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From Dr. Benoit Guénard
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To Whom It May Concern,

Evaluation of the PhD thesis of Mr. Jimmy Moses by Dr. Benoit Guénard

The thesis of Mr. Moses includes three main research chapter, including one already published within the journal *Biotropica* (congratulations!), a general introduction and a general summary. The central theme of the thesis is the study of ant communities along elevation gradients and their responses to several abiotic factors, mainly within one mountain range in Papua New Guinea, but also through the addition of other datasets (which to my understanding were collected by other researchers) in Ecuador and Tanzania.

Overall, the thesis and results presented are interesting and well-written. I also imagine that the conditions to conduct the fieldwork that has led to these results may have presented several challenges and I acknowledge the work involved here. I also appreciate that the work conducted in Papua New Guinea provides novel data and information on this rich and relatively poorly known fauna, and thus nicely complement the information currently available. **I have thus found the thesis at a satisfactory level to let the candidate pursuing his oral defence**.

I do have, however, a number of general comments that I would like to first express before moving on to more specific comments on particular points of the different chapters. The sampling of ant communities, while easy in appearance, is in fact more difficult that it seems and the use of particular sampling methods or the sampling effort invested for a particular treatment are both very likely to induce particular issues in the interpretation of the data. I believe that it is important in the different chapters of the thesis for these limitations and potential biases to be discussed further and for the interpretations to be presented with caution at the light of those. In particular, the use of baiting is well known to sample a non-random subset of ant species and it would be important for the author to acknowledge and discuss how those limitations could influence the results obtained. I also note that the author used accumulation curves in several chapters, with none of them showing indications of reaching an asymptote. What would then be the consequences of this in the interpretation of the results and confidence in their interpretations?

More information is also needed to fully understand the methodology used in the field. I provide more details below and in the PDF of the thesis attached. It is key for each of the methods used in the field to be detailed so those could eventually be replicated.

To fully assess a thesis or a manuscript, access to the data or the key information is needed. Unfortunately, the data provided in the different appendices fall short of this and I thus urge the author to provide a more detailed account of the data collected (see details below and in the attached PDF).

Spatio-temporal patterns are among the most difficult patterns to interpret and while I fully acknowledge the sampling efforts involved in measuring communities across two seasons; I believe that alternative hypotheses, in particular regarding those about inter-annual variation, have not been fully considered in chapter 3. I thus recommend to develop this further as I am not convinced that the design presented here allows to distinguish between these two distinct time frame.

As a result, this leads me to have several questions and also some hesitations on the conclusions reached as well on the methodology used to answer the questions addressed. There are also a number of contradictions or discrepancies between the different chapters that are not been addressed by the author. I strongly recommend the author to consider those in the final part of the thesis (summary) and to attempt to explain them.

Here are a series of questions that I would like the candidate to consider:

Q1- I would like to start with a first question about vocabulary used. Could you explain the differences between alien/exotic species and an invasive species? Why the use of particular vocabulary is important in ecology and conservation? Any indications from your work or previous studies conducted in PNG or nearby regions to justify the use of the invasive species terminology in your chapter 2? Why the effect of "invasive" species not considered in the chapter 1, while some species are obviously quite abundant.

Q2- Page 7 – Ants are often presented as easy to sample, but is it really true for community ecology studies? Are ants really easier than other insect groups to sample? Or do they present some other advantages that make them particularly suitable for ecological work? I would like you to develop this part a bit more because the sampling protocol and effort invested in ecological studies is absolutely key in establishing a certain level of confidence in the results then obtained.

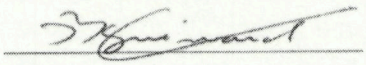
Q3- Page 174 - You observed some levels of variation in food exploitation between seasons. Would you also expect inter-annual variations (not tested here) and if so could it be more important than the seasonal variation observed? Ultimately your experiment has only one replicate for each season, and at the light of the idiosyncrasy observed in the different regions studied, it is not clear to me how you can detangle the effects of seasonality from the one of variation observed between years. Please explain and provide potential design to distinguish both?

Q4- Page 234 - Results for this study (chapter 3) also show that the mid-peak elevational pattern retrieved in the first two chapters was inconsistent at other sites, but also in PNG. How do you explain those differences? This also leads me to ask you the following question related to inconsistencies between your chapters. On page 53 – About *Anoplolepis gracilipes* - An exotic species which appears to be the most abundant of all species collected in your primary forest sites. Do you think this could have impacted the local native abundance and richness? This also contrasts with your results from chapter 2. How do you explain this?

Please contact Dr. Guénard for further information if necessary at bguenard@hku.hk

Best regards,

Dr. Benoit Guénard



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Jimmy Moses' PhD Thesis *"Ecology of ants along elevational rainforest gradients in the tropics"* reviewed by Tom. R Bishop

Overview

The candidate's thesis examines a fascinating topic: what controls the diversity, structure, and ecology of ant communities across tropical elevational gradients? The candidate rightly points out our relative lack of data and understanding on this broad issue and brings together a series of impressive datasets from Papua New Guinea, Ecuador, and Tanzania to tease apart the real drivers of community-level variation in this insect group.

The candidate has clearly completed a wealth of detailed field and lab work and has extended our exploration of an understudied location and fauna. I highly commend the candidate on this. The publication of the first chapter in *Biotropica* is impressive and this work is highly novel, in my view. It sheds new light on the factors that truly constrain tropical mountain ants and, potentially, ants across the globe. This impressive first chapter, however, is let down somewhat by the remainder of the thesis. The thesis is also on the short side – at least in my experience from the UK and South Africa. Throughout, there are three core issues:

(1) Detail: There is a lack of detail and rigour in the assessment and explanation of the literature. Firstly, there are key researchers in the field of ant ecology of relevance to the thesis that are hardly cited or not cited at all. Kaitlin Baudier, Jelena Bujan, Heraldo Vasconcelos and Julie Sheard are not cited and there are numerous papers from Tim Szewczyk, Christy McCain, and Mike Kaspari that are not explored. There is a lot more reading that can be done to support and extend the ideas in this thesis. Secondly, the candidate rarely unpacks or explains the ideas and results found in the literature to the reader. For example, there are many statements along the line of "previous work found that X is linked to Y", but often no explanation of the shape, direction, magnitude, variation, or consequences of these relationships is given. The candidate needs to convey this contextual information to the reader.

(2) Justification: The candidate does not clearly explain to the reader why we need to know these things about tropical ant communities. We are told that we do not know how ant communities respond to factors A and B, or X and Y, but we are rarely told *why* this is important and how it fits into the bigger picture. Some of this justification is touched on in the General Introduction, but greater detail and explanation is needed throughout the thesis. Linked to this, it seems that parts of the thesis are repetitive. For example, each of the chapters tests the link between richness and abundance patterns across elevation. This is not necessarily a problem – the datasets are different in each case – but it is not explained why we need to keep analysing the same pattern in different contexts.

(3) Conclusions: Some of the conclusions are not supported by the data and some are over-interpreted. I expand on these in more detail below.

Overall, I think that the data are good, and the analyses are largely fine (but see below). Work needs to be put in to the introductory and discussion sections to (1) properly place the work in context, (2) convince the reader why the work matters, and (3) to clearly explain *why* certain predictions and hypotheses are made. In my view, this is achievable with the data available, and I encourage the candidate to build more strongly on the impressive practical and analytical work that has taken place. Below, I provide my main thoughts on each chapter and detailed, but not exhaustive, line-by-line comments.

General Introduction

The General Introduction is extensive and covers a large range of topics. The topics are all relevant, but it is not immediately clear how they are supposed to be related. What links all these different topics? What is the main theme? The overarching question? This is not articulated well. I think a paragraph toward the beginning of the introduction outlining the big question (i.e., "understanding patterns of community diversity") before then diving into the various factors that might influence it would help to tie this chapter together.

Page 3: *"However, the insect patterns in species richness and abundance along tropical mountain slopes vary greatly ..."* *How* do they vary with geography and taxonomy? Are there consistent patterns?

Page 9: *"In particular, litter removal had a negative effect on the soil fauna..."* What is meant by a negative effect? A decrease in abundance? Richness? Both? More explanation needed. This is a common complaint I have with the thesis.

Page 11-12: *"However, only a handful studies were conducted at the level of whole communities along wide geographical gradients in the field and using multiple nutrients ..."* What did these studies find? This is an example of the kind of statement that needs to be explained further, if only briefly in this General Introduction.

Chapter 1

This chapter is already published by a reputable journal. Overall, this is a great piece of work illustrating how leaf litter can act as an important microclimatic buffer that influences community diversity patterns at different elevations. I do have some comments that may be useful to the candidate more broadly:

- Temperature was not analysed because it was correlated with elevation. Why not keep temperature and remove elevation? Temperature is surely the variable that actually influences the living world? It is even stated at one point that elevation is simply a proxy for thermal and other environmental change, so why not just use the field-collected temperature data?

- The R^2 values are relegated to the appendix (and this happens in the other chapters too). Why? The R^2s in this case are good and provide important contextual information to the reader about how well the models are capturing patters in the data. I would recommend bringing these statistics into the main text in the future.

Page 39: *"...leaf litter depth decreased monotonically with elevation..."* Figure 4 suggests that leaf litter is invariant to elevation – there is no trend. Am I missing something?

Chapter 2

This manuscript investigates whether the successional process for ants differs across an elevational gradient. This is a great idea but is let down by several issues in the methods and interpretation.

- There is little attempt to link existing empirical or theoretical literature to predictions or hypotheses about how successional and elevational gradients might interact (in fact, this is also a general criticism of the thesis). Instead, the literature is used to talk about them separately before predicting that the rate of succession will be lower at higher elevations. Why? What are the potential mechanisms that might lead to this prediction? The candidate does not explain to the reader why we might expect this pattern – except that a previous study found it. I would suggest cutting much of the text in the introduction focused on ants and PNG and putting in more text explaining the concepts behind this prediction and its source in the literature. Has there been research attempting to understand whether succession takes place at faster or slower rates across environmental gradients? How about forest succession from the tropics to the temperate zone? Might that not provide a baseline for what to expect here? More reading is required, and not just in the tropical ant literature.

- I am not at all convinced by the main conclusion that *"The successional ant communities converged slowly (insignificantly) in species composition with those in primary forest between 2015 and 2017."* First, the communities in the regenerating plots were not significantly different from each other, there is no evidence at all that they have "converged slowly" on the primary forest community (i.e., Figure 5). How can this conclusion be drawn and spoken about when the statistics clearly show that no change has taken place? Second, the time for regeneration has only been two years. I do not think it is reasonable to expect much regeneration over this timescale. This is acknowledged in the manuscript at points, but the conclusion that the "*study supports the assumption that spontaneous forest regeneration leads to a recovery of ant species diversity*" is still repeated. I think this is really overinterpreting the data.

Third, an assumption has been made that the ant community does not change significantly between the time periods. I do not think this is a safe assumption. The ant community will naturally vary through time – maybe by a little, maybe by a lot. Data on this natural, random variation through time is needed to put the temporal change occurring on the regenerating plots in context. For example, have the regenerating plots converged on the primary plots *over and above* the natural, background temporal variation in community composition? Or have the regenerating plots only changed by an amount comparable to this background variation? If they have changed more than the background variation, then that is evidence of community convergence or directional change through time. If they have only changed by an amount comparable to the background variation then we cannot distinguish between directional community change and random ecological drift. Personally, I have worked with data from Borneo and southern Africa which shows significant, and often large, temporal change in ant communities on pristine control plots. There is a wealth of this kind of evidence in the literature too, from ants and many other groups. I expect that the primary forest ant communities of PNG will fluctuate through time in a similar way.

I think it is critical for this kind of study to have estimates of the ant communities in the primary forest or control plots at *all* time points, not just the first one. Otherwise, it is conceptually impossible to disentangle whether the change on the regenerating plots is real and directed, or simply random ecological drift. If the samples for the primary forest plots in 2017 exist, I

strongly encourage the candidate to identify them and include the data here – even if the conclusions are unlikely to change.

Page 70: *"Hethcoat et al. (2019) found that ant community composition and species richness varied with both elevation and succession, and succession affected the communities more at lower than higher elevations."* How did they vary in this study? What were the proposed reasons for the difference at high elevations?

Page 71: Hypothesis 5 says that the rate of succession will be lower at higher elevations. Rate measured as what? Community change over time? This needs to be very explicit about what it is measuring. The similarity of communities between disturbed and primary plots will be higher at low elevations? Unclear.

Page 73: Not enough detail here on how far apart the sampling sites are and at which elevations.

Page 73: 1-3 years? More detail needed on this. Are the lower sites the ones that have been regenerating for longer?

Page 74: How was canopy height measured?

Page 75: The distance between regenerating and primary forest ant transects at each elevation is ~100 m. Is this enough for the plots to be independent?

Page 75: What time of day were the baits put out? For how long? This is critical information.

Page 78: How can a GLM be used when the data points are not independent? The regenerating transects/plots are present in the dataset twice, once from 2015 and once from 2017. This is pseudoreplication and a GLM is not appropriate. A mixed effects model is probably needed. Alternatively, the difference between the years 2017 and 2015 in terms of richness, occurrence, abundance, or community composition can be analysed. Of course, this requires the 2017 data for the primary forest plots.

Page 78: Why construct models with just elevation as a predictor if the goal is to test whether the diversity-elevation pattern depends on succession? This is not a problem *per se*, but the logic needs to be explained to the reader.

Page 90: Again, why are the R^2s in the appendix? These are important numbers and should be in the main text.

Page 92: *"The increasing trend in species richness through time in our study is however more likely to be driven by vegetation restoration towards a higher complexity and productivity than by an abrupt micro-climatic shift."* But how do you know whether this is due to succession or temporal changes driven by some other unrelated factor? This is where the primary forest community data from 2017 would be needed.

Chapter 3

This manuscript investigates how nutrient use by ant varies across three different elevational gradients on three continents. I commend the macroecological approach taken here – we need more of this kind of data collection and synthesis. However, the manuscript suffers from several of the general problems that I describe above in terms of detail and justification.

explanation needed. What are the wider consequences of changes in trophic guild composition?

Page 147: *"ecological stoichiometry rules"* What are these? Unclear.

Page 148: *"ant communities rather decreased with elevation"*. What does this mean? Ant communities decreased in terms of abundance, richness? Clear writing is needed to fully convey to the reader what is being spoken about.

Line 149: *"However, that study was conducted only in one season of the highest ant activity and it did not sample along elevational gradients."* No, but the Lasmar et al. (2021) study did sample a large range of climatic conditions. Is elevation special? Or is it just a model system where temperature (and other variables) varies a lot over a small spatial range?

Page 153: How exactly were $\delta^{15}N$ ratios used to assign species to functional groups? How were functional groups assigned for the PNG and Ecuador ants? The Peters paper only uses Tanzanian ants, so it cannot be used to assign feeding groups for the other areas, can it? Much more detail is needed on how this trophic assignment was done.

Page 154: AICc is not a "test". Be careful about phrasing it in this way.

Page 155: *"First, we calculated the proportion of baits occupied by ants as (i) the average per region, (ii) for each region separately, and (iii) between the two seasons."* Is this for each elevation? For each nutrient? Each nutrient at each elevation? It is not clear.

Page 169: *"Rather, we found that ant activity patterns varied between dry and wet season"* OK, so remind the reader what these patterns were. Was activity higher in the wet or the dry season?

Page 170 and 175: Ants are not endotherms. I am sure the candidate knows this and that it is a simple typo or mistake, but this really should have been picked up before.

Summary

The Summary chapter is short. There is no element of synthesis here. Rather, very brief summaries of each chapter are given, and a couple of future directions are explained. This Summary chapter should be used to highlight the general lessons that can be learnt from this thesis. What are the key themes of ant ecology that these chapters reveal that we did not previously know? What does this mean moving forward?

This might also be a good place to discuss how these ecological principles can be placed in an evolutionary context. Is PNG special and unique? Or would we expect ants everywhere to obey these rules?

Page 234*: "Although there was a slow convergence of succession in ant communities between the regenerating and primary species composition"* Again, I do not think this conclusion is supported by the data.

Questions for Defence Ceremony

(1) Chapter 1 suggests that in the lowlands, litter depth limits ant diversity as it provides microsites for nesting and foraging. In the highlands, temperature limits the ants, and so thin litter layers result in higher ant diversity. This is a believable story, but it is a correlation. How could this idea be tested further using either experiments or further observational data?

Further, I think the candidate has missed an opportunity to fully understand the data by only analysing them with elevation as the main predictor variable.

- It is not made clear what the real goal of this work is. Nutrient preferences may change, but what does this tell us about how the natural world works? There is little explanation in the introduction as to what the overarching goal is. There is one mention of how nutrients may shape the elevational distributions of ants, but why do we need to know about these elevational distributions? Again, more work is needed to place this study in a broader context and to explain to the reader why this is important.

- What are the hypotheses about the dry/wet seasons? Almost no detail or hypotheses are put forward as to *why* ant activity or nutrient preference may depend on season. What is the mechanism? Presumably, something related to rainfall or perhaps temperature? The candidate points out that other studies have found seasonal effects (or not) but does not really explain the reason for why this is the case.

- No information is given as to how the baits were laid out along the mountains in the main text. Were they all placed at the low elevations? The highest elevations? Were they distributed evenly across the elevational gradient? Which? It is critical that this information is in the main text, not hidden in the supporting information.

- There is a lot in the results about species richness and abundance across elevation. Is that what we are interested in? I thought we already knew this from Chapter 1 and from the other papers using the Ecuador and Tanzania data. Further, I thought the question was about nutrient use. Some more explanation is needed to justify why we need to know about species richness etc here, or perhaps these analyses can be removed or put into the supplementary material.

- The data are analysed here with elevation as the main predictor variable. This is fine in principle. However, later in the results and discussion in becomes clear that there is some idiosyncrasy in the findings. For example: *"Rather, there is a notable inconsistency in the relative use of different nutrients among the regions across elevations and seasons..."* Perhaps this inconsistency is driven by the fact that the elevations are not necessarily equivalent across the three sites.

The full data are not presented in the methods, but it seems that the elevation-temperature and elevation-rainfall relationships have different intercepts (i.e., the temperature or rainfall at 0 m a.s.l.) and slopes (i.e., the change in temperature or rainfall per m increase in elevation) at the different sites. Consequently, 1000 m a.s.l. may represent different thermal or rainfall conditions across the three mountains. Comparing elevations directly in this way may not be a fair test. Why not analyse with temperature and rainfall data as the explanatory variables instead? This may explain some of the inconsistency in the results and will generalise a lot more to other studies – 10°C is always 10°C, but 1000 m a.s.l. has different environmental conditions depending on where you are on the planet. These data may be available already or could be extracted from global climatic datasets like WorldClim or MicroClim. At the least, some argument against this approach should be provided if the candidate does not think it would be useful.

Page 146: *"commonly neglected in the studies of elevational gradients is the composition of trophic guilds"* Why does this matter? Why do we need to know? More unpacking and

(2) If you had *unlimited* resources and time, how would you design an experiment to test whether the rate of ant succession differed across environmental gradients?

(3) Do you think the elevational patterns in trophic guilds that you find would be the same across a latitudinal gradient? Why?

(4) What kinds of functional trait of phylogenetic data would you like to collect, and do you think these alternative measures of diversity would change the patterns that you observe?