

Genetic diversity and population structure of *Pyrus regelii* in South Kazakhstan based on ISSR markers

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Manuscript received: 22 May 2025. Revision accepted: 27 September 2025.

Abstract. Sitpayeva GT, Abdukhadyr A, Shmakov AI, Zverev NE, Koltunova AM, Yeszhanova AS, Shadmanova SH, Osmonali BB. 2025. Genetic diversity and population structure of *Pyrus regelii* in South Kazakhstan based on ISSR Markers. *Biodiversitas* 26: 4714-4729. *Pyrus regelii* is a relic wild pear species with a restricted distribution in the mountainous regions of South Kazakhstan. Despite its ecological and breeding importance, its genetic structure has never been systematically studied. This study provides the first comprehensive assessment of the genetic and morphological diversity of 5 natural populations from the Karatau and Kyrgyz Alatau ranges. A total of 12 ISSR primers were screened, with 6 highly polymorphic primers selected; these generated 113 loci, of which 112 were polymorphic (98.9%). The number of alleles per locus ranged from 3 to 7 (mean $N_a = 1.78$ per locus). Expected Heterozygosity (H_e) varied between 0.24-0.31, while Observed Heterozygosity (H_o) was consistently lower, indicating slight inbreeding. Shannon's information index ranged from 0.36-0.47. Pairwise F_{st} values reached 0.3451 between populations from different mountain systems, reflecting strong differentiation and limited gene flow across orographic barriers. STRUCTURE analysis ($K = 3$) and PCA confirmed three major genetic clusters, with signs of admixture in several populations. Morphological assessment showed variation in plant height (0.2-3.5 m), crown diameter (0.1-4.4 m), and leaf forms. Heterophyllous populations occurred only in the Kyrgyz Alatau, while Karatau populations were uniform with dissected leaves. The combined genetic and phenotypic evidence indicates adaptive variation that helps survival in arid and cold environments. However, fragmented distribution and isolation make the species vulnerable. Populations with unique alleles (Almalysay) and genetically isolated groups (Bozturgai, Berkara) should be prioritized for in situ conservation and monitoring. Ex situ measures, including seed banks and living collections, are necessary to capture the full spectrum of genetic diversity. Beyond conservation, the adaptive traits of *P. regelii* offer valuable genetic resources for breeding climate-resilient pear cultivars. Overall, this study establishes the first molecular baseline for *P. regelii* in Kazakhstan and offers a scientific basis for integrated strategies in conservation and sustainable utilization.

Keywords: Germplasm conservation, Kazakhstan pear, mountainous habitats, phenotypic variability, *Pyrus regelii*

INTRODUCTION

The genus *Pyrus* (Rosaceae) includes about 73 species, mainly distributed in the Northern Hemisphere, with introduced forms in the Southern Hemisphere (POWO) (Li et al. 2018). Its range spans from Western Europe to East Asia and is divided into two evolutionary groups: section *Pashia* (East Asia) and section *Pyrus* (Western Europe) (Zheng et al. 2014; Bandurko 2016; Korotkova et al. 2018; Shi et al. 2022; Yang et al. 2022; Cai et al. 2025; Yu et al. 2025). While earlier classification relied on morphology and geography, molecular methods now provide deeper insight into pear diversity (Sitpayeva et al. 2020; Sapkota et al. 2022; Sedov and Dolmatov 2022; Höfer and Flachowsky 2023; Bussmann et al. 2024; Mezhenksyj et al. 2024; Waite et al. 2024; Paniagua-Zambrana et al. 2025).

In Kazakhstan, two *Pyrus* species are recorded: *P. communis* and *P. regelii*. Globally, the genus includes about 30 species, three of which occur in Kazakhstan (Baitenov 2001). Only *P. regelii* grows wild in the country and

represents a valuable genetic resource for breeding drought- and frost-tolerant cultivars (Höfer and Flachowsky 2023). It is listed as Least Concern on the IUCN Red List and appears in the Red Data Book of Turkmenistan (Crowley 2021). In Kazakhstan, it is regionally protected and included in the Red Data Book of Zhambyl Region as a rare species (status 1E). Major threats stem from human activity, notably grazing, wood cutting, and habitat disturbance.

Ecologically, *P. regelii* is a xerophytic, drought-tolerant tree or shrub adapted to poor soils and slopes, serving as a key species in dry forest ecosystems and an indicator of biodiversity. As a wild relative of *Pyrus communis*, it provides valuable traits—drought and pest resistance, tolerance to extreme conditions—important for breeding, land restoration, and urban landscaping in arid regions (Waite et al. 2024). Globally, crop wild relatives are vital for food security under climate change (Global CWR project). Declining populations of *P. regelii* risk the loss of unique alleles essential for crop adaptation. Yet in

Kazakhstan, population-level ecological and genetic studies remain lacking, limiting conservation strategies.

Molecular genetic studies of other *Pyrus* species have successfully used ISSR markers to assess genetic variation and population structure due to their high polymorphism, reproducibility, and cost-effectiveness without requiring prior genomic information (Wang et al. 2022; Ali et al. 2024; Hayati et al. 2024; Liu et al. 2025). Compared with SSRs and SNPs, ISSRs are particularly suitable for rare, poorly studied species where large-scale genomic resources are unavailable (Liu et al. 2015; Xu et al. 2023; Gu et al. 2025; Guo et al. 2025). For example, ISSR analysis revealed significant intra- and interspecific variation in *P. pyrifolia*, *P. communis*, and related species, supporting its application in conservation genetics. Numerous studies have demonstrated the high efficiency of ISSR markers for assessing intraspecific polymorphism and clarifying phenetic relationships in the genus *Pyrus*, as well as their significant correlation with morphological traits. In *P. communis* cultivars, the ISSR method has proven to be technically simple, rapid, and cost-effective compared to RAPD and AFLP (Monte-Corvo et al. 2002). Similarly, in other wild fruit species such as *Malus sieversii*, the use of ISSR markers has proven effective in detecting hybrid forms and individuals occurring in natural populations (Shadmanova et al. 2019). These findings support the suitability of ISSR markers for investigating *P. regelii*.

Given the limited range, ecological specificity, and anthropogenic pressures on *P. regelii*, ISSR markers provide an efficient approach to identify genetic diversity hotspots, detect possible hybridization with *P. communis*, and establish priorities for in situ and ex situ conservation. This study aimed to: assess the genetic diversity of five

natural populations of *P. regelii* in southern Kazakhstan; determine the degree of differentiation and potential admixture among populations; and develop conservation recommendations integrating genetic and ecological data.

MATERIALS AND METHODS

Study area

Field expeditions in 2023-2024 sampled 5 natural populations of *P. regelii* from southern Kazakhstan (Zhambyl Region), with 10 individuals per population collected at intervals of at least 5-7 m to minimize the probability of sampling clones (Figure 1). In addition, 10 individuals of *P. communis* from the M.A. Lisavenko Research Institute of Horticulture of Siberia were included as an outgroup. The natural populations were located in the Kyrgyz Alatau (Merke, Kayyndy, Almalysay) and Karatau (Berkara, Bozturgai) mountain ranges.

The study area covers an altitude of 690-1146 m above sea level, with slopes of 30-50° and varying exposures. The climate of the region is strongly continental, with an average annual rainfall of 120-300 mm and average January/July temperatures from -46°C to +45°C. Seasonal precipitation is distributed unevenly with pronounced spring and autumn maxima. The soils are stony or gravelly with low moisture retention capacity. To conduct a comprehensive study of *P. regelii* populations, field geobotanical surveys were performed with geographic coordinates recorded through route-reconnaissance surveys using GPS navigation. The coordinates of the study points are shown in Table 1.

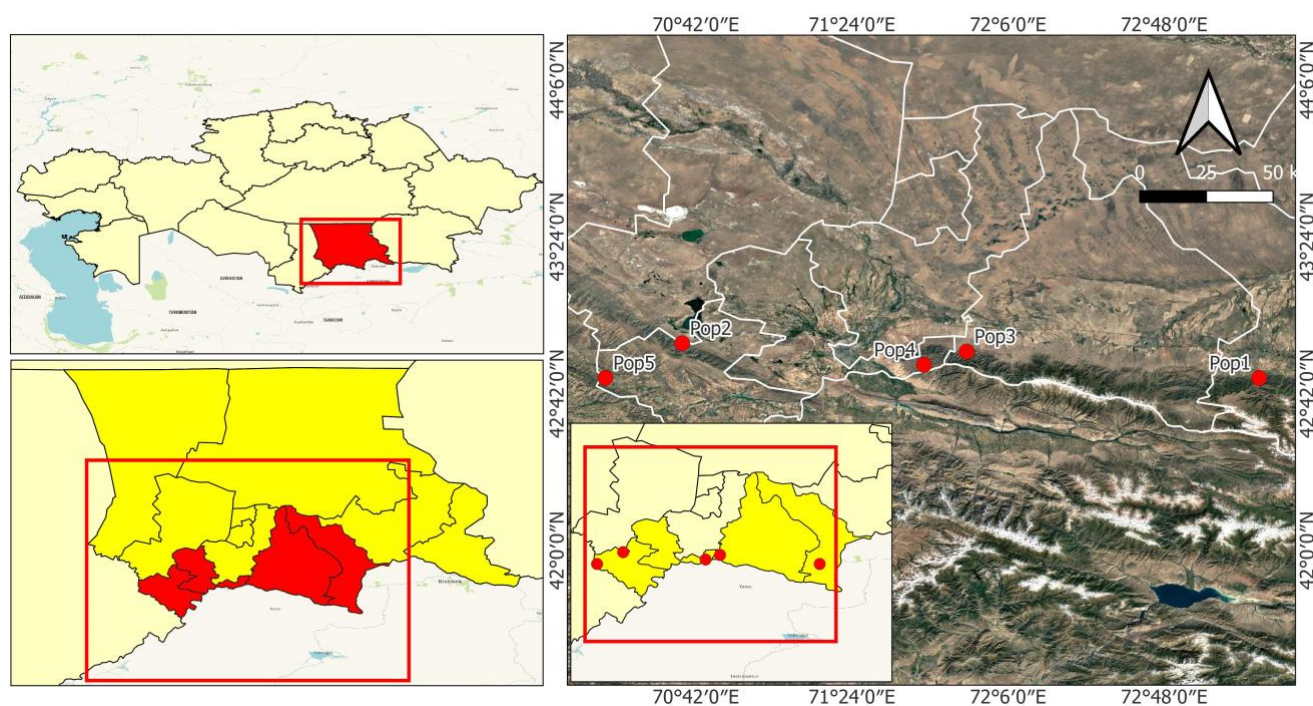


Figure 1. Site location map of *Pyrus regelii* populations in South Kazakhstan

Table 1. The localities and sample sizes of *Pyrus regelii*

| Population | Population abbreviations | Coordinates (lat/long) | Alt. (m) | Slope/exposure | Leaf blade shape | Number of samples |
|-------------------------------------|--------------------------|----------------------------|-----------|-----------------------|--|-------------------|
| Merke Gorge, rocky slope | Pop1 | 42.769694 N 73.226164 E | 1010-1076 | 45-50°, East | Leaves are either fully dissected, entire, or exhibit various intermediate forms of dissection | 10 |
| Berkara Gorge, rocky slope | Pop2 | 42.924824 N 70.6387 E | 613-700 | 40°, South-Eastern | Only dissected leaves | 10 |
| Kayindy Gorge, rocky slope | Pop3 | 42.887704 N 71.914564 E | 962-1020 | 45-50°, Western | Leaves are either fully dissected, entire, or exhibit various intermediate forms of dissection | 10 |
| Almalysay Gorge, rocky slope | Pop4 | 42.828456 N 71.723309 E | 1136-1153 | 30-40°, Southern | Only dissected leaves | 10 |
| Bozturgai River Valley, rocky slope | Pop5 | 42.769722 N 70.294722 E | 870-1002 | 30-40°, South-Eastern | Only dissected leaves | 10 |
| <i>Pyrus communis</i> | Outgroup | - | - | - | Entire | 10 |

Research objects

Pyrus regelii is a tree up to 8 m tall or a shrub with widely spreading branches covered in thorns (Figure 2). It is a xerophyte, and the leaves are variable in shape: *P. regelii* f. *koopmani* has deeply lobed leaves divided into narrow segments, often occurring in specimens that grow on rocks, and *P. regelii* f. *simplicifolia* is found on trees of larger size; most leaves are entire, and it grows in more humid places. In Kazakhstan, the species grows in the southern regions, particularly in the Kyrgyz Alatau (Zhailybayeva et al. 2023), Karatau, and Western Tien Shan mountain ranges (Baitenov 2001; Sitpayeva et al. 2020). Outside of Kazakhstan, *P. regelii* is also found in the mountainous regions of Kyrgyzstan, Uzbekistan, Tajikistan, and Turkmenistan (GBIF). It typically grows on rocky and gravelly mountain slopes and in the valleys of mountain rivers, and is endemic to Pamir-Alai and Western Tien-Shan (Central Asia).

According to Wintergoller and Zapryagaeva, *P. regelii* is a relic species of Central Asian subtropical savannas from the Paleogene. It is a dominant species in mountain savanna formations characterized by the presence of ephemerals and ephemerooids (Belousova et al. 2016). It is a wild relative of cultivated plants with food value (Sitpaeva et al. 2014). Stocks of the species are very limited and widely scattered over the area of growth, occurring as single specimens and small populations (Abdukhadyr et al. 2024). *Pyrus regelii* is considered a rare species in the Kyrgyz Alatau, and its conservation is of particular importance as a drought-resistant wild relative of cultivated plants (Ivashchenko 2015).

The Karatau and Western Tien Shan mountain ranges, where *P. regelii* occurs, are recognized as centers of endemism for both flora and fauna, and are characterized

by exceptionally high plant diversity. This species also serves as a food source for various animals and birds, and contributes to the prevention of soil erosion and landslides. It is therefore an important component of biological diversity within its native range. In addition, *P. regelii* has significant potential for breeding programs as a source of drought tolerance and winter hardiness genes. Its high adaptability and viability make it a promising candidate for developing new pear cultivars and serving as a rootstock in horticulture.

Ecological and morphological analysis

Population characteristics were evaluated following the methodology used in plant population biology, including describing population structure, age distribution, and vegetation density. For studying morphological traits, plant material was collected and preserved in herbaria. Morphometric analysis involved measuring plant height and crown diameter, which helped characterize the variation in habitus both within and among populations.

Molecular genetic analysis

DNA extraction

Fully developed, healthy leaves were collected, dried in silica gel, and stored at -20°C until DNA extraction. Approximately 10 mg of dried leaf tissue was crushed in a test tube by adding 500 µL of lysis buffer and 10 µL of protease solution. The DNA was isolated using the Diamond DNA Plant Kit (Altaibiotech, Russia) in accordance with the manufacturer's protocol. The amount and purity of DNA were determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA), and integrity was checked on a 1% agarose gel.



Figure 2. *Pyrus regelii* in the wild

ISSR marker selection

The study initially tested 12 ISSR primers (from published literature) but retained only 6 primers for final analysis. The selection was based on the following four key criteria: (i) Primers were required to produce clear, sharp, and repeatable bands in multiple PCR replicates. (ii) Primers generating smeared, faint, or inconsistent bands were discarded. (iii) Primers were evaluated based on their ability to detect high polymorphism across populations. A minimum threshold of 70% polymorphic loci per primer was applied (e.g., if a primer amplified 10 loci, at least 7 had to be polymorphic); primers with low polymorphism (<50%) were excluded to maximize resolution in genetic diversity assessment. (iv) Preference was given to primers with (CA)_n, (GA)_n, or (AGC)_n repeats, as these motifs are known to be highly polymorphic in *Pyrus* species (Monte-Corvo et al. 2001, 2002). (v) Primers amplifying multiple loci (10–25 bands per primer) were prioritized to capture sufficient genetic variation. Primers that failed to amplify or produced non-specific bands were excluded. Example: primer 17899B [(CA)₆GG] generated 24 polymorphic fragments, the highest among all primers, making it highly informative for population differentiation; primer UBC862 [(AGC)₅AGC] was selected due to its prior success in *Pyrus* studies (Liu et al. 2015), ensuring comparability with existing literature.

The selection of ISSR primers followed the commonly applied $\geq 70\%$ polymorphism threshold, which has been validated in multiple wild and cultivated *Pyrus* studies. For example, Monte-Corvo et al. (2001) reported 79.5% polymorphism in *P. communis*, confirming the reliability of this threshold, while Velicevici et al. (2023) observed 89% in cultivated pears, supporting its relevance for high-resolution analysis. Numerous recent studies have also demonstrated the effectiveness of ISSR markers in *Pyrus* and other species (Wang et al. 2017; Shadmanova et al. 2019; Nowicki et al. 2022; Abouseada et al. 2023; Hammam et

al. 2023; Upadyshev et al. 2023; Abdel-Sattar et al. 2024; Ali et al. 2024; Hayati et al. 2024; Liu et al. 2025). Thus, the primer selection in this study was thorough and scientifically justified, ensuring adequate discriminatory power, as shown by the distinct genetic clustering in STRUCTURE and PCA analyses. While future studies might add SSRs or SNPs to complement ISSRs, the current method is solid and appropriate for evaluating *P. regelii* population-level diversity.

PCR amplification

Polymerase Chain Reaction (PCR) was performed using the ready-to-use BioMaster HS-Taq PCR mix (Biolabmix, Russia) with a total volume of 20 μ L and a final primer concentration of 400 nM. Amplification was carried out in a BioRad My Cycler thermocycler using the following program: denaturation at 95°C for 20 sec, primer annealing at 55°C for 45 sec, and elongation at 72°C for 90 sec (13 cycles). This was followed by 25 cycles with the following conditions: denaturation at 95°C for 20 sec, primer annealing at 44°C for 30 sec, and elongation at 72°C for 90 sec. Amplification products were separated in a 2% agarose gel at 130 V for 3 hours. Ethidium bromide was used as a dyeing agent, and only clear and repetitive DNA fragments were selected for analysis.

Data analysis

The binary presence/absence matrix obtained from ISSR profiles (1: present, 0: absent) was used as the basis for all further calculations. Basic genetic diversity parameters, including the Percentage of Polymorphic Loci (PPL) and Fixation Index (F_{st}), were estimated in GENEPOP v4.7.5 (Raymond and Rousset 1995; Rousset 2008). The fixation index F_{st} was calculated using the weighted analysis of variance method of Weir and Cockerham (1984). A Mantel test (Mantel 1967) for isolation by distance was also performed in GENEPOP, correlating pairwise F_{st} values

with the natural logarithm of geographic distances between populations. The results indicated no significant isolation by distance, as the correlation was weak ($r = 0.157$). Population structure was investigated using two complementary approaches. First, Principal Component Analysis (PCA) was performed in GenAlEx v6.5 (Peakall and Smouse 2006). Pairwise genetic distance matrices were calculated for all individuals from the binary dataset, standardized by covariance, and projected into reduced dimensions to visualize clustering patterns. Correlation analysis, graphical visualization of results, and a heatmap illustrating the clustering of populations were obtained using ClustVis (Metsalu and Vilo 2015). Second, Bayesian clustering analysis was carried out in STRUCTURE v2.3.4 (Pritchard et al. 2000), applying the admixture model with correlated allele frequencies to account for possible gene flow. The analysis was run with a burn-in of 20,000 iterations followed by 100,000 MCMC iterations. The run length (20,000 burn-in, 100,000 MCMC) was chosen as sufficient based on convergence diagnostics and consistency across independent runs, in line with prior studies (Porrás-Hurtado et al. 2013). The number of clusters (K) ranged from 2 to 6, with three independent runs for each K value to ensure consistency. Higher values of K were not considered because the preliminary runs showed unstable clustering, and a biological interpretation outside this range did not make sense. The STRUCTURE output was subsequently analyzed in STRUCTURE Harvester (Earl and Von Holdt 2012) to determine the optimal K value using the Evanno method (Evanno et al. 2005), which calculates a ΔK statistic based on the rate of change in the log probability of the data [LnP(D)] between consecutive K values and identified a distinct peak in the ΔK distribution. Finally, genetic relationships among populations were assessed via cluster analysis based on the Unweighted Pair-Group Method with Arithmetic mean (UPGMA) in NTSYSpc (Rohlf 2018; Sevindik et al. 2023). The dendrogram was constructed using Nei's genetic distance matrix, allowing visualization of affinities and genetic separation between populations. Similar work has been carried out using analogous methods for rare species in Kazakhstan (Vesselova et al. 2022; Kubentayev et al. 2023; Osmonali et al. 2023; Dimeyeva et al. 2024; Imanbayeva et al. 2024, 2025; Vdovina et al. 2024).

RESULTS AND DISCUSSION

Ecological and morphological analysis

In the areas of natural growth of *Pyrus regelii* on the territory of the Karatau ridge, steep slope forms of relief, formed by dense rocks of the Carboniferous system, prevail. A thin cover of fine-grained diluvial deposits overlaps these rocks. The soil cover is characterized by uniformity and is represented by poorly developed mountain-steppe xeromorphic soils, often with outcrops of dense crystalline rock on the surface. The limited thickness of the soil profile, combined with the steepness of the slopes, makes this area unsuitable for farming. Currently, these areas are

used mainly as low-productive pastures in the early spring period.

The Kyrgyz Alatau, located between the rivers Chu and Talas, is part of the mountain system of the Northern Tien Shan and is characterized by a complex orographic structure. The northern macro-slope of the massif is characterized by steep, intensively dissected by erosion relief forms covered mainly with shrub vegetation. The soil cover in these areas is represented by mountain dark chestnut and mountain-steppe underdeveloped soils with a high content of crushed stone and low-profile thickness. The northern slope plume was formed as a result of debris flows and alluvial-deluvial processes that led to the formation of cones of removal associated with ancient and modern river systems descending from the Kyrgyz Alatau. The foothill plain, a significant part of the area, is composed of stony-pebble formations overlain by strata of cartilaginous loess-like loams of various thicknesses, providing a diverse geological makeup.

A low level of moisture characterizes the region's soils due to limited precipitation, which results in low productivity of agricultural crops. Due to natural and relief conditions, the area under consideration belongs to the zone of selective land use, where farming is possible only in limited areas of intermountain valleys. Most of the territory is used as summer pastures for cattle grazing. The process of collecting morphological and ecological data on *P. regelii*, whose representatives inhabit geographically diverse areas, has identified specific features characteristic of the species were identified. The results of biometric measurements of *P. regelii* showed considerable variation in the biometric sizes of the population's individuals. The highest average tree height was recorded in the Merke population—2.0 m—while the maximum value of the average crown diameter was observed in the Bozturgay population—2.1 m. The lowest mean values of tree height were recorded in the Berkara population—1.1 m—and the minimum mean crown diameter was recorded in the Kayindy and Berkara populations—1.4 m (Table 2). These data reveal clear differences in the phenotypic characteristics of plants, which may indicate the influence of local environmental conditions on the species' morphology.

In the populations of the Kyrgyz Alatau in the Merke and Kayindy gorges, *P. regelii* is characterized by pronounced heterophyllia by morphological features, and two forms (*P. regelii* f. *simplicifolia*, *P. regelii* f. *koopmani*) were identified (Figures 3.A, 3.B). A survey of areas adjacent to these populations shows that in one case, there is an orchard of varietal pears (*P. communis*) not far from *P. regelii*. In another population in the gorge, there is a self-seeded pear with entire leaves and pear-shaped fruit. The trees growing in these gorges show morphological variability of leaf laminae, represented by three main types: fully dissected, entire, and with leaves of different combinations of dissection, as shown in Figure 3. Phenotypic analysis of *P. regelii* leaves in the populations of Almalyay (Kyrgyz Alatau) (Figure 4.A), the Berkara Gorge, which is located on the northern side of the Karatau Mountains (Figure 4.B), and the Bozturgay Gorge, situated at a considerable distance from the latter but on the southern side of the same mountain range (Figure 4.C),

exclusively revealed individuals with pinnately dissected leaf blades, identifiable as *P. regelii* f. *koopmani* (Figure 3.A).

Molecular genetic analysis

The present study revealed significant polymorphism of ISSR markers in *P. regelii* species. The samples of *P. communis* (out) were used as an external control group, which allowed a comparative analysis of interspecific

differences. As shown in Table 3, six inter-microsatellite primers (ISSR Inter-Simple Sequence Repeats), selected based on preliminary screening for their high ability to detect polymorphism, were used for DNA analysis. The use of ISSR markers, known for their high reproducibility and informativeness, enabled an effective assessment of genetic diversity within and between populations of the studied species.

Table 2. Results of biometric studies of *Pyrus regelii* populations

| Pop | Proportion of leaf forms (%) | | Soil | Plant height (m) | | Crown diameter (m) | | | |
|------|---|--|---|------------------|-----|--------------------|-----|-----|-----|
| | <i>Pyrus regelii</i> f. <i>koopmani</i> | <i>Pyrus regelii</i> f. <i>simplicifolia</i> | | Mid | Min | Max | Mid | Min | Max |
| | | | | | | | | | |
| Pop1 | 80 | 20 | Stony and gravelly | 2.0 | 0.7 | 3.5 | 2.0 | 0.4 | 4.4 |
| Pop2 | 100 | 0 | Stony and gravelly | 1.1 | 0.5 | 2.0 | 1.4 | 0.6 | 2.0 |
| Pop3 | 60 | 40 | Stony and gravelly with exposed large rocks | 1.3 | 0.2 | 2.2 | 1.4 | 0.1 | 3.0 |
| Pop4 | 100 | 0 | Stony and gravelly | 1.5 | 0.7 | 2.0 | 1.5 | 0.7 | 2.8 |
| Pop5 | 100 | 0 | Stony and gravelly | 1.9 | 1.3 | 2.5 | 2.1 | 1.2 | 3.0 |



Figure 3. Leaf blade forms of *Pyrus regelii*. A. *Pyrus regelii* f. *koopmani*, B. *Pyrus regelii* f. *simplicifolia*, and C. various leaf forms



Figure 4. Morphological uniformity of *Pyrus regelii* leaves: A. Almalysay population (Kyrgyz Alatau), B. Berkara population (Karatau), and C. Bozturgay population (Karatau)

Based on the results of molecular genetic studies using 6 ISSR primers, 113 loci were amplified. The number of polymorphic fragments amounted to 112. All six primers used in the study exhibited a high level of polymorphism, and the most notable differences were observed in *P. communis* group 6, used as the outgroup. In *P. regelii*, the percentage of polymorphic fragments ranged from 95.0% (17899A) to 100% (17899B, HB12, UBC862, ISSR1, and M27). In *P. communis*, polymorphism values were more variable, ranging from 61.9% (HB12) to 100% (UBC862 and M27). The highest number of polymorphic sites was obtained with primer 17899B. Additionally, four unique amplified fragments were detected in *P. communis*, while approximately 70% of fragments amplified in *P. regelii* were absent in *P. communis*.

The fragment sizes ranged from 200 to 2000 bp, reflecting the broad diversity of ISSR profiles generated. In a similar previous study using other ISSR primers on five cultivars of cultivated pear, the percentage of polymorphism was 89% (Velicevici et al. 2023), and the differentiation matrix between the extreme varieties was 27.45% and 78.43%; in this study, the percentage of polymorphism was 98.9%. The difference in the results is due to the use of a different pool of primers and the choice of research subjects. The differentiation matrix between the extreme populations was 89.15% and 65.49%. Population genetic analysis of five populations of *P. regelii* showed variability in geographic dispersal. Based on the presence/absence data of amplified fragments, a population differentiation matrix was generated using the level of genetic variability (*F_{st}*) in the GENETPOP program (Table 4). Pairwise comparison of *F_{st}* values between populations located on different mountain systems consistently exceeds 0.30, indicating significant genetic separation.

The *F_{st}* values among the five populations of *P. regelii* ranged from 0.1085 (Merke-Berkara) to 0.3451 (Berkara-Bozturgay), indicating moderate to high genetic differentiation. The minimum differentiation suggests a possible historical or ongoing gene flow between the Merke and Berkara populations. At the same time, the maximum value points to pronounced isolation of the

Bozturgay population from Berkara even within the same mountain system (Karatau), likely due to orographic barriers, slope exposure, and habitat fragmentation. Overall, Bozturgay exhibited the greatest average genetic distance from other populations, confirming its status as the most genetically distinct group.

Genetic diversity metrics were recalculated from the ISSR dataset using GenAEx v6.5 (Peakall and Smouse 2006) and POPGENE (Yeh et al. 1999). In GenAEx, Expected Heterozygosity (*H_e*), Observed Heterozygosity (*H_o*), and Shannon's information index (*I*) were computed; *H_e* was estimated under Hardy-Weinberg equilibrium, *H_o* reflected the actual frequency of heterozygotes, and *I* quantified allelic richness and evenness. Allelic richness (*N_a*) and effective number of alleles (*N_e*) were standardized for sample size using rarefaction in POPGENE.

As shown in Table 5, all populations exhibited moderate diversity (*H_e* = 0.24-0.31), with Almalysay showing the highest values. The slight deficit of heterozygotes (*H_o* < *H_e*) suggests limited inbreeding, possibly due to restricted gene flow across mountainous habitats. Shannon's *I* (0.36-0.47) indicated uneven allele distributions, consistent with isolation-by-distance patterns. These values are comparable to those of other wild *Pyrus* species, which confirms the reliability of diversity estimates based on ISSR markers.

Compared to cultivated *Pyrus*, *P. regelii* displays lower diversity, likely due to its relict status and habitat fragmentation. Conservation efforts should prioritize Almalysay's unique alleles, while assisted gene flow could mitigate inbreeding in Berkara. Thus, the genetic diversity of *P. regelii* populations was found to be moderate (*H_e* = 0.24-0.31), which corresponds to the expected values for long-lived, outcrossing tree species. The Almalysay population demonstrated the highest diversity, likely reflecting its ecological isolation and the preservation of unique alleles, whereas Berkara was characterized by the lowest values, indicating reduced variability. In all populations, *H_o* was lower than *H_e*, suggesting a deficit of heterozygotes, most likely caused by limited pollen flow and partial inbreeding under conditions of geographic and ecological fragmentation.

Table 3. Characteristics of ISSR primers used for genetic analysis of *Pyrus regelii* and *Pyrus communis*

| Primer name | Nucleotide sequence (5'-3') | Number of alleles | | Polymorphism (%) | | Polymorphism (%) | |
|-------------|-----------------------------|----------------------|----------------------|----------------------|-----------------------|-----------------------|-----------------------|
| | | <i>Pyrus regelii</i> | <i>Pyrus regelii</i> | <i>Pyrus regelii</i> | <i>Pyrus communis</i> | <i>Pyrus communis</i> | <i>Pyrus communis</i> |
| 17899A | (CA) ₆ AG | 20 | 19 | 95.0% | 20 | 17 | 85.0% |
| 17899B | (CA) ₆ GG | 24 | 24 | 100.0% | 24 | 17 | 70.8% |
| HB12 | CACCACCACGC | 21 | 21 | 100.0% | 21 | 13 | 61.9% |
| UBC862 | (AGC) ₅ AGC | 12 | 12 | 100.0% | 20 | 20 | 100.0% |
| ISSR1 | (AC) ₈ T | 19 | 19 | 100.0% | 19 | 17 | 89.5% |
| M27 | (GA) ₈ C | 17 | 17 | 100.0% | 17 | 17 | 100.0% |

Note: Four unique amplified sequences were detected in *P. communis*. Approximately 70% of fragments amplified in *P. regelii* were absent in *P. communis*. The fragment sizes ranged from 200 to 2000 bp. The highest number of polymorphic sites was obtained using primer 17899B

Table 4. Differentiation matrix (pairwise F_{st} values) of *Pyrus regelii* populations

| Population | Pop 1 (Merke) | Pop 2 (Berkara) | Pop 3 (Kayindy) | Pop 4 (Almalysay) | Pop 5 (Bozturgay) |
|-------------------|---------------|-----------------|-----------------|-------------------|-------------------|
| Pop 1 (Merke) | - | | | | |
| Pop 2 (Berkara) | 0.1085 | - | | | |
| Pop 3 (Kayindy) | 0.1564 | 0.2953 | - | | |
| Pop 4 (Almalysay) | 0.2469 | 0.1492 | 0.2007 | - | |
| Pop 5 (Bozturgay) | 0.2696 | 0.3451 | 0.2418 | 0.1977 | - |

Note: Values below the diagonal represent pairwise F_{st} estimates, while diagonal values indicate within-population variation

Table 5. Analysis of general indicators of genetic diversity

| Pop. | Na | Ne | He | Ho | I |
|-----------|-----------|-----------|-----------|-----------|-----------|
| Merke | 1.82±0.12 | 1.51±0.09 | 0.28±0.03 | 0.25±0.04 | 0.42±0.05 |
| Kayindy | 1.76±0.11 | 1.48±0.08 | 0.26±0.03 | 0.23±0.03 | 0.39±0.04 |
| Almalysay | 1.91±0.14 | 1.59±0.10 | 0.31±0.04 | 0.27±0.05 | 0.47±0.06 |
| Berkara | 1.68±0.10 | 1.42±0.07 | 0.24±0.02 | 0.21±0.03 | 0.36±0.04 |
| Bozturgay | 1.73±0.11 | 1.46±0.08 | 0.25±0.03 | 0.22±0.03 | 0.38±0.04 |
| Overall | 1.78±0.12 | 1.49±0.08 | 0.27±0.03 | 0.24±0.04 | 0.40±0.05 |

Note: Na: Observed number of alleles, Ne: Effective number of alleles, He: Expected heterozygosity, Ho: Observed heterozygosity, I: Shannon's Information Index

To analyze the structure of genetic diversity among the studied samples, a dendrogram was constructed using the Unweighted Pair Group Method with Arithmetic mean (UPGMA), based on the genetic distance matrix (Figure 5). The analysis revealed a clear separation of samples into several major clusters corresponding to the P1-P5 populations, as well as a separate cluster including samples from the outgroup. Populations P1, P2, and P3 formed relatively compact groups with minimal intragroup genetic distances, indicating a high degree of genetic relatedness between their representatives. The P4 and P5 population samples showed greater genetic heterogeneity, which may indicate the presence of a subpopulation structure or recent genetic exchange processes. The outgroup of samples was clearly separated from the main sample and formed a separate cluster, which confirmed their significant genetic distance and presumed belonging to another species (*P. communis*). The genetic distances between major clusters were substantial, confirming the presence of a distinct structure among the studied samples. The constructed dendrogram clearly demonstrated the presence of a clear population structure among the studied specimens, and the compactness of the clusters formed by populations P1, P2, and P3 indicated a relatively high degree of genetic integrity within these groups. This may reflect both limited gene flow between them and possible descent from a narrow common genetic pool. At the same time, the high heterogeneity observed among the samples of the P4 and P5 populations is probably related to historical processes of migration, introgression, or a wider ecological amplitude of these groups' habitat. The genetic isolation of the outgroup confirms the taxonomic distance between *P. regelii* and *P. communis*. This clustering is consistent with the F_{st} matrix data and confirms the genetic distinctness of the Karatau

and Kyrgyz Alatau populations. Overall, the results of the UPGMA analysis highlight the complex genetic structure of the studied sample and indicate the need for further comprehensive research using additional molecular markers to understand genetic relationships within and between populations better.

In the NTSYSpc dendrogram, the populations were divided into three clusters (Figure 6). The general cluster is formed by Kyrgyz Alatau populations from the Merke and Kayindy gorges, corresponding to morphological and geographical data. Genetic differentiation between *P. regelii* populations was pronounced: the highest F_{st} value (0.3451) was recorded between the Bozturgai (Karatau Mountains) and Merke (Kyrgyz Alatau) populations. The UPGMA dendrogram confirmed this separation, revealing a clear distinction between the populations confined to the Karatau and Kyrgyz Alatau. Additionally, a Mantel test for isolation by distance was performed with the GENEPOP program, and the results indicated that populations are not isolated by distance, as the correlation between genetic differences and the logarithm of geographic distance was very weak at 0.157. Mantel's test showed a weak but positive correlation between genetic and geographic distance ($r = 0.157$), but the absence of a linear relationship indicates the predominant role of orographic barriers such as the Chui intermountain basin. Although significant, the low r value indicates that geographic distance alone does not explain observed genetic structure. Effective seed dispersal is limited by topography: seeds of *P. regelii* are dispersed mainly by gravity, less frequently by birds (Pavlov 1947), which drastically reduces the probability of long-distance gene exchange. These data indicate the existence of isolation by environment and relief, not only by distance.

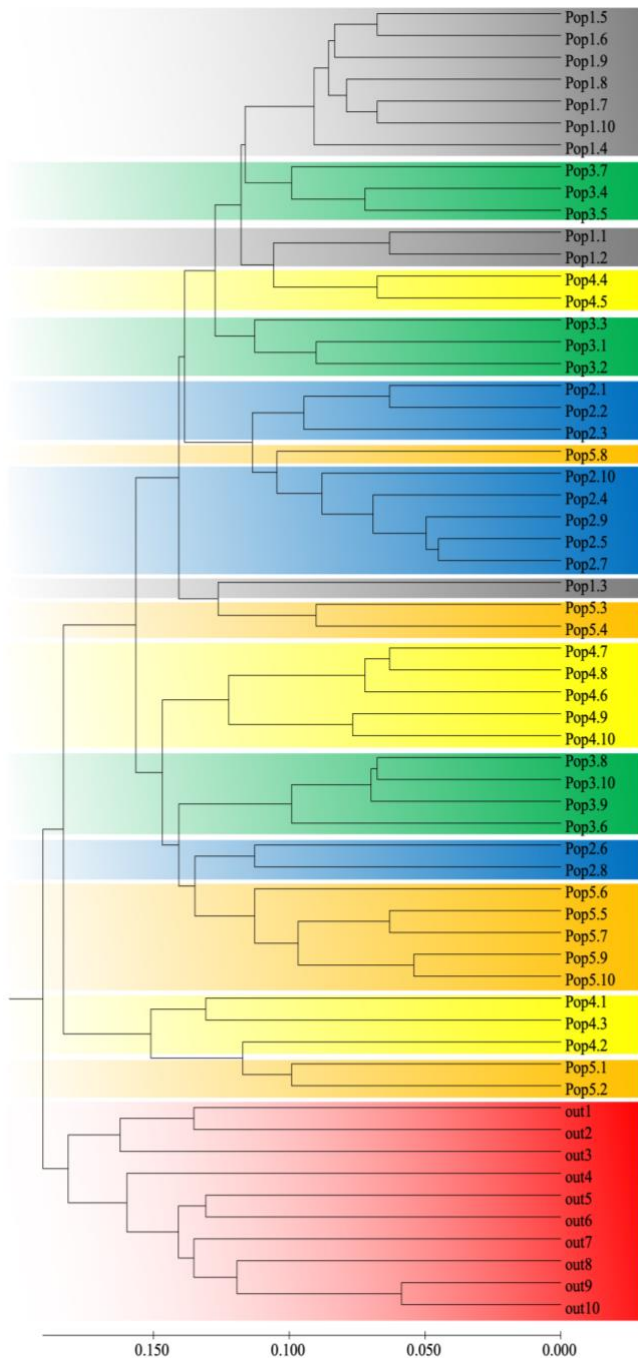


Figure 5. UPGMA dendrogram of *P. regelii* populations based on ISSR markers. Pop1: Merke, Pop2: Berkara, Pop3: Kayindy, Pop4: Almalysay, Pop5: Bozturgay (10 individuals per population). Out: *Pyrus communis* (10 individuals)

Leaves of *P. regelii* in these populations exhibited three types of dissection: fully dissected, whole, and intermediate forms. The population of the Almalysay Gorge of the Kyrgyz Alatau formed a separate branch, corresponding to both morphological (fully dissected leaf) and geographical data. This is probably due to the strong geographical isolation of this population formed by the gorge's mountain massifs, and consequently, the lack of possibility of overpollination. Populations formed a separate cluster from the Berkara and Bozturgai gorges. Trees of these populations had dissected

leaf plates without entire and intermediate forms. This, along with another factor—distance from the other three populations—allowed them to form a separate stable group. *P. communis* was used as an external group and formed a separate cluster.

The Principal Component Analysis (PCA) plot illustrates the genetic relationships among the five populations and the outgroup based on the first two principal components (PC1 = 15.9%, PC2 = 12.5%), which together account for 28.4% of the total variance (Figure 7). Pop1 and Pop2 populations partially overlap but are distinguishable, while Pop3 occupies an intermediate position, overlapping with both Pop1 and Pop2, indicating a possible gene flow or common origin. Pop4 and Pop5 show greater variance, indicating higher intrapopulation genetic diversity. The outgroup is clearly separated from all other populations, indicating its distinct genetic background. The clustering pattern observed reflects the genetic structure and relationships between the populations, consistent with their geographic divergence. Ellipses indicate 95% confidence intervals for each population. This pattern mirrors the STRUCTURE results ($K = 3$), confirming the presence of three main genetic clusters.

Analysis with the STRUCTURE program determined the most likely number of clusters (K) by calculating the logarithm of the probability of the data for each value of K . The highest probability (ProbK = 1.00) was recorded at $K = 3$ (Figure 8).

The Evanno method implemented in the STRUCTURE Harvester was used to determine the optimal K value for the genotyped *Pyrus* lines. According to the clustering model, the Evanno test showed that at $K = 3$, the ΔK value was 33.781, corresponding to the highest logarithmic probability and confirming the presence of three main groups (subpopulations) in the collection (Figure 9). The concordance among the F_{st} values, dendrogram clustering, and STRUCTURE results underscores the reliability of the identified population structure.

In addition, the population structure was analyzed to examine genetic composition and differentiation among populations. STRUCTURE histograms illustrate genetic clustering of individuals from six groups (Pop1-Pop5 and the outgroup) at K values from 2 to 6. When $K = 2$, individuals belong to two clusters. Pop1, Pop2, and (partially) Pop3 show high affiliation with the first cluster, while Pop4, Pop5, and the outgroup are mostly associated with the second cluster. Signs of admixture are already evident in Pop3, Pop4, and Pop5. At $K = 3$, defined as the optimal number of clusters by ProbK and DeltaK criteria, populations show a clearer genetic structure. Pop1 and Pop2 form a separate cluster, the outgroup stands out as an independent cluster, and Pop3, Pop4, and Pop5 show significant mixing, indicating complex genetic relationships consistent with PC analysis. At higher K values, an additional substructure appears, especially within Pop3, Pop4, and Pop5, reflecting their genetic heterogeneity, while Pop1 and Pop2 remain relatively homogeneous. The outgroup also forms a separate cluster, but with insignificant impurity signals, suggesting complex relationships consistent with PCA results (Figure 10).

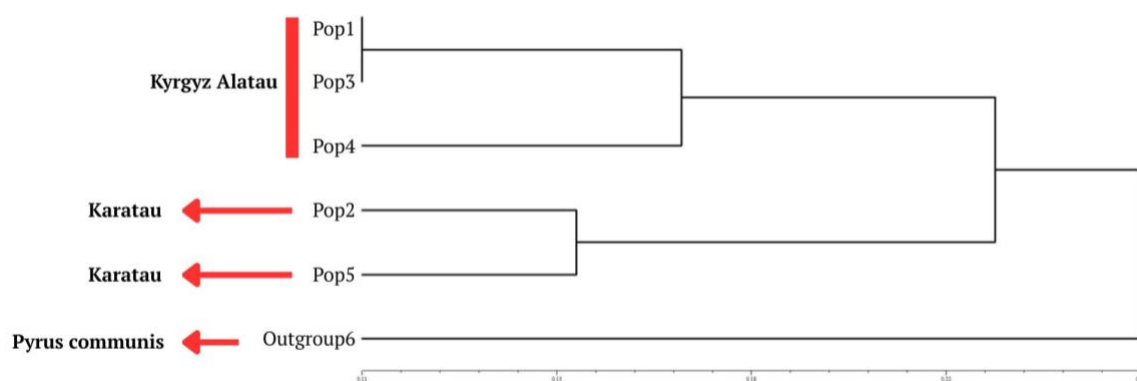


Figure 6. Clustering of 6 populations based on analysis of a two-dimensional trait matrix using the NTSYSpc program. Pop1: Merke, Pop2: Berkara, Pop3: Kayindy, Pop4: Almalysay, Pop5: Bozturgay. Outgroup6: *Pyrus communis*

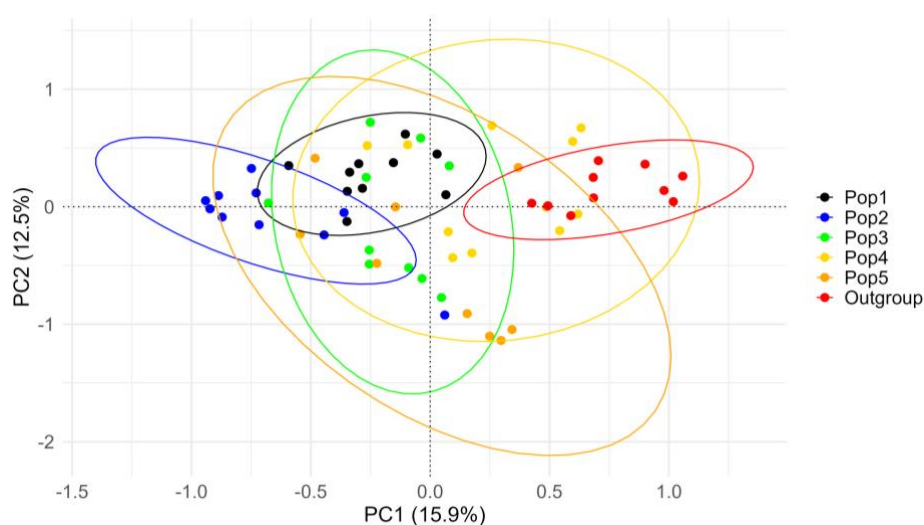


Figure 7. Principal Component Analysis (PCA)

Discussion

Genetic structure and geographic differentiation

The combined molecular, morphological, and ecological analyses provide a comprehensive understanding of the population structure and adaptive variation in *Pyrus regelii*. ISSR marker analysis revealed exceptionally high polymorphism (PPL = 98.9%), with Expected Heterozygosity (H_e) ranging from 0.24 in Berkara to 0.31 in Almalysay, and Shannon's Diversity Index (I) between 0.36 and 0.47. These values indicate moderate to high genetic diversity, which is typical for long-lived, outcrossing species (Hamrick and Godt 1996) but higher than levels reported for cultivated *Pyrus* (Velicevici et al. 2023).

Population differentiation was pronounced ($F_{st} = 0.1085-0.3451$), with the highest divergence between Bozturgay (Karatau Mountains) ($F_{st} = 0.3451$) and Merke (Kyrgyz Alatau). STRUCTURE analysis identified three main genetic clusters ($\Delta K = 33.781$ at $K = 3$), while PCA revealed partial overlap between neighboring populations, suggesting occasional but limited gene flow. Although the first two PCA axes explain only 28.4% of the variance, such

values are typical for analyses based on ISSR markers, where variation is distributed across many loci. This indicates that additional ecological and orographic factors may contribute to population differentiation, reinforcing the need to interpret PCA jointly with STRUCTURE and clustering results. The Mantel correlation between genetic and geographic distance was weak ($r = 0.157$), indicating that topographic and ecological barriers—particularly the arid Chu intermountain basin—are more influential than linear distance in restricting dispersal.

Similar isolation patterns have been observed in *P. ussuriensis* (Li et al. 2021), *P. hopeiensis* (Katayama et al. 2016). Studies of *P. communis* and its wild relatives using molecular markers (e.g., AFLP, SNP) have revealed a similar pattern: the presence of stable genetic clusters even at relatively small distances between populations (Yang et al. 2025). Similarly, molecular surveys of *Malus sieversii*, another relict fruit species of Central Asia, revealed moderate within-population diversity and strong between-population differentiation, attributed to habitat fragmentation and orographic barriers (Shadmanova et al. 2019).

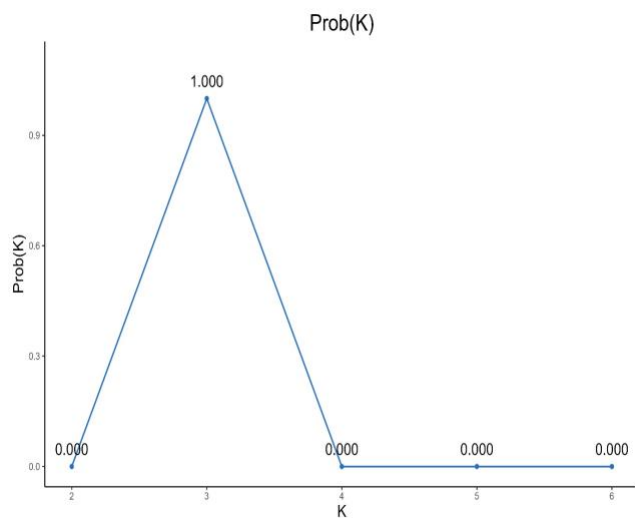


Figure 8. Clustering of *Pyrus regelii* populations using STRUCTURE (K = 2-6). Highest likelihood was at K = 3

In particular, the settlement of populations within isolated mountain areas (gorges of the Karatau and Kirghiz Alatau ranges) (Figure 2), as well as their remoteness from each other in the presence of significant orographic barriers, contribute to limited gene exchange and the formation of isolated gene pools. High F_{st} values (>0.30) between Karatau and Kyrgyz Alatau populations indicate substantial divergence, UPGMA clustering further supported this subdivision, with Merke and Kayindy forming a distinct Kyrgyz Alatau cluster, while Berkara and Bozturgay grouped separately within the Karatau range. As shown in the UPGMA diagram, the absence of clear clustering of pear forms according to their geographical distribution suggests intrapopulation inbreeding, which is characteristic of woody species of the Rosaceae family. The limited overlap between populations in PCA space and the admixture signals observed in STRUCTURE indicate that occasional gene flow takes place, but its level is not enough to unify populations. Similar patterns have been seen in other plant species: for example, in *Ranunculus subrigidus* on the Tibetan Plateau, environmental fragmentation has been found to restrict gene flow and encourage isolation by distance (Wu et al. 2019). The same type of mechanisms also operate in coniferous species of Europe, such as *Abies alba* and *Larix decidua*, where geography has a significant impact on population structure (Mosca et al. 2014). Thus, *P. regelii* maintains genetic variability typical of wild Rosaceae, despite its restricted range and ecological specialization.

However, isolation by environment plays an equally important role. *P. regelii* populations occur on slopes with varying exposures, slopes, heights above sea level, and soil types, which form local ecological niches. These differences can exert directional breeding pressure and facilitate adaptation to specific environmental conditions, thereby

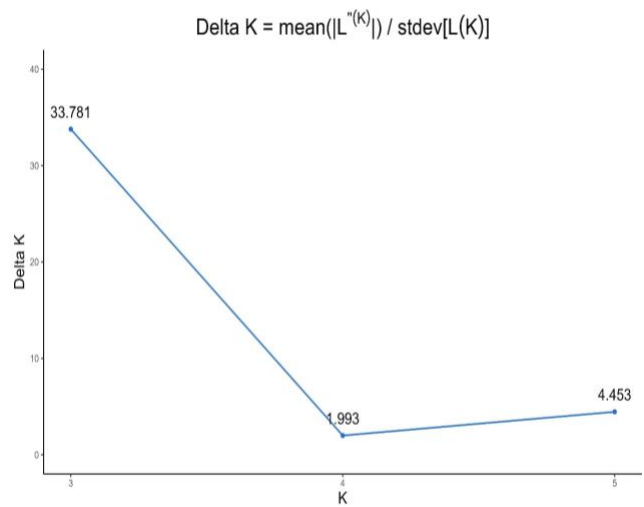


Figure 9. Analysis with the STRUCTURE Harvester program (ΔK distribution based on Evanno method showing optimal clustering at K = 3)

enhancing genetic divergence. An actual example is *Calibrachoa heterophylla* in eastern South America, where environmental factors (precipitation distribution and relief) have no less significant influence on population structure than geography (Silva-Arias et al. 2021). The observed differentiation is not solely due to spatial separation. The ecological heterogeneity of habitats—including differences in slope exposure, soil moisture, and altitude—likely imposes divergent selective pressures that enhance adaptive divergence among populations. Such environmentally driven patterns have been demonstrated in other montane species (e.g., *Cephalotaxus oliveri*) (Wang et al. 2016). Finally, in a number of plant species, such as *Caragana microphylla*, it has been proven that both mechanisms - isolation by distance and isolation by environment - can act simultaneously, forming a complex population structure (Xu et al. 2017). Thus, it can be assumed that in the case of *P. regelii*, both mechanisms operate simultaneously, and the identified genetic clusters are a reflection of both spatial separation and adaptive processes occurring in their habitat.

Morphological variation and its genetic correlations

Morphological data revealed clear phenotypic differentiation among populations. In the Kyrgyz Alatau (Merke, Kayindy, Almalysay), heterophyllous individuals with both *P. regelii* f. *koopmani* (pinnately dissected leaves) and f. *simplicifolia* (entire leaves) were recorded, with the Merke population showing the highest morphological heterogeneity (80% dissected vs. 20% entire leaves). In contrast, all individuals in the Karatau populations (Berkara, Bozturgay) exhibited exclusively dissected leaves, indicating morphological uniformity.

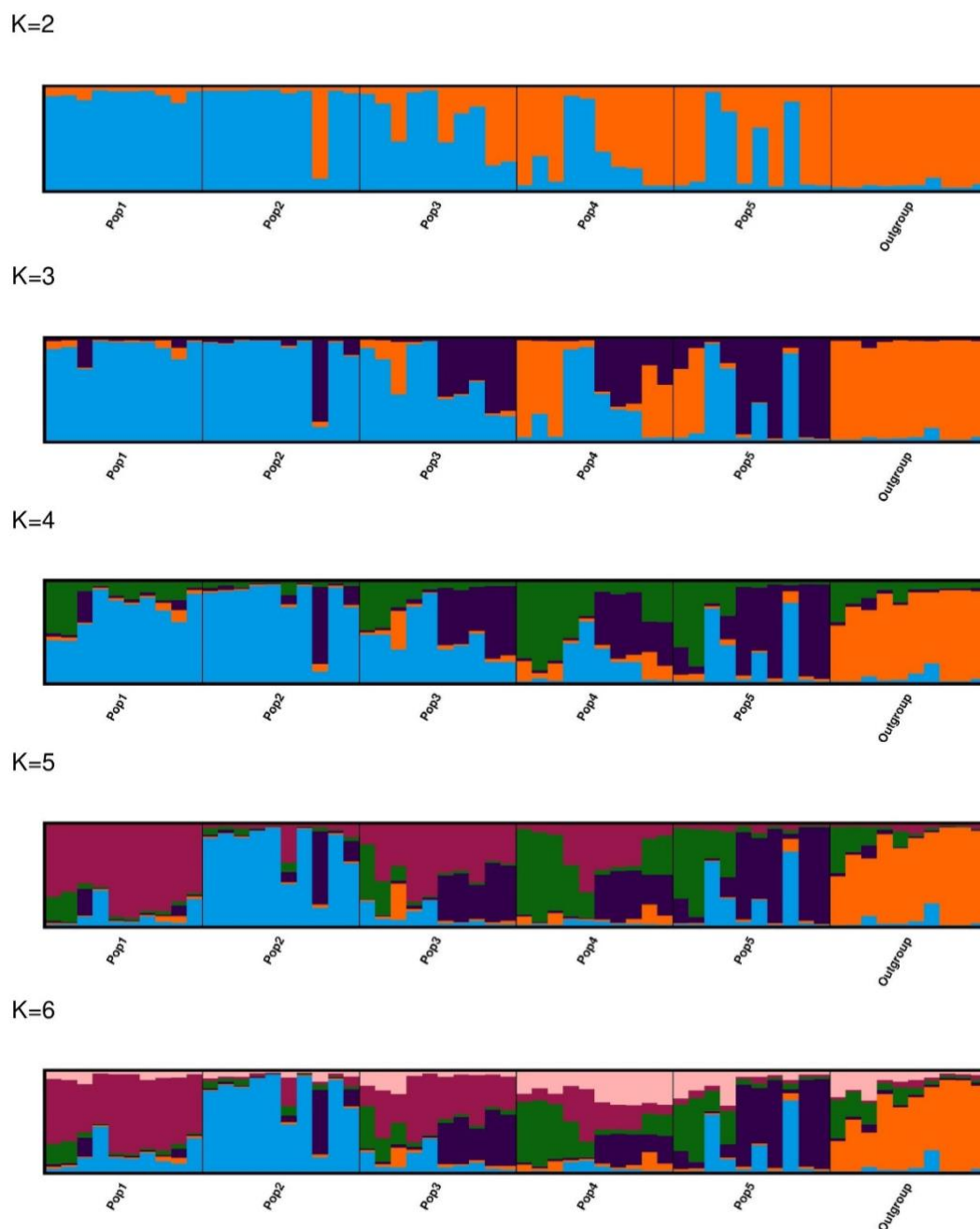


Figure 10. STRUCTURE histograms illustrating the genetic clustering of individuals from six groups (Pop1-Pop5 and Outgroup) for K values from 2 to 6

This morphological pattern closely parallels genetic clustering

The morphologically mixed Merke and Kayindy populations form a genetically related group with moderate diversity ($He \approx 0.26-0.28$) and signs of admixture, likely reflecting historical or ongoing gene flow, possibly influenced by proximity to *P. communis* orchards.

The morphologically uniform Karatau populations (Berkara, Bozturgay) correspond to highly differentiated genetic clusters ($F_{st} > 0.30$) with lower diversity ($He = 0.24-0.25$), suggesting long-term isolation and adaptation to more arid, rocky slopes.

Almalysay, although morphologically uniform (100% dissected leaves), shows the highest genetic diversity ($He = 0.31$), suggesting that environmental heterogeneity within its high-altitude microhabitats preserves allelic richness despite morphological consistency. These findings indicate that both local ecological conditions and broader evolutionary processes, such as hybridization within the genus *Pyrus*, may underlie the observed genetic structure of *P. regelii*. The intensive process of pear shape formation in Central Asia was established long ago, with the presence of numerous interspecific double and triple hybrids (*P. regelii* \times *P. communis* \times *P. serotina*, among others). Studies

confirm that spontaneous interspecific hybridization within the Rosaceae family is not only widespread but also an evolutionarily significant process contributing to genetic diversity and the formation of new taxa (Wu et al. 2024). Two subfamilies within the Rosaceae family include groups of multiple genera, which are almost entirely integrated into reticulate systems based on both natural and artificial hybridization data. However, the results of the present study demonstrate a stable genetic distinctiveness of *P. regelii*.

Ecological constraints and adaptation

The distinct environmental conditions of the Karatau and Kyrgyz Alatau ranges—differences in slope steepness, soil depth, stoniness, and moisture availability—appear to drive both morphological and genetic divergence. Biometric measurements further demonstrated phenotypic divergence, with Merke individuals reaching the greatest heights (2.0 m) and Bozturgay trees displaying the widest crown diameters (2.1 m), while Berkara trees remained the smallest. These morphological differences likely represent local adaptations to contrasting soil depth, slope stability, and moisture regimes in the two mountain systems.

The obtained data on the pristine conditions of *P. regelii* on the territory of the Karatau Range and the Kyrgyz Alatau indicate a high level of ecological selectivity of this species due to extreme orographic and soil-climatic conditions. Plants grow on steep, erosion-dissected slopes where soils are characterized by low thickness and poor water availability. These features create a unique ecological niche for xeromorphic woody species, including *P. regelii*. Similar adaptation tendencies to arid conditions are observed in other representatives of the region's xerophytic flora. Thus, according to the results of a study on *Capparis spinosa* (*C. herabasea*) growing in the Kyrgyz Alatau, it was found that under conditions of moisture deficit, the plant demonstrates pronounced anatomical and morphological rearrangements (the plant can alter the structure of leaves, stems, and roots when faced with drought) (Mamurova et al. 2021). In particular, an increase in the volume of xylem and fibro-vascular elements, as well as the expansion of the transit zone between the root and stem, contributes to the increased water absorption and water-retention capacity of tissues, which is noted.

Conservation implications

The integration of genetic, morphological, and ecological findings allows for targeted conservation recommendations:

Merke population (Pop1) - Genetically homogeneous with high morphological diversity. In situ conservation is recommended, with regular monitoring of environmental conditions and genetic diversity. Despite being located within the State Nature Reserve, it is exposed to strong anthropogenic pressure (grazing, recreation). To reduce risks of habitat degradation, conservation measures should include limiting livestock load and introducing seasonal attendance control.

Berkara population (Pop2) - Genetically isolated, morphologically uniform, and low in diversity. Requires urgent in situ and ex situ protection. Recommended actions

include systematic seed collection, desiccation, and cryopreservation in national seed banks, along with inclusion in regional rare species monitoring programs.

Kayindy population (Pop3) - Exhibits high morphological variability and partial genetic admixture with neighboring populations. This population may act as a bridge between genetic clusters and is therefore of special importance. It should be prioritized for long-term ecological and genetic monitoring, with micro-zoning of habitats to control anthropogenic impact. Seeds should be collected for ex situ storage and experimental planting in botanical gardens, as this population may serve as a valuable resource for studying hybridization and local adaptation.

Almalysay population (Pop4) - Genetically heterogeneous but morphologically uniform. Despite admixture signals, it preserves high allelic richness. Ex situ conservation is strongly recommended, with emphasis on seed collection for cryopreservation and establishment of living collections in botanical gardens. This will ensure representation of unique southern ecological niches in germplasm banks.

Bozturgay population (Pop5) - Morphologically stable but genetically heterogeneous, likely reflecting historical gene flow and adaptation to arid habitats. The pronounced genetic isolation of this population warrants priority conservation. Both in situ habitat protection and ex situ seed banking are necessary. Given its adaptation to extreme arid conditions, propagules from this population should be included in long-term storage facilities (cryopreservation, seed banks) and considered for restoration programs in arid landscapes.

Beyond population-level recommendations, it is important to emphasize the broader ecological and agricultural value of *P. regelii*. Despite the availability of well-studied cultivated pear forms, wild relatives, including *P. regelii*, represent a valuable source of genetic diversity and unique traits conferring resistance to abiotic stresses, making them promising for long-term breeding programs. This genetic variation can provide traits such as disease resistance, drought tolerance, and adaptability to changing climate conditions. By integrating genes from wild species, breeders can enhance the survival and productivity of cultivated pears under environmental pressures.

The conservation of *P. regelii* does not compete with the protection of more vulnerable species but complements it, as maintaining the genetic diversity of key ecosystem components contributes to the overall stability of biocenoses. Within its native range, *P. regelii* forms plant communities with Red Book species of Kazakhstan, such as *Tulipa greigii*, *Juno coerulea*, *Malus sieversii*, and *Spiraeanthus schrenkianus*, along with other rare and relict taxa. In addition, *P. regelii* plays an important role in preventing soil erosion on mountain slopes: its root system stabilizes soil and reduces the risk of landslides. It also provides food for numerous animals, including the Karatau argali and badger, while its seeds are consumed by various bird species.

At the same time, anthropogenic pressures such as seasonal livestock grazing and recreational activities (e.g., tourism in the Merke Gorge) intensify threats to natural populations. This underscores the need for establishing protected areas, controlling logging, and implementing sustainable land-use practices. Thus, the conservation of *P.*

regelii holds strategic significance not only for regional biodiversity but also for ensuring future food security under unpredictable climate change.

In the field of ex situ conservation of *P. regelii*, certain results have been achieved: a protocol for in vitro propagation and preservation has been developed (Dyussebekova et al. 2021). This approach is valuable for safeguarding unique genotypes and can serve as a complement to long-term conservation strategies, including seed banking and in situ preservation in natural habitats.

Limitations and future perspectives

While ISSR markers provided high-resolution insights into population structure, they are dominant markers and do not reveal co-dominant variation or locus-specific information. It's crucial to note that signals of admixture with *P. communis* detected in STRUCTURE cannot be confirmed without higher-resolution approaches. This underscores the urgent need for future studies to employ SNP genotyping, whole-genome sequencing, or chloroplast DNA markers to resolve questions of introgression and historical demography. Combining these advanced genomic tools with ecological niche modeling and common garden experiments would clarify the adaptive significance of genetic clusters and inform breeding programs.

In conclusion, this study provides the first comprehensive assessment of the genetic diversity and population structure of *P. regelii* in southern Kazakhstan using ISSR markers. The high level of polymorphism (98.9%) and clear genetic subdivision among populations reflect the combined effects of geographic isolation and ecological adaptation. Populations from the Karatau and Kyrgyz Alatau ranges from distinct genetic groups, with the Bozturgai and Berkara populations being particularly isolated. These findings highlight the urgent need for in situ conservation through establishing protected areas, managing grazing and tourism, and conducting long-term ecological and genetic monitoring. Complementary ex situ measures, including seed banks and living collections, are essential to preserve unique alleles and adaptive traits. Beyond conservation, *P. regelii* represents a valuable genetic resource for breeding programs. Its demonstrated tolerance to drought and low temperatures provides opportunities for developing new cultivars adapted to climate change. To maximize heterosis and maintain genetic integrity, breeding strategies should incorporate individuals from distinct genetic clusters, supported by molecular monitoring of both natural and ex situ collections. Overall, the results emphasize the ecological, evolutionary, and practical significance of *P. regelii* as a relic xerophytic species. Protecting its fragmented populations is essential not only for maintaining biodiversity and preventing soil erosion in fragile mountain ecosystems, but also for securing a unique gene pool with high potential for sustainable utilization. Given its restricted distribution and increasing anthropogenic threats, *P. regelii* should be prioritized for inclusion in the Red Book of Kazakhstan and considered within broader biodiversity conservation frameworks.

ACKNOWLEDGEMENTS

We express our gratitude to Elena Sergeevna Trosko, an employee of the M.A. Lisavenko Research Institute of Horticulture of Siberia, for providing the material of *Pyrus communis* for research.

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