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Morphometric analyses of the leaf variation within *Quercus* L. Sect. *Cerris* Loudon in Turkey

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Abstract: Oaks are classified heavily based on the leaf morphology. However, identification of specimens without acorns is usually controversial in *Cerris* section. Although members of *Cerris* section have a broad distribution area, there are only few taxonomic studies. Therefore, the current study is the first to show the discrimination of species in *Cerris* section based on leaf characters from Turkey. Discrimination among the members of *Cerris* section over Turkey (*Q. cerris* L. var. *cerris*, *Q. ithaburensis* Decne. subsp. *macrolepis* (Kotschy) Hedge and Yalt, *Q. brantii* Lindley, *Q. libani* Olivier, and *Q. trojana* P. B. subsp. *trojana*.) was aimed and variations within and among the species based on 15 qualitative leaf characters was presented. In this work we have studied the natural variability of these species by analysing leaf materials collected from 44 populations around Turkey. Cluster Analysis (CA) and Principal Component Analysis (PCA) were performed to assess intra-specific differentiation and to compare the distribution of variance in the individual and population level. The results showed that the leaf characters presented a good discrimination of five *Cerris* taxa in PCA at the population level, but the relationships between *Q. ithaburensis* subsp. *macrolepis* and *Q. brantii* showed complex groups in CA. Among the studied taxa, the highest variation was found within *Q. cerris* populations. In this work, we obtained discrimination of *Cerris* section species from Turkey based on leaf characters which is quite useful for those herbarium specimens without acorns and in other systematic observations.

Additional key words: cluster analysis, numerical taxonomy, oak, principal component analysis

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Introduction

The genus *Quercus* L. is one of the most abundant and economically-important genera of woody plants belonging to the Fagaceae family. It frequently exists as the dominant species in the Mediterranean area of the Northern Hemisphere (Manos et al. 2001; Govaerts and Frodin 1998). *Quercus* genus contains about 531 species in the last check list released by Govaerts and Frodin (1998) and is distributed across the Americas, Asia, Malaysia, Europe and North Africa. In Turkey, *Quercus* genus is represented with 23 taxa from three different Sections: *Quercus*, *Ilex* Loudon, and *Cerris* Loudon (Hedge and Yaltırık 1982; Yaltırık 1984). In the Flora of Turkey the *Cerris* section has five species including three infraspecific taxa, namely *Q. cerris* L. var. *cerris*, *Q. cerris* L. var. *austriaca*, (Willd) Loudon, *Q. ithaburensis* subsp. *macrolepis*, (Kotschy)

Hedge and Yalt, *Q. brantii* Lindley, *Q. libani* Olivier, *Q. trojana* P. B. and *Q. trojana* subsp. *yaltırikii* Ziel. et al. (Hedge and Yaltırık 1984; Zielinski et al. 2006).

Oaks are woody, long-lived and wind-pollinated species. Therefore, they can spread across wide geographic regions and so they show high levels of variation (Kremer and Petit 1993; Hokanson et al. 1993; Bacilieri et al. 1996). Since breeding barriers between *Quercus* species are extremely weak, oaks living in mixed populations show hybridization behaviour either in the same or in different sections (Bacilieri et al. 1996; Manos et al. 1999; Samuel 1999).

The taxonomy of genus *Quercus* is based on morphological traits mainly, which are crucial to differentiating species within the genus (Dupouey and Badeau 1993; Bruschi et al. 2000). Seed characters of oaks – seed size especially – shows high level of variation within the same species in some taxa (Anagiotos et al. 2012). Therefore, leaf characters are important in the classification of oaks (Stace 1989) and in the determination of the limits of species (Jensen et al. 1984). Leaf characters are used for classification of most oaks and they are also one of the most important indicators in identification of hybrid samples. Vegetative characters are used reluctantly in groups where reproductive characters are not helpful in classification (Stace 1989).

Due to hybridization problems in oaks, most of the species in Turkey and all distributed countries have taxonomic problems. Because of environmental variations, some of the *Quercus* taxa are mixed together and their delimitations are difficult to determine. To solve these problems, it is necessary to determine the boundaries of taxa (Borazan and Babaç 2003). Another important reason for the variation among oak species is varying environmental factors in different geographical regions (Petit et al. 1997). Morphological variations are usual within the same species living in different geographical regions and having distinct ecological factors. Discovering the reasons for variations is very important in *Quercus* taxonomy. Beside the other factors, variations may most probably be caused by ecological or genetic factors (Davis and Heywood 1963).

The distributions of *Q. ithaburensis* subsp. *macrolepis* and *Q. brantii* over Turkey are geographically separated by the Anatolian Diagonal (Davis 1971; Ekim and Güner 1986; Borazan and Babaç 2003; Uslu et al. 2011; Uslu and Bakış 2012). A similar geographical isolation mechanism was also reported between *Q. trojana* and *Q. libani* by Borazan and Babaç (2003) and Uslu and Bakış (2012). *Q. ithaburensis* subsp. *macrolepis* and *Q. brantii* can be discriminated from each other according to their fruit characteristics and their geographical distribution. However, fruitless samples can be problem. Some nomenclature and typification problems still remain in *Q. ithaburensis* complexes (Hedge and Yaltırık 1982). Morphometric analyses are generally used to demonstrate discrimination among the operational taxonomic units (OTUs). Therefore, we aimed to show discrimination among the members of *Cerris* section in Turkish Flora and to present variations within and among the species. Identification of *Q. ithaburensis* – *Q. brantii*, and *Q. libani* – *Q. trojana*, and some forms of *Q. brantii* – *Q. libani* based on leaves are usually confused when the herbarium specimens are missing acorns. In order to solve this problem, and achieve mentioned aims, morphometric leaf characters were analysed by the most frequently used multivariate statistics techniques: Cluster Analysis and Principal Component Analysis.

Material and Methods

Botanical Specimens: A total of 260 tree specimens (55 from *Q. brantii*, 55 from *Q. cerris*, 50 from *Q. ithaburensis* subsp. *macrolepis*, 52 from *Q. libani*, and 48 from *Q. trojana* subsp. *trojana*) were collected from 44 populations that are belong to *Cerris* section over Turkey (Appendix 1, Fig. 1) for the analyses. Populations were studied at the species level and infraspecific taxa were considered with belonging species. For each population, number of trees varied between ten and fifteen. For each tree, eight to ten leaves were randomly selected. To avoid seasonal and positional variations, samples were collected from different branches at approximately the same height and location where leaf growth had stopped (Blue and Jensen 1988).

For each character, mean values of each population were calculated. A total of fifteen morphometric characters were used as in previous studies of Jensen (1980; 1988; 1989), Stace (1989), Borazan and Babaç (2003) and Zúñiga et al. (2009). The morphological characters employed in this study and their explanations are presented in Figure 2.

This point forward, the taxa *Q. ithaburensis* subsp. *macrolepis*, *Q. cerris* var. *cerris* and *Q. trojana* subsp. *trojana* will be referred to as *Q. ithaburensis* and *Q. cerris* and *Q. trojana,* respectively.

Morphometric Analysis: For each character, data ranges of each taxon were tabulated in order to show their discriminative influence. Both cluster and principal component analysis techniques were used. Morphometric analyses were performed by Minitab version 16. A basic data matrix of 44 populations by 15 characters was created for this purpose. The data was standardized by using the linear transformation algorithm functions in Minitab program in order to reduce the effects of different measurement scales in different characters. To do this, the mean and the standard deviation of each character were used.

We computed a dissimilarity matrix using Euclidean distance coefficients (Dunn and Everitt 1982; Abbot et al. 1985) for the Cluster Analysis. A dendrogram was therefore produced using the unweighted pair group arithmetic averages method (UPGMA). Principal Component Analysis was performed with standardized data using a correlation matrix (Abbot et al. 1985).

Results

In this study, samples from 44 populations over Turkey belonging to the *Cerris* section of *Quercus* were analysed with Cluster Analysis and Principal Component Analysis. Two data matrices, 15 characters by 44 OTUs and 15 characters by 260 OTUs were created based on populations and tree specimens respectively.

For the aim of discrimination of taxa for each single character representation, minimum and maximum average values have been tabulated in Table 1. NPLB has been one of the best discriminative characters. It separated taxa into three distinct groups clearly: *Q. libani*; *Q. trojana – Q. brantii*; and *Q. ithaburensis – Q. cerris*. Another valuable character to discriminate the studied taxa, IBPS, resulted in three groups similarly: *Q. brantii; Q. ithaburensis – Q. cerris*; *Q. libani – Q. trojana*. Although many characters revealed that *Q. cerris*, *Q. brantii* and *Q. ithaburensis* have been grouped together, some characters such as ICWI and DBW have discriminated *Q. cerris* from *Q. ithaburensis* and *Q. brantii* clearly. On the other hand, in most cases *Q. trojana* has formed a complex with *Q. libani*. A few characters, LBW, LBL, and DBW, were able to discriminate these two taxa.

In the CA three main groups were obtained by a phenon line at a 5.08 dissimilarity level (Fig. 3). The phenon line was drawn according to variance decomposition for the optimal classification with reasonable values such as 43.96% within classes and 56.04% among classes. The first group was composed of the two clusters of *Q. trojana* and *Q. libani*. In the second group, CA was not able to form separate clusters for *Q. brantii* and *Q. ithaburensis*. Only *Q. brantii* populations from Adıyaman – Nemrut Mountain (BRN283 and BRN 284) and from Elazığ – Sivrice and Baskil (BRN287 and BRN286) were combined in the first subgroup. The second subgroup contained four different small groups in which three belong to *Q. ithaburensis* and one to the *Q. brantii* populations. One of these small groups covered the populations of conserved *Q. ithaburensis* old trees (ITH122, ITH130 and ITH198) which were located in the North Aegean coastal region. The second small group was composed of populations in which trees were sparsely distributed in cultivated areas. The third group was composed of populations of *Q. cerris* only (Fig. 3).

The dendrogram (Fig. 3) which produced as a result of CA was unable to group the *Q. brantii* and *Q. ithaburensis* populations separately. When we looked at the habitat information of samples, we have seen that, populations of sparsely distributed trees in cultivated areas were located separately from the populations of conserved small woodlands. Populations BRN283, BRN284, BRN286, and BRN287 are representatives of small forest habitats which had clustered separately from the remaining *Q. brantii* populations that are composed of sparsely distributed trees. This is more or less the same for the *Q. ithaburensis* populations. Furthermore, the main differences between *Q. ithaburensis* and *Q. brantii* are the size dependent characters in the dataset.

Two PCA plots, at population level and at individual (tree) level, are presented in figure 4 and figure 5 respectively. Components in the PCA plot of populations revealed 72% of total variation approximately and 65% in the PCA plot of tree specimens. PCA based on the populations' data gives a clear cut discrimination of all five *Cerris* taxa from the remaining taxa and *Q. trojana* and *Q. libani* could be discriminated using either one of the factors. On the other hand, one would need both first and second factors to discriminate the remaining taxa. *Q. libani* formed a dense distribution of samples while the remaining taxa – especially *Q. cerris* and *Q. ithaburensis* – were scattered loosely. Similar to the dendrogram produced by cluster analysis, *Q. trojana* and *Q. libani* were plotted closely on the left hand side of the plot, *Q. cerris* was at the opposite side and *Q. ithaburensis* and *Q. brantii* were located at the middle. However, the introgression between *Q. ithaburensis* and *Q. brantii* samples were resolved in PCA (Fig. 4).

The PCA based on tree samples (Fig. 5) was plotted to show the variations among the individual tree specimens. The central locations of each taxon were plotted and found to be similar to PCA plot of populations. However, the samples showed an introgressive distribution in Figure 5. It is not an easy task to perform a clear cut discrimination of taxa in this case. The most distinct taxa were *Q. libani* and *Q. trojana,* while the other three taxa showed overlapping distributions (Fig. 5).

Discussion

Comparison of taxa ranges within the characters (Table 1) had shown that most of them were separated at least into two groups. This indicated that chosen characters were also informative solely. The main subtraction from these comparisons would be the grouping of *Q. brantii – Q. cerris – Q. ithaburensis* complex and *Q. libani – Q. trojana* complex separately. Results of CA (Fig. 3) and PCA (Figs 4, 5) have also

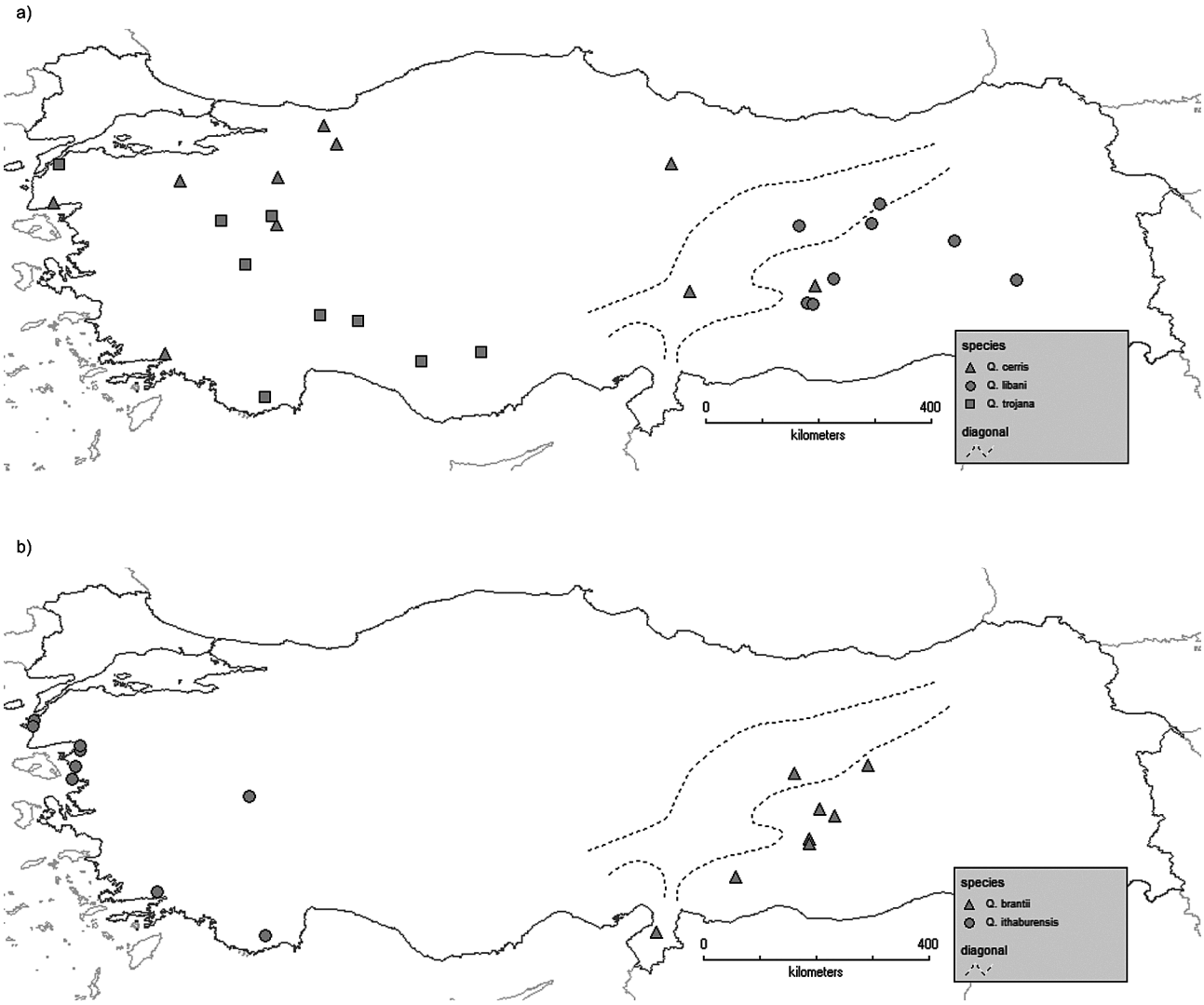

Fig. 1. Geographical distributions of Section *Cerris* populations belonging to a) *Q. cerris, Q. libani, Q. trojana,* b) *Q. brantii,* and *Q. ithaburensis* according to the Anatolian Diagonal (presented with dotted line)

supported these complexes. This was most probably caused by the size depending property of leaf characters, since most of the characters were based on measurements.

On the other hand, character NPLB gave a good discrimination with three groups: *Q. libani*; *Q. trojana – Q. brantii*; *Q. ithaburensis – Q. cerris*. Contrarily, *Q. libani* and *Q. trojana* have been grouped closely according to size dependent characters. Therefore, using combination of characters would give the best discriminations among the taxa as seen in results of CA (Fig. 3) and PCA (Figs 4, 5).

In both the CA and PCA results, *Q. trojana* and *Q. libani* were grouped as separate clusters. This result was significant, since those two taxa were distinguished according to their fruits generally rather than their leaves (Hedge and Yaltırık 1982; Yaltırık 1984). Section members were clearly grouped within the PCA plots and clustered in the CA dendrogram, with exceptions of *Q. brantii* and *Q. ithaburensis* in the CA. In both graphs, *Q. cerris* were placed away from *Q. trojana* and *Q. libani*. This was most possibly caused by the primitive structure of leaf venation in *Q. trojana* and *Q. libani* but not in *Q. cerris,* as mentioned in Yaltırık (1984) and Kasaplıgil (1992). Leaf venations in primitive forms were pinnate type; where secondary veins paired opposite as in *Q. trojana* and *Q. libani*. The other venation types that are found in other taxa were called derived in this study. The two remaining taxa, *Q. brantii* and *Q. ithaburensis*, were placed in the middle since they are at the transition between primitive and derived forms. This feature also causes *Q. libani* and *Q. trojana* to form a dense distribution of samples while the remaining taxa, especially *Q. cerris,* were scattered loosely due to low variation rates in primitive leaves and high variation rates in more derived forms.

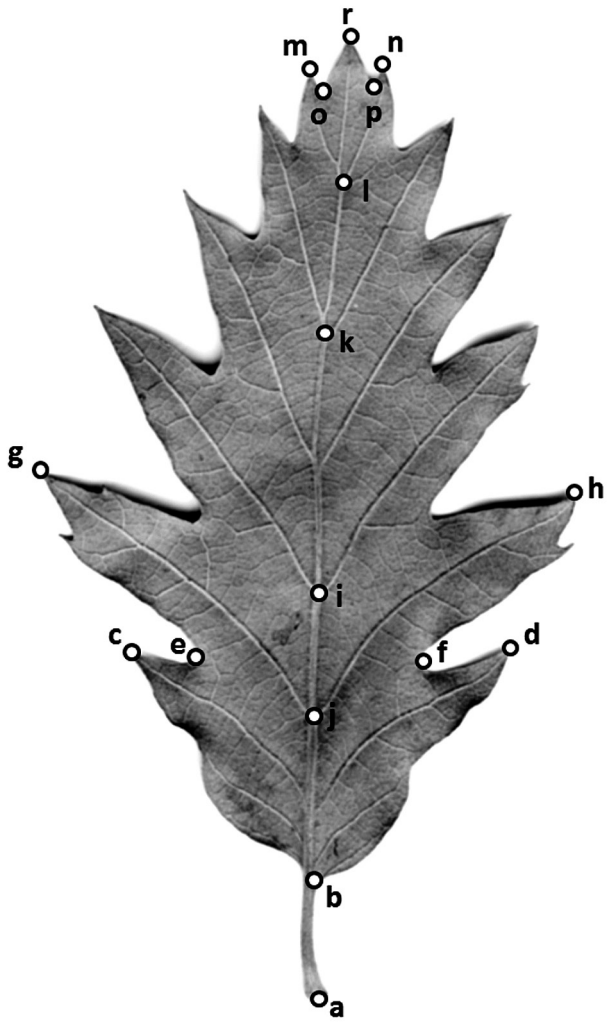

Fig. 2. Graphical representation of landmark points used for morphometric measurements and the list of morphological characters scored from leaves. **NPLB:** Total number of primary leaf lobes*; **LBL:** Leaf blade length (r–b); **LBW:** Leaf blade width (at the widest point) (g–h); **DBW:** The distance between the widest point and the leaf base (i–b); **BLW:** Basal lobe pair width* (c–d); **ALW:** Apical lobe pair width* (m–n); **DAB:** The distance between the apical lobe pair and basal lobe pair at the right side of the leaves* (d–n); **ICVI:** Interval between approximately central vein intersections (i–j); **IAVI:** Interval between apical vein intersections (k–l); **IBPS:** Interval between basal pair of sinuses* (e–f); **IAPS:** Interval between apical pair of sinuses* (o–p); **PTL:** Petiole length (a–b); **LBL/LBW:** Leaf blade length / Leaf blade width; **BLW / IBPS:** Basal lobe pair width / Interval between basal pair of sinuses*; **ALW / IAPS:** Apical lobe pair width / Interval between apical pair of sinuses*. (* For the leaf types such as *Q. libani* and *Q. trojana*, the tooth measurements are scored instead of lobes)

On the other hand, hybridization is very common in the eastern part of the Anatolian Diagonal between *Q. brantii* and the taxa of *Quercus* section members, especially with *Q. infectoria* subsp. *boissieri* (Zohary 1973; Menitsky 2005; Yaltırık 1984; Kasaplıgil 1992). Some of these hybrids and their leaf characters are very similar to those of *Q. ithaburensis* (Yaltırık 1984; Kasaplıgil 1992). However, *Q. brantii* and *Q. ithaburensis* taxa were separated from one another in terms of geographical distribution. Although, samples of *Q. ithaburensis* populations appeared in the *Q. brantii* and *Q. cerris* groups in the CA results, they actually form a separate fifth group in PCA's (Figs. 4–5). Although the general thought that *Q. brantii* are distributed in

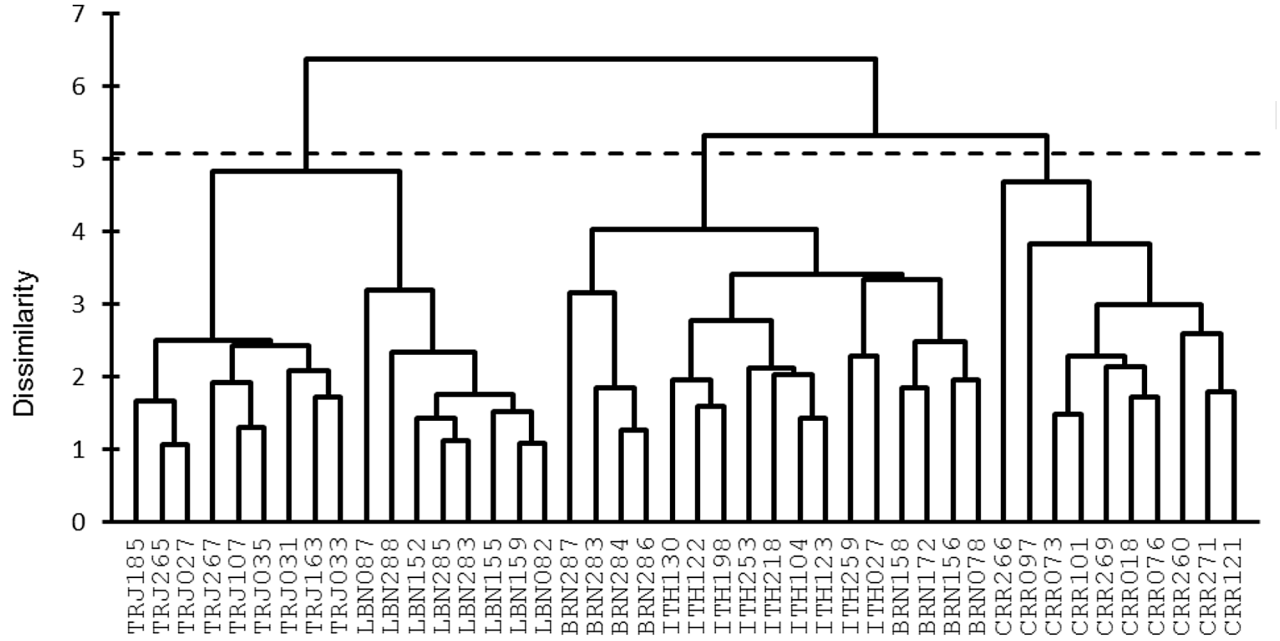

Fig. 3. Dendrogram of populations generated by Cluster Analysis using the UPGMA method

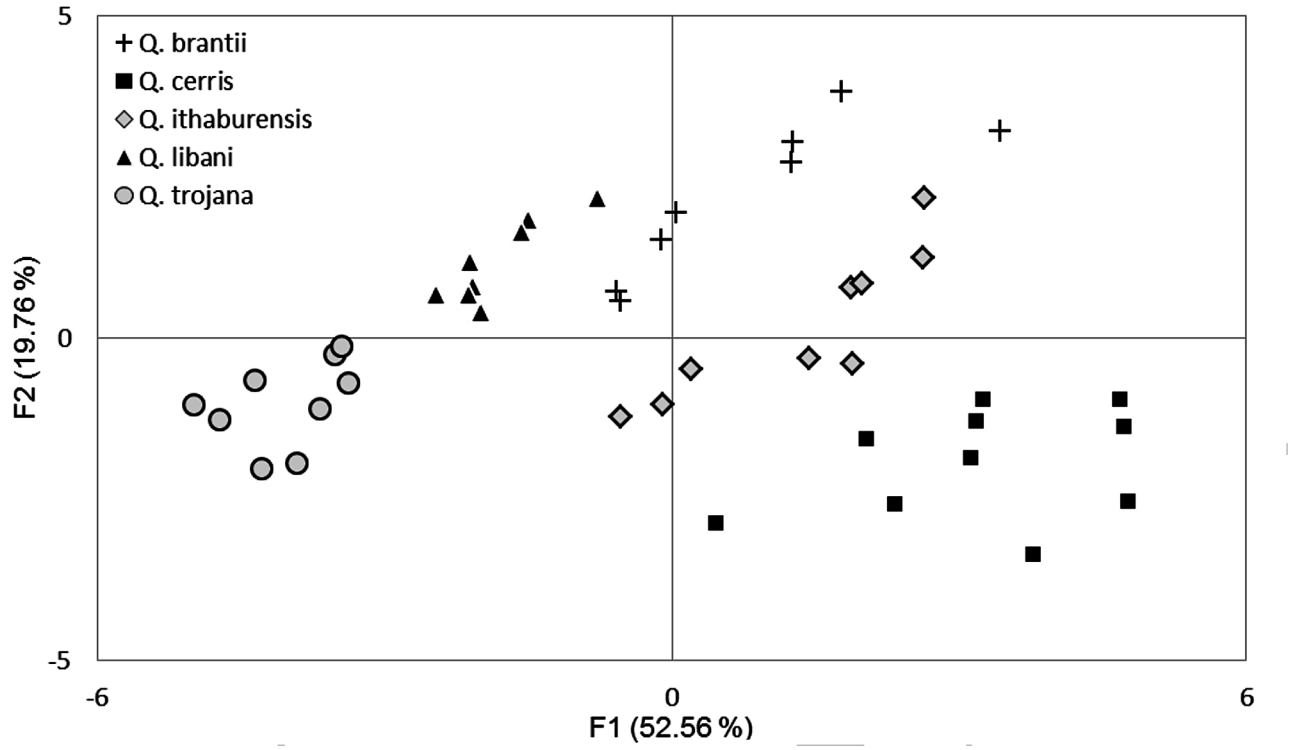

Fig. 4. Plot of distributions of populations generated by PCA, according to components 1 and 2

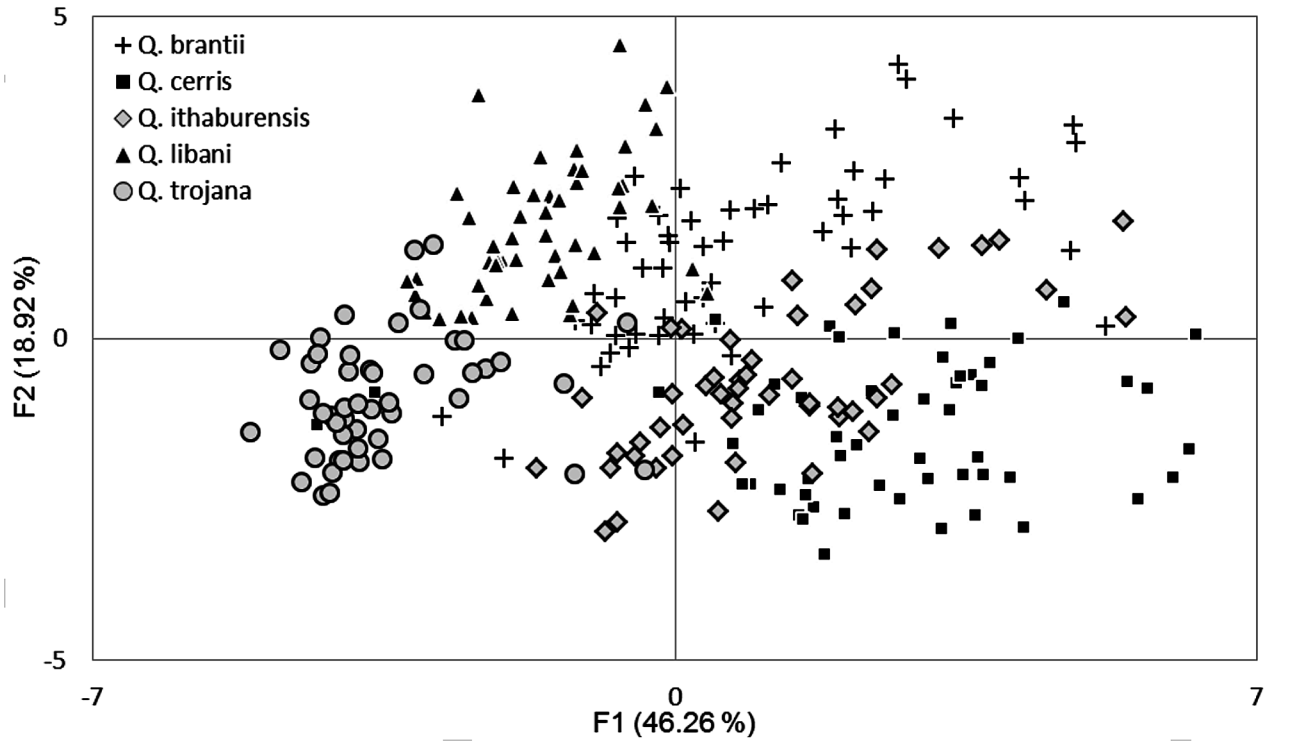

Fig. 5. Plot of distributions of tree specimens within populations generated by PCA, according to components 1 and 2

Table 1. Comparison of minimum-maximum averages of leaf characters for the studied taxa

Characters*\Taxa	Q. brantii	*Q. cerris* var. *cerris*	*Q. ithaburensis* subsp. *macrolepis*	Q. libani	*Q. trojana* subsp. *trojana*
NPLB	16.56–22.18	10.43–15.28	12.83–15.60	23.52–27.74	18.70–24.12
LBL	6.32–8.47	7.05–9.31	5.39–7.94	7.87–9.12	4.48–6.64
LBW	3.07–5.24	3.57–4.75	2.92–4.64	2.54–3.37	1.77–2.44
DBW	2.94–3.64	3.65–5.29	2.40–3.44	2.88–3.78	1.93–2.92
BLW	3.06–4.56	2.35–2.92	2.56–3.68	2.12–2.73	1.47–2.01
ALW	0.60–1.23	0.82–1.64	0.81–1.33	0.43–0.53	0.33–0.48
DAB	4.86–7.28	5.35–7.14	3.68–6.82	6.21–7.72	3.89–5.36
ICVI	0.63–1.06	0.94–1.77	0.84–1.30	0.65–0.80	0.45–0.89
IAVI	0.65–1.17	1.09–1.87	0.78–1.32	0.51–0.70	0.45–0.67
IBPS	2.68–4.03	0.97–2.16	1.99–3.20	1.38–2.07	1.14–1.84
IAPS	0.33–0.96	0.46–0.90	0.49–0.83	0.27–0.42	0.21–0.48
PTL	1.01–1.43	0.57–1.25	1.00–1.67	0.97–1.52	0.37–0.79
LBL/LBW	1.47–2.13	1.84–2.36	1.68–2.11	2.76–3.32	2.59–3.31
BLW/IBPS	1.06–1.31	1.32–1.99	1.13–1.42	0.67–1.37	0.80–1.15
ALW/IAPS	1.16–1.86	1.74–2.74	1.33–1.80	1.25–1.81	1.08–1.78

*See Fig. 2 for the character codes.

the west and *Q. ithaburensis* in the east of the Anatolian Diagonal, we do not observed such separation during our field trips. We believe that false identifications were caused by the herbarium specimens that are not having fruit parts.

Q. cerris, which is well-known for its irregular leaf shapes, has the highest level of variations on leaf characters in PCA (Fig. 5). Schwarz's classification of oaks in Anatolia supports this result (Schwartz 1993). *Q. cerris* is such a complex group and is one of the species in Section *Cerris* of Turkish Flora with infraspecific taxa. In addition, *Q. cerris* has a potential to hybridize with other taxa (Kasaplıgil 1992; Schwartz 1993; Conte et al. 2007; Bellarosa et al. 2005).

Current classification and identification of the section is mainly based on the leaf and fruit characters. Leaves are particularly significant as the availability of fruits depends on the seasons (Stace 1989; Jensen et al.1984). The biennial maturation of fruits as characteristic to *Cerris* section (Hedge and Yaltırık 1982) also makes studies based on fruit morphology difficult. In this study, we have conducted the most comprehensive morphometric analysis of leaves belong to the Turkish *Cerris* to date.

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Appendix 1

Geographical information of OTUs belongs to Section *Cerris*. Bold characters indicate population numbers, and the others are province name, GPS coordinates, and altitude, respectively

Q. brantii – **BRN156:** Tunceli, 39° 11.540 N – 39° 42.114 E, 1025 m. **BRN158:** Elazığ, 39° 03.650 N 38° 30.024 E, 1135 m. **BRN172:** Hatay, 36° 28.514 N – 036° 16.735 E, 500 m. **BRN078:** Gaziantep, 37° 22.709 N – 037° 33.114 E, 780 m. **BRN284:** Adıyaman, 37° 94.765 N – 038° 75.966 N, 1410 m. **BRN283:** Adıyaman, 37° 91.929 N – 038° 75.966 E, 870 m. **BRN287:** Elazığ, 38° 22.052 N – 039° 10.101N, 1440 m. **BRN286:** Elazığ, 38° 27.634 N – 038° 53.891 E, 1020 m. *Q. cerris* var. *cerris* – **CRR018:** Malatya, 38° 13.624 N – 038° 51.004 E, 1045 m. **CRR260:** Muğla, 37° 08.141 N – 028° 18.215, 650 m. **CRR266:** Çanakkale, 39° 34.484 N – 026° 30.340 E, 340 m. **CRR269:** Bolu, 40° 31.455 N – 031° 04.985 E, 640 m. **CRR271:** Düzce, 40° 49.916 N – 030° 53.400 E, 260 m. **CRR074:** Tokat, 40° 12.481 N – 036° 30.590 E, 1140 m. **CRR076:** Kahramanmaraş, 38° 07.976 N – 036° 49.134 E, 1125 m. **CRR097:** Bilecik, 39° 58.940 N – 030° 07.890 E, 890 m. **CRR101:** Kütahya, 39° 12.910 N – 030° 07.410 E, 1110 m. **CRR121:** Bursa, 39° 55.761 N – 028° 32.657E, 120 m. *Q. ithaburensis* subsp. *macrolepis* – **ITH104:** Uşak, 38° 55.761 N – 029° 40.485 E, 760 m. **ITH123:** Balıkesir, 39° 25.467 N – 026° 55.435 E, 140 m. **ITH122:** Balıkesir, 39° 30.036 N – 026° 55.990 E, 10 m. **ITH253:** İzmir, 38° 57.266 N – 026° 48.313 E, 130 m. **ITH259**: Muğla, 37° 80.059 N – 028° 10.388 E, 580 m. **ITH218:** Çanakkale, 39° 54.438 N – 026° 10.741 E, 30 m. **ITH198:** İzmir, 39° 10.503 N – 026° 51.387 E, 120 m. **ITH130:** Çanakkale, 39° 49.153 N – 026° 51.388 E, 121 m. **ITH127:** Antalya, 36° 25.862 N – 029° 55.469 E, 500 m. *Q. libani* – **LIB152:** Tunceli, 39° 33.300 N 39° 53.403 E, 1500 m. **LIB155:** Tunceli, 39° 14.059 N – 039° 45.040 E, 1030 m. **LIB159:** Erzincan, 39° 12.552 N 38° 35.229 E, 930 m. **LIB087:** Bingöl, 38° 58.110 N – 041° 05.685 E, 1490 m. **LIB285:** Adıyaman, 37° 56.833 N – 038° 43.149 E, 1670 m. **LIB283:** Adıyaman, 37° 56.139 N – 038° 48.180 E, 890 m. **LIB288:** Elazığ, 38° 21.280 N – 039° 09.002E, 1440 m. **LIB082:** Bitlis, 38° 19.529 N – 042° 06.577 E, 1640 m. *Q. trojana* subsp. *trojana* – **TRJ267:** Çanakkale, 40° 11.642 N – 026° 035.124 E, 225 m. **TRJ265:** Uşak, 38° 34.259 N – 029° 36.303E, 825 m. **TRJ163:** Karaman, 37° 09.275 N – 033° 25.659 E, 1100 m. **TRJ185:** Kütahya, 39° 21.732 N – 030° 02.756 E, 970 m. **TRJ033:** Konya, 37° 38.985 N – 031° 26.805 E, 1180 m. **TRJ105:** Kütahya, 39° 17.287 N – 029° 13.584 E, 760 m. **TRJ031:** Isparta, 37° 44.842 N – 030° 49.269 E, 1300 m. **TRJ027:** Muğla, 36° 25.862 N – 029° 55.469 E, 500 m. **TRJ035:** Konya, 37° 00.682 N – 032° 28.312 E, 1470 m.