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Phylogenetic analysis of the relict species *Dryopteris filix-mas* (L.) Schott. by the chloroplast gene (*rbcL*) and features of modern ontogenesis on the Mangistau Peninsula, Kazakhstan

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ABSTRACT

The article presents the study results of the populations of the relict species *Dryopteris filix-mas* (L.) Schott on the territory of the Tyubkaragan Peninsula in the Mangistau region, Kazakhstan. Studies have shown that this species is found among the blackberry-grass community's rocks, indicating its adaptation to the specific habitat conditions in this region. The work also presents the morphometric parameters of *D. filix-mas*, such as shoot length and average leaf size. The data obtained indicate that the studied populations are young and have a high self-renewal capacity. Analysis of the morphometric characteristics of generative individuals showed that the measured parameters are within the typical range for this species, which confirms its good condition and viability. Observations of the population were carried out for two years (2023-2024), which allows us to draw more substantiated conclusions about the dynamics of the *D. filix-mas* population. Currently, the population is in a satisfactory condition due to the high proportion of generative individuals and maximum density, a positive characteristic of sustainability. The population was found in shaded areas among rocks. Morphometric analysis showed that the length of the leaf blade in adult plants varied from 47 to 52 cm, while the number of spore-bearing leaves reached 4 per plant. The height of the plants ranged from 12 to 17 cm in young plants up to 57 cm in adults. The studied population consists mainly of generative individuals, contributing to its high self-renewability. Genetic analysis based on the chloroplast gene *rbcL* showed a high level of genetic variability, confirming the population's adaptation to specific conditions of the region. Nevertheless, the state of the population is assessed as threatened with extinction, which raises serious concerns. A factor contributing to this state is the dependence on the preservation of habitats and the sustainability of ecosystems in which this species grows. In this regard, the article recommends continued monitoring and control of the status of *D. filix-mas* populations to ensure their conservation and avoid potential extinction. These study results highlight the need for an active approach to protecting this relict species and the importance of further research to study the factors affecting the status of populations and the possibility of their restoration in the context of climate change and anthropogenic impact. The recommendation to implement sustainable methods of protection and monitoring will contribute to protecting *D. filix-mas* and maintaining biological diversity in the region.

Keywords: Plant, *Dryopteris filix-mas* (L.) Schott, Mangystau Peninsula, Ontogenetic structure, Morphometric parameters, Age composition, RBCL.
Article type: Research Article.

INTRODUCTION

Ferns are among the most ancient of the higher vascular plants, originating approximately 405 million years ago in the Devonian period of the Paleozoic era. In the late Paleozoic and early Mesozoic eras, tree ferns dominated vegetation formation on Earth. Although modern ferns retain considerable diversity, they remain among the most

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ancient perennials. One of the large genera is the genus *Dryopteris*, which includes many species distributed worldwide, especially in temperate zones. The most common species include *D. carthusiana*, widely found in Europe, North America and Asia, often on damp soils in forests and near water bodies. *D. cristata* in North America and Europe prefer marshy areas, moist forests, and shaded areas. *D. affinis* is found in Europe and Asia, mainly in shady and wet woods. *D. marginalis* grows in North America and prefers rocky soils and shady slopes. *D. filix-mas*, one of the best-known and most widespread species, is found in Europe, Asia, and North America; it prefers shady forests and damp places. These species of shield ferns are among the most common and are adapted to various climatic and environmental conditions (Kosakivska *et al.* 2016). Research on *D. filix-mas* has been conducted over several centuries, covering various aspects of its biology, genetics, and distribution. The species is a large plant with gracefully curved leaves, characteristic of many fern species, and a petiole structure with a tufted arrangement on the rhizome. Due to these features, *D. filix-mas* is easily recognisable in the natural environment. Scientists worldwide have significantly contributed to developing knowledge about this species, which has deepened our understanding of its evolutionary processes, ecological role and interactions with other organisms in ecosystems. Of particular interest are studies focusing on its adaptation to different climatic conditions and the mechanisms of its reproduction and dispersal, which allow the species to survive in various habitats (Lovis & Hick 2017). Modern research continues to this day, bringing new data on the genetic diversity of *D. filix-mas* and its ecosystem functions, such as its contribution to maintaining soil moisture and improving the structure of forest ecosystems. An important aspect is the conservation of biodiversity, which makes this species a subject of protection in some regions. As a result, the study of *D. filix-mas* deepens our understanding of this unique plant and contributes to developing effective strategies for its conservation and sustainable use in nature. Various botanists, biologists, and ecologists have studied *D. filix-mas*. The species was first described in the 18th and 19th centuries by Carl Linnaeus in 1753, who classified it in his work "Species Plantarum". The English botanist William Jackson Hooker (1846-1864) published "Species Filicum," describing several fern species. American botanist Asa Gray (1889) also included *D. filix-mas* in his "Manual of the Northern United States" (Schneider *et al.* 2005). Over the years, significant contributions to the study of the male fern (*D. filix-mas*) have been made by both foreign and Russian scientists. Among the foreign researchers, the American botanist Merritt Lindsay Fernald stands out, who studied the flora of North America, including ferns, in the 1950s. In 1960, Ralph McCarthy published research on the genus *Dryopteris* in the American Fern Journal, expanding knowledge of the ferns of the western United States (Shefferson & Roach 2001). In 1997, British botanist Christopher Nigel Page published the book “The Ferns of Britain and Ireland”, which became an essential source of the flora of these regions (Page, & Sheffield 2001). In 2000, Greer K. Greer and B. K. McCarthy studied the genetic diversity of *D. filix-mas*. In 2001, Barbara Jo Hoshizaki and Robbin K. Moran published the “Fern Grower's Manual”, providing a practical guide to growing ferns. In 2006, Alan R. Smith and colleagues proposed a new classification of modern fern species, which brought significant changes to the taxonomy of this group of plants (Cobb 1904; Barrington 1993; Marques *et al.* 2005). In Russia, there have also been many studies of the male fern. In the 19th century, Ivan Fyodorovich Schmalgauzen was one of the first to study ferns in Russia systematically (Shmalhausen 1866). At the beginning of the 20th century, Alexander Alfonsovich Fedchenko studied the flora of Siberia and Central Asia, including ferns, which allowed him to expand his understanding of the vegetation of these regions (Fedchenko 1910; Shishkin 1934-1964). In the 1930s and 1940s, Boris Konstantinovich Shishkin studied plant taxonomy, including ferns, which improved their classification. In the 1950s and 1960s, Elena Konstantinovna Gan actively studied the diversity of ferns in Russia, making important discoveries about their distribution (Shishkin 1934-1964). Modern Russian botanists continued these studies at the beginning of the 21st century. In the 2000s, Kozlov studied ferns, including *D. filix-mas*, in various regions of Russia (Yusupova 2022). In the 2010s, Anna Vladimirovna Chubarova studied ferns' ecological and geographic distribution, paying particular attention to their genetic diversity (Chubarova 2010). All these scientists made a significant contribution to the development of fern science, which allowed us to deepen our understanding of the biology, ecology, and distribution of the male fern in Russia and abroad. In Kazakhstan, *D. filix-mas* has been studied by various scientists over different periods. The beginning of the 21st century has seen an increase in publications devoted to studying *D. filix-mas* populations in multiple ecosystems of Kazakhstan. These works cover many aspects of the study of the male fern, from floristry and ecology to genetic features. The first description of this species, including the male fern, was presented in the multi-volume publication of N.V. Pavlov, "Flora of Kazakhstan" (Volumes 1-5) in 1956-1966, which contains information about ferns growing in the territory of Kazakhstan (Pavlov 1956-1966). Scientific research conducted by the famous scientist Rusanov in 1926 revealed two species of ferns in the flora of Mangistau: *Cystopteris fragilis* and *D. filix-mas* at the foot of the Bugurstan ravine, located in the northwest of Ustyurt (Catalog of rare and endangered plant species of the Mangistau region). However, the data presented needed to be more comprehensive to form a full-fledged scientific base (Ivanov 2015). For example, Sagandykov M.K., in his article "Flora and Vegetation of Northern Kazakhstan" (2002), included ferns in the study of the flora of this territory. Nurmukhamedova K.T., in her work "Ecological and Biological Features of Ferns in the Foothills of Alatau" (2008), highlighted the ecological and biological characteristics of ferns growing in the foothills of Alatau (Sagandykov 2002; Nurmukhamedova 2008). In the article by Aitbaev Zh.K., "Genetic diversity of ferns of the genus *Dryopteris* in the mountainous regions of Kazakhstan" (2011), a study was conducted on the genetic diversity of ferns of this genus, including *D. filix-mas*, in the mountainous regions of the country. Zhumabaeva A.T., in the article "Study of populations of *D. filix-mas* in various ecosystems of Kazakhstan" (2015), described populations of *D. filix-mas* in different ecosystems of the republic (Aitbaeva 2011). In 2023, during an expedition to study the flora of the Tyubkaragan Peninsula in the Mangistau Lowland, new habitats of the male shield fern were discovered for the first time in the area of Cape Zhygylgan. These findings were recorded in two shaded regions of 10-12 m^2 between stone boulders 10-15 m high. This discovery is of great scientific importance since the male shield fern is a rare relict species listed in the "Red Book of Kazakhstan" (2014) and is endangered. *D. filix-mas* is found in the Tyubkaragan-Gornomangyshlak district, which includes Gorny Mangyshlak and the Tyubkaragan Peninsula. Cape Zhygylgan is located on the Tyubkaragan Peninsula. This geobotanical region, which has a marine border, is characterized by a highly dissected relief and includes isolated massifs of Permian and Triassic rocks. The soil-forming materials are represented by Jurassic and Cretaceous deposits. The rocks of this region are also home to rare and endangered species listed in the Red Book of Kazakhstan and the Mangistau region, such as *Morus alba* and *Crataegus ambigua*. Genetic studies of *D. filix-mas* also include an analysis of the genetic diversity of this species in the various ecosystems where it occurs, which is quite rare. Analyses performed using SSR markers and other molecular methods (Red Book of Kazakhstan 2014; Zhumabaeva 2015; Imanbayeva *et al.* 2024) showed a high level of genetic variability, indicating the possibility of resistance to changing environmental conditions. In particular, the works of Greer and McCarthy (2000) showed that populations of *D. filix-mas* have a significant genetic structure, which is essential for planning measures to preserve this relict species. The phylogenetic position of *D. filix-mas* has been clarified by molecular studies, including analyses of the chloroplast gene *rbcL*, which have shown its close relationship to other species of the genus *Dryopteris*. Molecular phylogenies based on the sequences of various genes, such as *rbcL*, allow for a more precise determination of the phylogenetic relationships within the family Dryopteridaceae. These studies have deepened our understanding of the evolutionary relationships among ferns and have led to a revision of the taxonomy of the genus *Dryopteris* (Orazov *et al.* 2024). Further studies highlight the importance of *D. filix-mas* as a species that plays a crucial role in maintaining biodiversity. Studying its evolutionary and ecological characteristics and ability to adapt to climate change is also essential. The importance of *D. filix-mas* lies in its status as a rare relict species, which not only adds uniqueness to the vegetation cover of the region, but also serves as an indicator of the ecosystem's overall health. Its presence signals specific habitat conditions and biodiversity levels and provides valuable information on the ability of flora to adapt to changing climate conditions. *D. filix-mas* conservation is critical in maintaining ecological balance and preserving biological diversity in Kazakhstan. This species' high sensitivity to environmental changes makes it an essential object for monitoring the state of natural ecosystems. This brings to the discussion the need to protect its natural habitats and support programs to restore threatened populations. Implementing effective conservation strategies will contribute to the conservation of this species and maintain the health and sustainability of the ecosystems in which it lives (Koblanova *et al.* 2024).

MATERIALS AND METHODS

Study area

The study area is located in the western part of Kazakhstan near the Caspian Sea in the Mangyshlak region, including the Tyubkaragan Peninsula. The natural conditions of Mangyshlak form a desert type of vegetation, where xerophytes make up 65.8% of the total floristic diversity, numbering 679 species belonging to 63 families and 284 genera. More than half of the flora of the region is five family's characteristics of the Iran-Turan desert subzone of the Sahara-Gobi region: Chenopodiaceae - 91 species (14.6%), Asteraceae - 78 species (12.5%), Brassicaceae - 62 species (10.0%), Poaceae - 60 species. Additionally, five joint families such as Boraginaceae, Caryophyllaceae, Scrophulariaceae, Polygonaceae, and Lamiaceae, are typical of desert areas and, together with the previous ones, make up 70.9% of the flora of the region. The largest genera are *Astragalus* (19 species), *Artemisia* (18 species), and *Salsola* (15 species), which play a vital role in the formation of the plant landscape and act as edificators, dominants and codominants within plant communities, among which the genera *Anabasis*, *Artemisia*, *Agropyron* and *Astragalus* can be distinguished. The "Red Book of Kazakhstan" and the "Catalogue of Rare and Endangered Plant Species of the Mangistau Region" include 40 species found in the arid conditions of the region, including *Dryopteris filix-mas* and *Cystopteris fragilis*, which are classified as extremely rare and relict for Mangistau. *D. filix-mas* populations are located on the territory of Cape Zhygylgan, an impressive mountain range with a massive depression of an almost perfect round shape, surrounded by stones. This place, known to travelers as the "Gap," differs from other capes on the Caspian Sea coast by rocky slopes strewn with stone fragments, some of which are the size of a small house. The arrangement of the stones looks so chaotic that it creates the impression of a recent earthquake. The maximum height of the cape is 200 m, and the diameter of the depression reaches 4 km. Significant areas here are occupied by ridges with eroded slopes, where rare plant communities of subshrubs and shrubs grow, such as *Rhamnus sintenisii, Atraphaxis replicata, Caragana grandiflora, Convolvulus fruticosus* and *Astragalus turcomanicus*. On flat tops with rocky soils grow ephemerides (*Catabrosella humilis*, *Poa bulbosa*) and wormwoods (*Artemisia lerchiana*, *A. gurganica*). Also, here are perennial grasses (*Stipa caspia*, *Agropyron fragile*) and various prophetic plants (*Galium humifusum*, *Haplophyllum obtusifolium*, *Inula multicaulis*, etc.). On the gravelly slopes, the vegetation is denser, with a predominance of wormwoods and the participation of grasses and ephemeroids. In the lower parts of the slopes are distributed communities of tasbiyurgun and wormwood (*Artemisia terrae-albae,* and *A. gurganica*). The ridges are separated by dry rocky riverbeds, where you can find caragana-gurgan-wormwood communities, as well as various herbaceous perennials and annuals (*Allium albanum*, *A. delicatulum*, *Arenaria serpyllifolia*, *Medicago caerulea, Meristotropis triphylla, Onopordum acanthium, Cardaria draba, Chorispora tenella, Convolvulus arvensis, Lappula spinocarpos,* and *Lycopsis orientalis*).

Floristic methods

The study of plant populations on the territory of the Tyubkaragan Peninsula in the Mangistau Region was carried out by the route method during the vegetation period in the first half of July 2023-2024. The plant community was described by generally accepted geobotanical methods. To study the ontomorphological features of the species and the current state of populations, generally accepted methods and approaches developed in the framework of previous studies were used (Robotnov 1950; Uranov & Serebryakova 1976; Denisova *et al.* 1986; Zhivotovsky 2001; Zhukova 2013). During the study of individuals of various ontogenetic states, measurements of such morphometric parameters as shoot height, number of leaves on a shoot, leaf length and width, and other characteristics were carried out. Measurements were carried out selectively since the spread of morphometric data in plants was insignificant. Morphological descriptions of plants belonging to different ontogenetic states were made using the terminology, and approaches reflected in the works of P.Yu. Zhmylev and other researchers. It is important to note that understanding the morphology and ontomorphology of *D. filix-mas* is of crucial importance for studying its adaptations to environmental conditions, as well as for developing methods for its protection and conservation in the context of climate change and anthropogenic impact (Uranov & Serebryakova 1976; Red Book of Kazakhstan 1981; Aralbay *et al.* 2006; Zhukova & Polyanskaya 2013). The collected herbarium material was transferred to the Herbarium Fund of the Mangistau Experimental Botanical Garden for further research and conservation.

Genetic methods

Fresh leaves of *D. filix-mas* plants from the four populations studied were used for DNA extraction. Ten reference plant samples were selected from each population for each marker to study genetic diversity. Total DNA was isolated from ground leaf powder using the cetyltrimethylammonium bromide (CTAB) protocol with double chloroform purification (Maniatis *et al.* 1982). DNA quality and concentration were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and 1% agarose gel electrophoresis. The DNA concentration was normalized to the working concentration for further analysis. The buffers were prepared according to existing guidelines (Rogers & Bendich 1985). To isolate DNA, a method using cetyltrimethylammonium bromide was used (Sanger 1997). DNA sequencing was performed using the Sanger method using a BigDye™ Terminator v 3.1 Cycle Sequencing Kit (BLAST 2024). The data on the primary structure of the DNA fragments under study were analyzed through the National Center for Biotechnology Information (NCBI) genetic information database using the BLAST+ 2.15.0 (Basic Local Alignment Search Tool) program (Klein *et al.* 1994). For the barcoding of the plants selected for the study, we chose DNA sequences of the chloroplast genome, such as *rbcL* (Tamura *et al.* 2011). Table 1 shows the sequence of the primer used in the study.

Table 1. Description of genetic primers *rbcL* used in studying *Dryopteris filix-mas* plant populations.

Name	Sequence 5′–3′	Locus for Barcode
rbcLa_F	ATGTCACCACAAACAGAGACTAAAGC	*rbcL*
rbcLa_R	GTAAAATCAAGTCCACCRCG	*rbcL*

The obtained DNA sequences were edited, and adjacent sequences (from 193 to 570 bp) were assembled using MEGA version 5; the obtained DNA sequence information was then used for fundamental evolutionary analysis, determination of evolutionary distances and phylogenetic reconstructions by UPGMA and NJ. The calculated statistical description of nucleotides of each group of *D. filix-mas* sequences allows us to estimate the nucleotide diversity in each population. The index considers both richness (the number of unique nucleotides) and the uniformity of their distribution.

RESULTS

Floristic and botanical analysis

The study of the peninsula revealed valuable findings of *Dryopteris filix-mas* populations. This species is characterised by pinnately divided leaves surrounded by scaly residual covers. The leaves are richly green and can reach significant sizes, which gives the plant aesthetic appeal. Dense, pinnate leaves characterise the common fern and reach a height of 50 to 90 cm. Sporangia, responsible for reproduction, is located on the underside of the leaves in the form of brown round dots. The *D. filix-mas* population prefers moist, shaded microecosystems with well-drained soils containing moderate nutrients. In its natural range, this species can contribute to the biodiversity of ecosystems, providing shelter and food resources for various species of fauna. *D. filix-mas* exhibits several adaptations that enable it to thrive in multiple ecosystems. These adaptations include regenerating after injury and resistance to pathogens and pests, significantly increasing its chances of survival in the wild. As a result of expeditionary research conducted on the Tupkaragan Peninsula in the Mangistau Region, the ontogenesis of this species was studied. The material for the study was collected in the area of the Zhygylgan failure, with the specified coordinates N 44037/352//, E. 50049/220//, and an altitude of 902 meters above sea level. In the community, four sprouts are noted, connected with the gametophyte. It consists of a microscopic rhizome and embryonic roots. Juvenile sporophyte (j) is not associated with the gametophyte. The aboveground stage numbers 18 pcs. The height is 11 cm, and the width is 3.5 cm; immature sporophyte (im). The top of the front has a pointed shape. The absolute age of plants is approximately five years. The height of the plants varies from 12 to 17 cm, and the width from 5 to 9 cm. The population of *D. filix-mas* was found among the rocks in a fern-blackberry community. The projective cover is 75%. The community also contains *Nepeta cataria* L. and *Verbacum songarica* Schrenk (Fig. 1). This discovery contributes to the study of the ontogenesis of this species and its interaction with the environment. It was revealed that the ontogenesis of *D. filix-mas* includes 11 age states, the characteristics of which are reflected in Table 1. The rhizome consists of dead fronds fused, each of which is an annual increment. When studying the populations of the rarest in the desert zone. *D. filix-mas*, in field conditions, only the features associated with the above-ground part of the plant were used so as not to harm the entire population. Such features include the ratio of the length and width of the "leaf blade" of the frond and, in some cases, the presence of spores. To go through all the stages of the significant life cycle, the fern requires climatic conditions corresponding to its ecological amplitude, which is extremely rare in the desert zone. The height of the plants, on average, fluctuates between 22 and 65 cm. The diameter is from 67 to 85 cm. The leaf blade is light green, oblong-elliptical in outline, twice pinnately dissected. The leaf petiole is densely covered with rusty-brown scales. The number of adult leaves on individuals was from 5 to 8 pieces. The number of young leaves in the areas was five fronds per individual.


Fig. 1. *Nepeta cataria* L., *Verbacum songarica* Schrenk.

Table 1. Some characteristics of the identified age states of *Dryopteris filix-mas.*

Age status	Absolute age (years)	Number of adventitious roots	Rhizome length (mm)	Rhizome thickness(mm)	Plant height (mm)	Diameter (mm)	Length of leaf blade (mm)	Leaf blade width (mm)	Leaflet length (avg.; mm)	Leaflet width (avg.; mm)	Presence of a spore-bearing leaf
im	Up to 5 years	Up to 7	Up to 3	up to 1.2-1.7	6	1.5	2.1	1.6	1.1	0.4	-
j	5-7	Up to 9	Up to 5	1.7-2	7.5	3.5	4	2.4	1.5	0.8	-
v	7-9	Up to 12	Up to 7	up to 2.5	12	21	29	14	7	1.7	-
Sp1	7-10	Up to 13	Up to 9	up to 3.5	53	44	47-52	24	12	2.1	4
Sp2	15-17	Up to 15	Up to 12	up to 4	69	85	68-82	37	21	4	2

Thus, four sprouts are noted, which are connected with the gametophyte. It consists of a microscopic rhizome and embryonic roots. Juvenile sporophyte (j) has no connection with the gametophyte. Above-ground stage, numbers 18 pcs—height 11 cm, width 3.5 cm—aboveground plant. The top of the front has a pointed shape. The absolute age of plants reaches approximately five years. Plant height from 12 to 17 cm, width from 5-9 cm.

Fig. 2. Immature sporophyte (im).

The virginile sporophyte (v) is also characterised by the pointed shape of the frond apex. The height of virginile plants of *D. filix-mas* varies from 11 to 38 cm, providing information about the growth and development of this species—middle-aged spore-bearing sporophyte (Sp1), which has spores. The age of such plants is from 16 to 18 years. The height of middle-aged sporophytes varies from 33 to 57 cm; the diameter is 87 cm. The length of the leaf is approximately 47-52 cm, and its width is 37 cm. The size of the leaf petiole is 15 cm, and the number of spore-bearing leaves in these plants is four pcs.

Fig. 3. Age states of *Dryopteris filix-mas.*

In these desert conditions, *D. filix-mas* does not form senile and sub-senile individuals, and mass drying of the plant occurs in the Sp2 state. This coenopopulation was formed less than 30 years ago and has not yet reached a mature state. The nature of the spectrum may be due to the survival and development of spores in a particular community. These data on the structure and characteristics of different stages of development of *D. filix-ma*s help to understand its biological features and the reasons for changes in its growth and development. However, when studying the populations of the rarest in the desert zone in the field, only the features associated with the above-ground part of the plant can be used so as not to damage the entire population. Such features include the ratio of the length and width of the "leaf blade" of the frond and, in some cases, the presence of spores. Further research will clarify the limits of variation of the features studied.

Fig. 4. Middle-aged spore-bearing sporophyte (Sp1).

Fig. 5. Leaves with spores.

Results of genetic analysis

Genetic studies of the *rbcL* gene resulted in the sequences of several species of ferns of the genus *Dryopteris* being obtained and analysed. In particular, the sequence of the studied sample under PP978677 (*D. filix-mas*) was uploaded to the international database. The five most common species on our planet were selected for phylogenetic analyses, including the sample we are studying: *D. filix-mas* is one of the most famous and widespread species. It is found in Europe, Asia, and North America. It prefers shady forests and damp places. *D. carthusiana* (Carthusian shield fern) is widespread in Europe, North America, and Asia and is often found on moist soils in forests and the banks of reservoirs. *D. cristata* grows in marshy places, preferring damp forests and shaded areas. *D. affinis* (Related shield fern) is a species found in Europe and Asia, mainly in shady and wet woods. *D. marginalis* (Marginal shield fern) - grows in North America. It prefers rocky soils and shady slopes. Below is a summary table of nucleotide frequencies (T, C, A, G) for five fern species, including the *D. filix-mas* sample under study. The descriptive results of the obtained sequences are presented in Table 2. Several conclusions can be drawn from the table:

Similarity between species: All the *Dryopteris* fern species examined have similar nucleotide (T, C, A, G) percentages in their *rbcL* gene sequences. The percentage differences in nucleotide pairs are minor, indicating their close genetic relationship. For example, the nucleotide percentages of *D. filix-mas* (PP978677) and *D. affinis* are very similar, confirming their phylogenetic relationship.

Nucleotide variations: Although the average nucleotide percentages are relatively stable among all samples (e.g., average values for T: 25.64%, C: 23.96%, A: 26.08%, G: 24.32%), minor deviations are observed among individual species. *D. marginalis* has the most pronounced deviation in C content (23%), which may indicate specific evolutionary adaptations or mutations.

Conserved positions: At positions T-1, C-1, A-1 and G-1 (the first positions in the sequence), the nucleotide percentages are pretty similar between species, suggesting the presence of conserved regions in the *rbcL* gene that may be important for maintaining the functionality of the protein.

Sample under study (*D. filix-mas*): The sequence uploaded under number PP978677 (*D. filix-mas*) has a nucleotide distribution that is close to the average of all samples, confirming its typical position among other *Dryopteris* species.

Table 2. Statistical description.

Sequences	T(U)	C	A	G	Total	T-1	C-1	A-1	G-1	Post #1	T-2	C-2	A-2	G-2	Post #2	T-3	C-3	A-3	G-3	Post #3
KF186503.1 *Dryopteris cristata*	25.2	24.4	26.4	24	500	24.5508982	27.5449102	28.742515	19.1616766	167	29.9401198	28.1437126	25.748503	16.1676647	167	21.0843373	17.4698795	24.6987952	36.746988	166
AY268849.1 *Dryopteris affinis*	25.6	24	25.8	24.6	500	23.9520958	28.1437126	28.742515	19.1616766	167	32.3353293	25.748503	23.9520958	17.9640719	167	20.4819277	18.0722892	24.6987952	36.746988	166
EF463181.1 *Dryopteris marginalis*	26.6	23	26	24.4	500	23.9520958	28.1437126	28.742515	19.1616766	167	5.3293413	22.754491	24.5508982	17.3652695	167	20.4819277	18.0722892	24.6987952	36.746988	166
KF186505.1 *Dryopteris carthusiana*	25.2	24.4	26.4	24	500	24.5508982	27.5449102	28.742515	19.1616766	167	29.9401198	28.1437126	25.748503	16.1676647	167	21.0843373	17.4698795	24.6987952	36.746988	166
PP978677.1 *Dryopteris filix-mas*	25.6	24	25.8	24.6	500	23.9520958	28.1437126	28.742515	19.1616766	167	32.3353293	25.748503	23.9520958	17.9640719	167	20.4819277	18.0722892	24.6987952	36.746988	166
Avg.	25.64	3.96	26.08	24.32	500	24.1916168	27.9041916	28.742515	19.1616766	167	31.9760479	26.1077844	24.7904192	17.1257485	167	20.7228916	17.8313253	24.6987952	36.746988	166

This also indicates that this species is genetically close to other common fern species.

Phylogenetic relationships: Based on the comparative analysis, it can be assumed that the species *D. filix-mas*, *D. affinis* and *D. carthusiana* have the closest genetic relationships since their percentage distribution of nucleotides is maximally similar. *D. marginalis*, judging by minor deviations in the percentage content of nucleotides, may stand further on the phylogenetic tree. Thus, the data in the table confirm the close genetic relationship of the studied fern species of the *Dryopteris* genus and help to determine their evolutionary relationships. These relationships are pressed in the form of a phylogenetic tree in Fig. 6. Based on the phylogenetic trees constructed by the UPGMA and NJ methods, it can be concluded that both methods confirm close evolutionary relationships between the species of the genus *Dryopteris*. In particular, the species *D. filix-mas* and *D. affinis* form a close clade, indicating their genetic similarity and close evolutionary relationship. The species *D. cristata* and *D. carthusiana* are also grouped, reflecting their common origin. *D. marginalis* occupies a more isolated position in both phylogenetic schemes, indicating its greater genetic distance from the other species. The difference between the UPGMA and NJ trees is in the length of the branches: in the NJ method, they are longer, which reflects more accurate genetic distances between the species. In general, both methods confirm the reliability of the data and demonstrate the evolutionary relationships of the studied fern species. Both methods confirm that *D. filix-mas* and *D. affinis* are genetically the most closely related species. *D. cristata* and *D. carthusiana* also shows close relationships, while *D. marginalis* is more distant in the phylogenetic tree.

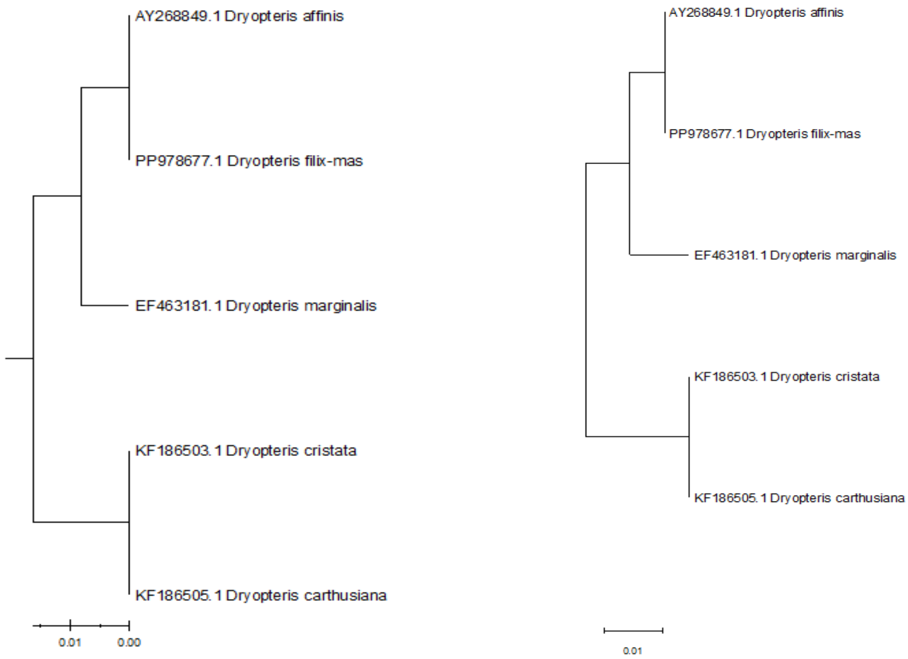

Fig. 6. Phylogenetic tree exhibiting genetic relationship of the studied fern species of the *Dryopteris* genus to determine their evolutionary relationships using UPGMA and NJ.

CONCLUSION

Dryopteris filix-mas was discovered on the Tyubkaragan Peninsula in the Mangistau Region. This species lives among rocks, which indicates its adaptation to specific habitat conditions. The study showed that the population is young but has a high capacity for self-renewal. As a result of our population studies, an age spectrum of the *D. filix-mas* population was constructed. Based on the data obtained, it can be concluded that this population belongs to the type of normal young cenopopulations. *D. filix-mas*, although a widespread plant, is still of scientific interest in the context of its morphology, anatomy, ecology and potential medical use. Due to its ability to adapt to various environmental conditions, this species of ferns contributes to preserving biodiversity and sustaining ecosystems. The state of the community in the specified climatic conditions is assessed as favourable, but there is a threat from the anthropogenic factor. To protect and preserve the *D. filix-mas* population, it is essential to protect it from human impact and create a natural monument. Studying the ontogenesis of ferns helps to reveal the mechanisms of species adaptation to specific habitat conditions, identify its survival strategy and tactics, and assess the sustainability of the population in this phytocenosis. This will allow us to understand better how the plant interacts with the environment, what adaptive mechanisms it uses to survive in difficult conditions, and what changes may affect its conservation in the future. All these aspects are essential for developing effective strategies for preserving and managing biodiversity in this ecosystem. The threat is the anthropogenic factor. It is necessary to protect it from penetration and monitor it. The study of morphometric characteristics (length of shoots, leaf size, etc.) showed that the parameters are within the typical range for this species. This confirms the excellent condition of the population. Population studies over two years (2023-2024) have established that the population consists mainly of generative individuals. This is a positive factor for the sustainability of the species. However, the absence of mature or old plants indicates the youth of the population, which may be less than 30 years old. Genetic analysis using the chloroplast gene *rbcL* showed a high level of genetic variability, which may indicate good adaptation of the population to changing environmental conditions. In phylogenetic analysis, *D. filix-mas* showed a close relationship with other species of *Dryopteris*: *D. filix-mas*, *D. affinis* and *D. carthusiana*, have the closest genetic relationships since their percentage distribution of nucleotides is most similar. Despite the satisfactory state of the population, species extinction is threatened due to anthropogenic factors and dependence on the preservation of habitats. It was recommended to continue monitoring and protection of this relict species. To preserve the population of *D. filix-mas* regarding climate change and anthropogenic impact, it is necessary to protect the habitats from human impact and continue monitoring. It is also recommended that conservation methods be actively implemented to preserve the region's biological diversity. Thus, the description of the ontogenesis of the fern allows us to reveal the mechanisms of adaptation of the species to specific habitat conditions, its strategy and survival tactics, and to assess the stability of the coenopopulation in this phytocenosis.

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