

ABANT İZZET BAYSAL UNIVERSITY
THE GRADUATE SCHOOL OF NATURAL AND APPLIED
SCIENCES
DEPARTMENT OF BIOLOGY



PHYLOGENETIC ANALYSIS AND PHYLOGEOGRAPHIC
STRUCTURE OF *CYNIPS QUERCUSFOLII*
(HYMENOPTERA: CYNIPIDAE) AS INFERRED FROM
MTDNA AND NDNA SEQUENCES

DOCTOR OF PHILOSOPHY

SERDAR DİNÇ

BOLU, DECEMBER 2017

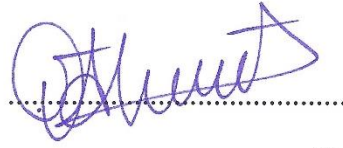
APPROVAL OF THE THESIS

PHYLOGENETIC ANALYSIS AND PHYLOGEOGRAPHIC STRUCTURE OF *CYNIPS QUERCUSFOLII* (HYMENOPTERA: CYNIPIDAE) AS INFERRED FROM MTDNA AND NDNA SEQUENCES submitted by **Serdar DİNÇ** in partial fulfillment of the requirements for the degree of **Doctor of Philosophy** in **Department of Biology, The Graduate School of Natural and Applied Sciences of ABANT İZZET BAYSAL UNIVERSITY** in **29/12/2017** by

Examining Committee Members

Signature

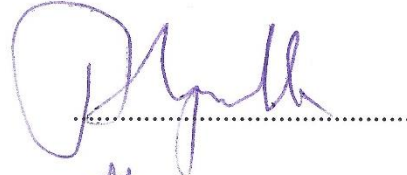
Supervisor
Prof. Dr. Serap MUTUN
Abant İzzet Baysal University



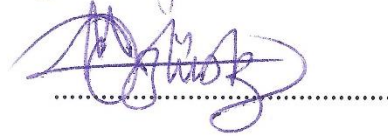
Member
Prof. Dr. Mustafa ÜNAL
Abant İzzet Baysal University



Member
Prof. Dr. Süleyman AKBULUT
Düzce University



Member
Assoc. Prof. Dr. Mehmet ÖZTÜRK
Abant İzzet Baysal University



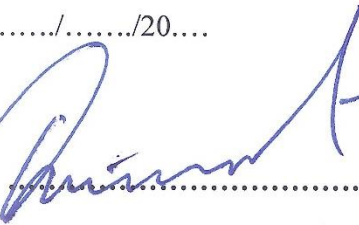
Member
Assist. Prof. Dr. Meral KEKEÇOĞLU
Düzce University



Graduation date:

...../...../20....

Assoc. Prof. Dr. Ömer ÖZYURT



Director of Graduate School of Natural and Applied Sciences

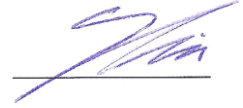


To my lovely wife Fatoş and my beautiful daughter Doğa...

DECLARATION

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Serdar DİNÇ



ABSTRACT

PHYLOGENETIC ANALYSIS AND PHYLOGEOGRAPHIC STRUCTURE OF *CYNIPS QUERCUSFOLII* (HYMENOPTERA: CYNIPIDAE) AS INFERRED FROM MTDNA AND NDNA SEQUENCES

PHD THESIS

SERDAR DİNÇ

**ABANT İZZET BAYSAL UNIVERSITY GRADUATE SCHOOL OF
NATURAL AND APPLIED SCIENCES
DEPARTMENT OF BIOLOGY
(SUPERVISOR: PROF. DR. SERAP MUTUN)**

BOLU, DECEMBER 2017

Cynips quercusfolii (Hymenoptera: Cynipidae) is a widespread oak gall wasp species distributed in the Palearctic region including Turkey. Individuals reared from asexual generation galls of the species were used to: i) reveal genetic variation present in the Turkish populations of *C. quercusfolii*, ii) search for the geographic structuring of the genetic diversity, iii) assess population demographic structure by exposing population expansion/retractions, iv) reconstruct phylogenetic and phylogeographic structure, v) test if the geographic barriers shaped phylogeographic and phylogenetic structure of the species, vi) effects of the climatic fluctuations on the genetic structure of the species, and vii) estimate times of divergence between major lineages of the species.

For these purposes, a 433 bp fragment of mitochondrial DNA cytochrome b gene and the entire ITS2 region nuclear DNA were amplified and sequenced from 294 individuals representing 38 populations of *C. quercusfolii*. All sequences generated 125 mtDNA haplotypes and 30 ITS2 alleles. Haplotype/allele and nucleotide diversity estimates indicated the existence of high genetic variation in the Turkish populations. Nonetheless, lower nucleotide diversity versus higher haplotype diversity implied that populations have gone through some population expansion and retraction in the past. Phylogenetic and phylogeographic analyses through performing maximum parsimony, maximum likelihood, and Bayesian approaches indicated that there is geographic structuring of the haplotypes considering the location of major barriers. Employing conventional molecular clock to the cytochrome b gene data to estimate times of divergence showed that *C. quercusfolii* split from the outgroup species in the Pliocene epoch, and during the Pleistocene climatic and environmental fluctuations played a key role in the structuring of the major clades and subclades. Overall, current findings of this thesis demonstrate that climatic oscillations and geographic barriers have left their imprints on *C. quercusfolii* in Turkey.

KEYWORDS: *Cynips quercusfolii*, Climatic oscillations, Geographic barrier, Phylogeography, Population demography.

ÖZET

CYNIPS QUERCUSFOLII' NİN (HYMENOPTERA: CYNIPIDAE) FİLOGENETİK ANALİZİ VE FİLOCOĞRAFİK YAPISININ MTDNA VE NDNA SEKANS YOLUYLA ARAŞTIRILMASI

DOKTORA TEZİ

SERDAR DİNÇ

ABANT İZZET BAYSAL ÜNİVERSİTESİ FEN BİLİMLERİ ENSTİTÜSÜ BİYOLOJİ ANABİLİM DALI (TEZ DANIŞMANI: PROF. DR. SERAP MUTUN)

BOLU, ARALIK - 2017

Cynips quercusfolii (Hymenoptera: Cynipidae) Türkiye de dahil olmak üzere Palearktik bölgede geniş yayılış gösteren bir gal arısı türüdür. Bu tez çalışmasında eşeysiz jenerasyon gallerinden elde edilen bireyler i) *C. quercusfolii* türünün ülkemiz populasyonlarının sahip olduğu genetik çeşitliliği tespit etmek, ii) genetik çeşitliliğin coğrafik dağılımını belirlemek, iii) populasyonların genişleme/daralma durumlarına bakarak populasyon demografik yapısını ortaya koymak, iv) türün filogenetik ve filocoğrafik yapılanmasını araştırmak, v) coğrafik bariyerlerin türün filocoğrafik ve filogenetik yapısı üzerine etkisini belirlemek, vi) türün genetik yapısı üzerine iklimsel dalgalanmaların etkisinin olup olmadığını test etmek, vii) türün ana soy hatlarının ayrışma zamanlarını tahmin etmek için kullanılmıştır.

Bu amaçla *C. quercusfolii* türünün 38 populasyonunu temsil eden 294 bireyin mitokondri genomundan 433 bç sitokrom b geni ile çekirdek genomundan ITS2 bölgesi çoğaltılarak dizilenmiştir. Dizilen örneklerden 125 mtDNA haplotipi ve 30 ITS2 aleli belirlenmiştir. Haplotip/alel ve nükleotid çeşitlilik analizleri ülkemiz populasyonlarında yüksek bir genetik çeşitliliğinin olduğunu ortaya koymaktadır. Bununla beraber, nükleotid çeşitliliğinin haplotip çeşitliliğine oranla daha düşük olması populasyonların geçmişte daralma/genişleme geçirdiklerini ifade etmektedir. Maksimum parsimoni, maksimum olasılık ve Bayesian yaklaşımların uygulandığı filogenetik ve filocoğrafik analizler soy hatlarının ülkemizdeki ana coğrafik bariyerlerle ilintili bir yapılanma gösterdiğini ortaya koymuştur. Sitokrom b gen verilerine klasik moleküler saat uygulaması yapıldığında *C. quercusfolii* türünün dışgrup haplotiplerinden Pliosen'de ayrıldığı, Pleistosen boyunca oluşan iklimsel ve çevresel değişimlerin türün ana klad ve altkladlarının ayrışmasında etkili olduğu belirlenmiştir. Bu tez ile elde edilen sonuçlar coğrafik bariyerler ve iklimsel değişikliklerin *C. quercusfolii* türünün ülkemizdeki yapılanmasına etkili olduğunu göstermektedir.

ANAHTAR KELİMELER: *Cynips quercusfolii*, Coğrafik bariyer, Filocoğrafya, İklimsel dalgalanmalar, Populasyon demografisi.

TABLE OF CONTENTS

	<u>Page</u>
ABSTRACT	v
ÖZET.....	vi
TABLE OF CONTENTS.....	vii
LIST OF FIGURES	ix
LIST OF TABLES	x
LIST OF ABBREVIATIONS AND SYMBOLS	xi
1. INTRODUCTION	1
1.1 Gall Inducing Organisms and Hypotheses to Explain Galling	1
1.2 Gall Formation and Development	3
1.3 Oak Gall Wasp Diversity and the Significance for Turkey.....	4
1.4 The Cherry Gall: <i>Cynips quercusfolii</i> (Linnaeus, 1758)	6
1.5 Paleohistory and Paleogeography of Turkey	8
1.6 Turkey: A Biodiversity Hotspot and Refuge Area	11
1.7 Molecular Markers in Phylogeographic and Phylogenetic Studies.....	14
1.8 Phylogeographic Studies Utilized Genetic Markers on Oak Gall Wasps in Turkey	17
2. AIM AND SCOPE OF THE STUDY	21
3. MATERIALS AND METHODS.....	22
3.1 Sampling of the Species.....	22
3.2 DNA Isolation.....	25
3.3 PCR Amplification, Purification and Sequencing	26
3.3.1 Mitochondrial Cytochrome b Gene	27
3.3.2 Amplification of Nuclear ITS2 Region	28
3.4 Purification and Sequencing	29
3.5 Data Analysis.....	29
3.5.1 Alignment of Sequences and Haplotype/Allele Determination	29
3.5.2 Populations Genetic Diversity Estimates	29
3.5.3 Detection of Population Genetic Structure and Population Demographic Analysis	32
3.6 Phylogenetic and Phylogeographic Analysis.....	32
3.6.1 Maximum Likelihood (ML), Maximum Parsimony (MP) and Bayesian Analysis	32
3.6.2 Network Analysis	33
3.6.3 Analysis Related to the Geographic Distribution of the Genetic Variation	34
3.6.4 Estimation of Time of Divergence	35
4. RESULTS.....	36
4.1 Sequence Data Evaluation of the Mitochondrial Marker: Cytochrome b (Cyt b) Gene	36

4.1.1	Genetic Diversity Estimates of the mtDNA Cytochrome b Gene of <i>Cynips quercusfolii</i>	36
4.1.2	Demographic Structure of <i>C. quercusfolii</i> Populations Based on Cyt b Gene.....	67
4.2	Assesment of the Nuclear Marker: Internal transcribed Spacer 2 (ITS2) Region	74
4.2.1	Genetic Diversity Estimates of the ITS2 Segment of <i>Cynips quercusfolii</i>	74
4.3	Demographic Structure of <i>C. quercusfolii</i> Populations Based on ITS2 Region	84
4.4	Phylogenetic and Phylogeographic Analyses on <i>C. quercusfolii</i> mtDNA cyt b Gene Analysis	90
4.4.1	Maximum Parsimony and Maximum Likelihood Analysis Results....	90
4.4.2	Bayesian Analysis Results and Times of Divergence.....	95
4.5	nDNA ITS2 Allele Analysis.....	97
5.	DISCUSSION.....	103
5.1	Genetic Diversity of <i>C. quercusfolii</i> in Turkey	103
5.2	Population Demographic Analyses: Did <i>C. quercusfolii</i> Populations Expanded or Diminished?	106
5.3	Possible Effects of Climate and Topography on <i>C. quercusfolii</i> Lineages	109
6.	CONCLUSION.....	112
7.	REFERENCES	113
8.	CURRICULUM VITAE.....	129

LIST OF FIGURES

	<u>Page</u>
Figure 1.1. Parthenogenetic generation galls of the cherry gall, <i>C. quercusfolii</i>	8
Figure 1.2. The location of the Anatolian Diagonal and the approximate location of the three phylogeographic regions in Turkey	13
Figure 1.3. Insect mitogenome	16
Figure 1.4. The nuclear ribosomal operon system which includes the non-coding ITS1 and ITS2 region.	17
Figure 3.1. Sampling sites for <i>C. quercusfolii</i>	23
Figure 3.2. a) An adult individual of <i>C. quercusfolii</i> . b) The exit hole of the adult after hatching.....	24
Figure 3.3. Isolated DNA photograph of <i>C. quercusfolii</i> specimens.....	26
Figure 3.4. Amplified cyt b gene segment of mtDNA samples from <i>C. quercusfolii</i> individuals.....	27
Figure 3.5. Amplified ITS2 segment of nDNA from <i>C. quercusfolii</i> individuals.....	28
Figure 3.6. Chromatogram of cytb region of mtDNA (433 bp).	30
Figure 3.7. Chromatogram of ITS2 region of nDNA (449 bp)	31
Figure 4.1. AMOVA analysis of cyt b data	66
Figure 4.2. Mismatch distribution of cyt b haplotypes	68
Figure 4.3. Mismatch distribution of populations with their own haplotypes for cyt b data set	70
Figure 4.4. Correlograms showing spatial genetic correlation coefficient (r) as a function of geographic distances in the entire study area based on cyt b	73
Figure 4.5. AMOVA analysis of ITS2 data.	83
Figure 4.6. Mismatch distribution of ITS2 alleles	85
Figure 4.7. Mismatch distribution of populations with their own haplotypes for ITS2 data set.....	86
Figure 4.8. Correlograms showing spatial genetic correlation coefficient (r) as a function of geographic distances in the entire study area based on ITS2 data.	89
Figure 4.9. Maximum parsimony 50% majority rule consensus tree of the cyt b haplotypes of <i>C. quercusfolii</i>	91
Figure 4.10. Maximum Likelihood 50% majority rule consensus tree of the cyt b haplotypes of <i>C. quercusfolii</i>	92
Figure 4.11. Results of the network analysis that was produced by Hapstar 0.7 from the mitochondrial Cyt B haplotypes	94
Figure 4.12. Beast consensus tree of cytb haplotypes.....	96
Figure 4.13. Maximum parsimony 50% majority rule consensus tree of the ITS2 alleles of <i>C. quercusfolii</i>	98
Figure 4.14. Maximum likelihood 50% majority rule consensus tree of the ITS2 alleles of <i>C. quercusfolii</i>	100
Figure 4.15. Bayesian consensus tree of ITS2 alleles	101
Figure 4.16. Results of the network analysis that was produced by Hapstar 0.7 from the nuclear ITS2 alleles.	102

LIST OF TABLES

	<u>Page</u>
Table 3. 1. Sampled localities of <i>C. quercusfolii</i>	24
Table 4.1. Total 125 cyt b haplotypes of the mitochondrial cyt b gene of <i>C. quercusfolii</i>	37
Table 4.2. Maximum composite likelihood estimates of the pattern of nucleotide substitutions for the cyt b region of <i>C. quercusfolii</i> . Rates of different transitional substitutions are shown in bold and those of transversional substitutions are shown in italics.	43
Table 4.3. Polymorphic amino acid sites of <i>C. quercusfolii</i> cyt b 125 haplotypes	44
Table 4.4. Frequency of amino acids used in <i>C. quercusfolii</i> cyt b haplotypes. All frequencies are averages over all sequences. Relative synonymous codon usage is given in parentheses following the codon frequency. (RSCU: Relative Synonymous Codon Usage).....	45
Table 4.5. Cyt b haplotypes and their frequencies in <i>C. quercusfolii</i> populations.....	47
Table 4.6. Pairwise comparison of 125 <i>C. quercusfolii</i> haplotypes. Net nucleotide differences are shown below diagonal and the percentage of the differences are presented above diagonal.	52
Table 4.7. Sample size, haplotype number, haplotype (h) and nucleotide (π) diversities for the cyt b gene and ITS2 region.	62
Table 4.8. Cytochrome b region F_{ST} analysis results showing pairwise genetic variations between populations. + : $p = 0.001$, -: not significant.	64
Table 4.9. Fu's F_S ve Tajima's D values calculated for each <i>C. quercusfolii</i> population for cyt b. * $P \leq 0.01$, ** $P \leq 0.001$	68
Table 4.10. Thirty alleles of the ITS2 region of <i>C. quercusfolii</i> . Only polymorphic character sites were shown.	75
Table 4.11. Maximum composite likelihood estimates of the pattern of nucleotide substitutions for the ITS2 region of <i>C. quercusfolii</i> . Rates of different transitional substitutions are shown in bold and those of transversional substitutions are shown in italics.	75
Table 4.12. ITS2 allele frequencies in <i>C. quercusfolii</i> populations.	78
Table 4.13. Pairwise comparison of 30 <i>C. quercusfolii</i> alleles. Net nucleotide differences are shown below diagonal and the percentage of the differences are presented above diagonal.	79
Table 4.14. ITS2 region F_{ST} analysis results showing pairwise genetic variations between populations. + : $p = 0.001$, -: not significant.	80
Table 4.15. Fu's F_S ve Tajima's D values calculated for each <i>C. quercusfolii</i> population for ITS2. * $P \leq 0.01$, ** $P \leq 0.001$	85

LIST OF ABBREVIATIONS AND SYMBOLS

°C	: Celsius degree
A	: Adenine
AIC	: Akaike information criteria
AMOVA	: Analysis of molecular variance
BEAST	: Bayesian Evolutionary Analysis Sampling Trees
BEAUTI	: Bayesian Evolutionary Analysis Utility
bp	: Base pair
C	: Cytosine
CI	: Consistency index
CytB	: Cytochrome b
ddH₂O	: Double-distilled water
dNTP	: Deoxynucleotide triphosphate
EDTA	: Ethylenediaminetetraacetic acid
EUR	: Euro
G	: Guanine
GenAlEx	: Genetic analysis in excel
GPS	: Geographic positioning system
GTR	: General Time Reversible
h	: Haplotype diversity
HI	: Homoplasy index
HKY	: Hasegawa, Kishino and Yano
HPLC	: High performance liquid chromatography
Hri	: Harpending's raggedness index
I	: Proportion of Invariable Sites
IAA	: Indole acetic acid
IGS	: Intergenic spacer
IR	: Irano
ITS2	: Internal transcribed spacer 2
Kb	: Kilobase
ln	: log-likelihood
lsu RDNA	: Large subunit ribosomal DNA
mA	: Microampere
MACA	: Most ancient common ancestor

MCMC	: Markov Chain Monte Carlo
MED	: Mediterranean
MgCl₂	: Magnesium chloride
ML	: Maximum likelihood
mM	: Milimolar
MP	: Maximum parsimony
MRCA	: Most recent common ancestor
mtDNA	: Mitochondrial DNA
MYA	: Millions years ago
nDNA	: Nuclear DNA
ng	: Nanogram
PAUP	: Phylogenetic analysis using parsimony program
PCR	: Polymerase chain reaction
pmol	: Picomolar
r	: Raggedness ratio
RFLP	: Restriction fragment length polymorphism
RI	: Retention index
rpm	: Revolutions per minute
SIB	: Siberian
SSD	: Sum of squared deviations
ssu RDNA	: Small subunit ribosomal DNA
svg	: Scalable vector graphics
T	: Thymine
TBE	: Tris-Borate-EDTA
ti	: Transition
TUR	: Turanian
tv	: Transversion
U	: Uracil
UK	: The United Kingdom
UV	: Ultraviolet
Γ	: Gamma
λ	: Lambda DNA
μl	: Microliter
π	: Nucleotide diversity

ACKNOWLEDGEMENTS

I would like to express my special appreciation and thanks to my supervisor Prof. Dr. Serap MUTUN for her guidance, continuous support, for her patience (if there is any left), motivation, and immense knowledge. I am also thankful for allowing me to grow as a research scientist, you have been a tremendous mentor.

I would also like to thank Prof. Dr. Mustafa ÜNAL and Assist. Prof. Dr. Meral KEKEÇOĞLU for their suggestions and comments. I also would like to thank my other committee members for their suggestions.

My special thanks go to my family. Words cannot express how grateful I am to my mother Filiz DİNÇ, my father Cihat DİNÇ mother-in law Sevgi KORKMAZ, father-in-law İDRİS KORKMAZ, for all the sacrifices that you have made on my behalf. I also would like to thank Figen ASLAN and Niğmet ASLAN for their financial and spiritual supports in the tough times. Your prayer for me was what sustained me thus far.

I would also like to thank all my friends, especially Cemalettin ÖZARSLAN, Erhan ÇİMEN and İsmail YURTSEVENYENER who supported me in writing, and incited me to strive towards my goal. My special thanks go to Ercan ÜNLÜ and his lab members for experimental support.

This study was supported by the Abant İzzet Baysal University, Grant No: 2011.03.01.380.

1. INTRODUCTION

A variety of interrelations are observed among different organismal groups in a broad spectrum from mutualistic to parasitic relationships. One of the most intricate associations accepted as a specific type of parasitism is seen between plants and some other organisms resulting in anomalous plant growths known as galls (Shorthouse et al., 2005). Since ancient times galls have drawn attention as remarkable structures, and have been used for different purposes. The first records about galls were noted by Theophrastus of Eressus' (371-286 BC) botanical works where he described several types of gall species (Senn, 1942; Thanos, 2005). Throughout the middle centuries galls were utilized to make gall ink, which is composed of gallic acid and iron. Gall ink was often used for art, to pen literary on parchments, and to write official documents. Leonardo da Vinci, Rembrandt and Van Gogh used gall ink for their drawings and notes, Bach composed his symphonies, and Thomas Jefferson used to write the Declaration of Independence using gall ink (Boggs, 2016; Kolar et al., 2010). In fact, most of the human history was penned with gall ink. Galls have also been used for leather tanning and medical treatments in many cultures around the world. Sumerians, Babylonians and Egyptians used galls for treatment of several diseases such as typhoid, dysentery and malaria (Ekici, 1975). In Chinese medicine, gall materials were used for dysentery, ulcers and hemorrhoids (Djakpo and Yao, 2010). Indians used poultices of ground gall nuts on sores, cuts and burns. Further uses of galls to cure diarrhea, cholera and gonorrhea were reported from diverse cultures (Umachigi et al., 2008).

1.1 Gall Inducing Organisms and Hypotheses to Explain Gallling

Various groups of organisms from insects, nematodes, fungi, bacteria to viruses can induce galls on different plant taxa (Dreger-Jauffret and Shorthouse, 1992; Mani, 1964). Plant galls have attracted great attention from the scientific point of view to understand gall morphology, gall induction and the gall- inducing organisms, evolution and adaptive significance of galling. At first, galls were

perceived as nonsense structures having no function and no value at all for both plants and gallers. Afterwards, it has been argued that galls might have adaptive value only for plants, while other hypotheses stated that both plants and the gallers gain benefits from each other in a mutualistic manner (Price et al., 1987). On the contrary, recent studies propose that galls are important formations primarily for the galling organism; giving harm to plants so that they try to get rid of gallers' attack with the least damage, through giving responses to gallers by restrained growth and reproduction (Stone and Schönrogge, 2003).

Among other gall causing organisms insects lead with nearly 13000 species from Thysanoptera, Heteroptera, Homoptera, Coleoptera, Lepidoptera, Diptera, and Hymenoptera. Gallling within and among insect orders is thought to be evolved several times resulting in great diversity in gall structure and morphology. Recent evidence proves that gallers are parasites of the host plants and control most aspects of gall development (Price, 2005). Nevertheless, various species are responsible for inducing galls of galler-specific morphologies at different parts of the same host plant at the same season of a year (Stone et al., 2002).

Gall inducing organisms, as the parasites of plants, benefit from galls through several ways (Harper et al., 2004; Shorthouse et al., 2005; Stone and Cook, 1998). Three relevant hypotheses were used to explain benefits of the galling to the inducers: the nutritive hypothesis, the microenvironment hypothesis, and the enemy hypothesis. The first explains that galls provide nutrients indispensable for developing offspring inside the larval chamber. Histochemical and cytological studies revealed that the tissue surrounding the organism is quite high in nutritive substances, but also very low regarding defensive phenolic compounds such as tannin concentration (Price et al., 1987). Gall induction also triggers the modification of plant cell contents, and thus distributed plant tissues by galler convert the host plant to a suitable habitat for the developing larvae (Price et al., 1987; Stone and Cook, 1998). The microenvironment hypothesis states that gall tissues play a key role to protect the galling insect from unfavorable environmental conditions such as desiccation. Researchers suggest that the hygrothermal stress, referring to both humidity and temperature, is one of the factors that results in selective adaptation of gallers. The underlying reason behind this suggestion is that, the gall structure itself

is not an insulator, but a barrier. During the high and low temperature periods, plants keep up these shifts with a little time lag that prevent the impacts of sudden temperature changes to plants as well as for the galls. Another aspect is to maintain proper humidity level for the galler and to prevent direct effects of harsh physical environmental factors such as snow, rain, ice, and sun (Stone et al., 2002; Stone and Schönrogge, 2003). The Enemy hypothesis states that galls are used as shelters by developing organism against natural enemies such as non-specialist predators and pathogens. Most of the researches have agreed to accept that the enemy concept covers most of the approaches representing evolutionary process and diversification of gallers through the interactive relation of plants and gall inducer (Bailey et al., 2009; Hayward and Stone, 2005).

1.2 Gall Formation and Development

Gall formation itself is a subject of great interest; it has three major stages as initiation, growth, and maturation (Stone et al., 2002). Gall-forming insects release gall-inducing stimuli; plant hormone production is initiated by the induction caused by either mechanical or salivary and ovipositor secretions of gall-inducing females (Stone and Schönrogge, 2003). Initiation phase begins after oviposition of female onto the meristematic tissue of the proper location of the host plants, and the gall induction begins (Mapes and Davies, 2001). During the early phase of gall formation indole acetic acid (IAA), auxins, zeatin and other cytokinins known as plant chemicals play significant role. The host plants give responses to galling with necrosis of cells neighboring the egg, and the cells below the necrosis site start to proliferate (Giron et al., 2016). This cell proliferation eventually ends up with a surrounding of the developing egg, and the process continues with cell differentiation and hypertrophy. Cell number is increased until gall reaches its maturity where growth is completed (Stone and Schönrogge, 2003; Yamaguchi et al., 2012).

In developed galls, larval chamber is surrounded by nutritive tissue. As the surrounding nutrition-rich tissue is consumed entirely by the developing larva the neighboring vacuolated parenchyma begins transforming into nutritive tissue. Over the vacuolated parenchyma, there is a layer of inner parenchyma which is destined to

become nutritive tissue. Inner parenchyma is further covered by outer layer of parenchyma and a protective hard layer of sclerenchyma. Galls are connected to the host plant vascular system with vascular bundles to receive nutrients which are essential to gall development (Stone et al., 2002).

A fully-grown gall can be examined from two perspectives: external gall morphologies and internal gall structure. Recent studies have revealed that variation in external morphological characters is evolutionarily associated with an effort to escape from enemy attacks (Stone and Cook, 1998). External gall morphological characters are observed in a wide array from sticky outer surface to being non-sticky, from hairy or spiny extensions to smooth surface, from soft tissue surface to hard gall surface, from being attached onto the host tissue to being detachable (Ronquist and Liljeblad, 2001). It is thus suggested that outer gall morphology is the extended phenotypes of gallers' genes since developing larvae's genes take the control of gall formation, and this in turn leads to high outer gall diversity seen in galls. Gall phenotype evolution researches further proposed that being sticky is a synapomorphic (shared-derived) character state compared with non-sticky state, meanwhile spinelessness is an ancestral form than having spines (Ronquist and Liljeblad, 2001). Despite great variation observed in external gall morphologies internal gall structure show one difference among species: the number of larval chamber. Galls may be either with single larval chamber (unilocular), or many larval chambers (multilocular) can be present in a single gall. Multilocular galls may have up to 100 larval chambers in a gall (Rokas et al., 2003). Multilocularity is accepted as a derived character state over the unilocularity (Stone and Cook, 1998).

1.3 Oak Gall Wasp Diversity and the Significance for Turkey

Among the most known and well-studied insect taxa such as aphids (Homoptera: Aphididae), gall midges (Diptera: Cecidomyiidae), and sawflies (Hymenoptera: Tenthredinidae), oak gall wasps are the second largest radiating group after gall midges (Bihari et al., 2011; Resh et al., 2009; Stone and Schönrogge, 2003). Oak gall wasps from the family Cynipidae are grouped under superfamily Cynipoidea. The subfamily Cynipinae composed of eight tribes comprises gall

forming phytophagous wasps in addition to inquilines from the Synergini tribe: Synergini includes an estimated number of 176 species with no ability to induce their own galls and their larvae feed on galls of other cynipids. Gall inducers from tribe Aylacini forms galls on herbaceous plants while others from the tribes Diplolepidini, Eschatocerini, Pediaspidini, Paraulacini and Qwaqwaiini induce galls on plant families including Rosaceae, Fabaceae, Sapindaceae, Nothofagus, Salicaceae, respectively (Ronquist et al., 2015).

Oak gall wasps are from the tribe Cynipini with 1360 defined species classified in 40 genera distributed world-wide (Liljeblad and Ronquist, 1998; Liljeblad et al., 2011). They show wide distribution in the moderate regions of the Northern Hemisphere. Biogeographic and fossil evidence together with molecular data suggest that the major lineages of oak gall wasps are thought to diverge in Central America along with their oak hosts (Ronquist et al., 2015). Approximately 700 species of oak cynipids are found in the Nearctic region, while 200 species are found in the Palearctic region (Hayward and Stone, 2006).

Gall inducing cynipids show great adaptation through adaptive radiation to oak hosts with alternation between sexual and asexual generation (heterogony). Heterogony is directly associated with host alternation (heteroecy) (Stone et al., 2002). Among the eight tribes, alternation between asexual and sexual generation was only described in the Cynipini and Pediaspidini (Pujade-Villar et al., 2001). Among the Cynipini members *Cynips*, *Andricus*, *Neuroterus*, and *Biorhiza* are known with the life cycle of sexual and following asexual or parthenogenetic (agamic) generation: Sexual generation galls are formed in spring or early summer in Cerris section oaks while parthenogenetic generation galls are produced in fall in the members of section Quercus oaks. Sexual generation galls are often inconspicuous and small; however, parthenogenetic galls are quite conspicuous and larger in their size. Heterogony and heteroecy are suggested to evolve as a response to intraspecific competition. Considering biology and distribution of both host trees and their wasp parasites, oak gall wasps are proposed to cospeciate along with their hosts (Ronquist and Liljeblad, 2001).

In Turkey, all four heteroecious genera are present and both host taxa are commonly found in a wide range. There are 18 different oak taxa and their possible

hybrids representing *Quercus*, *Cerris*, and *Ilex* sections (Kasaplıgil, 1992; Yaltırık, 1984). According to the 2014 data of the Republic of Turkey Ministry of Forestry and Water Affairs General Directorate of Forestry, Turkey has nearly 30% of forestry area, of which nearly 6 476 277 hectares are composed of oak trees. Thus, oaks and oak gall wasps are important components of the Turkish biodiversity.

Although Cynipidae fauna of Turkey have been poorly studied, Boedenheimer (1941) recorded 21 species from 5 genera. Acatay (1943) and Alkan (1952) recorded 24 species from 5 genera, Baş (1973) reported 34 species from 5 genera, and Kıyak et al. (2008) stated the presence of 30 species from 7 genera. Melika and Stone (2001) defined a new Cynipidae species from Iran and Turkey, and Melika et al. (2004) described a new species from Turkey, Greece and Iran. A checklist of Turkish Cynipidae published by Katılmış and Kıyak (2008) reported the presence of 81 species in Turkey, and with recent studies, the number of cynipid species in Turkey increased to 90 (Kemal and Koçak, 2010; Mutun and Dinç, 2011). Five new species and two new records from Turkey were described by Dinç et al. (2014) and Mutun et al. (2014) for the first time for the Turkish cynipid fauna. In a recent study, chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu (1951) (Hymenoptera: Cynipidae), was reported from Turkey as an important pest species with significant economic damage given by the species where it shows distribution (İpekdağ et al., 2015).

1.4 The Cherry Gall: *Cynips quercusfolii* (Linnaeus, 1758)

Cynips quercusfolii (Linnaeus, 1758) (Hymenoptera: Cynipidae) is an oak gall wasp species with a large distribution all over the territory of East and Central Europe from the Iberian Peninsula, Iran, Russia, middle flow of Ural River, Transcaucasus and Transcarpathian Region (Melika, 2006). Of the six *Cynips* species reported from Turkey, *Cynips quercusfolii* Linnaeus (1758) is a heterogonous species with an alternation between sexual and asexual generation. Sexual generation galls are produced on section *Cerris* oaks, and the asexual generation galls are produced on the section *Quercus* (white oaks) members. Sexual generation galls of *C. quercusfolii* are formed on *Q. virgiliana* (Dalla Torre and Kieffer, 1910). Asexual

generation galls of *C. quercusfolii* are formed on the underside of the leaves of white oak species including *Quercus petraea* (Matt.) Liebl., *Q. pubescens* Wild., *Q. robur* L. and *Q. frainetto* (Ionescu, 1973).

The life cycle of the species is quite complicated: first sexual generation deep-red galls appear in spring and become dark violet when mature. Females and males of the sexual generation individuals emerge in early July, after fertilization females use the veins on the underside of oak leaves for an oviposition site. Middle veins of the leaves are preferred for oviposition since it is crucial that middle veins of the middle lobes of oak leaves are generally the largest part of the leaves where high flow through of nutritional and assimilate materials, which in turn can increase subsequent survival chance of galls with the larva inside them (Giertych et al., 2013).

In late autumn, parthenogenetic females induce galls often on small adventitious buds and on the trunk or larger branches and rarely on dormant buds (Melika, 2006). Parthenogenetic generation galls are round, monolocular leaf galls, which are developed on *Q. petraea*, *Q. macranthera* Fisch. & C. A. Mey. Ex Hohen, *Q. pubescens*, *Q. robur*, and *Q. infectoria* Olivier. Asexual generation galls are initially green, later pale red and become yellowish brown when they are mature. Galls are solitary or occurring sometimes in groups of 2-3 (Figure 1.1 a and b). The gall usually falls with the leaves, but may dehisce independently (Melika, 2006). When adults are hatched from the galls through an exit hall, winged females are released singly.

The size of asexual *C. quercusfolii* females ranges from 3.5 mm to 3.9 mm, lateral side of head and mesosoma have setae, and overall body color is generally dark brown with some yellowish spots and black. While the antennae, veins of wings and legs are dark brown, the color of lower face, metanotum, propodeum, parallel lines of scutum, ventral mesosoma and metasoma is black (Giertych et al., 2013; Zargaran et al., 2011,).

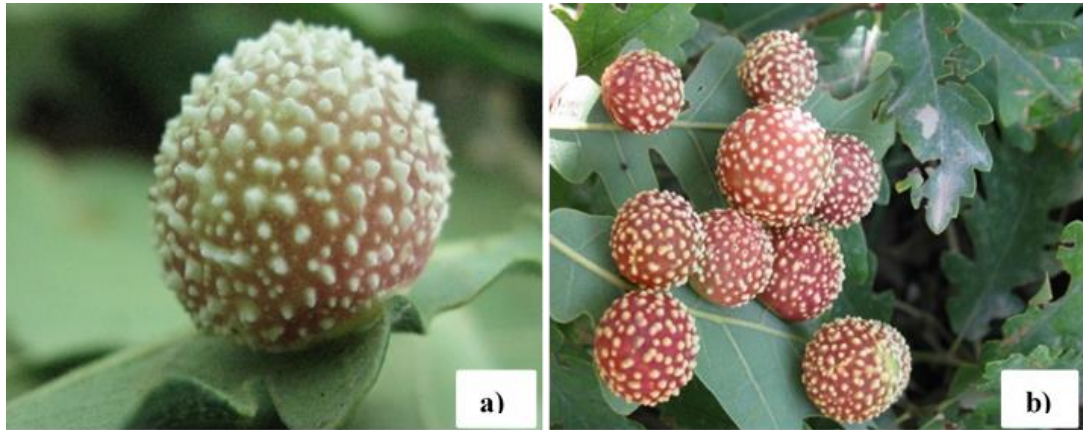


Figure 1.1. Parthenogenetic generation galls of the cherry gall, *C. quercusfolii*. a) Solitary gall, b) A group of galls.

1.5 Paleohistory and Paleogeography of Turkey

Turkey, more specifically Anatolia and Thrace, is located on the Alpine Himalayan orogeny and situated between 36-42° north latitude and between 26-45° east longitude. It is characterized by a very complex geologic history and it is one of the most active places in the world in terms of the recent geological and climatological events. The climatic changes that occurred during the glacial and interglacial periods, fractures, transgression, regression and folding due to volcanic and tectonic activities are the most crucial factors in the formation of complex topographical and climatological structure of Turkey (Okay, 2008).

The landmass today we call it as Turkey is originally composed of the three main plates from north to south; the Pontides, the Anatolides-Taurides and the Arabian platform. Formation of the area and these main continental fragments joined together in a prolonged period (Bozkurt, 2001). Between 290-245 million years ago (MYA) during Jura and the following Permian time there was only a single continental landmass on Earth; the Pangaea. It split into Gondwanaland in the south and Laurasia in the north, and Anatolia is known to have some affinities to both subcontinental masses. The Pontides composed of three terranes (Strandja, Sakarya and Istanbul) have Laurasian origin, and they have been joined into a single mass later around the middle Cretaceous times (Moix et al., 2008). The southern part of the Anatolides-Taurides show affinities to Gondwana, and separated from the

Gondwana in the Triassic times. At the beginning of the Triassic period (245-210 MYA), Karakaya marginal sea, also known as Karakaya complex, has developed on the subduction zone of the old ocean named Paleo-Tethys. In the Triassic period, the present eastern Mediterranean began to appear and this formation extending from the east to the Himalayas started to separate the Cimmerian continent. Cimmerian continent is consisted of parts of today's Turkey, Iran, Afghanistan, Tibet, and some vicinity areas. Cimmeria uplifted from the Gondwanan shores of the Paleo-Tethys Ocean during the Carboniferous-earliest Permian time (Şengör and Yılmaz, 1981). In the Jurassic period (210-145 MYA), the Cimmerian continent started to disintegrate, and two branches of Neo-Tethys ocean appeared. While northern branch of Neo-Tethys, including the İzmir-Ankara, the Intra-Pontide and the Inner Tauride oceans, separated the Anatolide - Tauride platform from Eurasia and isolated Sakarya continent, the southern branch separated this platform from the Gondwana land.

Northern and southern branch of Neo-Tethys reached their largest size in the beginning of Cretaceous period (145-65 MYA) and two oceans started to contract by subduction beneath the Eurasia, Sakarya and Anatolide-Tauride platforms throughout the late Cretaceous. On the other hand, southern side of Eurasia, the present Black Sea began to open (Rögl, 1999). The main formation of the contemporary Turkey continued through the Miocene and Oligocene. At the end of the Mesozoic time African continent in the south collided with the northern land masses of Europe and Asia. The collision initiated Alpine orogenic events and associated events giving rise to the Alps, the Hellenic and the Dinaric Mountains in Yugoslavia, Albania and Greece and the Taurus mountains the south and North Anatolian mountain lines in the north of Turkey around the end of the Mesozoic (65- 70 MYA). During Miocene, sediments that were present under the sea began to lift up and the altitudinal uplift approximated near 1000 meters in Anatolia. Meanwhile, the central part of Anatolia was lowland and covered by a lake system. Regressive and trans-regressive cycles of this time resulted in the exchange of fauna between Turkey and Europe, and Africa repeatedly during Miocene (Çıplak, 2004).

The uplifting continued through the Oligocene (34- 23 MYA) and Miocene (23-5 MYA), followed by the major formation of the mountain ranges and their first steps were taking place in the region. At the same time, Neo-Tethys came to an end

in Oligocene with the collision of the Eurasian and African plates. During these ongoing events initiation of the Taurus mountains as part of the Alp-Himalayan orogenic belt and the closure of the Neo-Tethys resulted in the formation of the Anatolian formations (Karadenizli, 2011). In the early and by mid Miocene except few massive in the Northwestern and Southeastern Anatolia (e.g. Bitlis massive) most parts of Anatolia were under sea. By mid-Miocene with the continuation of the uplifting of the terrane, the eastern part of Anatolia became to have higher altitude compared with the central and western Anatolia. Meanwhile, as the remnant of the Tethys Sea, the Mediterranean Sea was fully formed, humidity and the vegetation were already changing the environmental conditions of the area. Main reshaping of Anatolia and Thrace throughout the Miocene (Aquitainian (23-20.4 MYA), Burdigalian (20.4-16 MYA), Langhian (16-13.8 MYA), Serravallian (13.8- 11.6 MYA), Tortonian (11.6-7.2 MYA), and Messinian (7.2-5.3 MYA), and a common aridity crisis occurred at the end of the Miocene with the Messinian crisis. The partial Messinian crisis was initiated by the closure of the Gibraltar letting the desiccation of the Mediterranean which is characterized by some faunal exchanges between Anatolia and Greece.

The reformation of Turkish fauna and flora together with the paleogeographic and geologic events continued through Pliocene (5-2.6 MYA). The flooding of the Mediterranean to the northern part over the Aegean part and to the current Black Sea caused the Black Sea to have higher salinity. These ongoing events affected organisms that already settled in some island-like lands here in this period causing isolation of those populations.

The contemporary Turkish diversity regarding both species and subspecies number and the genetic variation exist in those taxa are greatly affected by the ongoing events of the Quaternary period (1.8 MYA-current). The Quaternary divided into two main epochs as the Pleistocene (1.8-0.01MYA) and the Holocene (0.01 MYA–current time). The Pleistocene with four major glacial ages (Günz (800.000-600.000 YA), Mindel (500.000-200.000 YA), Riss (200.000-130.000 YA) Würm (115.000- 11.700 YA), and the interglacial periods between each had the greatest impact on the Turkish faunal elements as well as in the genetic composition of those taxa (Çıplak 2003). Although the Anatolian faunal composition was shaped most by

these ongoing events the Anatolian geography and topography were not changing much, except the coastal shape changes and the opening and the closing of the Bosphorus and Dardanelles several times (Demirsoy, 1999).

1.6 Turkey: A Biodiversity Hotspot and Refuge Area

Turkey is one of the world's 35 biodiversity hotspots, and it is known with its high plant and animal species richness in addition to endemic taxa (Gür, 2016). Nearly 11.000 plant and 80.000 animal species are estimated to exist in Turkey, and the true number is assumed to be much higher than these numbers (Demirsoy, 2002). One responsible factor for this high species richness in Turkey is partly explained by the presence of three phytogeographic regions as the Caucasus, Irano-Anatolian, and the Mediterranean (OGM, 2014).

The richness is also attributed to the fact that Turkey is geographically located at a significant transect between Europe and Asia (Şekercioğlu et al., 2011). In relation to these factors, Turkey has different climatic and ecological zones. Rainy-humid temperate boreal climate is dominant in the north while eremial climatic characteristic that is hot and arid desert climate is observed in the south-eastern Turkey. Cold and arid Siberian climate is seen in the eastern part, and the Mediterranean climate which is warm and dry in the summers and rainy and warm in the winters is seen in the west and the southwest of Turkey. Unique tectonic and paleogeographic history, its placement between the temperate and subtropical regions at the junction of three continental masses, diverse climatic regimes exist in Turkey (Demirsoy, 1996).

Another factor created high species diversity in Anatolia is the mountain ranges (Şekercioğlu, 2011). In addition to high individual elevations, four mountain ranges acting as belts are significant barriers in the formation of the Anatolian species richness as well as the Anatolian phylogeography: the Taurus mountains in the south separates the Mediterranean from the inner steppe environments, the northern Anatolian mountains is a zone of separation of the Black Sea coast from the inner Anatolia, the western Anatolian mountains is a geographic barrier separating

the west from the inner Anatolia, and the Anatolian Diagonal running from the northeastern part of Turkey through the southeastern Anatolia (Davis, 1971).

First discovered by Cullen, and then referred to by Davis in 1971 based on the distribution of plant species, the Anatolian Diagonal runs from the southern part of Gümüşhane and Bayburt towards the southwestern part of Turkey. It makes a bifurcation around the Mediterranean part; one leads to the Amanos Mountains, and another one of the split leads to the Central Taurus Mountains. Thus, these natural barriers have been proven to play significant role in distribution of species richness and geographical distribution of the Anatolian flora and fauna (Çıplak et al., 1993; Çıplak, 2008; Davis, 1971, Ekim and Güner, 1986). The Anatolian Diagonal thus divides the Turkish lands into the eastern and western parts due to the altitudinal differences on both sides. The central lowland in the western part of Anatolia is generally below 1500 m while the eastern highland of Anatolia is over 1500 m. The Anatolian Diagonal acted as a geographic barrier for species, and shaped the dispersion of genetic lineages, therefore it has been recognized as a boundary between western/central and eastern Anatolian faunas and floras (Gülkaç and Yüksel, 1999; Kaya et al., 2015; Korkmaz et al., 2014). Therefore, in this thesis the terms “eastern” and “western” population used afterwards refer to this general division (Figure 1.2). According to the two competing hypotheses on the effects of this belt to create such a break focused on either the physical and climatic differences between the east and the west (Davis, 1971), and ecological and climatic components playing more significant role (Ekim and Güner, 1986). In any case, the fact that the Anatolian Diagonal is a significant barrier between eastern and western/central part of Turkey producing such current pattern observed today.

Turkey is also expected to be much more strongly affected by climatic fluctuations of the glacial periods. It has been suggested that the Mediterranean Basin terranes were under the effect of those ongoing climatic changes than any other parts of the world (Petit et al., 2005). One of the major response of species to the counteracting effects of the glacial cycles are retraction/contraction and expansion of the species range during the glacial and interglacial periods, respectively. During the prolonged cooling times of the glacial cycles populations/species retract their range, and as just the opposite they expand when the environmental conditions are more

suitable for survival (Hewitt, 2000). Even some populations may have been long isolated in protected areas during the cold periods and then expand their range afterwards (Hewitt, 1999). The effects of the glacial period-associated climatic fluctuations were felt greatly especially in the central and northern parts of Europe; many plant and animal species have either disappeared or survived in shelter (refuge) areas (Schmitt, 2007).

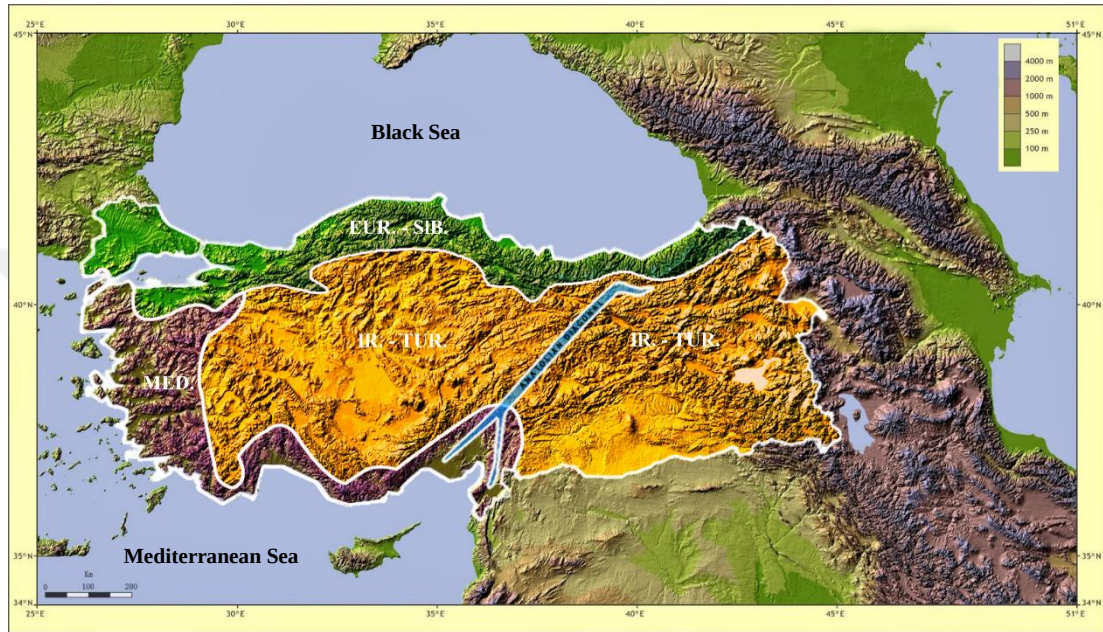


Figure 1.2. The location of the Anatolian Diagonal and the approximate location of the three phytogeographic regions in Turkey. Euro-Siberian (EUR. – SIB.), Mediterranean (MED.) and Irano-Turanian (IR.-TUR.)

During the last glacial periods, most parts of Turkey was never covered by glaciers except the northernmost region of Anatolia and the tops of the individual mountains (Demirsoy, 1996), thus allowing those species to utilize some protected areas as shelter areas in Anatolia. According to Hewitt (1996), shelter areas in Turkey, together with those in Europe, the Balkans, the Iberian Peninsula and Italian refugia, allowed species to survive against harsh environmental conditions, and taxa survived in refugia expanded their range after the cooler times (Hewitt, 2000). These expansion-contraction events have taken place several times during glacial and interglacial cycles of the Pleistocene explained later under the ‘expansion-contraction model’ (Proven and Bennet, 2008). In fact, Turkey is a suitable candidate for searching the counter effects of Quaternary climatic cycles, and thus have been

taking great attention especially in the last two decades. Several animal and plant species were investigated regarding the effects of the Late Quaternary glacial-interglacial cycles including ground squirrels (Gündüz et al., 2007; Gür, 2013), grasshoppers (Çıplak, 2004, 2008), frogs (Özdemir et al., 2014), mountain frogs (Veith et al., 2003), lizards (Bellati et al., 2011), and plants species (Ansell et al., 2011). During the glacial cycles, Anatolia was not a homogenous refugium, in fact refugium within refugium hypothesis has been proposed to explain the existence of several distinct refugia in Turkey (Bilgin, 2011; Çıplak, 2008).

Turkey is also suggested to be a genetic source for the European populations (Dubey et al., 2007). Indeed, Turkey is the center of origin for oak gall wasp taxa that are currently distributed in Europe, and deserve the name of the ‘cradle of the oak gall wasps’ (Rokas et al., 2003). It was clearly shown that the Anatolian populations of *Andricus quercustozae* (Bosc, 1792) constituted the center of dispersal origin of the species, stayed in a prolonged time in Anatolia, and after the glaciation time it expanded its range out of Turkey (Out of Anatolia hypothesis).

1.7 Molecular Markers in Phylogeographic and Phylogenetic Studies

Twentieth century have witnessed increasingly rapid development of molecular techniques used in systematics, molecular evolution, and phylogeography. We owe this rapid pace of progress to the use of either single or a combination of molecular markers to investigate populations, species and taxa at higher categories for resolving population genetics, the processes that created the contemporary population genetic structure of species, the pattern to draw general conclusions, phylogenetics and the spatial distribution of genetic variation across the sampled area. Thus, these novel methods and application allowed researchers to assess differences in a more detailed and precise way (Grechko, 2002; Yang and Rannala, 2012).

Specifically, the field of Phylogeography, which is concerned with the examination of the relationships between geography and mitochondrial DNA (mtDNA) genealogies arose as a new and fruitful area in Biology (Avise et al., 1987; Hickerson et al., 2010). Phylogeography as a field utilized molecular markers in a

broad spectrum of animal taxa (Chapco, 1997; Strange and Burr, 1997). By doing that, it reconstructed the history of diversity and speciation patterns, and has proven to be useful in taxonomic and evolutionary studies including distribution of genetic diversity across the range of a species and structuring of the genetic variation presenting diverse types of specific patterns (Avice, 1994; Bermingham and Moritz, 1998).

Mitochondrial DNA has been often used as a molecular marker for phylogenetic and phylogeographic studies of both populations and species, and has great impact in entomological studies (Cameron, 2014). Many researches have been conducted on phylogeography of various groups of animals including oak gall wasps where they showed that mtDNA haplotypes were indeed geographically clustered into different groupings depicting some general patterns (Challis et al., 2007; Clemente et al., 2000; Dinç and Mutun, 2011; Horn et al., 2006; Mutun, 2010; Sert et al., 2005; Stone et al., 2007; Stone et al., 2008). Mitochondrial DNA also known as the mitogenome or mtgenome is maternally inherited circular genome ranging from 15.0 to 17.0 kilobases (Kb), although sizes of 14-26 Kb have been found in different insect species due to differences in the copy number of short, tandemly repeated sequences or the duplication or deletion of sequences (Crozier and Crozier, 1993; Lemire, 2005). In animals, the mitochondrial genome contains 37 genes and a control region that is, in insects, called as the A+T- rich region (Harrison, 1989; Zhang et al., 1995). Among the 37 genes, there are 13 protein encoding genes, 22 tRNA genes and 2 ribosomal RNA genes (Harrison, 1989; Moritz et al., 1987) (Figure 1.3). Gene order variation occurs both between and within animal phyla, but within insects tRNA genes are known to vary in position both between the orders Diptera and Hymenoptera (Crozier and Crozier, 1993).

The evolutionary subdivision of a species is most likely to be resolved by studying mtDNA variation (Boore, 1999). Since mtDNA has high mutation rate compared to single-copy nuclear DNA, and under equilibrium conditions its maternal inheritance gives it an effective population size one quarter that of nuclear DNA. Therefore, population differentiation is expected to occur more rapidly for mtDNA than nuclear DNA making it to be more sensitive indicator of population structuring (Ballard and Pichaud, 2014; Galtier et al., 2009). Moreover, mtDNA is not subject to

recombination and variants are inherited as haplotype clones which can be ordered phylogenetically in a way which reflects the evolutionary genealogy of the variants (Arias et al., 2006). However, it has been recognized that molecular studies should not rely exclusively on only a set of sequences such as mtDNA. Covering different genes in a molecular study enhances the possibility of convergence to the correct outcome and phylogenetic tree (Crozier, 1990). Thus, mtDNA and nuclear genes are used together very efficiently to study population genetic structure, current and historical population sizes, dispersal routes, center of origin, evolutionary relationships of organisms, gene flow and population differentiation, molecular evolution, phylogenetics and phylogeography studies (Avice, 1994; Bermingham and Avice, 1986).

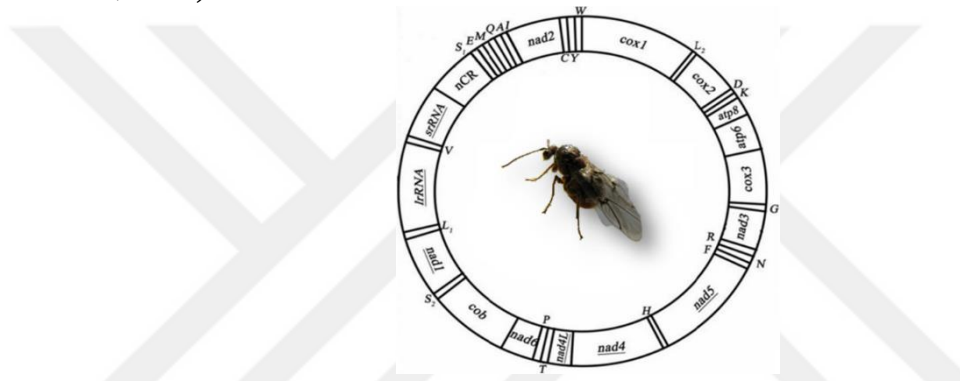


Figure 1.3. Insect mitogenome.

Internal transcribed spacer regions (ITS) from nuclear DNA are found in the 18S, 5.8S and 28S segments of ribosomal DNA (rDNA) (Buburuzan et al., 2007) (Figure 1.4). Owing to its rapid rate of evolution and lower amount of evolutionary constraint acting on such non-functional sequences such as ITS region make the region very suitable for studies that are associated with closely related species, identification of cryptic species and phylogeographic studies (Kumari and Subbotin, 2012; Mukabayire et al., 1999; Wagener et al., 2006; Wörheide et al., 2002; Yajie Ji, 2003). Therefore, application of at least one nuclear and a mitochondrially encoded gene segment as a combination provides more robust results compared to using only a single gene region. For example; general pattern for the commonly used mitochondrial cytochrome b gene (cyt b) resolves intermediate nodes and tips quite well, but provides less resolution at deeper nodes and those deeper nodes are better resolved by nuclear genes (Cook et al., 2002). Thus, a combination of mtDNA cyt b

gene and nuclear DNA (nDNA) ITS2 region were applied as a combination to achieve the goals of the thesis.



Figure 1.4. The nuclear ribosomal operon system which includes the non-coding ITS1 and ITS2 region.

1.8 Phylogeographic Studies Utilized Genetic Markers on Oak Gall Wasps in Turkey

With advances in the use of molecular techniques, especially sequencing of the genes or non-coding DNA regions researches on oak gall wasps in the western Palearctic and specifically in Turkey have been accelerated in the last two decades. Utilizing mainly cyt b gene sequence data from mitogenome at population- species level and ITS region were heavily applied to test some hypotheses relevant to the phylogenetics and phylogeography of oak gall wasps.

First detailed study using the molecular markers to test the origin of the oak gall wasp species, *Andricus quercustozae* distributed across the western Palearctic including Turkey used cyt b gene sequencing and protein electrophoresis data. Results indicated that Turkey was the center of origin and it dispersed out of Anatolia towards Europe allowing the scientists to use the term for Anatolia as ‘the cradle of oak gall wasps’. The Anatolian populations incorporated higher genetic diversity compared to the European populations, letting the scientists to accept Anatolia as one of the refuge areas for the oak gall wasps during the Pleistocene glacial cycles. Moreover, the Turkish refugium carried much higher genetic variation when the three other refugia (the Iberian, Italian and the Balkans) of Europe were taken into consideration (Rokas et al., 2003a).

A Palearctic-wide conducted investigation on 62 oak gall wasp species sampled also from Turkey covered the sequence analysis to search for the possible shelter areas of oak gall wasps. Refuge specific haplotypes have been recovered from Turkey in all clades (*Andricus kollari* (Hartig, 1843), *A. hartigi* (Hartig, 1843), *A.*

quercucalidis (Burgsdorf, 1783), *Andricus grossularia* Giraud 1859, *Andricus foecundatrix* (Hartig, 1840), and *Cynips* genus). Haplotypes detected in the Turkish populations formed a distinct clade structure being separated by high support values from the rest of haplotypes found in Italy/Hungary (Rokas et al., 2003b).

Longitudinal range expansion was studied in *A. coriarius* (Hartig, 1843) sampled from a large part of Palearctic covering a range from Spain, Italy, Croatia, Hungary, Slovakia, Greece, Lebanon, Turkey to Iran. Both nuclear and mitochondrial sequences data provided an evidence for the presence of a cryptic species distributed in the eastern part of its range covering Iran and Turkey. Further, the presence of three distinct lineages originated in the east; one lineage gave rise to all European populations dispersing from the eastern part of the sampled area around 1.6 MYA (Challis et al., 2007).

Oak gall wasp studies have not only revealed the natural dispersal of gall wasps from Anatolia to Europe, but also human-mediated dispersal was disclosed. On the colonization of northern Europe by *A. kollari* proved that human mediation was responsible for the dispersal of some oak gall wasp species. The findings of the study revealed that populations in France were colonized from Italy and Hungary, however the populations in the United Kingdom (UK) and neighboring parts of coastal northern France shared an eastern origin indicating Turkey and vicinity. In fact, it is known that during the Ottoman Empire time tons of galls were sold to different European countries, particularly to England as trade goods from the eastern Mediterranean around the 1830s (Stone et al., 2007).

Intraspecific genetic variation and the distribution of genetic diversity across Turkey were investigated in the gall wasp *A. caputmedusae* (Hartig, 1843). A large proportion of the mitogenome was digested with the restriction enzymes, and the obtained haplotype pattern was used to draw some conclusions on the population genetic structure of the species. Study conducted on 26 populations revealed a conspicuous genetic break between both sides of the Anatolian Diagonal. A clear pattern in the geographic distribution of haplotypes as east/in the Anatolian Diagonal, southeast/near the west of the Anatolian Diagonal, and west of the Anatolian Diagonal was apparent, and the data further provided evidence for the significance of geographic barriers to create a genetic discontinuity across the range

of a species. In the study, the effects of the last glacial periods and associated climatic fluctuations were also implied (Mutun, 2010).

Intraspecific genetic diversity and the phylogenetic relationships of *Andricus lucidus* haplotypes representing nine populations from the eastern part of Turkey showed high haplotype and nucleotide diversity in all populations with the formation of two main clades. The clade separation was associated with the topography and the effects of the environment coinciding with the Maraş Triangle (Mutun, 2011).

The use of PCR-RFLP method to assess the variation in *A. quercustozae* showed the existence of three main groupings of haplotypes: a mixture of east and west, west, and east. The placement of the east/west mixed haplotypes at the basal position in the phylogenetic tree implied that the species was quite commonly distributed once in Turkey. The study showed that *A. quercustozae* haplotypes grouped geographically, and for this clustering topography and the evolutionary history of Turkey were responsible (Dinç and Mutun, 2011).

Assessment of genetic variation in the sampled populations of Turkey was studied in *A. gallaetinctoriae*. Across Turkey 13 sampled populations, eight were either in or east of the Anatolian Diagonal, and five were in the west. The 15-defined haplotype displayed high genetic variation meanwhile indicating the physical factors to shape current distribution of this diversity (Mutun et. al., 2013).

Similarly, *A. lignicola* was investigated through sequencing mitochondrial cyt b gene from 15 populations across Anatolia. Eighteen distinct sequences of the species constituted two major clade structures. Furthermore, the existence of a cryptic species within *A. lignicola* (Hartig, 1843) was proposed by phylogenetic analysis. The genetic divergence values up to 10.6% were used to support the species separation. The most divergent lineages were split from each other as high as 46 substitutions. Genetic diversity estimates revealed the existence of a cryptic species (Mutun and Karagözoğlu, 2015).

Furthermore, both nuclear and mitochondrial sequence data of *Trigonaspis synaspis* (Hartig, 1841) from 21 populations used to unveil phylogeography of the species produced 50 cyt b haplotypes and 15 ITS2 alleles, respectively. High

mitochondrial DNA diversity compared to the nuclear DNA was conspicuous. Phylogenetic and phylogeographic analyses proved the importance of the Quaternary climatic fluctuations in Turkey. The pre-Pleistocene oscillations started around 2.4 MYA and the subsequent fluctuations greatly shaped the phylogenetic structure of the species. During the Pleistocene glaciation times, several lines of diversifications in the *T. synaspis* lineages were seen clearly (Mutun and Atay, 2015). Finally, a recent review of the Turkish oak gall wasps, a general pattern on the phylogeographic structure of the species in Turkey was put forward explaining the significant effects of the geographic barriers for shaping the contemporary structure of the oak gall wasps (Mutun, 2016).



2. AIM AND SCOPE OF THE STUDY

DNA sequences from two independently transmitted genomes were used in this thesis:

1. to reveal haplotype and nucleotide diversity, average genetic diversity of the *C. quercusfolii* across its distribution range in Turkey.
2. to figure out the geographic positioning of genetic variation of *C. quercusfolii* in Turkey.
3. to estimate times of divergences between and among major genetic lineages of the *C. quercusfolii*.
4. to investigate if Pleistocene had any effects on the population genetic structure of the *C. quercusfolii* to create some types of unique phylogeographic sub-structuring.
5. to search for the possible effects of the topography and other geographic factors to create the current population genetic structure of the *C. quercusfolii* in Turkey.
6. to draw some general conclusions on the Turkish oak gall wasps, so that information obtained with this thesis can be used to enlighten the other taxa showing similar distribution.

3. MATERIALS AND METHODS

3.1 Sampling of the Species

To achieve main goals of this thesis, it was necessary to collect as many specimens as possible from a high number of localities as far as the thesis budget and time would allow. Thus, through conducting detailed sampling of the species it was possible to reveal the exact distribution of the species in Turkey.

Only asexual generation galls were used in this thesis. For collecting parthenogenetic generation galls several collecting trips were conducted during the late summer and early fall seasons of 2010-2014. After expedition trips, 294 *Cynips quercusfolii* galls were collected from 38 locations that covered all distribution range of the species in Turkey (Figure 3.1). All relevant information including type of host plant, coordinates and altitude were noted for each location (Table 3.1). A Garmin model Geographic Positioning System (GPS) was used for recording coordinates. Despite all the collecting efforts no galls were found at some visited localities due to the absence of proper oak host plants, or in certain cases no adults would be reared successfully from the galls.

After collecting galls, they were labelled and placed in the insulated plastic bags and brought to the Molecular Zoology Laboratory, Abant İzzet Baysal University, Bolu. For rearing adults, galls were put in labelled jars, and covered by tulle for insulation, kept at room temperature and checked daily for the emergence of adults. In Figure 3.2 newly hatched adult and exit whole were shown (Figure 3.2 a and b, respectively). Hatched adults were stored at -80 °C until DNA isolation. *Cynips divisa* Hartig (1840) and *C. longiventris* Hartig (1840) individuals collected from different localities (Table 3.1) were used as outgroups in all phylogenetic analyses.

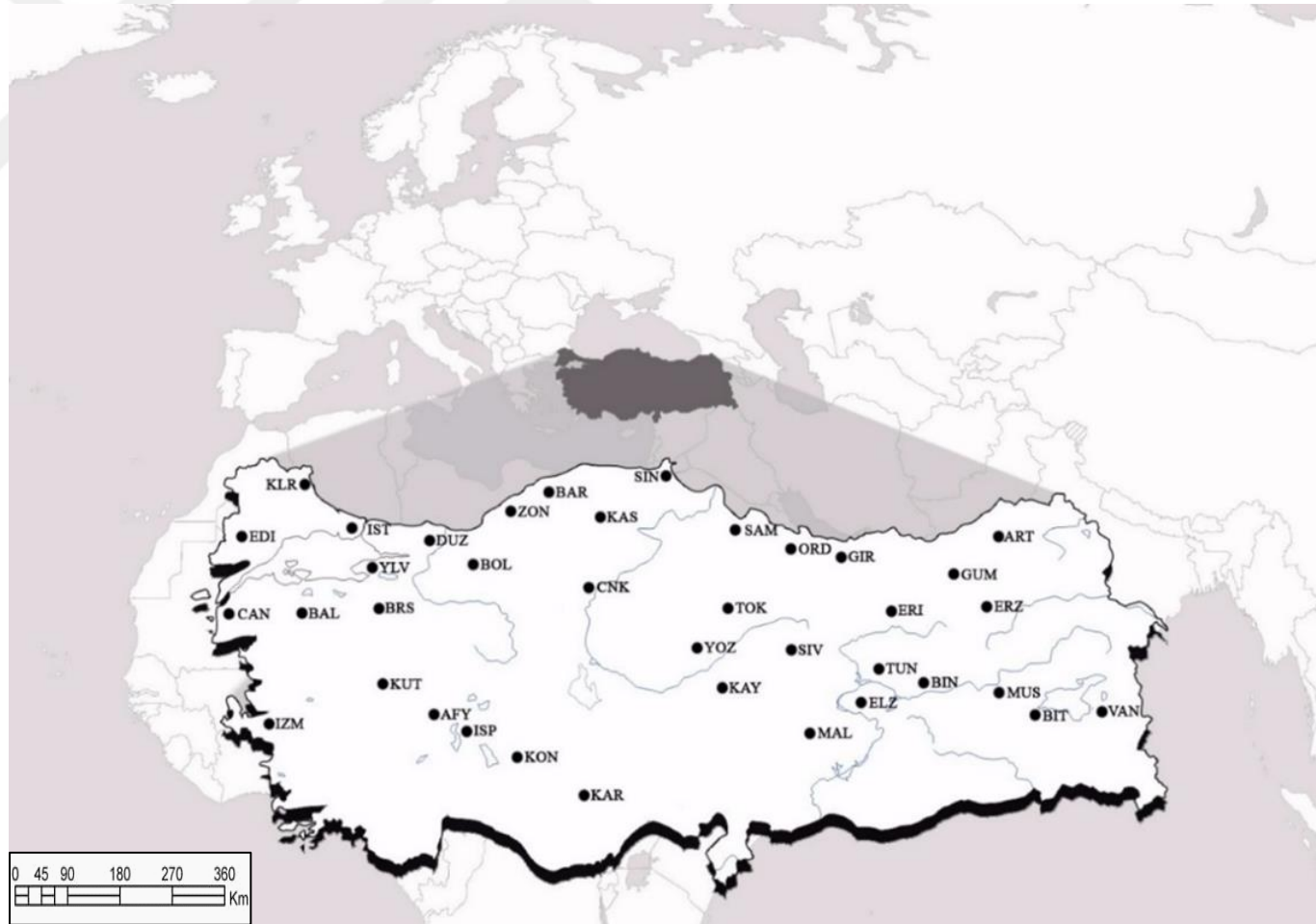


Figure 3.1. Sampling sites for *C. quercusfolii*. Population abbreviations and coordinates are shown in Table 1.

Table 3. 1. Samples localities of *C. quercusfolii*, abbreviation of each locality, coordinates, sample size, and the host at each location. Two outgroup information is also provided at the bottom of the Table.

<i>Cynips quercusfolii</i>							
Population	Abbreviation	Coordinates	Sample	Population	Abbreviation	Coordinates	Sample
AFYON	AFY	N 38° 46.621' E 30° 41.384'	10	İZMİR	IZM	N 39° 25.547' E 27° 06.827'	10
ARTVİN	ART	N 41° 26.068' E 42° 15.590'	2	KARAMAN	KAR	N 37° 39.117' E 33° 16.049'	10
BALIKESİR	BAL	N 39° 82.883' E 27° 81.699'	10	KASTAMONU	KAS	N 41° 27.204' E 33° 15.061'	11
BARTIN	BAR	N 41° 57.585' E 32° 62.565'	1	KAYSERİ	KAY	N 38° 37.063' E 36° 23.143'	11
BİNGÖL	BIN	N 38° 92.856' E 40° 36.798'	12	KIRKLARELİ	KLR	N 41° 85.992' E 27° 81.336'	10
BİTLİS	BIT	N 38° 43.688' E 42° 55.733'	10	KONYA	KON	N 36° 98.244' E 32° 40.465'	10
BOLU	BOL	N 40° 99.628' E 32° 06.246'	10	KÜTAHYA	KUT	N 39° 45.540' E 30° 25.914'	10
BURSA	BRS	N 40° 54.139' E 29° 27.262'	9	MALATYA	MAL	N 38° 99.107' E 37° 81.619'	3
ÇANAKKALE	CAN	N 39° 92.080' E 27° 27.564'	10	MUŞ	MUS	N 38° 64.304' E 41° 75.369'	11
ÇANKIRI	CNK	N 40° 71.351' E 33° 01.644'	10	ORDU	ORD	N 40° 45.983' E 37° 38.665'	2
DÜZCE	DUZ	N 40° 54.330' E 31° 10.246'	2	SAMSUN	SAM	N 41° 00.933' E 35° 87.766'	11
EDİRNE	EDI	N 41° 27.295' E 26° 78.507'	7	SİNOP	SIN	N 41° 80.846' E 34° 63.325'	10
ELAZIĞ	ELA	N 38° 57.990' E 38° 86.239'	5	SİVAS	SIV	N 39° 46.451' E 38° 00.067'	6
ERZİNCAN	ERI	N 39° 83.621' E 38° 75.296'	10	TOKAT	TOK	N 40° 48.323' E 36° 95.483'	1
ERZURUM	ERZ	N 39° 86.495' E 40° 62.626'	4	TUNCELİ	TUN	N 39° 59.013' E 39° 86.258'	1
GİRESUN	GIR	N 40° 26.610' E 38° 94.630'	10	VAN	VAN	N 38° 08.991' E 43° 09.806'	10
GÜMÜŞHANE	GUM	N 40° 26.209' E 39° 46.407'	11	YALOVA	YLV	N 40° 54.139' E 29° 27.262'	10
ISPARTA	ISP	N 37° 73.587' E 30° 83.298'	10	YOZGAT	YOZ	N 40° 10.859' E 35° 81.030'	1
İSTANBUL	IST	N 41° 19.981' E 28° 94.030'	10	ZONGUDAK	ZON	N 41° 42.168' E 32° 17.702'	3

<i>Cynips divisa</i> (Outgroup 1) Cyt b with 3 Haplotypes				<i>Cynips longiventris</i> (Outgroup 2) Cyt b with 2 Haplotypes			
Population	Abbreviation	Coordinates	Sample	Population	Abbreviation	Coordinates	Sample
ÇANKIRI	CNK	N 40° 71.351' E 33° 01.644'	2	EDİRNE	EDI	N 41° 27.295' E 26° 78.507'	3
TOKAT	TOK	N 40° 48.323' E 36° 95.483'	1				

<i>Cynips divisa</i> (Outgroup 1) ITS2 with 2 Allels				<i>Cynips longiventris</i> (Outgroup 2) ITS2 with 1 Allele			
Population	Abbreviation	Coordinates	Sample	Population	Abbreviation	Coordinates	Sample
ÇANKIRI	CNK	N 40° 71.351' E 33° 01.644'	2	EDİRNE	EDI	N 41° 27.295' E 26° 78.507'	3
TOKAT	TOK	N 40° 48.323' E 36° 95.483'	1				

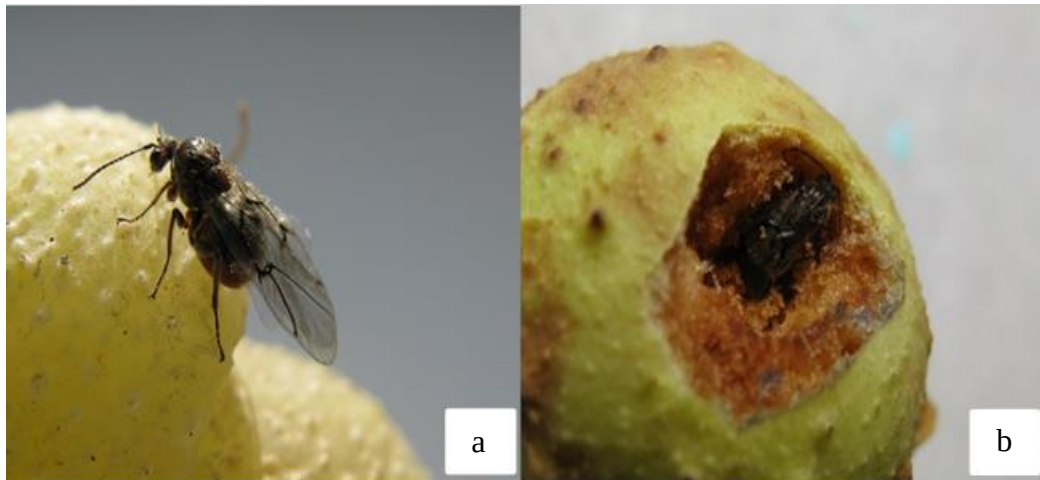


Figure 3.2. a) An adult individual of *C. quercusfolii*. b) The exit hole of the adult after hatching.

3.2 DNA Isolation

Total genomic DNA was isolated from single adult specimens using the DNeasy Tissue Kit (QIAGEN) following the manufacturer's instructions as following:

- a) Individual gall wasps were placed in 1.5 ml microcentrifuge tubes and kept at Thermo Fisher Scientific model -80 °C ultra-low freezer. For macerating insect into smaller pieces each specimen was overlaid with 180 µl ATL buffer and 20 µl of proteinase K (Sigma P2308). Sterile pellet pestles made of glass were used for grinding samples.
- b) The tubes were vortexed with Velp Scientifica model vortex and the samples were incubated overnight at 56 °C in a shaking JP Selecta SA model water bath until tissue was completely lysed.
- c) The incubated samples were vortexed for 15 seconds and 200 µl of AL buffer was added to the samples and mixed thoroughly. After adding 200 µl of 100% ethanol (Sigma 32221) the tubes were mixed again thoroughly.
- d) The samples were transferred into a DNeasy Mini spin column in a 2 ml collection tubes and centrifuged by Nüve NF800R model centrifuge at 8000 rpm for 1 minute.
- e) After discarding the flow-through the spin columns were placed in a new 2 ml collection tubes and 500 µl of buffer AW1 was added to each tube and centrifuged for 1 minute at 14000 rpm and the same steps were repeated for obtaining clean sample.
- f) After final centrifugation step, the spin columns were transferred into a new microcentrifuge tube. For elution, 200 µl of buffer AE was added to the samples and incubated for 5 minutes at room temperature.
- g) In the final stage, samples were centrifuged for 1 minute at 8000 rpm. Collected liquid carrying DNA sample was either kept on ice or at -20 °C until agarose gel electrophoresis.

For ensuring DNA extraction, 5 µl of extracted DNA samples were added to 3 µl of DNA loading dye, and the mixture was run on 1% agarose gel containing ethidium bromide (Sigma E5134). As the tank running buffer, 1X TBE (0,089 M Tris (Sigma T1503), 0,089 M Boric acid (Sigma B6768), 0,001 M disodium EDTA (Sigma E5134)) was used, and the gel was electrophoresed for 1 hour at 100 volts (V) and 50 mA using Consort E452 model gel electrophoresis power supply. In each gel 1 Kb λ molecular marker (Sigma D3937) was used to check the size of DNA. Samples were visualized using Ultra-Lum Ultra Cam A650 model UV transilluminator system and photographed with Canon Powershot a650 model camera. An agarose gel photograph of DNA isolated from some *C. quercusfolii* individuals was shown in Figure 3.3.

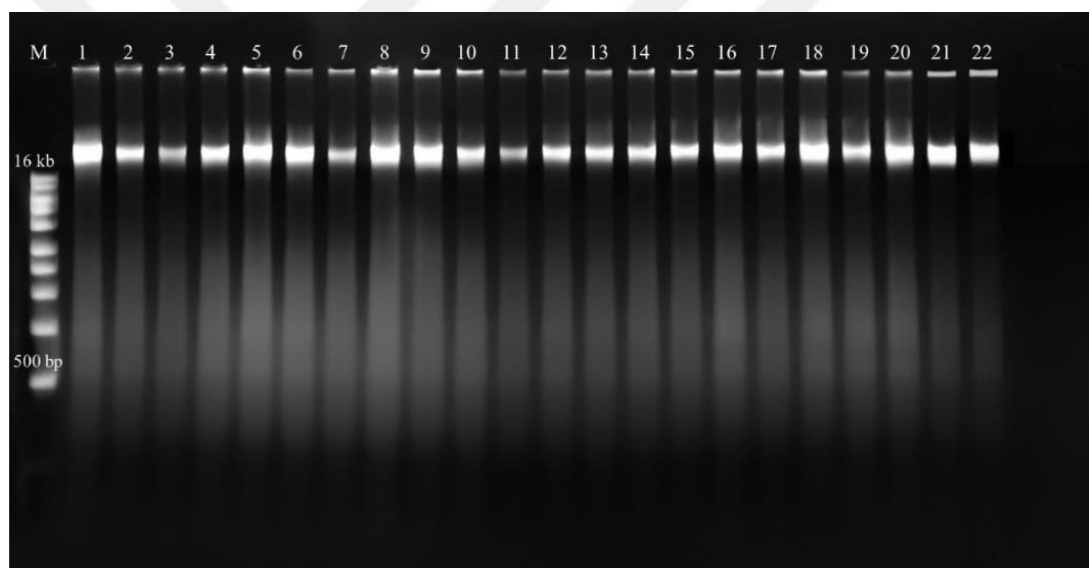


Figure 3.3. Isolated DNA photograph of *C. quercusfolii* specimens. M: 1 Kb marker, 1-10: Sinop, 11-20: Giresun, 21-22: Ordu.

3.3 PCR Amplification, Purification and Sequencing

Both mitochondrial cytochrome b gene region and internal transcribed spacer of nuclear genome were amplified using a Bioer model thermal cycler. Primers for both regions were HPLC purified and purchased from various companies. Promega (M8290), Sigma (D1806) and Vivantis (PL1202) brand Taq polymerase packs were

used including DNA polymerase, dNTP, MgCl₂ and PCR buffer. The detailed amplification procedures were given below separately for both DNA regions.

3.3.1 Mitochondrial Cytochrome b Gene

A pair of primers (CB1 and CB2) that were used previously in other Hymenoptera species were used to amplify a 433 basepair (bp) fragment of mitochondrial cytochrome b gene (Francisco et al., 2001; Moretto and Arias, 2005). Primer sequences were as follows: CB-J-10933 (CB1) 5'- TATGTACTACCATGAG GACAAATATC-3', and CB-N-11367(CB2) 5'- ATTACACCTCCTAATTTATTA GGAAT-3' (Simons et al., 1994). All PCR reactions were carried out in 40 µl volumes containing 1.6 µl of the total DNA extraction, 4.0 µl 10X PCR buffer (Promega), 3.2 µl MgCl₂ (25mM), 0.8 µl dNTPs (2mM each), 0.56 µl of each primer (20µM) and 0.56 µl of Taq DNA Polymerase (Vivantis). Thermocycling was performed using the following program: 3 min at 94 °C, 35 cycles of 30 secs at 94 °C, 1 min at 50 °C, 2 min at 72 °C, and a final extension step of 10 min at 72 °C.

Verification of PCR products were carried out on 1% agarose gels buffered with 1X TBE, stained with ethidium bromide, and visualized under UV light with 1 Kb λ molecular marker. Amplicons of the mitochondrial cyt b gene segment were shown in Figure 3.4. All amplified products were kept -20 °C until sequencing step.

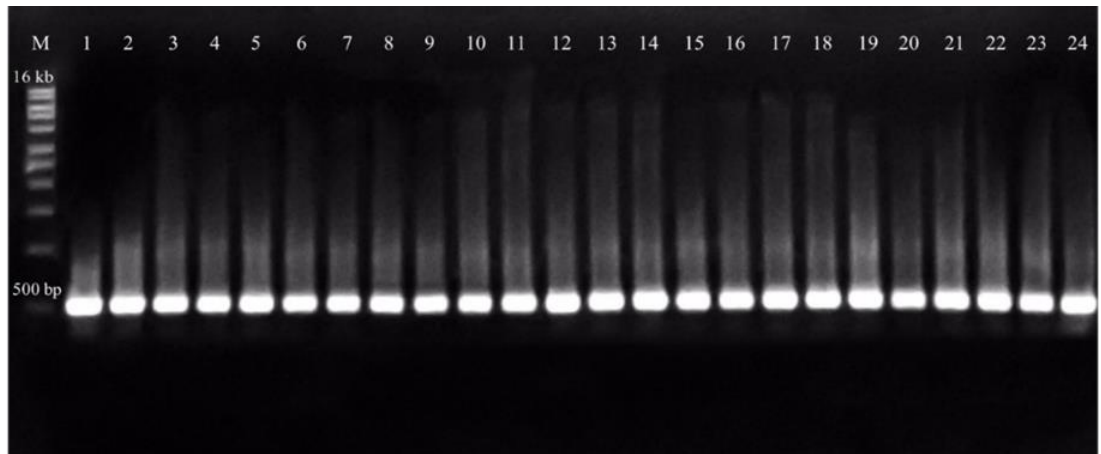


Figure 3.4. Amplified cyt b gene segment of mtDNA samples from *C. quercusfolii* individuals. M: 1 Kb marker, 1-10: Bolu, 11-20: Bitlis, 21-22: Artvin. 23-24 Düzce.

3.3.2 Amplification of Nuclear ITS2 Region

Size of the amplified PCR products for ITS2 region was 395 base pairs (bp). Following primers spanning the ITS2 region were used for amplification: ITS2f forward 5'-ATGAACATCGACATTTTCGAACGCATAT- 3' and ITS2r 5'-TTCTTTTCCTCCGCTTAGTAATATGCTTAA- 3' (Ji et al., 2003). All PCR reactions were carried out in 40 µl volumes containing, 10 µl 5X buffer, 5 µl MgCl₂ (25mM), 3 µl dNTPs (10mM), 1 µl of each primers (20pmol), 0.5 µl template DNA and 0.5 µl Taq polymerase (Vivantis). 19 µl of ddH₂O were added to each reaction to complete final volume of 40 µl. XP Thermal cycler was used for amplification reactions.

Temperature rates, times and cycles were set as follows: 5 min at 95 °C for first denaturation; 35 cycles of 40 seconds at 95 °C for denaturation, 40 seconds at 52.2°C for annealing, 45 seconds at 72 °C for elongation and 5 minutes at 72 °C for last elongation. PCR products were checked using 1% agarose gel with the 1 Kb λ DNA molecular marker (Sigma, DO428). A representative example of amplification results of the nDNA ITS2 region was shown was Figure 3.5.

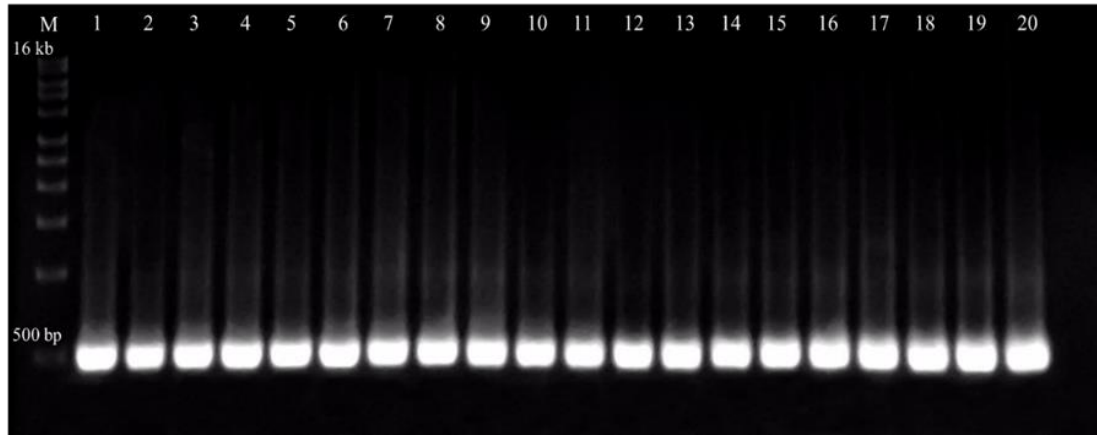


Figure 3.5. Amplified ITS2 segment of nDNA from *C. quercusfolii* individuals M: 1 Kb marker, 1-10: Kütahya, 11-20: Karaman.

3.4 Purification and Sequencing

Amplicons were sent to a Company (Macrogen, South Korea) for purification and sequencing reactions in a cycle sequencing using Perkin-Elmer BigDye Terminator chemistry on an ABI automated sequencer (Applied Biosystems). Approximately 50 ng of amplified product was sequenced in both directions for reducing the possibility of base calling errors.

3.5 Data Analysis

3.5.1 Alignment of Sequences and Haplotype/Allele Determination

Sequences were obtained from the company in the form of chromatograms (Figure 3.6 and 3.7). Chromatograms were first checked by eye, and sequences were aligned, if necessary, trimmed with the program BioEdit 7.2.5 (Hall, 1999). Similar sequences were collapsed into haplotypes/alleles through using the program GenAIEx 6.5 (Peakall and Smouse, 2012). To ensure that the obtained sequences were genuine mitochondrial DNA sequences, not numts (pseudogenes), several points have been given special attention such as the absence of stop codons and nonsense mutations. Protein coding fragment of mitochondrial cyt b gene was translated into amino acids in DnaSP 5.10.1 (Librado and Rozas, 2009) software using invertebrate amino acid translations for further testing of the sequences.

3.5.2 Populations Genetic Diversity Estimates

In genetic diversity estimation frequently applied approaches were used as the calculations of the number of polymorphic sites (S), nucleotide (π) and haplotype (h) diversity Nei (1987), the number of substitutions and pairwise nucleotide differences (k) (Tajima, 1983), and population differentiations in the form of F_{ST} using Arlequin 3.5.2.2 (Excoffier et al., 2010) and DnaSP 5.10.1 (Librado and Rozas, 2009) software packages. In the search of genetic diversity estimates of populations/species, haplotype diversity refers to the probability that two randomly

drawn alleles are different from each other, while nucleotide diversity is described as the average number of nucleotide differences in pairwise DNA sequence comparisons.

Haplotype/allele frequencies in each population were also checked both by counting, and the Arlequin program. In addition to searching the tendencies of nucleotide and amino acid uses in sequences, the presence of selection pressure and any deviations from the neutrality were checked through calculating Tajima's *D* (Tajima, 1989) and Fu's *F_s* (Fu, 1997) values implemented in Arlequin program. Analysis was conducted through 1000 permutations and the statistical significance was accepted as $p \leq 0.05$.

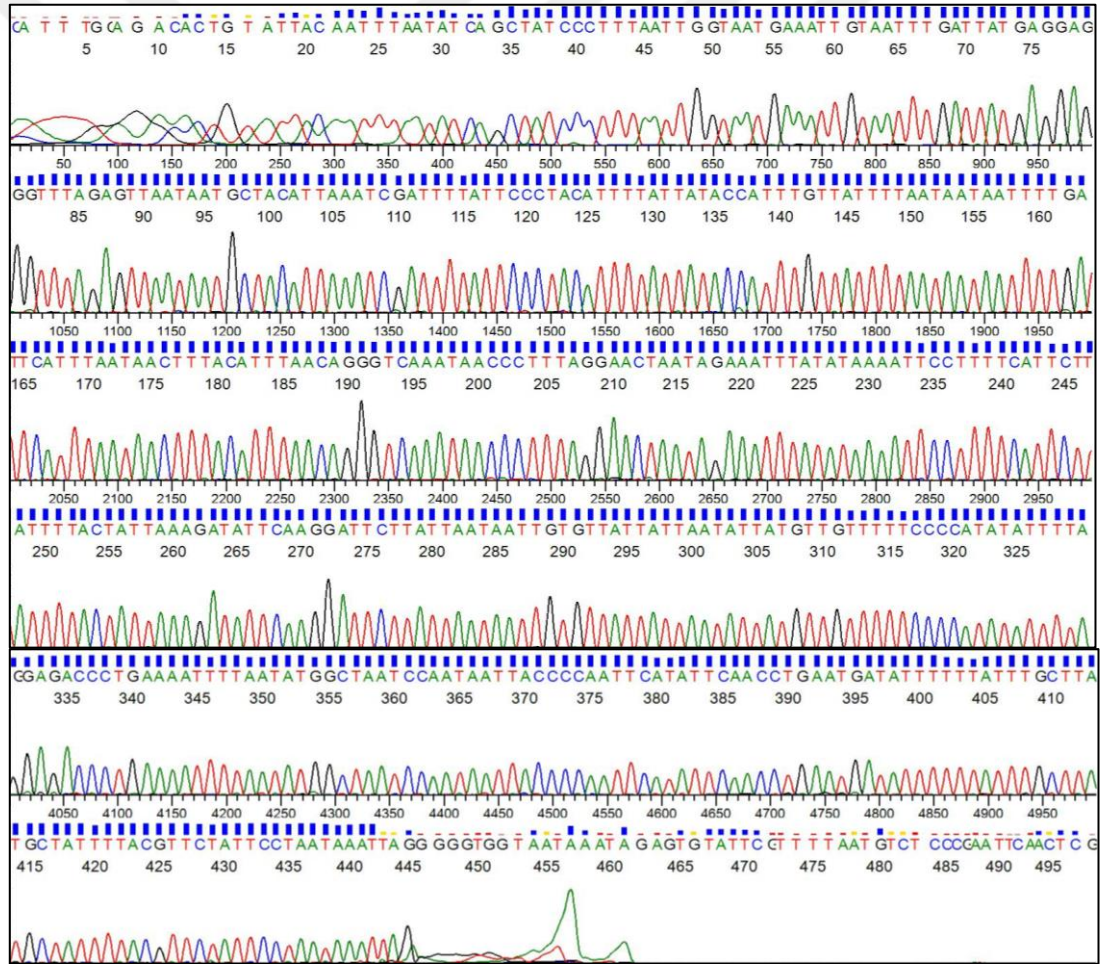


Figure 3.6. Chromatogram of cytb region of mtDNA (433 bp), obtained from sequencing of *C. quercusfolii* sampled from Afyon population.

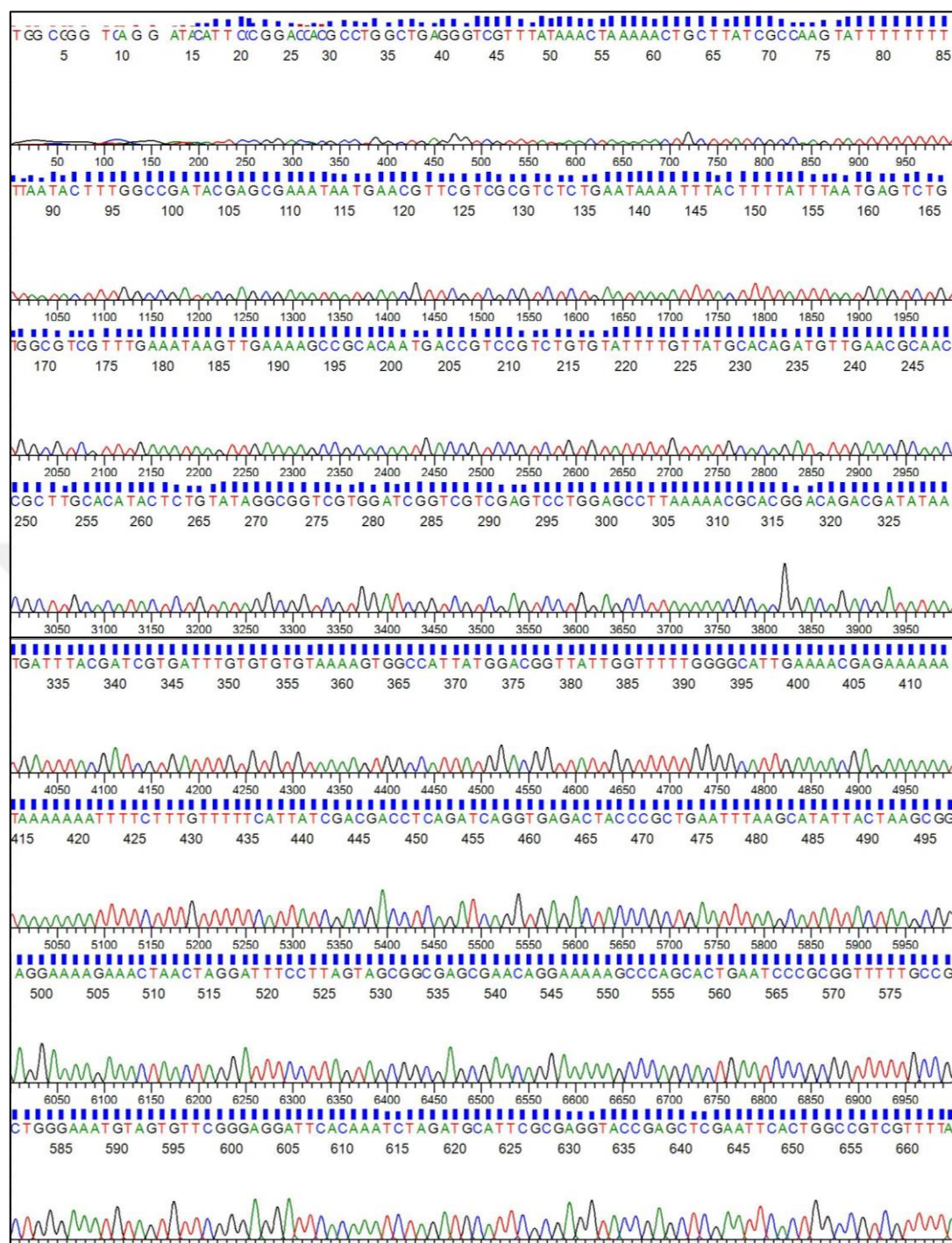


Figure 3.7. Chromatogram of ITS2 region of nDNA (449 bp), obtained from sequencing of *C. quercusfolii* from Afyon population.

3.5.3 Detection of Population Genetic Structure and Population Demographic Analysis

Both past and current factors are known to affect population size changes over time. Individual responses of populations as well as the taxon itself across the studied range to changes in environmental conditions such as climatic oscillations and any other naturally selecting factors may have been observed in the population size changes. Thus, population demographic analyses may unravel signatures of the past events that were important to create the contemporary population genetic structure (Avice, 2000). Demographic analyses were employed based on mismatch distribution to measure and estimate data to understand what factors affected population structure and dynamics. Possible factors can be environmental fluctuations, dispersal ability of the species, spatially heterogeneous environments such as heterogeneous topography of Turkey, genetic drift, and migration events which are the causes of fluctuations in gene frequency in a population. Population demographic analyses were conducted through pairwise differences between haplotypes/alleles. For disclosing population, demographic structures over time different population models (i.e. population expansion versus constant population size) were checked for the *C. quercusfolii* populations to see whether they were expanding, or they were at the demographic equilibrium. The raggedness index (r) (Harpending, 1994), Tajima's D (Tajima, 1989b), and Fu's and Li's D^* (Fu and Li, 1993) were also calculated to examine the deviations from neutrality.

3.6 Phylogenetic and Phylogeographic Analysis

3.6.1 Maximum Likelihood (ML), Maximum Parsimony (MP) and Bayesian Analysis

Resolving phylogenetic relationships among haplotypes/alleles requires construction of phylogenetic trees. Maximum Parsimony (MP), Maximum Likelihood (ML), and Bayesian analysis were applied to sequence data with PAUP*4.0b10 (Swofford, 2002), and MrBayes 3.2.6 software packages (Ronquist

and Huelsenbeck, 2003), respectively. MP analysis was performed under the heuristic search options, using TBR branch-swapping algorithm and 1000 replicates of random addition of taxa. MP analysis was employed with 1000 bootstrap replicates to assess support of branches (Swofford, 2002). In reconstructing the ML tree, the determination of the most fit model to the data set JModeltest 2 computer program was used (Darriba et al., 2012; Guindon and Gascuel, 2003). The substitution models suggested for the analyzed fragments were HKY+I+ Γ for cyt b and GTR+I for ITS. JModeltest calculated $-\ln = 1959.4363$ for the HKY+I+ Γ model, and $-\ln = 497.2462$ for the GTR+I as the best fit models to the current data sets. The analysis involved four simulations of Markov Chains, 100 million generations with trees being sampled every 1000 generations and a burn-in of 25%. The software tool TRACER version 1.3.1 (Rambout and Drummond, 2003) was used to observe the parameters and to determine the number of trees need to reach stationary (burn-in). After discarding burn-in trees and evaluating convergence, the remaining samples were retained for generating 50% majority rule consensus trees and calculating mean, variance, 95% credibility intervals and posterior probabilities.

3.6.2 Network Analysis

Haplotype network analysis is used to analyze sequence data sets due to insufficiency of conventionally applied methods of maximum parsimony and maximum likelihood to reconstruct the genealogical relationships of haplotypes. Because traditional methods assume that ancestral haplotypes are no longer in the population. These methods may also assume no recombination and for an accurate reconstruction they need large numbers of variable characters (Clement et al., 2000). Haplotype networks are more efficient way of resolving evolutionary relationships of haplotypes at the intraspecific level as migration (gene flow) may lead reticulate rather than hierarchical or treelike structure between/among populations. Furthermore, internal nodes in networks may provide more information through hypothetical haplotype connections (Posada and Crandall, 2001). Networks let one sequence to unite several times without bifurcating and give genealogical information about the divergence of population. In addition, reticulations may imply the effects of conflicting phylogenetic signals. Therefore, a haplotype network

analysis was constructed using the program HapStar Version 0.5 (C) (Teacher and Griffiths, 2011). For building a haplotype network, the haplotype data matrix was transferred to Arlequin program to compute standard AMOVA analysis and obtain a distance matrix to get pairwise difference between haplotypes to build a minimum spanning tree. The resulting svg file was transferred to Inkscape 0.91 (www.inkscape.org) program to visualize the network.

3.6.3 Analysis Related to the Geographic Distribution of the Genetic Variation

Since almost none of the species are evenly distributed and the geographic distance between/among populations are differing then there might be a correlation between geographic distance and the genetic distance between populations. GenAIEx 6.5 program also used to analyze the relationship between population pairwise genetic similarity and geographic distances using a spatial autocorrelation multivariate approach. Positive autocorrelation signals or similarity in haplotypes sampled in neighboring sites, may be due to spatial patterns shaped by natural selection or neutral processes and are expected when gene flow is limited to short distances (Epperson, 1990). A monotonic decrease in the autocorrelation coefficient from significant positive values at large distances across multiple loci can be indicative of directional dispersal events (Bertorelle and Barbujani, 1995; Stone and Sunnucks, 1993).

AMOVA analysis using Arlequin 2.0 program (Excoffier et al. 1992) was used to reveal the partitioning of genetic variation at different hierarchical levels across the species range and further if there are any geographical groupings present across the sampled area in Turkey. This method estimates the proportion of genetic variation at among groups, among populations within groups, and within each population. For seeing the exact partitioning of the genetic variation at different hierarchical levels different grouping schemes have been tried in addition to the clades that have been obtained in phylogenetic trees and network analysis. AMOVA with statistical significance was determined by permutation analyses to partition

genetic variation at different levels. Statistical significance was determined by 1000 permutations.

3.6.4 Estimation of Time of Divergence

Divergence times and their confidence intervals were estimated using a Bayesian Markov Chain Monte Carlo (MCMC) coalescent method implemented in BEAST version 1.5.2 (Drummond and Rambaut, 2007). Dates of the divergence were inferred using HKY model with G and Γ following the uncorrelated relaxed lognormal clock as implemented in BEAST. A 2.11% divergence/ MYA/ lineage (Papadopoulou, 2010; Pons and Vogler, 2005) was applied to the age determination. Both MRCAs (most recent common ancestors) and MACAs (most ancient common ancestors) were calculated and operators were optimized (Hayward and Stone, 2006) using BEAUTI. BEAST was run for 100 million generations sampling every 1000 and the convergence to stationary and the effective sample size (ESS) of model parameters were checked by TRACER. The maximum clade credibility tree built with TREEANNOTATOR discarding initial 25% samples as burn-in. FIG-TREE version 1.3.1 (Rambaut, 2009) was used to visualize the results.

4. RESULTS

4.1 Sequence Data Evaluation of the Mitochondrial Marker: Cytochrome b (Cyt b) Gene

4.1.1 Genetic Diversity Estimates of the mtDNA Cytochrome b Gene of *Cynips quercusfolii*

None of the sequences of the *C. quercusfolii* mitochondrial cytochrome b gene possessed neither stop codons nor non-sense mutations. Sequences of 294 specimens representing 38 localities produced 125 haplotypes (GenBank Accession number MG650475 - MG650599) (Table 4.1). Monomorphic sites were omitted; thus Table 4.2 shows only polymorphic sites. The number of monomorphic sites were 340. There were 93 substitutions where at 10 sites multiple hits (55, 127, 214, 322, 325, 343, 382, 391, 421, and 430). Of the remaining substitutions 30 were singleton (found in only one individual).

The number of parsimony informative characters was 53, and the remaining characters were parsimony uninformative. The nucleotide frequencies were 32.93%, 43.26%, 13.01%, and 10.80% for A, T, C, and G, respectively. Maximum composite likelihood estimates of the pattern of nucleotide substitutions showed that transitional substitutions were higher where the highest number of changes occurred between C/T (48.92) followed by G/A (21.24) (Table 4.2). The transition transversion rate ratios were $k_1 = 15.811$ (purines) and $k_2 = 27.729$ (pyrimidines). The overall transition/transversion ratio bias was $R = 8.629$, where $R = [A * G * k_1 + T * C * k_2] / [A + G] * (T + C)$.

Table 4. 1. Total 125 cyt b haplotypes of the mitochondrial cyt b gene of *C. quercusfolii*. Only polymorphic character sites are shown.

	34	40	43	46	47	49	55	58	64	67	70	79	82	85	91	94	97	100	103	107	124	125	127	142	146	166	173	188	193	196	202	205	208	209	214	216	217	220	223	226	232	238	247	250	253	256	
H1	A	T	C	T	T	A	T	T	T	A	T	A	A	G	A	T	T	T	T	T	C	C	A	A	A	G	T	T	A	G	T	C	T	T	A	C	T	T	A	T	T	T	T	T	T		
H2	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H3	G	C	T	.	.	.	.	.	.	.	.	.	G	.	.	.	.	C	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H5	G	C	T	.	.	.	.	.	.	.	.	.	G	A	.	.	.	C	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H6	G	C	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H7	G	.	T	.	.	.	.	.	.	.	C	.	.	A	.	.	.	C	C	.	T	.	C	.	G	A	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	
H8	G	C	T	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H9	G	C	T	.	.	.	.	.	.	.	.	.	G	A	.	.	.	.	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H10	G	C	T	.	.	.	.	.	.	.	.	.	G	A	.	.	.	.	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H11	G	C	T	.	.	.	.	.	.	.	.	.	G	A	.	.	.	.	.	.	T	.	T	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H12	G	C	T	.	.	.	.	.	.	.	.	.	G	A	.	.	.	.	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H13	G	C	T	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H14	G	C	T	.	.	.	.	.	.	.	.	.	G	A	.	.	.	C	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.
H15	G	.	T	.	.	.	.	.	.	.	C	.	.	A	.	.	C	.	.	T	.	C	.	G	A	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.
H16	G	.	T	.	.	.	.	.	.	C	.	.	.	A	.	.	C	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H17	G	.	T	.	.	.	.	.	.	C	.	.	.	A	.	.	C	.	.	T	.	C	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H18	.	.	T	.	C	.	.	.	.	C	.	.	.	A	.	.	.	.	.	.	T	.	C	G	A	.	.	T	.	.	T	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.
H19	G	.	T	.	.	.	.	.	.	C	.	.	.	A	.	.	C	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H20	G	.	T	.	.	.	.	.	.	C	.	.	.	A	.	.	C	C	.	T	.	C	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H21	G	.	T	.	.	.	.	.	.	C	.	.	.	A	.	.	C	C	.	T	.	C	.	G	A	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H22	G	.	T	.	.	.	.	.	.	C	.	.	.	A	.	.	C	.	.	T	.	C	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H23	G	.	T	.	.	.	.	.	.	C	.	.	.	A	.	.	C	.	.	T	.	C	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H24	G	.	T	.	.	.	.	.	.	C	.	.	.	A	.	.	C	.	.	T	.	C	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H25	G	.	T	.	.	.	.	.	.	.	.	.	.	A	.	.	C	.	.	T	.	C	.	G	A	.	.	.	A	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H26	G	C	T	.	.	.	.	.	.	.	.	G	A	G	.	.	C	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H27	G	C	T	.	.	.	C	.	.	.	.	G	A	.	.	.	C	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H28	G	C	T	.	.	.	C	.	.	.	.	G	A	.	.	.	C	.	.	T	.	T	.	G	A	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H29	G	C	T	.	.	.	.	.	.	.	.	G	A	.	.	.	C	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H30	G	C	T	.	.	.	.	.	.	.	.	G	A	.	.	.	C	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H31	G	C	T	.	.	.	.	.	.	.	.	G	A	.	.	.	C	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H32	G	C	T	.	.	.	.	.	.	.	.	G	A	.	.	.	C	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.		
H33	G	C	T	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	T	.	C	.	G	A	.	.	C	.	.	T	.	.	G	.	.	A	.	.	.	C	.	.	C	.		
H34	G	C	T	.	.	.	.	.	.	.	.	G	A	.	.	.	C	.	.	T	.	T	.	G	A	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H35	G	C	T	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	T	.	C	.	G	A	.	.	C	.	.	T	.	.	G	.	.	.	.	.	.	.	.	.	C	.		
H36	G	C	T	.	.	.	.	.	.	.	.	G	A	.	.	.	.	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H37	G	C	T	.	.	.	.	.	.	.	.	G	A	.	.	.	.	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H38	G	C	T	.	.	.	.	.	.	.	.	G	A	.	.	.	C	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H39	G	C	T	.	.	.	.	.	.	.	.	G	A	.	.	.	C	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H40	G	C	T	.	.	.	.	.	.	.	.	G	A	.	.	.	C	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H41	G	C	T	.	.	.	.	.	.	.	.	G	A	.	.	.	.	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H42	G	C	T	.	.	A	.	.	.	.	.	G	A	.	.	.	C	.	.	T	.	T	.	G	A	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H43	G	C	T	.	.	.	.	C	.	.	.	G	A	.	.	.	C	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H44	.	.	T	.	C	.	.	.	.	C	.	.	.	A	.	.	.	.	.	.	T	.	C	G	A	.	.	T	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H45	G	.	T	.	C	.	.	.	.	C	.	.	.	A	.	.	.	.	.	.	T	.	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H46	G	.	T	.	.	.	.	.	.	C	.	.	.	A	.	.	C	.	.	T	.	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H47	G	.	T	.	.	.	.	.	.	C	.	.	.	A	.	.	C	C	.	T	.	C	.	G	A	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H48	G	.	T	.	.	.	.	.	.	C	.	.	.	A	.	.	.	.	.	T	.	C	.	G	A	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	
H49	G	.	T	.	.	.	.	.	.	C	.	.	.	A	.	.	.	C	.	T	.	C	.	G	A	.	.	.	.	.	.	.	C	.	T	.	.	.	.	.	.	.	.	.	.	.	
H50	G	.	.	.	.	.	.	.	.	C	.	.	.	A	.	.	C	C	C	T	.	T	.	G	A	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	

Table 4.1 (cont'd).

	34	40	43	46	47	49	55	58	64	67	70	79	82	85	91	94	97	100	103	107	124	125	127	142	146	166	173	188	193	196	202	205	208	209	214	216	217	220	223	226	232	238	247	250	253	256	
H51	G	.	T	.	.	.	A	.	.	.	C	.	.	A	.	.	.	C	.	.	T	.	C	.	G	A	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	
H52	.	.	T	.	.	.	A	.	.	.	C	.	.	.	.	.	C	.	.	.	T	.	T	.	G	A	.	.	.	.	.	T	.	.	G	.	.	.	.	C	.	.	.	A	.	.	
H53	.	.	T	.	.	.	A	.	.	.	C	.	.	.	.	.	C	.	.	.	T	.	T	.	G	A	.	.	.	.	.	T	.	.	G	.	.	.	.	C	.	.	.	A	.	.	
H54	.	.	T	.	.	.	A	.	.	.	.	.	.	A	.	.	C	.	.	.	T	.	C	.	G	A	.	.	.	.	.	T	.	.	G	.	.	.	.	C	.	.	.	.	.	.	
H55	G	.	T	.	.	.	A	.	.	.	C	.	.	A	.	.	.	C	C	.	T	.	C	.	G	A	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	
H56	G	.	T	.	.	.	A	.	.	.	C	.	.	A	.	.	.	C	C	.	T	.	C	.	G	A	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	
H57	G	.	T	.	.	.	A	.	.	.	.	.	.	A	.	.	C	.	.	.	T	.	C	.	G	A	.	.	.	.	.	T	.	.	G	.	.	.	.	C	.	.	.	.	.	.	
H58	G	.	T	.	.	.	A	.	.	.	C	.	.	A	.	.	C	C	.	T	.	C	.	G	A	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H59	G	.	T	.	.	.	A	.	.	.	C	.	.	A	.	.	C	C	.	T	.	C	.	G	A	.	.	.	.	A	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	
H60	G	.	T	.	.	.	A	.	.	.	C	.	.	A	.	.	C	.	.	.	T	.	C	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H61	G	.	T	.	.	.	.	.	.	.	.	.	.	A	.	C	.	.	C	.	T	.	C	.	G	A	.	.	.	.	A	.	.	C	.	.	.	.	.	.	.	.	.	.	A	.	.
H62	G	.	T	.	.	.	A	.	.	.	C	.	.	A	.	.	C	C	.	T	.	C	.	G	A	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H63	G	.	T	.	.	.	.	.	.	.	C	.	.	A	.	.	C	C	.	T	.	C	.	G	A	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H64	G	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	C	.	T	.	C	.	G	A	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	C	.	.	.
H65	G	.	T	.	.	.	.	.	.	.	C	.	.	A	.	.	C	C	.	T	.	C	.	G	A	.	.	.	.	.	.	.	C	C	.	.	.	.	.	.	.	.	.	.	.	.	.
H66	G	.	T	.	.	.	.	.	.	.	C	.	.	A	.	.	C	.	.	T	.	C	.	G	A	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C
H67	.	.	T	.	.	.	.	.	.	.	C	.	.	.	.	.	C	C	.	T	.	C	.	G	A	.	.	.	.	A	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	C
H68	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	T	.	C	.	G	A	.	C	.	.	.	T	.	.	G	.	.	.	.	C	.	.	.	C	.	.	
H69	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	T	.	C	.	G	A	.	C	.	.	.	T	.	.	G	.	.	.	.	C	.	.	.	C	.	.	
H70	G	C	T	.	.	.	.	.	.	.	.	.	G	A	.	.	C	.	.	T	.	T	.	G	A	.	.	.	.	C	.	.	.	.	G	.	.	.	.	C	.	.	.	C	.	.	
H71	G	C	T	.	.	.	.	.	.	.	.	.	.	A	.	C	C	C	.	T	.	T	.	G	A	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H72	G	C	T	.	.	.	.	.	.	.	.	.	.	A	.	C	C	C	.	T	.	T	.	G	A	.	.	.	A	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H73	G	C	T	.	.	.	.	.	.	.	.	.	.	A	.	C	C	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H74	.	C	T	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	T	.	C	.	G	A	.	C	.	.	.	T	.	.	G	.	.	.	.	C	.	.	.	C	.	.	
H75	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H76	G	C	T	.	.	.	.	.	.	.	.	.	G	.	.	.	C	.	.	T	.	T	.	G	A	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H77	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H78	G	C	T	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H79	.	.	T	.	.	.	.	.	.	.	C	.	.	A	.	.	C	.	.	.	T	.	C	.	G	A	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
H80	G	C	T	.	.	.	.	.	.	.	.	.	G	A	.	.	C	.	.	T	.	T	.	G	A	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H81	G	C	T	.	.	.	.	.	.	.	.	.	G	A	.	.	C	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	.	G	.	.	C	.	.	.	.	.	.	.	.	.	
H82	G	.	T	.	.	.	.	.	.	.	C	.	.	A	.	.	C	C	.	T	.	C	.	G	A	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H83	.	.	T	.	.	.	.	.	.	.	C	.	.	A	.	.	.	C	.	.	T	.	C	.	G	A	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
H84	.	.	T	.	.	.	A	.	.	.	C	.	.	A	.	.	.	.	.	.	T	.	C	.	G	A	.	.	.	A	.	T	.	.	G	.	.	.	.	C	.	.	.	C	.	.	
H85	.	.	T	.	.	.	A	.	.	.	C	.	.	A	.	.	.	.	.	.	T	.	C	.	G	A	.	.	.	A	.	.	.	G	.	.	.	.	C	.	.	.	C	.	.	C	.
H86	G	.	T	.	.	.	.	.	.	.	.	.	.	A	.	.	C	.	.	T	.	C	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H87	G	C	T	.	.	.	.	.	.	.	.	.	.	A	.	.	C	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H88	G	C	T	.	.	.	.	.	.	.	.	.	G	A	.	.	C	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H89	G	C	T	.	.	.	.	.	.	.	.	.	G	A	.	.	C	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H90	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H91	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H92	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	T	.	C	.	G	A	.	C	.	.	.	T	.	.	G	.	.	.	.	C	.	.	.	C	.	.	
H93	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	C	C	.	.	T	.	C	.	G	A	.	.	.	.	.	T	.	.	G	.	.	.	.	C	.	.	.	.	.	.	
H94	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	T	.	C	.	G	A	.	C	.	.	.	T	.	.	G	.	.	.	.	.	.	.	.	.	.	.	
H95	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	T	.	C	.	G	A	.	C	.	.	.	T	.	.	G	.	.	.	.	C	.	.	.	.	C	.	
H96	G	C	T	.	.	.	.	.	.	.	.	.	G	A	.	.	C	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H97	G	.	T	.	.	.	.	.	.	.	.	.	G	A	.	.	C	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H98	G	C	.	.	.	.	.	.	.	.	.	.	G	A	.	.	C	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H99	G	C	T	.	.	.	.	.	.	.	.	G	A	.	.	.	C	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H100	G	.	T	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	

Table 4.1 (cont'd).

	34	40	43	46	47	49	55	58	64	67	70	79	82	85	91	94	97	100	103	107	124	125	127	142	146	166	173	188	193	196	202	205	208	209	214	216	217	220	223	226	232	238	247	250	253	256	
H101	G	.	T	.	.	.	.	.	.	.	.	.	G	A	.	.	.	C	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H102	G	C	T	.	.	.	.	.	.	.	.	.	G	A	.	.	.	C	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H103	G	.	T	.	.	.	.	.	.	.	C	.	.	A	.	.	.	C	.	.	T	.	C	.	G	A	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H104	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	T	.	C	.	G	A	.	C	.	.	.	T	.	.	G	.	.	.	.	.	C	.	.	.	.	C	.
H105	G	.	T	A	.	T	.	.	.	.	C	.	.	A	.	.	.	C	.	.	T	.	C	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H106	G	.	T	.	.	.	.	.	.	.	C	.	.	A	.	.	.	C	C	.	T	.	C	.	G	A	.	.	.	A	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	
H107	G	.	T	.	.	.	.	.	.	.	C	.	.	.	.	.	.	C	C	.	T	.	C	.	G	A	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H108	G	C	T	.	.	.	.	.	.	.	.	.	.	A	.	.	.	C	.	.	T	.	T	.	.	A	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H109	G	.	T	.	.	.	.	.	.	.	C	.	.	A	.	.	.	C	.	.	T	.	C	.	G	A	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H110	G	.	T	.	.	.	.	.	.	.	C	.	.	.	.	.	.	C	C	.	T	.	C	.	G	A	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H111	G	C	T	.	.	.	.	.	.	.	.	.	G	.	.	.	.	C	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H112	G	C	T	.	.	.	.	.	.	.	.	.	.	A	.	.	.	C	.	.	T	.	T	.	G	A	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H113	G	C	T	.	.	.	.	.	.	.	.	.	G	A	.	.	.	C	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H114	G	C	T	.	.	.	.	.	.	.	.	G	.	.	.	.	.	C	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H115	G	.	T	.	.	.	.	.	.	.	.	.	.	A	.	.	.	C	.	.	T	.	C	.	G	A	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H116	.	.	T	.	.	.	.	.	.	.	C	.	.	A	.	.	.	C	C	.	T	.	C	.	G	A	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H117	.	.	T	.	.	.	.	.	.	.	.	.	.	A	.	.	.	C	.	.	T	.	C	.	G	A	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H118	G	.	T	.	.	.	.	.	.	.	C	.	.	.	.	.	.	C	C	.	T	.	C	.	G	A	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.
H119	G	.	T	.	.	.	A	.	.	.	C	.	.	A	.	.	.	C	C	.	T	.	C	.	G	A	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H120	G	.	T	A	.	T	.	.	.	.	C	.	.	A	.	.	.	C	.	.	T	.	C	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H121	G	.	T	.	.	.	.	.	.	.	C	.	.	A	.	.	.	C	C	.	T	.	C	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H122	G	C	T	.	.	.	.	.	.	.	.	.	G	A	.	.	.	.	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.
H123	G	C	T	.	.	.	.	.	.	.	.	.	G	A	.	.	.	.	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H124	G	C	T	.	.	.	A	.	.	.	.	.	G	A	.	.	.	C	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H125	G	C	T	.	.	.	.	.	.	.	.	.	G	A	.	.	.	C	.	.	T	.	T	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Table 4.1 (cont'd).

	262	268	271	275	277	280	281	283	287	293	295	302	305	307	313	315	319	321	322	325	328	329	335	337	340	343	344	346	352	357	358	361	364	374	375	376	382	385	391	394	400	403	413	421	427	430	433	
H1	T	T	T	G	A	C	T	A	A	G	G	T	A	A	T	G	T	C	C	A	T	A	G	A	C	T	G	A	T	T	G	T	T	A	C	C	T	T	A	T	A	T	G	T	A	T	T	
H2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H3	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	C	.	.	T	C	.	.	.	.	.	.	.	.	A	.	
H4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H5	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	C	.	.	.	A	.	C	.	.	T	C	.	.	.	.	.	.	.	.	A	.	
H6	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	C	.	.	.	C	.	.	.	.	.	.	.	.	A	.	
H7	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	C	.	.	T	.	.	.	.	.	.	.	.	.	A	.	
H8	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	A	.	C	.	.	T	C	.	.	.	.	.	.	.	.	A	.	
H9	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	C	.	.	T	.	.	.	.	.	.	.	.	.	A	.	
H10	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	C	.	.	T	C	.	.	.	.	.	.	.	.	A	.	
H11	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	C	.	.	T	C	.	.	.	.	.	.	.	.	A	.	
H12	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	C	.	.	T	C	.	.	.	G	.	.	.	.	A	.	
H13	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	C	.	.	T	C	.	.	.	.	.	.	.	.	A	.	
H14	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	C	.	.	.	A	.	C	.	.	T	C	.	.	.	.	.	.	.	.	A	.	
H15	C	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	C	.	.	T	.	.	.	.	.	.	.	.	.	.	A	.	
H16	C	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	C	.	.	T	.	.	.	.	.	.	.	.	.	.	A	.	
H17	C	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	C	.	.	T	.	.	.	.	.	.	.	.	.	.	A	.	
H18	.	C	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	A	T	C	G	.	.	T	.	.	.	.	A	.	C	.	.	T	.	.	C	.	.	.	.	.	.	.	A	.	
H19	C	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	C	.	.	T	.	.	C	.	.	.	.	.	.	.	A	.	
H20	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	C	.	.	T	.	.	C	.	.	.	.	.	.	.	A	.	
H21	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	C	.	.	T	.	.	C	.	.	.	.	.	.	.	A	.	
H22	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	A	.	.	A	.	C	.	.	T	.	.	.	.	.	.	.	.	.	.	A	.	
H23	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	C	.	.	T	.	.	.	.	.	.	.	.	.	.	A	.	
H24	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	C	.	.	T	.	.	.	.	.	.	.	.	.	.	A	.	
H25	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	A	.	.	.	A	.	C	.	.	T	.	.	.	.	.	.	.	.	.	A	.	A	
H26	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	C	.	.	.	A	.	C	.	.	T	C	.	.	.	.	.	.	.	.	.	A	.	
H27	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	C	.	.	.	A	.	C	.	.	T	C	.	.	.	.	.	.	.	.	.	A	.	
H28	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	C	.	.	T	C	.	.	.	.	.	.	.	.	.	A	.	
H29	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	A	.	C	.	.	T	C	.	.	.	.	.	.	.	.	.	A	.	
H30	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	C	.	.	.	A	.	C	.	.	.	C	.	.	.	.	.	.	.	.	.	A	.	
H31	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	C	.	.	.	C	A	.	C	.	.	T	C	.	.	.	.	.	.	.	.	A	.	
H32	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	A	.	C	.	.	T	C	.	.	.	.	.	.	.	.	G	.		
H33	.	C	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	A	.	.	.	.	T	.	A	.	.	A	.	C	.	.	.	.	.	G	.	.	.	C	.	.	.	A	.	
H34	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	C	.	.	T	C	.	.	.	.	.	.	.	.	.	A	.	
H35	.	C	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	A	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	
H36	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	C	.	.	.	C	.	.	.	.	.	.	.	.	.	A	.	
H37	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	C	.	.	T	C	.	.	.	.	.	.	.	.	.	A	.	
H38	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	C	.	.	.	A	.	C	.	.	T	C	.	.	.	.	.	.	.	.	.	A	.	
H39	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	C	.	.	.	A	.	C	.	.	T	C	.	.	.	.	.	.	.	.	.	A	.	
H40	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	C	.	.	T	C	.	.	.	.	.	.	.	.	.	A	.	
H41	.	.	.	.	.	T	.	.	G	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	C	.	.	T	C	.	.	.	.	.	.	.	.	.	A	.	
H42	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	C	.	.	T	C	.	.	.	.	.	.	.	.	.	A	.	
H43	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	C	.	.	.	A	.	C	.	.	T	C	.	.	.	.	.	.	.	.	.	G	.	
H44	.	C	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	A	T	C	G	.	.	T	.	.	.	A	.	.	.	T	.	C	.	.	.	.	.	.	.	.	A	.		
H45	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	A	.	
H46	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	A	.	
H47	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	.	.	T	.	.	C	.	.	.	.	.	.	.	.	A	.	
H48	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	G	.	.	A	.	C	.	.	T	.	C	.	.	.	.	.	.	.	A	.	
H49	.	.	.	.	.	.	G	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	C	.	.	T	.	.	C	.	.	.	.	.	.	.	A	.	
H50	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	G	.	.	A	.	C	.	.	.	C	.	.	.	.	.	.	.	.	A	.	

Table 4.1 (cont'd).

	262	268	271	275	277	280	281	283	287	293	295	302	305	307	313	315	319	321	322	325	328	329	335	337	340	343	344	346	352	357	358	361	364	374	375	376	382	385	391	394	400	403	413	421	427	430	433	
H51	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	A	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	A	.
H52	C	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	A	.	C	.	.	G	.	.	.	.	.	A	.	.	.	T	.	.	.	.	.	.	.	.	.	C	.	A	.	
H53	C	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	A	.	C	.	.	G	.	.	.	.	.	A	.	.	.	.	.	.	.	G	.	.	.	.	C	.	A	.		
H54	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	A	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	G	.	.	.	C	.	A	.		
H55	.	.	.	.	.	.	.	.	.	.	A	.	T	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	C	.	.	T	.	.	.	.	.	.	.	.	.	A	.		
H56	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	C	.	.	T	.	.	.	.	.	.	.	.	.	A	.		
H57	.	C	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	A	.	.	.	.	.	T	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	.	C	.	A	.		
H58	.	.	.	.	.	.	.	.	.	.	A	.	T	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	C	.	.	T	.	.	.	.	.	.	.	.	.	A	.		
H59	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	C	.	.	T	.	.	.	.	.	.	.	.	.	A	.		
H60	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	C	.	.	T	.	.	.	.	.	.	.	.	.	A	.		
H61	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	A	.	.	.	A	.	C	.	.	T	.	.	.	.	.	.	.	.	.	A	.		
H62	.	.	.	.	.	.	.	.	.	.	A	.	.	.	C	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	C	.	.	T	.	.	C	.	.	.	.	.	.	A	.		
H63	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	G	.	A	.	C	.	.	T	.	.	C	.	.	.	.	.	.	A	.		
H64	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	G	.	A	.	C	.	.	T	.	.	C	.	.	.	.	.	.	A	.		
H65	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	A	.	C	.	.	T	.	.	C	.	.	.	.	.	.	.	A	.		
H66	C	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	C	.	.	.	G	.	.	.	T	.	.	.	A	.	C	.	.	T	.	.	.	.	.	.	.	.	.	.	A	.		
H67	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	G	.	A	.	C	.	.	T	.	.	C	.	.	.	.	.	.	A	.		
H68	.	C	.	A	.	T	.	.	.	.	A	.	.	G	.	T	.	.	A	.	.	.	.	.	T	.	A	.	.	A	.	C	.	.	T	.	.	G	.	.	A	C	G	.	A	.		
H69	.	C	.	.	.	T	.	.	.	.	A	.	.	G	.	T	.	.	A	.	.	.	.	.	T	.	A	.	.	A	.	C	.	.	T	.	.	G	.	.	A	C	G	.	A	.		
H70	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	C	.	.	T	.	C	.	.	.	.	.	.	.	A	.		
H71	.	.	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	A	.	.	.	A	.	.	.	A	.	C	.	.	T	.	.	.	.	.	.	.	.	.	A	.		
H72	.	.	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	A	.	.	.	A	.	.	.	A	.	C	.	.	T	.	.	.	.	.	.	.	.	.	A	.		
H73	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	A	.	.	A	.	.	.	A	.	C	.	.	T	.	.	.	.	.	.	.	.	.	A	.		
H74	.	C	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	A	.	.	.	.	T	.	.	A	.	.	A	.	.	.	T	.	.	.	G	.	.	.	C	.	.	A	.		
H75	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	T	.	.	.	.	.	.	.	.	G	.			
H76	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	C	.	.	T	.	C	.	.	.	.	.	.	.	A	.		
H77	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	C	.	.	T	.	C	.	.	.	.	.	.	A	.		
H78	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	T	T	.	.	.	.	.	T	.	.	.	.	.	A	.	C	.	.	T	.	C	.	.	.	.	.	.	A	.		
H79	C	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	C	.	.	.	G	.	.	.	.	.	.	.	.	A	.	C	.	.	T	.	.	.	.	.	.	.	.	.	A	.		
H80	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	C	.	.	T	.	C	.	.	.	.	.	.	.	A	.		
H81	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	C	.	.	A	.	C	.	.	T	.	C	.	.	.	C	.	.	.	.	A	.		
H82	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	C	.	.	T	.	.	C	.	.	.	.	.	.	A	.		
H83	C	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	C	.	.	.	G	.	.	.	T	.	.	.	.	A	.	.	.	.	T	.	.	.	.	.	.	.	.	.	A	.		
H84	.	.	.	G	T	.	.	.	.	.	A	.	.	.	C	.	.	.	A	.	C	.	.	.	.	A	.	.	.	A	.	A	.	.	.	.	.	.	.	.	.	.	.	.	A	.		
H85	.	.	.	G	T	G	.	.	.	.	A	.	.	.	C	.	.	.	A	.	C	.	.	.	.	A	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.		
H86	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	
H87	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	C	.	.	T	.	C	.	.	.	.	.	.	.	A	.		
H88	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	T	.	C	.	.	.	.	.	.	.	A	.		
H89	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	G	.	.	.	.	T	C	.	.	.	A	.	C	.	.	T	.	C	.	.	.	.	.	.	.	A	.		
H90	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	
H91	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	
H92	.	C	.	A	.	T	.	.	.	.	A	.	.	.	.	.	.	.	A	.	.	.	.	.	T	.	.	.	.	A	.	.	.	T	.	.	.	G	.	.	.	C	.	A	.			
H93	.	C	.	A	.	T	.	.	.	.	A	.	.	.	.	.	.	.	A	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	T	.	.	.	.	.	.	C	.	A	.			
H94	.	C	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.		
H95	.	C	.	A	.	T	.	.	.	.	A	.	.	.	.	.	.	.	A	.	.	.	.	.	T	.	.	.	.	A	.	.	.	T	.	.	.	.	.	.	.	C	.	A	.			
H96	.	.	.	.	.	T	.	.	.	A	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	C	.	.	.	A	.	.	.	.	T	.	C	.	.	.	.	.	.	.	A	.		
H97	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	T	.	C	.	.	.	.	.	.	.	A	.		
H98	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	A	.	C	.	.	T	.	C	.	.	.	.	.	.	.	A	.		
H99	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	C	.	.	.	A	.	.	.	.	T	.	C	.	.	.	.	.	.	.	A	.		
H100	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	T	.	C	.	.	.	.	.	.	.	.	A	.	

Table 4.1 (cont'd).

	262	268	271	275	277	280	281	283	287	293	295	302	305	307	313	315	319	321	322	325	328	329	335	337	340	343	344	346	352	357	358	361	364	374	375	376	382	385	391	394	400	403	413	421	427	430	433
H101	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	C	.	.	.	.	A	.	C	.	.	T	C	.	.	.	.	C	.	.	.	A	.
H102	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	C	.	.	T	C	.	.	.	.	.	.	.	.	A	.
H103	C	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	A	.
H104	.	C	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	A	.	.	.	.	.	T	.	A	.	.	.	A	.	.	T	.	.	.	.	.	.	.	.	.	.	.	A	.
H105	C	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	T	.	.	.	A	.	.	.	.	.	A	.	
H106	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	A	.	T	.	.	.	.	A	.	C	.	.	T	.	C	.	.	.	.	.	.	.	.	A	.
H107	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	C	.	.	T	.	C	.	.	.	.	.	.	.	.	A	.
H108	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	C	.	.	T	C	.	.	.	.	.	.	.	.	.	A	.
H109	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	G	.	.	A	.	C	.	.	T	.	C	.	.	.	.	.	.	.	A	.
H110	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	A	.	C	.	.	T	.	C	.	.	.	.	.	.	.	.	A	.
H111	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	C	.	.	.	A	.	C	.	.	T	C	.	.	.	.	.	.	.	.	.	A	.
H112	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	C	.	.	T	C	.	.	.	.	.	.	.	.	.	A	.
H113	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	C	.	.	C	.	A	.	C	.	.	T	C	.	.	.	.	.	.	.	.	A	.
H114	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	C	.	.	.	A	.	C	.	.	T	A	.	.	.	.	.	.	.	.	.	A	.
H115	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	C	.	.	T	.	C	.	.	.	.	.	.	.	.	A	.
H116	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	C	.	.	T	.	C	.	.	.	.	.	.	.	.	A	.
H117	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	C	.	.	T	.	.	.	.	.	.	.	.	.	.	A	.
H118	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	G	.	.	A	.	C	.	.	T	.	.	.	.	.	.	.	.	.	A	.
H119	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	C	.	.	T	.	C	.	.	.	.	.	.	.	.	A	.
H120	C	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	C	.	.	T	.	.	.	.	.	.	.	.	.	.	A	.
H121	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	G	.	.	A	.	C	.	.	T	.	C	.	.	.	.	.	.	.	A	.
H122	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	A	.	C	.	.	T	C	.	.	.	.	.	.	.	.	.	A	.
H123	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	C	.	.	.	A	.	C	.	.	T	C	.	.	.	.	.	.	.	.	.	A	.
H124	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	C	.	.	C	.	.	.	.	.	.	.	.	.	.	A	.
H125	.	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	A	.	C	.	.	T	C	.	.	.	.	.	.	.	.	.	A	.

Table 4. 2. Maximum composite likelihood estimates of the pattern of nucleotide substitutions for the cyt b region of *C. quercusfolii*. Rates of different transitional substitutions are shown in bold and those of transversional substitutions are shown in italics.

	A	T	C	G
A	–	<i>1.76</i>	<i>0.53</i>	6.96
T	<i>1.34</i>	–	14.72	<i>0.44</i>
C	<i>1.34</i>	48.92	–	<i>0.44</i>
G	21.24	<i>1.76</i>	<i>0.53</i>	–

Amino acid translations were shown in Table 4.3. Out of 144 amino acids 18 (12.5%) were showing substitutions. Thirty-seven substitutions (39.7%) occurred at third codon position, three substitutions (3.2%) were observed at second codon position, and fifteen (16.1%) were at first codon position. There was a tendency to use some amino acids more than others (Table 4.4). Leucine (L) and isoleucine (I) were in highest frequency (19). Amino acid replacements occurred at sites 16 (from leucine to phenylalanine), 49 (from valine to isoleucine), 58 (from leucine to methionine), 72 (from threonine to isoleucine), 92 (from glycine to serine) and 94 (from leucine to valine), 96 (from methionine to valine), 98 (from valine to methionine), 102 (from methionine to leucine), 105 (from cysteine to phenylalanine), 107 (from serine to phenylalanine), 110 (from isoleucine to valine), 112 (from glycine to serine), 115 (from glutamic acid to lysine), 119 (from methionine to threonine), 127 (from isoleucine to methionine), and 138 (from alanine to threonine). There was only a single multiple hit at amino acid site 125 (from threonine to serine and isoleucine).

Table 4. 3. Polymorphic amino acid sites of *C. quercusfolii* cyt b 125 haplotypes.

Haplotypes	Pos.																Haplotypes	Pos.																		
	AA																	AA																		
	1	4	5	7	9	9	9	1	1	1	1	1	1	2	3	8		1	4	5	7	9	9	9	1	1	1	1	1	1	2	3	8			
AA	L	I	L	T	G	L	M	V	M	C	S	I	G	E	M	T	I	A	L	I	L	T	G	L	M	V	M	C	S	I	G	E	M	T	I	A
H1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H65	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H2	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H66	.	V	.	.	.	.	.	.	.	.	V	.	.	.	.	.		
H3	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H67	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H4	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H68	.	V	.	.	S	.	.	.	.	.	.	.	K	.	I	.	.	
H5	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H69	.	V	.	.	.	.	.	.	.	F	.	.	.	K	.	I	.	T
H6	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H70	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H7	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H71	.	V	.	.	.	.	.	.	.	.	.	.	S	.	.	.	.	
H8	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H72	.	V	.	.	.	.	.	.	.	.	.	.	S	.	.	.	.	
H9	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H73	.	V	.	.	.	.	.	.	.	.	.	.	S	.	.	.	.	
H10	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H74	.	V	.	.	.	.	.	.	.	.	.	.	.	K	.	I	.	.
H11	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H75	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H12	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H76	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H13	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H77	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H14	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H78	.	V	.	.	.	.	.	.	.	.	F	.	.	.	.	.	.	
H15	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H79	.	V	.	.	.	.	.	.	.	.	.	V	.	.	.	.	.	
H16	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H80	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H17	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H81	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H18	.	V	.	.	.	.	.	.	.	.	V	.	.	.	.	.	.	H82	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H19	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H83	.	V	.	.	.	.	.	.	.	.	.	V	.	.	.	.	.	
H20	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H84	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H21	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H85	.	V	.	.	.	V	.	.	.	.	.	.	.	.	.	.	.	
H22	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H86	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H23	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H87	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H24	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H88	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H25	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H89	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H26	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H90	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H27	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H91	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H28	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H92	.	V	.	.	S	.	.	.	.	.	.	.	.	.	.	I	.	
H29	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H93	.	V	.	.	S	.	.	.	.	.	.	.	.	.	.	.	I	
H30	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H94	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H31	.	V	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	H95	.	V	.	.	S	.	.	.	.	.	.	.	.	.	I	.		
H32	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H96	.	V	.	.	.	.	M	.	.	.	.	.	.	.	.	.		
H33	.	V	.	.	.	.	.	.	.	.	.	.	K	.	.	.	.	H97	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.		
H34	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H98	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H35	.	V	.	.	.	.	.	.	.	.	.	.	K	.	.	.	.	H99	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.		
H36	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H100	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H37	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H101	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H38	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H102	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H39	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H103	.	V	M	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H40	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H104	.	V	.	.	.	.	.	.	.	.	.	.	.	K	.	I	.	
H41	.	V	.	.	.	.	V	.	.	.	.	.	.	.	.	.	.	H105	F	V	.	.	.	.	.	.	.	.	.	.	.	.	K	.	I	
H42	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H106	.	V	.	.	.	.	.	.	.	.	.	.	S	.	.	.	.	
H43	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H107	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H44	.	V	.	.	.	.	.	.	.	.	V	.	.	.	.	.	.	H108	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H45	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H109	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H46	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H110	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H47	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H111	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H48	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H112	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H49	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H113	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H50	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H114	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	M	.	
H51	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H115	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H52	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	S	.	H116	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H53	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H117	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H54	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H118	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H55	.	V	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	H119	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H56	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H120	F	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H57	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H121	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H58	.	V	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	H122	.	V	.	I	.	.	.	.	.	.	.	.	.	.	.	.	.	
H59	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H123	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H60	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H124	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H61	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H125	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
H62	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.																			
H63	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.																			
H64	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.																			

Table 4. 4. Frequency of amino acids used in *C. quercusfolii* cyt b haplotypes. All frequencies are averages over all sequences. Relative synonymous codon usage is given in parentheses following the codon frequency. (RSCU: Relative Synonymous Codon Usage).

Codon	Count	RSCU	Codon	Count	RSCU
UUU (F)	10.6	1.76	UCU (S)	2.9	2.9
UUC (F)	1.5	0.24	UCC (S)	0.9	0.91
UUA (L)	19.8	5.66	UCA (S)	2.2	2.14
UUG (L)	0.1	0.02	UCG (S)	0	0
CUU (L)	0.5	0.13	CCU (P)	4.5	2.02
CUC (L)	0.4	0.13	CCC (P)	0.4	0.18
CUA (L)	0.2	0.06	CCA (P)	4	1.8
CUG (L)	0	0	CCG (P)	0	0
AUU (I)	18	1.9	ACU (T)	4.8	2.41
AUC (I)	1	0.1	ACC (T)	0.2	0.08
AUA (M)	8.1	1.81	ACA (T)	3	1.51
AUG (M)	0.8	0.19	ACG (T)	0	0
GUU (V)	2	1.59	GCU (A)	4.2	2.83
GUC (V)	0	0.04	GCC (A)	0.7	0.49
GUA (V)	2.9	2.33	GCA (A)	1	0.68
GUG (V)	0.1	0.04	GCG (A)	0	0
UAU (Y)	5.7	1.91	UGU (C)	2	1.98
UAC (Y)	0.3	0.09	UGC (C)	0	0.02
UAA (*)	0	0	UGA (W)	3	1.49
UAG (*)	0	0	UGG (W)	1	0.51
CAU (H)	4.8	1.92	CGU (R)	0	0.05
CAC (H)	0.2	0.08	CGC (R)	0	0
CAA (Q)	1.9	1.94	CGA (R)	1.9	3.86
CAG (Q)	0.1	0.06	CGG (R)	0	0.1
AAU (N)	8.7	1.58	AGU (S)	0	0
AAC (N)	2.3	0.42	AGC (S)	0	0.01
AAA (K)	2	2	AGA (S)	2	2.03
AAG (K)	0	0	AGG (S)	0	0.01
GAU (D)	1.7	1.69	GGU (G)	0.9	0.44
GAC (D)	0.3	0.31	GGC (G)	0	0
GAA (E)	2.9	1.95	GGA (G)	4.4	2.2
GAG (E)	0.1	0.05	GGG (G)	2.7	1.35

The frequency of 125 cyt b haplotypes was given in Table 4.5. With the highest H5 (N=38) was shared among 11 localities (Afyon, Balıkesir, Bolu, Bursa, Çankırı, İstanbul, İzmir, Kastamonu, Kırklareli, Sinop, Yalova). The next highly observed haplotype was H15 (N= 26) detected in Bingöl, Bitlis, Kayseri, Tunceli, Muş, and Van. Among 125 haplotypes there was a high number of singletons (N= 80), however the number of private haplotype (N= 8) was low. For instance, H7 (N= 2) was found only in the Artvin population, H43 (N= 7) in the Edirne population, H125 (N= 3) in the Zonguldak population. Edirne and Zonguldak localities were fixed with respect to their haplotypes where only a single type of sequence was observed in both.

The pairwise comparisons to determine sequence differences between each *C. quercusfolii* haplotype ranged between 0.2% and 7.5% (Table 4.6). Between 85 cases there was one base pair difference (0.2%) while the most divergent haplotypes were H69 (from Isparta population, N=1) and H72 (from İstanbul population, N=1) with a 30 basepair difference (7.5%). The next highest pairwise difference was 29 base pair observed between H68 (from Isparta) - H72 (from İstanbul) - H114 (from Sinop), the third highest difference was 28 base pairs in two pairwise comparisons: between H69 (from Isparta population) - H32 (from Bursa, Çankırı, and İstanbul population), and H64 (from Gümüşhane and Sinop population), - H85 (from Kayseri).

Table 4. 5. Cyt b haplotypes and their frequencies in *C. quercusfolii* populations.

	AFY	ART	BAL	BAR	BIN	BIT	BOL	BRS	CAN	CNK	DUZ	EDI	ELA	ERI	ERZ	GIR	GUM	ISP	IST	IZM	KAR	KAS	KAY	KLR	KON	KUT	MAL	MUS	ORD	SAM	SIN	SIV	TOK	TUN	VAN	YLV	YOZ	ZON	Total	
H1	1																																							1
H2	3																																							3
H3	2																			6						2														10
H4	1																				1						2													2
H5	2		2				6	1		3								2	2		6		3								4					7				38
H6	1																																							1
H7		2															1																							3
H8			2																																					2
H9			1						3																															4
H10			2						4	2															1											1				10
H11			1																																					1
H12			1																																					1
H13			1																																					1
H14				1																																				1
H15					8	3																	1					7							1	6				26
H16					1																																			1
H17					1																																			1
H18					1																																			1
H19					1																																			1
H20						1											1																							2
H21						1								4			4																							9
H22						1																																		1
H23						1																						1						1						3
H24						1																						1						1						3
H25						2																																		2
H26							1																																	1
H27							1																	1																2

Table 4. 5 (cont'd).

	AFY	ART	BAL	BAR	BIN	BIT	BOL	BRS	CAN	CNK	DUZ	EDI	ELA	ERI	ERZ	GIR	GUM	ISP	IST	IZM	KAR	KAS	KAY	KLR	KON	KUT	MAL	MUS	ORD	SAM	SIN	SIV	TOK	TUN	VAN	YLV	YOZ	ZON	Total
H28							1												1																				2
H29							1	1											1			2		1															6
H30								1																															1
H31								2																															2
H32								1		1									1																				3
H33								1																															1
H34								1											1																				2
H35								1																															1
H36									2																														2
H37									1																														1
H38										1														1															2
H39										1																													1
H40										1											1								1		3								6
H41										1																													1
H42											2																												2
H43												7																											7
H44													3																										3
H45													1																										1
H46													1																										1
H47														1																									1
H48														3																									3
H49														1																									1
H50														1																									1
H51															1																								1
H52															2																								2

Table 4. 5 (cont'd).

	AFY	ART	BAL	BAR	BIN	BIT	BOL	BRS	CAN	CNK	DUZ	EDI	ELA	ERI	ERZ	GIR	GUM	ISP	IST	IZM	KAR	KAS	KAY	KLR	KON	KUT	MAL	MUS	ORD	SAM	SIN	SIV	TOK	TUN	VAN	YLV	YOZ	ZON	Total	
H53															1																									1
H54																1																								1
H55																2																								2
H56																1																								1
H57																1																								1
H58																1																								1
H59																1																								1
H60																1																								1
H61																1																								1
H62																1																								1
H63																	1																			2				3
H64																	1																1							2
H65																	1																							1
H66																	1																							1
H67																	1																							1
H68																		9																						9
H69																		1																						1
H70																			1																					1
H71																			1																					1
H72																			1																					1
H73																			1																					1
H74																				2																				2
H75																					3																			3
H76																					2																			2
H77																					1																			1
H78																					1																			1

Table 4. 5 (cont'd).

	AFY	ART	BAL	BAR	BIN	BIT	BOL	BRS	CAN	CNK	DUZ	EDI	ELA	ERI	ERZ	GIR	GUM	ISP	IST	IZM	KAR	KAS	KAY	KLR	KON	KUT	MAL	MUS	ORD	SAM	SIN	SIV	TOK	TUN	VAN	YLV	YOZ	ZON	Total	
H79																					1																			1
H80																						2																		2
H81																						1																		1
H82																							2																	2
H83																							6																	6
H84																							1																	1
H85																							1																	1
H86																								1																1
H87																								1																1
H88																								1																1
H89																								1																1
H90																									1															1
H91																									1															1
H92																									3															3
H93																									1															1
H94																								1																1
H95																									2															2
H96																									1															1
H97																										2														2
H98																									1															1
H99																									1															1
H100																										1														1
H101																										1														1
H102																										1														5
H103																											2													2
H104																											1													1

Table 4. 5 (cont'd).

	AFY	ART	BAL	BAR	BIN	BIT	BOL	BRS	CAN	CNK	DUZ	EDI	ELA	ERI	ERZ	GIR	GUM	ISP	IST	IZM	KAR	KAS	KAY	KLR	KON	KUT	MAL	MUS	ORD	SAM	SIN	SIV	TOK	TUN	VAN	YLV	YOZ	ZON	Total
H105																												1											1
H106																												1											1
H107																													1										1
H108																														1									3
H109																															3								1
H110																															1								1
H111																															1								1
H112																															1								1
H113																																1							1
H114																																1							1
H115																																	1						1
H116																																	1						1
H117																																	1						1
H118																																	1						1
H119																																		1					1
H120																																		1					1
H121																																			1				1
H122																																				1			1
H123																																				1			1
H124																																					1		1
H125																																						3	3
N	10	2	10	1	12	10	10	9	10	10	2	7	5	10	4	10	11	10	10	10	10	11	11	10	10	10	3	11	2	11	10	6	1	1	10	10	1	3	294
Hap _{Req}	6	1	7	1	5	7	5	8	4	7	1	1	3	5	3	9	8	2	9	3	7	4	5	8	7	8	2	5	2	6	5	6	1	1	4	4	1	1	

Table 4. 6. Pairwise comparison of 125 *C. quercusfolii* haplotypes. Net nucleotide differences are shown below diagonal and the percentage of the differences are presented above diagonal.

	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15	H16	H17	H18	H19	H20	H21	H22	H23	H24	H25	H26	H27	H28	H29	H30	H31	H32	H33	H34	H35	H36	H37	H38	H39	H40
H1		0.005	0.041	0.002	0.046	0.034	0.041	0.039	0.039	0.041	0.039	0.044	0.039	0.049	0.036	0.036	0.039	0.059	0.041	0.041	0.044	0.038	0.036	0.034	0.046	0.049	0.049	0.046	0.044	0.044	0.049	0.046	0.056	0.046	0.051	0.039	0.039	0.044	0.049	0.044
H2	2		0.041	0.002	0.041	0.034	0.036	0.039	0.034	0.036	0.039	0.039	0.039	0.044	0.031	0.031	0.034	0.054	0.036	0.036	0.039	0.033	0.031	0.029	0.041	0.044	0.044	0.041	0.039	0.039	0.044	0.041	0.056	0.041	0.051	0.034	0.034	0.039	0.044	0.039
H3	17	17		0.039	0.005	0.007	0.021	0.007	0.007	0.005	0.007	0.007	0.002	0.007	0.021	0.019	0.019	0.051	0.019	0.021	0.024	0.019	0.016	0.019	0.031	0.007	0.007	0.009	0.007	0.007	0.007	0.012	0.041	0.005	0.031	0.007	0.007	0.007	0.007	0.002
H4	1	1	16		0.044	0.031	0.039	0.036	0.036	0.039	0.041	0.041	0.036	0.046	0.034	0.034	0.036	0.056	0.039	0.039	0.041	0.036	0.034	0.031	0.043	0.046	0.046	0.044	0.041	0.041	0.046	0.044	0.054	0.044	0.049	0.036	0.036	0.041	0.046	0.041
H5	19	17	2	18		0.012	0.021	0.012	0.007	0.005	0.007	0.007	0.007	0.002	0.021	0.019	0.019	0.051	0.019	0.021	0.024	0.016	0.016	0.019	0.029	0.002	0.002	0.002	0.002	0.002	0.007	0.046	0.005	0.036	0.007	0.007	0.002	0.002	0.002	
H6	14	14	3	13	5		0.024	0.009	0.009	0.007	0.009	0.009	0.005	0.014	0.024	0.021	0.021	0.049	0.021	0.024	0.026	0.021	0.019	0.021	0.029	0.014	0.014	0.016	0.014	0.009	0.014	0.019	0.034	0.012	0.024	0.005	0.009	0.014	0.014	0.009
H7	17	15	9	16	9	10		0.029	0.019	0.021	0.024	0.024	0.024	0.024	0.009	0.012	0.007	0.046	0.012	0.005	0.002	0.007	0.005	0.007	0.019	0.024	0.024	0.026	0.024	0.024	0.024	0.029	0.049	0.021	0.039	0.024	0.024	0.024	0.024	0.019
H8	16	16	3	15	5	4	12		0.009	0.007	0.009	0.009	0.005	0.014	0.029	0.026	0.026	0.051	0.026	0.029	0.031	0.026	0.024	0.026	0.034	0.014	0.014	0.012	0.009	0.014	0.014	0.014	0.041	0.012	0.034	0.009	0.005	0.014	0.014	0.009
H9	16	14	3	15	3	4	8	4		0.002	0.005	0.005	0.005	0.009	0.019	0.016	0.016	0.043	0.016	0.019	0.021	0.016	0.014	0.016	0.024	0.009	0.009	0.012	0.009	0.009	0.009	0.014	0.039	0.007	0.029	0.005	0.005	0.009	0.009	0.005
H10	17	15	2	16	2	3	9	3	1		0.002	0.002	0.002	0.007	0.021	0.019	0.019	0.046	0.019	0.021	0.024	0.019	0.016	0.019	0.026	0.007	0.007	0.009	0.007	0.007	0.007	0.012	0.041	0.005	0.031	0.002	0.002	0.007	0.007	0.002
H11	16	16	3	17	3	4	10	4	2	1		0.005	0.005	0.009	0.024	0.021	0.021	0.049	0.021	0.024	0.026	0.021	0.019	0.021	0.029	0.009	0.009	0.009	0.014	0.044	0.007	0.034	0.005	0.005	0.009	0.009	0.005	0.009	0.005	
H12	18	16	3	17	3	4	10	4	2	1	2		0.005	0.009	0.024	0.021	0.021	0.049	0.021	0.024	0.026	0.021	0.019	0.021	0.029	0.009	0.009	0.012	0.009	0.009	0.009	0.014	0.044	0.007	0.034	0.005	0.005	0.009	0.009	0.005
H13	16	16	1	15	3	2	10	2	2	1	2	2		0.009	0.024	0.021	0.021	0.049	0.021	0.024	0.026	0.021	0.019	0.021	0.029	0.009	0.009	0.009	0.014	0.039	0.007	0.029	0.005	0.005	0.009	0.009	0.005	0.009	0.005	
H14	20	18	3	19	1	6	10	6	4	3	4	4	4		0.024	0.021	0.021	0.054	0.021	0.024	0.026	0.019	0.019	0.021	0.031	0.005	0.005	0.012	0.005	0.005	0.005	0.009	0.044	0.007	0.034	0.009	0.009	0.005	0.005	0.005
H15	15	13	9	14	9	10	4	12	8	9	10	10	10	10		0.002	0.002	0.046	0.007	0.009	0.012	0.007	0.005	0.002	0.024	0.024	0.024	0.026	0.024	0.024	0.024	0.029	0.044	0.021	0.039	0.024	0.024	0.019	0.024	0.019
H16	15	13	8	14	8	9	5	11	7	8	9	9	9	9	1		0.005	0.046	0.005	0.012	0.014	0.009	0.007	0.005	0.026	0.021	0.021	0.024	0.021	0.021	0.021	0.026	0.046	0.019	0.041	0.021	0.021	0.016	0.021	0.016
H17	16	14	8	15	8	9	3	11	7	8	9	9	9	9	1	2		0.044	0.005	0.007	0.009	0.005	0.002	0.005	0.026	0.021	0.021	0.024	0.021	0.021	0.021	0.026	0.046	0.019	0.036	0.021	0.021	0.021	0.021	0.016
H18	24	22	21	23	21	20	19	21	18	19	20	20	22	19	19	18		0.041	0.041	0.044	0.043	0.041	0.044	0.043	0.054	0.054	0.054	0.054	0.054	0.054	0.054	0.059	0.062	0.051	0.056	0.049	0.049	0.054	0.054	0.046
H19	17	15	8	16	8	9	5	11	7	8	9	9	9	9	3	2	2	17		0.007	0.009	0.009	0.007	0.009	0.031	0.021	0.021	0.024	0.021	0.021	0.021	0.026	0.051	0.019	0.041	0.021	0.021	0.021	0.021	0.016
H20	17	15	9	16	9	10	2	12	8	9	10	10	10	10	4	5	3	17	3		0.002	0.007	0.005	0.007	0.024	0.024	0.024	0.026	0.024	0.024	0.024	0.029	0.049	0.021	0.039	0.024	0.024	0.024	0.019	
H21	18	16	10	17	10	11	1	13	9	10	11	11	11	11	5	6	4	18	4	1		0.009	0.007	0.009	0.021	0.026	0.026	0.029	0.026	0.026	0.026	0.031	0.051	0.024	0.041	0.026	0.026	0.026	0.026	0.021
H22	16	14	8	15	7	9	3	11	7	8	9	9	9	8	3	4	2	18	4	3	4		0.002	0.005	0.021	0.019	0.019	0.024	0.019	0.019	0.019	0.024	0.046	0.019	0.036	0.021	0.021	0.019	0.019	0.016
H23	15	13	7	14	7	8	2	10	6	7	8	8	8	8	2	3	1	17	3	2	3	1		0.002	0.024	0.019	0.019	0.021	0.019	0.019	0.019	0.024	0.044	0.016	0.034	0.019	0.019	0.019	0.019	0.014
H24	14	12	8	13	8	9	3	11	7	8	9	9	9	9	1	2	2	18	4	3	4	2	1		0.021	0.021	0.021	0.024	0.021	0.021	0.021	0.026	0.041	0.019	0.036	0.021	0.021	0.016	0.021	0.016
H25	19	17	13	18	12	12	8	14	10	11	12	12	12	13	10	11	11	23	13	10	9	9	10	9		0.031	0.031	0.031	0.031	0.031	0.031	0.036	0.046	0.026	0.044	0.029	0.029	0.026	0.031	0.029
H26	20	18	3	19	1	6	10	6	4	3	4	4	4	2	10	9	22	9	10	11	8	8	9	13		0.005	0.012	0.005	0.005	0.005	0.009	0.049	0.007	0.039	0.009	0.009	0.005	0.005	0.005	
H27	20	18	3	19	1	6	10	6	4	3	4	4	4	2	10	9	22	9	10	11	8	8	9	13	2		0.012	0.005	0.005	0.005	0.009	0.049	0.007	0.039	0.009	0.009	0.005	0.005	0.005	
H28	19	17	4	18	4	7	11	5	5	4	5	5	5	5	11	10	10	23	10	11	12	10	9	10	13	5	5		0.007	0.012	0.012	0.012	0.051	0.005	0.041	0.012	0.007	0.012	0.007	
H29	18	16	3	17	1	6	10	4	3	4	4	4	4	2	10	9	22	9	10	11	8	8	9	13	2	3		0.005	0.005	0.005	0.049	0.007	0.039	0.009	0.005	0.005	0.005	0.005		
H30	18	16	3	17	1	4	10	6	4	3	4	4	4	2	10	9	22	9	10	11	8	8	9	13	2	2	5	2		0.005	0.009	0.044	0.007	0.034	0.005	0.009	0.005	0.005	0.005	
H31	20	18	3	19	1	6	10	6	4	3	4	4	4	2	10	9	22	9	10	11	8	8	9	13	2	2	5	2	2		0.009	0.049	0.007	0.039	0.009	0.009	0.005	0.005	0.005	
H32	19	17	5	18	3	8	12	6	6	5	6	6	6	4	12	11	11	24	11	12	13	10	10	11	15	4	4	5	2	4	4		0.054	0.012	0.044	0.014	0.009	0.009	0.009	0.009
H33	23	23	17	22	19	14	20	17	16	17	18	18	16	18	18	19	19	25	21	20	21	19	18	17	19	20	20	18	20	22		0.046	0.009	0.039	0.044	0.044	0.049	0.044		
H34	19	17	2	18	2	5	9	5	3	2	3	3	3	3	9	8	21	8	9	10	8	7	8	11	3	3	2	3	3	5	19		0.036	0.007	0.007	0.007	0.007	0.002		
H35	21	21	13	20	15	10	16	14	12	13	14	14	12	14	16	17	15	23	17	16	17	15	14	15	18	16	16	17	16	14	16	18	4	15		0.029	0.034	0.039	0.039	

Table 4. 6 (cont'd).

	H41	H42	H43	H44	H45	H46	H47	H48	H49	H50	H51	H52	H53	H54	H55	H56	H57	H58	H59	H60	H61	H62	H63	H64	H65	H66	H67	H68	H69	H70	H71	H72	H73	H74	H75	H76	H77	H78	H79	H80
H1	0.044	0.049	0.049	0.054	0.034	0.031	0.044	0.041	0.046	0.044	0.038	0.054	0.054	0.046	0.046	0.044	0.051	0.046	0.046	0.038	0.049	0.049	0.046	0.044	0.046	0.046	0.044	0.059	0.067	0.044	0.049	0.051	0.044	0.056	0.007	0.044	0.009	0.044	0.041	0.041
H2	0.039	0.044	0.044	0.049	0.029	0.026	0.039	0.036	0.041	0.039	0.033	0.054	0.054	0.041	0.041	0.038	0.046	0.041	0.041	0.033	0.043	0.044	0.041	0.044	0.041	0.041	0.044	0.059	0.067	0.039	0.043	0.046	0.038	0.056	0.007	0.044	0.009	0.044	0.036	0.041
H3	0.007	0.007	0.009	0.051	0.026	0.019	0.024	0.026	0.031	0.031	0.024	0.051	0.051	0.041	0.026	0.024	0.036	0.026	0.026	0.019	0.029	0.029	0.026	0.029	0.026	0.031	0.029	0.054	0.057	0.007	0.024	0.026	0.016	0.046	0.041	0.002	0.034	0.007	0.036	0.009
H4	0.041	0.046	0.046	0.051	0.031	0.029	0.041	0.039	0.044	0.041	0.036	0.051	0.051	0.043	0.043	0.041	0.049	0.043	0.046	0.046	0.044	0.041	0.044	0.044	0.041	0.041	0.056	0.064	0.041	0.046	0.049	0.041	0.054	0.005	0.041	0.007	0.041	0.039	0.044	
H5	0.007	0.007	0.005	0.051	0.026	0.019	0.024	0.026	0.031	0.031	0.024	0.056	0.056	0.041	0.026	0.024	0.036	0.026	0.026	0.019	0.026	0.029	0.026	0.034	0.026	0.031	0.034	0.059	0.062	0.007	0.021	0.024	0.014	0.051	0.046	0.007	0.039	0.012	0.036	0.009
H6	0.009	0.014	0.016	0.049	0.024	0.021	0.026	0.024	0.029	0.029	0.026	0.044	0.044	0.034	0.029	0.026	0.029	0.029	0.029	0.021	0.026	0.031	0.029	0.031	0.029	0.034	0.031	0.046	0.049	0.014	0.026	0.029	0.019	0.039	0.034	0.009	0.026	0.009	0.039	0.016
H7	0.024	0.024	0.026	0.046	0.016	0.009	0.007	0.009	0.009	0.014	0.007	0.054	0.054	0.039	0.005	0.002	0.034	0.009	0.005	0.007	0.016	0.007	0.005	0.012	0.005	0.019	0.012	0.057	0.059	0.024	0.021	0.024	0.024	0.054	0.041	0.024	0.034	0.024	0.024	0.026
H8	0.009	0.014	0.016	0.051	0.029	0.026	0.031	0.029	0.034	0.039	0.031	0.046	0.046	0.041	0.034	0.031	0.036	0.034	0.034	0.026	0.031	0.036	0.034	0.036	0.034	0.039	0.036	0.054	0.057	0.009	0.026	0.029	0.019	0.046	0.039	0.009	0.031	0.009	0.039	0.012
H9	0.005	0.009	0.012	0.043	0.019	0.016	0.021	0.019	0.024	0.029	0.021	0.049	0.049	0.034	0.024	0.021	0.029	0.024	0.024	0.016	0.021	0.026	0.024	0.031	0.024	0.029	0.031	0.051	0.054	0.009	0.021	0.024	0.014	0.044	0.039	0.009	0.031	0.014	0.034	0.012
H10	0.002	0.007	0.009	0.046	0.021	0.019	0.024	0.021	0.026	0.031	0.024	0.051	0.051	0.036	0.026	0.024	0.031	0.026	0.026	0.019	0.024	0.029	0.026	0.034	0.026	0.031	0.034	0.054	0.057	0.007	0.024	0.026	0.016	0.046	0.041	0.007	0.034	0.012	0.036	0.009
H11	0.005	0.009	0.012	0.049	0.024	0.021	0.026	0.024	0.029	0.034	0.026	0.054	0.054	0.039	0.029	0.026	0.034	0.029	0.029	0.021	0.026	0.031	0.029	0.036	0.029	0.034	0.036	0.057	0.059	0.009	0.026	0.029	0.019	0.049	0.044	0.009	0.036	0.014	0.039	0.007
H12	0.005	0.009	0.012	0.049	0.024	0.021	0.026	0.024	0.029	0.034	0.026	0.054	0.054	0.039	0.029	0.026	0.034	0.029	0.029	0.021	0.026	0.031	0.029	0.036	0.029	0.034	0.036	0.057	0.059	0.009	0.026	0.029	0.019	0.049	0.044	0.009	0.036	0.014	0.039	0.012
H13	0.005	0.009	0.012	0.049	0.024	0.021	0.026	0.024	0.029	0.034	0.026	0.049	0.049	0.039	0.029	0.026	0.034	0.029	0.029	0.021	0.026	0.031	0.029	0.031	0.029	0.034	0.031	0.051	0.054	0.009	0.026	0.029	0.019	0.044	0.039	0.005	0.031	0.009	0.039	0.012
H14	0.009	0.009	0.007	0.054	0.029	0.021	0.026	0.029	0.031	0.034	0.026	0.054	0.054	0.039	0.029	0.026	0.034	0.029	0.029	0.021	0.029	0.031	0.029	0.036	0.029	0.034	0.036	0.057	0.059	0.009	0.024	0.026	0.016	0.049	0.049	0.009	0.041	0.014	0.039	0.012
H15	0.024	0.024	0.026	0.041	0.012	0.005	0.012	0.014	0.019	0.024	0.007	0.043	0.044	0.034	0.014	0.012	0.034	0.014	0.014	0.007	0.026	0.016	0.014	0.021	0.014	0.009	0.021	0.051	0.059	0.024	0.031	0.034	0.024	0.049	0.036	0.024	0.029	0.024	0.014	0.026
H16	0.021	0.021	0.024	0.041	0.012	0.005	0.014	0.016	0.021	0.014	0.009	0.041	0.041	0.036	0.016	0.014	0.036	0.016	0.016	0.009	0.029	0.019	0.016	0.024	0.017	0.012	0.024	0.054	0.062	0.021	0.029	0.031	0.021	0.051	0.036	0.021	0.029	0.021	0.016	0.024
H17	0.021	0.021	0.024	0.044	0.014	0.007	0.014	0.012	0.016	0.021	0.009	0.046	0.046	0.036	0.012	0.009	0.031	0.012	0.012	0.005	0.024	0.014	0.012	0.019	0.012	0.012	0.019	0.054	0.057	0.021	0.029	0.031	0.021	0.051	0.039	0.021	0.031	0.021	0.016	0.024
H18	0.049	0.054	0.056	0.005	0.033	0.041	0.044	0.041	0.046	0.054	0.049	0.059	0.059	0.051	0.051	0.049	0.046	0.046	0.051	0.043	0.054	0.049	0.046	0.054	0.046	0.046	0.049	0.064	0.067	0.054	0.062	0.064	0.054	0.062	0.059	0.054	0.056	0.051	0.046	0.056
H19	0.021	0.021	0.024	0.041	0.016	0.009	0.014	0.012	0.016	0.016	0.014	0.046	0.046	0.041	0.016	0.014	0.036	0.016	0.016	0.009	0.029	0.014	0.012	0.019	0.012	0.016	0.019	0.059	0.062	0.021	0.029	0.031	0.021	0.057	0.041	0.021	0.034	0.021	0.021	0.024
H20	0.024	0.024	0.026	0.041	0.016	0.009	0.007	0.009	0.009	0.014	0.012	0.054	0.054	0.039	0.009	0.007	0.034	0.009	0.009	0.007	0.021	0.007	0.005	0.012	0.005	0.019	0.012	0.057	0.059	0.024	0.026	0.029	0.024	0.054	0.041	0.024	0.034	0.024	0.024	0.026
H21	0.026	0.026	0.029	0.044	0.019	0.012	0.005	0.007	0.007	0.012	0.009	0.056	0.057	0.041	0.007	0.005	0.036	0.012	0.007	0.009	0.019	0.005	0.002	0.009	0.002	0.021	0.009	0.059	0.062	0.026	0.024	0.026	0.026	0.057	0.044	0.026	0.036	0.026	0.026	0.029
H22	0.021	0.021	0.021	0.043	0.014	0.007	0.014	0.012	0.016	0.021	0.009	0.051	0.051	0.036	0.012	0.009	0.031	0.012	0.012	0.005	0.019	0.014	0.012	0.019	0.012	0.016	0.019	0.054	0.056	0.021	0.024	0.026	0.016	0.051	0.039	0.021	0.031	0.021	0.021	0.024
H23	0.019	0.019	0.021	0.041	0.012	0.005	0.012	0.009	0.014	0.019	0.007	0.049	0.049	0.034	0.009	0.007	0.029	0.009	0.009	0.002	0.021	0.012	0.009	0.016	0.009	0.014	0.016	0.051	0.054	0.019	0.026	0.029	0.019	0.049	0.039	0.019	0.029	0.019	0.019	0.021
H24	0.021	0.021	0.024	0.038	0.009	0.002	0.009	0.012	0.016	0.021	0.005	0.046	0.046	0.031	0.012	0.009	0.031	0.012	0.012	0.005	0.024	0.014	0.012	0.019	0.012	0.012	0.019	0.049	0.057	0.021	0.029	0.031	0.021	0.046	0.034	0.021	0.026	0.021	0.016	0.024
H25	0.029	0.033	0.033	0.051	0.026	0.024	0.016	0.024	0.024	0.034	0.021	0.056	0.056	0.036	0.024	0.021	0.036	0.029	0.019	0.026	0.012	0.026	0.024	0.031	0.024	0.034	0.026	0.054	0.062	0.034	0.026	0.024	0.029	0.051	0.046	0.029	0.038	0.034	0.039	0.031
H26	0.009	0.009	0.007	0.054	0.029	0.021	0.026	0.029	0.034	0.034	0.026	0.059	0.059	0.044	0.029	0.026	0.039	0.029	0.029	0.021	0.029	0.031	0.029	0.036	0.029	0.034	0.036	0.062	0.064	0.009	0.024	0.026	0.016	0.054	0.049	0.009	0.041	0.014	0.039	0.012
H27	0.009	0.007	0.007	0.054	0.029	0.021	0.026	0.029	0.034	0.034	0.024	0.056	0.056	0.041	0.026	0.024	0.036	0.026	0.026	0.019	0.029	0.029	0.029	0.036	0.029	0.034	0.036	0.062	0.064	0.009	0.024	0.026	0.016	0.054	0.049	0.009	0.041	0.014	0.039	0.012
H28	0.012	0.012	0.014	0.056	0.031	0.024	0.029	0.031	0.036	0.036	0.029	0.056	0.056	0.046	0.031	0.029	0.041	0.031	0.026	0.024	0.029	0.034	0.031	0.039	0.031	0.036	0.034	0.065	0.067	0.007	0.024	0.021	0.016	0.057	0.046	0.007	0.039	0.016	0.036	0.005
H29	0.009	0.009	0.007	0.054	0.029	0.021	0.026	0.029	0.034	0.034	0.026	0.054	0.054	0.044	0.029	0.026	0.039	0.029	0.029	0.021	0.029	0.031	0.029	0.036	0.029	0.034	0.036	0.062	0.064	0.005	0.019	0.021	0.012	0.054	0.044	0.009	0.036	0.014	0.034	0.007
H30	0.009	0.009	0.007	0.054	0.029	0.021	0.026	0.029	0.034																															

Table 4. 6 (cont'd).

	H81	H82	H83	H84	H85	H86	H87	H88	H89	H90	H91	H92	H93	H94	H95	H96	H97	H98	H99	H100	H101	H102	H103	H104	H105	H106	H107	H108	H109	H110	H111	H112	H113	H114	H115	H116	H117	H118	H119	H120	H121	H122	H123	H124	H125
H1	0.054	0.046	0.044	0.056	0.059	0.031	0.041	0.041	0.049	0.009	0.005	0.054	0.049	0.039	0.051	0.046	0.039	0.041	0.046	0.034	0.046	0.041	0.038	0.049	0.043	0.049	0.041	0.041	0.044	0.041	0.044	0.044	0.044	0.049	0.044	0.039	0.041	0.036	0.041	0.046	0.043	0.044	0.044	0.041	0.046
H2	0.049	0.041	0.039	0.051	0.053	0.026	0.036	0.036	0.044	0.009	0.005	0.054	0.049	0.039	0.051	0.041	0.034	0.036	0.041	0.034	0.041	0.036	0.033	0.049	0.038	0.044	0.041	0.041	0.039	0.041	0.044	0.039	0.044	0.044	0.034	0.036	0.031	0.041	0.041	0.038	0.039	0.039	0.039	0.036	0.041
H3	0.012	0.026	0.034	0.056	0.054	0.016	0.005	0.005	0.007	0.044	0.039	0.049	0.034	0.034	0.046	0.009	0.007	0.009	0.009	0.007	0.009	0.005	0.024	0.044	0.029	0.029	0.021	0.009	0.024	0.026	0.002	0.007	0.007	0.005	0.019	0.026	0.016	0.021	0.026	0.024	0.024	0.007	0.007	0.009	0.005
H4	0.051	0.044	0.041	0.054	0.056	0.029	0.039	0.039	0.046	0.007	0.002	0.051	0.046	0.036	0.049	0.044	0.036	0.039	0.044	0.031	0.044	0.039	0.036	0.046	0.041	0.046	0.039	0.044	0.041	0.039	0.041	0.041	0.046	0.041	0.036	0.039	0.034	0.039	0.044	0.041	0.041	0.041	0.041	0.038	0.044
H5	0.007	0.026	0.034	0.054	0.051	0.016	0.005	0.005	0.002	0.049	0.044	0.054	0.039	0.039	0.051	0.005	0.007	0.005	0.005	0.012	0.005	0.005	0.024	0.049	0.029	0.029	0.026	0.009	0.024	0.031	0.002	0.007	0.002	0.005	0.019	0.026	0.016	0.026	0.026	0.024	0.024	0.007	0.002	0.009	0.005
H6	0.019	0.029	0.036	0.049	0.051	0.014	0.007	0.012	0.014	0.036	0.031	0.041	0.036	0.026	0.039	0.016	0.014	0.016	0.016	0.009	0.016	0.012	0.026	0.036	0.031	0.031	0.024	0.012	0.026	0.029	0.009	0.009	0.014	0.012	0.021	0.029	0.019	0.024	0.029	0.026	0.026	0.009	0.009	0.012	0.012
H7	0.029	0.005	0.021	0.054	0.056	0.009	0.017	0.021	0.024	0.044	0.039	0.051	0.036	0.036	0.049	0.026	0.019	0.026	0.026	0.024	0.021	0.021	0.012	0.046	0.016	0.012	0.005	0.021	0.007	0.009	0.024	0.019	0.024	0.024	0.007	0.005	0.014	0.005	0.005	0.012	0.007	0.024	0.024	0.026	0.021
H8	0.019	0.034	0.041	0.051	0.048	0.024	0.012	0.012	0.014	0.041	0.036	0.049	0.039	0.036	0.046	0.016	0.014	0.012	0.016	0.009	0.016	0.007	0.031	0.044	0.036	0.036	0.029	0.016	0.031	0.029	0.009	0.014	0.014	0.012	0.026	0.034	0.024	0.029	0.034	0.031	0.031	0.009	0.009	0.012	0.007
H9	0.014	0.024	0.031	0.049	0.046	0.014	0.007	0.007	0.009	0.041	0.036	0.046	0.036	0.031	0.044	0.012	0.009	0.012	0.012	0.009	0.012	0.007	0.021	0.041	0.026	0.026	0.024	0.012	0.021	0.029	0.009	0.009	0.009	0.016	0.024	0.014	0.024	0.024	0.021	0.021	0.005	0.005	0.012	0.007	
H10	0.012	0.026	0.034	0.051	0.048	0.016	0.005	0.005	0.007	0.044	0.039	0.049	0.039	0.034	0.046	0.009	0.007	0.009	0.009	0.007	0.009	0.005	0.024	0.044	0.029	0.029	0.026	0.009	0.024	0.031	0.007	0.007	0.007	0.009	0.019	0.026	0.016	0.026	0.026	0.024	0.024	0.002	0.002	0.009	0.005
H11	0.014	0.029	0.036	0.054	0.051	0.019	0.007	0.007	0.009	0.046	0.041	0.051	0.041	0.036	0.049	0.012	0.009	0.012	0.012	0.009	0.012	0.007	0.026	0.046	0.031	0.031	0.029	0.007	0.026	0.034	0.009	0.009	0.009	0.012	0.021	0.029	0.019	0.029	0.029	0.026	0.026	0.005	0.005	0.012	0.007
H12	0.014	0.029	0.036	0.054	0.051	0.019	0.007	0.007	0.009	0.046	0.041	0.051	0.041	0.036	0.049	0.012	0.009	0.012	0.012	0.009	0.012	0.007	0.026	0.046	0.031	0.031	0.029	0.012	0.026	0.034	0.009	0.009	0.009	0.012	0.021	0.029	0.019	0.029	0.029	0.026	0.026	0.005	0.005	0.012	0.007
H13	0.014	0.029	0.036	0.054	0.051	0.019	0.007	0.007	0.009	0.041	0.036	0.046	0.036	0.031	0.044	0.012	0.009	0.012	0.012	0.005	0.012	0.007	0.026	0.041	0.031	0.031	0.024	0.012	0.026	0.029	0.005	0.009	0.009	0.007	0.021	0.029	0.019	0.024	0.029	0.026	0.026	0.005	0.005	0.012	0.007
H14	0.005	0.029	0.036	0.051	0.049	0.019	0.007	0.007	0.005	0.051	0.046	0.051	0.036	0.036	0.049	0.007	0.009	0.007	0.007	0.014	0.007	0.007	0.026	0.046	0.031	0.029	0.029	0.012	0.026	0.034	0.005	0.009	0.005	0.007	0.021	0.029	0.019	0.029	0.026	0.026	0.009	0.005	0.012	0.007	
H15	0.029	0.014	0.012	0.049	0.051	0.009	0.017	0.016	0.024	0.039	0.034	0.046	0.031	0.031	0.044	0.021	0.014	0.026	0.021	0.019	0.021	0.002	0.041	0.007	0.016	0.014	0.021	0.012	0.019	0.024	0.012	0.014	0.014	0.014	0.014	0.014	0.014	0.014	0.014	0.014	0.014	0.014	0.014	0.014	0.014
H16	0.026	0.017	0.014	0.051	0.054	0.012	0.014	0.014	0.021	0.039	0.034	0.049	0.034	0.034	0.046	0.019	0.012	0.024	0.019	0.016	0.019	0.019	0.005	0.044	0.009	0.019	0.016	0.019	0.014	0.021	0.021	0.016	0.021	0.021	0.014	0.016	0.016	0.016	0.016	0.009	0.014	0.021	0.021	0.024	0.019
H17	0.026	0.012	0.014	0.051	0.054	0.007	0.014	0.019	0.021	0.041	0.036	0.049	0.034	0.034	0.046	0.024	0.016	0.024	0.024	0.021	0.019	0.019	0.005	0.044	0.009	0.014	0.012	0.019	0.009	0.016	0.021	0.016	0.021	0.021	0.009	0.012	0.012	0.012	0.012	0.005	0.009	0.021	0.021	0.024	0.019
H18	0.059	0.046	0.044	0.061	0.069	0.046	0.046	0.051	0.051	0.062	0.056	0.059	0.049	0.049	0.056	0.056	0.049	0.056	0.056	0.049	0.051	0.051	0.049	0.054	0.054	0.048	0.046	0.051	0.043	0.051	0.054	0.049	0.054	0.054	0.044	0.041	0.036	0.051	0.046	0.048	0.043	0.049	0.049	0.056	0.049
H19	0.026	0.012	0.019	0.056	0.059	0.012	0.014	0.019	0.021	0.044	0.039	0.054	0.039	0.039	0.051	0.024	0.016	0.024	0.024	0.021	0.019	0.019	0.009	0.049	0.014	0.014	0.012	0.019	0.009	0.016	0.021	0.016	0.021	0.012	0.009	0.012	0.016	0.016	0.012	0.009	0.009	0.021	0.021	0.024	0.019
H20	0.029	0.005	0.021	0.054	0.056	0.009	0.017	0.021	0.024	0.044	0.039	0.051	0.036	0.036	0.049	0.026	0.019	0.026	0.026	0.024	0.021	0.021	0.012	0.046	0.016	0.007	0.005	0.021	0.007	0.009	0.024	0.019	0.024	0.024	0.007	0.005	0.014	0.009	0.005	0.012	0.002	0.024	0.024	0.026	0.021
H21	0.031	0.002	0.024	0.056	0.059	0.012	0.019	0.024	0.026	0.046	0.041	0.054	0.039	0.039	0.051	0.029	0.021	0.029	0.029	0.026	0.024	0.024	0.014	0.049	0.019	0.009	0.002	0.024	0.005	0.007	0.026	0.021	0.026	0.026	0.005	0.002	0.016	0.007	0.002	0.014	0.005	0.026	0.026	0.029	0.024
H22	0.024	0.012	0.019	0.046	0.049	0.007	0.014	0.019	0.019	0.041	0.036	0.049	0.034	0.034	0.046	0.021	0.016	0.021	0.021	0.016	0.019	0.009	0.044	0.014	0.014	0.012	0.019	0.009	0.016	0.019	0.016	0.019	0.019	0.009	0.012	0.012	0.012	0.012	0.009	0.009	0.021	0.019	0.024	0.019	
H23	0.024	0.009	0.016	0.049	0.051	0.005	0.012	0.016	0.019	0.039	0.034	0.046	0.031	0.031	0.044	0.021	0.014	0.021	0.021	0.019	0.016	0.016	0.007	0.041	0.012	0.012	0.009	0.016	0.007	0.014	0.019	0.014	0.019	0.019	0.007	0.009	0.009	0.009	0.009	0.007	0.007	0.019	0.019	0.021	0.016
H24	0.026	0.012	0.014	0.046	0.048	0.007	0.014	0.014	0.021	0.036	0.031	0.044	0.029	0.029	0.041	0.019	0.012	0.024	0.019	0.016	0.019	0.019	0.005	0.039	0.009	0.014	0.012	0.019	0.009	0.016	0.021	0.016	0.021	0.021	0.009	0.012	0.012	0.012	0.012	0.009	0.009	0.021	0.021	0.024	0.019
H25	0.036	0.024	0.036	0.043	0.046	0.024	0.026	0.026	0.031	0.043	0.043	0.049	0.038	0.033	0.046	0.029	0.024	0.034	0.029	0.024	0.029	0.031	0.026	0.046	0.031	0.026	0.024	0.026	0.026	0.029	0.031	0.024	0.031	0.031	0.021	0.024	0.024	0.024	0.024	0.031	0.026	0.029	0.026	0.036	0.031
H26	0.009	0.029	0.036	0.056	0.054	0.019	0.007	0.007	0.005	0.051	0.046	0.057	0.041	0.041	0.054	0.007	0.009	0.007	0.007	0.014	0.007	0.007	0.026	0.051	0.031	0.031	0.029	0.012	0.026	0.034	0.005	0.009	0.005	0.007	0.021	0.029	0.019	0.029	0.029	0.026	0.026	0.009	0.005	0.012	0.0

Table 4. 6 (cont'd).

	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15	H16	H17	H18	H19	H20	H21	H22	H23	H24	H25	H26	H27	H28	H29	H30	H31	H32	H33	H34	H35	H36	H37	H38	H39	H40
H51	16	14	10	15	10	11	3	13	9	10	11	11	11	11	3	4	4	20	6	5	4	4	3	2	9	11	10	12	11	11	11	13	19	10	17	11	11	9	11	9
H52	22	22	21	21	23	18	22	19	20	21	22	22	20	22	18	17	19	24	19	22	23	21	20	19	23	24	23	23	22	22	24	24	18	23	20	20	20	22	24	22
H53	22	22	21	21	23	18	22	19	20	21	22	22	20	22	18	17	19	24	19	22	23	21	20	19	23	24	23	23	22	22	24	24	16	23	20	20	20	22	24	22
H54	19	17	17	18	17	14	16	17	14	15	16	16	16	16	14	15	15	21	17	16	17	15	14	13	15	18	17	19	18	16	18	20	8	17	12	14	16	16	18	16
H55	19	17	11	18	11	12	2	14	10	11	12	12	12	12	6	7	5	21	7	4	3	5	4	5	10	12	11	13	12	12	12	14	22	11	18	12	12	12	12	10
H56	18	16	10	17	10	11	1	13	9	10	11	11	11	11	5	6	4	20	6	3	2	4	3	4	9	11	10	12	11	11	11	13	21	10	17	11	11	11	9	
H57	21	19	15	20	15	12	14	15	12	13	14	14	14	14	14	15	13	19	15	14	15	13	12	13	15	16	15	17	16	14	16	18	8	15	8	12	14	16	16	14
H58	19	17	11	18	11	12	4	14	10	11	12	12	12	12	6	7	5	19	7	4	5	5	4	5	12	12	11	13	12	12	12	14	20	11	16	12	12	12	12	10
H59	19	17	11	18	11	12	2	14	10	11	12	12	12	12	6	7	5	21	7	4	3	5	4	5	8	12	11	11	12	12	12	14	22	9	18	12	12	12	12	10
H60	16	14	8	15	8	9	3	11	7	8	9	9	9	9	3	4	2	18	4	3	4	2	1	2	11	9	8	10	9	9	9	11	19	8	15	9	9	9	9	7
H61	20	18	12	19	11	11	7	13	9	10	11	11	11	12	11	12	10	22	12	9	8	8	9	10	5	12	12	12	12	12	12	14	21	10	17	11	11	12	12	11
H62	20	18	12	19	12	13	3	15	11	12	13	13	13	13	7	8	6	20	6	3	2	6	5	6	11	13	12	14	13	13	13	15	23	12	19	13	13	13	11	
H63	19	17	11	18	11	12	2	14	10	11	12	12	12	12	6	7	5	19	5	2	1	5	4	5	10	12	12	13	12	12	12	14	22	11	18	12	12	12	12	10
H64	18	18	12	17	14	13	5	15	13	14	15	15	13	15	9	10	8	22	8	5	4	8	7	8	13	15	15	16	15	15	15	17	23	14	19	15	15	15	13	
H65	19	17	11	18	11	12	2	14	10	11	12	12	12	12	6	7	5	19	5	2	1	5	4	5	10	12	12	13	12	12	12	14	22	11	18	12	12	12	12	10
H66	19	17	13	18	13	14	8	16	12	13	14	14	14	14	4	5	5	19	7	8	9	7	6	5	14	14	14	15	14	14	14	16	20	13	18	14	14	12	14	12
H67	18	18	12	17	14	13	5	15	13	14	15	15	13	15	9	10	8	20	8	5	4	8	7	8	11	15	15	14	15	15	15	17	23	12	19	15	15	15	13	
H68	24	24	22	23	24	19	23	22	21	22	23	23	23	21	21	22	22	26	24	23	24	22	21	20	22	25	25	26	25	23	25	27	5	24	9	21	23	23	25	23
H69	27	27	23	26	25	20	24	23	22	23	24	24	24	24	25	23	27	25	24	25	23	22	23	25	26	26	27	26	24	26	28	8	25	10	22	24	26	26	24	
H70	18	16	3	17	3	6	10	4	4	3	4	4	4	4	10	9	9	22	9	10	11	9	8	9	14	4	4	3	2	4	4	4	20	3	16	4	2	4	4	2
H71	20	18	10	19	9	11	9	11	9	10	11	11	11	10	13	12	12	25	12	11	10	10	11	12	11	10	10	10	8	10	10	10	23	10	19	11	9	10	10	9
H72	21	19	11	20	10	12	10	12	10	11	12	12	12	11	14	13	13	26	13	12	11	11	12	13	10	11	11	9	9	11	11	11	24	9	20	12	10	11	11	10
H73	18	16	7	17	6	8	10	8	6	7	8	8	8	7	10	9	9	22	9	10	11	7	8	9	12	7	7	7	5	7	7	7	20	7	16	8	6	7	7	6
H74	23	23	19	22	21	16	22	19	18	19	20	20	18	20	20	21	21	25	23	22	23	21	20	19	21	22	22	23	22	20	22	24	2	21	6	18	20	20	22	20
H75	3	3	17	2	19	14	17	16	16	17	18	18	16	20	15	15	16	24	17	17	18	16	15	14	19	20	20	19	18	18	20	18	23	19	21	16	16	18	20	18
H76	18	18	1	17	3	4	10	4	4	3	4	4	2	4	10	9	9	22	9	10	11	9	8	9	12	4	4	3	4	4	4	6	18	1	14	4	4	4	4	2
H77	4	4	14	3	16	11	14	13	13	14	15	15	13	17	12	12	13	23	14	14	15	13	12	11	16	17	17	16	15	15	17	15	20	16	18	13	13	15	17	15
H78	18	18	3	17	5	4	10	4	6	5	6	6	4	6	10	9	9	21	9	10	11	9	8	9	14	6	6	7	6	6	6	8	17	5	14	6	6	6	6	4
H79	17	15	15	16	15	16	10	16	14	15	16	16	16	16	6	7	7	19	9	10	11	9	8	7	16	16	16	15	14	16	16	16	22	15	20	16	14	14	16	14
H80	17	17	4	18	4	7	11	5	5	4	3	5	5	5	11	10	10	23	10	11	12	10	9	10	13	5	5	2	3	5	5	5	21	2	17	5	3	5	5	3
H81	22	20	5	21	3	8	12	8	6	5	6	6	2	12	11	11	24	11	12	13	10	10	11	15	4	4	7	4	4	4	6	20	5	16	6	6	4	4	4	4
H82	19	17	11	18	11	12	2	14	10	11	12	12	12	12	6	7	5	19	5	2	1	5	4	5	10	12	12	13	12	12	12	14	22	11	18	12	12	12	12	10
H83	18	16	14	17	14	15	9	17	13	14	15	15	15	15	5	6	6	18	8	9	10	8	7	6	15	15	15	16	15	15	15	17	21	14	19	15	15	13	15	13
H84	23	21	23	22	22	20	22	21	20	21	22	22	22	21	20	21	21	25	23	22	23	19	20	19	18	23	22	21	21	21	23	23	18	21	18	20	20	21	23	22
H85	24	22	22	23	21	21	23	20	19	20	21	21	21	20	21	22	22	28	24	23	24	20	21	20	19	22	21	20	20	20	22	22	21	20	21	19	19	20	22	21
H86	13	11	7	12	7	6	4	10	6	7	8	8	8	8	4	5	3	19	5	4	5	3	2	3	10	8	8	9	8	6	8	10	16	7	12	6	8	8	8	6
H87	17	15	2	16	2	3	7	5	3	2	3	3	3	3	7	6	6	19	6	7	8	6	5	6	11	3	3	4	3	3	3	5	17	2	13	3	3	3	3	1
H88	17	15	2	16	2	5	9	5	3	2	3	3	3	3	7	6	8	21	8	9	10	8	7	6	11	3	3	4	3	3	3	5	17	2	15	3	3	1	3	1
H89	20	18	3	19	1	6	10	6	4	3	4	4	4	2	10	9	9	21	9	10	11	8	8	9	13	2	2	5	2	2	4	20	3	16	4	4	2	2	2	
H90	4	4	18	3	20	15	18	17	17	18	19	19	17	17	16	16	17	25	18	18	19	17	16	15	18	21	21	18	19	19	19	24	18	22	17	17	19	21	19	
H91	2	2	16	1	18	13	16	15	15	16	17	17	15	19	14	14	15	23	16	16	17	15	14	13	18	19	19	18	17	17	19	17	22	18	20	15	15	17	19	17
H92	22	22	20	21	22	17	21	20	19	20	21	21	19	21	19	20	20	24	22	21	22	20	19	18	20	23	23	24	23	21	23	25	5	22	9	19	21	21	23	21
H93	20	20	14	19	16	15	15	16	15	16	17	17	15	15	13	14	14	20	16	15	16	14	13	12	16	17	17	18	17	17	19	9	16	11	17	17	15	17	15	
H94	16	16	14	1																																				

Table 4. 6 (cont'd).

	H41	H42	H43	H44	H45	H46	H47	H48	H49	H50	H51	H52	H53	H54	H55	H56	H57	H58	H59	H60	H61	H62	H63	H64	H65	H66	H67	H68	H69	H70	H71	H72	H73	H74	H75	H76	H77	H78	H79	H80
H51	11	9	12	18	6	3	4	5	7	9		0.046	0.046	0.031	0.007	0.005	0.031	0.012	0.007	0.005	0.024	0.009	0.012	0.019	0.012	0.016	0.019	0.054	0.062	0.026	0.029	0.031	0.026	0.051	0.039	0.026	0.031	0.026	0.021	0.029
H52	22	22	25	22	18	19	21	22	23	24	19		0.005	0.029	0.054	0.051	0.034	0.049	0.054	0.046	0.056	0.057	0.059	0.062	0.059	0.049	0.056	0.046	0.054	0.054	0.061	0.064	0.054	0.044	0.054	0.054	0.046	0.051	0.043	0.056
H53	22	22	25	22	18	19	21	22	23	24	19	2		0.024	0.054	0.051	0.034	0.049	0.054	0.046	0.056	0.057	0.059	0.062	0.059	0.049	0.057	0.041	0.049	0.054	0.062	0.064	0.054	0.039	0.054	0.054	0.046	0.051	0.044	0.056
H54	16	16	19	19	15	14	15	16	17	20	13	12	10		0.039	0.036	0.009	0.034	0.039	0.031	0.041	0.041	0.044	0.051	0.044	0.039	0.046	0.021	0.029	0.044	0.051	0.054	0.044	0.019	0.046	0.044	0.039	0.041	0.039	0.046
H55	12	10	13	21	9	6	5	6	6	8	3	22	22	16		0.002	0.034	0.005	0.005	0.007	0.021	0.007	0.009	0.016	0.009	0.024	0.016	0.062	0.064	0.029	0.026	0.029	0.029	0.059	0.046	0.029	0.038	0.029	0.031	
H56	11	9	12	20	8	5	4	5	5	7	2	21	21	15	1		0.031	0.007	0.002	0.005	0.019	0.005	0.007	0.014	0.007	0.021	0.014	0.059	0.062	0.026	0.024	0.026	0.026	0.057	0.044	0.026	0.036	0.026	0.026	0.029
H57	14	14	17	19	15	14	15	14	15	18	13	14	14	4	14	13		0.029	0.034	0.026	0.036	0.036	0.039	0.046	0.039	0.039	0.046	0.026	0.029	0.039	0.046	0.049	0.039	0.024	0.051	0.039	0.044	0.036	0.044	0.041
H58	12	10	13	19	9	6	7	8	8	10	5	20	20	14	2	3	12		0.009	0.007	0.026	0.012	0.014	0.021	0.014	0.019	0.021	0.056	0.059	0.029	0.031	0.033	0.029	0.054	0.046	0.029	0.038	0.029	0.024	0.031
H59	12	10	13	21	9	6	5	6	6	8	3	22	22	16	2	1	14	4		0.007	0.016	0.007	0.009	0.016	0.009	0.024	0.012	0.062	0.064	0.029	0.026	0.024	0.029	0.059	0.046	0.024	0.039	0.029	0.029	0.026
H60	9	7	10	18	6	3	6	5	7	9	2	19	19	13	3	2	11	3	3		0.024	0.009	0.012	0.019	0.012	0.016	0.019	0.054	0.056	0.021	0.029	0.031	0.021	0.051	0.039	0.021	0.031	0.021	0.021	0.024
H61	11	13	13	22	12	11	8	9	9	13	10	23	23	17	9	8	15	11	7	10		0.024	0.021	0.029	0.021	0.036	0.024	0.059	0.062	0.031	0.019	0.016	0.021	0.056	0.049	0.026	0.041	0.031	0.041	0.029
H62	13	11	14	20	10	7	4	5	5	7	4	23	23	17	3	2	15	5	3	4	10		0.007	0.014	0.007	0.026	0.014	0.065	0.067	0.031	0.029	0.031	0.031	0.062	0.049	0.031	0.041	0.031	0.031	0.034
H63	12	12	13	19	9	6	3	2	4	4	5	24	24	18	4	3	16	6	4	5	9	3		0.007	0.005	0.024	0.007	0.062	0.064	0.029	0.026	0.029	0.029	0.059	0.046	0.029	0.039	0.029	0.029	0.031
H64	15	15	16	22	12	9	6	5	7	5	8	25	25	21	7	6	19	9	7	8	12	6	3		0.012	0.031	0.009	0.065	0.067	0.036	0.034	0.036	0.036	0.062	0.044	0.031	0.041	0.031	0.036	0.039
H65	12	12	13	19	9	6	3	4	4	6	5	24	24	18	4	3	16	6	4	5	9	3	2	5		0.024	0.012	0.062	0.064	0.029	0.026	0.029	0.029	0.059	0.046	0.029	0.039	0.029	0.029	0.031
H66	14	14	15	17	9	6	9	10	12	14	7	20	20	16	10	9	16	8	10	7	15	11	10	13	10		0.031	0.057	0.064	0.034	0.041	0.044	0.034	0.054	0.046	0.034	0.039	0.034	0.005	0.036
H67	15	15	16	20	12	9	6	5	7	7	8	23	23	19	7	6	19	9	5	8	10	6	3	4	5	13		0.059	0.062	0.036	0.034	0.031	0.036	0.057	0.044	0.026	0.036	0.031	0.031	0.034
H68	23	23	26	24	22	21	22	23	24	27	22	19	17	9	25	24	11	23	25	22	24	26	25	26	25	23	24		0.012	0.062	0.070	0.073	0.062	0.007	0.059	0.057	0.051	0.054	0.057	0.065
H69	24	24	27	27	25	24	25	24	25	28	25	22	20	12	26	25	12	24	26	23	25	27	26	27	26	26	25	5		0.064	0.072	0.075	0.064	0.014	0.067	0.059	0.059	0.057	0.065	0.067
H70	4	4	5	22	12	9	11	12	14	14	11	22	22	18	12	11	16	12	12	9	13	13	12	15	12	14	15	25	26		0.021	0.024	0.014	0.054	0.044	0.009	0.036	0.014	0.034	0.007
H71	11	11	11	25	15	12	10	13	13	13	12	25	25	21	11	10	19	13	11	12	8	12	11	14	11	17	14	28	29	9		0.002	0.007	0.062	0.049	0.026	0.041	0.026	0.041	0.024
H72	12	12	12	26	16	13	11	14	14	14	13	26	26	22	12	11	20	14	10	13	7	13	12	15	12	18	13	29	30	10	1		0.009	0.064	0.051	0.024	0.044	0.029	0.044	0.021
H73	8	8	8	22	12	9	11	12	14	14	11	21	21	17	12	11	15	12	12	9	9	13	12	15	12	14	15	25	26	6	3	4		0.054	0.044	0.019	0.036	0.019	0.034	0.016
H74	20	20	23	23	21	20	21	22	23	26	21	18	16	8	24	23	10	22	24	21	23	25	24	25	24	22	23	3	6	22	25	26	22		0.057	0.049	0.049	0.046	0.054	0.057
H75	18	20	19	22	14	13	18	17	19	18	16	22	22	19	19	18	21	19	19	16	20	20	19	18	19	19	18	24	27	18	20	21	18	23		0.044	0.007	0.044	0.041	0.046
H76	4	4	5	22	12	9	11	12	14	14	11	22	22	18	12	11	16	12	10	9	11	13	12	13	12	14	11	23	24	4	11	10	8	20	18		0.036	0.009	0.039	0.007
H77	15	17	16	21	11	10	15	14	16	17	13	19	19	16	16	15	18	16	16	13	17	17	16	17	16	15	21	24	15	17	18	15	20	3	15		0.036	0.034	0.039	
H78	6	6	7	21	12	9	11	12	14	14	11	21	21	17	12	11	15	12	12	9	13	13	12	13	12	14	13	22	23	6	11	12	8	19	18	4	15		0.039	0.016
H79	16	16	17	17	11	8	11	12	14	16	9	18	18	16	12	11	18	10	12	9	17	13	12	15	12	2	13	23	26	14	17	18	14	22	17	16	14	16		0.036
H80	5	5	6	23	13	10	12	13	15	15	12	23	23	19	13	12	17	13	11	10	12	14	13	16	13	15	14	26	27	3	10	9	7	23	19	3	16	7	15	
H81	6	6	5	24	14	11	13	14	15	16	13	24	24	18	14	13	16	14	14	11	14	15	14	17	14	16	17	25	26	6	12	13	9	22	22	6	19	8	18	7
H82	12	12	13	19	9	6	3	4	4	6	5	24	24	18	4	3	16	6	4	5	9	3	2	5	2	10	5	25	26	12	11	12	12	24	19	12	16	12	13	
H83	15	15	16	16	10	7	10	11	13	15	8	19	19	15	11	10	17	9	11	8	16	12	11	14	11	1	12	22	25	15	18	19	15	21	18	15	15	1	16	
H84	22	22	24	23	19	20	21	22	23	26	19	18	18	12	22	21	14	20	20	19	19	21	24	27	24	22	23	19	22	22	23	22	20	18	23	22	20	23	20	21
H85	21	21	23	26	20	21	22	23	24	27	20	21	21	15	23	22	17	23	21	20	20	22	25	28	25	25	24	22	25	21	24	23	21	21	24	21	24	23	20	20
H86	8	8	9	19	7	4	7	6	8	8	5	20	20	12	6	5	10	6	6	3	9	7	6	9	6	8	9	19	20	8	11	12	8	18	13	8	10	8	10	9
H87	3	3	4	19	9	6	8	9	11	11	8	21	21	15	9	8	13	9	9	6	10	10	9	12	9	11	12	22	23	3	8	9	5	19	17	3	14	3	13	4
H88	3	3	4	19	9	6	8	11	13	13	8	21	21	15	11	10	15	11	11	8	12	12	11	14	11	11	14	22	25	3	10	11	7	19	17	3	14	5	13	4
H89	4	4	3																																					

Table 4. 6 (cont'd).

	H81	H82	H83	H84	H85	H86	H87	H88	H89	H90	H91	H92	H93	H94	H95	H96	H97	H98	H99	H100	H101	H102	H103	H104	H105	H106	H107	H108	H109	H110	H111	H112	H113	H114	H115	H116	H117	H118	H119	H120	H121	H122	H123	H124	H125		
H51	0.031	0.012	0.019	0.046	0.049	0.012	0.019	0.019	0.026	0.041	0.036	0.049	0.034	0.034	0.046	0.024	0.016	0.029	0.024	0.021	0.024	0.024	0.009	0.044	0.014	0.019	0.012	0.024	0.009	0.016	0.026	0.021	0.026	0.026	0.009	0.012	0.016	0.012	0.016	0.012	0.007	0.014	0.026	0.026	0.024	0.024	
H52	0.059	0.059	0.046	0.043	0.051	0.049	0.051	0.051	0.059	0.056	0.051	0.041	0.036	0.031	0.039	0.056	0.049	0.056	0.056	0.044	0.056	0.051	0.046	0.041	0.051	0.059	0.054	0.056	0.056	0.054	0.054	0.054	0.059	0.054	0.056	0.054	0.044	0.054	0.054	0.051	0.056	0.054	0.054	0.046	0.054		
H53	0.059	0.059	0.046	0.043	0.051	0.049	0.051	0.051	0.059	0.056	0.051	0.036	0.036	0.031	0.039	0.056	0.049	0.056	0.056	0.044	0.056	0.051	0.046	0.041	0.051	0.059	0.054	0.056	0.056	0.054	0.054	0.054	0.059	0.054	0.057	0.054	0.044	0.054	0.054	0.051	0.056	0.054	0.054	0.046	0.054		
H54	0.044	0.044	0.036	0.029	0.036	0.029	0.036	0.036	0.044	0.049	0.044	0.016	0.016	0.016	0.019	0.041	0.034	0.046	0.041	0.034	0.041	0.041	0.036	0.021	0.041	0.044	0.044	0.041	0.041	0.049	0.044	0.039	0.044	0.044	0.036	0.039	0.024	0.044	0.039	0.041	0.041	0.039	0.039	0.036	0.039		
H55	0.034	0.009	0.026	0.054	0.056	0.014	0.021	0.026	0.029	0.049	0.044	0.057	0.041	0.041	0.054	0.031	0.024	0.031	0.031	0.029	0.026	0.026	0.016	0.051	0.021	0.016	0.009	0.026	0.012	0.014	0.029	0.024	0.029	0.029	0.012	0.009	0.019	0.009	0.005	0.016	0.012	0.029	0.029	0.026	0.026		
H56	0.031	0.007	0.024	0.051	0.054	0.012	0.019	0.024	0.026	0.046	0.041	0.054	0.039	0.039	0.051	0.029	0.021	0.029	0.029	0.026	0.024	0.024	0.014	0.049	0.019	0.014	0.007	0.024	0.009	0.012	0.026	0.021	0.026	0.026	0.009	0.007	0.016	0.007	0.002	0.014	0.009	0.026	0.026	0.024	0.024		
H57	0.039	0.039	0.041	0.033	0.041	0.024	0.031	0.036	0.039	0.054	0.049	0.021	0.016	0.016	0.019	0.041	0.034	0.041	0.041	0.034	0.036	0.036	0.036	0.021	0.041	0.039	0.039	0.036	0.036	0.044	0.039	0.034	0.039	0.039	0.031	0.039	0.024	0.039	0.034	0.036	0.036	0.034	0.034	0.031	0.034		
H58	0.034	0.014	0.021	0.049	0.056	0.014	0.021	0.026	0.029	0.049	0.044	0.051	0.036	0.036	0.049	0.031	0.024	0.031	0.031	0.029	0.026	0.026	0.016	0.046	0.021	0.016	0.014	0.026	0.016	0.019	0.029	0.024	0.029	0.029	0.016	0.014	0.014	0.014	0.009	0.016	0.012	0.029	0.029	0.026	0.026		
H59	0.034	0.009	0.026	0.049	0.051	0.014	0.021	0.026	0.029	0.044	0.044	0.057	0.041	0.041	0.054	0.031	0.024	0.031	0.031	0.029	0.026	0.026	0.016	0.051	0.021	0.012	0.009	0.021	0.012	0.014	0.029	0.019	0.029	0.029	0.012	0.009	0.019	0.009	0.005	0.016	0.012	0.029	0.029	0.026	0.026		
H60	0.026	0.012	0.019	0.046	0.049	0.007	0.014	0.019	0.021	0.041	0.036	0.049	0.034	0.034	0.046	0.024	0.016	0.024	0.024	0.021	0.019	0.019	0.009	0.044	0.014	0.014	0.012	0.019	0.009	0.016	0.021	0.016	0.021	0.021	0.009	0.012	0.012	0.007	0.009	0.009	0.021	0.021	0.019	0.019			
H61	0.034	0.021	0.039	0.046	0.048	0.021	0.024	0.029	0.029	0.046	0.046	0.054	0.044	0.039	0.051	0.031	0.026	0.031	0.031	0.026	0.026	0.029	0.029	0.049	0.033	0.024	0.021	0.024	0.024	0.026	0.029	0.021	0.029	0.029	0.019	0.021	0.021	0.021	0.021	0.029	0.024	0.026	0.024	0.034	0.029		
H62	0.036	0.007	0.029	0.051	0.054	0.016	0.024	0.029	0.031	0.051	0.046	0.059	0.044	0.044	0.057	0.034	0.026	0.034	0.034	0.031	0.029	0.029	0.019	0.054	0.024	0.014	0.007	0.029	0.009	0.012	0.031	0.026	0.031	0.031	0.009	0.007	0.021	0.012	0.002	0.019	0.009	0.031	0.031	0.029	0.029		
H63	0.034	0.005	0.026	0.059	0.061	0.014	0.021	0.026	0.029	0.049	0.044	0.057	0.041	0.041	0.054	0.031	0.024	0.031	0.031	0.029	0.026	0.026	0.016	0.051	0.021	0.012	0.005	0.026	0.002	0.005	0.029	0.024	0.029	0.029	0.007	0.005	0.019	0.005	0.005	0.016	0.002	0.029	0.029	0.031	0.026		
H64	0.041	0.012	0.034	0.067	0.069	0.021	0.029	0.034	0.036	0.046	0.041	0.059	0.044	0.044	0.057	0.039	0.031	0.034	0.039	0.031	0.034	0.034	0.034	0.024	0.054	0.029	0.019	0.007	0.034	0.009	0.007	0.031	0.031	0.036	0.031	0.014	0.012	0.026	0.007	0.012	0.024	0.009	0.036	0.036	0.039	0.034	
H65	0.034	0.005	0.026	0.059	0.061	0.014	0.021	0.026	0.029	0.049	0.044	0.057	0.041	0.041	0.054	0.031	0.024	0.031	0.031	0.029	0.026	0.026	0.016	0.051	0.021	0.012	0.005	0.026	0.007	0.009	0.029	0.024	0.029	0.029	0.007	0.005	0.019	0.009	0.005	0.016	0.007	0.029	0.029	0.031	0.026		
H66	0.039	0.024	0.002	0.054	0.061	0.019	0.026	0.026	0.034	0.049	0.044	0.051	0.036	0.036	0.049	0.031	0.024	0.036	0.031	0.029	0.031	0.031	0.012	0.046	0.016	0.026	0.024	0.031	0.021	0.029	0.034	0.029	0.034	0.034	0.021	0.024	0.019	0.024	0.014	0.016	0.021	0.034	0.034	0.036	0.031		
H67	0.041	0.012	0.029	0.056	0.059	0.021	0.029	0.034	0.036	0.041	0.041	0.054	0.039	0.039	0.051	0.039	0.031	0.031	0.039	0.039	0.031	0.034	0.034	0.024	0.049	0.029	0.014	0.007	0.029	0.009	0.007	0.031	0.026	0.036	0.031	0.014	0.007	0.021	0.007	0.012	0.024	0.009	0.036	0.036	0.039	0.034	
H68	0.062	0.062	0.054	0.046	0.054	0.046	0.054	0.062	0.062	0.057	0.005	0.019	0.019	0.007	0.059	0.051	0.065	0.059	0.046	0.059	0.059	0.054	0.009	0.059	0.062	0.057	0.059	0.059	0.062	0.057	0.059	0.059	0.062	0.057	0.057	0.062	0.057	0.054	0.057	0.041	0.057	0.062	0.059	0.059	0.057		
H69	0.064	0.064	0.062	0.054	0.061	0.049	0.057	0.062	0.064	0.070	0.064	0.016	0.031	0.026	0.019	0.067	0.059	0.067	0.067	0.054	0.062	0.062	0.062	0.016	0.067	0.064	0.059	0.062	0.062	0.064	0.059	0.059	0.064	0.059	0.057	0.059	0.044	0.059	0.064	0.062	0.062	0.059	0.062	0.059			
H70	0.014	0.029	0.036	0.054	0.051	0.019	0.007	0.007	0.009	0.046	0.041	0.057	0.041	0.041	0.054	0.012	0.009	0.007	0.012	0.014	0.012	0.002	0.026	0.051	0.031	0.031	0.029	0.012	0.026	0.029	0.009	0.009	0.009	0.012	0.021	0.029	0.019	0.029	0.029	0.026	0.026	0.009	0.009	0.007	0.007		
H71	0.029	0.026	0.044	0.056	0.059	0.026	0.019	0.024	0.024	0.051	0.046	0.064	0.049	0.049	0.062	0.026	0.026	0.021	0.026	0.031	0.026	0.019	0.034	0.059	0.038	0.029	0.026	0.024	0.029	0.026	0.024	0.024	0.024	0.024	0.026	0.026	0.026	0.026	0.034	0.029	0.026	0.024	0.024	0.024			
H72	0.031	0.029	0.046	0.054	0.056	0.029	0.021	0.026	0.026	0.049	0.049	0.067	0.051	0.051	0.064	0.029	0.029	0.024	0.029	0.034	0.029	0.021	0.036	0.062	0.041	0.026	0.029	0.021	0.031	0.029	0.036	0.026	0.029	0.039	0.034	0.039	0.039	0.026	0.024	0.019	0.029	0.029	0.036	0.031	0.029	0.026	0.026
H73	0.021	0.029	0.036	0.049	0.051	0.019	0.012	0.016	0.016	0.046	0.041	0.057	0.041	0.041	0.054	0.019	0.019	0.014	0.019	0.024	0.019	0.012	0.026	0.051	0.031	0.026	0.029	0.016	0.026	0.029	0.016	0.014	0.016	0.016	0.021	0.029	0.019	0.029	0.029	0.026	0.026	0.019	0.016	0.016			
H74	0.054	0.059	0.051	0.043	0.051	0.044	0.046	0.046	0.054	0.059	0.054	0.007	0.021	0.016	0.009	0.051	0.049	0.057	0.051	0.044	0.057	0.051	0.051	0.007	0.056	0.059	0.054	0.051	0.057	0.059	0.049	0.049	0.054	0.049	0.051	0.054	0.039	0.054	0.059	0.057	0.057	0.049	0.049	0.051	0.049		
H75	0.054	0.046	0.044	0.056	0.059	0.031	0.041	0.041	0.049	0.007	0.002	0.054	0.049	0.039	0.051	0.046	0.039	0.041	0.046	0.034	0.046	0.041	0.039	0.049	0.043	0.049	0.041	0.046	0.044	0.041	0.044	0.044	0.049	0.044	0.039	0.041	0.036	0.041	0.046	0.044	0.044	0.044	0.041	0.046			
H76	0.014	0.029	0.036	0.054	0.051	0.019	0.007	0.007	0.009	0.041	0.041	0.051	0.036	0.036	0.049	0.012	0.009	0.012	0.012	0.009	0.012	0.007	0.026	0.046	0.031	0.026	0.024	0.007	0.026	0.029	0.005	0.005	0.009	0.007	0.021	0.029	0.019	0.024	0.029	0.026							

Table 4. 6 (cont'd).

	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15	H16	H17	H18	H19	H20	H21	H22	H23	H24	H25	H26	H27	H28	H29	H30	H31	H32	H33	H34	H35	H36	H37	H38	H39	H40
H101	19	17	4	18	2	7	9	7	5	4	5	5	5	3	9	8	8	21	8	9	10	7	7	8	12	3	3	6	3	3	5	21	4	17	5	5	3	3	3	
H102	17	15	2	16	2	5	9	3	3	2	3	3	3	3	9	8	8	21	8	9	10	8	7	8	13	3	3	2	1	3	3	3	19	2	15	3	1	3	3	1
H103	16	14	10	15	10	11	5	13	9	10	11	11	11	11	1	2	2	20	4	5	6	4	3	2	11	11	11	12	11	11	11	13	19	10	17	11	11	9	11	9
H104	20	20	18	19	20	15	19	18	17	18	19	19	17	19	17	18	18	22	20	19	20	18	17	16	19	21	21	22	21	19	21	23	5	20	5	17	19	19	21	19
H105	18	16	12	17	12	13	7	15	11	12	13	13	13	13	3	4	4	22	6	7	8	6	5	4	13	13	13	14	13	13	13	15	21	12	19	13	13	11	13	11
H106	20	18	12	19	12	13	5	15	11	12	13	13	13	12	7	8	6	20	6	3	4	6	5	6	11	13	13	12	13	13	13	15	22	10	18	13	13	13	13	11
H107	17	17	9	16	11	10	2	12	10	11	12	12	10	12	6	7	5	19	5	2	1	5	4	5	10	12	12	13	12	12	12	14	20	11	16	12	12	12	10	
H108	17	17	4	18	4	5	9	7	5	4	3	5	5	5	9	8	8	21	8	9	10	8	7	8	11	5	5	4	5	5	5	7	19	2	15	5	5	5	3	
H109	18	16	10	17	10	11	3	13	9	10	11	11	11	11	5	6	4	18	4	3	2	4	3	4	11	11	11	12	12	11	11	13	21	10	17	11	11	11	9	
H110	17	17	11	16	13	12	4	12	12	13	14	14	12	14	8	9	7	21	7	4	3	7	6	7	12	14	14	13	12	14	14	14	22	13	18	14	12	14	14	12
H111	18	18	1	17	1	4	10	4	4	3	4	4	2	2	10	9	9	22	9	10	11	8	8	9	13	2	2	5	2	2	2	4	18	3	14	4	4	2	2	2
H112	18	16	3	17	3	4	8	6	4	3	4	4	4	4	8	7	7	20	7	8	9	7	6	7	10	4	4	3	4	4	4	6	18	1	14	4	4	4	4	2
H113	20	18	3	19	1	6	10	6	4	3	4	4	4	2	10	9	9	22	9	10	11	8	8	9	13	2	2	5	2	2	2	4	20	3	16	4	4	2	2	2
H114	18	18	2	17	2	5	10	5	4	4	5	5	3	3	10	9	9	22	9	10	11	8	8	9	13	3	3	6	3	3	3	5	18	4	14	5	5	3	3	3
H115	16	14	8	15	8	9	3	11	7	8	9	9	9	9	5	6	4	18	4	3	2	4	3	4	9	9	9	10	9	9	9	11	19	8	15	9	9	9	7	
H116	17	15	11	16	11	12	2	14	10	11	12	12	12	12	6	7	5	17	5	2	1	5	4	5	10	12	12	13	12	12	12	14	22	11	18	12	12	12	12	10
H117	15	13	7	14	7	8	6	10	6	7	8	8	8	8	6	7	5	15	7	6	7	5	4	5	10	8	8	9	8	8	8	10	16	7	12	8	8	8	8	6
H118	17	17	9	16	11	10	2	12	10	11	12	12	10	12	6	7	5	21	7	4	3	5	4	5	10	12	12	13	12	12	12	14	20	11	16	12	12	12	10	
H119	19	17	11	18	11	12	2	14	10	11	12	12	12	12	6	7	5	19	5	2	1	5	4	5	10	12	11	13	12	12	12	14	22	11	18	12	12	12	12	10
H120	18	16	10	17	10	11	5	13	9	10	11	11	11	11	3	4	2	20	4	5	6	4	3	4	13	11	11	12	11	11	11	13	21	10	17	11	11	11	11	9
H121	18	16	10	17	10	11	3	13	9	10	11	11	11	11	5	6	4	18	4	1	2	4	3	4	11	11	11	12	11	11	11	13	21	10	17	11	11	11	11	9
H122	18	16	3	17	3	4	10	4	2	1	2	2	2	4	10	9	9	20	9	10	11	9	8	9	12	4	4	5	4	4	4	6	18	3	14	2	2	4	4	2
H123	18	16	3	17	1	4	10	4	2	1	2	2	2	2	10	9	9	20	9	10	11	8	8	9	11	2	2	5	2	2	2	4	18	3	14	2	2	2	2	2
H124	17	15	4	16	4	5	11	5	5	4	5	5	5	5	11	10	10	23	10	11	12	10	9	10	15	5	4	4	3	3	5	5	19	4	15	3	3	5	5	3
H125	19	17	2	18	2	5	9	3	3	2	3	3	3	3	9	8	8	20	8	9	10	8	7	8	13	3	3	4	3	3	3	5	18	2	15	3	3	3	3	1

Table 4. 6 (cont'd).

	H41	H42	H43	H44	H45	H46	H47	H48	H49	H50	H51	H52	H53	H54	H55	H56	H57	H58	H59	H60	H61	H62	H63	H64	H65	H66	H67	H68	H69	H70	H71	H72	H73	H74	H75	H76	H77	H78	H79	H80
H101	5	5	4	21	11	8	10	11	13	13	10	23	23	17	11	10	15	11	11	8	11	12	11	14	11	13	14	24	25	5	11	12	8	23	19	5	16	7	15	6
H102	3	3	4	21	11	8	10	11	13	13	10	21	21	17	11	10	15	11	11	8	12	12	11	14	11	13	14	24	25	1	8	9	5	21	17	3	14	5	13	2
H103	11	11	12	18	6	3	6	7	9	11	4	19	19	15	7	6	15	7	7	4	12	8	7	10	7	5	10	22	25	11	14	15	11	21	16	11	13	11	7	12
H104	19	19	22	20	18	17	18	19	20	23	18	17	17	9	21	20	9	19	21	18	20	22	21	22	21	19	20	4	7	21	24	25	21	3	20	19	17	18	19	22
H105	13	13	14	20	8	5	8	9	11	13	6	21	21	17	9	8	17	9	9	6	14	10	9	12	9	7	12	24	27	13	16	17	13	23	18	13	15	13	9	14
H106	13	13	14	20	10	7	6	7	5	9	8	24	24	18	7	6	16	7	5	6	10	6	5	8	5	11	6	25	26	13	12	11	11	24	20	11	17	13	13	12
H107	12	12	13	19	9	6	3	4	4	6	5	22	22	18	4	3	16	6	4	5	9	3	2	3	2	10	3	23	24	12	11	12	12	22	17	10	14	10	12	13
H108	5	5	6	21	11	8	10	11	13	13	10	23	23	17	11	10	15	11	9	8	10	12	11	14	11	13	12	24	25	5	10	9	7	21	19	3	16	5	15	2
H109	11	11	12	18	8	5	4	1	5	5	4	23	23	17	5	4	15	7	5	4	10	4	1	4	3	9	4	24	25	11	12	13	11	23	18	11	15	11	11	12
H110	14	14	15	21	11	8	5	4	6	6	7	22	22	20	6	5	18	8	6	7	11	5	2	3	4	12	3	25	26	12	11	12	12	24	17	12	14	12	12	13
H111	4	4	3	22	12	9	11	12	14	14	11	22	22	18	12	11	16	12	12	9	12	13	12	13	12	14	13	23	24	4	10	11	7	20	18	2	15	4	16	5
H112	4	4	5	20	10	7	9	10	12	12	9	22	22	16	10	9	14	10	8	7	9	11	10	13	10	12	11	23	24	4	9	8	6	20	18	2	15	4	14	3
H113	4	4	3	22	12	9	11	12	14	14	11	24	24	18	12	11	16	12	12	9	12	13	12	15	12	14	15	25	26	4	10	11	7	22	20	4	17	6	16	5
H114	5	5	4	22	12	9	11	12	14	14	11	22	22	18	12	11	16	12	12	9	12	13	12	13	12	14	13	23	24	5	10	11	7	20	18	3	15	5	16	6
H115	9	9	10	18	8	5	4	3	5	7	4	23	23	15	5	4	13	7	5	4	8	4	3	6	3	9	6	22	23	9	10	11	9	21	16	9	13	9	11	10
H116	12	12	13	17	9	6	3	4	4	6	5	22	22	16	4	3	16	6	4	5	9	3	2	5	2	10	3	23	24	12	11	12	12	22	17	12	14	12	10	13
H117	8	8	9	15	9	6	7	8	10	12	7	18	18	10	8	7	10	6	8	5	9	9	8	11	8	8	9	17	18	8	11	12	8	16	15	8	12	8	8	9
H118	12	12	13	21	9	6	5	4	6	6	5	22	22	18	4	3	16	6	4	5	9	5	2	3	4	10	3	23	24	12	11	12	12	22	17	10	14	10	12	13
H119	12	10	13	19	9	6	3	4	4	6	3	22	22	16	2	1	14	4	2	3	9	1	2	5	2	10	5	25	26	12	11	12	12	24	19	12	16	12	12	13
H120	11	11	12	20	8	5	8	7	9	11	6	21	21	17	7	6	15	7	7	4	12	8	7	10	7	7	10	24	25	11	14	15	11	23	18	11	15	11	9	12
H121	11	11	12	18	8	5	4	3	5	5	6	23	23	17	5	4	15	5	5	4	10	4	1	4	3	9	4	24	25	11	12	13	11	23	18	11	15	11	11	12
H122	2	4	5	20	10	9	11	10	12	14	11	22	22	16	12	11	14	12	12	9	11	13	12	15	12	14	15	23	24	4	11	12	8	20	18	4	15	6	16	5
H123	2	4	3	20	10	9	11	10	12	14	11	22	22	16	12	11	14	12	12	9	10	13	12	15	12	14	15	23	24	4	10	11	7	20	18	4	15	6	16	5
H124	5	3	6	23	13	10	12	13	15	13	10	19	19	15	11	10	13	11	11	8	14	12	13	16	13	15	16	24	25	3	10	11	7	21	17	5	14	7	15	4
H125	3	3	4	20	11	8	10	11	13	13	10	22	22	16	11	10	14	11	11	8	12	12	11	14	11	13	14	23	24	3	10	11	7	20	19	3	16	3	15	4

Table 4. 6 (cont'd).

	H81	H82	H83	H84	H85	H86	H87	H88	H89	H90	H91	H92	H93	H94	H95	H96	H97	H98	H99	H100	H101	H102	H103	H104	H105	H106	H107	H108	H109	H110	H111	H112	H113	H114	H115	H116	H117	H118	H119	H120	H121	H122	H123	H124	H125	
H101	3	9	14	22	21	7	4	4	3	20	18	22	16	16	21	4	3	3	4	4	5		0.009	0.024	0.049	0.029	0.029	0.026	0.014	0.024	0.031	0.007	0.012	0.007	0.009	0.019	0.026	0.016	0.026	0.026	0.024	0.024	0.012	0.007	0.014	0.009
H102	5	11	14	21	20	7	2	2	3	18	16	22	16	16	21	4	3	2	4	4	5	4		0.024	0.049	0.029	0.029	0.026	0.009	0.024	0.026	0.007	0.007	0.007	0.009	0.019	0.026	0.016	0.026	0.026	0.024	0.024	0.007	0.007	0.005	0.005
H103	13	7	6	21	22	5	8	8	11	17	15	20	14	14	19	10	7	12	10	9	10	10		0.044	0.009	0.019	0.016	0.024	0.014	0.021	0.026	0.021	0.026	0.026	0.014	0.016	0.016	0.016	0.009	0.014	0.026	0.026	0.029	0.024		
H104	21	21	18	15	18	15	18	18	21	21	19	4	8	6	3	20	17	22	20	15	20	20	18		0.049	0.051	0.046	0.049	0.049	0.051	0.046	0.046	0.051	0.046	0.044	0.046	0.031	0.046	0.051	0.049	0.049	0.046	0.046	0.049	0.046	
H105	15	9	8	23	24	7	10	10	13	19	17	22	16	16	21	12	9	14	12	11	12	12	4	20		0.024	0.021	0.029	0.019	0.026	0.031	0.026	0.031	0.031	0.019	0.021	0.021	0.021	0.005	0.019	0.031	0.031	0.034	0.029		
H106	14	5	12	22	23	7	10	12	13	19	19	23	17	17	22	14	11	14	14	13	12	12	8	21	10		0.012	0.024	0.014	0.016	0.031	0.021	0.031	0.031	0.014	0.012	0.021	0.016	0.012	0.019	0.009	0.031	0.031	0.034	0.029	
H107	14	2	11	24	25	6	9	11	12	18	16	21	15	15	20	13	10	13	13	10	11	11	7	19	9	5		0.026	0.007	0.005	0.024	0.024	0.029	0.024	0.007	0.005	0.019	0.005	0.005	0.016	0.007	0.029	0.029	0.031	0.026	
H108	7	11	14	21	22	7	2	4	5	18	18	22	16	16	21	6	5	6	6	7	6	4	10	20	12	10	11		0.024	0.031	0.012	0.002	0.012	0.014	0.019	0.026	0.016	0.026	0.026	0.024	0.024	0.012	0.012	0.014	0.009	
H109	13	3	10	23	24	5	8	10	11	19	17	22	16	16	21	12	9	12	12	11	10	10	6	20	8	6	3	10		0.007	0.026	0.021	0.026	0.026	0.005	0.007	0.016	0.007	0.007	0.014	0.005	0.026	0.026	0.029	0.024	
H110	16	4	13	24	25	8	11	13	14	18	16	23	17	17	22	15	12	13	15	12	13	11	9	21	11	7	2	13	3		0.029	0.029	0.034	0.029	0.012	0.009	0.024	0.005	0.009	0.021	0.007	0.034	0.034	0.031	0.031	
H111	4	12	15	23	22	8	3	3	2	19	17	21	15	15	20	3	4	3	3	4	3	3	11	19	13	13	10	5	11	12		0.009	0.009	0.005	0.002	0.021	0.029	0.019	0.024	0.029	0.026	0.026	0.009	0.005	0.012	0.007
H112	6	10	13	20	21	6	1	3	4	17	17	21	15	15	20	5	4	5	5	6	5	3	9	19	11	9	10	1	9	12	4		0.009	0.012	0.016	0.024	0.014	0.024	0.024	0.021	0.021	0.009	0.009	0.012	0.007	
H113	4	12	15	23	22	8	3	3	2	21	19	23	17	17	22	3	4	3	3	6	3	3	11	21	13	13	12	5	11	14	2	4		0.007	0.021	0.029	0.019	0.029	0.024	0.026	0.026	0.009	0.005	0.012	0.007	
H114	5	12	15	23	22	8	4	4	3	19	17	21	15	15	20	4	5	4	4	5	4	4	11	19	13	13	10	6	11	12	1	5	3		0.021	0.029	0.019	0.024	0.029	0.026	0.026	0.012	0.007	0.014	0.009	
H115	11	3	10	21	22	3	6	8	9	17	15	20	14	14	19	10	7	10	10	9	8	8	6	18	8	6	3	8	2	5	9	7	9	9		0.007	0.012	0.012	0.007	0.014	0.009	0.021	0.021	0.024	0.019	
H116	14	2	9	22	23	6	9	11	12	18	16	21	15	15	20	13	10	13	13	12	11	11	7	19	9	5	2	11	3	4	12	10	12	12	3		0.014	0.009	0.005	0.016	0.007	0.029	0.029	0.031	0.026	
H117	10	8	7	16	19	4	5	7	8	16	14	15	9	9	14	9	6	9	9	8	7	7	7	13	9	9	8	7	7	10	8	6	8	8	5	6		0.019	0.019	0.016	0.016	0.019	0.019	0.021	0.016	
H118	14	4	11	24	25	6	9	11	12	18	16	21	15	15	20	13	10	13	13	10	11	11	7	19	9	7	2	11	3	2	10	10	12	10	5	4	8		0.009	0.016	0.007	0.029	0.029	0.031	0.026	
H119	14	2	11	22	23	6	9	11	12	20	18	23	17	17	22	13	10	13	13	12	11	11	7	21	9	5	2	11	3	4	12	10	12	12	3	2	8	4		0.016	0.007	0.029	0.029	0.026	0.026	
H120	13	7	8	23	24	5	8	10	11	19	17	22	16	16	21	12	9	12	12	11	10	10	4	20	2	8	7	10	6	9	11	9	11	11	6	7	7	7		0.014	0.026	0.026	0.029	0.024		
H121	13	3	10	23	24	5	8	10	11	19	17	22	16	16	21	12	9	12	12	11	10	10	6	20	8	4	3	10	2	3	11	9	11	11	4	3	7	3	3	6		0.026	0.026	0.029	0.024	
H122	6	12	15	22	21	8	3	3	4	19	17	21	17	15	20	5	4	5	5	4	5	3	11	19	13	13	12	5	11	14	4	4	4	5	9	12	8	12	12	11	11		0.005	0.012	0.007	
H123	4	12	15	21	20	8	3	3	2	19	17	21	17	15	20	3	4	3	3	4	3	3	11	19	13	13	12	5	11	14	2	4	2	3	9	12	8	12	12	11	11	2		0.012	0.007	
H124	7	13	16	19	18	7	4	4	5	18	16	22	18	16	21	6	5	4	6	7	6	2	12	20	14	14	13	6	12	13	5	5	5	6	10	13	9	13	11	12	12	5	5	0.009		
H125	5	11	14	22	21	7	2	2	3	20	18	21	15	16	20	4	3	4	4	5	4	2	10	19	12	12	11	4	10	13	3	3	3	4	8	11	7	11	11	10	10	3	3	4		

Estimation of genetic diversity was first conducted through calculations of average values for the species, and secondly haplotype and nucleotide diversity for each population were estimated, and the results were presented in Table 4.7. Average haplotype diversity (h) for *C. quercusfolii* was $h = 0.7328 \pm 0.0955$, and nucleotide diversity was $\pi = 0.0102 \pm 0.0068$, respectively. In Table 4.7 the localities with only a single specimen were not considered for the evaluation of the haplotype and nucleotide diversity where their haplotype diversity was indicated as 1 and nucleotide diversity was indicated as 0.0, or vice versa. Therefore, only those localities with larger sample size ($N > 2$) were given in detail hereinafter. Most of the populations showed high degree of haplotypic diversity; for instance, İstanbul and Giresun populations (each with $N=10$) $h = 0.9778$, followed by Bursa ($h = 0.9722$), Kütahya ($h = 0.9556$), Balıkesir and Kırklareli (each with $h = 0.9333$). Considering haplotype richness Sivas was the leading population where out of 6 sequenced individuals had a unique sequence. Other than Sivas, Balıkesir, Bitlis, Bursa, Çankırı, Giresun, İstanbul, Karaman, Kırklareli, Konya, and Kütahya also possessed high haplotype richness. Regarding nucleotide diversity the highest value was detected in Konya ($\pi = 0.0275$) followed by Karaman ($\pi = 0.0267$), and Kayseri ($\pi = 0.0246$). Most of the other populations also implied high level of nucleotide diversity values (Table 4.7).

Table 4. 7. Sample size, haplotype number, haplotype (h) and nucleotide (π) diversities for the cyt b gene and ITS2 region.

Populations	Size	CYT B N _{HAP}	ITS2 N _{ALEL}	CYT B		ITS2		Populations	Size	CYT B N _{HAP}	ITS2 N _{ALEL}	CYT B		ITS2	
				h	π	h	π					h	π	h	π
AFYON	10	6	1	0.8889 +/- 0.0754	0.0229 +/- 0.0129	0.0000 +/- 0.0000	0.0000 +/- 0.0000	İZMİR	10	3	5	0.6222 +/- 0.1383	0.0172 +/- 0.0099	0.6667 +/- 0.1633	0.0169 +/- 0.0105
ARTVİN	2	1	2	0.0000 +/- 0.0000	0.0000 +/- 0.0000	1.0000 +/- 0.5000	0.0132 +/- 0.0153	KARAMAN	10	7	3	0.9111 +/- 0.0773	0.0267 +/- 0.0149	0.5111 +/- 0.1643	0.0022 +/- 0.0023
BALIKESİR	10	7	4	0.9333 +/- 0.0620	0.0057 +/- 0.0038	0.5333 +/- 0.1801	0.0080 +/- 0.0057	KASTAMONU	11	4	1	0.6909 +/- 0.1276	0.0047 +/- 0.0032	0.0000 +/- 0.0000	0.0000 +/- 0.0000
BARTIN	1	1	1	1.0000 +/- 0.0000	0.0000 +/- 0.0000	1.0000 +/- 0.0000	0.0000 +/- 0.0000	KAYSERİ	11	5	3	0.7091 +/- 0.1366	0.0246 +/- 0.0137	0.5818 +/- 0.1420	0.0025 +/- 0.0025
BİNGÖL	12	5	4	0.5758 +/- 0.1634	0.0088 +/- 0.0053	0.4545 +/- 0.1701	0.0028 +/- 0.0026	KIRKLARELİ	10	8	4	0.9333 +/- 0.0773	0.0064 +/- 0.0041	0.6444 +/- 0.1518	0.0082 +/- 0.0058
BİTLİS	10	7	1	0.9111 +/- 0.0773	0.0114 +/- 0.0068	0.0000 +/- 0.0000	0.0000 +/- 0.0000	KONYA	10	7	3	0.9111 +/- 0.0773	0.0275 +/- 0.0153	0.7333 +/- 0.0764	0.0032 +/- 0.0029
BOLU	10	5	2	0.6667 +/- 0.1633	0.0031 +/- 0.0023	0.2000 +/- 0.1541	0.0017 +/- 0.0020	KÜTAHYA	10	8	3	0.9556 +/- 0.0594	0.0076 +/- 0.0048	0.6444 +/- 0.1012	0.0076 +/- 0.0054
BURSA	9	8	3	0.9722 +/- 0.0640	0.0193 +/- 0.0112	0.4167 +/- 0.1907	0.0057 +/- 0.0044	MALATYA	3	2	1	0.6667 +/- 0.3143	0.0277 +/- 0.0216	0.0000 +/- 0.0000	0.0000 +/- 0.0000
ÇANAKKALE	10	4	5	0.7778 +/- 0.0907	0.0023 +/- 0.0019	0.8000 +/- 0.1001	0.0211 +/- 0.0127	MUŞ	11	5	5	0.6182 +/- 0.1643	0.0051 +/- 0.0034	0.7636 +/- 0.1066	0.0055 +/- 0.0042
ÇANKIRI	10	7	7	0.9111 +/- 0.0773	0.0050 +/- 0.0034	0.9111 +/- 0.0773	0.0170 +/- 0.0105	ORDU	2	2	2	1.0000 +/- 0.5000	0.0230 +/- 0.0242	1.0000 +/- 0.5000	0.0044 +/- 0.0062
DÜZCE	2	1	2	0.0000 +/- 0.0000	0.0000 +/- 0.0000	1.0000 +/- 0.5000	0.0398 +/- 0.0419	SAMSUN	11	6	3	0.8364 +/- 0.0887	0.0123 +/- 0.0072	0.6182 +/- 0.1038	0.0027 +/- 0.0026
EDİRNE	7	1	4	0.0000 +/- 0.0000	0.0000 +/- 0.0000	0.7143 +/- 0.1809	0.0046 +/- 0.0039	SİNOP	10	5	3	0.8000 +/- 0.1001	0.0084 +/- 0.0052	0.3778 +/- 0.1813	0.0017 +/- 0.0020
ELAZIĞ	5	3	2	0.7000 +/- 0.2184	0.0194 +/- 0.0126	0.4000 +/- 0.2373	0.0035 +/- 0.0035	SİVAS	6	6	3	1.0000 +/- 0.0962	0.0101 +/- 0.0067	0.6000 +/- 0.2152	0.0029 +/- 0.0029
ERZİNCAN	10	5	3	0.8000 +/- 0.1001	0.0072 +/- 0.0046	0.3778 +/- 0.1813	0.0016 +/- 0.0019	TOKAT	1	1	1	1.0000 +/- 0.0000	0.0000 +/- 0.0000	1.0000 +/- 0.0000	0.0000 +/- 0.0000
ERZURUM	4	3	2	0.8333 +/- 0.2224	0.0234 +/- 0.0162	0.6667 +/- 0.2041	0.0029 +/- 0.0033	TUNCELİ	1	1	1	1.0000 +/- 0.0000	0.0180 +/- 0.013	1.0000 +/- 0.0000	0.0000 +/- 0.0000
GİRESUN	10	9	4	0.9778 +/- 0.0540	0.0180 +/- 0.0103	0.7111 +/- 0.1175	0.0037 +/- 0.0032	VAN	10	4	2	0.6444 +/- 0.1518	0.0072 +/- 0.0046	0.2000 +/- 0.1541	0.0008 +/- 0.0013
GÜMÜŞHANE	11	8	3	0.8909 +/- 0.0918	0.0083 +/- 0.0051	0.5636 +/- 0.1340	0.0024 +/- 0.0024	YALOVA	10	4	6	0.5333 +/- 0.1801	0.0023 +/- 0.0019	0.8444 +/- 0.1029	0.0187 +/- 0.0115
ISPARTA	10	2	3	0.2000 +/- 0.1541	0.0023 +/- 0.0019	0.7111 +/- 0.0860	0.0031 +/- 0.0029	YOZGAT	1	1	1	1.0000 +/- 0.0000	0.0000 +/- 0.0000	1.0000 +/- 0.0000	0.0000 +/- 0.0000
İSTANBUL	10	9	4	0.9778 +/- 0.0540	0.0124 +/- 0.0074	0.6444 +/- 0.1518	0.0148 +/- 0.0093	ZONGUDAK	3	1	1	0.0000 +/- 0.0000	0.0000 +/- 0.0000	0.0000 +/- 0.0000	0.0000 +/- 0.0000
				CYT B								ITS2			
Average h=				0.7328 +/- 0.0955				Average h=				0.5865 +/- 0.1402			
Average π =				0.0102 +/- 0.0068				Average π =				0.0058 +/- 0.0048			

Genetic differentiations between populations were performed through conducting pairwise F_{ST} analysis (Table 4.8.). Pairwise F_{ST} values between Artvin, Bartın, Düzce, Edirne, Tokat, Tunceli, Yozgat, and Zonguldak were 1.000 which indicated the complete differentiation. These comparisons were omitted from further consideration since these populations were either represented by a single specimen or only one type of haplotype was detected in these localities; so they would not provide reliable estimation. Thus, when the remaining pairwise comparisons were considered to reveal the real nature of differentiation, the highest and statistically significant F_{ST} values were observed in the comparisons between Isparta and Balıkesir ($F_{ST} = 0.92247$), Bolu ($F_{ST} = 0.95212$), Çanakkale ($F_{ST} = 0.96134$), Erzincan ($F_{ST} = 0.91360$), Gümüşhane ($F_{ST} = 0.90239$), and Kastamonu ($F_{ST} = 0.93758$), respectively.

To detect the geographic allocation of the genetic diversity at different hierarchical level across the sampled range several AMOVA analyses were conducted with a statistical significance of 20000 permutations and results of three of these trials were shown in Figure 4.1 a, b, and c. The highest among group differentiation (Figure 4.1 a) was found when Isparta population was compared with all other populations (54%), the second highest value (Figure 4.1 b) was found when each population was considered singly as 44.65%, and the third (Figure 4.1 c) was found as 41.45% when all populations were grouped under 6 clusters (Group1: Erzurum, Group2: Elazığ, Group3: Isparta, Konya, Group4: Afyon, Karaman, Bolu, Yalova, Group5: Balıkesir, Bartın, Bursa, Çanakkale, Çankırı, Düzce, Edirne, İstanbul, İzmir, Kastamonu, Kırklareli, Kütahya, Ordu, Samsun, Sinop, Yozgat, and Zonguldak, Group6: Artvin, Bingöl, Bitlis, Erzincan, Giresun, Gümüşhane, Kayseri, Malatya, Muş, Sivas, Tokat, Tunceli, and Van.

Table 4. 8. Cytochrome b region F_{ST} analysis results showing pairwise genetic variations between populations. + : $p=0.001$, -: not significant.

	AFY	ART	BAL	BAR	BIN	BIT	BOL	BRS	CAN	CNK	DUZ	EDI	ELA	ERI	ERZ	GIR	GUM	ISP
AFY		+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+
ART	0.36989		+	-	-	-	+	+	+	+	-	+	+	-	-	-	-	+
BAL	0.34204	0.78142		-	+	+	+	+	-	+	+	+	+	+	+	+	+	+
BAR	0.07165	1.00000	0.30864		-	-	-	-	-	-	-	-	-	-	-	-	-	-
BIN	0.45278	0.42268	0.69201	0.64482		+	+	+	+	+	+	+	+	+	+	+	+	+
BIT	0.38980	0.06928	0.62478	0.51416	0.13453		+	+	+	+	+	+	+	+	+	+	+	+
BOL	0.43780	0.87479	0.37182	0.20261	0.73820	0.67435		-	+	-	+	+	+	+	+	+	+	+
BRS	0.23932	0.43628	0.13130	-0.57292	0.52264	0.43874	0.09137		+	-	-	+	+	+	+	+	+	+
CAN	0.38174	0.89983	0.07602	0.71605	0.73605	0.67155	0.61147	0.22367		+	+	+	+	+	+	+	+	+
CNK	0.37778	0.79992	0.14436	0.04348	0.69457	0.62654	0.05437	0.05693	0.35036		+	+	+	+	+	+	+	+
DUZ	0.30633	1.00000	0.49640	1.00000	0.69510	0.60850	0.66520	0.08129	0.75687	0.49179		+	+	+	-	+	+	+
EDI	0.50193	1.00000	0.68837	1.00000	0.79735	0.74329	0.71000	0.31637	0.86891	0.59630	1.00000		+	+	+	+	+	+
ELA	0.44187	0.53998	0.71247	0.51724	0.52524	0.51579	0.77877	0.53771	0.76105	0.73260	0.63494	0.81149		+	+	+	+	+
ERI	0.52137	0.18591	0.74583	0.73446	0.50236	0.29293	0.80312	0.57876	0.79346	0.76062	0.77375	0.85694	0.63179		+	+	-	+
ERZ	0.47921	0.58112	0.74302	0.47186	0.62741	0.59935	0.80622	0.54448	0.79650	0.76849	0.61301	0.83597	0.50567	0.71290		+	+	+
GIR	0.39525	-0.03408	0.57251	0.36405	0.35905	0.16811	0.62013	0.40562	0.60232	0.58379	0.43262	0.67187	0.48454	0.27350	0.46965		+	+
GUM	0.52205	-0.01799	0.73762	0.70000	0.44699	0.23225	0.77998	0.57255	0.78482	0.74489	0.74168	0.83147	0.62792	0.03735	0.70383	0.23951		+
ISP	0.76437	0.96134	0.92247	0.95671	0.88513	0.86415	0.95212	0.78399	0.95341	0.93283	0.96134	0.97708	0.85289	0.91360	0.81716	0.79502	0.90239	
IST	0.31973	0.55298	0.26134	-0.12500	0.60210	0.50688	0.16598	0.09470	0.38009	0.16667	0.29278	0.46149	0.64245	0.64206	0.66189	0.47944	0.62633	0.87597
IZM	0.25000	0.48084	0.14764	-0.24444	0.53792	0.46823	0.25739	-0.00142	0.26820	0.18079	0.13223	0.43831	0.55414	0.60090	0.55004	0.43374	0.58591	0.77410
KAR	-0.04322	0.30459	0.36751	0.09549	0.39777	0.34496	0.46201	0.26668	0.41231	0.40969	0.29992	0.49637	0.39636	0.48527	0.41652	0.36268	0.47365	0.72916
KAS	0.42350	0.81845	0.31783	-0.01818	0.71993	0.65592	-0.02120	0.08493	0.53791	0.06470	0.55890	0.62347	0.76245	0.78127	0.78729	0.61171	0.76087	0.93758
KAY	0.35753	0.17274	0.55011	0.28970	0.19236	0.18713	0.58209	0.40716	0.57340	0.55097	0.43565	0.61732	0.34325	0.38517	0.39043	0.23818	0.33302	0.73155
KON	0.34546	0.72826	0.26030	-0.11111	0.63756	0.56011	0.00641	0.03459	0.43787	0.00444	0.41860	0.55128	0.70111	0.72065	0.73875	0.53180	0.69943	0.91942
KLR	0.34243	0.46249	0.57547	0.32323	0.54534	0.49349	0.63988	0.39977	0.60327	0.60407	0.46783	0.65170	0.44246	0.59967	0.35149	0.43780	0.59839	0.38046
KUT	0.29849	0.69887	0.14706	0.05397	0.61961	0.54827	0.21700	0.09393	0.33538	0.08148	0.36542	0.58223	0.67215	0.70546	0.70516	0.53103	0.68818	0.90496
MAL	0.27429	0.22581	0.63376	0.12195	0.18294	0.18177	0.72114	0.32356	0.71083	0.65562	0.44186	0.77972	0.33395	0.50776	0.31122	0.22470	0.46945	0.78873
MUS	0.49756	0.55222	0.76692	0.78214	-0.00308	0.11020	0.81484	0.57055	0.82237	0.77006	0.81128	0.87933	0.64310	0.56258	0.70567	0.37436	0.48613	0.92484
ORD	0.11550	0.00000	0.38262	-0.42857	0.35270	0.10374	0.55097	0.04202	0.59009	0.41523	0.28571	0.77918	0.40713	0.26026	0.44568	0.06599	0.17724	0.91155
SAM	0.27965	0.45969	0.21686	-0.01379	0.51180	0.41168	0.25242	0.11168	0.33350	0.20048	0.24642	0.50821	0.60270	0.55073	0.63943	0.40599	0.53183	0.86362
SIN	0.33594	0.63974	0.19218	-0.22222	0.61265	0.52729	0.04156	0.04332	0.36807	0.01566	0.32099	0.48632	0.67461	0.66667	0.71401	0.49961	0.64486	0.90278
SIV	0.34560	-0.01538	0.65385	0.54483	0.26531	0.03432	0.72367	0.40909	0.73165	0.66444	0.64324	0.80995	0.50912	0.20275	0.59322	0.10811	0.10561	0.89339
TOK	0.31019	1.00000	0.78908	1.00000	0.45455	0.09899	0.88109	0.39113	0.90707	0.80870	1.00000	1.00000	0.41667	-0.08046	0.41063	-0.44856	-0.23750	0.96016
TUN	0.12865	1.00000	0.74603	1.00000	-0.90909	-0.41587	0.85731	0.27404	0.88642	0.76344	1.00000	1.00000	0.27586	0.48634	0.28655	-0.00285	0.36129	0.95305
VAN	0.47222	0.31862	0.72863	0.70231	0.04278	0.05244	0.77668	0.53649	0.78241	0.73169	0.74570	0.84014	0.59969	0.38585	0.66873	0.29267	0.30055	0.90814
YLV	0.43058	0.90196	0.26852	0.36111	0.74439	0.67999	0.05644	0.09091	0.53535	-0.03276	0.72813	0.77105	0.78194	0.80868	0.81576	0.62332	0.78864	0.95734
YOZ	-0.00337	1.00000	0.45894	1.00000	0.67505	0.56908	0.66111	-0.19841	0.74444	0.48837	1.00000	1.00000	0.54348	0.75328	0.39303	0.32567	0.72308	0.95851
ZON	0.32252	1.00000	0.33780	1.00000	0.69155	0.61186	0.59798	0.10415	0.70078	0.38356	1.00000	1.00000	0.66156	0.77704	0.68731	0.51120	0.74419	0.96487

Table 4. 8 (cont'd).

	IST	IZM	KAR	KAS	KAY	KON	KLR	KUT	MAL	MUS	ORD	SAM	SIN	SIV	TOK	TUN	VAN	YLV	YOZ	ZON
AFY	+	+	-	+	+	+	+	+	-	+	-	+	+	+	-	-	+	+	-	+
ART	+	+	+	+	-	+	+	+	-	+	-	+	+	-	-	-	-	+	-	-
BAL	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+	+	-	+
BAR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BIN	+	+	+	+	+	+	+	+	-	-	-	+	+	+	-	-	-	+	-	+
BIT	+	+	+	+	+	+	+	+	-	+	-	+	+	-	-	-	-	+	-	+
BOL	+	+	+	-	+	-	+	+	+	+	+	+	-	+	-	-	+	-	-	+
BRS	-	-	+	-	+	-	+	-	+	+	-	+	-	+	-	-	+	-	-	+
CAN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+
CNK	+	+	+	-	+	-	+	-	+	+	-	+	-	+	-	-	+	-	-	+
DUZ	-	-	-	+	+	+	+	+	-	+	-	+	-	+	-	-	+	+	-	-
EDI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+
ELA	+	+	+	+	+	+	+	+	-	+	-	+	+	+	-	-	+	+	-	+
ERI	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+	+	-	+
ERZ	+	+	+	+	+	+	+	+	-	+	-	+	+	+	-	-	+	+	-	-
GIR	+	+	+	+	+	+	+	+	-	+	-	+	+	+	-	-	+	+	-	+
GUM	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+	+	-	+
ISP	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+
IST		+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	+	+	-	-
IZM	0.21926		+	+	+	+	+	+	-	+	-	+	+	+	-	-	+	+	-	+
KAR	0.33525	0.25140		+	+	+	+	+	-	+	-	+	+	+	-	-	+	+	-	+
KAS	0.12063	0.24912	0.44925		+	-	+	+	+	+	-	+	+	+	+	-	+	-	-	+
KAY	0.48064	0.42506	0.29344	0.57557		+	+	+	-	+	-	+	+	+	+	-	+	+	-	+
KON	0.13187	0.16576	0.37747	0.03884	0.50342		+	-	+	+	-	+	-	+	-	-	+	-	-	+
KLR	0.55590	0.37248	0.29800	0.63378	0.37972	0.57887		+	-	+	+	+	+	+	-	-	+	+	-	+
KUT	0.19192	0.11074	0.31952	0.18417	0.49151	0.07744	0.54533		+	+	-	+	-	+	-	-	+	+	-	+
MAL	0.52081	0.33144	0.20098	0.69391	0.05285	0.59030	0.16298	0.54886		+	-	+	+	+	-	-	+	+	-	-
MUS	0.65374	0.58786	0.43666	0.78832	0.22891	0.71237	0.57825	0.69241	0.27754		+	+	+	+	-	-	-	+	-	+
ORD	0.17138	0.02820	0.07410	0.48283	0.15754	0.30000	0.34686	0.23403	0.03774	0.48163		-	-	-	-	-	-	+	-	-
SAM	0.08236	0.15532	0.28201	0.19094	0.42209	0.14474	0.52797	0.14207	0.44623	0.56543	-0.04919		-	+	-	-	+	+	-	+
SIN	0.12988	0.11640	0.36216	0.06419	0.49239	-0.02946	0.56848	0.06213	0.55492	0.68103	0.12424	0.10296		+	-	-	+	-	-	-
SIV	0.50270	0.42402	0.29035	0.69256	0.17082	0.58856	0.45301	0.57060	0.20992	0.31019	-0.05495	0.37344	0.52408		-	-	-	+	-	+
TOK	0.54622	0.45098	0.22297	0.82769	0.03140	0.73795	0.39843	0.70698	-0.02857	0.62462	-0.66667	0.44528	0.64401	-0.01538		-	-	-	-	-
TUN	0.49533	0.33333	-0.00676	0.79259	-0.41205	0.66533	0.24613	0.61046	-0.89474	-0.87692	-0.42857	0.37447	0.58801	-0.01538	1.00000		-	-	-	-
VAN	0.61461	0.55593	0.41719	0.75308	0.21541	0.67255	0.56202	0.65676	0.26086	0.01658	0.31034	0.51305	0.63242	0.14961	0.36889	-0.57778		+	-	+
YLV	0.23907	0.23661	0.46154	0.10107	0.58554	0.04040	0.63589	0.19753	0.73142	0.82611	0.58115	0.28347	0.03916	0.73796	0.90873	0.88889	0.78685		-	+
YOZ	0.01818	-0.00901	0.00192	0.50222	0.31059	0.36869	0.34554	0.29551	0.18182	0.80323	-0.25000	0.03607	0.29487	0.58750	1.00000	1.00000	0.72797	0.75661		-
ZON	0.25506	0.13043	0.31408	0.47455	0.46242	0.33746	0.50964	0.27411	0.53846	0.80984	0.38776	0.19512	0.23077	0.65803	1.00000	1.00000	0.74694	0.65081	1.00000	

4.1.2 Demographic Structure of *C. quercusfolii* Populations Based on Cyt B Gene

Deviations from neutrality and population size changes over time can be deduced from several analyses. The Harpending's raggedness index calculated for *C. quercusfolii* was $r = 0.0049$, but not significant. When populations were checked individually only Kayseri population's Hri value was statistically significant ($Hri = 0.0360^*$). Fu's F_s ($-107,743$, $P < 0.00$) and Tajima's D ($-1,09653$, $P > 0.10$) values calculated for all populations across the whole sampled range were not statistically significant. However, when the analysis was narrowed into individual localities statistically significant values were indicated by some populations (Table 4.9). Negative significant Tajima's D values were calculated for Bingöl ($D = -1.7205^*$) and Isparta ($D = -1.7411^*$) populations. Regarding Fu's F_s Balıkesir (Fu's $F_s = -2.5895^*$), Çanakkale (Fu's $F_s = -0.8305^*$), İstanbul (Fu's $F_s = -3.3361^*$), Kırklareli (Fu's $F_s = -3.8244^*$), Kütahya (Fu's $F_s = -3.2237^*$), and Sivas (Fu's $F_s = -2.4199^{**}$).

Mismatch analysis of all pairwise combinations for the cyt b mitochondrial DNA haplotypes was estimated and the result is shown in Figure 4.2. The mismatch distribution including all samples was indicating a multimodal profile for the *C. quercusfolii* haplotypes; however, the results were not significantly supported. Therefore, each population (with more than one specimen) was further analyzed separately to obtain more detailed picture of the species across its range in Turkey and to go through into deeper population histories. All resultant graphs can be seen in Figure 4.3. Populations that showed unimodal profile were Balıkesir, Çanakkale, Kütahya, Malatya, Ordu, and Yalova, however only Balıkesir, Çankırı, Kütahya, and Yalova populations' unimodal mismatch distributions were statistically supported. Afyon, Bingöl, Bitlis, Bolu, Bursa, Çankırı, Elazığ, Erzincan, Erzurum, Giresun, Gümüşhane, İstanbul, İzmir, Karaman, Kastamonu, Kayseri, Kırklareli, and Konya populations showed multimodal profile.

Table 4. 9. Fu's F_S ve Tajima's D values calculated for each *C. quercusfolii* population for cyt b. * $P \leq 0.01$, ** $P \leq 0.001$.

Pop.	Fu's F_S	Tajima's D	Hri	SSD	Pop.	Fu's F_S	Tajima's D	Hri	SSD
AFY	2.1165	2.2443	0.1822	0.1010	IZM	6.6069	0.0276	0.4429	0.5372
ART	0.0000	0.0000	0.0000	0.0000	KAR	1.2189	1.0182	0.1175	0.0709
BAL	-2.5895*	-0.5171	0.0479	0.0029	KAS	0.8098	-0.6003	0.1616	0.0622
BAR	0.0000	0.0000	0.0000	0.0000	KAY	4.4619	0.1864	0.0360*	0.1380
BIN	1.4041	-1.7205*	0.1258	0.4260	KLR	-3.8244*	-1.2789	0.1081	0.0293
BIT	-0.7583	0.7599	0.0849	0.3704	KON	1.2867	0.2552	0.0661	0.0539
BOL	-1.5071	-1.4928	0.1397	0.0329	KUT	-3.2237*	0.1785	0.1116	0.0467
BRS	-1.4237	-0.2483	0.0470	0.0448	MAL	4.4998	0.0000	1.0000	0.4645
CAN	-0.8305*	-0.1297	0.2400	0.0347	MUS	-0.1552	-1.4911	1.0000	0.4542
CNK	-2.9681	-0.9579	0.0622	0.0053	ORD	2.3025	0.0000	0.0000	0.0000
DUZ	0.0000	0.0000	0.0000	0.0000	SAM	0.8918	0.1940	0.1028	0.06718
EDI	0.0000	0.0000	0.0000	0.0000	SIN	0.7939	-1.4208	0.0893	0.0280
ELA	3.4053	1.2185	0.4700	0.2082	SIV	-2.4199**	0.0280	0.1511	0.0719
ERI	0.4232	-0.8694	0.2296	0.0753	TOK	0.0000	0.0000	0.0000	0.0000
ERZ	2.6519	-0.6976	0.7500	1.0555	TUN	0.0000	0.0000	0.0000	0.0000
GIR	-2.2481	-0.37038	0.0301	0.0286	VAN	1.6772	0.4999	0.2380	0.1161
GUM	-2.40896	-1.51984	0.1104	0.0373	YLV	-0.8305	-0.1297	0.0962	0.3802
ISP	2.1972	-1.7411*	0.7200	0.0577	YOZ	0.0000	0.0000	0.0000	0.0000
IST	-3.3361*	0.4175	0.0518	0.1402	ZON	0.0000	0.0000	0.0000	0.0000

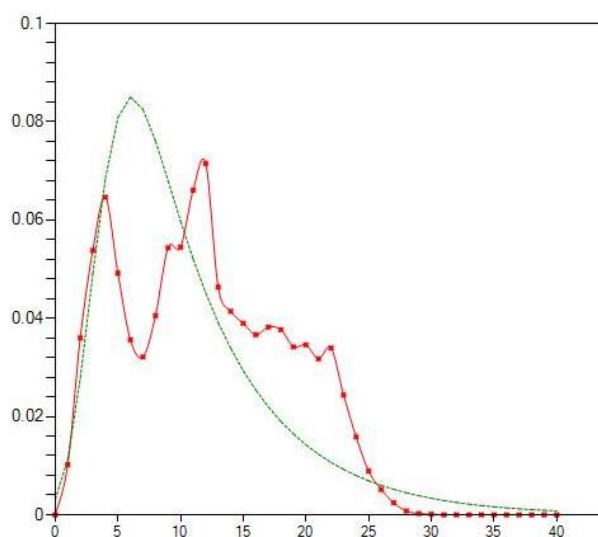


Figure 4.2. Mismatch distribution of all pairwise combinations for the cyt b haplotypes. The observed distribution represented by red line, and the expected frequencies depicted by green dotted line.

The results of the spatial genetic autocorrelation analysis across the study area for distance classes of 100 and 150 km with 95% confidence intervals were shown in Figure 4.4. The correlogram for the 100 km-distance class size showed positive and significant values of r at 100 km ($r = 0.436$, $p < 0.001$) and 400 km ($r = 0.101$, $p < 0.001$), with an x-axis intercept at 485,6 km (Figure 4.4a). The correlogram for the 150 km distance class size detected positive and significant values of r at 150 km ($r = 0.388$, $p < 0.001$), and 450 km ($r = 0.047$, $p < 0.001$) but significantly negative values for distances beyond the x-axis intercept 523,4 km (Figure 4.4b). These results imply that populations of *C. quercusfolii* separated by distances of less than 100 km are more genetically similar to each other are higher than expected by chance, while genetic differentiation between populations separated by more than ca. 500 km is higher than expected by chance.

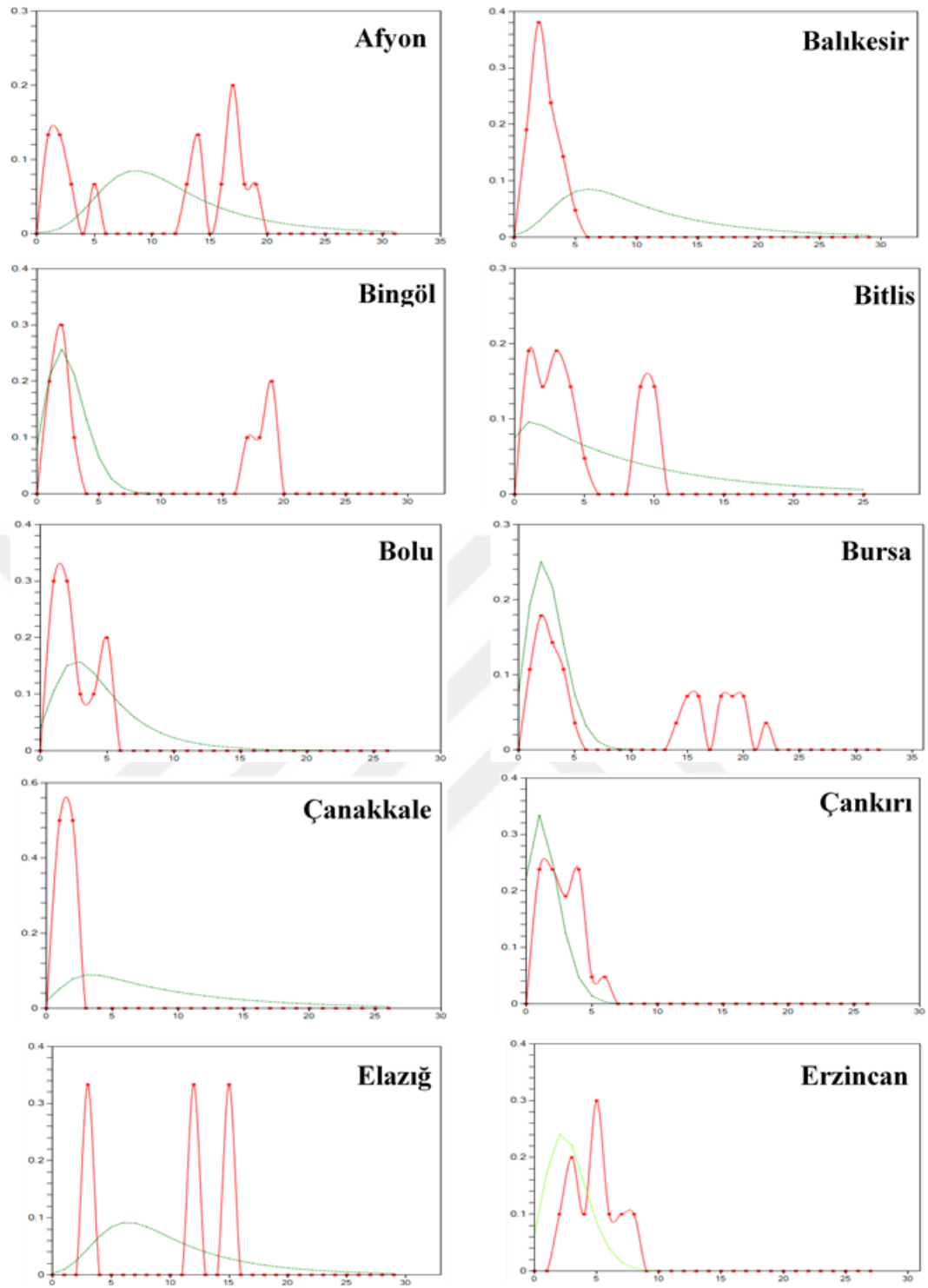


Figure 4.3. Mismatch distribution of populations with their own haplotypes for cyt b data set. The observed distribution represented by red line, and the expected frequencies depicted by green line.

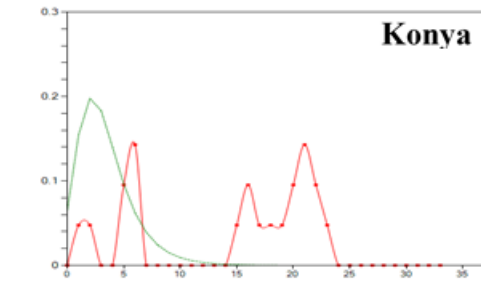
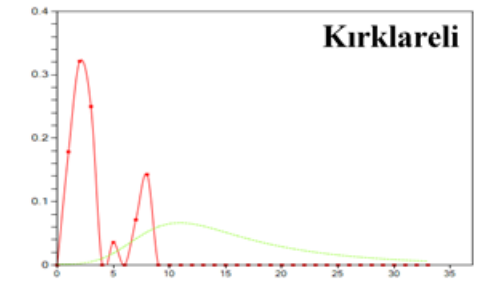
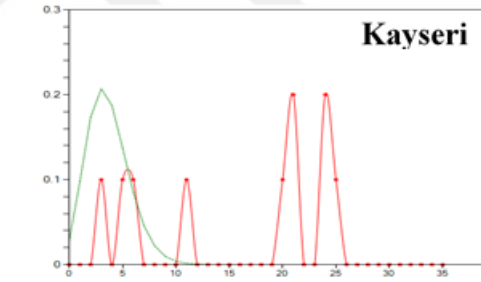
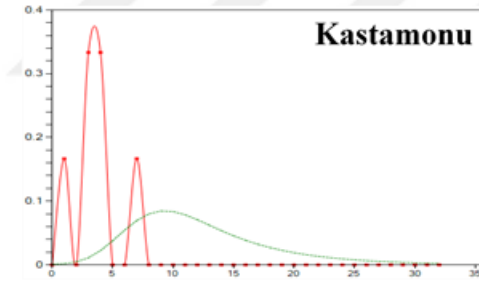
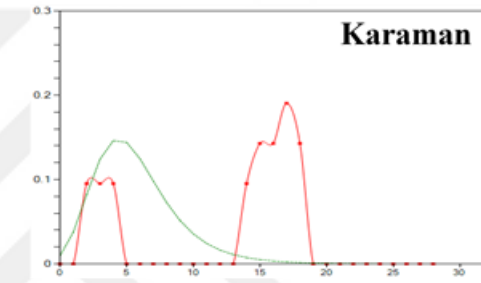
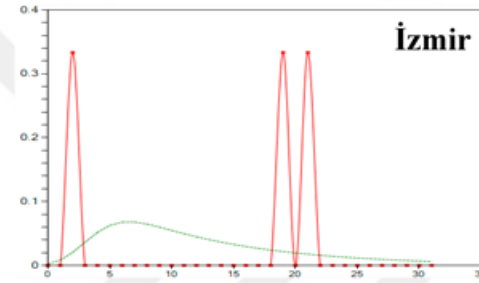
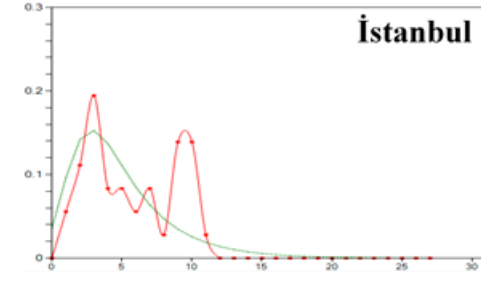
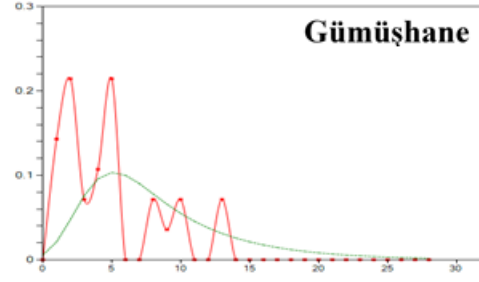
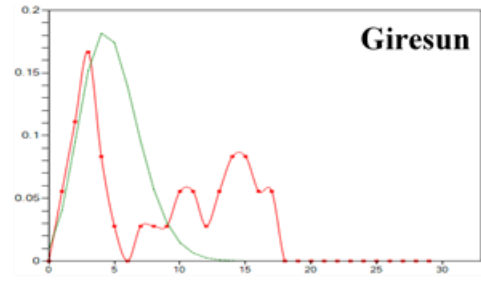
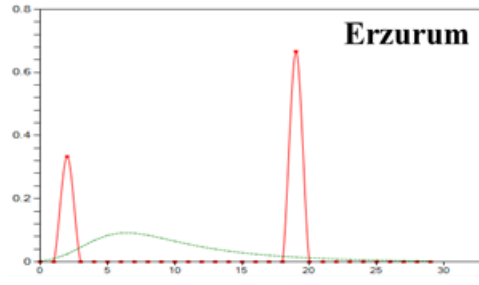


Figure 4.3 (cont'd).

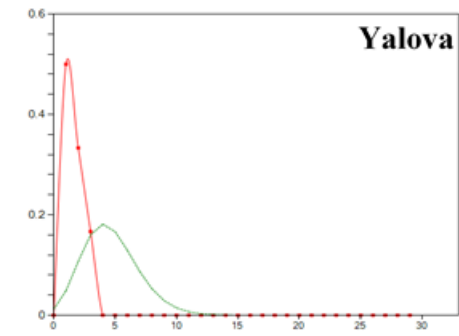
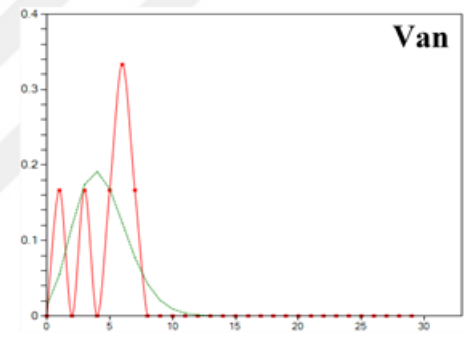
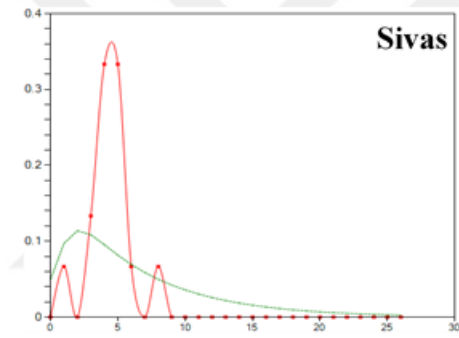
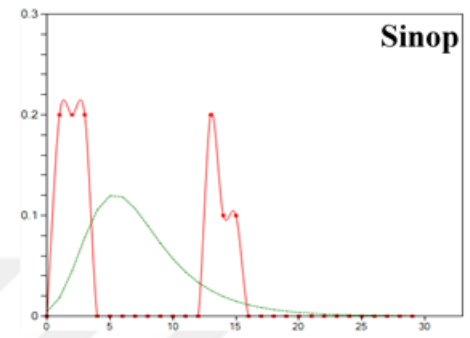
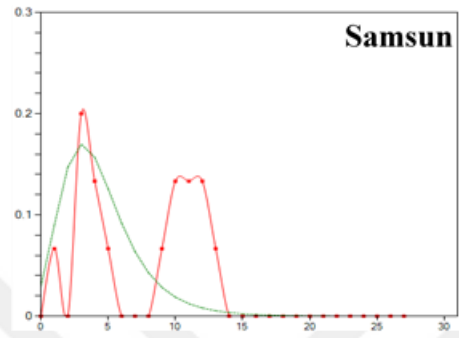
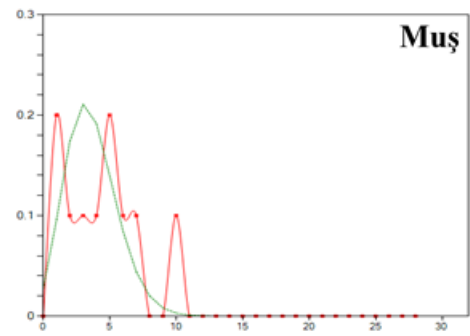
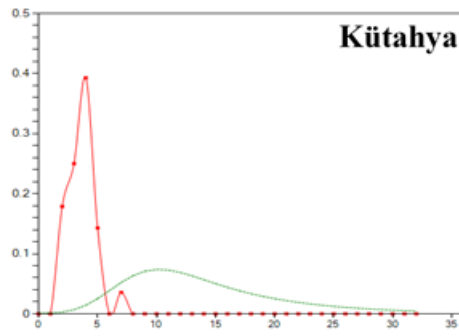


Figure 4.3 (cont'd).

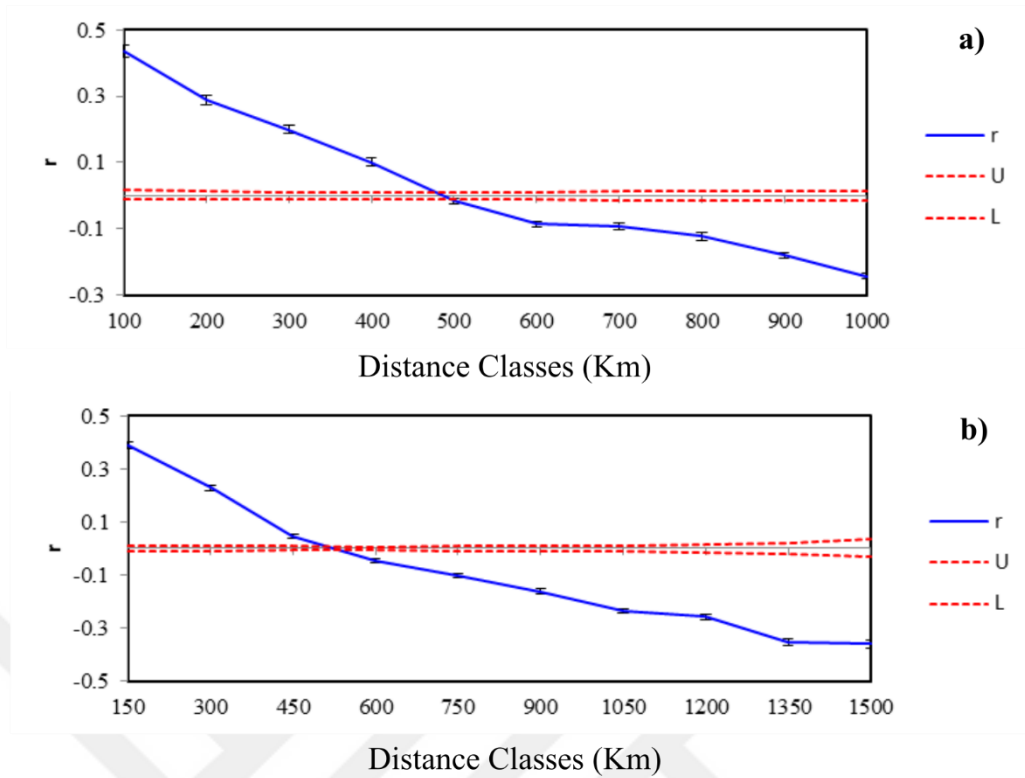


Figure 4.4. Correlograms showing spatial genetic correlation coefficient (r) as a function of geographic distances in the entire study area based on cyt b. a) 100 km distance class, b) 150 km distance class. Dashed lines indicate upper (U) and lower (L) boundaries of 95% confidence intervals under the null hypothesis of no geographic structure is present. 95% bootstrapped confidence intervals are shown around each of the calculated r value.

4.2 Assessment of the Nuclear Marker: Internal transcribed Spacer 2 (ITS2) Region

4.2.1 Genetic Diversity Estimates of the ITS2 Segment of *Cynips quercusfolii*

The amplified ITS2 fragment of *C. quercusfolii* after trimming 5.8S and 28S rDNA sequences from both flanking ends were 395 bp, and a representative electropherogram was presented in Figure 3.6. No length variation was observed for the ITS2 segment. Thirty alleles were determined when all 294 sequences were collapsed into alleles (GenBank Accession number MG661529- MG661558).

In ITS2 alleles 384 characters were monomorphic, while 11 sites were polymorphic (Table 4.10). There were 10 parsimony informative and one parsimony uninformative site (multiple hit at site 217). Nucleotide frequencies were 28.61%, 30.75%, 18.14%, and 22.49% for A, T, C, and G, respectively. Most of the substitutions occurred between A and G (33.13%), and the least was between A/C and G/C (%3.7) (Table 4.11). The transition and transversion rate ratios were $k_1 = 5.672$ (purines) and $k_2 = 0$ (pyrimidines). There were 6 transitions at sites 155, 163, 185, 188, 204, and 226, and 4 transversions at sites 165, 174, 176, and 190. Transition/transversion bias was $R = 1.461$. The average number of nucleotide differences was $k = 4.560$.

Table 4. 10. Thirty alleles of the ITS2 region of *C. quercusfolii*. Only polymorphic character sites were shown.

	155	163	165	174	176	185	188	190	204	217	226
A1	G	G	G	A	G	G	G	T	G	G	A
A2	.	.	C	.	.	.	.	.	A	T	.
A3	.	.	.	.	.	.	.	.	.	T	.
A4	A	A	C	.	.	A	A	.	A	T	.
A5	A	.	.	.	.	.	.	.	.	T	.
A6	.	.	.	.	.	.	.	.	A	T	.
A7	.	.	.	.	.	.	.	.	.	A	.
A8	.	.	.	.	.	.	.	.	A	A	.
A9	.	.	.	.	.	A	A	A	.	A	G
A10	A	A	C	C	C	A	A	.	A	T	.
A11	.	.	.	.	.	.	A	.	.	.	.
A12	A	A	C	C	C	.	A	.	A	T	.
A13	A	A	C	C	C	.	A	.	.	.	.
A14	A	A	C	C	.	.	.	.	.	.	.
A15	A	.	C	C	C	.	.	.	.	T	.
A16	A	.	.	.	.	.	.	.	.	.	.
A17	.	.	.	.	.	.	.	.	.	T	G
A18	.	.	C	.	C	.	.	.	.	T	.
A19	.	.	.	.	.	.	.	.	.	.	G
A20	A	.	.	.	.	A	A	.	.	T	.
A21	A	A	C	.	C	A	A	.	A	T	.
A22	A	A	C	C	C	A	A	.	A	.	.
A23	A	A	C	.	.	.	.	.	A	T	.
A24	A	A	C	.	C	A	A	.	.	.	.
A25	.	.	.	.	.	.	A	.	.	T	.
A26	.	.	C	.	.	.	.	.	.	T	.
A27	.	.	.	.	.	.	.	.	.	A	G
A28	.	A	.	.	C	.	.	.	.	.	.
A29	.	.	.	.	C	.	.	.	A	T	.
A30	A	A	C	.	.	.	A	.	A	.	.

Table 4. 11. Maximum composite likelihood estimates of the pattern of nucleotide substitutions for the ITS2 region of *C. quercusfolii*. Rates of different transitional substitutions are shown in bold and those of transversional substitutions are shown in italics.

	A	T	C	G
A	—	6.28	3.7	26.04
T	5.84	—	0	4.59
C	5.84	0	—	4.59
G	33.13	6.28	3.7	—

The frequency of ITS2 alleles was given in Table 4.12. The highest allele frequency was detected for A1 (N= 128), which was shared among Afyon, Artvin, Balıkesir, Bolu, Bursa, Çanakkale, Çankırı, Düzce, Edirne, Elazığ, Erzincan, Erzurum, Giresun, Gümüşhane, Isparta, İzmir, Karaman, Kastamonu, Kayseri, Kırklareli, Konya, Kütahya, Muş, Samsun, Sivas, Tokat, Yalova, Yozgat, and Zonguldak. The next highly observed allele A7 with a frequency of 71 shared among 16 populations. The third allele (A3) was found as a shared allele among 24 localities (N= 49). The number of singleton allele was 18, and there were 9 private alleles. There were fixed alleles in some populations; for example, Afyon and Bitlis were represented by 10 specimens each, but with only a single allele (A1, and A7, respectively), and Kastamonu represented by 11 samples all showing only a single allele (A1).

The pairwise comparisons to determine sequence differences between each *C. quercusfolii* alleles ranged between 1 bp and 10 bp (0.4% and 4.7%) (Table 4.13). Between 26 cases there was one base pair difference, while the most divergent alleles with 10- bases difference were between 5 cases. The most differing alleles were between A9 (from Bursa) and A12 (from Çanakkale), A10 (from Çanakkale, Çankırı, Düzce, İzmir, and Yalova), and A19 (from Giresun), A10 and A27 (from Van), A17 (from Düzce and Muş), A22 (from İzmir), and A22 (from İzmir) and A27 (from Van).

The average allelic diversity (h) of *C. quercusfolii* was $h = 0.5865 \pm 0.1402$ and the nucleotide diversity was $\pi = 0.0058 \pm 0.0088$, respectively (Table 4.7). The highest allelic diversity was possessed by Çankırı ($h = 0.9111$), followed by Yalova ($h = 0.844$), and Çanakkale ($h = 0.800$). However, the highest nucleotide diversity was shown by Çanakkale ($\pi = 0.0211$), Yalova ($\pi = 0.0187$), and Çankırı ($\pi = 0.0170$). Accordingly, the allelic richness is highest in Çankırı (N= 10, $N_{all} = 7$), and Yalova (N= 10, $N_{all} = 6$).

Genetic differentiations between populations using the ITS2 tests were employed through pairwise F_{ST} analysis (Table 4.14). The highest and statistically significant F_{ST} values were found in the comparisons between Van and Bolu ($F_{ST} = 0.75000$), Sinop ($F_{ST} = 0.75000$), Düzce ($F_{ST} = 0.72421$), Elazığ ($F_{ST} = 0.72856$),

Artvin ($F_{ST} = 0.69925$), respectively. All other values were showing lower level of differentiation.



Table 4. 12. ITS2 allele frequencies in *C. quercusfolii* populations.

	AFY	ART	BAL	BAR	BIN	BIT	BOL	BRS	CAN	CNK	DUZ	EDI	ELA	ERI	ERZ	GIR	GUM	ISP	IST	IZM	KAR	KAS	KAY	KLR	KON	KUT	MAL	MUS	ORD	SAM	SIN	SIV	TOK	TUN	VAN	YLV	YOZ	ZON	N _{hap}
A1	10	1	7				9	7	4	3		4		1		3	7	4	6	6	2	11	7	6	3	5		1		4	8		1			4	1	3	128
A2		1																																				1	
A3			1		1			1	1	2		1	4	1	2	1	1	2	1		1		2	2	4	4	3	1	1	6	1	4				1			49
A4			1																					1														2	
A5			1		1															1																		3	
A6				1			1					1																										3	
A7					9	10								8	2	5	3	4			7		2		3			5	1	1		1		1	9			71	
A8					1																																	1	
A9								1																														1	
A10									3	1	1									1															2			8	
A11									1																						1							2	
A12									1																													1	
A13										1																												1	
A14										1																												1	
A15										1																												1	
A16										1		1																										2	
A17											1																	3										4	
A18													1															3										1	
A19																	1							1														2	
A20																			1																			1	
A21																			2																			2	
A22																				1																		1	
A23																				1																		1	
A24																																						1	
A25																										1												1	
A26																											1											1	
A27																															1					1		1	
A28																																	1					1	
A29																																				1		1	
A30																																				1		1	
Total	10	2	10	1	12	10	10	9	10	10	2	7	5	10	4	10	11	10	10	10	10	11	11	10	10	10	3	11	2	11	10	6	1	1	10	10	1	3	294
N _{all}	1	2	4	1	4	1	2	3	5	7	2	4	2	3	2	4	3	3	4	5	3	1	3	4	3	3	1	5	2	3	3	3	1	1	2	6	1	1	

Table 4. 13. Pairwise comparison of 30 *C. quercusfolii* alleles. Net nucleotide differences are shown below diagonal and the percentage of the differences are presented above diagonal.

	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15	A16	A17	A18	A19	A20	A21	A22	A23	A24	A25	A26	A27	A28	A29	A30
A1		0.013	0.004	0.032	0.009	0.009	0.004	0.009	0.023	0.042	0.004	0.037	0.027	0.018	0.022	0.004	0.009	0.013	0.004	0.018	0.037	0.037	0.023	0.027	0.009	0.009	0.009	0.009	0.013	0.023
A2	3		0.009	0.018	0.013	0.004	0.013	0.009	0.032	0.027	0.018	0.023	0.032	0.023	0.018	0.018	0.013	0.009	0.018	0.023	0.023	0.032	0.009	0.032	0.013	0.004	0.018	0.023	0.009	0.018
A3	1	2		0.028	0.004	0.004	0.004	0.009	0.023	0.037	0.009	0.032	0.032	0.023	0.018	0.009	0.004	0.009	0.009	0.014	0.032	0.042	0.018	0.032	0.004	0.004	0.009	0.013	0.009	0.027
A4	7	4	6		0.023	0.023	0.032	0.027	0.032	0.009	0.027	0.013	0.023	0.023	0.027	0.027	0.033	0.028	0.037	0.013	0.004	0.013	0.009	0.013	0.023	0.023	0.037	0.032	0.027	0.009
A5	2	3	1	5		0.009	0.009	0.013	0.027	0.032	0.013	0.027	0.027	0.018	0.013	0.004	0.009	0.013	0.013	0.009	0.027	0.037	0.013	0.027	0.009	0.009	0.013	0.018	0.013	0.023
A6	2	1	1	5	2		0.009	0.004	0.027	0.032	0.013	0.027	0.037	0.027	0.023	0.013	0.009	0.013	0.013	0.018	0.027	0.037	0.013	0.037	0.009	0.009	0.013	0.018	0.004	0.023
A7	1	3	1	7	2	2		0.004	0.018	0.042	0.009	0.037	0.032	0.023	0.022	0.009	0.009	0.013	0.009	0.018	0.037	0.042	0.023	0.032	0.009	0.009	0.004	0.013	0.013	0.028
A8	2	2	2	6	3	1	1		0.023	0.037	0.014	0.032	0.037	0.027	0.027	0.014	0.013	0.018	0.014	0.023	0.032	0.037	0.018	0.037	0.013	0.013	0.009	0.018	0.009	0.023
A9	5	7	5	7	6	6	4	5		0.041	0.018	0.046	0.042	0.042	0.041	0.028	0.018	0.032	0.018	0.018	0.037	0.042	0.042	0.032	0.018	0.027	0.013	0.032	0.032	0.037
A10	9	6	8	2	7	7	9	8	9		0.037	0.004	0.013	0.023	0.018	0.037	0.042	0.028	0.047	0.023	0.004	0.004	0.018	0.013	0.032	0.032	0.047	0.032	0.027	0.018
A11	1	4	2	6	3	3	2	3	4	8		0.032	0.023	0.023	0.027	0.009	0.013	0.018	0.009	0.013	0.032	0.032	0.027	0.023	0.004	0.013	0.014	0.013	0.018	0.018
A12	8	5	7	3	6	6	8	7	10	1	7		0.009	0.018	0.014	0.032	0.037	0.023	0.042	0.027	0.009	0.009	0.013	0.018	0.027	0.027	0.042	0.027	0.023	0.013
A13	6	7	7	5	6	8	7	8	9	3	5	2		0.009	0.013	0.023	0.037	0.023	0.032	0.027	0.018	0.009	0.023	0.009	0.027	0.027	0.037	0.018	0.032	0.013
A14	4	5	5	5	4	6	5	6	9	5	5	4	2		0.013	0.013	0.027	0.023	0.023	0.027	0.027	0.018	0.013	0.018	0.027	0.018	0.027	0.018	0.032	0.013
A15	5	4	4	6	3	5	5	6	9	4	6	3	3	3		0.018	0.023	0.009	0.027	0.023	0.023	0.023	0.018	0.023	0.023	0.013	0.027	0.023	0.018	0.027
A16	1	4	2	6	1	3	2	3	6	8	2	7	5	3	4		0.013	0.018	0.009	0.013	0.032	0.032	0.018	0.023	0.013	0.013	0.014	0.013	0.018	0.018
A17	2	3	1	7	2	2	2	3	4	9	3	8	8	6	5	3		0.013	0.004	0.018	0.037	0.047	0.023	0.037	0.009	0.009	0.004	0.018	0.013	0.032
A18	3	2	2	6	3	3	3	4	7	6	4	5	5	5	2	4	3		0.018	0.023	0.023	0.032	0.018	0.023	0.013	0.004	0.018	0.013	0.009	0.027
A19	1	4	2	8	3	3	2	3	4	10	2	9	7	5	6	2	1	4		0.023	0.042	0.042	0.027	0.032	0.013	0.013	0.004	0.013	0.018	0.028
A20	4	5	3	3	2	4	4	5	4	5	3	6	6	6	5	3	4	5	5		0.018	0.027	0.023	0.018	0.009	0.018	0.023	0.027	0.023	0.023
A21	8	5	7	1	6	6	8	7	8	1	7	2	4	6	5	7	8	5	9	4		0.009	0.013	0.009	0.027	0.028	0.042	0.027	0.023	0.013
A22	8	7	9	3	8	8	9	8	9	1	7	2	2	4	5	7	10	7	9	6	2		0.023	0.009	0.037	0.037	0.047	0.027	0.032	0.013
A23	5	2	4	2	3	3	5	4	9	4	6	3	5	3	4	4	5	4	6	5	3	5		0.023	0.023	0.014	0.027	0.023	0.018	0.009
A24	6	7	7	3	6	8	7	8	7	3	5	4	2	4	5	5	8	5	7	4	2	2	5		0.027	0.027	0.037	0.018	0.032	0.013
A25	2	3	1	5	2	2	2	3	4	7	1	6	6	6	5	3	2	3	3	2	6	8	5	6		0.009	0.013	0.018	0.013	0.023
A26	2	1	1	5	2	2	2	3	6	7	3	6	6	4	3	3	2	1	3	4	6	8	3	6	2		0.013	0.018	0.013	0.023
A27	2	4	2	8	3	3	1	2	3	10	3	9	8	6	6	3	1	4	1	5	9	10	6	8	3	3		0.018	0.018	0.033
A28	2	5	3	7	4	4	3	4	7	7	3	6	4	4	5	3	4	3	3	6	6	6	5	4	4	4		0.013	0.023	
A29	3	2	2	6	3	1	3	2	7	6	4	5	7	7	4	4	3	2	4	5	5	7	4	7	3	3	4	3		0.027
A30	5	4	6	2	5	5	6	5	8	4	4	3	3	3	6	4	7	6	6	5	3	3	2	3	5	5	7	5	6	

Table 4. 14. ITS2 region F_{ST} analysis results showing pairwise genetic variations between populations. + : $p=0.001$, -: not significant.

	AFY	ART	BAL	BAR	BIN	BIT	BOL	BRS	CAN	CNK	DUZ	EDI	ELA	ERI	ERZ	GIR	GUM	ISP
AFY		-	-	-	+	+	-	-	+	+	+	-	+	+	+	+	-	+
ART	0.68750		-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-
BAL	0.08889	-0.08501		-	+	+	-	-	-	-	-	-	+	+	-	-	-	-
BAR	1.00000	-1.00000	0.17172		-	-	-	-	-	-	-	-	-	-	-	-	-	-
BIN	0.70660	0.46188	0.31888	0.65289		-	+	+	+	+	+	+	+	-	-	+	+	-
BIT	1.00000	0.79452	0.46405	1.00000	0.02665		+	+	+	+	+	+	+	-	-	+	+	+
BOL	0.00000	0.36047	0.00794	0.77778	0.57364	0.81818		-	+	+	+	-	+	+	+	+	-	+
BRS	0.03392	0.12495	-0.02914	0.44048	0.36983	0.52835	-0.01739		-	+	+	-	+	+	+	-	-	-
CAN	0.35435	-0.09005	0.14063	-0.22507	0.37173	0.43122	0.30406	0.23395		-	-	-	-	+	-	+	+	+
CNK	0.28807	-0.08922	0.06798	-0.03904	0.34337	0.41751	0.24206	0.17875	-0.04133		-	-	-	+	-	+	+	+
DUZ	0.76804	-0.20000	0.35005	-1.00000	0.64312	0.76804	0.67290	0.48191	-0.22642	-0.04506		-	-	+	-	+	+	+
EDI	0.14587	0.01302	-0.10068	0.38889	0.40325	0.65221	-0.03664	-0.03295	0.18769	0.11709	0.45368		+	+	-	-	-	-
ELA	0.80907	0.13649	0.20712	0.42857	0.50437	0.80907	0.61098	0.36594	0.17940	0.15512	0.30603	0.33057		+	-	+	+	+
ERI	0.79012	0.55182	0.31250	0.80117	-0.05263	0.05556	0.61111	0.34579	0.37127	0.34028	0.67775	0.43061	0.59108		-	-	+	-
ERZ	0.81221	0.18367	0.12130	0.55556	0.02703	0.56989	0.53566	0.20895	0.19246	0.16122	0.34815	0.22222	0.17981	0.10224		-	-	-
GIR	0.39683	0.27497	0.10515	0.57778	0.11488	0.29630	0.25033	0.06365	0.30590	0.24858	0.57782	0.12317	0.40919	0.05983	0.03614		-	-
GUM	0.21056	0.33651	0.03363	0.70476	0.34996	0.59880	0.06820	-0.02009	0.31659	0.24668	0.65353	0.01705	0.50390	0.33305	0.26506	-0.00389		-
ISP	0.40741	0.27019	0.06863	0.60494	0.15926	0.40741	0.22840	0.05874	0.29630	0.23565	0.59559	0.07764	0.37824	0.12186	0.01060	-0.08025	-0.03006	
IST	0.20106	-0.15922	-0.03556	-0.15709	0.28585	0.37860	0.13863	0.06176	-0.00660	-0.01124	0.04016	0.01843	0.11217	0.28205	0.09155	0.16667	0.15105	0.14566
IZM	0.20370	-0.18806	-0.00794	-0.19444	0.30270	0.38351	0.14875	0.10183	-0.04369	-0.07076	-0.01687	0.03613	0.15392	0.30000	0.12898	0.19262	0.17595	0.17874
KAR	0.68056	0.45792	0.23747	0.73099	-0.01428	0.14815	0.49939	0.25086	0.34705	0.30644	0.64876	0.32359	0.52381	-0.08401	0.04139	-0.02694	0.20049	0.01434
KAS	0.00000	0.71053	0.10159	1.00000	0.71734	1.00000	0.01000	0.04573	0.37084	0.30420	0.78618	0.16304	0.82077	0.79936	0.82470	0.41333	0.22500	0.42390
KAY	0.18594	0.29756	0.00334	0.68000	0.38090	0.63138	0.03375	-0.03066	0.30568	0.23347	0.64104	-0.03085	0.45603	0.37569	0.25069	0.03351	-0.08281	-0.01497
KON	0.06667	-0.10245	-0.09788	0.15152	0.31316	0.45098	-0.01190	-0.05909	0.15249	0.09059	0.34116	-0.09460	0.19355	0.29861	0.10604	0.07785	0.01531	0.05229
KLR	0.47619	0.22705	0.06732	0.54167	0.19622	0.47619	0.28270	0.10470	0.28427	0.22673	0.56811	0.07916	0.23913	0.19485	-0.08381	0.00141	0.06291	-0.06209
KUT	0.13333	-0.04966	-0.08401	0.21212	0.31017	0.45833	0.04762	-0.02206	0.14776	0.07653	0.35127	-0.06719	0.12313	0.29630	0.07173	0.09233	0.03366	0.04514
MAL	1.00000	0.25000	0.14702	1.00000	0.52542	1.00000	0.69595	0.33281	0.15243	0.13442	0.25000	0.31657	-0.13208	0.68204	0.25000	0.40501	0.54084	0.37824
MUS	0.49198	0.19991	0.16898	0.34286	0.12483	0.29464	0.37276	0.17772	0.29114	0.25600	0.45671	0.20191	0.18498	0.12972	-0.10017	0.04378	0.20290	0.06482
ORD	0.87805	-0.14286	-0.02473	0.33333	-0.11628	0.68750	0.51168	0.08263	0.04968	0.01440	0.00000	0.09506	0.04110	-0.00457	-0.60000	-0.11399	0.18145	-0.14198
SAM	0.49920	0.24293	0.05365	0.57500	0.37252	0.64727	0.28780	0.12864	0.29196	0.23183	0.59358	0.05925	0.18891	0.40073	0.06471	0.14631	0.14881	0.06348
SIN	0.00000	0.41696	0.00794	0.80000	0.57928	0.81818	-0.05263	-0.04601	0.30030	0.23661	0.67290	0.00795	0.61098	0.61111	0.53566	0.25033	0.06820	0.22840
SIV	0.78261	0.16176	0.17434	0.50000	0.38563	0.74359	0.57113	0.31827	0.22260	0.18888	0.41425	0.28712	-0.09701	0.47668	0.00000	0.30213	0.42081	0.25888
TOK	0.00000	-1.00000	-0.82222	1.00000	0.45455	1.00000	-1.00000	-0.95833	-0.29129	-0.42387	-0.63636	-0.83333	0.42857	0.58025	0.33333	-0.20635	-0.55000	-0.18519
TUN	1.00000	-0.50000	-0.07190	1.00000	-0.90909	0.00000	0.63636	0.02083	-0.13757	-0.16498	-0.63636	0.18519	0.42857	-0.88889	-0.33333	-0.40741	0.22500	-0.18519
VAN	0.90909	0.69925	0.43827	0.90476	0.02511	0.00000	0.75000	0.47999	0.42119	0.40523	0.72421	0.59173	0.72856	0.03704	0.39249	0.23203	0.53133	0.34921
YLV	0.26820	-0.16913	0.05797	-0.28620	0.32224	0.39365	0.21013	0.15626	-0.07921	-0.04778	-0.11216	0.09842	0.13858	0.32026	0.14185	0.23360	0.23160	0.22082
YOZ	0.00000	-1.00000	-0.82222	1.00000	0.45455	1.00000	-1.00000	-0.95833	-0.29129	-0.42387	-0.63636	-0.83333	0.42857	0.58025	0.33333	-0.20635	-0.55000	-0.18519
ZON	0.00000	0.25000	-0.10612	1.00000	0.58621	1.00000	-0.18421	-0.16209	0.15243	0.08401	0.40000	-0.08962	0.63746	0.68204	0.61290	0.19775	0.01635	0.20923

Table 4. 14 (cont'd).

	IST	IZM	KAR	KAS	KAY	KON	KLR	KUT	MAL	MUS	ORD	SAM	SIN	SIV	TOK	TUN	VAN	YLV	YOZ	ZON
AFY	-	-	+	-	-	-	+	-	+	+	+	+	-	+	-	-	+	+	-	-
ART	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
BAL	-	-	+	-	-	-	-	-	-	+	-	-	-	+	-	-	+	-	-	-
BAR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BIN	+	+	-	+	+	+	+	+	+	-	-	+	+	+	-	-	-	+	-	+
BIT	+	+	-	+	+	+	+	+	+	+	-	+	+	+	-	-	-	+	-	+
BOL	-	-	+	-	-	-	+	-	+	+	+	+	-	+	-	-	+	+	-	-
BRS	-	-	+	-	-	-	-	-	+	+	-	+	-	+	-	-	+	-	-	-
CAN	-	-	+	+	+	-	+	-	-	+	-	+	+	-	-	-	+	-	-	-
CNK	-	-	+	+	+	-	+	-	-	+	-	+	+	+	-	-	+	-	-	-
DUZ	-	-	+	+	+	+	+	+	-	-	-	+	+	-	-	-	+	-	-	-
EDI	-	-	+	+	-	-	-	-	-	+	-	-	-	+	-	-	+	-	-	-
ELA	-	-	+	+	+	+	-	-	-	+	-	-	+	-	-	-	+	-	-	+
ERI	+	+	-	+	+	+	-	+	+	-	-	+	+	+	-	-	-	+	-	+
ERZ	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+
GIR	+	+	-	+	-	-	-	-	+	-	-	-	+	+	-	-	+	+	-	-
GUM	-	+	-	-	-	-	-	-	+	+	-	-	-	+	-	-	+	+	-	-
ISP	-	+	-	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-
IST		-	+	+	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-
IZM	-0.07452		+	+	+	-	+	-	-	+	-	+	-	-	-	-	+	-	-	-
KAR	0.23885	0.26052		+	+	+	-	+	+	-	-	+	+	+	-	-	-	+	-	+
KAS	0.21614	0.21883	0.69315		-	+	+	+	+	+	+	+	-	+	-	-	+	+	-	-
KAY	0.13270	0.16342	0.24763	0.20000		-	-	-	+	+	-	-	-	+	-	-	+	+	-	-
KON	-0.01997	0.01235	0.22295	0.07876	-0.01543		-	-	-	+	-	-	-	-	-	-	+	-	-	-
KLR	0.13371	0.17770	0.09823	0.49231	0.03679	0.05109		-	-	-	-	-	+	-	-	-	+	+	-	-
KUT	-0.02599	0.02191	0.22068	0.14712	-0.00590	-0.08434	0.02116		-	+	-	-	-	-	-	-	+	-	-	-
MAL	0.06636	0.11401	0.58080	1.00000	0.48184	0.13043	0.18919	0.07363		-	-	-	+	-	-	-	+	-	-	-
MUS	0.17515	0.22105	0.09564	0.50714	0.19493	0.13120	0.02113	0.12800	0.10071		-	-	+	-	-	-	+	+	-	+
ORD	-0.06703	-0.02371	-0.09375	0.88832	0.16286	-0.04338	-0.26246	-0.08355	0.25000	-0.28751		-	+	-	-	-	-	-	-	-
SAM	0.13148	0.18254	0.30063	0.51429	0.08101	0.03615	-0.04627	-0.00595	0.11094	0.10079	-0.06888		+	-	-	-	+	+	-	-
SIN	0.13066	0.15556	0.49939	0.01000	0.03375	-0.01190	0.28270	0.03030	0.69595	0.36423	0.51168	0.28780		+	-	-	+	+	-	-
SIV	0.13051	0.17191	0.40351	0.79471	0.37370	0.15994	0.10241	0.10692	-0.15385	0.07948	-0.16418	0.08179	0.57113		-	-	+	-	-	+
TOK	-0.59788	-0.59259	0.36111	0.00000	-0.60000	-0.86667	-0.04762	-0.73333	1.00000	0.01429	0.00000	0.02857	-1.00000	0.42857		-	-	-	-	-
TUN	-0.24280	-0.23297	-0.70370	1.00000	0.28889	-0.09804	-0.04762	-0.08333	1.00000	-0.38000	-1.00000	0.32000	0.63636	0.33333	1.00000		-	-	-	-
VAN	0.36508	0.37153	0.11111	0.91354	0.56719	0.41948	0.41667	0.43137	0.85623	0.22740	0.38596	0.58737	0.75000	0.65079	0.81818	-1.00000		+	-	+
YLV	-0.05556	-0.08423	0.28809	0.28416	0.21906	0.06843	0.21238	0.06597	0.11190	0.23873	-0.00927	0.21761	0.21547	0.17972	-0.46360	-0.21270	0.38272		-	-
YOZ	-0.59788	-0.59259	0.36111	0.00000	-0.60000	-0.86667	-0.04762	-0.73333	1.00000	0.01429	0.00000	0.02857	-1.00000	0.42857	0.00000	1.00000	0.81818	-0.4636		-
ZON	-0.00177	0.00077	0.53410	0.00000	-0.00763	-0.12601	0.28571	-0.06557	1.00000	0.31498	0.64706	0.32328	-0.18421	0.61783	0.00000	1.00000	0.85623	0.06403	0.00000	

Several schemes of grouping for the AMOVA analyses for the ITS2 region were tested, and few results were presented in Figure 4.5 a, b, and c. The highest genetic variation (48.32%) was found in the first trial where populations were grouped into two clusters as Group1: Düzce, Group2: all other populations (Figure 4.5 a). In the second trial scheme (Figure 4.5 b), there was still a very high amount of genetic variation (41,54%) when all populations were grouped into three groups (Group1: Bartın, Group2: Düzce, and Group3: all other populations). One group test (Figure 4.5 c) where all populations were included as separate groups indicated that 22, 46% of genetic variation was divided among populations meanwhile 77.54% of diversity was observed within populations.



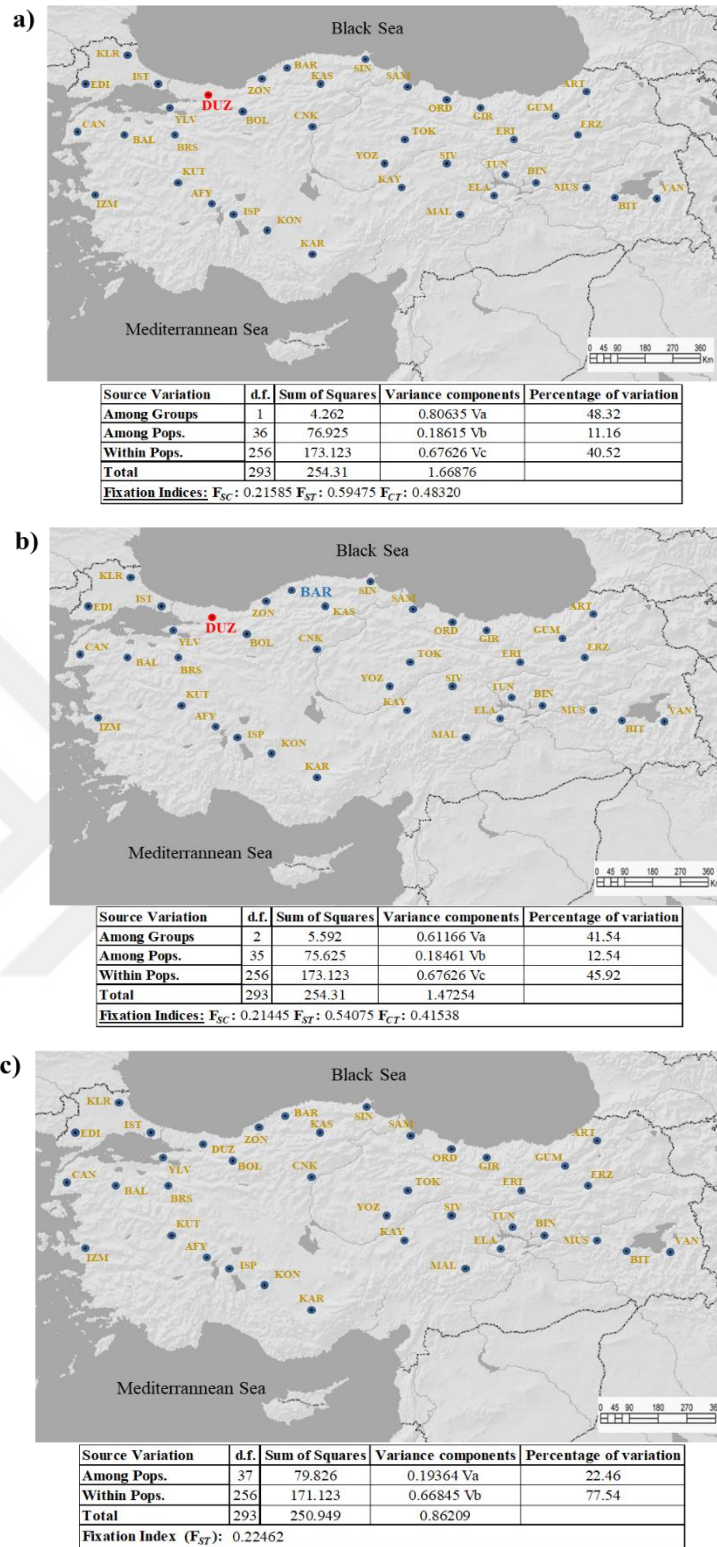


Figure 4.5. AMOVA analysis of ITS2 data. a) Düzce vs. other populations c) 38 population comparison b) Düzce and Bartın vs. other populations (color coded on the map).

4.3 Demographic Structure of *C. quercusfolii* Populations Based on ITS2 Region

The Harpending's raggedness index calculated for *C. quercusfolii* ITS2 region was $r = 0.1940$, but it was not significant. When populations were checked individually Isparta, Konya, Muş, and Samsun populations' H_{ri} values were statistically significant ($H_{ri} = 0.0360^*$). Fu's F_s ($-107,743$, $P < 0.00$) and Tajima's D ($-1,09653$, $P > 0.10$) values calculated for all populations were not statistically significant. In addition to overall estimations covering all localities, each population was also searched for the significant estimates where several populations were either positively or negatively significant (Table 4.15). Bursa population showed a significant positive value (Fu's $F_s = 0.8670$) but the Tajima's D was negatively significant (Tajima's $D = -1.2298$). In Çanakkale population both Fu's F_s (Fu's $F_s = 1.4503$) and Tajima's D (Tajima's $D = 2.1972$) were positive and significant. Similarly, İstanbul (Fu's $F_s = 1.8374$), İzmir (Fu's $F_s = 0.8939$), and Yalova (Fu's $F_s = 0.0035$) populations indicated positive and significant Fu's F_s values. Remaining populations did not produce significant estimates.

Mismatch distribution analysis for all samples produced a multimodal graph indicating demographic equilibrium (Figure 4.6). However, individual mismatch distribution analyses conducted for each population separately showed differences. Balıkesir, Bursa, Çanakkale, Çankırı, İstanbul, İzmir, Kırklareli, Kütahya, and Yalova populations indicated multimodal profile, however Artvin, Bingöl, Bolu, Düzce, Edirne, Elazığ, Erzincan, Erzurum, Giresun, Gümüşhane, Isparta, Karaman, Kayseri, Konya, Muş, Ordu, Samsun, Sinop, Sivas, and Van populations showed unimodal profile (Figure 4.7).

Table 4. 15. Fu's F_S ve Tajima's D values calculated for each *C. quercusfolii* population for ITS2. * $P \leq 0.01$, ** $P \leq 0.001$.

Pop.	Fu's F_S	Tajima's D	Hri	SSD	Pop.	Fu's F_S	Tajima's D	Hri	SSD
AFY	0.0000	0.0000	0.0000	0.0000	IZM	0.8939*	0.8820	0.2000	0.0821
ART	1.0986	0.0000	0.0000	0.0000	KAR	-0.7393	1.1417	0.2617	0.0245
BAL	0.3876	-1.1163	0.1506	0.3654	KAS	0.0000	0.0000	0.0000	0.0000
BAR	0.000	0.0000	0.0000	0.0000	KAY	-0.4224	1.6998	0.3652	0.0466
BIN	-1.5898	-1.1790	0.8830	0.0015	KLR	0.4402	-1.4664	0.1530	0.0407
BIT	0.0000	0.0000	0.0000	0.0000	KON	-0.1005	2.7513	0.7555**	0.1387
BOL	0.5862	-1.4008	0.7200	0.0514	KUT	1.6373	-1.2685	0.2261	0.0577
BRS	0.8670*	-1.2298*	0.2878	0.2608	MAL	0.0000	0.0000	0.0000	0.0000
CAN	1.4503*	2.1972*	0.1585	0.1210	MUS	-1.4990	0.7695	0.2899*	0.0554
CNK	-1.3873	0.9126	0.0483	0.0177	ORD	0.0000	0.0000	0.0000	0.0000
DUZ	2.1972	0.0000	0.0000	0.0000	SAM	-0.3140	1.9569	0.4380*	0.0627
EDI	-1.3898	-0.6540	0.0952	0.00673	SIN	-1.1639	-1.4008	0.1827	0.0057
ELA	1.0404	-0.9725	0.6800	0.3199	SIV	-0.8584	-1.1319	0.2400	0.0373
ERI	-1.2626	0.1759	0.2024	0.0054	TOK	0.0000	0.0000	0.0000	0.0000
ERZ	0.5400	1.6329	0.5555	0.0898	TUN	0.0000	0.0000	0.0000	0.0000
GIR	-1.2304	0.6273	0.2987	0.0512	VAN	-0.3393	-1.1117	0.4000	0.3310
GUM	-0.47889	1.57128	0.3338	0.0399	YLV	0.0035*	1.4631	0.0696	0.0219
ISP	-0.1561	2.5903	0.6839*	0.1208	YOZ	0.0000	0.0000	0.0000	0.0000
IST	1.8374*	0.8050	0.2459	0.1134	ZON	0.0000	0.0000	0.0000	0.0000

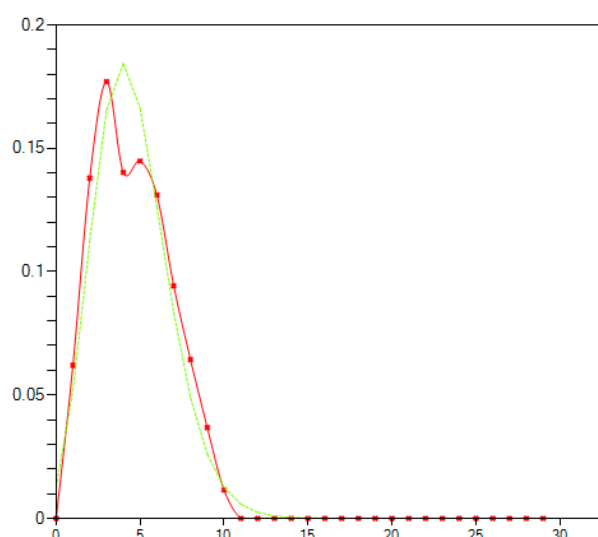


Figure 4.6. Mismatch distribution of all pairwise combinations for the ITS2 alleles. The observed distribution represented by red line, and the expected frequencies depicted by dotted green line.

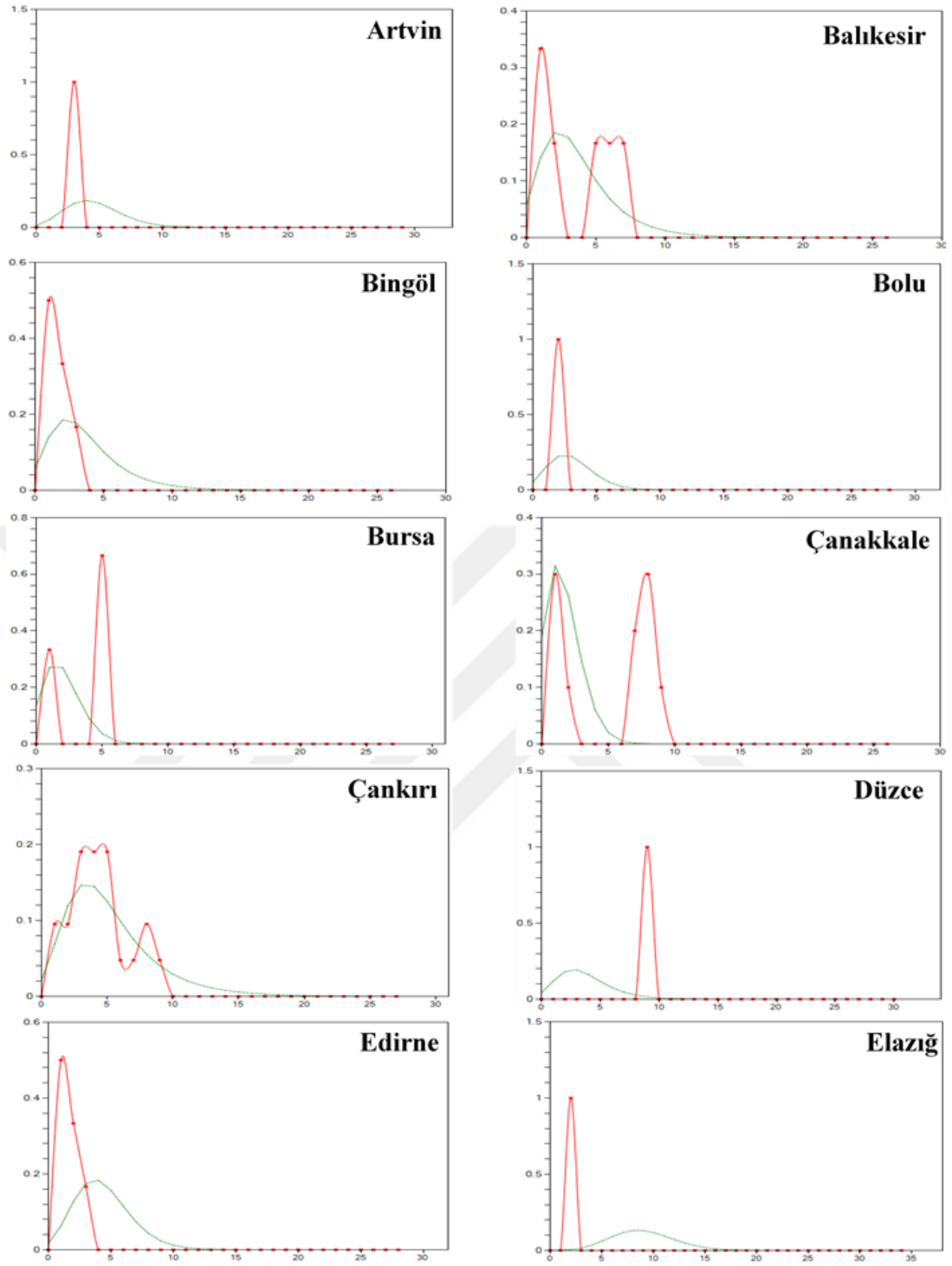


Figure 4.7. Mismatch distribution of populations with their own haplotypes for ITS2 data set. The observed distribution represented by red line, and the expected frequencies depicted by green line.

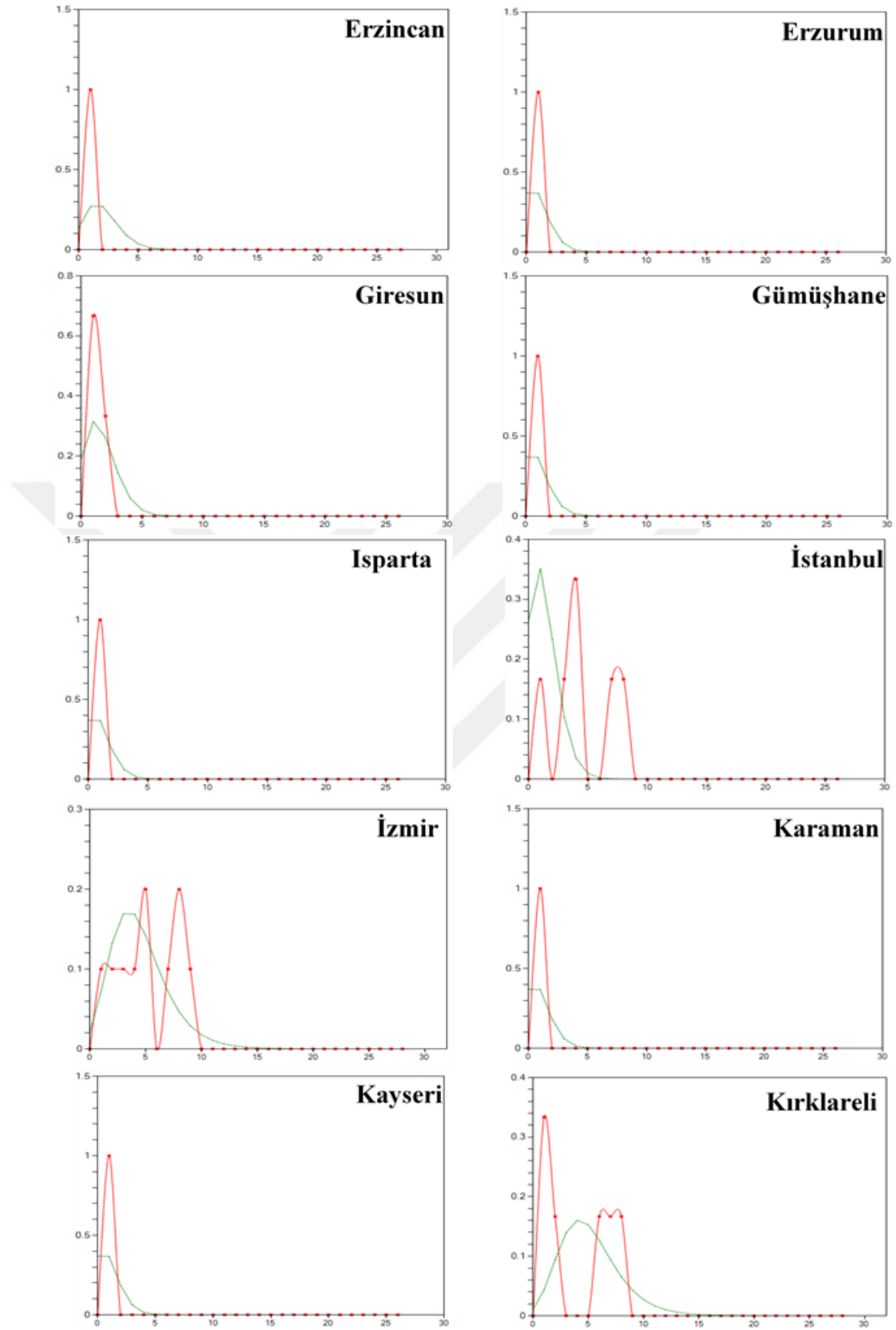


Figure 4.7 (cont'd).

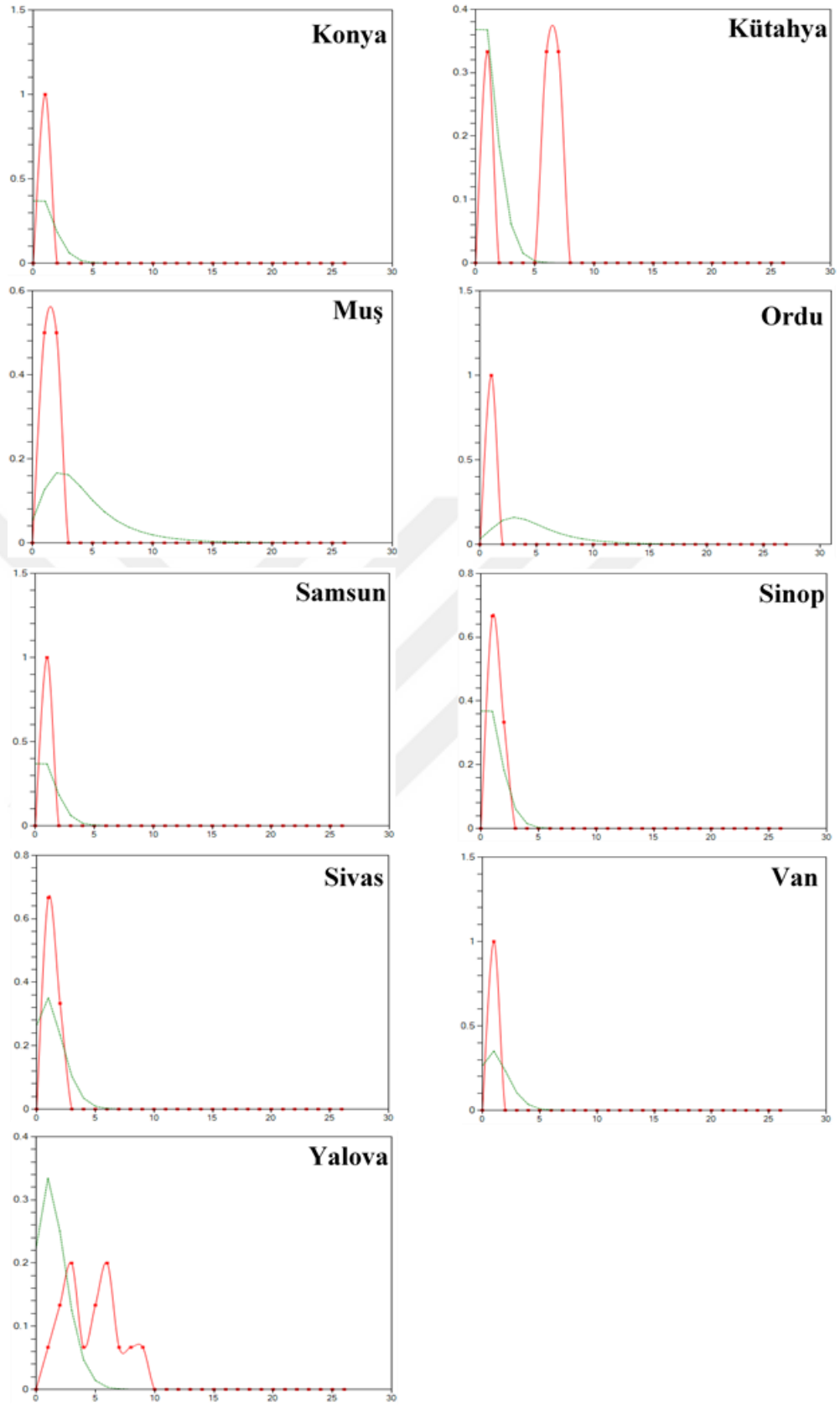


Fig 4.7 (cont'd).

The results of the spatial genetic autocorrelation analysis across the study area for distance classes of 100 and 150 km with 95% confidence intervals were shown in Figure 4.8. The correlogram for the 100 km-distance class size showed positive and significant values of r at 100 km ($r = 0.157$, $p < 0.001$) and 500 km ($r = 0.013$, $p < 0.001$), with an x-axis intercept at 543,7 km (Figure 4.8a). The correlogram for the 150 km distance class size detected positive and significant values of r at 150 km ($r = 0.139$, $p < 0.001$) and 450 km ($r = 0.029$, $p < 0.001$) but significantly negative values for distances beyond the x-axis intercept 555,7 km (Figure 4.8b). These results imply that populations of *C. quercusfolii* separated by distances of less than 100 km are more genetically similar to each other are higher than expected by chance, while genetic differentiation between populations separated by more than ca. 500 km is higher than expected by chance.

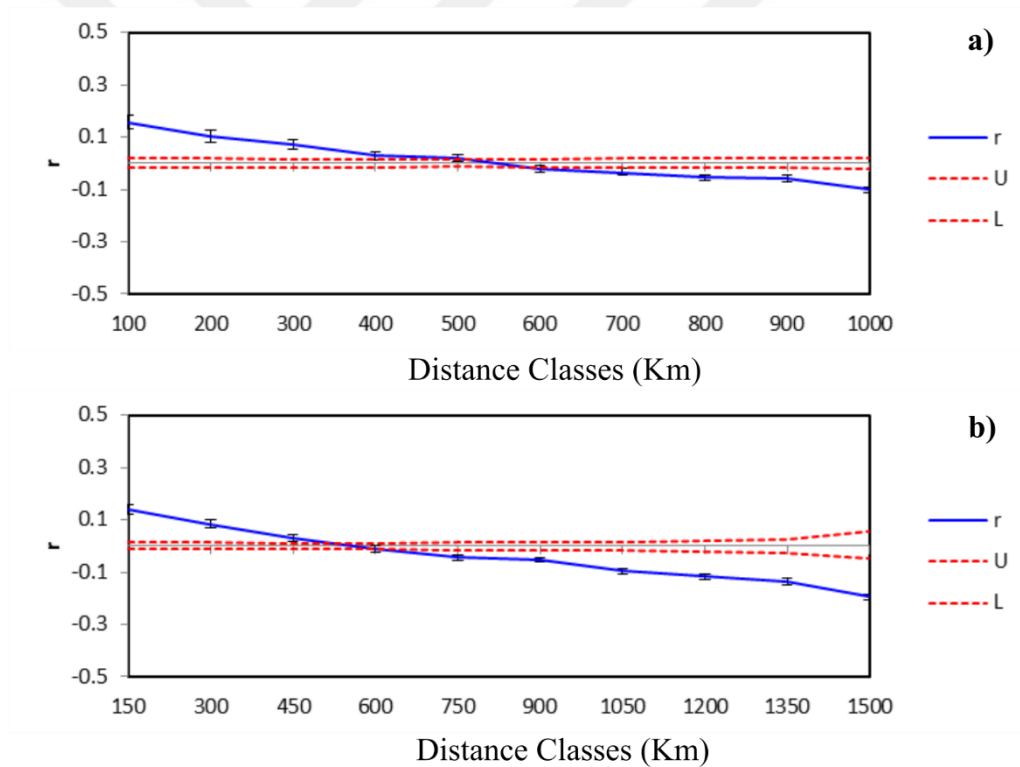


Figure 4.8. Correlograms showing spatial genetic correlation coefficient (r) as a function of geographic distances in the entire study area based on ITS2 data. a) 100 km distance class, b) 150 km distance class. Dashed lines indicate upper (U) and lower (L) boundaries of 95% confidence intervals under the null hypothesis of no geographic structure is present. 95% bootstrapped confidence intervals are shown around each of the calculated r value.

4.4 Phylogenetic and Phylogeographic Analyses on *C. quercusfolii* mtDNA cyt b Gene Analysis

4.4.1 Maximum Parsimony and Maximum Likelihood Analysis Results

Maximum parsimony, maximum likelihood, and the Bayesian approach were performed using 125 *C. quercusfolii* haplotypes, and 5 distinct haplotype sequences representing two outgroup species were included in all analyses to designate the character states of ingroup haplotypes. Maximum parsimony analysis produced 281 equally parsimonious trees, a 50% majority rule consensus tree was provided in Figure 4.9. The consistency index (CI) of the consensus tree was 0.423, the retention index (RI) was 0.828, and homoplasy index (HI) was 0.577. All branches in the phylogenetic tree were supported by high bootstrap values. In MP tree H117 from Sivas population was the most basally located haplotype which was sister to A and B (subclade B1 + subclade B2) clades. Clade A basal to both B clades contained haplotypes sampled from Karaman, Konya, Afyon, Kayseri, Erzurum, Giresun, Malatya, Isparta, İzmir, and Bursa. B clade divided into two subclades as B1 and B2. B1 subclade was comprised of Bolu, Giresun, Kırklareli, Bitlis, Muş, Sivas, Bingöl, Elazığ, Malatya, Kayseri, Muş, Tunceli, Van, Gümüşhane, Karaman, Erzurum, Artvin, Tokat, Erzincan, Ordu, Samsun, and Sinop. B2 subclade was İstanbul, Kırklareli, Samsun, Zonguldak, Karaman, Düzce, Çankırı, Ordu, Sinop, Bursa, Afyon, İzmir, Kütahya, Konya, Yalova, Edirne, Balıkesir, Bolu, Kastamonu, Bartın, Çanakkale, and Yozgat.

JModeltest determined HKY+I+G mutation model as the best fit model to the cyt b data set with $-\ln L = 1959.4367$ with AICc value 5154.6262. Transition/transversion ratio was set to $ti/tv = 6.2386$ where $p\text{-inv} = 0.5880$, and the gamma shape parameter was 0.5280. All values were introduced into the ML analysis implemented in the PAUP program, and a consensus tree of 131 maximum likelihood trees is given in Figure 4.10. The 50% majority rule consensus tree was with $CI = 0.420$, $RI = 0.825$, and $HI = 0.579$. Most of the branches were supported by high bootstrap values. The cyt b haplotypes formed two main clades, of which the basally located clade was composed of haplotypes from Konya, Giresun, Kayseri,

Bursa, Erzurum, Isparta, Malatya, and İzmir. The sister clade was well-structured where the H117 from Sivas was the most basal haplotype to all other haplotypes.

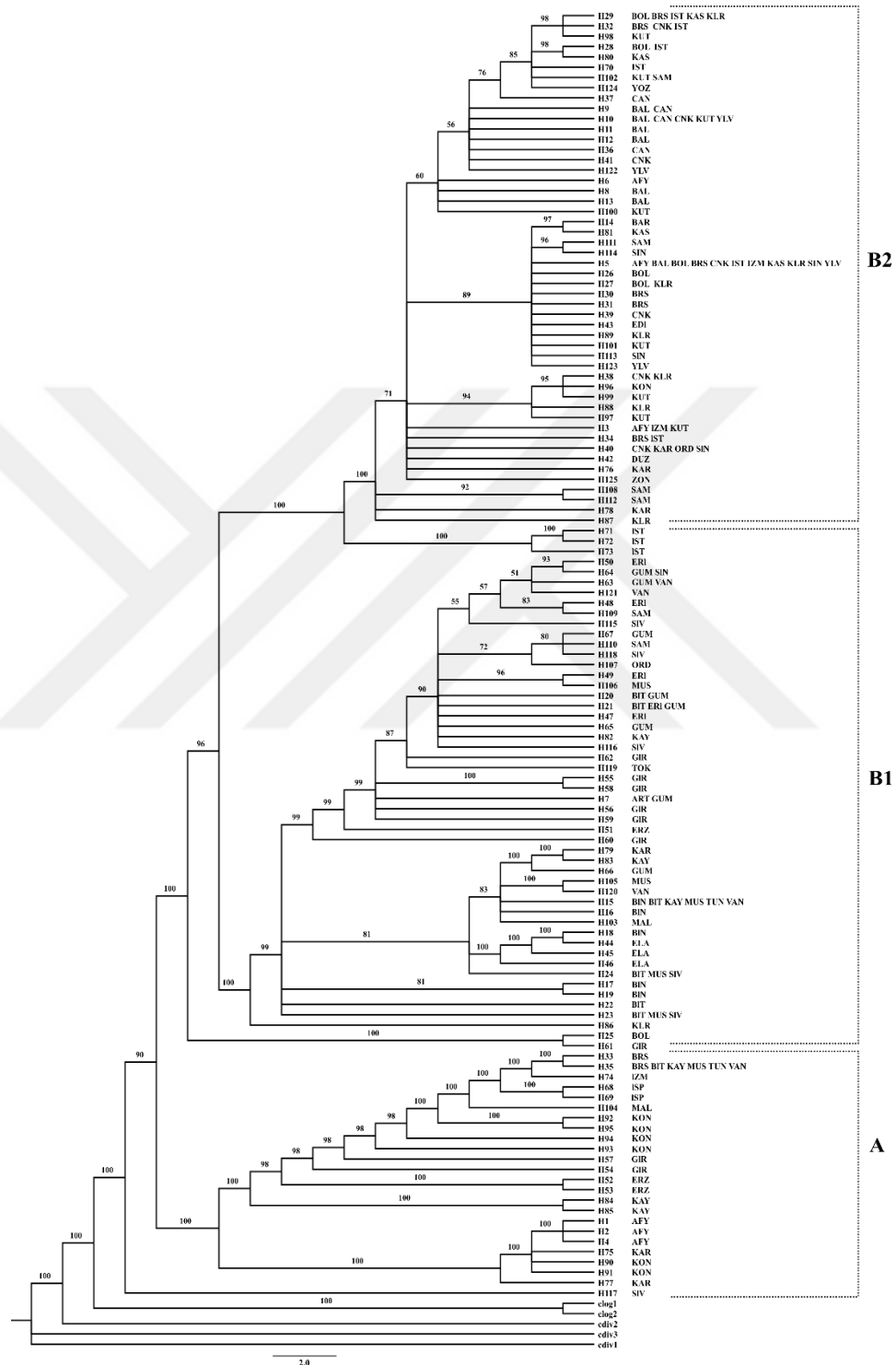


Figure 4.9. Maximum parsimony 50% majority rule consensus tree of the cyt b haplotypes of *C. quercusfolii*. The consensus tree is a resulting tree of 281 subtrees with a consistency index CI= 0.423 and RI= 0.828. Supporting bootstrap values are shown over relevant branches.

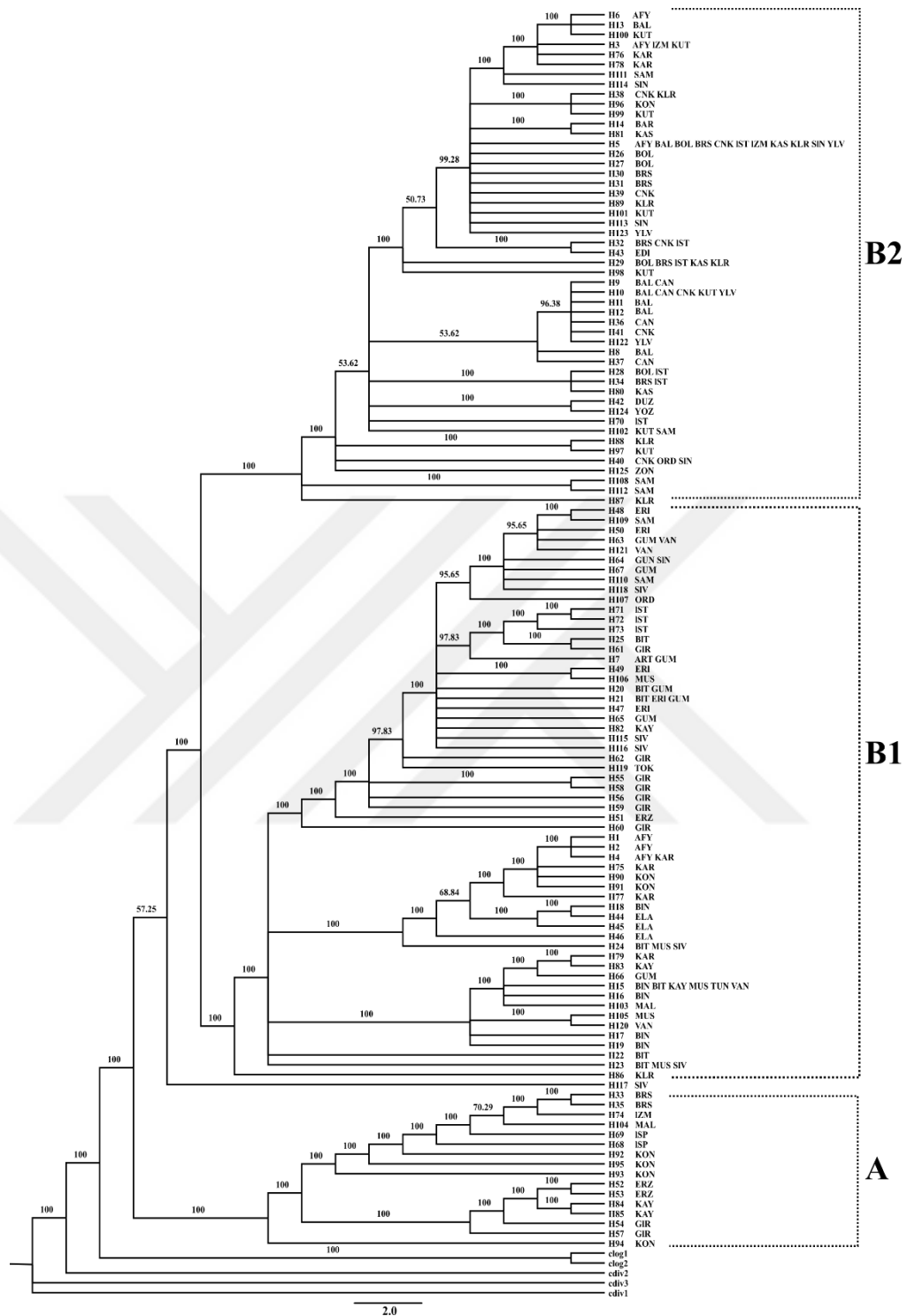


Figure 4.10. Maximum Likelihood 50% majority rule consensus tree of the cyt b haplotypes of *C. quercusfolii* ($-\ln L = 1959.4367$). The consensus tree is a resulting tree of 131 subtrees with a consistency index $CI = 0.420$ and $RI = 0.825$. The bootstrap values over 50% support are shown on relevant branches.

The remaining haplotypes further formed two main clades (Subclades B1 + B2); the basal subclade (subclade B1) was consisted of haplotypes from Samsun, Kırklareli, Zonguldak, Kütahya, Çankırı, Karaman, Ordu, Sinop, İstanbul, Yozgat, Düzce, Kastamonu, Bursa, Bolu, Çanakkale, Yalova, Balıkesir, Edirne, Konya, Bartın, Afyon and İzmir. The subclade B2 had a Kırklareli haplotype (H86) at its the most basal part. The remaining haplotypes represent Bitlis, Van, Muş, Bingöl, Malatya, Kayseri, Karaman, Gümüşhane, Kayseri, Tunceli, Erzurum, Giresun, Tokat, Sivas, Erzincan, Ordu, Samsun, İstanbul, Artvin, Elazığ, Karaman, Konya, and Afyon.

A minimum spanning network to resolve possible reticulations and to display evolutionary relationships among haplotypes was presented in Figure 4.11. The color-coded populations and the percentages of the haplotypes shared between/among populations were indicated in the network. Likewise, reticulate events and hypothetical haplotypes connecting presently detected haplotypes have been shown in the network. Some notorious star shaped haplotype groupings were well-marked; H24, a haplotype shared among Bitlis, Muş, and Sivas, was connected to haplotype H15 from Bingöl, Bitlis, Kayseri, Muş, Tunceli, and Van (connected to mostly eastern based haplotypes in one haplogroups). Meanwhile, H24 was also connected to H46 from Elazığ population which was further connected to an Elazığ haplotypes then it was connected to two hypothetical haplotype groups; one of which was linked to two eastern haplotypes in an order of haplotypes from Elazığ and Bingöl. On the other hand, H46 was also connected to some hypothetical haplotype lines leading to Karaman (H77) and a centrally placed haplotype (H91) from Konya population. H40 was the central haplotype to some other haplotypes from Karaman, İzmir, Kütahya, Konya, and Afyon. The other remarkable star phylogeny includes H23 (found in Bitlis, Muş, and Sivas) which was directly connected to H24 (an eastern originated haplotype represented in Bitlis, Muş, and Sivas). H23 was connected to a large star shaped haplogroup, of which, after a hypothetical haplotype, H20 (found in Bitlis and Gümüşhane) was further connected to H21 (from Bitlis, Erzincan, and Gümüşhane). In this haplotype group H21 and H63 (from Van and Gümüşhane) were connected to haplotypes detected mostly in the eastern and northeastern part of Anatolia.

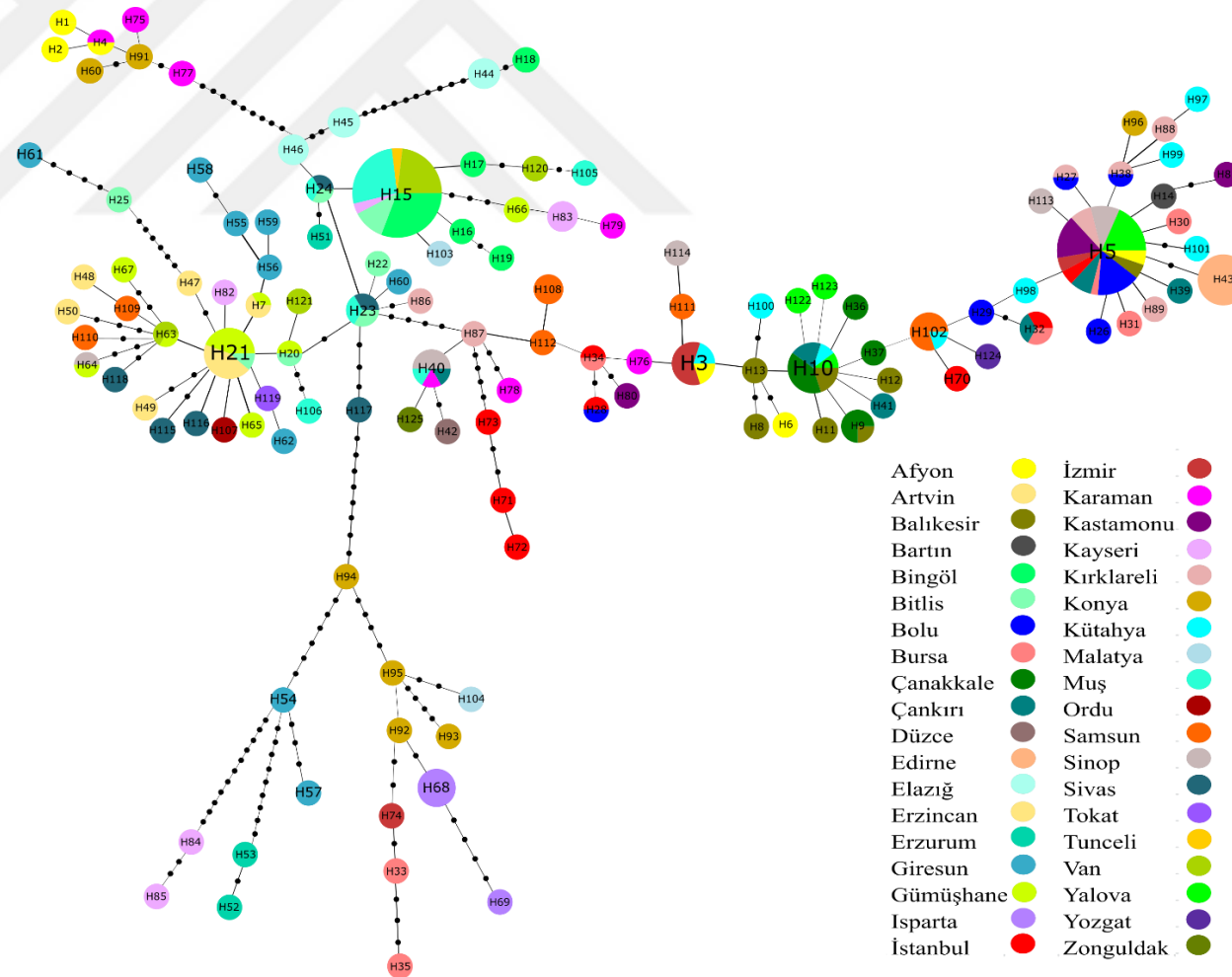


Figure 4.11. Results of the network analysis that was produced by Hapstar 0.7 from the mitochondrial Cyt B haplotypes. Color-coded populations are shown in the circles.

H23 was also connected to large lineage groups through some hypothetical haplotypes. In one of the haplotype group three hypothetical haplotypes were linked to a Sivas haplotype (H117) which was connected to some other haplotypes via a Konya haplotype (H94) and then to haplotypes mainly from Konya, Bursa, İzmir, Isparta, Giresun, Malatya, Kayseri, and Erzurum. But the appearance of loop indicating a reticulation in this haplotype group require further attention. There may be a convergent or a hybridization event went along this line. The second connection part to H23 via several hypothetical haplotypes was to H87 (from Karaman, Ordu, and Çankırı). This haplotype was connected to H10 through some haplotypes. On this site of the haplotype network two major haplogroups were notorious; the first one having H10 as the central haplotype to all other haplotypes was largely from the western part of Turkey. The second haplogroup carrying H5 at the center was also consisted of 16 unique haplotypes all from the central and western part of Turkey.

4.4.2 Bayesian Analysis Results and Times of Divergence

Obtained divergence times and posterior probability values were shown on the branches of the tree. The resulting tree was given in Figure 4.12. The ingroup haplotypes were diverged from the outgroup haplotypes near 4.91 MYA (95% HPD interval 1.8694-5.8393). The ingroup haplotypes were divided into two sister clades (Clade A+B) around 3.51 MYA (Piacenzian glaciation age). The A clade was composed of haplotypes from Bingöl, Elazığ, Konya, Erzurum, Kayseri, Giresun, Bursa, Isparta, İzmir, and Malatya. In this clade, after the first divergence from the sister clade (Clade B), a series of divergences have taken place ranging from 2.51 MYA (Gelasian glaciation age) to 150.000 YA (in the end of Riss glacial period). These chains of events resulted in several subsequent divergences in lineage A which began in pre-Pleistocene, and continued through the Pleistocene. The first radiation started around the Gelasian glaciation ended up with the separation of the two basal haplotypes (H44 and H18) from Bingöl and Elazığ from the rest of the haplotypes.

The B clade diverged around 2.29 MYA (Early Gelasian age) into two subclades where the basal one was composed of only a single haplotype from Sivas (H117). Nearly 2.02 MYA (Late Gelasian age) the second in-clade divergence

occurred resulting in two large subclades (B1+B2). The subclade B1 included a small basal clade composed of haplotypes from Afyon, Konya, and Karaman. The larger part of the B1 clade was composed of haplotypes from Bitlis, Kırklareli, Muş, Sivas, Elazığ, Bingöl, Van, Malatya, Tunceli, Gümüşhane, Karaman, Kayseri, Erzincan, Samsun, Tokat, Giresun, Erzurum, Ordu, and Artvin.

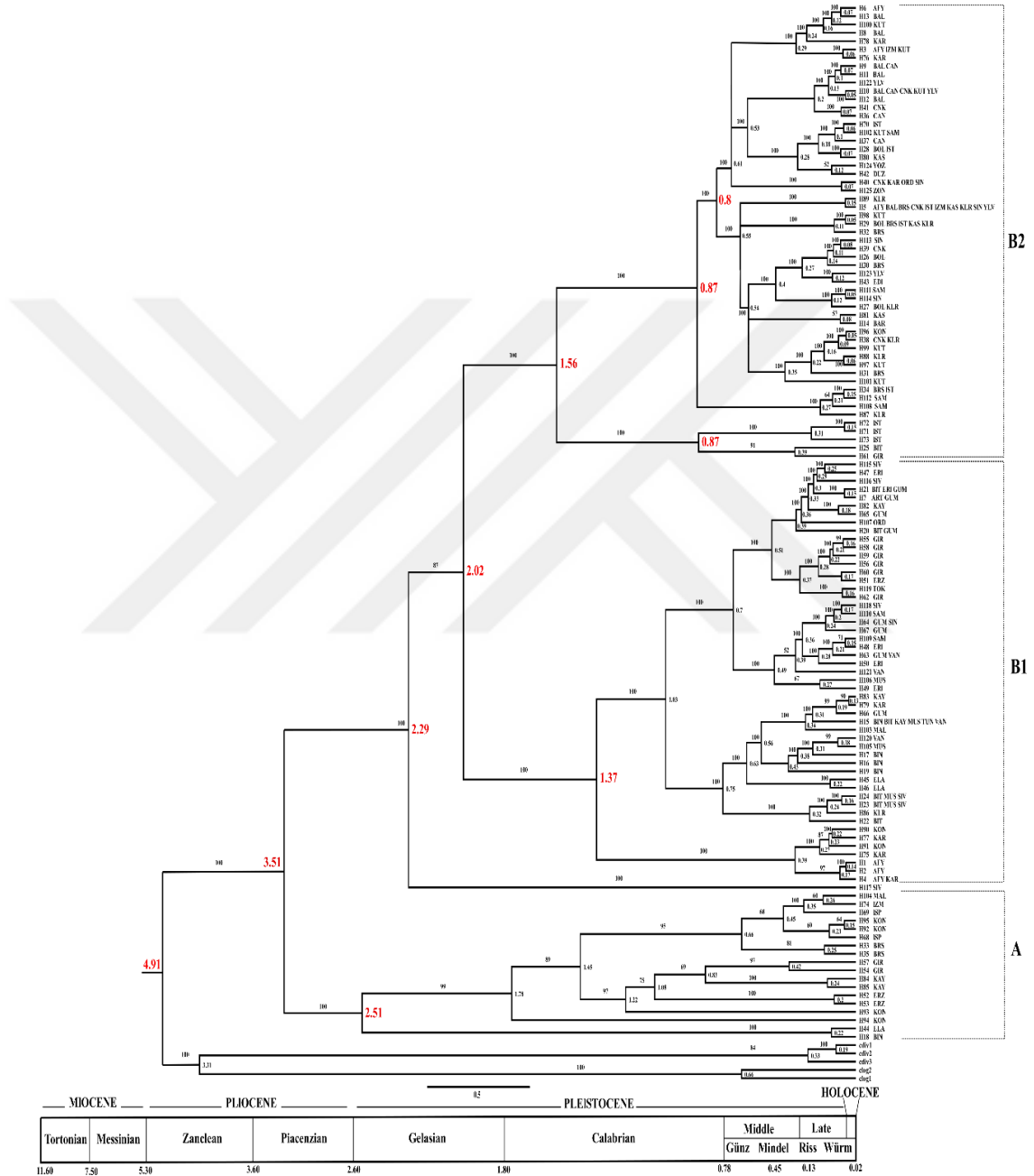


Figure 4.12. Beast consensus tree of cytb haplotypes with the node ages (tMRCA) and posterior probabilities are shown the interior part of each related node. Posterior probability values are also given at nodes.

Ongoing diversification events (from 1.37 MYA to 130.000 YA) along this subclade seem to take place from middle to Late Calabrian (1.8 MYA-780.000 YA) through Early Riss (130.000 YA). The B2 subclade seems to diverge around 1.56 MYA (Middle Calabrian age) was also composed of two clades. The basal one was diverged nearly 870.000 YA (Late Calabrian) consisted of haplotypes representing Bitlis, Giresun, and İstanbul. The second clade was also divided into two around 870.000 YA (Late Günz): the first haplogroup includes Bursa, İstanbul, Samsun, and Kırklareli haplotypes while the second consists of Afyon, Balıkesir, Bolu, Bursa, Çanakkale, İstanbul, İzmir, Kastamonu, Kırklareli, Sinop, Yalova, Çankırı, Kütahya, Samsun, Edirne, Bartın, Karaman, Konya, Zonguldak, Ordu, Düzce, and Yozgat. Ongoing events started around Late Calabrian (87.000 YA) to Late Würm (50.000 YA) appears to affect all other minor diversification events along the members of this lineage from middle to particularly late Pleistocene.

4.5 nDNA ITS2 Allele Analysis

Alleles of *C. quercusfolii* ITS2 region were used for maximum parsimony, maximum likelihood, and Bayesian analysis with the same outgroups. Thirty alleles produced 40 parsimonious trees, and the consensus tree generated through 50% majority rule was shown in Figure 4.13. The consensus tree is with CI= 0.350, RI= 0.726, and HI= 0.650. Mostly polytomic structure was observed in the tree; 14 alleles found in Yalova, Muş, İstanbul, Giresun, Kırklareli, Düzce, Çankırı, Edirne, Çanakkale, Sinop, Bingöl, Bartın, Bolu, Balıkesir, and İzmir were placed in the large polytomic part. In addition, geographically the most largely distributed alleles were also placed in this polytomic part: A1 (N= 128) was found as a shared common allele in Afyon, Artvin, Balıkesir, Bolu, Bursa, Çanakkale, Çankırı, Edirne, Erzincan, Giresun, Gümüşhane, Isparta, İstanbul, İzmir, Karaman, Kastamonu, Kayseri, Kırklareli, Konya, Kütahya, Muş, Samsun, Sinop, Tokat, Yalova, Yozgat, and Zonguldak. A7 (N= 71) was shared allele among Bingöl, Bitlis, Erzincan, Erzurum, Giresun, Gümüşhane, Isparta, Karaman, Kayseri, Konya, Muş, Ordu, Samsun, Sivas, Tunceli, and Van. A3 (N= 49) was found in Balıkesir, Bingöl, Bursa, Çanakkale, Çankırı, Edirne, Elazığ, Erzincan, Erzurum, Giresun, Gümüşhane, Isparta, İstanbul, Karaman, Kayseri, Kırklareli, Konya, Kütahya, Malatya, Muş, Ordu, Samsun, Sinop,

Sivas, and Yalova. In the polytomic part two alleles from Bursa and Van formed a monophyletic group. The only main clade was generated in the MP tree includes alleles from Sivas, Elazığ, Artvin, İzmir, Yalova, Balıkesir, Kırklareli, İstanbul, Çanakkale, Çankırı, Düzce, and Kütahya. A remarkable point of this clade was its inclusion of two alleles from Elazığ and Sivas at the most basal part of the clade.

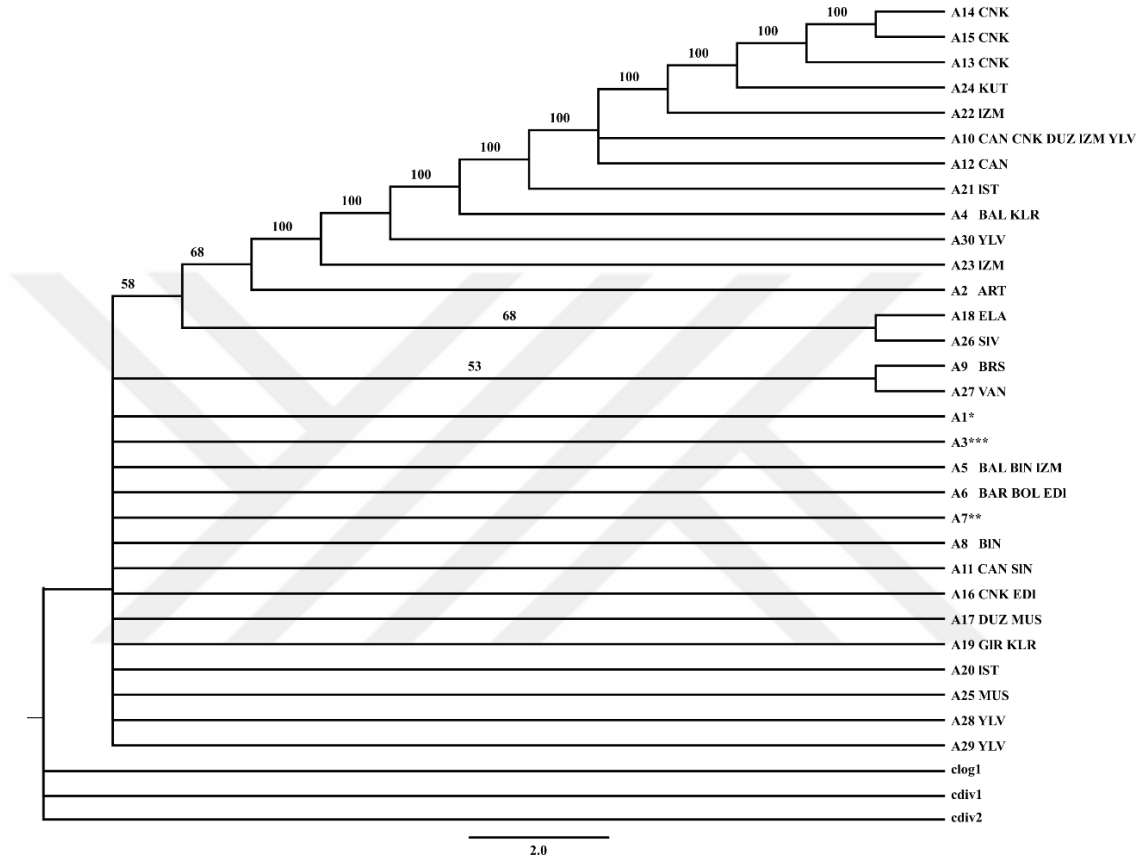


Figure 4.13. Maximum parsimony 50% majority rule consensus tree of the ITS2 alleles of *C. quercusfolii*. The consensus tree is a resulting tree of 40 subtrees with a consistency index CI= 0.350 and RI= 0.726. The bootstrap values that are 50% support are shown over relevant branches.

For ML analysis of the ITS2 region GTR+I was determined as the best fit model to data set with $-\ln L = 497.2462$ with AICc value 1166.419. The ML tree was the consensus tree of 32 best found trees with CI= 0.333, RI= 0.705, and HI= 0.667. The maximum likelihood generated a more structured tree compared to maximum parsimony (Figure 4.14). Basal alleles showed polytomy where a Bingöl, and A7 were basal alleles to all others. In the next clade with polytomy alleles from Yalova, Çankırı, Çanakkale, Sinop, Edirne, in addition to common allele A1. In ML tree unlike MP tree A18 and A26 formed a small monotypic group. Likewise, alleles A5, A20, A6, A29 were in the polytomic part of the MP, however they formed small monophyletic groups in the ML tree (A6+A29, A5+A20). Similarly, A18 and A26 alleles were monophyletic in the MP tree, but they were unresolved in the ML. All other allelic relationships were the same with those of MP.

Nuclear ITS2 data set was run for 50 million generations with a sample frequency of 1000 generation and a twenty million generation burn-in and the resulting consensus tree was shown in Figure 4.15. The Bayesian analysis of the ITS2 region produced mostly polytomic relationships among alleles without much resolution. At the basal part of the polytomy there were 19 alleles including the most three common alleles. The small polytomic part included 11 alleles representing Balıkesir, Kırklareli, Çanakkale, Düzce, İzmir, Yalova, Çankırı, İstanbul, and Kütahya.

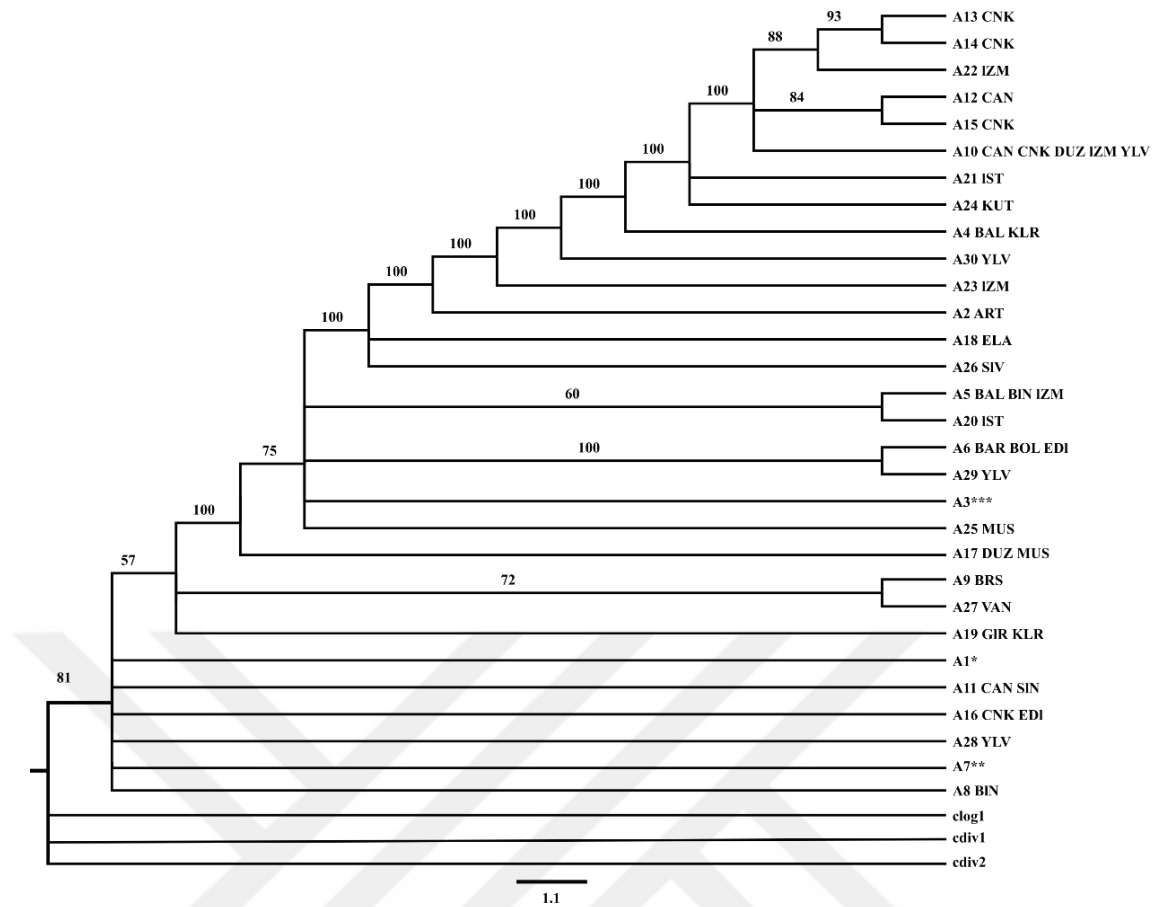


Figure 4.14. Maximum likelihood 50% majority rule consensus tree of the ITS2 alleles of *C. quercusfolii* (-lnL= 497.2462). The consensus tree is a resulting tree of 32 subtrees with a consistency index CI= 0.333 and RI= 0.705. The bootstrap values that are 50% support support are shown over relevant branches.

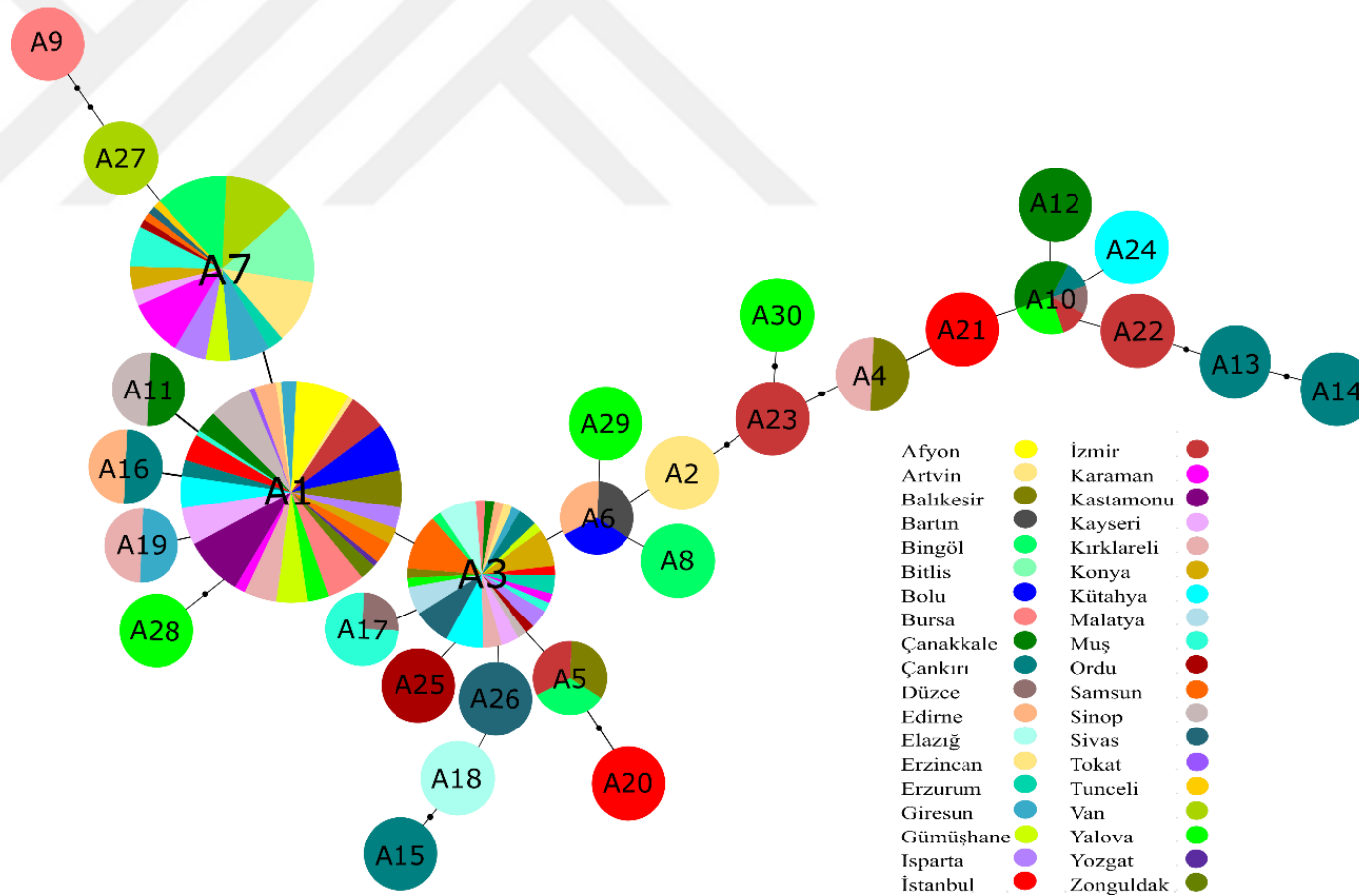


Figure 4.16. Results of the network analysis that was produced by Hapstar 0.7 from the nuclear ITS2 alleles. Color-coded populations are shown in the circles.

5. DISCUSSION

5.1 Genetic Diversity of *C. quercusfolii* in Turkey

Insect mitogenome is anti-G biased, in other words it is rich in A+T nucleotides. Absence of non-sense or stop codons in the protein coding genes of mitogenome are the proves of the sequences being a genuine one, not the pseudogenes (Zhang and Hewitt, 1996). Mitochondrial genome also shows bias toward having more transitional substitutions than transversional mutations (Brown et al., 1979). These characteristic features are used to prove that one's sequence is mitogenome in its origin. In *C. quercusfolii* the percentage of A and T (78%), the absence of both non-sense and stop mutations or indels, and tr/tv ratio (6.2386) proved that all 125 haplotypes are mitochondrial copies.

In this thesis, I examined genetic variability using both a 433 bp- fragment of mtDNA cyt b gene and the entire 397 basepair nDNA ITS2 region to test the hypotheses on *C. quercusfolii* regarding genetic diversity and other related topics. Both regions have been frequently employed as a combination to disclose genetic diversity at the intraspecific level as well reconstruction phylogenetic relationships among haplotypes/alleles in insects (Espionzo et al., 2013; Martines et al., 2006). Two hundred ninety-four *C. quercusfolii* samples generated 125 cyt b haplotypes, and 30 ITS2 alleles. High haplotype number for the cyt b gene is notorious, where 79 haplotypes showed only a single base pair difference in pairwise comparisons. The number of ITS2 alleles with only 30 distinct sequences was low, but it is not uncommon since comparable results from other oak gall wasp species of Turkey have been reported (Mutun and Atay, 2015; Mutun, 2016).

In addition to the presence of high haplotype number in *C. quercusfolii*, singleton and private haplotype numbers were also high: there were 80 singletons with only a single nucleotide substitution in pairwise comparisons, and 8 private haplotypes in cyt b gene data, and 18 singletons and 9 private alleles in the ITS2 data. The occurrence of high number of both singletons and private haplotypes/alleles may be a sign of recent derivation. High ratio of recently derived haplotypes with one or few substitutions in pairwise comparisons is suggested to be an indication of young sequences in the data set implying expanding populations (Crandall and Templeton, 1993). Under the light of this general acceptance, current data sets obtained from both mitochondrial and nuclear DNA region of *C. quercusfolii* can be explained by their more recent population expansion events where many of the sequences give rise to though that they did not have enough time to expand their geographic range yet.

Haplotype diversity is the probability of two samples randomly drawn from the population with differences in their sequences, however nucleotide diversity is the average number of nucleotide differences per site between the comparisons among DNA sequences (Nei, 1987). In *C. quercusfolii*, populations displayed high haplotypic ($h = 0.7328$ for the cyt b gene, and $h = 0.5865$ for the ITS2 region) diversity. Considering each population separately, Sivas was remarkable, where out of 6 sequenced individuals 6 unique haplotypes were determined, with a haplotypic diversity of 1.000 for the cyt b gene, and 3 alleles with an h value of 0.600 for the ITS2 region. Sivas was shown to be the edge population in a grasshopper species (Korkmaz et al., 2010). It seems that Sivas is also an important location for *C. quercusfolii* genetic variation; it may be related with its geographic location since it is located as one of the closest sampled population on the western part of the mountain zone sharing more affinity with the eastern populations. Additionally, some other populations also showed high haplotypic diversity as well as high haplotype number; Giresun and İstanbul populations had 9 haplotypes out of 10 sequenced samples with high level of haplotype diversity (0.9778), followed by Bingöl, Balıkesir, Çankırı Kırklareli, Kütahya, Bursa, and Konya. Considering allelic diversity Çankırı and Yalova were also with the highest allelic diversity (Tablo 4.8). Overall, excluding small sample sizes in some localities, populations of the study species displaying high genetic diversity in many populations are congruent with the

reports from other oak gall wasp taxa including *Andricus coriarius* (Challis et al., 2007), *A. quercustozae* (Rokas et al., 2003), *A. lucidus* (Mutun, 2011), *A. caputmedusae* (Mutun, 2010), *A. quercustozae* (Dinç and Mutun, 2011), *A. lignicolus* (Mutun and Karagözoğlu, 2015), *T. synaspis* (Mutun and Atay, 2015), and *A. curtisii* (Mutun, 2016).

Considering nucleotide diversity estimates the highest value was observed in two central Anatolian populations (Konya and Karaman). Konya population was also detected with its high nucleotide diversity in *T. synaspis* and *A. curtisii* (Mutun and Atay, 2015; Mutun, 2016). This may imply that some factors might have been acting on the area in the past causing some substitutional changes in *C. quercusfolii* sequences. With the current data further conclusion cannot be drawn, therefore it requires more detailed research.

It is also notable that in *C. quercusfolii* nucleotide diversity was lower than haplotype diversity where average nucleotide diversity values for the cyt b gene and ITS2 region are $\pi = 0.0102$, and $\pi = 0.058$, respectively. It is proposed that high haplotype diversity compared to low nucleotide diversity may be an indication of rapid population expansion from small effective population in the past (Avise, 2000; Zhang et al., 2006). In the light of this current results on *C. quercusfolii* supporting this proposition were further scrutinized in the population demographic history part.

Nevertheless, genetic variation estimates in *C. quercusfolii* was either higher than those observed in the European populations of several other oak gall wasp species or very close to the uppermost value. For instance, in a study covered many Palearctic populations in *A. coriarius* nucleotide diversity was $\pi = 0.00529$ in Iran, and $\pi = 0.00644$ in Lebanon, however, $\pi = 0.01554$ was in the Turkish populations (Challis et al., 2007). When compared with other Turkish oak gall wasp species, *C. quercusfolii* genetic diversity estimates are within the range of intermediate to high level. In *A. quercustozae* the highest genetic diversity was found in Turkey within a range of 0.2- 4.2%, in Italy it ranged between 0.12- 0.17% (Rokas et al., 2003). Other oak gall wasp species covered only the Turkish populations from various species revealed high diversity estimates as well. In *A. lucidus* haplotype and nucleotide diversity were 0, 8089 and 0, 115542 (Mutun, 2011) and in *A. caputmedusae* they were 0, 4631 and 0, 101214, respectively (Mutun, 2010). High variation is not only

observed in oak gall wasps, but also reported in plants (Ansell et al., 2011), shrew (Dubey et al., 2007), mouse (Michaux et al., 2004), Anatolian mountain frog (Veith et al., 2003), fishes (Hrberk et al., 2002), ground squirrels (Gündüz et al., 2007), and grasshoppers (Korkmaz et al., 2010).

5.2 Population Demographic Analyses: Did *C. quercusfolii* Populations Expanded or Diminished?

The evolutionary history of species and evolutionary forces that are effective in specific genes can be revealed based on DNA sequence comparisons within and between species (Ramos and Rozas, 2002). With the advances of population genetics, detecting and analyzing evolutionary footprints in DNA sequences allows establishment of the population demographic structure such as population size changes. Past biogeographic or climatic changes as well as habitat fluctuations are reflected on those particularly host dependent species' genetic variation when within and among population comparisons are surveyed. For instance, past habitat (host) destruction may result in diminished population leading to population bottleneck and lowered genetic variation on one hand, or at the other extreme hand habitat expansion may cause the parasitic species to expand its range together with the host.

Organisms bear the effects of historical fluctuations on their genome, and through studying different combination of genes from the genome one may be able to disclose population size changes either in the form of constant or expanding/contracting populations accordingly. The patterns of pairwise differences between haplotypes often forms a unimodal graph of samples from expanding populations, however samples drawn from populations at demographic equilibrium produce a multimodal pattern of numerous sharp peaks (Rogers and Harpending, 1992). Tajima's *D* neutrality test and Fu and Li tests were not significant for all populations analyzed together as a single unit indicating that these populations are not deviated significantly from neutrality (Kimura, 1983).

As it was mentioned briefly in the genetic diversity estimates, the first sign of a rapid demographic expansion from a small effective population size was observed in the combination of high haplotype diversity and low nucleotide diversity in *C.*

quercusfolii. Statistical tests originally developed for testing selective neutrality of mutations are now also used to search for the signs of population growth/decline (Ramos-Onsins and Rozas, 2002). Therefore, these tests were also implemented in *C. quercusfolii* data sets to assess population size changes. In *C. quercusfolii* mismatch distribution analysis for the haplotypes/alleles across the whole sampling area and each population individually, where the population size suitable, were examined through Tajima's D and Fu's F_S values, Harpending's raggedness index and SSD to detect population expansion/contractions. The mismatch analysis of cyt b gene data set where all populations included in the analysis (Figure 4.2) generated a multimodal profile with $H_{ri} = 0.16676$, but it was not statistically significant, indicating the population expansion. A multimodal profile in mismatch distribution imply decreasing or structured population size while a ragged distribution indicates how the lineage was widespread (Excoffier et al., 1992; Rogers and Harpending, 1992). The ITS2 data set produced a nearly bimodal profile (Figure 4.6) with an average of $H_{ri} = 0.21439$ and it is non-significant indicates diminishing population size.

When each population was separately tested more details would have been drawn from the analyses. Negative non-significant Tajima's D (-0.5171) was obtained for Balıkesir population indicating the presence of rare haplotypes (4 rare haplotypes/7 total haplotypes) and Fu's F_S (-2.5895*) was significantly negative indicating an excess of rare haplotypes over what would be expected under neutrality in an expanding population supporting result was also implied by the mismatch distribution (Figure 4.3). Çanakkale population showed negative and significant Fu's F_S (-0.8305*) and non-significant Tajima's D (-0.1297) indicating excess rare haplotypes but the excess was not statistically significant where neutral evolution was rejected for Çanakkale population. Mismatch distribution analysis also support population expansion in Çanakkale. In Kütahya with unimodal mismatch profile supporting population expansion with negative and significant Fu's F_S value (-3.2737*) and non-significant positive Tajima's D value, which indicate a bias toward intermediate frequency of haplotypes. Sivas population was also remarkable regarding its non-significant H_{ri} (0.1511) and SSD (0.0719), significant negative Fu's F_S (-2.4199*) and mismatch profile imply unimodal profile. Kırklareli population should also be mentioned with respect to its statistically non-significant

Tajima's D (-1.2789*), significant Fu's F_S (-3.8244), non-significant positive Hri (0.1081) and SSD (0.0293); all indicating population expansion with rare haplotypes. The non-smoothness of the raggedness of the mismatch profile indicate population expansion with mismatch being contracted and then being widespread again in the past. In Yalova population expansion was supported by unimodal mismatch distribution, and non-significant Tajima's D (-0.1297) and Fu's F_S (-0.8305) values implying the presence of rare alleles with neutral evolution. On the other hand, Bolu, Giresun, Gümüşhane, and Muş are with multimodal profile showing non-significant negative Tajima's D and Fu's F_S values. Some populations (Afyon, Bitlis, Elazığ, Isparta, İstanbul, Karaman, Kayseri, and Malatya) were with non-significant positive Tajima's D and Fu's F_S , and significant Hri, and SSD values and multimodal profile may indicate stable to diminishing populations. In most cases it has been proven that Fu's F_S is a more powerful analysis compared to Tajima's D test (Ramos-Onsins and Rozas, 2002; Jong et al., 2011). This may explain some of the discrepancies between the significance differences between Tajima's D and Fu's F_S values.

On the other hand, relevant population demographic analyses performed on the ITS2 region showed some differences between the results obtained through the *cyt b* gene. For instance, ITS2 analysis revealed that most populations (Artvin, Bingöl, Bolu, Düzce, Edirne, Elazığ, Erzincan, Erzurum, Giresun, Gümüşhane, Isparta, Karaman, Kayseri, Konya, Muş, Ordu, Samsun, Sinop, Sivas, and Van) show unimodal profile indicating population expansion, meanwhile Balıkesir, Bursa, Çanakkale, Çankırı, İstanbul, İzmir, Kırklareli, Kütahya, and Yalova showed multimodal population profile indicating structured populations. Similarities between the results of both ITS2 and *cyt b* were observed in Bursa, Çankırı, İstanbul, İzmir, and Kırklareli where these populations imply stable to diminishing population sizes.

Each population of a species can react differently, even populations of different species existing in the same area may give different responses to the same environmental conditions (Avise, 2000), likewise different genes even within the same individual can display different patterns of mutation rates (Brown, 1981; Palumbi et al., 2001; Quenouille et al., 2004). Nuclear genome is biparentally inherited, recombination is apparent, and it is possible to test the effects of population size through using specified genes with distinct mutation rates compared

with the mitogenome (Hoy, 1994; Zhang and Hewitt, 2003). Therefore, due to the differences between the inheritance and mutation patterns of the nDNA ITS2 region and maternally inherited mtDNA cyt b gene may shed light into different time zones due to their coalescent time difference. Alternately, some of the differences can result from balancing selection on a locus located in the vicinity although hitchhiking in mitogenome is rare but still possible (Ruiz-Pessini et al., 2004).

5.3 Possible Effects of Climate and Topography on *C. quercusfolii* Lineages

Assessment of contemporary population genetic structure and geographic structuring of the lineages have been made possible now with the recent advances utilizing molecular markers and different algorithms to analyze variety of data sets (Kuchta and Meyer, 2001; Loxdale and Lushai, 1998; Parker et al., 1998). One can analyze effects of the topographic barriers to gene flow through searching a pattern on the geographic distribution of genetic lineages (Avise, 2000). Phylogenetic and network analyses of the *C. quercusfolii* data sets displayed a common pattern that has been observed in the Turkish oak gall wasp species. The cyt b gene tree topologies displayed a basal haplogroup (Clade A) that is a mix of the haplotypes representing East, West and Central Anatolian populations indicating a widespread range of the species in the past. A division (around 2.29 MYA) in the B clade was notorious; where B1 subclade was predominated by the haplotypes from eastern populations, and the B2 was dominated by the haplotypes from western populations considering the location of the major mountain chain, the Anatolian Diagonal, in Turkey. Network analysis results were also supported similar splits (Figure 4.11). In the ITS2 trees the placement polytomic alleles, and then grouping of the alleles representing more westerly sampled localities point out almost similar separation. It is noteworthy to mention that the structured part of the ITS2 tree topology was mostly composed of alleles from the western populations. Less resolution provided by the ITS2 region may be explained by different modes of inheritance, mutation rates, selection pressures, and thus shedding light into different time frames (Shaw, 2002; Zhang and Hewitt, 2003). Nevertheless, as an overall assessment, *C. quercusfolii* sequences exhibited geographic grouping of the genetic lineages that was correlated with the

location of the Anatolian Diagonal support previous findings (Mutun, 2010; Dinç and Mutun, 2011; Rokas et al., 2003; Mutun and Atay, 2015; Mutun, 2016). Similar pattern was also shown by plants and animals (Çıplak, 2004; Ekim and Güner, 1986).

Through employing molecular clock (Johns and Avise, 1998) one can estimate timing of divergence, and historical factors possibly created current pattern can be revealed (Avise, 2000). Past geologic events such orogenic activities resulting in topographic changes, and climatic oscillations between cold and warm periods are some of the factors shaping organismal distributions. One of the most significant historical factor is the climatic fluctuations during Pleistocene that have ended up with either dividing species range or shaping phylogenetic structure of lineages (Hewitt, 2000). Those past oscillations are observed in the genomic components of taxa as signatures of the past leading species to either expand their range when the environmental conditions are suitable, and retract during the glacial times (Hewitt, 2001). While organisms with repeated contractions/expansions, and isolation in refugia during the unsuitable times maintain high intraspecific diversity (Weir and Schluter, 2007), responses can also be observed in their phylogenetic structuring (Avise, 2004).

Although Turkey was not under ice sheets, indirect effects of the glacial cycles and related fluctuations have left the signatures of the past events on the phylogenetic structure of species (Çıplak, 2008). Current findings of this thesis imply that these events also left their imprints on the geographic distribution of genetic lineages. The presence of three major clades in all consensus trees of the cyt b gene data set support strong structured genetic lineages. Despite minor differences, mostly congruent tree topologies produced by MP, ML, and the Bayesian approach for the cyt b data set. In the trees, it appears that the separation of *C. quercusfolii* from other two *Cynips* species used as outgroups occurred nearly 4.91 MYA which dates to the early Zanclean age (5.33-3.60 MYA) of Pliocene epoch (5.33-2.58 MYA). The first split (3.51 MYA) of *C. quercusfolii* coincides with the Piacenzian age of Pliocene producing Clade A and Clade B. While Pliocene epoch is characterized by warm, wet and relatively stable conditions in the western Palearctic (Atkinson et al., 2007), Anatolia was with more severe conditions (Demirsoy, 1999). It seems that during the fluctuations of Late Pliocene around 2.51 MYA influenced Clade A, and

subsequently a series of Pleistocene splits were observed in clade A creating some structures. A main split from Sivas ancestral lineage represented by H117 seem to give rise to B clade predating Pleistocene epoch (late Gelasian time – split age 2.29 MYA). Subsequent split around 2.02 MYA ended up with the formation of two main lineages as B1 and B2. After Calabrian age, from middle to late Pleistocene, four main glacial ages (Günz between 676-621 ka, and interglacial 563-478 ka, Mindel between 478-424 ka, interglacial 424-374 ka, Riss between 200-130 ka, and interglacial 130-115 ka, and Würm 110-12 ka) have been distinguished. It was notorious that haplotypes grouped in the B clade were dominantly found in the eastern part of the central Anatolia- northern Anatolia, and in the eastern Anatolia. This separation coincides well with the prePleistocene changes.

Ancestral lineage giving later rise to subclade B1 and subclade B2 seem to react to the changes accordingly throughout Pleistocene oscillations. Response given by the subclades B1 and B2 began in the middle Calabrian and continued middle through late Pleistocene. Those ongoing splitting events were also conspicuously effective in the last four glacial times. Divergence estimates indicate that after the interglacial time predating Günz seem to initiate major splitting events in both B1 and B2 subclades. Each of the following glacial times covering Mindel, Riss, and finally Würm appeared to shape more current structuring in *C. quercusfolii* through forming more shallow branching in the lineages. These current estimations are in accord with the findings of some previous works in other animal groups from Anatolia (Çıplak 2004a, 2004b, 2008; Kaya and Çıplak, 2016), and in oak gall wasps (Mutun and Atay, 2015; Mutun, 2016; Rokas et al., 2003).

In conclusion, applying molecular clock to cyt b gene sequence data revealed that glacial times, especially Pleistocene, left remarkable imprints on the oak gall wasp species *C. quercusfolii*. Climatic fluctuations together with heterogeneous topography of Turkey seems to be very effective in shaping the distribution of the genetic lineages of *C. quercusfolii* in Turkey.

6. CONCLUSION

In this study, examination of *Cynips quercusfolii* through analyzing sequences from mitogenome and nuclear genome revealed that:

1. The species has high genetic variation across the sampled area in Turkey. The average genetic diversity estimates are either higher or within the range of other oak gall wasp species studied in Turkey.
2. High haplotype and low nucleotide diversity were observed both in cyt b gene and ITS2 region. This finding implies a population expansion, however there were some diminishing populations.
3. Phylogenetic and phylogeographic analyses employed on cyt b gene and ITS2 region show that genetic lineages are geographically structured mainly into western and eastern dominated groups.
4. Reconstructing evolutionary relationships among haplotypes/alleles to reveal possible reticulations, network analysis displayed congruent results with phylogenetic trees.
5. *Cynips quercusfolii* ingroup haplotypes split from other *Cynips* species used as outgroups in prePliocene.
6. Current results clearly indicate that the contemporary structuring of *C. quercusfolii* genetic lineages have been significantly influenced by the climatic and environmental fluctuations during Pliocene and Pleistocene.

Turkey as one of the major hotspot area in the world located at the center of three phytogeographic regions, three continents, with complicated paleogeographic history, heterogenous topography, and relatively mild climate during the climatic fluctuations supports high species and genetic diversity. *Cynips quercusfolii* as part of the Turkish fauna also displays significant level of genetic diversity supported by the current findings. I believe that with the current results on *C. quercusfolii* and more detailed works on other oak gall wasps and animal species will shed more light into the importance of Turkey as a place ‘cradle of the genetic diversity’.

7. REFERENCES

- Acatay A (1943) “İstanbul ve Çevresi ve Bilhassa Belgrad Ormanındaki Zararlı Orman Böcekleri, Mücadeleleri ve İşletme Üzerine Tesirleri”, Yüksek Ziraat Enstitüsü Çalışmaları, 142: 107-120, Ankara.
- Alkan B (1952) “Türkiye’nin Zoosesid (Zoocecid)’ leri (Kökeni Hayvansal Bitki Uurları) Üzerinde Çalışmalar II”, Ankara Üniversitesi Ziraat Fakültesi 1952 Yıllığı, 17: 259-279, Ankara.
- Ansell SW, Stenoien HK, Grundmann M, Russell SJ, Koch MA, Schneider H and Vogel JC (2002) “The Importance of Anatolian Mountains as The Cradle of Global Diversity in *Arabis Alpine*, A Key Arctic-Alpine Species”, Annals of Botany, 108: 241-252.
- Arias MC, Brito RM, Francisco FDO, Moretto G, Oliveirac FFD, Silvestrea D and Sheppard WS (2006) “Molecular Markers as A Tool for Population and Evolutionary Studies of Stingless Bees”, Apidologie, Springer Verlag, 37 (2): 259-274.
- Atkinson RJ, Rokas A and Stone GN (2007) “Longitudinal Patterns in Species Richness and Genetic Diversity in European Oaks and Oak Gallwasps”, In: Weiss S, Ferrand N (eds) Phylogeography of Southern European Refugia, 127-151.
- Avise JC, Arnold J, Ball RM, Bermingham E, Lamb T, Neigel JE, Reeb CA, and Saunders NC (1987) “Intraspecific Phylogeography: The Mitochondrial DNA Bridge Between Population Genetics and Systematics”, Annual Review of Ecology, Evolution, and Systematics, 18:489-522.
- Avise JC (1994) Molecular Markers, Natural History and Evolution, Chapman and Hall, New York.
- Avise JC (2000) Phylogeography: The History and Formation of Species, Harvard University Press, Cambridge.
- Avise JC (2004) Molecular Markers, Natural History and Evolution, Second Edition, Sinauer, Sunderland.
- Bailey R, Schönrogge K, Cook JM, Melika G, Cso’ ka G, Thuro’ czy C and Stone GN (2009) “Host Niches and Defensive Extended Phenotypes Structure Parasitoid Wasp Communities”, PLoS Biology, 7(8): e1000179.
- Ballard JWO and Pichaud N (2014) “Mitochondrial DNA: More than an Evolutionary by Stander”, Functional Ecology, 28: 218–231.

- Baş R (1973) “Türkiye’de Orman Ağaçlarında Zarar Yapan Zar Kanatlılar (Hymenoptera) Üzerine Araştırmalar” [Investigation of Harmful Hymenopteran (Hymenoptera) in Forests in Turkey]. T.C. Orman Bakanlığı, Orman Genel Müdürlüğü, 570(23): 75-124.
- Bellati A, Pellitteri-Rosa D, Sacchi R, Nistri A, Galimberti A, Casiraghi M, Fasola M and Galeotti P (2011) “Molecular Survey of Morphological Subspecies Reveals New Mitochondrial Lineages in *Podarcis Muralis* (Squamata: Lacertidae) from the Tuscan Archipelago (Italy)”, Journal of Zoological Systematics and Evolutionary Research, 49: 240-250.
- Bermingham E and Avise JC (1986) “Molecular Zoogeography of Freshwater Fishes in the Southeastern United States”, Genetics, 113:939-965.
- Bermingham E and Moritz C (1998) “Comparative Phylogeography: Concepts and Applications”, Molecular Ecology, 367-369.
- Bertorelle G and Barbujani G (1995) “Analysis of DNA Diversity by Spatial Autocorrelation”, Genetics, 140: 811-819.
- Bihari P, Sipos B, Melika G, Fehér B, Somogyi K, Stone GN and Péntes Z (2011) “Western Palaearctic Phylogeography of an Inquiline Oak Gall Wasp, *Synergus umbraculus*”, Biological Journal of the Linnean Society, 102(4): 750–764.
- Bilgin R (2011) “Back to the Suture: The Distribution of Intraspecific Genetic Diversity In and Around Anatolia”, International Journal of Molecular Sciences, 12(6): 4080-4103.
- Boedenheimer FS (1941) “Türkiye’de Ziraat ve Ağaçlara Zararlı Olan Böcekler ve Bunlarla Savaş Hakkında Bir Etüt”, 300-308, Bayur matbaası, Ankara.
- Boggs (2016) “Plant Galls Unveiled – Part 1: Gall Types”, Tree Care Industry, 27(4): 26-34.
- Bozkurt E (2001) “Neotectonics of Turkey – a Synthesis”, Geodinamica Acta, 14: 3-30.
- Boore JL (1999) "Animal Mitochondrial Genomes", Nucleic Acids Research, 27(8):1767-1780.
- Brower AVZ (1994) “Rapid Morphological Radiation and Convergence Among Races of The Butterfly *Heliconius Erato*, Inferred from Patterns of Mitochondrial DNA Evolution”, Proceeding of the National Academy of Sciences USA, 91: 6491–6495.
- Brown WM, George MJR and Wilson AC (1979) “Rapid Evolution of Animal Mitochondrial DNA”, Proceedings of the National Academy of Sciences, USA, 76: 1967-1971.

- Brown WN (1981) "Mechanisms of Evolution in Animal Mitochondrial DNA", *Annals of the New York Academy of Sciences*, 361: 119-34.
- Buburuzan L, Gorgan LD and Bara II (2007) "Types of DNA Used in Speciation and Phylogeny Studies", *Analele Stiintifice ale Universitatii, Alexandru Ioan Cuza, Sectiunea Genetica și Biologie Moleculara*, TOM VIII: 25-30.
- Cameron SL (2014) "Insect Mitochondrial Genomics: Implications for Evolution and Phylogeny", *Annual Review of Entomology*, 59: 95-117.
- Challis RJ, Mutun S, Nieves-Aldrey JL, Preuss S, Rokas A, Aebi A, Sadeghi E, Tavakoli M and Stone GN (2007) "Longitudinal Range Expansion and Cryptic Eastern Species in the Western Palaearctic Oak Gallwasp *Andricus Coriarius*", *Molecular Ecology*, 16 (10): 2103-2114.
- Chao A (1984) "Nonparametric Estimation of the Number of Classes in a Population", *Scandinavian Journal of Statistics*, 11(4): 265-270.
- Chapco W (1997) "Molecular Evolutionary Genetics in Orthopteroid Insects", Pp. 337–354. In: Gangwars K, Muralidharan MC and Muralidharan M (eds): *Bionomics of Grasshoppers, Katydid and Their Kins*. CAB International Press.
- Clement M, Posada D and Crandall KA (2000) "TCS: a Computer Program to Estimate Gene Genealogies", *Molecular Ecology*, 9(10): 1657-1660.
- Cook JM, Rokas A, Pagel M and Stone GN (2002) "Evolutionary Shifts Between Host Oak Sections and Host-Plant Organs in *Andricus* Gallwasps". *Evolution*, 56(9): 18.
- Crandall KA and Templeton AR (1993) "Empirical Tests of Some Predictions from Coalescent Theory with Applications to Intraspecific Phylogeny Reconstruction", *Genetics*, 134(3): 959-969.
- Crozier RH (1990) "From Population Genetics to Phylogeny: Uses and Limits of Mitochondrial DNA", *Australian Systematic Botany*, 3: 111-124.
- Crozier RH and Crozier YC (1993) "The Mitochondrial Genome of the Honeybee *Apis mellifera*: Complete Sequence and Genome Organization" *Genetics*, 133: 97-117.
- Çıplak B, Demirsoy A and Bozcuk AN (1993) "Distribution of Orthoptera in Relation to the Anatolian Diagonal in Turkey", *Articulata*, 8(1): 1-20.
- Çıplak B (2003) "Distribution of Tettigoniinae (Orthoptera, Tettigoniidae) Bush-Crickets in Turkey: The Importance of the Anatolian Taurus Mountains in Biodiversity and Implications for Conservation", *Biodiversity and Conservation*, 12 (1): 47-64.

- Çıplak B (2004a) “Biogeography of Anatolia: The Marker Group Orthoptera”, *Memorie Della Societa Entomologica Italiana*, 82(2): 357-372.
- Çıplak B (2004b) “Systematics, Phylogeny and Biogeography of *Anterastes* (Orthoptera, Tettigonidae, Tettigoniinae): Evolution Within a Refugium”, *Zoologica Scripta*, 33: 19-44.
- Çıplak B (2008) “The Analogy Between Interglacial and Global Warming for The Glacial Relicts in a Refigium: A Biogeographic Perspective for Conservation of Anatolian Orthoptera”, In Fattorini S. (ed): *Insect Ecology and Conservation, Research Sign Post Kerala, India*, 135-163.
- Darriba D, Taboada Gl, Doallo R and Posada D (2012) “Jmodeltest 2: More Models, New Heuristics and Parallel Computing”, *Nature Methods*, 9(8): 772
- Davis PH (1974) “Distribution Patterns in Anatolia With Particular Reference to Endemism”, In: Harper, Hedge (eds.), *Plant life of Southwest Asia*, 15-27.
- Demirsoy A (1999) *Genel ve Türkiye Zoocoğrafyası*, Meteksan Basımevi, Ankara.
- Demirsoy (2002) *Genel ve Türkiye Zoocoğrafyası, "Hayvan Coğrafyası"*, Meteksan Basımevi, Ankara.
- Djakpo O and Yao W (2010) “*Rhus chinensis* and *Galla chinensis* – Folklore to Modern Evidence: Review, *Phytotherapy Research*, 24: 1739-1747.
- Dinç S and Mutun S (2011) “PCR-RFLP Variation of the Oak Gallwasp, *Andricus quercustozae* (Bosc, 1792) (Hymenoptera: Cynipidae) from Turkey”, *Turkish Journal of Entomology*, 35(1): 47-58.
- Dinç S, Mutun S and Melika G (2014) “A new species of *Andricus* Hartig Oak Gall Wasp from Turkey (Hymenoptera: Cynipidae, Cynipini)” *North-Western Journal of Zoology*, 10 (1): 122-127.
- Dreger-Jauffret F and Shorthouse JD (1992) Diversity of gall-inducing insects and their galls, in: J. D. Shorthouse & O. Rohfritsch (eds.). *Biology of Insect-Induced Galls*, 8-33. Oxford University Press, New York.
- Drummond AJ and Rambaut A (2007) “BEAST: Bayesian Evolutionary Analysis by Sampling Trees” *BMC Evolutionary Biology*, 7: 214.
- Drummond AJ, Suchard MA, Xie D and Rambaut A (2012) “Bayesian Phylogenetics With BEAUti and the BEAST 1.7”, *Molecular Biology and Evolution*, 29(8): 1969–1973.
- Dubey S, Cosson JF, Vohralik V, Krystufek B, Diker E and Vogel P (2007) “Molecular Evidence of Pleistocene Bidirectional Faunal Exchange Between Europe and The Near East: The Case of the Bicoloured Shrew (*Crocidura leucodon*, Soricidae)”, *Journal of Evolutionary Biology*, 20: 1799-1808.

- Dupanloup I, Schneider S and Excoffier L (2002) "A Simulated Annealing Approach to Define the Genetic Structure of Populations", *Molecular Ecology*, 11(12):2571-81.
- Ekici M (1975) "Andricus (Cynips) gallaetinctoriae (Oliver) (Mazı Arısı) Üzerine Araştırmalar" OAE Yayınları, Tek. Bült. Serisi, 66: 155, Ankara.
- Ekim T and Güner A (1986) "The Anatolian Diagonal: Fact or Fiction?", *Proceedings of the Royal Society of Edinburgh*, 89: 69-77.
- Epperson BK (1990) "Spatial Autocorrelation of Genotypes Underdirectional Selection", *Genetics*, 124: 757-771.
- Espinoza B, Martínez-Ibarra JA, Villalobos G, De La Torre B, Laclette JP and Martínez-Hernández F (2013) "Genetic Variation of North American Triatomines (Insecta: Hemiptera: Reduviidae): Initial Divergence Between Species and Populations of Chagas Disease Vector", *The American Journal of Tropical Medicine and Hygiene*, 88(2): 275-284.
- Excoffier L, Smouse PE and Quattro JM (1992) "Analysis of Molecular Variance Inferred from Metric Distances Among DNA Haplotypes Application to Human Mitochondrial DNA Restriction Data", *Genetics*, 131: 479-491.
- Excoffier L and Lischer HEL (2010) "Arlequin Suite Ver 3.5: A New Series of Programs to Perform Population Genetics Analyses Under Linux and Windows", *Molecular Ecology Resources*, 10: 564-567.
- Fransisco F O, Silvestre D and Arias MC (2001) "Mitochondrial DNA Characterization of Five Species of *Plebeia* (Apidae: Meliponini): RFLP and Restriction Maps", *Apidologie*, 32: 323-332.
- Fu YX and Li WH (1993) "Statistical Tests of Neutrality of Mutations", *Genetics*, 133:693-709.
- Fu YX (1997) "Statistical Tests of Neutrality Against Population Growth, Hitchhiking and Background Selection", *Genetics*, 147:915-925.
- Galtier N, Nabholz B, Glemin S and Hurst GDD (2009) "Mitochondrial DNA as a Marker of Molecular Diversity: a Reappraisal", *Molecular Ecology*, 18: 4541-4550.
- Giertych M, Jagodziński A and Karolewski P (2013) "Spatial distribution of *Cynips quercusfolii* (Hymenoptera: Cynipidae) galls on leaves and within the crowns of oak trees", *European Journal of Entomology*, 110(4): 657-661.
- Giron D, Huguet E, Stone GN and Body M (2016) "Insect-Induced Effects on Plants and Possible Effectors Used by Gallling and Leaf-Mining Insects to Manipulate Their Host-plant, *Journal of Insect Physiology*, 84: 70-89.

- Gebiola M, Lopez-Vaamonde C, Nappo AG and Bernardo U (2014) “Did the Parasitoid *Pnigalio mediterraneus* (Hymenoptera: Eulophidae) Track the Invasion of the Horse Chestnut Leafminer?”, *Biological Invasions*, 16: 843-857.
- Grechko VV (2002) “Molecular DNA Markers in Phylogeny and Systematics”, *Russian Journal of Genetics*, 38(8); 851–868.
- Guindon S and Gascuel O (2003) “ A simple, Fast, and Accurate Algorithm to Estimate Large Phylogenies by Maximum Likelihood”, *Systematic Biology*, 52(5):696-704.
- Gülkaç M and Yüksel E (1999) “Türkiye’deki Spalax Tür ve Alttürlerinin Dağılımına ve Türleşmesine Coğrafik İzolasyonun Etkisi”, *Turkish Journal of Zoology*, 2: 491-496.
- Gündüz I, Jaarola M, Tez C, Yenyurt C, Polly PD and Searle JB (2007) “Multigenic and Morphometric Differentiation of Ground Squirrels (*Spermophilus scuiridaem* Rodentia) in Turkey, with a Description of a New Species”, *Molecular Phylogenetics and Evolution*, 43: 916-935.
- Gür H (2013) “The Effects of the Late Quaternary Glacial-Interglacial Cycles on Anatolian Ground Squirrels: Range Expansion During the Glacial Periods?”, *Biological Journal of the Linnean Society*, 109: 13-32.
- Gür H (2016) “The Anatolian Diagonal Revisited: Testing the Ecological Basis of a Biogeographic Boundary”, *Zoology in the Middle East*, 62(3): 189-199.
- Hall TA (1999) “BioEdit: A User Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT”, *Nucleic Acids Symposium Series*, 41: 95-98.
- Harpending HC (1994) “Signature of Ancient Population Growth in a Low-Resolution Mitochondrial DNA Mismatch Distribution”, *Human Biology*, 66:591-600.
- Harper LJ, Schonrögge K, Lim KY, Francis P and Lichtenstein CP (2004) “Cynipid galls: Insect-Induced Modifications of Plant Development Create Novel Plant Organs”, *Plant, Cell & Environment*, 27: 327-335.
- Harrison RG (1989) “Animal Mitochondrial DNA as a Genetic Marker in Population and Evolutionary Biology”, *Trends in Ecology and Evolution*, 4: 6–11.
- Hayward A and Stone GN (2005) “Oak Gall Wasp Communities: Evolution and Ecology”, *Basic Applied Ecology*, 6: 435-443.
- Hayward A and Stone GN (2006). “Comparative Phylogeography Across Two Trophic Levels: The Oak Gallwasp *Andricus kollari* and Its Chalcid Parasitoid *Megastismus Stigmatizans*”, *Molecular Ecology*, 15: 479-489.

- Hewitt GM (1996) "Some Genetic Consequences of Ice Ages, and Their Role in Divergence and Speciation", *Biological Journal of the Linnaean Society*, 58 (3): 247-276.
- Hewitt GM (1999) "Post-Glacial Re-Colonization of European Biota", *Biological Journal of the Linnaean Society*, 68: 87-112.
- Hewitt GM (2000) "The Genetic Legacy of the Quaternary Ice Ages", *Nature*, 405: 907-913.
- Hewitt GM (2001) "Speciation, Hybrid Zones and Pylogeography or Seeing Genes in Space and Time", *Molecular Ecology*, 10, 537–549.
- Hickerson MJ, Carstens BC, Cavender-Bares J, Crandall KA, Graham CH, Johnson JB, Rissler L, Victoriano PF and Yoder AD (2010) "Phylogeography's Past, Present, and Future: 10 Years After Avise, 2000", *Molecular Phylogenetics and Evolution*, 54: 291-301.
- Horn A, Roux-Morabito G, Lieutier F and Kerdelhue C (2006) "Phylogeographic Structure and Past History of the Circum-Mediterranean Species *Tomicus destruens* Woll (Coleoptera: Scolytinae)", *Molecular Ecology*, 15: 1603-1615.
- Hoy MA (1994) "Insect Molecular Genetics: An Introduction to Principles and Applications", Academic Press Inc., 546, CA, USA.
- Hrbek T, Küçük F, Frickey T, Stölting KN, Wildekamp RH and Meyer A (2002) "Molecular Phylogeny and Historical Biogeography of the *Apahnius* (Pisces, Cyprinodontiformes) Species Complex of Central Anatolia, Turkey", *Molecular Phylogenetics and Evolution*, 25: 125-137.
- Ionescu AM (1973) "Biologia Galelor (Monografie Cecidologică). Editura Academiei Republicii Socialiste România, București.
- Ipekdağ K, Pamuk P, Emin A, Koçluk M, Aytar F, Kuzucu AS and Aksu Y (2015) "Recent Invasion of the Chestnuts in Western Turkey by the Chestnut Gall Wasp, *Dryocosmus kuriphilus*, and Early Management Practices by Using the Parasitoid Wasp, *Torymus sinensis*", IUFRO Conference, Population Dynamics and Integrated Control of Forest Defoliating and Other Insects, Sopot, Poland.
- Ji YJ, Zhang DX and He LJ (2003) "Evolutionary Conservation and Versatility of a New Set of Primers for Amplifying the Ribosomal Internal Transcribed Spacer Regions in Insects and Other Invertebrates", *Molecular Ecology Notes*, 3: 581-585.
- Johns GC and Avise JC (1998) "A Comparative Summary of Genetic Distances in the Vertebrates from the Mitochondrial Cytochrome b Gene", *Molecular Biology and Evolution*, 15(11): 1481-1490.

- Jong AM, Wahlberg N, Eijik MA, Brakfield PM and Zwan BJ (2011) "Mitochondrial DNA Signature for Range-Wide Populations of *Bicyclus anynana* Suggest a Rapid Expansion from Recent Refugia", Plos One, 6 (6): 1-5.
- Kalinowski ST (2004) "Counting Alleles with Rarefaction: Private Alleles and Hierarchical Sampling Designs", Conservation Genetics, 5: 539-543.
- Karadenizli L (2011) "Oligocene to Pliocene Palaeogeographic Evolution of the Çankiri-Çorum Basin, Central Anatolia, Turkey", Sedimentary Geology, 237:1-29.
- Kasaplıgil B (1992) "Türkiye'nin Geçmişteki ve Bugünkü Meşe Türleri", T. C. Orman Bakanlığı Orman Genel Müdürlüğü, 675: 64.
- Katılmış Y and Kıyak S (2008) "Checklist of Cynipidae of Turkey, with a New Genus Record", Journal of Natural History, 42 (31-32): 2161-2167.
- Kaya S, Boztepe Z and Çıplak B (2015) "Phylogeography of the *Poecilimon luschani* Species Group (Orthoptera, Tettigonidae): a Radiation Strictly Correlated with Climatic Transitions in the Pleistocene", Zoological Journal of the Linnean Society London, 173.
- Kaya S and Çıplak B (2016) "Budding Speciation via Peripheral Isolation: The *Psorodonotus venosus* (Orthoptera, Tettigoniidae) Species Group Example", Zoologica Scripta, 45: 521-537.
- Kemal M and Kocak AÖ (2010) "Winter Trips to South Van Lake and the Cynipid Galls on Oaks (Hymenoptera, Cynipidae)", Cesa News, 56: 1-66.
- Kıyak S, Kılıç T and Katılmış Y (2008) "A Contribution to the Knowledge of the Cynipini (Cynipidae: Hymenoptera) Fauna of Turkey", Munis Entomology and Zoology, 3(1): 523-535.
- Kimura M (1983) The Neutral Theory of Molecular Evolution, Cambridge University Press, 367, Cambridge.
- Kolar J, Stofa A, Strli M, Pompeb M, Pihlar B, Budnar M, Sim C and Reissland B (2010) "Historical Iron Gall ink Containing Documents Properties Affecting Their Condition", Analytica Chimica Acta, 555: 167-174.
- Korkmaz M, Lunt DH, Çıplak B, Değerli N and Başıbüyük HH (2014) "The Contribution of Anatolia to European Phylogeography: The Centre of Origin of the Meadow Grasshopper, *Chorthippus parallelus*", Journal of Biogeography, 41: 1793-1805.
- Kuchta SR and Meyer D (2001) "A Genealogical View of Geographical Variation", Molecular Ecology, 10: 2569-2576.

- Kumari S and Subbotin SA (2012) “Molecular Characterization and Diagnostics of Stubby Root and Virus Vector Nematodes of the Family Trichodoridae (Nematoda: Triplonchida) Using Ribosomal RNA Genes”, *Plant Pathology*, 61(6): 1021-1031.
- Lemire B (2005) “Mitochondrial Genetics”, WormBook Ed. The *C. elegans* Research Community, WormBook.
- Librado P and Rozas J (2009) “DnaSP v5: A Software for Comprehensive Analysis of DNA Polymorphism Data”, *Bioinformatics*, 25: 1451-1452.
- Liljeblad J and Ronquist F. (1998) “A Phylogenetic Analysis of Higher-Level Gallwasp Relationships (Hymenoptera: Cynipidae)”, *Systematic Entomology*, 23: 229-252.
- Liljeblad J, Nieves-Aldrey JL, Naser S and Melika G (2011) “Adding Another Piece to the Cynipoid Puzzle: The Description of a New Tribe, Genus and Species of Gall Wasp (Hymenoptera: Cynipidae) Endemic to the Republic of South Africa”, *Zootaxa* 2806: 35-52
- Loxdale HD and Lushai G (1998) “Molecular Markers in Entomology”, *Bulletin of Entomological Research*, 88: 577-600.
- Maddison WP (1996) “Molecular Approaches and the Growth of Phylogenetic Biology in Molecular Zoology: Advances, Strategies, and Protocols”, Edited by J. D. Ferraris and S. R. Palumbi. Wiley-Liss, Inc.
- Mani MS (1964) “Ecology of Plant Galls, The Hague.
- Manni F and Guerard E (2004) “Barrier vs. 2.2. Manuel of the User” Population Genetics Team, Museum of Mankind (Musée de l'Homme), Paris.
- Mapes CC and Davies PJ (2001) “Cytokinins in the Ball Gall of *Solidago altissima* and in the Gall Forming Larvae of *Eurosta solidaginis*”, *New Phytologist*, 151: 203-212.
- Martínez FH, Villalobos GC, Cevallos AM, Torre P, Laclette JP, Alejandre-Aguilar R and Espinoza B (2006) “Taxonomic Study of the *Phyllosoma* Complex and Other Triatomine (Insecta: Hemiptera: Reduviidae) Species of Epidemiological Importance in the Transmission of Chagas Disease: Using ITS-2 and mtCytB Sequences”, *Molecular Phylogenetics and Evolution*, 41: 279-287.
- Melika G and Stone GN (2001) “A New Species of Cynipid Gall Wasp from Turkey (Hymenoptera: Cynipidae)”, *Folia Entomologica Hungarica*, 62: 127-131.
- Melika G, Ross-Farre, P, Penzes ZS and Pujade-Villar J, (2004) “UFO Abei Melika et Pujade-Villar (Hymenoptera: Cynipidae: Synergini) New Genus and New Species from Japan”, *Acta Zoologica Academiae Scientiarum Hungaricae*, 51(4): 313-327.

- Melika G (2006) *Gallwasps of Ukraine: Cynipidae*, Schmalhausen Institute of Zoology, National Academy of Sciences, 646, Ukraine.
- Michaux JR, Libous R, Paradis E and Filippucci MG (2004) “Phylogeographic History of the Yellow-Necked Fieldmouse (*Apodemus flavicollis*) in Europe and in the Near and Middle East”, *Molecular Phylogenetics and Evolution*, 32: 788-792.
- Moix P, Beccaleto L, Heinz K, Hochard C, Rosselet F and Stampfli G (2008) “A New Classification of the Turkish Terranes and Sutures and Its Implication for The Paleotectonic History of the Region”, *Tectonophysics*, 451: 7-39.
- Monmonier MS (1973), “Maximum-Difference Barriers: An Alternative Numerical Regionalization Method”, *Geographical Analysis*, 5: 245–261.
- Moretto G and Arias MC (2005) “Detection of Mitochondrial DNA Restriction Site Differences Between the Subspecies of *Melipona quadrifasciata* Lepeletier (Hymenoptera: Apidae: Meliponini)”, *Neotropical Entomology*, 34(3): 381-385.
- Moritz C, Dowling TE and Brown WM (1987) “Evolution of Animal Mitochondrial DNA: Relevance for Population Biology and Systematics”, *Annual Review of Ecological Systematics*, 18: 269-292.
- Mukabayire O, Boccolini D, Lochouart L, Fontenille D and Besansky NJ (1999) “Mitochondrial and Ribosomal Internal Transcribed Spacer (ITS2) Diversity of the African Malaria Vector *Anopheles funestus*”, *Molecular Ecology*, 8: 289-297.
- Mutun S (2010) “Intraspecific Genetic Variation and the Phylogeography of the Oak Gallwasp *Andricus caputmedusae* (Hymenoptera: Cynipidae): Effects of the Anatolian Diagonal”, *Acta Zoologica Academiae Scientiarum Hungaricae*, 56 (2): 153-172.
- Mutun S (2011) “Intraspecific Genetic Diversity and Phylogeography of the Oak Gall Wasp *Andricus lucidus* (Hymenoptera: Cynipidae) Populations in Anatolia”, *Turkish Journal of Zoology*, 35 (4): 559-570.
- Mutun S, Dinç S (2011) “Contributions to the Gallwasp (Hymenoptera: Cynipidae) fauna of Turkey with one new record”, *Journal of Applied Biological Science*, 5(3): 83-85.
- Mutun S, Dinç S, Atay G and Karagözoğlu H (2013) “Assessment of Genetic Variation in the Anatolian Populations of *Andricus gallaetinctoriae* (Hymenoptera: Cynipidae)”, *Journal of Applied Biological Sciences*, 7 (3): 84-89.
- Mutun S, Dinç S, Bozsó M and Melika G (2014) “Four New Species of *Andricus* Hartig Oak Gallwasp From Turkey (Hymenoptera: Cynipidae, Cynipini)”, *Zootaxa*, 3760(2): 241-259.

- Mutun S and Atay G (2015) “Phylogeography of *Trigonaspis synaspis* (Hymenoptera: Cynipidae) from Anatolia Based on Mitochondrial and Nuclear DNA Sequences”, *European Journal of Entomology*, 112 (2): 259-269.
- Mutun S and Karagözoğlu H (2015) “Investigation of Genetic Variation Among Turkish Populations of *Andricus lignicola* Using Mitochondrial Cytochrome b Gene Sequence Data”, *Turkish Journal of Zoology*, 39 (5): 721-733.
- Mutun S (2016) “Molecular Diversity and Phylogeography of *Andricus curtisii* (Hymenoptera, Cynipidae) in Turkey”, *Biochemical Systematics and Ecology*, 67: 74-85.
- Nei M (1987) *Molecular Evolutionary Genetics*, Columbia University Press, New York.
- Nei S and Nei M (1987) “The Neighbor-Joining Method: a New Method for Reconstructing Phylogenetic Trees”, *Molecular Biology and Evolution*, 4:406-425.
- Okay AI (2008) *Geology of Turkey: A synopsis*. Anschnitt, 21: 19-42.
- Özdemir N, Gül S, Poyarkov NA, Kutrup B, Tosunoglu M and Doglio S (2014) "Molecular Systematics and Phylogeography of *Bufotes variabilis* (syn. *Pseudepidalea variabilis*) (Pallas, 1769) in Turkey", *Turkish Journal of Zoology*, 38: 412-420
- Palumbi SR, Cipriano F, Hare MP (2001) “Predicting Nuclear Gene Coalescence from Mitochondrial Data: the Three-Times Rule”, *Evolution*, 55(5): 859-868.
- Papadopoulou A, Anastasiou I and Vogler AP (2010) “Revisiting the Insect Mitochondrial Molecular Clock: the Mid-Aegean Trench Calibration”, *Molecular Biology and Evolution*, 27(7): 1659-72.
- Parker PG, Snow AA, Schug MD, Booton GC and Fuerst PA (1998) “What Molecules Can Tell Us About Populations: Choosing and a Molecular Marker”, *Ecology*, 79(2): 361-382.
- Peakall R and Smouse PE (2012) “GenAlEx 6.5: Genetic Analysis in Excel. Population Genetic Software for Teaching and Research-an Update”, *Bioinformatics*, 28: 2537-2539.
- Petit RJ, Duminil J, Fineschi S, Hampe A, Salvini D and Vendramin GG (2005) “Comparative Organization of Chloroplast, Mitochondrial and Nuclear Diversity in Plant Populations”, *Molecular Ecology*, 14: 689–701.
- Pons J and Vogler AP (2005) “Complex Pattern of Coalescence and Fast Evolution of a Mitochondrial rRNA Pseudogene in a Recent Radiation of Tiger Beetles”, *Molecular Biology Evolution*, 22:991–1000.

- Posada D and Crandall KA (2001) "Intraspecific Gene Genealogies: Trees Grafting into Networks", *Trends in Ecology and Evolution*, 16: 37-45.
- Price PW, Fernandes GW, Waring GL (1987) "Adaptive Nature of Insect Galls", *Environmental Entomology*, 16:15-24.
- Price P (2005) "Adaptive Radiation of Gall-inducing Insects", *Basics Ecological Applications*, 6: 413-421.
- Provan J and Bennett KD (2008) "Phylogeographic Insights into the Cryptic Glacial Refugia", *Trends in Ecology and Evolution*, 23 (10): 564-571.
- Pujade-Villar J, Bellido D, Segú G and Melika G (2001) "Current state of Knowledge of Heterogeny in Cynipidae (Hymenoptera, Cynipoidea)", *Sessió Conjunta d'Entomologia de l'Institut*, 11: 85–105.
- Quenouille B, Bermingham E and Planes S (2004) "Molecular Systematics of the Damselfishes (Teleostei: Pomacentridae): Bayesian Phylogenetic Analyses of Mitochondrial and Nuclear DNA sequences", *Molecular Phylogenetics and Evolution*, 31: 66-88.
- Rambout A and Drummond AJ (2003) Tracer. Version 1.3.1, Available from <http://evolve.zoo.ox.ac.uk/>.
- Rambout A (2009) FigTree. Version 1.3.1, Available from <http://tree.bio.ed.ac.uk/software/figtree/>.
- Ramos-Onsins SE and Rozas J (2002) "Statistical Properties of New neutrality tests Against Population Growth", *Molecular Biology and Evolution*, 19: 2092-2100.
- Resh VH and Carde RT (2009) *Encyclopedia of Insects*, Second Edition, Academic Press.
- Rogers A and Harpending H (1992) "Population Growth Makes Waves in the Distribution of Pairwise Differences", *Molecular Biology and Evolution*, 9: 552 -569.
- Rokas A, Atkinson RJ, Webster LMI, Csöka G and Stone GN (2003a) "Out of Anatolia: Longitudinal Gradients in Genetic Diversity Support an Eastern Origin for a Circum-Mediterranean Oak Gallwasp *Andricus quercustozae*", *Molecular Ecology*, 12: 2153-2174.
- Rokas A, Melika G, Abe Y, Nieves-Aldrey J-L, Cook JM and Stone GN (2003b) "Lifecycle Closure, Lineage Sorting, and Hybridization Revealed in a Phylogenetic Analysis of European Oak Gallwasps (Hymenoptera: Cynipidae: Cynipini) Using Mitochondrial Sequence Data", *Molecular Phylogenetics and Evolution*, 26: 36-45.

- Ronquist F and Liljeblad J (2001) "Evolution of the Gall Wasp-Host Plant Association", *Evolution*, 55: 2503-2522.
- Ronquist F and Huelsenbeck JP (2003) "MrBayes 3: Bayesian Phylogenetic Inference Under Mixed Models", *Bioinformatics*, 19:1572-1574.
- Ronquist F, Nieves-Aldrey JL, Buffington ML, Liu Z, Liljeblad J and Nylander JAA (2015) "Phylogeny, Evolution and Classification of Gall Wasps: the Plot Thickens", *Plos One*, 10(5): e0123301.
- Rögl F (1999) "Mediterranean and Paratethys Paleogeography During the Oligocene and Miocene. In J. Agusti, L. Rook and P. Adrews (eds.). *Hominoid Evolution and climatic change in Europe Vol. 1: the evolution of the Neogene terrestrial ecosystems in Europe*, pp 8-22, Cambridge University Press, Cambridge U.K.
- Ruiz-Pessini E, Mishmar D, Brandon M, Procaccio V and Wallace DC (2004) "Effects of Purifying and Adaptive Selection on Regional Variation in Human mtDNA", *Science*, 303: 223-226.
- Schmitt T (2007) "Molecular Biogeography of Europe: Pleistocene Cycles and Postglacial trends", *Frontiers in Zoology*, 4:11.
- Senn G (1942) "Oak Galls in the Historia Plantarum of Theophrastus", *Earth and Environmental Science Transactions of The Royal Society of Edinburgh*, 60(2): 343-354.
- Sert H, Suchentrunk F and Erdoğan A (2005) "Genetic Diversity within Anatolian Brown Hares (*Lepus europaeus* Pallas, 1778) and Differentiation among Anatolian and European Populations", *Mammalian Biology*, 70(3): 171-186.
- Shaw M, Murrell A and Barker SC (2002) "Low intraspecific Variation in the rRNA Internal Transcribed Spacer 2 (ITS2) of the Australian Paralysis Tick, *Ixodes holocyclus*", *Parasitology Research*, 88: 247–252.
- Shorthouse JD, Wool D and Raman A (2005) "Gall-inducing Insects- Nature's Most Sophisticated Herbivores", *Basic and Applied Ecology*, 6: 407-411.
- Simon, C, Fıratı F, Beckenbach A, Crespi B, Liu H and Flook P (1994) "Evolution, Weighting, and Phylogenetic Utility of Mitochondrial Gene Sequences and Compilation of Conserved Polymerase Chain Reaction Primers", *Annals of the Entomological Society of America*, 87(6): 651-701.
- Stone G and Sunnucks P (1993) "Genetic Consequences of an Invasion Through a Patchy Environment – the Cynipid Gall Wasp *Andricus quercuscalicis* (Hymenoptera: Cynipidae)", *Molecular Ecology*, 2: 251-268.
- Stone GN and Cook JM (1998) "The Structure of Cynipid Oak Galls: Patterns in The Evolution of an Extended Phenotype", *Proceedings of the Royal Society of London B*, 265: 979-988.

- Stone GN, Atkinson RJ, Brown G and Rokas A (2002) “The Population Genetic Consequences of Range Expansion: a Review of Pattern and Process, and the Value of Oak Gallwasps as a Model System”, *Biodiversity Sciences*, 10 (1): 80-97.
- Stone GN and Schrönrogge K (2003) “The Adaptive Significance of Insect Gall Morphology”, *Trends in Ecology & Evolution*, 18: 512-522.
- Stone GN, Challis RJ, Atkinson RJ, Csoka G, Hayward A, Melika G, Mutun S, Preuss S, Rokas A, Sadeghi E and Schönrogge K (2007) “The Phylogeographical Clade Trade: Tracing the Impact of Human-Mediated Dispersal on the Colonization of Northern Europe by the Oak Gallwasp *Andricus kollari*”, *Molecular Ecology*, 16 (13): 2768-2781.
- Stone GN, Atkinson RJ, Rokas A, Nieves-Aldrey JS, Melika G, Acs Z, Csoka G, Hayward A, Bailey R, Buckee C and McVean GAT (2008) “Evidence for Widespread Cryptic Sexual Generations in Apparently Purely Asexual *Andricus gallwaps*”, *Molecular Ecology*, 17: 652-665.
- Strange RM and Burr BM (1997) “Intraspecific Phylogeography of North American Highland Fishes: A Test of The Pleistocene Vicariance Hypothesis”, *Evolution*, 51: 885–897.
- Swofford DL (2002) PAUP: Phylogenetic Analysis Using Parsimony ver. 4.0b10, Sinauer Associates, Sunderland, Massachusetts.
- Şekercioğlu ÇH, Anderson S, Akçay E, Bilgin R, Can ÖE, Semiz G, Tavşanoğlu Ç, Yokeş MB, Soyumert A, İpekdağ K, Sağlam İK, Yücel M and Dalfes HN (2011) “Turkey’s globally important biodiversity in crisis”, *Biological Conservation*, 144: 2752-2769.
- Şengör AMC and Yılmaz Y (1981) “Tethyan evolution of Turkey: A plate tectonic approach”, *Tectonophysics*, 75:181-241.
- Tajima F (1983) “Evolutionary Relationship of DNA Sequences in Finite Populations”, *Genetics*, 105: 437-460.
- Tajima F (1989a) “Statistical Method for Testing the Neutral Mutation Hypothesis by DNA Polymorphism”, *Genetics*, 123: 585-595.
- Tajima F (1989b) “The Effect of Change in Population Size on DNA Polymorphism”, *Genetics*, 23(3): 597-601.
- Tamura K, Stecher G, Peterson D, Filipowski A and Kumar S (2013) “MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0, *Molecular Biology and Evolution*, 30: 2725-2729.
- Teacher AGF and Griffiths DJ (2011) “HapStar: Automated Haplotype Network Layout and Visualisation”, *Molecular Ecology Resources*, 11(1): 151-153.

- Thanos CA (2005) "Theophrastus on Oaks", *Botanical Chronology*, 18(1): 29-36.
- Umachigi SP, Jayaveera KN, Ashok Kumar CK, Kumar GS, Vrushabendr swamye BM and Kishore Kumar DV (2008) "Studies on Wound Healing Properties of *Quercus infectoria*", *Tropical Journal of Pharmaceutical Research*, 7 (1): 913-919.
- Veith M, Schmidtler J, Kosuch J, Baran I and Seitz A (2003) "Paleoclimatic Changes Explain Anatolian Mountain Frog Evolution: A Test for Alternating Vicariance and Dispersal Events", *Molecular. Ecology*, 12: 185-199.
- Wagener B, Reineke A, Löhr B, and Zebitz CPW (2006) "Phylogenetic Study of *Diadegma* Species (Hymenoptera: Ichneumonidae) Inferred from Analysis of Mitochondrial and Nuclear DNA Sequences", *Biological Control*, 37: 131-140.
- Wörheide G, Hooper JNA and Degnan BM (2002) "Phylogeography of Western Pacific *Leucetta* 'chagosensis' (Porifera: Calcarea) from Ribosomal DNA Sequences: Implications for Population History and Conservation of the Great Barrier Reef World Heritage Area (Australia)", *Molecular Ecology*, 11: 1753-1768.
- Weir JT and Schluter D (2007) "The Latitudinal Gradient in Recent Speciation and Extinction Rates of Birds and Mammals", *Science*, 315(5818): 1574-1576.
- Yajie J, Dexing Z and Lijun H (2003) "Evolutionary Conservation and Versatility of a New Set of Primers for Amplifying the Ribosomal Internal Transcribed Spacer Regions in Insects and Other Invertebrates", *Molecular Ecology Notes*, 3(4): 581.
- Yaltırık F (1984) *Türkiye Meşeleri Teşhis Kılavuzu*, Yenilik basımevi, İstanbul.
- Yang Z and Rannala B (2012) "Molecular Phylogenetics: Principles and Practice", *Nature Reviews Genetics*, 13(5): 303-314.
- Zargarán MZ, Safaralizadeh MH, Pourmirza AA and Valizadegan O (2011) "Effect of Cardinal Directions on Gall Morphology and Parasitization of the gall wasp, *Cynips quercusfolii*", *Journal of Insect Science*, 11:169.
- Zhang DX, Szymura JM and Hewitt GM (1995) "Evolution and structural conservation of the control region of insect mitochondrial DNA", *Journal of Molecular Evolution*, 40: 382-391.
- Zhang DX and Hewitt GM (1996) "Nuclear integrations: challenges for mitochondrial DNA markers", *Trends in Ecology and Evolution*, 11: 247-251.
- Zhang DX and Hewitt GM (2003) "Nuclear DNA analyses in genetic studies of populations: practice, problems and prospects", *Molecular Ecology*, 2: 563-584.

Zhang J, Cai Z and Huang L (2006) “Population Genetic Structure of Crimson Snapper *Lutjanus erythropterus* in East Asia, Revealed by Analysis of the Mitochondrial Control Region”, ICES Journal of Marine Science, 63: 693-704.



8. CURRICULUM VITAE

Name SURNAME : Serdar DİNÇ

Place and Date of Birth : İstanbul 14.02.1983

Universities : Abant İzzet Baysal University

Bachelor's Degree : Abant İzzet Baysal University

MSc Degree : Abant İzzet Baysal University

e-mail : dinc_s@ibu.edu.tr

Address : Abant İzzet Baysal University

List of Publications :

1. Dinç S and Mutun S (2011) “PCR-RFLP Variation of the Oak Gallwasp, *Andricus quercustozae* (Bosc, 1792) (Hymenoptera: Cynipidae) from Turkey”, Turkish Journal of Entomology, 35(1): 47-58.
2. Mutun S, Dinç S (2011) “Contributions to the Gallwasp (Hymenoptera: Cynipidae) fauna of Turkey with one new record”, Journal of Applied Biological Science, 5(3): 83-85.
3. Mutun S, Dinç S, Atay G and Karagözoğlu H (2013) “Assessment of Genetic Variation in the Anatolian Populations of *Andricus gallaetinctoriae* (Hymenoptera: Cynipidae)”, Journal of Applied Biological Sciences, 7 (3): 84-89.
4. Dinç S, Mutun S and Melika G (2014) “A new species of *Andricus* Hartig Oak Gall Wasp from Turkey (Hymenoptera: Cynipidae, Cynipini)” North-Western Journal of Zoology 10 (1): 122-127.
5. Mutun S, Dinç S, Bozsó M and Melika G (2014) “Four New Species of *Andricus* Hartig Oak Gallwasp From Turkey (Hymenoptera: Cynipidae, Cynipini)”, Zootaxa 3760(2): 241-259.

6. Mutun S., Dinç S. (2015). Twelve oak gallwasp species (Hymenoptera, Cynipidae) New to the Turkish Fauna. Turkish Journal of Zoology. 39(5): 962-964.

Projects :

1. Serap Mutun, Serdar Dinç (2011-2014). *Cynips quercusfolii* (Linnaeus, 1758) (Hymenoptera: Cynipidae)'nin mitokondriyal DNA sitokrom b geni çeşitliliği. Abant İzzet Baysal Üniversitesi, BAP. 30.000 TL. (Researcher).
2. Serap Mutun, Serdar Dinç (2011-2014). Ülkemiz meşe mazı arısı (Hymenoptera: Cynipidae) tür çeşitliliğinin araştırılması. Abant İzzet Baysal Üniversitesi, BAP. 30.000 TL. (Researcher).
3. Serap Mutun, Serdar Dinç (2016- cont'd). *Cynips quercus* (Hymenoptera: Cynipidae) türünün ülkemizdeki filocoğrafik yapılanması ve populasyon genetik yapısının belirlenmesi. Abant İzzet Baysal Üniversitesi, BAP 30.000 TL. (Researcher)

Presentation in Scientific Meetings :

1. Dinç S., Mutun S. (2009). Meşe Mazı Arısı *Andricus quercustozae* (Hymenoptera: Cynipidae)'nin Anadolu'nun Genetik Çeşitliliğine Katkısı: PCR-RFLP Varyasyonu. IX. Ulusal Ekoloji ve Çevre Kongresi, Nevşehir. (Poster Presentation).
2. Mutun S., Dinç S. (2009). Anadolu'nun Meşe Mazı Arısı (Hymenoptera: Cynipidae: Cynipini) Tür Çeşitliliği. IX. Ulusal Ekoloji ve Çevre Kongresi, Nevşehir. (Poster Presentation).
3. Mutun S., Dinç S., Karagözoğlu H. (2011). Bazı *Andricus* türlerinin DNA kesim farklılıkları yoluyla belirlenmesi. X. Ulusal Ekoloji ve Çevre Kongresi, Çanakkale. (Poster Presentation).
4. Mutun S., Dinç S. (2011). Bolu ili Cynipidae çeşitliliği. X. Ulusal Ekoloji ve Çevre Kongresi, Çanakkale. (Poster Presentation).
5. Karagözoğlu H., Dinç S., Mutun S. (2011). *Andricus lignicolus* Hartig, 1840 (Hymenoptera: Cynipidae) türünün ülkemizdeki bazı populasyonlarında genetik çeşitliliğin araştırılması. X. Ulusal Ekoloji ve Çevre Kongresi, Çanakkale. (Poster Presentation).

6. Mutun S., Dinç S. (2011). Meşe gal arıları: Türkiye'nin genetik çeşitliliği üzerine katkıları. X. Ulusal Ekoloji ve Çevre Kongresi, Çanakkale. (Poster Presentation).
7. Dinç S., Mutun S., Karagözoğlu H., Atay G. (2012). *Andricus truncicolus*'da larval odacık sayısı ve genetik farklılaşma. 21. Ulusal Biyoloji Kongresi, İzmir. (Poster Presentation).
8. Atay G., Dinç S., Karagözoğlu H., Mutun S. (2012). *Andricus infectoria*'da genetik varyasyonun İç ve Batı Anadolu populasyonlarında dağılımı. 21. Ulusal Biyoloji Kongresi, İzmir. (Poster Presentation).
9. Karagözoğlu H., Dinç S., Atay G., Mutun S. (2012). *Andricus glutinosus*'un Batı Anadolu populasyonlarındaki genetik varyasyonu. 21. Ulusal Biyoloji Kongresi, İzmir. (Poster Presentation).
10. Dinç S., Mutun S. (2014). Genetic Diversity of an Oak Dependent Gallwasp Species: Preliminary Analyses of *Cynips quercusfolii* (Hymenoptera: Cynipidae) from Turkey. IUFRO, Antalya, Türkiye. (Oral Presentation).
11. Mutun S., Dinç S. (2014). Beauty or the Beast? An Association Between Oak and Oak Gallwasps with Special Emphasis on Turkish Cynipid Species Richness. IUFRO, Antalya, Türkiye. (Oral Presentation).
12. Dinç S., Mutun S. (2016). Estimation of Genetic Diversity in the Turkish Populations of *Cynips quercusfolii* using mitochondrial cytochrome b gene sequence data. SEAB-Symposium on EuroAsian Biodiversity Antalya, Türkiye. (Oral Presentation).
13. Mutun S., Dinç S., Özarslan C. (2017). More to come on oak gall wasps: preliminary analysis on *Cynips quercus* mtDNA cyt b gene. Ecology 2017 International Symposium Kayseri, Türkiye. (Poster Presentation)