

*We want to thank reviewer #1 for their comments and providing constructive feedback on our manuscript. Below we address all the comments with our replies in italics.*

Reviewer #1: This manuscript uses cultures of two Thaumarchaeotal strains to investigate growth temperature and phase on OH-GDGT distributions and OH-GDGT-based proxies. This is an important piece of work in the context of the emergence of OH-GDGTs as a GDGT-based temperature indicator, especially in cold water settings. The paper is clear and well written with nicely presented figures, and thoroughly reported results. I mostly only have a few very minor suggested comments/changes, but did feel like the manuscript was missing an 'implications' or 'summary' section to tie the results together and put them into a wider context. Could the authors comment on what they see as the key implications of interspecies variability in OH-GDGTs with changes in growth temperature for OH-GDGT-based temperature proxies?

*We thank the reviewer for the positive feedback on our manuscript and we agree that including an Implications/ Summary section would be a valuable addition. Therefore, we will add this in the revised manuscript.*

Specific comments below:

Line 23. Add 'the' before natural.

*We will change this in the revised manuscript.*

Line 32. Add a comma between 'moieties' and 'and'.

*We will change this in the revised manuscript.*

Line 43. Add 'the' before Black Sea.

*We will change this in the revised manuscript.*

Line 141. Correct format for tex86oh.

*We will change this in the revised manuscript.*

Figure 3: Is there a reason purple shaded boxes haven't been added to c) and g)? It would be good to see how these results compare to global core top data sets for RI isoGDGTs.

*The reason why mean and standard deviations in purple shaded boxes were not presented for  $RI_{isoGDGTs}$  is because in this study we calculated the ring index of isoGDGTs based on Equation 5 according to (Pearson et al., 2004) where isoGDGT-4 was included. However, most studies in literature have not reported the isoGDGT-4 data and therefore, mean and standard deviation of  $RI_{isoGDGTs}$  could not be calculated for global marine surface sediments to present here.*

Line 347: Extent rather than extend.

*We will make this modification.*

Discussion: It feels like an implications section is missing here, that sums up the results and discussion and distills out the key points and implications of these findings for future OH-GDGT- based research. I recommend adding a paragraph to this effect before the conclusions.

*We will add this as mentioned in the general comments above.*

## **References**

*Pearson A., Huang Z., Ingalls A. E., Romanek C. S., Wiegel J., Freeman K. H., Smittenberg R. H. and Zhang*

C. L. (2004) Nonmarine crenarchaeol in Nevada hot springs. *Appl. Environ. Microbiol.* **70**, 5229–5237.