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Assessment of *Cousinia mindshelkensis* populations (Asteraceae) at the Karatau Ridge, South Kazakhstan

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Abstract. *Kenesbay A, Kurmantayeva A, Sitpayeva G, Shmakov A, Koltunova A, Kulymbet K. 2025. Assessment of* Cousinia mindshelkensis *populations (Asteraceae) at the Karatau Ridge, South Kazakhstan. Biodiversitas 26: 6174-6187. Cousinia mindshelkensis* is a narrow-localized endemic species, spread by small populations in the central part of the Karatau ridge, Western Tien Shan, and is included in the Kazakhstan Red Book. This study aimed to assess the current state of *C. mindshelkensis* populations in the Karatau range based on morphological, ecological, and molecular characters. Four populations (P1-P4) were investigated. Morphological data was analyzed based on test plots with 10-15 individuals from each population, while ecological analysis included habitat description. Molecular data based on iPBS and ITS markers were carried out using the STRUCTURE program, cluster and phylogenetic analysis. Morphological traits showed high variability. Plants from P1 and P2 were the smallest, P4 was characterized by intensive vegetative growth with reduced flowering, whereas P3 demonstrated the best reproductive performance. The highest density occurred in P1 and P2 of 4.1±0.9 and 1.6±0.79 individuals/m², respectively. Ecological analysis indicated that the species inhabits petrophytic, mixed-herbaceous-woody-shrubby, and herbaceous-shrubby communities. Genetic diversity assessed by 3 iPBS markers was an average of 61.2% with the highest presented by iPBS2373 of 87.5% polymorphism and phylogenetic analysis confirmed all samples belong to a single clade. These results highlight the necessity of continuous monitoring and justify the inclusion of *C. mindshelkensis* among priority targets for biodiversity conservation in the region.

Keywords: *Cousinia mindshelkensis*, ecological, Karatau Range, Kazakhstan, molecular

INTRODUCTION

The genus *Cousinia* was described by A.N. Cassini in 1827, based on *C. carduiformis*. However, the species name had to be later replaced with a priority, namely *C. orientalis*, as this plant had been described earlier (1805) as *Carduus orientalis* (Cherneva 1983). De Candolle's processing became the first summary of the genus. The genus belongs to the polymorphic genera of the family Asteraceae, and is one of the largest genera of the tribe Cardueae. It has more than 600 species distributed in Southwest and Central Asia (POWO 2025). Cherneva (1962, 1974) for the first time provides information on the species diversity of the genus *Cousinia,* a typical representative of the mountainous flora of Iran-Turan.

There are 56 species of *Cousinia* recorded in Kazakhstan, 35 of them in the Karatau Range (Western Tien Shan). Of the total number of Karatau *Cousinia,* 5 species are endemic to the Karatau flora, of which 2 species are relict narrow-range species (Kurmantayeva 2003; Red Book of Kazakhstan 2014). The *Cousinia* species diversity center stands out in Karatau, in the area of the Karatau massif of the highest peaks (Bessaz) (Kamelin 1990). This allows us to consider the genus *Cousinia* as a representative taxonomic group for the characterization of the Karatau. The species of the section *Lopholepis* are recorded only in the central part of Karatau (Mynzhilki), indistinctly separated from each other by races of a narrowly endemic relict cycle of species, such as *C. mindshelkensis* and *C. gomolitzkii*. *Cousinia* species confined to this part of Karatau are exclusively petrophytes.

The studies of the genus *Cousinia* in Central and Southwestern Asia focused mainly on morphological, ecological, and molecular characters. Thus, the Cynaroideae section, comprising 89 species, was analyzed in terms of morphological and genetic diversity (Atazadeh et al. 2020). The endemic species *Cousinia calocephala*, distributed across 14 Iranian provinces from Elburz to the Zagros, was analyzed using ISSR markers. Thirteen populations showed pronounced morphological and genetic differentiation: ANOVA confirmed high morphological variability, and AMOVA revealed 94% of genetic diversity among populations and 6% within them. Three ecotypes were identified, highlighting the adaptive influence of local conditions (Atazadeh et al. 2020).

According to Karimov et al. (2025), the chloroplast genomes of 6 *Cousinia* species ranged from 152,553 to 152,619 bp and exhibited a conserved quadripartite structure. Nucleotide diversity analysis revealed 412

polymorphic sites, with variable regions (trnE-rpoB, rbcL, ycf1) useful for species differentiation. These findings highlight the greater resolving power of chloroplast genome data compared to morphological traits.

Retrotransposon markers of the binding site between primers are highly effective in assessing the genetic diversity of plant populations, including endemic species in Kazakhstan. They were applied as markers for research on *Allium ledebourianum* (Raizer and Khapilina 2020; Khapilina et al. 2021) and *Tulipa* (Salybekova et al. 2025). The iPBS method offers high-resolution genotyping for detecting intraspecific polymorphism, as demonstrated in studies on Asteraceae (Ali et al. 2019; Bonchev and Vassilevska-Ivanova 2020). The ITS region of nuclear ribosomal DNA has proven highly informative for phylogenetic analyses and species delimitation in *Cousinia* (Atazadeh et al. 2020). However, integrative studies combining morphology, ecology, and molecular data (iPBS and ITS) remain scarce. This work provides the first comprehensive evaluation of populations of the rare endemic *C. mindshelkensis* in the Karatau. This study aimed to assess the current state of *C. mindshelkensis* populations in the Karatau range based on morphological, ecological, and molecular characters.

MATERIALS AND METHODS

Study area

Field studies were conducted in the central part of Karatau, Turkestan Region, South Kazakhstan. During the study, four populations (P1-P4) were identified of *C. mindshelkensis* (Figure 1, Table 1). Populations of *C. mindshelkensis* have been recorded in the Kishikarakuys, Itmuryin, and Karaungir gorges (Kenesbay et al. 2024). The areas are located at altitudes of 1,200 to 1,450 m asl, mainly on the southern and southwestern slopes with rocky and gravelly substrate. Monitoring of the phenological phases of *C. mindshelkensis* was carried out during the growing season from July to September in 2020-2021, which ensured high accuracy in determining the stages of plant development.

Table 1. Collection sites of *Cousinia mindshelkensis* samples for molecular genetic analysis

Population	Location	Altitude (m asl)	Latitude (N)	Longitude (E)	Collection number
1	Kazakhstan, Turkestan Region, Sozak District, Karatau, Kishikarakuis Gorge	1046	43°50′54″	68°31′55″	AA: 0003091
2	Kazakhstan, Turkestan Region, Sozak District, Karatau, Kishikarakuis Gorge	1063	43°50′50″	68°36′52″	AA: 0003092
3	Kazakhstan, Turkestan Region, Sozak District, Karatau, Itmuryn Gorge	1220	43°49′40″	68°40′45″	AA: 0003093
4	Kazakhstan, Turkestan Region, Sozak District, Karatau, Karaungir Gorge	1130	43°49′41″	68°42′23″	AA: 0003094

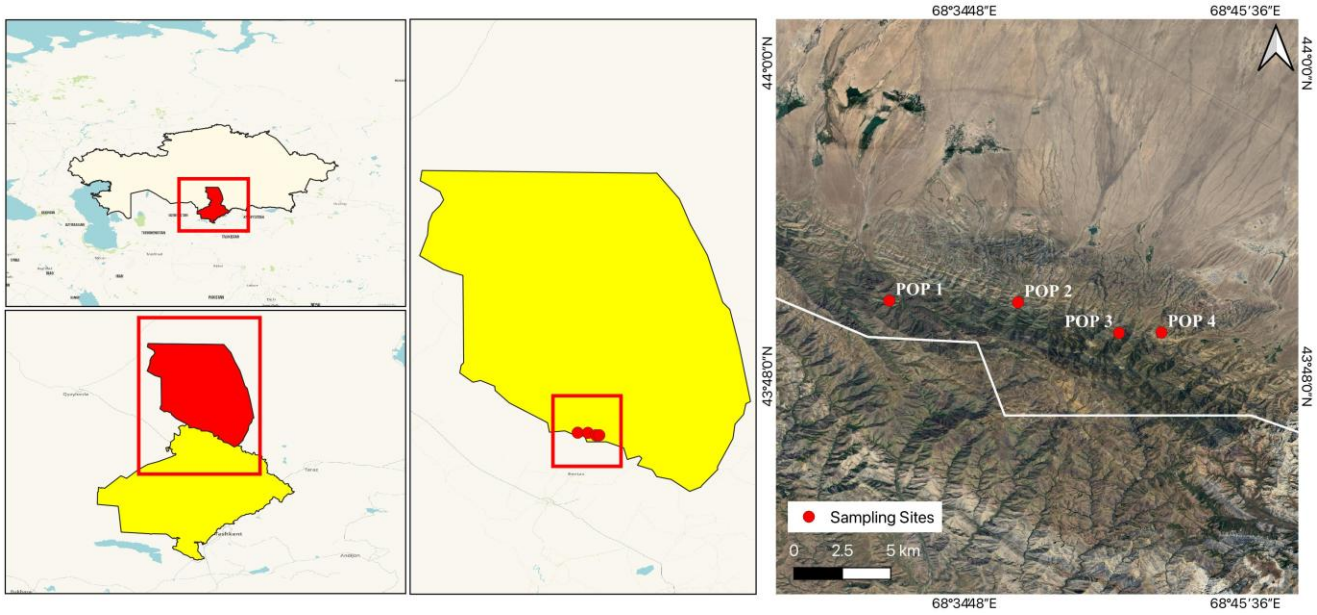

Figure 1. The distribution of four populations of *Cousinia mindshelkensis* in the Karatau Range, Turkestan Region, South Kazakhstan

Plant materials

Plant materials were collected from the natural populations. In the course of scientific research, the herbarium material stored at the Institute of Botany and Phytointroduction (AA), the herbarium fund of Al-Farabi KazNU, the Central Herbarium of the Institute of Botany of the Academy of Sciences of the Republic of Uzbekistan (TASH), the Digital Herbarium of Moscow State University (National Bank-Depository of Living Systems), the herbarium of higher plants of the V.L. Komarov Botanical Institute of the Russian Academy of Sciences, literary data, and our own research. During the work, 4 herbarium specimens of *C. mindshelkensis* were collected from 4 populations. The collected herbarium was transferred to the herbarium collection of the Institute of Botany and Phytointroduction under the collector numbers No. 0003091, No. 0003092, No. 0003093, No. 0003094 (Table 1). In the molecular study, 32 samples of *C. mindshelkensis* were used. Leaf samples for analysis were collected in the Kishikarakuis, Itmuryin, and Karaungir gorges (Table 2). Eight samples from each population were selected for analysis.

Procedures

Morphological observation

During monitoring, the following phenological phases were recorded, i.e., development of generative shoots, budding, flowering, fruiting, and death of above-ground shoots. Phenological stages were recorded visually, through direct field observation of individual specimens in the study areas (Figure 2). Field studies were carried out using the route reconnaissance method, while systematic ecological research was conducted with the morphological-geographical approach (Pavlov 1966; Baitenov et al. 1972). Population characteristics of *C. mindshelkensis* were assessed by standard geobotanical methods (Figure 2). To evaluate morphological variability (Begenov et al. 2015; Mukhitdinov et al. 2016; Zoteeva 2019; Bazarov et al. 2023), ten 1×1 m sample plots were established in each population, for a total of 40 plots (Erzhapova and Alikhadzhiev 2015; Naumov and Kirpichev 2017; Rumyantsev et al. 2023). For the morphological study, 10-15 individuals from each population were measured. Given the rarity and conservation status of the species, the number of individuals studied was limited to minimize the impact on natural populations while maintaining statistically representative data.

Vegetative traits included basal leaf length (BLL), width of the rosette leaves (WRL), and number of teeth on the basal leaves (TBL). Stem leaf parameters such as the number of stem leaves (NSL), stem leaf length (SLL), and stem leaf width (SLW) were also recorded. Generative traits encompassed generative shoot height (GHF), inflorescence height and width (HL, HW), flower length (FL), and the number of flowers per inflorescence (NF). All collected data were processed using standard descriptive statistics (mean±standard deviation), and comparative analysis was conducted to identify trait variation among populations (Erzhapova and Alikhadzhiev 2015; Fomina et al. 2019). Morphometric data and macroimages were obtained using a Levenhuk DTX RC3 remote-controlled microscope.

Ecological observation

Ecological data were collected in situ during population surveys using standard geobotanical methods, including description of habitat conditions (topography, soil, and moisture), assessment of vegetation cover, and identification of associated plant species (Seledec and Probatova 2022).

Genetic analysis

DNA isolation: DNA isolation was performed using the Diamond DNA Plant kit (LLC Altaibiotech, Russian). DNA extraction was performed according to the manufacturer's protocol.

DNA amplification: The iPBS primers were used to detect polymorphism among *C. mindshelkensis* samples. Additionally, the Internal Transcribed Spacer (ITS) region of ribosomal DNA was sequenced to provide a complementary, sequence-based phylogenetic context with higher resolution for clarifying interspecific relationships. The amplification reaction was carried out at the enterprise using ready-made PCR mixtures BioMaster HS-Taq PCR (Biolabmix, Russia) in a volume of 15 μL and a final primer concentration of 400 nM. BioMaster HS-Taq PCR Mix is a ready-to-use master mix containing: Taq DNA polymerase, dNTPs (200 μM each), $MgCl_2$ (1.5-2.5 mM, optimized), Reaction buffer (pH ~8.3-8.5), and primers synthesized by Evrogen LLC (Russia) were used for the analysis.

Three iPBS markers, i.e., iPBS2373 (5'-GAACTTGCTCCGATGCCA-'3), iPBS2386 (5'-CTGATCAACCCA-'3), and iPBS2239 (5'-ACCTAGGCTCGGATGCCA'-3) were selected for further analysis of interpopulation variation (Dorogina and Zhmud 2020). These primers successfully generated DNA amplicons with polymorphic loci. The PCR protocol included: an initial denaturation at 95°C for 3 minutes; followed by 30 cycles at 95°C for 15 seconds for denaturation, with annealing at 57°C for 30 seconds, extension at 72°C for 60 seconds; and a final extension at 72°C for 5 minutes.

Figure 2. Habitat and morphology of flowers of the first population of *Cousinia mindschelkensis* in the Karatau Range, Turkestan Region (South Kazakhstan)

Amplification of the ITS region, forward primer F (5′-TCGGATTGGTCTCGCTCTG-3′) and reverse primer R (5′-GCGTTCCAAGATGTCGATGTTC-3′) primers were used (Kutsev et al. 2014). DNA concentration was determined using the MaxLife personal gene analyzer H100 (Barnaul, Russia). Amplification was conducted in a programmable thermal cycler (Thermal Cycler) under the following conditions: initial DNA denaturation for 3 minutes at 94°C, followed by 35 cycles consisting of three steps: 30 seconds at 94°C for denaturation, 30 seconds at 57°C for annealing, and 1 minute at 72°C for extension. A final extension step was carried out for 10 minutes at 72°C, followed by cooling to 10°C for 10 minutes.

Electrophoresis and DNA sequencing: Amplification products were separated using 1.5% agarose gel electrophoresis in 1xTAE buffer at 80 V, stained with ethidium bromide (10 μg/mL). DNA fragments were visualized under UV light using a Gel IX20 Imager (INTAS, Göttingen, Germany) and documented with a Mitsubishi P93D thermal printer (Mitsubishi Electric Corporation, Tokyo, Japan). DNA sequencing was performed in the Bioengineering Laboratory of Altai State University using a 3500XL automated gene analyzer (Applied Biosystems, USA). The required amount of DNA fragment (0.3-0.5 pmol) was evaporated to dryness and dissolved in a prepared master mix consisting of 1-2 μL of BigDye 3.1 reagent (Applied Biosystems, USA), 1X sequencing buffer (Nimagen, USA), and additional components for complex regions, in a total volume of 40 μL. The Sanger reaction temperature profile consisted of a denaturation step for 3 min at 95°C, followed by 50-70 incubation cycles (25 sec at 95°C, 10 sec at 45-50°C, 3 min at 60°C), a final reaction heating for 5 min at 98°C, and storage at 4°C until the purification step. Sanger reactions were evaporated, dissolved in 25 μL of highly deionized Hi-Di formamide, and analyzed on a 3500XL automated gene analyzer. The resulting sequencing data were processed using Sequence Analysis v.6 software.

Data analysis

The data set was subjected to statistical analysis to assess morphological variability between populations. Descriptive statistics (mean, standard deviation, minimum, maximum) were calculated for each feature (Zhukova and Minets 2021; Ghaffari et al. 2022). Pearson correlation coefficients were used to evaluate the relationship between the main morphological parameters. Statistical analysis of morphological parameters of populations, including the Pearson correlation, was performed using the R-Studio platform (R-Studio Team 2020). The Pearson correlation coefficient was used as a measure of the relationship between variables, and the level of statistical significance was set at $P<0.05$ (Wanda et al. 2021; Zhuikova and Popova 2022).

Ecological data were analyzed through a comparative assessment of habitat characteristics, including soil conditions, topography (slope and aspect), and vegetation composition. Descriptive statistics were used to quantify key ecological parameters, and habitat similarity between populations was assessed through comparative analysis.

For the iPBS data, amplified fragments were scored as a binary matrix (1 for presence, 0 for absence of a band). Standard genetic diversity indices, including the number of alleles (Na), effective number of alleles (Ne), Shannon's Information Index (I), Hennig's diversity (h), and the percentage of polymorphic loci (PPL) were calculated using program PopGene (Freitas et al. 2018; Tonkin-Hill et al. 2019; Raizer and Khapilina 2020; Zulfahmi et al. 2021; Yildiz and Arbizu 2022). Population structure was inferred using a Bayesian clustering approach in the STRUCTURE software. The optimal number of clusters (K) was determined by the ΔK method (Buglova and Nuzhdina 2018; Pérez-Vargas et al. 2020).

Phylogenetic relationships were reconstructed using two methods Maximum Parsimony analysis was performed in PAUP* 4.0b10 using a heuristic search with 100 random addition replicates and TBR branch swapping (Minaeifar et al. 2015; Cheng et al. 2016; Medvedeva et al. 2023; National Center for Biotechnology Information 2025). Gaps were treated as missing data. Branch support was assessed using 1,000 bootstrap pseudo-replicates (Banchi et al. 2020; Kolter and Gemeinholzer 2021). Bayesian analysis was conducted in MrBayes 3.1.23, running two independent MCMC analyses for 10 million generations, sampling every 100 generations. The first 25% of trees were discarded as burn-in, and a majority-rule consensus tree was built from the remaining trees to obtain posterior probabilities (Kutlunina and Ermoshin 2017; Dorogina and Zhmud 2020; Nyamgerel et al. 2023).

RESULTS AND DISCUSSION

Morphological characteristics

According to our observations, *C. mindshelkensis* is a prickly cushion-like xerophilous perennial plant. The height of plants varies from 25 to 63 cm, under favorable habitat conditions (Figure 3). Stems are erect, simple, slightly branching in the upper part, white cobwebby; leaves are denser below, thinly gray-tomentose. Basal leaves are narrowly lanceolate, basal leaves reaches 5.33-13.66, along the edge of the basal leaves, there are spaced-notched-spiny teeth with a size of 9.45-29.3, stem leaves decrease in height upwards 1-4.8 cm. Basil is spherical to ovoid, the basket reaches 1.2-2.5. Slightly cobwebby above. The bracts, except for the inner ones, are densely leathery, oblong-ovate or lanceolate, keeled in the upper part, ending at the apex with a strong, protruding, yellow point; the corollas of the flowers are whitish or pale pink; the achenes are oblong to inversely ovate.

Analysis of morphological data from four geographically isolated populations of *C. mindshelkensis* (P1-P4) in the Karatau revealed marked variability in both vegetative and generative traits. The highest values of generative height (GHF) were recorded in population P3, which is probably due to more favorable microenvironmental conditions or genetic characteristics of this group. At the same time, populations P1 and P2 show more modest indicators (31.51±4.52 cm and 33.29±3.45 cm, respectively). Traits BLL, WRL, and TBL also varied:

individuals from population P2 had the longest basal leaves (11.13±1.26 cm) and the maximum number of teeth on them (TBL: 23.75±3.13), while in population P1 these indicators were minimal.

Table 2. Population-level variation in morphological parameters of *Cousinia mindshelkensis* studied

Morphological parameters	Population							
	P1		P2		P3		P4	
	Mean±SD	Min/Max	Mean±SD	Min/Max	Mean±SD	Min/Max	Mean±SD	Min/Max
GHF (cm)	31.51±4.52	25-40	33.29±3.45	28-40	48.01±9.2	31-63	42.93±6.36	34-51
SL (cm)	32.73±4.35	25-39	31.95±3.54	25-36	24.4±2.03	20-28	22.95±2.36	20-28
NS (cm)	3.8±2.14	1-7	2.37±1.29	1-5	2.13±0.92	1-4	1.89±0.1	1-3
BLL (cm)	7.08±0.75	5.33-8.01	11.13±1.26	8.83-13.66	8.99±1.47	6.5-12.33	9.31±0.82	7.6-10.5
WRL (cm)	1.04±0.2	0.8-1.5	1.48±0.35	1-2	1.03±0.49	0.5-2.3	1.02±0.07	0.9-1.25
TBL (cm)	10.8±0.98	9.45-13.1	23.75±3.13	17.2-29.3	20.36±2.09	16.7-24.22	17.66±2.24	13.5-20.7
NSL (cm)	9.93±1.87	7-15	17.62±1.89	14-20	14.62±3.63	7-20	20.04±3.24	15-27
SLL (cm)	3.77±0.61	2.8-4.8	4.12±1.02	2-6.33	2.04±1.15	1-6	3.29±0.42	2.33-4
SLW (cm)	0.6±0.22	0.35-1.2	0.76±0.47	0.35-1.9	0.52±0.15	0.38-1	0.81±0.28	0.5-1.33
TSL (cm)	11.37±1.21	8.4-13.4	13.37±1.85	10.6-16	8.67±1.48	7.1-11.8	10.61±1.34	8.3-14.25
HL (cm)	1.39±0.13	1.2-1.6	1.78±0.25	1.2-2.1	2.03±0.24	1.6-2.5	0	0
HW (cm)	1.79±0.18	1.5-2	1.15±0.18	0.9-1.5	1.37±0.21	1-1.7	0	0
FL (cm)	0.77±0.75	0-1.5	0.89±0.9	0-3	1.25±0.44	0-1.6	0	0
NF (cm)	2.61±1.24	1-5	0.47±0.74	0-2	2.93±2.22	0-7	0	0
NoS (cm)	2.54±1,12	1-4	2.48±1.18	1-5	1.81±0.87	1-3	3.2±1.15	1-5
SL (cm)	27.83±7.11	20-48	26.85±4.79	20-34	31.77±7.7	17-49	30.62±5.18	22-38

Note: GHF: Generative height at flowering (cm), SL: Stem length, NS: Number of stems, BLL: Basal leaf length (cm), WRL: Width of root leaves, TBL: Teeth on basal leaves, NSL: Number of stem leaves, SLL: Stem leaf length (mm), SLW: Stem leaf width (mm), TSL: Teeth on stem leaves, HL: Head length (cm), HW: Head width (cm), FL: Flower length (cm), NF: Number of flowers, NoS: Number of shoots, SL: Shoot length (cm)

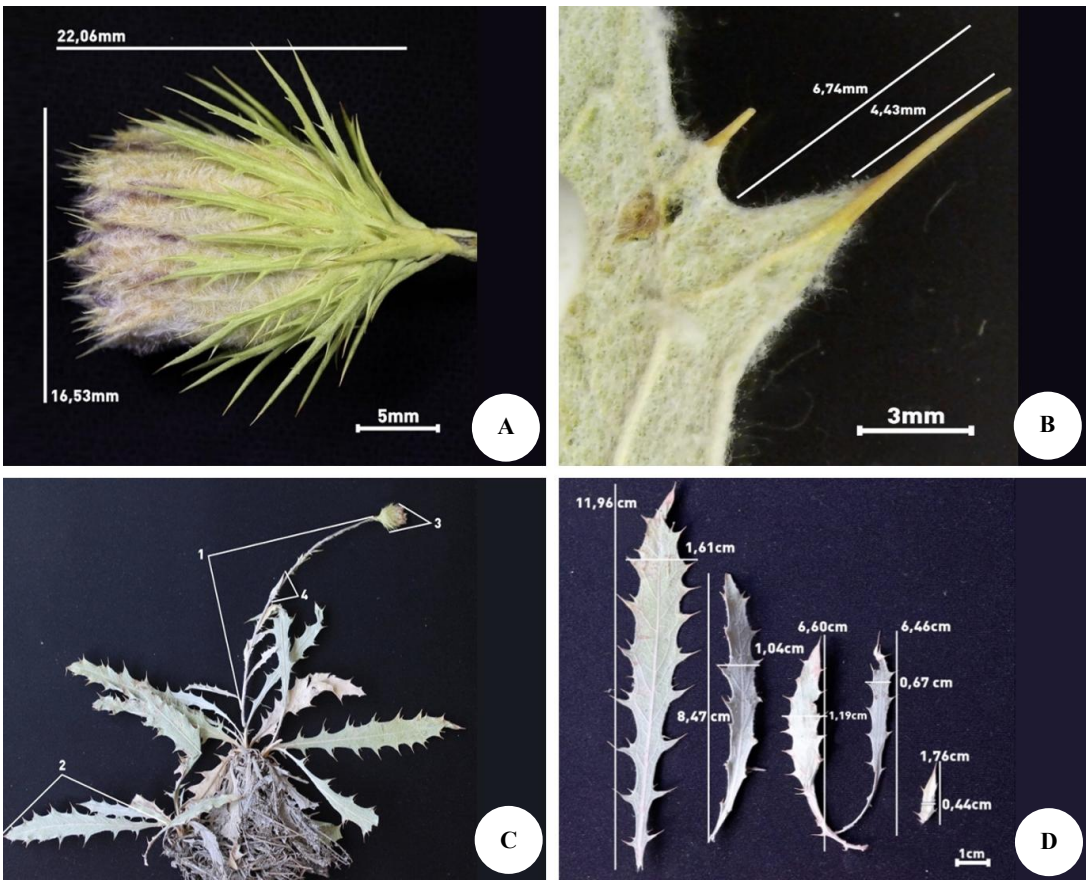

Figure 3. Morphological characteristics of *Cousinia mindshelkensis* in the studied populations: A: Flower, B: Length of teeth, C: Habitus (1: Stem, 2: Basal leaves, 3: Flower, 4: Stem leaves), D: Leaves

The characteristics of stem leaves (NSL, SLL, SLW) show similar trends: the largest number of leaves and their sizes were recorded in P4, despite the overall decrease in the generative activity of this population. This may indicate its vegetative orientation during the growing season. Generative traits, including inflorescence size (HL, HW), flower length (FL), and number of flowers (NF), are only present in populations P1-P3. The absence of these data in P4 is apparently due to phenological asynchrony or unfavorable conditions that affected flowering and fruiting. The largest values of generative structures were recorded in P3, indicating higher reproductive activity (Table 2).

There was a high positive correlation between the number of flowers and the number of shoots in all populations (Figure 4). This is logical, since each shoot is a potential inflorescence axis, and an increase in their number increases the reproductive potential of the plant. In populations 1 and 2, a positive correlation was found between stem length and number of flowers, which may indicate better development of generative organs in tall plants. At the same time, in populations 3 and 4, negative correlations between these traits were found, which may be associated with the redistribution of resources: Plants invest in stem growth at the expense of flower formation. The correlation between the length of the baskets (inflorescences) and the length of the flowers varies depending on the population.

In most populations (except for population 3), a negative correlation is observed, which may be explained by limited resources: As the length of the baskets increases, plants expend more energy on their formation, while the size of the flower decreases. Only in the third population is a positive correlation observed, which may indicate a genetically determined tendency toward a parallel increase in these traits.

The negative correlation between the height of generative individuals at flowering and the number of teeth on basal leaves, observed in populations 2, 3, and 4, may be an adaptive response to stressful conditions (Figure 4). When resources are scarce, plants may redistribute energy in favor of generative growth, reducing the development of vegetative structures. This reflects a survival strategy that prioritizes reproduction. In the first population, there are predominantly positive correlations between traits related to growth and branching, indicating stable and uniform plant development under favorable conditions. In the second population, significant negative correlations were found, including between height and number of leaves, which may indicate the presence of stress factors or differences in the life strategies of individuals. In populations 3 and 4, there is a strong correlation between root traits and above-ground biomass, which may be a manifestation of adaptation to arid environmental conditions, where it is important to ensure sufficient water supply through a developed root system (Figure 4).

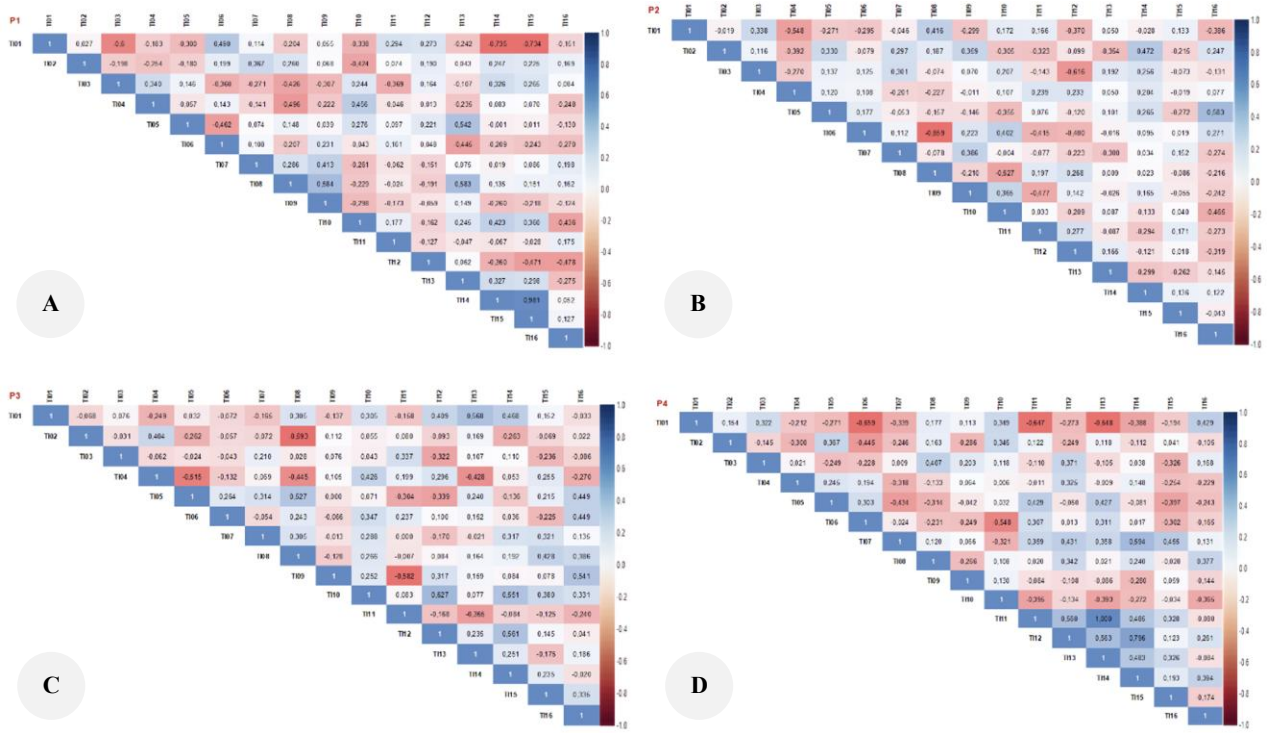

Figure 4. Correlation analysis of morphological traits in four populations of *Cousinia mindshelkensis*. A: Population 1 (P1), B: Population 2 (P2), C: Population 3 (P3), D: Population 4 (P4). Note: Tl01: Height of generative individuals at flowering (pcs), Tl02: Stem length, Tl03: Number of stems, Tl04: Length of basal leaves, Tl05: Width of basal leaves, Tl06: Number of teeth on basal leaves, Tl07: Number of stem leaves, Tl08: Length of stem leaves (mm), Tl09: Width of stem leaves (mm), Tl10: Number of teeth on stem leaves, Tl11: Length of baskets, Tl12: Width of baskets, Tl13: Length of flowers, Tl14: Number of flowers, Tl15: Number of shoots, Tl16: Length of shoots

Ecological characteristics

Cousinia mindshelkensis populations in the central part of the Karatau Range are presented in Figure 5. The first population (P1) was recorded in the Kishikarakuys Gorge at 1,046 m asl, on a north-facing rocky slope (35°) with gravelly substrate and exposed rock outcrops. Rockiness 70%. Petrophytic diverse grass-tree-shrub community. Total projective cover is 50-60%. The density of *C. mindshelkensis* in the habitat varies from a few to 60 specimens/m^2. The species is associated with several tree species, i.e., *Spiraea hypericifolia, Pyrus regelii*, *Malus domestica*, *Acer tataricum*; shrubs, i.e., *Crataegus turkestanica*, *Crataegus songarica*, *Cotoneaster laxiflorus*, *Ephedra equisetina*; and various grass species, i.e., *C. mindshelkensis*, *Artemisia sublessingiana*, *Achillea millefolium*, *Origanum vulgare*, *Ferula tenuisecta*, *Hypericum scabrum.*

The second population of *C. mindshelkensis* (P2) is located on the southern slope of the Kishikarakuys Gorge at an altitude of 1,063 m asl. It is found at the top of a rocky slope. The vegetation cover consists of a tree and shrub community. The total projective cover of the plant is 50-60%. The species is associated with several tree species, i.e., *S. hypericifolia*, *P. regelii*; Shrubs, i.e., *E. equisetina*, *Prunus erythrocarpa, Lonicera tatarica*, *Cotoneaster multiflorus*; and the herb species is *Artemisia mucronulata*.

The third population (P3) is located in the Itmuryin tract at an altitude of 1,250 m asl. It is a large rocky slope with a northeast exposure and a 34° incline. Total projective cover is 60-80%. It forms a mixed grass and shrub community. The species is associated with several shrub species, i.e., *E. equisetina*, *Ephedra intermedia*; mixed grasses, i.e., *Cotoneaster karatavicus* (height 1.5 m), *Acantholimon linczevskii*, *Echinops tschimganicus*, *Ziziphora clinopodioides*, and *Dracocephalum nutans* (height 7-30 cm).

The fourth population (P4) is located in the Karaungir tract at an altitude of 1,130 m asl on the northern slope. It is a grass-herb-shrub community. The total projective cover is 60%. The species is associated with several shrub species, i.e., *C. laxiflorus*, *Cotoneaster oliganthus*; herb species, i.e., *Androsace maxima*, *Festuca valesiaca*, *Stipa caucasica, E. tschimganicus*, *D. nutans*, *A. linczevskii*, and *Galium verum*.

The study showed that the highest population density of *C. mindshelkensis* was recorded in population 1 (4.1±0.9 individuals/m^2) and population 2 (1.6±0.79 individuals/m^2). This indicates favorable conditions for the growth of *C. mindshelkensis* perhaps better light, humidity, or soil structure. The lowest density is observed in population 1 (1.5±0.29 individuals/m^2) and in populations 4 to 1 (1.3±0.27 individuals/m^2). This may indicate a less stable or degraded population, or the beginning of colonization of the territory. The errors show a variation in density within populations, which may be due to microrelief, differences in vegetation cover, or other local factors.

The study characterized four populations of the species based on the distribution of individuals by ontogenetic states. Juvenile (j), immature (im), and virginal (v) individuals are absent in all populations. The absence of juvenile and virginal individuals is associated with the phenological phase of the plants-the harvest was carried out during flowering, and the young are poorly visible or have not yet developed. The age structure is shifted towards generative and aging stages. Virginal (v) plants are present only in population 3 (6.67%), whereas they are absent in the remaining populations.

Young generative individuals (g1) were found in populations 3 (94.1%) and 4 (23.1%), indicating a possible recent generation of new mature individuals, especially in population 3, where they make up the bulk of the population. The remaining populations (1 and 2) lack g1, which may indicate a break in the reproduction process. The average generative individuals (g2) predominate in populations 1-3 (38.9%, 66.1%, and 71.6%, respectively), but in population 4 their proportion is significantly lower (15.3%). This may indicate favorable conditions for a stable functioning adult population in the first three samples. Old generative (g3), sub-senile (ss), and senile (s) individuals are present in all populations, indicating the presence of aging population elements. The largest proportion of old generative individuals was observed in population 1 (66.7%), while in population 4, this indicator was significantly reduced (23.1%). Subsenile and senile plants are most pronounced in populations 1-3, where their total proportion reaches 90.5-74.59%, which may indicate an aging structure and a possible lack of full-fledged renewal. A significant presence of these stages was also noted in population 4 at 38.4%.

Molecular analysis

Genetic distance analysis revealed clear patterns of differentiation among the four populations of *C. mindshelkensis* (Table 3). The pairwise genetic distances ranged from 0.069 to 0.298, indicating varying levels of genetic similarity. The smallest genetic distance (0.069) was observed between Population 2 and Population 4, suggesting a high degree of genetic similarity and potentially recent gene flow or a shared ancestral origin between these two groups. Conversely, the largest genetic distance (0.298) was found between Population 3 and Population 4, highlighting them as the most genetically divergent pairs within the species. This pronounced differentiation suggests effective reproductive isolation, which could be driven by geographical barriers or ecological factors. Population 4 exhibited the most distinct genetic profile, with relatively high genetic distances to all other populations: 0.239 (to Pop. 1), 0.298 (to Pop. 3), and despite being the closest, 0.069 (to Pop. 2). This consistent pattern confirms that Population 4 is the most isolated and genetically unique. These patterns of genetic distance were further supported by the subsequent cluster analysis, which visually grouped the populations according to their pairwise dissimilarities.

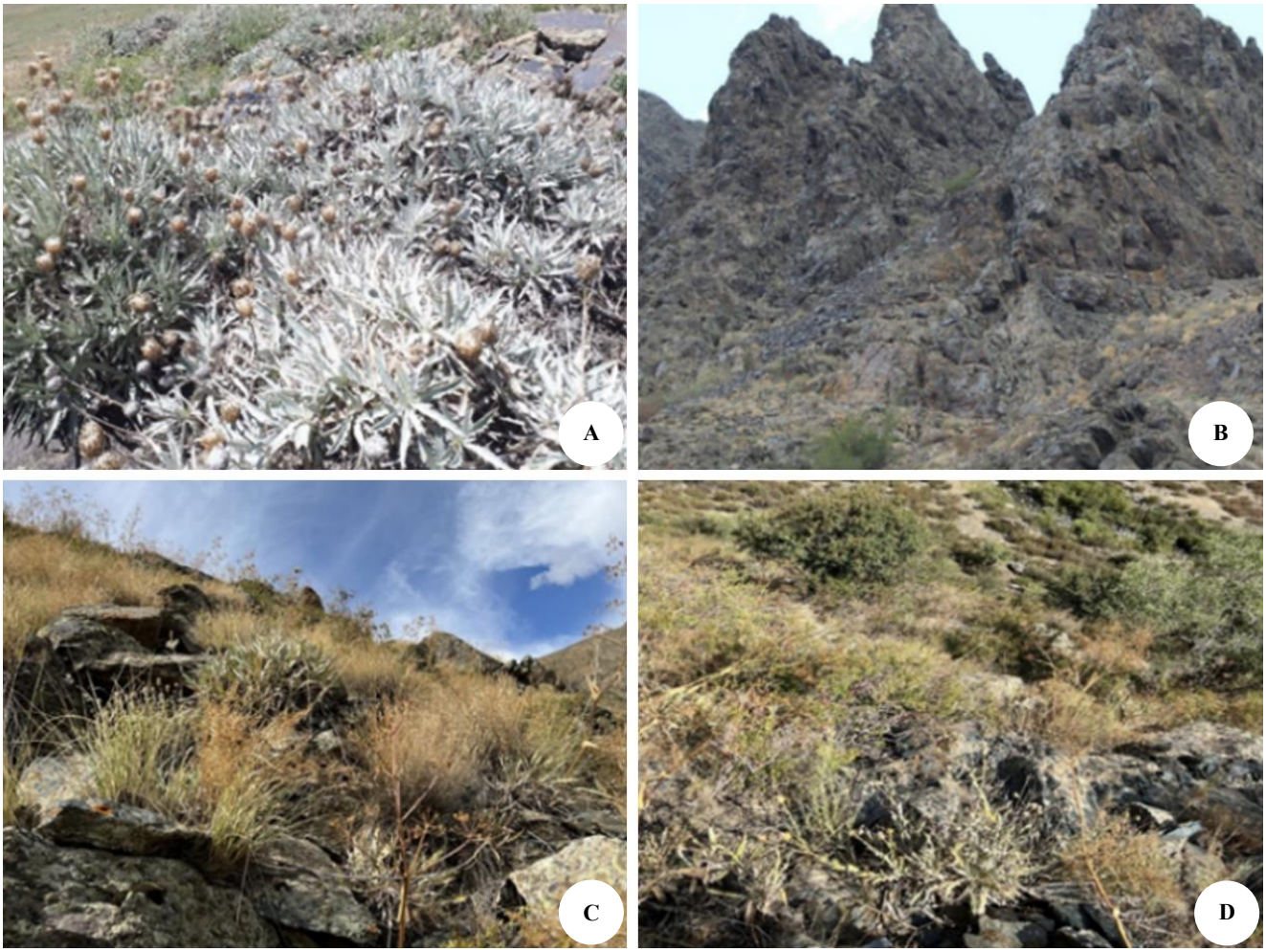

Figure 5. Ecological conditions of *Cousinia mindshelkensis* populations in the Karatau. A. Dense population of *Cousinia mindshelkensis* on rocky slopes (Pop. 1), B. Typical rocky landscape (Pop. 2), C. Habitat with sparse vegetation on steep rocky slopes (Pop. 3), D: Shrub-herbaceous community (Pop. 4)

Table 3. Genetic distances between populations of *Cousinia mindshelkensis*

Population	1	2	3	4
1	0.000			
2	0.145	0.000		
3	0.141	0.205	0.000	
4	0.239	0.069	0.298	0.000

Three highly informative iPBS markers (iPBS2373, iPBS2386, and iPBS2239) were used to assess the intraspecific genetic diversity of *C. mindshelkensis*. All analyzed loci were biallelic (Na: 2), as expected for dominant markers, but demonstrated markedly different levels of informativeness. The primer iPBS2239 was the most effective in capturing diversity, showing the highest values for the effective number of alleles (Ne: 1.676), Shannon's index (I: 0.520), and Nei's gene diversity (Nei: 0.364). A similarly high level of polymorphism was detected by primer iPBS2373, which yielded the highest percentage of polymorphic loci (PPL: 87.5%) and robust values for other indices (Ne: 1.543, I: 0.479, Nei: 0.320). In contrast, primer iPBS2386 showed comparatively limited variation (Ne: 1.146, I: 0.128, Nei: 0.086; PPL: 23.53%), indicating locus-specific constraints or conservation in certain genomic regions.

The average values across all loci (Ne: 1.455, I: 0.360, Nei: 0.257, and PPL: 61.2%) confirmed moderate to high levels of genetic diversity in *C. mindshelkensis* populations. These results, with two of the three markers being highly informative, highlight the potential adaptive capacity of the species and provide a valuable basis for future population genetic assessments and conservation planning (Table 4).

Cluster analysis

The genetic relationships among populations of *C. mindshelkensis* is visualized in a dendrogram (Figure 6). The dendrogram were reconstructed based on a matrix of pairwise genetic distances (Table 3). The analysis identified two primary, well-supported genetic clusters. The first cluster comprises populations 3 and 4, which

exhibit the smallest pairwise genetic distance (d: ~0.05-0.10, based on scale) and form a highly supported clade, indicating a recent common ancestry and a high level of gene flow between them. The second cluster consists of populations 1 and 2. Despite being grouped together, the considerable length of the branches leading to each population within this cluster suggests significant genetic differentiation between them, which is consistent with the relatively large pairwise distance estimated from the matrix (d: ~0.20-0.25).

Population structure

The genetic structure of *C. mindshelkensis* populations was analyzed using iPBS marker data and a model-based clustering approach implemented in STRUCTURE v2.2. The results showed that the highest ΔK value corresponded to K: 2, indicating the presence of two major genetic clusters among the selected individuals (Figure 7.B). The STRUCTURE histogram shows a clear division: individuals on the left side of the graph mainly belong to cluster 1 (red), while those on the right belong to cluster 2 (green). This division indicates a relatively clear genetic separation between the two population groups, with only a small proportion of individuals showing mixed ancestry. The presence of individuals with mixed ancestry indicates limited gene flow between clusters or recent divergence. ΔK values remained relatively low and stable from K: 2 to K: 8 (Figure 7.A), indicating weak or limited population structure at these clustering levels. However, at K: 9, there was a sharp and noticeable peak in ΔK, suggesting that this value represents the optimal number of genetic clusters in the dataset. The magnitude of this peak reflects a significant improvement in the explanatory power of the model at K: 9.

Phylogenetic analysis

The ITS region of nuclear ribosomal DNA was successfully amplified and sequenced for all *C. mindshelkensis* specimens and reference taxa. Sequence lengths ranged from 613 to 643 bp (mean 631 bp), with an aligned length of 658 bp. GC content ranged from 51.4% to 57.2% (mean 54.3%). The alignment contained 18 indels and 104 variable sites, of which 78 were informative for parsimony. Phylogenetic analysis was performed using maximum parsimony (PAUP* 4.0b10), Bayesian inference (MrBayes 3.1.2), and neighbor-joining (Figure 8). Both MP and BI revealed consistent topologies with moderate homoplasy and high support values, clustering *C. mindshelkensis* specimens according to geographic populations. NJ analysis grouped the species into two distinct clades. All *C. mindshelkensis* specimens had identical nucleotide sequences. The sequence data have been deposited in GenBank (NCBI 2025, https://www.ncbi.nlm.nih.gov/). The sequence data have been deposited in GenBank (NCBI 2025, https://www.ncbi.nlm.nih.gov/). The samples were assigned the following ID numbers: coll. Number 0003091 (Pop1): PP109398, coll. Number 0003092 (Pop2): PP109399, coll. Number 0003093 (Pop3): PP109400, and coll. Number 0003094 (Pop4): PP109401. Analysis of ITS sequences from *C. mindshelkensis* samples showed identical nucleotide sequences in all samples.

Table 4. iPBS-based assessment of genetic diversity in natural populations of *Cousinia mindshelkensis*

Locus	Na	Ne	I	Nei	PPL (%)
iPBS2373	2	1.543	0.479	0.320	87.5
iPBS2386	2	1.146	0.128	0.086	23.53
iPBS 2239	2	1.676	0.520	0.364	81.82
Mean	2	1.455	0.360	0.257	61.2

Note: Na: Observed number of alleles, Ne: Effective number of alleles, Nei: Nei gene diversity, I: Shannon information index, PPL: Percentage of polymorphic loci

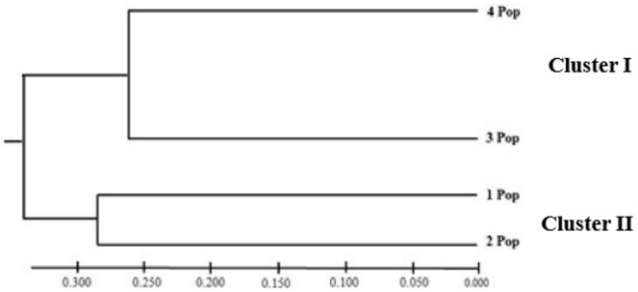

Figure 6. Dendrogram of genetic relationships of four populations of *Cousinia mindshelkensis* constructed by the UPGMA method based on the genetic distance matrix

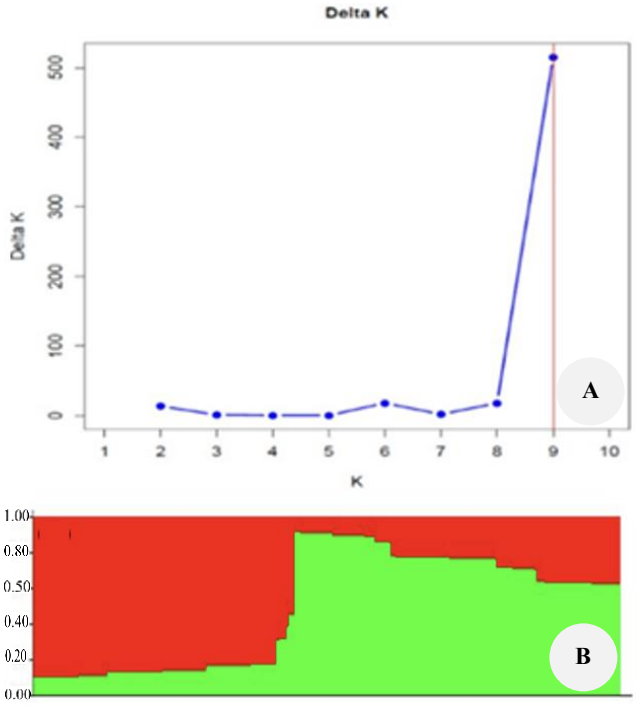

Figure 7. Structural analysis of 4 populations of *Cousinia mindshelkensis*. A: Change in (delta K) probability for K: 1-9, B: Genetic structure of genotypes obtained using STRUCTURE software based on iPBS marker datasets (iPBS2373, iPBS2386, and iPBS 2239)

The phylogenetic relationships of *C. mindshelkensis* and related taxa based on ITS sequencing data presents three main clades with high posterior probability (PP) and bootstrap support (BS) values were identified (Figure 8). All *C. mindshelkensis* specimens (populations 1-4) were grouped into clade II, which corresponds to section *Lopholepis*. This grouping of these specimens is strongly supported, confirming the monophyly of the species. Clade I includes *Cousinia sarawschanica*, *C. coerulea*, and *C. badghysi*, belonging to the sections Alpinae, Homalochaete, and Badghysia, respectively. The formation of the clade has high statistical support, indicating its phylogenetic stability. The grouping of species confined mainly to high-altitude and subalpine ecotopes probably reflects convergent adaptations to the harsh conditions of the high-altitude biotopes of Central Asia, characterized by low temperatures, high insolation, and limited moisture. Clade II is represented by all studied populations of *C. mindshelkensis* (Pop1-Pop4) belonging to the section *Lopholepis* and the species *C. gomolitzkii*. The clade is characterized by maximum support (PP: 1.00), which confirms its taxonomic integrity and high degree of intraspecific genetic relatedness. The close phylogenetic position of *C. gomolitzkii* indicates a possible common evolutionary origin and the preservation of a number of morphological and ecological characteristics associated with its confinement to rocky and gravelly slopes and a continental climate.

Clade III is the most representative and includes species from the sections Stenoloma, Leucocaulon, Kopetdagia, Olgaeanthe, Abolinia, Molles, Alpinae, Microcarpae, and others. The clade is characterized by morphological and ecological diversity, with support for individual branches ranging from moderate to high (PP: 0.59-0.93; BS: 57-100). The species represented are confined to a wide range of ecotopes, from foothill steppes and desert slopes to high-altitude meadows and rocky biotopes. This clade was probably formed as a result of several independent adaptive radiations caused by the colonization of various ecological niches and the heterogeneity of the region's orographic conditions.

Sequences from each population were developed, and the analysis showed that the nucleotide sequences were identical and, based on morphological criteria, all collected materials were classified as one species, *C. mindshelkensis.* We did not find any differences in morphological characteristics between individuals from different populations (Figure 8).

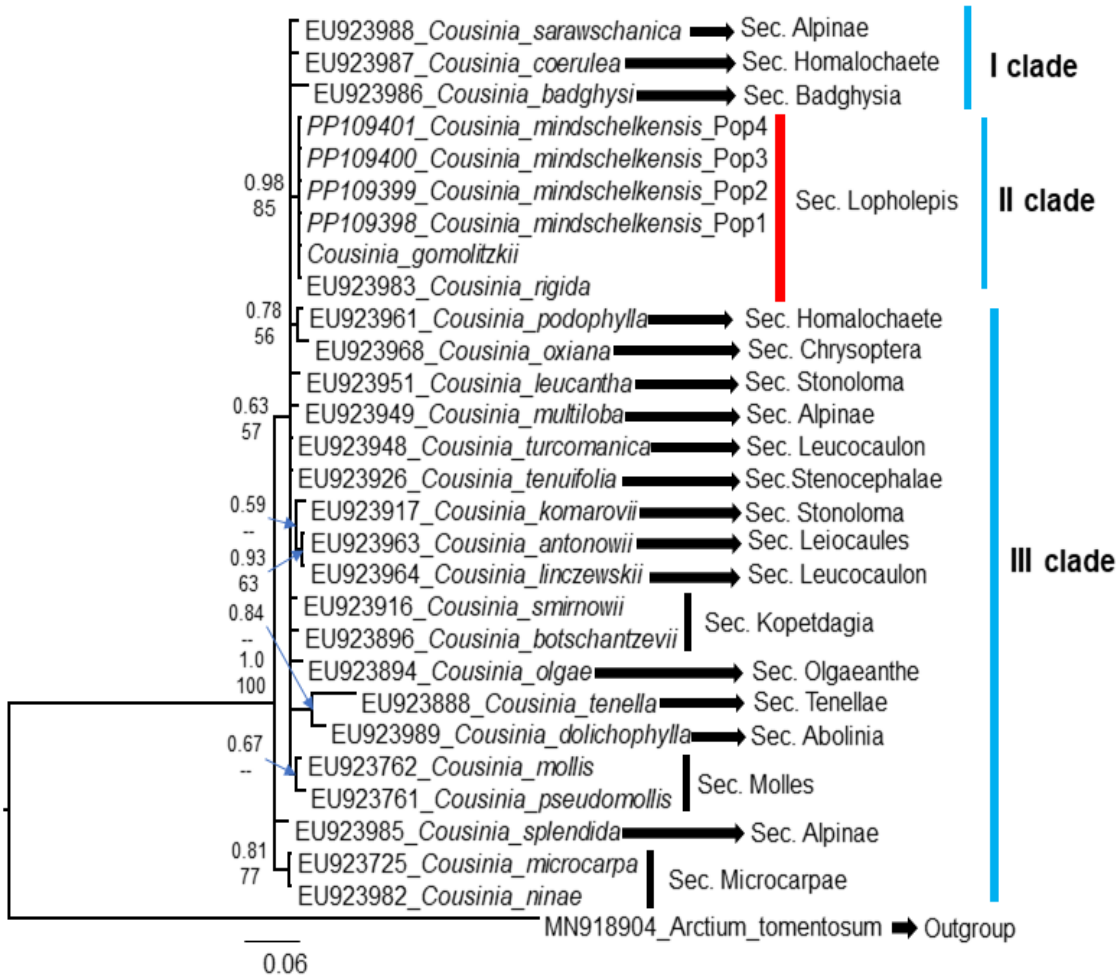

Figure 8. Phylogenetic chronology of the genus *Cousinia*, compiled on the basis of ITS sequences

Discussion

The results of a comprehensive study confirm the high ecological and genetic specificity of *C. mindshelkensis*, an endemic species of Karatau listed in the Red Book of the Republic of Kazakhstan (2014). An analysis of morphological traits in four isolated populations of *C. mindshelkensis* (P1-P4) revealed significant interpopulation variability, reflecting the influence of both environmental and genetic factors. The most pronounced generative development was observed in population 3, likely due to more favorable microclimatic conditions, while populations 1 and 2 were characterized by moderate growth and flowering rates. Population 4 exhibits a bias toward vegetative development, which may indicate an adaptive response to stressful environmental conditions or specific features of its ontogenetic structure.

A correlation analysis of the morphological characteristics of *C. mindshelkensis* revealed the relationship between plant growth, the number of leaves, shoots, and flowers. These results are consistent with similar observations of related species of Asteraceae, in which growth and reproductive characteristics have also demonstrated interdependence in various environmental conditions. Under optimal environmental conditions, individuals demonstrated intensive vegetative and reproductive development, which is consistent with the patterns described by Minaeifar (2025) and in more arid conditions, *C. mindshelkensis* devoted more resources to root growth. This suggests that the plant is trying to survive due to the increased development of the underground part, which scientists call one of the forms of adaptation-the so-called "reserve" or compensatory growth (Ebel et al. 2020). This trend has also been recorded in other xerophytic or mountain-endemic species, indicating that drought stress usually leads to prioritization of roots as a survival mechanism (Ivashchenko 2019, 2021). This result ensures the stability of populations in conditions of unstable availability of moisture and may explain the stability of *C. mindshelkensis* in habitats with different slopes. These results not only confirm the conclusions reached earlier, but also complement them with new data on morphological plasticity and local adaptation of plants.

We studied four populations growing in the Kishikarakuys, Itmuryn, and Karaungir gorges on various slopes and heights (1046-1250 m asl). All populations are confined to rocky, gravelly slopes with different exposures and are characterized by a variety of phytocenoses, from petrophytic mixed-herbaceous, woody, and shrubby associations (Sitpayeva et al. 2020, 2021). Our results are consistent with previous observations indicating that *C. mindshelkensis* occurs in isolated populations with highly variable densities depending on altitude and habitat conditions. Similar to the 1989 results, we also recorded populations with limited or absent evidence of effective reproduction, reflected by a predominance of mature and aging individuals. However, consistent with data from more favorable habitats (e.g., Bayaldyr, 1650 m), one of the studied populations (Population 3) contained young reproductive individuals, suggesting episodic regeneration under favorable environmental conditions. Overall, the observed age structure highlights both the vulnerability of the species at the lower limit of its range and its potential for recovery in optimal habitats.

In contrast to the earlier study by Ivashchenko (2020, 2024) and Tastanbekova et al. (2025), which described the distribution of *C. mindshelkensis* (1,100-2,000 m) and significantly higher population densities (up to 10-15 specimens/m^2 in the studied areas), our results indicate a lower abundance (on average 0.14-0.2 specimens/m^2), which may indicate that mature generative and generative individuals predominate, while young (juv and virg) individuals are relatively smaller than mature individuals. This distribution indicates a decrease in the potential for natural regeneration and a possible regression of local populations. Phytocenotic analysis showed that in all the studied sites, *C. mindshelkensis* coexists with typical satellites described by Ivashchenko, including *Tulipa alberti*, *Poa bulbosa*, *E. intermedia*, *F. tenuisecta*, *Thymus karatavicus*, etc. This indicates the high specificity of the ecological niches of the species and its dependence on the stability of local petrophytic and phryganoid communities. The presence of other rare and endemic species in communities, such as *Eryngium karatavicum* and *Tanacetum mindshelkense* underlines the importance of protecting the biodiversity of these ecosystems.

Genetic analysis using iPBS markers demonstrated that only three out of nine tested primers (iPBS2373, iPBS2386, and iPBS2239) provided stable and reproducible amplification, confirming their suitability for population-level studies of *C. mindshelkensis*. Such selective primer efficiency has also been reported in other endemic and rare taxa, emphasizing the strong species-specific nature of iPBS polymorphism and its dependence on genome architecture and retrotransposon distribution. Among the three primers, iPBS2373 exhibited the highest level of polymorphism and generated the greatest number of distinct DNA fragments, which is consistent with its reported high variability in *Allium altaicum* and *Linum usitatissimum* (Sheikina et al. 2019). In contrast, iPBS2386 showed reduced polymorphism (80%) and lower band complexity, possibly reflecting genomic constraints or decreased retrotransposon activity typical of xerophytic representatives of Asteraceae.

The mean polymorphism level of 85% obtained across the three selected primers indicates high genetic informativeness and a considerable degree of variability within *C. mindshelkensis* populations. Comparable or even slightly higher values have been reported for other narrowly distributed species of Central Asia, which may reflect historical isolation, habitat fragmentation, and ongoing microevolutionary differentiation (Herrando-Moraira 2020). The exclusion of six primers due to poor reproducibility further underscores the need for preliminary validation when applying iPBS markers to species lacking reference genomes (Herrando-Moraira et al. 2019).

The analysis of genetic distances revealed moderate to high differentiation among the studied populations. Population 4, although genetically close to population 2, showed marked divergence from populations 1 and 3, likely resulting from both geographic separation and

adaptation to distinct environmental conditions. The relatively high genetic distance between populations 3 and 4 (0.298) supports the assumption of restricted gene flow, a common feature of species with fragmented mountain habitats, whereas the low distance between populations 2 and 4 (0.069) may indicate occasional gene exchange through pollination or seed dispersal along slopes and valleys. These findings align with STRUCTURE results showing partial admixture despite clear cluster segregation.

Population genetic structure analysis revealed a bimodal pattern, with the most pronounced ΔK peak at K: 2, indicating two major genetic clusters corresponding to geographically or ecologically differentiated population groups. This pattern mirrors that observed in other narrowly endemic taxa inhabiting heterogeneous landscapes, where limited connectivity and topographic variation promote cluster formation (Vanijajiva and Pornpongrungrueng 2020; Yildiz and Arbizu 2022). A secondary ΔK peak at K: 9 suggests additional hierarchical substructuring within populations, possibly driven by microhabitat heterogeneity or local adaptation (Dierckxsens et al. 2017; Gao et al. 2018; Laface et al. 2022). Overall, the genetic data indicate that *C. mindshelkensis* maintains a complex population structure shaped by both historical fragmentation and limited contemporary gene flow.

Phylogenetic analysis based on the ITS region, confirmed the monophyly and high genetic homogeneity of *C. mindshelkensis* populations (Medvedeva et al. 2023). All samples from four populations showed identical nucleotide sequences and were clustered into a single, well-supported clade, indicating strong species cohesion, regardless of their geographic origin. This result aligns with the observations of Atazadeh et al. (2020), where several species of *Cousinia* with limited distributions were also found to exhibit low intraspecific variability within ITS regions, despite inhabiting different ecological zones. The placement of *C. mindshelkensis* alongside *C. gomolitzkii* and *C. rigida* in the same clade supports its taxonomic affiliation with the *Lopholepis* section, which was also discussed in earlier works by Cherneva (1962) and later refined by Rechinger (1953). These findings are consistent with historical morphological classifications and indicate congruence between molecular and classical taxonomy, reinforcing the robustness of the current sectional boundaries within the genus. The identical ITS sequences in *C. mindshelkensis* across populations suggest a recent divergence or limited accumulation of mutations in this region, which is common among narrowly endemic species with restricted gene flow. Despite this homogeneity, the species forms a clearly defined monophyletic group, making ITS a reliable marker for species-level identification, though insufficient for uncovering intraspecific variation.

Comparative analysis with GenBank sequences revealed that our samples cluster closely with previously deposited accessions from *C. mindshelkensis*, further validating species identification. These results not only confirm the taxonomic status of the studied individuals but also highlight the utility of nrITS markers in genus-wide phylogenetic assessments (Xing et al. 2019; Kalouti et al. 2022; Yun and Kim 2022; He et al. 2023; Imanbayeva et al. 2024; Minaeifar 2025). The molecular genetic data obtained indicate a complex population structure of *C. mindshelkensis*, formed under the influence of both historical fragmentation of the range and modern introgression processes (Paşayeva et al. 2020; Perezhogin et al. 2021; Shevcov and Nazarec 2024). These results are important for the development of programs for the conservation of the species' genetic diversity and the sustainable management of its populations.

In conclusion, the complex analysis of *C. mindshelkensis* populations revealed significant morphological and genetic differentiation associated with the ecological conditions of the Karatau mountains. Variability of vegetative and generative signs indicates high morphological plasticity and adaptation to various microhabitats. Correlation analysis confirmed the functional relationship between plant size and reproductive potential, reflecting adaptive strategies under environmental stress. Molecular data showed a high level of polymorphism and the presence of two main genetic clusters with limited gene flow between populations. These results indicate that both ecological heterogeneity and partial genetic isolation form the structure of the population. In general, *C. mindshelkensis* demonstrates significant adaptive potential, despite its narrow distribution, which is crucial for its long-term preservation.

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