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.

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 63-64) 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.

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.

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 98-104) 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. We also added some lines to the Discussion (Lines 313-319) interpreting our results in the context of the different evolutionary patterns conflated by 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. 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.

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 436-440).

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. Therefore, we fitted our SIMMAP model of ancestral reconstruction under the agnostic assumption that a generalist diet poses unique morphological challenges as distinct as each specialization is from each other. We do not impose any *a priori* constraints on the model over what state transition rates are permissible or weighted to be more or less likely. In other words, 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.

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 349-354 of the Discussion indicating the different biases inherent to the sampling methods used in the literature from which our data are derived.

Dietary information was compiled from the literature (and published datasets) and thus it contains data from a variety of sources, collected using a variety of methods in different geographic locations across different spatio-temporal scales. Other than Purcell (1981), none of the published data sources for siphonophore diets includes an assessment of prey availability. 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 358-367) 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.

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.

Yours sincerely,

Alejandro Damian-Serrano