

Wood anatomical relationships within *Abies* spp. from the Mediterranean area: a phyletic approach

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Abstract

An analysis was made of the wood anatomy of seven species, one subspecies and two varieties of the genus *Abies* from the Mediterranean area (*A. alba*, *A. borisii-regis*, *A. cephalonica*, *A. cilicica*, *A. nordmanniana*, *A. nordmanniana* subsp. *equi-trojani*, *A. numidica*, *A. pinsapo*, *A. pinsapo* var. *marocana* and *A. pinsapo* var. *tazaotana*) in order to find phyletically relevant features to help clarify and complement the evolutionary patterns based on molecular studies. The wood structure within the genus was qualitatively similar, except for specific features characteristic of certain provenances. However, the wood biometry allowed the different taxa to be grouped in accordance with their anatomical similarity. *A. alba*, *A. pinsapo*, *A. pinsapo* var. *marocana* and *A. pinsapo* var. *tazaotana* possess biometric features which distinguish them from the other Mediterranean firs. Furthermore, *A. numidica* showed biometric features which distinguish it from the eastern firs (*A. borisii-regis*, *A. cephalonica*, *A. cilicica*, *A. nordmanniana* and *A. nordmanniana* subsp. *equi-trojani*) and place it closer to certain Iberian populations of *A. pinsapo*. The maximum ray height in number of cells, frequency of rays with more than 30 cells and tracheid length can be regarded as specific patterns of the Mediterranean fir phylum.

Key words: Anatomy, biometry, evolution, Mediterranean firs, phylum.

Resumen

Anatomía de la madera de *Abies* spp. del área mediterránea: aproximación filética

En este trabajo se ha analizado la anatomía de la madera de siete especies, una subespecie y dos variedades del género *Abies* procedentes del área mediterránea (*A. alba*, *A. borisii-regis*, *A. cephalonica*, *A. cilicica*, *A. nordmanniana*, *A. nordmanniana* subsp. *equi-trojani*, *A. numidica*, *A. pinsapo*, *A. pinsapo* var. *marocana* y *A. pinsapo* var. *tazaotana*) con el fin de buscar caracteres filéticamente relevantes que ayuden a clarificar y complementar los patrones evolutivos basados en estudios moleculares. Las maderas estudiadas fueron cualitativamente similares, exceptuando ciertos caracteres propios de algunas procedencias. No obstante, la biometría de la madera permitió agrupar los diferentes taxones según su afinidad anatómica. *A. alba*, *A. pinsapo*, *A. pinsapo* var. *marocana* y *A. pinsapo* var. *tazaotana* mostraron caracteres biométricos afines que los distinguen del resto de abetos mediterráneos. Por otro lado, *A. numidica* mostró caracteres biométricos que lo distinguen de los abetos orientales (*A. borisii-regis*, *A. cephalonica*, *A. cilicica*, *A. nordmanniana* y *A. nordmanniana* subsp. *equi-trojani*) y lo asemejan a determinadas poblaciones ibéricas de *A. pinsapo*. La altura máxima de radios en número de células, la frecuencia de radios de más de 30 células y la longitud de traqueidas, pueden ser considerados como patrones específicos del phylum de los abetos mediterráneos.

Palabras clave: Abetos mediterráneos, anatomía, biometría, evolución, phylum.

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Received: 14-05-09. Accepted: 17-06-09.

Introduction

The Mediterranean firs, consisting of eight species, one subspecies and two varieties (Farjon, 2001), form a particularly homogeneous clade. They can be divided into two clearly distinct groups (Fig. 1): one in the north, with protruding bracts, comprising *A. alba* Miller, *A. borisii-regis* Mattfeld, *A. cephalonica* Loudon, *A. nebrodensis* (Lojac.) Mattei, *A. nordmanniana* (Steven) Spach and *A. nordmanniana* (Steven) Spach subsp. *equi-trojani* (Asch. & Sint. ex Boiss.) Coode & Cullen; and another in the south, with included bracts, distributed as far as the Caucasus, comprising *A. pinsapo* Boissier, *A. pinsapo* var. *marocana* (Trabut) Ceballos & Bolaños, *A. pinsapo* var. *tazaotana* (Cózar ex Huguet del Villar) Pourtet, *A. numidica* de Lannoy ex Carrière and *A. cilicica* (Antoine & Kotschy) Carrière (Flous, 1936). Chloroplast DNA variation showed that the Mediterranean firs form a clade with closely related species which are very different from the Asian and North American species (Xiang et al., 2002; Kormutak et al., 2004).

Various hypotheses have been put forward as to how the genus *Abies* evolved, on the basis of several features such as morphological and anatomical (Dallimore and Jackson, 1923; Mattfeld, 1930; Flous, 1936, 1937; Turill in Klæhn and Winieski, 1962), botanical (Pignatti, 1978; Quézel, 1978; Medus and Pons, 1980; Soto, 1998) and ecological (Arbez, 1969; Quézel et al., 1980; Mayer, 1981), in addition to pollen analysis (Aytug, 1959), phenolic composition (Granados and Rossell,

1966), terpene composition (Koedam, 1981; Fady, 1990), DNA analysis (Vicario et al., 1995; Parducci and Szmidt, 1999; Ziegenhagen et al., 2005), and allozyme analysis (Parducci et al., 2001). Fady et al. (1992) and Scaltsoyiannes et al. (1999) proposed a monophyletic evolutionary hypothesis for the genus *Abies* in the Mediterranean, according to which an ancient progenitor fir existed at the beginning of the Miocene in the Balkan Peninsula, in the Aegean, between the Ionian Sea and Turkey. During the speciation period of *Abies* spp. an adaptive projection of the ancient progenitor took place, resulting in the formation of *A. alba* and *A. nordmanniana* to the north and *A. cephalonica* to the south in response to the wide range of ecological features, while the central area remained occupied by the ancient progenitor. At the end of the Pliocene and during the Pleistocene, species such as *A. cephalonica*, *A. alba*, *A. nordmanniana*, *A. cilicica* and *A. pinsapo* became differentiated (Scaltsoyiannes et al., 1999).

Although it has been demonstrated that wood anatomy constitutes an important source of phylogenetic information which should be taken into consideration in systematics studies (Lens et al., 2007), the establishment of evolutionary patterns based on the wood structure of conifers has been studied very little (Jeffrey, 1905; Chamberlain, 1935; Flous, 1936; Greguss, 1955; Carlquist, 1975). The lack of defined evolutionary patterns may be due, among other reasons, to the homogeneity of conifer wood, which is particularly accentuated in the genus *Abies*. In fact, together with

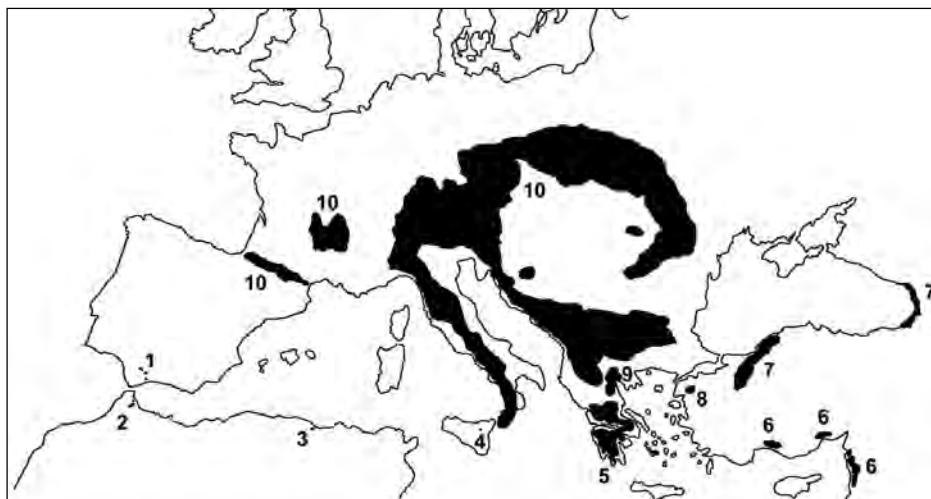


Figure 1. Distribution of the genus *Abies* in the Mediterranean. 1: *A. pinsapo*. 2: *A. pinsapo* var. *marocana* and *A. pinsapo* var. *tazaotana*. 3: *A. numidica*. 4: *A. nebrodensis*. 5: *A. cephalonica*. 6: *A. cilicica*. 7: *A. nordmanniana*. 8: *A. nordmanniana* subsp. *equi-trojani*. 9: *A. borisii-regis*. 10: *A. alba*.

Pseudolarix, the genus *Abies* shows the simplest structure of the Pinaceae (Greguss, 1955), although at the same time it includes the largest number of primitive features of all the Abietae (*Abies*, *Cedrus*, *Tsuga*, *Pseudolarix*) (Jeffrey, 1905).

The objective of this study was to define anatomical similarities and differences between the wood of the Mediterranean firs, in order to establish an evolutionary hypothesis related to the wood structure using the most accepted phylogenetic patterns.

Materials and methods

The samples of *A. alba* and *A. pinsapo* were collected in their natural forests: *A. alba* in the Martinier-La Mascarina forest in the municipality of Forcayo El Cornato, in the province of Huesca; *A. pinsapo* in the three natural areas of the species in the south of Spain (Sierra del Pinar de Grazalema, in the province of Cádiz and Sierra Bermeja and Sierra de las Nieves, in the province of Málaga); and *A. pinsapo* var. *marocana* and var. *tazatana* in Talassemtane National Park (Morocco), from mounts Magot and Tazaout, respectively.

Five trees more than 70 years old, representative of the forest, were felled in each zone. In the case of *A. pinsapo* the natural forests were located using the publications by Ceballos and Martín Bolaños (1930) for the province of Cádiz and by Ceballos and Vicioso (1933) for the province of Málaga. In the case of the Moroccan forests, the studies of Ceballos and Martín Bolaños (1928) and Sánchez Cózar (1946) were used.

The samples of *A. borisii-regis*, *A. cephalonica*, *A. cilicica*, *A. nordmanniana*, *A. nordmanniana* subsp. *equi-trojani* and *A. numidica* came from the wood collections of the following institutions: Centro de Investigación Forestal - Instituto Nacional de Investigaciones Agrarias (INIA) in Spain; Institut für Holzbiologie und Holzschutz - Bundesforschungsanstalt für Forst- und Holzwirtschaft (BFH) in Germany; Royal Botanic Gardens, Kew (Kew) in the United Kingdom; and Center for Wood Anatomy Research - Forest Products Laboratory (MADw) in the United States.

The wood samples were prepared for observation by means of light microscopy and scanning electron microscopy SEM mod. JEOL JSM-6380. The samples for light microscopy were prepared following the usual methods of softening, cutting and mounting, and were observed both without staining and after staining with safranin and Sudan 4 in order to make the resin red

(Jane, 1970). The anatomical descriptions were made in accordance with the IAWA Committee (2004).

The measurements of tracheid diameter, tracheid length, tracheid pit diameter, height and frequency of rays and number and size of cross-field pits were made on the light microscopy slides using the WinCell image analysis program. The tracheid length was measured following Ladell's light method (Ladell, 1959). In the case of the samples collected from their natural areas, the biometry was done on 5 microslides from each tree, in all cases on mature wood from a basal disc between rings 70 and 100. On each slide 35 random measurements were made. The number of pits per cross-field was measured on the five slides from each tree, on ten different rays. The height and frequency of the rays were measured on all the rays contained in one square millimeter in the tangential section.

For the samples from collections, 35 random measurements for each feature were also made on a single slide, following the same criteria.

The most frequent average ray height (n° cells and μm) was obtained by fitting the distribution of the ray height frequency to a normal distribution curve, with the maximum of this distribution constituting the most frequent value. For the analysis of the other variables measured, the Lilliefors normality test and Bartlett's homogeneity of variance test were applied ($\alpha = 0.05$). Because at least one of the conditions was not met in all cases, the Kruskal-Wallis test was used to compare the different *Abies* populations. The mean values were compared through LSD tests ($\alpha = 0.05$).

A principal component analysis (PCA) was performed in order to observe possible groupings among the different *Abies* taxa according to their wood biometry. This method extracts a set of uncorrelated variables as linear combinations of the original variables, reducing the dimensionality of data while preserving most of the variance. The new variables, called components, are arranged in order of decreasing variance and in this way those with the highest variance are termed PCs. All the parameters measured (Table 1), including the maximum ray height (n° cells), were used as input variables. The PCs were displayed graphically as scatter plots of the scores, in order to observe any groupings in the dataset. In a PCA, coefficients by which the original variables must be multiplied to obtain the PCs are called loadings. The numerical value of a loading for a given variable in a PC shows how much the variable has in common with the component (Massart et al., 1988). In this way, loading values were used to ascertain the main anatomical

Table 1. Biometry of *Abies* spp. in the Mediterranean

Southern group	<i>A. cilicica</i>		<i>A. numidica</i>	<i>A. pinsapo</i>			<i>A. pinsapo</i> var. <i>marocana</i>	<i>A. pinsapo</i> var. <i>tazaotana</i>
	INIA0008	BFH 14036	Kew 18393	Grazalema	Sierra Bermeja	Sierra de las Nieves	Talassetane	Tazaout
¹ Tracheid diameter (µm)	38.1 ± 6.6 (27.9-53.7)	39.3 ± 5.6 (28.0-54.1)	38.9 ± 4.1 (28.7-44.6)	46.9 ± 9.3 (23.4-76.0)	44.4 ± 9.8 (22.7-71.3)	44.9 ± 10.8 (25.3-73.4)	62.0 ± 6.3 (42.5-76.7)	51.4 ± 7.3 (30.1-67.2)
¹ Tracheid pit diameter (µm)	15.5±1.6 (11.8-18.9)	15.7±1.1 (13.7-18.0)	16.2±1.7 (12.0-19.5)	18.5 ± 1.8 (12.7-24.6)	20.2 ± 1.7 (16.0-25.3)	18.6 ± 2.7 (7.5-28.6)	21.2 ± 2.1 (10.5-26.0)	20.7 ± 2.6 (9.8-29.6)
¹ Tracheid length (µm)	3377±972 (1900-6166)	2905±395 (2200-3500)	2593 ± 452 (1833-3900)	2591 ± 793 (1335-4625)	3154 ± 861 (1500-7250)	2946 ± 934 (1420-6167)	3405 ± 1255 (1500-5670)	3518 ± 976 (1550-7500)
² Most frequent average ray height (n° cells)	9 (1-23)	9 (1-22)	9 (1-17)	8 (1-40)	4 (1-36)	5 (1-50)	7 (1-25)	7 (1-30)
³ Most frequent average ray height (µm)	169.1±10.3	157.1±6.8	165.5 ± 12.6	169.7 ± 24.5	95.3 ± 8.9	114.0 ± 12.2	134.0 ± 15.9	133.7 ± 12.4
¹ N° rays/mm ²	27.4 ± 1.5 (25-29)	22.2 ± 5.4 (18-31)	30.6 ± 3.4 (27-35)	24.4 ± 4.0 (18-32)	23.6 ± 2.9 (17-30)	23.8 ± 4.6 (13-32)	23.5 ± 3.2 (18-31)	22.9 ± 3.8 (16-31)
¹ Largest cross-field pit diameter (µm)	5.5±0.8 (3.7-7.5)	5.3±0.6 (3.6-6.7)	7.5±1.7 (4.7-10.7)	7.5 ± 1.2 (4.7-10.9)	7.1 ± 1.2 (3.6-11.3)	6.6 ± 1.1 (3.5-9.8)	7.2 ± 1.1 (1.0-11.1)	7.2 ± 1.1 (3.4-10.7)
¹ Smallest cross-field pit diameter (µm)	4.0±0.4 (2.9-4.8)	4.1±0.5 (3.0-5.2)	5.2±1.4 (2.1-8.5)	4.4 ± 1.2 (1.9-7.3)	4.2 ± 1.1 (1.3-7.5)	3.8 ± 0.9 (1.7-6.8)	2.6 ± 0.9 (1.1-5.2)	3.0 ± 0.9 (0.9-6.0)
¹ N° pits per cross-field	2.0 ± 0.8 (1-4)	1.8 ± 0.7 (1-3)	1.4 ± 0.5 (1-2)	1.6 ± 0.7 (1-4)	1.9 ± 0.6 (1-4)	1.9 ± 0.7 (1-4)	1.8 ± 0.6 (1-3)	1.9 ± 0.6 (1-4)
¹ N° rays/mm ² > 30 cells	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.025 ± 0.041	0.002 ± 0.009	0.021 ± 0.047	0.0 ± 0.0	0.004 ± 0.017

¹ $\bar{x} \pm \sigma$ (range) ² (range) ³ $\bar{x} \pm \sigma$

Table 1. Biometry of *Abies* spp. in the Mediterranean (continued)

Northern group	<i>A. alba</i>	<i>A. borisii-regis</i>	<i>A. cephalonica</i>	<i>A. nordmanniana</i>		<i>A. nordmanniana</i> subsp. <i>equi-trojani</i>
	Pyrenees	MADw 17846	MADw 17845	MADw 10536	BFH 14031	BFH 14034
¹ Tracheid diameter (µm)	50.4 ± 7.6 (27.3-74.6)	26.6 ± 3.4 (19.1-33.3)	35.3 ± 9.0 (18.5-56.7)	36.7 ± 7.6 (24.0-54.9)	36.8 ± 6.1 (25.6-47.2)	38.0 ± 6.6 (26.0-50.5)
¹ Tracheid pit diameter (µm)	22.1±2.3 (17.7-28.6)	19.5±1.0 (17.0-21.7)	17.1±1.4 (13.3-20.7)	16.4±1.2 (14.1-19.3)	18.0±1.1 (15.5-20.3)	17.5±1.4 (13.9-20.0)
¹ Tracheid length (µm)	2813 ± 721 (1536-4750)	3820 ± 1336 (2056-7500)	2798±654 (1682-4625)	3064±539 (2056-4100)	3032±744 (1654-4750)	3474±660 (2278-4875)
² Most frequent average ray height (n° cells)	5 (1-48)	8 (1-26)	7 (1-26)	8 (1-18)	8 (1-18)	6 (1-14)
³ Most frequent average ray height (µm)	158.9 ± 14.1	131.3±7.8	127.5±1.7	140.1±10.1	142.6±6.3	107.7±7.7
¹ N° rays/mm ²	30.6 ± 5.2 (18-42)	22.5 ± 2.1 (20-26)	21.8 ± 3.0 (18-26)	29.2 ± 4.0 (23-34)	24.2 ± 2.4 (21-27)	27.2 ± 2.4 (24-30)
¹ Largest cross-field pit diameter (µm)	7.1±1.0 (4.1-9.6)	5.8±0.7 (3.9-7.1)	5.4±0.7 (3.9-7.0)	4.8±0.6 (3.6-6.3)	5.8±0.7 (4.3-7.2)	5.3±0.6 (3.8-6.6)
¹ Smallest cross-field pit diameter (µm)	3.3±1.4 (1.1-6.8)	4.8±0.7 (3.3-6.3)	4.1±0.5 (3.1-5.3)	3.4±0.5 (2.5-4.6)	4.3±0.5 (3.3-5.3)	4.4±0.5 (2.8-5.4)
¹ N° pits per cross-field	1.8 ± 0.6 (1-4)	1.8 ± 0.9 (1-4)	1.7 ± 0.7 (1-3)	1.9 ± 0.7 (1-3)	1.6 ± 1.6 (1-3)	2.3 ± 0.9 (1-4)
¹ N° rays/mm ² > 30 cells	0.040 ± 0.054	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

¹ $\bar{x} \pm \sigma$ (range) ² (range) ³ $\bar{x} \pm \sigma$

variables related to the localization of the samples with respect to the PCs in the scatter plot of the scores.

The statistical calculations were done with the MATLAB V.6.5 Release 13 program. The ray height frequency distribution curves were fitted using Curve Fitting Toolbox Version 1.1 (R13) from the same program, for an interval of confidence of 95%.

Results

Anatomical description

The wood anatomy of the different taxa studied was in general qualitatively similar. Therefore, the following description refers to all the Mediterranean firs except in

the case of particular anatomical features observed in certain provenances.

Cross section. The sapwood and heartwood are indistinct. The growth ring boundaries are distinct (Fig. 2a). In some species the fresh wood is fetid, e.g. *A. pinsapo*. Tracheids are rectangular or hexagonal in outline in earlywood and rectangular in latewood. Tracheid pitting in tangential wall, generally in tracheids near the growth ring boundary (Fig. 2b). Axial parenchyma present but sparse, generally in marginal position, in isolated cells with high cellular content (Fig. 2c). Resin canals absent. Occasional presence of traumatic resin canals (Fig. 2d.1), surrounded by subsidiary cells with high cellular content (Fig. 2d.2) and crystals. Organic deposits in axial tracheids adjacent to rays in the Grazalema provenance of *A. pinsapo* (Fig. 2e). Tori

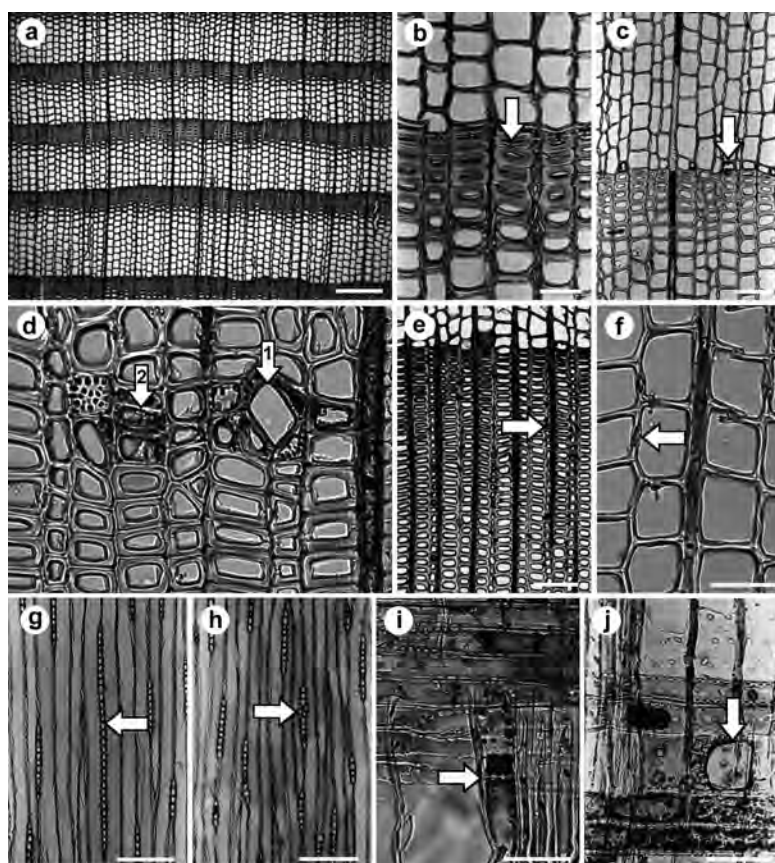


Figure 2. (a) Growth rings distinct and abrupt transition (*A. nordmanniana* subsp. *equi-trojani*) (Scale bar 250 μ m); (b) tracheid pitting in tangential walls of tracheids (*A. nordmanniana* subsp. *equi-trojani*) (Scale bar 50 μ m); (c) axial parenchyma marginal and scarce (*A. pinsapo*) (Scale bar 100 μ m); (d.1) traumatic resin canals; (d.2) subsidiary cells of traumatic resin canals, with high cell content (*A. pinsapo*) (Scale bar 50 μ m); (e) tracheids with organic deposits (*A. pinsapo*) (Scale bar 150 μ m); (f) tori well defined (*A. nordmanniana*) (Scale bar 50 μ m); (g) ray height (n° cells) high (*A. alba*) (Scale bar 150 μ m); (h) partially biseriata rays (*A. numidica*) (Scale bar 150 μ m); (i) axial parenchyma with nodular transverse end walls (*A. borisii-regis*) (Scale bar 50 μ m); (j) degenerate cells in rays (*A. pinsapo*) (Scale bar 50 μ m).

well defined (Fig. 2f), not scalloped, but extensions present.

Tangential section. Maximum ray height (n° cells) high in the western-most firs, particularly in *A. alba* and *A. pinsapo* (Fig. 2g). Rays almost exclusively uniseriate, as partially biseriate rays constitute less than 10% of the total (Fig. 2h).

Radial section. Transverse end walls of axial parenchyma cells nodular (Fig. 2i). Presence of degenerate cells in marginal rows of rays (Fig. 2j) in *A. pinsapo* from Grazalema and Sierra de las Nieves. Tracheid pitting in radial walls of earlywood predominantly biseriate, in opposite arrangement (Fig. 3a). Warty layer present (Fig. 3b). Horizontal walls of ray parenchyma cells distinctly pitted (Fig. 3c) and end walls nodular (Fig. 3d.1). Cross-field pitting taxodioid (Fig. 3d.2). Indentures present. (Fig. 3e) Prismatic calcium oxalate crystalline inclusions in ray parenchyma cells, located in both marginal and interior rows of rays (Fig. 3f).

Biometric study

The biometric characterization of the different fir groups is shown in Table 1.

For all the variables analyzed with the Kruskal-Wallis test, the provenance factor was significant ($P < 0.01$). For the tracheid diameter, the Spanish and Moroccan pinsapo firs and *A. alba* showed values significantly higher than the other Mediterranean firs ($P < 0.05$). Similarly, the Moroccan pinsapo firs showed larger tracheid diameters than their Spanish counterparts ($P < 0.05$). In terms of the tracheid pit diameter, *A. pinsapo* from Sierra Bermeja, the Moroccan firs, *A. alba* and *A. borisii-regis* form a distinct group from the others, with significantly higher values ($P < 0.05$) than *A. numidica*, the Grazalema and Sierra de las Nieves pinsapo firs, *A. nordmanniana*, *A. nordmanniana* subsp. *equi-trojani*, *A. cephalonica* and *A. cilicica*. In relation to the tracheid length, *A. numidica* showed significantly lower values ($P < 0.05$) than the

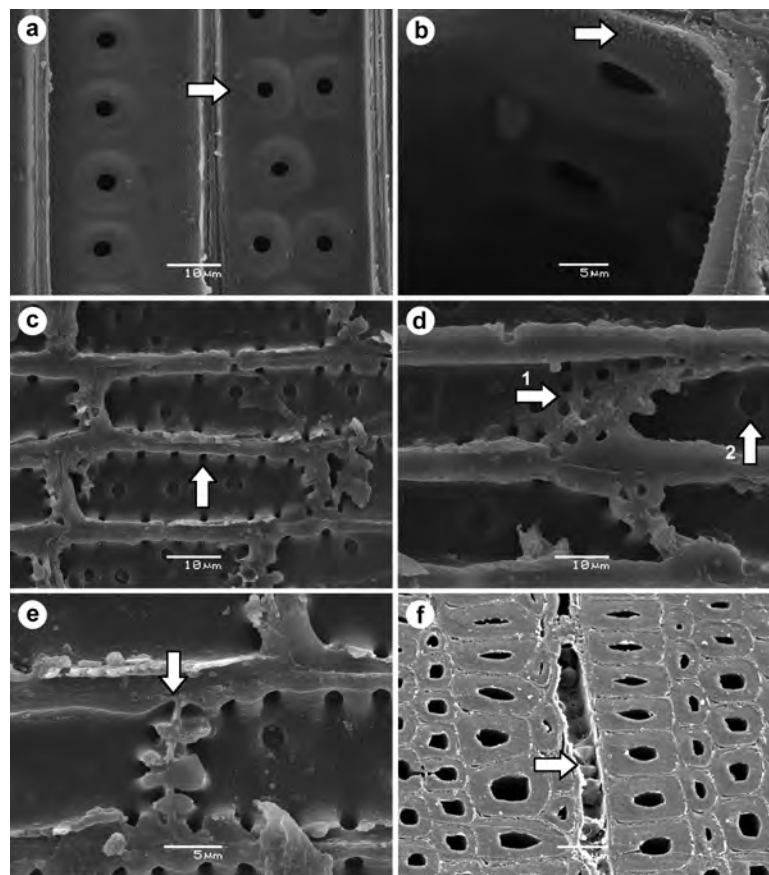


Figure 3. (a) Biseriate tracheid pitting in opposite arrangement (*A. cilicica*); (b) warty layer (*A. cephalonica*); (c) horizontal walls of ray parenchyma cells distinctly pitted (*A. borisii-regis*); (d.1) end walls of ray parenchyma cells nodular; (d.2) taxodioid pitting (*A. pinsapo* var. *marocana*); (e) indenture (*A. pinsapo* var. *tazaotana*); (f) crystals in ray parenchyma cells (*A. borisii-regis*).

other provenances, with the exception of the Grazalema provenance of *A. pinsapo*, and the Grazalema and Sierra de las Nieves pinsapo firs showed shorter tracheids than the Moroccan firs ($P < 0.05$), while the Sierra Bermeja firs occupy an intermediate position between the two groups. The most frequent average ray height (n° cells) showed maximum values in *A. cilicica* and *A. numidica*. There is a very obvious difference between the most frequent ray height in the Grazalema provenance of *A. pinsapo* (8 cells) and the Sierra Bermeja and Sierra de las Nieves pinsapo firs (4 and 5 cells, respectively). In the case of the maximum ray height (n° cells), two different groups of *A. pinsapo* can be distinguished: on the one hand, the Grazalema and Sierra de las Nieves pinsapo firs, with 40 and 50 cells respectively, and on the other, the Sierra Bermeja pinsapo firs, with 36 cells, and the two Moroccan varieties, with 25 in var. *marocana* and 30 in var. *tazaotana*. These values place the Grazalema and Sierra de las Nieves wood closer to the biometry of the rays of *A. alba* (48 cells) than to that of the Moroccan firs. With regard to the most frequent average ray height (μm), the high values observed in *A. cilicica*, *A. numidica*, *A. pinsapo* from Grazalema and *A. alba* are notable in comparison with the other populations. The number of rays per mm^2 in *A. numidica* and *A. alba* was significantly higher ($P < 0.05$) than the values for the Spanish and Moroccan *A. pinsapo* and for *A. borisii-regis* and *A. cephalonica*. In terms of the largest cross-field pit diameter, the Spanish and Moroccan *A. pinsapo*, in addition to *A. alba* and *A. numidica*, showed higher values ($P < 0.05$) than the other populations studied. The smallest cross-field pit diameter in *A. numidica* was larger than in the other taxa ($P < 0.05$), except for *A. borisii-regis*, while in the case of the Moroccan varieties of *A. pinsapo* it was smaller than in the other taxa ($P < 0.05$), with the exception of *A. nordmanniana*. As regards the number of pits per cross-field, the Grazalema *A. pinsapo* showed significantly lower values than the other Spanish *A. pinsapo* and the Moroccan varieties ($P < 0.05$), but similar values to *A. numidica*. Lastly, all the eastern firs, including *A. alba*, with the exception of *A. pinsapo* var. *marocana*, had rays with more than 30 cells.

Principal component analysis

The PCA of the anatomical variables was used to obtain the scatter plot of the scores from the first two PCs (Fig. 4). PC1 groups the Iberian Peninsula firs, including *A. alba*, in the positive semi-axis with the

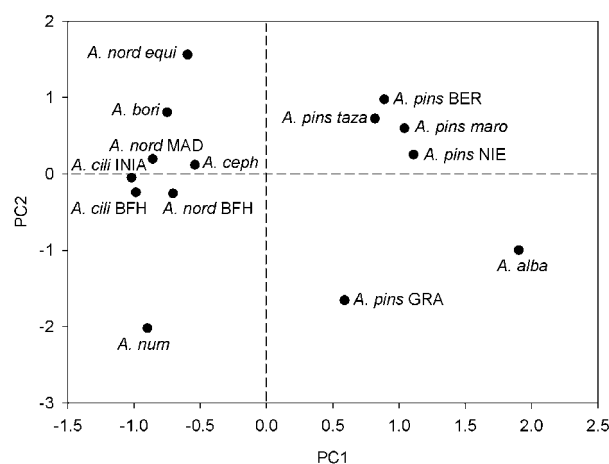


Figure 4. Scatter plot of principal component analysis scores of wood parameters from different Mediterranean *Abies* taxa. Abbreviations: *A. bori* = *A. borisii-regis*; *A. ceph* = *A. cephalonica*; *A. cili INIA* = *A. cilicica* from INIA collection; *A. cili BFH* = *A. cilicica* from BFH collection; *A. nord BFH* = *A. nordmanniana* from BFH collection; *A. nord MAD* = *A. nordmanniana* from MAD collection; *A. nord equi* = *A. nordmanniana* subsp. *equi-trojani*; *A. num* = *A. numidica*; *A. pins GRA* = *A. pinsapo* from Grazalema; *A. pins BER* = *A. pinsapo* from Sierra Bermeja; *A. pins NIE* = *A. pinsapo* from Sierra de las Nieves; *A. pins maro* = *A. pinsapo* var. *marocana*; *A. pins taza* = *A. pinsapo* var. *tazaotana*.

Moroccan firs, and groups the other Mediterranean firs in the negative semi-axis. PC2 separates *A. numidica* and the Grazalema provenance of *A. pinsapo*, which have the most negative scores, from the other Mediterranean firs, placing *A. alba* in an intermediate position between the two groups.

The loadings of PC1 and PC2 are shown in Table 2. The parameters selected because of their high influence in PC1 according to their loadings (absolute value >

Table 2. Loadings of the factors with the greatest influence in the principal component analysis

PC	Variance (%)	Variable	Factor loading
1	36.2	Tracheid pit diameter	0.86
		Maximum ray height (n° cells)	0.82
		Tracheid diameter	0.77
		Most frequent average ray height (n° cells)	-0.74
		N° rays/ $\text{mm}^2 > 30$ cells	0.72
		2	25.3
Tracheid length	0.80		
N° pits per cross-field	0.79		

0.70) were: tracheid pit diameter, maximum ray height (n° cells), tracheid diameter, most frequent average ray height (n° cells) and number of rays per mm² with more than 30 cells. The parameters selected because of their high influence in PC2 according to their loadings (absolute value > 0.70) were: most frequent average ray height (µm), tracheid length and number of pits per cross-field.

Discussion

This study shows that the biometry of *Abies* wood from the Mediterranean Arc allows the different taxa to be grouped and distinguished according to their anatomical similarity. The grouping observed in the positive semi-axis of PC1 between the Spanish and Moroccan pinsapo firs (Fig. 4) coincides with the molecular similarity seen between these taxa in earlier studies with allozyme markers (Scaltsoyiannes et al., 1999) and chloroplast DNA markers (Parducci and Szmidt, 1999). The position of *A. alba* in relation to the PC1 axis, grouped with the Spanish and Moroccan provenances of *A. pinsapo*, coincides with the clade observed using allozyme markers (Scaltsoyiannes et al., 1999), but not with the results observed using DNA markers, in which *A. alba* is placed closer to the eastern firs (Parducci and Szmidt, 1999; Ziegenhagen et al., 2005). However, in *A. alba* high intraspecific variability has been observed, with unique haplotypes which distinguish it from the other eastern firs (Vendramin et al., 1999; Parducci et al., 2001; Ziegenhagen et al., 2005). The distribution of *A. alba* in Europe is very wide, encompassing the principle mountain areas of central and southern Europe (Fig. 1). The current geographical distribution of forest species in Europe is a result of post-glacial recolonization, which began around 13,000 ago years from the glacier refuges in the south of Europe (Huntley, 1990). The Pyrenees were likely to have been a glacier refuge of *A. alba*. After the glacial period, the populations in this area were isolated from the others, as suggested by the major genetic differences with neighboring populations (Vendramin et al., 1999). In view of this, phyletic studies which relate *A. alba* to other species of the genus should take into account the provenance of the population studied. Given the anatomical proximity observed between *A. alba* from the Pyrenees and *A. pinsapo*, particularly the Grazalema population, it would be interesting to study whether common genetic factors exist between them. For exam-

ple, a common haplotype has been found to exist between *A. alba* and *A. numidica* (Parducci et al., 2001), despite the fact that they belong to two different subsections, suggesting contact between the two species in the past.

The species *A. nordmanniana*, *A. cephalonica* and *A. cilicica* appear very close anatomically on the scatter plot of the PCA scores (Fig. 4), corresponding to their genetic proximity observed using chloroplast DNA markers (Parducci and Szmidt, 1999). Near these species is *A. borisii-regis*, a taxon regarded as a natural hybrid between *A. alba* and *A. cephalonica* (Scaltsoyiannes et al., 1999). The genetic isolation of the Pyrenees populations of *A. alba* could explain why *A. borisii-regis* is positioned closer to *A. cephalonica* than to *A. alba*. *A. numidica* is far from this group according to PC2, in which it is located near the Grazalema provenance of *A. pinsapo*. Chloroplast DNA markers (Parducci and Szmidt, 1999) and mitochondrial markers (Ziegenhagen et al., 2005) place *A. numidica* genetically close to *A. pinsapo*, as they share haplotypes. It is possible that the Grazalema population, which in PC2 is positioned far from the other *A. pinsapo* provenances, has a closer phyletic relation to *A. numidica* than the other populations of *A. pinsapo* and its Moroccan varieties do. The closeness between the two groups is seen in the similar values for most frequent average ray height, tracheid length and largest cross-field pit diameter.

The finding of *Abies* fossils in different parts of the Iberian Peninsula has enabled some hypotheses on the phylogeny of *Abies* in Western Europe to be validated, in particular that established between the Pyrenees and the pinsapo fir enclaves. The fact that some of the fossil findings are similar to the firs now existing in the same region implies a certain degree of stability in the past and present geographical distribution of the genus in the Mediterranean since the Miocene (Viguié and Gausson, 1928). Liu (1971) and Parducci (2000) proposed that the arrival of *Abies* in southern Spain and north Africa occurred through the Pyrenees. The route followed the Thetic bridges from the southeast of France → Majorca → Granada → Sierra de las Nieves → Sierra de Grazalema → Sierra Bermeja, reaching as far as Morocco, to the mountainous regions of Tazaout and Talassemrane, and the Babor mountains in Algeria (Fig. 5). On allowing this route and the direction of the southern phylum as that proposed by Dubois-Ladurantie (1941), which establishes from most primitive to most evolved as *A. pinsapo* → *A. pinsapo* var. *marocana* → *A. pinsapo* var.

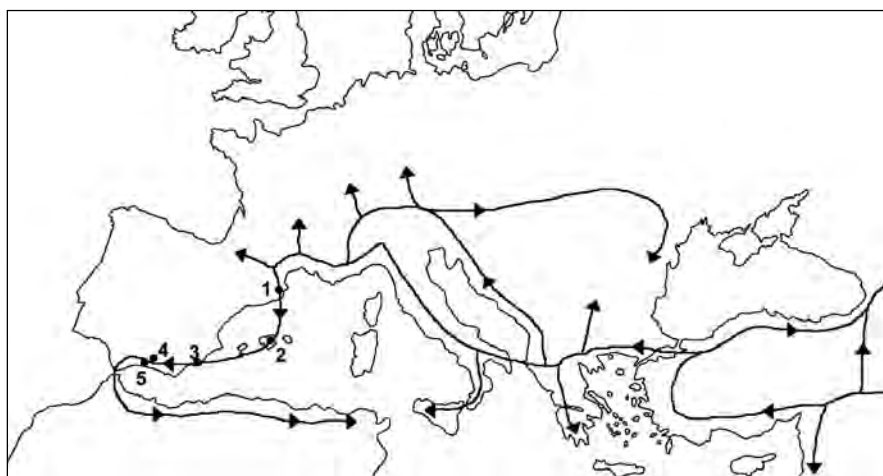


Figure 5. Migratory routes of *Abies* in the Mediterranean (Liu, 1971; Parducci, 2000) modified for the route in the Iberian Peninsula in accordance with fossil findings. 1: *A. saportana* Rerolle. Santa Eugenia, Coll del Saig, Cerdaña (Lérida). Late Miocene (Dubois-Ladurantie, 1941); 2: *Abies ramesi*. Majorca Island. Early Miocene (Depape, 1928); 3: *Abietites latisquamosus* Ludw. Shrine of Saint Eulalia, Totana (Murcia). Tertiary (Areitio, 1874); 4: *A. pinsapo*. Las Ventanas Cave (Granada). Late Quaternary (Carrión et al., 2001); 5: *Abies* spp. Padul (Granada). Late Pleistocene (Menéndez and Florschütz, 1964).

tazaotana → *A. numidica* → *A. cilicica*, it can be accepted that the speciation of *Abies* occurred after fragmentation of its territory. The fragmentation of the area of *A. pinsapo* into isolated populations must have caused reproductive isolation between them due to the already limited dispersal of pinsapo fir pollen (Arista and Talavera, 1994), and this situation may have been the start of the speciation of these populations and their subsequent morphological (Arista and Talavera, 1995) and genetic differentiation (Pascual et al., 1993). This in turn gave rise in the different species to a specific wood structure related to their antiquity rather than geographical proximity, as suggested by the fact that *A. pinsapo* var. *tazaotana*, although geographically much closer to *A. pinsapo* var. *marocana*, is nonetheless botanically closer to *A. numidica*. (Esteban et al., 2007).

Although there is no obvious relation between phyletic status and tracheid length, with the exception of Araucariaceae in comparison with the other conifer genera (Carlquist, 1975), tracheid length could be used as a phyletic pattern within the group of western firs. The fact that the tracheids are longer in the Moroccan firs (Table 1) than in the Spanish pinsapo firs was expected, as there is a correlation between plant size and tracheid length (Carlquist, 1975), and the Moroccan firs are larger. Only the pinsapo firs from Sierra Bermeja, although not located in their ecological optimum, are close to the Moroccan firs in terms of tracheid length. Thus it appears that tracheid length could be used as a

phyletic pattern in the pinsapo firs and that the Sierra Bermeja provenance therefore occupies an intermediate phyletic position between the Spanish pinsapo firs and the Moroccan firs. This situation shows that, within certain limits, the geographical location did not influence the morphological speciation of the Moroccan firs. Similarly, the tracheid length places *A. numidica* closer to the Spanish pinsapo firs than to the Moroccan varieties. Some researchers maintain that the populations of *A. pinsapo*, *A. pinsapo* var. *marocana* and *A. numidica* could even have formed a single species before the final separation of the Iberian Peninsula from the north of Africa at the end of the Tertiary (Sánchez Cózar, 1946; Bocquet et al., 1978; Jeanmonod and Bocquet, 1981; Blanca, 1993). This shared past prior to speciation may have been the common vector of the southern phylum.

Dubois-Ladurantie (1941) used tracheid diameter as a phyletic pattern in the American firs. However, this biometric feature is so strongly influenced by ecological conditions that it is very difficult to distinguish between the influence of such conditions and that of speciation. In the case of *A. pinsapo*, the larger tracheid diameters in the Moroccan provenances, which are statistically different from their Iberian counterparts, are probably determined by the better ecological conditions in the Rif. In fact, the Sierra Bermeja wood, corresponding to the least favorable ecological conditions, shows the smallest tracheid diameter of all the provenances.

The maximum height of the rays and their frequency can be used to characterize the *Abies* species (Castellarnau, 1880; Wieseheugel, 1932; Phillips, 1948; Greguss, 1955; Peraza, 1964). The biometry of the rays in *Abies* appears to provide evolutionary patterns. The maximum ray height (n° cells) allows two clades to be distinguished in *A. pinsapo*, coinciding with those established on the basis of tracheid length; firstly that of Sierra de Grazalema-Sierra de las Nieves, and secondly that of the Moroccan firs, while the Sierra Bermeja firs occupy an intermediate position. The wood of the samples from Sierra de las Nieves-Sierra de Grazalema presents a very similar structure to *A. alba* in terms of the maximum ray height in number of cells and the frequency of rays with more than 30 cells, although in *A. alba* rays with more than 30 cells are more frequent. The Moroccan provenances do not have this very high ray structure, despite their location in the ecological optimums of pinsapo fir forests, and therefore a relation between maximum ray height and ecological conditions is ruled out.

In general, the wood structure of the two Moroccan varieties is similar to the Iberian Peninsula firs, particularly the Sierra Bermeja provenance. Once again, this provenance could well present an intermediate situation, in phyletic terms, between the Grazalema and Sierra de las Nieves provenances, on the one hand, and the Moroccan firs on the other, in the same way as *A. nebrodensis* does between *A. alba* and *A. numidica* (Ducci et al., 1999; Parducci et al., 2001; Conte and Cristofolini, 2003).

The wood structure of the Moroccan firs is different from that of *A. numidica*, as seen in the separate position of the latter in the scatter plot of the PCA scores and in the values shown in Table 1. These results coincide with the distance observed between the two groups in their allozyme composition (Scaltsoyiannes et al., 1999). This distance may be imputed, in part, to extended isolation of the species and also to its restricted distribution and subsequent genetic displacement (Flous, 1936; Scaltsoyiannes et al., 1999). The same genetic divergence has also been observed between the populations of *Abies pinsapo* and var. *marocana* through the study of the inheritance of isozyme variations in their seed tissues (Pascual et al., 1993).

The wood samples of *Abies* spp. studied were in general qualitatively similar, except for particular features of certain provenances, as in the case of the organic deposits observed in the axial tracheids adjacent to the rays in the Grazalema provenances of *A. pinsapo*. The deposits, similar to those described in Araucariaceae (Jones, 1912; Patton, 1927; Heady et al., 2002), may be

associated with a large amount of resin in the ray parenchyma which then passes into the interior of the tracheid lumen through the cross-field pitting (Heady et al., 2002; Esteban et al. 2005). The presence of resin canals should not be regarded as diagnostic, as they appear in *Abies* as a response to wounding (Anderson, 1897; Jeffrey, 1905; Vierhapper, 1910; Rhoads, 1923; Chamberlain, 1935; Jane, 1970). However, the calcium oxalate crystals observed in the ray parenchyma of all the taxa, in both the marginal and submarginal rows, should be regarded as characteristic of *Abies* wood (Greguss, 1955; Jane, 1970; Core et al., 1979; IAWA Committee, 2004). All the species studied lack ray tracheids. Although they are recorded by some authors in *A. balsamea* (Penhallow, 1907), *A. homolepis* (Thompson, 1912) and *A. veitchii* (Jane, 1970), the presence of ray tracheids in *Abies* spp. is associated with wounding (Jeffrey, 1917; Chamberlain, 1935; Phillips, 1948). The degenerate cells in marginal position observed in *A. pinsapo* from Grazalema and Sierra de las Nieves have been described as a characteristic element of the genus *Abies* (Thompson, 1912).

Conclusions

The results obtained in this study show the potential of wood biometry in *Abies*, in conjunction with earlier molecular studies, for studying phyletic relations between taxa.

In the same way that features such as the stomata position, general cone shape, bract morphology, bud shape etc. can determine evolution into a phylum or clade and be constant in another phylum or clade (Flous, 1936; Dubois-Ladurantie, 1941), the features which determine evolution in wood can also establish a clade. The correlation between wood structure and climate conditions can make it difficult to use certain anatomical features (e.g. tracheid diameter) in studies on phyletic evolution. However, it was seen that geographical proximity was not a determining factor in the values of certain anatomical variables (e.g. ray height, tracheid length and number of pits per cross-field). Three main conclusions can be drawn from this study: *i*) the Mediterranean firs can be classified according to their wood anatomy into two large clades: the Spanish populations (including *A. alba*) and the Moroccan populations, on the one hand, and the rest of the Mediterranean populations on the other; *ii*) the most frequent average ray height, tracheid length and largest cross-field pit

diameter place the populations of *A. pinsapo* from Grazalema and *A. numidica* close together; and *iii*) the maximum ray height in number of cells, frequency of rays with more than 30 cells, and tracheid length can be regarded as specific patterns of the southern phylum of the western Mediterranean firs.

Acknowledgments

The authors wish to thank the following individuals and institutions:

Forestry Engineers Miguel A. Martín Casillas and José López Quintanilla for assistance during the trip to view *A. pinsapo* in Sierra de Las Nieves and Sierra Bermeja Natural Sites, in the province of Málaga, and Sierra de Grazalema Natural Park, in the province of Cádiz.

The public organizations of the Royaume du Maroc Haut Commissariat aux Eaux et Forêts et à la Lutte contre la Désertification, Direction Régionale des Eaux et Forêts du Rif à Tetouan, Service Provincial des Eaux et Forêts du Chefchaouen. In particular, Amrani Mohamed, Ingénieur en Chef des Eaux et Forêts du Rif à Tetouan and Assim ElHoussain, Chef du District du Chefchaouen, who provided guided access to the forests in Talassemrane and Tazaout (Morocco).

The Huesca Forest Service for providing assistance in the collection of samples of *A. alba* in mount Martinier-La Mascarina, in the municipality of Forcayo El Cornato.

Instituto Nacional de Investigaciones Agrarias, Institut für Holzbiologie und Holzschutz, Royal Botanic Gardens - Kew and Center for Wood Anatomy Research, for sending samples from their wood collections.

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