Supervisor review of PhD thesis by Daniel Souto-Vilarós '*Why so specious? The role of pollinators and symbionts in plant population structure and speciation along elevational gradients*'.

Daniel was my first doctoral student as a primary supervisor. Before coming to the Czech Republic Daniel's research had a focus on plant-pollinator interactions. He had worked on mating systems in *Solanum* and pollination of *Passiflora* species by swordtailed hummingbirds in the genus *Ensifera*. He graduated to working on figs and fig wasps in the long hot summer of 2015, funded by Grant Agency of the Czech Republic (grant number 15□24571S). It was not long before Daniel left for the field. By October 2015 we were in Australia getting lost on the campus of University of Western Sydney, as Daniel honed the keen sense of navigation that he would need for his upcoming adventure in the forests of Papua New Guinea.

While in the field Daniel demonstrated an excellent sense of natural history, quickly grasping the basics of the fig-wasp system and building excellent working relationships among villagers and assistants. His research started to take shape and he led the long-term sampling of fig and fig wasp samples that was to form the basis of his PhD thesis. It is clear from his Introduction that Daniel has an excellent understanding of the wider evolutionary issues to which his thesis connects, namely the origin and maintenance of biodiversity through species interactions. It is not long before his driving passion is revealed: pollinator-mediated plant diversification. Daniel demonstrates a wide understanding of pollination in general, and connects to his study system through highly mature and well written prose, the quality of which remains exceptionally high throughout the thesis. A further consideration in insect speciation is their bacterially determined 'extended phenotype', and Daniel gives a clear and well-reasoned introduction to what can be a conceptually demanding subject.

In the following five data chapters Daniel connects these various themes together to form a logical and well-structured thesis. In **Chapter I** he presents a paper from early in his PhD, on which he worked as part of team of Czech and New Guinean researchers. This paper used shallow sequencing (microsatellites) to establish genetic differentiation in two species of fig along the Mt Wilhelm elevational gradient, Daniel made a big contribution to this paper through both laboratory and written work. It sets the scene for the rest of the thesis, where he presents work on which he took the lead.

Figs are only part of the story here, and in **Chapter II** Daniel draws on an impressive amount of work (in the laboratory, field and office) to present the first comparative study of gene flow in both figs and their corresponding pollinating wasps. His central hypothesis is one of limitation to geneflow along the elevational gradient, as wasps struggle to adapt to a steep gradient and fall into distinct elevational populations. This hypothesis is tested across a 'speciation continuum' representative of the New Guinean fig flora as a whole. Daniel builds on existing evidence that fig wasps speciate faster than their hosts, and argues that a breakdown of 'one-to-one' specificity may be a natural part of the speciation process in this system. This demonstrates an ability to think beyond the focal system to connect to a wider evolutionary framework. As is always the case, some of the really difficulties are hidden in the text. The details of field work, incredible fig DNAses and tiny quantities of wasp DNA are not presented, and while Daniel was supported by a strong team, it is unfair not to give him due credit for learning many advanced programming methods from scratch. It is a

testament to his perseverance that this manuscript is currently in revision for a major ecological journal.

Chapter III is, however, a published paper. Here Daniel led a team of researchers in a multidisciplinary effort to uncover some of the mechanisms behind reproductive isolation between species of fig. In a remarkable paper that Daniel combines several data sets seamlessly and gives a convincing discussion of how volatile organic carbons, trait matching and elevation all play a role in the diversification of species. Such insights were also a central part of a review paper not included in this thesis (Chemical cues and genetic divergence in insects on plants: conceptual cross pollination between mutualistic and antagonistic systems ST Segar, M Volf, M Sisol, NA Pardikes, **D Souto-Vilarós** (2018). *Current Opinion in Insect Science*).

As the candidate explores the mechanisms responsible for diversification in this system he goes beyond interacting mutualists to consider third parties, *Wolbachia* in **Chapter IV** and non-pollinating fig wasps in **Chapter V**). This inclusion of third parties is a recognition that ecological networks are evolving entities. Seemingly pair-wise interactions are often not so. Integration of advanced analytical techniques and a solid ecological data set allows the candidate to build on previous chapters to offer a mechanism by which bacteria may facilitate speciation through cytoplasmic incompatibility. While the natural history of figs and fig wasps does not lend itself to manipulative experiments, the obvious follow up here, the data do support a pattern of parapatric speciation mediated by an endosymbiont, I hope that this manuscript sees publication in the near future.

Finally, the candidate establishes a solid set of expectations for how non-pollinating parasites are distributed across fig ecotypes and species in **Chapter V**. Non-pollinating wasps impose fitness costs on pollinators, but also provide a tractable system for studying community betadiversity and niche partitioning. Here Daniel explores both fitness costs and biotic trends through the counting of fig contents and morphological measurements. He provides support for some general trends in ecology, such as the mid-domain peak in species richness, but also shows that seed set can decline at higher elevations. This work is an important test of trends in insect abundance, but also provides the baseline data needed for further exploration of network structure and mutualism stability across this elevational gradient.

In summary, Daniel's thesis represents a large and well conducted body of research that makes a considerable contribution to the field of insect and plant speciation. He has demonstrated an ability to formulate, test and evaluate hypotheses using the scientific method and is a truly independent student. Indeed, as should be the case I no longer consider him a student at all. In my opinion, Daniel Souto-Vilarós has demonstrated in this work that he is clearly ready to defend this thesis, and be awarded his PhD.

Newport, Shropshire, UK, 10th of June 2019.