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Phylogenetic Analysis of Some Taxa Belonging to the Lamiaceae Family in Bitlis Province Using RAPD-PCR Technique

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ABSTRACT

This study examined the relationship between 54 taxa of 21 genera belonging to the family Lamiaceae, which grow naturally in Bitlis province. Genetic similarities between taxa were determined by RAPD-PCR technique. According to the results, the genera *Phlomis* L., *Lamium* L., *Ballota* L., *Stachys* L., and *Sideritis* L. in the subfamily of *Lamioideae* were supported by the morphological systematics, whereas the genera *Marrubium* L. separated from the group. It was observed that taxa belong to the genera *Nepeta* L., *Lallemantia* Fisch. & C.A. Mey, *Melissa* L., *Prunella* L., *Origanum* L., *Satureja* L., *Clinopodium* L., *Cyclotrichium* (Boiss.) Manden. & Scheng., *Mentha* L., and *Salvia* L. from the subfamily *Nepeteoideae* supported the morphological system, but *Ziziphora clinopodioides* Lam. taxa showed difference. According to the similarity matrix, the similarity was found mostly between *Clinopodium vulgare* L. subsp. *arundanum* (Boiss.) Nyman and *Clinopodium graveolens* subsp. *rotundifolium* (Pers.) Govaerts with the rate of 0.955 and between *Salvia verticillata* L. subsp. *verticillata* and *Salvia verticillata* subsp. *amasiaca* (Freyn & Bornm.) Bornm. with the rate of 0.934.

Bitlis İli Lamiaceae Familyasına Ait Bazı Taksonların RAPD-PCR Tekniği Kullanılarak Filogenetik Analizi

Araştırma Makalesi

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ÖZ

Bu çalışmada, Bitlis ilinde doğal olarak yetişen Lamiaceae familyasına ait 21 cinse ait 54 takson arasındaki ilişki incelenmiştir. Taksonlar arasındaki genetik benzerlikler RAPD-PCR tekniği ile belirlendi. Elde edilen sonuçlara göre, *Lamioideae* alt familyasında yer alan *Phlomis* L., *Lamium* L., *Ballota* L., *Stachys* L. ve *Sideritis* L. cinslerinin morfolojik sistematiği ile desteklendiği; *Marrubium* L. cinsinin ise gruptan ayrıldığı belirlendi. *Nepeteoideae* alt familyasından *Nepeta* L., *Lallemantia* Fisch. & C.A. Mey, *Melissa* L., *Prunella* L., *Origanum* L., *Satureja* L., *Clinopodium* L., *Cyclotrichium* (Boiss.) Manden. & Scheng., *Mentha* L. ve *Salvia* L. cinsine ait taksonların morfolojik sistemi desteklediği, ancak *Ziziphora clinopodioides* Lam. taksonlarının farklılık gösterdiği gözlendi. Benzerlik matrisine göre benzerlik en çok 0.955 oranı ile *Clinopodium vulgare* L. subsp. *arundanum* (Boiss.) Nyman ve *Clinopodium graveolens* subsp. *rotundifolium* (Pers.) Govaerts ve 0.934 oranı ile *Salvia verticillata* L. subsp. *verticillata* ve *Salvia verticillata* subsp. *amasiaca* (Freyn

& Bornm.) Bornm. arasında bulunmuştur.

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Introduction

Plants that have existed since the beginning of life on earth have always been a matter of curiosity throughout human history and have been used for food, medicine, shelter, weapons, etc. used for the purposes. Plant species, which are also abundant in our country, are actively used in many areas of life (Güner and Ekim, 2014).

Türkiye is located at the intersection point of three phytogeographical regions: Europe-Siberia, Mediterranean, and Iran-Turan. Lamiaceae is distributed in a wide variety of habitats, from Hawaii to Northeast Asia, from the Himalayas to the Arctic, to Australia, Africa, and the Americas (Erdem et al., 2017, Zaman et al., 2022). The family Lamiaceae, which is one of the most important families in this rich geography and has 46 genera and more than 725 species in Türkiye, has spread around the world with approximately 250 genera and 7825 taxa (Harley et al., 2004, Jamzad, 2013, Rattray and Wyk, 2021; Elmas et al., 2021).

The plant taxa belonging to the Lamiaceae family are important medicinally and commercially because of their antitumor, antioxidant, antimicrobial, and anti-inflammatory effects and since they have an important place in the floristic diversity of Türkiye and are subject of interest by ethnobotanists (Luo et al., 2019). *Lavandula* L., *Melissa* L., *Mentha* L., *Origanum* L., *Rosmarinus* L., *Salvia* L., *Satureja* L., and *Thymus* L. used as curatives against gastrointestinal disorders, hypoglycemia, respiratory disorders, and as cardiotonic and antihypertensives (Khoury et al., 2016; Rattray and Wyk, 2021).

The classification of plants was done based on morphological observations until recently. Nowadays, taxonomists are more interested to separate plant species based on molecular systematics, which give more precise results and aid morphological diagnosis with precise convenience in classification.

Plant phylogeny has gained significant momentum, especially in the last few years. These developments have played an essential role in determining kinship, taxonomic classification, and genetic diversity especially, among plant species and populations.

There are problems in the morphological and biochemical classification of plants that are phenotypically close to each other but genotypically distant. For this reason, some DNA markers have been widely applied to analyze plant genetic diversity, detect genetic modification, and determine species classification (Bui et al., 2022). One of the techniques used for this purpose is the RAPD-PCR technique.

In this study, it was aimed to investigate the phylogenetic relationship of 54 taxa belonging to the Lamiaceae family in Bitlis Province with RAPD-PCR technique.

Material and Method

Table 1. Taxa belonging to Lamiaceae collected in Bitlis Province

Taxa	Locality	Voucher and Specimen code
***Ajuga chamaepitys* (L.) Schreb. subsp. *chia* (Schreb.) Arcang.**	Bitlis:4 km after from Küçüksu, Roadside, Slopes, 1750 m, 10.06.2014	M. Kurşat & S. Topdemir 1016
***Teucrium orientale* L. var. *glabrescens* Hausskn. ex Bornm.**	Bitlis: Northern slopes of Mount Kambos, 1800-1950 m, 18.07.2014	M. Kurşat & S. Topdemir 1048
***Teucrium chamaedrys* L. subsp. *sinuatum* (Celak.) Rech.f.**	Bitlis: Northern slopes of Mount Kambos, 1800-1950 m, 03.07.2014	M. Kurşat & S. Topdemir 1031
***Teucrium polium* L. subsp. *polium* L.**	Bitlis: Northern slopes of Mount Kambos, 1800-1950 m, 03.07.2014	M. Kurşat & S. Topdemir 1030
***Scutellaria albida* L. subsp. *condensata* (RECH. FIL.) EDMONDSON**	Bitlis: Northern slopes of Mount Kambos, 1800-1950 m, 12.06.2014	M. Kurşat & S. Topdemir 1017
***Scutellaria orientalis* L. subsp. *orientalis* L.**	Bitlis: Ağaçköprü village, 1350-1450 m, 14.07.2014	M. Kurşat & S. Topdemir 1044
***Phlomis lanceolata* BOISS. ET HOHEN.**	Bitlis: Between Tatvan and Hizan, 4 km after from Küçüksu, Roadside, Slopes, 1750 m, 10.06.2014	M. Kurşat & S. Topdemir 1015
***Phlomis kurdica* RECH. FIL.**	Bitlis: Northern slopes of Mount Kambos, 1750 m, 03.07.2014	M. Kurşat & S. Topdemir 1032
***Lamium garganicum* L. subsp. *striatum* (Sm.) Hayek**	Bitlis: Eastern Slope of Kambos Mountain, Rocky, 1900 m, 23.04.2014	M. Kurşat & S. Topdemir 1001
***Lamium macrodon* BOISS. ET HUET**	Bitlis: South of Kambos Mountain, Slopes, Oak, 1650 m, 15.03.2014	M. Kurşat & S. Topdemir 1000
***Lamium album* L.**	Bitlis. Bitlis Eren University Campus, 1950 m, 10.05.2014	M. Kurşat & S. Topdemir 1003
***Ballota nigra* L. subsp. *kurdica* P.H.Davis**	Bitlis: Tatvan, Hanelma village and its surroundings, 1750 m, 14.06.2014	M. Kurşat & S. Topdemir 1024
***Marrubium parviflorum* FISCH. ET MEY. subsp. *parviflorum* FISCH. ET MEY.**	Bitlis: Between Tatvan and Hizan, 4 km after from Küçüksu, Roadside, Slopes, 1750 m, 11.07.2014	M. Kurşat & S. Topdemir 1043
***Marrubium astracanicum* JACQ.**	Bitlis: North slope of Kambos Mountain, 1850 m, 12.06.2013	M. Kurşat & S. Topdemir 1018
***Sideritis vulcanica* HUB.-MOR.**	Bitlis: North slope of Kambos Mountain, 1800-1950 m, 12.06.2014	M. Kurşat & S. Topdemir 1019
***Stachys balansae* BOISS. ET KOTSCHY**	Bitlis: North slope of Kambos Mountain, in the creek, 1850 m, 12.06.2014	M. Kurşat & S. Topdemir 1020
***Stachys spectabilis* CHOISY EX DC.**	Bitlis: North slope of Kambos Mountain, 1800-1950 m, 12.06.2014	M. Kurşat & S. Topdemir, 1021
***Stachys megalodonta* HAUSSKN. ET BORNM. EX P. H. DAVIS subsp. *mardinensis* BHATTACHARJEE**	Bitlis: North slope of Kambos Mountain, 1750 m, 14.06.2014	M. Kurşat & S. Topdemir, 1023
***Stachys iberica* BIEB subsp. *stenostachya* (BOISS.) RECH. FIL.**	Bitlis: Bitlis Eren University Campus, 1950 m, 04.07.2014	M. Kurşat & S. Topdemir, 1038
***Stachys iberica* BIEB subsp. *georgica* RECH. FIL.**	Bitlis: North slope of Kambos Mountain, 1850 m, 24.06.2014	M. Kurşat & S. Topdemir 1028
***Stachys annua* (L.) L. subsp. *annua* (L.) L. var. *lycaonica* BHATTACHARJEE**	Bitlis: Ağaçköprü village, 1350-1450 m, 25.05.2014	M. Kurşat & S. Topdemir 1007
***Stachys lavandulifolia* VAHL.**	Bitlis: Bitlis Eren University Campus, 1850-1950 m, 17.07.2014	M. Kurşat & S. Topdemir 1046
***Melissa officinalis* L. subsp. *officinalis* L.**	Bitlis: Ağaçköprü village, 1350-1450 m, 18.07.2014	M. Kurşat & S. Topdemir 1047
***Nepeta italica* L.**	Bitlis: South of Mount Kambos, 1240-1650 m 06.06.2014	M. Kurşat & S. Topdemir 1011
***Nepeta nuda* L. subsp. *albiflora* (BOISS.) GAMS**	Bitlis: Bitlis Eren University Campus, 2000 m, 17.06.2014	M. Kurşat & S. Topdemir 1013
***Nepeta trachonitica* POST**	Bitlis: South of Mount Kambos, 1650 m, 28.05.2014	M. Kurşat & S. Topdemir 1009
***Nepeta macrosiphon* BOISS.**	Bitlis: Northern slopes of Mount Kambos, Streamside, 1800 m, 08.07.2014	M. Kurşat & S. Topdemir 1041
***Nepeta transcaucasica* GROSSH.**	Bitlis: Nemrut Crater Lake road, Serinbayır	M. Kurşat & S.

	village and its surroundings, 2080 m, 30.05.2014	Topdemir 1010
***Lallemantia canescens* (L.) FISCH. ET MEY.**	Bitlis: Nemrut Crater Lake road-Between Ahlat, roadside, step, 2380 m, 14.07.2014	M. Kurşat & S. Topdemir 1045
***Lallemantia peltata* (L.) FISCH. ET MEY.**	Bitlis: Bitlis Eren University Campus, 1950 m, 27.05.2014	M. Kurşat & S. Topdemir 1008
***Prunella vulgaris* L.**	Bitlis: Ağaçköprü village, 1450 m, 18.07.2014	M. Kurşat & S. Topdemir 1042
***Origanum acutidens* (HAND.-MAZZ.) IETSWAART**	Bitlis: Ağaçköprü village and streamside, 1400 m, 26.07.2013	M. Kurşat & S. Topdemir 1052
***Origanum vulgare* L. subsp. *gracile* (C. KOCH) IETSWAART**	Bitlis: Ağaçköprü village, 1450 m, 18.07.2014	M. Kurşat & S. Topdemir 1051
***Satureja hortensis* L.**	Bitlis: Tatvan-Ahlat highway, Adabağ village and its surroundings, 1900 m, 22.09.2014	M. Kurşat & S. Topdemir 1053
***Clinopodium vulgare* L. subsp. *arundanum* (BOISS.) NYMAN**	Bitlis: East of Mount Kambos, 1400-1600 m, 18.06.2014	M. Kurşat & S. Topdemir 1027
***Clinopodium graveolens* (M.Bieb.) Kuntze subsp. *rotundifolium* (Pers.) Govaerts**	Bitlis: Bitlis Eren University Campus, 1950 m, 06.06.2014	M. Kurşat & S. Topdemir 1012
***Cyclotrichium glabrescens* (BOISS. ET KOTSCHY EX RECH. FIL.) LEBLEBİCİ**	Bitlis: Northern slopes of Mount Kambos, rocky, 1950 m, 07.07.2014	M. Kurşat & S. Topdemir, 1039
***Thymus kotschyanus* BOISS. ET HOHEN.**	Bitlis: Bitlis Eren University Campus, 1950 m, 04.07.2014	M. Kurşat & S. Topdemir 1036
***Mentha longifolia* (L.) HUDSON subsp. *typhoides* (BRIQ.) HARLEY**	Bitlis: Bitlis Eren University Campus, 1950 m, 04.07.2014	M. Kurşat & S. Topdemir 1037
***Ziziphora capitata* L.**	Bitlis: Bitlis Eren University Campus, 1950 m, 17.05.2015	M. Kurşat & S. Topdemir 1006
***Ziziphora clinopodioides* LAM.**	Bitlis: Bitlis Eren University Campus, 1850 m, 08.07.2014	M. Kurşat & S. Topdemir 1040
***Salvia macrochlamys* BOISS. ET KOTSCHY**	Bitlis: East of Mount Kambos, 1650 m, 18.06.2014	M. Kurşat & S. Topdemir 1025
***Salvia trichoclada* BENTHAM**	Bitlis: South of Mount Kambos, 1750 m, 15.05.2014	M. Kurşat & S. Topdemir 1004
***Salvia multicaulis* VAHL.**	Bitlis: East slope of Kambos Mountain, 1550 m, 23.04.2014	M. Kurşat & S. Topdemir 1002
***Salvia sclarea* L.**	Bitlis: Exit of Bitlis, Industrial Environment, 1550 m, 15.07.2014	M. Kurşat & S. Topdemir 1049
***Salvia frigida* BOISS.**	Bitlis: Bitlis Eren University Campus, 1850-1950 m, 15.05.2014	M. Kurşat & S. Topdemir 1005
***Salvia poculata* NAB.**	Bitlis: South of Mount Kambos, 1650 m, 17.06.2014	M. Kurşat & S. Topdemir 1026
***Salvia odontochlamys* HEDGE**	Bitlis: Northern slopes of Mount Kambos, 1800-1950 m, 03.07.2014	M. Kurşat & S. Topdemir 1033
***Salvia virgata* JACQ.**	Bitlis: Ağaçköprü village and streamside, 1350-1450 m, 26.07.2014	M. Kurşat & S. Topdemir 1050
***Salvia nemorosa* L.**	Bitlis: Güroymak, 1250 m, 08.06.2014	M. Kurşat & S. Topdemir 1022
***Salvia verticillata* L. subsp. *verticillata* L.**	Bitlis: Northern slopes of Mount Kambos, 1800-1950 m, 03.07.2014	M. Kurşat & S. Topdemir 1034
***Salvia verticillata* L. subsp. *amasiaca* (FREYN ET BORNM.) BORNM.**	Bitlis: Northern slopes of Mount Kambos, 1800-1950 m, 03.07.2014	M. Kurşat & S. Topdemir 1035
***Salvia candidissima* VAHL. subsp. *candidissima* VAHL.**	Bitlis: Between Tatvan and Hizan, 4 km after from Küçüksu, Roadside, Slopes, 1750 m, 10.06.2014	M. Kurşat & S. Topdemir 1014
***Salvia limbata* C. A. MEYER**	Bitlis: Ahlat, Seljuk Cemetery and Surroundings, 1650 m, 25.06.2014	M. Kurşat & S. Topdemir 1029

Plant samples used in the study

As the study materials, 21 genera of the family Lamiaceae grew in Bitlis Province and 54 taxa belonging to these genera were collected in vegetation periods between 2014-2015. Nine volumes of

the work titled “Flora of Turkey and the East Aegean Island’s (Davis, 1965-1985), Flora of Turkey and the East Aegean Island’s Supply. Vol: 10 vol. (Davis, 1988) Flora of Turkey and the East Aegean Island. Vol: 11 (Güner et al., 2000) were used for identification of the plants. The voucher samples are preserved in the Herbarium of Bitlis Eren University (Table 1).

DNA isolation

Fresh samples were crushed in liquid nitrogen to break down the cell wall and isolate total genomic DNA (nuclear and chloroplast DNA). This isolation step is essential for obtaining clean and pure DNA (Bozarı et al., 2014). The isolation process was performed with the Geneaid DNA Isolation Kit.

RAPD-PCR procedures

The samples were screened for RAPD variation using the standard supplied 10-base operon primers: For a master mixture, pure water (880 µL), 10xbuffer (150 µL), deoxynucleoside triphosphates (30 µL), magnesium chloride (60 µL), and Taq polymerase (25 µL) were prepared. The PCR samples contained a total of 30 µL including 24 µL of the master mixture, 3 µL of primer, and 3 µL of DNA. Fifty oligonucleotide primers were screened, and among them, 9 primers were selected and used for further studies. Sequences (5’→3’) from primers 1 to 9 utilized were GGACTGGAGT (OPL-1), CAGGCCCTTC (OPL-2), AGGTGACCGT (OPL-3), CCCGGATGGT (OPL-4), GTGTGCCCCA (OPL-5), GTCGCCGTCA (OPL-6), CAGCACCAGG (OPL-7), CCGCCTAGTC (OPL-8), and GGTCCCTGAC (OPL-9), respectively (Morden and Loeffler, 1999).

The thermal cycle was prepared as follows: 4 min. at 94°C; 40 cycles (for each step); 45 sec. at 94°C, 45 sec. at 36°C, 60 sec. at 72°C; 1 cycle (for each step); 8 min. at 72°C, and then brought down to 4°C.

Data analysis

Genetic analysis was performed on the photos taken with the gel imaging system. The presence (1) and the absence (0) of the bands were counted. After the data matrix was transferred to the computer environment, it was analyzed by using SPSS IBM Statistic Version 22 program. Binary-Jaccard criteria were chosen as the measure to calculate. Since this analysis is based on the determination of proximity and genetic similarity between species, this option is preferred in the present study.

Results and Discussion

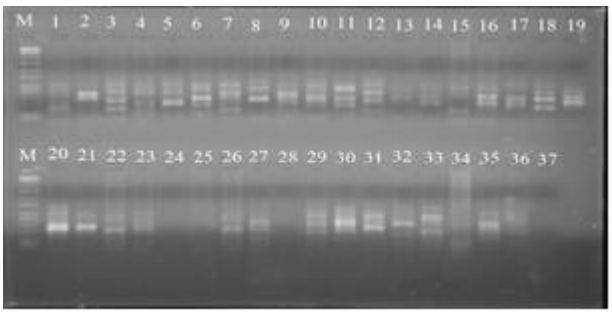

Figure 1. Gel image of OPL 1 primer.

M: Marker, 1: *A. chamaepitys* subsp. *chia***,** 2: *T. orientale* var. *glabrescens*, 3: *T. chamaedrys* subsp. *sinuatum*, 4: *T. polium* subsp. *polium*, 5: *S. albida* subsp. *condensata*, 6: *S. orientalis* subsp. *orientalis*, 7: *P. lanceolata*, 8: *P. kurdica*, 9: *L. garganicum* subsp. *striatum* var. *striatum*, 10: *L. macrodon*, 11: *L. album*, 12: *B. nigra* subsp. *kurdica*, 13: *M. parviflorum* subsp. *parviflorum*, 14: *M. astracanicum*, 15: *S. vulcanica*, 16: *S. balansae*, 17: *S. spectabilis*, 18: *S. megalodonta* subsp. *mardinensis*, 19: *S. iberica* subsp. *stenostachya*, 20: *S. iberica* subsp. *georgica*, 21: *S. annua* subsp. *annua* var. *lycaonica*, 22: *S. lavandulifolia*, 23: *M. officinalis* subsp. *officinalis*, 24: *N. italica* , 25: *N. nuda* subsp. *albiflora*, 26: *N. trachonitica*, 27: *N. macrosiphon*, 28: *N. transcaucasica*, 29: *L. canescens*, 30: *L. peltata*, 31: *P. vulgaris*, 32: *O. acutidens*, 33: *O. vulgare* subsp. *gracile*, 34: *S. hortensis*, 35: *C. vulgare* subsp. *arundanum*, 36: *C. graveolens* subsp. *rotundifolium*, 37: *C. glabrescens*.

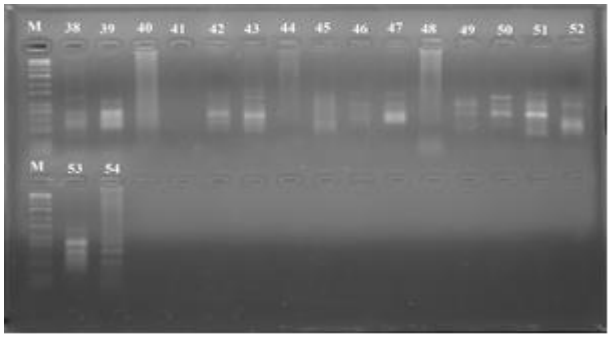

Figure 2. Gel image of OPL 1 primer. (Continued)

M: Marker**,** 38: *T. kotschyanus*, 39: *M. longifolia* subsp. *typhoides* 40: *Z. capitata*, 41: *Z. clinopodioides*, 42: *S. macrochlamys*, 43: *S. trichoclada*, 44: *S. multicaulis*, 45: *S. sclarea*, 46: *S. frigida*, 47: *S. poculata*, 48: *S. odontochlamys*, 49: *S. virgata*, 50: *S. nemorosa*, 51: *S. verticillata* subsp. *verticillata*, 52: *S. verticillata* subsp. *amasiaca*, 53: *S. candidissima* subsp. *candidissima* 54: *Salvia limbata*.

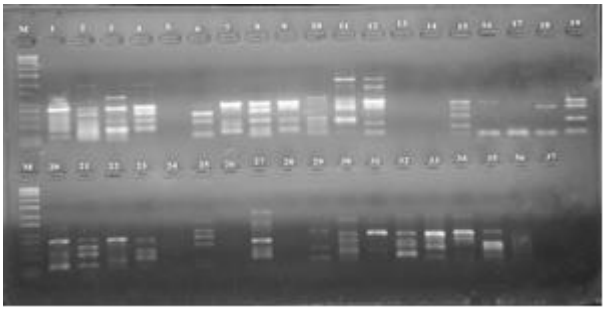

Figure 3. Gel image of OPL 2 primer.

M: Marker, 1: M: Marker, 1: *A. chamaepitys* subsp. *chia***,** 2: *T. orientale* var. *glabrescens*, 3: *T. chamaedrys* subsp. *sinuatum*, 4: *T. polium* subsp. *polium*, 5: *S. albida* subsp. *condensata*, 6: *S. orientalis* subsp. *orientalis*, 7: *P. lanceolata*, 8: *P. kurdica*, 9: *L. garganicum* subsp. *striatum* var. *striatum*, 10: *L. macrodon*, 11: *L. album*, 12: *B. nigra* subsp. *kurdica*, 13: *M. parviflorum* subsp. *parviflorum*, 14: *M. astracanicum*, 15: *S. vulcanica*, 16: *S. balansae*, 17: *S. spectabilis*, 18: *S. megalodonta* subsp. *mardinensis*, 19: *S. iberica* subsp. *stenostachya*, 20: *S. iberica* subsp. *georgica*, 21: *S. annua* subsp. *annua* var. *lycaonica*, 22: *S. lavandulifolia*, 23: *M. officinalis* subsp. *officinalis*, 24: *N. italica*, 25: *N. nuda* subsp. *albiflora*, 26: *N. trachonitica*, 27: *N. macrosiphon*, 28: *N. transcaucasica*, 29: *L. canescens*, 30: *L. peltata*, 31: *P. vulgaris*, 32: *O. acutidens*, 33: *O. vulgare* subsp. *gracile*, 34: *S. hortensis*, 35: *C. vulgare* subsp. *arundanum*, 36: *C. graveolens* subsp. *rotundifolium*, 37: *C. glabrescens*.

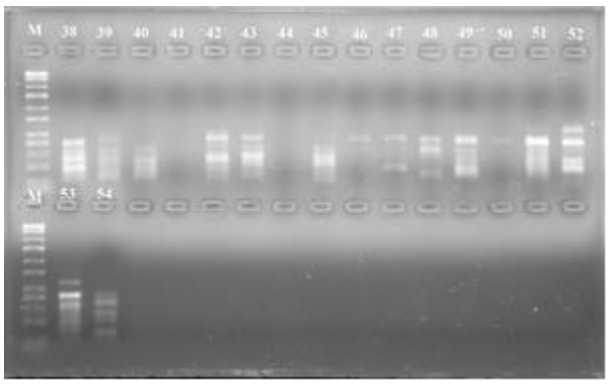

Figure 4. Gel image of OPL 2 primer. (Continued)

M: Marker**,** 38: *T. kotschyanus*, 39: *M. longifolia* subsp. *typhoides* 40: *Z. capitata*, 41: *Z. clinopodioides*, 42: *S. macrochlamys*, 43: *S. trichoclada*, 44: *S. multicaulis*, 45: *S. sclarea*, 46: *S. frigida*, 47: *S. poculata*, 48: *S. odontochlamys*, 49: *S. virgata*, 50: *S. nemorosa*, 51: *S. verticillata* subsp. *verticillata*, 52: *S. verticillata* subsp. *amasiaca*, 53: *S. candidissima* subsp. *candidissima* 54: *Salvia limbata*.

Figure 5. The dendrogram of genetic relationships among taxa belonging to the Lamiaceae family

The rapid development of molecular biology, one of the sub-branches of biology, has great importance in plant systematics. Molecular systematic studies can easily find a solution for species that are problematic during identification during molecular identification (Kochieva et al., 2006; Al-Rawashdeh, 2011; Özcan et al., 2015).

The genetic relationships of the taxa belonging to the Lamiaceae spread in Bitlis province were included in the study and investigated by using the RAPD-PCR technique. The literature studies revealed that taxa belonging to the family Lamiaceae were not examined at the family level by using RAPD-PCR technique. In this study, it was understood that RAPD-PCR gave reliable results in genetic studies.

As seen in the dendrogram shown in Figure 5, the taxa belonging to the *Teucrium* were found parallel to the morphological classification made according to Davis (1982). *T. chamaedrys* subsp. *sinuatum* and *T. polium* subsp. *polium* taxa showed similarity at the rate of 0,908.

The *T. orientale* var. *glabrescens*, which is slightly more distant than the expected morphological affinity, is close to *T. chamaedrys* subsp. *sinuatum* at the rate of 0.862, while its proximity rate to *T. polium* subsp. *polium* was 0.836. *A. chamaepitys* subsp. *chia* (0.625), the only taxon of *Ajuga*, which is the closest genus to *Teucrium*, showed proximity to *T. orientale* var. *glabrescens* with the highest similarity rate, and it was observed that it formed a separate group with this genus. This supports the morphological taxonomy. Özcan et al. (2015) revised the genus *Teucrium* with the ITS, nrDNA technique. The researchers identified the species *T. sirnakense* L'Hér. Özcan and Dirmenci, which are very close to *T. melissoides* Boiss & Hausskn and *T. scordium* L., during their study in Şırnak Province. It was observed that morphological characters, as well as molecular data, were used to determine a new taxon.

Scutellaria albida subsp. *condensata* showed the highest similarity to *Scutellaria orientalis* subsp. *orientalis* with a 0.700 similarity coefficient. In addition, it was observed that they were included in the same genetic group with the *Teucrium* and *Ajuga* genera (Figure 5). It was observed that this genetic grouping supports the morphological classification made by Davis (1982). While *Ajuga* and *Teucrium* are involved in the Ajugoideae subfamily, *Scutellaria* is involved in the Scutellarioideae subfamily. Safikhani et al. (2018) In Iran, 42 taxa belonging to the subgenus *Apeltanthus* and *Scutellaria* were searched using the nrDNA ITS and trnL-F sequences. They reported that in both ITS and trnL-F trees, there were two main branches within the genus, corresponding to the two subgenus *Scutellaria* and *Apeltanthus*. Consequently, they proposed revising the cross-section classification of both *Apeltanthus* and *Scutellaria* subspecies. Again, in a similar study, Chiang et al. (2012) conducted the phylogenetic analysis of *Scutellaria* taxa, which are endemic to Taiwan, using nuclear and chloroplast DNA markers. As a result, by uncovering multiple sources of Taiwanese *Scutellaria* species and the endemic species, especially *S. indica* L., *S. austrotaiwanensis* C.X. Xie & T.C. Huang confirmed that the "indica group" consisting of *S. tashiroi* Hayata and *S. playfairii* Kudô was rapid and novel speciation.

The similarity rate between *Phlomis lanceolata* and *Phlomis kurdica* was 0.908, and the two species were genetically very close to each other as expected. Sarkhail et al. (2014) The genetic distance range between different *Phlomis* species in Iran was calculated as 316-988. In fact, the furthest genetic distance (d = 0.990) was observed between *P. bruguieri* and *P. olivieri* species. The distance between *P. anisodonta* and *P. persica* (d = 0.988), as well as *P. persica* and *P. anisodonta* (d = 0.988), the closest distance (d = 316) was observed for *P. persica* and *P. olivieri*. Similar to the present study, Yüzbaşıoğlu and Dadandı (2008b), using the same technique, used randomly amplified polymorphic DNA markers to determine the genetic relationships among the species of the Dendrophlomis subdivision. Twenty members of twelve *Phlomis* taxa were analyzed with 14 selected primers and reported that they produced 85 RAPD bands. The researchers stated that the genetic distances ranged from 0.133 (between *P. amanica* Vierh. and *P. monocephala* P.H. Davis) to 0.494 (between *P. chimerae* Boissieu and *P. lunariifolia* Sm.) and divided the UPGMA tree into two main groups based on the distances.

While the genetic distance measured between the three taxa belonging to the *Lamium* is 0.838 between *L. garganicum* subsp. *striatum* var. *striatum* and *L. macrodon*, it is 0.787 between *L. garganicum* subsp. *striatum* var. *striatum* and *L. album*. The genetic distance between *L. macrodon* and *L. album* is 0.789. As can be seen from the numeric data, these results supported the morphological classification, with the highest similarity between *L. garganicum* subsp. *striatum* var. *striatum* and *L. macrodon* (0,838). It was observed that the genus *Lamium* made a group among itself and merged with the closest *Phlomis* (at the highest rate of 0.556). Krawczyk and Sawicki (2013) investigated the molecular evolution rates of rpoS genes and evaluated them as a phylogenetic marker in the genus *Lamium* (Lamiaceae). As a result of the analysis, the researchers concluded that genes differed in the level of variation, intragenic mutation rate, phylogenetic informativeness, and the effect of these mutations on the properties of encoded peptides. Also they reported that rpoS genes were reliable phylogenetic markers useful in reconstructing the connections of species belonging to the same genus.

In the present study, *Ballota nigra* subsp. *kurdica* was not evaluated within its own genus because it is the only taxon of its genus, and its proximity to members of the genera *Lamium* and *Phlomis* was calculated. It was observed as the furthest member to its group with its similarity rate of 0.506 and the similarity rate to *L. album* with 0.379 and *P. lanceolata*. The data of the present study are parallel to the morphological classification made according to Davis (1982). Bendiksby et al. (2011) stated that the genus *Ballota* is polyphyletic; it is similar to other Lamioideae genera but does not come from a common ancestor. Scheen et al. (2010) stated that *Ballota* taxa should be placed in the genus *Acanthoprasium*, but because they were not included in the analysis of *B. frutescens* in Europe, they were reluctant to suggest this taxonomic change.

M. parviflorum subsp. *parviflorum* and *M. astracanicum* taxa showed an affinity at the rate of 0.900 according to the similarity matrix shown in Figure 5. However, although the *Marrubium* members belong to the Lamioideae subfamily, they formed groups alone and remained distant from other

subfamily members. In their study, Scheen et al. (2010) stated that it is not possible to say whether or not *Marrubium* is still monophyletic in its limited form based on the current molecular phylogeny. The researchers did not mind that although the status of *Marrubium* and *Ballota* is still unresolved, *Marrubium*s and *Ballota* stayed in the same subfamily. On the other hand, Bendiksby et al. (2011) argued that *Marrubium* appeared monophyletic, but *Ballota* and *Marrubium* should be studied in more detail to solve the general limitations. These results were similar to the *Marrubium* of the present study. This situation brings to mind the possibility of migration, mutation, geographical distance, and natural selection.

Since *Sideritis vulcanica* is the only member of its genus among the collected samples, intra-genus evaluation could not be made. However, the similarity of *Sideritis vulcanica* to *Stachys megalodon* subsp. *mardinensis* with at the rate of 0.523 and the fact that both genera belong to the Lamioideae subfamily reveals the justification of this similarity. Bendiksby et al. (2011) It has been reported that the genera *Ballota, Lagopsis, Lamium, Leonotis, Leonurus, Leu-cas, Microtoena, Moluccella, Otostegia, Phlomoides, Sideritis, Stachys,* and *Thuspeinanta* are not monophyletic. Similarly, our results, determined that *Sideritis* was not monophyletic alone but was closely related to *Stachys*. The data of the present study support the morphological classification based on the location of the genus *Sideritis* in the Flora of Turkey (Davis, 1982).

The genetic affinity of *Stachys*, the second-largest genus among the collected plant samples, was particularly important in this study. It was observed that *S. megalodonta* subsp. *mardinensis* and *S. iberica* subsp. *stenostachya* taxa (Figure 5) formed a group and show proximity at the rate of 0.889. *S. iberica* subsp. *georgica* and *S. lavandulifolia* formed a separate group by showing similarity at the rate of 0.893. *S. iberica* subsp. *stenostachya* and *S. iberica* subsp. *georgica* were expected to show the highest similarity to each other. But it was observed that they stayed away with a slight difference with the rate of 0.807.

The fact that this rate was too low did not raise any doubts about its place in morphological systematic. It was observed that *Stachys balansae* was most closely associated with *S. lavandulifolia* with a similarity matrix of 0.847. *S. spectabilis* formed a group with *S. annua* subsp. *annua* var. *lycaonica* with the a rate of 0.857. The fact that *Stachys* formed a group among themselves and later formed a separate group with the genera *Phlomis, Lamium,* and *Ballota* proved the morphological systematics made for the subfamily Lamioideae. Kochieva et al. (2006), who reported a similar result to the present study, made a molecular analysis of 14 species belonging to the genus *Stachys* collected by ISSR and RAPD. As a result of this analysis, they refined the systematically accepted phylogenetic positions of some *Stachys* species. As a result of molecular data, *S. lanata* Jacq. and *S. byzantina* K. Koch concluded that species were synonymous while they stated that the taxa of *S.sieboldii* Miq. and *S. affinis* Bunge were separate species. Such and similar studies have provided serious data on molecular classification techniques and the location of plant taxa.

It was observed that the *Melissa officinalis* subsp. *officinalis* showed the highest similarity to *S. poculata*, one of the members of the genus *Stachys*, which it unites in the Nepetoideae because it is the only taxon of its genus among the plant samples collected.

The genus *Nepeta*, known as the Cat Mint, has a particular taxonomic importance for us (Güner et al., 2012). As seen in Figure 5, the genus *Nepeta* formed a separate group within itself. In this group, *Nepeta italica* was most closely related to *N. transcaucasica* with the rate of 0.815, *N. nuda* subsp. *albiflora* and *N. macrosiphon* with a rate of 0.814 and formed a separate group. Although *N. trachonitica* was later attached to both groups, it was most closely related to *N. transcaucasica* with a rate of 0.615. Al-Qurainy et al. (2014) strengthened the nuclear and chloroplast gene locus to define and preserve the identity of this species to enhance the DNA barcode and phylogenetic study of *N. deflersiana* Schweinf. ex Hedge. In addition, the researchers made phylograms of *N. deflersiana* and other *Nepeta* species from the GenBank database. As a result, they placed *N. deflersiana* in the same class as *N. insaurica* Hedge with a boot value of 99%. Kaufmann and Wink (1994) examined 41 species of Nepetoideae subfamily with rbcL specific primers. They stated that it was compatible with the classical systematics. The results are compatible with these studies.

L. canescens and *L. peltata*, two members of the genus *Lallemantia*, were found to form a group among themselves by showing similarity at the rate of 0.843 (Figure 5). After confirmation with this morphological classification was revised and the classification of *Prunella vulgaris*, of the *Prunella*, was noted closest to *Lallemantia* and included in the group with with similarity rate of 0.660 and renamed as *Lallemantia canescens*. Morphologically, *Lallemantia* and *Prunella* genera are included in the subfamily *Nepetoideae* and supported by the created dendrogram. Koohdar et al. (2016) investigated the genetic variability and population structure of samples collected by *Lallemantia royleana* Benth from 11 geographic populations. Genetic diversity parameters were determined in these populations. It has been reported that there is some gene change among the studied populations and populations based on morphological characters compatible with the NJ molecular data tree of UPGMA dendrogram.

The similarity rate of 0.923 between *Origanum acutidens* and *O. vulgare* subsp. *gracile* proved that the *O. vulgare* subsp. *gracile* was genetically close to *O. vulgare* and showed compatibility with the morphological systematics (Figure 5). Tonk et al. (2010) determined genetic variation by using DNA (RAPD) markers in their studies with 14 *O. onites* L. They reported that thyme clones were basically divided into three main groups by clustering, and the genetic similarity values between the samples ranged between 0.49 and 0.73. This indicated that genetic variation was low. The high similarity rate in the present study increased the similarity coefficient.

The similarity matrix between *Clinopodium vulgare* subsp. *arundanum* and *C. graveolens* subsp. *rotundifolium* was 0.955, showing that the two taxa were very close to each other (Figure 5). As seen in Figure 5, the combination of these two species with *Satureja hortensis* by forming a group between them supported the morphological place of the subfamily Nepetoideae. *Satureja hortensis* was found

similar to *C. vulgare* subsp. *arundanum* with the rate of 0.569. Drew and Sytsma (2012) conducted phylogenetic analysis of *Menthinae* sub-tribe species using cpDNA and nrDNA techniques. Therefore, they found three main levels within the Menthinae subtribe; (1) a clad including *Acinos* Miller, *Bystropogon* L'Hér., *Clinopodium* and *Ziziphora*, (2) *Micromeria* and *Mentha arvensis*, (3) a taxon restricted to a new alias candidate. These researchers contributed to molecular classification and the location of the Menthinae subtribe. The results were supported by the findings of Drew and Sytsma (2012).

Since *Cyclotrichium glabrescens* is the only member of its genus among the samples examined, no intrageneric classification was made. Therefore, *C. glabrescens* showed similarity to *Ziziphora clinopodioides* at the rate of 0.321, as seen in Figure 5, when its proximity to other taxa was examined. This is an expected affinity since *Cyclotrichium* and *Ziziphora* genera were in the Nepetoideae subfamily. But the genus *Cyclotrichium* based on the Flora of Turkey is more closer to the *Thymus* and *Mentha* taxa. Dirmenci et al. (2010) analyzed the genus *Cyclotrichium* from morphological, phylogenetic, and cytogenetic aspects. The researchers reported that all species of the genus were examined for their morphological characters and core ribosomal ITS (internal transcribed spacers) DNA sequences, but they did not participate in ITS sequence analysis (morphological examination was made only from the type sample) since *C. hausknechtii* (Bunge) Manden & Scheng. As a result, it has been concluded that *Cyclotrichium* is a different genus within the Nepetoideae subfamily with its distinctive morphological, phylogenetic, and cytogenetic features. Considering the intrageneric phylogenetics, *Cyclotrichium* is divided into three groups: 1. *C. niveum* (Boiss.) Manden. & Scheng, 2. *C. origanifolium* (Labill.) Manden. & Scheng and 3. the remaining six species. As a result, they empasize that the genus *Cyclotrichium* was the closest to the genera *Clinopodium* and *Mentha*. However, in the present study, the taxon belonging to the genus *Cyclotrichium* was found closer to the genus *Ziziphora*.

In *Thymus kotschyanus* and *Mentha longifolia* subsp. *typhoides*, they are the only representatives of their genus among the examples in the present study. The similarity between *Thymus kotschyanus* and *Mentha longifolia* subsp. *typhoides* has been found to be 0.661. The morphological affinities of *Thymus* and *Mentha* genera were genetically supported (Figure 5). Apostolova et al. (2016) applied the ISSR technique to determine the genetic similarities between the *Mentha* species they collected in Bulgaria and stated that the primers tested were appropriate for the evaluation of genetic relationships between genotypes in the *Mentha* and the performed ISSR technique would be easily applied. The researchers stated that the *Mentha* taxa were appropriate for comparing with morphological data in the dendrogram they created within the genus. Yousefi et al. (2015) collected 13 *Thymus* taxa from different geographical regions of Iran and one from England (*T. vulgaris* L.). They analyzed them by Randomly Replicated Polymorphic DNA (RAPD) markers using 20 primers to explore genetic polymorphism. It was reported that a total of 510 bands were detected from 20 RAPD primers and 483 of them (94.31%) gave polymorphic bands. The researchers performed the UPGMA cluster analysis

using Jaccard similarity coefficients based on RAPDs. The dendrogram they obtained from the method divided 14 thyme taxa into four main groups. Again, the researchers reported that the distribution based on basic coordinate analysis (PCoA) revealed four groups in the biplot and confirmed the results of the clustering method with some minor discrepancies.

The similarity rate of both *Ziziphora* species in the present study was observed as 0.396. *Z. capitata* was close to *T. kotschyanus* with 0.485 and to *Mentha longifolia* subsp. *typhoides* with 0.500 (Figure 5). Another *Ziziphora* species, *Z. clinopodioides*, was linked to the *Nepeta* group and showed similarity of 0.448 to *N. transcaucasica*. Making a conclusion that supports this situation, Tabaripour et al. (2020), 69 individuals were collected from 19 randomly selected populations belonging to the *Z. clinopodioides*. In addition, the combination of morphological and molecular data of plants collected from 5 geographical regions was compared. Both analyses revealed a high level of intra-population variability, and the classification of provinces did not reveal any subspecies among species.

Salvia, the most crowded genus of the present study and known as sage, are also important in terms of morphology and genetics. *Salvia verticillata* subsp. *verticillata* and *Salvia verticillata* subsp. *amasiaca* are expected to be very close genetically. The fact that they form a group that supports this expectation and the proximity rate of these two taxa is 0.934 which supports the expectation regarding morphological classification and proves the reliability of genetic classification. According to the dendrogram in Figure 5, *Salvia nemorosa* formed a group with *Salvia candidissima* subsp. *candidissima* and *Salvia limbata*, and *Salvia nemorosa* was close to *Salvia candidissima* subsp. *candidissima* by 0.915 and *Salvia limbata* by 0.914. Although this group was later linked to the group formed by *Salvia verticillata* subsp. *verticillata* and *Salvia verticillata* subsp. *amasiaca*, the similarity between *Salvia verticillata* subsp. *verticillata* and *Salvia candidissima* subsp. *candidissima* was found to be 0.917 (Figure 5). *Salvia odontochlamys* was similar to *Salvia virgata* with the rate of 0.897 and *Salvia nemorosa* at a rate of 0.897 (Figure 5). This is similar to morphological systematics in the Flora of Turkey. *Salvia poculata* was found to be equally close to all three species (*Salvia odontochlamys, Salvia virgata,* and *Salvia nemorosa)* at the rate of 0.862 and was included in the same group with these species. This suggests that the RAPD primers we have were randomly linked, and these primers were linked to the same site in some way.

Salvia trichoclada and *Salvia sclarea* were found to form a separate group by showing proximity of 0.883. *Salvia macrochlamys* is linked to this group by showing proximity to *Salvia sclarea* with a rate of 0.841. On the other hand, *Salvia multicaulis* was found to be 0.741 close to *Salvia frigida.* This group was later linked to other taxa of the *Salvia* genus as the furthest taxa with this rate. However, this has collected the *Salvia* taxa together. This contributed to the morphological systematics. In their study, Sözen and Yücel (2015) obtained a parallel result with the present study and stated that the data obtained in terms of genetic relationship of 4 *Salvia* species that are endemic in their study were compatible with morphological data and the RAPD-PCR technique is an appropriate technique for determining the genetic relationship. Öncü et al. (2015) applied a newly developed capillary gel

electrophoresis (CGE) with the determination of RAPD-PCR products following dynamic coating with hydroxyethyl cellulose method and a PCR purification cleaning procedure for some *Salvia* (sage) species to separate fourteen standard DNA fragments. The developed CGE has been successfully applied in ten different Turkish *Salvia* (sage) species (*S. bracteata* Banks & Sol., *S. candidissima* Vahl, *S. ceratophylla* L., *S. dichroantha* Stapf (endemic), *S. forskahlei* L., *S. fruticosa* Mill., *S. sclarea* L., *S. tomentosa* Mill., *S. verticillata* L. and *S. viridis* L.). According to the phylogenetic analysis results, *S. fruticosa* and *S. dichroantha* were the most distant genetically, while *S. bracteata* and *S. fruticosa* were reported as the most similar species. When Figure 5 was examined, it was indicated that the connections between the genera are parallel to the subfamily systematics. This situation is parallel to the morphological systematic affinity of *Salvia*, *Melissa*, *Nepeta*, *Lallemantia*, *Prunella*, *Origanum*, *Satureja*, *Clinopodium*, *Cyclotrichium*, *Thymus*, *Mentha* and *Ziziphora* genera in Nepetoideae. However, while *Melissa officinalis* subsp. *officinalis*, the only member of the *Melissa*, should be closer to the *Nepeta*, its proximity to *Stachys* has brought the possibility of a systematic change in its place. When Figure 5 was examined, it was seen that *Phlomis*, *Lamium*, *Ballota*, *Marrubium*, *Sideritis*, and *Stachys* genera, which are affiliated to Lamioideae subfamily, first formed a group within their own genus and then as subfamily. It is thought that the *Marrubium*, which does not comply with this situation, has moved away from the Lamioideae due to the possibilities of migration, mutation, geographical distance, and natural selection. According to the dendrogram in Figure 5, it was seen that members of the *Scutellaria* belonging to the Scutellarioideae subfamily formed a group.

According to the dendrogram in Figure 5, it was observed that the *Ajuga* and *Teucrium* genera of the Ajugoideae subfamily first showed proximity within themselves and then between the genera.

Conclusion

In this study, the proximity relationship between 21 genera belonging to the family Lamiaceae and 54 taxa belonging to these genera was revealed within the geographical limits of Bitlis Province. This will be the first study at Bitlis region and Lamiaceae family with RAPD-PCR technique. Upon comparison of the results obtained in genetic analysis in the family Lamiaceae, determined potential places of the species in the Flora of Türkiye, their locations, proximity relations, biodiversity, place in genetic systematics and distribution areas that will make essential contributions in the the scientific world.

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Statement of Conflict of Interest

Authors have declared no conflict of interest.

Author's Contributions

The contribution of the authors is equall.

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