

A genetic and morphometric insight on the Sardinian white oaks populations (subgen. *Quercus*, sect. *Quercus*).

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Introduction

Several white oak species (subgen. *Quercus* sect. *Quercus*) have been identified in Sardinia Island at present. Among them, *Quercus ichnusa* (Mossa, Bacchetta, et Brullo) is regarded as an endemic species of the island (Mossa et al. 1999). In addition, other pubescent oak species (*Q. amplifolia* Guss., *Q. congesta* C.Presl., *Q. dalechampii* Ten., *Q. virgiliana* (Ten.) Ten) have been reported in some floristic and vegetation studies (Pignatti et al. 2017; Bacchetta et al. 2004). Recent taxonomic revisions, considering the aforementioned white oak taxa in other areas of the Italian Peninsula, consider these taxa as included in the general variability of *Q. pubescens* subsp. *pubescens* (Di Pietro et al. 2016, 2020 a, b, 2021; Fortini et al. 2022). This is also the position of the checklist of the Italian vascular flora (Bartolucci et al. 2024). However, a comprehensive study, integrating both morphological and genetic data across the various deciduous oak taxa described so far for the Sardinia Island, is still lacking.

In this study, 279 adult trees from 14 populations representing seven white oak taxa were analyzed (Fig. 1). In Sardinia six populations were sampled: two of *Q. congesta*, two of *Q. ichnusa*, and two of *Q. virgiliana*. For the genetic comparison, five populations of *Q. pubescens* subsp. *pubescens* from the Italian Peninsula, Spain, and Croatia and three populations of *Q. frainetto*, *Q. petraea*, and *Q. robur* were also included.

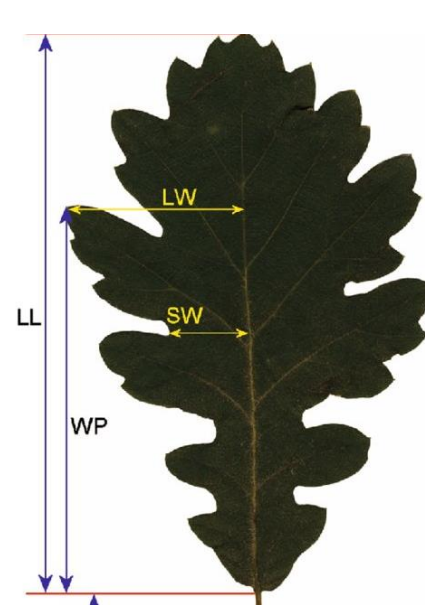


Fig. 2. Morphological leaf traits analysed in this study.

Materials and Methods

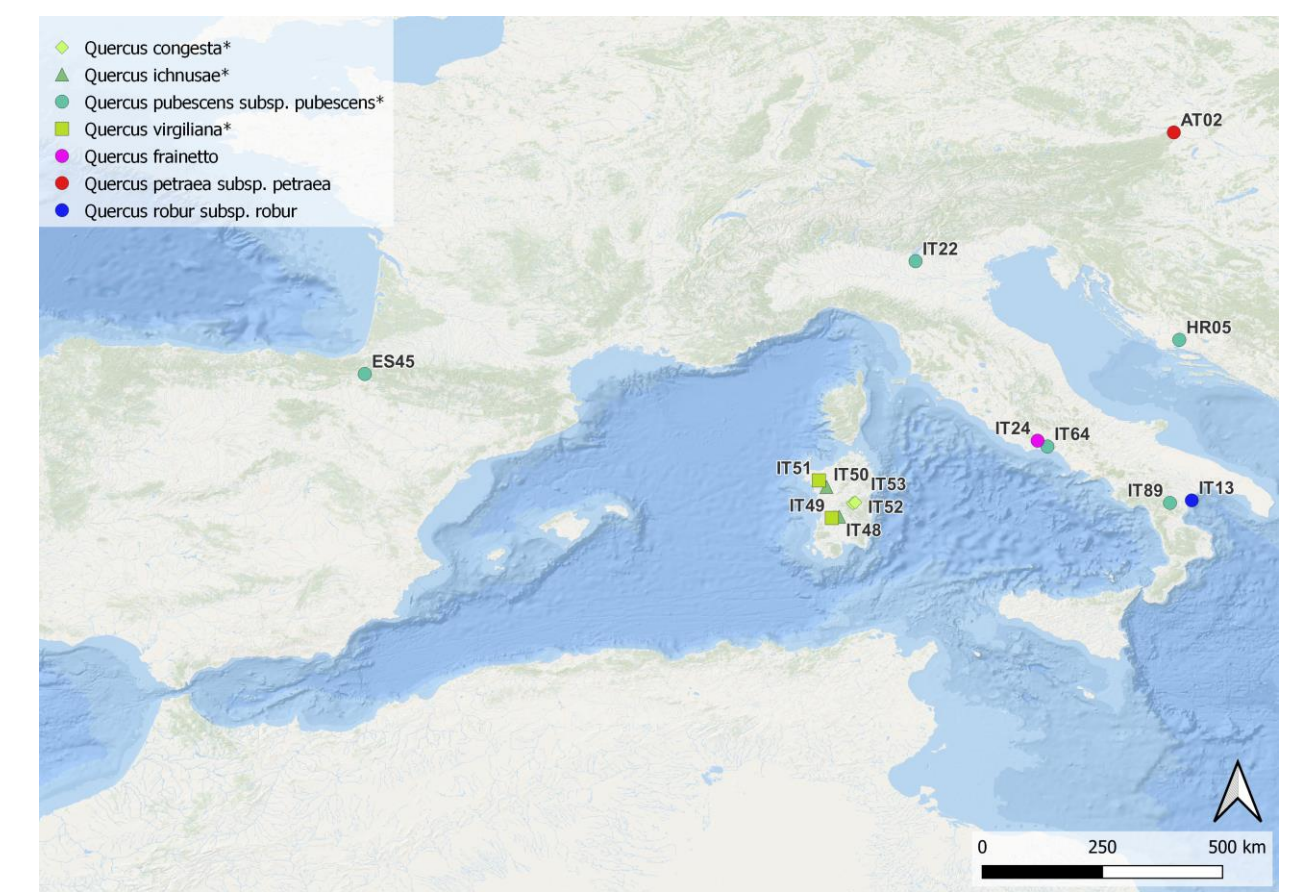


Fig. 1. Distribution map of the 14 populations of *Quercus* analysed at nuclear microsatellite markers. Taxa with asterisk (*) in the legend belong to the *Q. pubescens* collective group.

Genetic diversity and differentiation within and among populations were assessed using ten EST-SSR markers. For each population, genetic diversity indices were calculated, and population genetic structure was evaluated using Bayesian clustering methods. Genetic relationships among samples were further investigated through multivariate analyses, including UPGMA and Principal Coordinates Analysis (PCoA). In addition, the six Sardinian populations were subjected to a morphological analysis, in which twelve leaf traits were measured to explore patterns of morphological variation (Fig. 2).

Results

Results from the nuclear microsatellite markers analyses

According to Puechmaile (2016), the most likely number of genetic clusters was established in five (K=5). In the bar plot, the five clusters are depicted with distinct colours. The genetic clusters 1, 2, and 5 are mainly characterized by individuals from the populations of *Q. robur* (blue), *Q. petraea* (red), and *Q. frainetto* (magenta). In contrast, the gene pools associated with clusters 3 and 4 are distributed among the 11 populations belonging to the *Q. pubescens* group (Fig. 3).

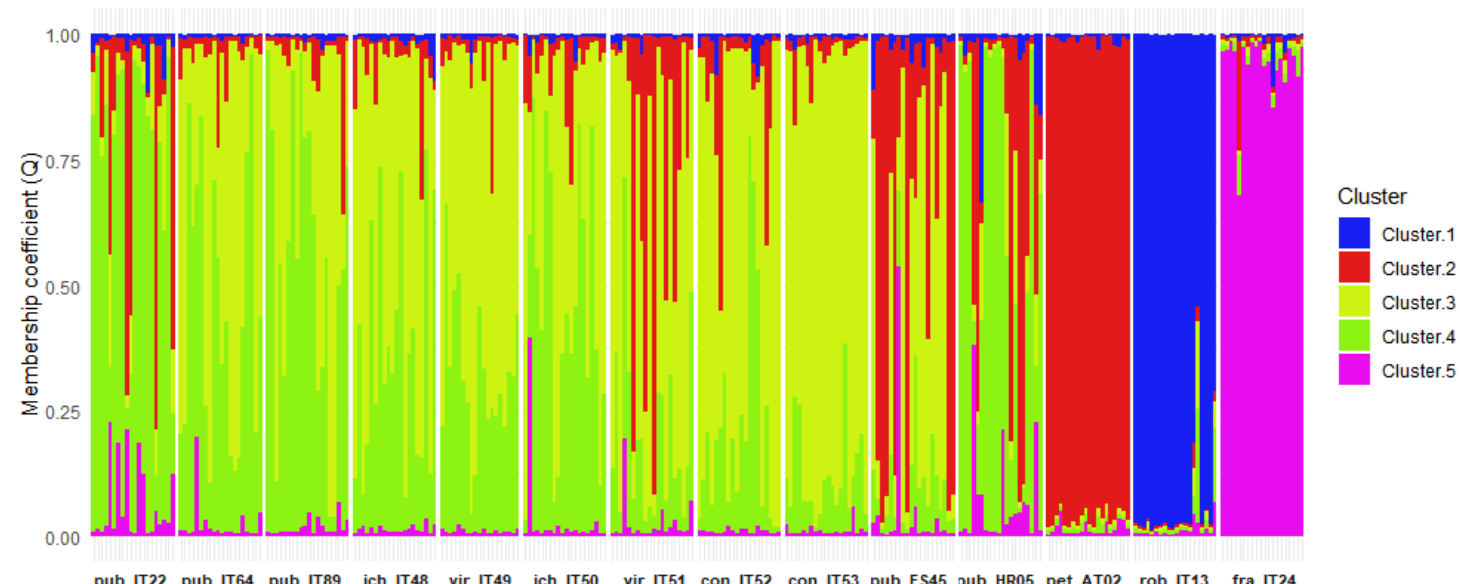


Fig. 3. Bar plot of STRUCTURE assignment results at K=5. Different colours represent each genetic cluster, while each vertical bar represents an individual with its membership coefficients (Q) across clusters. Populations are separated by white vertical lines.

In the UPGMA tree based on Nei's genetic distance, *Q. robur* and *Q. frainetto* were clearly distinct with strong support (BS=100%). *Q. petraea* was moderately separated from the *Q. pubescens* group (BS=62%). Among the *Q. pubescens* populations several weakly supported subclusters were identified. The six Sardinian populations did not group strictly by taxonomy but instead showed genetic similarity to Spanish and especially central-southern Italian *Q. pubescens* subsp. *pubescens* populations (Fig. 4).

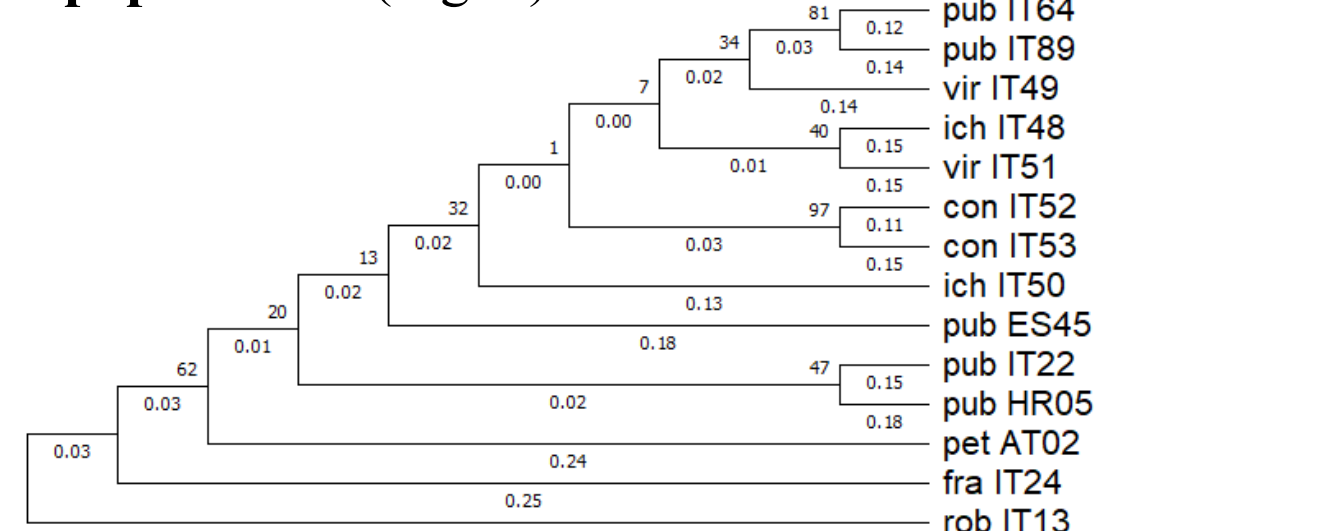


Fig. 4. UPGMA tree constructed using Nei's minimum genetic distance (1973) among the 14 *Quercus* populations. The horizontal branches indicate evolutionary changes measured in genetic divergence (number below). Bootstrap support values (from 1000 replicates) are displayed beside the branches.

The PCoA structure revealed a clear separation along the three axes between samples from the three populations of *Q. frainetto*, *Q. petraea*, and *Q. robur*, and those belonging to the *Q. pubescens* collective group. Furthermore, no separation emerged between the six Sardinian populations, based on their taxonomic identity (Fig. 5).

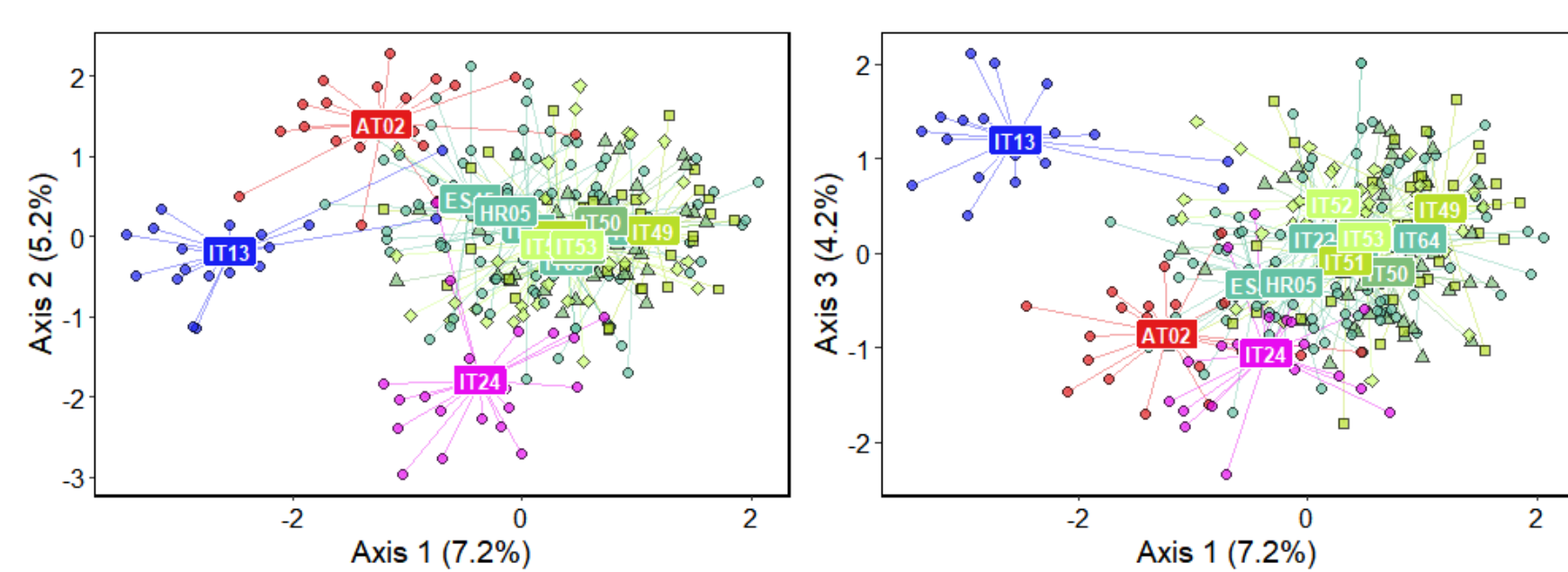


Fig. 5. Three-dimensional Principal Coordinate Analysis (PCoA) scatterplots of the 14 *Quercus* populations genotyped at 10 microsatellite loci. PCoA1 vs. PCoA2 (on the left) and PCoA1 vs. PCoA3 (on the right).

Results from the morphometric analyses

The nonparametric Kruskal-Wallis test revealed significant differences among the six Sardinian populations for several leaf morphological traits ($p < 0.05$), indicating variation in these characteristics across the studied populations. Post-hoc pairwise comparisons using Dunn's test with Bonferroni correction identified specific population pairs with statistically significant differences (Fig. 6).

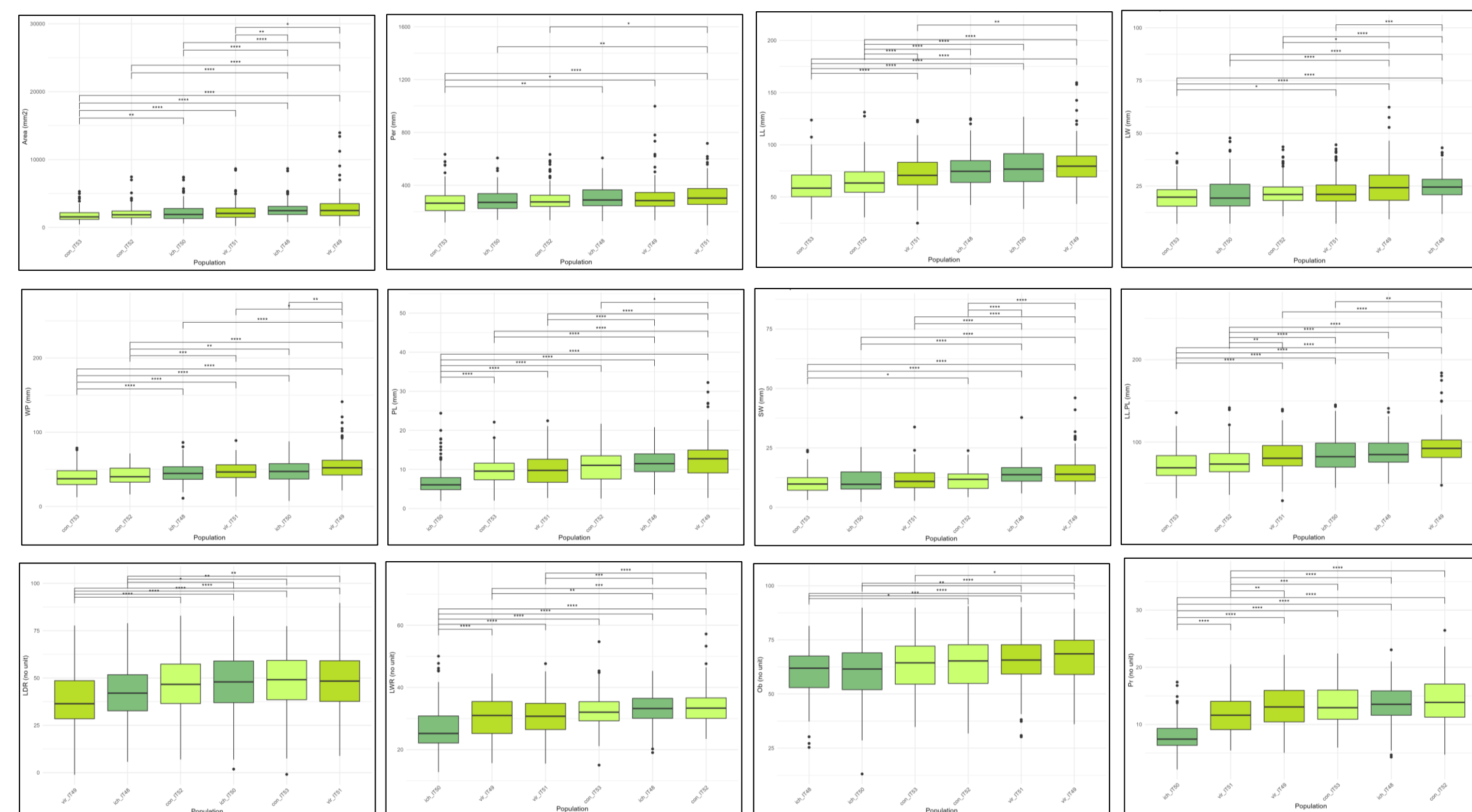


Fig. 6. Boxplots displaying the distribution of leaf morphological traits across six Sardinian populations of *Quercus*. Significant differences between pairs of populations, assessed with Bonferroni-corrected pairwise Dunn's tests after Kruskal-Wallis tests, are shown on the plots. Boxes represent the interquartile range (IQR), and the median is shown as the central line. Whiskers extend to 1.5 times the IQR, with outliers displayed as individual points. Colours denote different taxa. Area: leaf area; Per: leaf perimeter; LL: lamina length; PL: petiole length; LL+PL: lamina and petiole length; LW: lobe width; SW: sinus width; WP: widest point; LDR: lobe depth ratio; LWR: lobe width ratio; Ob: lamina shape or overbity; Pr: petiole ratio.

Discussion and Conclusion

Our results indicate that a clear genetic differentiation occurs among the four European white oak "macrospecies" (*Q. frainetto*, *Q. petraea*, *Q. pubescens*, and *Q. robur*). On the contrary, the genetic variation within the *Q. pubescens* complex is relatively low and it was found to be primarily influenced by the geographic distance between populations rather than their (real or presumed) taxonomic identity.

In particular, the Sardinian populations do not show a genetic structure consistent with the taxonomic diversification currently reported in some floristic and phytosociological studies. A similar result emerged from the morphometric analysis, in which an extensive overlap in leaf morphological trait values were found among the different populations investigated.

References

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