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Phylogenetic relationships among Lamiaceae species from Aydin (Türkiye), based on *rbc*L sequences

Original Article

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Abstract:

In this study, phylogenetic analyses were performed using chloroplast *rbc*L sequences from *Ajuga chamaepitys, Thymbra capitata, Lamium moschatum, Marrubium vulgare, Stachys cretica, Teucrium polium*, and *Vitex agnus-castus* species distributed in Aydın province, Türkiye. After isolating DNA from the samples, PCR amplification was carried out using the *rbc*LaF and *rbc*LaR primers. Both forward and reverse sequences were edited using BioEdit 7.2.3, FinchTV 1.4.0, and Sequencher 5.4.6 software. The corrected sequences were converted to protein sequences, and phylogenetic analyses were conducted using MEGA 6.0 software. The *rbc*L proteins have been motif analysed and their 3D structure revealed. As a result, the phylogenetic tree constructed with sequences obtained from National Center for Biotechnology Information (NCBI) showed that *Lamium* and *Marrubium* species formed a clade, while *Sideritis* and *Stachys* species formed another one. Additionally, the species of *Thymbra*, *Vitex*, *Ajuga*, and *Teucrium* each formed distinct groups. In the analyses involving only these seven species, the genetic distance matrix revealed that the closest species were *Ajuga chamaepitys* and *Vitex agnus-castus* (0.009), while the most distantly related species were *Teucrium polium* and *Thymbra capitata* (0.043). Also, the nucleotide diversity was calculated to be $\pi = 0.025278$. Overall, the *rbc*L sequence results were instrumental in elucidating the phylogenetic relationships within the Lamiaceae family.

Key words:
Lamiaceae, phylogeny, *rbc*L, Türkiye

Apstrakt:

Filogenetski odnosi između vrsta iz porodice Lamiaceae iz Ajdina (Turska) na osnovu *rbc*L sekvenci

U ovoj studiji su, korišćenjem hloroplastnih *rbc*L sekvenci *Ajuga chamaepitys*, *Thymbra capitata*, *Lamium moschatum*, *Marrubium vulgare*, *Stachys cretica*, *Teucrium polium* i *Vitex agnus-castus*, vrsta prisutnih u provinciji Ajdin, Turska, sprovedene filogenetske analize. Nakon izolacije DNK iz uzoraka, izvršena je PCR amplifikacija koristeći *rbc*LaF i *rbc*LaR prajmere. Obe sekvence (forward i reverse) uređene su korišćenjem softvera BioEdit 7.2.3, FinchTV 1.4.0 i Sequencher 5.4.6. Korigovane sekvence konvertovane su u proteinske sekvence, a filogenetske analize sprovedene korišćenjem softvera MEGA 6.0. *rbc*L proteini su analizirani na motive, a otkrivena je njihova 3D struktura. Kao rezultat, filogenetsko stablo konstruisano sa sekvencama dobijenim iz Nacionalnog centra za biotehnološke informacije (NCBI) pokazalo je da su *Lamium* i *Marrubium* formirale jednu, a *Sideritis* i *Stachys* drugu kladu. Dodatno, *Thymbra*, *Vitex*, *Ajuga* i *Teucriu*m formirale su zasebne grupe. U analizama koje su uključivale samo navedenih sedam vrsta, matrica genetičke distance je pokazala da su najbliže vrste *Ajuga chamaepitys* i *Vitex agnus-castus* (0,009), dok su najudaljenije vrste bile *Teucrium polium* i *Thymbra capitata* (0,043). Takođe, izračunati diverzitet nukleotida iznosi $\pi = 0,025278$. Pokazano je da su *rbc*L sekvence ključne u razjašnjavanju filogenetskih odnosa unutar porodice Lamiaceae.

Ključne reči:
Lamiaceae, filogenija, *rbc*L, Turska

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Introduction

Türkiye is located in three phytogeographic regions and is considered a globally important center of biodiversity (Başer & Kırımer, 2018; Yıldırım et al., 2024). The Lamiaceae family in Türkiye is

represented by 787 taxa across 48 genera, including 608 species and 179 subspecies and varieties (Behçet & Cengiz, 2023). Notable genera within this family include *Calamintha* Mill., *Mentha* L., *Salvia* L., *Satureja* L., *Scutellaria* L., *Stachys* L., *Plectranthus* L'Hér., *Hyptis* Jacq., *Teucrium* L., *Vitex* L., *Thymus* L., and *Nepeta* L. (Mamadalieva et al., 2021; Kilinç et al., 2022). Many members of the Lamiaceae family are rich in essential oils, aromatic compounds, and secondary metabolites, making them significant in fields such as medicine, pharmaceuticals, cosmetics, and food production (Agostin et al. 2009; Hilooğlu et al. 2016; Bekut et al. 2018). Additionally, these species are known for their antimicrobial (Ricci et al., 2005), antioxidant (Özgen et al., 2006), anti-influenza (Protsenko et al., 2022), anticancer (Kilinç et al., 2022), and antimutagenic (Martínez-Rocha et al., 2008) properties.

The angiosperm chloroplast (cpDNA) genome ranges from 107 to 218 kb and is organised as a circular DNA molecule with a highly conserved tetragonal structure. Most of the cpDNA sequence encodes protein-coding genes, transfer RNA (tRNA), and ribosomal RNA (rRNA) (Lorenzana & Rico, 2024). Chloroplast DNA sequences are effective and reliable for creating DNA barcodes for plants, helping to resolve confusion in morphological identification (Furan, 2024). In plants, the *mat*K, *ndh*F, *rbc*L, *rpo*B, *rpo*C1, *psb*A-*trn*H, *psb*K-*psb*L, *trn*L-F and *atp*F-*atp*H regions of cpDNA sequences have been proposed as barcode gene regions (Manjarres-Hernández & Morillo-Coronado, 2023; Chen et al., 2024; Sevindik et al., 2024). Among these gene regions, *rbc*L is recognized as an universal barcode gene that is ideal for plant species discrimination studies due to its high amplification efficiency and low mutation rate. This low mutation rate makes the *rbc*L gene suitable for detailed studies of intraspecific genetic and phylogenetic diversity (Pere et al., 2023). In this study, cpDNA *rbc*L sequences were used for the phylogenetic analysis of several Lamiaceae species distributed in the Aydin province of Türkiye.

Materials and Methods

Plant sampling and DNA extraction technique

Ajuga chamaepitys (L.) Schreb., *Thymbra capitata* (L.) Cav, *Lamium moschatum* Mill., *Marrubium vulgare* L., *Stachys cretica* L., *Teucrium polium* L., and *Vitex agnus-castus* L. species were collected from the South Campus of Aydın Adnan Menderes University, Faculty of Agriculture (37°45'35.85"N, 27°45'16.93"E) and brought to the laboratory for herbarium preparation. Herbarium numbers of the species are given in **Tab. 1**. Genomic DNA was isolated from the fresh green leaves using a commercial kit (GeneMark Cat No: DP022).

Protocols and methods for polymerase chain reaction (PCR) and sequence analysis

The information on the primer sequences, PCR protocol, and PCR components used in this study is provided in **Tab. 2.** The PCR protocol was carried out according to Sevindik et al. (2024). The polymerase chain reaction products were visualized using 1.0% agarose gel electrophoresis. The PCR results were sent to Triogen Biotechnology (Istanbul, Türkiye) for Sanger sequencing analysis. For the analysis of both forward and reverse sequences, contig sequences were generated using BioEdit 7.2.3 (Hall, 1999), FinchTV 1.4.0 and Sequencher 5.4.6 software. The resulting sequences were then blasted at NCBI to check their similarity levels. Species were uploaded to NCBI and accession numbers were obtained (**Tab. 1**).

Phylogenetic and protein 3D structure analysis

Sequences of some species from the Lamiaceae family, obtained from The National Center for Biotechnology Information

Table 1. Herbarium number, accession number, A+T, G+C (%) and total length (bp) of cpDNA *rbc*L sequences in Lamiaceae species

Species	Herbarium number	Accession number	A+T	G+C	Total
Ajuga chamaepitys	LKR#1600	PQ303556	55.8	44.2	536
Lamium moschatum	LKR#1606	PQ303557	55.8	44.2	536
Marrubium vulgare	LKR#1603	PQ303558	56.5	43.5	538
Stachys cretica	LKR#1604	PQ303559	56	44	539
Teucrium polium	LKR#1601	PQ303560	56.3	43.7	535
Thymbra capitata	LKR#1612	PQ306792	56	44	536
Vitex agnus castus	E. Sevindik 1203	PQ303555	56.2	43.8	535
Avg.			56	44	536.4

Table 2. Primers, PCR components, and PCR protocols

Primer name, 5'-3' sequences and references	PCR components	PCR Protocols
*rbc*La-F: 5'-ATGTCACCACAAACAGAGACTAAAGC-3' (Levin, 2013)	1 µL genomic DNA 1 µL primer (forward), 1 µL primer (reverse), 5 µL master mix (PCR buffer, 2 Mm $MgCl_2$, dNTP, 0.75 U Taq DNA polymerase) and 17 µL dH_2O	95°C, 1 min; 35× (95°C, 30 s; 51°C, 30 s; 68°C, 1 min); 68°C, 5 min.
*rbc*La-R: 5'-GTAAAATCAAGTCCACCRCG-3' (Kress et al., 2009)		

(NCBI), were aligned using the MEGA 6.0 program (Tamura et al., 2013). A neighbour-joining (NJ) phylogenetic tree (Saitou & Nei, 1987) was constructed using the same software. The phylogenetic tree was evaluated using the bootstrap test on a resampling of 1000 replicates (Felsenstein, 1985). Moreover, the nucleotide composition, genetic distance matrix, and nucleotide diversity of the *rbc*L sequences were determined. The *rbc*L sequences were converted to amino acid sequences using the DNA-to-protein conversion tool in MEGA 6.0 software, and the amino acid content was analyzed using the same program. Furthermore, the protein sequences were modeled in 3D using SWISS-MODEL (https://swissmodel.expasy.org/interactive). To analyze all domains and conserved protein motifs, Protein BLAST was employed in conjunction with MEME (http://memesuite.org/doc/fasta-format.html) (Bailey et al., 2009).

Results and discussion

The *rbc*L sequences ranged from 535 to 539 bp, with an average A+T content of 56.0% and a G+C ratio of 44.0% (**Tab. 1**). According to the genetic distance matrix, the closest species were identified as *Ajuga chamaepitys* and *Vitex agnus-castus* (0.009), while the most distant species were *Teucrium polium* and *Thymbra capitata* (0.043) (**Tab. 3**). Tajima's Neutrality Test indicated that the nucleotide diversity was π=0.025278. Additionally, transition/transversion rates for purines and pyrimidines, as well as the general transition/transversion rates independent of any base group, were calculated for the studied species and presented in **Tab. 4**. It is shown that although the most important change is in pyrimidine bases, this situation is more balanced for purine bases than for pyrimidine bases. In this study, sequences of several species, including

Table 3. Pairwise genetic distance matrix obtained from cpDNA *rbc*L sequences

Species	1	2	3	4	5	6	7
Ajuga chamaepitys	-						
Lamium moschatum	0.027	-					
Marrubium vulgare	0.021	0.013	-				
Stachys cretica	0.023	0.021	0.017	-			
Teucrium polium	0.017	0.039	0.035	0.033	-		
Thymbra capitata	0.029	0.033	0.035	0.037	0.043	-	
Vitex agnus castus	0.009	0.023	0.019	0.021	0.027	0.025	-

Table 4. Maximum Composite Likelihood estimates of nucleotide substitution patterns

	A	T	C	G
A	-	4.1	3.11	**11.1**
T	4.13	-	**19.78**	3.35
C	4.13	**26.07**	-	3.35
G	**13.66**	4.1	3.11	-

Lamium purpureum L., *Lamium maculatum* (L.) L., *Lamium album* L., *Lamium amplexicaule* L., *Vitex negundo* var. *incisa* (Lam.) C.B.Clarke, *Vitex zeyheri* Sond. ex Schauer, *Vitex trifolia* L., *Stachys byzantina* K.Koch, *Stachys germanica* L., *Stachys alpina* L., *Ajuga parviflora* Benth., *Ajuga bracteosa* f. alba Gürke, *Ajuga ciliata* Bunge, *Teucrium chamaedrys* L., *Teucrium scordium* L., *Teucrium scorodonia* L., *Marrubium aschersonii* Magnus,

Marrubium peregrinum L., *Marrubium incanum* Desr. and *Thymbra spicata* L. were obtained from NCBI, and the neighbor-joining (NJ) phylogenetic tree was constructed. The phylogenetic analysis identified the following groups: *Marrubium* and *Lamium* species (bootstrap value 55%) *Stachys* and *Sideritis* species (bootstrap value 97%), *Vitex* species (bootstrap value 79%), *Ajuga* species (bootstrap value 78%), *Teucrium* species (bootstrap value 54%), and *Thymbra spicata* and *Thymbra capitata* species (bootstrap value 100%) (**Fig. 1**).

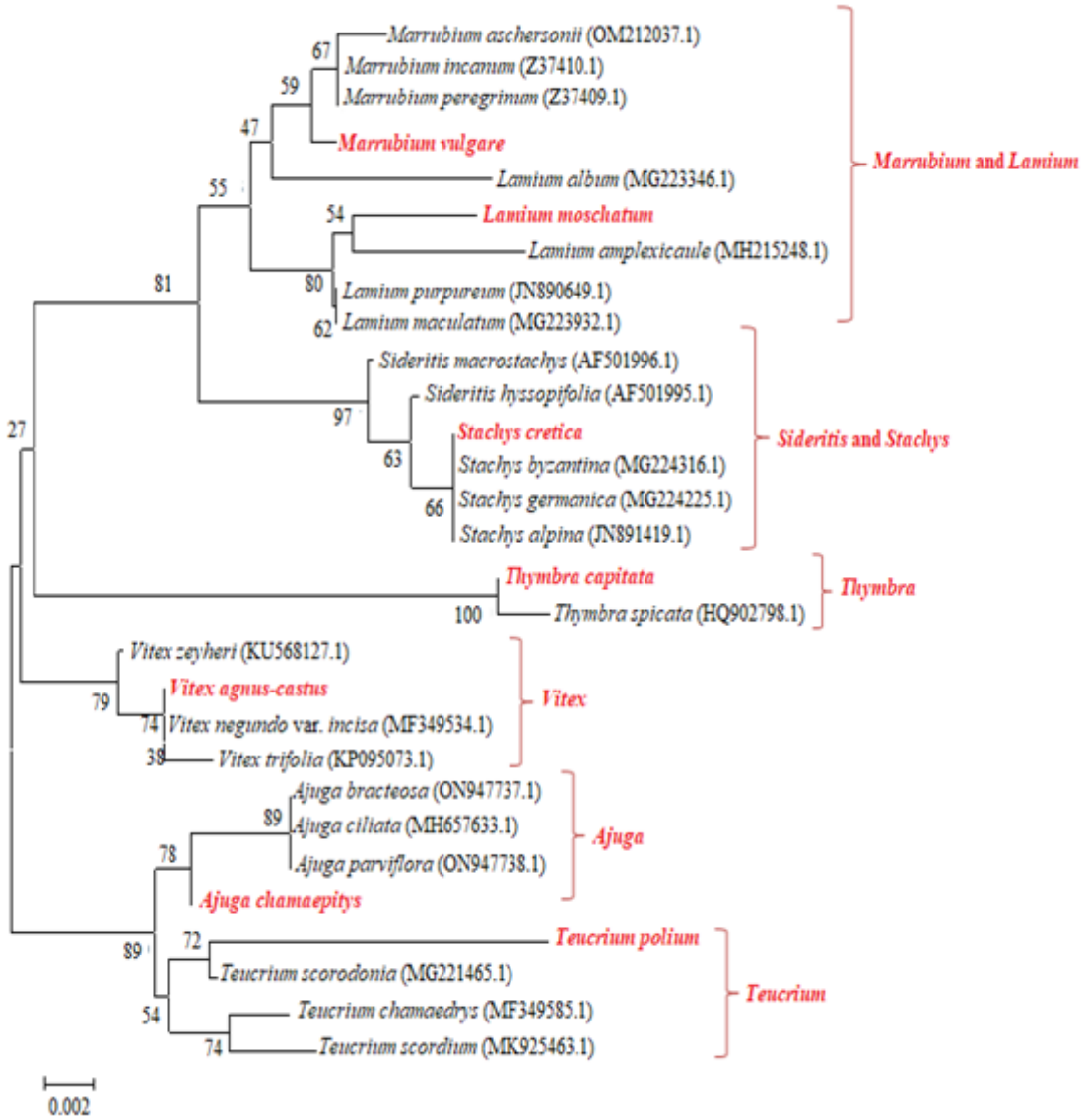

Fig. 1. The Neighbour-Joining tree generated using cpDNA *rbc*L sequences of Lamiaceae species, with sequences retrieved from NCBI

The *rbc*L-based, phylogenetic tree showed clear genus-level clustering. The average amino acid composition of rbcL proteins was analyzed using the MEGA 6.0 program and graphically represented in **Fig. 2**. Glycine was the most abundant amino acid (9.53%), while methionine was the least abundant (0.56%). Five conserved motifs within the *rbc*L protein sequences were identified using the MEME program (**Fig. 3**). All species contain motifs 1, 2, 3, 4 and 5, which are associated with protein structure and function. Some peptide motifs play important roles in protein protein-protein interactions (Filiz & Tombuloğlu, 2014). The 3D structure of *rbc*L proteins was modeled using the SWISS-MODEL program and is represented in **Fig. 4**. The three-dimensional structure of proteins facilitates drug design. It helps to understand protein function and active sites (Filiz & Koç, 2014). In their SDS-PAGE analysis, Ahmed and Al-Sodany (2019) identified *Teucrium polium* along with *Micromeria imbricata* and *Salvia deserti*, as well as *Marrubium*

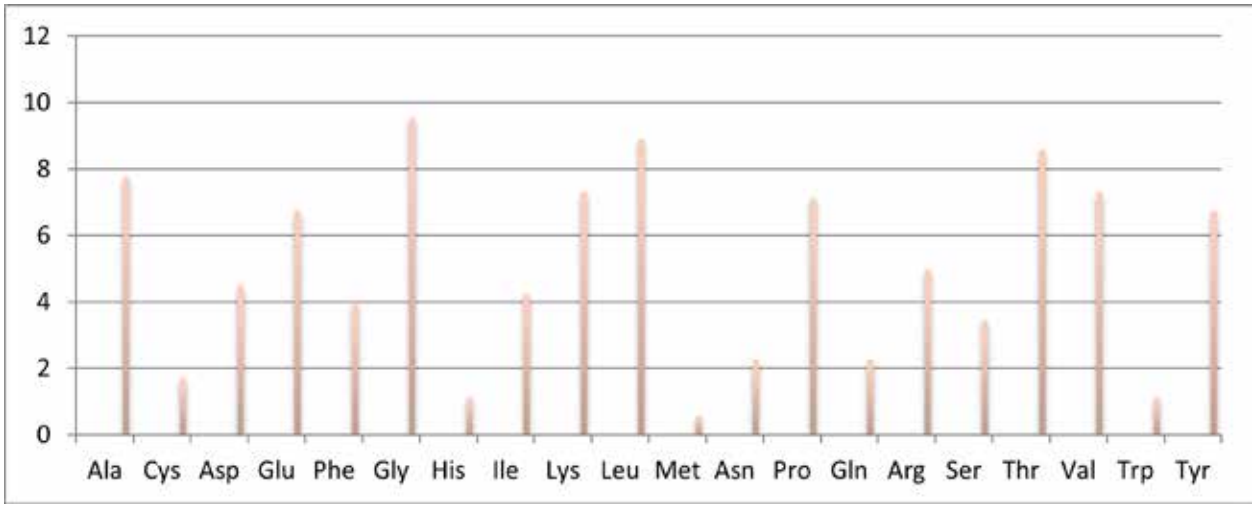

Fig. 2. Average amino acid composition of *rbc*L proteins

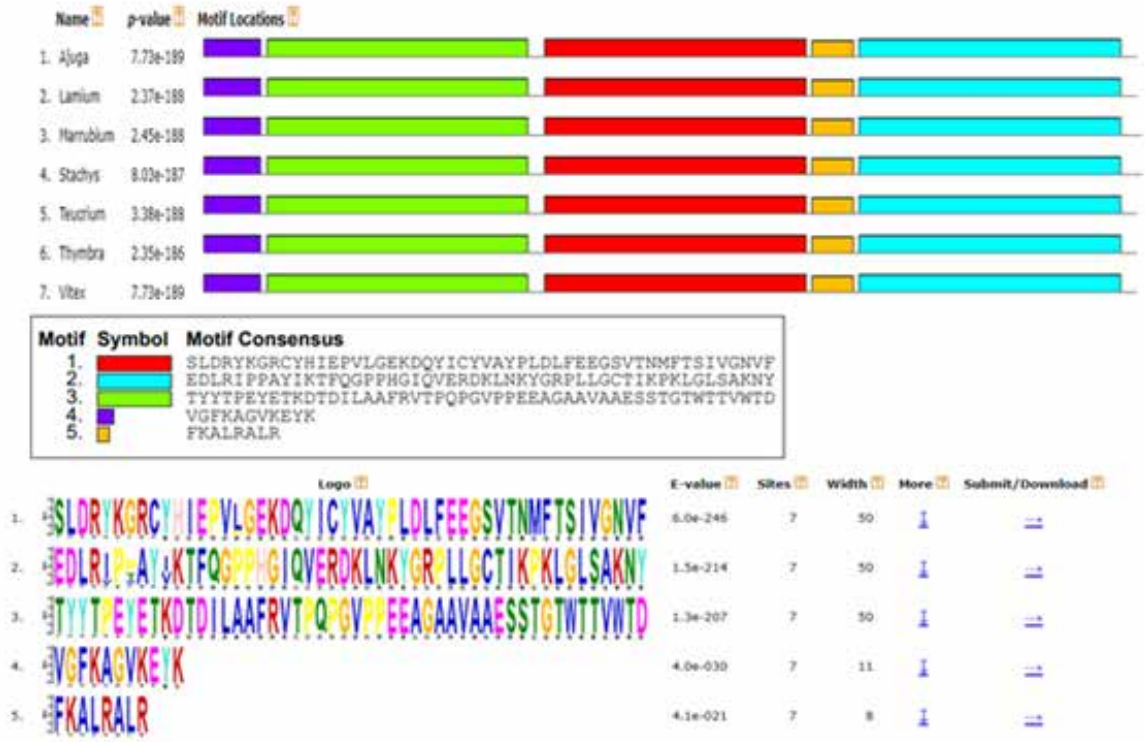

Fig. 3. Combined block diagrams of conserved protein motifs in *rbc*L proteins, as determined by MEME

vulgare with *Otostegia fruticosa*. In the RAPD and ISSR dendrogram, *Teucrium polium* and *Marrubium vulgare* were clustered together. Similarly, in their RAPD-PCR analysis, Topdemir et al. (2024) grouped *Lamium*, *Stachys*, and *Sideritis* species together, *Teucrium* and *Ajuga* species together, while *Marrubium* species formed a separate group. In our results, *Marrubium, Lamium, Sideritis* and *Stachys* were grouped together in a large group. This was supported by 81% of the bootstrap values. In the study by Ayaz et al. (2020), cpDNA *rps*14 sequence analysis placed *Marrubium vulgare* with *Lamium album* and *Lamium amplexicaule.* In our study, the NCBI obtained *Lamium album* sequence was also grouped with *Marrubium* species. Bendiksby et al. (2011) reported that the genus *Lamium* is not monophyletic. In the phylogenetic tree constructed with the *trn*L intron, the *trn*L-F spacer, the *rps*16 intron and the *mat*K sequences, the *Sideritis* and *Stachys* species appeared together, indicating that *Sideritis* and *Stachys* are not monophyletic genera. Our study similarly found that *Stachys* and *Sideritis* species formed a single group. Also, according to Bendiksby et al. (2011), *Marrubium* species were

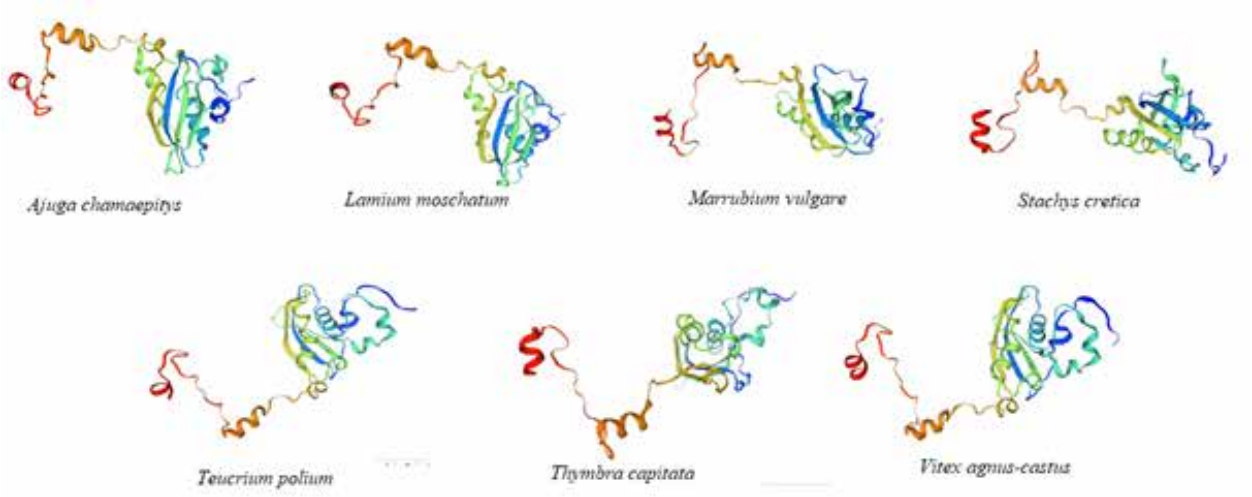

Fig. 4. The comprehensive 3D structures of *rbc*L proteins from Lamiaceae species, constructed using SWISS-MODEL

determined as a sister group with *Ballota* species. Wagstaff et al. (1998) explored the phylogenetic relationships of several Lamiaceae taxa using chloroplast *rbc*L and *ndh*F sequences. In the most parsimonious phylogenetic tree derived from the combined *rbc*L and *ndh*F data sets, *Marrubium vulgare* was grouped with *Lamium purpureum*, while *Vitex agnus-castus* was grouped with *Petitia domingensis*. Dundar et al. (2013) suggested that nine subspecies of *Stachys cretica* form a polyphyletic rather than monophyletic group based on nrDNA ITS analysis. They also observed that some *Sideritis* species clustered with *Stachys* species belonging to different sections.

Conclusion

In this study, phylogenetic analysis based on the chloroplast *rbc*L sequence was performed on seven species (*Ajuga chamaepitys, Thymbra capitata, Lamium moschatum, Marrubium vulgare, Stachys cretica, Teucrium polium*, and *Vitex agnus-castus*). The genetic distance matrix revealed that the closest species were *Ajuga chamaepitys* and *Vitex agnus-castus* (0.009), while the most distantly related species were *Teucrium polium* and *Thymbra capitata* (0.043). Also, the nucleotide diversity was determined to be π=0.025278. In the neighbour-joining phylogenetic tree, *Lamium* and *Marrubium* species appeared together, and *Sideritis* and *Stachys* species appeared together. *Thymbra, Vitex, Ajuga* and *Teucrium* species were grouped at the genus level. This study suggested that cpDNA *rbc*L analyses could be used in phylogenetic analyses at the genus level. The *rbc*L sequence results have significantly contributed to elucidating the phylogenetic relationships within the Lamiaceae family.

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References

Agostini, F., **Santos, C. A.**, **Rossato, M.**, **Márcia, R. P.**, **Paula, L. S.**, **Serafini, L. A.**, **Molon, R.**, & **Patrick, M.** (2009). Essential oil yield and composition of *Lamiaceae* species growing in southern Brazil. *Brazilian Archives of Biology and Technology*, *52*, 473–478. https://doi.org/10.1590/S1516-89132009000200026

Ahmed, S. M., & **Al-Sodany, Y. M.** (2019). Authentication of ecological, biochemical and molecular features for some *Lamiaceae* species from Saudi Arabia. *Egyptian Journal of Botany*, *59*(3), 581-594. https://doi.org/10.21608/ejbo.2019.6144.1246

Ayaz, A., **Zaman, W.**, **Saqib, S.**, **Ullah, F.**, & **Mahmood, T.** (2020). Phylogeny and diversity of *Lamiaceae* based on rps14 gene in Pakistan. *Genetika*, *52*(2), 435-452. https://doi.org/10.2298/GENSR2002435A

Bailey, T. L., **Boden, M.**, **Buske, F. A.**, **Frith, M.**, **Grant, C. E.**, **Clementi, L.**, & ... **Noble, W. S.** (2009). MEME SUITE: Tools for motif discovery and searching. *Nucleic Acids Research*, *37*(suppl_2), W202-W208. https://doi.org/10.1093/nar/gkp335

Başer, K., & **Kırımer, N.** (2018). Essential oils of Anatolian *Lamiaceae* – An update. *Natural Volatiles and Essential Oils*, *5*(4), 1-28.

Bekut, M., **Brkić, S.**, **Kladar, N.**, **Dragović, G.**, **Gavarić, N.**, & **Božin, B.** (2018). Potential

of selected *Lamiaceae* plants in anti (retro) viral therapy. *Pharmacological Research*, *133*, 301-314. https://doi.org/10.1016/j.phrs.2017.12.016

Bendiksby, M., **Thorbek, L.**, **Scheen, A. C.**, **Lindqvist, C.**, & **Ryding, O.** (2011). An updated phylogeny and classification of *Lamiaceae* subfamily *Lamioideae*. *Taxon*, *60*(2), 471–484. https://doi.org/10.1002/tax.602015

Behçet, L., & **Cengiz, H.** (2023). On the presence and distribution of *Lycopus exaltatus* L.f. (*Lamiaceae*) in Türkiye. *KSU Journal of Agriculture and Nature*, *26*(6), 1253-1258. https://doi.org/10.18016/ksutarimdoga.vi.1239934

Chen, Z., **Gao, L.**, **Wang, H.**, & **Feng, S.** (2024). Molecular identification and phylogenetic analysis of *Cymbidium* species (*Orchidaceae*) based on the potential DNA barcodes matK, rbcL, psbA-trnH, and internal transcribed spacer. *Agronomy*, *14*(5), 933. https://doi.org/10.3390/agronomy14050933

Dündar, E., **Akçiçek, E.**, **Dirmenci, T.**, & **Akgün, Ş.** (2013). Phylogenetic analysis of the genus *Stachys* sect. *Eriostomum* (*Lamiaceae*) in Turkey based on nuclear ribosomal ITS sequences. *Turkish Journal of Botany*, *37*(1), 14-23. https://doi.org/10.3906/bot-1203-26

Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, *39*(4), 783–791. https://doi.org/10.1111/j.1558-5646.1985.tb00420.x

Filiz, E., & **Tombuloğlu, H.** (2014). *In silico* analysis of DREB transcription factor genes and proteins in grasses. *Applied Biochemistry and Biotechnology*, *174*, 1272-1285. https://doi.org/10.1007/s12010-014-1093-x

Filiz, E., & **Koç, İ.** (2014). *In silico* sequence analysis and homology modeling of predicted beta-amylase 7-like protein in *Brachypodium distachyon* L. *Journal of BioScience and Biotechnology*, *3*(1), 61–67.

Furan, M. A. (2024). Species identification and germplasm conservation of *Origanum* based on chloroplast genes. *Genetic Resources and Crop Evolution*, *71*(3), 1101-1112. https://doi.org/10.1007/s10722-023-01679-5

Hilooğlu, M., **Yücel, E.**, **Kandemir, A.**, & **Sözen, E.** (2016). Endemik *Teucrium leucophyllum* (*Lamiaceae*) tohumlarında *in vitro* çimlendirme çalışmaları. *Sinop Üniversitesi Fen Bilimleri Dergisi*, *1*(1), 53-61.

Kilinç, B. Ö., **Gödelek, D.**, **Süfer, Ö.**, **Saygideğer Demir, B.**, **Sezan, A.**, **Saygideğer, Y.**, & **Bozok, F.** (2022). Essential oils from some *Lamiaceae* plants: Antioxidant and anticancer potentials besides thermal properties. *Chemistry & Biodiversity*, *19*(10), e202200418. https://doi.org/10.1002/cbdv.202200418

Kress, W. J., **Erickson, D. L.**, **Jones, F. A.**, **Swenson, N. G.**, **Perez, R.**, **Sanjur, O.**, & **Bermingham, E.** (2009). Plant DNA barcodes and a community phylogeny of a tropical forest dynamic plot in Panama. *PNAS*, *106*, 18621–18626. https://doi.org/10.1073/pnas.0909820106

Levin, R. A., **Wagner, W. L.**, **Hoch, P. C.**, **Nepokroef, M.**, **Pires, J. C.**, & **Zimmer, E. A.**, et al. (2003). Family-level relationships of *Onagraceae* based on chloroplast rbcL and ndhF data. *American Journal of Botany*, *90*(1), 107–115. https://doi.org/10.3732/ajb.90.1.107

Lorenzana, G. P., & **Rico, Y.** (2024). Complete chloroplast genomes of three copal trees (*Bursera*: *Bullockia*): Comparative analysis and phylogenetic relationships. *Molecular Biology Reports*, *51*(1), 1-11. https://doi.org/10.1007/s11033-024-09304-z

Mamadalieva, N. Z., **Akramov, D. K.**, **Wessjohann, L. A.**, **Hussain, H.**, **Long, C.**, **Tojibaev, K. S.**, & ... **Wink, M.** (2021). The genus *Lagochilus* (*Lamiaceae*): A review of its diversity, ethnobotany, phytochemistry, and pharmacology. *Plants*, *10*(1), 132. https://doi.org/10.3390/plants10010132

Manjarres-Hernández, E. H., & **Morillo-Coronado, A. C.** (2023). Phylogenetic determination of *Chenopodium quinoa* based on the chloroplast genes rbcL and matK. *International Journal of Agronomy*, *2023*(1), 6681851. https://doi.org/10.1155/2023/6681851

Martínez-Rocha, A., **Puga, R.**, & **Hernández-Sandoval, L.**, et al. (2008). Antioxidant and antimutagenic activities of Mexican oregano (*Lippia graveolens* Kunth). *Plant Foods for Human Nutrition*, *63*, 1–5. https://doi.org/10.1007/s11130-007-0061-9

Özgen, U., **Mavi, A.**, **Terzi, Z.**, **Coşkun, M.**, & **Houghton, P. J.** (2006). Antioxidant properties of some medicinal *Lamiaceae* (Labiatae) species. *Pharmaceutical Biology*, *44*(2), 107-112. https://doi.org/10.1080/13880200600592061

Pere, K., **Mburu, K.**, **Muge, E. K.**, **Wagacha, J. M.**, & **Nyaboga, E. N.** (2023). Molecular discrimination and phylogenetic relationships of *Physalis* species based on ITS2 and rbcL DNA barcode sequences. *Crops*, *3*(4), 302-319. https://doi.org/10.3390/crops3040027

Protsenko, M. A., **Mazurkova, N. A.**, & **Filippova,**

E. I., et al. (2022). Anti-influenza activity of extracts from plants of the *Lamiaceae* family. *Russian Journal of Bioorganic Chemistry*, *48*, 1534–1541. https://doi.org/10.1134/S1068162022070238

Ricci, D., **Fraternale, D.**, **Giamperi, L.**, **Bucchini, A.**, **Epifano, F.**, **Burini, G.**, & **Curini, M.** (2005). Chemical composition, antimicrobial and antioxidant activity of the essential oil of *Teucrium marum* (*Lamiaceae*). *Journal of Ethnopharmacology*, *98*(1-2), 195-200. https://doi.org/10.1016/j.jep.2005.01.022

Saitou, N., & **Nei, M.** (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, *4*(4), 406–425. https://doi.org/10.1093/oxfordjournals.molbev.a040454

Sevindik, E., **Korkom, Y.**, & **Murathan, Z. T.** (2024). Evaluating DNA barcoding using cpDNA matK and rbcL for species identification and phylogenetic analysis of *Prunus armeniaca* L. (*Rosaceae*) genotypes. *Genetic Resources and Crop Evolution*, *71*, 1825-1835. https://doi.org/10.1007/s10722-023-01748-9

Topdemir, S., **Kürşat, M.**, & **Bozarı, S.** (2024). Phylogenetic analysis of some taxa belonging to the family *Lamiaceae* in Bitlis Province using RAPD-PCR technique. *Osmaniye Korkut Ata University Journal of the Institute of Science*, *7*(2), 563-580. https://doi.org/10.47495/okufbed.1266779

Wagstaff, S. J., **Hickerson, L.**, **Spangler, R.**, **Reeves, P. A.**, & **Olmstead, R. G.** (1998). Phylogeny in *Labiatae* s.l., inferred from cpDNA sequences. *Plant Systematics and Evolution*, *209*, 265-274.

Yıldırım, M., **Tuğ, G. N.**, & **Yaprak, A. E.** (2024). Analyses of genetic diversity and population structure of endemic and endangered species *Sideritis gulendamii* (*Lamiaceae*) and implications for its conservation. *Genetic Resources and Crop Evolution*, 1-15. https://doi.org/10.1007/s10722-024-01907-6