Reassessment of the previous decision on Puntin et al. 2024 preprint

I have carefully examined the previous reviews, decision letters, additional information provided by the previous recommender, the authors' appeal letter and the current draft (https://www.biorxiv.org/content/10.1101/2023.08.23.554380v3). I acknowledge that the previous review rounds appear to have clearly contributed to the preparation of the current improved draft, and have pinpointed the shortcoming of the data that the study presents. However, I identified that some of the suggestions that were made and incorporated along the way have slightly diverged the focus of the paper away from the main topic and the strongest potential of the existing data. Some of these suggestions from the previous reviewer and recommender unfortunately appear to be stemming from misunderstanding of the study procedures and misconception of the main message that the study was designed to deliver by the author. As such, I am in support for reversing the decision of rejecting this article from the consideration for recommendation. You can also refer to my comments on the review history, appeal letter, and the previous recommender's comments attached, showing how I came to this conclusion. Here I present my views on the main three areas; (1) the effect of menthol-induced coral bleaching on coral associated microbiota, (2) the effect of geographic locations and aquarium facilities on the coral microbiota, and (3) the effect of long-term aquarium-based captivity on the coral microbiota; and where this study can be placed in each area. The preprint at the current form can merit a revision before it is recommended for publication.

Therefore I also provided my review of the current manuscript and made recommendations for changes. I hope these strengthen the article's clarity along the authors' intentions, as well as its scientific contributions.

Thank you for the thorough examination of our manuscript and the previous review history. We truly appreciate the effort and time you dedicated to providing this in-depth revision and constructive feedback. Your insights have strengthened our manuscript and clarified our main goals and findings.

We are grateful for the opportunity to further revise our work, especially considering the challenging circumstances during its development (e.g., COVID-19). We have carefully addressed all comments and suggestions, making the necessary amendments to the manuscript and supplementary materials, which we hope are now satisfactory for recommendation.

Below, we provide detailed responses to each comment (text in blue). Please note that the line numbers referenced here refer to the tracked changes version of the manuscript ("bioRxiv_ms_v4_tracked.docx").

1) The menthol-bleaching effect on the coral microbiota: The main study focus, and the only one topic with barely enough supporting data in my view, is the effect of menthol-induced coral bleaching on coral-associated bacterial community compositions. Observations were made using 5 coral colonies (3 sourced from Red Sea and kept in a German aquarium for 7 months, and 2 sourced from Hong Kong and kept in a HK aquarium for 3 months), each of which were physically separated to generate 6 clonal polyps. From each colony, 3 polyps were kept in the aquarium without menthol, and 3 were subjected to menthol bleaching to remove most (if not all) algal symbionts. Microbial 16S rRNA gene sequences were then obtained from these corals, with n = 3 polyps per treatment per colony, times 5 colonies examined. However, a few polyps did not

generate enough data (Fig 1), thus there were only 2 colonies that allowed between-treatment comparisons on 3 samples-vs.-3 samples statistically, resulting in most treatment-based comparisons being on average pictures across all colonies (Fig2, Fig3B), which in my opinion were not very convincing due to the colony-level variability in the microbial compositions responded to menthol bleaching. Considering the lack of sufficient data, I believe the previous reviews were fair to characterize this study to be a preliminary result (as inserted in the abstract as well). Nevertheless, when we look within each colony, while non-bleached polyps showed similar microbial compositions, microbiomes of bleached polyps were in general more diverse. Comparing across the 5 colonies, this non-bleached vs. bleached difference (i.e. apparent 'shift'; noting that microbiomes before the experiment was not obtained) appear in a non-directional, stochastic manner. I agree that this follows patterns expected from dysbiosis, as the authors interpreted. As data presented are barely minimal due to missing samples, this interpretation should however be presented as suggestion that requires verification through follow up studies. This should be reflected in the statements on this topic throughout the paper.

We appreciate your recognition of the study's focus and detailed critique of our data presentation. We acknowledge that the small sample size and the absence of before-and-after comparisons, which was caused by the restrictions imposed by the ongoing COVID-19 pandemic, limit the strength of our conclusions. Specifically, while our data indicate a potential alignment with patterns of dysbiosis, the variability observed at the colony level and the missing data points necessitate caution in interpretation.

In response to the comments, we have revised the manuscript to present our findings as preliminary. We emphasize that the observed shifts in microbial community composition following menthol bleaching suggest dysbiosis, but this interpretation requires further validation in future studies.

To address the specific concerns, we have made the following changes:

- **Abstract**: "Nevertheless, the changes in community composition ~~observed in both facilities~~ overall appeared to be stochastic ~~and indicative~~ suggesting ~~of~~ a dysbiotic state."
- **Results**
    - L208: "Menthol-bleaching seemed to elicit ~~elicited~~ stochastic changes in the microbial communities"
    - L209: "Changes in community composition between symbiotic states showed different~~d~~ trends ~~for coral colonies from the two regions~~ across the two facilities, however dissimilarity was generally higher in menthol bleached polyps. ~~For the Red Sea, there was no clear distinction in bacterial community composition between symbiotic and bleached polyps in terms of location and dispersion in the ordination space, when considering all colonies together (PERMANOVA, F = 0.76, PERMDISP2, F = 0.77, P > 0.05; Fig. 3C). For Hong Kong, the microbial communities of symbiotic and bleached polyps were significantly different (PERMANOVA, F = 4.0, P < 0.01; Fig. 3D), while the difference in dispersion was (just marginally) not statistically significant (PERMDISP2, F = 5.05 , P = 0.057). However, w~~While symbiotic polyps clustered by colony for both Red Sea and Hong Kong (indicating similar microbial communities, Fig.3A), bleached polyps showed no such clear grouping (Red Sea,

Fig.3C) or larger scattering compared to symbiotic polyps (Hong Kong, Fig.3D), and collectively had a significantly higher within-colony dissimilarity (P Mann-Whitney = 0.0001, Fig. 3A,B, Fig. S3), seemingly ~~. This~~ indicating~~es~~ random changes in the communities of the menthol-bleached polyps."

- **Discussion**
  - L299: "**Menthol bleaching seemingly led to stochastic changes in the microbiome of Galaxea**. Menthol bleaching ~~was~~ appeared to be associated with changes in the bacterial communities that differed between individual polyps and produced stochastic configurations."
  - L292: "A ~~The~~ stochastic response to bleaching aligns well with the concept of an obligatory nature of the coral-algal symbiosis."
- **Conclusions**
  - L462: "Overall, menthol bleaching appeared to induce~~d~~ stochastic changes in the microbiome, seemingly indicating dysbiosis."

2) The location effect on the stochastic shift of microbiome upon menthol bleaching: Authors may point out that the shifting patterns of microbiota induced by menthol bleaching differ across colonies; however in my view this difference cannot scientifically be attributed to the location or facility difference without having sufficient replicate colonies in Hong Kong (currently n=2 colonies; a third colony may change the whole picture, which we cannot know). There are also other factors to be considered and examined than the difference in the locations and facilities, which include host coral phylogenetic lineages, as the studied coral species is known to be polyphyletic containing cryptic lineages (e.g. Wepfer et al. 2020; cited in the manuscript though missing from the reference list; please check the reference list for other items, too). As such, the abstract statement "response of the bacterial microbiome to menthol bleaching varied between the two facilities", the paragraph "Microbial community dissimilarity patterns differed by geographic origin of colonies" (L187-) and the paragraph starting with "Changes in community compositions between symbiotic states differed for coral colonies from the two regions." (L196-) are questionable; I recommend these to be removed, as highlighting this aspect diverges the focus from the main objective of the study ((1) above). Specifically for the result paragraph L196-, it targets to address the apparent 'changes' between treatments, which in my opinion should best consider within each colony given the intercolony variability, but all bleached and all non-bleached polyps across colonies were examined together to point out the different patterns between locations (i.e. presence/absence of treatment based clustering; Fig3C, Fig3D); this is an inappropriate approach to address the main question, as all polyps were not independent samples. I see the non-directional 'shifts' across 5 colonies, and a treatment-based clustering in Hong Kong may well not have formed if a third colony showed another change direction, which we cannot know. Similarly in the discussion paragraph starting from L301, location-based contrast can be speculative - Pointing out the HK responses being more 'uniform' needs to be backed up by more than two sample colonies. The discussion on location-based differences overall should reflect the speculative nature cautiously – the discussion paragraph L301- should be removed (or substantially reduced its weight), also L406-407.

Thank you for the detailed feedback regarding the location effect on the microbiome shifts upon menthol bleaching. We acknowledge the concerns about attributing observed differences to location or facility without sufficient replicate colonies and the need to consider additional factors.

We agree with the issue of low replication and the fact that focusing on unsupported differences between facilities might divert attention from the main objective of the study. However, rather than completely removing the relevant paragraphs as suggested, we have chosen to shorten and reduce them. We believe there is value in reporting such patterns or trends as they highlight the potential importance of rearing conditions in shaping the coral microbiome, which is a critical aspect of model organism research.

We have made the following revisions to the manuscript:

- **Abstract**: "Symbiotic polyps (clonal replicates) from the same colony had similar microbiomes, which were distinct from those of other colonies despite co-culturing in shared aquaria. A pattern of seemingly differential ~~The~~ response of the bacterial microbiome to menthol bleaching ~~varied~~ between the two facilities emerged, warranting further investigation into the role of rearing conditions. Nevertheless, the changes in community composition ~~observed in both facilities~~ overall appeared to be stochastic ~~and indicative~~ suggesting ~~of~~ a dysbiotic state."
- **Results**
  - Removed the whole subsection at former L187 (now L200) titled "Microbial community dissimilarity patterns differed by geographic origin of colonies"
  - L209 (former L196): "Changes in community composition between symbiotic states showed different~~d~~ trends ~~for coral colonies from the two regions~~ across the two facilities, however dissimilarity was generally higher in menthol bleached polyps. ~~For the Red Sea, there was no clear distinction in bacterial community composition between symbiotic and bleached polyps in terms of location and dispersion in the ordination space, when considering all colonies together (PERMANOVA, F = 0.76, PERMDISP2, F = 0.77, P > 0.05; Fig. 3C). For Hong Kong, the microbial communities of symbiotic and bleached polyps were significantly different (PERMANOVA, F = 4.0, P < 0.01; Fig. 3D), while the differnce in dispersion was (just marginally) not statistically significant (PERMDISP2, F = 5.05 , P = 0.057). However, w~~While symbiotic polyps clustered by colony for both Red Sea and Hong Kong (indicating similar microbial communities, Fig.3A), bleached polyps showed no such clear grouping (Red Sea, Fig.3C) or larger scattering compared to symbiotic polyps (Hong Kong, Fig.3D), and collectively had a significantly higher within-colony dissimilarity (P Mann-Whitney = 0.0001, Fig. 3A,B, Fig. S3), seemingly ~~. This~~ indicate~~s~~ random changes in the communities of the menthol-bleached polyps."
- **Discussion**
  - L331 (former L301) was heavily shortened and toned down: "The response of the bacterial microbiome to menthol bleaching seemed to differ~~ed~~ between the two facilities. ~~For Red Sea corals, in the nMDS-ordination space on microbial community composition (Fig. 3), bleached polyps moved towards the center of the plot to the other symbiotic colonies rather than spreading in any random direction.~~

~~This, taken together with the observation that alpha diversity did not increase after bleaching (as otherwise expected with dysbiosis (Zaneveld et al. 2017)), points towards a "captivity" effect. Since the polyps were maintained together in filtered seawater in closed systems, their exposure to novel bacteria was limited, and bacteria shed by the other polyps likely constituted the predominant source of "novel" associates. In contrast, the response of the Hong Kong corals to menthol bleaching was directional and more uniform.~~ While we cannot draw definitive conclusions due to low replication, it is possible that differences in patterns of microbial response to menthol bleaching were linked to differences in rearing conditions. ~~We hypothesize that this latter might reflect a new stable state of the tank water, rather than of the holobiont.~~ While ~~rearing conditions~~ these were largely replicated between facilities, feed type, tank volume and filtration systems differed. ~~Feed can introduce bacteria into the system (Hartman et al. 2020), and uneaten portions could promote microbial growth. Such effects would have been amplified by the smaller volume of the containers used in Hong Kong compared to Red Sea. However, it should be noted that our experimental design did not allow us to directly test these hypotheses.~~ We therefore suggest that future studies incorporate an adequately replicated "facility" factor in their design, as well as food and seawater samples to better characterize ~~the influence of rearing conditions on the host microbiome~~ these aspects."

- **Conclusions**
  - L463-465: "The response of the bacterial microbiome to bleaching differed in trend between the two facilities, possibly reflecting differences in rearing conditions, which remain to be addressed."

Additionally, the aspect of cryptic lineages has been discussed in the context of similarity/dissimilarity between symbiotic colonies (L397-409), as detailed in our replies to the specific comment later in this document.

We have also double-checked the reference list to ensure all cited works, including Wepfer et al. (2020), are accurately included.

3) The 'captivity' effect: Authors may note that the microbiome of aquaria-kept corals (without menthol bleaching) are less diverse than what is reported from field-based studies from the same taxonomic coral species; however this should not be their scientific claim without examining wild-vs-captivity corals in the same study using the identical methodology, performing due scientific rigor. PCR primers, sequencing depths, identification of OTUs or ASVs, assignment of taxonomic affiliations differ among studies, making the microbial diversity based on reported numbers of taxa being difficult to compare directly. In addition, comparing microbiome of the studied coral species from different regions may be problematic due to the potential of cryptic species as mentioned above, which may well form host lineage-specific microbiomes. While I agree with the previous reviews that the lack of wild coral data in this study prevents proper examination in this aspect, it appears that this was never the authors' intended area of interest in this manuscript. Therefore I do not support that the lack of new wild coral data in this study to form the absolute reason to reject this paper, but rather suggest this aspect to be significantly 'toned down'. Accordingly, I suggest removing the abstract sentence "the coral-associated microbiomes were composed of relatively

few bacterial taxa, when compared to literature reports from wild corals.", sentences in L326-334 ("In fact…."), and a concluding sentence L404- "gain initial insights into the potential effects of long-term captivity in this coral species."; also rephrase L408 "composed of fewer taxa than reported for the wild corals" to fit better with statements on the potential advantage of simple multipartite symbiosis study models.

Thank you for the insightful comments on the 'captivity' effect. We agree that the comparison between microbiomes of aquarium-kept corals and those reported from field-based studies cannot be made rigorously without identical methodologies and direct comparison within the same study. However, irrespective of these differences (e.g., considering ASVs or OTUs and sequencing techniques), we observed objectively simple microbiomes in all *G. fascicularis* polyps (e.g., a symbiotic polyp harbored only 10 ASVs) and we think that we are also not doing justice to our data by not talking about this at all. Therefore, we have reframed the relevant sections to emphasize the simplicity of these microbiomes and their potential advantages for holobiont research. We have also made it clear that any explanations or causes are speculative, as these insights might be valuable for guiding future research efforts on this topic.

We therefore amended the manuscript as follows:

- **Abstract**: "the coral-associated microbiomes were composed of relatively few bacterial taxa (10-78 ASVs), ~~when compared to literature reports from wild corals.~~"
- **Results**:
    - L188-194: "A small number of ASVs dominated the bacterial communities, where the 3 and 9 most abundant ASVs accounted for > 25 % and > 50 % of the total number of sequences, respectively (Fig. 2B). Evenness was on average lower among the symbiotic polyps. Specifically, in the symbiotic samples, the 5 (for Red Sea) and 4 (for Hong Kong) most abundant ASVs account for > 50 % of total reads, while in the bleached samples it took 7 (for Red Sea) and 12 (for Hong Kong) ASVs to pass the 50 % relative abundance threshold. Of note, microbiomes were made of a small number of bacterial taxa, ranging from 10 to 78 ASVs, and with symbiotic RS1 polyps having the smallest number of ASVs (10, 11, and 13 respectively)."
- **Discussion**
    - L349: the first paragraph of the subsection title "The microbiome of long-term aquarium-reared Galaxea fascicularis" was largely rephrased and re-organized and it now reads as follows: "The *G. fascicularis* polyps hosted simple microbiomes which were composed of a relatively small number of bacterial taxa (10-78 ASVs). While direct comparisons across studies that employed different PCR, sequencing, and analysis pipelines should generally be avoided, such numbers still appear small compared to previous characterizations of wild *G. fascicularis* from the South China Sea which reported 646-1,459 OTUs (Li et al. 2013) and compared to most other coral species, which typically harbor 100s to 1000s of bacterial taxa (e.g., Ziegler et al. 2016; Hernandez-Agreda et al. 2018; Pollock et al. 2018; Galand et al. 2023). We are unable to tell whether captivity caused a reduction in bacterial diversity as we lack direct comparison with the original wild colonies. However, we suspect that captivity favored a simplification of the microbiome, as stable and homogenous

environmental conditions decrease both chances and need for the association with functionally and taxonomically diverse microbial partners. In fact, decreases in metabolic diversity and species richness have consistently been reported for tropical reef-building corals reared in closed systems (Kooperman et al. 2007; Vega Thurber et al. 2009; Pratte et al. 2015; Damjanovic et al. 2020), and for the anemone Aiptasia already after a few days of captivity (Hartman et al. 2020). Such effects could have been exacerbated by the use of filtered seawater during the bleaching phase, which largely reduced the pool of available microbes (Dungan et al. 2021b), and by the reduced structural complexity of the polyps that, compared to colonies, provide fewer micro-environments and ecological niches (Morrow et al. 2022, Putnam et al. 2017)."

- L384: "Regardless of the causes, ~~Although some may see this simplification of the microbiome as a problematic artefact associated with captive corals,~~ simple (or simplified) microbiomes present the opportunity to identify essential associates and facilitate the development of microbial manipulation protocols to unravel holobiont functioning (Jaspers et al. 2019; Puntin et al. 2022b). ~~While the majority of studies report corals as hosting complex and rich microbial communities, the key functional players still remain elusive (Jaspers et al. 2019; Barreto et al. 2021)~~. Culturing corals in sterile seawater may help to limit the horizontal acquisition of transient microbes and thus favor proliferation of core or stable members for detailed characterization (Dungan et al. 2021b). Also, a simple ~~simplified~~ microbiome facilitates ~~further~~ the elimination of bacterial populations to produce gnotobiotic or axenic hosts, which could subsequently be re-inoculated to produce a range of host-bacteria combinations to test microbial functions and inter-partner dynamics (Fraune et al. 2015; Murillo-Rincon et al. 2017; Jaspers et al. 2019; Taubenheim et al. 2020). Reduced microbial complexity—whether due to captivity or other factors—~~in captivity~~ might therefore provide advantages for these specific experimental approaches with the Galaxea model."

- **Conclusions**
  - L460 (former L404): "In this study, we provided the first baseline assessment of the response of the Galaxea bacterial microbiome to menthol bleaching~~, and gain initial insights into the potential effects of long-term captivity in this coral species~~."
  - L465: "~~Bacterial communities of the captive Galaxea colonies were composed of fewer taxa than reported for wild corals, which is in line with decreasing microbial diversity of many captive organisms. Nevertheless~~ Despite co-culturing in shared aquaria, symbiotic polyps originating from different colonies maintained distinct community assemblies. ~~, and showed~~ This suggest links to host and/or Symbiodiniaceae identity ~~which~~ that warrant further investigation. Bacterial communities of the captive Galaxea colonies were composed of a small number of taxa. A simple ~~simplified~~ microbiome could facilitate both characterization and experimental manipulation, and guide the identification of essential ("core") members among the retained associates."

Again, the last two aspects (the location and captivity effects) should be handled as speculative notions and removed or toned down clearly as such, certainly not to be claimed as established

observations in the abstract or the conclusions section, for the reasons highlighted above. By doing so, I hope that the focus of the study, i.e. the pattern of bleaching effect on coral microbiota, is further emphasised and comes clearer. I found the finding of stochastic changes upon menthol bleaching insightful and representing a step forward in manipulative experiments in coral symbiosis, and thus I believe there is a merit to publish this dataset.

In addition, compositions of algal symbionts data were further limited (8 polyps out of 15 nonbleached polyps studied, and 2 out of the 8 samples have 20~30 sequences each, cf. 1,085~1,669 in other), and were not able to contribute to decipher the effect of algal symbiont loss on bacterial community differences (in terms of what initial algal symbionts the bleached corals may have had). In fact, no statistical testing was conducted on this aspect above general pattern description (L253-). While I acknowledge that algal composition could have formed an important faucet to discuss the menthol-bleaching effect, the data presented were preliminary at most. I'd recommend removing this aspect from the main body (methods/results) and cite the existing data in a supplementary material while speculate this effect within the discussion, as one of the area that need further studies on (such that is written currently).

Thank you for highlighting the limitations regarding the algal symbiont composition data and its impact on deciphering bacterial community differences. We removed the part pertaining to the characterization of Symbiodiniaceae community from the Materials and Methods and Results sections (at L116, L127, L133-134, L172-176, and L272-282) and edited sentence in the Discussion at L410-415: "Besides host genotype, Symbiodiniaceae community composition ~~observed herein~~ could also explain differences in bacterial community composition between ~~Red Sea~~ colonies (Littman et al. 2010; Bernasconi et al. 2019). To investigate this aspect, we also characterized the Symbiodiniaceae communities of the same polyps (Supplementary Materials and Methods). While only a small proportion of samples were successfully sequenced, we ~~could identify~~ noticed emerging patterns of Symbiodiniaceae-bacteria co-occurrence that warrant further investigation (Fig. S5)."

All the information that was removed from the main manuscript is now available in the Supplementary Materials and Methods document, which now reads as follows:

"**Characterization of Symbiodiniaceae communities of *G. fascicularis* polyps**

Sampling and Symbiodiniaceae community analysis

The characterization of Symbiodiniaceae communities was performed using the same polyps' DNA extract employed for the 16S rRNA amplicon sequencing (characterization of bacterial communities) and sequenced in the same sequencing run (see main manuscript for details).

Symbiodiniaceae ITS2 region was amplified using the primers SYM_VAR_5.8S2 and SYM_VAR (Hume et al. 2018), and raw ITS2 sequencing data were analyzed using the SymPortal workflow remote instance (Hume et al. 2019). ITS2 sequencing produced poor results and it was only possible to characterize eight of the symbiotic samples (6 Red Sea, 2 Hong Kong; see Fig. 1). Of these, two did not pass quality check (i.e., they had < 200 sequences/genus) and SymPortal could not predict ITS2 type profiles. Therefore, we report (post-MED) ITS2 sequences.

Results

All colonies of *Galaxea fascicularis* were dominated by *Cladocopium* spp. symbionts. Symbiodiniaceae composition was consistent in polyps from each colony and differed by region. *Cladocopium* spp. ITS2 sequences accounted for > 92 % of the sequences in all samples but one (RS2, 76 %) (Fig. S5). Of these, C1 was by large the dominant ITS2 sequence (~ 33 – 80 % relative abundance). Sequences C1b, C41, and C41f were exclusively and consistently found in polyps of one Red Sea colony (RS3), where they collectively accounted for ~ 26 % of reads. *Durusdinium* spp. sequences were found in only one colony from the Red Sea (RS2), where they accounted for 4 % and 24 % of the sequences, and of which the most abundant sequences were D1 and D4 (Fig. S5). One Red Sea sample (RS3) also hosted sequence A1 (genus *Symbiodinium*) at ~ 5 % abundance. In both Hong Kong colonies C1 was the dominant sequence (68 – 80 %), with C1c present in lower abundance (15 – 20 %) (Fig. S5)."

Minor and more specific points:

L24 "tripartite interactions"; seeing that bacteria here refers to diverse communities consisted of many species, it is not technically a 'tripartite' relationships, but more like a highly complex multipartite interactions. The same applies to L51 "three partners".

Thank you for pointing this out. We amended the text accordingly:

- L24: "A growing body of knowledge suggests a central role of complex multipartite ~~tripartite~~ interactions between bacteria, Symbiodiniaceae, and the coral host in nutrition, health and fitness"
- L51: "untangling the complexity in the holobiont requires detailed knowledge of the interrelationships that consider all ~~three~~ partners"

L59: What seems missing here is what is known about the direct effect of menthol on bacteria in general. Menthol is known to inhibit wide variety of bacteria, and can select certain members in the community, such that known in cigarette associated microbiome. It is worth mentioning potential direct effects of menthol on bacterial communities associated with corals, not only by indirect impact through the lack of symbiotic algae, for a more balanced view.

Thank you for emphasizing the importance of discussing the direct effects of menthol on bacterial communities, which we incorporated as follows (L60-62): "Yet, its impact on their bacterial fraction remains unknown. Menthol is known to have antimicrobial activity against several human pathogens (Trombetta et al. 2005; Mahzoon et al. 2022) and can select against certain bacteria, thus impacting microbial community composition, as seen in other contexts (Chopyk et al. 2017)."

L69-: It would be a good courtesy to outline the volume and water filtration systems in the Ocean2100 facilities, in comparisons to HK, as these may be important information regarding microbial environments.

We included additional information to better contextualize the microbial environments (L72-74): "[…] and transported to the Ocean2100 aquarium facility at Justus Liebig University Giessen (Germany) where they were maintained in a 7,000 L closed aquarium system composed of several tanks (100-265 L) including a technical tank fitted with protein skimmer, active charcoal filter, phosphate adsorber, algal refugium (*Chaetomorpha* sp.) with reverse light cycle, and calcium reactor (Schubert and Wilke 2018)."

L71: The unit for salinity is missing. E.g. ppt.

We recognize that the use of the 'ppt' or '‰' is intuitive and often adopted to report salinity as the mass of dissolved salts per volume of water. However, technically, salinity should be expressed as practical salinity which is dimensionless (ratio of two conductivities). This is also referred to as "Practical Salinity of 1978" following the year of its official proposal as standard international unit (see relative Unesco documentation, from page 57 https://unesdoc.unesco.org/ark:/48223/pf0000065031/PDF/065031engb.pdf.multi)

L113: it is worth mentioning how the corals appeared after 13 days post the menthol treatment day, as this can be critical to interpret bacterial compositions data.

Thank you for noting this aspect, which is however mentioned in the Materials and Methods (L95-98: "Bleaching was assessed in the Red Sea polyps by visual inspection under a fluorescence stereomicroscope (Leica MZ16 F) 10 days after the menthol treatment, when algal cells were not detectable in any polyp. At the same time point, Hong Kong polyps also appeared fully bleached under microscopic inspection (Olympus Optical, mod. CHK at 400×).").

For clarity, we also amended L119 (former L113) as follows: " were sampled on the 13th day after the menthol treatment when they visually appeared completely bleached and otherwise healthy".

L121: Was the tissue dissociated from the skeleton before proceeding to DNA extraction? How was this performed?

This is indeed a relevant aspect. The polyps were crushed, and skeleton and tissue were together placed in the manufacturer's extraction/lysis buffer. The subsequent extraction process was carried out according to the manufacturer's instructions. During the early stages of the protocol, a column which functioned as a filter effectively removed any remaining skeletal fragments from the solution.

We amended the relative section accordingly (L128): "DNA was extracted using the Qiagen DNeasy 96 Blood & Tissue kit from crushed polyps (containing skeletal and tissue material) with a~~.A~~bout 10 mg of coral tissue per sample ~~was used~~ as starting material."

L281- "This surprisingly included Symbiodiniaceae-associated bacteria that we were expecting to be reduced after the physical removal of Symbiodiniaceae (Fig. S5; Supplementary Materials and Methods)." This seems to come out of blue in the discussion, and should be mentioned in Results. Also, menthol-induced bleaching is not 'physical removal' of the algal symbionts.

To address this, the Results section was amended as follows (L221-225) "Additionally, no bacterial taxa (neither at ASV nor at bacterial family level) showed significantly different relative abundance between symbiotic and bleached samples, neither across the whole data set nor by geographic origin (i.e., considering Red Sea and Hong Kong samples separately) (Mann-Whitney *U* test with Benjamini-Hochman correction, all $P > 0.05$). This includes bacteria previously reported to be associated with Symbiodiniaceae cultures (Fig. S4; Supplementary Materials and Methods).".

Please note that the lack of significantly different relative abundance between treatments, that was previously reported in the Results at L248-251, has now been removed to avoid redundancy ("~~No single taxon (neither at ASV nor at bacterial family level) was significantly different in relative abundance between symbiotic and bleached polyps, regardless of whether samples from the Red~~

~~Sea and Hong Kong were considered together or separately (Wilcoxon test with Benjamini-Hochman correction, all P > 0.05)~~").

Additionally, we added in Materials and Methods (L165-168) : "The differential relative abundance of bacterial taxa between symbiotic states (symbiotic, bleached) was tested at the ASV and family level across the whole data set and by geographic origin (Red Sea, Hong Kong) using the Mann-Whitney U test with Benjamini-Hochman correction for multiple comparison."

We also removed "physical" at L305 which now reads: "This surprisingly included Symbiodiniaceae-associated bacteria that we were expecting to be reduced after the ~~physical~~ removal of Symbiodiniaceae".

Around L300: Again, I feel that one missing aspect to discuss here is about the direct effect of menthol on bacterial communities. The text so far implies bacterial shift is solely due to the loss of algal symbionts, but this may well be rather indirect impact of menthol, as I indicated above. How bacterial composition may have been affected by menthol-'selection' and how long the impact to last etc., would be an important area to discuss.

To incorporate this aspect, we added a paragraph at L312-321: "The lack of significant changes in bacterial taxa abundance between symbiotic and menthol-bleached polyps is surprising also given the known antimicrobial activity of menthol. Despite its effectiveness against various bacteria (i.e., *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus* spp., *Lactobacillus* sp.) and its demonstrated selective activity on cigarette microbiomes (Trombetta et al. 2005; Chopyk et al. 2017; Mahzoon et al. 2022), direct impact of menthol on coral-associated bacteria may have been limited. Factors such as concentration, exposure time, and the varying susceptibility of bacterial species, alongside interactions with the coral environment and other microorganisms, likely contribute to the lack of an observable effect. These aspects, as well as further exploration of onset, duration, and specific mechanisms of action of menthol represent important areas for future research to elucidate its impact on coral-associated microbial communities."

L350: In my opinion this suggests the presence of cryptic species (spp.) within a region; as Wepfer et al. (2020) demonstrates.

We agree that the paraphyletic nature of the currently accepted *G. fascicularis* species and the existence of cryptic lineages complicate any interpretation of host phylogenetic effect on the associated microbiome. The work by Wepfer and colleagues (2020) is indeed well worth a more elaborated mention. We have therefore amended the paragraph (L397-409) as follows: "Interestingly, the coral colonies tested here maintained distinct bacterial microbiomes even after long-term co-culturing, which supports a degree of host genotype effects controlling the microbiome composition, as previously reported from Hydrozoan and other coral species in the field field (Pollock et al. 2018; Dubé et al. 2021)~~(Dubé et al. 2021)~~. Surprisingly, the microbiome of one Red Sea colony was highly similar to that of Hong Kong colonies. This appears counterintuitive as colonies from the Red Sea and Hong Kong may belong to different *Galaxea* lineages (*sensu* Wepfer et al. 2020), and considering the ~~despite~~ large differences in ~~geographic and~~ environmental conditions at their origin. While t~~T~~hese colonies were also maintained in separate facilities, ~~but~~ rearing conditions were similar at both locations (i.e., temperature, salinity, illumination) and ~~. In addition to the host phylogenetic basis of microbiome composition (Pollock et al. 2018), the similarity in environmental conditions~~ may have induced convergence of microbial community composition (Dubé et al. 2021).

On the other hand, *G. fascicularis* is a polyphyletic species that contains several morphologically cryptic lineages (Wepfer et al. 2020) and the dissimilarity between colonies from the Red Sea could reflect host phylogentic differences (i.e., cryptic species within the same region).”