

Dear Richard Ree,

Thank you very much for your positive appreciation of our work. We provided below replies to the comments and reviews. We followed most of them, including all additional analyses that were suggested. We apologize for not providing the new version with tracked changes. The manuscript had been modified before this option was actually available on PCI. We have submitted the revised version of the article on BioRxiv (doi: <https://doi.org/10.1101/148189>).

We look forward to your reply and thank you in advance for your consideration of our manuscript.

Yours sincerely,

Dr Nicolas Chazot
Department of Biology
Lunds Universitet
Sölvegatan 37
223 62, Lund, Sweden

REPLY TO REFEREES

Edited by Richard Ree

The influence of environmental change over geological time on the tempo and mode of biological diversification is a hot topic in biogeography. Of central interest are questions about where, when, and how fast lineages proliferated, suffered extinction, and migrated in response to tectonic events, the waxing and waning of dominant biomes, etc. In this context the dynamic conditions of the Miocene have received much attention, from studies of many clades and biogeographic regions.

Here, Chazot *et al.* [1] present an exemplary analysis of butterflies (tribe Ithomiini) in the Neotropics, examining their diversification across the Andes and Amazon. They infer sharp contrasts between these regions in the late Miocene: accelerated diversification during orogeny of the Andes, and greater extinction in the Amazon associated during the Pebas system, with interchange and local diversification increasing following the Pebas during the Pliocene.

Two features of this study stand out. First is the impressive taxon sampling (340 out of 393 extant species). Second is the use of ancestral range reconstructions to compute per-lineage rates of colonization between regions, and rates of speciation within regions, through time. The latter allows for relatively fine-grained comparisons across the 2 fundamental dimensions of historical biogeography, space and time, and is key to the main results described above.

The method resonated with me because (full disclosure) I performed a similar analysis in a study showing evidence for uplift-driven diversification in the Hengduan Mountains of China [2]. It's not without caveats -- the most important probably being that extinction is not accounted for in any meaningful sense. More discussion of these would benefit the paper.

>> In the previous version we provided a brief discussion of the shortcomings of not accounting for extinction for ancestral reconstruction of biogeographic areas, which could be an explanation for the relative incongruence we observed between our BioGeoBEARS and BiSSE inferences in some background lineages. We now also refer to published papers on the topic, (Sanmartin and Meseguer 2016, Lieberman 2002), discussing the shortcomings with regard to extinction, and suggests some improvements (p. 12, l. 385-397). However, we feel that an extensive discussion of the biases incurred by heterogeneous extinction in inferring ancestral areas goes beyond the scope of our paper. We propose the hypothesis that extinction in the western Amazonia is influencing our ancestral state reconstruction but we cannot prove such effect.

Also, it is not stated how ancestral ranges at nodes were converted to dispersal events in time, whether at the crowns or stems, or along branches somehow.

**>> The time at which the dispersal events occurred between two nodes was the middle of the branch connecting the two nodes. In Material and Methods (p21, l.695-697) it now reads :
“When a descendent node had a range different from the ancestral node, we considered the middle of the branch connecting these nodes as the timing of the event and recoded it.”**

As noted by the other reviewers, the study makes use of a variety of comparative methods for inferring diversification and geographic range evolution but does not include some that would seem to make sense (e.g., BAMM for inferring shifts in diversification). These omissions are not explained, or rather, the rationale for selecting the methods used is not made clear. In addition to BAMM, the HiSSE model came to mind.

>> Concerning BAMM: We followed the suggestion and decided to perform and include the results of an analysis using BAMM on the Ithomiini (p.7, l. 219 – 222 ; p.19, l. 512 - 618). We report the detailed results as supporting information S8. We decided to mainly focus on the method proposed by Morlon et al. (2011) because they showed in the original paper that when assuming heterogeneity of both speciation AND extinction across clades and time the accuracy of the estimate of past dynamics of diversification largely increased. They demonstrated the power of the method by applying it to the Cetacean phylogeny and recovered a pattern of diversification very close to what is known from the fossil record, including the pattern of declining diversity during the last 10 Ma. No other method so far has been able to recover past dynamics of diversification with such accuracy, including BAMM. BAMM makes the assumption that extinction rate is constant through time, meaning that a decline of diversity cannot be recovered, which proved to be a wrong assumption in the case of Cetacean and also potentially for Ithomiini butterflies. Besides, BAMM has been recently criticized from a more conceptual point of view (Moore et al. 2016). Although Rabosky et al. (2017) have replied to these critics, we feel more comfortable in primarily discussing inferences obtained from a method that has proven to be powerful and so far never put into question.

Concerning HiSSE: We fitted the model HiSSE (Beaulieu & O’Meara 2016) on our dataset, on both the whole tree and the coregroup, similarly to ClaSSE. We fitted seven HiSSE models and four models equivalent to BiSSE for direct comparison (p.20, l. 641 – 648 ; p.8, 239 - 244). The results support the presence of a hidden character, and therefore no evidence of a direct relationship between the Andes or non-Andean region and diversification rate. This confirms our original results, where we did not identify any strong relationship between diversification and the distribution of lineages (Andean or non-Andean), and suggested in the discussion that only some clades probably show increasing diversification in the Northern-Andes specifically but that Amazonian lineages (i.e. non-Andean) also radiated with potentially increased diversification rates (see discussion p.13, l. 420 – 423 and p.14-15, l. 449 - 474). Detailed HiSSE results are shown in supporting information S9.

Also, details are missing such as: why was both ClaSSE and BiSSE used to study the trait "Andean/non-Andean"?

>> ClaSSE allows comparing cladogenetic (at nodes) and anagenetic (along branches) state changes (transitions), whereas BiSSE only accommodates anagenetic changes. In our case, ClaSSE appears more appropriate, because transitions (shift from Andean to non-Andean distribution, and vice-versa) may be associated with speciation events as they entail shifts in multiple ecological dimensions (climate, hostplant, mimicry ring...). However ClaSSE does not implement ancestral state reconstruction. To infer ancestral distribution, we therefore had to use BiSSE models that were equivalent to ClaSSE, but with anagenetic instead of cladogenetic

rates. Both models are highly congruent in terms of parameter estimates. We have now added more information at different places across the manuscript:

p8, l.227 – 228 “Such models estimate character state-dependent rates of speciation, extinction and cladogenetic state transitions (i. e., occurring at nodes).”

P8, l.245 – 247. “Ancestral state inference is not implemented in ClaSSE. Instead, we used the BiSSE 82 model, in which transitions occur only along branches. To infer ancestral states on the whole tree, we fitted the BiSSE models corresponding to the best-fitting ClaSSE models”

Aside from greater clarification/justification of the methods, which I think would amount to relatively minor revisions, the paper is otherwise nicely written and thoughtfully analyzed, and represents a very nice contribution to our understanding of the effects of Miocene/Pliocene environmental change on the evolution of Neotropical biodiversity.

Reviewed by Delano Lewis, 2017-08-15 21:25

The investigation of events in the past is a challenging feat, but one that must be attempted with the best tools available in order to understand basic questions of neotropical diversity. To do this, various informed models have to be proposed and then tested with the best fit model that explains current diversity adopted until more data or better methods prove otherwise. The authors looked at the Andean uplift and the ensuing retreat of the Pebas ecosystem and the effect that the resulting landscape turnover had on neotropical butterfly radiation within the tribe Ithomiini. To do this they inferred a time calibrated phylogeny from molecular data set consisting of 87% of the known species (340 of 393) within the tribe, then tested several models of diversification rates, and finally to use the best fit models to infer the historical biogeography for the tribe.

The English and grammar of the paper appear adequate, although, it should be noted that depending on the journal, British Standard or American Standard may have to be adopted. I have no other comments on grammar other than to point out that on page 3 line 20 "During the late Miocene, during the Andean uplift" may better read with "and" instead of a comma. Also on page 6 line 21 that reads "One was at the root of and the core-group" seems to need modification as it seems the statement includes an error. It is recommended that both these statements be revisited.

>> Both points have now been addressed in the new version of the ms

After going through the introduction, I was expecting to go straight into the material and methods and had to search until they were found after the author contributions at the end of the paper. Due to the length and often times technicality of the cutting edge methods used in these complex analyses, journals often ask that they be lessened in length which often times dilutes them to useless banter. It is commendable that they authors chose to include them in the body of the paper, albeit in an usual position, but it is being suggested that a general paragraph that names the methods used without going into the details be included in the normal position, and that the more detailed discourse be included as one of the appendices. This would greatly reduce the length of the paper and those of us who are more technically minded will give the paper enough scrutiny to find the relevant information where we are directed to look.

>> The methods appear at the end to comply with the formatting requirements of journals where we may submit our ms in the future. But we do agree that the methods were presented too succinctly in the previous version. We now provide more methodological details before presenting each part of the result section.

With regards to the appendices, it is being suggested that they be reviewed and numbered correctly. It was a bit confusing to see two S1s and S8s, no S2, S3, S6, S7 or S9 and four S10s. I do realize that the

missing information, for the most part, is contained within the file labelled Supplemental Information, however it is suggested that the file be named based on all the supplemental information that is contained therein.

>> In have revised the supporting Information. In the previous version all the large figures were separated from the document describing the different supporting information. We have now gathered everything together into a single document.

On to more technical aspects, the authors should state categorically how they avoided much readily available data on species-rich subtribes from influencing (biasing) the calculation of the diversification rates.

>> The sampling fraction is fairly homogeneous throughout the tree: at the subtribe level, it ranges from 0.71 (for the smallest subtribe – 5 out of 7 extant species) to 0.94 for a medium-sized subtribe (29 out of 31 extant species). The sampling fraction of the 8 remaining subtribes ranges from 0.80 to 0.88, and all genera are represented by at least 50% of the species. Moreover, most of the diversification analyses implemented account for missing taxa. We are therefore confident that our sampling strategy does not bias the results.

It is commendable that there was a good link made with food-plant evolution in this group as the retreat of the Pebas most certainly influenced food plant radiation; this was one the first questions that was raised in my mind and it is good that the authors included this in their model developments.

>> Ithomiini have shifted from ancestral Gentianale food-plants to Solanaceae, and this shift may well have contributed to the diversification of the tribe, as proposed by Willmott and Freitas (2006). Two papers have actually attempted to test this hypothesis (Peña and Espeland 2010, Fordyce 2010), using correlative approaches (testing for a shift in diversification rate associated with the hostplant shift, and testing for density-dependent diversification rate – a signature of adaptive radiation –, respectively). But the results of both papers are unreliable, due to methodological flaws. In the first case, the use of the method BiSSE was inappropriate, because the shift to Solanaceae occurred only once (Rabosky and Goldberg 2015). In the second case, the estimation of total Ithomiini diversity, requested to perform the density dependence analysis, was wrong. Moreover, both approaches are highly correlative and indirect (there is no use of food-plant information per se), and even if they were applied correctly, they do not provide a proper test of the hypothesis that Ithomiini diversification has been driven by adaptive radiation on food-plants. So, although there is strong suspicion that this may be so, this hypothesis remains highly speculative (we are working on assembling a database based on our own field observation and on published records to properly test this hypothesis, but the task is challenging due to the diversity of the group and of their food-plants). Finally, we feel this discussion extends beyond the scope of our paper, which is focused on the (dramatic!) impact of past geological events. Major geological changes obviously trigger commensurate ecological changes (local paleo climates, hostplant and predator community...), but we have hardly any information on such changes.

Therefore, in response to your comment, we now mention in two instances, the possible role of food-plants on Ithomiini diversification, in association with the uplift of the Andes and the demise of Pebas, but we don't go any further as there is no proper test or data yet to support this hypothesis, and this is not the main focus of our paper. It now reads:

p.12, l. 372 – 375 : “Diversification was perhaps partly facilitated by an early shift to a new and diverse hostplant family, the Solanaceae, which is particularly diverse in the Neotropics (Willmott and Freitas 2006). This however, remains speculative and is confounded with the simultaneous colonization of the Neotropics.”

p.14, l. 457 – 460. “Lineages that dispersed into the Northern Andes and Amazonia after the demise of the Pebas system probably benefited from a large range of free ecological niches, including a diversity of host-plants that had already diversified or that radiated concomitantly.”

The inclusion of multiple sources of data as well as much of that data as possible makes for robust analyses and it was evident that the authors spent considerable time building a framework that validated the conclusions made.

>> Thank you for appreciating our work!

Caution should be exercised, however, in making a general statement about the neotropical region and perhaps limit these statements to the specific group that was investigated. In the Discussion on page 11 lines 6 - 8 when the authors state that "dynamics of multiple landscape transformations have determined the dynamics of speciation in the Neotropical region." It is suggested that they limit statements to the group that they investigated as the events outlined may not have had the same effect, if any, on other groups of organisms. A modification of that statement to reflect this is suggested.

>> This statement has been modified and now only refers to the Ithomiini (p. 11, l. 354)

In general, I thought the paper was well written, the analysis, though quite technical, seemed robust enough to tease out a model that most likely explains the diversity of the tribe Ithomiini in the Andes and Amazon regions. The tables and diagrams are clear and adequately present the information that their legends say they do and are tastefully enough for the manuscript.

>> Thank you.

Reviewed by anonymous reviewer, 2017-08-21 16:49

This manuscript presents the first species-level phylogeny of the Ithomiini (clear-wing butterflies). They then use this phylogeny to investigate the potential role of geophysical changes in South America in the Miocene and Pliocene (specifically the filling and draining of the Pebas wetland in present-day Western Amazon and the uplift of the Andes) in broad-scale macroevolutionary patterns in ithomiines. The authors examined this question thoroughly using several different biogeographic and macroevolutionary modeling frameworks. However, I have several concerns I would want to see addressed before I would consider recommending this manuscript, in addition to suggesting another editing pass be made to address grammatical errors and to capitalize the "g" in "BioGeoBEARS".

>> We now capitalize the G of BioGeoBEARS

First, the phylogeny used in this study was time-calibrated using secondary calibration points and host-plant divergence times due to a lack of in-group fossil data. While such a practice is fairly standard practice among studies of fossil-poor taxonomic groups, it compounds uncertainty in date estimation. The authors would do well to report the error bars from their time calibrations and consider the implications of this uncertainty in their discussion and conclusions.

We have added credibility intervals in the Results section describing the timing of divergence of subtribes and the origin of Ithomiini (p.6, l. 156 - 172). Besides, uncertainty surrounding divergence time estimates were taken into account in our analyses of the dispersal rates inferred from BioGeoBEARS. Since these rates were computed into 1Ma intervals this might indeed affect our results. One ancestral state estimation was performed on each of the 100 trees randomly sampled from the posterior distribution of our analysis on the timing of divergence and rates computed on each of these estimations (p.21, l. 692 - 694). The detailed results, including boxplots indicating the amount of variation in the pattern we reported can be found Supplementary Information S11.

Furthermore, estimating what can be interpreted as extinction rates from lineages without fossil representation can prove methodologically problematic (see Rabosky, 2010 in Evolution), and the authors should also consider this in their discussion as a probable source of error.

>> The question of extinction rate estimates from molecular phylogenies is a matter of strong debate. Rabosky (2010) indeed argued that extinction should not be estimated from molecular phylogenies. Theoretically however, this should be possible. It was demonstrated by Nee et al. (1994a,b) and Ricklefs (2007) and it was confirmed using simulations (Paradis 2004; Morlon et al. 2011). It is true that many studies have found extinction rates of 0, including for clades for which extinction occurred and it is also true that the pull-of-the-present from which the fraction of extinction had to be estimated is not found in all phylogenies. But Morlon (2014) argues that “this bias in the estimation of extinction rates arises from deviations from the underlying model assumptions, for example, when the underlying assumption of rate homogeneity is violated”. Morlon et al. (2011), which describes the method we use here on Ithomiini demonstrated that, when assuming heterogeneous rates of speciation AND extinction through time AND across clades, extinction can be recovered accurately. Hence the answer to the question of extinction is far from being straightforward.

Yet we stayed cautious in our interpretation of the potential pattern of extinction, suggesting that at least net diversification rates (speciation minus extinction) in background lineages were very low (we use “increasing relative extinction rate”) compared to the core-group. This is clearly identified by all four methods used. We propose the hypothesis that extinction may have driven this low diversification rate since both Morlon et al (2011)’s method and TreePar recovered a pattern of declining diversity. In conclusion it reads (p.15, l. 479 - 482): “Our findings suggest that the ecological turnover that first accompanied the expansion of the Pebas system has led to a decline of diversification, potentially driven by increasing extinction, in early lineages adapted to the ecological conditions that existed during the Oligocene in the upper-Amazon region.” And we “call for further investigations on the role of the Pebas in driving extinction during the Miocene” (p.15, l. 482 - 483).

Second, the authors use a hybrid macroevolutionary modeling method (implementing Medusa, Morlon et al 2011, and TreePar) to identify and quantify diversification rate shifts to account for differences in assumptions among the models. They do this in order to infer the number and position of rate shifts, as well as model how rates may vary between shift points. This study would be greatly improved if it were compared to the results of modeling these processes using BAMM (BAMM 2.5.0; Rabosky

2014), which can both infer likely rate shift points as well as quantify those shifts. While it is true that BAMM has come under criticism lately (Moore et al., 2016 in PNAS), many of these concerns have been addressed in subsequent literature (i.e. Rabosky et al 2017 in Systematic Biology).

>> Performing an analysis with BAMM in addition to our results has been suggested twice. We followed the suggestion and decided to perform an analysis using BAMM on the Ithomiini (p.7, l. 219 – 222 ; p.19, l. 512 - 618). Detailed results can be found in the supporting information S8. In our reply to the editor we provided some arguments justifying our methodological choice that we wish to share here as well.

We decided to mainly focus on the method proposed by Morlon et al. 2011 because they showed in the original paper that when assuming heterogeneity of both speciation AND extinction across clades and time the accuracy of the estimate of past diversification dynamics largely increased. They demonstrated the power of the method by applying it to the Cetacean phylogeny and recovering a pattern of diversification very close to what is known from the fossil record, including the pattern of declining diversity during the last 10 Ma. No other method so far has been able to recover past dynamics of diversification with such accuracy, including BAMM. BAMM makes the assumption that extinction rate is constant through time, which proved to be a wrong assumption in the case of Cetacean and potentially for Ithomiini butterflies as well. Besides, BAMM has been recently criticized from a more conceptual point of view (Moore et al. 2016). Although Rabosky et al. (2017) have replied to these critics, we feel more comfortable in primarily discussing inferences obtained from a method that has proven to be powerful and so far, never called into question.

Finally, the authors state that species were classified as Andean or non-Andean base on GPS coordinates and elevations, but do not state what the sources of this information were, nor what the criteria for classification were. The repeatability of this study would be greatly enhanced by the inclusion of this information

>> We now refer to several published distribution and elevational data, as well as unpublished databases (museum collections and our own field collections). We use the same criteria as in our previous publications (e.g., Elias et al. 2009, Chazot et al. 2016). Specifically, species can be unambiguously classified to either of the two categories, because Andean species are never found in the lowlands, whereas species that occur in the lowlands and that sometimes also occur at the Andean foothills never occur above ca. 800m, and are therefore classified as non-Andean. Species that do not occur in the Andean region (e.g., Atlantic Forest) are obviously considered as non-Andean. All of this is now clearly stated in the Methods section (p.19, l.623 - 628) and the geographic assignment indicated in Supporting Information S1 B.

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