Submission date. 17/03/2026

Associate editor's decision after peer review (08/07/2026).
Dear Dr. Young:
Manuscript ID NI-2026-0038 entitled "Gonadal development and anal fin morphogenesis in Cnesterodon decemmaculatus (Pisces, Poeciliidae)" which you submitted to the Neotropical Ichthyology, has been reviewed. The comments of the reviewer(s) are included at the bottom of this letter.
The reviewer(s) have recommended publication, but also suggest some minor revisions to your manuscript. Therefore, I invite you to respond to the reviewer(s)' comments and revise your manuscript.
To revise your manuscript, log into https://mc04.manuscriptcentral.com/ni-scielo and enter your Author Center, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Revision." Your manuscript number has been appended to denote a revision.
You may also click the below link to start the revision process (or continue the process if you have already started your revision) for your manuscript. If you use the below link you will not be required to login to ScholarOne Manuscripts.
*** PLEASE NOTE: This is a two-step process. After clicking on the link, you will be directed to a webpage to confirm. ***
https://mc04.manuscriptcentral.com/ni-scielo?URL_MASK=3d1edd42f417427b91283dd62697c605
You will be unable to make your revisions on the originally submitted version of the manuscript. Instead, revise your manuscript using a word processing program and save it on your computer. Please also highlight the changes to your manuscript within the document by using the track changes mode in MS Word or by using bold or colored text.
Once the revised manuscript is prepared, you can upload it and submit it through your Author Center.
When submitting your revised manuscript, you will be able to respond to the comments made by the reviewer(s) in the space provided. You can use this space to document any changes you make to the original manuscript. In order to expedite the processing of the revised manuscript, please be as specific as possible in your response to the reviewer(s).
IMPORTANT: Your original files are available to you when you upload your revised manuscript. Please delete any redundant files before completing the submission.
Because we are trying to facilitate timely publication of manuscripts submitted to the Neotropical Ichthyology, your revised manuscript should be submitted before 08-Aug-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. Elizete Rizzo
Associate Editor, Neotropical Ichthyology
ictio@icb.ufmg.br

Anonymous reviewer #1
Recommendation. Minor Revision
Comments.
A portion of your work focuses on the morphological description of male and female gonads. However, the size of the images does not allow for the observation of certain details that are essential for tracking their development, such as the blood vessels mentioned in Figure 1.

In this regard, I have two suggestions:

1. To avoid increasing the total number of figures in your manuscript, you could opt to include insets with higher magnification of key sections. These could feature cysts at different developmental stages, spermatozeugmata, and the various ovarian developmental stages you identified (such as oogonia, primary oocytes, and cortical alveoli oocytes), similar to the presentation in Figures 3G and 3H.
2. To include representative magnifications of each developmental phase alongside the images of developing testes and ovaries and their corresponding graphs. This approach would involve reorganizing your figures and increasing the number of images. I leave this to your consideration.

You possess highly valuable morphological material that could be better utilized by employing higher magnifications and providing more explicit descriptions.
Finally, the literature review I suggest is intended to provide greater precision in the presentation of your data and to complement your work.

Anonymous reviewer #2
Recommendation. Accept
Comments.
Dear authors.
Thanks for getting me in this review, I really enjoyed reading it.
Definitely, C. decemmaculatus should have its monument at the 9 de Julio Avenue. With every new paper, it make its stance as an excellent neotropical animal model for assays of all kinds, due to how highly informative and versatile it is.
As someone who is familiar with the females of this species, I think your study highlights a very interesting and powerful point, in addition to those discussed in the article: females reach mature phenotypic size, at least regarding the anal fin, before developing vitellogenic oocytes. I'd like to share a brief thought on this for future research.
The fact that females can look visibly mature might play an important role in intraspecific relationships. Poeciliids, like many atherinomorphs, are teleosts where vision and external appearance are highly significant in mating. The anal fin in C. decemmaculatus female is situated immediately next to the urogenital depresion and so the gonopore. In an apparently mature female the anal fin could be a visual cue for males. Even more, this apparent external maturity might also be linked to an early capacity to store sperm in the gonoduct.

Author's Rebuttal Letter (05/08/2026).
RESPONSE TO REVIEWERS
Ms. No. NI-2026-0038: Gonadal development and anal fin morphogenesis in Cnesterodon decemmaculatus (Cyprinodontiformes, Poeciliidae). Neotropical Ichthyology.
Dear Editor and reviewer,
Thank you very much for your positive concluding remarks regarding the manuscript and your constructive comments to help improve it. Please, find below the detailed replies to all your comments.
Editor's comments:
The reviewer(s) have recommended publication, but also suggest some minor revisions to your manuscript. Therefore, I invite you to respond to the reviewer(s)' comments and revise your manuscript.
Reply: We respond point-by-point to the reviewer's comments.
Reviewer's comments:
Reviewer: 1
Recommendation: Minor Revision
Comments:

A portion of your work focuses on the morphological description of male and female gonads. However, the size of the images does not allow for the observation of certain details that are essential for tracking their development, such as the blood vessels mentioned in Figure 1.
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You possess highly valuable morphological material that could be better utilized by employing higher magnifications and providing more explicit descriptions.
Finally, the literature review I suggest is intended to provide greater precision in the presentation of your data and to complement your work.
Additional Questions:
Does the manuscript contain new and significant information to justify publication?: Yes
Does the Abstract (Summary) clearly and accurately describe the content of the article?: Yes
Is the problem significant and concisely stated?: Yes
Are the methods described comprehensively?: Yes
Are the interpretations and conclusions justified by the results?: Yes
Is adequate reference made to other work in the field?: Yes
Is the language acceptable?: Yes
Length of article is: Adequate
Number of tables is: Adequate
Number of figures is: Adequate
Do you have any conflict of interest to declare?: No
If yes, please provide details below:
Rating:
Interest: 1. Excellent
Quality: 2. Good
Originality: 1. Excellent
Overall: 1. Excellent
I confirm I have not uploaded the author manuscript to any third party tool and have not used a generative AI tool to produce this reviewer report.: Confirm
General comments to reviewer 1:
We sincerely thank the reviewer for the thorough evaluation of our manuscript, the very positive assessment of its scientific value, and the constructive suggestions helped us improve both the presentation and interpretation of our findings. We appreciate the recognition of the morphological material presented in this study and agree that a more detailed visualization of key histological features improves the interpretation of gonadal development.
Following the reviewer's recommendation, we revised Figures 1 and 2 to better highlight diagnostic structures of the different gonadal developmental stages. We incorporated higher magnification views of representative histological features, including spermatozeugmata, oogonia, primary oocytes, and cortical alveoli oocytes, and expanded the corresponding descriptions in the text and figure legends where appropriate.

We also carefully considered the reviewer's suggestions regarding terminology and additional literature. The manuscript was revised accordingly to improve anatomical precision, consistency with current reproductive morphology terminology, and the clarity of several descriptions throughout the Results and Discussion sections.
Reviewer: 2
Recommendation: Accept
Comments:
Dear authors.
Thanks for getting me in this review, I really enjoyed reading it.
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Are the methods described comprehensively?: Yes
Are the interpretations and conclusions justified by the results?: Yes
Is adequate reference made to other work in the field?: Yes
Is the language acceptable?: Yes
Length of article is: Adequate
Number of tables is: Adequate
Number of figures is: Adequate
Do you have any conflict of interest to declare?: No
If yes, please provide details below:
Rating:
Interest: 1. Excellent
Quality: 1. Excellent
Originality: 1. Excellent
Overall: 1. Excellent
I confirm I have not uploaded the author manuscript to any third party tool and have not used a generative AI tool to produce this reviewer report.: Confirm
General comment to reviewer 2:
We sincerely thank the reviewer for the highly positive evaluation of our manuscript and for the encouraging comments regarding the value of Cnesterodon decemmaculatus as a Neotropical model species.
We agree that the apparent external maturation of females before the occurrence of vitellogenic oocytes may have important implications for reproductive biology, including male visual recognition of potential mates and the possibility of reproductive interactions prior to full ovarian maturation. These potential implications could be

investigated in future studies. We appreciate the reviewer's thoughtful interpretation of our results and the insightful perspective provided.

Reviewer: 3
General comments to reviewer 3:
We sincerely thank the reviewer for the careful reading of our manuscript and for the constructive comments and suggestions. We particularly appreciate the observations regarding gonadal terminology, which helped us improve the anatomical precision and consistency of the manuscript. Following the reviewer's recommendations, we revised the terminology used to describe early gonadal development throughout the text, tables, figures, and figure legends, and we incorporated additional literature to strengthen the discussion of gonadal morphology and development in poeciliid fishes. We also addressed all editorial and textual suggestions.
Specific comments to reviewer 3:
Changes suggested according to the Lines number of the MS. In green: additions. In blue: questions? In yellow: word related to indication.
Line
30 Males are identified
Reply: We agree with the reviewer's suggestion and the text was accordingly modified. Please see Line 30 (Page 2) in the Revised Manuscript.
47 Los machos se identifican inequívocamente
Reply: The text was accordingly modified. Please see Line 47 (Page 3) in the Revised Manuscript.
65 environmental factors can play key roles
Reply: The text was accordingly modified. Please see Line 65 (Page 4) in the Revised Manuscript.
181 lobular structures?
184 spermatic lobules?
185 of the testes lobes. ?
187 testes lobes,?

Comment explaining this aspect:
When you mention lobes (as primordia of testes, they are the early stage of testes) may confuse the terminology, especially taking account that the testis structure is lobular. Then, I suggest talking about testes instead of lobules. This aspect most be revised and changed in all the MS (text and images and captions).
From lines 305 to 310 the morphology of the testicular structure of poeciliid species is explained (as atherinomorphs). It is a proper and clear paragraph. The lobular structure is well described. There, it is mentioned:
305- 310: The organization of the testis germinal epithelium varies among teleost fishes, whose testes are lobular and classified into two types based on the distribution of spermatogonia: restricted and unrestricted (Grier et al., 1980; Parenti, Grier, 2004). In atherinomorph fishes such as C. decemmaculatus, the testis exhibits a restricted lobular organization with spermatogonia confined to the testicular periphery and an orderly progression of germ cell development within the lobules (Uribe et al., 2015).
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199 independent lobar structures (also talking about females) then, change to: independent ovarian structures
210 fusion of both ovarian lobes, change to: fusion of both ovaries,
Reply (Comments 181, 184, 185, 187, 199 and 210): We thank the reviewer for these valuable observations. Because all of these comments relate to the use of the terms "lobe", "lobar", and "lobular" in the description of early gonadal development, we address them together.

We agree that the original terminology could lead to confusion, particularly because the term lobular is appropriately used later in the manuscript to describe the histological organization of the testis in atherinomorph fishes, whereas several of the structures originally referred to as "lobes" actually correspond to paired gonadal structures rather than true anatomical lobes.

Following the reviewer's observation, the terminology was revised throughout the manuscript, including the Results section, Table 1, figure legends, and corresponding discussion. In males, references to "lobular structures", "spermatic lobules", and "testes lobes" were replaced by descriptions referring to "paired testicular structures" and "sperm ducts", according to the developmental stage being described. Likewise, in females, references to "lobar structures", "gonadal lobes", and "ovarian lobes" were replaced by "paired ovarian structures" and by descriptions of their subsequent fusion into a "single saccular ovary" after formation and closure of the ovarian cavity.

These revisions ensure consistency between the developmental descriptions and the anatomical terminology currently used for poeciliid gonads, while maintaining the distinction between the paired gonadal primordia observed during early ontogeny and the restricted lobular organization characteristic of the mature testis.

In addition, we reviewed and expanded the Discussion section to incorporate literature on gonadal development and morphology in poeciliid fishes (Mazzoni et al., 2014; Olivera-Tlahuel et al., 2017; Razmi et al., 2023; Uribe et al., 2015, 2019; Vicentini et al., 2010). Please see the revised Table 1, figure legends, and Results (Pages 9-10) and Discussion sections (Pages 14-17) in the Revised Manuscript.

References: Mazzoni TS, Grier HJ, Quagio-Grassiotto I. 2014. Male gonadal differentiation and the paedomorphic evolution of the testis in Teleostei. Anat Rec. 297:1137-1162. https://doi.org/10.1002/ar.22915

Olivera-Tlahuel C, Villagrán-Santa Cruz M, Moreno-Mendoza NA, Zúñiga-Vega JJ. Morphological structures for potential sperm storage in poeciliid fishes. Does superfetation matter? J Morphol. 2017; 278:907–18. https://doi.org/10.1002/jmor.20684

Razmi K, Tran NK, Patil JG. 2023. Gonad ontogeny and sex differentiation in a poeciliid, Gambusia holbrooki: Transition from a bi- to a mono-lobed organ. Biology. 12:731. https://doi.org/10.3390/biology12050731

Uribe MC, Grier HJ, Mejía-Roa V. 2015. Comparative testicular structure and spermatogenesis in bony fishes. Spermatogenesis. 4:e983400. https://doi.org/10.4161/21565562.2014.983400

Uribe MC, Rosa Cruz G, García Alarcón A, Campuzano Caballero JC, Guzmán Bárcenas MG. 2019. Structures associated with oogenesis and embryonic development during intraovarian gestation in viviparous teleosts (Poeciliidae). Fishes. 4:35. https://doi.org/10.3390/fishes4020035

Vicentini CA, Muñoz ME, Vicentini IBF. Ultrastructural aspects of spermatogenesis in Phalloceros caudimaculatus (Teleostei, Poeciliidae). Int J Morphol. 2010; 28(3):951–6. https://doi.org/10.4067/S0717-95022010000300047

213 Stage 4 (Fig. 2G). As in the other stages, add the oocyte stages seen here.

Reply: We thank the reviewer for this observation. Following the reviewer's suggestion, we expanded the Stage 4 description to explicitly indicate the oocyte developmental stages observed at this stage, maintaining consistency with the descriptions provided for the other ovarian developmental stages. Please see the revised Table 1 and Results section (Lines 216-217, Page 10) in the Revised Manuscript.

Reviewers' comments in the manuscript

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Page 4, Line 74: In this case, it would be more appropriate to use the term "birth" rather than "hatching", given that we are dealing with viviparous species.

Reply: We agree with the reviewer's comment. These modifications are highlighted in the Revised Manuscript with the changes marked. Please see Line 74 (Page 4) in the

Revised Manuscript.
Page 6, Line 123: It is better to use the term "stained" instead of "colored".
Reply: We have modified the text according to the reviewer's comment. These modifications are highlighted in the Revised Manuscript with the changes marked. Please see Line 123 (Page 6) in the Revised Manuscript.
Page 6, Lines 127–128: Were the fish euthanized using this anesthetic method? Since the specimens were subsequently stained with Alizarin Red and Alcian Blue, it is assumed they were not revived. This detail should be explicitly clarified in the text.
It remains to be specified whether the fish were fixed following anesthesia. Provided that the protocol is identical to the one previously mentioned, repetition is not required.
Reply: We thank the reviewer for pointing out this ambiguity. The fish used for growth monitoring and morphometric measurements were only anesthetized by immersion in ice-cold water and were subsequently returned to the rearing tanks. In contrast, fish selected for histological processing or whole-mount skeletal staining were euthanized with an overdose of benzocaine prior to fixation and subsequent processing. To clarify this distinction, the Materials and Methods section has been revised accordingly. Please see Lines 127–133 (Pages 6–7) in the Revised Manuscript.
Page 6, Line 128: Why do you indicate the sexing of organisms based on secondary sexual characteristics merely as a possibility, considering it is a fundamental aspect of your study? Was it not possible to sex the organisms at some stage? If so, how did you obtain these data?
Reply: We thank the reviewer for this comment. The expression "when possible" was used because individuals in the early stages of development cannot be reliably sexed based solely on external secondary sexual characteristics. During the Undifferentiated Anal Fin (UAF) stage, males and females exhibit the same external anal fin morphology and therefore cannot be distinguished phenotypically. Sex identification based on secondary sexual characters only becomes possible after the appearance of gonopodial primordium in males and the differentiation of the adult female phenotype in larger individuals. Individuals that could not be sexed externally were subsequently classified through the combined analysis of gonadal histology, anal fin morphology, and morphometric parameters. To clarify this point, the text has been revised. Please see Lines 129–130 (Page 6) in the Revised Manuscript.
Page 7, Line 133: Could the authors clarify why rays 3 and 5 were excluded from this morphometric parameter?
Reply: We thank the reviewer for this comment. Rays 3, 4, and 5 exhibit the most pronounced elongation during gonopodium development. Among them, ray 4 was selected because it provides a consistent measure of gonopodial elongation and has been used in previously proposed morphometric indices in poeciliids, including the Gonopodial Index (GI) and the Elongation Index (EI), thereby facilitating comparisons with other species. Ray 6 was selected as a reference ray because it does not undergo the marked elongation observed in rays 3–5 and its growth is primarily associated with overall body growth. Consequently, the combination of rays 4 and 6 allows a biologically meaningful quantification of relative gonopodial development. The rationale for selecting these rays has been clarified in the Materials and Methods section. Please see Lines 147–152 (Page 7) in the Revised Manuscript.
Page 7, Line 136: Double staining with Alizarin Red and Alcian Blue..
Reply: We modified the sentence following the reviewer's suggestion. Please see Lines 140–142 (Page 7) in the Revised Manuscript.
Page 7, Line 139: Replace "Pictures" per "Micrographs"
Reply: The word "Pictures" was replaced by "Micrographs". Please see Line 144 (Page 7) in the Revised Manuscript.
Page 7, Line 145 (Eq. 2): What was the criterion for considering only rays 4 and 6 when calculating the elongation index?

Reply: We thank the reviewer for this comment. The selection of rays 4 and 6 followed the same rationale described above. Ray 4 was chosen because it exhibits pronounced elongation during gonopodium development and has been previously employed in morphometric studies of poeciliids. Ray 6 was selected as a reference ray because it does not show the marked elongation observed in rays 3-5 and its growth is primarily associated with overall body growth. Therefore, the ratio between rays 4 and 6 provides a biologically meaningful estimate of relative gonopodial elongation. This rationale has been clarified in the Materials and Methods section. Please see Lines 147-152 (Page 7) in the Revised Manuscript.

Page 7, Lines 145-146 (Eq. 2 and 3): Are the elongation and gonopodium indices novel proposals, or are they adapted from previous studies?

Reply: We thank the reviewer for this valuable comment. Neither the Elongation Index (EI) nor the Gonopodial Index (GI) are novel metrics proposed in the present study. Both indices are adaptations of morphometric approaches previously employed in poeciliid fishes to quantify gonopodial development during normal growth, environmental monitoring, or following exposure to chemical compounds. In particular, the biological relevance of the Elongation Index was originally demonstrated by Angus et al. (2001) in Gambusia affinis and later applied by Doyle and Lim (2002) in Gambusia holbrooki. Originally, Angus et al. (2001) explained: "Since anal fin ray 6 does not elongate during formation of the gonopodium, we used the ratio of lengths of ray 4 (which is in the center of the gonopodium) and ray 6 as an index of elongation". Brockmeier et al. (2013) applied this index to demonstrate that the anabolic-androgenic steroid 17β-trenbolone induce anal fin elongation in G. affinis and G. holbrooki. Similarly, the use of ray 4 length relative to body size (GI) has been employed as an indicator of gonopodial development in poeciliids. Both indices (GI and EI) were also applied in previous studies on Cnesterodon decemmaculatus (Young et al., 2017, 2020).

The novel contribution of the present study is the development of the Gonopodium Development Index (GDI), which integrates EI, GI, and qualitative descriptors of gonopodial differentiation into a single metric of anal fin development. To avoid ambiguity, we have clarified this point in the revised manuscript and explicitly indicate that the GDI represents a novel index proposed in this study. Please see Line 160 (Page 8) and Lines 384-386 (Page 17) in the Revised Manuscript.

References: Angus RA, McNatt HB, Howell WM, Peoples SD. Gonopodial development in normal male and 11-ketotestosterone-treated female mosquitofish (Gambusia affinis): A quantitative study using computer image analysis. Gen Comp Endocrinol. 2001; 123(2):222–34. https://doi.org/10.1006/gcen.2001.7669

Brockmeier EK, Ogino Y, Iguchi T, Barber DS, Denslow ND. Effects of 17β-trenbolone on Eastern and Western mosquitofish (Gambusia holbrooki and G. affinis) anal fin growth and gene expression patterns. Aquatic Toxicol. 2013; 128–9:163–70. https://doi.org/10.1016/j.aquatox.2012.12.007

Doyle CJ, Lim R. The effect of 17β-estradiol on the gonopodial development and sexual activity of Gambusia holbrooki. Environ Toxicol Chem 2002; 21(12):2719–24. https://doi.org/10.1002/etc.5620211227

Young BJ, Cristos DS, Crespo DC, Somoza GM, Carriquiriborde P. Effects of 17α-ethinylestradiol on sex ratio, gonadal histology and perianal hyperpigmentation of Cnesterodon decemmaculatus (Pisces, Poeciliidae) during a full-lifecycle exposure. Ecotox Environ Safe. 2020; 205:111176. https://doi.org/10.1016/j.ecoenv.2020.111176

Young BJ, Lopez GC, Cristos DS, Crespo DC, Somoza GM, Carriquiriborde P. Intersex and liver alterations induced by long-term sublethal exposure to 17α-ethinylestradiol in adult male Cnesterodon decemmaculatus (Pisces: Poeciliidae). Environ Toxicol Chem. 2017; 36(7):1738–45. https://doi.org/10.1002/etc.3547

Page 9, Line 180: When comparing the Results section with Table 1, I noticed that the morphological descriptions for each developmental stage are more detailed in the table

than in the text. Therefore, I suggest the following alternatives:
1. Omit Table 1 and integrate all of its information into the Results section;
2. Move the data regarding fish size for each stage to Table 1, while keeping the morphological descriptions in the Results text.
A similar approach should be applied to the section on ovarian development.
Reply: We thank the reviewer for this helpful suggestion. We agree that the original presentation contained some redundancy between the Results section and Table 1. Following the reviewer's second recommendation, the fish size data associated with each gonadal developmental stage were transferred to Table 1, while the morphological descriptions were retained in the Results section. The same modification was applied to both testicular and ovarian development. This reorganization reduced redundancy and improved the clarity and readability of the manuscript. Please see Table 1 and Lines 190–220 (Pages 9–10) in the Revised Manuscript.
Page 9, Line 184: Could you clarify what is meant by 'the appearance of the spermatic lobes'? It would be more appropriate to describe these morphological changes explicitly in order to provide more precise information.
Reply: We agree that the term "spermatic lobes" was imprecise and could lead to confusion with the testicular lobes. The defining morphological feature of this developmental stage is the appearance of sperm ducts within each testis, accompanied by an increase in testicular size. This interpretation is consistent with descriptions of early testicular development in Gambusia affinis (Koya et al., 2003). Accordingly, the description of Stage 2 has been revised to reflect this morphological change more accurately. Please see the revised Table 1 and Lines 192–193 (Page 9) in the Revised Manuscript.
Reference: Koya Y, Fujita A, Niki F, Ishihara E, Miyama H. Sex differentiation and pubertal development of gonads in the viviparous mosquitofish, Gambusia affinis. Zool Sci. 2003; 20(10):1231–42. https://doi.org/10.2108/zsj.20.1231
Page 9, Line 185: Please clarify the distinction between "spermatic lobes'" and "testes lobes" (lobular testes). The current phrasing makes it difficult to discern if you are referring to different structures or using them synonymously.
Reply: We thank the reviewer for this observation. We agree that the original terminology was ambiguous and could lead to confusion regarding the structures being described. In the revised manuscript, the term "spermatic lobes" was replaced by "sperm ducts", which more accurately reflects the morphological feature observed at this developmental stage. In addition, the reference to "testes lobes" was removed and replaced by a description of the increase in overall testicular size. These changes clarify the distinction between the developing sperm ducts and the paired testicular structures in which they occur. Please see Lines 190–195 (Page 9) in the Revised Manuscript.
Page 9, Line 187: Could you please clarify whether the fusion you describe occurs among the testicular lobes themselves or with the central spermatic duct? This distinction is crucial, as in teleost fish, the tubular testis type differs significantly from the lobular type, with anastomosis being a defining feature exclusively present in tubular testes. I highly recommend reviewing Mazzoni et al., (2014) [https://doi.org/10.1002/ar.22915], and Uribe et al. (2015) [https://doi.org/10.4161/21565562.2014.983400], to ensure an accurate comparative terminology regarding these testicular structures.
Reply: We thank the reviewer for this important observation and for directing us to the works of Mazzoni et al. (2014) and Uribe et al. (2015). We agree that the original terminology could lead to confusion regarding the nature of the fusion process described in Stage 3. Our intention was not to describe an anastomosis among seminiferous structures nor the formation of a tubular testis. Rather, the observed process corresponds to the fusion of the paired developing testes into a single testicular organ while maintaining the restricted spermatogonial testis organization characteristic of atherinomorph fishes. To avoid ambiguity and to maintain consistency with the terminology used for restricted lobular testes in atherinomorph fishes, we revised the

corresponding descriptions in Table 1 and the Results and the Discussion sections. We also incorporated the suggested literature to improve the anatomical interpretation of the developing testis. Please see the revised Table 1, and Results (Lines 190–196, Page 9) and Discussion sections (Lines 307–309, Page 14; Lines 318–327, Page 14–15) in the Revised Manuscript.

References: Mazzoni TS, Grier HJ, Quagio-Grassiotto I. Male gonadal differentiation and the paedomorphic evolution of the testis in Teleostei. Anat Rec. 2014; 297:1137–62. https://doi.org/10.1002/ar.22915

Uribe MC, Grier HJ, Mejia-Roa V. Comparative testicular structure and spermatogenesis in bony fishes. Spermatogenesis. 2015; 4:e983400. https://doi.org/10.4161/21565562.2014.983400

Page 9, Line 192: I suggest including a high-magnification image of the spermatozeugmata, as this would strengthen the morphological aspect of your work.

Could you describe the arrangement of the spermatozoa within the spermatozeugmata? I believe it is important to highlight that, in this species, spermatozoa aggregate prior to leaving the testis.

Reply: We thank the reviewer for this valuable suggestion. We agree that a higher-magnification image of the spermatozeugmata strengthens the morphological characterization of testicular development. Accordingly, a high-magnification inset showing representative spermatozeugmata has been incorporated into Figure 1.

We also expanded the description of Stage 5 and the Discussion section to better characterize these structures. Spermatozeugmata were observed within the efferent ducts, indicating that spermatozoa aggregate before leaving the testis. In these structures, the spermatozoa are organized with their heads oriented toward the periphery of the spermatozeugmata and their flagella directed toward the center. This pattern is consistent with previous descriptions in poeciliid fishes. Please see revised Figure 1, Table 1, and Results (Lines 201–202, Page 10) and Discussion sections (Lines 339–344, Pages 15–16) in the Revised Manuscript.

Page 10, Line 199: I suggest replacing “lobar” with “'sacculated” to better describe the hollow, sac-like anatomy of the teleost ovary.

While the term “lobes” in ovaries or testes usually implies that the gonad is divided into distinct sections, the ovary actually undergoes a fusion process—as described in stage 3. Therefore, I believe it is more appropriate to describe the ovary as “two sacculated structures” rather than two lobes.

Reply: We thank the reviewer for this valuable observation. We agree that the term "lobar" could be misleading because it may suggest subdivisions of an already formed ovary rather than the paired ovarian structures observed during early development. The ovarian structures observed in Stage 1 are now described as paired ovarian structures, and the subsequent developmental stages explicitly refer to the fusion of the paired ovaries and the formation of the ovarian cavity, ultimately resulting in the single saccular ovary characteristic of poeciliid fishes.

We added literature in the Discussion section to support the terminology used (Uribe et al., 2019) and link the current state of the art between morphological characteristics and sex-specific expression of genes involved in gonadal differentiation in other poeciliid species (Razmi et al., 2023). Please see Table 1, and Results (Lines 205, 214 and 216, Page 10) and Discussion sections (Line 354 and 357, Page 16) in the Revised Manuscript.

References: Razmi K, Tran NK, Patil JG. Gonad ontogeny and sex differentiation in a poeciliid, Gambusia holbrooki: Transition from a bi- to a mono-lobed organ. Biology. 2023; 12:731. https://doi.org/10.3390/biology12050731

Uribe MC, Rosa Cruz G, García Alarcón A, Campuzano Caballero JC, Guzmán Bárcenas MG. Structures associated with oogenesis and embryonic development during intraovarian gestation in viviparous teleosts (Poeciliidae). Fishes. 2019; 4(2):35. https://doi.org/10.3390/fishes4020035

Page 10, Line 210: Replace “ovarian lobes” per “ovaries”
Reply: The terminology was revised throughout the manuscript to avoid the use of “ovarian lobes”. The corresponding descriptions now refer to paired ovarian structures. Please see revised Table 1, and Results (Lines 205 and 214, Page 10) and Discussion sections (Line 354, Page 16) in the Revised Manuscript.
Page 10, Line 213: Two options:
1) It was characterized by a single, round ovary.
2) It was characterized by a single, round, sacculated ovary.
Reply: We thank the reviewer for this suggestion. Following this recommendation and considering the ovarian morphology described for poeciliid fishes, the description of Stage 4 was revised. As mentioned above, the ovary is now described as a single saccular ovary with an enlarged ovarian cavity, emphasizing both the characteristic cystovarian organization and the developmental features observed at this stage. Please see revised Table 1 and Line 216 (Page 10) in the Revised Manuscript.
Page 11, Line 232: Could the authors clarify whether this refers to stage 1 or stage 2?
Reply: We thank the reviewer for identifying this inconsistency. We agree that this statement refers to Gonopodial Stage 1 rather than Stage 2. The text has been corrected accordingly. Please see Line 236 (Page 11) in the Revised Manuscript.
Page 13, Line 287: Figure 5 is cited five times prior to the first and only mention of Figure 4. Consequently, I suggest swapping the numbering of these two figures.
Reply: We thank the reviewer for this observation. We agree that the original ordering of Figures 4 and 5 could be confusing, since Figure 5 was cited multiple times before the first citation of Figure 4. Following the reviewer’s suggestion, the figure numbering has been revised and the figures were renumbered accordingly throughout the manuscript. Please see Lines 265, 268, 277, 284, 287 and 291 (Pages 12-13) in the Revised Manuscript and revised Figure Legends.
Page 14, Line 303: In the study species, the testes and ovaries do not form two lobes; instead, they are two well-defined gonadal structures positioned contralaterally, taking into account that during embryonic development, the ovaries fuse to form a single structure.
Reply: We thank the reviewer for this important observation. As described above, we agree that the term “lobes” was not appropriate in this context. The text has been revised to refer to “two distinct gonadal structures”, which more accurately reflects the morphology observed during early development. The revised text also emphasizes the subsequent fusion process described for poeciliid gonads. Please see Lines 307-308, 326 and 354 (Pages 14-16) in the Revised Manuscript.
Page 14, Line 307: I suggest including the following citation, "Uribe et al., 2015", since this work presented the most recent classification of the testicular structure in teleost fish.
Reply: The reference Uribe et al. (2015) has been added to the sentence describing the current classification of teleost testicular organization based on the distribution of spermatogonia. Please see Line 318 (Page 14) in the Revised Manuscript.
Reference: Uribe MC, Grier HJ, Mejia-Roa V. Comparative testicular structure and spermatogenesis in bony fishes. Spermatogenesis. 2015; 4:e983400. https://doi.org/10.4161/21565562.2014.983400
Page 14, Line 308: To prevent confusion or misinterpretation of the data, I consider it preferable to explicitly state “restricted spermatogonial testis type,” thereby clarifying that the spermatogonia are restricted, rather than the lobules.
Reply: We thank the reviewer for this important suggestion. We agree that the term “restricted spermatogonial testis type” more accurately reflects the current classification of teleost testicular organization and avoids the potential misunderstanding that the restriction applies to the lobules themselves. Accordingly, the terminology was revised throughout the manuscript and the testis of C. decemmaculatus is now described as a

restricted spermatogonial testis type, following Uribe et al. (2015). Please see Line 319 (Page 15) in the Revised Manuscript.
Reference: Uribe MC, Grier HJ, Mejia-Roa V. Comparative testicular structure and spermatogenesis in bony fishes. Spermatogenesis. 2015; 4:e983400. https://doi.org/10.4161/21565562.2014.983400
Page 15, Line 326: The ovaries are not bilobed; they are paired but fuse together during their development.
Reply: As mentioned in previous replies, we agree that the term "bilobular ovary" was not appropriate in this context. The text has been revised to describe the ovary at birth as paired ovarian structures, and the subsequent developmental process leading to their fusion into a single saccular ovary is now explicitly explained. Please see Line 326 (Page 16) in the Revised Manuscript.
Page 16, Line 342: What are the morphological characteristics these authors mention? How does this information relate to your research?
Reply: We thank the reviewer for this helpful observation. We agree that the original statement was too general and did not clearly indicate which morphological changes distinguish adult from juvenile males or how this information relates to the present study. Therefore, the text was revised to specify that adult males can be distinguished from juveniles by the progressive transformation of the anal fin into a functional gonopodium during sexual maturation. We also clarify that these morphological changes provided the basis for the quantitative and qualitative characters used to describe gonopodial morphogenesis in C. decemmaculatus. Please see Lines 375-376 (Page 17) in the Revised Manuscript.
Page 16, Line 347: Regarding the morphological measurements, what led to the decision of utilizing only the 4th and 6th anal fin rays? Did these specific parameters show a higher discriminatory power compared to the others?
Reply: We agree that the rationale for selecting anal fin rays 4 and 6 should be clarified. This issue was addressed in our response to the reviewer's comment on Page 7, Line 145 (Eq. 2), where we explain the biological basis for selecting these rays and their previous use in poeciliid morphometric studies. The corresponding clarification was incorporated into the Materials and Methods section (Lines 147-152, Page 7) in the Revised Manuscript.
Page 34, Figure Legends.
Figure 1: Replace "hatching" per "parturition"
Reply: We agree that the term "hatching" is not appropriate for a viviparous species. Following the reviewer's suggestion, "hatching" was replaced by "parturition". In addition, the terminology referring to reproductive events was revised throughout the manuscript to ensure consistency with the viviparous reproductive mode of the species. Please see the revised Figure 1 legend.
Figure 2: Replace "birth" per "parturition or the newborn stage"
Reply: As Figure 1 legend, we replaced "birth" by "parturition" in the figure legend. Please see the revised Figure 2 legend.
File: Figures
Page 1, Figure 1-E: Within the testicular structure, interstitial cells (Ic) are located between the lobules or seminiferous tubules; therefore, I suggest adjusting the pointer line for this label.
Reply: We thank the reviewer for this observation. We agree that the position of the label could be improved. The pointer line was adjusted so that it clearly indicates the interstitial tissue (It) of the testis. In addition, the original label "interstitial cells (Ic)" was replaced by "interstitial tissue (It)" because the structure identified corresponds to the interstitial compartment rather than to individual cells. This compartment consists of connective tissue containing interstitial (Leydig) cells, and blood vessels, as described for teleost testes, including poeciliid species (Uribe et al., 2015). Please see the revised

Figure 1.
Reference: Uribe MC, Grier HJ, Mejia-Roa V. Comparative testicular structure and spermatogenesis in bony fishes. Spermatogenesis. 2015; 4:e983400. https://doi.org/10.4161/21565562.2014.983400
File: Legend_tables.
Figure 1, comment 1: Replace "hatching" per "parturition, birth, or the newborn stage"
Reply: As mentioned above, we agree that the term "hatching" was replaced by "parturition". Please see the revised Figure 1 legend.
Figure 1, comment 2: Replace "tubules" per "lobules"
Reply: We thank the reviewer for this observation. We agree that the term "tubules" was not the most appropriate descriptor for the structure observed during this stage of testicular development. After revisiting the histological interpretation and relevant literature on poeciliid gonadal development, the terminology was revised to "sperm ducts", which more accurately reflects the developing ductal structures observed within each paired testicular structure. This interpretation is consistent with descriptions of early testicular development in Gambusia affinis (Koya et al., 2003). Accordingly, the figure legend and the corresponding text in the Results section were revised. Please see revised Figure 1 legend and Line 192 (Page 9) in the Revised Manuscript.
Reference: Koya Y, Fujita A, Niki F, Ishihara E, Miyama H. Sex differentiation and pubertal development of gonads in the viviparous mosquitofish, Gambusia affinis. Zool Sci. 2003; 20(10):1231–42. https://doi.org/10.2108/zsj.20.1231
Figure 3: Please see line 136 in the Material and Methods section.
Reply: The Figure 3 legend was revised to ensure consistency with the Materials and Methods section. Please see the revised Figure 3 legend and Lines 140-142 (Page 7).
Table 1, comment 1: Replace "lobar" per "lobular"
Reply: As mentioned above, we agree that the term "lobar" was not appropriate to describe the morphology observed at this developmental stage. Because the gonads are represented by two separate paired structures rather than true lobes, the terminology was revised throughout the manuscript and table to "paired testicular structures", ensuring consistency with the description used in the Results and Discussion sections. Please see the revised Table 1.
Table 1, comment 2: Please see lines 184-185 and 187 in the Results section.
Reply: We thank the reviewer for this observation. We agree that the original terminology could lead to confusion regarding the nature of the structures observed during early testicular development. Following the revisions made in the Results and Discussion sections, the descriptions in Table 1 were updated to maintain consistent terminology throughout the manuscript. References to "spermatic lobules" and "testes lobes" were replaced by descriptions referring to "sperm ducts" and paired testicular structures, respectively. In addition, this terminology was used for the subsequent fusion of paired testicular structures into a single testis. Please see the revised Table 1.
Table 1, comment 3: Replace "lobar" per "sacculated"
Reply: As mentioned above, we agree that the term "lobar" was not appropriate because it could suggest subdivisions of a single ovary rather than the paired ovarian structures observed during early development. In Stage 1, the term "paired ovarian structures" was adopted to describe the two independent ovarian structures, whereas the term "single saccular ovary" was used in later stages after fusion of the paired ovaries and formation of the ovarian cavity. Please see the response to the previous reviewer's comment (Page 10, Line 199).
Table 1, comment 4: Please see line 199 in the Results section.
Reply: We thank the reviewer for this observation. Consistent with the revisions introduced in the Results section, terminology referring to "gonadal lobes" and "ovarian lobes" was replaced throughout Table 1 by descriptions based on paired ovarian structures and their subsequent fusion into a single saccular ovary. This revision

maintains consistency between the descriptions of ovarian development modified in Results, Discussion, and Table 1. Please see the response to the previous reviewer's comment (Page 10, Line 199).
Table 1, comment 5: Please see line 213 in the Results section.
Reply: We thank the reviewer for this observation. Consistent with the revision of the ovarian terminology throughout the manuscript, the Stage 4 description in Table 1 was updated to refer to the ovary as a single sacculated structure with an enlarged ovarian cavity. Please see the response to the previous reviewer's comment (Page 10, Line 199).

Associate editor's decision after peer review (19/08/2026).
Dear Dr. Young:
Manuscript ID NI-2026-0038.R1 entitled "Gonadal development and anal fin morphogenesis in Cnesterodon decemmaculatus (Cyprinodontiformes, Poeciliidae)" which you submitted to the Neotropical Ichthyology, has been reviewed by editor.
The editor has recommended publication, but also suggest some minor revisions to your manuscript. Therefore, I invite you to respond to the editor' 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=1693b0bd65df4cb8953eca811d110629
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 19-Sep-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. Elizete Rizzo
Associate Editor, Neotropical Ichthyology
ictio@icb.ufmg.br

Author's Rebuttal Letter (07/09/2026).

Dear Editor,

Thank you very much for your positive evaluation of our manuscript and for your constructive comments to help improve its quality.

Editor's general comments:

The editor has recommended publication, but also suggest some minor revisions to your manuscript. Therefore, I invite you to respond to the editor' comments and revise your manuscript.

Reply: Please find below our point-by-point responses to all editor's comments.

Editor's specific comments:

Your work is very interesting, and the revised version (R1) has been improved in accordance with the reviewers' recommendations. However, a few points still require your attention:

1. Gonadal development stages are better described in Table 1 than in the Results text. However, since Table 1 is very long, it could be split into Table 1 (for testes) and Table 2 (for ovaries). Please include the histology images in the table, just as the fish size graphs were included. A sentence citing the tables can be added to the Results section, removing the text describing the gonadal development stages and thus eliminating redundancy.

Reply: We thank the Editor for this helpful suggestion. We agree that the detailed descriptions of gonadal development stages were largely redundant between the Results section and Table 1. Following the Editor's recommendation, the original table was divided into Table 1 (testicular development) and Table 2 (ovarian development). In addition, references to the corresponding histological micrographs were incorporated into each developmental stage, allowing direct association between the diagnostic morphological characteristics summarized in the tables and the representative histological images presented in Figures 1 and 2. The detailed stage-by-stage descriptions were removed from the Results section and replaced by concise summary paragraphs referring to Tables 1 and 2, thereby eliminating redundancy and improving readability. Please see the revised Tables 1 and 2 and the corresponding Results section in the Revised Manuscript (Pages 9-10, Lines 189-203).

2) In the Results and in the table, you describe five gonadal development stages: 1, 2, 3, 4, and 5. However, in Fig. 4, you refer to them as S1, S2, S3, S4, and S5. Please standardize the terminology used for the stages—either 1, 2...5 or S1, S2...S5. Although this is a minor detail, it could hinder the reader's understanding.

Reply: We agree that the use of both "Stage 1-5" and "S1-S5" to designate the same gonadal developmental stages could be confusing for readers. The terminology was standardized throughout the manuscript. The notation "Stage 1-5" is now used consistently in the Results section, Tables, and Figure 4. Please see the revised Figure 4 and corresponding text in the Revised Manuscript (Page 12, Lines 251, 260, 266 and 270).

3) As pointed out by the reviewers, the magnification of the histology images was low, especially regarding the organization of germ cells at each stage of testicular development. The insert in the testis figure (Fig. 1) was insufficient to show the organization of spermatozoa within the spermatozeugmata. Therefore, I suggest adding another column of high-magnification histology images (400x or 1000x)—particularly for males—to better illustrate germ cell development at each testicular stage. If you have the histology slides, obtaining new images and adding a column of higher-magnification images to better describe the testicular development stages should not be difficult. Note that the current image resolution is not high enough to clearly show the testicular germ cells.

Reply: We agree that additional high-magnification images will improve the visualization of gonadal development and the identification of diagnostic cellular features. Therefore,

representative high-magnification micrographs were incorporated into Figures 1 and 2. For the testis, additional images illustrating spermatocytes, spermatids, and spermatozeugmata were included to better characterize germ cell development and the organization of spermatozoa within spermatozeugmata. For the ovary, representative images of oogonia, primary oocytes, and cortical alveoli oocytes were incorporated to improve the visualization of oogenetic development. These additions provide a clearer histological characterization of the developmental stages, and complement the descriptions presented in the Results section and Tables 1 and 2. Please see the revised Figures 1 and 2 and their corresponding legends.

4) Please pay close attention to the figure captions and labels. For example, I did not find "T" and "IC" in the legend for Figure 1. Furthermore, the testicular parenchyma includes both germ cells and somatic cells. Therefore, "S: testicular somatic cells (parenchyma)" is incorrect.

Reply: We agree that the term “parenchyma” was not appropriate in this context because the testicular parenchyma includes both germinal and somatic cellular components. Accordingly, “parenchyma” was removed from the Figure 1 legend, and the label now refers specifically to testicular somatic cells.

In addition, we carefully rechecked all abbreviations and labels used in Figure 1 and its corresponding legend. The abbreviation referring to the interstitial compartment had already been revised during the previous round of review, where the original label “interstitial cells (Ic)” was replaced by “interstitial tissue (It)” to better reflect the histological structure identified. Similarly, the original label “seminiferous tubules (T)” was replaced by “sperm ducts (D)”. All figure labels and legend abbreviations were verified and updated where necessary to ensure full consistency between the figure and its legend. Please see the revised Figure legends.

Associate editor’s decision after peer review (09/09/2026).

Dear Dr. Young:

It is a pleasure to accept your manuscript entitled "Gonadal development and anal fin morphogenesis in Cnesterodon decemmaculatus (Cyprinodontiformes, Poeciliidae)" 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. Elizete Rizzo

Associate Editor, Neotropical Ichthyology

ictio@icb.ufmg.br

Neotropical Ichthyology

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