Submission date. 15/10/2025

Associate editor's decision after peer review (30/01/2026).
Dear Mr. Haddad:
Manuscript ID NI-2025-0179 entitled "Phenotypic variability in populations of Phalloceros uai (Cyprinodontiformes: Poeciliidae) distributed in the Quadrilátero Ferrífero, Brazil, with comments on feeding and reproduction habits" 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=833c96047c4548ee8b5527dad07353af
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 07-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. Paulo Lucinda
Associate Editor, Neotropical Ichthyology
lucinda@uft.edu.br

Anonymous reviewer #1
Recommendation. Major Revision
Comments.
The objective of Manuscript NI-2025-017 seems to be to verify if the specimens of Phalloceros from the rio Doce basin are conspecific with specimens of Phalloceros uai from the São Francisco basin. As such, this study is hardly worth publishing, because the presence of P. uai in the rio Dove basin is already reported in the literature.

However, the study includes some information about DNA sequences, variation in the number of lateral spots, occurrence of superfetation, and some information about diet. This ancillary information may eventually justify publication of a modified version of the manuscript.

Unfortunately, the results are few and poorly presented, and the manuscript requires major revision. The most relevant information of general interest would be the resulst of an appropriate analysis of the sequences of the Folmer fragment to the COI mitochondria gene. However, these sequences are not presented, and neither submitted to a public database. Additionally, the "results" of the molecular study are restricted to only two sentences (lines 24 to 28, on p.7), one of which mistakenly presents a photograph of an electrophoresis gel used for quality control as it were a kind of result. Submission of DNA sequences to a public database is a mandatory requirement in scientific publications. Although GeneBank is usually accepted as an adequate repository by most journals, I strongly recommend submitting the sequencing data to BoldSystems, including the electrophoregram(trace) .ab1 files. BoldSystems is the most appropriate database for COI-based DNA barcodes, includes (superseding) GeneBank data, and allows for the inclusion of the .ab1 files. Inclusion of the ab1 files will ensure verifiability of the results, mitigating the obvious lack of experience of the authors in handling DNA sequences.

The methodology must clearly demonstrate if the sequencing was unidirectional or bidirectional, the details of any corrections of automated base-calls ((see comments below), and the final sequences (which obviously is not the 750 bp estimate presented in Results). The analysis should minimally include a neighbor-joining tree demonstrating all individual samples and their geographic origin.

A large number of issues is listed below according to their order of appearance in the text, associated with page and line nubers as they appear in the Review PDF generated by Neotropical Ichthyology. Most of these require considerable attention, and go way beyond simple text corrections.

ABSTRACT

p.1, l.39: "Phalloceros uai, endemic to the Rio das Velhas basin,": This is incorrect! The species is widely distributed in various drainages!

p.1, l.40-42: "However, its occurrence in adjacent basins and presence of different phenotypes have been reported". The argumentative tone ("However") is based solely on the false information provided in the previous sentence. Additionally, abstract should NOT be argumentative. They should be simply informative.

p.1, l.42-43: "Given discrepancies in geographic distribution, morphological and genetic recognition,". What, exactly, are these "discrepancies". Abstracts should provide objective information, and the mentioned "discrepancies" are not clearly explained even in main text of the manuscript. The literature provides ample information about the geographic distribution of the species and does not mention any discrepancies about its widespread distribution.

p.1, l.45: "Quadrilátero Ferrífero": This geographic name appears only here and in the title of the manuscript, but does not seem to have any relevance for the actual subject of the manuscript. Few people know the limits of this region, and mentioning it in the title and in abstract is more likely to cause confusion than enlightenment for potential readers. I suggest removing it from the title and the Abstract.

p.1, l.45: "integrative taxonomic approach": Integrative taxonomy is a vague term to designate the use of more than one type of data. The abstract must objectively specify what kinds of data were actually "integrated", and avoid such vague parlance.

p.1, l.51: "Osteological structures were analyzed via diaphanization": In the manuscript, I did not see any osteological descriptions based on this method. On the contrary, a photograph of the gonopodium presented in the supplemental material is very bad and demonstrates complete disregard for the expected quality that should be associated with the method. At least, delete from the Abstract.

p.1, l.54: The Abstract should primarily focus on the results of the study. However, the presentation of results on start in line 54, after more than half of the text was dedicated to methods. And even so, the results are presented in very terse manner. For example, in line 54 a short and peremptory sentence states "Six lateral spot phenotypes were identified". What is the significance of this information? The next sentence states that "No significant differences were found …". It is not even clear what is the frame of reference for the "difference".
p.2, l.5: "Females showed viviparity": This is not a result of the study. It is just a trivial information about poeciliids in general.
p.2, l.5-7: "the diet was … items." Poor language.
p.2, p.7: "In conclusion": The Abstract should not follow the structure of a discussion. It may objectively present the conclusion of the study, but it should not be structured as a discussion. Furthermore, including the "population" from Córrego do Jaque in Phalloceros uai hardly qualifies as a "conclusion", because that stream is the type locality of the species! How could it be different?
p.2, l.7: "showed no": Poor language. How can something show something that is absent?
p.2., l.10: "despite deviations from the square lateral spot autapomorphy": In addition to being poorly written, this phrase is unintelligible for persons that read the Abstract without reading the main text of the manuscript. Even then, it makes little sense. How can a population "deviate" from a "spot"? The reference to the spot as an "autapomorphy" even contradicts the results of the study, because those results demonstrate the spot described by Lucinda is NOT an autapomorphy of the species.
RESUMO:
See problems already mentioned above.
INTRODUCTION
p.3, l.33-40: There are four problems in the way the diagnosis of P. uai is presented: (1) contrary to what is stated, only one of the characters is an autapomorphy, (2) the lateral square spot is not large, and (3) the other two characters are not autapomorphic nor exclusive of the species and are shared with nine other species, and (4) the source of the diagnosis must be cited.
p.3, l.50-52: "described as endemic": Lucinda never said the species was "endemic". He just said that the species is geographically distributed in the rio das Velhas. Note that endemism is property of areas; it is not a property of species. In any case the source of this statement must be cited. The sentence also has two grammar errors: the coma is misused in "Originally, described", and the sentence requires a conjunction (or some other structural improvement) because it conflates "species" (in the first part) with geographic "distribution" (in the second part) without proper verbal structure. Additionally, the difference between "Velhas River" and "Velhas River channel" is not clear and makes the sentence further confusing.
p.3, l.53: "Furthermore, ": This is overly argumentative. Delete.
p.4, l.7: "Given these divergences": Which divergence? Paragraphs should not start with imprecise references to other paragraphs, and the previous paragraph does not mention any "divergence" in current knowledge. The citations in the previous paragraphs are complementary; they are NOT "divergent".
p.4.. l.7-9: ", morphological and genetic identification," This fragment seems completely out of place. What is the issue about morphology? Genetic identifications was not even mentioned in the previous text. Delete or explain the actual problem with identification.
p.4, l.11: "true status": Truth is not a subject of scientific investigation! Change "true" to an adjective that properly describes the "status" that is actually being investigated, or at least, provide a hypothesis to be tested.
p.4, 21-32. Please explain the geography and the logic behind the choice of these sites. I suppose that the sites are related to the São Francisco and the Doce river basins, but

these basins are not mentioned, and most readers will not be familiar the names of streams listed here. More importantly, as presented this is not a description of the "Study area". Instead, it is just a list of four localities.
p.4, l. 37: "Specimens were collected": OK, but this is NOT part of the "Study area" section.
p.5, l.13: "All statistical analyzes were performed using R": What were the actual statistical analyses. Which statistical packages/modules were used to perform these analyses? Please not that R is a programing language. It is not a statistical program. Also, note that "analyses" and "analyzes" have different meanings.
p.5, l.44: Change the non-sensical "Sixteen specimens (Tab. S1) were used for molecular analyses." to "DNA sequences of 16 specimens (Tab. S1) were analyzed".
p.5, l.: Delete the colon in "containing: 2 μL"
IMPORTANT: It is necessary to inform if the amplicons were sequenced in a single direction (which) or bot. Also, were the sequences visually inspected and edited to eliminate bad or ambiguous base-calls. It is also necessary to inform if the sequences were trimmed to a single maximum length. If so, what was the length of the final alignment?
p.6, l.36-38: "1x1, 1x2, 1x3, 2x2, 2x3 and 3x3": Please explain the meaning of the ""x" in these formulas. Also: I noticed that the second number is always grater than the left one: This have something to do with the left/right side of the body? It would also be nice to include frequency and/or modal values for each formula.
p.6, l.41-43: "height from 0.87 – 11.47 mm": This is meaningless. The relationship hight/SL would be much more informative.
p.7, l. 3: "Linear regression analysis demonstrated a positive correlation between male gonopodium length": I seriously doubt that the regression of gonopodial length on fish size is linear. The gonopodial growth curve probably has a strong inflexion during the onset of reproductive maturation. Perhaps the apparent linear relationship is related to the fact that small and immature individuals were not included in the sample. In Fig. 4, it is evident that most points of small individuals are below the line, and the largest specimens are above the line. The analysis is biased by the large number of individuals between 19 and 23 mm. I recommend texting a couple of non-linear regressions, such as the sigmoid or logistic curves. Also, the manuscript must explain the criterion for choosing individual sizes in the sample because the specimen size distribution is =obviously not homogeneious.
p.7, l.7-9: "ANOVA revealed no significant differences in the mean gonopodium length among different populations". This is a non-sensical analysis, because absolute gonopodial length is very likely confounded by specimen size variation. A more meaningful comparison would be based on relative size of the gonopodium (e.g. gonopodium/SL or gonopodium/TL).
p.7, l.24-28: This two-sentence paragraph about DNA seems out of place in the middle of morphological analyses. More importantly, the first sentence does not present any results. The size of fragments resulting from PCR is a methodological detail that does not belong here and is hardly worth mentioning in the manuscript. The size of the fragment is obviously a mixture of primers and amplified segments. The Ivanova primer are not based on Phalloceros DNA and even include plasmid sequences! The relevant (but missing!) information should be the final edited segment, after removal of the primer portions, and trimming of the unreliable extremities down to a common length (which is usually 655 bp).
p.7, l.30-35: This paragraph contains only tree sentences about stomach contents. The information provided here seems very superficial, without any interesting analysis. What is the relationship between diet and geography? What is the point of sampling various different sites located hundreds of km apart and in different river basins, if all that is reported here is that the they eat mosquitos and some sand? Such meager information is hardly worth publishing.

p.8, l.3: “Phalloceros uai was originally described as endemic to Córrego do Jaque,” No! Lucinda never suggested that the córrego do Jaque was na area of endemism! He merely described the species a sample collected there. The source of a single sample has nothing to do with endemism.
p.8, l.7-17: Most of this text repeats information already presented in the introduction. The Discussion should focus on results and avoid duplication of well-established knowledge already presented in the introduction.
p.8, l.19: “Despite these expanded records,” This is a nonsensical argumentation, and does not have any connection with the remainder of the sentence. Delete.
p.8, l.28: “other specimens”: “specimens” are not “phenotypes”. Rewrite.
p.8, l. 32-39: “The absence of diverse phenotypes at the type locality might indicate a singular variation within that population, potentially driven by larger evolutionary processes (genetic or ecological), as described for males of the Poecilia wingei Poeser, Kempkes & Isbrücker 2005 (Prazdnikov, 2021), or it could be attributed to the limited number of specimens collected thus far.” This is complete speculation. The arguments go “all over the place”, from lack of specimens to “larger evolutionary processes”. Which processes? What exactly was described for males of Poecilia? This paragraph does not provide any objective information. The work of Poeser on populations has been severely criticizes by other authors. Please spare readers of all this nonsense.
p.9, l.14: “are from before”: This is not good English. Rewrite.
p.10, l.8-26: The first two sentences in this paragraph constitute a “discussion about nothing”. Discussing lack of knowledge, impossibility of evaluation, lack of relationship, etc. without actual data about hormones and environmental conditions, is a waste of readers’ time. The third sentence (“Nevertheless, despite the phenotypic differences, other morphological characters were consistent with original diagnosis”) lacks any logical relationship with the other two, is addition to being vague and uninformative about the characters. This entire paragraph should be deleted.
p.11, l.3-5: Aranha & Caramaschi (1991) did not mention Phalloceros uai! Author must provide source of this claim. Otherwise, this false statement must be corrected.
p.11, l.10: “Phalloceros uai is a viviparous”: OK, but this statement and the following ones are unrelated to the previous two sentences in the paragraph. There is no logic in discussing the literature about feeding habits and placental structure. More importantly, the entire paragraph does not discuss any result from the current study. The reference to the species in line 10 is trivial, and the reference in line 3 is falsely attributed to (Aranha & Caramaschi, 1999). This long paragraph, spanning lines 3 to 29 is unrelated to the study and should be deleted in its entirety.
p.11, l.31-35: The source of these statements about evolution and occurrence of superfetation must be cited. Obviously, this information was not produced in the present study.
p.11, l.35-37: “Furthermore, …” This is overly argumentative! In fact, his sentence is just a simplified repetition of was just said in the previous sentence!
p.11, l.49: “Except for P. caudimaculatus, Pollux et al. (2014) suggested that poecilids …”: The grammatical structure of this sentence is confusing. The exceptionality should be logically related to the poeciliids, instead of being presented as an exception to Pollux himself. In fact, there is no exception here! Both males and females of Phalloceros caudimaculatus, as other species of Phalloceros, lack “striking colors and ornamentation” associated with sexual dimorphism and are precisely an example of what was suggested by Pollux!
p.11, l.49: “Despite the limited knowledge of courtship behavior in P. uai reproduction, the other attributes were verified for the species”: This phrase is entirely non-sensical. What is the relationship between “limited knowledge” and verification of attribute? More importantly, what are “the other attributes”?
p.11, l.56: “the occurrence of superfetation was confirmed across all analyzed

populations": OK, but is this all that can be said about the results of the study, after an entire page commenting the literature? The Discussion should be focused on actual results and the extent of comments about the literatures should be proportional to the relevance of results actually presented.
p.12, l.3: "Based on the data available and obtained data": What are the "data available"? Minimally a formal citation of these data must be presented. Also, the order of the adjectives and the noun is different in the reference to the two sets of data, which is an infringement of grammatical rules.
p.14, l.33: "Ver Bras Zool.": This is not the correct abbreviation.
TABLES:
TABLE 1. Number of Phalloceros Eigenmann, 1907 specimens collected at each locality,
categorized by phenotype based on the number of lateral spots.
TABLE 2. Morphometric data for male, female and juvenile specimens of Phalloceros uai
Lucinda, 2008 from the upper Doce and upper São Francisco drainages, MG. N = Number of
specimens. ⊡ = Mean. md = Median. dp = Standard deviation.
TABLE 3. Total number and frequency of stomach contents separated by food item and sex. N =
Number of stomachs found with food items.
TABLE 4. Number of Phalloceros uai Lucinda, 2008 embryos by locality.
FIGURES
FIGURE 1. Sampling sites of Phalloceros populations in the upper Rio Doce and Upper São
Francisco drainages, MG.
**** All toponyms in the map are barely visible. A larger font must be used.
**** The meaning of the black line must be explained.
**** The limits of the São Francisco and the rio Doce must be shown.
**** A guidance map showing the general relationships of the two basins with the Atlantic Ocean would be more useful than a map of Brazil and its states. Keep in mind thar NI is about the Neotropical Region and has an audience that is much broader than Brasil.
FIGURE 2. Left lateral view of adult specimens of Phalloceros uai Lucinda, 2008. (A) Males.
(B) Females. Scale in millimeters.
**** Catalog numbers and locality source of these specimens must be presented.
FIGURE 3. Schematic drawing of Phalloceros uai Lucinda, 2008 phenotypes showing the
variation in the number of lateral spots on each side of the same specimen.
**** These drawings must be associated with the codes used in the text. Additionally, the presence of a greater number of spots on the right is obvious, but it is not clear if this is a natural phenomenon, or just an artifact resulting from an arbitrary decision to omit phenotypes with greater number of spots on the left side of the body. Please, keep in mind that sinistrorsal versus dexterous asymmetry is a major factor in the evolution of Phalloceros, as already described in various publications and even mentioned in this manuscript. Importantly, there is no point in drawing a schema, if the various instances are not labelled and cannot be properly referred in the Methods and Results.
FIGURE 4. Correlation between total length (mm) of males versus and their gonopodium length
(mm).
**** This not a "correlation"! This is a regression graph.
**** Fonts are too small.

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FIGURE 5. Schematic drawing of the gonopodium of Phalloceros uai in lateral view illustrating
rays 5, 4 and 3, with the anterior region on the left. Ray 5 canoe-shaped, ray 4p exhibits a
serrated structure, and ray 3 possesses spines and extensions.
**** "Ray 5 canoe-shaped": I don´t see the shape of a canoe in this drawing. Is this feature actually visible? It should be labeled. Also, ray R5 has two branches, but it is not clear which branch has this shape.
**** "Ray 5 canoe-shaped,": a verb is missing here.
**** What is the meaning of "extensões"? This is not the language used in the manuscript, nor it and acceptable anatomical description.
**** "possesses spines and extensions": The spines are not visible, nor labeled, in the drawing.
FIGURE 6. Set of embryos taken from a Phalloceros uai Lucinda, 2008 female surrounded by a
thin membrane. Scale 1 cm.
**** Size and locality of the specimen should be listed. Collection catalog number, if available, should also be listed.
FIGURE 7. Phalloceros uai Lucinda, 2008 embryos phenotype frequency in the final stages
(40) separated by locality and mother's phenotype.
**** The meaning of the phenotype codes must be explained. Also, the relationship between the codes and side (left versus side) must be explained: In cases of asymmetry were the left and right side counts lumped into a single category? Please keep in mind that laterality is a major evolutionary factor in Phalloceos evolution as demonstrated in various publications.
**** The lettering in the graph is barely visible. A larger font must be used.
p.26: This image requires a legend. Also, the letters are also barely visible. The fonts should have a minimal final size of 10 points in an A4 page format.
p.27: This image requires a legend. Also the specimen is so poorly preserved that it is hardly worth showing in a publication. Normally, the internal skeleton of gonopodial is easily visible in transmitted light. If all specimens are this bad, they should be cleared and stained. Also, the quality of the photograph is far from the possibility of the M205A microscope mentioned in the methods. The photograph should show the details of the terminus of the gonopodium, using Z-stacking multifocal techniques available in software from the microscope manufacturer of freely available on the Internet.
p.28: This image requires an explaining legend. The image was incorrectly mentioned in Results, but is not a result! Instead, it is just a gel produced by electrophoresis during a methodological quality control. It is not used for the production of the actual sequences.
p.29: This table requires an explanatory title. Here I can see the meaning of the "phenotype" codes, which is lacking in the main text. More importantly, it becomes evident that some codes lump together different phenotypes, and are therefore no actual "pehnotypes"! For example, in Santa Rita Durão, it is obvious that there are more spots on the left side than on the right side of the body. Minimally, the authors must perform a Chi-square statistical test to demonstrate that the predominance of spots on the left side is not statistically significant, before lumping the phenotype into a single category.
p.31: This table requires an explanatory title. Also, the height should be presents as a relative proportion of SL, instead of absolute values in millimeters.
p.32: This table presents values of Nannodrop readings used to evaluate DNA quality. These values are not results, nor are they used to produce actual the results. This table is useless, even as a supplementary material. Delete. The actual sequences should shpuld be presented instead.
p.33: This table requires an explanatory title. More importantly, these results must

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be partitioned according their geographical origin. The collections sites are located hundreds of km apart, and these results are probably confounding the effects of the entomofaunas from the different environments instead of describing the feeding habits of Phalloceros uai.
p.34: This table requires an explanatory title.
p.35: This table is poorly formatted, and requires an explanatory title.
p.37: Presenting genetic distances with 10 decimal cases is nonsense! Only three decimal cases should be used (which is equivalent to 0,1 % difference in genetic distance). In addition to conferring meaning to the values, the elimination of not significant digits would greatly improve the size and the readability of this table.

Anonymous reviewer #2
Recommendation. Major Revision
Comments.
Dear Editor,
The manuscript titled "Phenotypic variability in populations of Phalloceros uai (Cyprinodontiformes: Poeciliidae) distributed in the Quadrilátero Ferrífero, Brazil, with comments on feeding and reproductive habits" reports morphological polymorphisms in specimens from the Quadrilátero Ferrífero and expands the known distribution of P. uai in Minas Gerais. This is an original study that provides new information relevant to the species' diagnosis, as well as novel insights into its ecology, given that data on feeding and reproduction are presented.
My main concern is that the study does not investigate the shape of the lateral spots in the examined material. The text associates the species' "original morphotype" with the presence of a single black lateral spot, but Phalloceros uai is specifically and autapomorphically diagnosed by a wide, square-shaped lateral spot in large specimens (sensu Lucinda, 2008). If there are color polymorphisms among specimens from the Quadrilátero Ferrífero, it is important to clarify whether the diagnostic character of the species varies. Note that the lateral spots illustrated in one of the figures (unnumbered, beginning with "Schematic drawing...") are oval in shape, not square. Please include a section in the Results and Discussion addressing the square-shaped morphology of the lateral spot, as originally proposed in the species diagnosis.
In addition to the unclear description of spot shape, another point that raises doubts about the correct identification of this species as P. uai is the left-curved position of the urogenital papilla in the female specimen with three spots shown in the figure beginning with "Left lateral view of adult specimens...". As described by Lucinda (2008), a crucial diagnostic feature of P. uai is a right-curved urogenital papilla that is also positioned on the right side of the body (thus not visible in left lateral view). It should be clarified whether this is an anomalous specimen or whether the photograph was taken from the right side and then mirrored.
From the molecular perspective, the study concludes that all samples from the Quadrilátero Ferrífero belong to the same species, but it is not clear why this species is identified as Phalloceros uai. I suggest explicitly indicating which sequence was used as a reference (e.g., a topotype or a sequence from a specimen with a single square-shaped spot).
Other issues requiring attention before the manuscript can proceed to another round of review:
• In the file I received, the figures are not numbered.
• Please read the several comments I inserted directly in the PDF.
I congratulate the authors on their work and wish them success in the revision process.
Sincerely,
Igor Souto-Santos

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Author's Rebuttal Letter (27/01/2026).

Dear Dr. Lucinda,

Thank you for your time and for the constructive evaluation of our manuscript. We sincerely appreciate the insightful comments provided by both reviewers. We believe that the revisions made in response to these suggestions have significantly improved the quality and clarity of the paper.

In the following document, we outline our detailed, point-by-point responses to each editorial and reviewer remark. We hope that the changes we have made fully resolve the issues raised and that the revised manuscript meets the requirements for publication.

Best regards,

Eric Bruschi Haddad

PhD Candidate

University of Louisiana at Lafayette

Reviewer 1

Recommendation: Major Revision

Comments:

The objective of Manuscript NI-2025-017 seems to be to verify if the specimens of Phalloceros from the rio Doce basin are conspecific with specimens of Phalloceros uai from the São Francisco basin. As such, this study is hardly worth publishing, because the presence of P. uai in the rio Dove basin is already reported in the literature. However, the study includes some information about DNA sequences, variation in the number of lateral spots, occurrence of superfetation, and some information about diet. This ancillary information may eventually justify publication of a modified version of the manuscript.

Unfortunately, the results are few and poorly presented, and the manuscript requires major revision. The most relevant information of general interest would be the resulst of an appropriate analysis of the sequences of the Folmer fragment to the COI mitochondria gene. However, these sequences are not presented, and neither submitted to a public database. Additionally, the "results" of the molecular study are restricted to only two sentences (lines 24 to 28, on p.7), one of which mistakenly presents a photograph of an electrophoresis gel used for quality control as it were a kind of result. Submission of DNA sequences to a public database is a mandatory requirement in scientific publications. Although GeneBank is usually accepted as an adequate repository by most journals, I strongly recommend submitting the sequencing data to BoldSystems, including the electrophoregram(trace) .ab1 files. BoldSystems is the most appropriate database for COI-based DNA barcodes, includes (superseding) GeneBank data, and allows for the inclusion of the .ab1 files. Inclusion of the ab1 files will ensure verifiability of the results, mitigating the obvious lack of experience of the authors in handling DNA sequences.

The methodology must clearly demonstrate if the sequencing was unidirectional or bidirectional, the details of any corrections of automated base-calls ((see comments below), and the final sequences (which obviously is not the 750 bp estimate presented in Results). The analysis should minimally include a neighbor-joining tree demonstrating all individual samples and their geographic origin.

A large number of issues is listed below according to their order of appearance in the text, associated with page and line nubers as they appear in the Review PDF generated by Neotropical Ichthyology. Most of these require considerable attention, and go way beyond simple text corrections.

We appreciate the comments. We believe our paper is relevant for publication, since it is the first to report the phenotypic variability in specimens of Phalloceros uai, confirm and expand geographic distribution of the species within the state of Minas Gerais, presents relevant diagnostic information and provides ecological information for the species.

At the time of submission, we had not yet uploaded the sequences to GenBank. We have since uploaded the sequences and updated the accession numbers accordingly in the manuscript.
BOLD Systems mirrors sequence data deposited in GenBank, with many BOLD records linked to existing GenBank accession numbers and periodically synchronized between the two databases. Consequently, depositing sequences directly in GenBank already provides public accessibility and long-term preservation.
The molecular methodology has been updated as requested to make them more detailed.
As recommended by the reviewer, we included a phylogenetic analysis. We opted to use an exhaustive Maximum Likelihood (ML) search rather than a stepwise Neighbor-Joining (NJ) approach because NJ relies on distance matrices, which can reduce the amount of information used and may result in less accurate tree reconstruction. In contrast, ML evaluates the full sequence data under an explicit evolutionary model, providing a more robust and reliable phylogenetic inference.
For text corrections we have taken into consideration each comment and replied below.
ABSTRACT
p.1, l.39: “Phalloceros uai, endemic to the Rio das Velhas basin,”: This is incorrect! The species is widely distributed in various drainages!
We appreciate the reviewer's correction. We have revised the Abstract and the Introduction to accurately reflect the species' distribution. Our study formally extends its documented distribution to the Paraopeba River basin.
p.1, l.40-42: ”However, its occurrence in adjacent basins and presence of different phenotypes have been reported”. The argumentative tone (“However”) is based solely on the false information provided in the previous sentence. Additionally, abstract should NOT be argumentative. They should be simply informative.
The text has been edited as requested.
p.1, l.42-43: “Given discrepancies in geographic distribution, morphological and genetic recognition,”. What, exactly, are these “discrepancies”. Abstracts should provide objective information, and the mentioned “discrepancies” are not clearly explained even in main text of the manuscript. The literature provides ample information about the geographic distribution of the species and does not mention any discrepancies about its widespread distribution.
We heavily edited the Abstract to account for the reviewer comments.
p.1, l.45: “Quadrilátero Ferrífero”: This geographic name appears only here and in the title of the manuscript, but does not seem to have any relevance for the actual subject of the manuscript. Few people know the limits of this region, and mentioning it in the title and in abstract is more likely to cause confusion than enlightenment for potential readers. I suggest removing it from the title and the Abstract.
We removed the region from the title and added a paragraph describing the region known as Quadrilatero Ferrifero for the readers.
p.1, l.45: “integrative taxonomic approach”: Integrative taxonomy is a vague term to designate the use of more than one type of data. The abstract must objectively specify what kinds of data were actually “integrated”, and avoid such vague parlance.
The text has been edited as requested.
p.1, l.51: “Osteological structures were analyzed via diaphanization”: In the manuscript, I did not see any osteological descriptions based on this method. On the contrary, a photograph of the gonopodium presented in the supplemental material is very bad and demonstrates complete disregard for the expected quality that should be associated with the method. At least, delete from the Abstract.
The text has been edited as requested. We added more photographs of the gonopodium from different specimens to the supplemental material. Although the reviewer believes that our photo is not good enough for the clear and stain method, we believe that the process was sufficient to evaluate the structures necessary for the diagnosis of the

specimens.
p.1, l.54: The Abstract should primarily focus on the results of the study. However, the presentation of results on start in line 54, after more than half of the text was dedicated to methods. And even so, the results are presented in very terse manner. For example, in line 54 a short and peremptory sentence states "Six lateral spot phenotypes were identified". What is the significance of this information? The next sentence states that "No significant differences were found …". It is not even clear what is the frame of reference for the "difference".
The text has been edited for further clarification. The six identified lateral-spot phenotypes (1x1, 1x2, 2x1, 2x2, 2x3, and 3x3) represent the main phenotypic divergence from the original species description by Lucinda (2008). These phenotypes constitute a central finding of our study, as they directly address the taxonomic uncertainty surrounding Phalloceros uai.
p.2, l.5: "Females showed viviparity": This is not a result of the study. It is just a trivial information about poeciliids in general.
The text has been edited to remove this information.
p.2, l.5-7: "the diet was … items." Poor language.
The text has been edited to remove poor language.
p.2, p.7: "In conclusion": The Abstract should not follow the structure of a discussion. It may objectively present the conclusion of the study, but it should not be structured as a discussion. Furthermore, including the "population" from Córrego do Jaque in Phalloceros uai hardly qualifies as a "conclusion", because that stream is the type locality of the species! How could it be different?
The text has been edited as requested. The Córrego do Jaque population, the type locality, served as the reference population for Phalloceros uai, and the first author examined the holotype and paratypes in MRNJ.
p.2, l.7: "showed no": Poor language. How can something show something that is absent?
The text has been edited to remove poor language.
p.2., l.10: "despite deviations from the square lateral spot autapomorphy": In addition to being poorly written, this phrase is unintelligible for persons that read the Abstract without reading the main text of the manuscript. Even then, it makes little sense. How can a population "deviate" from a "spot"? The reference to the spot as an "autapomorphy" even contradicts the results of the study, because those results demonstrate the spot described by Lucinda is NOT an autapomorphy of the species.
The text has been edited as requested for clarification. We mean that specimens from other populations were identified as Phalloceros uai, even though they show phenotypes different from those described in Lucinda's (2008) original diagnosis.
RESUMO:
See problems already mentioned above.
We heavily edited the Resumo to account for the reviewer comments.
INTRODUCTION
p.3, l.33-40: There are four problems in the way the diagnosis of P. uai is presented: (1) contrary to what is stated, only one of the characters is an autapomorphy, (2) the lateral square spot is not large, and (3) the other two characters are not autapomorphic nor exclusive of the species and are shared with nine other species, and (4) the source of the diagnosis must be cited.
This paragraph has been edited as requested. It now reads: "Phalloceros uai Lucinda, 2008, is autapomorphically diagnosed by a wide, square lateral spot in larger specimens and can be further distinguished from its congeners by … (Lucinda, 2008)"
p.3, l.50-52: "described as endemic": Lucinda never said the species was "endemic". He just said that the species is geographically distributed in the rio das Velhas. Note that endemism is property of areas; it is not a property of species. In any case the source of

this statement must be cited.
This is a valid point. We removed all phrases about endemism.
The sentence also has two grammar errors: the coma is misused in "Originally, described", and the sentence requires a conjunction (or some other structural improvement) because it conflates "species" (in the first part) with geographic "distribution" (in the second part) without proper verbal structure. Additionally, the difference between "Velhas River" and "Velhas River channel" is not clear and makes the sentence further confusing.
The text has been edited as requested for clarification. It now reads: "Phalloceros uai was originally described from Córrego do Jaque and an unnamed creek from Jabuticatubas (das Velhas River basin). Subsequent studies expanded its known distribution within this basin to include the Cipó River and the main channel of the das Velhas River (Alves et al., 2008; Leal et al., 2011). Specimens identified as P. uai were reported from the Piracicaba River (Vieira et al., 2015), and Santo Antônio River drainages (Santos & Britto, 2021), within the Doce River basin. These reports have prompted further investigation of the species' distribution in adjacent basins."
p.3, l.53: "Furthermore, ": This is overly argumentative. Delete.
The text has been edited as requested. See above.
p.4, l.7: "Given these divergences": Which divergence? Paragraphs should not start with imprecise references to other paragraphs, and the previous paragraph does not mention any "divergence" in current knowledge. The citations in the previous paragraphs are complementary; they are NOT "divergent".
We thank the reviewer for this comment. We agree with the suggestion and have substantially revised and edited the paragraph accordingly.
p.4.. l.7-9: ", morphological and genetic identification," This fragment seems completely out of place. What is the issue about morphology? Genetic identifications was not even mentioned in the previous text. Delete or explain the actual problem with identification.
The text has been edited to remove genetic identification as requested. See above.
The morphological differences we observed were purely phenotypical. All analyzed populations shared the same gonopodial and urogenital anatomy as originally described by Lucinda (2008).
p.4, l.11: "true status": Truth is not a subject of scientific investigation! Change "true" to an adjective that properly describes the "status" that is actually being investigated, or at least, provide a hypothesis to be tested.
Where previously read "true status" now reads reassess the taxonomic status of P. uai populations.
MATERIALS AND METHODS
p.4, 21-32. Please explain the geography and the logic behind the choice of these sites.
We sampled four different sites to cover all basins (Doce River basin and Sao Francisco River basin) and major rivers around the type locality, including the type locality itself. We also incorporated localities previously reported by other researchers as containing populations considered to be P. uai..
I suppose that the sites are related to the São Francisco and the Doce river basins, but these basins are not mentioned, and most readers will not be familiar the names of streams listed here.
The text has been revised so that each drainage is now followed by its corresponding basin in parentheses.
More importantly, as presented this is not a description of the "Study area". Instead, it is just a list of four localities.
The authors believe that a detailed physical description of each sampling site is unnecessary, as it would not contribute relevant information to the main manuscript's objectives. However, we added a figure of the type locality to supplementary material.
p.4, l. 37: "Specimens were collected": OK, but this is NOT part of the "Study area" section.

The subtitle of the section was modified to “Sampling”.
p.5, l.13: “All statistical analyzes were performed using R”: What were the actual statistical analyses. Which statistical packages/modules were used to perform these analyses? Please not that R is a programing language. It is not a statistical program. Also, note that “analyses” and “analyzes” have different meanings.
We conducted two statistical analyses: linear regression and ANOVA. Linear regression was used to examine the relationship between gonopodium length and total length in males. In contrast, ANOVA was used to determine whether mean gonopodium length differed significantly among populations. Both analyses can be carried out using base R functions, without the need to install any additional packages. For example, linear regression can be performed with lm(formula, data), and ANOVA can be run using anova(model). The ggplot2 package was used only to improve the visualization of the graphs; it was not required for performing the statistical analyses themselves.
The text has been edited as requested. It now reads: “Linear regression models were utilized to assess the relationship between gonopodium length and total length in males, while ANOVA were utilized to test if there were statistically significant differences between the mean gonopodium length of the populations. All statistical analyses were performed in default R (R Core Team, 2021).”
p.5, l.44: Change the non-sensical “Sixteen specimens (Tab. S1) were used for molecular analyses.” to “DNA sequences of 16 specimens (Tab. S1) were analyzed”.
The text has been edited as requested.
p.5, l.: Delete the colon in ”containing: 2 µL”
The text has been edited as requested.
IMPORTANT: It is necessary to inform if the amplicons were sequenced in a single direction (which) or bot.
Amplicons were sequenced in both directions. The text was edited to contain this information.
Also, were the sequences visually inspected and edited to eliminate bad or ambiguous base-calls.
All sequences were manually edited to remove primers, to remove indels and deletions and eliminate bad or ambiguous nucleotides from poor sequencing.
It is also necessary to inform if the sequences were trimmed to a single maximum length. If so, what was the length of the final alignment?
The text has been altered to include the following sentence: “The DNA sequence consisted of a 650 bp fragment.”
RESULTS
p.6, l.36-38: “1x1, 1x2, 1x3, 2x2, 2x3 and 3x3”: Please explain the meaning of the “”x” in these formulas. Also: I noticed that the second number is always grater than the left one: This have something to do with the left/right side of the body? It would also be nice to include frequency and/or modal values for each formula.
X means left versus right side of the specimen. We added the phrase: “Phenotypes were defined by the number of lateral spots on each side of the body (left × right), e.g., 1 × 1 indicates one spot on both sides.” for better understanding. Frequency of each phenotype can be found in Table 1 of the paper.
p.6, l.41-43: “height from 0.87 – 11.47 mm”: This is meaningless. The relationship hight/SL would be much more informative.
We added height/SL as requested.
p.7, l. 3: “Linear regression analysis demonstrated a positive correlation between male gonopodium length”: I seriously doubt that the regression of gonopodial length on fish size is linear. The gonopodial growth curve probably has a strong inflexion during the onset of reproductive maturation. Perhaps the apparent linear relationship is related to the fact that small and immature individuals were not included in the sample.
Our data met the assumption of normality, as indicated by the residual plots shown in

Supplementary Figure 2 (formerly Fig. S1). Indeed, immature specimens were excluded from the analysis because the main goal of the study was to evaluate differences between populations and the gonopodium was a key structure for assessing these differences. Including immature individuals, would introduce ontogenetic variation that could confound population-level comparisons and obscure patterns relevant to the objectives of the study.

In Fig. 4, it is evident that most points of small individuals are below the line, and the largest specimens are above the line. The analysis is biased by the large number of individuals between 19 and 23 mm.

The high number of individuals in the 19 – 23 mm size range is random. The mean total length was 22.83 mm and the median was 21.69 mm, indicating a size distribution centered around this interval. We therefore consider this size range to be representative of the typical body size of males in the sampled populations.

I recommend texting a couple of non-linear regressions, such as the sigmoid or logistic curves. Also, the manuscript must explain the criterion for choosing individual sizes in the sample because the specimen size distribution is =obviously not homogeneious.

We evaluated alternative curve fits (logistic and sigmoid) and other statistical models (Poisson and GMM). However, none provided a better fit to the data than the normal distribution. Residuals plots indicated that the assumptions of linear regression were met. The Normal Q-Q plot shows residuals closely following the reference line, consistent with normally distributed errors; skewed or peaked data would deviate from this pattern. The scale location plot displays an approximately horizontal trend. And the residuals leverage plot revealed no influential outliers or funnel shaped patterns, indicating homoscedasticity.

p.7, l.7-9: “ANOVA revealed no significant differences in the mean gonopodium length among different populations”. This is a non-sensical analysis, because absolute gonopodial length is very likely confounded by specimen size variation. A more meaningful comparison would be based on relative size of the gonopodium (e.g. gonopodium/SL or gonopodium/TL).

ANOVA (analysis of variance) which compares differences among means within a sample. Given that our objective was to assess interpopulation differences as a criterion for evaluating species delimitation. ANOVA was used to test for significant differences in mean gonopodium length among populations.

As requested by the reviewer, we run two more ANOVA utilizing the relative size of the gonopodium and neither was statistically significant.

“Gonopodium/TL”

Analysis of Variance Table

Response: Gon/TL

Df Sum Sq Mean Sq F value Pr(>F)

Locality 3 0.000536 0.00017859 0.2892 0.8332

Residuals 438 0.270496 0.00061757

“Gonopodium/SL”:

Analysis of Variance Table

Response: Gon/SL

Df Sum Sq Mean Sq F value Pr(>F)

Locality 3 0.00050 0.00016508 0.1593 0.9237

Residuals 438 0.45398 0.00103648

If we don’t use ANOVA, we will have four separate average and median for each population:

Gon/TL Gon/SL

Avg Med Avg Med

Site 1: 0.35 0.35 0.44 0.44

Site 2: 0.35 0.35 0.44 0.44

Site 3: 0.34 0.35 0.43 0.43
Site 4: 0.34 0.34 0.44 0.43
p.7, l.24-28: This two-sentence paragraph about DNA seems out of place in the middle of morphological analyses. More importantly, the first sentence does not present any results. The size of fragments resulting from PCR is a methodological detail that does not belong here and is hardly worth mentioning in the manuscript. The size of the fragment is obviously a mixture of primers and amplified segments.
We moved the phrase "PCR amplification resulted in fragments of approximately 750 bp" to methods as requested.
The size of the PCR amplification informs the reader that our sequences were well amplified and sequenced to support our molecular analysis.
The Ivanova primer are not based on Phalloceros DNA and even include plasmid sequences!
The primer described by Ivanova et al. (2007) was selected because of its high universality, allowing successful amplification across multiple fish species, and because it has been recently used with success by Oliveira et al. (2024).
The relevant (but missing!) information should be the final edited segment, after removal of the primer portions, and trimming of the unreliable extremities down to a common length (which is usually 655 bp).
The text has been altered to include the following sentence: "The DNA sequence consisted of a 650 bp fragment." as commented above.
p.7, l.30-35: This paragraph contains only tree sentences about stomach contents. The information provided here seems very superficial, without any interesting analysis. What is the relationship between diet and geography? What is the point of sampling various different sites located hundreds of km apart and in different river basins, if all that is reported here is that the they eat mosquitos and some sand? Such meager information is hardly worth publishing.
No dietary differences were observed between populations, indicating that these fish maintain a consistent diet within populations.
The text has been edited as requested and now reads: "Diet did not differ between females and males, nor among populations (Tab. 3, Tab. S4)." with the inclusion of a new supplementary table to support our data.
We emphasize that data on feeding habits and natural history are fundamental to ichthyology. While such ecological studies may be perceived as less technically complex than modern phylogenomics, they provide the essential biological context necessary to understand species evolutionary roles and support applied conservation sciences. We acknowledge that natural history data in isolation may have limited scope; however, within this manuscript, they serve a critical role. By providing the first account of these traits for P. uai, these data complement our taxonomic and molecular findings, offering a more holistic characterization of the species across its expanded distribution.
DISCUSSION
p.8, l.3: "Phalloceros uai was originally described as endemic to Córrego do Jaque," No! Lucinda never suggested that the córrego do Jaque was na area of endemism! He merely described the species a sample collected there. The source of a single sample has nothing to do with endemism.
We removed the phrase about endemism.
p.8, l.7-17: Most of this text repeats information already presented in the introduction. The Discussion should focus on results and avoid duplication of well-established knowledge already presented in the introduction.
The authors agree and have edited the discussion to avoid repetition.
p.8, l.19: "Despite these expanded records," This is a nonsensical argumentation, and does not have any connection with the remainder of the sentence. Delete.
We removed "Despite these expanded records,".

p.8, l.28: “other specimens”: “specimens” are not “phenotypes”. Rewrite.
The text has been edited as requested.
p.8, l. 32-39: “The absence of diverse phenotypes at the type locality might indicate a singular variation within that population, potentially driven by larger evolutionary processes (genetic or ecological), as described for males of the Poecilia wingei Poeser, Kempkes & Isbrücker 2005 (Prazdnikov, 2021), or it could be attributed to the limited number of specimens collected thus far.” This is complete speculation.
We agree with the reviewer that the proposed explanations for the lack of phenotypic variation at the type locality were speculative. Our intention was not to provide a definitive causal explanation, but rather to highlight an intriguing observation that warrants further investigation. Following the suggestion, we have removed the speculative discussion regarding evolutionary processes and thyroid hormones. The text now focuses on the reported findings as a basis for future studies on the drivers of phenotypic diversity in P. uai.
The arguments go “all over the place”, from lack of specimens to “larger evolutionary processes”. Which processes? What exactly was described for males of Poecilia? This paragraph does not provide any objective information. The work of Poeser on populations has been severely criticizes by other authors. Please spare readers of all this nonsense.
We thank the reviewer for the comment. Poeser was cited exclusively as the original author of the species description, in accordance with standard taxonomic practice, and not as a source of population-level analyses or interpretations. We did not rely on, discuss, or evaluate Poeser’s work on populations elsewhere in the manuscript. Therefore, the citation serves only a nomenclatural purpose. To avoid any potential misunderstanding, we have clarified this point in the revised version of the manuscript.
p.9, l.14: “are from before”: This is not good English. Rewrite.
The text has been edited to improve clarity and conciseness.
p.10, l.8-26: The first two sentences in this paragraph constitute a “discussion about nothing”. Discussing lack of knowledge, impossibility of evaluation, lack of relationship, etc. without actual data about hormones and environmental conditions, is a waste of readers’ time. The third sentence (“Nevertheless, despite the phenotypic differences, other morphological characters were consistent with original diagnosis”) lacks any logical relationship with the other two, is addition to being vague and uninformative about the characters. This entire paragraph should be deleted.
Paragraph removed.
p.11, l.3-5: Aranha & Caramaschi (1991) did not mention Phalloceros uai! Author must provide source of this claim. Otherwise, this false statement must be corrected.
We thank the reviewer for pointing out the potential ambiguity in this sentence. Our intention was not to imply that Aranha & Caramaschi (1991) specifically analyzed the diet of Phalloceros uai, but rather that the carnivorous habit reported for P. uai is consistent with dietary observations made for other species of Phalloceros. We have revised the sentence to make this distinction explicit and avoid any misinterpretation.
p.11, l.10: “Phalloceros uai is a viviparous”: OK, but this statement and the following ones are unrelated to the previous two sentences in the paragraph. There is no logic in discussing the literature about feeding habits and placental structure. More importantly, the entire paragraph does not discuss any result from the current study. The reference to the species in line 10 is trivial, and the reference in line 3 is falsely attributed to (Aranha & Caramaschi, 1999). This long paragraph, spanning lines 3 to 29 is unrelated to the study and should be deleted in its entirety.
We thank the reviewer for this comment. We edited the paragraph.
p.11, l.31-35: The source of these statements about evolution and occurrence of superfetation must be cited. Obviously, this information was not produced in the present study.

p.11, l.35-37: “Furthermore, …” This is overly argumentative! In fact, his sentence is just a simplified repetition of was just said in the previous sentence!
p.11, l.49: “Except for P. caudimaculatus, Pollux et al. (2014) suggested that poecilids …”: The grammatical structure of this sentence is confusing. The exceptionality should be logically related to the poeciliids, instead of being presented as an exception to Pollux himself. In fact, there is no exception here! Both males and females of Phalloceros caudimaculatus, as other species of Phalloceros, lack “striking colors and ornamentation” associated with sexual dimorphism and are precisely an example of what was suggested by Pollux!
p.11, l.49: “Despite the limited knowledge of courtship behavior in P. uai reproduction, the other attributes were verified for the species”: This phrase is entirely non-sensical. What is the relationship between “limited knowledge” and verification of attribute? More importantly, what are “the other attributes”?
p.11, l.56: “the occurrence of superfetation was confirmed across all analyzed populations”: OK, but is this all that can be said about the results of the study, after an entire page commenting the literature? The Discussion should be focused on actual results and the extent of comments about the literatures should be proportional to the relevance of results actually presented.
We thank the reviewer for the six insightful comments above. We fully agree with the suggestions and have revised the paragraph, accordingly, integrating it into the Introduction to improve clarity, relevance, and logical flow.
p.12, l.3: “Based on the data available and obtained data”: What are the “data available”? Minimally a formal citation of these data must be presented. Also, the order of the adjectives and the noun is different in the reference to the two sets of data, which is an infringement of grammatical rules.
We thank the reviewer for highlighting this issue and have revised the paragraph to address the comments.
p.14, l.33: “Ver Bras Zool.”: This is not the correct abbreviation.
The text has been edited and it now reads: “Rev Bras Zool.”.
TABLES:
TABLE 1. Number of Phalloceros Eigenmann, 1907 specimens collected at each locality,
categorized by phenotype based on the number of lateral spots.
TABLE 2. Morphometric data for male, female and juvenile specimens of Phalloceros uai
Lucinda, 2008 from the upper Doce and upper São Francisco drainages, MG. N = Number of
specimens. □ = Mean. md = Median. dp = Standard deviation.
TABLE 3. Total number and frequency of stomach contents separated by food item and sex. N =
Number of stomachs found with food items.
TABLE 4. Number of Phalloceros uai Lucinda, 2008 embryos by locality.
FIGURES
FIGURE 1. Sampling sites of Phalloceros populations in the upper Rio Doce and Upper São
Francisco drainages, MG.
**** All toponyms in the map are barely visible. A larger font must be used.
**** The meaning of the black line must be explained.
**** The limits of the São Francisco and the rio Doce must be shown.
**** A guidance map showing the general relationships of the two basins with the Atlantic Ocean would be more useful than a map of Brazil and its states. Keep in mind thar NI is about the Neotropical Region and has an audience that is much broader than Brasil.

The figure has been altered as requested.
FIGURE 2. Left lateral view of adult specimens of Phalloceros uai Lucinda, 2008. (A) Males.
(B) Females. Scale in millimeters.
**** Catalog numbers and locality source of these specimens must be presented.
The figure legend has been altered as requested.
FIGURE 3. Schematic drawing of Phalloceros uai Lucinda, 2008 phenotypes showing the
variation in the number of lateral spots on each side of the same specimen.
**** These drawings must be associated with the codes used in the text. Additionally, the presence of a greater number of spots on the right is obvious, but it is not clear if this is a natural phenomenon, or just an artifact resulting from an arbitrary decision to omit phenotypes with greater number of spots on the left side of the body. Please, keep in mind that sinistrorsal versus dexterous asymmetry is a major factor in the evolution of Phalloceros, as already described in various publications and even mentioned in this manuscript. Importantly, there is no point in drawing a schema, if the various instances are not labelled and cannot be properly referred in the Methods and Results.
The figure and figure legend has been altered as requested.
FIGURE 4. Correlation between total length (mm) of males versus and their gonopodium length
(mm).
**** This not a "correlation"! This is a regression graph.
**** Fonts are too small.
The figure and figure legend has been altered as requested.
FIGURE 5. Schematic drawing of the gonopodium of Phalloceros uai in lateral view illustrating
rays 5, 4 and 3, with the anterior region on the left. Ray 5 canoe-shaped, ray 4p exhibits a
serrated structure, and ray 3 possesses spines and extensions.
**** "Ray 5 canoe-shaped": I don´t see the shape of a canoe in this drawing. Is this feature actually visible? It should be labeled. Also, ray R5 has two branches, but it is not clear which branch has this shape.
**** "Ray 5 canoe-shaped,": a verb is missing here.
**** What is the meaning of "extensões"? This is not the language used in the manuscript, nor it and acceptable anatomical description.
**** "possesses spines and extensions": The spines are not visible, nor labeled, in the drawing.
The figure and figure legend has been altered as requested.
FIGURE 6. Set of embryos taken from a Phalloceros uai Lucinda, 2008 female surrounded by a
thin membrane. Scale 1 cm.
**** Size and locality of the specimen should be listed. Collection catalog number, if available, should also be listed.
The figure legend has been altered as requested.
FIGURE 7. Phalloceros uai Lucinda, 2008 embryos phenotype frequency in the final stages
(40) separated by locality and mother's phenotype.
**** The meaning of the phenotype codes must be explained.
We added the phrase "Phenotypes were determined by counting lateral spots on the left and right sides independently (e.g., 1x1 indicates one spot on both sides). This approach allows for the characterization of both symmetrical and asymmetrical patterns." to the Materials and Methods.
Also, the relationship between the codes and side (left versus side) must be explained: In

cases of asymmetry were the left and right side counts lumped into a single category? Please keep in mind that laterality is a major evolutionary factor in Phalloceos evolution as demonstrated in various publications.
Left and right sides were scored separately; phenotypes were determined by independently counting lateral spots on each side (e.g., 1×1 indicates one spot on each side). This approach captures both symmetrical and asymmetrical patterning.
**** The lettering in the graph is barely visible. A larger font must be used.
The figure has been altered as requested.
p.26: This image requires a legend. Also, the letters are also barely visible. The fonts should have a minimal final size of 10 points in an A4 page format.
The figure has been altered as requested.
p.27: This image requires a legend. Also the specimen is so poorly preserved that it is hardly worth showing in a publication. Normally, the internal skeleton of gonopodial is easily visible in transmitted light. If all specimens are this bad, they should be cleared and stained. Also, the quality of the photograph is far from the possibility of the M205A microscope mentioned in the methods. The photograph should show the details of the terminus of the gonopodium, using Z-stacking multifocal techniques available in software from the microscope manufacturer of freely available on the Internet.
We added legend as requested: "Figure S4. Gonopodium of males of Phalloceros uai Lucinda 2008. (A) DZUFMG 160 – 1x1 Phenotype. (B) DZUFMG 159 – 2x3 Phenotype. (C) DZUFMG 160 – 2x1 Phenotype. (D) DZUFMG 161 – 2x1 Phenotype. All specimens possessed a left hook on the gonopodium, an asymmetrical gonopodial appendage (right wider than the left), a canoe-shaped ray 5, a serrated structure on ray 4p, and radius 3 with spines and extensions, consistent with original description."
We added three more gonopodiums to support our conclusions from different phenotypes and localities.
All images were captured using an M205A microscope. Brightness was adjusted to enhance visualization. With these modifications, we believe all structures necessary for the diagnosis of the specimens are clearly visible.
p.28: This image requires an explaining legend. The image was incorrectly mentioned in Results, but is not a result! Instead, it is just a gel produced by electrophoresis during a methodological quality control. It is not used for the production of the actual sequences.
The figure legend has been added, and the figure has been moved to the Materials and Methods.
p.29: This table requires an explanatory title. Here I can see the meaning of the "phenotype" codes, which is lacking in the main text. More importantly, it becomes evident that some codes lump together different phenotypes, and are therefore no actual "pehnotypes"! For example, in Santa Rita Durão, it is obvious that there are more spots on the left side than on the right side of the body. Minimally, the authors must perform a Chi-square statistical test to demonstrate that the predominance of spots on the left side is not statistically significant, before lumping the phenotype into a single category.
The table title is presented on page 12. As requested, we performed a chi-square test. The populations from Santa Rita Durão and Brumadinho were selected because the test requires an expected frequency of at least five observations per category. All analyses were conducted in R.
The chi-square test results were: $\chi^2 = 0.0162$, df = 1, p = 0.8987. Therefore, we do not reject the null hypothesis, indicating no association between sampling site and phenotype (1x2 vs. 2x1 lateral spots).
For clarity, we removed the row labeled "Phenotype" from the table.
p.31: This table requires an explanatory title. Also, the height should be presents as a relative proportion of SL, instead of absolute values in millimeters.
The table title is presented on page 13. The table has been altered as requested.
p.32: This table presents values of Nannodrop readings used to evaluate DNA quality.

These values are not results, nor are they used to produce actual the results. This table is useless, even as a supplementary material. Delete. The actual sequences should shpuld be presented instead.
The table is presented to clarify to the reader the quality of our sequences. We added two rows to the table (Museum voucher and GenBank access number).
The table is presented as Supplementary Material (Table S1).
p.33: This table requires an explanatory title. More importantly, these results must be partitioned according their geographical origin. The collections sites are located hundreds of km apart, and these results are probably confounding the effects of the entomofaunas from the different environments instead of describing the feeding habits of Phalloceros uai.
The table title is presented on page 13. An additional table has been included in the supplementary material to address the reviewer's request. As previously noted, diet did not differ between females and males, nor among populations just variations between local food availability.
p.34: This table requires an explanatory title.
The table title is presented on page 13.
p.35: This table is poorly formatted, and requires an explanatory title.
The table title has been added, and the table was formatted for better clarification.
p.37: Presenting genetic distances with 10 decimal cases is nonsense! Only three decimal cases should be used (which is equivalent to 0,1 % difference in genetic distance). In addition to conferring meaning to the values, the elimination of not significant digits would greatly improve the size and the readability of this table.
The table was formatted for better clarification.
Reviewer 2's comments
Recommendation: Major Revision
The manuscript titled "Phenotypic variability in populations of Phalloceros uai (Cyprinodontiformes: Poeciliidae) distributed in the Quadrilátero Ferrífero, Brazil, with comments on feeding and reproductive habits" reports morphological polymorphisms in specimens from the Quadrilátero Ferrífero and expands the known distribution of P. uai in Minas Gerais. This is an original study that provides new information relevant to the species' diagnosis, as well as novel insights into its ecology, given that data on feeding and reproduction are presented.
Dear Dr. Souto-Santos, we appreciate your comments.
My main concern is that the study does not investigate the shape of the lateral spots in the examined material. The text associates the species' "original morphotype" with the presence of a single black lateral spot, but Phalloceros uai is specifically and autapomorphically diagnosed by a wide, square-shaped lateral spot in large specimens (sensu Lucinda, 2008). If there are color polymorphisms among specimens from the Quadrilátero Ferrífero, it is important to clarify whether the diagnostic character of the species varies. Note that the lateral spots illustrated in one of the figures (unnumbered, beginning with "Schematic drawing...") are oval in shape, not square. Please include a section in the Results and Discussion addressing the square-shaped morphology of the lateral spot, as originally proposed in the species diagnosis.
We agree that Phalloceros uai was originally autapomorphically diagnosed by a wide, square-shaped lateral spot in large specimens (sensu Lucinda, 2008) and as presented in our paper (Figure 2 – Left lateral view…) the spot is square-shaped. We thank the reviewer for noting the discrepancy in the shape of the lateral spots in the unnumbered figure (Figure 3 - Schematic drawing…). We will revise the illustration to accurately represent the observed morphology. In addition, we have clarified a section to the Results and Discussion addressing the variation between the originally described square-shaped spots.
In addition to the unclear description of spot shape, another point that raises doubts

about the correct identification of this species as P. uai is the left-curved position of the urogenital papilla in the female specimen with three spots shown in the figure beginning with “Left lateral view of adult specimens...”. As described by Lucinda (2008), a crucial diagnostic feature of P. uai is a right-curved urogenital papilla that is also positioned on the right side of the body (thus not visible in left lateral view). It should be clarified whether this is an anomalous specimen or whether the photograph was taken from the right side and then mirrored.
All specimens were photographed in anatomical position (left lateral view). 3 spots phenotypes were very rare to collect in every population, so in correlation, good specimens for figures were even harder. You are correct that the female 3 spots in the figure is an anomalous specimen. Figure legend was updated to compensate for this error.
Although this specimen does not exhibit the diagnostic features of Phalloceros uai, all males display an asymmetrical gonopodial appendage, a canoe-shaped ray 5, a serrated structure on ray 4p, and a ray 3 with spines and extension consistent with the original description. Moreover, all sequences from the populations we analyzed differed 2% or less different from the type locality sequences and showed greater divergence from other species.
From the molecular perspective, the study concludes that all samples from the Quadrilátero Ferrífero belong to the same species, but it is not clear why this species is identified as Phalloceros uai. I suggest explicitly indicating which sequence was used as a reference (e.g., a topotype or a sequence from a specimen with a single square-shaped spot).
This is a valid point that merits careful consideration. We have added additional text to the Results and Discussion to address this. A total of 29 specimens were collected from the type locality (Table 1), and three of these were sequenced and treated as the reference for Phalloceros uai.
• In the file I received, the figures are not numbered.
This was a mistake in our end. We have updated all figures, tables, supplement material and legends.
• Please read the several comments I inserted directly in the PDF.
The text has been edited as requested.
“Individuals smaller than the smallest male with a developed gonopodium were classified as juveniles”
Comment: então foram considerados como adultos exemplares relativamente grandes sem genitalia desenvolvida? Exemplares que não atingiram a maturidade sexual nao sao adutos”
This is a valid point. We agreed with the reviewer and opted to remove the phrase from the paper.

Associate editor’s decision after peer review (11/03/2026).
Dear Mr. Haddad:
Manuscript ID NI-2025-0179.R1 entitled "Phenotypic variability in populations of Phalloceros uai (Cyprinodontiformes: Poeciliidae) with comments on feeding and reproduction habits" 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=a18fa3db5a85450198b478255f8f553f
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 11-May-2026. If it is not possible for you to submit your revision by this date, we may have to consider your paper as a new submission.
Once again, thank you for submitting your manuscript to the Neotropical Ichthyology and I look forward to receiving your revision.
Sincerely,
Dr. Paulo Lucinda
Associate Editor, Neotropical Ichthyology
lucinda@uft.edu.br
Entire Scoresheet:
Reviewer: 1
Recommendation: Major Revision
Comments:
This is a revised version of the manuscript. The new version is significantly improved. However, several new mistakes were introduced in the new text. Also, some problems listed in the previous review were only partially fixed, and still persist in the new edited text.
An important issue is related to the definition of each “phenotype”. This concept appears as the first word in the title, and seems to represent a major theoretical aspect of the study. The phenotypes are defined as pairs of counts of lateral spots on each side to the body. However, the number of phenotypes listed in Results (six phenotypes) is different from the number of phenotypes (nine) illustrated in Figure 3. In the Response to the reviews of the first version of the manuscript, it was explained that the difference is not statically significant, but the explanation is not clearly presented in the new manuscript. More importantly, detailed consideration of laterality in number of spots is necessary because both males and females of Phalloceros show considerable asymmetry of genitalia and sexual behavior. Considering that the concept of phenotypic variability is based mostly on the counting of spots and that it constitutes a central issue in the study, the manuscript should handle the discrepancies in the definition of the “phenotypes” in a better way.
I noted that additional material has been added to the Introduction and the Discussion. This extensive material is interesting, but creates an imbalance between

the limited data presented in Results and the remainder of the text. Accordingly, I suggest that the authors try to reduce reference to general ideas that have little direct connection with the data actually produced in the study, in order to reduce this imbalance.
Specific problems in the new version are listed below according to page and line numbers in the PDF for Review:
ABSTRACT
p.1, l.39: Change “squared” to ”square”.
p.1, l.44: “central-southern of Minas Gerais”: Grammar error.
p.1, l.47: “measured and gonopodium”: Comma is required here.
p.1, l.47: “features was examined”: Grammar error.
p.1, l.49: “analysis utilized COI gene”: This language style follows Brazilian Portuguese colloquial style. Text requires revision of grammar and style by a proficient English writer.
p.1, l.49: “while”: Incorrect use of conjunction ‘while’. Additionally, “molecular analysis” and visceral evaluation are unrelated methods and should be presented in separate (and more informative) sentences.
p.1, l.49-51: “stomach contents and gonads were evaluated”: The meaning of “evaluated” is not clear here.
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p.2, l.7: “despite deviations from the square lateral spot autapomorphy”: This statement remains problematic as in the previous version of the manuscript. The authors responded that “The text has been edited as requested for clarification”, but this obscure statement remains exactly as it was in the previous version! What exactly are these “deviations”? On p.1, l.51, it is stated that the spots in the examined specimens are large and square, which is the diagnostic shape listed by Lucinda (2008).
p.2, l.7-9: What is “The observed variation in lateral spot patterns”? I suppose that that the authors meant to refer to the variation in the number of lateral spots, but a simple count is not a “pattern”. Actually, variation in numbers is the opposite of a pattern! A number is not a pattern. By definition a pattern is something that is constant. Something that is variable in number does not constitute a pattern.
RESUMO
See comments about the Abstract.
Keywords
p.2, l.48: Keywords are meant to find articles that are highly relevant for a search, but some of the keywords (e.g., “carnivore”, “phenotype”) are next to useless as bibliographic search terms.
INTRODUCTION
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p.3, l.17-19: “urogenital papilla structure”: Change to “structure of gonopodium and female urogenital papilla”, to avoid the use of a noun (which already has an adjective) as an adjective.
p.3, l.19: Delete “simple”.
p.3, l.24 and everywhere else in the text: Check proper use of comma in reference citations.
MATERIAL AND METHODS
p.5, l.34: Change “two millimeters mesh” to “two-millimeter mesh”. (Further improvement is possible).
p.5, l.49-50: “counting lateral spots on the left and right sides independently (e.g., 1x1 indicates one spot on both sides).” This is quite confusing. Figure 3 shows 2/3 and 3/2

as different "phenotypes", but the Results treat the as a single phenotype! If these two "phenotypes" are treated as a the same thing, what is the meaning of "counting lateral spots on the left and right sides independently"? This statement does not make sense. More importantly, Phalloceros are known for exhibiting asymmetry in reproductive structures in both males and females. Treating left counts and right counts as the same thing violates the of the basic definition of a phenotype in a genus where species are differentiated based on the laterality of the gonopodial appendices and of the genital papilla.
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p.7, l.7-8: "PCR amplification resulted in fragments of approximately 750 bp (Fig. S2)." This information is misplaced. Amplification results should be listed before the description of the sequencing step.
p.7, l.14: Change "The DNA" to "the final trimmed DNA".
RESULTS
p.7, l.47-49: "different phenotypes. (1x1, 1x2, 1x3, 2x2, 2x3 and 3x3 lateral spots) (Fig. 2, Fig. 3)" This is directly contradicted by Figure 3. Figure 3 shows nine different phenotypes. If spot are relevant for sexual display, and the species exhibits strong asymmetry on both the gonopodial appendages, and in the female urogenital papilla, a "phenotype" with more spots on the right side cannot be equated with a "phenotype" with more spots on the left side. This is an important consideration, because the theoretical framework of the manuscript depends on the correct definition os a "phenotype".
p.8, l.3-5: "Total length (TL) ranged from 8.21 – 48.60 mm, standard length (SL) from 6.67 – 42.17 mm, and height/SL from 0.13 – 0.35": These data are in contradiction with those in Table 2. According to Table 2, the smallest SL is 7.53 mm (not 6.67 mm!), and the Heigth/SL proportion varies between 0.12 (in juveniles) and 0,52 (in females). These contradictions do not inspire confidence in the data. In the previous version of the manuscript the text reported a minimum body depth of only 0.87 mm. This is less than one millimeter! Also, the great range of relative body height implies that females may vary more than 3,7 times in body depth, and that they may be more than 4 times taller than juveniles, which seems a surprisingly large difference. I strongly recommend a careful revision of the raw data.
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p.9, l.27: "described to do Jaque stream": This is both a language and a logical error. Species are not described "to" a stream"! They are just described based on specimens. The geographic origin of the type specimens is irrelevant for the morphological description. The geographic origin of the holotype may be relevant for establishing a type locality, but that is quite different from saying that a species is described for

a river! This awkward construction is actually a bad translation of a common error committed by Brazilian Portuguese speakers, who incorrectly use the Portuguese preposition "para" (e.g., "a espécie foi descrita para o rio Amazonas", o que é uma aberração semântica). Additionally, translating "Córrego do Jaque" makes no sense. Note that Lucinda (2008) published in English but did not translate the name of the stream. I suggest changing the text to "described based on specimens from Córrego do Jaque". The translation of Rio das Velhas, in line 31 and 32, is also problematical. and is not even consistently done. The name of this river is translated in two different forms (!): "the das Velhas river", e "the Velhas River", both are odd translations that are not normally used by English-speaking persons. These toponyms should not be translated.
p.9, l.43-45: "All analyzed populations of Phalloceros share the same diagnostic characteristics of the gonopodium and urogenital papilla described by Lucinda (2008) for Phalloceros uai." This sentence should be deleted because it is both non-sensical and uninformative. It just says that all populations of P. uai share the diagnostic characters of P. uai, which is a truism! To hide the obvious redundancy the authors listed the populations as "All analyzed populations of Phalloceros" which is misleading: they are not population of the entire genus! Delete the sentence.
p.9, l.45: Delete "However,". There is no controversy here.
p.9, l.47: Change "register" to "record".
p.9, l.48-52: "The only exception to our findings is the work of Thomaz et al. (2019), who reported specimens without lateral spots in the das Velhas River": To state that their work is "the only exception to our findings" is an overstatement. Simply state that those authors reported the presence of specimens without spot in the region. That is just an observation; it is not an "exception" to anything.
p.10, l.12: "This pattern": What exactly is the "pattern" here? The term "pattern" is misused throughout the manuscript, without regard to its actual meaning. Here the lack of variation in the number of spots occurs only once, in a single locality. A single (!) occurrence does not constitute a "pattern". All uses of the word "pattern" must be replaced by a simple description of the actual observation. If there is an actual "pattern", the authors must explain what is this "pattern" and why they consider it as something of sufficient generality to regard it as a "pattern" of some sort.
p.10, l.15: "For instance, experimental data": Experimental data are NOT examples of the natural phenomena mentioned in the previous sentence.
p.10: l.20-22: "which constrains a more comprehensive understanding of the population phenotypic breadth": ?????? This is gibberish.
p.10, p.30: "Therefore, ": Non sequitur.
p.10, l.51: "vertical bars … were not recorded in either live or fixed specimens." This is false. Vertical bars are clearly visible on the male on the top of Fig. 2!
p.11, l.15: Delete "on" in "conferred on such".
Figure 3: The nine "phenotypes" depicted in this illustration must be labeled according to the names of their names in the study. For example which combination is 3x2 and which is 2x3? More importantly, the nine phenotypes in this illustration are treated as six phenotypes in the main text! This poor definition of what constitute a "phenotype" undermines the theoretical basis of the study.
Figure 4: The number of data pairs from each locality must be stated, and the geographic provenance of the data must be explicitly stated. Specifically, is the regression based on specimens from the four different sampling sites?
Figure 5: There are two problems here: (1): What is the meaning of "canoe-shaped"? Ray 5 is NOT shaped as a canoe! The canoe-shaped structure must be clearly labeled in the drawing. (2) The rays are mislabeled. For example, in the larger drawing the ray branch bearing a spine is labeled and R4a, but is labelled as R3 in the smaller figure. This problem must be corrected.
Figure 6: I suppose that "35 mm" refers to SL, but it is not stated in the legend.

Figure 7: (1) What is an "embryos phenotype frequency". I suppose that the authors meant to refer to a "embryonic frequency" or "fecundity", but that is not what is written. The graph does NOT show a "phenotype frequency". The frequency bars are grouped by phenotype categories, but they do not represent the frequency of phenotypes. The phenotype frequency is represented in Table 1, not here!
Figure S1. (1) This is a photograph of the Córrego do Jaque, but it is not necessarily a photograph of the type locality. The type locality was a spot in the in this Córrego, but it may not be the spot that was photographed. The photograph shows the sample locality for this study. (2) "from do Jaque stream," change to "in the Córrego do Jaque". DO not tranlate local toponyms.
Tabel S2: What are this "dissected" specimens? What were they dissected for? They were dissected for the food study? Or were they females dissected for the gonadal study? The purpose of the dissection must be stated in the tittle of the table.
Reviewer: 2
Recommendation: Minor Revision
Comments:
Dear Editor,
***I have reviewed the revised version of the manuscript entitled "Phenotypic variability in populations of Phalloceros uai (Cyprinodontiformes: Poeciliidae) distributed in the Quadrilátero Ferrífero, Brazil, with comments on feeding and reproductive habits." The authors have addressed most of the reviewers' comments. Overall, the manuscript has improved and is now closer to meeting the standards of Neotropical Ichthyology.
***In this second round, the main point to highlight is a discrepancy between the six phenotypes reported in the results (phenotypes 1×1, 1×2, 1×3, 2×2, 2×3, and 3×3) and those nine represented in figure 3, which also includes the additional patterns 2×1, 3×1, and 3×2. The authors should carefully verify how many phenotypes (6 or 9) were identified in the study. According to Tab 1, it seems that nine is the correct number, but authors should double check it.
***An important technical issue concerns the nomenclature of gonopodial elements. In the Methods section, the authors state that they followed Rosen & Gordon (1953), as modified by Lucinda & Reis (2005); however, they use terminology that is not found in these references. For example, they employ the term "R3 spine" to refer to a non-osseous structure illustrated at the tip of R4a (Figure 5), which is obviously inconsistent. More inconsistancies can be found in the attached PDF.
***Several additional minor revisions are still necessary, as detailed along the annotated PDF.
Sincerely,
Igor Souto-Santos

Author's Rebuttal Letter (11/05/2026).
Dear Dr. Lucinda,
Thank you for your time and for your thoughtful evaluation of the revised version of our manuscript. We greatly appreciate the constructive feedback provided during this second round of review.
We believe that the revisions made in response to these comments have further improved the clarity and overall quality of the manuscript. In the accompanying document, we provide a detailed, point-by-point response to all editorial and reviewer comments.
We hope that the revisions adequately address the remaining concerns and that the manuscript is now suitable for publication.
Eric Bruschi Haddad
PhD Candidate
University of Louisiana at Lafayette
Reviewer 1

Recommendation: Major Revision
Comments:
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The text has been revised to address all grammatical issues as requested.
To avoid confusion on the term pattern and deviations, we used "variation in the

number of lateral spots".
RESUMO
See comments about the Abstract.
Keywords
p.2, l.48: Keywords are meant to find articles that are highly relevant for a search, but some of the keywords (e.g., "carnivore", "phenotype") are next to useless as bibliographic search terms.
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p.7, l.14: Change "The DNA" to "the final trimmed DNA".
The text has been revised to address all grammatical issues as requested.
We clarified what the authors classified at different phenotypes.
RESULTS
p.7, l.47-49: "different phenotypes. (1x1, 1x2, 1x3, 2x2, 2x3 and 3x3 lateral spots) (Fig. 2, Fig. 3)" This is directly contradicted by Figure 3. Figure 3 shows nine different phenotypes. If spot are relevant for sexual display, and the species exhibits strong asymmetry on both the gonopodial appendages, and in the female urogenital papilla, a "phenotype" with more spots on the right side cannot be equated with a "phenotype" with more spots on the left side. This is an important consideration, because the theoretical framework of the manuscript depends on the correct definition os a

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We appreciate the reviewer's careful examination of our data. After rechecking the original measurements, we confirm that the discrepancies noted arise from minor errors in the text, primarily due to rounding and transcription inaccuracies. These have now been corrected. Importantly, Table 2 has been thoroughly verified against the raw dataset and contains accurate values. We have revised the manuscript to ensure full consistency between the text and the table.
p.8, l.5: Change "larger sizes and heights" to "larger sizes and body depth".
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The text has been revised to address all grammatical issues as requested.
All rivers and streams were given their original Portuguese names (e.g. Rio Paraopeba, Rio das Velhas, Córrego Caraça, Córrego do Jaque).
p.9, l.43-45: "All analyzed populations of Phalloceros share the same diagnostic characteristics of the gonopodium and urogenital papilla described by Lucinda (2008) for Phalloceros uai." This sentence should be deleted because it is both non-sensical and uninformative. It just says that all populations of P. uai share the diagnostic characters of P. uai, which is a truism! To hide the obvious redundancy the authors listed the populations as "All analyzed populations of Phalloceros" which is misleading: they are not population of the entire genus! Delete the sentence.
p.9, l.45: Delete "However,". There is no controversy here.

p.9, l.47: Change “register” to “record”.
p.9, l.48-52: “The only exception to our findings is the work of Thomaz et al. (2019), who reported specimens without lateral spots in the das Velhas River”: To state that their work is “the only exception to our findings” is an overstatement. Simply state that those authors reported the presence of specimens without spot in the region. That is just an observation; it is not an “exception” to anything.
The paragraph has been revised to address all issues as requested.
p.10, l.12: “This pattern”: What exactly is the “pattern” here? The term “pattern” is misused throughout the manuscript, without regard to its actual meaning. Here the lack of variation in the number of spots occurs only once, in a single locality. A single (!) occurrence does not constitute a “pattern”. All uses of the word “pattern” must be replaced by a simple description of the actual observation. If there is an actual “pattern”, the authors must explain what is this “pattern” and why they consider it as something of sufficient generality to regard it as a “pattern” of some sort.
p.10, l.15: “For instance, experimental data”: Experimental data are NOT examples of the natural phenomena mentioned in the previous sentence.
p.10: l.20-22: “which constrains a more comprehensive understanding of the population phenotypic breadth”: ?????? This is gibberish.
The paragraph has been revised to address all issues as requested.
p.10, p.30: “Therefore, ": Non sequitur.
p.10, l.51: “vertical bars … were not recorded in either live or fixed specimens.” This is false. Vertical bars are clearly visible on the male on the top of Fig. 2!
Thanks for the comment.
These markings are subtle and not as well-defined or prominent as the distinct vertical bars typically seen in other species of Phalloceros or in guppies.
p.11, l.15: Delete “on” in “conferred on such”.
The text has been revised to address all grammatical issues as requested.
Figure 3: The nine “phenotypes” depicted in this illustration must be labeled according to the names of their names in the study. For example which combination is 3x2 and which is 2x3? More importantly, the nine phenotypes in this illustration are treated as six phenotypes in the main text! This poor definition of what constitute a “phenotype” undermines the theoretical basis of the study.
We thank the reviewer for pointing this out. In our study, phenotypes were defined as ordered pairs of lateral spots, with the left and right sides counted independently. This approach treats asymmetric combinations (e.g., 2×3 and 3×2) as distinct phenotypes, which is why we report nine phenotypes in Figure 3 and Table 1.

Figure 4: The number of data pairs from each locality must be stated, and the geographic provenance of the data must be explicitly stated. Specifically, is the regression based on specimens from the four different sampling sites?
The legend has been revised.
Figure 5: There are two problems here: (1): What is the meaning of “canoe-shaped”? Ray 5 is NOT shaped as a canoe! The canoe-shaped structure must be clearly labeled in the drawing. (2) The rays are mislabeled. For example, in the larger drawing the ray branch bearing a spine is labeled and R4a, but is labelled as R3 in the smaller figure. This problem must be corrected.
We thank the reviewer for carefully examining Figure 5 and for pointing out these issues. We have revised the figure accordingly.
Figure 6: I suppose that “35 mm” refers to SL, but it is not stated in the legend.
Figure 7: (1) What is an “embryos phenotype frequency”. I suppose that the authors meant to refer to a “embryonic frequency” or “fecundity”, but that is not what is written. The graph does NOT show a “phenotype frequency”. The frequency bars are grouped by phenotype categories, but they do not represent the frequency of phenotypes. The

phenotype frequency is represented in Table 1, not here!
The legend has been revised.
Figure S1. (1) This is a photograph of the Córrego do Jaque, but it is not necessarily a photograph of the type locality. The type locality was a spot in the in this Córrego, but it may not be the spot that was photographed. The photograph shows the sample locality for this study. (2) "from do Jaque stream," change to "in the Córrego do Jaque". DO not tranlate local toponyms.
The legend has been revised.
Tabel S2: What are this "dissected" specimens? What were they dissected for? They were dissected for the food study? Or were they females dissected for the gonadal study? The purpose of the dissection must be stated in the tittle of the table.
The legend has been revised.
Reviewer: 2
Recommendation: Minor Revision
Comments:
Dear Editor,
***I have reviewed the revised version of the manuscript entitled "Phenotypic variability in populations of Phalloceros uai (Cyprinodontiformes: Poeciliidae) distributed in the Quadrilátero Ferrífero, Brazil, with comments on feeding and reproductive habits." The authors have addressed most of the reviewers' comments. Overall, the manuscript has improved and is now closer to meeting the standards of Neotropical Ichthyology.
We sincerely thank the reviewer for their positive evaluation of our revised manuscript and for acknowledging the improvements made. We appreciate the time and effort dedicated to reviewing our work, and we are pleased that the manuscript is now closer to meeting the standards of the journal.
***In this second round, the main point to highlight is a discrepancy between the six phenotypes reported in the results (phenotypes 1×1, 1×2, 1×3, 2×2, 2×3, and 3×3) and those nine represented in figure 3, which also includes the additional patterns 2×1, 3×1, and 3×2. The authors should carefully verify how many phenotypes (6 or 9) were identified in the study. According to Tab 1, it seems that nine is the correct number, but authors should double check it.
The paragraph has been revised to address all issues as requested.
***An important technical issue concerns the nomenclature of gonopodial elements. In the Methods section, the authors state that they followed Rosen & Gordon (1953), as modified by Lucinda & Reis (2005); however, they use terminology that is not found in these references. For example, they employ the term "R3 spine" to refer to a non-osseous structure illustrated at the tip of R4a (Figure 5), which is obviously inconsistent. More inconsistancies can be found in the attached PDF.
We thank the reviewer for carefully examining Figure 5 and Figure S3 for pointing out these issues. We have revised the figures accordingly.
***Several additional minor revisions are still necessary, as detailed along the annotated PDF.
The text has been revised to address all grammatical issues as requested.
Sincerely,
Igor Souto-Santos

Associate editor's decision after peer review (14/09/2026).
Dear Mr. Haddad:
It is a pleasure to accept your manuscript entitled "Phenotypic variability in populations of Phalloceros uai (Cyprinodontiformes: Poeciliidae) with comments on feeding and reproduction habits" 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. Paulo Lucinda
Associate Editor, Neotropical Ichthyology
lucinda@uft.edu.br
Entire Scoresheet:
Reviewer: 1
Recommendation: Major Revision
Comments:
This is the third version of the manuscript. The manuscript readability and language have been much improved, but confrontation of the main text with supplementary material reveals major flaws in the data. Contrary to what is said in the main text, Table S3 shows unacceptably high values of genetic distance and Fig. S4. These problems are evident on page 8 of the review PDF and are detailed below. They indicate the existence of major errors in the data, possibly related to poor data editing or data analysis. This manuscript cannot be published with these analytic errors and acceptance must be conditioned to public availability of the raw sequencing files, specifically the *.ab1 trace files. I strongly recommend their inclusion in the BoldSystems public database, which

is the standard repository for DNA barcoding data, but other public repositories are also available. Trace data files allow for the verification of the interpretation of the electropherograms and their consistency with the final data matrix. The data matrix is probably erroneous, but it is not currently available to reviewers for verification.

The problem with the DNA sequence analyses also highlights an imbalance between the Results and the remaining sections of the manuscript. The manuscript text has 12 pages (45 in total), but only two are dedicated to Results. More attention should be dedicated to the details of the actual results of the study. For example, only two (!) sentences are dedicated to describing the genetic results (p.8, lines 38 to 46). Likewise, only three sentences are dedicated to the description of the stomach analyses (p.8, lines 49,46).

Specific coments are presented below in order of appearance in the text:

p.1, l.47: "features were examined using clearing and staining." This is nonsensical. The features of cleared and stained gonopodia may be examined "using" a microscope or some other imaging device, but they cannot the examined "using clearing and staining". Clearing and staining is a method for staining specimens, it is not a means of "examination".

p.1, l.52-54: "No anatomical differences were observed in the gonopodium, and no significant differences in gonopodium length were detected". This is unnecessarily repetitive, as lines 44-46 state that gonopodial length is a morphological trait (and no other morphological trait is listed. Additionally, "variation" should be used instead of "differences", because there is no explicit comparison of paired items.

p.3, l.14: "distributed across watersheds in southern and southeastern South America": Phalloceros is absent in most of southern South America. No species of Phalloceros (or any other poeciliid) was ever found in Chile or in Patagonia.

p.4, l.3: "evidence suggest": Grammar error.

p.4, l.5-7: "the common ancestor ... lost and re-acquired multiple times throughout its lineage (Pollux et al., 2014)." Pollux et al. never said that the placenta was lost in the common ancestor! And the family does not constitute a single "lineage".

p.4, l.15: "focus species": Change to "focal species".

p.5, l.3-21: The description of sampling localities must inform in which country the collecting sites are situated. In fact, Brazil was never mentioned in the Introduction.

p.5, l.36: "adults … and juveniles' categories"> Grammar error.

p.6, l.5: Change "between" to "among".

p.6, l.7: "default R": ????? What is the meaning of default here?

p.7,l.3-5: "These sequences plus others available for Phalloceros species …" What are these other sequences? Where are they listed? Why were they edited? How were they used?

p.7, l.12-15: "Additionally, P. harpagos was excluded in the analysis because, at the time, it showed significant taxonomic uncertainty, and was considered a potential species complex, as later clarified (Oliveira et al., 2024; Souto-Santos et al., 2025)." The sampling never included P. harpagos! What is the point of excluding something that was never included? What is the meaning of "at the time" here? No "time" was ever mentioned in relation the the molecular analysis!

p.8, l.3: "abdomen dilation": Here and in several other instances in the text (e.g., "gonopodium length"), nouns are used as adjectives. Change to "abdominal dilation" here and use the proper adjectival form in all similar cases elsewhere in the text,

p.8, l.38: "…distance… were…": Grammar error.

p.8, l.38-42: "Mean genetic distance based on the K2P model were less than 1% among P. uai sequences, including those from specimens collected at the type locality, and exceeded 1% between P. uai and its congeners (Tab. S3)". Something is very wrong here. Almost all distances in Table S3 are greater 1%. Some are extremely great. For example, the distance between samples DZUFMG171 2x1 and DZUFMG171 1x1 is

4.1%. Such high distance values between samples from the same locality are not natural and are indicative of major errors in the data.
p.8, l.41-47: "Our maximum–likelihood phylogenetic analyses based on COI recovered all P. uai sequences as a monophyletic group, further supporting the conclusion that all analyzed populations belong to P. uai (Fig. S4).": The analysis does NOT support the conclusion. Monophyly cannot be tested with a dataset that includes only 6 of more than 20 species of Phalloceros. In fact, Fig. S4 shows that 8% of the trees are NOT monophyletic, because of the unusually high divergence of among the sequences. The long terminal branches in Fig. S4 demonstrate that each individual specimen diverges by about 3% from the common ancestor of P. uai which is absurd. Figure S4 only demonstrates that the data has serious problems, possibly the result of alignment errors.
p.19, l.3-5: "The authors confirm that the data supporting the findings of this study are available within the article and the accompanying supplementary material.": This is not true. The DNA sequences are not available in the article, nor in the Supplementary material. Additionally the electropherograms (Sanger traces, *.ab1 files) must be made available in a public repository, such as the BoldSystems which is the main repository for DNA Barcoding projects with COI gene. Considering the major inconsistencies between the text, Table S3 and Fig. S4., public availability of the raw data file in must be mandatory prior to manuscript acceptance.
Reviewer: 2
Recommendation: Accept
Comments:
Dear Editor,
I have reviewed the manuscript for the third time. The authors have addressed the main issues raised in the previous rounds, and the manuscript has improved considerably. In particular, the inconsistency regarding the number of phenotypes has been corrected, with the text now consistently reporting nine phenotypes.
I only note that the nomenclature issue concerning the gonopodium has not been fully resolved. In Figure 5, the structure indicated as "Spine" is still present in the same position that I previously identified as problematic, as it does not appear to correspond to the terminology used in the cited references. Although the text has been revised, I recommend that the authors check this label once more before publication.

This is a minor issue and does not affect my overall assessment of the manuscript. I therefore recommend acceptance of the manuscript for publication, provided that the authors make this final correction to Figure 5.
Sincerely,
Igor Souto-Santos

Neotropical Ichthyology

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SBI Official Journal of the Sociedade Brasileira de Ictiologia