Review of Chris Dahl's Doctoral Thesis '**A cross-continental comparison of fruit-and seed-feeding insects in the rainforests of Panama, Thailand and Papua New Guinea**'

Reviewer: Jiri Dolezal, Czech Academy of Sciences

The thesis of Mr Chris Dahl consists of four articles, three of which have been published in international, peer-reviewed Journals. Chris Dahl is the first author of two of them, the first published in Biotropica, and the unpublished manuscript. He substantially contributed to the second and fourth article published in Journal of Biogeography and Entomological Science. The four articles are preceded by a general introduction and followed by a general discussion. Overall, the published papers are of high quality and the manuscript have the potential to eventually be published in international, peer-reviewed Journals. The thesis clearly demonstrates Chris Dahl's talents and abilities in field data collection, laboratory work, data analyses and paper writing, all needed to independently conduct successful ecological research.

In the **General Introduction**, Chris gives an overview of his thesis and its different parts putting them into the relevant context of history and distribution of tropical forests, plant and insect diversity, plant fruit syndromes and dispersal modes and insect seed predation. He also concisely and competently summarizes the important differences between an experimental exclusion approach and individual-based species-specific quantitative approach for assessing the importance of seed predators for forest dynamics.

In the last part of General Introduction, we learn that the thesis deals with an important and interesting topic of mapping and classifying differences in seed and fruit trait syndromes among thousands of tropical woody species and insects feeding on them in extremely species rich tropical environments on three continents using extensive collection of plant, insect and vegetation plot data, with the general aim to explore how predispersal seed and fruit predation by attacking insects might control plant abundances and maintain high tree diversity in tropical forests through density dependent feedback mechanisms and how the relationships between insect predation and fruit diversity is modulated by abiotic filters such as water availability and seasonality, or biotic factors related to taxonomic and phylogenetic diversity of host plant species.

Much appreciated is an attempt to see these questions in whole complexity by conducting a cross-continental comparison of fruit- and seed-syndromes and their feeding insects in the rainforests of Panama, Thailand and Papua New Guinea that differ in history of plant-insect coevolution and environmental factors driving them. It is also important to mention that the authors employed a large body of different methods for data collection and evaluation including DNA extractions, multivariate ordination, phylogenetic analyses etc.

In the **Paper 1** (Chapter 1), Chris and co-authors report on the results from three Forest Global Observatory Plots in Neotropical (BCI, Barro Colorado Island in Panama), Oriental (KHC, Khao Chong, Thailand) and Australian (WANang, Papua New Guinea) regions and show a new classification of tropical forest woody species (mostly trees) into eight fruit trait syndromes based on fruit morphology, particularly fruit color and fleshiness, mesocarp thickness and the number of seeds. While fruit syndromes and colors were evolutionary flexible traits at higher taxonomic levels capable of influencing seed predators and dispersers assemblages, differences in floral composition and plant phylogeny among the three regions explained largest proportion in trait variation, with the Panamanian forest having a distinct distribution of syndromes due to more pronounced dry period and prevalence of non-

fleshy (dry) fruits as compared to humid forests in Thailand and Papua New Guinea. The prevalence of dry fruits in BCI seems to drive the overall differences between the three compared tropical forest sites and we can learn from the discussion and supplementary material that dry fruits are mostly associated with drought tolerant legume trees, and shrubs and lianas that prevail in the Panama BCI plot. Hence, how much of the total variation in fruit syndromes among and within sites could be explained by differences in life forms, and how much would life form spectra explained the total variation after accounting for evolutionary inertia, or what would be the net (marginal) effect after accounting for all potentially confounding factors, i.e. after removing differences in phylogeny, water availability and seasonality among sites?

In the **Paper 2** (Chapter 2), Chris and co-authors compare the same three ForestGEO sites and their fruit and seed syndromes as in the first paper to analyze possible convergence in faunal composition, species richness and guild structure among > 1600 insect species feeding on fruits of almost 1200 tropical woody species at the intercontinental scale. Most insect species reared from fruit samples were pulp eaters, followed by scavengers and seed eaters. The drier and more seasonal Panama BCI forest had more (ca. 44%) seed eaters than the wetter Papua or Thailand forest sites (ca. 13%) where pulp eaters prevailed due to dominance of flashy fruits among tree species. The BCI forest had the lowest plant species richness, with a high proportion of lianas and shrubs and their dry fruits, the highest insect species richness reared from fruit samples, but the lowest seed and plant species predation attacks as compared to the other two wetter sites. Much more variation in insect faunal composition was explained by host phylogeny than host life form and fruit traits. The fruit syndromes (dry versus flashy) explained most variation among traits, followed by seed length and the number of seeds. Because of large differences in most community parameter tested (inset species richness, guild structure, attack rates), Chris and co-authors concluded that there is low overall convergence in fruit- and seed-feeding insect assemblages at the intercontinental scale. While I liked the methodological approach of this chapter and its concise and informative introduction, I found the presentation of the results a bit difficult to follow but this has also much to do with the complex nature of the different methods. I am wondering if the applied variance partitioning tests were designed to assess the net (marginal) effect of each plant variables (i.e. life form and fruit traits) after accounting for host phylogeny?

In **Manuscript 3** (Chapter 3), Chris and co-authors focus on the effects of rainfall seasonality on the abundance of Coleoptera and Lepidoptera insect seed predators and their plant-frugivore interaction networks at the three rainforest plots introduced in the chapters 1 and 2. In particular, they explored if diversity and host specificity in plant-frugivore food webs vary between dry and wet seasons. The study is built on massive amount of fruit and insect data sampled over 3-4 year period from ca 11,000 individual trees belonging to 349 tree species, yielding >18,000 frugivore insect specimens. This is probably the largest study of this sort ever done in the tropics! Both Coleoptera and Lepidoptera frugivore insect diversities were higher in the drier and more seasonal Panama tropical forest with the prevalence of dry fruits compared to the wet forests of Thailand and Papua New Guinea with prevalence of flashy fruits. Concerning seasonal changes in seed predator abundance, only in the Thailand forest had both species groups increased abundance with a greater amount of rainfall during the wet period. Other conclusion about dry season being characterized by lower frugivore insect diversity and lower abundance of attacked fruits in all three tropical regions (see Abstract), is not well supported by the data and I did not find a figure supporting this statement (even though Table 1 shows significant (P=0.043) seasonal differences in richness of insect families). Rather than this, we learn from the discussion that

there is a lack of strong seasonality in frugivore insect assemblages, which is explained by medium to low rainfall seasonality of the studied tropical regions. Authors also speculate about asynchronous fruiting pattern among abundant coexisting tree species securing diversity of food resources all year round as a possible reason for low seasonal differentiation in seed- and fruit-feeding assemblies. This result is supported by insignificant differences in most interaction web parameters between the dry and wet seasons. While I am convinced about the results and interpretation, I think there is still open space for further analyses that would better link seasonal (a)synchrony in fruiting pattern with seasonal variation in frugivorous insect composition using e.g. co-correspondence analysis, together with host phylogeny effects. Quantifying dependency of insect assemblages on fruit syndrome assemblies after taking variation in host plant phylogeny into account might bring some additional needed insights into structuring forces. There are some inconsistencies that should be clarified during the defense (see pg 111). Despite the lack of significant differences in vulnerability between seasons, taxa and sites (Table 1) it is claimed that Lepidoptera networks had lower vulnerability in dry than wet season due to absence of rare interactions during dry season as related to lower fruit and insect abundances (compare Table 1 with Figure 5). There are also some typos that should be fixed before submitting the manuscript to international, per-reviewed journal.

In the published **Paper 4** (Chapter 4), Chris and co-authors report on insect assemblages attacking seeds and fruits in the Khao Chong (KHC) forest in southern Thailand. As the previous three studies, this one is also built on robust insect and fruit data collected from 357 liana and tree species during three years to test the predicted relationship between host plant abundance and predators attacks proposed by the Janzen-Connell hypothesis. Unlike prediction, insect feeding on seeds and fruits had a limited impact on host abundance in studied forest. Insect densities were low, as was the number of confirmed insect pests, and heavily attacked tree species (mostly from Annonaceae, Myristicacea, Sapindaceae and Fabaceae families) were not notably less abundant than other tree species. Authors conclude that insects feeding on seeds and fruits have little effect on observed levels of host abundance in this forest.

In the Summery, the topics of the previous chapters are concisely summarized.

According to my experience and opinion the thesis of Chris Dahl fulfils all formal requirements (respecting length, structure, style, publications, content and quality) of a PhD dissertation. Chris clearly demonstrated his ability to carry out an independent scientific research, and present its results to a international scientific community, and also, to draw from his results conclusions that can have direct management consequences to protect tropical forest and its diversity. I would also like to stress that the thesis reflects only one part of Chris Dahl's publication activity. He has coauthored 15 other scientific publications, the four of which as the first author!. Moreover, several poster and oral presentations were given by the applicant at international scientific conferences.

In conclusion, I have no hesitation in recommending this work for the award of the PhD qualification. The thesis provides a body of work that is a good example of how much field and lab work in ecology can contribute to the field when it is well thought out, conducted, analyzed and interpreted. The novel results will not only serve as valuable tests of existing hypotheses but should also go on to inspire new work in ecology both experimental and theoretical.

Yours sincerely,

Ceske Budejovice, 12.12.2019

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Reviewer's statement / PhD thesis of Chris Dahl

To the School of Doctoral Studies in Biological Sciences, Faculty of Science, University of South Bohemia in Ceské Budejovice

As the Thesis Reviewer appointed by the Faculty, I wish to submit the following evaluation on the thesis work submitted by PhD Candidate Chris Dahl, *A cross-continental comparison of fruit- and seed-feeding insects in the rainforests of Panama, Thailand and Papua New Guinea*.

The thesis consists of four chapters, along with a joint introduction and a summary part. All chapters represent joint work with multiple coauthors. The thesis includes a summary of the Candidate's independent contribution, and written confirmation by the supervisors of the Candidate's major contribution. Hence, there is no doubt that the thesis and the Candidate's contribution thereto meets the international requirements for a PhD dissertation.

Three of the chapters have already been published in good international journals. One of the chapters (III) is yet to be published, but is included in the thesis as an advanced manuscript draft. While it is evident that the published manuscripts (I, II and IV) have been honed to a more advanced level than the previously-unpublished parts (i.e. Introduction, Chapter III and Summary), the full thesis is of high international quality, and it is probably only a matter of time before the last manuscript is accepted for publication.

In terms of its scope, the thesis is one of the most impressive initiatives in modern community ecology that I have come across. Where studies of fruit-

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feeding insect communities to date have focused on single localities, this thesis takes on the monumental task of a cross-site and cross-continental comparisons. This is the first attempt at establishing general patterns across the tropics by comparable methods. It builds on tons of fruits sampled, thousands of plant species and next to 100,000 insects. As such, it makes an unparalleled contribution to our understanding of tropical rainforests.

The work of the Candidate not only focuses on an important topic, but advances the state in its subject field through a quantum leap. The thesis has deep roots in the past 50 years of ecology, and in the proposal that hyperdiverse communities may be structured by the extent to which their component species share or do not share natural enemies. Where past work has mainly focused on herbivores, this thesis applies the concepts to the type of plant-feeding where they really matter, i.e. to the direct consumption of plant embryos in fruits. In doing so, the dissertation brings together commendable natural history and species-level detail with advanced quantitative analysis and conceptual synthesis. Thus, the work is impressive in terms of both its scope and its novelty. The methods used have been adequately chosen, well implemented and are clearly presented. I am deeply impressed by the outcome, and congratulate the Candidate on his fine work.

As the Reviewer is asked to define a few questions for which the Candidate needs some time to prepare, I would ask the Candidate to prepare to respond to the following queries:

(1) In Chapter I, the Candidate proposes a series of fruit syndromes, derived from plausible assumptions regarding what fruit traits should affects insects how. Yet, in later chapters (e.g. Chapter II), the Candidate finds that these syndromes are rather weak predictors of insect-fruit interactions in general, and of attack rates in particular. If this is the case, then might one not question the ecological relevance of the syndromes defined? (Please argue in favor of the syndromes defined.)
(2) As pointed out by the Candidate, the traits of fruits will evolve in the crossfire between several selections pressures. On the one hand, there is the need to be dispersed, on the other, there is the need to avoid fruit predation etc. If the Candidate was tasked with designing an optimal fruit (i.e. a fruit conferring maximal fitness to the plant species) for each of his three rain forests, then what would the respective fruits look like and why?
(3) In the quest for patterns of similarity across communities built from large and diverse sets of species, there is always the concern that similarity may result not from convergence as such, but from phylogenetic constraints causing related species to share both traits and ecological associations. Throughout his thesis, the Candidate uses an elaborate set of approaches to

test for phylogenetic imprints. Could the candidate walk the audience through the *rationale* (not details) of the approaches taken, and the bottom line reached in terms of where phylogeny matters and how.

(4) The Candidate states that his work, as now completed, offers relevant guidance to the methods and sample sizes needed to document plant–frugivore networks in complex ecosystems. Could he summarize this guidance in a nutshell, for other researchers to follow?

(5) The Candidate makes repeated reference to the Janzen-Connell hypothesis, and ends his summary by saying that his work has importance for the testing of this hypothesis as a factor maintaining high plant diversity in tropical forests. Could he succinctly summarize his main findings from this very perspective, and whether they support or refute the hypothesis? If the current answer is inconclusive, then what should we do next to finally answer this question?

Uppsala, December 5, 2019

Tomas Roslin, Professor of Insect Ecology