Comparative wood anatomy in Abietoideae (Pinaceae)

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This study, which includes 51 species and six genera of subfamily Abietoideae (Pinaceae), assesses the systematic significance of the wood structure in this group. In particular, the presence of normal and traumatic resin canals, the ray structure and the axial parenchyma constitute phylogenetically informative features. Comparative wood anatomy of Abietoideae clearly supports the monophyly of the genera *Abies–Cedrus–Keteleeria–Nothotsuga–Pseudolarix–Tsuga*, all of which have axial parenchyma with nodular transverse end walls in the regions of growth ring boundaries, crystals in the ray parenchyma and pitted horizontal and nodular end walls of ray parenchyma cells. Axial resin canals support a subdivision of the subfamily into two groups: *Abies, Cedrus, Pseudolarix* and *Tsuga*, without axial resin canals, and *Keteleeria* and *Nothotsuga*, with axial resin canals and a specific arrangement of traumatic axial resin canals. © 2009 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2009, **160**, 184–196.

ADDITIONAL KEYWORDS: *Abies – Cedrus – Keteleeria – Nothotsuga – Pseudolarix* – taxonomy – *Tsuga*.

INTRODUCTION

Pinaceae include a total of 225 species grouped into 11 commonly accepted genera (*Abies* Mill., *Cathaya* Chun & Kuang, *Cedrus* Mill., *Keteleeria* Carrière, *Larix* Mill., *Nothotsuga* Hu ex C.N.Page, *Picea* A.Dietr., *Pinus* L., *Pseudolarix* Gordon, *Pseudotsuga* Carrière and *Tsuga* Carrière) distributed throughout the world (Farjon, 2001). The wood structure of Pinaceae has been widely studied (Castellarnau, 1883; Jeffrey, 1905; Penhallow, 1907; Phillips, 1948; Greguss, 1955; Jacquiot, 1955; Kukachka, 1960; Lin, Hu & Wang, 1995; Lin, Liang & Farjon, 2000; Esteban *et al.*, 2002). Anatomical features of the secondary xylem of the family have led to discussion of the taxonomic position of its genera, although in most cases this only took into account the presence or absence of resin canals (Bannan, 1936; Greguss, 1955; Jain, 1976; Fahn, 1979; Wu & Hu, 1997; Lin *et al.*, 2000).

Pilger (1926) divided Pinaceae into three subfamilies on the basis of the leaf arrangement, the presence or absence of short shoots and the shape of the leaflet stalk and the pulvinus: Pinoideae (*Pinus*), Laricoideae (*Cedrus*, *Larix* and *Pseudolarix*) and Abietoideae (*Abies*, *Cathaya*, *Keteleeria*, *Picea*, *Pseudotsuga* and *Tsuga*). Jeffrey (1987) divided Pinaceae into two: the presence of resin canals in the seeds and cleavage polyembryony supported monophyly of *Abies–Cedrus–Keteleeria–Pseudolarix–Tsuga* and the presence of resin canals in the secondary xylem and leaves having endodermis with thickened Casparian strips supported monophyly of *Cathaya–Larix–Picea–Pinus–Pseudotsuga*. On the basis of the morphological structure of vegetative and reproductive organs, Frankis (1989) distinguished four subfamilies of Pinaceae in a widely accepted classification (Farjon, 1990): Pinoideae (*Pinus*), Piceoidae (*Picea*), Laricoideae (*Cathaya*, *Larix* and *Pseudotsuga*) and Abietoideae (*Abies*, *Cedrus*, *Keteleeria*, *Nothotsuga*, *Pseudolarix* and *Tsuga*). Wu & Hu (1997) divided Pinaceae into three groups: always with resin canals (*Cathaya*, *Larix*, *Picea*, *Pinus and Pseudotsuga*), always without resin canals (*Abies*, *Cedrus*, *Pseudolarix and Tsuga*), and with/without resin canals (*Keteleeria*). Subsequently, Lin *et al.* (2000), in their study on resin canals in *Keteleeria*, after noting the permanent presence of axial resin canals in mature wood in all the species of this genus and confirming the pattern of traumatic resin canals, proposed a classification of

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Pinaceae into three groups: with horizontal and axial resin canals (*Cathaya*, *Larix*, *Picea*, *Pinus* and *Pseudotsuga*), with axial resin canals only (*Keteleeria* and *Nothotsuga*) and without resin canals (*Abies*, *Cedrus*, *Pseudolarix* and *Tsuga*).

Molecular systematic studies of Pinaceae also confirm several clades. Using *rbcL* sequences, Wang, Han & Hong (1998a) determined that, in the group *Abies–Keteleeria–Pseudolarix–Tsuga*, *Abies* is associated with *Keteleeria* and *Pseudolarix* is closely associated with *Tsuga*. Price, Olsen-Stojkovich & Lowenstein (1987) placed *Keteleeria* in the *Abies* group on the basis of immunology studies.

Pinaceae include the only genera of conifers with normal resin canals in the secondary xylem, both axial and radial. As this is one of the most distinctive features of the secondary xylem anatomy of the family, it supports a number of clades. However, other features including the structure of the rays or axial parenchyma can provide further criteria for establishing clades.

In subfamily Abietoideae, the genus with the largest number of species is *Abies*, with 48, followed by *Tsuga* (9), *Cedrus* (4), *Keteleeria* (3) and the monospecific *Nothotsuga* and *Pseudolarix*. Numerous studies have been conducted on the wood anatomy of the genera of Abietoideae, but the results were not compared at the subfamily level or used to establish clades.

The sapwood and heartwood of the six genera of Abietoideae are indistinct, although some authors have considered that differences can be observed in fresh wood of *Abies* (Beauverie, 1910; Record & Hess, 1943). For example, Sargent (1902) distinguished *A. nobilis* A.Dietr. and *A. magnifica* A.Murray from the other American firs on the basis of the reddish colour of their heartwood, Record (1934) proposed a subdivision of the American species on the basis of the heartwood colour and density and Phillips (1948) assigned *A. alba* Mill. and *A. balsamea* (L.) Mill. to the light-coloured heartwood group with low relative density and included *A. nobilis, A. magnifica* and *A. pindrow* Royle in another group with higher density and a yellowish–brown heartwood with shades of red. However, the difference observed between the heartwood and sapwood colour in fresh wood in species of Abietoideae should not be considered diagnostic (Esteban *et al.*, 2007).

Cedrus is the only genus with a characteristic odour which distinguishes it from the other genera of Abietoideae (Phillips, 1948; Peraza, 1964; Esteban & Guindeo, 1989). In some species of *Abies*, such as *A. pinsapo* Boiss., the fresh wood has a foetid odour, which disappears once the wood is dried (Esteban *et al.*, 2007).

Growth rings are distinct, although the transition from earlywood to latewood can be either abrupt

(IAWA Committee, 2004), gradual (Greguss, 1955) or semi-abrupt (Core, Côté & Day, 1979) and, although this variation depends on the climatic conditions, Wiesehuegel (1932) used the gradual transition of the wood in *Abies* as an identifying feature of *A. grandis* (Douglas ex D.Don) Lindl., *A. venusta* K.Koch and *A. concolor* Lindl. ex Hildebr. Kukachka (1960) distinguished eastern and western species of *Tsuga* on the basis of a more abrupt transition in the eastern species. However, these differences are so influenced by the ecological conditions that they should not be taken into account for diagnostic purposes (Esteban *et al.*, 2003).

Abies, *Cedrus*, *Pseudolarix* and *Tsuga* have no resin canals and *Keteleeria* (Phillips, 1948; Lotova, 1975; Esteban, Guindeo & de Palacios, 1996) and *Nothotsuga* (Lin *et al.*, 1995) have axial resin canals only. However, the presence of traumatic axial resin canals in tangential rows as a result of wounding is common in *Abies* (Anderson, 1897; Jeffrey, 1905; Chamberlain, 1935; Jane, 1970), *Tsuga* (IAWA Committee, 2004) and *Pseudolarix* (Wu & Hu, 1997) and in *Cedrus* they are both axial and radial (Pearson & Brown, 1932). Penhallow (1907) regarded the resin canals in *A. concolor*, *A. bracteata* (D.Don) Poit., *A. nobilis* and *A. firma* Siebold & Zucc. as having been formed in normal wood. Vierhapper (1910) also recorded them in *A. concolor*, *A. nobilis* and *A. bracteata*. Jeffrey (1905) made a thorough study of the resin canals in *Abies* spp. in different parts of the tree and concluded that they were absent from the wood of *Abies*, except in the wood of the reproductive axis of some species (*A. grandis*, *A. magnifica*, *A. apollonis* Link) and in the first annual ring of vigorous branches of mature trees (*A. magnifica*). De Bary (1884) in Jeffrey (1905) reported the presence of a resin canal in the centre of the primary root wood and considered it a feature common to *Abies* and *Cedrus*. Strasburger (1891) also reported it in *Pseudolarix*. Jeffrey (1905) and Chamberlain (1935) confirmed, without exceptions, the presence of this canal in *Abies* spp.

In all genera of Abietoideae, the horizontal walls of the ray parenchyma cells are distinctly pitted and the end walls are nodular (Phillips, 1948; Greguss, 1955; Jane, 1970; Lin *et al.*, 1995). These features are of diagnostic value in identifying *Abies* spp. (IAWA Committee, 2004).

Phillips (1948) reported the presence of indentures throughout Pinaceae. However, they are not obvious in *Cedrus*, *Keteleeria* (Phillips, 1948) and *Nothotsuga* (Lin *et al.*, 1995). Greguss (1955) reported indentures in a large number of *Abies* spp.

Some researchers have used ray height to distinguish between species. Kleeberg (1885) studied ray height in some species of *Abies*, recording the follow-

ing maximum heights: *A. cephalonica* Loudon 24 cells, *A. alba* 26, *A. religiosa* (Kunth) Schltdl. & Cham. 6, *A. pinsapo* 14, *A. nordmanniana* Spach 20, *A. grandis* 16, *A. equi-trojani* (Asch. & Sint. ex Boiss.) Mattf. 20 and *A. balsamea* 15, whereas Wiesehuegel (1932) regarded the low ray height of *A. lasiocarpa* (Hook.) Nutt. and *A. arizonica* Merriam (< 15 cells) as sufficient grounds to distinguish these two species from the others. However, the greater ray height in *A. alba* enables it to be distinguished from *A. pinsapo* (Peraza, 1964), although Esteban *et al.* (2007) established that the ray heights of *A. pinsapo* from Grazalema are similar to those of *A. alba* in the Pyrenees. A high number of ray cells (> 30) is a feature of some *Abies* spp. and is regarded as a specific feature of the genus (IAWA Committee, 2004).

Partially biseriate rays are relatively frequent in species of Abietoideae but do not exceed 10% of the total. Kleeberg (1885) reported them in *A. cephalonica*, Wiesehuegel (1932) in *A. amabilis* Douglas ex J. Forbes, *A. concolor, A. grandis, A. venusta, A. nobilis* and *A. magnifica*, and Greguss (1955) and Panshin & De Zeeuw (1970) in *A. magnifica*. Jane (1970) described rays with > 30 cells, frequently biseriate, in *Abies* spp.

Phillips (1948) recorded ray tracheids in all Pinaceae except *Abies, Keteleeria* and *Pseudolarix*. Prior to this, Kleeberg (1885) and Strasburger (1891) noted the absence of ray tracheids in *Abies* spp. The IAWA Committee (2004) reported them to be rare in *Abies* and *Pseudolarix*. Ray tracheids are frequent in *Cedrus* and *Tsuga* (Core *et al.*, 1979) and Lin *et al.* (1995) reported their presence in *Nothotsuga*. Although a direct relation exists in *Abies* spp. between the presence of ray tracheids and wounding (Jeffrey, 1917; Chamberlain, 1935; Phillips, 1948), some species, albeit rarely, can contain normal ray tracheids. Penhallow (1907) found them in *A. balsamea*, Thompson (1912) in *A. homolepis* Siebold & Zucc. and *A. veitchii* Lindl. and Jane (1970) in *Abies* spp. Wiesehuegel (1932) also recorded ray tracheids in *A. balsamea*, but did not specify whether they were associated with wounding.

Cross-field pitting is taxodioid in *Abies* spp. (Phillips, 1948; Greguss, 1955; Jane, 1970; Esteban *et al.*, 2002; IAWA Committee, 2004), although piceoid pitting is also observed in latewood (Schweingruber, 1990). In *Cedrus*, pitting is taxodioid or piceoid (Esteban *et al.*, 2002), although cupressoid pitting has also been observed (Phillips, 1948). Cross-field pitting is piceoid (Esteban *et al.*, 2002) and cupressoid (Greguss, 1955) in *Keteleeria*, taxodioid (Greguss, 1955) and cupressoid (Lin *et al.*, 1995) in *Nothotsuga*, piceoid (Esteban *et al.*, 2002) and taxodioid (Greguss, 1955) in *Pseudolarix* and piceoid and cupressoid (Esteban *et al.*, 2002) in *Tsuga*.

Tracheid pitting has been observed in the tangential walls of *Abies*, particularly in latewood (Wiesehuegel, 1932; Jacquiot, 1955; Peraza, 1964). Tracheid pitting in the radial walls is uniseriate or biseriate. In *Abies*, biseriate pitting is usually opposite in arrangement, whereas in *Cedrus* and *Keteleeria* it is alternate. Disc-shaped tori in earlywood pitting are well defined in all genera of Abietoideae (IAWA Committee, 2004), with extensions in *Abies concolor* (Greguss, 1955), *A. sachalinensis* Mast. (Sano, Kawakami & Ohtani, 1999) and *Abies* spp. (Willebrand, 1995) and scalloped in *Cedrus* spp. (Phillips, 1948; Peraza, 1964; Esteban & Guindeo, 1989). In *Pseudolarix*, transitional forms of scalloped tori have been observed occasionally (Willebrand, 1995).

Organic deposits have been described in the tracheids adjacent to the rays only in *A. pinsapo* (Esteban *et al.*, 2007). Some species of *Abies* and *Cedrus* have a warty layer in the inner layer of the secondary wall (S3 or tertiary wall) (IAWA Committee, 2004).

Axial parenchyma is frequent but sparse in *Abies, Cedrus, Keteleeria, Pseudolarix* and *Tsuga*, normally distributed along the growth ring boundaries in single cells, in the first row of earlywood or in the last row of latewood (IAWA Committee, 2004). However, in some taxa [*A. cephalonica* and *A. pinsapo* var. *tazaotana* (S.Cózar ex Villar) Pourtet] the arrangement is diffuse, with axial parenchyma cells distributed throughout the growth ring. The transverse end walls of axial parenchyma cells of all genera of Abietoideae are markedly nodular (Phillips, 1948), with more conspicuous nodules in tangential sections (Yatsenko-Khmelevsky, 1954).

Castellarnau (1880) recorded calcium oxalate crystals in the ray parenchyma in *A. alba*. Wiesehuegel (1932) used the presence of these crystals as a diagnostic feature to differentiate *A. magnifica*, to which he attributed regular occurrence of crystals, from *A. concolor* and *A. venusta*, in which he regarded the occurrence as variable. Crystals are not present in the rest of the American firs apart from *A. fraseri* (Pursh) Poir. and *A. grandis*, in which they occur occasionally.

Phillips (1948) recorded calcium oxalate crystals in *A. grandis* and *Cedrus* and noted that they are relatively rare in *Keteleeria* and *Pseudolarix*. Chrysler (1915) observed them in *C. libani* A. Rich. Greguss (1955) regarded the presence of calcium oxalate crystals as a special feature in *Abies* to be taken into consideration when identifying species. He found the crystals not only in the ray parenchyma cells, but also in the axial parenchyma (*A. veitchii*). Jane (1970) found calcium oxalate crystals in marginal ray parenchyma cells in *Abies* spp. Core *et al.* (1979) stated that the occurrence of rhomboidal and rectangular calcium oxalate crystals is common in *Abies* spp. and that they are sometimes used as a diagnostic feature.

Table 1. Institutions to which the samples studied belong

Country	Institution	Reference
Spain	Cátedra de Tecnología de la Madera Escuela Técnica Superior de Ingenieros de Montes Universidad Politécnica de Madrid	ETSIM
	Instituto Nacional de Investigaciones Agrarias Consejo Superior de Investigaciones Científicas	INIA
UK	Jodrell Laboratory Royal Botanic Gardens, Kew	Kew
USA	Center for Wood Anatomy Research Forest Products Laboratory	MADw SJRw
	Smithsonian Institution National Museum of Natural History	USw
Germany	Institut für Holzbiologie und Holzschutz Bundesforschungsanstalt für Forst- und Holzwirtschaft	BFH
France	CIRAD-Forêt	CIRAD
the Netherlands	Nationaal Herbarium Nederland Universiteit Utrecht	UN
Japan	Forestry and Forest Products Research Institute University of Kyoto	TWTw KU

Esteban *et al.* (1996) recorded the presence of crystals in the axial parenchyma cells of *A. numidica* de Lannoy ex Carrière. Lin *et al.* (1995) observed them in the ray parenchyma cells of *Nothotsuga*. The IAWA Committee (2004) reported the presence of prismatic crystals in the marginal and submarginal ray cells of some *Abies* spp.

The objective of this study was to compare the anatomical features of Abietoideae xylem, not only with regard to resin canals, as in previous studies, but also taking into account the structure of rays, tracheids and axial parenchyma and discussing the systematic position of the genera included in the subfamily and in Pinaceae.

MATERIAL AND METHODS

The material used in this study came from the wood collections of several research centres (Table 1). The 51 species, two subspecies and two varieties of the six genera of Abietoideae studied are listed in Table 2, with references to the collections to which they belong. In the case of *A. pinsapo*, samples were collected from each of the three natural areas of distribution in Spain and the two natural areas in Morocco.

Microscopic slides were prepared following the usual methods of softening, microtome cutting to a thickness of 10–15 µm, staining and mounting. Samples were observed both without staining and after staining with safranine and Sudan 4 to colour resin red (Jane, 1970). Descriptions were made in accordance with the recommendations of the IAWA Committee (2004).

Samples were observed by means of light microscopy and scanning electron microscopy (SEM), model JEOL JSM-6380. The SEM samples were prepared following the method described by Heady & Evans (2000).

RESULTS

The features observed in the species of Abietoideae studied are summarized in Table 3.

DISCUSSION

RESIN CANALS

Xylem analysis revealed that *Abies*, *Cedrus* and *Tsuga* have no normal resin canals (Fig. 1), but traumatic resin canals resulting from wounding were observed (Fig. 2). Similarly, in *Pseudolarix*, although no resin canals were observed in the present study, they have been recorded as a response to wounding (Wu & Hu, 1997) (Fig. 3). *Cedrus* is the only genus capable of forming traumatic axial and radial resin canals (Figs 4, 5) and some authors have placed *Cedrus* in an intermediate position between *Pinus* and *Abies* (Jeffrey, 1905). In *Keteleeria*, only axial resin canals were observed (Fig. 6), which concurs with the findings of Lin *et al.* (2000). The absence of resin canals in some species of this genus (Jain, 1976; Jeffrey, 1987; Wu & Hu, 1997) probably corresponds

Table 2. List of the species, subspecies and varieties studied, with references to the collections from which the samples were obtained

Species	Collection/number
Abies alba Mill.	ETSIM(X1530)
A. amabilis Douglas ex J.Forbes	ETSIM(X1216)
A. balsamea (L.) Mill.	INIA(X2173)
A. borisii-regis Mattf.	MADw17846
A. bracteata (D.Don) Poit.	MADw18347
A. cephalonica Loudon	BFH7079; INIA(X1592); MADw17845
A. cilicica (Antoine & Kotschy) Carrière	BFH14036; INIA(X2084); MADw17376; UN362
A. concolor Lindl. ex Hildebr.	INIA(X1577); INIA(X1594)
A. delavayi Franch.	Kew18361
A. durangensis Martínez	USw32800
A. firma Siebold & Zucc.	CIRAD11458ch
A. fraseri (Pursh) Poir.	BFH 5552; MADw771; USw14814
A. grandis (Douglas ex D.Don) Lindl.	ETSIM(X1526)
A. guatemalensis Rehder	INIA(X1593)
A. hickelii Flous & Gaussen	SJRw37237
A. holophylla Maxim.	ETSIM(X1155)
A. homolepis Siebold & Zucc.	MADw4428; TWTw1348; TWTw9276; TWTw11943; TWTw18725; USw23684
A. kawakamii (Hayata) T.Itô	BFH 7243; MADw2006; USw21248
A. koreana E.H.Wilson	INIA(X2171)
A. lasiocarpa (Hook.) Nutt.	BFH12097; BFH11392; MADw25431; USw14492
A. lasiocarpa (Hook.) Nutt. subsp. *arizonica* (Merriam) A.E.Murray	BFH11383; INIA(X1576)
A. magnifica A.Murray	MADw6307; USw14485
A. mariesii Mast.	BFH9103; BFH7230; MADw20334; TWTw598; TWTw9347; TWTw18156; TWTw18691; TWTw20555; TWTw21799; USw24492
A. nephrolepis Maxim.	CIRAD22366ch
A. nordmanniana Spach	MADw10536; MADw39459; UN314
A. nordmanniana Spach subsp. *equi-trojani* (Asch. & Sint. ex Boiss.) Coode & Cullen	BFH14031; BFH14034; MADw49056
A. numidica de Lannoy ex Carrière	Kew18393; UN192
A. pindrow Royle	Kew18394
A. pinsapo Boiss.	ETSIM(X2175); ETSIM(X2176); ETSIM(X2177)
A. pinsapo Boiss. var. *tazaotana* (S.Cózar ex Villar) Pourtet	ETSIM(X2267)
A. pinsapo Boiss. var. *marocana* (Trab.) Ceballos & Martín Bol.	ETSIM(X2268)
A. procera Rehder	BFH11428; MADw44891; USw19149;
A. religiosa (Kunth) Schltdl. & Cham.	CIRAD(X1218)
A. sachalinensis Mast.	CIRAD11459ch
A. sibirica Ledeb.	Kew18406
A. spectabilis Spach	Kew18417
A. veitchii Lindl.	Kew18422
A. vejarii Martínez	MADw25208; USw32803
Cedrus atlantica Manetti	Kew18423
C. brevifolia Elwes & Henry	ETSIM(X0952)
C. deodara (Roxb. ex Lambert) G.Don	Kew70743
C. libani A. Rich.	Kew18453
Keteleeria davidiana Beissn.	USw18469
K. evelyniana Mast.	MADw42042
K. fortunei Carrière	Kew18462

Table 2. *Continued*

Species	Collection/number
Nothotsuga longibracteata (W.C.Cheng) H.H.Hu ex C.N.Page	MADw32679
Pseudolarix amabilis Rehder	Kew18924
Tsuga canadensis Carrière	ETSIM(X1515)
T. caroliniana Engelm.	Kew18950
T. chinensis (Franch.) Pritzel in Diels	CIRAD26180 ch
T. diversifolia (Maxim.) Mast.	BFH 621; BFH 7081; BFH 9112; MADw48033; MADw26122; TWTw593; TWTw11949; TWTw18088; TWTw18152; TWTw18665; TWTw20551
T. dumosa Eichl.	Kew18953
T. heterophylla Sarg.	ETSIM(X1239)
T. mertensiana (Bong.) Carrière	INIA(X1397)
T. sieboldii Carrière	KU(X0382)

Table 3. Summary of features of Abietoideae based on the species studied

		Abies	*Cedrus*	*Keteleeria*	*Nothotsuga*	*Pseudolarix*	*Tsuga*
General	Heartwood colour	−	−	−	−	−	−
	Growth ring boundaries distinct	+	+	+	+	+	+
	Distinct odour	−	+	−	−	−	−
Tracheids	Organic deposits in heartwood tracheids	(+)	−	−	−	−	−
	Torus extensions	(+)	−	−	−	−	−
	Scalloped torus	−	+	−	−	−	−
	Warty layer	+	+	−	−	−	+
Axial parenchyma	Present (marginal)	+	+	+	+	+	+
	Transverse end walls nodular	+	+	+	+	+	+
Rays	Ray tracheids present	(+)	+	−	+	(+)	+
	Traumatic ray tracheids	+	+	−	−	+	+
	Horizontal walls of ray parenchyma cells pitted	+	+	+	+	+	+
	End walls of ray parenchyma cells nodular	+	+	+	+	+	+
	Indentures	+	+	+	+	+	+
	Piceoid	+	+	+	−	+	+
	Cupressoid	−	(+)	+	+	−	+
	Taxodioid	+	+	−	+	+	−
Intercellular canals	Axial resin canals	−	−	+	+	−	−
	Traumatic axial resin canals	+	+	+	+	+	+
	Radial resin canals	−	−	−	−	−	−
	Traumatic radial resin canals	−	+	−	−	−	−
Mineral inclusions	Crystals	+	+	+	+	+	+

+, present; −, absent; (+) occasional.

to descriptions of juvenile or branch wood. *Nothotsuga* and *Keteleeria* have axial resin canals only (Lin *et al.*, 1995) and in both genera traumatic resin canals resulting from wounding appear in tangential rows and/or randomly in solitary arrangement (Lin *et al.*, 2000). This arrangement differs from that in traumatic resin canals of *Abies* and *Tsuga* (Jeffrey, 1905; Bannan, 1936) or *Cedrus* (Wu & Hu, 1997), in which they are invariably arranged in tangential rows. Lin *et al.* (1995) suggested that, because of the presence of
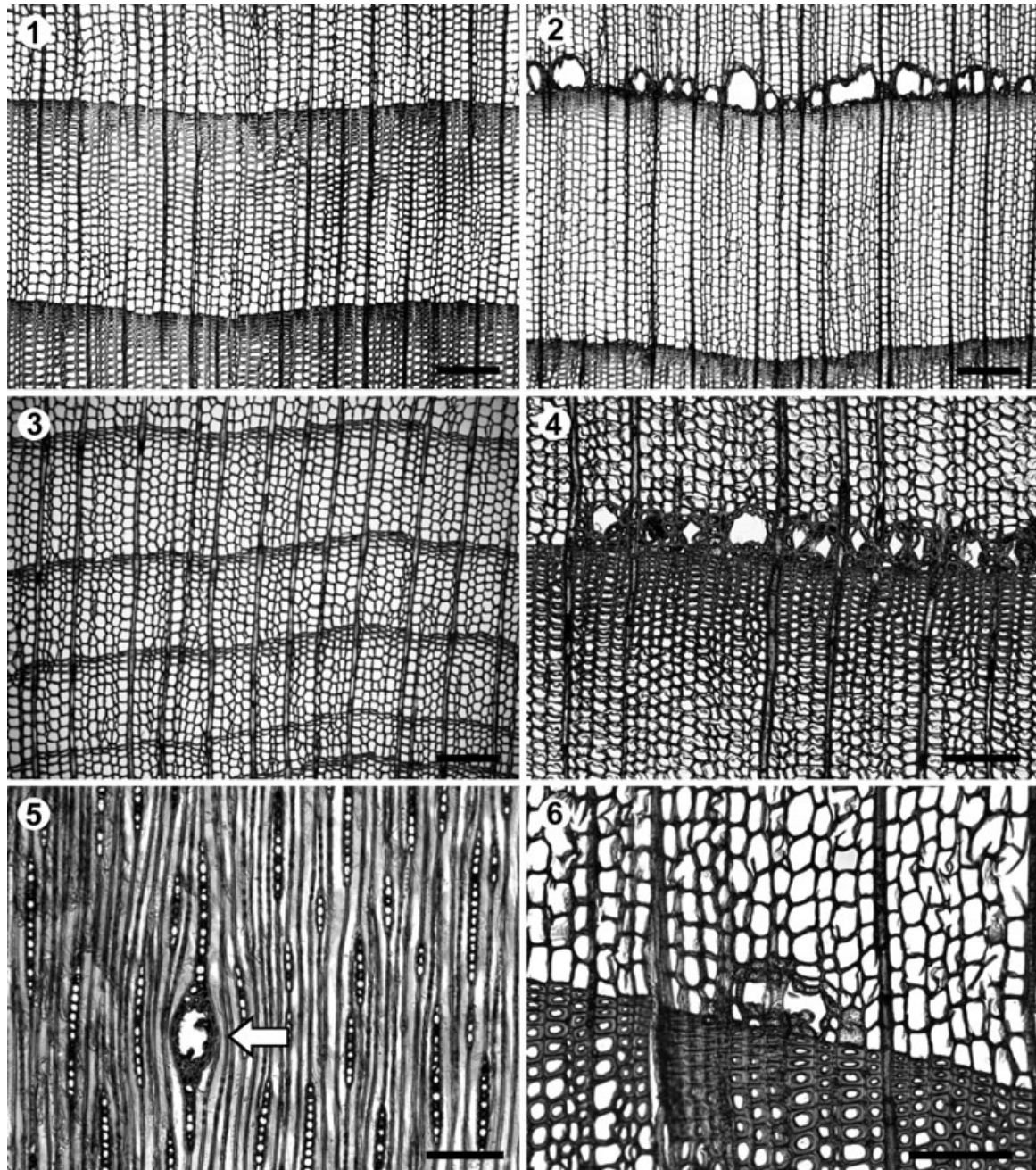
Figures 1–6. Resin canals. Fig. 1. Wood without resin canals (*Abies spectabilis*) (scale bar, 250 µm). Fig. 2. Traumatic axial resin canals (*Abies veitchii*) (scale bar, 250 µm). Fig. 3. Wood without resin canals (*Pseudolarix amabilis*) (scale bar, 250 µm). Fig. 4. Traumatic axial resin canals (*Cedrus brevifolia*) (scale bar, 150 µm). Fig. 5. Traumatic horizontal resin canals (*Cedrus brevifolia*) (scale bar, 150 µm). Fig. 6. Axial resin canals (*Keteleeria evelyniana*) (scale bar, 100 µm).

axial resin canals in the latewood and of resin cavities in the bark, *Nothotsuga* and *Keteleeria* have a high number of affinities. Therefore, based on the presence of resin canals, Abietoideae form two distinct groups: *Abies, Cedrus, Pseudolarix* and *Tsuga*, in which resin canals are absent, and *Keteleeria* and *Nothotsuga*, which possess axial resin canals.

RAYS

Ray structure has been used for grouping several families of conifers (Holden, 1913). Ray tracheids are a regular feature of all Pinaceae with resin canals, except for *Tsuga* (Fig. 7) and *Cedrus* (Fig. 8), which lack resin canals but frequently have ray tracheids (IAWA Committee, 2004). Nonetheless, in the two
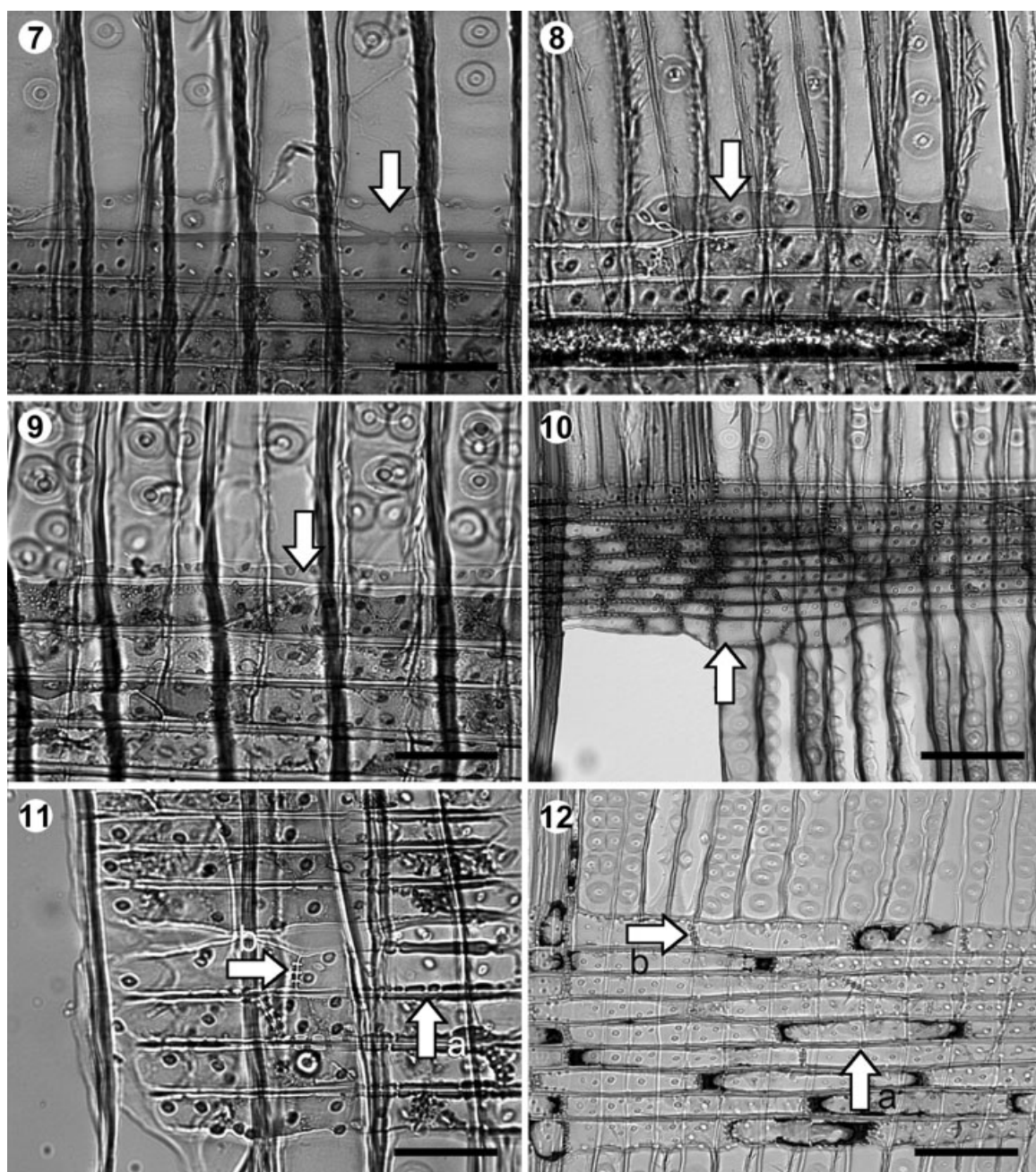
Figures 7–12. Rays. Fig. 7. Ray tracheid (*Tsuga diversifolia*) (scale bar, 50 µm). Fig. 8. Ray tracheid (*Cedrus atlantica*) (scale bar, 50 µm). Fig. 9. Ray tracheid (*Nothotsuga longibracteata*) (scale bar, 50 µm). Fig. 10. Degenerated cells (*Abies fraseri*) (scale bar, 100 µm). Fig. 11. a, horizontal walls of ray parenchyma cells with distinct pitting. b, nodular end walls (*Abies alba*) (scale bar, 50 µm). Fig. 12. a, horizontal walls of ray parenchyma cells with slight pitting. b, nodular end walls (*Pseudolarix amabilis*) (scale bar, 100 µm).

genera with axial resin canals, *Keteleeria* and *Nothotsuga*, the occurrence of ray tracheids is diagnostic. Ray tracheids were not observed in *Keteleeria*, but they are frequent in *Nothotsuga* (Fig. 9). In *Abies* and *Pseudolarix*, the presence of ray tracheids is less frequent and according to some authors they are associated with wounding, as in the occurrence of traumatic resin canals (Jeffrey, 1917; Chamberlain, 1935; Phillips, 1948). In the same way that traumatic resin canals are formed in *Abies, Cedrus, Keteleeria, Nothotsuga, Pseudolarix* and *Tsuga*, traumatic ray tracheids are also formed in these genera except for

Keteleeria and *Nothotsuga*. This was previously recorded by Holden (1913), who confirmed the absence of ray tracheids as a response to wounding in *Keteleeria*, but also noted the occurrence of axial traumatic resin canals.

Some authors have put forward phyletic hypotheses on the presence of traumatic ray tracheids. Jeffrey (1908) regarded the sporadic occurrence of ray tracheids associated with wounding simply as ancestral evidence of the original abundance of this feature in Abietineae. Penhallow (1907) interpreted the occurrence of ray tracheids in some species of *Abies* (*A. fraseri*, *A. nordmanniana*, *A. cephalonica*, *A. grandis*, *A. firma*) as a record of ancestral forms in the genus. Thompson (1910) observed the presence of abundant ray tracheids in root wood of *A. amabilis*. According to Thompson (1912), the fact that ray tracheids appear as a response to wounding in some species of *Abies* (*A. amabilis* and *A. concolor*) implies that they are an ancestral feature of the genus.

Ray tracheids are normally found in contact with either degenerated cells or ray parenchyma cells. Degenerated cells should be regarded as characteristic of *Abies* (Fig. 10), although their presence was also confirmed in this study in the species of *Cedrus*, which concurs with Chrysler (1915).

Taking into account the presence of non-traumatic ray tracheids, Abietoideae can thus be divided into two groups: those with ray tracheids (*Cedrus, Nothotsuga* and *Tsuga*) and those without (*Abies, Keteleeria* and *Pseudolarix*). However, in the second group, genera with ray tracheids of traumatic origin (*Abies* and *Pseudolarix*) and without ray tracheids (*Keteleeria*) co-occur.

In terms of the ray parenchyma cells, the six genera of Abietoideae studied have horizontal walls with simple pits. The pitting density varies between genera, being numerous in *Abies* (Fig. 11) and scarce in *Pseudolarix* (Fig. 12) and should be regarded as a distinguishing feature for Abietoideae, particularly in the case of *Abies* (Esteban *et al.*, 1996; IAWA Committee, 2004). The same occurs with the end walls of ray parenchyma cells, all of which have nodules that are developed to a greater (*Abies*) or lesser (*Pseudolarix*) extent. These two features therefore support the monophyly of Abietoideae. Lastly, cross-field pitting, an essential feature in the identification of conifer wood (Phillips, 1948; Peraza, 1964; Esteban *et al.*, 2002; IAWA Committee, 2004), is variable in Abietoideae (Table 3).

TRACHEIDS

No major differences were observed in the features associated with tracheids. Tracheid pitting in radial walls is normally uniseriate and when it is biseriate the arrangement is opposite (Figs 13, 14) and rarely alternate. In *Abies*, tori with extensions occur occasionally and, in *Cedrus*, scalloped tori are found, which were not observed in any other genus of Abietoideae (Fig. 15). Scalloped tori were observed in all *Cedrus* spp. studied and constitute a diagnostic feature at the genus level.

In *A. pinsapo* from the Sierra de Grazalema, the occurrence of tracheids with organic deposits was observed, but this should not be regarded as a characteristic feature of *Abies*, as in the case of Araucariaceae (*Araucaria* Juss., *Agathis* Salisb.) (Esteban *et al.*, 1996, 2005) because it was not observed in other species of the genus (Fig. 16).

All the genera of this subfamily share the common feature of tracheid pitting in the tangential wall of the tracheids located on the growth ring boundary. Pitting is abundant and is generally located in the first cell rows of the latewood (Fig. 17). A warty layer was only observed in *Abies*, *Cedrus* and *Tsuga* (Fig. 18). The presence of trabeculae is also frequent in all the genera (Fig. 19).

AXIAL PARENCHYMA

All the genera studied have sparse axial parenchyma in the regions of the growth ring boundaries (Fig. 20), invariably with nodular transverse end walls (Fig. 21). This is a regular feature of Abietoideae and supports the monophyly of this subfamily.
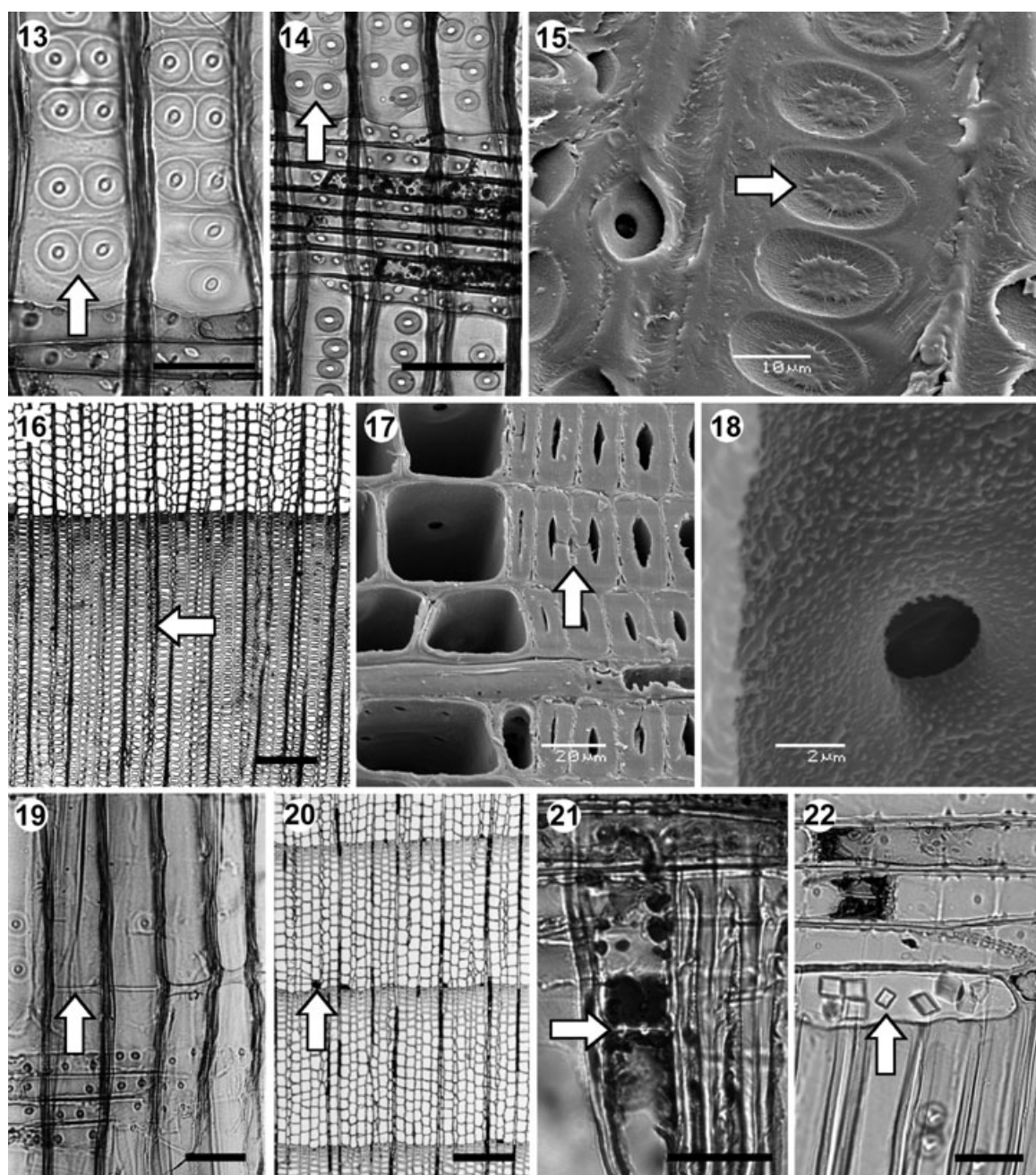
CRYSTALS

The presence of crystals was observed in all genera of Abietoideae (Fig. 22), normally occurring in the marginal and submarginal ray cells, both in normal and degenerated ray parenchyma cells. Degenerated cells are always marginal. Crystals are also frequent in the subsidiary cells of traumatic axial resin canals.

Although Greguss (1955), Jane (1970), Core *et al.* (1979), Esteban *et al.* (1996) and the IAWA Committee (2004) reported the presence of crystals as a regular feature in *Abies*, this feature can clearly be extended to the rest of the genera in Abietoideae. The presence of crystals therefore supports the monophyly of this subfamily.

PHYLOGENY

Molecular phylogenetic studies using the second intron in the mitochondrial gene *nad1* established that Pinaceae can be distinctly separated from the other families of conifers, which constitute a monophyletic group (Gugerli *et al.*, 2001). This coincides with the fact that resin canals in conifers are only present in the secondary xylem of Pinaceae: both

Figures 13–22. Tracheids, axial parenchyma cells and crystals. Fig. 13. Biseriate pitting in opposite arrangement (*Abies hickelii*) (scale bar, 50 µm). Fig. 14. Biseriate pitting in opposite arrangement (*Keteleeria evelyniana*) (scale bar, 100 µm). Fig. 15. Scalloped torus (*Cedrus atlantica*). Fig. 16. Organic deposits in tracheids (*Abies pinsapo*) (scale bar, 250 µm). Fig. 17. Tracheid pitting in tangential wall (*Tsuga diversifolia*). Fig. 18. Warty layer (*Abies pinsapo*). Fig. 19. Trabeculae (*Abies equi-trojani*) (scale bar, 50 µm). Fig. 20. Marginal axial parenchyma (*Abies pinsapo*) (scale bar, 250 µm). Fig. 21. Nodular transverse end wall of axial parenchyma cells (*Abies pinsapo*) (scale bar, 50 µm). Fig. 22. Crystals in ray parenchyma cells (*Pseudolarix amabilis*) (scale bar, 50 µm).

normal and traumatic in *Cathaya*, *Keteleeria*, *Larix*, *Nothotsuga*, *Picea*, *Pinus* and *Pseudotsuga* and only traumatic in *Abies*, *Cedrus*, *Pseudolarix* and *Tsuga*. Something similar happens in the case of ray trache-ids, which are characteristic of Pinaceae with normal resin canals but also occur, although less frequently, in other Pinaceae, in which case they are of traumatic origin. Although ray tracheids also occur in some

genera of Cupressaceae (*Cupressus* L., *Sequoia* Endl. and *Thujopsis* Siebold & Zucc.) (Phillips, 1948), their presence in these genera is rare.

Other features such as pitted horizontal walls and nodular end walls of the ray parenchyma cells, cross-field pitting, indentures, crystals and axial parenchyma in the regions of the growth ring boundaries do not justify Pinaceae as a separate group from the other conifers, given that other families of conifers also show these features. Therefore, only resin canals and ray tracheids justify Pinaceae as a separate group from the other conifers.

Phylogenetic analyses of plastid DNA (*matK* and *rbcL*) (Gernandt *et al.*, 2008) support two monophyletic subfamilies, Abietoideae and Pinoideae, corresponding better with the general structure of the wood in the two subfamilies. Wang, Tank & Sang (2000), in their study of intergeneric relations of Pinaceae using sequences of plastid *matK*, mitochondrial *nad5* and nuclear *4CL*, established a three-genome phylogeny consisting of the clade *Larix–Picea–Pinus–Pseudotsuga*, similar to the one found by Hart (1987) based on cladistic analysis, and that found by Price *et al.* (1987) based on immunological comparisons. The four genera in this clade have normal resin canals, both axial and radial, as does *Cathaya*.

Wang, Han & Hong (1998b), using PCR–restriction fragment length polymorphism (RFLP) analysis of plastid *trnK*, concluded that *Cathaya* is distinct from the other genera. Liston *et al.* (2003) showed that *Cathaya, Picea* and *Pinus* form an unresolved trichotomy. Their analysis provided support for the proposal by some researchers to treat this monotypic genus as another species of *Pseudotsuga* (Greguss, 1972). On the basis of its wood, *Cathaya* belongs to the clade *Cathaya–Larix–Picea–Pinus–Pseudotsuga* clade and, on the basis of its thick-walled epithelial cells, it belongs to the *Cathaya–Larix–Picea–Pseudotsuga* clade, somewhat further from *Pinus*, which has thin-walled epithelial cells.

Wang *et al.* (1998a) concluded that the clade *Abies–Keteleeria–Pseudolarix–Tsuga* is well resolved. These findings are similar to those obtained by Liston *et al.* (2003) based on immunological approaches plus nuclear and organellar DNA sequences. According to these studies, *Nothotsuga–Pseudolarix–Tsuga* form a clade sister to *Abies–Keteleeria*. Of the six genera included in Abietoideae, only the position of *Cedrus* is problematic. Molecular evidence supports this genus as a sister group of the rest of the family, resulting in Abietoideae being paraphyletic (Gernandt *et al.*, 2008). This coincides with the peculiar presence of traumatic radial resin canals in *Cedrus*, which are unique to this genus. In fact, based on the presence of this type of resin canal, *Cedrus* differs from the normal behaviour in the clade *Abies–Keteleeria–Nothotsuga–Pseudolarix–Tsuga*, including only species which produce traumatic axial resin canals. Moreover, *Cedrus* has scalloped tori, another unique feature of this genus.

Despite these distinctive features of *Cedrus*, all genera included in Abietoideae show common features in their secondary xylem which could justify the subfamily: pitted horizontal walls and nodular end walls of the ray parenchyma cells, indentures, axial parenchyma in the regions of the growth ring boundaries and presence of crystals in the ray parenchyma cells. The last two features are rare in other families of conifers.

The inclusion of Abietoideae in Pinaceae as a subfamily is justified by molecular analysis (Gernandt *et al.*, 2008). On the basis of the structure of the xylem, the subfamily is justified primarily by the presence of normal resin canals, in this case only axial, in *Keteleeria* and *Nothotsuga* and by the presence of traumatic resin canals in all the genera.

CONCLUSIONS

In conclusion, comparative wood anatomy is supportive of subfamily Abietoideae, proposed by Frankis (1989) and Farjon (1990), for *Abies*, *Cedrus*, *Keteleeria*, *Nothotsuga*, *Pseudolarix* and *Tsuga*, and the subdivision of Abietoideae, proposed by Lin *et al.* (2000), into two groups, one consisting of *Abies*, *Cedrus*, *Pseudolarix* and *Tsuga* and the other of *Keteleeria* and *Nothotsuga*, on the basis of the presence of axial resin canals in the two genera and the arrangement of the traumatic resin canals as a result of wounding, appearing in tangential rows and/or randomly in solitary arrangement. Molecular phylogenetic studies support the monophyly of the clade formed by *Abies*, *Keteleeria*, *Nothotsuga*, *Pseudolarix* and *Tsuga*, with only the inclusion of *Cedrus* remaining problematic.

ACKNOWLEDGEMENTS

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