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## ***Interactive comment on “Iron oxide deposits associated with the ectosymbiotic bacteria in the hydrothermal vent shrimp Rimicaris exoculata” by L. Corbari et al.***

### **Anonymous Referee #1**

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#### General comments

This manuscript provides a thorough investigation of the structure and composition of iron oxides formed in the branchial chamber of a hydrothermal vent shrimp and their association with ectosymbiotic bacteria. As reported in the introduction, the formation of iron oxides in association with this shrimp has been the focus of several articles. In a previous work Corbari and co-workers described the successive stages leading to the formation of this crust, along with bacterial colonisation following the moulting process (Corbari et al. 2008). A more complete analysis of the mineral composition and ultrastructure of the oxide-bacteria layer is provided in this new article. This is the first

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time bacteria-associated iron oxides are described in relation to ectosymbiotic bacteria. The imagery, mineralogical and chemical data reported in this paper are of high quality and the presentation of the results is clear. Significant information on the properties of these mineral are provided, both on the composition of oxides and their association with micro-organisms (see details below). There is no doubt this article provides important new insights to address the question of the contribution of ectosymbiotic bacteria in the formation of the iron oxides and should be recommended for publication in Biogeosciences. There is however some weaker points in the introduction and the discussion which deserve improvement in order to strengthen the conclusion.

### Specific comments

There are several main outcomes from this study that may be underlined.

1- The mineralogical composition (2I-ferrihydrite) and the inclusion of inorganic complexes in the mineral structure are consistent with those described by others for typical biogenic oxides. No particular features are observed which might have resulted from the particular conditions of oxide formation in the branchial chamber of the vent shrimp.

2- The tight association of the oxides with a single bacterial morphotype (rods), and their appearance only after this morphotype is observed on the cuticle, are consistent with the fact that mineral formation is mediated by particular representatives of the ectosymbiotic community. This is the first unambiguous indication of the role of microbes in the formation of oxides in the Rimicaris branchial chamber.

3- Perhaps the most original and interesting point of this article rely on the spatial distribution of oxides and bacteria, showing an inverse correlation of cells and minerals throughout the crust. Microbes and oxides tends to accumulated in distinct layers of the crust suggesting that products of the metabolisms are drove away from the cells. Although distinct to the template process described in Chan et al. 2004 (i.e. it does not involve stalk formation), this mechanism would have a similar protection role from cell encrustation.

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4- Additionally, an important question is raised in this article concerning the potential influence of the sample preservation method on the oxide composition, as indicated by distinct Fe/O ratios. Artefacts are suggested for the glutaraldehyde-fixed samples, while the frozen samples would be closer to the original material. If confirmed, this will constitute an important recommendation to future studies.

To fully appreciate the importance of these results on the symbiotic association, however, further explanations or a more rigorous discussion are needed on the following points:

1- The overarching question for this work is the potential role of iron oxidising bacteria as an energy-source for the shrimp. A more comprehensive statement of the information that underlies this hypothesis is needed. Particularly, it is unclear why previous assumptions concerning sulfide-oxidizing bacteria should be reconsidered. This would allow the reader to better appreciate the originality of this model.

2- Quantitative estimates of the contribution of inorganic compounds stabilizing the oxide structure are important as these ligands are expected to influence the surface properties of the oxide. As indicated in Table 3, they have been calculated under the assumption of most probable occurrence. More details should be provided on this assumption and on its relevance to hydrothermal conditions. Conditions of mineral formation in hydrothermal systems differ significantly from those of natural aquatic systems in equilibrium with the atmosphere. As an example, iron sulfides (FeS, FeS<sub>2</sub>) are abundant in hydrothermal environments and may therefore be deposited in the branchial chamber. The authors did not consider these environmental contaminants as potential components of the crust. Rejecting this hypothesis should be more robustly constrained in the discussion. If this is not possible, the uncertainty arising from the possible occurrence of these minerals on the average composition of the oxide should be considered.

3- A main conclusion of this paper is that bacteria must participate to the oxide forma-

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tion. As presented above, there are clear arguments in the manuscript to support this assumption. There is however some confusion in the discussion regarding this idea. The assertion that results indicate the biogenic origin of the iron formed (abstract) is in contradiction with the indication that both biotic and abiotic mineral particles coexist in the *R. exoculata* ectosymbiosis (p1839). In fact, the results are consistent with a bacterial influence but do not prove that all oxides in the branchial chamber are biogenic. As indicated by Fortin and Langley (2005), iron oxides formed in association with microorganisms display physicochemical characteristics that are similar to their abiotic counterpart. 2I-Ferrihydrite is among the minerals formed by these microbes but cannot constitute a proof of biologically-mediated formation as it can also be produced in abiotic conditions (Majzlan et al. 2004 *Geochimica et Cosmochimica Acta*, Vol. 68, No. 5, pp. 1049-1059). The authors should acknowledge this more clearly in the discussion.

Furthermore, iron oxidizing microbes are known to compete with their own by-products as iron oxidation is autocatalytic. Rentz et al. 2007 *Environ. Sci. Technol.*, 41, 6084-6089 have shown that iron oxides in bacterial mats are composed of both biogenic and abiotic products. In their review Fortin and Langley (2005) conclude: it remains very difficult to differentiate biogenic oxides from those formed as a result of abiotic reaction in natural samples containing neutrophilic iron-oxidisers. What is particularly lacking in this article is a more explicit mention of the potential heterogeneity of oxides in the crust and a discussion of the limits of the averaging approach used. This is particularly important to decipher whether the difference in Fe/O ratios results of an artefact due to preservation or simply reflect microscale variation in the oxide composition resulting of different formation processes.

In conclusion, a more cautious discussion of the results with regard to the complexity of processes leading to the formation of bacteria-associated oxides should be proposed. Particularly, it would deserve a clearer definition of what is meant here by: biogenic origin.

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Specific comments: p1827\_line9: Longitude for the Rainbow site is missing.  
p1838\_line7: U nor Ur p1838\_line8: Remove: with p1838\_line16: occur p1839\_line14:  
missing verb? p1842\_line 26: replace: element by compounds or molecule p  
1843\_lines 13-16: Unclear. P1855\_line 5 and table: Format for SO42- PO43-

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