MOLECULAR PHYLOGENETIC ANALYSES OF *JUNIPERUS* L. SPECIES IN TURKEY AND THEIR RELATIONS WITH OTHER JUNIPERS BASED ON cpDNA

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ABSTRACT

MOLECULAR PHYLOGENETIC ANALYSES OF *JUNIPERUS* L. SPECIES IN TURKEY AND THEIR RELATIONS WITH OTHER JUNIPERS BASED ON cpDNA

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Evolutionary relationships within and among two sections and three subsections of *Juniperus* species (Section Juniperus, subsections Juniperus, Oxycedrus and Caryocedrus, Section Sabina) naturally distributed in Turkey were investigated with molecular variations of chloroplast DNA (cpDNA).

This study revealed the phylogenetic relation of 66 individuals from 7 native Turkish *Juniperus* L. species based upon DNA sequence of *trnL* intron (*trnL5'-L3'*) , *trnL3'-F(GAA)* (*trnL-F* intergenic spacer), *trnV* intron and *matK* (*maturase kinase*) of chloroplast DNA (cpDNA) regions. Furthermore, *Juniperus* species obtained from GenBank and one *Cupressus sempervirens* L. species as outgroup were included to determine the evolutionary relation of Turkish Junipers with other *Juniperus* L. species.

The results of the study indicated that *Juniperus* L. species in Turkey were classified properly at section, species and even population level. Especially the species of section Juniperus gave correlated results with previous morphological classifications such that *J. communis* L. and *J. drupacea* Labill. which are known as blue seeded

Juniperus L. species were diverged from red seeded *J. oxycedrus* L. At species level, some populations were divergent. For instance, *J. oxycedrus* L. from Kastamonu Kayalı Köyü, *J. foetidissima* Willd. from Eskişehir Çatacık gave different haplotype patterns from other members of the species. For *J. oxycedrus* L. from Kastamonu Kayalı Köyü the divergence might be due to geographic isolation; however, *J.foetidissima* Willd. might vary through gene flow from other *Juniperus* L. species in the same location. In fact, *J. foetidissima* Willd. from Eskişehir Çatacık did not show close pattern with other *J. foetidissima* Willd. (Section Sabina) samples, but showed relationship with section Juniperus.

To figure out phylogenetic relationships among *Juniperus* L. species distributed in Turkey and in other regions of the World, DNA sequences of studied regions of foreign samples were obtained from the NCBI database and were evaluated with DNA sequence of Turkish species used in the curent study. The samples of Section Juniperus gave expected results but including New World species of Section Sabina lead to dispersed allocation with Old World species of the same section. New World Sabina section distributed with different subclusters within Old World Sabina section. The result can be concluded as New World members of Section Sabina has not been well resolved yet and possessed close relation with Old World Sabina section.

The evolutionary time have shown that *mat*K region has more recent divergence than *trn* region. Moreover, subsection Juniperus and Oxycedrus showed the closest relation followed by subsection Caryocedrus. The evolution of *Juniperus* L. date back to more than 20 million years which was probably at Oligocene Miocene boundry. The geography of origin of *Juniperus* L. was probaby Eurasia such that New World species of Section Sabina diverged from other sections of *Juniperus* L. and probably evolved seperately after Median – Tethyan belt seperation.

Key words: *Juniperus* L., Phylogeny, Divergence Time, *trnL*, *trnL3'-F(GAA)*, *trnV*, *matK*, cpDNA

ÖZ

KLOROPLAST GENOMUNA GÖRE TÜRKİYE'DEKİ *JUNIPERUS* L. TÜRLERİNİN MOLEKULER FİLOGENETİK ANALİZİ VE DİĞER ARDIÇ TÜRLERİ İLE İLİŞKİSİ

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Türkiye'de doğal olarak dağılım gösteren 2 seksiyon ve 3 alt-seksiyonun (Seksiyon Juniperus, alt-seksiyon Juniperus, Oxycedrus ve Caryocedrus, Seksiyon Sabina) kendi içinde ve birbirleri ile olan evrimsel ilişkisi kloroplast DNA kullanılarak elde edilen moleküler varyasyonlar kullanılarak belirlenmiştir.

7 adet doğal Türkiye ardıcından elde edilen 66 bireyin filogenetik ilişkisi kloroplast DNA'nın *trnL* intron (*trnL5'-L3'*) , *trnL3'-F(GAA)* (*trnL-F* intergenik boşluk), *trnV* intron ve *mat*K (maturaz kinaz) bölgelerine bağlı olarak ortaya çıkartılmıştır. Çalışmanın ikinci kısmı olarak Türkiye' deki ardıçların diğer ardıç türleri ile evrimsel ilişkisini belirlemek aracıyla GeneBank' dan elde edilen ardıç türleri ile dış grup olarak 1 adet *Cupressus sempervirens* L. türü çalışmaya dahil edilmiştir.

Çalışmanın sonuçları Türkiye'deki ardıç türlerinin seksiyon, tür ve hatta popülasyon seviyesinde düzgün dağılım gösterdiğini ortaya çıkarmıştır. Özellikle mavi tohumlu olarak bilinen *J. communis* L. ve *J. drupacea* Labill. türlerinin kırmızı tohumlu *J. oxycedrus* L. türünden ayrılması Juniperus seksiyonuna ait türlerin daha önce yapılmış morfolojik sınıflandırmalarla uyumlu sonuçlar verdiğini göstermiştir. Tür

seviyesinde bazı popülasyonlar farklı şekilde ayrılmıştır. Örneğin, Kastamonu Kayalı Köyü'nden *J. oxycedrus* L. ve Eskişehir Çatacık'dan *J. foetidissima* Willd. türün diğer üyelerinden farklı haplotip desenleri ortaya çıkarmıştır. Kastamonu Kayalı Köyü' nden elde edilen *J. oxycedrus* L. için muhtemelen coğrafik izolasyondan dolayı farklılık görülmüşken, Eskişehir Çatacık'dan elde edilen *J. foetidissima* Willd. türündeki farklılık diğer ardıç türleri ile meydana gelen gen akışından dolayı ortaya çıkmış olabilir. Gerçekten de Eskişehir Çatacık'dan toplanan *J. foetidissima* Willd. diğer *J. foetidissima* Willd. (Sabina Seksiyonu) türleri ile hiçbir yakınlık göstermemiş olup Juniperus seksiyonu ile ilişki göstermiştir.

Türkiye' de dağılım gösteren Juniperus türleri ile dünya üzerindeki diğer yerlerdeki türlerin filogenetik ilişkisini çözmek amacıyla çalışılan gen bölgelerine ait NCBI veritabanından yabancı örnekler toplanmış ve mevcut çalışma sonucunda Türkiye'den elde edilmiş DNA zincirleri ile birlikte değerlendirilmiştir. Juniper seksiyonuna ait örnekler beklenen sonuçları vermişken Sabina Seksiyonun Yeni Dünya türlerinin eklenmesi Eski Dünya türleri ile dağınık bir ayrım yapmasına neden olmuştur. Yeni Dünya türleri Eski Dünya türlerinin içinde alt gruplar oluşturmuş olup bu durum Yeni Dünya türleri ile Eski Dünya türlerini henüz tamamen ayrılmadığını ve halen evrimsel olarak yakın ilişkili olduğunu göstermektedir.

Evrimsel zaman matK bölgesinin trn bölgesinden daha yakın zamanda evrimleştiğini göstermektedir. Ayrıca Juniperus ve Oxycedrus altseksiyonları en yakın ilişkiyi göstermiş ve bunu Caryocedrus subseksiyonu takip etmiştir. Ardıçların evrimleşmesi 20 milyon yıldan daha fazla yıl önce olduğunu göstermiş olup bu zaman; Oligocene Miocene bağlantısına denk gelmektedir. Ardıçların ilk oluştuğu coğrafya muhtemelen Avrasya üzerinde olmuştur ve Sabina seksiyonunun yeni dünya türleri Median – Tethyan bağlantısının ayılmasından sonra diğer ardıç seksiyonlarında ayrı olarak evrimleşmiştir.

Anahtar Kelimeler: *Juniperus* L., Filogeni,Ayrılma zamanı, *trnL, trnL3'-F(GAA), trnV, matK,* cpDNA

to my family and my love…

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LIST OF ABBREVIATIONS

AMOVA Analysis of Molecular Variance

BLAST Basic Local Alignment Search Tool

cpDNA Chloroplast DNA

CTAB Cetyl Trimethyl Ammonium Bromide

DNA Deoxyribonucleic Acid

dNTP Deoxyribonucleotide triphosphate

EDTA Ethylenediaminetetraaceticacid disodium salt

ETOH Ethanol

$\mathbf{F_{st}}$ Fixation Index

ITS Internal Transcribed Spacer Region

matK The maturase Kinase

MEGA Molecular Evolutionary Genetic Analysis

mtDNA mitochondrial DNA

MUSCLE Multiple Sequence Comparison by Log – Expectation

NCBI National Center for Biotechnology Information

NJ Neighbour-joining

nDNA nuclear DNA

ORF Open Reading Frame

PCR Polymerase Chain Reaction

RAPD Random Amplification of Polymorphic DNA

rbcL Large subunit of Rubisco

rDNA Ribosomal DNA

RFLP Restriction Fragment Length Polymorphism

RNA Ribonucleic Acid

rpL Ribosomal Protein L gene

rpS Ribosomal Protein S gene

Sect. Section

Sp. Species

Subsp. Subspecies

TBE Tris-Borate-EDTA

T- Coffee Tree-based Consistency Objective Function For alignment Evaluation

TE Tris EDTA

trn Transfer Ribonucleic Acid Region

t-RNA Transfer Ribonucleic Acid

TÜBİTAK The Scientific and Technological Research Council of Turkey

Var. Variety

CHAPTER 1

INTRODUCTION

1.1. Biology and Evolution of *Juniperus*

The species of *Juniperus* L. are widely distributed throughout the northern hemisphere. It is naturally located from the Arctic regions, to south of tropical Africa and to the mountains of Central America (Thorne, 1972; Farjon,2005; Adams, 2011) (Figure 1.1). Almost all species grow in the northern hemisphere except for *J. procera* Hochst. ex Endl. which extends mountains of east Africa in southern hemisphere (Adams and Demeke, 1993) (Figure 1.1). The genus is monophyletic (Little, 2006; Adams, 2011). The number of *Juniperus* L. species varies depending on studies such that Farjon (2001) reported 52 species, while Adams (2011) indicated the presence of 67 species. The *Juniperus* L. are divided into two sections and three subsections. However, which species belonging to which sections is still not clear (Mao *et al*., 2010). The main sections are Juniperus and Sabina. The subsections belonging to section Juniperus are Juniperus, Oxycedrus and Caryocedrus although Caryocedrus is accepted as different section in some studies (Adams, 1993; Mao et al., 2010).

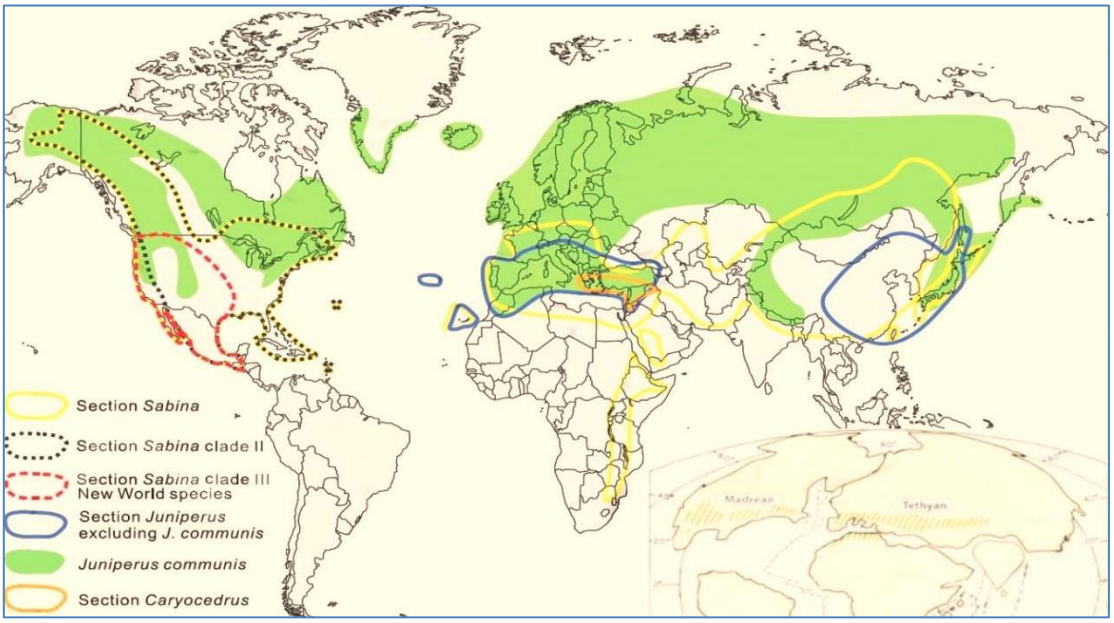

Figure 1.1. The distribution of *Juniperus* L. and hypothesized Madrean – Tethyan vegetation belt (Map from Mao *et al*., 2010)

Sect. Caryocedrus is restricted to the eastern Mediterranean region. *J. drupacea* Labill. the only species in this section, was probably differentiated about 20 million years ago (Adams, 2011). Sect. Juniperus like sect. Caryocedrus is not known from the fossil record in North America. Only it appears in Europe and Asia from the middle Miocene onwards (Straus, 1952; Negru, 1972). Section Sabina possesses pattern of geographic differentiation including the Himalaya and Tibetan Plateau, North America, the central Asia Europe, Africa and the Mediterranean. The fossil records for sect. Sabina date from the Eocene／Oligocene boundary (Kvacek, 2002) in Europe. It also dates from the late Oligocene to early Miocene in North America (Axelrod, 1956, 1987, 1991; Wolfe, 1964), These are related with the hypothesis that *Juniperus* was the part of Madrean-Tethyan vegetation belts (Axelrod, 1975) by the late Oligocene. Therefore they should have dispersed from one side to the other. It has more than 60 species according to recent studies (Adams, 2014) and classified in terms of their geography and morphology.

1.1.1. Morphology of *Juniperus*

Juniperus L. species possess "fruit" or "berry" like fleshy female cones where the scales are fused. Dispersal of these reproductive components by birds and small mammals (Santos *et al*., 1999) makes *Juniperus* L. be distributed in long distances (Adams, 2011).

Juniperus L. are evergreen shrubs or trees. Leaves are alternating opposite pairs in 4 ranks or in alternating whorls of 3. Pollen cones are with 3-7 pairs or trios sporophylls, each of which has 2-8 pollen sacs. Seed cones become mature in 1 – 2 years except *J. communis* L. whose cones mature in 3 years. The shape of the cones is spherical, ovoid and berrylike. Some of them have sweet taste although many of them are bitter and resinous. The choromosome number is 2n=22 except for *J. chinensis* L. which has tetraploid chromosome (4n=44) (Adams, 2011).

1.1.2. Taxonomy of *Juniperus*

Juniperus are of the cypress family Cupressaceae. The scientific classification is as follows:

Kingdom: Plantae

Division: Pinophyta

Class: Pinopsida

Order: Pinales

Famliy: Cupressaceae

Genus: *Juniperus* L.

- **Section *Juniperus*:** The species of this section possess needle like leaves in whorls of three, and jointed at the base.

    - **Subsect. *Juniperus*:** The species of this subsection are generally considered as northern and far eastern group with blue or blue-black mature seed cones (Figure 1.2a). Their cones are composed of 3 separate seeds and the needles have with one stomatal band. They are dioecious, with male and female cones on different trees. Cones are axillary on shoot, on a very short peduncle appearing sessile (Adams,

2011). The species of this subsection are *Juniperus communis* L. (Common Juniper) (Figure 1.2a), *Juniperus communis* subsp. *alpina* (Alpine Juniper), *Juniperus conferta* Parl. (Shore Juniper) (syn. *J. rigida* var. *conferta*) and *Juniperus rigida* Siebold & Zucc. (Temple Juniper or Needle Juniper).

- **Subsect. *Oxycedrus*:** The species of this subsection are the members of the genus from the Mediterranean Region. Their cones contain 3 separate seeds with red, reddish brown or reddish-purple color. Needles have two stomatal bands (Figure 1.2b). *Juniperus brevifolia* (Seub.) Antoine (Azores Juniper), *Juniperus cedrus* Webb & Berthel. (Canary Islands Juniper), *Juniperus deltoides* Adams (Eastern Prickly Juniper), *Juniperus formosana* Hayata (Chinese Prickly Juniper), *Juniperus lutchuensis* Koidz. (Ryukyu Juniper), *Juniperus macropoda* Boiss. (Pashthani Juniper), *Juniperus navicularis* Gand. (Portuguese Prickly Juniper), *Juniperus macrocarpa* Sibth. & Sm. (*J. oxycedrus* L. subsp. *macrocarpa*) (Large-berry Juniper) and *Juniperus oxycedrus* L. (Western Prickly Juniper or Cade Juniper).

- ***S*ubsect. *Caryocedrus*:** This subsection is distributed only in Greece, Syria, Lebanon, Israel and Turkey. However, the most extensive distribution is from south of the Taurus Mountains to north of Syria (Vidakovic, 1991; Farjon, 2005). The only member of this subsect. is *Juniperus drupacea* Labill. (Syrian Juniper). Species forms 10- 20 m (even 40 m) tall brown- grey big trees. Its cones with 3 seeds fuse together. Needles have two stomatal bands (Figure 1.2c). The tree of *J. drupacea* Labill is possibly the tallest Juniper exist in Kalekaya Village of Kahramanmaraş Province in Turkey (Karaca, 1994). According to Adams and Demeke (1993), this subsection is probably the most primitive one of the genus. In some studies, this section has been considered as separate genus (Florin, 1963) whereas DNA analysis showed it as the member of the genus *Juniperus* L. (Adams and Demeke, 1993).

- **Section *Sabina*:** The species of this section are considered as the most advanced section which possesses the highest species diversity. They are identified with their scale leaves and their adult leaves are generally similar to those of *Cupressus* L. species such that they are in opposite pairs or whorls of three. Moreover, the juvenile needle-like leaves are not jointed at the base. They have fleshy and nutrious female cones (Figure 1.2d, e, f, g). Different from other *Juniperus* L., some members of this section are monoecious (Adams, 2011). The section can be divided into several groups based on phenology, cone characteristics and leaf margin form (Adams, 1993). However, since this separation is not well defined, Mao *et al*., (2010) divided the section as Old World and New World species.

Table 1.1. Scientific and Common Names of Old World species of *Juniperus* L. in Section Sabina (Mao et al., 2010)

Scientific Name	**Common Name**	**Scientific Name**	**Common Name**
Juniperus chinensis L.	Chinese Juniper	*Juniperus pingii* var. *miehei*	
Juniperus chinensis var. *sargentii*	Sargent's Juniper	*Juniperus pingii* var. *wilsonii*	
Juniperus chinensis L. var. *tsukusiensis masummune*		*Juniperus procera* Hochst. ex Endl.	East African Juniper
Juniperus chinensis var. k*aizuka*		*Juniperus procumbens* (Siebold ex. Endl.) Miquel	Ibuki Juniper
Juniperus chinensis var. k*aizuka*		*Juniperus pseudosabina* Fisch. & C.A. Mey.	Xinjiang Juniper
Juniperus chinensis var. *procumbens*		*Juniperus recurva* Buch. – Ham. ex D. Don	Himalayan Juniper
Juniperus chinensis var. g*lobosa*		*Juniperus recurva* var. *butanica*	
Juniperus chinensis var. a*urea*		*Juniperus recurva* var. *coxii*	Cox's Juniper
Juniperus convallium Rehder & E.H. Wilson	Mekong Juniper	*Juniperus sabina* L. (Figure 1.2f)	Savin Juniper
Juniperus excelsa M. Bieb. (Figure 1.2e)	Greek Juniper	*Juniperus sabina* var. *davurica*	Daurian Juniper
Juniperus excelsa var. *polycarpos*	Persian Juniper	*Juniperus saltuaria* Rehder & E.H. Wilson	Sichuan Juniper
Juniperus foetidissima Willd. (Figure 1.2d)	Stinking Juniper	*Juniperus semiglobosa* Regel	Russian Juniper
*Juniperus indica*Bertol.	Black Juniper	*Juniperus squamata* Buch. –Ham. ex D. Don	Flaky Juniper
Juniperus komarovii Florin	Komarov's Juniper	*Juniperus thurifera* L.	Spanish Juniper
Juniperus phoenicea L. (Figure 1.2g)	Phoenician Juniper	*Juniperus tibetica* Kom.	Tibetan Juniper
Juniperus pingii Cheng ex Y. de Ferré	Ping Juniper	*Juniperus wallichiana* Hook. f. & Thomas. ex Brandis	Himalayan Black Juniper
Juniperus pingii var. *chengii*			

Table 1.2. Scientific and Common Names of New World species of *Juniperus* L. in Section Sabina (Mao et al., 2010)

Scientific Name	**Common Name**	**Scientific Name**	**Common Name**
Juniperus angosturana R.P. Adams	Mexican One-seed Juniper	*Juniperus jaliscana* Martinez	Jalisco Juniper
Juniperus ashei J.Buchholz	Ashe Juniper	*Juniperus monosperma* (Engelm.) Sarg.	One-seed Juniper
Juniperus arizonica (Syn: *J.coahuilensis* var. *arizonica* or *J. erythrocarpa* var. *coahuilensis*)	Redberry Juniper, Roseberry Juniper	*Juniperus monticola* Martinez	Mountain Juniper
Juniperus barbadensis L.	West Indies Juniper	*Juniperus occidentalis* Hook.	Western Juniper
Juniperus bermudiana L.	Bermuda Juniper	*Juniperus occidentalis* subsp. *australis*	Sierra Juniper
Juniperus blancoi Martinez	Blanco's Juniper	*Juniperus osteosperma* (Torr.) Little	Utah Juniper
Juniperus californica Carr.	California Juniper	*Juniperus pinchotii* Sudw.	Pinchot Juniper
Juniperus coahuilensis Martinez Gaussen ex R.P. Adams	Coahuila Juniper	*Juniperus saltillensis* M.T. Hall	Saltillo Juniper
Juniperus comitana Martinez	Comitán Juniper	*Juniperus scopulorum* Sarg.	Rocky Mountain Juniper
Juniperus deppeana Steud.	Alligator Juniper	*Juniperus standleyi*Steyerm.	Standley's Juniper
Juniperus durangensis Martinez	Durango Juniper	*Juniperus virginiana* L.	Eastern Juniper or Eastern Redcedar
Juniperus flaccida Schtdl.	Mexican Weeping Juniper	*Juniperus virginiana* subsp.*silicicola*	Southern Juniper
Juniperus gamboana Martinez	Gamboa Juniper	*Juniperus zanonii* (proposed by Adams, 2010)	
Juniperus horizontalis Moench	Creeping Juniper		

According to Adams (2014), Section Sabina is divided into 5 groups. These are

- Serrate leaf margins, western hemisphere
- One seed/ cone, turbinate or ellipsoidal shaped seed cones
- Multi-seeded Eastern Hemisphere
- One or more seeds / cone Eastern Hemisphere
- One or more seeds / cone Western Hemisphere

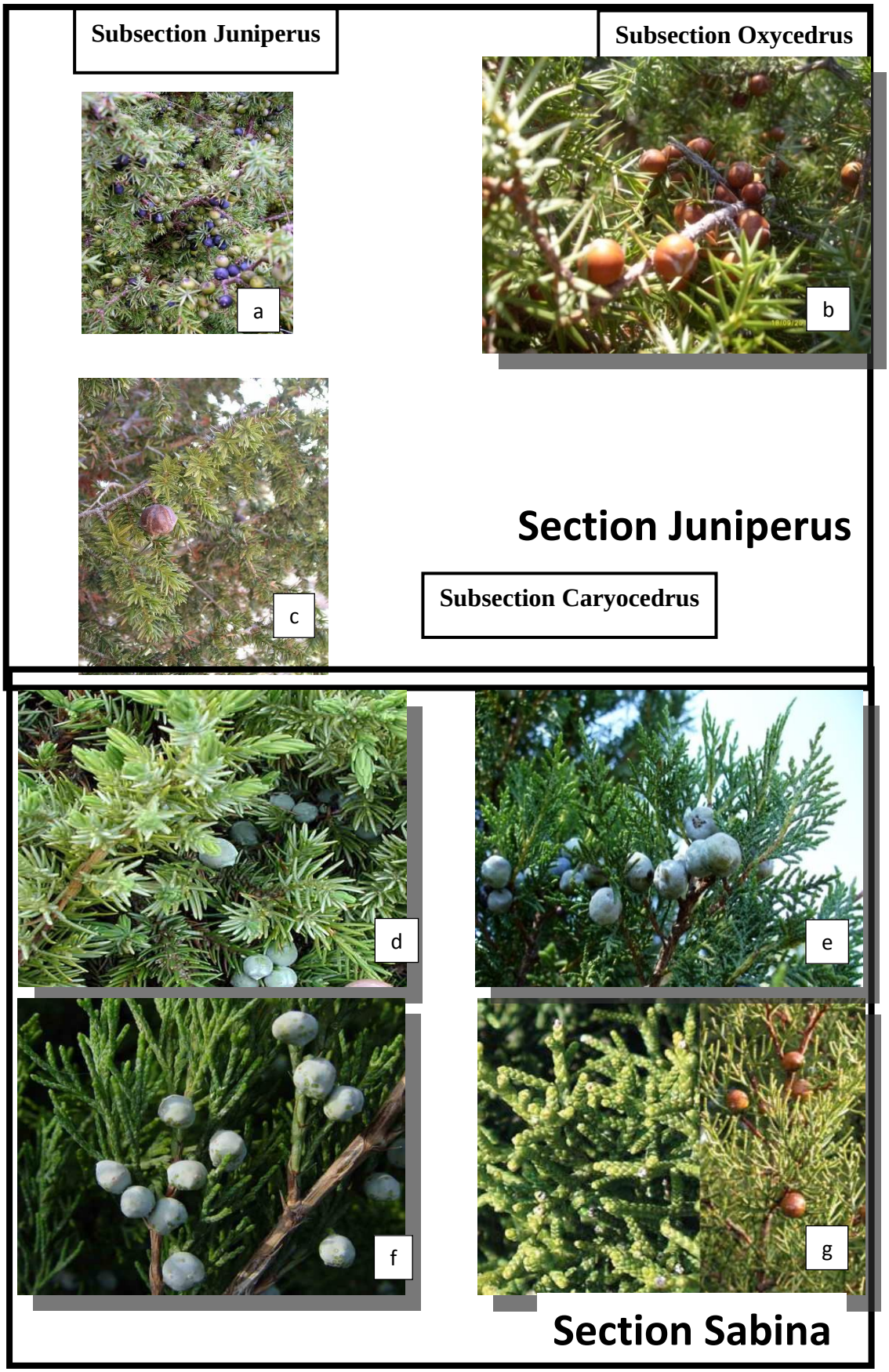

Figure 1.2. Sprout and Cone of some *Juniperus* species (a) *J. communis* L., (b) *J. oxycedrus* L., (c) *J. drupacea*. Labill, (d) *J. foetidissima* Willd, (e) *J. excelsa* M. Bieb., (f) *J. sabina* L., (g) *J.phoenicea* L.

1.1.3. *Juniperus* species in Turkey

Juniperus L. are widely distributed (Figure 1.3) and can be found naturally almost all regions of Turkey (General Directorate of Forestry, 2009; 2012). They are important economical and genetic resources. *Juniperus* L. have the third highest range of distribution (after *Pinus* L. and *Abies* L.) (575 315 ha) in Turkey (General Directorate of Forestry, 2012) as forest trees.There are 8 species naturally distributed in the country. These are *Juniperus communis* L. (section Juniperus, subsection Juniperus), *Juniperus oxycedrus* L. (section Juniperus, subsection Oxycedrus), *Juniperus drupacea* Labill. (section Juniperus, subsection Caryocedrus), *Juniperus excelsa* M. Bieb. (section Sabina), *Juniperus phoenicea* L. (section Sabina), *Juniperus foetidissima* Willd. (section Sabina), *Juniperus sabina* L. (section Sabina) and *J. oblonga* Beib. (Section Sabina). (Davis, 1966, 1988, 2001; Anşin and Özkan, 1993). Among these species, *J. oxycedrus* L. is widely distributed throughout the country whereas *J. phoenicea* L. is found mostly in the Southwestern Anatolia and *J. foetidissima* Willd. is widely distributed in Central and southern regions of Turkey. *Juniperus* L. in Turkey are also very diverse at subspecies and variety level such that there are three subspecies of *J. communis* L. (subsp. *hemisphaerica*, subsp. *communis*, subsp. *nana*, var. *saxatilis* Pall.) (Davis, 1966, 1988, 2001; Güner *et al*., 2012), three subspecies of *J. oxycedrus* L. (subsp. *macrocarpa* Sibth. & Sm., subsp. *oxycedrus* and subsp. *oxycedrus*, subsp. *oxycedrus* var. *spilinanus* Yalt., Eliçin & Terzioğlu, f. *yaltirikiana* M.Avcı & Ziel. and subsp. *procera*) (Tümen & Hafızoğlu, 2003; Güner *et al*., 2012) (Figure 1.3) and two subspecies of *J. excelsa* (subsp. *excelsa* and subsp. *polycarpos* K. Koch) (Davis, 2001; Güner *et al*., 2012).

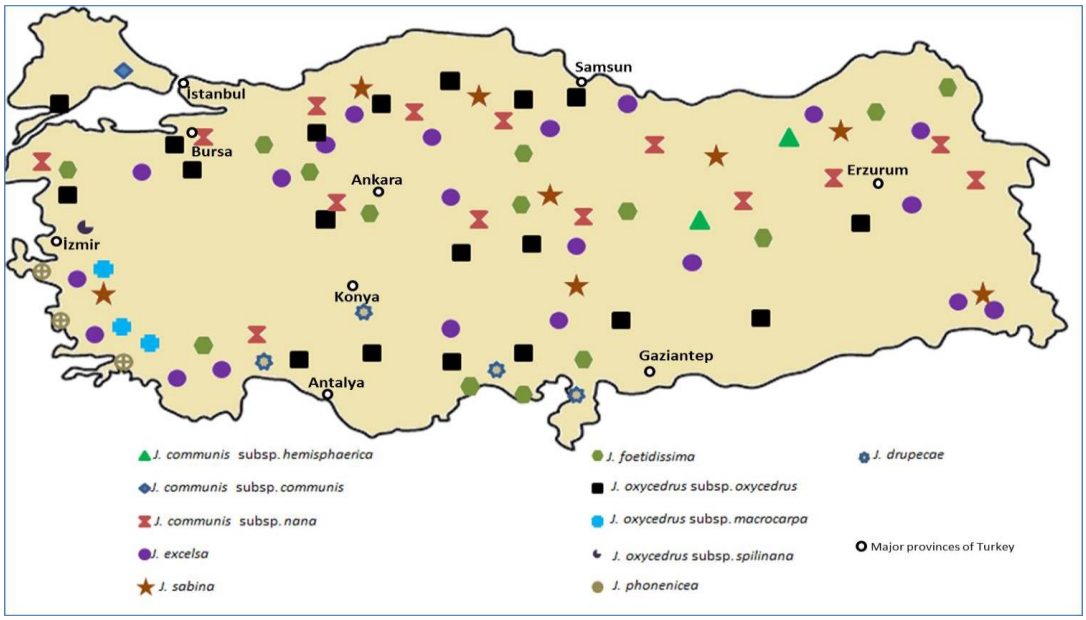

Figure 1.3. The map showing the natural distribution of *Juniperus* L. species in Turkey (adopted from: Tümen & Hafızoğlu, 2003)

1.1.4. Uses of *Juniperus*

Juniperus L. species are very important around the World due to the presence of extractive components and utilization of them in medicine and pharmacology. Not only in the World, but also in Turkey they are highly used for their wood, cones and leaves. Roots are important remedy for pain, cough, rheumatism, tuberculosis etc whereas cones and leaves are used for antispetic purposes. *Juniperus* L. species are rich in essential oil, tannin, flavanoid, resin, lignin and triperten (Hegnauer, 1986). Cones and leaves are also used in medicine and cosmetics in dermatologic disorders and as a stimulant. The female cones (berry like seeds) of *Juniperus communis* L. are used in making gin (Baytop, 1984). In antiquity, when the medicinal science was not developed, *Juniperus* L. were known as panacea, diuretic and sudorific. Not only used internally, but also they were used externally directly on to the skin of patients. Currently, they are still important against inflamation, head ache, diabetes, digestive troubles, bronchitis, asthma, kidney and urinary track infectios, hepatitis, sciatic, rheumatism, respiratory tract diseases, sinusitis, liver diseases and metabolism disorders (Koç, 2002; Gürkan, 2003). The wood of Junipers is being used for making furniture and paneling. It is also a good fuel wood, burning clean with little smoke and ash. (Herbst, 1978). *Juniperus excelsa* M. Bieb. wood is highly used in tuberculosis and hepatitis (Tümen ve Hafızoğlu, 2003).

1.2. The Phylogenetic Analysis with cpDNA

Molecular systematics covers a number of approaches in which phylogenetic relationship are determined using information from DNA of the interested organisms. The DNA barcoding approach is gaining importance in species identification (Hebert *et al*., 2003). It is mainly performed by using small fragment of DNA sequences of the genome. The purpose is to make contribution in ecological and conservation studies when traditional taxonomic identification is not feasible. Intra- and interspecific genetic divergences are assessed by using pairwise calculations (Meyer and Paulay, 2005) and phylogenetic analyses were performed to look for species monophyly (Lahaye *et al*., 2008). Molecular systematics and phylogenetic analyses usually start with DNA sequence analysis. The phylogenetic classification is advantageous because:

(1) determination of which information best provide natural relationships is not intuitive;

(2) by using either the same or different data including other genes or categories of information, the analysis could be repeated by other researchers and

(3) as new data emerged, it could be updated particularly from studies of chromosome organization and morphology and other traits determination by the genes that code for them (Graham and Wilcox, 2000).

In recent years, DNA data is used for the establishment of a classification as they become more widely available for many species. For plants, there are three basic types of DNA sequence data system which are nuclear (nDNA), chloroplast (cpDNA), and mitochondrial (mtDNA) (Simpson, 2006). The nuclear genome is not used in systematic botany frequently due to having a complex and highly repetitive characteristic. Since its structure, size, configuration, and gene order changes rapidly, the mitochondrial genome is used at the species level. However, cpDNA is more advantageous over other regions. In plant total DNA, it is a relatively abundant component. Thus it is easy in extraction and analysis. Also it contains primarily single copy genes where it provides extensive background for molecular information on the chloroplast genome. Therefore, molecular data obtained from the chloroplast

DNA are more useful over other molecular regions for phylogenetic reconfiguration in plant systematics (Liang, 1997).

Another important property of cpDNA for especially the conifers is its inheritance. cpDNA is inherited paternally in many conifers such as *Pseudotsuga* L., *Picea* L., *Pinus* L., *Larix* L., *Sequoia* L., and *Calocedrus* L. (Neale *et a*L., 1986; Neale and Sederof, 1988; Wagner *et a*L., 1992; Dong *et al*., 1992; Szmidt *et al*., 1987; Neale *et al*.,1989; Neale *et al*., 1991) although there are some non-paternal inheritences (Neale *et al*., 1986; Szmidt *et al*., 1987; Wagner *et al*. 1989; Neale *et al*. 1991; Dong *et al*., 1992). Previous studies of Cupressaceae (including *Juniperus* L.) (Neale *et al*., 1989, 1991; Mogensen 1996; Kondo, *et al*. 1998; Hwang *et al*., 2003), showed that cpDNA is also paternally inherited in members of this family.

Moreover, because of their mutational complexity and lack of representativeness, the classification based on whole genome may provide biased estimates of nucleotide diversity and thus may also give rise to incorrect estimates of genetic subdivision. However, with cpDNA, there is an effect only a single genetic locus, and usually only one or very few repetitive, polymorphic regions in the genome. The conservative rate of structural change and nucleotide substitution in conifer cpDNA makes it suitable for determining interspecific and intergeneric relationships (Hipkins *et al*., 1995). There are many studies dealing with plant phylogeny in which cpDNA variants have been detected (Stine *et al*., 1989; Stine and Keathley, 1990; Wang, 1990; Ponoy *et al*. 1994; Nelson *et al*., 1994).

1.2.1. Transfer Ribonucleic Acid Region (*trn*) of cpDNA

For the inference of plant phylogenies, cpDNA molecular regions are the primary source of data (Baldwin, 1992; Baldwin *et al*., 1995 Álvarez and Wendel, 2003). Moreover, since noncodinh regions are less functional than coding regions, they provide greater levels of variation for phylogenetic analyses. Therefore, they were easily used for lower level taxonomic studies (Gielly and Taberlet, 1994). For example, among the non-coding regions, the t-RNA (*trn*L-*trn*F$^{(GAA)}$ and *trn*V) are the most widely explored cpDNA fragment due to their extensive utilization in phylogenetic relationships at the levels below family (Taberlet *et al*.,1991; Kelchner, 2000). The *trn*L-F$^{(GAA)}$ region is composed of *trn*L$^{(UAA)}$ gene and *trn*L-F$^{(GAA)}$ intergenic spacer region. The *trn*L$^{(UAA)}$ gene consists of two highly conserved exons

which are divided by a group I intron. These intronic types are identified as an intergenic spacer which is characterized by a highly conserved core structure encoding the active site. In plants, the *trn*L intron generally displays sequence conservation in the regions flanking both *trn*L exons. However, the central part is highly used due to

- having flanked region by relatively conservative coding regions
- its moderate size,
- ease in amplification and sequencing (Bogler & Ortega, 2004).

The $tRNA^{UAC}$ (*trn*V) region, which is known as group II intron (Keller and Michel, 1985), has been first sequenced by Deno *et al*. (1982). The *trn*L-*trn*F and *trn*V regions present a quite high substitution rate in many plant taxa (Bayer and Starr, 1998; Bakker *et a*L.,2000; Mansion and Struwe, 2004). The t-RNA regions of the *trn*L and *trn*L - *trn*F and the region *trn*V are suitable for evolutionary studies due to; (i) th possession of the conserved *trn* genes and several hundred base pairs of non-coding regions, (ii) the high rate of mutations in the single-copy regions, and (iii) the absence of gene rearrangements among many species (Wolfe *et al*., 1987).

Not only *trn* regions, but also many nuclear, chloroplast or mitochandrial regions were studied in taxa of gymnosperms including *rbc*L and a single new nuclear smallsubunit (nuSSU) rDNA sequence (Chaw *et al*., 1997), RAPD studies in *Juniperus* (Adams, 2000), *mat*K and *chl*L gene of Taxodiaceae and Cupressaceae (Kusumi *et al*., 2000), *nad*5-4 region of *Abies* L. (Liepelt *et al*., 2002; Ziegenhagen *et al*., 2005), *ITS* region of Cycads (Bogler & Ortega, 2004) and Zamiaceae (Gonzalez & Vovides, 2002), *trn*D-*trn*T, *trn*S-*trn*G regions in *Cupressus* (Tingting *et al*., 2010), *cox*1, *nad*5 a/b intron, *trn*fM–*trn*S, *trn*T–*trn*F, *trn*C–*trn*D and *pet*G–*psa*J of *Pseudotsuga* (Wei *et al*., 2010) and so on. Moreover, despite their low evolutionary rate, cpDNA RFLPs (restriction fragment length polymorphisms) are used to detect variations at the population level They are also used for phylogeographic studies at both the interspecific and intraspecific level (Demesure *et al*. 1995; King & Ferris 1998; Dutech *et al*. 2000; Gao *et al*., 2007). Frequently used cytoplasmic DNA fragment in phylogeny of gymnosperm are chloroplast *trn*T-*trn*F (Wei and Wang, 2003), 50*rps*12-*rpL*20, *psb*B *psb*H and *rpL*16 intron (Shaw *et al*., 2005), and mitochondrial *nad*1 intron 2 (Won and Renner, 2005) and *nad*5 intron 1 (Jaramillo-

Correa *et al*., 2004). The three intergenic spacers *trn*T(UGU) - *trn*L(UAA), *trn*L(UAA)-*trn*F(GAA), and the *trn*L(UAA) intron in the *trn*T-F region of cpDNA have been widely used in the studies of phylogenetic relationships at inter- or intraspecific level due to a fast rate of evolution (Fujii *et al*., 1995, 1996; Böhle *et al*., 1996; Gielly *et al*., 1996; Bakker *et al*., 2000; Fukuda *et al*., 2001). Non-coding sequences tend to evolve faster than coding sequences. Hence, they usually provide information to get a phylogenetic tree. Hence, the *trn* regions were selected to realize phylogenetic relations among species of *Juniperus* L.

1.2.2. The Maturase Kinase (*mat*K) Gene

The *mat*K gene is an open reading frame (ORF) that encodes a maturase, a protein, used in RNA splicing (Neuhaus and Link, 1987; Wolfe *et al*, 1992). It is located within the intron of *trn*K (Lysine (UUU) gene) gene which possesses a group II intron that encodes the *mat*K (Hausner *et al*, 2006). These introns, which are found in eubacteria, archea and the organelles of fungi, plants, and algae, are mobile elements and have self-splicing abilitiy (Bonen and Vogel, 2001; Lambowitz and Zimmerly, 2004; Hausner *et al*, 2006). However, the *trn*K intron differs from other group II introns because of its encoding function (Hausner *et al*., 2006). For the construction of plant phylogenies, the *mat*K gene has been used as an indicator due to rapid evolution of the ORF's (e.g., Hilu and Liang, 1997; Kelchner, 2002; Hausner *et al*, 2006). There are various studies, which include family, genera and species levels, *mat*K gene sequence is used in phylogenetic analysis. In the study concerning conifers the studies with *mat*K included generally the genus *Pinus* (Wang *et al*., 1999; Quinn *et al*., 2002; Gernandt *et al*., 2003,2005; Zhang and Li, 2004; Eckert and Hall, 2006; Liston *et al*., 2007; Tsutsui *et al*., 2009; Flores-Renteria *et a*L., 2013; Hernandez-Leon *et al*., 2013). Also *Picea* (Germano and Klein, 1999; Quinn *et al*., 2002; Ran *et al*., 2010), *Cedrus* L., *Abies* L., *Keteleeria* L. (Quinn *et al*., 2002), *Tsuga* L. (Quinn *et al*., 2002; Havill *et al*., 2008), *Pseudotsuga* L., *Nothotsuga* L., *Larix* L., *Pseudolarix* L., (Quinn *et al*., 2002), and *Cathaya L.* (Quinn *et al*., 2002; Ran et al., 2010) have been studied. The *mat*K was shown to have higher variation than any other studied chloroplast genes. The variation was slightly higher at the 5' region than that at the 3' region although in general there is approximate even distribution observed throughout the entire gene. Also the gene might provide high phylogenetic information having high proportion of transversion (a change from

purine to a pyrimidine, or vice versa). These factors emphasize the utilization of the *mat*K gene in systematic studies. Moreover, it is suggested that comparative sequencing of *mat*K is appropriate for phylogenetic analysis at subfamily, family, genera and species levels (Tanaka *et al*., 1997).

CHAPTER 2

JUSTIFICATION OF THE STUDY

Juniperus L. is among most important tree species in Turkey. They have wide distribution and cover more than 550,000 ha of the country (General Directorate of Forestry, 2012). Under these circumstances, they also become economically important species and have a potential of desired hereditary features to be improved. For instance, they have been very important raw material in cosmetics, medicine and pharmacy for centuries (Tümen and Hafızoğlu, 2003). Moreover, they are disease resistant, insect tolerant and have high adaptive variation (Van Haverbeke and King, 1990) However, in contrast to this situation, very little genetic information or research present on *Juniperus* L. species. Moreover, their natural distribution area is reduced gradually due to anthrophogenic factors. There are numerous studies dealing with magnitude and pattern of variation in natural populations of *Juniperus* L. (Adams, 2000; Adams *et al*., 2003, 2005; Mao *et al*., 2010; Rumeu *et al*., 2011; Adams, 2012). There are also several studies at higher taxonomic levels indicating the evolutionary location of *Juniperus* L. within family of Cupressaceae (Gadek *et al*., 2000) or between close relatives of the genus (Kusumi *et al*., 2000). The studies indicated the existence of high genetic diversity within and among populations. For example, the study carried out by Adams (2000) using RAPD markers and leaf essential oils indicated that the species are separated clearly from each other. Moreover, Mao *et al*. (2010) indicated the wide range of distribution of *Juniperus* L. as a result of both long dispersal and migration across land bridges. They stated the origination of the genus as Eurasia from Eocene to Oligocene. In Turkey, the studies related with *Juniperus* L. were generally performed with isozyme polymorphism (Boratynski *et al*., 2009), microsatellites (Douaihy *et al*., 2011; 2012), heritability (Yücedağ *et al*., 2010), RAPD analysis (Adams, 2000) and DNA sequencing of ITS

region of nrDNA (Adams *et al*., 2006). However, there is no extensive phylogenetic studies for Turkish Junipers. Thus, evolutionary relationship of *Juniperus* in Turkey at species, genus and higher taxonomic levels is needed to be further explored to understand the evolutionary basis of this divergence. Extensive sampling of *Juniperus* L. and studying evolutionarily conserved regions of chloroplast genome, especially *trn* and *mat*K regions could be very useful to address the question of evolutionary divergence and divergence times within genus. Moreover, this study will relatively shed light on the general overview of *Juniperus* L. phylogeny and the place of Turkish *Juniperus* L. in the phylogeny. Since the combined molecular data-set to understand phylogenetic relationships among *Juniperus* L. species are rare, in the current study, four different chloroplast regions were utilized to construct phylogenetic relation of *Juniperus* L. genus.

CHAPTER 3

OBJECTIVES OF THE STUDY

The main objective of this study was to state the diversity and the evolutionary relationships among and within two sections and three subsections *Juniperus* L. genus that are naturally distributed in Turkey with the use of sequence data from 3 non-coding *trn* and *matK* regions of cpDNA.

The specific objectives of the study were:

1) To estimate molecular diversity and evolutionary divergence of Turkish *Juniperus* with other *Juniperus* L. on database.

2) To construct a molecular phylogenetic tree using DNA sequence data from *trn*L, *trn*L-F, *trn*V and *mat*K regions of cpDNA for Turkish *Juniperus* species along the other *Juniperus* L. of Old and New World using the available sequence data for *trn* and *mat*K from the databases.

CHAPTER 4

MATERIALS AND METHODS

4.1. Plant Material

In this study, all plant materials whose DNA sequences were utilized for analysis were collected from natural populations of *Juniperus* L. in Turkey. Additionally, the sequence data of *trn* and *mat*K from the NCBI GenBank database were obtained given the availabilities. For each species, at least 5 samples from different locations were utilized to have sufficient figuration of the genus. After sampling, the leaves were kept in small bags containing dry silica gel pellets at -20^{o}C.

4.1.1. *Juniperus* L. Species in Turkey

Juniperus species were sampled from natural stands in the period of 2011-2012. DNA has been obtained from needles for all samples. Tissue samples (needles) of *Juniperus oxycedrus* L. were obtained from 17 trees coming from 6 populations and that of *Juniperus drupacae* Labill. were obtained from 7 trees from a single population. Tissue samples of *Juniperus excelsa* M. Bieb. were sampled from 11 trees from 4 populations. The needle samples of *Juniperus foetidissima* Willd. were sampled in 2 populations. Tissue samples of *Juniperus communis* subsp. *nana* were obtained from 11 trees from a single location. Tissue samples of *Juniperus sabina* L. and *Juniperus phoenicea* L. were sampled from 12 and 5 trees, respectively coming from a single location. Detailed information on studied population is given in Table 4.1 and Figure 4.1.

Table 4.1. Geographic and topographic information of studied *Juniperus* L. species

Sections/Subsections	Taxa	Codes	Locations of Populations	Latitude (N)	Longitude (E)	Altitude (m)	Number of Trees
Sect. Juniperus Subsect. Juniperus	*J. communis subsp.nana*	CKI	Kastamonu-Ilgaz	41°22'26"	33°46'16"	1200	11
Sect. Juniperus Subsect. Oxycedrus	*J .oxycedrus* L.	OMA	K.maraş-Andırın	37°36'21"	36°19'51"	1350	3
		OMT	K.maraş-Tekir	37°52'58"	36°37'17"	1300	1
		OKK	Kastamonu-Kayalı	41°27'05"	33°53'01"	700	5
		OTC	Taşburun-Çatacık	39°55'54"	31°08'15"	1200	2
		OMS	Manisa-Sipil Dağı	38°33'01"	27°25'06"	1300	3
		OIC	Izmir-Çeşme	36°59'00''	27°23'00''	3-5	1
Sect. Juniperus Subsect. Caryocedrus	*J. drupacae* Labill.	DMA	K.maraş-Andırın	37°36'21"	36°19'51"	1350	7
Sect. Sabina	*J. excelsa* M. Bieb.	EMT	K.maraş-Tekir	37°52'58"	36°37'17"	1300	4
		EKP	Kayseri-Pınarbaşı	38°43'19"	36°23'27"	1500	1
		ETC	Taşburun-Çatacık	39°55'54"	31°08'15"	1200	3
		EMS	Manisa-Sipil Dağı	38°33'01"	27°25'06"	1300	2
	J.foetidissima Willd.	FMT	K.maraş-Tekir	37°52'58"	36°37'17"	1300	4
		FTC	Taşburun-Çatacık	39°55'54"	31°08'15"	1200	2
	J. sabina L.	SMS	Manisa-Sipil Dağı	38°33'01"	27°25'06"	1300	12
	J. phoenicea L.	PMB	Mugla-Bodrum	37°01'25''	27°21'05''	50	5

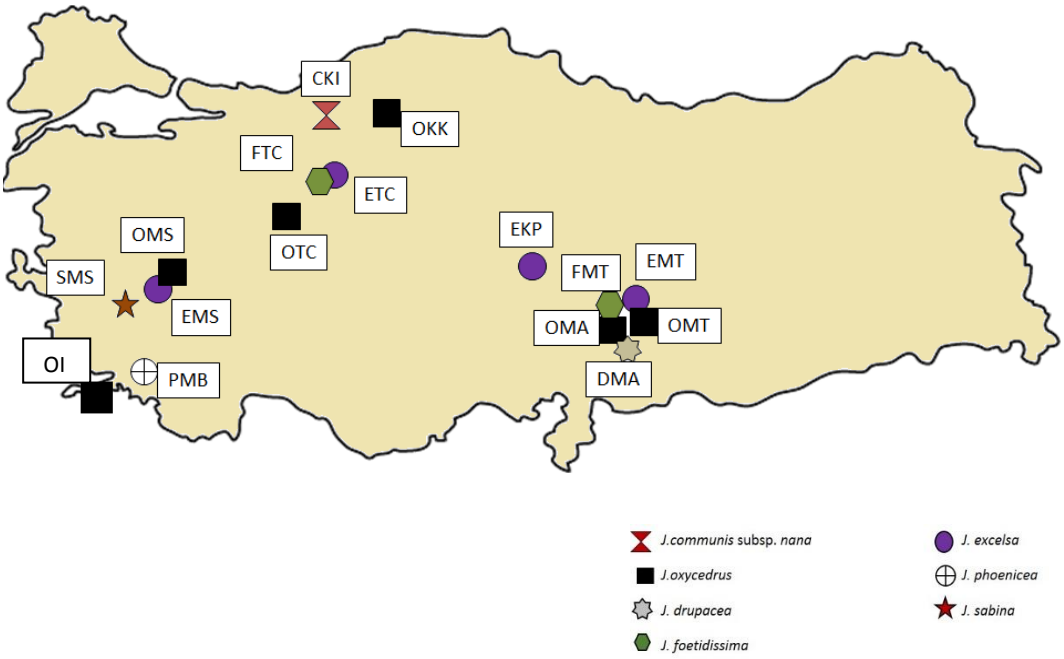

Figure 4.1 Locations of sampled *Juniperus* species. The explanations for the codes given in Table 2

4.2. DNA Extraction and Quantification Procedures

DNA extractions were performed as described in Doyle & Doyle (1990) for needle tissues of *Juniperus* L. by some modifications. The modified procedure was as follows:

1. For each sample, about 100 mg needle tissue was put in autoclaved mortar and grinded by liquid -80^{o} C nitrogen.
2. After obtaining powder – like structure of needles, 500 µl extraction buffer (2XCTAB) was added and grounding process was repeated.
3. Liquid mixture was poured into 1.5 mL eppondorf tubes and about 50 µl β – mercaptoethanol was added to the tubes. Then the tubes were vortexed and incubated at 65°C for at least one hour.
4. After incubation, mixture was centrifuged at 13000 rpm for 15 minutes.
5. The supernatant of the mixture was transfered to new tubes and 500 µl of chloroform: isoamyl alcohol (24:1) were added and mixed by gently shaking tubes.
6. The mixture was again centrifuged at 13000 rpm for 15 minutes.

7. The supernatant of the mixture was transferred into new eppendorf tubes and 500 µl of cold isopropanol was added.
8. Tubes were incubated at -20^{o}C overnight. Then, they were again centrifuged at 13000 rpm for 10 minutes. Total DNA settled down at the bottom of the tubes.
9. The supernatant was discarded very carefully and DNA pellet has been obained. 70% EtOH were used twice to clean and remove remnant from DNA. Tubes were allowed to dry for 15-20 minutes until pellet looked dry.
10. The DNA pellets were kept in 100 µl TE (Tris EDTA). Dissolved DNA was diluted to 10 ng/µl for PCR reactions. The DNAs were stored at -20^{o}C. The compositions of buffers and solutions used during DNA isolation protocol were given in Table 4.2.

The quantification of total DNA amount was carried out by using Thermo Fisher Scientific Inc. NanoDrop 2000 Spectrophotometer Version 1.4.1. By running in 0.8% agarose gel electrophoresis, the presence and quality of the DNA were also checked. DNA yields per megagametophyte varied from 500 to 5000 ng. All sample DNAs were diluted to 3 ng/µl for Polymerase Chain Reaction (PCR) application.

Table 4.2. Buffers and solutions used during DNA isolation from fresh leaf tissue

Buffers/ Solutions	Concentrations and Contents
2 X CTAB	2 gr CTAB (Cetyl trimethylammonium bromide)
	10 ml (pH : 8.0) Tris HCl (Tris(hydroxymethyl)aminomethane hydrochloride)
	4 ml (pH:8.0) 0.5M EDTA (Ethylenediaminetetraaceticacid disodium salt)
	28 ml 5M NaCl is completed upto 100 ml with dH_2O
β - Mercaptoethanol	35 ml β-Mercaptoethanol is completed upto 500 ml with dH_2O
Chloroform – Isoamyl alcohol	24:1
Ethanol	70 % in dH_2O
TE Buffer	10 M Tris HCl
	10 M EDTA

4.2.1. Primer Design and PCR Conditions

The tRNA regions used in this study are composed of the intron of *trn*L (Leu) gene, a flanking intergenic spacer, i.e. *trn*L–*trn*F and intron of *trn*V (Val). Three sets of primers were used to amplify the studied tRNA region in PCR. The primer sequences for the non coding *trn*L region of tRNA were 5′ CGA AAT CGG TAG ACG CTA CG 3′ (Forward) and 5′ GGG GAT AGA GGA CTT GA AC 3′ (reverse) while the primer sequences for *trn*L – *trn*F intergenic region of tRNA were GGT 5′ TCA AGT CCC TCT ATC CC 3′ (forward) and 5 ATT TGA ACT GGT GAC ACG AG 3′ (reverse) (Taberlet *et al*.,1991). For the *trn*V region, the primers of 5′ GTA GAG CAC CTC GTT TAC AC 3′ (forward) and 5′ CTC GAA CCG TAG ACC TTC TC 3′ (reverse) were adapted from (Wang *et al*., 1999) (Figure 4.2).

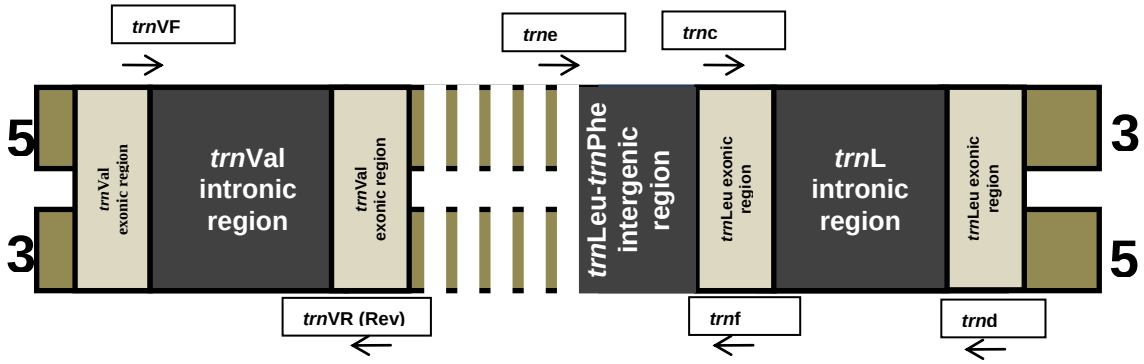

Figure 4.2. Studied *trn* regions of cpDNA (Taberlet *et al*., 1991 ; Wang *et al*., 1999). The vertical boxes shows the locations of exon while the studied intronic regions were indicated in square boxes

Similarly, the amplification of the *mat*K region have been performed using specific primers. For the amplification of this region, primer design were done by using Primer 3 version 0.4.0 (Rozen and Skaletsky, 2000), CLC Main Workbench 6.0 software package (CLC Bio, Inc.) and NCBI Primer designing tool. Since matK is a region with more than 1500 bp, the appropriate primer pairs were designed by dividing the region into two. For the amplification of the *mat*K region of Juniper species were J1F 5′ TTC CAA CTA GAT CGC ACC AT 3′ (Forward) and J1R 5′ ATT CCA AAG GAA CAG GGA GA 3′ (Reverse) primer pairs for the first half and J2F 5′ CTA CTC AAT TCA TCC GGA AA 3′ (Forward) and J2R 5′ CCT AAT TGT TCT CGA ACT ACA C 3′ (Reverse) primer pairs for the second half were used (Figure 4.3).

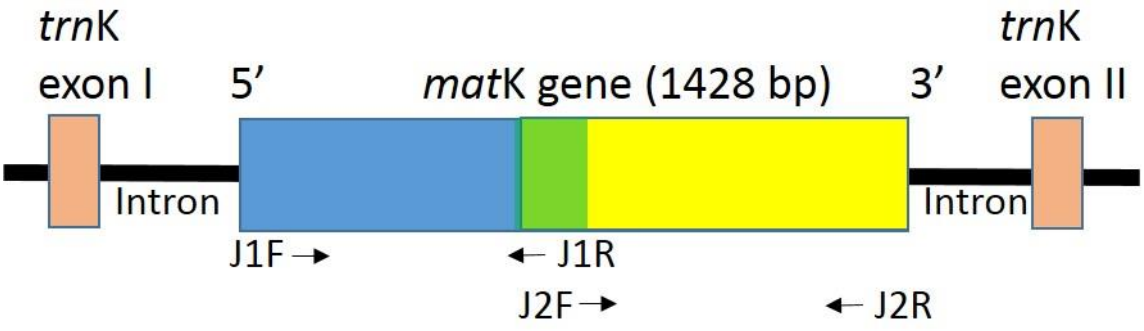

Figure 4.3. *trn*K gene and the studied *mat*K region of cpDNA. Blue part shows the first half of the amplified region with J1F (Forward) and J1R (Reverse) primers and the yellow part is the second half amplified with the J2F (Forward) and J2R (Reverse) primers. The green part is the overlapped part where both primer pairs amplified.

PCR reactions were performed in 50 µL total volume. For the optimization of PCR conditions, different concentrations of template DNA, primer, $MgCl_2$, dNTP were tested. The details of optimization experiments for *trn* and *mat*K regions of *Juniperus* were given in Table 4.3.

Table 4.3. Tested PCR conditions of *trn* and *mat*K regions for *Juniperus* species

10X Buffer	$MgCl_2$ (25 mM stock solution)	dNTP (10 mM each)	Primer pairs (100µM)	*Taq* DNA polymerase	DNA	Optimized cpDNA *trn* and *mat*K Regions
5.0	5.0	1.0	0.5 + 0.5	0.5	2.0	***Juniperus trn*cd**
5.0	6.0	2.0	1.0 + 1.0	0.5	2.0	***Juniperus trn*ef**
5.0	5.0	2.0	1.0 + 1.0	0.5	2.0	***Juniperus mat*K1 and *mat*K2**
5.0	6.0	1.0	0.5 + 0.5	0.5	2.0	***Juniperus trn*V**

Optimized PCR conditions for both studied *trn* and *mat*K regions had 2.0 µL of template DNA (7.5 ng/µL). For the PCR mixture for *trn*L of *Juniperus* species, there were 1X of 10X buffer (750 mM Tris.HCl pH: 8.8, 200 mM $(NH_4)_2SO_4$; MBI Fermentas, Lithuania); 0.5 µL (1 unit) of *Taq* DNA polymerase (Fermentas, Ontorio, Canada); 0.2 mM of dNTP mix (Fermentas, Ontorio, Canada); 2.5 mM $MgCl_2$ and 50 pmol of each primer. For *trn*L-F primer, 1X of 10X buffer; 0.5 µL (1 unit) of *Taq* DNA polymerase, 0.4 mM of dNTP mix, 3.0 mM $MgCl_2$ and 100 pmol of each primer were used. For the *trn*V primers, the PCR conditions were optimized that there were 2.0 µL template DNA, 1X of 10X buffer, 0.5 µL (1 unit) of *Taq* DNA polymerase, 0.2 mM of dNTP mix, 3.0 mM $MgCl_2$ and 50 pmole of each primer. Moreover, for the *mat*K regions, the PCR mixtures contained 1X of 10X buffer, 0.5 µL (1 unit) of *Taq* DNA polymerase, 0.4 mM of dNTP mix, 2.5 mM $MgCl_2$ and 100 pmol of each primer. The optimized PCR cycles for the amplification of *trn* and *mat*K regions were given in Table 4.4.

4.2.2. Agarose Gel Electrophoresis

One percent of agarose gels has been prepared by dissolving and boiling the agarose with 1X TBE (from 1 liter of 5X stock solution: 54 g of Tris base – 27.5 g of Boric acid – 20 ml of 0.5 M EDTA pH 8.0) buffer. The solution was poured into a horizontal gel tray in which the combs had been previously inserted. Then the agarose solution was left in tray for polymerization. After polymerization, 1X TBE buffer was poured into the electrophoresis apparatus and combs were removed cautiously to obtain wells. All samples were mixed with 6X DNA loading dye (Fermentas) seperately and loaded into each well. Agarose gels were run at 100 - 120 V for 40 – 60 minutes. After completion of the electrophoresis, it was stained with ethidium bromide and the bands were visualized by direct examination of the gel under UV light. If interested bands were amplified clearly, they were used for DNA sequencing.

Table 4.4. Optimized thermal cycler program used for amplification of *trn* and *mat*K regions of chloroplast genome of *Juniperus* L. species

Amplified Region	Temperature (^{o}C)	Duration	Number of cycles	Purpose
***trn* regions of Juniper species**	95	1 minute	1	Initial denaturation
	94	30 seconds	30	Internal denaturation
	55	30 seconds		Annealing
	72	50 seconds		Extension
	72	5 minutes	1	Final extension
***mat*K region of Juniper species**	94	5 minutes	1	Initial denaturation
	94	1 minute	30	Internal denaturation
	60	1 minute		Annealing
	72	2 minutes		Extension
	72	3 minutes	1	Final extension

4.2.3. Sequencing, Data Collection and Analysis of Sequence Data

The purification and sequencing reactions for both forward and reverse primers of *trn*L, intergenic spacer *trn*L – *trn*F, *trn*V and *mat*K regions were carried out in the Refgen Biotechnology facilities (Middle East Technical University, Teknokent, Ankara). An ABI 310 Genetic Analyzer (PE applied Biosystem) automatic sequencer was used for sequencing of amplified DNA products. After data collection, the sequences from the forward and reverse primers were aligned and checked both manually and using the DNA Baser v3.5.3 software (2012) for accuracy of the base-call. Both manual check and utilization of the software gave the sequenced region were about 10-20 bp shorter than the regions themselves due to the trimming of the regions. For optimum assemblage, the word size were arranged as almost 20 bases, sample identity of 60% to detect mismatches and local alignments with minimum overlap were set up size. In order to obtain reliable sequences, base quality (QV) were arranged as minimum 35 or higher. During pairwise alignment in order to provide decision for giving gap or mismatch penalty, minimum QV value were adjusted as 25. For multiple aligment procedure, MUSCLE (Multiple Sequence Comparison by Log Expectation) tool (Edgar, 2004) were used since it has several advantages over Clustal W and T-coffee. MUSCLE tools iteratively uses pair-wise alignment to refine the tree (combining sequences, and breaking profiles into separate nodes). Furthermore, it repeats until converges or until max iterations are reached. Moreover, it is 3000 X faster than other tools (Edgar, 2004). Important phylogenetic and molecular evolutionary statistics such as total nucleotide length (bp), GC content (%), nucleotide deletion and insertion, conserved and variable sites, parsimony informative sites, transition/transversion (tr/tv) ratio and nucleotide diversity of the sequences were calculated with the MEGA 5.2.2 software (Tamura *et al*., 2012).

Gaps, which are obtained during the alignment of homologous regions of sequences, represent deletions or insertions (indels). For this study, when computing distances, complete deletion method was used. In the complete deletion option, all of the sites were deleted from the data analysis. This option is generally desirable because different regions of DNA or amino acid sequences evolve under different evolutionary forces. The data sets of DNA sequences were edited in *.mas (MEGA Alignment Sequence) extension file format and collected and organized in *.meg

(MEGA Data Format) extension file format so that it could be analyzed with MEGA (Molecular Evolutionary Genetics Analysis) 5.2.2 software (Tamura *et al*., 2012). The sequence statistics, containing nucleotide frequencies, transition/transversion (tr/tv) ratio and variability in different regions of the sequences were calculated.

4.2.3.1. The Genetic Distance between Taxa

Distance estimates attempt to estimate the mean number of changes per site since 2 species (sequences) split from each other. P – Distance, which is simply known as counting the number of differences, may underestimate the amount of change - especially if the sequences are very dissimilar - because of multiple hits. To try to get better estimations, a model which includes the factors that give information about the evolution of the sequences, can be used. Genetic distances among taxa were detected by using Maximum Likelihood statistical method. In each taxon of outgroup as well as the other *Juniperus* L. sequences from GeneBank and Turkish Junipers were included. All positions with less than 95% site coverage were eliminated. That is, fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position.

4.2.3.2. Haplotype Frequency Analysis, Analyses of Molecular Variance (AMOVA, Estimation of F_{st} Values and Molecular Clock Estimation

In order to get more powerful discrimination between samples, haplotype (i.e. combination of alleles at one or more loci) analysis has been performed.The frequency estimation of all possible haplotypes by maximum likelihood methods has been conducted by using DnaSP v5 (Librado and Rozas, 2009). Similarly to evaluate the amount of population genetic structure Analyses of Molecular Variance (AMOVA) have been performed with Arlequin 3.5.1.3 (Excoffier *et al*.,1992, 2005). The genetic structure indices using information on allelic content of haplotypes, as well as their frequencies have been estimated (Excoffier *et al*., 1992, 2005). The information on differences in allelic content between haplotypes is entered as a matrix of Euclidean squared distances. The significance of the covariance components associated with different possible levels of genetic structure (within sections and among sections in this study) has been tested using non-parametrix

permutation procedures (Excoffier *et al*., 1992, 2005). The type of method and permutation are Distance matrix computation with Tamura & Nei model (Tamura and Nei, 1993). Finally, the pairwise Fst's have been estimated to obtain genetic distances btween sections with the application of a slight transformation to linearize the distance with section's divergence time (Reynolds *et al*., 1983; Slatkin, 1995).

The molecular evolutionary clock was first used and described by Zuckerkandl and Pauling (1965). This clock provides estimation for the time of divergence of species by using nucleotide differences in DNA sequences. Assuming the evolution of two or more lineages at constant rate, the number of variations among two samples would be straightforward since they diverged from their common ancestor (Futuyma, 2005). Therefore, we can estimate the time of divergence by the rate of nucleotide variations between DNA sequences of taxa. To calculate the rate of molecular evolution, the number of parsimony informative sites in the sequenced DNA region is used. The following equation was used to estimate molecular clock for *Juniperus* genus.

$$\text{Molecular Clock} = \frac{k}{\text{mutation rate}}$$

Where k is equal to:

$$k = -\left(\frac{3}{4}\right)\ln\left(1 - \frac{4}{3}d\right)$$

The d in the above equation was calculated as:

$$d = \frac{Variable\ Site}{Total\ Number\ of\ Base\ Pairs\ Sequenced}$$

In the equation, *d:* the number of substitutions per base pair; *k:* the substitutions since divergence time. In this study, for *trn* and *mat*K regions, this value was estimated separately. As the mutation rate $2x10^{-9}$ of plant cpDNA was used as a constant value (Pevsner, 2009).

4.2.3.3. Molecular Diversity and Phylogenetic Analysis of Juniperus L. Based on Sequence Data of trn and matK Regions

The differentiation among species from each other was analyzed by obtaining relevant sequences from NCBI by using BLAST (Basic Local Alignment Search Tool) for 3 non-coding regions of *trn* and *mat*K. Since the number of studies dealing with *trn*V region were considerably lower than studies in other two *trn* regions, some samples from GeneBank for *trn*V were not available for the analysis (Appendix 1).

4.2.3.4. Construction of Phylogenetic Trees

Phylogenetic trees are important to show the evolutionary relations between various species or other groups of organisms having a common ancestor. These phylogenetic relationships of genes or organisms are shown in a tree with either a rooted or an unrooted tree. During construction of a phylogenetic tree, the bootstrap test (Camin & Sokal, 1965), might be applied. Thereby, the reliability of a given branch pattern is verified by examining the frequency of the occurence of the branch in a large number of trees. During ascertaining the reliability, permutations with replacement is held. If the bootstrap value for a given interior branch is 95% or higher, then the topology at that branch is considered "correct". If the value is between 50 and 95 %, the topology is considered informative (Nei and Kumar, 2000). Two phylogenetic trees were constructed using MEGA 5.2.2 with Neighbour Joining method for both *trn* and *mat*K regions.

CHAPTER 5

RESULTS

5.1. Amplification of the t-RNA and *mat*K Regions of the Chloroplast DNA

For all three regions of non coding *trn* (*trn*L, *trn*L-F and *trn*V) and *mat*K regions, highly qualified and clear single bands were observed. All experiments and data collection for Turkish Juniper species were performed by the current study while for other members of the genus, the interested sequences were obtained from GenBank.

Since some of the taxa obtained from GenBank did not have sequences for all three regions, they were excluded in analysis. Indeed the general idea behind obtaining the sequences from database was based upon their geographic location and closeness of the species to the studied taxa.

5.2. Molecular diversity of studied cpDNA regions

In the present study, 66 individuals from 7 native Turkish Juniper species based on 3 *trn* and 1 *mat*K loci were recorded. In order to elucidate some of the conflincting results, for phylogenetic tree construction, combination of 3 *trn* regions together were also considered additional to analyses of 3 *trn* regions seperately. The reason is related with the fact that the length of a genetic region is somehow important for the phylogenetic relations between species. For instance, among the analysis performed, the most plausible result has been obtained in *mat*K region because it has more than 1400 bp while other regions were 350-600bp. To be more precise, the number of observed dissimilarity is calculated as proportion against the sequence length. Thus, for example if there is 5 % difference, it is only slightly changed in to 5.17 % in 1400 bp *mat*K region. However, in shorter sequences this proportion would be for example, 50% and the change in distance would be about %87 (Jukes and Cantor,

1969). Within the light of this information, after combining 3 *trn* regions the length was 1274 bp while total numbers of parsimony informative sites has been 25.

5.2.1. Molecular Diversity Statistics of Turkish Junipers Species in *trn* and *mat*K regions

Conserved, variable and parsimony informative sites, total nucleotide length (bp), GC content (%), number of deleted/inserted nucleotides, number of sequences, nucleotide diversity and transition/transversion (tr/tv) ratio were calculated by using the MEGA program for each section of Turkish Junipers. All of these mentioned molecular diversity parameters were calculated for two sections and three subsections of the genus as well as for all species by combining data from two sections. For all three *trn* regions included, the gene diversity in within Turkish Junipers have found as 0.8834 ± 0.0133. The result has been similar for *mat*K region which was 0.8824 ± 0.0151.

5.2.1.1. trnL Region of cpDNA

The length of the *trn*L intron region ranged from 315 to 329 bp, after the alignment of all samples of *Juniperus*. However, especially *J. oxycedrus* L. species possessed the length of 315 due to deletion in DNA sequence between 176th and 189th bp except *J. oxycedrus* subsp. *macrocarpa* from İzmir Çeşme. Moreover, there is also deletion covering all members of section Sabina between 209th and 214th bp which make its length shorter (323 bp) (Table 5.1). GC content (%) of each section and the total sample were almost the same (about 40 %) (Table 5.1). For *trn*L intronic region, there were 6 variable sites. All were parsimony informative.

Considering transition and transversion sites, if a purine is substituted by another purine (Adenine vs Guanine) or a pyrimidine by another pyrimidine (Thymine vs Cytosine), it is called transition (si). However, if a purine is substituted by a pyrimidine or *vice versa* (Adenine vs. Thymine or Guanine vs. Cytosine), this situation is called tranversion (sv). The transition and transversion of total *Juniperus* taxon were 68.13 and 31.87 %, respectively which indicate the si/sv rate equal to 2.03. This rate (R) is calculated using the equation

$R=[A \times G \times k_1 + T \times C \times k_2] / [(A + G) \times (T + C)]$ where k_1 is si/sv rate ratios for purines and k_2 for pyrimidines.

High insertion/deletion numbers were observed in subsection Oxycedrus where total 14 deletion sites were found. Conversely, in subsection Caryocedrus and Juniperus, the insertions were observed that made the length of this region for the taxon be longer than remaining taxa.

Table 5.1. The estimated molecular diversity parameters based on *trn*L intron of cpDNA for each section of the studied Turkish Juniper species

	Sect. Juniperus subsect. Juniperus	**Sect. Juniperus subsect. Oxycedrus**	**Sect. Juniperus subsect. Caryocedrus**	**Sect. Juniperus**	**Sect. Sabina**	**Total**
Number of Species	1	1	1	3	4	7
Number of Sequences	11	15	7	33	33	66
Total Length (bp)	329	315	329	329	323	329
GC Content (%)	39.5	39.4	39.5	39.4	40.1	39.8
Conserved Sites	329	315	329	325	321	324
Variable Sites within taxa	0	2	0	3	3	6
Parsimony Informative Sites within taxa	0	0	0	3	3	6
Transitional Pairs	33.33	53.54	33.33	36.46	99.64	68.13
Transversional Pairs	66.67	46.44	66.67	63.54	0.36	31.87
Transition/Transversion (si/sv)(R) ration	0.491	1.008	0.491	0.500	298.11	2.03
Number of Insertion	6	6	6	6	0	6
Number of Deletion	0	14	0	14	6	15

*5.2.1.2. trn*L-F *Region of cpDNA*

The molecular diversity parameters have been obtained for *trn*L-F region and provided in Table 5.2. The length of the *trn*L-F region was very variable both between and within subsections. In fact, the indel pattern of this region was different from other studied *trn* regions such that the region did not showed correlation in terms of insertions and deletions. Instead, species within section and populations within species showed different indel patterns. For example, there were high amount of indel in subsection Oxycedrus. The differences were observed at geographic level such that *J. oxycedrus* L. from Kastamonu Province were 21 bp shorter than the species from other locations where *J. oxycedrus* L. were sampled. Moreover, *J. oxycedrus* subsp. *macrocarpa* from İzmir Çeşme possessed an insertion between 167th and 192nd region which was not found in other *J. oxycedrus* L. species. In Section Sabina, high rate of indel was observed in *J. phoenicea* L.. In Subsection Juniperus, 8 insertions were obtained. However, the nucleotide contents were not as diverse as indel numbers. The only difference was due to the presence of C base instead of G at 25th position, A base instead of C at 148th position, G base instead of T at 233rd positon, C base instead of T at 291st position and A base instead of G in 357th positon. These substitutions were very useful for phylogenetic analyses not only at section and species level, but also at population level. As in *trn*L region, there was no parsimony informative sites in subsections Juniperus and Caryocedrus.

There were total of 6 variable sites and all of them were parsimony informative. The highest variability has been observed in the Section Juniperus due to the presence of different subsections. The GC content was similar among sections and was about 30%. Transition and transversion rates were 48.55 % and 51.45 %, respectively and hence 0.814 overall transition/transversion bias (R) have been obtained.

Table 5.2. The estimated molecular diversity parameters based on *trn*L-F intergenic region of cpDNA for each section of the studied Turkish Juniper species

	Sect. Juniperus subsect. Juniperus	Sect. Juniperus subsect. Oxycedrus	Sect. Juniperus subsect. Caryocedrus	Sect. Juniperus	Sect. Sabina	Total
Number of Species	1	1	1	3	4	7
Number of Sequences	11	15	7	33	33	66
Total Length (bp)	285	280-301	280	301	275-282	301
GC Content (%)	31.9	30.4	31.0	33.1	31.3	32.0
Conserved Sites	285	310	280	308	284	308
Variable Sites within taxa	0	1	0	4	3	6
Parsimony Informative Sites within taxa	0	1	0	4	3	6
Transitional Pairs	33.34	99.65	33.33	75.56	52.24	48.55
Transversional Pairs	66.66	0.35	66.67	24.44	47.76	51.45
Transition/Transversion (si/sv)(R) ration	0.43	241.82	0.424	2.67	0.945	0.814
Number of Insertion	8	31	0	31	7	27
Number of Deletion	29	10	24	29	24	33

5.2.1.3. trnV Intronic Region of cpDNA

The length of the region was ranged from 522 to 524 bp. GC content was almost the same among sections (36%). The most variable Section was Sabina while others were highly conservative. The indels were considerably lower than that in other studied *trn* regions. Main substitution has been observed in Section Sabina whereas in other subsections there was no substitution. Hence the transition/transversion rate were around 0.5 (Table 5.3). At subsection level, there were no variable and parsimony informative sites. The variation has been at section level. There were 9 and 6 variable sites in sections Juniperus ans Sabina, respectively. All of them were parsimony informative.

The variability among the sections was relatively high. There were total of 13 variable sites all of which were parsimony informative. The highest variability has been observed in the Section Juniperus. The GC content was varied from 35.2 % to 36.0 %. Transition and transversion rates as well as R value were quite extensive such that 64.86 % transition, 35.14 % transversion and hence 1.71 overall transition/transversion bias (R) have been obtained.

Table 5.3. The estimated molecular diversity parameters based on *trn*V intron of cpDNA for each section of the studied Turkish Juniper species

	Sect. Juniperus subsect. Juniperus	**Sect. Juniperus subsect. Oxycedrus**	**Sect. Juniperus subsect. Caryocedrus**	**Sect. Juniperus**	**Sect. Sabina**	**Total**
Number of Species	1	1	1	3	4	7
Number of Sequences	11	15	7	33	33	66
Total Length (bp)	523	523	523	523	524	524
GC Content (%)	35.2	35.4	36.0	35.4	36.0	35.8
Conserved Sites	523	523	523	515	518	512
Variable Sites within taxa	0	0	0	9	6	13
Parsimony Informative Sites within taxa	0	0	0	9	6	13
Transitional Pairs	33.23	33.34	33.34	68.53	33.60	64.86
Transversional Pairs	66.77	66.66	66.66	31.47	66.40	35.14
Transition/Transversion (si/sv)(R) ration	0.46	0.46	0.46	5.69	0.44	1.71
Number of Insertion	0	0	1	1	2	3
Number of Deletion	2	2	2	2	3	3

5.2.1.4. Maturase Kinase (matK) Region of cpDNA

Total of 68 sequences were available for *mat*K region. The sequence of the region, which is about 1430 bp, starts with ATG and ends with AGA. The studied sequences of Turkish *Juniperus* possessed 5 bp more sequence at 5′, but 15 bp less sequence at 3′ due to unreliability of this part of the region. Hence, it has been trimmed during alignment. The total number of sequnces was ranged from 1416- 1428 due to high rate of indel along the sequence. The indel pattern was similar to that of *trn*L-F region, but not as explicit as that region. The insertion and deletions have not been observed for all sections, but rather at species level. For example, in section Sabina, *J. sabina* L. species possessed 9 bp deletion just after the beginning of the sequence, between 226 - 231 bp and between 1386 - 1391 bp although other members of the section did not have this kind of pattern. Similarly at 211st bp, *J. phoenicea* L. showed 6 bp insertion. Interestingly, the same pattern of insertion did not present in the other species of the section. *J. phoenicea* L. also had the insertion between 782 – 787th positions which also did not present in other species.

The variability among the sections was also quite high. There were total of 35 variable sites all of which were parsimony informative. The highest variability has been observed in the Section Sabina. The GC content was similar among sections. 32.4 % GC content was observed when all Juniper species are considered. Transition and transversion rates as well as R value were quite extensive such that 62.87 % transition, 37.13 % transversion and hence 1.446 overall transition/transversion bias (R) have been obtained.

At section/subsection level, there was no variable site within section Juniperus. The length was 1416 bp. The GC content was 32.3 %. There were 2 variable sites in Subsection Oxycedrus, all of which were parsimony informative. There were 12 deletion in both subsection Juniperus and Oxycedrus, but no insertion was found. The transition rate of subsection Oxycedrus was much higher than transversion rate. The change was between Cytosine ↔ Thymine at 622nd and 625th base positions. There were no variable sites within subsection Caryocedrus, but there were 6 bp insertion between 211st – 216th base positions. Finally, considering section Sabina, the results were much more challenging than any other sections discussed above because the variations was not only due to at section level but also at species level.

Among species of the section, *J.sabina* L. and *J.phoenicea* L. were the most variable ones (Table 5.4).

Table 5.4. The estimated molecular diversity parameters based on *mat*K region of cpDNA for each section of the studied Turkish Juniper species

	Sect. Juniperus subsect. Juniperus	**Sect. Juniperus subsect. Oxycedrus**	**Sect. Juniperus subsect. Caryocedrus**	**Sect. Juniperus**	**Sect. Sabina**	**Total**
Number of Species	1	1	1	3	4	7
Number of Sequences	11	16	7	34	34	68
Total Length (bp)	1416	1416	1422	1422	1419	1428
GC Content (%)	32.3	32.3	32.1	34.3	32.6	32.4
Conserved Sites	1416	1414	1422	1417	1389	1393
Variable Sites within taxa	0	2	0	5	30	35
Parsimony Informative Sites within taxa	0	2	0	5	30	35
Transitional Pairs	33.33	99.63	33.33	62.69	84.69	62.87
Transversional Pairs	66.67	0.37	66.67	37.31	15.31	37.13
Transition/Transversion (si/sv)(R) ration	0.44	243.82	0.44	1.42	4.78	1.45
Number of Insertion	0	0	6	6	12	12
Number of Deletion	12	12	6	12	21	12

5.2.2. Genetic Divergence within and among sections/subsections of Turkish *Juniperus*

In the analysis of estimates of average evolutionary divergence within sections/subsections, 66 nucleotide sequences for *trn* and 68 sequences for *mat*K region were utilized. The total length was 309 for *trn*L region. Moreover, the length of studied regions was 267 for *trn*L-F, 522 for *trn*V and 1395 bp for *mat*K regions. Genetic divergence data within sections was provided in Table 5.5. In all regions, Sections Sabina and Oxycedrus showed divergence and Sections Sabina and Oxycedrus were divergent with respect to *trn* and *mat*K regions. To make the

divergence within taxa more conspicuous, the divergence among species of section Sabina has also been considered. In *trn*L region the divergence was highest within Section Sabina than other sections. The divergence in subsection Oxycedrus was due to *J.oxycedrus* subsp. *macrocarpa* and *J. oxycedrus* from Kastamonu Kayalı Köyü. For section Sabina the divergence within section was due to the presence of 4 different species. Especially, sample of *J. foetidissima* from Eskişehir Çatacık and *J. phoenicea* caused this difference.

Table 5.5. Estimated Average Nucleotide Diversity over Sequence Pairs within Sections/Subsections of Turkish *Juniperus*

Section / Subsection	Distance ± Standard Error	Regions
Subsection Juniperus	0.0000	*trn*L
Subsection Oxycedrus	0.0009 ± 0.0006	
Subsection Caryocedrus	0.0000	
Section Sabina	0.0017 ± 0.0011	
Subsection Juniperus	0.0000	*trn*L-F
Subsection Oxycedrus	0.0019 ± 0.0018	
Subsection Caryocedrus	0.0000	
Section Sabina	0.0043 ± 0.0024	
Subsection Juniperus	0.0000	*trn*V
Subsection Oxycedrus	0.0000	
Subsection Caryocedrus	0.0000	
Section Sabina	0.0036 ± 0.0016	
Subsection Juniperus	0.0000	*mat*K
Subsection Oxycedrus	0.0005 ± 0.0004	
Subsection Caryocedrus	0.0000	
Section Sabina	0.0106 ± 0.0023	

By using number of substitutions per site from averaging over all sequence pairs among sections, genetic divergences among sections were also estimated. In addition to section analysis, to calculate overall divergence, whole Turkish Juniper data has also been used that is, sections were not taken into consideration. The results have been provided in Table 5.6. When DNA sequences of *trn*L region was considered, genetic divergence between Sabina and Oxycedrus sections was greater (0.0075 ± 0.0046) than those values between other section combinations. There has been no genetic divergence between Caryocedrus and Juniperus sections (Table 5.6).

Table 5.6. Genetic divergence of Turkish Junipers among sections based on studied cpDNA regions. Standard error estimate(s) were obtained by a bootstrap procedure (500 replicates). The least distances are shown with green and the most diverged taxa combinations are shown with blue

Section / Subsection	Juniperus	Oxycedrus	Caryocedrus	Overall	Regions
Juniperus				0.0032 ± 0.0017	*trn*L
Oxycedrus	0.0065±0.0046				
Caryocedrus	0.0000	0.0065±0.0046			
Sabina	0.0010±0.0007	0.0075±0.0046	0.0010±0.0007		
Juniperus				0.0059 ± 0.0026	*trn*L-F
Oxycedrus	0.0062±0.0044				
Caryocedrus	0.0075±0.0052	0.0062±0.0046			
Sabina	0.0081±0.0046	0.0060±0.0035	0.0081±0.0047		
Juniperus				0.0087 ± 0.0026	*trn*V
Oxycedrus	0.0135±0.0053				
Caryocedrus	0.0096±0.0043	0.0136±0.0051			
Sabina	0.0117±0.0044	0.0157±0.0053	0.0024±0.0011		
Juniperus				0.0087 ± 0.0019	*mat*K
Oxycedrus	0.0007±0.0006				
Caryocedrus	0.0022±0.0013	0.0018±0.0011			
Sabina	0.0119±0.0029	0.0113±0.0027	0.0109±0.0027		

*trn*L-F region was not as informative as *trn*L region because there is no considerable close relationship between sections with respect to this region. However, genetic divergence between Sabina and other three sections was higher than any other combinations which is compatible with its morphological classification (Table 5.6). Nonetheless, the closest sections were found to be Sabina and Oxycedrus (0.0060 ± 0.0035) regarding *trn*L-F region. The sequence analysis of *trnV* intron region did not reveal similar results with those of *trnL* intron and *trnL-F* regions. The Caryocedrus subsection showed no difference with Subsection Juniperus in *trn*L region. Moreover, another close relationship has been found between Sabina and Caryocedrus (0.0024 ± 0.0011). The most divergent groups were the Sabina and Oxycedrus sections (0.0157 ± 0.0053) with respect to *trn*V region. Finally, the data from *mat*K region indicated that the closest sections were Juniperus and Oxycedrus whereas the most distant ones were Sections Sabina and Juniperus. When all studied regions were considered, Section Sabina showed the most distant relationship with other sections as expected.

Furthermore, the genetic distances among species in each section were also analysed in order to reveal the relationship between each taxa combinations. Species divergence analyses were given in Table 5.7 for each *trn* regions. Accordingly, in *trn*L region, generally the most distant taxa combination was *J.phoenicea* L. and *J.oxycedrus* L. (0.0131 ± 0.0061). Moreover, *J. oxycedrus* L. was the second most distant taxon to the other species. The remaining combinations showed no divergence at all. With respect to *trn*L-F region, *J.foetidissima* Willd. and *J.sabina* L. had no divergence; however, the remaining taxa were all far from each other. This result has also been observed in analyses at section level. Particularly, *J.phoenicea* L. had the most distant relation with other species as it was also observed in *trn*L region. Analyses with *trn*V region have revealed the close relationship between *J.drupacea* Labill. and *J.foetidissima* Willd. (0.0013 ± 0.0008) whereas *J.oxycedrus* L. and *J.excelsa* M. Bieb. have been the most distant taxa (0.0175 ± 0.0059). Finally, according to *mat*K region, *J.oxycedrus* L. was the closest to *J.communis* L. (0.0007 ± 0.0006); while, the most diverged taxa combination was *J.phoenicea* L. and *J.sabina* L. (0.0241 ± 0.0062).

Table 5.7. Genetic divergence between species within each section by using 4 different cpDNA regions. Standard error estimate(s) were obtained by a bootstrap procedure (500 replicates). The least distances are shown with green and the most diverged taxa combinations are shown with blue.

Subsections	Species	*J.communis* L.	*J.oxycedrus*	*J.drupacea* Labill.	*J.sabina* L.	*J.excelsa* M. Bieb.	*J.foetidissima* Willd.	Regions
Juniperus	*J. communis* L.							
Oxycedrus	*J. oxycedrus* L.	0.0000						
Caryocedurs	*J. drupacea Labill.*	0.0000	0.0065±0.0045					
Sabina	*J. sabina* L.	0.0000	0.0065±0.0045	0.0000				*trn*L
	J. excelsa M. Bieb.	0.0000	0.0065±0.0045	0.0000	0.0000			
	J.foetidissima Willd.	0.0000	0.0000	0.0000	0.0000	0.0000		
	J. phoenicea L.	0.0065±0.0042	0.0131±0.0061	0.0065±0.0042	0.0068±0.0042	0.0065±0.0042	0.0065±0.0042	
Juniperus	*J. communis* L.							
Oxycedrus	*J. oxycedrus* L.	0.0062±0.0043						
Caryocedurs	*J. drupacea Labill.*	0.0075±0.0050	0.0062±0.0045					
Sabina	*J. sabina* L.	0.0075±0.0051	0.0062±0.0045	0.0075±0.0051				*trn*L-F
	J. excelsa M. Bieb.	0.0075±0.0050	0.0062±0.0044	0.0075±0.0051	0.0075±0.0050			
	J.foetidissima Willd.	0.0075±0.0051	0.0062±0.0045	0.0075±0.0051	0.0000	0.0075±0.0050		
	J. phoenicea L.	0.0113±0.0064	0.0051±0.0040	0.0113±0.0066	0.0038±0.0036	0.0113±0.0065	0.0038±0.0036	
Juniperus	*J. communis* L.							
Oxycedrus	*J. oxycedrus* L.	0.0135±0.0049						
Caryocedurs	*J. drupacea Labill.*	0.0096±0.0041	0.0136±0.0051					
Sabina	*J. sabina* L.	0.0118±0.0046	0.0157±0.0054	0.0021±0.0020				*trn*V
	J. excelsa M. Bieb.	0.0135±0.0050	0.0175±0.0059	0.0038±0.0025	0.0056±0.0032			
	J.foetidissima Willd.	0.0084±0.0036	0.0129±0.0049	0.0013±0.0008	0.0034±0.0022	0.0051±0.0028		
	J. phoenicea L.	0.0116±0.0045	0.0155±0.0055	0.0019±0.0018	0.0040±0.0027	0.0058±0.0032	0.0032±0.0020	
Juniperus	*J. communis* L.							
Oxycedrus	*J. oxycedrus* L.	0.0007±0.0006						
Caryocedurs	*J. drupacea Labill.*	0.0022±0.0013	0.0018±0.0011					
Sabina	*J. sabina* L.	0.0215±0.0056	0.0207±0.0053	0.0202±0.0053				*mat*K
	J. excelsa M. Bieb.	0.0045±0.0020	0.0040±0.0019	0.0036±0.0019	0.0171±0.0046			
	J.foetidissima Willd.	0.0109±0.0036	0.0103±0.0033	0.0099±0.0031	0.0144±0.0037	0.0073±0.0027		
	J. phoenicea L.	0.0064±0.0025	0.0059±0.0023	0.0055±0.0021	0.0241±0.0062	0.0067±0.0026	0.0109±0.0035	

5.3. Molecular Phylogenetic Analyses Including Juniper Species from Database

In the preceding section, the molecular phylogenetic relations of 7 Turkish Juniper species have been discussed based on *mat*K and 3 non-coding *trn* region of cpDNA. The rest of the study included the phylogenetic relationships of Turkish *Juniperus* L. with other Juniper species obtained form GenBank.

5.3.1. Molecular Phylogenetic Relation of Turkish *Juniperus* with other Juniper species

As mentioned before, *Juniperus* L., which include more than 60 species, are composed of Section Juniperus with subsections Juniperus, Oxycedrus and Caryocedrus and Section Sabina. The sequences from 4 studied molecular regions have been obtained for almost all Juniperus species from GenBank (Appendix 1). However, considering the reliability of the sequnces and their geographic and taxonomic relations to Turkish *Juniperus* L., some of sequences were not included for the analyses. The sequences from *trn* and *mat*K regions of *Cupressus sempervirens* L. have been added to the analysis as an outgroup taxa.

For *trn*L region, the length of the sequence has been ranged from 304- 330 bp. In Sections Juniperus subsections Juniperus, Oxycedrus and Caryocedrus, there were insertions at 209th – 214th bp which was also observed in Turkish *Juniperus* L. of *J.communis* L., *J.oxycedrus* L. and *J.drupacea Labill*. When *J.macrocarpa* Sibth. & Sm. from GenBank and *J.oxycedrus* subsp. *macrocarpa* from Turkey were excluded, subsection Oxycedrus including *J.oxycedrus* L. obtained in Turkey possessed 14 bp deletion at positions of 176-189th bp. Morover, *J. oxycedrus* subsp. *macrocarpa* showed significant differences from other *J. oxycedrus* L. species from Turkey. Indeed, it showed similarity with *J. macrocarpa* Sibth. & Sm. obtained from GenBank. Table 5.8A showed the parsimony informative sites and indels of selected *Juniperus* based on *trn*L intronic region. GC content did not change much and it was 39.9 %. The variable sites were 18, but only 11 of them were parsimony informative.

Table 5.8. Substitutions and indels in the DNA sequences of *trn* and *mat*K regions for Juniper sections. Deletions have been shown with red dash. Parsimony informative sites were in green color. Numbers above the columns depicted the position of nucleotide within the sequence. The samples that were obtained in GeneBank have been highlighted with blue. A) trnL intronic region, B) *trn*L-F intergenic spacer region. (In Sections column J: Juniperus, O: Oxycedrus, C: Caryocedrus, SOW: Sabina Old World Species, SNW: Sabina New World Species), C) *trn*V intronic region, D) *mat*K region

A) *trn*L intronic region

Sections	Species	6	50	172	176	177	178	179	180	181	182	183	184	185	186	187	188	189	209	210	211	212	213	214	239	321	325
Juniperus	*Juniperus_communis var. communis*	G	A	A	T	G	G	A	T	T	G	G	A	T	A	C	A	A	A	A	A	A	T	**G**	C	**G**	C
	Juniperus_conferta	G	A	A	T	G	G	A	T	T	G	G	A	T	A	C	A	A	A	A	A	A	T	T	C	**G**	C
	Juniperus_rigida	G	A	A	T	G	G	A	T	T	G	G	A	T	A	C	A	A	A	A	A	A	T	T	C	-	-
	J.communis ssp nana(Turkey)	G	A	A	T	G	G	A	T	T	G	G	A	T	A	C	A	A	A	A	A	A	T	**G**	C	A	-
Oxycedrus	*Juniperus_deltoides*	G	A	**C**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	A	A	A	T	T	**T**	A	C
	Juniperus_macrocarpa	G	A	A	T	G	G	A	T	T	G	G	A	T	A	C	A	A	A	A	A	A	T	T	C	**G**	-
	Juniperus_navicularis	**A**	A	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	A	A	A	T	T	**T**	**G**	C
	Juniperus_oxycedrus_var._oxycedrus	**A**	A	**C**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	A	A	A	T	T	**T**	A	C
	J.oxycedrus (Turkey)	G	A	**C**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	A	A	A	T	T	**T**	A	-
	J.oxycedrus ssp.macrocarpa (Turkey)	G	A	A	T	G	G	A	T	T	G	G	A	T	A	C	A	A	A	A	A	A	T	T	C	A	-
Caryocedrus	*Juniperus_drupacea* Labill	G	A	A	T	G	G	A	T	T	G	G	A	T	A	C	A	A	A	A	A	A	T	**G**	C	.	C
	J.drupacea Labill.*(Turkey)*	G	A	A	T	G	G	A	T	T	G	G	A	T	A	C	A	A	A	A	A	A	T	**G**	C	A	-
Sabina Old World Species	*Juniperus_chinensis*	G	A	A	T	G	G	A	T	T	G	G	A	T	A	C	A	A	-	-	-	-	-	-	C	**G**	C
	Juniperus_excelsa M. Bieb.	G	A	A	T	G	G	A	T	T	G	G	A	T	A	C	A	A	-	-	-	-	-	-	C	**G**	C
	Juniperus_phoenicea L.	G	**G**	A	T	G	G	A	T	T	G	G	A	T	A	C	A	A	-	-	-	-	-	-	C	**G**	C
	Juniperus_sabina_var._arenaria	**A**	A	A	T	G	G	A	T	T	G	G	A	T	A	C	A	A	-	-	-	-	-	-	C	**G**	C
	Juniperus_semiglobosa	**A**	A	A	T	G	G	A	T	T	G	G	A	T	A	C	A	A	-	-	-	-	-	-	C	**G**	C
	J.foetidissima Willd. *(Turkey)*	G	A	A	T	G	G	A	T	T	G	G	A	T	A	C	A	A	-	-	-	-	-	-	C	A	-
	J.excelsa M. Bieb. *(Turkey)*	G	A	A	T	G	G	A	T	T	G	G	A	T	A	C	A	A	-	-	-	-	-	-	C	A	-
	J.sabina L. *(Turkey)*	G	A	A	T	G	G	A	T	T	G	G	A	T	A	C	A	A	-	-	-	-	-	-	C	A	-
	J.phoenicea L. *(Turkey)*	G	**G**	A	T	G	G	A	T	T	G	G	A	T	A	C	A	A	-	-	-	-	-	-	C	**G**	-
Sabina New World Species	*Juniperus_monosperma*	G	A	A	T	G	G	A	T	T	G	G	A	T	A	C	A	A	-	-	-	-	-	-	C	**G**	C
	Juniperus_monticola	G	A	A	T	G	G	A	T	T	G	G	A	T	A	C	A	A	-	-	-	-	-	-	C	**G**	C
	Juniperus_occidentalis	G	A	A	T	G	G	A	T	T	G	G	A	T	A	C	A	A	-	-	-	-	-	-	C	**G**	C
	Juniperus_virginiana	**A**	A	A	T	G	G	A	T	T	G	G	A	T	A	C	A	A	-	-	-	-	-	-	C	**G**	C
Outgroup	*Cupressus sempervirens* L.	G	A	A	T	G	G	A	T	T	G	G	A	T	A	C	A	A	-	-	-	-	-	-	C	**G**	C

In *trn*L-F region (Table 5.8B), the number of variable sites were 42 bp but only 19 of them were parsimony informative. The lenght of these samples ranged between 200-315 bp. This difference was due to possession of insertions in some species which were not observed in any other member of the same section. For example, between 159- 188th bases, there were 30 bp insertion that were only obtained in *J.macrocarpa* Sibth. & Sm. and *J. oxycedrus* subsp. *macrocarpa* from Turkey. The most remarkable case has been seen in Subsection *Juniperus* where 30 bp insertion between 236-261 bp has been observed in the members especially in *J.oxycedrus*. Suprisingly, this situation were not seen as common even at species level such that samples obtained in Kastamonu Province did not possess this insertion but others had. Similarly, Subsection Oxycedrus also had another different case such that between 110 – 118 bp a deletion, which were not present other members of the genus, has been obtained. These two exceptional indels also proclaim the divergence of this subsection.

The *trn*V region have showed 4 indels in total. For instance at 53rd bp, there was an insertion in Section Sabina except *J. monticola* Martinez. and *J. foetidissima* Willd. from Eskisehir Province. Moreover, at 25th bp there was another insertion specific to Section Caryocedrus (Table 5.8C). The total parsimony informative sites were 19 bp in selected samples among 27 variable sites. At 11th bp position, this diversity was variable within section level such that within subsection Juniperus, the change was A to T; however, this change was A to G at section Caryocedrus and Sabina. Only *J.foetidissima* Willd. from Eskisehir Province has changed into A to T. At subsection Oxycedrus, species sampled from Turkey did not changed at this base, but only the species obtained from GeneBank showed the substitution from A to T.

Table 5.8. Continued. B) *trn*L-F intergenic spacer region

Sect	Species	25	91	92	93	94	95	110	111	112	113	114	115	116	117	118	134	148	204	205	206	207	208	211	212	213	233	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	291	357
	J.communis var.communis	G	T	C	T	T	T	T	G	T	A	T	A	T	A	A	C	C	-	-	-	-	-	T	T	C	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	G
	J.communis var.saxatilis	G	T	C	T	T	T	T	G	T	A	T	A	T	A	A	C	C	-	T	A	T	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	G
	J.rigida	G	T	C	T	T	T	T	G	T	A	T	A	T	A	A	C	C	-	T	A	T	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	G
J	*J.communis ssp nana (Turkey)*	G	T	C	T	T	T	T	G	T	A	T	A	T	A	A	C	C	-	-	-	-	-	T	T	C	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	G
	J.deltoides	G	T	C	T	T	T	-	-	-	-	-	-	-	-	-	C	C	-	T	A	-	-	-	-	-	T	T	A	T	A	A	A	T	G	T	A	T	T	A	T	T	T	A	T	T	G	T	A	T	A	A	A	C	G
	J.macrocarpa	G	T	C	T	T	T	T	G	T	A	T	A	T	A	A	C	C	-	T	A	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	G
	J.navicularis	G	T	C	T	T	T	-	-	-	-	-	-	-	-	-	-	C	-	T	A	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	G
	J.oxycedrus var.oxycedrus	G	T	C	T	T	T	-	-	-	-	-	-	-	-	-	-	C	-	T	A	-	-	-	-	-	T	T	A	T	A	A	A	T	G	T	A	T	T	A	T	T	T	A	T	T	G	T	A	T	A	A	A	C	G
O	*J.oxycedrus (Turkey)*	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	C	-	T	A	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	G
	J.oxycedrus (Turkey)	G	T	C	T	T	T	-	-	-	-	-	-	-	-	-	-	C	-	T	A	-	-	-	-	-	T	T	A	T	A	A	A	T	G	T	A	T	T	A	T	T	T	A	T	T	G	T	A	T	A	A	A	C	G
	J.oxycedrus ssp. macrocarpa (Turkey)	G	T	C	T	T	T	T	G	T	A	T	A	T	A	A	C	C	-	T	A	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	G
	J.drupacea Labill	G	-	-	-	-	-	T	G	T	A	T	A	T	A	A	C	C	-	T	A	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	A
C	*J.drupacea Labil (Turkey)*	G	-	-	-	-	-	T	G	T	A	T	A	T	A	A	C	C	-	T	A	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	A
	J.excelsa	C	-	-	-	-	-	T	G	T	A	T	A	T	A	A	C	C	-	T	A	T	A	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	G
	J.phoenicea	G	-	-	-	-	-	T	G	T	A	T	A	T	A	A	C	C	-	T	A	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	G
	J.sabina var.arenaria	G	-	-	-	-	-	T	G	T	A	T	A	T	A	A	C	C	A	T	A	T	A	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	G	T	G
	J.semiglobosa	G	-	-	-	-	-	T	G	T	A	T	A	T	A	A	C	C	A	T	A	T	A	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	G
SOW	*J.thurifera*	C	-	-	-	-	-	T	G	T	A	T	A	T	A	A	C	C	-	T	A	T	A	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	G
	J.foetidissima (Turkey)	C	-	-	-	-	-	T	G	T	A	T	A	T	A	A	C	C	-	T	A	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	G
	J.excelsa (Turkey)	G	-	-	-	-	-	T	G	T	A	T	A	T	A	A	C	A	-	T	A	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	G
	J.sabina (Turkey)	C	-	-	-	-	-	T	G	T	A	T	A	T	A	A	C	C	-	T	A	T	A	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	G
	J.phoenicea (Turkey)	C	T	C	T	T	T	-	-	-	-	-	-	-	-	-	-	C	-	T	A	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
SNW	*J.monosperma*	G	-	-	-	-	-	T	G	T	A	T	A	T	A	A	C	C	-	T	A	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	G
	J.virginiana	G	-	-	-	-	-	T	G	T	A	T	A	T	A	A	C	C	A	T	A	T	A	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	G
	Cupressus sempervirens	G	-	-	-	-	-	T	G	T	A	T	A	T	A	A	C	C	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	G

Table 5.8 Continued. C) trnV intronic region

Sections	Species	9	10	11	14	15	16	25	29	30	31	45	53	80	200	247	272	388	427	457	491
Juniperus	*Juniperus communis* L. *var.communis* L.	-	A	T	C	A	T	-	T	A	T	A	-	A	G	G	T	G	A	A	A
	Juniperus conferta	-	A	T	C	A	T	-	T	A	T	A	-	G	G	C	T	G	A	A	A
	J.communis L. *ssp nana (Turkey)*	-	A	T	C	A	T	-	T	A	T	A	-	A	G	G	T	G	A	A	A
Oxycedrus	*Juniperus deltoides*	G	A	T	C	A	T	-	A	A	T	A	-	G	G	G	T	A	A	A	A
	Juniperus macrocarpa	G	A	T	C	A	T	-	A	A	T	A	-	G	G	G	T	A	A	A	A
	Juniperus oxycedrus. var.oxycedrus	G	A	T	C	A	T	-	A	A	T	A	-	G	G	G	T	A	A	A	A
	J.oxycedrus (Turkey)	-	G	A	T	C	T	-	A	A	T	A	-	G	G	G	T	A	A	A	A
	J.oxycedrus ssp.macrocarpa (Turkey)	-	A	T	C	A	T	-	A	A	T	A	-	G	G	G	T	A	A	A	A
Caryocedrus	*Juniperus drupacea* Labill.	-	A	G	C	A	T	T	A	A	T	A	-	G	G	G	C	G	A	G	A
	J.drupacea Labill.(Turkey)	-	A	G	C	A	-	T	A	A	T	A	-	G	G	G	C	G	A	G	A
Sabina Old World Species	*Juniperus chinensis*	-	A	G	C	A	T	-	A	A	T	C	T	G	G	G	C	G	A	G	A
	Juniperus excelsa M. Bieb.	-	A	G	C	A	T	-	A	A	T	A	T	G	G	G	C	G	A	G	A
	Juniperus phoenicea L.	-	A	G	C	A	T	-	A	C	T	A	T	G	G	G	C	G	A	G	A
	Juniperus sabina L. *var.arenaria*	-	A	G	C	A	T	-	A	A	T	A	T	G	A	G	C	G	A	G	T
	Juniperus semiglobosa	-	A	G	C	A	T	-	A	A	T	A	T	G	G	G	C	G	A	G	T
	J.foetidissima Willd. *(Turkey)*	-	A	G	C	A	T	-	A	A	T	A	T	G	G	G	C	G	A	G	A
	J.foetidissima Willd. *(Turkey)*	-	A	T	C	A	-	-	A	A	T	A	-	G	G	G	C	G	A	A	A
	J.excelsa M. Bieb. *(Turkey)*	-	A	G	C	A	-	-	A	A	C	A	T	G	G	G	C	G	T	G	A
	J.sabina (Turkey)	-	A	G	C	A	-	-	A	A	T	C	T	G	G	G	C	G	A	G	A
	J.phoenicea L. *(Turkey)*	-	A	G	C	A	T	-	A	C	T	A	T	G	G	G	C	G	A	G	A
Sabina New World Species	*Juniperus monosperma*	-	A	G	C	A	T	-	A	A	T	A	T	G	G	G	C	G	A	G	A
	Juniperus monticola	-	A	G	C	A	T	-	A	A	T	A	-	G	G	G	C	G	A	G	A
	Juniperus virginiana	-	A	G	C	A	T	-	A	A	T	A	T	G	A	G	C	G	A	G	T
Outgroup	*Cupressus sempervirens*	-	G	A	C	A	T	-	A	A	T	A	-	G	G	G	C	G	A	G	A

Table 5.8 Continued. D) *mat*K region

Sections	Species	12	13	14	15	16	17	18	22	159	174	211	212	213	214	215	216	226	227	228	229	230	231	240	276	278	283	348	355	449	595	600
Juniperus	*J.communis subsp.nana (Turkey)*	A	A	C	T	T	C	C	G	C	C	-	-	-	-	-	-	C	A	A	T	T	A	A	A	G	C	C	T	T	C	A
	Juniperus communis var. communis	A	A	C	T	T	C	C	G	C	C	-	-	-	-	-	-	C	A	A	T	T	A	A	A	G	C	C	T	T	C	A
	Juniperus conferta	A	A	C	T	T	C	C	G	C	C	-	-	-	-	-	-	C	A	A	T	T	A	A	A	G	C	C	T	T	C	A
Oxycedrus	*J.oxycedrus (Turkey)*	A	A	C	T	T	C	C	G	C	C	-	-	-	-	-	-	C	A	A	T	T	A	A	A	G	C	C	T	T	C	A
	J.oxycedrus ssp.macrocarpa (Turkey)	A	A	C	T	T	C	C	G	C	C	-	-	-	-	-	-	C	A	A	T	T	A	A	A	G	C	C	T	T	C	A
	Juniperus deltoides	A	A	C	T	T	C	C	G	C	C	-	-	-	-	-	-	C	A	A	T	T	A	A	A	G	C	C	T	T	C	A
	Juniperus formosana var. formosana	A	A	C	T	T	C	C	G	C	C	-	-	-	-	-	-	C	A	A	T	T	A	A	A	G	C	C	T	T	C	A
	Juniperus oxycedrus	A	A	C	T	T	C	C	G	C	C	-	-	-	-	-	-	C	A	A	T	T	A	A	A	G	C	C	T	T	C	A
Caryocedrus	*J. drupecae (Turkey)*	G	A	C	T	T	C	C	G	A	C	A	G	A	A	T	A	C	A	A	T	T	A	A	A	G	C	C	T	T	A	A
	Juniperus drupacea	G	A	C	T	T	C	C	G	A	C	A	G	A	A	T	A	C	A	A	T	T	A	A	A	G	C	C	T	T	A	A
Sabina Old World Species	*J.sabina (Turkey)*	-	-	-	-	-	-	-	G	A	T	-	-	-	-	-	-	-	-	-	-	-	-	G	A	A	C	C	T	T	C	G
	J.excelsa (Turkey)	G	A	C	T	T	C	C	G	A	C	-	-	-	-	-	-	C	A	A	T	T	A	G	A	A	C	C	T	T	C	G
	J.excelsa. (Turkey)	G	A	C	T	T	C	C	G	A	C	-	-	-	-	-	-	C	A	A	T	T	A	G	A	A	C	C	T	T	C	A
	J. phoenicea (Turkey)	G	A	C	T	T	C	C	G	A	C	A	G	A	A	T	A	C	A	A	T	T	A	A	A	G	C	C	T	T	C	G
	J. foetidissima (Turkey)	G	A	C	T	T	C	C	G	A	C	-	-	-	-	-	-	C	A	A	T	T	A	G	A	A	C	C	T	T	C	G
	J. foetidissima (Turkey)	G	A	C	T	T	C	C	G	A	C	-	-	-	-	-	-	C	A	A	T	T	A	G	A	A	C	C	T	T	C	G
	Juniperus chinensis	-	-	-	-	-	-	-	G	A	T	-	-	-	-	-	-	-	-	-	-	-	-	G	A	A	C	C	T	T	C	G
	Juniperus excelsa	G	A	C	T	T	C	C	G	A	C	-	-	-	-	-	-	C	A	A	T	T	A	G	A	A	C	C	T	T	C	G
	Juniperus phoenicea	G	A	C	T	T	C	C	G	A	C	-	-	-	-	-	-	C	A	A	T	T	A	G	A	G	C	C	T	T	C	G
	Juniperus sabina. var. arenaria	G	A	C	T	T	C	C	G	A	C	A	G	A	A	T	C	C	A	A	T	T	A	G	A	G	C	C	G	T	C	G
	Juniperus thurifera	-	-	-	-	-	-	-	G	A	T	-	-	-	-	-	-	-	-	-	-	-	-	G	A	A	C	C	T	T	C	G
Sabina New World Species	*Juniperus monosperma*	G	A	C	T	T	C	C	T	A	C	-	-	-	-	-	-	C	A	A	T	T	A	G	C	G	A	A	T	G	C	G
	Juniperus monticola	G	A	C	T	T	C	C	T	A	C	-	-	-	-	-	-	C	A	A	T	T	A	G	C	G	A	C	T	G	C	G
	Juniperus occidentalis	C	A	C	T	T	C	C	T	A	C	-	-	-	-	-	-	C	A	A	T	T	A	G	C	G	A	A	T	G	C	G
	Juniperus virginiana	G	A	C	T	T	C	C	G	A	C	A	G	A	A	T	C	C	A	A	T	T	A	G	A	G	C	C	G	T	C	G
Outgroup	*Cupressus sempervirens*	G	A	C	T	T	C	C	G	A	C	-	-	-	-	-	-	C	A	A	T	T	A	G	A	G	C	C	T	T	C	A

Table 5.8D (Continued)

Sections	Species	625	639	696	705	782	783	784	785	786	787	809	893	959	972	974	975	976	1013	1035	1039	1095	1099	1122	1132	1163	1231	1232	1272	1284	1291	1292
Juniperus	*J.communis subsp.nana (Turkey)*	C	G	T	T	-	-	-	-	-	-	G	T	C	T	-	-	-	T	G	G	T	C	T	C	G	G	G	G	C	A	G
	Juniperus communis var. communis	T	G	T	T	-	-	-	-	-	-	G	T	C	T	-	-	-	T	G	G	T	C	T	C	G	G	G	G	C	A	G
	Juniperus conferta	T	G	T	T	-	-	-	-	-	-	G	T	C	T	-	-	-	T	G	G	T	C	T	C	G	G	G	G	C	A	A
Oxycedrus	*J.oxycedrus (Turkey)*	T	G	T	T	-	-	-	-	-	-	G	T	C	T	-	-	-	T	G	G	T	C	T	C	G	G	G	G	C	A	G
	Juniperus deltoides	T	G	T	T	-	-	-	-	-	-	G	T	C	T	-	-	-	T	G	G	T	C	T	C	G	G	G	G	C	A	G
	Juniperus formosana var. formosana	T	G	T	T	-	-	-	-	-	-	G	T	C	T	-	-	-	T	G	G	T	C	T	C	G	G	G	G	C	A	G
	Juniperus oxycedrus	T	G	T	T	-	-	-	-	-	-	G	T	C	T	-	-	-	T	G	G	T	C	T	C	G	G	G	G	C	A	G
Caryocedrus	*J. drupecae (Turkey)*	T	G	T	T	-	-	-	-	-	-	G	T	C	T	-	-	-	T	G	G	T	C	T	C	G	G	G	G	C	A	G
	Juniperus drupacea	T	G	T	T	-	-	-	-	-	-	G	T	C	T	-	-	-	T	G	G	T	C	T	C	G	G	G	G	C	A	G
Sabina Old World Species	*J.sabina (Turkey)*	T	A	T	C	-	-	-	-	-	-	G	T	C	C	-	-	-	G	G	G	G	C	T	C	G	G	G	G	T	G	G
	J.excelsa (Turkey)	T	G	T	T	-	-	-	-	-	-	G	T	C	T	-	-	-	T	G	G	T	C	T	C	G	G	G	G	C	A	G
	J. phoenicea (Turkey)	T	G	T	T	A	A	C	A	A	A	G	C	C	T	-	-	-	T	A	A	T	C	T	C	G	G	G	G	C	A	G
	J. foetidissima (Turkey)	T	A	T	T	-	-	-	-	-	-	G	T	C	T	-	-	-	G	G	G	T	C	T	C	G	G	G	G	C	A	G
	J. foetidissima (Turkey)	T	A	T	T	-	-	-	-	-	-	G	T	C	C	-	-	-	G	G	G	T	C	T	C	C	G	G	G	T	G	G
	Juniperus chinensis	T	A	T	C	-	-	-	-	-	-	G	T	C	T	-	-	-	T	G	G	T	C	T	C	G	G	G	G	C	A	G
	Juniperus excelsa	T	A	T	T	-	-	-	-	-	-	G	T	C	C	-	-	-	G	G	G	T	C	T	C	C	G	G	G	T	G	G
	Juniperus phoenicea	T	G	T	T	A	A	C	A	A	A	G	C	C	T	-	-	-	T	A	A	T	C	T	C	G	G	G	G	C	A	G
	Juniperus sabina var. arenaria	T	G	C	T	-	-	-	-	-	-	G	T	G	C	-	-	-	G	G	G	G	C	T	C	G	G	G	G	C	A	G
	Juniperus thurifera	T	A	T	C	-	-	-	-	-	-	G	T	C	T	-	-	-	T	G	G	T	C	T	C	G	G	G	G	C	A	G
Sabina New World Species	*Juniperus monosperma*	T	G	T	T	-	-	-	-	-	-	A	T	C	T	C	A	A	T	G	G	T	T	C	T	G	T	T	A	C	A	G
	Juniperus monticola	T	G	T	T	-	-	-	-	-	-	G	T	C	T	-	-	-	T	G	G	T	T	C	T	G	T	T	G	C	A	G
	Juniperus occidentalis	T	G	T	T	-	-	-	-	-	-	A	T	C	T	C	A	C	T	G	G	T	T	C	T	G	T	T	A	C	A	G
	Juniperus virginiana	T	G	C	T	-	-	-	-	-	-	G	T	C	C	-	-	-	G	G	G	G	C	T	C	G	G	G	G	C	A	G
Outgroup	*Cupressus sempervirens*	T	G	T	T	-	-	-	-	-	-	G	T	C	T	-	-	-	T	G	G	T	C	T	C	G	G	G	G	C	A	G

5.8D (Continued)

Sections	Species	1306	1307	1342	1347	1375	1377	1386	1387	1388	1389	1390	1391	1396	1397	1399	1400	1401	1402	1403	1404	1405	1406	1407	1408	1420	1428
Juniperus	*J.communis subsp.nana (Turkey)*	T	T	G	A	C	G	A	C	A	A	A	A	T	T	C	A	A	A	A	G	A	A	A	G	C	**T**
	Juniperus communis var. communis	T	T	G	A	C	G	A	C	A	A	A	A	T	T	C	A	A	A	A	G	A	A	A	G	C	**T**
	Juniperus conferta	T	T	G	A	C	G	A	C	A	A	A	A	T	T	C	A	A	A	A	G	A	A	A	G	C	**T**
Oxycedrus	*J.oxycedrus (Turkey)*	T	T	G	A	C	G	A	C	A	A	A	A	T	T	C	A	A	A	A	G	A	A	A	G	C	**T**
	Juniperus deltoides	T	T	G	A	C	G	A	C	A	A	A	A	T	T	C	A	A	A	A	G	A	A	A	G	C	**T**
	Juniperus formosana var. formosana	T	T	G	A	C	G	A	C	A	A	A	A	T	T	C	A	A	A	A	G	A	A	A	G	C	**T**
	Juniperus oxycedrus	T	T	G	A	C	G	A	C	A	A	A	A	T	T	C	A	A	A	A	G	A	A	A	G	C	**T**
Caryocedrus	*J. drupecae (Turkey)*	T	T	G	A	C	G	A	C	A	A	A	A	T	T	C	A	A	A	A	G	A	A	A	G	C	**T**
	Juniperus drupacea	T	T	G	A	C	G	A	C	A	A	A	A	T	T	C	A	A	A	A	G	A	A	A	G	C	**T**
Sabina Old World Species	*J.sabina (Turkey)*	T	T	G	A	C	G	-	-	-	-	-	-	T	T	C	A	A	A	A	G	A	A	A	G	C	**T**
	J.excelsa (Turkey)	T	T	G	A	**T**	G	A	C	A	A	A	A	T	T	C	A	A	A	A	G	A	A	A	G	C	**T**
	J. phoenicea (Turkey)	T	T	G	A	C	G	A	C	A	A	A	A	T	T	C	A	A	A	A	G	A	A	A	G	C	**T**
	J. foetidissima (Turkey)	T	T	G	A	C	G	A	C	A	A	A	A	T	T	C	A	A	A	A	G	A	A	A	G	C	**T**
	J. foetidissima (Turkey)	T	T	G	A	C	G	A	C	A	A	A	A	T	T	C	A	A	A	A	G	A	A	A	G	C	**T**
	Juniperus chinensis	T	T	G	A	C	G	A	C	A	A	A	A	T	T	C	A	A	A	A	G	A	A	A	G	C	**T**
	Juniperus excelsa	T	T	G	A	**T**	G	A	C	A	A	A	A	T	T	C	A	A	A	A	G	A	A	A	G	C	**T**
	Juniperus phoenicea	T	T	G	A	C	G	A	C	A	A	A	A	T	T	C	A	A	A	A	G	A	A	A	G	C	**T**
	Juniperus sabina var. arenaria	**A**	T	G	A	C	G	-	-	-	-	-	-	T	T	C	A	A	A	A	G	A	A	A	G	C	**T**
	Juniperus thurifera	T	T	G	A	C	G	A	C	A	A	A	A	T	T	C	A	A	A	A	G	A	A	A	G	C	**T**
Sabina New World Species	*Juniperus monosperma*	T	**G**	**C**	**G**	C	**A**	A	C	A	A	A	A	**C**	**A**	**T**	-	-	-	-	-	-	-	-	-	**T**	C
	Juniperus monticola	T	**G**	**C**	A	C	**A**	A	C	A	A	A	A	T	-	-	-	-	-	-	-	-	-	-	-	**G**	C
	Juniperus occidentalis	T	**G**	**C**	**G**	C	**A**	A	C	A	A	A	A	**C**	**A**	**T**	-	-	-	-	-	-	-	-	-	**T**	C
	Juniperus virginiana	**A**	T	G	A	C	G	A	C	A	A	A	A	T	T	C	A	A	A	A	G	A	A	A	G	C	**T**
Outgroup	*Cupressus sempervirens*	T	T	G	A	C	G	A	C	A	A	A	A	T	T	C	A	A	A	A	G	A	A	A	G	C	C

According to analyses based upon *mat*K region, there were 82 parsimony informative sites which indicates the presence of high amount of diversity in the *mat*K region. The rate of indels were considerably high especially among Old World Species of Section Sabina. Within other sections/subsections, the indels were similar with each other except in one region of subsection Caryocedrus where AGAATA insertion existed between 211-216th bp (Table 5.8D).

The nucleotide diversity and genetic distance of *Juniperus* L. at section level based on Kimura 2-parameter best fit nucleotide substituiton model with 0.95 fraction of evolutionary invariable sites for *trn*L and Tamura 3-parameter model for other 3 regions (*trn*L F, *trn*V and *mat*K) have been shown in Table 5.9. Accordingly, in *trn*L region, the highest diverstiy has been found in Section Sabina of the New World Species (0.0047 ± 0.0026) followed by Subsection Oxycedrus (0.0036 ± 0.0021) whereas there was no diversity within Subsections Juniperus and Caryocedrus. The *trn*L-F region also revealed the similar results such that Subsections Juniperus and Caryocedrus had no or little diversity. However, most of the diversity has been detected in both Old (0.0050 ± 0.0021) and New World Species (0.0049 ± 0.0018) of Section Sabina, respectively and Subsection Oxycedrus (0.0057 ± 0.0031) (Table 5.9).

Table 5.9. Estimated Average Evolutionary Divergence of all *Juniperus* L. species over Sequence Pairs *trn* and *mat*K regions

Sections/Subsections	Distance ± Standard Error	Regions
Juniperus	0.0000	*trn*L
Oxycedrus	0.0036 ± 0.0021	
Caryocedrus	0.0000	
Sabina Old World Species	0.0018 ± 0.0009	
Sabina New World Species	0.0047 ± 0.0026	
Juniperus	0.0007 ± 0.0007	*trn*L-F
Oxycedrus	0.0057 ± 0.0031	
Caryocedrus	0.0000	
Sabina Old World Species	0.0050 ± 0.0021	
Sabina New World Species	0.0049 ± 0.0018	
Juniperus	0.0010 ± 0.0007	*trn*V
Oxycedrus	0.0045 ± 0.0018	
Caryocedrus	0.0000	
Sabina Old World Species	0.0034 ± 0.0013	
Sabina New World Species	0.0029 ± 0.0013	
Juniperus	0.0006 ± 0.0004	*mat*K
Oxycedrus	0.0004 ± 0.0003	
Caryocedrus	0.0000	
Sabina Old World Species	0.0087 ± 0.0014	
Sabina New World Species	0.0128 ± 0.0020	

Regarding *trn*V reigon, the only subsection with no diversity was Caryocedrus. The highest diversity has been found in Section Oxycedrus (0.0045 ± 0.0018) followed by Section Sabina of the Old World Species (0.0034 ± 0.0013) (Table 5.9). Finally, *mat*K region, which was analysed with Tamura 3-parameter model with 0.22 Gamma correction, revealed that most diverse group was New World Species of Section Sabina (0.0128 ± 0.0020), followed by Old World Species (0.0087 ± 0.0014). However, the other subsections of genus *Juniperus* showed little or no diversity at all (Table 5.9).

The genetic distance among each species have shown that (Table 5.10) there were no divergences between subsection Juniperus and subsection Caryocedrus with regard to the *trn*L regions. This results were also observed in the analyses using sequences of only Turkish *Juniperus* L. (Table 5.6). Section Sabina New World species which were indeed considered as a sister group for this study showed the highest divergence especially in the combination with Subsection Oxycedrus. Once again, similar to the results of Table 5.6, subsection Oxycedrus was the most distantly related to other groups. Pursuant to *trn*L-F region, Section Sabina Old and New World species which were the closest sections. However, the highest divergence was found between Section Juniperus subsection Juniperus and Subsection Caryocedrus. Indeed, Subsection Juniperus was found as the most divergent section regarding *trn*L-F region. Interestingly, the closest relationship was between Sabina New World species and subsection Caryocedrus regarding the *trn*V region. The most diverged taxon was again subsection Oxycedrus as it was evident in previous regions. Finally, *mat*K region has indicated the similar results with the ones obtained from analyses of Turkish *Juniperus* L. Accordingly, the closest relationship was between subsection Juniperus and Oxycedrus where as the highest divergence was between in New World Species and Old World species of Section Sabina (Table 5.10).

Table 5.10. Genetic divergence among sections based on studied regions. Standard error estimate(s) were obtained by a bootstrap procedure (500 replicates). Within the genus *Juniperus* L., the least distances are shown with green and the most diverged taxa combinations are shown with blue. (SOW: Section Sabina Old World Species and SNW: Section Sabina New World Species)

Section / Subsection	Juniperus	Oxycedrus	Caryocedrus	SOW	SNW	Overall	Regions
Juniperus							
Oxycedrus	0.0063±0.0039						
Caryocedrus	0.0000	0.0063±0.0039				0.0041 ± 0.0016	*trn*L
SOW	0.0010±0.0005	0.0073±0.0039	0.0010±0.0005				
SNW	0.0031±0.0017	0.0091±0.0041	0.0031±0.0017	0.0040±0.0019			
Outgroup	0.0107±0.0057	0.0137±0.0052	0.0073±0.0046	0.0073±0.0049	0.0104±0.0052		
Juniperus							
Oxycedrus	0.0089±0.0058						
Caryocedrus	0.0115±0.0078	0.0096±0.0064				0.0065 ± 0.0023	*trn*LF
SOW	0.0087±0.0055	0.0062±0.0032	0.0083±0.0056				
SNW	0.0084±0.0055	0.0066±0.0032	0.0081±0.0056	0.0053±0.0016			
Outgroup	0.0059±0.0054	0.0040±0.0031	0.0055±0.0055	0.0028±0.0012	0.0025±0.0009		
Juniperus							
Oxycedrus	0.0106±0.0039						
Caryocedrus	0.0099±0.0041	0.0117±0.0044				0.0077 ± 0.0021	*trn*V
SOW	0.0116±0.0042	0.0135±0.0044	0.0020±0.0008				
SNW	0.0117±0.0042	0.0135±0.0044	0.0018±0.0008	0.0037±0.0012			
Outgroup	0.0199±0.0061	0.0179±0.0056	0.0119±0.0047	0.0139±0.0048	0.0135±0.0047		
Juniperus							
Oxycedrus	0.0006±0.0004						
Caryocedrus	0.0019±0.0010	0.0016±0.0010				0.0085 ± 0.0012	*mat*K
SOW	0.0071±0.0017	0.0067±0.0016	0.0066±0.0015				
SNW	0.0167±0.0031	0.0163±0.0030	0.0161±0.0030	0.0180±0.0028			
Outgroup	0.0219±0.0045	0.0215±0.0044	0.0213±0.0044	0.0257±0.0048	0.0351±0.0056		

5.3.2. Haplotype Frequency Analysis, Analyses of Molecular Variance (AMOVA), Estimation of F_{st} Values and Molecular Clock Estimation

According to analysis based on studied *trn* regions of *Juniperus* L. species obtained from Turkey, relative observed haplotype frequencies have been obtained and shown in Figure 5.1 as a haplotype tree obtained with maximum parsimony method. The results indicated that there were 10 haplotypes in *Juniperus* L. obtained from Turkey. This value raised to 55 when all available sequences of Juniper species were included. Among Turkish Junipers, there were 26 haplotype variations starting from 9^{th} position and ended at 1232^{nd} position. Consequently, there were two main clusters. First one was composed of *J.oxycedrus* L. populations which revealed a relationship with *J. oxycedrus* L. subsp. *macrocarpa* and *J.communis* L. The second cluster included *J.drupacea Labill*. and the members of Section Sabina. However, within second cluster different populations of *J. foetidissima* Willd. showed different allocation in the tree. The result from *mat*K analysis has shown that there were 11 haplotypes among 68 Turkish Juniper samples (Figure 5.2). The haplotype number increased to 43 among 122 samples when all Juniper sequences were included. The haplotype tree of *mat*K region gave very similar results in terms of clustering except for *J.foetidissima* Willd.. One sample of *J .foetidissima* Willd. from Kahramanmaras possessed different haplotype than other *J.foetidissima* Willd. samples.

Analysis of Molecular Variance (AMOVA) (Weir and Cockerham, 1984; Excoffier *et al*., 1992; Wier, 1996) were performed for both Turkish *Juniperus* L. and *Juniperus* L. obtained from GeneBank for 3 *trn* and *mat*K regions. The results for *trn* regions indicated that for both Turkish *Juniperus* L. and the analyses with all Juniper species included that there were high rate of variation among sections. Especially for Turkish *Juniperus* L. more than 75 % of variations were due to the differences among sections. However, when all other Juniper species were included, the variation due to within section became more evident (Table 5.11). The *mat*K results have shown that when only Turkish *Juniperus* L. and all other *Juniperus* L. species were compared, the variation within sections were higher in Turkish Junipers than in all Junipers combined (Table 5.11).

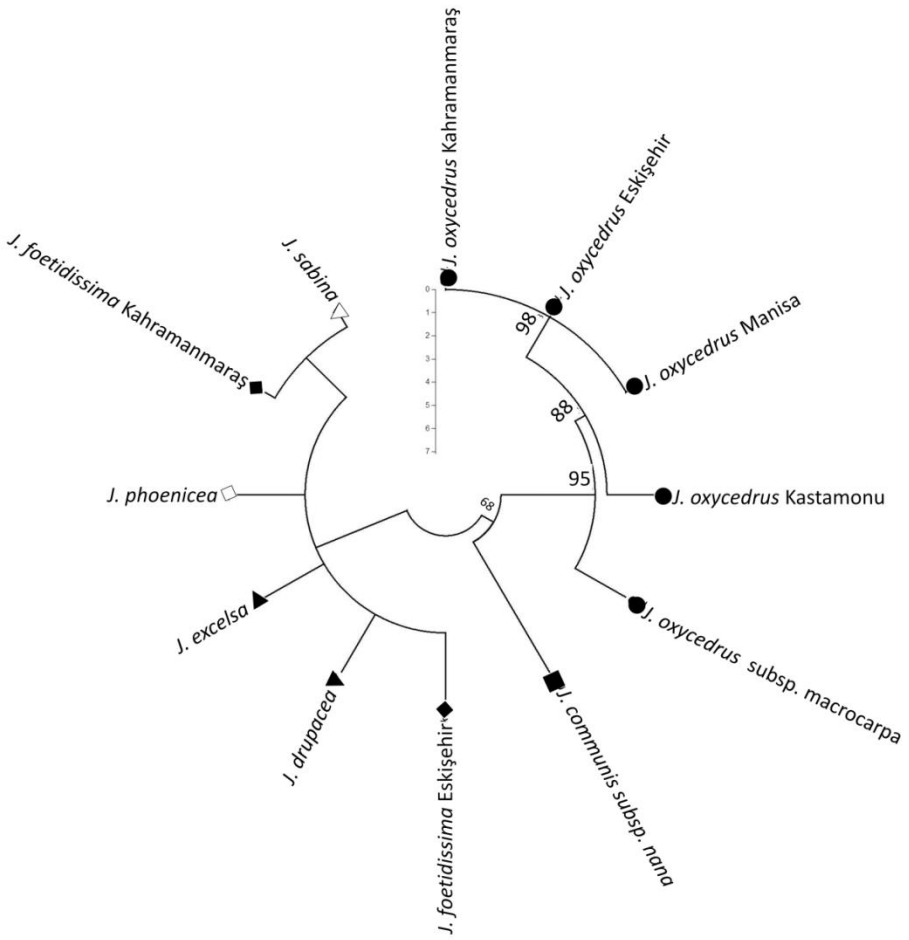

Figure 5.1. The relationship of Turkish Junipers based on haplotype pattern of 3 *trn* regions

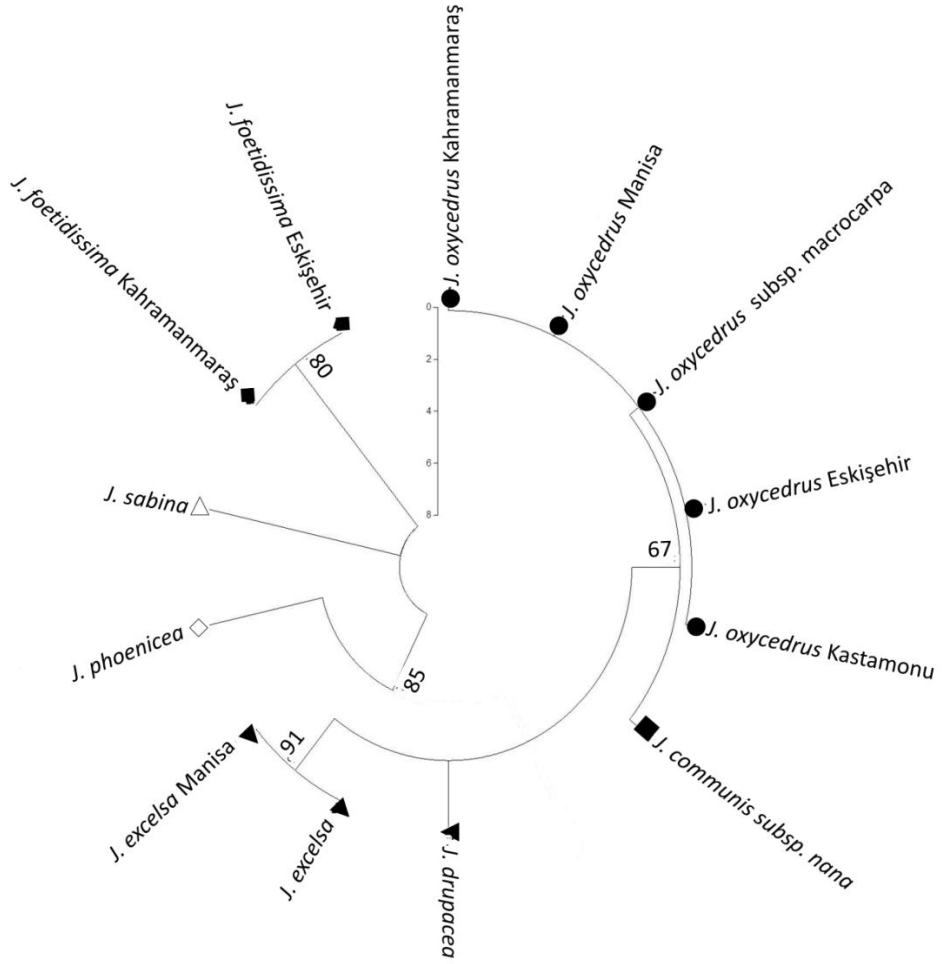

Figure 5.2. The relationship of Turkish Junipers based on haplotype pattern of *mat*K region

Table 5.11. Analysis of Molecular Variance (AMOVA) based on *trn* and *mat*K regions of Turkish *Juniperus* L. and *Juniperus* L. obtained from GeneBank

Source of Variation	Degrees of Freedom	Sum of Squares	Variance Components	Percentage of Variation	F_{st}
Turkish *Juniperus* (3 studied *trn* regions)					
Among Sections	3	204.073	4.588 Va	75.82	0.758
Within Sections	62	90.737	1.46 Vb	24.18	
Total	65	294.809	6.05		
Turkish *Juniperus* + *Juniperus* obtained from GeneBank (3 studied *trn* regions)					
Among Sections	4	314.847	3.53 Va	61.11	0.611
Within Sections	115	258.403	2.25 Vb	38.89	
Total	120	573.251	5.78		
Turkish *Juniperus* (*mat*K)					
Among Sections	3	141.063	2.93 Va	47.34	0.473
Within Sections	64	208.909	3.26 Vb	52.66	
Total	67	349.972	6.20		
Turkish *Juniperus* + *Juniperus* obtained from GeneBank (*mat*K)					
Among Sections	4	347.341	3.81 Va	45.35	0.454
Within Sections	117	536.693	4.59 Vb	54.65	
Total	121	884.034	8.39		

F_{st} values have been analyzed by using Tamura and Nei method (1993). All calculations were significant with p value lower than 0.050. Accordingly, for both *trn* and *mat*K regions of Turkish *Juniperus* L., most variations were due to between sections. However, Fst values were lower in Section Sabina which indicated the the variation due to within this section has been significant (Table 5.12). When other *Juniperus* L. from database have been added, Sabina species from both Old World and New World showed high variation in *trn* and *mat*K regions of species within secitons. If these results were interpreted with AMOVA results, it is clear that the variation within sections were mainly due to contribution of Section Sabina species.

Table 5.12. Comparisons of pairs of Juniper samples (sections pairwise F_{st} values) by using Tamura & Nei Distance Method (F_{st} p values < 0.0050)

Sect. Juniperus Subsect. Juniperus					
Sect. Juniperus Subsect. Oxycedrus	0.6848 0.6305 0.5653 0.3246				
Sect. Juniperus Subsect. Caryocedrus	1.0000 1.0000 0.7789 0.9466	0.6391 0.9511 0.5373 0.9583			
Section Sabina Old World species	0.5087 0.3413 0.4633 0.2711	0.3254 0.3500 0.5200 0.2732	0.4751 0.3593 0.2762 0.3129		
Section Sabina New World species	0.6853 0.5438	0.6060 0.5669	0.5517 0.4907	0.1269 0.4185	
	Sect. Juniperus Subsect. Juniperus	Sect. Juniperus Subsect. Oxycedrus	Sect. Juniperus Subsect. Caryocedrus	Section Sabina Old World species	Section Sabina New World species

trn regions of Turkish *Juniperus* L.

*mat*K region of Turkish *Juniperus* L.

trn regions of whole *Juniperus* L.

*mat*K region of whole *Juniperus* L.

Finally, molecular clock estimation analysis has been performed. According to Axelrod (1975), *Juniperus* L. was the part of Madrean-Tethyan vegetation belts by the late Oligocene. Moreover, Section Caryocedrus is restricted to the eastern Mediterranean region. *J. drupacea* Labill., the only species in this section, was probably differentiated about 20 million years ago (Adams, 2011). To support these hypotheses, divergence time of *Juniperus* L. samples was calculated at section / subsection level (Table 5.13). Different species of *Juniperus* L. from both Turkey and GenBank were used to estimate the evolutionary divergence time of *Juniperus* L. based on sequences of 3 *trn* and *matK* regions. Table 5.13 indicated the number of parsimony informative sites, total length of the region, *d* and *k* values, and molecular clock times.

New World species of Section Sabina diverged from other members of genus between 7.7 – 14.3 million years ago based on *trn* regions and between 15.9 – 26.8 million years ago based upon *mat*K region. Considering other sections, the most recent divergence time was found between subsection Juniperus and subsection Oxycedrus followed by subsection Juniperus and subsection Caryocedus. Generally divergence time of *mat*K region is more recent than *trn* region for all sections. This indicated the much slower evolution of *mat*K region than *trn* regions.

By using all these findings and the constructed phylogenetic trees based on each studied region, it obvious that New World species has been diverged from other species of *Juniperus* and dispersed from Europe – Asia.

Table 5.13. Molecular Clock Estimations for *Juniperus* species based on 3 *trn* and *mat*K regions

Juniperus Genus Sections	**Molecular Regions**	**# of parsimony informative sites**	**Length of regions (bp)**	***d***	***k***	**MCE (mya)***
Subsection Juniperus - Oxycedrus	*trn*	19	~1117	0.0170	0.01721	8.6
	*mat*K	3	~1416	0.0021	0.00212	1.1
Subsection Juniperus – Caryocedrus	*trn*	11	~1116	0.0099	0.00992	4.9
	*mat*K	5	~1418	0.0035	0.00353	1.8
Subsection Juniperus – Section Sabina Old World Species	*trn*	25	~1103	0.0227	0.02302	11.5
	*mat*K	42	~1413	0.0297	0.03033	15.2
Subsection Juniperus – Section Juniperus New World Species	*trn*	25	~1115	0.0224	0.02276	11.4
	*mat*K	46	~1414	0.0325	0.03326	16.6
Subsection Oxycedrus – Caryocedrus	*trn*	19	~1116	0.0170	0.01722	8.6
	*mat*K	5	~1418	0.0035	0.00353	1.8
Subsection Oxycedrus – Section Sabina Old World Species	*trn*	27	~1104	0.0245	0.02486	12.4
	*mat*K	42	~1413	0.0297	0.03033	15.2
Subsection Oxycedrus – Section Sabina New World Species	*trn*	31	~1115	0.0278	0.02833	14.2
	*mat*K	46	~1414	0.0325	0.03326	16.6
Subsection Caryocedrus – Section Sabina Old World Species	*trn*	19	~1101	0.0173	0.01746	8.7
	*mat*K	40	~1413	0.0283	0.02886	14.4
Subsection Caryocedrus – Section Sabina New World Species	*trn*	17	~1114	0.0153	0.01542	7.7
	*mat*K	44	~1415	0.0311	0.03176	15.9
Section Sabina Old World Species – Sabina New World Species	*trn*	31	~1103	0.02811	0.028645	14.3
	*mat*K	73	~1412	0.0517	0.05357	26.8
All Juniperus – Cupressus (Outgroup)	*trn*	51	~1250	0.0408	0.04195	20.9
	*mat*K	78	~1431	0.0545	0.05659	28.3

* Molecular Clock Estimation (Million Years Ago)

5.4. Construction of Phylogenetic Trees

5.4.1. Phylogenetic Trees based on *trn* and *mat*K regions of Turkish *Juniperus*

Considering Turkish *Juniperus* L. based upon studied *trn* regions, the evolutionary history was inferred by using the Neighbour Joining method with 0.22 Gamma Correction was set to the Tamura 3-parameter model option (Tamura, 1992). Section Sabina New World species have been considered as sister group during the phylogenetic tree construction. The optimal tree with the sum of branch length 0.053 has been shown in Figure 5.3. The analysis involved nucleotide sequences from Turkey and GenBank, and 1 *Cupressus sempervirens* L. as an outgroup. All positions containing gaps and missing data were eliminated. There were a total of 966 positions in the final dataset. Accordingly, the taxa showed arranged distribution at species level. However, there were some exceptions such that *J. foetidissima* Willd. from Eskişehir Çatacık showed divergence from *J. foetidissima* Willd. Kahramanmaraş Tekir populations. Moreover, *J. oxycedrus* L. subsp. *macrocarpa* Sibth. & Sm. diverged from other *J. oxycedrus* L. species and revealed a common ancestory. Within *J.oxycedrus* L., the divergence was at population level such that *J. oxycedrus* L. from Kastamonu Kayalı Köyü showed divergence from *J. oxycedrus* L. from other populations sampled in Turkey. The most diverged species from Turkey have been found as *J.phoenicea* L.. After including other *Juniperus* L. from GenBank, 3 clusters were found: the one that contained *J. communis* L., *J. conferta*, *J. rigida* and *J. formosana*. The first three were belong to subsect. Juniperus; however, *J. formosana* has been claimed to be included in subsect. Oxycedrus. Still it showed ancestral relation with other species of the cluster. The second cluster composed of *J. oxycedrus* L., *J. deltoides, J. macrocarpa, J. brevifolia* and *J. navicularis*. Like *J. formosana* in previous cluster, *J. macrocarpa* (including J. oxycedrus L. subsp. *macrocarpa* from Turkey) revealed an ancestral relation with other members of the cluster. All the members were included in subsect. Oxycedrus. The third cluster contained the remaining members of the taxon. *J. foetidissima* Willd. from Eskisehir Çatacık showed an diverged relation in the cluster and other members revealed an arranged distribution (Figure 5.3). Moreover, New World species of Section Sabina revealed a dispersed allocation in the tree.

For *mat*K region, the optimal tree with the sum of branch length = 0.09 was shown in Figure 5.4. The rate variation among sites was modeled with a gamma distribution (shape parameter = 0.06). There were total of 1383 positions in the final dataset. According to the region, the clustering became much more reasonable such that species from Section Sabina diverged from the members of Section *Juniperus* (Figure 5.4). In *mat*K region especially, *J. oxycedrus* L. and *J.communis* L. made a cluster together with relatively low bootstrap values (57 %). However, members of old world species of Section Sabina lined together and formed another cluster. As in *trn* regions some species New World Sabina showed dispersed formation on the tree. Although *J. drupacea* Labill. made another cluster, it showed again closed relation with *J. communis* L. and *J. oxycedrus* L..

For both *trn* and *mat*K regions, the members of the Section Juniperus showed clear separate groups on the tree. However, the species of Section Sabina are still far from clear separation to the section in the tree.

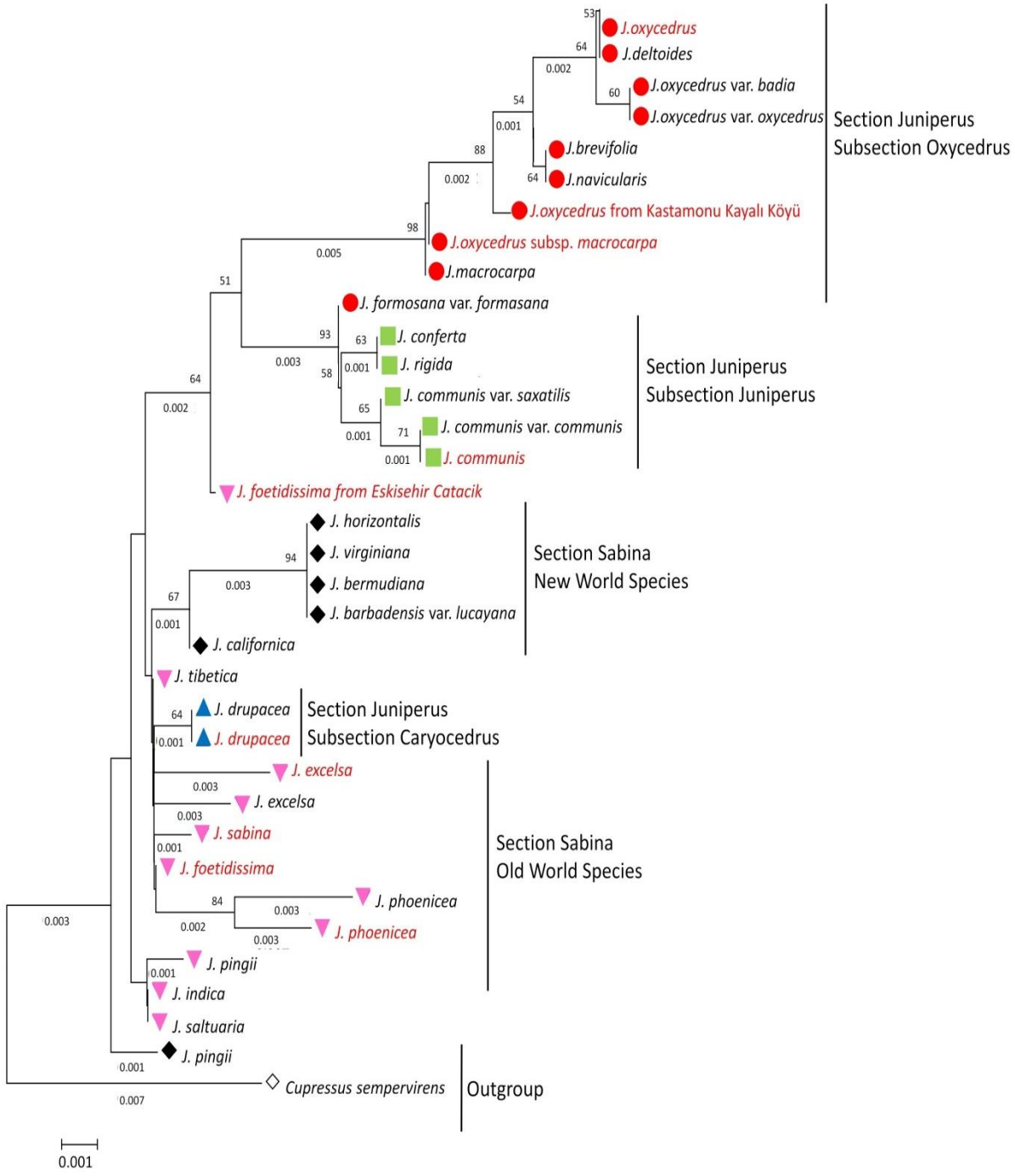

Figure 5.3. Phylogenetic tree of *Juniperus* L. based on 3 studied *trn* regions with Neighbour Joining method. The percentage of trees (≥ 50) in which the associated taxa clustered together has been shown above the branches. Moreover, the tree has drawn to scale, with branch lengths (≥ 0.001) measured in the number of substitutions per site (below the branches). The taxa shown in red are the ones sampled in Turkey. Others (in black) were obtained from GenBank

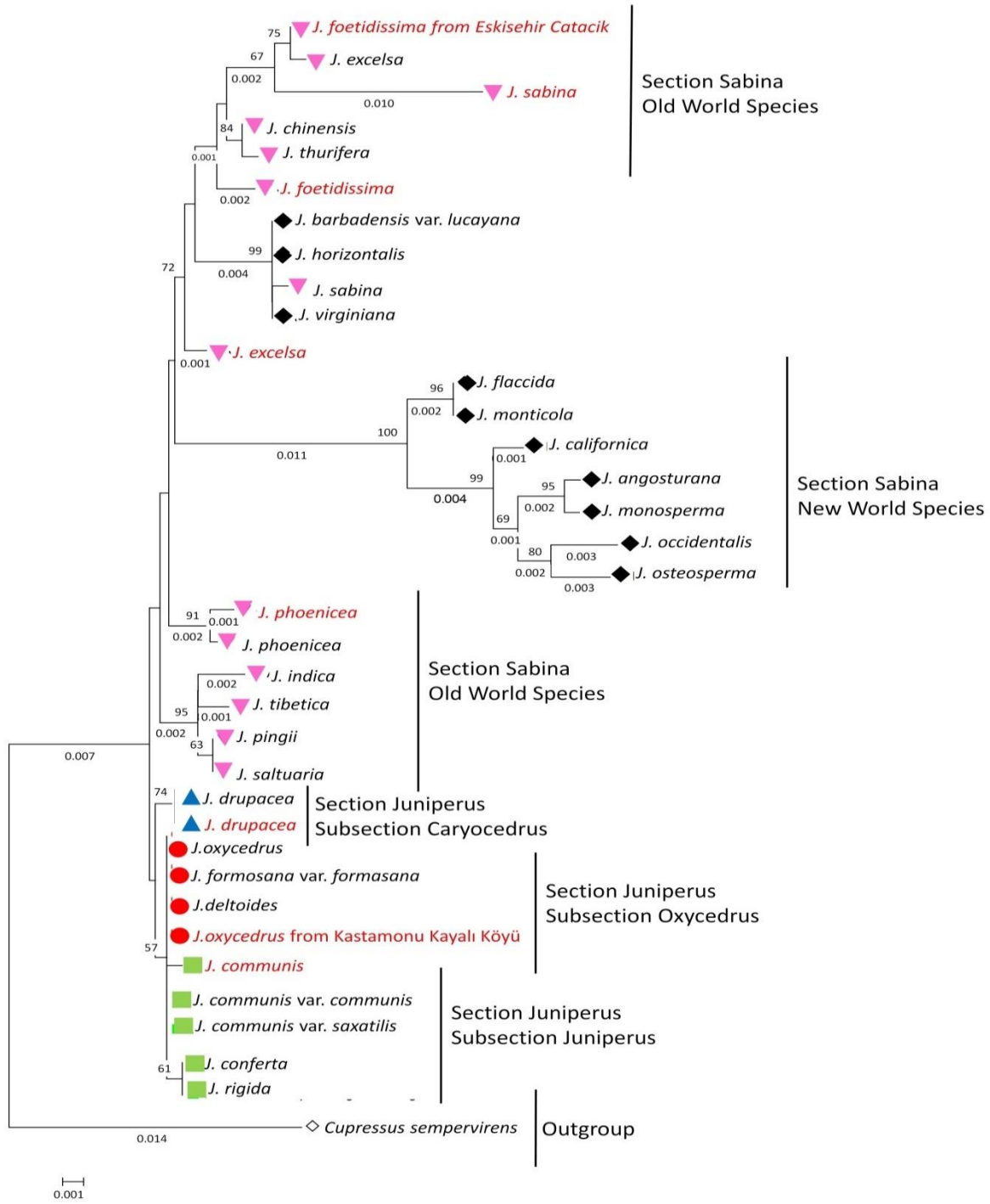

Figure 5.4. Phylogenetic tree of *Juniperus* L. based on *mat*K region with Neighbour Joining method. The percentage of trees (≥ 50) in which the associated taxa clustered together has been shown above the branches. Moreover, the tree has drawn to scale, with branch lengths (≥ 0.002) measured in the number of substitutions per site (below the branches). The taxa shown in red are the ones sampled in Turkey. Others (in black) were obtained from GenBank

CHAPTER 6

DISCUSSION

The cpDNA genes have very low rates of sequence divergence due to catalytic properties and formation of secondary structures (Kushel *et al*., 1990). Therefore they are more useful for evolutionary studies at higher taxonomic level (Taberlet, 1991).

6.1. Molecular Diversity Analysis of *Juniperus*

There were total 66 samples from Turkey with 329 bp *trn*L, 301 bp *trn*L-F and 524 bp *trn*V length. According to previous studies, *trn*L region varied from 316 to 330 bp, 200 to 315 bp in *trn*L – F region and from 517 to 525 bp in *trn*V region (Kusumi *et al*., 2000; Little, 2006; Mao *et al*., 2010; Opgenoorth *et al*., 2010; Rumeu *et al*., 2011). For *mat*K region, the length of the site ranged from 1416 to 1428 bp which were also in the range of previous studies (Gadek *et al*., 2000; Kusumi *et al*., 2000; Little, 2006; Fazekas *et al*., 2008; Mao *et al*., 2010; Bruni *et al*., 2012; De Mattia *et al*., 2012; Yang *et al*., 2012; Hong *et al*., 2014). For all studied regions, the variable sites were all parsimony informative and the highest variation was found in *mat*K region and *trnV* region of the *trn* regions. The gene diversity was relatively high for all genes. In all *Juniperus* species combined analyses, there were 49 parsimony informative sites in *trn* region and 82 sites in *mat*K region. Based on *mat*K region molecular diversity statistics showed correlation with previous study of Kusumi *et al*. (2000) such that the G/C content was about 33% in the published study and it was 32.4% in the current study. Furthermore, there are 6 indels with total 33 bp lengths. Transition / transversion ratio has been determine as 1.45 which has been detected in 1.74 in the study of Kusumi *et al*. (2000). When comparing the current results with

the same study for *trn*L region, G/C content has been found 34%. However, for Turkish *Juniperus* it has been determined as about 39 %. Moreover, the transition / tranversion ratio was also found more as 2.03. According to evolutionary divergence within each section, samples from Turkey and all members of the genus gave the similar results such that Section Sabina and Section Oxycedrus possessing the highest variation. For Turkey, Species of Section Sabina is composed of *J.foetidissima* Willd., *J.excelsa* M. Bieb., *J.sabina* L. and *J.phonicea* L. The section Juniperus Subsection Oxycedrus is composed of solely *J.oxycedrus* L. Although there is only one species exist in the current section for Turkey, the probable reason of this significant variation might be due to related with high rate of biogeographic distribution such that this species of *Juniperus* L. is widely distributed and native across the Mediterranean region from Morocco and Portugal, north to southern France, east to westernmost Iran, and south to Lebanon and Israel (Farjon, 2005). The regions where *J. oxycedrus* L. has been obtained in Turkey were Kastamonu, Kahramanmaraş, Manisa and Eskişehir provinces. According to some sources, *J.oxycedrus* was also seperated into several sepcies as *J.oxycedrus, J.navicularis* and *J.deltoides* (Adams, 2000) among which *J.oxycedrus* and *J.deltoides* are exist naturally in Turkey. Especially in Eskişehir province, another species *J.deltoides* was also exist. According to Adams *et al*. (2005), the morphological and genetically studies with ITS sequence genetic data is revealed similar results that two species are infact different from each other. Similarly, there are several subspecies of *J.oxycedrus* which are morphologically similar, but they are genetically different. This issue is called cryptic species, that is, species are showing similarity in morphology, but difference in genetics.

A deletion between 176^{th} – 189^{th} regions in *trn*L region of *J.oxycedrus* which was reported by other studies (Rumeu *et al*., 2011). Conversely, *J. oxycedrus* L. subsp. *macrocarpa* contained the insertion of TGGATTGGATACAA in the same region (Mao *et al*., 2010). Considering the variablity in section Sabina, the deletion between 209^{th} – 214^{th} bases has also been obtained in species from GeneBank (Mao *et al*., 2010). According to Mao *et al*. (2010), relationships among members of Section Sabina were mostly unresolved due to indels. This could be the reason for the variability of Turkish species as well. That might be also the reason why this section

is the most variable one for the comparisons made among Turkish species and whole genus.

In *trn*L-F region, there were two different haplotypes within species of *J. oxycedrus* L.. The population from Kastamonu province possessed deletion between 236th – 261st regions. This issue was also reported in *J. oxycedrus* L. subsp. *macrocarpa* from İzmir Çeşme and *J. macrocarpa* from GenBank. However, the insertion found in *J. macrocarpa* between 110th – 118th bp could not been observed in the population from Kastamonu province. This result might be due the fact, as Adams *et al*. (2005) indicated, cryptic speciation between *J. oxycedrus* L. and *J. macrocarpa*, which are morphologically almost identical but genetically, distinct species. Cryptic speciation as well as introgression between species were also suspected from the diversity found in the *trn*L-F region of subsection Oxycedrus.

6.1.1. Genetic Distances of *Juniperus*

According to distance between each taxon, the closest relationship has been identified between Section Juniperus Subsections Juniperus, Oxycedrus and Caryocedrus which contain species *J.communis* L., *J.oxycedrus* and *J.drupacea* Labill. from Turkey, respectively. Indeed Section Caryocedrus normally seperated from other sections but showed close relationship with Subsection Juniperus morphologically according to Mao *et al*. (2010). Although *J. drupacea* Labill. is restricted to the Mediterranean and generally used as a functional outgroups (Adams, 2000; Adams *et al*., 2003), *J.drupacea* Labill. and *J.communis* L. were both considered as blue seed cones and seperated from sebsection Oxycedrus which were classified as group with red - seed cones (Adams and Schwarzbach, 2012). The reliability of this result should be further explored by using several other markers as well by increasing the species number in section Juniperus. For all studied molecular regions, Section Sabina was clearly diverged from other *Juniperus* species (Adams *et al*., 2006, 2007; Mao *et al*., 2010; Adams and Schwarzbach, 2011, 2012). After including all *Juniperus*, New World species of Section Sabina showed the most divergent taxon. According to Adams (2011), species of Sabina form 5 different clades which contain both Old World and New World species. All the clades are paraphyletic to one another. Moreover, Maximum Parsimony analyses suggested that all three sections comprised a monophyletic group, with sections Juniperus and

Sabina sister to one another. Within section Sabina, almost all cpDNA clades were supported as monophyletic, but one clade (clade IV including the species *J. procumbens, J.chinensis, J. excelsa* and *J. procera*) was paraphyletic with respect to others (Mao *et al*., 2010).

At species level for almost all molecular regions, *J. phoenicea* L. showed the diverged relationship with other species. Adams and Schwarzbach (2012) reported that *J. phoenicea* L. is loosely associated with other members of Section Sabina. According to Boratynski *et al*. (2009), there have been high level of genetic differentiation in *J. phoenicea* L. in the Mediterranean region which implicated the geographic effect on species distinctness. Moreover, Adams and Schwarzbach (2012) indicated the geographic isolation on this species. Also, when Section Sabina were classified based on Adams (2011), 5 clades have been obtained. For Turkish *Juniperus, J. sabina* L. belongs to Clade III, *J. excelsa* M. Bieb. is included in clade IV and *J. phoenicea* L. is considered as the member of Clade V in which *J. phoenicea* L. is the only species in the group (Adams, 2011) and showed diverged relationship with other *Juniperus* (Mao *et al*., 2010).

6.2. Haplotype Frequency Analysis, AMOVA, Estimation of F_{st} Values and Divergence Times

According to studied gene regions, haplotype differences were observed in both intraspecific and interspecific levels. In *trn* regions, the interpopulational haplotype differences were found in *J. oxycedrus* L. and *J. foetidissima* Willd. such that *J. oxycedrus* L. from Kastamonu (Kayalı Köyü) showed the difference from other populations of *J. oxycedrus* L.. For *J. foetidissima* Willd., this difference has been between populations from Kahramanmaraş Tekir and Eskişehir Çatacık. Moreover, there were haplotype differences at intrapopulation level in *mat*K region of *J. foetidissima* Willd.. Also *J. oxycedrus* L. and *J.excelsa* M. Bieb. from Manisa Spil Dağı showed different haplotype composition. There were similar results obtained for Juniper species such that *J.przewalskii* possessed 6 haplotypes at *trn*T-F region (Zhang *et al*., 2005). *Juniperus osteosperma* also showed several different haplotype compositions (Terry *et al*., 2000). *J. sabina* L. from China (Guo *et al*., 2010), *J.brevifolia* from Azores island (Rumeu *et al*., 2011) and *J. oxycedrus* L. subsp.

macrocarpa from the Mediterranean region (Juan *et al*., 2012) showed different haplotype variations. The reason of these results might be commonly due to allopatric dispersion of the populations and recent colonization of the populations as a result of genetic drift (Slatkin, 1987). Moreover, a combined effect of cpDNA introgression and complex lineage sorting was inferred to explain the pattern of cpDNA variation (Widmer and Baltisberger, 1999). Combining this result with Fst values and AMOVA indicated that much of the variation were due to Sabina species. The significant ($P<0.05$) variation among sections suggests limited gene flow across them. This result has been detected in *trn* regions of both Turkish Junipers and all Junipers from GenBank. However, in terms of *mat*K region, much of the variation was seen within sections. The significant variation within sections and species supported by the strong differentiation of populations (Sertse *et al*., 2011). Anthropogenic gene transportation might also have contributed to the relatively high genetic diversity. The observed low diversity in a population suggests that anthropogenic activities leading to heavy population disturbances can affect the genetic composition of the species considerably. Anthropogenic activities therefore appear to be potential threats for the loss of genetic information particularly in spatially isolated small populations where genetic drift is possible. Gene flow from larger populations possibly enhances diversity in disturbed neighboring populations (Sertse *et al*., 2011). In Fst results (Table 5.14), the less differentiation was seen between Section Caryocedrus and Section Sabina Old World Species. Low variation among these sections could be caused by efficient gene flow (Pospiskova and Bartakova 2004).

Molecular Clock Estimation anaylsis revealed that New World species of Section Sabina diverged from other members of genus much earlier than other species of the genus. The most recent divergence time was found to be between subsection Juniperus and subsection Oxycedrus followed by subsection Juniperus and subsection Caryocedrus. Morever, divergence of *mat*K was more recent than that of *trn* region. The studies have shown that Section Sabina possesses pattern of geographic differentiation including the Himalaya and Tibetan Plateau, North America, the central Asia Europe, Africa and the Mediterranean. The fossil records for section Sabina date from the Eocene ⁄ Oligocene boundary (Kvacek, 2002) in Europe. It also dates from the late Oligocene to early Miocene in North America

(Axelrod, 1956, 1987, 1991; Wolfe, 1964), These are related with the hypothesis that *Juniperus* was the part of Madrean-Tethyan vegetation belts (Axelrod, 1975) by the late Oligocene. Therefore they should have dispersed from one side to the other. Section Caryocedrus is restricted to the eastern Mediterranean region. *J. drupacea* Labill., the only species in this section, was probably differentiated about 20 million years ago (Adams, 2011). Sect. Juniperus like sect. Caryocedrus is not known from the fossil record in North America. Only it appears in Europe and Asia from the middle Miocene onwards (Straus, 1952; Negru, 1972).

6.3. Construction of Phylogenetic Trees

Phylogenetic tree with NJ tree topology showed that some of the species made clusters with respect to *trn* regions, but some had diverged allocation in the tree. As previously stated *J. foetidissima* Willd., *J. excelsa* M. Bieb., *J. sabina* L., and *J. phoenicea* L. belong to Old World Species of Section Sabina. The current phylogenetic tree showed that Turkish *J.excelsa* M. Bieb., *J. sabina* L. and *J. phoenicea* samples made a sister relationship as expected in terms of *trn* region. However, populations of *J.foetidissima* Willd. from Eskişehir Çatacık revealed a different allocation in the tree and gave close relationship with Section Juniperus. Recently, documentation of intraspecific variation in cpDNA has become increasingly common. It has been considered as ‘‘chloroplast capture’’ following genetic exchange across species boundaries (Mason-Gamer, *et al*., 1995; Bain and Jansen, 1996). There were more than 100 cases of intraspecific variation in cpDNA possibly due to hybridization and introgression (Rieseberg and Wendel, 1993; Rieseberg 1995). The intraspecific variation in cpDNA is potentially indicative of hybridization between species. (Soltis, *et al*., 1992). Additional support for introgression has been provided by concordance in the geographic distribution and relationships suggested by independently evolving characters (e.g., cpDNA, nuclear ribosomal DNA, and morphology) (Wendel and Albert, 1992; Rieseberg, 1995; Rieseberg *et al*., 1996). For example, Terry *et al*. (2000) analyzed the introgression between *J.osteosperma* and *J.occidentalis* based on *trn*L and *trn*L-F regions and indicated three possible hypothesis for the cpDNA haplotype variation: (1) ancestral polymorphism inheritance; (2) intraspecific polymorphism; and (3) hybridization. Ancestral polymorphism is least likely because the members of *J.foetidissima* Willd. did not diverged considerably to form sister groupings. Other possible explanation

for this difference is due to mutation and formation of different cpDNA haplotypes. However, studying the cpDNA alone is not ehough for the presence of mutation and haplotype polymorphism. Gene flow between distinct lineages might be supported for this study due to the fact that biogeographic distribution of the population of *J. foetidissima* Willd. with other *Juniperus* in the same location (*J.oxycedrus* from Eskisehir Catacik) might have been provided the hypothesis. This geographic pattern in genetic variation would be expected if cytoplasmic introgression with *J. oxycedrus* L. which has also been obtanied in Eskişehir Çatacık. Unique, but similar mutation with *J.oxycedrus* in *trn*V region might have provided this difference.

Members of *J.oxycedrus* from Kastamonu Kayalı Köyü showed divergence from other *J.oxycedrus* populations in Turkey. Especially, the insertion of 26 bp occurred in *trn*L-F which was also present in *J.oxycedrus* and *J.deltoides* from GenBank. Previous studies of other species of Cupressaceae (Neale *et al*. 1989, 1991; Mogensen 1996; Kondo *et al*. 1998; Hwang *et al*. 2003) show that cpDNA is paternally inherited in members of this family. According to Zhang *et al*. (2005) if the same is true for *Juniperus*, population differentiation for cpDNA would be less indicative than if the genome were maternally inherited, assuming that pollens in the species are dispersed more widely than seeds (Ennos *et al*. 1999). Moreover, early studies of evolutionary change in chloroplast DNA (cpDNA) indicated limited variability within species (Neale *et al*., 1986; Birky, 1988). This finding was attributed to low rates of sequence evolution. However, documentation of intraspecific variation in cpDNA has become increasingly common and attributed in many cases (Terry *et al*., 2000). As a result, it could be concluded that there might be some intraspecific variation. This might be due to geographic barriers within species. Moreover, it is also possible that pollen, even in the absence of such barriers, is not naturally dispersed far in *Juniperus* L. (Zhang *et al*., 2005). More probably these two populations of *J.oxycedrus* L. were dispersed previously and became fixed and reproductively isolated in certain places. For the convenience of this result more molecular region should and will be included.

Furthermore, *J. drupacea* Labill. which is considered as a functional outgroup, showed a close relationship with *J. excelsa* M. Bieb.. When the nucleotide differences were analyzed, except for couple of differences of *J.drupacea* Labill., both species showed very little nucleotide divergences. Normally, if such an issue is

the case in sympatric populations, it might be tought as a probable introgression which is observed in Juniper species (Terry *et al*., 2000). However, the sampled populations are kilometers away far from each other. Hence, the remaining possible explanation for this closeness might be due to inheritance of ancestral polymorphism and intraspecific convergent polymorphism. The former indeed may not be the case because if it were so, there would also be intraspecific variation. However, for these two species there were one haplotype for each. The latter case is related with convergent evolution which might occur either through parallel mutation or differential homogenization and concerted evolution (Jorgensen and Cluster, 1988). Hence there may be a convergent evolution of *J. drupacea* Labill. and *J. excelsa* M. Bieb. with respect to *trn* regions. Within section Juniperus, almost all subsections seems to be monophyletic, although relationships between sections were not clearly resolved. Section Juniperus comprised 2-3 subclades in which one was clearly composed of Subsection Oxycedrus. According to Adams (2011) subsection Oxycedrus was corresponding to the red seed cone groups which are different from blue seed cones that comprised *J. communis* L. and *J. drupacea* Labill.. After including *Juniperus* sequence from database, the separation was mainly still based upon section level. In *trn* regions subsections of section Juniperus were diverged properly. However, the members of Section Sabina showed relatively dispersed allocation in the phylogenetic tree. Especially, New World species of the section showed divergence patterns. As indicated before, the evolutionary classification of this section is still far from being clear (Adams, 2011).

Considering *mat*K region, according to Fazekas *et al*. (2008) *mat*K region is a suitable barcoding region for the plants. It is a good DNA barcode region because of its rapid evolution (Hilu and Liang, 1997). Indeed, the phylogenetic tree in terms of *mat*K region gave good resolution for the members of section Juniperus. However, as in *trn* region, the members of Section Sabina did not give clear divergence. The Old World and New World species unresolved classification including Turkish Junipers. As recently discussed at the Fourth International Barcode of Life Conference (www.dnabarcodes2011.org), the *matK* amplification system requires some improvements (i.e. the definition of clade-specific primers (which has been performed during the study), or the identification of universal combinations of primers), in order to be effective when applied as a universal DNA barcode region

for plants (Bruni *et al*., 2012; DeMattia *et al*., 2012). In general, if there is a number of closely related species present, the combination of for example *rbcL+matK* is more effective in identifying plant species. The results were much more reasonable than trn region such that Section Sabina Old World Species including Turkish Junipers made clustering on the tree. *J. foetidissima* Willd., *J. excelsa* M. Bieb. and *J.sabina* L. from Turkey were separated with good resolution but *J. phoenicea* L. diverged differently. The reason might be due to the fact that it is a tree native to coastal sites of Mediterranean and distributed throughout a narrow range with scattered populations (Meloni *et al*., 2005). This is reflected also in the many uncertainties about the presence of intraspecific taxa, based upon morphological (Gaussen, 1968), biochemical (LeBreton and Thivend, 1981) and molecular (Adams et al., 2002) evidence. According to study of Boratynski *et al.* (2009), there are the high levels of differences and a long period of isolation at even population level of *J. phoenicea* L.. This state was also previously proposed by Lebreton and Rivera (1989). Hence, the different divergence of *J. phoenicea* L. was probably due to the geographical isolation. Considering New World Species of section Sabina, altough there were some unexpected divergences, most members of the section showed reliable clusters with 100 % bootstrap value. At section Juniperus, the subsections made groupings and subsection Caryocedrus gave sister relationship with subsection Juniperus and Oxycedrus.

As a result of phylogenetic tree analyses with *trn* and *mat*K regions, it is obvious that the members of section Juniperus gave expected results despite some differences at population level. However, the species from Section Sabina (especially the New World Species) are needed to be further studied to get better resolution.

CHAPTER 7

CONCLUSION

In accordance with the aim of this study, three non-coding regions of *trn* and *mat*K regions of cpDNA have been utilized and the magnitude and pattern of molecular diversity of *Juniperus* species in Turkey and their evolutionary relationships with other *Juniperus* L. have been investigated.

The constructed molecular phylogenetic trees revealed the phylogenetic relationships among Juniper species of Turkey. Accordingly, the divergence of section Juniperus is obvious such that the members of the section showed expected pattern in phylogenetic analysis. Main clusters were composed of Section Juniperus and Section Sabina. *J. drupacea* gave close relationship with subsection Juniperus and Oxycedrus. Among Turkish Section Sabina species, the highest divergence was obtained in *J. phoenicea*.

The obtained intraspecific variation in *J. oxycedrus* L. and *J. foetidissima* Willd. was probably due to the geographic isolation and gene flow between different species of Juniperus.

The members of Section Sabina should be further studied by using several other molecular regions because some samples obtained both from Turkey and from database did not show clear resolution and some species diverged unexpectedly. The main reason for these results may be due to recent colonization of the populations and anthropogenic effects. Moreover, the effect of geographic isolation through seed and limitedpollen dispersal might cause to the formation of different haplotypes

section, species and even population level. This distant relation was revealed as a result of both indels and nucleotide substitutions through the sequenced DNA.
Moreover, the highest diversity was also obtained in this section for both *trn* and *mat*K regions.

In the current study, indels in the DNA sequence of each studied region have been obtained with different frequency. There was a consistency between the numbers of inserted or deleted nucleotides at the section and species level. Thus these cpDNA regions could be used for separation of one section from the others. Therefore, each of the studied region was useful to construct phylogenetic relations, which had high quality of resolution at the section level.

To understand evolutionary relationships between Turkish Junipers and the species from other regions of the world, the DNA sequences of studied regions of cpDNA were gathered from GenBank and were evaluated together. The New World species of Section Sabina scattered in the phylogenetic tree. New World Section Sabina group were nested within a different subcluster, which was located in the main clade produced by samples of Old World Section Sabina samples.

The molecular clock analysis also showed that the New World Species of section Sabina diverged from other members of the genus about 15 - 20 million years ago. This indicated the fact that New World species evolved from other species of genus *Juniperus* and differentiated in a distinct manner.

Additional taxa and sequences from other useful DNA regions of cpDNA regions may provide further insights to understand phylogenetic relationships among Juniperus species not only at the section, but also at the species level.

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APPENDIX A

GENBANK ACCESSION NUMBERS OF SAMPLES OBTAINED FOR MOLECULAR DIVERSITY ANALYSIS

Table A.1 GenBank accession numbers of *trn* regions

GeneBank Accession No	**Species Name**	**Location**	**Authors**
HM024549	*Juniperus angosturana*	Mexico	Mao and Liu, 2010
HM024550	*Juniperus ashei*	USA	
HM024575	*Juniperus barbadensis var.lucayana*	Jamaica	Mao and Liu, 2010
HM024551	*Juniperus bermudiana*	Bermuda	
JF950948	*Juinperus brevifolia*	Portugal	Rumeu *et al*., 2011
HM024552	*Juniperus blancoi*	Mexico	Mao & Liu, 2010
HM024553	*Juniperus californica*	USA	
HM024554	*Juniperus chinensis*	China	
HM024555	*Juniperus coahuilensis var. arizonica*	Mexico	
HM024557	*Juniperus communis* L. *var. communis* L.	France	
HM024559	*Juniperus communis* L. *var. saxatilis*	Pakistan	
HM024556	*Juniperus comitana*	Mexico	
HM024591	*Juniperus conferta*	Japan	
HM024560	*Juniperus convallium*	China	
HM024561	*Juniperus deltoides*	Turkey	

Table A.1 (Cont'd) GenBank accession numbers of *trn* regions

HM024563	*Juniperus drupacea* Labill.	Greece	Mao & Liu, 2010
HM024565	*Juniperus excelsa* M. Bieb.	Turkey	
HM024566	*Juniperus flaccida*	Mexico	
HM024567	*Juniperus formosana var formosana*	China	
HM024568	*Juniperus formosana var mairei*	China	
HM024569	*Juniperus gamboana*	Mexico	
HM024570	*Juniperus gaussenii*	China	
HM024571	*Juniperus gracilior*	Dominican Republic	
AY988222	*Juniperus indica*	Himalaya	Little, 2006
HM024572	*Juniperus horizontalis*	Canada	Mao & Liu, 2010
HM024574	*Juniperus komarovii*	China	
HM024576	*Juniperus microsperma*	China	
HM024577	*Juniperus monosperma*	USA	
HM594864	*Juniperus macrocarpa*	Spain	Juan *et al*., 2011
HM024578	*Juniperus monticola*		Mao & Liu, 2010
JF950972	*Juniperus navicularis*	Portugal	Rumeu *et al*., 2011
HM024580	*Juniperus osteosperma*	USA	Mao & Liu, 2010
HM024579	*Juniperus occidentalis*	USA	
JF950981	*Juniperus oxycedrus* L. *var. badia*	Mediterrenean	Rumeu *et al*., 2011
JF950985	*Juinperus oxycedrus var. oxycedrus*		
HM024582	*Juniperus phoenicea* L.	France	Mao & Liu, 2010
HM024583	*Juniperus pinchotii*	USA	

Table A.1 (Cont'd) GenBank accession numbers of *trn* regions

HM024584	*Juniperus pingii*	China	Mao & Liu, 2010
HM024585	*Juniperus polycarpos*	Pakistan	
HM024586	*Juniperus procera*	Ethiopia	
HM024587	*Juniperus procumbens*	Japan	
HM024588	*Juniperus przewalskii*	China	
AB029868	*Juniperus rigida*		Kusumi *et al*., 2000
HM024593	*Juniperus sabina* L. *var. arenaria*	China	Mao & Liu, 2010
HM024596	*Juniperus saltillensis*	Mexico	
HM024597	*Juniperus saltuaria*	China	
HM024600	*Juniperus semiglobosa*	China	
HM024601	*Juniperus squamata*	China	
HM024616	*Juniperus tibetica*	China	
HM024603	*Juniperus thurifera*	Spain	
HM024605	*Juniperus virginiana*	USA	
HM023899	*Cupressus sempervirens*	Croatia	

Table A.2 GenBank accession numbers of *mat*K regions

GeneBank Accession No	**Species Name**	**Location**	**Authors**
HM024009	*Juniperus angosturana*	Mexico	Mao *et al*., 2010
HM024010	*Juinperus ashei*	USA	
HM024035	*Juniperus barbadensis var. lucayana*	Jamaica	
HM024011	*Juniperus bermudiana*	Bermuda	
HM024012	*Juniperus blancoi*	Mexico	
HM024013	*Juniperus californica*	USA	
HQ245896	*Juniperus chinensis*	China	Yang *et al*., 2012
HM024015	*Juniperus coahuilensis*	Mexico	Mao *et al*., 2010
HM024017	*Juniperus communis* L. *var. communis* L.	France	
HM024019	*Juniperus communis* L. *var. saxatilis*	China	
HM024016	*Juniperus comitana*	Mexico	
HM024051	*Juniperus conferta*	Japan	
HM024020	*Juniperus convallium*	China	
HM024050	*Juniperus coxii*	China	
HM024021	*Juniperus deltoides*	Turkey	
HM024022	*Juniperus deppeana*		
HM024024	*Juniperus durangensis*	Mexico	
HM024023	*Juniperus drupacea* Labill.	Greece	
HM024024	*Juniperus excelsa* M. Bieb.	Turkey	
HM024026	*Juniperus flaccida*	Mexico	
HM024027	*Juniperus formosana var formosana*	China	
HM024028	*Juniperus formosana var mairei*	China	

Table A.2 (Cont'd) GenBank accession numbers of *mat*K regions

HM024029	*Juniperus gamboana*	Mexico	Mao *et al*., 2010
HM024030	*Juniperus gaussenii*	China	
HM024031	*Juniperus gracilior*	Dominician Republic	
HM024032	*Juniperus horizontalis*	Canada	
HM024033	*Juniperus indica*	Nepal	
HM024034	*Juniperus komarovii*	China	
HM024036	*Juniperus microsperma*	China	
HM024037	*Juniperus monosperma*	USA	
HM024038	*Juniperus monticola*		
HM024039	*Juniperus occidentalis*	USA	
HM024040	*Juniperus osteosperma*	USA	
HM024041	*Juinperus oxycedrus*	France	
HM024042	*Juniperus phoenicea* L.	France	
HM024043	*Juniperus pinchotii*	USA	
HM024044	*Juniperus pingii*	China	
HM024045	*Juniperus polycarpos*	Pakistan	
HM024046	*Juniperus procera*	Ethiopia	
HM024047	*Juniperus procumbens*	Japan	
HM024048	*Juniperus przewalskii*	China	
HM024049	*Juniperus pseudosabina*	China	
HM024052	*Juniperus rigida var.rigida*	Japan	
HM024051	*Juniperus rigida var.conferta*	Japan	
HM024054	*Juniperus sabina* L. *var. davurica*	China	

Table A.2 (Cont'd) GenBank accession numbers of *mat*K regions

HM024053	*Juniperus sabina* L. *var. arenaria*	China	Mao *et al*., 2010
HM024056	*Juniperus saltillensis*	Mexico	
HM024057	*Juniperus saltuaria*	China	
HM024060	*Juniperus semiglobosa*	China	
HM024059	*Juniperus scopulorum*	USA	
HM024061	*Juniperus squamata*	China	
HM024064	*Juniperus tibetica*	China	
HM024063	*Juniperus thurifera*	Spain	
HM024065	*Juniperus virginiana*	USA	
HM023994	*Cupressus sempervirens*	Croatia	

APPENDIX B

CHROMATOGRAM STRUCTURES FOR EACH STUDIED REGIONS

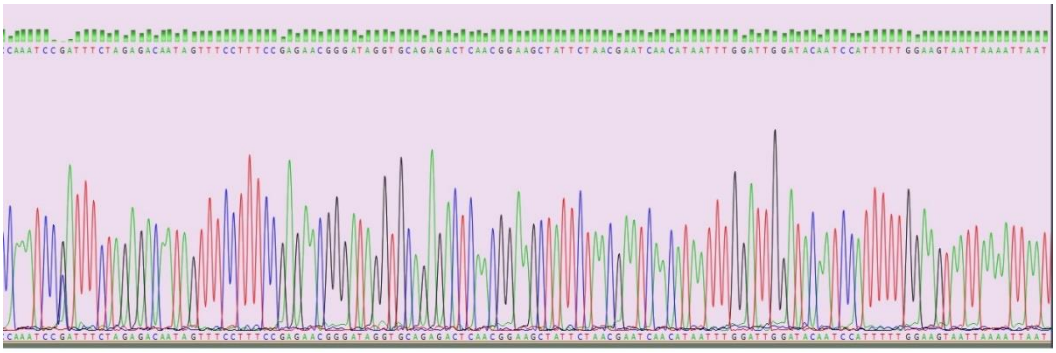

Figure B.1 An example of chromatogram for *trnL5'-L3'* (*trnL* intron)

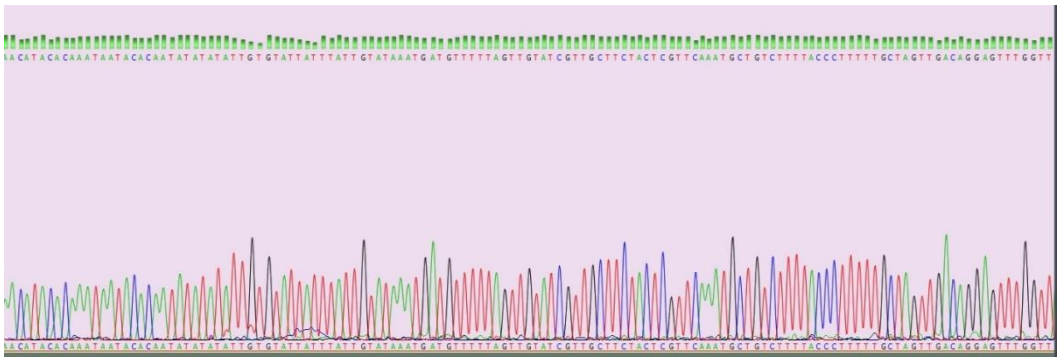

Figure B.2 An example of chromatogram for *trnL3'-F*$^{(GAA)}$ (*trnL-F* intergenic spacer)

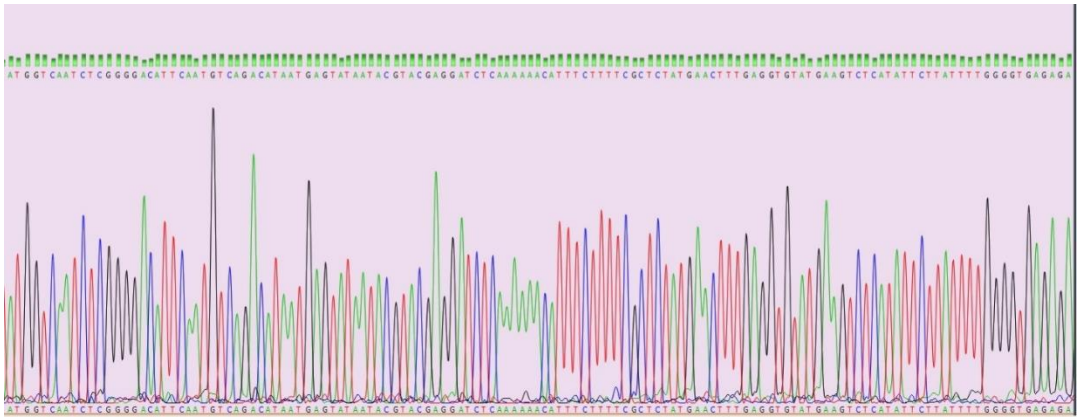

Figure B.3 An example of chromatogram for *trnV* intron

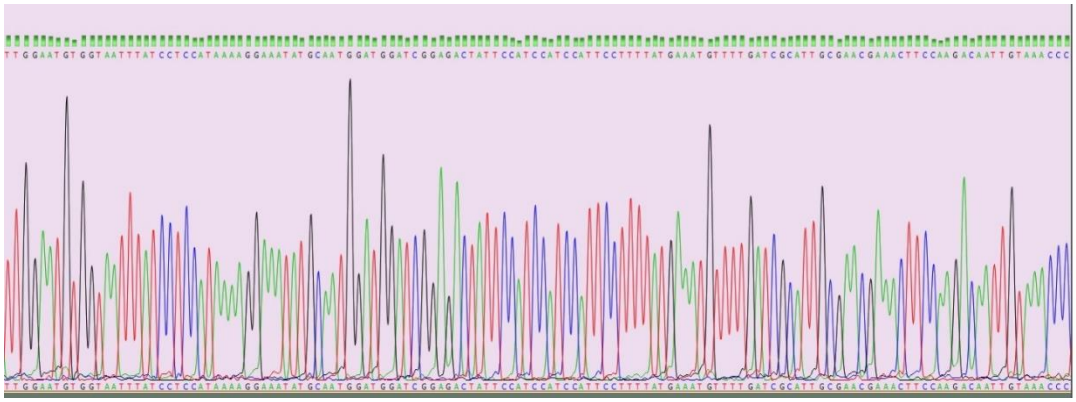

Figure B.4 An example of chromatogram for *matK (Maturase Kinase)*

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Education

Degree	Department / Program	University	Year
Bachelor	Faculty of Art and Science / Biological Sciences	Middle East Technical University	2006
Master of Science	Faculty of Art and Science / Biological Sciences	Middle East Technical University	2006 – 2009
Doctorate	Faculty of Art and Science / Biological Sciences	Middle East Technical University	2009 – 2015

Master of Science Thesis Title and Supervisor

Thesis Title: The Phylogenetic Analysis of *Pinus nigra* Arnold Subspecies *pallasiana* varieties with respect to noncoding *trn* Regions of Chloroplast Genome
Supervisor: Prof. Dr. Zeki KAYA

Doctorate Thesis Title and Supervisor

Thesis Title: Molecular Phylogenetic Analyses of *Juniperus* Species in Turkey and Their Relations with Other *Juniperus* based on cpDNA

Supervisor: Prof. Dr. Zeki KAYA

Academic and Professional Experience

Position	Place	Year
Research Assistant	Faculty of Art and Science / Biological Sciences Middle East Technical University	2006-2013
Deputy Expert	The Ministry of Forestry and Water Affairs, General Directorate of Nature Conservation and National Parks, Department of Biological Diversity	2013 –

Language Skills

English (Advance)

French (Upper Intermediate)

Spanish (Basic)

Computer Skills

- DNA and protein database analyses programmes
- MEGA 5.2
- Arlequin 3.5.
- POPGENE v1.31
- GDA 1.1 Genetic Data Analysis Software
- BioBayes v1.3
- mrbayes 3.1.2

- RAMAS Metapop v5 and Ecolab v2.0
- Web – based Database Programmes (NCBI, ENSEMBL, UCSC etc.)
- Statistical Packages (SPSS, R, Minitab, Excel Macro)
- Windows and Mac Operating system and Office Programmes
- Object Oriented Programming
- NTsys
- arcGIS v10 (Esri's Geographic Information System)
- Patch Analyst statistical package

Positions in Projects

1. The effect of *Hyperikum perforatum* and the effect of antidepressants on nicotine usage by using laboratory mouse, Gulhane Military Medical Academy, **Internship**, Completed, 2005.
2. The Phylogenetic Analysis of *Pinus nigra* Arnold Subspecies *pallasiana* varieties with respect to Noncodıng *trn* Regions of Chloroplast Genome, Scientific Research Fund of Middle East Technical University, (Project of Master of Science) **Researcher**, Completed, 2009.
3. The Phylogenetic Analysis of *Picea orientalis* (Oriental Spruce) Populations form Noertheastern Turkey with respect to Non – coding *trn* and *mat*K Regions of Chloroplast Genome, TUBITAK Project, TOVAG-107O684 and Scientific Research Fund of Middle East Technical University, **Researcher,** Completed, 2011.
4. The Evolutionary Relationship of Oak Species in Turkey based on *mat*K Region of cpDNA and *ITS* Region of Nuclear Genome. TUBITAK Project TOVAG-108O723, **Assistant Researcher**, Completed, 2012.
5. Molecular Phylogenetic Analyses of *Juniperus* Species in Turkey and Their Relations with Other *Juniperus* based on cpDNA, Scientific Research Fund of Middle East Technical University, (Doctorate Thesis Project), **Researcher,** Completed, 2009 – 2013.

6. Genetic Characterization of Turkish Black Poplar Populations – The Genetical Sources and Development of Black Poplar Breeding Programme, TUBITAK Project TOVAG 110O570, **Asistant Researcher**, Ongoing, 2012 – .
7. The Revision of *Scrophularia* L. Genus (Scrophulariaceae) in Turkey, TUBITAK Project, 112T140, **Asistant Researcher**, Ongoing, 2013 – .
8. National Biological Diversity Inventory and Monitoring Project, The Ministry of Forestry and Water Affairs, General Directorate of Nature Conservation and National Parks, Department of Biological Diversity, **Depuity Expert**, Ongoing, 2014 – .
9. Project for Determination of Plant Species to be Submerged Under The Dam Reservoir, The Ministry of Forestry and Water Affairs, General Directorate of Nature Conservation and National Parks, Department of Biological Diversity, **Depuity Expert**, Ongoing, 2014 – .

Awards

- 2002 – 2003 Presidency High Honour (as a top student)
- 2003 – 2004 Presidency High Honour (as a top student)
- 2004 – 2005 Presidency High Honour (as a top student)
- 2005 – 2006 Presidency High Honour and Graduated (as a second best student)

Publications and Preprints

A. <u>Papers Published in or Prepared for International Journals:</u>

A1. Gülsoy, A. D., Gülsoy, A. M., Çengel, B. ve Kaya, Z. 2014. The Evolutionary divergence of *Pinus nigra* subspecies *pallasiana* and its varieties based on non-coding *trn* regions of chloroplast genome. Turk.J.Bot. 38: 627 – 636.

A2. Gülsoy, A. M., Temel, F., Gülsoy, A. D., ve Kaya, Z. 2012. Evolutionary divergence of *Picea orientalis* with respect to non-coding *trn* and *mat*K regions of chloroplast genome. Turk. J. Bot. (Presented to Journal).

A3. Gülsoy, A.D., Gülsoy, A.M., Duman, H., ve Kaya, Z. 2012. Molecular phylogenetic analysis of Turkish oak species based on nuclear internal transcribed spacer (*ITS*) region. (In Preparation)

A4. Gülsoy, A.D., Gülsoy, A.M., Duman, H. ve Kaya, Z. 2012. Molecular phylogenetic analysis of Turkish oak species based on maturase K (*mat*K) region of chloroplast genome (In Preparation)

A5. Gülsoy, A.D., Duman, H. ve Kaya, Z. 2012. Molecular phylogenetic analysis of Turkish *Juniperus* species based on maturase K (*mat*K) and *trn* regions of chloroplast genome (In Preparation)

A6. Ulusal Biyolojik Çeşitlilik İzleme ve Değerlendirme Raporu 2014-2015. The Ministry of Forestry and Water Affairs, General Directorate of Nature Conservation and National Parks (In Preparation)

B. Posters and Oral Presentations:

B1. Gülsoy, A.D., Gülsoy A.M., Çengel B. ve Kaya Z. 2009. The Evolutionary Divergence of *Pinus nigra* Arnold Subspecies *pallasiana* Varieties Based On Non-Coding *trn* Regions Of Chloroplast Genome. In: International Symposium on Health Informatics and Bioinformatics, Nisan, 2009, Ankara Türkiye

B2. Gülsoy, A.M., Temel, F., **Gülsoy, A.D.**, ve Kaya, Z.2010.The phylogenetic analysis of *Picea orientalis* populations from northeastern Turkey with respect to non-coding *trn* regions of chloroplast genome. In: International Symposium on Biology of Rare and Endemic Plant Species (BIORARE-2010)-Biyoinformatik Çalıştayı, Mayıs 26-29, 2010, Fethiye, Muğla, Türkiye PP22, P. 71.

B3. Gülsoy, A.D., Gülsoy, A.M., Çengel, B., Şiklar, S. ve Kaya, Z.2010. The phylogenetic analysis of *Pinus nigra* arnold subspecies *pallasiana* varieties with respect to non-coding *trn* regions of chloroplast genome. In: International Symposium on Biology of Rare and Endemic Plant Species (BIORARE-2010)-Bioinformatic Workshop, Mayıs 26-29, 2010, Fethiye, Muğla, Türkiye, OPWII3, P. 47.

B4. Gülsoy, A.D., Gülsoy, A.M., Duman, H., ve Kaya, Z. 2012. Molecular phylogenetic analysis of Turkish oak species based on nuclear internal transcribed spacer (*ITS*) region. The Second International Symposium on the Biology of Rare and Endemic Plant Species, Nisan 24-27, 2012, Fethiye, Muğla-Turkey, P. 5

B5. Gülsoy, A.D., Gülsoy, A.M., Duman, H. ve Kaya, Z. 2012. Molecular phylogenetic analysis of Turkish oak species based on maturase K (*mat*K) region of chloroplast genome.The Second International Symposium on the Biology of Rare and Endemic Plant Species, Nisan 24-27, 2012, Fethiye, Muğla-Türkiye, P. 38

B6. Gülsoy, A.D., Temel, F., Gülsoy, A.M., ve Kaya, Z. 2012. Molecular Phylogeny Of *Juniperus* Species In Turkey Based On Non-Coding *trn* Region Of cpDNA. The Second International Symposium on the Biology of Rare and Endemic Plant Species, Nisan 24-27, 2012, Fethiye, Muğla-Türkiye, P. 38

B7. Gülsoy, A.D., Dizkirici, A., Gülsoy, A.M., Kansu, Ç., Duman, H., ve Kaya, Z. 2012. Genetics of Turkish oaks: Importance of conservation. The Second International Symposium on the Biology of Rare and Endemic Plant Species, Nisan 24-27, 2012, Fethiye Muğla-Turkey, P. 1.

B8. Kaya, Z., **Gülsoy, A.D.**, Gulsoy, M. ve Duman, H. 2012. The Molecular Phylogeny of Turkish Oaks from the Cerris section of *Quercus* genus, The 21st Biodiversity and Evolution International Symposium by DBG, 16-20 Eylül 2012, Mainz, Almanya , P 31, P 115.

B9. Kaya, Z., **Gülsoy, A.D.**, Gülsoy, M. ve Duman, H. 2012. The Molecular Phylogeny of Turkish Oaks from the Cerris section of *Quercus* genus, IUFRO-Genetics of Fagaceae and Nothofagaceae, Ekim 9 - 12, 2012, Bordeaux, Fransa

B10. Kaya, Z., **Gulsoy, A.D.**, Ulug, A., Wegrzyn, J. ve Neale, D. 2013. SNP Diversity of Candidate Genes Encoding Cellulose and Lignin Biosynthetic Enzymes in Populus nigra Clone Bank in Turkey: Its Implications for Conservation and Breeding. XXI. Plant and Animal Genome Conference, Ocak 12-16, 2013, San Diego, CA, USA PO760, p255.

C. Translated International Books or Chapters in the Books:

C1. Simpson, M. G. 2012. Plant Systematics Second Edition. Translated: **Gülsoy, A.D.** and Kaya Z. Chapter 19: Bitki Sistematiğinde Türler ve Koruma pp.649-668.

C2. Pevzner, P. ve Shamir, R. 2011. Bioinfomatics For Biologists First Edition. Translated: **Gülsoy, A.D.** and Kaya, Z. Chapter 12: Hadas-Libeskind, R. Figs, Wasps, Gophers and Lice: A Computational Exploration of Coevolution