INTRA- AND INTERPOPULATION RELATIONSHIPS AND TAXONOMIC STATUS OF *PINUS NIGRA* ARNOLD IN CROATIA ACCORDING TO MORPHOLOGY AND ANATOMY OF NEEDLES

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ABSTRACT

Intra- and interpopulational relationships and taxonomic status of six natural black pine populations (*Pinus nigra* Arnold) from Croatia were analyzed. Special attention in this research was paid to the establishment of taxonomic status and distribution of stenoendemic Dalmatian black pine (*Pinus nigra* Arnold subsp. *dalmatica* (Vis.) Franco). Three morphological and twenty-one anatomical traits of needles were used as well as analysis by the methods of multivariate statistics. Most of the researched individuals showed strong population affiliation, whereas a clinal transition of morphological and anatomical traits of needles was determined among the populations. The opinion of the existence of three subspecies of black pine on the territory of Republic of Croatia (*Pinus nigra* Arnold subsp. *nigra*, *Pinus nigra* Arnold subsp. *dalmatica* (Vis.) Franco and *Pinus nigra* Arnold subsp. *illyrica* (Vid.) Fukarek) was confirmed. For each of the three subspecies new ranges were represented. Four Croatian populations were included in the stenoendemic subspecies of the Dalmatian black pine (Brač, Hvar, Grobnik and Pelješac), and a new distribution, broken in two, was suggested. Since the Dalmatian black pine has been reduced to several populations only, and there are some signs of continuous diminishing of its range, this research points out the need of making an action plan to preserve all natural populations of this subspecies.

KEY WORDS: black pine, Croatia, intra- and interpopulation relationships, intraspecies taxonomy, distribution, endemic plant, conservation.

INTRODUCTION

The European black pine (*Pinus nigra* Arnold) is distributed along the Mediterranean Basin. In accordance with the distinctive traits of that area the black pine has a very discontinuous range and it is extremely variable in the morphological, anatomical and physiological traits. Although taxonomic researches within this species have had a long tradition (e.g. Olzabal 1915; Ronninger 1924; Georgescu 1937; Schwartz 1938; Delevoy 1949; Svoboda 1953; Vidaković 1953, 1957, 1991; Fukarek 1958; Gaussen et al. 1964, 1993; Ivanov 1971; Arbez et al. 1974; Nikolić and Tucić 1983; Scaltsoyiannes et al. 1994; Raffii et al. 1996), the variability mentioned above, the large area of distribution and the absence of systematic research of the whole range resulted in intraspecies taxonomy, classification and chorology that are still unclear (Vidaković 1991; Barbéro et al. 1998). Although the classification which divides black pine into five subspecies (*P. nigra* Arnold subsp. *dalmatica* (Vis.) Franco, *P. nigra* Arnold subsp. *laricio* (Poiret) Maire, *P. nigra* Arnold subsp. *nigra*

(*P. nigricans* Host), *P. nigra* subsp. *pallasiana* (Lamb.) Holmboe and *P. nigra* subsp. *salzmannii* (Dunal) Franco) is mostly used today (Gaussen et al. 1964, 1993; Jalas and Suominen 1988), it greatly simplifies the real intraspecific picture of this species, especially in the eastern, insufficiently studied part of its range.

All the past classifications of the black pine on the territory of the Republic of Croatia (e.g. Schwartz 1938; Svoboda 1953; Vidaković 1957, 1991; Fukarek 1958; Gaussen et al. 1964, 1993; Jalas and Suominen 1988; Price et al. 1998), directly or indirectly, took over their main information exclusively from the first description of the Dalmatian black pine (Visiani 1842), or from the only one comparative taxonomic research of the black pine in this area (Vidaković 1953). In accordance with these two studies, two (*P. nigra* subsp. *nigra* and *P. nigra* subsp. *dalmatica*) (Fig. 1a) or three (*P. nigra* subsp. *nigra*, *P. nigra* subsp. *dalmatica* and *Pinus nigra* Arnold subsp. *illyrica* (Vid.) Fukarek) (Fig. 1b) subspecies of the black pine have been distinguished in this region up to now.

Fig. 1. a) Distribution of different black pine subspecies in Croatia adapted from Jalas and Suominen (1988), ● – *Pinus nigra* Arnold subsp. *nigra*, ▲ – *Pinus nigra* Arnold subsp. *dalmatica* (Vis.) Franco; b) Distribution of different black pine subspecies in Croatia adapted from Fukarek (1958).

Since the biggest problem with the Visiani's research is coming to the conclusion about taxonomic relationships without the sufficient comparative material, whereas the flaw of the Vidaković's research is comparison of the populations that are too different in the number of researched individuals (in some cases particular populations were represented with just one individual), the taxonomic status as well as distribution of certain taxa of the black pine on the territory of Croatia call for revision.

The intention of this work was to determine intra and interpopulational relationships among six all-natural populations of the black pine from Croatia using the morphological and anatomical traits of leaf and the methods of multivariate statistics, in order to evaluate their taxonomic status and distribution. Special care was taken that all the known populations of the Dalmatian black pine were included in this research. The new information about this subspecies, widely known from many classifications (e.g. Schwartz 1938; Svoboda 1953; Vidaković 1957, 1991; Fukarek 1958; Gaussen et al. 1964, 1993; Price et al. 1998), is specially precious because it is stenoendemic for this region and it has been included recently, as the only black pine subspecies, in the preliminary list of endangered conifers of the world (Fajron et al. 1993).

MATERIALS AND METHODS

All the natural populations considered today to belong to the Dalmatian black pine (the islands of Brač and Hvar) or to be of the transitional character (Pelješac peninsula and Biokovo mountain) (Vidaković 1953, 1957; Fukarek 1958, Domac 1965; Trinajstić 1986; Rauš et al. 1992) were included in this research. The populations from Grobnik and Velika Paklenica, which are considered to be either Illyric black pine (Vidaković 1953, 1957) or Austrian black pine (Horvat 1956; Gaussen et al. 1964, 1993), were also included. The population of the Austrian black pine from the Mödlinger Klause locality in Austria was used as

a control group. The plant material from Croatia was collected in March 1996, while that from Austria in July 1997 (Fig. 2).

The 61 individuals of the black pine were included in the research. Every individual was represented by 30 needles of a fertile branch (10 needles aged one year, 10 needles aged two years and 10 needles aged three years). Three morphological and 21 anatomical traits were measured for every needle (Table 1). The chosen morphological and anatomical traits were based on the traits recommended in Vidaković's research (1953), but unlike that research, qualitative traits that showed to be very dependent on the sub-

Fig. 2. Geographical position of the analyzed Croatian black pine populations. AUS – Austria (10), BIO – Biokovo (8), BRA – Brač (10), GRO – Grobnik (9), HVA – Hvar (8), PAK – Velika Paklenica (9), PEL – Pelješac (7). Numbers in brackets denote the number of sampled individuals.

TABLE 1. Morphological and anatomical characters of needles, their eigenvectors for the first three PC axes (for 61 black pine individuals), eigenvalues, their percentage and cumulative percentages.

No.	Characters	PC axes		
		1	2	3
1	Length of needle (mm)	0.07	0.61	-0.36
2	Width of needle (µm)	-0.04	-0.80	-0.53
3	Height of needle (µm)	-0.13	-0.65	-0.67
4	Number of stomata	-0.52	-0.48	-0.23
5	Number of resin ducts	-0.59	-0.77	0.09
6	Average number of the cells that surround resin duct	-0.38	-0.04	0.71
7	Average diameter of the resin ducts (µm)	-0.16	-0.46	0.66
8	Average height of epidermal cell on the flat side of the needle (µm)	0.46	-0.04	0.57
9	Average height of epidermal cell on the convex side of the needle (µm)	0.24	0.50	0.39
10	Average height of epidermal cell in the corners of the needle (µm)	-0.30	0.40	0.19
11	Number of hypodermal layers on the flat side of the needle – minimum	-0.86	0.36	-0.07
12	Number of hypodermal layers on the flat side of the needle – maximum	-0.82	-0.06	0.08
13	Number of hypodermal layers on the convex side of needle – minimum	-0.67	0.39	-0.11
14	Number of hypodermal layers on the convex side of the needle – maximum	-0.60	-0.55	0.22
15	Number of hypodermal layers in the corners of the needle – minimum	-0.60	0.28	0.04
16	Number of hypodermal layers in the corners of the needle – maximum	-0.35	-0.16	-0.01
17	Number of mesophyll layers on the flat side of the needle – minimum	-0.85	0.29	0.31
18	Number of mesophyll layers on the flat side of the needle – maximum	-0.81	-0.32	0.35
19	Number of mesophyll layers on the convex side of the needle – minimum	-0.81	0.15	0.26
20	Number of mesophyll layers on the convex side of the needle – maximum	-0.84	-0.09	0.25
21	Number of transfusion parenchyma layers on the phloem side – minimum	-0.55	0.61	0.01
22	Number of transfusion parenchyma layers on the phloem side – maximum	-0.59	0.90	-0.05
23	Number of transfusion parenchyma layers on the xylem side – minimum	-0.83	-0.06	-0.41
24	Number of transfusion parenchyma layers on the xylem side – maximum	-0.74	-0.19	-0.33
Var. (%)		32.29	18.20	16.43
Cum.var. (%)		32.29	50.49	66.92
Eigen values		8.40	4.73	4.27

jective understanding of a researcher (the roundness of hypoderm on the convex side of a needle, the number of cells around a resin duct) as well as traits that showed to be uniform and therefore not informative in the regional limitation to the territory of Croatia (the number of sclerenchyma layers above, between and under the vascular bundles) were thrown out. On the other hand, entirely new traits were included in the research that showed to be especially valuable for differentiating populations with a very close relationships (the length of needle, the number of assimilation parenchyma layers, the number of cells surrounding resin ducts and the diameter of resin ducts) (Table 1).

All the needle traits, apart from the length of needle, were measured from the transversal cross-section in the thickest part of needle (between the lower third and a half of needle) using the light microscope and the ocular micrometer.

The arithmetical means of 30 needles measurement for every feature per individual were taken as a valid value for every individual (OTU). The row data matrix with 61 OTU-s (individuals) and 24 variables were standardized by z-score method (Sneath and Sokal 1973). The standard data matrix was the input matrix for the cluster and principal component analyses (PCA) (Sneath and Sokal 1973; Clifford and Stephanson 1975; Everitt 1978, 1981).

The cluster analysis was done by calculating the average taxonomic distance. The clustering method was UPGMA (Unweighted pair-group method, arithmetic average). The results were presented as a dendrogram.

PCA was carried out by computing eigenvalues and eigenvectors from the variables correlation matrix. The three dimensions were retained. The eigenvectors matrix

and the eigenvalues, their percentages, and their cumulative percentages were listed. The results, the spatial relationships between the individuals, were presented in the form of a 3D-scatter diagram. All the calculations were done by the NTSYS-PC package (Rohlf 1990).

RESULTS

The results of the hierarchical cluster analysis are shown in the dendrogram (Fig. 3). On the basis of the measured traits, most of the individuals showed strong population affiliation and built separated clusters. The population from island Hvar showed as the most variable (the four out of the eight individuals do not show any population affiliation). The populations from Grobnik, Brač, Hvar and Pelješac showed the biggest mutual resemblance ($D=1.25$). Transitional character from these populations towards the populations from Austria ($D=1.45$) and Velika Paklenica ($D=1.65$) was showed by the population from the mountain Biokovo ($D=1.30$).

The results of the PCA analysis are shown in Fig. 4. The first three PC axes contain 67% of the cumulative variability. Grouping of the researched populations into four groups could be seen (Fig. 4, groups A-D). Although in the PCA analysis majority of the individuals show population affiliation, transitional forms are revealed more strongly than in the cluster analysis (e.g. Biokovo population /group D/), as well as a close connection of certain populations (e.g. populations from Hvar, Brač, Grobnik, Pelješac /group A/). Moreover, with some exceptions (Grobnik population), it

Fig. 3. UPGMA dendrogram based on 24 quantitative morphological and anatomical leaf variables of 61 black pine individuals (individuals from population: 1 – Brač, 2 – Hvar, 3 – Pelješac, 4 – Biokovo, 5 – Velika Paklenica, 6 – Grobnik, 7 – Austria; A-D – population groups).

was possible to trace continuous transition from the northernmost population from Austria (group B) and Velika Paklenica (group C), through the population from Biokovo (group D), to the southernmost populations from the peninsula Pelješac and the islands of Brač and Hvar (group A). It was also possible to trace continuous transition, especially along the third PC axis, from the population Biokovo (elevation 1000-1200 m) through the populations of Pelješac (elevation 700-900 m), Grobnik (elevation 500-600 m), Brač (elevation 400-700 m), to the population of Hvar (elevation 300-600 m). The most isolated population proved to be the population from Velika Paklenica (group C).

The results of the PCA and cluster analysis agree in large part. The groups established with PCA (Fig. 4, groups A-D) that could also be seen on the dendrogram were marked in the same way on the dendrogram (Fig. 3).

For the separation of groups along the first PC axis (group A and the most individuals of group D from groups B and C) the most significant features were: the number of hypodermal layers on the flat side of needle, the number of parenchyma layers on the both sides of the transversal cross-section of needle and the number of transfusion parenchyma layers on the xylem side. For the separation of groups along the second PC axis (group B from group C) the length, the height and the width of needles, as well as the number of resin ducts and number of transfusion parenchyma layers on the phloem side proved to be especially valuable traits. Within group A, which was the only one consisting of a higher number of populations (Brač,

Hvar, Grobnik and Pelješac), the populations separated particularly well along the third PC axis. In that case the width of needle, the number of cells surrounding resin ducts and the diameter of resin ducts showed to be the most meritorious (Table 1).

DISCUSSION

Unlike previous researches (Visiani 1842; Vidaković 1953) and because of a wish to get as credible data as possible, only the natural populations of black pine from Croatia with a well-balanced number of specimens as well as the control population from Austria were included in this research. Because of the same reason the obtained results of measuring were analyzed by means of multivariate statistics (Sneath and Sokal 1973; Clifford and Stephenson 1975; Everitt, B. 1978, 1981). Among the all-morphological and anatomical traits used in this study, the traits especially valuable for determination were picked out (Table 1). These traits, in a conceivable future research of the whole black pine range, could be one of the valuable tools in the final solution of intraspecific indistinctiveness of this species. In that case, the traits from this research that contributed mostly to the variability along the first and along the second PC axis could be used for separation of populations on the subspecies or variety level.

Both multivariate analyses, cluster and PCA, gave the identical picture of intra and interpopulational relationships (Figs 3 and 4). The defined strong population affiliation of the researched individuals (individuals of the same population constitute more or less the same cluster or group) points to a certain isolation of populations resulting probably from the markedly discontinuous range of this species (Strand 1957; Fukarek 1958; Vidaković 1991; Barbéro et al. 1998), whereas interpopulational relationships point to a clinal change of morphological and anatomical traits (the

Fig. 4. PCA scatter diagram based on the correlation of 24 morphological and anatomical leaf variables of 61 black pine individuals (individuals from population: ★ – Brač, ● – Hvar, ☼ – Pelješac, □ – Biokovo, ○ – Velika Paklenica, ◇ – Grobnik, ◆ – Austria; A-D – population groups).

Fig. 5. Distribution of black pine taxa on the territory of Republic of Croatia based on results obtained by this research. A – *Pinus nigra* subsp. *dalmatica*, B – *Pinus nigra* subsp. *nigra*, C – *Pinus nigra* subsp. *illyrica*, D – transitional population among three sub-species marked as A, B and C.

defined dependence on geographic position and the height above the sea-level of populations) characteristic for anemophilous and anemochorous woody species of the continental spread (Strand 1957; Gaussen et al. 1964, 1993; Leding 1998).

The obtained interpopulational relationships and the grouping of populations into four groups (Fig. 3 and 4; groups A-D) make possible the distinction of three taxa of black pine and transitional forms between them. All three taxa can be marked as the taxa with the same taxonomic rank. After the results of this research, it is also possible to define the distribution of the determined taxa in this area more exactly than it has ever been possible before (Fig. 5).

The individuals from the populations of Pelješac, Brač, Hvar and Grobnik constitute the first taxon (Fig. 5A) that matches mostly the previously described Dalmatian black pine (*Pinus nigra* Arnold subsp. *dalmatica* (Vis) Franco) (Visiani 1842). As compared to the previous views on the spread of the Dalmatian black pine (Visiani 1842; Vidaković 1953, 1957; Fukarek 1958, Domac 1965; Trinajstić 1986; Rauš et al. 1992), this is the first time that the population from Grobnik is included in this taxon, and at the same time it is the taxon's northernmost habitat. Up to now this population has been categorized as the Illyric black pine, on the basis of only one taxonomic research that was based on two individuals only (Vidaković 1953), or has been categorized as the Austrian black pine, without a taxonomic research and only by geographical affiliation (Horvat 1956; Rauš et al. 1992; Gaussen et al. 1993). Relying on the obtained data, but also on the literature data, it is possible to explain this disjunction of the Dalmatian black pine in two different ways. In the first explanation the population from Grobnik would be a part of artificially planted forests of the Dalmatian black pine, also registered on Kvarner islands of Cres, Lošinj and Susak (Vidaković 1953). In that case, the native habitats of

the Dalmatian black pine, consistently to the generally accepted opinion (Vidaković 1953, 1957; Fukarek 1958, Domac 1965; Trinajstić 1986; Rauš et al. 1992), would be connected only with the islands and the coastal zone of south Dalmatia (the islands of Brač, Hvar and peninsula Pelješac), whereas populations in the northern Croatian Littoral, including Grobnik, would be of anthropogenic origin. The second explanation is based on the data that emphasize the natural origin of the Grobnik population (Horvat 1956; Fukarek 1958). If it is really so, the same population could be considered as the native population of the Dalmatian black pine in the disjunctive part of its range. In that case, the whole Eumediterranean belt of Croatia could be attributed as the primary range of the Dalmatian black pine. Although the range defined in this way is primarily bigger than has been considered so far, it should be emphasized that this primary distribution, with the disappearance of the Dalmatian pine from the northern Dalmatia, was secondary divided into two parts (Fig. 5A). This interruption of the range of the Dalmatian black pine in two distant parts emphasizes more the negative trend in the evolution of this subspecies, which is probably heading towards its disappearance.

The population from the mountain Biokovo, as a transitional population with the morphological and anatomical features of all established taxa (Fig. 3 and 4, group D), strongly indicates the presence of the second taxon. According to the researched morphological-anatomical traits, this population is probably the final southern part of the range of the *Pinus nigra* Arnold subsp. *nigra* (Fig. 5B), which spreads from the populations from Italy and Austria to Slovenia and western Croatia and gradually loses clear diagnostic characteristics in transitional morphological and anatomical traits on mountain Biokovo (Fig. 5D).

The population from Velika Paklenica represents the third taxon (Fig. 5C). This population has shown high morphological-anatomical specific quality (Fig. 3 and 4, group C), with no transitional morphological-anatomical traits towards the other analyzed populations, as thought so far (Vidaković 1953, 1957). The comparison of the obtained results with the literature data (Fukarek 1958) show that what we have here is most likely the Illyric black pine (*Pinus nigra* Arnold subsp. *illyrica* (Vid.) Fukarek) spread mostly in the neighboring Republic of Bosnia and Herzegovina. According to the obtained results, the population from Velika Paklenica represents the final western part of this taxon's range, which drives like a wedge between the ranges of the Austrian and Dalmatian black pine and together with them forms the transitional population on mountain Biokovo (Fig. 5).

Due to the choice of populations, limited mostly to the Dalmatian black pine, this research gives some indications of the ranges of the Austrian and Illyric black pine on the Croatian territory. For a detailed description of the ranges of these taxa it is necessary to undertake a new research with higher number of populations from the northwestern part of Croatia, but also from the neighboring Republic of Bosnia and Herzegovina. In the same way, for the purpose of the final defining of the natural range of the Dalmatian black pine, it is necessary to fully clarify the origin of this taxon on the islands and the coast of the northern part of the Adriatic. In this research, the status of the Dalmatian black pine as a separate and stenoendemic subspecies was

definitely confirmed. Since the Dalmatian black pine has been reduced to several populations only, and there are some signs of continuous diminishing of its range, this research absolutely justifies the inclusion of this taxon in the plans of preserving the world endangered conifers (Farjon et al. 1993), but also points out the need of making an action plan to preserve this taxon which (regrettably) is not at present a part of a strategy of preserving the biological and landscape diversity of the Republic of Croatia (Martinić 2000).

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WEWNĄTRZ- I MIĘDZYPOPULACYJNE ZALEŻNOŚCI
ORAZ STATUS TAKSONOMICZNY *PINUS NIGRA* ARNOLD W CHORWACJI
W OPARCIU O MORFOLOGIĘ I ANATOMIĘ IGIEŁ

STRESZCZENIE

Badano sześć naturalnych populacji *Pinus nigra* z Chorwacji. Specjalną uwagę zwrócono na określenie statusu taksonomicznego oraz na rozmieszczenie dalmatyńskiej sosny czarnej (*Pinus nigra* Arnold, podgatunek *dalmatica* (Vis.) Franco). Analizowano trzy morfologiczne i 21 anatomicznych cech igieł metodami analizy wielu zmiennych. Większość osobników wykazywała silne powinowactwo populacyjne. Ponadto stwierdzono stopniowe zmiany w cechach między populacjami. Potwierdzono występowanie trzech podgatunków sosny czarnej na terytorium Republiki Chorwackiej i określono nowe zakresy ich rozmieszczenia. Obserwuje się stałe zmniejszanie się obszaru występowania sosny czarnej, ograniczonej już tylko do kilku populacji. Praca wskazuje na konieczność wszczęcia działań w celu ochrony wszystkich naturalnych populacji tego podgatunku.

SŁOWA KLUCZOWE: sosna czarna, Chorwacja, zależności wewnątrz- i międzypopulacyjne, taksonomia wewnątrzgatunkowa, rozmieszczenie.