

**BRYOPHYTE FLORA OF THE KARKARALY REGION (KARAGANDY REGION, KAZAKHSTAN):  
SPECIES COMPOSITION AND HABITAT DISTRIBUTION****Rabiga Uakhit<sup>1</sup>, Nurassyl Manapov<sup>1,2</sup>, Aldina Nasredin<sup>2</sup>, Abai Alash<sup>1,2</sup>, Ainura Smagulova<sup>2</sup>, Ikimat Taiwo<sup>3</sup>, Sara Bekkuzhina<sup>1,3</sup>, Vladimir Kiyan<sup>1</sup>**<sup>1</sup>Laboratory of Biodiversity and Genetic Resources, LLP “National Center of Biotechnology”, Astana, Kazakhstan;<sup>2</sup>Department of Biotechnology and Microbiology, L.N. Gumilyov Eurasian National University, Astana, Kazakhstan;<sup>3</sup>Department of Microbiology and Biotechnology, S. Seifullin Kazakh Agrotechnical Research University, Astana, Kazakhstan;

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**ABSTRACT**

Bryophytes represent an important yet insufficiently studied component of terrestrial biodiversity in Central Kazakhstan. This study investigates the species composition and phylogenetic relationships of mosses in Karkaraly National Park (Karaganda Region), a forest–steppe enclave within the arid landscapes of the Kazakh Uplands. A total of 29 moss samples were collected and analyzed using an integrative approach combining microscopic morphological examination and molecular identification based on nuclear ribosomal *ITS1* and *ITS2* markers. Nine species belonging to seven families and four orders were identified, including *Ceratodon purpureus*, *Syntrichia ruralis*, *Dicranum muehlenbeckii*, *Dicranum schljakovii*, *Polytrichum juniperinum*, *Polytrichum piliferum*, *Sanionia uncinata*, *Pylaisia polyantha* and *Abietinella abietina*. Phylogenetic analysis using the Maximum Likelihood method confirmed species-level identification and revealed consistent clustering of samples within their respective genera. The detected assemblage reflects the ecological heterogeneity of the Karkaraly Mountains, where microclimatic variation supports both xerophytic and mesophilous moss species. This study represents one of the first integrative analyses combining morphological and molecular (*ITS1* and *ITS2*) approaches to identify mosses in Central Kazakhstan. The results provide novel baseline data on species composition and phylogenetic relationships of bryophytes in the Karkaraly region and highlight its role as a refugium for both xerophytic and mesophilous taxa within a semi-arid landscape.

**Keywords:** bryophytes, moss diversity, Karkaraly National Park, *ITS* phylogeny, molecular identification, Kazakhstan.**1. INTRODUCTION**

Bryophytes (mosses, liverworts, and hornworts) are among the earliest land plant lineages [1] and constitute an essential component of terrestrial biodiversity [2, 3]. Despite their small size, bryophytes play key ecological roles by regulating soil moisture, stabilizing substrates, mediating nutrient cycling, and forming microhabitats for microorganisms and invertebrates [4]. Their distribution is strongly governed by microclimatic conditions, substrate type, and moisture availability, making them sensitive indicators of environmental heterogeneity and landscape history [5].

Kazakhstan occupies a central position in Eurasia and encompasses a wide range of ecosystems, including steppe, semi-desert, montane forests, and wetlands. Bryophyte diversity within the country reflects this environmental heterogeneity, but it remains insufficiently studied compared to that of vascular plants. The foundational synthesis of bryophytes in the region is provided by the monograph Bryophytes of Middle Asia and Kazakhstan [6], which remains the principal taxonomic reference for the national bryoflora. Subsequent contributions have expanded the known species list through targeted floristic surveys and the documentation of new national and regional records, indicating that bryophyte diversity in Kazakhstan remains incompletely inventoried [6, 7].

Central Kazakhstan (Saryarka, Kazakh Uplands) is dominated by steppe landscapes characterized by arid to semi-arid climatic conditions and strong continentality. Of particular importance is the presence of isolated wetland and seepage habitats in the Karkaraly Mountains, including a relict Sphagnum site reported as one of the southernmost occurrences of sphagnum-dominated communities in Kazakhstan [8–10]. These habitats function as refugia for moisture-depend-

ent bryophyte taxa that are otherwise rare or absent in the surrounding steppe-dominated landscapes [11]. Such refugial systems are of high biogeographical value, as they may preserve relict populations and contribute to regional bryophyte diversity under ongoing climatic aridization.

Karkaraly State National Nature Park is located in Central Kazakhstan, in the eastern Kazakh Uplands, in the Karaganda Region, in the Karkaraly District. The total area of Karkaraly Park is 112,120 hectares and encompasses the Karkaraly and Kent mountain forest massifs. The massif is characterized by the isolation of its mountain groups and its landscape asymmetry, with the northern mountains steeper and more heavily vegetated. The terrain is a system of ridges that form a network of rocky crests and peaks, separated by deep gorges, intermontane valleys, and gently rolling plains [12].

Central Kazakhstan (Saryarka, Kazakh Uplands) is characterized by a strongly continental climate and predominantly steppe landscapes, where bryophyte communities are highly patchy and largely restricted to favorable microhabitats. The average annual temperature for 2010–2025 is +3.4°C, with average air temperatures of -12.6°C in January and +18.7°C in July. Average annual precipitation from 2010 to 2025 is 310 mm [13]. Average wind speed is 1.4 m/s, with a predominantly westerly direction. In open steppe environments, xerophytic and desiccation-tolerant terricolous mosses such as *Syntrichia*, *Tortula*, *Pterygoneurum*, *Bryum*, and *Didymodon* [14, 15] dominate exposed soils and biological soil crusts, while granite outcrops support saxicolous taxa including *Grimmia*, *Schistidium*, and *Hedwigia* [15, 16]. The Karkaraly Mountains (Karagandy Region), functioning as a forest–steppe enclave within this arid matrix, increase habitat heterogeneity by providing shaded forest floors, tree bark, de-

caying wood, and localized seepage zones that host mesophilous and epiphytic species such as *Hypnum*, *Brachythecium*, *Plagiomnium*, and *Orthotrichum*, as well as moisture-dependent genera including *Sphagnum* and *Aulacomnium* [17-19]. This gradient from xerophytic steppe assemblages to forest and hygrophilous communities underscores the biogeographical significance of Karkaraly as a regional refugium for bryophyte diversity in Central Kazakhstan [20-22].

The present study aims to comprehensively characterize the moss flora of Karkaraly National Park through integrative morphological (microscopic) and molecular identification, in order to clarify species composition, distribution patterns, and the ecological significance of bryophytes within this forest-steppe ecosystem.

## 2. MATERIALS AND METHODS

### 2.1 Mosses Collection Method

The material for the study was collected in the Karkaraly National Park in the Karaganda region (Figure 1, Table 1), at elevations of 800-900 m above sea level. Moss samples (29 samples) were collected manually to avoid mechanical damage to the plants and minimize contamination by soil particles and foreign plant debris. For each sample, the aboveground moss layer, including rhizoids, was collected, and excess substrate was removed. Samples were placed in individual paper envelopes labelled with the sample number, date, and collection location. During collection, location coordinates and

substrate type (soil, rock, wood) were recorded. Prior to collection, samples were stored in a dry, shaded place at room temperature or at 4°C.

### 2.2 Microscopic examination

Prior to microscopic analysis, dried material was rehydrated in distilled water for 5–10 minutes to restore leaf expansion and cellular structure. Macromorphological characters were examined using a light microscope ( $\times 10$ – $\times 40$  magnification), including growth form (tufts, cushions, mats), branching pattern, leaf arrangement, coloration, and presence or absence of sporophytes [4]. For detailed anatomical study, leaf fragments were mounted in distilled water or glycerol and examined under a light microscope ( $\times 100$ – $\times 400$  magnification). The following diagnostic characters were analyzed: leaf shape and apex morphology; margin characteristics (entire, dentate, recurved); costa structure and length; laminal cell shape, size, and wall thickness; presence of papillae; differentiation of basal versus median cells; and, where present, sporophyte features such as capsule morphology, peristome structure, and seta characteristics. In selected cases, transverse sections of leaves were prepared manually using a razor blade to assess costa anatomy and internal cell stratification.

Morphological identification was carried out using Bryophyte Portal – Online Identification Tools (<https://bryophyte-portal.org/portal/taxa/index.php>). Nomenclature was verified using Tropicos (Missouri Botanical Garden) (<https://www.tropicos.org/home>) and World Flora Online databases (<https://www.worldfloraonline.org/>).

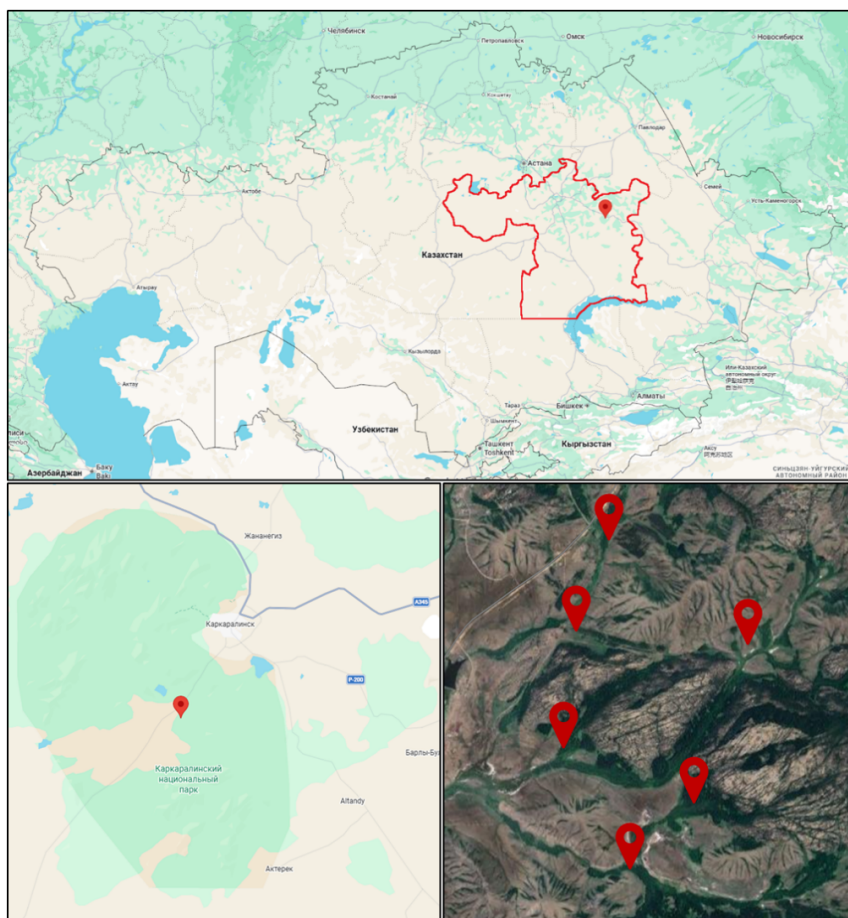


Figure 1 - Map of Kazakhstan with a highlighted collection point. Karaganda region, Karkaraly, Karkaraly National Park. (49.29981, 75.43117)

Table 1. Locations where the moss specimens were collected

№	Location	Vegetation	Mosses species collected
1	49.352204, 75.424597	Steppe (dominant landscape) Surrounding plains Dry grasslands	<i>Ceratodon purpureus</i> <i>Polytrichum juniperinum</i> <i>Polytrichum piliferum</i> <i>Syntrichia ruralis</i>
2	49.307379, 75.374752	Forest (locally developed) Pine forests Dry grasslands	<i>Dicranum schljakovii</i> <i>Dicranum muehlenbeckii</i> <i>Sanionia uncinata</i> <i>Abietinella abietina</i> <i>Pylaisia polyantha</i>
3	49.313743, 75.509990	Forest (locally developed) Pine forests Dry grasslands	<i>Dicranum schljakovii</i> <i>Dicranum muehlenbeckii</i> <i>Sanionia uncinata</i> <i>Abietinella abietina</i> <i>Pylaisia polyantha</i>
4	49.283944, 75.385243	Rocky and mountain habitats Granite outcrops Thin soils Forest (locally developed) Pine forests	<i>Ceratodon purpureus</i> <i>Polytrichum juniperinum</i> <i>Polytrichum piliferum</i> <i>Dicranum muehlenbeckii</i> <i>Sanionia uncinata</i> <i>Pylaisia polyantha</i> <i>Syntrichia ruralis</i>
5	49.230563, 75.439318	Rocky and mountain habitats Mesic microhabitats Shaded ravines Stream valleys North-facing slopes	<i>Dicranum schljakovii</i> <i>Dicranum muehlenbeckii</i> <i>Sanionia uncinata</i> <i>Abietinella abietina</i> <i>Pylaisia polyantha</i>
6	49.223374, 75.409299	Steppe (dominant landscape) Surrounding plains Dry grasslands	<i>Syntrichia ruralis</i> <i>Ceratodon purpureus</i> <i>Polytrichum juniperinum</i> <i>Polytrichum piliferum</i>

### 2.3 DNA isolation

DNA was isolated using CTAB buffer (2% CTAB, 100 mM Tris-HCl, pH 8.0, 20 mM EDTA, 1.4 M NaCl, 0.2%  $\beta$ -mercaptoethanol, and 0.1 mg/ml proteinase K). Homogenized samples were resuspended in 700  $\mu$ l of preheated CTAB buffer and incubated at 60°C for 1 hour with gentle agitation. Afterward, 700  $\mu$ l of chloroform:isoamyl alcohol (24:1) was added, and the mixture was inverted for 2 minutes, then centrifuged at 14,000  $\times$  g for 10 minutes at 4°C. The upper phase was collected, and DNA was precipitated by adding isopropanol (about 380  $\mu$ l) and left overnight at -20°C. Following centrifugation at 14,000  $\times$  g for 15 minutes, the pellet was washed with cold 75% ethanol and centrifuged again. After removing ethanol, the pellet was air-dried, resuspended in 50  $\mu$ l TE buffer, and stored at -20 °C.

### 2.4 PCR and Sequencing

For PCR, we used the Biolabmix BioMaster HS-Taq PCR kit (2x), containing: (100 mM Tris-HCl, pH 8.5, 100 mM KCl, 2 mM of each dNTP, 4 mM MgCl<sub>2</sub>, 0.06 U/ $\mu$ l Taq DNA polymerase, 0.2% Tween 20, HS-Taq DNA polymerase stabilizers). The PCR reaction mixture consisted of 12.5  $\mu$ l of the Biolabmix BioMaster PCR kit, 1  $\mu$ l of primers, and 8-9  $\mu$ l of double-distilled water. The DNA concentration in the reaction mixture was adjusted to 50  $\mu$ g/ $\mu$ l. The primers used were

ITS1-moss (F: CAAGGTTTCCGTAGGTGAAC), (R: CAA-GAGCCAAGATATCCG3), ITS2-moss (F: CGGATATCTTG-GCTCTTG), (R: CCGCTTAGTGATATGCTTA) [25]. The PCR protocol was as follows: initial denaturation at 94°C for 1 min, followed by 30 cycles of 94°C for 1 min, 59°C for 1 min, 72°C for 1.5 min, and a final extension at 72°C for 5 min.

The PCR products were separated by electrophoresis on a 1% agarose gel. DNA fragments were visualized under a UV lamp in a GelDoc XR+ transilluminator (Biorad, Germany). They were then sequenced on an Applied Biosystems 3730xl DNA Analyzer. The obtained data were visualized using BioEdit v7.7.1.0. Species identification was performed in the BLAST Nucleotide database (blast.ncbi.nlm.nih.gov).

### 2.5 Phylogenetic analysis

To confirm the taxonomic placement of the obtained isolates, phylogenetic analysis was conducted. Multiple sequence alignment of *ITS1*, *ITS2* sequences was performed using the MUSCLE algorithm implemented in MEGA version 11 software. A phylogenetic tree was constructed using the Maximum Likelihood method to infer evolutionary relationships among the studied isolates and reference sequences [26]. The reliability of the phylogenetic tree topology was assessed by bootstrap analysis with 1000 replicates.

### 3. RESULTS

Overall, of the 29 collected moss samples from Karkaraly National Park, 9 species were identified using microscopic examination and two ribosomal gene markers (*ITS1* and *ITS2*).

The species compositions are presented (Figure 2) by two classes: *Polytrichopsida*, *Bryopsida*; two subclasses: *Dicranidae*, *Bryidae*; four orders: *Polytrichales*, *Pottiales*, *Dicranales*, *Hypnales*; and seven families: *Polytrichaceae*, *Pottiaceae*, *Dicranaceae*, *Ditrichaceae*, *Pylaisiaceae*, *Amblystegiaceae*, *Thuidiaceae*.

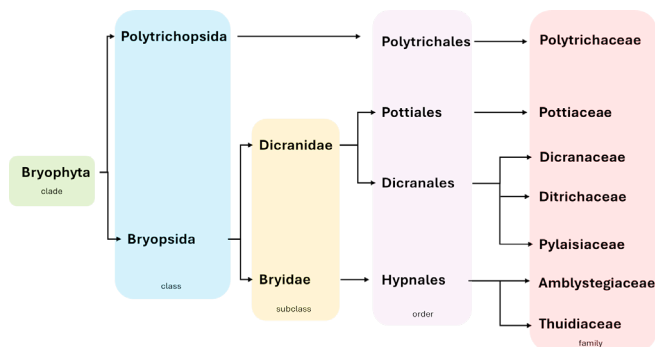


Figure 2 - Identified species of Bryophyta via microscopic-morphological examination

#### 3.1. Detailed description of the morphology of studied mosses

*Ditrichaceae* family – *Ceratodon purpureus*. Plants in open to dense tufts, turfs, or mats, green, dark green, brownish green, light green or yellow-green, usually darker proximally, often tinged reddish brown or purple. Stems (0.2-)1-3(-4) cm. Leaves (Figure 3C-H) crowded, erect-patent to contorted or somewhat crisped, rarely straight when dry, lanceolate, ovate-lanceolate, or triangular-lanceolate, 0.35-2.8 mm, margins recurved to near apex or rarely plane, irregularly serrate to uneven or smooth distally, apices acute to short-acuminate or, rarely, obtuse; costa strong, sub-percurrent to excurrent, sometimes as a long, smooth awn, medial laminal cells (6.5-)8-12(-14)  $\mu$ m, cell walls even, usually of medium thickness, often somewhat thicker and rounded at the cell angles. Seta (Figure 3B) 1-3(-4) cm, various shades of red, orange, or yellow.

Capsule oblong to long-cylindric (Figure 3B), (1-)2-2.5(-3) mm, smooth to strongly sulcate when dry; free to united at their nodes, finely papillose to spinulose-papillose, dark red and bordered to completely pale and absent borders. Spores (Figure 3E) (10-)11-14(-17)  $\mu$ m. Distributed almost throughout the world.

*Hypnaceae* family – *Pylaisia polyantha*. Plants: yellowish or whitish. Stems (Figure 4A): terete-foliate to flattened, somewhat regularly pinnate, branches 10 mm, creeping or rarely ascending and curved, subjulaceous; pseudoparaphyllia triangular, long-acuminate. Stem: and branch leaves are slightly differentiated. Stem leaves (Figure 4B, C): nearly straight, ovate-lanceolate, gradually narrowed to apex, concave, slightly plicate, 1.3-2 $\times$ 0.4-0.5 mm; margins plane, sometimes slightly involute distally; acumen long; costa double, short; alar cells 8–10 along margins, in 5–8 rows; medial laminal cells 50-80 $\times$ 4-5  $\mu$ m.

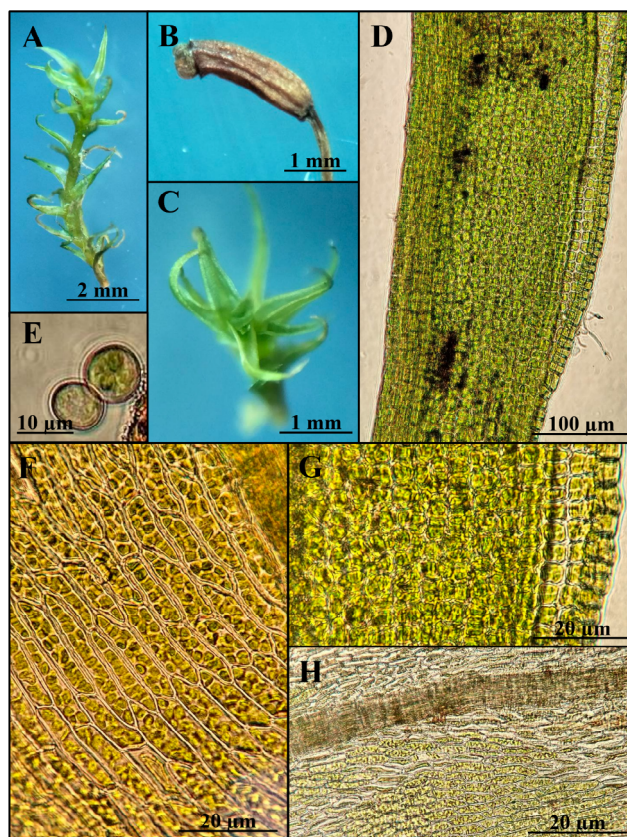


Figure 3 - *Ceratodon purpureus* morphological characteristics: A – stem; B – seta and capsule; C – spreading leaves; D- upper leaf; E – spores; F-H – leaf cells.

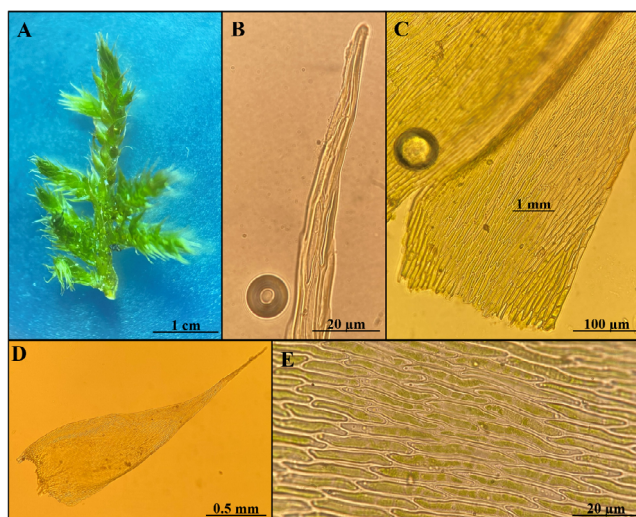


Figure 4 - *Pylaisia polyantha* morphological characteristics: A – stem; B – apex; C, D – leaves; E – leaf cells.

Branch leaves (Figure 4D) are narrowly ovate-lanceolate, shorter, 1.1-1.4 $\times$ 0.3-0.5 mm. Seta: 1–1.8 cm. *Pylaisia polyantha* is the type species of the genus; it is the most widely distributed of the species and is remarkably variable.

*Thuidiaceae* family – *Abietinella abietina*. Plants: dark green, yellowish brown, or dark brown, sometimes blackish tinged. Stems: to 12 cm; branches short, unequal, tapered; paraphyllia many. Stem (Figure 5A): leaves erect when dry, erect-spreading when moist, orange at insertion, plicate, 1.2–1.8 mm. Branch (Figure 5B): leaves erect when dry,

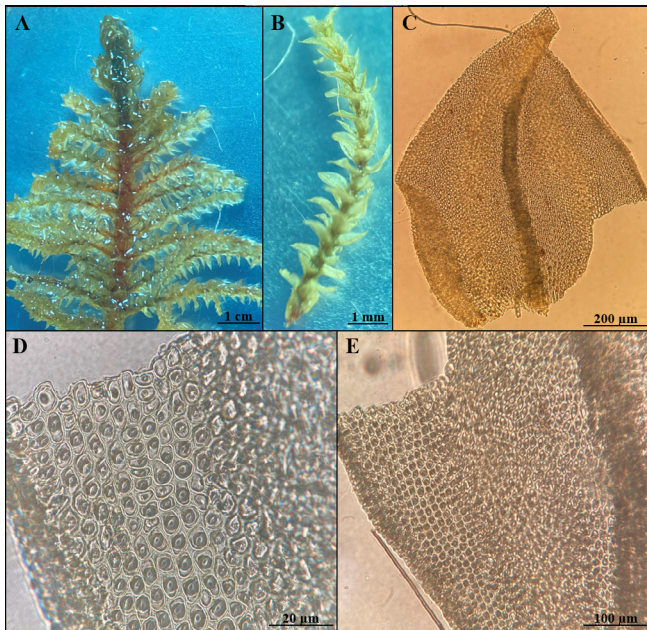


Figure 5 - *Abietinella abietina* morphological characteristics: A – stem; B – branch; C – upper leaf; D, E – leaf cells.

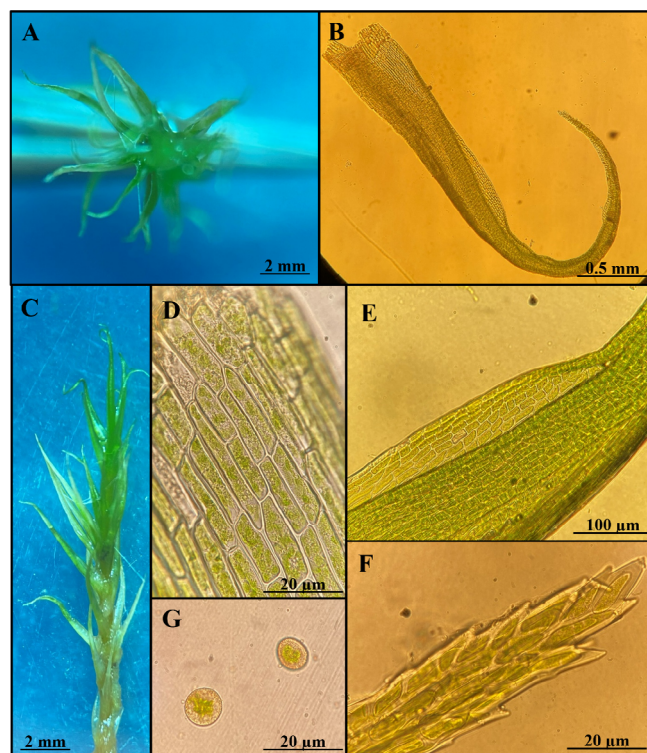


Figure 6 - *Dicranum muehlenbeckii* morphological characteristics: A – spreading leaves; B – basal leaf; C – stem; D-F – leaf cells; G – spores.

erect-spreading when moist, 0.6–0.7 mm; costa strong. Perichaetial: leaves to 4 mm (Figure 5C, D, E).

*Dicranaceae* family – *Dicranum muehlenbeckii*. Plants in dense tufts, green to yellowish green, dull. Stems (Figure 6C) 3–7 cm, densely tomentose with reddish brown rhizoids. Leaves (Figure 6; B-F) erect-spreading, strongly cirrate to crisped when dry, smooth, (4–)5–6.5(–8)×0.5–1 mm, concave below, tubulose above, lanceolate, acute; margins entire below, slightly serrate to entire above; laminae 1-stratose; costa excurrent, 1/6–1/4 the width of the leaves at base,

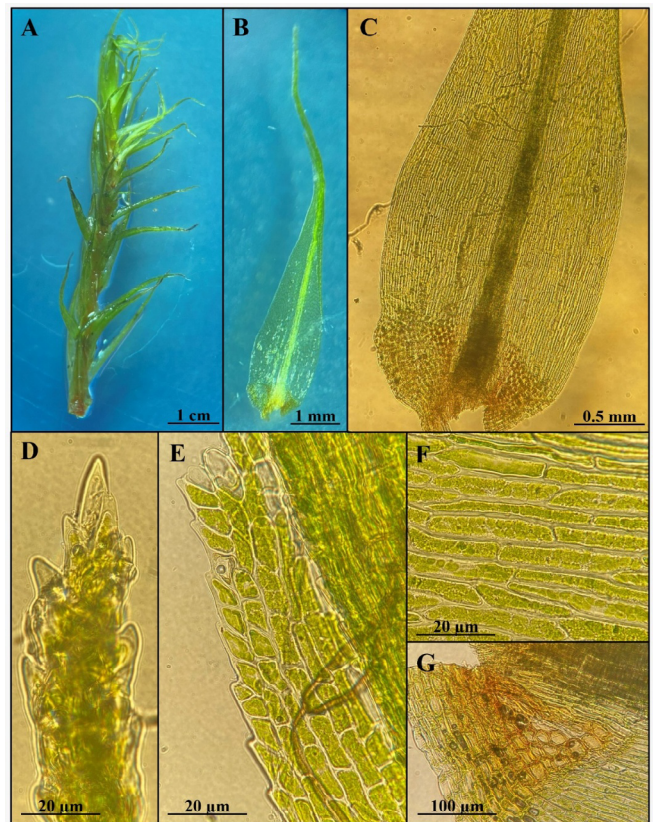


Figure 7 - *Dicranum schljakovii* morphological characteristics: A – stem; B, C – upper leaf; D – apex; E-G – leaf cells.

smooth or weakly toothed above on abaxial surface, abaxial ridges absent, with a row of guide cells, two stereid bands extending to the apex, adaxial and abaxial epidermal layers of cells differentiated; cell walls between lamina cells slightly bulging; leaf cells smooth to slightly rough above on abaxial surface (Figure 6E); differentiated, sometimes extending to costa; proximal laminal cells rectangular (Figure 6D), pitted, (19–)37–55(–73)×(5–)9–12(–14) μm; distal laminal cells short, irregularly quadrate-rectangular, not pitted, (7–)11–12(–23)×(6–)8–9(–13) μm.

Capsule (Figure 6G) 2–4 mm, long-cylindric, arcuate and inclined to ± straight and nearly erect, smooth, striate when dry, yellowish brown; operculum 1.5–2.5 mm. Spores 14–24 μm.

*Dicranaceae* family – *Dicranum schljakovii*. Mosses forming dark green or brownish, almost dense tufts. The stem (Figure 7A) is 0.5–15 cm tall, often tomentose.

The leaves usually face unilaterally and are sickle-shaped, less commonly diverging (Figure 7B, C). Dry leaves are sometimes curly or twisted. The leaf blade (Figure 7E) is linear-lanceolate, contracted into a grooved or tubular apex. The vein is narrow or fairly wide (Figure 7F). The cells at the leaf base angles are often brown (Figure 7G).

*Polytrichaceae* family – *Polytrichum juniperinum*. Plants: small to medium to fairly robust, gray-green to bluish green to reddish brown with age, in loose tufts, often forming extensive patches. Stems: (1–)4–5(–10) cm, simple, brownish tomentose only near the base. Leaves (Figure 8C-F): 3–6(–8) mm, densely imbricate, ± erect and almost straight when dry, erect-spreading to widely spreading when moist; sheath oblong-rectangular, yellowish, tapering to the blade; blade

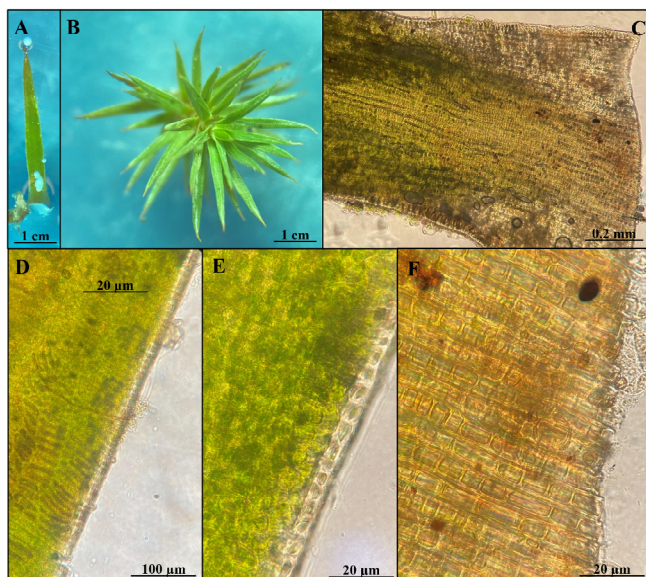


Figure 8 - *Polytrichum juniperinum* morphological characteristics: A – leaf; B – stems spreading leaves; C-E – leaves, F – leaf cells.

slender, bluish green and nitid, rather flat, with sharply infolded margins; entire or minutely crenulate, membranous and transparent, enclosing the lamellae and overlapping towards the apex; costa usually somewhat toothed distally, excurrent, forming a subulate, toothed awn, the awn reddish brown throughout or slightly decolorate at tip; lamellae bluntly crenate in profile, 6–8 cells high, the marginal cells in cross-section ovate to pyriform, thick-walled, ending in a distinct knob, smooth or rarely faintly papillose, the marginal cells of lateral lamellae (enclosed by the overlapping margins) ovoid and less strongly thickened; sheath cells (Figure 8E) 70–100 × 6–10 µm, narrowly rectangular (4–6:1), narrower toward the margin; cells (Figure 8F) of the broad marginal lamina transversely elongate, very thick-walled.

Sexual: condition dioicous; perigonial rosettes yellowish to reddish green; perichaetial leaves longer than the foliage leaves, long-sheathing, the blade almost obsolete, ending in a slender yellowish or hyaline awn.

*Polytrichaceae* family – *Polytrichum piliferum*. Plants: small to medium, glaucous green to reddish brown, in loose tufts. Stems: (0.5–)1–4 cm tall, rather wiry, unbranched, comose at the tips, whitish tomentose only near the base. Leaves: (2–)3–4 mm, erect, straight and slightly incurved when dry, erect-spreading when moist; sheath ovate, ± contracted to the blade; blade linear-lanceolate, turgid, with sharply infolded margins, the leaf apex abruptly contracted to the base of the awn; marginal lamina 5–8 cells wide (Figure 9D), 1-stratose, membranous, entire to finely serrulate toward the apex, enclosing the lamellae and overlapping in distal half or more; costa typically smooth abaxially, long-excurrent as a spinulose-toothed, hyaline awn; lamellae in profile crenulate-dentate to serrulate, with crenulations directed towards the leaf apex, (4–)6–8 cells high, the marginal cells in section conic to distinctly pyriform, terminating in a distinct knob, the marginal cells of the lateral lamellae ovoid, thinner-walled; sheath cells 60–80 × 10–15 µm, elongate-rectangular (4–6:1); cells of marginal lamina transversely elongated, ± irregular and sinuous, smaller toward the margins and obliquely oriented, especially approaching the apex, thick-walled (Figure 9G, H).

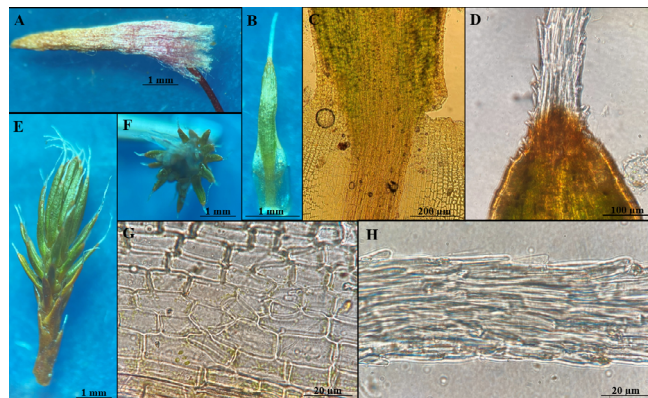


Figure 9 - *Sanionia uncinata* morphological characteristics: A – calyptra and seta; B-D – upper leaves; E – stem; F – spreading leaves; G, H – leaf cells.

Seta (Figure 9A): 1–3 cm, stout, flexuose, reddish brown. Calyptra (Figure 9A): dirty white to light brown, enclosing the capsule. Spores: 9–12 µm.

*Amblystegiaceae* family – *Sanionia uncinata*. Plants: small to medium-sized. Stems (Figure 10C): ± pinnate. Stem: leaves circinate (Figure 10D), falcate, or rarely ± straight, plicate or strongly plicate, rarely not plicate, 0.4–1.1 mm wide; base rounded-triangular or ovate; margins plane or rarely partly recurved distally, denticulate or finely denticulate distally; apex long- or very long-acuminate (Figure 10A); costa in bottom of shallow, wide-angled fold (or not in fold); alar region transversely triangular, transition to supra-alar cells sudden, supra-alar cells quadrate to rectangular, chlorophyllose, walls thin or slightly incrassate, eporose, region equal in size to or smaller than alar region; apical laminal cells with distal ends occasionally prorate abaxially (Figure 10B, F). Perichaetia: with inner leaves gradually narrowed to apex, margins finely denticulate to denticulate distally, apex long-acuminate (Figure 10E).

Capsule: horizontal or rarely erect to inclined; exothecial cells ± isodiametric, in 1–3 rows; exostome unspecialized, teeth long, broad basally, border widened at transitional zone

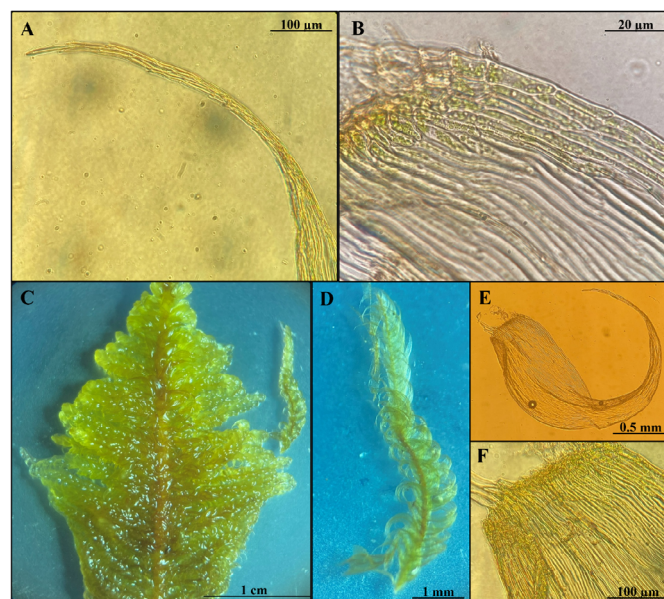


Figure 10 - *Sanionia uncinata* morphological characteristics: A – leaf; B – leaf cells; C – stem; D – leaf; E, F – upper leaf.

in pattern of external tooth; endostome unspecialized, in recently dehiscid capsules pale, brownish to yellowish, basal membrane constituting 36–45% total endostome height, processes perforated only along midline, cilia well developed.

*Pottiaceae* family – *Syntrichia ruralis*. Stems (Figure 11D): 5–15 mm. Leaves (Figure 11 A): clasping at base, in-folded and twisted around the stem when dry, wide-spreading (in smaller forms) to squarrose-recurved when moist, lingulate-ovate, 1.5–3.5 × 0.75–1.25 mm, canaliculate to keeled; margins tightly revolute in the proximal 7/8 or more, entire; apices emarginate to acute; costa excurrent into a serrate (or occasionally only faintly serrulate), hyaline awn that is red (Figure 11C) or sometimes broadly hyaline at base, weakly to strongly papillose on the abaxial surface and often serrate near the apex because of projecting cell ends, red-brown; basal cells abruptly differentiated (Figure 11E), narrowly rectangular, 35-70(-90)×11-18 μm, quadrate to narrowly rectangular at the margins; distal cells quadrate to polygonal, 8–12 μm, with 3–6 papillae per cell, bulging, somewhat obscure. Specialized asexual reproduction is absent.

Sexual: condition dioicous. Seta: red, 5–10 mm. Capsule: red-brown, 2–3.5 mm, straight, with an abrupt neck; operculum 1.25–1.75 mm, brown; peristome ca. 1.25 mm, the upper divisions twisted ca. 2 turns, red, the basal membrane white,

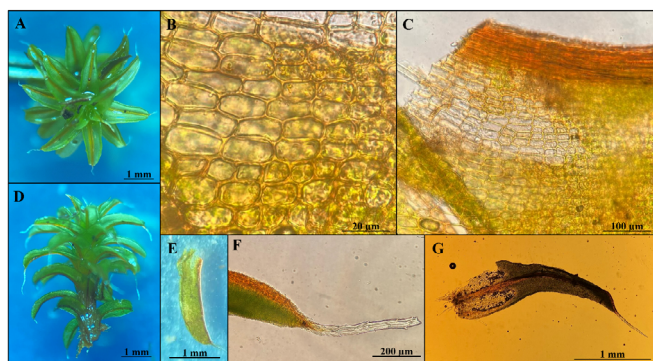


Figure 11 - *Syntrichia ruralis* morphological characteristics: A – spreading leaves; B, C – leaf cells; D – stem; E – basal leaf; F, G – adult leaf.

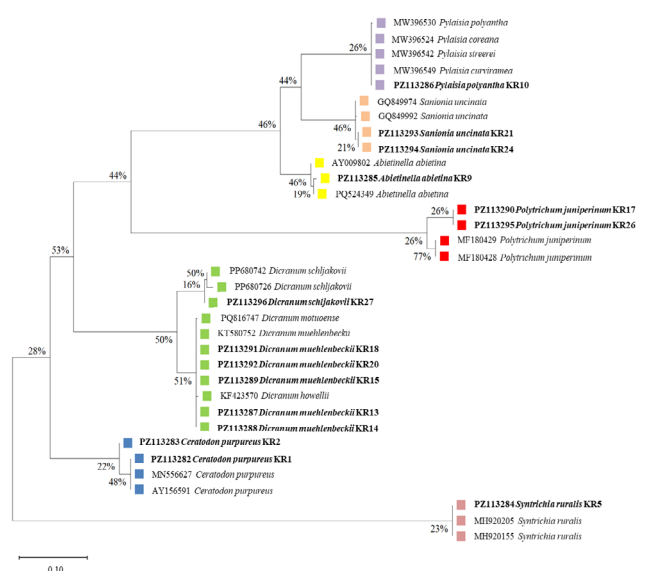


Figure 12 - Maximum likelihood phylogenetic tree of mosses based on *ITS1*. Bold text refers to samples from this study

about 1/3 the total length. Spores: 11–15 μm, papillose.

*Bioinformatic analysis.* Phylogenetic relationships among moss species collected from Karkaraly National Park (Central Kazakhstan) were reconstructed using the Maximum Likelihood (ML) method based on two nuclear ribosomal markers, *ITS1* and *ITS2*. All sequences included in the analyses were generated from specimens obtained in the present study. Overall, both datasets produced similar tree topologies and confirmed the taxonomic identity of the analyzed moss species (Figure 12).

In the *ITS1* phylogenetic reconstruction, *Polytrichum juniperinum* samples (KR17, PZ113290; KR26, PZ113295) formed a distinct clade with 63% bootstrap support, clearly separated from other taxa included in the analysis. A separate cluster was formed by representatives of the genus *Dicranum*, where *Dicranum schljakovii* (KR27, PZ113296) grouped together with multiple samples of *Dicranum muehlenbeckii* (KR13, KR14, KR15, KR18, and KR20). Internal bootstrap values within this clade ranged from 38% to 51%, reflecting moderate support for relationships among these closely related taxa.

The *ITS1* tree also revealed a cluster containing *Sanionia uncinata* (KR21, PZ113293 and KR24, PZ113294) with 60% bootstrap support, while *Abietinella abietina* (KR9, PZ113285) appeared as a sister lineage within the same broader cluster. *Pylaisia polyantha* (KR10, PZ113286) occupied an independent branch in this part of the tree. The two samples of *Ceratodon purpureus* (KR1, PZ113282 and KR2, PZ113283) grouped together with 38% bootstrap support, whereas *Syntrichia ruralis* (KR5, PZ113284) formed a separate branch within the upper cluster. No external outgroup

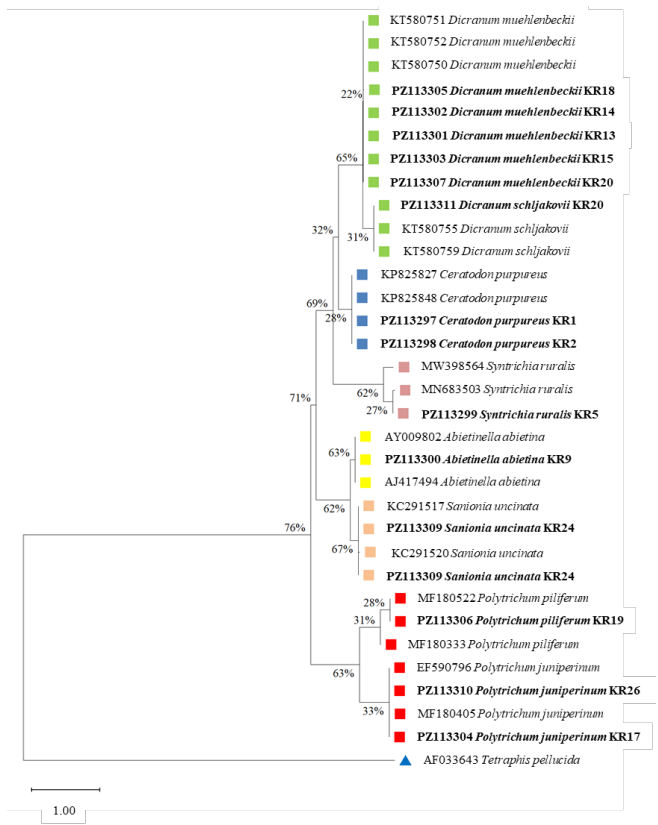


Figure 13 - Maximum likelihood phylogenetic tree of mosses based on *ITS2*. Bold text refers to samples from this study. Blue triangle is an outgroup.

was included in the *ITS1* analysis; therefore, the tree is presented as unrooted, and relationships are interpreted based on clustering patterns among the studied taxa.

In the *ITS2* phylogenetic reconstruction, *Dicranum muehlenbeckii* samples (KR13, KR14, KR15, KR18, and KR20) clustered together with reference sequences (KT580750-KT580752), forming a relatively well-defined clade with moderate bootstrap support (65%). The sample *Dicranum schljakovii* (KR20) grouped with corresponding GenBank sequences (KT580755 and KT580759), although with lower internal support (31-32%), indicating close but weakly resolved relationships within the genus *Dicranum* (Figure 13).

The two samples of *Ceratodon purpureus* (KR1 and KR2) formed a cluster together with reference sequences (KP825827 and KP825848), supported by 69% bootstrap, confirming their genetic similarity. Similarly, *Syntrichia ruralis* (KR5) grouped with reference sequences (MW398564 and MN683503), forming a distinct clade with bootstrap values ranging from 27% to 62%. The samples of *Abietinella abietina* (KR9) clustered with reference sequences (AY009802 and AJ417494) with moderate support (63%), while *Sanionia uncinata* (KR24) grouped with corresponding GenBank sequences (KC291517 and KC291520) with bootstrap support of 62-67%.

The *Polytrichum* lineage formed a separate cluster, where *Polytrichum piliferum* (KR19) grouped with reference sequences (MF180522 and MF180333), although with relatively low support (28–31%). The samples of *Polytrichum juniperinum* (KR17 and KR26) clustered together with reference sequences (EF590796 and MF180405), forming a distinct clade with moderate bootstrap support (63%). The tree was rooted using *Tetraphis pellucida* (AF033643) as an outgroup, which formed a clearly separated basal lineage.

## DISCUSSION

The moss species recorded in this study reflect the ecological heterogeneity of the Karkaraly Mountains, which function as a forest–steppe enclave within the arid landscapes of Central Kazakhstan [27, 28]. The presence of both acrocarpous taxon's, such as *Ceratodon purpureus*, *Syntrichia ruralis*, and *Dicranum* species [29-31], and pleurocarpous taxon's, including *Sanionia uncinata*, *Abietinella abietina*, and *Pylaisia polyantha* [32, 33], indicates a mosaic of habitats ranging from dry exposed soils and rocky substrates to shaded forest floors and moist microhabitats. Such diversity reflects Karkaraly's role as a regional refugium for bryophyte diversity, where microclimatic variation created by mountain relief supports species that would otherwise be absent from surrounding steppe ecosystems.

The combination of morphological identification and molecular confirmation using *ITS1* and *ITS2* markers allowed reliable identification of eight moss species belonging to seven families and four orders [25]. The congruence between morphological traits and phylogenetic clustering demonstrates that the integrative approach applied here is effective for the identification of bryophytes in regions where taxonomic expertise and reference collections remain limited [34].

The phylogenetic analyses based on the *ITS1* and *ITS2* nuclear ribosomal regions provided molecular support for the

taxonomic identification of moss species collected from Karkaraly National Park. In both datasets, samples belonging to the same species consistently clustered together, confirming the reliability of morphological identification performed in this study [35].

Across both phylogenies, clear clustering was observed for *Polytrichum juniperinum*, *Ceratodon purpureus*, *Abietinella abietina*, and *Sanionia uncinata*, with moderate bootstrap support, reflecting stable genetic differentiation of these taxa. The genus *Dicranum* formed a distinct lineage in both trees; however, relatively low to moderate bootstrap values within this clade suggest limited resolution of *ITS* markers for closely related species such as *D. muehlenbeckii* and *D. schljakovii*. A similar pattern was observed for *Syntrichia ruralis*, where low internal support likely reflects low sequence divergence within the species.

The *ITS2* dataset, which included an external outgroup (*Tetraphis pellucida*), allowed more reliable interpretation of broader phylogenetic relationships compared to the unrooted *ITS1* tree. The separation of pleurocarpous mosses (*Sanionia*, *Abietinella*, *Pylaisia*) from acrocarpous taxa (*Dicranum*, *Ceratodon*, *Syntrichia*) and the distinct placement of the *Polytrichum* lineage are consistent with current understanding of moss phylogeny and support the evolutionary validity of the obtained topology.

Despite overall agreement between markers, several nodes across both trees showed moderate or low bootstrap support, indicating that *ITS* regions alone have limited resolving power for deeper or recently diverged lineages. This limitation is well recognized in bryophyte systematics and suggests that the inclusion of additional molecular markers (e.g., plastid loci or genomic data) would improve phylogenetic resolution.

The pleurocarpous mosses *Sanionia uncinata* and *Abietinella abietina* formed a separate lineage in both analyses, reflecting their evolutionary divergence from the predominantly acrocarpous taxa included in this study. The grouping of the two *Sanionia uncinata* and *Abietinella abietina* samples, *ITS2* marker, further supports the accuracy of their species identification [36].

Although both *ITS1* and *ITS2* markers successfully distinguished the studied species, several internal nodes exhibited moderate or relatively low bootstrap support, indicating limited resolution of deeper phylogenetic relationships. This result is consistent with previous studies suggesting that nuclear ribosomal *ITS* regions are effective for species-level discrimination in bryophytes but may provide limited phylogenetic resolution at higher taxonomic levels [37, 38].

The present study contributes to bryophyte research in Central Kazakhstan by providing one of the first integrative datasets that combine morphological identification with molecular phylogenetic analysis using *ITS1* and *ITS2* markers. While previous studies in Kazakhstan have primarily focused on floristic inventories and taxonomic records, molecular confirmation of species identity remains limited. In this context, our results not only validate species identification but also establish a molecular reference framework for the region's bryophytes. Furthermore, the study demonstrates that the Karkaraly Mountains function as a biogeographical transition zone, where steppe, forest, and rocky habitats support distinct bryo-

phyte assemblages. The coexistence of xerophytic species (*Syntrichia*, *Ceratodon*, *Polytrichum*) and mesophilous taxa (*Dicranum*, *Sanionia*, *Abietinella*) highlights the role of microhabitat heterogeneity in maintaining bryophyte diversity under semi-arid conditions.

Thus, this work provides not only new regional records but also a framework for future ecological and molecular studies of bryophytes in Kazakhstan, particularly in the context of climate-driven aridization and biodiversity conservation.

### AUTHOR CONTRIBUTIONS

VK and RU: conceptualization, study design, data validation, and writing—original draft preparation. VK, AS, RU, AN, AA: collection of biological material. RU, AN, AA and NM: data curation and laboratory experiments. RU and VK: data analysis. VK: funding acquisition. RU, AS, and VK: revising and final approval of the manuscript. All authors contributed to the article and approved the submitted version.

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**ФЛОРА МОХООБРАЗНЫХ КАРКАРАЛИНСКОГО РЕГИОНА (КАРАГАНДИНСКАЯ ОБЛАСТЬ, КАЗАХСТАН): ВИДОВОЙ СОСТАВ И РАСПРОСТРАНЕНИЕ****Рабиға Уахит<sup>1</sup>, Нұрасыл Манапов<sup>1,2</sup>, Альдина Насредин<sup>2</sup>, Абай Алаш<sup>1,2</sup>, Айнура Смағұлова<sup>1</sup>, Икимат Тайво<sup>3</sup>, Сара Беккужина<sup>1,3</sup>, Владимир Киян<sup>1\*</sup>**<sup>1</sup>Лаборатория биоразнообразия и генетических ресурсов, ТОО «Национальный центр биотехнологии», Астана, Казахстан.<sup>2</sup>Кафедра биотехнологии и микробиологии, Евразийский национальный университет им. Л.Н. Гумилёва, Астана, Казахстан.<sup>3</sup>Кафедра микробиологии и биотехнологии, Казахский агротехнический исследовательский университет им. С. Сейфуллина, Астана, Казахстан.

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**АБСТРАКТ**

Мохообразные представляют собой важный, но недостаточно изученный компонент наземного биоразнообразия Центрального Казахстана. В данном исследовании изучаются видовой состав и филогенетические взаимоотношения мхов в Каркаралинском национальном парке (Карагандинская область) – лесостепном анклав среди засушливых ландшафтов Казахского мелкосопочника. Всего было собрано 29 образцов мхов, которые были проанализированы с использованием интегративного подхода, сочетающего микроскопическое морфологическое исследование и молекулярную идентификацию на основе ядерных рибосомных маркеров *ITS1* и *ITS2*. Было выявлено девять видов, относящихся к семи семействам и четырём порядкам, включая *Ceratodon purpureus*, *Syntrichia ruralis*, *Dicranum muehlenbeckii*, *Dicranum schljakovii*, *Polytrichum juniperinum*, *Polytrichum piliferum*, *Sanionia uncinata*, *Pyloisia polyantha* и *Abietinella abietina*. Филогенетический анализ, выполненный методом максимального правдоподобия (Maximum Likelihood), подтвердил идентификацию на уровне видов и показал устойчивую кластеризацию образцов внутри соответствующих родов. Обнаруженный видовой комплекс отражает экологическую гетерогенность Каркаралинских гор, где микроклиматические вариации поддерживают существование как ксерофитных, так и мезофильных видов мхов. Данное исследование представляет собой один из первых интегративных анализов, объединяющих морфологические и молекулярные (*ITS1* и *ITS2*) подходы для идентификации мхов в Центральном Казахстане. Полученные результаты предоставляют новые базовые данные о видовом составе и филогенетических взаимоотношениях мохообразных в Каркаралинском регионе и подчёркивают его роль как рефугиума для ксерофитных и мезофильных таксонов в условиях полусухого ландшафта.

**Ключевые слова:** мохообразные, разнообразие мхов, Каркаралинский национальный парк, филогения *ITS*, молекулярная идентификация, Казахстан.

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**ҚАРҚАРАЛЫ АУДАНЫНЫҢ (ҚАРАҒАНДЫ ОБЛЫСЫ, ҚАЗАҚСТАН) МҮКТӘРІЗДІЛЕР ФЛОРАСЫ: ТҮРЛІК ҚҰРАМЫ ЖӘНЕ ТАРАЛУЫ****Рабиға Уахит<sup>1</sup>, Нұрасыл Манапов<sup>1,2</sup>, Альдина Насредин<sup>2</sup>, Абай Алаш<sup>1,2</sup>, Айнура Смағұлова<sup>1</sup>, Икимат Тайво<sup>3</sup>, Сара Беккужина<sup>1,3</sup>, Владимир Киян<sup>1\*</sup>**<sup>1</sup>Биоалуантүрлілік және генетикалық ресурстар зертханасы, «Ұлттық биотехнология орталығы» ЖШС, Астана, Қазақстан<sup>2</sup>Биотехнология және микробиология, Л.Н. Гумилёв атындағы Еуразия ұлттық университеті, Астана, Қазақстан<sup>3</sup>Микробиология және биотехнология кафедрасы, С. Сейфуллин атындағы қазақ агротехникалық зерттеу университеті, Астана, Қазақстан.

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**АБСТРАКТ**

Орталық Қазақстанның құрлықтық биоалуантүрлілігінің маңызды, бірақ жеткілікті зерттелмеген компоненттерінің бірі – мүктәрізділер. Бұл зерттеуде Қарағанды облысындағы Қарқаралы ұлттық паркінде орналасқан мүктердің түрлік құрамы мен филогенетикалық байланыстары зерттеледі. Бұл аймақ – Қазақтың ұсақ шоқыларының құрғақ ландшафттары арасындағы орманды-дала анклавы. Жалпы алғанда 29 мүк үлгісі жиналып, олар интегративті тәсіл арқылы зерттелді. Зерттеу микроскопиялық морфологиялық талдауды және *ITS1* мен *ITS2* ядролық рибосомалық маркерлеріне негізделген молекулалық идентификацияны біріктірді. Нәтижесінде төрт қатарға және жеті тұқымдасқа жататын тоғыз түр анықталды. Олардың қатарына *Ceratodon purpureus*, *Syntrichia ruralis*, *Dicranum muehlenbeckii*, *Dicranum schljakovii*, *Polytrichum juniperinum*, *Polytrichum piliferum*, *Sanionia uncinata*, *Pyloisia polyantha* және *Abietinella abietina* жатады. Максималды ықтималдық әдісі (Maximum Likelihood) арқылы жүргізілген филогенетикалық талдау түр деңгейіндегі анықтауды растады және үлгілердің өздеріне тиісті туыстар ішінде тұрақты түрде топтасатынын көрсетті. Анықталған

түрлік құрам Қарқаралы тауларының экологиялық әртүрлілігін көрсетеді, мұнда микроклиматтық айырмашылықтар ксерофитті және мезофильді мүк түрлерінің қатар тіршілік етуіне мүмкіндік береді. Бұл зерттеу Орталық Қазақстандағы мүктерді анықтау үшін морфологиялық және молекулалық (*ITS1* және *ITS2*) тәсілдерді біріктіретін алғашқы интегративті талдаулардың бірі болып табылады. Алынған нәтижелер Қарқаралы өңіріндегі мүк тәрізділердің түрлік құрамы мен филогенетикалық байланыстары туралы жаңа бастапқы деректерді ұсынады және жартылай құрғақ ландшафт жағдайында ксерофитті және мезофильді таксондар үшін оның рефугиум ретіндегі рөлін көрсетеді.

**Түйін сөздер:** мүктәрізділер, мүк алуан түрлілігі, Қарқаралы ұлттық паркі, *ITS* филогениясы, молекулалық идентификация, Қазақстан.