Evolution on continental and oceanic islands in the Aegean Archipelago: insights from the *Roucela* clade (*Campanula*, Bellflowers)

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INTRODUCTION

The *Roucela* complex is a group of 12 rare, mostly narrow endemic bellflower species found primarily in the Aegean Archipelago[1] (Fig. 1). Little is known about plant evolution in these continental island systems[2,5]. This region has a complex, but well understood, climatic and geologic history with numerous sea-level changes and tectonic events causing connections and disconnections between islands and the mainland through time.

Here we utilize molecular dating, diversification, and niche modeling techniques to test hypotheses regarding historical divergence and better understand current distributions of bellflowers in the region. We conclude that diversification of the group on continental islands is the result of tectonic events and sea-level changes causing the break up of an ancient landmass connecting present day Greece and Turkey. Conversely, dispersal and *in situ* diversification seem to be driving the patterns we observe on the oceanic island of Cyprus. Furthermore, we find evidence that the shift from sub-tropical to a Mediterranean climate may have had a negative impact on these species.

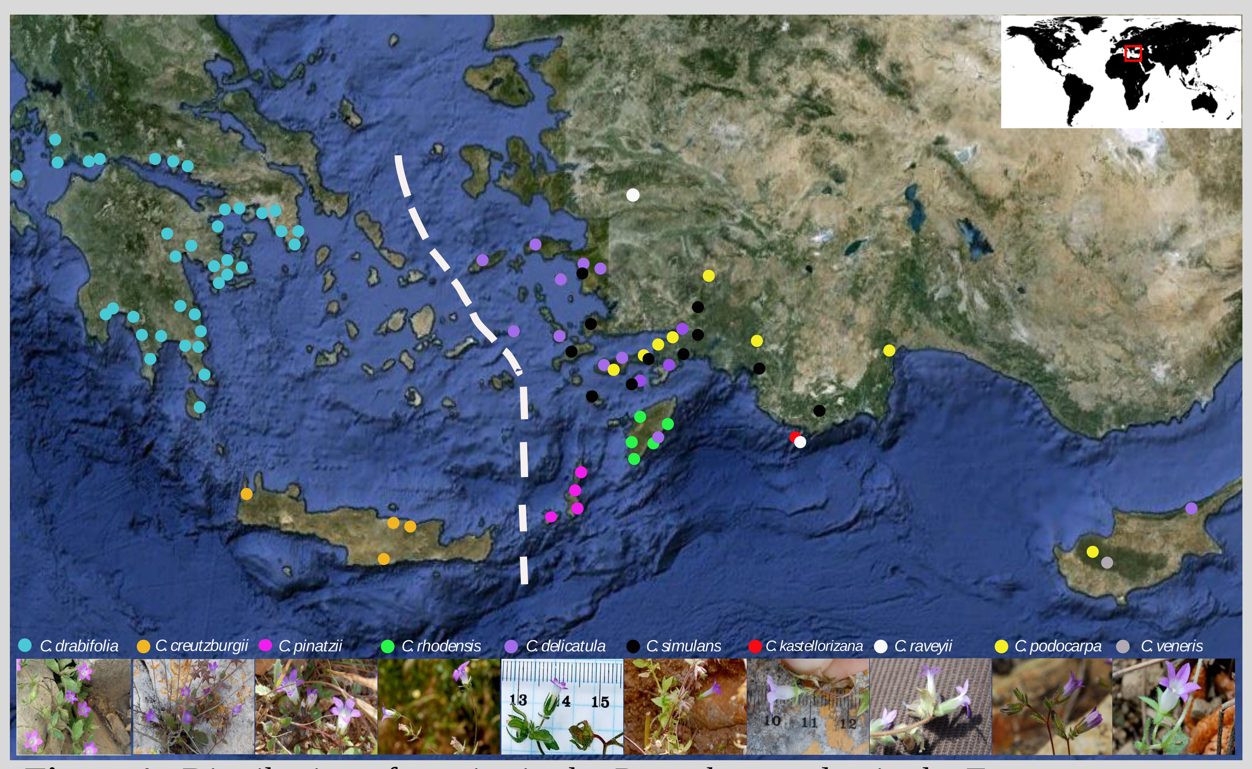

Figure 1. Distribution of species in the *Roucela* complex in the Eastern Aegean. Dashed line represents mid-Aegean Trench.

MATERIALS & METHODS

- Taxa were collected from the Aegean Archipelago, Cyprus, and the mainland of Greece (Fig. 1). Turkish taxa were sampled from herbarium material.
- Five plastid markers and two nuclear loci were sequenced.
- Dating analyses were carried out with BEAST[3] within the context of the larger Campanuloideae clade in order to utilize a fossil calibration and a root prior.
- Niche modeling analyses were based on field observations, species occurrence data gathered from GBIF, and herbarium specimens and analyzed with Maxent[4].
- Diversification analyses were conducted using the R packages APE, LASER, and GEIGER.

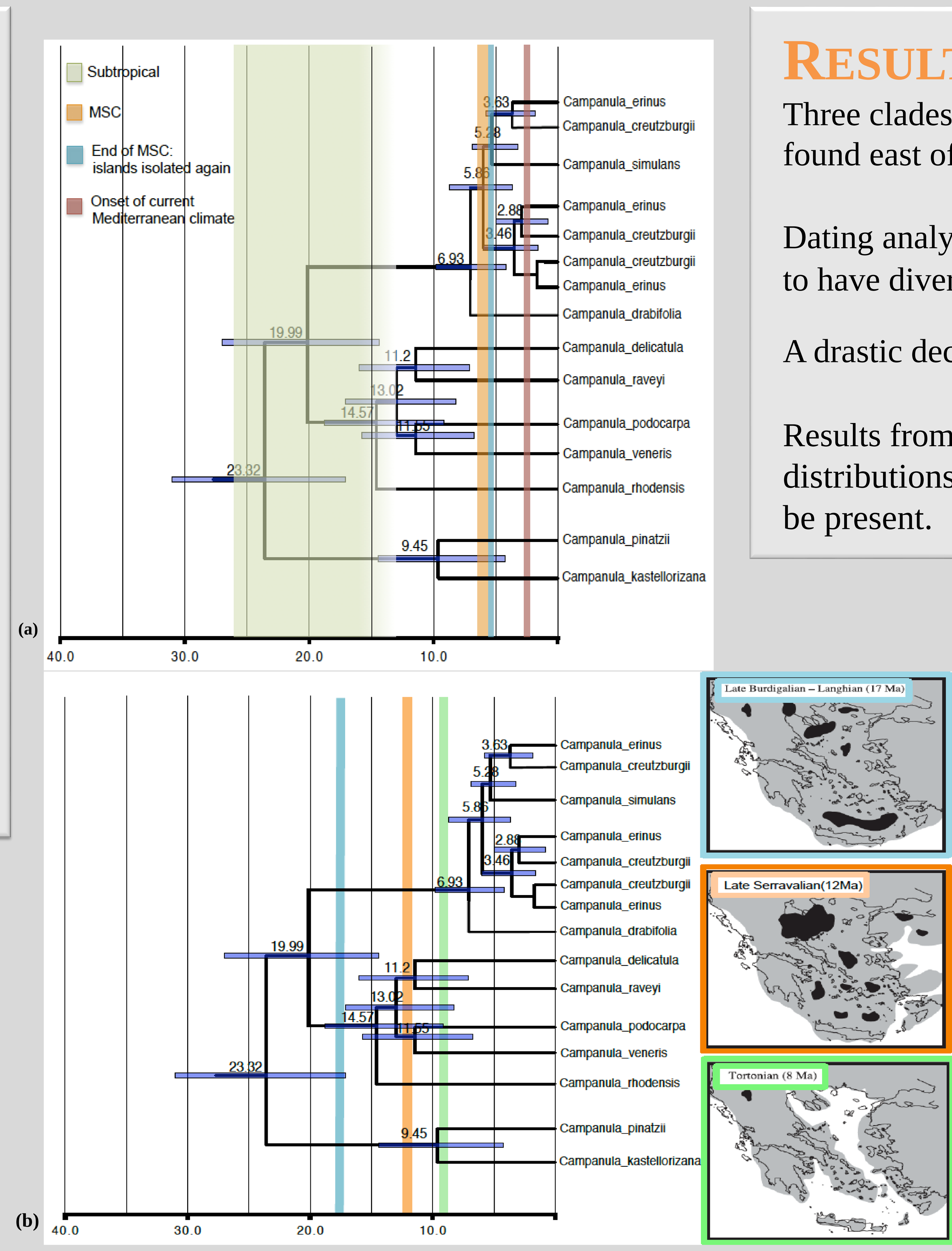

Figure 2. Divergence times estimated for the *Roucela* complex with BEAST. Major climatic (a) and geologic (b) events in the Mediterranean Basin have been overlaid onto the chronograms. Horizontal blue bars represent 95% highest posterior density intervals.

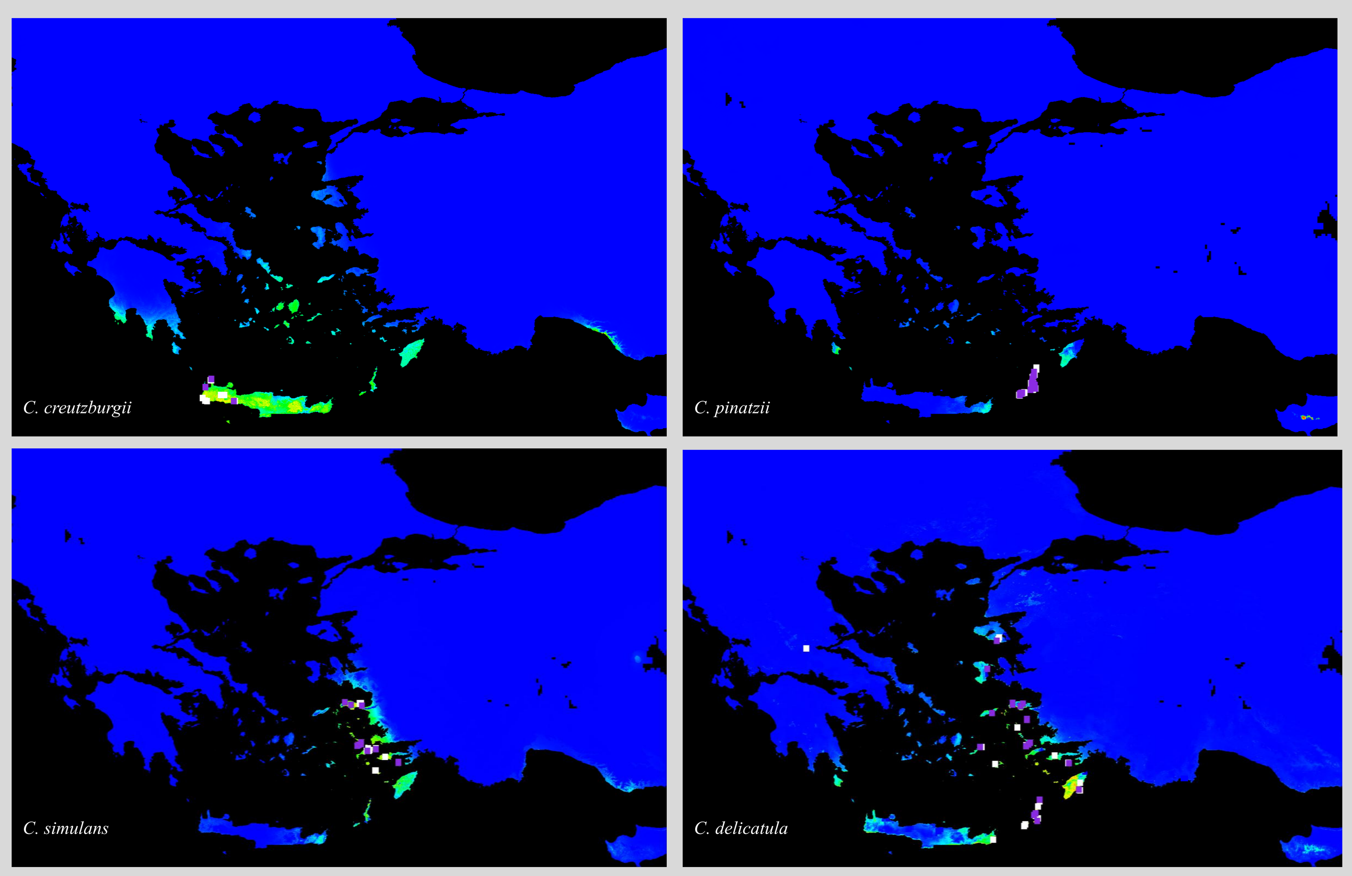

Figure 3. Distribution modeling maps for three *Roucela* species. Purple/white dots represent current localities. Green/yellow colors indicate suitable habitats – potential areas each species could colonize based on climatic variables. In all cases, potential distributions exceed realized distributions for these species.

RESULTS

Three clades are recovered within a monophyletic *Roucela* (Fig. 2). A grade composed exclusively of taxa found east of the mid-Aegean trench is sister to a clade that includes primarily taxa found west of the trench.

Dating analyses found the *Roucela* clade to have originated approximately 23 MYA. The eastern clades appear to have diversified 14-9 MYA while diversification of the western clade began approximately 6 MYA.

A drastic decrease in diversification rate beginning *ca*. 8 MYA was inferred (Fig. 4).

Results from niche modeling analyses, based on climatic data, indicate potential distributions exceed realized distributions for all tested species (Fig. 3) – suggesting dispersal limitations (either current or historical) may be present.

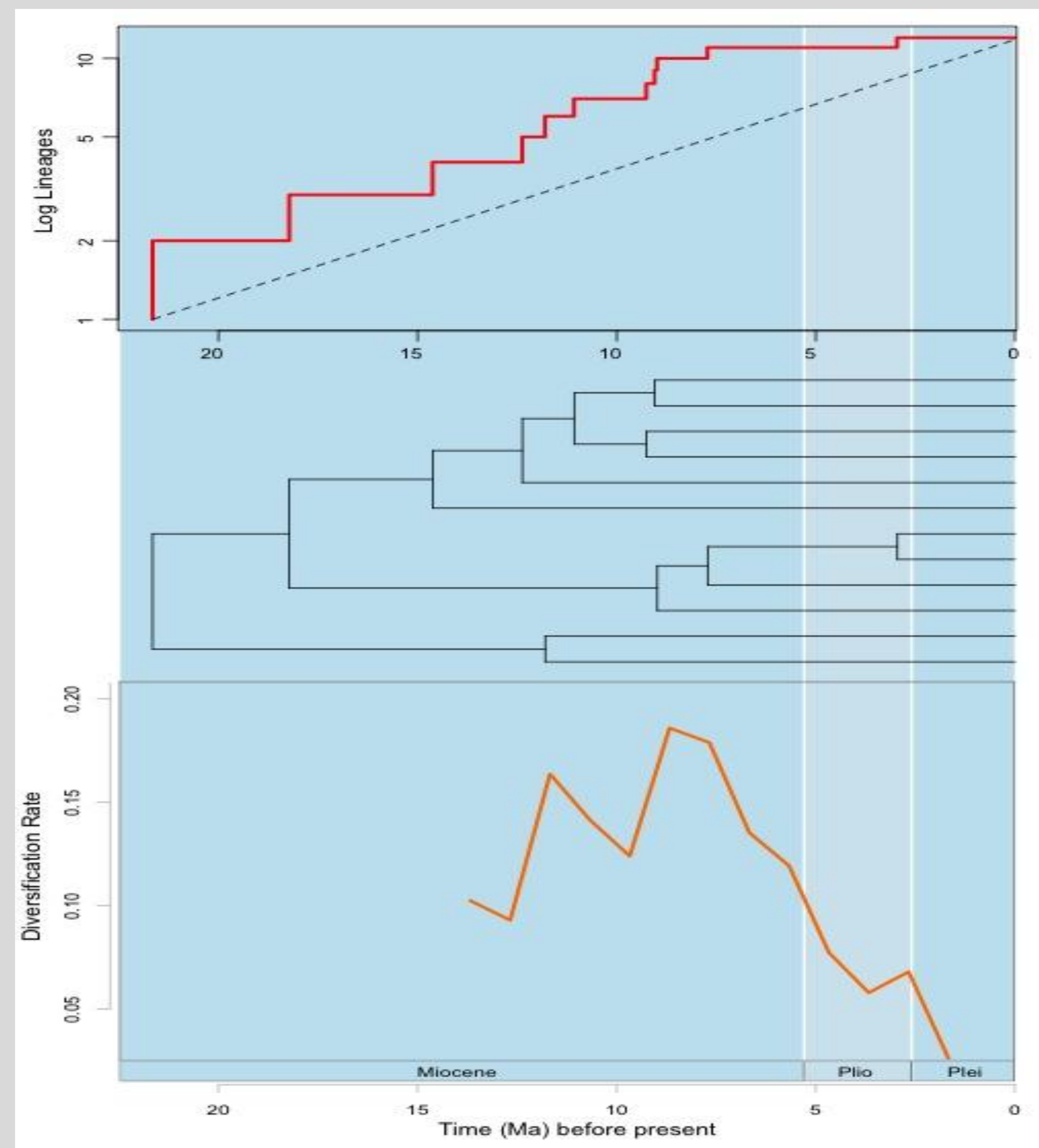

Figure 4. Results from diversification analyses for the Roucela clade. Upper panel shows the lineage-through-time plot for the group and the lower panel illustrates how the diversification rate has changed through time for this clade. This graph highlights the dramatic decrease in diversification beginning approximately 8 MYA – corresponding to major climatic shifts in the Mediterranean Basin.

SIGNIFICANCE

Contrary to numerous past studies, the onset of the Mediterranean climate has not promoted diversification in the *Roucela* complex and, in fact, may be negatively affecting these species. This finding has important implications for conservation as recent climate models predict a trend towards decreased precipitation in the Mediterranean Basin – potentially increasing the risk for extinction of these taxa as the region departs further from it's historically sub-tropical climate.

CONCLUSIONS

Molecular dating analyses (Fig. 2) illuminate specific past geological events that have been important in driving diversification within this group. Island isolation caused by the break up of the Aegean landmass during the Mid-Miocene is likely responsible for divergence of the eastern taxa (Fig. 2b) while the Messinian Salinity Crisis seems to have played a role in the evolution of species occurring west of the trench (Fig. 2a). The presence of three species on the oceanic island of Cyprus is the result of two dispersal events and one *in situ* speciation event.

We find the *Roucela* clade to be much older than predicted by often made claims that speciation in the Mediterranean is largely recent and driven by the onset of the Mediterranean climate (2 MYA). Conversely, we inferred a decrease in diversification rate from 8 MYA to the present (Fig. 4), during the shift from sub-tropical to a Mediterranean climate, suggesting either decreased rates of diversification or increased extinction during this period.

LITERATURE CITED

[1]Carlström, A. (1986) A revision of the *Campanula drabifolia* complex. *Willdenowia*, **15**, 375-387. [2]Cellinese, N., Smith, S.A., Edwards, E.J., Kim, S.-T., *et al.* (2009) Historical biogeography of the endemic Campanulaceae of Crete. *Journal of Biogeography*, **36**, 1253-1269. [3]Drummond, A.J., Suchard, M.A., Xie, D. & Rambaut, A. (2012) Bayesian phylogenetics with beauti and the beast 1.7. *Molecular Biology and Evolution*, **29**, 1969-1973. [4]Phillips, S.J., Anderson, R.P. & Schapire, R.E. (2006) Maximum entropy modeling of species geographic distributions. *Ecological Modelling*, **190**, 231-259. [5]Mansion, G., Parolly, G., Crowl, A., *et al.* (2012) How to handle speciose clades? Massive taxon-sampling as a strategy towards illuminating the natural history of the bell flowers (Campanula, Campanuloideae). *PLoS ONE*, **7**.