STUDY ON GENETIC DIVERISTY AND HYBRIDISATION OF THE CRETAN DATE PALM *Phoenix theophrasti* IN THE NATURAL PALM GROVES OF CRETE, GREECE, INCLUDED IN THE LIFE PHOENIX PROJECT

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1. Introduction

The genus *Phoenix* (Arecaceae family) comprises a group of palms that are among the most recognizable and culturally significant in the Old World. Despite their widespread use and iconic appearance, species within this genus are notably difficult to distinguish because they share a very similar overall morphology (Barrow 1998). As a result, taxonomic delimitation has been historically challenging. Today, most authors recognize approximately 14 species within the genus, though some debate persists due to the high degree of morphological overlap and the frequent occurrence of natural hybrids.

Phoenix species are distributed across a broad geographical range extending from the Atlantic Islands (the Canary Islands and Cape Verde), Madagascar, the Mediterranean Basin (including Crete, Turkey, and the Iberian Peninsula), and further east into the Arabian Peninsula and southern parts of Asia. Their habitats typically include semi-arid to arid environments, where their remarkable tolerance to drought, salinity, and high temperatures allows them to thrive. Some species, such as *Phoenix theophrasti* in Crete and southwestern Turkey or *Phoenix reclinata* in sub-Saharan Africa have more restricted and fragmented distributions, while others like *Phoenix dactylifera* have expanded widely due to cultivation.

A notable feature of the genus is its high ability for hybridization (Gros-Balthazard, 2013). Both natural and human-mediated hybridization events have been documented, particularly involving *P. dactylifera* (Flowers et al., 2019) and island endemics such as *P. canariensis* (Saro et al. 2015, 2019, 2024). In many regions, the introduction of ornamental palms or agricultural cultivars has led to genetic introgression, posing a threat to the integrity of local endemic taxa. This tendency to hybridisation complicates species identification, disrupts unique gene pools, and may ultimately threaten the conservation of narrow-range species.

Phoenix theophrasti (Cretan date palm) is a rare and endemic species of south-eastern Aegean Archipelago with the largest populations found on Crete. The species is distributed mainly along sandy beaches, river mouths, and other coastal habitats. Its natural populations are small and often isolated, ranging from only a few individuals to a few dozen, with the largest stand located in Vai, in eastern Crete. Although these habitats are included in the Natura 2000 network, *P. theophrasti* continues to face pressures from human activity, climate-driven drought, and, in the case of the Vai palm forest, the presence of exotic *Phoenix* species planted nearby. *As with all Phoenix species, P. theophrasti* is also capable of forming viable hybrids with both *P. dactylifera* and *P. canariensis*. While hybridization is thought to be rare in

most natural populations due to their isolation, the coexistence of cultivated *Phoenix* species near Vai raises concerns regarding the genetic purity of the native palm forest. In this context, the LIFE *Phoenix* project conducted a genetic study to evaluate both the genetic diversity and the possible presence of hybrids within the project's six populations of *P. theophrasti* on Crete.

2. Materials and Methods

Fieldwork was carried out between March and mid-May 2024 across all six natural palm groves included in the LIFE *Phoenix* project. The sampling strategy reflected the size of each population: fifty specimens were collected from Vai, whereas only three to four specimens were collected from the very small stands in Martsalo gorge and Chrysoskalitisa respectively. In total, 96 individuals were sampled and analyzed (Fig. 1, Table 1).

1. Vai
2. Agios Nikitas
3. Martsalo
4. Preveli
5. Souda Sellion
6. Chrysoskalitissa

Figure 1: Map of Crete showing the six palm populations that are included in LIFE Phoenix project. Samples were collected from all populations

Palm grove Site	Number of specimens collected
Vai	50
Agios Nikitas	7
Martsalo	5
Preveli	28
Souda Sellion	4
Chrysoskalitissa	2

Table 1*:* Number of sampled specimens from each of the six palm grove areas included in the project.

For each specimen, an identification code and basic ecological data (height, coordinates, substrate) were recorded following the project's protocol for leaf collection and DNA extraction. From each palm, 3-4 leaflets were cut from a single leaf. The height of the palms can limit sampling, therefore in such circumstances smaller individuals were selected and sampled. Each sample was further cut into smaller pieces and placed in zip bags with silica gel to ensure preservation. Morphological checks were also performed during field visits to detect any palms with atypical features suggesting hybrid origin. DNA extraction, analysis of genetic variation within Cretan populations as well as hybridization analysis were conducted by ADN Laboratories in France, using 17 microsatellite markers. The results were analyzed using two different methodologies, STRUCTURE and PCA.

3. Results

3.1 Genetic Diversity

The study for the genetic diversity found that *P. theophrasti* exhibits very low genetic variation compared to other *Phoenix* species. This is characteristic of species with small, fragmented populations and highlights a potential vulnerability to environmental change. Nevertheless, some degree of genetic differentiation was detected among populations. Population structure analysis indicated that the optimal number of genetic clusters was two (K=2) (Fig.2)

Principal Coordinate Analysis (PCoA) identified two loosely formed clusters showing the greatest genetic distance between the Vai and Preveli populations (Fig. 3). However, this differentiation was insufficient to form clearly distinct genetic clusters, as shown by both PCoA and STRUCTURE analyses.

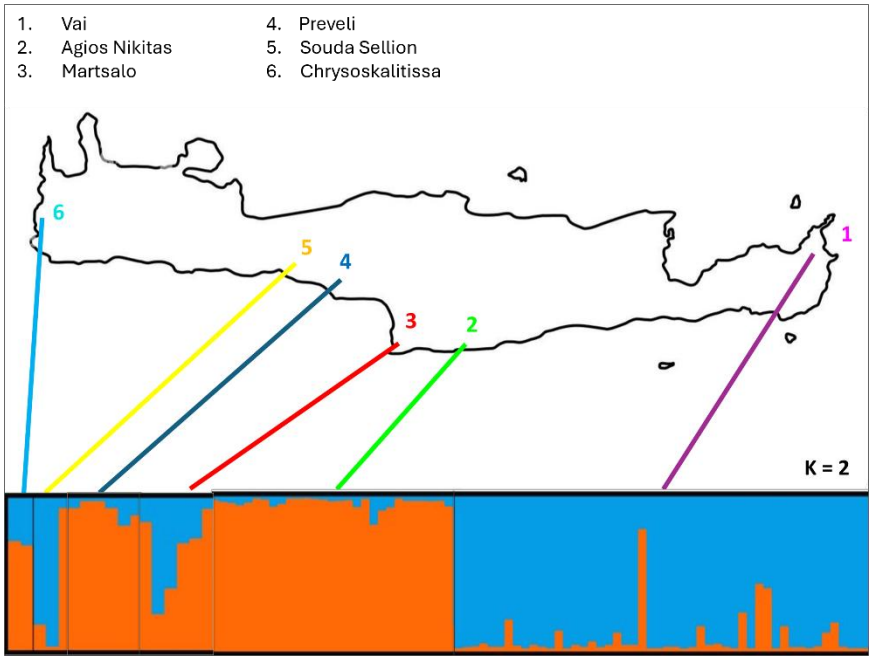

Figure 2: Genetic diversity analysis using STRUCTURE and PCoA. Two main groups are shown: The first in blue and the second in orange. Vertical bar of each cluster indicates the participation coefficient for each individual. The two-color bars represent genotypes with mixed characteristics

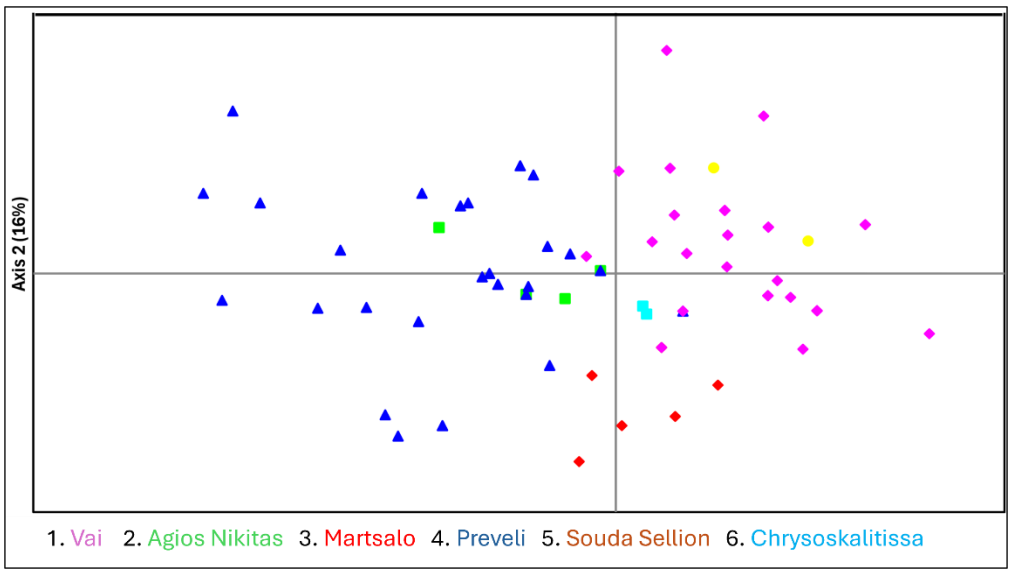

Figure 3: PCoA analysis Populations are indicated with different colours and shapes. There is an indication of two loosely clusters of the two largest populations (Vai and Preveli), whereas the rest are mixed.

3.2 Hybridization Assessment

Despite the presence of non-native *Phoenix* species in several areas—particularly near Vai—the genetic analyses revealed no evidence of hybridization between *P. theophrasti* and either *P. canariensis* or *P. dactylifera*. All 96 analysed specimens were confirmed as pure *P. theophrasti*.

4. Conclusions

The genetic study conducted under the LIFE *Phoenix* project provides critical insights into the status of *Phoenix theophrasti*. Although genetic diversity found to be low and populations – especially the isolated ones- may be at risk of reduced adaptive capacity, some degree of genetic differentiation exists between populations according to both analyses (STRUCTURE and PCoA). The first cluster mainly includes "Preveli" population, while Vai population forms the second cluster. The remaining populations exhibited mixed genetic composition, suggesting possible historical gene flow or geographical barriers that did not result in complete genetic differentiation. PCoA further strengthened the separation into two main groups, confirming the STRUCTURE results. The detection of two distinct genetic groups in Crete suggests that populations of *P. theophrasti* are not genetically homogeneous but display some degree of differentiation, thus, preserving genetic diversity is critical for the long-term survival and adaptability of the species.

Additionally, hybridization analysis showed that all six palm populations retain strong genetic integrity with no indication of hybridization events. Despite proximity to cultivated Phoenix species in Vai area, there was no sign of hybridization. Although the analyses detected no evidence of hybridisation between *Phoenix theophrasti* and other *Phoenix* species, the limited sampling covered only 50 individuals out of an estimated 5,000 in the Vai population. Hence, the presence of rare, localized hybrid individuals cannot be excluded.

These findings underscore the importance of conserving all remaining populations of *P. theophrasti*. Preserving genetically unique and geographically isolated stands is essential for maintaining the species' evolutionary potential and long-term resilience.

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