Dear Editor,

Thank you for evaluating and considering our manuscript titled "**Shaped to kill: The evolution of siphonophore tentilla for specialized prey capture in the open ocean**" for publication in PNAS.

We have highlighted our comments and responses are in blue and actionable comments from the reviewers in **bold**. In addition, we pasted the new text together wit our responses to facilitate the cross-referencing process.

_____

Reviewer #1:

This is one of the most interesting and well-written manuscripts I've reviewed in a long time. In it, the authors track the macroevolution of predation in the colonial siphonophores and find the surprising pattern that new predation strategies evolve from specialized ancestors. Theory based on solitary organisms predicts that specialists are dead-ends because the internal coadaptation required to become specialized constraints further evolution. Because siphonophores are colonial with a modular bauplan, the evolution of zooid types may be less constrained.

We thank Reviewer #1 for this encouraging feedback.

Because the ability for siphonophores to overcome typical constraints as they evolve specialized prey capture seems to arise due to their modular growth, **it would help to clarify if the tentilla is a part of the gastrozooid or a separate zooid itself given that it has modular growth as well.** This will help us understand if the result of this paper arises from the modular growth of siphonophores (in the case that tentilla are zooids) or if gastrozooids are less integrated than typical solitary organisms. It's always a challenge to translate phenomena seen in colonial inverts into ones known from solitary animals.

Great point. We modified the text in the Introduction (Lines 86-87) to clarify this. The tentillum is indeed a part of the tentacle, which is a part of gastrozooid, which as an individual zooid has an extremely modular bauplan, where changes to tentacle structures are decoupled from the rest of the zooid's anatomy.

Lines (86-87): "Siphonophore colonies have a modular body plan with different zooids specialized for different tasks. This modularity extends also within the feeding gastrozooids, which carry modular structures on the tentacle that are exclusively used for prey capture: the tentilla."

Reviewer #2:

On the face of it, this is a very compelling, generally interesting, and well-presented comparative analysis on the relationships between the co-evolution of feeding structures of siphonophore cnidarians and prey specialization. The main result, which is framed as a contradiction to the orthodoxy that specialization on a narrow range of prey (or host plants or habitats, etc.) is an evolutionary dead-end, seems to be contradicted by their data, which suggests that prey specialization may actually revert to generalization.

We thank Reviewer #2 for this positive feedback and illustrative summary of our main findings.

It's important to acknowledge that the concept of "evolutionary dead-end" is itself contentious, and can mean short lineage longevity, a failure for subsequent lineage radiation/diversification, as well as its meaning here, namely that, "specialized lineages are unlikely to evolve (back) into generalists or to shift the resources for which they are specialized." I think that the paper would be improved with a little more extensive discussion of this issue.

We agree that the concept of "evolutionary dead-end" can mean a variety of evolutionary outcomes related to lineage longevity, diversification, and niche breadth conservatism. We expanded the Introduction (Lines 121-127) to distinguish the specific uses of this term and clarify that we are exclusively referring to the ability of specialist lineages to give rise to feeding generalists or other specialists.

Lines 121-127: "Specialization in resource acquisition and use has often been presented as an evolutionary 'dead-end' (12–16). The concept of a 'dead-end' can be problematic because it conflates very different macroevolutionary patterns. These patterns can pertain to the clade, such as higher extinction rates or lower diversification rates, or to the evolutionary lock-in of lineages to particular attributes. Here we exclusively focus on this last sense, in which feeding specialization can be considered a 'dead-end' if lineages that are feeding specialists do not give rise to feeding generalists or specialists on other prey. "

We also added some lines to the Discussion (Lines 315-322) interpreting our results in the context of the different evolutionary patterns conflated by this term.

Lines 315-322: "These results show how an ancestor specialized for feeding on a particular prey can still give rise to multiple lineages and to novel feeding guilds, including generalists. For example, we find that, Eucladophora, a large clade of siphonophores that contains the majority of extant species and diverse feeding strategies, arose from a most recent common ancestor that was a specialist on large crustacean prey. Though 'evolutionary dead-end' is a problematic term that could apply to several evolutionary patterns, this result is inconsistent with multiple specific uses of this term."

In any case, the main explanation for this finding is that siphonophores are modular organisms, and that modules (in this case tentacles and their tentilla and nematocysts) that are involved in prey capture can evolve freely of functional constraints at the organismal level. This stands in contrast to the evolution of various kinds of trophic specialization in unitary organisms, which generally require integration of multiple systems in order to be efficient and effective, and that constrain the evolution of feeding specialization (and its reversibility). If true, this is an important finding, and begs the question of whether other clades of modular organisms, including other cnidarians, bryozoans, colonial ascidians and especially pelagic tunicates, are comparably released from the constraints of functional integration of parts, and can specialize without a penalty of becoming an evolutionary dead-end.

We agree with these statements and address these questions below in response to the Generality of Results comment.

 I am far from an expert on either the structural and functional aspects of siphonophore tentilla, or the phylogenetics of this group. But the paper does a really nice job cataloging the diversity of the relevant morphological characters and their apparent associations with different prey types. This in and of itself is a major advance in our understanding of the trophic and functional ecology of this group of gelatinous zooplankton. I am also far from an expert on the phylogenetic and comparative methods that are the analytical foundations of this paper, and the methods are absolutely critical to the robustness of the conclusions. Nevertheless, previous papers, both phylogenetic and functional, on siphonophores by Damian-Serrano and Dunn

establish a strong foundation for this paper. Overall, I found this paper compelling and fascinating. It explores a major question in the ecology and evolution of trophic specialization in a rigorous phylogenetic framework, and though necessarily incomplete, it is far and away the most important and imaginative paper I've read in a long time on the evolutionary ecology of gelatinous zooplankton.

We are very grateful to Reviewer #2 for their kind comments.

Some suggestions:

Ancestral state reconstruction (Lines 170-177): Character-state analysis failed to clarify whether generalist or specialist feeding was the ancestral character state. If real, this is a little weird and makes me wonder whether some of the feeding guild designations actually capture the generalist/specialist categorization.

Given that the phylogenetic placement of siphonophores among the hydroidoline hydrozoans is currently unresolved, we lack an adequate outgroup to robustly ground the siphonophore MRCA's ancestral state of feeding guild. Given the information available and the particular phylogenetic topology (the first split divides fish specialist Cystonectae from the Codonophora MRCA, reconstructed as large-crustacean specialist), it is not mathematically possible to conclude whether the siphonophore MRCA was a fish specialist or a crustacean specialist. However, this does not change our conclusions which are based on the evolutionary transitions observed beyond the MRCA.

I'm not familiar enough with the feeding ecology of siphonophores to know whether consumers of large crustaceans are more specialized than consumers of small crustaceans (or other types of prey). In other words, **I wonder whether some of the conclusions of this paper (that there is no clear directionality from generalist to specialist) may partly be terminological. This does not invalidate any of the results and conclusions, but for the open-water zooplankton novice like myself, it would make the paper more accessible.**

This is a great point and we agree that it needs to be clarified in the text. We have now added an explanation in the Methods section (Lines 442-446).

As far as we know, nobody is familiar enough with the feeding ecology of siphonophores to have strong priors on what directionality the character state transitions of feeding guild should have. We let the data (and the phylogeny) drive the values in the character state transition rate matrix which shows that generalists are likely a derived state from crustacean-specialist ancestors.

Lines 442-446: "When reconstructing the evolutionary history of feeding guilds, we fitted our SIMMAP model under the agnostic assumption that a generalist diet poses morphological challenges that are as distinct as each specialization is from each other. Thus, we do not impose any *a priori* constraints or weighting in the model for what state transition rates are permissible, letting the data determine the parameters."

Genetic correlations: I'm not sure why the paper uses the term "genetic correlations" when referring to the correlated evolution of traits. This could be made clearer.

We agree with Reviewer 2 that the usage of this term is inappropriate - its use was a mistake on our part . We have replaced it throughout the manuscript with the more appropriate term "evolutionary correlations".

Dietary analyses and ascertainment bias: Again, I am far from an expert on dietary analysis, and **was not clear how the paper assessed prey availability, how that varied in space and**

time, and disentangled that from the observed diet. It would also be useful if it were clearer how many samples, over what temporal and spatial scales, prey availability, and diet were assessed for each species used in the analysis, and how, or if, sampling may have had an impact on the results and their interpretation. The authors explicitly acknowledge some of these challenges beginning on line 330, and discuss it in a few lines. But this could be a major source of bias, and though difficult to circumvent in open-water systems like this that are extremely difficult to sample, could potentially (though I doubt it) make the results much less clear.

We share these concerns, and limitations in the data on prey availability in particular set boundaries on what we could and could not do in this study. There were many things we would have liked to address, such as a widespread analysis of prey selectivity including deep-sea species, but couldn't because of this constraint. This reviewer comment makes it clear that we did not sufficiently explain how we accommodated these limitations. Here are changes we made in this draft to make it more explicit:

- We expanded the supplementary data file `literature_diet_data.tsv` to include more information on the provenance of the observations, such as location, number of samples, sampling method. The VARS data all come from the Monterey Bay and Gulf of California using direct observations by remotely operated vehicles.

- We also expanded the limitations section in Lines 349-356 of the Discussion indicating the different biases inherent to the sampling methods used in the literature from which our data are derived.

Lines 349-356: "When interpreting these results, it is important to remember that diet is also dependent on environmental prey availability, which was not available in most of the sources we used (except Purcell 1981 & Purcell 1984). We integrated published dietary data collected using different methods bearing different inherent biases. Gut content inspections (used in the majority of our literature sources) are very effective at detecting small hard-bodied prey, but can fail to detect rapidly-digested soft-bodied prey. ROV observations can be biased towards large (often gelatinous) prey, and can easily oversee smaller prey items."

For the species studied in Purcell (1981), we took not only the gut contents but also the prey availability data into account (we calculated selectivity as Ivlev's D, which we used to assess some of the evolutionary correlations) when establishing their feeding guilds.

Generality of results: It's not clear if other clades of non-deep-sea siphonophores, or other clades of modular gelatinous zooplankton like salps, exhibit similar patterns. This seems important, especially the question of whether the phenomenon is limited to environments/ habitats that are exceptionally predictable in space and time (if, in fact, they are), and whether only predators (like what?) should exhibit comparable patterns (or would suspension feeders like salps exhibit comparable patterns of correlated evolution?).

This is a key question. We added a paragraph that addresses the generality of these findings in the Discussion section (Lines 360-370) by delineating specific testable hypotheses. We agree that salps are a really interesting system to compare to siphonophores, but we feel that we don't have the space in the manuscript to do them justice if we bring them up, especially since most people are unfamiliar with the biology of these animals.

Lines 360-370: "We hypothesize that siphonophores are able to evolve from specialization for one prey type to other states due to their prey-capture apparatus being extremely independent from the rest of the bodies in terms of location and function. We also hypothesize that the homogeneous midwater environment they live in favors the evolution of extreme morphological

adaptations for prey capture. Testing these hypotheses will be interesting in its own right, and also give a better sense of how generalizable our results are beyond siphonophores. It is important to note that our hypotheses only apply to organisms with access to a broad-enough diversity of resources (such as prey, or hosts) on which to specialize, and only when said resources pose distinct challenges that require anatomical modifications. Otherwise, there would not be enough variation to detect these patterns in the first place."

Here we flesh out our thoughts on the specific questions posed by Reviewer #2 based on our hypothesis framework -- i.e. whether (1) all colonial animals (such as salps), (2) all animals evolving in predictable habitats, and/or (3) only predators (as opposed to suspension feeders) should exhibit comparable patterns in the evolution of their trophic niche and feeding apparatus.

Salps exhibit modularity as distinct colony members (in blastozooid chains) and in their life cycle (alternation of oozoid and blastozooid stages). However, all the functions carried out by the blastozooid salp chain (sexual reproduction, feeding, locomotion…) are integrated within each one of the zooids. In this way, we expect that salp zooids should present evolutionary patterns more akin to those of solitary organisms. In line with our response to Reviewer 1's comment, our discussion on modularity is far more driven by the spatial and functional segregation of tentilla (a sub-part of the gastrozooid zooid) than by the sub-specialized body plan of the siphonophore colony as a whole. We do believe that more broadly, similar patterns should be expected in siphonophores at the colony level in relation to the evolution of other aspects of their ecological niche.

We do believe that all animals evolving in predictable habitats (such as the deep midwater layers) are more likely to evolve extreme adaptations to feeding, since they are less constrained by a rapidly changing abiotic environment. However, animals with an integrated body plan (i.e. fishes) would still face the constraints posed by external body shape disfiguration and internal organ displacement.

Whether only predators (as opposed to other consumers like suspension-feeders) should be expected to present these patterns depends on the mapping of variation in form to variation in function. The number of ways to adequately perform an ecological task (such as capturing prey, or retaining edible particles) greatly depends on the complexity and variability of challenges within the task. The morphological state space required to optimally retain suspended particles is extremely narrow due to the very specific and invariable physical challenges of the task. This usually leads to widespread convergent evolution across clades since there are only a few ways to accomplish the task (i.e. building a fine mucus net). Predators and parasites are different from other consumers because their feeding requires interacting with different organisms, which poses a widely variable set of challenges across the range of potential prey they can adapt to exploit. This is exacerbated in the zooplankton, since the communities are composed of animals of many different phyla, with extremely different body plans and anti-predator defenses. Therefore, we believe that predatory and parasitic clades are more likely to present the variation required to even detect the patterns that we describe in this work.

_____

Thank you all again for your time.

Yours sincerely,

Alejandro Damian-Serrano