Phylogenetic Analysis of the Genus *Pistacia* (Anacardiaceae)

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Abstract

Pistacia is an economically important genus because it contains the pistachio crop, *P. vera,* which has edible seeds of considerable commercial importance. The evolutionary history of the genus and the taxonomic relationships among the species are controversial and not well understood. This study that has been conducted on this genus to refine taxonomic and evolutionary relationship utilizing different types of data (including morphology, anatomy and molecular) The studied species were the following: *Pistacia aethiopica* J. O. Kokwaro, *P. atlantica* Desf., *P. chinensis* Bunge, *P. eurycarpa* Yaltirik, *P. falcata* Becc. ex Martelli, *P. integerrima* Stew. ex Brand., *P. khinjuk* Stocks, *P. lentiscus* L., *P. mexicana* HBK, *P. mutica* Fisch. & Mey., *P. palaestina* Boiss., *P. terebinthus* L., *P. texana* Swingle, *P. vera* L., and *P. weinmannifolia* Poiss. ex Franch. Phylogenetic analysis based on morphological data strongly supported the monophyly of *Pistacia*. The genus divided into two monophyletic groups. One group (Section *Pistacia*) contains *P. atlantica*, *P. chinensis*, *P. eurycarpa*, *P. falcata*, *P. integerrima*, *P. khinjuk*, *P. mutica, P. palaestina*, *P. terebinthus,* and *P. vera* while the other group (Section *Lentiscus*) contains *P. aethiopica, P. lentiscus, P. mexicana*, *P. texana*, and *P. weinmannifolia*. In anatomical analysis, all species had anomocytic stomata. In most species, the stomata density was higher on the abaxial surface than the adaxial. The ratio of abaxial to adaxial stomatal density varied from 0.0 to 1.7. Stomatal distribution may provide insights into how *Pistacia* species evolve in terms of leaf anatomy and respond to different climatic changes. Stomatal distribution changed (losing stomata on either surface) as the genus moved into regions of higher rainfall. This study revealed leaflets of *P. vera*, which have random orientation, were isobilateral, while leaflets of the other species were dorsiventral and were oriented horizontally. RAPD analysis showed that *P. khinjuk* and *P. vera* are very close species. This study provides more insights into understanding the evolution, taxonomy and genetics of this economically important genus.

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Mohannad AL-Saghir
June 2006

DEDICATION

To My Dear Lovely Wife
BUROUJ

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Chapter 1

Pistacia: A Review of Taxonomic Classification

Introduction

Pistacia L. belongs to the family Anacardiaceae (cashew family), order Sapindales (Zohary, 1952; Pell, 2004). It contains nine species and five subspecies according to the current study. Species are xerophytic trees, deciduous or evergreen and dioecious, up to 8-10 m high. Leaves are pinnately-compound, with (1)2-32 broad, elliptical to round-ovate leaflets. Buds are single, apical, and usually vegetative. In both male and female trees, flowers are apetalous, subtended by 1-3 small bracts and 2-7 bracteoles and borne in racemes or panicles. Male flowers have 4-5 anthers inserted on a disc. Female flowers have a short, 3-fid style. The species are wind-pollinated. The fruit is a drupe.

Pistacia is believed to have originated in Central Asia 80 million years ago (Parfitt and Badenes, 1997; Kafkas and Perl-Treves, 2001). Two centers of diversity have been described. One comprises the Mediterranean region of Europe, Northern Africa, and the Middle East (Appendix A, Chapter 2). The second comprises West and Central Asia (Appendix A, Chapter 2) (Kafkas and Perl-Treves, 2002; Kafkas et al., 2002). The species of the genus occur naturally from North Africa to the Philippines and in Honduras and Mexico to Texas (Appendix A, Chapter 2).

Few systematic studies have been published on this important genus. The first complete classification of the genus was published by Zohary (1952). Today, there are many questions about Zohary's taxonomic treatment, the status of many of the species, and the accuracy of dividing the genus into four sections. This disagreement is mainly because of little information being available on the phylogeny of the genus. Previous phylogenetic studies were hampered by the small sampling size, weak representation of the species, and poor resolution of the methods used. Consequently, the studies failed to resolve the relationships between species within the genus. Moreover, species of *Pistacia* easily form interspecific hybrids, suggesting close relationships, which limit the previous studies and make the actual level of speciation and relationships within the genus unclear. A better understanding of these relationships is also needed to make the species more useful for plant improvement or genetic studies.

A comprehensive taxonomic revision, utilizing different types of data, is urgently needed for *Pistacia* in order to clarify the phylogenetic relationships between the species and to characterize the collective germplasm. This will provide a framework and guidelines for plant improvement and help to preserve the genetic resources of this important crop, especially since these genetic resources are under threat by extensive forest cutting in their native countries (especially in the Mediterranean countries, and Central and Western Asia).

Taxonomic History

Linnaeus was the first to establish the genus. In his *Species plantarum*, Linnaeus (1753) recognized three species of *Pistacia*: *P. lentiscus*, *P. terebinthus*, and *P. vera*. Prior to Linnaeus, Tournefort (1700) considered *P. lentiscus* a distinct genus *Lentiscus*, while he classified *P. terebinthus* and *P. vera* under the genus *Terebinthus*. Desfontaines (1799) described *P. atlantica* as a new *Pistacia* species. Humboldt, Bonpland and Kunth, (1824) described *P. mexicana* as a new species. De Candolle (1825) in his revision of the genus, recognized the two latter species, *P. atlantica* Desf. and *P. mexicana* H.B.K. Bunge (1835) described *P. chinensis* as a new species; Fischer and Meyer (1838) described *P. mutica* as a new species; Boissier (1849) described *P. palaestina* as a new species; Stockmans (in Stockmans and Hooker, 1852) added *P.*

khinjuk as a new species. Marchand (1869), in his study of Terebinthaceae, reviewed the genus and recognized four species: *P. chinensis* Bunge, *P. mutica* Fisch. & Mey., *P. palaestina* Boiss., and *P. khinjuk* Stocks. Stewart (in Stewart and Brandis, 1874) described *P. integerrima* as a new *Pistacia* species.

The first monograph of the genus was provided by Engler (1883), who listed eight species: P. *atlantica, P. chinensis, P. khinjuk, P. lentiscus*, *P. mexicana, P. mutica*, *P. terebinthus*, and *P. vera*. However, Engler did not suggest any sectional subdivision of the genus. After Engler, several species were added by different authors. Martelli (1886) added *P. falcata*, Franchet (1886) described *P. weinmannifolia* and Swingle (1920) described *P. texana.*

At present, the most complete taxonomic study is that of Zohary (1952), who included 11 species in the genus and divided them into four sections: *Lentiscella* Zoh. **(**containing *P. mexicana* HBK and *P. texana* Swingle**)**; *Eu Lentiscus* Zoh. **(**containing *P. lentiscus* L., *P. saporte* Burnat, and *P. weinmannifolia* Poiss. ex Franch.**)**; *Butmela* Zoh. **(**containing *P. atlantica* Desf.**)**; and *Eu Terebinthus* Zoh. **(**containing *P. chinensis* Bunge, *P. khinjuk* Stocks., *P. palaestina* Boiss., *P. terebinthus* L., and *P. vera* L.**).**

Yaltirik (1967a, 1967b) classified *Pistacia* species in Turkey and described a new species, *P. eurycarpa* . This had been called *P. atlantica* var. *kurdica* by Zohary (1952). Zohary considered *P. palaestina* as a separate species, whereas Yaltirik (1967a, 1967b) retained it as a variety of *P. terebinthus*. Kokwaro described a new species from East Africa, *P. aethiopica***,** in Kokwaro and Gillett (1980). Sixty binomials for *Pistacia* were found in the International Plant Names Index (IPNI), 16 of which represent valid taxa (all those listed in this section) The hybrids will be further discussed in the taxonomic section of this study (Chapter 6).

Why is *Pistacia* important?

Pistacia vera L. (cultivated pistachio) is by far the most economically important species in the genus. It has edible seeds and considerable commercial importance. The other species grow in the wild and their seeds are used as a rootstock seed source and sometimes are used for fruit consumption, oil extraction, or soap production.

The pistachio is native to the arid zones of Central Asia (Appendix A, Chapter 2, Map 4); it has been cultivated for 3000-4000 years in Iran and was introduced into Mediterranean Europe by Romans at the beginning of the Christian Era (Crane, 1978). Pistachio cultivation extended westward from its center of origin to Italy, Spain, and other Mediterranean regions of Southern Europe, North Africa, and the Middle East, as well as to China, and more recently to the United States and Australia (Maggs, 1973; Hormaza et al., 1994, 1998). *Pistacia vera* is the only species in this genus that is successfully grown in orchards; it produces edible seeds large enough to be commercially acceptable. Pistachios are adapted to a variety of soils and are probably more tolerant of alkaline and saline soil than most tree crops (Tous and Ferguson, 1996). Moreover, Pistachios thrive in hot, dry desert-like conditions.

Pistachios are utilized mostly in the shell, for fresh consumption; processed uses include candy, baked goods, and ice cream. They also have folklore, medicinal and non- food uses such as toothache relief. The resin is used in Europe and North America; gum (dried resin) is used as a blood-clotting agent in Europe and the Middle East. Husks are used in India for dying and tanning. Pistachios have been reported as a remedy for scirrhus and sclerosis of the liver, abscesses, poor circulation, and other medical problems (Tous and Ferguson, 1996). Pistachio wood is good for carving, cabinetry, and firewood as is that of other species of *Pistacia.* Currently, Iran, the

United States, Turkey, and Syria are the main Pistachio producers in the world, contributing over 90% of the world production (FAO 2002).

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Chapter 2

Morphological Analysis

Introduction

Morphological characters provide a source of data for taxonomic research on different plant taxa and may help determine the direction and the course of evolution and provide the distinctions between species (Benson, 1962). Only a few studies have been published on the phylogenetic relationships between *Pistacia* species. These studies are mostly based on morphological characters. Leaf characteristics and fruit morphology were the main diagnostic traits used by Zohary (1952) to distinguish between the various species he recognized.

AL-Yafi (1978) divided *P. atlantica* into four subspecies based on their leaf morphologies. Kokwaro described a new species from East Africa, *P. aethiopica*, in Kokwaro and Gillett (1980), based on leaf morphology and tree size. Lin et al. (1984) characterized leaf morphology, photosynthesis, and leaf conductance of nine *Pistacia* species (*P. atlantica*, *P. chinensis*, *P. integerrima*, *P. khinjuk*, *P. lentiscus*, *P. mexicana*, *P. mutica*, *P. terebinthus*, *P. texana*, *P. vera*, and *P. weinmannifolia*). EL-Oqlah (1996) described *Pistacia* species in Jordan morphologically and anatomically (*P. atlantica*, *P. lentiscus*, and *P. palaestina)*. Kafkas and Perl-Treves (2001) addressed the taxonomic relationships and genetic variation of wild *Pistacia* germplasm in Turkey using morphological data and random amplified polymorphic DNA (RAPD). *Pistacia atlantic*a, *P. eurycarpa,* and *P. terebinthus*, the common wild species in the flora of Turkey, were included in this study. In addition, many varieties of *P. vera* were added for comparison. The morphological analysis revealed that among the species studied the closest species to *P. vera* is *P. eurycarpa*. Kafkas et al. (2002) characterized morphologically and surveyed the germplasm of three wild *Pistacia* species in Turkey

(*P. atlantica*, *P. eurycarpa*, and *P. terebinthus*). All genotypes displayed high diversity at the inter- and intra-specific level. Barazani et al. (2003) assessed the genetic variability of Mediterranean *P. lentiscus* genotypes by morphology, composition of essential oils, and RAPD. High polymorphism in morphological parameters was found among accessions, with no significant differences in relation to geographical origin or gender.

The objective of the present study was to clarify and provide more insights into understanding the taxonomic relationships among *Pistacia* species using morphological data. The study included 15 described species of the genus (Table 2.1). It was based on analysis of a wide range of herbarium material from the Field Museum, Chicago, Illinois, USA (F); Missouri Botanical Garden, St. Louis, Missouri, USA (MO); Royal Botanic Garden, Edinburgh, UK (E); Royal Botanic Gardens, Kew, UK (K); and Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA (VPI).

Four- hundred-twenty eight specimens were observed. The broad spectrum of the collections should ensure the inclusive distribution of all taxa. Thirty three samples represent four wild *Pistacia* species collected by the author during his trip to Jordan in June 2004. This trip was made in order to observe the plants in the field. Various literature sources were used to pinpoint and refine the taxonomy and the localities of specimens included.

Rhus aromatica, *R. copallina, R. glabra* and *Schinus molle* were included as an out-group to the genus based on recent studies that highlight the close relationship of *Rhus* and *Schinus* to *Pistacia* (Miller et al. 2001; Pell, 2004).

Description of *Pistacia* Species and Scoring Morphological Characters

Thirty characters were examined. Only 19 (1 habitat and 18 leaf characters) were used for phylogenetic analysis because these were the only characters found to be polymorphic among *Pistacia* species. The species were described with respect to tree and leaf morphology based on Benson (1979) using *Pistacia* descriptors developed by the International Plant Genetic Resources Institute (IPGRI 1998). Ten characters of 19 were qualitative (Table 2.2) and 9 were quantitative (Table 2.3). Each character was scored for up to 84 samples of each species.

Phylogenetic Analysis

Neighbor joining and parsimony analyses were performed on the morphological data using PAUP 4.0 b10 (Swofford, 2003). A heuristic search was performed by RANDOM stepwise addition (10,000 replicates) with tree bisection-reconstruction (TBR) branch swapping to find the most parsimonious tree. From the resulting trees, the majority rule consensus was calculated. Robustness and topology of the trees were evaluated using the consistency index (CI) to measure the level of homoplasy in the data (how cleanly the data fit the tree). The retention index (RI) was measured to determine the amount of synapomorphy on the tree. Robustness and reliability of the resulting phylogenies were measured by bootstrap (1,000 replicates with full heuristic searches). The stepwise distance matrix which measures the relatedness between the species (Kimura, 1980) was computed between all species. The data were analyzed with a neighbor joining approach.

Results

To investigate the relationships between the *Pistacia* species on the basis of morphological data, 19 phenotypic characters were coded as discrete states. All characters used were polymorphic and very useful in distinguishing between and separating the different species.

All the morphological characters used were parsimony informative characters. Parsimony analysis produced one maximally parsimonious tree of 49 steps, with a consistency index (CI) of 0.69 and a retention index (RI) of 0.75 (Fig. 2.1). This tree was consistent with the tree produced by neighbor joining analysis (Fig. 2.2). However, there were differences between the bootstrap support values of the different branches of the two trees and the relationships among species within each clade.

The morphological data used in the two analyses strongly supported the monophyly of *Pistacia*. The genus divided into two monophyletic groups: One group (Section *Pistacia*) contains *P. atlantica*, *P. chinensis*, *P. eurycarpa*, *P. falcata*, *P. integerrima*, *P. khinjuk*, *P. mutica, P. palaestina*, *P. terebinthus,* and *P. vera* while the other group (Section *Lentiscus*) contains *P. aethiopica, P. lentiscus, P. mexicana*, *P. texana*, and *P. weinmannifolia* (Figs. 2.1, 2.2).

Both analyses show that Section *Pistacia* contains three sister groups, the first group with *P. atlantica*, *P. mutica* and *P. eurycarpa*, the second with *P. chinensis*, *P. falcata*, and *P. integerrima* and the third group contains *P. khinjuk*, *P. palaestina*, *P. terebinthus*, and *P. vera*. The first group was monophyletic with the second group which had weak bootstrap support of 27% in the parsimony analysis and 35% in the neighbor joining analysis. Bootstrap support of the first group was 58% in the parsimony analysis and 88% in the neighbor joining analysis. *Pistacia atlantica* and *P. mutica* show a close relationship (56% in the parsimony analysis, 95% in the neighbor joining analysis). A branch appearing in 88% of the parsimony analysis bootstrap replicates separates *P. chinensis* from *P. falcata* and *P. integerrima* within the second group. *Pistacia falcata* and *P. integerrima* show a relationship with bootstrap support of 28%. A branch appearing in 91% of the neighbor joining analysis bootstrap replicates separates *P. integerrima* from *P. chinensis* and *P. falcata*. *Pistacia chinensis* and *P. falcata* show a relationship with 40% support.

Both analyses support the branch that separates the first group and the second group from the third group with bootstrap support of 46% in the parsimony analysis and 52% in the neighbor joining analysis. A branch appearing in 41% of the parsimony analysis bootstrap replicates separates *P. vera* and *P. khinjuk (*which are related with 33 % support) from *P. terebinthus* and *P. palaestina* (which are closely related with 81% support). A branch appearing in 56% of the neighbor joining bootstrap replicates separates *P. vera* from *P. khinjuk, P. terebinthus* and *P. palaestina*. A branch appearing in 47% of the neighbor joining bootstrap replicates separates *P. khinjuk* from *P. terebinthus* and *P. palaestina*. The latter two species are very closely related with 100 % support in the neighbor joining analysis. The only difference between the two analyses is the placement of P. *khinjuk* within the third group.

Section *Lentiscus,* as indicated by the two analyses, was also monophyletic. In the parsimony analysis, a branch appearing in 67% of the bootstrap replicates separates this section into two monophyletic groups, the first group contains *P. aethiopica*, *P. lentiscus* (both show a close relationship with bootstrap support of 80%) and *P. weinmannifolia*. The first two species were sister to *P. weinmannifolia* with moderate bootstrap support of 45%. The second group contains *P. mexicana* and *P. texana* which show a close relationship with bootstrap support of 70%.

In the neighbor joining analysis, a branch appearing in 71% of the bootstrap replicates separates the section into monophyletic groups. The first group contains *P. aethiopica* and *P. lentiscus* (which show a close relationship with bootstrap support of 82%) The second group contains *P. mexicana* and *P. texana* (which show a close relationship with bootstrap support of 97%) and *P. weinmannifolia* which is sister to the first two species with bootstrap support of 49%. The only difference between the two analyses is the placement of *P. weinmannifolia* between the two groups.

Discussion

This is the first comprehensive study to include this number of *Pistacia* species and a broad spectrum of herbarium specimens that represent each species. Both the maximally parsimonious tree and the neighbor joining tree resolve the relationships among *Pistacia* species based on the morphological data. The two trees support the monophyly of the genus as well as resolving the two sections of the genus *Pistacia* and *Lentiscus*. Section *Pistacia* species are large deciduous trees (rarely shrubs) while Section *Lentiscus* species are evergreen shrubs or small trees. Zohary (1952) divided the genus into four sections based on leaf and fruit morphology. In this study, I suggest a two sectional division of the genus based on leaf morphology. My results agree with Parfitt and Badenes's (1997) classification, which suggests the same two sectional division of the genus and with the results of cluster analyses made by Kafkas and Perl-Treves (2002), Katsiotis et al. (2003), and Golan-Goldhirsh et al. (2004). Moreover, the current study clearly defines the species status and the taxonomic relationships among the members of each section based on both analyses including the pairwise distances between all pairwise combinations of the species and the thorough morphological observation of a good number of specimens of each species (Table 2.4).

Within section *Pistacia, P. atlantica* was close and sister to *P. eurycarpa* (0.11 unit genetic distance) and *P. mutica*. While there was no genetic distance between *P. atlantica* and *P. mutica* (0.00 unit genetic distance) the two species are found on the same branch (Figs. 2.1, 2.2). Little morphological variation occurs in the specimens of the two species examined from different geographical areas. The measurements of all characters used were almost identical, and I found it difficult to distinguish between the two species. I investigated the original description of *P. mutica* by Fischer & Meyer (1883). It matches the description of *P. atlantica* (Desfontaines, 1779), and my analysis supports Zohary's (1952) determination that *P. atlantica* and *P. mutica* are the same species. Given the branch support in both parsimony and neighbor joining analyses (including genetic distance value) (Figs. 2.1, 2.2), I treat P. *mutica* as a synonym of *P. atlantica*. In addition, both have wide, odd-pinnate, membrananceous leaves with flattened petioles; the leaflets are 6-10, lanceolate, obtuse, and puberulent with a ciliated margin; the terminal leaflet is similar or smaller than the lateral ones; and the rachis is narrowly winged.

Zohary (1952) classified *P. eurycarpa* as a synonym of *P. atlantica* var. *kurdica* because of the presence of a leaf rachis wing. Yaltirik (1967a, 1967b), on the other hand, treated this plant as a different species based on leaf characters. In my analysis, *P. atlantica* was close and sister to *P. eurycarpa* (0.11 unit genetic distance) (Figs. 2.1, 2.2). I agree with Yaltirik's (1967a, 1967b) classification and observation of *P. eurycarpa* as a separate species (Figs. 2.1, 2.2) and not as a variety of *P. atlantica* as suggested by Zohary (1952) because the leaves are light-green on both sides (instead of being dark green above and pale below as in *P. atlantica*) and the fruits are depressed and larger (Yaltirik, 1967a, 1967b) which are confirmed by the current study. Furthermore, the leaflets (6.1-8.0 x 2.0-5.5 cm) are usually wider and thicker than and are never as numerous as in *P. atlantica* (3.0-7.0 x 0.5-2.5 cm) and the rachis wing is narrower or even absent. This treatment is supported by the phenotypic cluster analysis made by Kafkas and Perl-Treves (2001), in which the taxonomic placement of *P. eurycarpa* was identical to mine.

In both the parsimony analysis and the neighbor joining analysis (Figs. 2.1, 2.2), the closest relative of *P. vera* is *P. khinjuk*. According to the average genetic distances between the different species, *P. khinjuk* and *P. vera* were separated by 0.42 unit genetic distance. I consider *P. khinjuk* to be the closest relative to *P. vera* but a separate species. *P. khinjuk* has odd-pinnate membrananceous leaves, the leaflets are 2-6 and distinctly pinnately veined with an acuminate apex, while leaves in *P. vera* are trifoliolate or odd-pinnate and leathery, and the leaflets are 2-4, reticulate-veined with an obtuse or mucronulate apex.

According to both analyses, *P. palaestina* and *P. terebinthus* are closely related. They form a close pair and have been regarded as a single species (0.00 unit genetic distance). Engler (1883) the first to monograph *Pistacia*, considered *P. palaestina* as a variety of *P. terebinthus*. However, Zohary (1952) considered *P. palaestina* as a distinct species, because of two main distinctive characteristics: *P. palaestina* has mostly even-pinnate leaves and acuminate leaflets, while *P. terebinthus* has odd-pinnate leaves and obtuse or acute leaflets. Yaltirik (1967a) described two subspecies within *P. terebinthus*. The first, *P. terebinthus* subsp. *terebinthus* had odd-pinnate leaves with the terminal leaflet of the median leaves often as large as the lateral ones, and obtuse or ovate-oblong lateral leaflets. The second *P. terebinthus* subspecies had either even-pinnate and/or odd-pinnate leaves; the terminal leaflet of the median leaves was always smaller than the laterals or reduced to a bristle, and it had acuminate or oblong-lanceolate lateral leaflets. My results support Engler’s and Yaltirik’s classifications. The two entities are morphologically, ecologically, and genetically similar (Zohary, 2000; Kafkas and Perl-Treves 2002). I did not observe any morphological variation unique to either species. I have found it difficult to distinguish between *P. palaestina* and *P. terebinthus* specimens, and noticed that problem as well in Yaltirik's annotations of specimens. Leaves in both species are odd-pinnate or even-pinnate and membrananceous; the leaflets are 6-10, ovate to narrowly ovate with an acuminate or mucronate apex; the terminal leaflet (if present) is smaller than the laterals or reduced. Therefore I suggest that *P. palaestina* should be merged with *P. terebinthus* and become a synonym of the latter name.

P. chinensis is the most heterogeneous and complex species in the genus. Both analyses reveal that *P. chinensis*, *P. falcata*, and P. *integerrima* are close sister taxa. In the parsimony analysis (Fig. 2.1), *P. chinensis* was sister to *P. falcata* and *P. integerrima*. In the neighbor joining analysis (Fig. 2.2), *P. integerrima* was sister to *P. chinensis* (0.13 unit genetic distance) and *P. falcata* (0.13 unit genetic distance*)*. My results support Zohary's (1952) conclusion that theses names represent the same species. If one compares the specimens of the three species, he or she also would not fail to observe the close relationships between these taxa. The three species have large deciduous leaves which are 13.0-21.0 cm long and 11.4-18.2 cm wide, odd-pinnate or even-pinnate, membrananceous, with a flattened petiole; the leaflets are 10-14, 5.2-9.1 cm long and 1.0-2.0 cm wide, narrowly lanceolate, falcate, attenuate, and glabrous; the terminal leaflet (if present) is 2.0-4.0 cm long and 0.5-1.1 cm wide, smaller than the laterals or reduced; the rachis is not winged.

I suggest that *P. falcata* should be treated as a subspecies of *P. chinensis* not as a variety because their populations are separated geographically (Appendix A, Map 3). Leaves in *P. falcata* (13.0-21.0 cm long and 11.4-18.2 cm wide) are larger than in *P.*

chinensis (8.2-23 cm long and 8.0-20.0 cm wide). In *P. chinensis*, leaflets are 8-16 and lanceolate, while leaflets in *P. falcata* are 10-14, and narrowly lanceolate. Moreover, *P. falcata* leaflets have an elongated apex, abundant veins, and distinct narrow lanceolate leaflets.

Pistacia falcata is found in East Africa (Appendix A, Map 3). Its ancestors may have been transported to Madagascar and East Africa from India or China by migratory birds as hypothesized by Renner (2004) for Melastomataceae.

Anacardiaceae including *Pistacia* have fruits that are adapted for bird dispersal. The fruits are single-seeded. These fruits have a fleshy mesocarp, pseudarils, and seeds are high in fat and protein; they have thick endocarps that protect the seed from being crushed (Jordano, 1989). Birds probably discard the endocarp immediately after eating the mesocarp or pseudaril and/or void some of the endocarps intact later. This mode of dispersal has been suggested, based on these fruit adaptations, by Weeks et al. (2005) as the major mode of Burseraceae fruit dispersal and Burseraceae is the sister family of Anacardiaceae.

East African *Pistacia* could have been transported by fruits that may have been blown by the monsoon wind system via occasional storms between Africa, the Chagos Archipelago (half way between Africa and Indonesia) and India. Alternatively, fruits could have become lodged in floating vegetation as hypothesized by Renner (2004) for Melastomataceae.

I agree with Zohary's (1952) classification of *P. integerrima* as a subspecies of *P. chinensis* also. *P. integerrima* has large leaves, 11.0-22.0 cm long and 12.0-23.0 cm wide; the leaflets are 6-14, 6.0-11.5 cm long and 2.0-4.0 cm wide, and broadly lanceolate. In *P. chinensis* the leaves are 8.2-23.0 cm long and 8.0-20.0 cm wide; the leaflets are 8-16, 4.0-10.0 cm long and 0.9-2.4 cm wide, and lanceolate. Moreover, the distinct geographical distribution of this taxon (native to the western Himalayas) from *P. chinensis* subsp. *chinensis* is another piece of evidence to consider this plant as a subspecies not as a variety.

Within Section *Lentiscus,* in both analyses *P. lentiscus* and *P. aethiopica* form a close pair. Kokwaro in (Kokwaro and Gillett, 1980) described *P. aethiopica* from East Africa based on leaf morphology and tree size. My results support Zohary's classification of it as a variety of *P. lentiscus* (*P. lentiscus* var. *emarginata*). *P. aethiopica* has a distinct leafy narrow rachis wing, abundant large veins and an emarginate or mucronulate leaflet apex. Ancestors of this taxon may have reached East Africa in the same way as was hypothesized for *P. falcata* above (Appendix A, Map 7). Therefore, I suggest that this plant should be treated as a subspecies of *P. lentiscus* not as a variety given the above morphological variation and the distinct geographical distribution from *P. lentiscus* subsp. *lentiscus*.

In both analyses, *P. mexicana* and *P. texana* form a close pair and they are regarded as one species (0.00 unit genetic distance). Zohary (1952) and Parfitt and Badenes (1997) classified them as separate species. I suggest that *P. texana* and *P. mexicana* are the same species; *P. texana* should be merged with *P. mexicana* and the name becomes a synonym of the latter. They both have small leaves, 5.1-14.1 cm long to 1.8-5.3 cm wide, odd-pinnate, membrananceous with a flattened petiole; the leaflets are 10-32 small, 1.0-2.5 cm long and 0.3-1.0 cm wide, narrowly ovate, slightly falcate, distinctly mucronate, and glabrous; the terminal leaflet is 0.5-2.5 cm long and 0.3-1.0 cm wide, similar to or smaller than the lateral ones; the rachis is narrowly winged. *Pistacia mexicana* is the only New World species in the genus (Appendix A, Map 8). It is perhaps descended from an ancestor like the Asian P. *weinmannifolia* and its ancestors may have reached the New World via long distance dispersal or migration through a probable trans-Atlantic Tertiary Landbridge as hypothesized for Burseraceae by Weeks et al. (2005).

Pistacia weinmannifolia is sister to *P. mexicana* and *P. texana* (3.0 unit genetic distance) in the parsimony analysis while in the neighbor joining analysis it is sister to *P. lentiscus* (5.0 unit genetic distance) and *P. aethiopica* (3.0 unit genetic distance). Morphologically, *Pistacia weinmannifolia* is closer to *P. mexicana* than to *P. lentiscus* and *P. aethiopica.* Both have small, membrananceous leaves; the leaflets are small, ovate to narrowly ovate (rarely obovate), emarginate and are often alternate. This supports (but not strongly) the hypothesis that the ancestor of *P. mexicana* resembled P. *weinmannifolia*.

In comparison between the neighbor joining tree and the parsimony tree, there was a disagreement in placing a number of taxa such as *P. vera* and *P. integerrima* (Figs. 2.1, 2.2). This may be because of the different philosophy and assumptions of each method, treatment of the data (distance matrix in the neighbor joining method and discrete matrix in the parsimony method). The difference in the resolution of the morphological characters may account for this variation. The following characters could contribute to the difference: leaflet number, leaflet shape, leaflet apex shape and size of leaflet.

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Table 2.1: List of *Pistacia* species used in the current study.

Species Name
P. aethiopica
P. atlantica
P. chinensis
P. eurycarpa
P. falcata
P. integerrima
P. khinjuk
P. lentiscus
P. mexicana
P. mutica
P. palaestina
P. terebinthus
P. texana
P. vera
P.weinmannifolia

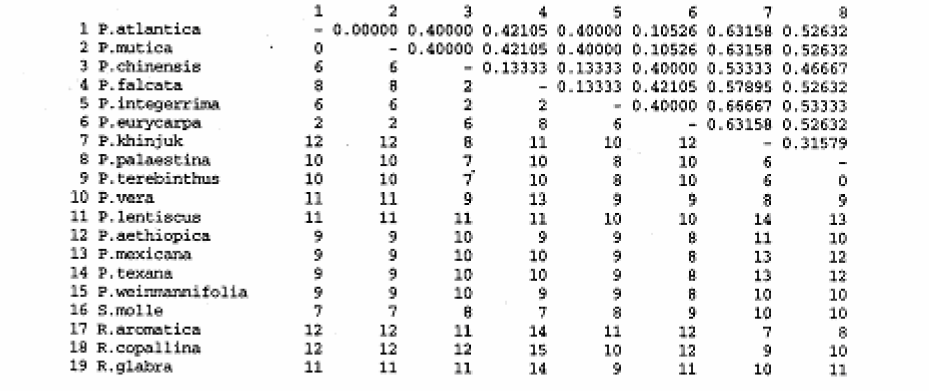
Table 2.2: Qualitative morphological characters used for phylogenetic analysis in this study.

	0	1	2	3
1-Growth habit	Shrub	Tree		
2-Leaf persistency	Evergreen	Deciduous		
3-leaf indumentum	Glabrous	Puberulent		
4-Leaf texture	Membranaceous	Leathery		
5-Leaf rachis wing	Absent	Present		
6-Lateral leaflet shape	Lanceolate, Narrowly or Lanceolate	Ovate, Narrowly or Broadly Ovate, Elliptical or Oboavte		
7-Lateral leaflet apex	Obtuse, Mucronulate, Emarginate	Acuminate	Attenuate	Mucronate
8-Terminal leaflet	Absent	Present		
9-Relative size of terminal leaflet to laterals.	Smaller	Similar	Larger	
10-Petiole shape	Flattened	Angled	Rounded	

Table 2.3: Quantitative morphological characters used for phylogenetic analysis in this Study.

	0	1	2	3	4
1-Leaf length (cm)	**2.0-7.0**	**7.1-12.0**	**12.1-17.0**	**17.1-1-22.0**	**22.1-30.0**
2-Leaf width (cm)	**1.0—6.0**	**6.1-11.0**	**11.1-16.0**	**16.1-23.0**	
3-Number of leaflet pairs	**1.0-3.0**	**4.0-6.0**	**7.0-9.0**	**10.0-12.0**	**13.0-20.0**
4-Lateral leaflet length (cm)	**1.0-4.0**	**4.1-8.0**	**8.1-12.0**		
5-Lateral leaflet width (cm)	**0.3-2.0**	**2.1- 4.2**	**4.3-8.0**		
6-lateral leaflet length/width ratio	**1.0-3.0**	**3.1-5**	**5.1-8.0**		
7-Terminal leaflet length (cm)	**1.0-4.0**	**4.1—8.0**	**8.1-12.0**		
8-Terminal leaflet width (cm)	**0.3-2.0**	**2.1- 4.2**	**4.3-8.0**		
9-Terminal leaflet length/width ratio	**1.0-3.0**	**3.1-5.0**	**5.1-8.0**		

Table 2.4: Stepwise morphological distance matrix.



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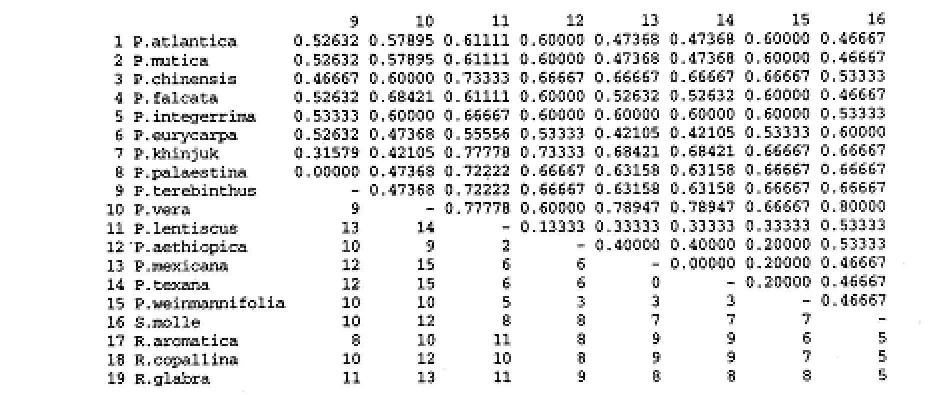
                             1       2       3       4       5       6       7       8
  1 P.atlantica              - 0.00000 0.40000 0.42105 0.40000 0.10526 0.63158 0.52632
  2 P.mutica                 0       - 0.40000 0.42105 0.40000 0.10526 0.63158 0.52632
  3 P.chinensis              6       6       - 0.13333 0.13333 0.40000 0.53333 0.46667
  4 P.falcata                8       8       2       - 0.13333 0.42105 0.57895 0.52632
  5 P.integerrima            6       6       2       2       - 0.40000 0.66667 0.53333
  6 P.eurycarpa              2       2       6       8       6       - 0.63158 0.52632
  7 P.khinjuk               12      12       8      11      10      12       - 0.31579
  8 P.palaestina            10      10       7      10       8      10       6       -
  9 P.terebinthus           10      10       7      10       8      10       6       0
 10 P.vera                  11      11       9      13       9       9       8       9
 11 P.lentiscus             11      11      11      11      10      10      14      13
 12 P.aethiopica             9       9      10       9       9       8      11      10
 13 P.mexicana               9       9      10      10       9       8      13      12
 14 P.texana                 9       9      10      10       9       8      13      12
 15 P.weinmannifolia         9       9      10       9       9       8      10      10
 16 S.molle                  7       7       8       7       8       9      10      10
 17 R.aromatica             12      12      11      14      11      12       7       8
 18 R.copallina             12      12      12      15      10      12       9      10
 19 R.glabra                11      11      11      14       9      11      10      11
```



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                             9      10      11      12      13      14      15      16
  1 P.atlantica        0.52632 0.57895 0.61111 0.60000 0.47368 0.47368 0.60000 0.46667
  2 P.mutica           0.52632 0.57895 0.61111 0.60000 0.47368 0.47368 0.60000 0.46667
  3 P.chinensis        0.46667 0.60000 0.73333 0.66667 0.66667 0.66667 0.66667 0.53333
  4 P.falcata          0.52632 0.68421 0.61111 0.60000 0.52632 0.52632 0.60000 0.46667
  5 P.integerrima      0.53333 0.60000 0.66667 0.60000 0.60000 0.60000 0.60000 0.53333
  6 P.eurycarpa        0.52632 0.47368 0.55556 0.53333 0.42105 0.42105 0.53333 0.60000
  7 P.khinjuk          0.31579 0.42105 0.77778 0.73333 0.68421 0.68421 0.66667 0.66667
  8 P.palaestina       0.00000 0.47368 0.72222 0.66667 0.63158 0.63158 0.66667 0.66667
  9 P.terebinthus            - 0.47368 0.72222 0.66667 0.63158 0.63158 0.66667 0.66667
 10 P.vera                   9       - 0.77778 0.60000 0.78947 0.78947 0.66667 0.80000
 11 P.lentiscus             13      14       - 0.13333 0.33333 0.33333 0.33333 0.53333
 12 P.aethiopica            10       9       2       - 0.40000 0.40000 0.20000 0.53333
 13 P.mexicana              12      15       6       6       - 0.00000 0.20000 0.46667
 14 P.texana                12      15       6       6       0       - 0.20000 0.46667
 15 P.weinmannifolia        10      10       5       3       3       3       - 0.46667
 16 S.molle                 10      12       8       8       7       7       7       -
 17 R.aromatica              8      10      11       8       9       9       6       5
 18 R.copallina             10      12      10       8       9       9       7       5
 19 R.glabra                11      13      11       9       8       8       8       5
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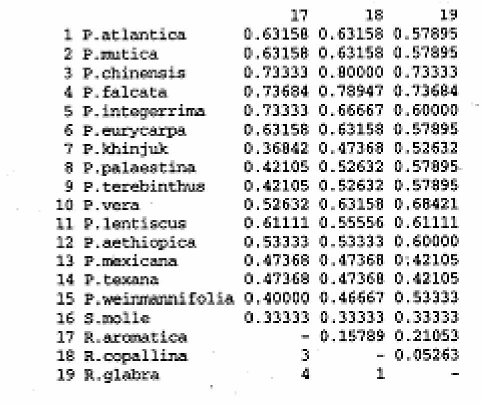


```
                            17      18      19
  1 P.atlantica        0.63158 0.63158 0.57895
  2 P.mutica           0.63158 0.63158 0.57895
  3 P.chinensis        0.73333 0.80000 0.73333
  4 P.falcata          0.73684 0.78947 0.73684
  5 P.integerrima      0.73333 0.66667 0.60000
  6 P.eurycarpa        0.63158 0.63158 0.57895
  7 P.khinjuk          0.36842 0.47368 0.52632
  8 P.palaestina       0.42105 0.52632 0.57895
  9 P.terebinthus      0.42105 0.52632 0.57895
 10 P.vera             0.52632 0.63158 0.68421
 11 P.lentiscus        0.61111 0.55556 0.61111
 12 P.aethiopica       0.53333 0.53333 0.60000
 13 P.mexicana         0.47368 0.47368 0.42105
 14 P.texana           0.47368 0.47368 0.42105
 15 P.weinmannifolia   0.40000 0.46667 0.53333
 16 S.molle            0.33333 0.33333 0.33333
 17 R.aromatica              - 0.15789 0.21053
 18 R.copallina              3       - 0.05263
 19 R.glabra                 4       1       -
```

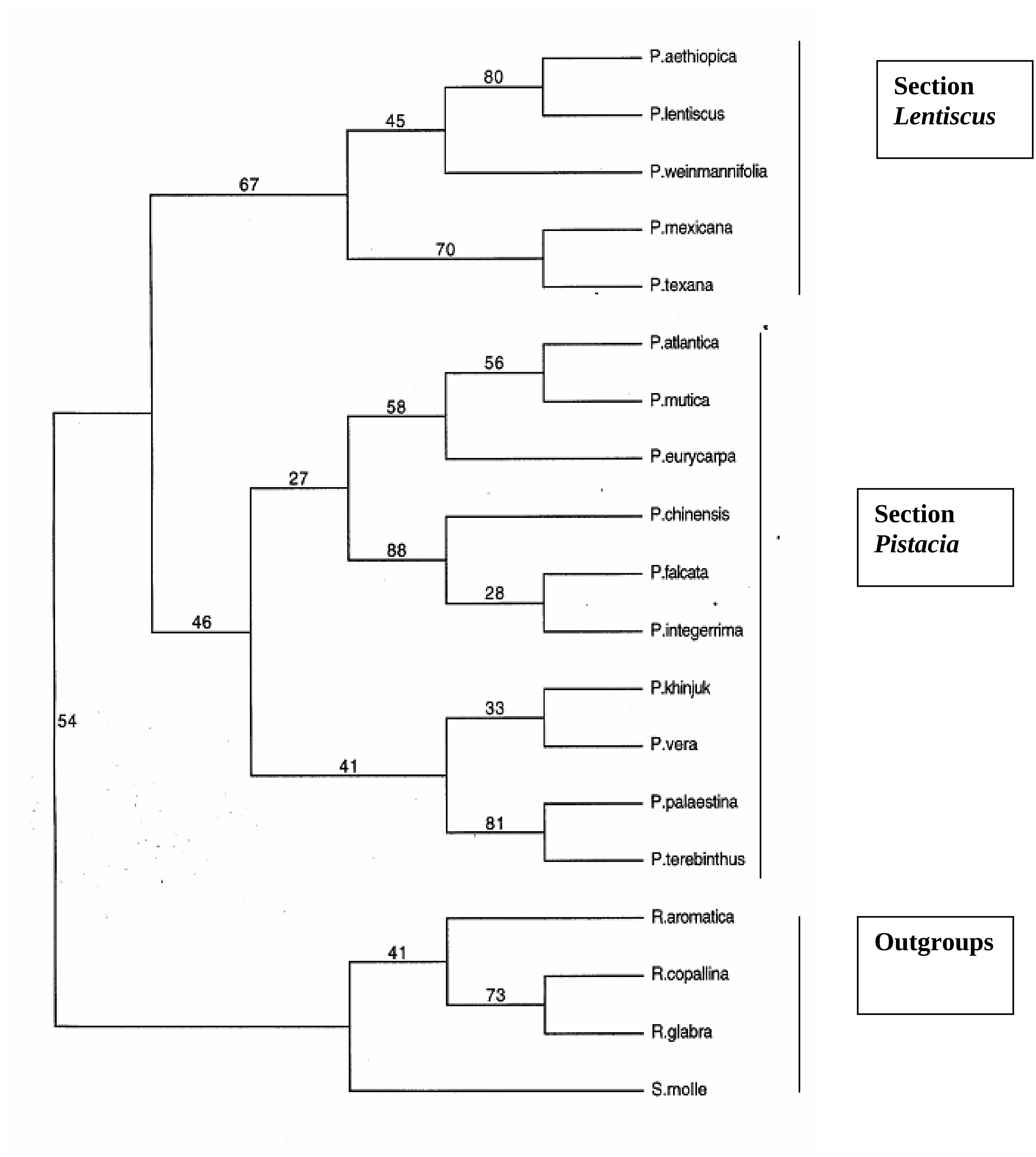


Figure 2.1: The majority rule consensus tree of 232 equally parsimonious tress generated with morphological data using parsimony analysis showing relationships of the *Pistacia* species with CI=0.69, RI=0.75 and tree length 49 steps. Numbers above the branches indicate the bootstrap values.

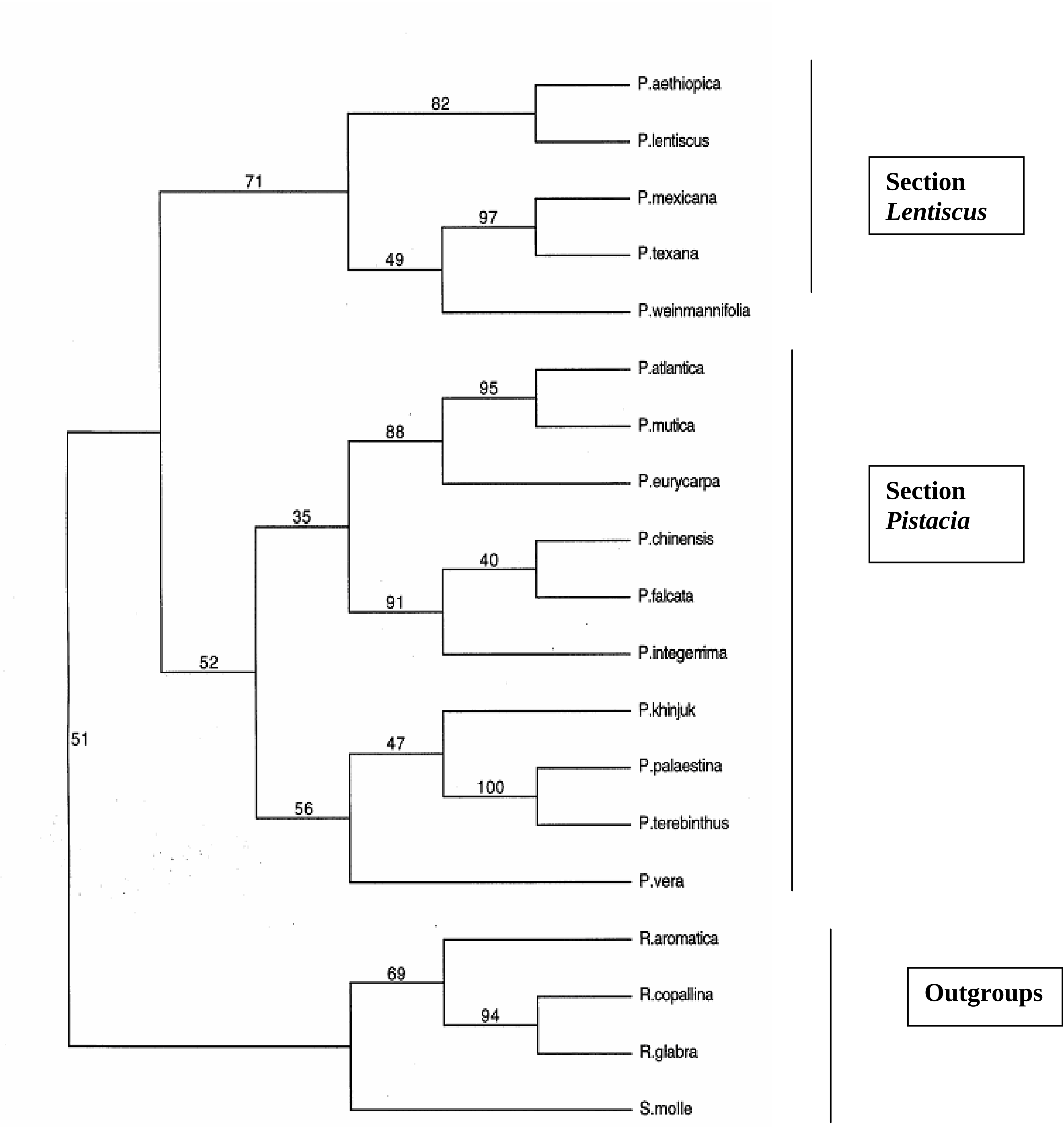


Figure 2.2: The majority rule consensus tree generated with morphological data using neighbor joining analysis showing relationships of the *Pistacia* species. Numbers above the branches indicate the bootstrap values.

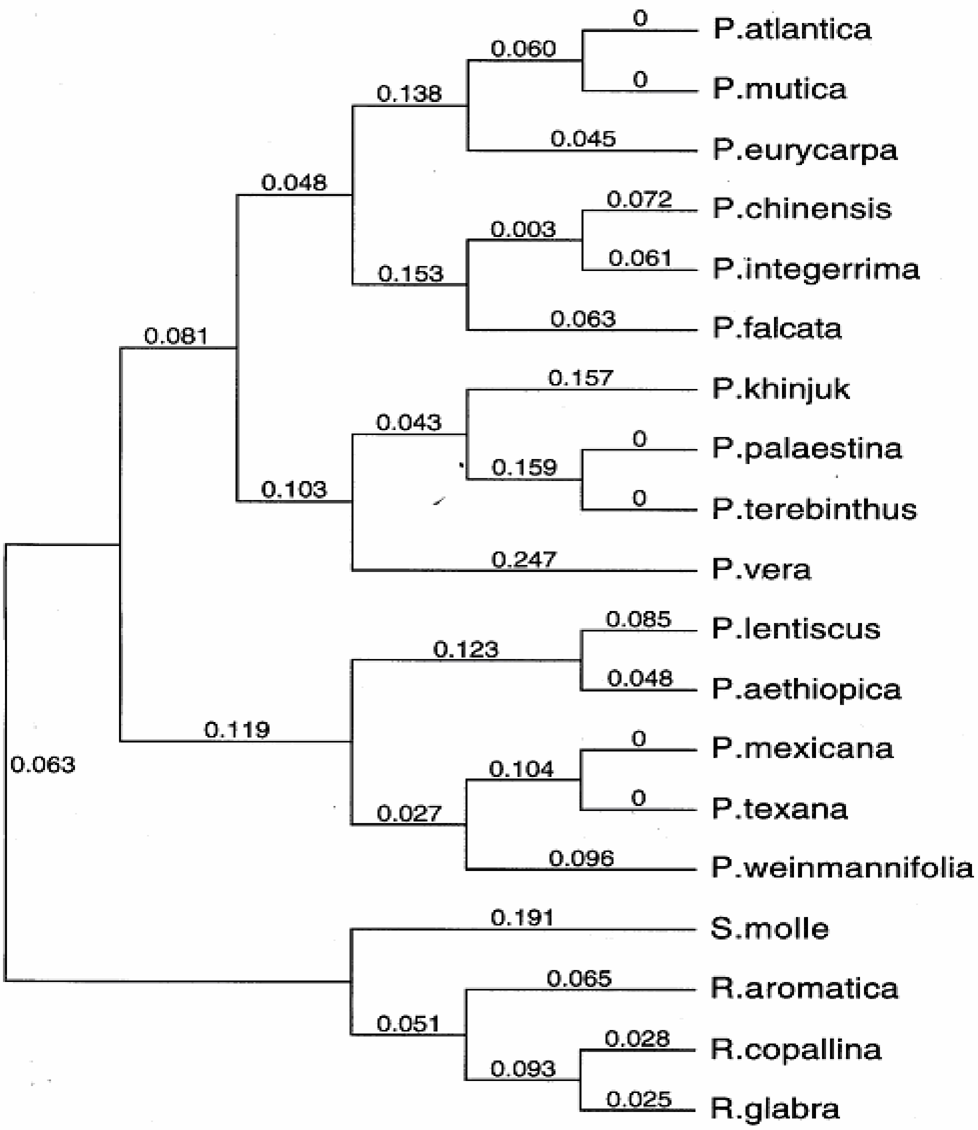


Figure 2.3: The majority rule consensus tree generated with morphological data using neighbor joining analysis showing relationships of the *Pistacia* species. Numbers above the branches indicate the branch lengths.

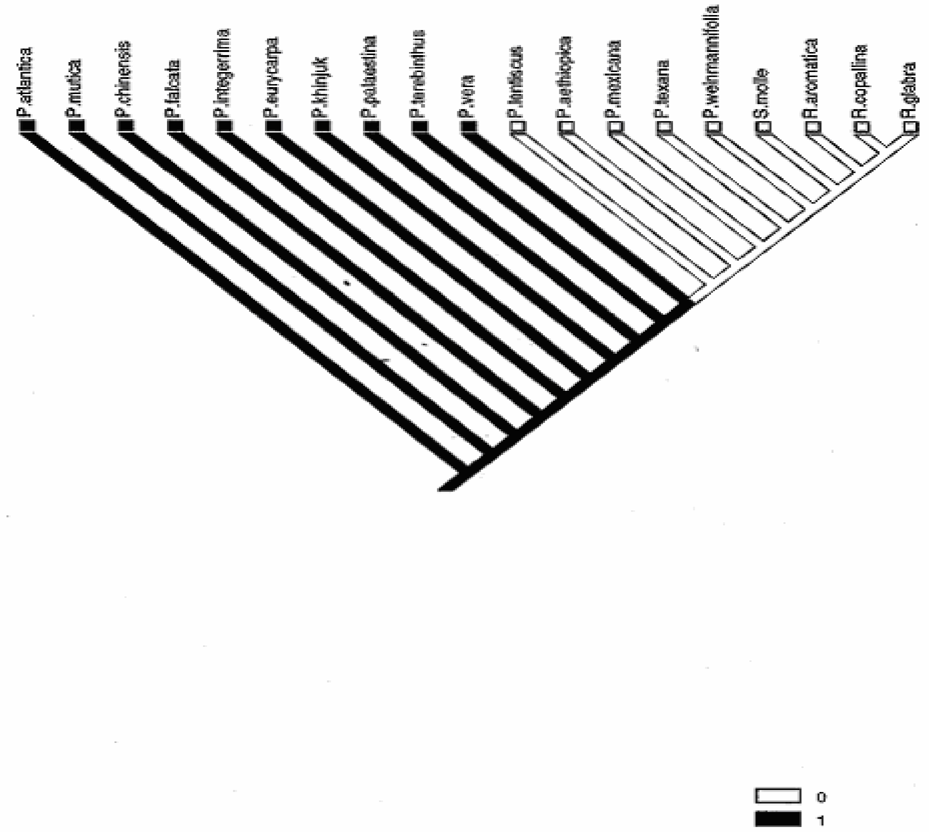
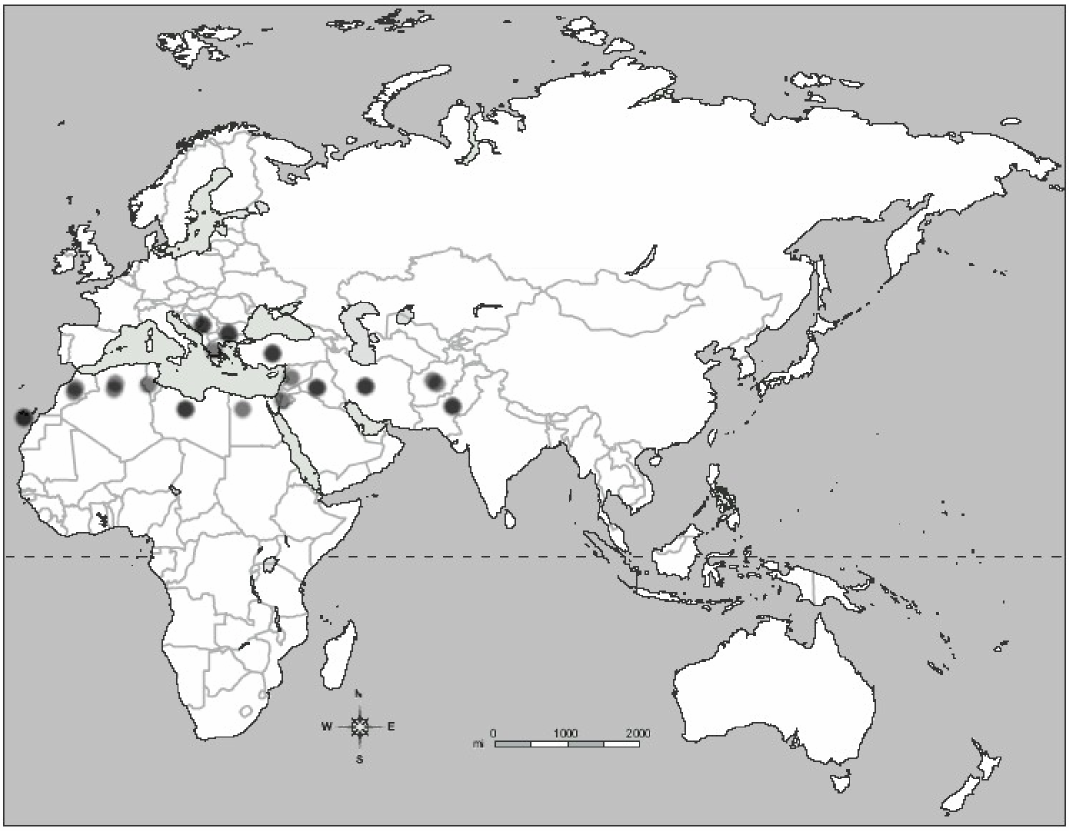


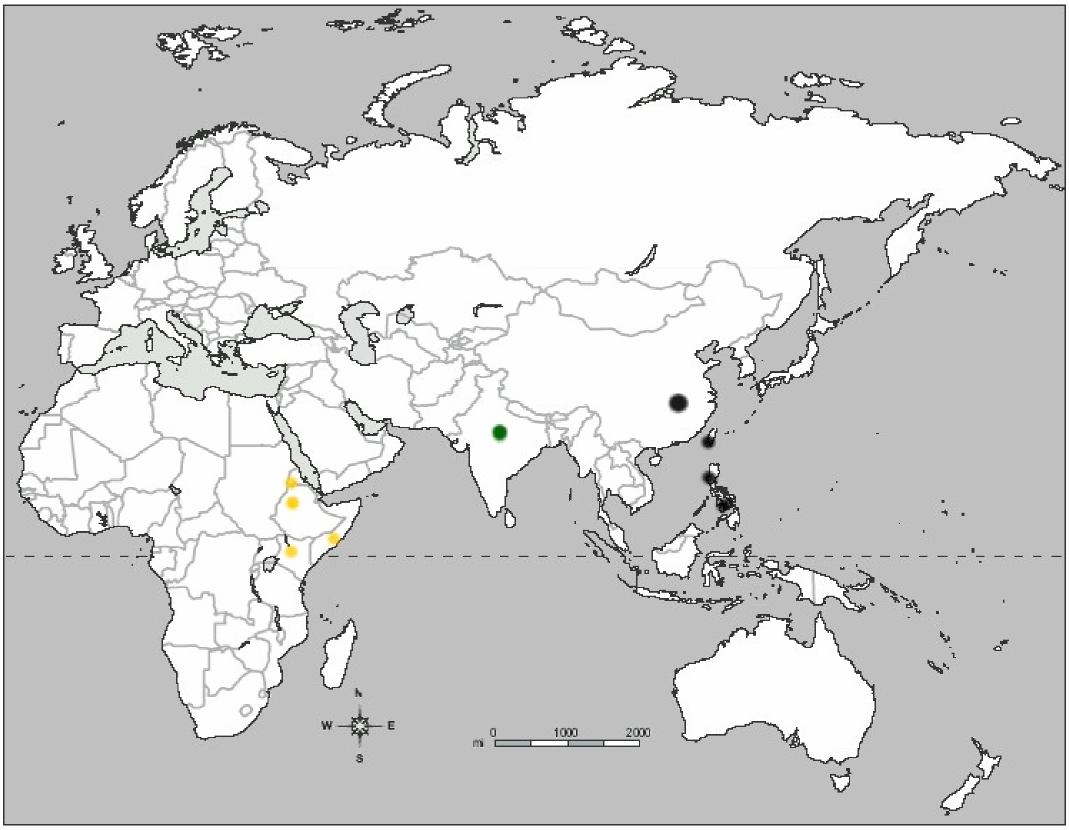
Figure 2.4: Mac Clade reconstruction of leaf persistency character among *Pistacia* species. 0=deciduous, 1= evergreen.

Appendix A: Distribution of *Pistacia* species based on the current study.



Map 1: Distribution of *P. atlantica* .

Map 2: Distribution of *P. eurycarpa*.

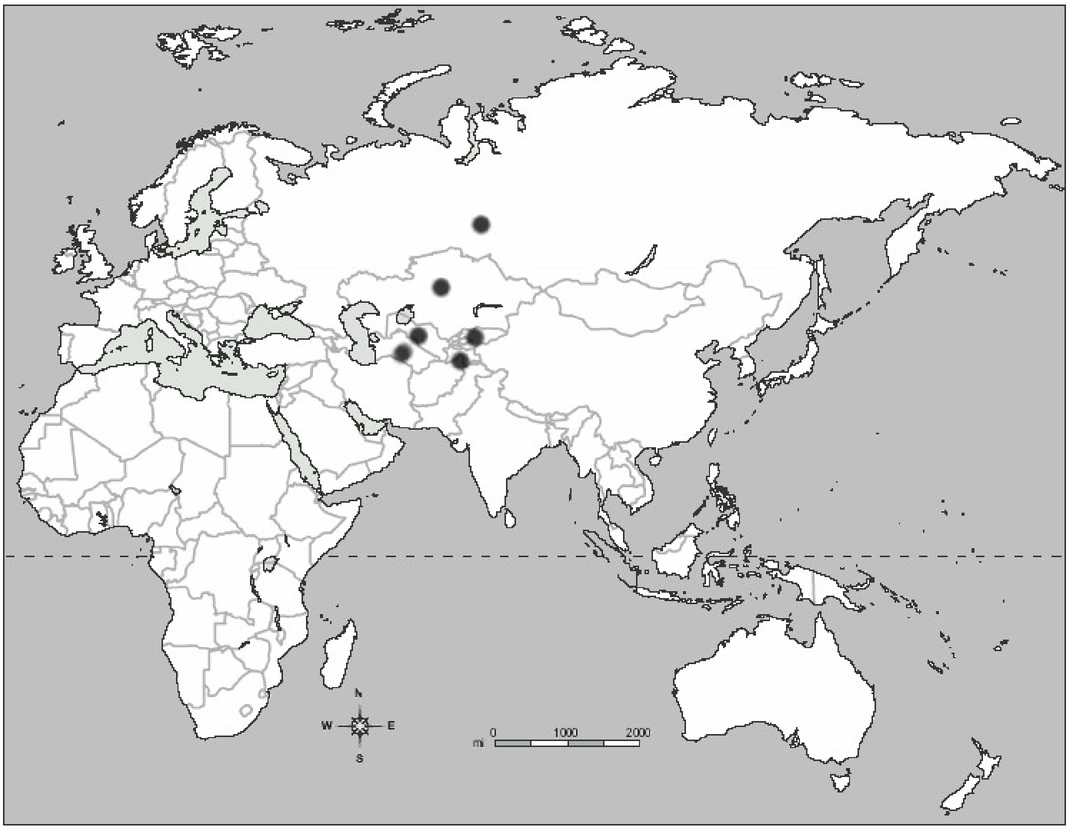


Map 3: Distribution of *P. chinensis.*

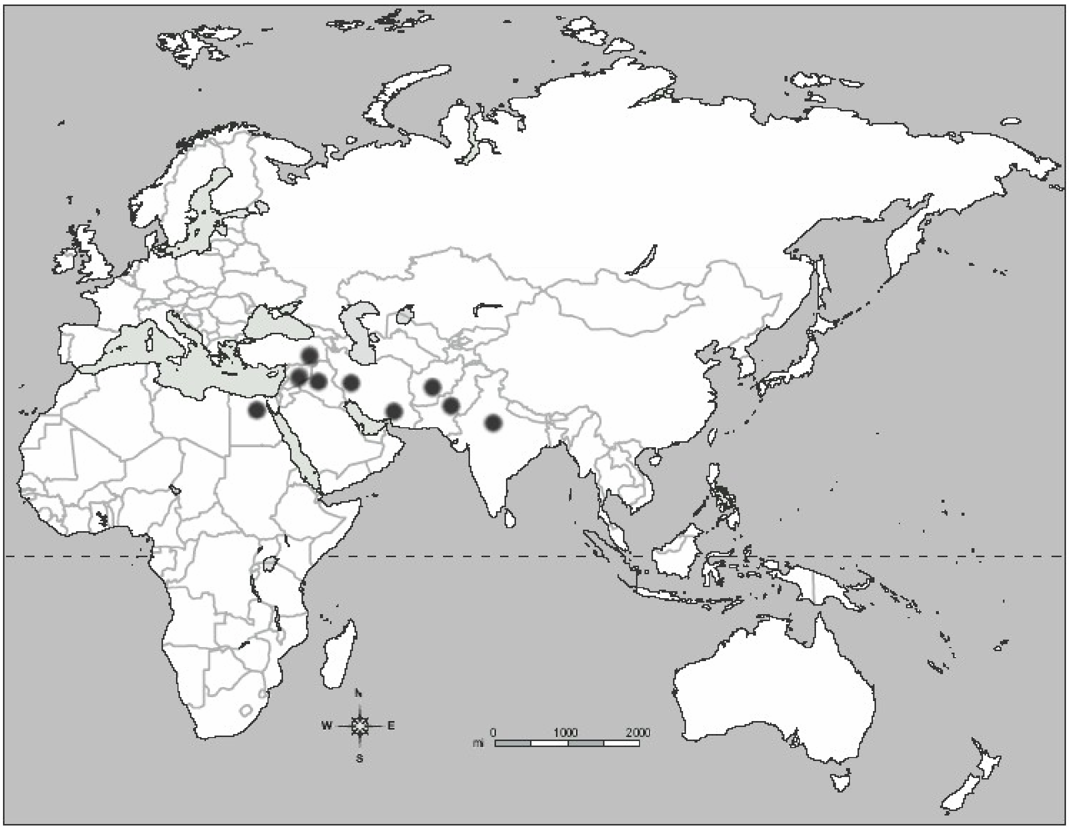
Black= *P. chinensis* subsp. *chinensis*

Yellow = *P. chinensis* subsp. *falcata*

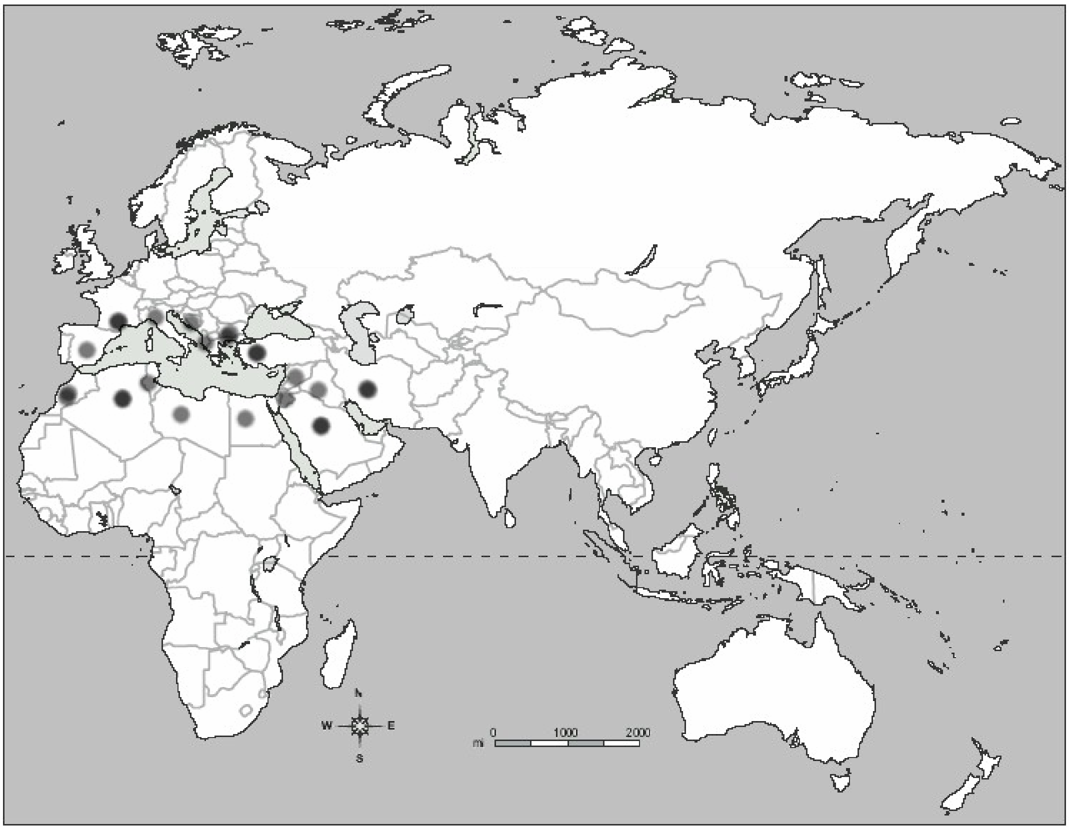
Green=*P. chinensis* subsp. *integerrima*



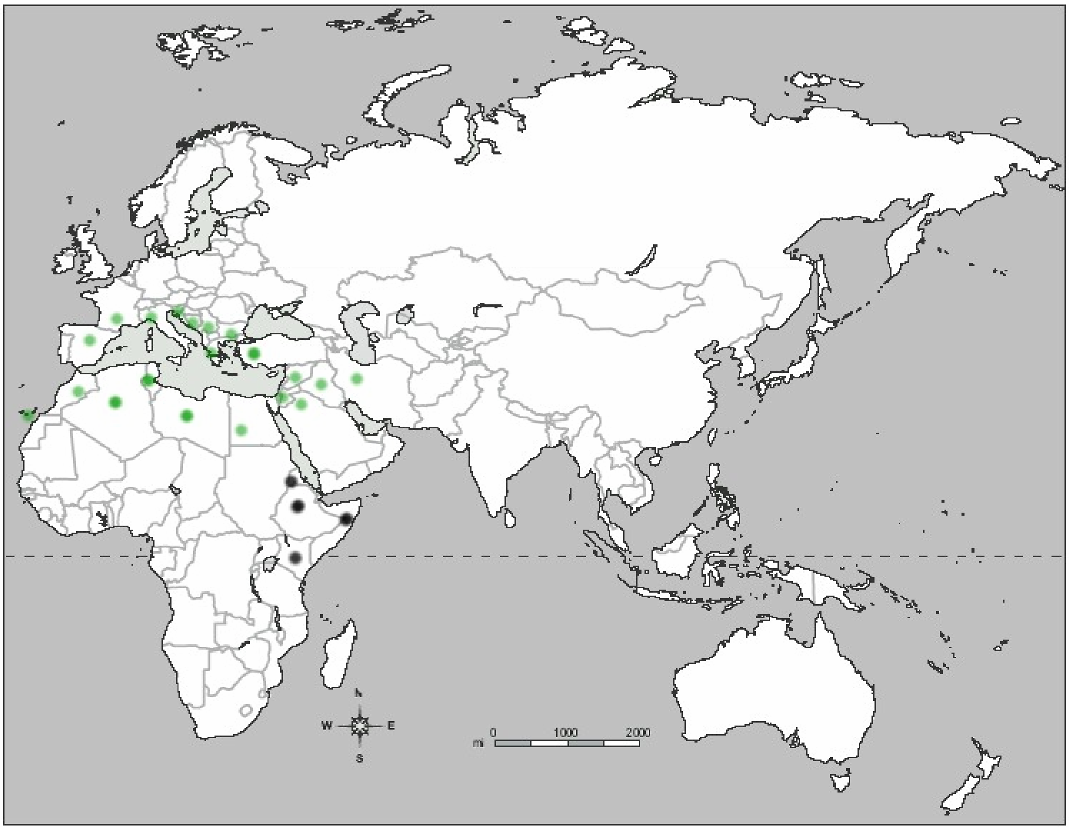
Map 4: Distribution of *P. vera.*



Map 5: Distribution of *P. khinjuk*.



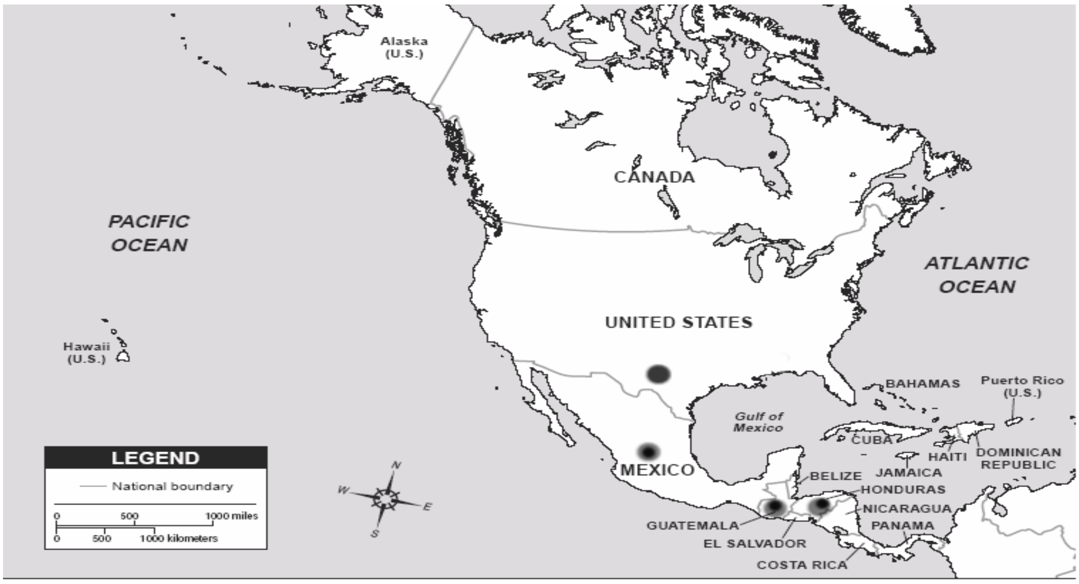
Map 6: Distribution of *P. terebinthus.*



Map 7: Distribution of *P. lentiscus.*

Black= *Pistacia lentiscus* subsp. *emarginata.*

Green= *Pistacia lentiscus* subsp. *lentiscus.*



Map 8: Distribution of *P. mexicana*.

Map 9: Distribution of *P. weinmannifolia.*

Chapter 3

Stomatal Distribution in *Pistacia* species (Anacardiaceae) *

Abstract

This study was carried out between the months of March and July 2005. *Pistacia* is an economically important genus because it has the pistachio crop, *P. vera,* which has edible seeds of considerable commercial importance. The evolutionary history of the genus (including the context of the evolution of the stomata) and the taxonomic relationship among the species are controversial and not well understood. This study was a part of a comprehensive phylogenetic study that has been conducted on this genus to refine taxonomic and evolutionary relationship utilizing different types of data (including morphology, cytology, anatomy and molecular) for a doctoral thesis. It aims to study the stomatal distribution in order to utilize this information for providing more insights into the evolutionary history of the stomata in the genus as well as the evolution and the taxonomy of genus itself. This study is the first one to report the stomatal distribution in all *Pistacia* species. The studied species were the following: *Pistacia aethiopica* J. O. Kokwaro, *P. atlantica* Desf., *P. chinensis* Bunge, *P. eurycarpa* Yaltirik, *P. falcata* Becc. ex Martelli, *P. integerrima* Stew. ex Brand., *P. khinjuk* Stocks, *P. lentiscus* L., *P. mexicana* HBK, *P. mutica* Fisch. and Mey., *P. palaestina* Boiss., *P. terebinthus* L., *P. texana* Swingle, *P. vera* L., and *P. weinmannifolia* Poiss. ex Franch. All species had anomocytic stomata. In most species, the stomata density was higher on the abaxial surface than the adaxial. The ratio of abaxial to adaxial stomatal density varied from 0.0

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to 1.7. Stomatal distribution may provide insights into how *Pistacia* species evolve in terms of leaf anatomy and respond to different climatic changes. The study indicates that the primitive anatomical condition in the genus is the occurrence of stomata on both adaxial and abaxial surfaces. Stomatal distribution changed (losing stomata on either surface) as the genus moved into regions of higher rainfall.

Introduction

Pistacia L. is a member of the family Anacardiaceae and consists of 11 species according to Zohary's classification (Zohary, 1952)**,** which is under question. Few systematic studies have been published on this important genus, the first complete classification of the genus was published by Zohary (1952). In his monograph, Zohary divided the genus into four sections: *Lentiscella* Zoh. **(**containing *P. mexicana* HBK. and *P. texana* Swingle**)**; *Eu Lentiscus* Zoh. **(**containing *P. lentiscus* L., *P. saportae* Burnat, and *P. weinmannifolia* Poisson**)**; *Butmela* Zoh. **(**containing *P. atlantica* Desf.**)**; and *Eu Terebinthus* Zoh. **(**containing *P. chinensis* Bunge., *P. khinjuk* Stocks, *P. palaestina* Bois., *P. terebinthus*, and *P. vera* L.)**.** *Pistacia vera*, commonly known as Pistachio, has edible seeds and considerable commercial importance. The other species grow in the wild and their seeds are used as rootstock seed sources and sometimes are used for fruit consumption, oil extraction, or soap production.

Pistacia is a xerophytic genus, which is shown by the presence of many adaptations to aridity, such as advanced development of palisade tissue and extensive root growth that allow *Pistacia* species like *P. atlantica* and *P. khinjuk* to grow in very harsh and dry areas with low rainfall (Spiegel-Roy et al., 1977; Lin et al., 1984). A single layer of thin walled epidermal cells characterized both leaflet surfaces of all species.The epidermal cells are covered with a relatively thick layer of cutin in *P. lentiscus*, *P. mexicana*, and *P. weinmannifolia*, but little or no cutin is observed in other species. All *Pistacia* species have no trichomes. Stomata and their guard cells are the major characteristic of epidermal cells. Stomata occur either on one, or the other, or both surfaces of the leaf (Spiegel-Roy et al., 1977; Lin et al., 1984; EL-Oqlah, 1996).

Stomata are small pores on the surface of leaves and stems, bounded by a pair of guard cells that control the exchange of gases, most importantly water vapor and CO2, between the interior of leaf and the atmosphere (Hetherington and Woodward, 2003). Gas exchange is regulated by controlling the aperture of the stomatal pore and the number of stomata that form on the epidermis. Environmental signals such as light intensity, the concentration of atmospheric carbon dioxide and endogenous plant hormones control stomatal aperture and development (Hetherington and Woodward, 2003). For example, plants under high wind conditions develop high stomatal density but stomatal aperture is small**.** Plants subjected to higher pCO_2 concentrations in the atmosphere decrease the number of stomata (Raven, 2002).

Rapid stomatal responses to environmental change plays a major role in maintaing the water movement from soil to plant. One study (Aasamaa et al., 2001) has demonstrated that stomatal size has a key role in this control and for six forest trees there is a clear negative relationship between the length of the stomatal pore and sensitivity to increasing drought. In these species larger stomata were slower to close and demonstrated a greater potential for hydraulic dysfunction under drought. Ferns from deep shade possess large stomata at low densities (Meidner and Mansfield, 1968) and in this natural environment, which may be cool and humid, it is found that truly shade-tolerant species often retain open stomata, even in deep shade, at least for early parts of the day (Allen and Pearcy, 2000). The constancy of the open stomata will minimize the impact of what would be slow opening limitations to photosynthesis during short-lived periods of sunlight, which are crucial for enhancing photosynthesis in this light-limited environment.

Small stomata can open and close more rapidly and their general association with high densities provides the capacity for rapid increases in the stomatal conductance of a leaf, maximizing CO_2 diffusion into the leaf during favourable conditions for photosynthesis (Raven, 2002). The effect of growth at elevated concentrations of CO_2 on stomatal density and stomatal index (the fraction of epidermal cells that are stomata) is one of the most intensively studied environmental controls on stomatal development. The reduction in stomatal density with CO_2 enrichment leads generally to a decrease in maximum stomatal conductance but an increase in the maximum rate of photosynthesis, at the elevated CO_2 concentration (Woodward et al., 2002).

The occurrence of stomata on both adaxial and abaxial surfaces of leaves of some species and not others is not readily attributed to any particular selection pressure under which the species may have originated. We suggest that this difference could be related to the ecological plasticity of *Pistacia* species to a wide range of environmental conditions. During the field trip in Jordan, plants of *P. atlantica* were found at 200 m below sea level**,** but the same species was also found at 1200 m above sea level. In addition, during this field trip P. *atlantica* was found in cold regions with high rainfall and in arid regions with low rainfall. This indicates the high level of plasticity of *Pistacia* species to live under radically different environmental conditions.

Few anatomical studies have been published on this genus. Grundwag and Werker (1976) described the wood anatomy of *Pistacia* species in Israel and Palestine

(*P. atlantica*, *P. khinjuk, P. lentiscus*, *P. palaestina*, *P.* X *saportae, P. terebinthus*, and *P. vera*) and Dong and Bass (1993) performed a similar study in China (*P. chinensis*, and *P. weinmannifolia*). Lin et al. (1984) characterized leaf morphology, photosynthesis, and leaf conductance of nine *Pistacia* species (*P. atlantica*, *P. chinensis*, *P. integerrima*, *P. khinjuk, P. lentiscus*, *P. mexicana*, *P. mutica*, *P. terebinthus*, *P. texana*, *P. vera*, and *P. weinmannifolia*). EL-Oqlah (1996) described *Pistacia* species in Jordan (*P. atlantica*, *P. lentiscus*, and *P. palaestina)* morphologically and anatomically. Castro-Díez et al. (1998) studied leaf morphology, leaf chemical composition**,** and stem xylem characteristics in two *Pistacia* (*P. lentiscus* and *P. terebinthus*) along a climatic gradient in a study area located in the NE quadrant of the Iberian Peninsula, which extended 350 km from the Atlantic coast to the middle Ebro Basin.

Pistacia is an economically important genus because it has the pistachio crop, *P. vera,* which has edible seeds of considerable commercial importance. The evolutionary history of the genus (including the context of the evolution of the stomata) and the taxonomic relationship among the species are controversial and not well understood. This study is a part of a comprehensive phylogenetic study that has been conducted on this genus to refine taxonomic and evolutionary relationship utilizing different types of data (including morphology, cytology, anatomy and molecular) for a doctoral thesis. It aims to study the stomatal distribution in order to utilize this information for providing more insights into the evolutionary history of the stomata in the genus as well as the evolution and the taxonomy of genus itself. This study is the first one to report the stomatal distribution in all *Pistacia*

Materials and Methods

This study was carried out between the months of March and July 2005. Leaves of *P. atlantica*, *P. khinjuk, P. lentiscus*, and *P. palaestina* were collected by the senior author during a field trip to Jordan in mid-summer 2004, while leaves of *P. aethiopica*, *P. chinensis*, *P. eurycarpa*, *P. falcata*, *P. integerrima*, *P. mexicana*, *P. mutica*, *P. terebinthus*, *P. texana*, *P. vera*, and *P. weinmannifolia* were obtained from herbarium specimens. Herbarium specimens were examined from the following herbaria: Field Museum, Chicago, Illinois, USA (F); Missouri Botanical Garden, St. Louis, Missouri, USA (MO); Royal Botanic Garden, Edinburgh, UK (E); Royal Botanic Gardens, Kew, UK (K); and Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA (VPI).

Stomata were counted from ten samples of each species included in this study**,** two from each specimen. For studying stomatal distribution, epidermal replicas of leaflets were made by coating the adaxial and abaxial surfaces with clear fingernail polish. The dried films were then peeled and mounted on slides. Replicas were observed using a BX61 Olympus microscope under 40 x magnification**. S**tomatal density was determined for each surface by randomly counting them for mm ^{2} (measured by micrometer) in 10 different fields in five different plants then the average was calculated.

This procedure that has been described in the literature as it is. There was no special procedure performed with the samples. No treatment of the herbarium specimens or specific fingernail polish at specific concentration was used.

Results

All species had anomocytic stomata (Fig.3.1, 3.2). Guard cells were not situated at the same level as adjacent epidermal cells and there were no subsidiary cells surrounding the guard cells. Our results disagree with the results of the study made by Lin et al (1984), who reported that all *Pistacia* species **(***P. atlantica*, *P. chinensis*, *P. integerrima*, *P. khinjuk, P. lentiscus*, *P. mexicana*, *P. mutica*, *P. terebinthus*, *P. texana*, *P. vera*, and *P. weinmannifolia***)** had actinocytic stomata. Stomatal density on both surfaces as well as the size of the stomata varies from species to species (Table 3.1). However, in most species, stomatal density was higher on the abaxial surface than on the adaxial. The ratio of abaxial to adaxial stomatal density varied from 0.0 to 1.7. In *P. atlantica*, *P. eurycarpa*, *P. mutica*, and *P. vera,* the stomata were observed to have low ratios on adaxial and abaxial surfaces with relatively large stomata. In these species, stomatal density on the adaxial surface was slightly higher than on the abaxial surface. In *P. chinensis*, stomata were observed on both adaxial and abaxial surfaces; stomatal density was notably higher on the adaxial surface with small stomata. Stomata were observed only on the adaxial surface in *P. falcata*, and *P. khinjuk* with large stomata. Stomata were observed only on the abaxial surface in *P. aethiopica, P. integerrima, P. lentiscus, P. mexicana, P. texana, P. palaestina, P. terebinthus,* and *P. weinmannifolia* and they have large stomata.

Discussion

Stomatal control of water loss allows plants to occupy habitats with fluctuating environmental conditions. Stomata first appeared in terrestrial land plants over 400 million years ago (Myr) (Edwards et al., 1998) and since then have changed markedly in size and density on plant surfaces.

Loss of stomata may have occurred in *Pistacia* species as an adaptation to changes in climate**,** from relatively even distribution of rainfall and temperature to seasonal variation with higher rainfall and warmer temperatures. This is supported by the presumably more advanced *Pistacia* species having smaller elongated leaflets with pointed shoot apices, which are more efficient for water removal from the leaf surface. This would be a useful adaptation as the genus moved into regions of higher rainfall (Parfitt and Badenes, 1997). We already elaborated on this relationship between the stomata and the climatic factors in the introduction.

Our morphological results (not shown here) indicate that *P. vera is* the most primitive species in the genus. So we suggest that the occurrence of stomata on both surfaces of leaf is the primitive state of this character and the occurrence on either surface is the advanced one in *Pistacia* species. The most common character trend in the genus was the occurrence of stomata only in abaxial surface in a low density and large size. There was a reversal of the character to the ancestral state in *P. atlantica*, *P. mutica* and *P. eurycarpa.* Moreover, our morphological results show that the genus can be divided into sections, *Lentiscus* and *Terebinthus*. Section *Terebinthus* contains the deciduous species (*P. atlantica*, *P. chinensis*, *P. eurycarpa, P. falcata*, *P. integerrima*, *P. khinjuk*, *P. mutica, P. palaestina*, *P. terebinthus* and *P. vera*) and the other group section *Lentiscus* contains the evergreen species (P. *aethiopica, P. lentiscus*, *P.*

mexicana P. texana, and *P. weinmannifolia*). We noticed that stomata were observed only in abaxial surface in section *Lentiscus* and in two deciduous species (*P. palaestina*, and *P. terebinthus*) in section *Terebinthus.* However, in the section *Terebinthus* there was a difference and no consistency in terms of occurrence of stomata. Moreover, this is consistent with our morphological results in that section *Lentiscus* was more homogeneous than *Terebinthus*. We suggest that the two deciduous sp that share the same character with evergreen because they inhabit the same environmental conditions (*P. lentiscus, P. palaestina* and *P. terebinthus* are the major elements of Mediterranean basin).

This study provides more data (stomatal distribution) that can be used in combination with morphological and molecular data to refine the taxonomic relationships among the different *Pistacia* species and map these differences into the phylogenetic tree of the genus. They may be used as key taxonomic traits to distinguish between the highly similar species of *Pistacia* (EL-Oqlah, 1996). For example**,** we found that there are anatomical differences between *P. khinjuk and P. vera* in terms of the occurrence of stomata (Table 3.1). In *P. khinjuk*, stomata were found only on the adaxial surface, while in *P. vera* they were found in both adaxial and abaxial surfaces. These differences occurred even though these two species are highly similar morphologically, so stomatal distribution can be used as a key taxonomic trait to distinguish between the two species.

Based on the correlation between the stomata and the wide range of the environmental conditions in which *Pistacia* species can grow, we suggest that the *Pistacia* species mainly inhabit humid and cool areas and are subjected to high wind condition.

Acknowledgements

The authors are grateful to the curators of the following herbaria, for their generous loans of herbarium specimens: Field Museum, Missouri Botanical Garden, Royal Botanic Garden, Edinburgh, and Royal Botanic Gardens, Kew. The authors are grateful to Prof. Erik Nilsen for allowing them to work in his well equipped lab which made this work possible.

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Table 3.1. Stomatal density (per mm²) on the adaxial and abaxial leaf surfaces of *Pistacia* species.

	Adaxial	Abaxial	Total	Ratio AdAb^{-1}
P. aethiopica	0.0	292.5	292.5	0
P. atlantica	242.5	150	392.5	1.6
P. chinensis	467.5	280	27.9	1.7
P. eurycarpa	235	137	372	1.7
P. falcata	242.5	0.0	242.5	---
P. integerrima	0.0	545	545	0
P. khinjuk	337.5	0.0	337.5	---
P. lentiscus	0.0	312.5	312.5	0
P. mexicana	0.0	225	225	0
P. mutica	210	165	350	1.3
P. palaestina	0.0	255	255	0
P. terebinthus	0.0	285	285	0
P. texana	0.0	225	225	0
P. vera	130	162.5	292.5	0.8
P.weinmannifolia	0.0	365	365	0

*Ad = Adaxial, Ab = Abaxial.

Figure 3.1: Paradermal sections of a *P. lentiscus* leaflet showing the a) adaxial surface, b) abaxial surface.

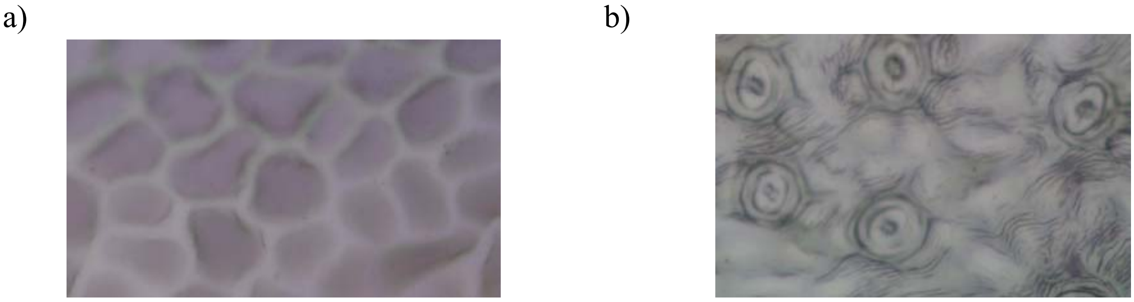
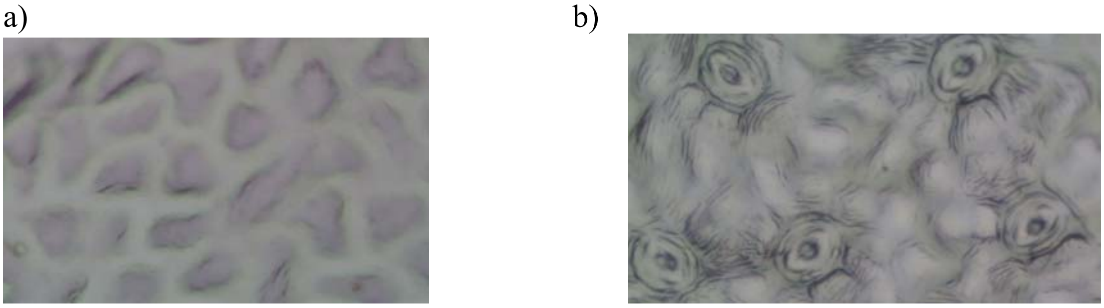


Figure 3.2: Paradermal sections of a *P. terebinthus* leaflet showing the a) adaxial surface, b) abaxial surface.



Chapter 4

Leaf Anatomy of *Pistacia* Species (Anacardiaceae) *

Abstract

Pistacia is an economically important genus because it contains the pistachio, *P. vera,* which has edible seeds of considerable commercial importance. The evolutionary history of the genus and the taxonomic relationships among the species are equivocal. This study is part of a comprehensive phylogenetic study that has been conducted on this genus in order to refine taxonomic and evolutionary relationships by utilizing different types of data (including morphological, cytological, anatomical and molecular). We aim to investigate the evolutionary and taxonomic relationships among *Pistacia* species. Here we present the first investigation of the leaf structure in all *Pistacia* species. The following species were studied: *Pistacia aethiopica* J. O. Kokwaro, *P. atlantica* Desf., *P. chinensis* Bunge, *P. eurycarpa* Yaltirik, *P. falcata* Becc. ex Martelli, *P. integerrima* Stew. ex Brand., *P. khinjuk* Stocks., *P. lentiscus* L., *P. mexicana* HBK, *P. mutica* Fisch. and Mey., *P. palaestina* Boiss., *P. terebinthus* L., *P. texana* Swingle, *P. vera* L., and *P. weinmannifolia* Poiss. ex Franch. Leaflets of *P. vera*, which have random orientation, were isobilateral, while leaflets of the other species were dorsiventral and were oriented horizontally.

*** Published as AL-Saghir, Mohannad G., Duncan M. Porter, and Erik T. Nilsen. 2006. Leaf Anatomy of *Pistacia* species (Anacardiaceae). Journal of Biological Sciences 6: 242-244.**

Introduction

Pistacia L. is a member of the family Anacardiaceae and consists of 11 species according to Zohary's classification (Zohary, 1952). Few systematic studies have been published on this important genus, the first complete classification of which was published by Zohary (1952). In his monograph, Zohary divided the genus into four sections: *Lentiscella* Zoh. **(**containing *P. mexicana* HBK and *P. texana* Swingle**)**; *Eu Lentiscus* Zoh. **(**containing *P. lentiscus* L., *P. saportae* Burnat, and *P. weinmannifolia* Poiss.**)**; *Butmela* Zoh. **(**containing *P. atlantica* Desf.**)**; and *Eu Terebinthus* Zoh. **(**containing *P. chinensis* Bunge, *P. khinjuk* Stocks., *P. palaestina* Boiss., *P. terebinthus* L., and *P. vera* L.)**.** *Pistacia vera*, commonly known as Pistachio, has edible seeds and considerable commercial importance. The other species grow in the wild and are used as rootstock sources and for fruit consumption, oil extraction, or soap production.

Few anatomical studies have been published on *Pistacia* species and those studies have not utilized all species in the genus. Grundwag and Werker (1976) described the wood anatomy of *Pistacia* species in Israel and Palestine (*P. atlantica*, *P. khinjuk, P. lentiscus*, *P. palaestina*, *P.* X *saportae, P. terebinthus*, and *P. vera*) and Dong and Bass (1993) performed a similar study in China (*P. chinensis* and *P. weinmannifolia***)**. Lin et al. (1984) characterized leaf morphology, photosynthesis, and leaf conductance of nine *Pistacia* species. **(***P. atlantica*, *P. chinensis*, *P. integerrima*, *P. khinjuk, P. lentiscus*, *P. mexicana*, *P. mutica*, *P. terebinthus*, *P. texana*, *P. vera*, and *P. weinmannifolia***)**. EL-Oqlah (1996) described *Pistacia* species in Jordan (*P. atlantica*, *P. lentiscus*, and *P. palaestina)* morphologically and anatomically. Castro-Díez et al. (1998) studied leaf morphology, leaf chemical composition**,** and stem xylem characteristics in two *Pistacia* species. (*P. lentiscus* and *P. terebinthus*) along a climatic gradient in a study area located in the NE quadrant of the Iberian Peninsula, which extended 350 km from the Atlantic coast to the middle Ebro Basin. AL-Saghir and Porter (2005) studied leaflet stomatal distribution in the genus.

The evolutionary history of the genus and the taxonomic relationships among the species are not well understood. This study is a part of a comprehensive phylogenetic study that has been conducted on this genus in order to refine taxonomic and evolutionary relationships by utilizing different types of data (including morphological, cytological, anatomical and molecular). We aimed to study the leaf structure in order to utilize this information for providing more insights into the evolutionary history and taxonomy of the genus. This study is the first to investigate internal leaf structure of all *Pistacia* species.

Materials and Methods

Leaves of *P. atlantica*, *P. khinjuk, P. lentiscus*, and *P. palaestina* were collected by the senior author during a field trip to Jordan in mid-summer 2004, while leaves of *P. aethiopica*, *P. chinensis*, *P. eurycarpa*, *P. falcata*, *P. integerrima*, *P. mexicana*, *P. mutica*, *P. terebinthus*, *P. texana*, *P. vera*, and *P. weinmannifolia* were obtained from herbarium specimens. Herbarium specimens were examined from the Field Museum, Chicago, Illinois, USA (F); Missouri Botanical Garden, St. Louis, Missouri, USA (MO); Royal Botanic Garden, Edinburgh, UK (E); Royal Botanic Gardens, Kew, UK (K); and Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA (VPI). Portions of leaflet lamina were cut into 4-10 mm^2 sections prior to rehydration. Tissues were rehydrated in a series of 25% alcohol, 10% alcohol, and distilled water. The rehydrated sections were stained in saturated aqueous safranin-O. The stained tissues were dehydrated in an alcohol series, 50%:50% alcohol and Xylene and 100% Xylene. The dehydrated tissues were infiltrated in a series of 50%:50% Xylene and paraffin oil and 100% paraffin oil followed by a second series of molten paraplast. The infiltrated tissues were embedded in paraffin in casting boats. Paraffin embedded blocks were sectioned transversely at 2 μm thickness with razor blades on a rotary microtome (MICROM Int. Walldorf, Germany). Slides were observed and photographed using an Olympus BX50 microscope with a 40x ocular.

Results

The cross sections of the leaflet lamina show a single layer of thin-walled epidermal cells on both leaflet surfaces of all species covered with a relatively thick layer of cutin in *P. aethiopica*, *P. lentiscus*, *P. mexicana*, and *P. weinmannifolia* (Fig.4.1c), but little or no cutin was observed in other species All *Pistacia* species have trichomes on their leaves, which has never been reported for the genus.

The leaflets of *P. vera* differ from those of other species (Fig.4.1a). The leaflets are randomly oriented and their adaxial and abaxial surfaces are similar. They are isobilateral, and the adaxial palisade cells are shorter than those of other species. The abaxial palisade consists of two layers of cells that appear slightly longer than the spongy mesophyll of other species. These cells are less densely packed than those of the adaxial layer of the same leaves.

Leaflets of the other species are dorsiventral in appearance (Fig.4.1b),. Their adaxial palisade consists of one layer that makes up most of the lamina thickness compared to that of *P. vera.* Many cells in the spongy tissue were palisade-like in appearance (i.e. long, narrow, and oriented perpendicular to the abaxial surface). However, they were intersperced with spongy mesophyll cells and not arranged in a dense palisade- like tissue. The abaxial spongy mesophyll consists of several layers, and the cells are very similar in appearance and density among all species except *P. vera*.

Discussion

Pistacia is a xerophytic genus, which is shown by the presence of many adaptations to aridity, such as advanced development of palisade tissue and extensive root growth. These adaptive traits allow species like *P. atlantica* and *P. khinjuk* to grow in very harsh and dry areas with low rainfall (Spiegel-Roy et al., 1977; Lin et al., 1984).

The current study indicates, excluding *P. vera*, that the leaflet internal anatomy of *Pistacia* species are homogenous. Our results agree with Lin et al. (1984). The similarity in leaf anatomy supports and the presence of trichomes on all species supports a close taxonomic relationship among the different species,

Our other morphological data (not shown here) indicate that *P. vera is* the most primitive species in the genus. We suggest that the isobilateral appearance of both leaflet surfaces is the primitive state of this character and the dorsiventral appearance is the advanced one in *Pistacia* species. There was no reversal to the ancestral state in this genus. Moreover, our other morphological results show that the genus can be divided into two sections, *Lentiscus* and *Terebinthus* (as described by Parfitt & Badenes 1997). Section *Terebinthus* contains the deciduous species *(P. atlantica*, *P. chinensis*, *P. eurycarpa, P. falcata*, *P. integerrima*, *P. khinjuk*, *P. mutica, P. palaestina*, *P. terebinthus* and *P. vera*) and section *Lentiscus* contains the evergreen species (*P. aethiopica, P. lentiscus*, *P. mexicana P. texana*, and *P. weinmannifolia*). The present study indicates a close relationship between the two sections and supports the monophyly of the genus (AL-Saghir and Porter, 2005).

This study provides more data that can be used in combination with morphological and molecular data to refine the taxonomic relationships among the different *Pistacia* species. Anatomical differences may be used as key taxonomic traits to distinguish between the highly similar species of *Pistacia* (EL-Oqlah, 1996). For example**,** we found that there are anatomical differences between *P. khinjuk and P. vera* in terms of their internal leaflet anatomy. The leaflets of *P. vera* are randomly oriented and isobilateral. The adaxial palisade cells are shorter than those of other species. The abaxial palisade consists of two layers of cells that appear slightly longer than those of other species. In contrast, the *P. khinjuk* leaflet is dorsiventral. The adaxial palisade is one-layered and makes up most of the lamina thickness compared to a much smaller portion in *P. vera.* Many cells in the spongy tissue were palisade-like in appearance. These differences were found even though the morphological traits of these two species are highly similar. Thus, the anatomical differences can be used as a key taxonomic trait to distinguish between the two species.

We have identified isobilateral leaves as an additional ancestral trait for *Pistacia*. Moreover, our anatomical evidence supports the monophyletic nature of *Pistacia* because all species have trichomes and all species except *P. vera* have dorsiventral leaves.

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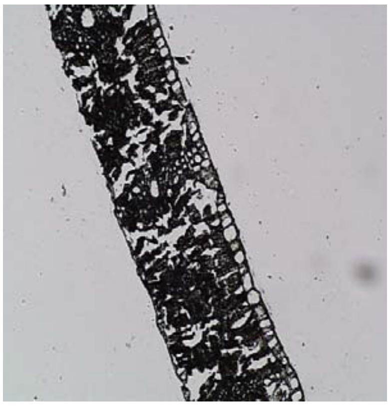
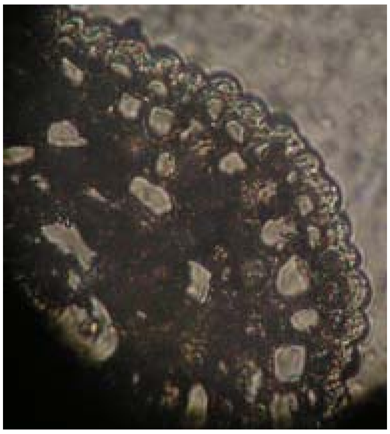
Figure 4.1: Cross sections of a) *P. vera* leaflet.

b) *P. atlantica* leaflet.



c) *P. mexicana* leaflet.



Chapter 5

RAPD Study of *Pistacia* Species

Introduction

It was planned to use DNA sequence analysis in this study. However, I found that Tingshuang Yi and Jun Wen from the department of Botany of the Field Museum of Natural History, Chicago (2005, personnel communication) have performed a 5-gene analysis of *Pistacia* to study its phylogeny and biogeography using both nuclear and chloroplast genes (NIA-i3 region, ITS4 and ITS5, trnC-trnD region, ndhF gene**,** and trnL-F regions), which was exactly what I had planned to do. Therefore, I switched to RAPD technology to study relationships in *Pistacia*.

Certain properties are desirable for a molecular marker such as highly polymorphic behavior, codominant inheritance, frequent occurrence in the genome, even distribution throughout the genome, selectively neutral behavior, easy access, easy and fast assay and high reproducibility (Weining and Henry, 1995). Examples of such DNA molecular markers are random amplified polymorphic DNA (RAPDs) (Welsh and McCelland, 1990; Williams et al., 1990) and amplified fragment length polymorphisms (AFLPs) (Vos et al., 1995). RAPD markers have proved to be good genetic markers to assay and evaluate the genetic diversity between and within the same species, populations and individuals (Warburton and Bliss, 1996). RAPD markers depend on the amplification of a DNA sequence by the polymerase chain reaction using only a single primer of arbitrary nucleotide sequence. The technique has proved to be fast and simple needs small quantities of template DNA, and detects relatively small amounts of genetic variation (Warburton and Bliss, 1996).

Molecular studies addressing the genus *Pistacia* are few. The pollen isozyme patterns of nine different enzymes in *P. lentiscus*, *P. terebinthus*, and *P. vera* were studied by Louskas and Pontikis (1979) in order to assay inter-specific relationships. They found a closer phylogenetic relationship between *P. terebinthus* and *P. vera* than between *P. lentiscus* and *P. vera*. Isozyme and DNA markers were used to distinguish and characterize the germplasm of *P. vera* (Hormaza et al., 1994, 1998; Dollo et al., 1995; Vezvaei, 1995; Barone et al., 1996; Dollo 1996; Caruso et al., 1998). Parfitt and Badenes (1997) were the first to provide a classification of 10 *Pistacia* species at the molecular level. They characterized these species based on chloroplast DNA profiles and subdivided the genus into two sections, *Terebinthus (P. atlantica, P. chinensis*, *P. khinjuk, P. integerrima, P. terebinthus*, and *P. vera*) and *Lentiscus* (*P. lentiscus, P. mexicana, P. texana*, and *P. weinmanniflolia*).

Kafkas and Perl-Treves (2001) addressed the taxonomic relationships and genetic variation of wild *Pistacia* germplasm in Turkey using morphological data and random amplified polymorphic DNA (RAPD). In the resulting molecular phylogeny, *Pistacia* species are clearly separated from each other. *Pistacia terebinthus* is the most divergent species, and the closest pair was *P. atlantica* and *P. eurycarpa.* Kafkas and Perl-Treves (2002) addressed the interspecific relationships in *Pistacia* based on RAPD fingerprinting. The following species were included: *P. atlantica, P. eurycarpa, P. khinjuk, P. lentiscus, P. mexicana, P. palaestina, P. terebinthus*, and *P. vera.* Phylogenetic analysis yielded two main groups: *P. atlantica, P. eurycarpa, P. khinjuk, P. integerrima,* and *P. vera* while *P. lentiscus, P. mexicana, P. palaestina*, and *P. terebinthus* formed the second group. The authors suggested that *P. palaestina* is a variety of *P. terebinthus*. This study showed that *P. eurycarpa* is a distinct species from *P. atlantica* rather than a variety of that species.

Katsiotis et al. (2003) used RAPD and amplified fragment length polymorphism (AFLP) to study the relationships of native and introduced *Pistacia* species in Greece. The AFLP and RAPD phenograms were comparable**,** with minor clustering differences. The results revealed two main groups one contains the evergreen species *P. lentiscus* and the resin-producing *P. lentiscus* cv. Chia (cultivated only on Island of Chios), and the other contains the deciduous trees *P. palaestina, P. terebinthus*, and *P. vera. Pistacia chinensis* was clustered either with the first group (by RAPD results) or with the second one (by AFLP results). Barazani et al. (2003) characterized the genetic variability of Mediterranean *P. lentiscus* genotypes by morphology, composition of essential oils, and RAPD. The RAPD dendrogram showed two main groups according to the species' geographical origin. One group included the Tunisian, Spanish, and Israeli accessions and the second group included Cyprian accessions. Golan-Goldhirsh et al. (2004) assessed the polymorphism among Mediterranean basin *Pistacia* species and accessions within the same species using RAPD and AFLP markers. Similar results were obtained by the two techniques. The RAPD and AFLP dendrograms showed two major groups with one group containing all the *P. lentiscus* accessions and the other group containing all other accessions. The latter group subdivided into two subgroups, one consisting of *P. palaestina* and *P. terebinthus*, the other consisting of *P. atlantica*, *P. khinjuk*, and *P. vera.*

Yi and Wen (2005, personnel communication) have performed a 5-gene analysis of *Pistacia* to study its phylogeny and biogeography using both nuclear and chloroplast genes (NIA-i3 region, ITS4 and ITS5, trnC-trnD region, ndhF gene**,** and trnL-F regions). They sampled all species of the genus and are preparing the manuscript with two additional collaborators. However, their study did not address the taxonomic relationships within the genus. The objectives of the present study were to investigate and clarify the taxonomic relationships among *Pistacia* species.

Materials and Methods

Plant Materials and DNA Extraction

The following four *Pistacia* species were included in this study: *P. khinjuk*, *P. lentiscus*, *P. terebinthus*, and *P. vera*. The seeds of only these four species were available. Seeds of these species were obtained from USDA, National Clonal Germplasm Repository (Davis, CA). *Rhus ovata* was used as an outgroup, the seeds being obtained from the Royal Botanic Gardens, Kew. DNA was extracted from the young leaves of greenhouse planted seedlings (4 weeks old) using a DNeasy Plant Mini kit (QIAGEN, Valencia, CA).

RAPD Amplification

Twenty-three random primers (10-mer) (Operon Technologies, Alameda, CA) of arbitrary sequence were used in this study (Table 5.1).

RAPD reactions were done in a total volume of 25 µl containing 60 ng of primer; 200 µM each of dATP, dCTP, dGTP and dTTP, 100 ng of DNA template, 2 mM $MgCL_2$ and 1.5 u of Taq DNA polymerase in 1X PCR buffer. DNA amplification was performed in a Biometra thermal cycler (Goettingen, Germany) in 0.2 ml PCR tubes programmed for initial denaturation at 95°C for 2 min followed by 43 cycles for 1 min at 95°C, at an annealing temperature of 37°C for 1 min and at 72°C for 2 min as an extension step. The final extension step was done for 5 min at 72°C and the reactions were kept at 4°C.

The RAPD-PCR amplified products were analyzed by gel electrophoresis in 1.5% ultrapure agarose in 0.5X TBE buffer stained with ethidium bromide (0.5µg /ml) at 125 volts for 5 hrs using a horizantal gel electrophoresis apparatus (Sigma Chemical Co. St. Louis, MO). The amplified products were visualized under UV light and photographed with a Gel documentary system. A 1kb ladder was used as a DNA standard to estimate the molecular weights of the amplified products.

Band Scoring and Phylogenetic Analysis

For each individual primer, PCR amplified products were designated. Data were scored on the basis of the presence or absence of the amplified products. If the product is present in a genotype, it was scored as 1, if absent, it was designated as 0. Only the clearest and strongest bands were used for phylogenetic analysis. Reproducibility of the patterns was tested by running the reactions in duplicates or in triplicates. Neighbor joining and parsimony analyses were performed on the RAPD data using PAUP 4.0 b10 (Swofford, 2003). A heuristic search was performed by RANDOM stepwise addition (10,000 replicates) with tree bisection-reconstruction (TBR) branch swapping to find the most parsimonious tree. From the resulting trees, the majority rule consensus was calculated. Robustness and topology of the trees were evaluated using the consistency index (CI) to measure the level of homoplasy in the data (how cleanly the data fit the tree). The retention index (RI) was measured to indicate the amount of synapomorphy on the tree. Robustness and reliability of the resulting phylogenies were measured by bootstrap (1,000 replicates with full heuristic searches). The step wise genetic distance matrix (Table 5.2) which measures the relatedness between the species (Kimura, 1980) was computed between all species. The data were analyzed with a neighbor joining approach.

Results

The RAPD technique was used to characterize the four *Pistacia* species and clarify the relationships between them. A total of 248 fragments was generated by 23 arbitrary sequence primers, and 139 bands out of these were polymorphic at the inter-specific level. Figure 5.1 indicates a representive example of RAPD markers detected in the four *Pistacia* species using primers OPA-09 and OPB-01.

Data were analyzed using the PAUP program, to obtain cladograms that depict the likely relationships among the species. All the RAPD characters used were parsimony informative characters. Parsimony analysis produced one maximally parsimonious tree of 34 steps, with a consistency index (CI) of 0.55 and a retention index (RI) of 0.67 (Fig. 5.2). This tree was consistent with the tree produced by neighbor joining analysis (Fig. 5.3). There were insignificant differences between the bootstrap support values of the different branches of the two trees and the relationships among species within each clade. Pair-wise genetic distances between all the species were calculated by the same program (Table 5.2).

According to these cladograms, the four *Pistacia* species are well separated from each other and the out group was well clustered from the *Pistacia* species. Two groups were resolved which were separated by a branch appearing in 87% of the bootstrap replicates in the parsimony analysis and 86% in the neighbor joining analysis. The first group includes *P. khinjuk*, *P. terebinthus* and *P. vera* while the second group comprises *P. lentiscus*. Within the first group, *P. khinjuk* and *P. vera* form a very close pair that is separated from *P. terebinthus* by a branch appearing in 99% of the bootstrap replicates in the parsimony analysis and 98% in the neighbor joining analysis.

Discussion

The results of the current study support the morphological study in terms of monophyly of the genus. Both analyses show that *P. khinjuk* and *P. vera* are very close species and *P. khinjuk* may be a descendant of *P. vera.* Two sections were revealed in the morphological study: Section *Pistacia* which contains deciduous species and Section *Lentiscus* which contains evergreen species. Two groups were obtained in the current study, the first group comprises *P. khinjuk*, *P. terebinthus* and *P. vera* (all species are deciduous trees and belong to Section *Pistacia*) and the second group comprises one species (*P. lentiscus*) which is an evergreen shrub and belongs to section *Lentiscus*. The current study confirmed the placement of the three species of the first group in Section *Pistacia* because they are genetically related and show a distant relationship with the other section represented by *P. lentiscus*.

Regarding the evolution of the four studied *Pistacia* species, Zohary (1952) hypothesized that *P. vera* is the most primitive species in the genus based on morphological characters (simple, odd-pinnate leaves, small number of leaflets per leaf, symmetrical leaflets, rounded leaflet apex, wingless petiole, highly branched panicles, deciduous character). Moreover, the very close relationship shown by the present study supports Zohary's hypothesis that *P. khinjuk* may be a descendent of *P. vera* .

As indicated earlier in this chapter, Parfitt and Badenes (1997) performed a phylogenetic study of ten *Pistacia* species based on an analysis of the chloroplast genome. They found that the closest species to *P. vera* is *P. khinjuk*, followed by *P. atlantica* and *P. terebinthus*. They could not discriminate *P. khinjuk* from *P. vera* because the chloroplast genome is very conserved within the genus, and they suggested that *P. khinjuk* and *P. vera* may be considered one species, despite the differences in morphology. Kafkas and Perl-Treves (2002) addressed the interspecific relationships in

Pistacia based on RAPD fingerprinting. The following species were included: *P. atlantica, P. eurycarpa, P. khinjuk, P. lentiscus, P. mexicana, P. palaestina, P. terebinthus*, and *P. vera*. Phylogenetic analysis yielded two main groups: *P. atlantica, P. eurycarpa, P. khinjuk, P. integerrima,* and *P. vera* while *P. lentiscus, P. mexicana, P. palaestina*, and *P. terebinthus* formed the second group. They showed again a close relationship between *P. khinjuk* and *P. vera*. The present study largely confirmed the relationships described by Parfitt and Badenes (1997) and Kafkas and Perl-Treves (2002).

Cytological Description

Cytogenetic studies addressing the genus *Pistacia* are rather few. These chromosome counts are under question because of frequent hybridization events in the genus, the close relationship between the species within the genus and the small size of the chromosomes. First, chromosome counts were reported for three species P. *atlantica* with 2n = 28, *P. lentiscus* with 2n = 24, and *P. vera* with 2n = 30 (Zohary, 1952). Ozbek and Ayfer (1957) reported the chromosome numbers of *P. vera*, *P. khinjuk*, and *P. terebinthus* as 2n=30, and of *P. atlantica* as 2n=28. Recently, Fasihi Harandi (1996), Fasihi Harandi and Ghaffari (2001), and Ghaffari and Fasihi Harandi (2002) studied the ecological distributions and karyotypes of Iranian *Pistacia* species. They gave the chromosome compliment of *P. atlantica* (including subspecies *cabulica*, *kurdica*, and *mutica*) as 2n = 28, *P. khinjuk* as 2n = 24, and. *P. vera* as 2n = 30. Vogt and Aparicio (1999) reported the chromosome number of *P. atlantica* as 2n=30.

Basrila et al. (2003) determined the chromosome numbers of four *Pistacia* species in root tip cells. Chromosome counts were performed for *P. atlantica*, *P. eurycarpa*, *P. terebinthus*, and *P. vera*, Chromosome numbers of all four species were found to be 2n=30.

These reported chromosome numbers tell us little about relationships of *Pistacia* species. Unfortunately, the cytological experiment in the present study has failed because of the unavailability of sufficient seeds of the studied species. I have collaborated with Dr. S. P. Tallury from the Department of Crop Science at North Carloina State University, Raleigh to conduct the cytological analysis but the experiment failed because it was very difficult to germinate the seeds.

From the literature review I do think there have been enough counts to confirm the chromosome numbers for *P. atlantica*, *P. terebinthus* and *P. vera.* I suggest that the basic number is X=15 for the genus. Reported diploid numbers of 24 and 28 probably result from reduction or are incorrect.

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Matus, I. A. and P. M. Hayes. 2002. Genetic diversity in three groups of barley germplasm assessed by simple sequence repeats. Genome 45: 1095-1106.

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Swofford D. L. 2003. PAUP*- Phylogenetic Analysis Using Parsimony, version 4.0b2a. Sunderland, MA, Sinauer Associates.

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Vogt, R. and A. Aparicio. 1999. Chromosome numbers of plants collected during Iter Mediterraneum IV in Cyprus. 11: 117-169.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. Van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper and M. Zabeau. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research 23: 4407-4414.

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Weining, S. and R. J. Henry. 1995. Molecular analysis of the DNA polymorphism of wild barley (*Hordeum spontaneum*) germplasm using the polymerase chain reaction. Genetic Resource and Crop Evolution 42: 237–281.

Welsh, J. and M. McCeleand. 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Research 18: 7213-7218.

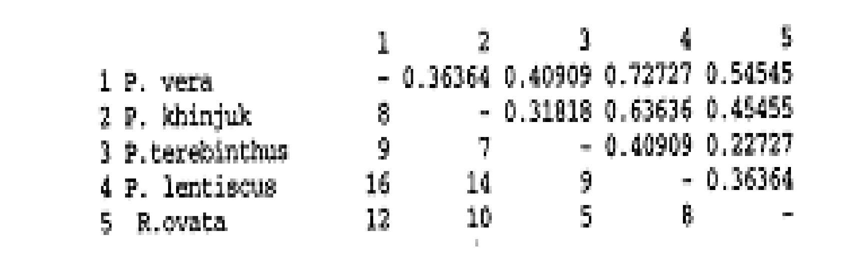
Williams, J. G. K., A. R. Kubelik, K. J. Livak, A. Rafalski and S.V.Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18: 6531-6535.

Zohary, M. 1952. A monographical study of the genus *Pistacia*. Palestine Journal of Botany Jerusalem Series 5: 187-228.

Table 5.1: Random primers used in this study to screen RAPD markers.

Primer	**Sequence 5` to 3`**
OPA-08	GTGACGTAGG
OPA-09	GGGTAACGCC
OPA-12	TCGGCGATAG
OPB-01	GTTTCGCTCC
OPC-02	GTGAGGCGTC
OPC-06	GAACGGACTC
OPC-11	AAAGCTGCGG
OPC-19	GTTGCCAGCC
OPD-05	TGAGCGGACA
OPD-07	TTGGCACGGG
OPD-10	GGTCTACACC
OPD-13	GGGGTGACGA
OPD-15	CATCCGTGCT
OPD-19	CTGGGGACTT
OPD-20	ACCCGGTCAC
OPE-01	CCCAAGGTCC
OPE-03	CCAGATGCAC
OPE-06	AAGACCCCTC
OPE-16	GGTGACGCAG
OPE-17	CTACTGCCGT
OPF-02	GAGGATCCCT
OPF-05	CCGAATTCCC
OPF-09	CCAAGCTTGG

Table 5.2: Pair wise genetic distance matrix.



	1	2	3	4	5
1 P. vera	-	0.36364	0.40909	0.72727	0.54545
2 P. khinjuk	8	-	0.31818	0.63636	0.45455
3 P.terebinthus	9	7	-	0.40909	0.22727
4 P. lentiscus	16	14	9	-	0.36364
5 R.ovata	12	10	5	8	-

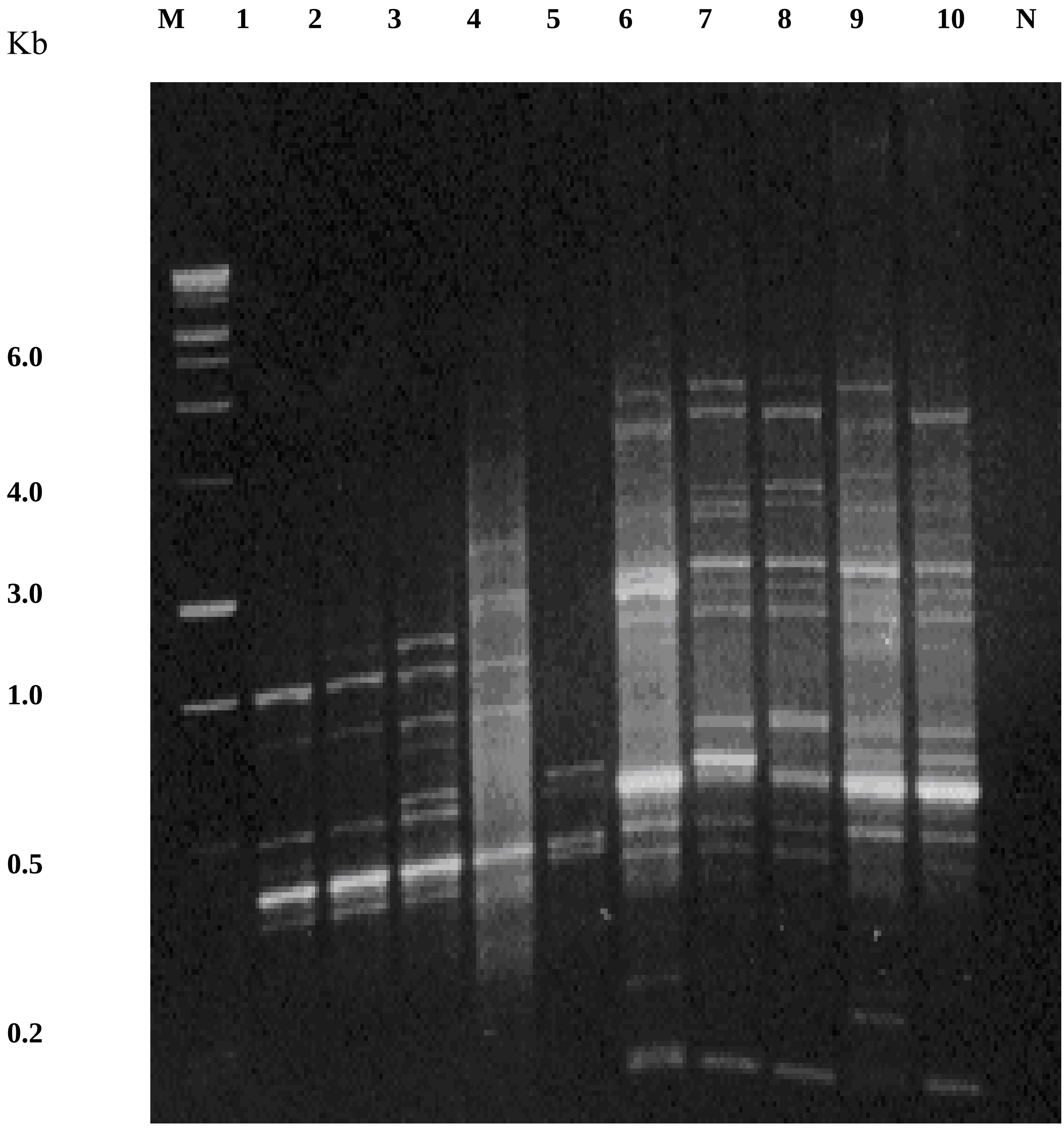


Figure 5.1: DNA amplification products of four *Pistacia* species. Lanes (1-5): RAPD patterns of DNA from *Pistacia vera*, *P. khinjuk*, *P. terebinthus, P. lentiscus* and *Rhus ovata* respectively using the primer OPA-09. Lanes (6-10): RAPD patterns of DNA from *P. vera*, *P. khinjuk*, *P. terebinthus, P. lentiscus* and *Rhus ovata* respectively using the primer OPB-01. Lane M is a 1 Kbp molecular weight marker, lane N is a negative control (amplification product lacking DNA template sample).

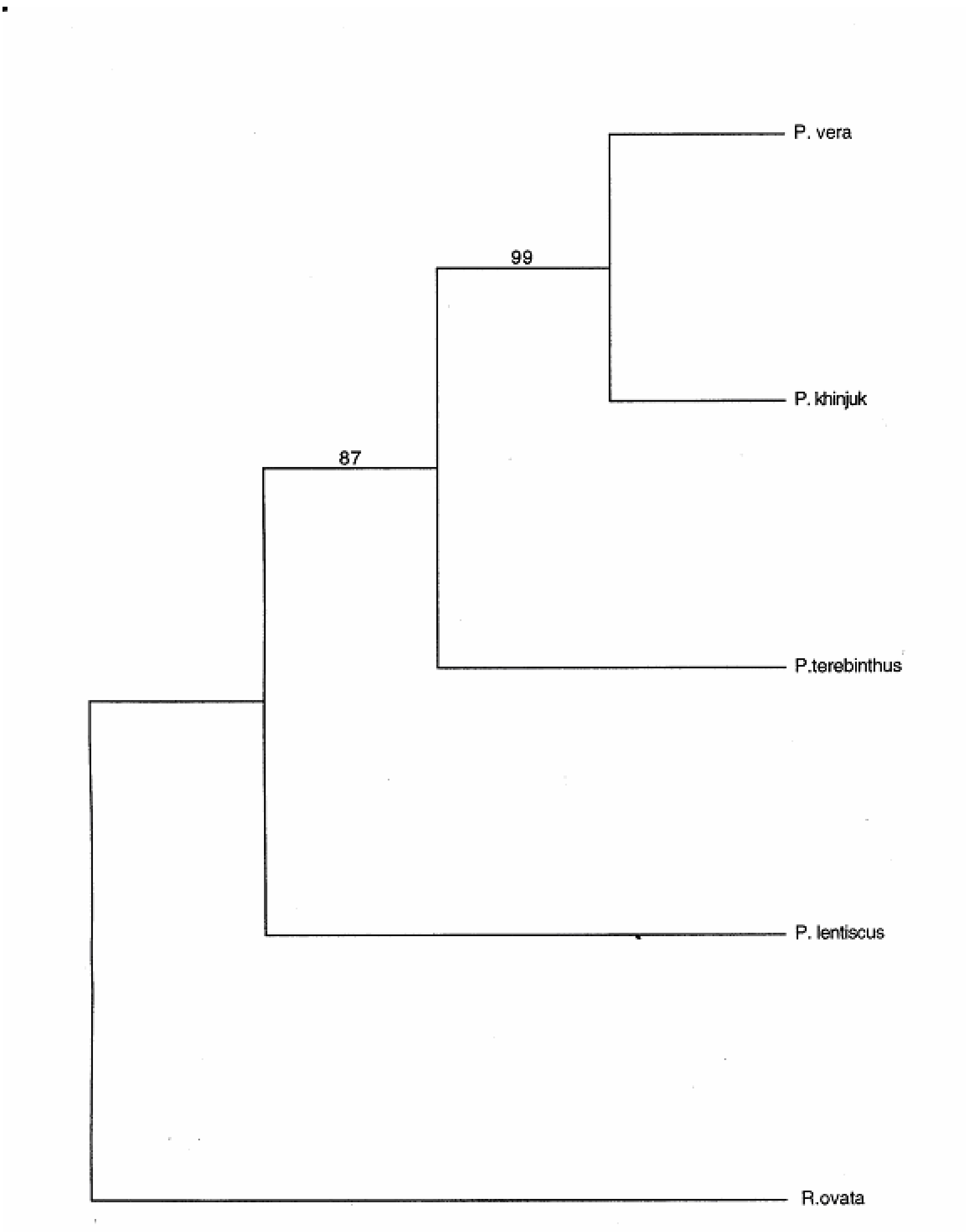


Figure 5.2: The majority rule consensus tree generated with RAPD data using parsimony analysis showing relationships of the *Pistacia* species with CI=0.55 and RI=0.67. Numbers above the branches indicate the bootstrap values.

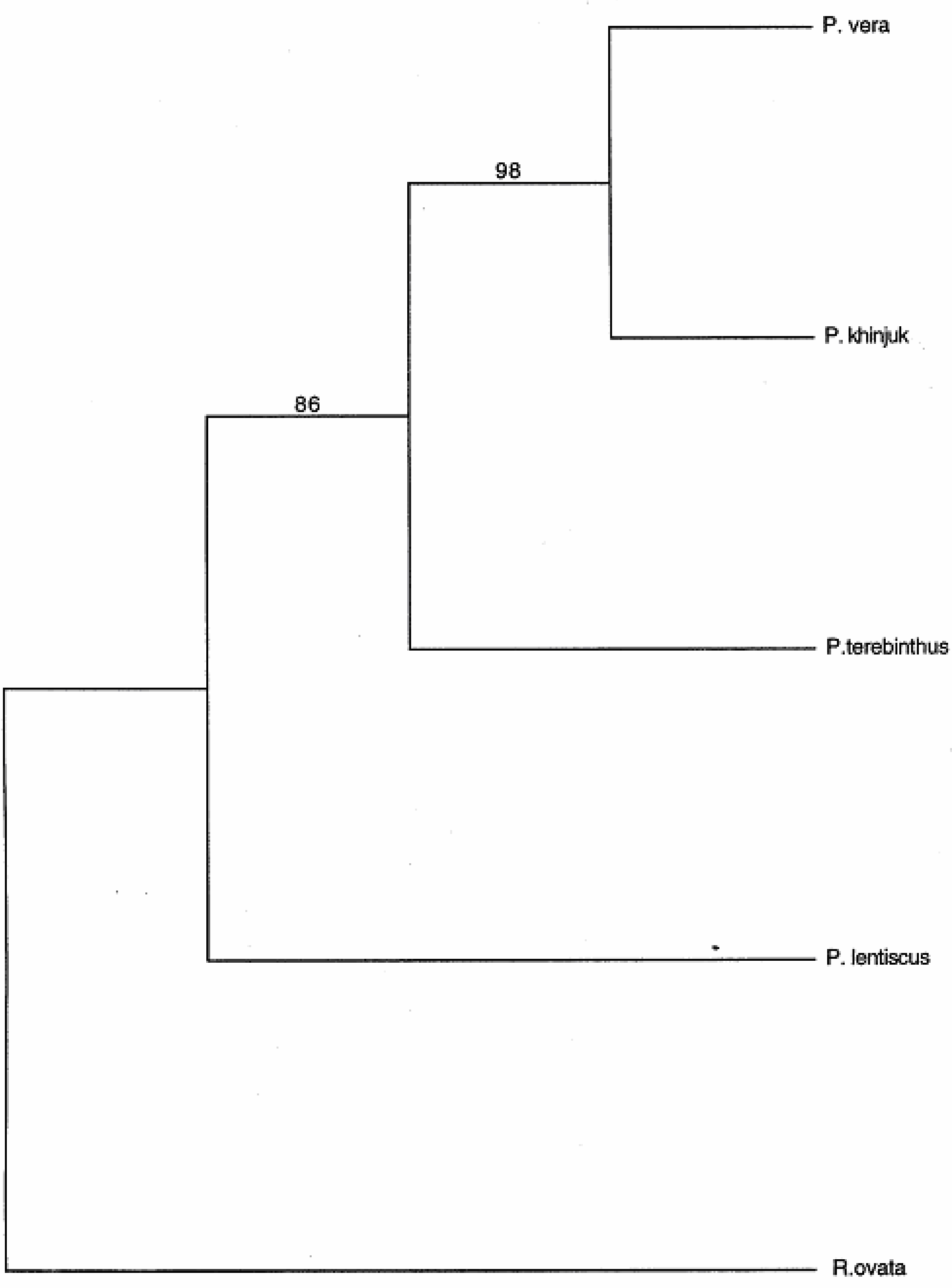


Figure 5.3: The majority rule consensus tree generated with RAPD data using neighbor joining analysis showing relationships of the *Pistacia* species. Numbers above the branches indicate the bootstrap values.

Chapter 6

Taxonomic Section

The Genus *Pistacia* and Its Sectional Subdivison

***Pistacia* L.,** Sp. Pl. 2: 1025. 1753.

Type species: *Pistacia vera* L. (Green, Prop. Brit. Bot.: 191. 1929), Lectotype: not designated (Reg. Veg. 127: 77. 1993).

Terebinthus P. Miller, Gard. Dict. Abr. ed. 4. 1754.

Evrardia Adanson, Fam. 2: 342. 1763.

Lentiscus O. Kuntze, Rev. Gen. 1: 152. 1891.

Dioecious trees or shrubs (rarely monoecious) with resins. Leaves alternate, deciduous or evergreen, usually pinnately compound rarely trifoliate or simple, membranous or leathery, stipules absent. Flowers in panicles or racemes, small, unisexual, apetalous, subtended by 1-3 small bracts and 2-7 bracteoles and wind pollinated. Staminate flowers with stamens 4-5, anthers inserted on disc. Pistillate flowers with pistil compound of 1-5 united carpels, locule 1, ovules usually 1 per locule and borne on axile placenta; ovary superior with a short 3-parted style; stigma capitate. Fruit a 1- seeded drupe.

Common names: Pistache, Pistachio (Zohary, 1952).

Key to *Pistacia*

1. Deciduous trees or shrubs

2. Leaf rachis wingless

3. Leaves leathery; leaflets obtuse or mucronulate, reticulate veined.………………………………………………………….. **1. *P. vera***

3. Leaves membrananceous; leaflets not as above

4. Leaflets 2-6 …………………………………………………**2. *P. khinjuk***

4. Leaflets 6-16

5. Leaflets acuminate…………………………………………**3. *P. terebinthus***

5. Leaflets attenuate

6. Leaflets mostly odd pinnate, narrowly lanceolate and falcate…… ………………………………………………**4.2. *P. chinensis* subsp. *falcata***

6. Leaflets even- pinnate

7. Leaflets broadly lanceolate.……..…**4.3. *P. chinensis* subsp. *integerrima***

7. Leaflets lanceolate ……………….**4.1. *P. chinensis subsp. chinensis.***

2. Leaf rachis winged

8. Leaflets dark green above, paler below; fruits as long as or longer than wide……………………………………………………**5. *P. atlantica***

8. Leaflets light green on both sides; fruits depressed, wider than long……………………………………………………**6. *P. eurycarpa***

1. Evergreen trees or shrubs

9. Leaves leathery

10**.** Leaflets 4-10, mucronate, lacking large distinct veins……………………….

…………………………………………….**7.1.** ***P. lentiscus*** **subsp.** ***lentiscus***

10. Leaflets 10, emarginate with large distinct veins…..............

…………………………………………….**7.2.** ***P. lentiscus*** **subsp.** ***emarginata***

9. Leaves membrananceous

11. Leaves even-pinnate, leaflets 10-21, often alternate……… **9.** ***P. weinmannifolia***

11. Leaves odd-pinnate, leaflets 10-32, never alternate, slightly falcate…

……………………………………………………………….**10.** ***P. mexicana***

Pistacia* L. sect. *Pistacia

Type species: *Pistacia vera* L. (Green, Prop. Brit. Bot.: 191. 1929).

Deciduous small trees or shrubs; leaves even- or odd-pinnate, rarely trifoliolate or simple; leaflets 4-24; rachis winged or wingless; drupes with bony endocarp. Includes the following species: *P. atlantica* Desf., *P. chinensis* Bunge, *P. eurycarpa* Yaltirik, *P. khinjuk* Stocks., *P. terebinthus* L., and *P. vera* L.

Butmela Zoh., Pal. J. Bot. 5: 195. 1952.

Eu Terebinthus Zoh., Pal. J. Bot. 5: 196. 1952.

Terebinthus Parf. & Bad., Proc. Natl. Acad. Sci. USA. 94: 7991. 1997.

Section *Butmela* is a taxonomic synonym based on *P. atlantica* Desf.

Section *Eu Terebinthus* is an illegitimate name, because the epithet in the name of a subdivision of a genus is not to be formed from the name of the genus to which it belongs by adding the prefix *Eu-* (ICBN, Art. 21.3).

Section *Terebinthus* was published without a Latin dignosis.

1-***Pistacia vera* L.**, Sp. Pl. 2 : 1025. 1753.

Lectotype: not designated (Reg. Veg. 127: 77. 1993). Probably one of the Linnean specimens in the herbarium of the Linnaean Society of London (L); described from a cultivated plant.

P. trifolia L., Sp. Pl. 2: 1025. 1753.

P. nigricans Crantz, Inst. Rei. Herb. 1: 184. 1766.

P. terebinthus Mill., Gard. Dict. ed. 8. n. 1. 1768.

P. variifolia Salisb., Prod. 171. 1796.

P. reticulata Willd., Sp. Pl. 4: 751. 1797.

P. macrophylla Pers., Syn. Pl. 2: 615. 1806.

P. officinarum W. T. Aiton, Hortus Kew. ed. 1. 3: 398. 1811.

P. badghysi K. P. Popov, Izv. Akad. Nauk Turkm. SSR, Biol. Nauk 5: 1978.

Trees to 10 m high. Leaves deciduous, large, 10.2-17.0 cm long, 8.4-16.0 cm wide, trifoliolate or odd-pinnate, leathery, petiole flattened. Leaflets 3-5, large, 4.0-8.0 cm long, 3.0-5.5 cm wide, with average ratio 1.6, ovate to broadly ovate, obtuse or mucronulate, puberulent, and reticulate-veined. Terminal leaflet 6.0-10.0 cm long, 3.0-8.0 cm wide, with average ratio 1.5, often larger than laterals. Rachis not winged, pubescent. Inflorescence paniculate; seed containing large cotyledons, edible, cultivated.

Common names: Pistachio, Pistache, Fistashka (Russia), Pista (Parsian), Pistacchio (Italia), Pistacier (French), Fustuq Halabi (Arabic), Botne (Hebrew) (Zohary, 1952). Green almond, Pistachier cultivé, Pistazie, Pistazienbaum, Pisutachio, Alfóncigo, Pistachero (GRIN-CA, 2006).

Specimens examined: AFGHANISTAN. Badghes, N.E of Herat, Qalai-Naw, 4500 ft. *C. Grey-Wilson & T. F. Hewer 1275,* 06/28/1971 (K). Faisabad, Meshed, Warduj Valley, 4500 ft. *P. Furse 6443*, 06/01/1964 (K). **IRAN**. Jugo Paigah, Kotal inter Pul-Khumri**,** 35°55'N 68°45'E, 1500 m. *K. H. Rechinger 33910*, 1967 (K). **RUSSIA**. S. loc. *N. Samokisch & N. Androssov 7428,* 30/08/1910 (K). S. loc. 8500-600 m. *s. coll. s. n.*, 13/05/1975 (K). S. loc. *s. coll. s. n.*, 1960 (E). S. loc. *s. coll. s. n.*, 1960 (MO). S. loc. *N.*

Belianina & G. Proakuriakova 165, 1975 (MO). S. loc. *s. coll. s. n,* 1967 (MO). **SYRIA**. S.loc., *s.coll. 169*, 1856 (F). **USA** (cultivated). California, Chico, USDA**.** *s. coll. s. n.***,** 10/08/1960 (F). **TURKEY**. Urfa, Urfa city, Samsat. *N. F. Miller 886,* 1983-1984 (K). **TURKIMENISTAN**. Western Kopet Dag. *D. Kurbanov 776,* 06/30/2001 (MO).

Geographical Distribution: Wild *P. vera* is native to temperate Asia, Afghanistan [North], Iran [North East], Kyrgyzstan, Tajikistan, Turkmenistan, Uzbekistan [Central Asia] (Chapter 2, Appendix A, Map 4).

2- ***Pistacia khinjuk*** **Stocks.**, Hook. Kew Journ. 4: 143. 1852.

Type: Beloochistan, *Stocks 719*. 1849 (K).

P. acuminta Boiss. & Buhse, Nouv. Mem. Soc. Nat. Mosc. 12: 53. 1860.

P. khinjuk var. *populifolia* Boiss., Fl. Or. 2: 7. 1872.

Rhus integerrima Wall., Cat.: 8474. 1828.

Rhus kakrasingee Royle., Bot. Himal. 3: 175. 1835.

P. khinjuk f. *heterophylla* Engl., DC. Monogr. Phanerog. 4: 290. 1883.

P. khinjuk var. *glabra* Engl., DC. Monogr. Phanerog. 4: 290. 1883.

P. khinjuk var. *microphylla* Boiss., Fl. Or. Suppl. 154. 1888.

P. khinjuk var. *glaberrima* Boiss., Fl. Or. Suppl. 154. 1888.

P. khinjuk var. *genuina* Bornmueller, Beih. Bot. Centralbl. 19: 224. 1906.

P. khinjuk f. *heterophylla* Bornmueller, Beih. Bot. Centralbl. 19: 224. 1906.

P. khinjuk var. *macrocarpa* Zoh., Pal. J. Bot. 5: 212. 1952.

Small trees to 7 m high. Leaves deciduous, large, 11.0-17.7 cm long, 8.0-15.5 cm wide, odd-pinnate, membrananceous, petiole angled or rounded. Leaflets 3-7, large, 4.5-8.5 cm long, 1.5-4.0 cm wide, with average ratio 2.3, ovate to broadly ovate, acuminate, pinnately-veined, and glabrous. Terminal leaflet 2.9-8.5 cm long, 1.2-4 cm wide, with average ratio 2.1, often larger than laterals. Rachis not winged.

Common names: Gulungoor, Gwun, Khinjuk, Shurumma (Afghanistan), Ushgai Buzgai (Baluchistan) (Zohary, 1952).

Specimens examined: AFGHANISTAN. Kurrum Valley. *J. E. T. Aitchison 234*, 04/1879 (K). **IRAQ**. Jarmo, along Wadi west of Camp. *H. Helbaek 1272*, 30/04/1955 (K). Mam, Mosul, 2750 ft. *s. coll. 1643*, 10/10/1931 (K). Kermanshah Bisitun, 1350-1650 m, 34°23'N 47°25'E. *M. Jacobs s. n.*, 23/06/1963 (K). Mam, Bikhair Mt., near Zakhe (90 Km NW of Mosul). *Ali Rawi 23063*, 02/07/1957 (K). Quest du Jaz Murian . Route vera Bandar Abbas, 530 m, 27°25'N 57°15'E. *J. Léonard 5889*, 4/28/1972 (K). Rowandag, 1500 ft. *s. coll. 2142*, 20/04/1932 (K). **JORDAN**. Bada, little Petra. *M. G. AL-Saghir JOR24*, 06/04/2004 (VPI), *M. G. AL-Saghir JOR25*, 04/06/2004 (VPI). Petra. *M. G. AL-Saghir JOR26*, 04/06/2004 (VPI), *M. G. AL-Saghir JOR 27*, 04/06/2004 (VPI). **PAKISTAN**. Baluchistan, 4000 ft. *J. Linczervski 3639*, 04/05/1889 (K). Western Pakistan, Baluchistan, Quetta, 1400 m. *J. Lamond 1245*, 14/05/1965 (E). Quetta. *J. Sinclair 2444*, 31/08/1942 (E). **SAUDI ARABIA**. Jabal Dallagh, Tabak, 4500 ft. *I. S. Collenette 710*, 23/04/1984 (K). Jabal Dallagh, Tabak, 4500 ft. *I. S. Collenette 4380*, 23/04/1984 (E). **TURKEY**. Siirt, 1646 m. *P. H. Davis 43285*, 05/19/1966 (K). Mardin, Gizre, 400m. *Davis 42652*, 05/07/1966 (K).

Geographical Distribution: Extends from southeastern Turkey to northern Syria, northern Iraq, the mountains of western and southern Iran, and from there through Pakistan, Baluchistan, and into eastern Afghanistan. Towards the west, this species penetrates into southern Jordan, Hijaz, south Sinai and the eastern desert of Egypt (Chapter 2, Appendix A, Map 5).

3-***Pistacia terebinthus* L.** , Sp. Pl. 2 : 1025. 1753.

Lectotype: not designated (Reg. Veg. 127: 77. 1993). Probably one of the Linnean specimens in the Herbarium of the Linnaean Society of London (L.).

P. vera Mill., Gard., Dict. ed. 8. n. 4. 1768.

P. terebinthus M. Bieb. Fl. Taur. Caucas. 2: 418. 1808.

P. therebinthus Scop., Fl. Carniol. ed. 2: 262. 1771.

P. crassifolia Salisb., Prod. 2:172. 1825.

P. palaestina Boiss., Diagn. Pl. Or. Nov. Ser.1, 9: 1. 1849.

P. mutica Raul ex Boiss., Fl. Orient. 2: 6. 1872.

P. terebinthus var. *angustifolia* Lee & Lam. ex Lamotte, Prodr. Plat. Centr. 180. 1876.

P. terebinthina St-Lag., Ann. Soc. Bot. Lyon 7: 132. 1880.

P. terebinthus subsp. *vulgaris* Engl., in DC. Monogr. Phanerog. 4: 288. 1883.

P. terebinthus subsp. *palaestina* (Boiss.) Engl., DC. Monogr. Phanerog. 4: 290. 1883.

P. terebinthus var. *macrocarpa* Zoh., Pal. Jour. Bot. 5: 209. 1952.

P. terebinthus var. *oxycarpa* Zoh., Pal. Jour. Bot. 5: 209. 1952.

Shrubs 2-3 m high, or small trees to 6 m high. Leaves deciduous, large, 10.0-19.0 cm long, 6.0-19.0 cm wide, odd-pinnate or even-pinnate, membrananceous, petiole rounded (rarely flattened). Leaflets 6-10, 3.5-8.0 cm long, 1.0-3.1 cm wide, with average ratio 2.7, ovate to narrowly ovate, acuminate or mucronate, and glabrous (rarely puberulent). Terminal leaflet (if present) 1.0-6.0 cm long, 0.5-2.0 cm wide, with average ratio 2.3, smaller than laterals or reduced. Rachis not winged.

Common names: Adharia (Arabic), Butum (Arabic), Ela (Hebrew), Scornabecco, Terebinte, Terebintha, Terebinto (Zohary, 1952). Cyprus-turpentine, Pistachier, Térébinthe, Terebinth, Turpentine-tree (GRIN-CA, 2006).

Specimens examined: ALGERIA. Below Ain N'Sour. 900-1000 m. *H. Davis & H. Peter 58367*, 05/30/1975 (E). Near Takesane Forest House, S.W. of Djelfa. 1400 m. *H. Davis & H. Peter 53383*, 08/06/1971 (E). **BALEARIC ISLANDS**. Mallorca, Gorg Blau, 500 m. *L .F. Ferguson & I. K. Ferguson 2472*, 13/07/1969 (MO). **CYPRUS**. Karaman, 300 m. *Davis 1451*, 18/07/1994 (K). **FRANCE**. *J. Stainton 1247*, 6/10/1952 (K). N of Pout St Espint?, 350 m. *P. A. Rawdon 216*, 09/13/1953(K). Cipiéres Commune, 580 m. *F. N. Hepper 9887*, 06/07/1994 (K). Lot, Commune of Martel. *S. coll. s. n.*, 1990 (K). **GREECE**. Bozdag, N. W. of Drama, 500 ft. *J. Stainton 7490* (K). **INDIA**. Dehia Dium? *J. S. Gamble 23726*, 08/1989? (K). **IRAQ**. Jebel Khatchra near Balad Sinjar. *H. Field & Y. Lazar 654*, 05/06/1934 (F). **JORDAN**. Ajloun, 1029 m. *M. G. AL-Saghir JOR05*, 24/05/2004 (VPI). Ajloun, 1032 m. *M. G. AL-Saghir JOR07*, 24/05/2004 (VPI). Nyma, 1017 m. *M. G. AL-Saghir JOR09*, 24/05/2004 (VPI). Ajloun City, 857 m. *M. G. AL-Saghir JOR11*, 24/05/2004 (VPI). Ajloun City, 740 m. *M. G. AL-Saghir JOR12*, 29/05/2004 (VPI). Jerash, 1048 m. *M. G. AL-Saghir JOR14a*, 29/05/2004 (VPI) *M. G. AL-Saghir JOR14b*, 05/29/2004 (VPI). Umm Qays, 275 m. *M. G. AL-Saghir JOR02*, 24/05/2004 (VPI). **LIBYA**. Tripolitania, Suk et Sebet, 300 m. *Mazzocehi 1217*, 28/04/1963 (K). **PALESTINE**. Gallica, Pyronees, Sorio. *s.coll. s.n.*, 20/06/1906_(F). Jerusalem. *F. Meyers 28*, 05/30/1902 (F), *F. Meyers 2028*, 04/12/1904 (F). Wadi Qarn Banks. *A. Eig & M. Zohary s.n.*, 30/05/1926 (MO). **SPAIN**. Jaén, Jabalcuz, Calizas, 750 m. *C. Fernández 860433*, 01/07/1986 (K). S. loc. 200-500 m. *s. coll. s. n.* (K). S. loc., 230-500 m. *s. coll. s. n.*, 1867? (K). **SYRIA**. Antiochiae, 800 m. *J. E. Dinsmore 20392*, 08/06/1938 (K). Aley, 800 m. G. Samuelsson 1116, 19/05/1939 (MO). **TURKEY**. Adapazari, W of Adapazari. *Davis & Coode 39181*, 08/08/1962 (E). Antalye, Pisidie, 1100 m. *P. H. Davis 15779*, 27/07/1969 (E). Antayla, Termessos, 900 m, 36°59'N 30°30'E. *M. P. Frankis 8925*, 25/09/1989 (E). Gumshane, Near Torul. *M. Pitman & A. Wickham 23*, 10/09/1993 (K). Sinop, above Gerze, 300 m. *O. Polunin 25,010*, 09/07/1954 (E).

Geographical distribution: Native to the Mediterranean basin (Chapter 2, Appendix A, Map 6).

4-***Pistacia chinensis* Bunge**, Mem. Div. Sav. Acad. St. Petersb. 2: 89. 1835.

Type: "Peking leg. *Bunge*, in Herb, Petropol. 1883." (Zohary, 1952, p. 215).

Trees to 25 m high. Leaves deciduous, large, 8.2-23.0 cm long, 8.0-23.0 cm wide, even-pinnate, membrananceous, petiole angled or flattened. Leaflets 6-16, long, 4.0-12.0 cm long, 0.9-4.0 cm wide, with ratio 4.1, lanceolate, attenuate, and glabrous. Rachis not winged.

4.1-***Pistacia chinensis* subsp. *chinensis***

Type: “Peking leg. *Bunge*, in Herb, Petropol. 1883.” (Zohary, 1952, p. 215).

P. formosana Matsum., Bot. Mag. Tokyo 15: 40. 1901.

P.chinensis f. latifoliata Loesener, Beib. Bot. Jahrb., 34, 75: 49. 1904.

P. philippinensis Merr. & Rolfe, Philipp. Jour. Sci. 3: 107. 1908.

Trees to 25 m high. Leaves deciduous, large 8.2-23 cm long, 8.0-20.0 cm wide, even-pinnate, membrananceous, petiole angled or flattened. Leaflets 8-16, long, 4.0-10.0 cm long, 0.9-2.4 cm wide, with average ratio 4.1, lanceolate, attenuate, and glabrous. Rachis not winged. Drupes globular, apiculate with bony endocarp.

Common names: Huang-lien-shur or Huang-m-ya shu, Ransinbok (Formosa) (Zohary, 1952). Chinese pistachio (GRIN-CA, 2006).

Specimens examined: CHINA. Chekiaeg. *R. C. Ching son.,* 21/06/1924 (K). Hing Jiangau. *W. Z. Fang & Y. K. Hsiung 7939*, 10/10/1979 (MO). Gansu, Shuijiawan, bikou. *X. Wang 159*, 26/05/1992 (MO). Guangxi, Fushi Town, Liuliao *P. J. Cribb et al., 485*, 10/05/1997 (K). Guangxi, Fushi Town, Liuliao, 500 m. *P. J. Cribb et al. 485*, 10/05/1997 (K). Jiangau, Yun-Tai-anan, 200 m. *Yao 8484*, 22/09/1981 (MO). Jiangau. *Yao 8535*, 11/02/1982 (MO). Kowloon, *Tai-Hung Tung et al.514*, 27/08/2002 (K). Kowloon, *Tai-Hung Tung et al. 514*, 27/08/2002 (K). Kwangsi, Kweilin, 220 m. *P. P. Wan & K. S. Chow 79169*, 1979 (K). Kwangtung, S of Linchow, Yang Shan District. *T. M. Tsui 495*, 07/09/1932 (F). Kwangtung. *C. Ford 308*, 1888 (K). S. loc. *C. Cooper s. n.*, 1884 (K). Lunan Xian, Changhu, 1850-2000 m. *Sino-American Botanical Expedition* *to China 1581*, 08/02/1984 (MO). Nanhsi, Hsiang, 150 m. *W. P. Leu 2011*, 04/04/1993 (E). Near Peking. *S. W. Bushell s.n.*, 1874 (F). Shanghai. *C. Cooper 275*, 1873 (K). Shanghai. *s. coll. 275*, 04/1881 (E). Shantung Prov., Pao Shan, near Tsinanfu, 200 m. *C. Y. Chiao 3152*, 20/09/1930 (F). Western China. *E. H. Wilson 3363*, 09/01/1933 (K). Yunnan, Cao Xi Temple, Anning, 42 km S. of Kunming, 2025 m. *s. coll. 127*, 1981 (K). W Yunnan, Cao Xi Temple, Anning, 40 km W of Kunming, 2025 m. *s. coll. 126,* 29/04/1981 (E). S. loc. *T. Ceming 93159*, 06/03/1993 (MO). S. loc. *H. H. Chung 6228*, NA (F). S. loc. 700m. *L. Hanlin 793*, 23/09/1958 (MO). S. loc. *C. Z. Jixin 205*, 06/16/1936 (MO). *Jurong group of Nanjing Foresty University Expedition 7064*, 1978 (MO). *Naingu 42986*, 19/11/1931 (MO). S. loc. 1200 m. *L. W. R. Price 858*, 11/7/1912 (K). *Qinghua. 5072*, 19/10/1979 (MO). S. loc. Z. *Shaoyao s. n.*, 05/07/1958 (MO). S. loc. *Z. Shaoyao 2218*, NA (MO). S. loc. 680 m. *L. Tianwei 230*, 05/27/1962 (MO). S. loc. *L. Tianwei & Z. Zhaofen 258*, 06/16/1993 (MO). S. loc. *H. Xianyu 30203*, 29/08/1958 (MO). S. loc. *Ye Huagu 730*, 1982 (MO). S. loc. *H. L. Yin 6062*, 07/02/1996 (MO). S. Loc. *L. Yongshan. 96296*, 08/05/1996 (MO). S. Loc. *Zhao 53*, 08/28/1996 (MO). S. loc. *L. Zhiyou 2072*, 12/06/1984 (MO). **TAIWAN**. Ilan Hsian, Chi-Lan Shan, 1200 m, 24°38'48""N 121°28'40""E. *S. Clarke et al. 339*, 11/11/1993 (E). Taipei city, Taipei Botanical Garden. *C. K. Liou 148,* 05/17/1996 (E). **USA** (cultivated). California, Berkeley, the Garden of Anson & Anita Blake. *N. F. Bracelin 2283*, 09/09/1942 (F), S. loc. 300 m. *L. Linhan & H. Guanzhou s. n.*, 24/10/1963 (MO). S. loc. *Z. Zhiying 14018*, 15/08/1959 (MO).

N. *F. Bracelin 2597*, 25/06/1944 (F).

Geographical Distribution: Native to China, Taiwan, and the Philippines (Chapter 2, Appendix A, Map 3).

4.2-***Pistacia chinensis* Bunge subsp. *falcata* (Becc. ex Martelli) Rech. f.**, Fl. Iranica 63: 8. 1969.

Type: Eritrea, “Keren Sul Mte Deban fra, 4500-5500 m. *Beccari 273*, 08/1870 (FI)” (Zohary, 1952, p. 217).

P. falcata Becc. ex Martelli, Fl. Bogos. 24. 1886.

Rhus falcata Penz., Atti Congr. Bot. Geneva p. 337. 1893.

P. chinensis var. *falcata* (Becc. ex Martelli) Zoh., Pal. J. Bot. 5 : 217. 1952.

Trees to 7 m high. Leaves deciduous, large, 13.0-21.0 cm long, 11.4-18.2 cm wide, odd-pinnate or even-pinnate, membrananceous, petiole flattened. Leaflets 10-14, long, 5.2-9.1 cm long, 1.0-2.0 cm wide, with ratio 5.5, narrowly lanceolate, falcate, attenuate, and glabrous. Terminal leaflet (if present) 2.0-4.0 cm long, 0.5-1.1 cm wide, with average ratio 4.3, smaller than laterals or reduced. Rachis not winged.

Specimens examined: EGYPT. W. Haikwal, G. Elba, E. desert. *s. coll. 57/884,* 09/17/1936(K). **ERITREA**. Amasen, Monte Zagher, 2600 m. *A. Pappi 5308*, 20/05/1902 (F). Shoa Prov., Caribaldi Pass, 28 km W. of Metahara, 1100m. *J. W. Ash 2360*, 02/10/1974 (MO). Shoa Prov., just W of Garibaldi Pass, 1260 m. *W. Burger 3618*, 02/01/1963 (F). Shoa, Metahara-Nazret.1450 m. *M. Thulin et al. 3875*, 05/21/1980 (MO). Shoa Prov., 5 km E. of Wolenchetti, 1300 m. *W. Burger 3695*, 2/16/1965 (F). **ETHIOPIA**. S.W. of Awash station, 700 m. *W. de Wilde 10484*, 1966 (MO). **SAUDI ARABIA**. Abha, 2050 m. *U. Baierle & P. König 82,* 04/8/1982 (E).

Geographical Distribution: East Africa (Eritrea, Ethiopia, Kenya, and Somalia) anf Saudi Arabia (Chapter 2, Appendix A, Map 3).

4.3-***Pistacia chinensis* Bunge subsp. *integerrima* (Stew. ex Brand.) Rech**. **f**., Fl. Iranica 63: 8. 1969.

Type: "Syntypi numerosi e Pakistan occid" (Rechinger, 1969).

P. integerrima Stew. ex Brand., For. Fl. NW. Ind. 122. 1874.

P. khinjuk var. *stocksii* Engl., DC. Monogr. Phaner. 4: 291. 1883.

P. chinensis var. *integerrima* (Stew. ex Brand.) Zoh., Pal. J. Bot. 5: 216. 1952.

Tree to 25 m high. Leaves deciduous, large, 11.0-22.0 cm long, 12.0-23.0 cm wide, even-pinnate, membrananceous, petiole angled or flattened. Leaflets 6-14, long, 6.0-11.5 cm long, 2.0-4.0 cm wide, with average ratio 3.4, broadly lanceolate, attenuate, and glabrous. Rachis not winged.

Common names: Gusgu, Kakrangche, Kakrat, Kakring, Kakroi, Kanger, Kanroi, Masua (Afghanistan), Sarawan, Shue, Tungu (Zohary, 1952).

Specimens examined: PAKISTAN. Karachi, 600-1200 m. *s.coll. 2775*, 05/07/1968 (MO). Kashmir, 2500m. *s. coll. 28117*, 28/06/1876 (K). North West Fronter Province**,** Hazara, Bisian. *M. Shah & D. Khan 1808*, 24/04/1978 (K). N. W. F. P. Hazara, Manchora. *M. Shaukat & N. Ahmad 266*, 14/05/1976 (K). N. W. F. P, Swat, Miadam, 6000 m. *M. Shah & D. Khan 1073,* 29/05/1976 (MO). Punjab, Rawalpindi, Margalla Hills. *I. Dar et al. 01*, 14/04/1976 (MO). Punjab, Islamabad, Rawalpindi. *Sarfraz Khan*

42 (K). Punjab, Rawalpindi, Pindora, Islamabad. *S. Khan & W. Rehman 50,* 16/04/1978 (MO). Punjab, Rawalpindi, Pindora, Islamabad. *S. Khan 42* (MO).

Geographical Distribution: Native to Afghanistan, Pakistan, Nepal, and India (Chapter 2, Appendix A, Map 3).

5- ***Pistacia atlantica* Desf.**, Fl. Atl. 2: 364. 1800.

Type: Probably Algeria *Desfontaines* (BM)(Zohary, 1952).

P. chia Desf., Tabl. Ecol. Bot. 199. 1804.

P. atlantica var. *latifolia* DC., Prod. 2: 61. 1825.

P. mutica Fisch. & Mey., Bull. Soc. Nat. Mosc. 4: 338. 1838.

P. cabulica Stocks., Hook. Kew. Jour. 4: 143. 1852.

P. choulettei Gand., Dec. Pl. Nov. 1: 44. 1875.

P. mutica subsp. *cabulica* (Stocks.) Engl. in DC. Monogr. Phanerog. 4: 287. 1883.

P. mutica f. multifidia Engl., DC. Monogr. Phanerog. 4: 287. 1883.

P. atlantica var. *cypricola* Lindb. f. Soc. Sci. Fenn. Arsbok 20B: N. 07. 1942.

P. mutica var. *cipricola* Lindb. f., Act. Soc. Sci. Fenn. Nov. Ser. B, 2 N. 07: 22. 1946.

P. *atlantica* subsp. *mutica* (Fisch. & Mey.) Rech. f., Fl. Iran. 63: 4. 1969.

Tree to 7 m high. Leaves deciduous, large, 8.0-17.6 cm long, 5.2-14.0 cm wide, odd-pinnate, membrananceous; petiole flattened. Leaflets 6-10, large, 3.0-7.0 cm long, 0.5-2.0 cm wide, with average ratio 3.5, lanceolate, obtuse, puberulent, and margin ciliated. Terminal leaflet 2.7-7.0 cm long, 0.5-2.0 cm wide with average ratio 3.5, same size or smaller than the laterals. Rachis narrowly winged.

Common names: Betoum, Butum (Zohary, 1952).

Specimens examined: AFGHANISTAN. Kurrum Valley. *J. E. T. Aitchison 361*, 1879 (K). Nuristan, Asmar Barikot, 900 m. *L. Edelberg 1639*, 1949 (K). Herat, 1800 m, 34°18'N 64°00'E. *K. H. Rechinger 19192* (MO). **AZERBAIDZHAN**. Shamlhes, 130 m. A. K. *Shvortsov s.n.*, 1982 (MO). **CANARY ISLANDS**. E. of Agaete, 100 m. *P. H. Davis 67350*, 04/18/1981 (E). Gomera, 600 m. *C. J. Pitard 545*, 1906 (MO). **CYPRUS**. Trimithia, Neophyton, 1000 m. S. Ecoyomides 1019, 25/07/1967 (K). **GEORGIA**. Vashlovani Reserve. *J. F. Gaskin 175*, 13/05/1999 (MO). **IRAN.** Nargeci. *T. Antonio et al 256*, 12/05/1975 (MO). **IRAQ.** Shaith Adi, 2500 ft. *S. coll. 3666*, 14/07/1933 (K). Sefin Dagh. *F. Karium 3940*0, 11/07/1972 (K). **JORDAN**. Umm Qays, 275 m. *M. G. AL-Saghir JOR1,* 29/05/2004 (VPI). Ajloun, 877 m *M. G. AL-Saghir JOR3*. 24/05/2004 (VPI) 960 m. *M. G. AL-Saghir JOR4*, 24/05/2004 (VPI) 1092 m. *M. G. AL-Saghir JOR6,* 24/05/2004 (VPI). Nayma, 1017 m. *M. G. AL-Saghir JOR8,* 29/05/2004 (VPI) *M. G. AL-Saghir JOR10,* 29/05/2004 (VPI). Baqqawaeh, Safawi. *M. G. AL-Saghir JOR15a*, 02/06/2004 (VPI). Wadi AL- Botom. Azraq. *M. G. AL-Saghir JOR16*, 06/02/2004 (VPI).Wadi AL- Botom. Sahaab. *M. G. AL-Saghir JOR17*, 02/ 06/2004 (VPI). Wadi AL- Botom. Sahaab, 610 m. *M. G. AL-Saghir JOR18,* 02/06/2004 (VPI) 617 m. *M. G. AL-Saghir JOR19,* 02/06/2004 (VPI). Bair AL Dabaghat, Shobak, 771 m. *M. G. AL-Saghir JOR 20*, 04/06/2004 (VPI) 771 m. *M. G. AL-Saghir JOR 21*, 04/06/2004 (VPI) *M. G. AL-Saghir JOR 22*, 04/06/2004 (VPI) *M. G. AL-Saghir JOR 23*, 04/06/2004 (VPI). Qasar Amra, Azraq Oasis. *L. Boulos et al. 7084*, 25/04/1974(K). Qasar Amra, 25 km S.W. of Azraq Oasis.*V. Tackholm et al. 9168*, 14/05/1976 (K). **LIBYA**. North of Senam, Ulaad Bveek, 900 m. *H. G. Keith 146*, 1957 (K). Tripolitania, 700-750 m. *N. Y. Sandwith 2780*, 25/04/1939 (K). Gebel Nafoussa, Tefren, 700 m.

Davis 49540 15/03/1970 (E). **MOROCCO**. Marrakech, Asni, Ijoukak, 1170 m, 31°01'N 8°09'W. *S. L. Jury 11959*, 07/10/1993 (K). Titeki, 1250 m. *P. & J. Davis 48863*, 28/03/1969 (E). *G. Mines et al s.n.*, 05/09/1968 (MO). Thlwane, 800 m. *J. Lewalle 9731*, 27/03/1981 (MO). **PAKISTAN**. Dutte. *R. R. Steward 831*, 23/08/1902 (K). Baluchistan, 6000 ft. *J. Lace s.n.*, 27/04/1888 (E). **PALESTINE**. Jagur. *M. Zohary s.n.,* 06/01/1951 (K). Lower Galilee. *M. Zohary s.n.,* 06/01/1951 (MO). Jerusalem. *J. B. Dinsmore 9339*, 29/03/1913 (F). Jerusalem. *F. Meyers 712*, 20/04/1903 (F). **RUSSIA.** Tauria, 100 m. *A. K. Shvortsov 82*, 25/07/1967 (MO). **TURKEY**. Antalya, Serik, Seldsjuk bridge, 30 m. *P. H. Davis 638*, 23/04/1959 (K). Dodecanese. Rhodes, near the Acropolis. *C. C .Townsend 71/73*, 18/04/1971 (K).

Geographical Distribution: North Africa, Canary Islands, Greece, Aegean Islands, Cyprus, Palestine, Jordan, Syrian Desert, Turkey, Ukraine, Russia, Iran, Afghanistan, and western Pakistan (Chapter 2, Appendix A, Map 1).

6- ***Pistacia eurycarpa* Yaltirik**, Notes Roy. Bot. Gard. Edinb. 28: 11. 1967.

Type: Turkey, Bitlis; *McNeill* 600, 16/07/1956 (holotype. E).

P. atlantica var. *kurdica* Zoh., Pal. J. Bot. 5: 206. 1952.

Shrubs or small trees to 5 m high. Leaves deciduous, large, 10.2-18.2 cm long, 9.0-13.5 cm wide, odd-pinnate, membrananceous, petiole flattened. Leaflets, 5-7, large, 4.5-6.5 cm long, 1.5-3.8 cm wide, with average ratio 2.3, lanceolate, obtuse, puberulent, margin ciliated to glabrous. Terminal leaflet 5.2-8.0 cm long, 2.5-4.0 cm wide, with ratio 2.1. Rachis narrowly winged.

Common names: Badwar, Butum, Gazwan, Giwan, Habbat, Khadra, Khayak (Zohary, 1952).

Specimens examined: IRAN. N. W. Zagros, 1700 m. *Fliegner & Simmone 501*, 22/10/1977 (K). N.W. Zagros, Sardasht, 1400 m. *Fliegner & Simmone 486,* 22/10/1977 (K). **KURDISTAN**. 1500 m. *K. H. Rechinger 49144*, 1974 (K). **TURKEY**. Fars, 70 Km of Naryriz, 1700 m. *M. H. Bokhari & J. R. Edmondson 2093,* 17/06/1977 (E). Hakkari, Yuksekova. *P. H . Davis 44665*, 06/08/1966 (E). Hakkari, Yuksekova. *C. R. Sperling, D. Eser & H. H. Gecit 6787*, 19/07/1985 (E).

Geographical distribution: Syrian Desert, northern Iraq, Kurdistan, Turkey, Iran, Afghanistan, and Armenia (Chapter 2, Appendix A, Map 2).

***Pistacia* sect. *Lentiscus* Al-Saghir, nom. nov.**

Type species: *P. lentiscus* L.

Folia persistentia, paripinnata vel imparipinnate; foliola 6-32 jugata; rhachis alata. Small trees or shrubs; leaves evergreen, even-pinnate or odd-pinnate; leaflets 6-32; rachis narrowly winged.

Includes the following species: *P. lentiscus* L., *P. mexicana* HBK, and *P. weinmannifolia* Poiss. ex Franch.

Section *Lentiscus* Parf. & Bad., Proc. Natl. Acad. Sci. USA. 94: 7987-7992. 1997. *Lentiscus* was published by Parfitt & Badnes without a Latin description.

7-***Pistacia lentiscus* L.**, Sp. Pl. 2: 1026. 1753.

Lectotype: Herb. Linn. No. 1170.8 (LINN) (Siddiqi in Jafri & El-Gadi (ed.), *Fl. Libya* 52: 3. 1978)

Small evergreen trees or shrubs 1-4 m high. Leaves small, 2.0- 10.0 cm long, 3.4-10.0 cm wide, frequently even-pinnate (rarely odd-pinnate), leathery, petiole flattened. Leaflets 4-10, small, 1.6-4.5 cm long, 0.4-1.7 cm wide, with average ratio 2.8, ovate to narrowly ovate, elliptic or elliptical (rarely obovate), mucronate (rarely mucronulate) and glabrous. Terminal leaflet (if present) 1.4-2.1 cm long, 0.4-0.9 cm wide, with average ratio 3.3, similar or smaller than laterals. Rachis narrowly winged. Drupes borne in short racemes.

7.1-*Pistacia lentiscus* **subsp.** ***lentiscus***

P. massliiensis Mill., Gard. Dict. ed. 8. n. 6. 1768.

P. lentiscus var. *massiliensis* (Mill.) Duham., ed. Nov. 4: 72. 1808.

P. lentiscus var. *angustifolia* DC., Prodr. 2: 65. 1825.

P. gummmifera Salisb., Prod. 2: 172. 1825.

P. lentiscus var. c*hia* Duham., Cat. Hort. Par. 1829.

P. lentiscus var. *latifolia* Coss., Not. Pl. Crit. 54. 1848.

P. brevifolia Gand., Dec. Pl. Nov. 1: 44. 1875.

P. multiflora Gand., Dec. Pl. Nov. 1: 44. 1875.

P. subfalcata Gand., Dec. Pl. Nov. 1: 44. 1875.

P. lentiscus f. *leptophylla* Albo., 1919.

Shrubs (rarely small trees) to 5 m high. Leaves evergreen, small, 2.0- 7.5 cm long 3.4-10.0 cm wide, frequently even-pinnate (rarely odd-pinnate), leathery with flattened petiole. Leaflets 4-10, small, 1.6-4.5 cm long 0.4-1.7 cm wide with average ratio 2.8, ovate to narrowly ovate, elliptic or elliptical (rarely obovate), mucronate (rarely mucronulate), and glabrous. Terminal leaflet (if present) 1.4-2.1 cm long 0.4-0.9 cm wide with average ratio 3.3, same size or smaller than laterals. Rachis narrowly winged.

Common names: Akind, Battum, Droug, Elath, Hamastiq, Lentisco, Lentischio, Lentisque, Ochinos, Saris, Sondro (Zohary, 1952). Almecegueiralentisco, Arbre au mastic, Chios mastictree, lentisque, Mastic, Mastixbaum (GRIN-CA, 2006).

Specimens examined: ALGERIA. Foret du Bainem, 1000 ft. *R. P. Croston s. n.*, 14/01/1978 (MO). S. loc. *A. Faure s. n.*, 27/07/1930 (MO). S. loc. *A. Faure s. n.*, 31/08/1930 (MO). S. loc. *P. Jamin? s. n.*, 05/1849 (F). *C. Remain? s. n.*, 14/04/1860

(F). **CANARY ISLANDS**. Coteau da Sinagiee, Alpes Mountains. *s. coll. 842*, 04/04/1862? (F). *Alice Carter Cook 523,* 04/1897 (F). Tajira Alta, 400 m. *D. Bramwell 1096,* 28/03/1969 (E). S. loc. *C. Kunkel 12438*, 01/02/1969 (MO). S. loc. *A. Cook 523*, 1897 (MO). **CYPRUS.** Hedges below village, 250 m. *P. H. Davis 1450*, 07/18/1994 (K). **FRANCE**. Herault, 400 m. *B. Summers et al 6511*, 10/06/1993 (MO). **GREECE**. Melos Cyclases, 300 m. *M. Young 393*, 19/04/1976 (K). Parga. *B. Verlourt 4129*, 08/2/1964 (K). Sotir prope Kymi. *S. coll. 38178, 05*/09/1969 (MO). **ITALY.** Calabria, Reggio di Calabria. *R. K. Brummitt et al. 5256*, 21/06/1965 (K). San Remo. *Metralfe s.n.*, 1931 (K). *Ramona Park 3* (F). Sardinia. *J. S. Gamble 28269,* 1907 (K). **JORDAN**. Jerash, Akhasheiba, 503 m. *M. G. AL-Saghir JOR13a*, 29/05/2004 (VPI) *M. G. AL-Saghir JOR13b*, 29/05/2004 (VPI). **LIBYA**. Wadi Kouf W. of Beida, 300 m. *Davis 201*, 28/03/1970 (E). **MOROCCO**. Beni-Mellal, 1300 m. *P. H. Davis 55163*, 13/07/1973 (MO). Imouzzerdes-Ida-Outanane to Oulma. 500 m. *P. & J. Davis 48503*, 03/21/1969 (E). Mogadol. *A. W. Tresthewy 120*, 1934 (K). Temara, 100 m. *J. Lewalle 9710*, 13/03/1981 (MO). S. loc. 200 m. *J. Lewalle 13877*, 23/06/1992 (MO). **PALESTINE**. Bet. Masir. *F. Meyers 815,* 09/02/1903 (F). Jerusalem. *J. E. Dinsmore 4815,* 05/03/1907 (F). S. loc. *P. H. Davis 3974*,16/02/1942 (K). Kiryath-Anavium, near Jerusalem. *M. Zohary 260* (E). Lower Galilae, top of mountain Tabor. *D. Jaffe & C. Shenkar s.n.*, 31/03/1954 (MO). Mount Carmel 200 m. *F. S. Meyers & J .E. Dinsmore 7126,* 29/03/1911 (F). Valley of Ajalon. *American colony 815*, NA (MO). **SOUTH MACEDONIA**. S. loc. *A. W. Hull et al. 2608*, 12/04/1934 (K). **SPAIN.** Alicante, Valencia, La Nucia. *C. Jongkind & R. M. Nieuwenhuis 795,* 04/10/1993 (MO). Alicante,Valencia, Finestrat. *C. Jongkind & R. M. Nieuwenhuis 772*, 04/08/1993 (MO). JAéN, Pozoalcón, en el desvio de la pista hacia el cortijo de Cerro Miguel, 900-1000 m. *E. Villanueva et al. s. n.*, 09/06/1987 (F).Prov. Murcia, Venta de los Pinos. *A. Schinini*

& S. Sánchez Garcia 30609, 05/24/1996 (F). Sierra de la Cruz. *s. coll. s. n.*, 24/07/1925 (K). S. loc. *E. A. Willmott. s. n.*, 1854 (K). S. Loc. *E. A. Willmott s. n.*, 1826 (K). S. Loc. *A. Roderguz s. n.*, 15/05/1953 (K). **SYRIA**. S. loc. *J. D. Hooker & D. Hanbury s. n.* , 09/10/1860 (F). **TUNISIA**. Gardimaou. *J. W. Jansen 166* 16/04/1965 (MO). 14 km from Maktar to Kairouan, 820 m. *Davis & Lamond 57146*, 05/03/1975 (E). Kroumirie, between Les Chenes and Ain Draham, 400 m. *Davis 70025*, 05/07/1984 (E). Tabarka, 600 m. *J. W. Jansen 564*, 14/06/1965 (MO). **TURKEY**. Aegean Islands, 800 ft. *Royal Liberty School 55*, 22/08/1963 (K). Antayla, Kiremi thaneler, 40 m. *E. Hennipman 136, 0*7/04/1959 (K). Okulu, Antayla. *S. coll. 8515*, 13/02/1966 (MO). **YUGOSLAVA.** Hrvatska, Maqui. *P. Frost-Olsen 1414*, 19/08/1977 (MO).

Geographical Distribution: Native to the Mediterranean basin (Chapter 2, Appendix A, Map 7).

7.2-***Pistacia lentiscus*** **L. subsp. *emarginata (*Engl.) AL-Saghir, comb. nov.**

Basionym: *P. lentiscus* L. var. *emarginata* Engl., DC. Monogr. Phanerog. 296. 1883.

Type: Somalia, Al Mts, *Hildebrandt 1531*, 1875 (BM).

Synonym: *P. aethiopica* J. O. Kokwaro, Kew Bull. 34: 755. 1980.

Trees (rarely shrubs) 5-12 m high. Leaves evergreen, small, 4.0-10.0 cm long, 3.8-8.4 cm wide, even-pinnate, leathery, petiole flattened or angled. Leaflets 6-10, small, 1.9-4.2 cm long, 0.6-1.6 cm wide, with average ratio 2.6, ovate to narrowly ovate (rarely obovate), emarginate, glabrous, with large distinct veins. Rachis narrowly winged.

Common names: Hamar, Heis, Sisaye, Ulaaso (Zohary, 1952).

Specimens examined: ETHIOPIA. Sidamo, Borana, 27 mi. S. E. of Negele, Sidamo, Borana, 34 km on the Negele-Filtu road, 1650 m. *M. Thulin et al. 3450*, 09/05/1980 (K). S. loc. 4850 ft. *H. F. Mooney 5607*, 22/01/1954 (K). Sidamo, 20 km S. of Neghelle along the road to Melka Guba, 1500 m. *F. M. Tadesse & K. Vollesen 3119*, 18/05/1982 (K). **KENYA**. Gendabi, W. slopes of Mt Hanang, 7500 ft. *P. J. Greenway 7690*, 10/02/1946(K). Kajiado District. *J. B. Gillett & F. Kariuki 18816*, 21/10/1969 (K). Marsabit District, 1600 m. *F. N. Hepper & P. M. L. Jaeger 697,* 21/11/1978 (K). Nairobi, 5000-6000 m. *J. Wilson 1017*, 1960 (K). Marsabit, Mt. Kulal, Gatab. 1600 m. *F. N. Hepper & P. M. L. Jaeger 6972*, 21/11/1978 (MO). Neghelle, 1200 m. *J. W. Ash 2447*, 13/04/1974 (MO). Nairobi. *L. Matlan 3124*, 17/05/1954 (K). Narok District. *J. C. Rammell 3488*, NA (K). Nairobi, 9 miles S. E. of Narok, 6200 m. *C. G. Trapnell 2339*, 23/07/1957 (K). **SOMALIA**. Gan Libah, Golis range, 5400 ft. *P. E. Glover & Gilliland 1162*, 16/07/1945 (K). Garad Forest Reserve, Brigavo. *P. Bally & R. Melville 16001*,17/01/1973 (MO). Libah Melemt, 5200-5950 m. *J. B. Gillett 4697*, 03/12/1932 (K). Surud Forest, 20-24 km N.W. of Erigavo. *P. R. O. Bally & R. Melville 16001*, 17/01/1973 (K). S. loc. 2300 m. *J. J. Lavranos & Howood 10359*, 17/01/1973 (E).

Geographical Distribution: Native to East Africa (Eritrea, Ethiopia, Kenya, and Somalia) (Chapter 2, Appendix A, Map 7).

8-***Pistacia mexicana* HBK**, Nov. Gen. et Sp. 7: 22, tab. 608 .1825.

Type: Mexico, Guerrero , Chilpancingo. *Bonpland*, 1825 (BM) (Swingle, 1920).

P. texana Swingle, Jour. Arnold. Arb. 2: 107. 1920.

Trees (rarely shrubs) to 6 m high. Leaves evergreen, small, 5.1-14.1 cm long, 1.8-5.3 cm wide, odd-pinnate, membrananceous, petiole flattened. Leaflets 10-32, small, 1.0-2.5 cm long, 0.3-1.0 cm wide, with ratio 2.7, narrowly ovate, slightly falcate, distinctly mucronate, and glabrous. Terminal leaflet 0.5-2.5 cm long, 0.3-1.0 cm wide, with ratio 2.5, same size or smaller than laterals. Rachis narrowly winged.

Common names: Copall (Zohary, 1952). Mexican Pistachio (GRIN-CA, 2006).

Specimens examined: GUATEMALA. Baja Verapaz, near and above Santa Rosa, 1500 m. *P. C. Standley 91089*, 04/04/1941 (F). Baja Verapaz, N of Santa Rosa. *P. C. Standley 69749*, 30/03/1939 (F). Estancia Grande, 600 m. *P. C. Standley 59171*, 12/08/1938 (F). Jalapa, along quebrada near Zapote, 1 mile N of San Pedro Pinula, 1500 m. *J. A. Steyermark 32975*, 12/09/1939 (F). Huehuetenango, Vicinity of Aguacatán near the spring of San Juan, 1600 m. *P. C. Standley 81370,* 27/12/1940 (F). Huenhuetenango, between San Seabstián H. and large peasco above town, 2000-2200m. *J. A. Steyermark 50518,* 13/08/1942 (F). Jalapa, Mountains along the road between Jalapa and San Pedro Pinula 1400-1800 m. *P. C. Standley 77021*, 11/12/1940 (F). Jalapa, W of San Pedro Pinula, 1000 m. *P. C. Standley 77124*, 11/12/1940 (F). **HONDURAS**. Morazán, Tegucigalpa. *Antonio Molina R. 7760,* 03/17/1957 (F). Morazán, entre la Quebrada Tarán y Valle de Angelon. *Antonio Molina R. 11094*, 08/03/1962 (F). Morazán, Rio Choluteca. *Antonio Molina R. 7789*, 03/24/1957 (F). Comayagua, Rancho Grande , San Luis, 4000ft. *J. B. Edwards p-269*, 05/22/1932 (F). Morazán, 1200 m. *A. Molina R. & L. O. Williams 1421*, 12/13/1946 (F). Morazán, near Suyapa, 1200 m. *P. C. Standley & L. O. Williams 1421*, 11/25-12/14/1946 (F). Suyapa, 1200m. *Antonio Molina R. 1206*, 10/7/1943 (F). Morazán, Quebrada Suyapa, Northeast deTeguci Galpa, Aldea de Suyapa, 1100 m. *A. Molina R. 2551*, 08/10/1949 (F). Morazán, Vicinity of Suyapa, 1100-1200 m. *P. C. Standley 12038*, 12/09/1948 (F). Morazán, Tegucigalpa, 1200 m. *A. Molina R7760*. 17/03/1957 (F). Morazán, Rio Choluteca, 722m. *A. Molina R.7789*, 24/03/1957 (F). Morazán, Entre la Quebrada Tarán y Valle de Angelon, 1300m. *A. Molina R. 11094*, 08/03/1962 (F). Morazán, Aldea de Suyapa, 1100m. *A. Molina 2551*, 08/10/1949 (MO). Francisco Morazan. *M. Erazo 86*, 05/07/1975 (MO). Olancho, San Francisco de la Paz, Gualaco, 700 m. *McCarter & Hughes4*, 04/04/1983 (MO). Olancho, Campo Abierto, 960 m. *R. Ramos 109*, 05/08/1984 (MO). **MEXICO**. Aguirrero, 1550 m. *M. C. Johnston et al. 11718*, 07/06/1973 (F). Amajac, de Atotonilco el Grande, 1900 m. *R. Hernández Magaa et al. 6016*, 05/07/1981 (MO). Chiapas, 800m. *D. E. Breedlove 24568*, 16/04/1972 (F). Chiapias, Ocozocoautla, Camino de Bajada del Macho, para el rancho La Cruz. *J. I. Calzada et al. 10098*, 08/05/1983 (F).Chiapas, 3 km north of Tuxtla Gutiérrez along road to El Sumidero, 1800 ft. *D. E. Breedlove 9567*, 04/07/1965 (F). Chiapas, Ixtapa, Mexican highway # 190 and road to Bochil and Simjovel, 3400 ft. *D. E. Breedlove 9624*, 04/10/1965 (F). Chiapas, Municipio of Amatenango del Valle, 5900 ft. *A. S. Ton 2144*, 14/03/1967 (F). Chiapas, 4 mi W of Comitán, 5750 ft. *M. C. Carlson 1994*, 26/04/1949 (F). Chiapas, 4 mi W of Comitán. *M. C. Carlson 1994,* 26/04/1949 (F). Chilpancingo, 1800 m. *W. Anderson & C. Anderson 4916*, 27-28-/07/1968 (MO). Chichihualco, 11 km al W de Xochipala, camino a Filo de Caballo, 1640 m. *E. Martinéz*

714, 12/04/1982 (F). Coahuila, 11 km W de Xochipala, camino a filo de Caballo, 1640 m. *E. Martínez 701*, 04/12/1982 (F). Coahuila, Sierra del Carmen, Rchos, 1200 m. *M. A. Carranza et al. 2107*, 20/08/1994 (MO). Coahuila, Sierra de la Paila, N side, on Fan just below Mina EL San Luis. *C. C. Parry 98*, 08/1878 (K). Coahuila, Castanos. *F. L. Wynd & C. H. Mueller 105*, 06/16/1936 (K). Coahuila, About 2 km SW of Rancho San Miguel, 575 m. *F. Chiang et al. 7502*, 02/06/1972 (UT). Coahuila, E side of Sierra de los Guajcs, 12 km W Hacienda de la Encantada. *R. M. Stewart 1509*, 10-16/09/1941 (F). Coahuila, E side of Sierra de los Guajcs, 12 km W Hacienda de la Encantada. *R. M. Stewart 1509*, 09/10-16/1941 (F). Coahuila, 4 km E of San Francisco, 1450 m. *F. Chiang et al. 7605*, 10/06/1972 (MO). Coahuila, Castanos. *F. L. Wynd & C. H. Mueller 105*, 06/16/1936 (K). Coahuila, Canon de la Gavia, 1250-2200 m. *M. C. Johnston et al. 12001*, 2-3/08/1973 (MO). Coahuila, 11 km W de Xochipala, camino a filo de Caballo. *E. Martínez 701*, 12/04/1982 (F). Coahuila, Muzquiz. *E. Marsh 91*, 1935 (F). Coahuila, Muzquiz. *E. Marsh 368,* 07/07/1936 (F). Coalcoman, 1260 m. *H. et a.l 12135*, 09/05/1938 (K). Guanajuato, Camino a los Cocos, 1100m. *E. Ventura & E. López 6664*, 17/04/1989 (MO). Guanajuato, Bajio, Rancho Beltran, S de Xichu, 1600 m. *E. Ventura & E. López 6782*, 15/06/ 1989 (MO). Hidalgo, Jacala, I km al N. de Jacala. *Abisai Garcia M. 3192*, 06/08/1987 (F). Hidalgo, Jacala, 4500 ft. *V. H. Chase 7445*, 13/07/1939 (MO). Hidalgo, Jacala, Jacala. *A. Garcia & J. Miller 3192*, 06/08/1987 (MO). Hidalgo, Jacala, Shady mountain Ravine, 4500 ft. *V. H. Chase 7445*, 13/07/1939 (F). Huajuco Cannon, 35 mi South of Monterrey, 3500 ft. *A. F. Wilson m11*, 29/03/1954 (F). Huehuetenango, Puente El Aguilar, along road east of San Rafael Pétzal, 1730 m. *P. C. Standley 81411*, 29/12/1940 (F). Jalisco, Tapalpa, Guadalajara. *E. Lott & J. A. Magallanes 387*, 06/21/1981 (MO). Jalisco, Tlajomulco, Cerro Viejo, 1850 m. *T. S. Cochrane et al. 12617*, 06/22/1991 (MO). Nuevo Leon, 8 km al SE de

Galeana, Sobre la Carretera a linaree, 1700 m. *J. Rzedowski 27177*, 24/03/1970 (F). Nuevo Leon, Villa Santiago along low ledges bordering stream way. *C. H. Mueller 2040*, 24/06/1935 (F). Metzquititlan, Barranca de Venados, 1800 m. *R. Hernández 3615*, 08/06/1979 (MO). Monterrey. *A. F. Wilson m11,* 29/03/1954 (F). Neuvo leon, Sampagos. *M. T. Edwards 286*, 21/06/1937 (F). Neuvo Leon**,** Huajuco Canyon, 35 mi S of Coahuila, Sierra de la Paila, N side, on fan just below Mina El Aguirrero. *M. C. Johnston et al. 11718*, 07/06/1973 (F). Near Fiscal, 1100 m. *P. C. Standley 80412*, 18/12/1940 (F). Nuevo Leon, Mpio. Montemorelos, 750 m. *T. F. Patterson et al. 7098*, 09/05/1992 (UT). Nuevo Leon, 15 mi of Galeana, 45-5000 ft. *C. H & M. T. Mueller 630*, 30/03/1934 (F). Nuevo Leon, Mountains near Monterrey. *C. H. & M. T. Mueller 333*, 1933 (F). Nuevo Leon, Monterrey. *C. G. Pringle s. n.,* 1935 (F). Puebla, 5500 ft. *C. G. Pringle 8553*, 1901 (F). Oaxaca, Ixtlan, 2000 m. *R. C. Trigos & D. Lorence 2327*, 27/05/1983 (MO). Oaxaca, Cerro El Ramón, de El Rodeo, 2200 m. *A. García 2421*, 070/8/1986 (MO). Oaxaca, Ixtlan, Ixtlan de Juárez. *R. C. Trigos & D. Lorence 635*, 03/04/1981 (MO). Oaxaca, Sierra de San Felipe del Agua. *A. Saynes et al. 11,* 05/24/1984 (MO). Oaxaca, W del entronque de la carr, 1480 m. *R. Torres et al. 452*, 05/16/1982 (MO). Oaxaca, Canada de Carrizalillo, Cerro Verde Mpio. Tepelmeme, 1600-1820 m. *P. Tenorio 6976*, 17/07/1984 (F). Oaxaca, Albarradas, Hierve el Agua, 1760 m. *R. Gereau et al 20381*, 05/02/1986 (MO). Oaxaca, Etla, San Jerónimo, Barranca Ceniza,1500-1600 m. *A. Salinas et al. 6906*, 05/30/1992 (MO). Oaxaca, Telixtlahuaca, Etla,500-1625 m. *A. Salinas et al. 6882,* 29/05/1992 (MO). Oaxaca, Juxtlahuaca, Santos Reyes Tepejillo, 1510 m. *J. I. Calzada 20923*, 04/19/1996 (MO). Oaxaca, Carretera, Cuicatlan-Telixtlahuaca, 1800 m. *E. Hernández 16746*, 05/04/1978 (F). Oaxaca, 1900 m. *C. Conzatti s. n.,* 19/05/1802 (F). San Luis. *C. C. Parry 98*, 08/1878 (K). Oaxaca, Hierve el Agua, East of Mitla, 1730 m. *S. C. Caran 5*, 23/04/1989

(UT). Queretaro, Canon del Rio Moctezuma, 1120 m. *S. Zamudio et al. 1138*, 28/04/ 2000 (UT). Quiché. *José Ignacio Aguilar 1157*, 1942 (F). Puebla, Mpio, Caltepec, Agua Socoya, Barranca, 2070 m. *P. Tenorio 19998*, 14/03//2001 (MO). Puebla. *J. N. Rose 5858*, 08/01/1901 (K). San Antonio, 9000 m. *H. et al. 10258*, 06/01/1937 (MO). 35 mi E of San Luis Potosi & 8 mi of San Francisco on Mex. 70, 5500 ft. *L. Byrne 4643*, 06/05/1979 (F). San Miguel. *F. Gonzalez et al. 734*, 03/23/1980 (MO). San Luis Potosi, Minsas de San Rafael. *C. G. Purpus 5362*, NA (F). Tamaulipas, Vicinity of San Miguel. *H. H. Bartlett 10700*, 29/07/1930 (F). Tenejapa, Arroyo Jeshab, 800 m. *A. Méndez 5874*, 04/20/1983 (MO). Tuzamapan-Huatusco, Antes Rio Pescado, 600 m. *A. Vovides, I. Calzada 665,* 09/05/1981 (F). Veracruz, 4 km antes de Acultzingo Rumbo a Orizaba, 2000 m. *N. A. Gomez-Pompa 2222*, 07/28/1971 (F). Ver., Coatepec, Cerro de Achichuca sobre carr. Mpio. Unam, Iztacala, 2025 m. *J. I. Calzada 24053*, NA (K). Zitacuaro, Zitacuaro.*H. et al 11983*, 21/06/1983 (MO). **USA**. Texas. Val Verde Co., Hidden Trail Canyon, branching NW off Rio Grande. *A . M. Powell 3055*, 02/18/1977 (UT).

Geographical Distribution: Native to Texas, Mexico, Guatemala, and Honduras (Chapter 2, Appendix A, Map 8).

9- ***Pistacia weinmannifolia* Poiss. ex Franch.**, Bull. Soc. Bot. Fr. 33: 467. 1886.
Type: China, Yunnan au dessus da Tapintze, près Tali. *Delavay 562*, 1883 (P).

P. coccinea Coll. & Hemsl., Jour. Linn. Soc. Bot. 28: 36. 1890.
P. malayana M. R. Hend., Gard. Bull. Straits Settlements. 7: 97. 1933.
P. weinmannifolia var. *malayana* (M. R. Hend.) Zoh., Pal. J. Bot. 5: 202. 1952.

Trees (rarely shrubs) to 3-20 m high. Leaves evergreen, small, 5.0-10.2 cm long, 2.0-6.4 cm wide, even-pinnate, membrananceous petiole flattened or angled. Leaflets 10-22, small, 1.0-3.2 cm long, 0.5-1.6 cm wide, with ratio 2.6, ovate to narrowly ovate (rarely obovate), emarginate, and glabrous, often alternate. Rachis narrowly winged.

Specimens examined: CHINA. Guizhou, Xingyi, Po Gang. *Z. H. Tsi et al. 53,* 13/03/1996 (K). Yunnan, Baihualing, eastern side of Gaoligong Shan Region, 1460 m. *Bao Shan Xian & Mangkuan Xiang 13953* (F). Yunnan, Chuxiong Xian, Long tang, Kunming.1820 m. *B. Bartholomew et al. 1251,* 25/07/1984 (E). Yunnan, Heqin. *Y. B. Lou 43,* 07/01/1996 (K) Yunnan, Lijiang Hillside. *S. Y. Hu & Y. C. Kong 117*, 07/07/1999 (K). Yunnan, 2500 m. *E. E. Maire 4*, 1923 (K). Yunnan, 4500 m. *A. Henry 9600 B*, 1898 (K). Yunnan, Kunming, 2025 m. *s. coll. 127*, 29/04/1981 (K). S. loc. *R. P. Soulie s. n.* 1891 (K). Guangxi, Huanjiang, Mulungtown, 960 m. *P. J. Cribb et al. 433*, 07/05/1997 (K). Western China. *E. H. Wilson 3367*, 2003 (K). Tibet. *S. coll. 62* (k). Yunnan, Ma Chang. *D. Dulcouse 6236* (K). Guizhou, Xingyi, Po Gang, 1100 m. *Z. H. Tsi et al. 52,* 03/13/1996 (K). Yunnan. *S. coll. 1244*, 12/13/1904 (E). Yunnan, 900-1000 ft. *G. Forrest 10153* (E). Yunnan, Kunming, 2025 m. *Sino-British Expedition 127,* 29/04/ 1981 (E). Yunnan. *D. Chu 1218,* 1988 (MO). **VIETNAM**. Ha Siang, 1250 m. *s. coll. 50390*, 04/04/2000 (MO).

Geographical distribution: Native to West China, Vietnam, East Tibet, Burma, and Malaya (Chapter 2, Appendix A, Map 9).

Hybrids

Pistacia X *narbonnensis* L., Sp. Pl. 2: 1025. 1735.

P. X *nemausensis* Req. ex Planch., Bull. Soc. Bot. Fr. 11: xlvi. 1864.

P. X *cappadocica* Planch., Bull. Soc. Bot. Fr. 11: xlvi. 1864.

Hybrid between *P. terebinthus* and *P. vera*; native to the Mediterranean Basin.

P. *lentisco-terebinthus* Sap. & Mar. Ann. Sci. Nat. Ser. 5. Bot. 14: 2. 1871.

P. X *hybrida* Gasparr. ex Parl., Fl. Ital. 5. 383. 1873.

P. *lentiscus* X *terebinthus* Engl., DC. Monogr. Phanerog. 4: 286. 1883.

P. X *saportae* Burnat, Fl. Alpes. Marit. 2: 54. 1896.

P. *saportae* var. *oxycarpa* Zoh., Pal. Jour. Bot. 5: 203. 1952.

Hybrid between *P. lentiscus* and *P. terebinthus*; native to the Mediterranean Basin.

Excluded species

P. *americana* Mill., Gard. Dict. ed. 8. n. 7. 1768 = *Simarouba glauca* Cronquist (Simaroubaceae)

P. *fagaroides* Willd., Enum. Hort. Berol. Suppl. 66. 1814 = *Bursera fagaroides* (HBK) Engl. (Burseraceae)

P. *occidentalis* Baill., Adansonia, xi. 181. 1874 = *Dacryodes hexandra* Griseb. (Burseraceae).

P. *oleosa* Lour., Fl. Cochinch. 2: 615. 1790 = *Schleicheria trijuga* Willd. (Sapindaceae)

P. *simarbura* L., Sp. Pl. 2: 1026. 1753 = *Bursera gummifera* L. (Burseraceae).

P. *vitex* Steud. Nom. 1: 627. 1821 = *Vitex pubescens* Heyne ex Wall. (Lamiaceae).

Chapter 7

Pistacia Evolution

Zohary (1952) notes that, based on fossil evidence, *P. lentiscus* originated 40 million years ago, and the genus as a whole probably developed more than 80 million years ago. This conclusion is questionable because Anacardiaceae pollen and wood first appear in the Paleocene epoch, 65 to 55 million years ago (Hsu, 1983; Muller, 1984; Pell, 2004) and is found throughout the world. The origin for the order in which the Anacardiaceae occurs, Sapindales, dates back approximately 84 to 65 million years before present (Magallón and Sanderson, 2001; Wikström et al., 2001).

Based on this information and the fossil records of the genus, I hypothesize that *Pistacia* originated in the Paleocene epoch. Because Anacardiaceae is pantropical in distribution and North and South America represent major diversification centers of the family and given the geographical distribution of *Pistacia*, I postulate that ancestral species of *Pistacia* came from North America. This hypothesis is supported by *Pistacia* fossil records from the Paleocene of Wyoming and Colorado (Edwards and Wonnacott, 1935). Migration may have taken place from western Laurasia (North America) to eastern Laurasia (Europe and Asia) ending up in Central Asia via Europe where the genus radiated within Asia (West Asia and Mediterranean Basin) as hypothesized by Weeks et al. (2005) for the Burseraceae. This migration may have been facilitated by the boreotropical land bridge (Tiffney, 1985; Tiffney and Manchester, 2001), which spanned the North Atlantic during the Early to Middle Eocene. Global temperatures during the Eocene were highest during this time period and tropical vegetation is known to have occurred in this land corridor (Wolfe, 1978; Zachos et al., 2001). "Cooler temperatures during the Middle Eocene extirpated frost intolerant taxa in this region and the physical land connections disappeared sometime afterward" (Weeks et al., 2005, P.98). This vicariant scenario for *Pistacia* is indirectly supported by the localities of *Pistacia* fossils from the Early Eocene in England and Russia (Weeks et al., 2005). Following the migration of ancestral *Pistacia* into the Old World, *Pistacia* appears to have dispersed and radiated within continental Africa during the Middle Eocene (44 Ma). The spread of *Pistacia* to India and Southeast Asia appears to have occurred in relatively recent geologic time (5.0 Ma), perhaps due to a northeasterly range expansion of *Pistacia* coincident with the establishment of arid habitat in East Africa (Potts and Behrensmeyer, 1992; de Menocal, 1995).

The Oligocene origin of the American species, *Pistacia mexicana,* from a southeastern Asian ancestor (like *P. weinmannifolia*) may be due to long distance dispersal or migration through a probable trans-Atlantic Tertiary Landbridge, as hypothesized by Weeks et al. (2005) for Burseraceae.

I suggest that the genus extended its distribution range away from Central Asia to West Asia and the Mediterranean basin, East Africa and the New World species by passive dispersal mediated by wind, water, birds or even by people. This is supported by evolution toward a smaller seed with a hard endocarp paralleling a change in reproductive strategy from distribution by ground squirrels (burying the seed, as with walnuts and oaks) to bird- or wind- mediated distribution, which would require a seed capable of passing through a bird's gut or being blown by the wind. (Jordano, 1989). Species in both Section *Lentiscus* and Section *Pistacia*, which diverged relatively early, have evolved smaller leaves with more leaflets and a winged rachis and smaller hard seeds although these monophyletic groups probably evolved independently and gradually. Smaller elongated leaflets with pointed shoot apices also are more efficient for water removal from the leaf surface compared with simple rounded leaves and are more adequate for wind pollination. This would be a useful adaptation as the genus moved into higher rainfall regions (Parfitt and Badenes, 1997).

Li and Tanimura (1987) suggest that differences in mutation rates among organisms may be more a function of generation time than DNA repair rates. *Pistacia* species have a long generation cycle, at least 10 years to first flowering, and a life-span estimated to be as much as 400 years in some cases. The average replacement cycle for pistachio is probably between 50 and 200 years in the wild, so it is not surprising that *Pistacia* has evolved much more slowly than the annual species used to derive standard mutation rate estimates (Parfitt and Badenes, 1997).

Zohary hypothesized *P. khinjuk* to be directly descended from *P. vera*, a hypothesis that can not be supported or rejected so far. Zohary also considered *P. khinjuk* and *P. vera* to be the most primitive, each showing nine characters be attributed to primitive species: simple leaves, odd-pinnate leaves, small number of leaflets per leaf, symmetrical leaflets, rounded leaflet apex, simple petiole (no wings), highly branched panicles, deciduous character, and large fruit. This is consistent with a Central Asian center of diversity for the genus because the natural range for *P. vera* spans this region. *Pistacia* species based on *P. khinjuk* and *P. vera* are the only ones with large edible drupes. Both have a similar somewhat unique three-leaflet odd-pinnate leaf. RAPD markers in this study show that *P. khinjuk* and *P.vera* form a very close pair, accordingly, I suggest that *P. khinjuk* is indeed a direct descendant of *P.vera*.

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Education

2002- 2006: Virginia Tech, Blacksburg, VA. Doctoral program in Biological Sciences. Dissertation entitled: "Phylogenetic analysis of the genus *Pistacia* (Anacardiaceae)" Advisor: Professor Duncan M. Porter.

1999-2002: Yarmouk University, Irbid, Jordan. Master of Science in Biological Sciences. Thesis: Analysis Of Genetic Diversity of Wild Barley (*Hordeum spontanenum* C. Koch) Populations in Northern Jordan (Ajloun area) using DNA-Based Markers (AFLP and RAPD). Advisor: Professor Hanan I. Malkawi.

1995-1999: Jordan University of Science and Technology, Irbid, Jordan. Bachelor of Science in Biological Sciences.

Professional Experience

- Research Assistant Fellowship. The Correspondence of Charles Darwin Project, Cambridge University Library and the American Council of Learned Societies [2005-2006].

- Graduate Teaching Assistantship, Virginia Tech, Blacksburg, VA [9/2002 to 2006]
  - Laboratory Instructor for Principles of Biology Lab for Non Biology Majors.
  - Laboratory Instructor for Biological Principles Lab for Biology Majors.
  - Laboratory Instructor for Plant Taxonomy Lab.
  - Laboratory Instructor for Molecular Biology Lab.

- Graduate Teaching Certificate, Virgina Tech [2006]
- Nominated for Graduate Student Teaching Excellence Award for 2006 (Virginia Tech).
- Instructor. Department of Biological Sciences, Virginia Tech. Taught Princples of Biology for Biology majors[Summer 2006]

Grants Received

- Graduate Research Development Program grant, Virginia Tech [April 2003] ($250).
- Virginia Academy of Science grant, Virginia [July 2003] ($1180).
- Sigma Xi grant, Virginia [December 2003] ($500).
- Travel award from John Clayton Chapter of the Virginia Native Plant Society [October 2003] ($250).
- Graduate Research Development Program grant, Virginia Tech [December 2004] ($ 250).
- Earl Core Student Award from Southern Appalachian Botanical Society, Virginia [April 2005] ($250).
- Virginia Academy of Science grant, Virginia [August 2005] ($1180).