Reviewer's statement on the PhD thesis by Philip T. Butterill: „Tri-trophic interactions of host plants, gall-forming insects and their parasitoids in the tropical rainforests of Papua New Guinea" submitted for defense at the University in South Bohemia, České Budějovice on 13 May 2015

Plant galls and their insect inducers represent a relatively frequent object of scientific studies addressing general evolutionary and ecological questions such as coevolution, adaptive radiation, as well as biochemical and histological processes associated with the formation of galls at the cell level. Attractive topics for insect gall research in recent decades included also revealing global biodiversity patterns for gall-makers and their possible explanations: how many species of gall-inducing organisms (insects) are there worldwide? Are the gall-inducing species distributed evenly or do they concentrate in some biomes? Some interesting hypotheses have been proposed such as the „hypothesis of the harsh environment" by Fernandes & Price (1988) predicting more species in dry habitats with sclerophyllous vegetation especially in the subtropics than in more humid habitats such as tropical rainforests. However, their testing has been difficult because of a paucity of methodically well-founded field studies, particularly from tropical forests.

The PhD thesis by Philip T. Butterill deals predominantly with the community ecology of gall-forming insects in tropical rainforests, focusing especially on a detailed description of the species richness and host specifity of both the gall-inducers and their parasitoids in two regions of Papua New Guinea. The thesis also analyses various factors, such as plant traits, phylogeny and succession, that could drive or explain the observed patterns.

The thesis benefited from the unique infrastructure of the New Guinea Binatang Research Center in the lowland rainforest region in the Madang province and good contacts with local people also in another region of Papua, the Finisterre Mountains. Based on the information given in the thesis, Philip Butterill led the fieldwork in both regions which was done in two periods, each lasting more than 8 months of stays in rainforests – besides personal physical resistance, he must have proven excellent organization skills in leading a team of local parataxonomists and assistants who helped him to collect a prolific material of galls from a wide array of identified tree species. Next stage of the PhD project obviously included rearing of adult insects (gall-formers and their parasitoids) from galls and their sorting and identification to higher systematic groups and morphospecies which also must have been quite demanding given the amount of material collected and the fact that most of this work probably had to be done on the site. The sampling was designed in a slightly different way between the lowland and mountain studies but in both cases, the design was logical and suitable for testing the hypotheses which were clearly formulated for each of the individual studies within the PhD project. Especially the montane rainforest study is remarkable and unique for its sampling method – felling of defined, replicated areas of primary and secondary forests which allowed a quantitative census of galls on all present trees both in understorey and canopy, the latter being usually inaccessible for sampling of galls (except for a very few studies which used e.g. cranes for sampling in the canopy) but suspected to be highly species-rich and interesting.

Again based on the information on the authors‘ contributions to individual studies included in the thesis, Philip Butterill himself analyzed all the community data using an array of statistical methods, most of which are far from trivial, which also proves he is well-acquainted with and can practically use many specialized software packages, especially in the R environment. As far as I can judge, he correctly used species‘ accumulation curves and estimator functions to estimate the real species richness on the collecting sites and the efficiency of his sampling, used uni- and multivariate statistics including e.g. regression trees, parametric and non-parametric tests, and corrected his statistical tests for phylogeny of the host plants wherever this was relevant. In addition, he obviously also mastered recently developed statistical tools for analyses of quantitative food webs.

Main scientific results of the thesis are concentrated in three core papers on the galling insect community ecology. One of them was already published in the Ecological Entomology journal and surely had to pass a tough peer-review there. Two other papers are presented as manuscripts. Their quality is very high – both manuscripts are clearly and concisely written and contain deep analyses of unique data leading to many interesting and novel facts. In my eyes, both manuscripts are well-prepared for submission to some reknowned scientific journal (have the authors already done this in between?) and I have no doubts they have very good chances to be accepted very soon or with some minor revisions (any news about their current status?).

Being myself an insect taxonomist, I acknowledge the effort Philip Butterill obviously made to identify the taxa collected and that he entered into contact with specialists for the individual insect groups to formally describe at least some of the taxa. Papua New Guinea is one of the least explored parts of the world for gall-inducing insects and the great majority of the material collected during the PhD project very probably belong to undescribed or at least imperfectly known taxa. Not using the huge material of insects collected within such a well-run project which would be probably logistically very difficult for anybody to repeat in near future and with many available supplementary data on the host plants, morphology of galls, hosts for parasitoids etc., also for taxonomic studies would really be a pitty. So I consider as great that the thesis also includes another two published papers, one in the Austral Entomology journal, the other one in Zootaxa, which include formal descriptions of two new species of cecidomyid gall-midges and one of a cynipid gall-wasp, to which Philip Butterill contributed as a co-author. This is only a very small fraction from the number of the species collected but surely a good start. Probably some additional taxonomic papers are being prepared for publication – I am myself aware of one additional manuscript on gall-making psyllids submitted to the Journal of Natural History which has not been included in the thesis. Is there some more taxonomic work on the way perhaps also in other groups?

Altogether, the thesis is thus composed of five individual papers, three published in international peer-reviewed scientific journals and two manuscripts. For the three core, community ecology papers (one published, two manuscripts), Philip Butterill is the first and corresponding author. The papers are introduced in the thesis by a common introductory text setting up the thesis into a wider context of the current ecological research and defining the aims and objectives of the thesis. Main results of the whole thesis are summarized on four pages at the end. Both the introduction and the summary are relatively short but concisely and clearly written and frame the individual papers in a way that is usually expected for a PhD thesis.

I have read the whole thesis with interest and failed to find any major methodological imperfections or incorrect opinions in interpretation of results. The references cited in the PhD thesis as well as in the individual papers are quite exhaustive and up-to-date with perhaps no very important publications omitted which would be relevant to the topic.

To conclude: The thesis undoubtedly brings original knowledge on ecology and zoology which has already been partly published in international professional journals or probably will be so in a near future. From the formal point of view, the thesis fulfills all requirements for awarding a doctoral degree in the study programme of Zoology at the University of South Bohemia. I fully recommend the thesis to be defended.

Brno, 6 May 2015

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Questions:

1. Galls can be of very different forms and complexity from simple deformations or swellings of parts of the host plant, through open pit galls to large, closed, structurally complex galls. What was your working definition of a gall in your studies? (what did you consider as a gall and what not?).
2. In Chapter 2, you mention the gall apparency as an important factor influencing the species richness of the parasitoids. Obviously, you formally distinguished some basic morphological categories of galls in your studies as you give three categories of gall physical apparency (low, medium and high) in Chapter 2: Figure 5, but I failed to find any definition of these categories in your thesis. Could you specify your criteria, please? Was there some pattern in distribution of different morphological types of galls depending on the taxonomy of the gall-inducing insects or on the taxonomy of the host-plants? E.g. some insect taxonomic group causing exclusively or predominantly only some kind of gall form, or some types of galls occurring only/missing in some group of plants?
3. In Chapter 1, Table S3 you give numbers of morphospecies of gall-formers from individual higher systematic groups of insects for the lowland region of Papua. A similar table is not provided for Chapter 3 dealing with the montane rainforest. Was the taxonomic composition of the insect gall-forming community similar in the mountains to the lowland, i.e. also dominanted by cecidomyiid gall-midges as seems to be usual in tropical latitudes? Did you observed any notable general differences between the lowland and montane galling community? Was there some overlap in tree species studied/gall morphotypes/insect morphospecies recorded between the two regions?
4. In Chapter 2, p. 52, you write your parasitoid network probably included also facultative or obligate hyperparasitoids that you did not distinguish for reasons that are easy to understand. But do you have an idea what could be approximate proportions of hyperparasitoid to parasitoid species numbers based on some better-studied gall systems, e.g. from the Palaearctic region? Do you expect a same narrow host specifity for hyperparasitoids as for primary parasitoids?
5. Your estimate of the global species richness of gall-forming insects between 460,000 and 1,000,000 species appears as very high compared to previous estimates in literature as well as relatively in proportion to the total number of arthropod species worldwide recently estimated in Hamilton et al. and Basset et al. (2010, 2011, 2012) as 6.1 million species. This would mean that between as much as 7.5 and 16% of arthropods in the world are gall-makers. But you yourself characterize the tropical rainforest gall-forming insect guild as relatively species poor (e.g. in the title of your Ecological Entomology paper, Chapter 1). Could you comment on this controversy and give some more details on your global estimate, please?

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STATEMENT

May 9, 2015

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Statement on the PhD thesis of M.Sc. Philip T. Butterill

The PhD thesis work of Philip T. Butterill ambitiously targets very important and timely research questions: how diverse are herbivorous insects and their associated parasitoids in tropical rainforests, what influences herbivore diversity on different plant taxa and in different successional stages and vertical strata of forests, and how are tropical herbivore–parasitoid networks structured? The thesis focuses on gall-inducing insects, which are highly suited for these kinds of analyses in poorly known parts of the world: (1) species can be sampled in masses by collecting plant modules, (2) although the species themselves may be undescribed, they can be sorted to operational morphospecies based on distinct gall morphologies and host-plant identity, and (3) gallers and their parasitoids are often relatively easy to rear to the adult stage. As a consequence, thorough statistical analyses are in many cases possible, which allows for interesting comparisons to previous surveys of gall-inducing insects and their enemies in other parts of the world, or to studies on other insect herbivore guilds in Papua New Guinea.

The thesis consists of five research articles and an introductory chapter summarizing the results of the separate papers. Articles 1 and 3 are hypothesis-driven analyses of factors determining the diversity of galling insects. In article 3, Butterill and coauthors use the combined data of these two papers to estimate the global diversity of gall-inducing insects, and argue that current estimates are too low and may reflect lack of study in tropical areas. Article 2 is most likely the most comprehensive quantitative analysis of a tropical tritrophic food web done to date, and demonstrates a high level of specialization in a diverse galler–parasitoid network. The last two articles (4 and 5) contain descriptions of three new gall-inducing species that were found during sampling for the studies on the diversity of gall inducers in lowland and montane rainforests. Although basic taxonomy is nowadays unfortunately often seen as slightly unfashionable, these descriptive papers constitute a logical part of the PhD thesis, and add to our knowledge of tropical insect diversity.

Butterill is first author in three articles, and second author in two. According to the description of author contributions, Butterill has had a central role in planning, data collection and analysis, and writing of the

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articles. Of the articles, three have been published in established peer-reviewed journals, and two are still at the manuscript stage. However, like the already-published papers, the manuscripts are of a very high quality, and undoubtedly can also be published in international peer-reviewed journals.

The overall quality of the PhD thesis project and the resultant thesis are high. The sampling strategies employed are extensive, and have required the assistance of many field assistants. Statistical analyses are thorough and appropriate, and Butterill and coauthors have made a laudable effort to control for many potentially confounding factors, such as phylogenetic non-independence, sampling effort, and effects of rarity and abundance.

Based on the above, I conclude that the work of Philip Butterill clearly fulfills the requirements of a PhD thesis, and I recommend that the thesis can be defended publicly. In the Appendix, I have listed seven main questions that could be discussed during the defence, along with smaller questions of a more technical nature.

Joensuu, Finland, May 9, 2015

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Appendix

Main questions for the PhD thesis defence of Philip T. Butterill:

1. In articles 1–3, morphologically similar galls collected from single plant species are assumed to represent a single galler species. This assumption of gall morphotype × plant species as a proxy for (taxonomic) galler species is realistic and is used in many studies, especially in the case of poorly known but extremely species-rich galler–plant systems in tropical regions. However, explain the potential caveats applying to this assumption, and how potential deviations from it could influence your results and inferences. In Article 1, gallers were also DNA barcoded to check or confirm their species status, so describe the differences that were found, and their potential effect on the results, especially on estimates of species richness.

2. Explain *why* and *how* you have used information on the phylogeny of the sampled host plants in the statistical analyses of Articles 1 and 2 (and 3).

3. Article 1 investigates the effects of different plant traits on galler species richness, and Article 2 (among other things) tests the effects of different gall traits on the number of associated parasitoid species. Discuss these analyses in light of observations that (1) herbivore species richness (and possibly also parasitoid diversity) often depends on the abundance of their hosts, and (2) plant and galler traits tend to be phylogenetically conserved.

4. Article 2 presents a species-rich galler-parasitoid food web with very complex interactions. Which factors could explain parasitoid sharing among galler species in the focal food web (Figs. 1 and 4)? Do specific parasitoid families or genera exhibit preferences for specific galler species or taxa? More generally, how do specialist *vs.* generalist parasitoids differ in their (1) ecological and (2) evolutionary effects?

5. Inferences of species richness and food-web structure are always complicated by differences in sampling intensity, especially in tropical environments where many rare/singleton species tend to be present. Articles 1–3 use sophisticated statistical methods to investigate the effects of sample size (and host abundance) on estimates of species richness and numbers of gallers per host-plant species (Articles 1 and 3), and on indices of food-web structure and specialization in galler–parasitoid associations (Article 2). Explain the main conclusions of the 'robustness' analyses used in these three articles.

6. Especially in species-rich multitrophic study systems, all plant, herbivore, and parasitoid species and their interactions cannot possibly be surveyed at the same time. What are the potential consequences of the unobserved parts of 'total' food webs (other plants, herbivore species, herbivore guilds, etc.) for different indices describing food-web structure (*e.g.*, Table 1 in Article 2)?

7. Articles 4 and 5 describe three new galler species that were found during the community sampling for Articles 1–3. How many additional galler and parasitoid species found and reared during these studies await description, or were the other species known from previous surveys?