ABANT IZZET BAYSAL UNIVERSITY

THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

DEPARTMENT OF BIOLOGY

GEOGRAPHIC DISTRIBUTION OF GENETIC DIVERSITY OF *CYNIPS DIVISA* (HYMENOPTERA: CYNIPIDAE) IN TURKEY

MASTER OF SCIENCE

ERHAN ÇİMEN

BOLU, MAY 2018

APPROVAL OF THE THESIS

GEOGRAPHIC DISTRIBUTION OF GENETIC DIVERSITY OF *CYNIPS DIVISA* (HYMENOPTERA: CYNIPIDAE) IN TURKEY submitted by **Erhan ÇİMEN** in partial fulfillment of the requirements for the degree of **Master of Science** in **Department of Biology, The Graduate School of Natural and Applied Sciences of ABANT IZZET BAYSAL UNIVERSITY in 11/05/2018** by

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Erhan ÇİMEN

ABSTRACT

GEOGRAPHIC DISTRIBUTION OF GENETIC DIVERSITY OF *CYNIPS DIVISA* (HYMENOPTERA: CYNIPIDAE) IN TURKEY
MASTER THESIS

ERHAN ÇİMEN

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

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Cynips divisa is an oak dependent gall wasp species with wide distribution range in the western Palearctic. Adults reared from asexual generation galls were used: i) to reveal genetic diversity of the species in Turkey, ii) to disclose geographic distribution of genetic diversity (intraspecific phylogeography) across the sampled range of the species, iii) to reveal genetic differentiation between and among populations, iv) to search for the possible factors that governed contemporary phylogeographic pattern, and v) evaluate findings of the thesis with the phylogenetic and phylogeographic results reported for other oak gall wasp species both from Turkey and the Palearctic region.

After total DNA isolation from 278 individuals collected from 22 populations, partial mitochondrial cytochrome b gene and the entire nuclear ITS2 region were amplified, sequenced, and analyzed. All sequences produced 115 haplotypes and 15 alleles. Populations of *C. divisa* displayed high diversity with average h= 0.81818, π= 0.01197, and h= 0,31623, π= 0,00167 for cyt b gene and ITS2 region, respectively. Phylogenetic analysis pointed to separation of *C. divisa* from its congenerics around Pliocene. Diversification estimates of main haplogroups show signals of major lineage divergences through Quaternary period. Moreover, splits resulted in more shallow structuring during more recent glacial times covering the last 780.000 years appear to play a key role in the geographic distribution of genetic diversity of *C. divisa* in Turkey.

KEYWORDS: *Cynips divisa*, Cynipidae, Genetic diversity, Geographic distribution, Oak gall wasp.

ÖZET

CYNIPS DIVISA (HYMENOPTERA: CYNIPIDAE)'NIN TÜRKİYE'DEKİ GENETİK ÇEŞİTLİLİĞİNİN COĞRAFİK DAĞILIMI

YÜKSEK LİSANS TEZİ

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Meşelere bağımlı olan *Cynips divisa* Palearktik bölgenin batısında geniş dağılış gösteren bir gal arısı türüdür. Bu çalışmada eşeysiz jenerasyon gallerinden elde edilen erginler i) türün ülkemizdeki genetik çeşitliliğini tespit etmek, ii) türün genetik çeşitliliğinin coğrafik olarak ne şekilde dağılış gösterdiğini belirlemek (tür içi filocoğrafya), iii) populayonların ne şekilde farklılaştığını belirlemek, iv) türün mevcut filogenetik ve filocoğrafik yapılanmasına neden olan muhtemel faktörleri araştırmak, v) elde edilen bulguların değerlendirilerek ülkemizde ve Palearktik'te çalışılan diğer gal arılarıyla karşılaştırılarak genel bir değerlendirilmesinin yapılması amacıyla kullanılmıştır.

Yirmi iki lokasyondan toplanan 278 ergin bireyden total DNA izolasyonu yapılarak mitokondriyal sitokrom b geninin bir kısmı ile çekirdek genomundan ITS2 bölgesinin tamamı çoğaltılmış, dizilenmiş ve analiz edilmiştir. Elde edilen dizilerden 115 haplotip ve 15 alel belirlenmiştir. Yüksek oranda genetik çeşitlilik sergileyen türün ortalama genetik çeşitliliği sitokrom b geni ve ITS2 bölgesi için sırasıyla h= 0.81818, π= 0.01197 ve h= 0,31623, π= 0,00167 olarak belirlenmiştir. Filogenetik bulgular *C. divisa* türünün dışgrup olarak kullanılan diğer cinsdeş türlerden Pliosen'de ayrıştığını ifade etmektedir. Türün kendi içindeki ana haplogruplarının farklılaşması ise Kuaterner dönemine işaret eder. Ülkemizdeki *C. divisa* türünün genetik çeşitliliğinin coğrafik dağılımını belirleyen temel etmenlerin özellikle son 780.000 yıllık zaman diliminde oldukça önemli olduğu tahmin edilmektedir, bu dönemin yansımalarını türün temel soy hatlarının gruplanmasında görmek mümkündür.

ANAHTAR KELİMELER: *Cynips divisa*, Cynipidae, Genetik çeşitlilik, Coğrafik dağılım, Meşe gal arısı.

TABLE OF CONTENTS

Page

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 Oak Gall Wasps (Hymenoptera, Cynipidae, Cynipini) 3
1.2 The Study Species: *Cynips divisa* (Hartig, 1840) 4
1.3 Why Turkey is important for Biodiversity? 5
1.4 Molecular Phylogeography: Commonly used Markers 7

2. AIMS AND SCOPE OF THE THESIS **9**

3. MATERIALS AND METHODS **10**

3.1 Sampling *Cynips divisa* 10
3.2 DNA Isolation 13
3.3 Polymerase Chain Reaction (PCR) 15
3.3.1 Amplification of the mtDNA Cytochrome b Gene 15
3.3.2 Amplification of the ITS2 Region 16
3.4 Sequencing 17
3.5 Data Analysis 18
3.5.1 Sequence Alingment and Determination of Unique Sequences 18
3.5.2 Genetic Diversity Indices 18
3.5.3 Phylogenetic and Phylogeographic Analysis, Times of Divergence 20

4. RESULTS **22**

4.1 Diversity Indices of *Cynips divisa* 22
4.1.1 Cytochrome b Gene Diversity 22
4.1.2 ITS2 Region Diversity 41
4.2 Population Demographic Analysis 46
4.2.1 Population Differentiation 55
4.3 Phylogenetic and Phylogeographic Analysis 60

5. DISCUSSION **72**

5.1 Genetic Diversity Estimates of the Turkish Populations of *Cynips divisa* 72
5.2 Historical Demography of *Cynips divisa* 74
5.3 Geographic Distribution of Genetic Lineages: Implications of Phylogenetic, Network and Statistical Analyses 76

6. CONCLUSION **80**

7. REFERENCES **81**

8. CURRICULUM VITAE 93

LIST OF FIGURES

Page

Figure 3.1. The study species, *C. divisa* gall. 11
Figure 3.2. Sampling sites for *C. divisa*. 11
Figure 3.3. Isolated DNA samples from *C. divisa* individuals. . 14
Figure 3.4. An amplified 433 basepairs fragment of the mitochondrial cyt b gene of *C. divisa*. 16
Figure 3.5. Amplified ITS2 segment of nDNA from *C. divisa* individuals. 17
Figure 3.6. A representative chromatogram of the cyt b gene of an Aydın sample. 19
Figure 3.7. A representative chromatogram of the ITS2 region of a Malatya sample. 20
Figure 4.1. Mismatch distribution of all pairwise combinations for the cytb haplotypes. 48
Figure 4.2. Mismatch distribution of populations with their own haplotypes for cyt b data set.. 49
Figure 4.3. Mismatch distribution of all pairwise combinations for the ITS2 alleles. 52
Figure 4.4. Mismatch distribution of populations with their own haplotypes for ITS2 data set. 53
Figure 4.5. MP 50% majority rule consensus tree of consensus tree of the cyt b haplotypes of *C. divisa*. 62
Figure 4.6. Maximum Likelihood 50% majority rule consensus tree of the cyt b haplotypes of *C. divisa*. 63
Figure 4.7. Beast consensus tree of cytb haplotypes with the node ages and posterior probability values are shown at relevant node. 65
Figure 4.8. Minimum spanning tree of the cyt b haplotypes of *C. divisa*. 67
Figure 4.9. Maximum parsimony 50% majority rule consensus tree of the ITS2 alleles of *C. divisa*. 69
Figure 4.10. Maximum likelihood 50% majority rule consensus tree of the ITS2 alleles of *C. divisa* (-lnL= 484.6991). 70
Figure 4.11. Minimum spanning tree of the ITS2 alleles of *C. divisa*. 71

LIST OF TABLES

Page

Table 3.1. Sampling localities, coordinates, and sampling size for each population of *C. divisa*. 12
Table 4.1. Polymorphic characters of 115 cyt b haplotypes of *C. divisa*. 23
Table 4.2. Maximum composite likelihood estimates for cyt b region. 27
Table 4.3. Polymorphic amino acid sites of *C. divisa*. 28
Table 4.4. Cytb haplotype frequencies in each location of *C.divisa*. 30
Table 4.5. Pairwise comparison of 115 *C. divisa* haplotypes. 33
Table 4.6. Sample size, haplotype number, haplotype (h) and nucleotide (π) diversity for cyt b gene and ITS2 region. 40
Table 4.7. ITS2 alles of *C. divisa.* Only polymorphic sites are shown. 41
Table 4.8. Maximum composite likelihood estimates of the pattern of nucleotide substitution for ITS region of *C. divisa*. 42
Table 4.9. ITS2 allele frequencies of *C.divisa*. 44
Table 4.10. Allelic differences ranged from 1 base (0.4%) to 7 bases (2.9%) 45
Table 4.11. Population demographic analysis of the cyt b region 47
Table 4.12. Population demographic analysis of the ITS2 region of *C. divisa* 52
Table 4.13. Fst analysis of cyt b region representing pairwise genetic variations between populations. 57
Table 4.14. Fst analysis of ITS2 region representing pairwise genetic variations between populations. 58
Table 4.15. AMOVA results of ITS2 segment with respect to different groupings. 59
Table 4.16. AMOVA results of cyt b gene where all populations were grouped separately. 59

LIST OF ABBREVIATIONS AND SYMBOLS

^{0}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
ddH_2O	: Double-distilled water
dNTP	: Deoxynucleotide triphosphate
EDTA	: Ethylenediaminetetraacetic acid
EtOH	: Ethanol
GTR	: General Time Reversible
G	: Guanine
h	: Haplotype diversity
HI	: Homoplasy index
HKY	: Hasegawa, Kishino and Yano
Hri	: Harpending's raggedness index
I	: Proportion of Invariable Sites
ITS2	: Internal transcribed spacer 2
Kb	: Kilobase
MACA	: Most ancient common ancestor
MCMC	: Markov Chain Monte Carlo
$MgCl_2$	: Magnesium chloride
ML	: Maximum likelihood
mg	: Milligram
mM	: Milimolar
mm	: Milimeter
MP	: Maximum parsimony
MRCA	: Most recent common ancestor
mtDNA	: Mitochondrial DNA

MYA : Million years ago

NaAce : Sodium acetate

NaCl : Sodium chloride

nDNA : Nuclear DNA

PAUP : Pylogenetic analysis using parsimony program

PCR : Polymerase chain reaction

Ph : Potential of hydrogen

r : Raggedness ratio

rpm : Revolutions per minute

SSD : Sum of squared deviations

SDS : Sodium Dodecyl Sulfate

T : Thymine

U : Uracil

UV : Ultraviolet

Vol : Volume

Γ : Gamma

λ : Lambda DNA

µl : Microliter

π : Nucleotide diversity

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1. INTRODUCTION

Earth houses millions of plant and animal species with outnumbered interactions between these two living groups. One of such interactions arose between oak trees and other organisms eventually ending up with the emergence of one of the most complicated and spectacular formations known as galls. Galls have attracted great attention since ancient times. The first mention of galls was by Theophrastus (371-286 BC), in his publication he talked about some gall species (Senn, 1942; Thanos, 2005). Gall materials have been used by diverse cultures. During middle centuries gall ink extracted from variety of galls were used for taking notes and writing some official documents. For instance, Leonardo da Vinci, Rembrandt and Van Gogh used gall ink in their art and paintings. Thomas Jefferson penned Declaration of Independence with gall ink (Kolar et al., 2006). In fact, some of the human history was put down on paper with gall ink. Galls were also used for medical treatments; Sumerians and Egyptians utilized galls for the cure of dysentery and malaria (Ekici, 1975). In human history, galls were used as commercial property, and were important part of the trade in some major trading centers such as Syria, Iraq and Lebanon in the eastern Mediterranean region, and according to rumor thousands of camel-loads of galls were sold in a regular trade (Niebuhr, 1780).

In more recent times, galls have got considerable interest from the scientific community due to complicated and amazing phenotypic variation and diversity exist in the galls and gall forming organismal groups. Researchers interested in answering questions regarding first correct definition of galls and then their formation. In early times, galls were thought as impotent organisms formed by plants to adapt against environmental fluctuations. Later, two competing hypotheses have been put forward to explain galls and galling process. In the first hypothesis, it was proposed that galls are nonsense organisms affected only plants and induced by galler; however, the second hypothesis suggested that the galls and plants have beneficial life conditions in a mutualistic way (Price et al., 1987). The most recent explanation stated that a gall is an abnormal tissue growth formed by the plant as a response to parasitic attack of some organisms such as nematodes, fungi, bacteria, viruses, mites, and insects (Dreger-Jauffret, 1992). Current recognition is that galls are structures resulting from

interaction between gall inducing organisms and plants, and in that relationship gallers are thought as parasites, and plants are the hosts trying to manage that parasitic attack with the least damage (Price, 2005).

Galls as abnormal vegetative swellings are formed in various parts of host plants such as branches, leaves, twigs, and roots. Their form ranges from a simple leaf-fold to very complex structures. Evolutionarily the most developed galls include one (uni- or monolocular galls) or many (multilocular galls) larval chambers. The larval chamber(s) is surrounded by a thin layer of nutritive tissue, an outer layer of parenchyma, and sclerenchyma (Stone and Schönrogge, 2003). Inner gall structure shows similarity as compared to the outer gall phenotype, which shows great variation: from sticky to non-sticky, hairy or spiny to smooth surface, soft and succulent to dry and heavily lignified forms (Stone and Cook, 1998). Variation in external gall phenotype is explained by defense of the gallers against possible parasitoid attack (Stone et al., 2002).

Despite variation to expound gall phenotype differences, inner gall similarities led scientists to come up with three competing hypotheses: the nutritive hypothesis, the microenvironment hypothesis, and the enemy hypothesis. The first hypothesis proposed that the gall provides nutrition to developing larva(e) inside the larval chamber. The tissue in the larval chamber is rich in nutrients necessary for developing offspring while it has low defensive value (Rohfritsch, 1992). In this case, gall inducer affects plant tissue to make convenient home for larva(e) as it modifies plant tissue (Price et al., 1987; Ronquist and Liljeblad, 2001). The second hypothesis states that gall plays a key role to protect larva(e) against external fluctuations in the surroundings. The last hypothesis argued that gall structures have changed its construction to reduce mortality by natural enemies (Cornell, 1983).

In all types of evolutionarily developed-galls, gall formation steps are similar: initiation, growth, and maturation. The galler release salivary or ovipository secretion and manipulate host plant to produce gall for the benefit of larva (Stone et al., 2002). Initiation phase begins with oviposition of female on the proper site of plant, immediately after oviposition initiation phase starts. Studies have shown that plant chemicals such as auxins, zeatin, indole acetic acid and other cytokinins are essential for gall formation. During the growth stage, neighboring cells of egg undergo

necrosis and begin to proliferate. When the proliferation process ends, maturation phase begins (Giron et al., 2016). During maturation phase, the gall acts as if it is a main source for mineral and photoassimilates (Bagatto and Shorthouse, 1994). In this period, outer gall tissues synthesize tannins and phenolics that are send away to the free-feeding larva(e) (Abe, 1995, 1997; Schönrogge et al., 1994). Further in the maturation phase, cell division rate is decreased, in this step larva grazes nutritive cells in the larval chamber, feeding continues until larva reaches sclerenchyma layer of the larval chamber. Meanwhile, intestine of larva, that was closed throughout entire stages, opens for defecation. At this stage, pupa starts to turn to adult. After reaching maturity adult makes an exit hole to climb out of the gall, and it leaves the gall (Shorthouse, 1993).

1.1 Oak Gall Wasps (Hymenoptera, Cynipidae, Cynipini)

Among other gall inducing organisms, insects are the dominating group with nearly 13000 described species (Lijleblad and Ronquist, 1998; Ronquist, 1999). Three major groups of gall inducing insects are aphids, gall midges, and gall wasps. Gall wasps under superfamily Cynipoidea (Hymenoptera: Cynipidae) are the second largest group after gall midges (Diptera: Cecidomyiidae) (Stone and Schönrogge, 2003). Cynipidae is divided into 5 tribes: Tribe Aylacini (herb gall wasps), Diplopedini (rose gall wasps), Eschatocerini (acacia gall wasps), Pediaspidini (sycamore fall wasps), and the Cynipini (oak gall wasps) (Ronquist, 1995, 1999). Members of the tribe Cynipini induce their galls on host plant family Fagaceae, particularly on oaks (*Quercus*) (Abe et al., 2007).

Oak gall wasps were proposed to coevolve and diverge together with their oak hosts. Recent studies suggested a diversification event of *Quercus* occurred in Eastern Asia (Acs et al., 2007), so the most probable origin of oak gall wasps might also be in East Asia (Abe et al., 2007). Together with their oak hosts, gall wasp origination might have had occurred most possibly during Paleocene (65-56 MYA) or Eocene (56-35 MYA) (Cannon and Manos, 2003). After distribution from Asia, it is assumed that one of the lineages dispersed into North America via the Beringian Land bridge (Thorne, 1993; White et al., 1997). Oaks occurred in North America

from the Eocene and Palearctic white oaks probably originated from North America (White et al., 1997). Oaks in the section Cerris and Quercus disperse to Western Palearctic through mountainside of Himalaya and temperate highlands of Central Asia during Pliocene about 5 MYA (Manos and Stanford, 2001), however there are some ongoing arguments on these matters. Despite some uncertainties in the origin of both oak hosts and oak gall wasps, as their obligate parasites, one point is clear; oak species diversity drives oak gall wasp species diversity (Abe et al., 2007).

With nearly 1360 species classified in 40 genera, oak gall wasps show worldwide distribution, however they are predominantly found in the northern hemisphere (Liljeblad et al., 2011; Liljeblad and Ronquist, 1998). However, species richness is higher in the Nearctic with approximately 700 species in 10 genera compared to the Palearctic with nearly 163 species in 10 genera (Rokas et al., 2003). Located in the western Palearctic Turkey is rich in oak taxa; it has 6.476.277 he oak forestry area with 20 oak species and their possible hybrids. This host richhness is paralleled with oak gall wasp diversity; a recent study reported that there are 148 gall wasp species in 25 genera from Turkey (Azmaz and Katılmış, 2017).

1.2 The Study Species: *Cynips divisa* (Hartig, 1840)

Cynips divisa (Hartig, 1840) (Hymenoptera: Cynipidae) is an oak gall wasp species with the common name red-pea gall or red currant gall. The species is distributed across East and Central Europe, Iberian Peninsula, Russia, and Caucasus (Melika, 2006). It shows widespread distribution in Turkey and prefers *Quercus petraea*, *Q. pubescens*, *Q. robur*, *Q. frainetto*, and *Q. macrenthera* as host (S. Mutun and E. Çimen Pers. Obs).

Cynips divisa is a heteroecious and heterogonic species with asexual and sexual generation. Sexual generation galls are pubescent with conical shape, and located on the margins of leaves, sometimes occurring on catkins. Its galls resemble lemon shape with hairy surface. Size of sexual generation galls of *C. divisa* is 3-3.5 mm x 2-2.5 mm on leaves, and 4-6 mm long on catkins. Initially galls are green and, later in the development they turn into reddish. Sexual generation galls develop in the spring and show up in May (Melika, 2006).

Asexual generation galls appear in globular shapes and located underside of leaves on the main or major lateral veins. They are found as solitary galls, up to 20 galls may occur on one leaf with small wart on the upper surface and flattened base. The size of asexual *C. divisa* gall is between 4-6 mm. Initially they appear greenish-yellow, and later they turn into reddish coloration, when they became mature they are shining and pale brown. Wall of the gall is thick and woody, its larval chamber is monocular. Asexual generation galls develop from June to September, and adults emerge in October/November or February /March of the following year (Melika, 2006).

1.3 Why Turkey is important for Biodiversity?

Biodiversity refers to the presence of variety of life forms on Earth and is considered at various levels of biological hierarchy from genes to species and ecosystems. Genetic, organismal and ecological diversity are all parts of biodiversity, and it is not distributed evenly over the world (Gaston and Spicer, 2013). Some parts in the world are with higher biologic diversity than the others, leading to some biodiversity hotspots. Turkey is currently accepted as one of the 36 hotspot areas in the world (Gür, 2016), bearing high number of plant and animal species with remarkable endemism observed in both insects (Çıplak and Demirsoy, 1995, 1996) and plants (Médail and Quézel, 1999). There are about 80.000 animal and 11.000 defined plant species in Turkey and 4000 plant taxa are endemic to Anatolia (Davis, 1984, 1988; Güner et al., 2000). The existence of such high species diversity is accompanied by substantial amount of genetic variation in the examined species thus far. This high biologic diversity in Turkey was explained by several factors.

Firstly, its unique location as a natural land bridge between Europe and Asia, and Africa allowed Turkey to have species from these continents, in fact several times faunal exchanges have occurred since Miocene (Demirsoy, 1999; Şekercioğlu et al., 2011). Secondly, the region where Turkey is located has complex geologic history which was shaped mainly by geological events such as transgression, tectonic and orogenesis activities (Okay, 2008; Rögl, 1999; Şengör and Yilmaz, 1981). Particularly, when Indo-Arabian Plate collided with Eurasian plate major mountain

ranges started to form, eventual formation of Turkey occurred around this and the subsequent times. Alps, Albania, Greece, the Dinaric and Hellenic Mountains of Yugoslavia and in Turkey Taurus Mountains were formed because of these ongoing collisions and orogenesis events (Bozkurt, 2001). Consequently, together with the Taurus Mountains, highlands of the eastern part of Anatolia, and some other individual mountains became remarkable and effective geographic barriers for many taxa creating species/lineage/genetic break for them (Çıplak et al., 1993; Davis, 1971; Ekim and Güner, 1986).

Thirdly, Turkey is situated on three phytogeographic regions (the Irano-Turanian, Mediterranean and the Euro-Siberian) (OGM, 2014). It is well-known that varied habitats are one of the key factors for supporting higher diversity. Moreover, heterogenous topography even in short geographic distances creating more diverse microhabitats is vital for biologic diversity, that is exactly what is observed in Turkey (Şekercioğlu et al., 2011).

Lastly, climatic oscillations of the Quaternary period (between 2,5 MYA-12.000 YA) were another factor shaping both species and lineages in Turkey. There were sixteen glacial periods in the last 2.4 MY (Webb and Bartlein, 1992). Particularly, the last four glacial periods (Günz, Mindel, Riss, and Würm) during Pleistocene influenced greatly faunal composition of Anatolia and surrounding area. During glacial cycles of the Quaternary period northern latitudes were covered by large glaciers, nonetheless the places that were not covered by ice sheets, temperature dropped drastically (Hewitt, 1996). Studies particularly conducted in the 20th century in the Palearctic area highlight the key role of Turkey as a shelter for those species distributed in northern latitudes. While Turkey as a place not covered by glaciers faced with some temperature decreases, it was with relatively mild climate still allowing many species to survive in the area, thus acting as a shelter for many plant and animal taxa. Therefore, in addition to the Iberian, Italian, and Balkans refugium, Turkey is accepted as the fourth refugium that allowed survival of species when species shifted their range to Anatolia during the cold periods of the glacial cycles (Hewitt, 1999).

1.4 Molecular Phylogeography: Commonly used Markers

Phylogeography as a field in biology refers to the geographic distribution of genetic lineages across the studied range of a taxon (Avise et al., 1987). Intraspecific phylogeography, is interested in the phylogeographic patterns and processes as it attempts to explain contemporary geographic structuring of genetic diversity across the sampled area of a species. This attempt takes historical and ecological factors into consideration that created current phylogeographic patterns. It is now well-known that either physical or ecological barriers to gene flow within the geographic range of a species is highly effective factors for creating such phylogeographic patterns (Avise, 2000).

Twentieth century has witnessed great advances in scientific methods and applications of molecular techniques, so that many mysterious questions related to life were begun to have some answers. As a matter of fact, utilization of some areas in a combined fashion such as molecular biology, phylogenetics, systematics and phylogeography helped us to study organisms in detail and to reveal their phylogeographic patterns (Chapco, 1987). Thus, one of the current trends is to use a combination of molecular markers in phylogeographic and phylogenetic studies. Accordingly, it is often preferred to use mitochondrial and nuclear DNA together to investigate relationships between populations, species and taxa (Avise, 2000).

In entomological studies including oak gall wasps (Atkinson et al., 2007; Cook et al., 2002; Liljeblad and Ronquist, 1998; Rokas et al., 2003; Stone et al., 2008; Stone et al., 2007) mitochondrial DNA has been used as molecular marker to better understand species and populations (Cameron, 2014), because mitochondrial DNA as a molecular marker provides resolution at the recent past as far back as 2 to most 5 million years (MY). This feature is provided by its clonality, neutrality, and clock-like mutation rate (Avise, 2004). Mitochondrial DNA is around 16-20 kilobases (Kb) in size, and maternally inherited circular genome with 37 genes coding for proteins and a A+T-rich region in insects (Harrison, 1989; Hoy, 2003). These 37 genes are consisted of 22 tRNA, 2 rRNA and 13 protein coding genes (Lemire, 2005). It does not show recombination and, there is no known repair mechanism (Caterino et al., 2000). Due to all these features, its effective population

size is one fourth of the nuclear DNA (Sun et al., 2009). One of the protein coding genes of mitochondrial genome is the cytochrome b gene (cytb) which is often employed in phylogeographic studies for revealing single nucleotide polymorphism, population genetic structure, and differentiation among populations of especially animals (Kartavtsev and Lee, 2006). Besides, insect specific primer pairs encompassing the gene is known, and it is one of the most frequently sequenced gene, so that necessary information for the taxon-wide comparisons is possible (Jermiin and Crozier, 1994).

Combining mtDNA cyt b gene data together with nuclear DNA (nDNA) is suggested over using only a single gene because both markers cover a larger time frame in the history of the taxon due to their different inheritance pattern and mutation rates (Parker et al., 1998). Since nDNA is biparentally inherited genome it undergoes recombination and contains many genes and non-coding regions that can serve different purposes in phylogenetic and molecular evolutionary studies (Loxdale and Lushai, 1998). On the other hand, using internal transcribed spacer 2 (ITS2) of nuclear ribosomal DNA has been proven as a useful molecular marker due to its power at the species and population level. In fact, it is used as a nuclear barcode in animals due primarily to its rapid pace of evolution (Ji et al., 2003). Using both makes it possible to compare their results to address some questions at the intraspecific and up to genus level, thus making both markers useful. Therefore, in this thesis using the two markers in combination was preferred over employing only a single gene.

2. AIMS AND SCOPE OF THE THESIS

The red pea gall, *Cynips divisa*, was chosen as the study material of this thesis because it is a widespread gall wasp species across Turkey, and there is no detailed study on this species yet. Its wide distribution makes this species a suitable candidate for studying molecular diversity, geographic distribution of the existing genetic variation, phylogeography, and population genetics. Therefore, major goals and main objectives of the thesis are: i) to reveal genetic diversity of the species in Turkey, ii) to disclose geographic distribution of genetic diversity (intraspecific phylogeography) across the sampled area, iii) to reveal genetic differentiation between and among its populations, iv) to search for possible factors that created contemporary phylogeographic and phylogenetic pattern (if there is any), and evaluate the results.

3. MATERIALS AND METHODS

3.1 Sampling *Cynips divisa*

Only asexual generation galls (Figure 3.1) were collected during the late summer and early fall seasons between 2010-2017. More than 300 galls were collected from 22 localities in Turkey (Figure 3.2, Table 3.1), however only 278 *C. divisa* adults were used in this thesis. Sampling size for some localities are low because either enough number of galls were not found at some visited sites or we were not able to rear adults from the galls that we collected.

After sampling from the field, galls were brought to the Molecular Zoology Laboratory, Abant İzzet Baysal University, in Bolu. They were put in separate jars and covered with tulle. They were hold at room temperature and checked daily for emerged adults. After hatching from the galls, adults were removed and transferred to deep freezer, and kept at -80 ºC until DNA isolation. *Cynips quercus* Fourcroy (1785) and *Cynips longiventris* Hartig (1840) were used as outgroups in all phylogenetic analyses.

Figure 3.1. The study species, *C. divisa* gall.

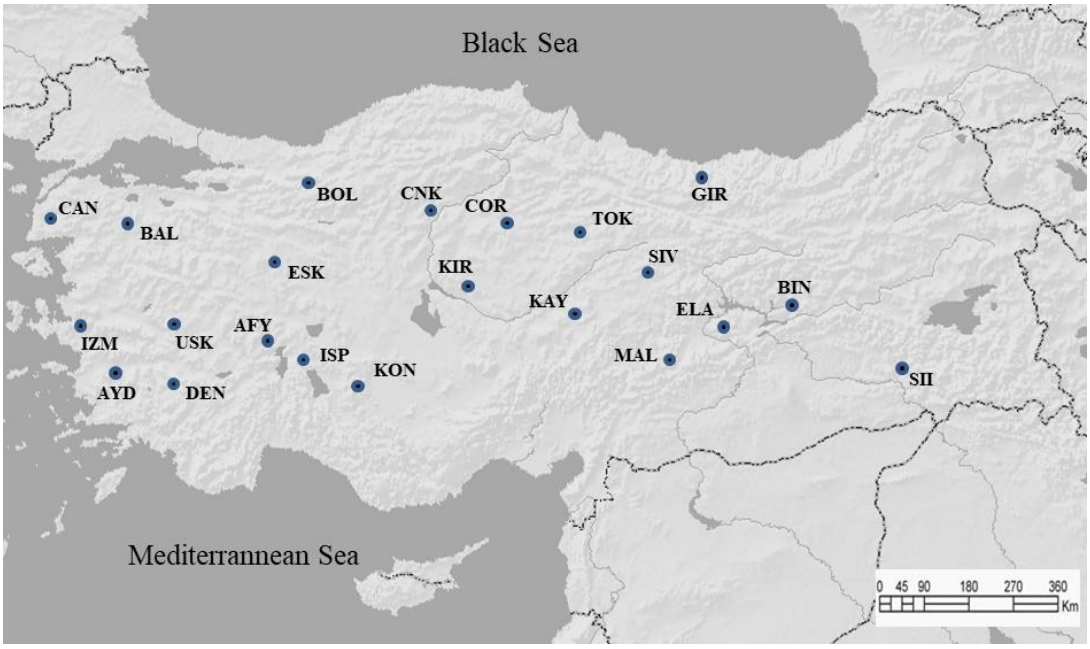

Figure 3.2. Sampling sites for *C. divisa*.

Table 3.1. Sampling localities, coordinates, and sampling size for each population of *C. divisa*.

Location	Coordinates	N	Location	Coordinates	N
Afyon	N 38° 795.79′ E 30° 058.71′	13	Giresun	N 40° 28.991′ E 38° 81.428′	11
Aydın	N 37° 563.55′ E 28° 356.33′	13	Isparta	N 37° 748.80′ E 31° 187.44′	7
Balıkesir	N 40° 175.97′ E 28° 087.54′	14	İzmir	N 38° 050.55′ E 28° 118.95′	5
Bingöl	N 38° 928.56′ E 40° 367.98′	15	Kayseri	N 38° 586.06′ E 36° 420.05′	20
Bolu	N 40° 57.086’ E 35° 48.006′	14	Kırşehir	N 39° 477.91′ E 33° 875.84′	16
Çanakkale	N 39° 583.86′ E 26° 526.60′	15	Konya	N 37° 549.39′ E 31° 462.94′	20
Çankırı	N 40° 50.220′ E 33° 25.260′	15	Malatya	N 38° 991.07′ E 37° 816.19′	6
Çorum	N 40° 206.19′ E 35° 219.03′	14	Siirt	N 38° 127.10′ E 41° 675.98′	5
Denizli	N 38° 047.30′ E 28° 782.22′	13	Sivas	N 39° 935.21′ E 37° 843.76′	14
Elazığ	N 38° 539.07′ E 38° 755.38′	13	Tokat	N 40° 44.712′ E 37° 31.269′	15
Eskişehir	N 39° 641.36′ E 31° 528.02′	5	Uşak	N 38° 534.06′ E 29° 664.65′	15

3.2 DNA Isolation

Total genomic DNA was isolated from single adult specimens using manual DNA isolation method routinely employed in entomology.

a) Individual gall wasps were put in 1.5 ml ependorf tubes separately and crushed gently, 200 µl of extraction buffer (10 mM Tris, pH 8.0, 2 mM EDTA, pH 8.0, 10 mM NaCl and 1% SDS) was added to each tube.
b) Specimens were chopped on ice and additional 800 µl of extraction buffer was added to each tube, and after adding ~0.4 mg of Proteinase K tubes were vortexed with Velp Scientifica model vortex.
c) Samples were incubated at 55 ºC in a JP Selecta SA model shaking water bath for overnight.
d) Next morning, tubes were taken from water bath and removed supernatant carefully and transferred into a new tube.
e) Some volume of phenol (lower level of buffer saturated with phenol) was added, and vortexed carefully, and upper part of the mixture was taken (This step was repeated twice).
f) Same volume of chloroform iso-amylalcohol (25:24:1) was added to each tube and the mix was vortexed and supernatant was taken.
g) Sodium acetate (3M, Ph:5.2) was added to the tubes as following: volume of sodium acetate = (volume of liquid + 4) + 2.25.
h) 100% EtOH was added to tubes as follow; Volume of 100% EtOH = (Vol of liquid + Vol of NaAce) x 2 and mixed well.
i) The tubes were incubated at -70 ºC for 60 min.
j) Centrifuged for 15 min at 14000 rpm and supernatant were poured out.
k) 1 ml of 70% EtOH was added to tubes, and spinned for 10 min at 10000 rpm, and poured out supernatant.
l) Depending on the size of pellet, DNA samples were dissolved with sterile double distilled water (e.g. 100µl of big pellet which covers bottom for tube; 3 µl for tiny pellets).
m) In the final stage, all DNA samples were kept on ice or -20 ºC until agarose gel electrophoresis.

A 5 µl of DNA sample was run on agarose gel to ensure DNA extraction and to check the quality of samples. For this purpose, each extracted DNA sample was run in 1% agarose gel electrophoresis with a 1 Kb Lambda DNA molecular marker (Sigma D3937). Running buffer contained 1X TBE (0,089 M), Tris (Sigma T1503), 0,089 M Boric acid (Sigma B6768), 0,001 M disodium EDTA (Sigma E5134). Gel was run for 1 hour at 100 volts (V) and 50 mA. An agarose gel photograph of DNA samples of *C. divisa* specimens was shown in (Figure 3.3).

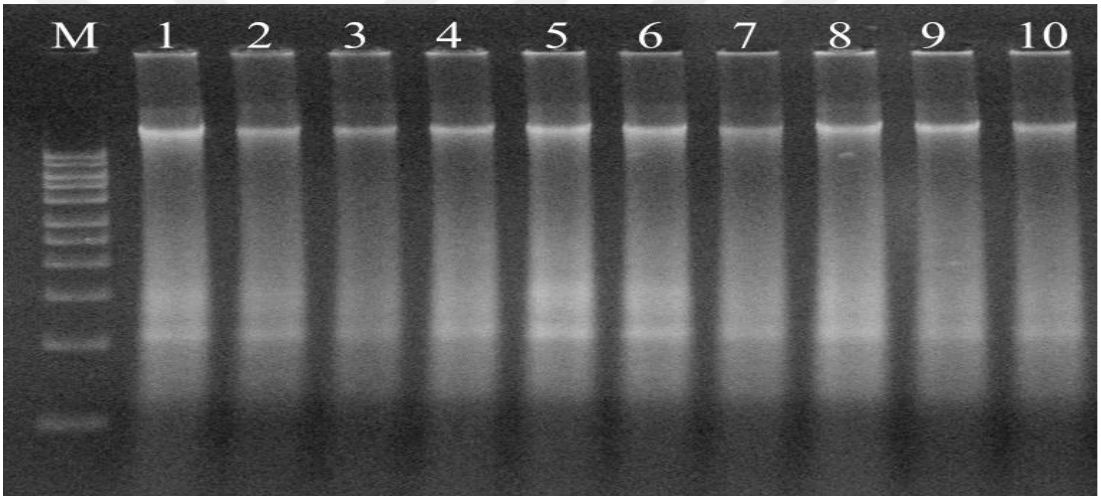

Figure 3.3. Isolated DNA samples from *C. divisa* individuals. M. 1 Kb Molecular marker, 1-3: Giresun, 4-7: Malatya, 8-10. Bingöl.

3.3 Polymerase Chain Reaction (PCR)

All amplification procedures were carried out in a Bioer XP model thermal cycler. Vivantis (PL1202) brand Taq polymerase packs were used for amplification, which include DNA polymerase, dNTP, $MgCl_2$, and PCR buffer. Detailed information for the amplification of both mitochondrial cyt b gene and the nuclear ITS2 region is as following.

3.3.1 Amplification of the mtDNA Cytochrome b Gene

After DNA isolation, 433 base pair of mitochondrial cyt b gene was amplified with CB1- 5'- TATGTACTACCATGAGGACAAATATC- 3' and CB2- 5'- ATTACACCTCCTAATTTATTAGGAAT- 3' primers (Simon et al. 1994, Stone et al. 2007). To amplify cyt b segment, each PCR reaction included 1.6 µl of the total DNA, 4.0 µl 10X PCR buffer (Promega), 3.2 µl $MgCl_2$ (25mM), 0.8 µl dNTPs (2mM each), 0.56 µl of each primer (20µM) 0.56 µl of Taq DNA polymerase (Vivantis), and 28.72 µl ddH_20 was added to each reaction to complete final volume of 40 µl. Temperature rates, times and cycles for cyt b region were set as follows: 3 min at 94 ºC, 35 cycles of 30 secs at 94ºC, 1 min at 50 ºC, 2 min at 72 ºC, and a final extension step of 10 min at 72 ºC. Same procedure was used for PCR negative controls (included everything, except DNA). PCR products were visualized on 1 % agarose gel buffered with Tris-Boric acid-EDTA (TBE), stained with ethidium bromide, and checked under UV light and photographed (Figure 3.4).

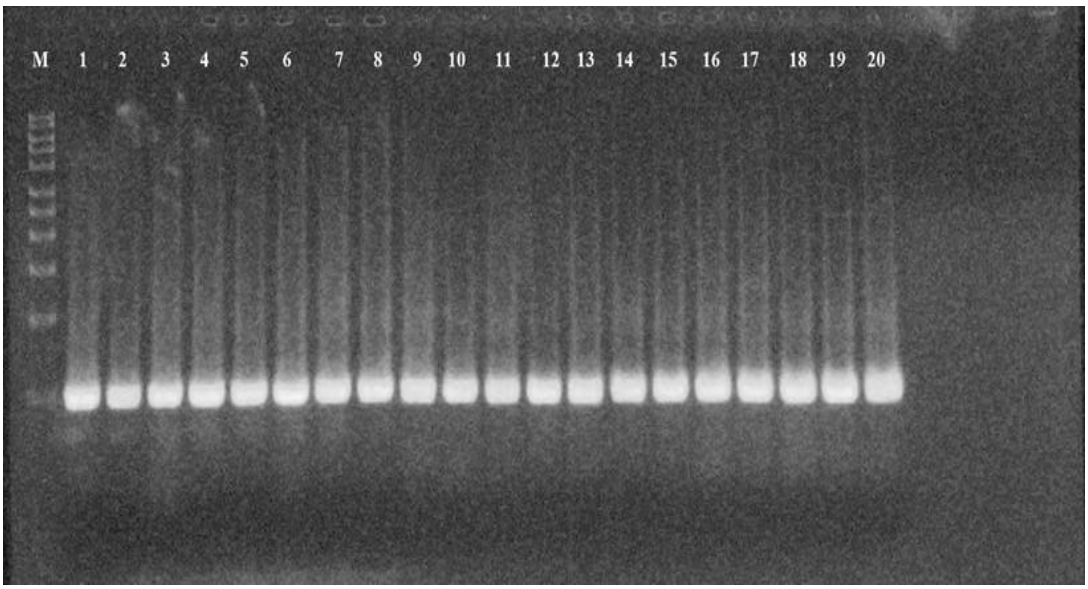

Figure 3.4. An amplified 433 basepairs fragment of the mitochondrial cyt b gene of *C. divisa*. M: 1 Kb marker, 1-3: Kayseri, 4-8: Giresun, 9-13: Siirt, 14-17: Malatya, 18-20: Sivas.

3.3.2 Amplification of the ITS2 Region

ITS2 region of nuclear DNA was amplified from the isolated DNA templates of *C. divisa* using MutITSintF 5′-GTTCGTCGCGTCTCTG-3′ MutITSintR 5′-CCGTCCATAATGGCCAC-3′ primers that were designed in our Laboratory. For the amplification of the ITS2 region 10 µl 5X buffer, 5 µl $MgCl_2$ (25mM), 3 µl dNTPs (10mM), 1 µl of each primers (20 pmol), 0.5 µl template DNA, 0.5 µl Taq polymerase (Vivantis) and 19µl ddH2O were added to each tube. Amplification conditions were as follows: 5 min at 95 °C for first denaturation; 35 cycles of 30 seconds at 94 °C for denaturation, 30 seconds at 52°C for annealing, 30 seconds at 72 °C for elongation, and 5 minutes at 72 °C for last elongation step. For ensuring amplification, PCR products were run in a 1% agarose gel with the 1 Kb λ DNA molecular marker (Sigma, DO428). A gel image is shown in Figure 3.5. All PCR products were kept on -20 °C until sequencing.

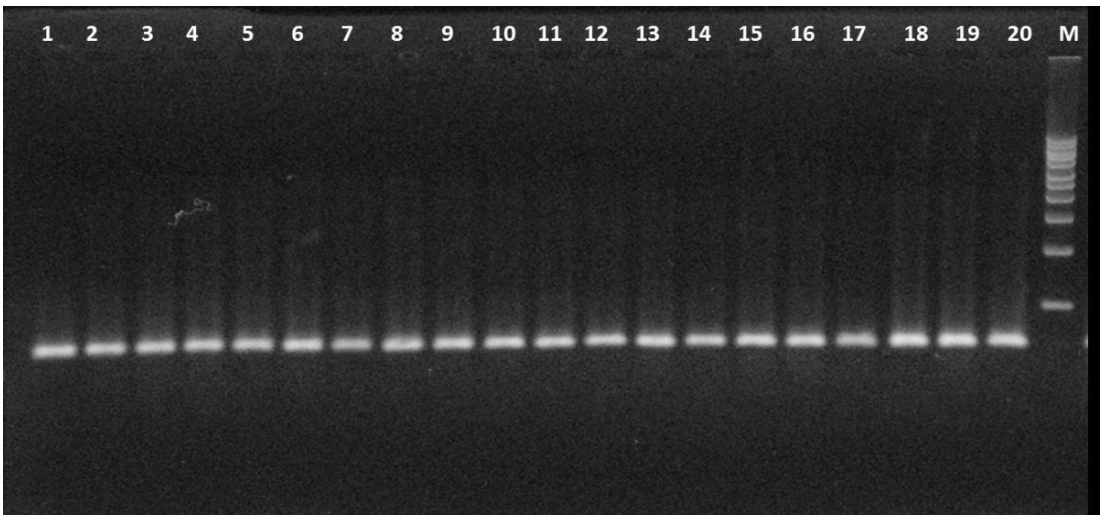

Figure 3.5. Amplified ITS2 segment of nDNA from *C. divisa* individuals M: 1 Kb marker, 1-8: Giresun, 9-14: Kayseri, 16-20: Malatya.

3.4 Sequencing

With great developments in recent years it is now possible to sequence large amount of DNA or even whole genome in short period of time (Loxdale and Lushai, 1998). Different companies are now available for providing sequencing service procurement. Therefore, PCR amplicons were sent to a company and used as template in the following cycle sequencing reactions using Perkin-Elmer BigDye Terminator chemistry on an ABI automated sequencer (Applied Biosystems). Both strands were sequenced in an attempt to minimize PCR artifacts, ambiguities and base-calling errors.

3.5 Data Analysis

3.5.1 Sequence Alingment and Determination of Unique Sequences

Chromatograms of both strands were first checked by eye for the presence of any misreading, subsequently sequences were aligned and trimmed with the program package Geneious 10.2 (Kearse et al., 2012). Unique sequences that could be collapsed into haplotypes/alleles were determined using DnaSP 5.10.1 program (Librado and Rozas, 2009). Representative chromatograms of the cyt b gene and ITS2 region are given in Figure 3.6 and Figure 3.7, respectively. Since cyt b gene is a product coding segment of the mitochondrial genome, all haplotypes were translated into amino acids using DnaSP 5.10.1 to detect non-sense mutations and internal stop codons.

3.5.2 Genetic Diversity Indices

Number of polymorphic sites (S), nucleotide (π) and haplotype (h) diversity (Nei, 1987) number of substitutions and pairwise nucleotide differences (k) (Tajima, 1983) were estimated separately both for the cyt b gene and the ITS region. Population differentiations were estimated using Arlequin 3.5.2.2 (Excoffier and Lischer, 2010) and DnaSP 5.10.1 programs (Librado and Rozas, 2009).

Population demographic analysis of *C. divisa* were conducted through mismatch distribution analysis as implemented in DnaSP 5.10.1 (Librado and Rozas, 2009). Mismatch analyses are based on the observed number of pairwise differences both against constant population size and sudden population expansion models (Rogers and Harpending, 1992). The raggedness index (r) (Harpending, 1994), Tajima's *D* (Tajima, 1989), and Fu's *Fs* (Fu and Li, 1993) were calculated to determine neutrality and the smoothness of pairwise mismatch plot. These tests are used frequently for testing any deviations from neutrality as combinations since Tajima's *D* is not as strong indicator as the Fu's *Fs*. Thus, all tests were run for all data sets of *C. divisa* to have more robust results.

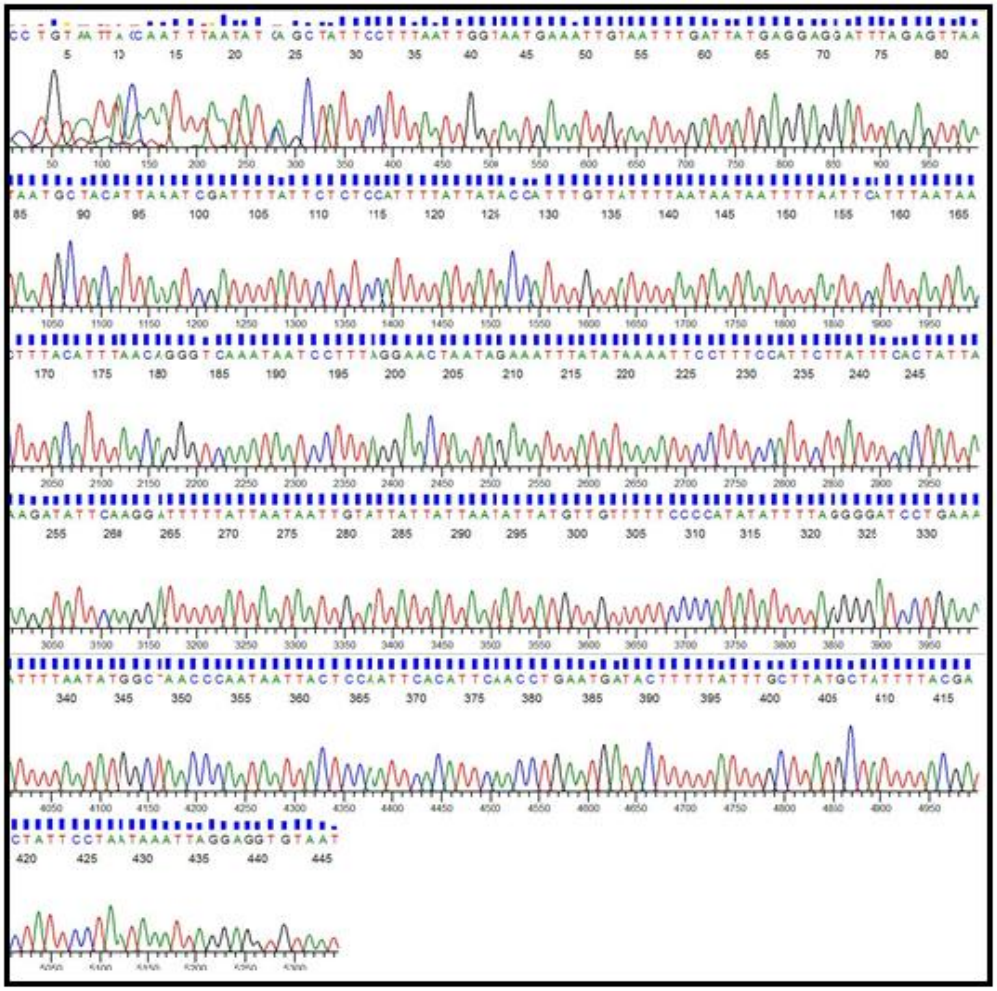

Figure 3.6. A representative chromatogram of the cyt b gene of an Aydın sample.

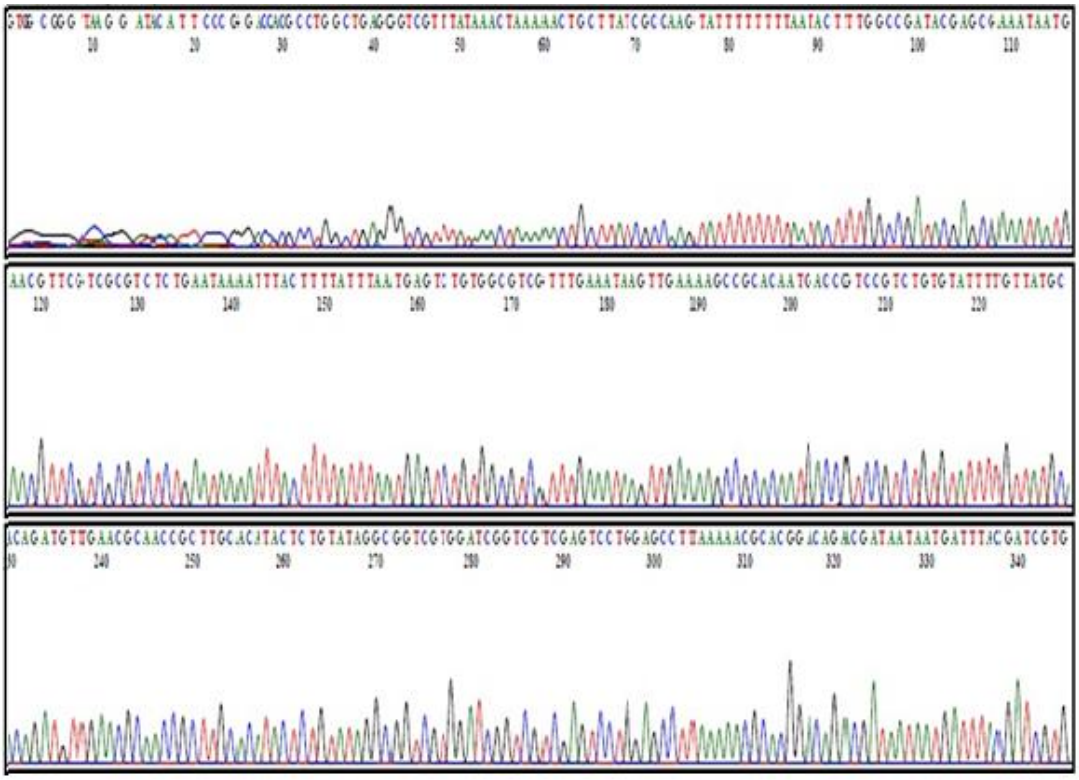

Figure 3.7. A representative chromatogram of the ITS2 region of a Malatya sample.

3.5.3 Phylogenetic and Phylogeographic Analysis, Times of Divergence

In all phylogenetic analysis 278 sequences of *C. divisa* were used to construct data matrices. Nucleotide frequencies, mean transition and transversion ratios were obtained from the PAUP*4.0 package (Swofford, 2002). Three different sets of phylogenetic analyses have been conducted on *C. divisa* data sets: maximum likelihood (ML), maximum parsimony (MP) using PAUP*4.0 package, and Bayesian-based inference implemented in MRBAYES 3.0 (Ronquist and Huelsenbeck, 2003). A heuristic search using TBR branch-swapping algorithm was applied in MP analysis with 1000 replicates of random addition of taxa (Swofford, 2002).

The best fit model of evolution was detected using JModeltest 0.1.1 (Posada and Crandall, 1998), and the obtained models for both regions were used in all likelihood-based analyses. Akaike information criterion (AICc) was used to determine the best-fit substitution model (Posada and Buckley, 2004) for the data in

the ML reconstructions. The substitution model suggested for the analyzed fragments by JModeltest were determined as GTR+I+G for both the cyt b and ITS2 regions.

Divergence times among major lineages together with their confidence intervals were extrapolated using a Bayesian Markov Chain Monte Carlo (MCMC) coalescent approach implemented in BEAST version 1.5.2 (Drummond and Rambaut, 2007). Cyt b gene was calibrated with 2.1% sequence divergence/ lineage/ MY (Papadopoulou et al., 2010). BEAST program was used for estimating divergence dates using GTR model with G and I, a relaxed molecular clock, following the uncorrelated relaxed lognormal clock. BEAST was run for 50 million generations sampling every 1000, and TRACER (Rambaut and Drummond, 2003) was used to check the convergence to stationary and the effective sample size (ESS) of model parameters. TREEANNOTATOR was utilized to reconstruct a maximum clade credibility tree after discarding the first 25% samples as burn-in FIG-TREE version 1.3.1 (Rambaut, 2009) to visualize the results.

Since phylogenetic analyses may not be adequate for revealing genealogical relationships at the population level (Posada and Crandall, 2001), a haplotype network was employed to *C. divisa* data sets. For this purpose, HapStar Version 0.5 (C) (Teacher and Griffiths, 2011) was used to draw networks of haplotypes and alleles. Additionally, AMOVA analysis was run for revealing genetic differentiation at different hierarchical levels for any haplotype/allele groupings. Arlequin 3.5.2.2 program was used to run AMOVA analysis (Excoffier and Lischer, 2010). The analysis was performed at three levels: among groups, among populations within groups, and within populations. Statistical significance was tested through 1000 permutations.

4. RESULTS

4.1 Diversity Indices of *Cynips divisa*

4.1.1 Cytochrome b Gene Diversity

The number of individuals used in this thesis is 278 representing 22 localities across Turkey. All sequences of *C. divisa* would be collapsed into 115 cytohrome b haplotypes. Gen Bank Accession numbers of the haplotypes are MH230991-MH231105. There was neither stop codons nor non-sense mutations in any of the haplotypes, which proved that they were genuine mtDNA sequences. Polymorphic sites are shown in Table 4.1. There were 99 polymorphic and 334 monomorphic sites. Fifty-four substitutions were found in only a single sequence. The number of parsimony informative characters was 45 and remaining characters were parsimony uninformative. All parsimony informative sites were used in subsequent analyses.

Transition/transversion rate ratios are k1 = 28.062 (purines) and k2 = 39.803 (pyrimidines). Overall transition/transversion bias is R = 12.945. Nucleotide frequencies in haplotypes were 33.27% A, 43.37% T, 12.71% C, 10.64% G, respectively. The highest maximum likelihood estimate of nucleotide substitutions were between C/T (47.11) and G/A (25.48) (Table 4.2). Codon positional changes were 98, of which 73 substitutions occurred at the 3rd codon position (74.48%) followed by first (18 substitutions, 18.36%), and second codon positions (7 mutations, 7.14%). There were 11 multiple hits in haplotypes.

Table 4.1. Polymorphic characters of 115 cyt b haplotypes of *C. divisa*.

	37	40	43	46	47	52	55	58	67	70	73	79	82	85	88	90	94	100	103	112	121	124	125	127	139	142	146	154	157	166	170	178	180	184	188	190	193	196	201	205	209	214	223	229	232	238	241	244	247	249
H1	A	T	T	T	T	T	T	T	A	C	A	A	A	A	T	G	T	C	T	T	T	T	C	C	A	A	G	A	A	A	C	A	C	A	T	A	A	G	A	T	T	A	A	A	T	T	T	T	C
H2	.	.	.	.	.	.	.	.	.	T	.	G	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.
H3	.	.	.	.	.	.	.	.	.	T	.	.	.	G	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.
H4	.	A	.	.	.	.	.	.	.	T	.	G	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.
H5	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	A	.	.	.	.	.	.	.	.	.	C	.	.
H6	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.
H7	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H8	.	.	.	.	.	.	.	.	.	T	.	G	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	G	.	.	.	.	.	C	.	.
H9	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.
H10	.	.	.	.	.	.	.	.	.	T	.	G	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.
H11	.	.	.	.	.	.	.	.	.	T	.	G	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.
H12	.	.	C	.	.	.	.	.	.	T	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.
H13	.	.	.	.	.	C	.	.	.	T	.	G	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	T
H14	.	.	.	.	.	.	.	.	.	T	.	G	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	C	.	.
H15	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	A	.	.	.	.	.	.	.	.	.	C	.	.
H16	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.
H17	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.
H18	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	T	A	.	.	A	.	T	G	.	.	.	.	.	A	.	.	.	A	T	.	A	.	.	.	.	.	.	.	.	.	.	.
H19	.	.	.	.	.	.	.	.	.	T	.	G	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.
H20	.	.	.	.	.	.	.	.	.	T	.	.	.	G	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.
H21	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	C	.	C	.	.
H22	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.
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Table 4.1 (cont'd).

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H61	.	.	.	.	.	.	.	.	.	T	.	G	.	.	.	T	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.
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H109	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	C	.	.	.	.	.	.	.	C	.	.
H110	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	C	.	.	.	.	.	.	.	C	.	.
H111	.	.	.	.	.	.	.	.	.	T	.	G	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.
H112	.	.	.	.	.	.	.	.	.	T	G	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	C	C	.	.
H113	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	C	.	.
H114	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.
H115	.	.	.	.	.	.	.	.	.	T	.	G	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.

Table 4.1 (cont'd).

	250	253	256	259	262	268	277	280	281	284	291	293	296	298	302	310	314	316	319	321	322	325	329	334	337	340	343	358	361	364	365	367	370	371	373	376	379	382	385	388	391	397	403	415	418	424	425	427	430
H1	T	T	C	T	C	T	A	T	T	C	T	G	T	A	T	A	T	T	C	T	C	A	G	A	A	T	T	A	T	T	C	A	A	A	T	T	A	T	T	T	A	A	T	T	T	T	T	A	A
H2	.	.	T	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	.	.	G	.	C	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.
H3	.	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.
H4	.	.	T	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	.	.	G	.	C	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.
H5	.	.	T	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	C	.	G	.	C	.	.	.	G	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.
H6	.	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	C	.	G	.	C	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.
H7	.	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	C	.	G	.	C	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.
H8	.	.	T	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	.	.	G	.	C	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.
H9	.	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	G	.	.	G	.	C	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.
H10	.	.	T	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	C	.	G	.	C	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.
H11	.	.	T	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	.	.	G	.	C	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.
H12	.	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	C	.	G	.	C	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.
H13	.	.	T	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	.	.	.	A	.	.	.	.	G	.	C	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.
H14	.	.	T	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	T	.	A	.	.	.	.	G	.	C	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.
H15	.	.	T	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	.	.	G	.	C	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.
H16	.	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.
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H18	.	.	T	.	T	.	.	.	.	T	.	.	C	.	.	.	.	.	T	C	A	.	A	.	.	.	.	.	.	.	T	G	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.
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H20	.	.	T	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	C	.	G	.	C	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.
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H26	.	.	T	.	T	.	.	.	.	.	.	.	.	.	.	.	G	C	T	C	.	.	A	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.
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H28	A	C	.	.	T	.	.	.	.	.	.	.	.	G	.	.	.	.	T	C	A	.	A	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.
H29	.	.	T	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	.	.	.	A	.	.	.	.	G	.	C	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.
H30	.	.	T	.	T	.	.	.	.	T	C	.	.	.	.	.	.	.	T	C	.	.	A	.	.	.	.	G	.	.	.	.	.	.	.	C	.	.	C	.	.	.	C	.	.	.	.	.	.
H31	.	.	.	.	.	.	.	C	.	T	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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H38	.	.	T	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	C	.	.	.	C	.	.	.	.	.	.
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H41	.	.	T	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	.	.	G	.	C	.	.	.	.	.	C	.	.	C	.	.	.	C	.	.	.	.	.	.
H42	.	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	C	.	G	.	C	.	.	G	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.
H43	.	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	.	.	G	.	C	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.
H44	.	.	T	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	.	.	G	.	C	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.
H45	.	.	T	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	.	.	G	C	C	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.
H46	.	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	G	C	.	G	.	C	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.
H47	.	.	T	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	.	.	.	A	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.
H48	.	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	C	A	.	.	C	.	G	.	C	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.
H49	.	.	T	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	.	.	G	.	C	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.
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H51	.	.	T	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.
H52	.	.	T	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	.	.	G	.	C	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.
H53	.	.	T	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	.	.	G	.	C	.	.	.	.	.	.	.	C	C	.	.	.	C	.	.	.	.	.	.
H54	.	.	T	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	.	.	G	.	C	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.
H55	.	.	T	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	.	.	G	.	C	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.
H56	.	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.
H57	.	.	T	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.
H58	.	.	T	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	.	.	.	A	.	.	.	.	G	.	C	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.
H59	.	.	T	.	T	.	.	.	.	T	.	.	.	.	A	.	.	.	T	C	.	.	A	.	.	C	.	G	.	C	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.
H60	.	.	T	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	.	.	G	.	C	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	C	.	.	.

Table 4.1 (cont'd).

	250	253	256	259	262	268	277	280	281	284	291	293	296	298	302	310	314	316	319	321	322	325	329	334	337	340	343	358	361	364	365	367	370	371	373	376	379	382	385	388	391	397	403	415	418	424	425	427	430
H61	.	.	T	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	.	.	G	.	C	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.
H62	.	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	C	.	.	.	C	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.
H63	.	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	G	C	.	.	.	C	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.
H64	.	.	.	.	T	.	.	C	.	T	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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H68	.	.	T	.	T	C	.	.	.	T	.	.	.	.	.	.	.	.	.	C	A	T	.	G	.	.	.	.	.	C	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.
H69	.	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	G	C	.	G	.	C	.	.	.	.	.	G	.	C	.	.	.	C	.	.	.	.	.	.
H70	.	.	T	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	G	C	.	G	.	C	.	.	.	.	.	.	C	C	.	.	.	C	.	.	.	.	.	.
H71	.	.	.	.	.	.	.	C	.	T	.	.	.	.	.	.	.	.	T	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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H73	.	.	T	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	.	.	G	.	C	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.
H74	.	.	.	.	.	.	.	C	.	T	.	.	.	.	.	.	.	.	.	C	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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H76	.	.	.	.	.	C	.	C	.	T	.	.	.	.	.	.	.	.	.	C	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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H83	.	.	T	.	T	.	.	.	A	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	C	.	G	.	C	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.
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H86	.	.	T	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	.	.	G	.	C	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.
H87	.	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	C	A	G	.	C	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.
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H90	.	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	C	.	G	.	C	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	G
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H97	.	.	.	.	.	.	.	C	.	T	.	.	.	.	.	G	.	.	.	C	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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H99	.	.	T	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	.	.	G	.	C	.	.	.	.	C	C	.	.	C	.	.	.	C	.	.	.	.	.	.
H100	.	.	.	.	.	.	.	C	.	T	.	A	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.
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H102	.	.	.	.	.	.	.	C	.	T	.	.	.	.	.	.	.	.	.	C	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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H106	.	.	T	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	.	.	.	A	.	.	.	.	G	.	C	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.
H107	.	.	T	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	.	.	G	.	C	.	.	.	.	.	.	.	.	C	.	.	G	C	.	.	.	.	.	.
H108	.	.	T	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	C	.	G	.	C	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.
H109	.	.	T	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	.	.	G	.	C	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.
H110	.	.	T	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	.	.	G	.	C	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.
H111	.	.	T	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	.	.	G	.	C	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.
H112	.	.	T	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	A	.	.	A	.	.	C	.	G	.	C	.	.	.	.	.	.	.	.	C	.	.	.	C	C	.	.	.	.	.
H113	.	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	C	.	G	.	C	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.
H114	.	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	C	.	.	.	C	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.
H115	.	.	T	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	T	C	.	G	A	.	.	.	.	G	.	C	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.

Table 4.2. Maximum composite likelihood estimates for cyt b region. Rates of different transitional substitutions are shown in bold and those of transversional substitutions are shown in italics.

	A	T	C	G
A	-	*1,18*	*0,35*	**8,15**
T	*0,91*	-	**13,81**	*0,29*
C	*0,91*	**47,11**	-	*0,29*
G	**25,48**	*1,18*	*0,35*	-

All haplotypes were translated into amino acids generating a total of 144 amino acids (Table 4.3). Considering insects' amino acid translations there were no indels (insertion-deletion) or non-sense amino acids. In the translated sequences, there were 34 replacement substitutions. Replacements occurred at site 30 (from serine to methionine), 49 (from valine to isoleucine), 51 (from leucine to phenylalanine), 57 (from histidine to asparagine), 60 (from threonine to serine), 67 (from asparagine to serine), 83 (from serine to phenylalanine), 94 (from leucine to methionine), 97 (from isoleucine to threonine), 98 (from valine to methionine), 101 (from leucine to methionine), 105 (from cysteine to glycine), 107 (from phenylalanine to serine), 110 (from valine to isoleucine), 122 (from proline to serine), 124 (from isoleucine to valine), There was one multiple hit at the amino acid replacement at the site 63 (from leucine to isoleucine and methionine). In *C. divisa* cyt b haplotypes it seems that there is a tendency to use leucine (LUUA= 19), followed by isoleucine (IAUU= 17), phenylalanine (FUUU=10), and methionine (MAUA= 8).

Table 4.3. Polymorphic amino acid sites of *C. divisa*.

	30	49	51	57	60	63	67	83	94	97	98	101	105	107	110	122	124		30	49	51	57	60	63	67	83	94	97	98	101	105	107	110	122	124
H1	**S**	**V**	**L**	**H**	**T**	**L**	**N**	**S**	**L**	**I**	**V**	**L**	**C**	**F**	**V**	**P**	**I**		**S**	**V**	**L**	**H**	**T**	**L**	**N**	**S**	**L**	**I**	**V**	**L**	**C**	**F**	**V**	**P**	**I**
H2	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.	**H59**	.	.	.	.	.	.	.	.	.	.	.	M	.	S	I	.	.
H3	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.	**H60**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.
H4	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.	**H61**	M	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.
H5	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	V	**H62**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.
H6	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.	**H63**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.
H7	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.	**H64**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.
H8	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.	**H65**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.
H9	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.	**H66**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.
H10	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.	**H67**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.
H11	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.	**H68**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.
H12	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.	**H69**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.
H13	.	.	.	.	.	.	.	F	.	.	.	.	.	.	I	.	.	**H70**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.
H14	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.	**H71**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.
H15	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.	**H72**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.
H16	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.	**H73**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.
H17	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.	**H74**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.
H18	.	.	.	N	.	I	.	.	.	.	.	.	.	S	I	S	.	**H75**	.	.	.	.	.	.	.	.	.	.	.	.	.	.	I	.	.
H19	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.	**H76**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.
H20	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.	**H77**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.
H21	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.	**H78**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.
H22	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.	**H79**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.
H23	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.	**H80**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.
H24	.	.	F	.	.	.	.	.	.	.	.	.	.	S	I	.	.	**H81**	.	.	.	.	.	.	.	.	.	.	.	.	.	.	I	.	.
H25	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.	**H82**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.
H26	.	.	.	.	.	.	.	.	.	.	.	.	G	S	I	.	.	**H83**	.	.	.	.	.	.	.	M	.	.	.	.	.	S	I	.	.
H27	.	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.	**H84**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.
H28	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.	**H85**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.
H29	.	I	.	.	.	.	.	.	.	.	.	.	.	.	I	.	.	**H86**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.
H30	.	.	.	.	.	.	.	.	.	T	.	.	.	S	I	.	.	**H87**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.
H31	.	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.	**H88**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.
H32	.	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.	**H89**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.
H33	.	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.	**H90**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.
H34	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.	**H91**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.
H35	.	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.	**H92**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.
H36	.	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.	**H93**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.
H37	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.	**H94**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.
H38	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.	**H95**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.
H39	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.	**H96**	.	.	.	.	.	.	.	.	.	.	.	.	.	.	I	.	.
H40	.	.	.	.	.	.	.	.	.	.	.	.	.	Y	I	.	.	**H97**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.
H41	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.	**H98**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.
H42	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.	**H99**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.
H43	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.	**H100**	.	.	.	.	.	.	.	.	.	.	M	.	.	S	.	.	.
H44	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.	**H101**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.
H45	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.	**H102**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.
H46	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.	**H103**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.
H47	.	.	.	.	.	.	.	.	.	.	.	.	.	.	I	.	.	**H104**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.
H48	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.	**H105**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.
H49	.	.	.	.	S	.	.	.	.	.	.	.	.	S	I	.	.	**H106**	.	.	.	.	.	.	.	.	.	.	.	.	.	.	I	.	.
H50	.	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.	**H107**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.
H51	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.	**H108**	.	.	.	.	.	.	S	.	.	.	.	.	.	S	I	.	.
H52	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.	**H109**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.
H53	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.	**H110**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.
H54	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.	**H111**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.
H55	.	.	.	.	.	M	.	.	.	.	.	.	.	S	I	.	.	**H112**	.	.	.	.	.	.	.	.	.	.	.	.	.	Y	I	.	.
H56	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.	**H113**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.
H57	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.	**H114**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.
H58	.	.	.	.	.	.	.	.	.	.	.	.	.	.	I	.	.	**H115**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.	.

Frequency of 115 haplotypes was shown in Table 4.4. The most frequent haplotype (H2) was shared among 42 individuals from 14 populations (Afyon, Aydın, Bingöl, Çanakkale, Çankırı, Çorum, Denizli, Eskişehir, Giresun, Kayseri, Kırşehir, Sivas, Tokat, and Uşak). The second most shared haplotype was H27 that was detected in 40 individuals collected from 9 localities (Bingöl, Bolu, Elazığ, Isparta, Konya, Malatya, Siirt, Sivas, and Tokat). Eighty-four haplotypes were singleton (found in only one individual), and 13 were private haplotype (found in only one population). For instance, H36 (N= 3) was found only in Bolu, H37 (N= 2) in Çanakkale, H42 (N= 3) in Çankırı, H69 (N=2) in Eskişehir, H79 and H80 (N=2) were found only in Kayseri. Some populations were with high haplotype number such as Konya (Nhap= 13/N= 20), Çankırı (Nhap= 12/N= 15), Bingöl (Nhap= 11/N= 15), Uşak (Nhap= 11/N= 15), and Çorum (Nhap= 10/N= 14). Meanwhile some other populations such as Bolu (Nhap= 3/N= 14) and Sivas (Nhap= 7/N= 14) had lower haplotype number considering their population size.

Pairwise comparisons used for designating sequence differences between haplotypes ranged from 0.2% to 7.3% (Table 4.5). There was 1 base pair difference (0.2%) in 75 pairwise comparisons, and there were 28 base pair differences between H28 (represented by one individual in Bingöl population) and H112 (represented by one individual in Uşak population). The second highest difference was between H28 (from Bitlis) - H40 (from Çanakkale) - H68 (from Elazığ) with 27 base pairs. The third highest pairwise difference was between H28 (from Bingöl), H59 (from Denizli), H75 (from Giresun) and H96 (from Konya) with 26 base pair-difference.

Table 4.4. Cytb haplotype frequencies in each location of *C. divisa*.

	AFY	AYD	BAL	BIN	BOL	CAN	CNK	COR	DEN	ELA	ESK	GIR	ISP	IZM	KAY	KIR	KON	MAL	SII	SIV	TOK	USK	N
H1	1																						1
H2	5	1		1		1	2	5	6		1	2			1	7				4	3	3	42
H3	1																					1	2
H4	1																						1
H5	1																						1
H6	1		1	1			1								2								6
H7	1																						1
H8	1																						1
H9	1																						1
H10		1																					1
H11		2														1						1	4
H12		2																					2
H13		1																					1
H14		1																					1
H15		1							1														2
H16		1		1		1														1		1	5
H17		3					1							4						1	1	2	12
H18			2																				2
H19			1																				1
H20			3																				3
H21			2																				2
H22			1			7					1				4	1							14
H23			1																				1
H24			1																				1
H25			1																				1
H26			1																				1
H27				4	10					7			1				7	2	1	5	3		40
H28				1																			1
H29				1																			1
H30				1																1			2
H31				1																			1
H32				1																			1
H33				2																			2
H34				1																			1
H35					1																		1
H36					3																		3
H37						2																	2
H38						1																	1
H39						1																	1
H40						1																	1
H41						1	1	1															3
H42							3																3
H43							1																1

Table 4. 4 (cont'd).

	AFY	AYD	BAL	BIN	BOL	CAN	CNK	COR	DEN	ELA	ESK	GIR	ISP	IZM	KAY	KIR	KON	MAL	SII	SIV	TOK	USK	N
H43							1																1
H44							1					1				3							5
H45							1																1
H46							1																1
H47							1																1
H48							1								1								2
H49							1																1
H50								1					5		4			1			1		12
H51								1															1
H52								1															1
H53								1															1
H54								1															1
H55								1															1
H56								1															1
H57								1							3						1		5
H58									1														1
H59									1														1
H60									1														1
H61									1														1
H62									1														1
H63									1													1	2
H64										2		2							1				5
H65										1													1
H66										1													1
H67										1													1
H68										1													1
H69											2												2
H70											1												1
H71												1											1
H72												1											1
H73												1											1
H74												1											1
H75												1											1
H76												1											1
H77													1										1
H78														1									1
H79															2								2
H80															2								2
H81															1								1
H82																1							1
H83																1							1
H84																1							1

Table 4. 4 (cont'd).

	AFY	AYD	BAL	BIN	BOL	CAN	CNK	COR	DEN	ELA	ESK	GIR	ISP	IZM	KAY	KIR	KON	MAL	SII	SIV	TOK	USK	N
H85																1							1
H86																	1						1
H87																	1						1
H88																	1						1
H89																	1						1
H90																	1						1
H91																	1						1
H92																	1						1
H93																	2						2
H94																	1						1
H95																	1						1
H96																	1						1
H97																	1						1
H98																		1					1
H99																		1					1
H100																		1					1
H101																			1				1
H102																			1				1
H103																			1				1
H104																				1			1
H105																				1			1
H106																					1		1
H107																					2		2
H108																					1		1
H109																					1		1
H110																					1		1
H111																						1	1
H112																						1	1
H113																						1	1
H114																						1	1
H115																						2	2
N	13	13	14	15	14	15	15	14	13	13	5	11	7	5	20	16	20	6	5	14	15	15	278
Nhaplotype	9	9	10	11	3	8	12	10	8	6	4	9	3	2	9	8	13	5	5	7	10	11	

Table 4.5. Pairwise comparison of 115 *C. divisa* haplotypes. Net nucleotide differences are shown above diagonal and percentage of the differences are presented below 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		15	13	16	17	13	12	16	13	15	14	15	16	16	15	11	14	21	16	15	15	14	14	16	11	15	3	21	15	16	4	5	5	16	6	4	15	14	12	17
H2	0,036		4	1	4	4	5	1	4	2	1	4	3	3	2	4	5	20	1	4	4	3	3	3	4	4	14	24	2	3	15	14	14	3	15	15	2	3	5	9
H3	0,031	0,009		5	6	4	5	5	4	6	5	4	7	7	4	2	5	20	5	4	4	3	3	7	6	4	12	22	6	7	13	10	12	5	15	13	4	5	5	9
H4	0,039	0,002	0,012		5	5	6	2	5	3	2	5	4	4	3	5	6	21	2	5	5	4	4	4	5	5	15	25	3	4	15	15	15	4	16	16	3	4	6	10
H5	0,041	0,009	0,014	0,012		4	5	5	6	4	5	4	7	7	2	6	5	20	3	4	4	3	5	7	6	6	16	24	6	7	17	16	14	7	17	17	6	7	5	9
H6	0,031	0,009	0,009	0,012	0,009		1	5	2	2	3	2	7	5	4	4	1	20	3	2	2	1	3	7	6	6	12	22	6	7	13	12	10	7	15	13	6	5	3	5
H7	0,029	0,012	0,012	0,014	0,012	0,002		6	3	3	4	3	8	6	5	5	2	19	4	3	3	2	4	8	7	7	11	21	7	8	12	11	9	8	14	12	7	6	4	6
H8	0,039	0,002	0,012	0,005	0,012	0,012	0,014		5	3	2	5	4	4	3	5	6	21	2	5	5	4	4	4	5	5	15	24	3	4	16	15	15	4	14	16	3	4	6	10
H9	0,031	0,009	0,009	0,012	0,014	0,005	0,007	0,012		4	3	4	7	5	4	4	1	20	5	4	4	3	3	7	6	6	12	22	6	7	13	12	12	7	15	13	6	5	5	5
H10	0,036	0,005	0,014	0,007	0,009	0,005	0,007	0,007	0,009		1	4	5	3	4	6	3	20	1	2	4	3	5	5	6	6	14	24	4	5	15	14	12	5	15	15	4	3	5	7
H11	0,034	0,002	0,012	0,005	0,012	0,007	0,009	0,005	0,007	0,002		5	4	2	3	5	4	19	2	3	5	4	4	4	5	5	13	23	3	4	14	13	13	4	14	14	3	2	6	8
H12	0,036	0,009	0,009	0,012	0,009	0,005	0,007	0,012	0,009	0,009	0,012		7	7	4	4	3	22	3	4	2	1	3	7	6	6	14	22	6	7	15	14	12	7	17	15	6	7	3	7
H13	0,039	0,007	0,017	0,009	0,017	0,017	0,019	0,009	0,017	0,012	0,009	0,016		6	5	7	8	23	4	7	7	6	6	6	7	7	17	27	3	6	18	17	17	6	18	18	5	6	8	11
H14	0,039	0,007	0,017	0,009	0,017	0,012	0,014	0,009	0,012	0,007	0,005	0,017	0,014		5	7	6	20	4	5	7	6	6	6	7	7	15	24	5	6	16	15	15	6	16	16	5	4	8	10
H15	0,036	0,005	0,009	0,007	0,005	0,009	0,012	0,007	0,009	0,009	0,007	0,009	0,012	0,012		4	5	18	3	4	4	3	3	5	4	4	14	22	4	5	15	14	14	5	15	15	4	5	5	9
H16	0,026	0,009	0,005	0,012	0,014	0,009	0,012	0,012	0,009	0,014	0,012	0,009	0,017	0,017	0,009		5	18	5	6	4	3	3	7	4	4	10	20	6	7	11	10	10	5	13	11	4	5	5	9
H17	0,034	0,012	0,012	0,014	0,012	0,002	0,005	0,014	0,002	0,007	0,009	0,007	0,019	0,014	0,012	0,012		21	4	3	3	2	4	8	7	7	13	23	7	8	14	13	11	8	16	14	7	6	4	4
H18	0,053	0,050	0,050	0,053	0,050	0,050	0,048	0,053	0,050	0,050	0,048	0,056	0,058	0,050	0,045	0,045	0,053		21	20	22	21	21	21	16	20	20	21	22	21	21	20	22	19	21	20	20	19	19	22
H19	0,039	0,002	0,012	0,005	0,007	0,007	0,009	0,005	0,012	0,002	0,005	0,007	0,009	0,009	0,007	0,012	0,009	0,053		3	3	2	4	4	5	5	15	25	3	4	16	15	13	4	16	16	3	4	4	8
H20	0,036	0,009	0,009	0,012	0,009	0,005	0,007	0,012	0,009	0,005	0,007	0,009	0,017	0,012	0,009	0,014	0,007	0,050	0,007		4	3	5	7	6	6	14	24	6	7	15	12	12	7	15	15	6	5	5	7
H21	0,036	0,009	0,009	0,012	0,009	0,005	0,007	0,012	0,009	0,009	0,012	0,005	0,016	0,017	0,009	0,009	0,007	0,056	0,007	0,009		1	3	7	6	6	14	24	6	7	15	14	12	5	17	15	6	7	3	7
H22	0,034	0,007	0,007	0,009	0,007	0,002	0,005	0,009	0,007	0,007	0,009	0,002	0,014	0,014	0,007	0,007	0,005	0,053	0,005	0,007	0,002		2	6	5	5	13	23	5	6	14	13	11	6	16	14	5	6	2	6
H23	0,034	0,007	0,007	0,009	0,012	0,007	0,009	0,009	0,007	0,012	0,009	0,007	0,014	0,014	0,007	0,007	0,009	0,053	0,009	0,012	0,007	0,005		6	5	5	13	23	5	6	14	13	13	6	16	14	5	6	4	8
H24	0,039	0,007	0,017	0,009	0,017	0,017	0,019	0,009	0,017	0,012	0,009	0,017	0,014	0,014	0,012	0,017	0,019	0,053	0,009	0,017	0,017	0,014	0,014		5	7	15	23	5	4	16	15	17	6	16	16	5	6	6	12
H25	0,026	0,009	0,014	0,012	0,014	0,014	0,016	0,012	0,014	0,014	0,012	0,014	0,016	0,017	0,009	0,009	0,017	0,040	0,012	0,014	0,014	0,012	0,012	0,012		6	10	20	6	5	11	10	12	7	11	11	6	7	3	11
H26	0,036	0,009	0,009	0,012	0,014	0,014	0,017	0,012	0,014	0,014	0,012	0,014	0,017	0,017	0,009	0,009	0,017	0,050	0,012	0,014	0,014	0,012	0,012	0,017	0,014		14	24	6	7	15	14	14	5	15	15	4	5	7	11
H27	0,007	0,034	0,029	0,036	0,039	0,029	0,026	0,036	0,029	0,034	0,031	0,034	0,041	0,036	0,034	0,024	0,031	0,050	0,036	0,034	0,034	0,031	0,031	0,036	0,024	0,034		22	16	15	1	2	2	15	3	1	14	13	11	17
H28	0,053	0,061	0,056	0,065	0,061	0,056	0,053	0,061	0,056	0,061	0,059	0,056	0,070	0,061	0,056	0,050	0,059	0,054	0,064	0,061	0,061	0,059	0,059	0,059	0,051	0,062	0,056		26	25	23	22	24	24	24	23	24	23	21	27
H29	0,036	0,005	0,014	0,007	0,014	0,014	0,017	0,007	0,014	0,009	0,007	0,014	0,007	0,012	0,009	0,014	0,017	0,056	0,007	0,014	0,014	0,012	0,012	0,012	0,014	0,014	0,039	0,067		5	17	16	16	5	17	17	4	5	7	10
H30	0,039	0,007	0,017	0,009	0,017	0,017	0,019	0,009	0,017	0,012	0,009	0,016	0,014	0,014	0,012	0,017	0,019	0,053	0,009	0,017	0,016	0,014	0,014	0,009	0,012	0,017	0,036	0,064	0,012		16	15	17	6	15	16	5	4	6	12
H31	0,009	0,036	0,031	0,036	0,041	0,031	0,029	0,039	0,031	0,036	0,034	0,036	0,044	0,039	0,036	0,026	0,034	0,053	0,039	0,036	0,036	0,034	0,034	0,039	0,026	0,036	0,002	0,059	0,041	0,039		3	3	16	4	2	15	14	12	18
H32	0,012	0,034	0,024	0,036	0,039	0,029	0,026	0,036	0,029	0,034	0,031	0,034	0,041	0,036	0,034	0,024	0,031	0,050	0,036	0,029	0,034	0,031	0,031	0,036	0,024	0,034	0,005	0,056	0,039	0,036	0,007		4	15	5	3	14	13	11	17
H33	0,012	0,034	0,029	0,036	0,034	0,024	0,021	0,036	0,029	0,029	0,031	0,029	0,041	0,036	0,034	0,024	0,026	0,056	0,031	0,029	0,029	0,026	0,031	0,041	0,029	0,034	0,005	0,061	0,039	0,041	0,007	0,009		15	5	3	14	13	11	15
H34	0,039	0,007	0,012	0,009	0,017	0,017	0,019	0,009	0,017	0,012	0,009	0,017	0,014	0,014	0,012	0,012	0,019	0,048	0,009	0,017	0,012	0,014	0,014	0,014	0,017	0,012	0,036	0,061	0,012	0,014	0,039	0,036	0,036		16	16	3	4	8	12
H35	0,014	0,036	0,036	0,039	0,042	0,036	0,034	0,034	0,036	0,036	0,034	0,041	0,044	0,039	0,036	0,031	0,039	0,053	0,039	0,036	0,041	0,039	0,039	0,039	0,026	0,036	0,007	0,062	0,042	0,036	0,009	0,012	0,012	0,039		4	15	13	14	20
H36	0,009	0,036	0,031	0,039	0,041	0,031	0,029	0,039	0,031	0,036	0,034	0,036	0,044	0,039	0,036	0,026	0,034	0,050	0,039	0,036	0,036	0,034	0,034	0,039	0,026	0,036	0,002	0,059	0,041	0,039	0,005	0,007	0,007	0,039	0,009		15	14	12	16
H37	0,036	0,005	0,009	0,007	0,014	0,014	0,017	0,007	0,014	0,009	0,007	0,014	0,012	0,012	0,009	0,009	0,017	0,050	0,007	0,014	0,014	0,012	0,012	0,012	0,014	0,009	0,034	0,061	0,009	0,012	0,036	0,034	0,034	0,007	0,036	0,036		3	7	11
H38	0,034	0,007	0,012	0,009	0,017	0,012	0,014	0,009	0,012	0,007	0,005	0,017	0,014	0,009	0,012	0,014	0,048	0,009	0,012	0,017	0,014	0,014	0,014	0,014	0,017	0,012	0,031	0,059	0,012	0,009	0,034	0,031	0,031	0,009	0,031	0,034	0,007		8	10
H39	0,029	0,012	0,012	0,014	0,012	0,007	0,009	0,014	0,012	0,012	0,014	0,007	0,019	0,019	0,012	0,012	0,009	0,048	0,009	0,012	0,007	0,005	0,009	0,014	0,007	0,017	0,026	0,053	0,017	0,014	0,029	0,026	0,026	0,019	0,034	0,029	0,017	0,019		8
H40	0,041	0,021	0,022	0,024	0,022	0,012	0,014	0,024	0,012	0,017	0,019	0,017	0,026	0,024	0,021	0,021	0,009	0,056	0,019	0,017	0,017	0,014	0,019	0,029	0,026	0,026	0,041	0,070	0,024	0,029	0,044	0,041	0,036	0,029	0,049	0,039	0,026	0,024	0,019	
H41	0,039	0,002	0,012	0,005	0,012	0,012	0,014	0,005	0,012	0,007	0,005	0,012	0,009	0,009	0,007	0,012	0,014	0,053	0,005	0,012	0,012	0,009	0,009	0,009	0,012	0,012	0,036	0,064	0,007	0,005	0,039	0,036	0,036	0,009	0,036	0,039	0,007	0,005	0,014	0,024
H42	0,036	0,009	0,009	0,012	0,009	0,005	0,007	0,012	0,009	0,009	0,012	0,005	0,017	0,017	0,009	0,009	0,007	0,056	0,007	0,009	0,005	0,002	0,002	0,007	0,017	0,014	0,014	0,034	0,061	0,014	0,017	0,036	0,034	0,029	0,017	0,041	0,036	0,014	0,017	0,007	0,017
H43	0,031	0,005	0,005	0,007	0,009	0,005	0,007	0,007	0,005	0,009	0,007	0,005	0,012	0,012	0,005	0,005	0,007	0,050	0,007	0,009	0,005	0,002	0,002	0,012	0,009	0,009	0,029	0,056	0,009	0,012	0,031	0,029	0,029	0,012	0,036	0,031	0,009	0,012	0,007	0,017
H44	0,034	0,002	0,007	0,005	0,007	0,007	0,009	0,005	0,007	0,007	0,005	0,007	0,009	0,009	0,002	0,007	0,009	0,048	0,005	0,007	0,007	0,005	0,005	0,009	0,007	0,007	0,031	0,059	0,007	0,009	0,034	0,031	0,031	0,009	0,034	0,034	0,007	0,009	0,009	0,019
H45	0,041	0,005	0,014	0,007	0,014	0,014	0,017	0,007	0,014	0,009	0,007	0,014	0,012	0,012	0,009	0,014	0,017	0,056	0,007	0,014	0,014	0,012	0,012	0,012	0,014	0,014	0,039	0,064	0,009	0,012	0,042	0,039	0,039	0,012	0,042	0,041	0,009	0,012	0,017	0,026
H46	0,036	0,014	0,014	0,017	0,014	0,005	0,007	0,017	0,005	0,009	0,012	0,009	0,021	0,017	0,014	0,014	0,002	0,056	0,012	0,009	0,009	0,007	0,012	0,021	0,019	0,019	0,034	0,061	0,019	0,021	0,036	0,034	0,029	0,021	0,041	0,036	0,019	0,017	0,012	0,012
H47	0,029	0,007	0,012	0,009	0,012	0,012	0,014	0,009	0,012	0,012	0,009	0,012	0,009	0,014	0,007	0,012	0,014	0,048	0,009	0,012	0,012	0,009	0,009	0,009	0,007	0,012	0,031	0,059	0,007	0,009	0,034	0,031	0,036	0,014	0,034	0,034	0,012	0,014	0,009	0,021
H48	0,036	0,009	0,009	0,012	0,009	0,005	0,007	0,012	0,009	0,009	0,012	0,005	0,017	0,017	0,009	0,009	0,007	0,056	0,007	0,009	0,005	0,002	0,007	0,017	0,014	0,014	0,034	0,062	0,014	0,017	0,036	0,034	0,029	0,017	0,042	0,036	0,014	0,017	0,007	0,017
H49	0,039	0,002	0,012	0,005	0,012	0,012	0,014	0,005	0,012	0,007	0,005	0,012	0,009	0,009	0,007	0,012	0,014	0,053	0,005	0,012	0,012	0,009	0,009	0,009	0,012	0,012	0,036	0,065	0,007	0,009	0,039	0,036	0,036	0,009	0,039	0,039	0,007	0,009	0,014	0,024
H50	0,009	0,036	0,031	0,039	0,036	0,026	0,024	0,039	0,031	0,031	0,034	0,031	0,044	0,039	0,036	0,026	0,029	0,053	0,034	0,031	0,031	0,029	0,034	0,039	0,026	0,036	0,002	0,059	0,041	0,039	0,005	0,007	0,002	0,039	0,009	0,005	0,036	0,034	0,024	0,039
H51	0,036	0,005	0,009	0,007	0,014	0,014	0,017	0,007	0,014	0,009	0,007	0,014	0,012	0,012	0,009	0,009	0,017	0,050	0,007	0,014	0,014	0,012	0,012	0,012	0,014	0,009	0,034	0,061	0,009	0,012	0,036	0,034	0,034	0,007	0,036	0,036	0,005	0,007	0,017	0,026
H52	0,034	0,002	0,012	0,005	0,012	0,012	0,014	0,005	0,012	0,007	0,005	0,012	0,009	0,009	0,007	0,012	0,014	0,048	0,005	0,012	0,012	0,009	0,009	0,009	0,007	0,012	0,031	0,059	0,007	0,009	0,034	0,031	0,031	0,009	0,034	0,034	0,007	0,009	0,009	0,024
H53	0,039	0,002	0,012	0,005	0,012	0,012	0,014	0,005	0,012	0,007	0,005	0,012	0,009	0,009	0,007	0,012	0,014	0,053	0,005	0,012	0,012	0,009	0,009	0,009	0,012	0,012	0,036	0,064	0,007	0,009	0,039	0,036	0,036	0,009	0,039	0,039	0,007	0,009	0,014	0,024
H54	0,034	0,002	0,012	0,005	0,012	0,012	0,014	0,005	0,012	0,007	0,005	0,012	0,009	0,009	0,007	0,007	0,014	0,048	0,005	0,012	0,012	0,009	0,009	0,009	0,007	0,012	0,031	0,059	0,007	0,009	0,034	0,031	0,031	0,009	0,034	0,034	0,007	0,009	0,014	0,024
H55	0,039	0,002	0,012	0,005	0,012	0,012	0,014	0,005	0,012	0,007	0,005	0,012	0,009	0,009	0,007	0,012	0,014	0,048	0,005	0,012	0,012	0,009	0,009	0,009	0,012	0,012	0,036	0,062	0,007	0,009	0,039	0,036	0,036	0,009	0,039	0,039	0,007	0,009	0,014	0,024
H56	0,029	0,012	0,007	0,014	0,017	0,012	0,014	0,014	0,012	0,017	0,014	0,012	0,019	0,019	0,012	0,002	0,014	0,048	0,014	0,017	0,012	0,009	0,009	0,019	0,012	0,012	0,026	0,053	0,017	0,019	0,029	0,026	0,026	0,014	0,034	0,029	0,012	0,014	0,014	0,024
H57	0,034	0,002	0,012	0,005	0,012	0,012	0,014	0,005	0,012	0,007	0,005	0,012	0,009	0,009	0,007	0,012	0,014	0,048	0,005	0,012	0,012	0,009	0,009	0,005	0,007	0,012	0,031	0,059	0,007	0,005	0,034	0,031	0,036	0,009	0,034	0,034	0,007	0,009	0,009	0,024
H58	0,034	0,007	0,017	0,009	0,017	0,017	0,014	0,009	0,017	0,012	0,009	0,017	0,009	0,014	0,012	0,017	0,019	0,053	0,009	0,017	0,017	0,014	0,014	0,014	0,017	0,017	0,036	0,064	0,007	0,014	0,039	0,036	0,036	0,014	0,039	0,039	0,012	0,014	0,019	0,026
H59	0,041	0,009	0,014	0,012	0,009	0,009	0,012	0,012	0,014	0,009	0,012	0,009	0,017	0,017	0,009	0,014	0,012	0,056	0,007	0,009	0,005	0,007	0,012	0,017	0,014	0,014	0,039	0,067	0,014	0,017	0,041	0,039	0,034	0,012	0,042	0,041	0,014	0,017	0,012	0,022
H60	0,039	0,002	0,012	0,005	0,012	0,012	0,014	0,005	0,012	0,007	0,005	0,012	0,009	0,009	0,007	0,012	0,014	0,053	0,005	0,012	0,012	0,009	0,009	0,009	0,012	0,012	0,036	0,064	0,007	0,009	0,039	0,036	0,036	0,009	0,039	0,039	0,007	0,009	0,014	0,024

Table 4. 5 (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	16	15	13	14	17	15	12	15	16	4	15	14	16	14	16	12	14	14	17	16	16	13	13	4	5	2	4	18	16	16	6	5	15	4	15	5	14	15	4	5
H2	1	4	2	1	2	6	3	4	1	15	2	1	1	1	1	5	1	3	4	1	1	4	6	13	14	13	15	19	7	5	13	14	2	13	2	14	5	2	13	16
H3	5	4	2	3	6	6	5	4	5	13	4	5	5	5	5	3	5	7	6	5	5	2	4	11	10	11	13	19	7	7	11	14	4	11	6	12	5	4	13	14
H4	2	5	3	2	3	7	4	5	2	16	3	2	2	2	2	6	2	4	5	2	2	5	7	14	15	14	16	20	8	6	14	15	3	14	3	15	6	3	14	17
H5	5	4	4	3	6	6	5	4	5	15	6	5	5	5	5	7	5	7	4	5	5	4	6	15	16	15	17	21	7	5	15	16	6	15	6	16	7	4	15	16
H6	5	2	2	3	6	2	5	2	5	11	6	5	5	5	5	5	5	7	4	5	5	2	2	11	12	11	13	19	3	3	11	14	6	11	6	12	3	4	13	12
H7	6	3	3	4	7	3	6	3	6	10	7	6	6	6	6	6	6	6	5	6	6	3	3	10	13	10	12	18	4	4	12	13	7	10	7	11	4	5	12	11
H8	2	5	3	2	3	7	4	5	2	16	3	2	2	2	2	6	2	4	5	2	2	5	7	14	15	14	16	20	8	6	14	15	3	14	3	15	6	3	14	17
H9	5	4	2	3	6	2	5	4	5	13	6	5	5	5	5	5	5	7	6	5	5	4	2	11	12	11	13	19	3	3	11	14	6	11	6	12	1	4	13	14
H10	3	4	4	3	4	4	5	4	3	13	4	3	3	3	3	7	3	5	4	3	3	4	4	13	14	13	15	19	5	3	13	14	4	13	4	14	5	4	13	14
H11	2	5	3	2	3	5	4	5	2	14	3	2	2	2	2	6	2	4	5	2	2	5	5	12	13	12	14	18	6	4	12	13	3	12	3	13	4	3	12	15
H12	5	2	2	3	6	4	5	2	5	13	6	5	5	5	5	5	5	7	4	5	5	2	4	13	14	13	15	21	5	5	13	16	6	13	6	14	5	4	15	14
H13	4	7	5	4	5	9	4	7	4	18	5	4	4	4	4	8	4	4	7	4	4	7	9	16	17	16	18	22	10	8	16	17	5	16	3	17	8	5	16	19
H14	4	7	5	4	5	7	6	7	4	16	5	4	4	4	4	8	4	6	7	4	4	7	7	14	15	14	16	19	8	6	14	15	5	14	5	15	6	5	14	17
H15	3	4	2	1	4	6	3	4	3	15	4	3	3	3	3	5	3	5	4	3	3	4	6	13	14	13	15	19	7	5	13	14	4	13	4	14	5	2	13	16
H16	5	4	2	3	6	6	5	4	5	11	4	5	5	3	5	1	5	7	6	5	5	2	4	9	10	9	11	19	7	7	9	12	6	9	6	10	5	4	11	12
H17	6	3	3	4	7	1	6	3	6	12	7	6	6	6	6	6	6	8	5	6	6	3	1	12	13	12	14	20	2	2	12	15	7	12	7	13	2	5	14	13
H18	21	22	20	19	22	22	19	22	21	21	20	19	21	19	19	19	19	21	22	21	21	20	20	19	22	19	21	25	21	21	21	20	20	19	22	20	20	20	19	22
H19	2	3	3	2	3	5	4	3	2	14	3	2	2	2	2	6	2	4	3	2	2	3	5	14	15	14	16	20	6	4	14	15	3	14	3	15	6	3	14	15
H20	5	4	4	3	6	4	5	4	5	13	6	5	5	5	5	7	5	7	4	5	5	4	4	13	12	13	15	19	5	3	13	14	4	13	6	14	5	4	13	14
H21	5	2	2	3	6	4	5	2	5	13	6	5	5	5	5	5	5	7	2	5	5	2	4	13	14	13	15	21	5	5	13	16	6	13	6	14	5	4	15	14
H22	4	1	1	2	5	3	4	1	4	12	5	4	4	4	4	4	4	6	3	4	4	1	3	12	13	12	14	20	4	4	12	15	5	12	5	13	4	3	14	13
H23	4	3	1	2	5	5	4	3	4	14	5	4	4	4	4	4	4	6	5	4	4	3	5	12	13	12	14	20	6	6	12	15	5	12	5	13	4	3	14	15
H24	4	7	5	4	5	9	4	7	4	16	5	4	4	4	4	8	2	6	7	4	4	7	9	14	15	14	16	22	10	8	14	15	5	14	5	15	8	5	14	17
H25	5	6	4	3	6	8	3	6	5	11	6	3	5	3	5	5	3	7	6	5	5	6	8	9	10	9	11	19	9	7	9	10	4	9	6	10	7	4	9	12
H26	5	6	4	3	6	8	5	6	5	15	4	5	5	5	5	5	5	7	6	5	5	4	6	13	14	13	15	19	9	7	13	14	6	13	6	14	7	4	13	16
H27	15	14	12	13	16	14	13	14	15	1	14	13	15	13	15	11	13	15	16	15	15	12	12	1	2	1	1	17	15	15	3	2	14	1	16	2	13	14	1	2
H28	25	24	22	23	25	24	23	24	25	23	24	23	25	23	24	21	23	25	26	25	25	22	22	21	24	21	22	27	25	25	23	24	24	21	26	22	22	24	23	24
H29	3	6	4	3	4	8	3	6	3	17	4	3	3	3	3	7	3	3	6	3	3	6	8	15	16	15	17	21	9	7	15	16	4	15	2	16	7	4	15	18
H30	2	7	5	4	5	9	4	7	4	16	5	4	4	4	4	8	2	6	7	4	4	7	9	14	15	14	16	22	10	8	14	15	5	14	5	15	8	5	14	17
H31	16	15	13	14	17	15	14	15	16	2	15	14	16	14	16	12	14	16	17	16	16	13	13	2	3	2	2	18	16	16	4	3	15	2	17	3	14	15	2	3
H32	15	14	12	13	16	14	13	14	15	3	14	13	15	13	15	11	13	15	16	15	15	12	12	3	2	3	3	17	15	15	5	4	12	3	16	4	13	14	3	4
H33	15	12	12	13	16	12	15	12	15	1	14	13	15	13	15	11	15	15	14	15	15	10	10	3	4	3	3	17	13	13	5	4	14	3	16	4	13	14	3	2
H34	4	7	5	4	5	9	6	7	4	16	3	4	4	4	4	6	4	6	5	4	4	5	7	14	15	14	16	19	10	8	14	15	5	14	5	15	8	5	14	17
H35	15	17	15	14	17	17	14	17	16	4	15	14	16	14	16	14	14	16	17	16	16	15	15	4	5	4	4	18	18	16	6	3	15	4	17	5	16	15	2	5
H36	16	15	13	14	17	15	14	15	16	2	15	14	16	14	16	12	14	16	17	16	16	13	13	2	3	2	2	16	16	16	4	3	15	2	17	3	14	15	2	3
H37	3	6	4	3	4	8	5	6	3	15	2	3	3	3	3	5	3	5	6	3	3	4	6	13	14	13	15	19	9	7	13	14	4	13	4	14	7	4	13	16
H38	2	7	5	4	5	7	6	7	4	14	3	4	4	4	4	6	4	6	7	4	4	5	5	12	13	12	14	18	8	6	12	13	5	12	5	13	6	5	12	15
H39	6	3	3	4	7	5	4	3	6	10	7	4	6	6	6	6	4	8	5	6	6	3	5	10	11	10	12	20	6	6	10	13	5	10	7	11	6	5	12	11
H40	10	7	7	8	11	5	9	7	10	16	11	10	10	10	10	10	10	11	9	10	10	7	5	16	17	16	18	22	4	6	16	19	11	16	10	17	6	9	18	17
H41		5	3	2	3	7	4	5	2	16	3	2	2	2	2	6	2	4	5	2	2	5	7	14	15	14	16	20	8	6	14	15	3	14	3	15	6	3	14	17
H42	0,012		2	3	6	4	5	2	5	13	6	5	5	5	5	5	5	7	4	5	5	2	4	13	14	13	15	21	5	5	13	16	6	13	6	14	5	4	15	14
H43	0,007	0,005		1	4	4	3	2	3	13	4	3	3	3	3	3	3	5	4	3	3	2	4	11	12	11	13	19	5	5	11	14	4	11	4	12	3	2	13	14
H44	0,005	0,007	0,002		3	5	2	3	2	14	3	2	2	2	2	4	2	4	3	2	2	3	5	12	13	12	14	18	6	4	12	13	3	12	3	13	4	1	12	15
H45	0,007	0,014	0,009	0,007		8	5	6	3	17	4	3	3	3	3	7	3	5	6	3	3	6	8	15	16	15	17	21	9	7	15	16	4	15	4	16	7	4	15	18
H46	0,017	0,009	0,009	0,012	0,019		7	4	7	13	8	7	7	7	7	7	7	9	6	7	7	4	2	13	14	13	15	21	3	3	13	16	8	13	8	14	3	6	15	14
H47	0,009	0,012	0,007	0,005	0,012	0,017		5	4	14	5	4	4	4	4	6	2	4	5	4	4	5	7	12	13	12	14	20	8	6	12	13	5	12	3	13	6	3	12	15
H48	0,012	0,005	0,005	0,007	0,014	0,009	0,012		5	13	6	5	5	5	5	5	5	7	4	5	5	2	4	13	14	13	15	20	5	5	13	16	6	13	6	14	5	4	15	14
H49	0,005	0,012	0,007	0,005	0,007	0,017	0,009	0,012		16	3	2	2	2	2	6	2	4	5	2	2	5	7	14	15	14	16	20	8	6	14	15	3	14	3	15	6	3	14	17
H50	0,039	0,031	0,031	0,034	0,041	0,031	0,034	0,031	0,039		15	14	16	14	16	12	14	16	15	16	16	11	11	2	3	2	2	18	14	14	4	3	15	2	17	3	14	15	2	1
H51	0,007	0,014	0,009	0,007	0,009	0,019	0,012	0,014	0,007	0,036		3	3	3	3	5	3	5	6	3	3	4	6	13	14	13	15	19	9	7	13	14	4	13	4	14	7	4	13	16
H52	0,005	0,012	0,007	0,005	0,007	0,017	0,009	0,012	0,005	0,034	0,007		2	2	2	6	2	4	5	2	2	5	7	12	13	12	14	18	8	6	12	13	1	12	3	13	6	3	12	15
H53	0,005	0,012	0,007	0,005	0,007	0,017	0,009	0,012	0,005	0,039	0,007	0,005		2	2	6	2	4	5	2	2	5	7	14	15	14	16	20	8	4	14	15	3	14	3	15	6	3	14	17
H54	0,005	0,012	0,007	0,005	0,007	0,017	0,009	0,012	0,005	0,034	0,007	0,005	0,005		2	4	2	4	5	2	2	5	7	12	13	12	14	20	8	6	12	13	3	12	3	13	6	3	12	15
H55	0,005	0,012	0,007	0,005	0,007	0,017	0,009	0,012	0,005	0,039	0,007	0,005	0,005	0,005		6	2	4	5	2	2	5	7	14	15	14	16	20	8	6	14	15	3	14	3	15	6	3	14	17
H56	0,014	0,012	0,007	0,009	0,017	0,017	0,014	0,012	0,014	0,029	0,012	0,014	0,014	0,009	0,014		6	8	7	6	6	3	5	10	11	10	12	20	8	8	10	13	7	10	7	11	6	5	12	13
H57	0,005	0,012	0,007	0,005	0,007	0,017	0,005	0,012	0,005	0,034	0,007	0,005	0,005	0,005	0,005	0,014		4	5	2	2	5	7	12	13	12	14	20	8	6	12	13	3	12	3	13	6	3	12	15
H58	0,009	0,017	0,012	0,009	0,012	0,021	0,009	0,017	0,009	0,039	0,012	0,009	0,009	0,009	0,009	0,019	0,009		7	4	4	7	9	14	17	14	16	20	10	8	16	15	5	14	3	15	8	5	14	17
H59	0,012	0,009	0,009	0,007	0,014	0,014	0,012	0,009	0,012	0,036	0,014	0,012	0,012	0,012	0,012	0,017	0,012	0,017		5	5	4	6	15	16	15	17	21	7	5	15	16	6	15	6	16	7	4	15	16
H60	0,005	0,012	0,007	0,005	0,007	0,017	0,009	0,012	0,005	0,039	0,007	0,005	0,005	0,005	0,005	0,014	0,005	0,009	0,012		2	5	7	14	15	14	16	20	8	6	14	15	3	14	3	15	6	3	14	17

Table 4. 5 (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
H1	10	16	18	12	15	17	14	5	16	15	4	5	4	5	4	15	5	7	17	5	7	4	5	4	5	14	16	15	14	15	16	18	14	13	16
H2	5	7	3	5	2	2	5	16	1	4	15	16	13	16	15	2	16	18	4	16	18	15	14	15	16	1	3	4	3	2	1	6	5	6	1
H3	3	7	7	1	4	6	5	14	5	4	11	14	11	14	13	6	14	16	8	14	16	13	12	13	14	5	5	6	5	4	5	8	5	4	5
H4	6	8	4	6	3	3	6	17	2	5	16	17	14	17	16	3	17	19	5	17	19	16	15	16	17	2	4	5	4	3	2	7	6	7	2
H5	7	7	3	7	4	6	5	18	5	4	17	16	15	16	17	6	18	20	8	18	20	17	16	17	16	5	5	4	5	4	5	6	3	6	5
H6	5	3	5	5	4	6	3	14	5	2	13	14	11	12	13	6	14	16	6	14	16	13	12	13	12	5	5	2	5	4	5	6	1	2	5
H7	6	4	6	6	5	7	4	13	6	3	12	13	10	11	12	7	13	15	7	13	15	12	11	12	11	6	6	3	6	5	6	7	2	3	6
H8	6	8	4	6	3	3	6	17	2	5	16	17	14	17	16	3	17	19	5	17	19	16	15	16	17	2	4	5	4	3	2	7	6	7	2
H9	5	3	7	5	4	6	5	14	5	4	13	14	11	14	13	6	12	16	6	14	16	13	12	13	14	5	5	4	5	4	5	8	3	4	5
H10	7	5	3	7	4	4	5	16	3	4	15	16	13	14	15	4	16	18	4	16	18	15	14	15	14	3	5	2	5	4	3	6	3	4	3
H11	6	6	4	6	3	3	6	15	2	5	14	15	12	15	14	3	15	17	3	15	17	14	13	14	15	2	4	3	4	3	2	7	4	5	2
H12	5	5	5	5	4	6	3	16	5	2	15	16	13	14	15	6	16	16	6	16	18	15	14	15	14	5	5	4	5	4	5	6	3	4	5
H13	6	10	6	8	5	5	8	19	4	7	18	19	16	19	18	1	19	19	7	19	21	18	17	18	19	2	6	7	6	5	4	8	8	9	4
H14	8	8	6	8	5	5	8	17	4	7	16	17	14	17	16	5	17	19	5	17	17	14	15	16	17	4	6	5	4	3	4	9	6	7	4
H15	5	7	3	5	2	4	5	16	3	4	15	14	13	16	15	4	16	18	6	16	18	15	14	15	16	3	3	4	3	2	3	6	3	6	3
H16	1	7	7	1	4	6	5	12	5	4	11	12	9	12	11	6	12	14	8	12	14	11	10	11	12	5	5	6	3	4	5	8	5	4	5
H17	6	2	6	6	5	7	4	15	6	3	14	15	12	13	14	7	13	17	7	15	17	14	13	14	13	6	6	3	6	5	6	7	2	3	6
H18	19	22	21	19	20	20	21	21	21	22	21	20	19	20	21	22	22	24	22	22	23	20	20	21	22	21	21	20	19	20	19	24	19	20	21
H19	6	6	2	6	3	3	4	17	2	3	16	17	14	15	16	3	17	19	5	17	19	16	15	16	15	2	4	3	4	3	2	5	4	5	2
H20	7	5	5	5	4	6	5	16	5	4	13	16	13	14	15	6	16	18	6	16	18	15	14	15	14	5	5	2	5	4	5	6	3	4	5
H21	5	5	5	5	4	6	3	16	5	2	15	16	13	14	15	6	16	18	8	16	18	15	14	15	14	5	5	4	5	4	5	6	3	4	5
H22	4	4	4	4	3	5	2	15	4	1	14	15	12	13	14	5	15	17	7	15	17	14	13	14	13	4	4	3	4	3	4	5	2	3	4
H23	4	6	6	4	3	5	4	15	4	3	14	15	12	15	14	5	15	17	7	15	17	14	13	14	15	4	4	5	4	3	4	7	4	5	4
H24	8	10	6	8	5	5	8	17	4	7	16	17	14	17	16	5	17	19	7	17	19	16	15	16	17	4	6	7	6	5	4	9	8	9	4
H25	5	9	7	5	4	6	5	12	5	6	11	12	9	12	11	6	12	14	8	12	14	11	10	11	12	5	5	6	3	4	5	8	7	8	5
H26	5	9	7	5	4	6	7	16	5	6	15	16	13	16	15	6	16	18	8	16	16	15	12	15	16	5	5	6	5	4	5	8	7	6	5
H27	11	15	17	11	14	16	13	2	15	14	1	2	1	2	3	16	2	4	16	2	6	1	2	1	2	15	15	14	13	14	15	18	13	12	15
H28	21	25	25	21	24	25	23	24	25	24	23	22	21	23	23	26	24	24	24	24	25	22	22	23	24	25	24	24	23	24	24	28	21	22	25
H29	5	9	5	7	4	4	7	18	3	6	17	18	15	18	17	2	18	20	6	18	20	17	16	17	18	1	5	6	5	4	3	7	7	8	3
H30	8	10	6	8	5	5	8	17	4	7	16	17	14	17	16	5	17	19	5	17	19	16	15	16	17	4	6	7	6	5	4	9	8	9	4
H31	12	16	18	12	15	17	14	3	16	15	2	3	2	3	4	17	3	5	17	3	7	2	3	2	3	16	16	15	14	15	16	19	14	13	16
H32	11	15	17	9	14	16	13	4	15	14	1	4	1	4	5	16	4	6	16	4	8	3	4	3	4	15	15	14	13	14	15	18	13	12	15
H33	11	13	15	11	14	16	11	4	15	12	3	4	3	2	5	16	4	6	16	4	8	3	4	3	2	15	15	12	13	14	15	16	11	10	15
H34	6	10	6	6	5	3	8	17	4	7	16	17	14	15	16	5	17	19	7	17	19	16	15	16	17	4	6	7	6	5	2	9	8	7	4
H35	14	18	18	14	15	17	16	5	16	17	4	5	4	5	6	17	5	7	16	5	9	4	5	4	5	16	16	15	14	15	16	19	16	15	16
H36	12	14	18	12	15	17	14	1	16	15	2	3	2	3	4	17	3	5	17	3	7	2	3	2	3	16	16	15	14	15	16	19	14	13	16
H37	5	7	5	5	4	4	7	16	3	6	15	16	13	16	15	4	16	18	6	16	18	15	14	15	16	3	5	6	5	4	3	8	7	6	3
H38	6	8	6	6	5	5	8	15	4	7	14	15	12	15	14	5	15	17	3	15	17	14	13	14	15	4	6	5	6	5	4	9	6	5	4
H39	6	6	6	6	5	7	2	13	6	3	12	13	10	11	12	7	13	15	9	13	15	12	11	12	11	6	6	5	6	5	6	7	4	5	6
H40	9	4	10	10	9	11	8	17	10	7	18	19	16	17	18	10	17	21	11	19	21	18	17	18	17	9	10	7	10	9	10	7	6	7	10
H41	6	8	4	6	3	3	6	17	2	5	16	17	14	17	16	3	17	19	3	17	19	16	15	16	17	2	4	5	4	3	2	7	6	7	2
H42	5	5	5	5	4	6	3	16	5	2	15	16	13	14	15	6	16	18	8	16	18	15	14	15	14	5	5	4	5	4	5	6	3	4	5
H43	3	5	5	3	2	4	3	14	3	2	13	14	11	14	13	4	14	16	6	14	16	13	12	13	14	3	3	4	3	2	3	6	3	4	3
H44	4	6	4	4	1	3	4	15	2	3	14	15	12	15	14	3	15	17	5	15	17	14	13	14	15	2	2	3	2	1	2	5	4	5	2
H45	7	9	5	7	4	4	7	18	3	6	17	18	15	18	17	4	18	20	6	18	20	17	16	17	18	3	3	6	5	4	3	8	7	8	3
H46	7	3	7	7	6	8	5	16	7	4	15	16	13	14	15	8	14	18	8	16	18	15	14	15	14	7	7	4	7	6	7	8	3	4	7
H47	4	8	6	6	3	5	6	15	4	5	14	15	12	15	14	3	15	17	7	15	17	14	13	14	15	2	4	5	4	3	4	6	6	7	4
H48	5	5	5	5	4	6	3	16	5	2	15	16	13	14	15	6	16	18	8	16	17	15	14	15	14	5	5	4	5	4	5	6	3	4	4
H49	6	8	4	6	3	3	6	17	2	5	16	17	14	17	16	3	17	19	5	17	19	16	15	16	17	2	4	5	4	3	2	7	6	7	2
H50	12	14	16	12	15	17	12	3	16	13	2	3	2	1	4	17	3	5	17	3	7	2	3	2	1	16	16	13	14	15	16	17	12	11	16
H51	5	9	5	5	4	4	7	16	3	6	15	16	13	16	15	4	16	18	6	16	18	15	14	15	16	3	5	6	5	4	3	8	7	6	3
H52	6	8	4	6	3	3	4	15	2	5	14	15	12	15	14	3	15	17	5	15	17	14	13	14	15	2	4	5	4	3	2	7	6	7	2
H53	6	8	4	6	3	3	6	17	2	5	16	17	14	17	16	3	17	19	5	17	19	16	15	16	17	2	4	5	4	3	2	7	6	7	2
H54	4	8	4	4	3	3	6	15	2	5	14	15	12	15	14	3	15	17	5	15	17	14	13	14	15	2	4	5	2	3	2	7	6	7	2
H55	6	8	4	6	3	3	6	17	2	5	16	17	14	17	16	3	17	19	5	17	19	16	15	16	17	2	4	5	4	3	2	7	6	7	2
H56	2	8	8	2	5	7	6	13	6	5	12	13	10	13	12	7	13	15	9	13	15	12	11	12	13	6	6	7	4	5	6	9	6	5	6
H57	6	8	4	6	3	3	6	15	2	5	14	15	12	15	14	3	15	17	5	15	17	14	13	14	15	2	4	5	4	3	2	7	6	7	2
H58	6	10	6	8	5	5	8	17	3	7	16	17	14	17	16	3	17	19	7	17	19	16	15	16	17	2	6	7	6	5	4	8	8	9	4
H59	7	7	5	7	4	6	5	18	5	4	17	18	15	16	17	6	18	20	8	18	20	17	16	17	16	5	5	4	5	4	5	6	5	6	5
H60	6	8	4	6	3	3	6	17	2	5	16	17	14	17	16	3	17	19	5	17	17	16	15	16	17	2	4	5	4	3	2	7	6	7	2

Tabl e 4. 5 (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
H61	0,039	0,002	0,012	0,005	0,012	0,012	0,014	0,005	0,012	0,007	0,005	0,012	0,009	0,009	0,007	0,012	0,014	0,053	0,005	0,012	0,012	0,009	0,009	0,009	0,012	0,012	0,036	0,065	0,007	0,009	0,039	0,036	0,036	0,009	0,039	0,039	0,007	0,009	0,014	0,024
H62	0,031	0,009	0,005	0,012	0,009	0,005	0,007	0,012	0,009	0,009	0,012	0,005	0,017	0,017	0,009	0,005	0,007	0,050	0,007	0,009	0,005	0,002	0,007	0,017	0,014	0,009	0,029	0,056	0,014	0,017	0,031	0,029	0,024	0,012	0,036	0,031	0,009	0,012	0,007	0,017
H63	0,031	0,014	0,009	0,017	0,014	0,005	0,007	0,017	0,005	0,009	0,012	0,009	0,021	0,017	0,014	0,009	0,002	0,050	0,012	0,009	0,009	0,007	0,012	0,021	0,019	0,014	0,029	0,056	0,019	0,021	0,031	0,029	0,024	0,017	0,036	0,031	0,014	0,012	0,012	0,012
H64	0,009	0,031	0,026	0,034	0,036	0,026	0,024	0,034	0,026	0,031	0,029	0,031	0,039	0,034	0,031	0,021	0,029	0,048	0,034	0,031	0,031	0,029	0,029	0,034	0,021	0,031	0,002	0,053	0,036	0,034	0,005	0,007	0,007	0,034	0,009	0,005	0,031	0,029	0,024	0,039
H65	0,012	0,034	0,024	0,036	0,039	0,029	0,031	0,036	0,029	0,034	0,031	0,034	0,041	0,036	0,034	0,024	0,031	0,056	0,036	0,029	0,034	0,031	0,031	0,036	0,024	0,034	0,005	0,061	0,039	0,036	0,007	0,005	0,009	0,036	0,012	0,007	0,034	0,031	0,026	0,041
H66	0,005	0,031	0,026	0,034	0,036	0,026	0,024	0,034	0,026	0,031	0,029	0,031	0,039	0,034	0,031	0,021	0,029	0,048	0,034	0,031	0,031	0,029	0,029	0,034	0,021	0,031	0,002	0,053	0,036	0,034	0,005	0,007	0,007	0,034	0,009	0,005	0,031	0,029	0,024	0,039
H67	0,009	0,036	0,031	0,039	0,041	0,031	0,029	0,039	0,031	0,036	0,034	0,036	0,044	0,039	0,036	0,026	0,034	0,053	0,039	0,036	0,036	0,034	0,034	0,039	0,026	0,036	0,002	0,056	0,041	0,039	0,005	0,007	0,007	0,039	0,009	0,005	0,036	0,034	0,029	0,044
H68	0,044	0,047	0,047	0,050	0,053	0,047	0,045	0,050	0,047	0,047	0,045	0,053	0,055	0,047	0,047	0,047	0,050	0,065	0,050	0,047	0,053	0,050	0,050	0,056	0,047	0,047	0,042	0,070	0,053	0,055	0,045	0,042	0,042	0,047	0,045	0,039	0,047	0,045	0,050	0,056
H69	0,039	0,017	0,017	0,019	0,017	0,007	0,009	0,019	0,007	0,012	0,014	0,012	0,024	0,019	0,017	0,017	0,005	0,053	0,014	0,012	0,012	0,009	0,014	0,024	0,021	0,022	0,036	0,064	0,021	0,024	0,039	0,036	0,031	0,024	0,044	0,039	0,022	0,019	0,014	0,009
H70	0,039	0,012	0,017	0,014	0,012	0,007	0,009	0,014	0,007	0,007	0,009	0,012	0,019	0,014	0,012	0,017	0,005	0,053	0,009	0,007	0,012	0,009	0,014	0,019	0,017	0,017	0,036	0,064	0,017	0,019	0,039	0,036	0,031	0,019	0,039	0,039	0,017	0,014	0,014	0,014
H71	0,014	0,031	0,026	0,034	0,036	0,026	0,029	0,034	0,026	0,031	0,029	0,031	0,039	0,034	0,031	0,021	0,029	0,053	0,034	0,031	0,031	0,029	0,029	0,034	0,021	0,031	0,007	0,059	0,036	0,034	0,009	0,012	0,012	0,034	0,014	0,009	0,031	0,029	0,024	0,039
H72	0,012	0,034	0,034	0,036	0,039	0,034	0,031	0,036	0,034	0,034	0,031	0,039	0,041	0,036	0,034	0,029	0,036	0,050	0,036	0,034	0,039	0,036	0,036	0,036	0,024	0,034	0,005	0,061	0,039	0,036	0,007	0,009	0,009	0,036	0,007	0,007	0,034	0,031	0,031	0,047
H73	0,036	0,005	0,009	0,007	0,014	0,014	0,017	0,007	0,014	0,009	0,007	0,014	0,012	0,012	0,009	0,014	0,017	0,051	0,007	0,009	0,014	0,012	0,012	0,012	0,009	0,014	0,034	0,062	0,009	0,012	0,036	0,029	0,034	0,012	0,036	0,036	0,009	0,012	0,012	0,026
H74	0,009	0,031	0,026	0,034	0,036	0,026	0,024	0,034	0,026	0,031	0,029	0,031	0,039	0,034	0,031	0,021	0,029	0,048	0,034	0,031	0,031	0,029	0,029	0,034	0,021	0,031	0,002	0,053	0,036	0,034	0,005	0,007	0,007	0,034	0,009	0,005	0,031	0,029	0,024	0,039
H75	0,036	0,005	0,014	0,007	0,014	0,014	0,017	0,007	0,014	0,009	0,007	0,014	0,007	0,012	0,009	0,014	0,017	0,056	0,007	0,014	0,014	0,012	0,012	0,012	0,014	0,014	0,039	0,067	0,005	0,012	0,041	0,039	0,039	0,012	0,042	0,041	0,009	0,012	0,017	0,024
H76	0,012	0,034	0,029	0,036	0,039	0,029	0,026	0,036	0,029	0,034	0,031	0,034	0,041	0,036	0,034	0,024	0,031	0,050	0,036	0,034	0,034	0,031	0,031	0,036	0,024	0,034	0,005	0,056	0,039	0,036	0,007	0,009	0,009	0,036	0,012	0,007	0,034	0,031	0,026	0,041
H77	0,034	0,012	0,012	0,014	0,017	0,007	0,009	0,014	0,002	0,012	0,009	0,012	0,019	0,014	0,012	0,012	0,005	0,050	0,014	0,012	0,012	0,009	0,009	0,019	0,017	0,017	0,031	0,056	0,017	0,019	0,034	0,031	0,031	0,019	0,039	0,034	0,017	0,014	0,014	0,014
H78	0,036	0,005	0,009	0,007	0,009	0,009	0,012	0,007	0,009	0,009	0,007	0,009	0,012	0,012	0,005	0,009	0,012	0,050	0,007	0,009	0,009	0,007	0,007	0,012	0,009	0,009	0,034	0,061	0,009	0,012	0,036	0,034	0,034	0,012	0,036	0,036	0,009	0,012	0,012	0,021
H79	0,009	0,031	0,031	0,034	0,036	0,031	0,029	0,034	0,031	0,031	0,029	0,036	0,039	0,034	0,031	0,026	0,034	0,048	0,034	0,031	0,036	0,034	0,034	0,034	0,021	0,031	0,002	0,059	0,036	0,034	0,005	0,007	0,007	0,034	0,005	0,005	0,031	0,029	0,029	0,044
H80	0,012	0,039	0,034	0,041	0,039	0,029	0,026	0,041	0,034	0,034	0,036	0,034	0,046	0,041	0,039	0,029	0,031	0,056	0,036	0,034	0,034	0,031	0,036	0,041	0,029	0,039	0,005	0,061	0,044	0,041	0,007	0,009	0,005	0,041	0,012	0,007	0,039	0,036	0,026	0,041
H81	0,024	0,012	0,007	0,014	0,017	0,012	0,014	0,014	0,012	0,017	0,014	0,012	0,014	0,019	0,012	0,002	0,014	0,048	0,014	0,017	0,012	0,009	0,009	0,019	0,012	0,012	0,026	0,053	0,012	0,019	0,029	0,026	0,026	0,014	0,034	0,029	0,012	0,014	0,014	0,021
H82	0,039	0,017	0,017	0,019	0,017	0,007	0,009	0,019	0,007	0,012	0,014	0,012	0,024	0,019	0,017	0,017	0,005	0,056	0,014	0,012	0,012	0,009	0,014	0,024	0,021	0,021	0,036	0,064	0,021	0,024	0,039	0,036	0,031	0,024	0,044	0,034	0,017	0,019	0,014	0,009
H83	0,044	0,007	0,017	0,009	0,007	0,012	0,014	0,009	0,017	0,007	0,009	0,012	0,014	0,014	0,007	0,017	0,014	0,053	0,005	0,012	0,012	0,009	0,014	0,014	0,017	0,017	0,041	0,064	0,012	0,014	0,044	0,042	0,036	0,014	0,044	0,044	0,012	0,014	0,014	0,024
H84	0,029	0,012	0,002	0,014	0,017	0,012	0,014	0,014	0,012	0,017	0,014	0,012	0,019	0,019	0,012	0,002	0,014	0,048	0,014	0,012	0,012	0,009	0,009	0,019	0,012	0,012	0,026	0,053	0,017	0,019	0,029	0,021	0,026	0,014	0,034	0,029	0,012	0,014	0,014	0,024
H85	0,036	0,005	0,009	0,007	0,009	0,009	0,012	0,007	0,009	0,009	0,007	0,009	0,012	0,012	0,005	0,009	0,012	0,050	0,007	0,009	0,009	0,007	0,007	0,012	0,009	0,009	0,034	0,061	0,009	0,012	0,036	0,034	0,034	0,012	0,036	0,036	0,009	0,012	0,012	0,021
H86	0,041	0,005	0,014	0,007	0,014	0,014	0,017	0,007	0,014	0,009	0,007	0,014	0,012	0,012	0,009	0,014	0,017	0,050	0,007	0,014	0,014	0,012	0,012	0,012	0,014	0,014	0,039	0,064	0,009	0,012	0,041	0,039	0,039	0,007	0,041	0,041	0,009	0,012	0,016	0,026
H87	0,034	0,012	0,012	0,014	0,012	0,007	0,009	0,014	0,012	0,012	0,014	0,007	0,019	0,019	0,012	0,012	0,009	0,053	0,009	0,012	0,007	0,005	0,009	0,019	0,012	0,017	0,031	0,059	0,017	0,019	0,034	0,031	0,026	0,019	0,039	0,034	0,017	0,019	0,005	0,019
H88	0,012	0,039	0,034	0,041	0,044	0,034	0,031	0,041	0,034	0,039	0,036	0,039	0,046	0,041	0,039	0,029	0,036	0,053	0,041	0,039	0,039	0,036	0,036	0,041	0,029	0,039	0,005	0,061	0,044	0,041	0,007	0,009	0,009	0,041	0,012	0,002	0,039	0,036	0,031	0,041
H89	0,039	0,002	0,012	0,005	0,012	0,012	0,014	0,005	0,012	0,007	0,005	0,012	0,009	0,009	0,007	0,012	0,014	0,053	0,005	0,012	0,012	0,009	0,009	0,009	0,012	0,012	0,036	0,065	0,007	0,009	0,039	0,036	0,036	0,009	0,039	0,039	0,007	0,009	0,014	0,024
H90	0,036	0,009	0,009	0,012	0,009	0,005	0,007	0,012	0,009	0,009	0,012	0,005	0,017	0,017	0,009	0,009	0,007	0,056	0,007	0,009	0,005	0,002	0,007	0,017	0,014	0,014	0,034	0,061	0,014	0,017	0,036	0,034	0,029	0,017	0,041	0,036	0,014	0,017	0,007	0,017
H91	0,009	0,036	0,026	0,039	0,041	0,031	0,029	0,039	0,031	0,036	0,034	0,036	0,044	0,039	0,036	0,026	0,034	0,053	0,039	0,031	0,036	0,034	0,034	0,039	0,026	0,036	0,002	0,059	0,041	0,039	0,005	0,002	0,007	0,039	0,009	0,005	0,036	0,034	0,029	0,044
H92	0,012	0,039	0,034	0,041	0,039	0,034	0,031	0,041	0,034	0,039	0,036	0,039	0,046	0,041	0,034	0,029	0,036	0,050	0,041	0,039	0,039	0,036	0,036	0,041	0,029	0,039	0,005	0,056	0,044	0,041	0,007	0,009	0,009	0,041	0,012	0,007	0,039	0,036	0,031	0,047
H93	0,009	0,031	0,026	0,034	0,036	0,026	0,024	0,034	0,026	0,031	0,029	0,031	0,039	0,034	0,031	0,021	0,029	0,048	0,034	0,031	0,031	0,029	0,029	0,034	0,021	0,031	0,002	0,053	0,036	0,034	0,005	0,002	0,007	0,034	0,009	0,005	0,031	0,029	0,024	0,039
H94	0,012	0,039	0,034	0,041	0,039	0,029	0,026	0,041	0,034	0,034	0,036	0,034	0,046	0,041	0,039	0,029	0,031	0,050	0,036	0,034	0,034	0,031	0,036	0,041	0,029	0,039	0,005	0,059	0,044	0,041	0,007	0,009	0,005	0,036	0,012	0,007	0,039	0,036	0,026	0,041
H95	0,009	0,036	0,031	0,039	0,041	0,031	0,029	0,039	0,031	0,036	0,034	0,036	0,044	0,039	0,036	0,026	0,034	0,053	0,039	0,036	0,036	0,034	0,034	0,039	0,026	0,036	0,007	0,059	0,041	0,039	0,009	0,012	0,012	0,039	0,014	0,009	0,036	0,034	0,029	0,044
H96	0,036	0,005	0,014	0,007	0,014	0,014	0,017	0,007	0,014	0,009	0,007	0,014	0,002	0,012	0,009	0,014	0,017	0,056	0,007	0,014	0,014	0,012	0,012	0,012	0,014	0,014	0,039	0,067	0,005	0,012	0,041	0,039	0,039	0,012	0,041	0,041	0,009	0,012	0,016	0,024
H97	0,012	0,039	0,034	0,042	0,044	0,034	0,031	0,041	0,029	0,039	0,036	0,039	0,046	0,041	0,039	0,029	0,031	0,056	0,041	0,039	0,039	0,036	0,036	0,042	0,029	0,039	0,005	0,061	0,044	0,041	0,007	0,009	0,009	0,041	0,012	0,007	0,039	0,036	0,031	0,041
H98	0,017	0,044	0,039	0,047	0,049	0,039	0,036	0,047	0,039	0,044	0,041	0,039	0,047	0,046	0,044	0,034	0,041	0,061	0,047	0,044	0,044	0,041	0,041	0,047	0,034	0,044	0,009	0,062	0,049	0,047	0,012	0,014	0,014	0,046	0,017	0,012	0,044	0,041	0,036	0,052
H99	0,041	0,009	0,019	0,012	0,019	0,014	0,016	0,012	0,014	0,009	0,007	0,014	0,016	0,012	0,014	0,019	0,017	0,056	0,012	0,014	0,019	0,017	0,017	0,017	0,019	0,019	0,039	0,061	0,014	0,012	0,041	0,039	0,039	0,017	0,039	0,041	0,014	0,007	0,021	0,026
H100	0,012	0,039	0,034	0,041	0,044	0,034	0,031	0,041	0,034	0,039	0,036	0,039	0,046	0,041	0,039	0,029	0,036	0,056	0,041	0,039	0,039	0,036	0,036	0,041	0,029	0,039	0,005	0,061	0,044	0,041	0,007	0,009	0,009	0,041	0,012	0,007	0,039	0,036	0,031	0,047
H101	0,017	0,044	0,039	0,047	0,049	0,039	0,036	0,047	0,039	0,044	0,041	0,044	0,052	0,041	0,044	0,034	0,041	0,059	0,047	0,044	0,044	0,041	0,042	0,047	0,034	0,039	0,014	0,065	0,049	0,047	0,017	0,019	0,019	0,047	0,022	0,017	0,044	0,041	0,036	0,052
H102	0,009	0,036	0,031	0,039	0,041	0,031	0,029	0,039	0,031	0,036	0,034	0,036	0,044	0,034	0,036	0,026	0,034	0,050	0,039	0,036	0,036	0,034	0,034	0,039	0,026	0,036	0,002	0,056	0,041	0,039	0,005	0,007	0,007	0,039	0,009	0,005	0,036	0,034	0,029	0,044
H103	0,012	0,034	0,029	0,036	0,039	0,029	0,026	0,036	0,029	0,034	0,031	0,034	0,041	0,036	0,034	0,024	0,031	0,050	0,036	0,034	0,034	0,031	0,031	0,036	0,024	0,029	0,005	0,056	0,039	0,036	0,007	0,009	0,009	0,036	0,012	0,007	0,034	0,031	0,026	0,041
H104	0,009	0,036	0,031	0,039	0,041	0,031	0,029	0,039	0,031	0,036	0,034	0,036	0,044	0,039	0,036	0,026	0,034	0,053	0,039	0,036	0,036	0,034	0,034	0,039	0,026	0,036	0,002	0,059	0,041	0,039	0,005	0,007	0,007	0,039	0,009	0,005	0,036	0,034	0,029	0,044
H105	0,012	0,039	0,034	0,041	0,039	0,029	0,026	0,041	0,034	0,034	0,036	0,034	0,046	0,041	0,039	0,029	0,031	0,056	0,036	0,034	0,034	0,031	0,036	0,041	0,029	0,039	0,005	0,061	0,044	0,041	0,007	0,009	0,005	0,041	0,012	0,007	0,039	0,036	0,026	0,041
H106	0,034	0,002	0,012	0,005	0,012	0,012	0,014	0,005	0,012	0,007	0,005	0,012	0,005	0,009	0,007	0,012	0,014	0,053	0,005	0,012	0,012	0,009	0,009	0,009	0,012	0,012	0,036	0,064	0,002	0,009	0,039	0,036	0,036	0,009	0,039	0,039	0,007	0,009	0,014	0,021
H107	0,039	0,007	0,012	0,009	0,012	0,012	0,014	0,009	0,012	0,012	0,009	0,012	0,014	0,014	0,007	0,012	0,014	0,053	0,009	0,012	0,012	0,009	0,009	0,014	0,012	0,012	0,036	0,061	0,012	0,014	0,039	0,036	0,036	0,014	0,039	0,039	0,012	0,014	0,014	0,024
H108	0,036	0,009	0,014	0,012	0,009	0,005	0,007	0,012	0,009	0,005	0,007	0,009	0,017	0,012	0,009	0,014	0,007	0,050	0,007	0,005	0,009	0,007	0,012	0,017	0,014	0,014	0,034	0,061	0,014	0,017	0,036	0,034	0,029	0,017	0,036	0,036	0,014	0,012	0,012	0,017
H109	0,034	0,007	0,012	0,009	0,012	0,012	0,014	0,009	0,012	0,012	0,009	0,012	0,014	0,009	0,007	0,007	0,014	0,048	0,009	0,012	0,012	0,009	0,009	0,014	0,007	0,012	0,031	0,059	0,012	0,014	0,034	0,031	0,031	0,014	0,034	0,034	0,012	0,014	0,014	0,024
H110	0,036	0,005	0,009	0,007	0,009	0,009	0,012	0,007	0,009	0,009	0,007	0,009	0,012	0,007	0,005	0,009	0,012	0,050	0,007	0,009	0,009	0,007	0,007	0,012	0,009	0,009	0,034	0,061	0,009	0,012	0,036	0,034	0,034	0,012	0,036	0,036	0,009	0,012	0,012	0,021
H111	0,039	0,002	0,012	0,005	0,012	0,012	0,014	0,005	0,012	0,007	0,005	0,012	0,009	0,009	0,007	0,012	0,014	0,048	0,005	0,012	0,012	0,009	0,009	0,009	0,012	0,012	0,036	0,061	0,007	0,009	0,039	0,036	0,036	0,005	0,039	0,039	0,007	0,009	0,014	0,024
H112	0,044	0,014	0,019	0,017	0,014	0,014	0,017	0,017	0,019	0,014	0,017	0,014	0,019	0,021	0,014	0,019	0,017	0,061	0,012	0,014	0,014	0,012	0,017	0,021	0,019	0,019	0,044	0,073	0,017	0,021	0,047	0,044	0,039	0,021	0,047	0,047	0,019	0,021	0,017	0,017
H113	0,034	0,012	0,012	0,014	0,007	0,002	0,005	0,014	0,007	0,007	0,009	0,007	0,019	0,014	0,007	0,012	0,005	0,048	0,009	0,007	0,007	0,005	0,009	0,019	0,017	0,017	0,031	0,053	0,017	0,019	0,034	0,031	0,026	0,019	0,039	0,034	0,017	0,014	0,009	0,014
H114	0,031	0,014	0,009	0,017	0,014	0,005	0,007	0,017	0,009	0,009	0,012	0,009	0,021	0,017	0,014	0,009	0,007	0,050	0,012	0,009	0,009	0,007	0,012	0,021	0,019	0,014	0,029	0,056	0,019	0,021	0,031	0,029	0,024	0,017	0,036	0,031	0,014	0,012	0,012	0,017
H115	0,039	0,002	0,012	0,005	0,012	0,012	0,014	0,005	0,012	0,007	0,005	0,012	0,009	0,009	0,007	0,012	0,014	0,053	0,005	0,012	0,012	0,009	0,009	0,009	0,012	0,012	0,036	0,064	0,007	0,009	0,039	0,036	0,036	0,009	0,039	0,039	0,007	0,009	0,014	0,024

Table 4. 5 (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
H61	0,005	0,012	0,007	0,005	0,007	0,017	0,009	0,012	0,005	0,039	0,007	0,005	0,005	0,005	0,005	0,014	0,005	0,009	0,012	0,005		5	7	14	15	14	16	20	8	6	14	15	3	14	3	15	6	3	14	17
H62	0,012	0,005	0,005	0,007	0,014	0,009	0,012	0,005	0,012	0,026	0,009	0,012	0,012	0,012	0,012	0,007	0,012	0,017	0,009	0,012	0,012		2	11	12	11	13	19	5	5	11	14	6	11	6	12	5	4	13	12
H63	0,017	0,009	0,009	0,012	0,019	0,005	0,017	0,009	0,017	0,026	0,014	0,017	0,017	0,017	0,017	0,012	0,017	0,021	0,014	0,017	0,017	0,005		11	12	11	13	19	3	3	11	14	8	11	8	12	3	6	13	12
H64	0,034	0,031	0,026	0,029	0,036	0,031	0,029	0,031	0,034	0,005	0,031	0,029	0,034	0,029	0,034	0,024	0,029	0,034	0,036	0,034	0,034	0,026	0,026		3	2	2	16	14	14	4	3	13	2	15	3	12	13	2	3
H65	0,036	0,034	0,029	0,031	0,039	0,034	0,031	0,034	0,036	0,007	0,034	0,031	0,036	0,031	0,036	0,026	0,031	0,041	0,039	0,036	0,036	0,029	0,029	0,007		3	3	19	15	15	3	4	12	3	16	4	13	14	3	4
H66	0,034	0,031	0,026	0,029	0,036	0,031	0,029	0,031	0,034	0,005	0,031	0,029	0,034	0,029	0,034	0,024	0,029	0,034	0,036	0,034	0,034	0,026	0,026	0,005	0,007		2	16	14	14	4	3	13	2	15	3	12	13	2	3
H67	0,039	0,036	0,031	0,034	0,042	0,036	0,034	0,036	0,039	0,005	0,036	0,034	0,039	0,034	0,039	0,029	0,034	0,039	0,041	0,039	0,039	0,031	0,031	0,005	0,007	0,005		18	16	16	4	3	15	2	17	3	14	15	2	3
H68	0,050	0,053	0,047	0,045	0,053	0,053	0,050	0,050	0,050	0,044	0,047	0,045	0,050	0,050	0,050	0,050	0,050	0,050	0,053	0,050	0,050	0,047	0,047	0,039	0,047	0,039	0,045		22	20	20	17	19	18	21	17	20	19	16	19
H69	0,019	0,012	0,012	0,014	0,022	0,007	0,019	0,012	0,019	0,034	0,021	0,019	0,019	0,019	0,019	0,019	0,019	0,024	0,017	0,019	0,019	0,012	0,007	0,034	0,036	0,034	0,039	0,055		4	14	17	9	14	9	15	4	7	16	15
H70	0,014	0,012	0,012	0,009	0,017	0,007	0,014	0,012	0,014	0,034	0,017	0,014	0,009	0,014	0,014	0,019	0,014	0,019	0,012	0,014	0,014	0,012	0,007	0,034	0,036	0,034	0,039	0,050	0,009		14	15	7	14	7	15	4	5	14	15
H71	0,034	0,031	0,026	0,029	0,036	0,031	0,029	0,031	0,034	0,009	0,031	0,029	0,034	0,029	0,034	0,024	0,029	0,039	0,036	0,034	0,034	0,026	0,026	0,009	0,007	0,009	0,009	0,050	0,034	0,034		5	13	4	15	5	12	13	4	5
H72	0,036	0,039	0,034	0,031	0,039	0,039	0,031	0,039	0,036	0,007	0,034	0,031	0,036	0,031	0,036	0,031	0,031	0,036	0,039	0,036	0,036	0,034	0,034	0,007	0,009	0,007	0,007	0,042	0,041	0,036	0,012		14	3	16	4	15	14	1	4
H73	0,007	0,014	0,009	0,007	0,009	0,019	0,012	0,014	0,007	0,036	0,009	0,002	0,007	0,007	0,007	0,017	0,007	0,012	0,014	0,007	0,007	0,014	0,019	0,031	0,029	0,031	0,036	0,047	0,021	0,017	0,031	0,034		13	4	14	7	4	13	16
H74	0,034	0,031	0,026	0,029	0,036	0,031	0,029	0,031	0,034	0,005	0,031	0,029	0,034	0,029	0,034	0,024	0,029	0,034	0,036	0,034	0,034	0,026	0,026	0,005	0,007	0,005	0,005	0,045	0,034	0,034	0,009	0,007	0,031		15	1	12	13	2	3
H75	0,007	0,014	0,009	0,007	0,009	0,019	0,007	0,014	0,007	0,041	0,009	0,007	0,007	0,007	0,007	0,017	0,007	0,007	0,014	0,007	0,007	0,014	0,019	0,036	0,039	0,036	0,041	0,053	0,021	0,017	0,036	0,039	0,009	0,036		16	7	4	15	18
H76	0,036	0,034	0,029	0,031	0,039	0,034	0,031	0,034	0,036	0,007	0,034	0,031	0,036	0,031	0,036	0,026	0,031	0,036	0,039	0,036	0,036	0,029	0,029	0,007	0,009	0,007	0,007	0,042	0,036	0,036	0,012	0,009	0,034	0,002	0,039		13	14	3	4
H77	0,014	0,012	0,007	0,009	0,017	0,007	0,014	0,012	0,014	0,034	0,017	0,014	0,014	0,014	0,014	0,014	0,014	0,019	0,017	0,014	0,014	0,012	0,007	0,029	0,031	0,029	0,034	0,050	0,009	0,009	0,029	0,036	0,017	0,029	0,017	0,031		5	14	15
H78	0,007	0,009	0,005	0,002	0,009	0,014	0,007	0,009	0,007	0,036	0,009	0,007	0,007	0,007	0,007	0,012	0,007	0,012	0,009	0,007	0,007	0,009	0,014	0,031	0,034	0,031	0,036	0,047	0,017	0,012	0,031	0,034	0,009	0,031	0,009	0,034	0,012		13	16
H79	0,034	0,036	0,031	0,029	0,036	0,036	0,029	0,036	0,034	0,005	0,031	0,029	0,034	0,029	0,034	0,029	0,029	0,034	0,036	0,034	0,034	0,031	0,031	0,005	0,007	0,005	0,005	0,039	0,039	0,034	0,009	0,002	0,031	0,005	0,036	0,007	0,034	0,031		3
H80	0,041	0,034	0,034	0,036	0,044	0,034	0,036	0,034	0,041	0,002	0,039	0,036	0,041	0,036	0,041	0,031	0,036	0,041	0,039	0,041	0,041	0,029	0,029	0,007	0,009	0,007	0,007	0,047	0,036	0,036	0,012	0,009	0,039	0,007	0,044	0,009	0,036	0,039	0,007	
H81	0,014	0,012	0,007	0,009	0,017	0,017	0,009	0,012	0,014	0,029	0,012	0,014	0,014	0,009	0,014	0,005	0,014	0,014	0,017	0,014	0,014	0,007	0,012	0,024	0,026	0,024	0,029	0,050	0,019	0,019	0,024	0,031	0,017	0,024	0,012	0,026	0,014	0,012	0,029	0,031
H82	0,019	0,012	0,012	0,014	0,021	0,007	0,019	0,012	0,019	0,034	0,021	0,019	0,019	0,019	0,019	0,019	0,019	0,024	0,017	0,019	0,019	0,012	0,007	0,034	0,036	0,034	0,039	0,050	0,009	0,009	0,034	0,041	0,021	0,034	0,021	0,036	0,009	0,017	0,039	0,036
H83	0,009	0,012	0,012	0,009	0,012	0,017	0,014	0,012	0,009	0,039	0,012	0,009	0,009	0,009	0,009	0,019	0,009	0,014	0,012	0,009	0,009	0,012	0,017	0,039	0,042	0,039	0,044	0,056	0,019	0,014	0,039	0,042	0,012	0,039	0,012	0,041	0,019	0,012	0,039	0,042
H84	0,014	0,012	0,007	0,009	0,017	0,017	0,014	0,012	0,014	0,029	0,012	0,014	0,014	0,009	0,014	0,005	0,014	0,019	0,017	0,014	0,014	0,007	0,012	0,024	0,021	0,024	0,029	0,050	0,019	0,019	0,024	0,031	0,012	0,024	0,017	0,026	0,014	0,012	0,029	0,031
H85	0,007	0,009	0,005	0,002	0,009	0,014	0,007	0,009	0,007	0,036	0,009	0,007	0,007	0,007	0,007	0,012	0,007	0,012	0,009	0,007	0,007	0,009	0,014	0,031	0,034	0,031	0,036	0,047	0,017	0,012	0,031	0,034	0,009	0,031	0,009	0,034	0,012	0,005	0,031	0,039
H86	0,007	0,014	0,009	0,007	0,009	0,019	0,012	0,014	0,007	0,041	0,009	0,007	0,007	0,007	0,007	0,017	0,007	0,012	0,014	0,007	0,007	0,014	0,019	0,036	0,039	0,036	0,041	0,050	0,021	0,017	0,036	0,039	0,009	0,036	0,009	0,039	0,017	0,009	0,036	0,044
H87	0,014	0,007	0,007	0,009	0,017	0,012	0,014	0,007	0,014	0,029	0,017	0,009	0,014	0,014	0,014	0,014	0,014	0,019	0,012	0,014	0,014	0,007	0,012	0,029	0,031	0,029	0,034	0,050	0,014	0,014	0,029	0,036	0,012	0,029	0,017	0,031	0,014	0,012	0,034	0,031
H88	0,041	0,039	0,034	0,036	0,044	0,039	0,036	0,039	0,041	0,007	0,039	0,036	0,041	0,036	0,041	0,031	0,036	0,041	0,044	0,041	0,041	0,034	0,034	0,007	0,009	0,007	0,007	0,042	0,041	0,041	0,012	0,009	0,039	0,007	0,044	0,009	0,036	0,039	0,007	0,009
H89	0,005	0,012	0,007	0,005	0,007	0,017	0,009	0,012	0,005	0,039	0,007	0,005	0,005	0,005	0,005	0,014	0,005	0,007	0,012	0,005	0,005	0,012	0,017	0,034	0,036	0,034	0,039	0,050	0,019	0,014	0,034	0,036	0,007	0,034	0,007	0,036	0,014	0,007	0,034	0,041
H90	0,012	0,005	0,005	0,007	0,014	0,009	0,012	0,005	0,012	0,031	0,014	0,012	0,012	0,012	0,012	0,012	0,012	0,017	0,009	0,012	0,012	0,005	0,009	0,031	0,034	0,031	0,036	0,053	0,012	0,012	0,031	0,039	0,014	0,031	0,014	0,034	0,012	0,009	0,036	0,029
H91	0,039	0,036	0,031	0,034	0,041	0,036	0,034	0,036	0,039	0,005	0,036	0,034	0,039	0,034	0,039	0,029	0,034	0,039	0,041	0,039	0,039	0,031	0,031	0,005	0,002	0,005	0,005	0,045	0,039	0,039	0,009	0,007	0,031	0,005	0,041	0,007	0,034	0,036	0,005	0,007
H92	0,041	0,039	0,034	0,036	0,044	0,039	0,036	0,039	0,041	0,007	0,039	0,036	0,041	0,036	0,041	0,031	0,036	0,041	0,044	0,041	0,041	0,034	0,034	0,007	0,009	0,007	0,007	0,047	0,041	0,041	0,012	0,009	0,039	0,007	0,044	0,009	0,036	0,039	0,007	0,009
H93	0,034	0,031	0,026	0,029	0,036	0,031	0,029	0,031	0,034	0,005	0,031	0,029	0,034	0,029	0,034	0,024	0,029	0,034	0,036	0,034	0,034	0,026	0,026	0,005	0,007	0,005	0,005	0,039	0,034	0,034	0,009	0,007	0,031	0,005	0,036	0,007	0,029	0,031	0,005	0,007
H94	0,041	0,034	0,034	0,036	0,044	0,034	0,036	0,034	0,041	0,002	0,039	0,036	0,041	0,036	0,041	0,031	0,036	0,041	0,039	0,041	0,041	0,029	0,029	0,007	0,009	0,007	0,007	0,044	0,036	0,036	0,012	0,009	0,039	0,007	0,044	0,009	0,036	0,039	0,007	0,005
H95	0,039	0,036	0,031	0,034	0,041	0,036	0,034	0,036	0,039	0,009	0,036	0,034	0,039	0,034	0,039	0,029	0,034	0,039	0,041	0,039	0,039	0,031	0,031	0,009	0,012	0,005	0,009	0,044	0,039	0,039	0,014	0,012	0,036	0,009	0,041	0,012	0,034	0,036	0,009	0,012
H96	0,007	0,014	0,009	0,007	0,009	0,019	0,007	0,014	0,007	0,041	0,009	0,007	0,007	0,007	0,007	0,017	0,007	0,007	0,014	0,007	0,007	0,014	0,019	0,036	0,039	0,036	0,041	0,053	0,021	0,017	0,036	0,039	0,009	0,036	0,005	0,039	0,017	0,009	0,036	0,044
H97	0,041	0,039	0,034	0,036	0,044	0,034	0,036	0,039	0,042	0,007	0,039	0,036	0,041	0,036	0,042	0,031	0,036	0,041	0,044	0,041	0,042	0,034	0,029	0,007	0,009	0,007	0,007	0,047	0,036	0,036	0,012	0,009	0,039	0,007	0,044	0,009	0,031	0,039	0,007	0,009
H98	0,047	0,044	0,039	0,041	0,049	0,044	0,041	0,044	0,047	0,012	0,044	0,041	0,047	0,041	0,047	0,036	0,041	0,047	0,049	0,047	0,047	0,039	0,039	0,012	0,014	0,012	0,012	0,052	0,047	0,046	0,017	0,014	0,044	0,012	0,049	0,014	0,041	0,044	0,012	0,014
H99	0,007	0,019	0,014	0,012	0,014	0,019	0,017	0,019	0,012	0,041	0,014	0,012	0,012	0,012	0,012	0,021	0,012	0,017	0,019	0,012	0,012	0,019	0,019	0,036	0,039	0,036	0,041	0,053	0,021	0,017	0,036	0,039	0,014	0,036	0,014	0,039	0,017	0,014	0,036	0,044
H100	0,041	0,039	0,034	0,036	0,044	0,039	0,036	0,039	0,041	0,007	0,039	0,036	0,041	0,036	0,041	0,031	0,036	0,041	0,044	0,041	0,041	0,034	0,034	0,007	0,009	0,007	0,007	0,047	0,041	0,041	0,012	0,009	0,039	0,007	0,044	0,009	0,036	0,039	0,007	0,009
H101	0,047	0,044	0,039	0,041	0,050	0,044	0,041	0,041	0,047	0,017	0,044	0,042	0,047	0,042	0,047	0,036	0,042	0,047	0,049	0,042	0,047	0,039	0,039	0,017	0,019	0,012	0,017	0,044	0,047	0,047	0,021	0,019	0,044	0,017	0,049	0,019	0,042	0,044	0,017	0,019
H102	0,039	0,036	0,031	0,034	0,041	0,036	0,034	0,036	0,039	0,005	0,036	0,034	0,039	0,034	0,039	0,029	0,034	0,039	0,041	0,039	0,039	0,031	0,031	0,005	0,007	0,005	0,005	0,042	0,039	0,039	0,009	0,007	0,036	0,005	0,041	0,007	0,034	0,036	0,005	0,007
H103	0,036	0,034	0,029	0,031	0,039	0,034	0,031	0,034	0,036	0,007	0,034	0,031	0,036	0,031	0,036	0,026	0,031	0,036	0,039	0,036	0,036	0,029	0,029	0,002	0,009	0,007	0,007	0,042	0,036	0,036	0,012	0,009	0,034	0,007	0,039	0,009	0,031	0,034	0,007	0,009
H104	0,039	0,036	0,031	0,034	0,041	0,036	0,034	0,036	0,039	0,005	0,036	0,034	0,039	0,034	0,039	0,029	0,034	0,039	0,041	0,039	0,039	0,031	0,031	0,005	0,007	0,005	0,005	0,044	0,039	0,039	0,009	0,007	0,036	0,005	0,041	0,007	0,034	0,036	0,005	0,007
H105	0,041	0,034	0,034	0,036	0,044	0,034	0,036	0,034	0,041	0,002	0,039	0,036	0,041	0,036	0,041	0,031	0,036	0,041	0,039	0,041	0,041	0,029	0,029	0,007	0,009	0,007	0,007	0,047	0,036	0,036	0,012	0,009	0,039	0,007	0,044	0,009	0,036	0,039	0,007	0,005
H106	0,005	0,012	0,007	0,005	0,007	0,017	0,005	0,012	0,005	0,039	0,007	0,005	0,005	0,005	0,005	0,014	0,005	0,005	0,012	0,005	0,005	0,012	0,017	0,034	0,036	0,034	0,039	0,050	0,019	0,014	0,034	0,036	0,007	0,034	0,002	0,036	0,014	0,007	0,034	0,041
H107	0,009	0,012	0,007	0,005	0,007	0,017	0,009	0,012	0,009	0,039	0,012	0,009	0,009	0,009	0,009	0,014	0,009	0,014	0,012	0,009	0,009	0,012	0,017	0,034	0,036	0,034	0,039	0,050	0,019	0,014	0,034	0,036	0,012	0,034	0,012	0,036	0,014	0,007	0,034	0,041
H108	0,012	0,009	0,009	0,007	0,014	0,009	0,012	0,009	0,012	0,031	0,014	0,012	0,012	0,012	0,012	0,017	0,012	0,017	0,009	0,012	0,012	0,009	0,009	0,031	0,034	0,031	0,036	0,047	0,012	0,007	0,031	0,034	0,014	0,031	0,014	0,034	0,012	0,009	0,031	0,034
H109	0,009	0,012	0,007	0,005	0,012	0,017	0,009	0,012	0,009	0,034	0,012	0,009	0,009	0,005	0,009	0,009	0,009	0,014	0,012	0,009	0,009	0,012	0,017	0,029	0,031	0,029	0,034	0,050	0,019	0,014	0,029	0,031	0,012	0,029	0,012	0,031	0,014	0,007	0,029	0,036
H110	0,007	0,009	0,005	0,002	0,009	0,014	0,007	0,009	0,007	0,036	0,009	0,007	0,007	0,007	0,007	0,012	0,007	0,012	0,009	0,007	0,007	0,009	0,014	0,031	0,034	0,031	0,036	0,047	0,017	0,012	0,031	0,034	0,009	0,031	0,009	0,034	0,012	0,005	0,031	0,039
H111	0,005	0,012	0,007	0,005	0,007	0,017	0,009	0,012	0,005	0,039	0,007	0,005	0,005	0,005	0,005	0,014	0,005	0,009	0,012	0,005	0,005	0,012	0,017	0,034	0,036	0,034	0,039	0,047	0,019	0,014	0,034	0,036	0,007	0,034	0,007	0,036	0,014	0,007	0,034	0,041
H112	0,017	0,014	0,014	0,012	0,019	0,019	0,014	0,014	0,017	0,041	0,019	0,017	0,017	0,017	0,017	0,021	0,017	0,019	0,014	0,017	0,017	0,014	0,019	0,041	0,044	0,041	0,047	0,058	0,022	0,017	0,036	0,044	0,019	0,041	0,017	0,044	0,022	0,014	0,041	0,044
H113	0,014	0,007	0,007	0,009	0,017	0,007	0,014	0,007	0,014	0,029	0,017	0,014	0,014	0,014	0,014	0,014	0,014	0,019	0,012	0,014	0,014	0,007	0,007	0,029	0,031	0,029	0,034	0,050	0,009	0,009	0,029	0,036	0,017	0,029	0,017	0,031	0,009	0,012	0,034	0,031
H114	0,017	0,009	0,009	0,012	0,019	0,009	0,017	0,009	0,017	0,026	0,014	0,017	0,017	0,017	0,017	0,012	0,017	0,021	0,014	0,017	0,017	0,005	0,005	0,026	0,029	0,026	0,031	0,047	0,012	0,012	0,026	0,034	0,019	0,026	0,019	0,029	0,012	0,014	0,031	0,029
H115	0,005	0,012	0,007	0,005	0,007	0,017	0,009	0,009	0,005	0,039	0,007	0,005	0,005	0,005	0,005	0,014	0,005	0,009	0,012	0,005	0,005	0,012	0,017	0,034	0,036	0,034	0,039	0,047	0,019	0,014	0,034	0,036	0,007	0,034	0,007	0,036	0,014	0,007	0,034	0,041

Table 4. 5 (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
H61	6	8	4	6	3	3	6	17	2	5	16	17	14	17	16	3	17	19	5	17	19	16	15	16	17	2	4	5	4	3	2	7	6	7	2
H62	3	5	5	3	4	6	3	14	5	2	13	14	11	12	13	6	14	16	8	14	16	13	12	13	12	5	5	4	5	4	5	6	3	2	5
H63	5	3	7	5	6	8	5	14	7	4	13	14	11	12	13	8	12	16	8	14	16	13	12	13	12	7	7	4	7	6	7	8	3	2	7
H64	10	14	16	10	13	15	12	3	14	13	2	3	2	3	4	15	3	5	15	3	7	2	1	2	3	14	14	13	12	13	14	17	12	11	14
H65	11	15	17	9	14	16	13	4	15	14	1	4	3	4	5	16	4	6	16	4	8	3	4	3	4	15	15	14	13	14	15	18	13	12	15
H66	10	14	16	10	13	15	12	3	14	13	2	3	2	3	2	15	3	5	15	3	5	2	3	2	3	14	14	13	12	13	14	17	12	11	14
H67	12	16	18	12	15	17	14	3	16	15	2	3	2	3	4	17	3	5	17	3	7	2	3	2	3	16	16	15	14	15	16	19	14	13	16
H68	20	20	22	20	19	20	20	17	20	21	18	19	16	18	18	21	19	21	21	19	18	17	17	18	19	20	20	19	20	19	19	23	20	19	19
H69	8	4	8	8	7	9	6	17	8	5	16	17	14	15	16	9	15	19	9	17	19	16	15	16	15	8	8	5	8	7	8	9	4	5	8
H70	8	4	6	8	5	7	6	17	6	5	16	17	14	15	16	7	15	19	7	17	19	16	15	16	15	6	6	3	6	5	6	7	4	5	6
H71	10	14	16	10	13	15	12	5	14	13	4	5	4	5	6	15	5	7	15	5	9	4	5	4	5	14	14	13	12	13	14	15	12	11	14
H72	13	17	17	13	14	16	15	4	15	16	3	4	3	4	5	16	4	6	16	4	8	3	4	3	4	15	15	14	13	14	15	18	15	14	15
H73	7	9	5	5	4	4	5	16	3	6	13	16	13	16	15	4	16	18	6	16	18	15	14	15	16	3	5	6	5	4	3	8	7	8	3
H74	10	14	16	10	13	15	12	3	14	13	2	3	2	3	4	15	3	5	15	3	7	2	3	2	3	14	14	13	12	13	14	17	12	11	14
H75	5	9	5	7	4	4	7	18	3	6	17	18	15	18	17	2	18	20	6	18	20	17	16	17	18	1	5	6	5	4	3	7	7	8	3
H76	11	15	17	11	14	16	13	4	15	14	3	4	3	4	5	16	4	6	16	4	8	3	4	3	4	15	15	14	13	14	15	18	13	12	15
H77	6	4	8	6	5	7	6	15	6	5	14	15	12	15	14	7	13	17	7	15	17	14	13	14	15	6	6	5	6	5	6	9	4	5	6
H78	5	7	5	5	2	4	5	16	3	4	15	16	13	16	15	4	16	18	6	16	18	15	14	15	16	3	3	4	3	2	3	6	5	6	3
H79	12	16	16	12	13	15	14	3	14	15	2	3	2	3	4	15	3	5	15	3	7	2	3	2	3	14	14	13	12	13	14	17	14	13	14
H80	13	15	17	13	16	18	13	4	17	12	3	4	3	2	5	18	4	6	18	4	8	3	4	3	2	17	17	14	15	16	17	18	13	12	17
H81		8	8	2	5	7	6	13	6	5	12	13	10	13	12	5	13	15	9	13	15	12	11	12	13	4	6	7	4	5	6	8	6	5	6
H82	0,019		8	8	7	9	6	15	8	5	16	17	14	15	16	9	15	19	9	17	19	16	15	16	15	8	8	5	8	7	8	9	4	5	8
H83	0,019	0,019		8	5	5	6	19	4	5	18	17	16	17	18	5	19	21	7	19	21	18	17	18	17	4	6	5	6	5	4	7	4	7	4
H84	0,005	0,019	0,019		5	7	6	13	6	5	10	13	10	13	12	7	13	15	9	13	15	12	11	12	13	6	6	7	4	5	6	9	6	5	6
H85	0,012	0,017	0,012	0,012		4	5	16	3	4	15	16	13	16	15	4	16	18	6	16	18	15	14	15	16	3	3	4	3	2	3	6	5	6	3
H86	0,017	0,021	0,012	0,017	0,009		7	18	3	6	17	18	15	16	17	4	18	20	6	18	20	17	16	17	18	3	5	6	5	4	1	8	7	8	3
H87	0,014	0,014	0,014	0,014	0,012	0,017		15	6	3	14	15	12	13	14	7	15	17	9	15	17	14	13	14	13	6	6	5	6	5	6	7	4	5	6
H88	0,031	0,036	0,047	0,031	0,039	0,044	0,036		17	16	3	4	3	4	3	18	4	6	18	2	8	3	4	3	4	17	17	16	15	16	17	20	15	14	17
H89	0,014	0,019	0,009	0,014	0,007	0,007	0,014	0,041		5	16	17	14	17	16	3	17	19	5	17	19	16	15	16	17	2	4	5	4	3	2	7	6	7	2
H90	0,012	0,012	0,012	0,012	0,009	0,014	0,007	0,039	0,012		15	16	13	14	15	6	16	18	8	16	18	15	14	15	14	5	5	4	5	4	5	6	3	4	5
H91	0,029	0,039	0,044	0,024	0,036	0,041	0,034	0,007	0,039	0,036		3	2	3	4	17	3	5	17	3	7	2	3	2	3	16	16	15	14	15	16	19	14	13	16
H92	0,031	0,041	0,041	0,031	0,039	0,044	0,036	0,009	0,041	0,039	0,007		3	4	5	18	4	6	18	4	8	3	4	3	4	17	17	16	15	16	17	20	13	14	17
H93	0,024	0,034	0,039	0,024	0,031	0,036	0,029	0,007	0,034	0,031	0,005	0,007		3	4	15	3	5	15	3	7	2	3	2	3	14	14	13	12	13	14	17	12	11	14
H94	0,031	0,036	0,041	0,031	0,039	0,039	0,031	0,009	0,041	0,034	0,007	0,009	0,007		5	18	4	6	18	4	8	3	4	3	2	17	17	14	15	16	15	18	13	12	17
H95	0,029	0,039	0,044	0,029	0,036	0,041	0,034	0,007	0,039	0,036	0,009	0,012	0,009	0,012		17	5	7	17	3	7	4	5	4	5	16	16	15	14	15	16	19	14	13	16
H96	0,012	0,021	0,012	0,017	0,009	0,009	0,017	0,044	0,007	0,014	0,041	0,044	0,036	0,044	0,041		18	18	6	18	20	17	16	17	18	1	5	6	5	4	3	7	7	8	3
H97	0,031	0,036	0,047	0,031	0,039	0,044	0,036	0,009	0,042	0,039	0,007	0,009	0,007	0,009	0,012	0,044		6	18	4	8	3	4	3	4	17	17	16	15	16	17	20	15	14	17
H98	0,036	0,047	0,052	0,036	0,044	0,049	0,041	0,014	0,047	0,044	0,012	0,014	0,012	0,014	0,017	0,044	0,014		18	6	10	5	6	5	6	19	19	18	17	18	19	22	17	16	19
H99	0,021	0,021	0,017	0,021	0,014	0,014	0,021	0,044	0,012	0,019	0,041	0,044	0,036	0,044	0,041	0,014	0,044	0,044		18	20	17	16	17	18	5	7	6	7	6	5	10	7	8	5
H100	0,031	0,041	0,047	0,031	0,039	0,044	0,036	0,005	0,041	0,039	0,007	0,009	0,007	0,009	0,007	0,044	0,009	0,014	0,044		8	3	4	3	4	17	17	16	15	16	17	20	15	14	17
H101	0,036	0,047	0,052	0,036	0,044	0,049	0,042	0,019	0,047	0,044	0,017	0,019	0,017	0,019	0,017	0,049	0,019	0,024	0,049	0,019		5	6	7	8	19	19	18	17	18	19	22	17	16	18
H102	0,029	0,039	0,044	0,029	0,036	0,041	0,034	0,007	0,039	0,036	0,005	0,007	0,005	0,007	0,009	0,041	0,007	0,012	0,041	0,007	0,012		3	2	3	16	16	15	14	15	16	19	14	13	16
H103	0,026	0,036	0,041	0,026	0,034	0,039	0,031	0,009	0,036	0,034	0,007	0,009	0,007	0,009	0,012	0,039	0,009	0,014	0,039	0,009	0,014	0,007		3	4	15	15	14	13	14	15	18	13	12	15
H104	0,029	0,039	0,044	0,029	0,036	0,041	0,034	0,007	0,039	0,036	0,005	0,007	0,005	0,007	0,009	0,041	0,007	0,012	0,041	0,007	0,017	0,005	0,007		3	16	16	15	14	15	16	19	14	13	16
H105	0,031	0,036	0,042	0,031	0,039	0,044	0,031	0,009	0,041	0,034	0,007	0,009	0,007	0,005	0,012	0,044	0,009	0,014	0,044	0,009	0,019	0,007	0,009	0,007		17	17	14	15	16	17	18	13	12	17
H106	0,009	0,019	0,009	0,014	0,007	0,007	0,014	0,041	0,005	0,012	0,039	0,041	0,034	0,041	0,039	0,002	0,041	0,047	0,012	0,041	0,047	0,039	0,036	0,039	0,041		4	5	4	3	2	6	6	7	2
H107	0,014	0,019	0,014	0,014	0,007	0,012	0,014	0,041	0,009	0,012	0,039	0,041	0,034	0,041	0,039	0,012	0,042	0,047	0,017	0,041	0,047	0,039	0,036	0,039	0,041	0,009		5	4	3	4	7	6	7	4
H108	0,017	0,012	0,012	0,017	0,009	0,014	0,012	0,039	0,012	0,009	0,036	0,039	0,031	0,034	0,036	0,014	0,039	0,044	0,014	0,039	0,044	0,036	0,034	0,036	0,034	0,012	0,012		5	4	5	6	3	4	5
H109	0,009	0,019	0,014	0,009	0,007	0,012	0,014	0,036	0,009	0,012	0,034	0,036	0,029	0,036	0,034	0,012	0,036	0,041	0,017	0,036	0,041	0,034	0,031	0,034	0,036	0,009	0,009	0,012		1	4	7	6	7	4
H110	0,012	0,017	0,012	0,012	0,005	0,009	0,012	0,039	0,007	0,009	0,036	0,039	0,031	0,039	0,036	0,009	0,039	0,044	0,014	0,039	0,044	0,036	0,034	0,036	0,039	0,007	0,007	0,009	0,002		3	6	5	6	3
H111	0,014	0,019	0,009	0,014	0,007	0,002	0,014	0,041	0,005	0,012	0,039	0,041	0,034	0,036	0,039	0,007	0,041	0,047	0,012	0,041	0,047	0,039	0,036	0,039	0,041	0,005	0,009	0,012	0,009	0,007		7	6	7	2
H112	0,019	0,021	0,017	0,021	0,014	0,019	0,017	0,049	0,017	0,014	0,047	0,049	0,041	0,044	0,047	0,017	0,049	0,055	0,024	0,049	0,055	0,047	0,044	0,047	0,044	0,014	0,017	0,014	0,017	0,014	0,017		7	8	7
H113	0,014	0,009	0,009	0,014	0,012	0,017	0,009	0,036	0,014	0,007	0,034	0,031	0,029	0,031	0,034	0,017	0,036	0,041	0,017	0,036	0,041	0,034	0,031	0,034	0,031	0,014	0,014	0,007	0,014	0,012	0,014	0,017		3	6
H114	0,012	0,012	0,017	0,012	0,014	0,019	0,012	0,034	0,017	0,009	0,031	0,034	0,026	0,029	0,031	0,019	0,034	0,039	0,019	0,034	0,039	0,031	0,029	0,031	0,029	0,017	0,017	0,009	0,017	0,014	0,017	0,019	0,007		7
H115	0,014	0,019	0,009	0,014	0,007	0,007	0,014	0,041	0,005	0,012	0,039	0,041	0,034	0,041	0,039	0,007	0,041	0,047	0,012	0,041	0,044	0,039	0,036	0,039	0,041	0,005	0,009	0,012	0,009	0,007	0,005	0,017	0,014	0,017	

Haplotype and nucleotide diversity for each *C. divisa* population was calculated and results were shown in Table 4.6. Average genetic diversity estimates were h= 0.81818 and π= 0.01197, for haplotype and nucleotide diversity, respectively. Most of *C. divisa* populations displayed high genetic diversity estimates where the highest haplotype diversity was in Siirt (h= 1.0000 +/- 0.1265) with 5 distinct haplotypes revealed by 5 specimens sequenced in this study. Remaining populations also showed substantial amount of diversity estimates. In fact, except İzmir and Bolu, all examined populations were with haplotype diversity greater than 0.500. Likewise, existing nucleotide diversity was striking for many *C. divisa* populations across the sampled area in Turkey. Most populations displayed nucleotide diversity estimate higher than 0.0100 where the highest nucleotide diversity was in Bingöl population (π= 0.0242), followed by Giresun (π= 0.01981), Balıkesir (π= 0.01966), and Sivas (π=0.01936).

Table 4.6. Sample size, haplotype number, haplotype (h) and nucleotide (π) diversity for cyt b gene and ITS2 region.

Population	Size	CYT B	ITS2	CYT B		ITS2		Population	Size	CYT B	ITS2	CYT B		ITS2	
		N_{HAP}	N_{ALEL}	h	π	h	π			N_{HAP}	N_{ALEL}	h	π	h	π
AFYON	13	9	2	0.8718 +/- 0.0913	0.0113 +/-0.0066	0.1538 +/- 0.1261	0.0006 +/- 0.0010	**GİRESUN**	11	9	2	0.9636 +/- 0.0510	0.01981 +/-0.0112	0.1818 +/- 0.1436	0.0007 +/- 0.0011
AYDIN	13	9	2	0.9359 +/- 0.0507	0.0093 +/-0.0056	0.3846 +/- 0.1321	0.0015 +/- 0.0017	**ISPARTA**	7	3	2	0.5238 +/- 0.2086	0.00967 +/- 0.0062	0.4762 +/- 0.1713	0.0019 +/- 0.0021
BALIKESİR	14	10	3	0.9451 +/- 0.0451	0.0196 +/- 0.0108	0.2747 +/- 0.1484	0.0016 +/- 0.0017	**İZMİR**	5	2	2	0.4000 +/- 0.2373	0.0046 +/- 0.0036	0.6000 +/- 0.1753	0.0024 +/- 0.0026
BİNGÖL	15	11	6	0.9333 +/- 0.0538	0.0242 +/- 0.0131	0.7048 +/- 0.1139	0.0075 +/- 0.0051	**KAYSERİ**	20	9	3	0.9053 +/- 0.0351	0.01796 +/- 0.0097	0.4684 +/- 0.1045	0.0021 +/- 0.0021
BOLU	14	3	3	0.4725 +/- 0.1358	0.0018 +/- 0.0015	0.4725 +/- 0.1358	0.0023 +/- 0.0022	**KIRŞEHİR**	16	8	2	0.8000 +/- 0.0916	0.0058 +/- 0.0037	0.3250 +/- 0.1251	0.0013 +/- 0.0015
ÇANAKKALE	15	8	1	0.7905 +/- 0.1049	0.0085 +/- 0.0051	0.000 +/- 0.0000	0.0000 +/- 0.0000	**KONYA**	20	13	2	0.8842 +/- 0.0666	0.01684 +/- 0.0091	0.1000 +/- 0.0880	0.0004 +/- 0.0008
ÇANKIRI	15	12	1	0.9619 +/- 0.0399	0.00818+/- 0.0049	0.000 +/- 0.0000	0.0000 +/- 0.0000	**MALATYA**	6	5	3	0.9333 +/- 0.1217	0.0173 +/- 0.0109	0.8000 +/- 0.1721	0.0040 +/- 0.0036
ÇORUM	14	10	2	0.8901 +/- 0.0807	0.0087 +/- 0.0052	0.1429 +/- 0.1188	0.0006 +/- 0.0009	**SİİRT**	5	5	1	1.0000 +/- 0.1265	0.0078 +/- 0.0056	0.0000 +/- 0.0000	0.0000 +/- 0.0000
DENİZLİ	13	8	2	0.8077 +/- 0.1131	0.0068+/- 0.0042	0.1538 +/- 0.1261	0.0006 +/- 0.0010	**SİVAS**	14	7	3	0.8242 +/- 0.0781	0.0193 +/- 0.0107	0.3846 +/- 0.1494	0.0020 +/- 0.0020
ELAZIĞ	13	6	5	0.7179 +/- 0.1279	0.0079 +/- 0.0048	0.7436 +/- 0.0866	0.0046 +/- 0.0036	**TOKAT**	15	10	2	0.9333 +/- 0.0449	0.0177 +/- 0.0098	0.3429 +/- 0.1278	0.0013 +/- 0.0016
ESKİŞEHİR	5	4	1	0.9000 +/- 0.1610	0.0097 +/- 0.0067	0.0000 +/- 0.0000	0.0000 +/- 0.0000	**UŞAK**	15	11	2	0.9524 +/- 0.0403	0.0099 +/- 0.0058	0.2476 +/- 0.1307	0.0009 +/- 0.0013
		CYT B								**ITS2**					
Average h		**0,81818 +/- 0,95722**						**Average h**		**0,31623+/- 0,10798**					
Average π		**0,01197+/- 0,00708**						**Average π**		**0,00167 +/- 0,00165**					

4.1.2 ITS2 Region Diversity

Size of the ITS2 region was determined as 395 base pairs for *C. divisa*. All sequences of 278 specimens generated 15 distinct alleles (GenBank Acession No: MH231106- MH231120). In alleles, 379 sites were monomorphic, and 16 sites were polymorphic, and 3 were parsimony informative. Polymorphic characters were shown in Table 4.7.

Nucleotide frequencies in alleles were 26.08%, 31.03%, 18.88%, and 24.02% for A, T, C, and G, respectively. Transition/transversion rate ratios were k1 = 6.968 (purines), and k2 = 0.992 (pyrimidines). Overall transition/transversion bias was R = 1.978. Based on maximum likelihood estimation, most of the substitutions occurred between A and G (30.36%), and the least was between A/C and G/C (%3.13) (Table 4.8).

Table 4.7. ITS2 alles of *C. divisa.* Only polymorphic sites are shown.

	61	64	70	85	98	103	170	172	180	193	199	200	203	232	247	248
A1	T	G	A	T	A	G	G	G	G	A	A	G	G	A	C	A
A2	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.
A3	.	.	.	C	.	.	.	.	.	.	.	.	C	.	.	.
A4	.	.	.	C	.	.	.	.	.	.	.	.	A	G	.	.
A5	.	.	.	C	G	T	.	.	.	.	.	.	.	G	.	.
A6	A	.	.	C	.	.	.	A	A	C	.	.	.	G	.	.
A7	.	.	.	C	.	.	.	.	.	.	.	.	.	G	.	.
A8	.	.	G	C	.	.	.	.	.	.	.	.	.	.	.	.
A9	.	.	.	C	.	.	.	.	.	.	.	A	A	G	.	.
A10	.	.	.	C	.	.	.	.	.	.	.	.	A	G	A	.
A11	.	.	.	C	.	.	.	.	.	.	C	.	.	T	.	.
A12	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.
A13	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	T
A14	.	.	.	C	.	.	A	.	.	.	.	.	.	.	.	.
A15	.	A	.	C	.	.	.	.	.	.	.	.	.	.	.	.

Table 4.8. Maximum composite likelihood estimates of the pattern of nucleotide substitution for ITS region of *C. divisa*. Rates of different transitional substitutions are shown in bold and those of transversional substitutions are shown in italics.

	A	T	C	G
A	-	*5,18*	*3,15*	**27,96**
T	*4,36*	-	**3,13**	*4,01*
C	*4,36*	**5,14**	-	*4,01*
G	**30,36**	*5,18*	*3,15*	-

Frequency of ITS alleles was shown in Table 4.9. Allele 1 (A1) was with the highest frequency shared among 182 individuals representing 20 different localities (Afyon, Aydın, Balıkesir, Bingöl, Bolu, Çanakkale, Çankırı, Çorum, Denizli, Eskişehir, Giresun, Isparta, İzmir, Kayseri, Kırşehir, Konya, Malatya, Sivas, Tokat, and Uşak). A2 was the second most frequent allele (N=67) found in 16 localities (Afyon, Aydın, Balıkesir, Bingöl, Bolu, Çorum, Denizli, Isparta, İzmir, Kayseri, Kırşehir, Konya, Malatya, Sivas, Tokat, and Uşak). Eleven of the 15 alleles were singleton, and there was no private allele.

Allelic differences ranged from 1 base (0.4%) to 7 bases (2.9%) (Table 4.10). In 11 cases, there was 1 base pair difference indicating recent substitutions. The highest pairwise difference (7 bp) occurred between A12 from Giresun and A6 from Bingöl. The following highest pairwise difference was 6 bp observed between A6 (from Bingöl) – A1 (from Afyon, Aydın, Balıkesir, Bingöl, Bolu, Çanakkale, Çankırı, Çorum, Denizli, Eskişehir, Giresun, Isparta, İzmir, Kayseri, Kırşehir, Konya, Malatya, Sivas, Tokat, and Uşak) – A3 (from Balıkesir), A5 (from Bingöl), A8 (from Bolu), A9 (from Elazığ), A10 (from Elazığ), A11 (from Elazığ), A13 (from Kayseri), A14 (from Malatya), and A15 (from Sivas).

Average allele (h) and nucleotide diversity for *C. divisa* ITS2 region were h= 0,31623 and π= 0,00167, respectively (Table 4.6). Most populations demonstrated high diversity where Malatya (h= 0.8000 +/- 0.1721) was followed by Elazığ (h= 0.7436 +/-0.0866) and Bingöl (h= 0.7048 +/- 0.1139). Accordingly, nucleotide diversity was highest in Bingöl (π= 0.0075 +/- 0.0051), and in Elazığ (π= 0.0046 +/-

0.0036). Considering allelic haplotype richness Malatya (N= 6, Nall=3) and Elazığ (N=13, Nall=5) populations were with relatively high number of alleles.

Table 4.9. ITS2 allele frequencies of *C. divisa*.

	AFY	AYD	BAL	BIN	BOL	CAN	CNK	COR	DEN	ELA	ESK	GIR	ISP	IZM	KAY	KIR	KON	MAL	SII	SIV	TOK	USK	N
A1	12	10	12	8	10	15	15	13	12		5	10	2	3	14	3	1	1		11	12	13	182
A2	1	3	1	3	3			1	1				5	2	5	13	19	3		2	3	2	67
A3			1																				1
A4				1						5													6
A5				1																			1
A6				1																			1
A7				1						5								1	5				12
A8					1																		1
A9										1													1
A10										1													1
A11										1													1
A12												1											1
A13															1								1
A14																		1					1
A15																				1			1
N	13	13	14	15	14	15	15	14	13	13	5	11	7	5	20	16	20	6	5	14	15	15	278
Nallele	2	2	3	6	3	1	1	2	2	5	1	2	2	2	3	2	2	3	1	3	2	2	

Table 4.10. Allelic differences ranged from 1 base (0.4%) to 7 bases (2.9%).

	A1	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**	**A9**	**A10**	**A11**	**A12**	**A13**	**A14**	**A15**
A1		1	2	3	4	6	2	2	4	4	3	1	2	2	2
A2	0,004		1	2	3	5	1	1	3	3	2	2	1	1	1
A3	0,008	0,004		2	4	6	2	2	3	3	3	3	2	2	2
A4	0,012	0,008	0,008		3	5	1	3	1	1	3	4	3	3	3
A5	0,016	0,012	0,016	0,012		6	2	4	4	4	4	4	4	4	4
A6	0,025	0,020	0,025	0,020	0,025		4	6	6	6	6	7	6	6	6
A7	0,008	0,004	0,008	0,004	0,008	0,016		2	2	2	2	3	2	2	2
A8	0,008	0,004	0,008	0,012	0,016	0,025	0,008		4	4	3	3	2	2	2
A9	0,016	0,012	0,012	0,004	0,016	0,025	0,008	0,016		2	4	5	4	4	4
A10	0,016	0,012	0,012	0,004	0,016	0,025	0,008	0,016	0,008		4	5	4	4	4
A11	0,012	0,008	0,012	0,012	0,016	0,025	0,008	0,012	0,016	0,016		4	3	3	3
A12	0,004	0,008	0,012	0,016	0,016	0,029	0,012	0,012	0,020	0,020	0,016		3	3	3
A13	0,008	0,004	0,008	0,012	0,016	0,025	0,008	0,008	0,016	0,016	0,012	0,012		2	2
A14	0,008	0,004	0,008	0,012	0,016	0,025	0,008	0,008	0,016	0,016	0,012	0,012	0,008		2
A15	0,008	0,004	0,008	0,012	0,016	0,025	0,008	0,008	0,016	0,016	0,012	0,012	0,008	0,008	

4.2 Population Demographic Analysis

Neutrality test and population size changes were tested by several analyses, and the results were shown in Table 4.11. When all populations were taken into consideration as a single unit the mean Tajima's *D* was -0,48287, but it was not statistically supported. All populations were also checked separately. Resulting Tajima's *D* analysis showed that Çorum (Tajima's *D* = - 1, 6675), Elazığ (Tajima's *D*= - 1, 99717), Isparta (Tajima's *D*= - 1, 46766), and Malatya (Tajima's *D*= - 1, 36789) populations were with negative and statistically significant values indicating population expansion. Other populations' Tajima's *D* values were either positive or negative, but they were not significant. Mean Fu's *Fs* was -0, 96544, but it was not significant. Although the mean was not significant some individual population estimations generated statistically significant values. For example, Çankırı (Fu's *Fs*= - 6, 64025), Çorum (Fu's *Fs*= -3, 58416), Siirt (Fu's *Fs*= - 2, 00426), and Uşak (Fu's *Fs*= - 3, 96493). Similarly, some populations produced either positive or negative Fu's *Fs* values, however they were not statistically significant. Neither average nor populations analyzed separately produced significant values for Harpending's raggedness index (Hri= 0, 12034) and SSD (SSD= 0, 26514).

Cyt b data was also analyzed for mismatch distributions to expose population demographies over time. First analysis included all pairwise combinations for the cyt b haplotypes and the resulting graph was shown in Figure 4.1. The resulting graph showed a multimodal profile which was not supported statistically. Thus, separate analysis for each population was conducted and the resulting graphs were shown in Figure 4.2. Almost all populations indicated a multimodal or bimodal profile.

Table 4.11. Population demographic analysis of the cyt b region. *: p≤ 0,005.

Population	Hri	SSD	Tajima's *D*	Fu's F_S	Population	Hri	SSD	Tajima's *D*	Fu's F_S
Afyon	0,13445	0,07019	-1,31594	-1,81538	**Giresun**	0,07273	0,07970	1,16343	-1,25283
Aydın	0,05178	0,01729	-0,42324	-2,44396	**Isparta**	0,20862	0,07915	-1,46766*	3,03724
Balıkesir	0,04190	0,03634	-0,35012	-0,89417	**İzmir**	0,68000	0,21713*	-1,12397	2,63906
Bingöl	0,05070	0,89016*	-0,43070	-0,79783	**Kayseri**	0,04033	0,03793	2,00602	1,42862
Bolu	0,16689	0,01921	-1,22200	0,37544	**Kırşehir**	0,03264	0,32966*	-1,36280	-2,03103
Çanakkale	0,08435	0,03186	-0,79437	-1,05590	**Konya**	0,01773	0,69931*	-0,53832	-2,08758
Çankırı	0,02776	0,00417	-0,70224	-6,64025*	**Malatya**	0,06667	0,04717	-1,36789*	0,34704
Çorum	0,09781	0,02830	-1,66756*	-3,58416*	**Siirt**	0,16000	0,06433	0,08298	-2,00426*
Denizli	0,04816	0,01513	-1,43731	-2,27640	**Sivas**	0,07463	0,05696	1,39288	2,07179
Elazığ	0,07133	0,01553	-1,99717*	0,26359	**Tokat**	0,04127	0,04508	0,77525	-0,76545
Eskişehir	0,43000	0,15853	0,66055	0,21161	**Uşak**	0,04780	0,02269	-0,50288	-3,96493*

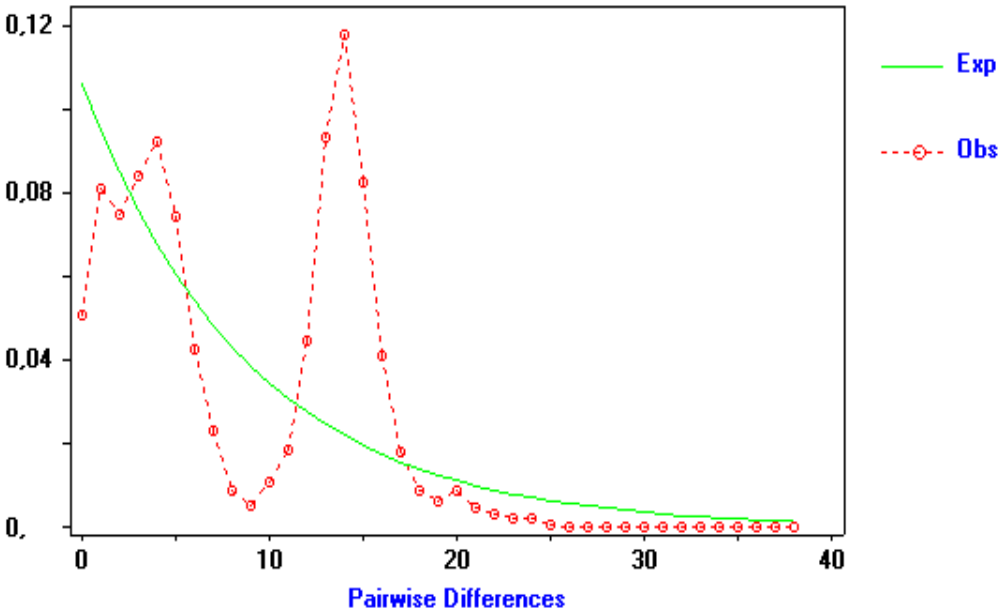


Figure 4.1. Mismatch distribution of all pairwise combinations for the cytb haplotypes. The observed distribution represented by red line, and the expected frequencies depicted by green dotted line.

ITS2 data set was also analyzed for estimating changes of *C. divisa* populations over time to figure out whether there are any deviations from neutrality. Relevant results were shown in Table 4.12. Results indicated that only two values were statistically significant. SSD value for the Bingöl population (SSD= 0,40243) was statistically significant indicating a population decline. Malatya population also showed statistically significant, but negative value implying a population expansion. All other estimates were not supported statistically.

Resulting graph of mismatch analysis of all pairwise combinations of ITS2 alleles, and for each population analyzed individually is provided in Figure 4.3 and Figure 4.4, respectively. All pairwise combinations produced a multimodal profile. Individual population graphs were unimodal, except for Bingöl population where only few small picks were observed.

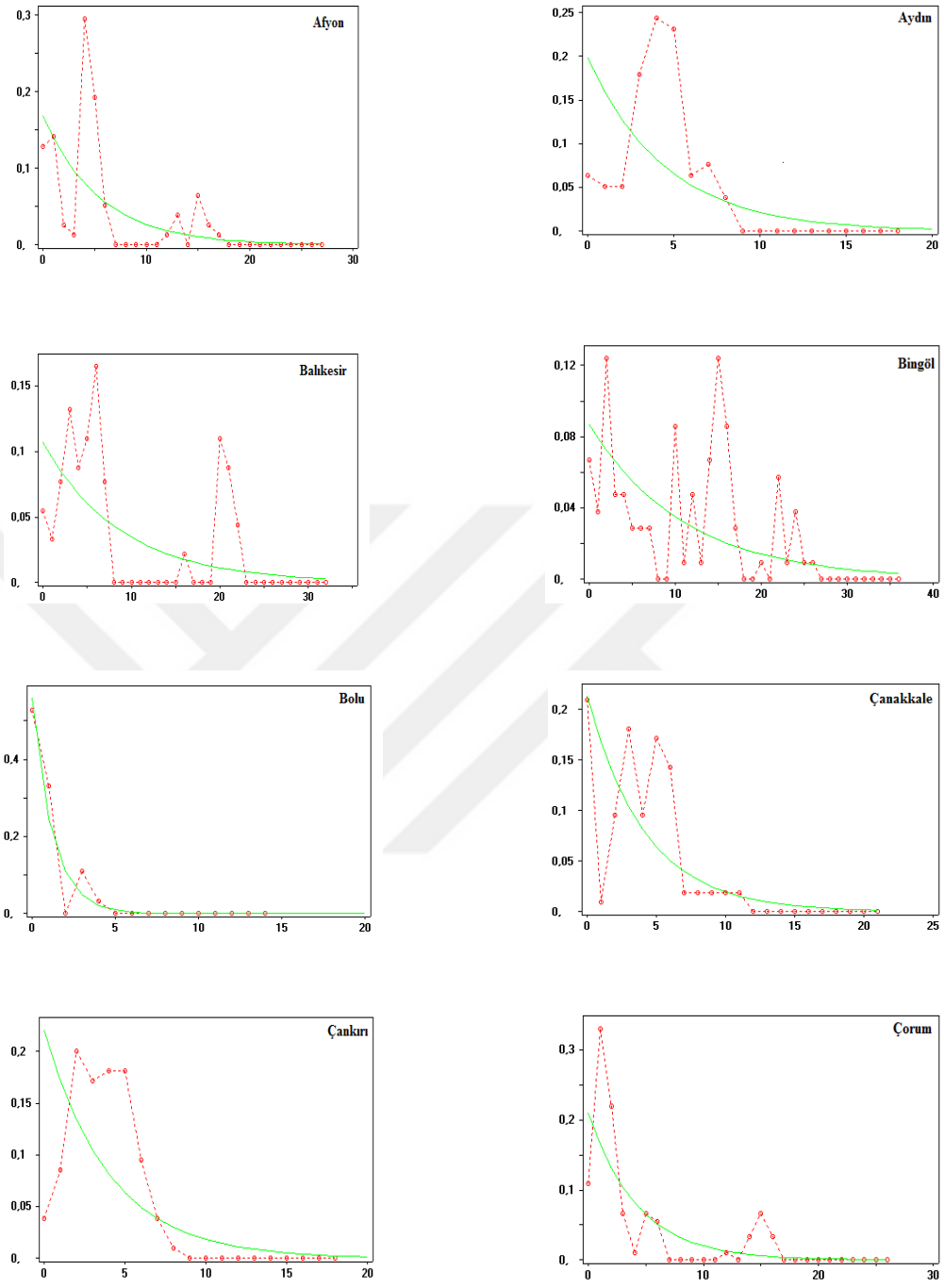
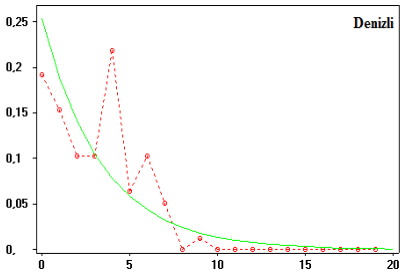
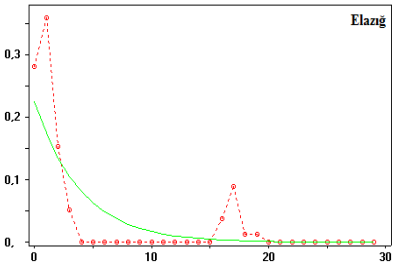
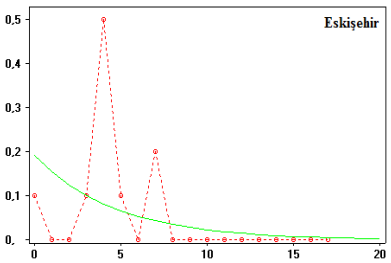
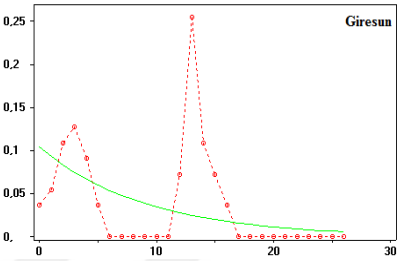


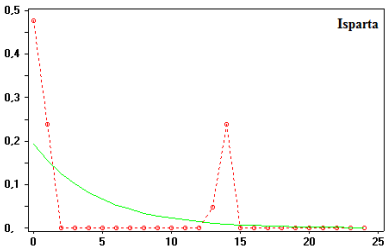
Figure 4.2. Mismatch distribution of populations with their own haplotypes for cyt b data set. The observed distribution represented by red line, and the expected frequencies depicted by green line.

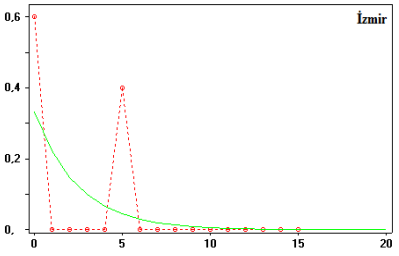


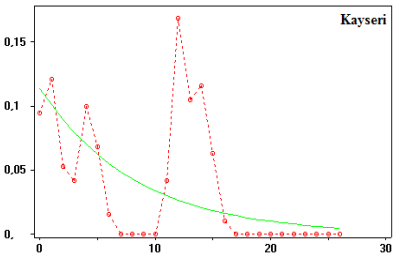












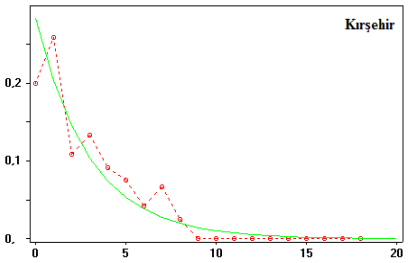


Figure 4.2 (cont'd)

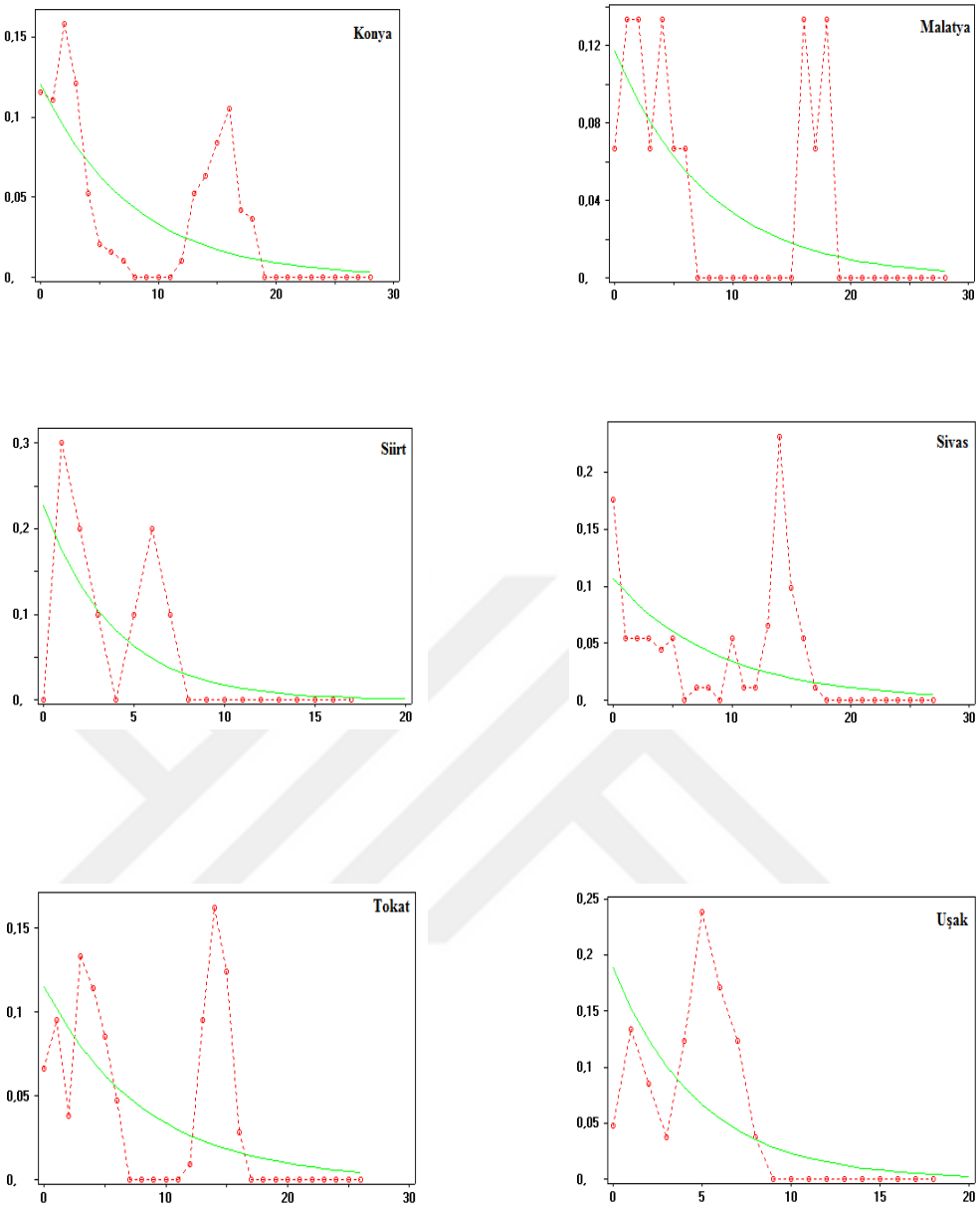


Figure 4.2 (cont'd)

Table 4.12. Population demographic analysis of the ITS2 region of *C. divisa*. *: p≤ 0,005.

Population	Hri	SSD	Tajima's *D*	Fu's *FS*	Population	Hri	SSD	Tajima's *D*	Fu's *FS*
Afyon	0,50296	0,02811	-1,14915	-0,53714	Giresun	0,43802	0,01756	-1,12850	-0,40988
Aydın	0,20118	0,00596	0,42560	0,68913	Isparta	0,22902	0,01718	0,55902	0,58867
Balıkesir	0,35672	0,00913	-0,95919	-0,85452	İzmir	0,40000	0,05428	1,22474	0,62615
Bingöl	0,03673	0,40243*	-1,19511	-0,97962	Kayseri	0,12723	0,00447	-0,08998	-0,06013
Bolu	0,10313	0,00088	-0,20057	-0,20707	Kırşehir	0,22813	0,00265	0,15575	0,55122
Çanakkale	0,00000	0,00000	0,00000	0,00000	Konya	0,65000	0,00006	-1,16439	-0,87930
Çankırı	0,00000	0,00000	0,00000	0,00000	Malatya	0,36000	0,06645	-1,23311	-1,81298*
Çorum	0,53061	0,00009	-1,15524	-0,59478	Siirt	0,00000	0,00000	0,00000	0,00000
Denizli	0,50296	0,02811	-1,14915	-0,53714	Sivas	0,15868	0,00072	-0,53247	-0,46544
Elazığ	0,11834	0,01020	-1,01207	-1,39819	Tokat	0,21633	0,00342	0,23502	0,59667
Eskişehir	0,43802	0,01756	0,00000	0,00000	Uşak	0,31610	0,28138	-0,39883	0,13336

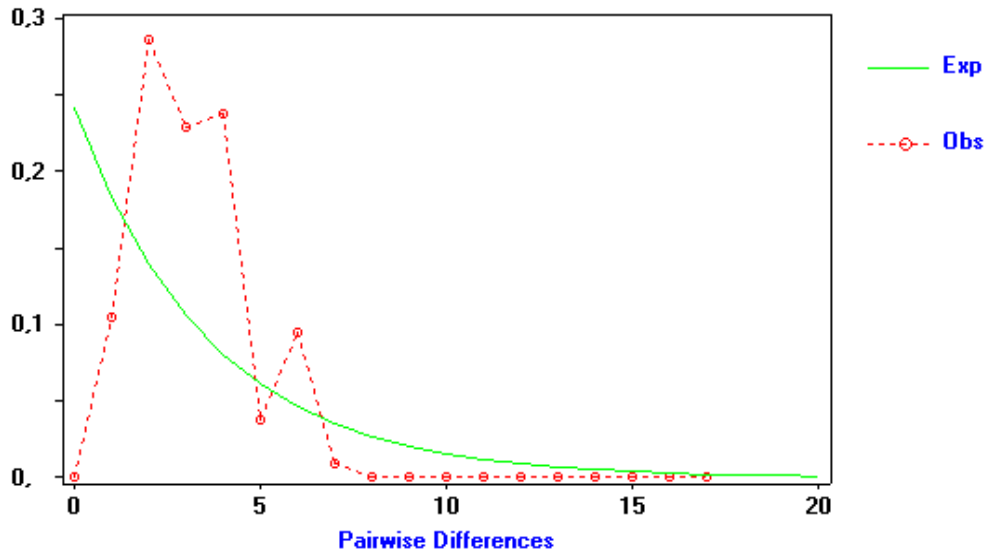


Figure 4.3. Mismatch distribution of all pairwise combinations for the ITS2 alleles.

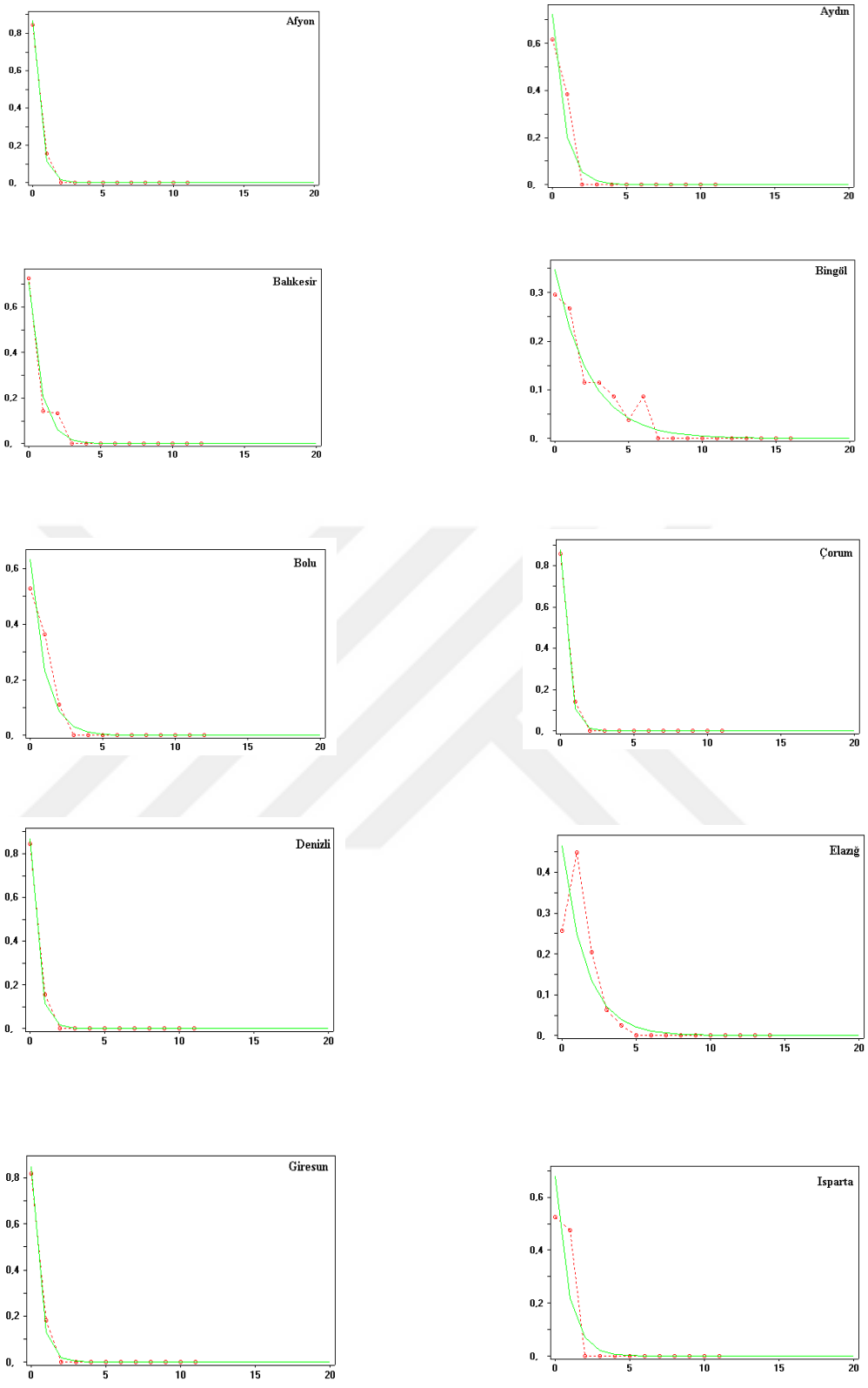


Figure 4.4. Mismatch distribution of populations with their own haplotypes for ITS2 data set. The observed distribution represented by red line, and the expected frequencies depicted by green line.

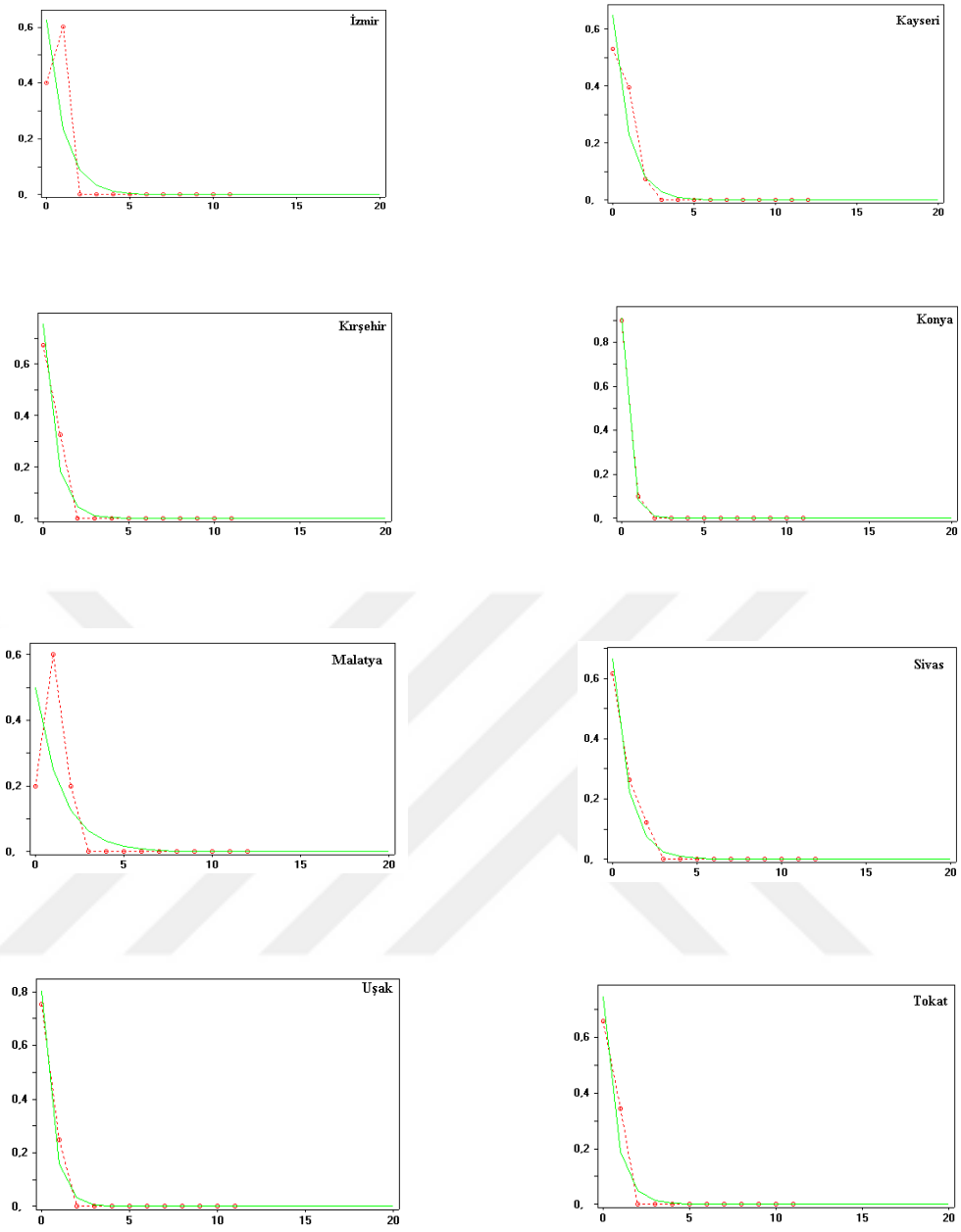


Figure 4.4 (cont'd)

4.2.1 Population Differentiation

Pairwise comparisons through calculating Fst values for all pairs of populations are given in Table 4.13 and Table 4.14 using cyt b and ITS2 data sets, respectively. Population differentiation for some pairwise comparisons were high. Regarding usefulness of both data sets for providing resolution it is assumed that the cyt b data may give more resolution for more recent population differentiations while ITS2 data may be more useful for more ancient differentiations (Slatkin, 1987).

Cyt b indicated that those geographically distant populations are distinct enough to produce a value over 0.500. For example, Bolu was different from most of the populations compared with the highest differentiation of Fst= 0.91950 (with İzmir), and the value was statistically significant. Bolu was also different from Çanakkale, Çankırı, Çorum, Denizli, Eskişehir, Kırşehir, Tokat, and Uşak populations. Similarly, Siirt population showed significant differentiation from Afyon, Aydın, Balıkesir, Çanakkale, Çankırı, Çorum, Denizli, Eskişehir, İzmir, and Kırşehir. İzmir population was also conspicuously different from Elazığ and Isparta. Elazığ population was also distinct from Aydın, Çanakkale, Çankırı, Çorum, and Denizli. On the contrary, it seemed that some populations were highly similar to each other, but these values were not supported statistically.

Likewise, some comparable differentiation results were obtained through ITS2 analysis. For instance, Siirt was one of the most distinct population from all other populations, except for Elazığ where the Fst was very low (Fst= 0.30962). However, Elazığ was different from some other populations with high statistical support. Konya population seem to show high differentiation from all other populations with high statistical support. Similarly, Kırşehir was significantly distinct from all other populations, except for Isparta and İzmir, for which the Fst values were not significant. For the ITS2 data set some populations were comparable with each other with high similarities. In the analysis results, there were some negative Fst values, but none of these were supported statistically, therefore they were not considered for further evaluation.

In addition to pairwise comparisons populations were also tested for revealing hierarchical distribution of genetic variation at different levels. Although several

grouping schemes were tested, but the result of the trial that generated highest and significant differentiation among groups was given in Table 4.15 and Table 4.16 for both the cyt b gene and the ITS2 region, respectively.

Based on the cyt b result, the highest genetic partitioning (36.61%) was among populations when all populations were introduced into the analysis as separate units. The separation was statistically significant, however substantial amount of genetic diversity still existed within population (63.39%). AMOVA analysis of populations with different grouping schemes using ITS2 data displayed highest among-group differentiation when population were clustered into two groups that were also obtained through the phylogenetic analyses of the same data set. Therefore, Bingöl, Elazığ, Malatya, and Siirt populations were grouped together and all other populations were taken as a single group. Percentage of variation was 47.76% for the among-group, 34.33% for within populations, and 17.90% for among populations within groups. All values were statistically supported.

Table 4.13. Fst analysis of cyt b region representing pairwise genetic variations between populations. +: p≤ 0.001, -: not significant.

	AFY	AYD	BAL	BIN	BOL	CAN	CNK	COR	DEN	ELA	ESK	GIR	ISP	IZM	KAY	KIR	KON	MAL	SII	SIV	TOK	USK
AFY		-	-	+	+	-	-	-	-	+	-	+	+	+	+	-	+	+	+	+	-	-
AYD	-0.01246		-	+	+	-	-	+	-	+	-	+	+	-	+	-	+	+	+	+	-	-
BAL	0.03642	0.04426		+	+	-	-	+	+	+	-	+	+	-	+	+	+	+	+	+	-	-
BIN	0.23953	0.30203	0.21111		+	+	+	+	+	+	+	-	-	+	-	+	-	-	-	-	-	+
BOL	0.78704	0.83064	0.68900	0.26572		+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
CAN	0.03145	0.03597	0.04961	0.31113	0.83180		-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-
CNK	0.00610	0.00284	0.06044	0.33904	0.84382	-0.00405		+	-	+	-	+	+	+	+	-	+	+	+	+	+	-
COR	0.00830	0.13120	0.13187	0.29403	0.82629	0.15971	0.14209		-	+	+	+	+	+	+	-	+	+	+	+	-	+
DEN	-0.01552	0.06997	0.10778	0.35296	0.87267	0.10962	0.07065	-0.00125		+	+	+	+	+	+	-	+	+	+	+	-	-
ELA	0.68198	0.73112	0.59493	0.16617	0.02269	0.73746	0.75261	0.72713	0.77743		+	+	-	+	+	+	-	-	-	+	+	+
ESK	0.12597	0.04823	0.04439	0.28510	0.88701	0.11633	0.10976	0.31815	0.28375	0.75127		+	+	-	+	+	+	+	+	+	-	-
GIR	0.20525	0.29604	0.19151	-0.03668	0.42646	0.31671	0.33367	0.24777	0.33308	0.28682	0.30798		+	+	-	+	-	-	+	-	-	+
ISP	0.61669	0.66805	0.49407	0.07752	0.25462	0.67626	0.69957	0.68648	0.74245	0.08803	0.66482	0.21231		+	+	+	-	-	+	-	+	+
IZM	0.21487	0.11407	0.09847	0.30173	0.91950	0.21080	0.19732	0.44045	0.42474	0.77734	-0.01974	0.34814	0.70946		+	+	+	+	+	+	+	-
KAY	0.16882	0.21553	0.13566	0.01487	0.46089	0.20066	0.23420	0.24768	0.28816	0.36144	0.20721	0.01776	0.21285	0.22780		+	+	+	+	-	-	+
KIR	-0.01596	0.06128	0.10543	0.37617	0.87761	0.10289	0.05400	0.02267	-0.03570	0.78974	0.29450	0.35682	0.76218	0.42910	0.30218		+	+	+	+	+	-
KON	0.43971	0.49665	0.39535	0.00244	0.12602	0.50301	0.52417	0.48814	0.54383	0.05339	0.48764	0.06150	0.00548	0.49939	0.13320	0.56096		-	-	-	+	+
MAL	0.53990	0.59930	0.44415	0.01709	0.13134	0.62101	0.64144	0.60353	0.66957	0.01197	0.57452	0.10597	-0.03174	0.61671	0.19419	0.69638	-0.04499		-	-	+	+
SII	0.67350	0.73135	0.55237	0.13230	0.26652	0.74309	0.75978	0.73289	0.79262	0.01189	0.75000	0.25094	0.13020	0.80714	0.33517	0.81090	0.06073	0.01557		-	+	+
SIV	0.21554	0.29193	0.20508	-0.04925	0.37161	0.30284	0.32618	0.26682	0.34139	0.25419	0.29485	-0.06110	0.15752	0.32230	-0.00583	0.36269	0.03486	0.07794	0.22527		-	+
TOK	0.02017	0.08931	0.05986	0.07867	0.59533	0.11713	0.11242	0.07380	0.11787	0.48687	0.14144	0.02107	0.39120	0.18909	0.02825	0.12343	0.24841	0.31483	0.45496	0.03606		-
USK	-0.02646	-0.03548	0.04145	0.29642	0.81258	0.00442	0.00016	0.09257	0.03546	0.71813	0.07893	0.28318	0.65690	0.15054	0.21154	0.03138	0.49063	0.59573	0.71688	0.28265	0.08496	

57

Table 4.14. Fst analysis of ITS2 region representing pairwise genetic variations between populations. +: p≤ 0.001, -: not significant.

	AFY	AYD	BAL	BIN	BOL	CAN	CNK	COR	DEN	ELA	ESK	GIR	ISP	IZM	KAY	KIR	KON	MAL	SII	SIV	TOK	USK
AFY		-	-	+	-	-	-	-	-	+	-	-	+	-	-	+	+	+	+	-	-	-
AYD	0.01087		-	-	-	-	-	-	-	+	-	-	-	-	-	+	+	+	+	-	-	-
BAL	-0.04281	-0.04360		+	-	-	-	-	-	+	-	-	+	-	-	+	+	+	+	-	-	-
BIN	0.13950	0.05709	0.09722		-	+	+	+	+	+	-	+	-	-	-	+	+	-	+	-	-	+
BOL	0.05222	-0.06103	-0.00962	0.04056		+	+	-	-	+	-	-	-	-	-	+	+	+	+	-	-	-
CAN	0.01141	0.18580	0.05772	0.21429	0.19371		-	-	-	+	-	-	+	-	+	+	+	+	+	-	-	-
CNK	0.01141	0.18580	0.05772	0.21429	0.19371	0.00000		-	-	+	-	-	+	-	+	+	+	+	+	-	-	-
COR	-0.07988	0.02333	-0.03550	0.15102	0.06478	0.00510	0.00510		-	+	-	-	+	-	-	+	+	+	+	-	-	-
DEN	-0.08333	0.01087	-0.04281	0.13950	0.05222	0.01141	0.01141	-0.07988		+	-	-	+	-	-	+	+	+	+	-	-	-
ELA	0.75714	0.69697	0.71084	0.37471	0.66340	0.80500	0.80500	0.76566	0.75714		+	+	+	+	+	+	+	+	-	+	+	+
ESK	-0.09551	0.04412	-0.05671	0.07821	0.05574	0.00000	0.00000	-0.09771	-0.09551	0.70982		-	+	-	-	+	+	+	+	-	-	-
GIR	0.00123	0.11226	0.02741	0.16336	0.13159	0.02941	0.02941	0.00247	0.00123	0.75424	-0.08911		+	-	+	+	+	+	+	-	-	-
ISP	0.59097	0.31189	0.40000	0.00698	0.19146	0.77612	0.77612	0.60914	0.59097	0.56724	0.61749	0.62370		-	-	-	-	-	+	-	-	+
IZM	0.20309	-0.07892	0.02136	-0.07143	-0.11700	0.51613	0.51613	0.22804	0.20309	0.58916	0.25000	0.29229	0.01619		-	-	+	-	+	-	-	-
KAY	0.06536	-0.05076	0.00556	0.05732	-0.04903	0.19226	0.19226	0.07697	0.06536	0.68320	0.07540	0.14580	0.18992	-0.11404		+	+	+	+	-	-	-
KIR	0.67782	0.47176	0.54188	0.12366	0.36257	0.79434	0.79434	0.68917	0.67782	0.63828	0.70944	0.70760	-0.08281	0.23847	0.34974		-	-	+	+	+	+
KON	0.86196	0.70569	0.74115	0.24997	0.59140	0.93982	0.93982	0.86691	0.86196	0.70381	0.91525	0.87551	0.16357	0.59888	0.56020	0.03719		-	+	+	+	+
MAL	0.58964	0.38209	0.45098	0.00633	0.28428	0.73214	0.73214	0.60793	0.58964	0.43814	0.53846	0.60621	-0.06061	0.10121	0.30017	-0.00119	0.11680		+	+	+	+
SII	0.94087	0.84325	0.84520	0.30962	0.76562	100.000	100.000	0.94417	0.94087	0.18296	100.000	0.93872	0.78328	0.81250	0.76234	0.79605	0.92314	0.53846		+	+	+
SIV	-0.00299	-0.06629	-0.03936	0.06629	-0.04525	0.12325	0.12325	0.00721	-0.00299	0.68772	-0.00374	0.07524	0.28923	-0.06461	-0.03344	0.45082	0.66702	0.36364	0.80243		-	-
TOK	-0.01269	-0.07432	-0.04893	0.08163	-0.04371	0.14286	0.14286	-0.00201	-0.01269	0.71792	0.01639	0.08779	0.36951	-0.03448	-0.03201	0.51454	0.73062	0.43462	0.85748	-0.05983		-
USK	-0.05957	-0.04303	-0.05600	0.11765	0.00023	0.07143	0.07143	-0.05220	-0.05957	0.74447	-0.04278	0.03798	0.49048	0.07821	0.01405	0.60615	0.80170	0.52553	0.89959	-0.03917	-0.05442	

Table 4.15. AMOVA results of cyt b gene where all populations were grouped separately.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations	21	470.538	1.56716 Va	36.61*
Within populations	256	694.577	2.71319 Vb	63.39*
Total	277	1165.115	4.28035	
Fixation Index	FST : 0.36613			

Table 4.16. AMOVA results of ITS2 segment with respect to different groupings. Populations were grouped into two groups. 1) Bingöl, Elazığ, Malatya, and Siirt, 2) All other populations.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	F-index
Among Groups	1	21.069	0.29141 Va	47.76*	0.47762
Among populaitons within groups	20	31.699	0.10924 Vb	17.90*	0.34273
Within populations	256	53.628	0.20949 Vc	34.33*	0.65666
Total	277	106.396	0.61013		

4.3 Phylogenetic and Phylogeographic Analysis

For revealing geographic structuring of the genetic variation in *C. divisa* populations in Turkey maximum parsimony, maximum likelihood and Bayesian approaches were employed on both cyt b gene and ITS2 region data sets separately.

MP analysis of the cyt b data matrix produced a consensus tree of 230 equal-length trees with a step of 230 (Figure 4.5). The CI was 0.5391, RI was 0.8503, and HI was 0.5989. Bootstrap values provided high statistical support for all branches in the tree. In the consensus tree, H18 (from Balıkesir) shows a polytomy with the outgroups and the remaining haplotypes. Those remaining haplotypes is divided into two main haplogroups. To facilitate understanding of the tree this large clade was named as A and B clades. While the A clade is more structured than the B clade both clades show some polytomic subclades. A Bingöl haplotype (H28) is placed as the basal haplotype to clades A and B. The basal haplotype of clade A is a Balıkesir haplotype (H25) while the basal haplotype of clade B is an Elazığ haplotype (H68).

The A clade is composed of four subclades: A1, A2, A3, and A4. In these subclades there are some small haplotype groups that either make monophyletic or polytomic groupings. A1 subclade consists of only 5 different haplotypes detected in Afyon, Uşak, Kırşehir, Aydın, Bingöl, Çanakkale, Sivas, and Kayseri. A2 subclade is a part of a polytomic haplogroup, where there is a Balıkesir haplotype and relatively large subclade that is composed of some smaller haplogroups. Subclades A3 and A4 are composed of haplotypes representing either central or the western part of the sampling area in Turkey. While A2 subclade is formed by 5 haplotypes (from Balıkesir, Bingöl, Konya, Uşak, Çanakkale, and Çorum) the A3 subclade is compromised by many haplotypes representing Denizli, Uşak, Aydın, Çankırı, Çanakkale, Konya, Afyon, and others.

On the other hand, Clade B is composed of two main lineages where a haplotype found in Elazığ seems to be sister to a polytomic haplogroup. In the polytomic part, a Siirt haplotype (H101), a monophyletic group composed of H95 (from Konya) and H100 (from Malatya), H66 (from Elazığ), H1 (from Afyon), and 27 more haplotypes representing most eastern, some central, northern and western sampling localities of *C. divisa* in Turkey formed polytomy.

ML analysis produced a consensus tree of 174 trees (Figure 4.6). CI was 0.519, RI was 0.838, and HI was 0.481. The ML analysis generated nearly the same results with the MP analysis, where H18 (from Balıkesir) was the most basally placed haplotype in both trees. The placement of other haplotypes at the basal part of the tree is similar to MP tree. The bootstrap values are high for all branches. The grouping of the main clades as Clade A and B composed of mostly the same haplotypes that were found in MP tree with high statistical support.

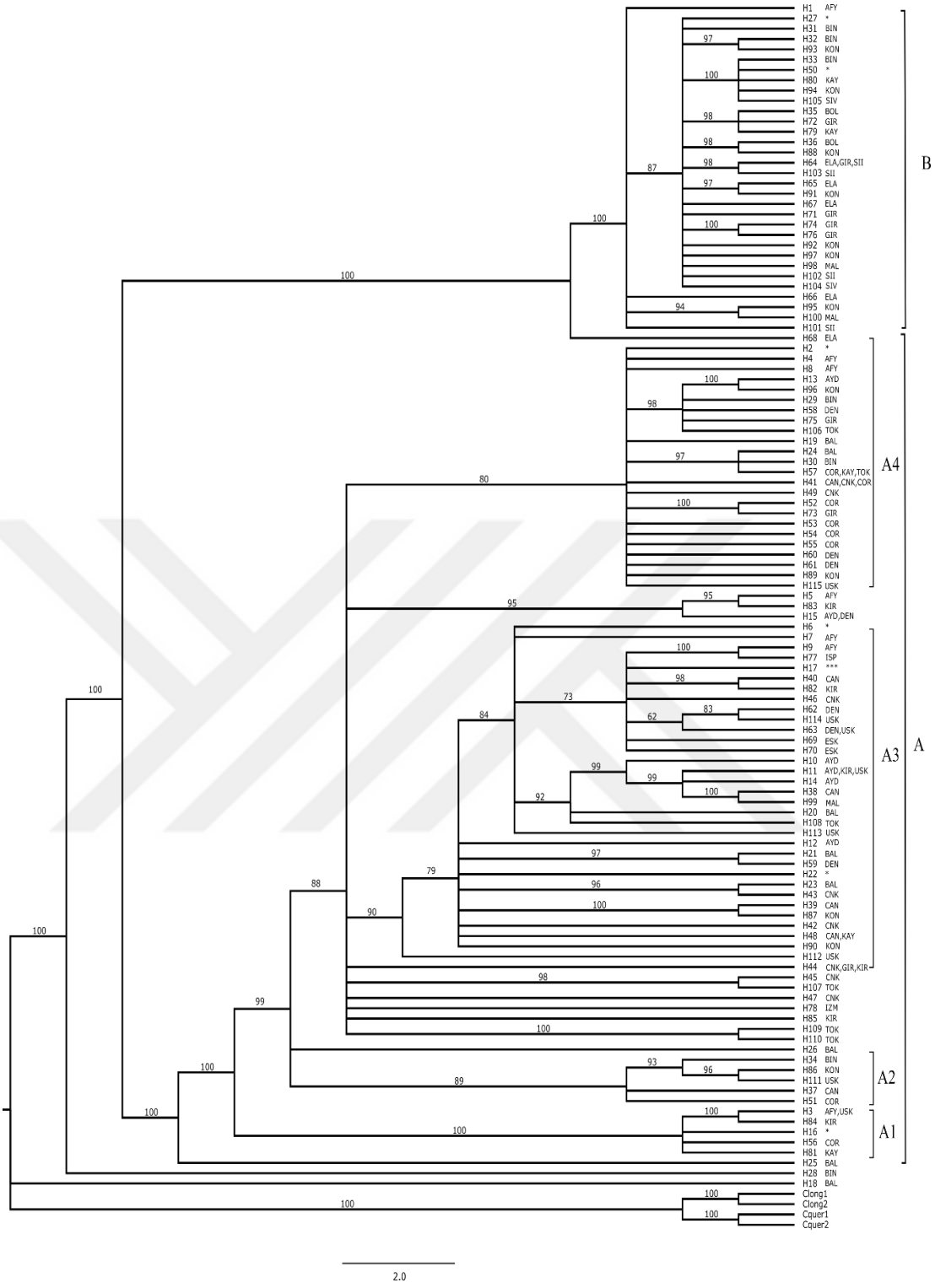


Figure 4.5. MP 50% majority rule consensus tree of consensus tree of the cyt b haplotypes of *C. divisa*. The bootstrap values that are 50% support are shown over relevant branches. *: Shared haplotypes found in several populations.

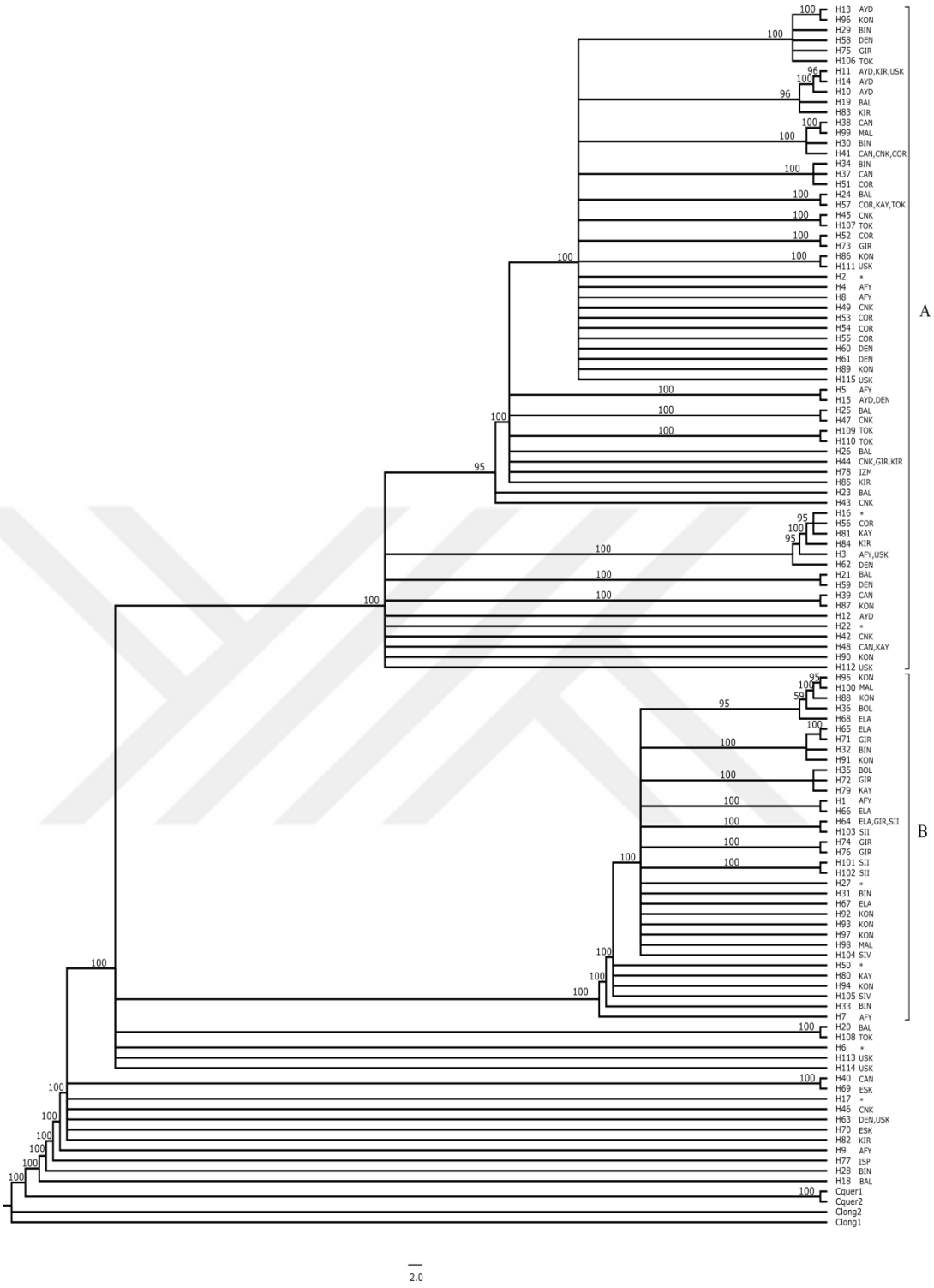


Figure 4.6. Maximum Likelihood 50% majority rule consensus tree of the cyt b haplotypes of *C. divisa*. The bootstrap values over 50% support are shown on relevant branches. *: Shared haplotype among many populations.

The Bayesian based Beast tree is shown in Figure 4.7. It was almost the same with MP and ML trees. Times of divergence of main lineages were also shown at the relevant nodes. The divergence estimates indicated that the ingroup haplotypes diverged from the outgroup haplotypes during Pliocene around 3.345 million years ago (MYA). The separation of the most basal haplotype (H18 from Balıkesir) from its sister clade which includes rest of the haplotypes, is at the nearly end of the Pliocene around 2.962 MYA. Likewise, a Bingöl haplotype (H28) at the basal part was diverged from the remaining haplotypes around 2.392 MYA at the Gelasian period of Pliocene. Further diversification events seem to continue during early Pleistocene. After this point in the tree there is much more structured appearance. Divergence of the remaining haplotypes into Clade A and B, most probably occurred around the beginning of the Pleistocene nearly 1.79 MYA. The A and B clades separated from each other about 1.509 MYA, and subsequent and ongoing diversification events throughout the Pleistocene seem to result in a series of deep to shallow structuring in *C. divisa*. The most recent diversification events caused shallow and small subclades in the last period of the Pleistocene, particularly during Günz, Mindel, Riss and Würm glaciation times.

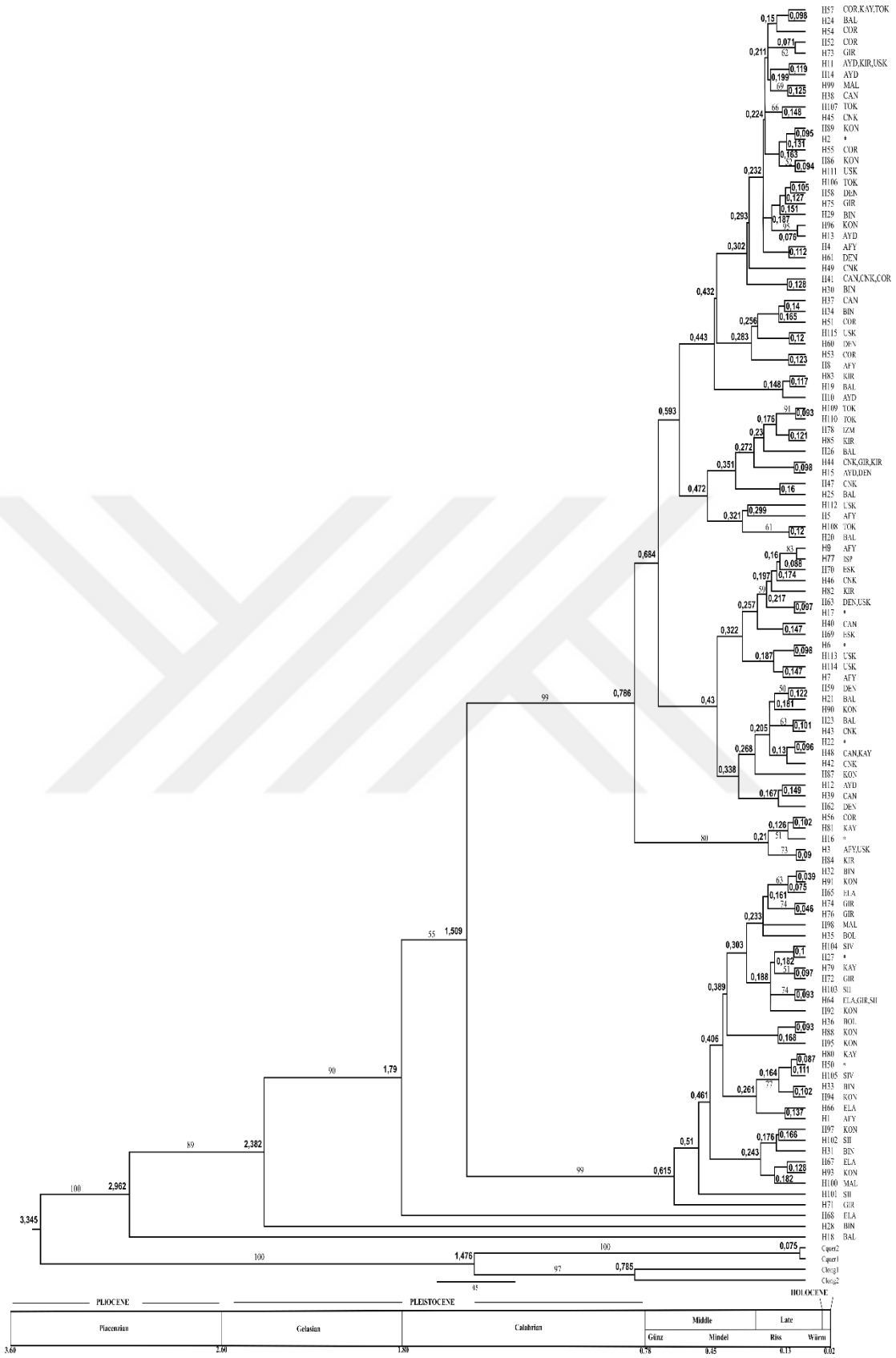


Figure 4.7. Beast consensus tree of cytb haplotypes with the node ages and posterior probability values are shown at relevant node.

Minimum spanning network for better resolving evolutionary relationships among haplotypes and visualize possible reticulations was conducted, and the result was shown in Figure 4.8. The network provided similar structuring with phylogenetic trees. There are several main haplogroups forming star like phylogeny in the network where three major groupings are apparent each with common haplotypes at its center. The first group includes H27 at the center, which is a shared and common haplotype found in 9 localities mainly from the eastern part of the sampling area. Moreover, 17 haplotypes directly and 15 haplotypes indirectly connected to H27 at this haplogroup. When evaluated altogether they are dominated by the haplotypes from the easterly located populations.

The first haplogroup relates with the second via H33 from Bingöl. More structured appearance is obvious in the second haplogroup, where there are several small star-like phylogenies. One of the most striking feature of this haplogroup is the presence of haplotypes from the western and central part of Turkey that dominate the entire group. In this cluster H6 (found in Afyon, Balıkesir, Bingöl, and Çankırı), H7 (found in Afyon), H22 (found in Balıkesir, Çanakkale, Eskişehir, Kayseri, and Kırşehir), H16 (found in Aydın, Bingöl, Çanakkale, Sivas, and Uşak), and H44 (found in Çankırı, Giresun, and Kırşehir) are located at the central part of the small haplotype group. Overall, one of the most striking characteristics of this haplogroup in general is that it is dominated by the haplotypes found at the central and western part of Turkey.

The second haplogroup is related with the third haplogroup through H44 and H8 (found in Afyon), and then to the most frequent haplotype H2 (found in 14 different localities). Except for Bingöl, Sivas, Tokat, and Kayseri, H2 was detected in all central and western localities. Overall, it is also apparent that the third haplogroup is governed by the west and central haplotypes.

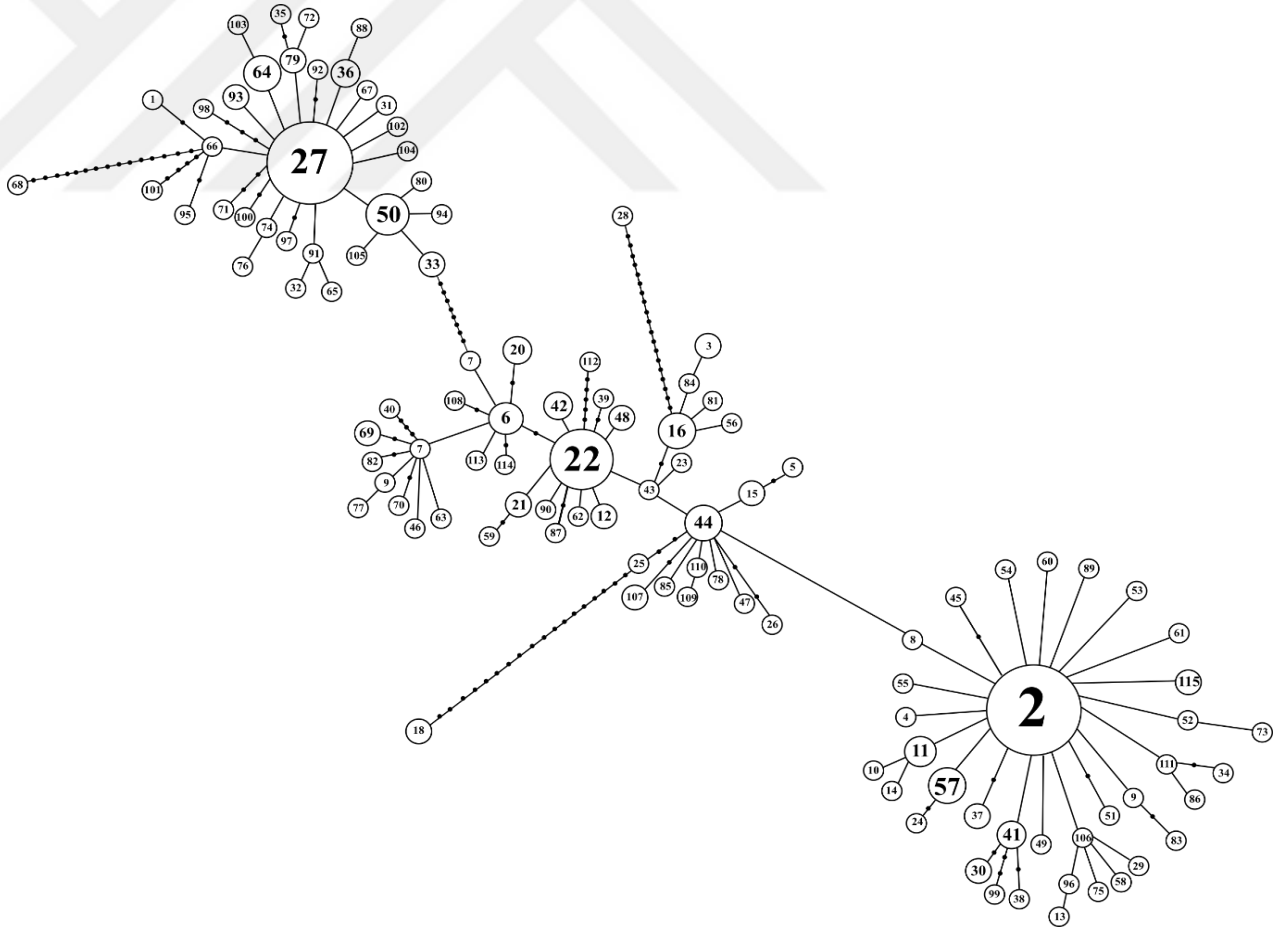


Figure 4.8. Minimum spanning tree of the cyt b haplotypes of *C. divisa*. Circle size is proportional to the frequency of alleles across the sampling area. ● indicates hypothetical alleles.

Fifteen alleles of *C. divisa* and the two outgroup alleles were used in both MP and ML analysis, and the resulting consensus trees were shown in Figure 4.9 and Figure 4.10. Both analyses generated the same tree where a large polytomy was present. The only large clade was formed by the alleles representing only the eastern localities (Bingöl, Elazığ, Malatya, and Siirt). A small monophyletic group consisted of two commonly shared alleles as A1 (shared among Afyon, Aydın, Balıkesir, Bingöl, Bolu, Çanakkale, Çankırı, Çorum, Eskişehir, Denizli, Giresun, Isparta, İzmir, Kayseri, Kırşehir, Konya, Malatya, Sivas, Tokat, and Uşak), and A12 (found only in Giresun). All the remaining alleles were placed in the polytomic part, and no relationship would be resolved.

Minimum spanning tree of the ITS2 alleles is shown in Figure 4.11. The most common allele (A1), shared among 20 populations out of 22 localities, is connected directly to A2 which is also a shared allele among 16 populations. Two-star phylogeny formations are observed in the network. The first includes A2 at the center, and it is connected to 8 other alleles including A1. In this first group Malatya (A14), Sivas (A15), Kayseri (A13), Elazığ (A11), Balıkesir (A3), and Bolu (A8) alleles are placed. The second star phylogeny has A7 at its center. A7 was detected as another common allele shared among Bingöl, Elazığ, Malatya, and Siirt. This second allele group is connected to alleles all of which are found only in east part of the sampled area of *C. divisa*.

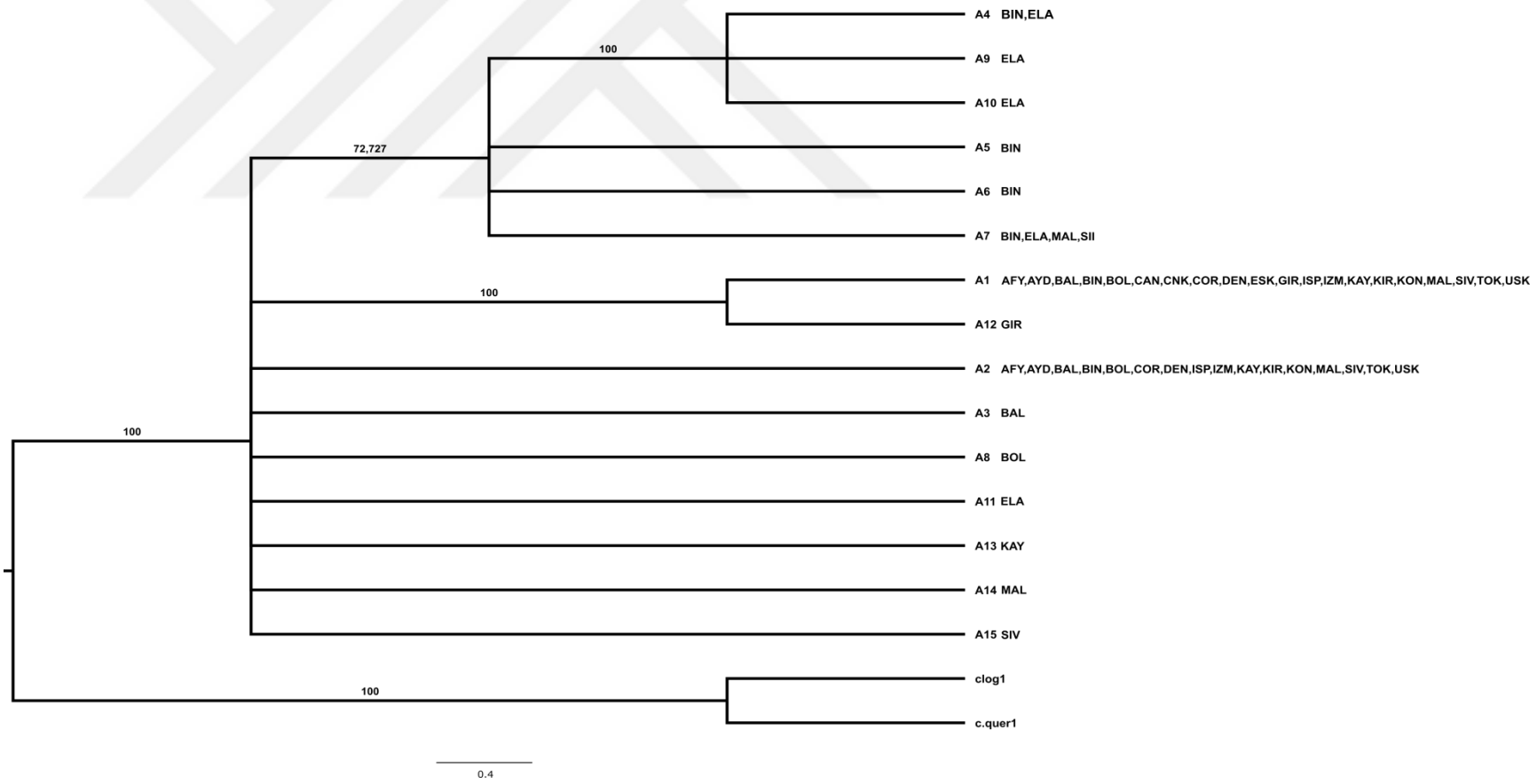


Figure 4.9. Maximum parsimony 50% majority rule consensus tree of the ITS2 alleles of *C. divisa*. The bootstrap values that are 50% support are shown over relevant branches.

69

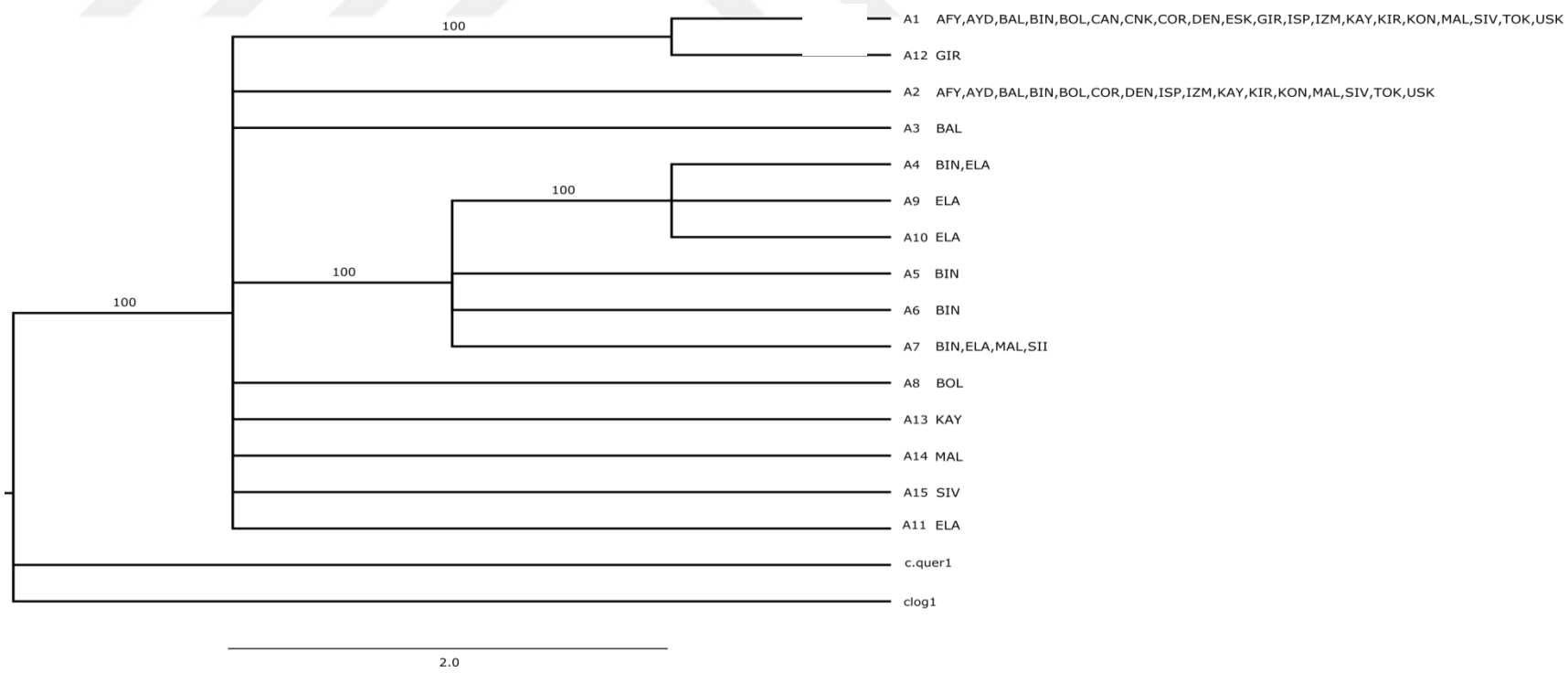


Figure 4.10. Maximum likelihood 50% majority rule consensus tree of the ITS2 alleles of *C. divisa* (-lnL= 484.6991). The consensus tree is a resulting tree of 3 subtrees. The bootstrap values that are 50% support support are shown over relevant branches.

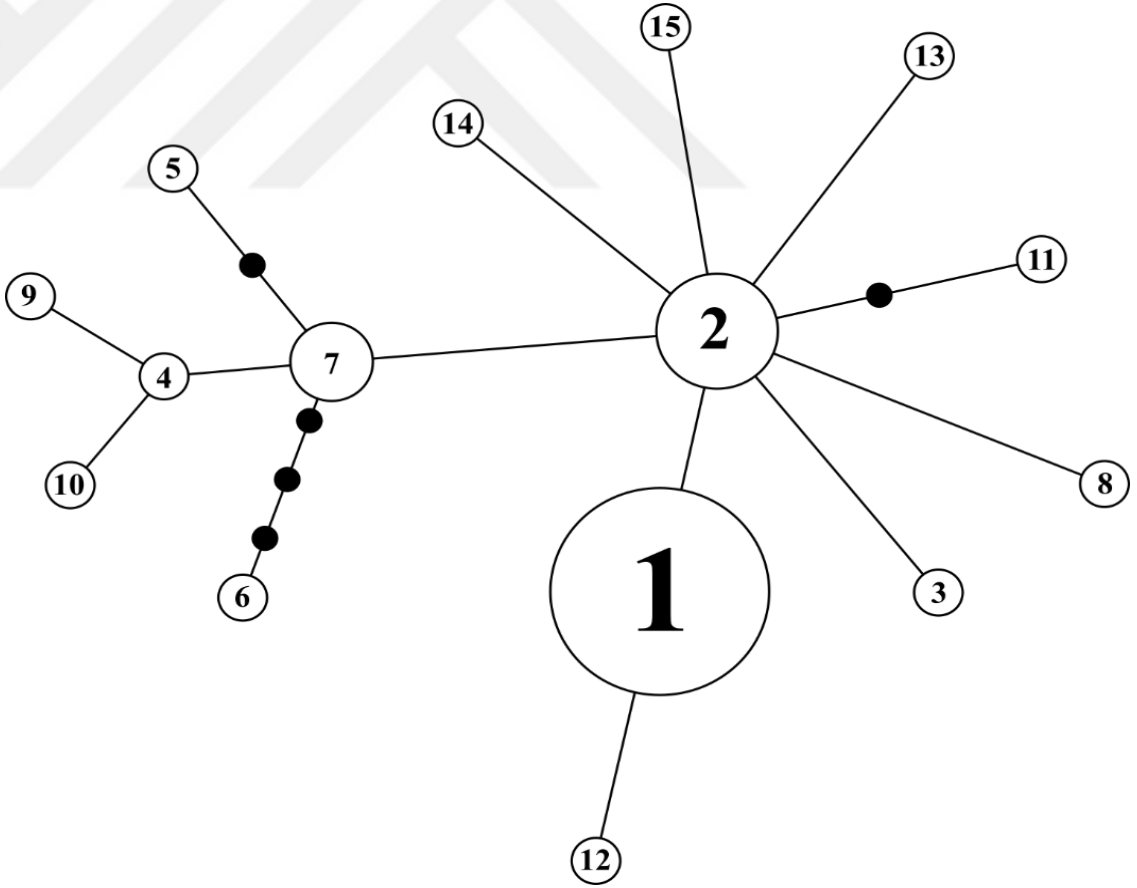


Figure 4.11. Minimum spanning tree of the ITS2 alleles of *C. divisa*. Circle size is proportional to the frequency of alleles across the sampling area. ● indicates hypothetical alleles.

5. DISCUSSION

5.1 Genetic Diversity Estimates of the Turkish Populations of *Cynips divisa*

One of the main concerns of molecular systematic studies utilizing mitochondrial genome is associated with nuclear counterparts (pseudogenes or numts) of mitogenes. For eliminating such possible problems, several points were checked in this thesis including high A+T content, absence of stop or non-sense mutations in the DNA and translated amino acid sequences (Zhang and Hewitt, 1996), and higher rate of transition versus transversion (Brown et al., 1979). *Cynips divisa* data set fits well with these criteria because A+T nucleotides (76.64% A+T versus 23.35% G+C) are high, there are no stop codons and non-sense mutations, and the number of transition is higher than transversions. These findings prove that the generated sequences in this study are in fact genuine mitochondrial genome.

Genetic diversity estimates of *C. divisa* was assessed through examination of two genomic regions each with different substitutional rate and evolutionary ratio. Thus, analyzing two separate DNA segments is assumed to shed light to different time zones in the history of the species (Quenouille et al., 2004). In this study, genetic diversity estimates were explored by means of estimating haplotype and nucleotide diversity since these two calculations are good estimators of the contemporary genetic variation. Haplotype or gene diversity refers to the possibility of two randomly sampled sequences being different from each other, however nucleotide diversity is the average number of nucleotide difference per site between two different sequences (Nei, 1987). In *C. divisa*, both cyt b gene and the ITS2 region produced high haplotype versus low nucleotide diversity where mean gene diversity was ~ 0.818 and 0.316, and the mean nucleotide diversity was ~ 0.0119 and 0.0016 for cyt b and ITS2, respectively. Furthermore, the number of singleton haplotypes and alleles are 84 out of 115 (73%) and 11 out of 15 (73%) for cyt b and ITS2 data. The number of private haplotypes is 13 out of 115 haplotypes (11%-without eliminating singletons from the entire number of haplotypes), 13 out of 31

(41.9% when singletons are eliminated from the entire number of haplotypes (115)). Current findings on *C. divisa* regarding high haplotype versus low nucleotide diversity and high number of singleton and private haplotypes/alleles may imply some fluctuations in population sizes in the past. Such pattern is often interpreted as recent population expansion after population declines (Avise, 2000). In fact, similar findings with respect to genetic diversity estimates and high number of singletons were reported from other gall wasp species studied from Turkey (Mutun, 2016; Mutun and Atay, 2015; Dinç, 2017).

While mean genetic diversity estimates are well-within the limits of other oak gall wasps from Turkey, each population genetic diversity estimates also display some congruencies with the results of other gall wasp taxa. In particular, estimates for those populations with genetic diversity greater than 0.500 are highly similar with those of other oak gall wasp taxa including *Andricus coriarius* (Challis et al., 2007), *A. quercustozae* (Rokas et al., 2003), *A. caputmedusae* (Mutun, 2010), *A. quercustozae* (Dinç and Mutun, 2011), *A. lucidus* (Mutun and Dinç, 2011), *A. lignicolus* (Mutun and Karagözoğlu, 2015), *T. synaspis* (Mutun and Atay, 2015), *A. curtisii* (Mutun, 2016), and *C. quercusfolii* (Dinç, 2017).

Mean diversity exposed by *C. divisa* in this thesis is higher than most other gall wasp species. For instance, genetic diversity estimates was h= 0,463, and π= 0,101 in *Andricus caputmedusae* (Mutun, 2010), h= 0,8089 and π= 0, 115 in *A. lucidus* (Mutun, 2011), h= 0, 450, and π= 0, 050 in *A. quercustozae* (Dinç and Mutun, 2011),and h= 0.855, π= 0.013 for the cyt b and h= 0.311 and π= 0.001 for the ITS2 region, respectively in *Trigonaspis synaspis* (Mutun and Atay, 2015). In *C. quercusfolii* genetic diversity was h= 0.732 and π= 0.010 for cyt b, and h= 0.5865 and π= 0.005 for the ITS2 region (Dinç, 2017). In *C. divisa*, the detected gnetic diversity values (h= 0.818 and π= 0.011 for cyt b gene, and h= 0.316 and π= 0.001 for ITS2 region, respectively disclose the richness of genetic variation in oak gall wasps currently showing distribution in Turkey.

High genetic diversity presently found in *C. divisa* is not only high for the Turkish gall wasp species but also it is strikingly higher than the European oak gall wasp species. A large-scale study incorporated many Palearctic populations of *A. coriarius* species nucleotide diversity was 0. 005 in Iranian populations, π=0.006 in

Lebanese populations, but diversity was 0.015 in the Turkish populations (Challis et al., 2007). Similarly, genetic diversity was ranging from 0.2% to 4.2% in Turkey, but it ranged between 0.12 and 0.17% in Italy for *A. quercustozae* (Rokas et al., 2003). Current findings of this thesis provide not only more robust data overall for the Turkish gall wasp diversity, but also for overall Turkish biodiversity. However, larger scale and detailed studies on Turkish oak gallwasps and other species are still necessary to complete the picture of biologic diversity in the western Palearctic.

5.2 Historical Demography of *Cynips divisa*

Studying current populations across the range of a species allows revealing historical population structure and the possible factors that might have possibly caused the contemporary pattern (Avise, 2000). Since both past and present factors are important to give the current shape and structuring of populations, one may find imprints of the historical factors that resulted in either stable populations through time or population bottlenecks or growth (Hewitt, 1996). Several statistical tests used to disclose population expansion or declines include mismatch analysis and calculations of Tajima's *D*, Fu's *Fs*, Harpending's raggedness index (Hri), and the sum of squared deviations (SSD). In *C. divisa,* mismatch analyses using all pairwise combinations of both cyt b and ITS2 data separately sign multimodal profile with several picks. While unimodal pattern in mismatch distribution graph, significant negative Tajima's *D* and Fu's *Fs*, smaller SSD and Hri values indicate population expansion, multimodal graph, significant positive Tajima's *D* and Fu's *Fs* larger SSD and Hri imply either declining or non-expanding populations (Rogers and Harpending, 1992). ITS2 graph with three picks, two shallow and one drastic pick, can be interpreted as more ancient, but a non-drastic population decline and subsequent expansion, followed by severe population decline near the more recent time, and keeping stable then. On the other hand, mismatch graph of cyt b data may be explained as population expansion event, followed by a non-drastic decline, and expansion again. After small growth a severe decline and subsequent population expansion occurred, and populations were then kept at smaller size but at equilibrium. Overall, both data sets imply structured populations that are presently at demographic equilibrium, however underwent some declines and expansions in the

past (Ramos-Onsins and Rozas, 2002). It seems that both data sets used in this study provided resolution for different time zones as would be expected for ITS2 providing information for the more ancient events and the cyt b for the more recent demographic processes.

Individually analyzed populations with respect to cyt b and ITS2 data sets revealed their own demographic histories. While ITS2 data implied population expansion for all populations except for Bingöl where it showed multimodal profile indicating equilibrium. It was also supported by its relatively high SSD value suggesting presently non-expanding population. Although Bingöl seems to be a non-expanding population today, haplotype network analysis indicated that it provided lineage(s) to the western populations. It seems that Bingöl provided haplotypes/lineages spread in more ancient past rather than more recent past. Other than Bingöl, all other populations of *C. divisa* showing unimodal profile are also supported by low SSD and Hri indices. Mostly negative Tajima's *D* and Fu's *Fs* values may also support these findings even though some of the values were not supported significantly. Discrepancies found sometimes among the statistical support between Tajima's *D* and Fu's *Fs* may be explained by Hri and SSD being more sensitive indicators as compared Tajima's *D* and Fu's *Fs* (Jong et al., 2011).

On the other side, cyt b data with multimodal profiles for almost all populations indicated structured populations with several declines and expansions. Since mtDNA cyt b data set provides more resolution on the more current times, it seems that some recent fluctuations in *C. divisa* populations happened as suggested by graphs. Among all populations it appears that Bingöl is the most structured population presently. This result is also supported by large SSD and negative, but non-significant Tajima's *D* and Fu's *Fs* values. In addition, some other populations such as Elazığ, Çankırı, Isparta, Malatya, Kırşehir, Siirt, Uşak, and Çorum show significant negative values suggesting population expansion. However, some differences between Tajima's *D* and Fu's *Fs* of few populations may be due to either different resolving power of both tests, or as an alternative severe and concurrent fluctuations in those population over time might have had occured (Zhang et al., 2006). Overall assessment of *C. divisa* population historical demography based on the cyt b data set proposes severe, concurrent and ongoing fluctuations resulted in the

structured population demographies for the species in Turkey. In fact, population size changes as a response to oscillations in the environment have long been known to affect geographic distribution of genetic diversity (Hewitt, 2000), and common for the Anatolian species (Bohlen et al., 2006; Bellati et al., 2011; Çıplak, 2004, 2008; Gündüz et al., 2007; Kornilios et al., 2012; Özdemir et al., 2014; Stöck et al., 2012; Veith et al., 2003). Moreover, findings of this thesis produced almost a similar pattern with several oak gall wasp species studied from Turkey (Dinç 2017; Mutun, 2016; Mutun and Atay, 2015) with some minor differences that may possibly be due to differential responses given by different species even if they show sympatric distribution (Avise, 2000).

5.3 Geographic Distribution of Genetic Lineages: Implications of Phylogenetic, Network and Statistical Analyses

In the last several decades, developments in computer algorithms combined with advances in molecular methods allowed scientists to explore species histories (Loxdale and Lushai, 1998) and geographic distribution of genetic lineages (Bermingham and Moritz, 1998; Hewitt, 2004; Hickerson et al., 2010; Kidd and Ritchie, 2006; Pyron, 2015; R Kuchta and Meyer, 2001). Based on all these advancements in the field, I attempted to uncover geographic distribution of genetic diversity and major lineages of *C. divisa* in Turkey and explain the processes and the pattern. Phylogenetic, network and statistical analyses on *C. divisa* produced two main clades with a Balıkesir haplotype (H18) being most basal to all other sequences recovered across the sampled area. This finding seems to be contradictory with previous findings from other oak gall wasp species studied so far in Turkey (stating generally an eastern haplotype being most basal to all others) (Challis et al., 2007; Mutun, 2010, 2016; Mutun and Atay, 2015; Rokas et al., 2003). Therefore, it is necessary to dig this result into deeper. One explanation would be that Balıkesir haplotype (found only in 2 individuals in Balıkesir) is indeed an old haplotype. On the contrary, the basal placement of H18 and its being polytomic with the outgroups and all other ingroup haplotypes may be due to either long branch attraction (LBA) or retention of ancestral polymorphism (Avise, 2000). LBA is a biological situation in which convergent or parallel changes may produce artifactual placement of

sequences (Anderson and Swofford, 2004). On one other hand, according to a common acceptance, ancestral and old haplotypes are often geographically more widespread (Crandall and Templeton, 1993; Neigel and Avise, 1993; Slatkin, 1991), and on the other hand LBA or H18 is the representative of an ancestral lineage that coales back to the more ancient times; thus, any of these may be responsible for the current situation of H18. Either exclusion of H18 from the analysis or more sampling from the locality would resolve this inexplicit situation. If the current placement of Balıkesir haplotype reflecting its old age then there might have been an ancient representative lineage there, and possibly in other nearby localities we were not able to sample them just by chance and not being able to sample them again by chance in none of the other localities sampled in this thesis.

The clade formation of all analyses generated nearly the same pattern for the cyt b data where the two main lineages roughly grouped haplotypes into Clade A dominated by the haplotypes mainly from central and western part of Turkey, and the Clade B which is dominated by both west and east part of Turkey. A more structured-appearance of the Clade A further grouped haplotypes into geographically significant and meaningful groupings. Similar clade formations and structured clades have been reported from other gall wasp species from Turkey. Regarding the general pattern apparent in the current findings of *C. divisa* coincide with the most results of other gallwasps species. For instance, in *A. caputmedusae* there were three major clades as west of the Anatolian Diagonal, southeast/ near west of the Anatolian Diagonal and east/ in the Anatolian Diagonal (Mutun, 2010). Similarly, in *A. quercustozae* there were three main clades as eastern clade, western clade, and eastern/ western clade (Dinç and Mutun, 2011). Nevertheless, current results found in *C. divisa* allow to draw a general conclusion since geographic grouping of the genetic lineages is correlated with the location of the major physical barriers located in Turkey, in turn providing further evidence for previous findings (Dinç and Mutun, 2011; Mutun, 2010, 2016; Mutun and Atay, 2015; Rokas et al., 2003). Such a pattern has also been reported for plants (Ansell et al., 2011; Ekim and Güner, 1986) and some other insects (Çıplak, 2004).

Besides, even though ITS2 seems to be less powerful marker as compared to the cyt b gene at the intraspecific level, it implied similar pattern where populations

from the east of Turkey were grouped together and all other populations fall into the polytomy. The time frame unveiled by ITS2 falls into older and more ancient times, and more recent times can be resolved by the cyt b data. Keeping this in mind, an overlap in the general pattern in geographic partitioning of the genetic diversity and lineages of *C. divisa* is striking and provide support for such general pattern to be drawn. Minor differences between the results of the two markers in the case of *C. divisa* may be due possibly to different inheritance modes, mutation rates, and selection pressure of the two marker genes (Shaw et al., 2002; Zhang and Hewitt, 2003).

Main clade and subclade ages through estimation of *C. divisa* predated the Pleistocene period. In fact, the first separation of the species from its congenerics seems to have occurred around 3.34 MYA in the middle Pliocene which is a period of environmental fluctuations (Hewitt, 2000). The estimated age of the basal haplotypes ranges from 2,99 MY to 2.38 MY. This range covers the end of the Pliocene and the early times of Pleistocene. Likewise, the two clades (A and B clades in MP and ML) appear to diverge from a linage around Elazığ (that was represented by H68) nearly 1.79 MYA which corresponds to early Pleistocene. Great deal of evidence has accumulated now regarding the effects of the Pleistocene glacial and interglacial changes of the Quaternary period (Bryson, 1974; Hewitt, 1996; Petit et al., 2005) shaping both geographic distribution of species and as well as genetic diversity and lineages (Hewitt, 2000), and it seems that it is also valid for *C. divisa*.

The footmarks of the historical events particularly climatic and environmental fluctuations are observed in current structuring of populations (Avise, 2004). Responses given by the species/populations/lineages as contraction and expansions result in the more and either deep or shallow structured phylogenies (Avise, 2004; Ferris et al., 1999; Templeton et al., 1990). It seems that the ongoing series of glacial and interglacial cycles have also left their signatures on the structured phylogeny of the *C. divisa* lineages through the Pleistocene. Although Turkey as a place that was never covered by ice sheets temperature drop was pronounced, and many species either shifted their range or contracted their populations. Continuing oscillation during the last glacial period also affected greatly vegetation of Turkey. A recent study showed that vegetation including oak hosts together with climate in Anatolia

and adjacent regions oscillated concurrently during the last glacial period (Şenkul and Doğan, 2013). Analysis on *C. divisa* denote where these fluctations lineages were shaped thoroughly from the beginning to the end of the Pleistocene. Divergence of Clade A and B occurred around 1.50 MYA ended up with a separation of some eastern and western lineages creating at the same time some haplogroup formations as also inferred from the haplotype minimum spanning network. A clear grouping of haplogroups indicated similar structured clade formations in the recent past as well as in the more ancient times. Splittings between the lineages of *C. divisa* continued through more recent history of the species in Turkey particularly during the end of the Calabrian time. But the dominating cycles resulting in the more recent structuring in *C. divisa* were during the last four glacial times as Günz (between 676-621 ka), Mindel (between 478-424 ka), Riss (between 200-130 ka), and Würm (110-12 ka). These diversification estimates support previous findings (Dinç, 2017; Çıplak, 2008; Kaya and Çiplak, 2016; Mutun, 2016; Mutun and Atay, 2015; Rokas et al., 2003).

As a general assessment, geographic distribution of genetic variation in *C. divisa* points out the importance of climatic changes in shaping genetic structure of the species. Findings of this thesis provide further evidence for Quaternary oscillations in those places that were not even covered by glaciers and show how they were markedly influencing species. While much more work is still necessary to reveal the outcomes of climatic fluctuations in the biological world, data obtained on *C. divisa* will be helpful to enlighten the past and it will facilitate to make inferences about past factors that resulted in the present Turkish biodiversity.

6. CONCLUSION

In this thesis, an oak gall wasp species, Cynips *divisa*, was explored for the purpose of revealing mainly geographic distribution of genetic diversity, and the possible reasons that resulted in the current pattern. Results point to:

1. *C. divisa* has substantial amount of genetic diversity in the Turkish populations as compared to the previously studied Turkish and European gall wasp taxa.

2. High haplotype versus low nucleotide diversity apparent for both regions designate population size changes over time, possibly bottleneck event(s) was followed by population expansion.

3. Phylogenetic, phylogeographic, network, and statistical analyses employed on both regions indicated that there is a geographic structuring of the population up to a certain level. Not as robust as some other previously examined oak gall wasp species, *C. divisa* displays haplogroup formations that are geographically significant.

4. *Cynips divisa* seems to have diverged around Pliocene from other *Cynips* species incorporated into the analysis as outgroups.

5. Formation of major clades and shallow subclades appear to be associated with climatic oscillations of Pleistocene. In particular, the last 780.000 years seem to play key role in shaping the major diversification events of the species in Turkey.

Current findings of this thesis emphasize importance of historical events that produced contemporary structure of a species. Among other historical factors, as also suggested by the results of *C. divisa*, climatic fluctuations are crucial for species/lineage divergences and clade formations. It is believed that results of this study will be helpful to draw a general pattern in the future both for oak gall wasps of Turkey as well as the Palearctic region.

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1. Çimen E, Mutun S (2017). Contribution of *Cynips divisa* on the Turkish Genetic Diversity. Ecology 2017 International Symposium Kayseri, Türkiye. (Poster Presentation)