

Submission date. 18/02/2026

Associate editor's decision after peer review (24/04/2026).

Dear Mr. MEJIA DE LOAYZA:

Manuscript ID NI-2026-0024 entitled "Past connection and isolation of Rineloricaria (Siluriformes: Loricariidae) across southeastern Brazilian coastal drainages: evidence for palaeodrainage connectivity and description of a new species" 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 minor 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.

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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.

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When submitting your revised manuscript, you will be able to respond to the comments made by the reviewer(s) 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 be as specific as possible in your response to the reviewer(s). 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 25-May-2026. 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 Melo

Associate Editor, Neotropical Ichthyology

melomar@usp.br

Anonymous reviewer #1**Recommendation.** Minor Revision

Comments. O manuscrito “Past connection and isolation of *Rineloricaria*...” revisa informação morfológica e molecular para propor uma nova espécie desse gênero em uma área de onde já se conhecem outras espécies endêmicas. O manuscrito está muito bem escrito e justifica apropriadamente a existência dessa nova linhagem. Poucos problemas de forma e estilo estão marcados em uma cópia do ms, que vai anexo. As principais questões do manuscrito são:

Página 3, linha 46, 53, 56 e outras: Em inglês, o descritor geográfico deve ser escrito em letra maiúscula. Em português não, mas a forma correta em inglês é inicial maiúscula (Paraíba do Sul River, não river; Itabapoana River, não Itabapoana river).

P 8, L 52: asterisco significa a contagem do holótipo, certo? Não dos espécimens-tipo.

P 11, L 22, 31, 38, etc: As citações dos corpos d’água estão uma mistura de inglês e português. Os autores devem escolher uma forma e manter-se com ela. Por exemplo, na linha 22 diz “Itapemirim River basin, Rio Castelo upstream...” A maioria do ms está em inglês, então sugiro usar Itapemirim River basin, Castelo River upstream...”. Nas linhas seguintes tem “Itabapoana river”, “Córrego do farol”, “Rio Preto”, “ribeirão das Flores”, etc.... Sem padronização de língua nem de iniciais maiúsculas ou minúsculas.

P 11, L 34, 39 etc: Siglas ou expressões que fazem sentido em português, não fazem em inglês. Assim, o que significa “Bom Jesus do Itabapoana na RJ-230”? Precisa colocar “Bom Jesus da Itabapoana on road RJ-230”. O mesmo logo abaixo, “160 m a oeste da BR-101”, deve ser 160 m W of road [or highway] BR-101”. Também, o que significa para um não-brasileiro, PCH? A sigla em inglês para Pequenas Central Hidrelétrica é SHP (Small Hydroelectric Powerplant).

P 15, L 39, 43: Marcas coloridas em peixes devem ser sempre: longitudinais, stripes; transversais no corpo, bars; e transversais nas nadadeiras, bands.

Figura 2: Use símbolos diferentes além das cores diferentes para, para facilitar a vida dos leitores daltônicos.

Figure 5: Indique o sexo do holótipo

Tabelas: Geral: Remova as cores das tabelas.

Tabela 5: O holótipo deve ser incluído no range. Além disso, algo nos chamou a atenção: As medidas preanal length e postanal length tem valores iguais para o holótipo, que é uma fêmea, e desvios padrão mais altos que a média para os parátipos. As duas coisas juntas sugerem que exista diferença significativa nessas medidas entre machos e fêmeas e, portanto, essas medidas deveriam ser apresentadas separadas para os dois sexos.

Fora esses detalhes, consideramos um excelente manuscrito.

Roberto Reis, Bruna Gomes e Dan Gonzalez-Kosasky

Anonymous reviewer #2**Recommendation.** Minor Revision

Comments. Uma boa adição aos conhecimentos da fauna e sua evolução nas bacias costeiras do Brasil. Espécie parece ter uma boa diagnose e é suportada por caracteres moleculares também.

Deixo varios comentários e correções direto no documento PDF em anexo.

Meus únicos comentários mais importantes são:

1) a PCA, que acho que deveria ser dado um tratamento para o tamanho? O eixo 1 é diretamente ligado ao tamanho dos exemplares, então o que ele tem ali é uma espécie com indivíduos grandes e outra com pequenos...

2) Ficou meio estranho essa separação entre morfotipo 1 e 2 a priori, sem muita explicação, e essa separação é usada na AMOVA e na PCA (porque não fazer uma PCA com bacias ou paleodrenagens por exemplo?). Acho que merece uma explicação melhor.. seria morfotipos baseado em um trabalho anterior ou nos caracteres morfológicos discretos que servem para a sua diagnose (filamento caudal por exemplo).

3) Tem o suporte que esse grupo (*R. steindachneri* + *R. capixaba*) forme um grupo monófilético, talvez *R. capixaba* seja mais proximamente relacionada com outra do sudeste?? mencionar isso, penso que é de outro trabalho (Mejia & Buckup, 2024?)

Author's Rebuttal Letter (19/05/2026).

Dear Editor,

Neotropical Ichthyology

We have revised manuscript ID NI-2026-0024 entitled "Past connection and isolation of Rineloricaria (Siluriformes: Loricariidae) across southeastern Brazilian coastal drainages: evidence for palaeodrainage connectivity and description of a new species" following the reviewers' comments and recommendations.

We appreciate the reviewers' constructive comments, which significantly improved the quality of the manuscript. All suggested changes and comments have been carefully considered and incorporated into the revised manuscript. Most suggestions involved improvements in clarity, standardization of terminology, and minor adjustments to figures and tables. These modifications are indicated in the tracked-changes version of the manuscript. Below, we provide a detailed, point-by-point response to all comments raised by the reviewers.

REVIEWER 1

We thank the reviewer for the careful reading of the manuscript and the positive evaluation. All suggestions were accepted and incorporated as detailed below.

Comment: Pagina 3, linha 46, 53, 56 e outras: Em inglês, o descritor geográfico deve ser escrito em letra maiúscula. Em português não, mas a forma correta em inglês é inicial maiúscula (Paraíba do Sul River, não river; Itabapoana River, não Itabapoana river).

Response: The manuscript was revised to standardize all geographic names following English conventions (e.g., "Paraíba do Sul River", "Itabapoana River").

Comment: P 8, L 52: asterisco significa a contagem do holótipo, certo? Não dos espécimens-tipo.

Response: The asterisks refer exclusively to counts of the holotype, not to all type specimens. This has been clarified in the Methods section to avoid ambiguity.

Comment: P 11, L 22, 31, 38, etc: As citações dos corpos d'água estão uma mistura de inglês e português. Os autores devem escolher uma forma e manter-se com ela. Por exemplo, na linha 22 diz "Itapemirim River basin, Rio Castelo upstream..." A maioria do ms está em inglês, então sugiro usar Itapemirim River basin, Castelo River upstram...". Nas linhas seguintes tem "Itabapoana river", "Córrego do farol", "Rio Preto", "ribeirão das Flores", etc.... Sem padronização de língua nem de iniciais maiúsculas ou minúsculas.

Response: The portuguese word "rio" has been translated to English (e.g., "Castelo River", "Itapemirim River basin"), ensuring consistency. Additionally, we changed the initials in all toponyms to upper case. However, the names of small streams are maintained as used in the local language, because they have no direct equivalents in the English language. A small stream is called a "brook", "beck", "burn", "gill" ou "ghyll", "nourne", "rill" in United Kingdom, and "creek", "brook" or "arroyo" in the United States, but these type of small streasm are not directly mapped in the words "córrego" and "ribeirão" as used locally in the studied region. We also believe that that main purpose of providing local toponyms is to allow their recognition in the field.

Comment: P 11, L 34, 39 etc: Siglas ou expressões que fazem sentido em português, não fazem em inglês. Assim, o que significa "Bom Jesus do Itabapoana na RJ-230"? Precisa colocar "Bom Jesus da Itabapoana on road RJ-230". O mesmo logo abaixo, "160 m a oeste da BR-101", deve ser 160 m W of road [or highway] BR-101". Também, o que significa para um não-brasileiro, PCH? A sigla em inglês para Pequenas Central Hidrelétrica é SHP (Small Hydroelectric Powerplant).

Response: All locality descriptions were revised to improve clarity for an international audience. Expressions originally written in Portuguese were translated and standardized in English (e.g., “on RJ-230 road”). The abbreviation “PCH” was replaced by “Small Hydroelectric Power Plant”, because the English acronym would be equally opaque non-anglophonic researchers.

Comment: P 15, L 39, 43: Marcas coloridas em peixes devem ser sempre: longitudinais, stripes; transversais no corpo, bars; e transversais nas nadadeiras, bands.

Response: The terminology was revised accordingly throughout the manuscript.

Comment: Use símbolos diferentes além das cores diferentes para, para facilitar a vida dos leitores daltônicos.

Response: Figure 2 was modified to include distinctly-shaped symbols in addition to color coding. Squares now represent *R. steindachneri* and circles represent *R. capixaba*, while colors continue to indicate the different river basins. The figure caption was revised accordingly.

Comment: Figure 5: Indique o sexo do holótipo

Response: The figure caption was revised to include this information.

Comment: Tabelas: Geral: Remova as cores das tabelas.

Response: All tables were reformatted to remove color.

Comment: Tabela 5: O holótipo deve ser incluído no range. Além disso, algo nos chamou a atenção: As medidas preanal length e postanal length tem valores iguais para o holótipo, que é uma fêmea, e desvios padrão mais altos que a média para os parátipos. As duas coisas juntas sugerem que exista diferença significativa nessas medidas entre machos e fêmeas e, portanto, essas medidas deveriam ser apresentadas separadas para os dois sexos.

Response: We thank the reviewer for carefully checking Table 5. The holotype was included in the morphometric dataset, as indicated in the table caption and by the sample size reported for each measurement ($n = 15$, corresponding to 14 paratypes plus the holotype). We identified, however, a typographical error in the table, where the initial digit “1” was inadvertently omitted from the standard length of specimen MNRJ 51332, resulting in “77.5” instead of “177.5” mm SL. This error has now been corrected.

We also clarify that the holotype is a male, not a female. Among the paratypes, some specimens show developed odontodes consistent with mature males, whereas others may represent females or immature individuals; however, reproductive organs were not examined to determine sex for all specimens with certainty. For this reason, and because the morphometric summary already includes the holotype and all measured paratypes, we consider that separating the morphometric data by sex would not be appropriate at this stage. The raw morphometric dataset, including preanal length and postanal length, was rechecked, and no anomalous values were detected. The raw data may be provided to the editor or reviewers if needed.

REVIEWER 2

We thank the reviewer for the constructive comments and positive evaluation of the manuscript. His comments are carefully addressed as follows.

Comment 1: a PCA, que acho que deveria ser dado um tratamento para o tamanho? O eixo 1 é diretamente ligado ao tamanho dos exemplares, então o que ele tem ali é uma espécie com indivíduos grandes e outra com pequenos...

Response: We thank the reviewer for this observation. The PCA was not conducted using raw morphometric measurements, but with size-adjusted data expressed as percentages of standard length (SL) and head length (HL). In addition, all variables were centered and standardized prior to the analysis, minimizing the influence of absolute body size and differences in variable scale. We have clarified these procedures in the Methods section as follows: “The PCA was conducted with size-adjusted multivariate morphometric data, with all measurements expressed as percentages of standard length (SL) and head

length (HL). Prior to the analysis, variables were centered and standardized to unit variance in R using the functions `scale()` and `prcomp()`, minimizing the influence of absolute body size and differences in measurement scale among variables.”

Furthermore, the specimens of *Rineloricaria steindachneri* from the Paraíba do Sul River basin included in the PCA correspond to the same morphometric dataset presented in Mejia, Buckup (2024). The size ranges of the compared groups broadly overlap (*R. capixaba*: 93.6–177.5 mm SL; *R. steindachneri*: 98.7–164.6 mm SL), indicating that the observed differentiation is not simply associated with one group containing consistently larger individuals than the other. This information has also been included in the Methods section.

Comment 2: Ficou meio estranho essa separação entre morfotipo 1 e 2 a priori, sem muita explicação, e essa separação é usada na AMOVA e na PCA (porque não fazer uma PCA com bacias ou paleodrenagens por exemplo?). Acho que merece uma explicação melhor.. seria morfotipos baseado em um trabalho anterior ou nos caracteres morfológicos discretos que servem para a sua diagnose (filamento caudal por exemplo).

Response: We thank the reviewer for this important observation. We agree that the rationale for separating morphotype 1 and morphotype 2 needed to be better explained. These groups were not defined arbitrarily, but represented operational taxonomic hypotheses based on the initial examination of discrete morphological characters observed among specimens historically identified as *Rineloricaria steindachneri*. These characters included mainly caudal-fin filament development, color pattern, and differences in body plate ornamentation, which were later evaluated together with morphometric and molecular evidence.

The PCA was used to test whether our initial morphometric and genetic variation was congruent with the results of a multivariate analysis that is not based on prior groups. Unlike Discriminant analysis, PCA does not include information about prior groups. Because the objective of the analyses was to evaluate whether populations previously identified as *R. steindachneri* represented a single entity or more than one diagnosable lineage, grouping specimens only by river basins or palaeodrainages would not directly test the taxonomic hypothesis proposed here. We have clarified this rationale in the Methods section.

Comment 3: Tem o suporte que esse grupo (*R. steindachneri* + *R. capixaba*) forme um grupo monofilético, talvez *R. capixaba* seja mais proximamente relacionada com outra do sudeste?? mencionar isso, penso que é de outro trabalho (Mejia & Buckup, 2024?)

Response: There is support for the close phylogenetic relationship between *R. steindachneri* and *R. capixaba*. Broader phylogenetic analyses including additional southeastern Brazilian species of *Rineloricaria* were conducted using an expanded molecular dataset and are presented in Mejia (2025, phd thesis). These analyses recover *R. capixaba* as closely related to *R. steindachneri*, rather than to another southeastern congener.

However, we did not include this broader phylogenetic reconstruction of *Rineloricaria* relationships in the present manuscript because the main objective here was to evaluate the taxonomic identity and population structure of specimens historically identified as *R. steindachneri*. In addition, including a genus-level COI tree would be partially redundant with the phylogenetic framework already presented in Mejia, Buckup (2024). More importantly, presenting a genus-wide phylogeny of *Rineloricaria* is outside the scope of a manuscript that is dedicated to the description of a single species. Therefore, we prefer to keep the manuscript focused on the delimitation, diagnosis, and phylogeographic structure of the two lineages treated here.

Sincerely,

Eduardo Mejia

(on behalf of all co-authors)

Associate editor's decision after peer review (27/05/2026).

Dear Eduardo Mejia and Paulo Buckup:

It is a pleasure to accept your manuscript entitled "Past connection and isolation of *Rineloricaria* (Siluriformes: Loricariidae) across southeastern Brazilian coastal drainages: evidence for palaeodrainage connectivity and description of a new species" in its current form for publication in the *Neotropical Ichthyology*.

I have two suggestions to your manuscript to be incorporated in the final version.

Page 1, lines 29–35. To avoid confusion with sea-level oscillations caused by tide changes, I believe this sentence would be better if rephrased as follows:

From "Sea-level oscillations along the southeastern Brazilian coast repeatedly exposed and inundated palaeodrainage systems, creating alternating phases of connectivity and isolation among adjacent river basins that may have structured diversification in freshwater fishes."

To "Marine transgressions and regressions repeatedly inundated and exposed the Southern Brazilian coast, creating alternating phases of connectivity and isolation among adjacent coastal paleodrainages, that may have structured freshwater fishes diversification."

Page 16. It is necessary to clearly state that the specific name is "A noun in apposition". 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 our Social Media Editor, Igor Souto-Santos, at icass.ufrrj@gmail.com within 30 days.

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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 send an e-mail to neoichth@nupelia.uem.br 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 Melo

Associate Editor, Neotropical Ichthyology

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Neotropical Ichthyology



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