

ANALYSIS OF PUBESCENT OAK (*QUERCUS PUBESCENS* WILLD.)  
BY MEANS OF CHLOROPLAST DNA (cpDNA)  
IN THE WESTERN PART OF THE BALKAN PENINSULA

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ABSTRACT

In the area of the western Balkans, xerothermal broad-leaf forests hold a very special position, the main species being pubescent oak (*Quercus pubescens* Willd.). The study comprises 36 populations of pubescent oak from Croatia, Bosnia and Herzegovina, Montenegro, Kosovo, Albania and Macedonia.

Genetic variability and population diversity was analysed on chloroplast DNA in order to determine to which haplotype an individual or studied population belongs, as well as how they are related.

For the detection of one haplotype analysed four sequences (AS, TF, DT and CD) on the chloroplasts DNA. The results of the analysis suggest that there are six different haplotypes of pubescent oak (2, 4, 5, 17, 31, 33) in the studied populations. Two more sub-haplotypes were found in haplotype 5 (a and b), but only in populations occurring in Croatia and Bosnia and Herzegovina.

The results suggest the great variability of pubescent oak in the researched area, which is generally characteristic of other species from the area of the Balkan Peninsula.

The results obtained by means of cpDNA analysis can help in the formation of seed zones. However, due to the strong selection pressure of unplanned logging, and the possible introduction of herbal material of unknown origin, it is necessary to find a sufficient number of autochthonous entities (i.e., populations of the researched species) as well as new suitable markers for their characterization.

The results of this paper constitute a scientifically recognised, partial examination of the origin of the starting forest reproductive material from the broader geographic area, thus serving future projects on its regeneration.

KEY WORDS: *Quercus pubescens*, pubescent oak, variability, cpDNA.

INTRODUCTION

Pubescent oak (*Quercus pubescens* Willd.) is one of the most important types of broad-leaf trees in the Mediterranean and sub-Mediterranean areas, including the western part of the Balkan Peninsula comprised of Croatia, Bosnia and Herzegovina, Montenegro, Kosovo, Albania and Macedonia (Trinajstić 1986). The pubescent oak in this area occurs in climatogenic forests together with the Oriental hornbeam (*Carpinus orientalis* Mill.), forming the forest community *Carpinetum orientalis adriaticum* = *dinaricum* (Horvat et al. 1974). According to Stefanović (1977) and

Trinajstić (1986), this type of forest covers huge areas in Istria and the islands of Krk and Cres, the Mediterranean and continental sub-Mediterranean parts of Croatia, Herzegovina, Montenegro and Albania. This type of pubescent oak forest can be found in three zones: warm, moderate and cold. In inland areas, this type of forest can be found even in the high Dinaric mountains, where on the southern warm slopes it reaches as high as 900 m above sea level, especially on the edges of Karst valleys. It also penetrates deep into the inland in the valleys of the major rivers of the Adriatic basin. It appears in the central parts of the Balkan peninsula as well, but only in dry, arid and, above all,

warm places. The xerothermal character of the forest is determined by the sub-Mediterranean climate, which is characterized by warm and dry summers, and specific orthographic and edaphic conditions that lend themselves to thermophilic vegetation (Stefanović et al. 1983).

Morphological variability of the pubescent oak in the area this study covers is very high, as is its taxonomy,

which has a large number of varieties (Jovanović 2000; Franjić et al. 2006). A number of varieties can be found in the literature and, according to some authors (Schwarz 1936; Pignatti 1997; Trinajstić 1974, 2007; Škvorc 2003), some of them should be treated as separate species (Uzunova et al. 1996; Bussotti and Grossoni 1997; Bruschi et al. 2000). This primarily refers to *Quercus brachyphylla* Kot-

TABLE 1. The studied populations of pubescent oak (*Quercus pubescens*), and chloroplasts haplotypes in western Balkans.

Ordinal number	Country	Population indicator	Population	No of individuals	Longitude	Latitude	Altitude (m)	Haplotype	Sub-haplotype
1		1	Avtovac	1	43°04'	18°32'	1000	5	–
2		3	Bileca	5	42°54'	18°24'	780	5	b
3	Bosnia and Herzegovina	8	Glamocko polje	4	43°58'	16°54'	1050	5/17	–
4		12	Kifino selo	4	43°17'	18°13'	1020	17	–
5		15	Popovo polje	1	42°44'	18°15'	320	5	a
6		21	Tjentiste	2	43°23'	18°46'	462	2/4	–
7		23	Vogosca	1	43°53'	18°21'	600	5	b
8		28	Jasenovo polje	5	42°51'	18°56'	845	4	–
9	Montenegro	30-a	Petrovac	3	44°33'	16°17'	700	5	–
10		30	Sv. Stefan	3	42°16'	18°53'	55	33	–
11		31	Tuzi	4	42°20'	19°20'	140	17	–
12	Serbia-Kosovo	33	Pristina	5	42°37'	21°11'	650	5	–
13		35	Bunic	5	44°41'	15°39'	693	2	–
14		40	Cepikuce	5	42°49'	17°49'	350	5	a
15		52	Imotski	6	43°27'	17°09'	–	5	b
16		55	Karadza	2	43°34'	16°49'	425	5	a
17		56	Karin	3	44°07'	15°35'	230	5	a
18		58	Konavle	4	42°33'	18°17'	160	5	–
19		66	Medvidja	3	44°06'	15°46'	503	5	a
20		71	Pakostane	3	43°54'	15°31'	58	17	a
21		73	Slivno-Raba	4	42°58'	17°31'	–	31/2	b
22	Croatia	76	Roski slap	3	43°52'	15°59'	–	2	–
23		77	Visovacka brina	3	45°48'	15°37'	67	5	b
24		83	Papratno	1	42°51'	17°38'	236	5	b
25		84	Smrđljivac-Kom	3	44°12'	16°03'	160	2	–
26		85	Staza	4	43°15'	17°06'	420	5	–
27		87	Sveti Ilija	5	43°00'	17°09'	–	31/5	a
28		89	Trilj	1	43°37'	16°45'	–	5	b
29		93	Vidova Gora	2	43°19'	16°36'	–	5	–
30		95	Vrlika	5	43°52'	16°29'	–	5	a
31		63	Ližljan	4	44°49'	13°58'	49	2	–
32	FYR Macedonia	100	Kicevo	2	41°29'	20°54'	690	5	–
33		101	Kratovo	3	42°09'	22°36'	–	5	–
34		106	Liqe I kuq	3	42°10'	20°15'	650	5	–
35	Albania	107	Kerpice	3	40°40'	20°10'	350	5	–
36		108	Bishnice	2	40°54'	20°25'	600	5	–
Total			36						117

schy 1862, *Q. dalechampii* Tenore 1830, *Q. virgiliana* (Ten.) Ten. 1835, *Q. congesta* C. Presl. 1822, *Q. sicula* Borzi in Lojac. 1907.

For centuries, this species has been exposed to anthropogenic activity in this region, leading to a change in forest structures as well as in species habitats (Begović 1960, 1978). The major reason for this is the continuous use of leaf litter for live stock in extensive cattle breeding, resulting in the creation of coppices of broad-leaf forests that persist to this day throughout the entire distribution area.

In the last 13,000 years, oaks have undergone constant dynamic movement, moving from their refugia in the south of Europe to Central, Western and Eastern Europe. This was caused by considerable climatic change, one of the most important factors in forest tree colonization. The best illustration of the dynamics of forest tree colonization was provided by the analysis of fossil pollen, effectively indicating the directions of colonization in Europe (Huntley and Birks 1983; Hewit et al. 1999). Recent molecular research involving cpDNA and mtDNA has built upon earlier studies. Thus, many assumptions resulting from pollen analyses have been confirmed at the molecular level in research on the geographic articulation of haplotype variability of oak related to the postglacial colonization of oaks (Dumolin et al. 1995; Dumolin-Lapegue et al. 1998; Ferris et al. 1993, 1995, 1998; Petit et al. 1993, 2002a, b; Slade et al. 2008). All investigations have confirmed the earlier results obtained in the analysis of fossil pollen. Enormous genetic diversity between researched populations was expected, particularly those close to old glacial oak refugia. Five of these survived the last ice age, and it is from these that oaks moved to the north to the present distribution area.

The aim of this study is to present the haplotype structure of pubescent oak in the area of the Western Balkans, as well as its glacial origin, applying molecular genetic analysis of pubescent oak populations by means of chloroplast DNA (cpDNA).

Apart from fundamental significance of this study, it can also be applied to the regeneration of degraded forests through control of the origin of seed and seedlings relevant to the

process of artificial regeneration of pubescent oak forests (forestry and planting of seeds), as well as by setting up genetic banks and archives applying in situ and ex situ methods.

## MATERIALS AND METHODS

Samples from 36 populations of the pubescent oak were collected in Croatia, Bosnia and Herzegovina, Montenegro, Serbia-Kosovo, Albania and Macedonia (Table 1, Fig. 1). Five to six tree individuals were collected, with the analysis successful in one to six samples per population. The samples were collected from relatively older trees, according to current methodology. Fresh buds in the resting period were used in the analysis.

Between five and ten buds per tree were used for the isolation of DNA depending on their size and quality. The isolation of the total DNA was performed by means of modified CTAB protocol according to Doyle and Doyle (1987), and the level of success of DNA isolation was measured by spectrometer. Upon successful extraction, a polymerase chain reaction (PCR) was begun.

Four fragments of chloroplast DNA were multiplied. For the amplification of every fragment, the pairs of primers shown in Table 2 were used.

Restrict enzyme digestion of cpDNA fragments AS and TF obtained by polymerase chain reaction (PCR) was conducted with *Hinf I* (Demesure et al. 1995; Taberlet et al. 1991) over a duration of five hours DT and CD with *Taq I* (Demesure et al. 1995) over a duration of three hours. Upon digestion, electrophoresis was carried out on an 8% polyacrylamid gel for one hour and ten or fifteen minutes, depending on the studied fragment and the AFLP technique applied. The fragments were scored and haplotypes determined according to the system of Petit et al. (2002a).

The bands obtained on gels were recorded and read using computers (Table 3).

The results were statistically processed using the haplont and haplodive software, which can be found on the web site [www.Pierroton.inra.fr/genetics/Labo/Software](http://www.Pierroton.inra.fr/genetics/Labo/Software).

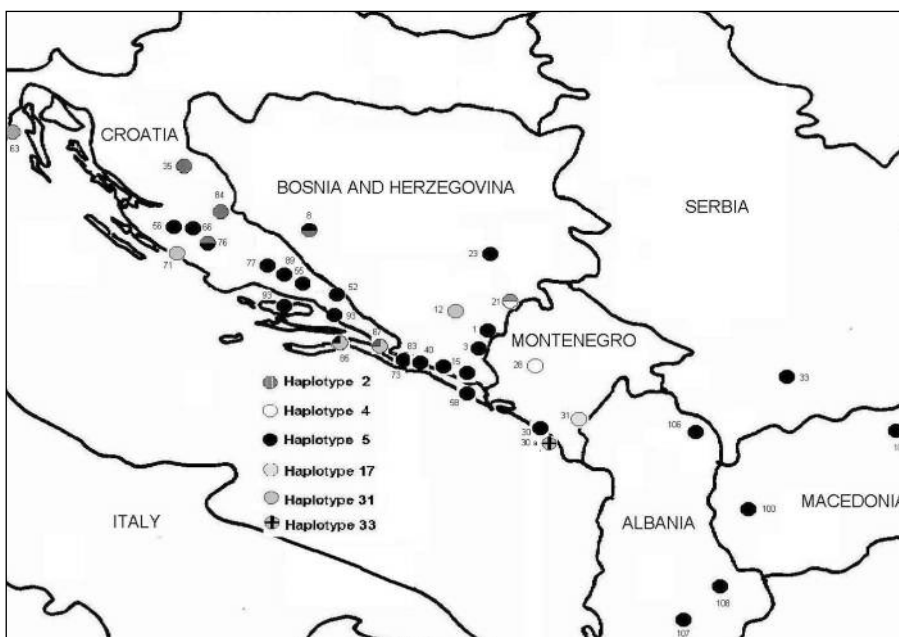


Fig. 1. The distribution of pubescent oak chloroplasts haplotypes in western Balkans.

TABLE 2. Basic details of the primers and restriction enzymes.

Fragments	Primers	Number of bases	Sequence (5'-3')	Annealing temperature ( $T_A$ ) in °C	Restrict enzyme
AS	<i>psa A</i> [PSI(P700 apo protein A1)]	22	5'-ACTTCTGGTTCGGCGAACGAA-3'	57.5	<i>Hinf I</i>
	<i>tm S</i> [tRNA-Ser(GGA)]	22	5'-AACCACTCGGCCATCTCTCCTA-3'		
DT	<i>tm D</i> [tRNA-Asp(GUC)]	19	5'-ACCAATTGAACTACAATCC-3'	54.5	<i>Taq I</i>
	<i>tm T</i> [tRNA-Thr(GGU)]	20	5'-CTACCACTGAGTTAAAAGGG-3'		
CD	<i>tm C</i> [tRNA-Cys(GCA)]	20	5'-CCAGGTCAAATCTGGGTGTC-3'	58.0	<i>Taq I</i>
	<i>tm D</i> [tRNA-Asp(GUC)]	20	5'-GGGATTGTAGTTCAATTGGT-3'		
TF	<i>tm T</i> [tRNA-Thr(UGU)]	20	5'-CATTACAAATGCGATGCTCT-3'	57.5	<i>Hinf I</i>
	<i>tm F</i> [tRNA-Phe(GAA)]	19	5'-ATTGAACTGGTGACACGAG-3'		

TABLE 3. The position of bands on the gels for the detected haplotypes.

Gel	DT	CD	AS	TF	Haplotype
Bands positions	91211	19221	142923	2020211	2
	11111	11231	162223	2020212	4
	11211	11231	162223	2020212	5a
	11211	11231	162223	2030213	5b
	11311	11231	142223	2020212	17
	21211	11231	162223	2020212	31
	11211	11231	152222	222212	33

Total variability ( $h_T$ ) was determined, as well as variability within the population ( $h_S$ ), haplotype differentiation irrespective of the relationship between haplotypes ( $G_{ST}$ ) with consideration of parental relations ( $N_{ST}$ ), as well as the optimal number of samples per population ( $n_{opt}$ ), according Pons and Petit (1995).

## RESULTS

In thirty-two populations, only one haplotype was found, whereas in four populations two haplotypes were found, as shown in Figure 1. In the 13 populations where haplotype 5 occurs, sub-haplotypes *a* and *b* were found. These were also found in haplotype 31. In mixed populations, haplotypes 2, 4, 5, 17 and 31 were present. Haplotypes 4, 5, 31 and 33 were probably also present, these being typical haplotypes of the Balkan peninsula. In nine populations haplotypes of the Apennine origin emerge, specifically haplotypes 2 and 17. A large number of pure populations suggests the relatively minor influence of man (i.e. interpopulation genetic flow affected by man).

Haplotype 2 was found only in the populations of Tjentište, Bunić, Slivno Raba, Roški slap, Smrdljivac Kom and Ližljan. This haplotype originated in the Apennines. In Western Croatia it can be found in pure populations, suggesting a direct link with the Apennine populations. In the central part of the researched area it exists both in isolation and in mixed populations. This haplotype is the most common in the north-west of this habitat (Petit et al. 2002a).

Haplotype 4 can be found only in one pure (Jasenovo polje) and one mixed population (Tjentište). It is a typical example of Balkan haplotypes, and spreads as far north as Denmark (Petit et al. 2002a).

Haplotype 5, which is the most common in this area, (Table 1 and Figs 1 and 2) is a typical Balkan haplotype,

although some studies (Petit et al. 2002a) point to the fact that it can also be found in Central Europe. It emerges in twenty-five populations, twenty-three of which are pure. Also, for this haplotype, sub-haplotypes *a* and *b* (Table 1) were established by the TF gel.

Haplotype 17 was found in four populations: Glamočko polje, Kifino selo, Tuzi and Pakošane. It is also of Apennine origin, and within these isolated habitats it spreads mainly to the north-west. Furthermore, the Glamočko polje population is of mixed origin, a combination of haplotypes 5 and 17.

Haplotype 31 was registered in two mixed populations affected by the Mediterranean climate, Slivno-Raba and Sveti Ilija populations, and sub-haplotypes were also found in each. This haplotype has also been registered on the Adriatic coast and in Romania. Haplotype 33 was only found in the Sveti Stefan population, and is a brand new haploty-

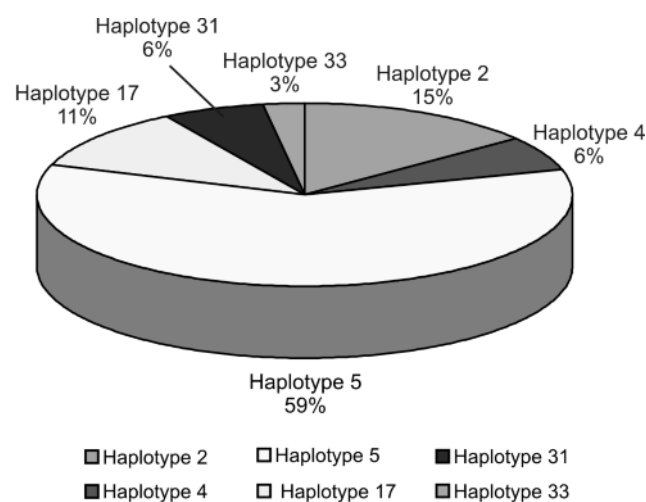


Fig. 2. Haplotypes structure of the studied populations.

TABLE 4. The parameters that show the portion of the units of each haplotype, parameter  $h_S$  shows the haplotypes variability contained within the population,  $h_T$  an overall haplotype variability within and between the populations,  $G_{ST}$  a part of variability which falls to variability between populations (data were obtained by statistical program *haplodiv*, and were used according to Pons and Petit (1995)).

Haplotype	2	4	5	17	31	33	Total
Total of indiv.	17	6	71	13	7	3	117
Share of indiv. %	14.52	5.13	60.68	11.12	5.98	2.53	100
Share of pop. %	3.34	0	3.15	1.92	2.67	0	6.5
$h_S$	0.0406	0	0.0410	0.0256	0.0346	0	0.0816
$h_T$	0.2522	0.0769	0.5099	0.2400	0.1160	0.0770	0.6578
$G_{ST}$	0.8390	1.0000	0.9195	0.8938	0.7004	1.0000	0.8759

pe. It may have been the result of local mutation and may not have spread from its refugia, or the analysis may have lacked precision. Because of this, the population should be studied in more detail.

The largest haplotype variability was observed in southern and central Dalmatia (Croatia, which is the meeting point of the Mediterranean and sub-Mediterranean floristic groups, i.e. climatic zones). Southern populations from Macedonia and Albania showed the consistent presence of haplotype 5, which is typical of the Balkan glacial refugia (Table 1 and Fig. 1).

Variability indicators in the studied populations had the following values: haplotype variability contained within populations ( $h_S$ ) was 0.0816, total intra- and inter-population haplotype variability ( $h_T$ ) was 0.6578, and variability accounting for inter-population variability ( $G_{ST}$ ) was 0.8759. The optimal number of samples per population ( $n_{opt}$ ) was 2.68 individuals, and the size of parental relations ( $N_{ST}$ ) was 0.0012, which is a very low figure (Table 4).

## DISCUSSION

Given the ecological and economic significance of this relatively valuable species, the amount of research that has been conducted so far on the countries of the western Balkans may be considered negligible. The results obtained in this study can, however, serve as guidelines for further research at this level, as the method applied in this study provides only a small portion of the populational genetic information, with a large part remaining inaccessible. Furthermore, the possible applications of haplotype variability in any ecological research are rather limited (Bachmann 1994).

Thanks to the use of genetic markers, inter- and intra-population differences, if any, become more obvious and distinct. The possible causes of these differences, apart from natural selection and anthropogenic activity, may also include developmental factors or processes of adjustment to specific ecological conditions, as well as mutation processes, which are likely to have caused the occurrence of haplotype 33 in the Sveti Stefan population.

The area of the Balkan Peninsula is very specific in terms of environmental conditions, with great diversity of climatic, edaphic, orographic and other factors within a small area, which directly leads to the differentiation of ecotypes. This is why experts believe that forest tree species from the Balkans display considerable variability

when compared to the same species from the north (Comps et al. 1991; Gömöry et al. 1999; Konnert and Bergmann 1995). Within a small area the studied populations of pubescent oak from the the western Balkans reveal great variability. The cause of this variability should, nevertheless, be sought in the crossing of routes during postglacial migration in this area, while in the southern part the material is homogenous, representing autochthonous oak refugia (Fineschi et al. 2002; Petit et al. 2002a, b).

If the basic methodological effects (i.e. the number of individuals in the sample, as well as the development of the species and anthropogenic activity over thousands of years) cannot be excluded, the differences observed between the populations of pubescent oak may suggest that adaptation processes in each of the studied populations may play a relevant role (Müller and Aas 1997). Therefore, on the basis of the results, the following conclusions can be drawn:

- The haplotype variability observed is not very characteristic of Western, Northern and Eastern Europe, but is more or less typical of the Balkan Peninsula (South-eastern Europe). It should be noted that a high level of variability was observed in central Dalmatia.
- Given the position of haplotype 31 and its distribution area in Europe, and given its possible adaptability in certain ecological conditions, its glacial refugia may well be sought in the area of southern Dalmatia in Croatia.
- Interestingly, haplotype 5 was the most common haplotype (Fig. 2) in that its occurrence in southern parts of the Balkan Peninsula was pure, particularly in Serbia-Kosovo, Albania and Macedonia, these being typical examples of the Balkan glacial refugia.
- Occurrence of haplotype 33, which has been registered nowhere else, may either be a consequence of mutational processes that took place in the Sveti Stefan population or of imprecisions during the population identification. Because of this, we believe that the analysis of this population should be repeated.

On the basis of the above, we may conclude that the existing differences between populations and their genetic differentiation were caused by postglacial migration and the crossing of these migrational routes. It is possible that the adaptability of certain genotypes to certain habitats was affected by specific selection processes (Gregorius 1991).

However, the results must be taken with certain reservations. When it came to full identification, the researchers encountered a dilemma over the sample size necessary to embrace the actual genetic diversity of the studied populations. It is a well-known fact that different increments in

the number of individuals in the population sample give correspondingly different ideas of genetic structure. As the sample increment increases, so does the likelihood that rare haplotypes will appear in a particular population.

Because of this, regeneration of the pubescent oak and the protection of its genofond (i.e. its diversity, considering its current state), will play an important role in the future. Regeneration itself is related to the solution of certain problems, from complicated nursery production due to special ecological requirements of the species to a poorly represented gene pool in the material produced. In view of this, the following facts should be considered with regard to artificial regeneration:

- Origin of seeds and seedlings: the seeds should have a genetic structure that will match the local populations selected on the basis of seed zones.
- Determining the optimal number of plants to be included in the processes of natural and artificial regeneration in order to end up with an appropriate number of plants that would represent the genetic structure of the population.
- The use of seeds collected from as many trees as possible that are evenly distributed in the population and of different age in order to reduce the possibly detrimental impact of inbreeding. This will help to preserve the genetic structure of the population.
- Maintaining maximal heterogeneity of newly planted trees and allowing natural selection in order to eliminate as many non-adapted individuals as possible.

## CONCLUSIONS

Across the region including Croatia, Montenegro, Kosovo, Albania and Macedonia, six haplotypes were found: 2, 4, 5, 17, 31 and 33, as well as subtypes of haplotypes 5 (a, b).

The greatest variability of haplotype was found in central Dalmatia. This could be the consequence of the localization of glacial refugia in the mouth of the Neretva river (haplotype 31) and probably near Skadar lake (haplotype 4).

The most common is haplotype 5, which has two sub-haplotypes. Haplotype 2 occurs in the west, and haplotype 17 is found in Montenegro, Bosnia and Herzegovina and Croatia. Haplotype 4 has an interesting distribution – from the Zeta to Komarnica rivers. Haplotype 33 was found in just one population (Sveti Stefan) in Montenegro.

Four haplotypes originate from the Balkan refugia; one can be found throughout the entire research area, while the others are more geographically structured.

Two haplotypes are of Apennine origin.

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## LITERATURE CITED

- BACHMANN K. 1994. Molecular markers in plant ecology, *New Phytologist* 126: 403-418.
- BEGOVIĆ B. 1960. Strani kapital u šumarskoj privredi Bosne i Hercegovine za vrijeme Otomanske vladavine. RADOVI V. pp. 0-274, Sarajevo. (in Bosnian)
- BEGOVIĆ B. 1978. Razvojni put šumske privrede u Bosni i Hercegovini u periodu Austrougarske uprave (1878-1918) sa posebnim osvrtom na eksploataciju šuma i industrijsku preradu drveta. DJELA – Anu –BiH. Knjiga 31, pp. 0-204, Sarajevo. (in Bosnian)
- BRUSCHI P., VENDRAMIN G.G., BUSSOTTI F., GROSSONI P. 2000. Morphological and molecular differentiation between *Quercus petraea* (Matt.) Liebl. and *Quercus pubescens* Willd. (Fagaceae) in northern and central Italy. *Ann. Bot.* 85: 325-333.
- BUSSOTTI F., GROSSONI P. 1997. European and Mediterranean oaks (*Quercus* L.; Fagaceae): SEM characterization of the micromorphology of the abaxial leaf surface. *Biol. J. Linn. Soc.* 124: 183-199.
- COMPS B., THIÉBAUT B., SUGAR I., TRINAJSTIĆ I., PLAZIBAT M. 1991. Genetic variation of the Croatian beech stands (*Fagus sylvatica* L.): spatial differentiation in connection with the environment. *Ann. For. Sci.* 48: 15-28.
- DOYLE J.J., DOYLE J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* 19: 11-15.
- DEMASURE B., SODZI N., PETIT R.J. 1995. A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. *Mol. Ecol.* 4: 129-131.
- DUMOLIN S., DEMASURE B., PETIT R.J. 1995. Inheritance of chloroplast and mitochondrial genomes in pedunculate oak investigated with an efficient PCR method. *Theor. Appl. Genet.* 91 (8): 1253-1256.
- DUMOLIN-LAPEGUE S., PEMONGE M.H., PETIT R.J. 1998. Association between chloroplast and mitochondrial lineages in oaks. *Mol. Biol. Evol.* 15 (10): 1321-1331.
- FERRIS C., OLIVER R.P., DAVY A.J., HEWITT G.M. 1993. Native oak chloroplast reveal an ancient divide across Europe. *Molecular Ecology* 2: 337-344.
- FERRIS C., OLIVER R.P., DAVY A.J., HEWITT G.M. 1995. Using chloroplast DNA to trace postglacial migration routes of oaks into Britain. *Mol. Ecol.* 4: 731-738.
- FERRIS C., KING R.A., VAINOLA R., HEWITT G.M. 1998. Chloroplast DNA recognizes three refugial sources of European oaks and suggests independent eastern and western immigrations to Finland. *Heredity* 80: 584-593.
- FINESCHI S, TAURCHINI D., GROSSONI P., PETIT R.J., VENDRAMIN G.G. 2002. Chloroplast DNA variation of white oaks in Italy. *For. Ecol. Manage.* 156: 103-114.
- FRANJIC J., LIBER Z., ŠKVORC Ž., IDŽOJTIĆ M., ŠOŠTARIĆ R. 2006. Morphological and molecular differentiation of the Croatian populations of *Quercus pubescens* Willd. (Fagaceae). *Acta Soc. Bot. Pol.* 75 (2): 123-130.
- GÖMÖRY D., PAULE L., BRUS R., ZHELEV P., TOMOVIĆ Z., GRAČAN J. 1999. Genetic differentiation and phylogeny of beech on the Balcan peninsula. *J. Evol. Biol.* 12: 746-754.
- GREGORIUS H.R. 1991. Gene Conservation and the Preservation of Adaptability. In Seitz, A., Loeschke, V. (eds), *Species Conservation: A Population-Biological Appgautschi@fo-wi.ethz.chroach*. Birkhäuser Verlag, Basel pp. 31-47.
- HORVAT I., GLAVAC S, ELLENBERG H. 1974. Vegetation Südosteuropas. *Geobotanica selecta*, Bd. VI Gustav Fischer Verlag, Stuttgart.
- HEWITT G.M., RACEY P.A., BACON P.J., DALLAS J.F. 1999. Post-glacial re-colonization of European biota. *Proc. Biol. J. Linn. Soc.* 68 (1-2): 87-112.
- HUNTLEY B., BIRKS H.J.B. 1983. An atlas of past and present pollen maps for Europe 0-13 000 years ago, Cambridge University Press, Cambridge pp. 73-90.
- JOVANOVIĆ B. 2000. Dendrologija. Univerzitetska štampa, Beograd. (in Serbian)
- KONNERT M., BERGMANN F. 1995. The geographical distribution of genetic variation of silver fir (*Abies alba*, Pinaceae) in relation to a migration history. *Plant Syst. Evol.* 196 (1-2): 19-30.

- MÜLLER B., AAS G. 1997. Species-specific variability of *Quercus pubescens* in central Europe. In: K.C. Steiner (ed.), Diversity and adaptation in oak species. Proceedings of the second meeting of IUFRO working party 2.08.05, Genetics of *Quercus*, October 12-17, 1997, University park, Pennsylvania USA pp. 132-140.
- PETIT R.J., KREMER A., WAGNER D.B. 1993. Geographic structure of chloroplast DNA polymorphisms in European oaks. *Theor. Appl. Genet.* 87: 122-128.
- PETIT R.J., BREWER S., BORDACS S., BURG K., CHEDDADI R., COART E., COTTRELL J., CSAIKL U.M., VAN DAM B.C., DEANS J.D., FINESCHI S., FINKELDEY R., GLAZ I., GOICOECHEA P.G., JENSEN J.S., KÖNIG A.O., LOWE A.J., MADSEN S.F., MÁTYÁS G., MUNRO R.C., POPESCU F., SLADE D., TABBENER H., DE VRIES S.M.G., ZIEGENHAGEN B., DE BEAULIEU J.L., KREMER A. 2002a. Identification of refugia and postglacial colonization routes of European white oaks based on chloroplast DNA and fossil pollen evidence. *For. Ecol. Manage.* 156 (1-3): 49-74.
- PETIT R.J., CSAIKL U.M., BORDACS S., BURG K., BREWER S., COART E., COTTRELL J., VAN DAM B.C., DEANS J.D., GLAZ I., DUMOLIN-LAPEGUE S., FINESCHI S., FINKELDEY R., GILLIES A., GOICOECHEA P.G., JENSEN J.S., KÖNIG A.O., LOWE A.J., MADSEN S.F., MÁTYÁS G., MUNRO R.C., PEMONGE M.H., POPESCU F., SLADE D., TABBENER H., TAURCHINI D., DE VRIES S.M.G., ZIEGENHAGEN B., KREMER A. 2002b. Chloroplast DNA variation in European white oaks. *Phylogeography and patterns of diversity based on data from over 2600 populations.* *For. Ecol. Manage.* 156 (1-3): 5-26.
- PIGNATTI S. 1997. *Flora d'Italia 1.* Edagricole. Bologna. (in Italian)
- PONS O., PETIT R.J. 1995. Estimation, variance and optimal sampling of gene diversity, I. Haploid locus. *Theor. Appl. Genet.* 90: 462-470.
- SLADE D., ŠKVORC Z., BALLIAN D., GRAČAN J., PAPEŠ D. 2008. The chloroplast DNA polymorphisms of White Oaks of section *Quercus* in the Central Balkans. *Silvae Genet.* v. 57 (4-5): 227-234.
- STEFANOVIĆ V. 1977. *Fitocenologija sa pregledom šumskih fitocenozna Jugoslavije.* Zavod za udžbenike Sarajevo. (in Bosnian)
- STEFANOVIĆ V., BEUS V., BURLICA Č., DIZDAREVIĆ H., VUKOREP I. 1983. *Ekološko-vegetacijska rejonizacija Bosne i Hercegovine.* Šumarski fakultet Sarajevo, Posebna izdanja br. 17. (in Bosnian)
- SCHWARZ O. 1936. *Monographie der Eichen Europas und des Mittelmeergebietes.* Feddes Repert. Sonderdruck D pp. 1-200.
- ŠKVORC Ž. 2003. *Morfološka i genetička varijabilnost hrasta medunca (*Quercus pubescens* Willd.) i duba (*Q. virgiliana* (Ten.) Ten.) u Hrvatskoj.* Magistarski rad (Mscr.). Biološki odsjek PMF. Zagreb. (in Croatian)
- TABERLET P., GIELLY L., PAUTOU G., BUVET J. 1991. Universal primers for amplification of tree non-coding regions of chloroplast DNA. *Plant Mol. Biol.* 17: 1105-1109.
- TRINAJSTIĆ I. 1974. *Quercus* L. In I. Trinajstić (ed.), *Anali tiča flora Jugoslavije* 1(3). Institut za botaniku Sveučilišta u Zagrebu pp. 460-481. (in Croatian)
- TRINAJSTIĆ I. 1986. *Fitogeografsko raščlanjenje šumske vegetacije istočnojadranskog sredozemnog područja – polazna osnovica u organizaciji gospodarenja mediteranskim šumama.* *Glasnik za šumarske Pokuse, Posebno Izdanje 2:* 53-67. (in Croatian)
- TRINAJSTIĆ I. 2007. O problemu međusobnog razlikovanja hrastova *Quercus pubescens* Willd. i *Quercus virgiliana* (Ten.) Ten. *Šumarski List* 1/2: 57-60. (in Croatian)
- UZUNOVA K., PALAMAREV E., EHRENDORFER F. 1996. Anatomical changes and evolutionary trends in the foliar epidermis of extant and fossil Euro-Mediterranean oaks (Fagaceae). *Plant Syst. Evol.* 204: 141-159.