

Dedicated to the Professor Adam Boratyński

Differentiation of Mediterranean species of *Juniperus* from the *Sabina* section as a result of their migrations

Małgorzata Mazur*

Article info

Received: 8 Mar. 2021
Revision received: 23 Jul. 2021
Accepted: 23 Jul. 2021
Published: 31 Dec. 2021

Associate Editor

Elżbieta Cieślak

Abstract. The *Sabina* section is one of the three groups in the *Juniperus* genus and the most diverse. The variability of Mediterranean junipers from the *Sabina* section is related to their Tertiary and Pleistocene migrations and long-term isolations. Their contemporary taxonomic and geographic diversity was influenced by important events such as the migration of continents, the disappearance of Tethys, orogenic movements or the Messinian salinity crisis. The results of morphological measurements of seed cones, seeds and branchlets with leaves of 19 populations of *Juniperus phoenicea* complex, *J. excelsa* s.str., *J. thurifera* subsp. *thurifera* and subs. *africana*, *J. foetidissima* and *J. sabina* var. *sabina* and var. *balkanensis* were statistically compiled using univariate statistics and multivariate analysis. The most important characters differentiating the populations within the taxa were the thickness of the branchlet and the cone diameter, while between the taxa the ratio of cone diameter to the width of the seeds and the number of seeds per cone were used for speciation. *J. phoenicea* complex is distinguished from the other studied taxa by the greatest number of characters. *J. foetidissima*, *J. sabina* var. *sabina* and *J. canariensis* are characterized by the highest variability of morphological characters, while *J. excelsa* and *J. sabina* var. *balkanensis* – the lowest. The studies confirmed the ancient nature of the *J. phoenicea* complex in relation to other taxa from the *Sabina* section, as a result of an earlier detachment from the ancestor, and no loss of variability due to the effects of colonization and isolation in *J. canariensis*. In addition, the similarity of *J. sabina* and *J. thurifera* was demonstrated, which would confirm the descent from a common ancestor and similar migration routes from the center of Europe towards the Iberian Peninsula, as well as further differentiation of *J. thurifera* into subspecies caused by isolation due to the opening of the Strait of Gibraltar. The distinctiveness of *J. foetidissima* from all the other analyzed taxa was also confirmed, and some morphological similarity was shown, proving the original character of *J. excelsa* s.str. and its similarity to the *J. phoenicea* complex in this respect.

Key words: biogeography, biometry, *Cupressaceae*, plant morphology, plant variation

Introduction

Juniperus is the second most diverse genus of all the coniferous plants and the largest of the 30 members of the *Cupressaceae* family. It includes about 75 species (Farjon 2005; Adams 2014) of evergreen trees and shrubs of different heights and habitats (Zohary 1973; Browicz 1982; Adams 2014). The *Juniperus* and *Cupressus* genera and the *Hesperocyparis-Callitropis-Xanthocyparis* clade come from a common ancestor more than 60 million years ago, most likely from Asia, and the divergence of *Juniperus* and *Cupressus* was about 56 million years ago (Mao et al. 2019). The development of Mediterranean vegetation was associated with the displacement of continental plates and

the disappearance of Tethys (Popov et al. 2006; Ivanov et al. 2011). The diversification of the genus *Juniperus* started probably during the Eocene, in the Tethyan belt of vegetation in Eurasia, and further spread and evolution in Europe, Asia, Africa, and North America took place in the Oligocene, and the early Neogene, due to geological events that started with the collision of Arabia with Eurasia (Allen & Armstrong 2008). This event was accompanied by marine regressions and transgressions, orogeny and climate change, which caused, among other events, the creation of the Mediterranean Sea (Popov et al. 2006; Ivanov et al. 2011). The emergence of arid habitats with low rainfall were less suitable to other species (Axelrod 1975; Willis & McElwain 2002; Farjon 2005; Mao et al. 2010), thus resulting in more or less intense speciation processes in isolated lines of the genus (Mao et al. 2010). The closure of the Strait of Gibraltar and the Messinian

Department of Evolutionary Biology, Kazimierz Wielki University, Ossolińskich 12, 85-093 Bydgoszcz, Poland
ORCID: 0000-0003-0043-3706

* Corresponding author e-mail: mazur@ukw.edu.pl

salinity crisis in the Miocene (about 5.3 million years ago) allowed for further migration and exchange of flora between Europe, Africa and Asia (Bocquet et al. 1978; Verdu et al. 2003; Kovar-Eder et al. 2006; Favre et al. 2007). The history of the *Juniperus* was further influenced by the Pliocene climate cooling, Pleistocene cyclic glaciations, as well as human activities in recent centuries and competition with other species (Jimenez et al. 2003). High requirements for access to light and a pioneering nature make it easier for junipers to colonize new areas. Additionally, there are arbuscular mycorrhizal fungi, typical for dry and semi-dry areas (Sanguin et al. 2016), which may help them survive (Salvà-Cotarineu et al. 2021). For the *Juniperus* from the Iberian Peninsula and the Balkans, based on the pollen stratigraphy, spreading has been shown during the dry periods of the Late Pleistocene and Holocene and withdrawal in wetter periods, mainly due to competition with broadleaf trees (Carrión et al. 2001a, b, 2003, 2004, 2010; Tzedakis 2004). Migrations caused by climate change and often large distances created in this way between parts of the range of individual species, led to further differentiation. Long-term variability between populations at the molecular level may lead to the emergence of lower-rank taxa within the species, also identified by phenotypic characters. In several cases, the emergence of geographically isolated varieties, subspecies, and even species was confirmed, e.g., in the cases of *J. thurifera* s.lat. (Boratyński et al. 2013) and *J. phoenicea* s.lat. (Mazur et al. 2010, 2018; Romo et al. 2019).

The habitat requirements of junipers are the cause of small amounts of their preserved remains, because the dry habitats they choose do not favor the formation of macrofossils (Kvaček 2002). Moreover, in palynological studies, juniper pollen is identified only at the genus level (Carrión et al. 2003). Thus, the history of the evolution of junipers and possible migration routes or periods of isolation of species and populations in refuges can only be traced by studying their current molecular, morphological, and geographical diversity (Bennet et al. 1991; Petit et al. 2003).

The existence of three main *Juniperus* differentiation centers is presumed: the Mediterranean region, the desert of Mexico with the southwestern part of USA and central Asia (western China), while geographic distribution is mainly limited by climate, biotic interactions and dispersal ability (Mao et al. 2010; Adams 2014; Salvà-Cotarineu et al. 2021). It is assumed that the processes of hybridization and speciation still take place within the taxon, which increase its diversity (Farjon 2005; Mao et al. 2010, 2019; Adams 2014).

The genus *Juniperus* has been divided into three monophyletic sections: *Caryocedrus* (only one species *J. drupacea* in the Mediterranean region), *Juniperus* (syn: *Oxycedrus* Spach; 14 species in Mediterranean, East Asia, and one the circumboreal – *J. communis*) and *Sabina* (Miller) Spach (approx. 60 species in the Mediterranean, Asia, Africa and southwestern North America) (Farjon 2005; Adams 2014). *Sabina* is the least homogeneous of all sections and is divided into three or four groups (Mao et al. 2010; Adams & Schwarzbach 2013; Adams

2014), two of them with entire leaves: single-seeded junipers with turbinate cones of eastern hemisphere and multi-seeded of the eastern and western hemispheres (e.g.: *J. excelsa*, *J. foetidissima*, *J. thurifera* and *J. sabina*) and the next two groups with serrate leaves: junipers of North America and multi-seeded junipers with only slightly serrate leaves (treated as ‘pseudoserrate’) of the eastern hemisphere (*J. phoenicea*, *J. turbinata* and *J. canariensis*), which is the reason why the latter are included in the multi-seeded group with entire leaves.

The ancestor of the *Sabina* section was most likely from Asia (Mao et al. 2010). During the Eocene and the Oligocene, *Sabina* were widespread in the Madrean-Tethyan vegetation belts on three continents (Axelrod 1975; Wen & Ickert-Bond 2009; Adams 2014; Wen et al. 2016). The earliest *Sabina* divergence event probably took place in Central or Central and Western Europe, in the Oligocene, with the appearance of a multi-seeded juniper with slightly serrate leaves (*J. phoenicea* complex) (Mao et al. 2010). It is assumed that the ancestor of *J. phoenicea* s.lat. was part of the Tethian-Madrean flora (Axelrod 1975) and subsequently migrated to both American continents, then became the ancestor of a group of American serrated-leaved junipers (Adams & Schwarzbach 2013; Mao et al. 2019). The species included in the multi-seeded junipers with entire leaves, *J. excelsa*, *J. foetidissima*, *J. thurifera* and *J. sabina*, are believed to originate from the same ancestor and their differentiation may have occurred at the end of the Tertiary due to the cooling of the climate (Barbero et al. 1994; Jiménez et al. 2003; Marcysiak et al. 2007). As already suggested by Barbero and co-workers (1994) based on biometric and biochemical studies by *J. excelsa* and *J. thurifera*, the likely migration route during the Tertiary, from the central European ancestor, was two phyletic lines: through the submountain Alps regions to the Iberian Peninsula along the route northwest leading to the uprising of *J. thurifera* and to the northeast Balkans, which led to the creation of *J. excelsa*. Such migration pathways have been confirmed by *J. thurifera* molecular tests (Jiménez et al. 2003) and in the leaf oil comparisons (Adams et al. 2003). The isolation and survival of glacials in different refuges should have led to further differentiation between these species (Hewitt 1996, 1999, 2004).

Currently, the ranges of *J. excelsa* s.str. and *J. foetidissima* overlap, while the range of *J. thurifera* is isolated. The typical *J. excelsa* is a mountain taxon, which can create forests locally and occurs in southeastern Europe and southwestern Asia at elevations of 500–1500 m. It can also occur at lower altitudes of about 50–100 m, for example in the Crimea (Browicz 1982; Boratyński et al. 1992; Didukh 1992). *J. foetidissima* grows in areas similar to the typical *J. excelsa* and can sometimes be found together, growing in the mountains of the Eastern Mediterranean, in Europe and Asia, often on rocks or rocky slopes, from a Mediterranean to more continental climate (Browicz 1982; Boratyński et al. 1992; Farjon 2005; Adams 2014). *J. thurifera* grows in the mountains in the western part of the Mediterranean Region, in the Iberian Peninsula, French Alps and Corsica, in West

Europe and Atlas and Anti-Atlas in North Africa (Farjon 2005; Romo & Boratyński 2005; Boratyński et al. 2013). *J. foetidissima* is distinguished from typical *J. excelsa* and *J. thurifera* by a smaller number of seeds, larger seeds and thicker shoots with leaves (Farjon 2005; Marcysiak et al. 2007; Adams 2014). The distinctiveness of *J. foetidissima* is probably also partly due to its mountainous character and occurrence in higher altitudes, which limited its range and deepened the distance to the co-occurring *J. excelsa* (Browicz 1982; Boratyński et al. 1992; Didukh 1992; Farjon 2005; Marcysiak et al. 2007).

The distinction between taxa within the *J. excelsa* group is debatable. Two subspecies were assumed: *J. excelsa* subsp. *excelsa* and *J. excelsa* subsp. *polycarpus*, where the typical Greek juniper is characterized by slightly thinner twigs, smaller seed cones, and more seeds (Farjon 2005) and occurs in the western part of the range in a Mediterranean climate – in the Balkan Peninsula, Anatolia, Syria, Lebanon, Iran, and Crimea (Jalas & Suominen 1973; Browicz 1982; Boratyński et al. 1992; Farjon 2005; Douaihy et al. 2012). Latest research, mainly Adams and co-workers, based on leaf essential oils, RAPD, isoenzyme analysis, nrDNA and cpDNA assume the existence of the *J. excelsa* complex, which includes *J. excelsa*, *J. polycarpus* with two varieties (var. *polycarpus* and var. *turcomanica*) and *J. seravschanica* (Adams 2014, 2016; Hojjati et al. 2009, 2019).

The *Juniperus sabina* group is widely distributed from Spain, through Europe to Kazakhstan, Siberia, Mongolia, and China (Farjon 2005). In the isolated eastern part of the range, different contents of leaf terpenoids were noted (Adams et al. 2006a). Similar differences were shown by RAPD analyses (Adams et al. 2007) and, consequently, after further analyses of nrDNA and cpDNA, this part of the range is referred to as a separate taxon *J. davurica*, which have distinguished into varieties (Adams & Schwarzbach 2013). The typical taxon is characteristic of mountainous areas and its discontinuous range extending from the east of Mongolia to the Iberian Peninsula to the west (Browicz & Zieliński 1982; Farjon 2005; Zając & Zając 2009; Adams 2014). Adams and co-workers (2016) described a new variety, *J. sabina* var. *balkanensis* R. P. Adams et A. N. Tashev, from the Balkan Peninsulas (Bulgaria and Greece). Subsequent studies showed the presence of 'balkanensis' variety in western Turkey (Adams et al. 2017), Bosnia-Herzegovina, Italy, Croatia, Macedonia (Adams et al. 2018a) and Albania (Adams et al. 2018b). The new variety is reported as a trace of the old hybridization between the two species *J. sabina* var. *sabina* and *J. thurifera*, and differs from the typical one in having *J. thurifera*-like chloroplasts, but it is difficult to distinguish morphologically. Studies of 29 populations of *J. sabina*, including 13 var. *sabina* and 16 var. *balkanensis*, showed that all studied populations of 'balkanensis' are tetraploid, while 'sabina' are diploid (Farhat et al. 2019). The current geographic distribution of these three taxa does not coincide and *J. thurifera* does not occur in the Balkans (Farjon 2005; Romo & Boratyński 2005; Boratyński et al. 2013). Most likely the ranges of taxa or the ranges of their ancestors

were wider (Terrab et al. 2008; Mao et al. 2010), so it is possible that their geographical ranges coincided in the past (Farhat et al. 2019), while hybridization could have occurred during the Oligocen-Miocen migration of *J. thurifera* and *J. sabina* from the center of its creation to the Iberian Peninsula (Jimenez et al. 2003; Marcysiak et al. 2007). Cases of *J. sabina* var. *balkanensis* and *J. thurifera* occurring together in the French Alps have been documented (Farhat et al. 2020). Examination of several individuals with atypical morphology revealed allotriploid cases resulting from the hybridization between the tetraploid of *J. thurifera* and the diploid of *J. sabina*. The authors suggest that incomplete reproductive barriers may be enabling interspecies hybridization and even genetic introgression through further backcrossing.

Juniperus phoenicea complex now has a wide range, covering the entire Mediterranean region – from the Canary Islands, Atlas Mountains, and the Atlantic coast of Portugal in the west, through southern Europe, southwest Asia, to Jordan, Saudi Arabia, and Israel in the east, mainly growing in the sphere of influence mild Mediterranean climate, but also continental, e.g., in the Atlas Mountains (Jalas & Suominen 1973; Quezel & Pesson 1980; Browicz 1982; Boratyński et al. 1992; Charco 2001; Farjon 2005). Genetic research on the complex revealed inconsistent relationships between regions and migration history (Adams et al. 2002, 2006b, 2010, 2013; Meloni et al. 2006; Boratyński et al. 2009; Dzialuk et al. 2011). The latest genetic and biometric studies covering phenotypic characters did not reveal clear geographic trends, but confirmed the taxonomic distinctiveness of *J. phoenicea* s.str. and *J. turbinata* (Pinna et al. 2014; Jimenez et al. 2017; Mazur et al. 2018; Sanchez-Gomez et al. 2018) and gave rise to the distinction of the third species in the complex: *J. canariensis* (Romo et al. 2019). The history of the migration of the complex, after separating from its ancestor in the Oligocene, was indicated by Lebreton & Thivend (1981) and Lebreton & Rivera (1989). They conducted research on the content of procyanidins and prodelfinidines in leaves and showed its origin from the Iberian Peninsula. Contemporary geographical ranges of these three species, due to the choice of a different ecological niche, evolved as a result of isolation and adapting to local conditions (Salvà-Cotarineu et al. 2021). *J. phoenicea* s.str. is characterized by a narrower range than *J. turbinata*. It occurs inland, mainly in dolomite soils, in submountain and mountain positions in the Iberian Peninsula, southern France, and northwest Italy. It is a pioneering species, in various types of forest and shrub communities, adapted to the Mediterranean climate. It occurs in a wide range of bioclimates, from humid to semi-arid habitats, but also in a temperate climate on mountain slopes (Arrigoni 2012; Mazur et al. 2016; Salvà-Cotarineu et al. 2021). *J. turbinata* has a much wider range and may be found covering sandy coastal soils in regions around the Mediterranean Sea, colonizing sand dunes and rocks, and in the mountains of northwest Africa and southwest Asia, occurring up to 2,400 m in the High Atlas, creating open scrubland or forests. However, it most often grows up to 400 m above sea level, mainly

in the semi-arid to humid Mediterranean climate (Browicz & Zieliński 1982; Boratyński et al. 1992; Adams 2014; Mazur et al. 2016). Due to the relatively wide range of occurrence, the species shows great diversity. The most important, but not taxonomically significant, as in the case of *J. oxycedrus* (Boratyński et al. 2014), was noted between African and European populations, which confirms the importance of the Strait of Gibraltar. Slightly lower differentiation was found between European and Asian populations, which may be related to the intercontinental barrier in the form of the Aegean Sea, which was found to be more significant in the case of *J. drupacea* (Sobierajska et al. 2016). *J. canariensis* can be found in the Canary Islands, except the two driest islands of Lanzarote and Fuerteventura, and in isolated specimens in Madeira. It grows in an oceanic climate, where it forms patches, penetrating into shrub communities, at an altitude of up to 1,000 m (Fernández-Palacios et al. 2008; Romo & Salvà-Catarineu 2013; Adams 2014; Romo et al. 2019; Salvà-Cotarineu et al. 2021). Interestingly, *J. canariensis* has not shown a loss of genetic diversity due to the bottleneck or foundational effects during the colonization of Canary Islands, neither at the genetic level (Jimenez et al. 2017; Sanchez-Gomez et al. 2018) nor at the morphological level (Mazur et al. 2018).

The aim of this study was to trace the history of evolution and presumed migration routes of Mediterranean species of *Juniperus* from the *Sabina* section based on available published data and to check to what extent their taxonomic and geographical diversity, especially supported by genetic and biochemical research, is reflected in the morphology of cones, seeds, and shoots.

Materials and methods

Plant material

Material from biometric measurements of 19 populations of *Juniperus foetidissima*, *J. thurifera* subsp. *thurifera*, *J. thurifera* subsp. *africana*, *J. excelsa* s.str., *J. phoenicea*, *J. turbinata*, *J. canariensis*, *J. sabina* var. *sabina* and *J. sabina* var. *balkanensis*, analyzed for specific taxa groups in several previous studies (including three populations of *J. sabina* not yet published; see Table 1), has been re-analyzed. The aforementioned taxa have been thoroughly tested, but separately and on a different set of morphological characters of cones, seeds, and shoots. In this study, the output data from the measurements were analyzed statistically together and based on characters that proved important in distinguishing species. This made it possible to compare the morphological variability of characters both within and between taxa, which allowed for obtaining additional information to know the history of the migration and evolution of the *Sabina* section in the Mediterranean region.

Every population was represented by 14–36 individuals, 445 in total, and every individual by 10 cones and 10 one-year-old lateral branchlets. The set of 15 morphological characters of cones, seeds, and branchlets with leaves (Table 2) and measurement procedures of three new

populations used for this study were taken from earlier studies (Marcysiak et al. 2007; Mazur et al. 2018).

Statistical treatment

The unimodality and frequency distribution of each character was checked by the Shapiro-Wilk test. The discontinuous and quality character: the number of cone scale rows, which takes two values in *Juniperus*, section *Sabina*: 4 for decussate or 6 for ternete cones, was converted into the percentage of cones with 4 scale rows (CSR4). Before starting multivariate analyses the arcsine transformation was applied, in accordance with the procedure used earlier (Mazur et al. 2018). The homogeneity of variance was verified by Levene's test (Zar 1999; Sokal & Rohlf 2003). The correlation between 15 characters was checked with the Pearson's linear correlation coefficient to assess potential redundancy (Pearson 1900). Data were standardized to avoid possible effects of different types of characters used in the study. The arithmetic means, standard deviations, and variation coefficients of characters were calculated for each population and taxon. The influence of all characters on the differentiation of populations and taxa were verified using Tukey's *T*-test (Zar 1999). The taxonomic distance between nine taxa was pre-tested by the cluster analysis (Cattell 1943; Sokal & Rohlf 2003) and illustrated on the UPGMA dendrogram constructed on the Euclidean distances (Zar 1999). The results were verified using the discriminant function (McLachlan 2004), which allowed us to check the discriminatory power of each character and to verify the relationships between populations and detect the geographic patterns of differentiation within taxa. The dispersions of individuals from populations between the first two discriminant variables were shown on a scatterplot. All calculations were made using STATISTICA 13 (TIBCO Software Inc. 2017).

Results

The frequency distribution of the values of studied characters was unimodal and normal or close to in most populations and the variances were homoscedastic for most characters. The standardization of data improved obtaining these assumptions.

The values of the variation coefficient (VC) for most characters oscillate between 5.8% (the number of cone scales – CSN in *J. thurifera* subsp. *africana*) to 45.2% (the ratio SN/CSN for *J. foetidissima*). However, the VC values of two characters, the percent of decussate type of cones (CSR4) and the ratio describing the shape of seed cones (CL/CD), were very specific. The character CL/CD had a very low VC value, ranging from 2.1% (*J. phoenicea*) to 6.6% (*J. turbinata*). The VC value in all populations averaged 3.6%. The second character, CSR4, averaged 11.1% in all populations ranged between 0.0% (*J. thurifera* and *J. sabina*) and 56.8% (*J. canariensis*). The most variable characters for all analyzed taxa turned out to be those related to the number of seeds: SN/CSN, SN, and CD/SN, characterized by the average value of

Table 1. Sampled populations of *Juniperus* from the *Sabina* section.

| Taxon | Locality | Acronym | No. | Geographical coordinates | | Altitude [m a.s.l.] | Source of material |
|--|---|--------------------|--------|--------------------------|--------|------------------------|---|
| | | | | N | A | | |
| <i>J. foetidissima</i> | Crimea, Chuchel, NW of Yalta | FOE_UA | 20 | 34.233 | 44.667 | 1000 | Marcysiak et al. 2007 |
| <i>J. thurifera</i> subsp. <i>thurifera</i> | Greece, Pinods Oros, W of Kalambaka | FOE_GR | 19 | 21.367 | 39.700 | 1400 | Marcysiak et al. 2007 |
| | Spain, Zaragoza: Montes de la Retuerta de Pina, W of Bujaraloz | THUt_SP | 20 | -0.200 | 41.367 | 400 | Mazur et al. 2005, Marcysiak et al. 2007, Boratyński et al. 2013 |
| | France, Hautes Alpes, St. Crépin | THUt_FR | 36 | 6.500 | 44.933 | 1500 | Mazur et al. 2005, Marcysiak et al. 2007, Boratyński et al. 2013 |
| <i>J. thurifera</i> subsp. <i>africana</i> | Morocco, Middle Atlas, Jbel Bou Iblane, E of Talzemt, | THUa_MAI | 31 | -4.167 | 33.600 | 2200 | Boratyński et al. 2013 |
| <i>J. excelsa</i> s.str. | Morocco, High Atlas, Slopes above Tessaout (Toufrine) | THUa_MAI | 36 | -6.467 | 31.450 | 2500 | Boratyński et al. 2013 |
| | Bulgaria, The Struma river valley, near Gara Pirin, on the slopes | EXC_BG | 18 | 23.200 | 41.717 | 400-500 | Mazur et al. 2004, Marcysiak et al. 2007 |
| <i>J. sabina</i> | Ukraine, Crimea, S slopes above Yalta NE of Uchansu Falls | EXC_UA | 25 | 34.117 | 44.483 | 450-600 | Mazur et al. 2004, Marcysiak et al. 2007 |
| | Ukraine, Crimea, Chatyrdag – var. <i>sabina</i> | SABs_UA | 15 | 44.567 | 34.217 | 1250 | collected by Boratyński, Iszkulo, Lewandowski 2006 |
| | Spain, Sierra de Baza – var. <i>sabina</i> – var. <i>balkanensis</i> | SABs_SP SABb_SP | 9 5 | 37.467 | -2.733 | 2000 | collected by Boratyński and Boratyńska 2001 |
| <i>J. phoenicea</i> | Turkey, Sıpiil Dağı above Manisa – var. <i>balkanensis</i> | SABb_TR | 26 | 38.567 | 27.417 | 1450 | collected by Boratyński and Boratyńska 2005 |
| | Spain, Aragón, Nueno, Salto de Roldán | PHO_SP | 27 | 42.27 | -00.38 | 1000 | Mazur et al. 2003, Mazur et al. 2016, Mazur et al. 2018 |
| | Andorra, Coll de Jau, Sant Julià de Lòria | PHO_AD | 27 | 42.46 | 01.48 | 1150 | Boratyński et al. 2009, Dzialuk et al. 2011, Mazur et al. 2018 |
| <i>J. turbinata</i> | Spain, Andalucía, Playa de las Matalascañas | TUR_SP | 29 | 37.02 | -06.57 | 25 | Mazur et al. 2003, Boratyński et al. 2009, Dzialuk et al. 2011, Mazur et al. 2018 |
| | Sicily, Piano Pirrera near Acate (Ragusa) | TUR_IT | 30 | 14.433 | 37.017 | 130 | Mazur et al. 2016, Mazur et al. 2018 |
| <i>J. canariensis</i> | Morocco, High Atlas, Aït Lekak | TUR_MO | 30 | 31.26 | -07.83 | 1600 | Mazur et al. 2010, Mazur et al. 2018 |
| | Spain, Canary Islands, El Hierro, El Julan I | CAN_SP1 | 21 | 27.72 | -18.07 | 970 | Mazur et al. 2018 |
| | Spain, Canary Islands, Tenerife | CAN_SP2 | 31 | 28.56 | -16.25 | 200 | Mazur et al. 2018 |

No. – number of specimens.

Table 2. Average value (A) and variation coefficients (VC) of 15 analyzed characters of cones, seeds and leaves of nine *Juniperus* taxa from the *Sabina* section.

| Character | Character abbreviation | <i>J. foetidissima</i> | | <i>J. thurifera</i> subsp. <i>thurifera</i> | | <i>J. thurifera</i> subsp. <i>africana</i> | | <i>J. excelsa</i> | | <i>J. sabina</i> var. <i>sabina</i> | | <i>J. sabina</i> var. <i>balkanensis</i> | | <i>J. phoenicea</i> | | <i>J. turbinata</i> | | <i>J. canariensis</i> | |
|---|------------------------|------------------------|-------|---|-------|--|-------|-------------------|-------|-------------------------------------|-------|--|-------|---------------------|-------|---------------------|-------|-----------------------|-------|
| | | A | VC | A | VC | A | VC | A | VC | A | VC | A | VC | A | VC | A | VC | A | VC |
| Cone with 4 scale rows [%] | CSR4 | 97.92 | 4.24 | 100.00 | 0.00 | 100.00 | 0.00 | 99.77 | 1.53 | 100.00 | 0.00 | 100.00 | 0.00 | 78.06 | 21.69 | 90.85 | 15.28 | 49.92 | 56.85 |
| Length of cone [mm] | CL | 8.42 | 10.55 | 8.02 | 8.37 | 7.48 | 10.67 | 8.71 | 7.37 | 6.14 | 15.93 | 6.62 | 7.95 | 8.15 | 8.00 | 10.55 | 12.61 | 9.60 | 7.94 |
| Diameter of cone [mm] | CD | 8.39 | 10.58 | 8.32 | 8.48 | 7.30 | 11.20 | 8.77 | 8.29 | 6.07 | 17.02 | 6.71 | 7.21 | 8.52 | 7.53 | 9.98 | 10.00 | 9.89 | 7.71 |
| Cone scale number | CSN | 4.50 | 17.15 | 7.91 | 9.83 | 5.87 | 5.83 | 7.16 | 6.59 | 7.55 | 17.71 | 6.92 | 11.96 | 10.33 | 12.56 | 9.58 | 9.42 | 10.82 | 14.60 |
| Number of seeds per cone | SN | 1.56 | 32.85 | 3.58 | 22.08 | 1.33 | 26.67 | 5.87 | 20.05 | 1.76 | 27.33 | 2.82 | 18.15 | 7.79 | 15.40 | 6.48 | 15.56 | 3.76 | 21.53 |
| Length of seed [mm] | SL | 5.79 | 10.55 | 4.88 | 7.06 | 5.60 | 8.93 | 4.78 | 7.94 | 4.32 | 14.63 | 4.87 | 8.05 | 4.14 | 6.37 | 5.47 | 7.76 | 5.73 | 8.45 |
| Width of seed [mm] | SW | 5.12 | 10.99 | 3.62 | 10.64 | 4.85 | 12.32 | 2.99 | 9.96 | 3.34 | 22.41 | 3.18 | 10.11 | 2.58 | 10.19 | 3.02 | 12.23 | 3.76 | 9.18 |
| Number of leaves per 5-mm section of ultimate lateral branchlet | LN | 15.59 | 12.86 | 19.00 | 18.16 | 17.56 | 13.02 | 20.05 | 11.08 | 19.49 | 10.27 | 17.92 | 13.07 | 26.57 | 11.11 | 24.32 | 14.67 | 26.51 | 12.03 |
| Thickness of the ultimate lateral branchlet with leaves [mm] | BT | 1.10 | 7.93 | 0.82 | 7.70 | 0.68 | 7.25 | 0.73 | 9.85 | 0.85 | 5.95 | 0.93 | 6.09 | 0.95 | 11.98 | 0.83 | 9.42 | 0.71 | 9.10 |
| Ratios | CL/CD | 1.01 | 3.93 | 0.96 | 2.52 | 1.03 | 3.51 | 0.99 | 3.49 | 1.02 | 3.32 | 0.99 | 3.24 | 0.96 | 2.15 | 1.06 | 6.57 | 0.97 | 3.68 |
| | SL/SW | 1.14 | 10.70 | 1.38 | 9.35 | 1.17 | 8.92 | 1.62 | 7.39 | 1.33 | 9.93 | 1.56 | 9.41 | 1.64 | 9.43 | 1.85 | 11.05 | 1.54 | 6.53 |
| | CD/SN | 6.23 | 20.91 | 2.64 | 26.36 | 6.14 | 20.63 | 1.60 | 20.10 | 4.04 | 29.56 | 2.64 | 22.07 | 1.16 | 14.04 | 1.61 | 15.42 | 2.96 | 18.91 |
| | CD/SW | 1.66 | 12.17 | 2.37 | 9.60 | 1.53 | 10.72 | 2.98 | 8.65 | 1.87 | 12.14 | 2.16 | 8.57 | 3.37 | 9.95 | 3.37 | 10.50 | 2.67 | 9.40 |
| | CL/CSN | 2.12 | 23.37 | 1.03 | 8.80 | 1.29 | 14.02 | 1.24 | 10.51 | 0.82 | 6.78 | 0.98 | 11.57 | 0.85 | 8.73 | 1.14 | 16.94 | 0.99 | 11.63 |
| | SN/CSN | 0.40 | 45.17 | 0.46 | 20.81 | 0.23 | 26.28 | 0.83 | 17.73 | 0.83 | 39.32 | 0.25 | 19.79 | 0.80 | 13.70 | 0.69 | 15.60 | 0.39 | 26.80 |

VC in all compared taxa: 25.0%, 22.2% and 20.9%, respectively. In contrast, the most constant characters in this respect, not including CL/CD, were the thickness of branchlet (BT) and the length of seed (SL), with VC values: 8.4% and 8.9%, respectively (Table 2).

On average, all studied characters showed significant differences between the compared populations and taxa ($p \leq 0.01$). The pairs of taxa are distinguished by seven (*J. foetidissima* from *J. thurifera* subsp. *africana* and *J. sabina* var. *balkanensis* from *J. thurifera* subsp. *thurifera*) to 15 characters (*J. thurifera* subsp. *africana* from *J. phoenicea*) (Table 3), while pairs of populations belonging to the same taxa were distinguished by 1–8 characters (Table 4). The characters that most often distinguished pairs of taxa were SN and CSN (in 32 and 31 cases, respectively), whereas the characters least frequently able to distinguish pairs of taxa were CSR4 and CL/CD (in 20 and 21 cases, respectively). In the case of the population within taxa, the most different character was BT, which distinguished seven pairs of populations, while CSR4 and the ratio CD/SW were not distinguished from any pair (Table 4).

Many highly statistically significant correlations were found between the 15 analyzed characters. The most strongly correlated characters are those related to the size of cones CL and CD (0.94), SN and CD/SW (0.92), SN and SN/CSN (0.91). The weakest correlated characters in relation to the others turned out to be the ratios CL/CD and CL/CSN. To avoid redundancy of characters, nine characters with correlations ($r < 0.7$) were used for multivariate analyses (i.e. CSR4, CD, SL, SW, LN, BT, CL/CD, CL/CSN, SN/CSN).

The cluster analysis performed on average values of nine characters of nine taxa, showed a close relation of *J. sabina* and *J. thurifera* taxa, of which the African subspecies of *J. thurifera* was the greatest outlier, joining the others in 61% of the taxonomic distance. The quite close relation between *J. excelsa* and *J. phoenicea* complex, especially to *J. turbinata* (at 47% of taxonomic distance) was also shown. *J. foetidissima* turns out to be the most morphologically different, compared to all examined taxa (Fig. 1).

In the discriminant analysis, all nine characters had discriminant significance at a highly significant statistical level ($p < 0.01$). The greatest discriminating power had BT, CL/CSN and CD, with partial Wilks' lambda values of 0.20, 0.30 and 0.41, respectively (Table 5). The scatterplot was made in the space between the first two discriminant variables (U_1 & U_2), with 73.1% of the total variation. The width of seed (SW) and then two ratios: CL/CSN and SN/CSN had the greatest influence on U_1 (54.4% of variation). On the other hand, the character with the greatest impact on U_2 (18.7% of variation) was the thickness of branchlet (BT) (Table 5). Populations on the graph formed four groups. Two of them consisted of the populations of *J. foetidissima* and *J. thurifera* subs. *africana*. The third group was made up of *J. sabina* with both varieties and *J. thurifera* subsp. *thurifera* populations. The last and largest group was made up of the populations of *J. excelsa*, *J. canariensis*, *J. turbinata* and *J. phoenicea*, which seem to be quite homogeneous

Table 3. Numbers of characters differing at the statistically significant level ($p \leq 0.01$) of nine taxa of *Juniperus* from the *Sabina* section using Tukey *T*-test.

| Taxa | <i>Juniperus foe.</i> | <i>J. thur.</i> subsp. <i>thur.</i> | <i>J. thur.</i> subsp. <i>afr.</i> | <i>J. exc.</i> | <i>J. sab.</i> var. <i>sab.</i> | <i>J. sab.</i> var. <i>balk.</i> | <i>J. pho.</i> | <i>J. tur.</i> |
|---|-----------------------|-------------------------------------|------------------------------------|----------------|---------------------------------|----------------------------------|----------------|----------------|
| <i>J. thurifera</i> subsp. <i>thurifera</i> | 11 | . | . | . | . | . | . | . |
| <i>J. thurifera</i> subsp. <i>africana</i> | 7 | 12 | . | . | . | . | . | . |
| <i>J. excelsa</i> s.str. | 11 | 11 | 13 | . | . | . | . | . |
| <i>J. sabina</i> var. <i>sabina</i> | 11 | 8 | 10 | 10 | . | . | . | . |
| <i>J. sabina</i> var. <i>balkanensis</i> | 11 | 7 | 12 | 8 | 7 | . | . | . |
| <i>J. phoenicea</i> | 13 | 12 | 15 | 14 | 13 | 11 | . | . |
| <i>J. turbinata</i> | 14 | 13 | 14 | 13 | 13 | 13 | 13 | . |
| <i>J. canariensis</i> | 13 | 9 | 13 | 13 | 13 | 10 | 10 | 11 |

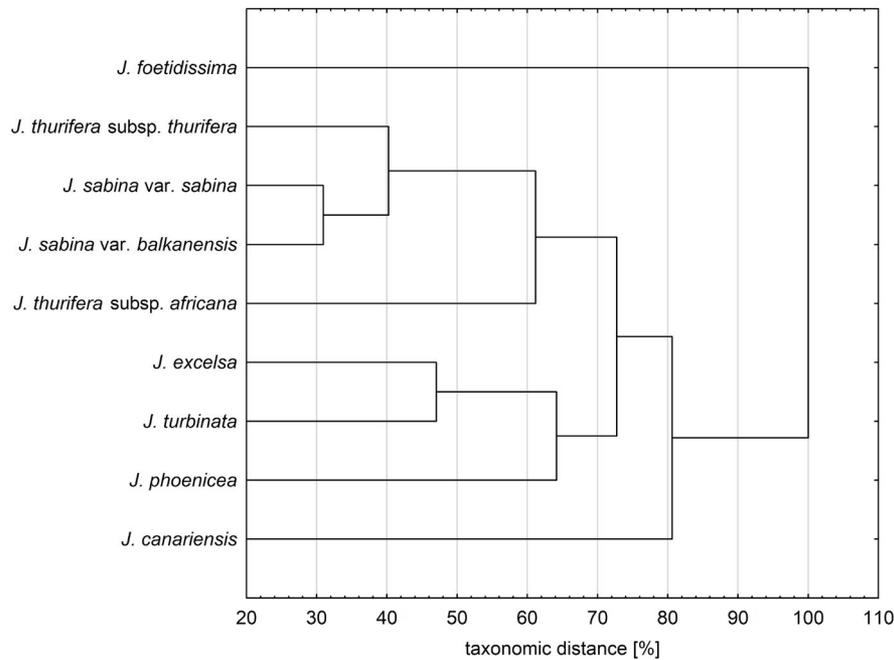


Figure 1. The UPGMA dendrogram, resulting from cluster analysis, made on nine characters of cones, seeds and branchlets with leaves, showing the taxonomic distance between nine *Juniperus* taxa from the *Sabina* section (set of nine characters as in Table 5).

Table 4. The list of characters differing at the statistically significant level ($p \leq 0.01$) from populations within taxa of *Juniperus* from the *Sabina* section using Tukey *T*-test.

| Populations | | Characters |
|-------------|----------|----------------------------------|
| FOE_UA | FOE_GR | BT, CD/SN, CL/CSN, SN/CSN |
| THU_t_SP | THU_t_FR | LN |
| THUa_MA1 | THUa_MA2 | SW, CL/CSN |
| EXC_BG | EXC_UA | CD, BT |
| SABs_UA | SABs_SP | CL, CD, CSN, SL, SW, CD/SN |
| SABb_SP | SABb_TR | CSN |
| SABb_SP | SABs_SP | CD/SN |
| SABs_UA | SABb_SP | CD, CSN |
| SABs_UA | SABb_TR | CL, CD, SL, BT |
| PHO_SP | PHO_AD | CSN, BT |
| TUR_SP | TUR_MO | SN, LN, BT, CL/CD, SN/CSN |
| TUR_SP | TUR_IT | CL, CD, SW, BT, SL/SW, CL/CSN |
| TUR_MO | TUR_IT | CD, SN, SL, SW, LN, CL/CD, SL/SW |
| CAN_SP1 | CAN_SP2 | BT |

Acronyms of populations as in Table 1, acronyms of character as in Table 2.

Table 5. The result of the discriminant function analysis made on nine characters of cones, seeds and branchlets with leaves from 19 *Juniperus* populations from the *Sabina* section.

| Characters | Partial Wilk's λ | F delete | P | U ₁ 54.4% | U ₂ 18.7% |
|------------|--------------------------|----------|------|----------------------|----------------------|
| CSR4 | 0.52 | 21.53 | 0.00 | 1.48 | 0.01 |
| CD | 0.41 | 33.47 | 0.00 | 3.14 | 0.01 |
| SL | 0.67 | 11.50 | 0.00 | 0.88 | 0.76 |
| SW | 0.52 | 21.75 | 0.00 | 11.01 | 1.28 |
| LN | 0.50 | 23.27 | 0.00 | 5.91 | 0.00 |
| BT | 0.20 | 93.17 | 0.00 | 0.62 | 14.10 |
| CL/CD | 0.50 | 22.91 | 0.00 | 0.02 | 0.03 |
| CL/CSN | 0.30 | 53.12 | 0.00 | 7.38 | 1.34 |
| SN/CSN | 0.49 | 24.05 | 0.00 | 6.88 | 3.08 |

p – the statistically significant level; U₁ and U₂ – the first and second discriminant variables; character abbreviations as in Table 2.

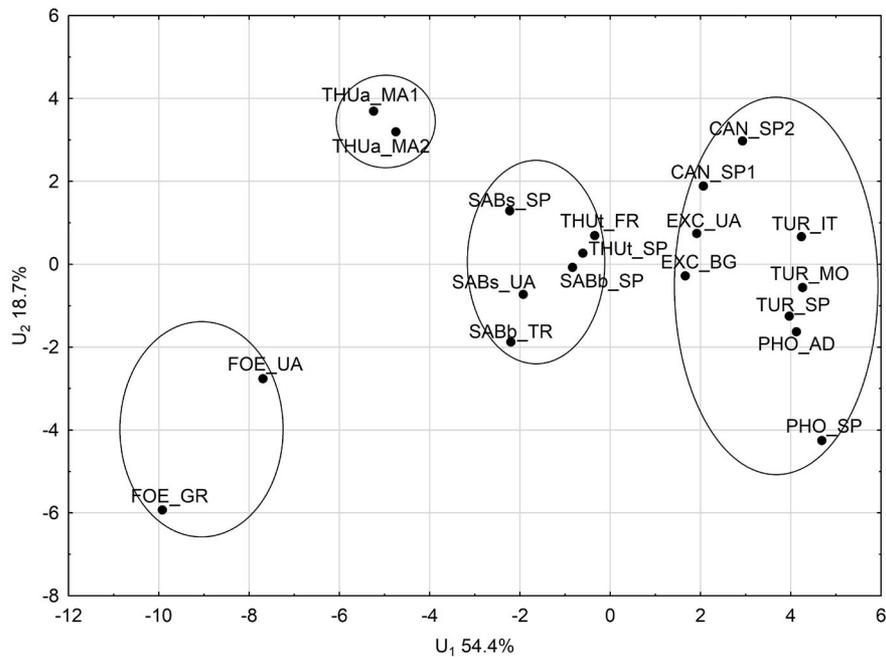


Figure 2. The scatterplot, resulting from discriminant analysis, made for 19 *Juniperus* populations from the *Sabina* section, between first two discriminant variables (U_1 & U_2), based on nine characters of cones, seeds and branchlets with leaves (populations abbreviations as in Table 1; set of nine characters as in Table 5).

in terms of U_1 , but differentiated in terms of U_2 (18.7%), where *J. canariensis* and *J. phoenicea*, especially from Spain (PHO_SP), turned out to be more distant (Fig. 2).

Discussion

Importance of morphological characters

All of the morphological characters of seed cones, seeds and shoots turned out to be important in previous studies on individual taxa of *Juniperus* (Mazur et al. 2004, 2018; Marcysiak et al. 2007; Douaihy et al. 2012; Boratyński et al. 2013) and therefore were also used to analyze the morphological variation in the *Sabina* section.

Based on the estimated values of the correlation, it was found that seed cones were not only highly statistically significantly correlated with each other and with the characters of the seeds, but also with the number of leaves per shoot (LN). Only the thickness of the final branch of twigs (BT) did not show such correlations with the other measured characters, unlike those described earlier, when analyzing individual species or complexes, where no correlation was shown between the characters of cone and seed or needle and branchlet (Marcysiak et al. 2007; Douaihy et al. 2012; Boratyński et al. 2013; Mazur et al. 2018). The percent of decussate type of cone (CSR4) was highly significantly, negatively correlated with the number of scales (CSN), significantly but less strongly negatively correlated with the number of seeds (SN), while CSN was positively and statistically highly correlated with SN.

In *J. sabina* and *J. thurifera* populations, all seed cones were decussate type (CSR4 – 100%), similarly in one population of *J. excelsa* from the Balkans (EXC_BG), and one of *J. foetidissima* from Greece (FOE_GR). On

the other hand, in the *J. phoenicea* complex, ternete cones were observed in each population and in some were the majority. In our comparisons, this happened in one population of *J. canariensis* – CAN_SP1 (CSR4 – 46.6%) (Table 2). From 9.6 to 10.8 scales per cone (CSN), were recorded in the *J. phoenicea* complex, while for the rest of the taxa it was 4.5–7.9 scales. The variation in the number of seeds per cone (SN) was similar because this character depends on the cone scale numbers (Schulz et al. 2003). The largest number of SN characterized *J. phoenicea* s.str. and *J. turbinata* (7.8 and 6.5, on average, respectively), while *J. canariensis* had a lower value (3.8, on average), but the largest size of seeds (SL and SW) compared to all taxa tested. More seeds were recorded in *J. excelsa* – 5.9 seeds per cone, on average, while the remaining taxa ranged from 1.6 to 3.6, on average. A larger number of seeds per cone and different patterns of their arrangement in the seed cone (Lemoine-Sebastian 1967; Schulz et al. 2003), as well as a greater number of scales and a significant share of ternete seed cones, which then underwent a certain simplification with evolution (Schulz et al. 2003), may confirm the suggestion that *J. phoenicea* complex seems to be the most primitive within the genus and that it could have given rise to the other junipers from the *Sabina* section (Farjon & Ortiz Garcia 2002, 2005; Schulz et al. 2003; Adams 2014; Mazur et al. 2018). The largest number of scales and the largest share of ternete cones may suggest that *J. canariensis* has the most ancestral character, while the lower number of seeds contradicts this (Mazur et al. 2018). When analyzing vegetative characters, the highest number of leaves per twig (LN) was also noted in the *J. phoenicea* complex, from 24.3 (*J. turbinata*) to 26.6 (*J. phoenicea*), on average, while in the remaining taxa it was 15.6–20.05. It is worth noting that in the case of this character, *J. excelsa* was also characterized by a high value,

20.05, on average. Similarly, this species was characterized by a large number of seeds and scales per cone (5.9 and 7.2, on average, respectively) (Table 2). The original character of *J. excelsa* can be taken into account for taxa from the Eastern Mediterranean.

Intra- and inter-taxa differences

The expected result is that more characters differed statistically significantly from taxa than individual populations within them, as demonstrated by numerous previous studies conducted on them (e.g., Boratyński et al. 2013; Mazur et al. 2018). The most common characters discriminating between populations within taxa were the thickness of the branchlet (BT, in 7 cases) and the cone diameter (CD, in 5 cases) (Table 4). Among others, these two characters were shown in earlier studies as significantly differentiating, e.g., within *J. excelsa* s.str. (Mazur et al. 2004), although the studies by Douaihy and co-workers (2012) on much larger material showed the importance of the cone diameter (CD) in differentiation between populations, but BT only in a few cases. In the present comparison, only the populations within *J. thurifera*, the majority of the population within *J. sabina* (only distinguish between SABs-UA with SABb-TR) and in one case the populations of *J. turbinata* (TUR-MOU with TUR-IT), the BT character did not distinguish statistically significantly. On the other hand, the CD character significantly distinguished the populations within *J. excelsa*, *J. turbinata* and one population of *J. sabina* (SABs-UA). Two analyzed characters did not distinguish the population within taxa: the share of the decussate type of cone (CSR4) and the ratio CD/SW (Table 4).

The *J. phoenicea* complex was highly statistically significantly distinguished from other taxa by the greatest number of characters, with a few exceptions; e.g., *J. canariensis* from *J. thurifera* subsp. *thurifera* and from *J. sabina* var. *balkanensis* have been distinguished only by 9 and 10 characters, respectively. *J. thurifera* subsp. *thurifera* differed from subsp. *africana* by 12 characters, while both subspecies from *J. sabina* var. *sabina* by 8 and 10 (respectively) (Table 3). Only 7 characters distinguished *J. sabina* var. *balkanensis* with *J. sabina* var. *sabina* and with *J. thurifera* subsp. *thurifera*, and *J. thurifera* subsp. *africana* with *J. foetidissima*. On the genetic level, the similarity between *J. sabina* var. *sabina* and *J. thurifera* s.lat. Adams and co-workers (2016) have already observed. Two characters – CSR4 and the ratio CD/SW, which turned out to be irrelevant in distinguishing the population within taxa, turned out to be very important in distinguishing between taxa. Especially the CD/SW made it possible to distinguish statistically significantly almost all taxa and turned out to be one of the most frequently differentiating characters, with the seeds number (SN) (Table 3). SN was also not frequent in distinguishing populations within taxa. It allowed us to distinguish only the populations of *J. turbinata* from Morocco (TUR-MO) from other populations (Table 4).

The two characters that were most significant in distinguishing within taxa, BT and CD, also had an important impact in discriminant analysis made between 19

populations tested (Table 4 and 5). Both the scatterplot and UPGMA dendrogram showed a similarity between *J. sabina* and *J. thurifera* subs. *thurifera*, as well as some similarity of *J. excelsa* to the *J. phoenicea* complex and the distinctiveness of *J. foetidissima* (Figs 1–2). Earlier studies, made on the same populations, but concerning selected groups compared here, showed the distinctiveness of *J. foetidissima* from *J. excelsa* s.str. and *J. thurifera* subsp. *thurifera* (Marcysiak et al. 2007), the distinctiveness of *J. thurifera* subspecies (Boratynski et al. 2013) as well as separateness of *J. canariensis* from other species of the *J. phoenicea* complex (Mazur et al. 2018). The similarity of *J. excelsa* to *J. turbinata* and other species of the *J. phoenicea* complex, visible in the scatterplot (Fig. 2), results from the second discriminant variable (U_2), which depends mainly on the branch thickness (BT), which had similar average values and variation coefficients values in both compared taxa (0.58–1.00 mm, 9.85% in *J. excelsa* and 0.68–1.00 mm, 9.42% in *J. turbinata*; Table 2). In the dendrogram, *J. excelsa* joins *J. turbinata* at 47% of the total taxonomic distance (Fig. 1). On the other hand, Tukey's *T*-test showed that these taxa are significantly differentiated by all the examined characters, except for the width of seeds (SW) and the ratio of the cone diameter to the number of seeds (CD/SN; Table 3).

Geographic variation and migration routes

The comparison of the average values of the variation coefficients (VC) of morphological characters between individual taxa of *Juniperus* showed the highest VC in *J. foetidissima* (15.6%), *J. sabina* var. *sabina* (15.5%) and *J. canariensis* (15.0%). On the other hand, the most permanent ones turned out to be *J. excelsa* and *J. sabina* var. *balkanensis* with VC 10.0% and 10.5%, on average, respectively (Table 2). The high level of variability of the analyzed populations of *J. foetidissima* has already been demonstrated in previous studies (Marcysiak et al. 2007), where the analysis of the same six populations (Table 1) and another four of *J. excelsa* s.str., *J. thurifera* subs. *thurifera* and *J. foetidissima*, based on a similar set of 14 morphological characters, also showed the highest VC of *J. foetidissima* (17.9%), and lower in *J. excelsa* s.str. and *J. thurifera* subsp. *thurifera* (15.3% each). These three taxa are assumed to be from the same ancestor (Barbero et al. 1994; Jimenez et al. 2003). However, the demonstrated morphological difference of *J. foetidissima* (Marcysiak et al. 2007) and its higher VC compared to the others, may indicate earlier divergence or other origin of this species, what has already suggested by Marcysiak and co-workers (2007). This is confirmed by slightly different ecological requirements of *J. foetidissima*, such as occurrence at higher altitudes and smaller areas than the typical *J. excelsa* (Browicz 1982; Boratyński et al. 1992; Didukh 1992; Farjon 2005). It can be assumed that *J. excelsa* s.str. and *J. thurifera* s.lat. are vicariate taxa in two currently isolated areas (Barbero et al. 1994), also *J. foetidissima* and *J. excelsa* can be treated as vicarious species (Jimenez et al. 2003). Studies conducted on the products of terpenoid metabolism have shown similarity of *J. foetidissima* to Central and East Asian

species and distinctiveness of *J. excelsa* and *J. thurifera*. However, the RAPD research demonstrated a closer relationship between *J. foetidissima* and *J. thurifera* than between *J. excelsa*, which joined the Central Asian taxa (Adams 1999). This is partially confirmed by the results of the discriminant analysis (Fig. 2), but these conclusions should be verified on a more extensive material (Farjon 2005). All three species are morphologically similar, but despite the sympatricity of the ranges of *J. excelsa* and *J. foetidissima*, which could indicate their close relationship, the morphological similarity of *J. excelsa* s.str. to *J. thurifera* and the distinctiveness of these two species from *J. foetidissima* was demonstrated (Marcysiak et al. 2007).

Further migrations of the *J. thurifera* population from the Iberian Peninsula, in the Tertiary, between the end of the Miocene and the end of the Pliocene, reached Africa. The populations of both continents were in contact before the opening of the Strait of Gibraltar, which is confirmed by the greater genetic similarity of the southern Peninsula populations to African populations (Carrion et al. 2001a; Jiménez et al. 2003). The emergence of the Strait of Gibraltar barrier after glacial periods blocked the flow of genes between Moroccan and European populations, influencing the geographic structure of *J. thurifera* variability (Terrab et al. 2008; Boratyński et al. 2013). Romo & Boratyński (2007) documented, primarily based on differences in the size of seed cones and the number of seeds inside, two subspecies: *J. thurifera* subsp. *thurifera*, found in the Mediterranean part of Europe, and *J. thurifera* subsp. *africana* (Maire) Romo & Boratyński, stat.nov. The distinction between the two subspecies was supported by the previously demonstrated differences in the content of prodelphinidine in the leaves (Gauquelin et al. 1988; Lebreton 1990), the essential oil composition and RAPD (Adams et al. 2003). Other morphological, biochemical and genetic studies conducted on the taxa of *J. thurifera* confirmed the existence of differences, confirming the correctness of distinguishing subspecies (Achak et al. 2008; Terrab et al. 2008; Boratyński et al. 2013). Studies on *J. thurifera* populations showed that this species is tetraploid, making it the only known species that has undergone speciation by genome duplication (polyploidization) (Romo et al. 2013).

Morphological studies of cones, seeds, and shoots of several populations of *J. excelsa* s.str. (Mazur et al. 2004) showed significantly less internal taxon differentiation than similar studies found in *J. thurifera* (Romo & Boratyński 2007; Boratyński et al. 2013), although some differences were found between the Crimean and Balkan populations, which was interpreted as the reason of long geographical isolation caused by the origin of the Crimean populations from a refugium other than the Balkan ones (Kornaś & Medwecka-Kornaś 2002; Yena et al. 2005). The obtained results may also suggest two different ways of their migration, or a different coefficient of morphological divergence in isolated populations, but did not show the reduction of variability of characters in the Crimean populations that might have happened as a result of geographic isolation (Mazur et al. 2004).

Douaihy and co-workers (2012) conducted a morphological versus molecular markers study, but they did not find the expected clear similarities. However, similarities have been shown on a morphological level between the populations of Greece, Turkey, and Crimea, which may be due to a common origin. The Black Sea level during the glaciation was much lower than today, which greatly facilitated the migration of plants along the coast from Anatolia to the Crimea and vice versa (Yena et al. 2005; Douaighy et al. 2012), while the presence of *J. excelsa* in the eastern Mediterranean during the LGM period (Magyari et al. 2008) indicates possible migrations between the Anatolian and Balkan peninsulas. Other routes of migration to the Crimean and Balkan peninsulas could have caused differences between the populations of *J. excelsa* from these two centers, which is confirmed by previous studies (Mazur et al. 2004). The taxonomy of this complex needs further research.

J. sabina as a typical mountain species is characterized by discontinuous range, but the connection of its parts probably took place many times in the Pleistocene period (Jiménez et al. 2003; Marcysiak et al. 2007; Boratyński et al. 2013), which may explain the high variability, at least on the morphological level. The explanation of the lower variability of the variety '*balkanensis*', which is a tetraploid and has a *J. thurifera*-like chloroplast (Adams et al. 2016; Farhat et al. 2019), requires further research. The authors provided several characters of leaves, cones, and number of seeds that may be useful to distinguish the new variety of *J. sabina* – '*balkanensis*' (Adams et al. 2016). In this study, a comparison of four *J. sabina* populations, including two classified as '*balkanensis*', showed a high similarity of all studied populations (Figs 1–2, Table 2), although Tukey's test showed that they were statistically significantly different from one to six characters (Table 4). A recent study of 27 populations of *J. sabina* (including three used in these comparisons, Table 1) also did not confirm the morphological differentiation between these two varieties (unpublished data). 18 morphological characters used in this study, showed high average VC values of characters for both varieties, higher than those for other taxa (19.9% in '*sabina*' and 19.1% in '*balkanensis*').

The study of 41 populations of *J. phoenicea* complex (including seven used for the present comparison, Table 1; Mazur et al. 2018) showed a higher VC value for *J. canariensis* (21.5%), and a lower value for *J. turbinata* (17.8%) and *J. phoenicea* (17.4%). Higher level of phenotypic variability of *J. phoenicea* s.lat., noted in previous research, found in the western part of the range, may support the theory of the European origin of the common ancestor of the complex, confirming its origin from the Iberian Peninsula, where it most likely diverged on *J. phoenicea* s.str. and *J. turbinata* (Lebreton & Rivera 1989; Lebreton & Perez de Paz 2001; Mao et al. 2010; Dzialuk et al. 2011; Adams et al. 2014 a,b; Mazur et al. 2018). The probable divergence of these two species took place quite early in the history of distinguishing the complex (Adams & Schwarzbach 2013). Whereas the separation of *J. canariensis* could have happened with the

formation of the Canary Islands, most likely not earlier than in the Miocene, as a result of colonization from the coast of the Iberian Peninsula (Fernández-Palacios et al. 2011). Thus, in the Canary Islands populations the level of variation should decrease, which, as noted by Mazur and co-workers (2018), did not take place, which the authors explain in two ways: either the Canary Islands are a type of Mediterranean flora enclave in which a high level of morphological variability has been preserved, or a high variability here was a secondary phenomenon that occurred after colonization. The high level of diversity in *J. canariensis* populations did not confirm the tendency to reduce genetic diversity in marginal populations (Hamrick et al. 1992), which was also demonstrated for other species, including junipers, e.g., for *J. drupacea* (Sobierajska et al. 2016).

Acknowledgments

I would like to thank Professor Adam Boratyński for the countless hours devoted to me during our work with junipers, for being a great promoter of my doctoral dissertation, for support and invaluable substantive help and technical tips at every stage of our cooperation and for the opportunity to work on so many valuable collections of junipers collected by Professor and Professor Krystyna Boratyńska.

References

- Achak, N., Romane, A., Alifriqui, M. & Adams, R. P. 2008. Effect of the leaf drying and geographic sources on the essential oil composition of *Juniperus thurifera* L. var. *africana* Maire from the Tensift – Al Haouz, Marrakech Region. *Journal of Essential Oil Research* 20: 200–204. <https://doi.org/10.1080/10412905.2008.9699990>
- Adams, R. P. 1999. Systematics of multi-seeded eastern hemisphere *Juniperus* based on leaf essential oils and RAPD DNA fingerprinting. *Biochemical Systematics and Ecology* 27: 709–725.
- Adams, R. P. 2014. *Junipers of the world: The genus Juniperus*. 4th ed. Trafford Publishing Co., Bloomington, IN.
- Adams, R. P. 2016. Two new cases of chloroplast capture in incongruent topologies in the *Juniperus excelsa* complex: *J. excelsa* var. *turcomanica* comb. nov. and *J. excelsa* var. *seravschanica* comb. nov. *Phytologia* 98: 219–231.
- Adams, R. P. & Schwarzbach, A. E. 2013. Phylogeny of *Juniperus* using nrDNA and four cpDNA regions. *Phytologia* 95: 179–187.
- Adams, R. P., Pandey, N., Rezzi, S. & Casanova, J. 2002. Geographic variation in the Random Amplified Polymorphic DNAs (RAPDs) of *Juniperus phoenicea*, *J. p.* var. *canariensis*, *J. p.* subsp. *eu-mediterranea*, and *J. p.* var. *turbinata*. *Biochemical Systematics and Ecology* 30(3): 223–229.
- Adams, R. P., Mumba, L. E., James, A. S., Pandey, R. N., Gauqueline, T. & Badri, W. 2003. Geographic variation in the leaf oils and DNA fingerprints (RAPDs) of *Juniperus thurifera* L. from Morocco and Europe. *Journal of Essential Oil Research* 15: 148–154. <https://doi.org/10.1080/10412905.2003.9712098>
- Adams, R. P., Nguyen, S. & Liu, J. 2006a. Geographic Variation in the Leaf Essential Oils of *Juniperus sabina* L. and *J. sabina* var. *arenaria* (E.H. Wilson) Farjon. *Journal of Essential Oil Research* 18: 497–502. <https://doi.org/10.1080/10412905.2006.9699152>
- Adams, R. P., Nguyen, S. & Achak, N. 2006b. Geographic variation in *Juniperus phoenicea* (Cupressaceae) from the Canary Islands, Morocco and Spain based on RAPDs analysis. *Phytologia* 88: 270–278.
- Adams, R. P., Schwarzbach, A. E., Nguyen, S., Morris, J. A. & Liu, J.-Q. 2007. Geographic variation in *Juniperus sabina* L., *J. sabina* var. *arenaria* (E. H. Wilson) Farjon, *J. sabina* var. *davurica* (Pall.) Farjon and *J. sabina* var. *mongolensis* R. P. Adams. *Phytologia* 89: 153–166.
- Adams, R. P., Rumeu, B., Nogales, M., & Fontinha, S. S. 2010. Geographic variation and systematics of *Juniperus phoenicea* L. from Madeira and the Canary Islands: SNPs from nrDNA and petN-psbM. *Phytologia* 92: 59–67.
- Adams, R. P., Boratyński, A., Arista, M., Schwarzbach, A. E., Leschner, H., Liber, Z., Minissale, P., Mataraci, T. & Manolis, A. 2013. Analysis of *Juniperus phoenicea* from throughout its range in the Mediterranean using DNA sequence data from nrDNA and petN-psbM: The case for the recognition of *J. turbinata* Guss. *Phytologia* 95: 202–209.
- Adams, R. P., Altarejos, J., Arista, M. & Schwarzbach, A. E. 2014a. Geographic variation in *J. phoenicea* var. *phoenicea* from throughout its range: Analysis of nrDNA and the petN-PsbM cp region. *Phytologia* 96: 247–251.
- Adams, R. P., Arista, M., Boratyński, A., Hoauri, H. H., Leschner, H., Liber, Z., Minissale, P., Sciandrello, S. & Mataraci, T. 2014b. Geographic variation in the leaf essential oil of *Juniperus turbinata* from throughout its range in the Mediterranean. *Phytologia* 96: 149–158.
- Adams, R. P., Schwarzbach, A. E. & Tashev, A. N. 2016. Chloroplast capture by a new variety, *Juniperus sabina* var. *balkanensis* R. P. Adams and A. N. Tashev, from the Balkan peninsula: A putative stabilized relictual hybrid between *J. sabina* and ancestral *J. thurifera*. *Phytologia* 98: 100–111.
- Adams, R. P., Boratyński, A., Mataraci, T., Tashev, A. N. & Schwarzbach, A. E. 2017. Discovery of *Juniperus sabina* var. *balkanensis* R. P. Adams and A. N. Tashev in western Turkey (Anatolia). *Phytologia* 99: 22–31.
- Adams, R. P., Boratyński, A., Marcysiak, K., Roma-Marzio, F., Peruzzi, L., Bartolucci, F., Conti, F., Mataraci, T., Schwarzbach, A. E., Tashev, A. N. & Siljak-Yakovlev, S. 2018a. Discovery of *Juniperus sabina* var. *balkanensis* R. P. Adams and A. N. Tashev in Macedonia, Bosnia-Herzegovina, Croatia and Central and Southern Italy and relictual polymorphisms found in nrDNA. *Phytologia* 100: 117–127.
- Adams, R. P., Farhat, P., Shuka, L. & Siljak-Yakovlev, S. 2018b. Discovery of *Juniperus sabina* var. *balkanensis* R. P. Adams and A. N. Tashev in Albania and relictual polymorphisms found in nrDNA. *Phytologia* 100: 187–194.
- Allen, M. B. & Armstrong, H. A. 2008. Arabia-Eurasia collision and the forcing of mid Cenozoic global cooling. *Palaeogeography, Palaeoclimatology, Palaeoecology* 265(1–2): 52–58. <https://doi.org/10.1016/j.palaeo.2008.04.021>
- Arrigoni, P. V. 2012. Miscellaneous notes about some taxa of the Italian flora. *Webbia* 67: 37–46. <https://doi.org/10.1080/00837792.2012.10670907>
- Axelrod, D. 1975. Evolution and biogeography of Madrean-Tethyan sclerophyll vegetation. *Annals of the Missouri Botanical Garden* 62: 280–334. <https://doi.org/10.2307/2395199>
- Barbero, M., Lebreton, Ph. & Quézel, P. 1994. Sur les affinités biosystématiques et phytoécologiques de *Juniperus thurifera* L. et de *Juniperus excelsa* Bieb. *Ecologia Mediterranea* 20: 21–37.
- Bennet, K. D., Tzedakis, P. C. & Willis, K. J. 1991. Quaternary refugia of north European trees. *Journal of Biogeography* 18: 103–115. <https://doi.org/10.2307/2845248>
- Bocquet, G., Widler, B. & Kiefer, H. 1978. The Messinian Model – A new outlook for the floristics and systematics of the Mediterranean area. *Candollea* 33(2): 269–287.
- Boratyński, A., Browicz, K. & Zieliński, J. 1992. *Chorology of trees and shrubs in Greece*. Institute of Dendrology of Polish Academy of Sciences, Kórnik.
- Boratyński, A., Lewandowski, A., Boratyńska, K., Montserrat, J. M. & Romo, A. 2009. High level of genetic differentiation of *Juniperus phoenicea* (Cupressaceae) in the Mediterranean region: Geographic implications and Evolution 277: 163–172. <https://doi.org/10.1007/s00606-008-0122-z>

- Boratyński, A., Jasińska, A. K., Marcysiak, K., Mazur, M., Romo, A. M., Boratyńska, K., Sobierajska, K. & Iszkulo, G. 2013. Morphological differentiation supports the genetic pattern of the geographic structure of *Juniperus thurifera* (Cupressaceae). *Plant Systematics and Evolution* 299: 773–784. <https://doi.org/10.1007/s00606-013-0760-7>
- Boratyński, A., Wachowiak, W., Dering, M., Boratyńska, K., Sękiel-wicz, K., Sobierajska, K., Jasińska, A. K., Klimo, M., Montserrat, J. M., Romo, A., Ok, T. & Didukh, Y. 2014. The biogeography and genetic relationships of *Juniperus oxycedrus* and related taxa from the Mediterranean and Macaronesian regions. *Botanical Journal of the Linnean Society* 174: 637–653. <https://doi.org/10.1111/boj.12147>
- Browicz, K. 1982. *Chorology of trees and shrubs in South-West Asia and adjacent regions*. Vol. 1. Polish Academy of Science, Institute of Dendrology, Poznań – Kórnik. Państwowe Wydawnictwo Naukowe, Poznań.
- Carrión, J. S., Andrade, A., Bennett, K. D., Navarro, C. & Munuera, M. 2001a. Crossing forest thresholds: inertia and collapse in a Holocene sequence from south-central Spain. *Holocene* 11: 635–653. <https://doi.org/10.1191/09596830195672>
- Carrión, J. S., Munuera, M., Dupré, M. & Andrade, A. 2001b. Abrupt vegetation changes in the Segura mountains of southern Spain. *Journal of Ecology* 89: 783–797. <https://doi.org/10.1046/j.0022-0477.2001.00601.x>
- Carrión, J. S., Yll, E. I., Walker, M. J., Legaz, A. J., Chain, C. & López, A. 2003. Glacial refugia of temperate, Mediterranean and Ibero-North African flora in south-eastern Spain: new evidence from cave pollen at two Neanderthal sites. *Global Ecology and Biogeography* 12(2): 119–129. <https://doi.org/10.1046/j.1466-822X.2003.00013.x>
- Carrión, J. S., Yll, E. I., Willis, K. J. & Sanchez, P. 2004. Holocene forest history of the eastern plateaux in the Segura Mountains (Murcia, southeastern Spain). *Review of Palaeobotany and Palynology* 132: 219–236. <https://doi.org/10.1016/j.revpalbo.2004.07.002>
- Carrión, J. S., Fernández, S., González-Sampériz, P., Gil-Romera, G., Badal, E., Carrión-Marco, Y., López-Merino, L., López-Sáez, J. A., Fierro, E. & Burjachs, F. 2010. Expected trends and surprises in the Lateglacial and Holocene vegetation history of the Iberian Peninsula and Balearic Islands. *Review of Palaeobotany and Palynology* 162: 458–475. <https://doi.org/10.1016/j.revpalbo.2009.12.007>
- Cattell, R. B. 1943. The description of personality: Basic characters resolved into clusters. *Journal of Abnormal and Social Psychology* 38: 476–506. <https://doi.org/10.1037/h0054116>
- Charco, J. 2001. *Güa de los árboles y arbustos del norte de África*. Agencia Española de Cooperación Internacional, Madrid.
- Didukh, Y. P. 1992. *Rastitelnyj pokrov Gornogo Kryma*. Naukova Dumka, Kiev.
- Douaihy, B., Sobierajska, K., Jasińska, A. K., Boratyńska, K., Ok, T., Romo, A., Machon, N., Didukh, Y., Dagher Kharrat, M. B. & Boratyński, A. 2012. Morphological versus molecular markers to describe variability in *Juniperus excelsa* subsp. *excelsa* (Cupressaceae). *Annals of Botany Plants* 013: 1–14. <https://doi.org/10.1093/aobpla/pls013:pls013>
- Działuk, A., Mazur, M., Boratyńska, K., Montserrat, J. M., Romo & A., Boratyński, A. 2011. Population genetic structure of *Juniperus phoenicea* (Cupressaceae) in the western Mediterranean Basin: Gradient of diversity on a broad geographical scale. *Annals of Forest Science* 68: 1341–1350. <https://doi.org/10.1080/14772000.2016.1257518>
- Farhat, P., Siljak-Yakovlev, S., Adams, R. P., Dagher Kharrat, M. B. & Robert, T. 2019. Genome size variation and polyploidy in the geographical range of *Juniperus sabina* L. (Cupressaceae). *Botany Letters* 166(2): 10. <https://doi.org/10.1080/23818107.2019.1613262>
- Farhat, P., Takvorian, N., Avramidou, M., Garraud, L., Adams, R. P., Siljak-Yakovlev, S., Dagher Kharrat, M. B. & Robert, T. 2020. First evidence for allotriploid hybrids between *Juniperus thurifera* and *J. sabina* in a sympatric area in the French Alps. *Annals of Forest Sciences* 77: 93. <https://doi.org/10.1007/s13595-020-00969-7>
- Farjon, A. 2005. *A monograph of Cupressaceae and Sciadopitys*. Royal Botanic Gardens, Kew.
- Farjon, A. & Ortiz Garcia, S. 2002. Towards the minimal conifer cone: Ontogeny and trends in *Cupressus*, *Juniperus* and *Microbiota* (Cupressaceae s.str.). *Botanisches Jahrbücher für Systematic* 124: 129–147.
- Farjon, A. & Ortiz Garcia, S. 2005. The early development of ovuliferous cones of Cupressaceae s.l. – a survey of the genera. In: Farjon, A. (ed.), *A Monograph of Cupressaceae and Sciadopitys*. pp. 27–46. Royal Botanic Gardens, Kew.
- Favre, E., François, L., Fluteau, F., Cheddadi, R., Thévenod, L. & Suc, J.-P. 2007. Messinian vegetation maps of the Mediterranean region using models and interpolated pollen data. *Geobios* 40: 433–443. <https://doi.org/10.1016/j.geobios.2006.12.002>
- Fernández-Palacios, J. M., Otto, R., Delgado, J. D., Arévalo, J. R., Naranjo, A., Artiles, F. G., Morici, C. & Barone, R. 2008. *Los bosques termofilos de Canarias*. Proyecto IFE04/NAT/ES/000064. Excmo. Cabildo Insular de Tenerife, Santa Cruz de Tenerife.
- Fernández-Palacios, J. M., de Nascimento, L., Otto, R., Delgado, J. D., García-del-Rey, E., Arévalo, J. R. & Whittaker, R. J. 2011. A reconstruction of Palaeo-Macaronesia, with particular reference to the long-term biogeography of the Atlantic island laurel forests. *Journal of Biogeography* 38: 226–246. <https://doi.org/10.1111/j.1365-2699.2010.02427.x>
- Gauquelin, T., Hassani, M. I. & Lebreton, P. 1988. Le Génévrier thurifère, *Juniperus thurifera* L. (Cupressaceae): analyse biométrique et biochimique; propositions systématiques. *Ecologia Meditteranea* 14: 31–42.
- Hamrick, J. L., Godt, M. J. W. & Sherman-Broyles, S. L. 1992. Factors influencing levels of genetic diversity in woody plant species. *New Forests* 6: 95–124.
- Hewitt, G. M. 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society* 58: 247–276. <https://doi.org/10.1111/j.1095-8312.1996.tb01434.x>
- Hewitt, G. M. 1999. Post-glacial re-colonization of European biota. *Biological Journal of the Linnean Society* 68: 87–112. <https://doi.org/10.1006/bijl.1999.0332>
- Hewitt, G. M. 2004. Genetic consequences of climatic oscillations in the Quaternary. *Philosophical Transactions of the Royal Society B: Biological Sciences* 359: 183–195. <https://doi.org/10.1098/rstb.2003.1388>
- Hojjati F., Zarre, S. & Assadi, M. 2009. Isoenzyme diversity and cryptic speciation in *Juniperus excelsa* (Cupressaceae) complex in Iran. *Biochemical Systematics and Ecology* 37: 193–200. <https://doi.org/10.1016/j.bse.2009.03.002>
- Hojjati, F., Sereshti, H. & Hojjati, M. 2019. Leaf essential oils and their application in systematics of *Juniperus excelsa* complex in Iran. *Biochemical Systematics and Ecology* 84: 29–34. <https://doi.org/10.1016/j.bse.2019.03.004>
- Ivanov, D., Utescher, T., Mosbrugger, V., Syabryaj, S., Djordjević-Milutinović, D. & Molchanoff, S. 2011. Miocene vegetation and climate dynamics in Eastern and Central Paratethys (Southeastern Europe). *Palaeogeography, Palaeoclimatology, Palaeoecology* 304: 262–275. <https://doi.org/10.1016/j.palaeo.2010.07.006>
- Jalas, J. & Suominen, J. (eds) 1973. *Atlas Florae Europaeae*. Vol. 2. The Committee for Mapping the Flora of Europe and Societas Biologica Fennica Vanamo, Helsinki.
- Jiménez, J. F., Werner, O., Sanchez-Gomez, P., Fernández, S. & Guerra, J. 2003. Genetic variations and migration pathway of *Juniperus thurifera* L. (Cupressaceae) in the western Mediterranean region. *Israel Journal of Plant Sciences* 51: 11–22. <https://doi.org/10.1560/ABR5-A6MP-5XEG-V0WF>
- Jiménez, J. F., Sánchez-Gómez, P., Cánovas, J. L., Hensen, I. & Aouisat, M. 2017. Influence of natural habitat fragmentation on the genetic structure of Canarian populations of *Juniperus turbinata*. *Silva Fennica* 51(1678): 1–14. <https://doi.org/10.14214/sf.1678>
- Kornaś, J. & Medwecka-Kornaś, A. 2002. *Geografia roślin*. Wyd. 2. Wydawnictwo Naukowe PWN, Warszawa.

- Kovar-Eder, J., Kvaček, Z., Martinetto, E. & Roiron, P. 2006. Late Miocene to Early Pliocene vegetation of southern Europe (7–4 Ma) as reflected in the megafossil plant record. *Palaeogeography, Palaeoclimatology, Palaeoecology* 238: 321–339. <https://doi.org/10.1016/j.palaeo.2006.03.031>
- Kvaček, Z. 2002. A new juniper from the Paleogene of Central Europe. *Feddes Repertorium* 113: 492–502. <https://doi.org/10.1002/fedr.200290001>
- Lebreton, P. 1990. La chimiotaxonomie des Gymnospermes. *Bulletin de la Société Botanique de France. Lettres Botaniques* 137(1): 35–46. <https://doi.org/10.1080/01811797.1990.10824864>
- Lebreton, P. & Pérez de Paz, P. L. 2001. Définition du Genévrier de Phénicie (*Juniperus* aggr. *phoenicea*), reconsidéré à ses limites biogéographiques: Méditerranée orientale (Crète et Chypre) et Atlantique (Iles Canaries). *Bulletin mensuel de la Société linnéenne de Lyon* 70: 73–92.
- Lebreton, P. & Rivera, D. 1989. Analyse du taxon *Juniperus phoenicea* L. sur des bases biochimiques et biométriques. *Naturalia Monspelienis, Serie botanique* 53: 17–41.
- Lebreton, P. & Thivend, S. 1981. Sur une sous-espèce du Genévrier de Phénicie *Juniperus phoenicea* L., définie à partir de critères biochimiques. *Naturalia Monspelienis, Serie botanique* 47: 1–12.
- Lemoine-Sébastien, C. 1967. L'inflorescence femelle des Juniperaie: Ontogénèse, structure, phylogénèse. *Travaux du Laboratoire Forestier de Toulouse* 7: 1–455.
- Magyari, E. K., Chapman, J. C., Gaydarska, B., Marinova, E., Deli, T., Huntley, J.P., Allen, J. R. M. & Huntley, B. 2008. The 'oriental' component of the Balkan flora: evidence of presence on the Thracian Plain during the Weichselian late-glacial. *Journal of Biogeography* 35: 865–883. <https://doi.org/10.1016/j.quascirev.2012.01.006>
- Mao, K., Hao, G., Liu, J., Adams, R. P. & Milne, R. I. 2010. Diversification and biogeography of *Juniperus* (*Cupressaceae*): Variable diversification rates and multiple intercontinental dispersals. *New Phytologist* 185: 332–342. <https://doi.org/10.1111/j.1469-8137.2010.03351.x>
- Mao, K., Ruhsam, M., Ma, Y., Graham, S. W., Liu, J., Thomas, Ph., Milne, R. I. & Hollingsworth, P. M. 2019. A transcriptome-based resolution for a key taxonomic controversy in *Cupressaceae*. *Annals of Botany* 123: 153–167. <https://doi.org/10.1093/aob/mcy175>
- Marcysiak, K., Mazur, M., Romo, A., Montserrat, J. M., Didukh, Y., Boratyńska, K., Jasińska, A., Kosiński, P. & Boratyński, A. 2007. Numerical taxonomy of *Juniperus thurifera*, *J. excelsa* and *J. foetidissima* (*Cupressaceae*) based on morphological characters. *Botanical Journal of the Linnean Society* 155: 483–495. <https://doi.org/10.1111/j.1095-8339.2007.00730.x>
- Mazur, M., Boratyńska, K., Marcysiak, K., Gómez, D., Tomaszewski, D., Didukh, Y., Boratyński, A. 2003. Morphological variability of *Juniperus phoenicea* (*Cupressaceae*) from three distant localities on Iberian Peninsula. *Acta Societatis Botanicorum Poloniae* 72: 71–78.
- Mazur, M., Boratyńska, K., Marcysiak, K., Didukh, Y., Romo, A., Kosiński, P. & Boratyński, A. 2004. Low level of inter-populational differentiation in *Juniperus excelsa* M. Bieb. (*Cupressaceae*). *Dendrobiology* 52: 39–46.
- Mazur, M., Marcysiak, K., Boratyński, A., Boratyńska, K., Romo, A., Montserrat, J. M., Kosiński, P. 2005. Morphological variation of *Juniperus thurifera* L. (*Cupressaceae*) from four populations in South-Western Europe. In: Prus-Głowacki, W., Pawlaczyk, E. M. (eds), *Variability and evolution-new perspectives*, vol. 72, pp. 399–409. Ser. Biologia. Adam Mickiewicz University, Poznań.
- Mazur, M., Klajbor, K., Kielicz, W., Sowińska, M., Romo, A., Montserrat, J. M. & Boratyński, A. 2010. Intra-specific differentiation of *Juniperus phoenicea* in the western Mediterranean region revealed in morphological multivariate analysis. *Dendrobiology* 63: 21–31.
- Mazur, M., Minissale, P., Sciandrello, S. & Boratyński, A. 2016. Morphological and ecological comparison of populations of *Juniperus turbinata* Guss. and *J. phoenicea* L. from the Mediterranean region. *Plant Biosystems* 150: 313–322. <https://doi.org/10.1080/11263504.2014.994579>
- Mazur, M., Zielinska, M., Boratyńska, K., Romo, A., Salvà-Catarineu, M., Marcysiak, K. & Boratyński, A. 2018. Taxonomic and geographic differentiation of *Juniperus phoenicea* agg. based on cone, seed, and needle characteristics. *Systematics and Biodiversity* 16: 469–483. <https://doi.org/10.1080/14772000.2018.1439120>
- McLachlan, G. J. 2004. *Discriminant Analysis and Statistical Pattern Recognition*. John Wiley & Sons, New York.
- Meloni, M., Perini, D., Filigheddu, R. & Binelli, G. 2006. Genetic variation in five Mediterranean populations of *Juniperus phoenicea* as revealed by inter-simple sequence repeat (ISSR) markers. *Annals of Botany* 97: 299–304. <https://doi.org/10.1093/aob/mcj024>
- Pearson, K. 1900. On the criterion that a given system of deviations from the probable in the case of a correlated system of variables is such that it can be reasonably supposed to have arisen from random sampling. *Philosophical Magazine* 5(50): 157–175.
- Petit, R. J., Aguinalde, I., Beaulieu, J. L., Bittkau, C., Brewer, S., Cheddadi, R., Ennos, R., Fineschi, S., Grivet, D., Lascoux, M., Mohanty, A., Müller-Starck, G., Demdesure-Musch, B., Palmé, A., Martín, J. P., Rendell, S. & Vendramin, G. G. 2003. Glacial refugia: hotspots but not melting pots of genetic diversity. *Science* 300: 1563–1565. <https://doi.org/10.1126/science.1083264>
- Pinna, M. S., Grilloa, O., Mattanaa, E., Cañadasad, E. M. & Bacchetta, G. L. 2014. Inter- and intraspecific morphometric variability in *Juniperus* L. seeds (*Cupressaceae*). *Systematics and Biodiversity* 12: 211–223. <https://doi.org/10.1080/14772000.2014.904827>
- Popov, S. V., Shcherba, I. G., Ilyina, L. B., Neveeskaya, L. A., Paramonova, N. P., Khondrakian, S. O. & Magyar, I. 2006. Late Miocene to Pliocene palaeogeography of the Paratethys and its relation to the Mediterranean. *Palaeogeography, Palaeoclimatology, Palaeoecology* 238: 91–106. <https://doi.org/10.1016/j.palaeo.2006.03.020>
- Quezel, P. & Pesson, P. 1980. Biogéographie et écologie des conifères sur le pourtour méditerranéen. Gauthier-Villars, Paris. *Actualités d'écologie forestière* 205–255.
- Romo, A. & Boratyński, A. 2005. Chorology of *Juniperus thurifera* (*Cupressaceae*) in Morocco. *Dendrobiology* 54: 41–50.
- Romo, A. & Boratyński, A. 2007. Nomenclatural note on *Juniperus thurifera* subsp. *africana* (*Cupressaceae*). *Annales Botanici Fennici* 44: 72–75.
- Romo, A. M. & Salvà-Catarineu, M. 2013. Phytodiversity of the remnants of Canarian endemic juniper woodlands on El Hierro, Canary Islands / La phytodiversité dans les rémanents de la forêt endémique de genévrier d'El Hierro, îles Canaries. *Ecologia Mediterranea* 39: 191–199.
- Romo, A., Hidalgo, O., Boratyński, A., Sobierajska, K., Jasińska, A. K., Vallés, J. & Garnatje, T. 2013. Genome size and ploidy levels in highly fragmented habitats: the case of western Mediterranean *Juniperus* (*Cupressaceae*) with special emphasis on *J. thurifera* L. *Tree Genetics & Genomes* 9: 587–599. <https://doi.org/10.1007/s11295-012-0581-9>
- Romo, A., Mazur, M., Salvà-Catarineu, M. & Boratyński, A. 2019. A re-evaluated taxon: Genetic values and morphological characters support the recognition of the Canary Island juniper of the *phoenicea* group at a specific level. *Phytotaxa* 406(1): 64–70. <https://doi.org/10.11646/phytotaxa.406.1.3>
- Salvà-Catarineu, M., Romo, A., Mazur, M., Zielinska, M., Minissale, P., Dönmez, A. A., Boratyńska, K. & Boratyński, A. 2021. Past, present and future geographic range of the relict Mediterranean and Macaronesian *Juniperus phoenicea* complex. *Ecology and Evolution* 11: 5075–5095. <https://doi.org/10.1002/ece3.7395>
- Sánchez-Gómez, P., Jiménez, J. F., Cánovas, J. L., Vera, J. B., Hensen, I. & Aouissat, M. 2018. Genetic structure and phylogeography of *Juniperus phoenicea* complex throughout Mediterranean and Macaronesian regions: different stories in one. *Annals of Forest Science* 75: 75. <https://doi.org/10.1007/s13595-018-0741-7>
- Sanguin, H., Mathaux, C., Guibal, F., Prin, Y., Mandin, J-P., Gauque-lin, T. & Duponnois, R. 2016. Ecology of vertical life in harsh environments: The case of mycorrhizal symbiosis with secular cliff

- climbing trees (*Juniperus phoenicea* L.). *Journal of Arid Environments* 134: 132–135. <https://doi.org/10.1016/j.jaridenv.2016.07.008>
- Schulz, C., Jagel, A. & Stutzel, T. 2003. Cone morphology in *Juniperus* in the light of cone evolution in *Cupressaceae* s.l. *Flora* 198: 161–177. <https://doi.org/10.1078/0367-2530-00088>
- Sobierajska, K., Boratyńska, K., Jasińska, A., Dering, M., Ok, T., Douaihy, B., Dagher-Kharrat, M. B., Romo, A. & Boratyński, A. 2016. Effect of the Aegean Sea barrier between Europe and Asia on differentiation in *Juniperus drupacea* (*Cupressaceae*). *Botanical Journal of the Linnean Society* 180: 365–385. <https://doi.org/10.1111/boj.12377>
- Sokal, R. R. & Rohlf, T. J. 2003. *Biometry: The principles and practice of statistics in biological research*, Eight printing. Freeman W. H. and Comp, San Francisco.
- Terrab, A., Schoenswetter, P., Talavera, S., Vela, E. & Stuessy, T. F. 2008. Range-wide phylogeography of *Juniperus thurifera* L., a presumptive keystone species of western Mediterranean vegetation during cold stages of the Pleistocene. *Molecular Phylogenetics and Evolution* 48: 94–102. <https://doi.org/10.1016/j.ympev.2008.03.018>
- Tzedakis, P. C. 2004. The Balkans as prime glacial refugial territory of European temperate trees. In: Griffiths, H. I., Kryštufek, B. & Reed, J. M. (eds), *Balkan biodiversity*, pp. 49–68. Kluwer Academic Press, Dordrecht/Boston/London.
- Verdú, M., Davila, P., Garcia-Fayos, P., Flores-Hernandez, N. & Valiente-Banuet, A. 2003. ‘Convergent’ characters of mediterranean woody plants belong to pre-mediterranean lineages. *Biological Journal of the Linnean Society* 78: 415–427. <https://doi.org/10.1046/j.1095-8312.2003.00160.x>
- Wen, J. & Ickert-Bond, S. M. 2009. Evolution of the Madrean–Tethyan disjunctions and the North and South American amphitropical disjunctions in plants. *Journal of Systematics and Evolution* 47(5): 331–348. <https://doi.org/10.1111/j.1759-6831.2009.00054.x>
- Wen, J., Nie, Z-L. & Ickert-Bond, S. M. 2016. Intercontinental disjunctions between eastern Asia and western North America in vascular plants highlight the biogeographic importance of the Bering land bridge from late Cretaceous to Neogene. *Journal of Systematics and Evolution* 54(5): 469–490. <https://doi.org/10.1111/jse.12222>
- Willis, J. K. & McElwain, J. C. 2002. *The evolution of plants*. Oxford University Press, Oxford, New York.
- Yena, An., Yena, Al. & Yena, V. 2005. ‘Stankewicz pine’ in Crimea: some new taxonomical, chorological and paleolandscape considerations. *Dendrobiology* 53: 63–69.
- Zajac, M. & Zajac, A. 2009. *The geographical elements of native flora of Poland*. Laboratory of Computer Chorology, Institute of Botany, Jagiellonian University, Kraków.
- Zar, J. H. 1999. *Biostatistical analysis*. 4th ed. Prentice-Hall, New Jersey.
- Zohary, M. 1973. *Geobotanic foundation of the Middle East*. Gustav Fischer Verlag and Sweets and Zeitlinger, Stuttgart and Amsterdam.