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Enhancing *Quercus cerris* identification via DNA barcoding and new PCR primers

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INTRODUCTION

In an increasingly **urbanized** world, the significance of oak trees in city environments is becoming more pronounced. **Oaks** continue to offer essential ecological, aesthetic, and social benefits, yet they face unique challenges from urban stressors such as restricted root space and poor soil quality. Understanding the adaptability and resilience of oak roots is crucial, as roots are vital for nutrient and water absorption, and for anchoring trees in often-altered urban soils. However, studying roots in urban settings is challenging due to their underground nature and the complexities of the urban environment. Molecular techniques like **DNA barcoding** present new opportunities to distinguish tree species and explore the biological diversity associated with their **roots**. This study aims to apply DNA barcoding to identify oak species and their abiotic and biotic interactors in urban contexts. Specifically, we propose the design of **PCR primers** for species-specific DNA barcoding of ***Quercus cerris***.

METHODOLOGY

1 **Comparable sequences research**

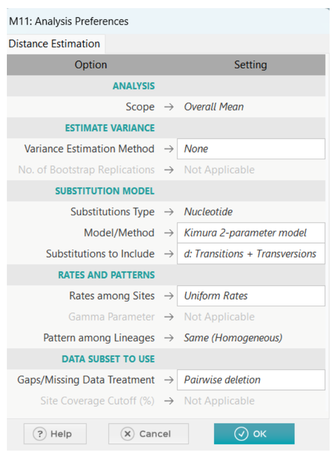
2 **Edit and sequence alignment**

3 **Analysis of sequence characteristics**

MEGA Molecular Evolutionary Genetics Analysis

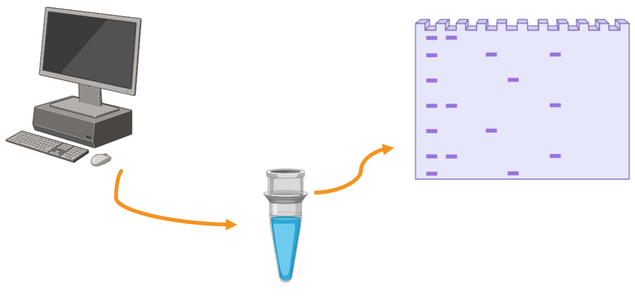
Determination of genetic divergence
+
Identification ability of candidate barcodes

4



Primer Design
(*In-silico* validation + *In-wet* validation)

5



RESULTS

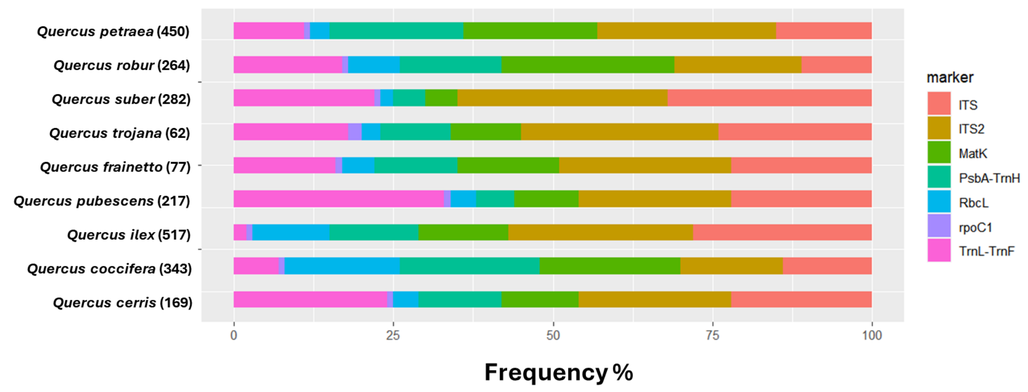


Figure 1. Count of nucleotide sequences for species and marker, in brackets the total number of sequences for species

Marker	RbcL	ITS	ITS2	PsbA-TrnH	MatK	rpoC1	TrnL-TrnF
Sequence length(bp)	310-1449	323-601	95-224	262-458	239-1515	500-554	135-389
Alignment length(bp)	1449	668	251	503	1515	619	404
GC content(%)	41.9-45.5	51.4-66.9	50.3-70.7	21.6-29.4	29.3-36.9	42.8-43.7	25.9-31.4
Conserved sites	1428	223	70	375	1480	594	289
Variable sites	21	420	171	87	35	4	107
Informative sites	15	296	133	35	23	2	61

Table 1. Sequence characteristics of candidate barcodes

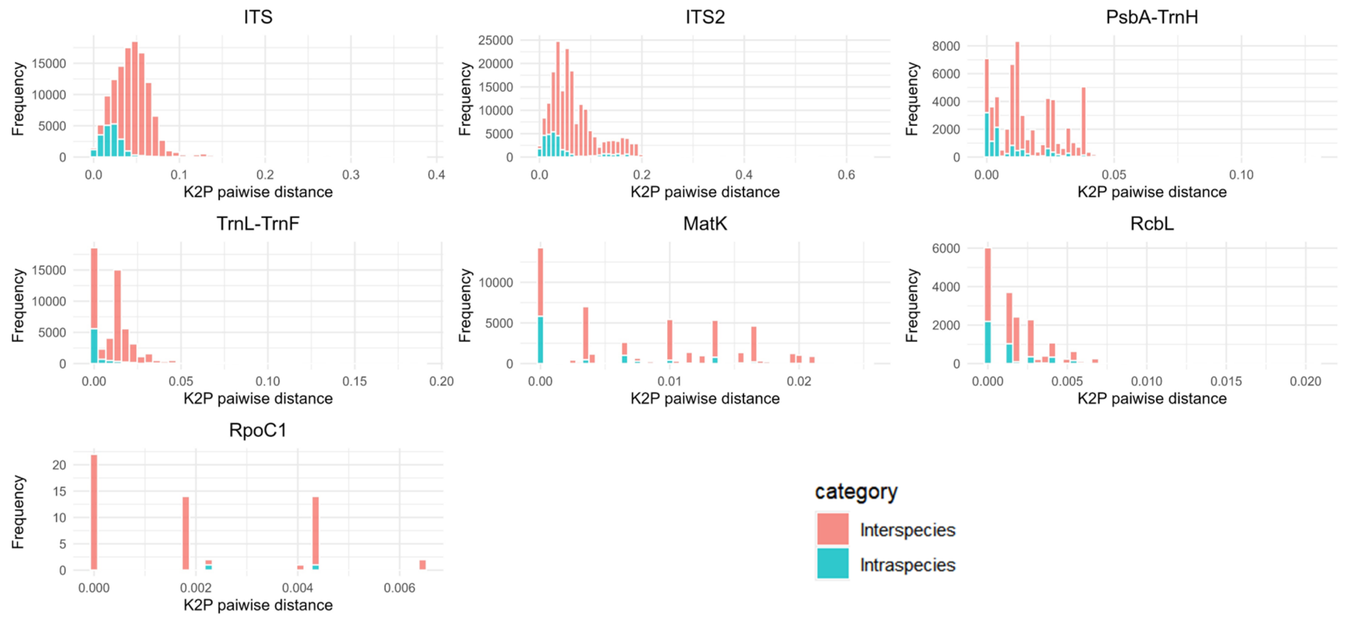


Figure 2. Distribution of K2P pairwise distances for candidate barcodes of *Quercus sp.*

marker1	marker2	W+	W-	N	results	p-value
ITS2	ITS	10429.5	2936.5	163	ITS2>ITS	5,335E-07
ITS	PsbA-trnH	10333	1448	153	ITS>PsbA-TrnH	5,755E-13
PsbA-trnH	TrnL-TrnF	662.5	822.5	54	PsbA-TrnH=TrnL-TrnF	0.4935
TrnL-TrnF	MatK	2015	400	69	TrnL-TrnF>MatK	0,001364
MatK	RbcL	774.5	171.5	43	MatK>RbcL	0.0002784
RbcL	rpoC1	153	0	17	RbcL>rpoC1	0.0003198

Table 2. Wilcoxon signed rank test for interspecific variations between different sequences

marker	ID	length(bp)	Tm(C°)	GC%	product length(bp)	*In-silico* validation	*In-wet* validation
ITS	Q.C-1180-ITS_F	17	62	82.35	361	✓	✓
	Q.C.-1532-ITS_R	18	62	72.22			
ITS2	Q.C.ITS2_M_F	17	68.57	88.24	181	✓	✓
	Q.C.ITS2_1120_R	22	68.7	68.18			
PsbA-TrnH	Q.C.-111-PsbA-trnH_F	23	64	39.13	408	✓	✗
	Q.C.-10-PsbA-trnH_R	19	64	68.42			

Table 3. *Q. cerris* primers designed and validated using automated bioinformatic tools

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