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Assessment of the Ph.D. Thesis of RNDr. František X. J. Sladeček

The thesis begins with a general introduction of 26 pages (including references) that provides the framework of the work. This one is based on four scientific papers, two published in Entomological Science (Chapter 1) and PlosOne (chapter 2), and two in the form of manuscripts (chapters 3 and 4).

Overall, on the positive side, the thesis shows good use and integration of relevant and up-to-date literature, some interesting ideas and research questions, an aptitude for field-based experimental work and is well written with minimal editorial errors. The candidate clearly shows a good understanding of the ecology of dung beetles and flies and the kind of new information needed to fill gaps in our knowledge, and shows good potential.

One of the strengths of the thesis is the study of the mechanisms of species coexistence in ephemeral habitats, how the species form transitory communities by minimizing competition. In Chapters 1 and 2, emphasis focuses on the quantitative ecology of dung-attracted flies and the relationships between dung beetles and flies, including adults and larvae. The study on the succession of dung-inhabiting predators (developed in Chapter 3) is original. Indeed, few works in literature are devoted to this aspect. Finally, another interesting point developed in this thesis (Chapter 4) is the study of the relationships between the succession of volatile compounds and the dynamics of the dung beetles and flies that are attracted.

This results make it possible to consider other hypotheses that could be explored in the future in order to define more finely all the mechanisms of coexistence of the species. These future developments will be discussed at the time of the defense of the thesis.

Detailed comments on the thesis chapters

Introduction

The candidate explains what are ephemeral habitats, and why they can provide a logistically very sustainable models for ecological studies. He explains why he will focus on the ecology of beetles and dipteran that inhabit such ephemeral habitats (particularly dung and carrion).

Some points raised in the introduction call for questions and more explanations.

Page 5, first paragraph. Why did you classify beetles and dipteran species into three functional groups: saprophages (i.e. coprophages and necrophages), omnivores and predator, amalgamating the diet of adults with that of larvae? It seems too arbitrary. The boundaries are not so clear, though in the text some comments were nuanced. In particular, why to make a particular category for omnivores, based on the diet of adults and larvae that may be different? Too well defined dichotomies only reflect a part of the reality as, for the same species, adults and their larvae can live in very different environments, with distinct life traits, and opposite diets, as if they were different species. For example, in Lepidoptera, most caterpillars are phytophagous (but some have very varied diets, including predation), while the adults (butterflies) are mainly nectarivorous. Another example, in the Syrphid dipteran, one can find larvae that according to the species can be predators or saprophages, with the adults that feed on flowers. Therefore, in invertebrates, and particularly in insects, adult and larval stages often need to be considered separately according to their diet.

In general, the dung beetles exploit mammal droppings and have developed multiple adaptations to use dung. This mode of feeding is not a primitive character, but it results from an ultra-specialization. If one examine the trophic regimes of all Scarabaeoidea, we can conclude that phytophagy and saprophagy are of a much older type than coprophagy, even if coprophagy is a very old process since fossil nests of dung beetles (coprolites) were found in late Cretaceous sediments. The trophic boundaries between families are not strictly marked and some genera or species provide a natural transition to other dietary patterns, while retaining most of the time ethological character of their group. Saprophagy or necrophagy observed in some highly evolved species should be considered as occupying an empty ecological niche or as an adaptation to original or difficult environment (where the droppings of large mammals are rare, or where competition with flies is weak). Thus, the Australian species *Onthophagus dunningi* is mycetophagous but its nesting mode is comparable to that of other *Onthophagus* species. Likewise, many Aphodius and their larvae prefer manure to excrement, or even soils rich in organic matter. Moreover, the larvae of some Aphodiinae feed on the living roots of plants. One can also mention the case of some Canthonina of the *Deltochilum* genus, which drifted towards necrophagy, whereas the close species remained coprophagous. Furthermore, *Lethrus* species (Geotrupidae) chew the fresh leaves of plants to make a paste with the consistency of dung, which serves as food for their larvae. The Australian *Cephalodesmius* species do the same with fresh plant debris fallen to the ground.

Page 11, last paragraph. Can you better explain the following sentence: "only ball-forming relocators have some affinity for higher temperature": why would this involve only rollers? The references you cited (see Krell et al) relate to African species. In temperate regions where rollers are in small numbers, are the tunnelers in the same case?

Other comments for introduction

Page 4, line 3: Ridsdill-Smith (and not Ridsdillsmith)

Page 22: MacArthur (and not Macarthur)

Chapter 1

From the results presented in this chapter, several questions arise. Artificial dung pats were formed from dung of permanently stalled cows. Do you know if cows had been treated previously (recently) with some veterinary medical products (e.g. deworming drugs)?

In chapter 2, you assumed that the fly communities would have been the same if you had used dung from animals that freely consumed fresh grass on pasture. It depends in part on the diet of stalled cows. Do you think that the fly communities on the surface of the pats are the exact reflection of what happens inside the dung pat? Did you consider the future of the offspring of these different species? That is to say, in the next generation, do you think the same spectrum of species could be observed (similar percentages of the species in relation to each other), when using for example emergence cages?

Chapter 2

Page 54, first paragraph. You mainly considered temporal segregation to explain the coexistence of flies and dung beetles. You also mentioned that the habitat could play a role of filter, but without quite exploring this very interesting way, which explains also a part of the coexistence of flies and dung beetles, especially in mosaic environments. Consider, for example, a very open pasture, with few isolated and scattered trees inside. If dung pats were disposed simultaneously in the open parts of the pasture, and in the shadow projected on the ground by each tree, two distinct communities of insects can be observed a few meters apart, one dominated by the dung beetles (open pasture), and the other by Dipteran (shaded part).

Did you notice similar differences in the micro-distribution of flies and dung beetles in the sites where you worked?

Chapter 3

This part focuses on the coexistence of dung-inhibiting predators.

Page 68, paragraph 1, line 7. The candidate wrote: "The trophic links are based on the size ratio of predator to prey, assuming that predator eats smaller prey/predator". I do not quite agree because this is not always the case. For example medium-sized histeridae species (adults) often attack preys larger than them (e.g. large *Onthophagus* species), provided that the preys do not move too much and that their integuments are poorly sclerotized. Similarly, the larvae of some Diptera (e.g. *Muscina stabulans*) kill large *Aphodius* larvae when immobile in the dung.

Page 84, end of third paragraph. A seasonal interchange of species was observed, the species of similar size being seasonally separated. Did you make the same observation with the dung beetles?

The trophic relationships established using statistical methods of data ordination are still partly theoretical. In particular, the large predators often are quite generalists and they prey on large prey ranges, with both small and large preys that are found most often by chance while browsing the galleries in the dung. The food groups interfere, as can be seen in Figure 2A, page 78.

Among the future directions of your research, did you plan to experimentally demonstrate the reality of the food webs highlighted in your work, offering to different predators of known size a fairly wide range of preys (of different sizes and different species)?

Chapter 4

The hypothesis of this section is that the succession of beetles and flies depends on the succession of volatile compounds emitted by the dung pats over time. This approach is very original and very promising because it allows to better understand the processes of succession of the species in dung pats as they age. However, a methodological question arises: beetles were sampled in 2009; flies were sampled in 2011 and 2012 (pages 120-122). In the meantime, the cows were not the same, their food could be have been modified. So it can be difficult to link directly the results of the analyzes of dung volatiles and the successions of dung-inhabiting beetles and flies. Volatile compounds in dung differ depend on the diet of the animals, i.e. fresh grass in the pasture, supplemented (or not) by hay or straw, or protein-rich meals that are added to the diet, especially in the case of dairy cows. Therefore in this chapter, it will be necessary to specify the diet of animals.

Page 120, paragraph 3 ("Materials and methods, volatile samplings"), it should be specified whether the dung pats that have been deposited in the field have been freely colonized by insects, or if the colonization was prevented by putting a net. Indeed the activity of insects modifies the gaseous emissions; the creation of tunnels accelerates the oxidation and the transformation of chemicals, as well as the rate of emission of volatile compounds. In the experiment described here, colonization of pats by insects was implicitly admitted, but it was necessary to wait until the discussion to be sure of that.

Page 124, section 2: it has been assumed that dung volatiles' succession did not change substantially between years and seasons. This assumption has not been verified.

However, despite these objections or requests for clarification, the results presented in this chapter provide new data that complement what was already known. In particular, the results showed a succession of dung-emitted volatiles (early and late successorial compounds), allowing insects to exercise a choice and structuring their assemblages. The possibility of a habitat-filtering scenario for dung-inhabiting insects seems compatible with the observations (page 131).

Conclusion of the assessment

In conclusion, the results and discussion presented in the thesis are very interesting and original, with promising and varied future research possibilities that deserve to be encouraged and supported.

Montpellier, November 8, 2017

Dr. Jean-Pierre Lumaret, Professor Emeritus

Opponent's Review. Thesis title: *Community ecology of insects inhabiting ephemeral habitats* by František X. J. Sládeček

Thesis of František X. J. Sládeček consists of short Introduction and four chapters (two published papers and two manuscripts). First part with Introduction is a detailed text, providing general background for ephemeral habitats and ecology of its inhabitants. It presents most of the questions treated further in subsequent chapters, providing broader context and links to general ecology questions. As a reader, I appreciate detailed discussion about niche-based coexistence of species vs. environmental filtering in ephemeral habitats. The intro also provide broad review of contemporary papers dealing with ecology of invertebrates in different ephemeral habitats. I have found no important recent paper missing on this list.

Second part contains four chapters, with two papers already published in IF journals, and two manuscripts, dealing with various aspects of ecology of dung-visiting Coleoptera and Diptera. All of them are written in clear and concise English, with minimum of typos (e.g., "eddisplay" instead of "display", p. 75; "tow" instead of "two", p. 76; "*Ontophagus joanne*" instead of "*joannae*", p. 98). All chapters have a classical structure of ecological papers. The hypotheses/research questions are clearly formulated, data were collected with sophisticated sampling design (usually minimizing effect of eventual spatial heterogeneity and actual weather conditions) and processed in an excellent way by statistical analysis, using advanced multivariate approaches (combination of ordinations, GLM/GAMM, clustering etc.). Results are usually balanced, with most important results (figures, tables) included in the text, and with additional analyses attached in supplementary files. Most of the texts also contain thorough discussion and detailed list of directions for future corresponding studies.

General remark. I am not sure if the two manuscripts (chapters III and IV) are already submitted in some biological journals. From other universities I know that this is a usual condition of an unpublished MS to be included in the thesis. I am not sure what are exact rules on this faculty/ study programme? I would like to hear more details on additional steps done since finishing the thesis (in mid-September, two month ago) – this question is probably no more relevant.

In the further text, I will include comments and questions concerning individual chapters.

- **Chapter I** focus on ecology of dung-visiting Diptera, studying succession on dung and seasonality using quantitative data.

Comments. Please, can you specify your skills in ID of Diptera species/morphospecies? I know you are able to identify the dung-inhabiting beetles, but not sure about flies. It is not clear to me, seeing Hana Šuláková as the second co-author (working in forensic entomology on Diptera as the main group of interest)? This is not commented within the paper.

Questions. 1. Can you comment on the CCA ordination presenting succession of adults of Diptera – why the first axis explained just 3.5% of variability?

2. Is any study on dung- or carrion-visiting Diptera available from other biomes than temperate with different seasonality pattern (e.g., tropics with dry/monsoon periods)? Or, can you expect different pattern also in temperate alpine habitats, with much shorter season?

• **Chapter II investigates different functional groups of beetles and flies inhabiting dung, and** compare temporal pattern: succession and seasonality of saprophages, omnivores and predators.

Questions. 3. You comment on differences between beetle omnivores from study plots in CZ and UK as possible artefacts of different collecting/extracting techniques. Is the same responsible for differences in beetle predators (compare Figs 3C, 3F)?

• **Chapter III** investigates the potential competitive and intra-guild predation of dung-inhabiting predators (sensu lato, incl. parasitoids etc.).

Comments. Question 2 is relevant also here.

Questions. 4. You stated that "one of us … weighted 20 adult individuals of each dung-inhabiting beetle species". But abundances were less than 20 spec. in many species (see Table S1) – you weighted specimens of the relevant species from different experiment(s)?

5. How you have associated males and females to some of the morphospecies, e.g. in some staphylinids (*Atheta* sp. 1 to 4; Table S1)? Based just on external morphology, or using male and female genitalia? Reliable identification in these aleocharines usually require study of male aedeagus or female spermatheca on microslides.

• **Chapter IV** investigate the successional dynamics of dung emitted volatile compounds, and correlations with beetle and flies abundances.

Questions. 6. Here you mentioned collecting of samples (mostly for beetles) also in very early spring (mid-April) or autumn (September/October). Similar samples were not included in previous studies (chapters 1–3). Can these samples contain some species with early spring/autumnal seasonality, missing in samples from other months, which can contain e.g. additional predators species to the list provided in chapter III?

Final Summary briefly repeated new results of the four previous chapters and put them into context at three levels: dung ecology, ecology of ephemeral habitats and general ecology.

Generally, I must admit that most of the questions are just marginal to the topics of the four chapters. This thesis belong to the best entomological texts I have ever chance to read and review.

In my opinion, the thesis of František X. J. Sládeček fulfilled fully the requested requirements and the candidate should be awarded with the title Ph.D.

Praha, November 14, 2017

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