



Review of Ph.D. Thesis

Mgr. Ludmila Rejlová: Evolutionary processes shaping the genus *Urtica* L. (*Urticaceae*) in Europe and adjacent areas

The thesis studies a widespread species *Urtica dioica* and its several close relatives. Despite *U. dioica* is well-known organism to everyone from the childhood and is ubiquitous (at least in Europe), its taxonomy and evolution remains partly unknown. The reasons may be phenotypic plasticity, dispersal by humans (that may have blurred the natural distribution of the taxa), as well as somehow lower attractivity of this plant and its habitat to botanists. This Ph.D. thesis thus aims on filling an important gap in our knowledge, based on really solid sampling (over the whole Europe plus Anatolia with northern Iran, more than 1300 populations and 7000 individuals) and combining several methods.

The thesis contains the introductory part (39 pages + 10 pages of references) and three papers ('Case studies'), of which two are already published in good quality journals included in the WOS (which also means that little work is left to me as a reviewer, as both were thoroughly reviewed during the publication process). The introduction is rather long (compared to other theses I have reviewed or read) and contains general description of the family and the genus, descriptions of the taxa within *U. dioica* complex and selected other taxa (island endemics), and a brief outline of evolutionary mechanisms, the breeding system, and seed dispersal mechanisms (sections 1–4). The last sections of the introduction summarize aims of the thesis, methodology and key results (sections 5–7), followed by a brief discussion of the results (sections 8–9).

The introduction reads well but the amount of information is somewhat unbalanced. Descriptions of the family and the individual *U. dioica* subspecies are quite detailed. On the other hand, an account of published phylogenies of the genus (as a backbone of any evolutionary study) is nearly missing. In the description of the genus, only *U. dioica* and several endemic taxa are mentioned, while other species widespread in Europe and western Asia (e.g. *U. cannabina*, *U. membranacea*, *U. pilulifera* or *U. urens*) are hardly named – I would expect mentioning them at least in a brief description of the genus (how many species it has, how many of them grow in the study area and which ones, how many of them are widely- and narrowly distributed, etc.). Similarly, the chapter 2.2 on evolutionary mechanisms (which is the key chapter in respect of the title of the thesis) is quite detailed and informative in general descriptions of the individual mechanisms (allopatric speciation, hybridization, polyploidy), while there are only two short paragraphs about *Urtica*. Some parts might be omitted or much shortened, because they are included in the papers (cases studies) – such as the chapter 2.4 on seed dispersal (nearly the same being in Case study III) and methods (that are described more in detail the published manuscripts). On the other hand, I like the chapter Discussion (section 8) that brings a summary of all published data (Case studies I + II) and discuss a few aspects that are not included in that papers (I guess because of the usual space limitations).

The first published paper (Case study I) is mainly a detailed cytogeographic study of *U. dioica*, supplemented with absolute genome size estimates and ecological niche modelling. I must again highlight the dense sampling, which allowed to capture the quite scattered occurrence of some taxa (such as the diploid subsp. *subinermis*) and provides enough detail in the possible contact areas (such as the northern Italy with the subsp. *pubescens*). The latter subspecies is quite different from other diploids in its habitat preferences – so the whole discussion (in this paper and elsewhere) on that diploids are found in more natural ('relict') habitats should in fact read 'diploids except for subsp.



pubescens). The study detected a relationship of the genome size and some environmental parameters, and I have one additional question about that topic, see below.

The second published paper (Case study II) includes a morphometric and molecular (target enrichment) study. The morphometric part clearly shows certain differentiation among diploids (with partial overlaps) as well as huge variation among tetraploids. In opposite, the molecular part did not provide enough resolution to separate the taxa within *U. dioica* complex (while other species, such as *U. kioviensis* and the Mediterranean taxa, are well separated). Several possible evolutionary causes are discussed; see one additional (rather technical) question to this below.

The manuscript (Case study III) is focussed on the Mediterranean endemic nettles. The introduction (review) is quite nice and summarizes necessary information on the Mediterranean area, history of island, the study species, as well as several features related to their evolution and dispersal. Minor polishing of the text would be beneficial, but this is natural for an unpublished (unreviewed) text. However, I strongly miss any particular evolutionary hypotheses/scenarios, and comparison with other Mediterranean taxa; see my third question for the defence. By the way, in which journal do you aim to publish this review, is it decided yet?

In summary. The Ph.D. thesis of Ludmila Rejlová brings new information about the studied genus *Urtica*, but my feelings are mixed. On one hand, it includes two papers published in good journal, in which the candidate is the first author. On the other hand, I would expect that general (introductory) part covered a broader context of the studies; the third manuscript is mainly descriptive and lacking the particular evolutionary hypotheses about studied plants and evolutionary conclusions. However, I highlight the positive part of my evaluation, and despite some criticism I recommend the thesis for defence. The candidate obviously took part in all stages of the work (i.e., field sampling, lab work, data analysis, paper writing) and the two published paper prove her ability to conduct and finalize the scientific research, which qualifies for the Ph.D. degree.

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Mgr. Petr Kouřecký, Ph.D.

Questions for the defence:

(1) Case study I. You modelled relationship of genome size or monoploid genome size (in case when both ploidies are included) with latitude, longitude and elevation. What is the rationale behind such analyses? Have you expected some gradient in genome size in Europe? If so, why, could it have some functional consequences? The use of the linear model implicitly means that the value is increasing or decreasing with the given parameter; is this a suitable model? Diploids include several isolated taxa – what is the reason to merge them into one category?

(2) Case study II. The HybSeq analysis included both diploids and tetraploids. Were the ploidies considered in computations? In my opinion, bifurcating tree may not be good representation, especially when some reticulations are expected (allotetraploids, as mentioned several times). May this be one of the reasons why the tree is rather unresolved?

(3) Case study III. Are there other genera in the Mediterranean/Macaronesian area that have a distribution similar to *Urtica*? If so, could we learn something from them, could the *Urtica* evolution be shaped by similar processes and history? If not, what makes *Urtica* so unique?