

# PINUS NIGRA ARN. AND THE INFRATAXON PINUS NIGRA SSP. BANATICA A SHORT REVIEW

MĂRIUCA MARANGOCI

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**Abstract:** Banat black pine is an infrataxon sometimes considered as subspecies, sometimes as variety of black pine, with natural spreading area in southwestern Romania, on Banat Mountains, from where it slightly extends into south of the Danube, in Serbia. The controversy surrounding the position of Banat black pine in southwestern Romania has been known since the last century. The Banat black pine population from this part of Romania has never been thoroughly studied at the molecular level. A future direction of study could be that variability analysis and genetic structure of individuals from natural population of *Pinus nigra* ssp. Banatica in southwestern Romania, using genetic markers.

## INTRODUCTION

*Pinus* genus belongs to the Pinaceae family; it has its origin in the northern hemisphere and grows in many types of forests in Europe, Asia, North Africa, North America and Central America. It is a diverse genus that includes over 100 species and dates back to the Mesozoic period (Keeley, 2012). Most species, about 70, are from North America and Central America. Another area with a wide variety of species, about 25, is located in Asia, especially in China (Price et al, 1998). According to European Flora taxonomy (Tutin et al, 1993), the *Pinus* genus is divided in two sub-genera: Diploxylon and Haploxylon. Research on chloroplast DNA analyses (Gernandt et al., 2005) also led to delimitation of two sub-genera: *Pinus* sub-genus (Diploxylon or Hard pines) respectively *Strobus* (Haploxylon or Soft pines), with several sections each. Black pine is included in *Pinus* section, *Pinus* subsection, which includes predominantly species with Eurasian, Mediterranean spreading area and others with North America and Cuba spreading area.

Black pine is one of the most important species in Europe, both economically and adaptively. The largest natural spreading area of black pine in Europe includes Mediterranean regions, on both side of the 40° parallel. It has its origin in the eastern half of the Iberian Peninsula, continuing eastwards across France, Italy, Balkan Peninsula, to the Asian part of Turkey. The southern limit of the spreading area is in north Africa, close to Gibraltar and Algeria. The northern limit at about 46° apart from the black pine in Banat and another island presence is reported in Crimea (Șofletea et Curtu, 2008). Black pine presents very wide variability at a species and exotype level, including many subspecies and varieties, with obvious structural genetic individualization, depending on the origin area and according to biochemical determination of isoenzymes (Bonnet et Bikay, 1978). Thus, we can distinguish: *Pinus nigra* ssp. *clusiana* Clem. et Arias (*Pinus salzmanni* Dunal), including black pine populations in North Africa, Spain and southern France; *Pinus nigra* ssp. *laricio* Poirlet (*Pinus nigra poiretiana* Asch. et Graebn), spread across Corsica (var. *corsicana*) and Calabria (var. *calabrica*); *Pinus nigra* ssp. *nigricans* Host, including populations in the central and eastern European distribution areas; *Pinus nigra* ssp. *pallasiana* Lamn, includes populations of black pine from Asia Minor; *Pinus nigra* ssp. *caramanica* Loud, from Crimea; *Pinus nigra* ssp. *banatica* (Born.) Novak (*Pinus nigra* var. *banatica* Endl. George. et Ion) – Banat black pine (Șofletea et Curtu, 2008) – an infrataxon sometimes considered as subspecies, sometimes as variety of black pine, with natural spreading area in southwestern Romania, on Banat Mountains, from where it extends moderately towards southern Danube, in Serbia. Therefore, in the Romanian specialty literature it is known as Banat black pine.

## TAXONOMIC CLASSIFICATION OF THE BANAT BLACK PINE

The controversial position of the Banat black pine in south-eastern Romania has been known since the last century. Boșcaiu and Boșcaiu (1999) mention that Rochel (1828) and Schwarzott (1831) announced for the first time the presence of the black pine in south-eastern Romania, on Domogled Mountain and on Danube valley, near Sviñața, under *Pinus pinaster* Auct. non Aiton. Thus the association of the Banat black pine with *Pinus pinaster* is inappropriate, considering that its spreading area is in the Mediterranean, where it is found especially in France, Spain, Portugal and North Africa. According to the morphological description, the maritime pine has longer needles than the Banat black pine, 10-22 cm,

slightly stinging, cones are longer than 8-18 cm, with bright reddish apophysis. Banat black pine has needles 8-12 cm long, dark green and cones are 6-10 cm long, with bright yellowish apophysis (Debazac, 1964).

Later, Heuffel (1858) described the same populations as *Pinus nigra laricio* auct. non Poiret (Boşcaiu et al, 1999). In relation to this classification, morphological characters of *Pinus nigra* ssp. *laricio* and *Pinus nigra* ssp. *banatica* are different; needles are 12-15 cm long, cones (4-7cm long) with brown-yellowish or bright reddish apophysis. Bud scales are light brown, whereas in Banat black pine, bud scales are whitish-gray (Debazac, 1964).

Subsequently, Romanian black pine populations, because of their peculiarities, are separately mentioned by some authors as the endemic taxon *Pinus nigra* subs. *banatica* (Endel ex Borb.) Novak. According to Georgescu (1936), these black pine populations should be treated as independent species, as *Pinus banatica* (Georgescu et Ionescu) (Boşcaiu et al, 1999). Morphologically, it differs from the typical *Pinus nigra* species through the pyramid-shaped crown of young trees, irregular, tabular shaped at middle and old age trees. Tendrils, yellowish-greenish, to purplish blue – green, cones yellow to dirty yellow – greenish (Şofletea et Curtu, 2008). Needles are very rigid and stinging (Negulescu et Săvulescu, 1965).

The same source (Boşcaiu and Boscaiu, 1999) debate Borza's (1947) mentions, who classify populations of black pine in Banat and Oltenia as *Pinus nigra* subsp. *pallasiana* (Lamb.) Holmboe. This classification was taken over in 1946 by Gausson et al, in the first edition of European Flora 1 and therefore maintained in the second edition of the same volume in 1993. Jalas et Suominen (1973) in the atlas of European Flora 2 classified almost all black pine species in Balkans as *Pinus nigra* subsp. *pallasiana*. Related to the association with *Pinus nigra* ssp. *pallasiana* the information about the spreading area of this species do not include populations in southeastern Romania, it is found in Greece, Turkey, Crimea and Bulgaria the latter being considered the furthest spreading area to the West (Isajev, 2004).

## SPREADING AREA AND PARTICULARITIES OF HABITATS OCCUPIED BY BANAT BLACK PINE

Sporopollinic spectra performed in Peştera lui Veterani (Veterans Cave) showed that Banat black pine from Tricule formed compact stands of pine since the last glaciation, 12,000-14,000 years ago. Endemic to Romanian Carpathians, with low demands and high resistance for arid and sunny hills, Banat black pine covered these resort areas a long time ago, before replanting other broad-leaved species. The existence of relic specimens of Banat black pine shows that the connection between Crimea and the southern Carpathian population might have been possible through Balkan Peninsula, not directly through Crimea (Matacă, 2005).

The natural spreading area of the species includes areas in Banat and Western Oltenia. It is commonly found on limestone cliffs from low altitudes ( about 150 m on Danube Valley) to full mountain areas (1500 m in Mehedinţi Mountains) (Şofletea et Curtu, 2008). In Banat: along the Cerna Valley, it starts at Pecinişca and Herculane to Corcoia, with interruption in Cheile Cernei (Cerna Gorge), on steep cliffs in the Domogledului peak ( Domogled, Şuşcu, Hurcu), on Culmea Desiminului (Desiminului Summit) and Arjanei on the right of Cerna (Clepeanc, Stone of Banitei); on Danube Valley at Tricule and on Trescovăţ cliffs (Sviniţa). In Olt: between Baloteşti and Păuneşti on Topolniţa valley; Izverna (Baia de Aramă), Runcu on Sohodol Valley of Runcu, on Cleanţul Cucului and Dosul Macrişului (Târgu Jiu) ( Săvulescu et al, 1952).

In 1958, Paskovski and Leandru described four types of forest where we can find Banat black pine as well (*Pinetum nigri banatica-orni*; *Quercetum-Pinetum nigri banatica-myrtilli*, *Pinetum nigri banatica-sessilis* and glades of black pine with shrubs).

Black pine with flowering ash on limestone (*Pinetum nigri banatica-orni*), includes altitudes of about 700-900 m, southern and south-eastern exposure, rocky slopes and groves, with superficial soil, and tithonic limestone sublayers. The stand is composed of black pine in variable consistency with an active rhythm of growth. The sub-stand is made of flowering ash and eglantine in smaller quantity. Black pine with oak species (*Quercetum-Pinetum nigri banatica-myrtilli*) on siliceous rocks: this type of forest is rare, found only in a spot on Cerna Valley and at Sviniţa. It can be found at altitudes of 250 – 300 m, on skeletal soil, very superficial with sublayers of siliceous rocks (granite in Cerna Valley, siliceous breccia at Sviniţa). Some specimens are 20 m tall, but most of them are shorter (12-14 m). A rare sub-stand is made of shrub specimens of sessile oak, Italian oak, Turkey oak, mountain ash, flowering ash, dribs and drabs of beech. Plants such as *Chrysozogon gryllus*, *Festuca duriuscula*, *Vulpia myurus*, *Genista pilosa*, *Asperula cynanchica*, *Hieracium pavichii*, *Achillea* sp. are also present; Cerna Valley resort is characterized by a conspicuous frequency of *Vaccinium myrtillus*, *Cytisus nigricans* and *Cytisanthus radiatus*, which cannot be found at Sviniţa where the plant cover is generally weak. In this area, the glade with wood of black pine can also be found at Trescovăţ Stone, not far from Sviniţa, the approximate altitude being 500-670 m. Here the pine is joined by sessile oak, flowering ash, and fewer beech, white beech, trembling poplar, silver linden, like shrubby or at most as short trees. Among shrubs, the most abundant is lilac. Mix of black pine with broad-leaved trees on limestone (*Pinetum nigri banatica-sessilis*), furrows steep slopes of Domogled and Şuşcu, being the lithological layer limestone. In the first altitudinal plant belt are species as sessile oak, European haze, linden, fewer birch are present, the second altitudinal plant belt come across of flowering ash, mountain ash, and rarely oriental hornbeam. The sub-stand is

composed of smoke tree, Turkey hunzel, blackthorn, cornel tree, red dogwood, rock buckthorn etc. Glades of black pine with shrubs (Pinetum nigri banatici-radiati, Pinetum nigri banatici-junipereum, Pinetum banaticum-Syringium nanae) were found on Cerna Valley and Oltenia, at altitudes of about 600-1000 m always on sunny slopes, with limestone sublayer. The sub-stand is represented by thickets of different shrubs (common juniper and black cytusus, dwarfish juniper and lilac, flowering ashes and lilac).

Doniță et al. (2005) identified and characterized in the Cernei Mountains' habitat R4218 – Southeast Carpathian glade – forests of black pine (*Pinus nigra* ssp. *banatica*) with *Genista radiata*, on limestone, rendzina soils, superficial, skeletal, saturated in bases, water balanced, with possible deficits in summer, eutrophic. Phytocoenosis within it are performed by European forest and sub-Mediterranean species. The altitudinal plant belt is made, in the upper level of black pine trees (*Pinus nigra* ssp. *banatica*), which do not form a finished layer, and in the lower altitudinal plant belt of flowering ash (*Fraxinus ornus*), Turkish hazel (*Corylus colurna*), silver linden (*Tilia platyphyllos*), sessile oak (*Quercus petraea*), Oriental Hornbeam (*Carpinus orientalis*); the shrub level is missing or it is poorly developed, made of *Cotoneaster integerrima*, *Cotinus coggygria*, *Cornus mas*, *Sorbus cretica*, *Rhamnus saxatilis*, local *Syringa vulgaris*, *Juniperus communis*. The plant cover and sub shrubs is dominated by *Carex humilis* and *Sesleria rigida*.

Located in the southwestern part of Romania, the natural spreading area of the Banat black pine is predominantly subject to the influence of western and southwestern atmospheric circulation. Western circulation, in the cold period, brings polar air masses or, rarely, tropical, maritime, favourable to mild winters with mostly heavy rainfall at low altitudes. During the summer, it causes a higher degree of thermal instability, evidenced by the frequency of the downpours, accompanied by electric discharge. (Buza et al., 1981).

#### **The Banat black pine as genetic resource and afforestation-reforestation material in Romania**

A stand of Banat black pine was selected for the production of forestry materials in the "qualified" category (fig. 1), with a total area of 83.20 ha, of which 60.40 ha represents the buffer zone, with altitudes ranging from 730 m to 970 m (Baile Herculane, Caraș-Severin County) (Pârnuță et al., 2011).

There were three preservation nucleus established for black pine of native origin, with a total area of 8.1 ha. The nucleus have been chosen from three district of Romania (Brașov, Caraș-Severin și Mehedinți) (Pârnuță et al., 2012).

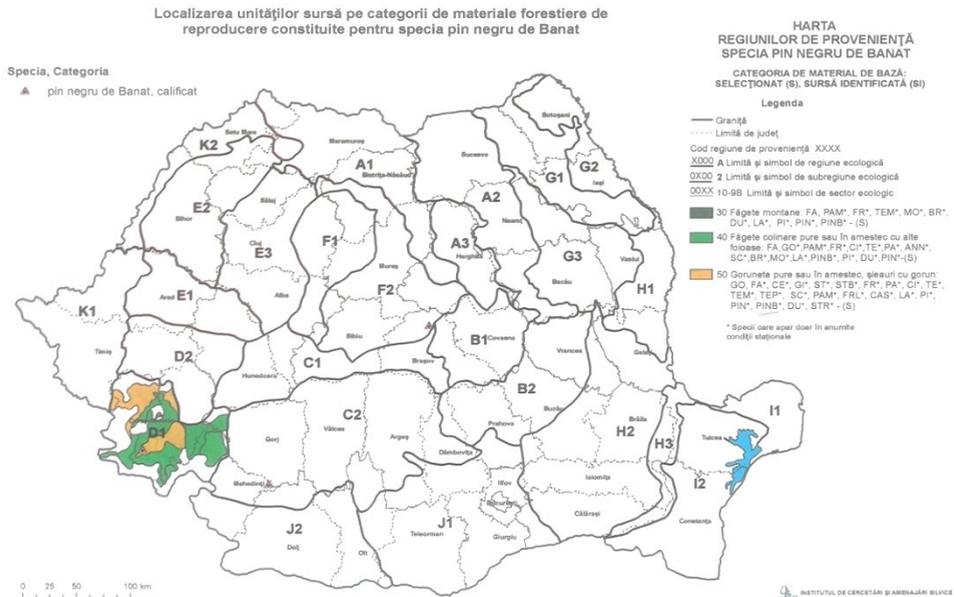


Fig.1. Location of the establishments of origin by categories of forest reproductive materials constituted for the Banat black pine (Pârnuță et al., 2011).

## GENETIC DATA ABOUT THE BLACK PINE

The black pine is a species that has been genetically analyzed over time through isoenzyme, terpene, cpDNA and core DNA studies. Genetic differentiation between subspecies of *Pinus nigra* species has been achieved with these analyzes.

Variation of isoenzymes was studied in 28 populations of *Pinus nigra*, 9 known subspecies and two unknown in Cyprus. With the help of the dendrogram showing relations between populations, studies were divided into three main categories: Corsican and Moroccan, Laricio and Cyprus and the rest (Nikolic et al., 1983). The geographic variation of the terpenoid composition of *Pinus nigra* was studied using 109 samples, representing 72 natural and 11 planted populations. Five geographical groups were distinguished: Spanish, continental French, Corsican, Calabrian and eastern (including Austria, the Balkans, Turkey and the Crimea). Results from terpenoid composition and from data on the anatomic, morphological and growth characteristics and on frequencies of terpenoids, flavonoids and allozymes support the use of Wheeler et al. classification system (Gerber, 1995). Genetic variation in five natural black pine populations in Austria, Bulgaria, Greece, Corsica (France) and Calabria (Italy) was analyzed by starch gel electrophoresis with 10 enzymatic systems. There was a clear differentiation between ssp. laricio (Calabria, Corsica) and austrian ssp. (Austria, Bulgaria), and the Greek population was more similar to the austrian ssp (Scaltsoyiannes et al., 1994). Three subspecies of *Pinus nigra* Arn. (ssps. nigra, salzmannii, laricio) were analyzed using 23 isoenzymatic loci for four morphologic characters (length, width, wing scar and weight/10 seeds). The dendrogram obtained using genetic distances among populations indicated the existence of 3 groups, corresponding to the 3 subspecies. The data clearly showed that the Corsican population is the furthest, with relative lack of genetic variation, perhaps due to its geographical isolation (Aguinagalde et al., 1997). The geographic variation of *Pinus nigra* terpenes in southwestern Europe was studied in 16 Corsica Herault (France) and Eastern Pyrenees (France and Spain) populations. Differences in quantitative content of the selected compounds which divided the populations in two basic geographic groups: on the one hand populations of Herault and the eastern Pyrenees and, on the other hand, populations of Corsica. Some trees, as well as populations, share similarities, although they are not part of the same geographical region. These analyzes confirm the hypothesis that afforestation of Herault and Eastern Pyrenees was also achieved with Corsica black pine (Bojovic et al., 2005). A study on canonical discrimination analysis (CDA) was conducted in Serbia to verify the hypothesis of intraspecific chemical separation of *Pinus nigra* (ssp. nigra, var. gocense, ssp. pallasiana, and var. banatica) taxons, based on terpenes. The division of seven natural populations of *Pinus nigra* resulted in 3 groups (ssp. nigra, ssp. pallasiana and var. banatica). Individuals in pallasiana group represented the smallest proportion in (E) - caryophyllene and terpinolene and richer in  $\alpha$ -humulene, and those in ssp. nigra var. banatica had the highest content of  $\alpha$ -pinene and myrcene (Sarac et al, 2014).

Genetic structure and genetic diversity were analyzed in 9 black pine populations in Bulgaria, using chloroplast microsatellite and terpene analyzes as markers. The cpSSP analyzes divided the black pine into four groups, and the most representative terpenes were  $\alpha$ -pinene, followed by  $\beta$ -pinene. The results suggest that the structural model of the genetic diversity of chloroplast DNA in black pine populations is the consequence of historical biogeographical processes (Naydenov et al, 2006). According to the hypothesis which states Western European populations survived during the last ice age rather than being recolonised in the post-glacial period was tested. Genetic variation was evaluated using a set of 10 chloroplastic DNA microsatellites. 311 specimens were analyzed, with 235 haplotypes revealing high level of diversity in most populations. With the help of bayesian analysis, 10 groups corresponding to 6 studied samples were differentiated. Temporal estimation places separation between the Alps and other regions about 150,000 years ago, and the most recent separation was found in southern France about 30,000 years ago. Analyzing these data, it was deduced that chloroplastic DNA of Western European populations is likely to have been present during the last ice age (Zara et Richard, 2006).

Eight black pine populations in Southern Spain and Northern Morocco were analyzed using ISSR markers. Analysis of main components shows the presence of two groups, while bayesian analysis revealed the presence of three groups. Low genetic diversity noticed in two of the five populations is probably a direct consequence of inadequate management because no genetic variability estimation was performed before forestry treatments. Testing genetic variability of populations before any management was recommended (Rubio-Moraga et al, 2012).

## CONCLUSIONS

In the past 200 years the Banat black pine in south-western Romania has been classified under various subspecies such as pallasiana, laricio and banatica. A future direction of study could be analysis of variability and genetic structure of individuals from natural populations of *Pinus nigra* ssp. banatica in southwest Romania, with the help of genetic markers.

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- 1 Transilvania University of Brașov, Faculty of Silviculture and Forest Engineering. Str. Șirul Beethoven, nr. 1, 500123 Brașov, Romania.  
 mariuca.marangoci@yahoo.com

