

# Testing the origins of the Balkan serpentine endemics in *Onosma* (Boraginaceae): A molecular phylogenetic approach

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## Abstract

Plant life on serpentine soils has been a topic of research for decades, but the evolutionary dynamics behind the origin of specialized lineages endemic to such a stressful habitat are still incompletely understood. This issue was addressed using the genus *Onosma* (Boraginaceae) as model system and a molecular phylogenetic approach. Nuclear DNA ITS sequences were generated for a broad sample of taxa including seven obligate endemics allopatrically distributed on the ophiolitic "islands" in the Balkans, along with accessions of serpentine-tolerant and non-tolerant species. Bayesian inference of phylogeny showed that European obligate endemics belong to at least five clades without direct relationships to each other. Lack of a common ancestor and of correlation between geographic and genetic distances suggested polyphyletic evolution of endemic species group. Preference for non-serpentine habitats is suggested as the ancestral condition but key preadaptive traits such as drought tolerance and ability to cope with high Mg soil concentrations may have fostered multiple events of colonization of ultramafics. Based on a dated tree calibrated with the known age of the Macaronesian clade of the sister genus *Echium*, such events can be placed towards the limit between the Pliocene and Pleistocene (ca. 2.5 mya).

## Introduction

With about 335 endemics of which 123 obligate, the Balkan peninsula is the main centre of diversity for serpentine flora in Europe (Stevanović et al., 2003). From an evolutionary point of view, however, little is known about the origins of such ecologically specialized lineages. Molecular phylogenetic studies have been recently performed in tribes Lithospermeae (Boraginaceae) and Alyssaceae (Brassicaceae) to understand whether serpentine specialization was the result of independent evolutionary events or an ecological synapomorphy inherited from a common ancestor (Cecchi and Selvi, 2009; Cecchi et al., 2010).

In the present paper we focus on a similar question using the genus *Onosma* as model system, since this large group of Boraginaceae includes the highest number of taxa able to grow on ultramafics of southeast Europe and Anatolia. Six obligate endemics and five facultative serpentinophytes occur in the rugged mountain areas of the southern Balkans. Their distribution ranges are largely allopatric, while close geographical proximity occurs with non-tolerant congeneric species. Such a condition represents an ideal system to investigate whether endemics share a common ancestry or originated from multiple speciation

events and different progenitors. Accordingly, we used nrDNA ITS sequences and a phylogenetic approach to open a window on relationships of serpentine endemics and on their possible age using recent dated phylogenies of Boraginaceae-Lithospermeae,

## Materials and methods

A sample of 31 accessions of *Onosma* including all known serpentine-tolerant species in Europe and a selection of west Anatolian and Aegean taxa was analysed for nr DNA ITS1 sequences. Plant material came from field collections by the authors and herbarium loans. Details of DNA isolation and amplification follow Cecchi et al. (2010).

After visual inspection of the sequences and their multiple alignment, a Bayesian inference of phylogeny was performed including additional 27 accessions of *Onosma* and 42 of the sister genus *Echium* that were retrieved from GenBank. Phylograms were rooted using members of *Moltkia* Lehm. and *Cerintho* L. (Lithospermeae) as outgroups. Details of the analysis follow Cecchi et al. (2010). A time frame for the main nodes in the Bayesian consensus phylogram was estimated using the most likely age of the Macaronesian clade of the sister genus *Echium* as calibration point (Mansion et al., 2009).

## Results

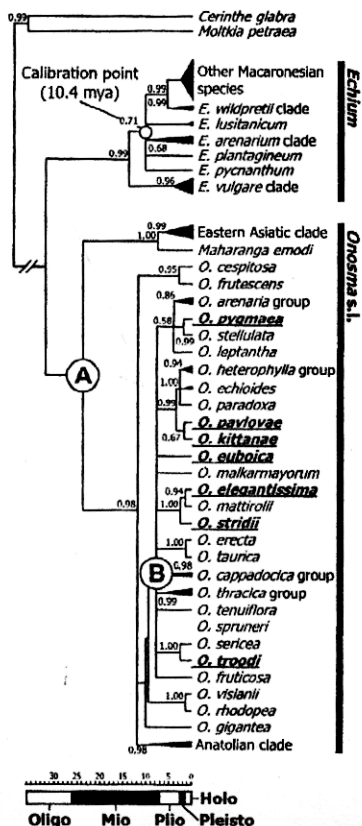


Figure 1. Dated bayesian tree from ITS1 alignment of *Onosma* and *Echium* (118 accessions). Serpentine obligate endemics are in bold and underlined. Numbers near nodes are Bayesian Posterior Probabilities.

The Bayesian consensus tree (Figure 1) showed monophyly of *Onosma* and its early split in the two main branches of east Asiatic and Tethyan species, that possibly diverged in the early Miocene (A; ca. 24 mya). ITS1 sequences within the latter group provided partial resolution of relationships due to a relatively low rate of variation. This suggests rapid radiation in the Euro-Mediterranean region, the bulk of which has likely occurred around the Mio-Pliocene limit (B). Obligate endemics are

distributed in at least five different lineages, four continental and one insular (*O. troodi*, Cyprus). None of them seem to have originated before about 2.5 mya. Serpentine clades also included facultative serpentinophytes and/or non-tolerant taxa growing on basic substrates, mainly limestone or Mg-rich soils such as dolomite. An example is the *O. elegantissima* clade that also included calcicolous *O. mattirolii*.

## Discussion

Lack of a common ancestor and of geographic cohesion in the serpentine clades suggested polyphyletic and polytypic evolution on the different outcrops in south-east Europe. Preference for non-serpentine habitats is suggested as the ancestral condition in *Onosma*, but constitutive preadaptive traits such as drought tolerance, and ability to cope with high Mg concentrations in the substrate have likely promoted multiple events of colonization of the ophiolitic outcrops. Age estimates suggest that divergence of the serpentine lineages has started on the Plio-Pleistocene limit, when the warm ophiolitic outcrops may have acted as "insular" refugia during the first cold climatic phases. Divergent adaptive evolution of the colonizing lineages has then proceeded under the combined effects of isolation and selective pressure of soil constraints.

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