

The *South African Journal of Science* follows a double-anonymous peer review model but encourages Reviewers and Authors to publish their anonymised review reports and response letters, respectively, as supplementary files after manuscript review and acceptance. For more information, see [Publishing peer review reports](#).

Peer review history for:

Stewart RD, van der Bank M, Davies JT. Unveiling South African insect diversity: DNA barcoding's contribution to biodiversity data. *S Afr J Sci.* 2024;120(5/6), Art. #16448. <https://doi.org/10.17159/sajs.2024/16448>

HOW TO CITE:

Unveiling South African insect diversity: DNA barcoding's contribution to biodiversity data [peer review history]. *S Afr J Sci.* 2024;120(5/6), Art. #16448. <https://doi.org/10.17159/sajs.2024/16448/peerreview>

Reviewer H: Round 1

Date completed: 11 October 2023

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

Conflicts of interest: None declared

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

Do you believe somebody with more methodological expertise in the area of this study than you have needs to review this?

Yes/No

If yes, can you suggest the type of expertise needed.

Not applicable

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:

The paper is a review of insect diversity in South Africa through DNA barcodes data. The paper is interesting and shows the gaps between studies carried out on insects across the different provinces of the country, as well as the differences in the representation of orders, families, genera and species in the BOLD database. The objectives were not clearly stated and there was a lack of useful information across some subchapters. I felt author(s) did not do an extensive review on some aspects that would have helped to understand the importance and the value of insects in the ecosystems. Enclosed are a few comments for improvement.

Page 1

L 8-10: Split into two sentences. For instance: from "Insects...to Earth." And from "They comprise...to decomposition services."

L9: including pollination, pest control...

L18: Please provide the full meaning of BOLD as it appears the first time. Example: BOLD = The Barcode of Life Data System

L21-23: Please rephrase. How can barcode records derive from Malaise traps? According to what I know, Malaise traps are among several collection methods of insects.

L23-25: Your conclusions are not clear to me. Have you made statistical analyses of studies using Malaise, entomological nets and other methods for the sampling of insects?

L30-31: Please improve the sentence. For instance: "Provinces greatly sampled include Gauteng, Mpumalanga and Limpopo, while the rest of provinces such as Eastern Cape, Free State, KwaZulu-Natal, Northern Cape, North West and Western Cape remains under-sampled".

L33-34: "Urgent investment" on what concretely? The idea sounds incomplete. Please rephrase

Page 2

L38-40: Reference 3, Zhang (2013) is old. Please search for up to date data to ascertain proportions reported. In case there is no recent manuscripts (which I doubt), you can still use continental or worldwide public database too for updating the data provide by Zhang (2013).

L40: Instead of "providing" rather use "including". Decomposition services of wastes? Plant material etc? Please clarify

L41-43: This sentence is hard to grasp. Bees are'nt part of insects? Please rephrase

L44: Reference 7 (Klein et al. 2003) is too old for such statement. Please use a most recent one.

As a note: there is an imbalance between the benefits and drawbacks of insects throughout the

introduction. Please develop a bit about their usefulness in the decomposition of agricultural wastes and pest control.

Nowhere, I have seen the aims or objectives of the review

Page 3

L65-66: Please rephrase. For instance: "Insects are particularly susceptible to environmental change, which makes them potential bio-indicators of ecosystem health"??

L67-68: Please add the reference

L64-76: Subchapter Global insect decline: You did not mention any trends about insect declines in Africa or Southern Africa or even South Africa. By not giving such information you are not adding any value to this review paper. In contrast, by adding such information, you will also facilitate the thematic transition to subchapter Importance of South Africa's insects.

Find below a paper on insect decline in South Africa. I am sure you may find more.

A survey of managed honey bee colony losses in the Republic of South Africa - 2009 to 2011. *Journal of Apicultural Research*. 53(1):35-42. DOI 10.3896/IBRA.1.53.1.03

L78: Subchapter Importance of South Africa's insects: The title of the subchapter does not fit with the content. For instance, I wonder whether coming up with the fact disease caused by certain insects is the reason of the subchapter "importance of South Africa's insects". Overall, the information provides are generalities

Please make a well structured subchapter with the addition of reader-friendly information and the use of recent literature.

For instance: L79-81, there is a recent paper on Diptera order precisely hoverflies from Free State province that you can use to update the information provide here. L86-89, you are not giving what species are used for biocontrol within South Africa. L87-89, please add recent references.

- Developmental performance of *Eristalis tenax* larvae (Diptera: Syrphidae): Influence of growth media and yeast addition during captive rearing. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, 1–11. <https://doi.org/10.1002/jez.2696>

Page 4

L128-130: Please add most recent references. For instance see the study below:

- Local-Scale DNA Barcoding of Afrotropical Hoverflies (Diptera: Syrphidae): A Case Study of the Eastern Free State of South Africa. *Insects* 2023, 14, 692.

L139-141: Elaborate a bit about the consequences of 'dark taxa'

As a note: Elaborated about how DNA barcoding assists in separating between species and within species.

Page 5

L147-148: What do you exactly mean? Please complete the sentence

L155-156: For the sake of information, report the records made by Myburgh as well

L166-167: As a recall, add the estimated from Scholtz and Chown.

As a note: Use the recent study by Kamdem et al. 2023 to update species diversity for South African's Diptera.

Fig 1. The figure exhibits the estimated number of species for South Africa or for Southern Africa. Please clarify.

Page 7

L204: "Herman Staude and Ross Stewart". Please add the year.

L220-221: 7.7% of 384 523 records is not 31 855. Please recheck the calculations. Do so for other

calculations here and there
L222: "See above". Where?

Page 8

L240: Subtitle Biomonitoring: Please add "South Africa" and "DNA barcoding"

L250: In South Africa or In South African

Conclusion: add more prospects, example in monitoring pests of agricultural products

Author response to Reviewer H: Round 1

The paper is a review of insect diversity in South Africa through DNA barcodes data. The paper is interesting and shows the gaps between studies carried out on insects across the different provinces of the country, as well as the differences in the representation of orders, families, genera and species in the BOLD database. The objectives were not clearly stated and there was a lack of useful information across some subchapters. I felt author(s) did not do an extensive review on some aspects that would have helped to understand the importance and the value of insects in the ecosystems. Enclosed are a few comments for improvement.

AUTHOR: We thank the reviewer for their detailed comments, which we address in our revised submission, and as outlined below.

L 8-10: Split into two sentences. For instance: from "Insects...to Earth." And from "They comprise...to decomposition services."

AUTHOR: L8-10: The sentence was split into two sentences as suggested. "Insects are one of the most species-rich groups on Earth. They comprise much of animal diversity and play a vital role in ecosystems, including pollination, pest control, and decomposition services."

L9: including pollination, pest control...

AUTHOR: L9 – The sentence was adjusted as suggested.

L18: Please provide the full meaning of BOLD as it appears the first time. Example: BOLD = The Barcode of Life Data System

AUTHOR: L18 – The full meaning for BOLD is now included: "Barcode of Life Data System"

L21-23: Please rephrase. How can barcode records derive from Malaise traps? According to what I know, Malaise traps are among several collection methods of insects.

AUTHOR: L21-22 – The sentence was reworked to explain better which sampling method was the most dominant trapping method: "Most of the barcoded records are derived from Malaise trap sampling in Gauteng, Mpumalanga and Limpopo, while the rest of South Africa remains poorly sampled."

L23-25: Your conclusions are not clear to me. Have you made statistical analyses of studies using Malaise, entomological nets and other methods for the sampling of insects?

AUTHOR: L24 – We apologise for the confusion, this sentence has been reworked to indicate that there needs to be a national sampling effort in general and not just Malaise trap sampling.

L30-31: Please improve the sentence. For instance: "Provinces greatly sampled include Gauteng, Mpumalanga and Limpopo, while the rest of provinces such as Eastern Cape, Free State, KwaZulu-Natal, Northern Cape, North West and Western Cape remains under-sampled".

AUTHOR: L31-33 – The sentence was changed to "Well-sampled provinces include Gauteng, Mpumalanga and Limpopo, while the Eastern Cape, Free State, KwaZulu-Natal, Northern Cape, North West and Western Cape remain under-sampled." We hope this was sufficient.

L33-34: "Urgent investment" on what concretely? The idea sounds incomplete. Please rephrase

AUTHOR: L34-35 – The sentence was changed to be more specific and indicate that there must be investments in: "in taxonomic expertise and biomonitoring".

L38-40: Reference 3, Zhang (2013) is old. Please search for up to date data to ascertain proportions reported. In case there is no recent manuscripts (which I doubt), you can still use continental or worldwide public database too for updating the data provide by Zhang (2013).

AUTHOR: L42 – The reference was updated and we now cite a study from 2018. (Stork NE. How Many Species of Insects and Other Terrestrial Arthropods Are There on Earth? *Annu Rev Entomol.* 2018;63(1):31–45. <https://doi.org/10.1146/annurev-ento-020117-043348>)

L40: Instead of “providing” rather use “including”. Decomposition services of wastes? Plant material etc?
Please clarify

AUTHOR: L42-43 – The sentence was reworked as suggested.

L41-43: This sentence is hard to grasp. Bees aren't part of insects? Please rephrase

AUTHOR: L44 – The sentence was rephrased to indicate that bees are included as pollinators of agricultural crops.

L44: Reference 7 (Klein et al. 2003) is too old for such statement. Please use a most recent one.

AUTHOR: L46 – a more recent reference was used, and we now cite a study from 2020. (Reilly JR, Artz DR, Biddinger D, Bobiwash K, Boyle NK, Brittain C, et al. Crop production in the USA is frequently limited by a lack of pollinators. *Proc R Soc B Biol Sci.* 2020;287(1931):2–9. [10.1098/rspb.2020.0922](https://doi.org/10.1098/rspb.2020.0922))

As a note: there is an imbalance between the benefits and drawbacks of insects throughout the introduction. Please develop a bit about their usefulness in the decomposition of agricultural wastes and pest control.

AUTHOR: L 50-56 – The introduction was restructured to help better balance the discussion on the benefits and drawbacks of insects.

Nowhere, I have seen the aims or objectives of the review

AUTHOR: L68-71 – The aim and objectives for the review paper have now been added at the end of the introduction.

L65-66: Please rephrase. For instance: “Insects are particularly susceptible to environmental change, which makes them potential bio-indicators of ecosystem health”??

AUTHOR: L74-75 – The sentence was rephrased as: “Insects are particularly sensitive to environmental change, and thus provide useful bio-indicators of ecosystem health”.

L67-68: Please add the reference

AUTHOR: L77 – A reference has been added to this sentence. (Hallmann CA, Sorg M, Jongejans E, Siepel H, Hofland N, Schwan H, et al. More than 75 percent decline over 27 years in total flying insect biomass in protected areas. *PLoS One.* 2017;12(10). [10.1371/journal.pone.0185809](https://doi.org/10.1371/journal.pone.0185809))

L64-76: Subchapter Global insect decline: You did not mention any trends about insect declines in Africa or Southern Africa or even South Africa. By not giving such information you are not adding any value to this review paper. In contrast, by adding such information, you will also facilitate the thematic transition to subchapter Importance of South Africa's insects.

Find below a paper on insect decline in South Africa. I am sure you may find more.

A survey of managed honey bee colony losses in the Republic of South Africa - 2009 to 2011. *Journal of Apicultural Research.* 53(1):35-42. DOI [10.3896/IBRA.1.53.1.03](https://doi.org/10.3896/IBRA.1.53.1.03)

AUTHOR: L73-96 – We agree with the reviewer, and have therefore restructured this section, now retitled Insect Decline. Here, we also highlight that the figure of an estimated 44 000 insect species is dated, and use a suggested article (Kamdem et al. 2023) to indicate how these figures are likely underestimates. We also refer to the observed loss in honey-bee colonies and how the butterflies in the Renosterveld vegetation are under threat because their natural vegetation is being destroyed.

L78: Subchapter Importance of South Africa's insects: The title of the subchapter does not fit with the content. For instance, I wonder whether coming up with the fact disease caused by certain insects is the reason of the subchapter “importance of South Africa's insects”. Overall, the information provides are generalities

AUTHOR: L86-96 – As we mentioned above, this section is now sunk into the Insect decline. While reworking this section, we also updated the references using more recent literature, as suggested below.

Please make a well structured subchapter with the addition of reader-friendly information and the use of recent literature. For instance: L79-81, there is a recent paper on Diptera order precisely hoverflies from Free State province that you can use to update the information provide here. L86-89, you are not giving what species are used for biocontrol within South Africa. L87-89, please add recent references.

- Developmental performance of *Eristalis tenax* larvae (Diptera: Syrphidae): Influence of growth media and yeast addition during captive rearing. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, 1–11. <https://doi.org/10.1002/jez.2696>

AUTHOR: L86-96 – As detailed above, this subsection was reworked and combined into a new broader section titled Insect Decline. Because we had to reduce some text to stay within word limits, we removed the specific discussion on biocontrol within South Africa, as this was only peripheral to our main focus.

L128-130: Please add most recent references. For instance see the study below:

- Local-Scale DNA Barcoding of Afrotropical Hoverflies (Diptera: Syrphidae): A Case Study of the Eastern Free State of South Africa. *Insects* 2023, 14, 692.

AUTHOR: L128-129 – The suggested article was added as it was a good example as to how DNA barcoding can discriminate between morphologically challenging taxa and has been included in this context.

L139-141: Elaborate a bit about the consequences of 'dark taxa'.

AUTHOR: L143-144 – We better described how 'dark taxa' lack taxonomic information, limiting the insight that could be gained on their ecological functions.

As a note: Elaborated about how DNA barcoding assists in separating between species and within species.

AUTHOR: L139-141 – A sentence was added describing how DNA barcoding "allows us to cluster samples by sequence similarity, essentially discriminating between OTUs in the absence of detailed taxonomic knowledge".

L147-148: What do you exactly mean? Please complete the sentence

AUTHOR: L150-151 – The sentence was restructured to make it clearer the contribution by Myburgh. "Myburgh et al.17 recently published on South Africa's contribution of insect records on BOLD by South Africa".

L155-156: For the sake of information, report the records made by Myburgh as well

AUTHOR: L159 – This value (56 392 records) is now included at the end of the sentence.

L166-167: As a recall, add the estimated from Scholtz and Chown.

AUTHOR: L171 – The estimated value by Scholtz and Chown (43 565) has been added, as suggested.

As a note: Use the recent study by Kamdem et al. 2023 to update species diversity for South African's Diptera.

AUTHOR: This study is a useful example to highlight that much of South Africa's insect diversity is underestimated (especially Diptera), and we cite it on lines 89-91.

Fig 1. The figure exhibits the estimated number of species for South Africa or for Southern Africa. Please clarify.

AUTHOR: Fig 1. (L497-499) – The figure legend was revised to make it clearer that the Number of BINs is from South Africa and the estimated numbers are from southern Africa, as reported by Scholtz and Chown.

L204: "Herman Staude and Ross Stewart". Please add the year.

AUTHOR: L209 – This was reworked to include the years for each person.

L220-221: 7.7% of 384 523 records is not 31 855. Please recheck the calculations. Do so for other calculations here and there.

AUTHOR: L224-226 – The percentages were correct, but the sentence was confusing as to what they represented. We have therefore reworked the sentence so that they it is clear to the reader that we are refereeing to the total 416 266 published records.

L222: "See above". Where?

AUTHOR: We see how this was ambiguous, so it was removed.

L240: Subtitle Biomonitoring: Please add "South Africa" and "DNA barcoding"

AUTHOR: L244 – We have changed the subtitle to "Biomonitoring in South Africa using DNA barcoding".

L250: In South Africa or In South African

AUTHOR: L254 – We now make clear that this study was for South Africa.

Conclusion: add more prospects, example in monitoring pests of agricultural products

AUTHOR: L273-277 – We have added content to highlight some of the additional prospects and benefits of extending DNA barcoding in South Africa. "A better knowledge of both the taxonomic and geographic distribution of insect diversity within South Africa would allow for more targeted conservation action to maintain the important ecological functions they provide. Combining DNA barcoding and Malaise trapping would also allow for improved monitoring of agricultural pests and pollinators, supporting food security."

Reviewer H: Round 2

Date completed: 01 February 2024

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

Conflicts of interest: None

Does the review fall within the scope of SAJS??

Yes/No

Is the review written in a style suitable for a non-specialist and is it of wider than only specialist interest?

Yes/No

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

Yes/No

Does the review provide a significantly novel perspective or significant recent advances in the field?

Yes/No

Is the objective of the review concisely stated?

Yes/No

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

Yes/No

Do current debates and points of contention receive appropriate coverage?

Yes/No/Not applicable

Are gaps in the literature adequately identified?

Yes/No/Not applicable

Does the review provide direction for future research?

Yes/No/Not applicable

Is the methodology and statistical treatment appropriate?

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

Are the interpretations and recommendations aligned with the objective?

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 concise and free of repetition and redundancies?

Yes/No

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

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:

I recognise the improvements and appreciate them compared to the first submitted version. Nevertheless, there is further need, some of it considerable. I have reread the paper entirely and my feeling is that authors MUST make the manuscript read by a third party.

The language of this paper needs to be professionally embellished to make the expression more authentic and convenient for the reader to understand. Several paragraphs still need to be corrected. Below are a few syntax and expressions errors.

-For instance, Abstract: please use past tense consistency in line 18, 20, 22.

-Abstract: Line 20: BIN stands for "Barcode Index Number" or "Barcoding Index Number" please recheck

-Line 21, please make a clear distinction of South Africa and southern Africa. Do so throughout the manuscript.

Introduction

-Line 43: rather mention "decomposition of organic materials" instead of restricting it to "plant decomposition" which of course is not complete

-Line 48: "...to half a million premature deaths annually..." in humans? Please make clearer

-Line 52: add references

-Introduction: paragraph 2, line 50-56. Please complete the information about their role in "decomposition of organic materials". One or two sentences could be enough

In the paper below you have a recent study of insect decomposition of organic matter from plant and animal origin. I had included this reference in previous comments:

-Developmental performance of *Eristalis tenax* larvae (Diptera: Syrphidae): Influence of growth media and yeast addition during captive rearing. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, 1–11. <https://doi.org/10.1002/jez.2696>

-Line 59: "... and insect pests that reduce agricultural crop yields" add a reference at the end of the sentence

-Line 58-59: " Millions of lives are lost annually to human diseases carried by insect vectors¹² and insect pests that reduce agricultural crop yields". Hard to grasp, please rephrase.

-Line 69-70: " We query the public database BOLD (Barcode of Life Data System) for insect records in South Africa and explore their current coverage".

Note: There is no mention of DNA barcode technology throughout your introduction. You did it only while stating the objectives. Also, nothing about insects of South Africa (for instance data of their benefits to agriculture in SA etc.). The most important aspects related to this topic should be clearly presented in order to provide a properly description of the state of art in this field and gaps. Moreover, the main objectives of this review should be clearly and detailed presented in few words. For instance which tool(s) did you use for quering on

BOLD. As far as I know there are numerous tools on BOLD for sometimes different purposes. You can remove some ge

Insect decline

-Line 82-83: not clear what you are saying. please rephrase

-Line 88-89: Please check for better data and regional public databases, such SANBI database etc. We never know!

-Line 87-97: move parts of this paragraph to the introduction section.

The taxonomic impediment, DNA barcoding and dark taxa

-Improve the outline of this subchapter. You could use an outline that links the thematic between taxonomic impediment, barcode technology and recent surge in species inventory

-Please define "taxonomic impediment".

<https://academic.oup.com/zoolinnean/article/193/2/381/6374389>

-Line 103-106: This is a nonsense to me. 80 000 undescribed insect species vs. estimated 44 000 insect species (line 88). How is this possible?

-Line 132-134: " Additional applications of DNA barcoding.... of illegal wildlife47,48". Remove this sentence.

Note: Move parts of this section to introduction section. For instance paragraph 111-120, and so on

Progress on insect barcoding in South Africa: This section should be reorganized

-Please rephrase, line 152-153: "Myburgh et al.17 recently published on South Africa's contribution of insect records on BOLD database..."

-Please say it differently, line 153-155: "D'Souza et al.25 added 339 193 insect records..."

-Line 152-155 : One could not start the section by providing such information. One could start by giving a short story on first insects barcodes from South Africa deposited on BOLD (number of barcodes, insect order, family, date etc.) and follow with the information provided in paragraph (line 156-163). Moreover, you could use this information (line 152-155) to conclude on fast growing number of barcodes deposited from South Africa.

-Line 156: please remove this bit of sentence

-Line 159: From Table 1, the total number of barcodes from South Africa is 416 211, while you reported 416 266 in text.

-Line 164-165: please make a clear distinction of South Africa and southern Africa.

-Line 165-166: " However, the taxonomy of southern African insects is constantly changing". This bit of sentence is not scientifically true.

-Line 172: Please rephrase

-Line 173-175: Please add the estimated number of species for each group. "However, we identify five orders with more BINs than known species for southern Africa (Hymenoptera = 9259 vs. 5273, Diptera = 7611 vs. 6243, Thysanoptera = 263 vs. 228, Plecoptera = 32 vs. 22, and Strepsiptera = 7 vs. 6; Fig. 1)".

Please make a clear distinction of South Africa and Southern Africa. It seems the study by Scholtz and Chown estimated the species diversity in Southern Africa which encompasses countries such Botswana, South Africa, Namibia, Eswatini etc., different from South Africa (solely The republic of South Africa). This means, the estimated number of species from South Africa must be retrieved from those of Southern Africa. Please check!!!

-Line 175-177: BE CAREFUL when you draw such conclusion! BOLD is a database where everyone (even not a taxonomist) can deposit sequences for a given organism. Therefore, discordance between BIN assignments and current taxonomy may occur. BIN assigned on BOLD must not be seen as the only reliable tool (or the only source) for species identification, reason we distinguish published (through an article or scientific communication) and unpublished records. You must deeper the reflexion and provide possible explanations (recurrent cases of cryptic diversity etc.) in accordance with data and their interpretation

Please consult the following resources that may help you learn more about BIN. there are numerous papers on the matter online: <https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0066213>

<https://cdnsiencepub.com/doi/full/10.1139/gen-2017-0096>

<https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0054921>

<https://link.springer.com/article/10.1007/s11033-022-08015-7>

<https://www.sciencedirect.com/science/article/abs/pii/S0031405608000449>

-Line 182-183: Please add values in parentheses

-Line 185-187: To make the thing clearer, please add relevant values in parentheses. Also add the estimated number of species when needed.

-Line 193-194: Rephrase and correct. Dipetra is an order not a family. The percentage of 49% is not true. 121 513 over 416 266 is 29%

-Line 198: How did you find this percentage of 88%

-Line 205: Have I missed figure 2? You are already citing figure 3

-Line 205-206: " South Africa's most significant contribution... the Kruger Malaise Program²⁵" do you mean the most significant contribution in a single submission? Otherwise explain me how 339 193 could be higher than 416 266.

-Line 207-208: Instead of "The majority of the sequences were composed of" use "The major contributors of the sequences were composed of"

-Line 209: add the estimated number of species

-Line 210-213: Rephrase please. "The subsequent most valuable contributions were ca. 29 400 and 15 884 specimens collected using Malaise traps in the West Rand district and the City of Johannesburg, respectively (H. Staude in 2012 and R. Stewart in 2017)"

-Line 214-217: 'highest' is superlative and use in a comparative manner, and cannot be used as such. Say it differently

-Line 217: You cannot cite fig 3 before fig 2. This section should be reorganized

-Line 218: avoid contracted form

-Line 228: Please state the method use to collect the rest of 8% Note: This section should be reorganized Biomonitoring in South Africa using DNA barcoding

Start by briefly define biomonitoring and its usefulness.

-Line 272-277: Draw the main conclusions about DNA barcoding contribution to the knowledge of South African biodiversity (insects) to date, before moving to future prospects and/or further needs.

Author response to Reviewer H: Round 1

Dear Authors, I recognise the improvements and appreciate them compared to the first submitted version. Nevertheless, there is further need, some of it considerable. I have reread the paper entirely and my feeling is that authors MUST make the manuscript read by a third party.

The language of this paper needs to be professionally embellished to make the expression more authentic and convenient for the reader to understand. Several paragraphs still need to be corrected. Below are a few syntax and expressions errors.

AUTHOR: We thank the reviewer for their constructive feedback, which we have addressed as detailed below (our comments and changes in the manuscript highlighted in yellow). We apologise for errors in our language and grammar in our previous submission. We have undertaken a thorough edit of the final manuscript to catch any remaining errors, and we hope you will find this new version much improved.

For instance, Abstract: please use past tense consistency in line 18, 20, 22.

AUTHOR: Line 17, 19, 20 – The tense has been adjusted.

Abstract: Line 20: BIN stands for “Barcode Index Number” or “Barcoding Index Number” please recheck

AUTHOR: Line 19-20 – The acronym has been corrected to “Barcode Index Number”.

Line 21, please make a clear distinction of South Africa and southern Africa. Do so throughout the manuscript.

AUTHOR: Line 71-74 – We now more clearly distinguish between these geographical locations and explain why the data from southern Africa is discussed: “However, there is currently no comprehensive modern classification of higher insect taxa available for South Africa. Records for southern Africa suggest there are ca. 44 000 insect species, encompassing 7750 genera, 569 families, and 25 orders, but these numbers are now more than 35 years old and have shown to be underestimates.”

Line 43: rather mention “decomposition of organic materials” instead of restricting it to “plant decomposition” which of course is not complete

AUTHOR: Line 43 – Thank you for this suggestion, this sentence now reads: “Insects play a vital role in ecosystems, including pollination, pest control, decomposition of organic materials, and nutrient cycling”

Line 48: “...to half a million premature deaths annually...” in humans? Please make clearer

AUTHOR: Line 48 – We have made it clearer that the deaths are in humans. “... half a million premature human deaths annually due to a shortage ... ”

Line 52: add references

AUTHOR: Line 52 – Added two references:

1. Brust GE, Wakil W, Qayyum MA. Chapter 8: Minor Pests. In: Sustainable Management of Arthropod Pests of Tomato. Elsevier Inc.; 2017. p. 183–214. 10.1016/B978-0-12-802441-6.00008-5
2. Bonning BC. Biotechnology and Insects. In: Encyclopedia of Insects. Second Edi. Elsevier Inc.; 2009. p. 105–8. 10.1016/B978-0-12-374144-8.00035-7

Introduction: paragraph 2, line 50-56. Please complete the information about their role in “decomposition of organic materials”. One or two sentences could be enough

AUTHOR: Line 54-58 – We have added information about the role of insects in the decomposition of organic matter and included the suggested citation. The sentences now reads: “Insect scavengers, detritivores, or filter-feeders on microbes also contribute importantly to decomposition¹². Thus many insects additionally help with nutrient cycling and transformation of living biomass into frass, cycling the Carbon and Nitrogen back into the soil¹³. This processes of breaking down the dead organic matter accounts for ca. 29% of forest deadwood decomposition¹⁴.”

In the paper below you have a recent study of insect decomposition of organic matter from plant and animal origin. I had included this reference in previous comments:

- Developmental performance of *Eristalis tenax* larvae (Diptera: Syrphidae): Influence of growth media and yeast addition during captive rearing. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, 1–11. <https://doi.org/10.1002/jez.2696>

- Line 59: “... and insect pests that reduce agricultural crop yields” add a reference at the end of the sentence

AUTHOR: Line 61 – A reference has been added to the end of this sentence.

1. Tudi M, Ruan HD, Wang L, Lyu J, Sadler R, Connell D, et al. *Agriculture Development, Pesticide*

Application and Its Impact on the Environment. Environ Research public Heal. 2021;18(1112):1–23.

Line 58-59: “ Millions of lives are lost annually to human diseases carried by insect vectors¹² and insect pests that reduce agricultural crop yields”. Hard to grasp, please rephrase.

AUTHOR: Line 60-61 – We have adjusted the sentence to read “Millions of lives are lost annually to both human diseases carried by insect vectors¹⁵ and insect crop pests which can significantly reduce agricultural yield¹⁶”. We hope that the reviewer is happy with this change

Line 69-70: “ We query the public database BOLD (Barcode of Life Data System) for insect records in South Africa and explore their current coverage”.

AUTHOR: Line 87-88 – The sentences has been changed following the reviewer’s recommendation.

Note: There is no mention of DNA barcode technology throughout your introduction. You did it only while stating the objectives. Also, nothing about insects of South Africa (for instance data of their benefits to agriculture in SA etc.). The most important aspects related to this topic should be clearly presented in order to provide a properly description of the state of art in this field and gaps. Moreover, the main objectives of this review should be clearly and detailed presented in few words. For instance which tool(s) did you use for querying on BOLD. As far as I know there are numerous tools on BOLD for sometimes different purposes.

AUTHOR: Line 70-85 – We have moved a paragraph on DNA barcoding into the introduction to better introduce this method and the BOLD system. We have also included a paragraph to provide an overview of the insects of southern Africa, clearly distinguishing between South Africa and southern Africa. We queried the public database within BOLD, downloaded the specimen data as a tab separated file and no additional BOLD tools were used.

Line 82-83: not clear what you are saying. please rephrase

AUTHOR: line 97-98 – We have reworked the sentence to read: “Given the current projected rates of decline across some insect taxa, it is likely that many species will go extinct before they can be formally classified”.

Line 88-89: Please check for better data and regional public databases, such SANBI database etc. We never know!

AUTHOR: Line 73-74 – We checked for better databases at national or regional level that cover the insect diversity and found that they refer to the same study we cite, also noting that it is outdated and underestimates the true diversity. We have added citations to this sentence to support this finding.

Line 87-97: move parts of this paragraph to the introduction section.

AUTHOR: Line 70-76 – This paragraph has been moved into the introduction section, as suggested.

Improve the outline of this subchapter. You could use an outline that links the thematic between taxonomic impediment, barcode technology and recent surge in species inventory.

Please define “taxonomic impediment”.

<https://academic.oup.com/zoolinnea/article/193/2/381/6374389>

AUTHOR: Line 110-111 – We now define “taxonomic impediment” and provide the relevant citation.

Line 103-106: This is a nonsense to me. 80 000 undescribed insect species vs. estimated 44 000 insect species (line 88). How is this possible?

AUTHOR: Line 116-117 – We have reworded the sentence to clarify that the estimate of 44 000 described insect species is outdated and (obviously) an underestimate; we use these numbers to illustrate that there are still likely many undescribed species in South Africa.

Line 132-134: “ Additional applications of DNA barcoding.... of illegal wildlife^{47,48}”. Remove this sentence.

AUTHOR: This has been removed, thank you.

Note: Move parts of this section to introduction section. For instance paragraph 111-120, and so on

AUTHOR: We have moved the paragraph introducing DNA barcoding into the introduction.

Progress on insect barcoding in South Africa: This section should be reorganized

- Please rephrase, line 152-153: “Myburgh et al.¹⁷ recently published on South Africa’s contribution of insect records on BOLD database...”

- Please say it differently, line 153-155: “D’Souza et al.²⁵ added 339 193 insect records...”

- Line 152-155 : One could not start the section by providing such information. One could start by giving a short story on first insects barcodes from South Africa deposited on BOLD (number of barcodes, insect order, family, date etc.) and follow with the information provided in paragraph (line 156-163). Moreover,

you could use this information (line 152-155) to conclude on fast growing number of barcodes deposited from South Africa.

AUTHOR: Line 152-161 – This entire section has been reworked to first discuss insect barcodes from South Africa, highlighting the contribution by Myburgh et al, and then comparing our records to those already present in BOLD.

Line 156: please remove this bit of sentence

AUTHOR: We have removed what we think the reviewer was suggesting “*recording information on locality (province and GPS coordinates) and taxonomic classification*”.

Line 159: From Table 1, the total number of barcodes from South Africa is 416 211, while you reported 416 266 in text.

AUTHOR: Line 154 – This has been checked and updated to show the amount in the table, 416 211. Thank you for this.

Line 164-165: please make a clear distinction of South Africa and southern Africa.

AUTHOR: We have carefully gone through the text to make clear the distinction between these two regions.

Line 165-166: “ However, the taxonomy of southern African insects is constantly changing”. This bit of sentence is not scientifically true.

AUTHOR: Line 163-164 – This sentence was adjusted and now reads “In 1995, Scholtz and Chown⁵⁵ noted that there had been changes to the taxonomy of southern African insects”. We hope this addresses the reviewers concern.

Line 172: Please rephrase

AUTHOR: Line 171-173 – This has been rephrased to read: “However, we identified five taxonomic orders where the number of BINs exceeds the known species count for southern Africa”. We hope that this improves the sentence.

Line 173-175: Please add the estimated number of species for each group. “However, we identify five orders with more BINs than known species for southern Africa (Hymenoptera = 9259 vs. 5273, Diptera = 7611 vs. 6243, Thysanoptera = 263 vs. 228, Plecoptera = 32 vs. 22, and Strepsiptera = 7 vs. 6; Fig. 1)”.

AUTHOR: Line 165-176 – The estimated number of species for each group has been added.

Please make a clear distinction of South Africa and Southern Africa. It seems the study by Scholtz and Chown estimated the species diversity in Southern Africa which encompasses countries such Botswana, South Africa, Namibia, Eswatini etc., different from South Africa (solely The Republic of South Africa). This means, the estimated number of species from South Africa must be retrieved from those of Southern Africa. Please check!!!

AUTHOR: As noted above, we have gone through the text to make sure that the terms South Africa and southern Africa are referred to appropriately.

Line 175-177: BE CAREFUL when you draw such conclusion! BOLD is a database where everyone (even not a taxonomist) can deposit sequences for a given organism. Therefore, discordance between BIN assignments and current taxonomy may occur. BIN assigned on BOLD must not be seen as the only reliable tool (or the only source) for species identification, reason we distinguish published (through an article or scientific communication) and unpublished records. You must deeper the reflexion and provide possible explanations (recurrent cases of cryptic diversity etc.) in accordance with data and their interpretation

Please consult the following resources that may help you learn more about BIN. there are numerous papers on the matter online: <https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0066213>
<https://cdnsiencepub.com/doi/full/10.1139/gen-2017-0096>
<https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0054921>
<https://link.springer.com/article/10.1007/s11033-022-08015-7>
<https://www.sciencedirect.com/science/article/abs/pii/S0031405608000449>

AUTHOR: We understand the taxonomic uncertainty around BINs and appreciate that DNA barcoding is not a substitute for good taxonomy. we have worked closely with Paul Herbert, Chief Executive Officer Scientific Director & CEO, iBOL, and the BOLD system, helping align BINs to known taxonomy. Here, we highlight how BINs identified on BOLD can be used in conjunction with taxonomy to help describe the richness of insect diversity in South Africa. We have included an additional sentence with some of the references you have suggested (line 145-146) addressing the fact that there is not always a one-to-one

match between barcode BINs species recognised by trained taxonomists.

Line 182-183: Please add values in parentheses

AUTHOR: Line 184-185 – Values have been added in parentheses

Line 185-187: To make the thing clearer, please add relevant values in parentheses. Also add the estimated number of species when needed.

AUTHOR: Line 190 – The values and estimates have been added in parentheses where relevant.

Line 193-194: Rephrase and correct. Diptera is an order not a family. The percentage of 49% is not true. 121 513 over 416 266 is 29%

AUTHOR: Line 186-187 – We have rephrased this to read: “Scholtz⁵⁹ noted that within Diptera there are approximately 150 families found in southern Africa, of these only 77 are currently on BOLD”.

Line 198: How did you find this percentage of 88%

AUTHOR: Line 190 – We now explain that the percentage is calculated from the 113 records out of the 129 total records for Plecoptera.

Line 205: Have I missed figure 2? You are already citing figure 3

AUTHOR: Line 196-197– We have adjusted the order of figures as well as the numbering so that it is correctly reflected in the text.

Line 205-206: “ South Africa’s most significant contribution... the Kruger Malaise Program²⁵” do you mean the most significant contribution in a single submission? Otherwise explain me how 339 193 could be higher than 416 266.

AUTHOR: Line 197-199– We have edited this to now read: “South Africa’s most significant contribution in a single submission to BOLD was the 339 193 specimens and ca. 260 000 sequences – generated from a single project, the Kruger Malaise Program”, we hope this is clearer.

Line 207-208: Instead of “The majority of the sequences were composed of” use “The major contributors of the sequences were composed of”

AUTHOR: Line 199 – We have reworded the beginning of this sentence, thank you

Line 209: add the estimated number of species

AUTHOR: Line 202 – We assume the Reviewer is refereeing to the “species count for southern Africa Hymenoptera”, we have added this value.

Line 210-213: Rephrase please. “The subsequent most valuable contributions were ca. 29 400 and 15 884 specimens collected using Malaise traps in the West Rand district and the City of Johannesburg, respectively (H. Staude in 2012 and R. Stewart in 2017)”

AUTHOR: Line 202-204 – Thank you, we have rephrased this sentence as suggested.

Line 214-217: ‘highest’ is superlative and use in a comparative manner, and cannot be used as such. Say it differently

- Line 205-208 – We have rephrased this sentence to now read: “It is notable that winter rainfall areas, such as the Cape Floristic region and Succulent Karoo hot spots (Western Cape and Northern Cape), have a rich bee diversity⁵⁸, but Hymenoptera within these two provinces remain poorly sampled (926 and 556 specimens respectively) compared to regions that have been sampled with Malaise traps (Fig. 3; Table 1)”.

Line 217: You cannot cite fig 3 before fig 2. This section should be reorganized

AUTHOR: Line 208,211 – We have adjusted the figure numbering.

Line 218: avoid contracted form

AUTHOR: Line 209 – We assumed that the reviewer was referring to the “Haven’t” on line 214. This has been changed to “have not”.

Line 228: Please state the method use to collect the rest of 8%

AUTHOR: Line 220 – We have added a line stating that the remaining 8% was not specified on BOLD.

Note: This section should be reorganized

Biomonitoring in South Africa using DNA barcoding

Start by briefly define biomonitoring and its usefulness.

AUTHOR: Line 241-242 – We now briefly describe that biomonitoring involves using biological organisms sensitive to pollutants, toxins, or other substances to assess environmental conditions.

Line 272-277: Draw the main conclusions about DNA barcoding contribution to the knowledge of South African biodiversity (insects) to date, before moving to future prospects and/or further needs.

AUTHOR: Line 265-268 – We have added a main conclusion stating that DNA barcoding has significantly

increased the overall data on insect diversity within South Africa, emphasizing underestimates of current taxonomic diversity, and highlighting barcoding knowledge gaps within provinces and taxonomic orders.

Reviewer H: Round 3

Date completed: 27 February 2024

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

Conflicts of interest: None

Does the review fall within the scope of SAJS??

Yes/No

Is the review written in a style suitable for a non-specialist and is it of wider than only specialist interest?

Yes/No

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

Yes/No

Does the review provide a significantly novel perspective or significant recent advances in the field?

Yes/No

Is the objective of the review concisely stated?

Yes/No

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

Yes/No

Do current debates and points of contention receive appropriate coverage?

Yes/No/Not applicable

Are gaps in the literature adequately identified?

Yes/No/Not applicable

Does the review provide direction for future research?

Yes/No/Not applicable

Is the methodology and statistical treatment appropriate?

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

Are the interpretations and recommendations aligned with the objective?

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 concise and free of repetition and redundancies?

Yes/No

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

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

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Yes/No

Comments to the Author:

Authors have revised manuscript following my previous comments and suggestions. I would like to add

minor comments:

-71-72 : add a reference

-103-104 : "The IUCN RedList (2023) has only evaluated ~1.2% (12 441) of described insects, and of those 25.9% are "data deficient". Please provide in brackets the number the number for 25.9%.

-273-276: Please avoid "we" or "our" here. Maybe rather use the passive 3rd person point of view. "In this review, it has been shown that the use of DNA barcoding significantly increased the overall data on insect diversity within South Africa, emphasizing underestimates of current taxonomic diversity. It highlighted barcoding knowledge gaps within provinces and taxonomic orders."

Author response to Reviewer H: Round 3

Authors have revised manuscript following my previous comments and suggestions. I would like to add minor comments:

AUTHOR: We thank the reviewer for their comments, which we have addressed as detailed below (our comments and changes in the manuscript highlighted in green).

71-72: add a reference

AUTHOR: 72 – We have added the reference:

1. Scholtz CH. The Higher Classification of Southern African Insects. Entomol Soc South Africa. 2016;24(2):545–55. <https://doi.org/10.4001/003.024.0545>

103-104: "The IUCN RedList (2023) has only evaluated ~1.2% (12 441) of described insects, and of those 25.9% are "data deficient". Please provide in brackets the number the number for 25.9%.

AUTHOR: Line 120 – We have added this value behind the brackets, thank you.

273-276: Please avoid "we" or "our" here. Maybe rather use the passive 3rd person point of view. "In this review, it has been shown that the use of DNA barcoding significantly increased the overall data on insect diversity within South Africa, emphasizing underestimates of current taxonomic diversity. It highlighted barcoding knowledge gaps within provinces and taxonomic orders."

AUTHOR: Line 265-267, 270 – We have changed the wording so that it is passive and removed words like "we" and "our".

Reviewer D: Rounds 1 and 2

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