Kastamonu Uni., Orman Fakültesi Dergisi, 2021, 21(1): 41-54 Research Article
Kastamonu Univ., Journal of Forestry Faculty
Doi:10.17475/kastorman.908630

Genetic Diversity Analysis of cpDNA in Turkish Abies Taxa

Yasemin CELIK ALTUNOGLU[1], Kerim GÜNEY[2*], Pinar BALOGLU[3], Mehmet Cengiz BALOGLU[1]

[1]Kastamonu University- Faculty of Engineering and Architecture- Department of Genetics and Bioengineering, Kastamonu, TURKEY
[2]Kastamonu University - Faculty of Forestry, Department of Forest Engineering- Kastamonu, TURKEY
[3]Kastamonu University - Research and Application Center, Kastamonu, TURKEY
[*]Corresponding Author: kguney@kastamonu.edu.tr

Received Date: 12.04.2020 Accepted Date: 01.03.2021

Abstract

Aim of study: Five Abies taxa naturally distributed in Turkey. *Abies nordmanniana* has three subspecies and *A. cilica* has two subspecies. In this study, we aimed to show phylogenetic relationships both in Turkish taxa and in other Abies taxa from around the world based on cpDNA regions, *trnR-trnN* and *rps18-rpl20* regions.

Material and methods: Following CTAB-based DNA isolation method, the relevant fir DNA regions were amplified and sequenced. Phylogenetic trees were constructed using maximum likelihood method with 1000 bootstrap replicates.

Main results: It was difficult to make distinctions among the Turkish *Abies* taxa based on the sequenced DNA regions. Based on *rps18-rpl20* phylogenetic tree, some members of *Abies cilicica* subsp. *isaurica, A. nordmanniana* subsp. *equi-trojani* and *A. cilicica* subsp. *cilicica* were in the same clade with *A. spectabilis* and *A. densa;* However, some members of *A. nordmanniana* subsp. *bornmuelleriana, A. cilicica* subsp. *isaurica* and *A. nordmanniana* subsp. *equi-trojani* were placed in a clade with *A. alba* placed near Turkey and *A. amabilis* known from North America.

Highlights: This study provides new insights into the distribution of cpDNA variation in *Abies* species in Turkey and the genetic variation between firs in Turkey and the rest of the world.

Keywords: Fir, Molecular Taxonomy, Chloroplast DNA Region

Türk Göknar Taksonlarında Genetik Çeşitlilik Analizleri

Öz

Çalışmanın amacı: Beş Abies taksonu Türkiye'de doğal olarak dağılmıştır. *Abies nordmanniana*' nın üç alt türü ve *A. cilica* 'nın iki alt türü vardır. Bu çalışmada, cpDNA bölgeleri, *trnR-trnN* ve *rps18-rpl20* bölgelerine göre hem Türk taksonlarında hem de dünyanın diğer Abies taksonlarında filogenetik ilişkileri göstermeyi amaçladık.

Materyal ve yöntem: CTAB metodu ile DNA izolasyonunun ardından ilgili göknar DNA bölgeleri çoğaltılmış ve dizilenmiştir. Filogenetik ağaçlar, 1000 tekrarlı maximum likelihood (maksimum olabilirlik) yöntemi kullanılarak oluşturulmuştur.

Temel sonuçlar: Türkiye'deki göknar taksonları arasında bu bölgeler bakımından ayrım yapmak zor gözükmektedir. *rps18-rpl20* dizileri temelindeki filogenetik ağaca dayanarak, bazı *Abies cilicica* subsp. *isaurica, A. nordmanniana* subsp. *equi-trojani* ve *A. cilicica* subsp. *cilicica* üyeleri *A. spectabilis* ve *A. densa* ile aynı sınıfta yer alırken, bazı *A. nordmanniana* subsp. *bornmulleriana, A. cilicica* subsp. *isaurica* ve *A. nordmanniana* subsp. *equi-trojani* üyeleri ise Türkiye'ye yakın yayılış gösteren *A. alba* ve Kuzey Amerika'da yayılışı bilinen *A. amabilis* ile aynı sınıfta yer almıştır.

Araştırma Vurguları: Mevcut çalışma, Türkiye'deki göknar türleri arasında cpDNA çeşitliliğinin dağılımına ve Türkiye göknarları ile dünyada yayılış gösteren diğer göknar türleri arasındaki genetik çeşitliliğe yeni bir ışık tutmaktadır.

Anahtar Kelimeler: Göknar, Moleküler Taksonomi, Kloroplast DNA Bölgesi

Citation (Atıf): Celık Altunoglu, Y., Guney, K., Baloglu, P., & Baloglu, M. C. (2021). Genetic Diversity Analysis of cpDNA in Turkish Abies Taxa. *Kastamonu University Journal of Forestry Faculty*, 21 (1), 41-54.

Introduction

Firs (*Abies* Mill.) are coniferous trees in the Pinaceae family which includes 51 species that are native to the Northern Hemisphere. They distribute naturally in the temperate and boreal regions of the Northern Hemisphere and are mainly found in the mountainous regions of North America, Central America, Europe, North Africa and Asia (Himalaya, South China, and Taiwan) (Li, 1975). The genus is restrained to the mountainous areas in the subtropical and temperate latitudes of the Northern Hemisphere (Farjon, 1990).

Fir tree species are found in many forest areas of Turkey. Five taxa, which are placed in two fir tree species (*Abies nordmanniana* and *Abies cilicica*) are naturally distributed in Turkey. Three of these five taxa are endemic. These taxa are *Abies nordmanniana* subsp. *nordmanniana* (distributed in the North of Kızılırmak in East Black Sea region), *A. nordmanniana* subsp. *bornmuelleriana* (distributed from the West of Kızılırmak to Uludağ in the east Blacksea region), *Abies nordmanniana* subsp. *equi-trojani* (distributed between the Çanakkale, Balıkesir and Bursa), *Abies cilicica* subsp. *isaurica* (distributed in the middle and West Taurus Mountains in the South Anatolia region) and *A. cilicica* subsp. *cilicica* (distributed in the East Taurus Mountains in the South Anatolia region). However, *A. nordmanniana* subsp. *bornmuelleriana* and *A. nordmanniana* subsp. *equi-trojani* cannot be morphologically distinguished. Therefore, these two taxa were combined under the name of ‘’Kazdagi Fir’’ in ‘‘Turkey Plant List (Vascular Plants)’’ (Güner et al., 2000; Güner et al., 2012).

Molecular genetic methods are widely used to analyze the conserved regions of the genome (Ateş, 2011). Molecular phylogenetic analysis using DNA sequence data enables molecular botanists to better define various taxonomic categories. Nuclear (nDNA), chloroplast (cpDNA) and mitochondrial (mtDNA) DNA sequences have been utilized to evaluate phylogenetic relationships in plants (Suyama et al., 2000; Xiang et al., 2004; Tozkar et al., 2009; Semerikova et al., 2011; Aguirre-Planter et al., 2012). However, some cpDNA regions are more informative and useful than other DNA regions for phylogenetic reconstructions (Liang, 1997). In addition, non-coding DNA regions might be more significant because of their rapid evolution than coding DNA regions. This property of non-coding DNA regions causes more variable characters and these DNA regions more useful for better construction and resolution of the phylogenetic tree (Wang et al., 1999).

Although there are many studies on the morphological classification of fir tree species (Kormutak et al., 2004; Hansen et al., 2005; Ziegenhagen et al., 2005; Xiang et al., 2009), a small number of molecular phylogenetic studies in the literature have examined *Abies* taxa in Turkey. These studies focused on the isozyme variation in a limited number of Anatolian fir populations (Şimşek, 1992; Gülbaba et al., 1996). To date, chloroplast *mat*K, some *trn* regions, and nuclear *ITS* DNA regions have been examined to determine the molecular differentiation of Turkish fir tree populations (Ateş, 2011; Özdemir Değirmenci, 2011; Tayanç et al., 2013). Analyses of chloroplast *rbcl* and mitochondrial *nad*5-4 regions were limited to Mediterranean firs (in the South Anatolia region) which were compared with other fir species from around the world (Kormutak et al., 2004; Ziegenhagen et al., 2005). In addition, microsatellite markers were also evaluated to uncover the genetic diversity of the fir populations in Turkey (Hansen et al., 2005; Kaya et al., 2008; Hrivnák et al., 2017; Tayanç et al., 2013).

In this study, we aim to show phylogenetic relationships in Turkish fir taxa based on two non-coding cpDNA markers, i.e. *trnR-trnN* and *rps18-rpl20*. In addition, we used other fir species from previous phylogenetic studies to show taxonomic and phylogenetic position of the Turkish taxa in large phylogeny.

Material and Methods

Plant Material

Samples of fir taxa distributed in Turkey were investigated to determine their phylogenetic relationships (Güner et al., 2012). The morphological characteristics of

taxa were used to identify different members of the genus *Abies*. Studied specimens were identified using Flora of Turkey (Davis, 1965). The geographical locations of sampled Turkish *Abies* taxa are shown in Table 1.

Table 1. Geographical locations of sampled Turkish *Abies* taxa

Sample name	Altitude (m)	Coordinate
Abies cilicica subsp. *isaurica*-1	1.119	36S 0415577 - UTM 4075471
Abies cilicica subsp. *isaurica*-3	1.182	36S 0415601 - UTM 4075497
Abies cilicica subsp. *isaurica*-4	1.189	36S 0415610 - UTM 4075480
Abies cilicica subsp. *isaurica*-6	1.216	36S 0415164 - UTM 4075536
Abies cilicica subsp. *isaurica*-8	1.232	36S 0415168 - UTM 4075502
Abies cilicica subsp. *isaurica*-9	1.415	36S 0419208 - UTM 4075834
Abies cilicica subsp. *isaurica*-10	1.413	36S 0419194 - UTM 4075801
Abies nordmanniana subsp. *nordmanniana*-1	1.800	37T 0732683 - UTM 4557703
Abies nordmanniana subsp. *nordmanniana*-2	1.864	37T 0732503 - UTM 4557508
Abies nordmanniana subsp. *nordmanniana*-5	1.768	37T 0732252 - UTM 4558371
Abies nordmanniana subsp. *bornmuelleriana*-2	2.023	36T 0560422 - UTM 4545271
Abies nordmanniana subsp. *bornmuelleriana*-3	2.025	36T 0560421 - UTM 4545209
Abies nordmanniana subsp. *bornmuelleriana*-5	1.992	36T 0560561 - UTM 4545721
Abies nordmanniana subsp. *bornmuelleriana*-10	1.938	36T 0561995 - UTM 4546679
Abies nordmanniana subsp. *bornmuelleriana*-11	1.903	36T 0562577 - UTM 4546607
Abies nordmanniana subsp. *equi-trojani*-1	844	35S 0507652 - UTM 4418903
Abies nordmanniana subsp. *equi-trojani*-2	814	35S 0507706 - UTM 4419155
Abies nordmanniana subsp. *equi-trojani*-5	797	35S 0507521 - UTM 4419451
Abies nordmanniana subsp. *equi-trojani*-7	784	35S 0506998 - UTM 4419749
Abies nordmanniana subsp. *equi-trojani*-8	782	35S 0506979 - UTM 4419750
Abies nordmanniana subsp. *equi-trojani*-10	765	35S 0506695 - UTM 4419740
Abies cilicica subsp. *cilicica*-1	1.500	36S 0710704 - UTM 4150712
Abies cilicica subsp. *cilicica*-5	1.396	36S 0710727 - UTM 4151006
Abies cilicica subsp. *cilicica*-10	1.275	36S 0710158 - UTM 4153077

DNA Isolation, PCR Amplification and Sequence Analysis

A modified cetyl trimethylammonium bromide (CTAB)-based method was used to isolate DNA from fresh needles (Saghai-Maroof et al., 1984). RNA from the extracted DNA solution was removed by RNAse enzyme (2 mg/mL) at 37 ˚C for 30 min. Concentration and purity of DNA were calculated using MultiscanGo Spectrophotometer (Thermo Scientific, USA). In addition, the DNA quality was checked by 2 % agarose gel and stored at -80 ˚C until use.

PCR reactions included 1.5 mM $MgCl_2$, 1.25 U Taq DNA polymerase enzyme, 5 µL 10x PCR buffer, 5µL 2 mM dNTP, 25 pmol/µL of each gene's specific forward and reverse primer, 100 ng of template DNA and distilled water to achieve a final volume of 50 µL. After testing for amplification efficiency, *trn*R-*trn*N and *rps18-rpl20* cpDNA regions were amplified by PCR. Reaction conditions for the *trn*R-*trn*N region were as follows: initial denaturation at 95 ˚C for 5 min and denaturation at 95 ˚C for 1 min, annealing at 61.4 ˚C for 1 min and extension at 72 ˚C for 1 min which was repeating 35 times, then final extension at 72 °C for 7 min. After the initial denaturation step at 95 ˚C for 5 min, the following three steps were repeated for 35 times for the *rps18-rpl20* region: denaturation at 95 ˚C for 1 min, then annealing at 50.1 ˚C for 1 min and extension at 72 ˚C for 1 min, then final extension step was at 72 ˚C for 7 min. The primer sequences used were as follows: *trn*R-*trn*N-F 5'GCCTGTAGCTCAGAGGATTA3', *trn*R-*trn*N-R 5'TCCTCAGTAGCTCAGTGGTA 3', and *rps18-rpl20*F 5'AGTCGATTTATTAGTGAGCA3', *rps18-rpl20*R 5'CTTCGTCGTTTGTGGATTAC 3' (Wang et al., 1999; Suyama et al., 2000). Amplification efficiency was checked with agarose gel electrophoresis and visualized by

gel imaging system (Vilbor Lourmat, France).

Forward and reverse sequence reads were compared for the most accurate DNA sequence in the studied DNA regions. PCR product purification and DNA sequencing were performed by Refgen Biotechnology (Ankara University, Teknokent, Ankara). ABI 310 Genetic Analyzer User' s Manual was used for sequence analysis. Sequencing was performed using the Big Dye Cycle Sequencing Kit (Applied Biosystems) with a ABI 310 Genetic Analyzer (PE Applied Biosystem, USA) automatic sequencer with forward and reverse primers of the relevant cpDNA regions.

Phylogenetic Tree Analysis

Finch TV software (Geospiza Inc.) was used to visualize DNA sequence results. SnapGene software (from GSL Biotech; available at snapgene.com) was used for contig forward and reverse sequences. Sequences obtained from *trnR-trnN* and *rps18-rpl20* sub-regions and sequences retrieved from NCBI were combined and analyzed to understand genetic diversity among Abies taxa. A phylogenetic tree was constructed by the maximum likelihood method with bootstrap analysis for 1000 replicates and JTT (Jones-Taylor-Thornton) substitution model after multiple sequence alignments by ClustalW using the MEGA 6 program (Tamura et al., 2013). For alignment analysis, ClustalW was used according to following parameters: pairwise alignment gap opening = 15, gap extension = 6.6 and multiple alignment gap-opening = 15, gap extension =6.7, delay divergent sequences = 30% and transition weight = 0.5. Alignments were controlled and visually examined. Gaps in the aligned sequence data were considered as missing data. Pairwise distances between *Abies* taxa distributed in Turkey and pairwise distances between Turkish *Abies* members and *Abies* members from around the world were compared. Phylogenetic trees were displayed using interactive Tree Of Life software (iTOL) (http://itol.embl.de) (Letunic & Bork, 2016). Turkish fir taxa were marked with ▼.

Keteleeria davidiana (JN935765.1) and *Picea abies* (AJ001025.1) were used as outgroups in the constructed phylogenetic trees (Semerikova & Semerikov, 2014). The total *trn*R-*trn*N sequences of 14 fir species and total *rps18-rpl20* sequences of 21 fir species were obtained from the NCBI database and used to evaluate phylogenetic relationships between fir species in Turkey and those from around the world (Table 2).

Table 2. *trn*R-*trn*N and *rps18-rpl20* sequences retrieved from NCBI.

***trn*R-*trn*N Sequences**		***rps18-rpl20* Sequences**	
Species Name	Accession Numbers	Species Name	Accession Numbers
A. fraseri	AB029699.1	*A. numidica*	AB019938.1
A. yuanbaoshanensis	JF276098.1	*A. fabri*	AB029709.1
A. lasiocarpa	AB029703.1	*A. firma*	AB029711.1
A. holophylla	AB029700.1	*A. homolepis*	AB029714.1
A. fabri	AB029696.1	*A. amabilis*	JN935712.1
A. forrestii	JF276116.1	*A. alba*	JN935710.1
A. fargesii	AB029697.1	*A. recurvata*	JN935726.1
A. mariesii	AB029704.1	*A. fraseri*	AB029712.1
A. chensiensis	JF276103.1	*A. balsamea*	JN935713.1
A. nephrolepis	JF276111.1	*A. bracteata*	JN935711.1
A. firma	AB029698.1	*A. vejarii*	JN935731.1
A. grandis	FJ514487.1	*A. sibirica*	KC597631.1
A. iowiana	FJ514486.1	*A. guatemalensis*	JN935748.1
A. concolor	FJ514485.1	*A. hickelii*	JN935733.1
		A. concolor	KC597675.1
		A. religiosa	JN935740.1

Table 2 (Continued)

***trn*R-*trn*N Sequences**		***rps18-rpl20* Sequences**	
Species Name	**Accession Numbers**	**Species Name**	**Accession Numbers**
		A. grandis	JN935717.1
		A. durangensis	JN935755.1
		A. densa	KC597651.1
		A. spectabilis	KC597650.1
		A. cilicica	KC597667.1

Results and Discussion

Turkish *Abies* taxa were classified in previous studies but there were inconsistences in the taxonomy because of the variable and complex morphological features of fir species. The *trn*R-*trn*N sequence analysis showed that, the total length of this region was 823 bp and GC content was 45.5 %. Computed pairwise (p) distances between the studied samples ranged between 0.000 and 0.077 (Supplementary Table 1). All Turkish fir taxa were in the same clade of the phylogenetic tree (Figure 1). Considering that the phylogenetic tree was constructed by the maximum likelihood method and low p distance values, it is difficult to make distinctions among Turkish *Abies* taxa members based on this cpDNA region. In the Türkiye Bitkileri Listesi (Damarlı Bitkiler (Güner et al. 2012), *A. nordmanniana* subsp. *bornmuelleriana* and *A. nordmanniana* subsp. *equi-trojani* were combined. Our findings support this taxonomic treatment based on the low p distance values compared with p distance values among the other Turkish fir taxa (Supplementary Table 1).

In addition, *Abies* taxa in Turkey were compared with other fir species from around the world to elucidate their phylogenetic relationship (Figure 2). The p distance values ranged between 0.000 and 1.227 (Supplementary Table 2). A grouping was observed between these groups. *Abies concolor*, *A. grandis* and *A. iowiana* were in the same clade (gray branch), mainly distributed around North America. *A. nordmanniana* subsp. *nordmanniana* members were in a different clade with some members of *A. cilicica* subsp. *isaurica* and *A. cilicica* subsp. *cilicica* in a one subgroup and *A. nordmanniana* subsp. *bornmuelleriana* and *A. nordmanniana* subsp. *equi-trojani* members in another subgroup. Other fir species aggregated in a different clade (yellow branch). The reason for this aggregation might be the absence of cpDNA *trn*R-*trn*N sequences from NCBI database in trees which are distributed in mainly Asia region.

The *rps18-rpl20* regions of cpDNA were compared between Turkish fir taxa and other fir species from around the world (Figure 3). The total length of this region was 513 bp and its GC content was 33.6%. According to the phylogenetic tree, *Abies* taxa members were closer to each other in the clade and low p values (varied between 0.000 and 0.063) promoted this aggregation (Supplementary Table 3). Two main clusters appeared when Turkish fir taxa and other fir species from around the world were compared (Figure 4). The p distance values were varied between 0.000 and 0.599 (Supplementary Table 4). *A. nordmanniana* subsp. *nordmanniana* was in the same cluster (green branch) as *A. numidica*. In another clade, different subgroups were observed. According to these subgroups, some members of *A. cilicica* subsp. *isaurica*, *A. nordmanniana* subsp. *equi-trojani* and *A. cilicica* subsp. *cilicica* were in the same clade (pink branch) as *A. spectabilis* (East Himalayan Fir) and *A. densa* (Bhutan Fir) which are known as a variety of *A. spectabilis* (*A. spectabilis* var. *densa*). However, some members of *A. nordmanniana* subsp. *bornmulleriana*, *A. cilicica* subsp. *isaurica* and *Abies nordmanniana* subsp. *equi-trojani* were found in the clade (yellow branch) with *A. alba* (European Silver Fir), which is distributed near Turkey and *A. amabilis* (Pacific Silver Fir) which is distributed in the Pacific Northwest of North America. Our chloroplast *rps18-rpl20* region results indicate that fir species exposed to similar

climate conditions aggregate in the same phylogenetic tree branch.

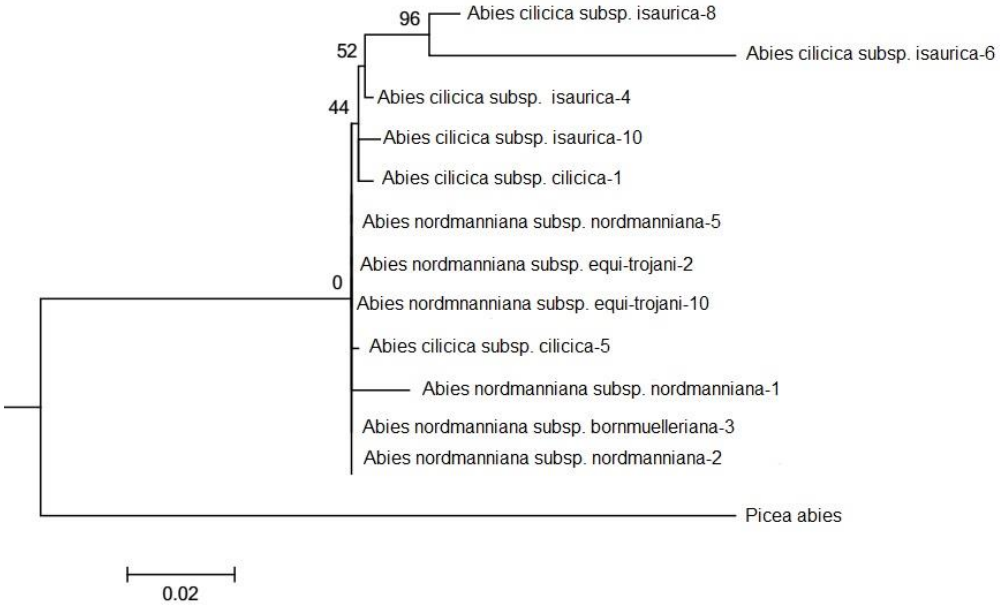

Figure 1. Phylogenetic tree based on the sequence of *trn*R-*trn*N DNA region among Turkish fir taxa members

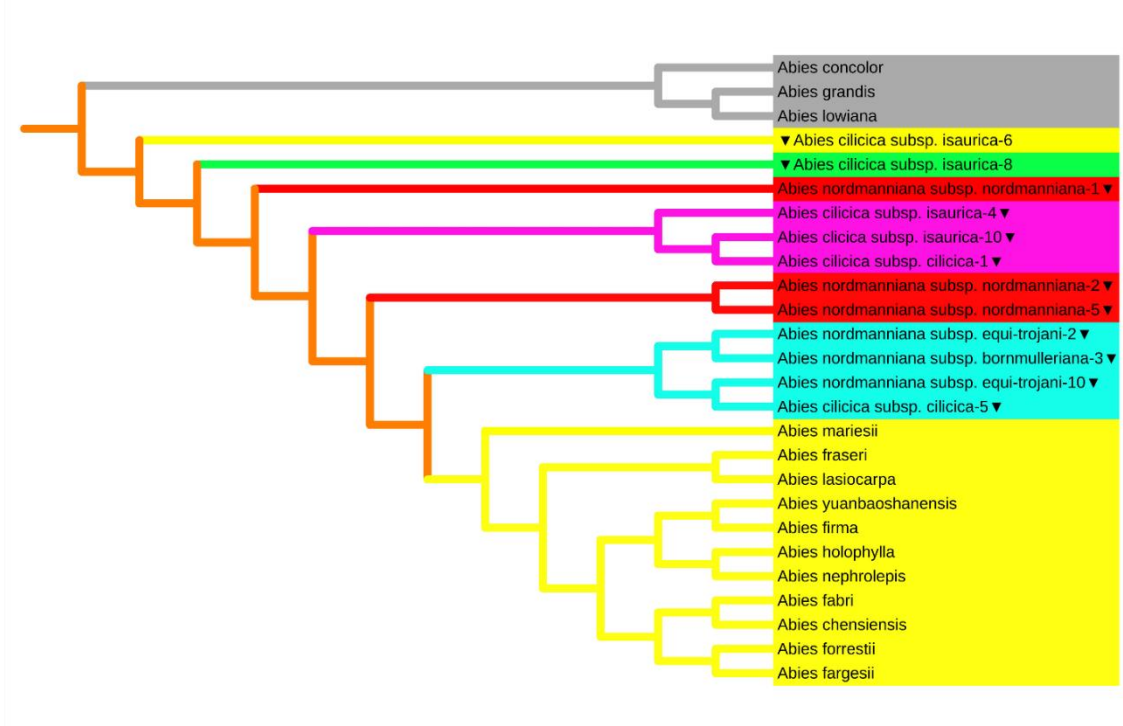

Figure 2. Phylogenetic tree based on the sequence of *trn*R-*trn*N DNA region among Turkish fir taxa members and other fir species from around the world

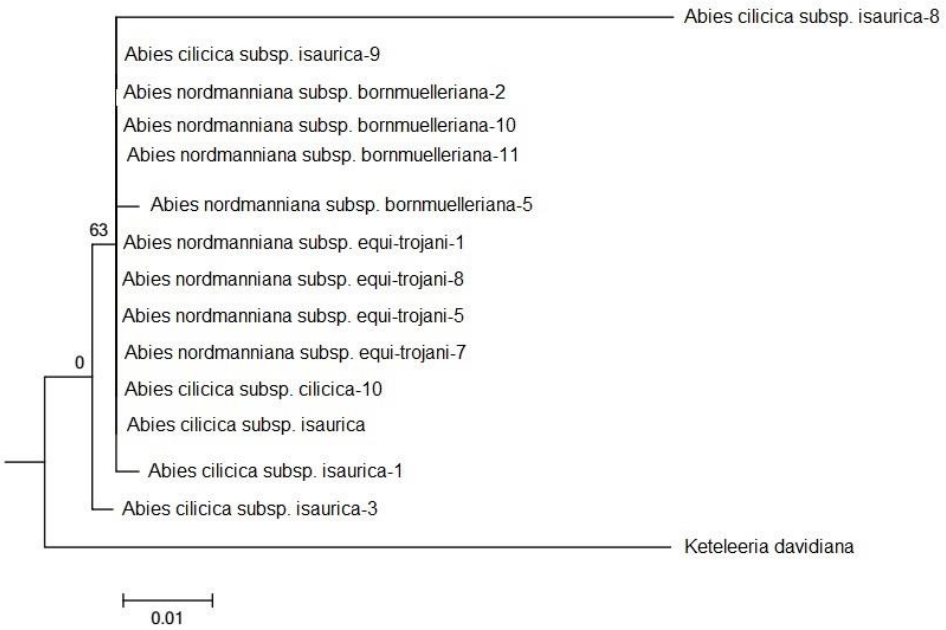

Figure 3. Phylogenetic tree based on the sequence of *rps18- rpl20* DNA region of Turkish fir taxa members.

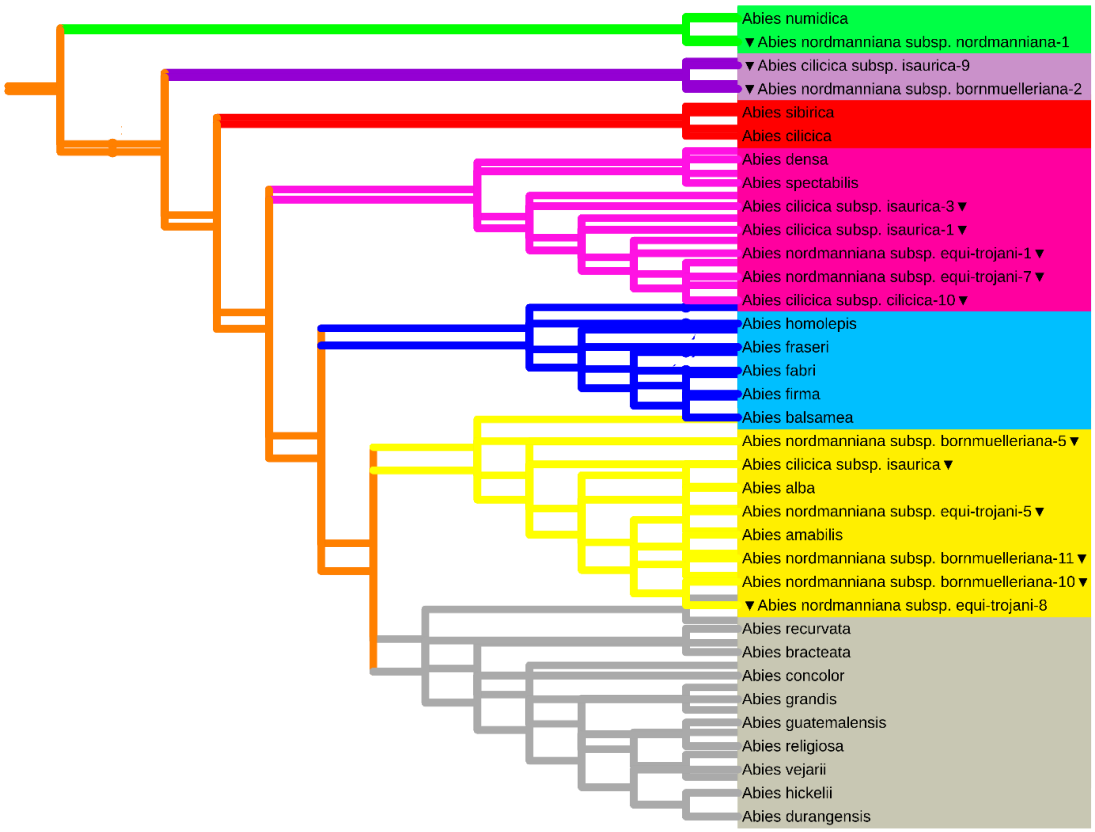

Figure 4. Phylogenetic tree based on the sequence of *rps18-rpl20* DNA region of Turkish fir taxa members and other fir species from around the world.

A genetic study of eight classified Mediterranean and one North American fir species shown that North American species (*A. concolor*) had the most divergent haplotypes (Parducci & Szmidt, 1999). According to a study conducted by Kormutak et al. (2004) Mediterranean firs including closely related species differed from both Asian and North American firs based on PCR-RFLP analysis of eight genes from cpDNA. In this study, Kormutak et al. (2004) reported that Mediterranean fir species has the lowest level polymorphism. In another study, Asian, North American and Mediterranean fir species were phylogenetically classified based on *trn*L and *trn*F region sequences. The Mediterranean fir species (European) *A. alba* and *A. nordmanniana* differed from other fir species based on their tandem repeat types (Isoda et al., 2000). In addition, the sequence analysis of the *trn*L region of Turkish fir taxa revealed the existence of a single clade, which includes Turkish-European fir species. In the same study, Turkish fir species were aggregated and separated from Asian-American fir species in a phylogenetic tree based on their *trn*F sequence analysis (Özdemir Değirmenci, 2011). A similar aggregation of Turkish fir species was also observed in our phylogenetic tree based on the sequences of the *trn*R-*trn*N regions. Another important finding was made by comparing the *mat*K1 region sequence of Turkish fir species. All Turkish fir taxa and *A. numidica* (Algerian fir) that grow in the Mediterranean phytogeographic region were found in the same clade, whereas other fir species from around the world dispersed into various phylogenetic clades (Ateş, 2011). In this study, *A. nordmanniana* subsp. *nordmanniana* was observed in a group with *A. numidica* based on the sequences of *rps*18- *rpl*20. A possible explanation for this might be their similar ecological requirements. Another important finding was that *A. cephalonica* (Greek Fir) which is closely related to *A. nordmanniana* (distributed in Northern Turkey) (Fady et al., 1992) was the closest species to the Turkish fir taxa with low distance rates (0.008) based on the ITS region sequence data (Tayanç et al., 2013). However, studied DNA regions in these last three studies were conserved and no differences were observed among Turkish fir taxa members (Ateş, 2011; Özdemir Değirmenci, 2011; Tayanç et al., 2013). Therefore, these results are in line with those of the previous studies. Xiang et al. (2009) analyzed internal transcribed spacer (ITS) region for 31 *Abies* species to examine phylogenic classification of the genus. Small and large sub-repeats were identified for Pinaceae and Abies, respectively. Four major groups, (1) Western North American monotypic section, (2) Eastern Asian-North American lineage, (3) Western North America group including species from Mexico and Eastern Asia and (4) Eurasian lineage group, were determined based on ITS regions. These results are consistent with data obtained from our study, although relationship among the four major clades remained unconvincingly resolved. This combination of findings supports the conceptual premise that Turkish fir members are in same phylogenetic clade when cpDNA or nDNA regions are used to construct a phylogenetic tree. However, fir taxa in Turkey phylogenetically differ from firs distributed in the Mediterranean and European regions and were commonly found in a distinct clade according to their cpDNA regions. Interestingly, our study shows that based on *rps18-rpl20* cpDNA, some members in Turkish fir taxa are in the same clade as *A. amabilis* located in the Pacific Northwest of North America.

Conclusion

Previous studies on fir species in Turkey have focused on their morphological differences and there are limited data on their molecular phylogenetic relationships. The current study provides new insights into the distribution of cpDNA variation for *Abies* taxa in Turkey and examines the genetic variation between Turkish fir species and other fir species distributed around the world. Based on two cpDNA markers, Turkish Abies taxa are not separated from one another. This may stem from recent divergence among the taxa and indicate their common ancestor. Therefore, further studies may shed lighter on the classification of *Abies* by comparing the maternally inherited

mtDNA and nDNA at low taxonomic level, which was suggested by previous biogeographic studies in coniferous species.

Ethics Committee Approval

N/A

Peer-review

Externally peer-reviewed.

Author Contributions

Conceptualization: Y.Ç.A., K.G., M.C.B.; Investigation: Y.Ç.A., K.G.; Material and Methodology: P.B., M.C.B., K.G.; Supervision: Y.Ç.A., K.G., M.C.B.; Visualization: P.B., Y.Ç.A.; Writing-Original Draft: P.B., Y.Ç.A., M.C.B.; Writing-review&Editing: P.B., Y.Ç.A., M.C.B., K.G.; Other: Y.Ç.A., K.G. All authors have read and agreed to the published version of manuscript.

Conflict of Interest

The authors have no conflicts of interest to declare.

Funding

This study has been supported within the scope of project numbered KÜBAP-01/2012-11 by Kastamonu University, Scientific Research Projects Coordination Department.

References

Aguirre-Planter, É., Jaramillo-Correa, J. P., Gómez-Acevedo, S., Khasa, D. P., Bousquet, J. & Eguiarte, L. E. (2012). Phylogeny, diversification rates and species boundaries of Mesoamerican firs (Abies, Pinaceae) in a genus-wide context. *Molecular Phylogenetics and Evolution*, 62(1), 263-274.

Ateş, M. A. (2011). Molecular phylogenetics of Turkish Abies (pinaceae) species based on matK gene regions of chloroplast genome (Master's thesis, Dept. of Biological Sciences, Middle East Technical University, Ankara).

Davis, P. (1985). PH 1965-1985. *Flora of Turkey* and the East Aegean Islands. 1-9. Edinburg: Edinburgh University Press.

Fady, B., Arbez, M. & Marpeau, A. (1992). Geographic variability of terpene composition in Abies cephalonica Loudon and Abies species around the Aegean: hypotheses for their possible phylogeny from the Miocene. *Trees-Structure and Function*, 6(3), 162-171.

Farjon, A. (1990). *Pinaceae*. Drawings and descriptions of the genera Abies, Cedrus, Pseudolarix, Keteleeria, Nothotsuga, Tsuga, Cathaya, Pseudotsuga, Larix and Picea. Königstein: Koeltz Scientific Books.

Gülbaba, A. G., Velioğlu, E., Özer, A. S., Doğan, B., Doerksen, A. H. & Adams, W. T. (1996). Population Genetic Structure of Kazdağı Fir (Abies equitrojani Aschers et Sint.), A Narrow Endemic to Turkey: Implications for in situ Conservation. In Proceedings of the International Symposium of In situ Conservation of Plant Genetic Diversity.

Güner, A., Özhatay, N., Ekim, T. & Başer, H. K. C. (2000). *Flora of Turkey and East Aegean Islands*: v.11 Supplement 2. Edinburgh: Edinburgh University Press.

Güner, A., Aslan, S., Ekim, T., Vural, M. & Babaç, M. (2012). *Turkey plant list (vascular plants)*. İstanbul: Nezahat Gökyiğit Botanical Garden and Flora Research Association Publication.

Hansen, O. K., Kjær, E. D. & Vendramin, G. (2005). Chloroplast microsatellite variation in Abies nordmanniana and simulation of causes for low differentiation among populations. *Tree Genetics & Genomes*, 1(3), 116-123.

Hrivnák, M., Paule, L., Krajmerová, D., Kulaç, Ş., Şevik, H., Turna, İ., ... & Gömöry, D. (2017). Genetic variation in Tertiary relics: The case of eastern-Mediterranean Abies (Pinaceae). *Ecology and Evolution*, 7(23), 10018-10030.

Isoda, K., Shiraishi, S. & Kisanuki, H. (2000). Classifying Abies species (Pinaceae) based on the sequence variation of a tandemly repeated array found in the chloroplast DNA trnL and trnF intergenic spacer. *Silvae Genetica*, 49(3), 161-164.

Kaya, Z., Skaggs, A. & Neale, D. B. (2008). Genetic Differentiation of Abies equi-trojani (Asch. & Sint. ex Boiss) Mattf. populations from Kazdağı, Turkey and the genetic relationship between Turkish Firs belonging to the Abies nordmanniana Spach Complex. *Turkish Journal of Botany*, 32(1), 1-10.

Kormutak, A., Vookova, B., Ziegenhagen, B., Kwon, H. Y. & Hong, Y. P. (2004). Chloroplast DNA variation in some representatives of the Asian, North American and Mediterranean firs (Abies spp*). Silvae Genetica*, 53(1-6), 99-104.

Letunic, I. & Bork, P. (2016). Interactive tree of life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. *Nucleic Acids Research*, 44(1), 242-245.

Li, H.L. (1975). *Flora of Taiwan*, Epoch publishing, 2, 316p., Taiwan.

Liang, H. (2003). The Phylogenetic Reconstruction of the grass family (Poaceae) using matK gene sequences (Doctoral dissertation, Virginia Polytechnic Institute, Blacksburg).

Özdemir Değirmenci, F. (2011). Molecular phylogenetic position of Turkish abies (pinaceae) based on noncoding trn regions of chloroplast genome (Master's thesis, Dept. of Biological Sciences, Middle East Technical University, Ankara).

Parducci, L. & Szmidt, A. (1999). PCR-RFLP analysis of cpDNA in the genus Abies. TAG *Theoretical and Applied Genetics*, 98(5), 802-808.

Saghai-Maroof, M.A., Soliman, K.M., Jorgensen, R.A. & Allard, R. (1984). Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proceedings of the National Academy of Sciences, 81(24), 8014-8018.

Semerikova, S.A., Semerikov, V.L. & Lascoux, M. (2011). Post-glacial history and introgression in Abies (Pinaceae) species of the Russian Far East inferred from both nuclear and cytoplasmic markers. *Journal of Biogeography*, 38(2), 326-340.

Semerikova, S.A. & Semerikov, V.L. (2014). Molecular phylogenetic analysis of the genus Abies (Pinaceae) based on the nucleotide sequence of chloroplast DNA. *Russian Journal of Genetics*, 50(1), 7-19.

Suyama, Y., Yoshimaru, H. & Tsumura, Y. (2000). Molecular phylogenetic position of Japanese Abies (Pinaceae) based on chloroplast DNA sequences. *Molecular Phylogenetics and Evolution*, 16(2), 271-277.

Şimşek, Y. (1992). Türkiye Orijinli Göknar Türlerinin Genetik Yapıları Üzerine Araştırmalar. Ormancılık Araştırma Enstitüsü, Teknik Bülten Serisi (221).

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, 30(12), 2725-2729.

Tayanç, Y., Çengel, B., Kandemir, G. & Velioğlu, E. (2013). Türkiye'de Yayılış Gösteren Göknar (Abies spp.) Populasyonlarının Genetik Çeşitliliği ve Filogenetik Sınıflandırması. Orman Genel Müdürlüğü Orman Ağaçları ve Tohumları Islah Araştırma Enstitüsü Müdürlüğü Yayınları, (47).

Tozkar, C.Ö., Önde, S. & Kaya, Z. (2009). The phylogenetic relationship between populations of marginally and sympatrically located Pinus halepensis Mill. and Pinus brutia Ten. in Turkey, based on the ITS-2 region. *Turkish Journal of Agriculture and Forestry*, 33(4), 363-373.

Wang, X.R., Tsumura, Y., Yoshimaru, H., Nagasaka, K. & Szmidt, A.E. (1999). Phylogenetic relationships of Eurasian pines (Pinus, Pinaceae) based on chloroplast rbcL, matK, rpl20-rps18 spacer, and trnV intron sequences. *American Journal of Botany*, 86(12), 1742-1753.

Xiang, Q.P., Xiang, Q.Y., Guo, Y.Y. & Zhang, X.C. (2009). Phylogeny of Abies (Pinaceae) inferred from nrITS sequence data. *Taxon*, 58(1), 141-152.

Xiang, Q.P., Xiang, Q.Y., Liston, A. & Zhang, X.C. (2004). Phylogenetic relationships in Abies (Pinaceae): evidence from PCR-RFLP of the nuclear ribosomal DNA internal transcribed spacer region. *Botanical Journal of the Linnean Society*, 145(4), 425-435.

Ziegenhagen, B., Fady, B., Kuhlenkamp, V. & Liepelt, S. (2005). Differentiating groups of Abies species with a simple molecular marker. *Silvae Genetica*, 54(1-6), 123-126.

Supplement Table 1. The p distances of Turkish fir taxa members based on the trnR-trnN DNA region.

	isau.4	*isau.8*	*isau.6*	*isau.10*	*nord.2*	*nord.5*	*equi.2*	*equi.10*	*cili.1*	*cili.5*	*nord.1*	*born.3*
Abies cilicica subsp. *isaurica-4*												
Abies cilicica subsp. *isaurica-8*	0.019											
Abies cilicica subsp. *isaurica-6*	0.070	0.063										
Abies clicica subsp. *isaurica-10*	0.007	0.023	0.071									
Abies nordmanniana subsp. *nordmanniana-2*	0.004	0.020	0.068	0.005								
Abies nordmanninana subsp. *nordmanniana-5*	0.004	0.020	0.068	0.005	0.000							
Abies nordmanniana subsp. *equi-trojani-2*	0.004	0.020	0.068	0.005	0.000	0.000						
Abies nordmanniana subsp. *equi-trojani-10*	0.004	0.020	0.068	0.005	0.000	0.000	0.000					
Abies cilicica subsp. *cilicica-1*	0.005	0.022	0.070	0.005	0.004	0.004	0.004	0.004				
Abies cilicica subsp. *cilicica-5*	0.005	0.022	0.070	0.007	0.001	0.001	0.001	0.001	0.005			
Abies nordmanniana subsp. *nordmanniana-1*	0.015	0.029	0.077	0.016	0.011	0.011	0.011	0.011	0.015	0.012		
Abies nordmanniana subsp. *bornmuelleriana-3*	0.004	0.020	0.068	0.005	0.000	0.000	0.000	0.000	0.004	0.001	0.011	
Picea abies	0.194	0.211	0.263	0.195	0.189	0.189	0.189	0.189	0.194	0.190	0.201	0.189

Supplement Table 2. The p distances of Turkish fir taxa members and other fir species based on the trnR-trnN DNA region.

	fra.	*yuan.*	*lasio.*	*holoph*	*fabri*	*forrst.*	*farge.*	*marie.*	*chen.*	*neph.*	*firma*	*grand.*	*iowi.*	*conco.*	*isau.4*	*isau.8*	*isau.6*	*isau.10*	*equi.2*	*equi.10*	*cili.1*	*cili.5*	*nord.1*	*born.3*
Abies fraseri																								
Abies yuanbaoshanensis	0.002																							
Abies lasiocarpa	0.002	0.004																						
Abies holophylla	0.002	0.000	0.004																					
Abies fabri	0.004	0.002	0.006	0.002																				
Abies forrestii	0.006	0.004	0.008	0.004	0.002																			
Abies fargesii	0.006	0.004	0.008	0.004	0.002	0.002																		
Abies mariesii	0.004	0.006	0.006	0.006	0.008	0.010	0.010																	
Abies chensiensis	0.004	0.002	0.006	0.002	0.000	0.002	0.002	0.008																
Abies nephrolepis	0.002	0.000	0.004	0.000	0.002	0.004	0.004	0.006	0.002															
Abies firma	0.002	0.000	0.004	0.000	0.002	0.004	0.004	0.006	0.002	0.000														
Abies grandis	1.084	1.087	1.087	1.087	1.087	1.084	1.078	1.078	1.087	1.087	1.087													
Abies lowiana	1.075	1.078	1.078	1.078	1.078	1.075	1.069	1.069	1.078	1.078	1.078	0.004												
Abies concolor	1.066	1.069	1.069	1.069	1.069	1.066	1.060	1.060	1.069	1.069	1.069	0.006	0.002											
Abies cilicica subsp. *isaurica-4*	0.012	0.014	0.014	0.014	0.016	0.018	0.018	0.012	0.016	0.014	0.014	1.071	1.062	1.053										
Abies cilicica subsp. *isaurica-8*	0.034	0.036	0.036	0.036	0.038	0.040	0.040	0.034	0.038	0.036	0.036	1.093	1.084	1.075	0.026									
Abies cilicica subsp. *isaurica-6*	0.094	0.096	0.096	0.096	0.098	0.100	0.100	0.094	0.098	0.096	0.096	1.227	1.216	1.206	0.085	0.078								
Abies clicica subsp. *isaurica-10*	0.012	0.014	0.014	0.014	0.016	0.018	0.018	0.012	0.016	0.014	0.014	1.080	1.071	1.062	0.004	0.024	0.080							
Abies nordmanniana subsp. *equi-trojani-2*	0.008	0.010	0.010	0.010	0.012	0.014	0.014	0.008	0.012	0.010	0.010	1.077	1.068	1.059	0.004	0.026	0.085	0.002						
Abies nordmanniana subsp. *equi-trojani-10*	0.008	0.010	0.010	0.010	0.012	0.014	0.014	0.008	0.012	0.010	0.010	1.077	1.068	1.059	0.004	0.026	0.085	0.002	0.000					
Abies cilicica subsp. *cilicica-1*	0.012	0.014	0.014	0.014	0.016	0.018	0.018	0.012	0.016	0.014	0.014	1.080	1.071	1.062	0.004	0.022	0.083	0.002	0.004	0.004				
Abies cilicica subsp. *cilicica-5*	0.008	0.010	0.010	0.010	0.012	0.014	0.014	0.008	0.012	0.010	0.010	1.077	1.068	1.059	0.004	0.026	0.085	0.002	0.000	0.000	0.004			
Abies nordmanniana subsp. *nordmanniana-1*	0.012	0.014	0.014	0.014	0.016	0.018	0.018	0.012	0.016	0.014	0.014	1.077	1.068	1.059	0.008	0.024	0.080	0.004	0.004	0.004	0.006	0.004		
Abies nordmanninana subsp. *bornmulleriana-3*	0.008	0.010	0.010	0.010	0.012	0.014	0.014	0.008	0.012	0.010	0.010	1.077	1.068	1.059	0.004	0.026	0.085	0.002	0.000	0.000	0.004	0.000	0.004	

Supplement Table 3. The p distances of Turkish fir taxa members based on the rps18-rpl20 DNA region.

	isau.8	*isau.3*	*isau.9*	*born.2*	*born.10*	*born.11*	*born.5*	*equi.1*	*equi.8*	*equi.5*	*equi.7*	*cili.10*	*isau*	*isau.1*
Abies cilicica subsp. *isaurica* -8														
Abies cilicica subsp. *isaurica* -3	0.060													
Abies cilicica subsp. isaurica-9	0.057	0.020												
Abies nordmanniana subsp. *bornmuelleriana* -2	0.055	0.020	0.002											
Abies nordmanniana subsp. *bornmuelleriana* -10	0.063	0.026	0.000	0.008										
Abies nordmanniana subsp. *bornmuelleriana* -11	0.055	0.020	0.002	0.004	0.007									
Abies nordmanniana subsp. *bornmuelleriana* -5	0.058	0.022	0.026	0.004	0.033	0.029								
Abies nordmanniana subsp. *equi-trojan* i-1	0.055	0.018	0.012	0.012	0.017	0.010	0.015							
Abies nordmanniana subsp. *equi-trojani* -8	0.055	0.020	0.009	0.002	0.015	0.011	0.022	0.012						
Abies nordmanniana subsp. *equi-trojani* -5	0.055	0.020	0.030	0.004	0.035	0.031	0.017	0.012	0.024					
Abies nordmanniana subsp. *equi-trojani* -7	0.056	0.020	0.006	0.002	0.009	0.007	0.032	0.012	0.009	0.028				
Abies cilicica subsp. *cilicica* -10	0.055	0.007	0.000	0.000	0.007	0.000	0.002	0.000	0.000	0.000	0.000			
Abies cilicica subsp. *isaurica*	0.055	0.020	0.024	0.002	0.031	0.028	0.002	0.013	0.020	0.015	0.030	0.000		
Abies cilicica subsp. i*saurica* -1	0.058	0.022	0.004	0.004	0.011	0.002	0.009	0.006	0.004	0.004	0.004	0.002	0.006	
Keteleeria davidiana	0.123	0.088	0.058	0.064	0.058	0.061	0.084	0.074	0.070	0.091	0.065	0.071	0.083	0.074

Supplement Table 4. The p distances of Turkish fir taxa members and other fir species based on the rps18-rpl20 DNA region.

	numi.	*fabri.*	*firma*	*homo.*	*amab.*	*alba*	*recur.*	*fras.*	*bals.*	*brac.*	*veja.*	*sibir.*	*guat.*	*hicke.*	*conc.*	*relig.*	*grand.*	*dura.*	*densa*	*spect.*	*cili.*	*isau.8*	*isau.3*	*isau.9*	*nord.1*	*born.2*	*born.10*	*Born.11*	*born.5*	*equi.1*	*equi.8*	*equi.5*	*equi.7*	*cili.10*	*isau.*	*isau.1*
numi.																																					
fabri.	0.552																																				
firma	0.552	0.002																																			
homo.	0.553	0.002	0.004																																		
amab.	0.545	0.006	0.007	0.007																																	
alba	0.545	0.004	0.006	0.006	0.002																																
recur.	0.547	0.009	0.011	0.011	0.004	0.006																															
fras.	0.549	0.002	0.004	0.004	0.007	0.006	0.011																														
bals.	0.545	0.000	0.002	0.002	0.006	0.004	0.009	0.002																													
brac.	0.547	0.013	0.015	0.015	0.007	0.009	0.007	0.015	0.013																												
veja.	0.545	0.009	0.011	0.011	0.007	0.009	0.007	0.011	0.009	0.011																											
sibir.	0.547	0.002	0.004	0.004	0.007	0.006	0.011	0.004	0.002	0.013	0.011																										
guat.	0.545	0.007	0.009	0.009	0.009	0.007	0.009	0.009	0.007	0.013	0.002	0.009																									
hicke.	0.547	0.011	0.013	0.013	0.009	0.011	0.009	0.013	0.011	0.013	0.002	0.013	0.004																								
conc.	0.547	0.011	0.013	0.013	0.009	0.011	0.009	0.013	0.011	0.011	0.002	0.009	0.004	0.004																							
relig.	0.544	0.007	0.009	0.009	0.007	0.007	0.007	0.009	0.007	0.011	0.000	0.009	0.000	0.002	0.002																						
grand.	0.547	0.009	0.011	0.011	0.011	0.009	0.011	0.011	0.009	0.015	0.004	0.011	0.002	0.006	0.006	0.002																					
dura.	0.549	0.011	0.013	0.013	0.009	0.011	0.009	0.013	0.011	0.011	0.002	0.013	0.004	0.000	0.004	0.002	0.006																				
densa	0.545	0.002	0.004	0.004	0.009	0.007	0.013	0.005	0.002	0.016	0.013	0.002	0.011	0.016	0.013	0.011	0.013	0.016																			
spect.	0.534	0.002	0.005	0.005	0.010	0.007	0.015	0.005	0.002	0.017	0.015	0.002	0.012	0.017	0.015	0.012	0.015	0.017	0.002																		
cili.	0.545	0.004	0.006	0.006	0.006	0.004	0.009	0.006	0.004	0.011	0.009	0.002	0.007	0.011	0.007	0.008	0.009	0.011	0.004	0.005																	
isau.8	0.538	0.068	0.070	0.070	0.060	0.063	0.065	0.071	0.068	0.065	0.068	0.065	0.070	0.070	0.065	0.068	0.073	0.071	0.083	0.088	0.063																
isau.3	0.532	0.030	0.032	0.032	0.023	0.025	0.027	0.032	0.030	0.027	0.030	0.030	0.032	0.032	0.030	0.030	0.034	0.032	0.031	0.032	0.027	0.063															
isau.9	0.550	0.007	0.009	0.009	0.002	0.004	0.006	0.009	0.007	0.007	0.009	0.006	0.011	0.011	0.007	0.009	0.013	0.011	0.009	0.010	0.004	0.057	0.022														
nord.1	0.319	0.586	0.586	0.587	0.584	0.584	0.582	0.584	0.582	0.584	0.584	0.583	0.584	0.586	0.585	0.583	0.584	0.585	0.595	0.599	0.583	0.575	0.570	0.585													
born.2	0.542	0.010	0.012	0.012	0.004	0.006	0.008	0.012	0.010	0.008	0.012	0.008	0.014	0.014	0.010	0.012	0.016	0.014	0.012	0.013	0.006	0.055	0.022	0.002	0.581												
born.10	0.549	0.007	0.009	0.009	0.002	0.004	0.006	0.009	0.007	0.006	0.009	0.007	0.011	0.011	0.009	0.009	0.013	0.009	0.009	0.010	0.006	0.065	0.029	0.002	0.584	0.010											
born.11	0.549	0.008	0.010	0.010	0.002	0.004	0.006	0.010	0.008	0.008	0.010	0.010	0.011	0.011	0.011	0.010	0.013	0.012	0.011	0.013	0.008	0.058	0.022	0.004	0.581	0.006	0.007										
born.5	0.545	0.032	0.034	0.034	0.027	0.029	0.030	0.035	0.032	0.032	0.034	0.034	0.036	0.036	0.036	0.034	0.038	0.037	0.041	0.045	0.032	0.060	0.024	0.028	0.584	0.006	0.033	0.029									
equi.1	0.543	0.020	0.022	0.022	0.014	0.016	0.018	0.022	0.020	0.018	0.022	0.020	0.024	0.024	0.022	0.022	0.026	0.024	0.022	0.027	0.018	0.058	0.020	0.014	0.579	0.014	0.019	0.011	0.017								
equi.8	0.544	0.015	0.017	0.017	0.010	0.012	0.013	0.017	0.015	0.015	0.017	0.017	0.019	0.019	0.019	0.017	0.021	0.019	0.021	0.023	0.015	0.058	0.022	0.011	0.579	0.004	0.015	0.011	0.022	0.013							
equi.5	0.551	0.036	0.038	0.038	0.031	0.033	0.034	0.039	0.036	0.036	0.038	0.038	0.040	0.040	0.040	0.038	0.042	0.039	0.046	0.051	0.036	0.058	0.022	0.032	0.591	0.006	0.035	0.031	0.017	0.013	0.024						
equi.7	0.546	0.013	0.015	0.015	0.008	0.010	0.011	0.015	0.013	0.013	0.015	0.013	0.017	0.017	0.015	0.015	0.019	0.012	0.014	0.018	0.011	0.059	0.022	0.008	0.581	0.004	0.009	0.009	0.034	0.012	0.011	0.030					
cili.10	0.526	0.009	0.012	0.012	0.002	0.005	0.007	0.012	0.009	0.007	0.009	0.009	0.012	0.012	0.009	0.009	0.014	0.012	0.009	0.013	0.007	0.058	0.009	0.002	0.567	0.002	0.009	0.002	0.005	0.000	0.002	0.002	0.000				
isau.	0.550	0.035	0.037	0.037	0.026	0.028	0.030	0.037	0.032	0.030	0.033	0.032	0.035	0.035	0.034	0.033	0.037	0.034	0.038	0.042	0.030	0.058	0.022	0.026	0.588	0.004	0.030	0.027	0.002	0.015	0.020	0.015	0.032	0.002			
isau.1	0.531	0.011	0.013	0.013	0.007	0.009	0.011	0.014	0.011	0.011	0.013	0.011	0.016	0.016	0.013	0.013	0.018	0.016	0.011	0.016	0.011	0.060	0.024	0.007	0.557	0.006	0.013	0.004	0.011	0.006	0.006	0.006	0.004	0.002	0.009		