing this work	reat work er, Mary
	Cheers Ina		
	Ina Smith, PhD		

Senior Research Scientist | Risk Evaluation and Preparedness Program | Health and Biosecurity | CSIRO, Clunies Ross St, Black Mountain ACT 2601 Ph. 02 6218 3579

Duplicate Email - Removed

	S	22	Document 45
	From: Sent: To: Cc: Subject:	Rob Daly <u>S22</u> Wednesday, 4 March 2020 11:06 AM Lindi Masson; Ben Coghlan David Anderson; Gilda Tachedjian; Joshua Hayward Adam Johnson; Dearnley, Megan (AAHL, Geelong A Christine Langer; Solymosi, Philip; Hille, Georg; And Werner; dimitri.konto <u>S22</u> Gary Crameri; H Poumbourios; Glenn.Marsh; Michelle Baker; Edward <ina.smith <u="">S22; Brendan Crabb; Professor Ja Tracy Parish; Margaret Hellard; Tachedjian Lab Lab; Re: PNAS MS# 2019 15400R Decision Notification</ina.smith>	; Muzza Work; Kohl, Claudia; AHL); Brianna Jesaveluk; reas; Cecilia Sanchez; Adam leidi Drummer; Andy l Holmes; Wang Linfa; imes Beeson; Angus Morgan; David Harrison
~	Well done Gilda and all involve	ed!	
(	From: Lindi Masson Date: Wednesday, 4 March To: Ben Coghlan Cc: David Anderson \$22 \$22	s22 2020 at 10:52 am s22 s22 Joshua Hayward Joshua Hayward S22 "Kohl, Claudia" s22 Adam Johnson	Muzza Work
2	s22 Brianna Jesaveluk	"Dearnley, Megan (AAHL, Geelong AAHL)" s22 Christine Langer	s22
3	s22 s22 Andreas s22 "d	"Solymosi, Philip"\$22\$22Cecilia Sanchez\$2dimitri.kontos\$22	"Hille, Georg" 22 Adam Werner , Gary Crameri
(	s22 s22 s22 s22 s22 s22 <rob.daly@burnet.edu.au> s22 s22 Dav Subject: Re: PNAS MS# 201</rob.daly@burnet.edu.au>	Heidi Drummers22"Glenn.Marsh"s22Edward Holmess22" <ina.smith< td="">s22Professor James Beesons22, Angus Morgans22Margaret Hellards22vid Harrisons22.9 15400R Decision Notification</ina.smith<>	, Andy Poumbourios , Michelle Baker Wang Linfa Brendan Crabb Rob Daly Tracy Parish Tachedjian Lab Lab

Fantastic news!! Well done to all of you!
On Wed, 4 Mar 2020 at 09:51, Ben Coghlan \_\_\_\_\_\_\_ s22 \_\_\_\_\_ wrote:
Yes, you've timed it well! Congrats all round.
On Wed, 4 Mar 2020 at 09:50, David Anderson \_\_\_\_\_\_ s22 \_\_\_\_\_ wrote:
Well done Gilda and team, a great effort and timely as well.
Cheers, David

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	s22
From: Sent: To:	Gilda Tachedjian \$22 Wednesday, 4 March 2020 3:31 PM Wang Linfa
Cc:	Joshua Hayward; Muzza Work; Kohl, Claudia; Adam Johnson; Dearnley, Megan (AAHL, Geelong AAHL); Brianna Jesaveluk; Christine Langer; Solymosi, Philip; Hille, Georg; Andreas; Cecilia Sanchez; Adam Werner; dimitri.konto 222 Crameri; Heidi Drummer; Andy Poumbourios; Glenn.Marsh; M Holmes; < Ina.Smith 222 Brendan Crabb; Professor James Beeson; David Anderson; Rob Daly Tachedjian Lab Lab; David Harrison
Subject:	Re: PNAS MS# 2019 15400R Decision Notification

Thanks Linfa, mission accomplished!

# Professor Gilda Tachedjian BSc (Hons) PhD

Yead, Life Sciences Discipline Group Head, Retroviral Biology and Antivirals Lab NHMRC Senior Research Fellow

# **Burnet Institute**

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	c
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s22	

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On 4 Mar 2020, at 11:58 am, Wang Linfa

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wrote:

Dare Gilda, Josh and all,

That is excellent and congratulations to all!

Yes it has been a long journey and all started with Mary's initial observation of a potential retrovirus in bats. My original drive was for you two sisters to have a "proper" collaboration so that we can have more Tachedjianx2 authored papers!

The timing is perfect as well.....Hope you are all ready for the medial questions!

Cheers,

LF

Linfa (Lin-Fa) Wang, PhD FTSE Professor & Director Programme in Emerging Infectious Diseases Duke-NUS Medical School

s22

	s22
From:	Kohl, Claudia
Sent:	Thursday, 5 March 2020 2:42 AM
To:	Gilda Tachedjian; Joshua Hayward; Muzza Work; Adam Johnson; Dearnley, Megan (AAHL, Geelong AAHL); Brianna Jesaveluk; Christine Langer; Solymosi, Philip; Hille, Georg; Nitsche, Andreas; Cecilia Sanchez; Adam Werner; dimitri.kontos@mh.org.au; Gary Crameri; Heidi Drummer; Andy Poumbourios; Glenn.Marsh; Michelle Baker; Edward Holmes; Wang Linfa; <ina.smith@csiro.au></ina.smith@csiro.au>
Cc:	Brendan Crabb; Professor James Beeson; David Anderson; Rob Daly; Angus Morgan;
Subject:	AW: PNAS MS# 2019-15400R Decision Notification

Document 45

Fantastic news! Thanks to all of you and cant wait to see it in print. All the best, Claudia

Duplicate Email - Removed

#### s22

From:	Gilda Tachedjian s22
Sent:	Thursday, 5 March 2020 9:39 AM
To:	Dearnley, Megan (AAHL, Geelong AAHL)
Cc:	Joshua Hayward; Muzza Work; Kohl, Claudia; Adam Johnson; Brianna Jesaveluk;
	Christine Langer; Solymosi, Philip; Hille, Georg; Andreas; Cecilia Sanchez; Adam
	Werner; dimitri.kontos S <sup>22</sup> Gary Crameri; Heidi Drummer; Andy
	Poumbourios; Marsh, Glenn (H&B, Geelong AAHL); Michelle Baker; Edward Holmes;
	Wang Linfa; Smith, Ina (H&B, Black Mountain); Brendan Crabb; Professor James
	Beeson; David Anderson; Rob Daly; Angus Morgan; Tracy Parish; Margaret Hellard;
	Ben Coghlan; Tachedjian Lab Lab; David Harrison
Subject:	Re: PNAS MS# 2019-15400R Decision Notification

Thanks Megan for your contributions.

Terrific to have your EM images of the virus in the paper!

cheers

(

Gilda

# Professor Gilda Tachedjian BSc (Hons) PhD

Head, Life Sciences Discipline Group Head, Retroviral Biology and Antivirals Lab NHMRC Senior Research Fellow

## **Burnet Institute**

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On 4 Mar 2020, at 9:18 am, Dearnley, Megan (AAHL, Geelong AAHL) wrote:

s22

## Well done everyone and pleasure to contribute!

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Document 47

	s22			
From:	s22 behalf of JGV			
Sent:	Monday, 30 March 2020 8:31 PM			
To:	Glenn A Marsh			
Subject:	:t: Decision on JGV D 20 00049 submitted to Journal of General Virology [EMID:c066d5d211ed05a7]			

You are being carbon copied ("cc:'d") on an e-mail "To" "Kate S Baker" s22								
CC: "Mary Tachedj	ian"	s22	Jennifer Barr"	s22	"Glenn A Marsh"			
s22		s22	"Gary C	rameri"	s22 Sandra			
Crameri"	s22	, "Ina Smith"	s22 "(	Clare EG Holmes"	s22			
"Richard Suu-Ire"	s22	, "Andres Fe	rnandez-Loras"	s22	"Andrew A			
Cunningham	s22	"James LN	Wood" s2	"Lin Fa V	Vang" s22			
s22			22					

# Manuscript number: JGV-D-20-00049

Title: Achimota pararubulavirus 3: a new bat-derived paramyxovirus of the genus Pararubulavirus Authors: Kate S Baker, BVSc PhD; Mary Tachedjian; Jennifer Barr; Glenn A Marsh; Shawn Todd; Gary Crameri; Sandra Crameri; Ina Smith; Clare EG Holmes; Richard Suu Ire; Andres Fernandez-Loras; Andrew A Cunningham; James LN Wood; Lin Fa Wang

Dear Dr Baker,

Thank you for submitting your manuscript to Journal of General Virology.

We have now received expert reviewer comments for your paper, and based on these comments and my assessment, I am sorry to inform you that your manuscript is not suitable for publication in *Journal of General Virology*.

# Editor comments:

JGV only considers manuscripts that are based on the identification of novel virus sequences if the manuscript provides novel insights into the biology of viruses. I agree with reviewer 1 that passaging attempts on two different cell lines do not justify the claim that virus growth has been studied on cell lines from different hosts and I thus do not see which insights into virus biology are being provided here. I thus haver to reject the manuscript.

The reviewers' comments can be found at the bottom of this email.

I hope you find this information helpful when submitting to another journal, and that you consider submitting to *Journal of General Virology* in the future.

Kind regards, **Prof Dr Silke Stertz** Editor, *Journal of General Virology* Microbiology Society | microbiologyresearch.org

Please let us know about your experience with our journals by completing this 2-minute survey: <u>www.surveymonkey.co.uk/r/7KFXMMT</u>

# **Reviewers' comments:**

Reviewer 1: The authors isolated a bat pararubulavirus on bat cells and characterized the genomic sequence. The new bat virus was genetically closely related to previously isolated bat viruses termed Achimota viruses 1 and 2.

The main problem of this short manuscript is its descriptive nature. The isolate is not used beyond passaging attempts on Vero cells which failed for unknown reasons. There is little to be learned from the genomic sequence per se. I suggest to enhance the biological significance of this study by passaging the isolate on several cell lines derived from bats and other animals. The repetitive reference to zoonotic pararubulaviruses is not necessary and likely overclaiming the true zoonotic potential of those viruses. Only Sosuga virus is known to have infected at least one individual and for Menangle virus there is indirect serologic evidence suggestive of human infection. This should be put in context throughout the manuscript.

Reviewer 2: This manuscript describes the detection and characterization of a so far unknown pararubulavirus in Straw colored fruit bats in Ghana. In general, the manuscript does lack clarity in some points in the Materials & Methods and Results sections, it should be carefully revised.

## Abstract

The sentence lines 38-41 seems incomplete, please revise.

#### Introduction

The first paragraph (II 49 59) is strongly focused on high impact zoonotic viruses such as coronaviruses, filoviruses and paramyxoviruses, but it needs to also be mentioned that bats can harbour a broad variety of other viruses of varying pathogenicity.

173: reference 11 is not the correct reference, it also is the same as reference 9, please correct.

## Materials & Methods, Results

Since this virus could only be propagated and identified after a subpassage, and even then could not be amplified from the original sample, the authors should critically discuss the possibility of a laboratory contamination. Also, the possibility of this virus originating from a different sample that U72 should be discussed.

#### Discussion

I 240-242: the close relation to other zoonotic viruses does not necessarily imply that this virus also has a zoonotic potential. This should be worded more carefully.

In compliance with data protection regulations, you may request that we remove your personal registration details at any time. (Remove my information/details). Please contact the publication office if you have any questions.

	s22
From:	s22 pnascentral.org
Sent:	Tuesday, 7 April 2020 3:42 AM
To:	gildat s22
Cc:	joshua.hayward <u>\$22</u> Mary.Tachedjian <u>\$22</u> KohlC <sup>\$22</sup> adam.johnson <u>\$22</u> megan.dearnle <u>\$22</u> brianna.jesaveluk <u>\$22</u> christine.lange <u>\$22</u> philip- daniel.solymosi <u>\$22</u> HilleG <u>\$22</u> NitscheA <u>\$22</u> cecilia.sanchez <u>\$22</u> awerner <u>\$22</u> dimitri.kontos <u>\$22</u>
	Gary.Crameri <u>\$22</u> glenn.marsh <u>\$22</u> ; michelle.baker <u>\$22</u> andy.poumbourios <u>\$22</u> ; hdrummer <u>\$22</u> edward.holme <u>\$22</u> linfa.wang <u>\$22</u> lna.Smith <u>\$22</u> gtachedjian <u>\$22</u>
Subject:	PNAS MS# 2019 15400B Publication Undate

2019 15400K Publication Up

Dear Prof. Tachedijan,

PNAS has scheduled publication of your article, "Infectious KoRV-related retroviruses circulating in Australian bats ," 2019 15400R, in Latest Articles the week of April 13, 2020. Your article may publish in Latest Articles any day during that week. The Latest Articles publication date is the official date of record.

PNAS will not publish your article until the production vendor, Sheridan Journal Services, has incorporated the changes you made on the proofs. If you have requested a second set of proofs, your article will not publish until you s22 have reviewed the edits. If you have questions about proofs, please contact (PNAS\_Specialist.djs

The press embargo on your article will lift on April 13, 2020 at 3:00 PM U.S. Eastern time. The embargo date is the earliest possible date that your article can publish. Embargoed copies of your accepted article will be available to journalists starting Wednesday, April 8, 2020, on a secure reporters only web site. Should you or your institution's public relations office have any press- or embargo-related questions, please contact the PNAS News Office at s22 or 202-334 1310. pnasnews

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If you must delay publication for a special reason, please notify the PNAS News Office immediately, no later than noon US ET on Tuesday, April 7, 2020.

PNAS automatically deposits the final, published version of all its content, regardless of funding, in PubMed Central (PMC) and makes it free at both PMC and PNAS within 6 months of publication. For release immediately on publication, the open access surcharge is \$1,300 for authors from institutions with a site license/open access membership. For more information, please see our editorial (https://www.pnas.org/cgi/content/full/102/15/5303). For information about the PNAS open access option, including fees and license details, please visit https://www.pnas.org/page/subscriptions/open access.

Best regards, **PNAS News Office**  s22

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Document 49

	s22	а. С
	From: Sent: To: Cc:	Wang Linfa s22 Tuesday, 8 September 2020 2:35 PM Barr, Jenn (H&B, Geelong ACDP); 'viruses@mdpi.com'; Kate Baker Tachedjian, Mary (H&B, Geelong ACDP); Marsh, Glenn (H&B, Geelong ACDP); Todd, Shawn (H&B, Geelong ACDP): Gary Crameri; Crameri, Sandra (AAH), Geelong
	Subject:	ACDP); Smith, Ina (H&B, Black Mountain); Holmes, Clare (AAHL, Geelong ACDP); Richard Suu-Ire; Andres Fernandez-Loras; Andrew Cunningham; James Wood RE: [Viruses] Manuscript ID: viruses-941748 - Submission Received
	Fingers crossed!	
	Linfa (Lin-Fa) WANG, PhD FTSE Professor Programme in Emerging Infection s22 Original Message From: Barr, Jenn (H&B, Geelong A Sent: Tuesday, 8 September 2020 To: 'viruses s22 Cc: Tachedjian, Mary (H&B, Geelong s22 Cc: Tachedjian, Mary (H&B, Geelong s22 Cc: Tachedjian, Mary (H&B, Geelong s22 Cc: Tachedjian, Mary (H&B, Geelong s22 Suu-Ire s22 Suu-Ire s22 Suu-Ire s22 Jam Subject: RE: [Viruses] Manuscript	ACDP) \$22 ACDP) \$22 0 6:59 AM Kate Baker < \$22 ong ACDP) \$22 Marsh, Glenn (H&B, Geelong ACDP) Shawn (H&B, Geelong ACDP) \$22 ; Gary Crameri ri, Sandra (AAHL, Geelong ACDP) \$22 ; Smith, Ina (H&B, ; Holmes, Clare (AAHL, Geelong ACDP) \$22 ; Smith, Ina (H&B, ; Holmes, Clare (AAHL, Geelong ACDP) \$22 Andrew Cunningham es Wood \$22 Wang Linfa \$22 i.D: viruses-941748 - Submission Received
	- External Email -	
_		
Ç	Good luck Kate!!!	
	Jenn	
	Original Message From: \$22 mdpi.com \$ Sent: Tuesday, 8 September 2020 To: Kate Baker \$22 Cc: Tachedjian, Mary (H&B, Geel \$22 Marsh Geelong ACDP) \$22 ACDP) \$22 Geelong ACDP) \$22 Geelong ACDP) \$22 Subject: [Viruses] Manuscript ID	22 On Behalf Of Editorial Office 0 12:19 AM ong ACDP) \$22 Barr, Jenn (H&B, Geelong ACDP) a, Glenn (H&B, Geelong ACDP) \$22 Todd, Shawn (H&B, Gary Crameri \$22 Crameri, Sandra (AAHL, Geelong ; Smith, Ina (H&B, Black Mountain) \$22 Holmes, Clare (AAHL, ; Richard Suu-Ire \$22 Andres Fernandez-Loras rew Cunningham \$22 James Wood \$22 Viruses-941748 - Submission Received

Dear Dr. Baker,

Thank you very much for uploading the following manuscript to the MDPI submission system. One of our editors will be in touch with you soon.

Journal name: Viruses

Manuscript ID: viruses-941748

Type of manuscript: Article

Title: Achimota pararubulavirus 3: a new bat-derived paramyxovirus of the genus Pararubulavirus Authors: Kate Baker \*, Mary Tachedjian, Jennifer Barr, Glenn Marsh, Shawn Todd, Gary Crameri, Sandra Crameri, Ina

Smith, Clare Holmes, Richard Suu-Ire, Andres Fernandez-Loras, Andrew Cunningham, James Wood, Lin-Fa Wang \* Received: 7 September 2020

E-mails: kbaker		s22	Mary.Tacheo	djian	lennifer.B	arr SZZ	Glenn.Marsh	522	
Shawn.Todd	s22	gary.Cran	neri s22	Sandra.Cram	eri s22	ha.Smith	s22		
clare.holmes	s22	suuire	s22	aferlasvet	s22	a.cunningham	s22	jlnw2 S2	22
linfa.wang	s22	Sub	omitted to see	ction: Animal V	iruses,			]	
https://www.mdpi.com/journal/viruses/sections/animal_viruses									

Viral Zoonoses and Global Public Health

https://www.mdpi.com/journal/viruses/special\_issues/viral\_zoonoses

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The following points were confirmed during submission:

1. Viruses is an open access journal with publishing fees of 2000 CHF for an accepted paper (see https://www.mdpi.com/about/apc/ for details). This manuscript, if accepted, will be published under an open access Creative Commons CC BY license (https://creativecommons.org/licenses/by/4.0/), and I agree to pay the Article Processing Charges as described on the journal webpage (https://www.mdpi.com/journal/viruses/apc). See https://www.mdpi.com/about/openaccess for more information about open access publishing.

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If you have any questions, please do not hesitate to contact the Viruses editorial office at viruses@mdpi.com

Kind regards,

Viruses Editorial Office



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S	22
From: Sent: To: Cc:	s22 behalf of Katarina Dragic Wednesday, 9 September 2020 12:01 AM Kate Baker Katarina Dragic; Mary Tachedjian; Jennifer Barr; Glenn Marsh; Shawn Todd; Gary Crameri; Sandra Crameri; Ina Smith; Clare Holmes; Richard Suu Ire; Andres Fernandez Loras; Andrew Cunningham; James Wood; Lin-Fa Wang; Viruses Editorial
Subject:	Office [Viruses] Manuscript ID: viruses 941748 Assistant Editor Assigned
Dear Dr. Baker,	
Your manuscript has been assi any questions related to your	gned to Katarina Dragic for further processing who will act as a point of contact for paper.

Journal: Viruses

Manuscript ID: viruses-941748

Title: Achimota pararubulavirus 3: a new bat derived paramyxovirus of the genus Pararubulavirus Authors: Kate Baker \*, Mary Tachedjian, Jennifer Barr, Glenn Marsh, Shawn Todd, Gary Crameri, Sandra Crameri, Ina Smith, Clare Holmes, Richard Suu-Ire, Andres Fernandez-Loras, Andrew Cunningham, James Wood, Lin Fa Wang \*

Received: 07 September 2020							
E-mails: kbaker	s22 Mary.	Tachedjian s22	Jennifer.Bar	Gle	nn.Marsh	s22	
Shawn.Todd S22	gary.Crameri	s22 Sandra.Cram	neri s22	na.Smit s	22		
clare.holme \$22	suuire s22	aferlasvet	s22 a.o	cunningham	s22 jlnw2	s22	
linfa.wang@duke-nus	s.edu.sg					- C	

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Best regards, Ms. Katarina Dragic Assistant Editor,

s22

1. C	Document
	s22
From:	s22 behalf of Katarina Dragic s22
Sent:	Monday, 21 September 2020 5:44 PM
То:	Kate Baker
Cc:	Mary Tachedjian; Jennifer Barr; Glenn Marsh; Shawn Todd; Gary Crameri; Sandra Crameri; Ina Smith; Clare Holmes; Richard Suu Ire; Andres Fernandez Loras; Andrew Cunningham: James Wood: Lin Fa Wang: Viruses Editorial Office
Subject:	[Viruses] Manuscript ID: viruses 941748 Major Revisions Until 20 October
Dear Dr. Baker,	
Thank you for submitting th	e following manuscript to Viruses:
Manuscript ID: viruses 9417 Type of manuscript: Article Title: Achimota pararubulav Authors: Kate Baker *, Mary Smith, Clare Holmes, Richan Received: 7 September 2020 E mails: kbaker \$22 Shawn.Todd \$22 gary clare.holmes \$22 Shawn.Todd \$22 gary clare.holmes \$22 https://www.mdpi.com/jou Viral Zoonoses and Global P https://www.mdpi.com/jou	48 rus 3: a new bat derived paramyxovirus of the genus Pararubulavirus Tachedjian, Jennifer Barr, Glenn Marsh, Shawn Todd, Gary Crameri, Sandra Crameri, Ina d Suu-Ire, Andres Fernandez-Loras, Andrew Cunningham, James Wood, Lin Fa Wang * Mary.Tachedjian \$22 Jennifer.Barr \$22, Glenn.Marsh \$22 Crameri \$22 Sandra.Cramer \$22 Ina.Smith \$22 r \$22 aferlasvet \$22 a.cunningham \$22 jInw2 \$22 Submitted to section: Animal Viruses, rnal/viruses/sections/animal_viruses ublic Health rnal/viruses/special_issues/viral_zoonoses
It has been reviewed by exp further. Please find your ma https://susy.mdpi.com/user	erts in the field and we request that you make major revisions before it is processed nuscript and the review reports at the following link: /manuscripts/resubmit/6b64fdb891ed4d524cd8555919739683

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Do not hesitate to contact us if you have any questions regarding the revision of your manuscript or if you need more time. We look forward to hearing from you soon.

Kind regards, Ms. Katarina Dragic Assistant Editor, \$22

Set:         Set:         Set:         Wednesday, 21 October 2020 231 AM         To:         Kate Baker         C:         May Tachedjian, Jennifer Barr, Glenn Marsh, Shawn Todd; Gary Crameri, Sandra Crameri; Ina Smith; Clare Holmes; Richard Suu Ire; Andres Fernandez Loras; Andrew Cunningham; James Wood; Lin Fa Wang         Subject:         Uriuses] Manuscript ID: viruses 941748         Type of manuscript: Article         Title: Achimot pararubulavirus 3: a new bat derived paramyxovirus of the genus Pararubulavirus         Authors: Kate Baker *, Mary Tachedjian, Jennifer Barr, Glenn Marsh, Shawn Todd, Gary Crameri, Sandra Crameri, Ina         Smith, Clare Holmes, Richard Suu-Ire, Andres Fernandez-Loras, Andrew Cunningham, James Wood, Lin-Fa Wang *         Received: 7 September 2020         E-mails: kbaker       \$922       gary. Chanded Suu-Ire, September 2020         E-mails: kbaker       \$922       aferlasvet       \$922       Jennifer Barr, September 2020         E-mails: kbaker       \$922       may, Tachedjia       \$222       Jennifer Barr, September 2020         E-mails: kbaker       \$922       sptomal/viruses/spcial_issues/viral_zonoses       \$22       Jennifer Barr, September 2020         E-mails: kbaker       \$922       jsg Submitted to section: Animal Vi		Document 52
Set:       Vednesday, 21 October 2020 2:31 AM         Sent:       Vednesday, 21 October 2020 2:31 AM         To:       Kate Baker         C:       May Tachedjian, Jennifer Barr, Glenn Marsh; Shawn Todd; Gary Cramer; Sandra Crameri; Ina Smith; Clare Holmes; Richard Suu Ire; Andres Fernandez Loras; Andrew Cunningham; James Wood; Lin Fa Wang         Subject:       [Viruses] Manuscript ID: viruses 941748         Dear Dr. Baker,       Thank you very much for resubmitting the modified version of the following manuscript:         Manuscript ID: viruses 941748       Type of manuscript: Article         Title: Achimota pararubulavirus 3: a new bat derived paramyxovirus of the genus Pararubulavirus         Authors: Kate Baker *, Mary Tachedjian, Jennifer Barr, Glenn Marsh, Shawn Todd, Gary Crameri, Sandra Crameri, Ina Smith, Clare Holmes, Richard Suu-Ire, Andres Fernandez-Loras, Andrew Cunningham, James Wood, Lin-Fa Wang *         Received: 7 September 2020       E-mails: kbaker       \$22         E-mails: kbaker       \$22       acrameri \$22       Jandra Crameri \$22         Shawn.Todd       \$22       guinted to section: Animal Viruses, https://www.mdpi.com/journal/viruses/sections/animal_viruses       \$22         Viral Zonoses and Global Public Health       https://susy.mdpi.com/journal/viruses/sections/animal_viruses       Yiral Zonoses and Global Public Health         Viruses Editorial Office       Yere in touch with you soon regarding progress of the manuscript.       \$22		s22
To: Kate Baker Mary Tachedjian; Jennifer Barr; Glenn Marsh; Shawn Todd; Gary Cramer; Sandra Crameri, Ina Smith; Clare Holmes; Richard Suu Ire; Andres Fernandez Loras; Andrew Cunningham; James Wood; Lin Fa Wang Subject: (Viruses) Manuscript ID: viruses 941748 Manuscript Resubmitted Dear Dr. Baker, (Viruses) Manuscript ID: viruses 941748 Manuscript Resubmitted Type of manuscript: Article Title: Achimota pararubulavirus 3: a new bat derived paramyxovirus of the genus Pararubulavirus Authors; Kate Baker * Mary Tachedjian, Jennifer Barr, Glenn Marsh, Shawn Todd, Gary Crameri, Sandra Crameri, Ina Smith, Clare Holmes, Richard Suu-Ire, Andres Fernandez-Loras, Andrew Cunningham, James Wood, Lin-Fa Wang * Received: 7 September 2020 Mary Tachedjian set 2 Sandra.Crameri Sole Janningham Sole Sole Sole Sole Sole Sole Sole Sole	From: Sent:	s22 behalf of Submission System s22 Wednesday, 21 October 2020 2:31 AM
C:       Mary Tachedjian; Jennifer Barr, Glenn Marsh; Shawn Todd; Gary Crameri; Sandra Crameri; Ina Smith; Clare Hollmes; Richard Suu Ire; Andres Fernandez Lora; Andrew Cunningham; James Wood; Lin Fa Wang         Subject:       [Viruses] Manuscript ID: viruses 941748         Dear Dr. Baker,       Thank you very much for resubmitting the modified version of the following manuscript:         Manuscript ID: viruses 941748       Type of manuscript: Article         Title: Achimota pararubulavirus 3: a new bat derived paramyxovirus of the genus Pararubulavirus Authors; Kate Baker *, Mary Tachedjian, Jennifer Barr, Glenn Marsh, Shawn Todd, Gary Crameri, Sandra Crameri, Ina Smith, Clare Holmes, Richard Suu-Ire, Andres Fernandez-Loras, Andrew Cunningham, James Wood, Lin-Fa Wang *         Received: 7 September 2020       Mary.Tachedjia       \$22       Ina.Smith, S22       Ina.Smith, \$22         Infa.wang       \$22       gary.Crameri       \$22       Sandra.Crameri       \$22         Infa.wang       \$22       gary.Crameri       \$22       Ina.Smith, \$22       Inw.2       \$22         Infa.wang       \$22       gary.Crameri       \$22       Inw.2 <th>To:</th> <td>Kate Baker</td>	To:	Kate Baker
Crameri; Ina Smith; Clare Holmes; Richard Suu Ire; Andres Fernandez Loras; Andrew Cunningham; James Wood; Lin Fa Wang Subject: [Viruses] Manuscript ID: viruses 941748 Manuscript Resubmitted Dear Dr. Baker, Thank you very much for resubmitting the modified version of the following manuscript ID: viruses 941748 Type of manuscript: Article Title: Achimota pararubulavirus 3: a new bat derived paramyxovirus of the genus Pararubulavirus Authors: Kate Baker *, Mary Tachedjian, Jennifer Barr, Glenn Marsh, Shawn Todd, Gary Crameri, Sandra Crameri, Ina Smith, Clare Holmes, Richard Suu-Ire, Andres Fernandez-Loras, Andrew Cunningham, James Wood, Lin-Fa Wang * Received: 7 September 2020 E-mails: kbaker \$22 gary. Crameri \$22 andra. Crameri \$22 lennifer.Barr \$22 Silenn.Marsh \$22 linfa.wang \$22 gary. Crameri \$22 andra. Crameri \$22 and cunningham \$22 jinw2 \$22 Linfa.wang \$22 gary. Crameri \$22 andra. Crameri \$22 and cunningham \$22 jinw2 \$22 Linfa.wang \$22 gary. Crameri \$22 andra. Crameri \$22 and cunningham \$22 jinw2 \$22 Linfa.wang \$22 gary. Crameri \$22 sandra. Crameri \$22 and cunningham \$22 jinw2 \$22 Linfa.wang \$22 gary. Crameri \$22 sandra. Crameri \$22 and cunningham \$22 jinw2 \$22 Linfa.wang \$22 gary. Crameri \$22 sandra. Crameri \$22 and cunningham \$22 jinw2 \$22 Linfa.wang \$22 gary. Crameri \$22 sandra. Crameri \$22 Linfa.wang \$22 gary. Crameri \$22 Lin	Cc:	Mary Tachedjian; Jennifer Barr; Glenn Marsh; Shawn Todd; Gary Crameri; Sandra
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Thank you very much for resubmitting the modified version of the following manuscript: Manuscript ID: viruses 941748 Type of manuscript: Article Title: Achimota pararubulavirus 3: a new bat derived paramyxovirus of the genus Pararubulavirus Authors: Kate Baker *, Mary Tachedjian, Jennifer Barr, Glenn Marsh, Shawn Todd, Gary Crameri, Sandra Crameri, Ina Smith, Clare Holmes, Richard Suu-Ire, Andres Fernandez-Loras, Andrew Cunningham, James Wood, Lin-Fa Wang * Received: 7 September 2020 E-mails: kbaker \$22 Mary.Tachedjia \$22 lennifer.Barr \$22 Glenn.Marsh \$22 clare.holmes \$22 suire \$22 a faclasvet \$22 a.c.unningham \$22 jinw2 \$22 linfa.wang \$22 isg Submitted to section: Animal Viruses, https://www.mdpi.com/journal/viruse/sections/animal_viruses Viral Zoonoses and Global Public Health https://swy.mdpi.com/journal/viruse/secial_issues/viral_zoonoses https://susy.mdpi.com/journal/viruse/secial_issues/viral_zoonoses https://susy.mdpi.com/journal/viruse/secial_issues/viral_zoonoses https://susy.mdpi.com/journal/viruse/secial_issues/viral_zoonoses https://susy.mdpi.com/journal/viruse/secial_issues/viral_zoonoses https://susy.mdpi.com/journal/viruse/secial_issues/viral_zoonoses https://susy.mdpi.com/journal/viruse/secial_issues/viral_zoonoses https://susy.mdpi.com/journal/viruse/secial_issues/viral_zoonoses https://susy.mdpi.com/journal/viruse/secial_issues/viral_zoonoses https://susy.mdpi.com/journal/viruse/secial_issues/viral_zoonoses https://www.mdpi.com/journal/viruses/ Factor integrates MDPI 	Dear Dr. Baker,	
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clare.holmes S22 suuire S22 aferlasvet S22 a.cunningham S22 jlnw2 S22 linfa.wang S22 sg Submitted to section: Animal Viruses, https://www.mdpi.com/journal/viruses/sections/animal_viruses Viral Zoonoses and Global Public Health https://www.mdpi.com/journal/viruses/special_issues/viral_zoonoses https://susy.mdpi.com/user/manuscripts/review_info/6b64fdb891ed4d524cd8555919739683 A member of the editorial office will be in touch with you soon regarding progress of the manuscript. Kind regards, MDPI  Viruses Editorial Office Postfach, CH-4020 Basel, Switzerland S22 https://www.mdpi.com/journal/viruses/	Title: Achimota pararu Authors: Kate Baker *, Smith, Clare Holmes, R Received: 7 Septembe E-mails: kbaker Shawn.Todd \$22	bulavirus 3: a new bat derived paramyxovirus of the genus Pararubulavirus Mary Tachedjian, Jennifer Barr, Glenn Marsh, Shawn Todd, Gary Crameri, Sandra Crameri, Ina ichard Suu-Ire, Andres Fernandez-Loras, Andrew Cunningham, James Wood, Lin-Fa Wang * r 2020 s22 Mary.Tachedjia s22 Jennifer.Barr s22 Glenn.Marsh s22 gary.Crameri s22 Sandra.Crameri s22 Ina.Smith s22
Viral Zoonoses and Global Public Health https://www.mdpi.com/journal/viruses/special_issues/viral_zoonoses https://susy.mdpi.com/user/manuscripts/review_info/6b64fdb891ed4d524cd8555919739683 A member of the editorial office will be in touch with you soon regarding progress of the manuscript. Kind regards, MDPI  Viruses Editorial Office Postfach, CH-4020 Basel, Switzerland \$22 https://www.mdpi.com/journal/viruses/	clare.holmes \$22 linfa.wang \$22 https://www.mdpi.com	suuire s22 aferlasvet s22 a.cunningham s22 jlnw2 s22 sg Submitted to section: Animal Viruses, m/journal/viruses/sections/animal_viruses
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From: Sent: To: Cc:	s22 Wednesday, 21 October 2020 5:38 PM Kate Baker Mary Tachedjian; Jennifer Barr, Glenn Marsh; Shawn Todd; Gary Crameri; Sandra Crameri; Ina Smith; Clare Holmes; Richard Suu-Ire; Andres Fernandez Loras; Andrew Cunningham; James Wood; Lin-Fa Wang; Viruses Editorial Office
Subject:	[Viruses] Manuscript ID: viruses-941748 - Revised Version Received
Dear Dr. Baker,	
Thank you very much for providi	ng the revised version of your paper:
Manuscript ID: viruses-941748 Type of manuscript: Article Title: Achimota pararubulavirus 3 Authors: Kate Baker *, Mary Tack Smith, Clare Holmes, Richard Sur Received: 7 September 2020 E-mails: kbaker <u>\$22</u> Shawn.Todd <u>\$22</u> gary.Crar clare.holmes <u>\$22</u> Shawn.Todd <u>\$22</u> gary.Crar clare.holmes <u>\$22</u> Sulfinfa.wang <u>\$22</u> Sulfittips://www.mdpi.com/journal/ Viral Zoonoses and Global Public https://www.mdpi.com/journal/ https://susy.mdpi.com/user/mai	8: a new bat-derived paramyxovirus of the genus Pararubulavirus hedjian, Jennifer Barr, Glenn Marsh, Shawn Todd, Gary Crameri, Sandra Crameri, Ina h-Ire, Andres Fernandez-Loras, Andrew Cunningham, James Wood, Lin-Fa Wang * Mary.Tachedjian \$22 Jennifer.Bar \$22 Glenn.Mars \$22 meri \$22 Sandra.Crameri \$22 Ina.Smith \$22 \$22 aferlasvet \$22 a.cunningham \$22 inw2 \$22 mitted to section: Animal Viruses, viruses/sections/animal_viruses Health viruses/special_issues/viral_zoonoses huscripts/review_info/6b64fdb891ed4d524cd8555919739683 r paper and will keep you informed about the submission status.

Kind regards,

Ms. Katarina Dragic Assistant Editor,

s22

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From:	James Wood
Sent:	Monday, 26 October 2020 7:02 PM
To:	'Katarina Dragić'; Kate Baker
Cc:	Mary Tachedjian; Jennifer Barr; Glenn Marsh; Shawn Todd; Gary Crameri; Sandra
	Crameri; Ina Smith; Clare Holmes; Richard Suu-Ire; Andres Fernandez Loras; Andrew
	Cunningham; Lin Fa Wang; Viruses Editorial Office
Subject:	RE: [Viruses] Manuscript ID: viruses-941748 - Minor Revisions Within 2 days
	Update

# Dear Ms Dragic

I am sure that none of us can speak for Dr Baker at this time, but I do know that she has been exceptionally busy this year assisting with the national response to COVID 19. I imagine that she may be spending some time with her family this week. I find it shocking that you are suggesting that the response cannot wait for the 3 extra working days that her automatic reply suggests she needs?

Please can you send me the contact details for the journal editor in chief as I would like to raise this with him or her Thank you

Regards

James Wood

\*\*\*\*\*\*

James Wood

Head of Department of Veterinary Medicine and Alborada Professor of Equine and Farm Animal Science University of Cambridge Madingley Road Cambridge

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Honorary Research Fellow, Institute of Zoology

Original Message From: Katarina Dragić	s22				
Sent: 26 October 2020	07:23				
To: Kate Baker	s22				
Cc: Mary Tachedjian	s22	Jennifer Barr	s22	; Glenn Marsh	
s22	Shawn Todd	s22 G	iary Crameri	s22	Sandra
Crameri	; Ina Smith	s22	Clare Holmes	s22	
Richard Suu Ire	s22 Andres	Fernandez-Loras	s22	; Andrew Cur	nningham
s22	James Wood	s22 Lin	Fa Wang	s22	Viruses
Richard Suu Ire s22	James Wood	s22 Loras	Fa Wang	; Andrew Cur s22	Viruses

# Editorial Office <viruses@mdpi.com>

Subject: [Viruses] Manuscript ID: viruses 941748 Minor Revisions Within 2 days Update

Dear Authors,

May this email finds you well.

Since we have received automatic reply from Corresponding Author Dr.

Kate Baker that she is on annual leave until the 1st November, can you please inform us will you be able to finish these Minor Revision in the next two days. Please let us know as soon as possible.

Looking forward to hearing from you.

Kind regards,

Ms. Katarina Dragic Assistant Editor,

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On 2020-10-26 05:04, Allison Yang wrote:

> Dear Dr. Baker,

>

> Thank you for submitting your manuscript:

>

> Manuscript ID: viruses-941748

> Type of manuscript: Article

> Title: Achimota pararubulavirus 3: a new bat-derived paramyxovirus of

> the genus Pararubulavirus

> Authors: Kate Baker \*, Mary Tachedjian, Jennifer Barr, Glenn Marsh,

> Shawn Todd, Gary Crameri, Sandra Crameri, Ina Smith, Clare Holmes,

> Richard Suu-Ire, Andres Fernandez-Loras, Andrew Cunningham, James

s22

> Wood, Lin-Fa Wang \*

> Received: 7 September 2020

> https://www.mdpi.com/journal/viruses/sections/animal viruses

> Viral Zoonoses and Global Public Health

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- > Assistant Editor

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	Document 57
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From: Sent: To: Cc:	allison.yan s22 Tuesday, 27 October 2020 3:29 PM Kate Baker Mary Tachedijan: Jennifer Barr: Glenn Marsh: Shawn Todd: Gary Crameri: Sandra
Subject:	Crameri; Ina Smith; Clare Holmes; Richard Suu Ire; Andres Fernandez-Loras; Andrew Cunningham; James Wood; Lin Fa Wang; Viruses Editorial Office; Katarina Dragic [Viruses] Manuscript ID: viruses 941748 - Accepted for Publication
Dear Dr. Baker,	
We are pleased to info	rm you that the following paper has been officially accepted for publication:
Type of manuscript: Ar Title: Achimota pararul Authors: Kate Baker *, Smith, Clare Holmes, R Received: 7 September	ticle bulavirus 3: a new bat-derived paramyxovirus of the genus Pararubulavirus Mary Tachedjian, Jennifer Barr, Glenn Marsh, Shawn Todd, Gary Crameri, Sandra Crameri, Ina ichard Suu-Ire, Andres Fernandez-Loras, Andrew Cunningham, James Wood, Lin-Fa Wang * 2020 \$22
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Kind regards, Prof. Dr. Olli Vapalahti Assoc. Prof. Dr. Tarja Sironen

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	Document 58
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From: Sent: To: Cc:	s22 Wednesday, 28 October 2020 7:56 PM Kate Baker Mary Tachedjian; Jennifer Barr; Glenn Marsh; Shawn Todd; Gary Crameri; Sandra
Subject:	Crameri; Ina Smith; Clare Holmes; Richard Suu-Ire; Andres Fernandez Loras; Andrew Cunningham; James Wood; Lin Fa Wang; Viruses Editorial Office [Viruses] Manuscript ID: viruses 941748 Final Proofreading Before Publication
Dear Dr. Baker,	
We invite you to proofread your that you will require no further o	manuscript to ensure that this is the final version that can be published and confirm hanges from hereon:
Manuscript ID: viruses-941748 Type of manuscript: Article Title: Achimota pararubulavirus Authors: Kate Baker *, Mary Tac Smith, Clare Holmes, Richard Sur Received: 7 September 2020	3: a new bat-derived paramyxovirus of the genus Pararubulavirus hedjian, Jennifer Barr, Glenn Marsh, Shawn Todd, Gary Crameri, Sandra Crameri, Ina J-Ire, Andres Fernandez-Loras, Andrew Cunningham, James Wood, Lin-Fa Wang *
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Supplementary and other additional files can be found at the second link. We look forward to hearing from you soon.

Kind regards,

Ms. Katarina Dragic Assistant Editor,

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From: Sent: To: Cc:	s22 Thursday, 29 C Kate Baker Mary Tachedjia Crameri: Ina Sr	on behalf of Sub October 2020 5:03 A an; Jennifer Barr; Gl mith: Clare Holmes:	enn Marsh; Shawn Te Richard Suu-Ire: App	s22 odd; Gary Crameri; Sand	ra
Subject:	Cunningham; J [Viruses] Manu	lames Wood; Lin-Fa uscript ID: viruses-9	a Wang 41748 - Manuscript I	Resubmitted	
Dear Dr. Baker,					
Thank you very much fo manuscript:	or resubmitting the modif	fied version of the	following		
Manuscript ID: viruses- Type of manuscript: Art Title: Achimota pararub Authors: Kate Baker *, I Smith, Clare Holmes, Ri Received: 7 September	941748 ticle pulavirus 3: a new bat-der Mary Tachedjian, Jennifer chard Suu-Ire, Andres Fer 2020	rived paramyxoviru r Barr, Glenn Marsl rnandez-Loras, And	s of the genus Parar 1, Shawn Todd, Gary Irew Cunningham, Ja	ubulavirus Crameri, Sandra Crame ames Wood, Lin-Fa War	eri, Ina Ig *
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Document 60

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From: Sent: To:		s22 on behalf of viruse s22 Friday, 30 October 2020 11:27 PM khaker s22 Many Tachedijan s22 Jennifer Barr s22
10.	8	Glenn.Marsh     s22     Shawn.Todd     s22     gary.Crameri     s22       Sandra.Crameri     s22     Ina.Smith     clare.holmes       suuire     s22     aferlasvet     s22
Cc: Subject:		billing <u>\$22</u> ; website <u>\$22</u> ; viruses <u>\$22</u> [Viruses] Manuscript ID: viruses 941748; doi: 10.3390/v12111236. Paper has been published.

Dear Authors,

We are pleased to inform you that your article "Achimota Pararubulavirus 3: A New Bat-Derived Paramyxovirus of the Genus Pararubulavirus" has been published in Viruses as part of the Special Issue Viral Zoonoses and Global Public Health and is available online:

Abstract: https://www.mdpi.com/1999-4915/12/11/1236

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Kind regards,

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Cc:	allison Yang
Subject:	Re: [Viruses] Manuscript ID: viruses-941748; doi: 10.3390/v12111236. Paper has been published.

Dear Dr. Wang,

C

Congratulations to you on the following publication in special issue Viral Zoonoses and Global Public Health

We hope that your experience publishes in Viruses is positive and wish that you would support this journal continuously with further submissions. As recurring authors to this issue, you will enjoy 20% discount on the publication fees if you would be interested to submit a second paper before the January deadline. You are also welcome to pass on the message (with discounts reserved) to colleagues who might be interested in making a contribution.

Thank you. We look forward to your further collaboration.

Kind regards, Allison

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Document 62

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From:	s22 on behalf of viruses s22
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Cc:	billing s22 website s22 viruses s22 dragic s22
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