



# Evidence from multivariate morphometric study of the *Quercus pubescens* complex in southeast Italy

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**ABSTRACT:** The name *Quercus pubescens* s.l. encompasses a complex of deciduous oak taxa with mainly southeast-European distribution and a large ecological niche. As the easternmost region of Italy, Apulia is rather isolated from a geographical and physiographical viewpoint and counts the highest number of oak species (10). In the taxonomic and phytosociological literature, the occurrence of several species belonging to the *Quercus pubescens* collective group is reported for this region. In order to verify if different sets of morphological characters are associated with different taxa, 24 populations of *Quercus pubescens* s.l. located in different ecological-geographical areas of Apulia were sampled. A total of 367 trees, 4254 leaves and 1120 fruits were collected and morphologically analysed. Overall, 25 morphological characters of oak leaves and fruits were statistically treated using both univariate and multivariate analysis. Nested ANOVA showed that leaves collected from a single tree exhibited a degree of morphological variability higher than that observed when comparing leaves coming from different trees of the same population and from different trees of different populations as well. Almost all the morphological characters analysed exhibited a continuous trend of variation so that none of them can be used as a character to discriminate between populations. Only leaf and fruit "size" and fruit petiole length emerged as slightly discriminating characters. Our results suggest that it is unlikely that more than one species belonging to the *Quercus pubescens* complex occurs in the Apulia region. Comparison between the Apulian populations and a genetically pure *Q. pubescens* population coming from a different area (the Molise region) strengthened the assumption as to the existence of a single species that can provisionally be classified under the name of *Q. pubescens* s.l.

**KEYWORDS:** fruit, genetic assignment, leaf, morphometric data, *Quercus*, statistical analysis, taxonomy

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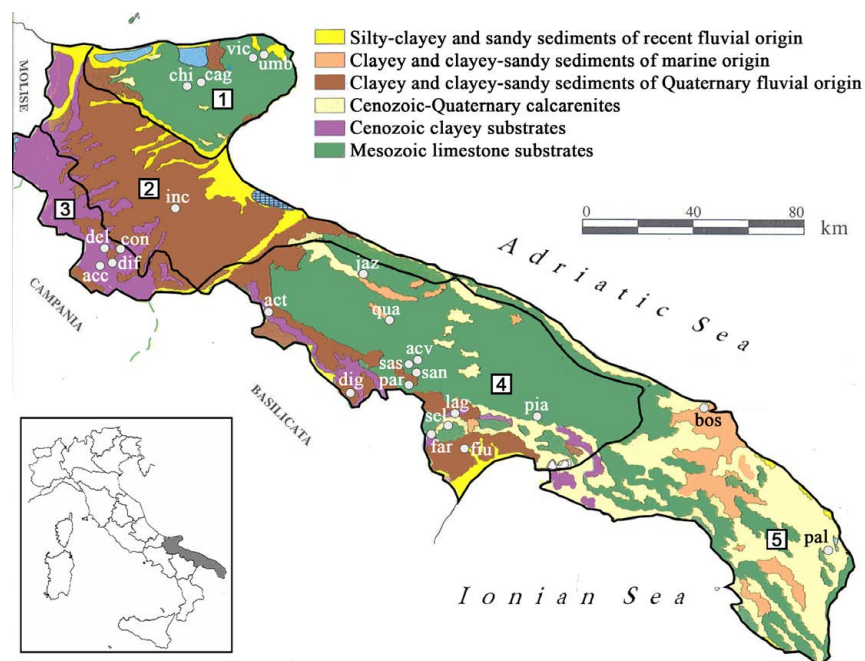
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## INTRODUCTION

Over the last 13,000 years oaks have experienced constant dynamic movement from their refugia in the south of Europe to Central, Western and Eastern Europe (BREWER

*et al.* 2002; LESBARRERES 2009). The marked climatic excursions associated with Quaternary glaciations led to the extinction of numerous oak taxa in Central Europe, whereas the milder climate enabled a higher number of oaks to survive in Southern Europe, especially within the

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**Figure 1.** Spatial distribution of the 24 populations sampled in the study area (Apulia region), three-letter codes as in Table 1. Code of physiographic units: 1 - Gargano promontory; 2 - Tavoliere delle Puglie; 3 - Daunian sub-Apennine area; 4 - Murgia plateau; 5 - Salento peninsula.

Mediterranean basin (PETIT *et al.* 2002). The taxonomic classification of oaks at the species level has always been based on observation of the macro-morphological characters of leaves and fruits (DUPOUEY & BADEAU 1993; VISCOSI *et al.* 2009a; VISCOSI *et al.* 2011; VISCOSI & FORTINI 2011), sometimes with the addition of ecological data or (especially in the last decade) micromorphological and molecular data (DUMOLIN-LAPÈGUE *et al.* 1997; BRUSCHI *et al.* 2000; BRUSCHI *et al.* 2003; SALVINI *et al.* 2008; FORTINI *et al.* 2009; FORTINI *et al.* 2013; FORTINI *et al.* 2015; VISCOSI *et al.* 2009b; CURTU *et al.* 2011; PANAHİ *et al.* 2012; YÜCEDAĞ & GAILING 2013). On the Italian peninsula, the number of oak species increases moving southwards, where the negative effects of Quaternary glaciations were only moderately experienced by the forest vegetation. Apulia is the easternmost region of the Italian peninsula. It is rather isolated from a geographical and physiographical point of view and characterised by a large number of endemic and amphi-Adriatic plant species and communities due to the trans-Adriatic land bridges which repeatedly connected the Apulian plate and the western Balkans during the Miocene (FRANCINI-CORTI 1966; FORTE *et al.* 2005; DI PIETRO & WAGENSOMMER 2008; TERZI *et al.* 2010).

Apulia is home to the greatest number of oaks in Italy (*Q. cerris* L., *Q. trojana* Webb, *Q. ithaburensis* subsp. *macrolepis* (Kotschy) Hedge & Yalt., *Q. ilex* L., *Q. suber* L., *Q. calliprinos* Webb, *Q. crenata* Lam., *Q. robur* L., *Q. pubescens* Willd., *Q. frainetto* Ten.), although there is still not complete agreement as to the precise number. According to some authors (BRULLO *et al.* 1999; BRONDI *et al.* 2004, 2010), other taxa, namely *Q. virgiliana* Ten., *Q. dalechampii* Ten. and *Q. amplifolia* Guss, should be added to the Apulian list of white oaks in place of *Q. pubescens*.

MISANO & DI PIETRO (2007) and DI PIETRO & MISANO (2009) instead referred solely to *Q. virgiliana* as occurring in the *Q. trojana* woods of southwest Apulia. In the last two decades, several works treating the taxonomy of the *Q. pubescens* complex have been published for Southern Europe. In Italy the taxonomic debate on the white oaks centers mainly around *Q. virgiliana* and *Q. dalechampii* (locus classicus: southern Italy), while *Q. amplifolia* (locus classicus: Sicily) is less considered. The point at issue in this debate is whether or not the afore-mentioned taxa are good species (see CONTI *et al.* 2005). As regards the *Q. pubescens*/*Q. virgiliana* dualism, some authors (BUSSOTTI & GROSSONI 1997; GOVAERTS & FRODIN 1998; CONTI *et al.* 2005) consider *Q. virgiliana* a doubtful taxon which should be assigned to *Q. pubescens* subsp. *pubescens*. In Croatia, TRINAJSTIĆ (2007) accepted the differentiation between *Q. pubescens* and *Q. virgiliana*, while ŠKVORC *et al.* (2005) and FRANJIĆ *et al.* (2006) confirmed the presence of *Q. pubescens* only. BALLIAN *et al.* (2010) likewise refer only to *Q. pubescens* in a study on chloroplast variability of the *Q. pubescens* complex in the western Balkans. JERSE & BATIĆ (2007) for Slovenia and SOFLETEA *et al.* (2011) and ENESCU *et al.* (2013) for Romania included *Q. virgiliana* in *Q. pubescens* on the basis of morphological and molecular analysis, while BORAZAN & BABAC (2003) for Turkey identified *Q. virgiliana* as a hybrid of *Q. petraea* and *Q. pubescens*. As regards the *Q. pubescens*/*Q. dalechampii* dualism, the situation is different. Some authors (FIORI 1923; BÉGUINOT 1927; SCHWARZ 1936-39, 1993) considered *Q. dalechampii* as belonging to the *Q. petraea* cycle, whereas others (ASCHERSON & GRAEBNER 1911; CAMUS 1938-1939; BRULLO *et al.* 1999) included it in the *Q. pubescens* cycle. The recent valid lectotypification of *Q. dalechampii* (DI PIETRO *et al.* 2012) definitively established

*Q. dalechampii* as belonging to the cycle of *Q. pubescens*.

*Quercus pubescens* s.l. woods are the most widespread deciduous forest type in the Apulian region and on the Italian peninsula as a whole. Their great ecological amplitude allows them to grow across a wide range of climatic and soil conditions, from the flooded depressions of the plains to the dry and rocky sea-facing slopes of the hilly and submontane belts. Notwithstanding this considerable potential, the Apulian *Q. pubescens* woods are limited to isolated stands at present, having been replaced by extensive olive groves and vineyards. The uncertainty as to the actual number of taxa belonging to the *Q. pubescens* complex represents a gap in our basic floristic and coenological knowledge and a limiting factor for resource management and environment-planning practices. The present paper is a contribution aimed at filling this gap by analysing morphological variation at different levels in several natural populations of *Q. pubescens* s.l. and comparing it with results obtained on a genetically pure population growing in the neighbouring Molise region.

## STUDY AREA

The Apulia region is situated at the southeastern tip of the Italian peninsula, where it is largely open to the Adriatic and Ionian Seas (Fig. 1). The region is prevalently level to slightly sloping, with more than 60% of the territory lying below 200 m a.s.l. The two mountainous areas, the Gargano promontory and the Daunian sub-Apennine area, do not exceed 1,150 m. According to BLASI & MICHETTI (2007), the bioclimate is mainly thermo-Mediterranean and meso-Mediterranean. Five physiographic units can be distinguished in the Apulia region (Fig. 1): the Daunian sub-Apennine area is composed of low mountains and gentle hills mainly developed on Cenozoic clayey and marly substrates. The Gargano promontory is a limestone massif with a succession of broad plains and low-lying hills jutting into the Adriatic Sea. The Tavoliere delle Puglie is a tableland developed on clayey-sandy Quaternary sediments of fluvial origin. The Murgia plateau is a Mesozoic limestone plateau typically characterised by extensive steppe-like grasslands and cut by very deep gorges locally called "gravines". The Salento peninsula forms the land border between the Ionian and Adriatic Seas and is mainly composed of Cenozoic calcarenites. From a phytogeographic viewpoint, the Apulia region is characterised by a high number of endemic and amph-Adriatic plant species (CONTI *et al.* 2005; LICHT 2008), especially in the dry grasslands and garrigues (BIANCO *et al.* 1988; FORTE *et al.* 2005; DI PIETRO & WAGENSOMMER 2008; DI PIETRO & MISANO 2010; TERZI *et al.* 2010; DI PIETRO & WAGENSOMMER 2014). The forest vegetation of the basal and hilly belts is characterised by evergreen woods (*Q. ilex* and *Pinus halepensis*) and thermophilous *Q. pubescens* s.l. woods. *Quercus calliprinos* and *Q. ithaburensis* subsp. *macrolepis* are restricted to southern Apulia in the

form of scattered shrublands. *Quercus frainetto* woods are extremely rare, while *Q. trojana* woods are abundant on the Murgia plateau, with *Carpinus orientalis* occurring on the bottom of the gravines. *Quercus cerris* occurs especially in the sub-montane belt of the Gargano promontory and the Daunian sub-Apennine area, together with mesophilous mixed *Ostrya capinifolia* or *Tilia-Acer* ravine woods, while beech is restricted to the central Gargano especially the Foresta Umbra site (HOFMANN 1961; BIONDI *et al.* 2004; BIONDI *et al.* 2008; DI PIETRO & MISANO 2009).

## MATERIALS AND METHODS

**Sampling** - *Quercus pubescens* s.l. populations occur in both the temperate and the Mediterranean bioclimatic regions at elevations of 40 to 1000 m a.s.l., where they form pure or mixed deciduous woods together with *Q. frainetto*, *Q. trojana* and *Q. cerris*. The research was carried out through widespread sampling of *Q. pubescens* s.l. populations, the collection sites covering the entire regional territory as uniformly as possible and being representative of a wide range of environmental conditions in terms of substrate and bioclimate (Fig. 1; Table 1, available as Appendix at <http://botanicaserbica.bio.bg.ac.rs/>). Leaves and acorns were collected from adult trees in autumn (from 8 to 16 individuals per population), randomly in the upper part of the crown. Voucher specimens are deposited in the herbarium of the University of Molise (IS) (THIERS 2015).

The number of leaves analysed for each individual ranged between 15 and 22. The leaves were pressed, dried and scanned using an Epson GT-15000 scanner with a resolution of 300 dpi, the abaxial surface facing upwards in the process, and measured with an ImageTool instrument (RASBAND 1997-2007). In total 17 leaf characters were assessed: six dimensional characters (area, perimeter, lamina length, petiole length, lobe width, sinus width), two counted characters (number of lobes, number of intercalary veins), four observed characters (abaxial and adaxial laminar pubescence, petiole pubescence evaluated on a standard area of 1 mm<sup>2</sup> using Kissling's grading system from 1 to 6, basal shape of the lamina using Kremer's index varying from 1 to 9) and five transformed characters (compactness, obversity, petiole ratio, lobe depth ratio, lobe width ratio) (Table 2, available as Appendix at <http://botanicaserbica.bio.bg.ac.rs/>). Fruit measurements were made using an electronic digital calliper (Maurer 93110). In total eight fruit characters were assessed: four dimensional characters (fruit axis length, cupule length, acorn length and acorn width) and four observed characters (scale shape, cupule edge regularity, type of scale, and scale gibbosity). In total 367 trees, 4254 leaves and 1120 fruits were collected in 24 stands. Since not all the individual trees were currently bearing fruits, three different matrices were prepared: matrix A (367 individuals × 17 leaf characters), matrix B (179 individuals with fruits × 17 leaf characters) and matrix B1 (179 individuals × 25 leaf+fruit characters).

Owing to the lack of genetic information concerning the individual Apulian oak trees sampled, a further data-set (matrix C: 422 individuals  $\times$  17 leaf characters) was prepared. This data-set was composed of the 367 Apulian individuals of matrix A added to 55 individual oaks growing in the neighbouring Molise region that previously were proved to be genetically homogeneous and identified as *Q. pubescens* (VISCOSI *et al.* 2012).

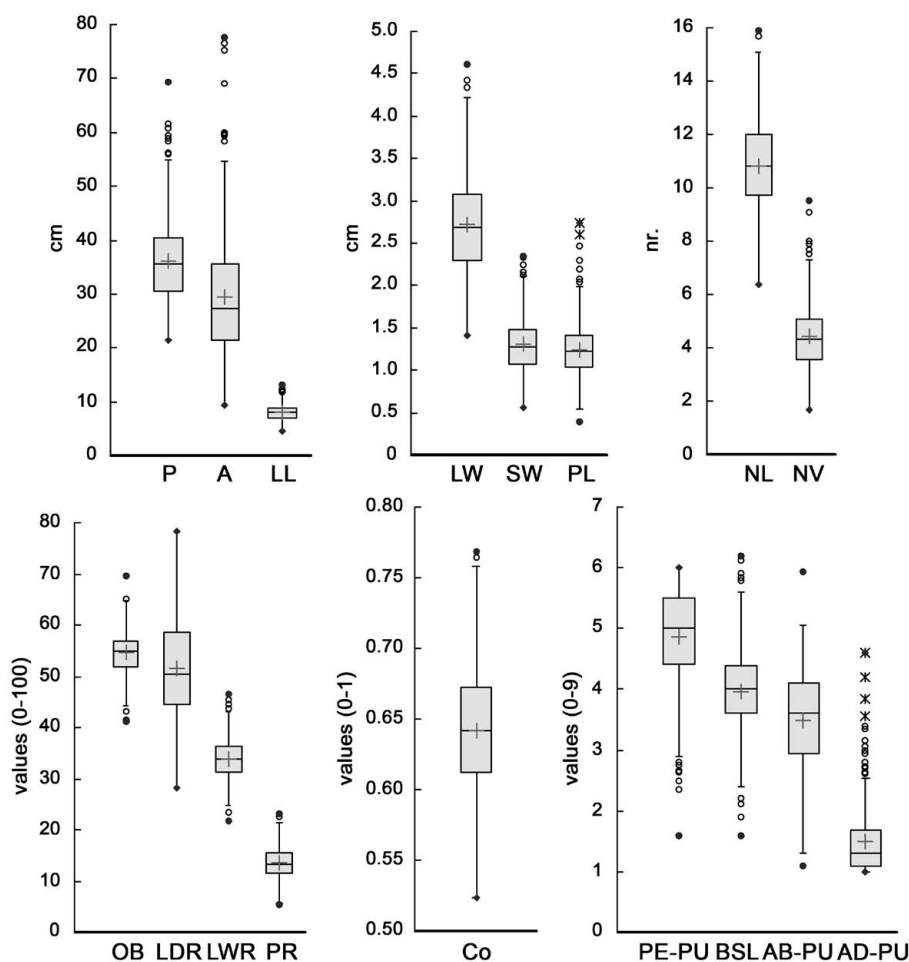
**Univariate analysis** - For each population the minimum, average and maximum values and the standard deviation (absolute and relative) were calculated for the 25 morphological characters. In order to graphically express the total range of variability of the pubescent oak populations, a single box plot was prepared for each of the leaf and fruit morphological characters. This analysis procedure was based on average values of the morphological characters calculated for each individual tree. The normal distribution of the variables (morphological characters) was tested using the Shapiro-Wilks test and any variables showing a non-normal distribution were subjected to logarithmic transformation. The partition of total variance of leaf morphology was evaluated through nested analysis of the variance (ANOVA) at three hierarchical levels: a: inter-populational (variance among different populations);

b: intra-populational (variance among the trees of a single population; and c: individual (variance among the leaves of a single tree).

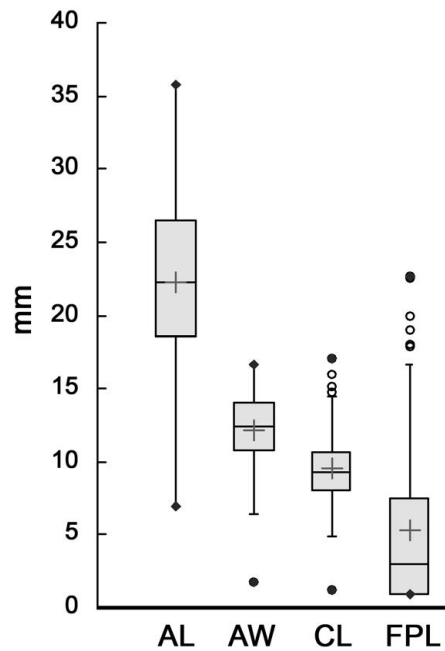
**Multivariate analysis** - A cluster analysis was performed on matrix A using Ward's minimum variance and the chord distance (this algorithm was used in all the herein presented cluster analysis). A discriminant function analysis (DFA) was performed to verify the real existence of groups previously identified by the cluster analysis. In order to reduce the effects of the different scale of measurements used for the variables, the data matrix was standardised by shifting variables so as to be centered at zero and scaling them to obtain a unit of variance.

A cluster analysis was performed on both matrix B1 and matrix B in order to verify whether the groups identified in the dendrogram based on leaf characters (matrix B) were also identifiable in the matrix including both leaf and fruit characters (matrix B1).

Next a cluster analysis and a DFA ordination analysing the 17 leaf morphological characters were performed on matrix C in order to verify whether the genetically homogeneous *Q. pubescens* individuals from Molise segregated as a distinct group or formed a mixed group including the Apulian oak individuals.



**Figure 2.** Box plots of leaf character variability calculated for the whole set of individual trees (367). The box plots show the minimum and maximum (filled diamonds), together with the first quartile ( $Q_1$ ), median (lines), mean (crosses), third quartile ( $Q_3$ ) and both limits beyond which values are considered anomalous. Values that are outside the  $[Q_1 - 3(Q_3 - Q_1); Q_3 + 3(Q_3 - Q_1)]$  interval are indicated with the asterisk symbol, while values that are in the  $[Q_1 - 1.5(Q_3 - Q_1); Q_1]$  or the  $[Q_3 + 1.5(Q_3 - Q_1); Q_3 + 3(Q_3 - Q_1)]$  intervals are given with the "o" symbol.



**Figure 3.** Box plots of fruit character variability calculated for the whole set of fruit-bearing individual trees (179). The box plots show the same things as in Fig. 2.

A spreadsheet modified to analyse more than 50 subgroups with up to 200 observations per subgroup was used to perform two-level nested ANOVA (McDONALD 2009). Box tests (chi-square asymptotic approximation and Fisher's asymptotic approximation) were performed in order to select the DFA type to be used. The Syntax 2000 program (PODANI 2001) was used only for the cluster analysis, while the XLSTAT program ver. 2014.1.03 (ADDINSOFT 1995-2014) was used for all the other statistical analysis.

## RESULTS

**Univariate analysis** - Among the leaf characters summarised in Table 3 (available as Appendix at <http://botanicaserbica.bio.bg.ac.rs/>), A, P and LL exhibited both the highest coefficient of variation and the highest correlation (Spearman's  $r_s$  values: A vs. P = 0.89; A vs. LL = 0.74; P vs. LL = 0.69). As regards the fruits, the high values of the relative standard deviation exhibited by FPL testify to the lack of a continuous gradient for this character. In addition, length of the cupule (CL) exhibited a very high coefficient of variation (Table 3, available as Appendix at <http://botanicaserbica.bio.bg.ac.rs/>), followed by acorn length (AL) and weight (AW). These two latter characters are strongly correlated (Spearman's  $r_s$  values: AL vs. AW 0.7), while no correlations were identified between length of the cupule (CL) and that of the fruit petiole (FPL).

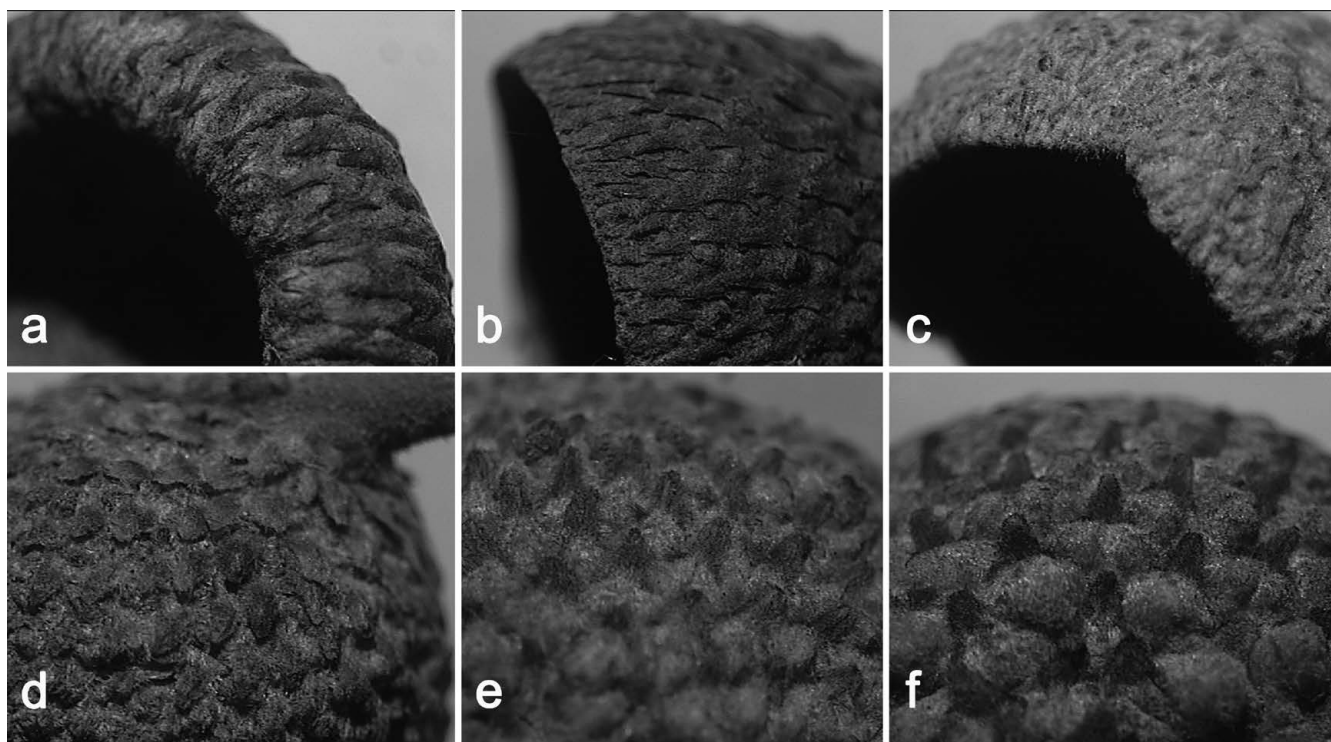
The box plots calculated for morphological characters of leaves collected from all the individual trees (Fig. 2) showed

that neither the "counted" characters (NV and NL) nor the "observed" characters (AD-PU, AB-PU and BSL) exhibited a significant range of variation. As regards the "transformed" characters, a high variability was exhibited only by LDR. Conversely, all the dimensional characters showed a wide range of variation. The box plots based on morphological variability of the fruits (Fig. 3) showed that length of the fruit petiole (FPL) (1-22.6 mm), acorn (AL) (7-35.8 mm) and cupule (CL) (4.85-17 mm) exhibited high variability.

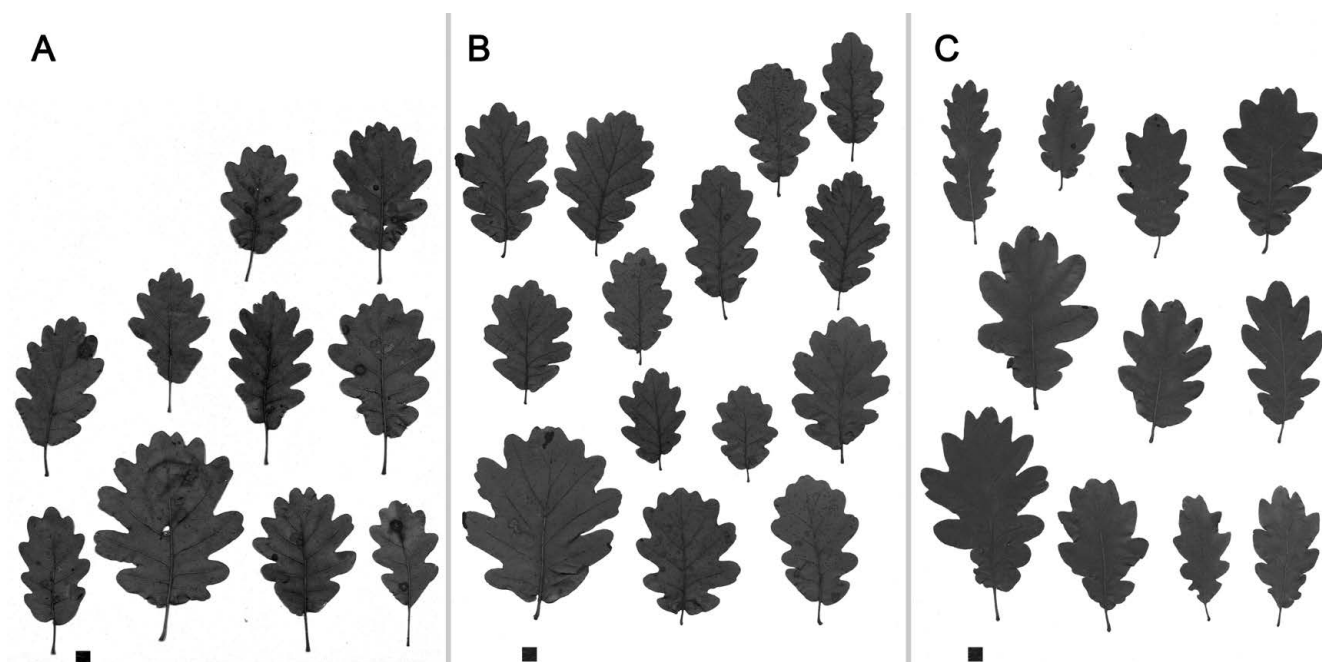
The cupules have a regular edge in 74% of the cases. The scales have a triangular shape in 67% of the cases and a pyriform shape in the remaining 33%. The colour of the tip of the scale is predominantly brown or red (48 and 46%, respectively), with a small percentage of black tips. Finally, gibbosity is present in only 21% of the scales (Fig. 4).

Nested ANOVA showed that the variability of morphological characters observed among leaves of a single individual tree (ranging between 41.31 and 87.59%) accounted for the greatest part of total variability. This is followed by the variability observed among leaves of trees belonging to the same population (9.42 - 42.08%) and by that observed among leaves of trees belonging to different populations (3.00 - 17.73%) ((Table 4, available as Appendix at <http://botanicaserbica.bio.bg.ac.rs/>)). The morphological variability among leaves of single trees was mainly due to LWR and LDR, although all the characters provided a significant contribution (Fig. 5). The differences observed among leaves of trees belonging to the same population were mainly due to leaf compactness (Co), petiole length (PL) and sinus width (SW). Finally, the morphological characters that mainly accounted for the variability observed among leaves of trees belonging to different populations were identified as leaf area (A), petiole length (PL), the lobe width ratio (LWR) and leaf length (LL).

**Multivariate analysis** - The cluster analysis of matrix A, which was based on leaf characters, produced a dendrogram showing three main clusters of individual trees exhibiting a very low degree of reciprocal dissimilarity ( $< 0.022$ ) (Fig. 6a). This result was also confirmed by the DFA diagram, where the three clusters previously identified on the dendrogram largely overlapped each other (Figs. 6b and 6c). Based on the average values of leaf characters, the three main clusters were morphologically characterised as follows. Cluster 1: obovate leaves with long sinus, small values of both leaf area and perimeter, pubescent. Cluster 2: ovate leaves with long sinus and moderate pubescence. Cluster 3: leaves quite similar in shape to those of cluster 1, but larger in area and perimeter and showing a lesser degree of pubescence (Fig. 7). No particular gradient, either geographic or ecological, was apparent observing the arrangement of individual trees on the dendrogram. The individual trees belonging to each single population exhibited a scattered distribution within all of the three major clusters without forming a particular grouping hypothesisable as being linked to the influence of some environmental parameters such as altitude or



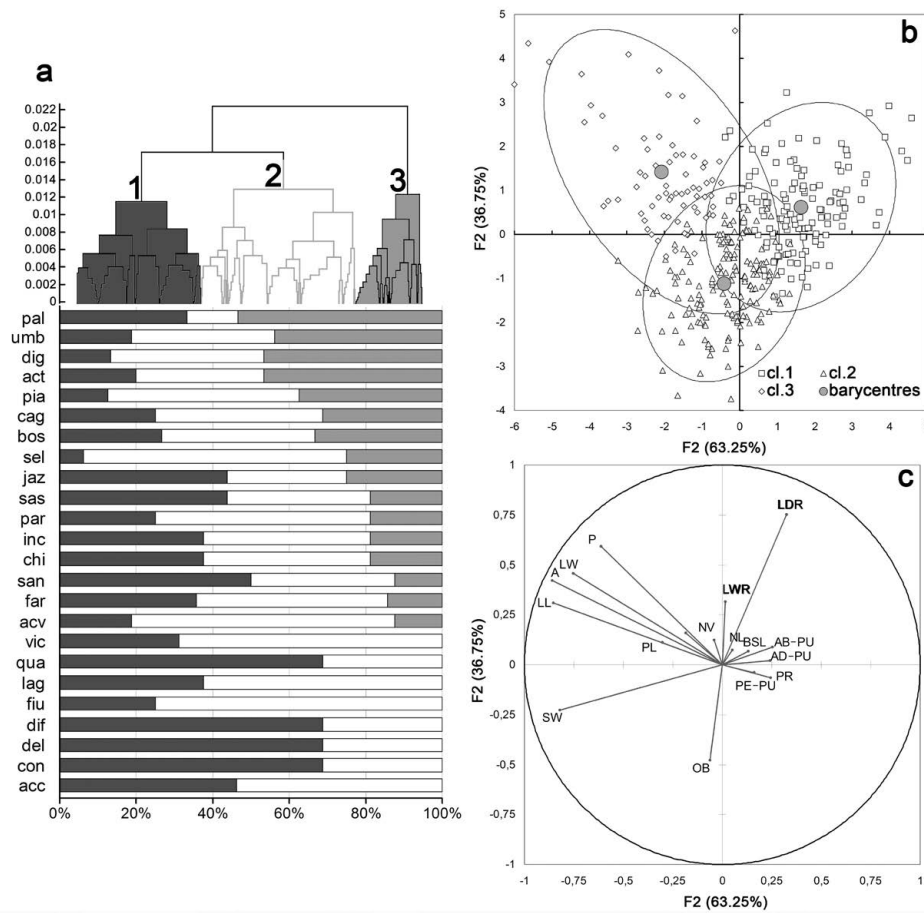
**Figure 4.** Cupule edge types: a) curved edge; b) regular edge; c) irregular edge. Cupule scale shapes: d) triangular; e) pyriform; f) pyriform with gibbosity.



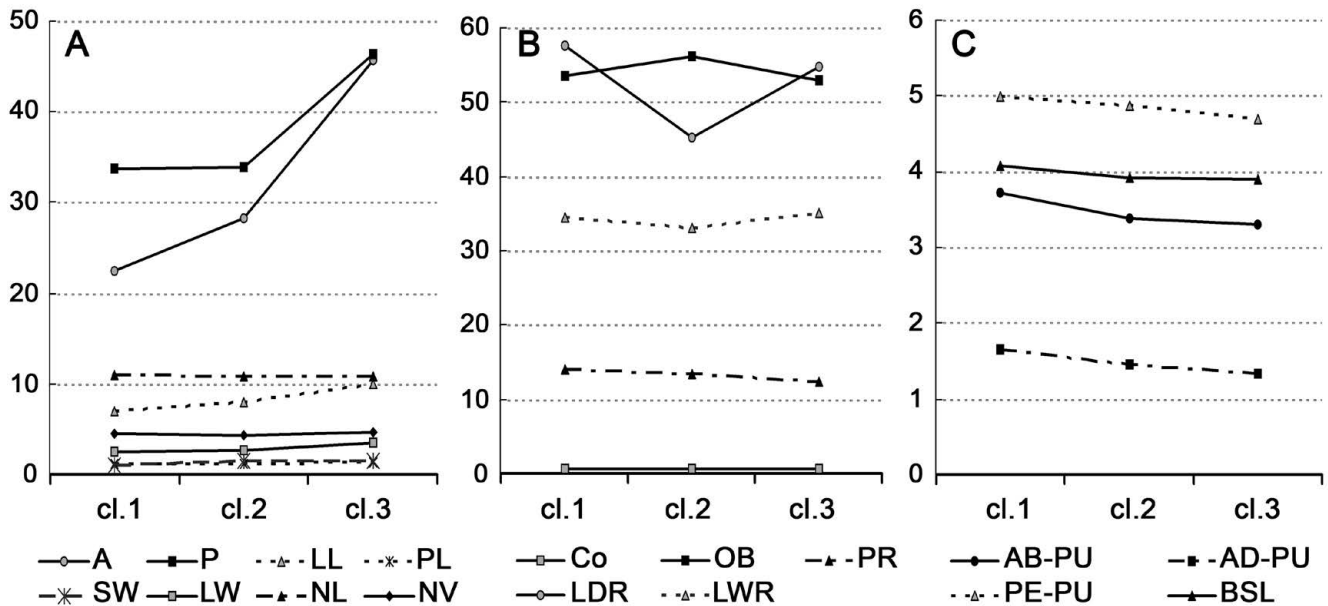
**Figure 5.** Example of leaf morphology variability in three individual trees coming from different oak populations (A: tree no. 8 of “act” site; B: tree no. 4 of “sel” site; C: tree no. 2 of “jaz” site). Black square = 1x1 cm.

bedrock type. Only from a bioclimatic viewpoint was a slight positive correlation observed in cluster 3, where the individual trees were mainly collected at sites characterised by a Mediterranean bioclimate.

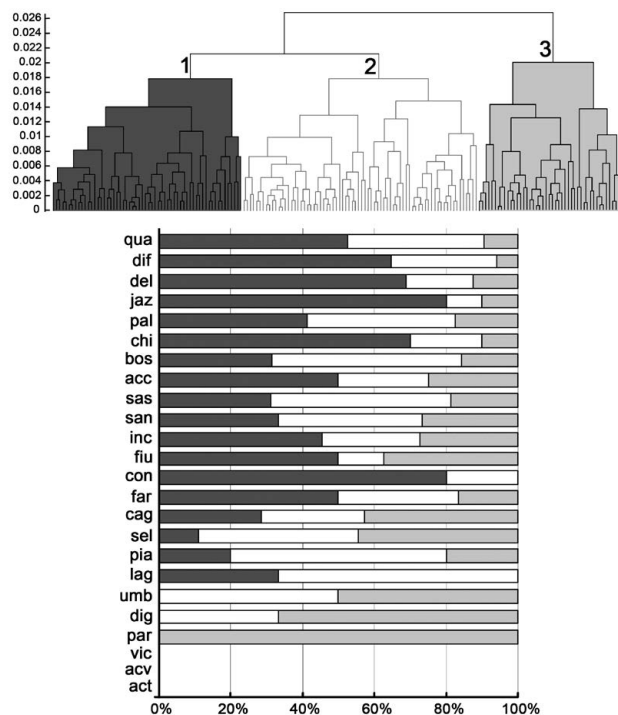
The occurrence of three main clusters showing a very low degree ( $< 0.026$ ) of reciprocal dissimilarity (Fig. 8) was also observable in the case of dendrograms deriving from cluster analysis of the two matrices composed of



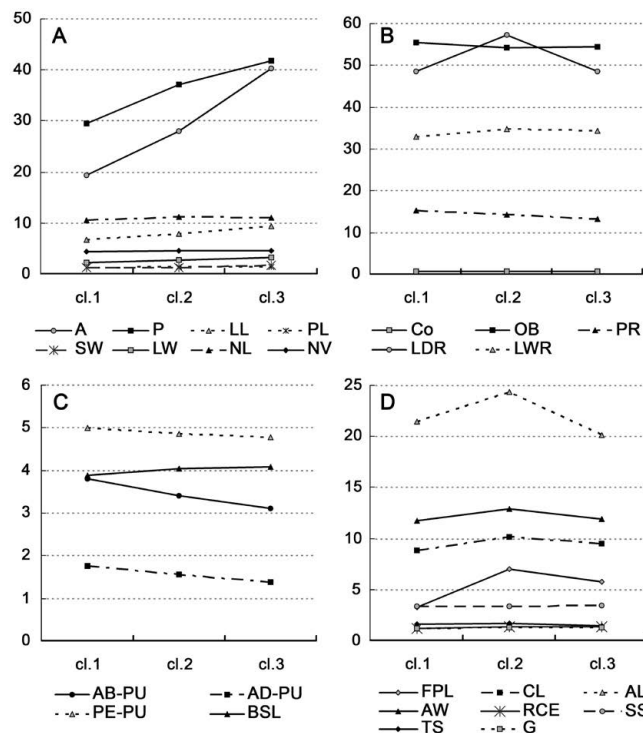
**Figure 6.** a) Upper part: Cluster analysis of matrix A based on mean values of leaf morphological characters. Lower part: Percentage of occurrence of each of the three clusters in each of the different sampled populations. b) DFA analysis of the 367 individual trees distributed within the first two quadratic discriminant functions (canonical correlations:  $F1 = 0.809$ ,  $F2 = 0.724$ ). The grey circles indicate barycentres of the three clusters previously identified by cluster analysis. c) Correlation circle showing the relationship between leaf morphological characters and discriminant factors.



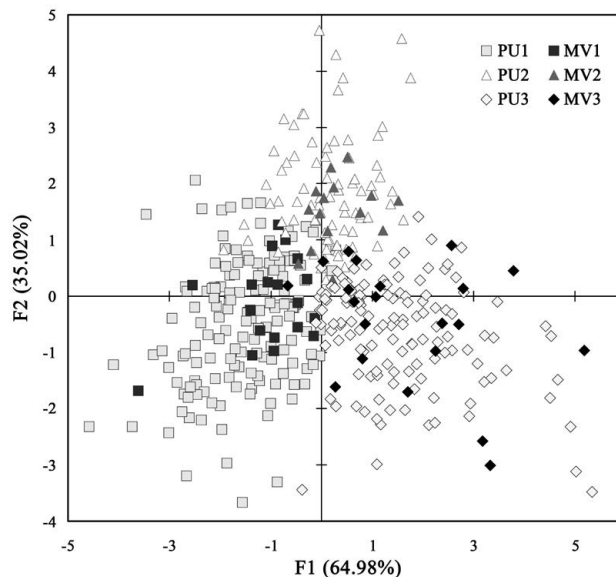
**Figure 7.** Average values of different leaf morphological characters in each of the three clusters obtained from cluster analysis of matrix A (A: dimensional/counted characters, B: transformed characters, C: observed characters).



**Figure 8.** Cluster analysis (using minimum variance and chord distance) of matrix  $B_1$  based on leaf and fruit characters. Percentage of occurrence of each of the three clusters in each of the different sampled populations.



**Figure 9.** Average values of leaf and fruit morphological characters (A: dimensional/counted characters, B: transformed characters, C: observed leaf characters; D: dimensional/observed fruit characters) in each of the three clusters obtained from cluster analysis of matrix  $B_1$ .



**Figure 10.** DFA analysis of the 422 Apulian (PU) + Molise (MV) individual trees distributed within the first two quadratic discriminant functions. The numbers 1, 2 and 3 refer to the three clusters derived from cluster analysis of matrix “C” (not shown in the paper).

leaf (B) and the leaf+fruit ( $B_1$ ) characters. The addition of fruit characters to a matrix based only on leaf characters produced an approximately 30% change in the cluster analysis results. Among the 179 individuals of matrix  $B_1$  (leaf + fruit characters), 129 remained in the same group identified on the dendrogram of matrix B (leaf characters only). On the basis of average values of leaf and fruit morphological characters (Fig. 9), it is possible to state the following facts regarding the three main clusters. Average values of the measured characters A, P and (to a lesser extent) LL increase passing from cluster 1 to cluster 3, while those of leaf pubescence (AD-PU, AB-PU) slightly decrease. The circular shape (CO) of leaves increases passing from cluster 1 to cluster 2 and decreases from cluster 2 to cluster 3. All the other characters exhibit quite similar values in all three clusters. The fruit non-dimensional characters (SS, TS, G) exhibit equivalent values in the three clusters, while those pertaining to acorn dimensions (AW, AL, CL, FPL) were slightly higher in cluster 2.

The DFA analysis based on matrix C, which was composed of individual trees from the Apulia region and the “genetically homogeneous” trees from the Molise region, did not exhibit any clear separation between these two regions (Fig. 10). The Molise individuals did not segregate as a single group, but were found to be randomly distributed within the three clouds of Apulian individuals. This distribution confirmed the result of cluster analysis performed on matrix “C” (dendrogram not shown), which displayed three main clusters, each of them composed of both Apulian and Molise individual trees. Accordingly, each of the three DFA clouds includes both Apulian and Molise individuals.



## DISCUSSION

The problems involved in the identification of downy oaks in Southern Europe are not solely confined to taxonomy, but also have repercussions in several other fields such as conservation biology, ecology, forestry, economics, etc. The choice of the Apulia region was strategic, since the available taxonomic and phytosociological literature regarding this area reports various species belonging to the *Q. pubescens* complex (*Q. amplifolia*, *Q. dalechampii*, *Q. pubescens*, *Q. virgiliana*) as playing a physiognomic guide role in some phytosociological associations (*Cyclamino-Quercetum virgilianae* Biondi *et al.* 2004, *Irido-Quercetum virgilianae* Biondi *et al.* 2004, *Stipo-Quercetum dalechampii* Biondi *et al.* 2004). However, the results that emerged from the present research do not seem to confirm any possible taxonomic differentiation, neither among the individual trees of a single population nor among the different pubescent-oak populations. In fact, no particular geographical or ecological gradient of variation of leaf and fruit morphological characters emerged from statistical analysis of the *Quercus pubescens* s.l. data-set. Individual trees sampled in temperate woods growing on clayey-marlstone substrates (northern Apulia) were classified together with individuals collected about 400 km further south in the thermo-Mediterranean woods of southern Apulia. At the same time, individual trees on fluvial deposits of the Tavoliere delle Puglie plain were classified together with those growing on conglomeratic substrates bordering the Murgia plateau and together with ones growing on the compact limestone of the deep gorges known as "Gravine".

A very low degree of reciprocal dissimilarity characterised the three clusters of individual oak trees derived from cluster analysis based exclusively on leaf characters, as well as the three clusters derived from cluster analysis based on leaf+fruit characters. The individual trees belonging to a single wood's population turned out to be uniformly distributed in all three clusters of the dendrogram, or at least in two of them.

The values of all the dimensional/counted, transformed and observed characters exhibited a more or less continuous trend of variation, with the result that no specific groups of characters related to specific groups of individual trees were identified. This morphological variability also applied to characters that are usually reported as diagnostic in taxonomy of the *Q. pubescens* complex, namely sinus width (*Q. dalechampii* vs. *Q. virgiliana*), lobe width (*Q. amplifolia* vs. *Q. dalechampii*) and basal shape of the lamina (*Q. amplifolia* vs. *Q. virgiliana*).

Morphological comparison between the dominant individual trees collected in populations of the aforementioned phytosociological associations characterised by different and well-established species of pubescent oak, such as *Q. virgiliana* or *Q. dalechampii* (BIONDI *et al.* 2004; BIONDI *et al.* 2010) revealed no evidence to support any taxonomic differentiation.

The continuous trend of variation of leaf and fruit characters in Apulian pubescent oaks suggests that this morphological variability could be attributable to other causes, such as hybridisation or phenotypic plasticity. Examples of phenotypic plasticity in *Quercus* are very common, especially regarding the leaves, while natural hybridisation and interbreeding of the resulting genotypes are known to be among the most important factors in the oak's evolution (BARTON 2001). It has been pointed out in several papers (BRUSCHI *et al.* 2000; ŠKVORC *et al.* 2005; CURTU *et al.* 2007, 2009; VISCOSI *et al.* 2012) that *Q. pubescens* is a white oak species which has been undergoing major processes of hybridisation and backcrossing with closely related and sympatric species such as *Q. petraea*, *Q. robur* and *Q. frainetto*. Many factors contribute to the high potential of *Q. pubescens* for hybridisation. BACILIERI *et al.* (1996) and SALVINI *et al.* (2008) showed that *Q. pubescens* pollen is able to remain viable for much longer than that of other white oak species. In the field of vegetation science, it is well-known that *Q. pubescens* exhibits an extremely wide ecological amplitude which allows it to play a major physiognomic role within a huge syntaxonomic range that includes *Quercetea pubescentis*, *Quercetum-Fagetum* and *Quercetum ilicis* (JAKUCS 1961; HORVAT *et al.* 1974; BARBERO & QUÉZEL 1977; BLASI *et al.* 2004; BRULLO *et al.* 2008; DI PIETRO *et al.* 2010). The existence of glacial refugia where different Apulian white oaks were forced to grow side by side during the cold Quaternary periods certainly favoured and intensified hybridisation processes (FINESCHI *et al.* 2002; PETIT *et al.* 2002).

The impossibility of establishing specific sets of diagnostic characters or taxonomic frameworks to classify the morphological diversity of Apulian pubescent oaks is confirmed by DFA ordination that also includes genetically homogeneous *Q. pubescens* s.l. individuals collected in the adjacent Molise region (VISCOSI *et al.* 2012). The fact that individual trees sampled in Molise did not form a clearly distinguishable group but were untidily distributed with Apulian individual trees on the DFA diagram not only confirmed the high variability of the *Q. pubescens* complex, it also indicated that not all oak trees with the same genotype necessarily have the same appearance. All of this evidence led us to provisionally assign all of the pubescent oak individuals collected in the study area to a single species, one that is characterised by high morphological variability.

The only morphological characters that at least slightly seem to follow a bioclimatic gradient are the dimensional characters of both the leaf and the acorn. To be specific, leaf "size" acts as the main discriminating character. The populations of group 3, which included ones from collection sites with Mediterranean bioclimates (both thermo-Mediterranean and meso-Mediterranean), were characterised by leaves and acorns that were on average larger and longer than those from trees of populations growing at sites with temperate bioclimates (clusters 1

and 2) (Fig. 2). However, this result cannot be directly used for taxonomic diagnosis. The leaves play a crucial role in survival and growth of a plant, and the variations of leaf size exhibited by the oak individuals sampled could have been influenced by variability of different environmental parameters (RODERICK *et al.* 2000; ROYER *et al.* 2008, KRÍSTKOVÁ *et al.* 2014). But the nested ANOVA analysis we conducted showed that most of the morphological variability exhibited by leaf characters in Apulian oaks lay in differences among leaves of the same individual tree rather than among leaves of different individual trees or among leaves from trees of different populations. If this result partially weakens the weight of environmental factors in producing morphological leaf-variation, at the same time it warns against superficial taxonomic classification based solely on the cursory observation and measurement of a few leaf characters. FORTINI *et al.* (2015) showed that the use of a mixed set of macro-morphological, micro-morphological and genetic characters was quite efficient in taxonomic discrimination among three oak species living in sympatry (*Q. petraea*, *Q. pubescens* and *Q. frainetto*). Such a multi-functional classification system is probably more in accordance with the multi-species concept (BURGER 1975; VAN VALEN 1976), which was proposed precisely to overcome the limits of applying the term “biological species” to taxa such as the oaks, orchids, brambles, etc., with a high propensity to hybridise.

We are aware that a study based only on morphometric analysis cannot lead to final conclusions about the identification of “good species” or hybrids. Nevertheless, if taxonomy and the resulting nomenclatural framework depend on our ability to understand any eventual morphological variation, the only possible approach is for us to accept that the morphological variation observed in the study area is consistent with the occurrence of one single species. As for the name of this species, more than one option is possible. In DI PIETRO *et al.* (2012), it emerged that the morphological diagnostic characters listed as differential by Tenore in the protologue of *Q. dalechampii* were not identifiable in the original material and scarcely discriminative in relation to some other similar oak taxa described for southern Italy. According to BRULLO *et al.* (1999), the species occurring in the basal and hilly belts of southern Italy is to be called *Q. virgiliana*, while *Q. pubescens* is considered as restricted to the temperate part of central and northern Italy. The same authors assert that *Q. amplifolia* occurs only in Sicily and southern Latium (central Italy) and not in Apulia. The most recent Floras and checklists (CONTI *et al.* 2005; EURO+MED PLANT BASE 2006) are more cautious and include both *Q. virgiliana* and *Q. amplifolia* in the variability of *Q. pubescens*. The morphological features of the Apulian specimens perfectly match those reported in the *Q. pubescens* Willd. protologue (WILLDENOW 1796) and

therefore there are no substantial reasons preventing the Apulian specimens collected in the present study from being assigned to *Q. pubescens*.

A woodlands coenological situation similar to that observed in the Apulia region (BIONDI *et al.* 2004; DI PIETRO & MISANO 2009) is known for the Dalmatian coast, where pure woods of *Q. virgiliana* were reported for the warmest areas (TRINAJSTIĆ 1990; VUKELIĆ, 2012). Bio-systematic studies on the *Q. pubescens* complex in Croatia (ŠKVORC *et al.* 2005) revealed a clear molecular and morphological differentiation between the Mediterranean populations of the south and the continental populations of the north. FRANJIC *et al.* (2006) identified the populations of southern Croatia as pure *Q. pubescens* and those of northern Croatia as “intermediate” populations due to introgression with *Q. petraea*. This hypothesis, which is reasonable for Croatia owing to its more northerly geographical location, is hardly adoptable for the Apulia region, as it is the only Italian region (together with the island of Sardinia) where *Q. petraea* is absent (CONTI *et al.* 2005). Nevertheless, a morphological-molecular comparison between the “pure” *Q. pubescens* population of southern Croatia and those of the Apulia region would be of great interest, especially in order to test the influence of such an important geographical barrier as the Adriatic Sea on both the gene flow and morpho-anatomical patterns of the oaks.

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**REZIME**

## Kompleks *Quercus pubescens* u jugoistočnoj Italiji - podaci multivarijantne morfometrijske studije

Romeo DI PIETRO, Piera DI MARZIO, Piero MEDAGLI, Giuseppe MISANO, Giuseppe N. SILLETTI, Robert P. WAGENSOMMER i Paola FORTINI

**I**me *Quercus pubescens* s.l. obuhvata kompleks listopadnih hrastova široke ekološke niše, uglavnom rasprostranjenih u jugoistočnoj Evropi. Apulija je, kao najistočnija oblast Italije, geografski i fiziografski prilično izolovana i u njoj se nalazi najviše vrsta hrastova (10). U taksonomskoj i fitocenološkoj literaturi, u ovoj oblasti se navodi prisustvo nekoliko vrsta iz grupe *Quercus pubescens*. Kako bi proverili da li se određeni morfološki karakteri mogu povezati sa pojedinim taksonima, sakupljen je biljni material iz 24 populacije *Quercus pubescens* s.l. iz ekološko-geografski različitih delova Apulije. Uzorkovan je i morfološki analiziran materijal sa ukupno 367 stabala i to 4254 listova i 1120 plodova. Ukupno 25 morfoloških karaktera vezanih za listove i plodove hrastova su statistički obrađeni pomoću univarijantne i multivarijantne analize. Nested ANOVA analiza je pokazala da se listovi unutar jednog drveta odlikuju većom morfološkom varijabilnošću u odnosu na varijabilnost listova između različitih stabala iz iste ili iz drugih populacija. Gotovo svi analizirani morfološki karakteri pokazuju kontinuirani trend variranja tako da nijedan ne može biti korišćen za razlikovanje između populacija. Pokazalo se da su samo "veličina" lista i ploda i dužina drške ploda blago diskriminantni karakteri. Naši rezultati ukazuju na to da je malo verovatno da u Apuliji postoji više od jedne vrste iz *Quercus pubescens* kompleksa. Poređenje apulijskih populacija sa genetički čistom populacijom iz druge oblasti (Molise) pojačava pretpostavku o postojanju jedne vrste koja se privremeno može klasifikovati kao *Q. pubescens* s.l.

**KLJUČNE REČI:** plod, genetička pripadnost, list, morfometrija, *Quercus*, statističke analize, taksonomija

**Table 1.** Characteristics of collection sites: **A:** site code of the collection; **B:** municipality and administrative province (BA=Bari, BR=Brindisi, FG=Foggia, LE=Lecce, TA=Taranto); **C:** number of individuals; **D:** number of individuals bearing fruits; **E:** altitude (m a.s.l.); **F:** lithology; **G** and **H:** geographical coordinates (latitude and longitude expressed in metres, UTM); **I:** bioclimatic region, thermotype and umbrotype according to BLASI & MICHETTI (2007) and based on the RIVAS-MARTÍNEZ (1997) indices.

| A   | B                          | C  | D  | E   | F                | G       | H      | I                          |
|-----|----------------------------|----|----|-----|------------------|---------|--------|----------------------------|
| acc | Deliceto 1 (FG)            | 13 | 12 | 640 | clayey marlstone | 4557220 | 526723 | Mesotemp. humid            |
| act | Spinazzola (BA)            | 15 | -  | 600 | conglomerates    | 4540867 | 597285 | MesoMedit. humid/sub-humid |
| acv | Acquaviva delle Fonti (BA) | 16 | -  | 200 | limestone        | 4524805 | 650840 | MesoMedit. dry             |
| bos | Brindisi (BR)              | 15 | 13 | 40  | clayey-sandy     | 4504890 | 744270 | ThermoMedit dry            |
| cag | Cagnano Varano (FG)        | 16 | 7  | 520 | limestone        | 4624669 | 567854 | MesoMedit. humid/sub-humid |
| chi | San Marco in Lamis (FG)    | 16 | 9  | 880 | limestone        | 4624043 | 561581 | Supratemp. humid/sub-humid |
| con | Deliceto 2 (FG)            | 16 | 4  | 550 | clayey marlstone | 4562416 | 534533 | Mesotemp. humid/sub-humid  |
| del | Deliceto 3 (FG)            | 16 | 15 | 560 | clayey marlstone | 4564231 | 529921 | Mesotemp. humid/sub-humid  |
| dif | Accadia (FG)               | 16 | 14 | 670 | clayey marlstone | 4558496 | 530175 | Mesotemp. humid/sub-humid  |
| dig | Gravina in Puglia (BA)     | 15 | 3  | 380 | conglomerates    | 4512469 | 618357 | MesoMedit. humid/sub-humid |
| far | Laterza 1 (TA)             | 14 | 5  | 420 | limestone        | 4497035 | 647716 | MesoMedit. humid/sub-humid |
| fiu | Castellaneta Marina (TA)   | 16 | 8  | 40  | clayey-sandy     | 4491270 | 658881 | ThermoMedit dry            |
| inc | Foggia (FG)                | 16 | 9  | 75  | fluvial deposit  | 4582747 | 553553 | MesoMedit. dry             |
| jaz | Ruvo di Puglia (BA)        | 16 | 9  | 320 | limestone        | 4551101 | 622237 | MesoMedit. dry             |
| lag | Laterza 2 (TA)             | 8  | 3  | 305 | calcarenites     | 4504016 | 656599 | MesoMedit. humid/sub-humid |
| pal | Palmariggi (LE)            | 15 | 10 | 40  | calcarenites     | 4446957 | 278106 | ThermoMedit dry            |
| par | Santeramo in Colle 1 (BA)  | 16 | 3  | 500 | limestone        | 4514201 | 647317 | MesoMedit. humid/sub-humid |
| pia | Martina Franca (TA)        | 16 | 4  | 460 | limestone        | 4502657 | 686693 | MesoMedit. humid/sub-humid |
| qua | Toritto (BA)               | 16 | 13 | 420 | limestone        | 4534971 | 632507 | MesoMedit. dry             |
| san | Santeramo in Colle 2 (BA)  | 16 | 13 | 470 | limestone        | 4518821 | 650238 | Mesotemp. humid/sub-humid  |
| sas | Santeramo in Colle 3 (BA)  | 16 | 12 | 490 | limestone        | 4523011 | 649110 | Mesotemp. humid/sub-humid  |
| sel | Laterza 3 (TA)             | 16 | 9  | 340 | limestone        | 4499728 | 654902 | MesoMedit. humid/sub-humid |
| umb | Vico del Gargano 1 (FG)    | 16 | 4  | 240 | limestone        | 4635677 | 587298 | MesoMedit. dry             |
| vic | Vico del Gargano 2 (FG)    | 16 | -  | 520 | limestone        | 4635503 | 582284 | Mesotemp. humid/sub-humid  |

**Table 2.** List of analysed leaf and fruit morphological characters.

| <b>Leaf variables</b>  |   | <b>code</b> |
|------------------------|---|-------------|
| Dimensional            | Area (cmq)  | A           |
|                        | Perimeter (cm)  | P           |
|                        | Lamina length (cm)  | LL          |
|                        | Petiole length (cm)   | PL          |
|                        | Sinus width (cm)  | SW          |
|                        | Lobe width (cm)   | LW          |
| Counted                | Number of lobes   | NL          |
|                        | Number of intercalary veins   | NV          |
| Transformed            | Compactness: $\sqrt{(4/\pi) \cdot \text{area}} / \text{major axis}$ | Co          |
|                        | Obversity (lamina shape): $WP/LL \cdot 100$                         | OB          |
|                        | Petiole ratio: $PL/(LL+PL) \cdot 100$                               | PR          |
|                        | Lobe depth ratio: $(LW-SW)/LW \cdot 100$                            | LDR         |
|                        | Lobe width ratio: $LW/LL \cdot 100$                                 | LWR         |
| Observed               | Abaxial laminar pubescence  | AB-PU       |
|                        | Adaxial laminar pubescence  | AD-PU       |
|                        | Petiole pubescence  | PE-PU       |
|                        | Basal shape of the lamina   | BSL         |
| <b>Fruit variables</b> |   | <b>code</b> |
| Dimensional            | Fruit petiole length (mm)   | FPL         |
|                        | Cupula length (mm)  | CL          |
|                        | Acorn length (mm)   | AL          |
|                        | Acorn width (mm)  | AW          |
| Observed               | Regularity of cupula edge   | RCE         |
|                        | Scale shape   | PSS         |
|                        | Type of scale   | TS          |
|                        | Gibbosity   | G           |







|    |   | acc | act | acv | bos | cag | chi | con | del | dif | dig | far | flu | inc | jaz | lag | pal | par | pia | qua | san | sas | sel | umbvic |  |
|----|---|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--------|--|
| TS | S | 0.5 |     |     | 0.5 | 0.0 | 0.5 | 0.5 | 0.5 | 0.0 | 0.0 | 0.4 | 0.5 | 0.4 | 0.0 | 0.4 | 0.0 | 0.5 | 0.5 |     | 0.4 | 0.4 | 1.2 |        |  |
|    | R | 0.1 |     |     | 0.1 | 0.0 | 0.2 | 0.1 | 0.1 | 0.1 | 0.0 | 0.0 | 0.1 | 0.1 | 0.1 | 0.0 | 0.1 | 0.0 | 0.2 | 0.1 |     | 0.1 | 0.1 | 0.4    |  |
|    | A | 1.5 |     |     | 2.0 | 2.1 | 1.7 | 1.5 | 1.7 | 1.7 | 1.0 | 1.6 | 1.3 | 1.0 | 1.4 | 1.0 | 2.3 | 1.3 | 1.5 | 1.8 |     | 1.3 | 1.6 | 1.7    |  |
|    | M | 2.0 |     |     | 2.0 | 3.0 | 2.0 | 2.0 | 2.0 | 3.0 | 1.0 | 2.0 | 2.0 | 1.0 | 2.0 | 1.0 | 3.0 | 2.0 | 2.0 | 2.0 |     | 3.0 | 3.0 | 3.0    |  |
|    | m | 1.0 |     |     | 2.0 | 2.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |     | 1.0 | 1.0 | 0.9    |  |
|    | S | 0.5 |     |     | 0.0 | 0.4 | 0.5 | 0.6 | 0.5 | 0.6 | 0.0 | 0.5 | 0.5 | 0.0 | 0.5 | 0.0 | 0.8 | 0.6 | 0.6 | 0.4 |     | 0.6 | 0.9 | 0.7    |  |
| G  | R | 0.3 |     |     | 0.0 | 0.2 | 0.3 | 0.4 | 0.3 | 0.4 | 0.0 | 0.3 | 0.4 | 0.0 | 0.4 | 0.0 | 0.4 | 0.4 | 0.4 | 0.2 |     | 0.5 | 0.6 | 0.4    |  |
|    | A | 1.0 |     |     | 1.3 | 1.0 | 1.2 | 1.0 | 1.1 | 1.2 | 1.0 | 1.4 | 1.3 | 1.1 | 2.0 | 1.3 | 1.0 | 1.0 | 1.4 |     | 1.3 | 1.3 | 1.4 |        |  |
|    | M | 1.0 |     |     | 2.0 | 1.0 | 2.0 | 1.0 | 2.0 | 2.0 | 1.0 | 1.0 | 2.0 | 2.0 | 2.0 | 2.0 | 1.0 | 1.0 | 2.0 |     | 2.0 | 2.0 | 2.0 |        |  |
|    | m | 1.0 |     |     | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 1.0 |     | 1.0 | 1.0 | 0.5    |  |
|    | S | 0.0 |     |     | 0.5 | 0.0 | 0.4 | 0.0 | 0.3 | 0.4 | 0.0 | 0.0 | 0.5 | 0.5 | 0.3 | 0.0 | 0.5 | 0.0 | 0.0 | 0.5 |     | 0.5 | 0.5 | 0.6    |  |
|    | R | 0.0 |     |     | 0.4 | 0.0 | 0.4 | 0.0 | 0.2 | 0.4 | 0.0 | 0.0 | 0.4 | 0.4 | 0.3 | 0.0 | 0.4 | 0.0 | 0.0 | 0.4 |     | 0.4 | 0.4 | 0.4    |  |

**Table 4.** Nested ANOVA analysis of leaf characters ( $p < 0.001$ ). **Fs** = F-statistic; % = percentages of the total variance of leaf characters accounted for at three hierarchical levels (individual trees from different populations, individual trees from the same population, leaves from the same tree).

|                         | <b>A</b>   |          | <b>P</b>  |          | <b>SW</b> |          | <b>LW</b>  |          | <b>LL</b> |          |
|-------------------------|------------|----------|-----------|----------|-----------|----------|------------|----------|-----------|----------|
|                         | <b>Fs</b>  | <b>%</b> | <b>Fs</b> | <b>%</b> | <b>Fs</b> | <b>%</b> | <b>Fs</b>  | <b>%</b> | <b>Fs</b> | <b>%</b> |
| <b>diff populations</b> | 8.25       | 17.73    | 5.69      | 12.27    | 3.51      | 7.01     | 8.02       | 15.46    | 6.69      | 14.15    |
| <b>same population</b>  | 8.89       | 33.37    | 9.78      | 36.09    | 9.18      | 38.53    | 7.04       | 29.01    | 8.48      | 33.73    |
| <b>leaves same tree</b> |            | 48.89    |           | 51.65    |           | 54.46    |            | 55.53    |           | 52.13    |
|                         | <b>LWR</b> |          | <b>Co</b> |          | <b>PL</b> |          | <b>LDR</b> |          | <b>PR</b> |          |
|                         | <b>Fs</b>  | <b>%</b> | <b>Fs</b> | <b>%</b> | <b>Fs</b> | <b>%</b> | <b>Fs</b>  | <b>%</b> | <b>Fs</b> | <b>%</b> |
| <b>diff populations</b> | 3.72       | 3.00     | 4.92      | 11.70    | 6.61      | 16.64    | 2.45       | 3.77     | 5.53      | 11.23    |
| <b>same population</b>  | 2.24       | 9.42     | 11.53     | 42.08    | 12.77     | 42.05    | 7.67       | 35.20    | 7.96      | 33.35    |
| <b>leaves same tree</b> |            | 87.59    |           | 46.23    |           | 41.31    |            | 61.02    |           | 55.41    |