Thank you for handling our manuscript and for the opportunity to submit a revised version for consideration for publication at Genome Biology and Evolution. We appreciate the reviewer's time and insightful comments and overall positive views of the manuscript. In this thoroughly revised manuscript, we now more directly emphasize the overall contributions of our study, while also working to improve the clarity and integration of the different components of our research. We have also worked to make the presentation more concise and have made a number of formatting changes to comply with the recommendations of GBE. Below we provide a point-by-point response to each of the reviewer comments. To facilitate your review, we have reproduced all reviewer comments below and our responses are indicated by ">>>*Response*". Page and line numbers refer to the lines in the tracked changes version of the manuscript, with specific text from the manuscript reproduced in "quotes" when appropriate. We have also uploaded a clean manuscript for review.

Thank you for your time and consideration,
Gregg Thomas, Jonathan Hughes, and Jeff Good
(on behalf of the authors)

Reviewer: 1

Comments to the Author
In this manuscript, the authors construct a species level phylogeny of murine rodents with more markers/taxa/robust methods than in the past and then investigate possible drivers of and impacts of discordance. Overall, the manuscript and the work are careful, clear, and rigorous and engage with the literature in this research area. I enjoyed reading it. I had two main concerns. The first is around framing. While the manuscript does a good job of referencing and discussing past work, it was less clear what specific contributions this work makes to the field beyond those works. The improved phylogeny is definitely a step forward and has relevance for anyone that works with mice/rats, but it is not necessarily of broad interest to the field. The investigation of discordance is positioned as the question of broader significance in the manuscript, and I think that argument could be strengthened. Second, I did not find the analyses w/r to recombination particularly compelling. I have a handful of minor comments.

>>> Thank you for your thoughtful feedback, which we feel has helped us sharpen the overall presentation, and better motivate and integrate the two different components of the study (the generation of a broader species tree and the analysis of discordance), and more clearly highlight the advances and limitations of our work with an emphasis on elements of the recombination results.

1. How does this work push the field forward?
While the significance of results regarding discordance is discussed in different parts of the paper, there is an opportunity to be more specific about how this work adds to the literature. To what extent are the drivers of and the impacts of discordance unknown (what specific questions need to be addressed vs. past work, empirical or simulations, etc.)? Why this system to address these questions? What do we learn that is new from this example? Examples:

a.There is a discussion about the lack of consensus on the relationship between discordance and recombination (91-96). Does one more study change where we are or address the lack of agreement among past studies or technical challenges in some way? Is there just a need for more empirical studies (yes)?

>>> We thank the reviewer and acknowledge that the justifications for our study could have been more directly stated throughout the manuscript. The relationships between recombination and discordance are likely to be complex and, perhaps system dependent to some extent, so there is indeed a need for more empirical research. However, we propose that very few studies have systematically explored these issues as we have using an empirical system with extensive genome resources. While we do not want to overstate our contributions beyond what is warranted, we also suggest that this more than is just another empirical study in a well-established subject area. Rather, our work is one of the few to explore these issues as the primary biological motivations of the study. A lack of a clear understanding of the evolution of recombination, and the consequences for this divergence for a broad array of evolutionary genetic questions remains a fundamental issue in our field.

To better make these points we have added clarifying statements throughout:

- *Lines 117-119 (Introduction). Pointing out the need for more studies of discordance in systems with sufficient resources. "Thus, it remains unclear how phylogenetic discordance scales locally across the genome as a function of recombination and the strength of linked selection, pointing to the need for empirical studies in systems with sufficient genomic resources to explore the causes of discordance."*
- *Lines 147-150 (Introduction). Summary statement on the advances of our work. "Collectively, our results advance understanding of how core features of genome biology influence underlying phylogenetic patterns, the extent to which established model system resources can be leveraged for broader phylogenetic studies, and the consequences of ignoring phylogenetic uncertainty"*
- *Lines 340-419 (Discussion). We have extensively rewritten the discussion of the relationship between recombination and patterns of ILS at different phylogenetic scales, which we also discuss in more detail below.*

b.One result from this work is that discordance is observed within genes with implications for molecular evolution analyses. This result is consistent with previous work that is cited, but how does this add to our understanding?

>>> Thanks for this comment. Actually, relatively few studies have explored this from a phylogenetic perspective. Indeed, much of the previous emphasis on the potential for this phenomenon pointed out that this potentially leads to modest effects within, for example, Drosophila (see Mendez and Hahn, 2016). So, while our work is consistent with previous empirical and simulation-based studies, this is among the first explorations of this issue in mammals, which is likely to be widespread across taxa. The central advance here is that we hope that through our quantification, we are bringing the issue to the forefront of both novice and experienced comparative genomicists' minds, so they are better able to handle it in their own analyses (e.g. by using gene trees instead of species trees). There is not at this time an elegant solution to the issue of recombination within genes, but a better appreciation of these issues will hopefully encourage caution when interpreting the results of gene-specific analyses of molecular evolution. In our revised manuscript, we provide a discussion of the central issues of ILS with respect to studies of molecular evolution and provide recommendations for avoiding these pitfalls when possible (Lines 457-470).

c.The discussion in 517-521 made me wonder how much including just one taxon from the group with least support rather than all three would have influenced the outcome of this work—

which again pushed me to think more about what are we learning from this particular empirical example and what questions does it suggest for the future?

>>> Yes, the sampling of the three Praomyini species drove much of the discordance in our study, but not all. To emphasize this, we have added text to the Discussion (Lines 356-358):

"These patterns illustrate how extensive discordance can arise even in a small sample of species and underscores that a single inferred species tree often may not capture the history of individual regions of the genome".

We understand the concerns of the review that a few species (three of the seven taxa in our sample) drive a majority of ILS in this study. However, we note that discordance often varies across nodes in the tree and that taxon sampling always plays a role. One practical outcome of this is that subsampling taxa to reduce discordance may be a feasible means of reducing the effects of discordance in studies of molecular evolution. We have added this suggestion to the Discussion (line 457-459):

"Our results also imply that studies of molecular evolution may benefit from approaches that reduce genome-wide levels of discordance, such as through post hoc pruning of species that disproportionately contribute to unresolved nodes."

d. Many readers won't be familiar with the cited work in 589-593. How does this expand on that? Phylogenetic discordance is documented and there have been cautionary papers in the past. The manuscript calls out the investigation of 1) the relationship between discordance and genome biology and 2) the extent of impacts of discordance on comparative studies as the major insights. I was left unsure of how much the work added to what was known in these two areas vs. more of a "call to arms" to push folks to adopt the recommended practices.

>>> We believe that our work will ideally contribute to both a specific understanding of the causes and consequences of discordance, while also bringing broader awareness of the issue. Much of the previous work has focused on verbal models supported by simulations, whereas we demonstrate these effects using a large novel empirical dataset, as clarified now in the Discussion (Lines 423-428):

"Previous studies have used simulations to show that tree misspecification can lead to incorrect placement of substitutions on branches, possibly leading to spurious results for tests of positive directional selection within empirical datasets (Hahn and Nakhleh 2016; Mendes and Hahn 2016). Here, we use empirical data in mice to show…."

We agree that discordance's effects on inferences of molecular evolution may still be unfamiliar to a wide range of researchers that, with advancing sequencing technology, may be delving into comparative genomics for their particular system of interest. This can be seen in the small sampling of papers we cited in following paragraphs that use a single species tree for such analyses. By explicitly showing how the choice of gene vs. species tree affects positive selection results, we hope our paper would be more than a cautionary paper and lead to a wider adoption of use of gene trees when appropriate. We have made our recommendation more explicit on lines 457-470.

2. 217-219, 530+ and throughout. I don't understand how estimates of recombination in one species (house mice) could be used to assess the relationship between recombination rate and phylogenetic discordance in seven taxa over 12MY of divergence.

>>> We have extensively clarified and rewritten the Results and Discussion on recombination and patterns of discordance, which hopefully clarify some of these issues. There are two major points to clarify. First, we note that while we emphasized the surprising result that there was not a strong association between discordance with recombination rates from mice, this might have been overstated. What we did not detect was an overall elevation of local discordance (differences in history between neighboring windows), but we did find a weak relationship between recombination rates and discordance with the overall species tree. These results do demonstrate some predictive power of using recombination rates from one species to inform discordance.

Second, although the reviewer's caution is likely warranted, several studies have used exactly this approach to yield interesting results. For example, Foley et al. (2023, Cell Genomics) used population-based estimates of recombination from 1 bat species to analyze patterns of discordance across 60 species of bats spanning approximately 10-12 million years of divergence (so similar evolutionary depth as our study, but with a lot more taxa and discordance, and likely less accurate estimates of recombination). They found an incredible pattern where discordance was extensive in regions of high recombination, but the species tree was only accurately recovered within areas of low recombination (a pattern they attribute to extensive introgression in that system). With this strong precedent we believe the practice of using recombination maps from one species to inform a phylogenetic study will likely increase in frequency. We have clarified this in the Discussion (see Lines 343-361). In particular, we note (on Line 374-377):

"Overall, the evolution recombination landscapes across closely related species remains an important empirical question in evolutionary genetics (Dapper and Payseur, 2017), especially as the generation of chromosome-scale genome assemblies continues to greatly outpace estimates of patterns of recombination within those genomes."

After reading the methods, I also wasn't that sure how this analysis was done or how it was informed by the co-linearity analysis.

>>> Our original idea was to use the high-quality maps from both mouse and rat to possibly infer some sort of ancestral recombination rate throughout the genome, however we found that, because the mouse and rat genomes lack long alignment blocks, this was not feasible. We were still able to use the mouse-rat alignment to assess the extent to which ancestral structural variation may have affected our analyses, which we now clarify in the text (lines 213-215).

For example, 630: This seems like a weak temptation to me. Not many taxa have good genetic maps and the idea that one map from one taxa would somehow be useful seems like a stretch to me?

>>> This is probably an appropriate level of skepticism but as explained above, this approach is already being implemented as researchers seek to understand the relationship between recombination and phylogenetic discordance (Foley et al. 2023). We also think the fact that there are so few good genetic maps may lead to the temptation. Likewise, while genetic cross or

pedigree-based maps will likely remain limited, population-based estimates of recombination are becoming increasingly easy to estimate from LD-based recombination maps. It is our impression that researchers tend to view recombination landscapes as another form of annotation that can be lifted across closely related species. The rate at which recombination rates evolve remains poorly understood and not widely appreciated within the field. We hope that our findings contribute to this growing area.

I needed more context. In general, more discussion of what we know about recombination rate variation would be very helpful to put this work in context. For example, do the genetic maps we have in mammals (ideally at these timescales) suggest that recombination rates are consistent across the chromosome at these scales? Are hotspots conserved? Is this approach meaningful?

>>> See discussion above, and and our revised Discussion of this topic (Lines 359-377):

"Given the fundamental role that recombination should play in shaping patterns of genetic variation within genomes, it is reasonable to assume that patterns of ILS should be broadly shaped by local recombination rate. We did not observe a clear relationship between local recombination rates in mice (M. musculus) and the degree of local phylogenetic discordance (i.e., phylogenetic similarity over 5 Mb intervals). However, we did find that regions of high recombination rate tended to be more discordant with the inferred species tree, in line with findings in mammals (Pease and Hahn 2013; Rivas-Gonzalez et al. 2023)(Foley et al. 2023) and Drosophila (Pease and Hahn 2013). Recombination rates evolve fairly rapidly both within (Kong et al. 2002; Cox et al. 2009; Stapley et al. 2017) and between mammalian species (Ptak et al. 2005; Stapley et al. 2017) due, in part, to the high turnover of hotspots due to the changing landscape of binding sites for PRDM9 (Baudat et al. 2010; Singhal et al. 2015). Similar to findings in great apes (Hobolth et al. 2007), our results suggest that high-resolution genetic maps from a single species provide some weak predictive value for understanding broader patterns of species tree discordance. However, these limited estimates may not be predictive of finer-scale patterns in a sample spanning over 12 million years of mammalian evolution (but see Foley et al. 2023). Overall, the evolution of recombination landscapes across closely related species remains an important empirical question in evolutionary genetics (Dapper and Payeur, 2017), especially as the generation of chromosome-scale genome assemblies continues to greatly outpace estimates of patterns of recombination within those genomes."

Minor comments:

408: "We also examine(add -d) regions of the genome centered on [removed so-called] recombination hotspots identified in M. musculus (add citation) and found that they had significantly slower rates of decay in similarity over genomic distance compared to windows that were not centered on hotspots"

>>> We thank the reviewer for pointing out these mistakes and have made the corrections.

422-427: More discussion of the difference in patterns around genes and UCEs would be interesting. I wanted to hear more about why decay may be faster around genes than UCE's but similarity is higher.

>>> We have decided to simplify these results and focus on the general patterns of discordance by feature. In doing so, we have removed the decay analysis as it yielded relatively weak results that were not entirely consistent. We are not sure how to interpret some of the results as noise or biological signal, and as such, we feel their inclusion just adds confusion to an already detail-rich analysis.

Our revised manuscript focuses more directly on the contrast between UCEs and other elements as this was the strongest signal in the data with respect to functional categories (line 256-265):

"In general, UCEs showed more local phylogenetic similarity among adjacent windows (i.e., less discordance) than regions surrounding recombination hotspots (p = 2.42e-12), coding genes (p = 4.65e-14), rapidly evolving coding genes (p = 1.56e-6), and windows that did not include any of these features (p = 5.02e-14; Fig. 5B). In contrast, protein-coding genes (including rapidly evolving genes) were indistinguishable from background rates of discordance observed in windows without annotated genomic features (Fig. 5B). Likewise, UCEs were also much more similar to the overall species tree when compared to any other feature (Fig. 5C). Unlike our test of local discordance, protein-coding genes also showed less species tree discordance than windows containing no features or recombination hotspots, but the effect was much less pronounced than observed at UCEs."

With expanded Discussion (lines 395-406):

"Consistent with this, we observed the lowest rates of local discordance (Fig. 5A) and overall gene tree/species tree discordance (Fig. 5B) near UCEs when compared to all other genomic features we studied. These results suggest that a history of recurrent purifying selection on UCEs (Katzman et al. 2007) strongly reduces patterns of discordance through a persistent local reduction in Ne. In contrast, protein coding genes showed rates of local discordance that were similar to background levels, even when considering genes rapidly evolving due to positive directional selection (Fig. 5!). However, both classes of genes did show less species tree discordance than background consistent with previous results (Scally et al. 2012; Rivas-Gonzalez et al. 2023), but this effect was much weaker than as observed at UCEs (Fig. 5B). Collectively, these data suggest that the frequency and strength of selection plays an important role in structuring patterns of incomplete lineage sorting across the genome over deeper evolutionary timescales."

479-495: This section is a little muddled. It seems to be arguing that phylogenetic analyses of Murine rodents (whether broad or limited to a single group) has been lacking approaches with more than a modest number of loci. It then argues that there is evidence for discordance even with such approaches. I am confused by the White citation. That work uses genome wide data and focuses on house mice specifically (a single species).

>>>The original intent was to argue both that previous phylogenetic work either used few loci to investigate many species or more loci to investigate particular genera, and that even with these more limited studies there were still indications of discordance. We agree however that, as written, the message is a bit confusing. As such, we have removed discussion of discordance from this section and stick to describing previous phylogenetic analyses.

530: Perhaps unsurprisingly? I would expect there to be enough variation in recombination rates among the lineages that a relationship between rates in one and overall patterns (dependent on

data from all the taxa) would not be a given. e.g. 539 wouldn't we expect there to be differences in analyses looking at population level data vs species level data?

>>> See above.

544-545: Yes it does.

>>> We attempted to address this assumption by comparing the mouse and rat genomes via whole genome alignment. The results from that analysis show reasonably conserved synteny between the two, at least marginally assuring us that our conclusions are not artifacts of structural variation.

559: on->in

>>> Corrected.

580: underestimate [of]

>>> Corrected.

583: It would be good to cite some examples to underscore the importance of this work in shaping the field with this warning/recommendation. I would not expect the use of UCEs in population level analyses except under very limited circumstances but am ready to be corrected. I think the earlier point about not using them to infer ancestral population sizes might be more relevant (but again some citations would help make the case).

>>> We agree UCEs should not be used for these purposes. Unfortunately, there are many examples of using these markers for population genetic inferences. For example:
https://peerj.com/articles/5735/
https://onlinelibrary.wiley.com/doi/full/10.1111/mec.16624
And many more.

We have clarified this section a little, along with our expanded discussion of how strongly influenced UCEs are by linked selection. Given the breadth of examples using UCEs as population genetic markers, we prefer not to provide a list of studies that have done this, rather noting this is clearly a concern. We have also added reference to the problems related to character reconstruction. See lines 413-419:

"Given this relationship, species tree inferences based on UCEs should likely not, for example, be extended to related population genetic parameters of interest (e.g., ancestral population sizes, estimates population genetic diversity), and could mislead the reconstruction of trait evolution across phylogenies (Avise and Robinson 2008; Hahn and Nakhleh 2016; Hibbins et al. 2023). Finally, despite the relative ease of generating UCE data, such markers are likely not suitable for genetic inferences within populations given the pervasive effects of linked selection."

Fig S6. Not sure if this is a file conversion issue but I had a very difficult time with this figure.

>>> We cannot see an issue on our end?

Reviewer: 2

Comments to the Author
In the manuscript "The genomic landscape, causes, and consequences of extensive phylogenomic discordance in Old World mice and rats," Thomas et al. investigate the relationships among gene/species tree discordance and chromosomal position, recombination rates, and selection. The implications of these relationships to phylogenetic methodology are great, and this topic is important especially as studies are designed and genomic approaches are being applied. The authors determine that the regions that are near each other produce less discordant gene trees. Surprisingly, they did not find recombination rate as a strong correlate with discordance, as would be expected especially given their first result that regional gene trees are less discordant than those based on loci that are further away. The authors also found that regions under selection were less discordant, presumably due to purifying selection.

>>> Thank you for the feedback. We hope our responses are satisfactory.

I will begin my review with a discussion of some of the larger issues and then finish with some minor points.

I struggled a bit in the Methods section to understand exactly why the authors were conducting particular experiments. Perhaps a larger overview of what they are trying to do would be helpful, or at least a topic sentence that explains their approach in a broader context would benefit the reader. I generally figured it out later in the paragraphs, but I had to spend the time to think about it and it should have been clearer.

>>> We thank the reviewer for pointing out the lack of clarity and narrative in the Methods section. We realize now that our presentation was a little disjointed. To address this, we have revised several sections to give an overview of our rationale and approach:

End of the Introduction (Lines 132-150):

"We combine these new genomes with previously sequenced rodent genomes and genomic resources from the M. musculus model system to study phylogenetic relationships within Murinae as well as the landscape of discordance along rodent chromosomes. We first inferred a species tree for these and other sequenced rodent genomes, focusing on signals derived from commonly used ultra-conserved elements (UCEs). We used these UCE data to infer a robust, time-calibrated phylogeny of sequenced murine rodents, providing a useful resource for future comparative studies within this important group. We then used a subset of whole genomes to study how phylogenetic discordance is related to species-level inferences of relatedness, recombination rate, and patterns of molecular evolution. Using genetic maps and functional annotation from the powerful house mouse system, we test several hypotheses linking spatial patterns of discordance to genetic drift, natural selection, and recombination. Finally, we show how the use of a single species-tree impacts gene-level inferences from common molecular evolution tests for natural selection in these species. Collectively, our results advance understanding of how core features of genome biology influence underlying phylogenetic patterns, the extent to which established model system resources can be leveraged for broader phylogenetic studies, and the consequences of ignoring phylogenetic uncertainty."

Results, transition between UCE tree and landscape of discordance (Line 177-180):

“Although congruent with previous works (Lecompte et al. 2008; Steppan and Schenk 2017), this dated UCE phylogeny provides context on the evolutionary timescale upon which we next describe the genomic landscape of phylogenetic discordance across a collection of murine genomes.”

Methods (Lines 511-514):

“We first set out to reconstruct a phylogeny of sequenced murine rodents to provide both a useful resource for future comparative genomic studies within this important group as well as a time-calibrated phylogeny to frame an in-depth analysis of phylogenetic discordance across a subset of murine whole genomes (see below).”

Methods (Lines 584-587):

“For the second part of our work, we wanted to quantitatively infer phylogenetic discordance across a subset of the murine genomes used to infer the species tree and relate that discordance to other features of the genome, such as recombination rate, proximity to genes, and rates of molecular evolution.”

It is unclear why the authors applied secondary calibrations when there is such a rich fossil record for the group, as they cited. We know that secondary calibrations are a problem and they seem quite unnecessary in this system. On line 503 the authors state that they applied four fossil calibration points, but this isn't true. They applied secondary calibration points.

>>> We have made better use of the available fossil record for Muridae and have accordingly updated our calibrations (new Table S3). We retain calibrations for both Apodemus and Mus, though we use more conservative maximum ages based on Kimura et al. (2015) and Aghova et al. (2018). Further, we have replaced our calibration for crown Murinae (which would include Phloemyini) with one for the MRCA of Mus/Arvicanthis. The secondary calibration for Rattus has also been replaced with a fossil calibration representing the MRCA of Otomyini and Arvicanthini Having re-performed the divergence time analysis, most node ages remain close to our previous estimates, though Rattus is inferred as slightly younger.

What was the purpose of aligning the mouse and rat genome? There is no discussion about this beyond a paragraph in the Methods starting on line 246.

>>> We aligned the mouse and rat genomes to get a general view of how structural variation may be affecting our conclusions. Since mouse and rat span the timescale of our subset of species for the discordance analysis, this alignment gives us a good idea of the broad levels of structural variation we can expect among our species, though of course lineage specific variation exists as well and will not be accounted for with this comparison. Initially, we were also hoping to somehow use recombination maps from both mouse and rat, however the lack of long alignment blocks between the two made this untenable. We now discuss the mouse and rat alignment in both the Results (lines 213-229) and the Discussion (lines 378-388).

The authors recovered 17,216 loci from M. musculus (line 286), but I was curious about the distribution of those loci across the genome. Were they mapped to assure that there weren't any positional biases? Also, was removing areas with low recombination (e.g., centromeres) considered?

>>> Their distribution is genome-wide with nearly 80% of all annotated genes recovered in all species. We did not filter out low recombination regions so as to not bias our results beyond what was recovered, and we did not explicitly remove centromeres, however since they are repeat-rich and difficult to align, they were mostly removed by the alignment filtering (see C panels in File S1, left side since mouse chromosomes are telocentric). Genes are not randomly distributed in the mouse genome so there are likely biases here that are already inherent to the biology of these genomes. Our approach was to use as many genes as possible, we are not sure how to further account for positional biases in these data. We are not sure how these biases would impact the results reported here.

The recombination analysis and results are all based on one lineage, that of M. musculus. In explaining why recombination wasn't locally associated with discordance, the authors cite rate heterogeneity, but it could also be caused by heterogeneity among lineages. If each lineage has a very different recombination rate (which could be due to difference in chromosome lengths, etc.), we would predict that we would never detect a relationship between discordance and recombination. Are there these types of data in Rattus that can be used as at least one additional measure of heterogeneity in recombination?

>>> We agree with the reviewer that lineage heterogeneity is likely a key driver of these results. As described above, we have thoroughly revised the text regarding recombination rates and discordance in the Discussion. See above and lines 359-377. We also attempted to incorporate the recombination maps from the rat genome in order to get some measure of how the rate has changed over the timing of our sample, however the lack of large alignment blocks between mouse and rat made this comparison prohibitively difficult for this study (see above regarding the mouse-rat whole genome alignment). Though some quantitative comparison between the maps of the two species would undoubtedly make for an interesting future study.

What were the parameters of the trimmomatic analysis?

>>> Illumiprocessor acts as an automated wrapper script around trimmomatic, which neither takes as input nor outputs trimmomatic parameters. This means we used the default trimmomatic options and now say so in the text (line 494).

It feels that the authors are overselling their results on line 627. The species and gene trees are unknown, all the authors can say with certainty is that they are different. So they can't really say that the species tree leads to false negative or false positive results, all they can say is that the gene trees result in different inferences. I agree with the authors that this gives me pause in using a single tree, but it isn't identifying what biases are occurring.

>>> We appreciate the reviewer's comment and agree that there is uncertainty here. We have made the assumption with our analyses that the gene tree is always accurate and the results using the gene tree are correct. Thus the use of the terms "false negative" and "false positive" are framed in this context. We also feel that while the absolute truth is not known here, we are comfortable assuming that the evolutionary history inferred from a specific region is a better representation of that local history than a genome-wide average. However, as the reviewer points out, this is not always the case. We have added a sentence that hopefully clarifies this point:

Lines 465-470: "While we used the simplifying assumption that the results from the gene tree are more likely to be correct, this may not always be the case given that errors can also occur

during gene tree inference. Still, our results confirm that the use of a single tree for such tests that rely on accurate estimation of substitution rates are likely to lead to both inaccurate inferences of positive selection and strongly encourage the use of individual gene trees for such analyses."

Do the unique topologies (summarized, for example, in Table 4) include unique polytomies?

>>> Polytomies are not explicitly represented in IQ-Tree, instead a minimum branch length of something like 0.000001 is set and all trees are bifurcating. So, in a sense, polytomies can be said to be randomly resolved. We find only 2,093 out of our 263,907 trees resolved with any branches having the minimum branch length, or fewer than 1% of trees, and only 1,247 of these short branches were internal (polytomies), likely meaning polytomies have little influence on our results.

Minor points:

>>> We thank the reviewer for catching these mistakes!

Line 304: Remove second instance of "of each"

>>> Corrected.

Line 452: There is no Figure 6A

>>> Corrected.

Lines 494-495: Fix sentence, perhaps a missing parenthesis?

>>> We have changed the sentence:

Lines 324-327: Other recent studies have expanded the number of loci used for phylogenetic inference, including the use of 1,245 exons (Roycroft et al. 2020) and 1,360 exons (Roycroft et al. 2021), but have focused mainly on the Hydromyini group.