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PROCEEDINGS B

Structural colours in diverse Mesozoic insects

Chenyang Cai, Erik Tihelka, Yanhong Pan, Ziwei Yin, Rixin Jiang, Fangyuan Xia and Diying Huang

Article citation details

Proc. R. Soc. B **287**: 20200301. http://dx.doi.org/10.1098/rspb.2020.0301

Review timeline

Original submission: Revised submission: Final acceptance: 11 February 2020 1 June 2020 9 June 2020 Note: Reports are unedited and appear as submitted by the referee. The review history appears in chronological order.

Review History

RSPB-2020-0301.R0 (Original submission)

Review form: Reviewer 1

Recommendation

Major revision is needed (please make suggestions in comments)

Scientific importance: Is the manuscript an original and important contribution to its field? Good

General interest: Is the paper of sufficient general interest? Excellent

Quality of the paper: Is the overall quality of the paper suitable? Excellent

Is the length of the paper justified? Yes

Should the paper be seen by a specialist statistical reviewer? No

Reports © 2020 The Reviewers; Decision Letters © 2020 The Reviewers and Editors; Responses © 2020 The Reviewers, Editors and Authors. Published by the Royal Society under the terms of the Creative Commons Attribution License http://creativecommons.org/licenses/ by/4.0/, which permits unrestricted use, provided the original author and source are credited Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report. No

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

Is it accessible? Yes Is it clear? Yes Is it adequate? Yes

Do you have any ethical concerns with this paper? No

Comments to the Author

The authors describe several examples of structural color in insects from mid-cretaceous Burmese amber. Through electron microscopy and optical modeling, they show that the color (at least of a few specimens) is likely original, showing the potential of amber for examining the evolution of structural color. The specimens are fantastic, the data are solid, the conclusions follow from the data for the most part, and the paper is clearly written. The objectives of the research, however, need clarification. What broad question(s) are the researchers addressing with these data? Is it a taphonomic or an evolutionary question, or simply a description of some interesting fossils? I give some suggestions for framing below, as well as a similar recent paper that should be discussed.

L48: Important to note that not all structural colors are metallic, e.g. non-iridescent blue colors in feathers.

L48: This sentence states that the oldest known original structural colors are ~47myo, but then cites a paper (Zhang et al. 2018) that shows a structural color from mid-cretaceous amber, ~100 myo. Moreover, they must have missed an even more recent paper showing detailed analyses of structural color in micropterigidae from amber of the same age (D'Alba et al. 2019 J.Roy.Soc. Interface 16:20190366). The sentence thus needs to be reformulated to explain the significance of the manuscript.

L53: This sentence is unclear. Is the purpose of the paper to explain the taphonomy of colors in amber? If so, has this been debated in the literature? The objectives of the paper need to be more clearly described.

L66: How were the specimens taxonomically identified?

L111: I appreciate the authors' examination of negative controls like this. Have they thought about examining a piece of cuticle from a specimen that is not likely to have had structural color in life? The expectation is that such a specimen would have no multilayers, and would provide a nice confirmation of their hypothesis that the multilayer causes the colors.

L138: it would be nice to compare the theoretical spectrum with the measured spectrum. Have the authors tried dissolving the amber away from the specimen with chloroform? This method was successfully employed by D'Alba et al. (2019, citation above).

L137: You can't really say the match is "perfect" without quantitative empirical color data.. even then, I doubt the match would be perfect. You could say something like it is "qualitatively similar."

L163: Unclear exactly why this is an important contribution to taphonomy of amber. Could you please provide more context from the literature?

L172: This statement about the RI remaining unchanged needs justification. Are you really

arguing that the biomolecular composition is the same after 99 million years in amber? This seems very unlikely. For one thing, amber has probably seeped into the cuticle. Perhaps the RI has not changed much, but the biomolecular composition likely has.

L203: This line seems to contradict L191, where you state that the silver cuticles were damaged. Why do you argue the silver color is original if most silver specimens are damaged?

Review form: Reviewer 2

Recommendation Accept with minor revision (please list in comments)

Scientific importance: Is the manuscript an original and important contribution to its field? Good

General interest: Is the paper of sufficient general interest? Good

Quality of the paper: Is the overall quality of the paper suitable? Good

Is the length of the paper justified?

Yes

Should the paper be seen by a specialist statistical reviewer? No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report. No

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

Is it accessible? Yes	
Is it clear? Yes	
Is it adequate? Yes	

Do you have any ethical concerns with this paper?

Yes

Comments to the Author

This is a very well written and useful study of the preservation potential of structural colours in amber fossils by using advanced imaging methods applicable to fossils with the objective of elucidating the material nature of fossils and the processes of fossilization and in particular colour preservation. As such a deeper understanding of the substance preservation of fossils offers innumerable benefits for the reconstruction of the history of life. My suggested changes are all minor and should be very straightforward and easy to make, such that I should not need to see a resubmission. Thus, I strongly support publication. My comments and suggested changes are made directly in the submitted typescript:

Review form: Reviewer 3 (Dany Azar)

Recommendation

Accept with minor revision (please list in comments)

Scientific importance: Is the manuscript an original and important contribution to its field? Good

General interest: Is the paper of sufficient general interest? Good

Quality of the paper: Is the overall quality of the paper suitable? Good

Is the length of the paper justified? Yes

Should the paper be seen by a specialist statistical reviewer? No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report. No

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

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Is it accessible?
Yes
Is it clear?
Yes
Is it adequate?
Yes
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Do you have any ethical concerns with this paper? No

Comments to the Author

A nice paper that makes light on the preservation of physical colours for insects preserved in amber. The paper is well structured and written in a good English. Comments are given directly on annotated MS. This paper is acceptable after minor revision as indicated in the annotated MS

Decision letter (RSPB-2020-0301.R0)

12-May-2020

Dear Dr Cai:

Your manuscript has now been peer reviewed and the reviews have been assessed by an Associate Editor. The reviewers' comments (not including confidential comments to the Editor) and the comments from the Associate Editor are included at the end of this email for your reference. As you will see, the reviewers and the Editors have raised some concerns with your manuscript and we would like to invite you to revise your manuscript to address them.

We do not allow multiple rounds of revision so we urge you to make every effort to fully address all of the comments at this stage. If deemed necessary by the Associate Editor, your manuscript will be sent back to one or more of the original reviewers for assessment. If the original reviewers are not available we may invite new reviewers. Please note that we cannot guarantee eventual acceptance of your manuscript at this stage.

To submit your revision please log into http://mc.manuscriptcentral.com/prsb and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions", click on "Create a Revision". Your manuscript number has been appended to denote a revision.

When submitting your revision please upload a file under "Response to Referees" - in the "File Upload" section. This should document, point by point, how you have responded to the reviewers' and Editors' comments, and the adjustments you have made to the manuscript. We require a copy of the manuscript with revisions made since the previous version marked as 'tracked changes' to be included in the 'response to referees' document.

Your main manuscript should be submitted as a text file (doc, txt, rtf or tex), not a PDF. Your figures should be submitted as separate files and not included within the main manuscript file.

When revising your manuscript you should also ensure that it adheres to our editorial policies (https://royalsociety.org/journals/ethics-policies/). You should pay particular attention to the following:

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Use of animals and field studies:

If your study uses animals please include details in the methods section of any approval and licences given to carry out the study and include full details of how animal welfare standards were ensured. Field studies should be conducted in accordance with local legislation; please include details of the appropriate permission and licences that you obtained to carry out the field work.

Data accessibility and data citation:

It is a condition of publication that you make available the data and research materials supporting the results in the article. Datasets should be deposited in an appropriate publicly available repository and details of the associated accession number, link or DOI to the datasets must be included in the Data Accessibility section of the article

(https://royalsociety.org/journals/ethics-policies/data-sharing-mining/). Reference(s) to datasets should also be included in the reference list of the article with DOIs (where available).

In order to ensure effective and robust dissemination and appropriate credit to authors the dataset(s) used should also be fully cited and listed in the references.

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If you have already submitted your data to dryad you can make any necessary revisions to your dataset by following the above link.

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All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI. Please try to submit all supplementary material as a single file.

Online supplementary material will also carry the title and description provided during submission, so please ensure these are accurate and informative. Note that the Royal Society will not edit or typeset supplementary material and it will be hosted as provided. Please ensure that the supplementary material includes the paper details (authors, title, journal name, article DOI). Your article DOI will be 10.1098/rspb.[paper ID in form xxxx.xxxx e.g. 10.1098/rspb.2016.0049].

Please submit a copy of your revised paper within three weeks. If we do not hear from you within this time your manuscript will be rejected. If you are unable to meet this deadline please let us know as soon as possible, as we may be able to grant a short extension.

Thank you for submitting your manuscript to Proceedings B; we look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Best wishes, Dr Maurine Neiman mailto: proceedingsb@royalsociety.org

Associate Editor Board Member: 1 Comments to Author: Dear Dr Cai, Three experts in the field have reviewed your manuscript. While the general comments are positive, I agree with the reviewers that some changes are required to improve the quality of the manuscript. Furthermore, one of the reviewers asked for clarifications on whether the fossils come from Myanmar areas that are under controls of the military and their accessibility (see http://vertpaleo.org/GlobalPDFS/SVP-Letter-to-Editors-FINAL.aspx).

Best wishes, Roberto Feuda

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)

The authors describe several examples of structural color in insects from mid-cretaceous Burmese amber. Through electron microscopy and optical modeling, they show that the color (at least of a few specimens) is likely original, showing the potential of amber for examining the evolution of structural color. The specimens are fantastic, the data are solid, the conclusions follow from the data for the most part, and the paper is clearly written. The objectives of the research, however, need clarification. What broad question(s) are the researchers addressing with these data? Is it a taphonomic or an evolutionary question, or simply a description of some interesting fossils? I give some suggestions for framing below, as well as a similar recent paper that should be discussed.

L48: Important to note that not all structural colors are metallic, e.g. non-iridescent blue colors in feathers.

L48: This sentence states that the oldest known original structural colors are ~47myo, but then cites a paper (Zhang et al. 2018) that shows a structural color from mid-cretaceous amber, ~100 myo. Moreover, they must have missed an even more recent paper showing detailed analyses of structural color in micropterigidae from amber of the same age (D'Alba et al. 2019 J.Roy.Soc. Interface 16:20190366). The sentence thus needs to be reformulated to explain the significance of the manuscript.

L53: This sentence is unclear. Is the purpose of the paper to explain the taphonomy of colors in amber? If so, has this been debated in the literature? The objectives of the paper need to be more clearly described.

L66: How were the specimens taxonomically identified?

L111: I appreciate the authors' examination of negative controls like this. Have they thought about examining a piece of cuticle from a specimen that is not likely to have had structural color in life? The expectation is that such a specimen would have no multilayers, and would provide a nice confirmation of their hypothesis that the multilayer causes the colors.

L138: it would be nice to compare the theoretical spectrum with the measured spectrum. Have the authors tried dissolving the amber away from the specimen with chloroform? This method was successfully employed by D'Alba et al. (2019, citation above).

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L163: Unclear exactly why this is an important contribution to taphonomy of amber. Could you please provide more context from the literature?

L172: This statement about the RI remaining unchanged needs justification. Are you really arguing that the biomolecular composition is the same after 99 million years in amber? This seems very unlikely. For one thing, amber has probably seeped into the cuticle. Perhaps the RI has not changed much, but the biomolecular composition likely has.

L203: This line seems to contradict L191, where you state that the silver cuticles were damaged. Why do you argue the silver color is original if most silver specimens are damaged?

Referee: 2

Comments to the Author(s)

This is a very well written and useful study of the preservation potential of structural colours in amber fossils by using advanced imaging methods applicable to fossils with the objective of elucidating the material nature of fossils and the processes of fossilization and in particular colour preservation. As such a deeper understanding of the substance preservation of fossils offers innumerable benefits for the reconstruction of the history of life. My suggested changes are all minor and should be very straightforward and easy to make, such that I should not need to see a resubmission. Thus, I strongly support publication. My comments and suggested changes are made directly in the submitted typescript:

Referee: 3

Comments to the Author(s)

A nice paper that makes light on the preservation of physical colours for insects preserved in amber. The paper is well structured and written in a good English. Comments are given directly on annotated MS. This paper is acceptable after minor revision as indicated in the annotated MS

Author's Response to Decision Letter for (RSPB-2020-0301.R0)

See Appendix A.

Decision letter (RSPB-2020-0301.R1)

09-Jun-2020

Dear Dr Cai

I am pleased to inform you that your manuscript entitled "Structural colours in diverse Mesozoic insects" has been accepted for publication in Proceedings B.

You can expect to receive a proof of your article from our Production office in due course, please check your spam filter if you do not receive it. PLEASE NOTE: you will be given the exact page length of your paper which may be different from the estimation from Editorial and you may be asked to reduce your paper if it goes over the 10 page limit.

If you are likely to be away from e-mail contact please let us know. Due to rapid publication and an extremely tight schedule, if comments are not received, we may publish the paper as it stands.

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Electronic supplementary material:

All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI.

Thank you for your fine contribution. On behalf of the Editors of the Proceedings B, we look forward to your continued contributions to the Journal.

Sincerely, Dr Maurine Neiman Editor, Proceedings B mailto: proceedingsb@royalsociety.org

Associate Editor: Board Member Comments to Author: The authors addressed carefully all the concerns raised by the reviewers, including the potential moratorium on the fossils. As a consequence, I am glad to recommend the manuscript for publication.

Appendix A

Dear Drs Neiman and Feuda,

Thanks you very much for handling our manuscript during the crazy time of COVID-19 outbreak. The comments and suggestions from three experts are very helpful for improving the quality of our paper. In the light of their advice, we have revised the manuscript accordingly. Detail point-to-point response can be found as follows:

Reply to Referee #1:

The authors describe several examples of structural color in insects from mid-cretaceous Burmese amber. Through electron microscopy and optical modeling, they show that the color (at least of a few specimens) is likely original, showing the potential of amber for examining the evolution of structural color. The specimens are fantastic, the data are solid, the conclusions follow from the data for the most part, and the paper is clearly written.

A: Thanks so much for your positive comments.

The objectives of the research, however, need clarification. What broad question(s) are the researchers addressing with these data? Is it a taphonomic or an evolutionary question, or simply a description of some interesting fossils? I give some suggestions for framing below, as well as a similar recent paper that should be discussed.

A: Thank you for bringing this to our attention. Yes, we agree that we should make the research objective clearer to readers. We have revised our narrative to focus on a key taphonomic question: what is the cause of the diverse metallic colours in amber-entombed insects? We are grateful to the reviewer for providing a very important reference (D'Alba et al. 2019 J.Roy.Soc. Interface 16:20190366) about this topic. Unlike the dull (non-metallic) colours as shown in D'Alba et al. (2019), our fossils directly preserved diverse metallic colours which are clearly visible without extraction or any further preparation, although in both cases the nanostructures can be preserved (as evidenced by further observations using SEM and TEM). Moreover, structural colours can be caused by multiple forms of bionanostructures: D'Alba et al. (2019) focused on scales (diffraction gratings) found on the body surface of micropterigids and some springtails; whereas we focused on the multilayer reflectors within the epicuticle of various insects (three orders), which also represents the most common form of structural colours in nature.

L48: Important to note that not all structural colors are metallic, e.g. non-iridescent blue colors in feathers.

A: Yes, that is true. To avoid confusions, we have changed 'structural colours [metallic colours] to 'metallic structural colours'.

L48: This sentence states that the oldest known original structural colors are ~47myo, but then cites a paper (Zhang et al. 2018) that shows a structural color from mid-cretaceous amber, ~100 myo. Moreover, they must have missed an even more recent paper showing detailed analyses of structural color in micropterigidae from amber of the same age (D'Alba et al. 2019 J.Roy.Soc. Interface 16:20190366). The sentence thus needs to be reformulated to explain the significance of the manuscript.

A: Yes, that is a good point. We have modified the Introduction section to highlight the scientific

importance of our manuscript. The structural colors in Zhang et al. (2018) are not visible before the application of modeling or 'reconstruction', so this paper is cited earlier in the introduction. We focus on 'visual visual metallic structural colours', so we re-organized the introduction and discussed the difference between D'Alba et al. (2019) and our paper. See also the response above.

L53: This sentence is unclear. Is the purpose of the paper to explain the taphonomy of colors in amber? If so, has this been debated in the literature? The objectives of the paper need to be more clearly described.

A: Yes, we have re-organised the introduction section and add an important reference you mentioned.

L66: How were the specimens taxonomically identified?

A: Good point. Now we added the identifier of the fossil insects (identified by C.C., the first author of the manuscript)

L111: I appreciate the authors' examination of negative controls like this. Have they thought about examining a piece of cuticle from a specimen that is not likely to have had structural color in life? The expectation is that such a specimen would have no multilayers, and would provide a nice confirmation of their hypothesis that the multilayer causes the colors.

A: Yes, good point. Actually we have considered this point and that is the reason why we put a metallic coloured wasp and a non-metallic brown ant as the first figure, as fig.1a. As clearly shown in the picture, two hymenopterans preserved in a single piece of amber. One with structural colour (as blue), whereas the other is dull coloured, just as seen in their modern counterparts.

L138: it would be nice to compare the theoretical spectrum with the measured spectrum. Have the authors tried dissolving the amber away from the specimen with chloroform? This method was successfully employed by D'Alba et al. (2019, citation above).

A: Yes, it is a good idea. We have tried to further prepare the amber and dissolve the amber to obtain the insect. However, as we mentioned above and in the paper, the causes of structural colours are so different between D'Alba et al. (2019) and this study. In D'Alba et al. (2019), the colours were caused by the nanostructures ON the scales on the body surface. By contrast, the colours in our specimens are caused by the multilayer reflectors IN the epicuticle, with the cooperation of refractive index. When two specimens of our tested samples were cut and processed using alcohol or even by water/air, the colours will turn into 'silver' (as shown in fig. 4 in our manuscript). We have discussed this unexpected observations in the manuscript. Thank you for the suggestion anyway!

L137: You can't really say the match is "perfect" without quantitative empirical color data.. even then, I doubt the match would be perfect. You could say something like it is "qualitatively similar." A: Yes, 'perfectly correlate with' is changed to 'are qualitatively similar to'.

L163: Unclear exactly why this is an important contribution to taphonomy of amber. Could you please provide more context from the literature? A: We have reworded this sentence to clarifying this. As another form of structural colours have also been reported from the same amber, we say 'our study provides new information about the taphonomy of amber inclusions'.

L172: This statement about the RI remaining unchanged needs justification. Are you really arguing that the biomolecular composition is the same after 99 million years in amber? This seems very unlikely. For one thing, amber has probably seeped into the cuticle. Perhaps the RI has not changed much, but the biomolecular composition likely has.

A: Yes, we have reworded this sentence. Now it is clear that the RI (refractive index) has not changed much.

L203: This line seems to contradict L191, where you state that the silver cuticles were damaged. Why do you argue the silver color is original if most silver specimens are damaged? A: Sorry for bringing this confusion... We did not intend to say that 'the silver cuticles were damaged', but we want to mention that 'the silver amber inclusions were either covered with air bubbles touching the body or their non-metallic body parts (e.g. legs) were slightly worn out, during preparation,...' We have modified L195 to avoid confusion.

Reply to Referee #2:

Comments to the Author(s)

This is a very well written and useful study of the preservation potential of structural colours in amber fossils by using advanced imaging methods applicable to fossils with the objective of elucidating the material nature of fossils and the processes of fossilization and in particular colour preservation. As such a deeper understanding of the substance preservation of fossils offers innumerable benefits for the reconstruction of the history of life.

My suggested changes are all minor and should be very straightforward and easy to make, such that I should not need to see a resubmission. Thus, I strongly support publication. My comments and suggested changes are made directly in the submitted typescript:

A: Thanks so much for your positive comments. See our detailed reply below.

[Detailed Comments Annotated on PDF]

1. also in Chiapas Mexican amber, there is a coloured bee: Wille, A. 1959. A new fossil stingless bee (Meliponini) from the amber of Chiapas, Mexico. Journal of Paleontology 33 (5): 849–852; I have seen several metallic green coloured samples with David Coty in MNHN- Paris A: Thank you for the information. This rare reference is now added.

2. this is a little exaggerated, better to delete A: OK, it is deleted.

3. Chenge 'derives' to 'are inclusions' A: OK, it is changed.

4. Change 'against' to 'with' A: OK, it is changed.

4. Change 'counterparts' to 'representatives'

A: OK, it is changed.

5. but also three-dimensional photonic crystals and diffraction gratings; Seago et al. (2009). Gold bugs and beyond: a review of iridescence and structural colour mechanisms in beetles (Coleoptera). J R Soc Interface. ; 6(Suppl 2): S165–S184.

A: We cited this reference later in the paper. In this context, we are intended to focus on 'Bright metallic colours'.

6. for me the silver colour is due that during preparation and with the stress a very thin layer of air is created between the insect and the amber. we can see the same phenomenon when we embed a metallic coloured insect in artificial resin. As long as the resin do not encounter stress (could be just a chock) the metallic colour is still. When stressed their will be a thin layer of air that turns metallic colour to silver (mirror effect). I have tried this experiment several times :) this hypothesis is true and it was verified in different types of amber from different origin! I guess that both hypotheses must be considered. So please change this paragraph accordingly A: Yes, it is good hypothesis .We have added this in the discussion. 'Alternatively, the silver colour

may be caused by a very thin layer of air created between the insect and the amber during preparation.'

7. you cannot conclude such assumption if the nanostructures are preserved. It has been proven at least in certain type of ambers that molecules undergo chemical changes even if the morphological structure is perfectly preserved.

A: OK, it is now deleted.

8. have you tried to extract the insect from the amber by using chemicals? it is a delicate process but feasible

A: This is a good idea, also mentioned by Reviewer #1. We have tried to further prepare the amber and dissolve the amber to obtain the insect. However, as we mentioned above and in the paper, the causes of structural colours are so different between D'Alba et al. (2019) and this study. In D'Alba et al. (2019), the colours were caused by the nanostructures ON the scales on the body surface. By contrast, the colours in our specimens are caused by the multilayer reflectors IN the epicuticle, with the cooperation of refractive index. When two specimens of our tested samples were cut and processed using alcohol or even by water/air, the colours will turn into 'silver' (as shown in fig. 4 in our manuscript). We have discussed this unexpected observations in the manuscript. Thank you for the suggestion anyway!

Reply to Referee #3:

Comments to the Author(s)

A nice paper that makes light on the preservation of physical colours for insects preserved in amber. The paper is well structured and written in a good English. Comments are given directly on annotated MS. This paper is acceptable after minor revision as indicated in the annotated MS A: Thanks so much for your positive comments.

[Detailed Comments Annotated on PDF]

1. Change '47 million' to '48 million...' A: OK, it is changed.

2. reference missing

A: Yes, thanks. Two important references have been added to this section.

3. in this context it would be necessary to discuss also the results from Stankiewicz, B.A., Briggs, D.E.G., Evershed, R.P., Flannery, M.B., Wuttke, M., 1997. Preservation of chitin in 25-Million-Year-Old fossils. Science 276, 1541-1543. A: OK, discussion and this reference are added to the manuscript.

4. should be mentioned in this context.

Cody, G. D., N. S. Gupta, D. E. G. Briggs, A. L. D. Kilcoyne, R. E. Summons, F. Kenig, R. E. Plotnick, and A. C. Scott. 2011. Molecular signature of chitin-protein complex in Paleozoic arthropods. Geology 39:255-258.

Ehrlich, H., Rigby, J.K., Botting, J.P., Tsurkan, M.V., Werner, C., Schwille, P., Petrášek, Z., Pisera, A., Simon, P., Sivkov, V.N., Vyalikh, D.V., Molodtsov, S.L., Kurek, D., Kammer, M., Hunoldt, S., Born, R., Stawski, D., Steinhof, A., Bazhenov, V.V., Geisler, T., 2013. Discovery of 505-million-year old chitin in the basal demosponge Vauxia gracilenta. Sci. Rep. 3, 3497.

A: OK, both references are added.

5. there is also a study on fossil butterfly wing scales from the Triassic / Jurassic boundary that show clearly Scanning electron microscopy (SEM) images that the scale surface comprises a regular 2-D array of concavities, and the transmission electron microscopy (TEM) images of these scales in cross-section reveal the multilayering that causes. van Eldijk, T.J.B., Wappler, T., Strother, P.K., van der Weijst, C.M.H., Rajaei, H., Visscher, H., van de Schootbrugge, B., 2018. A Triassic-Jurassic window into the evolution of Lepidoptera. Science Advances 4, e1701568.

A: Thank you for bringing this to our attention. We are aware of this paper when we prepared our manuscript. This paper is focused on the discovery of an old scale possibly belonging to Lepidoptera. In the original paper, the authors did not mentioned anything about structural colour, because they may aware of the fact that not all Lepidopteran scales will form structural colours.

6. as already proofed by Cody, G. D., N. S. Gupta, D. E. G. Briggs, A. L. D. Kilcoyne, R. E. Summons, F. Kenig, R. E. Plotnick, and A. C. Scott. 2011. Molecular signature of chitin-protein complex in Paleozoic arthropods. Geology 39:255-258. Ehrlich, H., Rigby, J.K., Botting, J.P., Tsurkan, M.V., Werner, C., Schwille, P., Petrášek, Z., Pisera, A., Simon, P., Sivkov, V.N., Vyalikh, D.V., Molodtsov, S.L., Kurek, D., Kammer, M., Hunoldt, S., Born, R., Stawski, D., Steinhof, A., Bazhenov, V.V., Geisler, T., 2013. Discovery of 505-million-year old chitin in the basal demosponge Vauxia gracilenta. Sci. Rep. 3, 3497.

A: As recommended by Reviewer #2, we have deleted discussion about the preservation of biomolecules, as this is out of the scope of this paper, which is focused on the cause of metallic structural colours. These two references are cited in the discussions as well.

7. Labeling is not clearly visible

A: Thanks. Now it is improved.

Thanks you again for time and patience!

Best regards Chenyang Cai