The study by Rousselle et al. investigates the relationship between the rate of adaptation (omega_a) and the effective population size across different time-scales. Fur this purpose, the authors collate newly generated and publicly available protein coding sequence re-sequencing data across 50 species belonging to ten divergent groups of animals. Based on this data set, the authors then estimate the rate of adaptation in these 50 species. Subsequent analysis of the relationship between the rate of adaptation and different proxies of the effective population size suggests a positive relationship at short time-scales, and no or a negative relationship at large time-scales.

The authors address a relevant question based on an impressive data set. Their findings are interesting, and are discussed from different angles. I have only a few major concerns with respect to data analysis, presentation and interpretation of results. In addition, I think the writing of the Introduction and Discussion as well as the order of the Results should be improved to better guide the reader.

Major remarks:

1) My biggest concern with respect to the presentation and interpretation of results is the inconsistency between Figures 1 and 2. Figure 1 reports a negative relationship between omega_a and pi_S at large time-scales, and Figure 2 reports positive relationships between omega_a and pi_S at short time-scales. However, while Figure 1 is based on all mutations, Figure 2 is based on GC-conservative mutations only. Looking up the respective of Figure 1 A and B for GC-conservative changes in the Supplementary Material, I once find a slightly positive and once a negative relationship between omega_a and pi_S at large time-scales, Figure S2 and S3, respectively. This leaves a somewhat dubious impression. Given that the main conclusion of the study is based on the contrast between relationships shown in Figures 1 and 2, I suggest the authors to consistently report results based on GC-conservative changes in the main text, and report results based on all mutations in the Supplementary Material. Moreover, significance levels of relationships should be reported throughout the main text and the Supplementary Material. It seems the authors intended to report significance levels by a star, but stars are absent throughout Figures S2 and S3. If this means all relationships are not significant, this should in addition be spelled out. If stars instead have been forgotten to add, they should be added. At present the statement starting on page 9, line 179, “Here again, the correlations, even if not significant, were in line with …” seems not well supported.
2) The authors seem to control for phylogenetic inertia in some of their analyses but in others not. It is not entirely clear to me why the authors choose to do so. I suggest the authors to consistently control for phylogenetic inertia.
3) In the discussion of their results, the authors state on page 17, line 355, “We do not see any particular reason why the gene sample would be biased with respect to virus interacting proteins in some specific groups, …” I suggest the authors to back up this statement by actually examining if the gene sample is biased with respect to virus interacting proteins in some specific groups.
4) In order to better guide the reader through the results, I suggest the authors to re-order results sections, and present section 5 directly after section 2. Sections 2 and 5 both

address the relationship between omega_a and the effective population size at large time-scales, and are both suggestive of a negative relationship. I think it would be nice to first address the relationship between omega_a and the effective population size at large time-scales from all different angles, and afterwards resolve the puzzle by the ANCOVA currently presented in section 3. Thus, my suggested order is 1, 2, 5, 3, 4.

5) In the opening of the Introduction, the authors explain that different theoretical models can predict either a positive or a negative relationship between omega_a and the effective population size. This is a very nice opening of the Introduction. However, I think it is important that underlying assumptions of different models are stated more explicitly. Specifically, instead of stating "one would intuitively expect" (page 2, line 33), the authors should clearly state, "under the assumption of a constant DFE one would expect". The assumption of a constant DFE is crucial to the positive relationship between omega_a and the effective population size, and is in clear contrast to other models discussed in the same paragraph (page 3, line 45). This is only one example. More generally, differences in the underlying assumptions of the different models should be stated more clearly. In addition, the authors mention that if $s >> 1/ N_e$, then mutations should accumulate roughly at rate $4N_e mu_a s$. It would be more accurate to say, if s is small and $N_e s >> 1$, then mutations should accumulate roughly at rate $4N_e mu_a s$. Besides, I also suggest shortening sentences throughout the Introduction. Some of the sentences span up to six lines, and could easily be split into two or three separate sentences in order to improve readability.

6) In the opening of the Discussion (section 1), the authors seem to emphasize that data have been generated as part of the present study. I don't think it is necessary to "sell" the study by emphasizing data generation. In my opinion, the value of the study rather lies in their interesting observations. I therefore suggest the authors to reduce the emphasis on data generation, but instead directly start by a summary of their main findings. Moreover, I think it is important to also in the opening of the Discussion clearly state that a fixed DFE across divergent taxa would be necessary in order to expect the same relationship across taxa. Section 2 of the Discussion seems rather technical and lengthy. Most of its content is actually already mentioned in the Results section. I suggest the authors to radically shorten this section. I think it would be more valuable to instead focus the Discussion on sections 3 and 4, and also strengthen the respective sections.

Minor remarks:

7) Page 3, line 63, the authors mention near-neutrality together with the original MK test. Note that the original MK test is based on the Neutral theory of molecular evolution not on the Nearly neutral theory of molecular evolution. This should be corrected.

8) Page 5, line 97, the sentence "Of note, the species sampled in this study …" comes a bit out of the blue, and might better be introduced in context of the next paragraph starting line 100. Besides, the wording "in this study" gives the impression the authors refer to the present study and should be replaced with "Galtier (18)".

9) Page 5, line 110, "In this study, we propose to test the effect of evolutionary scale on …" should be replaced with "In this study, we test the effect of evolutionary time-scale on …".

10) Could the author please comment on why the percentage of recovered among targeted transcripts was noticeably low in two of the earth worms?

11) Page 8, line 147, “ands called the diploid genotypes of individuals ar every coding position.” should be replaced with “and called the diploid genotypes of individuals for every coding position.”.

12) Page 8, line 148, “summed up” should be replaced with “summarized”.

13) Table S3, the same number of decimal digits should be reported throughout the table. Besides, a precision of 6 decimal digits seems not necessary.

14) The caption of Table S3 provides an explanation why #SNPs are not integers. Reading this explanation several times, I am still not able to understand it. I suggest to replace with a simpler explanation.

15) Page 12, line 223, “We were concerned that the correlation …” should be replaced with “We were concerned that the positive correlation …”.

16) Figure 3, the same legend is presented in each of the panels. However, not all of the species groups are represented in each of the panels. The legends should be updated accordingly.

17) Formatting of p-values should be consistent throughout the manuscript. I suggest consistent formatting as scientific numbers.