Peer Community In
Evolutionary Biology

Recent assembly of European biogeographic species pool

Fabien Condamine *based on peer reviews by 3 anonymous reviewers*

Joaquín Calatayud, Miguel Á. Rodríguez, Rafael Molina-Venegas, María Leo, José Luís Hórreo, Joaquín Hortal (2018) Pleistocene climate change and the formation of regional species pools. bioRxiv, ver. 1, peer-reviewed and recommended by Peer Community in Evolutionary Biology. 10.1101/149617

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Biodiversity is unevenly distributed over time, space and the tree of life [1]. The fact that regions are richer than others as exemplified by the latitudinal diversity gradient has fascinated biologists as early as the first explorers travelled around the world [2]. Provincialism was one of the first general features of land biotic distributions noted by famous nineteenth century biologists like the phytogeographers J.D. Hooker and A. de Candolle, and the zoogeographers P.L. Sclater and A.R. Wallace [3]. When these explorers travelled among different places, they were struck by the differences in their biotas (e.g. [4]). The limited distributions of distinctive endemic forms suggested a history of local origin and constrained dispersal. Much biogeographic research has been devoted to identifying areas where groups of organisms originated and began their initial diversification [3]. Complementary efforts found evidence of both historical barriers that blocked the exchange of organisms between adjacent regions and historical corridors that allowed dispersal between currently isolated regions. The result has been a division of the Earth into a hierarchy of regions reflecting patterns of faunal and floral similarities (e.g. regions, subregions, provinces). Therefore a first ensuing question is: "how regional species pools have been assembled through time and space?", which can be followed by a second question: "what are the ecological and evolutionary processes leading to differences in species richness among species pools?". To address these questions, the study of Calatayud et al. [5] developed and performed an interesting approach relying on phylogenetic data to identify regional and sub-regional pools of European beetles (using the iconic ground beetle genus *Carabus*). Specifically, they analysed the processes responsible for the assembly of species pools, by comparing the effects of dispersal barriers, niche similarities and phylogenetic history. They found that Europe could be divided in seven modules that group zoogeographically distinct regions with their associated faunas, and identified a transition zone matching the limit of the ice sheets at Last Glacial Maximum (19k years ago). Deviance of species co-occurrences across regions, across sub-regions and within each region was significantly explained, primarily by environmental niche similarity, and secondarily by spatial connectivity, except for northern regions. Interestingly, southern species pools are mostly separated by dispersal barriers, whereas northern species pools are mainly sorted by their environmental niches. Another important finding of Calatayud et al. [5] is that most phylogenetic structuration occurred during the Pleistocene, and they show how extreme recent historical events (Quaternary glaciations) can profoundly modify the composition and structure of geographic species pools, as opposed to studies showing the role of deep-time evolutionary processes. The study of biogeographic assembly of species pools using phylogenies has never been more exciting and promising than today. Catalayud et al. [5] brings a nice study on the importance of Pleistocene glaciations along with geographical barriers and niche-based processes in structuring the regional faunas of European beetles. The successful development of powerful analytical tools in recent years, in conjunction with the rapid and massive increase in the availability of biological data (including molecular phylogenies, fossils, georeferrenced occurrences and ecological traits), will allow us to disentangle complex evolutionary histories. Although we still face important limitations in data availability and methodological shortcomings, the last decade has witnessed an improvement of our understanding of how historical and biotic triggers are intertwined on shaping the Earth's stupendous biological diversity. I hope that the Catalayud et al.'s approach (and analytical framework) will help movement in that direction, and that it will provide interesting perspectives for future investigations of other regions. Applied to a European beetle radiation, they were able to tease apart the relative contributions of biotic (niche-based processes) versus abiotic (geographic barriers and climate change) factors.

References:

[1] Rosenzweig ML. 1995. Species diversity in space and time. Cambridge: Cambridge University Press.

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[3] Lomolino MV, Riddle BR, Whittaker RJ and Brown JH. 2010. Biogeography, 4th edn. Sinauer Associates, Inc., Sunderland, MA.

[4] Wallace AR. 1876. The geographical distribution of animals: with a study of the relations of living and extinct faunas as elucidating the past changes of the earth's surface. New York: Harper and Brothers, Publishers.

[5] Calatayud J, Rodríguez MÁ, Molina-Venegas R, Leo M, Hórreo JL and Hortal J. 2018. Pleistocene climate change and the formation of regional species pools. bioRxiv 149617 ver. 4 peer-reviewed by Peer Community In Evolutionary Biology. doi: [10.1101/149617](`https://doi.org/10.1101/149617`)

Reviews

Evaluation round #2

DOI or URL of the preprint: `https://doi.org/10.1101/149617`
Version of the preprint: 2

Authors' reply, 12 May 2018

Dear Dr. Condamine,

Thank you very much for handling our manuscript, the positive comments and the helpful suggestions. We reviewed the manuscript as requested and incorporated your suggestions. Specifically, we removed the ancestral estimation model comparison and used only results from the DEC model. To avoid expensive and unnecessary computation and based on previous analyses, we only used 1,000 randomly chosen phylogenetic hypotheses from each calibration scheme in new analyses. Results remained almost equal with same interpretations. Regarding, the discussion about details of ancestral range inferences, we still think that such interpretations may be at least controversial and most likely quite speculative. Indeed, the recent Ree & Sanmartín paper you pointed out has raised to some extent serious concerns about the underlying assumptions of probabilistic ancestral range estimation models in general. This, together with the violation of some other assumptions by our dataset and the difficulties in interpreting results from many phylogenetic hypotheses, persuaded us from making detailed interpretations from these analyses (which in any case would be secondary to the main idea of the study). We however agree with you and referee #1 of round 1 in that accessing and discussing more about the phylogenetic structure of Carabus regional faunas might shed light on new facts. Hence and to this aim, we conducted new analyses and explorations. Firstly, we computed the probability of pre- and post-Pleistocene nodes to have all descendant species grouped in each particular region. This analysis revealed that non-glaciated regions during LGM hold a small but larger number of related lineages (diversifying both before and after the beginning of the Pleistocene, see Fig.4b). This supports that more stable areas are more prone to accumulate related species, providing further and interesting results to the manuscript. Secondly, we plotted 100 phylogenies (from each calibration dataset) where nodes were coloured if all descendant species belong to the same region. Colours were based on the regions the species belong to. Exploration of these plotted phylogenies (that are now provided in an appendix) showed that most of the lineages that diversified before the Pleistocene and were clustered in the same region (especially in the central European region), correspond with alpine species (see Fig.4c). This observation suggests that species adapted to cold environment might have been able to better resist glacial conditions, which is a somehow speculative but still quite interesting idea. Moreover, this helps to respond to referee #1's previous questions about the characteristics that might have allowed some Carabus to overcome glaciations. In sum, even though we did not interpret ancestral state estimations, we believe that these new results had considerably improved the manuscript in the requested line of evidences.

We are also very grateful to the referee for revising again the manuscript, as well as for her/his positive comments and suggestions.

Decision by Fabien Condamine, posted 12 May 2018

Decision for MS. Calatayud et al. (`http://dx.doi.org/10.1101/149617`)

Dear authors,

Thank you again for soliciting the Peer Community in Evolutionary Biology to evaluate your study.

I have now received the feedback of one of the three reviewers for your preprint study (see below). You will see that the referee is very positive about the revision. In addition, the referee identified little mistakes/typos that should be corrected to improve the study. Overall, I agree with the reviewer that the study is both solid and interesting. As I said, I think the approach is thorough and original, which may be useful for further studies looking at effect of glaciation effects on regional species pools. The study is now strong on the methodological aspects like phylogenetic and dating analyses.

To summarize, the authors have addressed the major (6) points raised by the reviewers including: (1) The re-writing and re-structuring of the paper. This is now much clearer. (2) The hypotheses tested are better presented, and it's clear. (3) The phylogenetic and dating analyses have been re-performed based on the referee's comments. I really like the presentation of both calibration scenarios. (4) The downstream analyses have been redone with the newly generated time-calibrated phylogeny (comparing the Andujar et al.'s hypothesis and Deuve et al.'s one). (5) The results and discussion take into account the new phylogenetic/dating analyses including the uncertainty of your species placement with the grafting. (6) The results are now better put in the context of existing literature regarding Pleistocene climatic oscillations.

However, I have a last comment regarding the biogeographic analyses. The authors have used the now traditional and popular BioGeoBEARS approach to infer the ancestral states. I am not convinced by the use of the J parameter in DEC models and relatives. This parameter is a microevolutionary process and is not adapted for macroevolutionary inferences: if you think about founder-speciation event then it is more a population genetic event than a real macroevolutionary process. So how are we supposed to estimate such event at a macroevolutionary time-scale? To support my point of view, there is a paper showing that "DEC+J is a poor model of founder- event speciation, and statistical comparisons of its likelihood with DEC are inappropriate" (Ree & Sanmartín 2018, full reference below). This also applies to the DIVA-like and BayArea-like models. For simple inference of ancestral ranges on a fixed phylogeny, a DEC-based model may be defensible if statistical model selection is not used to justify the choice. So in other words, it means that DEC+J cannot be compared to DEC anymore. To have more clues about that, please look at the Ree's talk at an Evolution meeting: `https://www.youtube.com/watch?v=8Cf4hfZI3VY&ab_channel=EvolutionVideos` Ref: Ree R.H. & Sanmartin I. (2018) Conceptual and statistical problems with the DEC+J model of founder-event speciation and its comparison with DEC via model selection. J. Boigeogr., 45, 741-749. `https://doi.org/10.1111/jbi.13173`

So in summary, I would advise the authors to select one model (DEC or DIVA-like or BayArea-like) and stick to it. My preference will go to the classical DEC model as there are studies showing that it is a very robust model even under complex scenarios (Beeravolu & Condamine 2018, full reference below). I would suggest removing the model comparison that is now flawed. Ref: Beeravolu C.R. & Condamine F.L. (2018) An Extended Maximum Likelihood Inference of Geographic Range Evolution by Dispersal, Local Extinction and Cladogenesis. bioRxiv, `https://doi.org/10.1101/038695`

Last point, I would strongly suggest the authors to discuss in more details the results of the biogeographic inferences as suggested previously by the referee #1. I think it would bring interesting facts to the paper and for the discussion. It is currently lacking.

Based on the referees' comments and my last comments, I believe the manuscript will benefit from a slight revision but not followed by a third round of reviews. I hope you are not too disappointed and that you will resubmit a revised paper. For the moment, I do not recommend the study in PCi Evol. Biol. but if the biogeographic results are better explained, I will recommend the paper in PCi Evol. Biol.

Dr. Fabien Condamine, recommender for PCi Evol. Biol.

Reviewed by anonymous reviewer 3, 21 February 2018

I went through the manuscript several times for this second round of reviews and the manuscript looks very good to me now. The authors have taken into account most of reviewer's comment and I am happy with the replies they provided to my own comments. They actually did a great job since criticisms had been raised concerning the phylogeny. The authors have now proposed two different time-calibrated tree that seem to be well done and re-analysed everything with these two phylogenies. The phylogeny is indeed poorly resolved and the authors "grafted" species not sequenced but they largely took into account this uncertainty in the analyses. Hence, to my opinion the results are strong and the well discussed in the light of the recent Pleistocene climate changes.

I just spotted a few mistakes:

Line 436: "were not significant" L463: "regression" L518-519: maybe make more reference to the figures and table, in general for the discussion. L545: "interpreted in the light"?

Evaluation round #1

DOI or URL of the preprint: **10.1101/149617**

Version of the preprint: 1

Authors' reply, 13 February 2018

Download author's reply

Decision by Fabien Condamine, posted 13 February 2018

Revise

Dear authors,

Thank you for soliciting the Peer Community in Evolutionary Biology to assess your study.

We have now received the feedback of three reviewers for your preprint study (see below). You will see that the three referees bring up very interesting and useful comments as well as suggestions that I am sure will improve the study. Overall, I agree with the reviewers that the study has many merits and that the findings are interesting. I also think the approach proposed here is original and may be useful for further studies. However, the study suffers from some methodological issues. I think the main issues concern the phylogeny and dating analyses, but because these results are the cornerstone of the other analyses, the corresponding results may be inconclusive as it stands. The referees also felt that the manuscript suffers from a lack of clarity in several parts of the text. The manuscript would certainly benefit from a careful reading by a native English speaker.

To summarize, I have identified six major points raised by the reviewers that you would need to carefully address. This includes the following: (1) The writing and structure of the paper (in many places the referees have suggested to rephrase the sentence, clarify the meaning, or be more accurate); (2) Clarification of the hypotheses tested (this is an important part of the paper and it needs support from previous studies and the biology of the group); (3) Revising the phylogenetic and dating analyses based on the comments (e.g. using RAxML / MrBayes for the phylogeny and BEAST for the dating with a fixed topology if necessary); (4) Redoing the downstream analyses depending on the newly generated time-calibrated phylogeny (would be great to compare your results with the newly generated tree, and the results obtained with the Deuve et al.'s tree as suggested); (5) Revising the results and discussion based on that new analyses if any change has to be done and including the uncertainty of your results (confidence intervals or credibility intervals); (6) Emphasizing your results in the context of existing literature regarding Pleistocene climatic oscillations and associated effect on biodiversity.

Based on the referees' comments and my reading, I believe the manuscript will benefit from a revision and a second round of reviews. If you chose to resubmit a revised paper, please make a point-by-point reply to the comments (like for a traditional journal). For the moment, I do not recommend the study in PCi Evol. Biol. but if the revision is thorough (satisfies the reviewers) and the results still support the conclusions, I will be supportive for the paper as being recommended.

Dr. Fabien Condamine, recommender for PCi Evol. Biol.

Reviewed by anonymous reviewer 3, 28 November 2017

The manuscript entitled "Pleistocene climate change and the formation of regional species pools" seeks to statistically identify distinct biogeographic regions within the Palearctic region, focusing on the genus Carabus (Coleoptera), in order to test which variables explain best the species turnover across these regions, and whether they fit with the hypothesis the Pleistocene climatic variation affected the pattern of diversity. The authors focus on four (actually three) explanatory variables: i) the ecological niche (climatic niche + habitat), ii) geographic connectivity among regions and iii) phylogenetic relationships. The study does not bring new data and rely on sequences publicly available and distribution data from previous publications of the same authors. Globally, while the Material and Method section and the Results are well written, clear and concise, I found that the Introduction and Discussion are much more difficult to follow, some ideas would require to be better explained, in particular when tightly related to the hypotheses (see below). I really like the approach chosen to identify the biogeographic regions. It gives a real statistical support for describing the pattern of diversity.

However, I have more questions about the method used for reconstructing the phylogeny and interpretation of the ancestral range estimation (see details below).

Introduction L33. I find "determine" too strong. Also I am not sure about this sentence and the whole idea of this introductory paragraph. The authors cite for example Ricklefs & He (2016) as an example supporting the idea that "regional biota […] determine ecological and evolutionary processes […] at finer scale". But the reference above actually supports the idea that the PROCESSES acting at large scale and structuring the regional biota (speciation, extinction, dispersion) can affect local processes. This may sound like a detail but otherwise I do not see how the "regions biota" per se can affect local processes. I also think this clarification is extremely important since the conclusion of the paper (the last sentence) directly refers to this question of PROCESSES acting at large scale and potentially indirectly affecting the local scale. L55-59. I find these two sentences contradictory. Do the authors mean that it is well known that species/population ranges are the result of Pleistocene climate but we do not know how general this is? These sentences should be clarified. L.67-82. This part deserves to be clarified. It gives the framework of the study, and many of the patterns tested are based on this, yet it is confusing. For example l. 67-70, I do not understand the last part of the sentence. I don't know if this may help but a way of presenting some of the processes would be to talk about the balance (or ratio) between speciation and dispersal/adaption for a region. Variations in this ratio may be mediated by important barriers to dispersal (reducing dispersal) or climatic fluctuations (increasing dispersal) and lead to phylogenetic clustering or overdispersion. L70-71. Throughout the manuscript the authors use "phylogenetically constraint". To me this is an over-interpretation of the pattern called phylogenetic signal or phylogenetic conservatism (depending on the definition one wants to use). Using phylogenetic conservatism or signal would make things much more clear I think. Also, it is not the "species' resemblance" that is conserved but the climatic niche, which leads to a resemblance among closely related species. L85. The authors study the "European Carabus". Does that mean there are non-european Carabus? In other words, is Carabus endemic to the Palearctic? If not where are the other Carabus in the phylogeny, and how this may affect the results (ancestral range estimation in particular) and interpretation?

L99. "presumed low dispersal capacity": reference needed, especially since hypothesis H1 at least is based on this statement.

L103. Hypothesis H3. This is a question that came to me several times throughout the paper: do we need the Pleistocene climatic fluctuations to explain that lineages living at higher latitudes are adapted to cold environments? After all, temperatures decreasing toward the poles, one would expect to see lineages adapted to cold environments at higher latitudes compared to the Mediterranean basin. I think the authors should think of a way to distinguish between a "simple" latitudinal gradient effect and the Pleistocene climatic fluctuations.

Material and methods L168-172. I wonder: the authors do not give the sign of the expected correlation. Here, the only hypothesis is a positive correlation between niche distance and phylogenetic distance (leading to a phylogenetic conservatism). However one may expect a negative correlation, i.e. closely related species have very dissimilar niches, a pattern one may expect for example in the case of adaptive radiation. Was the test designed to detect both negative and positive correlations? L195-196. The sentence need to be revised. L215. Should remind what a "node" is here. L239-242. The sentence need to be revised. L256. Is there a specific reason for using kernel smoother instead of "polygons"? L287-296. The section about the molecular phylogeny is extremely short and would benefit from adding more information here, instead of simply referring the supporting information. The authors unlink the substitution models in the RAXML across regions (genes I presume) – supposing that each gene follows a different substitution model. However they consider only one clock, estimated only based on the branch length of the RAXML tree and time constraints. I do not understand the choice of using the chonos function for time-calibrating the tree, i.e. using a method that does not even use the molecular matrix to estimate one clock, while this information is available. To me seems like this is under-exploiting the information available. Applying a molecular clock is not simply rescaling a topology based on some constraints. I do not pretend that the results will drastically change but it seems to me like the method used here is quite weak, especially in the context of inferring historical biogeography, where the timing of events is particularly important. L287. Phylogenetic distances were computed from the time-calibrated tree? L323. Ancestral range estimation: Have the authors modified the adjacency matrix or multipliers? Is there a reason for not reducing the adjacent areas?

Results L414. Revise the supporting information numbering. L416. I do not understand which information allows the authors to conclude that colonization occurred "recently". Because there is no phylogenetic signal? L420. In 59% of cases BAYAREA+j has the lowest AIC. But I am curious, what is the mean AIC difference with the second best model? L422-425. The authors discuss the parameter j. However they do not providing any discussion about the fact that BAYAREA is chosen over other models such as DEC. BAYAREA models speciation event in which both descendant lineages inherit of the entire ancestral range, leading to speciation events that occurred over the entire distribution of the ancestral lineages. How does such model fit when interpreting the results? L419. Concerning the ancestral range estimation: I still think that a short description of the pattern is necessary. I understand that the authors are interested in assessing the role of recent climatic event, but the group is more than 20 my old (according to the phylogeny). I think the authors cannot really ignore the 20 my of diversification before the Pleistocene events. And I would like to see the distribution of species at the tips of the phylogeny, otherwise this information is not available and it will also help better understanding the regional structure. Should we also observe a directional pattern of dispersal events if Pleistocene climatic fluctuations affected speciation and dispersal? Shouldn't we observe recent northward colonizations? Do we?

Figure 4. Based on this time-calibrated tree, there seems to be parts of the tree that were clearly not affected (at least in terms of speciation) by Pleistocene climate changes. For example the clade including Tomocarabus, Diocarabus, Orinocarabus, Eurycarabus, Aulonocarabus, Pachystus (which is inferred to have occurred in the northern part for a long time) or the clade Archicarabus. Are there any hypotheses that may explain why some lineages have been more affected by Pleistocene climatic events than others?

Discussion L451. Sentence needs to be revised. L451-452. Could be interesting to give an example of how local diversity is affecting to clearly contrast with the result of this paper L460-462. Or simply a lesser geographic complexity? L480-482. Revise the sentence, which in itself cannot be understood. L490-491. Could this also be a consequence of the lower number of species in northern regions? L502. Then vicariant events should be recent; otherwise local diversification would have led to phylogenetic clustering. L516-519. Then where are these founder speciation events in the ancestral state estimation? This statement could be supported by some examples of such events (or event show it on the figure). L522-254. It seems like the authors are still discussing about the +j, but I do not see how this sentence related to the +j parameter. Some rewording is needed.

Reviewed by anonymous reviewer 1, 28 November 2017

The paper aims at depicting the processes that contribute to the formation of regional species pools. Using Carabus as a biological model, the authors used a network approach to characterise regional and subregional faunas across Europe. Similarities in species co-occurrence within these faunas were then modelled as a function of climatic niche and habitat similarities, spatial connectivity and phylogenetic distances. I'm not familiar with the analyses used in the paper, but I found the issue very interesting. I think that such studies should enable a better understanding of the origins and dynamics of regional biotas. My main concern was for the structure of the paper. The authors made the effort to define specific hypotheses (H1-H6) in the introduction, which they did not refer hereinafter, especially in the discussion. Moreover, the material and method section is very long. The Data origin section should be embedded in the other subsections for easy readability. If the structure of the paper is revised, I think that it deserves the label of PCI Evol Biol. Minor comments l.69-82: the link between the text and Fig. 1a is not obvious, because the terms used were not the same. Please clarify, or delete reference to Fig. 1a. l. 128: I have not found the map at `http://dataservice.eea.europa.eu` l. 134-142: the relevance of the environmental and geographical data concerning Carabus could be explained in this paragraph l. 165: "lineages" l. 175: "Further, the independent effects...": why "independent"? l. 177: " to geographic isolation (Fig. 1a.iii), whereas..." l. 261-269 were vegetation categories defined at the scale of each grid cell, or more finely? l. 273: "pixel" = grid cell? l. 369-386: what about the geographical consistency of submodules? Please choose between the terms “region/subregion” and “module/submodule” throughout the text to facilitate reading. l. 398-400: spatial connectivity was not significantly related to species co-occurrence in Modules 5 and 6. l. 408-409: “The effects of connectivity were stronger in southern regions (i.e., modules 1 and 3)”: in module 3 it was not stronger than in modules 4 and 7. l. 412-414, “Indeed, niche similarity and spatial connectivity…”: this sentence is confusing and may be deleted. l. 414: “Only niche similarities (mainly climate)”: were all the significant effects of niche similarities among regions and subregions related to climate? l. 415-417: “ as expected if…”: for instance here, the authors should refer more clearly to hypothesis H3. l. 424: “which could be seen as a signal of Pleistocene glaciations…”: or of more ancient events? l. 426 “the range contraction parameter”: named e (extinction) in the Table legend? l. 489: replace “stronger related” by “more strongly related”?

Reviewed by anonymous reviewer 2, 28 November 2017

In this study, Catalayud et al. look into how species pools of the genus Carabus are formed in response to Pleistocene climate change. I find the study interesting but I have some issues with parts of the methodology.

I am assuming the methods used to detect species pools and asses the determinants of species pools are sound. I have no expertise in these methods and the section describing them is a little tedious to read and understand.

The matrix design seems a little odd to me. I understand how the matrices are designed but I find it hard for example to understand why the connectivity matrix is designed based on solely topography and presence of water bodies. The slope and the presence of water bodies are not necessarily the most crucial factors determining Carabus beetle dispersal. A more inclusive view would be to take into account ecosystem/niche change/breaks. At the moment, this is decoupled in the different matrices. It is not clear to me if this makes sense in a biological way. Knowing the biology and distribution of Carabus in Western Europe, it is not clear to me why the authors chose the slope and presence of water bodies as proxies for barriers limiting dispersal.

Are the 16S alignments with Kalign and MUSCLE strictly identical? If not I don’t see why the authors mention the use of a criterion to select their alignments under different programs. They seem to select a better alignment in MUSCLE but decide to take a suboptimal one based on the criterion that they decided to use in the first place.

The phylogenetic and dating analyses could be improved. The dataset was not partitioned and was analyzed as a whole. This is most likely suboptimal. Furthermore, the backbone of the topology was constrained to reflect the one of Deuve et al. (2012). I don’t see a good rationale to do so, especially considering the rather low nodal support across Deuve et al.’s topology in the first place. The dating is equally odd, with no less than 21 secondary calibrations used to date the resulting RAxML topology. I am wondering why the authors did not directly use the chronogram from Deuve et al. and pruned the tips they were not interested in.

It should be added that the dating of Carabus origin is debated even though the literature shows at the moment a unique hypothesis (e.g. several studies by Andujar and colleagues). The latest dating of the beetle-tree-of-life (McKenna et al. 2015; Toussaint et al. 2017) clearly show that Carabinae split from Trachypachidae >150 million years ago. Based on this extremely old divergence, it seems unreasonable to assume an age for the crown of Carabinae at <50 Ma. Most dating studies focusing on Cychrini or Carabini have been based on substitution rates of mitochondrial genes or on biogeographical constraints which are well-known to potentially result in dramatically underestimated ages. This should be kept in mind when analyzing divergence times in Carabus and other related lineages. In their results and discussion, the authors mention the perfect fit of their dating and BioGeoBEARS analyses with the Pleistocene glaciations, emphasizing confidence intervals of tens of thousands of years. This is likely not conservative and could be highly biased considering how shaky the divergence times estimates within Carabus are likely to be.

The placement of taxa based on “taxonomic knowledge” is somewhat dubious and I am having a hard time understanding it. I am not really convinced by the method developed in Rangel et al. (2015). The phylogeny of Carabus is complex and the morphological affinity among species of certain groups has been largely challenged by molecular data in the past decade. Here, the authors place >30% of the species they study in clades of the (moderately to poorly supported) tree based on "taxonomic knowledge". This is not really appropriate in the absence of proper morphological justification, and all analyses based on the resulting topology are likely to be severely biased.

Overall, I feel that the topic of this study is important. Some results are very interesting, such as the grouping of 7 zoogeographical regions and the importance of niche similarity and spatial connectivity in shaping the co-occurrence of species. The different impact of factors in Northern and Southern regions is also very interesting. But I also find that while the authors use quite complicated methods, they pay less attention, to some extent, to the crucial steps required to build either their models (matrices) or datasets (phylogenetic tree and chronogram). They also barely mention any bias or a more balanced view that could originate from their results. For instance, the result that phylogenetic distances were not correlated with regional co-occurrence is likely biased by the suboptimal methodology used to build a chronogram in this study. Similarly, the authors are quite speculative in their discussion but seem to systematically not report any lack of statistical support or confidence intervals. For instance, they disregard the overall complete lack of nodal support in their phylogenetic tree (this is not even mentioned in the text, and not indicated in Fig. 4). The dating analyses are not discussed and there is not confidence interval provided. The likelihood of the BioGeoBEARS analyses is not given. The pattern presented in Fig 4 is one among 100 and has almost no resolution, this might be worth discussing. A final note is the absence of discussion in the context of existing literature regarding Pleistocene range contractions/shift, lineage diversification and faunal re-assembly. There is a large amount of studies looking into this topic using different methodologies, mostly derived from population genomics or phylogeography. This input would be interesting to underscore the importance of these new results in the field.