# Peer review history for:

Madupe PP, Munir F, Dickinson M, Taurozzi AJ, Mackie M, Tawane M, et al. Results from an *Australopithecus africanus* dental enamel fragment confirm the potential of palaeoproteomics for South African Plio-Pleistocene fossil sites. S Afr J Sci. 2025;121(1/2), Art. #18571. https://doi.org/10.17159/sajs.2025/18571

**HOW TO CITE:**
Results from an *Australopithecus africanus* dental enamel fragment confirm the potential of palaeoproteomics for South African Plio-Pleistocene fossil sites [peer review history]. S Afr J Sci. 2025;121(1/2), Art. #18571. https://doi.org/10.17159/sajs.2025/18571/peerreview

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**Reviewer 1: Round 1**
**Date completed:** 10 September 2024
**Recommendation:** Accept / Revisions required / **Resubmit for review** / Resubmit elsewhere / Decline / See comments
**Conflicts of interest:** None

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**Yes**/No

**Comments to the Author:**

*Scope of topic and language*

This paper rests essentially on the report of new enamel-derived palaeoproteomic data for an *Australopithecus africanus* tooth fragment, Sts 63, from Sterkfontein Member 4. The result extends the reach of this technique in time, compared to several individuals of *Paranthropus robustus* from Swartkrans in an earlier paper (as yet still only on Biorvix, peer-reviewed version unpublished), and they discuss the implications for application to the fossil record, especially for the South African hominin sites. This paper nicely complements the previous Swartkrans work. The scope is appropriate for the journal. There are, however, a number of issues of organisation of the paper, length & emphasis, and language, which need attention to make it more methodical, straightforward, and accessible. The 'reviewing-the-field' parts are far too long, while the results *of this study* are not discussed succinctly. The language wanders into the colloquial. These factors could make it difficult for the average SAJSci reader (and public) to follow.

*Content*

Significance

The results show that the ancient proteome for Sts 63 is more degraded than the Swartkrans specimens, and that this finding conforms to a pattern for closed system decay. It's in keeping with its greater age than the Swartkrans (ca <2Ma) and the Dmanisi, Georgia teeth (1.78Ma). If confirmed across sites of different ages and fossilisation conditions (this is as yet one tooth) it is a significant finding. Just as importantly the individual is securely identified as male, result which can potentially resolve some previously intractable problems in the fossil record and open pathways for new research. A good overview is provided about why secure identification of males is relatively straightforward but that of females is not, while identification of males nevertheless, amongst a cohort, allows default identification of females. They discuss the ways in which this new approach resolves problems of existing approaches of identification in the fossil record. In particular the issues that muddy interpretations of species identification based on morphology and context, time (or deposit) depth, and sexual dimorphism; these are all longstanding problems in the South African

dolomitic/karst sites where contexts are extremely broad in time (in Members), and difficult to compare within and between sites.

Title, abstract, points of interest summary

The title "The potential of palaeoproteomics in southern Africa – a perspective with new results on *Australopithecus africanus"* seems to me to be the wrong way round (similarly the abstract and structure of the body of the paper, see below). It diminishes the importance of the results for Sts 63 from Sterkfontein, which should be instantly recognisable for context and age if properly identified. The potential of palaeoproteomics is important, yes, *but should flow from the report on this new, older sample and then discuss how this new avenue can enhance understanding of the fossil record*. Strongly suggest a rewording to something like "Results for an *Australopithecus africanus* tooth fragment, Sts63, confirms the potential of palaeoproteomics to South African fossil sites"

Abstract: needs a bit of reordering to be more direct – what is/are the problem(s) in the SA fossil record, why would palaeoproteomics be useful, what are the results, implications and the "to do" list. This echoes a problem with the organisation of the paper itself - the order is not helpful. I found the last sentence of the abstract unhelpful, vague and inappropriate in a scientific abstract.

Strongly suggest ending the abstract with a line on the exciting potential shown, maybe even say why it's happening in *these* sites. And then provide some more solid ideas in the last section of the paper itself. Currently it's vague.

Significance: The statement needs to be tidied up, it reads like a hurried afterthought. Missing is what specimen (Sts 63), from where (Sterkfontein Mbr 4, ~age), the approach (palaeoproteomics from enamel), and what the results show - concisely. What does "comprehension of sexual dimorphism" even mean? The biological sex is determined with *confidence* (not accuracy).

Literature review.

The literature review is extensive but *very long* considering the nature of the paper (a report?). The section that explains how the Palaeoproteomics field developed could be significantly trimmed; presently it goes all the way back to first discoveries of proteins in fossils in 1935 and should most certainly be drastically shortened and references trimmed to what are the essential recent developments. What is missing are a few references to the long legacy of work on the tricky topic of sex determination/dimorphism in the South African sites (eg. Wolpoff and Thackeray, who have also pointed to its importance as an interference in species identification).

Methods and results

The organisation of "what we did by way of analysis" seems to have been subsumed within the section labelled "An initial study of the protein preservation of *Australopithecus africanus"* which seems rather inadequate. Why is it an 'initial study', why is only protein preservation mentioned in the heading? A firmer and more accurate heading is required, eg "The enamel proteome of *Australopithecus africanus Sts 63"*. Some language used to introduce the section is inappropriate (eg. results are *reported* not 'shared').
The section needs to be tightened up – to be concise and to the point about the sample, its context, problems associated and why/how a palaeoproteomic study can address them. This statement "Sexual dimorphism, as the primary explanation for the morphological variation is also a possibility" is very vague – it's not "*also* a possibility" but a strong rationale for carrying out the research.

In the methods, it is useful to point out that the "etching technique" is minimally invasive. Third paragraph is wordy. Rather: "To assess markers of diagenesis, we compared the AMELY-59 intensities as a function of AMELX-60 intensities with a reference set of previously determined enamel to indicate the expected correlation between FAA and THAA racemisation for closed-system enamel … ."

*Methods* and *Results* seem to be elided, so not clear. There are too many "possibilities" in this section, whereas for instance, the mention of the effects of greater age can be expressed as "consistent with

greater age…". They are, of course older than the Swartkrans specimens for a start. Note that the geomorphological complexity of the Sterkfontein site will likely contribute to greater variability in preservation amongst different specimens (when they are analysed). Use the statement "These are the first australopithecene palaeoproteomic data to demonstrate sex identification in individuals, with a more detailed analysis of the palaeoproteome currently underway" in the conclusion rather than its present spot. The text in the figures for this section is too small to read easily so need enlargement

That section ends abruptly by switching topics to "The current challenges of palaeoproteomics" which ends up substituting for a thorough discussion of the results themselves. Also for the purposes here they are "opportunities" not "challenges". In particular *the discussion must be better connected to the findings reported*. They are great results even if only a single specimen but together with the existing data from the Swartkrans study can be used to lead nicely into a Discussion. Suggest to avoid/alter this negative sentence: "However, all investigations come with caveats, and there are still technical limitations in the palaeoproteomic workflow" - it states the obvious, and it's not clear what is meant by "Palaeoproteomic workflow". The explanations in the next paragraph about why its more challenging to identify females is clear and useful for the less initiated reader. The sentence beginning 358-360 should also be in the methods even if noted here as well. I thought that pointing to genetic homoplasy or molecular convergence (L372- 4) is somewhat of a remote problem, not sure why it's brought up as a caveat at all.

I was puzzled by the placement of the Methods section, is it correct? The research permit number for the analysis of the specimen is cryptic; normally it's given in fuller context (eg research permit no. xyz was granted for abc analysis by the XY authority). Usually this information appears at the end of the paper (not sure about the SAJ Sci protocol).

References

The reference list is inordinately long mostly because it concentrates on every single development heading towards Palaeoproteomics since 1935. It's really not necessary; judicious pruning is required to the essentials – could easily be <60 refs. Missing as noted above are to issues associated with morphological sex identification (eg Wolpoff and Thackeray who deal with SA cases). Copeland et al (2011) provides a good example of why a better method for sexing carries such potential ie, the potential for examining differences in behaviour or residence pattern/male or female philopatry amongst hominins.

*Presentation*

As noted above the organisation, style of language, and its clarity, needs a lot of tightening up. It's too long, too detailed in some places, too brief in others. It tends to read in some places like an essay and there is repetition. The structure needs attention. The language used is frequently inappropriate for a research paper. It's unnecessarily long in several parts and requires attention for better flow. The authors need to pay attention to the concept and results *in this case* and join them up. Both figures are required, but they need labelling with a larger font to be readable, and be directly addressed in the text. These comments are included in comments above.

Scientific conduct

My one concern is that the permission permit may be abbreviated.

For editor

All the comments made above can be conveyed to the author(s). Here for sake of brevity I repeat the summary I gave at the beginning of the review:

Significance

The results show that the ancient proteome for Sts 63 is more degraded than the Swartkrans specimens, and this finding conforms to a pattern for closed system decay within enamel. It's in keeping with its greater age than the Swartkrans (ca <2Ma) and the Dmanisi, Georgia teeth (1.78Ma). If confirmed across sites of different ages and fossilisation conditions (this is as yet one tooth) it is a significant finding. Just as importantly the individual is securely identified as male, result which can potentially resolve some previously intractable problems in the fossil record and open pathways for new research. A good caveat

overview is provided about why secure identification of males is relatively straightforward but that of females is not while identification of males nevertheless, amongst a cohort, allows default identification of females. They discuss the ways in which this new approach resolves problems of existing approaches of identification in the fossil record. In particular the issues that muddy interpretations of species identification based on morphology and context, time (or deposit) depth, and sexual dimorphism, are all longstanding problems in the case of the South African dolomitic sites where contexts are broad in time (in Members), and difficult to compare within and between sites.

Recommendation

The results are significant, but the rather sprawling and unfocused structure and language tend to obscure the importance and potential. There are issues of organisation, length, language (not just wordy but in some cases inappropriate), over-referencing for a short report which diminishes its accessibility for a general scientific audience (could easily reduce to <60), I suggest Resubmit for Review. The problems identified above are more than just minor revisions. It needs to be much better organised and attention paid to the language and points given above.

**Author response to Reviewer 1: Round 1**

Scope of topic and language

This paper rests essentially on the report of new enamel-derived palaeoproteomic data for an Australopithecus africanus tooth fragment, Sts 63, from Sterkfontein Member 4. The result extends the reach of this technique in time, compared to several individuals of Paranthropus robustus from Swartkrans in an earlier paper (as yet still only on Biorvix, peer-reviewed version unpublished), and they discuss the implications for application to the fossil record, especially for the South African hominin sites. This paper nicely complements the previous Swartkrans work. The scope is appropriate for the journal. There are, however, a number of issues of organisation of the paper, length & emphasis, and language, which need attention to make it more methodical, straightforward, and accessible. The 'reviewing-the-field' parts are far too long, while the results of this study are not discussed succinctly. The language wanders into the colloquial. These factors could make it difficult for the average SAJSci reader (and public) to follow.

Abstract: needs a bit of reordering to be more direct – what is/are the problem(s) in the SA fossil record, why would palaeoproteomics be useful, what are the results, implications and the "to do" list. This echoes a problem with the organisation of the paper itself - the order is not helpful. I found the last sentence of the abstract unhelpful, vague and inappropriate in a scientific abstract.

Strongly suggest ending the abstract with a line on the exciting potential shown, maybe even say why it's happening in these sites. And then provide some more solid ideas in the last section of the paper itself. Currently it's vague.

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AUTHOR: Thank you for the constructive feedback we have now restructured the entire manuscript and significantly cut down the literature review

The title "The potential of palaeoproteomics in southern Africa – a perspective with new results on Australopithecus africanus" seems to me to be the wrong way round (similarly the abstract and

structure of the body of the paper, see below). It diminishes the importance of the results for Sts 63 from Sterkfontein, which should be instantly recognisable for context and age if properly identified. The potential of palaeoproteomics is important, yes, but should flow from the report on this new, older sample and then discuss how this new avenue can enhance understanding of the fossil record. Strongly suggest a rewording to something like "Results for an Australopithecus africanus tooth fragment, Sts63, confirms the potential of palaeoproteomics to South African fossil sites"

AUTHOR: We thank the referee for the feedback, we have now changed the title to "Results from an ***Australopithecus africanus*** dental enamel fragment confirm the potential of palaeoproteomics for South African Plio-Pleistocene fossil sites"

The statement needs to be tidied up, it reads like a hurried afterthought. Missing is what specimen (Sts 63), from where (Sterkfontein Mbr 4, ~age), the approach (palaeoproteomics from enamel), and what the results show - concisely. What does "comprehension of sexual dimorphism" even mean? The biological sex is determined with confidence (not accuracy).

AUTHOR: We thank the reviewer for the feedback we have now tidied up the significance and have now included important information and removed ambiguous language lines 24 to 28

Insert reviewer comment here: What is missing are a few references to the long legacy of work on the tricky topic of sex determination/dimorphism in the South African sites (eg. Wolpoff and Thackeray, who have also pointed to its importance as an interference in species identification).

AUTHOR: Thank you for picking up our oversight, we have now cited the work of Thackeray and Wolpoff on lines 45 to 47

Methods and results

The organisation of "what we did by way of analysis" seems to have been subsumed within the section labelled "<u>An initial study of the protein preservation of Australopithecus africanus" which seems rather inadequate. Why is it an 'initial study', why is only protein preservation mentioned in the heading?</u> A firmer and more accurate heading is required, eg "The enamel proteome of Australopithecus africanus Sts 63".

<u>Some language used to introduce the section is inappropriate (eg. results are reported not 'shared')</u>. The section needs to be tightened up – to be concise and to the point about the sample, its context, problems associated and why/how a palaeoproteomic study can address them. This statement "Sexual dimorphism, as the primary explanation for the morphological variation is also a possibility" is very vague – it's not "also a possibility" but a strong rationale for carrying out the research.

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Scientific conduct

My one concern is that the permission permit may be abbreviated.

AUTHOR: We have rearranged the sections starting with the methods section and made the language

concise and easy to follow. We also rectified our mistake of not clearly stating what Permit body awarded it; it's now clearly mentioned in the methods section. Following that is the results section with a clear communication of the results we obtained.

That section ends abruptly by switching topics to "The current challenges of palaeoproteomics" which ends up substituting for a thorough discussion of the results themselves. Also for the purposes here they are "opportunities" not "challenges". In particular the discussion must be better connected to the findings reported. They are great results even if only a single specimen but together with the existing data from the Swartkrans study can be used to lead nicely into a Discussion. Suggest to avoid/alter this negative sentence: "However, all investigations come with caveats, and there are still technical limitations in the palaeoproteomic workflow" - it states the obvious, and it's not clear what is meant by "Palaeoproteomic workflow". The explanations in the next paragraph about why its more challenging to identify females is clear and useful for the less initiated reader. The sentence beginning 358-360 should also be in the methods even if noted here as well. I thought that pointing to genetic homoplasy or molecular convergence (L372- 4) is somewhat of a remote problem, not sure why it's brought up as a caveat at all.

AUTHOR: We cleaned up the remainder of the text and structuring it as follows, in the discussion we have this subsection "A preliminary protein profile of A. africanus" where we contextualise the current finding in relation to the previous work done on ***Paranthropus*** and what it could mean for future work on investigating variation. Data availability has been moved to the methods section. Lastly, we have renamed the limitation section to The current limitations – and future opportunities of palaeoproteomics, and have rewritten and made more concise the paragraph on genetic homoplasy, admixture and ILS and referenced Degnan, J. H. & Rosenberg, N. A 2009 works on Gene tree discordance and phylogenetic inference, which details the challenges and how to better form phylogenies using gene trees.

References

The reference list is inordinately long mostly because it concentrates on every single development heading towards Palaeoproteomics since 1935. It's really not necessary; judicious pruning is required to the essentials – could easily be <60 refs. Missing as noted above are to issues associated with morphological sex identification (eg Wolpoff and Thackeray who deal with SA cases). Copeland et al (2011) provides a good example of why a better method for sexing carries such potential ie, the potential for examining differences in behaviour or residence pattern/male or female philopatry amongst hominins.

AUTHOR: We have significantly cut down on the reference list and added the suggested works by Wolpoff and Thackeray. We agree that Copelands work is a good example of better approaches for sexing fossils. It was extremely useful for the *Paranthropus* palaeoproteomics work (and future work) as one of the specimens (SK 835) had been analysed by Copeland. As it stands the palaeoproteomic work corroborates the work on isotopes and philopatry of these hominins.

**Reviewer 1: Round 2**

**Date completed:** 02 December 2024

**Recommendation: Accept** / Revisions required / Resubmit for review / Resubmit elsewhere / Decline / See comments

**Conflicts of interest:** None

Does the manuscript fall within the scope of SAJS?

**Yes**/No

Is the manuscript written in a style suitable for a non-specialist and is it of wider interest than to specialists alone?

**Yes**/No

Does the manuscript contain sufficient novel and significant information to justify publication?

**Yes**/No

Do the Title and Abstract clearly and accurately reflect the content of the manuscript?

**Yes**/No

Is the research problem significant and concisely stated?

**Yes**/No

Are the methods described comprehensively?

**Yes**/No

Is the statistical treatment appropriate?

Yes/No/Not applicable/**Not qualified to judge**

Are the interpretations and conclusions justified by the research results?

**Yes**/Partly/No

Please rate the manuscript on overall contribution to the field

Excellent/**Good**/Average/Below average/Poor

Please rate the manuscript on language, grammar and tone

Excellent/**Good**/Average/Below average/Poor

Is the manuscript succinct and free of repetition and redundancies?

**Yes**/No

Are the results and discussion confined to relevance to the objective(s)?

**Yes**/No

The number of tables in the manuscript is

Too few/**Adequate**/Too many/Not applicable

The number of figures in the manuscript is

Too few/**Adequate**/Too many/Not applicable

Is the supplementary material relevant and separated appropriately from the main document?

Yes/No/**Not applicable**

Please rate the manuscript on overall quality

**Excellent**/Good/Average/Below average/Poor

Is appropriate and adequate reference made to other work in the field?

**Yes**/No

Is it stated that ethical approval was granted by an institutional ethics committee for studies involving human subjects and non-human vertebrates?

Yes/No/**Not applicable**

If accepted, would you recommend that the article receives priority publication?

**Yes**/No

Are you willing to review a revision of this manuscript?

Yes/**No**

Select a recommendation:

**Accept** / Revisions required / Resubmit for review / Decline

With regard to our policy on 'Publishing peer review reports', do you give us permission to publish your anonymised peer review report alongside the authors' response, as a supplementary file to the published article? Publication is voluntary and only with permission from both yourself and the author.

**Yes**/No

**Comments to the Author:**

Very good job restructuring the paper!

One other point I suggest is to temper hopes of transferring this technique easily to East Africa. (Discussion lines 32 onwards) has a brief discussion about protein preservation outside the Cradle, ending with a mention of possibilities for East Africa (L 87). Just to point out 2 things: the preservation status of enamel in all the South African sites that are dolomitic/CaCO3 rich, is surprisingly good, certainly for isotopes and trace elements. As pointed out by Lee-Thorp & Sponheimer (this SAJ Sci volume) studies show limited reorganisation of the ions within the crystal lattices and little diagenesis (eg Sponheimer & Lee-Thorp 2006, Geochim Cosmochim Acta; 70: 1644-1654). The depositional environments in East Africa however are very different with consequences for enamel preservation/recrystallisation and intrusions (for ex. Kohn et al 1999. Altered states: effects of diagenesis on fossil tooth chemistry. Geochim Cosmochim Acta 63: 2737-2747). The point is that expanding palaeoproteomics to East African sites is unlikely to be straightforward and will need much exploration of preservation– we already know it's not as good as the SA sites.

**Author response to Reviewer 1: Round 2**

One other point I suggest is to temper hopes of transferring this technique easily to East Africa. (Discussion lines 32 onwards) has a brief discussion about protein preservation outside the Cradle, ending with a mention of possibilities for East Africa (L 87). Just to point out 2 things: the preservation status of enamel in all the South African sites that are dolomitic/CaCO3 rich, is surprisingly good, certainly for isotopes and trace elements. As pointed out by another paper in the special issue, studies show limited reorganisation of the ions within the crystal lattices and little diagenesis (eg Sponheimer & Lee-Thorp 2006, Geochim Cosmochim Acta; 70: 1644-1654). The depositional environments in East Africa however are very different with consequences for enamel preservation/recrystallisation and intrusions (for ex. Kohn et al 1999. Altered states: effects of diagenesis on fossil tooth chemistry. Geochim Cosmochim Acta 63: 2737-2747). The point is that expanding palaeoproteomics to East African sites is unlikely to be straightforward and will need much exploration of preservation– we already know it's not as good as the SA sites.

AUTHOR: We thank the reviewer for the constructive feedback, we have now added this sentence "However, there is currently no published hominin protein preservation data on fossils recovered from other sites in South Africa and no data from open-air fossil sites, particularly in eastern Africa, where the very different depositional environments are known to have detrimental diagenetic consequences for enamel preservation (Kohn et al. 1999). Expanding palaeoproteomic studies of enamel outside of the Cradle cave system context will therefore require considerable exploration of preservation, an important future avenues for research into African Plio-Pleistocene hominins." between line 40 to 43 to help caution the potential of palaeoproteomics in eastern Africa.

**Reviewer 2: Round 1**

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