REPUBLIC OF TURKEY
BİNGÖL UNIVERSITY
INSTITUTE OF SCIENCE

MOLECULAR PHYLOGENY OF THE GENUS *LALLEMANTIA* Fisch. & Mey. (LAMIACEAE) IN TURKEY

MASTER THESIS

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BIOLOGY

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This dissertation was accepted by the following committee on 02.01.2018 with the vote unity.

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Director of the institute

PREFACE

To begin with, I thank (Allah) for his blessing who made me able to complete and perform this study with success. I would like to thank to my supervisor Assist. Prof. Dr. Gülden KOÇAK who does not spare her help and knowledge and gives the necessary support for the completion of my studies during the course of the thesis. I am also very grateful to Assist. Prof. Dr. Murat KÜRŞAT for supplying plant materials and Assist. Prof. Dr. Alpaslan KOÇAK for his support and help.

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I must express my very profound gratitude to my parents and members of my family for providing me with unfailing support and continuous encouragement throughout my years of study and through the process of researching and writing this thesis. Finally, I am thankful to my colleagues and all others friends for their help and encouragement.

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ÖZET

Ballıbabagiller (Lamiaceae) çok yıllık bitkilerden olup 236 cins ve 7173 tür ile dünyada en geniş altıncı familyayı temsil eder. *Lallemantia* Fisch. & Mey. cinsi Lamiaceae familyasına ait olup dünyada *Lallemantia baldshuanica*, *Lallemantia canescens*, *Lallemantia iberica*, *Lallemantia peltata* ve *Lallemantia royleana* olmak üzere beş türü vardır. Bunlardan sadece *Lallemantia canescens*, *Lallemantia iberica* ve *Lallemantia peltata* Türkiye'de doğal yayılış göstermektedir.

Lallemantia türlerine ait moleküler sekans bilgilerine dayanan araştırmalar olsa da daha önce filogenetik ilişkilerine yönelik çalışmalara rastlanmamıştır. Bu çalışma ile Türkiye'de yayılış gösteren *Lallemantia* cinsine ait tüm türlerin nrDNA ITS ve cpDNA *trnT-F* bölgelerinin çoğaltılması ve analizleri amaçlanmıştır. *Lallemantia* türlerine ait DNA dizilemeleri sonuçları filogenetik ilişkileri ortaya koymak üzere MEGA 6.0 programı kullanılarak Maksimum Parsinomi Metodu ile incelenmiş ve filogenetik ağaçlar oluşturulmuştur. Sonuçlar Flora of Turkey'deki cins dağılımına uyumluluk gösterse de *Lallemantia* tür ayrımı ile uyuşmamaktadır. ITS bölgesine göre oluşturulan filogenetik ağaç, *L. peltata* ve *L. iberica*'nın, *trnL* (UAA) intron ve *trnL-F* bölgelerine göre oluşturulan ağaçlar ise *L. peltata* ile *L. canescens*'in yakın akrabalık içerdiğini göstermiştir. Sonuç olarak, nrDNA ile cpDNA sonuçları hem birbirini hem de morfolojik verileri desteklememektedir.

Anahtar Kelimeler: *Lallemantia*, nrDNA, cpDNA, ITS, *trnT-F*.

MOLECULAR PHYLOGENY OF THE GENUS *LALLEMANTIA* Fisch. & Mey. (LAMIACEAE) IN TURKEY

ABSTRACT

Lamiaceae the sixth largest angiosperm family contains 236 genera and approximately 7173 species. *Lallemantia* Fisch. & Mey. is one of the genus of Lamiaceae family in the world. It is composed of five species in the world; *Lallemantia baldshuanica*, *Lallemantia canescens*, *Lallemantia iberica*, *Lallemantia peltata* and *Lallemantia royleana*. From these *Lallemantia canescens*, *Lallemantia iberica* and *Lallemantia peltata* are naturally grown in Turkey.

There are some investigations on the molecular sequences of *Lallemantia*, however, phylogenetic relationship based on these sequences have not been done yet. By this study it is aimed to amplify the nrDNA ITS and cpDNA *trnT-F* region of *Lallemantia* species grown in Turkey. The DNA sequences were analyzed by MEGA 6.0 program and phylogenetic trees were constructed by Maximum Parsinomy Method. Results were congruent in the means of Flora of Turkey genus discrimination but they were not parallel to *Lallemantia* species separation. According to the phylogenetic tree constructed by the ITS region sequences *L. peltata* and *L. iberica*; according to the phylogenetic trees constructed by the *trnL* intron and *trnL-F* regions sequences *L. canescens* and *L. peltata* showed closer relationships. Thus not only the phylogenetic relationships of *Lallemantia* species are incompatible with discrimination of this genus but nrDNA and cpDNA phylogenetic trees are also incompatible with each other.

Keywords: *Lallemantia*, nrDNA, cpDNA, ITS, *trnT-F*.

1. INTRODUCTION

The Flora of Turkey contains 167 plant family, 1320 genera and 11.707 generic taxa and one third of this flora consists of aromatic and medicinal plants (Davis et al. 1988; Guner et al. 2001; Baser 2002). Flowering plants (angiosperms) are the largest and most diverse group in the plant kingdom (Borch et al. 2003). In Flora of Turkey Lamiaceae family includes 45 genera and 735 taxa (Davis 1978). Lamiaceae family members show worldwide distribution and the real area of habitation is Mediterranean basin but unlikely at high latitude or altitude (Heywood et al. 1996; Harley et al. 2004). Family is composed of annual, biennial or perennial aromatic or non-aromatic herbs, and includes subshrubs, shrubs and trees (Harley et al. 2004). Family members are widespread over Asia, Middle East and Europe and flowers stage from April to June (Ursu and Borcean 2012). Lamiaceae the sixth largest angiosperm family contains 236 genera and approximately 7173 species many of which are aromatic and medicinal in world (Harley et al. 2004; Dinc et al. 2009; Li et al. 2016; Jamzad 2012). The species of *Mentha* (perppermint), *Salvia* (sage), *Origanum* (oregano), *Thymus* (tyme) and *Rosmarinus* (rosemary) have usage in culinary purposes because of their essential oils (Harley et al. 2004). Among these Lamiaceae plant family is an important gene depository in Turkey (Kocabas and Karaman 2001).

Lallemantia Fisch. & Mey. is one of the genus of Lamiaceae family (Sharifi-Rad et al. 2014). The genus *Lallemantia* including herbaceous annual and biennial plants is characterized by simple leaves; a thyrsoid, spike-like or oblong, often interrupted inflorescence; ovate to rotund or sometimes linear, aristate-toothed bracteoles; and oblong, trigonous, smooth and mucilaginous nutlets (Harley et al. 2004). The genera *Lallemantia* is originated from Caucasian distributed from Turkey to Asia and also cultivated in Europe (Cao 1994; Harley et al. 2004; Govaerts et al. 2010). It is composed of five species in the world; *Lallemantia baldshuanica, Lallemantia canescens, Lallemantia iberica, Lallemantia peltata and Lallemantia royleana.*

From these *Lallemantia canescens, Lallemantia iberica* and *Lallemantia peltata* are naturally grown in Turkey (Kew 2009). These taxa have importance in economical and medicinal fields (Dinc et al. 2009).

Molecular phylogenetic studies have been frequently used to resolve generic delimitation and infrageneric classifications in many groups of plants (Watson et al. 2000; Masuda et al. 2009; Sonboli et al. 2011; Sonboli et al. 2012). The use of the ITS region in plant molecular systematics has been reviewed by Baldwin *et al.* (1995). The ITS region is now a widely used data source in molecular systematic studies of plants at lower taxonomic levels for three principal reasons.

First, the high copy number allows easy amplification of the region from total DNA. Second, the spacer sequences evolve rapidly and can therefore resolve lower level relationships better than slowly evolving genes, such as 18S and *rbc*L (Baldwin 1992; Baldwin et al. 1995; Baker et al. 1999). Third, the availability of several sets of universal (or near so) PCR primers working with a large diversity of taxonomic groups (White et al. 1990; Gardes and Bruns 1993). Besides the nrDNA, chloroplast DNA (cpDNA) sequence variations are widely used to investigate interspecific relationships among angiosperms and other plants (Taberlet et al. 1991). In chloroplast genome the *trnT-trnF* region is located in the large single-copy region, approximately 8 kb downstream of *rbcL*.

Three highly conserved transfer RNA genes [tRNA genes for threonine (UGU), leucine (UAA) and phenylalanine (GAA)] are found in tandem, separated by spacers of several hundred base pairs (bp). The high variability of the two spacers and the intron in *trnL* have led to the wide use of *trnT-trnF* sequences in addressing relationships at the species and genus levels (Borsch et al. 2003). Moreover, the region was quite informative in phylogenetic studies of families like Lamiaceae (Bendiksby et al. 2014).

1.1. General Characteristics of Lamiaceae

Description of Lamiaceae Family in Flora of Turkey (Davis 1982); Herbs or shrubs, usually glandular and aromatic; stems 4-angled or not. Leaves exstipulate, simple, sometimes pinnate, always opposite. Inflorescence basically of cymes borne in the axils of bracts or upper leaves and usually contracted to form false whorls (verticillasters); the latter may also be arranged to form 'spikes', heads, racemes or cymes. Flowers hermaphrodite, or male-sterile (functionally female) in gynodioecious plants. Bracts clearly different from leaves, or similar to them; bracteoles present or not. Calyx usually 5-lobed with an upper 3-toothed and lower 2-toothed part, rarely lobes or teeth 1 and 1 and 4, or calyx actinomorphic; veins 5-20. Corolla gamopetalous, zygomorphic and bilabiate with usually indistinctly 2-lobed upper lip (hood or galea), falcate, straight or ± concave, and 3-lobed lower lip (labellum); rarely upper lip reduced and lower lip 5-lobed, or with 1 upper and 4 lower lobes, or corolla actinomorphic. Stamens adnate to corolla, 4 and didynamous, or 2 (and staminodes usually present); posterior (upper) pair usually shorter than anterior (lower) pair; anther thecae 2- or 1-celled, parallel or divergent, rarely (in *Salvia*) separated by elongated connectives. Ovary superior, 2-carpellate and 4-ovulate, 4-lobed. Style gynobasic, rarely not, shortly bifid above. Fruit of four (rarely fewer) dry (very rarely fleshy) nutlets, mucilaginous on wetting (myxospermic) or not.

1.2. *Lallemantia* Fisch. & Mey.

Description of *Lallemantia* Genus in Flora of Turkey (Davis 1982); Annual and perennial herbs. Verticillaters subtended by floral leaves, forming an elongate oblong inflorescence; bracteoles prominently veined, aristatedentate. Calyx tubular, 15-veined, weakly 2-lipped, upper lip 3-dentate, middle lobe broader than lateral, sinuses with a thickened fold; lower lip 2-dentate, teeth lanceolate. Corolla 2-lipped; tube narrow, gradually widening from base; upper lip slightly galeate, 2-lobed, with 2 longitudinal folds within; lower lip 3-lobed, declinate, with broadly reniform middle lobe. Stamens 4; filaments arising from near base of tube; anthers with divergent thecae. Style with 2 unequal lobes. Nutlets oblong, 3-angled, smooth or finely punctate.

1. Bracteoles orbicular	**1. peltata**
1. Bracteoles distinctly longer than broad	
2. Annual; corolla 11-18 mm	**2. iberica**
2. Perennial; corolla 28-40 mm	**3. canescens**

1.2.1. *L. peltata* (L.) Fisch. & Mey.

Annual; stem erect, simple or branched, 15-40 cm. Lower leaves ovate or oblong, 40-55 x 7-12 mm, serrate, petiolate; upper leaves ± lanceolate to linear, 15-50 x 3-10 mm, weakly serrate to entire, subsessile. Bracteoles 7-10 x 6-9 mm (excl. awns), orbicular, ± truncate at base, reticulate-nerved beneath, ciliate-dentate, Calyx ± cylindrical, divided to less than 1/3, uppermost tooth obovate, lateral oblong; lower teeth lanceolate. Corolla violet-blue to pale bluish, less commonly white, 14-18 mm, tube ± equaling calyx. Fl. 5-7. Fallow fields, roadside, eroding slopes, in ravines, 1250-2500 m (Davis 1982).

1.2.2. *L. iberica* (Bieb.) Fisch. & Mey.

Similar to *L. peltata* but lowest leaves ovate, lamina to 18 x 10 mm, crenate; bracteoles 6-10 x 2-4 mm, ovate, cunate at base, teeth usually with very long capillate awns; corolla violet-blue (sometimes with white lower lip), pale blue or white, 11-15(-18) mm. Fl. 4-6. Roadside, slopes, fallow fields, weed of cultivated land, 500-2150 m (Davis 1982).

1.2.3. *L. canescens* (L.) Fisch. & Mey.

Perannial, ± greyish-canescent with very fine hairs, usually with numerous ascending to erect stems arising from a branched woody base, 20-50 cm tall. Lower leaves oblong-elliptic, 20-60 x 7-12 mm, long-petiolate, lamina crenate-dentate to subpinnatifid; upper leaves linear-lanceolate, to 80 x 8 mm, shortly petiolate to sessile. Bracteoles 8-11 x 2.5-3.5 mm (excl. awns), ciliate-dentate. Calyx ±cylindrical, divided to ¼, upper tooth ovate, lateral triangular; lower teeth lanceolate. Corolla violet, dark violet-blue, blue-purple, lilac or lavender-blue, 28-40 mm, tube distinctly exserted from calyx. Fl. 6-8. Fallow fields, hillsides, roadside banks, rocky igneous and limestone slopes & scree, 1300-3200 m (Davis 1982).

1.3. Molecular Systematics of Plants

Molecular systematics (phylogenetics) is the evolutionary history of organisms and it exhibits the relationships among related taxa as species, genera, family or higher groupings by using the structure and function of molecule (Yang and Rannala 2012). Molecular systematic analysis depends on the determination of changes in DNA sequences derived from nuclear or cytoplasm (mitochondria and chloroplast) and/or amino acid sequences data (Nei and Kumar 2000). Molecular systematics use different techniques to derive phylogenetic trees which are used to show the evolutionary history of related taxa depends on their molecular characteristics (Lio and Goldman 1998; Brown 2002).

1.3.1. DNA Sequences Used in Molecular Systematic

Different kinds of molecular data can be used in molecular systematics to investigate the evolutionary relationships of genes and organisms.

1.3.1.1. Nuclear DNA Sequences

Plant nuclear genome is organized into discrete chromosomes consist of DNA and associated proteins. The number of chromosomes and size of the plant genome show alteration among species with 2350-fold range from 63 to 149.000 Mbp (Heslop-Harrison and Schwarzacher 2011). The most important reasons of this type of diversity are based on a heritable condition named as polyploidy which possessing more than multiple copies of complete sets of chromosomes and in their origins more than 50% of angiosperms are polyploid (Heslop-Harrison and Schmidt 2007). The other reasons are mutations as duplications, deletions, and gene flow (Gören 2011) and amount of repetitive DNA in the genome (Harrison and Schmidt 2007). Plant nuclear genome is composed of genes (exons and introns) repetitive DNA sequences, regulatory elements and other low copy number sequences (Figure 1.1) (Harrison and Schmidt 2007).

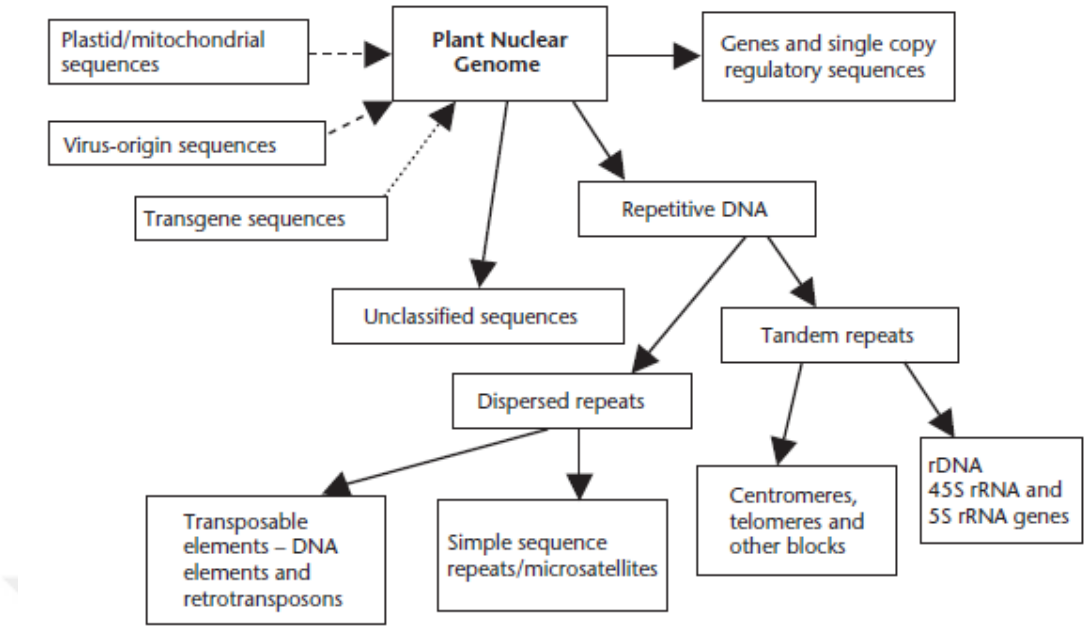

Figure 1.1. Plant genome components (Harrison and Schmidt 2007)

Generally evolution rate of nuclear DNA (nrDNA) is considered that slower than the cytoplasmic source of DNA except plant derived nrDNA, it is the fastest evolving among the three genomes that they contain (Brown et al. 1979, 1982; Wolfe et al. 1987). Higher rate of evolution is concluded by more variation and greater efficiency of sequencing effort (Small et al. 2004). This case introduces some advantages for using nrDNA in phylogenetic studies especially of low taxonomic levels (Small et al. 1998). Generally preferred nrDNA molecular data come from nuclear ribosomal DNA (rDNA) (Alvarez and Wendel 2003). In eukaryotes the rDNA is organized as tandem head to tail repeats. Each repeated units composed of a transcribed region consisting of 18S, 5.8S, 26S genes and an intergenic spacer (IGR) consisting of a non-transcribed spacer (NTS) and external transcribed spacers (ETS) (Alonso et al. 2014). Ribosomal RNAs are first transcribed as preRNA containing 5′ and 3′ETS and ITS-1 and ITS-2 sequences (Figure 1.2) (Tollervey and Kiss 1997).

In addition to conserved coding regions of plant genes some highly variable regions as the internal transcribed spacers (ITS-1 and ITS-2) of the 18S-5.8S-26S nuclear ribosomal cistron (Figure 1.2) become to dominate plant molecular phylogenetic studies comparing of closely related genera and species (Soltis et al. 1998; Alvarez and Wendel 2003).

Available data show that using of ITS sequences is convenient in the phylogenetic studies of angiosperms. ITS-1 and ITS-2 sequences are G+C rich and these parts are rather conserved among angiosperms (Hershkovitz and Zimmer 1996; Hershkovitz and Lewis 1996). According to Hershkovitz and Zimmer (1996) in all angiosperms 40% of the ITS-2 conserved and in angiosperms above the family level, 50% of the ITS-2 is alignable. ITS-1 and ITS-2 sequences are 300 bp, 5.8 gene sequences is 163-164 bp and so amplification of ITS sequences give 500-700 bp PCR products in angiosperms (Baldwin 1992; Baldwin et al. 1995). This type of a small size of the target DNA fragment increase efficiency during PCR (Alvarez and Wendel 2003). There are a set of universal primers that can be used for amplifying the ITS sequences from most plants (White et al. 1990). There are generally used two sets of primers are chosen for PCR studies. First set is AB101 (forward primer) compatible with 18S gene and AB102 (reverse primer) compatible with 26S gene (Douzery et al. 1999). Second set is ITS5 (forward primer) and ITS4 (reverse primer) (White et al. 1990).

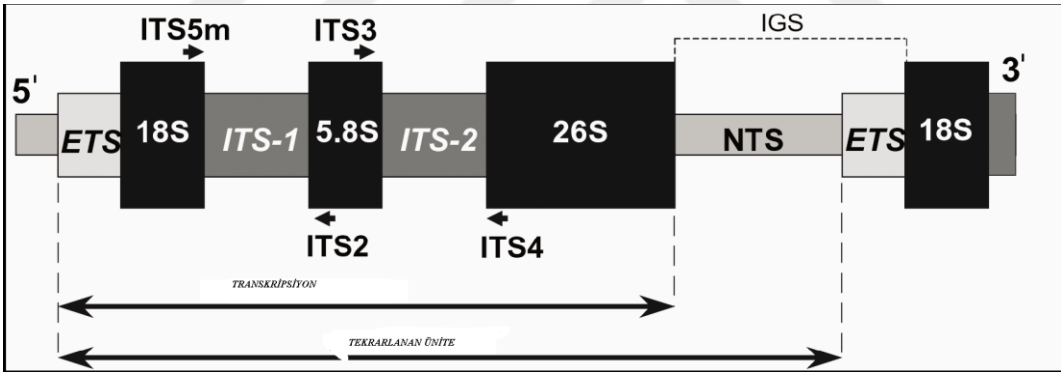

Figure1.2. Schematic representative of ITS-1 and ITS-2 regions (Saar et al. 2001)

1.3.1.2. Chloroplast DNA Sequences

Chloroplast genome of land plants is a small circular molecule ranging from 107 kb (*Cathaya argyrophylla*) to 218 kb (*Pelargonium*), composed of 120-130 genes, taking part in photosynthesis, transcription and translation. In each chloroplast there is more than one copy of genome. They contain their own double stranded DNA characterized by two inverted repeat segments (IR), one contain large (LSC) one small single-copy region (SSC) (Figure 1.3) (Soltis et al. 1998; Daniel et al. 2016).

Functionally chloroplast genome can be divided into three groups; non-coding regions, protein coding regions and introns (Clegg et al. 1994). Recent studies have showed that non-coding intergenic region which often include regulatory sequences indicate significant diversity (Daniel et al. 2006). Generally genes and introns of land plant genomes are conserved. Uncommonly in several plant species loss of introns have been reported (Jansen et al. 2007; Daniel et al. 2016). Also in chloroplast genomes of certain lineages land-plant structural rearrangement as loss of IR regions or entire gene families has been demonstrated. Also some studies demonstrate the presence of linear chloroplast genomes (Oldenburg and Bendrich 2004a; 2004b).

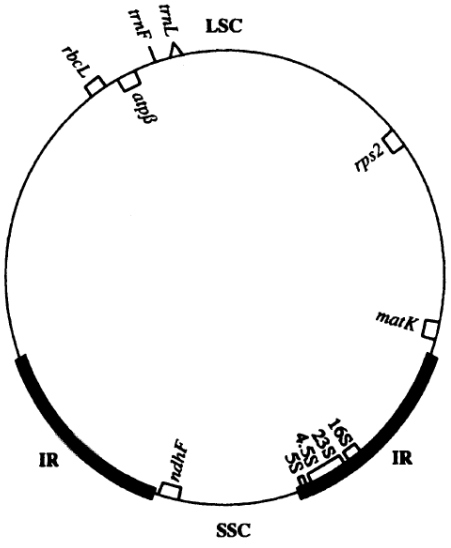

Figure 1.3. Diagram of chloroplast genome map (representative of most land plants) (Soltis et al. 1998)

Chloroplast DNA (cpDNA) sequences are widely used in phylogenetic studies especially in analyzing the interspecific relationship among angiosperms by some reasons (Taberlet et al. 1991). cpDNA Despite the nrDNA sequences, cpDNA sequences evolve slowly. This situation brings along serious limitation to uses of this molecule in intraspecific and population level. However, chloroplast genome size is small enough to examine the complete genome to undercover the relationships between the closely related taxa by some DNA analysis methods as restriction site analysis (Soltis et al. 1998; Daniel et al. 2016). cpDNA is structurally stable, inherited uniperantally in angiosperms, haploid and

thus non-recombinant except some examples and this features reduce the intraspecific variation (Small et al. 2004).

In molecular systematics ribulose bisphosphate carboxylase/oxygenase (RUBISCO) gene large fragment, *rbcL* sequences, NADH dehydrogenase subunit 5, *ndh*F sequences located between the SSC and IR regions and non-coding chloroplast sequences as tRNA genes intergenic spacer *trnL-F* regions are preferred generally (Baldwin 1992; Douzery et al. 1999; Bell et al. 2001; Alverez and Wendel 2003; Potter et al. 2007; Guo et al. 2011).

Chloroplast genome non coding sequences including the *trnL* (UAA) intron and the intergenic spacer *trnL* (UAA)-*trnF* (GAA) (Figure 1.4) have phylogenetic capacity to reveal the phylogeny and evolutionary relationship of intra-species to inter-family level (Xu and Ban 2004; Liu et al. 2006; Tsai et al. 2006).

Non-coding sequences have similar rates of evolution to that of some coding regions or faster than them. These regions length is small, they are usually shorter than 700 bp, *trnL* intron length approximately 350-600 bp and *trnL-F* spacer length approximately 120-350 bp depending on study group. This feature is an advantage to researcher to amplify and sequence of these regions (Soltis et al. 1998; Tsai et al. 2006).

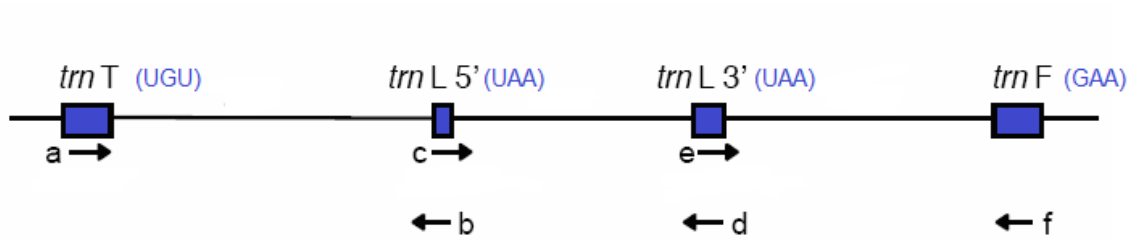

Figure 1.4. tRNA genes, intergenic noncoding chloroplast sequences and universal primers used to amplify these regions (Taberlet et al. 1991)

Figure 1.5 shows the uses of different molecular data come from nuclear, chloroplast or mitochondrial genome in taxonomic level.

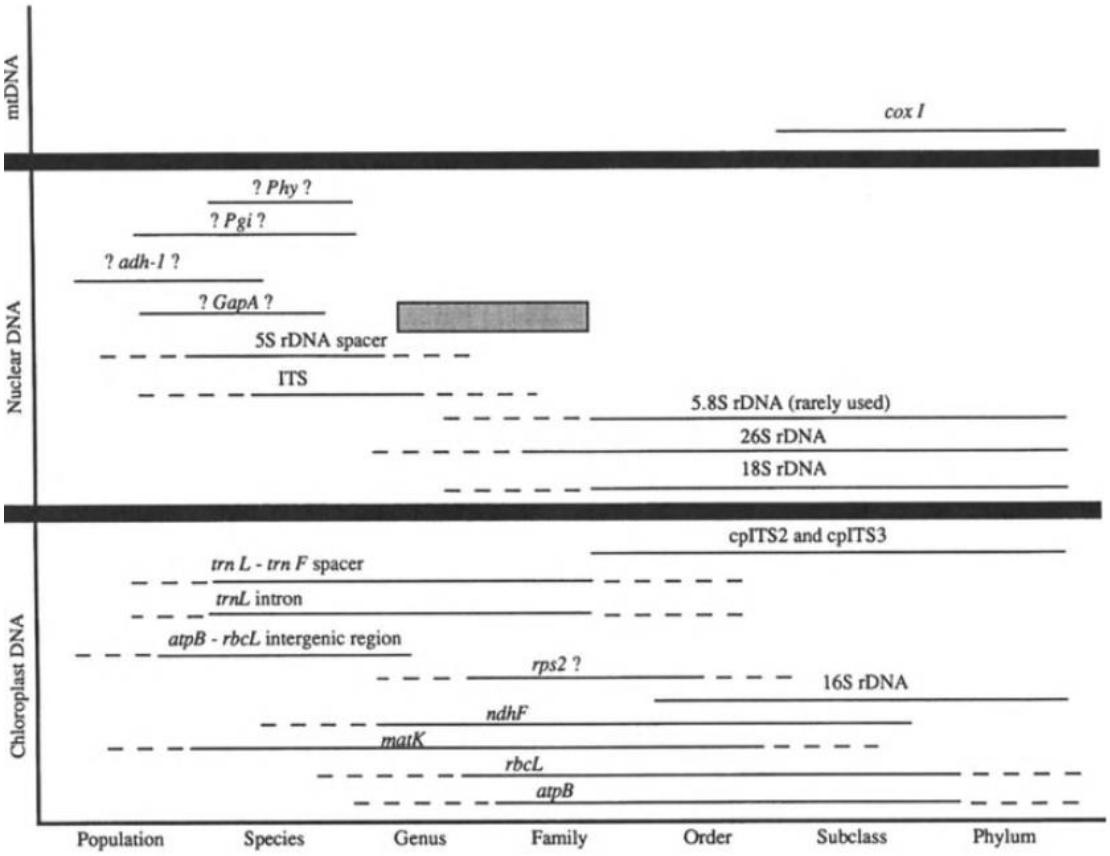

Figure 1.5. Taxonomic level of utility of Angiosperm chloroplast mitochondria, and nuclear DNA (Soltis and Soltis 1996)

1.3.2. DNA Sequencing

DNA sequencing is process of determining the order of nucleotides bases adenine, guanine, cytosine and thymine found in a strand of DNA. Nowadays DNA sequencing are common in biotechnology, biological systematics, medical diagnosis, virology and gene engineering.

Firstly, in 1973 Maxam and Gilbert sequenced 24 base pair by spot analysis and in 1975 Sanger Sequencing or namely chain termination method was developed by Fred Sanger and coworkers (Sanger and Coulson 1975). Today more easily applicable and automated methods are available (Bisht and Panda 2013).

1.3.2.1. Automated DNA Sequencing

Sanger sequencing (Sanger and Coulson 1975) creates the basic of the automated sequencing. The only difference in dye-terminator sequencing four dideoxynucleotide labelled with four different fluorescent dyes with different wavelengths other than radioactive isotopes. Automated sequencing provide faster and long chain sequencing and up to 384 DNA samples in a single run with using capillary electrophoresis. Automated system maintains separation, detection and recording of order of the nucleotide in the sample as fluorescent peak trace chromatograms (Munshi 2012).

1.3.3. Multiple Sequence Alignment and ClustalW

Multiple sequence alignment is the important tool to molecular modeling, database searching, and phylogenetic tree creation. Basically multiple sequence alignment is an alignment of 3 or more nucleotide or protein sequences. It gives more information than pair-wise alignment. One of the widely used multiple sequence alignment programs are Clustal series of programs which was firstly written by Des Higgins in 1988 and improved many times (Higgins and Sharp 1988). In the past versions UPGMA was used but now Neighbor-Joining (NJ) methods have been used to calculate the best match for the sample sequences, align them and find out similarities and differences among the sequences. ClustalW perform multiple sequence alignments with divergent DNA or protein sequences and produces biologically meaningful comparison. (Larkin et al. 2007). By phylograms evolutionary relationships can be seen.

1.3.4. Phylogenetic Analysis

Phylogeny purposes to reconstitute the history and relationship of taxonomic group of organisms according to their grade of similarity (Dereeper et al. 2008). One special type of phylogeny is the phylogenetic that compares the sequence homology of genes from several species, generates the genes trees and computes the historically distances by various computational methods (Paradis et al. 2004).

The statistical and bioinformatics outcomes are used in phylogenetic studies to construction of phylogenetic tree which is a dendrogram resembles the structure of tree illustrates proximity of different genes, species or organisms sharing common an ancestor (Baum 2008). By phylogenetic trees, relatively closed organisms, function and origin of a gene can be identified. In phylogenetic trees there are nodes and branches. Two adjacent nodes connect together by a branch. External and internal nodes represent extant taxa and hypothetical progenitors of operational taxonomic units known as last common ancestor respectively. Cluster emphasizes a group of taxa sharing a monophyletic origin. To build phylogenetic trees from molecular data different methods can be used. The most common methods are group as distance-based methods such as UPGMA method, Neighbor-joining method and character based methods such as Maximum Parsinomy, Maximum likelihood and Bayesian inference (Brown 2002; Lemey et al. 2009).

The aim of this study is to be the first report to display the systematic position of three *Lallemantia* species in Turkey. In this study we used the molecular data from the nuclear ITS region and we further included sequence information from the chloroplast non-coding regions (*trnT-trnF*) to provide a more comprehensive taxonomic and phylogenetic results and a more stable classification with using closely related outgroups.

2. LITERATURE REVIEW

Until today many studies about the molecular systematics of different genus of Lamiaceae have been realized. Among these studies a large scale chloroplast phylogeny of the Lamiaceae is remarkable in the means of shedding new lights on its subfamilial classification (Li et al. 2016). The other studies generally mention about in genus level relationships (Drew and Sytsma 2012; Chen et al. 2016; Roy et al. 2016; Bariotakis et al. 2016). Some other studies are also advert about molecular markers which can be used for phylogenetic studies of members of Lamiaceae family (Moja et al. 2016).

Generally in molecular phylogenetic studies the internal transcribed spacers (ITS) of the nuclear ribosomal DNA repeat (nrDNA) which are two regions of noncoding and relatively rapidly evolving DNA sequence that flank the very slowly evolving 5.8S ribosomal RNA gene are usually preferred. The region comprising the ITS and 5.8S gene has been used extensively for phylogenetic inference among relatively closely related species (Gonzalez et al. 1990; Lee and Taylor 1992; Baldwin 1992, 1993; Suh et al. 1993; Wojciechowski et al. 1993; Baldwin et al. 1995; Yuan et al. 1996). In addition to the nrDNA the use of chloroplast DNA (cpDNA) restriction site analysis and nucleotide sequence data have been used in the recognition and recircumscription of the Lamiaceae (Trusty et al. 2004).

There are also different publications in literature about *Lallemantia* which are about micromorphological analysis (Dinc et al. 2009), phytochemistry, antimicrobial activity (Dehaghi et al. 2016), antifungal activity (Hosseini and Shahidi 2016; Waller et al. 2017) and in vitro callus induction (Razavi et al. 2017) of *Lallemantia* taxa. The only one study is found in the literature Masoud *et al.* (2016) that mentioned about the population genetics, molecular phylogeny and biogeography of the genus *Lallemantia*. They studied molecular phylogenetic with inter-simple sequence repeat (ISSR) markers and inter-genic spacer of chloroplast genome rpl16. However they did not use nrDNA

ITS regions and cpDNA *trnT-trnF* sequences data for identification of relationship of species. So this study will provide a different perspective for the molecular phylogeny and relationship of the *Lallemantia*.

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Plant Materials

Plant material was obtained from silica-gel dried leaved of collected specimens in the wild. . *Lallemantia canescens* was collected from natural habitats in Nemrut Crater Lake, Bitlis; *Lallemantia peltata* was collected from Bitlis Eren University Campus; *Lallemantia iberica* was collected from Doğancık Village Baskil Elazığ. All specimens were collected in 2015. The other plant materials used in this study as outgroup were collected from different places previously and handled in different studies.

3.1.2. Glass and Plastics Materials, Chemicals, Enzymes and Kits

All of the glass and plastic materials as pipet tips, microcentrifuge and PCR tubes and other heat resistant materials were sterilized by using autoclave for 20 min. at 121°C before starting study. Chemicals, enzymes and kits and their suppliers are listed and given in Table 3.1.

Table 3.1. List of chemical and enzymes used and their suppliers

Chemical or Enzyme	Supplier
Agarose	Sigma Aldrich
Chloroform	Chemsolute
CTAB	Acros Organics
DNA Isolation Kit	Macherey-Nagel
EDTA	Bioshop
Ethanol	Merck
Ethidium Bromide	Vivantis
Glacial acetic acid	Fisher
HCl	Sigma-Aldrich

Table 3.1. (Continue) List of chemical and enzymes used and their suppliers

Chemical or Enzyme	Supplier
Isoamylalcohol	Fisher
6X loading buffer	ThermoScientific
2. mercaptoethanol	Acros Organics
Molecular size marker	Solis Biodye
NaCl	Sigma-Aldrich
NaOH	Sigma-Aldrich
Phenol:Chloroform:Isoamylalcohol	Acros Organics
Taq Polymerase	BioLabs
Tris	BioShop

3.1.3. Buffers and Solutions

3.1.3.1. Agarose Gel Preparation

In order to visualize DNA samples and PCR products 0.8% (w/v) and 1.2% (w/v) agarose gel were prepared. For these purpose 0.8 g or 1.2 g agarose and 2.0 mL 50X TAE buffer were added and dissolved in 100 mL distilled water and homogenized in microwave.

3.1.3.2. EDTA (0.5 M, pH 8.0)

For preparation of EDTA (ethylenediaminetetra acetic acid di-sodium salt) (0.5M and pH 8.0) 186.1 g of EDTA was weighed and added to 800 mL of distilled water. The pH was adjusted to 8.0 with NaOH and sterilized by autoclaving.

3.1.3.3. 50 X TAE Buffer

242 g of Tris base was dissolved in 600 mL distilled water and the pH was adjusted to 8.0 with 57.1 mL glacial acetic acid. After that 100 mL 0.5 M EDTA (pH 8.0) was added and the volume was adjusted to 1 liter. TAE buffer was diluted to 1X before use.

3.1.3.4. CTAB Buffer

2.0 g CTAB (hexadecyl trimethyl-amonium bromide), 10.0 mL 1 M Tris (pH 8.0), 4.0 mL 0.5 M EDTA (pH 8.0), 28.0 mL 5 M NaCl, 40.0 mL ddH_2O were added and pH was adjusted to pH 5.0 with HCl and made up to 100 mL with ddH_2O.

3.1.3.5. Tris (1.0 M, pH 8.0)

121. g Tris base was dissolved in 800 mL of H_2O. The pH was adjusted to 8.0 by adding 42 mL of HCl. Volume was adjusted to 1 L with ddH_2O.

3.1.3.6. 1X TE Buffer

10 mL Tris (1 M) and 2 mL EDTA (0.5 M, pH 8.0) were added to 988 mL ddH_2O.

3.1.4. Molecular Size Markers

100 bp DNA ladder was used for DNA fragment size determination shown in Figure 3.1. This ladder contains 13 DNA fragments and their sizes are ranging from 100 bp to 3,000 bp.

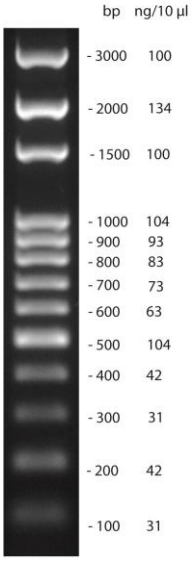

Figure 3.1. Molecular size marker

3.2. Methods

3.2.1. Total DNA Isolation from Plant Materials

Total genomic DNA isolation of the plant samples collected and sheltered in the silica gel were done by modified CTAB protocol (Doyle and Doyle 1987) or Nucleospin Plant Kit (Macherey-Nagel, Düren-Germany).

3.2.1.1. CTAB Protocol

Total genomic DNA was extracted by modified protocol of the cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle 1987) as mentioned below;

- 20 mg of plant tissue in silica gel was grinded and homogenized to a fine paste with liquid nitrogen using sterilized mortal and pastel.
- 1.5 mL CTAB was added and extract mixture was transferred to a 1.5 mL microcentrifuge tubes, mixed with 20 μL β-mercaptoethanol and vortexed. CTAB/plant extract mixture was incubated for about 30 min. at 65°C in a water bath and vortexed every 10 min.
- After incubation CTAB/plant extract mixture was centrifuged at 14,000 rpm for 15 min. Supernatant was transferred to clean 1.5 mL microcentrifuge tube and 0.8V Phenol:Chloroform:Isoamylalcohol (25:24:1) was added and centrifuged at 14,000 rpm for 12 min.
- Supernatant was transferred to clean 1.5 mL microcentrifuge tube, 0.8V Chloroform:Isoamylalcohol (24:1) was added and centrifuged at 14,000 rpm for 10 min.
- Supernatant was taken and 0.7V Isopropanol was added and mixed.
- Samples were incubated overnight at -20°C for precipitation of DNA.
- Pellets were washed with 70% cold ethanol and DNA pellets were air dried at room temperature and re-dissolved in 50 μL TE buffer.

3.2.1.2. DNA Isolation with NucleoSpin Kit

Total genomic DNA isolation was done by the direction of the kit procedure as mentioned below;

- 20 mg dry weight plant material was homogenized with liquid nitrogen using mortal and pastel.
- Powder was transferred to a new tube and 400 µL Buffer PL1 was added and mixture was vortexed thoroughly. 10 µL RNase A solution was added and mixed. The suspension was incubated for 30 min at 65°C in a water bath.
- NucleoSpin® Filter with violet ring was placed into a 2 mL collection tube and lysate was loaded onto the column and centrifuged for 2 min. at 14,000 rpm. Filter was discarded and flow-through was collected.
- 450 mL PC Buffer was added onto the flow-through and mixed by pipetting.
- NucleoSpin® Column with green ring was placed into a new 2 mL collection tube and 700 µL of sample was loaded onto the column and centrifuged for 1 min at 14,000 rpm. After centrifugation flow-through was discarded.
- 400 µL of Buffer PW1 was added to the column, centrifuged for 1 min. at 14,000 rpm and flow-through was discarded.
- 700 µL of Buffer PW2 was added to column, centrifuged for 1 min. at 14,000 rpm and flow-through was discarded.
- 200 µL of Buffer PW2 was added to the column, centrifuged for 2 min. at 14,000 rpm.
- Column was placed into a new 1.5 mL microcentrifuge tube. 50 mL Buffer PE at 65°C was pipetted on to the membrane and incubated 5 min at 65°C and then centrifuged for 1 min at 14,000 rpm to elute the DNA.

3.2.2. DNA Purity and Quantity Determination

To determine the DNA quantity absorbance value was estimated by measuring the absorbance at 260 nm in microplate reader (Molecular Devices, USA) and quantity of DNA was calculated by using the equality below;

dsDNA concentration (ng/µL) = OD_{260} x dilution factor x 50ng/µL

The purity of DNA was estimated by the ratio of absorbance value of 260 nm and 280 nm. A_{260}/A_{280} was calculated and DNA with the ratio of 1.8 was used for PCR.

3.2.3. Agarose Gel Electrophoresis

In order to visualize DNA samples and PCR products 0.8% (w/v) or 1.2% (w/v) agarose gel was prepared respectively. For gel solution preparation 0.8 g or 1.2 g agarose was weighed and added in 100 mL 1X TAE buffer and melted in a microwave until agarose was completely dissolved for approximately 3 min. When it cooled down to 50-55°C, 0.2-0.5 µg/mL ethidium bromide solution was added and mixed. Agarose gel was poured slowly into a gel tray with the well comb in place. For polymerization of the agarose gel it was let to sit for 20-30 min at room temperature. After polymerization of the gel the comb was removed and the tray was placed into the electrophoresis tank. Tank was filled with 1X TAE buffer. DNA samples or PCR products were mixed with 6X loading buffer and loaded into the wells. Molecular weight ladder was loaded into generally the first lane and the last lane of the gel. The gel was run at 5-10V/cm for 30-45 min. The gel was visualized with gel imaging system (Bio-Rad, Canada).

3.2.4. Polymerase Chain Reaction (PCR)

Primer sets that used in this study are universal primers. Whole region of nrDNA ITS region was amplified with ITS AB101 and ITS AB102 primers (Douzery et al. 1999). nrDNA ITS region also amplified with another set of primers namely; ITS4 and ITS5 (White et al. 1990) in some cases. Amplification of the three non-coding regions; *trnT* (UGU)-*trnL* (UAA) 5' exon, *trnL* (UAA) intron and *trnL* (UAA) 3' exon-*trnF* (GAA) were performed using the B48557-A49291; B49317-A49855 and B49873-A50272 primer sets respectively (Taberlet et al. 1991). Primer sequences are listed in Table 3.2.

Table 3.2. Sequences of the universal primers

Primer	Sequence of primer
ITS-AB101 (forward)	ACGAATTCATGGTCCGGTGAAGTGTTCG
ITS-AB102 (reverse)	TAGAATTCCCCGGTTCGCTCGCCGTTAC
ITS-4 (reverse)	TCCTCCGCTTATTGATATGC
ITS-5 (forward)	GGAAGTAAAAGTCGTAACAAGG
B48557 (forward)	CATTACAAATGCGATGCTCT
A49291 (reverse)	TCTACCGATTTCGCCATATC
B49317 (forward)	CGAAATCGGTAGACGCTACG
A49855 (reverse)	GGGGATAGAGGGACTTGAAC
B49873 (forward)	GGTTCAAGTCCCTCTATCCC
A50272 (reverse)	ATTTGAACTGGTGACACGAG

In Table 3.3. it is given the solutions used in PCR reactions. OneTaq 2X master mix standard buffer was preferred. After all solutions were added to PCR tubes, they were mixed by pipetting. Total volume was adjusted to 50 µL with sterile ddH_2O.

Table 3.3. Solutions used in PCR reactions

Solution	Quantity	Concentration
Standard buffer	25 µL	-
Forward primer	1 µL	10 µM
Reverse primer	1 µL	10 µM
Template DNA	3 µL	50 ng/ µL
Nuclease free water	to 50 µL	-

Amplification was performed by PCR Equipment (Sensoquest Labcycler). The PCR condition is shown in Table 3.4.

Table 3.4. PCR procedure and cycles

Step	Temperature	Time	Cycle number
Initial denaturation	95°C	5 min.	1 cycle
Denaturation	95°C	1 min.	
Annealing	60°C	1 min.	35 cycles
Extension	72°C	1 min.	
Last extension	72°C	6 min.	1 cycle

3.2.5. DNA Sequencing and Sequence Analysis

Amplified nrDNA ITS region and *trnL* and *trnL-F* region PCR products were sequenced by MedSanTek (İstanbul) using Applied Biosystems 3500 xL Genetic Analyzer. Sequences were aligned by using ClustalW (Thompson et al. 1994) software and checked visually.

3.2.6. Phylogenetic Analysis

Samples were analyzed under three data sets. First one composed of nrDNA ITS region, second one composed of the sequences from *trnL* intron region and last one composed of the sequences from region between *trnL-F*. Molecular diversity statistics for each data was analyzed by using Molecular Evolutionary Genetics Analysis software MEGA 6.0 (Tamura et al. 2013) and pylogenetic tree was constructed by Maximum Parsinomy Method.

4. RESULTS AND DISCUSSION

4.1. Experimental Strategies for Molecular Systematic Analysis of Genus *Lallemantia* (Lamiaceae) Grown in Turkey

The experimental strategy for studying molecular phylogeny of the Genus *Lallemantia* (Lamiaceae) grown in Turkey is shown in Figure 4.1.

In this study it was aimed to display the systematic relationship of the *L. canescens*, *L. iberica* and *L. peltata* which are grown in Turkey. For this purpose firstly total DNA isolations were done from dried plant leaves. Then, two sets of universal primers were used to amplify nrDNA ITS regions and three sets of universal primers were used to amplify cpDNA non-coding regions; region between *trnT* (UGU) and *trnL* (UAA) 5' exon; *trnL* (UAA) intron and intergenic spacer between the *trnL* (UAA) 3' exon and *trnF* (GAA). After amplification PCR fragments were sent to DNA sequencing. The data derived from sequenced PCR products were aligned using ClustalW (Thompson et al. 1994) software.

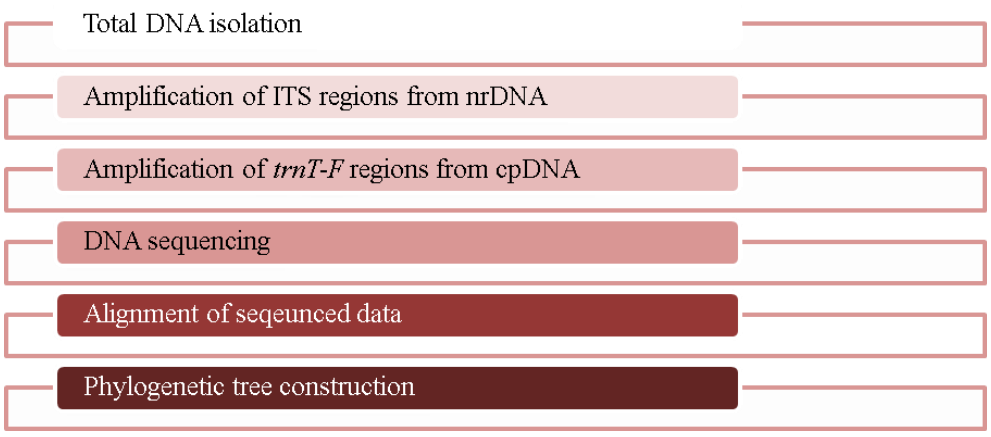

Figure 4.1. Flowchart of the experimental strategies

Variable sites, genetic distances, nucleotide diversity and parsimony-informative sites were computed by using Molecular Evolutionary Genetics Analysis software MEGA 6.0 (Tamura et al. 2013). Ultimately, phylogenetic tree was constructed by Maximum Parsinomy Method.

4.2. Isolation of Total DNA from Plant Samples

Total genomic DNAs were isolated from dried plant leaves (*Lallemantia canescens*, *Lallemantia iberica*, *Lallemantia peltata*, *Stachys iberica* subsp. *iberica*, *Lamium album*, *Nepeta fissa*, *Origanum acutidens*, *Thymus kotschyanus* var. *kotschyanus*, *Stachys kurdica* var. *kurdica*, *Satureja boissieri)* as described in the Materials and Methods Section 3.2.1. DNA samples were visualized on agarose gel electrophoresis as shown in Figure 4.2. Purified DNA samples concentration and purity was measured by spectrophotometer and calculated by formula described in the section 3.2.2.

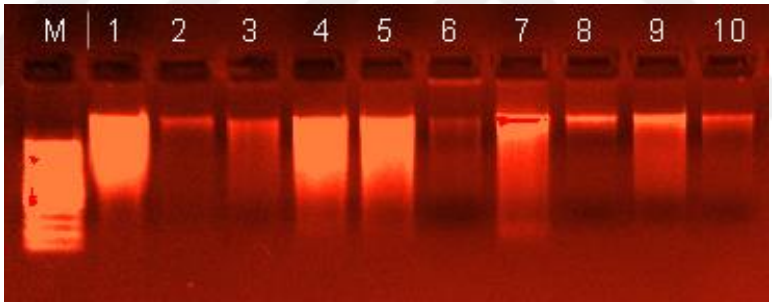

Figure 4.2. Electrophoresis of total genomic DNA isolated with NucleoSpin Kit from dried plant leaves in 1.0% agarose gel. 3 µl of each genomic DNA was electrophoresed. M: Molecular size marker (100 bp DNA ladder); 1. *L. canescens;* 2. *L. iberica*; 3. *L. peltata;* 4. *Stachys iberica* subsp. *iberica;* 5. *Lamium album;* 6. *Nepeta fissa;* 7. *Origanum acutidens;* 8. *Thymus kotschyanus* var. *kotschyanus;* 9. *Stachys kurdica* var, *kurdica*; 10. *Satureja boissieri*

4.3. PCR Amplification

4.3.1. Amplification of nrDNA ITS Regions

ITS1+5.8S rDNA+ITS2 regions of the plant samples were amplified using both AB101 and ITS5 forward primers and AB102 and ITS4 reverse primers. Regions amplified by these primers were illustrated in Figure 4.3. (White et al. 1990; Douzery et al. 1999).

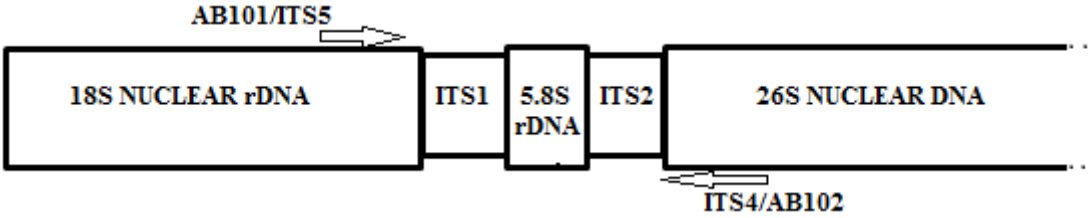

Figure 4.3. The schematic illustration of the amplified region by primers AB101/ITS5 and AB102/ITS4 (Baldwin 1992; Douzery et al. 1999)

To optimize PCR reactions, different annealing temperatures were tested. Amplification with AB101/AB102 primer set yielded PCR products nearly 800 bp and amplification with ITS5/ITS4 primer set yielded PCR products nearly 700-800 bp (Figure 4.4). *Origanum acutidens and Satureja boissieri* could not be amplified by both two sets of primers.

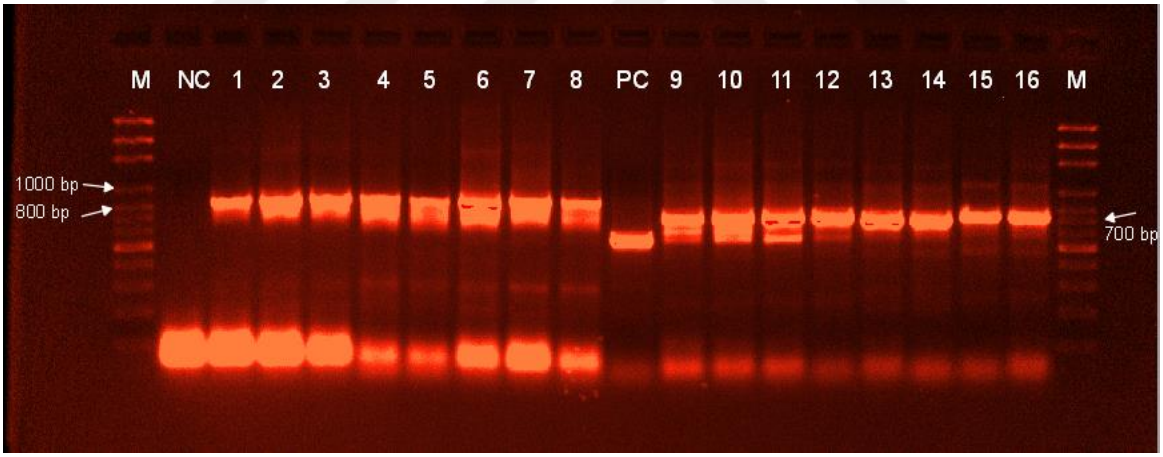

Figure 4.4. Electrophoresis of PCR products amplified with AB101/AB102 and ITS5/ITS4 primer sets in a 1.2% agarose gel. M: Molecular size marker (100 bp DNA ladder) NC: negative control; PC: positive control; lane 1-8 show PCR products amplified using AB101-AB102 and lane 9-16 show PCR products amplified using ITS5/ITS4 1. *L. canescens*; 2. *L. iberica*; 3. *L. peltata*; 4. *Stachys iberica* subsp. *iberica*; 5. *Lamium album*; 6. *Nepeta fissa*; 7. *Thymus kotschyanus* var. *kotschyanus*; 8. *Stachys kurdica var. kurdica*; 9. *L. canescens*; 10. *L. iberica;* 11. *L. peltata*; 12. *Stachys iberica* subsp. *iberica*; 13. *Lamium album*; 14. *Nepeta fissa*; 15. *Thymus kotschyanus* var. *kotschyanus*; 16. *Stachys kurdica* var. *kurdica*

4.3.2. Amplification of cpDNA *trnT-F* Regions

Polymerase chain reaction (PCR) of the three non-coding regions (Figure 4.5); *trnT* (UGU)-*trnL* (UAA) 5' exon, *trnL* (UAA) intron and *trnL* (UAA) 3' exon-*trnF* (GAA) were performed using the B48557-A49291; B49317-A49855 and B49873-A50272 primer sets respectively (Taberlet et al. 1991).

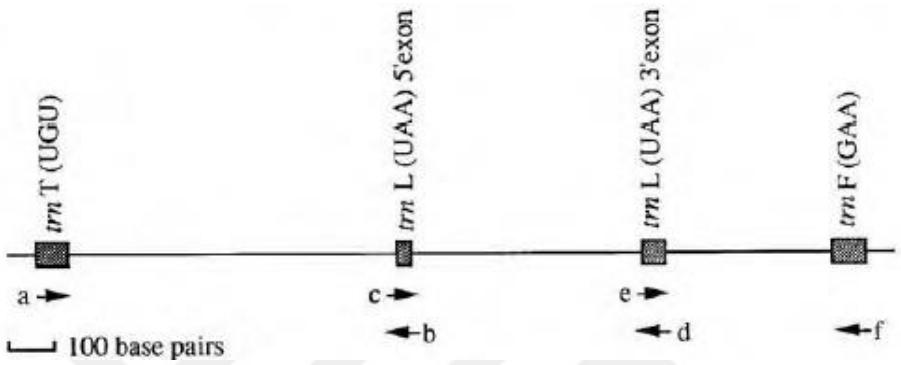

Figure 4.5. The schematic illustration of the amplified region by B48557-A49291. (a-b); B49317-A49855 (c-d) and B49873-A50272 (e-f). and the positions and directions of these universal primers 3' ends of the primers were indicated by tips of arrows (Taberlet et al. 1991)

To optimize PCR reactions, different annealing temperatures were tested. Amplification of regions between *trnT* (UGU) and *trnL* (UAA) 5' exon with primer B48557-A49291 set yielded no PCR with plant samples of *L. canescens, L. iberica, L. peltata,* on the contrary yielded nearly 600-700 bp PCR products with the plant samples *Stachys iberica* subsp. *iberica* and *Lamium album* (Figure 4.6). Therefore regions between *trnT* (UGU) and *trnL* (UAA) were not included in phylogenetic tree construction. Amplification of regions between *trnL* (UAA) 5' exon and *trnL* (UAA) 3' exon with primer B49317-A49855 set yielded products nearly 600 bp PCR products with *L. canescens, L. iberica, L. peltata, Stachys iberica* subsp. *iberica* and *Lamium album* (Figure 4.6). Amplification of regions between *trnL* (UAA) 3' exon and *trnF* (GAA) with primer B49873-A50272 set yielded between 400-500 bp PCR products with plant samples *L. canescens, L. iberica, L. peltata* and 300 bp PCR products with plant sample of *Stachys iberica* subsp. *iberica* and 400-500 bp PCR products with plant sample of *Lamium album* (Figure 4.6).

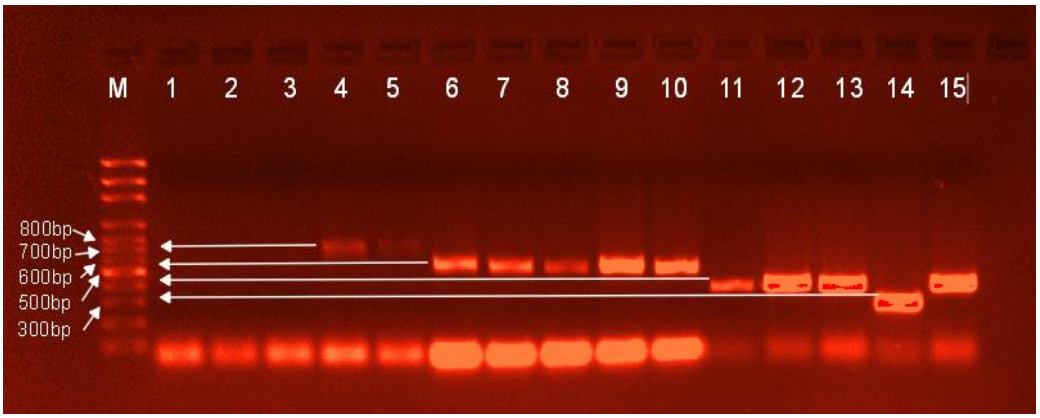

Figure 4.6. Electrophoresis of PCR products amplified with B48557-A49291; B49317-A49855 and B49873-A50272 primer sets in a 1.2% agarose gel. 5 µl of each PCR products was electrophoresed. M: Molecular size marker (100 bp DNA ladder). lane 1-5 show PCR products amplified by using B48557-A49291, lane 6-10 show PCR products amplified by using B49317-A49855 and lane 11-15 show PCR products amplified by using B49873-A50272. 1. *L. canescens;* 2. *L. iberica*; 3. *L. peltata*; 4. *Stachys iberica* subsp. *iberica*; 5. *Lamium album*; 6. *L. canescens*; 7. *L. iberica*; 8. *L. peltata* ; 9. *Stachys iberica* subsp. *iberica*; 10. *Lamium album*; 11. *L. canescens*; 12. *L. iberica*; 13. *L. peltata*; 14. *Stachys iberica* subsp. *iberica*; 15. *Lamium album.*

Amplification of regions between *trnL* (UAA) 5' exon and *trnL* (UAA) 3' exon with primer B49317-A49855 set yielded products nearly 500 bp PCR products with *Nepeta fissa*, and 400 bp with *Thymus kotschyanus* var. *kotschyanus* and *Stachys kurdica* var. *kurdica* (Figure 4.7). Amplification of regions between *trnL* (UAA) 3' exon and *trnF* (GAA) with primer B49873-A50272 set yielded between 400 bp PCR products with plant samples *Nepeta fissa* and 300 bp PCR products with *Thymus kotschyanus* var. *kotschyanus* and *Stachys kurdica var. kurdica* (Figure 4.7).

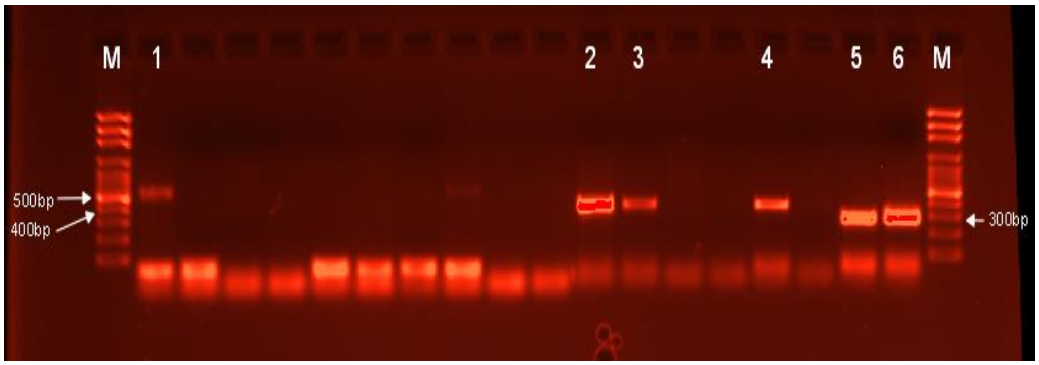

Figure 4.7. Electrophoresis of PCR products amplified with B48557-A49291; B49317-A49855 and B49873-A50272 primer sets in a 1.2% agarose gel. 5 µl of each PCR products was electrophoresed. M: Molecular size marker (100 bp DNA ladder). lane 1, 2 and 3 show PCR products amplified by using B49317-A49855 and lane 4, 5, and 6 show PCR products amplified by using B49873-A50272. 1. *Nepeta fissa*; 2. *Thymus kotschyanus* var. *kotschyanus*; 3. *Stachys kurdica* var. *kurdica;* 4. *Nepeta fissa*; 5. *Thymus kotschyanus* var. *kotschyanus*; 6. *Stachys kurdica* var. *kurdica.*

4.4. DNA Sequencing and Alignment

ITS and *trnL* intron and *trnL-F* regions were amplified belonging to plant samples. DNA sequencing of these PCR products was done by MedSanTek. Sequencing of each PCR product was done by unidirectional using forward primers. Sequences were converted to FASTA format and recorded in Note Pad. Raw data were checked visually by aligned using ClustalW (Thompson et al. 1994) software. Sequences and alignment results are shown in Appendix.

4.5. Phylogenetic Analysis

4.5.1. nrDNA and Phylogenetic Tree Analysis

ITS region includes; ITS1, 5.8SrDNA and ITS2 portions. Polymorphisms existing in ITS enable to compare of closely related genera and species. During phylogenetic analysis all sequences were aligned with both ClustalW by using Molecular Evolutionary Genetics Analysis software MEGA 6.0 (Tamura et al. 2013). Then, sequences were clustered and contigs were created by elimination of gap regions. Phylogenetic trees were constructed by character-based Maximum Parsimony Method (Figure 4.8). Number of parsimony-informative sites, transition, transversion, nucleotide diversity, and variable and conserved sites were computed and summarized in Table 4.1.

For construction of phylogenetic tree depends on sequences of nrDNA ITS region, *L. iberica, L. peltata, L. canescens* and outgroups; *Stachys iberica* subsp. *iberica, Stachys kurdica* var. *kurdica* and *Tanacetum vulgare and Tanacetum nitens* were used. According to discrimination of *Lallemantia* species in Flora of Turkey mentioned in Section 1.2. *L. peltata, L. iberica* and *L. canescens* are distinguished depending on their bracteoles. Bracteoles of *L. peltata* is orbicular however bracteoles of *L. iberica* and *L. canescens* are distinctly longer and broad and *L. iberica* is annual and its corolla is 11-18 mm and *L. canescens* is perennial and its corolla is 28-40 mm. On the contrary, the phylogenetic tree constructed by using Maximum Parsimony Method with nrDNA ITS sequence data results is not compatible with this discrimination. *L. iberica* and *L. peltata* relationship grade is more close to each other and *L. canescens* is separated from these as seen in

Figure 4.8. *Stachys iberica* subsp. *iberica* and *Stachys kurdica* var. *kurdica* which are belonging to Lamiaceae family constituted a cluster and were separated from branches of *Lallemantia*. The other out group of *Tanacetum vulgare and Tanacetum nitens* which belong to family Asteraceae were seen as outermost of all. The phylogenetic tree is compatible with taxonomic separation of genus in Flora of Turkey.

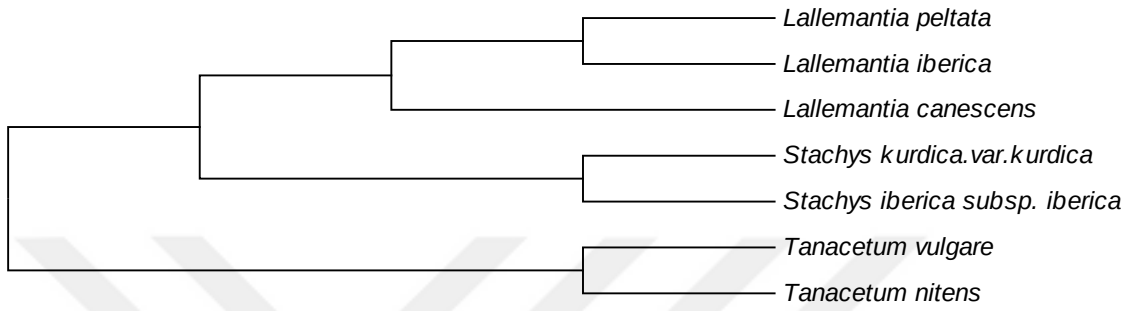

Figure 4.8. Maximum Parsinomy tree of nrDNA ITS region

Table 4.1. Numeric information of ITS

Features	**ITS**
Length of the aligned sequence (including all taxa with outgroup)	524
GC% content (including all taxa with outgroup)	58.3
Parsimony informative sites (including all taxa with outgroup)	251
Variable Sites	301
Conserved Sites	220

4.5.2. cpDNA and Phylogenetic Tree Analysis

4.5.2.1. *trnL* (UAA) intron and *trnL-F*

trnL (UAA) intron includes *trnL* (UAA) 5' exon – *trnL* (UAA) 3' exon portion. *trnL-F* includes region between *trnL* (UAA) 3' exon and *trnF* (GAA). The length of these regions varies among species. Sequences of *L. iberica, L. peltata, L. canescens, Stachys kurdica* var. *kurdica, Stachys iberica* subsp. *iberica, Tanacetum vulgare* and *Tanacetum nitens* were aligned by ClustalW program and gaps were eliminated and contigs were created. Maximum Parsimony Method was used to construct the phylogenetic trees (Figure 4.9 and 4.10). Number of parsimony-informative sites, transition, transversion,

nucleotide diversity and variable and conserved sites were computed and summarized in Table 4.2 and 4.3.

The phylogenetic tree constructed with cpDNA *trnL* intron and *trnL-F* sequences data results are not compatible with the discrimination of *Lallemantia* genus. *L. canescens* and *L. peltata* relationship grade is more close to each other and *L. iberica* is separated from the *L. canescens and L. peltata. Stachys iberica* subsp. *iberica* and *Stachys kurdica* var. *kurdica* which are belonging to Lamiaceae family constituted a cluster and were separated from branches of *Lallemantia*. *Tanacetum vulgare* and *Tanacetum nitens* which are from Asteraceae family constitutes outer group. The relationship founded out by cpDNA sequences of *L. canescens*, *L. peltata* and *L. iberica* is not abided to that of nrDNA data. On the other hand on the grade of genus discrimination emerging phylogenetic tree is not contracted to taxonomic separation of genus described in Flora of Turkey.

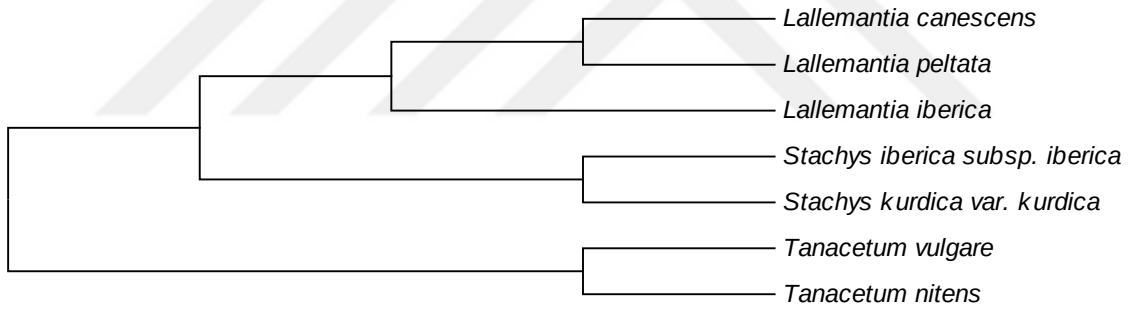

Figure 4.9. Maximum Parsinomy tree of cpDNA *trnL* (UAA) intron region

Table 4.2. Numeric information of *trnL* (UAA) intron

Features	***trnL* (UAA) intron**
Length of the aligned sequence (including all taxa with outgroup)	360
GC% content (including all taxa with outgroup)	34.1
Parsimony informative sites (including all taxa with outgroup)	85
Variable sites	270
Conserved sites	75

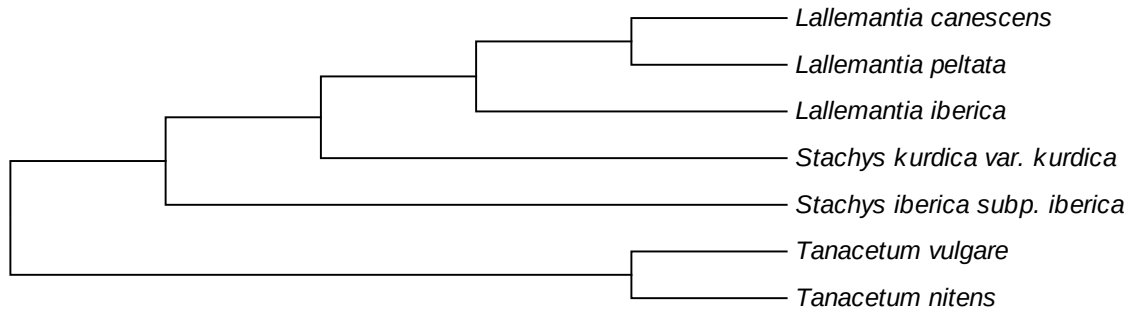

Figure 4.10. Maximum Parsinomy tree of *trnL-F* region

Table 4.3. Numeric information of *trnL-F*

Features	***trnL-F***
Length of the aligned sequence (including all taxa with outgroup)	351
GC% content (including all taxa with outgroup)	39.9
Parsimony informative sites (including all taxa with outgroup)	93
Variable sites	123
Conserved sites	228

5. CONCLUSION

In conclusion, in this study nrDNA ITS region (ITS1, 5.8SrDNA and ITS2) and cpDNA *trnT-F* region were analysed. The sequences obtained from these DNA regions were aligned and compared and used for construction of phylogenetic tree. The results were congruent in the means of Flora of Turkey genus discrimination. On the other hand they were not parallel to *Lallemantia* species separation. According to the nrDNA sequence data *L. peltata* and *L. iberica* showed closer relationship compared to *L. canescens.* On the contrary cpDNA both region sequence data illustrated that *L. canescens* and *L. peltata* were separated from the same node but *L. iberica* branched out of them. Thus not only the phylogenetic relationships of *Lallemantia* species are incompatible with discrimination of this genus but nrDNA and cpDNA phylogenetic trees are also incompatible with each other.

Until today there isn't any phylogenetic analysis on *L. peltata*, *L. canescens* and *L. iberica* found in Turkey. Although nrDNA ITS and cpDNA *trnL-F* regions were analysed in this study for the first time it only give us an idea about DNA sequence similarity and diversity of species and reflection of this comparison on the phylogenetic tree. For the more comprehensive results different markers from both nrDNA and cpDNA would be studied and compared in detail.

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APPENDIX

trnL (UAA) Intron Sequences

Lallemantia canescens

GATAACTTTCAAATTCAGAGAAACCCCGGAATTAAGAAAAAAGGGCAATCCTGAGC
CAAATCCTGTTTTCTCAAAACAAAGGTTCAAAAAACAACAAAAAGGATAGGTGCAG
AGACTCAATGGAAGCTGTTCTAACGAATGGAGTTGACTGCGCCGGTAAAGGAATCTT
TCCATGGAAATTTTAGAAAGCATGAAGGATAAACGCATCTATTGAATACAATATCAA
ATTTTTAATGTTGGCCCGAATCTGTTTTTTTTTTTTTTTAATATGAAAATAACAAAATT
TAATATGAAAATAAGTGGGAATTTATTTCACTTTGAAAAAAAAA

Lallemantia iberica

GATAACTTTCAATTCAGAGAAACCCCGGAATTAAGAAAAATGGGCAATCCTGAGCCA
AATCCTGTTTTCTCAAAACAAAGGTTCAAAAAACAACAAAAAGGATAGGTGCAGAG
ACTCAATGGAAGCTGTTCTAACGAATGGAGTTGACTGCGCCGGTAAAGGAATCTTTC
CATGGAAATTTTAGAAAGGATAAACGCATCTATTGAATACAATATCAAATTTTTAAT
GTTGGCCCGAATCTGTTTTTTTTTTTTATTTTAATATGAAAATAACAAAATAAGTGGG
AATTTATTTCACGTTGAAGAAAAAAAA

Lallemantia peltata

GATAACTTTCAAATTCAGAGAAACCCCGGAATTAAGAAAAATGGGCAATCCTGAGCC
AAATCCTGTTTTCTCAAAACAAAGGTTCAAAAAACAACAAAAAGGATAGGTGCAGA
GACTCAATGGAAGCTGTTCTAACGAATGGAGTTGACTGCGCCGGTAAAGGAATCTTT
CCATGGAAATTTTAGAAAGGATGAAGGATAAACGCATCTATTGAATACAATATCAAA
TTTTTAATGTTGACCCGAATCTGTTTTTTTTTTTTTTTAATATAAAAATAAGTGTGAAT
TTATTTCACCTTGAATAAAAAAAAAAA

Stachys iberica subsp. *iberica*

GATAACTTTCAAATTCAGAGAAACCCCGGAATTAATCAAAATGGGCGATCCTGATCC
AAATCCTGTTTTTTCAAAACAAAGGTTCAAAAAACCAAATAAAGGATAGGTGCAGAG
ACTCAACGGAAGCTGTTCTAACAAATGGAGTTTACTGCGTTGGTAGAGGAATCCTTT
CTAGGAAACTTCAAAAAGGATGAAGGATAAACGTATCTATCGAATACTATATCAAAT
GATTAATGATAGCCCGAATCCGTA

Stachys kurdica var. *kurdica*

GATAACTTTCAAATTCAGAGAAACCCCGGAATTAATCAAAATGGGCGATCCTGATCC
AAATCCTGTTTTTTCAAAACAAAGGTTCAAAAAACCAAATAAAGGATAGGTGCAGAG
ACTCAACGGAAGCTGTTCTAACAAATGGAGTTGACTGCGTTGGTAGAGGAATCCTTT
CTACGGAAACTTCAGAAAGGATGAAGGATAAACGTATCTATCGAATACTATATCAAA
TGATTAATGATGGCCCGAGTCCGTATTTTTAAATATGAAAAATAGAAGAATTGGTGT
GAATTGATTCTATAATTGAAGAAAAAA

Tanacetum vulgare

TTACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAAGAAAAATGGGCAA
TCCTGAGCCAAATCACGTTTTCCGAAAACAAACAAAGGTTCAGAAAGCGAAAAGAA
AAAAAAGATAGGTGCAGAGACTCGATGGAAGCTGTTCTAACGAATGGAGTTGATTGT
CTTACATTGGTAGAGGAATCCTTCTATCGAAACTTCAGAAAAGATGTCAGAAAAGAT
GAAGGATAAACCTGTATACATAATACAGAATTGAAGAAAGAATCAATCAAATATTC
ATTGATCAAAGATTCACTCCATAATCTGATAGATCTTTTGAAGAACTGATTAATCGGA
CGAGAATAAAGATAGAGTCCCGTTCTACATGTCAATACTGGCAACAATGAAATTTAT
AGTAATAGGAAAATCCGTCGATTTCAAAAATCATGAGGGTTCAAGTCTTTCTCTGAG
TGCCCCGGAAA

Tanacetum nitens

CGCTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAAGAAAAATGGGCAAT
CCTGAGCCAAATCACGTTTTCCGAAAACAAACAAAGGTTCAGAAAGCGAAAAGAAA
AAAAAGATAGGTGCAGAGACTCGATGGAAGCTGTTCTAACGAATGGAGTTGATTGTC
TTACATTGGTAGAGGAATCCTTCTATCGAAACTTCAGAAAAGATGTCAGAAAAGATG
AAGGATAAACCTGTATACATAATACAGAATTGAAGAAAGAATCAATCAAATATTCAT

TGATCAAAGATTCACTCCATAATCTGATAGATCTTTTGAAGAACTGATTAATCGGAC
GAGAATAAAGATAGAGTCCCGTTCTACATGTCAATACTGGCAACAATGAAATTTATA
GTAATAGGAAAATCCGTCGATTTCAAAAATCATGAGGGTTCAAGTCTTTCTATAATC
CCCGGGAAA

***trnL-F* Sequences**

Lallemantia canescens

TAGGGGTTCCAAATTCCCTTATCCTTCTAATTCCTTGACAAGCTTATTTTAGCGTAAA
TGACGGACTTTCTTTTATCACATGTGATATAGAATACACATTGCAAATAAAGCAAGG
AATGCCAATATGAATGAATTGCGTTGAAATTACAGGACTTGGAGAAAACTTTACAAT
CCCCCCCGTGTCCCTTTAATTGACATCGACTCCAGTCATCTAATAAAATGAGGGTGGG
ATGCTACATTGGAAATGGTCGGGATAGCTCAGCTGGTAGAGCAGAGGACTGAAAAT
CCTCGTGTCACC

Lallemantia iberica

TAGGGGTTCCAAATTCCCTTATCCTTCTAATTCCTTGACAAGCTTATTTTAGCGTAAA
TGACGGACTTTCTTTTATCACATGTGATATAGAATAGAATACACATTGCAAATAAAG
CAAGGAATGCCAATATGAATGAATAGCGTTGAAATTACAGGACTTGGATAAAACTTT
ACAATCCCCCCCCGTGTCCCTTTAATTGACATCGACTCCGTCATCTAATAAAATGAGGG
TGGGATGCTACATTGGAAATGGTCGGGATAGCTCAGCTGGTAGAGCAGAGGACTGA
AAATCCTCGTGTCCC

Lallemantia peltata

TAGGGGTTCCAAATTCCCTTATCCTTCTAATTCCTTGACAAGCTTATTTTAGCGTAAA
TGACGGACTTTCTTTTATCAAATGTGATATAGAATACACATTGCAAATAAAGCAAGG
AATGCCAATATGAATAGCGTTGAAATTACAGGACTTGGAGAAAACTTTACAATCCCC
CCCGTGTCCCTTTAATTGACATCGACTCCGTCATCTAATAAAATGAGGGTGGGATGCT
ACATTGGAAATGGTCGGGATAGCTCAGCTGGTAGAGCAGAGGACTGAAAATCCTCGT
GTCACCC

Stachys iberica subp. *iberica*

TTATCCCATCCCCCCTTAAGGAATCCCTATTTGAATAATTCACAATCAATAGATGCAG
GACAAAACTTTGTAATCCTGCCTGTCCCTTTAATTGACAGAGACTACAGTTATCCTAT
AAAATGAAGATGGGATGCTACATTGGGAATGGTCGGGATAGCTCAGCTGGTAGAGC
AGAGGACTGAAAATCCTCGTGTCCC

Stachys kurdica var. *kurdica*

TATTTACCCTATCCCCCTTCTTTTTCGTTAACGGTCCCAAATTCCCTTATCCTTCTGAT
TCTTTGACAAACGTATTTGGGCGTAAATGACTTTATCTTATCACATGTGATATAGAAT
ACACATTCCAAATGAAGCAATGAATGCCGATATGAATGAATAGCCTTGAAATTACAG
GACTCGGAGAAAACTTTGTAATCCCCCGTGTCCCTTTAATTGACATCGACTCCAGTCA
TCTAATAAAATGAGGGTGGGATGCTACATTGGAAATGGTCGGGATAGCTCAGCTGGT
AGAGCAGAGGACTGAAAATCCTCGTGTCACA

Tanacetum vulgare

ACACTGGCTCTATTCTTTATTGTATCCTTTTGATTTATCTTGTTTTTCGTTAGCGGTTCA
AAATTCCTTATCTTTCCCATTCACTACTCTTTATACAATTATACAAAAGGATCTGAGC
GGAAAAGCTGTTCTCTTATCACATCACACGGGATATATATGATACATGTACAAATGA
ATATCTTTGAGCAAGGAATCCCCGTGTGAATTATTCACGATCGATATTTTTATTCATA
CTGAAGTTATTCTTTTGCCAAATTATAGGACCTGGACGAGGCTTTGTAATACCCTTTC
AATTGACATAGACCCACGTTGTCTAGTAAAATGAAAATGAGGATGCGACATCAGGA
ATAGTTGGGATAGCTCAGTTGGTAGAGCAGAGGACTGAAAATCCTCGTGTCCCGGGT
CGGGAAAATAAAAA

Tanacetum nitens

CACTTGACTCTATTCTTTATTGTATCCTTTTGATTTATCTTGTTTTTCGTTAGCGGTTCA
AAATTCCTTATCTTTCCCATTCACTACTCTTTATGCAATTATACAAAAGGATCTGAGC
GGAAAAGCTGTTCTCTTATCACATCACACGGGATATATATGATACATGTACAAATGA
ATATCTTTGAGCAAGGAATCCCCGTGTGAATTATTCACGATCGATATTTTTATTCATA
CTGAAGTTATTCTTTTGCCAAATTATAGGACCTGGACGAGGCTTTGTAATACCCTTTC
AATTGACATAGACCCACGTTGTCTAGTAAAATGAAAATGAGGATGCGACATCAGGA

ATAGTTGGGATAGCTCAGTTGGTAGAGCAGAGGACTGAGAATCCTCGTGTCACCAGT
TGCGAAATAAAA

ITS Sequences

Lallemantia peltata

TGGGATGTTTATTAATCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATT
GTCGAAACCTGCAAAGCAGACCGCGAACCCGTGCGTAACGAACCGCGTGCGTCGCG
GCGTGGGGGCGACCCCCGTCGCGCCGCCGCGTCCCCGCCGGCGCCATCCCTCGGGCG
GCGTCGTGCGGGCTAACGAACCCCGGCGCGGAATGCGCCAAGGAAAACAGAAACGA
AGCGTCCGCCCCCCGCTCCCCGTCCGCGGAGCGTGCGGGGGACCGGCCGTCTATCAA
AATGTCATAACGACTCTCGGCAAAGGATATCTCGGCTCTCGCATCGATGAAGAACGT
AGCGAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGA
ACGCAAGTTGCGCCCGAAGCCATCAGGCCGAGGGCACGTCTGCCTGGGCGTCACGCA
TCGCGTCGCCCCCCCTCCATCGAGGCGGGGCGGATATTGGCCCCCCGTGCGTCCCGG
CGCGCGGCCGGCCCAAATGCGATCCCTCGGCGGCTCGTGTCGCGACCAGTGGTGGTT
GAACTCATCAATCTCTCAAGGTCGCGATCCCGTGCCGTCCGAACGGGCATCAACGAA
CGACCCAACGGCGTCGGGCCCCAGCGGCCCCGCGCCTTCGACCGCGACCCCAGTGCA
GGCAATACC

Lallemantia iberica

TTGTATGGTGATAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATT
GTCGAAACCTGCAAAGCAGACCGCGAACCCGTGCGTAACGAACCGCTTGCGTCGCG
GCGTGGGGGCGACCCCCGTCGCGCCGCCGCGCCCCTGCCGGCGCCATCCCTCGGGAG
GCGTCGTGCGGGCTAACGAACCCCGGCGCGGAATGCGCCAAGGAAAACAGAAACGA
AGCGTCCGCCCCCCGCTCCCCGTCCGCGGAGCGTGCGGGGGACCGGCCGTCTATCAA
AATGTCATAACGACTCTCGGCAAAGGATATCTCGGCTCTCGCATCGATGAAGAACGT
AGCGAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGA
ACGCAAGTTGCGCCCGAAGCCATCAGGCCGAGGGCACGTCTGCCTGGGCGTCACGCA
TCGCGTCGCCCCCCCTCCATCGAGGTGGGGCGGATATTGGCCCCCCGTGCGTCCCGG
CGCGCGGCCGGCCCAAATGCGATCCCTCGGCGGCTCGTGTCGCGACCAGTGGTGGTT
GAACTCATCAATCTCTCAAGGTCGCGATCCCGTGCCGTCCGAACGGGCATCAACGAA
CGACCCAAGGCGTCGGGCCCCAGCGGCCCCGCGCCTCGACCGCGACCCCAGTCAAGC
GAATAACCG

Lallemantia canescens

TGGAAAGTTAAAAAATCGTAACAAGGTCTCCGTAGGTGAACCTGCGGAAGGATCATT
GTCGAACCTGCAAAGCAATCCGCAAACCCGAACGAACCGCATCTCGCGCTCGGGGG
CGACCCGGCTAACACGCGGCAATGCCCAAGGAAAACACGAAGCTGCATCCTTAGTCC
CCGGGCGGGAAATAAACTTTCGGCAACGGATCTCTCGGTTCTGGCATCGATGAAGAA
CGTAGCGAAATGCGATAATTGGTTTGAATTGCAGAAGCCCGTGATCCATCGAGTCTT
TGAACCCAAGTTGCGCCCGAAGCCATGAGGCCGAGGGCACGTCTGCCTGGGCCGTCA
CGCATCGCGTCGCCCCCCCTCGCCGCGTGGGGCGGATTCCCCCGGTGGCGCCGGCCG
CGCGGCCGGCATGCGATCCCTTGGCGGCTCGTGTCGCGACCAGTGGTGGTTGAACTC
TCTCAAGGTCGCGATCCCGTGCCGTCCGAACGGGC

Stachys kurdica var. *kurdica*

TTTTTGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGCGCCCCGCTAACGAAATTCG
GGCGCGGAATGCGCCAAGGAAAACGAAATGGAGCGCTCCCCTCCCCCCGGCGCGCC
CCGTCCGCGGGGCGAACCGCGGGGAGACGGACGCCTATCGAATGTCTAAACGACTCT
CGGCAACGGATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAATGCGATACTT
GGTGTGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGA
AGCCATCAGGCCGAGGGCACGCCTGCCTGGGCGTCACGCATCGCGTCGCCCCCCACC
CCCCGGGGTGCCGGGGCGGAGATTGGCCCCCCGTGCGCAGCGATGCGCGCGGCCGG
CCCAAACCCGAATCCGCCGTCGACGCGCGTCGCGACCAGTGGTGGTTGAACCCTCAA
CTCGCGTGCTGTCGCGCCCCGCCGCGCCGTCGGTCCGGAGACCGCAGGGCCCAACGG
AGCGATCCACGGATCGCGCCCACGACCGCGACCCCAGGTCACCCGAATACGCG

Stachys iberia subsp. *iberica*

TTGTGGGTGTAACTTCTCTCTTACAAGGTTTCCGTAGGTGAACGTGCGGAAGGATCAT
TGTTGAAACCTGCAAAGCAGACCGCGAACACGTTCACAAAAAACAAAACCCGGAGC
CGCTGAGCGGGGGAGACCCCGGGAAGCGGCCCCGATAACGAACTCGGGCGCGGAAT
GCGCCAAGGAAAACGAAATGGAGCGCACCCGCCTCCCCCGAGCGCCCCGTCCGCGG
GGCGACGGGGGTGGAGAGGGACGCCTATCGAATGTCTAAACGACTCTCGGCAACGG
ATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAATGCGATACTTGGTGTGAAT
TGCAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCATTAG
GCCGAGGGCACGCCTGCCTGGGCGTCACGCATCGCGTCGCCCCCTCCCCCCCGCCTC
GGGGGGCGTTGGGGCGGAGATTGGCCCCCCGTGCGCAGCGATGCGCGCGGCCGGCC

CAAACACGAATCCGCCGTCGACGCAAACGTCGCGACCAGTGGTGGTTGAACCCTCAA
CTCGCGTGCTGTCGCGTCCCGATGCGCCGTCGGTCCGGAGACGAACGAACCCAATGG
AGCGATCGCGAATCGCGCCCACGACCGCGACCCCAGTCAGGCGATACCC

Tanacetum vulgare

CGGCGTCGCCTGACCTGGGGTCGCGGTCGAAGCGTCATCCTAAGATAACACATTGGG
GTATTTGAAGAGTTTTTCCTTGCGACTAACACAGAACAAAGAACGAGGGTTTTTACG
ACCACCACTAGTTCGTGCGTCCATCGAAGGGACTCCTATTTTGGCCAACCACACCAT
GAGCACGGGAGACCAATATCCGCCCCGAACAAAGATTTGTTGGGGGCGACGCGATG
CGTGACGCCCAGGCAGACGTGCCCTCGGCCAAAAGGCTTCGGGCGCAACTTGCGTTC
AAAAACTCGATGGTTCACGGGATTCTGCAATTCACACCAAGTATCGCATTTTGCTAC
GTTCTTCATCGATGCGTGAGCCGAGATATCCGTTGCCGAGAGTCGTTTGTGATTATAA
AGAAGCCACGTCTCATGAGCACACCGCGAACGGGGCAACATAAAACTAGCCTTCTTA
AGTTTAGTTTTCCTTGGCACACATTGTGCCGGGGGTTGTTATTGCGCCAATGACACAT
TCACCATGTCCAAAAGAACACAAGTAAATGCACATCGACAAAGCATCGAGAGGATC
AAACAAGTGCTTAATCCACTCGACGCTCGGTTGTTTTTACATGTTCGCGGGTCGTTCT
GCTTTGCAGGGTTCGACAATGATCCTTCCGCAGGTTCACCTACGGAAACCTTGTAATA
TTTTTTAGCGATGCAGTCACCA

Tanacetum nitens

ATGCTGTCCGCCTGACCTGGGGTCGCGGTCGAAGCGTCATCCGAAGACAACACATTG
GGGTATTTGAAGAGTTTTCCTTGCGATTAACACAGAACAAAGAACGAGGGTTTTTAC
GACCACCACTAGTTCGTGCGTCCATCGAAGGGACTCCTATTTTGGCCAACCACACCA
TGAGCACGGGAGACCAATATCCGCCCCCAACAGAGATTTGTTGGGGGCGACGCGAT
GCGTGACGCCCAGGCAGACGTGCCCTCGGCCAAAAGGCTTCGGGCGCAACTTGCGTT
CAAAAACTCGATGGTTCACGGGATTCTGCAATTCACACCAAGTATCGCATTTTGCTAC
GTTCTTCATCGATGCGTGAGCCGAGATATCCGTTGCCGAGAGTCGTTTGTTATTATAA
AGAAGCCACGTCCCATGAGCACACCGCGAACGGGGCAACATAAAACAAGCCTTCTT
AAGTTTAGTTTTCCTTGGCACACATTGTGCCGGGGGTTGTTATTGCGCCGATGATACA
TTCACCATGTCCAAAAGAACACAAGTAAATGCACACCGACAAGCATCGAGAGGATC
AAACAAGTGCTTAATCCACTCGACGCTCGGTTGTTTTTACGTGTTCACGGGTCGTTCT
GCTTTGCAGGGTTCGACAATGATCCTTCCGCAGGTTCACCTACGGAAACCTTGTCGCG
TTTTATTGTGCTGCGCGCGTAGCA

ClustalW Alignment of *trnL* (UAA) intron

```
L.iberica         GATAACTTTCAATT-CAGAGAAACCCCGGAATTAAGAAAAATGGGCAATCCTGAGCCAAA
L.peltata         GATAACTTTCAAATTCAGAGAAACCCCGGAATTAAGAAAAATGGGCAATCCTGAGCCAAA
L.canescens       GATAACTTTCAAATTCAGAGAAACCCCGGAATTAAGAAAAAAGGGCAATCCTGAGCCAAA
                  ************:* *************************:******************

L.iberica         TCCTGTTTTCTCAAAACAAAGGTTCAAAAAACAACAAAAAGGATAGGTGCAGAGACTCAA
L.peltata         TCCTGTTTTCTCAAAACAAAGGTTCAAAAAACAACAAAAAGGATAGGTGCAGAGACTCAA
L.canescens       TCCTGTTTTCTCAAAACAAAGGTTCAAAAAACAACAAAAAGGATAGGTGCAGAGACTCAA
                  ************************************************************

L.iberica         TGGAAGCTGTTCTAACGAATGGAGTTGACTGCGCCGGTAAAGGAATCTTTCCATGGAAAT
L.peltata         TGGAAGCTGTTCTAACGAATGGAGTTGACTGCGCCGGTAAAGGAATCTTTCCATGGAAAT
L.canescens       TGGAAGCTGTTCTAACGAATGGAGTTGACTGCGCCGGTAAAGGAATCTTTCCATGGAAAT
                  ************************************************************

L.iberica         TTTAGAAAG-------GATAAACGCATCTATTGAATACAATATCAAATTTTTAATGTTGG
L.peltata         TTTAGAAAGGATGAAGGATAAACGCATCTATTGAATACAATATCAAATTTTTAATGTTGA
L.canescens       TTTAGAAAGCATGAAGGATAAACGCATCTATTGAATACAATATCAAATTTTTAATGTTGG
                  *********       ****************************************.

L.iberica         CCCGAATCTGTTTTTTTTTTTTTATTTTAAT-----------ATGAAAATAACAAAATAAG
L.peltata         CCCGAATCTGTTTTTTTTTTTTTTTT--------------------AATATAAAAATAAG
L.canescens       CCCGAATCTGTTTTTTTTTTTTTTTTAATATGAAAATAACAAAATTTAATATGAAAATAAG
                  **********************:**                    ****: ********

L.iberica         TGGGAATTTATTTCACGTTGAAGAAAAAAAA---
L.peltata         TGTGAATTTATTTCACCTTGAATAAAAAAAAAA
L.canescens       TGGGAATTTATTTCACTTTGAAAAAAAAA-----
                  ** ************* ***** ******
```

ClustalW Alignment of *trnL-F*

```
L.canescens       TAGGGGTTCCAAATTCCCTTATCCTTCTAATTCCTTGACAAGCTTATTTTAGCGTAAATG
L.iberica         TAGGGGTTCCAAATTCCCTTATCCTTCTAATTCCTTGACAAGCTTATTTTAGCGTAAATG
L.peltata         TAGGGGTTCCAAATTCCCTTATCCTTCTAATTCCTTGACAAGCTTATTTTAGCGTAAATG
                  ************************************************************

L.canescens       ACGGACTTTCTTTTATCACATGTGATATAGAATA-----CACATTGCAAATAAAGCAAGG
L.iberica         ACGGACTTTCTTTTATCACATGTGATATAGAATAGAATACACATTGCAAATAAAGCAAGG
L.peltata         ACGGACTTTCTTTTATCAAATGTGATATAGAATA-----CACATTGCAAATAAAGCAAGG
                  ******************.***************     **********************

L.canescens       AATGCCAATATGAATGAATTGCGTTGAAATTACAGGACTTGGAGAAAACTTTACAATCCC
L.iberica         AATGCCAATATGAATGAATAGCGTTGAAATTACAGGACTTGGATAAAACTTTACAATCCC
L.peltata         AATGCCAATATGAAT----AGCGTTGAAATTACAGGACTTGGAGAAAACTTTACAATCCC
                  ***************    :********************** ****************

L.canescens       CCCCGTGTCCCTTTAATTGACATCGACTCCAGTCATCTAATAAAATGAGGGTGGGATGCT
L.iberica         CCCCGTGTCCCTTTAATTGACATCGACTCCG-TCATCTAATAAAATGAGGGTGGGATGCT
L.peltata         CCCCGTGTCCCTTTAATTGACATCGACTCCG-TCATCTAATAAAATGAGGGTGGGATGCT
                  *****************************. ***************************

L.canescens       ACATTGGAAATGGTCGGGATAGCTCAGCTGGTAGAGCAGAGGACTGAAAATCCTCGTGTC
L.iberica         ACATTGGAAATGGTCGGGATAGCTCAGCTGGTAGAGCAGAGGACTGAAAATCCTCGTGTC
L.peltata         ACATTGGAAATGGTCGGGATAGCTCAGCTGGTAGAGCAGAGGACTGAAAATCCTCGTGTC
                  ************************************************************

L.canescens       ACC-
L.iberica         CC--
L.peltata         ACCC
                  .*
```

ClustalW Alignment of ITS

```
L.peltata       --TGGGATGTTTATTAATCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTG
L.iberica       --TTGTATGGTGATAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTG
L.canescens     TTTGGAAAGTTAAAAAATCGTAACAAGGTCTCCGTAGGTGAACCTGCGGAGGGATCATTA
                  * * *:* * *::*.*********** ********************.********.

L.peltata       TCGAAACCTGCAAAGCAGACCGCGAACCCGTGCGTAACGAACCGCGTGCGTCGCGGCGTG
L.iberica       TCGAAACCTGCAAAGCAGACCGCGAACCCGTGCGTAACGAACCGCTTGCGTCGCGGCGTG
L.canescens     CTGAGTTAG--GGAGCAATCC-CGAACCT--------CCAACCCTTTGTG----------
                  **.: .   ..****.:** ******         * ****   ** *

L.peltata       GGGGCGACCCCCGTCGCGCCGCCG-CGTCCCCGCCGGCGCCATCCCTCGGGCGGCGTCGT
L.iberica       GGGGCGACCCCCGTCGCGCCGCCG-CGCCCCTGCCGGCGCCATCCCTCGGGAGGCGTCGT
L.canescens     --AACGCATCTCGTTGCTTCGGGGGCGACCCTGCCG-----TTCACGCGG----------
                  ..**.. * *** **  **  * ** *** ****     :**.* ***

L.peltata       GCGGGCTAACGAACCCCGGCGCGGAATGCGCCAAGGAAAACAGAAACGAAGCGTCCGCCC
L.iberica       GCGGGCTAACGAACCCCGGCGCGGAATGCGCCAAGGAAAACAGAAACGAAGCGTCCGCCC
L.canescens     ---------CATTCCCCCCGGAGG-------------------------TCATCAAAAC
                         *.::****   *.**                          *.**....*

L.peltata       CCCGCTCCCCGTCCGCGGAGCGTGCGGGGGACCGGCCGTCTATCAAAATGTCATAACGAC
L.iberica       CCCGCTCCCCGTCCGCGGAGCGTGCGGGGGACCGGCCGTCTATCAAAATGTCATAACGAC
L.canescens     ACTGCATCCTTACGTCGGAGTATAAAG-----------------TTAATTTAATAAAAC
                .* **: **  :*  ***** .*...*                 ::*: * *::*..**

L.peltata       TCTCGGCAAAGGATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAATGCGATACTT
L.iberica       TCTCGGCAAAGGATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAATGCGATACTT
L.canescens     TTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGT
                * **..***.****.*** ** *** *************** **************. *

L.peltata       GGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGC
L.iberica       GGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGC
L.canescens     AATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCCTGG
                ..*************** *.***** ******.************..******** .:*

L.peltata       CATCAGGCCGAGGGCACGTCTGCCTGGGCGTCACGCATCGCGTCGCCCCCCCTCCATCGA
L.iberica       CATCAGGCCGAGGGCACGTCTGCCTGGGCGTCACGCATCGCGTCGCCCCCCCTCCATCGA
L.canescens     TATTC--CGGGGGGCATGCCTGTTCGAGCGTCAT--TTCACCACTCAAGCCTCGCTTGGC
                 ** .  * *.***** * ***   *.******   :**.* :* *.. **   *:* *.

L.peltata       GGCGGGGCGGATATTGGCCCCCCGTGCGTCCCGGCGCGCGGCCGGCCCAAATGCGATCCC
L.iberica       GGTGGGGCGGATATTGGCCCCCCGTGCGTCCCGGCGCGCGGCCGGCCCAAATGCGATCCC
L.canescens     ATTGGG-------------CGTCGCGAGTCCCT---CGCG--CGCCTCAAAGTCTCCGGC
                .  ***             *  ** *.*****    ****  ** * ****  * .   *

L.peltata       TCGGCGGCTCGTGTCGCGACCAGTGGTGGTTGAACTCATCAATCTCTCAAGGTCGCGATC
L.iberica       TCGGCGGCTCGTGTCGCGACCAGTGGTGGTTGAACTCATCAATCTCTCAAGGTCGCGATC
L.canescens     TCGGCGATTCGT----CTCCCAGCGTTG-----TGGCAACTATTTCGCAGTGGAGT----
                ******. ****    * .**** * **     :  **:*:** ** **. * .*

L.peltata       CCGTGCCGTCCGAACGGGCATCAACGAACGACCCAACGGCGTCGGGCCCCAGCGGCCCCG
L.iberica       CCGTGCCGTCCGAACGGGCATCAACGAACGACCCAAGG-CGTCGGGCCCCAGCGGCCCCG
L.canescens     ----------------------------------------TCGGGTCGCGGGGCCGTTA
                                                        ***** * *.* * *   .

L.peltata       CGCCTTCGACCGCGACCCCAGTGCAGGCAATACC--
L.iberica       CGCCT-CGACCGCGACCCCAGTCAAGCGAATAACCG
L.canescens     AATCTTCAAAGGTGACCTCGGATCACGTAAGGTAAG
                .. ** *.*. * **** *.*: .*   ** . .
```

Clustal W Alignment of *trnL* (UAA)

```
L.canescens      ----GATAACTTTCAAATTCAGAGAAACCC----------------CGGAATTAAGAAAA
S.kurdica        ----GATAACTTTCAAATTCAGAGAAACCC----------------CGGAATTAATCAAA
L.iberica        -----GATAACTTTCAATTCAGAGAAACCC----------------CGGAATTAAGAAAA
L.peltata        ----GATAACTTTCAAATTCAGAGAAACCC----------------CGGAATTAAGAAAA
S.iberica        ----GATAACTTTCAAATTCAGAGAAACCC----------------CGGAATTAATCAAA
T.vulgare        GGTTCAAGTCCCTCTATCCCCAAAAAGACCGTTTGACTCCCTAATTCTTTATTGTTCCTT
                      .: :.  *  *:  *..*.**..**               *  :***.: ..::

L.canescens      AAGGGCAATCCTG-------------------------AGCCAAATCCTGTTTTCTCAAA
S.kurdica        ATGGGCGATCCTG-------------------------ATCCAAATCCTGTTTTTTCAAA
L.iberica        ATGGGCAATCCTG-------------------------AGCCAAATCCTGTTTTCTCAAA
L.peltata        ATGGGCAATCCTG-------------------------AGCCAAATCCTGTTTTCTCAAA
S.iberica        ATGGGCGATCCTG-------------------------ATCCAAATCCTGTTTTTTCAAA
T.vulgare        TTGATTTATCTTGTTTTTCGTTAGCGGTTCAAAATTCCTTATCTTTCCCATTCTCTTTAT
                 ::*.   *** **                         : . .::*** .** * * :*:

L.canescens      ACAAAGGTTCAAAAAACAACAAAAAGGATAGGTG-----------------CAGAGACTC
S.kurdica        ACAAAGGTTCAAAAAACCAAATAAAGGATAGGTG-----------------CAGAGACTC
L.iberica        ACAAAGGTTCAAAAAACAACAAAAAGGATAGGTG-----------------CAGAGACTC
L.peltata        ACAAAGGTTCAAAAAACAACAAAAAGGATAGGTG-----------------CAGAGACTC
S.iberica        ACAAAGGTTCAAAAAACCAAATAAAGGATAGGTG-----------------CAGAGACTC
T.vulgare        ACAATTATACAAAAGGATCTGAGCGGAAAAGCTGTTCTCTTATACATCACACGGGATATA
                 ****: .*:*****... . .:...*.*:** **                *.*..:.*.

L.canescens      AATGGAAGCTGTTCTAACGAATGGAGTTGACTGCG--------------------------
S.kurdica        AACGGAAGCTGTTCTAACAAATGGAGTTGACTGCG--------------------------
L.iberica        AATGGAAGCTGTTCTAACGAATGGAGTTGACTGCG--------------------------
L.peltata        AATGGAAGCTGTTCTAACGAATGGAGTTGACTGCG--------------------------
S.iberica        AACGGAAGCTGTTCTAACAAATGGAGTTTACTGCG--------------------------
T.vulgare        TATGATACATGTACAAATGAATATCTTTGAGCAAGGAATCCCCGTGTGAATTATTCACGA
                 :* *.:* .***:*:** .***. . ** *  ..*

L.canescens      CCGGTAAAG-GAATCTTTCCATGGAAATTTTAG----AAAGCATGAAGGATAAACGCATC
S.kurdica        TTGGTAGAGGAATCCTTTCTACGGAAACTTCAG----AAAGGATGAAGGATAAACGTATC
L.iberica        CCGGTAAAG-GAATCTTTCCATGGAAATTTTAG----AAAG-------GATAAACGCATC
L.peltata        CCGGTAAAG-GAATCTTTCCATGGAAATTTTAG----AAAGGATGAAGGATAAACGCATC
S.iberica        TTGGTAGAG-GAATCCTTTCTAGGAAACTTCAA----AAAGGATGAAGGATAAACGTATC
T.vulgare        TCGATATTTTTATTCATACTGAAGTTATTCTTTTGCCAAATTATAGGACCTGGACGAGGC
                   *.** :   *: * *:    .*::* *  :     ***         .*..*** . *

L.canescens      TATTGAATACAATATCAAATTTTTAATGTTGGCCCGAATCTGTTTTTTTTTTTTTTTAAT
S.kurdica        TATCGAATACTATATCAAATGATTAATGATGGCCCGAGTCCGTATTTTTAAATATGAAAA
L.iberica        TATTGAATACAATATCAAATTTTTAATGTTGGCCCGAATCTGTTTTTTTTTTTTTATTTTA
L.peltata        TATTGAATACAATATCAAATTTTTAATGTTGACCCGAATCTGTTTTTTTTTTTTTTT---
S.iberica        TATCGAATACTATATCAAATGATTAATGATAGCCCGAATCCGTA----------------
T.vulgare        TTTGTAATACCCTTTCAATTGACATAGACCCACGTTGTCTAGTAAAATGAAAATGAGGAT
                 *:*  ***** .*:****:* : ::* .   .*   .    **:

L.canescens      ATGAAAATAACAAAATTT----------AATATGAAAATAAGTGGGAATTTATTTCACTT
S.kurdica        ATAGAAG------------------------------AATTGGTGTGAATTGATTCTATAA
L.iberica        ATATGAA----------------------AATAACAAAATAAGTGGGAATTTATTTCACGT
L.peltata        ----------------------------AATATAAAAATAAGTGTGAATTTATTTCACCT
S.iberica        ------------------------------------------------------------
T.vulgare        GCGACATCAGGAATAGTTGGGATAGCTCAGTTGGTAGAGCAGAGGACTGAAAATCCTCGT


L.canescens      TGAAAAAAAAA-----
S.kurdica        TTGAAGAAAAAA----
L.iberica        TGAAGAAAAAAAA---
L.peltata        TGAATAAAAAAAAAAA
S.iberica        ----------------
T.vulgare        GTCACCAGTTCAAAT-
```

ClustalW Alignment of *trnL-F*

```
L.canescens      ------------------------------------------------------------
L.iberica        ------------------------------------------------------------
L.peltata        ------------------------------------------------------------
S.kurdica        -----TATTTACCCTATCCCCCTTCT-----------------------------------
S.iberica        ------------------------------------------------------------
T.vulgare        GGTTCAAGTCCCTCTATCCCCAAAAAGACCGTTTGACTCCCTAATTCTTTATTGTATCCT


L.canescens      ----------------------TAGGGGTTCCAAATTCCCTT----------------ATC
L.iberica        ----------------------TAGGGGTTCCAAATTCCCTT----------------ATC
L.peltata        ----------------------TAGGGGTTCCAAATTCCCTT----------------ATC
S.kurdica        ---------------TTTTCGTTAACGGTCCCAAATTCCCTT----------------ATC
S.iberica        -------------------------TTATCCCATCCCCCCTT----------------AAG
T.vulgare        TTTGATTTATCTTGTTTTTCGTTAGCGGTTCAAAATTCCTTATCTTTCCCATTCACTACT
                                         .* *.*:.  ** *:              *

L.canescens      CTTCTAATTCCTTGACAAGCTTATTTTAGCGTAAATGACGGACTTT-CTTTTATCACATG
L.iberica        CTTCTAATTCCTTGACAAGCTTATTTTAGCGTAAATGACGGACTTT-CTTTTATCACATG
L.peltata        CTTCTAATTCCTTGACAAGCTTATTTTAGCGTAAATGACGGACTTT-CTTTTATCAAATG
S.kurdica        CTTCTGATTCTTTGACAAACGTATTTGGGCGTAAAT----GACTTT-ATCTTATCACATG
S.iberica        ------------------------------------------------------------
T.vulgare        CTTTATACAATTATACAAAAGGATCTGAGCGGAAAAGCTGTTCTCTTATCACATCACACG


L.canescens      TGATATAGAATA----------CACATTGCAAATAAAGCAAGGAATGCCAATATGAATGA
L.iberica        TGATATAGAATAGAATA-----CACATTGCAAATAAAGCAAGGAATGCCAATATGAATGA
L.peltata        TGATATAGAATA----------CACATTGCAAATAAAGCAAGGAATGCCAATAT----GA
S.kurdica        TGATATAGAATA----------CACATTCCAAATGAAGCAATGAATGCCGATATGAATGA
S.iberica        ------------------------------------------GAATCCCTATTTGAATAA
T.vulgare        GGATATATATGATACATGTACAAATGAATATCTTTGAGCAAGGAATCCCCGTGTGAATTA
                                                  **** ** .* *     *

L.canescens      ATTGCGTTG-----------------------------------AAATTACAGGACTTGG
L.iberica        ATAGCGTTG-----------------------------------AAATTACAGGACTTGG
L.peltata        ATAGCGTTG-----------------------------------AAATTACAGGACTTGG
S.kurdica        ATAGCCTTG-----------------------------------AAATTACAGGACTCGG
S.iberica        TTCACAATC-----------------------------------AATAGATG----CAGG
T.vulgare        TTCACGATCGATATTTTTATTCATACTGAAGTTATTCTTTTGCCAAATTATAGGACCTGG
                 :* .* :*                                    **:: * .      **

L.canescens      AGAAAACTTTACAATCCCCCCCGTGTCCCTTTAATTGACATCGACTCCAGTCATCTAATA
L.iberica        ATAAAACTTTACAATCCCCCCCGTGTCCCTTTAATTGACATCGACTCCG-TCATCTAATA
L.peltata        AGAAAACTTTACAATCCCCCCCGTGTCCCTTTAATTGACATCGACTCCG-TCATCTAATA
S.kurdica        AGAAAACTTTGTAATCCCCCGTGT--CCCTTTAATTGACATCGACTCCAGTCATCTAATA
S.iberica        ACAAAACTTTGTAATCCTGCCTGT--CCCTTTAATTGACAGAGACTACAGTTATCCTATA
T.vulgare        ACGAGGCTTTGTAATACCCT---------TTCAATTGACATAGACCCACGTTGTCTAGTA
                 * .*..****. ***.*           ** ******** .*** ..  * .** :.**

L.canescens      AAATGAGGGTGGG-ATGCTACATTGGAAATGGTCGGGATAGCTCAGCTGGTAGAGCAGAG
L.iberica        AAATGAGGGTGGG-ATGCTACATTGGAAATGGTCGGGATAGCTCAGCTGGTAGAGCAGAG
L.peltata        AAATGAGGGTGGG-ATGCTACATTGGAAATGGTCGGGATAGCTCAGCTGGTAGAGCAGAG
S.kurdica        AAATGAGGGTGGG-ATGCTACATTGGAAATGGTCGGGATAGCTCAGCTGGTAGAGCAGAG
S.iberica        AAATGAAGATGGG-ATGCTACATTGGGAATGGTCGGGATAGCTCAGCTGGTAGAGCAGAG
T.vulgare        AAATGAAAATGAGGATGCGACATCAGGAATAGTTGGGATAGCTCAGTTGGTAGAGCAGAG
                 ******...**.* **** **** .*.***.** ************ *************

L.canescens      GACTGAAAATCCTCGTGTCACC----------
L.iberica        GACTGAAAATCCTCGTGTCCC-----------
L.peltata        GACTGAAAATCCTCGTGTCACCC---------
S.kurdica        GACTGAAAATCCTCGTGTCACA----------
S.iberica        GACTGAAAATCCTCGTGTCCC-----------
T.vulgare        GACTGAAAATCCTCGTGTCACCAGTTCAAAT
                 ******************.*
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ClustalW Alignment of ITS

```
S.kurdica       ------------------------------------------------------------
S.iberica       ------------------------------------------------------------
L.peltata       -----GGGGCCCGACGCCGT------------------------------------------
L.iberica       -----GGGGCCCGACGCCTT------------------------------------------
L.canescens     ------------CTTACCT------------------------------------------
P.mascula       TTCGCCGCTCGCGACGTCGCGAGAAGTCCACTGAACCTTATCATTTAGAGGAAGGAGAAG


S.kurdica       ---------------------------------------TGGGGTCGCG----GTCGTGG-
S.iberica       ------------------------------------------TCGCG----GTCGTGG-
L.peltata       ------TGGGTCGTTCGTTGATGCCCGTTCGGACGGCACGGGATCGCG----ACCTTGA-
L.iberica       ------GGG-TCGTTCGTTGATGCCCGTTCGGACGGCACGGGATCGCG----ACCTTGA-
L.canescens     ------TACGTGATCCGAGGTCACCTTTGAAGATTTAACGGCCCCGCG----ACCCGAA-
P.mascula       TCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTGTCGAA-CCTGCCTAGC-
                                                          **..    . *  .

S.kurdica       -GCGCGATCCGTGGATCGCTCCGTTGGGCC----CTGCGGTCTCCGGA------------
S.iberica       -GCGCGATTCGCG-ATCGCTCCATTGGGTT----CGTTCGTCTCCGGA------------
L.peltata       -GAGATTGATGAGTTCAACCACCACTGGTCGCGACACGAGCCGCCGAGGGATCGCA----
L.iberica       -GAGATTGATGAGTTCAACCACCACTGGTCGCGACACGAGCCGCCGAGGGATCGCA----
L.canescens     -CTCCACTGCGAAATAGTTGCCACAACGCTGGGAGACGAATCGCCGAG------------
P.mascula       AGAACGACCAGCGAACTTGTAAAAATGCTCGGGATGACGGAAGGCGTGAGCCTCTC----
                   .    * . :    ..                . .  ** .

S.kurdica       -------------CCGACGGCGCGGCGGGG------------CGCGACAG----------
S.iberica       -------------CCGACGGCGCATCGGGA------------CGCGACAG----------
L.peltata       -------TTTGGGCCGGCCGCGCGCCGGGA------------CGCACGGGGGGCCAATAT
L.iberica       -------TTTGGGCCGGCCGCGCGCCGGGA------------CGCACGGGGGGCCAATAT
L.canescens     -------------CCGGAG--ACTTTGAGG------------CGCGCGAGGGACTCG---
P.mascula       CTTCATCCCATGTCCGGTCGCGCCATACGTTGAGTCGCCCCTCGCACGATGTGCAGGGAA
                          ***.    .*   . *            ***.. .

S.kurdica       --------------------------------CACGCGAGTTGAGGGTTCAACCACCACT
S.iberica       --------------------------------CACGCGAGTTGAGGGTTCAACCACCACT
L.peltata       CCGCCCCG-----------------------CCTCGATGGAGGGGGGGCGACGCGATGCG
L.iberica       CCGCCCCA-----------------------CCTCGATGGAGGGGGGGCGACGCGATGCG
L.canescens     -CGACGCC-----------------------CAATGCCAAGCGAGGCTTGAGTGGTGAAA
P.mascula       GCGCCATTGTTCTGGTGTGCTCTCGGATTTACAACAACCCCCGGCGCAAACCGCGCCAAG
                                           .: ..    *. *    .   .  ..

S.kurdica       GGTCGCG---ACG--CGCG-------TCGACGGCGGATTCGGGTTTGGGCCGGCCG----
S.iberica       GGTCGCG---ACGTTTGCG-------TCGACGGCGGATTCGTGTTTGGGCCGGCCG----
L.peltata       TGACGCCCAGGCAGACGTG-------CCCTCGGCCTGATGGCTTCGGGCGCAACTTGCGT
L.iberica       TGACGCCCAGGCAGACGTG-------CCCTCGGCCTGATGGCTTCGGGCGCAACTTGCGT
L.canescens     TGACGCTCGAACAGGCATG-------CCCCC--CGGAATACCAGGGGGCGCAATGTGCGT
P.mascula       GAA--CTAAAACGAAAGA--GCATGCCCC-CGTTGCCCCGGCTTCGGGATGCGCGGGAGG
                 .:  *    .*.   .          *  *              **    .

S.kurdica       ------------------------------------------------------CGCGCATC
S.iberica       ------------------------------------------------------CGCGCATC
L.peltata       TCAA------AGACTCGATGGTTCACGGGATTCTGCAATTCACACCAAGTATCGCATTTC
L.iberica       TCAA------AGACTCGATGGTTCACGGGATTCTGCAATTCACACCAAGTATCGCATTTC
L.canescens     TCAA------AGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTC
P.mascula       TAATGTCTTCTTTTACATATCAAAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATC
                                                                 * *. :**

S.kurdica       GCTGCG------------------CACGGGGGGCCAATCTCCG-----------------
S.iberica       GCTGCG------------------CACGGGGGGCCAATCTCCG-----------------
L.peltata       GCTACGTTCTTCATCGATGCGAGAGCCGAGATATCCTTTGCCGAGAGTCG-----------
L.iberica       GCTACGTTCTTCATCGATGCGAGAGCCGAGATATCCTTTGCCGAGAGTCG-----------
L.canescens     GCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTTATTAAATTA
P.mascula       GATAAAGAACGTAGCGAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAATCACCG
                *.*...                  .*  *. .  . *    *
```

```
S.kurdica       ------------------------------------------------------------
S.iberica       ------------------------------------------------------------
L.peltata       --------------------------------TTATGACATTTTGATAGACGGCCGG---
L.iberica       --------------------------------TTATGACATTTTGATAGACGGCCGG---
L.canescens     ACTTTATACTCCGACGTAAGGATGCAGTGTTTTGATGACCTCCGGGGGGAATGCCGCGT-
P.mascula       AGTCTTTGAACGCAAGTTGCG--CCCAAAGCCTTTAGGCTGAGGGCACGTCTGCCTGGG-

S.kurdica       --------------------CCCCGGCACCCCGGGGGGTGGG------------------
S.iberica       --------------------CCCCAACGCCCCCCGAGGCGGGGGGGAGGGGG--------
L.peltata       ------------------TCCCCCGCACGCTCCGCGGACGGGGAGCGGGGGGCGGACGCTT
L.iberica       ------------------TCCCCCGCACGCTCCGCGGAC----------------------
L.canescens     -----GAACGGCAGGGTCGCCCCCGAAGCAACGAGATGCGTTCACAAAGGGTTGGAGGTT
P.mascula       CGTCACGTATCCCGTCGCACCCCCAACCCGTCCCAACTCGGGAATGATGGCTGGTGGGAG
                                    ***  .. *  *  ..

S.kurdica       ------------------------------------------------------------
S.iberica       ------------------------------------------------------------
L.peltata       CG-----------------TTTCTGTTTTCCT--------------------------T
L.iberica       ------------------------------------------------------------
L.canescens     CGGGATTGCTCCCTAACTCAGTAATGATCCCT--------------------------C
P.mascula       CGGATATTGGCCTCCCGTGTACTCGCGTTACG-GTTGGTCTAAAATCGAGC--CCCGAGC

S.kurdica       ------------------------------------------------------------
S.iberica       ------------------------------------------------------------
L.peltata       GGCGC-------------------------------------------------------ATTC
L.iberica       ------------------------------------------------------------
L.canescens     CGCAG-------------------------------------------------------GTTC
P.mascula       GACGA-ACGTCACGACAA-GTGGTGGTCTGTAATAGCTATTTCGTGTTGTGCGTTGTCTC

S.kurdica       ------------------------------------------------------------
S.iberica       ------------------------------------------------------------
L.peltata       CGCGCCGGGGT--------------------------------------------------
L.iberica       ------------------------------------------------------------
L.canescens     ACCTACGGAGA--------------------------------------------------
P.mascula       GTCGCCCGTGG-GAGCTCACA--GAGACCC-CAAAGCATCGTCA---CGATGATGC-ATC

S.kurdica       ---------------------------------------------
S.iberica       ---------------------------------------------
L.peltata       --TCGTTAGCCC--------------------------------
L.iberica       ---------------------------------------------
L.canescens     --CCTTGTTACGATTTTTT-----AACT--TTCCA---AAA-------
P.mascula       CATCGCGACCCCAG-GTCA-GCGGGACT--ACCCG-CTGAATTTAAGC
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CURRICULUM VITAE

She was born on March 1990 in Kalar, a city of Kurdistan region of Iraq. After she completed primary, secondary and high school in Kalar, Sulaymaniyah, in 2009 she started to study Biology in Sulaymaniyah University faculty of science, and graduated in 2013 holding bachelor degree of science, and now she is master student at Bingol University in Molecular Biology, Bingol, Turkey.