Review of PhD thesis of Daniel Souto-Vilarós

PhD thesis of Daniel Souto-Vilarós is modern type of theses comprising introductory review for subsequent chapters that came from two published and three manuscripts prepared for publication. Whole thesis is printed on 183 pages including short appendix and introductory review that covers 24 pages. Content of the thesis is summarized on pages 173–180.

Text of introduction is sufficiently well written, but separate chapters does not have a link. Though it is clear from presented publications, the introduction does not connect the publications clearly, but rather introduce each topic separately. The text is often hard to understand, especially when complicated information should be explained. I am not a native speaker and should not criticize the language, but I think that it is not as good as it could be. Anyway, the text is understandable and sufficiently well readable. I did not understand from your text how the figs are pollinated. If the female entrance of syconium is irreversible than figs should be pollinated within the same flower in monoecious fig species. Did I miss the information how the pollen is carried by agaonids among trees?

The name proposed as *Ceratosolen "kaironkensis"* is proposed as *nomen nudum*. If this is known fact, than it is undescribed species and the name *C. kaironkensis* should not be used at all, except mentioning the status. There is also no citation either in the introduction, or prepared publication (Chapter II). So, the question is, where the status appeared, and in which publication occurred the name? Both these information missing and should be presented.

Chapters I and II are dealing with genetic delimitation of fig tree species and their agaonid pollinators on elevation gradient. I appreciate use of various modern genetic markers for differentiation of populations and relatively robust data. Just the number of agaonid wasps is much lower than needed for population differentiation. The sampling should contain more than 20, but rather 50 unrelated individuals from each population. However, overall pattern should be generally similar, but probably not the same. Though the results suggest strong coevolution between agaonids and fig tree species. It is definitely not absolute and I expect that occasional effective cross pollination between fig species mediated by agaonid wasps occur as well as transitions between utilization of unrelated fig tree species. So, my question is what is known about non-specificity of pollinating agaonids and how frequent is it?

There are some minor formal problems:

Table 1 in Chapter II contains information about pollinator agaonid wasps; however, it is formally incorrectly used. *Kradibia wassae* is species and cannot contain information like sp. 1, sp. 2, because *K. wassae* will always be only a single species. Optimal solution is *Kradibia* sp. "wassae1", *Kradibia* sp. 1 aff. *wassae*, or similar.

Numbering of figures within Chapter II, but also in remaining parts of document is very erroneous. It does not reflect any logical system consistently and the numbering below figures does not correspond with text.

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Chapter III presents very interesting topic of chemical ecology regarding recognition of figs by agaonid wasps using a signal from fig volatile compounds. It has already been published in high quality impacted journal.

Chapter IV continues in searching for originators of fig tree species diversification. Now, in intracellular bacteria of the genus *Wolbachia* that are associated fig pollinating agaonid wasps. The text has rather moderate quality with numerous formal errors. The text is definitely not prepared for submission. Some descriptive parts are overly long, some parts hard to understand and some tests are missing. No evidence of cospeciation is presented and these tests should be added, though this might be obvious for you by bare eye. The theory, how *Wolbachia* is distributed within history of infected and recently diverged species with two different *Wolbachia* strains (pages 130-131), is quite interesting. If though generally and not only for fig wasps, it needs to be rethink in more general context. According to my knowledge parthenogenetic lineages keep *Wolbachia* much longer time than is typical for sexually reproduced species and this exception might not fit the general theory. I have to admit that I have not checked the literature and I might be not fully right. So, I would like to ask you for reaction to my contradiction, even I did not formulate a question here. It is actually important to add that proposed theory is otherwise clearly formulated and testable.

Built reasoning continues and refers to model at the Figure 3 in chapter IV (problem with logic of figure labelling continues). Firstly, it can be made easier to read and understand by graphical improvement and focusing to take home message that probably is population differentiation by different reproductive isolation mechanisms including these caused by *Wolbachia*. This is probably clear only to the authors and readers that read it five times. Also the text should be more focused and straightforward and then the figure will probably be unnecessary, because it is not a real model without the probability of each evolutionary pathway or speciation rate in each speciation category, or any other values. Generally this is nothing new here. Without data it is overly speculative, redundant, and should be deleted.

Chapter V study assemblages of pollinating and non-pollinating agaonid wasps along the elevational gradient. From the methods it is unclear to me how many female syconia were analysed. Only numbers for male syconia (Table 1) and female trees (Table 3) are presented. Methods and results are not well separated and need to be reworked. Information on the stage of syconia of *F. wassa* from 2,700 m is presented in the results and should be written in methods. It also does not say anything about missing number of analysed trees from this elevation (Table 3). Are male and female syconia mixed for the analyses? There is an information about male and female syconia in the methods, but no information about it in the results and figures. Though this chapter seems to be interesting, it is also quite unfinished and needs a lot of improvement before publication.

At the end, I would like to summarize that scientific and intellectual quality of research is high. However, the text is also unbalanced regarding quality of some chapters. Finally, I am recommending the thesis of Daniel Souto-Vilarós for defence.

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Report on the PhD thesis manuscript presented by Daniel Souto-Vilaros:

Why so specious? The role of pollinators and symbionts in plant population structure and speciation along elevational gradients.

The work presented in the thesis is impressive. It is the first assessment in the fig literature of genetic variation along altitudinal gradients. The first part is a general introduction to the questions addressed during the PhD. The questions are well presented and open on general questions.

The first chapter presents the first published investigation of *Ficus* and pollinating wasp spatial genetic structure and subdivision into distinct gene pools and species along an altitudinal gradient using next generation sequencing. Basically figs are structured into different subspecies or different species and fig pollinating wasps are structured into a series of species. Wasp have speciated more than the figs.

The second chapter presents receptive fig odour variation within and among species and variety, along the altitudinal gradient, and the wasp's behavioural response to these odours. There is actually very limited data available on such aspects in fig biology and, in that perspective, the work presented in the thesis is quite exceptional. Receptive fig odors differ between subspecies and species, but are more similar between closely related taxons than between or distantly related taxons, and wasps may show incomplete discrimination between closely related species.

The third chapter presents the prevalence and variation in *Wolbachia* strains hosted by different species or populations of pollinating wasps. The underlying hypothesis is that cytoplasmic incompatibilities induced by *Wolbachia* could facilitate speciation. Given the steepness of the ecological gradient associated with altitude, the results are once more unique as the variation is closely packed along the altitudinal gradient.

The fourth chapter deal with the altitudinal variation in non-pollinating fig wasp community composition. This data is also unique, but should probably be crosschecked by a fig wasp taxonomist (e.g. the presence of an *Apocrypta* in figs of *Ficus wassa*).

Given the quality and quantity of the work, including the data, the interpretations and the ideas presented in the manuscript, I can certify that the thesis can be accepted without hesitation.

The work opens up on a series of questions and fits into the very rapidly evolving knowledge on fig biology throughout the word. Much work has been published between 2017 and now, so that our whole thinking about the system is rapidly taking shape. This leads to the large number of questions I would have liked to ask Daniel Souto-Vilaros.

Some questions.

A difficult question is whether speciation is limiting *Ficus* (and pollinating wasp) species diversity or whether the ecological space available is limiting species diversity. What insights may continental hemi-epiphytic *Ficus* species floras provide on this question?

Is pollinator specialisation leading to speciation or is it a factor facilitating speciation driven by other forces?

Do data throughout the world suggest a general pattern of a single pollinator species throughout the range of a *Ficus* species or do they suggest generally multiple (parapatric and sometimes sympatric) species? How could this affect our vision of (co-)speciation?

You state: "Similarly, from the fig's perspective, selection should favour female figs to become more attractive to wasps in order to produce more seeds..." is this actually true? The quantity of energy invested in fig odor has been shown to be quite limited.

You state "Similarly, it is thought that volatile signatures are responsible for maintaining species specificity within the system since wasps entering the wrong tree would be detrimental to both parties". Is this actually true with the altitudinal variation in host species presence: can wasps be selected to avoid tree growing at a different altitude?

You state "Finally in order to sustain wasp populations, fig trees must produce fruits year-round". This is only true for monoecious figs. Do you have any indication of seasonal peaks in numbers of receptive female figs in your study species and of differences in peak receptivity period among sub-species or closely related species? This question is particularly pregnant for altitude forms.

Figure 5. Is attraction by odors of non-host fig surprising?

Is the statement "The inability of *Ceratosolen* sp. wasps to penetrate and successfully oviposit in F. microdictya figs represents the ultimate fitness cost from the wasp's perspective suggesting strong selection against making such a choice" really true or should it be modulated according to the frequency of the event?

You state "In general, selection should favour tolerance of *Wolbachia* by newly-forming species if the fitness costs of potential hybridization are high and species are in contact". I have difficulties defining a model in which this would actually work, could you define one?

I would like to finish by giving all my congratulations to this beautiful piece of work.

Professor, CNRS

Head of the Department InES at CEFE