Complex ecological interactions across a focus of cutaneous leishmaniasis in Eastern Colombia: novel description of Leishmania species, hosts and phlebotomine fauna

Claudia M. Sandoval-Ramírez, Carolina Hernández, Aníbal A. Teherán, Reinaldo Gutierrez-Marín, Ruth A. Martínez-Vega, Duvan Morales, Richard Hoyos-Lopez, Astrid Araque-Mogollón and Juan David Ramírez

Review History

RSOS-200266.R0 (Original submission)

Review form: Reviewer 1

Is the manuscript scientifically sound in its present form?
No

Are the interpretations and conclusions justified by the results?
No

Is the language acceptable?
Yes

Do you have any ethical concerns with this paper?
No
Have you any concerns about statistical analyses in this paper?
No

Recommendation?
Reject

Comments to the Author(s)
In this study, to elucidate transmission dynamics of cutaneous leishmaniasis in a limited endemic area, the authors analyzed ecology and feeding source of sand flies, and Leishmania species in sand flies. Although many data were presented and analyzed, it was unfocused, and more importantly, there are many issues for the data collection process that is the key for this study.

1. Detection of Leishmania DNA was performed using engorged female sand flies. However, vector research must be done with sand flies without blood in the gut. Vector species support specific parasite growth, in other words, parasites must be detected in the vector even after digestion of host blood. Therefore, Leishmania DNA detected in this study possibly derived from host blood at the best.
2. The positive rate of sand flies is impossibly high. I doubt contamination during sample preparation processes. If the authors believe this data is correct, it must be shown by dissection of sand flies. Dissection of only 10 sand flies will be enough if the results are correct.
3. As the ecology of sand flies, the authors focused on only flora of each area. However, temperature and humidity affect more on sand fly ecology. In addition, fauna is another important factor as the feeding source of sand flies, and it must be addressed in this kind of study.
4. Tables 1 and 2 should be moved to supplementary data.
5. Overall, the resolution of figures must be improved.
6. No sequence data was presented. The data must be shown as a supplementary data or, at least, deposited in GenBank.
7. The purpose of barcoding is unclear.
8. Discussion is too long and unfocused.

Review form: Reviewer 2

Is the manuscript scientifically sound in its present form?
Yes

Are the interpretations and conclusions justified by the results?
Yes

Is the language acceptable?
Yes

Do you have any ethical concerns with this paper?
No

Have you any concerns about statistical analyses in this paper?
No

Recommendation?
Accept with minor revision (please list in comments)
Comments to the Author(s)

The manuscript “Complex ecological interactions across a focus of cutaneous leishmaniasis in eastern Colombia” aimed to analyze the patterns of diversity, food preferences, and Leishmania species in the sand fly to elucidate the transmission dynamics in one focus of cutaneous leishmaniasis. The study was done in rural areas in the municipality of Arboledas in the state of Norte de Santander, Colombia.

It is an elegant and complete study about cutaneous leishmaniasis epidemiology and should be published.

Major concern

The study is very descriptive and did not, in my opinion, show graphically where the transmission cycles overlap. The actual figures (5 and 6) help to understand, but in fact, the transmission cycles could be presented by octopus and not by parasite, reservoir, and vector.

What cycles of what species of parasites are contained in an area. The authors limit themselves to saying that transmission scenarios in the country are more complex than previously thought. These are overlapping transmission cycles within the same ecological scenario. I was hoping that at the end of the manuscript, the authors would give an overview of what is happening. Would not there be a host or vector specificity? Would there be an anthropization of cutaneous leishmaniasis? Would the parasite mosaic theory or the Stockholm paradigm explain what is happening?

Minor concerns

1. In the introduction, the authors give a number that needs to be revised. According to Alvar and colleagues, 98 countries, 3 territories and 5 continents are affected by leishmaniasis (Alvar et al. 2012). The authors state that according to the World Health Organization (WHO), leishmaniasis is present in at least 88 countries in the world and cite Alvar et al, 2012.

2. Line 120, please revise. The collection was done in 3 nights and not in 3 days,

3. In lines 324 - 326, please delete the first paragraph. It is about materials and methods.

4. Inline 179, please delete the bold characters.

5. The references do not respect the rules of the nomenclature. The scientific name should be put in italics and the names of journals should be put as the guidelines.

6. The sentence of 364 is taken from the authors or from Urrutia et al. 2018?

7. Inline 385, what is the L? please give by the extender.

8. Inline 387, tables 1 and 2 are correct.

9. To what the authors attribute the fact that the authors did not find Lu longipalpis. Wouldn't it be because the vector has already been urbanized?

10. In line 509, I think that rote transmission is not the best word.

Review form: Reviewer 3

Is the manuscript scientifically sound in its present form?  
Yes

Are the interpretations and conclusions justified by the results?  
Yes

Is the language acceptable?  
Yes

Do you have any ethical concerns with this paper?  
No
Have you any concerns about statistical analyses in this paper?
No

Recommendation?
Accept with minor revision (please list in comments)

Comments to the Author(s)
Congratulations on the manuscript and the scientific work. The statistical analysis is strong and makes it possible to establish the conclusions clearly. Nevertheless it is necessary to fit some simple topics.
In line 28 and 36 the term “food preferences” is very broad. There is only evidence of blood intake. Adjustment is required.
In line 29 explain the ecoregion where the focus of leishmaniasis studied is located.
Specify the department where the two locations are located (Summary).
It is possible to better compare the diversity indices between the Localities (Macro approach) and between microenvironments (Micro approach). It is also necessary to place numeric values of the indexes (Summary).
In the line 44, can improve the redation of "circulating feeding sources of domestic animals". The term circulation is confused.
In the introduction it is necessary to include the abbreviation of Leishmania the first time that it is enunciated, to continue using it in the rest of the document.
In the line 97 to complement “Groves, North of Santander”
Update the reference of Galati 2016 and Galati 2003; by the keys of Galati 2018, on line 33 and Linea 38.
Because only visually blood-fed females were included. It is necessary to clarify the selection criteria and the way in which they interpret the result.
Line 169. Include Network 5.0 Reference
Line 174-200: Ecology of sand flies: The text is very extensive, it is suggested to summarize.
“Detection of feeding sources”. Modify title more specifically.

Decision letter (RSOS-200266.R0)

We hope you are keeping well at this difficult and unusual time. We continue to value your support of the journal in these challenging circumstances. If Royal Society Open Science can assist you at all, please don't hesitate to let us know at the email address below.

Dear Dr Ramírez,

The editors assigned to your paper ("Complex ecological interactions across a focus of cutaneous leishmaniasis in eastern Colombia") have now received comments from reviewers. We would like you to revise your paper in accordance with the referee and Associate Editor suggestions which can be found below (not including confidential reports to the Editor). Please note this decision does not guarantee eventual acceptance.

Please submit a copy of your revised paper before 20-May-2020. Please note that the revision deadline will expire at 00.00am on this date. If we do not hear from you within this time then it will be assumed that the paper has been withdrawn. In exceptional circumstances, extensions may be possible if agreed with the Editorial Office in advance. 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 Editors, 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.
To revise your manuscript, log into http://mc.manuscriptcentral.com/rsos 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. Revise your manuscript and upload a new version through your Author Centre.

When submitting your revised manuscript, you must respond to the comments made by the referees and upload a file "Response to Referees" in "Section 6 - File Upload". Please use this to document how you have responded to the comments, and the adjustments you have made. In order to expedite the processing of the revised manuscript, please be as specific as possible in your response.

In addition to addressing all of the reviewers' and editor's comments please also ensure that your revised manuscript contains the following sections as appropriate before the reference list:

- **Ethics statement (if applicable)**
  If your study uses humans or animals please include details of the ethical approval received, including the name of the committee that granted approval. For human studies please also detail whether informed consent was obtained. For field studies on animals please include details of all permissions, licences and/or approvals granted to carry out the fieldwork.

- **Data accessibility**
  It is a condition of publication that all supporting data are made available either as supplementary information or preferably in a suitable permanent repository. The data accessibility section should state where the article's supporting data can be accessed. This section should also include details, where possible of where to access other relevant research materials such as statistical tools, protocols, software etc can be accessed. If the data have been deposited in an external repository this section should list the database, accession number and link to the DOI for all data from the article that have been made publicly available. Data sets that have been deposited in an external repository and have a DOI should also be appropriately cited in the manuscript and included in the reference list.

If you wish to submit your supporting data or code to Dryad (http://datadryad.org/), or modify your current submission to dryad, please use the following link: http://datadryad.org/submit?journalID=RSOS&manu=RSOS-200266

- **Competing interests**
  Please declare any financial or non-financial competing interests, or state that you have no competing interests.

- **Authors’ contributions**
  All submissions, other than those with a single author, must include an Authors’ Contributions section which individually lists the specific contribution of each author. The list of Authors should meet all of the following criteria; 1) substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; 2) drafting the article or revising it critically for important intellectual content; and 3) final approval of the version to be published.

  All contributors who do not meet all of these criteria should be included in the acknowledgements.

  We suggest the following format:
  AB carried out the molecular lab work, participated in data analysis, carried out sequence alignments, participated in the design of the study and drafted the manuscript; CD carried out the statistical analyses; EF collected field data; GH conceived of the study, designed the study, 


coordinated the study and helped draft the manuscript. All authors gave final approval for publication.

- **Acknowledgements**
  Please acknowledge anyone who contributed to the study but did not meet the authorship criteria.

- **Funding statement**
  Please list the source of funding for each author.

Once again, thank you for submitting your manuscript to Royal Society Open Science and I look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Kind regards,

Anita Kristiansen
Editorial Coordinator

Royal Society Open Science
openscience@royalsociety.org

on behalf of Dr Krijn Paaijmans (Associate Editor) and Pete Smith (Subject Editor)
openscience@royalsociety.org

Associate Editor’s comments (Dr Krijn Paaijmans):
Comments to the Author:

Dear authors,

I agree with reviewers that your study about cutaneous leishmaniasis epidemiology in Colombia is elegant and provides a substantial contribution to our understanding of the interaction between sand flies and blood meal hosts.

The reviewers did, however, raise some concerns that need to be addressed. In particular, the comment of reviewer 1 about the detection of Leishmania DNA (using complete sandfly bodies) is worrying me, as parasites may have been present in the host blood meal, which does not mean the sandfly species is a competent vector. If this point cannot be addressed (due to the lack of additional samples for analysis), this caveat should be clearly highlighted in both the results and discussion. The remaining data on densities, blood meal analysis, etc. are still worth publishing (but focus on additional explanatory factors, beyond the flora of each area).

Finally, the length of the paper can be reduced and please provide an explanation at the end of the manuscript (see comments reviewers 2 and 3).

Comments to Author:

Reviewers' Comments to Author:
Reviewer: 1

Comments to the Author(s)
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10- In line 509, I think that rote transmission is not the best word.

Reviewer: 3

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“Detection of feeding sources”. Modify title more specifically.

Author's Response to Decision Letter for (RSOS-200266.R0)

See Appendix A.

Decision letter (RSOS-200266.R1)

We hope you are keeping well at this difficult and unusual time. We continue to value your support of the journal in these challenging circumstances. If Royal Society Open Science can assist you at all, please don’t hesitate to let us know at the email address below.

Dear Dr Ramírez:

On behalf of the Editors, I am pleased to inform you that your Manuscript RSOS-200266.R1 entitled "Complex ecological interactions across a focus of cutaneous leishmaniasis in eastern Colombia" has been accepted for publication in Royal Society Open Science subject to minor revision in accordance with the referee suggestions. Please find the referees' comments at the end of this email.
The reviewers and Subject Editor have recommended publication, but also suggest some minor revisions to your manuscript. Therefore, I invite you to respond to the comments and revise your manuscript.

• Ethics statement
If your study uses humans or animals please include details of the ethical approval received, including the name of the committee that granted approval. For human studies please also detail whether informed consent was obtained. For field studies on animals please include details of all permissions, licences and/or approvals granted to carry out the fieldwork.

• Data accessibility
It is a condition of publication that all supporting data are made available either as supplementary information or preferably in a suitable permanent repository. The data accessibility section should state where the article's supporting data can be accessed. This section should also include details, where possible of where to access other relevant research materials such as statistical tools, protocols, software etc can be accessed. If the data has been deposited in an external repository this section should list the database, accession number and link to the DOI for all data from the article that has been made publicly available. Data sets that have been deposited in an external repository and have a DOI should also be appropriately cited in the manuscript and included in the reference list.

If you wish to submit your supporting data or code to Dryad (http://datadryad.org/), or modify your current submission to dryad, please use the following link: http://datadryad.org/submit?journalID=RSOS&manu=RSOS-200266.R1

• Competing interests
Please declare any financial or non-financial competing interests, or state that you have no competing interests.

• Authors’ contributions
All submissions, other than those with a single author, must include an Authors’ Contributions section which individually lists the specific contribution of each author. The list of Authors should meet all of the following criteria; 1) substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; 2) drafting the article or revising it critically for important intellectual content; and 3) final approval of the version to be published.

All contributors who do not meet all of these criteria should be included in the acknowledgements.

We suggest the following format:
AB carried out the molecular lab work, participated in data analysis, carried out sequence alignments, participated in the design of the study and drafted the manuscript; CD carried out the statistical analyses; EF collected field data; GH conceived of the study, designed the study, coordinated the study and helped draft the manuscript. All authors gave final approval for publication.

• Acknowledgements
Please acknowledge anyone who contributed to the study but did not meet the authorship criteria.

• Funding statement
Please list the source of funding for each author.

Please note that we cannot publish your manuscript without these end statements included. We have included a screenshot example of the end statements for reference. If you feel that a given
heading is not relevant to your paper, please nevertheless include the heading and explicitly state that it is not relevant to your work.

Because the schedule for publication is very tight, it is a condition of publication that you submit the revised version of your manuscript before 28-May-2020. Please note that the revision deadline will expire at 00.00am on this date. If you do not think you will be able to meet this date please let me know immediately.

To revise your manuscript, log into https://mc.manuscriptcentral.com/rsos and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions". Under "Actions," click on "Create a Revision." You will be unable to make your revisions on the originally submitted version of the manuscript. Instead, revise your manuscript and upload a new version through your Author Centre.

When submitting your revised manuscript, you will be able to respond to the comments made by the referees and upload a file "Response to Referees" in "Section 6 - File Upload". You can use this to document any changes you make to the original manuscript. In order to expedite the processing of the revised manuscript, please be as specific as possible in your response to the referees.

When uploading your revised files please make sure that you have:

1) A text file of the manuscript (tex, txt, rtf, docx or doc), references, tables (including captions) and figure captions. Do not upload a PDF as your "Main Document".
2) A separate electronic file of each figure (EPS or print-quality PDF preferred (either format should be produced directly from original creation package), or original software format)
3) Included a 100 word media summary of your paper when requested at submission. Please ensure you have entered correct contact details (email, institution and telephone) in your user account
4) Included the raw data to support the claims made in your paper. You can either include your data as electronic supplementary material or upload to a repository and include the relevant doi within your manuscript
5) All supplementary materials accompanying an accepted article will be treated as in their final form. Note that the Royal Society will neither edit nor typeset supplementary material and it will be hosted as provided. Please ensure that the supplementary material includes the paper details where possible (authors, article title, journal name).

Supplementary files will be published alongside the paper on the journal website and posted on the online figshare repository (https://figshare.com). The heading and legend provided for each supplementary file during the submission process will be used to create the figshare page, so please ensure these are accurate and informative so that your files can be found in searches. 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.

Once again, thank you for submitting your manuscript to Royal Society Open Science and I look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Kind regards,

Anita Kristiansen
Editorial Coordinator

Royal Society Open Science
openscience@royalsociety.org
Reviewer comments to Author:

Please have the manuscript read through by a native English speaker to check / improve the language where necessary.

Author’s Response to Decision Letter for (RSOS-200266.R1)

See Appendix B.

Decision letter (RSOS-200266.R2)

We hope you are keeping well at this difficult and unusual time. We continue to value your support of the journal in these challenging circumstances. If Royal Society Open Science can assist you at all, please don’t hesitate to let us know at the email address below.

Dear Dr Ramírez,

It is a pleasure to accept your manuscript entitled "Complex ecological interactions across a focus of cutaneous leishmaniasis in eastern Colombia" in its current form for publication in Royal Society Open Science.

You can expect to receive a proof of your article in the near future. Please contact the editorial office (openscience_proofs@royalsociety.org) and the production office (openscience@royalsociety.org) to let us know if you are likely to be away from e-mail contact -- if you are going to be away, please nominate a co-author (if available) to manage the proofing process, and ensure they are copied into your email to the journal.

Due to rapid publication and an extremely tight schedule, if comments are not received, your paper may experience a delay in publication. Royal Society Open Science operates under a continuous publication model. Your article will be published straight into the next open issue and this will be the final version of the paper. As such, it can be cited immediately by other researchers. As the issue version of your paper will be the only version to be published I would advise you to check your proofs thoroughly as changes cannot be made once the paper is published.

Please see the Royal Society Publishing guidance on how you may share your accepted author manuscript at https://royalsociety.org/journals/ethics-policies/media-embargo/.

Thank you for your fine contribution. On behalf of the Editors of Royal Society Open Science, we look forward to your continued contributions to the Journal.

Best regards,
Lianne Parkhouse
Editorial Coordinator
Royal Society Open Science
openscience@royalsociety.org
on behalf of Dr Krijn Paaijmans (Associate Editor) and Pete Smith (Subject Editor)
openscience@royalsociety.org

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Appendix A

Bogotá D.C. May 7th, 2020

Doctors
Krijn Paaijmans (Associate Editor)
Pete Smith (Subject Editor)
openscience@royalsociety.org

Dear Doctors,

First of all, we want to thank your consideration with our manuscript. We have responded and applied the suggestions and comments raised by the reviewers that have substantially improved the quality of our manuscript. These can be found below

Reviewers' Comments to Author:
Reviewer: 1

Comments to the Author(s)
In this study, to elucidate transmission dynamics of cutaneous leishmaniasis in a limited endemic area, the authors analyzed ecology and feeding source of sand flies, and Leishmania species in sand flies. Although many data were presented and analyzed, it was unfocused, and more importantly, there are many issues for the data collection process that is the key for this study.

1. Detection of Leishmania DNA was performed using engorged female sand flies. However, vector research must be done with sand flies without blood in the gut. Vector species support specific parasite growth, in other words, parasites must be detected in the vector even after digestion of host blood. Therefore, Leishmania DNA detected in this study possibly derived from host blood at the best.

Response:

We agree with the reviewer, the main limitation of this study was the selection of engorged females for DNA Leishmania detection. However, other studies have used the same methodology (Jaouadi et al., 2018). In addition, the aim of this study was to determine ecological interactions among sandflies, Leishmania species, and hosts, for that reason we included only engorged females because we did not search to evaluate the vectorial capacity of Phlebotomine species.

To circumvent this comment, the next paragraph was added in the text:
“Although Leishmania DNA was detected in different species of phlebotomines, this detection does not necessarily reflect infection by the parasite since the females had fed. This was a limitation to detect the vectorial capacity of the phlebotomines species found in Arboledas.”

2. **The positive rate of sand flies is impossibly high. I doubt contamination during sample preparation processes. If the authors believe this data is correct, it must be shown by dissection of sand flies. Dissection of only 10 sand flies will be enough if the results are correct.**

*Response*

The positive rate of sand flies in this study was high probably because we detected DNA from *Leishmania* only in engorged females. In addition, other studies that include fed and not fed females had reported similar rates: Chargui et al.,2028 (3.4%), Gonzalez et al.,2017 (3.7%), Hashiguchi et al.,2019 (0.75-8.33%), Mhaidi et al.,2018 (2.51% - 7.27%) and Zorrilla et al.,2017 (6-8%). In addition, we used water as a negative control in DNA extraction and PCR assays. These controls were processed, and negative results were obtained in all assays. The dissection of sand flies is not possible because the complete body from insects were extracted. However, as we stated above, we detected *Leishmania* DNA, we never attempted to determine the transmission of the parasite of vectorial capacity of the phlebotomines studied.

3. **As the ecology of sand flies, the authors focused on only flora of each area. However, temperature and humidity affect more on sand fly ecology. In addition, fauna is another important factor as the feeding source of sand flies, and it must be addressed in this kind of study.**

*Response*

We agree with the reviewer but unfortunately we did not collect data about temperature, humidity, and fauna during the sampling. In addition, the locality had access problems (guerrillas and paramilitary areas – armed conflict areas) and do not exist official databases about these.

4. **Tables 1 and 2 should be moved to supplementary data.**

*Response*

The tables were included in supplementary data as suggested.

5. **Overall, the resolution of figures must be improved.**

*Response*
The resolution of the figures was improved.

6. No sequence data was presented. The data must be shown as a supplementary data or, at least, deposited in GenBank.

Response

The sequences were deposited in GenBank, the accession numbers are now provided in this version.

7. Discussion is too long and unfocused.

Response

We thank the comment of the reviewer. The discussion was shortened as suggested.

Reviewer: 2

Comments to the Author(s)
The manuscript “Complex ecological interactions across a focus of cutaneous leishmaniasis in eastern Colombia” aimed to analyze the patterns of diversity, food preferences, and Leishmania species in the sand fly to elucidate the transmission dynamics in one focus of cutaneous leishmaniasis. The study was done in rural areas in the municipality of Arboledas in the state of Norte de Santander, Colombia. It is an elegant and complete study about cutaneous leishmaniasis epidemiology and should be published.

Response

We deeply thank the comment of the reviewer

Major concern
The study is very descriptive and did not, in my opinion, show graphically where the transmission cycles overlap. The actual figures (5 and 6) help to understand, but in fact, the transmission cycles could be presented by octopus and not by parasite, reservoir, and vector. What cycles of what species of parasites are contained in an area. The authors limit themselves to saying that transmission scenarios in the country are more complex than previously thought. These are overlapping transmission cycles within the same ecological scenario. I was hoping that at the end of the manuscript, the authors would give an overview of what is happening. Would not there be a host or vector specificity? Would there be an anthropization of cutaneous leishmaniasis? Would the parasite mosaic theory or the Stockholm paradigm explain what is happening?
Response:

We do not have data about the transmission cycles and clinical cases of cutaneous leishmaniasis coordinates in Arboledas. However, we consider that the information of reservoirs, phlebotomines and *Leishmania* species circulating in Arboledas is very important for the study of this focus of cutaneous leishmaniasis but is necessary in the near future studies to collect samples from human cases for understanding the transmission dynamics of *Leishmania* species, for improving and focus the control strategies.

We thank the reviewer about the hypothesis of anthropization and mosaic theory that have been included in the discussion as well.

**Minor concerns**

1- In the introduction, the authors give a number that needs to be revised. According to Alvar and colleagues, 98 countries, 3 territories and 5 continents are affected by leishmaniasis (Alvar et al 2012). The authors state that according to the World Health Organization (WHO), leishmaniasis is present in at least 88 countries in the world and cite Alvar et al, 2012.

Response

The text was modified

2- Line 120, please revise. The collection was done in 3 nights and not in 3 days,

Response

The text was modified

3- In lines 324 - 326, please delete the first paragraph. It is about materials and methods.

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Response
The text was modified

6- The sentence of 364 is taken from the authors or from Urrutia et al. 2018?
Response

Deleted as suggested

7- Inline 385, what is the L? please give by the extender.
Response

The text was modified

8- Inline 387, tables 1 and 2 are correct.
9- To what the authors attribute the fact that the authors did not find Lu longipalpis. Wouldn't it be because the vector has already been urbanized?
10- In line 509, I think that wrote transmission is not the best word.
Response

The text was modified

Reviewer: 3

Comments to the Author(s)
Congratulations on the manuscript and the scientific work. The statistical analysis is strong and makes it possible to establish the conclusions clearly. Nevertheless it is necessary to fit some simple topics.
In line 28 and 36 the term “food preferences” is very broad. There is only evidence of blood intake. Adjustment is required.
In line 29 explain the ecoregion where the focus of leishmaniasis studied is located.
Specify the department where the two locations are located (Summary).
It is possible to better compare the diversity indices between the Localities (Macro approach) and between microenvironments (Micro approach). It is also necessary to place numeric values of the indexes (Summary).
In the line 44, can improve the redation of "circulating feeding sources of domestic animals". The term circulation is confused.
Response

We thank the valuable comments of the reviewer. The text was modified
In the introduction it is necessary to include the abbreviation of Leishmania the first time that it is enunciated, to continue using it in the rest of the document.

Response

The text was modified

In the line 97 to complement “Groves, North of Santander”

Update the reference of Galati 2016 and Galati 2003; by the keys of Galati 2018, on line 33 and Linea 38.

Response

We added reference in methods

Because only visually blood-fed females were included. It is necessary to clarify the selection criteria and the way in which they interpret the result.

Response

We added the selection criteria in the Discussion

Line 169. Include Network 5.0 Reference

Response

We added the link of the software

Line 174-200: Ecology of sand flies: The text is very extensive; it is suggested to summarize.

“Detection of feeding sources”. Modify title more specifically.

Response

The text was modified

Best regards

The authors
Dear Doctors,

We thank your soon response and positive decision. The manuscript was revised by a native English speaker from the EDANZ GROUP, please find below a screenshot of the certificate:

We hope that this new version meets the required criteria of the journal.

Best

The authors