

External Review
of the Ph.D. Thesis “**Influence of climatic fluctuations in Neogene on evolution of ecologically diverse plant genus: an example of *Hippophae* L. (Elaeagnaceae)**“
by MSc. Dongrui Jia

The present Ph.D. thesis of MSc. Dongrui Jia was conducted between 2010 and 2013 at the Charles University of Prague, Czech Republic, under the supervision of Dr. Igor V. Bartish.

This thesis (in English) is comprised of four Chapters (I–IV). Following Acknowledgements and an Abstract, the thesis starts with a detailed and well written Introduction (Chapter I), which covers general features of the focal study area, the Asian Qinghai-Tibetan Plateau (QTP) region, and introduces the target taxon, *Hippophae* L. (Elaeagnaceae) as a suitable model system for addressing timely questions in plant phylogeography and biogeography, in particular concerning the impact of geological (orogenic) and/or climatic changes on the origin, diversification and range shifts of components of the Eurasian temperate flora over Neogene time scales (Chapters II & III). Apart from these valuable insights at, respectively, species (*H. rhamnoides*) and genus/family (*Hippophae*/Elaeagnaceae) level, the thesis nicely illustrates the impact of Late Quaternary climate change on niche suitability and current patterns of within-population genetic diversity at lower (subspecies) taxonomic level (*H. r.* ssp. *sinensis*; Chapter IV), with potential implications for predicting the sustainability of extant species populations in the face of global warming. Personally, I would have liked the thesis to conclude with some more general remarks, e.g., by sketching frontiers of knowledge in the field (i.e. ‘Biogeography & Phylogeography of temperate plant species in China and adjacent areas’; see, e.g., Qiu et al., 2011), and prospects for future research. This discussion, however, could also form part of the upcoming defence.

In the following, I will outline my general observations and assessment for each chapter, along with a few critical remarks/questions.

Chapter I (Introduction) focuses on the geology, biodiversity, and phylogeography of the QTP region and adjacent areas, provides general information on the family Elaeagnaceae, and reviews previous systematic and molecular work on *Hippophae*. The respective sections testify to the author’s profound knowledge of the relevant literature. Clear highlights of this chapter are the meticulous up-to-date reviews on biogeographical and phylogeographic plant

studies so far conducted on and around the QTP (Table 1–2), which exemplify how molecular analyses have the potential to uncover significant correlations between different uplift stages of the plateau from the Miocene to the Quaternary and the ages of diversification and range dynamics of endemic lineages, including periods of hybridization and introgression following secondary contact. Given that especially the rate of molecular phylogeographic plant studies is growing tremendously in the study area at an ever-increasing rate [e.g., Qiu et al., 2011; but also see the recently published “Plants of China” (2013) by Hong De-Yuan & Stephen Blackmore, Science Press, Beijing], the importance of this up-dated review cannot be overstated, and I can imagine it has the potential to form the basis of a follow-up review, e.g., similar to that presented by Qiu et al. (2011).

Critical points/questions: (1) At the end of this chapter (‘Biogeographic hypotheses for *Hippophae*’), I would have expected a more detailed account of the specific biogeographic questions addressed for this highly interesting group, and how they will be tackled in the following chapters. Nevertheless, the information conveyed in this ‘Biogeographic hypotheses’ section was helpful and relevant. (2) On p.4 “the new-formed Mediterranean” climate is associated with the “Early Miocene”, but the bimodal Mediterranean climate regime we know today only began to appear during the Late Pliocene (c. 3.2 mya; see, e.g., Suc 1994; Thompson 2005: 18-26).

The content of **Chapter II**, which I am glad to see having been published by *New Phytologist*, and for which I have acted as reviewer of an earlier version (Dec. 2011), deals with the phylogeography of *H. rhamnoides*. This chapter viz. paper truly provides a significant contribution, if not milestone, to the field of Eurasian plant phylogeography. After all, this work features an impressive population sampling at the species’ range-wide scale (including previously missing samples/taxa from Europe; see Fig. 2-1), which then serves as a basis for state-of-the-art analyses, including molecular phylogenetic dating (e.g., BEAST), ancestral area reconstructions (DIVA), and mismatch distribution analyses (MDAs) of ITS and/or cpDNA haplotype variation to address and confirm – for the first time at any intra-specific level – the long-held ‘out-of-QTP hypothesis’. Overall, these interesting results imply (i) an origin of the species in the QTP region; and (ii) multiple ‘out-of-QTP’ dispersals events, accounting for much of the species’ expansion across Eurasia, most likely during the (Late) Pliocene/Quaternary.

Critical points/questions: (1) The observed topological incongruence between ITS vs. cpDNA trees (discussed on pp. 63/63) might have causes (at least in part) other than past

hybridization/introgression (e.g., incomplete lineage sorting). Nonetheless, some of these interpretations appear to be congruent with previous hypotheses, e.g., concerning the homoploid hybrid origin of *H. goniocarpa* and *H. litangensis* (e.g., Bartish et al., 2002), and thus should be valid (pp. 63/64). (2) I was still surprised that only one of the cpDNA clades (i.e. E) was interpreted to show significant range expansion (p. 58; Table 2-4; Fig. S2-2), while all other clades (A–D) have likewise non-significant SSD and H_{Rag} values, with D also showing a significantly negative F_S value (Table 2-4). It seems that the MDA results are a bit more complicated and toned down in favour of a mere visual evaluation of the mismatch distributions, an approach potentially prone to subjectivity (see earlier comment in review).

Chapter III has its emphasis on the phylogenetic relationships, ages, and routes of migration of all species and subspecies of the genus *Hippophae*, which has its centre of diversity in the QTP region, but – with a single species (*H. rhamnoides*) – also extends into Europe as well as areas further east of the QTP. By cautiously applying fossil-based clock calibrations, the genus is shown to be of relatively old age (Late Eocene/Early Oligocene) and, e.g., the origin of *H. rhamnoides* is dated to the Mid-Miocene. Interestingly, all constituent (sub)species apparently underwent a near simultaneous diversification much more recently, i.e. during the Late Pliocene/Pleistocene, a time period that is also identified as a phase of active migrations across the Eurasian highlands. Overall, many parts of this molecular biogeographic chapter have almost text-book quality, especially regarding the well-written Introduction (pp. 82–86), the careful selection of fossil data used for calibration and subsequent divergence time estimations, using both penalized likelihood (PL) and Bayesian approaches (M & M: p. 93–96), and the excellent Figure 3–5, illustrating the results of the combined dating and biogeographic analyses. In sum, this study supports Bobrov's (1962) original hypothesis that *H. rhamnoides* colonized Europe in a step-wise (east-to-west) fashion along Eurasian mountain ranges during the Late Tertiary (Late Miocene), probably in response to climate-induced deforestation providing ecological opportunities for a pioneer species.

Critical points/questions: (1) I missed a slightly more detailed discussion of this latter interpretation in light of the species' interesting ecology and what is known about Eurasian vegetation history, notwithstanding the in-depth literature coverage on pp. 112–113. (2) The chapter lacks a Conclusion paragraph, and thus misses the opportunity to place the highly interesting and solid results in a somewhat broader context. (III) p. 109: the scenario of homoploid hybrid speciation is perhaps too readily invoked as the sole explanation of

topological incongruence between cpDNA and nDNA trees (see also critique on Chapter II). (IV) p. 94: *Ficus* has no ‘achenes’ but essentially (compound) drupes.

The final **Chapter IV** explores current patterns of genetic (nSSR) diversity and structure in *H. rhamnoides* ssp. *sinensis* in light of past (LGM, LIG) and current patterns of niche suitability across its wide range from the eastern QTP region, across the Loess Plateau, to NE China. This work excels in taking advantage not only of the full range of well-established population genetic analytical tools (e.g., AMOVA, BARRIER, IBD, ALLELINSPEACE) in characterising these populations, but also links the genetic patterns identified with climate-based environmental factors and ENM-derived parameters of past/present niche suitability, stability and change (the latter of which have only rarely been employed yet). In brief, the results indicate that the conspicuously low allelic richness (A_R) of central (Loess Plateau) populations is most likely due to post-glacial range fragmentation (reduced gene flow, drift), resulting in a shallow west-east differentiation (e.g., AMOVA among-group differentiation c. 6%) along a west-to-east gradient (‘ecozone’) of variable/cold to stable/dry climatic conditions. This interpretation makes sense based on the data presented, which are carefully and critically explored [e.g., regarding potentially confounding effects of null alleles (pp. 148/149)], and, again, are very well illustrated (e.g., Figs. 4–3/4). Overall, this is a study I would want to recommend my students and colleagues of Molecular Ecology to read, given that the results reported have certainly wider implications beyond this particular study system in showing how contemporary patterns of within-population genetic diversity (viz. adaptive potential) are still influenced by past (LGM-to-present) changes in climate-related niche suitability, resulting in an overall pattern of isolation-by-environment (rather than geographic distance per se). A logic next-step would be to explore (with this model system) potential patterns of isolation-by-adaptation, at both non-neural (‘outlier’) loci and for heritable phenotypic traits.

Critical points/questions: (1) p.163: “... and our ecological niche modelling” – I don’t understand how the current ENMs support this notion of a west-to-east expansion. Also: it should simply read “Last Interglacial Period” (drop “in the Late Pleistocene”). (2) pp. 163–164: why are so many complicated scenarios invoked/discussed, and thus before all results (including ENM) are presented? Hence, the first part of the Discussion could be written more concisely. (3) Why are levels of genetic diversity not reduced in supposedly expanding peripheral (west and east) populations? Instead, could they represent long-term stable ones (i.e. in the QTP and the North East), and thus despite the ENM patterns? (4) The near-lack of niche suitability at the LIG is conspicuous – any explanation?

Final assessment

I have greatly enjoyed reading this thesis, which is very well and competently written. The basic concepts and relevant questions are addressed in a very comprehensive manner. I am not familiar with all analyses conducted and/or statistical programmes employed, but as far as I am able to judge: all analyses have been done very carefully, if not meticulously, and the results have been interpreted always very cautiously. The present thesis demonstrates the wide-ranging analytical and intellectual skills of MSc. Dongrui Jia in combining state-of-the-art phylogenetic, historical biogeographic, and molecular-ecological methodologies, together with a profound taxonomic-systematic and ecological knowledge on members of *Hippophae*, and a broad understanding of their Eurasian environs, particularly the QTP region and adjacent areas. In sum, the results obtained significantly further our understanding of the impact of historical landscape and climate changes on temperate plant evolution in Eurasia. I am looking forward seeing the work reported in Chapters III & IV to be published very soon.

In summary, the Ph.D. thesis of MSc. Dongrui Jia is excellent. Without reservation, I consider this thesis suitable for defence and grade it

“very good” (1.0).

This thesis, therefore, fulfils the criteria necessary for obtaining a Ph.D. degree.

Univ.-Prof. Dr. Hans Peter Comes

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