SCIENTIFIC HORIZONS

Journal homepage: https://sciencehorizon.com.ua
Scientific Horizons, 27(10), 136-147

UDC 581.15:581.6
Doi: 10.48077/scihor10.2024.136

Chorological analysis of species of the genus *Dianthus* in the South Caucasus region with an emphasis on the flora of Nakhchivan Autonomous Republic

Enzala Novruzova*
PhD in Biology
Nakhchivan State University
AZ7012, University Campus, Nakhchivan, Azerbaijan
https://orcid.org/0009-0003-3547-6025

Article's History:
Received: 12.03.2024
Revised: 15.08.2024
Accepted: 25.09.2024

Abstract. The purpose of the study was to conduct a chorological analysis of six species of the genus *Dianthus* in the Nakhchivan Autonomous Republic, using field and molecular genetic methods. The main focus was on their geographical distribution, population status, and genetic diversity. As a result of the study of six species of the genus *Dianthus*, significant differences were found in their geographical distribution, ecological niches, and adaptation to local conditions. Molecular genetic analysis showed a high level of genetic diversity among populations of *Dianthus nahcivanensis* and *Dianthus nakhchivanicus*, which confirmed their uniqueness and endemic status. It has also been proven that the genus *Dianthus* has a high species diversity and shows significant variations in its distribution depending on the geographical and climatic conditions of the region. The study used geobotanic methods to determine the habitats of species and analyse factors affecting their distribution. In particular, features of the habitat were identified in connection with the local conditions of the Nakhchivan Autonomous Republic, which included a significant number of endemic species. The genetic analysis performed using the polymerase chain reaction method and sequencing helped to identify the level of genetic diversity and establish phylogenetic relationships between populations. It was found that *Dianthus raddeanus*, *Dianthus parviflorus, Dianthus nakhchivanicus* and *Dianthus nahcivanensis* species are more vulnerable due to the impact of anthropogenic activities, in particular agriculture and grazing, which has led to a decrease in the area of their habitat. Modelling using the maximum entropy method showed potential zones of expansion of species habitats under favourable ecological conditions. The results obtained were important for understanding the ecological patterns of distribution of the genus *Dianthus* in the region and the development of strategies for preserving biodiversity in the face of climate change and anthropogenic pressure. Based on the study, it was recommended

Suggested Citation:
Novruzova, E. (2024). Chorological analysis of species of the genus *Dianthus* in the South Caucasus region with an emphasis on the flora of Nakhchivan Autonomous Republic. *Scientific Horizons*, 27(10), 136-147. doi: 10.48077/scihor10.2024.136.

*Corresponding author

to create or expand protected areas, limit anthropogenic impact, and conduct additional monitoring of the number and genetic structure of *Dianthus* populations

Keywords: endemics; distribution zone; genetic diversity; anthropogenic impact; biodiversity conservation

INTRODUCTION

Genus *Dianthus* (carnation) belongs to the family *Caryophyllaceae* and includes more than 300 species distributed mainly in temperate and subtropical zones of Eurasia. This genus is an important object of botanical research due to its considerable diversity and the presence of endemic and rare species in need of protection. In the South Caucasus region, in particular in the Nakhchivan Autonomous Republic, *Dianthus* is represented by a number of unique species that demonstrate significant ecological plasticity and genetic diversification. However, despite this, research on biogeography, ecology, and genetic diversity of representatives of the genus *Dianthus* in this region is limited.

In the scientific literature, it has been repeatedly noted that species of the genus *Dianthus* are of great evolutionary and ecological importance because of their ability to adapt to diverse ecosystems. Some species of this genus are widely distributed in Eurasia and North Africa, while others are narrowly endemic. In particular, studies have shown that endemic species such as *Dianthus nahcivanensis* and *Dianthus nakhchivanicus*, have limited geographical distribution and significant genetic isolation, which makes them vulnerable to environmental changes (Terlević *et al.*, 2022). In addition, studies of the chemical composition of extracts of these compounds can open up new opportunities for creating safer pharmaceutical products. Thus, M.Z. Mladenović *et al.*, (2023) study identified 275 components that were part of the flower extract, while 18 of them, as it was noted, were completely new compounds. In a more up-to-date 2024 paper, P.-J. Hou *et al.* (2024) discovered the medicinal properties of clove extract in the prevention of urolithiasis. A large study was also conducted by the A.K. Celik *et al.* (2024) research group, which revealed high anti-cancer and antioxidant activity of endemic species of *Dianthus*. In the Republic of Azerbaijan, researchers from the Institute of Plant Chemistry of the Academy of Sciences identified 6 compounds with *Dianthus helenae* which subsequently demonstrated anticonvulsant and nootropic effects (Yusupova *et al.*, 2021). All this may indicate a significant potential for further study of this genus not only in the framework of geobotanical research, but also in medical and pharmaceutical chemistry.

Previous studies of the Caucasus flora have shown that the South Caucasus region is an important centre of biodiversity with a large number of endemic species, including not only representatives of the genus *Dianthus*, but also many other families (Beker & Rączka, 2019; Kuljanishvili *et al.*, 2021). In particular, the Nakhchivan Autonomous Republic is a place of distribution of numerous endemic and rare plant species, which makes this region key for the protection of biodiversity. Given the climatic and geological features of the region, many species show high adaptation to harsh conditions such as lack of moisture, increased solar radiation, and stony soils (Elizbarashvili *et al.*, 2021). One of the key problems that arose in the study of the flora of the Caucasus is the influence of anthropogenic factors on natural ecosystems. A number of studies show that grazing, agriculture, and construction have a significant impact on the population status of many plant species, including members of the genus *Dianthus*.

However, since 2018, significant progress has been made in understanding the molecular genetics of species of the genus *Dianthus* (Lin *et al.*, 2022; Franzoni *et al.*, 2023). The use of modern methods of molecular genetic analysis allows researchers to determine the genetic structure of populations, the level of genetic diversification, and to identify relationships between similar species. In particular, studies using polymerase chain reaction (PCR) and DNA (deoxyribonucleic acid) sequencing methods have revealed unique genetic markers for rare and endemic species (Meng *et al.*, 2023). However, anthropogenic factors can lead to a significant decrease in the number and fragmentation of populations, which increases the risk of extinction of individual species. Vulnerable species growing in regions with significant anthropogenic impact showed low regenerative capacity and genetic diversity (Glibovytska *et al.*, 2024). Thus, the study of species of the genus *Dianthus* in Nakhchivan is an important contribution to understanding the biogeography and genetic structure of the plant world of the Caucasus. The results of this study can serve as a basis for developing strategies for the protection of rare and endemic plant species in the region, and contribute to further research on the genetic diversity of representatives of the genus *Dianthus*.

The purpose of this study was to carry out a comprehensive chorological analysis of six species of the genus *Dianthus* growing in the Nakhchivan Autonomous Republic, using both field and molecular genetic methods. Special attention was paid to the following species: *Dianthus raddeanus, Dianthus orientalis, Dianthus parviflorus, Dianthus elegans, Dianthus nahcivanensis,* and *Dianthus nakhchivanicus*, which are important components of the region's mountain ecosystems.

MATERIALS AND METHODS

In the course of the study, several aspects of the biology and ecology of six species of the genus *Dianthus*, which grew up in the Nakhchivan Autonomous Republic, were analysed. In addition to molecular genetic analysis, laboratory analysis and the use of modern methods of geographic information systems (GIS), a comprehensive field study was conducted, which included the collection of geographical data, soil characteristics, determination of microclimatic conditions, and assessment of the state of populations and anthropogenic impacts. The collected samples were identified at the species level using Caucasian flora determinants, after which they were dried and herbarised for further analysis in the laboratory. During the collection of samples, the main ecological parameters of habitats were recorded, in particular the type of soil, the level of illumination, air temperature, and relative humidity.

Field studies were conducted during two growing seasons (from May to October 2022 and 2023) in various environmental conditions of the Nakhchivan Autonomous Republic. Surveys were carried out by route expeditions, during which samples of plant material were collected from various types of habitats, such as highlands, foothills, and forest-steppe and steppe ecosystems. During field work, GPS devices were used to accurately determine the coordinates of species' growth sites. The following parameters were recorded in each growth site: geographical coordinates (latitude, longitude, altitude above sea level); soil type and its physical and chemical properties; microclimatic conditions (temperature, humidity, illumination); population status (density, number, degree of damage); presence of anthropogenic influences (degree of grazing, traces of land cultivation, development).

Herbarium collections from local and international botanical institutes and museums were also used to obtain more detailed data, which contained information about collection sites, morphological features, flowering periods, and other characteristics of species *Dianthus*. The herbarium of the National Institute of Azerbaijan provided access to important data on species collected in the country, including the Nakhchivan Autonomous Republic: herbarium samples of *Dianthus elegans* and *Dianthus parviflorus*, information on historical collection sites of species and their prevalence in mountainous areas, data on soil conditions and climatic characteristics of places where samples were collected. The herbarium of the Nakhchivan Institute of Botany was one of the key sources of information about endemic species of *Dianthus* from the region. The following data were used: herbarium samples of *Dianthus nahcivanensis* and *Dianthus nakhchivanicus*, sample collection sites, in particular exact coordinates and environmental conditions, morphological descriptions such as stem length, leaf shape, flower structure, information about flowering periods, which helped in determining the phenological phases of species.

Methods of chorological analysis were used to determine the distribution areas of species of the genus *Dianthus*. Habitat maps were created based on literature data, herbarium collections, and samples obtained during field surveys. For this purpose, geographic information systems (GIs) were used, which helped to combine various data sources and build maps of areas indicating high-altitude belts and habitat types. QGIS software was used for data analysis and visualisation. 1:50000 and 1:100000 scale topographic maps of the region were used to analyse the geographical distribution of species, and geological maps to determine the relationship between soil conditions, topography, and species distribution of *Dianthus*.

Data on the distribution of species were analysed using the maximum entropy model (MaxEnt), which helped to identify potential distribution zones of species considering environmental factors. Data on temperature, humidity, light, and other parameters that are critical for species survival in certain conditions were collected and processed. The model was calibrated based on 75% of the training data, and the remaining 25% was used to test the accuracy of the forecast. During field studies, soil samples were collected from different areas where the species grew. Each soil sample was collected at a depth of 10-15 cm, which is standard for analysing the surface layer of soil. Soil collection sites corresponded to the coordinates of species growth points of *Dianthus*.

The collected samples were dried at room temperature and then transported to a laboratory for further analysis. The laboratory analysis included several stages. First, the physical properties of the soil were determined by granulometric analysis to determine the content of sand, silt, and clay in the samples. The sieve analysis method and the hydrometric method were used to assess the mechanical composition of the soil. Soil pH values were also measured using an electronic pH meter. The Valkley-Black method (burning organic matter) was used to analyse the organic matter content. Nitrates, phosphates, and other mineral elements were determined by spectrophotometry. Atomic absorption spectrometry and colour testing methods were used to analyse the content of macro- and microelements such as nitrogen, potassium, and phosphorus. This helped to assess the potential of the soil to support plant growth. These data were used to establish correlations between soil types, their physical and chemical properties, and the state of populations of species in the genus *Dianthus*.

Molecular genetic analysis was performed to assess the genetic diversity of Dianthus species and their evolutionary relationships. For this purpose, the polymerase chain reaction (PCR) method with randomly amplified DNA (RAPD) and DNA sequencing analysis were used. DNA was isolated from the samples using

the standard CTAB (cetyltrimethylammonium bromide method) method, which is effective for isolating DNA in plant samples with a high content of polyphenols and polysaccharides. The quantity and quality of the isolated DNA were checked by spectrophotometry and agarose gel electrophoresis. The optimal DNA concentration index for subsequent manipulations was at least 50 ng/μL. A total of 48 samples were taken from different populations of each species, followed by DNA extraction using the QIAamp DNA Mini Kit in accordance with the manufacturer's instructions. PCR primers were developed based on chloroplast genes and nuclear markers, which made it possible to assess both chloroplast and nuclear genetic diversity. The PCR procedure was performed using a temperature cycle consisting of denaturation at 95°C, annealing of primers at 50-60°C, and elongation at 72°C. The number of PCR cycles was optimised to 35-40 to achieve the maximum amount of amplified product without losing the specificity of the reaction.

The samples were then sequenced using the Sanger method, and the results were analysed using MEGA X software suite to build phylogenetic trees and determine the level of genetic diversity of each species. The obtained DNA sequences were compared with National Center for Biotechnology Information (NCBI) (2024) databases to determine phylogenetic relationships and potential evolutionary lineages. PCR products were analysed by electrophoresis in 2% agarose gel using molecular weight markers to compare amplicon sizes. The obtained data from sequencing and PCR analysis of amplicons were used to assess the level of genetic diversity among populations. Indicators such as the Nei's genetic diversity index, heterozygosity level, and the number of alleles per locus were used. Calculations were performed using the GenePop and Arlequin programmes. To assess the impact of anthropogenic factors on the number and distribution of species, local residents, agricultural workers and environmentalists were interviewed, and satellite images of the territory over the past 20 years were analysed. The survey was conducted using standardised questionnaires that contained the following blocks of questions: general information on land use, the impact of grazing, anthropogenic load and development, nature conservation, and species conservation.

A total of 150 respondents were interviewed, including: 75 people, mostly farmers and landowners, who had many years of economic experience in the region, 50 people who worked directly in fields and pastures, with an average work experience of more than 15 years, 25 experts who participated in field research and observations in the Nakhchivan Autonomous Republic. The work experience of environmentalists ranged from 10 to 25 years, which provided a high level of expert assessment of anthropogenic impacts. Areas with active anthropogenic activity were also identified, in particular, agricultural land, grazing and development areas. Based on the collected data, indices of the impact of anthropogenic factors for each habitat of species of the genus *Dianthus* were calculated. The data was integrated into GIS, which helped to create risk maps for each species and assess the degree of extinction of populations. All the data obtained were subjected to statistical analysis using software. Variance analysis (ANOVA) was used to compare environmental parameters between different species and populations. Correlation analyses were used to analyse the relationship between genetic diversity and anthropogenic factors.

RESULTS

During the study, field and laboratory studies of six species of the genus *Dianthus* were conducted in the Nakhchivan Autonomous Republic, covering the collection of geographical, environmental, and genetic data for a comprehensive assessment of their biological and environmental characteristics. The results of the study showed that the species of the genus *Dianthus* in the South Caucasus region has an uneven distribution associated with the diverse environmental conditions present in this region. It was found that the greatest abundance and diversity of species is observed at altitudes from 1500 to 2500 metres above sea level, where species such as *Dianthus raddeanus* and *Dianthus orientalis* grow. In the flat and foothill areas, in particular in the Nakhchivan Autonomous Republic, species *Dianthus parviflorus* and *Dianthus elegans* were mainly found. These species have shown resistance to harsher climatic conditions, such as high temperatures and low precipitation (Table 1).

***Table 1**. Distribution of the main species of Dianthus in the region by climatic conditions*

Species of *Dianthus*	Height belt (m)	Latitude	Longitude	Soil type	Average annual temperature (°C)	Average precipitation (mm)
Dianthus raddeanus	1,200-1,400	39.240° N	45.420° E	Stony, gravel	10-15	600-800
Dianthus orientalis	1,500-1,700	39.300° N	45.450° E	Sandy loam	8-12	500-700
Dianthus parviflorus	1,100-1,300	39.200° N	45.480° E	Sandy, clay	12-18	200-400
Dianthus elegans	1,700-1,900	39.380° N	45.400° E	Forest, loamy	14-20	300-500
Dianthus nahcivanensis	1,400-1,600	39.400° N	45.360° E	Stony soils	14-24	
Dianthus nakhchivanicus	1,600-1,800	39.290° N	45.390° E	Mountain chernozems	14-22	

***Source:** created by the author*

Species *Dianthus elegans* was found on the highest mountain areas (1700-1900 m), while *Dianthus parviflorus* grew at lower altitudes (1100-1300 m). *Dianthus nakhchivanicus* grows at altitudes of 1600-1800 m, while *Dianthus nahcivanensis* recorded at altitudes of 1400-1600 m. Species of *Dianthus raddeanus*, *Dianthus parviflorus*, and *Dianthus orientalis* were found at altitudes from 400 to 1500 m, indicating a wider ecological habitat. These data showed that the species adapted to different ecological niches in the study region.

Microclimatic conditions in the growing areas varied depending on the altitude zone and location. Temperatures ranged from 18°C to 28°C during the growing season. The highest temperatures were recorded in populations *Dianthus raddeanus*, *Dianthus orientalis*, and *Dianthus parviflorus*, which grew in low-lying areas where the average daily temperature reached 28°C. In high-altitude populations of *Dianthus nahcivanensis* and *Dianthus nakhchivanicus,* the temperature conditions were more moderate, with an average of about 20-22°C. In terms of humidity, populations located at high altitudes had higher humidity levels (up to 70% in the morning), while low-lying populations were characterised by lower humidity levels (40-50%). Illumination in all places of growth was high, which met the specific environmental requirements of the genus *Dianthus* to the light.

The population status showed different levels of density and abundance for each species. *Dianthus parviflorus* showed the highest abundance, while *Dianthus elegans* had the lowest density. *Dianthus nahcivanensis* and *Dianthus nakhchivanicus* showed moderate levels of density and abundance, which indicated stable populations in their habitats (Table 2). Population assessment also included analysis of plant damage. Significant damage was recorded in populations of *Dianthus raddeanus* and *Dianthus orientalis* through grazing and trampling, which led to a decrease in the population size and damage to root systems.

***Table 2**. Status of Dianthus species populations in the South Caucasus region*

Species	Density (plants/m²)	Number (individuals)	Degree of damage (%)
Dianthus raddeanus	30-40	150-200	10-15
Dianthus orientalis	25-35	100-150	20
Dianthus parviflorus	40-50	250-300	10
Dianthus elegans	10-20	50-80	15
Dianthus nahcivanensis	20-30	120-180	10-12
Dianthus nakhchivanicus	15-25	80-100	12

***Source:** created by the author*

Dianthus elegans, which was recorded at altitudes up to 1900 m, was characterised by the ability to grow on red soils in more severe climatic conditions. The soils on which the species grew ranged from stony to red clay soil. Species *Dianthus elegans* was characterised by the highest content of organic matter and nitrogen in the soil, which explains its distribution on red soils. *Dianthus nahcivanensis* and *Dianthus nakhchivanicus* had a slightly lower content of organic substances and grew on stony and mountainous chernozems (Table 3).

***Table 3**. Soil types and their physical and chemical properties*

Species	Soil type	pH	Organic content (%)	Nitrogen (%)
Dianthus raddeanus	Stony soils	7	3.2.	0.14
Dianthus orientalis	Mountain chernozems	6.8	3.5	0.15
Dianthus parviflorus	Loam	7.1	2.8	0.12
Dianthus elegans	Red soils	7	4	0.18
Dianthus nahcivanensis	Stony soils	6.7	2.9	0.13
Dianthus nakhchivanicus	Mountain chernozems	6.9	3.7	0.16

***Source:** created by the author*

Analysis of the soils in which the studied species grew showed significant variability in physical and chemical properties. The soils in the areas of *Dianthus nahcivanensis* growth had high stoniness and low humus content (about 2.5%), which indicated that this species was adapted to poor conditions. The soils on which *Dianthus nakhchivanicus* grew were characterised by moderate stoniness and a humus content of 3.1-3.5%, which created more favourable conditions for growth. In species populations of *Dianthus raddeanus*, *Dianthus orientalis*, and *Dianthus parviflorus*, the soils were moderately sandy with a low content of nitrogen and organic matter, which indicated their adaptability to harsh environmental conditions.

As a result of the conducted chemical analysis of the soil for the study of six species of the genus *Dianthus* in the Nakhchivan Autonomous Republic, the following results were obtained regarding the content

of mineral elements and macronutrients in various soil samples. Soil samples were taken from growth sites of the species *Dianthus raddeanus*, *Dianthus orientalis*, *Dianthus parviflorus*, *Dianthus elegans*, *Dianthus nahcivanensis,* and *Dianthus nakhchivanicus*. Nitrogen content was relatively stable in all samples, with the highest rates found at growth sites of *Dianthus parviflorus* and *Dianthus nahcivanensis*. The potassium and phosphorus content were highest in the soils with *Dianthus parviflorus*, which may indicate more fertile soils in these areas (Table 4).

***Table 4**. Content of trace elements in the soils at the growth sites of the species of Dianthus*

Species	Nitrogen (mg/kg)	Potassium (mg/kg)	Phosphorus (mg/kg)
Dianthus raddeanus	8	300	15
Dianthus orientalis	7	250	12
Dianthus parviflorus	9	320	18
Dianthus elegans	7.5	280	14
Dianthus nahcivanensis	8.2	310	16
Dianthus nakhchivanicus	6.5	230	10

***Source:** created by the author*

The results obtained showed that the soils of the growth of species of the genus *Dianthus* have a neutral-alkaline pH, moderate organic matter content, and different levels of macro- and microelements. This indicated that the species of the genus *Dianthus* can adapt to different soil conditions, but there have been some advantages over more fertile and nutrient-rich areas. The conducted interview of local residents, agricultural workers and environmentalists helped to collect information on the impact of anthropogenic factors on species of the genus *Dianthus* in the Nakhchivan Autonomous Republic. The data obtained were presented in the form of tables for convenient evaluation of the results. According to the results of the interview, most agricultural workers used pesticides to treat their land, which contributed to a change in the flora and, possibly, a decrease in the number of some species of *Dianthus*. Environmentalists recorded a high proportion of changes in the flora of the region (Table 5).

***Table 5**. General information on land use*

Category of respondents	Number of respondents	Average duration of activity (years)	Pesticide use (%)	Changes in flora and soil (%)
Local residents	75	20	60	55
Agricultural workers	50	15	80	70
Environmentalists	25	18	N/A	80

***Source:** created by the author*

Grazing significantly affected vegetation, especially in regions with large livestock numbers. Environmentalists noted a significant decline in the populations of some species of *Dianthus* in such territories (Table 6).

***Table 6**. Impact of grazing*

Category of respondents	Number of respondents	Average number of livestock per farm	Vegetation changes (%)	Changes in soils (%)
Local residents	75	50	65	50
Agricultural workers	50	75	70	65
Environmentalists	25	N/A	85	70

***Source:** created by the author*

Anthropogenic activities, in particular development and changes in water resources, have caused a deterioration in the environmental situation. Environmentalists noted that new buildings have had a particularly negative impact on the growth areas of rare species of *Dianthus* (Table 7).

***Table 7**. Anthropogenic load and development*

Category of respondents	Number of respondents	New buildings nearby (%)	Changes in water resources (%)	Environmental degradation (%)
Local residents	75	40	30	50
Agricultural workers	50	55	45	60
Environmentalists	25	75	65	80

***Source:** created by the author*

Only a small proportion of local residents and agricultural workers were aware of the existence of rare and endemic species of *Dianthus* (Table 8), while environmentalists actively supported measures to protect them.

***Table 8**. Nature conservation and species conservation*

Category of respondents	Number of respondents	Knowledge of rare species (%)	Support for security measures (%)
Local residents	75	30	20
Agricultural workers	50	25	35
Environmentalists	25	95	90

***Source:** created by the author*

The survey showed that a small part of the local population showed knowledge of rare species, which indicates the need to raise awareness and implement conservation measures to preserve the local flora. Anthropogenic factors such as agricultural activity, grazing, and land-use changes were also found to have a significant impact on populations of rare and endemic species (Table 9). In areas with intensive grazing, significant vegetation damage has been observed, which may lead to a decrease in the number of species, such as *Dianthus nahcivanensis*, in the future.

***Table 9**. Influence of anthropogenic factors on population size*

Species	Type of anthropogenic impact	Impact level	Change in population (%)	Main threats
Dianthus orientalis	Grazing of livestock	Moderate	-20	Soil degradation, trampling
Dianthus raddeanus	Agriculture	Moderate	-25	Reduction of natural habitats
Dianthus parviflorus	Development	High	-40	Loss of habitat
Dianthus elegans	Agriculture	Low	-15	Limited competition with other species
Dianthus nahcivanensis	Intensive grazing	High	-50	Trampling, environmental pollution
Dianthus nakhchivanicus	Agriculture	High	-45	Loss of natural habitats

***Source:** created by the author*

Spectrophotometric analysis showed that the concentration of isolated DNA in all samples exceeded 50 ng/μL, which allowed the use of these samples for further PCR analysis. The quality of the isolated DNA was checked by agarose gel electrophoresis, where all samples showed clear bands, indicating no DNA degradation. For each of the six species of *Dianthus*, several genetic markers were amplified, in particular microsatellites or single sequence repeats (SSRs) and internally transcribed spacers (ITS). The optimal annealing temperature of the primers was 55°C for SSR markers and 52°C for its markers. After 35 PCR cycles, amplicons of the desired size were obtained, which was confirmed by the results of electrophoresis in 2% agarose gel. All the samples under study gave different amplification products, the dimensions of which coincided with those expected in previous studies.

The amplified DNA fragments were sequenced according to the Sanger method. The resulting DNA sequences were mapped to databases to identify species and verify the relationship between them. All six species were found to have characteristic genetic polymorphisms that clearly distinguished their genetic profiles. In general, the species *Dianthus nahcivanensis* and *Dianthus nakhchivanicus* they found a high level of genetic diversity compared to other species. This was due to the isolation of their populations, which adapted to specific growth conditions. Genetic diversity in these species

was concentrated within populations, which indicated their long-term existence in localised regions.

On the contrary, species *Dianthus raddeanus*, *Dianthus parviflorus*, and *Dianthus orientalis* exhibited lower levels of genetic diversity, indicating greater genetic isolation and possible historical "bottle necks". This indicated a decrease in genetic exchange between populations, which may be the result of anthropogenic factors, such as habitat fragmentation. Molecular genetic analysis of *Dianthus raddeanus* showed significant genetic diversity, which indicates the potential for adaptation and survival in changing environmental conditions. However, the identified local genetic isolates confirmed the need for more research on the impact of population fragmentation on the long-term conservation of the species.

Phylogenetic analysis showed the evolutionary relationships between populations of different species of the genus *Dianthus*. It was found that *Dianthus nahcivanensis* and *Dianthus nakhchivanicus* form separate clusters, which confirmed their genetic isolation from other species. They showed genetic closeness to each other, indicating a common origin or a long history of coevolution in isolation. Other species, such as *Dianthus raddeanus* and *Dianthus orientalis*, formed separate phylogenetic groups that also indicated a long evolutionary history and specific adaptations to different environmental conditions.

Genetic analysis determined the level of genetic diversity within and between populations, established phylogenetic relationships between species, and identified possible mechanisms of adaptation to different environmental conditions. Most species, such as *Dianthus orientalis* and *Dianthus raddeanus,* demonstrated a high level of genetic diversity (heterozygosity up to 0.75), which indicates their adaptive potential and high resistance to changes in environmental conditions (Table 4). Endemic species of *Dianthus nahcivanensis* and *Dianthus nakhchivanicus* had significantly lower rates of genetic diversity (heterozygosity up to 0.42), which indicates limited genetic variability (Table 10). This may be due to their isolation and narrow distribution area, which increases the risk of extinction due to environmental changes or anthropogenic factors.

***Table 10**. Genetic diversity of species of the genus Dianthus*

Species	Heterozygosity	Level of genetic diversification	Number of allelic variants
Dianthus orientalis	0.75	High	12
Dianthus raddeanus	0.68	High	10
Dianthus parviflorus	0.55	Average	8
Dianthus elegans	0.60	Average	9
Dianthus nahcivanensis	0.42	Low	6
Dianthus nakhchivanicus	0.40	Low	5

***Source:** created by the author*

A study of six species of the genus *Dianthus* in the Nakhchivan Autonomous Republic revealed significant genetic and ecological diversity, but all species were vulnerable to anthropogenic factors and climate change. Analysis of species habitats of *Dianthus* in the South Caucasus established that most species of this genus demonstrate highly specialised ecological requirements and have a limited habitat. This makes them vulnerable to environmental changes and anthropogenic impacts. Endemic species, such as *Dianthus nahcivanensis* and *Dianthus nakhchivanicus*, require special attention to the protection and preservation of their places of growth. The conducted research investigated in detail the chorological features of the species of the genus *Dianthus* in the South Caucasus, identified key factors affecting their distribution and conservation, and developed specific measures to preserve these species. The results can serve as a basis for further research and strategies for managing the conservation of plant biodiversity in the region.

DISCUSSION

The study of the six species of the genus *Dianthus*, which are common in the territory of the Nakhchivan Autonomous Republic, used a comprehensive approach to investigate their ecology, geographical distribution, population status, and genetic structure. The selected methods helped to obtain a holistic view of the ecological features and threats to the species under study. The main research methods were field surveys, analysis of physical and chemical properties of soils, study of microclimatic conditions of growth sites, and molecular genetic surveys to investigate the genetic diversity of populations.

Field studies have made it possible to determine the geographical coordinates of the growth sites of each species, including altitude above sea level, soil type, and microclimatic conditions (temperature, humidity, and light). These data were important for understanding each species' ecological niches and their adaptive potential. The analysis of the physical and

chemical properties of soils included the determination of acidity (pH), the concentration of basic minerals, and the content of organic substances. This approach revealed how soil conditions affect the growth and distribution of these species.

Studies of endemic plant species required special attention. The literature sources contain conflicting opinions about their adaptability to different climatic conditions. M. Behroozian's *et al.* (2020a) research team questioned the general assumption that endemic species have narrow ecological niches. Using environmental modelling and functional characteristics analysis, they found that some species of *Dianthus* demonstrate significant ecological variability and adaptation to different environmental conditions. This led to the assumption of their ability to occupy a variety of ecological niches, which goes against traditional ideas about endemics as highly specialised species (Behroozian *et al.*, 2020a).

In this study of *Dianthus* species, both specific and more general ecological traits characteristic of different populations were identified. The ability of endemic species to adapt and occupy wider ecological niches is partially substantiated, since some species, such as *Dianthus raddeanus* and *Dianthus nahcivanensis* indeed, demonstrated adaptation to various microclimatic conditions and changes in soil composition (Zubtsova *et al.*, 2019). These species can grow in different conditions of altitude and humidity, which confirms the idea of ecological plasticity. However, for some other species, such as *Dianthus parviflorus* and *Dianthus nakhchivanicus*, environmental specialisation remained prominent. They showed a significant dependence on specific environmental conditions, such as soil type and light levels, which is consistent with the traditional view of highly specialised endemics. Thus, the study partially confirmed Behroozian's thesis about the variability of ecological niches in some endemic species. However, it did not refute the opinion about the traditional highly specialised ecological strategy in certain populations. On the other hand, C.A.M. Marshall *et al.* (2022) study, which examined factors affecting plant endemism in two West African forest centres, focused on various environmental and climatic variables, and environmental characteristics such as altitude, forest type, climatic conditions, and anthropogenic impact. Climatic conditions, in particular precipitation, were found to be a significant predictor of endemism. The paper also highlighted the importance of protecting these forests due to their significant role in the conservation of rare and endemic species.

The South Caucasus region is characterised by the proliferation of zones with endemic plant crops (Nikolaishvili & Dvalashvili, 2015; Noroozi *et al.*, 2019). Although it is also possible to find among the literature sources of fauna research, in particular amphibians (Gasimova, 2021), with similar habitats, in the framework of this study, were rather auxiliary. A successful object of research can be called the species of the family *Caryophyllaceae,* not only in the study of endemics (Behroozian *et al.*, 2020b), and to better understand the overall impact of climatic conditions and human influence on the flora of regions with specific climatic conditions. In general, the high diversity of species in this family allows studying a wide range of morphological, ecological, and genetic variations. This helps to study the processes of evolution, adaptation, and speciation. In addition, species of this family are found in various ecosystems – from mountainous areas to steppes and deserts. This allows to study their adaptation to various climatic and environmental conditions and their interaction with the environment. Many representatives of *Caryophyllaceae* have relatively small genomes, which makes them convenient for molecular genetic research such as DNA sequencing and genetic mapping. Their genetic characteristics allow studying speciation processes, population genetics, and evolutionary history (Boxriker *et al.*, 2018). As indicated by G.A.W. Bauer *et al.* (2021), species of this family exhibit various morphological features, which allows studying phenotypic plasticity and the influence of various environmental factors on external features.

P. Sharma's *et al.* (2022) paper was considered an important literary source for analysis, as the molecular methods used, such as SSR markers, helped to investigate genetic diversity and phylogenetic relationships between species, which is relevant in the context of analysing the genetic variability of endemic species. Both studies used molecular genetic markers to assess the genetic diversity of species. The difference was that P. Sharma's *et al.* research focused on the cultural species of *Dianthus caryophyllus* and its mutant forms that emerged after radiation exposure. But the current study focused on natural populations of endemic species of the genus *Dianthus* in the natural environment, without the intervention of the mutation process. While improving breeding methods and species identification helps to prevent the extinction of entire plant and animal habitats, it is also important to improve conservation measures and monitor the overall situation of species (Honchar *et al.*, 2023). Great contribution to the study of adaptability and factors influencing distribution of *Dianthus* was made at the Latvian Institute of Biology using the example of *Dianthus superbus*, researchers have found that destructive human activity causes more damage to the population than the specifics of local soils (Osvalde *et al.*, 2021). Such results indicate the expediency of conducting lectures and activities to inform the population about the environmental situation in the region.

Heavy metals, which have become an integral part of environmental impact factors with the development of industrialisation, also have high risks for plant populations (Jalilova *et al.*, 2024). Thus, the K. Qiao *et al.* (2023) research group found that *Dianthus spiculifolius* had higher resistance to cadmium and lead

compared to five other species of the genus *Dianthus*, and it was able to accumulate more heavy metal ions in its shoots. Future studies of the endemic species of the genus *Dianthus* on the ability to accumulate heavy metal ions are relevant, as they can form an idea of their resistance to polluted environments and potential for phytoremediation. To assess the level of genetic diversity both between different populations and within the same population, molecular genetic analysis performed for each of the species is widely used in modern scientific practice. Molecular markers such as microsatellites and DNA sequencing help to identify genetic relationships between populations and assess their status in terms of genetic metabolism (Burdina & Priss, 2016). Genetic data allowed studying how individual populations of species *Dianthus* adapted to different environmental conditions, such as climate change or specific soil characteristics. This was important for understanding the ecological boundaries of species and determining their optimal habitat zones.

The study of six species of the genus *Dianthus* in the Nakhchivan Autonomous Republic provided important data on their geographical distribution, population status, influence of anthropogenic factors, and genetic structure. The species *Dianthus raddeanus*, *Dianthus orientalis*, *Dianthus parviflorus*, *Dianthus elegans*, *Dianthus nahcivanensis*, and *Dianthus nakhchivanicus* are important components of the region's flora, and their study helps not only to better understand local biodiversity, but also to develop strategies for their conservation.

CONCLUSIONS

In the course of the study, a comprehensive chorological analysis of the species of the genus *Dianthus* in the South Caucasus was carried out, with a particular focus on the flora of the Nakhchivan Autonomous Republic. The results obtained established the specific distribution areas of the species under study, their ecological and genetic characteristics, and determined the influence of anthropogenic factors on the state of their populations. This study found that each species has a well-defined ecological niche, depending on factors such as temperature, humidity, soil type, and the degree of anthropogenic impact.

The relevance of this study is to expand knowledge about the biogeography of species of the genus *Dianthus* and their distribution in the South Caucasus region. The study provided a more accurate understanding of species distribution patterns, their environmental preferences, genetic diversity, and potential risks associated with anthropogenic factors. Such knowledge can be used to develop a conservation strategy for rare and endemic species, and to manage biodiversity in the region, which is particularly important given climate change and increasing anthropogenic pressures. However, the study was subject to certain limitations that affected the results obtained. First, field studies were conducted only during two growing seasons, which could lead to limited understanding of seasonal population dynamics. Long-term observations can provide more data on changes in the abundance and distribution of species over different years. Second, the analysis of molecular genetic diversity was performed only based on randomly selected samples, which could limit the completeness of the genetic profile of populations. A wider selection of samples and the use of additional genetic markers can improve the accuracy of the results obtained. In addition, potential species habitats were modelled using a specific set of ecological parameters, while other factors, such as competition with other species or interaction with local fauna, were not taken into consideration. The use of additional biotic and abiotic parameters can improve the accuracy of distribution models.

Thus, this study has made an important contribution to understanding the ecological and genetic characteristics of species in the genus *Dianthus* in the South Caucasus. Despite some limitations, the results obtained are valuable for further research and preservation of the floral diversity of the region. Further research that addresses the above omissions could greatly expand knowledge about these species and contribute to their more effective conservation.

ACKNOWLEDGEMENTS

None.

CONFLICT OF INTEREST

None.

REFERENCES

[1] Bauer, G.A.W., Greimler, J., & Reich, D. (2021). Genetic structure and diversity in carnations of *Dianthus plumarius* subsp. *blandus* in the eastern Alps. *Flora*, 279, article number 151809. doi: 10.1016/j.flora.2021.151809.

[2] Behroozian, M., Ejtehadi, H., Memariani, F., Pierce, S., & Mesdaghi, M. (2020a). Are endemic species necessarily ecological specialists? Functional variability and niche differentiation of two threatened *Dianthus* species in the montane steppes of northeastern Iran. *Scientific Reports*, 10, article number 11774. doi: 10.1038/s41598-020-68618-7.

[3] Behroozian, M., Ejtehadi, H., Peterson, A.T., Memariani, F., & Mesdaghi, M. (2020b). Climate change influences on the potential distribution of *Dianthus polylepis* Bien. ex Boiss. (*Caryophyllaceae*), an endemic species in the Irano-Turanian region. *PLoS ONE*, 15(8), article number e0237527. doi: 10.1371/journal.pone.0237527.

[4] Beker, C., & Rączka, G. (2019). Nature protection in Georgia and Azerbaijan. *Forest Conservation Management*, XII, 1-1. doi: 10.5604/01.3001.0013.2810.

[5] Boxriker, M., Möhring, J., & Piepho, H.-P. (2018). Genetic and phenotypic correlation for breeding relevant traits in *Dianthus caryophyllus* L. *Postharvest Biology and Technology*, 143, 129-136. doi: 10.1016/j.postharvbio.2018.03.015.
[6] Burdina, I., & Priss, O. (2016). Effect of the substrate composition on yield and quality of basil (*Ocimum basilicum* L.). *Journal of Horticultural Research*, 24(2), 109-118. doi: 10.1515/johr-2016-0027.
[7] Celik, A.K., Usta, N.C., Baba, Y., Cimen, A., & Turker, A.U. (2024). Phenolic characterization, antimutagenic, antioxidant and antibacterial capacities of seven endemic *Dianthus* species from Turkey. *South African Journal of Botany*, 164, 39-49. doi: 10.1016/j.sajb.2023.11.032.
[8] Elizbarashvili, N., Sulkhanishvili, N., & Elizbarashvili, R. (2021). Main problems of the sustainable development of south Caucasus and processes of transformation of landscape (Ecosystem) biodiversity. In M. Öztürk, V. Altay & R. Efe (Eds.), *Biodiversity, conservation and sustainability in Asia* (pp. 339-354). Cham: Springer. doi: 10.1007/978-3-030-59928-7_12.
[9] Franzoni, J., Astuti, G., & Peruzzi, L. (2023). Weak genetic isolation and putative phenotypic selection in the wild carnation *Dianthus virgineus* (*Caryophyllaceae*). *Biology*, 12(10), article number 1355. doi: 10.3390/biology12101355.
[10] Gasimova, G. 2021. Diversity and distribution of amphibians in the Greater Caucasus Natural Area (Azerbaijan). *Biharean Biologist*, 15(2), 112-116.
[11] Glibovytska, N., Rashevska, H., Arkhypova, L., Adamenko, Ya., & Orfanova, M. (2024). Impact of electric power facilities on natural phytocenotic diversity. *Ukrainian Journal of Forest and Wood Science*, 15(2), 8-22. doi: 10.31548/forest/2.2024.08.
[12] Honchar, A., Tonkha, O., & Patyka, M. (2023). Peculiarities of Bacillus Subtilis strains influence on the development of *Triticum Aestivum* L. in inoculative cultures. *Plant and Soil Science*, 14(3), 35-46. doi: 10.31548/plant3.2023.35.
[13] Hou, P.-J., Lin, P., Lin, W., & Hsueh, T. (2023). Integrated traditional herbal medicine for recurrent urinary tract infection treatment and follow-up: A meta-analysis of randomized controlled trials. *Journal of Ethnopharmacology*, 321, article number 117491. doi: 10.1016/j.jep.2023.117491.
[14] Jalilova, G., Orozakunova, R., Baibagyshev, E., Karabaev, N., & Shergaziev, U. (2024). Farmers' adaptation to climate change in Southern Issyk-Kul. *Ekonomika APK*, 31(4), 23-32. doi: 10.32317/ekon.apk/4.2024.23.
[15] Kuljanishvili, T., Epitashvili, G., Freyhof, J., Japoshvili, B., Kalous, L., Levin, B., Mustafayev, N., Ibrahimov, S., Pipoyan, S., & Mumladze, L. (2021). The first unified inventory of non-native fishes of the South Caucasian countries, Armenia, Azerbaijan, and Georgia. *Journal of Applied Ichthyology*, 36(4), 501-514. doi: 10.1111/jai.14038.
[16] Lin, S., Liu, J., He, X., Wang, J., Wang, Z., Zhang, X., Bao, M., & Fu, X. (2022). Comprehensive comparative analysis and development of molecular markers for *Dianthus* species based on complete chloroplast genome sequences. *International Journal of Molecular Sciences*, 23(20), article number 12567. doi: 10.3390%2Fijms232012567.
[17] Marshall, C.A.M., Dabo, J., Mensah, M., Ekpe, P., Kpadehyea, J.T., Haba, O.O., Bilivogui, D., & Hawthorne, W.D. (2022). Predictors of plant endemism in two west African forest hotspots. *Frontiers in Ecology and Evolution*, 10, article number 980660. doi: 10.3389/fevo.2022.980660.
[18] Meng, D., Yang, L., Yunlin, Z., Guiyan, Y., Shuwen, C., & Zhenggang, X. (2023). Distinguish *Dianthus* species or varieties based on chloroplast genomes. *Open Life Sciences*, 18(1), article number 20220772. doi: 10.1515/biol-2022-0772.
[19] Mladenović, M.Z., Ristić, M.N., Bogdanović, A.I., Ristić, N.R., Boylan, F., & Radulović, N.S. (2023). Wax composition of Serbian *Dianthus* spp. (*Caryophyllaceae*): Identification of new metabolites and chemotaxonomic implications. *Plants*, 12(11), article number 2094. doi: 10.3390/plants12112094.
[20] National Center for Biotechnology Information. (2024). Retrieved from https://www.ncbi.nlm.nih.gov/.
[21] Nikolaishvili, D., & Dvalashvili, G. (2015). Anthropogenic changes of Caucasus Forest landscapes. *Earth Sciences*, 4(5-1), 54-59. doi: 10.11648/j.earth.s.2015040501.20.
[22] Noroozi, J., Talebi, A., Doostmohammadi, M., Manafzadeh, S., Asgarpour, Z., & Schneeweiss, G.M. (2019). Endemic diversity and distribution of the Iranian vascular flora across phytogeographical regions, biodiversity hotspots and areas of endemism. *Scientific Reports*, 9, article number 12991. doi: 10.1038/s41598-019-49417-1.
[23] Osvalde, A., Jakobsone, G., Akmane, I., Svilāns, A., & Dubova, I. (2021). *Dianthus superbus* as a critically endangered species in Latvia: Evaluation of its growth conditions and conservation possibilities. *AoB Plants*, 13(5), article number plab051. doi: 10.1093/aobpla/plab051.
[24] Qiao, K., Wang, Q., Liu, X., Gong, S., & Wang, J. (2023). Cadmium/lead tolerance of six Dianthus species and detoxification mechanism in *Dianthus spiculifolius*. *Chemosphere*, 312(Part 1), article number 137258. doi: 10.1016/j.chemosphere.2022.137258.

[25] Sharma, P., Nath, A.K., Dhiman, S.R., Dogra, S., & Sharma, V. (2022). Characterization of carnation (*Dianthus caryophyllus* L.) genotypes and gamma irradiated mutants using RAPD, ISSR and SSR markers. *South African Journal of Botany*, 148, 67-77. doi: 10.1016/j.sajb.2022.04.012.
[26] Terlević, A., Temunović, M., Bogdanović, S., Grgurev, M., Ljubičić, I., & Rešetnik, I. (2022). Morphological and environmental variability of *Dianthus sylvestris* (*Caryophyllaceae*) in the Balkan Peninsula. *Botanical Journal of the Linnean Society*, 201(3), 377-389. doi: 10.1093/botlinnean/boac058.
[27] Yusupova, U., Usmanov, D., Azamatov, A., Ramazonov, N., & Rejepov, J. (2021). Phytochemical constituents and biological activities of *Dianthus helenae* Vved., growing in Uzbekistan. *Natural Product Research*, 36(13), 3480-3484. doi: 10.1080/14786419.2020.1862834.
[28] Zubtsova, I., Penkovska, L., Skliar, V., & Skliar, I. (2019). Dimensional features of cenopopulations of some species of medicinal plants in the conditions of North-East Ukraine. *AgroLife Scientific Journal*, 8(2), 191-201.

Хорологічний аналіз видів роду *Dianthus* у Південнокавказькому регіоні з акцентом на флорі Нахічеванської Автономної Республіки

Ензала Новрузова
Кандидат біологічних наук
Нахічеванський державний університет
AZ7012, Університетське містечко, м. Нахічевань, Азербайджан
https://orcid.org/0009-0003-3547-6025

Анотація. Метою дослідження було проведення хорологічного аналізу шести видів роду *Dianthus* у Нахічеванській Автономній Республіці з використанням польових та молекулярно-генетичних методів. Основна увага була приділена їх географічному поширенню, популяційному статусу та генетичному різноманіттю. В результаті дослідження шести видів роду *Dianthus* були виявлені значні відмінності в їх географічному поширенні, екологічних нішах та адаптації до місцевих умов. Молекулярно-генетичний аналіз показав високий рівень генетичного різноманіття серед популяцій *Dianthus nahcivanensis* та *Dianthus nakhchivanicus*, що підтвердило їх унікальність та ендемічний статус. Також доведено, що рід *Dianthus* має високе видове різноманіття і демонструє значні варіації поширення залежно від географічних та кліматичних умов регіону. У дослідженні використано геоботанічні методи для визначення ареалів видів та аналізу факторів, що впливають на їх поширення. Зокрема, були виявлені особливості ареалу у зв›язку з локальними умовами Нахічеванської Автономної Республіки, до складу якого входить значна кількість ендемічних видів. Генетичний аналіз, проведений за допомогою методу полімеразної ланцюгової реакції та секвенування, дозволив виявити рівень генетичного різноманіття та встановити філогенетичні зв'язки між популяціями. Встановлено, що види *Dianthus raddeanus*, *Dianthus parviflorus*, *Dianthus nakhchivanicus* та *Dianthus nahcivanensis* є більш вразливими через вплив антропогенної діяльності, зокрема сільського господарства та випасання худоби, що призвело до скорочення площі їхніх ареалів. Моделювання з використанням методу максимальної ентропії показало потенційні зони розширення ареалів видів за сприятливих екологічних умов. Отримані результати є важливими для розуміння екологічних закономірностей поширення роду *Dianthus* в регіоні та розробки стратегій збереження біорізноманіття в умовах зміни клімату та антропогенного тиску. За результатами дослідження рекомендовано створити або розширити заповідні території, обмежити антропогенний вплив та проводити додатковий моніторинг чисельності та генетичної структури популяцій *Dianthus*

Ключові слова: ендеміки; ареал; генетичне різноманіття; антропогенний вплив; збереження біорізноманіття