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Genius loci ...

A REVIEW OF DOCTORAL (PH.D.) THESIS

REVIEWER: ĽUBOŠ MAJESKÝ, DEPARTMENT OF BOTANY, FACULTY OF SCIENCE, PALACKY UNIVERSITY IN OLOMOUC

AUTHOR: MGR. VOJTĚCH ZEISEK

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THESIS TITLE: TAXONOMIC PRINCIPLES, REPRODUCTIVE SYSTEMS, POPULATION GENETICS AND RELATIONSHIPS WITHIN SELECTED GROUPS OF GENUS *TARAXACUM* (ASTERACEAE)

SUPERVISOR: ASSOC. PROF., RNDR. JAN KIRSCHNER, CSC., INSTITUTE OF BOTANY, CZECH ACADEMY OF SCIENCES

Introduction

With a pleasure, I read Vojtěch Zeisek's doctoral thesis about the taxonomy, reproduction systems, population genetics and relationships of selected groups of genus *Taraxacum*, supervised by Assoc. prof. Jan Kirschner, which was carried out at the Institute of Botany of Czech Academy of Sciences in Průhonice (Prague). The overall aim of the reviewed thesis was to elucidate the taxonomy, reproductive biology, population genetics and relationships among selected groups of species using tools of modern molecular biology. Appropriate and correct taxonomical principles and their applying represent a highly desirable tool for all botanists (not only for that studying particular plant group), especially in such a tricky genus as *Taraxacum* is. Only careful consideration will lead to sustainable and reliable taxonomy, understandable for a broad scientific auditory but also for the public. The core of the thesis represents a compilation of five scientific papers in various stages of publication process (three already published, one accepted for publication and one prepared for submission) each one dealing with a different dandelion group. This compilation of research manuscripts is completed by introductory chapters (Part II, pages 15–50) dealing with the general issues and aspects relevant to the investigated genus.

The second part (Part II; p. 15–50) provide a brief introduction of problems associated with species recognition (species concepts), followed with the introduction of the genus *Taraxacum* and description of basic issues linked with the complexity of the genus (strong reticulated evolution, hybridity, polyploidy and presence of several reproduction modes). Another chapter is dedicated to an enumeration of molecular techniques used to study the genus. This is completed by comprehensive Table (Tab. 8.1., p. 36–42) listing studies of the genus *Taraxacum* which used various molecular and/or cytogenetic techniques.

The third part (Part III; p. 53–97) describes the genetic variation and genetic structure within the members of one rather exceptional group of dandelions *T*. sect. *Dioszegia*. The genetic variation and structure were inferred from 14 microsatellite loci among 115 individuals from 20 localities sampled mainly within the European range of the investigated section (but including several samples from Iran). The aim was to investigate the genetic "make-up" of selected taxa and to make a taxonomic revision of this group of dandelions. The investigation resulted in the recognition of four taxa: two at the rank of subspecies *T. serotinum* subsp. *serotinum* occupying the large area from the Balkan peninsula trough Central Europe up to Eastern Europe and *T. serotinum* subsp. *tomentosum* with restricted area in South-western Europe (S France, Spain and possibly Morocco); one at the variety rank: *T. serotinum* var. *iranicum* (restricted to Iran); the fourth taxon represents *T. haussknechtii* (restricted to the Republic of Macedonia and adjacent parts of Greece, Albania, Bulgaria, and Serbia). Based on SSR genotyping and former observations three recognized species are sexual allogamous and one (*T. serotinum* subsp. *tomentosum*) is sexual autogamous. The possible origin of this autogamous subspecies is further discussed and several possible scenarios are presented. This chapter is finished with the taxonomic treatment and revision of the section *T.* sect. *Dioszegia*.

The fourth part (Part IV, p. 99–137) represents an interesting "experiment" with the taxonomic concept used in the taxonomic treatment of the genus. The rationale behind this was to check the conformity of taxonomic treatment of the genus (by recognizing of different microspecies**–**polyploid apomictically reproducing taxa) among four different taraxacologists (specialist on the genus). The conformity was tested by means of microsatellite (SSR) genotyping. This experiment involved four world-renowned taraxacologist and nine selected microspecies, which were collected by the specialists across the large area of Europe. Genotyping by eight SSR loci revealed an almost perfect match of the involved specialists in used species concept. It also confirmed the previously suggested genotypic structure of agamospecies consisted of one dominant genotype and a few derived genotypes. The results proved that dandelion microspecies represent morphologically and genetically definable entities, and can be seen as evidence to advocate the taxonomic concept used for treating species diversity of the genus.

The fifth part (Part V, p. 139–184) is addressed to an Asiatic group of mostly mountain dandelions from the region of the West Himalaya frequently treated under the name of *T. stenolepium* or *T. sect. Orientalia*. The investigation has shown that the name *T. stenolepium* served as a trash-can for diverse taxa from the West Himalayan region. This part represents thus taxonomic reassessment of the above-mentioned taxon *T. stenolepium*, revision of the *T.* sect. *Orientalia* and description of a new group of dandelions *T.* sect. *Squamulosa*. The introduction of the new section is completed by an ITS phylogenetic analysis of the sexual species from all the relevant *Taraxacum* groups including newly described sexually reproducing taxon T*. stenotegulatum*. Phylogenetic analysis served as a tool advocating the independent position of the *T.* sect. *Squamulosa* among recognized sections.

The sixth part (Part VI, p. 185–216) describes a study of *T. bicorne*, a congener of widely analyzed an alternative rubber crop *T. koksaghyz*, as another source of rubber. Two populations of *T. bicorne* were analyzed by means of 13 selected SSR loci and KASP loci. Further, for phylogenetic assessment of the taxa, ITS locus was sequenced. Hybridization experiments with *T. koksaghyz* were performed and the rubber content measurements were

done in both related taxa to compare the potential for further breeding programs. The study is accompanied by detail taxonomic characterization of *T. bicorne*. Results showed and confirmed supposed close relatedness of *T. bicorne* and *T. koksaghyz*, high within-population genetic diversity in the two investigated populations. As expected the quantification of rubber content showed *T. koksaghyz* produce twice as much rubber than *T. bicorne*. However, lower rubber content does not disqualify *T. bicorne* from further breeding programs.

The seventh part (Part VII, p. 217–243) represents a taxonomic evaluation of one sexual (*T. bithynicum*) and one asexual (*T. pseudobithynicum*) dandelion taxa from the Mt. Uludağ, Turkey. A detailed taxonomic treatment of both taxa is provided, accompanied by chromosome counts, scanning electron microscopy of pollen and achenes and ITS phylogeny. *Taraxacum pseudobithynicum*, a triploid apomictic taxon, is described as a new for science. The sectional position of the investigated taxa remains unclear, but a certain relationship with *T*. sect. *Scariosa* is outlined and discussed.

Strengths of the thesis

The strength of the thesis can be seen in the properly taxonomically defined plant material, which was used for investigation. This is evidently thanks to the supervisor´s years of experience with the studied genus. The other strength is the selection of appropriate molecular methods and thorough analysis of acquired data.

Shortcomings

Though the majority of the core part of the thesis was already published I have much-facilitated work, because the research outputs were already reviewed and shortcomings had been corrected. I did not find any substantial shortfalls, methodological or interpretational.

Minor remarks

I consider the formatting of the thesis as badly chosen. The selected layout into parts and many chapters make the reading and orientation within the text uncomfortable and intricate. I consider introductory chapters dedicated to the general description of the genus *Taraxacum* (Part II, Chapters 7/8/9, p. 27 – 50) a bit illogically ordered. Chapters 7 and 9 are dealing with the genus *Taraxacum*, while Chapter 8 provides an overview of molecular techniques used in botanical research. This chapter (Chapter 8) should be the last one, together with the comprehensive table (Tab. 8.1). The chosen organization does not contribute to the fluent text flow. Moreover, too general statements appear in the text which needs a better explanation to be properly understood in the context.

Regarding the analysis of microsatellites, I think it should be always mentioned how the investigated loci were treated and analyzed; as allele sizes or number of repeats. This is important in connection to the nature of these loci and way how the variability in SSRs is acquired. Not appropriate selection during analysis may introduce substantial biases into results.

I found parts describing “Material and Methods” too brief lacking important information. This is especially relevant to the Parts V, VI, and VII. From the description, it is not clear how many samples were analyzed, what was the purpose of the statistical evaluation of e.g. genetic data, etc. More specific remarks are picked up below.

Majority of remarks listed below are related to the Part VI (p. 185–213), as this part suffers from several shortfalls.

Part VI, p.192: Information about cultivation is insufficient regarding the scope of the study, which was the determination of rubber content in the two investigated taxa. It is important to at least briefly describe the cultivation conditions, otherwise, the experiment is not relevant.

Part VI, p. 193: It should be written in the M&M section how many crosses were done with how many plants. It would be fine to use symbols for designation of a female and a male partner in the crosses. It would be good to write how the number of hybrid achenes was assessed regarding the possibility of forced autogamy. Otherwise, you cannot be sure about the hybrid origin.

Part VI, p. 193: I do not understand the rationale behind using KASP (CPT1-what it is?) loci for genetic analysis when it was nearly invariant. Probably, it was used to compare the similarity of rubber synthesis pathways, but in that case, it should not be used for computation of population statistics. Especially because these are coding genes, thus are not under neutral selection, etc. Another thing is that the nature of KASP loci is not sufficiently described. Readers do not know what it is, how these loci were evaluated (sequenced, genotyped by fingerprinting method, or in some other way).

Part VI, p.197: Calculation of inbreeding coefficient is not mentioned in the M&M section (how it was calculated, where, for which purpose). But it is referred to this coefficient in the results (p. 197, Fig. 29.3). Fig. 29.3 – how it is meant, per locus or multilocus estimates?-it is not clear from the graph.

Part VI, section 28.8: Why different numbers of individuals of *T. bicorne* vs. *T. koksaghyz* were used for rubber content measurements when the purpose of the study was to characterize the rubber content in *T. bicorne* in comparison to *T. koksaghyz.* From uneven sample set, general conclusions cannot be made. This is strengthened by the lack of other important information (see comments about cultivation and characteristic of the analyzed material). In this part characterization of plant material (roots) used for rubber content quantification (size, age, growing conditions, dry mass, etc.) is totally missing.

Part VI, section 30.1 (Discussion): I found this part of the discussion too long and unnecessary. It should be focused more on the interpretation of the results in the context of the scope of the study.

Department of Botany
Palacký University in Olomouc | Faculty of Science
Šlechtitelů 27 | CZ 783 71 Olomouc | Czech Republic
T: +420 585 634 801
botany@upol.cz | **www.botany.upol.cz/en**

Questions:

1) Part III – Why T. *serotinum* subsp. *serotinum* was not more evenly sampled, especially in the Balkan Peninsula, regarding its large distribution area? Majority of investigated samples of this taxon comes from boundary areas, and only six samples come from the main European range of the taxon. This is in contrast to *T. haussknechtii*, which was sampled only from the central area but not from peripheral parts. Could this sampling affect the calculation of population statistics (e.g. values of F_{ST}, allelic richness, MLG, etc.)?

2) Part V., p. 160: It is written that some of the sexual dandelion taxa originated from "polyploid resexualization". Please, could you explain how do you mean it? Can you describe the process of polyploid resexualization?

3) Part VII., p. 237: The proposed hypothesis about the hybrid origin of *T. pseudobithynicum* based on similarity of karyotypes is not appropriate together with the inferring of the number of satellite chromosomes (this cannot be inferred from the presented picture). If this taxon is of hybridogenous origin and comes from hybridization between *T. bithynicum* x unknown apomictic taxon, how you would explain the number of satellite chromosomes (keeping in mind that you do not refer to any satellite-chromosome in *T. bithynicum*)? Did you sequence ITS locus for *T. pseudobythinicum*?

Final conclusion

Taken as a whole I found the thesis of Vojtěch Zeisek interesting, thoroughly performed and well presented with a good output in the form of results published in good and standard scientific botanical journals. The thesis brings valued information about the genus *Taraxacum.* I think this reflects Vojtech' expertise in molecular methods, good orientation and performance in statistical methods, understanding of population and genetic theory and positive attitude to scientific research.

Therefore, I recommend the acceptance of Vojtěch Zeisek's doctoral thesis for the defense and to award the degree of Ph.D.

I would like to congratulate Vojtěch on this success and wish him a successful scientific career and fruitful private life.

With regards,
Ľuboš Majeský

V Olomouci, dňa 31. 08. 2018 **.....**

Department of Botany
Palacký University in Olomouc | Faculty of Science
Šlechtitelů 27 | CZ 783 71 Olomouc | Czech Republic
T: +420 585 634 801
botany@upol.cz | **www.botany.upol.cz/en**