nature portfolio

Peer Review File

Ancient proteins identify various Denisovan remains from Southwest China

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Parts of this Peer Review File have been redacted as indicated to remove third-party material.

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

Version 0:

Reviewer comments:

Referee #1

(Remarks to the Author)
This study describes proteomics analyses (primarily screening) of fragmentary fossils from Bianfu cave, Yunnan, China. Using ZOOMS, the authors identified three specimens, a proximal radius and two parietal fragments as human, and then performed proteomics to look in more detail at the collagen peptides preserved. All three specimens preserve a single amino acid polymorphism in the Collagen alpha-2 (I) chain that is diagnostic for Denisovans, found in all other Denisovan specimens as well. After this, they constructed Bayesian phylogenetic trees, further confirming the phylogenetic positions of the remains.
As the two parietal fragments are not very diagnostic, they only compared parietal thickness to other fossils, showing that they fall within the variability of most groups, but tend to be somewhat thicker than is typical for recent modern humans (at the very high end of recent variability). Next they focus on the radius fragment, which is also rather fragmentary, and propose that the medial orientation of the radial tuberosity, the large radial head and the long neck indicate archaic affinities.

The study is both novel and significant, describing new hominin material and using proteomics to show it is Denisovan.

I'm not going to comment on the proteomics methods and analyses, as these are outside my area of expertise, but I have some comments on the morphological analyses.

First, I think that it is necessary to provide more details on how the orientation of the radial tuberosity was really assessed. A qualitative description is inherently subjective, especially in the case of fragmentary remains. BFD771 only preserves part of the radial head and neck, and not much of the shaft besides the radial tuberosity. Looking at the photos, I ask myself whether this specimen can be reliably oriented allowing to assess the orientation of the tuberosity. I think that the tuberosity would clearly influence how you determine the anatomical axes (anteroposterior, mediolateral etc), and so using these to then quantify the orientation of the tuberosity is a circular argument. Trinkaus and Churchill (1988) the cited reference, used the position of the interosseous crest to quantify the orientation of the tuberosity, but the crest is not preserved here.

Another issue is the reconstruction of radial head diameter - the authors fitted a circle for this, but one of the typical features of Neanderthal radii is the anteroposteriorly elliptical radial head (e.g. De Groote, 2011) - if this is also the case here (and one should assume so, based on the other similarities the authors claimed to see), then fitting a circle to it would lead to an increased ML diameter.

Smaller things:

Line 188: as mentioned above, how sure can the authors be that ML and AP radial head diameters are equal?

Line 190: measuring radial neck length to 1/100 of a mm (22.43) is overinflating measurement precision.

Line 197-200: I am not convinced this is anything novel - every other site I am familiar with also removed clearly non-human fragments before attempting to do Zooms, if all they were looking for were human specimens. Nobody wants to waste money and time…

Figure 1 a: I am not sure the Mercator projection is well suited for this map, it strongly distorts as you go further from the equator.

Referee #2

(Remarks to the Author)
The manuscript extracts ancient proteins evidence from human bone fragments found in the Bianfu Cave in southwestern China and confirmed that the Bianfu Cave individuals are Denisovans. This provides clearer insights into the broad geographical distribution of Denisovans in East Asia. In particular, Bianfu Cave offers the first identified radius bone of Denisovans and enables deeper research and understanding of the Denisovan population and represents a significant breakthrough. I recommend the article for publication in Nature in the future, with the following comments and suggestions prior to publication:

1.The title mentions "population dispersal," but this issue does not appear to be addressed in the text. The authors could discuss the possible population composition across East Asia during the period approximately between 200 ka and 50 ka, as well as the potential distribution characteristics of Denisovans.

2.Line 32: It is not advisable to directly use the minimum U-series dates of the bone and its cement-like carbonate deposits as the age of the human remains. While this may be acceptable in the absence of in situ stratigraphic context, Bianfu Cave had undergone meticulous excavation and had detailed stratigraphic dating studies. The stratigraphic age is more accurate and should be used as the reliable age for the Denisovan fossil remains to avoid discrepancies and misunderstandings in chronological interpretation. Please use or cite the stratigraphic age.

3.Lines 210–212: The direct dating results of the human bones span both glacial and interglacial stages (MIS 6–5). In fact, these dates are likely underestimated, so discussing a span across glacial-interglacial stages based on them is inaccurate. Stratigraphic ages should also be used here. During the glacial phase of this period, human populations in East Asia were already widely distributed and showed expansion trends, for example, in the Nihewan Basin and the Qinling Mountains region, even in high-latitude areas. Therefore, during the glacial phase of this period, the Yunnan-Guizhou Plateau likely did not serve as a glacial refuge. Instead, its glacial forest-steppe environment might have been more suitable for the survival of large mammals. Therefore, this area is suitable for human hunting activities. Thus, its significance is not that of a "refuge," but rather a "hunting paradise."

Additional minor issues:
4. Line 72: "190–68 ka" – please indicate the source.
5. Lines 93–95: The manuscript provides the minimum ages of bones from layers 9 and 7 without further explanation. Although raw data are available in the supplementary materials, a brief explanation is needed, either in the main text or the supplementary materials.
6. Page 201: Avoid using the minimum age, the stratigraphic age is more accurate.
7. Line 461:Figure 1a: What does the green area represent? Elevation? This does not appear to be a Digital Elevation Model map. A scale bar for distance is also needed. Figure 1b: Why are layers 9 and 7 marked in red and blue, respectively? What is the difference? Please clarify. In the stratigraphic profile, the labels "Upper Cultural Layer" and "Lower Cultural Layer" can be removed, as they are not mentioned in the text. Removing them would make the stratigraphic diagram more consistent.
8.Lines 501–502: Please indicate the source of the ages.

Referee #3

(Remarks to the Author)
Key results:
The authors used mass-spectrometry based proteomics to confidently identify Denisovan bones from Yunnan province, confirming their expected presence in southwest China. Their cutting-edge methodological approach (a systematic morphological screening, followed by collagen fingerprinting), allowed the multidisciplinary research team to identify possible denisovan bones from a massive assemblage of non-diagnostic fragments, that would often be ignored from such sites, as their morphology carries no signature of their taxonomic identity. With shotgun proteomic sequencing, they confirm the identity of these bones as Denisovan, and in doing so, reveal the first long-bone of a Denisovan - a fragmentary radius, preserving an articular surface. This radius provides key insight into the temporal occupation of Denisovans in the Yunnan province as well as their possible post-cranial phenotype.
Validity:
We observe no significant flaws that should prohibit publication, but we do have minor recommendations, detailed in our line-by-line review below.
Originality and significance:
Conclusions are original. The results presented here are of great significance to the field of paleoanthropology due to the presence of a Denisovan post-cranial bone. It is my understanding that the only other known post-cranial bones are a phalanx and fragmentary, uninformative rib and long-bone fragments.
Data & methodology:
Methodology is sound and reproducible. The screening approach to reduce 68,000 specimens down to 22 specimens was effective, as was the following ZooMS approach. The data is also accessible.
Appropriate use of statistics and treatment of uncertainties:

Yes, appropriate
Conclusions:
Yes, they follow the data. The presentation of their data in the main and supplementary figures also permits swift interpretation for a reader. We only have limited suggestions on this, point, detailed below.
Suggested improvements:
The title could
Abstract should mention how many bones were used for ZooMS
The presence of so many low-abundance collagens needs to be further validated. We think some of those collagens with low deamidation scores could be misassigned, as potentially they present unresolved sequences.
References:
Manuscript utilizes previous literature appropriately.
Clarity and context:
The abstract is very good, but I think it needs to be more clear how many bones/fragments were used for ZooMS here, and shotgun sequencing
Inflammatory material:
No
Springer Nature is committed to diversity, equity and inclusion; please raise any concerns that may in your view have an impact on this commitment.
We have no concerns.
Please indicate any particular part of the manuscript, data, or analyses that you feel is outside the scope of your expertise, or that you were unable to assess fully.
The morphological analysis, biomechanics
Uranium-series dating
Please address any other specific question asked by the editor via email.
Line-by-line edits
Lines 1-2: As the title needs to be shortened anyway following the guidelines of the journal, we suggest removing "...and population dispersal". Even if this is a big implication for this project, we do not think it is discussed in this paper in such detail to warrant being in the title. If the title needs to be shorter (75 characters?), then we recommend taking either half (before or after the semi-colon).

Lines 28-31: This sentence should be split, so that the number of specimens morphologically-screened is clear, and also the number of specimens screened through ZooMS is clear. It is not clear at the moment, and we think it could be read to a layperson that 68k bones were screened using both techniques (which would be a lot for ZooMS).

Lines 33-36: We really like this!

Line 54: We think this sentence "diverse… amino acid variation among Denisovan individuals" is possibly true, but not necessarily, so maybe the authors could change it to reflect "known amino acid variation" or something similar.

Lines 54-55: We recommend replacing "likely" with a word that is less definite e.g., "possibly", "plausibly", etc

Lines 79-82: We really like this approach and think it is very productive for identification of such large assemblages.

Lines 105-106: Maybe we would remove the word "conclusively" here, and recommend rephrasing it, as there could be other potential non-Homo genera in Pleistocene Asia (e.g., Meganthropus, possibly) during the Middle Pleistocene. Maybe our point is a bit pedantic, so this probably works as long as "conclusively" is removed, without rephrasing, too.

Lines 117-118: While the latter statement is true, it appears odd to recover so many different collagenous proteins, without encountering even a single non-collagenous protein (please correct us, if we are wrong).
We are concerned, especially seeing Extended Data Fig 3., that many of the low-abundance collagens could alternatively represent modern contamination, possibly from human handling of the specimens, or that they could be poorly-fragmented spectra with misassigned sequences. The deamidation rates are very low for some of these (within the range for contaminants).
It is quite interesting that the most common collagens encountered in bone (to our knowledge, COL1A1, COL1A2, COL2A1, COL5A1, COL5A2) all display very high deamidation rates in these analyses. We thought initially that the low-deamidation rates for other collagens may be because they are not particularly abundant, so it could be just due to random variation from a small sample size. But, even some of these expected proteins (e.g., COL2A1, COL5A1, COL5A2) are not particularly abundant in certain samples, but still display high deamidation rates.
If the authors are adamant about reporting these in the protein and amino acid counts (which could be okay; maybe they are authentic), then we would recommend the authors add additional steps to their methods in Supplementary Text 2 for confirming the endogeneity of the proteome. In this case, we can recommend to BLAST any possible alternative sequences, when accounting for poorly-fragmented ion series (e.g., if the b- or y- ions do not split two amino acid residues, then those amino acids could be in either order) and isobaric residues (e.g., I vs L, deamQ vs E, deamN vs D). For the first point, to illustrate it better in Suppl. File 4, with the annotated MS/MS spectra, "MS/MS spectrum covering COL1A2 996K, Source file:20231123_23M731.raw, Scan: 5503" displays the sequence GETGPSGPVGPAGAVGPK, but it could also alternatively be EGTGSPGPVGPAGAVGPK. With the very high similarity between different collagen chains, we think this step would be necessary to confidently identify the low-abundance collagenous proteins and include them in reporting. Possibly, it does not change the identification of these collagens, but maybe it could.
Our apologies if perhaps this is already done to an extent, but we had some trouble figuring out what presented data follows

the “strict MS2 support” method outlined on line 694.
Ultimately, this is not the most important part of the manuscript, and the conclusions still follow the data presented without this, but we would rather err on the side of caution here, particularly when it comes to reporting the raw number of proteins and amino acids.

Lines 211-212: We think this hypothesis is super interesting. Is there anything else to add here? If not, that is okay, but we think this could be an interesting point for further elaboration if it can be contextualized in any pre-existing theory/data.

Line 564: IAA is quite reactive, and can overalkylate terminal lysines. We wonder if this could be an issue since the most informative SAP/variant call in this paper rests on a C-term K. For this reason, it would be nice to see more visualized spectra, particularly for those highly-scoring spectra for each sample with the informative K at the end of the sequence.

Figure 2: Could you label the y-axis with a more clear term, e.g., “sequencing depth”, “depth of coverage”, “# of peptides/PSMs/spectra”, or something along those lines.

Those are our suggestions. Congratulations to the authors on an exciting manuscript.

Ryan Sinclair Paterson and Benjamin J. Cragun

Referee #4

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports.

Referee #5

(Remarks to the Author)
The manuscript entitled “A Denisovan radius from Southwest China: ancient proteins reveal Denisovan post-cranial morphology and population dispersal” reports the discovery of two partial parietal fragments and a proximal radial fragment from Bianfu Cave (Heqing County, western Yunnan Province, southwestern China), dated to approximately 190–68 ka. The remains were identified as hominin through ZooMS and subsequently attributed to Denisovans using proteomic analyses. The authors provide a morphological description of the material and comparisons with Neanderthals and modern humans. This constitutes an important contribution to paleoanthropology, as confirmed Denisovan postcranial remains remain exceptionally rare. The identification of what may represent the first Denisovan long bone, together with additional cranial material, is therefore of considerable significance and has the potential to substantially expand our understanding of Denisovan morphology. Given the exceptional nature of the material, additional morphological, and possibly functional, information on these remains, in particular on the (first) Denisovan long bone would have been highly valuable. Indeed, no details on the methods applied to study the morphology of the bones are provided and the comparisons with other hominins are limited. Below I provide details, not in a particular order, on the major comments and suggestions on how to make the best of this discovery. I also include at the end of the review minor comments. It should be noted that I am not a specialist in paleoproteomics or ZooMS analyses, and I therefore do not feel qualified to provide a detailed assessment of the paleoproteomic analyses.

Goal of the study & morphology of the remains
Proximal radius
As currently written, the abstract and introduction state that the findings and notably the proximal radius deliver “crucial insights into postcranial adaptation” and that the discovery of additional postcranial remains will clarify morphological variation and evolutionary adaptation. However, while it is clear that the primary goal of the study is to identify Denisovan bones, the main focus of the paper lies on the methodological approach, namely ZooMS and proteomic analyses, rather than on functional morphological analyses of the remains. I found that there is therefore a discrepancy between the claims made in the abstract and introduction regarding “crucial insights into postcranial adaptation” and the actual morphological analyses presented, which could be developed at a higher level. Here more details and a few suggestions:

Line 179: the authors wrote: “In cross-section, the radial neck appears elliptical and exhibits a posterior buttress, represented by the relatively thick cortical bone area.” However, more information is needed:
- Where exactly was the cross-section extracted (and how was the location standardized or identified as homologous between individuals)?
- How does the cross-sectional shape compare to other hominin fossils? What does “relatively thick cortical bone” mean in quantitative terms? The authors need to measure cortical thickness and compare it at least to modern humans in order to justify describing the cortex as “thick”.
- Finally, an area cannot technically be “thick.” Thickness refers to a linear dimension (e.g., cortical thickness), not to an area. So “thick cortical bone area” is imprecise here.

Regarding the best-fit estimation methods (Fig. 4), it is possible that the mediolateral diameter could be overestimated and that the radial head could not appear spherical, as often observed in Neanderthals (see Fig. 4 where the AP diameter is larger than the ML diameter in Neanderthals). If the mediolateral diameter cannot be estimated reliably, then only the anteroposterior diameter should be estimated. Importantly, this would not change the overall conclusion: the diameter would still appear closer to that observed in Neanderthals than in modern humans.

“The radial neck is 22.43 mm long”: which methods were used to measure radial neck length? Was it measured as the distance between the radial tuberosity and the radial head? There is no information on the measurement protocol, and I think this section is needed.

“The radial tuberosity is markedly elevated and faces medially. This configuration is observed in 76.5% of late Neanderthals, whereas all of Homo antecessor, 80% of early Neanderthals, and 88.8% of H. sapiens exhibit an anteromedially oriented tuberosity”. If I understand correctly, the “early Neanderthal” group only includes Sima de los Huesos. In that case, I suggest stating “80% of the Sima de los Huesos sample”. How did you calculate radial tuberosity orientation? This is critical to ensure that your observations are comparable to previously published measurements. Did you use the methods detailed in Rodríguez et al. (2016)?

To better characterize the morphological variation of this new and unique specimen attributed to Denisovans, a more comprehensive quantitative assessment would be highly valuable. Several anatomical proportions could be explored in greater detail. For example, the relative height of the radial head with respect to the neck would be informative, as it may relate to joint stability and the mechanical constraints associated with pronation and supination. But also the fovea diameter (or the length of the articular surface) is a relevant measurement. Reporting such measurements, even descriptively, would enrich the functional interpretation. However, in addition to the descriptions currently provided, the authors could consider conducting a geometric morphometric (GM) analysis to situate the specimen within a broader comparative framework. Even if preservation is incomplete, landmark-based approaches might still allow meaningful comparisons with Neanderthals, Homo sapiens, and other relevant hominins, capturing the broader proportions but also all together the differences in development and shape of the several features currently only qualitatively described by the authors.
Cross-sectional analyses of the radial neck would also considerably strengthen the study. Cross-sectional geometric properties—such as second moments of area, polar moment of area (J), and section moduli (Z values)—have been widely reported in the literature and provide important insights into biomechanical loading and functional adaptation. These metrics could help contextualize the specimen in terms of habitual forearm loading patterns and potential behavioral implications.
Finally, while the manuscript focuses primarily on comparisons with contemporaneous groups, functional interpretations often benefit from a broader evolutionary framework. Including earlier hominins (e.g., australopiths) and, where appropriate, extant taxa such as chimpanzees would provide important context for strengthen the evolutionary and biomechanical interpretations.

parietal fragments
The authors also state that it is “likely that Denisovans exhibit substantial intra-population morphological variation” and that direct evidence from skeletal remains, especially long bones, is essential for robust morphological and behavioral reconstructions. However, this claim appears overstated in the context of the present study. The study, by definition, cannot address such questions on the basis of one isolated specimen. At the same time, the manuscript reports additional cranial remains, including parietal fragments, which are particularly valuable given the limited Denisovan fossil record. They could contribute meaningfully to documenting morphological variability. However, their broader significance for understanding Denisovan diversity is not clearly integrated into the introduction nor fully developed in the discussion. Indeed, only very limited information is provided regarding the parietal fragments. There is no description or discussion of observable features such as sulci or vascular impressions, nor are there detailed morphological comparisons with other Denisovan material, or with Neanderthals and Homo sapiens. Even if the preserved parietal fragments are small, they nevertheless offer an opportunity to explore this question, at least preliminarily. A more detailed anatomical description and explicit comparisons with available Denisovan specimens (whenever possible), as well as with Neanderthals and Homo sapiens, would considerably strengthen the manuscript and better align the results with the broader claims of intra-population morphological variations made in the introduction.

Prescreening
Several times the manuscript mentions that a prescreening based on size, cortical thickness, and surface features was used prior to ZooMS analyses. It is also stated that this prescreening is important given the cost and time requirements of ZooMS, and the authors present this as a new or improved protocol. However, if the authors want to develop and promote this approach, the detailed protocol and methods need to be presented and expanded: how many researchers performed the prescreening, what level of experience they had, and what explicit criteria were used (e.g., how did they assess cortical thickness, morphological diagnostic features etc…) are important information if the two-step screening approach should be used in the future to aid in the identification of more Denisovan long bones as stated by the authors.

Archaeological site and references
The authors mention that four human teeth were unearthed from the middle of sub-layer 7. However, I was unable to locate a reference announcing the discovery of these four teeth. Additional information is required here, particularly because the newly reported parietal fragment and radius attributed to Denisovans derive from the same layer. This association raises several important questions. What type of teeth are these (e.g., anterior, posterior)? Do they preserve diagnostic morphological features that allow taxonomic attribution? Could they potentially belong to Denisovans? When referring to “human” teeth, do the authors specifically mean Homo sapiens? If so, does this imply that different hominin groups were using the cave during a similar time period? Finally, is there a specific age estimate available for sub-layer 7?
Similarly, further clarification regarding the stone artifacts is necessary. No references are provided, and I was unable to identify accessible publications detailing these materials. In which layers were the lithic artifacts recovered? Even if attribution cannot yet be verified, were any of them found in the same layer as the Denisovan remains?
In addition, the U–Th dating of the fossils and its consistency with previously published chronologies for the site are of central importance. If robust, these results would suggest Denisovan occupation during both glacial and interglacial periods

and could lend support to hypotheses proposing a possible glacial refugium on the Yunnan–Guizhou Plateau. It is therefore essential to provide the relevant references for previous OSL and U-series dating at the site in order to assess whether the current chronological framework is sufficiently robust.

ZooMS & paleoproteomic analyses
The proximal radius (BFD771) is clearly hominin in morphology. While I fully appreciate the value of proteomic confirmation, I was surprised that this specimen appears to have been treated as part of a broad screening of fragmentary bones, given its diagnostic anatomical features. In this case, the element does not appear ambiguous from a morphological standpoint.
The authors emphasize the importance of ZooMS as a tool for identifying fragmentary hominin remains and thereby expanding the sample available for functional and evolutionary interpretation. However, in the specific case of BFD771, the morphology of the proximal radius is sufficiently distinctive that its identification as hominin should have been possible prior to proteomic screening. This raises a certain discrepancy in the narrative: on the one hand, ZooMS is presented as a powerful method for detecting otherwise unidentifiable fragmentary remains; on the other hand, the most informative and best-preserved postcranial elements—those that can meaningfully contribute to functional analyses—are precisely those that are morphologically diagnostic without requiring ZooMS for taxonomic identification. The authors state that “wider use of the two-step screening approach established in our study would aid in the identification of more Denisovan long bones in the future.” However, if a long bone requires a two-step screening approach for identification, it is likely to be either highly fragmentary or lacking in clear diagnostic morphology. Such remains may, by definition, preserve more limited functional information compared to more complete and morphologically identifiable elements.

Minor comments
In several instances throughout the manuscript, including in the abstract, it should be clearly stated that the parietal remains are fragmentary and that the long bone consists specifically of a proximal radial fragment.
Line 43: “particularly long bones”: why? Articular and epiphyses can be highly relevant for joint mobility and movement (e.g., in hand elements).
Line 78: typo “throughout”
Line 82: it would be great to provide the dates of the layers here.

Version 1:

Reviewer comments:

Referee #1

(Remarks to the Author)
I congratulate the authors on this exciting study. They addressed all of my previous comments in detail, and I don't think any further changes are necessary, except for a minor correction of a typo I noticed:
Extended Data Fig 9: Australopithecus Sediba -> sediba

Referee #2

(Remarks to the Author)
The authors have carefully and thoroughly addressed my previous comments, and I am satisfied with the revisions made. The chronological evidence presented in this study appears robust and supports the reliability of the fossil ages. This work substantially advances our understanding of Denisovan population dynamics in East Asia and carries significant scientific implications. I therefore recommend the manuscript for publication.

Referee #3

(Remarks to the Author)
The authors have appropriately responded to all key suggestions from the first round of reviews.

We have additionally reviewed the newly-incorporated proteomic RAW data and the workflow for validating ambiguous peptides. The data remains solid, with the conclusions appropriately following the data presented, and the data analysis additions are very well-suited for this purpose.

We thus have no major suggestions, and we congratulate the authors on a fantastic manuscript.

We have only very minor suggestions:
Main text
Line 136: “Homonidae” should be “Hominidae”
Line 949 “...every three amino acid…” should be “every three amino acids”

Supplementary Data 4

“Decision” column in supplementary table. I think “unreliable” vs “reliable” is fine, but i wonder if it would be preferable to change to "accepted"/"rejected" (or something else if the authors prefer), as I suppose this table reflects how the authors integrated these peptides/positions into downstream analysis, and it is still quite difficult at the moment to outright label any

peptides in palaeoproteomics as "unreliable" in a broader sense.

Supplementary
Line 53: “tremble” should be “TrEMBL”
There are some more typos in the newly added sections
Line 79, Line 93: “deamdation”
Line 93: “other archaic hominin as well” should be “...hominins…”

Ryan Sinclair Paterson and Benjamin Cragun

Referee #4

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports.

Referee #5

(Remarks to the Author)
The authors have carefully addressed my previous comments, and the manuscript has been substantially improved. The results are exciting and represent an important contribution to our understanding of Denisovan distribution and morphology. I particularly congratulate the authors for incorporating additional morphological observations and quantitative analyses, which considerably strengthen the study. I still have several comments that I believe would further improve the manuscript. I also provide a few minor comments aimed at improving clarity and readability.

Major comments
1. I think there are still some inaccuracies in the way the discovery and identification of the hominin remains are presented. As for the two teeth, the radius is morphologically identifiable as hominin and proteomic analyses can help to confirm its taxonomic attribution and identify it as Denisovans.
Several statements throughout the manuscript may therefore be misleading. For example:
Page 4, lines 92–93: the fossils are described as "unidentifiable mammalian fragments," which is not accurate the radius.
Page 4, line 97: the term "non-diagnostic bone fragments" is similarly problematic because the radius possesses morphological characteristics that allowed its identification as a hominin element prior to proteomic confirmation.

2. The authors correctly note that one parietal fragment derives from Layer 7 whereas the other comes from Layer 9. Given the observed morphological differences (in cortical thickness) between the two specimens, could these differences potentially reflect temporal change?
This possibility may be worth discussing, particularly because Layer 7 dates to the later part of MIS 6, when climatic conditions may have differed from those associated with Layer 9. While the current sample size obviously prevents firm conclusions, I am wondering whether acknowledging this possibility would provide a broader interpretive framework for the observed variation.

3. I think the discussion could be expanded regarding the functional significance of the proximal radial morphology.
The authors note that the Denisovan radius shares certain characteristics with Neanderthals, including its large proximal dimensions and likely medially oriented radial tuberosity. However, the functional implications of these features are not fully explored.
A large proximal radius and a medially oriented radial tuberosity are often interpreted as increasing the mechanical advantage of the biceps brachii during elbow flexion and forearm supination. The combination of these apparently Neanderthal-like features with an overall morphology and cross-sectional geometry that more closely resemble modern humans is particularly intriguing. It may suggest a distinct loading regime or behavioral pattern in Denisovans compared with both Neanderthals and modern humans. I encourage the authors to further discuss these potential functional implications.

4. Page 4, line 114: it would be helpful to specify in the main text exactly which materials were dated using Uranium-series methods. Because the dating information currently relies on a cross-citation, it is difficult for readers to understand how the chronology of the different layers was established and what material was directly dated.

Minor comments
• Title: the title emphasizes the radius and teeth, but the study also reports two parietal fragments. Should the cranial remains be mentioned more explicitly?
• Page 3, line 65: I suggest adding the term "functional" before "variation."
• Page 4, line 108: it is unclear what the authors mean by "higher taxonomic resolution." Please clarify.
• Page 9, line 240: it is unclear what is meant by "category" in this context.
• Page 9, line 241: it may be worth mentioning that microCT analyses could potentially help assess ontogenetic status. This could become important in future studies when evaluating whether the assemblage represents one or multiple individuals.
• Page 10, line 269: before discussing Denisovan diversity, we first need additional fossils and a more complete characterization of the Denisovan phenotype. I would suggest rephrasing accordingly.
• Page 10, line 283: I suggest replacing "found" with "identified."
• Page 10, line 283: I suggest replacing "our" with "these."

• Page 11, line 296: I am not sure that "morphological diversity" should be emphasized here. Given the current sample size, the primary contribution of this study is the identification and description of additional Denisovan remains and the documentation of their phenotype. Understanding Denisovan morphological diversity will require a substantially larger fossil record.

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Referees' comments:

Referee #1 (Remarks to the Author):

This study describes proteomics analyses (primarily screening) of fragmentary fossils from Bianfu cave, Yunnan, China. Using ZOOMS, the authors identified three specimens, a proximal radius and two parietal fragments as human, and then performed proteomics to look in more detail at the collagen peptides preserved. All three specimens preserve a single amino acid polymorphism in the Collagen alpha-2 (I) chain that is diagnostic for Denisovans, found in all other Denisovan specimens as well. After this, they constructed Bayesian phylogenetic trees, further confirming the phylogenetic positions of the remains.

As the two parietal fragments are not very diagnostic, they only compared parietal thickness to other fossils, showing that they fall within the variability of most groups, but tend to be somewhat thicker than is typical for recent modern humans (at the very high end of recent variability). Next they focus on the radius fragment, which is also rather fragmentary, and propose that the medial orientation of the radial tuberosity, the large radial head and the long neck indicate archaic affinities.

The study is both novel and significant, describing new hominin material and using proteomics to show it is Denisovan.

Response: Thanks for your concise summary and positive feedback for our work.

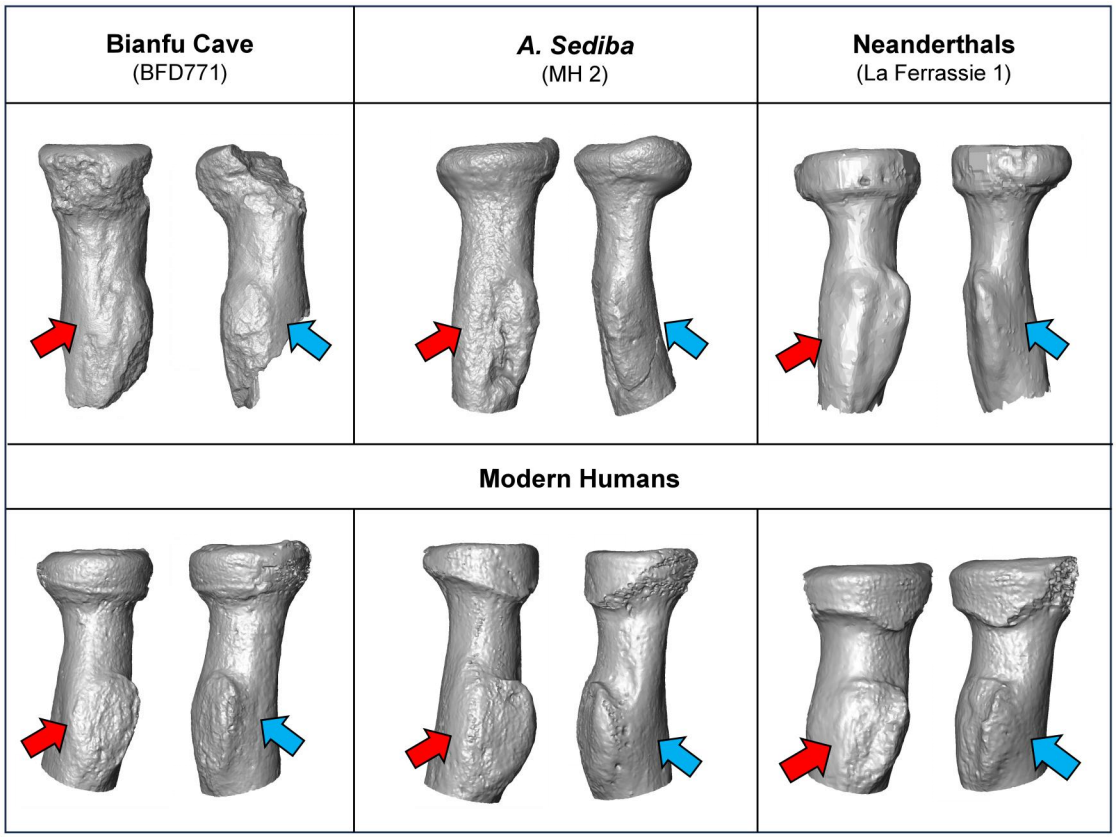
I'm not going to comment on the proteomics methods and analyses, as these are outside my area of expertise, but I have some comments on the morphological analyses.

First, I think that it is necessary to provide more details on how the orientation of the radial tuberosity was really assessed. A qualitative description is inherently subjective, especially in the case of fragmentary remains. BFD771 only preserves part of the radial head and neck, and not much of the shaft besides the radial tuberosity. Looking at the photos, I ask myself whether this specimen can be reliably oriented allowing to assess the orientation of the tuberosity. I think that the tuberosity would clearly influence how you determine the anatomical axes (anteroposterior, mediolateral etc), and so using these to then quantify the orientation of the tuberosity is a circular argument. Trinkaus and Churchill (1988) the cited reference, used the position of the interosseous crest to quantify the orientation of the tuberosity, but the crest is not preserved here.

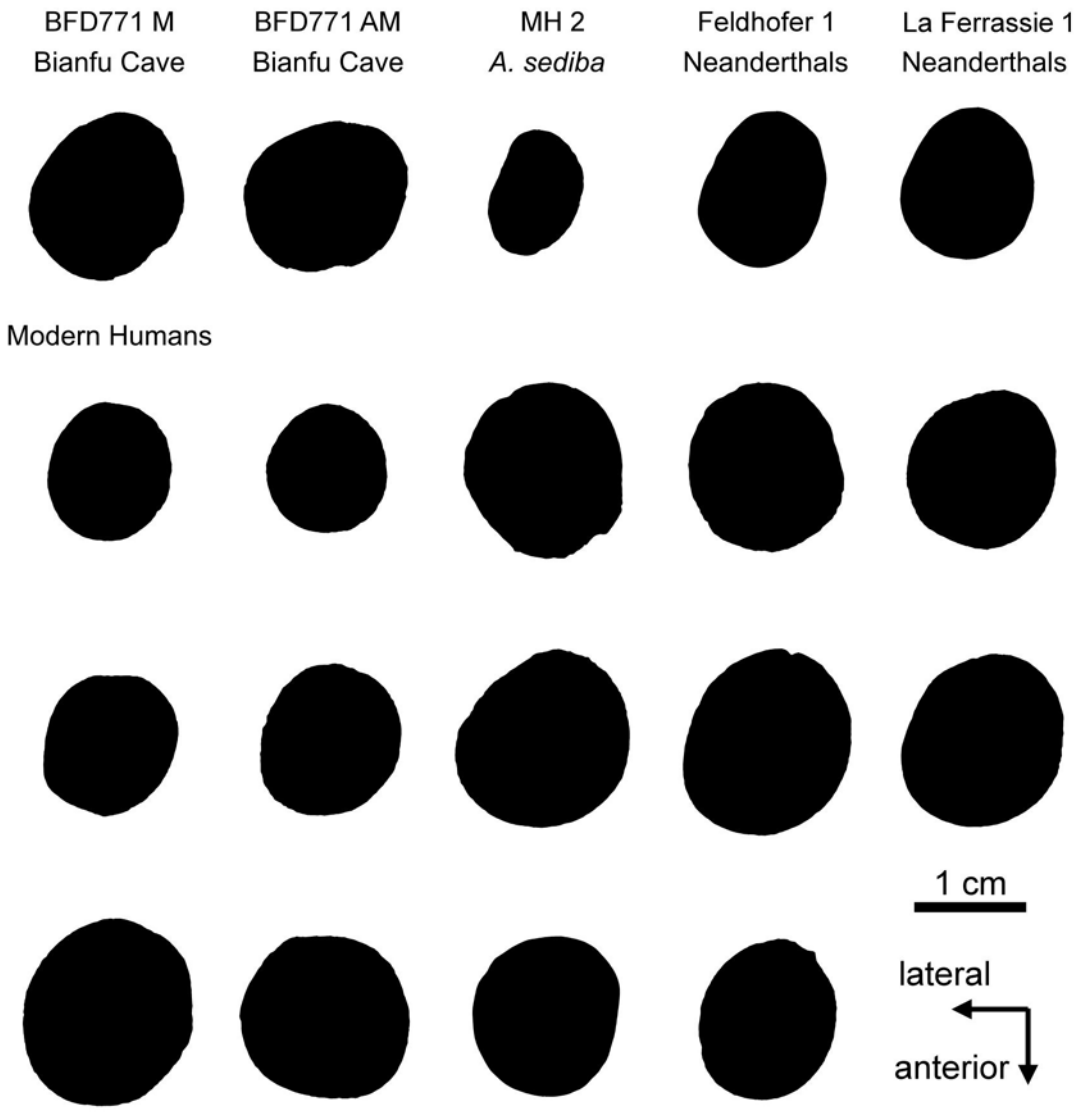
Response: Thank you for your constructive comment. In response, we have incorporated a detailed methodological description regarding the laterality determination and orientation of the radial tuberosity for the BFD radial fragment. Our analysis includes a comparative morphological

assessment of the radial tuberosity margin and the mid-neck cross-sectional geometry within a broad evolutionary framework, including modern humans, Neanderthals, and *Australopithecus*. This expanded comparison substantiates the classification of the BFD radius as a right-sided element with a medially oriented radial tuberosity. Consequently, we have refined the descriptions in the main text to reflect a more cautious interpretation and provided extensive documentation in the Extended Data Fig. 9, Supplementary Figs 12 and 13, to detail the diagnostic criteria employed.

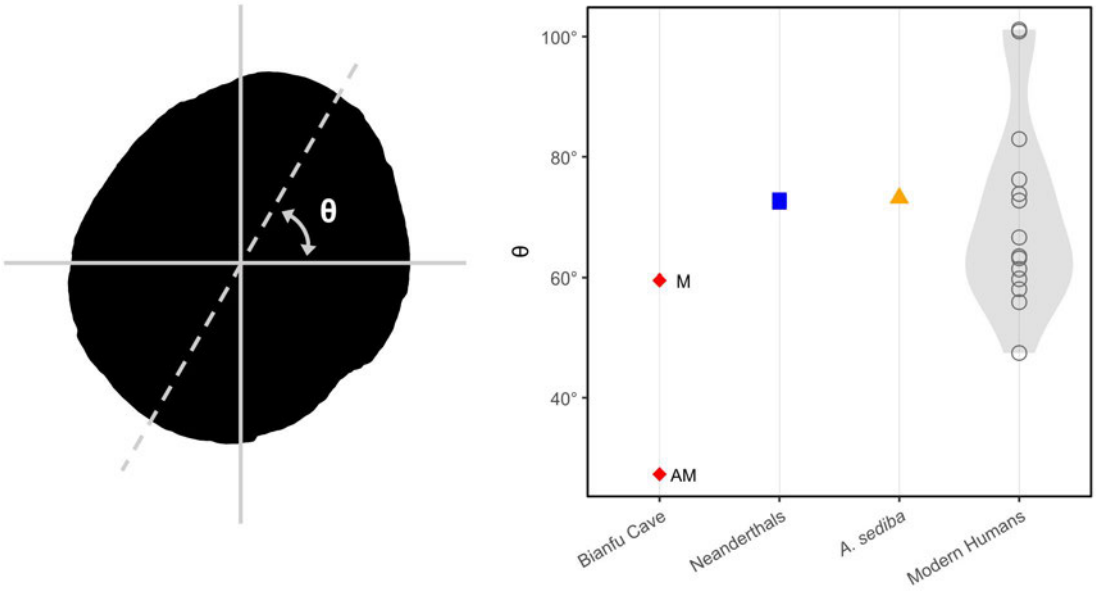
Main text: *Based on the marginal morphology of the radial tuberosity and the major axis orientation of the radial mid-neck cross-section, BFD771 is most likely a right radius with a medially oriented radial tuberosity (Extended Data Fig. 9, Supplementary Figs. 12 and 13).*



Extended Data Fig. 9. *Comparative morphology of the radial tuberosity. The proximal radius fossil from Bianfu Cave (BFD771) is shown within a comparative framework including Australopithecus sediba (MH 2), Neanderthals (La Ferrassie 1), and three modern humans. All comparative specimens are shown as right radii for comparative consistency, with the diaphysis distal to the radial tuberosity truncated to match the preservation of BFD771. Red arrows indicate the anterior margin of the radial tuberosity; blue arrows indicate the posterior margin. In all comparative specimens, the boundary between the tuberosity and the posterior diaphyseal surface is distinct, and the posterior diaphyseal surface is smooth. Conversely, the anterior margin of the tuberosity exhibits a gradual transition into the anterior diaphyseal surface without a distinct border, resulting in a structural elevation of the anterior diaphysis. BFD771 aligns with these morphological characteristics when oriented as a right radius.*



Supplementary Fig. 12. *Comparative cross-sectional analysis of the radial mid-neck. The proximal radius fossil from Bianfu Cave (BFD771) is compared against the right radii of Australopithecus sediba (MH 2), Neanderthals (Feldhofer 1 and La Ferrassie 1), and 14 modern humans. The intact preservation of comparative specimens enables precise alignment following standard anatomical orientation, with the interosseous crest defining the medial aspect. Cross-sections were extracted at the midpoint between the distal margin of the radial head and the proximal limit of the radial tuberosity. In the majority of comparative specimens, the major axis of the cross-section is oriented anterolaterally-posteromedially. BFD771 aligns with this morphology when oriented as a right radius. Two hypothetical orientations are modeled for BFD771: medial (M) and anteromedial (AM) positioning of the radial tuberosity.*



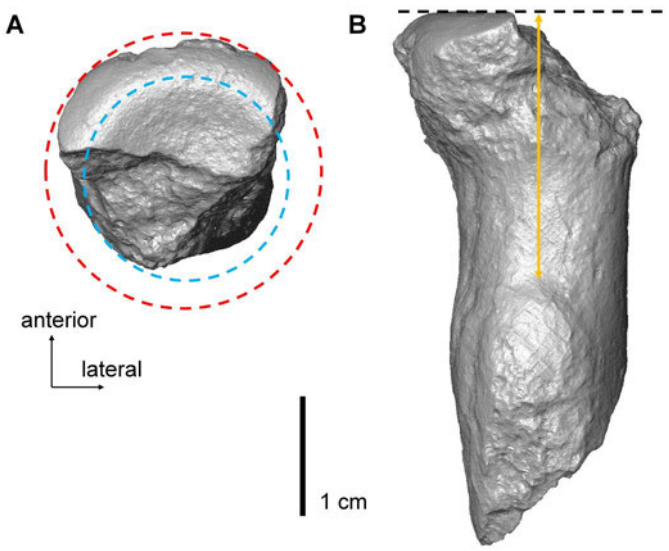
Supplementary Fig. 13. *Comparison of Theta (θ) in the radial mid-neck cross-section. The proximal radius fossil from Bianfu Cave (BFD771) is compared against the right radii of Australopithecus sediba (MH 2), Neanderthals (Feldhofer 1 and La Ferrassie 1), and 14 modern humans. Theta represents the orientation of maximum bending rigidity. Two hypothetical orientations for the radial tuberosity of BFD771 are present: medial (M) and anteromedial (AM). The medial orientation is considered most probable, as the major axis inclination of the BFD771 (AM) reconstruction represents an extreme outlier relative to the comparative specimens.*

Another issue is the reconstruction of radial head diameter - the authors fitted a circle for this, but one of the typical features of Neanderthal radii is the anteroposteriorly elliptical radial head (e.g. De Groote, 2011) - if this is also the case here (and one should assume so, based on the other similarities the authors claimed to see), then fitting a circle to it would lead to an increased ML diameter.

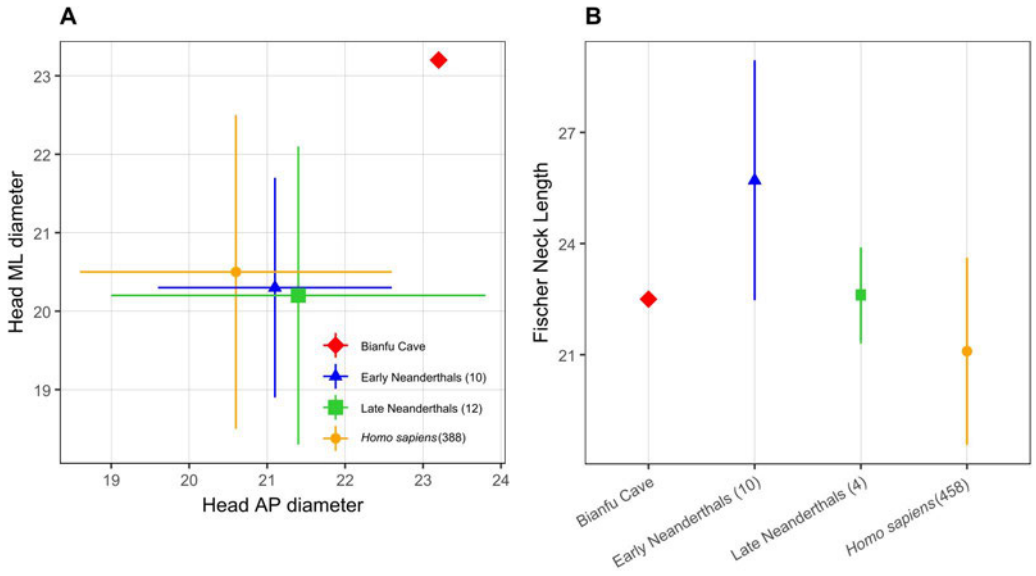
Response: Thank you for your comment. We acknowledge that the use of a best-fit circle is a simplification; its purpose was to provide a preliminary dimensional reference rather than a morphological reconstruction of the actual head shape, which we recognize is highly diagnostic for distinguishing Neanderthals from modern humans. We have revised the manuscript to explicitly state that this geometric fit serves as a dimensional proxy, and this restoration does not imply a circular morphology for the BFD specimen. To improve accuracy, we utilized the better-preserved radial fovea as a supplementary geometric constraint, resulting in a revised diameter of 23.2 mm.

Main text: *The radial head dimensions were estimated using best-fit circles corresponding to the preserved head margin (23.2 mm) and fovea articular edge (17.2 mm) (Supplementary Fig. 14A).*

Given the inherent limitations and limited taxonomic significance of reconstructing head size from fragmentary remains, we have relocated this analysis to the Supplementary Figs. 14 and 15.



Supplementary Fig. 14. *Osteometric measurement protocols for the proximal radius fossil from Bianfu Cave (BFD771). (A) Estimation of radial head (red) and fovea (blue) diameters. The radial head diameter is estimated using a best-fit circle constrained by the preserved anteromedial and anterolateral margins. The radial fovea diameter is estimated through a best-fit circle aligned with the path of the preserved articular edge. The foveal circle serves as a reliable geometric constraint, providing a secondary dimensional reference for the estimation of the radial head. (B) Radial neck length (Fischer neck length), defined as the distance between the superior medial margin of the radial head and the most proximal point of the radial tuberosity, following the definition of Rodríguez, et al.*



Supplementary Fig. 15. *Osteometric analysis of the proximal radius fossil from Bianfu Cave (BFD771). (A) Bivariate plot of radial head dimensions (anteroposterior versus mediolateral diameters). (B) Comparative analysis of radial neck length (Fischer neck length). Error bars indicate the mean ± one standard deviation. Comparative data are sourced from Rodríguez, et al.*

Smaller things:

Line 188: as mentioned above, how sure can the authors be that ML and AP radial head diameters are equal?

Response: Thank you for your comment. The equality of the ML and AP diameters results from our application of a best-fit circle, which was utilized as a direct method for providing a preliminary dimensional reference of the radial head. We believe this is necessary to provide the reader with an essential first-order estimate for the specimen's overall size. To address your concerns and prevent any misinterpretation, we have clarified our terminology regarding radial head dimensions. We now explicitly state that this geometric fit serves only as a preliminary approximation of size, and does not imply that the original morphology was perfectly circular. We also utilized the better-preserved radial fovea as a supplementary geometric constraint.

Main text: *The radial head dimensions were estimated using best-fit circles corresponding to the preserved head margin (23.2 mm) and fovea articular edge (17.2 mm) (Supplementary Fig. 14A).*

Line 190: measuring radial neck length to 1/100 of a mm (22.43) is overinflating measurement precision.

Response: Thank you for this suggestion. The initial measurements were conducted in Avizo 9.2 using the software's default decimal settings. In response to your comment, we have adjusted the decimal precision to 1/10.

Main text: *The fisher neck length (22.5 mm, Supplementary Fig. 14B) falls within the range of late Neanderthals and the upper portion of H. sapiens, and is comparatively shorter than those observed in early Neanderthals (Supplementary Fig. 15B).*

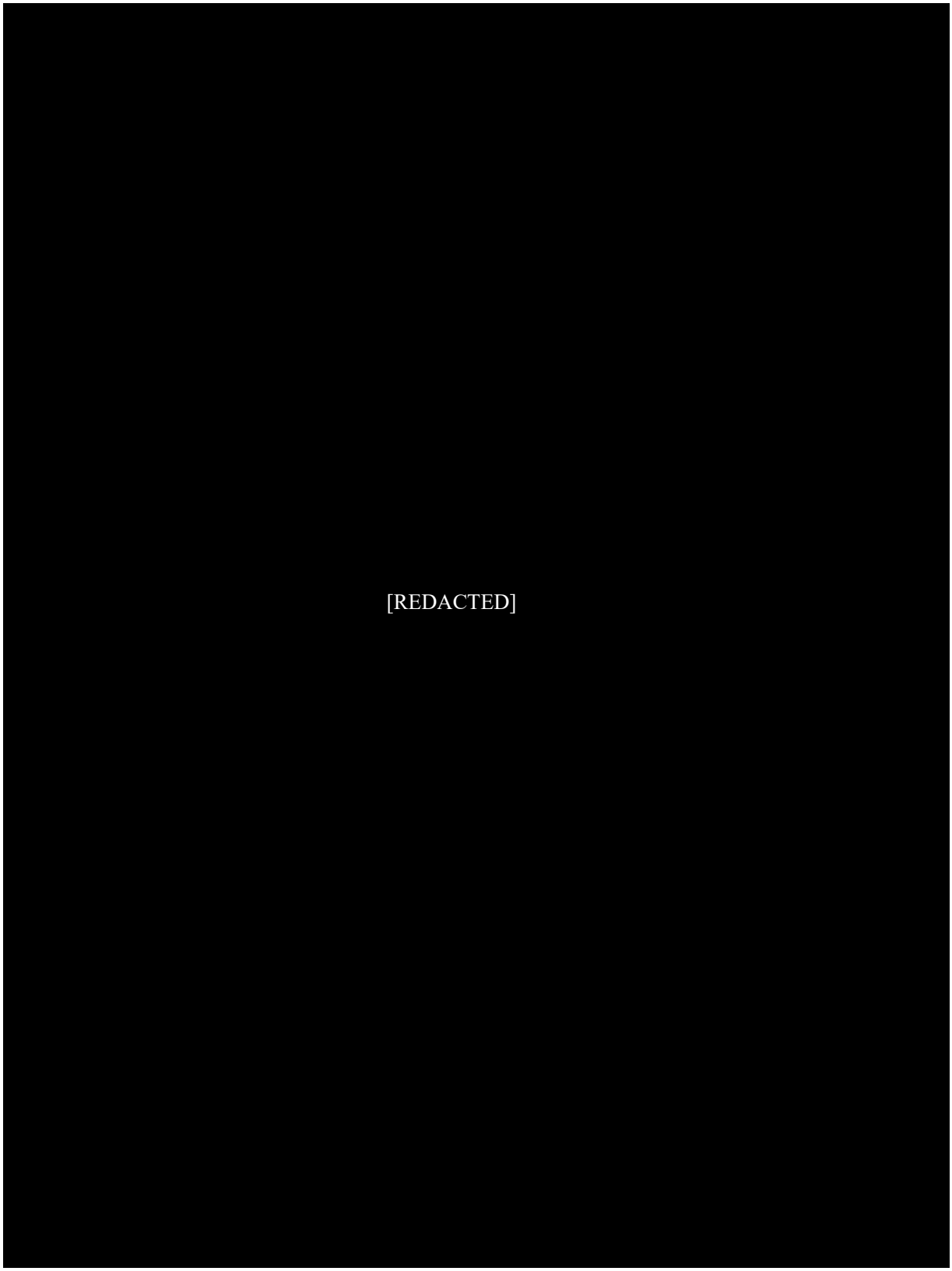
Line 197-200: I am not convinced this is anything novel - every other site I am familiar with also removed clearly non-human fragments before attempting to do Zooms, if all they were looking for were human specimens. Nobody wants to waste money and time…

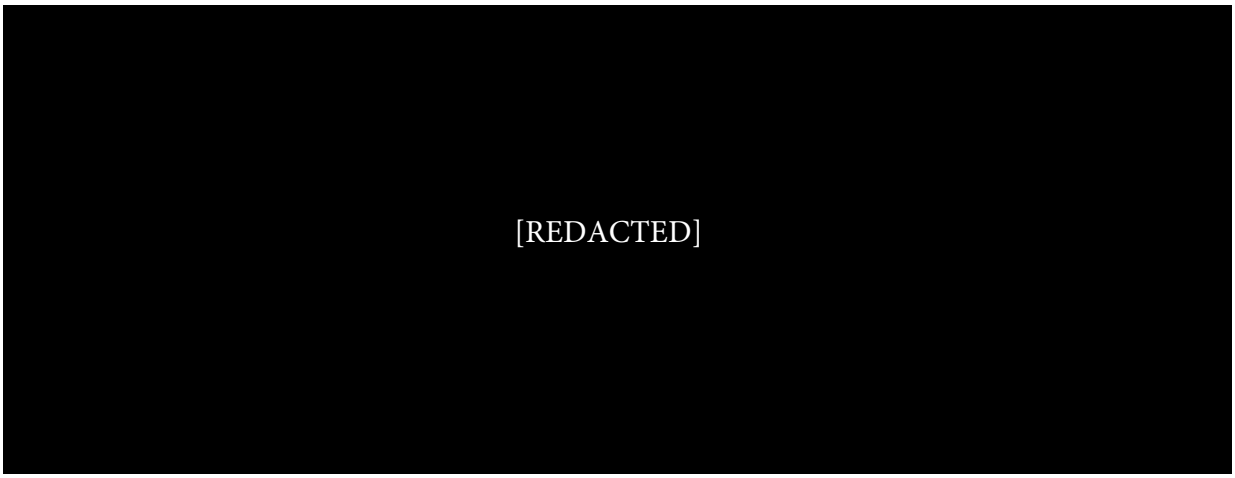
Response: Thank you for pointing this out. We now learn that some other sites also performed morphological prescreening before ZooMS. Therefore, we have revised the main text and described this "morphological prescreening + ZooMS" process as a regular approach without overemphasizing its innovation.

Main text: *Here we have performed a systematic morphological prescreening on more than 60,000 bone fragments from Bianfu Cave, and identified 22 candidate hominin specimens. Subsequent ZooMS and proteomic analyses confirmed three to be Denisovan remains, including two parietal bone fragments (BFD767 and BFD769) and one proximal radial fragment (BFD771).*

Figure 1 a: I am not sure the Mercator projection is well suited for this map, it strongly distorts as you go further from the equator.

Response: Thank you for your comment. We have revised the map accordingly. Now the map is projected using the Albers Equal-Area Conic projection with standard parallels at 22°N and 53°N and a latitude of origin at 37.5°N to minimize areal distortion across the latitudinal range of the study area (~15°N to ~65°N). Hope our revision could address your concern.




[REDACTED]

Referee #2 (Remarks to the Author):

The manuscript extracts ancient proteins evidence from human bone fragments found in the Bianfu Cave in southwestern China and confirmed that the Bianfu Cave individuals are Denisovans. This provides clearer insights into the broad geographical distribution of Denisovans in East Asia. In particular, Bianfu Cave offers the first identified radius bone of Denisovans and enables deeper research and understanding of the Denisovan population and represents a significant breakthrough. I recommend the article for publication in Nature in the future, with the following comments and suggestions prior to publication:

Response: Thanks for the concise summary and positive feedback to our work.

1.The title mentions "population dispersal," but this issue does not appear to be addressed in the text. The authors could discuss the possible population composition across East Asia during the period approximately between 200 ka and 50 ka, as well as the potential distribution characteristics of Denisovans.

Response: Thanks for your comment. We fully agree that "population dispersal" would be a big topic that could not be fully addressed in this manuscript. As suggested, we focused on Denisovans, and have added a discussion about the potential distribution characteristics of Denisovans in the main text. To make the title more consistent with our content, we have revised the title as "*Ancient proteins identify a Denisovan radius and teeth from Southwest China*". (Note: In the revision, we have performed additional proteomic analysis on two hominin teeth discovered during the excavation and also attributed them to Denisovans.)

Main text: *Combined with four hominin teeth, two of which (YHB3518 and YHB3075) were identified as Denisovan by proteomic analysis, these findings establish Bianfu Cave among the known Denisovan localities, second only to Denisova Cave in Siberia in its abundance of remains. This site lies along a major migration corridor linking the Qinghai-Tibet Plateau with East Asia, South Asia, and Southeast Asia, filling a critical geographic gap in the known distribution of Denisovans and revealing their presence across a vast range, from the northernmost reaches of Siberia and northeastern China, through the central Qinghai-Tibet Plateau, to the southeastern Taiwan Strait and now the southwestern Yunnan-Guizhou Plateau.*

2.Line 32: It is not advisable to directly use the minimum U-series dates of the bone and its cement-like carbonate deposits as the age of the human remains. While this may be acceptable in the absence of in situ stratigraphic context, Bianfu Cave had undergone meticulous excavation and had detailed stratigraphic dating studies. The stratigraphic age is more accurate and should be used as the reliable age for the Denisovan fossil remains to avoid discrepancies and misunderstandings in chronological interpretation. Please use or cite the stratigraphic age.

Response: Thanks for your comment. We have cited the stratigraphic age, which was conducted in another sister paper using a Bayesian age model combining the OSL and U-series dates.

Abstract: *This approach identified three hominin fossils, two parietal bone fragments and one proximal radial fragment, from layers dated to ~167-134 thousand years ago[1].*

1 Ruan, Q. et al. Denisovans from southwestern China and their subsistence strategies. Cross citation (2026).

3.Lines 210–212: The direct dating results of the human bones span both glacial and interglacial stages (MIS 6–5). In fact, these dates are likely underestimated, so discussing a span across glacial-interglacial stages based on them is inaccurate. Stratigraphic ages should also be used here. During the glacial phase of this period, human populations in East Asia were already widely distributed and showed expansion trends, for example, in the Nihewan Basin and the Qinling Mountains region, even in high-latitude areas. Therefore, during the glacial phase of this period, the Yunnan-Guizhou Plateau likely did not serve as a glacial refuge. Instead, its glacial forest-steppe environment might have been more suitable for the survival of large mammals. Therefore, this area is suitable for human hunting activities. Thus, its significance is not that of a "refuge," but rather a "hunting paradise."

Response: Thanks for your professional comment. We have used stratigraphic ages, ~167-134 ka, in the discussion, i.e., the hominin fossils occupied this site in the glacial phase (MIS 6). And we fully agree with you that human populations in East Asia were already widely distributed and showed expansion trends during this period. Based on the faunal and pollen analysis of Bianfu Cave, which was performed in another sister paper (cited in our manuscript), as well as the mammalian fauna from surrounding regions, the environment and fauna composition were also consistent with your interpretation. Thus, we revised the “refuge” to “hunting paradise” in our main text as you suggested.

Main text: *Our findings extend the Denisovan occupation to ~167-134 ka within Marine Isotope Stage (MIS) 6[1]. During this glacial period, the relatively warm climate and conifer-dominated forest or forest-steppe at Bianfu Cave and surrounding regions may have provided a stable supply of mammal resources, especially a predominance of medium- to large-sized herbivores[1,42-44]. This may have enabled the Yunnan-Guizhou Plateau to serve as a hunting paradise for hominins.*

1 Ruan, Q. et al. Denisovans from southwestern China and their subsistence strategies. Cross citation (2026).

42 Zhao, L. et al. New Discovery of Human Fossils and Associated Mammal Fauna from Mawokou Cave in Bijie, Guizhou Province of Southern China. Acta Anthropologica Sinica 35, 24-35 (2016).

43 Zhang, Z., Liu, J., Zhang, H. & Yuan, C. A pleistocene mammalian fauna from Panxian Dadong, Guizhou province. Acta Anthropologica Sinica 16, 209-220 (1997).

44 Schepartz, L., Stoutamire, S. & Bekken, D. Stegodon orientalis from Panxian Dadong, a Middle Pleistocene archaeological site in Guizhou, South China: Taphonomy, population structure and evidence for human interactions. Quaternary International 126-128, 271-282, doi:10.1016/j.quaint.2004.04.026 (2005).

Additional minor issues:

4.Line 72: "190–68 ka" – please indicate the source.

Response: We have added the citation.

5.Lines 93–95: The manuscript provides the minimum ages of bones from layers 9 and 7 without further explanation. Although raw data are available in the supplementary materials, a brief explanation is needed, either in the main text or the supplementary materials.

Response: Thanks for your comment. We have added a brief explanation in the main text: *Uranium-series dating provided minimum ages of ~102 ka for two samples from Layer 7 and ~143 ka for the parietal from Layer 9 (BFD769) (Materials and Methods, Supplementary Text 1, Extended Data Table 1). These ages are much younger than those of the corresponding layers, namely between 148-139 ka and 142-134 ka for Layer 7, and between 167-161 ka and 160-150 ka for Layer 9. Given that bones are open systems for Uranium-series dating, their ages may represent minimum ages, while the stratigraphic ages are more accurate.*

6.Page 201: Avoid using the minimum age, the stratigraphic age is more accurate.

Response: Thanks for your comment. We have used the more accurate stratigraphic age: *Our findings extend the Denisovan occupation to ~167-134 ka within Marine Isotope Stage (MIS) 6.*

7. Line 461:Figure 1a: What does the green area represent? Elevation? This does not appear to be a Digital Elevation Model map. A scale bar for distance is also needed. Figure 1b: Why are layers 9 and 7 marked in red and blue, respectively? What is the difference? Please clarify. In the stratigraphic profile, the labels "Upper Cultural Layer" and "Lower Cultural Layer" can be removed, as they are not mentioned in the text. Removing them would make the stratigraphic diagram more consistent.

Response: Thanks for your comment. We have revised Figure 1 accordingly. For Figure 1a, the new basemap uses cross-blended hypsometric tints from Natural Earth available at https://www.naturalearthdata.com/downloads/10m-cross-blend-hypso/10m-cross-blended-hypso-with-shaded-relief-and-water, where colors reflect a combination of elevation and environmental context (e.g., lowland warm-humid regions in green, lowland arid regions in brown, and highland regions in white). A scale bar is provided for distance reference. For Figure 1b, we just marked Layers 9 and 7 to show the stratigraphic layers containing Denisovan remains. Basically, they do not have much difference. So we have uniformed the color to blue. In the stratigraphic profile, the labels "Upper Cultural Layer" and "Lower Cultural Layer" have been removed as you suggested. As we have added new proteomic analysis on two hominin teeth discovered during the excavation to confirm their taxonomic attribution, we also added the photos of these teeth (YHB3518 and YHB3075) in Figure 1. Their labels were also added in Figure 1b.

[REDACTED]

[REDACTED]

8.Lines 501–502: Please indicate the source of the ages.

Response: We have added the citation.

Referee #3 (Remarks to the Author):

Key results:

The authors used mass-spectrometry based proteomics to confidently identify Denisovan bones from Yunnan province, confirming their expected presence in southwest China. Their cutting-edge methodological approach (a systematic morphological screening, followed by collagen fingerprinting), allowed the multidisciplinary research team to identify possible denisovan bones from a massive assemblage of non-diagnostic fragments, that would often be ignored from such sites, as their morphology carries no signature of their taxonomic identity. With shotgun proteomic sequencing, they confirm the identity of these bones as Denisovan, and in doing so, reveal the first long-bone of a Denisovan - a fragmentary radius, preserving an articular surface. This radius provides key insight into the temporal occupation of Denisovans in the Yunnan province as well as their possible post-cranial phenotype.

Validity:

We observe no significant flaws that should prohibit publication, but we do have minor recommendations, detailed in our line-by-line review below.

Originality and significance:

Conclusions are original. The results presented here are of great significance to the field of paleoanthropology due to the presence of a Denisovan post-cranial bone. It is my understanding that the only other known post-cranial bones are a phalanx and fragmentary, uninformative rib and long-bone fragments.

Data & methodology:

Methodology is sound and reproducible. The screening approach to reduce 68,000 specimens down to 22 specimens was effective, as was the following ZooMS approach. The data is also accessible.

Appropriate use of statistics and treatment of uncertainties:

Yes, appropriate

Conclusions:

Yes, they follow the data. The presentation of their data in the main and supplementary figures also permits swift interpretation for a reader. We only have limited suggestions on this, point, detailed below.

Response: Thanks for your detailed summary and great support for our work.

Suggested improvements:

The title could

Abstract should mention how many bones were used for ZooMS

Response: Thanks for your suggestion. We have revised accordingly.

Abstract: *To identify additional hominin long bones, we morphologically screened more than 60,000 bone fragments from Bianfu Cave in Southwest China, of which 76 were selected for ZooMS, and 17 for LC-MS/MS analysis.*

The presence of so many low-abundance collagens needs to be further validated. We think some of those collagens with low deamidation scores could be misassigned, as potentially they present unresolved sequences.

Response: Thanks for your constructive comment. We have confirmed the identification of low-abundance collagens according to your specific suggestions given below and the details are shown in the response for those suggestions.

References:

Manuscript utilizes previous literature appropriately.

Response: Thanks for your recognition.

Clarity and context:

The abstract is very good, but I think it needs to be more clear how many bones/fragments were used for ZooMS here, and shotgun sequencing

Response: Thanks for your suggestion. We have revised accordingly.

Abstract: *To identify additional hominin long bones, we morphologically screened more than 60,000 bone fragments from Bianfu Cave in Southwest China, of which 76 were selected for ZooMS, and 17 for LC-MS/MS analysis.*

Inflammatory material:

No

Springer Nature is committed to diversity, equity and inclusion; please raise any concerns that may in your view have an impact on this commitment.

We have no concerns.

Please indicate any particular part of the manuscript, data, or analyses that you feel is outside the scope of your expertise, or that you were unable to assess fully.

The morphological analysis, biomechanics

Uranium-series dating

Please address any other specific question asked by the editor via email.

Line-by-line edits

Lines 1-2: As the title needs to be shortened anyway following the guidelines of the journal, we suggest removing "...and population dispersal". Even if this is a big implication for this project, we do not think it is discussed in this paper in such detail to warrant being in the title. If the title needs to be shorter (75 characters?), then we recommend taking either half (before or after the semi-colon).

Response: Thanks for your comment. We fully agree that "population dispersal" would be a big topic that could not be fully discussed in this manuscript. To make the title more consistent with our content, we have revised the title as "*Ancient proteins identify a Denisovan radius and teeth from Southwest China*". (Note: In the revision, we have performed additional proteomic analysis on two hominin teeth discovered during the excavation and also attributed them as Denisovans.)

Lines 28-31: This sentence should be split, so that the number of specimens morphologically-screened is clear, and also the number of specimens screened through ZooMS is clear. It is not clear at the moment, and we think it could be read to a layperson that 68k bones were screened using both techniques (which would be a lot for ZooMS).

Response: Thanks for your suggestion. We have revised accordingly.

Abstract: *To identify additional hominin long bones, we morphologically screened more than 60,000 bone fragments from Bianfu Cave in Southwest China, of which 76 were selected for ZooMS, and 17 for LC-MS/MS analysis.*

Lines 33-36: We really like this!

Response: Thanks for your positive feedback.

Line 54: We think this sentence "diverse… amino acid variation among Denisovan individuals" is possibly true, but not necessarily, so maybe the authors could change it to reflect "known amino acid variation" or something similar.

Response: We have revised as "*known spatiotemporal distribution and amino acid variation among Denisovan individuals*".

Lines 54-55: We recommend replacing "likely" with a word that is less definite e.g., "possibly", "plausibly", etc

Response: Thanks for your comment. To address you and other reviewer's concerns and avoid overstatement, we have revised this sentence as "*Additionally, given the divergent Denisovan*

ancestry in modern populations and the known spatiotemporal distribution and amino acid variation among Denisovan individuals, a broader fossil record is needed to define Denisovan morphological diversity. ”

Lines 79-82: We really like this approach and think it is very productive for identification of such large assemblages.

Response: Thanks for your positive feedback.

Lines 105-106: Maybe we would remove the word “conclusively” here, and recommend rephrasing it, as there could be other potential non-Homo genera in Pleistocene Asia (e.g., Meganthropus, possibly) during the Middle Pleistocene. Maybe our point is a bit pedantic, so this probably works as long as “conclusively” is removed, without rephrasing, too.

Response: We have removed the word “conclusively” as you suggested.

Lines 117-118: While the latter statement is true, it appears odd to recover so many different collagenous proteins, without encountering even a single non-collagenous protein (please correct us, if we are wrong).

We are concerned, especially seeing Extended Data Fig 3., that many of the low-abundance collagens could alternatively represent modern contamination, possibly from human handling of the specimens, or that they could be poorly-fragmented spectra with misassigned sequences. The deamidation rates are very low for some of these (within the range for contaminants).

It is quite interesting that the most common collagens encountered in bone (to our knowledge, COL1A1, COL1A2, COL2A1, COL5A1, COL5A2) all display very high deamidation rates in these analyses. We thought initially that the low-deamidation rates for other collagens may be because they are not particularly abundant, so it could be just due to random variation from a small sample size. But, even some of these expected proteins (e.g., COL2A1, COL5A1, COL5A2) are not particularly abundant in certain samples, but still display high deamidation rates.

If the authors are adamant about reporting these in the protein and amino acid counts (which could be okay; maybe they are authentic), then we would recommend the authors add additional steps to their methods in Supplementary Text 2 for confirming the endogeneity of the proteome. In this case, we can recommend to BLAST any possible alternative sequences, when accounting for poorly-fragmented ion series (e.g., if the b- or y- ions do not split two amino acid residues, then those amino acids could be in either order) and isobaric residues (e.g., I vs L, deamQ vs E, deamN vs D). For the first point, to illustrate it better in Suppl. File 4, with the annotated MS/MS spectra, “MS/MS spectrum covering COL1A2 996K, Source file:20231123_23M731.raw, Scan: 5503” displays the sequence GETGPSGPVGPAGAVGPK, but it could also alternatively be EGTGSPGPVGPAGAVGPK. With the very high similarity between different collagen chains, we think this step would be necessary to confidently identify the low-abundance collagenous proteins and include them in reporting. Possibly, it does not change the identification of these collagens,

but maybe it could.

Our apologies if perhaps this is already done to an extent, but we had some trouble figuring out what presented data follows the “strict MS2 support” method outlined on line 694.

Ultimately, this is not the most important part of the manuscript, and the conclusions still follow the data presented without this, but we would rather err on the side of caution here, particularly when it comes to reporting the raw number of proteins and amino acids.

Response: Thanks very much for your constructive suggestions, which significantly help us improve the rigor of our study. According to your advice, we have added additional “permutation and unipept confirmation” steps in Supplementary Text 2 to validate the identifications of low-abundance collagens. Except the five most common collagens in bone or dentine (COL1A1, COL1A2, COL2A1, COL5A1, COL5A2), all the other collagens were considered low abundant. For each low-abundance collagen, all its PSMs were validated through the following steps (workflow shown in Supplementary Fig. 18).

(1) For each PSM, all the possible permutations (potential peptide candidates) isobaric to the annotated precursor are generated. These isobaric changes include two patterns you have suggested, one is the positional isomer caused by poorly-fragmented ion series, and the other is single residue isobaric switch. Besides the I/L isobaric pairs, we also considered three additional common pairs, i.e. deamidated Q [Q(+0.98)] vs E, deamidated N [N(+0.98)] vs D and py-Glu from E[E(-18.01)] vs py-Glu from Q[Q(-17.03)]. We take the spectrum you have mentioned above as an example, i.e. the “MS/MS spectrum covering COL1A2 996K, Source file:20231123_23M731.raw, Scan: 55035”, which displays the sequence GETGPSGPVGPAGAVGPK. However, after considering the coverage of y- and b- ions and the presence of isobaric amino acid residues in this sequences, seven alternative sequences would also be possible candidates, including GQTGSPGPVGPAGAVGPK, EGTGPSGPVGPAGAVGPK, EGTGSPGPVGPAGAVGPK, GQTGPSGPVGPAGAVGPK, QGTGPSGPVGPAGAVGPK, QGTGSPGPVGPAGAVGPK, GETGSPGPVGPAGAVGPK.

(2) BLAST all permutations to retrieve their possible matches in the entire database. Note that due to the low coverage of y- and b- ions or the presence of many isobaric amino acids in some PSMs, the number of permutations can be very large. To better balance the computational resources, PSMs with more than 10,000 permutations are considered too ambiguous and excluded while the PSMs with less than 10,000 permutations are retained for further validation. For each PSM, every permutation need to be BLASTed. Here we discard the common BLAST tools, which search against the entire NCBInr database, as it would be time-consuming. Instead, we used the more efficient pep2prot tool in Unipept (https://api.unipept.ugent.be/api/v2/pept2prot) to obtain all the UniProt entries in the entire UniProt database (including SwissProt and Tremble), which could match the given sequence.

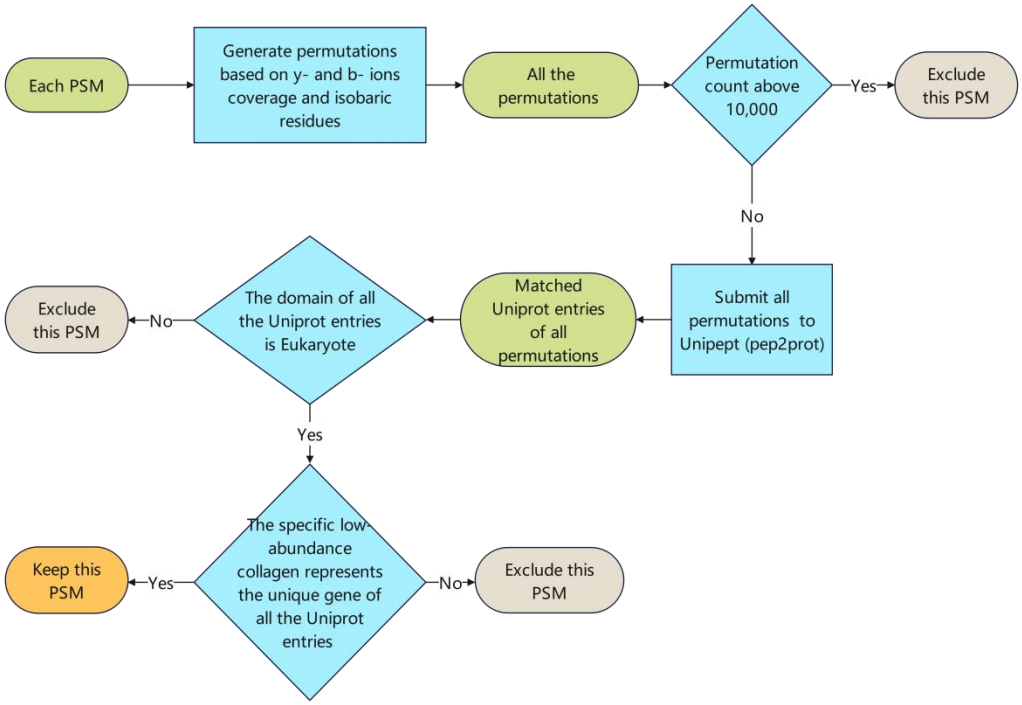
(3) For each PSM, summarize the matching results of all the permutations. If one of these Uniprot matches belongs to other protein (with different gene name from the low-abundance collagen, which may indicate incorrect identification) or domain other than Eukaryote such as Bacteria (possible contamination sources), this PSM is considered a possible misassignment, thus excluded from further calculations. PSMs that match unique gene name and species limited to Eukaryote

are considered reliable matches to this low-abundance collagen.

After these “permutation and unipept confirmation” processes, the reliable PSMs of each low-abundance collagen were retained for calculating the numbers of Q/N-containing peptides and related deamidation values. As the deamidation values were calculated based on intensity, only those peptides with valid intensity would be used in the calculation. Thus the numbers of Q/N-containing peptides with valid intensity were calculated. If at least two Q/N-containing peptides with valid intensity were identified in a single protein for deamidation calculation, we considered the values reliable. Combined with the results of other proteins, the endogenous proteins were determined based on the reliable deamidation values supported by at least two Q/N-containing peptides.

After this additional validation, only three low-abundance collagens were identified across five Bianfu Cave hominin samples, i.e. COL23A1 in the parietal BFD767, COL15A1 in parietal BFD769, and COL11A2 in tooth YHB3518. These collagens have also been reported in other hominin samples, such as Xiahe 2, Harbin, and Penghu. The details were provided in Supplementary Text 2， Extended Data Table 2, Extended Data Fig. 3, Supplementary Figs. 1-4. Compared with the original results, the identified proteins, peptides and amino acid coverage were less, but more reliable.

Regarding to “strict MS2 support”, it is one of the steps used to validate the informative SAPs within the *Homo* genus. The MS/MS spectrum for each supporting PSM of the specific SAP was manually inspected to check the fragmentary y- and b- ions surrounding the SAP. The “strict MS2 support” criterion requires at least two y- ions or two b- ions flanking the SAP. When the SAP is situated at the terminus of the peptide, PSMs with only one associated y- or b- ion were also considered to be “strict MS2 support”. For a reliable SAP identification, we require at least one peptide has “strict MS2 support” PSMs. This process is different from the “permutation and unipept confirmation” steps, which are suggested for the validation of low-abundance collagens.



Supplementary Fig. 18. *The workflow for the "permutation and unipept confirmation" steps to validate the identification of low-abundance collagens.*

Lines 211-212: We think this hypothesis is super interesting. Is there anything else to add here? If not, that is okay, but we think this could be an interesting point for further elaboration if it can be contextualized in any pre-existing theory/data.

Response: Thanks for your comment. But based on the update and suggestion from the other reviewer, we revised this part. Now we have used the more accurate stratigraphic ages, ~167-134 ka, in the discussion, i.e., the hominin fossils occupied this site in the glacial phase (MIS 6). And during this period, human populations in East Asia were already widely distributed and showed expansion trends. After incorporating the faunal and pollen analysis of Bianfu Cave, which was performed in another sister paper (cited in our manuscript), as well as the mammalian fauna from surrounding regions, we revised the "refuge" to "hunting paradise" in our main text for better elucidation.

Main text: *Our findings extend the Denisovan occupation to ~167-134 ka within Marine Isotope Stage (MIS) 6[1]. During this glacial period, the relatively warm climate and conifer-dominated forest or forest-steppe at Bianfu Cave and surrounding regions may have provided a stable supply of mammal resources, especially a predominance of medium- to large-sized herbivores[1,42-44]. This may have enabled the Yunnan-Guizhou Plateau to serve as a hunting paradise for hominins.*

1 Ruan, Q. et al. Denisovans from southwestern China and their subsistence strategies. Cross citation (2026).

42 Zhao, L. et al. New Discovery of Human Fossils and Associated Mammal Fauna from Mawokou Cave in Bijie, Guizhou Province of Southern China. Acta Anthropologica Sinica 35, 24-35 (2016).

43 Zhang, Z., Liu, J., Zhang, H. & Yuan, C. A pleistocene mammalian fauna from Panxian Dadong, Guizhou province. Acta Anthropologica Sinica 16, 209-220 (1997).

44 Schepartz, L., Stoutamire, S. & Bekken, D. Stegodon orientalis from Panxian Dadong, a Middle Pleistocene archaeological site in Guizhou, South China: Taphonomy, population structure and evidence for human interactions. Quaternary International 126-128, 271-282, doi:10.1016/j.quaint.2004.04.026 (2005).

Line 564: IAA is quite reactive, and can overalkylate terminal lysines. We wonder if this could be an issue since the most informative SAP/variant call in this paper rests on a C-term K. For this reason, it would be nice to see more visualized spectra, particularly for those highly-scoring spectra for each sample with the informative K at the end of the sequence.

Response: Thanks for pointing this out. To address your concern, we have checked the off-target alkylation in the two fractions that have been treated with IAA, i.e., the "acid insoluble fraction elastase" and "acid insoluble fraction trypsin" from the parietal BFD767. For the elastase fraction, Carbamidomethylation only occurred on Cystine (C), without any off-target alkylation. However, off-target alkylation was detected in the trypsin fraction, mainly on the N-terminal of peptides or Glutamic acid (E) in the peptides, with a small amount also occurring on Lysine (K). Only 3 PSMs underwent Carbamidomethylation modification at the C-terminal K, accounting for 1.40% of all Carbamidomethylation modifications, and all these 3 PSMs were from COL1A1. As for C-terminal K, over 98% remained unmodified, while only 0.05% underwent Carbamidomethylation modification. Thus, we can see that overalkylation has little impact on the identification of C-terminal K in our sample. So far, none of the COL1A2 996K-related spectra identified in five hominin samples have Carbamidomethylation modification at the K position. We have also provided more high-quality spectra in Supplementary Data 5.

Figure 2: Could you label the y-axis with a more clear term, e.g., "sequencing depth", "depth of coverage", "# of peptides/PSMs/spectra", or something along those lines.

Response: Thanks for your comments. We have labeled the y-axis as "Peptide Depth" for clarification.

Those are our suggestions. Congratulations to the authors on an exciting manuscript.

Response: We are very appreciated of your great support.

Ryan Sinclair Paterson and Benjamin J. Cragun

Referee #4 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports.

Response: We thank the reviewer for co-reviewing our manuscript and hope we have addressed all your concerns.

Referee #5 (Remarks to the Author):

The manuscript entitled “A Denisovan radius from Southwest China: ancient proteins reveal Denisovan post-cranial morphology and population dispersal” reports the discovery of two partial parietal fragments and a proximal radial fragment from Bianfu Cave (Heqing County, western Yunnan Province, southwestern China), dated to approximately 190–68 ka. The remains were identified as hominin through ZooMS and subsequently attributed to Denisovans using proteomic analyses. The authors provide a morphological description of the material and comparisons with Neanderthals and modern humans. This constitutes an important contribution to paleoanthropology, as confirmed Denisovan postcranial remains remain exceptionally rare. The identification of what may represent the first Denisovan long bone, together with additional cranial material, is therefore of considerable significance and has the potential to substantially expand our understanding of Denisovan morphology. Given the exceptional nature of the material, additional morphological, and possibly functional, information on these remains, in particular on the (first) Denisovan long bone would have been highly valuable. Indeed, no details on the methods applied to study the morphology of the bones are provided and the comparisons with other hominins are limited. Below I provide details, not in a particular order, on the major comments and suggestions on how to make the best of this discovery. I also include at the end of the review minor comments. It should be noted that I am not a specialist in paleoproteomics or ZooMS analyses, and I therefore do not feel qualified to provide a detailed assessment of the paleoproteomic analyses.

Response: Thank you for your comment. To address these concerns, we have incorporated a more detailed description of the comparative specimens and methodologies employed in the radial and parietal morphological analyses. Furthermore, we have expanded our comparative sample to include a broader taxonomic range, encompassing modern humans, Neanderthals, *Australopithecus*, and extant great apes. We have also integrated in-depth morphological analyses, including cross-sectional geometry, diffeomorphic surface matching, and cortical thickness colormaps, to provide a more comprehensive comparative framework. All the details were updated in the main text, Materials and Methods, Fig. 4, Extended Data Figs. 8 and 9, Supplementary Figs. 12-17, Supplementary Tables 1 and 2. We believe these additions significantly address your concerns and enhance the overall quality and reliability of this study.

Goal of the study & morphology of the remains

Proximal radius

As currently written, the abstract and introduction state that the findings and notably the proximal radius deliver “crucial insights into postcranial adaptation” and that the discovery of additional postcranial remains will clarify morphological variation and evolutionary adaptation. However, while it is clear that the primary goal of the study is to identify Denisovan bones, the main focus of the paper lies on the methodological approach, namely ZooMS and proteomic analyses, rather than on functional morphological analyses of the remains. I found that there is therefore a discrepancy between the claims made in the abstract and introduction regarding “crucial insights

into postcranial adaptation" and the actual morphological analyses presented, which could be developed at a higher level. Here more details and a few suggestions:

Response: Thanks for these suggestions. As suggested, given the fragmentary nature of our specimens and the lack of sufficient evidence to infer adaptation, we have modified it to a more conservative "*phenotype*" in the abstract. And the introduction section was also revised accordingly.

Beside, we have added more morphological analyses as you suggested, which have significantly improved the technical rigor of this study. Details regarding to your specific comments were shown below. We hope these additional analyses will address your concerns.

Line 179: the authors wrote: "In cross-section, the radial neck appears elliptical and exhibits a posterior buttress, represented by the relatively thick cortical bone area." However, more information is needed:

- Where exactly was the cross-section extracted (and how was the location standardized or identified as homologous between individuals)?

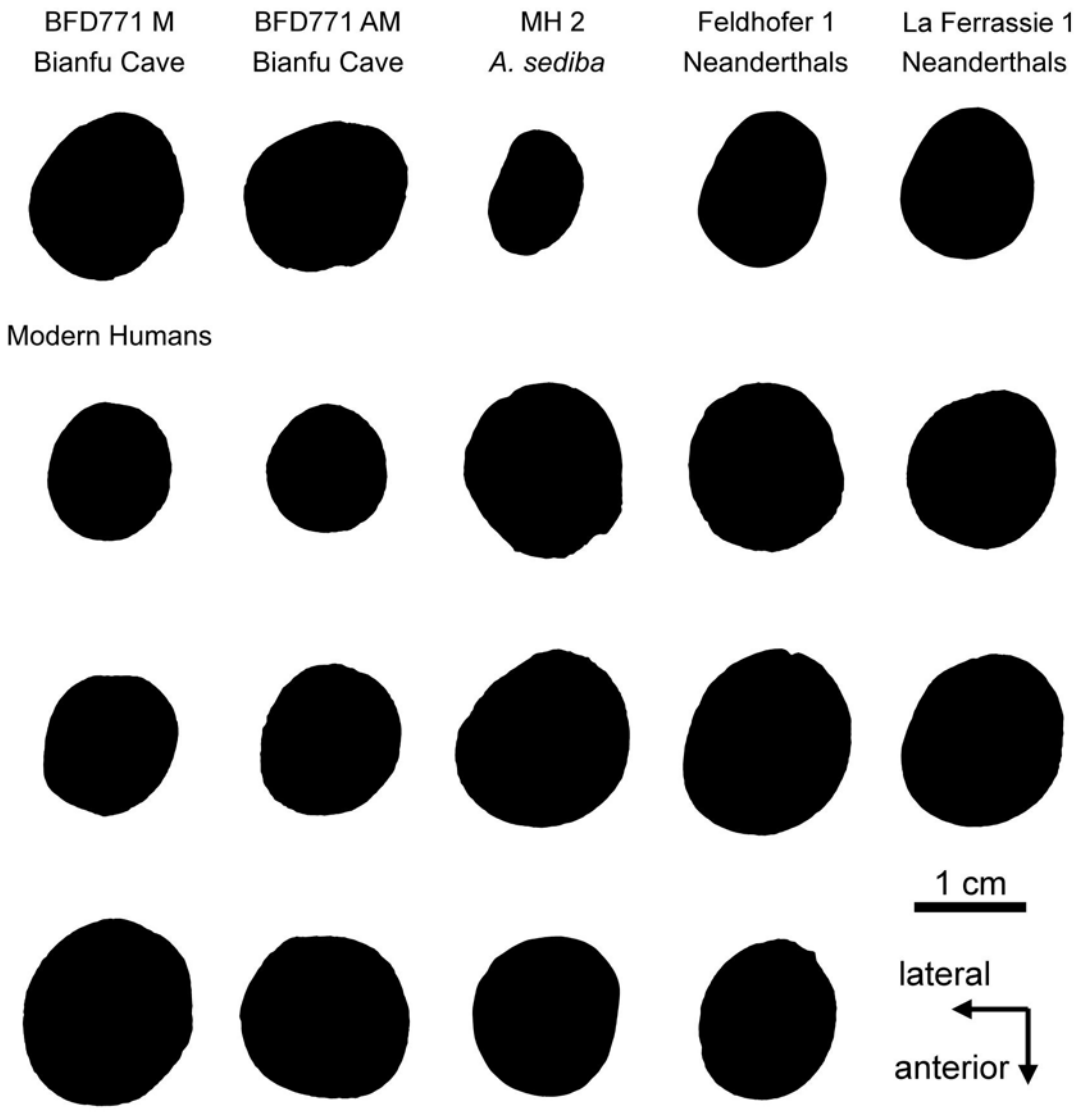
- How does the cross-sectional shape compare to other hominin fossils? What does "relatively thick cortical bone" mean in quantitative terms? The authors need to measure cortical thickness and compare it at least to modern humans in order to justify describing the cortex as "thick".

- Finally, an area cannot technically be "thick." Thickness refers to a linear dimension (e.g., cortical thickness), not to an area. So "thick cortical bone area" is imprecise here.

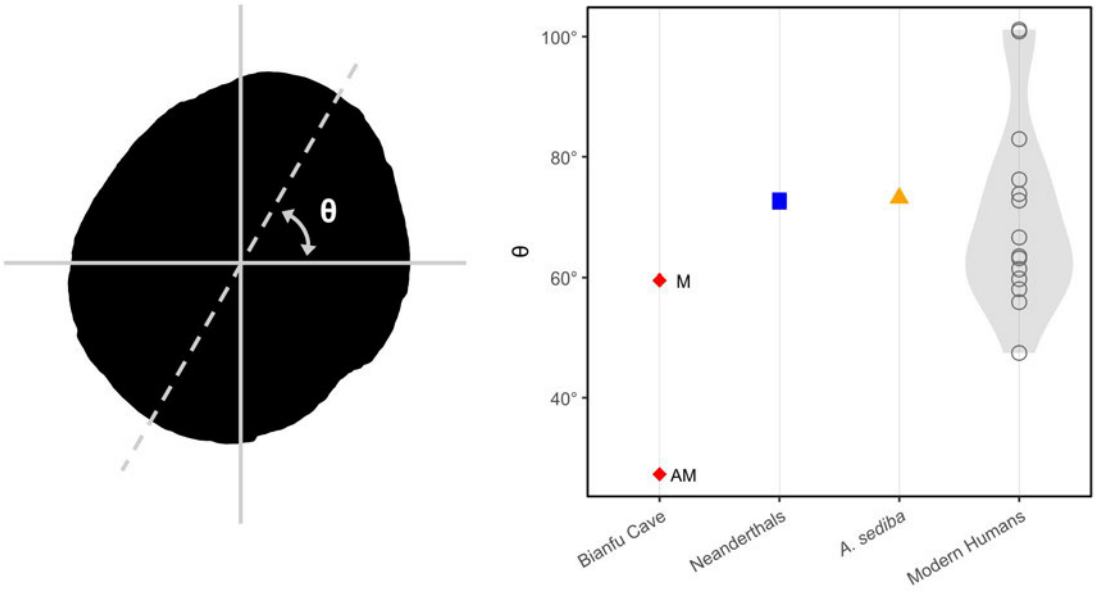
Response: Thank you for your comment. The analyzed cross-section represents the radial mid-neck, defined as the midpoint between the distal margin of the radial head and the proximal limit of the radial tuberosity. We agree that the terms 'thick' or 'thickness' lack precision in this context, as they were not quantitatively assessed. Consequently, we have removed these qualitative descriptors from the main text and incorporated a robust cross-sectional geometric analysis. This includes parameters such as total area (TA), polar moment of area (J) as a proxy for torsional rigidity, and bending rigidity shape ratios (Imax/Imin). These data are presented within a broad comparative framework encompassing modern humans, Neanderthals, *Australopithecus*, and extant great apes (Supplementary Figs. 12, 13, and 16).

Main text: *Based on the marginal morphology of the radial tuberosity and the major axis orientation of the radial mid-neck cross-section, BFD771 is most likely a right radius with a medially oriented radial tuberosity (Extended Data Fig. 9, Supplementary Figs. 12 and 13).*

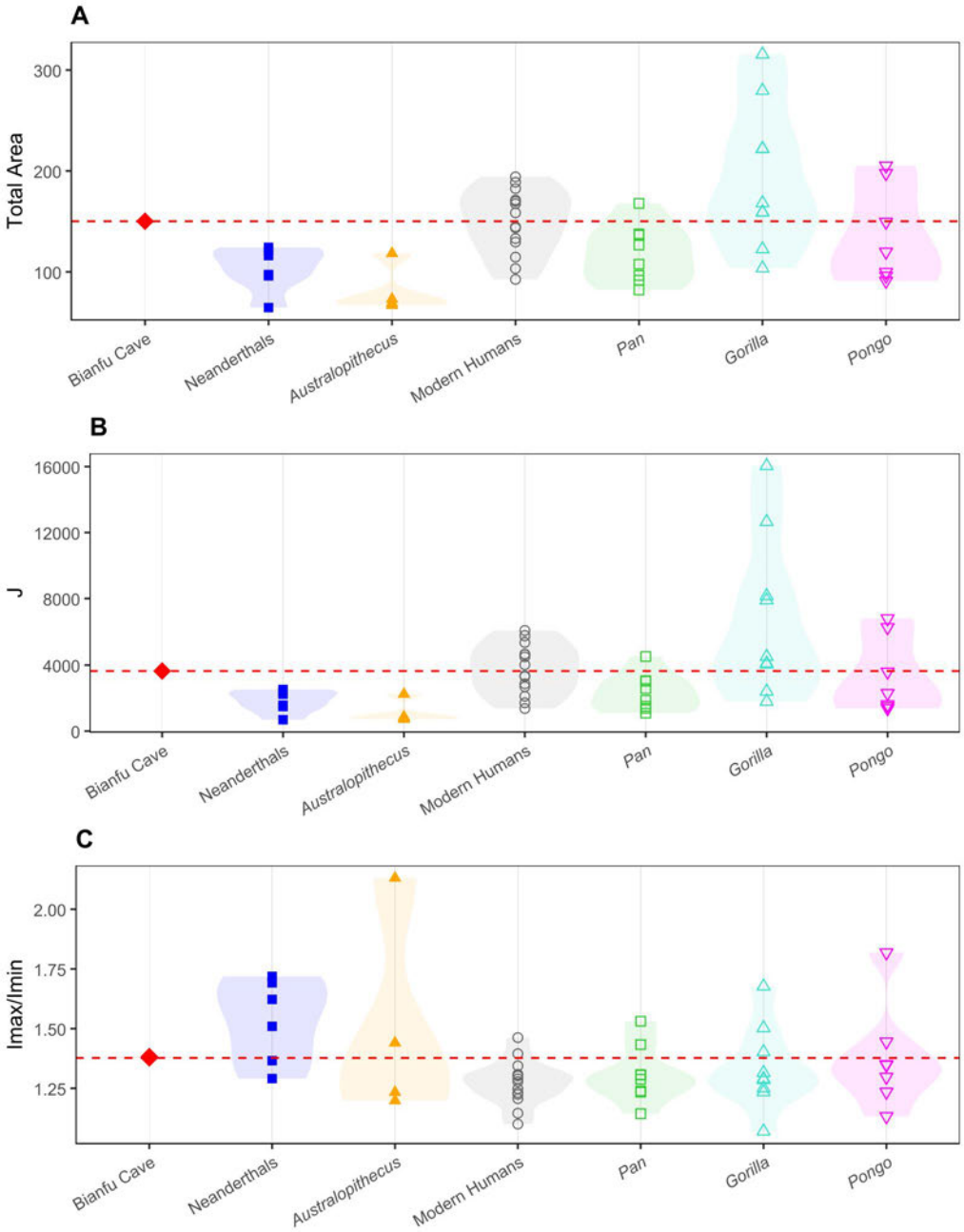
Cross-sectional geometric (CSG) analysis of the radial mid-neck reveals that BFD771 exhibits a higher total area and torsional rigidity than Neanderthals and Australopithecus, falling within the variation of both modern humans and extant great apes (Supplementary Fig. 16A and 16B). The cross-sectional shape, evaluated by the bending rigidity ratio, remains within the range of all comparative groups (Supplementary Fig. 16C).



Supplementary Fig. 12. *Comparative cross-sectional analysis of the radial mid-neck. The proximal radius fossil from Bianfu Cave (BFD771) is compared against the right radii of Australopithecus sediba (MH 2), Neanderthals (Feldhofer 1 and La Ferrassie 1), and 14 modern humans. The intact preservation of comparative specimens enables precise alignment following standard anatomical orientation, with the interosseous crest defining the medial aspect. Cross-sections were extracted at the midpoint between the distal margin of the radial head and the proximal limit of the radial tuberosity. In the majority of comparative specimens, the major axis of the cross-section is oriented anterolaterally-posteromedially. BFD771 aligns with this morphology when oriented as a right radius. Two hypothetical orientations are modeled for BFD771: medial (M) and anteromedial (AM) positioning of the radial tuberosity.*



Supplementary Fig. 13. *Comparison of Theta (θ) in the radial mid-neck cross-section. The proximal radius fossil from Bianfu Cave (BFD771) is compared against the right radii of Australopithecus sediba (MH 2), Neanderthals (Feldhofer 1 and La Ferrassie 1), and 14 modern humans. Theta represents the orientation of maximum bending rigidity. Two hypothetical orientations for the radial tuberosity of BFD771 are present: medial (M) and anteromedial (AM). The medial orientation is considered most probable, as the major axis inclination of the BFD771 (AM) reconstruction represents an extreme outlier relative to the comparative specimens.*



Supplementary Fig. 16. *Cross-sectional geometric (CSG) analysis of the mid-neck region of the proximal radius fossil from Bianfu Cave (BFD771). Cross-sections were extracted at the midpoint between the distal margin of the radial head and the proximal limit of the radial tuberosity. (A) Total area (TA), representing the area within the subperiosteal surface. (B) Polar moment of area (J), reflecting torsional and average bending rigidity. (C) The ratio of maximum to minimum second moments of area (Imax/Imin), representing the bending rigidity ratio and overall cross-sectional shape independent of orientation. Cross-sectional geometric parameters were calculated exclusively from the subperiosteal surface.*

Regarding the best-fit estimation methods (Fig. 4), it is possible that the mediolateral diameter could be overestimated and that the radial head could not appear spherical, as often observed in

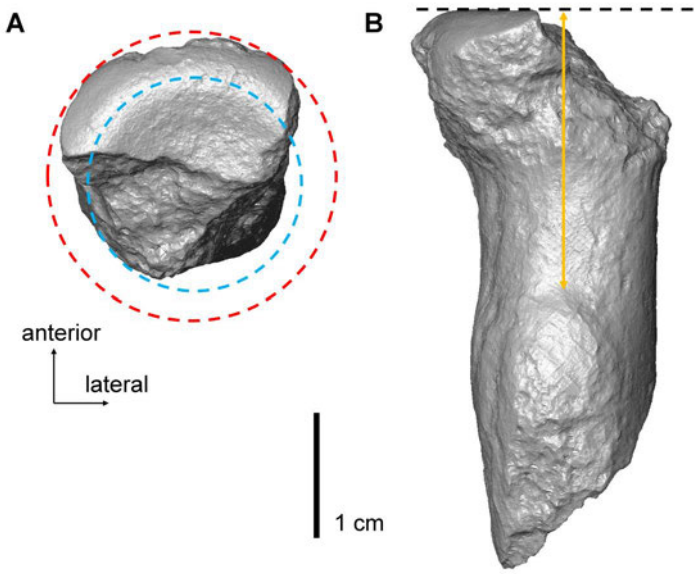
Neanderthals (see Fig. 4 where the AP diameter is larger than the ML diameter in Neanderthals). If the mediolateral diameter cannot be estimated reliably, then only the anteroposterior diameter should be estimated. Importantly, this would not change the overall conclusion: the diameter would still appear closer to that observed in Neanderthals than in modern humans.

Response: Thank you for your comment. The ML and AP radial head diameters are assumed to be equal because a best-fit circle was utilized as the most direct method to provide a first-order approximation of the BFD specimen's head size. We believe this is essential for providing readers with a fundamental impression of the radial dimensions.

Due to the fragmentary preservation of the BFD radial head in both the AP and ML aspects, a precise reconstruction of its original shape is not feasible. Consequently, we cannot definitively determine whether the specimen exhibits a modern human-like or Neanderthal-like morphology. A circle best-fitted to the preserved margin was therefore employed as a preliminary approach to quantify overall size.

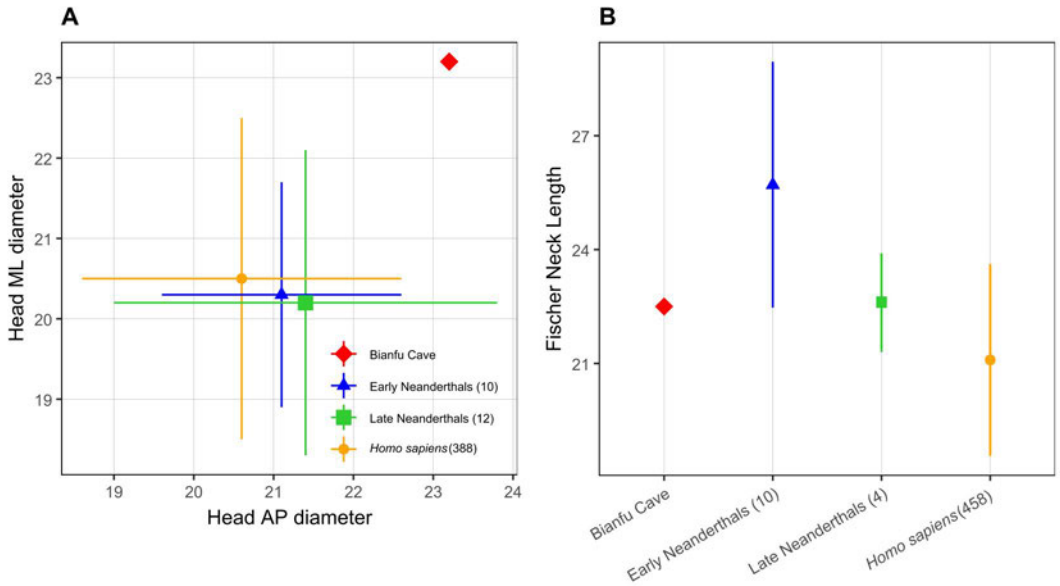
To address your concerns and prevent misinterpretation, we have refined our terminology to explicitly state that the circle serves only as a dimensional proxy, and does not imply a circular biological morphology (Supplementary Figs. 14A and 15A). To improve accuracy, we also utilized the better-preserved radial fovea as a supplementary geometric constraint, resulting in a revised diameter estimate of 23.2 mm for the radial head.

Main text: *The radial head dimensions were estimated using best-fit circles corresponding to the preserved head margin (23.2 mm) and fovea articular edge (17.2 mm) (Supplementary Fig. 14A).*



Supplementary Fig. 14. *Osteometric measurement protocols for the proximal radius fossil from Bianfu Cave (BFD771). (A) Estimation of radial head (red) and fovea (blue) diameters. The radial head diameter is estimated using a best-fit circle constrained by the preserved anteromedial and anterolateral margins. The radial fovea diameter is estimated through a best-fit circle aligned with the path of the preserved articular edge. The foveal circle serves as a reliable geometric constraint,*

providing a secondary dimensional reference for the estimation of the radial head. (B) Radial neck length (Fischer neck length), defined as the distance between the superior medial margin of the radial head and the most proximal point of the radial tuberosity, following the definition of Rodríguez, et al.



Supplementary Fig. 15. *Osteometric analysis of the proximal radius fossil from Bianfu Cave (BFD771). (A) Bivariate plot of radial head dimensions (anteroposterior versus mediolateral diameters). (B) Comparative analysis of radial neck length (Fischer neck length). Error bars indicate the mean ± one standard deviation. Comparative data are sourced from Rodríguez, et al.*

"The radial neck is 22.43 mm long": which methods were used to measure radial neck length? Was it measured as the distance between the radial tuberosity and the radial head? There is no information on the measurement protocol, and I think this section is needed.

Response: Thank you for the suggestion. In this study, we utilized the Fisher neck length, defined as the distance from the superior limit of the medial margin of the radial head to the most proximal point of the radial tuberosity. We have clarified this in the main text using "*The fisher neck length*". As you suggested, we have shown the osteometric measurement protocol in Supplementary Fig. 14B (see above), incorporated a formal definition of this metric, and provided the primary citation for the source publication within the figure caption.

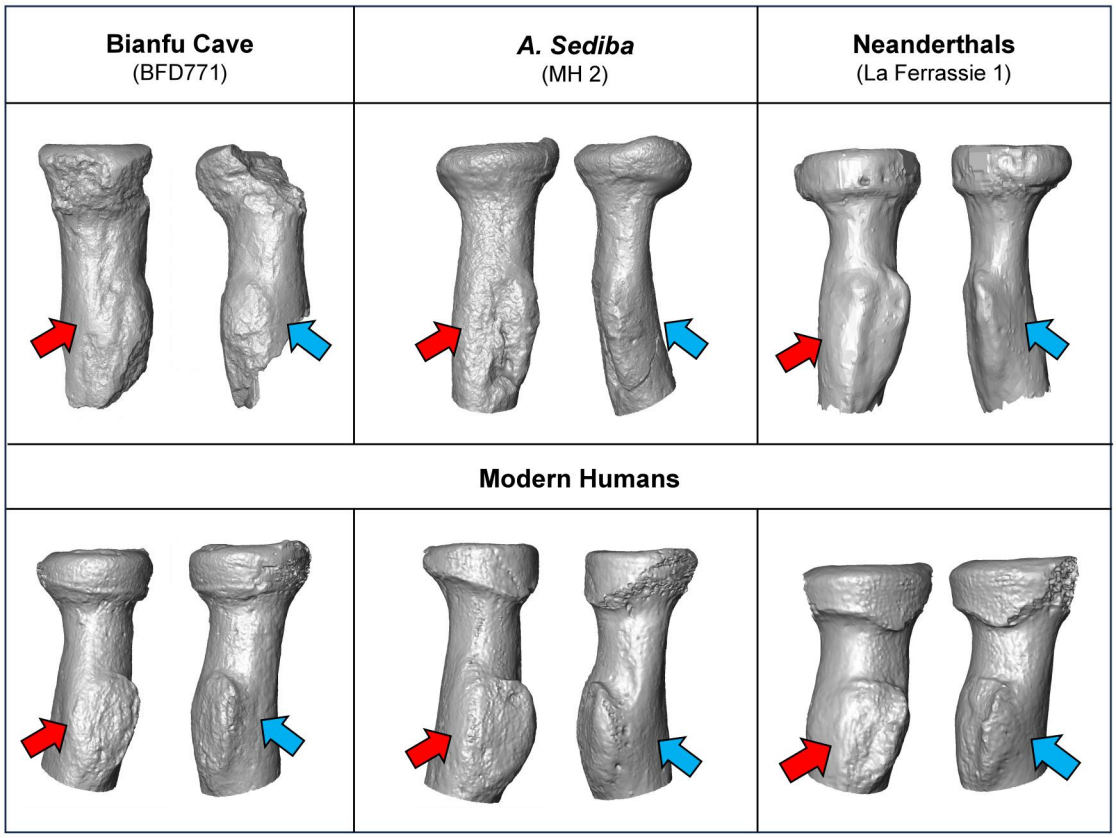
"The radial tuberosity is markedly elevated and faces medially. This configuration is observed in 76.5% of late Neanderthals, whereas all of Homo antecessor, 80% of early Neanderthals, and 88.8% of H. sapiens exhibit an anteromedially oriented tuberosity". If I understand correctly, the "early Neanderthal" group only includes Sima de los Huesos. In that case, I suggest stating "80% of the Sima de los Huesos sample". How did you calculate radial tuberosity orientation? This is critical to ensure that your observations are comparable to previously published measurements.

Did you use the methods detailed in Rodríguez et al. (2016)?

Response: Thank you for your suggestion. This sentence has been revised as follows: "*A medial orientation of the radial tuberosity is observed in 76.5% of late Neanderthals, as well as in Australopithecus afarensis (AL 288-1p) and H. habilis (OH 62). In contrast, an anteromedially oriented tuberosity is the predominant condition in Homo antecessor, early Neanderthals from Sima de los Huesos (80%), and H. sapiens (88.8%)*".

To address your concerns regarding the orientation of the radial tuberosity, we have incorporated a detailed methodological description regarding the laterality determination and orientation of the radial tuberosity for the BFD radial fragment. Our analysis includes a comparative morphological assessment of the radial tuberosity margin and the mid-neck cross-sectional geometry within a broad evolutionary framework, including modern humans, Neanderthals, and *Australopithecus*. This expanded comparison substantiates the classification of the BFD radius as a right-sided element with a medially oriented radial tuberosity. Consequently, we have refined the descriptions in the main text to reflect a more conservative interpretation and provided extensive documentation in the Extended Data Fig. 9, Supplementary Figs. 12 and 13 (see above), to detail the diagnostic criteria employed.

Main text: *Based on the marginal morphology of the radial tuberosity and the major axis orientation of the radial mid-neck cross-section, BFD771 is most likely a right radius with a medially oriented radial tuberosity (Extended Data Fig. 9, Supplementary Figs. 12 and 13).*



Extended Data Fig. 9. *Comparative morphology of the radial tuberosity. The proximal radius*

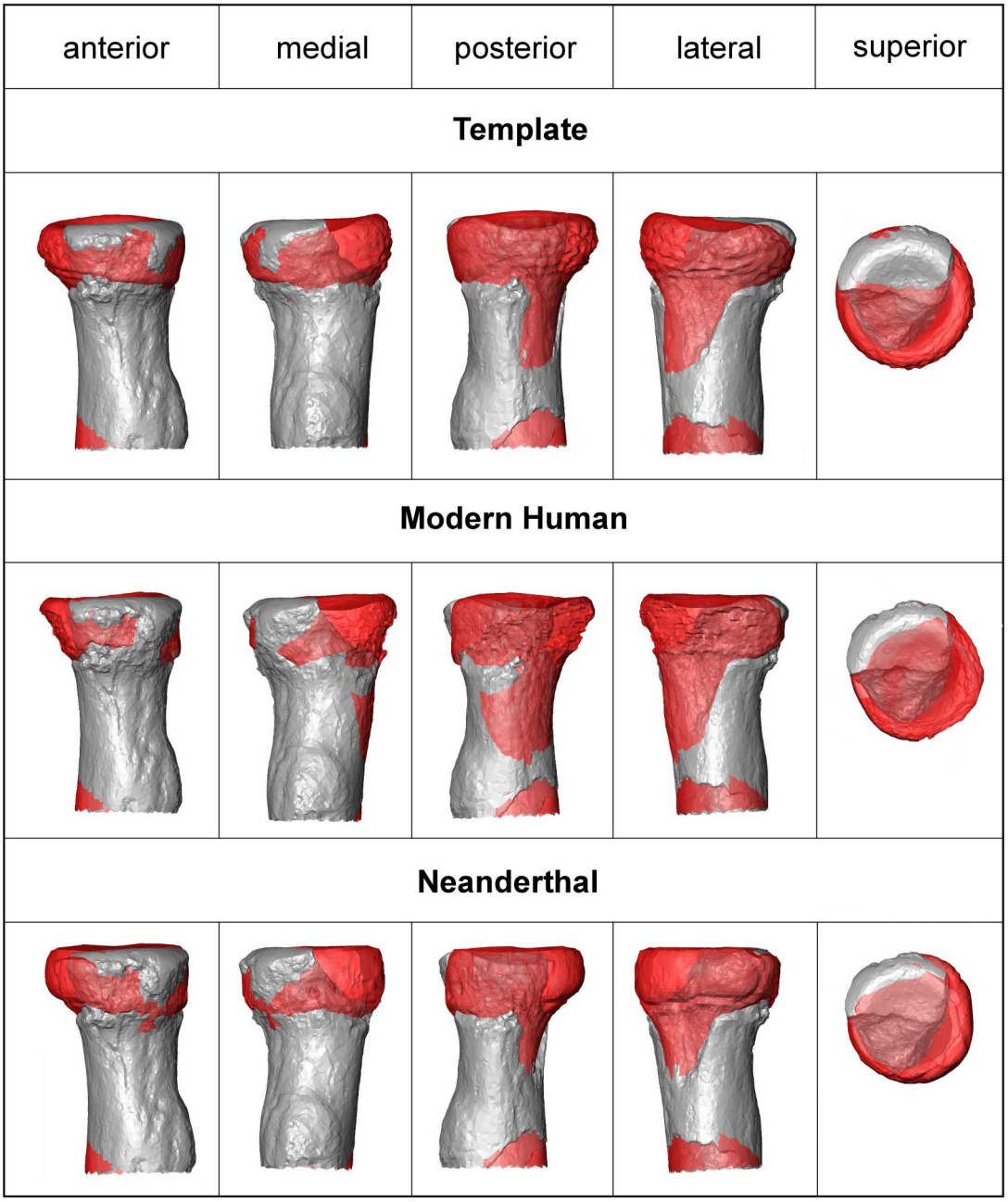
fossil from Bianfu Cave (BFD771) is shown within a comparative framework including Australopithecus sediba (MH 2), Neanderthals (La Ferrassie 1), and three modern humans. All comparative specimens are shown as right radii for comparative consistency, with the diaphysis distal to the radial tuberosity truncated to match the preservation of BFD771. Red arrows indicate the anterior margin of the radial tuberosity; blue arrows indicate the posterior margin. In all comparative specimens, the boundary between the tuberosity and the posterior diaphyseal surface is distinct, and the posterior diaphyseal surface is smooth. Conversely, the anterior margin of the tuberosity exhibits a gradual transition into the anterior diaphyseal surface without a distinct border, resulting in a structural elevation of the anterior diaphysis. BFD771 aligns with these morphological characteristics when oriented as a right radius.

To better characterize the morphological variation of this new and unique specimen attributed to Denisovans, a more comprehensive quantitative assessment would be highly valuable. Several anatomical proportions could be explored in greater detail. For example, the relative height of the radial head with respect to the neck would be informative, as it may relate to joint stability and the mechanical constraints associated with pronation and supination. But also the fovea diameter (or the length of the articular surface) is a relevant measurement. Reporting such measurements, even descriptively, would enrich the functional interpretation.

Response: Thank you for the suggestion. To address your concerns regarding the relative height of the radial head in proportion to the neck, we have incorporated a quantified diffeomorphic surface matching analysis within a broad comparative framework. This approach effectively captures this morphological variation and provides a more direct visual representation of these proportions. The diffeomorphic surface matching results suggest that BFD771 aligns more closely with modern humans due to its relatively short neck in proportion to the radial head. This configuration is distinct from the elongated necks characteristic of most Neanderthals and *Australopithecus*, both of which exhibit greater morphological consistency with extant great apes. These results have been updated in the main text, and details were provided in Fig. 4 and Supplementary Fig. 17.

Main text: *Diffeomorphic surface matching analysis, which quantitatively evaluates overall external shape independent of size and orientation, aligns the radius BFD771 more closely with modern humans due to its relatively short neck in proportion to the radial head. This configuration distinguishes it from the relatively elongated necks characteristic of most Neanderthals and Australopithecus, both taxa exhibiting greater morphological consistency with extant great apes (Fig. 4, Supplementary Fig. 17).*

Additionally, the fovea diameter was estimated using a best-fit circle applied to the articular fovea margin (Supplementary Fig. 14A, see above), and this measurement has been incorporated into the main text: *The radial head dimensions were estimated using best-fit circles corresponding to the preserved head margin (23.2 mm) and fovea articular edge (17.2 mm) (Supplementary Fig. 14A).*



Supplementary Fig. 17. *Virtual restoration protocols for the proximal radius fossil from Bianfu Cave (BFD771) for diffeomorphic surface matching analysis. The template represents the mean shape model derived from a taxonomically diverse comparative sample, including modern humans, Neanderthals, Australopithecus, and extant great apes. The template, a representative modern human, and Neanderthal La Ferrassie 1 were truncated to match the preserved anatomy of BFD771. Meshes were subsequently subjected to rigid body transformation and uniform scaling, followed by translation and rotation to achieve a best-fit alignment with BFD771.*

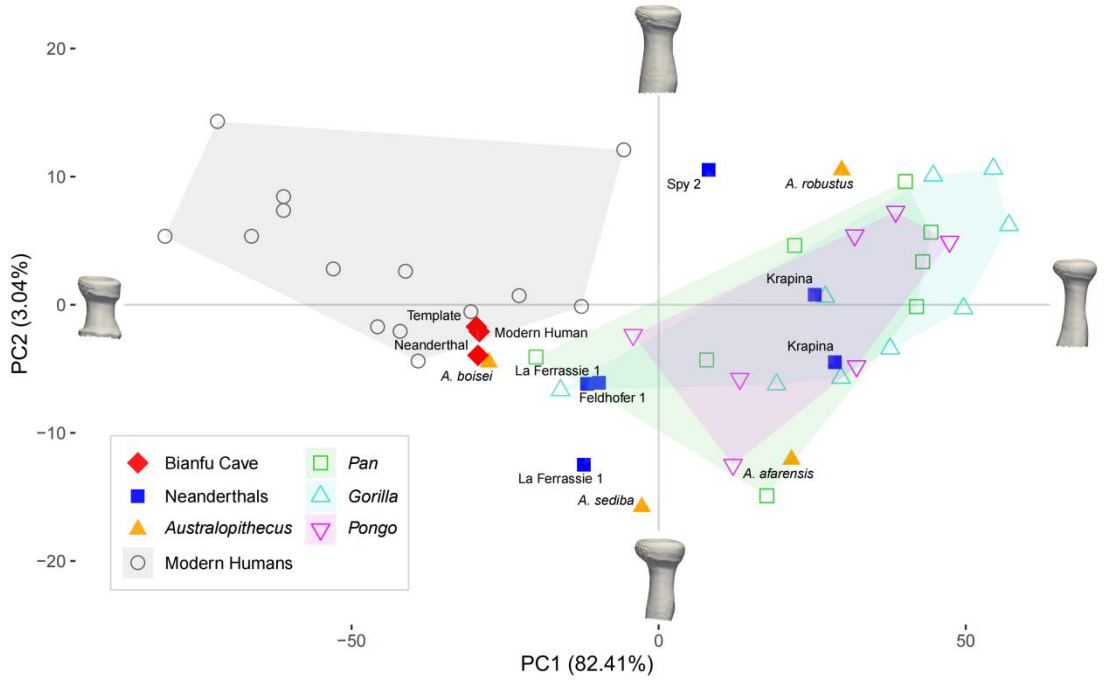


Figure 4. *Principal Component Analysis (PCA) of velocity vectors derived from diffeomorphic surface matching. The diffeomorphic framework removes orientation and size variation, isolating pure morphological shape information. To ensure precise alignment of the proximal radial segments across a broad taxonomic sample, the diaphysis distal to the most prominent projection of the radial tuberosity was truncated. The proximal radius fossil from Bianfu Cave (BFD771) was processed with three restoration protocols designed to capture a wider range of morphological variation and optimize restoration reliability. These protocols were based on the reference template representing the mean shape of all comparative specimens, modern humans, and Neanderthals, respectively (Supplementary Fig. 17). PC1 primarily captures the relative length of the radial neck in proportion to the radial head. PC2 primarily captures the relative dimensions of the radial head compared to the transverse dimensions of the proximal diaphysis.*

However, in addition to the descriptions currently provided, the authors could consider conducting a geometric morphometric (GM) analysis to situate the specimen within a broader comparative framework. Even if preservation is incomplete, landmark-based approaches might still allow meaningful comparisons with Neanderthals, Homo sapiens, and other relevant hominins, capturing the broader proportions but also all together the differences in development and shape of the several features currently only qualitatively described by the authors.

Response: Thank you for your suggestion. We fully recognize the importance of performing a comprehensive shape analysis of the proximal radial segment of the BFD specimen within a broader comparative framework. To address your concerns, we have incorporated a diffeomorphic surface matching analysis into the proximal radius segment. This method uses a landmark-free approach within the framework of diffeomorphometry, quantifying shape characteristics via deformation velocity vectors derived from a template of the sample's mean shape. This approach effectively bypasses the challenges associated with placing homologous landmarks on

fragmentary remains while providing statistical rigor and visualization capabilities comparable to traditional Geometric Morphometrics (GMM). Furthermore, we have expanded our comparative sample to include a broader taxonomic framework, encompassing modern humans, Neanderthals, *Australopithecus, Pan, Gorilla,* and *Pongo*. These results have been updated in the main text and details were provided in Fig. 4 and Supplementary Fig. 17 (see above).

Main text: *Diffeomorphic surface matching analysis, which quantitatively evaluates overall external shape independent of size and orientation, aligns the radius BFD771 more closely with modern humans due to its relatively short neck in proportion to the radial head. This configuration distinguishes it from the relatively elongated necks characteristic of most Neanderthals and Australopithecus, both taxa exhibiting greater morphological consistency with extant great apes (Fig. 4, Supplementary Fig. 17).*

Cross-sectional analyses of the radial neck would also considerably strengthen the study. Cross-sectional geometric properties—such as second moments of area, polar moment of area (J), and section moduli (Z values)—have been widely reported in the literature and provide important insights into biomechanical loading and functional adaptation. These metrics could help contextualize the specimen in terms of habitual forearm loading patterns and potential behavioral implications. Finally, while the manuscript focuses primarily on comparisons with contemporaneous groups, functional interpretations often benefit from a broader evolutionary framework. Including earlier hominins (e.g., australopiths) and, where appropriate, extant taxa such as chimpanzees would provide important context for strengthen the evolutionary and biomechanical interpretations.

Resposne: Thank you for the suggestion. We fully recognize the importance of cross-sectional geometric (CSG) analysis in providing insights into biomechanical loading and functional adaptation. To address your concerns, we have incorporated a CSG analysis of the radial mid-neck, defined as the cross-section at the midpoint between the distal margin of the radial head and the proximal limit of the radial tuberosity. We compared total area (TA), polar moment of area (J), and bending rigidity ratios (Imax/Imin) within a broad comparative framework including modern humans, Neanderthals, *Australopithecus, Pan, Gorilla,* and *Pongo*. Due to the limitations of accessing CT data for such a broad sample, the comparative dataset primarily consists of surface scans. Consequently, J and Imax/Imin were calculated based on subperiosteal properties (assuming a solid cross-section). While this approach does not account for endosteal (internal) cortical distribution, studies have shown that subperiosteal geometry remains a robust proxy for overall functional and structural information. These results have been updated in the main text and details were provided in Supplementary Fig. 16 (see above).

Main text: *Cross-sectional geometric (CSG) analysis of the radial mid-neck reveals that BFD771 exhibits a higher total area and torsional rigidity than Neanderthals and Australopithecus, falling within the variation of both modern humans and extant great apes (Supplementary Fig. 16A and 16B). The cross-sectional shape, evaluated by the bending rigidity ratio, remains within the range of all comparative groups (Supplementary Fig. 16C).*

parietal fragments

The authors also state that it is "likely that Denisovans exhibit substantial intra-population morphological variation" and that direct evidence from skeletal remains, especially long bones, is essential for robust morphological and behavioral reconstructions. However, this claim appears overstated in the context of the present study. The study, by definition, cannot address such questions on the basis of one isolated specimen. At the same time, the manuscript reports additional cranial remains, including parietal fragments, which are particularly valuable given the limited Denisovan fossil record. They could contribute meaningfully to documenting morphological variability. However, their broader significance for understanding Denisovan diversity is not clearly integrated into the introduction nor fully developed in the discussion. Indeed, only very limited information is provided regarding the parietal fragments. There is no description or discussion of observable features such as sulci or vascular impressions, nor are there detailed morphological comparisons with other Denisovan material, or with Neanderthals and Homo sapiens. Even if the preserved parietal fragments are small, they nevertheless offer an opportunity to explore this question, at least preliminarily. A more detailed anatomical description and explicit comparisons with available Denisovan specimens (whenever possible), as well as with Neanderthals and Homo sapiens, would considerably strengthen the manuscript and better align the results with the broader claims of intra-population morphological variations made in the introduction.

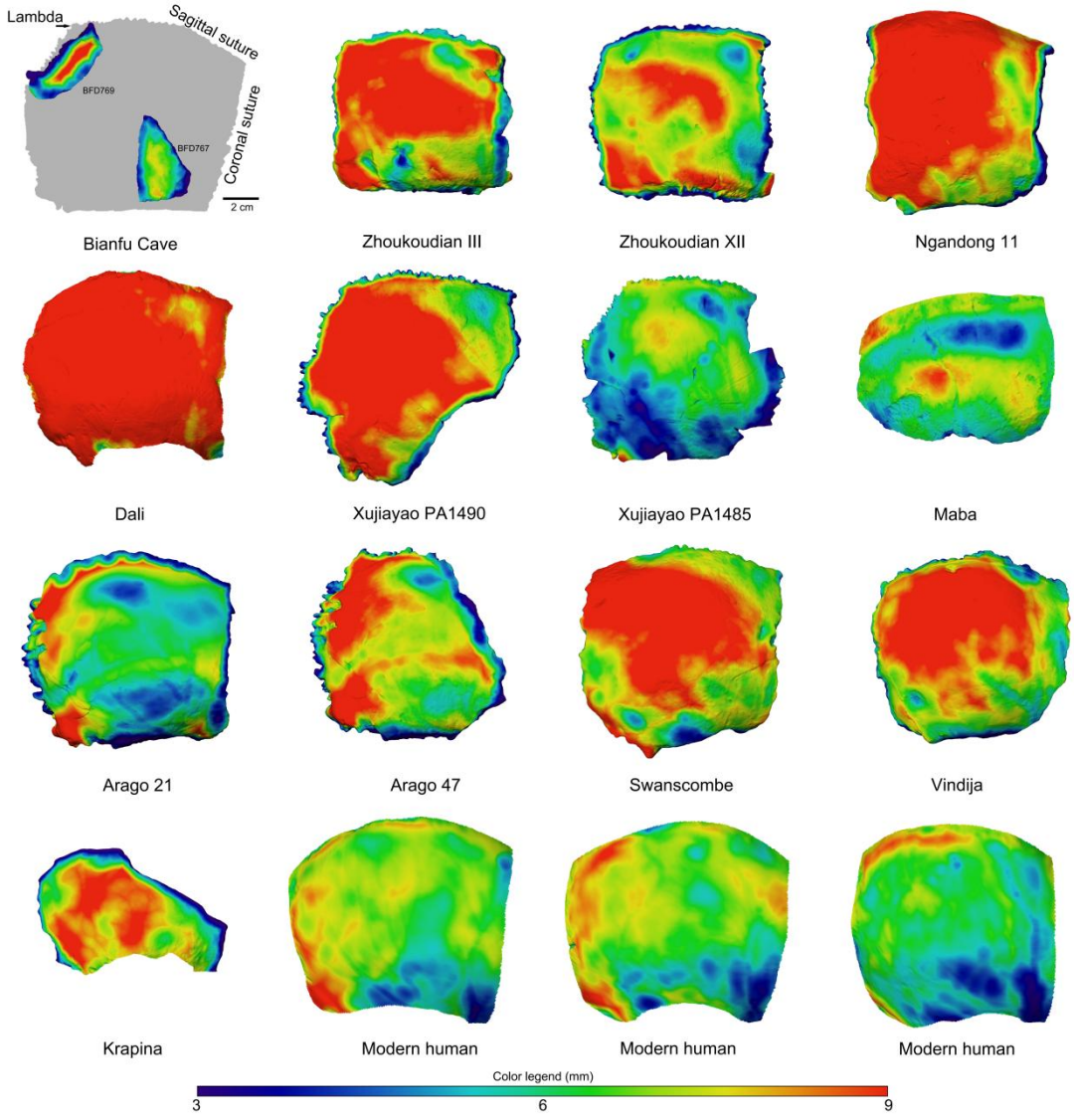
Response: Thanks for these suggestions. To avoid overstatement, we have revised "likely that Denisovans exhibit substantial intra-population morphological variation" to "*a broader fossil record is needed to define Denisovan morphological diversity*" since we agree that this conclusion cannot be securely made based on isolated and especially fragmental bone.

In the new manuscript, we have added more description of the parietal fragments and further pinpointed their anatomical positions based on the vascular impressions, sagittal sulcus, and cranial suture. In addition, apart from the value comparison of cranial vault thickness, we have incorporated colormap of the cranial vault thickness and compared with *H. erectus*, *H. heidelbergensis*, Neanderthals, and *H. sapiens* (see Extended Data Fig. 8).

New description morphology of the parietal was added as "*BFD767 is from the anterior portion of the right parietal's lateral wall. It exhibits a weak curvature, relatively smooth external surface, and two vascular impressions on the endocranial surface (identified as the two obelic branches of the middle meningeal vessels). BFD769 has a moderate curvature and represents a right parietal fragment next to the lambda, preserving a small portion of the superior sagittal sinus and the lambdoid suture.*"

New colormap and comparison analysis was added as "*In terms of the cranial vault thickness pattern, the parietal BFD767 resembles some specimens of H. heidelbergensis, Neanderthals, and East Asian late Middle Pleistocene archaic Homo (Xujiayao PA1485 and Maba), being thicker than that of H. sapiens but thinner than that of Asian late H. erectus (Extended Data Fig. 8). The parietal BFD769 is very thick and similar to that of Asian late H. erectus, H. heidelbergensis, and East Asian late Middle Pleistocene archaic Homo (Dali, Xujiayao PA1490), and is thicker than that of Neanderthals and H. sapiens (see also Fig. S4 in reference[68]). Considering both parietal fragments together, the cranial vault thickness pattern of Bianfu Cave hominins is most likely that*

of H. heidelbergensis and East Asian late Middle Pleistocene archaic Homo."



Extended Data Fig. 8. *The vault thickness of the two parietal fragments from Bianfu Cave (BFD767 and BFD769) and the comparative samples, including Asian late H. erectus (Zhoukoudian and Ngandong), East Asian late Middle Pleistocene archaic Homo (Dali, Xujiayao, and Maba), H. heidelbergensis (Arago and Swanscombe), Neanderthals (Vindija and Krapina), and recent modern humans.*

Prescreening

Several times the manuscript mentions that a prescreening based on size, cortical thickness, and surface features was used prior to ZooMS analyses. It is also stated that this prescreening is important given the cost and time requirements of ZooMS, and the authors present this as a new or improved protocol. However, if the authors want to develop and promote this approach, the

detailed protocol and methods need to be presented and expanded: how many researchers performed the prescreening, what level of experience they had, and what explicit criteria were used (e.g., how did they assess cortical thickness, morphological diagnostic features etc…) are important information if the two-step screening approach should be used in the future to aid in the identification of more Denisovan long bones as stated by the authors.

Response: Thanks for your suggestion. In this study, two of our co-authors, Song Xing and Yuhao Zhao, performed the morphological prescreening. They have been focused on hominin fossil study for over 5 years. And the prescreening process relied mainly on traditional morphological observation and measurement. We have added more details in Materials and Methods.

Materials and Methods: *After manual inspection and traditional morphological measurement of more than 60,000 non-diagnostic bone fragments based on bone size, cortical thickness, articular surface morphology, muscle attachment marks on long bone surface, vascular impressions on cranial internal surface, and so on, 22 fragments were considered as potential hominin remains.*

However, even though morphological prescreening is quite important, we now learn that some other sites also performed this process before ZooMS. Therefore, we have revised the main text and described this "morphological pre-screening + ZooMS" process as a regular approach without overemphasizing its innovation.

Main text: *Here we have performed a systematic morphological prescreening on more than 60,000 bone fragments from Bianfu Cave, and identified 22 candidate hominin specimens. Subsequent ZooMS and proteomic analyses confirmed three to be Denisovan remains, including two parietal bone fragments (BFD767 and BFD769) and one proximal radial fragment (BFD771).*

Archaeological site and references

The authors mention that four human teeth were unearthed from the middle of sub-layer 7. However, I was unable to locate a reference announcing the discovery of these four teeth. Additional information is required here, particularly because the newly reported parietal fragment and radius attributed to Denisovans derive from the same layer. This association raises several important questions. What type of teeth are these (e.g., anterior, posterior)? Do they preserve diagnostic morphological features that allow taxonomic attribution? Could they potentially belong to Denisovans? When referring to "human" teeth, do the authors specifically mean Homo sapiens? If so, does this imply that different hominin groups were using the cave during a similar time period? Finally, is there a specific age estimate available for sub-layer 7?

Similarly, further clarification regarding the stone artifacts is necessary. No references are provided, and I was unable to identify accessible publications detailing these materials. In which layers were the lithic artifacts recovered? Even if attribution cannot yet be verified, were any of them found in the same layer as the Denisovan remains?

Response: Thanks for your suggestion. We have added more details and related citation in Materials and Methods. Four hominin teeth were discovered, i.e. a lower left lateral incisor [I_2], a lower left fourth premolar [P_4] and two lower right second molars [M_2s], including anterior and posterior categories. According to the morphological analysis conducted in another sister article

(cited in our manuscript), the lower M_2s exhibit close morphological affinity with Xiahe and Laos Denisovans, as well as the East Asian late Middle Pleistocene archaic *Homo* from Hualongdong. To further confirm their taxonomic attribution, two of these teeth, a lower fourth premolar (YHB3518) and a lower second molar (YHB3075), were selected for proteomic analysis, and further attributed to Densovans as well. The results have been updated in the manuscript.

And we are sorry for the misunderstanding caused by referring to "human" teeth, here we have revised it to "*hominin*" teeth. The specific age estimate for Layer 7 and further clarification regarding the stone artifacts were also provided.

Materials and Methods: *Four hominin teeth were unearthed in the middle of Layer 7 (between 148-139 ka and 142-134 ka)[1], suggesting a high likelihood of finding additional hominin fossils in this site. The four hominin teeth included a lower left lateral incisor [I_2], a lower left fourth premolar [P_4] and two lower right second molars [M_2s]. Morphological analysis found the lower M_2s exhibit close morphological affinity with Xiahe and Laos Denisovans, as well as the East Asian late Middle Pleistocene archaic Homo from Hualongdong[1]. To further confirm their taxonomic attribution, two of these teeth, a lower fourth premolar (YHB3518) and a lower second molar (YHB3075) (Fig. 1C and 1D), were selected for proteomic analysis.An acid etch approach was conducted on the enamel while dentine powder was drilled for protein extraction, resulting in one enamel fraction and three dentine fractions for LC-MS/MS analysis.*

All cultural layers yielded lithic artifacts, totaling 1,366 stone artifacts, including both core and flake tools[1].

1 Ruan, Q. et al. Denisovans from southwestern China and their subsistence strategies. Cross citation (2026).

In addition, the U–Th dating of the fossils and its consistency with previously published chronologies for the site are of central importance. If robust, these results would suggest Denisovan occupation during both glacial and interglacial periods and could lend support to hypotheses proposing a possible glacial refugium on the Yunnan–Guizhou Plateau. It is therefore essential to provide the relevant references for previous OSL and U-series dating at the site in order to assess whether the current chronological framework is sufficiently robust.

Response: Thanks for your comment. We have cited the more accurate stratigraphic ages based on OSL and U-series dating, ~167-134 ka, in the discussion, i.e., the hominin fossils occupied this site in the glacial phase (MIS 6). And during this period, human populations in East Asia were already widely distributed and showed expansion trends. After incorporating the faunal and pollen analysis of Bianfu Cave, which was performed in another sister paper (cited in our manuscript), as well as the mammalian fauna from surrounding regions, we revised the "refuge" to "hunting paradise" in our main text for better elucidation.

Main text: *Our findings extend the Denisovan occupation to ~167-134 ka within Marine Isotope Stage (MIS) 61. During this glacial period, the relatively warm climate and conifer-dominated forest or forest-steppe at Bianfu Cave and surrounding regions may have provided a stable supply of mammal resources, especially a predominance of medium- to large-sized herbivores1,42-44.*

This may have enabled the Yunnan-Guizhou Plateau to serve as a hunting paradise for hominins.

1 Ruan, Q. et al. Denisovans from southwestern China and their subsistence strategies. Cross citation (2026).

42 Zhao, L. et al. New Discovery of Human Fossils and Associated Mammal Fauna from Mawokou Cave in Bijie, Guizhou Province of Southern China. Acta Anthropologica Sinica 35, 24-35 (2016).

43 Zhang, Z., Liu, J., Zhang, H. & Yuan, C. A pleistocene mammalian fauna from Panxian Dadong, Guizhou province. Acta Anthropologica Sinica 16, 209-220 (1997).

44 Schepartz, L., Stoutamire, S. & Bekken, D. Stegodon orientalis from Panxian Dadong, a Middle Pleistocene archaeological site in Guizhou, South China: Taphonomy, population structure and evidence for human interactions. Quaternary International 126-128, 271-282, doi:10.1016/j.quaint.2004.04.026 (2005).

ZooMS & paleoproteomic analyses

The proximal radius (BFD771) is clearly hominin in morphology. While I fully appreciate the value of proteomic confirmation, I was surprised that this specimen appears to have been treated as part of a broad screening of fragmentary bones, given its diagnostic anatomical features. In this case, the element does not appear ambiguous from a morphological standpoint.

The authors emphasize the importance of ZooMS as a tool for identifying fragmentary hominin remains and thereby expanding the sample available for functional and evolutionary interpretation. However, in the specific case of BFD771, the morphology of the proximal radius is sufficiently distinctive that its identification as hominin should have been possible prior to proteomic screening. This raises a certain discrepancy in the narrative: on the one hand, ZooMS is presented as a powerful method for detecting otherwise unidentifiable fragmentary remains; on the other hand, the most informative and best-preserved postcranial elements—those that can meaningfully contribute to functional analyses—are precisely those that are morphologically diagnostic without requiring ZooMS for taxonomic identification. The authors state that “wider use of the two-step screening approach established in our study would aid in the identification of more Denisovan long bones in the future.” However, if a long bone requires a two-step screening approach for identification, it is likely to be either highly fragmentary or lacking in clear diagnostic morphology. Such remains may, by definition, preserve more limited functional information compared to more complete and morphologically identifiable elements.

Response: Thanks for pointing this out. We recognize the limitations of ZooMS as you have suggested and we have revised the main text and described it as a regular approach without overemphasizing its innovation.

Main text: *Here we have performed a systematic morphological prescreening on more than 60,000 bone fragments from Bianfu Cave, and identified 22 candidate hominin specimens. Subsequent ZooMS and proteomic analyses confirmed three to be Denisovan remains, including two parietal bone fragments (BFD767 and BFD769) and one proximal radial fragment (BFD771).*

However, even though the bones with diagnostic anatomical features possibly could be identified as hominins before ZooMS screening, we still need further proteomic analysis to attribute them to Denisovans. Thus, here we revised the "wider use of the two-step screening approach established in our study would aid in the identification of more Denisovan long bones in the future." to "*similar proteomic analyses would aid in the identification of additional Denisovan long bones in the future.*"

Minor comments

In several instances throughout the manuscript, including in the abstract, it should be clearly stated that the parietal remains are fragmentary and that the long bone consists specifically of a proximal radial fragment.

Response: We have revised throughout the manuscript as you suggested.

Line 43: "particularly long bones": why? Articular and epiphyses can be highly relevant for joint mobility and movement (e.g., in hand elements).

Response: Thanks for pointing this out. We have removed "particularly long bones" in the main text.

Line 78: typo "throughout"

Response: Here we used "a high-throughput ZooMS approach" to explain that this approach could rapidly process a large number of samples simultaneously. Thus "throughput" is correct.

Line 82: it would be great to provide the dates of the layers here.

Response: We have provided the dates of the layers as you suggested.

Main text: *we classified 22 fragments as possible hominin remains, with 5 from the upper layers (~140-63 ka) and 17 from the lower layers (~195-134 ka, Fig. 1B)*[1].

1 Ruan, Q. et al. Denisovans from southwestern China and their subsistence strategies. Cross citation (2026).

Referees' comments:

Referee #1 (Remarks to the Author):

I congratulate the authors on this exciting study. They addressed all of my previous comments in detail, and I don't think any further changes are necessary, except for a minor correction of a typo I noticed:
Extended Data Fig 9: Australopithecus Sediba -> sediba
Response: Thanks for your positive feedback for our study. We have corrected the species name in the figure.

Referee #2 (Remarks to the Author):

The authors have carefully and thoroughly addressed my previous comments, and I am satisfied with the revisions made. The chronological evidence presented in this study appears robust and supports the reliability of the fossil ages. This work substantially advances our understanding of Denisovan population dynamics in East Asia and carries significant scientific implications. I therefore recommend the manuscript for publication.
Response: Thanks for your positive feedback for our study.

Referee #3 (Remarks to the Author):

The authors have appropriately responded to all key suggestions from the first round of reviews.

We have additionally reviewed the newly-incorporated proteomic RAW data and the workflow for validating ambiguous peptides. The data remains solid, with the conclusions appropriately following the data presented, and the data analysis additions are very well-suited for this purpose.

We thus have no major suggestions, and we congratulate the authors on a fantastic manuscript.
Response: Thanks for your positive feedback for our study.

We have only very minor suggestions:
Main text
Line 136: “Homonidae” should be “Hominidae”
Line 949 “...every three amino acid…” should be “every three amino acids”
Response: Thanks for pointing these out. We have corrected as you suggested.

Supplementary Data 4

“Decision” column in supplementary table. I think “unreliable” vs “reliable” is fine, but i wonder if it would be preferable to change to "accepted"/"rejected" (or something else if the authors prefer), as I suppose this table reflects how the authors integrated these peptides/positions into

downstream analysis, and it is still quite difficult at the moment to outright label any peptides in palaeoproteomics as "unreliable" in a broader sense.

Response: Thanks for your professional comments. We have changed "reliable"/"unreliable" to "Accepted"/"Rejected" as you suggested.

Supplementary

Line 53: “tremble” should be “TrEMBL”

There are some more typos in the newly added sections

Line 79, Line 93: “deamdation”

Line 93: “other archaic hominin as well” should be “...hominins…”

Response: We are sorry for these typos and corrected as you suggested. And we also checked the whole manuscript to correct similar typos.

Ryan Sinclair Paterson and Benjamin Cragun

Referee #4 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports.

Referee #5 (Remarks to the Author):

The authors have carefully addressed my previous comments, and the manuscript has been substantially improved. The results are exciting and represent an important contribution to our understanding of Denisovan distribution and morphology. I particularly congratulate the authors for incorporating additional morphological observations and quantitative analyses, which considerably strengthen the study. I still have several comments that I believe would further improve the manuscript. I also provide a few minor comments aimed at improving clarity and readability.

Major comments

1. I think there are still some inaccuracies in the way the discovery and identification of the hominin remains are presented. As for the two teeth, the radius is morphologically identifiable as hominin and proteomic analyses can help to confirm its taxonomic attribution and identify it as Denisovans.

Several statements throughout the manuscript may therefore be misleading. For example:

Page 4, lines 92–93: the fossils are described as "unidentifiable mammalian fragments," which is not accurate the radius.

Page 4, line 97: the term "non-diagnostic bone fragments" is similarly problematic because the radius possesses morphological characteristics that allowed its identification as a hominin element prior to proteomic confirmation.

Response: Thanks for your comments. We have removed the misleading words

"unidentifiable" and "non-diagnostic" throughout the manuscript to ensure accurate expression.

2. The authors correctly note that one parietal fragment derives from Layer 7 whereas the other comes from Layer 9. Given the observed morphological differences (in cortical thickness) between the two specimens, could these differences potentially reflect temporal change?

This possibility may be worth discussing, particularly because Layer 7 dates to the later part of MIS 6, when climatic conditions may have differed from those associated with Layer 9. While the current sample size obviously prevents firm conclusions, I am wondering whether acknowledging this possibility would provide a broader interpretive framework for the observed variation.

Response: Thanks for your comments. The observed variation in parietal thickness might reflect physical variations resulting from temporal or corresponding climatic change. However, the observed differences could also result from variations in anatomical regions (as is the case for two Bianfu Cave parietal fragments, see Extended Data Fig. 9), sex differences, or purely individual variation. It is difficult to determine which factor played the dominant role.

3. I think the discussion could be expanded regarding the functional significance of the proximal radial morphology.

The authors note that the Denisovan radius shares certain characteristics with Neanderthals, including its large proximal dimensions and likely medially oriented radial tuberosity. However, the functional implications of these features are not fully explored.

A large proximal radius and a medially oriented radial tuberosity are often interpreted as increasing the mechanical advantage of the biceps brachii during elbow flexion and forearm supination. The combination of these apparently Neanderthal-like features with an overall morphology and cross-sectional geometry that more closely resemble modern humans is particularly intriguing. It may suggest a distinct loading regime or behavioral pattern in Denisovans compared with both Neanderthals and modern humans. I encourage the authors to further discuss these potential functional implications.

Response: Thank you for your constructive suggestion. We have now expanded the functional interpretation of the Bianfu Cave proximal radius: *"Although the Denisovan proximal radius shares large proximal dimensions and a likely medially oriented radial tuberosity with Neanderthals, its overall external shape and mid-neck cross-sectional geometry align more closely with modern humans. Radial tuberosity orientation, neck length, head dimensions, and neck cross-sectional geometry are critical biomechanical features related to M. biceps brachii mechanical efficiency and proximal radial load-bearing capacity[43,45]. The mosaic pattern of these traits in the Bianfu Cave radius, relative to Neanderthals and modern humans, indicates differential biomechanical demands and behavioral adaptations in Denisovans."*

4. Page 4, line 114: it would be helpful to specify in the main text exactly which materials were dated using Uranium-series methods. Because the dating information currently relies on a cross-citation, it is difficult for readers to understand how the chronology of the different layers was established and what material was directly dated.

Response: Thanks for your comment. We have added the specific information about dating in the main text: "*Uranium-series (U-series) dating on three hominin bones and attached carbonates provided minimum ages of ~102 ka for two samples from Layer 7 and ~143 ka for the parietal from Layer 9 (BFD769) (Materials and Methods, Supplementary Text 1, Supplementary Table 1). These ages are much younger than those of the corresponding layers established by a Bayesian age model integrating optically stimulated luminescence (OSL) dating of sediment samples and U-series dating of speleothems, namely between 148-139 ka and 142-134 ka for Layer 7, and between 167-161 ka and 160-150 ka for Layer 9*[1]."

Minor comments

• Title: the title emphasizes the radius and teeth, but the study also reports two parietal fragments. Should the cranial remains be mentioned more explicitly?

Response: Thanks for your comment. We agree that the previous title did not reflect all the contents of this manuscript. Thus we have revised the title as "*Ancient proteins identify various Denisovan remains from Southwest China*" to better summarize the whole content and also fulfill the character limit of 75 for the title in Nature.

• Page 3, line 65: I suggest adding the term "functional" before "variation."

Response: Thanks for your comment. We have revised as you suggested.

• Page 4, line 108: it is unclear what the authors mean by "higher taxonomic resolution." Please clarify.

Response: Actually our point is that ZooMS can attribute the unidentifiable bone fragments to family or even species level, offering better taxonomic resolution. However, this resolution is not necessarily better when compared with the identifiable mammalian fauna at this site. Thus we have removed this phase to avoid misunderstanding.

• Page 9, line 240: it is unclear what is meant by "category" in this context.

Response: Sorry for this confusing expression. We have revised this sentence as "*BFD771 is the only Denisovan radius identified to date.*"

• Page 9, line 241: it may be worth mentioning that microCT analyses could potentially

help assess ontogenetic status. This could become important in future studies when evaluating whether the assemblage represents one or multiple individuals.

Response: Thank you for your comment. Actually micro-CT analysis has already been used to help assess ontogenetic status in our study, i.e. the age for the Bianfu Cave radius was estimate based on the fusion status of the proximal secondary ossification center. To make it clear, we have added this methodological description in main text: *"The robust cortical structure and fully fused proximal epiphysis, assessed through micro-CT analysis of the internal morphology, indicate that the individual was likely an adult or near-adult*[43]*."*

• Page 10, line 269: before discussing Denisovan diversity, we first need additional fossils and a more complete characterization of the Denisovan phenotype. I would suggest rephrasing accordingly.

Response: Thanks for your advise. We have revised "diversity" to "phenotype" accordingly. And we also checked the whole manuscript for similar relatively inaccurate expressions and rephrase them.

• Page 10, line 283: I suggest replacing "found" with "identified."
• Page 10, line 283: I suggest replacing "our" with "these."

Response: We have revised as you suggested.

• Page 11, line 296: I am not sure that "morphological diversity" should be emphasized here. Given the current sample size, the primary contribution of this study is the identification and description of additional Denisovan remains and the documentation of their phenotype. Understanding Denisovan morphological diversity will require a substantially larger fossil record.

Response: Thanks for your comment. We have revised "diversity" to "phenotype".