

Evolutionary history of Chinese cavefishes parallels paleogeoclimatic and river capture processes

Corresponding Author: Dr Jiang Zhou

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The manuscript presents a broad-scale phylogenetic and biogeographic analysis of cavefish diversification in southwestern China. However, the study shows substantial conceptual and methodological overlap with a recently published article in *Molecular Ecology* (DOI: 10.1111/mec.70038), which addresses a similar research question using comparable data types, analytical approaches and geographic focus. Both studies report similar timelines for cave colonization, diversification, and dispersal between major river basins (e.g., Yangtze and Pearl Rivers), and interpret their results within the same tectonic and climatic framework.

Although the present manuscript includes more taxa, it does not clearly articulate a distinct hypothesis or novel analytical perspective that differentiates it from previous work. The degree of overlap may limit its contribution to the field unless the authors clarify what new insights are provided beyond existing studies.

Reviewer #2

(Remarks to the Author)

The authors investigated the geologic history of the fantastic karst regions of southeast China through examining endemic cavefish phylogeography. The authors utilized phylogeographic and phylogenetic comparative methods (i.e., ancestral state reconstruction) to estimate the ages and evolutionary history of six fish families containing cavefishes. Overall, this was an enjoyable paper to read and will interest those concerned with caves, biogeography, phylogeography, geology, karst, and fish biology. I have one large methodology concern, and I have some suggestions to make the paper appeal to a wider audience.

Main Points

My main concern is the coding of the fish species as “cave” or not. For example, there are several *Sinocyclocheilus* species that in the supplement are coded as cave, despite being surface-dwelling. This is yet to mention the several species that are intermediate, spending some but not all their time in caves. If the authors would like to use “cave-obligate” vs. “non-cave-obligate”, this could be a simple solution. I would also encourage the authors to be very specific about how they define cave-obligate vs. not. Additionally, I am concerned that sister surface taxa were not included, which would skew several of the analyses— though I could be incorrect, I believe there are surface *Paranemachilus* that would potentially change some of the lineage divergence events. I would be happy to be incorrect here. But I would like either a justification for the coding scheme, or if there were inaccuracies, I would like the authors to revisit their coding and potentially rerun the analyses.

I believe the authors could expand on the introduction and discussion to put this paper in a greater context. There are a few biological variables that are not mentioned within the manuscript that I think potentially require attention. The authors do not mention groundwater or groundwater dispersal. Determining the history of groundwater availability on these timescales is currently unachievable, so I do not expect the authors to account for groundwater dispersal in the methods or anything to that effect, but I think I would like a mention that groundwater could play a role in the distribution of the cavefishes. The authors discuss the cavefishes dispersing across the Pearl and Yangtze Rivers; while not impossible, it may be unlikely. Additionally, ancestral state reconstructions most likely underestimate the number of independent colonization events, as it is possible that clades entirely made of cavefishes could have arisen from independent colonization events followed by surface ancestor extinction (e.g., *Sinocyclocheilus*). A mention of pre-adaptation being necessary for subterranean

colonization may also be warranted. I think some caveats such as these would be good to include in the paper. I think these would potentially fit into the discussion, but a mention of them in the introduction could work as well.

I would recommend a bit more in the introduction about the river basins in China, as those unfamiliar with the area might get lost. The Pearl River basin and its rivers are named in the materials and methods, but I think a mention in the introduction could allow the reader a better visualization. Additionally, the authors mention the need to examine cavefishes due to global climate change, but this needs detail—readers unfamiliar with cave biology may not understand the effect climate change will have on subterranean ecosystems. The conservation aspect of this paper is never mentioned again, though. I would recommend some expansion somewhere in the discussion. I believe it would be of benefit to explain somewhere the Stepwise and Late Orogeny hypotheses. I was unfamiliar with them and felt a little lost when they came up.

A paragraph at the beginning of the discussion that includes highlights of the discussion sections could be helpful to tie up the paper. Or possibly a conclusions paragraph. There are some ideas in the introduction that are not brought back up in the discussion that probably should be (e.g., global climate change impacting cave organisms).

Materials and Methods

Please see my concern about the coding of cavefishes. I cannot speak to the methodology of the dynamic meta-analysis of lineage diversification, but the phylogenetic and chronogram methodologies appear rigorous. Is there an International Animal Care and Use Committee protocol or something to that effect for the newly samples tissues?

Line-by-Line Edits

Line 37: Could the authors add “geologic” to “the evolutionary processes shaping natural landscapes...” or something similar? Reading this as an evolutionary biologist gave me some pause to understand what the authors were trying to say. It makes sense, I was just thinking about categorizing the evolutionary processes as non-biological.

Line 39–40: I strongly disagree with this statement and other cave biologists would as well. Caves are unequivocally not separated from the surface habitat; where caves are sometimes considered ecological islands is in comparison to one another. Caves are oftentimes not islands, though, and may be connected through conduits in the karst. I would recommend the authors reword this a little more carefully.

Line 48: This may be semantics, but I'm not sure that I would count this as a single phylogeny. Perhaps change this to something along the lines of greatest number of taxa? I'm not sure what the perfect way to say this is, but I'm not sure I'm comfortable with this being considered a single phylogeny.

Figure 1. This figure is incredibly busy. For someone not intimately familiar with the geography of southeast China, it took much too long to find the Pearl and Yangtze Rivers, and none of the other rivers are labeled. I really believe this figure needs to be broken up into two. One with the geology and geography and one with the fish and species richness. Additionally, please include the photo credits in the figure legend.

Version 2:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The authors have made substantial revisions to the manuscript and have carefully addressed most of the major concerns raised in the previous review. In particular, the differentiation from Mao et al. (2025), the clarification of ecological coding for cave-adapted fishes, and the harmonization of species numbers across different analyses have significantly improved the clarity and overall quality of the manuscript. However, a few issues still require further clarification.

1. The manuscript presents compelling temporal associations between cavefish diversification and major paleogeoclimatic and river-related events. However, in several places these associations are expressed in causal terms. The authors are encouraged to more clearly distinguish between correlation-based biological inference and direct causal interpretation, especially in the title, discussion and conclusions.
2. Line 362-368, section Estimation of divergence times, the manuscript states that divergence times were estimated in BEAST for “each dataset”, following the description of mitogenome sequencing, seven nuclear genes, and additional GenBank sequences. However, it is not entirely clear which sequence matrix (mitochondrial only, nuclear only, or concatenated mitochondrial + nuclear data) was actually used for the divergence time estimation. In addition, the manuscript specifies the clock model and tree prior, but the calibration strategy (e.g., fossil calibrations, secondary calibrations, or substitution rate priors) is not explicitly described in the main text.
3. The revised classification of troglomorphic versus non-troglomorphic taxa is now clearly described. However, the manuscript does not explicitly state whether the main conclusions are sensitive to this revised coding. A brief clarification on whether the overall patterns remain unchanged after re-coding would improve transparency.
4. Terms such as *in situ* appear frequently but are not always formatted consistently in italics throughout the manuscript. A final consistency check is suggested.

Reviewer #2

(Remarks to the Author)

I was one of the original reviewers of this manuscript. I am pleased with the changes that have been implemented and commend the authors. My last suggestion is that I would recommend moving the new paragraph comparing this manuscript to Mao et al. to later in the discussion instead of having it as the very first paragraph leading into the discussion.

Version 3:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The authors have carefully addressed all of my previous comments and revised the manuscript accordingly. I am satisfied with the revisions and have no further concerns.

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Response to the second Reviewer:

Dear Reviewer:

Thank you for your valuable comments on our manuscript, formerly titled "**Rise and Diversification of Cavefishes in the Karst Regions of Southwest China.**" The title has now been revised to "**Evolutionary trajectories and dynamics of cavefishes in the karst hotspot of southwest China.**" Your suggestions have been instrumental in refining our manuscript and will continue to guide our future research on cave fishes. We have thoroughly considered all of your comments and have made corresponding revisions to the text, with changes highlighted in **blue** in the original document. In addition, we apologize for the delayed response, which resulted from the need to incorporate updated datasets and conduct additional analyses to thoroughly address your comments. Below, we provide a point-by-point response to each of your remarks.

Overall comments: The authors investigated the geologic history of the fantastic karst regions of southeast China through examining endemic cavefish phylogeography. The authors utilized phylogeographic and phylogenetic comparative methods (i.e., ancestral state reconstruction) to estimate the ages and evolutionary history of six fish families containing cavefishes. Overall, this was an enjoyable paper to read and will interest those concerned with caves, biogeography, phylogeography, geology, karst, and fish biology. I have one large methodology concern, and I have some suggestions to make the paper appeal to a wider audience.

Response: Dear Reviewer, Thank you very much for your careful review of our manuscript and for your valuable comments. We were delighted to learn that you found our study "enjoyable to read" and that it will be of interest to researchers in fields such as speleology, biogeography, phylogeography, geology, karst science, and fish biology.

Your primary methodological concern and your suggestions for broadening the paper's appeal were crucial for improving the quality of our work. We have thoroughly revised and supplemented the original manuscript based on your feedback.

Regarding the specific questions and suggestions you raised, we have responded in detail one by one in the "Point-to-Point Response:" at the back of this document. We hope that our revisions and explanations adequately address your concerns and that the revised manuscript now meets the required standards for publication.

Once again, we sincerely appreciate the time and effort you have dedicated, and your contributions to enhancing the quality of our paper.

Point-to-Point Response:

Comment 1. My main concern is the coding of the fish species as "cave" or not. For example, there are several *Sinocyclocheilus* species that in the supplement are coded as cave, despite being surface-dwelling. This is yet to mention the several species that are intermediate, spending some but not all their time in caves. If the authors would like to use "cave-obligate" vs. "non-cave-obligate", this could be a simple solution. I would also encourage the authors to be very specific about how they define cave-obligate vs. not. Additionally, I am concerned that sister surface taxa were not included, which would skew several of the analyses— though I could be incorrect, I believe there are surface *Paranemachilus* that would potentially change some of the lineage divergence events. I would be happy to be incorrect here. But I would like either a justification for the coding scheme, or if there were inaccuracies, I would like the authors to revisit their coding and potentially rerun the analyses.

Response: Dear Reviewer, Thank you very much for your valuable comments regarding the coding of the "cave" attribute for the fish species. The issues you raised about the accuracy of ecological type classification for certain species (particularly within the genus *Sinocyclocheilus* and intermediate forms), as well as the potential impact of including sister taxa on the analysis results, are indeed crucial. We fully agree that clear definitions and precise coding are essential for the reliability of this study.

In response to your concerns, we have undertaken the following detailed revisions and provided additional clarifications:

(1) Clarification of Ecological Type Coding Criteria and Definitions. We have fully adopted your suggestion and explicitly implemented a dichotomous classification of ecological types in the revised manuscript (lines 46–49): "troglomorphic (stygobionts)" versus "non-troglomorphic (stygophiles)". This classification standard primarily references widely recognized authoritative literature in the field (e.g., DOI: 10.1111/j.1095-8649.2011.03066.x; DOI: 10.1016/B978-0-12-814124-3.00027-3).

Specifically: 1) Troglomorphic: Refers to species whose entire life cycle, or critical stages thereof, depends on the subterranean cave environment and which typically exhibit corresponding characteristic morphological adaptations (e.g., eye degeneration, loss of pigmentation); 2) **Non-troglomorphic:** Refers to species that utilize cave environments but are not strictly dependent on them and do not exhibit strong troglomorphic morphological features.

(2) Supplementation and Verification of Coding Basis. You correctly pointed out the complexity regarding the ecological types of some *Sinocyclocheilus* species, which may have surface populations. As a team long engaged in field investigations and taxonomic research on Chinese cave fishes (for instance, we have recently described several new cave fish species, such as *Sinocyclocheilus longicornus* [DOI: 10.3897/zookeys.1141.91501], two species in the genus *Karstsinnectes* [DOI: 10.11865/zs.2024204; DOI: 10.3897/zse.100.133964], and six new hypogean species of the genus *Triplophysa* [DOI: 10.3897/zookeys.1185.105499, etc.]), our initial coding integrated extensive field observations and literature records.

Nonetheless, to address your concerns and ensure transparency for all readers, we have systematically reviewed and updated the coding for all species. This review was based primarily on: 1) The original description literature of the species; 2) Authoritative regional monographs (e.g., Lan et al., 2013, Cave Fishes of Guangxi, China; Zhao & Zhang, Endemic fishes of *Sinocyclocheilus* (Cypriniformes: Cyprinidae) in China species diversity, cave adaptation, Systematics and Zoogeography); 3) The latest comprehensive

reviews (e.g., Ma et al., 2023, doi: 10.24272/j.issn.2095-8137.2022.484). All references supporting the ecological type coding have been meticulously added to Supplementary Table S1 for your and the readers' review and verification.

(3) Handling of the Genus *Paranemachilus* and Its Sister Taxa. Your attention to the genus *Paranemachilus* and its potential surface-dwelling sister taxa is highly professional. According to the latest taxonomic revisions (referencing DOI: 10.3897/zse.100.129520) and authoritative monographs (Lan et al., 2013, Cave Fishes of Guangxi, China;), species of the genus *Paranemachilus* are known to inhabit subterranean caves and connected waters near cave exits. Their ecological habits are classified as "non-troglophobic." Within our phylogenetic framework and taxonomic sampling, we have strived to include representative species within this genus. The current record did not identify the presence of unequivocal surface-dwelling sister taxa that would significantly alter the inferences of the lineage divergence events focused on in this paper. However, we fully agree that continuous updates to the analysis will be important as new taxa are discovered and phylogenetic relationships are further resolved.

Additionally, since the data required for our final analysis are the 95% confidence intervals of divergence times, the principle of the MDE method (which posits that diversification/dispersal events are more likely to occur when the corresponding divergence time intervals overlap) can, to some extent, mitigate the influence of closely related surface-dwelling sister species. Similar approaches have been widely used in previous studies, and their research outcomes have been published in high-quality journals (e.g., DOI: 10.1073/pnas.2207199119; DOI:10.1098/rspb.2021.1308; DOI: 10.1038/s41467-023-39555-6; DOI:10.1073/pnas.2322527121; DOI: 10.1093/nsr/nwaf157; DOI: 10.1038/ncomms12132; DOI: 10.1093/nsr/nwz035; DOI: 10.1093/nsr/nwaa263).

(4) Update of Data Analysis. Based on the aforementioned re-verification and coding of species ecological types, and considering the updated genetic data resulting from the recent publication of several new cave fish species by our team, we have re-calculated all relevant analyses in the manuscript using the revised coding scheme and the latest genetic dataset.

We believe these revisions directly address the core concerns you raised, significantly enhancing the accuracy and transparency of the species ecological type coding and ensuring the robustness of the analytical results. Once again, we sincerely thank you for your insightful comments, which have greatly helped us improve this manuscript.

Comment 2. I believe the authors could expand on the introduction and discussion to put this paper in a greater context. There are a few biological variables that are not mentioned within the manuscript that I think potentially require attention. The authors do not mention groundwater or groundwater dispersal. Determining the history of groundwater availability on these timescales is currently unachievable, so I do not expect the authors to account for groundwater dispersal in the methods or anything to that effect, but I think I would like a mention that groundwater could play a role in the distribution of the cavefishes. The authors discuss the cavefishes dispersing across the Pearl and Yangtze Rivers; while not impossible, it may be unlikely. Additionally, ancestral state reconstructions most likely underestimate the number of independent colonization events, as it is possible that clades entirely made of cavefishes could have arisen from independent colonization events followed by surface ancestor extinction (e.g., *Sinocyclocheilus*). A mention of pre-adaptation being necessary for subterranean colonization may also be warranted. I think some caveats such as these would be good to include in the paper. I think these would potentially fit into the discussion, but a mention of them in the introduction could work as well.

Response: We sincerely thank you for this insightful suggestion to broaden the context of our study. We have carefully considered the points raised and have revised the manuscript accordingly to provide a more comprehensive perspective.

(1) Role of Groundwater Dispersal. We fully agree that groundwater dynamics likely play a crucial role in cavefish distribution. In the revised manuscript, we have now explicitly acknowledged this factor. Specifically, we have added a statement in the [Discussion \(lines 159–161\)](#) noting that while direct historical reconstruction remains challenging, groundwater connections undoubtedly represent a significant potential pathway for dispersal that could influence observed distribution patterns.

(2) Inter-basin Dispersal between the Pearl and Yangtze Rivers. We appreciate your note on the likelihood of this dispersal route. Our interpretation was based on an ideal model framework. However, we have now strengthened this argument by citing relevant literature. We refer to a recent study on East Asian cyprinids (DOI: 10.1016/j.xinn.2023.100417) which provides evidence for faunal exchanges between these river basins, supporting the plausibility of such dispersal events for ancestral lineages.

(3) Underestimation of Independent Colonization Events. This is a critical point. We have incorporated this important caveat into the [Discussion \(lines 201–203\)](#). We now explicitly state that our Ancestral State Reconstruction might underestimate the number of independent cave colonization events. We discuss the possibility that clades now entirely composed of cavefish (using *Sinocyclocheilus* as an

example) could have originated from multiple independent colonizations followed by the extinction of their respective surface ancestors.

(4) Pre-adaptation for Subterranean Colonization. We thank you for suggesting this aspect. Following this advice, we have added a relevant passage in the manuscript **(lines 142–146)**. We discuss that the transition to cave life likely involved the evolution of innovative traits from ancestral precursors, which can be considered pre-adaptations or key innovations that facilitated subterranean colonization. This includes the regression of energy-costly structures and the enhancement of others, as highlighted in our study. In addition, we have also added relevant content in the preface **(see lines 47–49)**.

We believe these additions have significantly strengthened the manuscript by placing our findings within a broader evolutionary and ecological context and by acknowledging important limitations and complexities. Thank you for helping us improve the quality and impact of our work.

See lines 47–49:

Among vertebrates, the cavefishes are exemplary species exhibiting unique adaptations to subterranean life, the most notable being the degeneration or absence of pigmentation, eyes, and scales²¹.

See lines 142–146:

During this process, their ancestors innovatively evolved specialized morphological and genetic functions, exemplified by the regression of energy-consuming organs (e.g., eyes, scale, and pigmentation)^{38,39}, enhancement of energy-storage and sensory organs (e.g., the caudal adipose keel and the distinctive horns or humps in some cave-adapted *Sinocyclocheilus* species)³⁸⁻⁴⁰, and unique genetic regulatory mechanisms^{41,42}.

See lines 159–161:

Although the specific influences of cave and groundwater on cavefish diversification cannot be precisely reconstructed at present, their role in shaping dispersal, in situ diversification, and distribution patterns should not be underestimated.

See lines 201–203:

Finally, although our study reveals a significant coupling between paleoclimatic changes and bursts of cavefish diversification, incomplete sampling of both cave-adapted and surface-dwelling relatives, and extinction of surface lineages may influence the robustness of these findings.

Comment 3. I would recommend a bit more in the introduction about the river basins in China, as those

unfamiliar with the area might get lost. The Pearl River basin and its rivers are named in the materials and methods, but I think a mention in the introduction could allow the reader a better visualization. Additionally, the authors mention the need to examine cavefishes due to global climate change, but this needs detail—readers unfamiliar with cave biology may not understand the effect climate change will have on subterranean ecosystems. The conservation aspect of this paper is never mentioned again, though. I would recommend some expansion somewhere in the discussion. I believe it would be of benefit to explain somewhere the Stepwise and Late Orogeny hypotheses. I was unfamiliar with them and felt a little lost when they came up.

Response: We sincerely thank you for these constructive suggestions, which have helped us improve the clarity and contextual background of the manuscript. We have addressed each point as follows:

(1) Introduction of River Basins. Following your suggestion, we have added a brief description of the major river basins relevant to our study in the Introduction (lines 58–66). This provides readers, especially those unfamiliar with the geography of the region, with better spatial context for understanding the distribution of our study species. Furthermore, to enhance visual clarity, we have redrawn Figure 1 to more clearly illustrate the Pearl River basin and the distribution of its major tributaries.

(2) Impact of Climate Change on Subterranean Ecosystems. We agree that the link between climate change and threats to subterranean ecosystems needed elaboration. We have revised the relevant text in the Introduction (lines 55 – 57) to more clearly explain how global warming, through potential mechanisms like drought and reduced groundwater recharge, poses a significant threat to cavefish populations by degrading their highly specialized and fragile habitats.

(3) Conservation Implications. We thank you for highlighting the importance of the conservation aspect. While a detailed conservation plan falls outside the scope of this paper, we have now incorporated a statement in the Discussion (lines 181–184) that explicitly emphasizes the conservation urgency for cavefishes based on our findings and their inherent vulnerability.

(4) Explanation of the Stepwise and Late Orogeny Hypotheses. Hypotheses may not be familiar to all readers. To clarify this essential background, we have added a detailed legend to Figure 3 (specifically for panel 3H) that graphically and textually summarizes the key differences between the Stepwise and Late Orogeny hypotheses regarding the timing and pattern of Qinghai-Tibet Plateau uplift and their impacts on biotic diversification.

We believe these revisions have significantly improved the manuscript by providing better geographical and geological context, clarifying the conservation relevance, and making the study more accessible to a

broader audience. We are grateful for your valuable insights.

See lines 55 – 57:

In the context of global warming, potential droughts and diminished groundwater recharge in caves may irreversibly impact cavefish populations^{27,28}, underscoring the critical need to advance our understanding of subterranean biodiversity and its evolutionary origins.

See lines 58–66:

In China, cavefishes are primarily documented in the southwestern region (Fig. 1A), particularly in the karst areas of the upper Pearl River (including the Beipanjiang, Guijiang–Hejiang, Hongshui, Zuojiang–Youjiang, and Liujiang rivers), the upper Yangtze River (encompassing the Jinshajiang, Wujiang, and Yuanjiang rivers), and the Red River Basin²⁹ (Fig. 1B). However, despite being recognized as a biodiversity hotspot and conservation priority, the diversity of these subterranean fishes remains severely underestimated due to insufficient field surveys and systematic research (Table S1). Moreover, their evolutionary history is poorly understood. For instance, it remains unclear whether historically warm climates in geological time promoted diversification bursts or had the opposite effect. Therefore, integrated evolutionary analyses of multiple cavefish clades will help predict their response trajectories under global warming and inform adaptive conservation strategies and interventions.

See lines 181–184:

In other words, while long-term global temperature rise could theoretically promote cavefish diversification under idealized conditions, the current degradation of subterranean ecosystems and the need to balance conservation with sustainable human benefits render in situ protection of cavefishes urgently needed.

Comment 4. A paragraph at the beginning of the discussion that includes highlights of the discussion sections could be helpful to tie up the paper. Or possibly a conclusions paragraph. There are some ideas in the introduction that are not brought back up in the discussion that probably should be (e.g., global climate change impacting cave organisms).

Response: We thank you for the valuable suggestion to better synthesize the key findings of our study.

In response to the recommendation to include a paragraph at the beginning of the Discussion that highlights the main points, we have carefully reviewed the format of published papers in this journal. We noted that a standalone, introductory summary paragraph in the Discussion section is not a common convention.

Therefore, we have implemented the alternative approach you suggested. To enhance the structure and

clarity of the Discussion, we have added clear, summarizing topic sentences at the beginning of key paragraphs (see lines 162 and 254, and others). These sentences serve to guide the reader by immediately stating the core conclusion or purpose of the paragraph that follows, thereby effectively tying the different sections of our discussion together.

Regarding the point about revisiting the issue of "global climate change impacting cave organisms," which was raised in the Introduction, we have indeed addressed this in our response to Comment 3. The relevant text has been added to the Discussion to elaborate on this important aspect.

We believe these revisions have significantly improved the flow and coherence of the Discussion section by explicitly connecting our specific results to the broader themes introduced earlier in the paper.

Comment 5.

Materials and Methods

Please see my concern about the coding of cavefishes. I cannot speak to the methodology of the dynamic meta-analysis of lineage diversification, but the phylogenetic and chronogram methodologies appear rigorous. Is there an International Animal Care and Use Committee protocol or something to that effect for the newly samples tissues?

Response: We thank you for raising this important question regarding the ethical collection of the newly sampled tissues used in this study.

The tissue specimens utilized in this research were collected over an extended period, from 2010 to 2025. We would like to clarify that the vast majority of the cavefish species studied are not listed as protected animals under Chinese regulations; therefore, their collection did not require specific permits from relevant management authorities for most of the sampling period, particularly for the earlier years.

Nevertheless, strictly adhering to ethical principles in wildlife research, we deliberately avoided large-scale field collection throughout this project, especially for any potentially threatened species. A significant portion of the tissue samples was obtained through gifts and collaboration from fellow researchers, such as Prof. Li-Na Du and Mr. Jia-Hu Lan, who contributed materials from their own established collections.

In response to your suggestion, we have now included a dedicated "Ethics Statement" section in the final version of the manuscript (see lines 585–590, 325–327). This study was supported by the National Key R&D Program of China (Grant No. 2022YFF1301502, "Endangerment Processes and Mechanisms of Endangered Flagship Animals") and Strategic Priority Research Program of the Chinese Academy of

Sciences (CAS) (XDB31000000). For the majority of the species involved, samples were collected during biodiversity surveys conducted in southwestern China under the framework of this project. These surveys were carried out with the necessary permissions from the relevant local authorities, including forestry and agricultural bureaus.

We also fully recognize the conservation importance of the critically endangered species included in this study. To minimize potential impacts on these vulnerable populations, we deliberately designed our research approach through collaboration with Prof. Li-Na Du and Mr. Jia-Hu Lan, two respected experts in cave fish taxonomy and conservation. Within this cooperative framework, Prof. Du and Mr. Lan provided specimens of *Karstsinnectes longzhouensis*, *Troglonectes hechiensis*, *Karstsinnectes acridorsalis*, and *Troglonectes barbatus* in 2019, which had been previously collected during their authorized surveys following appropriate protocols. This methodology enabled us to acquire necessary research materials while avoiding additional sampling pressure on these threatened species. To ensure full transparency, we have explicitly acknowledged the contributions of both Prof. Du and Mr. Lan in the Acknowledgements section of our manuscript.

We believe these clarifications adequately address the ethical considerations you raised. Thank you again for your thorough review and valuable suggestions, which have undoubtedly improved the quality and completeness of our manuscript.

See lines 585–590:

Ethics Statement

All authors have approved the manuscript content, author list and order, and contribution statements. Sample collection complied with China's wildlife protection laws and regulations. The sampling procedures followed the National Standards of the People's Republic of China (GB/T 39760-2021; GB/T 35892-2018) and the Guidelines for the Care and Use of Laboratory Animals established by the Ethics Committee of Guizhou Normal University (Guiyang, Guizhou, China).

See lines 325–327:

All sampling procedures were performed with ethical consideration, whereby fin clips were collected non-invasively from fish anesthetized with Tricaine (150 mg/ml) that were subsequently revived and released, or were obtained only after natural death.

Comment 6.

Line-by-Line Edits

Line 37: Could the authors add “geologic” to “the evolutionary processes shaping natural landscapes...” or something similar? Reading this as an evolutionary biologist gave me some pause to understand what the authors were trying to say. It makes sense, I was just thinking about categorizing the evolutionary processes as non-biological.

Response: We thank you for this insightful suggestion. We agree that the original phrasing could be ambiguous from an evolutionary biology perspective. As suggested, we have revised the sentence in [Line 41](#) to explicitly acknowledge the role of geologic processes alongside evolutionary mechanisms. This revision more accurately frames the causal relationship between landscape evolution and biological diversification, and we believe it significantly improves the conceptual clarity of the introduction.

See lines 42–44:

The [geologic processes](#) shaping natural landscapes and their influence on evolutionary processes have profoundly influenced the diversification of the extant biota¹⁵⁻¹⁸, a concept now widely accepted in biogeography^{19,20}.

Comment 7.

Line 39–40: I strongly disagree with this statement and other cave biologists would as well. Caves are unequivocally not separated from the surface habitat; where caves are sometimes considered ecological islands is in comparison to one another. Caves are oftentimes not islands, though, and may be connected through conduits in the karst. I would recommend the authors reword this a little more carefully.

Response: We thank you for raising this important point regarding the conceptualization of caves as ecological islands. We fully agree that the original phrasing oversimplified the complex connectivity of cave systems. Following your recommendation, we have carefully reworded the statement to more accurately reflect the current understanding in cave ecology. The revised text ([see lines 45–47](#)) acknowledges that while caves can be conceptualized as ecological islands in specific comparative contexts, they remain hydrologically and ecologically connected through subsurface conduits within karst systems. This modification better aligns with the nuanced perspective shared by the cave biology community.

See lines 45–47:

[Caves can be considered as subterranean ecological islands with distinct characteristics, interconnected with surface environments through rivers or karst aquifers²¹](#), and paleogeoclimatic events have strongly influenced cave biodiversity¹³.

Comment 8.

Line 48: This may be semantics, but I'm not sure that I would count this as a single phylogeny. Perhaps change this to something along the lines of greatest number of taxa? I'm not sure what the perfect way to say this is, but I'm not sure I'm comfortable with this being considered a single phylogeny.

Response: We thank you for this pertinent observation regarding the terminology used to describe our phylogenetic reconstruction. We agree that referring to it as a "single phylogeny" could be misleading, as it indeed represents a comprehensive framework encompassing multiple taxonomic groups. Following your suggestion, we have revised the text in [lines 67–68](#) to more accurately reflect the nature of our dataset. The wording now emphasizes that our study incorporates the "greatest number of cavefish taxa" to date within its phylogenetic context, rather than characterizing it as a single phylogeny. This modification provides a more precise description of our methodological approach and phylogenetic scope.

See lines 67–68:

In this study, we compiled a species list of Chinese cavefishes and reconstructed the most extensive phylogeny to date, comprising 208 species in [ten phylogenetic trees](#), representing two orders, six families, and 22 genera.

Comment 9. Figure 1. This figure is incredibly busy. For someone not intimately familiar with the geography of southeast China, it took much too long to find the Pearl and Yangtze Rivers, and none of the other rivers are labeled. I really believe this figure needs to be broken up into two. One with the geology and geography and one with the fish and species richness. Additionally, please include the photo credits in the figure legend.

Response: We sincerely appreciate this valuable suggestion. In response, we have thoroughly revised and reconfigured **Figure 1** to enhance its clarity and interpretability. Specifically:

(1) Splitting the Original Figure. The original Figure 1 has been split into three distinct subfigures (now labeled as Figures 1A, 1B, and 1C) to separately present: Species richness and distribution of cavefish; Detailed visualization of river distribution; Geographical and geological context; Detailed structural and tectonic setting of the hotspot region.

(2) Improved Labeling and Accessibility. We have now clearly labeled the Pearl River and Yangtze River, along with other major rivers and geological features, to assist readers who may be less familiar with the regional geography.

(3) Inclusion of Photo Credits. All photo credits have been explicitly included in the revised figure legends, as requested.

(4) Additional Geological Context. Considering the unique geological history and fault systems characterizing the hotspot region, we have added a new panel (**Figure 1C**) to visualize key tectonic structures and their potential influence on cavefish diversification.

These changes are intended to make the figure set more intuitive, visually balanced, and informative for a broad audience. We believe the revised figures now more effectively communicate the spatial, geological, and biological patterns central to our study.

The revised Figure 1 and its legend are as follows.

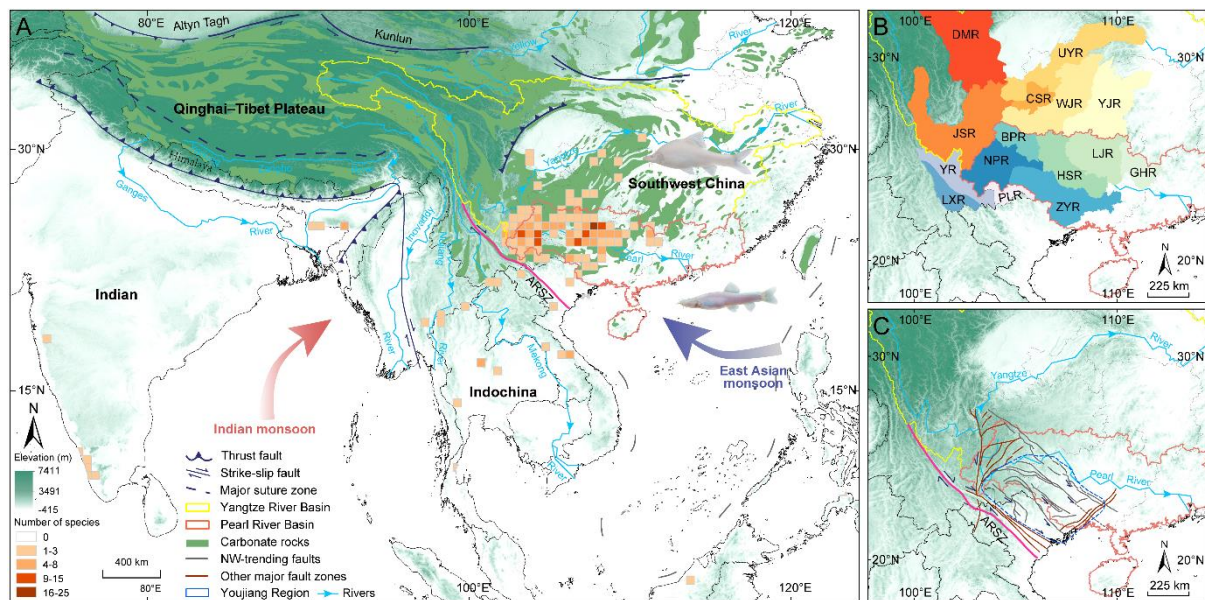


Fig. 1 Map of study area. **(A)** Spatial patterns of species diversity of cavefishes as measured by the number of species. The purple line denotes the Ailao Shan-Red River shear zone (ARSZ). The inset images show representative cavefish from southwestern China: *Sinocyclocheilus microphthalmus* (top) and *Troglonectes microphthalmus* (bottom). Photo by Mr. Jia-Hu Lan. The karst distribution was referenced from Bi⁸⁴. **(B)** Hydrology of the upper Yangtze and Pearl Rivers. Abbreviation: GHR, Guijiang-Hejiang River; LJR, Liujiang River; HSR, Hongshui River; ZYR, Zuojiang-Youjiang River, BPR, Beipanjiang River, NPR, Nanpanjiang River; UYR, upper Yangtze River; YJR, Yuanjiang River; WJR, Wujiang River; CSR, Chishui River; JSR, Jinshajiang River; DMR, Dadu-Minjiang River; PLR, Panlongjiang River; LXR, Lixianjiang River; YR, Yuan River. **(C)** Map of the Youjiang region showing the distribution of fault zones³⁷.

Response to the first Reviewer:

Dear Reviewer:

Thank you for your valuable comments on our manuscript, formerly titled "**Rise and Diversification of Cavefishes in the Karst Regions of Southwest China.**" The title has now been revised to "**Evolutionary history of Chinese cavefishes highlights how paleogeoclimatic and river capture processes shaped modern biodiversity.**" Your suggestions have been instrumental in refining our manuscript and will continue to guide our future research on cave fishes. We have thoroughly considered all of your comments and have made corresponding revisions to the text, with changes highlighted in **red** in the original document. In addition, we apologize for the delayed response, which resulted from the need to incorporate updated datasets and conduct additional analyses to thoroughly address your comments. Below, we provide a point-by-point response to each of your remarks.

Overall comment: The manuscript presents a broad-scale phylogenetic and biogeographic analysis of cavefish diversification in southwestern China. However, the study shows substantial conceptual and methodological overlap with a recently published article in *Molecular Ecology* (DOI: 10.1111/mec. 70038), which addresses a similar research question using comparable data types, analytical approaches and geographic focus. Both studies report similar timelines for cave colonization, diversification, and dispersal between major river basins (e.g., Yangtze and Pearl Rivers), and interpret their results within the same tectonic and climatic framework.

Although the present manuscript includes more taxa, it does not clearly articulate a distinct hypothesis or novel analytical perspective that differentiates it from previous work. The degree of overlap may limit its contribution to the field unless the authors clarify what new insights are provided beyond existing studies.

Response: Thank you for your thoughtful comments and for raising the important point regarding potential overlap with the recently published study (DOI: 10.1111/mec.70038). We appreciate the opportunity to clarify how our work differs from and builds upon existing research. Below, we provide a detailed point-by-point response to address your concerns.

(1) Taxonomic Scope: The study referenced (DOI: 10.1111/mec.70038) focuses exclusively on the genus *Sinocyclocheilus*. In contrast, our study encompasses a much broader taxonomic range, including representative species from 10 major clades of Chinese cavefishes. While there is some taxonomic overlap, our expanded sampling provides a more comprehensive and representative dataset, allowing us to explore large-scale evolutionary patterns across multiple clades.

(2) Data Type: The referenced study employs reduced-representation genomic data (RADseq). In our work, we utilize a multi-locus dataset by integrating available genetic markers for the 10 cavefish clades. This approach, based on sequence data, has been widely applied and validated in macroevolutionary and biogeographical studies, as

evidenced by its use in high-impact journals (e.g., DOI: 10.1073/pnas.2207199119; DOI:10.1098/rspb.2021.1308; DOI: 10.1038/s41467-023-39555-6; DOI:10.1073/pnas.2322527121; DOI: 10.1093/nsr/nwaf157; DOI: 10.1038/ncomms12132; DOI: 10.1093/nsr/nwz035; DOI: 10.1093/nsr/nwaa263). The difference in data type is driven by distinct research objectives and allows us to address questions at a broader phylogenetic scale.

(3) Analytical Methods: While both studies employ phylogenetic reconstruction—a standard and essential methodology in evolutionary biology—our analytical framework extends significantly beyond that of the referenced paper. The cited study primarily investigates mito-nuclear discordance and population genomics within *Sinocyclocheilus*, with an emphasis on introgression and lineage-specific diversification dynamics.

In contrast, our study introduces a novel, meta-analysis-like approach that: 1) Reconstructs ancestral habitats and biogeographical histories across multiple cavefish groups; 2) Examines temporal dynamics of species diversification and evaluates patterns across different river basins (e.g., origins, acceleration, and peak diversity); 3) Quantifies biotic interchange between the Yangtze and Pearl River basins, revealing for the first time the directionality and asymmetry of historical dispersal events. These analyses provide new insights into the historical connectivity between major river systems, which were not explored in the previous study.

(4) Geographic Focus: It is true that both studies concentrate on the karst regions of southwestern China, as this area represents a biodiversity hotspot for cavefishes. However, our study expands the biogeographical scope by explicitly comparing evolutionary patterns across multiple river basins and exploring large-scale regional dynamics.

(5) Research Questions and Hypotheses: The referenced article focuses on explaining phylogenetic conflict and assessing the contributions of geological, climatic, and biotic factors to the radiation of *Sinocyclocheilus*. Our study, however, aims to identify general evolutionary patterns common to multiple cavefish lineages. By integrating data from diverse groups, we propose a new three-stage model of cavefish evolution linked to karst development—a hypothesis not presented in previous work. Furthermore, we provide the first evidence of sequential and highly asymmetric dispersal between the Yangtze and Pearl Rivers, shedding light on the historical connectivity between these basins. This is not mentioned in the references.

In summary, while both studies address cavefishes evolution in southwestern China, our research differs in taxonomic breadth, data type, analytical methodology, and conceptual framework. From our perspective, we do not entirely dismiss the scientific value of the referenced study. On the contrary, the two studies can be seen as complementary in certain aspects. With this in mind, we have cited the scientific conclusions of the referenced paper in the Discussion section and have also pointed out the limitations of our own research (See lines 201 – 210, 292 – 294). We believe that the expanded scope and novel hypotheses presented in our manuscript offer significant and distinct contributions to the field.

We have carefully considered your comments and have revised the manuscript accordingly to better highlight these distinctions. Thank you once again for your valuable feedback.

Sincerely,

Tao Luo

Response to the Editor:

Dear Editor:

Thank you for your valuable comments on our manuscript, formerly titled "**Rise and Diversification of Cavefishes in the Karst Regions of Southwest China.**" The title has now been revised to "**Evolutionary history of Chinese cavefishes highlights how paleogeoclimatic and river capture processes shaped modern biodiversity.**" Your suggestions have been instrumental in refining our manuscript and will continue to guide our future research on cave fishes. We have thoroughly considered all of your comments and have made corresponding revisions to the text, with changes highlighted in **green** in the original document. In addition, we apologize for the delayed response, which resulted from the need to incorporate updated datasets and conduct additional analyses to thoroughly address your comments. Below, we provide a point-by-point response to each of your remarks.

Point-to-Point Response:

Comment 1. (1) Directly compare and discuss the novelty of your findings in light of the recent study from Mao et al (DOI: 10.1111/mec. 70038), as noted by Reviewer #1.

Response: We hope for your understanding regarding our manuscript. We have thoroughly and meticulously reviewed the research paper mentioned by the first reviewer. In fact, that study is based on entirely different research premises and draws distinct conclusions. The referenced paper (DOI: 10.1111/mec), which employs population genetics methods, primarily investigates the causes of mito-nuclear discordance in the cavefish genus *Sinocyclocheilus*, attributing it to gene flow between species. It also assesses population dynamics and morphological evolution during cave adaptation. From our perspective, we do not entirely dismiss the scientific value of the referenced study. On the contrary, the two studies can be seen as complementary in certain aspects. With this in mind, we have cited the scientific conclusions of the referenced paper in the Discussion section and have also pointed out the limitations of our own research (**See lines 203–212, 294–296**). In the responses below, we provide a detailed explanation of the differences between the two studies.

(1) Taxonomic Scope: The study referenced (DOI: 10.1111/mec.70038) focuses exclusively on the genus *Sinocyclocheilus*. In contrast, our study encompasses a much broader taxonomic range, including representative species from 10 major clades of Chinese cavefishes. While there is some taxonomic overlap, our expanded sampling provides a more comprehensive and representative dataset, allowing us to explore large-scale evolutionary patterns across multiple clades.

(2) Data Type: The referenced study employs reduced-representation genomic data (RADseq). In our work, we utilize a multi-locus dataset by integrating available genetic markers for the 10 cavefish clades. This approach, based on sequence data, has been widely applied and validated in macroevolutionary and biogeographical studies, as evidenced by its use in high-impact journals (e.g., DOI: 10.1073/pnas.2207199119; DOI:10.1098/rspb.2021.1308; DOI: 10.1038/s41467-023-39555-6; DOI:10.1073/pnas.2322527121; DOI: 10.1093/nsr/nwaf157; DOI: 10.1038/ncomms12132; DOI: 10.1093/nsr/nwz035; DOI: 10.1093/nsr/nwaa263). The difference in data type is driven by distinct research objectives and allows us to address questions at a broader phylogenetic scale.

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(4) Geographic Focus: It is true that both studies concentrate on the karst regions of southwestern China, as this area represents a biodiversity hotspot for cavefishes. However, our study expands the biogeographical scope by explicitly comparing evolutionary patterns across multiple river basins and exploring large-scale regional dynamics.

(5) Research Questions and Hypotheses: The referenced article focuses on explaining phylogenetic conflict and assessing the contributions of geological, climatic, and biotic factors to the radiation of *Sinocyclocheilus*. Our study, however, aims to identify general evolutionary patterns common to multiple cavefish lineages. By integrating data from diverse groups, we propose a new three-stage model of cavefish evolution linked to karst development—a hypothesis not presented in previous work. Furthermore, we provide the first evidence of sequential and highly asymmetric dispersal between the Yangtze and Pearl

Rivers, shedding light on the historical connectivity between these basins. This is not mentioned in the references.

In summary, while both studies address cavefishes evolution in southwestern China, our research differs in taxonomic breadth, data type, analytical methodology, and conceptual framework. We believe that the expanded scope and novel hypotheses presented in our manuscript offer significant and distinct contributions to the field.

We have carefully considered your comments and have revised the manuscript accordingly to better highlight these distinctions. Thank you once again for your valuable feedback.

Comment 2. (2) Address Reviewer #2's concerns regarding how cavefish are coded (which would involve rerunning these analyses), and include further comparisons to relevant surface taxa. We agree with the reviewer that it may be more appropriate to use the "cave-obligate" terminology throughout the text, rather than "cavefish" in many cases.

Response: Dear Editor, Thank you very much for your and second Reviewer valuable comments regarding the coding of the "cave" attribute for the fish species. As mentioned by the second reviewer, the concerns regarding the accuracy of ecological type classification for certain species (particularly in the genus *Sinocyclocheilus* and intermediate forms), along with the potential impact of including sister taxon pairs on the results, are indeed critical. We fully agree that clear definitions and precise coding are essential for the reliability of this study.

In response to your concerns, we have undertaken the following detailed revisions and provided additional clarifications:

(1) Clarification of Ecological Type Coding Criteria and Definitions. We have fully adopted your suggestion and explicitly implemented a dichotomous classification of ecological types in the revised manuscript (lines 46–49): "troglomorphic (stygobionts)" versus "non-troglomorphic (stygophiles)". This classification standard primarily references widely recognized authoritative literature in the field (e.g., DOI: 10.1111/j.1095-8649.2011.03066.x; DOI: 10.1016/B978-0-12-814124-3.00027-3).

Specifically: 1) **Troglomorphic:** Refers to species whose entire life cycle, or critical stages thereof, depends on the subterranean cave environment and which typically exhibit corresponding characteristic morphological adaptations (e.g., eye degeneration, loss of pigmentation); 2) **Non-troglomorphic:** Refers to

species that utilize cave environments but are not strictly dependent on them and do not exhibit strong troglomorphic morphological features. Therefore, in accordance with established practices in cavefish research, we have classified cavefish into two ecological types—"troglomorphic" and "non-troglomorphic"—and will adhere to this classification in subsequent studies.

(2) Supplementation and Verification of Coding Basis. You correctly pointed out the complexity regarding the ecological types of some *Sinocyclocheilus* species, which may have surface populations. As a team long engaged in field investigations and taxonomic research on Chinese cave fishes (for instance, we have recently described several new cave fish species, such as *Sinocyclocheilus longicornus* [DOI: 10.3897/zookeys.1141.91501], two species in the genus *Karstsinnectes* [DOI: 10.11865/zs.2024204; DOI: 10.3897/zse.100.133964], and six new hypogean species of the genus *Triplophysa* [DOI: 10.3897/zookeys.1185.105499, etc.]), our initial coding integrated extensive field observations and literature records.

Nonetheless, to address your concerns and ensure transparency for all readers, we have systematically reviewed and updated the coding for all species. This review was based primarily on: 1) The original description literature of the species; 2) Authoritative regional monographs (e.g., Lan et al., 2013, Cave Fishes of Guangxi, China; Zhao & Zhang, Endemic fishes of *Sinocyclocheilus* (Cypriniformes: Cyprinidae) in China species diversity, cave adaptation, Systematics and Zoogeography); 3) The latest comprehensive reviews (e.g., Ma et al., 2023, doi: 10.24272/j.issn.2095-8137.2022.484). All references supporting the ecological type coding have been meticulously added to **Supplementary Table S1** for your and the readers' review and verification.

Upon completing the new coding process, we incorporated recently published new species of cavefish and subsequently re-conducted the relevant analyses.

Comment 3. (3) From an editorial perspective, it seems that there are inconsistencies throughout the text about how many species are involved in this analysis. The Abstract states "We analyzed 525 species from 10 clades to reconstruct their diversification history", though other sections list far smaller numbers, as in:

Line 56 "A literature search obtained data for ~190 species of cave fishes"

Line 228: "343 occurrence sites for 205 species were included in the analysis"

Line 232: "In total, 85 tissue samples from 12 genera across three families were collected"

Please carefully review the text and clarify how many distinct species have been included as part of this work. It may also be helpful to include a supplementary table or data file specifying the number of species and list which ones were included in each analysis.

Response: Dear Editor, thank you very much for your thorough review and for pointing out the inconsistencies in the text regarding the number of species included in our analyses. We sincerely appreciate the opportunity to clarify these points.

We have carefully re-examined and updated the relevant data throughout the manuscript. The primary source of confusion stemmed from the different contexts in which species numbers were mentioned—referring respectively to the total number of species considered for distribution mapping, the subset used in downstream analyses, and the specific samples newly sequenced for this study.

Please find below a point-by-point response to the specific lines you highlighted:

(1) Regarding the statement in the Abstract: "We analyzed 525 species from 10 clades to reconstruct their diversification history"

Clarification: This number (525) refers to the total number of species encompassing both cave-dwelling species and their closely related surface-dwelling relatives across the 10 clades studied. This broad set was used as the initial framework for understanding large-scale diversification patterns. To avoid confusion, we have revised this sentence in the Abstract (lines 14-16) to more precisely reflect the scope of the analysis.

(2) Regarding Line 56: "A literature search obtained data for ~190 species of cave fishes"

Clarification & Correction: Based on our updated and comprehensive species inventory, the current number of recognized cavefish species within China is 199. We have corrected this number in the manuscript accordingly.

(3) Regarding Line 228: "343 occurrence sites for 205 species were included in the analysis"

Clarification & Correction: This section pertains to the data used for mapping species distributions

and diversity patterns. Our updated inventory lists 199 cavefish species within China and an additional 22 species from adjacent regions in Asia. To visualize the broader distribution pattern and highlight Southwest China as a diversity hotspot, we included these 221 species. For this combined set, we gathered a total of 370 occurrence sites.

Furthermore, for the subsequent analyses on diversity patterns and evolutionary history, we utilized a subset of 183 species. This represents 92% of the 199 known Chinese cavefish species, which we believe is a robust and comprehensive representation given the challenges in specimen collection.

(4) Regarding Line 232: “In total, 85 tissue samples from 12 genera across three families were collected”

Clarification: This figure is correct and refers specifically to the new genetic samples we collected, amplified, and sequenced for this study. These samples were subsequently used for the phylogenetic, divergence time, and biogeographical analyses presented in the paper.

To ensure full transparency and reproducibility: All relevant sequence data have been deposited in a public repository (DRYAD, <https://doi.org/10.5061/dryad.ht76hdrqx>; Zenodo, <https://doi.org/10.5281/zenodo.17578805>).

As you helpfully suggested, we have now explicitly stated the final number of species used in each specific analysis within the main text.

We have also provided detailed supplementary tables (**Tables S3–S12**) that list all species included in the various analyses, specifying which ones were used for distribution mapping, phylogenetic reconstruction, and other downstream analyses.

We hope these clarifications and the corresponding revisions in the manuscript satisfactorily address your concerns. Thank you again for your valuable feedback, which has significantly improved the clarity of our work.

Finally, we have added a dedicated "Conclusions" (**See lines 297–314**) section to provide a comprehensive summary of the article. This section synthesizes the key findings of our study, discusses their broader implications, and offers potential directions for future research.

Sincerely,

[Tao Luo/Corresponding Author Jiang Zhou]

On behalf of all co-authors

Response to the first Reviewer:

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Tao Luo

Overall comment: The manuscript presents a broad-scale phylogenetic and biogeographic analysis of cavefish diversification in southwestern China. However, the study shows substantial conceptual and methodological overlap with a recently published article in *Molecular Ecology* (DOI: 10.1111/mec. 70038), which addresses a similar research question using comparable data types, analytical approaches and geographic focus. Both studies report similar timelines for cave colonization, diversification, and dispersal between major river basins (e.g., Yangtze and Pearl Rivers), and interpret their results within the same tectonic and climatic framework.

Although the present manuscript includes more taxa, it does not clearly articulate a distinct hypothesis or novel analytical perspective that differentiates it from previous work. The degree of overlap may limit its contribution to the field unless the authors clarify what new insights are provided beyond existing studies.

Response: Thank you for your thoughtful comments and for raising the important point regarding potential overlap with the recently published study (DOI: 10.1111/mec.70038). We appreciate the opportunity to clarify how our work differs from and builds upon existing research. Below, we provide a detailed point-by-point response to address your concerns.

(1) Taxonomic Scope: The study referenced (DOI: 10.1111/mec.70038) focuses exclusively on the genus *Sinocyclocheilus*. In contrast, our study encompasses a much broader taxonomic range, including representative species from 10 major clades of Chinese cavefishes. While there is some taxonomic overlap, our expanded sampling provides a more comprehensive and representative dataset, allowing us to explore large-scale evolutionary patterns across multiple clades.

(2) Data Type: The referenced study employs reduced-representation genomic data (RADseq). In our work, we utilize a multi-locus dataset by integrating available genetic markers for the 10 cavefish clades. This approach, based on sequence data, has been widely applied and validated in macroevolutionary and biogeographical studies, as evidenced by its use in high-impact journals (e.g., DOI: 10.1073/pnas.2207199119; DOI:10.1098/rspb.2021.1308; DOI: 10.1038/s41467-023-39555-6; DOI:10.1073/pnas.2322527121; DOI: 10.1093/nsr/nwaf157; DOI: 10.1038/ncomms12132; DOI: 10.1093/nsr/nwz035; DOI: 10.1093/nsr/nwaa263). The difference in data type is driven by distinct research objectives and allows us to address questions at a broader phylogenetic scale.

(3) Analytical Methods: While both studies employ phylogenetic reconstruction—a standard and essential methodology in evolutionary biology—our analytical framework extends significantly beyond that of the referenced paper. The cited study primarily investigates mito-nuclear discordance and population genomics within *Sinocyclocheilus*, with an emphasis on introgression and lineage-specific diversification dynamics.

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(4) Geographic Focus: It is true that both studies concentrate on the karst regions of southwestern China, as this area represents a biodiversity hotspot for cavefishes. However, our study expands the biogeographical scope by explicitly comparing evolutionary patterns across multiple river basins and exploring large-scale regional dynamics.

(5) Research Questions and Hypotheses: The referenced article focuses on explaining phylogenetic conflict and assessing the contributions of geological, climatic, and biotic factors to the radiation of *Sinocyclocheilus*. Our study, however, aims to identify general evolutionary patterns common to multiple cavefish lineages. By integrating data from diverse groups, we propose a new three-stage model of cavefish evolution linked to karst development—a hypothesis not presented in previous work. Furthermore, we provide the first evidence of sequential and highly asymmetric dispersal between the Yangtze and Pearl Rivers, shedding light on the historical connectivity between these basins. This is not mentioned in the references.

In summary, while both studies address cavefishes evolution in southwestern China, our research differs in taxonomic breadth, data type, analytical methodology, and conceptual framework. From our perspective, we do not entirely dismiss the scientific value of the referenced study. On the contrary, the two studies can be seen as complementary in certain aspects. With this in mind, we have cited the scientific conclusions of the referenced paper

in the Discussion section and have also pointed out the limitations of our own research (See lines 230 – 239, 321 – 323). We believe that the expanded scope and novel hypotheses presented in our manuscript offer significant and distinct contributions to the field.

Based on the above considerations and in response to your suggestions, we have revised the manuscript to highlight both the key strengths of our study and its distinctions from the referenced work. !Specifically, we have added a new paragraph in the Discussion section (See lines 130 – 155) to elaborate on these aspects. Additionally, we have referenced the relevant literature in the Introduction (See lines 63 – 67) to provide appropriate context.

Revised Text

See lines 63–67:

Although several studies based on incomplete sampling and reduced-representation genomic data have revealed the important roles of paleoclimatic history and gene flow in driving the speciation and radiation of *Sinocyclocheilus*³⁰, their limited taxonomic scope still precludes a comprehensive understanding of the evolutionary history of Chinese cavefishes and the associated geomorphological evolution—such as river formation and connectivity.

See lines 131–155:

Divergent perspectives on the evolutionary history of Chinese cavefishes: A comparison with recent study

Cavefishes serve as excellent models for studying biogeographic patterns, with substantial evidence demonstrating their utility in revealing how paleogeographic events influenced speciation and adaptive evolution in subterranean ecosystems^{14,25,26,30-32}. However, focusing on a single taxonomic group provides limited capacity to fully unravel how paleoclimatic and geological processes influenced biodiversity evolution across broader macroevolutionary scales. A recent study by Mao et al.³⁰ applied advanced genomic approaches to illustrate the critical role of paleoclimatic history and gene flow in driving speciation and radiation in *Sinocyclocheilus*. While both our study and Mao et al.³⁰ confirm a strong association between paleoclimatic events and cavefish evolutionary history, key differences distinguish the two works. First, in terms of taxonomic sampling, Mao et al.³⁰ included 50 taxa, whereas our study incorporated 183 species (~92% of known extant species) by integrating newly sequenced and publicly available NCBI data—a 3.7-fold larger sample size that offers a more comprehensive perspective on the origins of China's cavefish diversity hotspot. Second, while Mao et al.³⁰ used reduced-representation genomic data and reported extensive gene flow leading to phylogenetic discordance in *Sinocyclocheilus*, our study employed mitochondrial markers. Although similar discordance may occur at major nodes, multiple studies have validated the utility of mitochondrial data for inferring diversification history^{13,19,20,33-37}, particularly when divergence time intervals overlap—providing stronger support for reconstructing historical events¹⁹, in contrast to the single-node calibration approach adopted by Mao et al.³⁰. Finally, beyond examining the influence of paleoclimatic drivers such as Qinghai–Tibet Plateau uplift and Asian monsoon evolution, our study also incorporated the effects of Indochina block extrusion, karst development, and river capture processes (see subsequent Discussion). By quantifying *in situ* diversification and dispersal events across basins, we reconstructed the evolutionary history of river drainage networks—aspects not fully explored in Mao et al. In summary, although methodological and analytical differences exist between the two studies, they offer complementary insights into the evolutionary history of Chinese cavefishes. Biological events not fully resolved in our study, for example, may be further clarified by the cross-species gene flow patterns identified

by Mao et al. Future phylogenomic studies with expanded taxon sampling will further enhance our understanding of speciation and diversification patterns in Chinese cavefishes.

See lines 230 – 239:

Finally, although our study reveals a significant coupling between paleoclimatic changes and bursts of cavefish diversification, incomplete sampling of both cave-adapted and surface-dwelling relatives, and [extinction of surface lineages](#) may influence the robustness of these findings. Additionally, the contribution of genetic factors such as gene flow to cavefish diversification remains incompletely understood—a key limitation of this work. For instance, previous studies have demonstrated that gene flow facilitated adaptive radiations in primates^{64,65}, voles⁶⁶, bats⁶⁷, QTP fishes⁶⁸, and cichlids⁶⁹. While phylogenomic analyses of *Sinocyclocheilus*³⁰ have revealed how gene flow and paleoclimatic shifts jointly promoted its radiation, it remains unclear whether similar mechanisms operate in other cavefish groups or what genomic features characterize introgressed regions. Thus, future phylogenomic studies encompassing broader taxonomic groups will be essential to advance understanding of the origins, diversification bursts, and adaptive radiation of the cavefish hotspot in the karst regions of southwestern China.

See lines 321 – 323:

However, the limited distribution of cavefishes across these two basins may affect the robustness of our findings. Future studies incorporating more diverse lineages would help strengthen and extend support for our conclusions.

Response to the Editor:**Dear Editor:**

Thank you for your valuable comments on our manuscript, formerly titled "**Rise and Diversification of Cavefishes in the Karst Regions of Southwest China.**" The title has now been revised to "**Evolutionary history of Chinese cavefishes highlights how paleogeoclimatic and river capture processes shaped modern biodiversity.**" Your suggestions have been instrumental in refining our manuscript and will continue to guide our future research on cave fishes. We have thoroughly considered all of your comments and have made corresponding revisions to the text, with changes highlighted in **green** in the original document. In addition, we apologize for the delayed response, which resulted from the need to incorporate updated datasets and conduct additional analyses to thoroughly address your comments. Below, we provide a point-by-point response to each of your remarks.

Sincerely,

Tao Luo

On behalf of all co-authors

Point-to-Point Response:

Comment 1. (1) Directly compare and discuss the novelty of your findings in light of the recent study from Mao et al (DOI: 10.1111/mec. 70038), as noted by Reviewer #1.

Response: We hope for your understanding regarding our manuscript. We have thoroughly and meticulously reviewed the research paper mentioned by the first reviewer. In fact, that study is based on entirely different research premises and draws distinct conclusions. The referenced paper (DOI: 10.1111/mec), which employs population genetics methods, primarily investigates the causes of mito-nuclear discordance in the cavefish genus *Sinocyclocheilus*, attributing it to gene flow between species. It also assesses population dynamics and morphological evolution during cave adaptation. From our perspective, we do not entirely dismiss the scientific value of the referenced study. On the contrary, the two studies can be seen as complementary in certain aspects. With this in mind, we have cited the scientific conclusions of the referenced paper in the Discussion section and have also pointed out the limitations of our own research (**See lines 203–212, 321–323**). In the responses below, we provide a detailed explanation of the differences between the two studies.

(1) Taxonomic Scope: The study referenced (DOI: 10.1111/mec.70038) focuses exclusively on the genus *Sinocyclocheilus*. In contrast, our study encompasses a much broader taxonomic range, including representative species from 10 major clades of Chinese cavefishes. While there is some taxonomic overlap, our expanded sampling provides a more comprehensive and representative dataset, allowing us to explore large-scale evolutionary patterns across multiple clades.

(2) Data Type: The referenced study employs reduced-representation genomic data (RADseq). In our work, we utilize a multi-locus dataset by integrating available genetic markers for the 10 cavefish clades. This approach, based on sequence data, has been widely applied and validated in macroevolutionary and biogeographical studies, as evidenced by its use in high-impact journals (e.g., DOI: 10.1073/pnas.2207199119; DOI:10.1098/rspb.2021.1308; DOI: 10.1038/s41467-023-39555-6; DOI:10.1073/pnas.2322527121; DOI: 10.1093/nsr/nwaf157; DOI: 10.1038/ncomms12132; DOI: 10.1093/nsr/nwz035; DOI: 10.1093/nsr/nwaa263). The difference in data type is driven by distinct research objectives and allows us to address questions at a broader phylogenetic scale.

(3) Analytical Methods: While both studies employ phylogenetic reconstruction—a standard and essential methodology in evolutionary biology—our analytical framework extends significantly beyond that of the referenced paper. The cited study primarily investigates mito-nuclear discordance and population genomics within *Sinocyclocheilus*, with an emphasis on introgression and lineage-specific diversification dynamics.

In contrast, our study introduces a novel, meta-analysis-like approach that: 1) Reconstructs ancestral habitats and biogeographical histories across multiple cavefish groups; 2) Examines temporal dynamics of species diversification and evaluates patterns across different river basins (e.g., origins, acceleration, and peak diversity); 3) Quantifies biotic interchange between the Yangtze and Pearl River basins, revealing for the first time the directionality and asymmetry of historical dispersal events. These analyses provide new insights into the historical connectivity between major river systems, which were not explored in the previous study.

(4) Geographic Focus: It is true that both studies concentrate on the karst regions of southwestern China, as this area represents a biodiversity hotspot for cavefishes. However, our study expands the biogeographical scope by explicitly comparing evolutionary patterns across multiple river basins and exploring large-scale regional dynamics.

(5) Research Questions and Hypotheses: The referenced article focuses on explaining phylogenetic conflict and assessing the contributions of geological, climatic, and biotic factors to the radiation of *Sinocyclocheilus*. Our study, however, aims to identify general evolutionary patterns common to multiple cavefish lineages. By integrating data from diverse groups, we propose a new three-stage model of cavefish evolution linked to karst development—a hypothesis not presented in previous work. Furthermore, we provide the first evidence of sequential and highly asymmetric dispersal between the Yangtze and Pearl Rivers, shedding light on the historical connectivity between these basins. This is not mentioned in the references.

In summary, while both studies address cavefishes evolution in southwestern China, our research differs in taxonomic breadth, data type, analytical methodology, and conceptual framework. We believe that the expanded scope and novel hypotheses presented in our manuscript offer significant and distinct contributions to the field.

We have carefully considered your comments and have revised the manuscript accordingly to better highlight these distinctions. Thank you once again for your valuable feedback.

For detailed responses, please refer to the reply to the first reviewer.

The revised text is as follows:

See lines 230 – 239:

Finally, although our study reveals a significant coupling between paleoclimatic changes and bursts of cavefish diversification, incomplete sampling of both cave-adapted and surface-dwelling relatives, and extinction of surface lineages may influence the robustness of these findings. Additionally, the contribution of genetic factors such as gene flow to cavefish diversification remains incompletely understood—a key limitation of this work. For instance, previous studies have demonstrated that gene flow facilitated adaptive radiations in primates^{64,65}, voles⁶⁶, bats⁶⁷, QTP fishes⁶⁸, and cichlids⁶⁹. While phylogenomic analyses of *Sinocyclocheilus*³⁰ have revealed how gene flow and paleoclimatic shifts jointly promoted its radiation, it remains unclear whether similar mechanisms operate in other cavefish groups or what genomic features characterize introgressed regions. Thus, future phylogenomic studies encompassing broader taxonomic groups will be essential to advance understanding of the origins, diversification bursts, and adaptive radiation of the cavefish hotspot in the karst regions of southwestern China.

See lines 321 – 323:

However, the limited distribution of cavefishes across these two basins may affect the robustness of our

findings. Future studies incorporating more diverse lineages would help strengthen and extend support for our conclusions.

Comment 2. (2) Address Reviewer #2's concerns regarding how cavefish are coded (which would involve rerunning these analyses), and include further comparisons to relevant surface taxa. We agree with the reviewer that it may be more appropriate to use the "cave-obligate" terminology throughout the text, rather than "cavefish" in many cases.

Response: Dear Editor, Thank you very much for your and second Reviewer valuable comments regarding the coding of the "cave" attribute for the fish species. As mentioned by the second reviewer, the concerns regarding the accuracy of ecological type classification for certain species (particularly in the genus *Sinocyclocheilus* and intermediate forms), along with the potential impact of including sister taxon pairs on the results, are indeed critical. We fully agree that clear definitions and precise coding are essential for the reliability of this study. For detailed responses, please refer to the reply to the second reviewer.

In response to your concerns, we have undertaken the following detailed revisions and provided additional clarifications:

(1) Clarification of Ecological Type Coding Criteria and Definitions. We have fully adopted your suggestion and explicitly implemented a dichotomous classification of ecological types in the revised manuscript (lines 46–49): "troglomorphic (stygobionts)" versus "non-troglomorphic (stygophiles)". This classification standard primarily references widely recognized authoritative literature in the field (e.g., DOI: 10.1111/j.1095-8649.2011.03066.x; DOI: 10.1016/B978-0-12-814124-3.00027-3).

Specifically: 1) **Troglomorphic:** Refers to species whose entire life cycle, or critical stages thereof, depends on the subterranean cave environment and which typically exhibit corresponding characteristic morphological adaptations (e.g., eye degeneration, loss of pigmentation); 2) **Non-troglomorphic:** Refers to species that utilize cave environments but are not strictly dependent on them and do not exhibit strong troglomorphic morphological features. Therefore, in accordance with established practices in cavefish research, we have classified cavefish into two ecological types—"troglomorphic" and "non-troglomorphic"—and will adhere to this classification in subsequent studies.

(2) Supplementation and Verification of Coding Basis. You correctly pointed out the complexity regarding the ecological types of some *Sinocyclocheilus* species, which may have surface populations. As a team long engaged in field investigations and taxonomic research on Chinese cave fishes (for instance, we

have recently described several new cave fish species, such as *Sinocyclocheilus longicornus* [DOI: 10.3897/zookeys.1141.91501], two species in the genus *Karstsinnectes* [DOI: 10.1186/zs.2024204; DOI: 10.3897/zse.100.133964], and six new hypogean species of the genus *Triplophysa* [DOI: 10.3897/zookeys.1185.105499, etc.]), our initial coding integrated extensive field observations and literature records.

Nonetheless, to address your concerns and ensure transparency for all readers, we have systematically reviewed and updated the coding for all species. This review was based primarily on: 1) The original description literature of the species; 2) Authoritative regional monographs (e.g., Lan et al., 2013, Cave Fishes of Guangxi, China; Zhao & Zhang, Endemic fishes of *Sinocyclocheilus* (Cypriniformes: Cyprinidae) in China species diversity, cave adaptation, Systematics and Zoogeography); 3) The latest comprehensive reviews (e.g., Ma et al., 2023, doi: 10.24272/j.issn.2095-8137.2022.484). All references supporting the ecological type coding have been meticulously added to **Supplementary Table S1** for your and the readers' review and verification.

Upon completing the new coding process, we incorporated recently published new species of cavefish and subsequently re-conducted the relevant analyses.

Comment 3. (3) From an editorial perspective, it seems that there are inconsistencies throughout the text about how many species are involved in this analysis. The Abstract states "We analyzed 525 species from 10 clades to reconstruct their diversification history", though other sections list far smaller numbers, as in:

Line 56 "A literature search obtained data for ~190 species of cave fishes"

Line 228: "343 occurrence sites for 205 species were included in the analysis"

Line 232: "In total, 85 tissue samples from 12 genera across three families were collected"

Please carefully review the text and clarify how many distinct species have been included as part of this work. It may also be helpful to include a supplementary table or data file specifying the number of species and list which ones were included in each analysis.

Response: Dear Editor, thank you very much for your thorough review and for pointing out the inconsistencies in the text regarding the number of species included in our analyses. We sincerely appreciate the opportunity to clarify these points.

We have carefully re-examined and updated the relevant data throughout the manuscript. The primary source of confusion stemmed from the different contexts in which species numbers were mentioned—referring respectively to the total number of species considered for distribution mapping, the subset used in downstream analyses, and the specific samples newly sequenced for this study.

Please find below a point-by-point response to the specific lines you highlighted:

(1) Regarding the statement in the Abstract: "We analyzed 525 species from 10 clades to reconstruct their diversification history"

Clarification: This number (525) refers to the total number of species encompassing both cave-dwelling species and their closely related surface-dwelling relatives across the 10 clades studied. This broad set was used as the initial framework for understanding large-scale diversification patterns. To avoid confusion, we have revised this sentence in the Abstract (**lines 13-16**) to more precisely reflect the scope of the analysis.

(2) Regarding Line 56: "A literature search obtained data for ~190 species of cave fishes"

Clarification & Correction: Based on our updated and comprehensive species inventory, the current number of recognized cavefish species within China is 199. We have corrected this number in the manuscript accordingly.

(3) Regarding Line 228: "343 occurrence sites for 205 species were included in the analysis"

Clarification & Correction: This section pertains to the data used for mapping species distributions

and diversity patterns. Our updated inventory lists 199 cavefish species within China and an additional 22 species from adjacent regions in Asia. To visualize the broader distribution pattern and highlight Southwest China as a diversity hotspot, we included these 221 species. For this combined set, we gathered a total of 370 occurrence sites.

Furthermore, for the subsequent analyses on diversity patterns and evolutionary history, we utilized a subset of 183 species. This represents 92% of the 199 known Chinese cavefish species, which we believe is a robust and comprehensive representation given the challenges in specimen collection.

(4) Regarding Line 232: “In total, 85 tissue samples from 12 genera across three families were collected”

Clarification: This figure is correct and refers specifically to the new genetic samples we collected, amplified, and sequenced for this study. These samples were subsequently used for the phylogenetic, divergence time, and biogeographical analyses presented in the paper.

To ensure full transparency and reproducibility: All relevant sequence data have been deposited in a public repository (DRYAD, <https://doi.org/10.5061/dryad.ht76hdrqx>; Zenodo, <https://doi.org/10.5281/zenodo.17578805>).

As you helpfully suggested, we have now explicitly stated the final number of species used in each specific analysis within the main text.

We have also provided detailed supplementary tables (**Tables S3–S12**) that list all species included in the various analyses, specifying which ones were used for distribution mapping, phylogenetic reconstruction, and other downstream analyses.

We hope these clarifications and the corresponding revisions in the manuscript satisfactorily address your concerns. Thank you again for your valuable feedback, which has significantly improved the clarity of our work.

Finally, we have added a dedicated "Conclusions" (**See lines 324–341**) section to provide a comprehensive summary of the article. This section synthesizes the key findings of our study, discusses their broader implications, and offers potential directions for future research.

The revised text is as follows:

See lines 13 – 16:

To elucidate their evolutionary origins and diversification history, we analyzed **183 cave species and their close relatives** from ten clades.

See lines 324 – 341:

Conclusions

This study compiled 199 currently recognized Chinese cavefishes, identifying two major hotspots. By integrating genetic data from 183 cave-adapted and related surface-dwelling species, we reconstructed a comprehensive phylogeny to explore their diversification history. To our knowledge, this represents the most taxonomically complete phylogeny of Chinese cavefishes to date. Our results indicate that cave colonization began in the middle Eocene (~44 Ma), with *in situ* diversification as the primary mode of speciation. This process started around 43.8 Ma, increased sharply by ~25 Ma, and exhibited two peaks at ~10 Ma and 2.4 Ma, with one valley at ~3.3 Ma. Diversification patterns varied across basins, showing staggered origins, asynchronous acceleration, and peak periods. We further revealed for the first time asynchronous origins and dispersal between the Pearl and Yangtze River basins, suggesting possible river capture events as early as the late Oligocene (~26 Ma), followed by repeated connections. Based on these findings, we delineate three stages of cavefish evolution and refine the three-stage model of karst development, reflecting responses to middle Eocene paleoclimatic changes influenced by QTP uplift. In summary, this work provides a valuable framework for understanding speciation in extreme environments and offers a compelling case of tectonics–climate–biosphere co-evolution. However, limitations such as incomplete taxon sampling and a lack of genome-scale data may affect phylogenetic inference and obscure the contribution of gene flow to diversification. Future studies should prioritize expanded taxonomic sampling and high-quality genomic resources to further clarify the diversification history, evolutionary patterns, and ecological adaptations of subterranean biotas.

Response to the second Reviewer:

Dear Reviewer:

Thank you for your valuable comments on our manuscript, formerly titled "**Rise and Diversification of Cavefishes in the Karst Regions of Southwest China**." The title has now been revised to "**Evolutionary trajectories and dynamics of cavefishes in the karst hotspot of southwest China**." Your suggestions have been instrumental in refining our manuscript and will continue to guide our future research on cave fishes. We have thoroughly considered all of your comments and have made corresponding revisions to the text, with changes highlighted in **blue** in the original document. In addition, we apologize for the delayed response, which resulted from the need to incorporate updated datasets and conduct additional analyses to thoroughly address your comments. Below, we provide a point-by-point response to each of your remarks.

Sincerely,

Tao Luo

On behalf of all co-authors

Overall comments: The authors investigated the geologic history of the fantastic karst regions of southeast China through examining endemic cavefish phylogeography. The authors utilized phylogeographic and phylogenetic comparative methods (i.e., ancestral state reconstruction) to estimate the ages and evolutionary history of six fish families containing cavefishes. Overall, this was an enjoyable paper to read and will interest those concerned with caves, biogeography, phylogeography, geology, karst, and fish biology. I have one large methodology concern, and I have some suggestions to make the paper appeal to a wider audience.

Response: Dear Reviewer, Thank you very much for your careful review of our manuscript and for your valuable comments. We were delighted to learn that you found our study "enjoyable to read" and that it will be of interest to researchers in fields such as speleology, biogeography, phylogeography, geology, karst science, and fish biology.

Your primary methodological concern and your suggestions for broadening the paper's appeal were crucial for improving the quality of our work. We have thoroughly revised and supplemented the original manuscript based on your feedback.

Regarding the specific questions and suggestions you raised, we have responded in detail one by one in the "Point-to-Point Response:" at the back of this document. We hope that our revisions and explanations adequately address your concerns and that the revised manuscript now meets the required standards for

publication.

Once again, we sincerely appreciate the time and effort you have dedicated, and your contributions to enhancing the quality of our paper.

Point-to-Point Response:

Comment 1. My main concern is the coding of the fish species as “cave” or not. For example, there are several *Sinocyclocheilus* species that in the supplement are coded as cave, despite being surface-dwelling. This is yet to mention the several species that are intermediate, spending some but not all their time in caves. If the authors would like to use “cave-obligate” vs. “non-cave-obligate”, this could be a simple solution. I would also encourage the authors to be very specific about how they define cave-obligate vs. not. Additionally, I am concerned that sister surface taxa were not included, which would skew several of the analyses— though I could be incorrect, I believe there are surface *Paranemachilus* that would potentially change some of the lineage divergence events. I would be happy to be incorrect here. But I would like either a justification for the coding scheme, or if there were inaccuracies, I would like the authors to revisit their coding and potentially rerun the analyses.

Response: Dear Reviewer, Thank you very much for your valuable comments regarding the coding of the "cave" attribute for the fish species. The issues you raised about the accuracy of ecological type classification for certain species (particularly within the genus *Sinocyclocheilus* and intermediate forms), as well as the potential impact of including sister taxa on the analysis results, are indeed crucial. We fully agree that clear definitions and precise coding are essential for the reliability of this study.

In response to your concerns, we have undertaken the following detailed revisions and provided additional clarifications:

(1) Clarification of Ecological Type Coding Criteria and Definitions. We have fully adopted your suggestion and explicitly implemented a dichotomous classification of ecological types in the revised manuscript (lines 47–51): "troglomorphic (stygobionts)" versus "non-troglomorphic (stygophiles)". This classification standard primarily references widely recognized authoritative literature in the field (e.g., DOI: 10.1111/j.1095-8649.2011.03066.x; DOI: 10.1016/B978-0-12-814124-3.00027-3).

Specifically: 1) Troglomorphic: Refers to species whose entire life cycle, or critical stages thereof, depends on the subterranean cave environment and which typically exhibit corresponding characteristic morphological adaptations (e.g., eye degeneration, loss of pigmentation); 2) **Non-troglomorphic:** Refers to

species that utilize cave environments but are not strictly dependent on them and do not exhibit strong troglomorphic morphological features.

(2) Supplementation and Verification of Coding Basis. You correctly pointed out the complexity regarding the ecological types of some *Sinocyclocheilus* species, which may have surface populations. As a team long engaged in field investigations and taxonomic research on Chinese cave fishes (for instance, we have recently described several new cave fish species, such as *Sinocyclocheilus longicornus* [DOI: 10.3897/zookeys.1141.91501], two species in the genus *Karstsinnectes* [DOI: 10.11865/zs.2024204; DOI: 10.3897/zse.100.133964], and six new hypogean species of the genus *Triplophysa* [DOI: 10.3897/zookeys.1185.105499, etc.]), our initial coding integrated extensive field observations and literature records.

Nonetheless, to address your concerns and ensure transparency for all readers, we have systematically reviewed and updated the coding for all species. This review was based primarily on: 1) The original description literature of the species; 2) Authoritative regional monographs (e.g., Lan et al., 2013, Cave Fishes of Guangxi, China; Zhao & Zhang, Endemic fishes of *Sinocyclocheilus* (Cypriniformes: Cyprinidae) in China species diversity, cave adaptation, Systematics and Zoogeography); 3) The latest comprehensive reviews (e.g., Ma et al., 2023, doi: 10.24272/j.issn.2095-8137.2022.484). All references supporting the ecological type coding have been meticulously added to Supplementary Table S1 for your and the readers' review and verification.

(3) Handling of the Genus *Paranemachilus* and Its Sister Taxa. Your attention to the genus *Paranemachilus* and its potential surface-dwelling sister taxa is highly professional. According to the latest taxonomic revisions (referencing DOI: 10.3897/zse.100.129520) and authoritative monographs (Lan et al., 2013, Cave Fishes of Guangxi, China;), species of the genus *Paranemachilus* are known to inhabit subterranean caves and connected waters near cave exits. Their ecological habits are classified as "non-troglomorphic." Within our phylogenetic framework and taxonomic sampling, we have strived to include representative species within this genus. The current record did not identify the presence of unequivocal surface-dwelling sister taxa that would significantly alter the inferences of the lineage divergence events focused on in this paper. However, we fully agree that continuous updates to the analysis will be important as new taxa are discovered and phylogenetic relationships are further resolved.

Additionally, since the data required for our final analysis are the 95% confidence intervals of divergence times, the principle of the MDE method (which posits that diversification/dispersal events are

more likely to occur when the corresponding divergence time intervals overlap) can, to some extent, mitigate the influence of closely related surface-dwelling sister species. Similar approaches have been widely used in previous studies, and their research outcomes have been published in high-quality journals (e.g., DOI: 10.1073/pnas.2207199119; DOI:10.1098/rspb.2021.1308; DOI: 10.1038/s41467-023-39555-6; DOI:10.1073/pnas.2322527121; DOI: 10.1093/nsr/nwaf157; DOI: 10.1038/ncomms12132; DOI: 10.1093/nsr/nwz035; DOI: 10.1093/nsr/nwaa263).

(4) Update of Data Analysis. Based on the aforementioned re-verification and coding of species ecological types, and considering the updated genetic data resulting from the recent publication of several new cave fish species by our team, we have re-calculated all relevant analyses in the manuscript using the revised coding scheme and the latest genetic dataset.

We believe these revisions directly address the core concerns you raised, significantly enhancing the accuracy and transparency of the species ecological type coding and ensuring the robustness of the analytical results. Once again, we sincerely thank you for your insightful comments, which have greatly helped us improve this manuscript.

In summary, based on the aforementioned content and recently published species, we have recoded the ecological types of the species in the revised manuscript (by adding a new "Cave Code" column in Table S1 to indicate the cave status of each species, i.e., Troglomorphic and Nontroglomorphic) and conducted corresponding analyses, including phylogenetic reconstruction, ancestral habitat assessment, biogeographic analysis, and diversification analysis. Based on these results, we have redesigned relevant figures and tables, with particular emphasis on the addition of Figure 4 as a summary of the evolutionary history of diversification. These findings have led to revisions in the Introduction, Methods, Results, and Supplementary Materials (**Fig. S1A, Fig. S1B, Fig. S1E, Fig. S3A, Fig. S3B, Fig. S3C; Table S1, Table S3, Table S4, Table S7, Table S13, Table S14, Table S15, Table S6, Table S17, Table S18, Table S19, and Table S20**). Additionally, the former **Table 2** has been moved to the Supplementary Materials as Table S18. Details of the revisions are provided below.

For the revised “Introduction”

See lines 47–51:

Among vertebrates, the cavefishes are exemplary species exhibiting unique adaptations to subterranean life, the most notable being the degeneration or absence of pigmentation, eyes, and scales²². Based on their

differential dependence on cave environments, cavefishes are divided into two ecological types: troglomorphic (typical, stygobionts) and non-troglomorphic (atypical, stygophiles)^{23,24}; a dichotomous classification scheme adopted here.

For the revised “Materials and Methods”

See lines 381–383:

The habitat status of each species was coded as cave-dwelling or epigeal according to established criteria^{23,24}, based on actual surveys and reports in the literature (Table S1).

For the revised “Table 1”

Table 1. Numbers and mean ages of cavefishes involved in various biogeographic events and their 95% confidence intervals.

Biogeographic event type	Number	Mean age (95% CI) (Ma)
Dispersal	49	10.0 (12.3–8.0)
<i>In situ</i> diversification	307	8.0 (8.9–7.2)
Migrations into caves	20	20.9 (27.8–14.7)
Migration outside the caves	190	8.2 (9.3–7.1)
Lineage divergences	2	19.9 (20.6–19.3)
From Pearl River dispersal to Yangtze River	7	11.3 (16.0–6.1)
From Yangtze River dispersal to Pearl River	6	6.2 (7.9–4.7)

For the revised “Figures”

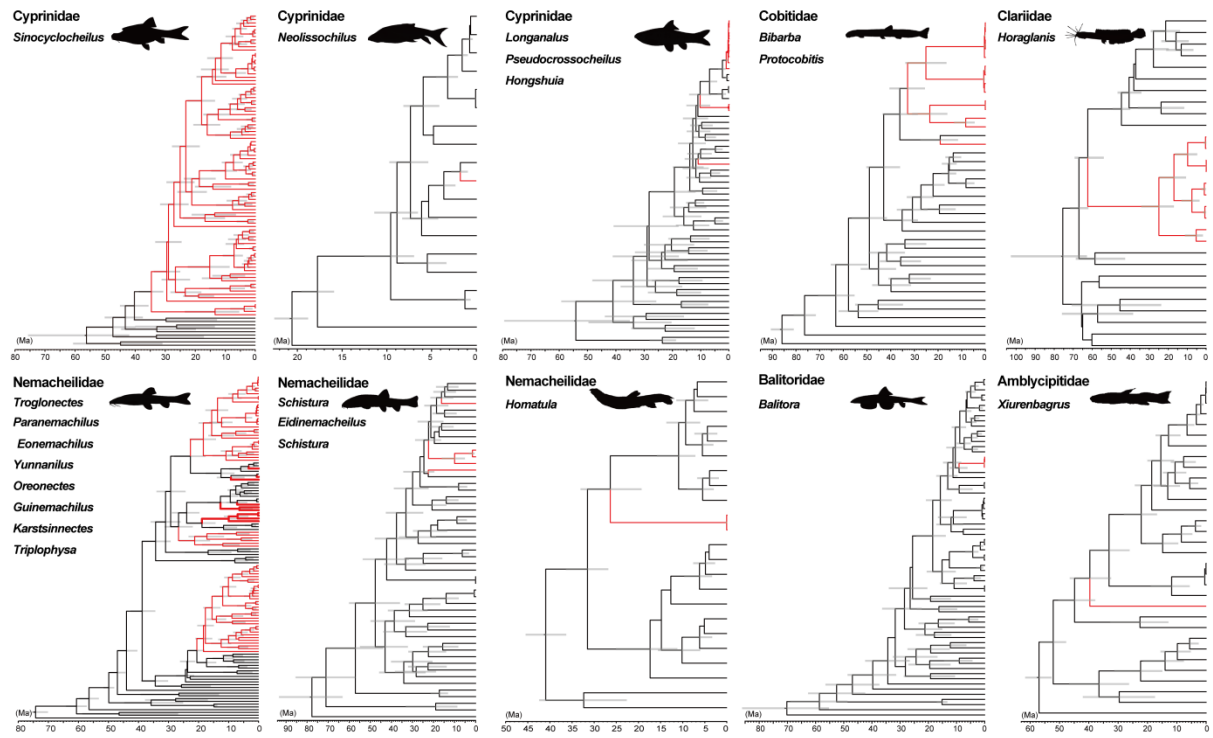


Fig. 2 An overview of the time tree used for the phylogeographic analysis. The red color indicates cave species.

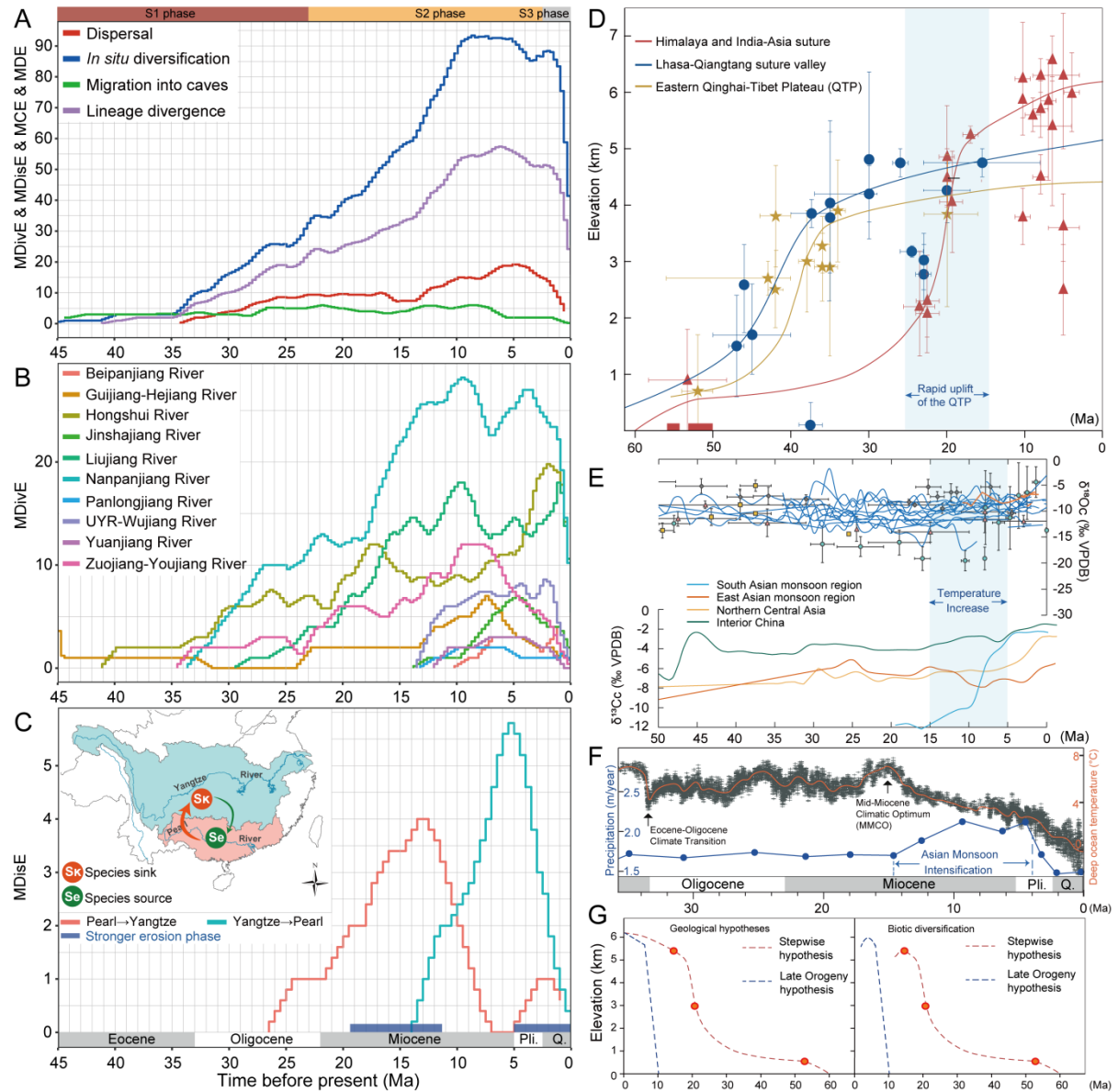


Fig. 3 Dynamic rates of Chinese cavefish diversification and the potential driving factors. **(A)** The rates of dispersal, *in situ* diversification, migration into caves, and lineage divergences through time. The S1–S3 phases were modified from the three-phase hypothesis of karst evolution in southern China proposed by [Che and Yu¹²](#). The grey rectangular boxes represent time frames that are not included in the three phases. **(B)** *In situ* diversification dynamics in different drainages, based on the maximum number of observed *in situ* diversification events per million years (MDivE). **(C)** Dispersal dynamics based on the observed maximum number of dispersal events per Ma (MDisE) between the Yangtze and Pearl River basins. **(D)** The stepwise uplift history of the Qinghai–Tibet Plateau (QTP), showing the Himalayas and the India–Asia suture, the Lhasa–Qiangtang suture valley, and the eastern QTP, modified from [Ding et al.²](#). **(E)** Palaeoclimate variation over the past ~50 Ma in Asia: stable oxygen ($\delta^{18}\text{O}_c$) and carbon isotopic composition ($\delta^{13}\text{C}_{\text{carb}}$) records as determined from aeolian silt sequence records, modified from [Lu et al.¹](#). VPDB (Vienna Pee Dee Belemnite). **(F)** Global temperature trends and East Asian monsoon conditions from the late Eocene to the present (mean annual precipitation at idealized CO_2 levels), modified from [Ding et al.¹¹](#). **(G)** The uplift history and expected

biotic assembly processes of the QTP since ~60 Ma, modified from Ding *et al.*⁶³ and Xu *et al.*²⁰. The Stepwise Hypothesis posits that the QTP initiated its uplift during the early Paleocene through a series of stepwise uplift events, with accelerated uplift occurring later during the Miocene (23–15 Ma) before reaching its present elevation. In contrast, the Late Orogeny Hypothesis proposes that the QTP began its major uplift in the early Miocene and reached the current elevations much later (~7.0–3.2 Ma).

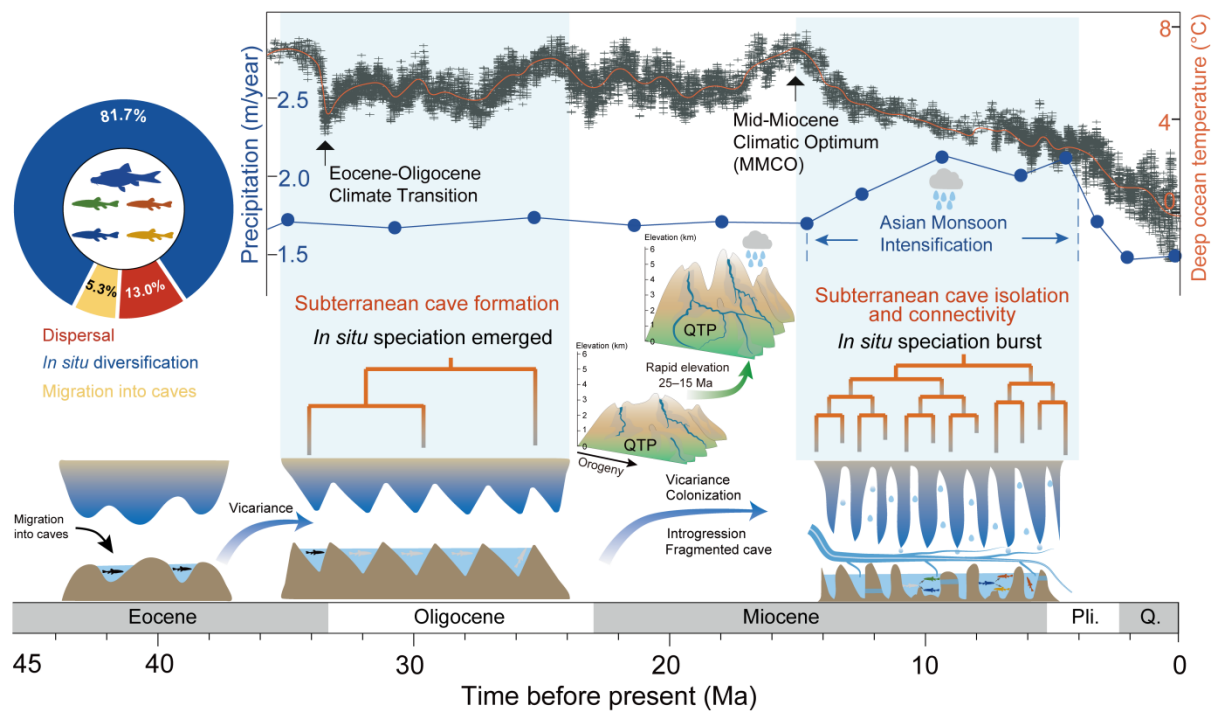


Fig. 4 Evolution of karst cavefish in southwestern China in relation to climatic and geological history. Top left: Major events associated with cavefish diversification and their relative contributions. Top: Evolution of global climate (represented by deep-sea oxygen isotope records⁹⁵ and estimated deep ocean temperatures (orange)⁹⁶) and East Asian monsoon conditions (mean annual precipitation at idealized CO₂ levels⁵⁶) from the late Eocene to the present, modified from Ding *et al.*¹¹. Bottom: Schematic representation of karst cave and cavefish formation across four evolutionary phases from the late Eocene to the present. The schematic uplift of the Qinghai–Tibet Plateau (QTP) illustrates how tectonic processes created extensive fragmented subterranean landscapes, providing ecological opportunities for *in situ* diversification of cavefish (modified from Ding *et al.*²). The phylogenetic diagram indicates the relative number of *in situ* speciation events (modified from Lu *et al.*³⁵). Abbreviation: Pli., Pliocene; Q., Quaternary.

For the revised “Table 1”

Table 1. Numbers and mean ages of cavefishes involved in various biogeographic events and their 95% confidence intervals.

Biogeographic event type	Number	Mean age (95% CI) (Ma)
Dispersal	49	10.0 (12.3–8.0)
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From Pearl River dispersal to Yangtze River	7	11.3 (16.0–6.1)
From Yangtze River dispersal to Pearl River	6	6.2 (7.9–4.7)

For the revised “Supplementary Materials”

Detailed information on the revised “Supplementary Materials” can be found in the documents uploaded to the journal system, including Fig. S1A, Fig. S1B, Fig. S1E; Fig. S3A, Fig. S3B, Fig. S3C; Table S1, Table S3, Table S4, Table S7, Table S13, Table S14, Table S15, Table S6, Table S17, Table S18, Table S19, Table S20.

Comment 2. I believe the authors could expand on the introduction and discussion to put this paper in a greater context. There are a few biological variables that are not mentioned within the manuscript that I think potentially require attention. The authors do not mention groundwater or groundwater dispersal. Determining the history of groundwater availability on these timescales is currently unachievable, so I do not expect the authors to account for groundwater dispersal in the methods or anything to that effect, but I think I would like a mention that groundwater could play a role in the distribution of the cavefishes. The authors discuss the cavefishes dispersing across the Pearl and Yangtze Rivers; while not impossible, it may be unlikely. Additionally, ancestral state reconstructions most likely underestimate the number of independent colonization events, as it is possible that clades entirely made of cavefishes could have arisen from independent colonization events followed by surface ancestor extinction (e.g., *Sinocyclocheilus*). A mention of pre-adaptation being necessary for subterranean colonization may also be warranted. I think some caveats such as these would be good to include in the paper. I think these would potentially fit into the discussion, but a mention of them in the introduction could work as well.

Response: We sincerely thank you for this insightful suggestion to broaden the context of our study. We have carefully considered the points raised and have revised the manuscript accordingly to provide a more comprehensive perspective.

(1) Role of Groundwater Dispersal. We fully agree that groundwater dynamics likely play a crucial role in cavefish distribution. In the revised manuscript, we have now explicitly acknowledged this factor. Specifically, we have added a statement in the [Discussion \(lines 188–190\)](#) noting that while direct historical reconstruction remains challenging, groundwater connections undoubtedly represent a significant potential pathway for dispersal that could influence observed distribution patterns.

(2) Inter-basin Dispersal between the Pearl and Yangtze Rivers. We appreciate your note on the likelihood of this dispersal route. Our interpretation was based on an ideal model framework. However, we have now strengthened this argument by citing relevant literature. We refer to a recent study on East Asian cyprinids (DOI: 10.1016/j.xinn.2023.100417) which provides evidence for faunal exchanges between these river basins, supporting the plausibility of such dispersal events for ancestral lineages.

(3) Underestimation of Independent Colonization Events. This is a critical point. We have incorporated this important caveat into the [Discussion \(lines 230 – 232\)](#). We now explicitly state that our Ancestral State Reconstruction might underestimate the number of independent cave colonization events. We discuss the possibility that clades now entirely composed of cavefish (using *Sinocyclocheilus* as an

example) could have originated from multiple independent colonizations followed by the extinction of their respective surface ancestors.

(4) Pre-adaptation for Subterranean Colonization. We thank you for suggesting this aspect. Following this advice, we have added a relevant passage in the manuscript ([lines 171–175](#)). We discuss that the transition to cave life likely involved the evolution of innovative traits from ancestral precursors, which can be considered pre-adaptations or key innovations that facilitated subterranean colonization. This includes the regression of energy-costly structures and the enhancement of others, as highlighted in our study. In addition, we have also added relevant content in the preface ([see lines 47–49](#)).

We believe these additions have significantly strengthened the manuscript by placing our findings within a broader evolutionary and ecological context and by acknowledging important limitations and complexities. Thank you for helping us improve the quality and impact of our work.

See lines 47–49:

[Among vertebrates, the cavefishes are exemplary species exhibiting unique adaptations to subterranean life, the most notable being the degeneration or absence of pigmentation, eyes, and scales²¹.](#)

See lines 171–175:

[During this process, their ancestors innovatively evolved specialized morphological and genetic functions, exemplified by the regression of energy-consuming organs \(e.g., eyes, scale, and pigmentation\)^{38,39}, enhancement of energy-storage and sensory organs \(e.g., the caudal adipose keel and the distinctive horns or humps in some cave-adapted *Sinocyclocheilus* species\)³⁸⁻⁴⁰, and unique genetic regulatory mechanisms^{41,42}.](#)

See lines 188–190:

[Although the specific influences of cave and groundwater on cavefish diversification cannot be precisely reconstructed at present, their role in shaping dispersal, in situ diversification, and distribution patterns should not be underestimated.](#)

See lines 230–232:

[Finally, although our study reveals a significant coupling between paleoclimatic changes and bursts of cavefish diversification, incomplete sampling of both cave-adapted and surface-dwelling relatives, and extinction of surface lineages may influence the robustness of these findings.](#)

Comment 3. I would recommend a bit more in the introduction about the river basins in China, as those unfamiliar with the area might get lost. The Pearl River basin and its rivers are named in the materials and methods, but I think a mention in the introduction could allow the reader a better visualization. Additionally, the authors mention the need to examine cavefishes due to global climate change, but this needs detail—readers unfamiliar with cave biology may not understand the effect climate change will have on subterranean ecosystems. The conservation aspect of this paper is never mentioned again, though. I would recommend some expansion somewhere in the discussion. I believe it would be of benefit to explain somewhere the Stepwise and Late Orogeny hypotheses. I was unfamiliar with them and felt a little lost when they came up.

Response: We sincerely thank you for these constructive suggestions, which have helped us improve the clarity and contextual background of the manuscript. We have addressed each point as follows:

(1) Introduction of River Basins. Following your suggestion, we have added a brief description of the major river basins relevant to our study in the Introduction ([lines 58 – 70](#)). This provides readers, especially those unfamiliar with the geography of the region, with better spatial context for understanding the distribution of our study species. Furthermore, to enhance visual clarity, we have redrawn Figure 1 to more clearly illustrate the Pearl River basin and the distribution of its major tributaries.

(2) Impact of Climate Change on Subterranean Ecosystems. We agree that the link between climate change and threats to subterranean ecosystems needed elaboration. We have revised the relevant text in the Introduction ([lines 55 – 57](#)) to more clearly explain how global warming, through potential mechanisms like drought and reduced groundwater recharge, poses a significant threat to cavefish populations by degrading their highly specialized and fragile habitats.

(3) Conservation Implications. We thank you for highlighting the importance of the conservation aspect. While a detailed conservation plan falls outside the scope of this paper, we have now incorporated a statement in the Discussion ([lines 210 – 213](#)) that explicitly emphasizes the conservation urgency for cavefishes based on our findings and their inherent vulnerability.

(4) Explanation of the Stepwise and Late Orogeny Hypotheses. Hypotheses may not be familiar to all readers. To clarify this essential background, we have added a detailed legend to Figure 3 (specifically for panel 3H) that graphically and textually summarizes the key differences between the Stepwise and Late Orogeny hypotheses regarding the timing and pattern of Qinghai-Tibet Plateau uplift and their impacts on biotic diversification.

We believe these revisions have significantly improved the manuscript by providing better geographical

and geological context, clarifying the conservation relevance, and making the study more accessible to a broader audience. We are grateful for your valuable insights.

See lines 55 – 57:

In the context of global warming, potential droughts and diminished groundwater recharge in caves may irreversibly impact cavefish populations^{27,28}, underscoring the critical need to advance our understanding of subterranean biodiversity and its evolutionary origins.

See lines 58 – 70:

In China, cavefishes are primarily documented in the southwestern region (Fig. 1A), particularly in the karst areas of the upper Pearl River (including the Beipanjiang, Guijiang–Hejiang, Hongshui, Zuojiang–Youjiang, and Liujiang rivers), the upper Yangtze River (encompassing the Jinshajiang, Wujiang, and Yuanjiang rivers), and the Red River Basin²⁹ (Fig. 1B). However, despite being recognized as a biodiversity hotspot and conservation priority, the diversity of these subterranean fishes remains severely underestimated due to insufficient field surveys and systematic research, and their evolutionary history remains poorly understood (Table S1). Although several studies based on incomplete sampling and reduced-representation genomic data have revealed the important roles of paleoclimatic history and gene flow in driving the speciation and radiation of *Sinocyclocheilus*³⁰, their limited taxonomic scope still precludes a comprehensive understanding of the evolutionary history of Chinese cavefishes and the associated geomorphological evolution—such as river formation and connectivity. Furthermore, it remains unclear whether historically warm climates in geological time promoted diversification bursts or had the opposite effect. Therefore, integrated evolutionary analyses of multiple cavefish clades will help predict their response trajectories under global warming and inform adaptive conservation strategies and interventions.

See lines 210–213:

In other words, while long-term global temperature rise could theoretically promote cavefish diversification under idealized conditions, the current degradation of subterranean ecosystems and the need to balance conservation with sustainable human benefits render in situ protection of cavefishes urgently needed.

Comment 4. A paragraph at the beginning of the discussion that includes highlights of the discussion sections could be helpful to tie up the paper. Or possibly a conclusions paragraph. There are some ideas in the introduction that are not brought back up in the discussion that probably should be (e.g., global climate change impacting cave organisms).

Response: We thank you for the valuable suggestion to better synthesize the key findings of our study.

In response to the recommendation to include a paragraph at the beginning of the Discussion that highlights the main points, we have carefully reviewed the format of published papers in this journal. We noted that a standalone, introductory summary paragraph in the Discussion section is not a common convention.

Therefore, we have implemented the alternative approach you suggested. To enhance the structure and clarity of the Discussion, we have added clear, summarizing topic sentences at the beginning of key paragraphs (see lines 191 and 83, and others). These sentences serve to guide the reader by immediately stating the core conclusion or purpose of the paragraph that follows, thereby effectively tying the different sections of our discussion together.

Regarding the point about revisiting the issue of "global climate change impacting cave organisms," which was raised in the Introduction, we have indeed addressed this in our response to Comment 3. The relevant text has been added to the Discussion to elaborate on this important aspect.

We believe these revisions have significantly improved the flow and coherence of the Discussion section by explicitly connecting our specific results to the broader themes introduced earlier in the paper.

See line 191:

In situ diversification dynamics of Chinese cavefishes were driven by phased karst development.

See line 283:

The Pearl River and Yangtze River may have become connected as early as the late Oligocene.

Comment 5.

Materials and Methods

Please see my concern about the coding of cavefishes. I cannot speak to the methodology of the dynamic meta-analysis of lineage diversification, but the phylogenetic and chronogram methodologies appear rigorous. Is there an International Animal Care and Use Committee protocol or something to that effect for the newly samples tissues?

Response: We thank you for raising this important question regarding the ethical collection of the newly sampled tissues used in this study.

The tissue specimens utilized in this research were collected over an extended period, from 2010 to 2025. We would like to clarify that the vast majority of the cavefish species studied are not listed as protected

animals under Chinese regulations; therefore, their collection did not require specific permits from relevant management authorities for most of the sampling period, particularly for the earlier years.

Nevertheless, strictly adhering to ethical principles in wildlife research, we deliberately avoided large-scale field collection throughout this project, especially for any potentially threatened species. A significant portion of the tissue samples was obtained through gifts and collaboration from fellow researchers, such as Prof. Li-Na Du and Mr. Jia-Hu Lan, who contributed materials from their own established collections.

In response to your suggestion, we have now included a dedicated “Ethics Statement” section in the final version of the manuscript ([see lines 616–521, 354–356](#)). This study was supported by the National Key R&D Program of China (Grant No. 2022YFF1301502, “Endangerment Processes and Mechanisms of Endangered Flagship Animals”) and Strategic Priority Research Program of the Chinese Academy of Sciences (CAS) (XDB31000000). For the majority of the species involved, samples were collected during biodiversity surveys conducted in southwestern China under the framework of this project. These surveys were carried out with the necessary permissions from the relevant local authorities, including forestry and agricultural bureaus.

We also fully recognize the conservation importance of the critically endangered species included in this study. To minimize potential impacts on these vulnerable populations, we deliberately designed our research approach through collaboration with Prof. Li-Na Du and Mr. Jia-Hu Lan, two respected experts in cave fish taxonomy and conservation. Within this cooperative framework, Prof. Du and Mr. Lan provided specimens of *Karstsinnectes longzhouensis*, *Troglonectes hechiensis*, *Karstsinnectes acridorsalis*, and *Troglonectes barbatus* in 2019, which had been previously collected during their authorized surveys following appropriate protocols. This methodology enabled us to acquire necessary research materials while avoiding additional sampling pressure on these threatened species. To ensure full transparency, we have explicitly acknowledged the contributions of both Prof. Du and Mr. Lan in the Acknowledgements section of our manuscript.

We believe these clarifications adequately address the ethical considerations you raised. Thank you again for your thorough review and valuable suggestions, which have undoubtedly improved the quality and completeness of our manuscript.

See lines 616–621:

Ethics Statement

All authors have approved the manuscript content, author list and order, and contribution statements. Sample collection complied with China's wildlife protection laws and regulations. The sampling procedures followed the National Standards of the People's Republic of China (GB/T 39760-2021; GB/T 35892-2018) and the Guidelines for the Care and Use of Laboratory Animals established by the Ethics Committee of Guizhou Normal University (Guiyang, Guizhou, China).

See lines 354–356:

All sampling procedures were performed with ethical consideration, whereby fin clips were collected non-invasively from fish anesthetized with Tricaine (150 mg/ml) that were subsequently revived and released, or were obtained only after natural death.

Comment 6.

Line-by-Line Edits

Line 37: Could the authors add “geologic” to “the evolutionary processes shaping natural landscapes...” or something similar? Reading this as an evolutionary biologist gave me some pause to understand what the authors were trying to say. It makes sense, I was just thinking about categorizing the evolutionary processes as non-biological.

Response: We thank you for this insightful suggestion. We agree that the original phrasing could be ambiguous from an evolutionary biology perspective. As suggested, we have revised the sentence in **Line 42 – 44** to explicitly acknowledge the role of geologic processes alongside evolutionary mechanisms. This revision more accurately frames the causal relationship between landscape evolution and biological diversification, and we believe it significantly improves the conceptual clarity of the introduction.

See lines 42–44:

The **geologic processes** shaping natural landscapes and their influence on evolutionary processes have profoundly influenced the diversification of the extant biota¹⁵⁻¹⁸, a concept now widely accepted in biogeography^{19,20}.

Comment 7.

Line 39–40: I strongly disagree with this statement and other cave biologists would as well. Caves are unequivocally not separated from the surface habitat; where caves are sometimes considered ecological islands is in comparison to one another. Caves are oftentimes not islands, though, and may be connected

through conduits in the karst. I would recommend the authors reword this a little more carefully.

Response: We thank you for raising this important point regarding the conceptualization of caves as ecological islands. We fully agree that the original phrasing oversimplified the complex connectivity of cave systems. Following your recommendation, we have carefully reworded the statement to more accurately reflect the current understanding in cave ecology. The revised text ([see lines 45–47](#)) acknowledges that while caves can be conceptualized as ecological islands in specific comparative contexts, they remain hydrologically and ecologically connected through subsurface conduits within karst systems. This modification better aligns with the nuanced perspective shared by the cave biology community.

See lines 45–47:

Caves can be considered as subterranean ecological islands with distinct characteristics, interconnected with surface environments through rivers or karst aquifers²¹, and paleogeoclimatic events have strongly influenced cave biodiversity¹³.

Comment 8.

Line 48: This may be semantics, but I'm not sure that I would count this as a single phylogeny. Perhaps change this to something along the lines of greatest number of taxa? I'm not sure what the perfect way to say this is, but I'm not sure I'm comfortable with this being considered a single phylogeny.

Response: We thank you for this pertinent observation regarding the terminology used to describe our phylogenetic reconstruction. We agree that referring to it as a "single phylogeny" could be misleading, as it indeed represents a comprehensive framework encompassing multiple taxonomic groups. Following your suggestion, we have revised the text in [lines 71–72](#) to more accurately reflect the nature of our dataset. The wording now emphasizes that our study incorporates the "greatest number of cavefish taxa" to date within its phylogenetic context, rather than characterizing it as a single phylogeny. This modification provides a more precise description of our methodological approach and phylogenetic scope.

See lines 71–72:

In this study, we compiled a species list of Chinese cavefishes and reconstructed the most extensive phylogeny to date, comprising 208 species in [ten phylogenetic trees](#), representing two orders, six families, and 22 genera.

Comment 9. Figure 1. This figure is incredibly busy. For someone not intimately familiar with the geography

of southeast China, it took much too long to find the Pearl and Yangtze Rivers, and none of the other rivers are labeled. I really believe this figure needs to be broken up into two. One with the geology and geography and one with the fish and species richness. Additionally, please include the photo credits in the figure legend.

Response: We sincerely appreciate this valuable suggestion. In response, we have thoroughly revised and reconfigured **Figure 1** to enhance its clarity and interpretability. Specifically:

(1) Splitting the Original Figure. The original Figure 1 has been split into three distinct subfigures (now labeled as Figures 1A, 1B, and 1C) to separately present: Species richness and distribution of cavefish; Detailed visualization of river distribution; Geographical and geological context; Detailed structural and tectonic setting of the hotspot region.

(2) Improved Labeling and Accessibility. We have now clearly labeled the Pearl River and Yangtze River, along with other major rivers and geological features, to assist readers who may be less familiar with the regional geography.

(3) Inclusion of Photo Credits. All photo credits have been explicitly included in the revised figure legends, as requested.

(4) Additional Geological Context. Considering the unique geological history and fault systems characterizing the hotspot region, we have added a new panel (**Figure 1C**) to visualize key tectonic structures and their potential influence on cavefish diversification.

These changes are intended to make the figure set more intuitive, visually balanced, and informative for a broad audience. We believe the revised figures now more effectively communicate the spatial, geological, and biological patterns central to our study.

The revised Figure 1 and its legend are as follows.

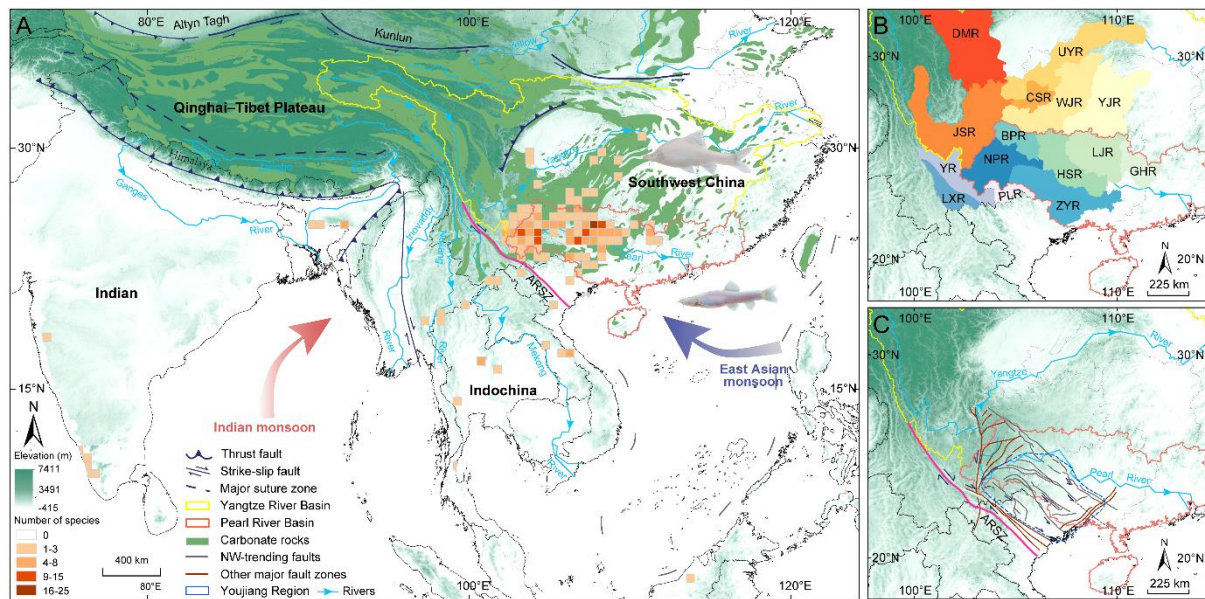


Fig. 1 Map of study area. **(A)** Spatial patterns of species diversity of cavefishes as measured by the number of species. The purple line denotes the Ailao Shan-Red River shear zone (ARSZ). The inset images show representative cavefish from southwestern China: *Sinocyclocheilus microphthalmus* (top) and *Troglonectes microphthalmus* (bottom). Photo by Mr. Jia-Hu Lan. The karst distribution was referenced from Bi⁸⁴. **(B)** Hydrology of the upper Yangtze and Pearl Rivers. Abbreviation: GHR, Guijiang-Hejiang River; LJR, Liujiang River; HSR, Hongshui River; ZYR, Zuojiang-Youjiang River, BPR, Beipanjiang River, NPR, Nanpanjiang River; UYR, upper Yangtze River; YJR, Yuanjiang River; WJR, Wujiang River; CSR, Chishui River; JSR, Jinshajiang River; DMR, Dadu-Minjiang River; PLR, Panlongjiang River; LXR, Lixianjiang River; YR, Yuan River. **(C)** Map of the Youjiang region showing the distribution of fault zones³⁷.

Response to the second Reviewer:

Dear Reviewer:

Thank you for your valuable comments on our manuscript, formerly titled "**Evolutionary history of Chinese cavefishes highlights how paleogeoclimatic and river capture processes shaped modern biodiversity.**" We hope you are aware that we have followed the suggestions of the first reviewer and revised the title. The revised title is "**Evolutionary history of Chinese cavefishes parallels paleogeoclimatic and river capture processes**". Below, we provide a point-by-point response to each of your remarks.

Sincerely,

Tao Luo

On behalf of all co-authors

Overall comments: I was one of the original reviewers of this manuscript. I am pleased with the changes that have been implemented and commend the authors. My last suggestion is that I would recommend moving the new paragraph comparing this manuscript to Mao et al. to later in the discussion instead of having it as the very first paragraph leading into the discussion.

Response: Thank you for your suggestion. We have followed your advice and moved the comparative content regarding Mao et al. to the end of the Discussion section. You can find these changes in the revised manuscript. For detailed information, see lines 309–332.

See lines 306 – 329:

Divergent perspectives on the evolutionary history of Chinese cavefishes: A comparison with recent study

Cavefishes are recognized as valuable models for biogeographic studies, with substantial evidence supporting their utility in revealing how paleogeographic events have shaped speciation and adaptive evolution in subterranean ecosystems^{14,25,26,30,41,76}. However, investigations focused on a single taxonomic group may have limited capacity to fully reconstruct how paleoclimatic and geological processes have influenced biodiversity evolution across broader spatial and temporal scales.

Our study builds upon and extends prior work in several key dimensions. For instance, a recent phylogenomic study by Mao et al.³⁰ provided critical insights into the roles of paleoclimate and gene flow in the radiation of the genus *Sinocyclocheilus*. While both studies document a strong association between

paleoclimatic events and cavefish evolutionary history, our work expands the scope in notable ways. First, we incorporated a significantly broader taxonomic sample (183 species, representing ~92% of known extant Chinese cavefishes) by integrating newly sequenced and publicly available data, offering a more comprehensive perspective on the origins of the regional cavefish diversity hotspot. Second, while Mao et al.³⁰ employed reduced-representation genomic data to reveal complex gene flow patterns within *Sinocyclocheilus*, our study utilized mitochondrial markers. Multiple studies have validated the utility of mitochondrial data, particularly with comprehensive node calibration, for inferring deep diversification histories and biogeographic events^{13,19,20,36,77-80}. Third, beyond shared focal drivers like QTP uplift and monsoon evolution, our analytical framework explicitly incorporated the effects of additional geophysical processes—including Indochina extrusion, phased karst development, and river capture events—and quantitatively reconstructed drainage network evolution through in situ diversification and dispersal metrics. In summary, the methodological and analytical differences between these studies provide complementary insights into the evolutionary history of Chinese cavefishes. Patterns such as cross-species gene flow identified at the genomic level may help explain biological events not fully resolved by phylogenetic methods alone³⁰. Future phylogenomic studies with expanded taxon sampling across diverse cavefish lineages will be essential to integrate these perspectives and further elucidate the mechanisms of speciation and diversification in this unique fauna.

Response to the first Reviewer:

Dear Reviewer:

Thank you for your valuable comments on our manuscript, formerly titled "**Evolutionary history of Chinese cavefishes highlights how paleogeoclimatic and river capture processes shaped modern biodiversity.**" The title has now been revised to "**Evolutionary history of Chinese cavefishes parallels paleogeoclimatic and river capture processes**". Your suggestions have been instrumental in refining our manuscript and will continue to guide our future research on cave fishes. We have thoroughly considered all of your comments and have made corresponding revisions to the text, with changes highlighted in **red** in the original document. Below, we provide a point-by-point response to each of your remarks.

Sincerely,

Tao Luo

On behalf of all co-authors

Overall comment: The authors have made substantial revisions to the manuscript and have carefully addressed most of the major concerns raised in the previous review. In particular, the differentiation from Mao et al. (2025), the clarification of ecological coding for cave-adapted fishes, and the harmonization of species numbers across different analyses have significantly improved the clarity and overall quality of the manuscript. However, a few issues still require further clarification.

Response: Thank you for your careful assessment of our manuscript and for the opportunity to further improve it. We sincerely appreciate your acknowledgment of the substantial revisions we have made. We have now carefully addressed the four additional points raised in your latest comments with detailed modifications to the manuscript. Thank you again for your constructive feedback, which has been invaluable in enhancing the clarity and rigor of our work.

Point-to-Point Response:

Comment 1. 1. The manuscript presents compelling temporal associations between cavefish diversification and major paleogeoclimatic and river-related events. However, in several places these associations are expressed in causal terms. The authors are encouraged to more clearly distinguish between correlation-based biological inference and direct causal interpretation, especially in the title, discussion and conclusions.

Response: We thank the reviewer for this crucial comment regarding the distinction between correlation and causation. We fully agree that our data demonstrate temporal associations, and direct causal claims would be over-

interpretation. We have now carefully revised the manuscript throughout, particularly in the title, discussion and conclusion sections. We have replaced definitive causal language with more cautious phrasing that highlights the strong temporal coincidence and proposes plausible mechanistic links without asserting direct causation. We believe these revisions significantly strengthen the scientific rigor and clarity of our argument.

The revised title is as follows:

Evolutionary history of Chinese cavefishes parallels paleogeoclimatic and river capture processes

Comment 2. 2. Line 362-368, section Estimation of divergence times, the manuscript states that divergence times were estimated in BEAST for “each dataset”, following the description of mitogenome sequencing, seven nuclear genes, and additional GenBank sequences. However, it is not entirely clear which sequence matrix (mitochondrial only, nuclear only, or concatenated mitochondrial + nuclear data) was actually used for the divergence time estimation. In addition, the manuscript specifies the clock model and tree prior, but the calibration strategy (e.g., fossil calibrations, secondary calibrations, or substitution rate priors) is not explicitly described in the main text.

Response: Dear Reviewer, thank you for raising these important points regarding the clarity of our divergence time estimation methodology. We sincerely appreciate your careful reading, as precise reporting of analytical procedures is indeed essential for reproducibility. We have now revised the manuscript to address both of your concerns.

(1) Clarification of sequence matrices used. As you correctly noted, the composition of available genetic markers varied across taxa in our dataset. To ensure robust phylogenetic reconstruction and divergence time estimation, we utilized three main types of sequence matrices, depending on the available data for each specific lineage comparison: 1) concatenated mitochondrial + nuclear genes (Tables S4, S7, S8, S9, S11); 2) concatenated mitochondrial genes only (Tables S5, S6, S10); 3) mitochondrial Cyt *b* gene only (Table S12). We have clarified this in the revised text (**lines 364–367**) to explicitly state which matrix type was used for each major analysis, directing readers to the corresponding supplementary tables for full details.

(2) Description of calibration strategy. We agree that the calibration methodology should be explicitly stated. We have now added a clear description in the main text. For our BEAST analyses, we employed a strategy combining fossil calibrations and secondary calibrations to derive time-calibrated phylogenies (**lines 370–372**). The specific calibration points (e.g., fossil constraints, their justified age priors, and sources for secondary calibrations) are now detailed in the newly added **Supporting Material** (also referenced in the main text), ensuring transparency and allowing for critical evaluation.

We believe these revisions have significantly improved the clarity and completeness of the methodology section concerning divergence time estimation. Thank you again for your valuable suggestions.

Revised Text

See lines 366–377:

Estimation of divergence times

Sequence alignment was performed with PhyloSuite v.1.2.3⁸¹ using MAFFT v.7.520⁸² for each gene fragment, followed by manual adjustments by MEGA v.7.0⁸³. **To ensure robust phylogenetic reconstruction and divergence time estimation, we utilized three main types of sequence matrices, depending on the available data for each specific lineage comparison: 1) concatenated mitochondrial + nuclear genes (Tables S4, S7, S8, S9, and S11); 2) concatenated mitochondrial genes only (Tables S5, S6, and S10); 3) mitochondrial Cyt *b* gene only (Table S12).** PartitionFinder v.2.1.1 was used to identify the best-fit evolutionary model⁸⁴ under the Bayesian information criterion (BIC), where each gene segment was defined as a partition. For each dataset, the divergence times were estimated in BEAST v.2.4.7⁸⁵ using the uncorrelated relaxed molecular clock, a Yule prior, and the best-fit model. **For our BEAST analyses, we employed a strategy combining fossil calibrations and secondary calibrations to derive time-calibrated phylogenies (see the Supporting Material for more details).** Tracer v.1.7.1⁸⁶ was used to assess an appropriate burn-in fraction and a sufficient effective sample size (> 200) .

Comment 3. 3. The revised classification of troglomorphic versus non-troglomorphic taxa is now clearly described. However, the manuscript does not explicitly state whether the main conclusions are sensitive to this revised coding. A brief clarification on whether the overall patterns remain unchanged after re-coding would improve transparency.

Response: Thank you for pointing this out, and we apologize for not addressing it sufficiently in our previous response.

In the revised manuscript, we have not only provided clearer definitions for "troglomorphic" and "non-troglomorphic" taxa based on the latest taxonomic research and field surveys but have also included newly described cavefish species. These data updates have had some impact on our analytical results, mainly reflected in:

(1) Changes in MDE curve shape. The updated species data led to differences in the peak height, timing of peaks, and the curve shape in specific basins (such as the Pearl River-Yangtze River basin) compared to the previous version of the MDE curve. This reflects the influence of more comprehensive data on biodiversity evolution estimates.

(2) Robustness of the main conclusions. Despite the changes in the details mentioned above, the core conclusions and overall patterns of our study remain unchanged after the data update. This includes the main findings

regarding the biodiversity hotspots, diversity evolution, and the driving role of environmental factors for Chinese cavefishes.

It is important to clarify that regarding the coding criteria for "troglomorphic" traits, we have strictly adhered to widely accepted definitions in the field (as indicated by the references, DOI: 10.1111/j.1095-8649.2011.03066.x; DOI: 10.1016/B978-0-12-814124-3.00027-3) from the initial study design. Therefore, the previous revision primarily involved a clearer description of taxon definitions and data expansion, rather than a change in the fundamental principles of classification coding.

These analysis results based on the latest data have further prompted us to emphasize, in the Discussion section of the previously revised manuscript (lines 215 – 217, 304 – 305, 327 – 329, 341 – 345), the importance of continuously improving species information in future research to achieve more stable and precise pattern recognition.

We appreciate your review and hope this clarification addresses your question.

See lines 215 – 217:

Thus, future phylogenomic studies encompassing broader taxonomic groups will be essential to advance understanding of the origins, diversification bursts, and adaptive radiation of the cavefish hotspot in the karst regions of southwestern China.

See lines 304 – 305:

Future studies incorporating a broader range of subterranean and surface-dwelling lineages would help to test, strengthen, and extend the proposed framework.

See lines 327 – 329:

Future phylogenomic studies with expanded taxon sampling across diverse cavefish lineages will be essential to integrate these perspectives and further elucidate the mechanisms of speciation and diversification in this unique fauna.

See lines 341 – 345:

However, limitations such as persistent gaps in taxon sampling and the current lack of genome-scale data may affect phylogenetic resolution and obscure the role of processes like gene flow. Future studies should prioritize expanded taxonomic sampling and generate high-quality genomic resources to further elucidate the diversification history, evolutionary mechanisms, and ecological adaptations of these unique subterranean biotas.

Comment 4. 4. Terms such as *in situ* appear frequently but are not always formatted consistently in italics throughout the manuscript. A final consistency check is suggested.

Response: Thank you for your valuable suggestion. We have carefully searched the entire manuscript to identify and standardize the formatting of terms such as "*in situ*". All instances have been revised to ensure consistent italicization throughout. We appreciate your attention to detail and the opportunity to improve the manuscript's consistency.