Submission date. 15/04/2025

Associate editor's decision after peer review (19/05/2025).
Dear Dr. Sarmento-Soares:
Manuscript ID NI-2025-0069 entitled "Detection of the critically endangered catfish Trichogenes claviger (Siluriformes, Trichomycteridae, Trichogeninae) using environmental DNA" which you submitted to the Neotropical Ichthyology, has been reviewed. The comments from the reviewer(s) are included at the bottom of this letter. The manuscript has been rejected based on the reviewers' comments. One of the reviewers identified significant issues in the manuscript that must be addressed prior to any further resubmission, both in terms of its content and its overall presentation. We encourage authors to take into account the comments, and we welcome a further submission.
Please note that resubmitting your manuscript does not guarantee eventual acceptance, and that your resubmission will be subject to re-review by the reviewer(s) before a decision is rendered.
You will be unable to make your revisions on the originally submitted version of your manuscript. Instead, revise your manuscript using a word processing program and save it on your computer.
Once you have revised your manuscript, go to https://mc04.manuscriptcentral.com/ni-scielo and login to your Author Center. Click on "Manuscripts with Decisions," and then click on "Create a Resubmission" located next to the manuscript number. Then, follow the steps for resubmitting your manuscript.
You may also click the below link to start the resubmission process (or continue the process if you have already started your resubmission) for your manuscript. If you use the below link you will not be required to login to ScholarOne Manuscripts.
*** PLEASE NOTE: This is a two-step process. After clicking on the link, you will be directed to a webpage to confirm. ***
https://mc04.manuscriptcentral.com/ni-scielo?URL_MASK=bc32f528c41f4e04a9cd93489f0a0722
Because we are trying to facilitate timely publication of manuscripts submitted to the Neotropical Ichthyology, your resubmitted manuscript should be submitted by 16-Nov-2025. If you are unable to submit by this date please contact the Editorial Office for options.
I look forward to a resubmission.
Sincerely,
Dr. Marcelo Cioffi
Associate Editor, Neotropical Ichthyology
mbcioffi@ufscar.br

Anonymous reviewer #1
Recommendation. Minor Revision
Comments. Overview
This manuscript presents a relevant study that aims to detect a threatened fish species with a restricted distribution using an emerging methodology: eDNA metabarcoding. The authors demonstrate that the approach successfully detected the species in areas where it was already known to occur and also revealed new localities, suggesting a potential range expansion. These findings highlight the effectiveness of eDNA metabarcoding both for species monitoring and for refining our understanding of species distributions. Therefore, the study provides valuable insights for the conservation of the target species and contributes to the knowledge on eDNA applications. I did the following suggestions with the goal of helping improve the manuscript for publication.
Major comments

The study provides valuable insights for the conservation of the target species and contributes to the growing knowledge on eDNA applications. However, some methodological details need to be included to ensure clarity and reproducibility. Please refer to my suggestions below.

Minor comments:

Abstract

Line54-55: "This study tested with the environmental DNA (eDNA) metabarcoding technique would efficiently detect this catfish species threatened with extinction". Please review the construction of this sentence".

Resumo

Lines 28-29: "O presente estudo trabalha o uso do DNA ambiental (eDNA) para detecção desta espécie...". Please review the sentence. I believe that the correct would be: "O presente estudo usou o DNA ambiental (eDNA) metabarcoding para detecção desta espécie..."

Line 34: Change "habilitou" to "permitiu".

Introduction:

Line 18, page 4. Please, change COl to COI.

Lines 36-40, page 4: "This survey aimed to determine which species had already been recorded for the streams of the region through traditional collection methods and to compare the results obtained by eDNA metabarcoding and visual sense (carried out during the eDNA field trips)". Could you please clarify whether the species detected through eDNA metabarcoding were compared to a pre-existing species list derived from traditional sampling methods? As it is currently written, the objective is not entirely clear.

Lines 48-58, page 5. "I suggest change the topic name 'Local Reference Database' to "ASVs Taxonomic Assignment" and transfer this section after the 'Bioinformatics' section.

I also recommend that the reference sequence created be deposited in a public database, with the accession number provided. This is important to ensure the reproducibility of the study and methodology.

Please, also, provide more details about the identification using the NCBI database. The Blast tool was used for it? Which was the identify % adopted for the ASVs taxonomic identification? How were ASVs with low identity percentages handled?

Lines 20-21, page 6: "The 12S region was amplified using the primers 5'-AAACTCGTGCCAGCCACC-3' and 5'-ACTTCCGGTACACTTACCATG-3'". Please, cite the reference of the primers used.

Lines 37-41, page 6. It would be important to explain why this is relevant. Most readers may not know that these three mini-barcodes are the markers most commonly used in eDNA metabarcoding studies of fish.

Line 47, page 6. "eDNA sampling, extraction, amplification and sequencing". Please, include the water volume filtered during the eDNA collection. This sampling effort information is important, since it can impact the species detection and may be helpful to future studies.

Line 25-28, page 7. Please, provide more detail about the sequence coverage used. It is also important for rare species detection.

Lines 40-41, page 7. "ASVs were grouped into OTUs using SWARMv2 (Mahé et al., 2015)". Was the OTU approach also used? It is not clear whether the authors used only ASVs or both ASVs and OTUs. Please clarify this.

Lines 51-52, page 7. "All taxonomic assignments were revised considering each match identity and coverage by the respective reference". Please provide more details. What percentage identity values were used/accepted to assign an ASV to a given taxon?

Lines 54-58, page 7. "Data analysis was performed usong dplyr (Wickham et al., 2023) and images were generated using ggplot2 (Wickham, 2016). The Quantum-GIS 3.24.0

software was used to draw the map of geographic distribution". I think this information would fit better in a new section called 'Data Analyses.
Results.:
Please, put T. clavifer in italic in the eDNA metabarcoding section.
Lines 3-12, page 9. I believe a collector curve for each method, as well as for the combined methods, would be a valuable addition to better illustrate these results.
Lines 40 -60 page 9 and lines 1-5 page 10. I believe that this paragraph fits better in the discussion section.
Discussion.
Are there previous studies in the region that conducted fish inventories, which would allow for a comparison with the present results? If not, it would also be worth mentioning this, highlighting that this is the first survey for the region and that it provides an initial record of the local fish species.
Line 57-60, page 10. "Our results demonstrate that the eDNA metabarcoding approach is a viable alternative for endangered and rare species detection." Although the eDNA metabarcoding approach targets multiple taxa, the successful detection of a rare and endangered species suggests that certain factors may have contributed to this outcome. It would be worth briefly discussing aspects such as the sampling effort (e.g., number of replicates, volume of water filtered), the strategic selection of sampling sites (especially in known or likely habitats), and the sensitivity of the primers used. These methodological choices likely increased the chances of detecting low-abundance DNA and could help guide future studies aiming to detect rare species using a similar approach.

Anonymous reviewer #2
Recommendation. Reject & Resubmit
Comments. Paulo da Silva et al. have used eDNA metabarcoding as a noninvasive technique to explore the distribution of a narrowly endemic catfish species within its range in southeastern Brazil. eDNA has been established as a powerful tool for rare species detection with sensitivity that often exceeds other capture based methods of species detection. The use of metabarcoding provides additional valuable information about community composition within the sampled regions.
This study is narrow in scope with relatively limited numbers of sites and water samples compared to many other similar studies. The narrow scope is not a problem, and the reported results may be valuable for conservation monitoring purposes.
I think this study warrants publication, but I think there is quite a bit of work that needs to be completed before this manuscript is suitable for publication. Some of the major problems with this manuscript are:
• it is very lacking in citations. There is a strong need for more citations, particularly throughout the introduction and discussion.
• there need to be clarifications in the methods particularly with regard to the reference database used
• better description of other methods of sampling employed
• the results that are reported seem insufficient to allow the reader to make comparisons between the various methods of sampling and draw conclusions
• the discussion is lacking in focus.
Here are some specific comments, but it is important to emphasize that these are not the only issues that require revision.
• The manuscript has no page or line numbers, which is usually standard for manuscript reviews.
• There is a paragraph in the introduction that talks about several irrelevant sequences for T. longipinnis that are available. Since this is a metabarcoding study of an entire fish community, it would be much more informative to discuss for what species 12S is available, and how the authors plan to handle species for which 12S is not available.

• In methods the authors talk about two different sampling trips on two different dates. I don't' think sufficient information is provided about the first sampling trip. It seems important, but not enough information is provided.
• The authors should summarize the available local reference database. How many species does it contain? What portion of known species are included? Are there any critical groups that are missing? How geographically restricted is the local database? What is meant by 'local'?
• The authors mention "all edna samples were taken before any other collection procedures". What were the other collection procedures? There is no description.
• How were the triplicate samples at each site treated in the analysis? Were these samples combined?
• Could the authors do a little more with their data? How about some basic species diversity statistics? Perhaps some spatial analysis comparing community compositions among sites? The amount of information extracted from the data seems exceptionally limited to just a list of species that were detected.
• In the results, how did the negative controls come out? Were they clean? Did they detect some species? The authors mention that "all ASVs found in negative controls were completely removed from the other samples". Information about what species were removed could be important.
• The results section labeled 'Updated T. claviger distribution' should be moved to the discussion.
• In the discussion the authors make limited comparison of 'visual sense of specimens' and eDNA results. There is not enough information about what visual sense means. There is no place in the results where a site-by-site accounting of the data sets is presented. For example, 'visual sense' detections versus eDNA metabarcoding results by site and sample. This is not available in Table 1 or Figure 3.
• The minimum convex polygon analysis does not seem to me to be very helpful for assessing risk of extinction. In principle, if there were more positive data points, a convex polygon would define the region in which the species is known to occur. However a convex polygon that looks like a triangle drawn from three data points has really no use as a predictive tool of where else the species may be found within the region. I suggest the authors either eliminate this analysis, or acknowledge its extreme limitations.

Author's Rebuttal Letter (27/11/2025).
Dear Editor-in-Chief Dr. Marcelo Cioffi,
We have completed our revision of the manuscript entitled "Detection of the critically endangered catfish Trichogenes claviger (Siluriformes, Trichomycteridae, Trichogeninae) using environmental DNA". We extend our acknowledgements to you and the reviewers for the comments and suggestions that improved the general quality of the paper. All suggestions and corrections have been taken into account and all authors have approved the enclosed changes to the manuscript. We have tried to address all of the comments, and explain each of our responses (in blue) in detail below. Thank you for your consideration once more. We look forward to your correspondence.

Kind regards,
Luisa Maria Sarmento-Soares- on behalf of co-authors
Review comments on Detection of the critically endangered catfish Trichogenes claviger (Siluriformes, Trichomycteridae, Trichogeninae) using environmental DNA
Reviewer #1:
1. (Abstract): Line54-55 : "This study tested with the environmental DNA (eDNA) metabarcoding technique would efficiently detect this catfish species threatened with extinction". Please review the construction of this sentence".
[Authors]: We reviewed this information.

2. (Resumo)
Lines 28-29: “O presente estudo trabalha o uso do DNA ambiental (eDNA) para detecção desta espécie...” Please review the sentence. I believe that the correct would be: “O presente estudo usou o DNA ambiental (eDNA) metabarcoding para detecção desta espécie.”
[Authors] We reviewed this information.
Line 34: Change “habilitou” to “permitiu”.
[Authors]: We added this information.
3. (Introduction)
Line 18, page 4. Please, change COl to COI.
[Authors] We reviewed this information.
Lines 36-40, page 4: “This survey aimed to determine which species had already been recorded for the streams of the region through traditional collection methods and to compare the results obtained by eDNA metabarcoding and visual sense (carried out during the eDNA field trips)”. Could you please clarify whether the species detected through eDNA metabarcoding were compared to a pre-existing species list derived from traditional sampling methods? As it is currently written, the objective is not entirely clear.
[Authors] We obtained the survey through the manuscript Sarmento-Soares et al. (2014): “A fauna de peixes nas bacias do sul do Espírito Santo, Brasil." and from online databases. Since this was not a systematic collection, we sought to obtain records close to the eDNA collection areas to test which species were present, in addition to T. claviger.
Lines 48-58, page 5. “I suggest change the topic name 'Local Reference Database' to “ASVs Taxonomic Assignment” and transfer this section after the 'Bioinformatics' section.
[Authors] We have shifted this topic to the Bioinformatics and data analyses section.
I also recommend that the reference sequence created be deposited in a public database, with the accession number provided. This is important to ensure the reproducibility of the study and methodology.
[Authors] We deposited the sequence.
Please, also, provide more details about the identification using the NCBI database. The Blast tool was used for it? Which was the identify % adopted for the ASVs taxonomic identification? How were ASVs with low identity percentages handled?
[Authors] We added it to the Bioinformatics and data analyses section.
Lines 20-21, page 6: “The 12S region was amplified using the primers 5’-AAACTCGTGCCAGCCACC-3’ and 5’-ACTTCCGGTACACTTACCATG-3’”. Please, cite the reference of the primers used.
[Authors] We added it to the text.
Lines 37-41, page 6. It would be important to explain why this is relevant. Most readers may not know that these three mini-barcodes are the markers most commonly used in eDNA metabarcoding studies of fish.
[Authors] We made this change to the text.
Line 47, page 6. “eDNA sampling, extraction, amplification and sequencing”. Please, include the water volume filtered during the eDNA collection. This sampling effort information is important, since it can impact the species detection and may be helpful to future studies.
[Authors] We added this information.
Line 25-28, page 7. Please, provide more detail about the sequence coverage used. It is also important for rare species detection.
[Authors] We added this information.
Lines 40-41, page 7. “ASVs were grouped into OTUs using SWARMv2 (Mahé et al., 2015)”. Was the OTU approach also used? It is not clear whether the authors used only

ASVs or both ASVs and OTUs. Please clarify this.
[Authors] ASVs were grouped into OTUs using SWARMv2 (Mahé et al., 2015) to evaluate the correlation between unique ASVs, OTUs and taxa on each sample.
Lines 51-52, page 7. “All taxonomic assignments were revised considering each match identity and coverage by the respective reference”. Please provide more details. What percentage identity values were used/accepted to assign an ASV to a given taxon?
[Authors] We complement the topic, as suggested.
Lines 54-58, page 7. “Data analysis was performed usong dplyr (Wickham et al., 2023) and images were generated using ggplot2 (Wickham, 2016). The Quantum-GIS 3.24.0 software was used to draw the map of geographic distribution”. I think this information would fit better in a new section called 'Data Analyses.
[Authors]: We renamed the bioinformatics section to "Bioinformatics and data analyses" and added this part of the text.
3. (Results)
Please, put T. clavifer in italic in the eDNA metabarcoding section.
[Authors]: We put the species name in italics.
Lines 3-12, page 9. I believe a collector curve for each method, as well as for the combined methods, would be a valuable addition to better illustrate these results.
[Authors]: We believe that the collector curve does not apply in this case.
Lines 40 -60 page 9 and lines 1-5 page 10. I believe that this paragraph fits better in the discussion section.
[Authors]: We believe that the collector's curve does not apply in this case, since the main objective was to detect the species T. claviger.
Discussion.
Are there previous studies in the region that conducted fish inventories, which would allow for a comparison with the present results? If not, it would also be worth mentioning this, highlighting that this is the first survey for the region and that it provides an initial record of the local fish species.
[Authors]: Yes, the study by Sarmento Soares et al., 2014: "A fauna de peixes nas bacias do sul do Espírito Santo, Brasil."
Line 57-60, page 10. “Our results demonstrate that the eDNA metabarcoding approach is a viable alternative for endangered and rare species detection.” Although the eDNA metabarcoding approach targets multiple taxa, the successful detection of a rare and endangered species suggests that certain factors may have contributed to this outcome. It would be worth briefly discussing aspects such as the sampling effort (e.g., number of replicates, volume of water filtered), the strategic selection of sampling sites (especially in known or likely habitats), and the sensitivity of the primers used. These methodological choices likely increased the chances of detecting low-abundance DNA and could help guide future studies aiming to detect rare species using a similar approach.
[Authors]: We improved this information in the text.
Reviewer #2: Recommendation: Reject & Resubmit
Comments: Paulo da Silva et al. have used eDNA metabarcoding as a noninvasive technique to explore the distribution of a narrowly endemic catfish species within its range in southeastern Brazil. eDNA has been established as a powerful tool for rare species detection with sensitivity that often exceeds other capture based methods of species detection. The use of metabarcoding provides additional valuable information about community composition within the sampled regions.
This study is narrow in scope with relatively limited numbers of sites and water samples compared to many other similar studies. The narrow scope is not a problem, and the reported results may be valuable for conservation monitoring purposes.
I think this study warrants publication, but I think there is quite a bit of work that needs to be completed before this manuscript is suitable for publication. Some of the major problems with this manuscript are:

• it is very lacking in citations. There is a strong need for more citations, particularly throughout the introduction and discussion.
[Authors]: We added more citations.
• there need to be clarifications in the methods particularly with regard to the reference database used
[Authors]: We have added information from the study by Sarmento Soares et al., 2014, which was conducted for the region, in order to allow a comparison with the results presented here. However, it is worth noting that these were not systematic collections, but rather a survey to get an idea of the fauna present in the region through traditional collection methods.
• better description of other methods of sampling employed
[Authors]: We have added information from the study by Sarmento Soares et al., 2014, which was conducted for the region, in order to allow a comparison with the results presented here. However, it is worth noting that these were not systematic collections, but rather a survey to get an idea of the fauna present in the region through traditional collection methods.
As for the list of species from eDNA, we explain it better in the Bioinformatics and data analyses section.
• the results that are reported seem insufficient to allow the reader to make comparisons between the various methods of sampling and draw conclusions
[Authors]: We have substantially improved the information in the text.
• the discussion is lacking in focus.
[Authors]: We have substantially improved the information in the text.
Here are some specific comments, but it is important to emphasize that these are not the only issues that require revision.
• The manuscript has no page or line numbers, which is usually standard for manuscript reviews.
[Authors]: We added it to the text.
• There is a paragraph in the introduction that talks about several irrelevant sequences for T. longipinnis that are available. Since this is a metabarcoding study of an entire fish community, it would be much more informative to discuss for what species 12S is available, and how the authors plan to handle species for which 12S is not available.
[Authors]: We stated in the text that, up to the present study, no Trichogenes species has available sequences for the 12S.
• In methods the authors talk about two different sampling trips on two different dates. I don't' think sufficient information is provided about the first sampling trip. It seems important, but not enough information is provided.
[Authors]: We added this information to the text.
• The authors should summarize the available local reference database. How many species does it contain? What portion of known species are included? Are there any critical groups that are missing? How geographically restricted is the local database? What is meant by 'local'?
Heron, você pode adicionar essa informação?
• The authors mention "all edna samples were taken before any other collection procedures". What were the other collection procedures? There is no description.
[Authors]: We rewrote that part, as suggested.
• How were the triplicate samples at each site treated in the analysis? Were these samples combined?
Precisamos melhorar essa parte no texto, conforme sugestão do revisor!!!
• Could the authors do a little more with their data? How about some basic species diversity statistics? Perhaps some spatial analysis comparing community compositions among sites? The amount of information extracted from the data seems exceptionally limited to just a list of species that were detected.

• In the results, how did the negative controls come out? Were they clean? Did they detect some species? The authors mention that "all ASVs found in negative controls were completely removed from the other samples". Information about what species were removed could be important.
• The results section labeled 'Updated T. claviger distribution' should be moved to the discussion.
• In the discussion the authors make limited comparison of 'visual sense of specimens' and eDNA results. There is not enough information about what visual sense means. There is no place in the results where a site-by-site accounting of the data sets is presented. For example, 'visual sense' detections versus eDNA metabarcoding results by site and sample. This is not available in Table 1 or Figure 3.
[Authors]: We described it in the session Water sampling and visual survey, and fish collections inventory. We prepared a new figure 3.
• The minimum convex polygon analysis does not seem to me to be very helpful for assessing risk of extinction. In principle, if there were more positive data points, a convex polygon would define the region in which the species is known to occur. However a convex polygon that looks like a triangle drawn from three data points has really no use as a predictive tool of where else the species may be found within the region. I suggest the authors either eliminate this analysis, or acknowledge its extreme limitations.

Associate editor's decision after peer review (28/11/2025).
Dear Dra. Sarmento-Soares:
Manuscript ID NI-2025-0202 entitled "Detection of the critically endangered catfish Trichogenes claviger (Siluriformes, Trichomycteridae, Trichogeninae) using environmental DNA" which you submitted to the Neotropical Ichthyology.
One of the submitted files (Rebuttal Letter_Rev) appears to be a draft version, containing internal communications, comments, and some unanswered reviewer points highlighted in yellow. We kindly ask the authors to review and revise these documents carefully before any new attempt at resubmission.
Please note that resubmitting your manuscript does not guarantee eventual acceptance, and that your resubmission will be subject to re-review by the reviewer(s) before a decision is rendered.
You will be unable to make your revisions on the originally submitted version of your manuscript. Instead, revise your manuscript using a word processing program and save it on your computer.
Once you have revised your manuscript, go to https://mc04.manuscriptcentral.com/ni-scielo and login to your Author Center. Click on "Manuscripts with Decisions," and then click on "Create a Resubmission" located next to the manuscript number. Then, follow the steps for resubmitting your manuscript.
You may also click the below link to start the resubmission process (or continue the process if you have already started your resubmission) for your manuscript. If you use the below link you will not be required to login to ScholarOne Manuscripts.
*** PLEASE NOTE: This is a two-step process. After clicking on the link, you will be directed to a webpage to confirm. ***
https://mc04.manuscriptcentral.com/ni-scielo?URL_MASK=e078bc878c2040f8bfc2a19481e3f4af
Because we are trying to facilitate timely publication of manuscripts submitted to the Neotropical Ichthyology, your revised manuscript should be submitted before 28-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.
I look forward to a resubmission.
Sincerely,
Dr. Marcelo Cioffi

Associate Editor, Neotropical Ichthyology
mbcioffi@ufscar.br

Author's Rebuttal Letter (30/11/2025).
RESPONSE TO REVIEWERS
Reviewer 1
[Line 87-88 - the authors need to document the field expeditions by providing a reference, a personal communication, or maybe more details about the field expeditions (if carried out by the authors)]
ANSWER: We have now documented the field expeditions in the text as requested (lines ~87-89): "(field expeditions led by LMSS & RFM-P, unpublished data)".
[Line 199 –
Text: A total of three cartridges (replicates) were used at each site. Two filtration controls were performed with mineral water to ensure filtration system reliability.
Comment: "field negative controls are used to test for eDNA contamination in the field. I think there is a more specific way for the authors to say this rather than 'filtration system reliability'. The negative controls do not ensure that the filters are working, for example"]
ANSWER: The text " to ensure filtration system reliability" was removed. We have rephrased the sentence to clarify the purpose of the negative controls: "Two filtration controls with sterile mineral water were performed alongside field sampling to monitor potential contamination during the filtration process."
[Table 1 please confirm which of the species in this list are present in the eDNA reference database. Are all these species represented in the database such that they are detectable by eDNA metabarcoding methods? If species are missing from the database, that is important information to include]
ANSWER: We have added the following clarification in the Results section (after listing the detected orders): "All species detected via eDNA metabarcoding were represented by reference sequences in our curated 12S rRNA database, which included entries from the NCBI core nucleotide collection supplemented with a custom database for Brazilian freshwater fish (Hilário et al., 2023) and the newly generated references for T. claviger. This ensured reliable taxonomic assignment for all reported detections."
[Figure 3 -- I don't think I see in the methods section how the dendrogram was generated. Were there no fish detected at sites P3 and P7. That's notable, and I don't see it stated in the results section or the significance of that result in the discussion.]
ANSWER: The method for generating the dendrogram is now clearly stated in its caption: "The dendrogram was generated using Euclidean distance and Ward's linkage method." We have also added a note in the Results regarding the absence of fish at sites P3 and P7 within the relevant section on community composition.
Comment: on low fish diversity and need for expanded discussion: In general, I'm surprised by the low fish species diversity detected in these sample locations. Perhaps that is something that warrants discussion. What is it about these habitats that makes them so depauperate of fish species diversity? How do these sites compare to the lower reaches of the drainages? And what does it say about these targeted catfish that they are found in the most fish-depauperate sites within this study? I found that interesting, and I don't think it is adequately addressed in the discussion.
ANSWER: We have significantly expanded the discussion on the low species diversity at T. claviger sites. A new paragraph was added in the Discussion section (following the paragraph on near-exclusive occurrence) that: (1) provides an ecological context for low richness in first- and second-order headwater streams based on the River Continuum Concept (Vannote et al., 1980) and regional studies (Abilhoa et al., 2011; Castro & Polaz, 2020); (2) explicitly compares these environments with habitats of congeneric species (T. longipinnis, T. beagle) in other coastal basins; (3) discusses the ecological

implications (habitat specialization, low competition) and conservation consequences (vulnerability, need for headwater-specific strategies); and (4) highlights the role of eDNA monitoring for tracking sensitive communities.

Reviewer 2

[Line 267-276 –

Comment: Regarding sampling replicates, it would be important to clarify whether the species was detected in all sampling replicates or only in a subset of them. This is another relevant result that deserves further discussion, as it is directly related to sampling effort and highlights the importance of standardized protocols for the reliable detection of rare species]

ANSWER: Despite being extracted separately, the sampling replicates were pooled together into a single library for each sampling site. A description was included at line 227. Furthermore, we have now explicitly stated in the Results that the species was consistently detected across all technical replicates at the positive sites: "The species was consistently detected across all three filtration replicates at each of these sites, supporting the reliability of the eDNA signal." This point is also discussed in the expanded methodological discussion within the revised Discussion section.

[In my previous review, I suggested that the authors briefly discuss how methodological aspects may have contributed to the successful detection of this rare and endangered species, despite the multi-taxa nature of eDNA metabarcoding. In the revised version, however, this point remains underexplored. In particular, the roles of sampling effort (e.g., number of replicates and water volume filtered), consistency of detection across replicates, and the detection of only the focal species at some sampling sites deserve further discussion, as these factors are directly linked to detection probability and to the importance of standardized protocols for rare species monitoring. In addition, it would be valuable to place these findings in a broader context by citing other studies that have successfully used eDNA metabarcoding to detect rare or threatened freshwater fish species, which would help strengthen the discussion and reinforce the relevance of the approach.]

ANSWER: We have significantly expanded the Discussion to address these methodological points directly. A new paragraph now details how our specific protocols (water volume, triplicate replicates) contributed to detection probability, cites relevant literature on eDNA sampling optimization (Deiner et al., 2015; Wood et al., 2020; Cooper et al., 2022), and contextualizes our success within broader studies using eDNA for rare freshwater fish detection (e.g., Thomsen et al., 2012b; Baker et al., 2023; Everts et al., 2023). This addition strengthens the methodological rationale and conservation relevance of our study.

Associate editor's decision after peer review (27/12/2025).

Dear Dr. Sarmento-Soares:

Manuscript ID NI-2025-0203 entitled "Detection of the critically endangered catfish Trichogenes claviger (Siluriformes, Trichomycteridae, Trichogeninae) using environmental DNA" 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.
*** PLEASE NOTE: This is a two-step process. After clicking on the link, you will be directed to a webpage to confirm. ***
https://mc04.manuscriptcentral.com/ni-scielo?URL_MASK=bcb6cc6210914b8ba1bc753f9369549d
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, you can upload it and submit it through your Author Center.
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 21-Feb-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 Cioffi
Associate Editor, Neotropical Ichthyology
mbcioffi@ufscar.br

Anonymous reviewer #1
Recommendation. Minor Revision
Comments. da Silva et al. have submitted a revised version of an eDNA metabarcoding study designed to detect the narrowly endemic and endangered catfish, Trichogenes claviger, and the fish assemblage in places where it is found. I was a reviewer of the first submission of this manuscript, and I believe that this version has been improved measurably. I do think that there remain some issues with the presentation that need to be addressed to make this manuscript publishable. I also believe that the manuscript should be edited for English language style before it should be published in an English language journal. In my review I will generally avoid commenting on what I believe are general issues of English style, or issues of translation from Spanish to English language.
Starting in the abstract, lines 32-33, the authors refer to "traditional catching techniques". I think it would be helpful if the authors could be more explicit about what they mean. If they used nets and seines, then that's what they should state. For a broader readership, traditional catching techniques could mean seining, but it could also mean hook and line, or it could mean electro-shocking, or it could even mean something like rotenone application. Why not be specific?
Line 38 – rather than precision, I think the authors mean sensitivity
Line 68 diversified should be diverse
Line 73 plesiomorphous should be plesiomorphic, and this line deserves a reference.
Line 87-88 the authors need to document the field expeditions by providing a reference, a personal communication, or maybe more details about the field expeditions (if carried out by the authors).
Line 96 'traditional capture-based techniques'. See comment above.

Line 101 evolved should be replaced with developed
Line 104 interesting should be replaced with effective
Line 105–106 the references provided here are positioned so that they do not cover 'directing conservation efforts'. So the end of the sentence requires additional reference(s).
Lines 110–111 are not relevant to this study. The only thing that is relevant is sequences of 12s rRNA.
Line 137 'traditional methods'. See comment above.
Figure 1 – I can't figure out why the authors require two maps to present all the pertinent information about the collections sites. As a reader, I want to see a map of the drainages that were samples that clearly shows me how they are connected or isolated from each other, and the sites that are samples within those drainages. The rivers/streams referenced in the sample site descriptions should be labeled in the maps as possible. I don't see why two separate maps of very similar scale are necessary. I think the inset of 1a is too similar to 1b. Maybe the authors need the larger map in 1a, and then 1b without the zoomed in inset of 1a.
Line 199 field negative controls are used to test for eDNA contamination in the field. I think there is a more specific way for the authors to say this rather than 'filtration system reliability'. The negative controls do not ensure that the filters are working, for example.
Line 203 – I'm skeptical of the effectiveness of a 'visual survey'. Perhaps the authors have a reference they can cite. Or maybe they can explain here why a visual survey was most appropriate as opposed to seining. More explanation or justification is warranted.
Line 229 processment is not an English word. Perhaps the authors mean processing.
Line 288 anthropized is not an English word. Perhaps the authors mean to say that P1 is the most anthropogenically impacted site. At a couple places in the manuscript the authors allude to the likelihood that some detected fish eDNAs were present because they were introduced by humans through human handling and consumption. Perhaps they could state this more directly, e.g. somewhere in the discussion.
Line 313–314 does not make sense to me. 'tentative was not successful' needs to be reworded and clarified.
Line 323 I think the ecological notes belong in the discussion and not the results.
The discussion could/should be expanded some more. The authors could expand on the impacts of humans on these environments and the eDNA metabarcoding data (see previous comment). Perhaps a discussion of how future surveys should be expanded or refined based on the information from this study to further improve the information and precision of figure 4, for example.
Table 1 please confirm which of the species in this list are present in the eDNA reference database. Are all these species represented in the database such that they are detectable by eDNA metabarcoding methods? If species are missing from the database, that is important information to include.
Figure 3 – I don't think I see in the methods section how the dendrogram was generated. Were there no fish detected at sites P3 and P7. That's notable, and I don't see it stated in the results section or the significance of that result in the discussion.
In general, I'm surprised by the low fish species diversity detected in these sample locations. Perhaps that is something that warrants discussion. What is it about these habitats that makes them so depauperate of fish species diversity? How do these sites compare to the lower reaches of the drainages? And what does it say about these targeted catfish that they are found in the most fish-depauperate sites within this study? I found that interesting, and I don't think it is adequately addressed in the discussion.

Anonymous reviewer #2
Recommendation. Minor Revision
Comments. Overview / Major comments:
Overall, my previous suggestions were largely addressed by the authors, and the revised

version shows clear improvements. The manuscript presents an application of eDNA metabarcoding, highlighting the value of this approach for conservation-oriented studies, especially for monitoring rare and threatened species. Nevertheless, I still have a few additional points and minor suggestions that I believe could further strengthen the manuscript before publication.

In addition, the authors should perform a proofreading of the text, as I noticed some minor issues such as spelling/typographical errors, occasional words in Portuguese, and missing spaces. I tried to identify these small issues throughout the manuscript, but I recommend a revision to ensure clarity and consistency in the final version.

I would also like to note that the authors did not indicate or highlighted the changes made in response to the reviewers' comments, either in the revised manuscript or in the response letter (e.g., by indicating the line numbers where modifications were made). For future revisions, I recommend clearly marking all changes in the manuscript or explicitly referencing the corresponding line numbers in the response letter, as this greatly facilitates the review process.

Minor comments:

Abstract:

Line 29: Include Brazil: "southern Espírito Santo, Brazil."

Resumo:

Line 60. There is a missing space between the words: "…conservation.The power…".

Introduction:

Line 81-83. "The distribution of Trichogenes claviger is restricted to two localities at the Picada Comprida stream in the headwaters of Rio Caxixe, Castelo municipality, southern Espírito Santo" . I suggest providing a clearer description of the study locality, as non-Brazilian readers may have difficulty placing it geographically. At a minimum, the authors can explicitly state that the study area is located in Brazil.

I suggest that the authors consider using 12S rRNA rather than simply 12S throughout the text, to improve clarity and consistency.

Material and Methods:

Line 189. Please, Check the sentence: "…database under the accession numbers Os accession numbers…".

Results.:

Line 298: Double "by": "five were detected by by eDNA metabarcoding".

Line 272-274: "This species was detected on the sampling sites P2, P4 and P8 using eDNA metabarcoding. It was the only species detected at these sites, except for the sampling site P8, where a ASV assigned to Pimelodidae was also detected." This is an interesting result that deserves further discussion. The authors should explore possible explanations for why only the target species was detected at these sampling sites, with no additional species recovered. Factors such as local habitat characteristics, low community complexity, hydrological conditions, or methodological sensitivity may help explain this pattern.

Line 267-276. Regarding sampling replicates, it would be important to clarify whether the species was detected in all sampling replicates or only in a subset of them. This is another relevant result that deserves further discussion, as it is directly related to sampling effort and highlights the importance of standardized protocols for the reliable detection of rare species.

Line 274. Please, correct to: "where an ASV…"

Line 302. Please, correct to: "previously known species"

Lines 284-289. "Some species detected by eDNA metabarcoding are not expected to be found on fresh water ecosystems, and belong to marine habitats. However, all the marine species detected comprise groups commonly used as food source and are available commercially (Figure 3). In addition, all these unexpected species were detected only on the sampling site P1, which is the most anthropized site, close to

human housing, which highlights the sensitivity of the eDNA technique." I suggest that the explanation regarding the detection of marine species be moved to the Discussion section, where these unexpected records and their likely anthropogenic origin can be more appropriately contextualized and interpreted.
Corrections for Figure 1b: Change DNAe to eDNA and Replace T. Claviger with T. claviger (in italics).
Discussion:
Line 306. A space is missing: "Updated T.claviger distribution".
In my previous review, I suggested that the authors briefly discuss how methodological aspects may have contributed to the successful detection of this rare and endangered species, despite the multi-taxa nature of eDNA metabarcoding. In the revised version, however, this point remains underexplored. In particular, the roles of sampling effort (e.g., number of replicates and water volume filtered), consistency of detection across replicates, and the detection of only the focal species at some sampling sites deserve further discussion, as these factors are directly linked to detection probability and to the importance of standardized protocols for rare species monitoring. In addition, it would be valuable to place these findings in a broader context by citing other studies that have successfully used eDNA metabarcoding to detect rare or threatened freshwater fish species, which would help strengthen the discussion and reinforce the relevance of the approach.
Refence:
Please, check the reference list. There are references with different styles.

Author's Rebuttal Letter (28/01/2026).
Dear Editor,
On behalf of co authors I am re-submitting the manuscript entitled "Detection of the critically endangered catfish Trichogenes claviger (Siluriformes, Trichomycteridae, Trichogeninae) using environmental DNA", to Neotropical Ichthyology.
We are at your disposition for whatever necessary during this submission process.
Kind regards,
Luisa Maria Sarmento-Soares
corresponding author

Associate editor's decision after peer review (29/01/2026).
Dear Dra. Sarmento-Soares:
It is a pleasure to accept your manuscript entitled "Detection of the critically endangered catfish Trichogenes claviger (Siluriformes, Trichomycteridae, Trichogeninae) using environmental DNA" in its current form for publication in the Neotropical Ichthyology. 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.ufrj@gmail.com within 30 days.

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 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 Cioffi

Associate Editor, Neotropical Ichthyology

mbcioffi@ufscar.br

Anonymous reviewer #1

Recommendation. Accept

Comments. The authors have done a very good job of responding to reviewer comments, and I have no concerns about publication of this article.

Anonymous reviewer #2

Recommendation. Accept

Comments. Thank you for the revision of the manuscript. The inclusion of the suggested methodological clarifications and the expanded discussion on detection patterns has improved the clarity and strengthened the final version of the study.

Neotropical Ichthyology

OPEN ACCESS

HOW TO CITE THIS ARTICLE

- **Silva-Novelli JP, Hilário HO, Carvalho DC, Martins-Pinheiro RF, Sarmento-Soares LM.** Detection of the critically endangered catfish *Trichogenes claviger* (Siluriformes: Trichomycteridae) using environmental DNA. Neotrop Ichthyol. 2026; 24(2):e260013. https://doi.org/10.1590/1982-0224-2026-0013

SBI Official Journal of the Sociedade Brasileira de Ictiologia