Review of PhD thesis of Paolo Biella

PhD thesis of Paolo Biella is written in the modern style of theses consist of introductory review for subsequent chapters that came from published and for publication prepared manuscripts. Whole thesis is printed on 196 pages including short appendix and introductory review is 26 pages long. The body of thesis stands on five chapters that came from two published and three unpublished manuscripts. Content of the thesis is summarized on pages 188–190.

In the introductory part, various important topics of pollination biology are described in detail. Generally, I am satisfied by the introduction, maybe it is because I am not specialist in pollination and pollination networks. However, I am specialist in pollinators and their behavior and see the problematics from a different angle. Of course, interactions between pollinators and plants are important, however, I do not see the reason for a view of conservation of pollinator-plant interaction based on current data. We cannot extract important connections between pollinators and plants at satisfactory level and experience of field entomologist, could be more valuable for conservation topics. It is important to direct attention of researchers to physiological and neurological constraints of pollinators in flower/plant choice. Neurological constraints of specialization could closely be connected to ecological fitting of many pollinators to various plant species without prediction of their affinity and possibility of rewiring of the network without or with minimal loss of connections. It is important to note that abundance of species is very important factor for detection of effective rewiring. And decades of research would be needed for understanding thy system on even regional level in current pollinator decline era. Of course, author of the thesis have not omitted this topic, however I would appreciate more critical review of current research of pollination biology. My first question is, whether candidate agree, or disagree with my opinion and how he would implement ecological fitting theory to pollination ecology?

As in the above paragraph, I would rather see some information about features and ability of pollinator individuals for selection of flowers to be more connected to information on pollination ecology. Again, some biological constraints that are general, but also that are different between species, modulate behavior of all the network system. So, when the author used phenology term without details I would like to ask what modulate phenology and what form different phenology in different species. The term is rigid. Can also candidate decompose phenology to "subordinate components"? Could be theoretically possible to explain some features of pollination networks by these "subordinate components" and without phenology?

Chapter I is dealing with connection of conservation biology and plant-pollinator interactions (hopefully this is not oversimplification). I fell that there would be really needed to conserve some important network parts that are important for stability of the system and survival of (rare) species connected in the network. However, seeing *Bombus* (*Psithyrus*) *rupestris* as a member of local hub suggest something weird or even wrong. This species is social parasitic and highly polylectic. This parasitic species cannot be more common than the host and in principal should not be real dominant. From my experience, this species is abundant in male sex in the end of summer on flowers with easily accessible nectar. I see males of this species as a stump on nectar source, rather a species that is important for sta-

bility of highly speciose pollinator community, which should be conserved. My question is, whether the result of the analysis is biased by number of *B. rupestris* males as I suggested above? Could we discuss, what should be the important features of species included in hub, which is worth of conservation? Of course, I am aware of that there should be more different views of the community. The hubs are only the most abundant associations and in my point of view, they are not worth of conservation, simply because they are most common. And important connections, either plants, or pollinators that are associated with rare species are hidden. Sex of pollinator is also important for the significance of the species in the pollination web (at least for Hymenoptera). I would like to ask, how often pollination biologists consider different significance of sexes in bees? Why adult bee females should be more valuable for the systems and in the evolutionary-ecology view?

Chapter II is directed to ecology and prediction of potential distribution of endangered Alpine species of *Bombus*. I appreciate complexity of the research with a lot of details on the species acquired using standard and modern methods. Transfer of males among localities is suggested as a conservation action. However, the transfer could also be negative for multiple reasons. What are the reasons not to do it? How could you test, whether population need introduction of alien males?

Chapter III presents nice experiment simulating situation under population decline. I really like the design of this experiment. I also appreciate utilization of NGS methods for pollen determination. Though I understand practical reasons for use of *Bombus terrestris* for this research, it is however one of the worst model species for such a nice experiment. It is a species that is abundant and currently not under significant treat. Removing of half of workers from the colony may not cause real problem for that colony for more than a few days. I also think that this does not mimic real environmental stress that came to species under population decline. Of course, this experiment show, how the species behave during foraging under small and large colony, or may be higher and lower density of individuals. Weak results are not surprising. Could candidate present improvement of the experimental design to mimic environmental stress that happens to species treated by recent environmental changes?

Chapters IV and V show experiment mimicking local extinction or loss of dominant plant species and the effect to plant-pollinator interactions. I consider both these chapters very important research, because they show and describe problems of plant-pollinator interactions, at least for dominant species at both sides of network. It particularly answers my questions from the beginning of my review. I keep them laid as it was a reaction to introductory review.

Overall, I found thesis of Paolo Biella highly intellectually stimulating and it was a pleasure to read it. I am recommending the thesis for defense and I am certain that Paolo Biella deserves PhD title without any doubt.

In Prague, 10.1.2019

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External Examiner's Report on "A conservation perspective on the mechanisms that influence plant-pollinator interactions" by Paulo Biella

General comments

The thesis provides a useful contribution to the understanding of plant-pollinator interactions by i) examining responses to ecological network structure by manipulating plant and insect communities; ii) using advances in DNA metabarcoding to examine changes in diet breadth, insect visitation rates etc. and iii) considering the importance of temporal dynamics. There was also novelty in describing species interactions in high altitude habitats for the first time. Despite the title, the conservation perspectives were rather tenuous and it was not at all clear how the results of the thesis could be specifically used by land managers. For these approaches to be widely adopted, there needs to be better linkages between the biological relevance of the descriptors chosen and how these could be used beyond biomonitoring. For example, there was no evaluation of how or why network robustness might be useful for conservation.

There are a number of major omissions that should be addressed in the thesis. One is the issue of sampling completeness in network ecology is such a hot topic at the moment but this is barely touched open, yet could have dramatic impacts on network structure metrics in some of the chapters. Another is the issue of contamination when conducting metabarcoding studies and how this might impact upon subsequent interaction data. Finally, the choice of network descriptors for some chapters was never fully explained. What was the biological relevance of choosing them (e.g. connectance)? At the very least, these could be discussed in the context of limitations of the studies.

Overall the structure of the thesis is coherent and the rationale suitably explained at the start of the thesis. However, there are some referencing formatting errors (the author needs to be consistent throughout), spelling/grammatical

mistakes and the thesis could be improved by using a figure/table format attributable to each chapter (e.g. Figure 1.1 in chapter 1 and so on).

For the *viva voce* I would like to press the candidate on the following questions:

1. **What do measures such as truncated power-law degree distribution, nestedness and modularity tell us specifically about the biology of plant-pollinator networks?**
2. **How might the proposed definitions of specialisation/generalism be affected by the sampling completeness of a network?**
3. **How representative is the sudden loss of dominant plants and/or pollinating insects in nature. What are the implications for the studying of network robustness?**
4. **Define species 'importance' in a network context**
5. **How might the results of the network analyses best inform conservation practice?**

Below I provide specific comments and questions for each chapter, some of which I may select during the *viva voce.*

Introduction
This has a coherent structure and helps to provide the overall context of what might appear as otherwise disparate chapters. It sets the context well before presenting the work of each chapter. Key references are used appropriately.

Chapter 1
This chapter not only provides a novel study of species-interactions in the Northern Apennine, but indicates that it is possible to identify key species/hubs within ecological networks. It was surprising that only one or two sentences on the conservation applications were included, given the title of the thesis!

Chapter 2
After persuading the reader of the importance of studying communities and their interactions in previous chapters, it was surprising that this chapter considered a single-species approach (in particular the conservation recommendations) and the analyses seemed somewhat out of kilter with other chapters. Nevertheless, it served to consider the difficulties of studying rare species, how to include them in wider network analyses and how environmental change might affect their distribution.

Chapter 3
This chapter uses DNA metabarcoding to construct interaction networks from pollen pellets and then examines how reducing bee numbers affects interactions and network metrics. Combined, this is a novel approach and there will be a lot of interest in this work by network and pollination ecologists. This chapter could benefit from a greater appreciation of the potential impacts of contamination on outputs and subsequent network structure, particularly given there were no observed changes in dietary breadth. I would like to question the candidate on **whether this really is the case or an artefact of the lab protocols?** I would also

like to discuss the pros and cons of using ROC. It is unclear whether the candidate used count data of DNA reads as a proxy for pollen abundance? I would like a thorough explanation of how and why quantitative network descriptors were used. **What are the fitness consequences for plants in the study system?** How might this approach be rolled out to answer other questions in ecology and Evolution. **How might one use phylogenetically-structured networks in an adaptive network context?**

Chapter 4
This is one of few hypothesis-led chapters in the thesis. Manipulating plant-pollinator assemblages in the field is rare and an excellent way of examining more theoretical robustness measures. It was unclear what the purpose of the pilot study was for in the UK, particularly given the more rigorous study and interesting results from the Czech study?

Chapter 5
Linked to the comments in Chapter 4, this provides clear evidence of an effect on network structure when key plants are removed, but I would like clarification on the biological reasons **why the candidate chose these specific network metrics?** I will explore with the candidate what is meant by network rewiring, the extent in empirical species-interaction networks and what the consequences might be for both plant and animal populations, including how this might be measured.

Summary
I am satisfied with the overall conclusions of the thesis but would like to discuss the next steps and future directions of this research.

Yours sincerely

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