

Round #1

Decision

by Corinne Vacher, 2019-04-12 22:58

Manuscript: <https://peerj.com/preprints/27519/>

Revision requested

Dear Dr. Dittami, Thank you for submitting your article to PCI Ecology. I have now received two reviews of your manuscript, which you will find below. Both reviewers greatly appreciated your work, which is an extremely comprehensive and well-written review. I invite you to take into account all their comments and submit a revised version to PCI Ecology.

1. In particular, the two reviewers suggest that the section on the evolution of holobionts be strengthened and better integrated with the rest of the study.

We agree that the former section on the evolution of holobionts was weak. To avoid any artificial split, we have now linked the evolutionary aspects of the first part of the manuscript more tightly to the historical perspectives and added the work of Guimarães et al, underlining the importance of non-interacting species in shaping the co-evolution of mutualistic communities. We reinforced the question of evolution in the abstract and conclusion, and mention examples of models for evolution of photosymbiosis. Furthermore, as we state in paragraph 5 of the history of the marine holobiont concept, the questions of evolution and function are tightly tied together; thus we now briefly point out the importance of these inter-connected questions also in the section on the development of model systems and in the section of holobiont assembly and regulation.

2. I also suggest that throughout the manuscript, what distinguishes marine holobionts from terrestrial holobionts in terms of functioning, study methods and challenges, should be better highlighted.

The two main differences between marine and terrestrial holobionts we have identified are the high level of connectedness of habitats and the diversity of phylogenetic lineages (Figure 1). They are discussed in the third paragraph of the first section, mentioned in the conclusion, and were now added to the abstract.

Many of the marine specificities are related to these two observations, notably the fact that different specific actors and models are important and that the interactions observed in the marine environment have therefore different impacts on biogeochemical cycles, ecosystem services linked to marine environment. For instance, it is likely that the large phylogenetic diversity occurring in the sea will increase the complexity and chemodiversity of host-

symbiont interactions (an idea now added in the bacterial gardening section – holobiont assembly and regulation). We now also mention limitations with regard to reference databases and knowledge on gene function for the more “exotic” marine lineages (tools section) along with the need to optimize techniques for different lineages and to identify and develop representative keystone marine holobiont models.

Nevertheless, while throughout this manuscript we attempt to describe marine systems and specific challenges, the important concepts and the methods used to elucidate holobiont interactions are similar or even the same across ecosystems. This is why we end up drawing more parallels to terrestrial systems than highlighting fundamental differences, e.g. when we state in the “challenges” section that « Better understanding the factors that shape the composition of holobionts is highly relevant for marine organisms given that, despite a highly connected and microbe-rich environment, most marine hosts display a high specificity for their microbiota and even patterns of phyllosymbiosis for some associations (Kazamia et al. 2016; Brooks et al. 2016; Pollock et al. 2018). »

3. Future lines of research could also be more clearly stated in the summary and conclusion, with an emphasis on those specific to marine holobionts. I hope that these comments will allow you to further improve the manuscript and look forward to receiving the revised version.

Thank you. Examples of future lines of research have been added to the abstract and the conclusion.

Yours sincerely, Corinne Vacher

Reviews

Reviewed by Aurélie Tasiemski, 2019-04-03 15:33

The review well written by Simon M. Dittami et al gives a very interesting state of the art as well as the methods and perspectives about the concept of holobionts transposed to marine organisms. This review emerged from the brainstorming of the main participants of a workshop recently organized on this topic. This work also underlines the major limits which make the holobiont concept still puzzling and challenging to decipher even more for uncultivable species with numerous genes/molecules with still unknown functions.

1. I think that some point of discussion should be addressed: do the authors think that unicellular and pluricellular organisms can be considered the same way in terms of plasticity/evolution and also in terms of methodologies?

Most of the principles apply to both unicellular and multicellular hosts, but there are indeed some restrictions, notably when it comes to their ecological role (dissemination vectors – this is now stated in the manuscript). There are also fewer examples of chemical gardening of microbes in unicellular hosts, but the same processes are still likely to occur (now added to the manuscript), and some differences in terminology (e.g. the term phycosphere used specifically for unicellular photosynthetic eukaryotes) are now included in the manuscript. In terms of methods, at least in marine biology single-cell genomics is used essentially for unicellular organisms (now mentioned in the manuscript), but the other methods can be adapted (in theory) to any holobiont system. As for plasticity and evolution, the main factor appears to be the functional stability of the system and not the size of the host.

2. Also the authors do not mention the differences in between ecto and endosymbiosis and their respective impact on the genome evolution and adaptation, plasticity of both partners. The definition of endosymbiosis should be better detailed (intra, extracellular, strict...with some examples) and the ectosymbiosis should be defined.

The definition of endosymbiosis has been expanded in the glossary and ectosymbiosis as well as examples were added. We now also put more emphasis on the different “intensities” of symbioses throughout the “The (marine) history of the holobiont concept” section and explicitly mention endo- and ectosymbiosis.

3. Do the authors think that the term “long lasting interactions” is finally well adapted to the holobiont concept if we take into consideration that along the life cycle of an individual (and its varying environment) the symbiotic community changes?

More or less long lasting', yes, but lasting anyway - otherwise selective evolutionary influence decreases accordingly. Lasting may also mean - occurring in each generation, even shortly; this was now added to the definition of symbiosis / holobiont and we changed long-lasting to lasting.

Reviewed by Sophie Arnaud-Haond, 2019-03-21 17:48

This manuscript, “the community perspective on the concept of marine holobionths: state-of-the-art, challenges, and future directions”, has been written by a working group created during the workshop on marine holobionths in March 2018. The manuscript builds on the evolution of the holobiont concept in the research landscape where new generation sequencing allowed unravelling the extent of long-

term relationships between hosts and their diverse microbiota across the tree of life. The authors aimed at discussing the way concepts in research and marine holobionts evolved those last years to highlight the need of a better understanding of those complex systems, and detail the roadmap they forecast considering the technological and conceptual challenges this represents. To that extent, this is a very timely and interesting topic. The main objectives appear to be the promotion of an increased a focus/scale up of research at the level of the holobionts, and in fine of a better integration of manipulative experiments on models with large-scale inventories and analysis of interactions/co-occurrence. I found this perspective and opinion manuscript very interesting, but also very dense. I make several comments here below that I hope can be useful.

Main comments The team chose a “short” format that, because rather well documented, is also rather dense.

1. Some of the sections may gain in being structured in subsections, or rephrased for the reader to follow better the lines of thoughts and connection among sentences and examples. This is particularly true for the sections on models and emerging methodologies.

We can see the usefulness of this and at the same time, given the short nature of the paper, three different levels of headlines seemed too much. Rather than adding headlines we have therefore now opted for highlighting important keywords for each paragraph/section which will also help the reader navigate the text. We have also modified the paragraph structure where appropriate.

2. Section on “marine holobionts models” may be easier to follow, and possibly more accurate with a clear distinction between works on symbiosis (i.e. the observation and study of bilateral associations), versus their extension to the analysis of the complexity and function of a holobiont system as a unit (in a similar way Margulis is approaching this in its 1990 article). The detailed descriptions of symbiotic models may this way naturally reflect the reasoning that led to the development and increasing used of the holobionts concept.

Thank you. This section was reorganized as suggested.

3. The part on evolution of holobionts seemed to me slightly disconnected from the rest of the MS mostly dealing with ecological approaches and consequences of the holobionts structure and function. As it stands, it is too short to render justice to the many aspects to consider and change in our approaches, to integrate the holobionts concept to evolutionary theories and studies (compared to subsequent sections mostly dealing with ecological studies). If remaining in the MS, this section may gain in being better integrated with the following ones (meaning those should also be expanded). Another option would be to keep the focus of the MS on ecology and skip that part.

Indeed, an extensive discussion of the evolution of holobionts would probably fill an entire review paper, and we agree that the evolution section does not do justice to this fact. As described in more detail in the response to the editor, we have now linked the evolutionary aspects of the first part of the manuscript with the historical perspectives but also strengthened the evolutionary questions in a few other parts of the paper.

4. The part on marine holobionts functioning relates much more to the determinants and mechanisms of association and dislocation of partners, than to their respective functions in an ecosystem, maybe another title would be more appropriate?

We have now changed the title of this section to “Marine holobiont assembly and regulation”

5. The way the two main axes of research are presented in figure 3 and discussed pages 9 and 10 is very interesting and offers a very clear view of the extent of the gap that needs to be bridged. Would it be interesting to consider the use of large-scale studies describing complex systems over large spatial and temporal scales to identify new models for experimental approaches in order to help bridging that gap? How could this be done on the basis of large networks describing complex systems of “Russian dolls” in nature? This may interestingly link to the section on emerging methodologies and the use of “-omics” techniques? In relation to this, the figure 3 is interesting but may be more complete and offer a better global view of the scope and conclusions of this article if completed to illustrate the way new technologies will help bridging the identified gap. It may also include the illustration of the way such changes in paradigm and approaches can have a cascade of consequences on the applications detailed in the last section.

In our opinion one of the key factors towards bridging this gap is indeed the establishment of new relevant laboratory model systems that can be used to test the hypotheses derived from environmental studies, generated by new technologies. We fully agree that network analyses of these large data sets will be very useful to identify relevant keystone models. A corresponding new node was now added to figure 3, and the idea explained in the “Integrating marine model systems with large-scale studies” section. We are aware of the fact that, the more complex the interactions get, the more difficult it is to establish suitable models, which will only be a proxy of the ecological reality.

6. Line 427 to 429, and later: A significant amount of literature has been published those last years on marine systems including not only corals or sponges but also macrophytes, showing the importance of taking into account the microbial compartment to understand the ecology and evolution of the host. How could management plans to fight against some invasive species, or

conserve of declining ones similarly take into account and manipulate microbiota, reform cautions wisely discussed in the end of that section?

We are not sure we fully understood how the reviewer would like us to amend the manuscript, but discuss this topic in the “Ecosystem services and holobionts in natural and managed systems” section. We fully agree that we need to remain cautious about the potential ecological impacts of holobiont transfers or manipulations (regarding both host and microbial components).

7. Finally, this manuscript is mostly written on an ecological (rather than evolutionary) perspective. Following that line, it seems to me that an interesting add to this opinion article would be a discussion on the accuracy of the now exploding amount of habitat models and forecasts for habitat shifts (particularly under global change scenario) most often only taking into account the abiotic compartment and ignoring the biotic one.

Indeed, to our knowledge there are only very few models that consider biotic interactions in ecological modeling (some on mixotrophy but no holobiont yet) but this can also be explained by the fact that so far, we have very little understanding of the functioning of holobionts. This lack is now explicitly mentioned in the last sentence of the “Challenges and opportunities in marine holobiont research” section and indirectly in the conclusion/perspectives (improvement of habitat models). We now also cite Bell et al 2018, as one example of a study that includes the biotic compartment, but where more precise information on the biotic interactions would be needed.

8. Minor comments and suggestions: Line 120 As opposed to vertical transmission, I may suggest that the arisen transmission is just the acquisition of the associated microbiome from the environment (which process can be continuous and independent of the emergence of new generation?).

« New generation » was now removed from the definition of horizontal transmission, but we would prefer to keep « horizontal » rather than « arisen » transition in our glossary as the former is much more widely used.

9. Line 159-161 and on: the authors offer brief and interesting history of the research lines that led to coin the term holobionts. This article from Margulis should be cited as 673-670 (not just 673). Despite the term holobionts is introduced in the legend of 2 figures, most discussion in this particular article relate to the difference between symbiosis and mutualism, analogy between symbiosis and sex, and symbiosis as a main source of novelty (compared to gradual changes; in the sense of Mereschkovsky). May be this is a semantic distinction, yet the way this is phrased in the 1990 article relates symbiosis to evolutionary novelty rather than describing it as the way “evolution mainly work...”. Is it possible that the description of the proposition of Margulis (lines

159-161) in the present manuscript would be more related to its book “Symbiosis as a Source of Evolutionary Innovation” (1991) that is most often cited as the origin of this term?

We now cite the 1991 book as suggested

10. Line 156: Is widespread (I is missing)

This was corrected

11. Line 210-213: sentence difficult to understand

The sentence was shortened and simplified

12. Line 328 to 332 the sentence is unclear.

The second half of the sentence was deleted rendering it easier to follow.

13. Line 352 biology is a rather vague term here, and it may be worth here to detail the need for physiology and biochemistry?

« biology » was replaced by « physiology, biochemistry » as requested