Species diversity and phylogenetic relationships within the tribe Mentheae (Lamiaceae) in Uzbekistan using ITS sequence data

E Nikitina¹, Kh Khalbekova²

¹Institute of Botany, Academy of Sciences of Uzbekistan, 100125, Tashkent, Durmon yuli street, 32
²Institute of Bioorganic Chemistry, Academy of Sciences of Uzbekistan, M. Ulugbek street, 83

E-mail: elenanikita2013@rambler.ru

Abstract: Regular monitoring and comprehensive study of natural populations rare endangered species, both by classical taxonomy and molecular genetic analysis methods are of considerable importance all over the world. This work has focused at the research of species diversity flora of Uzbekistan using combinations of DNA markers. Currently, there are no universal DNA site for plant species identification. Pairwise sequence alignments and multiple sequence alignments are made using ClustalW program. DNA sequence data were used to verify the taxonomic identity and phylogenetic aspects of the studied representatives the tribe Mentheae (Lamiaceae) collected in Uzbekistan territory. The phylogenetic analyzes of this tribe was reconstructed for 44 species, representing 19 genera using nuclear ribosomal DNA internal transcribed spacer region (nrITS), containing ITS1 and ITS4 adopted as an important basis, since it can provide a high resolution of relationships. 15 consensus ITS gene sequences were obtained for studied species in this work and 29 published molecular sequence data from GenBank were used. Dendrograms were constructed using hierarchical clustering with the software Mega X. The parsimony analyses were resulted in an accurate consensus tree. Three major clades was identified within the tribe Mentheae. So, this paper demonstrates the successfully usefulness of DNA technology as a tool for genetic inventory at the species diversity assessment of rare endangered plants.

1. Introduction

The conservation of the species diversity of wild flora in Uzbekistan is the main task. The peculiar climatic conditions, different zones, high-altitude belts- all those aspects determine the phenotypic diversity of the flora of Uzbekistan. The groups of rare, endemic and relict species most adequately reflect the directions of migration flows during flora formation and speciation processes in limited areas. The extraordinary diversity of morpho-ecological characteristics, in turn, complicates families taxonomy. The taxa identification, as well as the assessment of phylogenetic relationships among species are major problems for modern biology.

Currently, the flora of Uzbekistan accounts approximately 4385 species of wild vascular plants including a significant number of medicinal, essential oil, ornamental and globally important species [1].
A significant number, about 400, are relict, endemic, rare species to this area (10%–12% of flora), and 378 are considered national endemics [2].

The rare and endangered species are characterized by a low survive ability under climate change conditions and anthropogenic factors, that leads to loss of valuable genotypes and biodiversity decreasing. Thus, taking into account the mentioned factors, inventory is an extremely important task and reliable method for identifying is urgently needed.

The main goal of DNA barcoding is species identification using short genetic markers (DNA fragments) to determine the belonging of the unknown organism to a certain taxon, to place it in an existing classification, but not to establish new phylogenetic relationships. This technology is successfully used to identify rare and endangered species, to study invasive and alien species. In connection with the development and improvement of modern molecular technologies, the number of DNA barcodes is constantly growing, and therefore, one of the main task is the selection of optimal DNA markers that the most satisfying the requirements and objectives of the research [3-5].

So, the purpose of this research is species diversity assessment in Uzbekistan regions using the DNA barcoding method. The important species of the tribe Mentheae (Lamiaceae) growing in Uzbekistan were sampled as objects in this study.

2. Materials and Methods
The research materials are wild plant species of the Mentheae Dumort. tribe, Nepetoideae (Dumort.) Luerss. subfamily (Lamiaceae Martinov, Labiatae Juss.). In the flora of Uzbekistan, the Mentheae tribe is represented by 20 genera, including approximately 95 species (Table). The important and rare 15 species of Salvia, Dracocephalum, Nepeta, Lallemantia, Ziziphora genera are sampled for this study.

The materials for this study were collected in different geographical regions of Uzbekistan [6]. Fresh, silica gel dried leaf material were used for analyze. As well as herbarium specimens material stored in the collection of the National Herbarium of Uzbekistan, (TASH) were studied in this research.

The total DNA was isolated from fresh and herbarium plant materials using commercial set GeneJET Plant Genomic DNA Purification Kit (Thermo Scientific, USA) according to the manufacturer's protocol, with some modifications for herbarium specimens. A phylogenetic analysis of studied species was performed based on the obtained nucleotide sequences of the ITS nuclear region. Amplification of the nuclear ITS region was carried out with a C1000 Touch Thermal Cycler (BioRad, USA), by using primers of the forward and reverse primer sets (ITS1, 5’-tcctagttgaacctgcgg-3’; ITS4, 5’-ticctcgcgttggatgatgc-3’) as internal primers. A fragments of target marker ranged from ~600 to ~700 b.p. The PCR products were purified with an Exonuclease I and Shrimp Alkaline Phosphatase (Thermo Fisher Scientific, USA). Termination reaction performed using a commercial kit Brilliant Dye Terminator v3.1 Cycle Sequencing Kit (Nimagen, Netherlands). Determination of the nucleotide sequence was performed on a genetic analyzer ABI 3500 DNA Analyzer (Applied Biosystems, USA) in a forward and reverse directions. Sequence chromatograms viewed and evaluate in Sequencer v.4.5116. Alignment of sequences pairs conducted in the BioEdit Sequence Alignment Editor v.7.0.01. The resulting consensus DNA sequences are saved in FASTA format. 31 sequences of the ITS region used for a phylogenetic dendrogram construction were download from the GenBank database. The region for comparing sized 610 b.p.

The nucleotide sequences alignment was performed using the ClustalW v.2.0.117 algorithm [7]. The hierarchical clustering was inferred using the Neighbor-Joining method [8]. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches [9]. The evolutionary distances were computed using the Maximum Composite Likelihood method [10] and are in the units of the number of base substitutions per site. The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). This analysis involved 46 nucleotide sequences. There were a total of 447 positions in the final dataset.

Additional the Maximum Likelihood method with General Time Reversible model were used [11]. Initial tree for the heuristic search were obtained automatically by applying the Maximum Parsimony
method. A discrete Gamma distribution with Invariant sites (G+I) was used to model evolutionary rate differences among sites. Evolutionary analyses were conducted in MEGA X [12].

3. Results and discussion

Lamiaceae are interesting due to a huge amount of biologically active compounds with a wide spectrum of action. Almost all species are medicinal plants and are widely used both in folk medicine and in pharmacology. It is consistently recognized 236 genera, and comprising more than 7000 species [13].

According to Harley et al., [14] the tribe Mentheae consists of nearly half of subfamily Nepetoideae, including approximately 65 genera and 2300 species, and delimitates into three subtribes: Menthinae, Nepetinae and Salviinae. The representatives of 20 genera included in the tribe Mentheae are grow in the different geographical regions, mountainous and foothill area of Uzbekistan: Clinopodium L., Hyssopus L., Mentha L., Micromeria Benth., Origanum L., Satureja L., Thymus L., Ziziphora L. (subtribe Menthinae Endl.); Dracophyllum L., Drepanocaryum Pojark., Lallemantia Fisch. & C.A. Mey., Nepeta L. (subtribe Nepetinae Coss. & Germ.); Hymenocrater Fisch. et Mey., Kudrjaschevia Pojark., Melissa L., Lophanthus Adans., Rosmarinus L., Salvia L. (subtribe Salviinae Endl.), Lycopus L. (subtribe Lycopinae B. T. Drew & Sytsma) (Table 1).

| №  | Taxa, collected date                        | Distribution in Uzbekistan, coordinates, altitude                               |
|----|---------------------------------------------|---------------------------------------------------------------------------------|
| 1  | Acinos rotundifolius Pers. current name Clinopodium graveolens subsp. rotundifolium (Pers.) Govaerts | Tien-Shan, Pamir-Alay, Kopet-Dag mountains                                       |
| 2  | Clinopodium integerrimum Boriss.            | Chuy, Fergana valley, Tien-Shan, Pamir-Alay, Kopet-Dag mountains               |
| 3  | Micromeria popovii (B. Fedtsch. & Gontsch.) Vved. | Karshi steppe, Pamir-Alay                                                      |
| 4  | Mentha arvensis L.                          | Syr-Darya, Amu-Darya rivers, Fergana valley, Zeravschan river, Alatau, Tien-Shan, Pamir-Alay |
| 5  | Mentha longifolia (L.) Huds. var. asiatica (Boriss.) Rech.f. | All over Central Asia                                                          |
| 6  | Mentha pamiroalaica Boriss.                 | Pamir-Alay (Nuratau, Zeravschan, Hissar, Kuhting ridges)                       |
| 7  | Origanum tyttanthum Gontsch.                | Tashkent chul, Fergana valley, Tien-Shan, Pamir-Alay                           |
| 8  | Satureja hortensis L.                       | Vicinity of cities and towns: Tashkent, Fergana, Andijan                       |
| 9  | Thymus dmitrievae Gamajun.                  | Tien-Shan                                                                      |
| 10 | Thymus insertus Klokov                      | Tien-Shan, Pamir-Alay                                                          |
| 11 | Thymus seravschanicus Klokov                | Fergana valley, Tien-Shan, Pamir-Alay                                          |
| 12 | Thymus subnervosus Vved., Nabiev & Tulyag.  | Pamir-Alay, endemic of Uzbekistan, Nuratau mountains                           |
| 13 | Ziziphora capitata L.                       | Tien-Shan (Karatau, Kuramin ridges), Pamir-Alay (Alay, Zerabschan, Hissar ridges) Kopet-Dag |
| 14 | Ziziphora clinopodioides Lam.* (2019)       | Syr-Darya river, Alatau, Tien-Shan, Pamir-Alay, Kopet-Dag, Nuratau 40.513754N 66.743058E, 1269m |
| 15 | Ziziphora interrupta Juz.                   | Surkhan-Sherabad valley, Tien-Shan, Pamir-Alay                                 |
| 16 | Ziziphora pamiroalaica Juz.                 | Surkhan-Sherabad valley, Zeravschan river valley, Tien-Shan, Pamir-Alay        |
| No.  | Species Name                     | Author                     | Location                                                                 |
|------|----------------------------------|----------------------------|---------------------------------------------------------------------------|
| 17.  | *Ziziphora pedicellata*          | Pazij & Vved.*             | Tashkent chul, Tien-Shan, Kuramin ridge 41.114383N 70.716559E, 1785m     |
| 18.  | *Ziziphora persica*              | Bunge                      | Pamir-Alay (Zeravschan, Hisarr, Kuhitang), Kopet-Dag                      |
| 19.  | *Ziziphora saffrutcosa*          | Pazij & Vved.*             | Tien-Shan (Kuramin, Mogoltau ridges), Pamir-Alay (Turkestan, Malguzar, Hisarr ridges) 40.4156N 68.93654E, 1116m |
| 20.  | *Ziziphora tenuior L.*           | (2019)                     | Syr-Darya river, Kyzylkum, Karakum, Amudarya river valley, Tien-Shan, Pamir-Alay 39.600281N 68.370566E, 2219m |
|      | **Subtribe Lycopinae**           |                            |                                                                           |
| 21.  | *Lycopus europeanus*             | L.                         | Syr-Darya river valley, Kyzylkum, Amu-Darya river valley, Fergana valley, Pamir-Alay, Kopet-Dag |
| 22.  | *Lycopus exaltatus*              | L.                         | Syr-Darya river, Tien-Shan, Pamir-Alay (Zeravschan, Hisarr ridges), Kopet-Dag |
|      | **Subtribe Nepetinae**           |                            |                                                                           |
| 23.  | *Dracocephalum adylovii*         | I.I. Malzev*               | Pskem river valley. Endemic of Uzbekistan, Western Tian-Shan 42.0831N 70.4848E, 2100m |
| 24.  | *Dracocephalum bipinnatum*       | Rupr.                      | Tien-Shan (Alatau, Karatau, Chatkal, Fergana ridges), Pamir-Alay (Hisarr ridges) |
| 25.  | *Dracocephalum discolor*         | Bunge                      | Dzhungar Alatau, Tien-Shan (Chatkal, Kuramin ridges), Pamir-Alay           |
| 26.  | *Dracocephalum diversifolium*    | Rupr.                      | Tien-Shan (Chatkal, Kuramin, Fergana ridges) Pamir-Alay (Alay, Zeravschans, Hisarr ridges) |
| 27.  | *Dracocephalum formosum*         | Gontsch.                   | Pamir-Alay (Hisarr ridge). Endemic Of the Western Pamir-Alay               |
| 28.  | *Dracocephalum imberbe*          | Bunge                      | Tien-Shan (Alatau ridge), Pamir-Alay(Alay ridge)                           |
| 29.  | *Dracocephalum integrifolium*    | Bunge                      | Western Tien-Shan (Alatau, Chatkal, Pskem, Kuramin, Fergana), Pamir-Alay (Alay, Hisarr, Turkestanridges). Endemic of Uzbekistan |
| 30.  | *Dracocephalum karataviense*     | Pavl. & Roldugin            | Tien-Shan (Talass, Alatau Karatau, Chatkal)                                |
| 31.  | *Dracocephalum komarovii*        | Lipsky                     | Tien-Shan (Ugam, Chatkal, Kuramin ridges), Pamir-Alay (Turkestan, Zeravschan ridges) |
| 32.  | *Dracocephalum nodulosum*        | Rupr.                      | Tien-Shan (Alatau, Chatkal, Kuramin, Fergana ridges), Pamir-Alay (Alay, Nuratau ridges), Alay valley |
| 33.  | *Dracocephalum nuratavicum*      | Adylov* (2019)             | Pamir-Alay (Nuratuiu ridge). Endemic of Uzbekistan, Nuratuiu mountains. Endemic of Uzbekistan 40.3109N 66.4429E, 1335m |
| 34.  | *Dracocephalum nutans*           | L.                         | Tien-Shan (Alatau, Ugam, Pskem Chatkal, Fergana ridges)                    |
| 35.  | *Dracocephalum oblongifolium*    | Regel                      | Tien-Shan (Talass, Alatau, Ugam, Pskem, Chatkal ridges), Pamir-Alay (Lalay, Turkestan, Zeravschan, Hisarr ridges) |
| 36.  | *Dracocephalum scrobiculatum*    | Regel                      | Pamir-Alay (Turkestan, Zeravschan, Hisarr ridges)                          |
| 37.  | *Dracocephalum spinulosum*       | Popov                      | Tien-Shan (Ugam, Pskem, Chatkal ridges)                                   |
| 38.  | *Dracocephalum stamineum*        | Kar. & Kir.                | Tien-Shan (Alatau, Chatkal, Fergana ridges), Pamir-Alay (Alay, Nuratau, Zeravschan, Hisarr ridges) |
| No. | Species Name                                      | Habitat Description                                                                                                                                 |
|-----|-------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------|
| 39. | *Drepanocaryum* *sewerzowii* (Regel) Regel      | Kyrgyz (Aktau mountains), Amu-Darya, Zeravshan rivers valley, Fergana valley, Tien-Shan, Pamir-Alay (Alay, Nuratau, Zeravshan ridges)               |
| 40. | *Hymenocrater incisodentatus* Boriss.            | Pamir-Alay (Hissar, Kuhitang, ridges). Endemic of South-Western Pamir-Alay                                                                       |
| 41. | *Hyssopus seravschanicus* (Dubj.) Pazij          | Fergana valley, Tien-Shan (Alatau ridges), Pamir-Alay (Nuratau, Zeravshan, Hissar ridges)                                                         |
| 42. | *Kudrjaschevia jacubi* (Lipsky) Pojark.          | Pamir-Alay (Zeravshan, Hissar, Kuhitang ridges)                                                                                                 |
| 43. | *Lallemantia royleana* (Benth.)*^* (2019)        | Syr-Darya river valley, Usjurt, Tien-Shan (Alatau), Pamir-Alay (Alay, Nuratau, Hissar, Western Pamir, Kuhitang, Babatag ridges) 38.28218N, 66.22975E, 759 m |
| 44. | *Lophanthus ouroumitanensis* (Franch.) Kochk. & Tzukerv. | Pamir-Alay (Alay, Hissar, Kuhitang ridges)                                                                                                       |
| 45. | *Lophanthus schrenkii* Levin                    | Alatau, Tien-Shan (Alatau, Pskem, Chatkal ridges)                                                                                               |
| 46. | *Lophanthus shtschourowskianus* (Regel) Lipsky  | Tien-Shan (Fergana ridge), Pamir-Alay (Alay, Turkestan, Nuratau ridges)                                                                             |
| 47. | *Lophanthus subnivalis* Lipsky                   | Pamir-Alay (Alay, Zeravshan, Hissar ridges)                                                                                                       |
| 48. | *Lophanthus tschimganicus* Lipsky                | Tien-Shan (Pskem, Ugam, Chatkal, Kuramin ridges)                                                                                                 |
| 49. | *Lophanthus virescens* (Lipsky) Kochk.           | Pamir-Alay (Alay, Zeravshan, Hissar, Kuhitang ridges)                                                                                             |
| 50. | *Nepeta alatavica* Lipsky                       | Tien-Shan (Alatau, Ugam, Pskem, Chatkal ridges)                                                                                                 |
| 51. | *Nepeta badachschanica* Kudrjasch.              | Western Pamir                                                                                                                                     |
| 52. | *Nepeta bracteata* Benth.                       | Pamir-Alay (Turkestan, Zeravshan, Kuhitang ridges), West Pamir                                                                                   |
| 53. | *Nepeta bucharica* Lipsky                       | Tien-Shan (Alatau, Chatkal, Fergana ridges), Pamir-Alay (Zeravshan, Hissar ridges)                                                               |
| 54. | *Nepeta cataria* L.                             | Syr-Darya, Amu-Darya rivers valley, Tien-Shan (Karatau, Ugam, Pskem, Chatkal, Kuramin ridges), Pamir-Alay (Alay, Nuratau, Zeravshan, Hissar, Kuhitang ridges) |
| 55. | *Nepeta formosa* Kudrjasch.                     | Tien-Shan (Karatau, Chatkal ridges), Pamir-Alay (Alay, Hissar, Darvaz ridges)                                                                     |
| 56. | *Nepeta kokanica* Regel                         | Tien-Shan (Karatau, Chatkal ridges), Pamir-Alay (Alay, Turkestan, Zeravshan, Hissar, Darvaz ridges)                                                |
| 57. | *Nepeta lipsyi* Kudrjasch.                      | Pamir-Alay                                                                                                                                         |
| 58. | *Nepeta mariae* Regel                           | Tien-Shan, Pamir-Alay                                                                                                                                |
| 59. | *Nepeta maussarifi* Lipsky                      | Pamir-Alay (Zeravshan, Hissar ridges)                                                                                                               |
| 60. | *Nepeta micrantha* Bunge                        | Syr-Darya river valley, Kyzylkum, Alatau, Tien-Shan, Western Pamir-Alay                                                                             |
| 61. | *Nepeta olgae* (Regel) (2020)                   | Syr-Darya river valley, Kyzylkum, Fergana valley, Surkhan-Sherabad valley. Endemic of Central Asia 37.960280N 66.798898E, 1430 m               |
| 62. | *Nepeta pamirensis* Franch.                     | Pamir-Alay (Alay, Hissar, Pamir ridges)                                                                                                            |
| 63. | *Nepeta pannonica* L.                           | Tien-Shan, Pamir-Alay (Alay, Zeravshan, Hissar ridges)                                                                                              |
| 64. | *Nepeta podostachys* Benth.                     | Pamir-Alay (Alay, Turkestan, Zeravshan, Hissar, Darvaz ridges)                                                                                     |
| 65. | *Nepeta pungens* (Bunge) Benth. | Ustjurt, Kyzylkum, Dzhungar Alatau, Tien-Shan (Karatau, Kuramin, Mogoltau mountains), Pamir-Alay (Alay, Turkestan, Nuratau, Zeravschan, Hissar ridges) |
| 66. | *Nepeta santoana* Popov | Pamir-Alay (Alay, Turkestan, Zeravschan ridges) |
| 67. | *Nepeta satureiodes* Boiss. | Ustjurt, Kyzylkum, Fergana valley, Tien-Shan (Karatau, Kuramin ridges), Pamir-Alay (Alay, Turkestan, Zeravschan, Darvaz ridges), Western Pamir |
| 68. | *Nepeta subhastata* Regel | Pamir-Alay (Alay, Turkestan ridges). Endemic of the Northern Pamir-Alay |
| 69. | *Nepeta ucranica* L. | Tien-Shan (Alatau, Karatau, Karzhantau, Chatkal, Kuramin ridges), Pamir-Alay (Alay, Turkestan ridges) |

**Subtribe Salviinae**

| 70. | *Melissa officinalis* L. | Tien-Shan (Alatau, Karatau, Karzhantau, Ugam, Pskem, Chatkal Mts), Pamir-Alay (Alay, Zeravschan, Hissar ridges), Kopet-Dag |
| 71. | *Salvia aequidens* Botsch. | Pamir-Alay (Turkestan, Nuratau ridges). Endemic of Uzbekistan, North-Western Pamir-Alay |
| 72. | *Salvia aethiopis* L. | Tien-Shan, Pamir-Alay (Zeravschan, Hissar, Kuhitang ridges), Kopet-Dag |
| 73. | *Salvia ariana* Hedge* (2013) | Kuhitang, Actau, Kopet-Dag, Aysun-tau 38.2220N 67.0239E, 1230 m |
| 74. | *Salvia bucharica* Popov* (2019) | Pamir-Alay (Zeravschan, Hissar, Kuhitang, Babatag ridges) 38.1705N 66.4527E, 1380m |
| 75. | *Salvia desert*a Schangin | Tien-Shan (Alatau, Karatau, Karzhantau, Chatkal, Kuramin ridges), Pamir-Ala (Alay, Nuratau ridges) |
| 76. | *Salvia drobovi*i Botsch. | Pamir-Alay (Zeravschan, Hissar ridges). Endemic of Uzbekistan, Western Pamir-Alay |
| 77. | *Salvia glabricaulis* Pobed. | Tien-Shan (Kuramin ridge), Pamir-Alay (Turkestan ridge) |
| 78. | *Salvia insignis* Kudrjasch. | Pamir-Alay (Babatag ridge). Endemic of the South Pamir-Alay |
| 79. | *Salvia komarovii* Pobedjasch. | Pamir-Alay (Zeravschan ridge). Endemic of the South Pamir-Alay |
| 80. | *Salvia korolkovii* Regel & Schmalh.* (2019) | Tien-Shan (Karazhantau, Ugam, Pskem, Koksu, Chatkal ridges) Rare endemic of the Western-Tien Shan 41.3033N 69.5854E, 1500m |
| 81. | *Salvia lilacinocoeulea* Nevski | Pamir-Alay (Hissar, Baysuntau, Kuhitang). Endemic of the South-Western Pamir Alay |
| 82. | *Salvia macrosiphon* Boiss. | Syrdarya, Zeravschan, Kashkadarya, Pamir-Alay (Alay, Turkestan, Nuratau, Zeravschan, Hissar) |
| 83. | *Salvia margaritae* Botsch.* (1979) | Pamir-Alay (Samarqand mountains, Hissar ridge). Rare narrow endemic of the Alay ridge 39.9767N 71.8130E, 1544m |
| 84. | *Salvia nemorosa* L. | Aral and Caspian deserts |
| 85. | *Salvia sarawsvchanica* Regel & Schmalh. | Pamir-Alay (Nuratau, Turkestan, Zeravschan, Hissar, Kuhitang ridges) |
| 86. | *Salvia sclarea* L.* (2019) | Tien-Shan (Alatau, Karatau, Ugam, Pskem, Chatkal, Kuramin ridges), Pamir-Alay (Alay, Turkestan, Nuratau, Zeravschan, Kopet-Dag) 41.6743N 69.9613E, 3409 m |
| No. | Species                        | Distribution                                                                 |
|-----|--------------------------------|------------------------------------------------------------------------------|
| 87. | *Salvia spinosa* L.* (2013)    | Kyzylkum outlier mountains, Tashkent chul, Zeravshan, Kashkadarya rivers valley, Pamir-Alay, Kopet-Dag |
| 88. | *Salvia submutica* Botsch. & Vved.* (2019) | Pamir-Alay (Nuratau ridge). Rare narrow relict endemic of Nuratau 40.3109N 66.4008E, 1490m |
| 89. | *Salvia tianschanica* Makhm.   | Tien-Shan (Chatkal, Kuramin ridges), Bashkyzysay. Rare endemic of Uzbekistan, Tien-Shan |
| 90. | *Salvia turcomanica* Pobed.    | Pamir-Alay (Hissar ridges), Kopet-Dag |
| 91. | *Salvia virgata* Jacq.         | Tien-Shan (Alatau, Karatau, Ugam, Pskem, Chatkal, Kuramin ridges, Pamir-Alay (Alay, Turkestan, Nuratau, Zeravshan, Hissar ridges) |
| 92. | *Perovskia angustifolia* Kudrj. | Tien-Shan (Chatkal, Kuramin, Mogoltau), Pamir-Alay (Alay, Nuratau, Hissar ridges) |
| 93. | *Perovskia botschantzzevi* Kovalevsk. & Kocz. | Tien-Shan (Chatkal, Fergana, Kuramin, Mogoltau ridges), Pamir-Alay (Nuratau, Kuhitang ridges) |
| 94. | *Perovskia kudrjaschevii* Gorschk. & Pjat. | Tien-Shan (Karzhantau, Chatkal ridges), Pamir-Alay (Nuratau, Turkestan, Hissar, Kuhitang ridges) |
| 95. | *Perovskia scrophulariifolia* Bunge | Tien-Shan (Fergana ridge), Pamir-Alay (Alay, Turkestan, Nuratau, Zeravshan, Hissar, Kuhitang ridges) |

* - the species used in this work

Most of them are source of biologically active compounds and used as antioxidant, antiseptic, immunomodulatory, antimicrobial in traditional medicine and pharmaceutics. Thus, *Ziziphora* [15-17], *Salvia* [18], *Hymenocrater* [19], *Dracocephalum* [20] and other representatives of Lamiaceae characterized by a high content of polysaccharides, polyphenols, alkaloids and other carbohydrates [21-23].

The world flora accounts more than 70 species of the genus *Dracocephalum*, Central Asia flora-26 species, 16 species are expected to grow in Uzbekistan [24]. The fund of the National Herbarium of Uzbekistan (TASH) submitted by herbarium specimens of 32 taxa collected in Central Asia.

The genus *Lallemantia* accounts five species native to Central and Southwestern Asia. In the flora of Uzbekistan the genus represents by single species *Lallemantia royleana*.

The genus *Salvia* includes approximately 1000 species distributed throughout the world [25-28], 25 species are currently accepted for the flora of Uzbekistan. The National Herbarium of Uzbekistan (TASH) contains over 1800 herbarium specimens belong to 43 species of the genus *Salvia*.

The genus *Nepeta* is also a large genus of *Mentheae* and accounts approximately 300 species, distributed in Eurasia [29]. It represents by 19 described species in Uzbekistan [30].

According to modern data, the genus *Ziziphora* includes from 25 to 30 accepted taxa, which are distributed in the Mediterranean and Central Asia [13,15,31]. 8 taxa of *Ziziphora* are named for the territory of Uzbekistan. Its species are of prime importance in different fields of pharmaceutical, chemical, medicinal, traditional and folk medicines [15-17].

The results of hierarchical clustering are presented by a constructed dendrogram obtained by the Neighbor-Joining (NJ) algorithm, which is congruent with Maximum Likelihood (ML) method, using MEGAX program; the reliability assess of the resulting phylogenetic tree topology was obtained with Bootstrap analysis (1000 replicates). The resulting graphic image was visualized using the MX:TreeExplore program. A phylogenetic analysis of 14 taxa was performed based on the obtained nucleotide sequences of the *ITS* nuclear region.

It shows the phylogenetic relationships of some genera within the tribe *Mentheae*. The *ITS* region is commonly used to identify plants of different geographical origins for its high variability properties. It is able to provide high resolution to understand the genera classification within one tribe. 32 sequences of the *ITS* region used for a phylogenetic dendrogram construction were download from the GenBank database (Figure 1). The region of target marker for comparing sized 610 b.p.
**Figure 1.** Hierarchical clustering of some genera within the tribe *Mentheae*, constructed according to the sequence comparison of the ITS region by Neighbor-Joining and Maximum Likelihood methods. Bootstrap support (above 50%) for 1000 replicates is shown above branches.

Based on the analysis of Drew B. [32], species *Lavandula dentate, Lavandula multifida* were used as an outgroup. The genus belongs to the tribe *Ocimeae*, which is closely related *Mentheae*, within subfamily *Nepetoideae* (*Lamiaceae*) [14].

The dendrogram was constructed using nuclear region ITS data. So, the phylogenetic tree shows phylogenetic relationships of some genera within the tribe *Mentheae*, and revealed a clear division of the tribe into four main clades by subtribes, with a high Bootstrap value: *Menthinae; Nepetinae; Salviinae; Lycopinae* (Figure).

According Harley R. [14], *Mentheae* is subdivided into three subtribes, *Salviinae, Nepetinae* and *Menthinae*. The later studies by Drew B. [32], had based upon morphological and molecular evidence,
identifies additional two new subtribes Lycopinae B. T. Drew & Sytsma, subtribe nov. TYPE: Lycopus L. and Prunellinae (Dumortier) B. T. Drew & Sytsma, TYPE: Prunella L.

The genera quantity various from approximately 60 [33], 65 [14] to 73 [25] genera. Mentheae are characterized by stamens divergent or ascending (not decline), a distinctly 2-lipped corolla, symmetric disc (if asymmetric and anterior lobe elongate, then corolla distinctly 2-lipped), and nutlets with an areolate abscission scar [33].

The species of Thymus, Origanum, Micromeria, Sataureja, Mentha, Clinopodium, Ziziphora genera are grouped into one clade belonging to subtribe Menthinae. It is broken into two major well-supported subclades. Mentha, Clinopodium, Ziziphora genera is grouped in a subclade as a sister to subclade with Origanum, Micromeria, Thymus, Satureja Mediterranean genera [32]. This clade is characterized by reticulate pollen grains and the circular shape of the abscission scar with an expanded area on the nutlets [34].

The represents of Dracocephalum, Hissopus, Lallemanatia, Hymenocrater, Drepanocaryum, Nepeta genera are nested within subtribe Nepetinae. Nepeta, Drepanocaryum, Hymenocrater, Lophanthus form one subclade. It is probably originated in the Mediterranean/Central Asia with spread to East Asia region. Dracocephalum, Hissopus, Lallemanatia are included in subclade with a much broader distribution [2]. Dracocephalum and Hissopus show a close relationship.

Other Menthinae genera such as Melissa, Perovskia, Salvia are considered as potential members of separate group. Melissa officinalis, Perovskia abrotanoides, Salvia scrophularifolia form subclade, and close sister related to Salvia, that as a part embedded within subtribe Salviinae. According authors [25,26,35], Salvia L. is a highly polyphyletic genus. Our ITS dataset also provides evidence on the clearly polyphyletic nature of Salvia genus consisting separate subclades. It comprises two distinct lineages including Salvia species with different sister group and additional genera. It is revealed, that Salvia genus is non- monophyletic, and consists of two major subclades. Thus, S. bucharica, S. korolkovii, S. submutica are grouped into Salvia subgenus, S. arriana, S. spinosa, S. sclarea, S. aethiopis, S. sarawshanchica, S. macrosiphon are formed Sclarea subgenus. S. margaritae belonging to Macrosphace subgenus is placed separately in a highly supported branch.

In our study Lycopus europaeus forms a separate moderately supported clade of Lycopinae subtribe. According Drew B. [32], based on cpDNA, nrDNA, morphological (two stamens, a unique pericarp structure) analyses, has support subtribal status for Lycopus L.

So, our hierarchical clustering within Mentheae has revealed two strongly supported monophyletic large clades: Salviinae and Nepetinae + Menthinae subtribes, and one separate Lycopinae subtribe.

These results consistent with the classical classification of the tribe Mentheae proving the taxonomic position of taxa according to their morphological characters [14,34,36].

Relationships within Mentheae have been greatly clarified based on molecular evidence. Thus, the currently accepted subtribes are Salviinae, is sister to Nepetinae, Menthinae, Prunellinae and Lycopinae [32,33,37]. The monophyletic nature of Mentheae and its division are also supported by our analyses.

4. Conclusions
Thus, the results obtained in this research demonstrates the effectiveness of using DNA sequences of nuclear ITS regions as an approaches for the diversity assessing of rare endangered plant species growing on the territory of Uzbekistan.

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6. References
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