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Evaluation

of the Ph.D. thesis of Mgr. Tomas Urfus entitled

„Evolutionary mechanisms and relationships among taxa of genus *Pilosella*"

The present PhD thesis of Tomas Urfus consists of five chapters, of which three represent already published contributions and two are in the state of manuscripts presumably prepared for publication. While three of the chapters (1-3) deal with morphological and cytological variation in *Pilosella* species, chapters 4 and 5 present results of morphological and cytological studies in the genus *Picris.* The PhD thesis further consists of a general introduction of 11 pages and a conclusion section of three pages. In total the thesis 170 pages long; it is written completely in English.

The general introduction aims at the presentation of the rational behind the selection of the study group and the levels and techniques of investigation. It introduces the study group (actually only the study group of the genus *Pilosella* and only in a few final sentences the genus *Picris*) and gives an overview of the evolutionary importance of polyploidy, hybridisation, and agamospermy. Notwithstanding the good and rather comprehensive treatment of these topics with regards to content, the reader of the thesis is somewhat lost concerning the understanding the aim of the thesis. This is mainly due to the fact that the author does not manage to describe the rationale behind the selection of two study groups. This is only stated as an aim of the thesis (p. 14: "Comparison of different variation patterns in related taxa"), but this aim is never addressed thoroughly throughout the thesis and especially not in the general discussion section, for

which a comparison of microevolutionary patterns seen in *Picris* vs. *Pilosella* would be expected at latest by the reader.

The first chapter of the thesis is a manuscript dealing with a multivariate statistical analysis in a hybridising population of *Pilosella officinarum* and *P. piloselloides* subsp. *bauhinii.* Two data sets were analysed with one representing the maternal plants from the field and the second being the progeny of those plants. The number of plants and characters studied are impressive, the statistical methodology is straightforward, the results are well presented, but the discussion section and the linguistics are very poor. These last two points render the manuscript of this study unpublishable in the present form.

The second chapter is an already high-ranked (Annals of Botany) published contribution on the cytogeography of *Pilosella officinarum.* The paper is not first-authored by the PhD candidate, and according to his statements on page 1 of his thesis he mainly contributed to the collecting of plants analysed (from 192 populations), the flow-cytometric analyses, and (only partly) the writing of the manuscript. The conclusions of the paper were that penta- and hexaploid cytotypes of the species arose independently in Europe with the apomictic plants having colonised the deglaciated areas.

The third chapter is again an unpublished manuscript addressing the morphological variation assessed by multivariate statistical analyses in *Pilosella officinarum,* but now not only in a single hybrid population but on a larger part of its distributional range. The variables measured and the multivariate statistical methods used are very similar to the methods used in chapter 1. The author found some morphological differences paralleling ploidy levels (tetra-, penta-, vs. hexaploids) and breeding systems in hexaploids (apomicts vs. sexual individuals). He concludes that the latter is an argument for the independent formation of these lineages and that these different lineages may deserve acknowledgement in the taxonomy of the species. Again, the writing abilities and the discussion of the own data with findings of other authors, which should be done in a discussion section of a paper, is quite poor (some citations are even missing from the list of the literature cited! e.g., Reisch & al. 2003, Parisod & Besnard 2007). While the data are surely worth publishing, this chapter would again need some considerable editing (both technically and with regards to reasoning) before it is publishable in an international journal.

The forth chapter of the PhD thesis is again an already high-ranked (Journal of Biogeography) published paper. Again, the PhD candidate is not the first author of the paper and was mainly contributing to the publication by his flow-cytometrical analyses of plant material. The astonishing fact about this contribution is that it is not dealing with the genus *Pilosella* but with *Picris hieracioides.* The same is true for the fifth chapter to which the same observations apply (i.e., paper first-

authored by another person, contribution of the PhD candidate on the methodical level, *Picris*). Any connections with the three first chapters of the thesis are not discernable.

The final chapter of the present thesis is considered to be the conclusion section of the thesis. Actually, it only gives summaries of the individual chapters and it does not provide any discussion of the data in a more comprehensive and inclusive fashion. If the aim of the whole thesis was a comparison of consequences of different evolutionary strategies realised in *Pilosella* and *Picris* (as stated in the introduction and under the aims of the study!) this would be the place to do that comparison! But this is not even tried by the author. The reader only learns that "the *Picris* comparative studies revealed highly distinct microevolutionary pattern[s?] compared to [the] genus *Pilosella."* and that *Picris* is not an apomictically evolving genus! Therefore, this chapter is not a discussion of the results of the different chapters but it is only an enlarged summary of the chapters. The chance for giving an argument for the inclusion of members of two genera into the study is missed completely here!

It is hard to judge on the quality of the present PhD thesis properly being an external reviewer. It is a compilation of manuscripts done by the candidate and publications of other authors, for which the candidate admittedly contributed considerable amounts of data, but for which he is not the first or corresponding author. Being ignorant about the detailed conditions for submissions and successful acceptance of PhD theses at Charles University in Prague I can only assume that this constellation passes the expectations that are defined as prerequisites for acceptance of a PhD thesis. According to our standards here in Regensburg University I can only state that a comparable work would be hardly accepted as PhD thesis because (a) contributions that were not done independently by the candidate are in the majority (three out of five chapters), (b) the remaining chapters that are first-authored by the candidate (along with the introduction and conclusion section) are not published yet and/or are of strikingly worse quality (linguistically, but also concerning scientific reasoning under incorporation of citations providing a theoretical or practical background) than the remaining chapters, and (c) there is no apparent coherency of chapters dealing with *Pilosella* and those dealing with *Picris.* In respect to the last point it would have been much better for the PhD thesis if the candidate would have narrowed down the focus of his thesis to the first three chapters dealing with *Pilosella.* This would have allowed him a much more stringent argumentation when putting data together and discussing them in a (much more comprehensive) general discussion section. With the adding of the two contributions on *Picris* the focus of the thesis is considerably watered-down and distracts both the author and the reader from a more thorough understanding of the main topic of the thesis. Narrowing down the presentations to the three chapters on *Pilosella* would have allowed the author to work more

thoroughly towards a better quality of the chapters under his first-authorship. Consequently, in its present form the PhD thesis of T. Urfus may be considered acceptable as PhD thesis only with great benevolence. The extensive methodical contributions to studies done and published under other first-authorships (chapters 2, 4, and 5) definitively deserve acknowledgement but obstruct an unhindered view on the author's scientific autonomy and his capabilities in scientific writing and reasoning.

Assuming that acceptance of the present PhD thesis was already decided on by the PhD committee of Charles University in Prague while taking into account the local rules governing PhD requirements, I am adding questions to the defendant in the following according to your ´rules and customs´ that you kindly have sent with your invitation to evaluate the thesis:

1) By studying morphological and cytological variation in two members of the Compositae-Cichorieae with different modes of reproduction you are aiming at a comparison of consequences following from these different modes on microevolutionary processes leading to differentiation within taxa on different spatial scales. In what respect the selection of the two study systems *Pilosella* and *Picris* is suited to answer that question and could allow some more general conclusions? Why did you prefer this two-genera-system over a system within a single genus (e.g., *Pilosella*) with more closely related species being representatives of the one or the other mode?

2) You have shown by a morphometric analysis that hybridisation between *Pilosella piloselloides* subsp. *bauhinii* and *P. officinarum* leads to hybrids that were treated under the species names *P. brachiata* and *P. leptophyton* and that all of these taxa form a intensively interbreeding hybrid swarm, in which plants with all different reproductive modes participate. What does this tell you about the genetic background of these different reproductive modes and the genetic background of the surveyed morphological data? Were there any correlations between reproductive mode and morphology revealed? How are your results of the hybrid swarm fitting to the results you got from the study of morphological variation in *P. officinarum* on a larger geographical scale (chapter 3)?

3) You attribute the morphological variation observed in sexual types of *P. officinarum* to common hybridisation of this species with other *Pilosella* species. Why is this statement not contradicting your findings of chapter 1, where you found also plants with other reproductive modes participating in the formation of hybrid swarms? You found a strong correlation of morphology with ploidy level in *P. officinarum.* Do you think that these morphological differences between cytotypes are rather due to different selection pressures acting on the cytotypes or are more due to a nucleotypic

effect? What kind of experiments would you propose to find out whether the one or the other is more reasonable? Is there any evidence for an interrelation between nucleotypic effects and natural selection? In general, what could be a model for the interrelationship of nucleotypic effects, natural selection, and genome sizes?

4) Hybridisation plays a very important role in plat evolution. What could be the different outcomes of hybrids formed between to plant species? It was reasoned (mostly on theoretical grounds) that hybridisation may play an important role in the selection of stronger prezygotic isolation barriers between two not yet completely isolated species/taxa (the so-called reinforcement process). What is the reasoning behind this reinforcement mechanism? What are arguments and observations in favour of the realisation of a reinforcement mechanism in natural plant populations? What arguments are found against its realisation? Are there any evidences from your study system whether a reinforcement process is acting upon the hybridising species?

5) The evolutionary potential of agamic complexes is sometimes considered as being restricted as compared with that of sexual groups, sometimes it was called "a blind alley of evolution" (Darligton 1939). Are there more recent findings arguing against that view? What do your findings in *Pilosella* contribute to this discussion? What about the fate of agamic complexes in general and of the *Pilosella* agamic complex in particular when considerable climate changes will change the abiotic and biotic selection regimes in the habitats of these taxa? Are there any observations or indications that climate change already has changed the selection regimes in *Pilosella* populations and that sexual forms are gaining advantages over the asexual ones?

Finally, I would like to state that despite the considerable shortcomings of the present PhD thesis I consider it suitable for a defence and that its quality fulfils the criteria necessary to obtain a PhD degree. I am aware of the fact that I do not have to propose any marking for the present paper. However, with all the facts listed that I would consider considerable shortcomings I would suggest that this should be taken into account if a marking is done by the Board of Examiners.

Regensburg (Germany), December 2, 2011

Prof. Dr. Christoph Oberprieler