

Submission date. 19/08/2025

Associate editor's decision after peer review (29/09/2025).

Dear Mr. Araújo:

Manuscript ID NI-2025-0148 entitled "Where are we going to live? Population genomics emits warning signals about endangered Amazonian rheophilic loricariids under habitat loss" which you submitted to the Neotropical Ichthyology, has been reviewed. The comments of the reviewer(s) are included at the bottom of this letter.

The reviewer(s) have recommended publication, but also suggest some revisions to your manuscript. Therefore, I invite you to respond to the reviewer(s)' comments and revise your manuscript.

To revise your manuscript, log into <https://mc04.manuscriptcentral.com/ni-scielo> and enter your Author Center, 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.

You may also click the below link to start the revision process (or continue the process if you have already started your revision) for your manuscript. If you use the below link you will not be required to login to ScholarOne Manuscripts.

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You will be unable to make your revisions on the originally submitted version of the manuscript. Instead, revise your manuscript using a word processing program and save it on your computer. Please also highlight the changes to your manuscript within the document by using the track changes mode in MS Word or by using bold or colored text. Once the revised manuscript is prepared, upload BOTH versions (the tracked and a clean) and submit them through your Author Center.

When submitting your revised manuscript, you will be able to respond to the comments in the space provided. You can use this space to document any changes you make to the original manuscript. In order to expedite the processing of the revised manuscript, please reply POINT TO POINT all the suggestions of the reviewers and be as specific as possible in your response to the comments.

IMPORTANT: Your original files are available to you when you upload your revised manuscript. Please delete any redundant files before completing the submission.

Because we are trying to facilitate timely publication of manuscripts submitted to the Neotropical Ichthyology, your revised manuscript should be submitted before 29-Nov-2025. If it is not possible for you to submit your revision by this date, we may have to consider your paper as a new submission.

Once again, thank you for submitting your manuscript to the Neotropical Ichthyology and I look forward to receiving your revision.

Sincerely,

Dr. Marcelo Cioffi

Associate Editor, Neotropical Ichthyology
mbcioffi@ufscar.br

Anonymous reviewer #1

Recommendation. Minor Revision

Comments. Dear Editor and Authors,

The manuscript by Leonardo et al., entitled "Where are we going to live? Population genomics emits warning signals about endangered Amazonian rheophilic loricariids under habitat loss", provides an important assessment on population genomics of three *Baryancistrus* species from the Tocantins-Araguaia River basin. The authors studied

demographic history and correlated data with potential conservation efforts for the species. After analysing the manuscript and the supplementary material provided, besides the sampling size being a little small for some of the analyses, I believe that the methods and conclusions are well drawn to address this issue, and the manuscript thus deserves publication after minor revisions, aiming to enhance the presentation of data and correct some incorrect formatting/small typos. Please refer to the attached document for small typos, and see below some general considerations:

Please note that according to Neotropical Ichthyology instructions, the first time a species name appears in the text, it should be followed by the descriptor name and year. Check this throughout the whole manuscript to ensure formatting consistency.

In Fig. 1, the species name *Baryancistrus* sp. "Araguaia" is fully italicized, please remove italics from "sp. "Araguaia".

For Fig. 3, the description for Habitat amount in 1985/2020 sometimes overlaps with the graph, which makes reading difficult when the picture is in a small size. Increase spacing on text from graphs to better visualization. Also, increase the size or thickness of the numbers in the graph (0, 0.25, 0.5, etc). Maybe it's better to put them outside the graph, connected by lines with the graph intersections.

Anonymous reviewer #2

Recommendation. Major Revision

Comments. I congratulate the authors on a generally much improved manuscript. However, I still feel that it requires more work. There are a large number of topics discussed, many analyses, and how they are interpreted in the discussion requires a more structured textual approach to help readers to follow the logic being used.

Most of the discussion is better than before... but could do with minor adjustments in a similar manner to those indicated specifically for L431–438 – I would suggest a rewrite of the discussion with clear subheadings for topics.

The subheadings in the methods could be better defined/grouped and repeated in the discussion to answer the specific questions raised as objectives at the end of the introduction.

A good model might be:

Subtitle:

Sentence 1 – Raise concept citing examples (e.g. what causes homogeneity?),

Sentence 2 – describe what statistics would support one result or another (i.e. the different possible causes),

Sentence 3 – state how the new results fit into this,

Sentence 4 – describe implications based on real world issues in the region with references.

Specific points:

L112 – limited sample numbers?

L213 – Frichot and François!

L215 – François et al missing from references

L337 – The whole paragraph needs to make clear what sample size/area is being discussed in the text. Analyses within regions just above showed no IBD but Fig 4 treats the combined R1 and R2 but not other upstream sites... and this needs to be clear to the reader without having to reinterpret results using figures and maps.

L 374– Specifically – to L 380 – repeating methods unnecessarily. The rest of the paragraph continues to repeat some results but does better at integrating them with literature as a discussion.

L428–429 – How do the findings show IBD changes over time?? This is not made clear.

L431–438 – Lots of technical language and word choices that make this section quite hard to follow in simple terms. It really needs to be made much clearer for the reader what each term means and how the results actually show these. – What is Demographic

inertia, and how would these or any other kind of results (homogeneity combined with what other information) allow people generally to distinguish it from panmixia?? It is explained in a long block of text afterwards, but the structure of the whole section is just patchy. The authors still need to work on making text read in a more logical step-by-step manner, introducing concepts and then explaining how the data in the study system shows on or other result.

Author list of the supplementary material is different from the main manuscript!

Various Refs are inconsistent between text and bibliography (not all are in the supplementary material):

Bruno
Crivellari
Frankham
Haddad
IBAMA
.....others

Author's Rebuttal Letter (07/11/2025).

Dear academic editor

Dr. Marcelo Cioffi

Associate Editor, Neotropical Ichthyology

Thank you for your email enclosing the reviewers' comments and for the opportunity to submit a revised version of the manuscript (Ref: NI-2025-0148).

The authors thank the journal editorial and the reviewers for the time and effort that you dedicated to evaluating our manuscript.

We are grateful for the comments, questions, and suggestions made to our manuscript, which were carefully considered. We hope that our responses can satisfy what has been requested.

The ddRAD sequencing generated in this study and the corresponding VCF files are available on <https://doi.org/10.5281/zenodo.16970398>.

We are available to consider future comments or suggestions if necessary, to improve the manuscript so that it can be published in this prestigious Journal.

The responses or comments of all lines cited refer to the revised manuscript file with tracked changes. We are also providing a clean version of the revised manuscript without highlights or tracked changes.

Best regards

Sincerely,

Felipe Araújo

Corresponding author

Researcher

Museu Paraense Emílio Goeldi (MPEG)

Response to General Comments

Dear Reviewers,

We sincerely thank the reviewers for their thorough and constructive evaluation of our manuscript. Your insightful comments have been invaluable in improving the clarity, precision, and focus of this technical note. We have carefully considered each of your points and have made extensive revisions to address them.

Below, we detail how we have addressed your general comments:

Response to General Comments from Reviewer 1

1. (Page , line -) Reviewer: In Fig. 1, the species name *Baryancistrus* sp. "Araguaia" is fully italicized, please remove italics from "sp. "Araguaia".

Our Action: We appreciate the reviewer's careful attention to taxonomic detail. The figure has been corrected: we removed the italics from "sp." and "Araguaia" so that only the genus name, *Baryancistrus*, is italicized. This is now in accordance with standard

zoological nomenclature for undescribed species.

2. (Page , line -) Reviewer: For Fig. 3, the description for Habitat amount in 1985/2020 sometimes overlaps with the graph, which makes reading difficult when the picture is in a small size. Increase spacing on text from graphs to better visualization. Also, increase the size or thickness of the numbers in the graph (0, 0.25, 0.5, etc). Maybe it's better to put them outside the graph, connected by lines with the graph intersections.

Our Action: Thank you for the helpful suggestion. Figure 3 was improved to enhance readability. Labels ('Habitat amount in 1985/2020' and 'Null model') were repositioned with increased spacing from the triangular plots to avoid overlapping, and the axis labels (0, 0.25, 0.5, 0.75, 1) were enlarged and thickened for better visibility at smaller sizes. These changes improve clarity without altering the data representation.

Response to General Comments from Reviewer 2

3. (Page 12 - 17, line 431 - 438) Reviewer: Most of the discussion is better than before... but could do with minor adjustments in a similar manner to those indicated specifically for L431-438 - I would suggest a rewrite of the discussion with clear subheadings for topics.

Our Action: The original sentence at lines 431-438, which discussed "apparent genetic homogeneity," has been revised. We have adopted the term "seemingly" for precision and to reflect that the observed patterns may be an artifact of demographic history (e.g., recent expansion), rather than indicative of significant contemporary connectivity, aligning with the caution required for this species. The revised statement now reads: : "The seemingly genetic homogeneity in certain regions may therefore be an artifact of demographic history (such as a recent population expansion), rather than indicative of significant contemporary connectivity."

This revision addresses the reviewer's concern regarding interpretive wording, providing a more precise and scientifically cautious tone throughout the manuscript.

In addition, the same adjustment was consistently applied in subsequent sections where the wording "apparent genetic homogeneity" occurred, ensuring coherence and clarity throughout the manuscript.

4. (Page 12- 17, line -) Reviewer: I would suggest a rewrite of the discussion with clear subheadings for topics.

Our Action: We greatly appreciate the reviewer's constructive suggestion regarding the organization of the manuscript. We agree that incorporating clear subheadings significantly improves the readability and logical flow of the Discussion section.

We respectfully retained the current structure of the Methods section, as it already meets field standards for clarity and reproducibility. The existing organization already adheres to the field's standards for clarity and reproducibility and does not compromise the overall quality or integrity of the study's design. Therefore, we believe no further intervention is necessary in the Methods subheadings.

Regarding the Discussion section, we have fully implemented the suggestion to align the content with the core objectives. We have restructured the existing text into five thematically consistent subheadings, as listed below:

1. Introduction and Analytical Framework
2. Large-Scale Population Structure and Homogeneity
3. Fine-Scale Genetic Divergence and Environmental Drivers
4. Temporal Scale, Demographic Artifacts and Anthropogenic Impacts
5. Genetic Erosion and Conservation Measures

Crucially, we have revised the internal logic of these sections to reflect the structured model suggested by the reviewer: each subsection now begins by establishing the core concept and statistical basis for our findings, followed by a clear statement of how our new results fit into the existing literature, concluding with the implications for regional conservation. This ensures the Discussion follows a clear, logical progression, significantly improving the overall coherence and thematic organization of the

manuscript.

5. (Page 4, line - 112) Reviewer: limited sample numbers?

Our Action: Our method selection was specifically based on the need for a technique that is robust to the challenges of sampling wild populations, such as our rheophilic species, where sample sizes often show variability across sites and the quality of field-collected biological material can be uneven.

To provide a more accurate and scientifically justifiable statement, we have revised the text to ensure we do not imply that our final total sample size was insufficient for the statistical power required.

The revised text at L112 now reads:

"ddRADSeq is a cost-effective, reference-free approach that generates thousands of SNPs and is particularly useful for studies where the available sample size is moderate or variable, or where the quality of biological material is compromised (Nazareno *et al.*, 2017; Allendorf *et al.*, 2010; Khoshkholgh *et al.*, 2020)."

This revision clarifies that ddRADSeq is an ideal and established tool for handling sample variability, thereby preempting concerns about the statistical adequacy of our final dataset, as supported by the robust results presented in our study.

6. (Page 7, line 213) Reviewer: Replace "Frichot and François, 2016" for "Frichot, François, 2015".

Our Action: We thank the reviewer for identifying the incorrect citation format and year for the Frichot reference. This was a transcription and formatting error.

1. **Correction of Year and Author Format:** The correct publication year for the Frichot and François article is 2015, not 2016. The format has also been corrected to strictly comply with the journal's required style for two authors (e.g., comma and space, no "and"). The citation now correctly reads: (Frichot, François, 2015).

2. **Scope of Correction:** We confirm that this specific correction (year and format) has been applied systematically to all instances of this citation throughout the entire manuscript text.

We are confident that all instances of the Frichot, François (2015) citation are now accurate and conform to the journal's guidelines.

7. (Page 7, line 215) Reviewer: François *et al* missing from references.

Our Action: We thank the reviewer for pointing out this omission.

1. **Reference Addition:** We confirm that the reference has now been added to the References list. The full entry is:

François O, Martins H, Caye K, Schoville SD. Controlling false discoveries in genome scans for selection. *Molecular Ecology*; 2016; 25:454–469. <https://doi.org/10.1111/mec.13513>

2. **In-Text Correction (L215):** The in-text citation at Line 215, which is used to support theoretical/methodological background, has been checked and corrected to strictly follow the journal's required format for more than two authors. The citation now reads: (François *et al.*, 2016).

The reference is now included and the in-text citation is compliant with the journal's guidelines.

8. (Page 7, line 337) Reviewer: The whole paragraph needs to make clear what sample size/area is being discussed in the text. Analyses within regions just above showed no IBD but Fig.4 treats the combined R1 and R2 but not other upstream sites... and this needs to be clear to the reader without having to reinterpret results using figures and maps.

Our Action: We appreciate the reviewer's critical feedback on the lack of clarity regarding the spatial scale and sample set used in this paragraph. We agree that the distinction between regional (separate R1 or R2) and global (combined R1 + R2) analyses was not explicitly stated, which previously required the reader to infer this from figures and maps.

Action Taken: We have revised the paragraph (L343 onwards) to clearly define the sample set being analyzed for the IBD correlations (L349–350) and to clarify the contrast with the regional analyses (L351).

1. Clarity on Global Analysis (L348): We inserted a clear introductory phrase to establish that the initial results (Kernel density, IBD correlation) refer to the combined dataset.

- New text at L348: "When analyzing the combined R1 and R2 reaches (global scale), a significant correlation was found..."

2. Clarity on Regional Analysis (L351): We added language to reinforce the distinction for the separate analyses.

- New text at L351: "In contrast, at the regional level (i.e., analyzing R1 and R2 separately), this correlation was not significant..."

This revision ensures the reader has immediate clarity on the spatial scope of the analysis throughout the entire paragraph.

9. (Page 12, line 374 – 380) Reviewer: – repeating methods unnecessarily. The rest of the paragraph continues to repeat some results but does better at integrating them with literature as a discussion.

Our Action: We thank the reviewer for pointing out the unnecessary repetition of Methods and objectives at the beginning of the discussion (L375–L380), and for allowing us to identify a critical inconsistency in the autocorrelation results (L387).

Action Taken:

1. L375–L386 (Discussion Opening): We have maintained the strong opening phrase ("Here, we present the first..."). However, the subsequent text (up to L386) was condensed and completely rewritten to remove the detailed repetition of methods and objectives. The paragraph now immediately focuses on stating the main conclusion of the study (i.e., contrasting genetic structure patterns).

2. L387–L388 (Inconsistency Corrected): The sentence regarding the "weak but significant structure at fine scales (up to 12 km)" (L387–388) was removed, as it contradicted the observed strong IBD pattern in *B. longipinnis* (Fig. 4).

3. Scientific Rigor: The phrase justifying the power of our genomic data was updated to include the highly relevant citation:

- Revised Text: "...a pattern often obscured by less powerful molecular markers (Skey et al., 2023) but clearly visible with our genomic data."

4. Integration: The autocorrelation section was replaced with a concise statement ("The spatial autocorrelation analysis confirmed these contrasting spatial genetic patterns,") which accurately reflects our findings and restores scientific precision to the discussion flow.

We ensure the revised paragraph now functions as a focused, interpretive discussion, effectively integrating our results with the literature.

10. (Page 14, line 428 – 429) Reviewer: How do the findings show IBD changes over time?? This is not made clear.

Our Action: We thank the reviewer for this important clarification request. We acknowledge that our study represents a snapshot in time, and thus our findings cannot show IBD changing over time directly.

We have revised the text (L435–436) to clarify that our statement is an inference based on the species' ecology and vulnerability, rather than a direct finding from temporal data:

1. We explicitly linked the strong IBD found in *B. longipinnis* with the existing evidence of its contracting distribution area and habitat loss.

2. The revision now clarifies that this combination suggests the population is highly sensitive to further environmental changes.

3. The final sentence is rephrased to state that this high sensitivity implies the IBD pattern is likely not static and may fluctuate temporally (Junge et al., 2011), arguing for the species' ecological vulnerability rather than claiming we measured the change itself.

This addresses the reviewer's concern by grounding the temporal interpretation in the ecological and demographic context of the species.

Response to Reviewers (annotated print - attached)

Journal: Neotropical Ichthyology

Manuscript ID: NI-2025-0148

Reviewer: 2

Q1. (Page 4, line 97) Reviewer: The sentence "Baryancistrus longipinnis" should be replaced by "Baryancistrus longipinnis (Kindle, 1985)"

Response: Thank you for pointing out this mistake. We changed the format.

Q2. (Page 4, line 101) Reviewer: Replace "B. niveatus" for "Baryancistrus niveatus (Castelnau, 1855)".

Response: Thank you for pointing out this mistake. We changed the format.

Q3. (Page 6, line 161-163) Reviewer: The sentence: "A third undescribed species (Baryancistrus sp. "Araguaia") was collected in the Rio Tocantins basin (n=6 individuals) but was not considered in the landscape genetics analyses due to low sample size, with only its population genetic diversity parameters being estimated." should be moved from lines 172-174 to lines 165-167 in the manuscript.

Response: Thank you for this comment. We agree with the reviewer's suggestion to moved the phrase to lines 165-167.

Q4. (Page 7, line 208) Reviewer: The sentence "data missing of 40%" should be replaced by "40% data missing".

Response: Thank you for pointing out this mistake. We changed the format.

Q5. (Page 7, line 218) Reviewer: Replace "Frichot and François, 2016" for "Frichot, François, 2015".

Response: Thank you for pointing out this mistake. We changed the format.

Q6. (Page 8, line 236) Reviewer: Replace "indexes" for "indices".

Response: Thank you for pointing out this mistake. We changed the format.

Q7. (Page 9, line 287) Reviewer: Replace "Supplementary file" for "supplementary file".

Response: Thank you for pointing out this mistake. We have corrected the capitalization of "Supplementary file" to "supplementary file" throughout the entire text.

Q8. (Page 11, line 332) Reviewer: For better reading, include between parenthesis the AICc scores. Do the same in the description of results for Region 2 below.

Response: Thank you for this suggestion. To quantify the "superior fit" and "similarly low AICc scores" and improve readability, we revised the text to specify that models with $\Delta AICc \leq 2$ were considered the set of top-ranking models with substantial empirical support. We applied this change consistently to the description of results for both Region 1 and Region 2 of both species (B. longipinnis and B. niveatus).

Q9. (Page 11, line 337-338 and line 340-342) Reviewer: Move to discussion or delete the sentences "These results underscore the importance of habitat quality and environmental gradients in shaping genetic differentiation in this region" and "highlighting their role as key drivers of genetic structure within complex aquatic habitats areas characterized by pronounced spatial heterogeneity and dynamic physicochemical factors".

Response: Thank you for this feedback. We agree that these concluding statements were misplaced within the section and disrupted the flow.

Both sentences, "These results underscore the importance of habitat quality and environmental gradients in shaping genetic differentiation in this region" and "highlighting their role as key drivers of genetic structure within complex aquatic habitats areas characterized by pronounced spatial heterogeneity and dynamic physicochemical factors", have been moved and integrated into the main discussion paragraph (now lines 506-510).

This relocation ensures that these statements function as a proper synthesis of our landscape genetics findings, improving the overall coherence of the manuscript.

Q10. (Page 12, line 370-372) Reviewer: Move to discussion or delete the sentence "These

findings underscore the importance of incorporating temporal and spatial habitat dynamics into conservation strategies aimed at maintaining genetic diversity.”

Response: Thank you for this suggestion. We agree that this statement represents a key implication of our study and was misplaced within the results section.

The sentence “These findings underscore the importance of incorporating temporal and spatial habitat dynamics into conservation strategies aimed at maintaining genetic diversity.” has been moved to the final part of the Discussion (now lines 494–495), specifically at the conclusion of the paragraph detailing the genetic impacts of habitat loss. This relocation ensures that the statement functions as a proper synthesis of our findings, improving the overall coherence and flow of the manuscript.

Associate editor’s decision after peer review (02/12/2025).

Dear Mr. Araújo:

It is a pleasure to accept your manuscript entitled "Where are we going to live? Population genomics emits warning signals about endangered Amazonian rheophilic loricariids under habitat loss" in its current form for publication in the *Neotropical Ichthyology*.

The manuscript has been re-assessed by two independent reviewers, both of whom consider the revisions satisfactory and recommend the paper for publication. One reviewer has raised a few minor comments that should be addressed during the proof stage, not requiring an additional round of revision, nevertheless.

Congratulations for the acceptance of your article, and be aware on the following topics:

1. Publication Fee

NI will charge a publication fee if none of the co-authors is an active SBI member. This measure is essential to strengthen SBI and thus ensure the continuity of our journal, scientific society, and biannual meetings. SBI is not limited to Brazilians but is open to anyone interested in freshwater and marine Neotropical fishes. More details on SBI are available at <https://www.sbi.bio.br/>. Please email tesouraria.sbi@gmail.com to confirm whether any of your co-authors is a current SBI member and to activate your SBI membership if needed. Otherwise, if you will cover the publication fee of R\$1.000, please inform us at the same email. For authors outside Brazil, the fee will be converted to US dollars based on the official exchange rate on the date of payment. The only exceptions to this fee are invited articles.

2. Science Communication and Social Media

NI actively promotes published articles to both academic colleagues and the general public, including science journalists. To support this, we create social media posts and require images and/or videos of fish related to your work. If your article does not include such images, please send a photo of a representative fish species, preferably alive in its natural habitat. If you do not have your own photo, you may provide a link to an online image, along with the source, author, and, if applicable, authorization for its use. We also publish video summaries of articles in Portuguese on our Instagram (@neoichth). We ask you to designate one author to record a short video using a mobile phone, following the attached instructions. The video should include visual materials (photos, graphics) and a script for subtitles to enhance accessibility. Please email the completed material to neoichth@nupelia.uem.br within 30 days. For now, please provide the name and email address of the author responsible for recording the video.

Additionally, if your article is taxonomic in nature and has been submitted to Zoobank, it is your responsibility to update the manuscript's status on Zoobank once it has been published.

All of the above information and materials are mandatory for the publication of your article, including the scientific dissemination component, which is crucial in the current climate of science denial and misinformation. If you have any questions, please feel free

to contact us at neoichth@nupelia.uem.br.

Please respond to this e-mail within five working days to let us know you are aware of all the important points mentioned above.

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

Sincerely,

Dr. Marcelo Cioffi

Associate Editor, Neotropical Ichthyology

mbcioffi@ufscar.br

Anonymous reviewer #1

Recommendation. Accept

Comments. The revised version of the manuscript satisfactorily addresses all my previous comments and has been further improved following the suggestions of Reviewer 2. I therefore recommend acceptance of the manuscript.

Anonymous reviewer #2

Recommendation. Accept

Comments. Accept with minor issues.

Overall, a much improved manuscript requiring only minor modifications for format, spelling and placement.

L257-276 – This is presented as a list with “:”, so all number subunits should end with “;”, and the last item (7) should start with “and”

L261-263 – There appear to be a number of missing references “()”. This will need to be corrected in the reference list as well of course.

L366-367 – Belongs to the discussion.

L438 – Close parentheses on reference

Various smaller formatting and spelling issues in the references – especially see Skey and Simonsen references regarding formatting. Tyers – 2 Ps in Applications,

Table 2 – spelling error – ‘Valeu’ instead of ‘value’

Neotropical Ichthyology



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HOW TO CITE THIS ARTICLE

- Leonardo MG, Lanes ECM, Araújo FAA, Cruz VP, D’Oliveira Pavan SE, Monteiro ILP, Quaresma TE, Oliveira C, Akama A. Where are we going to live? Population genomics emits warning signals about endangered Amazonian rheophilic loricariids under habitat loss. *Neotrop Ichthyol.* 2026; 24(2):e250148. <https://doi.org/10.1590/1982-0224-2025-0148>



Official Journal of the
Sociedade Brasileira de Ictiologia