Submission date. 07/07/2025

Associate editor's decision after peer review (06/08/2025).
Dear Dr. Vicari:
Manuscript ID NI-2025-0119 entitled "Cytogenetic and molecular characterization of Parodon (Characiformes: Parodontidae) species from the Amazon basin reveals a new Molecular Operational Taxonomic Unit" 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.
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=779c9b3588f24b4bb21c909f0cef574e
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 06-Sep-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,
Prof. Claudio Oliveira
Associate Editor, Neotropical Ichthyology
claudio.oliveira@unesp.br

Anonymous reviewer #1
Recommendation. Minor Revision
Comments. The manuscript "Cytogenetic and molecular characterization of Parodon (Characiformes: Parodontidae) species from the Amazon basin reveals a new Molecular Operational Taxonomic Unit" compared Parodon species using cytogenetic and genetic markers, aiming the molecular species delimitation. The data and discussion presented

are highly relevant to understanding the Parodontodia family. The text is objective and clear, however needs adjustments, as desbribed below:
Resumo/Resumen – The Resumo presented is in the English language. Provide the translation to Portuguese or Spanish.
Page 10, line 8 to 15 - The information presented in this paragraph would fit better in the introduction.
Page 10, line 15 to 20 – This paragraph should be removed. It did not bring anything relevant to the discussion.
Page 12, line 48 to 54 – The authors mentioned that the Parodon suborbitalis complex does not constitute a monophyletic group in evolutionary terms. In this context, I suggest that the authors include a brief discussion on what the cytogenetic data reveal about this complex.
Page 13, line 25 to 29 - The information that P. cf. buckleyi is an undescribed species was only addressed in the conclusion paragraph of the paper. I suggest that this information be explored in the discussion before being mentioned in the conclusion paragraph and present more arguments that support this statement.
Figure 1 – The figure legend should make clear which points were sampled in the present work.
Figures 1 and 5 – The colors used to represent the species in figure 1 are different from the colors chosen in figure 5. I suggest that each species be identified by the same color in both figures to create a visual identity and facilitate the reader's understanding.

Anonymous reviewer #2
Recommendation. Major Revision
Comments. I am reviewing the manuscript entitled "Cytogenetic and molecular characterization of Parodon (Characiformes: Parodontidae) species from the Amazon basin reveals a new Molecular Operational Taxonomic Unit." The study adopts an integrative approach, combining cytogenetic and molecular data to investigate species delimitation within the genus Parodon in the Amazon basin. The methods employed are appropriate and well-executed, the results are clearly presented, and the identification of a MOTU not yet formally described adds originality and relevance to the work.
Despite the overall quality of the manuscript, some aspects require minor improvements, such as the explicit formulation of hypotheses or research questions, as well as a deeper integration of molecular, cytogenetic, and morphological data. Below, I provide my detailed recommendations by section.
Introduction
The authors provide an adequate contextualization of the diversity within the genus Parodon and the challenges associated with species delimitation. However, the introduction lacks clearly stated hypotheses or research questions. It is recommended that the authors explicitly present the hypothesis(es) or guiding research question(s) in a clear and objective manner to reinforce the investigative focus of the study.
The introduction concludes with a generic statement ("In this context, we described cytogenetically two Parodon species..."), which could be revised to better reflect the scientific gap addressed and the intended contribution of the research.
Additionally, the concept of MOTU (Molecular Operational Taxonomic Unit), although mentioned in the title and abstract, is not introduced or contextualized in the introduction. It would be important to include a brief explanation of what MOTUs are and emphasize their relevance in the context of integrative taxonomy, especially for groups with conserved morphology such as Parodon.
Another point that deserves attention is the absence of a dedicated paragraph

highlighting the importance of cytogenetics in taxonomy. Although the review of karyotypic diversity in Parodontidae is informative and well-supported, the introduction could be strengthened by making clearer the role of chromosomal data within the study's framework. It is recommended to clarify how cytogenetic and molecular data complement each other in the delimitation of MOTUs, promoting a more integrative and cohesive approach.

Specific Comments

The abstract is written in English, whereas it should be in Portuguese. Please adjust it to comply with the journal's formatting guidelines. Page 5, line 32: Please remove the mention of “Tab. 1,” as it does not apply to the context presented. Methodology The COI sequences were properly deposited in GenBank (with accession numbers provided); however, it is also recommended that the sequences be submitted to the BOLD Systems platform. This repository is widely recognized as the primary reference for DNA barcoding studies, offering additional tools for data analysis and visualization. Depositing the sequences in BOLD not only facilitates access and reproducibility but also provides complementary evidence, such as the identification of cryptic diversity, taxonomic incongruences, and automated classifications (e.g., SPLIT, MERGE, MATCH, and MIXTURE categories). It is worth noting that the manuscript itself refers to species retrieved from BOLD, which highlights an inconsistency in the choice of repository for sequence submission. The manuscript states that the analyzed populations exhibit morphological characteristics consistent with the Parodon suborbitalis complex; however, it does not describe which characteristics were observed or what procedures were used for this morphological identification. It is recommended that the authors include a brief description of the criteria and methods used for the morphological identification of specimens, especially since the discussion emphasizes morphological similarity as part of the taxonomic challenge. In addition, it is unclear whether the morphological identification was performed by one of the authors or by a specialist in the taxonomy of the group. Please clarify who was responsible for the identification. Finally, the manuscript does not describe the criteria used for selecting the thresholds and evolutionary models in the species delimitation analyses (ABGD and ASAP). It is important to provide this methodological information to ensure the reproducibility and transparency of the results. It is recommended to indicate the minimum number of metaphases analyzed per specimen for each procedure (karyotype, FISH, banding), as this is a standard practice in cytogenetic studies. This information is essential for validating the reliability of the chromosomal data presented.

Results

The section presents the main findings of the study; however, more detailed explanations are lacking to reinforce the interpretations and the integration between the cytogenetic and molecular data.

The analysis of the COI gene and the haplotype network is presented briefly, without any clear contribution from the nucleotide and haplotype diversity indices. Since these indices are neither discussed nor used to support the study's conclusions, it is recommended that they be removed from the manuscript.

Additionally, the figure showing the haplotype network contains overlapping colors, which compromises its readability and the interpretation of results. Given that the focus of the study is species (lineage) delimitation rather than population structure, the genetic distance table alone sufficiently demonstrates differentiation between taxa.

An additional suggestion is to convert the genetic distance matrix into a heatmap figure, which would make the visualization of relationships between taxa clearer and more informative.

Minor Revisions

In the sentence “Sequences were checked and corrected in Geneious v7.1.9

software (Kearse et al., 2012) and deposited in GenBank", it would be more appropriate to state that electropherograms were reviewed and corrected, as this is the standard practice prior to sequence assembly and submission. Please check this detail on page 7, line 10.
Page 7, lines 26–27: Nucleotide and haplotype diversity indices were calculated, but the results were not discussed or interpreted in a meaningful way in the results section. In this context, it is recommended to remove these analyses from the manuscript, as they do not clearly contribute to the study's objectives.
Reinforcing a point previously mentioned: since the introduction highlights morphological differences, the results section is expected to include a brief summary of the morphological observations made.
Suggestion: include a table (or a clearer textual explanation) linking the identified MOTUs to previously recognized morphological taxa. This would help reinforce the hypothesis of cryptic diversity. If no marked morphological differences were observed between Parodon suborbitalis and Parodon sp., this absence of phenotypic diagnosis should also be explicitly mentioned, as it provides important support for the presence of cryptic lineages.
Discussion
The discussion lacks a clearer integration of the morphological, cytogenetic, and DNA barcoding evidence with the species delimitation methods employed in the study. It is recommended that these approaches be triangulated to strengthen the argument and demonstrate how the data complement one another in delimiting the observed lineages.
Although chromosomal evolution is discussed in some depth, the authors could expand this section by emphasizing how the integration of cytogenetic, morphological, and molecular data can help clarify the taxonomy of complex groups. It would be relevant to include examples from the literature that illustrate this integrative approach and to discuss more clearly what the data obtained in this study reveal in this context.
In addition, it would be useful for the authors to highlight which of the cytogenetic markers used (e.g., rDNA localization, heterochromatin, FISH, among others) were the most informative in delineating the lineages, going beyond the simple description of the number and location of detected probes.
Although the manuscript mentions that the specimens exhibit morphological characteristics consistent with Parodon suborbitalis, the discussion does not address whether there was congruence or incongruence between the morphological and molecular data. This potential agreement or discrepancy should be explored, as it is essential for supporting the hypothesis of cryptic diversity.
Another aspect that could be expanded is the biogeographic implications of the observed lineages. A brief discussion of the geographic distribution of the sampled populations and potential geographic barriers or historical events that may have contributed to the observed divergence would enrich the discussion.
Finally, considering the hypothesis that one of the lineages may represent a species not yet formally described, it is recommended to discuss the conservation implications, particularly regarding the need for the formal recognition of evolutionarily significant units (ESUs) in the context of biodiversity management and regional conservation efforts.

Author's Rebuttal Letter (10/09/2025).
Dear Dr. Cláudio Oliveira
Associate Editor, Neotropical Ichthyology
I received my manuscript ID NI-2025-0119, entitled "Cytogenetic and molecular characterization of Parodon (Characiformes: Parodontidae) species from the Amazon

basin reveals a new Molecular Operational Taxonomic Unit." indicated for resubmission after a revised version. The comments and suggestions made by the reviewers are really helpful and constructive. We carefully address all issues mentioned in the reviewers' reports in a point-to-point response and provide suitable rebuttals of any criticisms that we disagreed with. All changes to the manuscript were indicated in a separate revised manuscript file with tracked changes mode in MS Word. I hope that my revision satisfies your criticisms and suggestions.
Sincerely,
Marcelo Ricardo Vicari
Reviewer: 1
Comments:
The manuscript "Cytogenetic and molecular characterization of Parodon (Characiformes: Parodontidae) species from the Amazon basin reveals a new Molecular Operational Taxonomic Unit" compared Parodon species using cytogenetic and genetic markers, aiming the molecular species delimitation. The data and discussion presented are highly relevant to understanding the Parodontodia family. The text is objective and clear, however needs adjustments, as desbribed below:
Resumo/Resumen – The Resumo presented is in the English language. Provide the translation to Portuguese or Spanish.
R: We have added the Portuguese version of the abstract in the Resumo section.
Page 10, line 8 to 15 - The information presented in this paragraph would fit better in the introduction.
Page 10, line 15 to 20 – This paragraph should be removed. It did not bring anything relevant to the discussion.
R: We have moved the entire paragraph to the introduction section and reorganized the information on Parodon buckleyi populations from the Machado River sub-basin to reinforce the possibility that specimens identified as P. cf. buckleyi are also a new MOTU.
Page 12, line 48 to 54 – The authors mentioned that the Parodon suborbitalis complex does not constitute a monophyletic group in evolutionary terms. In this context, I suggest that the authors include a brief discussion on what the cytogenetic data reveal about this complex.
R: We provide more details on cytogenetic data for species grouped within the P. suborbitalis complex in the discussion, although only a small number of species have been described cytogenetically.
Page 13, line 25 to 29 - The information that P. cf. buckleyi is an undescribed species was only addressed in the conclusion paragraph of the paper. I suggest that this information be explored in the discussion before being mentioned in the conclusion paragraph and present more arguments that support this statement.
R: We discussed the possibility of P. cf. buckleyi being an undescribed species in other parts of the discussion.
Figure 1 – The figure legend should make clear which points were sampled in the present work.
R: We added a marker to the collection sites of Parodon sp. and P. cf. buckleyi on the map and described them in the figure legend.
Figures 1 and 5 – The colors used to represent the species in figure 1 are different from the colors chosen in figure 5. I suggest that each species be identified by the same color in both figures to create a visual identity and facilitate the reader's understanding.
R: We have changed the colors of the collection sites and used the same pattern for species in the haplotype network.
Thank you for the comments!
Reviewer 2:
Comments:

I am reviewing the manuscript entitled “Cytogenetic and molecular characterization of Parodon (Characiformes: Parodontidae) species from the Amazon basin reveals a new Molecular Operational Taxonomic Unit.” The study adopts an integrative approach, combining cytogenetic and molecular data to investigate species delimitation within the genus Parodon in the Amazon basin. The methods employed are appropriate and well-executed, the results are clearly presented, and the identification of a MOTU not yet formally described adds originality and relevance to the work.

Despite the overall quality of the manuscript, some aspects require minor improvements, such as the explicit formulation of hypotheses or research questions, as well as a deeper integration of molecular, cytogenetic, and morphological data. Below, I provide my detailed recommendations by section.

Introduction

The authors provide an adequate contextualization of the diversity within the genus Parodon and the challenges associated with species delimitation. However, the introduction lacks clearly stated hypotheses or research questions. It is recommended that the authors explicitly present the hypothesis(es) or guiding research question(s) in a clear and objective manner to reinforce the investigative focus of the study.

R: We provide more details in the last paragraph of the introduction regarding the hypothesis and objective of the manuscript.

The introduction concludes with a generic statement (“In this context, we described cytogenetically two Parodon species...”), which could be revised to better reflect the scientific gap addressed and the intended contribution of the research.

R: This information was rewritten to better fit the manuscript objective.

Additionally, the concept of MOTU (Molecular Operational Taxonomic Unit), although mentioned in the title and abstract, is not introduced or contextualized in the introduction. It would be important to include a brief explanation of what MOTUs are and emphasize their relevance in the context of integrative taxonomy, especially for groups with conserved morphology such as Parodon.

R: We have described the MOTU concept in the introduction of the manuscript.

Another point that deserves attention is the absence of a dedicated paragraph highlighting the importance of cytogenetics in taxonomy. Although the review of karyotypic diversity in Parodontidae is informative and well-supported, the introduction could be strengthened by making clearer the role of chromosomal data within the study's framework. It is recommended to clarify how cytogenetic and molecular data complement each other in the delimitation of MOTUs, promoting a more integrative and cohesive approach.

R: We have reinforced the importance of cytogenetics of Parodontidae in the discussion section.

Specific Comments

The abstract is written in English, whereas it should be in Portuguese. Please adjust it to comply with the journal's formatting guidelines.

R: We have added the Portuguese version of the abstract in the Resumo section.

Page 5, line 32: Please remove the mention of “Tab. 1,” as it does not apply to the context presented.

R: We removed the Tab. 1 mention in this line.

Methodology

The COI sequences were properly deposited in GenBank (with accession numbers provided); however, it is also recommended that the sequences be submitted to the BOLD Systems platform. This repository is widely recognized as the primary reference for DNA barcoding studies, offering additional tools for data analysis and visualization. Depositing the sequences in BOLD not only facilitates access and reproducibility but also provides complementary evidence, such as the identification of cryptic diversity, taxonomic incongruences, and automated classifications (e.g., SPLIT, MERGE,

MATCH, and MIXTURE categories). It is worth noting that the manuscript itself refers to species retrieved from BOLD, which highlights an inconsistency in the choice of repository for sequence submission.
R: We recognize the importance of the BOLD System repository and are familiar with all the tools available on the site for DNA barcode analysis. To avoid duplicate sequences in multiple databases, we currently prefer not to submit sequences to the BOLD system at this moment. We also know that BOLD periodically extracts sequences from the GenBank database and incorporates them into its own database. Therefore, when sequences are published in the GenBank database, they will automatically be incorporated into the BOLD database.
The manuscript states that the analyzed populations exhibit morphological characteristics consistent with the Parodon suborbitalis complex; however, it does not describe which characteristics were observed or what procedures were used for this morphological identification. It is recommended that the authors include a brief description of the criteria and methods used for the morphological identification of specimens, especially since the discussion emphasizes morphological similarity as part of the taxonomic challenge.
In addition, it is unclear whether the morphological identification was performed by one of the authors or by a specialist in the taxonomy of the group. Please clarify who was responsible for the identification.
R: The specimens were identified by Drª. Carla S. Pavanelli, one of the authors of the manuscript and a specialist in the taxonomy of the group. We have added information on how it was conducted in the material and methods. We recognize that in-depth morphological study of the specimens is still necessary, and highlight this information in the discussion. However, morphological characterization of the specimens, which were analyzed cytogenetically and molecularly, was not the objective of this manuscript.
Finally, the manuscript does not describe the criteria used for selecting the thresholds and evolutionary models in the species delimitation analyses (ABGD and ASAP). It is important to provide this methodological information to ensure the reproducibility and transparency of the results.
R: We used the K80 TS/TV model for distance mode in the ABGD and ASAP, due to the limited evolutionary model available for analysis on the websites (only JC69 and K80), we assumed that Ts and Tv have different rates at a specific ratio (K80 model), rather than equal frequency (JC69 model). The other parameters were left at their default settings, and the results were interpreted as suggested by the software developers.
It is recommended to indicate the minimum number of metaphases analyzed per specimen for each procedure (karyotype, FISH, banding), as this is a standard practice in cytogenetic studies. This information is essential for validating the reliability of the chromosomal data presented.
R: We analyzed at least 30 metaphases for each species for classical cytogenetic techniques, and at least 20 metaphases for each probe in FISH experiments. We included this information in the manuscript.
Results
The section presents the main findings of the study; however, more detailed explanations are lacking to reinforce the interpretations and the integration between the cytogenetic and molecular data.
The analysis of the COI gene and the haplotype network is presented briefly, without any clear contribution from the nucleotide and haplotype diversity indices. Since these indices are neither discussed nor used to support the study's conclusions, it is recommended that they be removed from the manuscript.
R: We removed the nucleotide and haplotype diversity indices from the manuscript.
Additionally, the figure showing the haplotype network contains overlapping colors, which compromises its readability and the interpretation of results. Given that the focus

of the study is species (lineage) delimitation rather than population structure, the genetic distance table alone sufficiently demonstrates differentiation between taxa.
R: We changed the colors of the collection sites (figure 1) and in the haplotype network (figure 5), as suggested by reviewer 1, for better visualization of the data.
An additional suggestion is to convert the genetic distance matrix into a heatmap figure, which would make the visualization of relationships between taxa clearer and more informative.
R: We have included the heatmap of the genetic distance matrix in Figure 5 and have also included the table regarding the genetic distance matrix as supplementary material.
Minor Revisions
In the sentence "Sequences were checked and corrected in Geneious v7.1.9 software (Kearse et al., 2012) and deposited in GenBank", it would be more appropriate to state that electropherograms were reviewed and corrected, as this is the standard practice prior to sequence assembly and submission. Please check this detail on page 7, line 10.
R: We corrected the information in the manuscript.
Page 7, lines 26–27: Nucleotide and haplotype diversity indices were calculated, but the results were not discussed or interpreted in a meaningful way in the results section. In this context, it is recommended to remove these analyses from the manuscript, as they do not clearly contribute to the study's objectives.
R: We removed the nucleotide and haplotype diversity indices of the manuscript.
Reinforcing a point previously mentioned: since the introduction highlights morphological differences, the results section is expected to include a brief summary of the morphological observations made.
Suggestion: include a table (or a clearer textual explanation) linking the identified MOTUs to previously recognized morphological taxa. This would help reinforce the hypothesis of cryptic diversity. If no marked morphological differences were observed between Parodon suborbitalis and Parodon sp., this absence of phenotypic diagnosis should also be explicitly mentioned, as it provides important support for the presence of cryptic lineages.
R: The main goal of this study is to relate molecular differentiation among Parodon representatives. The observed differences in mitochondrial sequences were useful for detecting Molecular Operational Taxonomy Units among Amazonian Parodon lineages. A phenotypic diagnosis and a morphological species description should be the goal of a broad study of Amazonian Parodontidae.
Discussion
The discussion lacks a clearer integration of the morphological, cytogenetic, and DNA barcoding evidence with the species delimitation methods employed in the study. It is recommended that these approaches be triangulated to strengthen the argument and demonstrate how the data complement one another in delimiting the observed lineages.
R: We rewrote some sentences and reorganized the discussion topic to better integrate the results.
Although chromosomal evolution is discussed in some depth, the authors could expand this section by emphasizing how the integration of cytogenetic, morphological, and molecular data can help clarify the taxonomy of complex groups. It would be relevant to include examples from the literature that illustrate this integrative approach and to discuss more clearly what the data obtained in this study reveal in this context.
R: We included some examples from the literature that illustrate the integrative approach among morphology, cytogenetic, and DNA barcode for the integrative taxonomy.
In addition, it would be useful for the authors to highlight which of the cytogenetic markers used (e.g., rDNA localization, heterochromatin, FISH, among others) were the most informative in delineating the lineages, going beyond the simple description of the number and location of detected probes.
R: We rewrite some sentences in the discussion to highlight which chromosomal

markers were most informative to differentiate the species analyzed.
Although the manuscript mentions that the specimens exhibit morphological characteristics consistent with Parodon suborbitalis, the discussion does not address whether there was congruence or incongruence between the morphological and molecular data. This potential agreement or discrepancy should be explored, as it is essential for supporting the hypothesis of cryptic diversity.
R: The species analyzed were identified as part of the P. suborbitalis complex, due mainly by the color pattern and the geographic origin.
Another aspect that could be expanded is the biogeographic implications of the observed lineages. A brief discussion of the geographic distribution of the sampled populations and potential geographic barriers or historical events that may have contributed to the observed divergence would enrich the discussion.
R: We have included a brief discussion related to potential barriers that could have contributed to the divergence into Parodon genus.
Finally, considering the hypothesis that one of the lineages may represent a species not yet formally described, it is recommended to discuss the conservation implications, particularly regarding the need for the formal recognition of evolutionarily significant units (ESUs) in the context of biodiversity management and regional conservation efforts.
R: We included a brief discussion on the impact of the discovery of new species and the necessity of biodiversity management and efforts for conservation of the species.

Associate editor's decision after peer review (27/10/2025).
Dear Dr. Vicari:
Manuscript ID NI-2025-0119.R1 entitled "Cytogenetic and molecular characterization of Parodon (Characiformes: Parodontidae) species from the Amazon basin reveals a new Molecular Operational Taxonomic Unit" 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.
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=9065b352198e4a52996ad06090a2d2af
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 27-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,
Prof. Claudio Oliveira
Associate Editor, Neotropical Ichthyology
claudio.oliveira@unesp.br

Anonymous reviewer #1
Recommendation. Accept
Comments. (There are no comments.) [Corrections were made directly in the manuscript and were included in the attached file.]

Anonymous reviewer #2
Recommendation. Minor Revision
Comments. Dear authors,
The manuscript presents an integrative cytogenetic and molecular analysis of Parodon species from the Amazon basin, addressing long-standing taxonomic challenges within the "Parodon suborbitalis complex." The study combines classical cytogenetics, FISH mapping, and COI-based molecular delimitation to provide novel insights into chromosomal evolution, species boundaries, and biogeographic relationships in Parodontidae. Overall, it represents a strong and well-structured contribution to Neotropical fish systematics.
In general, the manuscript reads well after the applied corrections. However, the title could be improved to better highlight the taxonomic novelty and the integrative nature of the study. The current title is clear but somewhat descriptive; emphasizing the discovery of a cryptic lineage would increase its impact and visibility. Please consider one of the following alternatives:
Integrative cytogenetic and molecular evidence reveals a new cryptic lineage within the Parodon suborbitalis complex (Characiformes: Parodontidae)
A new cryptic Parodon lineage revealed by integrative cytogenetic and molecular analyses in the Amazon basin
The following actions were undertaken throughout the document:
English language editing – correction of grammar, punctuation, and syntax to improve fluency and readability while maintaining the original scientific meaning.
Ethics approval statement – standardized the wording and confirmed the need to verify the correct protocol number (Protocol No. 06/2019).
Discussion structure – the final paragraph was identified as a natural Conclusion and is recommended to be presented as a separate section for greater clarity and impact.
Implementation of edits – all corrections and editorial adjustments have been incorporated in the attached version marked with Track Changes.
Please review the tracked-changes document carefully to verify and approve all suggested modifications prior to submission.

Author's Rebuttal Letter (30/10/2025).
Dear Dr. Cláudio Oliveira
Associate Editor, Neotropical Ichthyology

I received my manuscript ID NI-2025-0119-R1, entitled "Cytogenetic and molecular characterization of Parodon (Characiformes: Parodontidae) species from the Amazon basin reveals a new Molecular Operational Taxonomic Unit." indicated for resubmission after a revised version. The reviewers' comments and suggestions are really helpful and constructive. We carefully address all issues mentioned in the reviewers' reports in a point-to-point response. All changes to the manuscript were indicated in a separate revised Word file with tracked changes enabled. I hope that my revision satisfies your criticisms and suggestions.
Sincerely,
Marcelo Ricardo Vicari
Reviewer 1:
Comments:
(There are no comments.)
Reviewer 2:
Comments:
Dear authors,
The manuscript presents an integrative cytogenetic and molecular analysis of Parodon species from the Amazon basin, addressing long-standing taxonomic challenges within the “Parodon suborbitalis complex.” The study combines classical cytogenetics, FISH mapping, and COI-based molecular delimitation to provide novel insights into chromosomal evolution, species boundaries, and biogeographic relationships in Parodontidae. Overall, it represents a strong and well-structured contribution to Neotropical fish systematics.
In general, the manuscript reads well after the applied corrections. However, the title could be improved to better highlight the taxonomic novelty and the integrative nature of the study. The current title is clear but somewhat descriptive; emphasizing the discovery of a cryptic lineage would increase its impact and visibility. Please consider one of the following alternatives:
Integrative cytogenetic and molecular evidence reveals a new cryptic lineage within the Parodon suborbitalis complex (Characiformes: Parodontidae)
A new cryptic Parodon lineage revealed by integrative cytogenetic and molecular analyses in the Amazon basin
R: We have changed the manuscript title to highlight the discovery of a cryptic lineage in Parodontidae.
The following actions were undertaken throughout the document:
English language editing – correction of grammar, punctuation, and syntax to improve fluency and readability while maintaining the original scientific meaning.
R: We reviewed the English in the manuscript and corrected the text according to suggestions and the Grammarly assistant. Thank you!
Ethics approval statement – standardized the wording and confirmed the need to verify the correct protocol number (Protocol No. 06/2019).
R: The protocol number for the use and studies of the animals analyzed in this manuscript is correct. Approval for tissue sampling of the Parodon species used in this study was obtained in 2019, and captures occurred between 2019 and 2021.
Discussion structure – the final paragraph was identified as a natural Conclusion and is recommended to be presented as a separate section for greater clarity and impact.
R: In fact, the final paragraph looks like a conclusion, however the Neotropical Ichthyology journal does not permit a separate “Conclusion” section in the manuscript. According to the Instructions to authors, “Do not provide a separate Conclusion section. However, we encourage highlighting conclusions as the last paragraph(s) of the Discussion.”
Implementation of edits – all corrections and editorial adjustments have been incorporated in the attached version marked with Track Changes.

R: We checked the edits suggested by the reviewer and incorporate them into the manuscript.
Please review the tracked-changes document carefully to verify and approve all suggested modifications prior to submission.
R: We checked and approved the reviewer's suggestion in the manuscript.
Thank you!

Associate editor's decision after peer review (13/01/2026).
Dear Dr. Vicari:
Manuscript ID NI-2025-0119.R2 entitled "Integrative cytogenetic and molecular evidence reveals a new cryptic lineage within the "Parodon suborbitalis complex" (Characiformes: Parodontidae)" 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.
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 13-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,
Prof. Claudio Oliveira
Associate Editor, Neotropical Ichthyology
claudio.oliveira@unesp.br

Anonymous reviewer #1
Recommendation. Accept
Comments. (There are no comments.)

Anonymous reviewer #2
Recommendation. Accept
Comments. The study is highly relevant within the framework of integrative taxonomy and will significantly contribute to a better understanding of evolutionary processes in the genus Parodon. The following suggestions are offered:
*Conceptual clarity:
State the main objective explicitly in the introduction and link integrative taxonomy directly to the focal group (Parodontidae and the "P. suborbitalis complex").
Define MOTU and other technical terms (e.g., K2P, FN, Myr) at first mention.
*Terminology and consistency:
Standardize terminology for sex chromosomes (e.g., consistently use "proto-sex chromosomes" or "putative sex chromosomes").
Ensure consistent capitalization and spelling of repetitive elements and transposons (Helitron, Tc1-Mariner, EnSpm, pPh2004).
*Caution in taxonomic and evolutionary claims:
Soften strong taxonomic statements (e.g., "represents an undescribed species") by using "supports the hypothesis" or "is consistent with the interpretation that…".
Emphasize that confirmation of new species, especially for P. cf. buckleyi, requires material from the type locality and additional evidence.
*Biogeography and monophyly
Make the link between Andean/Neotropical geological history and lineage divergence more explicit.
When stating that the "P. suborbitalis complex" is not monophyletic, clearly relate this to the phylogenetic criteria and the recovered clades.
*Style and language:
Shorten very long sentences and avoid multiple nested clauses; prefer clear, direct scientific prose.
Use consistent verb tenses: past for results ("showed", "revealed") and present/present perfect for general interpretations ("indicate", "suggest").
Avoid redundancy and ensure each paragraph has a clear central message (e.g., cytogenetic patterns, molecular divergence, biogeography).
*Methods and limitations
Highlight more clearly that the phylogeny is based on a single mitochondrial gene and that multilocus/phylogenomic data are needed to test the robustness of the inferred relationships.
Explicitly reinforce that combining cytogenetics and DNA barcoding is a justified and previously validated approach for revealing cryptic diversity in Parodontidae.
*Suggestion for figures:
In Figure 2, it would be advisable to use software to confirm the chromosomal morphology of the subtelocentric pairs and, consequently, to revise the karyotype formula. The chromosomes show good morphology and resolution, but the karyotype arrangement would be improved if homologous pairs were positioned closer together, which would better highlight the quality of the results.

Author's Rebuttal Letter (04/02/2026).
Dear Dr. Cláudio Oliveira
Associate Editor, Neotropical Ichthyology
I received my manuscript ID NI-2025-0119-R2, entitled "Cytogenetic and molecular characterization of Parodon (Characiformes: Parodontidae) species from the Amazon

basin reveals a new Molecular Operational Taxonomic Unit." indicated for resubmission after a revised version. The reviewers' comments and suggestions are really helpful and constructive. We carefully address all issues mentioned in the reviewers' reports in a point-to-point response. All changes to the manuscript were indicated in a separate revised Word file with tracked changes enabled. I hope that my revision satisfies your criticisms and suggestions.
Sincerely,
Marcelo Ricardo Vicari
Reviewer: 1
Recommendation: Accept
Comments:
(There are no comments.)
Reviewer: 2
Recommendation: Accept
Comments:
The study is highly relevant within the framework of integrative taxonomy and will significantly contribute to a better understanding of evolutionary processes in the genus Parodon. The following suggestions are offered:
*Conceptual clarity:
State the main objective explicitly in the introduction and link integrative taxonomy directly to the focal group (Parodontidae and the “P. suborbitalis complex”).
R: The main objective of the manuscript was rewritten in the introduction to link integrative taxonomy to the P. suborbitalis complex.
Define MOTU and other technical terms (e.g., K2P, FN, Myr) at first mention.
R: We checked all technical terms abbreviate and defined them at first mention.
*Terminology and consistency:
Standardize terminology for sex chromosomes (e.g., consistently use “proto-sex chromosomes” or “putative sex chromosomes”).
R: We checked the terminology for sex chromosomes and standardized the proto-sex chromosome terminology.
Ensure consistent capitalization and spelling of repetitive elements and transposons (Helitron, Tc1-Mariner, EnSpm, pPh2004).
R: We checked and standardized the spelling of transposable and repetitive elements.
*Caution in taxonomic and evolutionary claims:
Soften strong taxonomic statements (e.g., “represents an undescribed species”) by using “supports the hypothesis” or “is consistent with the interpretation that…”.
R: We altered some sentences to avoid this interpretation.
Emphasize that confirmation of new species, especially for P. cf. buckleyi, requires material from the type locality and additional evidence.
R: This information was reinforced in the conclusion of the manuscript.
*Biogeography and monophyly
Make the link between Andean/Neotropical geological history and lineage divergence more explicit.
When stating that the “P. suborbitalis complex” is not monophyletic, clearly relate this to the phylogenetic criteria and the recovered clades.
R: The discussion about the non-monophyletic P. suborbitalis complex was revised according to the phylogenetic tree presented and genetic distances. Besides that, a link between phylogeny and biogeography was implemented in this round of revision.
*Style and language:
Shorten very long sentences and avoid multiple nested clauses; prefer clear, direct scientific prose.
R: We are looking to implement a direct scientific language in this revision.
Use consistent verb tenses: past for results (“showed”, “revealed”) and present/present

perfect for general interpretations (“indicate”, “suggest”).
R: We checked and corrected verb tenses in the results and discussion sections.
Avoid redundancy and ensure each paragraph has a clear central message (e.g., cytogenetic patterns, molecular divergence, biogeography).
R: We summarize some of the cytogenetic discussion paragraphs, avoiding redundancy, result descriptions, and repetitions, and organizing each paragraph into a central message.
*Methods and limitations
Highlight more clearly that the phylogeny is based on a single mitochondrial gene and that multilocus/phylogenomic data are needed to test the robustness of the inferred relationships.
R: We reinforced in the conclusion that the present study was based on one mitochondrial molecular marker, and more markers were necessary to infer the relationship among Parodon.
Explicitly reinforce that combining cytogenetics and DNA barcoding is a justified and previously validated approach for revealing cryptic diversity in Parodontidae.
R: This information was reinforced in the 6th paragraph of the discussion.
*Suggestion for figures:
In Figure 2, it would be advisable to use software to confirm the chromosomal morphology of the subtelocentric pairs and, consequently, to revise the karyotype formula. The chromosomes show good morphology and resolution, but the karyotype arrangement would be improved if homologous pairs were positioned closer together, which would better highlight the quality of the results.
R: Figures 2 and 3 were reorganized for better visualization of chromosome pairs. Regarding the morphology of the chromosomes, they were characterized following the chromosome arm ratio proposed by Levan et al. (1964) and arranged in the karyotypes according to the latest studies of Parodon cytogenetics.
Thank you!

Associate editor’s decision after peer review (06/02/2026).
Dear Dr. Vicari:
It is a pleasure to accept your manuscript entitled "Integrative cytogenetic and molecular evidence reveals a new cryptic lineage within the “Parodon suborbitalis complex” (Characiformes: Parodontidae)" 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 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,

Prof. Claudio Oliveira
Associate Editor, Neotropical Ichthyology
claudio.oliveira@unesp.br

Neotropical Ichthyology

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HOW TO CITE THIS ARTICLE

- **Azambuja M, Oliveira EA, Sassi FMC, Nogaroto V, Moreira-Filho O, Pavanelli CS, Vicari MR.** Integrative cytogenetic and molecular evidence reveals a new cryptic lineage within the "*Parodon suborbitalis* complex" (Characiformes: Parodontidae). Neotrop Ichthyol. 2026; 24(2):e250119. https://doi.org/10.1590/1982-0224-2025-0119

SBI Official Journal of the Sociedade Brasileira de Ictiologia