This document outlines and explains the reasons behind our appeal against the decision to reject the manuscript titled "The bacterial microbiome of symbiotic and menthol-bleached polyps of *Galaxea fascicularis* in captivity".

During the review process, we have identified factual errors and unfair treatment that significantly impacted the decision to reject our work. These are outlined and expanded below.

Please note that text in italics in quotation marks are direct quotes from the recommender and reviewers.

To briefly summarize, the manuscript focuses on the emerging model organism *Galaxea fascicularis* for coral symbiosis research, in the context of further establishing it as model system. The Galaxea model represents a useful tool to unravel the complexity of the coral holobiont through the ‘disassembling’ of its components (animal host, algal symbiont, bacteria, etc., as demonstrated in previous publications). As a first step, the removal of the algal symbiont (Symbiodiniaceae) can be effectively achieved through menthol bleaching. However, the effects of menthol on other essential components of the holobiont (i.e., bacteria) have not yet been investigated. We therefore replicated the menthol bleaching protocol in two facilities and characterized the bacterial microbiome of both symbiotic (untreated) and menthol bleached polyps (clonal replicates) of *G. fascicularis* that were maintained in captivity (aquaria) for several months. This study therefore provides valuable first insights into the bacterial microbiome of menthol bleached corals of this aquarium reared model species. The results showed that menthol treatment was generally associated with random changes in the bacterial communities, however the response differed between the two facilities, highlighting the importance of small but relevant differences in rearing conditions. The study did not aim to rigorously test the effect of captivity itself (e.g., by drawing direct comparison between wild and captive corals). In addition, a pattern of microbial simplification was observed supporting reports that showed similar effects in other coral species and captive animals more broadly.

While we appreciated the time and effort that the reviewers and recommender dedicated to this evaluation, we argue that they did not provide a factually correct and unbiased assessment of our work. Below is the breakdown of each point:

**Factual errors made by the reviewers or recommender that had a major impact on the decision**:

The recommender used incorrect information with regards to a) the time frames of captivity vs. duration of the experiment, b) the bacterial taxa identified, and c) the current state of the literature.

1. The recommender and reviewer 2 wrongly understood the **duration** of time spent in **captivity** (aquarium-rearing) of our corals before the experiment took place, which is used to justify their decision to reject our manuscript.

Specifically, the recommender finds our ‘captivity effect’ hypothesis and relative discussion “*highly speculative*”, while he finds the alternative hypothesis proposed by Reviewer 2 as “*more compelling: that the* ***10-14 days of captivity*** *sufficiently impacted and weakened the bacterial community associated with symbiotic Red Sea polyps to make the effects of bleaching barely perceptible.*”. However, the corals in the facility were **maintained in captivity** for **several months** before the start of the experiment (see manuscript at lines 68-70, and abstract: “*G. fascicularis* […] that were **long-term aquarium-reared** […]”). The recommender confused the total duration of the captivity at the time of the experiment (several months) with the time allowed to recover from fragmenting (10-14 days)—both described in the Materials and Methos section. Importantly, all coral colonies used for the experiment are still alive and well in the long-term rearing facility four years later. In fact, that is the whole purpose of establishing a model system that can be investigated under strictly constrained conditions. The hypothesis that captivity itself severely weakened the coral before the menthol treatment appears therefore unsupported.

1. Speculative interpretation of the role of associated bacteria: “*This idea gains support from the significant presence of a* ***putative coral pathogen*** *(****Alteromonas*** *spp.) within the core microbiome of captive Galaxea.*”. This is also factually incorrect, as members of the genus *Alteromonas* may provide broad functions in the coral holobiont: members of *Alteromonas* are common coral-associates found in almost every coral-associated microbiome, some members are also candidate probiotics with suspected beneficial functions for the coral, and others are suspected pathogens, as we reviewed in our manuscript well supported by literature (see lines 372-374 and 384-389). The presence of *Alteromonas* spp. therefore cannot be used as evidence for the alleged “*impacted and weakened*” state of the corals’ bacterial community prior to menthol bleaching.

Additionally, the recommender’s interpretation that *Alteromonas* spp. abundance should be taken as in **indicator of underlying stress** in our corals because of its presence in the core microbiome is **not supported by our data**: if *Alteromonas* was positively correlated with stress, then its abundance should have been higher in the menthol bleached samples (even assuming that captivity itself caused significant stress, menthol bleaching would have unquestionably caused additional stress). However, as visible from figure 4 (lines 241:244), the abundance of *Alteromonadaceae* both increased (RS1, RS3) and decreased (RS2, HK1, HK2) in bleached samples. A similar pattern can be seen in Figure 5 (lines 272-276) for the abundance of the core members of the microbiome ASV\_003 and AVS\_008, both *Alteromonas* spp. Hence, the role of *Alteromonas* spp. in the coral holobiont, and in the Galaxea holobiont, remains to be clarified.

1. Reviewer 2 suggests a new interpretation of what drives the bacteria-Symbiodiniaceae association, which is however based on a misinterpretation of the studies that we referenced. The statement that “*the 3 external studies were based on non-bleached corals and clearly confirmed this assumption*” is plain wrong, as these are based on Symbiodiniaceae algae cultures, as explicitly reported in our Supplementary Material and Methods section.

Other sections of the review also lack clarity or misinterpret the current state of the literature (other peer-reviewed studies as well as our data), showing a lack of attention or expertise in coral microbial ecology throughout the review. For instance, the sentence “*Unfortunately, the authors have already dismissed this notion (Line 349), arguing that the reduction or simplification of the microbiome is not an issue associated with captive corals' simplified microbiomes*” is self-contradictory and does not correctly interpret our point of discussion. Captive animals have simplified microbiomes, and corals are no exception.

**Unfair treatment and/or bias in the review process**

Our concerns regarding the fairness of the review process are based on the following points: d) the reviewers and recommender do not point out any objective methodological flaw regarding the primary objective of our study; e) neither of the reviewers clearly recommends a rejection (indeed one says that the work in its current form could be published); f) unclear arguments from the recommender effectively misled our efforts.

1. There are **no standing critiques regarding** the methodology, the analytical approach, the transparency and reproducibility of the analysis, and the presentation of the **primary objective** of this study (i.e., to investigate the bacterial microbiome of symbiotic and menthol bleached polyps of the emerging coral model *Galaxea fascicularis*, as per the title). We present our results in the most clear and transparent way. The criticisms rather focused on possible alternative interpretations and on secondary aspects (the ‘captivity effect’) which however cannot be substantiated with the present data.
2. **Neither of the reviewers recommend a rejection**. Such a decision seems therefore based primarily on the recommender’s opinion and interpretation of our work, which we showed above to be based on false premises.

In summary: **Reviewer 1** is satisfied with how we addressed the points raised in the first round of revision, and gives the green light for publication. **Reviewer 2** points out how the study limitations “*are not completely insurmountable ; it is not impossible that authors overcome them, by continuing to dig the interpretation of their data and polish the main message.*”.

1. The rejection decision is based on our inability to provide additional data to address a **secondary** aspect of our investigation (the ‘captivity effect’). Hence completely dismissing the primary objective of our work: to describe the bacterial microbiome of symbiotic and menthol bleached polyps of captive *Galaxea fascicularis* (see: **title**). We did not conceive this experiment to investigate how captivity affects the bacterial microbiome (the ‘captivity effect’). For that, samples from wild and captive corals need to be sequenced together. As we cannot travel in time, the recommender basically requests that we run a completely new experiment **to address a different question**.

To summarize, not a single one of the arguments put forward in this round of revisions is valid on closer inspection, and we hope that you will reverse the decision to reject our manuscript.