Local- versus broad-scale environmental drivers of continental *β*-diversity patterns in subterranean spider communities across Europe

Stefano Mammola, Pedro Cardoso, Dorottya Angyal, Gergely Balázs, Theo Blick, Hervé Brustel, Julian Carter, Srećko Ćurčić, Samuel Danflous, László Dányi, Sylvain Déjean, Christo Deltshev, Mert Elverici, Jon Fernández, Fulvio Gasparo, Marjan Komnenov, Christian Komposch, L'ubomír Kováč, Kadir Boğaç Kunt, Andrej Mock, Oana Teodora Moldovan, Maria Naumova, Martina Pavlek, Carlos E. Prieto, Carles Ribera, Robert Rozwałka, Vlastimil Růžička, Robert S. Vargovitsh, Stefan Zaenker and Marco Isaia

Review History

RSPB-2019-1579.R0 (Original submission)

Review form: Reviewer 1 (Ingi Agnarsson)

Recommendation
Accept with minor revision (please list in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?
Excellent

General interest: Is the paper of sufficient general interest?
Excellent

Quality of the paper: Is the overall quality of the paper suitable?
Excellent

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
Yes

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
No

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

> **Is it accessible?**
> N/A
>
> **Is it clear?**
> N/A
>
> **Is it adequate?**
> N/A

Do you have any ethical concerns with this paper?
No

Comments to the Author
This is an excellent 'big data' paper making fundamental advances in the study of cave biodiversity and its drivers. The data are thoroughly analysed and the paper is very well written. I have only a few minor comments:

"Conversely, troglobiont species rarely occurred in more than two caves (mean±s.d. = 2.15±2.70; range = 1–21)."
Additionally, keep in mind that species identified based on morphological characters alone, may represent species complexes in caves as demonstrated by DNA data in various studies (e.g. Esposito et al. 2015, several Hedin papers). So the real number of caves per species may be lower than indicated here.
Mention total number of caves in results

In the caves I'm most familiar with it is clear that bat guano has a major effect on cave biota as bats dramatically increase the energy input in caves. I am wondering if the authors have some data on bat presence/abundance in these caves.

Sincerely, Ingi Agnarsson

Review form: Reviewer 2

Recommendation
Accept with minor revision (please list in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?
Good

General interest: Is the paper of sufficient general interest?
Good

Quality of the paper: Is the overall quality of the paper suitable?
Good

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
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In my point of view, the research question addressed is especially interesting and appropriate for the scope of this journal. This manuscript can potentially reach a broad audience of ecologists in general and it is of special interest for arachnologists and speleologists. In summary, I found this manuscript to be very well written and the development of the main ideas particularly easy to follow. The authors have nicely posed the research question and adequately put into context the

model organisms and theoretical background. Most part of the methods and results are well described and the discussion is interesting.

On the other hand, despite the potential of the study, I have two major doubts concerning the current version of the manuscript that I recommend be either addressed or clarified prior to publication. They are related to choices in the statistical analyses that remained unclear to me, which, I suspect, could have affected the observed patterns.

(i) first, the authors detected no significant role of local variables on both subsets of cave-dwelling spider communities. They claim (Page 14; lines 25-29) that the influence of local features would be primarily detected on species abundance, which allegedly is not the case of the dataset used (simple presence/absence of species). However, in Page 10 (lines 2-6), they do not describe on the 'nature' of the dataset (i.e., whether it is abundance- or incidence-based). I suggest the authors inform it.

(ii) second, although I am not a specialist in all the statistical properties of GDM, in the statistical analyses subsection, the authors stated that the pairwise dissimilarity matrices employed in the GDM were computed with the Bray-Curtis dissimilarity coefficient. To my knowledge, this coefficient is adequate to quantitative data (i.e., abundance-based) (not to incidence matrices). Although I agree with the choice to weight the metrics by species richness, the justification provided ('particularly suited for detecting underlying ecological gradients'; 'routinely used in generalized dissimilarity modeling') are not consistent. Therefore, unless there is a particular (and yet undescribed) procedure that justifies the usage of a semi-metric index on an incidence data, I highly recommend informing it (or re-ran the tests based on adequate coefficients). I was particularly circumspect regarding this choice, which made me question whether it could have affected the observed result.

Minor comments:
1. There are results on alpha diversity, but they are completely overlooked in the discussion. My suggestion is to briefly address this result.

2. the two last sentences in the first paragraph of the Discussion (Page 13; lines 4-9) are too broad and focus artifacts not related to the discussion of the main results; they seem unnecessary for the section. They become important in view of the requirements of this journal on manuscript length.

Decision letter (RSPB-2019-1579.R0)

13-Sep-2019

Dear Dr Mammola:

Your manuscript has now been peer reviewed and the reviews have been assessed by an Associate Editor. The reviewers' comments (not including confidential comments to the Editor) and the comments from the Associate Editor are included at the end of this email for your reference. All of us find quite a lot to like about your manuscript. However, as you will see, the reviewers and the AE have raised some concerns and we would like to invite you to revise your manuscript to address them (detailed comments are below).

We do not allow multiple rounds of revision so we urge you to make every effort to fully address all of the comments at this stage. If deemed necessary by the Associate Editor, your manuscript

will be sent back to one or more of the original reviewers for assessment. If the original reviewers are not available we may invite new reviewers. Please note that we cannot guarantee eventual acceptance of your manuscript at this stage.

To submit your revision please log into http://mc.manuscriptcentral.com/prsb and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions", click on "Create a Revision". Your manuscript number has been appended to denote a revision.

When submitting your revision please upload a file under "Response to Referees" - in the "File Upload" section. This should document, point by point, how you have responded to the reviewers' and Editors' comments, and the adjustments you have made to the manuscript. We require a copy of the manuscript with revisions made since the previous version marked as 'tracked changes' to be included in the 'response to referees' document.

Your main manuscript should be submitted as a text file (doc, txt, rtf or tex), not a PDF. Your figures should be submitted as separate files and not included within the main manuscript file.

When revising your manuscript you should also ensure that it adheres to our editorial policies (https://royalsociety.org/journals/ethics-policies/). You should pay particular attention to the following:

Research ethics:
If your study contains research on humans please ensure that you detail in the methods section whether you obtained ethical approval from your local research ethics committee and gained informed consent to participate from each of the participants.

Use of animals and field studies:
If your study uses animals please include details in the methods section of any approval and licences given to carry out the study and include full details of how animal welfare standards were ensured. Field studies should be conducted in accordance with local legislation; please include details of the appropriate permission and licences that you obtained to carry out the field work.

Data accessibility and data citation:
It is a condition of publication that you make available the data and research materials supporting the results in the article. Datasets should be deposited in an appropriate publicly available repository and details of the associated accession number, link or DOI to the datasets must be included in the Data Accessibility section of the article (https://royalsociety.org/journals/ethics-policies/data-sharing-mining/). Reference(s) to datasets should also be included in the reference list of the article with DOIs (where available).

In order to ensure effective and robust dissemination and appropriate credit to authors the dataset(s) used should also be fully cited and listed in the references.

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If you have already submitted your data to dryad you can make any necessary revisions to your dataset by following the above link.

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All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI. Please try to submit all supplementary material as a single file.

Online supplementary material will also carry the title and description provided during submission, so please ensure these are accurate and informative. Note that the Royal Society will not edit or typeset supplementary material and it will be hosted as provided. Please ensure that the supplementary material includes the paper details (authors, title, journal name, article DOI). Your article DOI will be 10.1098/rspb.[paper ID in form xxxx.xxxx e.g. 10.1098/rspb.2016.0049].

Please submit a copy of your revised paper within three weeks. If we do not hear from you within this time your manuscript will be rejected. If you are unable to meet this deadline please let us know as soon as possible, as we may be able to grant a short extension.

Thank you for submitting your manuscript to Proceedings B; we look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Best wishes,
Dr Sarah Brosnan
Editor, Proceedings B
mailto: proceedingsb@royalsociety.org

Associate Editor
Board Member: 1
Comments to Author:
This study comparing the relative influence of local- and broad-scale environmental variables on cave-dwelling spider communities across the European continent has now received two reviews from experts in the field and I have read the MS myself. Examining the role of local habitat features in driving broad-scale β-diversity patterns is novel in part because of the challenges of comparing broad biological patterns without the confounding effect of local habitat features (something overcome by examining cave-dwelling communities of spiders). I agree that the MS was of potential broad interest. Nonetheless, more information is required and there are at least two significant caveats that would have to be addressed with revision to support the conclusions. First, more information is needed on the dataset to inform whether the conclusions are actually supported by the data, namely with respect to the local variable (Referee 2). I tend to agree that this should be relatively easy to accommodate, but may have impacts to the study. Second, I agree that further justification is warranted for the quantitative approach (e.g., Bray-Curtis dissimilarity coefficient, Referee 2). This aspect may require further analyses to demonstrate support for the findings. Finally, there are number of minor points raised from both Referees that should be addressed to increase the clarity of the study. For all of these reasons, I am recommending revision to address these important points raised in the review.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)
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"Conversely, troglobiont species rarely occurred in more than two caves (mean±s.d. = 2.15±2.70; range = 1–21)."
Additionally, keep in mind that species identified based on morphological characters alone, may represent species complexes in caves as demonstrated by DNA data in various studies (e.g. Esposito et al. 2015, several Hedin papers). So the real number of caves per species may be lower than indicated here.
Mention total number of caves in results

In the caves I'm most familiar with it is clear that bat guano has a major effect on cave biota as bats dramatically increase the energy input in caves. I am wondering if the authors have some data on bat presence/abundance in these caves.

Sincerely, Ingi Agnarsson

Referee: 2

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On the other hand, despite the potential of the study, I have two major doubts concerning the current version of the manuscript that I recommend be either addressed or clarified prior to publication. They are related to choices in the statistical analyses that remained unclear to me, which, I suspect, could have affected the observed patterns.

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Minor comments:
1. There are results on alpha diversity, but they are completely overlooked in the discussion. My suggestion is to briefly address this result.

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Author's Response to Decision Letter for (RSPB-2019-1579.R0)

See Appendix A.

Decision letter (RSPB-2019-1579.R1)

04-Oct-2019

Dear Dr Mammola

I am pleased to inform you that your manuscript entitled "Local versus broad scale environmental drivers of continental beta diversity patterns in subterranean spider communities across Europe" has been accepted for publication in Proceedings B.

You can expect to receive a proof of your article from our Production office in due course, please check your spam filter if you do not receive it. PLEASE NOTE: you will be given the exact page length of your paper which may be different from the estimation from Editorial and you may be asked to reduce your paper if it goes over the 10 page limit.

If you are likely to be away from e-mail contact please let us know. Due to rapid publication and an extremely tight schedule, if comments are not received, we may publish the paper as it stands.

If you have any queries regarding the production of your final article or the publication date please contact procb_proofs@royalsociety.org

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Electronic supplementary material:
All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI.

Thank you for your fine contribution. On behalf of the Editors of the Proceedings B, we look forward to your continued contributions to the Journal.

Sincerely,

Dr Sarah Brosnan
Editor, Proceedings B
mailto: proceedingsb@royalsociety.org

Appendix A

Associate Editor

This study comparing the relative influence of local- and broad-scale environmental variables on cave-dwelling spider communities across the European continent has now received two reviews from experts in the field and I have read the MS myself. Examining the role of local habitat features in driving broad-scale β-diversity patterns is novel in part because of the challenges of comparing broad biological patterns without the confounding effect of local habitat features (something overcome by examining cave-dwelling communities of spiders). I agree that the MS was of potential broad interest. Nonetheless, more information is required and there are at least two significant caveats that would have to be addressed with revision to support the conclusions. First, more information is needed on the dataset to inform whether the conclusions are actually supported by the data, namely with respect to the local variable (Referee 2). I tend to agree that this should be relatively easy to accommodate, but may have impacts to the study. Second, I agree that further justification is warranted for the quantitative approach (e.g., Bray-Curtis dissimilarity coefficient, Referee 2). This aspect may require further analyses to demonstrate support for the findings. Finally, there are number of minor points raised from both Referees that should be addressed to increase the clarity of the study. For all of these reasons, I am recommending revision to address these important points raised in the review.

RESPONSE:
Thank you for handling this submission and for the useful guidance on how to revise the manuscript. As detailed in the point-by-point responses below, we have fully taken into consideration the constructive comments by the two referees. To facilitate the assessment of this revision, we have highlighted changes in the main text in steal blue.

Regarding the Bray-Curtis dissimilarity issue raised by the referees, it is actually easier that it might seem at a first look. This because it is just a matter of terminology. Bray Curtis index is based on abundance data, while when dealing with presence/absence data, Soerensen index is preferred. Indeed, the two indices are very similar. In fact, if a Bray-Curtis dissimilarity is applied to presence/absence data, the result is the same as if you had applied Sorensen index;in other words, the two indices behave identically. We have called it Bray-Curtis through the text because this is the way in which the distance measure is named in the R package 'gdm'. However, we agree that this might generate unnecessary confusion in a reader, and thus we have now consistently called it Sørensen in the text.

We believe that the manuscript has greatly improved as a result of the constructive

comments received in the previous submission. We are confident that it will considered suitable for publication in Proceedings B.

>>>>>> Referee: 1

Comments to the Author(s)

This is an excellent 'big data' paper making fundamental advances in the study of cave biodiversity and its drivers. The data are thoroughly analysed and the paper is very well written.

RESPONSE: Thank you for spending time in revising our manuscript and for your positive attitude toward our work.

I have only a few minor comments:

"Conversely, troglobiont species rarely occurred in more than two caves (mean±s.d. = 2.15±2.70; range = 1–21)."

RESPONSE: Done.

Additionally, keep in mind that species identified based on morphological characters alone, may represent species complexes in caves as demonstrated by DNA data in various studies (e.g. Esposito et al. 2015, several Hedin papers). So the real number of caves per species may be lower than indicated here.

RESPONSE: Very nice point! It is certain true that there should be some cryptic species diversity in our dataset. Yet, quite remarkably, it has been recently demonstrated that this is not a significant shortcoming in macroecological studies of subterranean species, since overlooked cryptic species diversity is generally homogeneously distributed along environmental gradients (Eme et al., 2018). We have now briefly mentioned this caveat in the discussion.

Eme, D., Zagmajster, M., Delić, T., Fišer, C., Flot, J. F., Konecny‑Dupré, L., ... & Malard, F. (2018). Do cryptic species matter in macroecology? Sequencing European groundwater crustaceans yields smaller ranges but does not challenge biodiversity determinants. *Ecography*, *41*(2), 424-436.

Mention total number of caves in results

RESPONSE: Done.

In the caves I'm most familiar with it is clear that bat guano has a major effect on cave biota as

bats dramatically increase the energy input in caves. I am wondering if the authors have some data on bat presence/abundance in these caves.

RESPONSE: This is actually an interesting idea for a potential study modelling the distribution of guanobionts species in relation to the distribution of most common bat species. Unfortunately, we currently lack distribution data of most bat species. Anyway, we believe that such an analysis would be more suited for a tropical context, because bat guano is a significant driver of subterranean diversity in tropical caves, where roosting bat colonies can be numerically abundant and the consequent deposition of guano substantial (so-called "guano caves"). It has been demonstrated (e.g. Ferreira & Martins 1998) that in guano caves, spiders proved to be influenced by the distribution of guano, because of the use of guanobiont arthropods as prey. On the other hand, European caves behave quite differently because the availability of guano (if any) is typically very reduced and spatially localized. *Ipso facto*, a similar parameter has never been considered in continental macroecological analyses of subterranean species in Europe.

Ferreira, R. L., & Martins, R. P. (1998). Diversity and distribution of spiders associated with bat guano piles in Morrinho cave (Bahia State, Brazil). *Diversity and distributions*, 235-241.

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RESPONSE: Thank you for spending time in revising our manuscript and for your positive

attitude toward our work.

On the other hand, despite the potential of the study, I have two major doubts concerning the current version of the manuscript that I recommend be either addressed or clarified prior to publication. They are related to choices in the statistical analyses that remained unclear to me, which, I suspect, could have affected the observed patterns.

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RESPONSE: Thank you for highlighting this important point. This information was indeed only available in Appendix S1, where we described in great details all variables in the dataset. We have now clearly specified the nature of the species data also in the main text.

(ii) second, although I am not a specialist in all the statistical properties of GDM, in the statistical analyses subsection, the authors stated that the pairwise dissimilarity matrices employed in the GDM were computed with the Bray-Curtis dissimilarity coefficient. To my knowledge, this coefficient is adequate to quantitative data (i.e., abundance-based) (not to incidence matrices). Although I agree with the choice to weight the metrics by species richness, the justification provided ('particularly suited for detecting underlying ecological gradients'; 'routinely used in generalized dissimilarity modeling') are not consistent. Therefore, unless there is a particular (and yet undescribed) procedure that justifies the usage of a semi-metric index on an incidence data, I highly recommend informing it (or re-ran the tests based on adequate coefficients). I was particularly circumspect regarding this choice, which made me question whether it could have affected the observed result.

RESPONSE: Thank you for raising this important point that might have generated confusion in a number of readers of this paper. The problem here is not a statistical one, but rather a matter of terminology.

Bray-Curtis can be applied to presence/absence data, but in this case is more typically known as Sørensen index. See, e.g., this discussion:

https://www.researchgate.net/post/What_is_the_difference_between_Bray-Curtis_Similarity_Sorensen_Distance_and_Bray-Curtis_Index

We used the term Bray-Curtis through the text because this is the way in which the distance measure is named in the R package 'gdm'. Thanks to your comment, we now see that this might generate unnecessary confusion in a reader. Thus, we have now consistently used the term Sørensen.

Minor comments:
1. There are results on alpha diversity, but they are completely overlooked in the discussion. My suggestion is to briefly address this result.

RESPONSE: True. We now started the discussion with a brief discussion alpha diversity results.

2. the two last sentences in the first paragraph of the Discussion (Page 13; lines 4-9) are too broad and focus artifacts not related to the discussion of the main results; they seem unnecessary for the section. They become important in view of the requirements of this journal on manuscript length.

RESPONSE: We agree. We removed this.