

# **Taxonomic Portfolio**

**‘The Pinaceae family and *Pinus halepensis* M.’**

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## Introduction

The plant kingdom is one of the two generally accepted divisions of the living world, the other being the animal kingdom (Chinery, 1995). It includes familiar organisms like trees, flowers, herbs and ferns. Aristotle in the 4<sup>th</sup> century BC was the first to describe and classify different species of flora and fauna (Howes, 1997). He divided all living things between plants, which generally do not move or have sensory organs, and animals. In Linnaeus' system, these became the kingdoms Vegetabilia (later Plantae) and Animalia (www.Wikipedia, the <http://en.wikipedia.org/wiki/Plant>). Theophrastus, follower of Aristotle produces the 'Enquiry into Plants', a detailed description and classification of plants and their properties.

Among the plants, trees are the most complex and successful plants on earth. They have been around for 370 million years and quite likely will be around for many millions of years to come. Today, they cover almost a third of the earth's dry land and comprise more than 80.000 different species ranging from small Arctic willows that are just a few inches high to the lofty giant redwoods, which stand at an amazing 113 m/368 ft. (Russel & Cutler 2003).

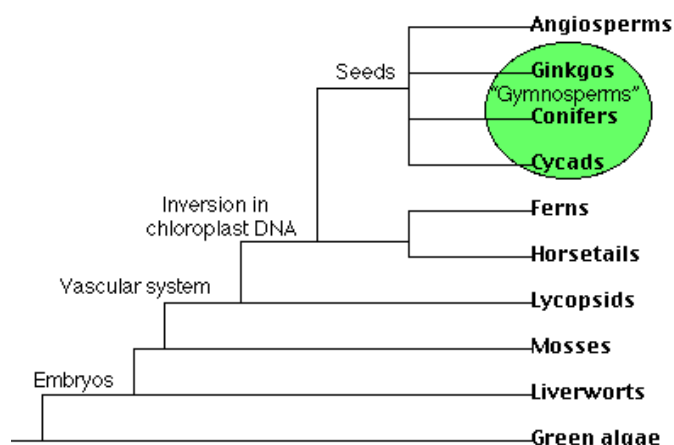
Trees are the oldest living organisms on earth. In California, USA, there are Bristlecone pines which are known to be over 4.500 years old and in the United Kingdom there are yew trees of a similar age (Russel & Cutler 2003). Ever since the first primates appeared in the Palaeocene epoch, 65 million years ago, trees have played an integral part in human development (Russel & Cutler 2003).

They have always played crucial economic role, providing timber for housing, shipbuilding, paper, furniture and chemicals such as medicines and dyes, as well as food. They shelter and provide homes for many species of birds, mammals and insects, and provide conditions for other plant life to thrive. Even more importantly, they help to regulate the world's climate. Without trees there would be no life on earth (Russel & Cutler 2003).

Herodotus in 450 BC was aware of the importance of intraspecific variation in tree species, although he did not know the word "genetic".

Current concepts of systematics and taxonomy of plants are based on their phylogenetic characteristics (phylogenetic classification, cladistics), rather than their morphological characteristics (phenetics), mainly used in the past.

In terms of evolution it is thought that plants are assigned to a single **clade**; that is, a natural grouping based on the belief that they have all evolved from a common ancestor.

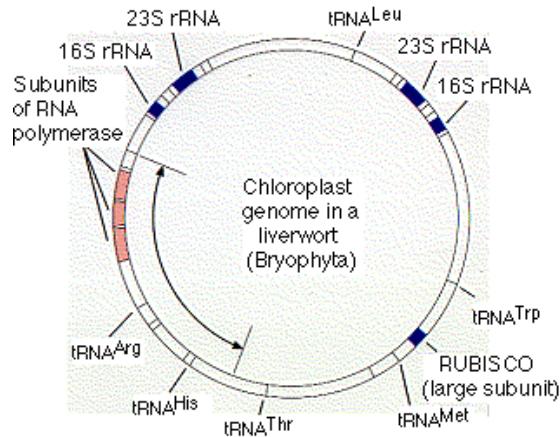


Green algae never develop more than a few differentiated types of cells and their fertilized eggs do not develop into an embryo. The ancestors of these organisms were the most primitive members of the clade. In other words, organisms that we would put in this division were probably the ancestors of all the other plants.

Embryophyta (liverworts and mosses) are fairly simple plants that do produce a number of differentiated cell types and whose fertilized egg develops into a distinct embryo. However, they have neither vascular tissue nor woody tissue and thus never grow very large.

Vascular plants are those with **xylem** and **phloem** running through their roots, stems, and leaves. Spermatophyta (seed plants) are by far the most diverse lineage among vascular plants. They all share in

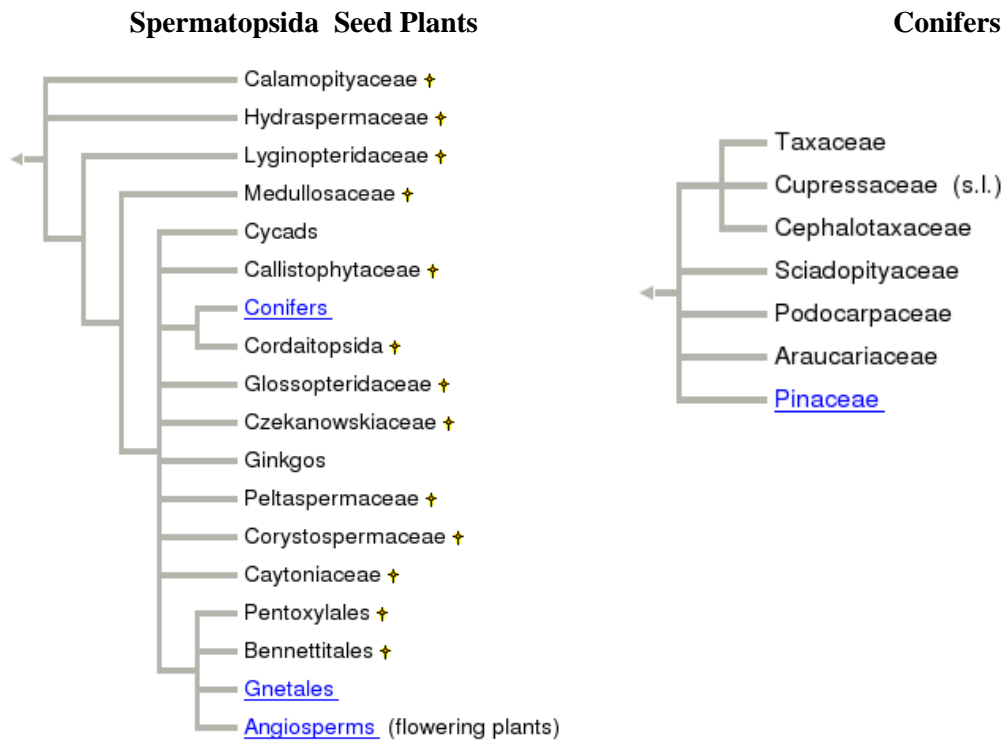
common the production of wood. A difference in the chloroplasts genome distinguishes lycopsids, ferns and horsetails from gymnosperms and angiosperms (see figure below).



The diagram (based on the work of Ohyama, K. et al., Nature 322:572, 1986 and Linda A. Raubeson and R. K. Jansen, Science 225:1697, 1992) shows the genome of the first chloroplast DNA to be sequenced, that of the liverwort *Marchantia polymorpha*. It contains 121,024 base pairs encoding 128 genes. The short lines indicate a few of the tRNA genes, some of which are labeled. The order of the genes between the arrows (~6:30 to ~10:00) is also found in the lycopsids. But in all other vascular plants, this region is inverted and the order of the genes is precisely reversed. This provides further evidence that the other vascular plants belong to a separate clade.

Most familiar are the multicellular land plants, called embryophytes. They include the green plants, which they include the spermatopsida vascular plants (seed plants with full systems of leaves, stems, and roots). Early seed plants are referred to as gymnosperms (naked seeds), as the seed embryo is not enclosed in a protective structure at pollination, with the pollen landing directly on the embryo. Conifers belong to gymnosperms and are the dominant group of trees in most forested areas of the Pacific Northwest.

The following diagrams (adopted from Wikipedia, the free encyclopedia, <http://www.wikipedia.org/wiki/Plant>) provides the classification of seed plants in general and conifers more specifically.



Containing group: Embryophytes

Containing group: *Spermatopsida*

## Current Concepts in the Classification of Plants

The introduction of genetics in plant science revealed that plant systematic diversity is clearly the result of evolution and has changed the principles of classification of plants. It seems that morphological characteristics (phenetics) is not the main indicator of species resemblance or dissimilarity. Genetic elements confirm the degree of closeness to a common ancestor between species (phylogeny). In a Phylogenetic system specific characters are highlighted as having overriding importance in defining a taxon. Those which have evolved 'recently' and are shared by the taxon and indicate it to be a true monophyletic group (clade). Thus, a group composed of an ancestor and all its descendants is known as a *monophyletic group* (mono=single; phylum=lineage), or a clade. We can recognise a monophyletic group because of the shared derived characters of the group (=synapomorphies); characters which arose in the ancestor of the group and are present in all of its members. It means that phylogenetic relationships: groupings of species, genera, families etc., reflect their evolutionary history.

Phylogenetic taxonomists often work with incomplete set of characters and have to construct the most likely evolutionary relationship between plants. Application of molecular methods of identification and complex statistical models, in order to choose between possible 'trees' and identify the one which has involved the least convergence and so the least amount of evolution, establishes the principle of **parsimony** (Judd *et al.*, 2002).

As a result of all the above, taxonomy of plant species tends to be difficult, uncertain and sometimes invalid.

## Pinaceae

Pinaceae are the largest and both economically and ecologically the most important family of conifers. Species in the largest genera – *Abies*, *Picea*, and *Pinus*–are the primary components of many forests in cooler and colder regions of the Northern Hemisphere.

Especially, pine trees are commercially among the most important of species used for timber in temperate and tropical regions of the world. Many are grown as a source of wood pulp for paper manufacture. This is because they are fast-growing softwoods that can be planted in relatively dense stands, they are tolerant of poor soils and relatively arid conditions, making them popular in reforestation and because their acidic decaying needles may inhibit the growth of other competing plants in the cropping areas. The resin of some species is important as the source of turpentine. Some species have large seeds, called pine nuts that are harvested and sold for cooking and baking. Some pines are used for christmas trees, and pine cones are also widely used for christmas decorations. Many pines are also very attractive ornamental trees planted in parks and large gardens. A large number of dwarf cultivars have been selected, suitable for planting in smaller gardens ([www.wikipedia.org/wiki/Plant](http://www.wikipedia.org/wiki/Plant)).

*Pinus*, with over 100 species, is the largest genus of conifers and the most widespread genus of trees in the Northern Hemisphere. The natural distribution of pines ranges from arctic and subarctic regions of Eurasia and North America south to subtropical and tropical (usually montane) regions of Central America and Asia. Pines are also extensively planted in temperate regions of the Southern Hemisphere (Price 1995).

The family name derives from the ancient Latin *Pinus*, and in turn from the Greek *Pitys*, for pines. In the past (pre-19th century) pines were often known as *fir*, from Old Norse *fyrre*, by way of Middle English *firre*. The Old Norse name is still used for pines in some modern north European languages: in Danish, *fyr*, in Norwegian, *furu*, and *Föhre* in parts of Germany, but in modern English, "fir" is now restricted to *Abies* and *Pseudotsuga*. Other unrelated European names include German *Kiefer* (the most widely used name in Germany), Swedish *tall*, Dutch *den*, Finnish *mänty*, Russian *sosna*, Bulgarian and Serbo-Croat *bor*, and Greek *pitys*. Korean people call it "Sonamu".

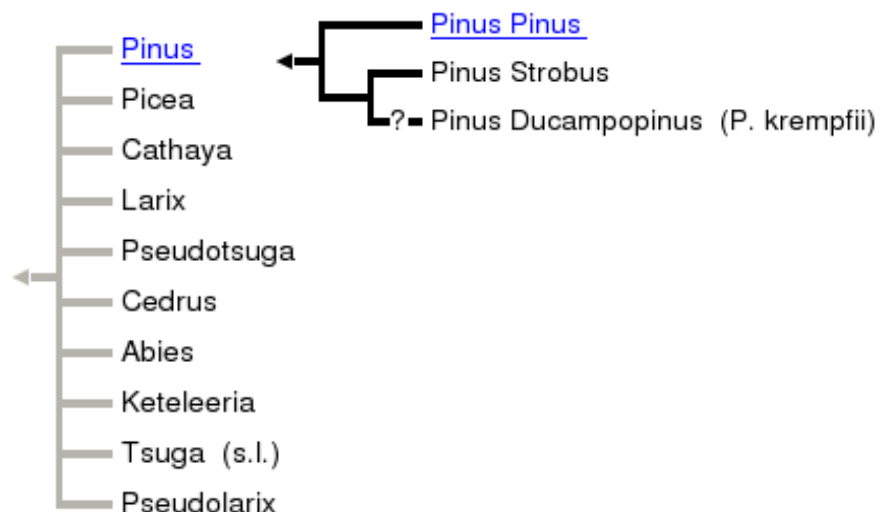
## Evolution, classification, and distribution of pinaceae.

The first gymnosperms (naked seeds) arose in the Middle Devonian (~365 million years ago). Fossilized cones have shown that ancestors of *Pinaceae* family evolved by the mid-Jurassic (~160 million years ago). The family originally included all conifers but is now restricted to a clearly monophyletic group united by characters seen in the mature seed cones: bract-scale complexes consisting of well-developed scales that are free from the subtending bracts for most of their length, two inverted ovules on the adaxial face of each scale, and a (usually obvious) seed wing developed from the cone scale (Thieret at the [Flora of North America web site](#) 10-12-2004). Cone and seed characters further serve to discriminate the four subfamilies, according to Frankis (1989):

1. Cones biennial, rarely triennial, with each year's scale growth distinct, forming an umbo on each scale. Cone scale base broad, concealing the seeds fully from abaxial view. Seed without resin vesicles. Seed wing holding the seed in a pair of claws. Leaves with primary stomatal bands adaxial (above the xylem) or equally on both surfaces. **Subfamily Pinoideae** (*Pinus*)
2. Cones annual, without a distinct umbo. Cone scale base broad, concealing the seeds fully from abaxial view. Seed without resin vesicles, blackish. Seed wing holding the seed loosely in a cup. Leaves with primary stomatal bands adaxial (above the xylem) or equally on both surfaces. **Subfamily Piceoideae** (*Picea*)
3. Cones annual, without a distinct umbo. Cone scale base broad, concealing the seeds fully from abaxial view. Seed without resin vesicles, whitish. Seed wing holding the seed tightly in a cup. Leaves with primary stomatal bands abaxial (below the phloem vessels) only. **Subfamily Laricoideae** (*Larix*, *Cathaya*, *Pseudotsuga*)
4. Cones annual, without a distinct umbo. Cone scale base narrow, with the seeds partly visible in abaxial view. Seed with resin vesicles. Seed wing holding the seed tightly in a cup. Leaves with primary stomatal bands abaxial (below the phloem vessels) only. **Subfamily Abietoideae** (*Abies*, *Cedrus*, *Pseudolarix*, *Keteleeria*, *Nothotsuga*, *Tsuga*)

*Pinaceae* is divided into 10 or 11 commonly accepted genera (Rushforth, 1987): *Abies*(31), *Cathaya*(1), *Cedrus*(13), *Keteleeria*, *Larix*(20), *Picea*(24), *Pinus*(111), *Pseudolarix*(2), *Pseudotsuga*(4) and *Tsuga*(6), while Page (1988) accepts a furthermore genera, *Nothotsuga*(1) .

### Pinaceae



containing group: *conifers* (adopted from Liston and Price)

### Genetic variation in pinacea and pines

Pines are considered as among the most genetically variable of all species as revealed by measures of quantitative genetic variation and by diversity at allozyme loci .

Variation at neutral loci may be governed mostly by mutation and drift. Further, the level of differentiation between populations at neutral loci depends on a balance between migration and genetic drift. Pollen-mediated gene flow among adjacent populations is effective in preventing population differentiation. The homogenizing effect of gene flow can be seen in the distribution of allele frequencies in pines, the high estimates of migration, and the low values for population differentiation. The proportion of total genetic diversity that exists between populations is often less than 5 %, which means that more than 95 % of total variation is within populations. Some pine species with disjunct populations and restricted gene flow have more genetic diversity among populations: for example 16-27 % for *P. radiata* and 22 % for *P. muricata* (Price *et al.*, 1995).

In quantitative traits, the level of genetic variation should depend on a balance between mutation and selection, or between different selection pressures. When the trait has an influence on the survival or reproduction of the individual (e.g. cold tolerance), the pattern of variation will be clinal due to the strong selection. Thus, when there is diversifying selection, the balance between selection and migration can result in considerable genetic differences between populations. Hence, neutral loci do not necessarily predict patterns of variation in traits that are subjected to differential selection (Price *et al.*, 1998).

Molecular data imply a basal split between the Pinaceae and a clade including all of the other conifers. Numerous features - including inversion of the ovules, prominent terminal seed wing, pattern of proembryogeny, proteintype sieve cells plastids, and the absence of biflavonoid compounds-establish the monophyly of Pinaceae. The family is not phylogenetically close to other extant conifer groups and is probably the sister group to the remaining conifers (Judd *et al.*, 2002).

In the study of Gugerli *et al.* (2001) the second intron in the mitochondrial gene *nad1* was surveyed using PCR, DNA sequencing, or Southern hybridization in 323 species (313 genera, 212 families) of seed plants. The intron was absent in all 22 species (22 genera, 8 families) of non-Pinaceae conifers studied, in *Welwitschia mirabilis*, and in seven angiosperms. Whereas absence of the intron in seven angiosperms and *Welwitschia* is likely due to seven independent losses when evaluated against the recently published multigene phylogenies, the lack of the intron in all non-Pinaceae conifers was explained by a single loss. These data suggested that the non-Pinaceae conifers represent a monophyletic group. This study also conducted a phylogenetic analysis of seed plants using a combined data set of the partial exon and intron sequences of *nad1* generated from this study and published sequences of mitochondrial *cox1* and small subunit (SSU) rDNA, chloroplast *rbcL*, and nuclear 18S rDNA. The results supported the split of conifers into two groups: Pinaceae and non- Pinaceae conifers.

According to strongly congruent structural and seed protein immunological data divide Pinacea into two subfamilies, Abetoideae and Pinoideae. Abietoideae include *Abies*, *Cedrus*, *Keteleeria*, *Pseudolarix*, and *Tsuga*, while *Cathaya*, *Larix*, *Picea*, *Pinus*, and *Pseudotsuga* make up Pinoideae.

Phylogenetic analysis of three genes –*mat k* from the chloroplast, mitochondrial *nad5*, and nuclear *4CL*- agree with this fundamental partition of the family except that *Cedrus* is sister to the remainder of the family. The Pinoideae are supported by several synapomorphies (absence of resin canals in the seed coat, absence of a narrowed, pedicillate base of the cone scales, and presence of two resin canals in the vascular cylinder of the young taproot) and contain two clades, *Pseudotsuga* plus *Larix*, and *Cathaya*, *Picea* plus *Pinus*. Abietoideae in the molecular phylogeny has two sets of sister taxa, *Abies* plus *Keteleeria* and *Pseudolarix* plus *Tsuga*. The monotypic genus *Nothotsuga* is very closely related to *Tsuga* and is best treated as a species of *Tsuga*.

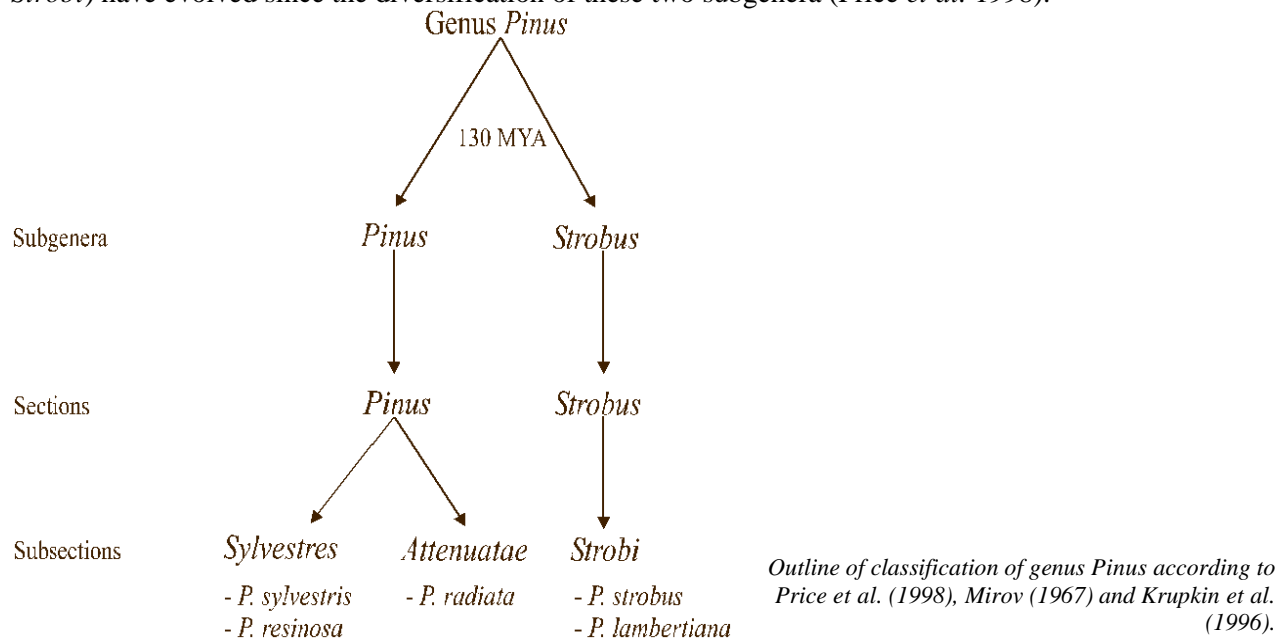
More than half the species in the *Pinaceae* are included in the genus *Pinus* (111 species) (Price *et al.* 1998). *Pinus* is highly distinct in its leaves, which are clustered in groups of usually two to five, and its cone scales, which are apically thickened and often armed with a pickle. This genus also has the longest fossil record of extant Pinaceae, extending back to Jurassic or early Cretaceous.

Most classifications of *Pinus* recognize two major lineages: subgenus **Pinus** and **Strobus**.

During the early part of the Cretaceous (nearly 130 million years ago) (Millar 1993), pines diversified into **two monophyletic subgenera**, *Pinus* (diploxyton or hard pines, with two fibro vascular bundles in the needles) or *Strobus* (haploxyton or soft pines, with one fibrovascular bundle in the needle) (Mirov 1967, Richardson & Rundel 1998). This division is consistent with data from wood anatomy and

secondary chemistry, and is supported in recent molecular phylogenetic studies (Strauss & Doerksen 1990; Wang & Szmidt 1993).

Several sections (e.g. *Strobus* and *Pinus*) and further subsections (e.g. *Sylvestres*, *Attenuatae* and *Strobi*) have evolved since the diversification of these two subgenera (Price *et al.* 1998).



As it was mentioned before the two universally accepted subgenera are the typical pines in subgenus *Pinus* and the white pines in subgenus *Strobus*. But in some classifications one or more of the species in subgenus *Strobus* are split off into a third subgenus *Ducampopinus*. The Vietnamese species, *P. krempfii* possesses unusual flattened needles and a unique wood anatomy. Based on these distinctive features, the species has been considered a third subgenus in the influential classification of Little & Critchfield (1969). (Again each of the subgenera can be further subdivided into a number of sections and subsections). On the other hand, secondary product chemistry and rbcL sequences suggest that *P. krempfii* should be considered a member of subgenus *Strobus*.

Taking a broad view of subgenus *Ducampopinus* (Huxley *et al.* [eds.], 1992), the subgenera are distinguished by the following criteria (Richardson 1998):

**Subgenus *Pinus* L. TYPICAL or HARD PINES.** About 73 species. Cone scales with umbo dorsal and mucronate, scales with a sealing band adjacent to the apophysis where the scales meet on the closed cone. Seedwing articulate (easily separated from the seed) to weakly adnate [not easily separated]. Leaf vascular bundle double. Fascicles with 2-6 leaves, stomata more or less equally distributed on all surfaces; sheath mostly persistent.

**Subgenus *Ducampopinus* (A. Cheval.) de Ferré ex Critchf. & Little (syn. genera *Caryopitys* Small; *Ducampopinus* A. Cheval.) PINYON, LACEBARK & FOXTAIL PINES.** About 20 species. Cone scales with umbo dorsal and mucronate, scales without a sealing band. Seedwing articulate. Leaf vascular bundle single. Fascicles with 1-5 leaves, stomata all or most on inner faces, rarely equal; sheath deciduous to partly or fully persistent.

**Subgenus *Strobus* Lemmon (syn. genera *Apinus* Necker; *Strobus* Opiz) WHITE or SOFT PINES.** About 23 species. Cone scales with umbo terminal and not mucronate, scales without a sealing band. Seedwing strongly adnate. Leaf vascular bundle single. Fascicles with 5 leaves, stomata all or most on inner faces, rarely equal; sheath deciduous.

Several molecular studies have documented a large genetic distance or branch length between the two subgenera (Strauss and Doerksen, 1990; Wang and Szmidt, 1993). However, these studies were restricted in taxonomic sampling and/or geographic scope.

The following table (adopted from the study on *Pinus* phylogeny, by Liston *et al.*, 1999) provides information on genetic characteristics and classification of *Pinus* species. The study was based on nuclear ribosomal DNA Internal Transcribed Spacer (ITS) region sequences.

### Sampled *Pinus* Species and Outgroups

<i>Pinus</i> classification and species	GenBank accession no.	Voucher information <sup>a</sup>
<b>Subgenus <i>Pinus</i></b>		
<b>Section <i>Pinus</i></b>		
Subsect. <i>Pinus</i> (5/17) <sup>b</sup>		
<i>P. kesiya</i> Gordon	AF037004	Thailand, X. R. Wang 960 (no voucher)
<i>P. resinosa</i> Aiton	AF037002	Hoyt Arboretum, OR, Liston 975-2 (OSC)
<i>P. sylvestris</i> L.	AF037003	Inst. Forest Gen., CA, Strauss V35 (no voucher)
<i>P. thunbergii</i> Parlatores	AF037025	Hoyt Arboretum, OR, Liston 1001 (OSC)
<i>P. tropicalis</i> Morelet	AF037005	A. Leiva, Havana Bot. Garden, Cuba (OSC)
Subsect. <i>Pinaster</i> Endlicher emend. Frankis <sup>c</sup> (5/6)		
<i>P. canariensis</i> C. Smith	AF037022	Inst. Forest Gen., CA, Strauss 44/10 (no voucher)
<i>P. halepensis</i> Miller	AF037007	Pinetum M. Marti´nez, Chapingo, Mexico (no voucher)
<i>P. merkusii</i> Junghuhn & de Vriese	AF037006	Thailand, X. R. Wang 956 (no voucher)
<i>P. pinaster</i> Aiton	AF037024	Peavy Arboretum, Liston 1005 (OSC)
<i>P. roxburghii</i> Sargent	AF037021	Pinetum M. Marti´nez, Chapingo, Mexico (no voucher)
Subsect. <i>Pineae</i> Little & Critchfield (1/1)		
<i>P. pinea</i> L. “New World hard pines”	X87935	Marrocco <i>et al.</i> (1996)
Subsect. <i>Contortae</i> Little & Critchfield (2/4)		
<i>P. contorta</i> Loudon	AF037014	Mt. Shasta, CA, Oliphant S-13 (OSC)
<i>P. virginiana</i> Miller	AF037015	Greer Gardens, OR, Liston 1011 (OSC)
Subsect. <i>Australes</i> Loudon (1/11)		
<i>P. echinata</i> Miller	AF037016	Inst. Forest Gen., CA, Strauss 80/24 (no voucher)
Subsect. <i>Ponderosae</i> Loudon <sup>d</sup> (6/17)		
<i>P. coulteri</i> D. Don	AF037013	Rancho Santa Ana Bot. Gard., CA, Liston 997 (OSC)
<i>P. douglasiana</i> Marti´nez	AF037012	Pinetum M. Marti´nez, Chapingo, Mexico, Alvarez-Buylla 7 (CHAPA 38972)
<i>P. durangensis</i> Marti´nez	AF037010	Pinetum M. Marti´nez, Chapingo, Mexico, Alvarez-Buylla 4 (CHAPA 38971)
<i>P. hartwegii</i> Lindley	AF037008	Pinetum M. Marti´nez, Chapingo, Mexico, Alvarez-Buylla 68 (CHAPA 38973)
<i>P. montezumae</i> Lambert	AF037009	Pinetum M. Marti´nez, Chapingo, Mexico, Alvarez-Buylla 29 (CHAPA 38952)
<i>P. ponderosa</i> P. & C. Lawson	AF037011	Inst. Forest Gen., CA, Strauss 81/39 (no voucher)
Subsect. <i>Attenuatae</i> Van der Burgh (1/3)		
<i>P. attenuata</i> Lemmon	AF037020	Hoyt Arboretum, OR, Liston 1007 (OSC)
Subsect. <i>Oocarpae</i> Little & Critchfield emend. Price, Liston, and Strauss (2/10)		
<i>P. patula</i> Schltdl. & Cham.	AF037019	Inst. Forest Gen., CA, Strauss 14/79C (no voucher)
<i>P. teocote</i> Schltdl. & Cham.	AF037018	Pinetum M. Marti´nez, Chapingo, Mexico, Alvarez-Buylla 17 (CHAPA 38978)
Subsect. <i>Leiophyllae</i> Loudon (2/2)		
<i>P. leiophylla</i> Schiede & Deppe	AF037017	Pinetum M. Marti´nez, Chapingo, Mexico, Alvarez-Buylla 14 (CHAPA 38967)
<i>P. lumholtzii</i> Robinson & Fernald	AF037026	Pinetum M. Marti´nez, Chapingo, Mexico, Alvarez-Buylla 13 (CHAPA 38977)

### Subgenus *Strobis* Lemmon

### Section *Parrya* Mayr



Subsect. *Balfourianae* Engelm. (1/3)

*P. aristata* Engelm. AF037000 Hoyt Arboretum, OR, Liston 980-2 (OSC)

Subsect. *Krempfianae* Little & Critchfield (1/1)

*P. krempfii* Lecomte AF036980 Vietnam, Ickert-Bond 268 (ASU)

Subsect. *Cembroides* Engelm. (6/11)

*P. cembroides* Zuccarini AF036997 Pinetum M. Marti´nez, Chapingo, Mexico, Alvarez-Buylla 86 (no voucher)

*P. culminicola* Andresen & Beaman AF036995 Pinetum M. Marti´nez, Chapingo, Mexico, Alvarez-Buylla 107 (CHAPA 38954)

*P. johannis* Robert AF036999 Pinetum M. Marti´nez, Chapingo, Mexico, Alvarez-Buylla 106 (no voucher)

*P. maximartinezii* Rzedowski AF036994 Pinetum M. Marti´nez, Chapingo, Mexico, Alvarez-Buylla 67 (CHAPA 38982)

*P. nelsonii* Shaw AF037001 Pinetum M. Marti´nez, Chapingo, Mexico, Alvarez-Buylla 143 (CHAPA 38964)

*P. pinceana* Gordon AF036998 Pinetum M. Marti´nez, Chapingo, Mexico, Alvarez-Buylla 31 (CHAPA 38956)

Subsect. *Rzedowskianae* Carvajal (1/1)

*P. rzedowskii* Madrigal & Caballero AF036996 Michoacan, Mexico, Quijada s.n. (no voucher)

Subsect. *Gerardianae* Loudon (2/3)

*P. bungeana* Endl. AF036992 Hoyt Arboretum, OR, Liston 1003 (OSC)

*P. gerardiana* D. Don AF036993 Inst. Forest Gen., CA, Strauss 25/25 (no voucher)

Section *Strobis*

Subsect. *Strobi* Loudon (8/21)

*P. ayacahuite* Schldtl. AF036981 Pinetum M. Marti´nez, Chapingo, Mexico, Alvarez-Buylla 111 (no voucher)

*P. chiapensis* (Marti´nez) Andresen AF036988 Inst. Forest Gen., CA, Strauss 2/57 (no voucher)

*P. kwangtungensis* Chen AF036985 Washington Park Arboretum, WA, Liston 1027 (OSC)

*P. lambertiana* Douglas AF036990 CA, Wilson 8266 (OSC)

*P. parviflora* Siebold & Zucc. AF036984 Greer Gardens, OR, Liston 1000 (OSC)

*P. peuce* Grisebach AF036989 Washington Park Arboretum, WA, Liston 1028 (OSC)

*P. strobis* L. AF036982 Inst. Forest Gen., CA, Strauss 32/37 (no voucher)

*P. wallichiana* Jackson AF036991 Inst. Forest Gen., CA, Strauss 36/28 (no voucher)

Subsect. *Cembrae* Loudon (3/5)

*P. albicaulis* Engelm. AF036983 Hoyt Arboretum, OR, Liston 982-2 (OSC)

*P. cembra* L. AF036987 Greer Gardens, OR, Liston 1010 (OSC)

*P. pumila* (Pallas) Regel AF036986 U.S. Nat. Arboretum, DC, Liston 1014 (OSC)

Outgroups

*Cathaya argyrophylla* Chung & Kuang AF036978 Silba 5587b (B)

*Picea mexicana* Marti´nez U24251 Klein and Smith (1996)

<sup>a</sup> Herbarium acronyms follow Holmgren *et al.* (1990).

<sup>b</sup> The number of species sampled and total number of species in each subsection. The arrangement of subsections follows Price *et al.* (1998).

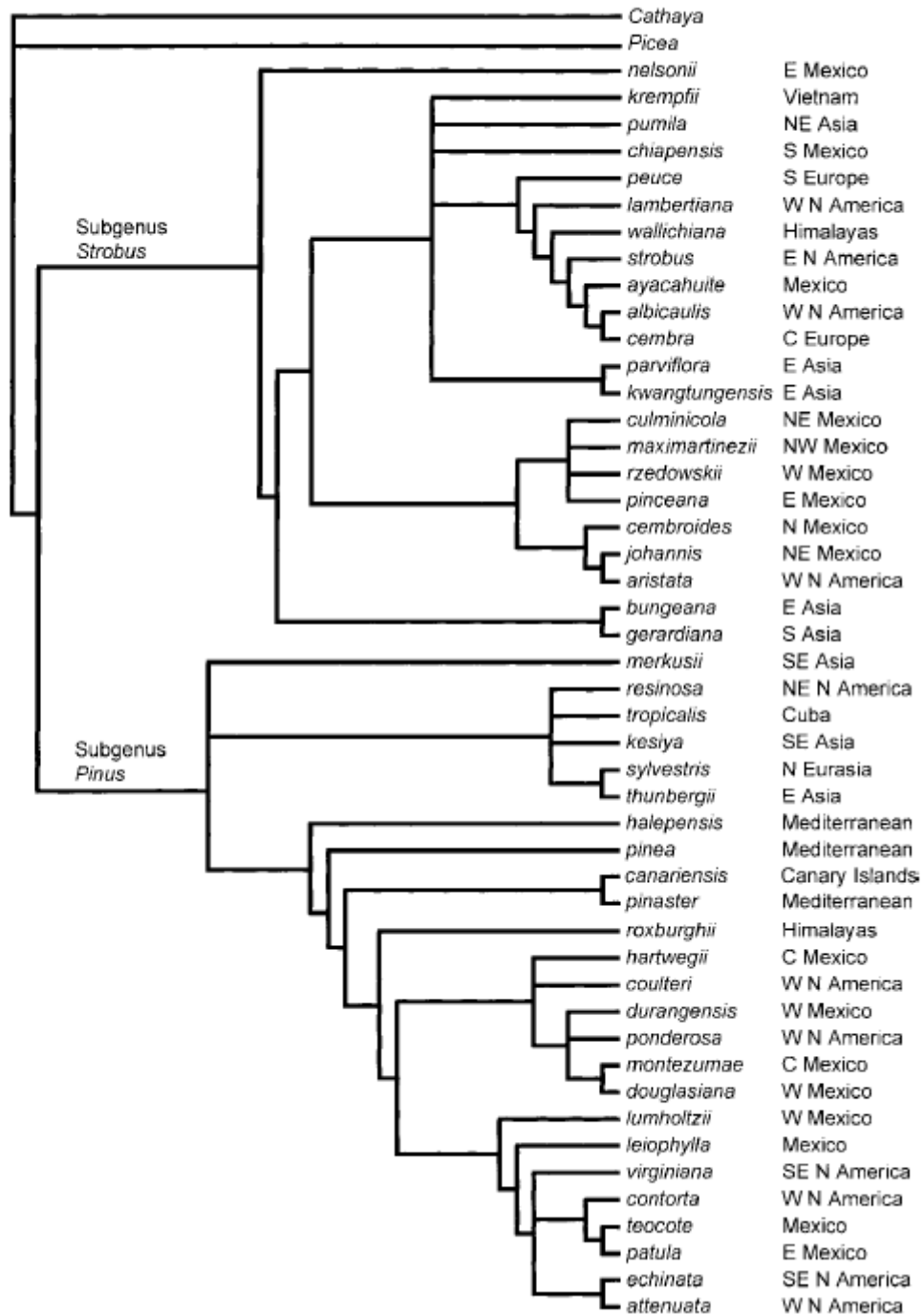
<sup>c</sup> Includes Subsect. *Canarienses* Loudon and Subsect. *Halepenses* Van der Burgh (see Frankis, 1993).

<sup>d</sup> Includes Subsect. *Sabinianae* Loudon (see Price *et al.*, 1998). (Liston *et al.*, 1999)

(From: Liston *et al.*, 1999)

The study of Liston *et al.* (1999) on phylogenetics of *Pinus*, suggested the division of *Pinus* as shown in the following diagram. The two subgenera and the generalized geographic range of each sampled species are indicated:

ITS REGION PHYLOGENY OF *Pinus*



(From: Liston *et al.*, 1999)

The genetic distance between the subgenera, at least between *Pinus* and *Strobos*, may be as large as or larger than that between e.g. *Keteleeria* Carrere, *Cedrus* Trew and *Abies* Miller (Price *et al.*, 1998), and if strict genetic criteria were used they should perhaps be treated at generic rank. However, the pines as a whole form a clear **monophyletic** unit and retention as a single genus remains the best treatment. The nomenclatural upheaval of splitting the genus would also be a serious problem. Of the subgenera, *Strobos* is uniform with only small morphological differences, while *Pinus* is more variable as well as being larger. *Ducampopinus* as circumscribed above shows the greatest morphological differentiation despite its small

number of species; many show relictual distributions and its species have been regarded by some as ancestral to the rest of *Pinus* (e.g. Farjon 1984, Klaus 1989).

Much work is being done with problematic taxa. Considerable chemotaxonomic and genetic data are available on the genus, but coverage is far from comprehensive (Richardson 1998).

A recent subdivision of the genus is (Price *et al.* 1998) [with a few amendments]:

### Subgenus *Pinus*

Section <i>Pinus</i> , Subsection <i>Pinus</i>	<i>P. densata</i> , <i>P. densiflora</i> , <i>P. heldreichii</i> , <i>P. hwangshanensis</i> , <i>P. kesiya</i> , <i>P. luchuensis</i> , <i>P. massoniana</i> , <i>P. mugo</i> , <i>P. nigra</i> , <i>P. resinosa</i> , <i>P. sylvestris</i> , <i>P. tabuliformis</i> , <i>P. thunbergii</i> , <i>P. tropicalis</i> , <i>P. yunnanensis</i>
Section <i>Pinea</i> , Subsection <i>Pinaster</i> Loudon	<i>P. brutia</i> , <i>P. canariensis</i> , <i>P. halepensis</i> , <i>P. latteri</i> , <i>P. merkusii</i> , <i>P. pinaster</i> , <i>P. roxburghii</i>
Section <i>Pinea</i> , Subsection <i>Pineae</i> Little & Critchfield	<i>P. pinea</i>
Section <i>Trifoliis</i> , Subsection <i>Contortae</i> Little & Critchfield	<i>P. banksiana</i> , <i>P. contorta</i>
Section <i>Trifoliis</i> , Subsection <i>Australes</i> Loudon	<i>P. caribaea</i> , <i>P. clausa</i> , <i>P. cubensis</i> , <i>P. echinata</i> , <i>P. elliottii</i> , <i>P. glabra</i> , <i>P. occidentalis</i> , <i>P. palustris</i> , <i>P. pungens</i> , <i>P. rigida</i> , <i>P. serotina</i> , <i>P. taeda</i> , <i>P. virginiana</i>
Section <i>Trifoliis</i> , Subsection <i>Ponderosae</i> Loudon	'Sabinianae Group': <i>P. coulteri</i> , <i>P. sabiniana</i> , <i>P. torreyana</i> 'Ponderosa Group': <i>P. arizonica</i> , <i>P. durangensis</i> , <i>P. engelmannii</i> , <i>P. jeffreyi</i> , <i>P. ponderosa</i> , <i>P. washoensis</i> 'Montezumae Group': <i>P. devoniana</i> , <i>P. hartwegii</i> , <i>P. montezumae</i> 'Pseudostrobus Group': <i>P. douglasiana</i> , <i>P. maximinoi</i> , <i>P. pseudostrabus</i>
Section <i>Trifoliis</i> , Subsection <i>Oocarpae</i> Little & Critchfield	'Attenuata Group': <i>P. attenuata</i> , <i>P. muricata</i> , <i>P. radiata</i> 'Oocarpa Group': <i>P. greggii</i> , <i>P. jaliscana</i> , <i>P. oocarpa</i> , <i>P. patula</i> , <i>P. praetermissa</i> , <i>P. pringlei</i> , <i>P. tecunumanii</i> 'Teocote Group': <i>P. herrerae</i> , <i>P. lawsonii</i> , <i>P. teocote</i>
Section <i>Trifoliis</i> , Subsection <i>Leiophyllae</i> Loudon	<i>P. leiophylla</i> , <i>P. lumholtzii</i>

### Subgenus *Ducampopinus*

Section <i>Ducampopinus</i> , Subsection <i>Krempfianae</i> Little & Critchfield	<i>P. krempfii</i>
Section <i>Gerardiana</i> , Subsection <i>Gerardianae</i> Loudon	<i>P. bungeana</i> , <i>P. gerardiana</i> , <i>P. squamata</i>
Section <i>Parryana</i> ,	<i>P. nelsonii</i>

Subsection <i>Nelsoniae</i> Van der Burgh	
Section <i>Parryana</i> , Subsection <i>Rzedowskianae</i> Carvajal	<i>P. maximartinezii</i> , <i>P. pinceana</i> , <i>P. rzedowskii</i>
Section <i>Parryana</i> , Subsection <i>Cembroides</i> Engelmann	<i>P. cembroides</i> , <i>P. culminicola</i> , <i>P. discolor</i> , <i>P. edulis</i> , <i>P. johannis</i> , <i>P. juarezensis</i> , <i>P. monophylla</i> , <i>P. orizabensis</i> , <i>P. remota</i>
Section <i>Parryana</i> , Subsection <i>Balfourianae</i> Engelmann	<i>P. aristata</i> , <i>P. balfouriana</i> , <i>P. longaeva</i>

### Subgenus *Strobus*

Section <i>Strobus</i> , Subsection <i>Strobi</i> Loudon	<i>P. amamiana</i> , <i>P. armandii</i> , <i>P. ayacahuite</i> , <i>P. bhutanica</i> , <i>P. chiapensis</i> , <i>P. dalatensis</i> , <i>P. fenzeliana</i> , <i>P. flexilis</i> , <i>P. lambertiana</i> , <i>P. monticola</i> , <i>P. morrisonicola</i> , <i>P. parviflora</i> , <i>P. peuce</i> , <i>P. pumila</i> , <i>P. strobiformis</i> , <i>P. strobus</i> , <i>P. wallichiana</i> , <i>P. wangii</i>
Section <i>Strobus</i> , Subsection <i>Cembrae</i> Loudon	<i>P. albicaulis</i> , <i>P. cembra</i> , <i>P. koraiensis</i> , <i>P. sibirica</i>

Many studies try to correlate *Pinus* species depending on similarities or differences of specific loci in their genome. The study of Wang et al. (1999) analysed the sequence divergence of chloroplast *rbcL*, *matK*, *trnV* intron, and *rpl20-rps18* spacer regions was analyzed among 32 *Pinus* species and representatives of six other genera in Pinaceae. The total aligned sequence length is 3570 bp. Of the four sequences examined, *matK* evolved much faster than *rbcL* in *Pinus* and in other Pinaceae genera. The two noncoding regions did not show more divergence than the two coding regions, especially within each *Pinus* subgenus. Phylogenetic analyses based on these four sequences gave consistent results and strongly supported the monophyly hypothesis for the genus *Pinus* and its two recognized subgenera. *Pinus krempfii*, the two-flat-needle pine endemic to Vietnam, was placed in subgen. *Strobus* and showed closer affinity to subsect. *Gerardianae*. The ancient character of sect. *Parrya* is further confirmed. However, monophyly of the sect. *Parrya* is not supported by our data. Among the Eurasian pines of subgen. *Pinus*, Mediterranean pines formed one clade and the Asian members of subsect. *Sylvestres* formed another. The Himalayan *P. roxburghii* showed considerable divergence from all the other hard pines from both regions. *Pinus merkusii* was distinctly separated from all the Asian members of subsect. *Sylvestres*.

Genetic diversity of *Pinus halepensis* Mill. was analysed in nine populations (six Spanish populations and one each from Tunisia, France and Greece). Twenty four RAPD loci were amplified with 60 megagametophyte DNA samples from each population. Populations' contribution to Nei gene diversity and to allelic richness were calculated. Results showed higher within population genetic variation. The results obtained suggests that RAPD markers are valuable for the estimation of genetic diversity in *P. halepensis* and for the study of the divergence among population, allowing to think that eastern Mediterranean populations of *P. halepensis* have undergone a different history from those of the western Mediterranean area (Gomez et al., 2001).

### Genetic variation between *Pinus* species based on chemical products

The high degree of genotypic variability observed in a number of *Pinus* species is reflected in the biochemical variability, which is usually studied at the levels of terpene composition and isozyme variation. The volatile constituents, particularly monoterpenes, have been extensively studied, since it has been demonstrated that the monoterpene composition besides some non-genetic variabilities related to the environment, is dependent upon the plant's genotype and can be used for taxonomic purposes. The chemical composition and the interspecific variation of various *Pinus* species volatiles have been the sub-

ject of numerous studies. The bulk of the work though has been focused on North American and Western European species and only a limited number of chemically oriented reports dealt with southeastern Mediterranean pine species (Roussis *et al.*, 1995).

**Terpenoids** are the largest family of plant metabolites with the skeletons consisting of different numbers of five-carbon isoprene units joined together. All plants produce terpenoids, some of which are primary metabolites including those found in chlorophyll, steroids, carotenoids, and growth regulators such as abscissic acid and gibberelline. Isoprenoids classified as secondary compounds include monoterpenoids (C10), sesquiterpenoids (C15) and diterpenoids (C20) that are constituents of essential oils and resin. Recently, these secondary metabolites have received great attention regarding their multiple contributing effects on community and ecosystem properties. Conifers are prolific producers of oleoresin that can be constitutive and induced or newly synthesised in response to biotic stresses. The relative proportions (percentages) of constitutive monoterpenes in mature tissues (which make up the constitutive monoterpene profile or the constitutive mixture) are strongly inherited and analyses of constitutive monoterpene mixtures have found many applications in chemotaxonomy. A strong variation was evident among *P. halepensis* Mill., *Pinus brutia* Ten. and their artificial hybrid *P. brutia* x *P. halepensis*. Variations in the constitutive mixture of monoterpenoids have important consequences in the chemical defence of plants since monoterpenoids show antimicrobial, fungistatic and insecticidal properties and act as antifeedant deterrents to mammals herbivores. Certain constitutive monoterpene profiles in slash pine (*Pinus elliotii* Engelm. var. *elliottii*), loblolly pine (*Pinus taeda* L.) clones and jack pine families (*Pinus banksiana* Lamb.) tended to be less susceptible to fusiform rust than other chemotypes. In addition, induced defence chemistry plays an important role in tree survival; in general, the largest proportionate accumulation in the most toxic monoterpenes can occur in response to attacks by insects and fungi (Michelozzi 1998).

Similarly a Greek study (Roussis *et al.*, 1995) analysed the chemical composition of the **volatile metabolites from needles** of *Pinus halepensis* Miller, *P. brutia* Tenore, *P. nigra* Arnold, *P. pinea* Linnaeus and *P. canariensis* Sweet and Sprengel, grown in natural habitats in Attiki, Greece. The leaves were selected as the chemical source because it is recognized that the basic tissue responsible for the production of **terpenes** is the needle resin canal epithelium and so is expected to exhibit smaller tree-to-tree variation than other plant parts. Fifty metabolites were detected, characterized and quantified on the basis of their retention data and mass spectra, as constituents of the essential oils. As expected the majority of the identified metabolites were found to be monoterpenes and sesquiterpenes.

Two groups G1 (*P. brutia*) and G2 (*P. pinea*, *P. nigra*, *P. canariensis* and *P. halepensis*), were initially recognized. The G1 group was characterized by high amounts of fl-pinene, a relatively minor constituent in the G2 group. The large amounts of limonene in *P. pinea* oil were responsible for the discrimination of these populations within the G2 group. Within their cluster the populations of *P. canariensis* were discriminated on the basis of a large germacrene D contribution. In the *P. nigra* and *P. halepensis* populations cluster, the most significant discrimination factor was ~t-pinene; this terpenoid being present in large amounts only in the *P. nigra* oils. Five chemotypes corresponding to each of the five investigated pine species were recognized. Although the foliage chemical constituents of the five investigated pine species appear to be good separators and distinct chemotypes characteristic of the pine species are recognized, they fail to reproduce the evolutionary groups. The observed deviation of the hierarchical structure from the generally accepted morpho-geographical taxonomic scheme is high. Similar failures to discriminate between major subgeneric evolutionary lines on the basis of leaf terpenoid metabolites have been reported for North American Haploxyton and Diploxyton pines. It seems that high congruence between terpene-based and morphological classification can be achieved only at subspecific taxonomic levels and introgressions between closely related species e.g. *P. halepensis* and *P. brutia* (Roussis *et al.*, 1995).

A distinctive feature of Pinaceae plants (Gymnospermae) is also, the presence of a significant amount of non- methylene-interrupted polyenoic (NMIP) **fatty acids** (FAs) in lipids obtained from the leaves (needles) and seeds. According to Wolf *et al.*, 1997, for example the taxonomy of *Pinus* species could base on the seed oil fatty acid compositions.

The most interesting NMIP acid found in the seeds of Pinaceae is **pinolenic acid** (18:3 A5,9,12). It takes part in eicosanoid biosynthesis, influences blood pressure [9] and is used in a dietary nutrition. The presence of uncommon NMIP acids in the total lipids of Pinaceae allows data on FA composition to be

used for the chemometric comparison of a number of plant species by multivariate statistical analysis. Therefore, the step-by-step study of FAs in Pinaceae is important not only for plant biochemistry, but also for plant chemosystematization. The differences among Pinaceae species hardly depend on climatic and geographic factors, but are species-specific (Imbs & Pham 1995).

In another study (Gerson & Kelsey 2004) foliage samples from 10 taxa of the genus *Pinus* occurring in Mexico and the southwestern US were extracted for **piperidine alkaloids**. Eight (*P. cembroides*, *P. durangensis*, *P. engelmannii*, *P. leiophylla*, *P. leiophylla* var. *chihuahuana*, *P. lumholtzii*, *P. monophylla*, and *P. ponderosa*) contained alkaloids; only a trace was detected in *P. ponderosa* var. *arizonica*; and no alkaloids were found in *P. discolor*. Relative concentrations of the two end products of piperidine synthesis were consistent among taxa within subsections: the *Leiophyllae* produced euphococcinine exclusively; the *Ponderosae* produced primarily pinidine; and the *Cembroides* produced appreciable quantities of both. Alkaloid profiles of *P. leiophylla* and *P. l. chihuahuana* were very similar, and in agreement with classification of *chihuahuana* as a variety of *P. leiophylla*. Among the *Ponderosae*, *P. p. arizonica* was unique in its lack of alkaloids, suggesting species-level distinction from *P. ponderosa* may be appropriate. The absence of alkaloids in *P. discolor*, contrary to its close relative *P. cembroides*, provides further evidence towards designation as a separate species. Alkaloids in a subset of seedlings subjected to water stress did not differ from controls.

A chemosystematic study based on needle **flavonoids** (Kaunduna *et al.*, 1997), was carried out on 324 trees from six populations of *Pinus halepensis*, six of *Pinus brutia* and one of *Pinus eldarica*. Two main flavonoid classes were identified and measured by HPLC: (pro)anthocyanins and flavonols. Relative (pro)anthocyanin contents were constant from one taxon to another, while flavonol aglycones, in particular quercetin and isorhamnetin, allowed complete discrimination of the three taxa. In agreement with previous studies, needle flavonoids show that *P. halepensis* and *P. brutia* are two distinct species. Surprisingly, *P. brutia* subsp. *brutia* was found to be more distant from *P. brutia* subsp. *eldarica* than from *P. halepensis*. Concerning their phylogenetic relationships, needle flavonoids bring chemical evidence of the common origin of the three pines studied. Early divergence of the progenitor resulted in the appearance of contemporary *P. halepensis* and *P. brutia*; subsequent evolution of *P. brutia* gave *P. eldarica*. On the basis of the phylogenetic results, it was proposed to maintain *P. eldarica* as a subspecies of the *P. brutia* complex.

Flowers and/or cones of seven species of the family Pinaceae were subjected to qualitative and quantitative examination for **anthocyanins**. Cyanidin- and peonidin-3-glucoside were found in the general *Pinus*, *Picea* and *Larix*. Male flowers of *Picea abies* contained, in addition, small amounts of delphinidin-3-glucoside (2%) and some unknown compounds (2%). Species belonging to *Abies* contained the 3-monoglucosides of delphinidin, petunidin, cyanidin, malvidin and peonidin. This is the first identification of methoxylated anthocyanins in the Pinaceae, and no petunidin- or malvidin-derivative has previously been reported to occur outside the Angiospermae (Andersen 1992).

### **Geographic distribution of Pinaceae family**

Pinaceae are mainly distributed in the Northern Hemisphere. Three or four genera grow only in eastern Asia; one *Cedrus* is confined to North Africa, the Near East, Cyprus, and the Himalayas and the remaining six genera (the major genera) all occur widely in the Northern Hemisphere (Judd *et al.* 2002). However, the family ranges from warm temperate climates to the limit of tree growth above the Arctic Circle, from permanently water saturated soils to well-drained soils, and from sea level to alpine habitats up to 4800 m above sea level in eastern Tibet (Judd *et al.* 2002). Native to all continents and some oceanic islands of the northern hemisphere, chiefly in boreal, temperate, or mountainous tropical regions; reaching its southernmost distribution shortly below the Equator in southeast Asia (Sumatra), (Mirov 1967).

In terms of evolution it has to be mentioned, that after the diversification of the subgenera *Pinus* and *Strobilus* pines migrated throughout the middle latitudes of the northern hemisphere super-continent, Eurasia. Major environmental changes in the early Cretaceous led to a splitting of several subsections of *Pinus* into northern refugial populations in western Siberia, mid-latitude populations in eastern Asia, and southern refugial populations in other parts of Asia and Europe. Intensive mountain building events together with climate changes created the environmental heterogeneity that drove the radiation of pine taxa in several areas which became secondary centres of diversification of *Pinus* (e.g. Mexico and north-eastern

Asia). At the end of the Eocene (55-37 MYA) the genus *Pinus* diversified further due to climatic changes (Richardson 1998). The impact of the Eocene had the effect of dissecting the genus and concentrating pines into widely disjunct regions. During the Pleistocene (1.7 - 0.01 MYA) pine populations and species shifted first south, then north following the glacial and interglacial periods. The climatic fluctuation at the Pleistocene may have played important role in speciation or at least the preservation of distinctive genotypes (Richardson 1998). The last 10000 years after the last glacial period have shaped the current distribution of pines.

Pine species have a major economic value as sources of timber, pulp and other products. Pine species are an important part of the ecosystem. In northern Europe and Asia the taiga or boreal forest forms the most extensive area of coniferous forest in the world. However, different historical, ecological and genetic factors have interacted to determine the limits of distribution of individual pine species. The extremes of distribution patterns are represented by widespread species such as Scots pine (*Pinus sylvestris*), and highly localized endemic species such as radiata pine (*Pinus radiata*).

For example, Scots pine (subgenus *Pinus*) has the largest geographic distribution of any pine, ranging from the Scottish Highlands along the Atlantic to the Pacific coast of eastern Siberia. In addition, Scots pine has relict populations from the Pleistocene in the Mediterranean region and central Europe (Mirov 1967). It ranges from latitudes 70° N in Norway and Finland to 37° N in Spain, and elevations from sea level to 2600m. With such a wide range of biogeographic and ecological distribution, it is not surprising to find that Scots pine is highly plastic and contains considerable amount of morphological and physiological variability (Mirov 1967).

Red pine (*Pinus resinosa*) belongs to the subgenus *Pinus* and is closely related to Scots pine. Red pine is a native of the northeast part of the American continent and it occurs locally also in northeast West Virginia (Mirov 1967). Red pine is very uniform morphologically and it has very little genetic variation. Despite the current large range, the low level of variation observed in red pine could have resulted from one or a series of population bottleneck(s) in the past. However, chloroplast microsatellites have revealed substantial amount of genetic diversity in red pine.

Radiata pine (*Pinus radiata*)-member of the subgenus *Pinus*- once ranged more widely, but now has only five distinct natural populations along the coast of central California and two island populations off the coast of Baja California. The mainland populations have hundreds of thousands of trees, while the island populations are much smaller. In particular, the Guadalupe population is close to extinction with less than 400 trees. The extant populations of radiata pine are disjunct relicts of much larger ancestral populations. According to Axelrod (1980, 1981), fragmentation has occurred within the last 8000 years. Millar (1997) suggested that repeated cycles of population growth and fragmentation have occurred at least in the last two million years. The island populations, Cedros and Guadalupe, are well separated from mainland populations. Cedros seems to have separated from mainland up to 10 million years ago and it is assumed that radiata pine has colonized the Guadalupe Island 1-4 MYA (Axelrod 1980). In the case of radiata pine there is good historical evidence that populations have gone through bottlenecks in the past (Axelrod 1980 and 1981), while the reduction of the population size of Guadalupe population is a recent phenomenon. Radiata pine is the most widely cultivated exotic conifer, mainly in Australia, New Zealand and Chile. Breeding programmes in these countries have resulted in substantial genetic improvement (e.g. gains in stem volume) (Lavery & Mead 1998). Nowadays, radiata pine is a target for conservation efforts, and the accurate determination of inbreeding levels and genetic structure of natural populations would help refine conservation strategies.

Eastern white pine (*Pinus strobus*) is a member of the subgenus *Strobus*. It extends from Newfoundland to northern Georgia in northern America. It thus currently has a large effective population size. Eastern white pine is an ecologically and economically important forest species in the area (Mirov 1967).

Sugar pine (*Pinus lambertiana*) is the member of the subgenus *Strobus*. The effective population size of sugar pine is large. It is distributed from western Oregon through Sierra Nevada to California. Sugar pine is considered a very important timber species due to the quality of wood (Mirov 1967).

## Major botanical features, particularly diagnostic features

### Description of Pinaceae family

This is the largest family of gymnosperms. Trees or shrubs, often emitting strong fragrances from bark and or leaves; resin canals present in wood and leaves. Branches whorled or opposite (rarely alternate). Leaves simple linear to needlelike (rarely narrowly ovate), spiral but often appearing 2-ranked by twisting of leaf base to bring most of the leaves into one plane, clustered or fascicled in groups or 2-5 in *Pinus* sessile or short-petioled, on long shoots or tightly clustered on short shoots, persistent (deciduous in *Larix* and *Pseudotsuga*). Microsporangiate strobili with spirally arranged, bilaterally symmetrical microsporophylls; microsporangia 2 on the abaxial microsporophyll surface; pollen grains with 2saccae (saccae absent in *Larix*, *Pseudotsuga*, and all but two species of *Tsuga*) and 2 prothallial cells. Cones with spirally arranged, flattened bract scale complexes; scales persistent (deciduous in *Abies*, *Cedrus*, and *Pseudotsuga*), bracts free from the scale, longer than the cone scale to much shorter than the cone scale; maturing in 2(3) years; ovule 2, inverted (micropyle directed toward the cone axis) on the adaxial cone scale surface; archegonia few per ovule, not clustered. Seeds with a long, terminal wing derived from tissue of the cone scale (wing reduced or absent in some species of *Pinus*); embryo straight. Cotyledons 2-18. (Judd et al 2002).

### Morphology of Pines

The bark of most pines is thick and scaly, but some species have thin, flaking bark. The branches are produced in regular "pseudowhorls", actually a very tight spiral but appearing like a ring of branches arising from the same point. Many pines are *uninodal*, producing just one such whorl of branches each year, from buds at the tip of the year's new shoot, but others are *multinodal*, producing two or more whorls of branches per year. The new spring shoots are sometimes called "candles"; they are light-colored and point upward at first, later darken and spreading outward. These "candles" offer foresters a means to evaluate fertility of the soil and/or vigour of the trees.

### Foliage

Pines have four types of leaves. Seedlings begin with a whorl of 4-20 *seed leaves* (cotyledons), followed immediately by *juvenile leaves* on young plants, 2-6 cm long, single, green or often blue-green, and arranged spirally on the shoot. These are replaced after six months to five years by *scale leaves*, similar to bud scales, small, brown and non-photosynthetic, and arranged like the juvenile leaves; and the adult leaves or *needles*, green (photosynthetic), bundled in clusters (*fascicles*) of (1-) 2-5 (-6) needles together, each fascicle produced from a small bud on a dwarf shoot in the axil of a scale leaf. These bud scales often remain on the fascicle as a basal sheath. The needles persist for 1.5-40 years, depending on species. If a shoot is damaged (e.g. eaten by an animal), the needle fascicles just below the damage will generate a bud which can then replace the lost growth.

### Cones

Pines are mostly monoecious, having the male and female cones on the same tree, though a few species are sub-dioecious with individuals predominantly, but not wholly, single-sex. The male cones are small, typically 1-5 cm long, and only present for a short period (usually in spring, though autumn in a few pines), falling as soon as they have shed their pollen. The female cones take 1.5-3 years (depending on species) to mature after pollination, with actual fertilization delayed one year. At maturity the cones are 3-60 cm long. Each cone has numerous spirally arranged scales, with two seeds on each fertile scale; the scales at the base and tip of the cone are small and sterile, without seeds. The seeds are mostly small and winged, and are anemophilous (wind-dispersed), but some are larger and have only a vestigial wing, and are bird-dispersed (see below).

At maturity, the cones usually open to release the seeds, but in some of the bird-dispersed species (e.g. Whitebark Pine), the seeds are only released by the bird breaking the cones open. In others, the *fire*



*climax pines*, the seeds are stored in closed cones for many years until a forest fire kills the parent tree; the stored seeds are then released in huge numbers to re-populate the burnt ground.

### **Ecology**

Pines grow well in acid soils, some also on calcareous soils; most require good soil drainage, preferring sandy soils, but a few, e.g. Lodgepole Pine, will tolerate poorly drained wet soils. A few are able to sprout after forest fires, e.g. Canary Island Pine. Some species of pines, e.g. Bishop Pine, need fire to regenerate and their populations slowly decline under fire suppression regimes. Several species are adapted to extreme conditions imposed by elevation and latitude; see e.g. Siberian Dwarf Pine, Mountain Pine, Whitebark Pine and the bristlecone pines.

### **Remarks**

In many areas *Pinus* is a forest dominant, either early successional or longer-lived, persisting in the late successional forest. Certain fire successional species, have a "grass stage," i.e., the stem of the young seedling elongates little during the first several years (meanwhile developing a large taproot) and bears many long, curved leaves, the plant then resembling a bunchgrass. Other species have cones that are long persistent and remain closed, opening only when heated by wildfire; seeds are released soon after, once the fire is out.

Most pines are fire-adapted, meaning that the recurrence of fire permits pines to maintain a dominant role in forest successions that lead to dominance by non-pines. The precise nature of this fire adaptation varies widely, with some pines tolerant of frequent low-intensity fires and others tending to produce high fuel accumulations that permit stand-destroying fires, after which the pines regenerate quickly. In habitats with infrequent or no fire, pines tend to occur either on extremely poor sites such as serpentine barrens or lithosols, or on extremely wet sites such as bogs. Their low shade tolerance typically precludes growth beneath a closed forest canopy. Many species are very drought tolerant (Price 1995).

## List of pines by region

### European & Mediterranean pine species (some extend into Asia):

- *P. brutia* – Turkish Pine
- *P. canariensis* – Canary Island Pine
- *P. cembra* – Swiss Pine
- *P. halepensis* - Aleppo Pine
- *P. heldreichii* – Bosnian Pine
- *P. mugo* – Mountain Pine
- *P. nigra* – European Black Pine or Austrian Pine
- *P. peuce* – Makedonian Pine
- *P. pinaster* - Maritime Pine
- *P. pinea* - Stone Pine
- *P. sylvestris* - Scots Pine

### Asian pine species:

- *P. amamiana* - Yakushima White Pine
- *P. armandii* - Chinese White Pine
- *P. bhutanica* - Bhutan White Pine
- *P. bungeana* - Lacebark Pine
- *P. dalatensis* - Vietnamese White Pine
- *P. densata* - Sikang Pine
- *P. densiflora* - Japanese Red Pine
- *P. fenzeliana* - Hainan White Pine
- *P. gerardiana* - Chilgoza Pine
- *P. henryi* - Henry's Pine
- *P. hwangshanensis* - Huangshan Pine
- *P. kesiya* - Khasi Pine
- *P. koraiensis* - Korean Pine
- *P. krempfii* - Krempf's Pine
- *P. latteri* - Tenasserim Pine
- *P. luchuensis* - Luchu Pine
- *P. massoniana* - Masson's Pine
- *P. merkusii* - Sumatran Pine
- *P. morrisonicola* - Taiwan White Pine
- *P. parviflora* - Japanese White Pine
- *P. pumila* - Siberian Dwarf Pine
- *P. roxburghii* - Chir Pine
- *P. sibirica* – Siberian Pine
- *P. squamata* - Qiaojia Pine
- *P. tabuliformis* - Chinese Red Pine
- *P. taiwanensis* - Taiwan Red Pine
- *P. thunbergii* - Japanese Black Pine
- *P. wallichiana* - Blue Pine
- *P. wangii* (syn. *P. kwangtungensis*) - Guangdong White Pine
- *P. yunnanensis* - Yunnan Pine

**North American pine species:**

**Canada & USA, except for close to the Mexican border:**

- *P. albicaulis* - Whitebark Pine
- *P. aristata* - Rocky Mountains Bristlecone Pine
- *P. attenuata* - Knobcone Pine
- *P. balfouriana* - Foxtail Pine
- *P. banksiana* - Jack Pine
- *P. clausa* - Sand Pine
- *P. contorta* - Lodgepole Pine
- *P. coulteri* - Coulter Pine
- *P. echinata* - Shortleaf Pine
- *P. edulis* - Colorado Pinyon
- *P. elliotii* - Slash Pine
- *P. flexilis* - Limber Pine
- *P. glabra* - Spruce Pine
- *P. jeffreyi* - Jeffrey Pine
- *P. lambertiana* - Sugar Pine
- *P. longaeva* - Great Basin Bristlecone Pine
- *P. monophylla*-*Single-leaf Pinyon\** - Western White Pine
- *P. muricata* - Bishop Pine
- *P. palustris* - Longleaf Pine
- *P. ponderosa* (syn. *P. washoensis*) - Ponderosa Pine
- *P. pungens* - Table Mountain Pine
- *P. radiata* - Monterey Pine or Radiata Pine
- *P. reflexa* - Southwestern White Pine
- *P. remota* - Texas Pinyon or Papershell Pinyon
- *P. resinosa* - Red Pine
- *P. rigida* - Pitch Pine
- *P. sabineana* - Gray Pine, Foothill Pine or Digger Pine
- *P. serotina* - Pond Pine
- *P. strobus* - Eastern White Pine
- *P. taeda* - Loblolly Pine
- *P. torreyana* - Torrey Pine
- *P. virginiana* - Virginia Pine

**Southern Arizona & New Mexico, Mexico, Central America & Caribbean:**

- *P. apulcensis* - Apulco Pine
- *P. arizonica* - Arizona Pine
- *P. ayacahuite* - Mexican White Pine
- *P. caribaea* - Caribbean Pine
- *P. cembroides* - Mexican Pinyon
- *P. chiapensis* - Chiapas White Pine
- *P. cooperi* - Cooper's Pine
- *P. cubensis* - Cuban Pine
- *P. culminicola* - Potosí Pinyon
- *P. devoniana* (syn. *P. michoacana*) - Michoacan Pine
- *P. durangensis* - Durango Pine
- *P. engelmannii* - Apache Pine
- *P. estevezii* - Estevez's Pine
- *P. gordoniana* (syn. *P. douglasiana*) - Gordon's Pine
- *P. greggii* - Gregg's Pine
- *P. hartwegii* - Hartweg's Pine
- *P. herrerae* - Herrera's Pine
- *P. hondurensis* (syn. *P. caribaea* var. *hondurensis*) - Honduras Pine
- *P. jaliscana* - Jalisco Pine
- *P. johannis* - Johann's Pinyon
- *P. lawsonii* - Lawson's Pine
- *P. leiophylla* - Chihuahua Pine
- *P. lumholtzii* - Lumholtz's Pine
- *P. maximartinezii* - Big-cone Pinyon
- *P. maximinoi* (syn. *P. tenuifolia*) - Thinleaf Pine
- *P. montezumae* - Montezuma Pine
- *P. nelsonii* - Nelson's Pinyon
- *P. occidentalis* - Hispaniolan Pine
- *P. oocarpa* - Egg-cone Pine
- *P. patula* - Patula Pine
- *P. orizabensis* - Orizaba Pinyon
- *P. pinceana* - Weeping Pinyon
- *P. praetermissa* - McVaugh's Pine
- *P. pringlei* - Pringle's Pine
- *P. pseudostrobus* - Smooth-bark Mexican Pine
- *P. quadrifolia* - Parry Pinyon
- *P. rzedowskii* - Rzedowski's Pine
- *P. strobiformis* - Chihuahua White Pine
- *P. tecunumanii* - Tecun Uman Pine
- *P. teocote* - Ocote Pine
- *P. tropicalis* - Tropical Pine

### Economic Uses - Products:

*“The Woodcutters and the Pine Tree”*

*“Some woodcutters were splitting a pine tree, and thanks to the wedges that they had made of its wood, they split it easily. And the pine tree said: “I dread less the axe that cuts me than the wedges which came from me.” (Aesop’s Fables).*

Pines are economically important for their timber, pulp, tar, and turpentine. When the world was tied together by sail, pines often assumed strategic importance as naval stores, thereby influencing patterns of Western colonialism. They were the first timber resource exploited in much of North America. They have long been a principal source of timber for all purposes, including firewood, construction and woodworking. They continue to be a leading genus in agroforestry production, dominating plantations in the U.K. (*P. contorta*, *P. nigra*), New Zealand (*P. radiata*) and Brazil (*P. elliottii*).

Stone pines (*P. pinea* and the species in subsection *Cembroides*) have an edible seed gathered by indigenous peoples (and sometimes commercially) and often comprising a major seasonal food source. By many accounts, they are also an aphrodisiac (Santerson 2000). Pines are also popular ornamentals.

Many pines have been also used to produce **turpentine**, a semi-fluid, yellow or brownish resin (oleoresin). **Resin** is a secretion formed in special resin canals or passages of plants, from many of which, such as, for example, pine trees, it is exuded in soft drops, hardening into solid masses in the air. Pine resin flows on the external surface of a tree after a wound is inflicted to form a protective coat that seals the wound to pathogenic microorganisms and prevents loss of sap. To obtain resin commercially, a tapping cut is made in the pine bark and the resin drops are collected into buckets or bags. It can also be extracted from almost all plants by treatment of the tissue with alcohol.

The principal products of pine resin are **rosin** and **turpentine oil**. The most significant hard resin from a commercial point of view is rosin, which is obtained by distillation of pine resin. Rosin is used in paper glue and soap manufacturing, as a constituent of varnishes and paints, and for coating strings of musical instruments (Grieve 1984). Oil of turpentine is also produced by pine resin distillation.

**Colophony** is also derived from pines. Colophony is a sticky, amber –coloured material of variable composition. It is named Colophon, an ancient town on the west coast of Turkey. Synonyms are kolophonium (Germany), colophane (France), colofonia (Spain), kolofonium Sweden and gum rosin or wood rosin (USA).

**Gum rosin** is derived from the oleoresin of living trees (notably *Pinus palustris* and *P. caribaea*). Wood resin is obtained from distillates of pine stumps. Tall oil rosin (tall is Swedish for pine) is derived by fractional distillation of crude tall oil, a by-product of wood pulping in paper manufacture. Rosins contain roughly 90% resin acids , notably abietic acid and its isomers (pimaric acids).

Turpentine consists of an average of 20% of the oleo-resin. Turpentine, rosin and derivatives of these are known collectively as gum naval stores (and the turpentine and rosin as gum turpentine and gum rosin, respectively). This distinguishes them from turpentine and rosin which have been recovered as by-products from chemical pulping of pines and which are referred to as sulphate naval stores; and wood naval stores, which are similar materials obtained from aged pine stumps. Neither sulphate nor wood naval stores are discussed further.

Traditionally, turpentine has been employed as a solvent or cleaning agent for paints and varnishes and as for shoe polish (Grieve 1984) and this is still often the case today, particularly in those countries where the pine trees are tapped. There are also some specialized uses, in the pharmaceutical industry, for example since it also has medical properties. Most turpentine nowadays, however, is used as a source of chemical isolates which are then converted into a wide range of products. Many of these, including the biggest single turpentine derivative, synthetic pine oil, are employed for fragrance and flavour use, although there are also many important non-aromatic applications such as polyterpene resins. Pine oil is used in disinfectants, cleaning agents and other products having a "pine" odour. Derivatives such as isobornyl acetate, camphor, citral, linalool, citrinellal, menthol and many others are used either in their own right or for the elaboration of other fragrance and flavour compounds. Many of the odours and flavours in use today, which are associated with naturally occurring oils, may well be derived, instead, from turpentine. A few of the minor

constituents of turpentine, such as anethole, are employed for fragrance or flavour use without the need for chemical modification.

Certain resins are obtained in a fossilized condition, amber being the most notable instance of this class; The resins which are obtained as natural exudations are in general mixtures of different, peculiar acids, named the resin acids, which dissolve in alkalis to form resin soaps, from which the resin acids are regenerated by treatment with acids. They are closely related to the terpenes, with which they occur in plants and of which they are oxidation products. Examples of resin acids are abietic (sylvic) acid,  $C_{20}H_{30}O_2$ , occurring in colophony, and pimaric acid,  $C_{20}H_{35}O_2$ , a constituent of gallipot resin. Abietic acid can be extracted from colophony by means of hot alcohol; it crystallizes in leaflets, and on oxidation yields trimellitic acid, isophthalic acid and terebic acid. Pimaric acid closely resembles abietic acid into which it passes when distilled in a vacuum; it has been supposed to consist of three isomers.

Resins when soft are known as oleo-resins, and when containing benzoic acid or cinnamic acid they are called balsams. Other resinous products are in their natural condition mixed with gum or mucilaginous substances and known as gum resins. The general conception of a resin is a noncrystalline body, insoluble in water, mostly soluble in alcohol, essential oils, ether and hot fatty oils, softening and melting under the influence of heat, not capable of sublimation, and burning with a bright but smoky flame. A typical resin is a transparent or translucent mass, with a vitreous fracture and a faintly yellow or brown colour, non-odorous or having only a slight turpentine odor and taste. Many compound resins, however, from their admixture with essential oils, have distinct and characteristic odours.

Those species of *Pinus* that are currently tapped, and the countries that are known to utilize them for this purpose, are listed below. Where tapping is solely or mainly carried out on natural stands, as distinct from plantations, this is indicated by (n).

#### Species that are currently tapped.

<i>Pinus elliottii</i> Engelm. (slash pine)	USA, Brazil, South Africa, Zimbabwe, Kenya
<i>P. pinaster</i> Aiton (maritime pine)	Portugal (n)
<i>P. massoniana</i> D. Don (Masson pine)	People's Republic of China
<i>P. merkusii</i> (Merkus pine)	Indonesia, Thailand (n)
<i>P. caribaea</i> Morelet (Caribbean pine)	South Africa, Kenya
<i>P. roxburghii</i> Sarg (syn. <i>P. longifolia</i> Roxb. ex Lambert) (chir pine)	India (n), Pakistan (n)
<i>P. oocarpa</i> Schiede	Mexico (n), Honduras (n)
<i>P. sylvestris</i> L. (Scots pine)	Former Soviet Union (n)
<i>P. radiata</i> D. Don (Monterey pine)	Kenya
<i>P. halepensis</i> Miller (Aleppo pine)	Greece (n)
<i>P. brutia</i> Ten.	Turkey

### Medicinal Uses

The best known pharmaceutical products of this family are essential oils and balsams, which are typically found in scizogenic excretion ducts of the leaves as well as in excretion pores of wood and bark. Both are rich in monoterpenoids such as  $\alpha$ -pinene and borneol. Colophony is particularly rich in terpenoids (including diterpenoids such as abietic acid). Other widely reported groups of compounds from members of this family are flavonoids, condensed tannins and lignans (e.g. pinoresinol) (Heinrich et al. 2004). The turpentine has medical properties and can be used as stimulant, antispasmodic, astringent, and anti-phthogenic. It is also antiseptic, diuretic, rubefacient and vermifuge (Grieve 1984). It is a valuable remedy used internally in the treatment of kidney and bladder complaints and can be used both internally and as a rub and steam bath in the treatment of rheumatic affections (Grieve 1984). It is being used for diseases of the respiratory mucous membranes and respiratory complaints such as coughs, colds, influenza and tuberculosis (Grieve 1984). Externally it is used for a variety of skin complaints, wounds, sores, burns, boils etc and is used in the form of liniment plasters, poultices, herbal steam baths and inhalers (Grieve1984).

### The hierarchy of taxonomic ranks of *Pinus halepensis* M.

<b>KINGDOM</b>	Plantae
Subkingdom	Embryobionta
<b>DIVISION</b>	Tracheophyta
Subdivision	Spermatophytina
<b>CLASS</b>	Pinopsida (Coniferopsida)
Subclass	Dicotyledonidae
<b>ORDER</b>	Pinales (Conirerales)
Suborder	
<b>FAMILY</b>	Pinaceae
Subfamily	Pinoideae
Tribe	
Subtribe	
<b>GENUS</b>	<i>Pinus</i>
Subgenus	Pinus
Section	Pinea
Subsection	Pinaster
Series	
Subseries	
<b>SPECIES</b>	<i>halepensis</i>
Subspecies	
Variety	
Form	
Subform	

## *Pinus halepensis* M

**DIAGNOSTIC FEATURES** (From Flora Europea and Stace 1997).

### **1. Family (Pinaceae) Diagnostic Features**

- Evergreen or deciduous resiniferous trees, rarely shrubs.
- Vegetative buds with brown bud –scales
- Leaves borne spirally, +- entire, linear to needle –like , borne singly on long –shoots or in clusters of 2 –many on short-shoots.
- Monoecious
- Male sporangia 2 per sporophyll
- Female cones with spirally arranged cone-scale bearing 2 ovules
- Fruit a usually woody cone.
- Seeds winged and becoming detached from cone-scale at maturity
- Cone- scales becoming woody, each with a distinct bract below it.

Distinguished from other coniferous families by the combination of spirally borne, very narrow leaves in scaly buds, and ovules borne in cones in which each cone- scale has a distinct bract below it.

Some genera bear short lateral stems (short-shoots) of very limited length, in addition to the normal extension shoots (long shoots).

“Twigs” refers to stems of the previous 1-2 years’ growth.

### **2. Genus (*Pinus*) Diagnostic Features:**

- Evergreen trees or shrubs with regularly whorled branches.
- Twigs of two kinds: long shoots bearing scale –like leaves
- Deciduous short shoots bearing acicular leaves in clusters of 2-5(-8).
- Resin –canals 2 or more, varying in position.
- Male flowers catkin-like, clustered at the base of the young twigs.
- Cones ripening in the second or third year, eventually falling in their entirety, cylindrical to ovoid
- Ovuliferous scales woody
- Bracts minute
- The exposed part of scales (apophysis) with a prominent protuberance (umbo), usually ending in a spine or prickle.

### **3. Species (*Pinus halepensis* M.) Diagnostic Features:**

- Up to 20 m
- Crown rounded
- Trunk and branches often crooked
- Bark silver-grey, becoming reddish-brown and deeply fissured.
- Twigs glabrous, remaining light grey for many years.
- Buds non resinous.
- Leaves 60-150 x 0.70 mm , in pairs, slender, clear green, with 1-3 layers or hypodermal cells
- Resin canals 3-8, submarginal (sometimes with 1-2 median).
- Cone 5 -12 x 4 cm, shining, brown.
- Apophysis convex
- Peduncle 1-2 cm, recurved.
- Seed c. 7 mm
- Wing c. 20 mm





### Physical Characteristics

The name *Pinus halepensis* is derived from the word *Pinus* which was the Roman name for pine and from the word *halepensis* -*halepicus* –a – um from Aleppo, northern Syria (Gledhill 2002).

Aleppo pine is a stout tree (at maturity) rarely exceeding 20 m high, with twisting branches and fine twigs and a bole with a girth up to 5m.

The bark is smooth, silvery grey and shining at first, becoming reddish-brown, fissured and scaly on boles. The shoots are grey- glaucous and glabrous, remaining grey for many years but eventually becoming green. The winter buds are conical, c. 8 mm long, with fringed scales and often relexed at the tips.

The leaves are 6-15 cm x 0.7 mm, occurring in pairs, persisting for c. 2 years, slender, clear green, urved, twisted above, with minutely toothed margins and a short horny apex; there are 3-8 submarginal or central resin canals.

The cones are 5-12 x 4cm, ovate conical, solitary or in groups of 2-3, spreading or deflexed, reddish, occurring on thick scaly peduncles up to 2 cm long.

The scales are shiny, about 2.5mm long; the exposed part is convex. The seeds are approximately 7mm long with a wing up to 2 cm long.

[www.dipbot.unict.it/orto/0089-1.html](http://www.dipbot.unict.it/orto/0089-1.html)



[www.botanical-online.com/fotospinushalapensis.htm](http://www.botanical-online.com/fotospinushalapensis.htm)

The flowers are monoecious (individual flowers are either male or female, but both sexes can be found on the same plant) and are pollinated by wind. The plant not is self-fertile.

*Pinus halepensis* prefers light (sandy) and medium (loamy) soils, requires well-drained soil and can grow in nutritionally poor soil. It prefers also, acid, neutral and basic (alkaline) soils and can grow in very alkaline soil. It cannot grow in the shade. It seems to be one of the most drought – tolerant pine species (Atzmon N. 2004, et al.) and can tolerate drought. A species common to the countries surrounding the Mediterranean it is commonly planted in hot dry places to check soil erosion and as a windbreak.

### **Habitats and Possible Locations**

Rocky places and hills by the sea (Polunin O.1969).

#### **Geographic distribution :**

Balearic Islands, Corsica, Crete, France, Greece, Italy, Poland, Sardinia, Sicily, Spain, Yugoslavia.



This distribution map was compiled by members of the EUFORGEN Conifers Network based on an earlier map published by W.B. Critchfield & E.L. Little, Jr., Geographic Distribution, of the Pines of the World, USDA Forest Service Misc. Publication 991, 1966 (<http://dendrome.ucdavis.edu/Image/Rangemap/>) and was published in: Fady, B., H. Semerci and G.G. Vendramin. 2003. EUFORGEN Technical Guidelines for genetic conservation and use for Aleppo pine (*Pinus halepensis*) and Brutia pine (*Pinus brutia*). International Plant Genetic Resources Institute, Rome, Italy, 6 pages

### **Economic – other Uses:**

Aleppo pine is a fairly wind-tolerant tree, which can be used in shelterbelt plantings (Huxley 1992). A tan or green dye is obtained from the needles (Grae 1974) and a tannin is obtained from the bark (Polunin 1969). The wood is used for rough construction (Polunin 1969). The “Greek turpentine” is obtained from the stems of Aleppo pine (Uphof 1959, Usher 1974, Hill 1952). The resins are obtained by tapping the trunk, or by destructive distillation of the wood (Grieve 1984). The resin from the trunk is used for chewing and for flavouring wine (Kunkel 1974, Huxley 1992) and a vanillin flavouring is obtained as a by-product of other resins that are released from the pulpwood (Huxley 1992). The Greeks traditionally cut down an Aleppo pine every year, decorating it with flowers and ribbons in honor of the god Attis. This custom is believed to have been adopted by the Europeans in honor of Christ, making the Aleppo the first Christmas tree.

### **Cultivation details**

Thrives in a light well-drained sandy or gravelly loam (Chittendon 1951, Bean 1981) Dislikes poorly drained moorland soils. Established plants are very tolerant of drought (Rushforth 1987, Huxley 1992), they succeed in poor chalky soils and also in poor dry sandy soils (Taylor 1990). Fairly wind-resistant (Huxley 1992). Plants are not very successful in Britain (Chittendon 1951) They are tender when young but are then fully hardy (Bean 1981). Plants are strongly outbreeding, self-fertilized seed usually grows poorly (Huxley 1992). They hybridize freely with other members of this genus (Huxley 1992). Leaf secretions inhibit the germination of seeds, thereby reducing the amount of plants that can grow under the tree. Plants in this genus are notably susceptible to honey fungus (Huxley 1992.)

### **Propagation**

It is best to sow the seed in individual pots in a cold frame as soon as it is ripe if this is possible otherwise in late winter. A short stratification of 6 weeks at 4°C can improve the germination of stored seed (McMillan-Browse 1985). Plant seedlings out into their permanent positions as soon as possible and protect them for their first winter or two (Bean 1981). Plants have a very sparse root system and the sooner they are planted into their permanent positions the better they will grow. Trees should be planted into their permanent positions when they are quite small, between 30 and 90cm (Huxley 1992). We actually plant them out when they are about 5 - 10cm tall. So long as they are given a very good weed-excluding mulch they establish very well. Larger trees will check badly and hardly put on any growth for several years. This also badly affects root development and wind resistance (Huxley 1992). Cuttings: this method only works when taken from very young trees less than 10 years old. Use single leaf fascicles with the base of the short shoot. Disbudding the shoots some weeks before taking the cuttings can help. Cuttings are normally slow to grow away (Rushforth 1987).

### **Text Key for *Pinus halepensis* Miller (Aleppo Pine).**

- Plants dispersal by seeds  $\mapsto$  6 (Seed plants)  
6 Carpels and endosperm absent  $\mapsto$  7 **Gymnosperms**  
7 Vessels absent; leaves usually spiral, rarely opposite or joined at base  $\mapsto$  8  
8 Sperm not flagellate, transported to ovule by pollen tube; leaves simple, small, and scale-like to larger and linear, with resin canals in most species **Conifers**  
(From: *Plant Systematics a phylogenetic approach 2002*)  
1. Leaves dark green, hard, narrow and usually spine-tipped, scale-like or needle like (except Gingo); veins parallel; usually annual growth in whorls; males and females always on separate trees; never with petals; fruit a cone or berry-like **Gingo, conifers & taxads**  $\mapsto$  2  
2. Leaves scale-like, linear or lanceolate, but never fan-shaped  $\mapsto$  3  
3. Leaves protruding scales, or not scale-like  $\mapsto$  9  
9. Leaves opposite or spirally arranged, neither in rosettes nor spurs  $\mapsto$  12  
12. Fruits cones, never berry-like, with obvious scales  $\mapsto$  15  
15. Leaves spirally arranged on the shoots  $\mapsto$  18  
18 Leaves linear or linear-lanceolate, never more than 5 mm wide  $\mapsto$  19  
19 Leaves without 2 obvious white bands on the lower surface  $\mapsto$  22  
22 Leaves in bundles of 2, 3, or 5 **Pinus (Pines)**  
1. Leaves in pairs  $\mapsto$  13  
13. Shoots grey glaucous in 1<sup>st</sup> year.  $\mapsto$  14  
14 Seeds with well-developed wings  $\mapsto$  15  
15 Leaves less than 1 mm wide; resin canals submarginal ***halepensis***.  
(From Hamlyn Guide Trees of Britain and Europe)

### **Glossary:**

**Carpel:** functional unit of the female organ of a flower; may be separated or joined, resulting in a partitioned ovary.

**Glaucous :** covered with a bluish or whitish layer

**Lanceolate:** Broad, but tapering to a point at both ends, like the blade of a lance

**Linear:** narrow and more or less parallel-sided

**Rosettes** (leaves not in Rosettes ): leaves not spread in horizontal plane from a short axis at ground level.

**Whorl:** the arrangement in which leaves, petals etc all arise at the same point on an axis , encircling it.

## Other pine species that are distributed in Greece:

### *Pinus pinea* L

*Pinus pinea* L. (Stone pine or Umbrella Pine)

**Crown:** A stout, parasol-shaped tree up to 30 m tall.

**Bark:** The bark on old trees is reddish –grey with deep longitudinal fissures, flaking to leave reddish-orange patches.

**Shoots:** Glabrous, greyish –green, becoming brown in maturity.

**Buds:** Winter buds are 0.5-1.2 cm long, with reflexed scales.

**Leaves:** 10-20 cm x c.1.5-2 mm., occurring in pairs persisting for 2-3 years, slightly twisted, acute with 12 stomatic lines on the outer surface and 6 on the inner surface; the resin canals are marginal.

**Cones:** 8-14 x 10 cm, ripening shining brown after 3 years, the exposed part of the scale weakly pyramidal.

**Seeds:** 1.5-2 x 0.7-1.1cm with a wing less than 1 cm long.

**Habitat:** A native of light sandy soils in Mediterranean Europe.

**Distribution:** Al, Bl, Co, Cr, Fr, Gr, It, Pl, Sa, Si, Sp, Tu, Yu.

**Uses:** the rich oily seeds are eaten raw or roasted and used as flavouring; the timber is used locally for furniture.

### *Pinus heldreichii* Christ

**Crown:** A tree up to 20 m tall with a rounded pyramidal shape.

**Bark:** The trunk is ashy-gey, flaking to leave yellowish patches.

**Shoots:** Glabrous but glaucous in the first year, then brown.

**Buds:** Green not resiniferous

**Leaves:** Needles green 6-9 cm x c.1.5mm., occurring in pair, rigid, more or less spine tipped.

**Cones:** 7-8 x 2.5mm brown and slightly shining; the exposed part of the scale is flat with a very short straight prickle.

**Seeds:** 7 mm long with a wing c.2.5 cm long.

**Habitat:** A resistant montane species occurring on limestone in the Balkan Peninsula.

**Distribution:** Al, Gr, Yu.

### *Pinus sylvestris* L. (Scots Pine)

**Crown:** A tree up to 35-40 m long, pyramid- shaped or conical when young, becoming flat topped or rounded with age, with the branches sparsely arranged high up on the trunk.

**Bark:** at the base of the trunk reddish or grey – brown and cracked; on the upper trunk and branches orange-red to pink, and scaly.

**Shoots:** hairless and pale green, becoming brown.

**Buds:** cylindrical, dark brown or red.

**Leaves:** Blue grey or blue green needles, often twisted, growing in pairs, 3-7 cm long. Needles last from 3-6 years.

**Male cones:** small, yellow and rounded, clustered near the tips of the shoots in early summer.

**Female cones:** pink and globular when fertilize, becoming green and turning down on the stem during the next year. Mature third –year cones, 3-8 cm long, are grey –brown, ovoid and pointed.

**Habitat:** Mainly in lowlands, but in mountains to over 2000m. Tolerates a range of soils; hardy, not sensitive to drought. In Europe its natural habitat is in the more extreme sites(for example those on very dry, poor sandy, sometimes boggy soils) . Semi – shade or light –loving species;deep-rooting.

**Distribution;**Native over much of Europe and northern and western Asia, the Scots pine is very widely planted for its valuable timber; it also makes an attractive hardy ornamental tree, thriving in light acid soils. Grows to 35-40 m.

**Uses:** yields a good multi –purpose resinous timber with reddish heart –wood and pale brown sapwood.

*Pinus mugo* Turra (Mountain Pine)

**Crown:** A shrub or small tree with many crooked irregularly spreading branches.

**Bark:** The bark is grey-black and scaly.

**Buds:** Ovoid- cylindrical and very resiniferous.

**Leaves:** 3-8 cm x 1.5-2 mm., occurring in pairs, bright green, curved, rigid persisting 5 years or more.

**Cones:** Subterminal, solitary or in groups of 2-3, ovate-conical, up to 5cm long x 1.5-2.5 cm wide; the exposed part of the scale is flat, or convex above and concave below.

**Seeds:** Small.

**Habitat:** A common species to the mountains of C. Europe and the Balkan Peninsula. It is frequently planted for sand binding and shelter in N.Europe.

**Distribution:** Al, Au, Bu, Cz, Fr, Ge, Gr, It, Po, Rs, Ru, Sw, Yu.

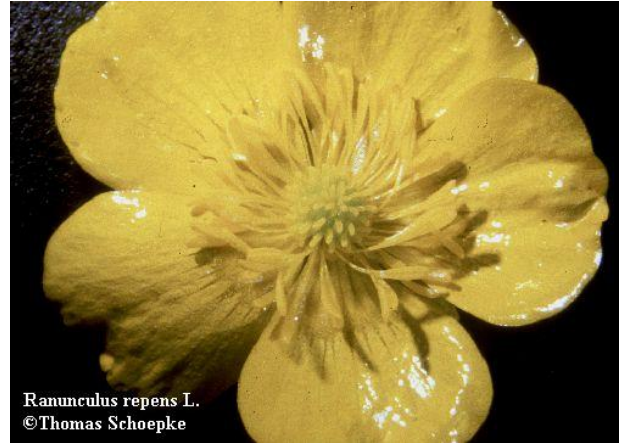
### **My key of pine species identification :**

Key features in identifying pines are leaves (occurrence, size, colour), shape of trees, shape of crowns, bark and cones (shape, size, colour) .

1. Leaves evergreen needle-like, not single, clustered or in whorls  $\mapsto$  2
1. Leaves evergreen, needle-like single  $\mapsto$  *Pinus monophylla*
2. Leaves in pairs with very toothed margins  $\mapsto$  5
2. Leaves very long, in pairs  $\mapsto$  6
3. Leaves in pairs, (not very long) 2.5-8 cm blue to bluish –green, glaucous = *P. sylvestris*  
Leaves 2.5-8 occur on a shrub then = *P. mugo*  
Leaves 6-9 cm very dark green ; when bark very ashygrey = *P. heldreichii*
4. Leaves in bundles of 5  $\mapsto$  *P. peuce*  
Leaves in bundles of 3 -5  $\mapsto$  7
5. Leaves in pairs 6-15 cm long clear green, curved , twisted, with toothed margins where when holding the middle between thumb and index finger and moving the fingers from the needle's apex towards the base, we get the sensation of a rough surface whereas moving our fingers towards the opposite direction the needle's surface feels smooth  $\mapsto$  = *P. halepensis*  
Leaves 10-15 cm long stout, straight or curved with not so toothed margins = *Pinus nigra*.
6. Leaves in pairs, 10-20 cm; crown spherical; 20-25 m tree high; bark very reddish grey with deep longitudinal fissures especially on old trees with big cones 8-14 cm long= *P. pinea*  
Leaves in pairs 10-25 cm long , crown wide flat, tree height , with cones 8-22 cm long 40 m = *P. pinaster*
7. Leaves in clusters of 3, 10-15 long  $\mapsto$  *Pinus radiata*
7. Leaves in clusters of 3, 15-30 cm long  $\mapsto$  *Pinus ponderosa*

## Formal description of *Ranunculus repens* L. (Ranunculaceae)

Perennial herb, with long, stout fibrous roots, and stolons which root at the nodes and an +- erect flowering stems to 60 cm; basal leaves triangular-ovate in outline, the usually pubescent-hairy leaves are divided into 3 lobes, each of which is again divided into 3 toothed segments, the middle one long-stalked and borne above the 2 laterals; Flowers medium-sized, 5–15 mm in diameter or length; borne on a stalk; bisexual; actinomorphic; glossy, yellow 20-30 accros; furrowed, hairy pedicels, 6-10 cm in length, support the flowers in a more or less erect position; corolla composed of 5 free, glossy, golden yellow petals (16 x 12 mm), and the calyx of 5 free, hairy sepals (9x4 mm); sepals not reflexed; achenes 2.5-3.8 mm long, 2–2.8 mm wide, glabrous, smooth, with short curved beak ; 2n=32; on the upper surface of the petal base, situated pocket-like nectary; anthers 2.25 mm, filaments up to 5.5 mm; stamens 25–40; vary in length; ovary: 1 mm hypogynous Carpels apocarpous; 35–60; ovule:0.3 mm; group of achenes: 4x4 mm; the carpels and stamens are attached to a cone-shaped and hairy receptacle.



### Flora formula :

K5 C5 A G1-

### Chromosome information

2n = 14, or 32; FNA.

### Distribution

Northern hemisphere distribution: all continents except Africa and Antarctica. North American phytogeography: introduced. Abundance in the province: common. Northern hemisphere: Newfoundland, Labrador, St. Pierre and Miquelon, Canada, United States, Eurasia. Canada: Nfld., Lab., N.S., P.E.I., N.B., Que., Ont., Alta., B.C., Yukon. USA: Middle Eastern States, North Midwest States, South Midwest States, North Rockies, Southern Rockies, California, Alaska. Newfoundland distribution: throughout; Labrador distribution: western, or southern.

### Ecology and habitat

Margins of ponds, disturbed areas (lawns, roadsides, etc.), meadows.

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