



*Krystyna Boratyńska, Dominik Tomaszewski, Josep M. Montserrat, Sławomir Marek, Adam Boratyński**

Taxonomic position of *Pinus ceciliae* (Pinaceae) endemic for Balearic Islands as revealed on needle characteristics

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Abstract: The Cecilian pine (*Pinus ceciliae*) is endemic to Balearic islands (Spain). It is a small taxon, sometimes treated as synonym of Aleppo pine (*P. halepensis*), to which is closely related, differing mainly with dense crown shape and upright branches. The other characteristics, which differ between *P. ceciliae* and *P. halepensis* concern the cone scale and needle length only. We examined biometrically needles of Cecilian pine from Mallorca (5 tress) and Menorca (9 trees) islands, and compared them to Aleppo pine populations represented by 30 trees from each island. Each tree was represented by 5 needles, and they were studied with respect to 17 morphological and anatomical characteristics. We detected that needles of the Cecilian pine were smaller, but only when compared to the Aleppo pine from the same island. In general, this difference was also observed in the number of resin canals, number of stomata and stomatal rows. Interestingly, the proportions of the needle dimensions pattern were similar in both taxa. In conclusion we stated the results support the taxonomic rank of Cecilian pine as a variety, *Pinus halepensis* var. *ceciliae* (Llorens & L.Llorens) L.Llorens, Fl. Països Catalans, 1: 197 (1984).

Keywords: biometry, discrimination analysis, *Pinus halepensis*, Principal Component Analysis

Addresses: K. Boratyńska, Institute of Dendrology, Polish Academy of Sciences, Parkowa 5, 62-035 Kórnik, e-mail: borkrys@man.poznan.pl

A. Boratyński, Institute of Dendrology, Polish Academy of Sciences, Parkowa 5, 62-035 Kórnik, e-mail: borata@man.poznan.pl

D. Tomaszewski, Institute of Dendrology, Polish Academy of Sciences, Parkowa 5, 62-035 Kórnik, e-mail: dominito@man.poznan.pl

Jose M. Montserrat, Jardín Botánico de Barcelona, Parc de Montjuïc, Dr. Font i Quer 2, 08038 Barcelona, e-mail: jmmontserrat@ibb.csic.es

S. Marek, Żabikowska street, 62-030 Luboń, Poland

*corresponding author

Introduction

Pinus ceciliae Llorens & L.Llorens (1984: Folia Bot. Misc. 1984, 4: 55) (Pinaceae Spreng. ex F.Rudolphi, nom. cons.) was described as a species by Antoni and

Leonard Llorens in 1972 (Llorens & Llorens, 1972; Llorens L., 1984), but their taxonomic range has been reduced to the variety of Aleppo pine *P. halepensis* Mill. var. *ceciliae* (Llorens & L.Llorens) L.Llorens (Llorens, 1979; Rosselló et al., 1992). The characteristics that

distinguish the Cecilian pine specimens from typical *P. halepensis* are: 1) upright branches arranged at an angle of approximately 15° , which results in the formation of a narrow tree crown, and 2) smaller cones compared to *P. halepensis*, with convex scale apophysis (Llorens & Llorens, 1972; Llorens, 1979, 1984; Laguna Lumbreras, 2000; Núñez Vázquez, 2013a,b). In addition, needles of the Cecilian pine are shorter (Fig. 1), a lighter green and its wood is softer compared to typical *P. halepensis* (Llorens, 1979; Núñez Vázquez, 2013a,b).

The crown shape of Cecilian pine resembles a pyramidal or compact form of *P. nigra* J.F. Arnold and *P. brutia* Ten., reported from Anatolia (see Krüssmann, 1972; Vidaković, 1991; Yücel, 1997; Yaltirik & Boydak, 2000; Boydak, 2001; Tosun, 2003; Ferrer &

Laguna Lumbreras, 2010). However, the systematic status of the forms mentioned above has yet to be fully accepted, and they were treated as synonyms of *P. nigra* species or typical subspecies of *P. brutia* (Frankis, 2000). The status of *P. ceciliae* or *P. halepensis* var. *ceciliae* is unclear and the taxon name has been included in *P. halepensis* (Amaral Franco, 1986; Farjon, 2017). Lately, Cecilian pine was proposed as restricted, local taxon with the variety rank, known only from Balearic Islands (Bolòs & Vigo, 1984; Rosselló et al., 1992; Rosselló & Sáez, 2000).

The resin of the Aleppo pine has been used for production of tar by dry distilling its wood, a practice performed in the western Mediterranean since the Neolithic period (Orengo et al., 2013). Tar was indispensable for waterproofing objects like boats, shoe



Fig. 1. Twigs of *Pinus halepensis* (up) and *P. ceciliae* (down) from the Barcelona Botanic Garden (photo J.M. Montserrat)

soles, water-resistant tools, among others. The resin-rich pines were also used to illuminate the streets before the introduction of modern street lamps.

A pine tree selected for its greater quantity of resin is easy to cut because of its softer wood (Llorens, 1979), which possibly grows faster and seems to be a good choice for tar production. It should be kept in mind that in the Balearic Islands, there are few tree species available, and in the past, most of craftworks, house and boat buildings and woodworks were made using materials obtained from local resources. Locally, this pine (Menorca, Pere Fraga, pers. comm.) is known as ‘pi teier’, which means resinous pine and is also known by the carpenters of Ciutadella (Menorca).

The Cecilian pine has been reported to be from Mallorca, Menorca, Ibiza and Cabrera (Núñez Vázquez, 2013a,b). It grows there as single trees dispersed among *P. halepensis* forests (Rosselló et al., 1992) mainly on private terrains. Currently, *P. ceciliae* has been reported in about 100 natural localities of (Núñez Vázquez, 2007, 2008, 2013a,b), and 160 places where it was planted. The relatively low number of localities and individuals in particular localities put this taxon among endangered taxa. Currently it is protected as a species ‘of special interest’ (CAB, 1992). Urbanisation is cited as a factor which puts it at risk of extinction, but frequent fires and the general absence of seedlings and saplings are mentioned as well (Núñez Vázquez, 2008, 2013a).

The known differences between *P. ceciliae* and *P. halepensis* are connected mainly with crown shape (e.g. López Gonzalez, 2001), but field observations have also showed that the leaf longevity on *P. ceciliae* was always less than two years, while on *P. halepensis* functional needles were observed in 2-year-old and even older dolichoblasts (Fig. 1). The detailed needle morphology and anatomy, however, have not been studied. Thus, the aim of this study was to compare the needle morphological and anatomical characteristics of the Cecilian pine with typical *P. halepensis*, using biometrical methods and statistical analyses. We expected to detect the needle characters, which could support the distinctiveness of Cecilian pine and support their taxonomic position as a variety.

Material and methods

Material collection

For the present study, we provisionally used the name *P. ceciliae*, and their taxonomic status was not presumed. The study area included two localities of this taxon, one from Mallorca and one from Menorca (Table 1). The sampling was conducted on 20 and 21 September 2004 in degraded *P. halepensis* forest communities on the limestone rock or on the substratum developed from limestone. The Cecilian pine did not form compact stands. Their individuals were dispersed in the forest of typical Aleppo pine.

Five two-year-old dwarf shoots were gathered from the sunny side of a crown of five trees in Mallorca and nine in Menorca. Each tree was represented by five needles, each needle taken from different dwarf shoots. *P. ceciliae* was characterised by data from 70 needles. For comparison, two populations of typical forms of *P. halepensis* were sampled from the same places. Each sample consisted of 150 needles taken from 30 individuals (5 needles per individual) (Table 1). The needle length was measured immediately after sampling. Subsequently, the needles were preserved in 70% alcohol and kept in a fridge until further preparations were made (Boratyńska & Bobowicz, 2000, 2001).

Analysed characteristics

The needle preparation methods, the set of characteristics analysed and measurement methods were adopted from investigations on Mediterranean pine and fir species (Boratyńska & Bobowicz, 2000, 2001; Bağcı & Babaç, 2003; Jasińska et al., 2014; Boratyńska et al., 2015). Finally, 12 needle characteristics were measured and/or evaluated, and an additional five resulted from recalculations (Table 2).

The number of stomata rows and stomata on a 2 mm long section from the central part of a needle (SRC, SRF, SNC and SNF in Table 2) were counted under a stereo microscope at magnification of 40×. Measurements of the anatomical characteristics (NW, NT, RC, WE, TE, TH and DVB in Table 2) were

Table 1. Plant material of *Pinus ceciliae* (C) and *P. halepensis* (H) from Mallorca (MA) and Menorca (ME)

Taxon	Code	Location	Latitude [°] N	Longitude [°] E	Altitude [m]	Number of specimens (needles)
<i>Pinus ceciliae</i>	CMA	Mallorca, N of Cala Pi	39.4106	2.8544	77	5(25)
	CME	Menorca, Sant Tomàs	39.9124	4.0457	15	1(5)
			39.9193	4.0386	59	1(5)
			39.9140	4.0518	76	5(25)
			39.9406	4.0418	126	2(10)
<i>Pinus halepensis</i>	HMA	Mallorca, N of Cala Pi	39.4106	2.8544	77	30(150)
	HME	Menorca, Sant Tomàs	39.9140	4.0518	76	30(150)

Table 2. Average values, standard deviation (SD) and homogenous groups (a, b, c) of analyzed needle characteristics of *Pinus ceciliae* populations (CMA and CME) and *P. halepensis* populations (HMA and HME) and significance of differences (*P*) between them as revealed by General Linear Model (GLM) Bonferroni test and the Kruskal-Wallis non-parametric test for the characters with a skewed distribution (SRF, RC and NS)

Character	Code	Average (SD) homogenous group				P values (only statistically significant)							
		CMA	CME	HMA	HME	CMA/ CME	CMA/ HMA	CMA/ HME	CME/ HMA	CME/ HME	HMA/ HME		
Needle length [mm]	NL	49.4 (±3.51) c	68.8 (±10.75) ab	65.8 (±12.71) a	79.2 (±11.87) b	0.026	0.032	0.000				0.000	
Number of stomata rows on convex (abaxial) side of needle	SRC	5.9 (±0.61) ab	5.6 (±0.42) a	6.0 (±0.61) ab	6.3 (±0.53) ab					0.032			
Number of stomata rows on flat (adaxial) side of needle	SRF	2.2 (±0.22) a	2.2 (±0.28) a	2.2 (±0.35) a	2.3 (±0.36) a								
Number of stomata on 2 mm long section of needle on convex side	SNC	16.7 (±0.55) b	15.3 (±0.79) a	14.4 (±0.87) a	15.0 (±0.86) a	0.027	0.000	0.000					
Number of stomata on 2 mm long section of needle on flat side	SNF	16.8 (±0.57) a	15.4 (±1.12) ab	14.9 (±1.14) b	15.9 (±1.00) a		0.004					0.004	
Width of needle [μ m]	NW	948 (±68.26) a	962 (±91.08) a	972 (±68.75) a	1050 (±71.12) b			0.028		0.018		0.000	
Thickness of needle [μ m]	NT	587 (±40.26) a	606 (±62.14) a	624 (±46.58) a	660 (±42.48) b			0.011		0.019		0.024	
Number of resin canals	RC	5.5 (±0.54) a	6.2 (±0.21) a	6.3 (±1.02) a	6.3 (±0.76) a								
Width of epidermal cells [μ m]	WE	16.6 (±1.85) a	17.2 (±1.26) a	17.9 (±1.13) a	18.2 (±1.42) a								
Thickness of epidermal cells [μ m]	TE	14.4 (±1.44) a	16.7 (±2.47) ab	16.1 (±1.31) ab	16.8 (±1.48) b			0.015					
Thickness of hypoderms cells [μ m]	TH	13.2 (±1.58) a	14.8 (±1.49) ab	15.4 (±1.44) bc	16.2 (±1.09) c			0.006		0.000		0.039	
Distances between the vascular bundles [μ m]	DVB	33.5 (±5.97) a	29.1 (±8.89) a	34.9 (±6.75) a	35.4 (±8.61) a								
Stomatal rows ratio (characters SRC/SRF)	SRR	2.8 (±0.25) a	2.7 (±0.31) a	2.8 (±0.38) a	2.8 (±0.29) a								
Stomatal number ratio (characters SNC/SNF)	SNR	1.0 (±0.06) ab	1.0 (±0.05) b	1.0 (±0.05) ab	0.9 (±0.04) a					0.038			
Marquet's coefficient (characters DVB×NW/NT)	MAR	54.1 (±9.47) a	46.4 (±14.47) a	54.4 (±10.26) a	56.5 (±14.34) a								
Needle thickness/width ratio (characters NT/NW)	NS	0.6 (±0.01) a	0.6 (±0.02) a	0.6 (±0.03) a	0.6 (±0.02) a								
Thickness/width of epidermal cells (characters TE/WE)	ES	0.9 (±0.06) a	1.0 (±0.09) a	0.9 (±0.07) a	0.9 (±0.07) a								

taken on the scaled photographs of cross sections from the central part of the needle. An optical microscope, Olympus BX 53-F with an Olympus DP27 camera and cellSens Standard software were used for the measurements.

Statistical analyses

The normality of the frequency distribution of every character and the homoscedasticity of the variances were tested using Shapiro–Wilk’s W-test and Brown–Forsythe’s test, respectively. The data were standardised before multivariate statistical analyses (Zar, 1999) to avoid the possible influence of different characteristic types.

To evaluate significant differences between *P. ceciliae* and *P. halepensis* from Mallorca and Menorca (four groups), the General Linear Model (GLM) Bonferoni test for characters with normal distribution and non-parametric Kruskal–Wallis test for characters with skewed distribution were used. A discrimination function analysis with a forward stepwise model (FSDA) was applied to identify the discrimination power of each character and eliminate possible redundant ones, as well as to describe the relationships between *P. ceciliae* and *P. halepensis* and to detect differences between particular individuals of both taxa (Sokal & Rohlf, 1995). Additionally, these relations were verified using Principal Component Analysis (PCA) and cluster analysis after the Ward method. The statistical analyses were carried out using STATISTICA v. 9 (StatSoft PL).

Results

Most of the characteristics had normal distributions, with only the three following traits exhibiting a skewed distribution: number of stomatal rows on the flat side of the needle, the number of resin canals and the needle thickness/width ratio (SRE, RC and NS, respectively). Some characters had non-homoscedastic variances, which caused the discrimination analyses results to be unreliable.

More than a half of the characters evaluated in this study differed among four sets of compared material. *Pinus ceciliae* differed significantly from *P. halepensis* in several needle characteristics, but the highest numbers of differences between the species were found in pairs from the same island (Table 2). On both islands, significant differences between compared taxa were found only in the thickness of hypodermis cells (TH), which was greater in *P. halepensis* than in *P. ceciliae* and did not differ between populations of the second species. On Mallorca, the number of stomata (SNC and SNF) and hypodermis thickness (TH), and to a lesser degree needle length

(NL), revealed significantly lower values ($P \leq 0.05$) in the needles of *P. ceciliae* than in *P. halepensis*. On Menorca, the number of stomatal rows on the convex needle side (SRC), needle width and thickness (NW, NT), thickness of hypodermis cells (TH) and stomatal number ratio (SNR) were all significantly different ($P \leq 0.05$) between *P. ceciliae* and *P. halepensis* (Table 2), in which larger values were observed for the characters in *P. halepensis*.

Interestingly, the needles of *P. ceciliae* from Mallorca (CMA) were shorter and had a greater number of stomata on the convex side than the needles from Menorca (CME) ($P \leq 0.05$). A larger number of characters differentiated the populations of *P. halepensis* from both islands, and generally values of the characters from Menorca were greater (Table 2).

The results also revealed small but significant differences between populations of *P. ceciliae* from Mallorca and Menorca, and relatively high between populations of *P. halepensis* from these islands (Table 2).

Of all of the characteristics evaluated the FSDA distinguished NL, SNC and TH as the distinguishing characteristics between *P. ceciliae* and *P. halepensis* with the highest power. SRE, SNF, NT, WE, TE, DVB, MAR and SRR were excluded from the analysis model. The first discriminant variable U_1 , responsible for about 63% of the total variation, was determined mostly by NL, TH, SNC, NW and SNR, while the second variable U_2 , which covered nearly 27% of the total variation, was influenced mostly by SNC, NS and NW. Individuals of *P. ceciliae* from Mallorca appeared to be different from populations of *P. halepensis* and *P. ceciliae* from Menorca (Fig. 2). *Pinus ceciliae* individuals were dispersed among individuals of *P. halepensis*. The classification matrix also confirmed

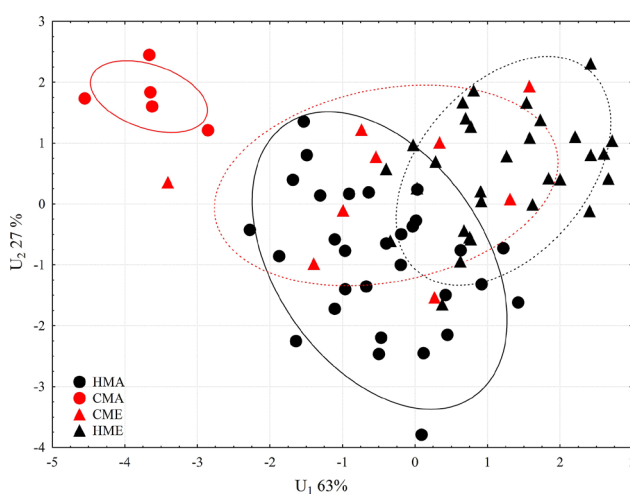


Fig. 2. Dispersion of *P. ceciliae* samples from Mallorca (CMA) and Menorca (CME) and *P. halepensis* samples from Mallorca (HMA) and Menorca (HME) (with 95% confidence intervals for both compared taxa/islands) in 2-D space between two first discriminant variables responsible for nearly 90% of variation

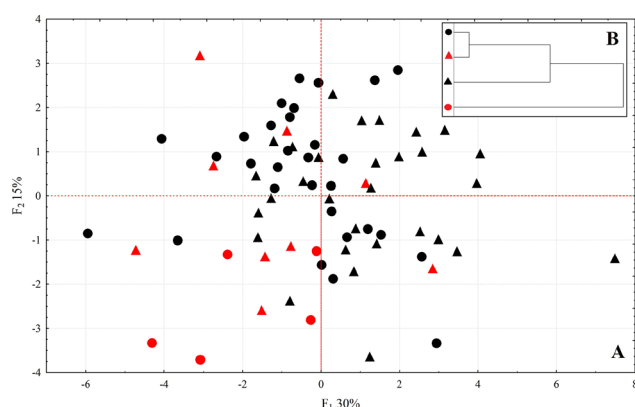


Fig. 3. Dispersion of individuals of *Pinus ceciliae* and *P. halepensis* in the space between two first principal components of PCA (A) and clustering of populations of *P. ceciliae* and *P. halepensis* after the Ward method (B)

a close connection of the Menorcan population of *P. ceciliae* to *P. halepensis*. Surprisingly, the population of *P. ceciliae* from Menorca and *P. halepensis* from Mallorca appeared to be the closest with a relatively small, but still significant square Mahalanobis' distance value of 3.8 ($P = 0.013$). The remaining square of Mahalanobis' distance combinations between populations were significantly large, with the greatest values between *P. ceciliae* from Mallorca and *P. halepensis* from Mallorca and Menorca, (17.5 and 25.5, respectively).

The dispersion of individuals between the first two principal components and clustering of populations showed similar results (Fig. 3A, B). Both analyses confirmed distinctiveness of *P. ceciliae* from Mallorca, while all individuals (except one) representing this taxon from Menorca, intermingled with individuals of typical *P. halepensis*, placed at the margin of this species variation space (Fig. 3A).

Discussion

The *Pinus ceciliae* description was based on a limited number of specimens from Mallorca. It was described as a tree with a compact crown, needles shorter than that of typical *P. halepensis* and smaller cones with protuberant umbo on cone scales (Llorens & Llorens, 1972; Llorens, 1979; Núñez Vázquez, 2007, 2013a,b). Our data confirmed *P. ceciliae* individuals from Mallorca have a significantly smaller needle length (NL), but not individuals from Menorca, which have shorter (insignificantly) needles compared to the local Menorcan population of *P. halepensis*. At the same time, the needles from Menorcan individuals of *P. ceciliae* are longer (insignificantly) than needles of Mallorcan *P. halepensis*. This finding could indicate that needles of the *P. ceciliae* are shorter than *P. halepensis* needles, only on the same island.

The needle length of *P. halepensis* needles varies from about 60 to 120 mm (Coode & Cullen, 1965; Werker & Fahn, 1969; Meikle, 1977; Boulli et al., 2001; García Esteban et al., 2010), sometimes even up to 150 mm (Amaral Franco, 1986). In that context, needles from Mallorca appeared to be relatively short, while those from Menorca were also small, but closer to reported values (Table 2). In comparison with the data from the whole geographic range of *P. halepensis*, the needles of *P. ceciliae* are shorter on Mallorca, while on Menorca, the values resembled values reported from northern Morocco (Boulli et al., 2001, Table 2).

The needle length positively correlated with the altitude of the Atlas Mountains of Morocco (Boulli et al., 2001). This implicates a possible influence of environmental conditions, such as high temperatures, drought and low level of precipitation during vegetation period in the lowest locations. In our study, the specimens from both *P. ceciliae* and *P. halepensis* came from low locations, but the conditions on the Mallorca could have worsened as a result of human influence, as the island is highly urbanised.

Regarding other characteristics, the needle width (NW) for *P. halepensis* has been reported to vary from 0.7 to 1.0 mm (Amaral Franco, 1986). Our data (Table 2), which were based on needle cross-section measurements, revealed widths that were generally around 1 mm and indicated slightly smaller values for *P. ceciliae*, but significant differences were found between two species only on the Menorca island. The same trend was observed for the needle thickness (NT).

The number of resin canals on the cross-section of the *P. ceciliae* needle varied between 4 and 8, with an average of 5.8. The mean value for the number of resin canals was approximately 6.3 (2–10) in *P. halepensis*. We found no difference in the number of resin canals between *P. ceciliae* and *P. halepensis*. The number of resin canals observed in our study is similar to that reported for *P. halepensis* from the central and western Mediterranean region (Vidaković, 1953; Werker & Fahn, 1969; Kivimäenpää et al., 2010).

The thickness of hypodermis (TH) was significantly lower in the *P. ceciliae* needles compared to *P. halepensis* needles; however, this character also appeared to be useful for distinguishing between species when comparing material from the same island (Table 2). Such data have not been analysed until now; however, in *P. halepensis*, the hypodermis is composed of two or even three cell-layers (Vidaković, 1953, 1991). Vidaković (1953, 1991) found a relationship between the number of hypodermis layers and the number of resin canals in two types of *P. halepensis* on the Balkan Peninsula. The first type had needles with 4–8 resin canals and 1–2 layers of hypodermis cells, while the second type had a smaller number of resin

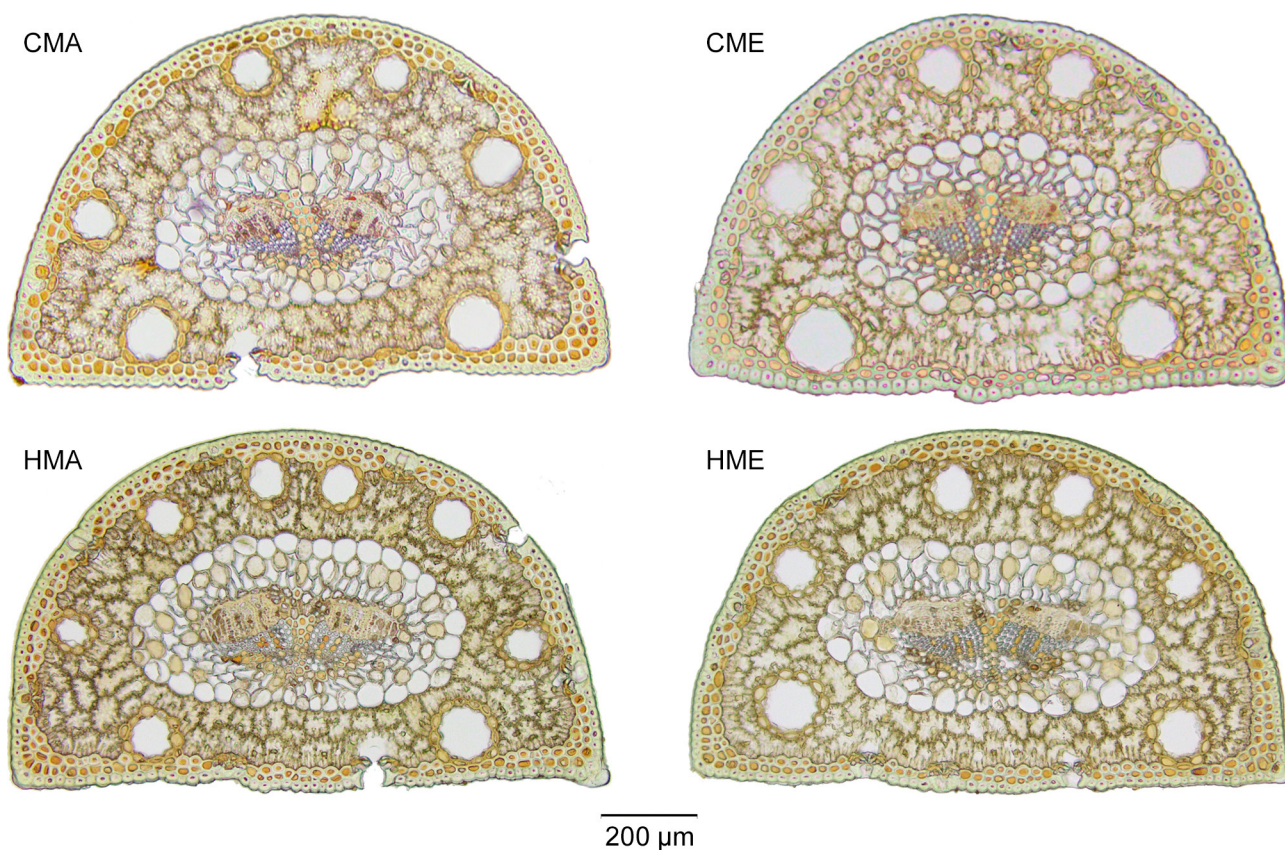


Fig. 4. Cross-sections of needles of *Pinus ceciliae* and *P. halepensis*; acronyms as in Table 1 (photo K. Boratyńska)

canals (usually less than 4) and 2–4 hypodermis layers. Our data showed 1–2 layers of hypodermis cells, with some additional hypodermal cells in the corners of the needle cross-section in both *P. ceciliae* and *P. halepensis* (Fig. 4).

The number of stomata rows appeared to be significantly lower, only on the convex side (SRC) of the needles in *P. ceciliae* from Menorca than in the needles of *P. halepensis*, while in the Mallorcan populations of both taxa, the number of stomata rows was nearly the same (Table 2) – indicating an unstable nature of this characteristic. The number of stomata rows in the needles of *P. halepensis* had not been examined until now. However, the values observed in our study correspond, to some degree, with the number of stomata detected on the edges of the needle cross-section of *P. halepensis* from Mallorca (6) and Menorca (6.7) (Kivimäenpää et al., 2010).

The differences between *P. halepensis* and *P. ceciliae* were also found in the cone characteristics. *Pinus ceciliae* cones have scales with highly visible, procumbent apophyses, while the apophyses of *P. halepensis* cone scales are generally flat (Laguna Lumbreras, 2000). However, the cone of *P. halepensis* could have also scales with more procumbent, ‘raised’ apophyses (Vidaković, 1953, 1991; Ayari et al., 2014). We only had a few *P. ceciliae* cones, which made it impossible to perform a biometrical study. Despite this fact, it

should be noted that the *P. ceciliae* cones had procumbent apophyses on their scales, while the apophyses of *P. halepensis* were flat.

In summary, significant differences were observed in the tree crowns of *P. ceciliae* and typical *P. halepensis*, but there were no substantial differences observed in the needles. *Pinus ceciliae* needles were smaller (NL, NW, NT), but only when compared with the samples from the same island. Additionally, in general, no significant differences were observed in the number of resin canals, stomata and stomatal rows. Interestingly, the proportions of the needle dimensions pattern are similar in both taxa. However, the study was performed on a relatively limited number of samples of *P. ceciliae*, and for that reason the presented results should be treated with some caution. Despite this limitation, in our opinion, Cecilia pine taxonomic rank is a variety, *P. halepensis* Mill. var. *ceciliae* (Llorens & Llorens) Llorens ex O. Bolòs & Vigo, Fl. Països Catalans, 1: 197 (1984), as proposed lately by Núñez Vázquez (2013a). The infraspecific position is proved by relatively low level of differences between this taxon and typical *P. halepensis*, local geographic distribution and probable origin from long lasting selection (Llorens, 1979).

Pinus halepensis var. *ceciliae* is an important element of *P. halepensis* variation and shall be conserved. Failure to recognition of the infraspecific taxa without

critical evaluation using modern methods could lead to its' disappearance, and consequently, to loss of part of Mediterranean biodiversity (e.g. Hamilton & Reichard, 1992; Dagnino et al., 2017). In this context, the conservation status of *P. halepensis* var *ceciliae* 'taxon of special interest' (CAB, 1992) is justified. Establishing of plantations to preserve the individuals representing this variety would be one of possible active protection.

Nomenclatural note

Pinus halepensis Mill. var. *ceciliae* (Llorens & L. Llorens) L. Llorens ex O. Bolòs & Vigo, Fl. Països Catalans, 1: 197 (1984)

Homotypic Names:

- *Pinus ceciliae* Llorens & L.Llorens, Folia Bot. Misc. 4: 55 (1984), *basion*.
- *Pinus halepensis* var. *ceciliae* (Llorens & L.Llorens) L.Llorens ex Rosselló, Cubas & N.Torres, Candollea 47: 67 (1992), *comb. superfl.*
- *Pinus halepensis* Mill. var. *ceciliae* (Llorens & L. Llorens) L. Llorens, Mediterranea, 3: 116 (1979), *comb. inval.*
- *Pinus halepensis* subsp. *ceciliae* (Llorens & L.Llorens) Silba, J. Int. Conifer Preserv. Soc. 16: 22 (2009).

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