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To whom it may concern

Vienna, 31st of October 2022

Review of the Ph.D. thesis of Ludmila Rejlova “Evolutionary Processes Shaping the Genus *Urtica* L. (Urticaceae) in Europe and Adjacent Areas.”

I do not know of any existing conflict of interest.

Brief summary of the thesis...

Ludmila Rejlova’s thesis aims to shed light on the geographical distribution patterns, taxonomy, evolutionary history, and gene flow between certain cytotypes among *Urtica dioica* subspecies and various related species from Europe including the subspecies *kurdistanica*, which is native to West Asia. Therefor she applied a wide spectrum of approaches, which are 1) cytological (relative and absolute genome size and chromosome counts) 2) morphological (morphometry of leaves supported by appropriate statistical data analysis), 3) ecological preferences (Bioclim) and 4) phylogenetic (genome skimming) investigations. In an extensive survey, 7012 samples from 1317 populations were used in the cytogeographical evaluation (Table S1), and, subsequently, a selected subset of 279 samples was subjected to the phylogenetic, morphological and more detailed cytological approaches.

...and its results

- In *U. dioica* the tetraploid cytotype ($2n=2x=52$) is widespread and predominates (87%) the diploid cytotype (except in Po Plain/Italy). In very rare cases even odd numbers of chromosome sets (3x and 5x) were identified in adult members of mixed populations. Interestingly, a survey of *U. dioica* fruits showed higher incidence of deviating ploidy levels in embryos and endosperm.

- The average absolute monoploid genome size did not vary significantly among the diploid subspecies of *U. dioica* (except *U. d. ssp. cypria*), but was slightly lower in the tetraploid subspecies. In contrast, the closely related species varied significantly from *U. dioica*. In case of *U. d. ssp. cypria*, genome size deviation plus molecular data support the elevation to the species rank. Similar applies to *U. bianorii*, which was previously incorrectly classified as a subset of *U. atrovirens*.
- The cytogeographic analysis revealed a narrower ecological niche and a preference to lower elevations and human-unaffected sites in the diploid subspecies compared to the tetraploids (again except the Po Plain/Italy).
- The finding of distinct clusters at morphological variation analysis among the diploid subspecies was not supported by the phylogenetic evaluation. However, the tetraploids merged with diploids but showed a clear differentiation of individuals from the Middle East dissecting the tetraploid sample set into two parts.

Commentary on individual aspects of the thesis, specific comments on the professional, linguistic and formal aspects of the thesis

The thesis consists of two main parts, i.e. 1st “General Chapters” and 2nd “Case Studies” (one yet unpublished and two already published papers – all of them with LR as first author). The first part serves as a frame, which summarizes the case studies in a synergetic manner.

The thesis is clearly structured, the discussion and conclusions are logical and sound, results were adequately documented, the text is leaded by a compelling introduction.

The linguistic quality is excellent, easy to read, concise and homogenous throughout the entire thesis.

The thesis is a comprehensive work in respect of sample size and approaches and will provide a valuable basis in further explorations of this fascinating plant group in many respects, e.g. - in order to name only two - to unravel the phylogeny of this for long time overlooked plant group, or to learn about general modes of colonization.

There are merely minor problems within this thesis concerning typos or other technical issues (see below).

Therefore, I warmly recommend the thesis for the defence.

Dr. rer. nat. Eva M. Temsch

Questions:

- 1) The study points to the need of reference material in both, the genome size measurement as well as for the ploidy level assessment. You demonstrated nicely that chromosome counts in ploidy level reference individuals are inevitable (see *U. cypria*). Why and how did you select *Bellis perennis* and the used C-value as the reference standard for genome size measurement? The paper: "Reference standards in flow cytometry with a focus on plant nuclear DNA content measurements – overview and best practices. Cytometry Part A, <https://doi.org/10.1002/cyto.a.24495>" will probably help you to develop a short discussion dealing with the mode of selection as well as the pros and cons of exactly this species and the C-value used as the genome size reference.

- 2) There are already published C-values in the genus *Urtica* (see <https://cvalues.science.kew.org/>). They are not at all mentioned in your thesis. Why? And how would they fit into your findings? Set a short discussion up.

- 3) On page 22, you wrote: "Currently, the estimated proportion of recent polyploids (neopolyploids) among angiosperms is 33–35% (Wood et al., 2009; Rice et al., 2019). Within other groups, most polyploids can be found among sporophyte plants (90–95%). On the other hand, polyploidy is very rare in gymnosperms, even absent in certain groups." There is obviously a taxon called "sporophyte plants" addressed in this paragraph. I am bound to say, I never heard from such a taxon and I do not exactly know what you are meaning. However, I've got a notion that you either addressed "sporophytes" or "spore plants". Discuss why both terms must not be used to define a certain taxon in a scientific paper. The paper: "Greilhuber J., Doležel J. (2009) 2C or not 2C: a closer look at cell nuclei and their DNA content. *Chromosoma* 118: 391-400." will help you to discuss this issue.

Technical comments:

“Zhengyia” in “*Urtica + Hesperocnide*, Zhengyia T. Deng, D. G. Zhang & H. Sun“ must be written in italic letters.

Page 82 (already published paper): „percentageof“. A missing blank.

Although the term “DNA ploidy” was correctly applied throughout the manuscript, the respective terminology paper citation is missing: “Suda J. et al. (2006) Ploidy level versus DNA ploidy level: an appeal for consistent terminology. *Taxon* 55 (2): 447–450“. In contrast to a journal paper, a thesis is not strictly limited in regard of the number of pages, therefore I suggest to cite such papers.

The following sentence on Page 21: „Polyploidy is the state of a cell or organism in which more than two sets of chromosomes are present (from a molecular point of view, traces of genome duplication, i.e., paralogous genes).“ is incorrect in regard of haplophasic organisms. I suggest to rather write „Polyploidy is the state in which more than one or two sets of chromosomes are present (from a molecular point of view, traces of genome duplication, i.e., paralogous genes) in haplophasic or diplophasic organisms, respectively. If merely a fraction of an organism’s cells is polyploid, the organism is called “endopolyploid”. Read: Greilhuber et al. (2005) The origin, evolution and proposed stabilization of the terms ‘Genome Size’ and ‘C-Value’ to describe nuclear DNA contents. *Annals of Botany* 95:255-260 as well as Greilhuber J., Doležel J. (2009) 2C or not 2C: a closer look at cell nuclei and their DNA content. *Chromosoma* 118: 391-400.

Some entries of the reference chapter are in a wrong sequence: “**Thompson** K & Grime JP. 1979..., **Thomson** JD & Barrett SCH. 1981..., **Thompson** JD & Lumaret R. 1992”. Check if there are more of such cases.