Revision round #1

Decision for round #1 : *Revision needed*

Dear Dr. Wörheide,

Although all the reviewers found your manuscript valuable and interesting, they have provided several comments for improvement. Therefore, I recommend revising your preprint following the reviewers' suggestions

Best regards,

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*by **Javier del Campo**, 02 Dec 2024 12:52*
Manuscript: https://doi.org/10.1101/2024.09.05.611358
version: 1

Our responses will be written in purple. We will address some general comments that were raised by more than one reviewer.

1) Several reviewers had trouble locating the supplemental information. All of those figures should have been available on bioRxiv as a formatted PDF (with the figures embedded as PNG). All of the original figures generated from R were ALSO available as PDFs on the github repo in the folder "**supplements_for_paper**", but not in any order. However, we have now uploaded the complete supplementary material file as it appears now also on bioRxiv
2) The data tables used to make the figures were always on the github repo, in the folder "**summary_data**". This may not have been obvious to any user, so we have changed the readme to make that clearer. We did not feel it was necessary to upload a supplemental table with the paper, since it is better for readers to just use the repo.
3) Several reviewers raised points regarding estimations of the timing of the speciation. These were from timetree and were NOT available for all species pairs, so we did not include that as a formal analysis. Ignoring the issues of whether the fossils themselves are correctly identified (as family, order, etc), or that the molecular clock analysis is accurate, the problem of using molecular clocks as the basis to estimate is specifically that the differences in protein identity between the species pairs are not linear, meaning

that the accumulation of differences in proteins is not “clock like” because of naturally differing rates of mutations.

Review by Jean-Baptiste Ledoux, 11 Oct 2024 15:32

In this manuscript, entitled “Genomics change are varied across congeneric species pairs”, Francis and collaborators look for a general role of macro- and micro-synteny breaking in speciation across the animal kingdom. To reach this objective, they “examined macro- and micro-synteny in congeneric pairs with chromosome-level genome assemblies representing six animal phyla” adding the newly annotated Acropora hyacinthus genome (l. 70-80 p.2). Following different quality check and data filtering steps, they generated ortholog pairs and quantify micro- and macro-synteny between pair of congeneric species based on home-made bioinformatics pipelines.
Regarding micro-synteny, the Authors found that most species pairs differ in synteny but show relatively high sequence identity; that macro- and microsynteny together tend to decrease with speciation but can be decoupled in many species’ pairs. They discussed the low occurrence of obvious karyotype differences in their dataset.
Accounting for contrasted level of divergence between species within pair, the Authors suggest that the pattern of proteins identity values are similar across species pairs. They discussed how the multiple genetic trajectories of genetic change among congeneric species revealed by their study precludes the definition of species delimitations based on patterns of micro- or macro-synteny.
Interestingly, they also provided and discussed some of the limitations of their study, including quantification of synteny blocks or estimations of the rates of chromosome rearrangements.

As a conclusion, the Authors stated that “genomic changes inferred from speciation are variable and contextual and that synteny should not be used at present as a measure of divergence or speciation ».

My skills in comparative genomics and synteny analyses are relatively limited, so I hope this review to be constructive and relevant.
I found the paper very interesting and clear, even for a non-specialist. The study is quite ambitious and well-conducted. The authors did a great job to involve their work in the broad context of speciation.
Accordingly, the conclusions, i.e. lack of general link between macro-micro-synteny breaking patterns and speciation, are of interest for a broad readership going beyond the comparative geneticists’ community.

Here are some minor comments that hopefully can improve the current version of the MS:

1) The introduction is well written and interesting drawing the broad “speciation” context. Albeit, I would expect a bit more details

regarding current knowledge on synteny patterns. For instance, on l. 36 to 55 on page 2, maybe more context should be added to the different examples. Reading (quickly!) Jiang et al. 2019, it is not clear to me how the Authors reached the conclusion that, "extensive syntenic blocks, of tens to hundreds of genes, have been found in octocorals (Jiang et al. 2019) but not medusozoans (Jiang et al. 2019)".

The ref should have been "Khalturin et al. 2019", this has been changed. Broadly, this context is not known outside of a handful of examples, like spawning vs viviparous, marine vs terrestrial.

2) l.5-20 p.3: This paragraph may be improved a bit. It is not clear whether all the insect and mamal pairs involved species from different genus. I suggest to mention this dataset in the Material and Methods. Then regarding the two processes suggested by the Authors, it is not clear whether those are exclusive or not (I guess not) and at stake both in mammals and insects (Ok for insects but do we really have a significant loss of synteny or decrease of protein identity in mammals compared to other species pairs?).

Our measurement captures the known differences in protein identity in mammals, as first discussed in the mouse genome project in 2004 (Watterson et al). Human vs. other apes has a decrease in synteny, but not much change in protein identity.

3) Figure 2: Can the Authors justify a bit more the choice of the 4 species pairs represented in the figure? As mentioned, I am not a specialist but I found the identification of red/blue boxes in C and D difficult to understand and poorly justified. It seems to me that many other cells showed similar dot patterns. Maybe, the legend can be improved a bit to facilitate the understanding of the figure.

It was kind of just a mix to show varied levels of rearrangements and scrambling. The other 18 species pairs are on the online repo, some are very boring looking.

4) The discussion on the limitations of the study and particularly on the lack of divergence similarity among pairs within group is interesting. There are different papers addressing this topic with a population genetics perspective. They can be of interest to complement the reasoning of the Authors with some "microevolutionary perspectives" (see for instance: Roux C, Fraïsse C, Romiguier J, Anciaux Y, Galtier N, Bierne N (2016) Shedding Light on the Grey Zone of Speciation along a Continuum of Genomic Divergence. PLoS Biol 14(12): e2000234. doi:10.1371/journal.pbio.2000234; see also https://doi.org/10.1016/j.tree.2020.03.002 or Mérot, C., Stenløkk, K.S.R., Venney, C., Laporte, M., Moser, M., Normandeau, E., Árnyasi, M., Kent, M., Rougeux, C., Flynn, J. M., Lien, S., & Bernatchez, L. (2023). Genome assembly, structural variants, and genetic differentiation between lake whitefish young species pairs

(Coregonus sp.) with long and short reads. Molecular Ecology, 32, 1458-1477. https://doi.org/10.1111/mec.16468).

Not a question, but thank you for the references.

5) l.35-37 p.4: "Like the relationship with protein identity, we found that both macrosynteny and microsynteny together tend to decrease with speciation" The meaning of this sentence is not clear. What does speciation mean here? Are the Authors talking about divergence between species within each pair (but see l.14-16 p.5)?

We meant that macrosynteny and microsynteny correlate. We have changed this sentence.

6) I was wondering whether or not it may be of interest to include another layer in the pairwise comparisons by considering, in the different analyses, species pairs which are found in sympatry vs. species pairs found in allopatry. The Authors touched this topic with their comment on Cassostrea species (l. 10-14 p.6).

An interesting, but difficult topic to address with the current data. We know that a few of the pairs are, but not enough to go beyond anecdotes.

7) The paragraph entitled "Distribution of protein identify value…" is a bit hard to follow. The link between the expectations (l.78-82 p.4) and the results (l.1-11 p.5) is unclear and could be improved. Some sentences may also be rephrased. I am probably misunderstanding the reasoning of the Authors but my feeling is that "As mutations accumulate (Figure 3B,C,E,F), very few proteins remain completely identical" is a bit tautologic. More mutation more differences… hmmm? I am probably missing something.

Our apologies, this was confusing. We tried to reword this paragraph.

Title and abstract
Does the title clearly reflect the content of the article? Yes,
Does the abstract present the main findings of the study? Yes

Introduction
Are the research questions/hypotheses/predictions clearly presented? Yes
Does the introduction build on relevant research in the field? Yes, but I would like to have a better picture of existing knowledge regarding patterns of synteny.

Materials and methods
Are the methods and analyses sufficiently detailed to allow replication by other researchers? Yes
Are the methods and statistical analyses appropriate and well described? Yes but see comments

Results
In the case of negative results, is there a statistical power analysis (or an adequate Bayesian analysis or equivalence testing)? Not relevant
Are the results described and interpreted correctly? Yes

Discussion
Have the authors appropriately emphasized the strengths and limitations of their study/theory/methods/argument? Yes
Are the conclusions adequately supported by the results (without overstating the implications of the findings)? Yes

Review by anonymous reviewer 1, 17 Oct 2024 08:14

This study comprehensively analyzed the micro- and macro-synteny, as well as protein identity, of 1-to-1 orthologs in congeneric species pairs across diverse non-model phyla. By examining these factors, the authors investigated their potential role in speciation. Their findings revealed a correlation between protein identity and microsynteny, but also identified disparities in the conservation of macro- and microsynteny. Moreover, they observed variations in synteny conservation, suggesting a lack of a singular genomic trajectory during speciation.

Although limited by the relatively small dataset of available chromosome-level genome assemblies at the time, this research offers valuable insights into key concepts relevant to future studies on genomic variation and speciation. While the authors were unable to pinpoint a specific genomic feature to delineate species boundaries, they propose that future investigations, addressing limitations such as accurate divergence time estimation independent of protein identity, may uncover discernible patterns. Additionally, this study provides a foundation for future research by bringing together several key concepts and definitions that will be essential for understanding genomic variation in relation to speciation.

I enjoyed reading the manuscript and I only have a few questions and suggestions.

In general, renaming the supplementary figures in the the repositories (github, zenodo) would make following the manuscript easier. Additionally, it seems that they do not appear in the correct order.

Please see above.

In Introduction:

- In page 2, line 52 you mention that you use genomes representing six animal phyla, however I was only able to find five phyla (Mollusca, Chordata, Echinodermata, Arthropoda and Cnidaria). Which would be the sixth?

That was Porifera, which was removed from this version, so you are right, only five.

In Methods - Datasets from NCBI:

- Which is Supplemental Figure 1?

This was the schematic of the analysis, see above.

- Adding a supplementary table (expanding Table 1) with all of the genomes used including basic features such as their taxonomic classification, genome ID, assembly and annotation strategies (if available) and genomic features such as genome size, number of chromosomes, % of Ns and BUSCO completeness would be very useful.

Some of these data (size, chromosomes, etc) were already present in the main data table on the repo. We have made it clearer where to find that table on the repo in the readme file of the repo.

In Methods - Generation of ortholog pairs:

- In page 3, line 28: Shouldn't "For each species" be "For each pair of species"?

Yes, pairs, this has been changed.

- Which is Supplemental Figure 1? Is it the same as the one referenced before in the previous section?

- Why was Crasstostrea used as all vs all and not chose one reference to compare against the others like in Drosophila or Ephinephelus?

In Figure 2, *Crassostrea* is only used twice, meaning the 3-way comparison was only for Figure 3. In the first version, we did all-v-all, but we removed this.

- Which is Supplemental Figure 5? Check the order of Supplemental Figures appearances.

Order is fixed.

- Which test was performed to support the following claim: "The frequency of gaps by species is examined in Supplemental Figure 5 but does not appear to cause any obvious bias."

No test, the point was more that having a lot of gaps could lead to (probably) systematically higher protein identity if divergent regions were excluded. This probably is the case to some extent, but is impractical/impossible to fix with the current data.

In Methods - Quantification of microsynteny in genome pairs:

- Please label the Supplementary Figures in the repositories.

We'll do this on the final version after acceptance, to avoid any changing of numbers.

In Methods - Quantification of macrosynteny:

- For identifying homologous chromosomes, you only consider a one to one relation between chromosomes in species? This approach would not detect chromosome fusions and fissions. While most of the pairs of species you assessed have the same number of chromosomes this approach could miss fusions and fissions that led to the same number of chromosomes. This would be even more difficult in species with a different number of chromosomes.

Right, that is exactly the point, any deviation of that is counted as “not macrosyntenic”.

In Results - Microsynteny changes more frequently than macrosynteny:

- In Page 4, line 65. How do you distinguish between a fusion and a fission in both Daphnia and Crassostrea without using a third species?

The shuffling pattern is more consistent with a recent fusion or split, but we agree that this is imprecise, so we changed the wording to “fusion/fission”.

- Page 4, line 67, Crassostea should be Crassostrea.

Fixed. Thank you.

In Results - Distribution of protein identity values is similar across species pairs:

- Page 4, line 73. Which is the 23rd pair?

Was removed in this version, changed to 22 pairs.

· Title and abstract

o Does the title clearly reflect the content of the article? [ ] Yes, [X] No (please explain), [ ] I don't know

I believe that the title could be improved to better describe the type of genomic changes that are assessed. Also, it should specify that the study is only considering metazoa.

The title is already too long as it is, so we feel we cannot add more qualifier words.

o Does the abstract present the main findings of the study? [X] Yes, [ ] No (please explain), [ ] I don’t know

· Introduction

o Are the research questions/hypotheses/predictions clearly presented? [X] Yes, [ ] No (please explain), [ ] I don’t know

o Does the introduction build on relevant research in the field? [X] Yes, [] No (please explain), [ ] I don’t know

· Materials and methods

o Are the methods and analyses sufficiently detailed to allow replication by other researchers? [X] Yes, [ ] No (please explain), [ ] I don’t know

o Are the methods and statistical analyses appropriate and well described? [X] Yes, [ ] No (please explain), [ ] I don’t know

· Results

o In the case of negative results, is there a statistical power analysis (or an adequate Bayesian analysis or equivalence testing)? [ ] Yes, [ ] No (please explain), [ ] I don’t know

o Are the results described and interpreted correctly? [X] Yes, [ ] No (please explain), [ ] I don’t know

In general, however I have minor questions regarding the chromosome fusions and fissions.

See above.

· Discussion

o Have the authors appropriately emphasized the strengths and limitations of their study/theory/methods/argument? [X] Yes, [ ] No (please explain), [ ] I don’t know

o Are the conclusions adequately supported by the results (without overstating the implications of the findings)? [X] Yes, [ ] No (please explain), [ ] I don't know

Review by anonymous reviewer 2, 09 Oct 2024 05:42

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Summary
The recent explosion in the availability of chromosome-level genomes permits extensive genomic comparisons beyond the restricted model taxa in which they were previously conducted. The preprint by Francis et al. compares macrosynteny, microsynteny and protein evolution between 22 pairs of congeneric species belonging to five phyla. The authors report correlations between these three variables but do not find a "universal path" to species divergence. The manuscript was a pleasure to read and represents a valuable contribution to the field. In particular, the possibility of using genomic signatures for taxon delimitation is a pressing issue to address. I do not have any major concerns about the manuscript but I do have some intermediate level comments and minor comments, which I list below.

I preface the below comments by saying that the Supplementary Figures appeared not to be available on BioRxiv and though a link to a GitHub repository is provided and files to remake the figures are available, I could not find a document that presented them with their captions. If they are indeed available and I have missed them, I apologise, and suggest making their location more obvious. If not, please make them accessible – it would be most convenient as a PDF on BioRxiv. Please also note that the link to the Github repository is incorrect in the Data Availability section of the BioRxiv preprint (dash between Pal and Muc).

Our apologies, see above.

Intermediate-level comments
(1) The logic behind adding the 'Insect' and 'Mammal' comparisons is currently unclear and needs more explanation. If I understand correctly, these are "to remake the comparisons of previous studies", at least in the case of the mammals, but the insects are not mentioned in any part of the methods section.

The insects were used since that was done in a previous study, that we reference. The mammals are used since many people are familiar with mammals, using ones found in many other evolutionary studies. We could have added more, but then the study is about insects or mammals, instead of about animals.

o Which species are used and how many?
This has been clarified in the methods.

o How distantly related are they?
Different insect orders.

o Is it valid to add these comparisons in to the main dataset or are they too different?o Are these comparisons taken into account in the main conclusions of the results

section (e.g. “microsynteny broadly correlates with protein identity across animals”)?

Yes and no, this was replicating previous analysis that had looked at species, but also more broadly, since that was what was available at the time of the Zdobnov 2007 study.

The addition or not of these comparisons appears to make a significant impact on the conclusions and therefore should be fully explained and justified. The datasets themselves need something equivalent to table 1 to readers can study their composition.
A significant portion of the first sections of the Results section is devoted to the discussion of these groups. This is not consistent with the relative weight placed on them in the methods section. I wonder if it would be easier for the reader to follow if the main questions of the paper were first addressed without these extra comparisons and then there was a separate section specifically discussing these additions and their importance.

This was replicating a study that we cite several times. It actually made the paper harder to read by having a separate section for those, so they are mixed in.

(2) I find the ‘loose correlation’ reported as shown in Figure 1C to be unconvincing to the eyeball test, especially if the ‘insect’ pairs, which as far as I can tell are separate from the main dataset, are removed. Did you do any statistical test to show that there is indeed a relationship between these two variables?

Not enough species pairs currently to do any meaningful stats. Check back in 5 years.

(3) As in point (2), the statement that “microsynteny changes more frequently than macrosynteny” is equivocal (and to me very logical and expected) but seems only very weakly supported by the data in the plot, again with no statistical assessment. Is there any other data supporting this on top of Figure 1D? Can statistical tests be used to test the claim?

This was argued by Lv et al. 2011, and is observed elsewhere.

(4) “Distribution of protein identity values is similar across species pairs” is again a very clear statement which the evidence immediately available to the reader does not clearly support. On the contrary, the species shown in Figure 3 seem to have highly variable distributions. Indeed, as you say that species “may all be at different stages of divergence”, would it not be tremendously surprising for the distribution of protein identity values to be similar?

Fair point, in SFig 7, they look very similar, but in the histograms, they are varied. We changed the text to say varied distributions.

Though as said above I could not access the Supplementary Figures so this information may be there, it is quite difficult for the reader to make any assessments of broad trends when the main text figure contains only 2 of the 17 genera studied. I wonder whether there is a way of summarizing this data across all 17 genera to make it easier to assess broad patterns? For instance, could you compare the mean protein identity to pairwise divergence time for allspecies? This would then allow you to see whether pairs diverge in protein identity at different
rates. I know in a later section you discuss the pitfalls of using divergence times in this context and I understand the reluctance to open up that particular can of worms, but it strikes me that even rough estimates of divergence times between each pair of species would add significant context to the analyses.

This was in the supplement.

Minor comments

P1L72 – Implies that synteny-breaking genome rearrangements are not a type of mutation. Surely they are indeed a type of mutation, albeit distinct from point mutations, indels etc.?

We see your point, but there isn’t a shorter way of saying this. The Muller model is not specific about point mutations, so we can’t just specify that. The point was more that changes in gene order can change fitness of hybrids. Is that a mutation? Sort of, but I’m not sure people call it that.

P2L10 – This sentence is quite dismissive considering others have argued that macrosynteny changes play a major role in speciation (E.g. Augustijnen et al. 2024 Sci Adv. https://doi.org/10.1126/sciadv.adl0989.) This may be worth briefly discussing or at least Rephrasing.

Fair point, we think two different time scales are being discussed, one within population, the other between populations. We tried to specify that the models discussed translocations, not fusions.

P3L27 (1) – This is not a criticism but simply a question. I am interested in the thinking behind your method of orthology assignment. Why not use a highly-cited and robust program like OrthoFinder which would achieve many of the described steps automatically?

OrthoFinder is fine, but tries to do a lot more, our method was simpler and faster.

P3L27 (1) – How many orthologues did you have per species pair in your final dataset? If this data is not currently available, it should be reported. Could be added to Table 1 or as Supplementary.

This was in the supplement.

P4L63 – Can you explain in more detail how chromosome fusions and splits are distinguished?

As above, we changed the wording.

P7L42 – where in Figure 1 is there evidence of 'a strong correlation between protein identity and divergence time'?

This was moved to supplement, our apologies for the confusion.

Fig. 1A 'enes' -> 'genes'

Fixed.

General questions from PCI

• Did you read the "guide for reviewers"? (see the Help menu of the thematic PCI or the dedicated blog post) YES
• Is the manuscript well written? YES
• Is the description of the rationale and methods clear and comprehensive? YES
• Are there flaws in the design of the research? NO
• Are there flaws in the analysis? NO
• Are there flaws in the interpretation of results? POSSIBLY – my comments request further explanation of some results.
• Do you have concerns about ethics or scientific misconduct? NO
• Did you detect a spin on the results, discussion or abstract? (a spin is a way of twisting the reporting of results such that the true nature and range of the findings are not faithfully represented, https://doi.org/10.1073/pnas.1710755115) NO
• Is something critical missing? NO

Evaluation of the various components of the article

Title/abstract/introduction

• Does the title clearly reflect the content of the article? YES
• Does the abstract present the supported findings of the study concerned and no other? YES
• Does the introduction clearly explain the motivation for the study? YES
• Is the research question/hypothesis/prediction clearly presented? YES
• Does the introduction build on relevant recent and past research performed in the field? YES

Materials and Methods

• Are the methods and analysis described in sufficient detail to allow replication by other researchers? YES
• Is the experimental plan consistent with the questions? YES
• Are the statistical analyses appropriate? Further analyses required.
• Have you evaluated the statistical scripts and program codes? NO

Results
• Have you checked the raw data and their associated description? NA
• Have you run the data transformations and statistical analyses and checked that you get the same results? NA
• To the best of your ability, can you detect any obvious manipulation of data (e.g. removal)? NO
• Do the statistical results strongly support the conclusion (p< 10-3 or BF>20)? NA
• In the case of negative results, was a statistical power analysis (or an appropriate Bayesian analysis) performed? NA
• Did the authors conduct many experiments but retain only some of the results? NO
Discussion
• Do the interpretations of the analysis go too far? ? POSSIBLY – my comments request further explanation of some results.
• Are the conclusions adequately supported by the results? ? POSSIBLY – my comments request further explanation of some results.
• Does the discussion take into account relevant recent and past research performed in the field? YES
• Did the authors test many hypotheses but consider only a few in the discussion? NO
References
• Are all the references appropriate? YES
• Are the necessary references present? MOSTLY
• Do the references seem accurate? YES
Tables and figures
• Are the tables and figures clear and comprehensive? YES
• Are all the tables/figures useful? YES
• Are there too many/too few tables and figures? NO
• Do the tables and figures have suitable captions such that they can be understood without having to read the main text? YES

Review by anonymous reviewer 3, 28 Oct 2024 15:13

All in all, the authors have done an excellent job exploring the effects of speciation on genome structure. Congratulations on an excellent manuscript! I am looking forward to seeing this in print and I will definitely be adding it to my own citation library. I have a few comments below that I think would drastically improve the manuscript with very little work. These are personal opinions and I think the work is well-written and impactful as-is and other reviewers may not share these same thoughts.

Does the title clearly reflect the content of the article?

Yes. The title accurately reflect the content of the article. However, the author could stand to be a bit more specific as "genomic changes" is nonspecific and the title could likely improved slightly for higher impact.

Does the abstract present the main findings of the study?

Yes.

Are the methods and analyses sufficiently detailed to allow replication by other researchers?

Yes.

Are the methods and statistical analyses appropriate and well described?

Yes.

In the case of negative results, is there a statistical power analysis (or an adequate Bayesian analysis or equivalence testing)?

Yes.

Are the results described and interpreted correctly?

Yes.

Have the authors appropriately emphasized the strengths and limitations of their study/theory/methods/argument?

Mostly yes. My only concern is that the authors do not emphasize enough that they are working with species pairs that are diverged by highly variable amounts of time and are often not sister species. They do mention this on lines 34-45 on page 7 and 5-9 on page 6 but I think this needs to be even more thoroughly discussed at varying points in the manuscript since the observed processes might not directly be the result of speciation (which might be better observed in sister species pairs). The definition of a genus is as arbitrary as the definition of a species (which the authors do write about extensively in the introduction, maybe expand and describe how genera are also extremely arbitrary?) and some of the congeneric pairs are diverged by hundreds of millions of years (e.g. Daphnia) while others are diverged by only a few million (e.g. Drosophila) and are not sister (e.g. Daphnia pulex and Daphnia magna). The number of appropriate genomic resources available on NCBI *specifically for sister species* are scant (there are

some good sister species references in other repositories but I strongly understand the desire to use NCBI over external databases) so I believe the authors have done an excellent job putting together an important paper with what is available. But I would specifically highlight that future work should explore the micro/macrosyntenic and identity differences between *sister* species since that could end up showing different results than seen here.

A bit beyond the goal of the paper, but we recall an anecdote from one colleague that thought that genera kind of represent an ecological niche, but that speciation is just the noise from mutations on top of that. Whether any of the ranks translate to something biological is still unclear.

I think all of my concerns would be alleviated by a few additional sentences that expand upon the limitations and potentially adding in a simple divergence time phylogeny (I don't actually want the authors to *run* the phylogenetic analysis, but instead put together a stylized figure that pulls from many different papers and depicts the divergence time and sister/non-sister relationships of the included taxa. Maybe even some kind of icon to depict the kind of speciation? e.g. allopatric vs sympatric).

We specifically did NOT want to do this kind of analysis, because of how sparse and messy the data are.

Are the conclusions adequately supported by the results (without overstating the implications of the findings)?

Yes. The only change that I might make is to line 77 of page 7 where I would change species-pairs to sister species-pairs.

Changed.