



FREDERICK RESEARCH CENTRE

Vulnerability of narrow endemic *Cedrus brevifolia* from Cyprus: Detection of genes and phenotypic trait diversity linked to adaptation

Project Code: DIDAKTOR/0609/13

Deliverable 4



Submission of three publications to international journals.

Vulnerability of narrow endemic *Cedrus brevifolia* from Cyprus: Detection of genes and phenotypic trait diversity linked to adaptation

The European Commission in 2013 published the “Research and Innovation performance in EU Member States and Associated Countries” which indicates that in terms of research output, Cyprus had the fourth lowest number of scientific publications in the EU, in 2010. The current project’s aim of raising the research profile of Cypriot researchers, and of the scientific committee in Cyprus in general, is achieved by the publication of scientific results to pre-reviewed journals. The project DIDAKTOR/069/13 aims to submit three original articles to pre-reviewed scientific journals.

	<u>Article 1</u>	
Proposed title	Annotation of adaptive genes from de novo transcriptome assembly for the island endemic vulnerable species of <i>Cedrus brevifolia</i> Henry (Pinaceae)	
Scope	The current manuscript presents in detail the work flow for choosing a subset of gene fragments located in the low repeated fraction of the <i>Cedrus</i> genome, including polymorphism previously detected in <i>Cedrus atlantica</i> . Thus, a valid sequencing transferability between the congener <i>Cedrus</i> spp. (i.e. <i>Cedrus brevifolia</i>) could be a key parameter conditioning the success of the experiment. Two major considerations were adopted in genes fragment transferability: (i) selecting those located in coding and low repeated regions and (ii) filtering them according to the number of homologous sequences found within genome/transcriptome assemblies. The present work aims to present a protocol of annotation and re-sequencing of the genes fragments for non-model species characterised by high ecological significance. The expected results from this study will be powerful tools in the area of molecular markers for further ecological and genetic studies of an endemic island species such as the <i>Cedrus brevifolia</i> .	
Software		<u>Statistical analysis / Analysis level</u>
	<i>CodonCode Aligner software</i>	Coding sequences / sequences per sample
	<i>BioEdit Sequence Alignment Editor</i>	Alignment of gene / whole sample size
	<i>DnaSP V.5</i>	Calculation of SNP / Indel absolute value

	<u>Article 2</u>	
Proposed title	Assessment of morpho-anatomic characteristics of <i>Cedrus brevifolia</i> Henry needles: a tool for	

	detecting species local adaptation.		
Scope	This manuscript is the first in-depth investigation on the morpho-anatomical properties and the patterns of the needles in the natural population of <i>Cedrus brevifolia</i> in Cyprus (investigation at the population level). The ultimate goal is to extend the knowledge on the ecological adaptation processes adopted by this precious and vulnerable species in response to different geographical locations.		
Software		<u>Statistical analysis</u>	<u>Analysis level</u>
	SPSS	Traits variance	Within sampling plots / Within site
		ANOVA	Among groups (sites and sampling plots)
		Euclidean distance	
	NTSYS-PC v. 2.0	Visualization of the classification patterns of pairwise Euclidean distance carried out by the unweighted pair group method with arithmetic average (UPGMA) for both morphological and anatomical traits.	Among sampling plots
	FactoMineR	Principal component analysis (PCA) - original traits were grouped and evaluated in terms of their participation in forming the new independent components.	Morphological traits Anatomical traits
		Creation of scatter-plot based on the most variable PCA axis.	Among sampling plots Among sites
	GENELAND	MCMC: a unifying model for estimating the number of panmictic groups and locating their spatial boundaries based on the morphological and anatomical traits.	Sampling plots Tripylos site
Statistica v.7	Discriminant function analysis; - CART-style exhaustive search for univariate splits used as a split selection	Assessment at the ecological level according to the altitude	

		method, with the Gini measure of goodness of fit and FACTstyle direct stopping as a stopping rule with the fraction of objects set to 0.15.	range and the diversity patterns of morpho-anatomical traits. - For sampling plots and site level.
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	<u>Article 3</u>		
Proposed title	Potential for evolutionary and demographic responses to gene diversity for narrow endemic tree species: the case of <i>Cedrus brevifolia</i>.		
Scope	This manuscript aims to investigate population genetic aspects and population evolution factors that influence the gene diversity and population structure of <i>Cedrus brevifolia</i> . For the implement of these purposes the following parameters are addressed: (i) the estimation of nucleotide diversity in the natural population of <i>C. brevifolia</i> and particularly within and among the five nowadays identified regimes of this population, (ii) to discover SNPs in each of the putative candidate genes (those that may be under natural selection) and to determine whether the genes found can potentially be under selection and (iii) to assess the genetic diversity within and among the sampling plots (and sites).		
Software	DnaSP v.5	<u>Statistical analysis</u>	<u>Analysis level</u>
		Gene diversity	- Nucleotide diversity θ_w and π - Pairwise nucleotide differences among sequences - Heterogeneity of sequence variation across loci
		Neutrality test	Statistical analyses were conducted to identify genes or amino acid sites departing from the standard neutral model

			<p>of evolution.</p> <ul style="list-style-type: none"> - Ewens–Watterson F - Tajima's D - Fay and Wu's normalized H - Kelly's Zns
		Linkage Disequilibrium	<p>Among SNPs for each gene:</p> <ul style="list-style-type: none"> - Total sample size - Per site
	PHASE	Reconstruction of the haplotype (allele) phases from unphase data. This haplotype reconstruction was conducted using a coalescent-based Bayesian method to infer the haplotypes.	Gene level
	Fstat	Observed number of alleles / allelic richness / expected heterozygosity / inbreeding coefficient	Within-plots allele diversity
		Two-sided test	Among sites
	Structure 2.3.4	MCMC: a unifying model for estimating the number of panmictic groups based on genetic data.	<p>Sampling plots</p> <p>Tripylos site</p>
	Arlequin 3.5	observed heterozygosity	<p>Within sampling plots</p> <p>Within site</p>
		AMOVA	Among groups (sites and sampling plots)
		F_{ST} pairwise distance	Sampling plots