Article

Morphology, Phytochemistry, and Genetics-Based Analysis of Endemic Species Belonging to *Allium* sect. *Schoenoprasum* (Amaryllidaceae) from the Kazakhstan Altai

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Posted Date: 17 November 2025

doi: 10.20944/preprints202511.1040.v1

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Abstract

The genus *Allium* L. (Amaryllidaceae) comprises ecologically flexible species widespread across mountain ecosystems, yet the relationships among morphology, environment, and genetics within section *Schoenoprasum* in Central Asia remain poorly understood. This study investigated four taxa – *A. ledebourianum*, *A. ivasczenkoae*, *A. ubinicum*, and *A. schoenoprasum* – from the Kazakhstan Altai to assess their morphological variation, ecological preferences, phytochemical activity, and genetic relationships. Populations occurred on gentle chernozem slopes under humid, nutrient-rich conditions and showed stable regeneration dominated by young individuals. Morphometric analyses revealed pronounced interspecific differentiation: *A. ledebourianum* attained the greatest height and umbel size, whereas *A. ubinicum* was smallest but possessed proportionally larger floral organs. Principal component analysis explained 94% of total variance, distinguishing *A. ubinicum* and *A. schoenoprasum* from the remaining taxa. Floral traits correlated significantly with temperature, moisture, and soil reaction, indicating strong environmental influence on phenotype. Extract assays showed variable bioactivity, with *A. ubinicum* displaying the highest antioxidant potential (IC_{50} = 88 μL) and highest cytotoxicity (LC_{50} of 5.9 μg/mL), while *A. ledebourianum* shows no antioxidant activity and the lowest toxicity (LC_{50} of 10.9 μg/mL). Phylogenetic reconstruction using *matK*, *rbcL*, and *psbA–trnH* chloroplast markers confirmed close affinity between *A. ledebourianum* and *A. ivasczenkoae*, while *A. ubinicum* formed a distinct lineage. Together, morphological, ecological, and molecular data highlight the Kazakhstan Altai as a center of diversification for section *Schoenoprasum*. These results emphasize the adaptive plasticity of endemic *Allium* species and their potential as sources of valuable bioactive compounds, underscoring the importance of conserving genetically and morphologically diverse populations in mountain ecosystems.

Keywords: *Allium ledebourianum*; *Allium ivasczenkoae*; *Allium ubinicum*; *Allium schoenoprasum*; demographic structure; phenotypic plasticity; bioactive compounds; molecular markers

1. Introduction

The genus *Allium* L. (Amaryllidaceae) is among the most species-rich and ecologically diverse groups of monocots, comprising more than 1000 taxa distributed across temperate and subtropical regions of the Northern Hemisphere [1]. This genus includes globally important crops such as onion (*A. cepa*), garlic (*A. sativum*), and leek (*A. porrum*), valued not only as vegetables and spices but also as sources of bioactive compounds with wide-ranging pharmacological properties [2,3]. Wild relatives of cultivated *Allium* species also play key ecological roles and represent an important genetic reservoir for crop improvement and adaptation to environmental stress [4,5].

Among Central Asian ecoregions, the Kazakhstan Altai stands out as a biodiversity hotspot, harboring a high concentration of endemic and relict plant species [6,7]. This mountainous system forms part of the Altai–Sayan floristic region, characterized by high levels of endemism and a mosaic of habitats shaped by complex topography and climatic gradients. Within this system, the genus *Allium* demonstrates exceptional adaptive radiation, with over 100 species documented across Kazakhstan, several of which are endemic or narrowly distributed [8–10]. Recent studies in the neighboring Kyrgyz Alatau documented 36 *Allium* species representing 13 subgenera, including 13 narrow endemics, some of which are listed in national Red Data Books and the IUCN Red List [11]. These findings highlight the exceptional concentration of diversity and rarity in Central Asian mountain systems, reinforcing the likelihood that the Kazakhstan Altai harbors similarly high levels of underexplored endemic diversity [12,13]. However, despite this richness, population-level, ecological, and molecular data for Altai endemics remain scarce, limiting conservation and taxonomic resolution.

Within the genus, section *Schoenoprasum* Dumort. Is of particular interest due to its ecological specificity and economic potential. This section includes the well-known chive (*Allium schoenoprasum* L.), a perennial herb widely cultivated for culinary use and noted for its antioxidant and anti-inflammatory activities [14,15]. Recent phytochemical studies have highlighted the richness of phenolic compounds in *A. schoenoprasum*, linking them to strong antioxidant capacity and potential applications in nutraceuticals [16]. However, its wild relatives in Central Asia remain poorly characterized in terms of morphology, ecology, and genetics, despite their likely role as reservoirs of adaptive and phytochemical diversity.

The Kazakhstan Altai harbors several endemic species of section *Schoenoprasum*, yet their taxonomic status, morphological differentiation, and ecological roles remain inadequately explored. While preliminary floristic surveys and checklists confirm their presence, comprehensive population-level and integrative studies are scarce [10,17]. Recent work has provided valuable insights into seed morphology of section *Schoenoprasum* endemics in Eastern Kazakhstan [18], but no study to date has combined ecological, morphometric, phytochemical, and molecular datasets to evaluate species limits and adaptation within this group.

Research on Kazakhstan's *Allium* flora has expanded considerably in recent decades, particularly in taxonomy, phylogenetics, and population genetics. Several new and cryptic *Allium* taxa have been described from Central Asia [19–21], underscoring the genus's underestimated diversity and taxonomic complexity. However, most molecular studies have focused on economically important groups such as *A. cepa* or *A. altaicum*, leaving section *Schoenoprasum* underrepresented in comparative or phylogenomic analyses [22–26].

Phytochemically, *Allium* species are rich in sulfur-containing compounds, flavonoids, and phenolics, which underlie their antimicrobial, antioxidant, and anticancer properties [27–29]. Studies on Kazakhstan endemics have revealed unique phenolic profiles with cosmeceutical and pharmacological potential [30], but the extent of intraspecific variation and environmental modulation of these compounds remains unknown.

Ecologically, *Allium* species often serve as indicators of alpine and subalpine habitat conditions [31]. Population studies from neighboring regions highlight the vulnerability of endemic *Allium* taxa to habitat degradation and overharvesting [32,33]. In the Kazakhstan Altai, however, integrated data connecting demography, ecology, and molecular diversity are still lacking, constraining effective conservation planning.

Despite growing research, major knowledge gaps persist for endemic section *Schoenoprasum* species of the Kazakhstan Altai. Specifically, their population dynamics, morphological plasticity, bioactive potential, and genetic differentiation remain unquantified. Addressing these aspects is essential for resolving species boundaries, assessing adaptive evolution, and informing conservation priorities.

Several endemic *Allium* species of the Kazakhstan Altai are listed in the Red Data Book of Kazakhstan, underscoring their vulnerability and the urgent need for conservation-oriented research [11]. Beyond their ecological significance, wild relatives of section *Schoenoprasum* represent valuable genetic resources for breeding programs, particularly for traits such as drought and cold tolerance that are increasingly relevant under climate change [5,34]. The Altai region itself holds exceptional biogeographic importance, functioning as a floristic bridge between Central Asia, Siberia, and Mongolia, and serving as a center of speciation for many taxa [7]. Despite this, section *Schoenoprasum* remains globally underexplored compared with crop-related sections such as *Cepa* [35,36] and *Porrum* [37,38]. Recent advances in phylogenomics and DNA barcoding [19–21] now provide powerful tools for resolving complex *Allium* lineages, highlighting the timeliness and novelty of our integrative approach.

Therefore, this study integrates population ecology, morphology, phytochemistry, and genetics to establish a comprehensive baseline for endemic section *Schoenoprasum* species in the Kazakhstan Altai and to elucidate the ecological and genetic mechanisms driving their differentiation.

2. Materials and Methods

2.1. Field Expeditions

The objects of the study were 6 populations of 4 species of the genus *Allium* section *Schoenoprasum* collected in the territory of the Kazakhstan Altai from June to August in 2023 and 2024 (Figure 1).

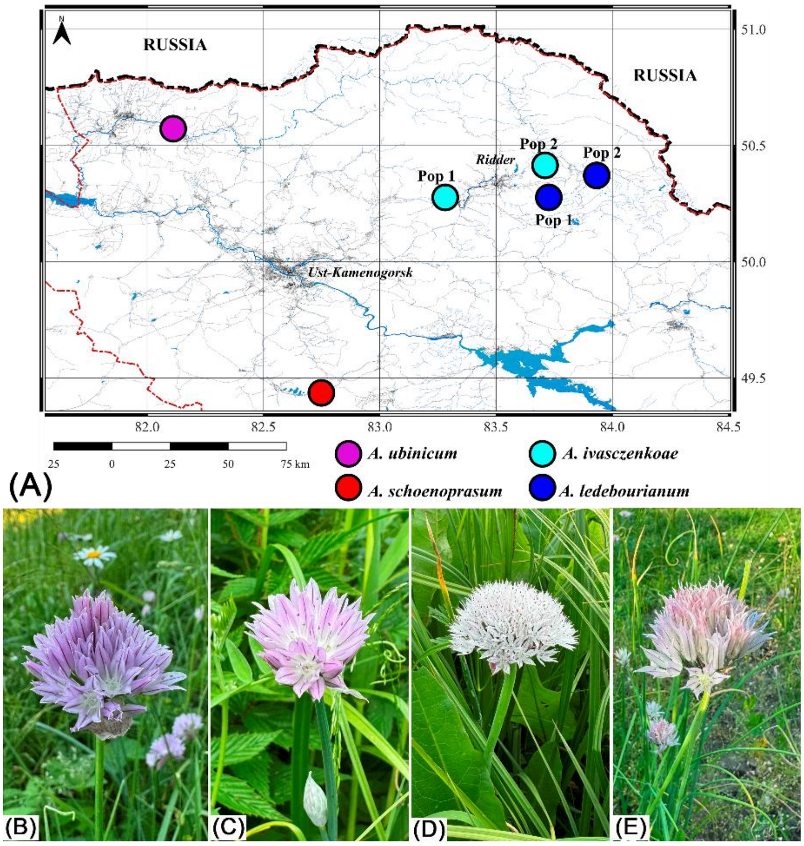

Figure 1. Geographical locations of collection points in Kazakhstan Altai (A) and photos of flowers of collected 4 species from the genus Allium section *Schoenoprasum*: *A. schoenoprasum* (A), *A. ivasczenkoae* (B), *A. ledebourianum* (C), and *A. ubinicum* (D).

doi:10.20944/preprints202511.1040.v1

A. ivasczenkoae and *A. ubinicum* are narrow endemics of the Kazakhstan Altai, *A. ledebourianum* is a regional endemic of the Altai, and *A. schoenoprasum* is a widespread species in the region.

Field surveys in the Kazakhstan Altai were conducted using route–reconnaissance methods appropriate for heterogeneous terrain and large spatial scales [39]. Populations were located in representative habitats according to species-specific ecological preferences [40]. Site conditions were evaluated following Ellenberg's ecological indicator scales (EIV) [41], with assessments of light (L), temperature (T), moisture (M), soil reaction (R), nutrient availability (N), and salinity (S). L was measured using a luxmeter UNI-T UT383S (UNI-T, China) under standardized daytime conditions (between 10:00 and 14:00 h) on cloud-free days to minimize temporal variation. Soil samples were collected from the root zone of representative plants and analyzed for R, S, and N in the laboratory. These clarifications ensure that environmental data reflect consistent site conditions. M and T were recorded with a thermohygrometer RGK-TH 30 (RGK, Russia). R (pH) was determined using a pH meter Yieryi HH-1000 (Yieryi, China). N was estimated based on soil fertility characteristics, including organic matter content and visible vegetation cover, while S was evaluated according to soil type and the presence of halophytic plant species.

Individuals were assigned to ontogenetic stages following the criteria of Jones C.G. [42], based on the structure of the shoot system, bulb morphology, and presence of reproductive organs.

2.2. Morphometric Analysis

In the field, morphological and morphometric traits of 30 adult plants per population were studied using eight parameters: plant height (cm), number of stems per clump (count), number of leaves per clump (count), stem length (cm), stem diameter at base (mm), flower stalk length (mm), leaf length (cm), and leaf width (mm). To describe floral morphology, a random sample of 30 flowers was collected from each population [43].

Inflorescences were collected in June 2024. Flowers were preserved in a mixture of ethanol, glycerol, and distilled water (3:2:1) at 16 °C. Only fully developed and healthy specimens were used for analysis. Morphological descriptions focused on diagnostic characters common to flowers of each species within section *Schoenoprasum*. Floral morphometric measurements were taken using a stereomicroscope (Magus A18T, Russia).

The main morphometric parameters of the flower were studied: petal length (mm), petal width (mm), stamen length (mm), pistil column length (mm), ovary length (mm), ovary width (mm), anther length (mm), and anther width (mm) (Figure 2).

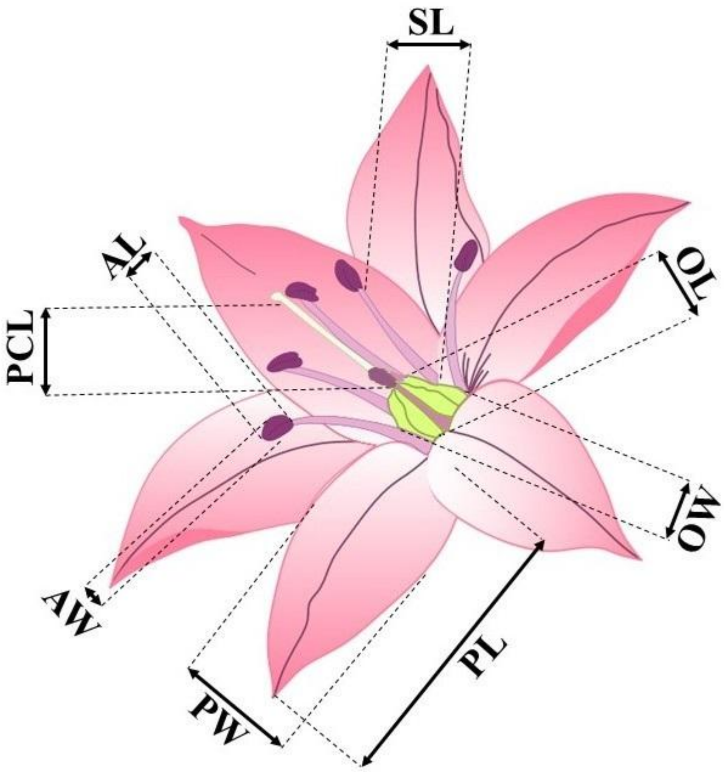

Figure 2. Diagram of the general structure of *Allium* flower with an indication of the studied morphometric parameters. PL – petal length, PW – petal width, SL – stamen length, PCL – pistil column length, OL – ovary length, OW – ovary width, AL – anther length, AW – anther width.

2.3. Analyses of Cytotoxicity and Antioxidant Activity

Cytotoxic activity of *Allium* extracts was assessed using the brine shrimp (*Artemia salina*) lethality assay [44]. Extracts were prepared using Soxhlet extraction with ethanol and concentrated under reduced pressure at ≤ 45 °C. Stock solutions were prepared at 1 mg/mL in ethanol (1000 µg/mL). Working concentrations were obtained by dilution in artificial seawater to final concentrations of 1, 5, 10, 25, and 50 µg/mL.

Each concentration was tested in triplicate, with 20 nauplii per replicate, and the total volume per test tube was 2 mL. The solvent control consisted of artificial seawater containing 0.5% (v/v) ethanol, matching the highest solvent fraction used in test wells. A separate distilled-water control was also included for reference.

After 24 h of exposure, the numbers of live and dead nauplii were recorded. Percent mortality was calculated using the formula:

$$Mortality\ (\%) = \left(\frac{number\ of\ dead\ nauplii}{initial\ number\ of\ live\ nauplii}\right) \times 100$$

LC_{50} values (concentration causing 50% mortality) and their 95% confidence intervals were calculated using probit regression in GraphPad Prism (v. 9.0). χ^2, p and slope parameters were recorded.

Antioxidant activity of *Allium* samples was determined using the standard 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay [45,46]. A 0.1 mM ethanolic solution of DPPH was freshly prepared and kept in the dark to prevent photodegradation. Test extracts were prepared as 1000 µg/mL stock solutions in ethanol, and aliquots of 10, 20, 30, 40, and 50 µL were added to 2 mL of the DPPH solution. Antioxidant activity of *Allium* samples was determined using the standard 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay [46]. A 0.1 mM ethanolic solution of DPPH was freshly prepared and kept in the dark to prevent photodegradation. Test extracts were prepared as 1000 µg/mL /mL stock solutions in ethanol, and aliquots corresponding to final concentrations of 5, 10, 15, 20, and 25 µg/mL were added to 2 mL of the DPPH solution. The mixtures

doi:10.20944/preprints202511.1040.v1

were thoroughly vortexed and incubated at room temperature in the dark. The decrease in absorbance was monitored at 517 nm using a UV-5100 spectrophotometer (Metash, China) equipped with a 3 mL quartz cuvette and a 1 cm path length. Measurements were taken at 5 min intervals up to 30 min to capture the kinetics of radical scavenging. Antioxidant activity was expressed as the percentage of DPPH inhibition relative to the control (ethanol + DPPH without extract) using the formula:

$$Inhibition\ (\%) = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 100$$

IC_{50} values (concentration required to inhibit 50% of the DPPH radicals) were calculated to compare the antioxidant efficiency of the samples.

2.4. Genetic Analysis

Fresh leaves were collected from each plant, immediately dried to prevent degradation, and stored at –80 °C until further use. Samples were maintained under these conditions to preserve DNA integrity and prevent enzymatic or microbial activity prior to extraction.

Genomic DNA was extracted from leaf tissue using a modified CTAB protocol [47], followed by chloroform–isoamyl alcohol (24:1) purification and precipitation with cold isopropanol. DNA integrity was verified by electrophoresis on 1% agarose gels, and concentration and purity were determined with a NanoDrop 2000 spectrophotometer (Thermo Scientific, Singapore).

Three chloroplast markers were selected for genetic analysis: *MatK* and *RbcL* (coding genes commonly used for plant barcoding) and the *PsbA–TrnH* intergenic spacer (highly variable). PCR amplification was performed on a T100 thermocycler (Bio-Rad, USA) in 25 µL reactions containing 1× buffer, 2.0 mM $MgCl_2$, 0.2 mM each dNTP, 0.4 µM of each primer, 1 U Taq DNA polymerase (Thermo Scientific, USA), and 20–50 ng of template DNA. Thermal cycling conditions were as follows: *MatK* – 94 °C for 3 min; 35 cycles of 94 °C for 30 s, 50 °C for 30 s, 72 °C for 1 min; final extension 72 °C for 5 min. *PsbA–TrnH* – same profile with annealing at 54 °C. *RbcL* – same profile with annealing at 52 °C. PCR products were checked on 1.5% agarose gels with ethidium bromide. Amplicons were purified with the QIAquick PCR Purification Kit (Qiagen, Germany) and sequenced bidirectionally on an ABI 3730xl DNA Analyzer (Applied Biosystems, USA). Sequence editing and assembly were performed in Geneious Prime v.2023 (www.geneious.com/features/prime). Alignments were generated with MUSCLE and manually refined. For each marker, nucleotide composition (A, T, G, C), alignment length, and mean values with standard deviations were calculated in MEGA X (www.megasoftware.net).

Phylogenetic trees were constructed using the Neighbor-Joining (NJ) method with the Kimura 2-parameter (K2P) evolutionary model. Node reliability was evaluated through bootstrap analysis with 1000 pseudoreplicates. Analyses were conducted independently for each marker and for the combined dataset. Dendrogram visualization and design were performed using FigTree v.1.4.4 (www.tree.bio.ed.ac.uk/software/figtree).

2.5. Statistics

All data analyses were performed using standard statistical and bioinformatic software. Morphometric parameters were summarized as means ± standard deviations and coefficient of variation (CV). Principal component analysis (PCA), correlation analysis, and hierarchical clustering (sectional dendrograms) were conducted in RStudio v.1.3.1093 (www.posit.co/download/rstudio-desktop) using the packages factoextra, corrplot, dendextend, cluster, and ggplot. Diagrams of the general floral structure and morphometric parameters were prepared in AutoCAD LT 2024 (Autodesk Inc., USA), which provides precise control over geometric shapes and proportions, ensuring accuracy in the illustration of diagnostic morphological features.

3. Results

3.1. Populational and Ecological Features

An assessment of plant communities containing *A. ledebourianum, A.ivasczenkoae, A. ubinicum,* and *A. schoenoprasum* in the Kazakhstan Altai showed that the studied populations are restricted to humid habitats with constant light exposure and nutrient-rich soils. The soils are predominantly mountain chernozems, and the terrain is generally flat, with slopes rarely exceeding 10°. No signs of diseases or pest damage were observed in the examined plants. The populations were in good condition, exhibiting dispersal tendencies. Age structure analysis indicated that the populations are demographically diverse, comprising individuals from all developmental stages (Figure 3).

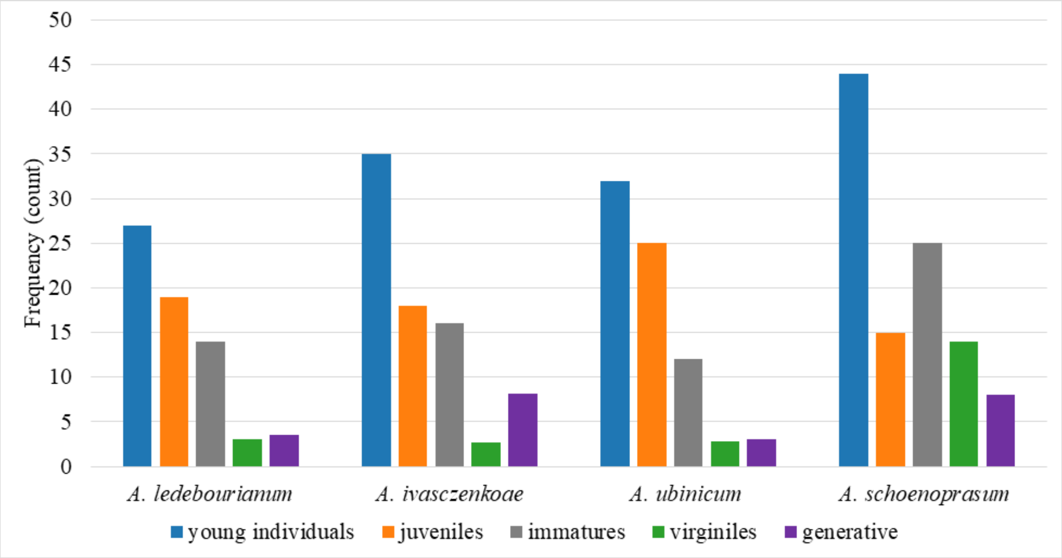

Figure 3. Population structure of four *Allium* species from the section *Schoenoprasum* based on the frequency of individuals across five ontogenetic stages.

In all four species studied, young individuals represented the most abundant ontogenetic stage, accounting for the largest proportion of each population. In *A. ledebourianum*, young plants predominated, followed by juveniles and immatures, while virginile and generative individuals were rare. *A. ivascenkoae* exhibited a similar pattern, with young individuals dominating, moderate numbers of juveniles and immatures, and few virginile and generative individuals. In *A. ubinicum*, young and juvenile individuals were most common, whereas immature, virginile, and generative plants were infrequent. *A. schoenoprasum* showed the highest number of young individuals overall, along with substantial proportions of immatures and juveniles, and smaller cohorts of virginile and generative plants. Collectively, these results indicate that populations of all species are dominated by early developmental stages, while later stages occur less frequently, reflecting species-specific demographic structures within the sampled habitats.

The associated flora of the studied *Allium* plant communities comprised 124 species belonging to 76 genera and 34 families (Table S1). Characteristic species list included *Juncus compressus* Jacq., *Calamagrostis epigejos* (L.) Roth, *Deschampsia cespitosa* (L.) P. Beauv., *Trollius altaicus* C.A. Mey., *Filipendula ulmaria* (L.) Maxim., *Geum rivale* L., *Salix viminalis* L., *Veronica longifolia* L., and *Angelica decurrens* (Ledeb.) B. Fedtsch. The most species-rich families were Poaceae, Asteraceae, and Cyperaceae.

Comparison with regional floristic lists indicated that these communities are most similar in composition and dominant families to the flora of the West Altai Nature Reserve (Table 1).

Table 1. Comparison of leading families of vascular flora communities associated with *Allium* section *Schoenoprasum* across four geographic regions (plant communities with the participation of the *Schoenoprasum* section in the Kazakhstan Altai, Koktau Mountains, West Altai Reserve, and General Kazakhstan Altai).

doi:10.20944/preprints202511.1040.v1

Family	Plant communities with the participation of the *Schoenoprasum* section in the Kazakhstan Altai		Plants of Koktau Mountains		Plants of West Altai Reserve		Plants of Kazakhstan Altai (general)	
	Number of species/ Percentage of the Total number of species, %	Number of genera/ Percentage of the Total number of genera, %	Number of species/ Percentage of the Total number of species, %	Number of genera/ Percentage of the Total number of genera, %	Number of species/ Percentage of the Total number of species, %	Number of genera/ Percentage of the Total number of genera, %	Number of species/ Percentage of the Total number of species, %	Number of genera/ Percentage of the Total number of genera, %
Poaceae	18/14.5	11/14.4	64/7.7	28/8.1	101/12.7	32/9.4	308/12.6	62/8.9
Asteraceae	12/9.6	10/13.1	129/15.5	44/12.7	100/12.6	45/13.3	324/13.3	82/11.9
Cyperaceae	11/8.8	2/2.6	32/3.8	4/1.1	34/4.3	5/1.4	96/3.9	9/1.3
Ranunculaceae	9/7.2	6/7.9	38/4.5	14/4.0	34/4.3	15/4.4	103/4.2	26/3.7
Salicaceae	9/7.2	2/2.6	16/1.9	2/0.6	27/3.4	2/0.5	56/2.3	2/0.3
Rosaceae	9/7.2	8/10.5	48/5.8	20/5.8	45/5.7	18/5.3	109/4.5	28/4.0
Fabaceae	7/5.6	3/3.9	47/5.6	15/4.3	37/4.5	11/3.2	183/7.5	24/3.5
Apiaceae	6/4.8	5/6.5	23/2.7	15/4.3	22/2.8	16/4.7	71/2.9	40/5.8
Amaryllidaceae	5/4.0	1/1.3	15/1.8	1/0.3	14/1.8	1/0.3	45/1.8	1/0.15
Geraniaceae	3/2.4	1/1.3	8/0.9	2/0.6	5/0.6	2/0.5	12/0.4	2/0.3
Total	89/71.7	49/64.4	420/50.7	145/41.8	419/52.4	147/43.5	1307/53.6	276/40.1

In *Schoenoprasum*-associated communities in the Kazakhstan Altai, Poaceae (14.5%), Asteraceae (9.6%), and Cyperaceae (8.8%) dominated. This mirrors patterns in the Koktau Mountains, West Altai Reserve, and broader Kazakhstan Altai flora, where Poaceae (7.7–12.6%), Asteraceae (11.9–15.5%), and Cyperaceae (3.8–9.6%) prevailed. The top 10 families comprised 71.7–81.0% of species across datasets, reflecting similar dominant family structures.

As for the ecological conditions, four *Allium* species from the section *Schoenoprasum* in the Kazakhstan Altai revealed distinct ecological preferences across key environmental gradients, as quantified by Ellenberg Indicator Values (EIVs) (Table S2). EIVs revealed that all studied species of section *Schoenoprasum* are predominantly heliophilous to semi-shade tolerant, with light preferences ranging from L = 7 to 8.5; the highest light demand was recorded for *A. ubinicum* (L = 8.5), whereas *A. ivasczenkoae* exhibited the lowest value (L = 7). Temperature preferences indicated adaptation to montane-temperate conditions (T = 4–5.5), with *A. ledebourianum* showing the strongest cool indication (T = 4) and *A. ubinicum* together with *A. schoenoprasum* reflecting moderate heat requirements (T = 5.5). Moisture preferences corresponded to fresh to well-moistened soils (M = 7–9), with *A. schoenoprasum* displaying the highest wetness value (M = 9) characteristic of flood meadows, while *A. ledebourianum* (M = 7.5) and *A. ubinicum* (M = 7) indicated moderately moist habitats. Soil reaction values (R = 5–5.5) suggested tolerance of slightly acidic to near-neutral conditions, with *A. ivasczenkoae* and *A. schoenoprasum* showing the broadest range. Nutrient preferences ranged from moderately to strongly nutrient-rich sites (N = 4.5–8), with *A. ledebourianum* exhibiting the highest demand (N 8) and *A. ubinicum* the lowest (N = 4.5). Salinity values (S = 0–4) confirmed that all taxa are glycophytes, with *A. ubinicum* (S = 3) and *A. schoenoprasum* (S = 3.5) showing the lowest tolerance, indicating strict confinement to non-saline habitats.

3.2. Morphometric Characteristics of Plants and Flowers

The second step was the assessment of the morphometric and quantitative traits of reproductive individuals of the four studied species based on eight parameters (Table 2).

Table 2. Morphometric parameters of *A. ledebourianum*, *A.ivasczenkoae*, *A. ubinicum*, and *A. schoenoprasum*.

Traits	Species

		A. ledebourianum	*A. ivasczenkoae*	*A. ubinicum*	*A. schoenoprasum*
Plant height (cm)	min-max	86 – 110	54 – 61.5	30 – 50	49 – 71
	M±SD	94.18±5.55	58.45±1.72	39.85±3.96	57.8±4.58
	CV, %	8.89	4.18	14.09	11.23
Number of stems per clump (count)	min-max	2 – 8	1 – 4	1 – 6	2 – 7
	M±SD	4.56±0.92	2.7±0.74	3.31±0.62	4.06±0.68
	CV, %	38.35	39.23	39.05	31.73
Number of leaves per clump (count)	min-max	68 – 150	18 – 42	7 – 15	6 – 10
	M±SD	96.5±17.04	31±5.69	11.5±2.02	7.6±1.29
	CV, %	24.17	26.03	25.01	24.18
Stem length (cm)	min-max	83 – 95	49 – 61	32 – 44	41 – 63
	M±SD	88.5±2.58	56.3±2.7	37.35±2.83	53.25±5.41
	CV, %	4.13	6.8	10.74	14.41
Stem diameter at base (mm)	min-max	5 – 8	3 – 4	2 – 4	3 – 5
	M±SD	6.4±0.59	3.6±0.36	3.1±0.4	3.6±0.49
	CV, %	13.17	14.34	18.31	19.42
Flower stalk length (mm)	min-max	44 – 86	35 – 58	19 – 33.5	30 – 41.5
	M±SD	65.7±8.10	42.85±4.72	28.7±3.04	33.8±2.86
	CV, %	17.48	15.62	15.03	12
Leaf length (cm)	min-max	86 – 104	36 – 70	38 – 54	20 – 57
	M±SD	93.6±3.72	54.45±7.86	43.15±4.61	40.1±7.46
	CV, %	5.63	20.47	15.13	26.4
Leaf width (mm)	min-max	7 – 12	2 – 5	3 – 5	2 – 3
	M±SD	9±1.12	3.8±0.65	3.7±0.47	2.5±0.37
	CV, %	18.88	24.18	18.24	21.08

M – the mean value of the trait, SD – standard deviation, min-max – minimal and maximal values, CV – coefficient of variation.

Plant height ranged 30–110 cm, highest in *A. ledebourianum* and lowest in *A. ubinicum*. Stem number per clump and leaf number were highest in *A. ledebourianum*. Leaf length averaged 83–95 cm in *A. ledebourianum* vs. 32–44 cm in *A. ubinicum*. Stem base diameter and flower stalk length varied moderately. Leaf width ranged 2–12 mm, with greatest variability in A. *ledebourianum*. CV showed moderate-high variation, especially in leaf number and height.

The morphometric characteristics of inflorescences were analyzed across four *Allium* species and presented in Table 3.

Table 3. Morphometric characteristics of inflorescences.

Trait		Species			
		A. ledebourianum	*A. ivasczenkoae*	*A. ubinicum*	*A. schoenoprasum*
Inflorescence length (mm)	min-max	25.5 – 38	16 – 23.5	20 – 25	21 – 30
	M±SD	31.25±3.22	20.15±1.49	23.2±1.36	23.6±2.35
	CV, %	14.61	10.53	8.33	14.15
Inflorescence diameter (mm)	min-max	40.5 – 56	20.5 – 33.5	22 – 36	26 – 41
	M±SD	49.2±3.39	27±3.1	28.6±3.39	34.3±3.71
	CV, %	9.78	16.3	16.82	15.37
Number of flowers per inflorescence (count)	min-max	61 – 93	18 – 22	16 – 30	24 – 44
	M±SD	74.16±11.87	20.5±1.37	19.37±3.50	35.17±7.19
	CV, %	16	6.72	22.14	20.45

M – the mean value of the trait, SD – standard deviation, min-max – minimal and maximal values, CV – coefficient of variation.

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Inflorescence length varied significantly among species, with *A. ledebourianum* exhibiting the widest range and *A. ivasczenkoae* the shortest. Inflorescence diameter showed a similar trend, with the largest in *A. ledebourianum* and the smallest in *A. ivasczenkoae*. The number of flowers per inflorescence was also highest in *A. ledebourianum* (74.16 ± 11.87) and lowest in *A. ivasczenkoae* (20.5 ± 1.37), indicating pronounced interspecific differences in floral density. The highest variability among these morphometric traits was observed for *A. schoenoprasum.*

Of particular importance in the morphometric analysis of adult reproductive individuals is the flower, the principal reproductive organ of the plant. Assessment of its structure revealed consistent and distinctive differences in morphology (Figure 4, Table 4).

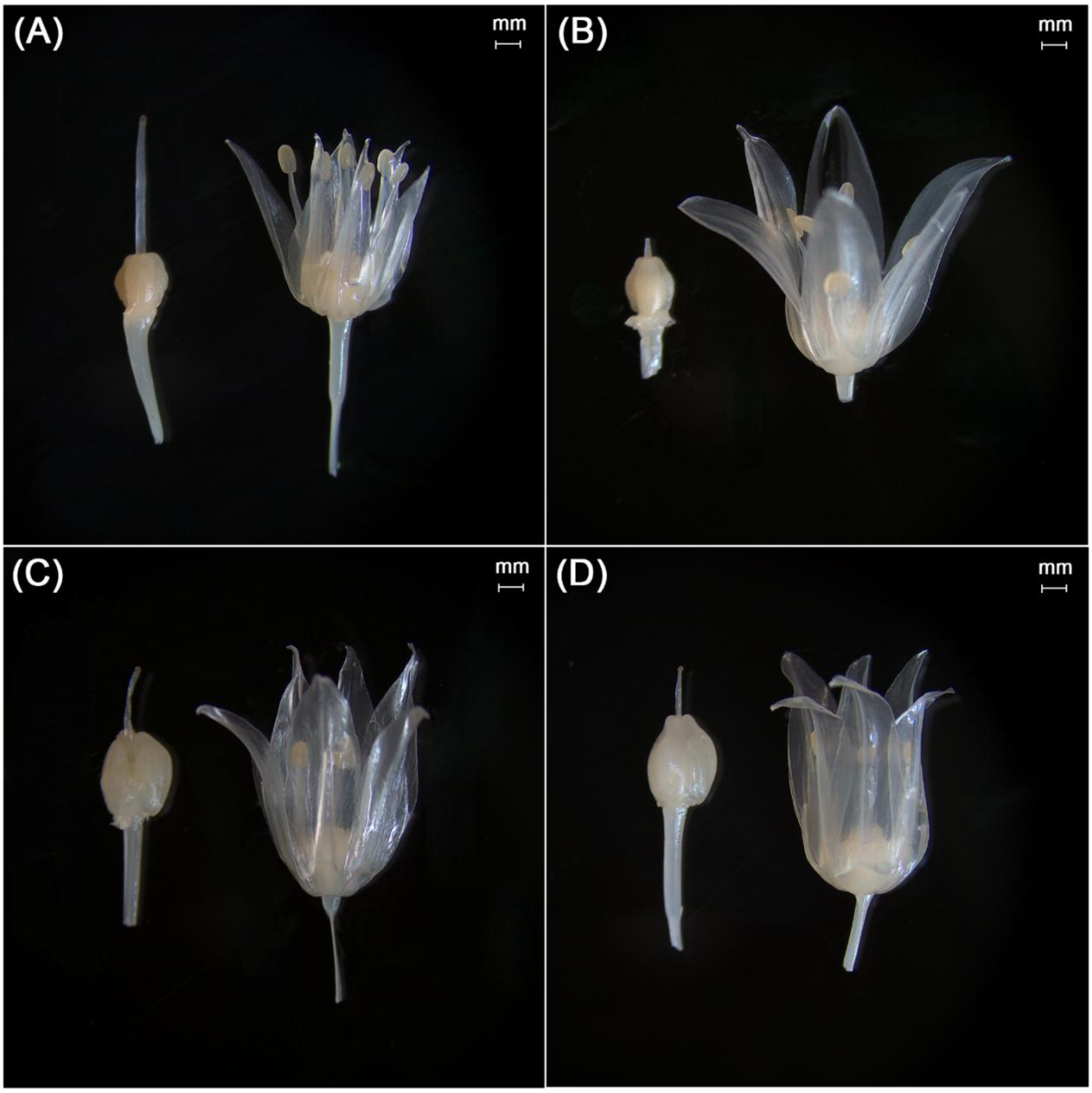

Figure 4. Flowers (on the right side) and ovary (on the left side) of species of the section *Schoenoprasum*. *A. ledebourianum* (A), *A. ivasczenkoae* (B), *A. ubinicum* (C), *A. schoenoprasum* (D).

Raw floral morphometry data is provided in Table S3. Floral morphology of the studied species showed that, although *A. ledebourianum* possesses the largest inflorescences, *A. ubinicum* and *A. ivasczenkoae* produce the largest individual flowers. Populations of *A. ledebourianum* were characterized by comparatively long stamens (5.99–6.76 mm), whereas *A. ubinicum* exhibited a relatively robust ovary (3.69 mm) (Table 4).

Table 4. Morphometric parameters of flowers in the studied *Allium* species.

Value	Petal	Ovary	Anther

	Length (mm)	Width (mm)	Stamen length (mm)	Pistil column length (mm)	Length (mm)	Width (mm)	Length (mm)	Width (mm)
Allium ubinicum Kotukhov								
M±SD	11.982±0.438	3.258±0.154	5.129±0.221	3.439±0.179	3.687±0.352	2.701±0.245	1.211±0.045	0.649±0.028
CV, %	9.554	12.356	11.270	13.399	25.002	23.802	9.646	11.281
P%	1.744	2.256	2.058	2.488	4.564	4.346	1.761	2.060
Allium schoenoprasum L.								
M±SD	10.657±0.226	3.163±0.137	4.232±0.287	3.089±0.231	2.513±0.164	2.003±0.120	1.124±0.034	0.641±0.036
CV, %	5.560	11.391	17.749	19.580	17.142	15.668	7.894	14.808
P%	1.015	2.079	3.240	3.574	3.129	2.860	1.441	2.703
Allium ledebourianum Schult. & Schult.f.								
M±SD	6.923±0.196	2.391±0.086	6.756±0.326	6.808±0.618	2.194±0.070	2.044±0.086	1.263±0.039	0.679±0.031
CV, %	7.429	9.438	12.657	13.723	8.398	10.990	8.006	11.909
P%	1.356	1.723	2.311	4.339	1.533	2.006	1.462	2.174
Allium ivasczenkoae Kotukhov								
M±SD	11.459±0.344	3.519±0.077	4.861±0.387	4.069±0.318	2.438±0.039	2.119±0.065	1.399±0.044	0.853±0.030
CV, %	7.840	5.716	20.858	11.808	4.222	7.992	8.243	9.222
P%	1.431	1.044	3.808	3.734	0.771	1.459	1.505	1.684

M±SD – mean values ± standard deviation, CV – coefficient of variation, P% – the relative error of the sample mean

A. ubinicum and *A. ivasczenkoae* exhibited larger petal sizes compared to the smaller overall dimensions of *A. ledebourianum*. *A. schoenoprasum* showed intermediate characteristics, with notable variability in ovary and anther sizes. Variability and error metrics across species suggest reliable measurements with natural dispersion, highlighting distinct morphological profiles among the *Allium* species studied.

Despite the observed diversity in floral morphology among species of section *Schoenoprasum*, the flowers of individuals from the Kazakhstan Altai can be standardized for systematic and taxonomic purposes. Morphometric analysis enabled the development of diagrams representing the mean dimensions of floral structures (Figure 5).

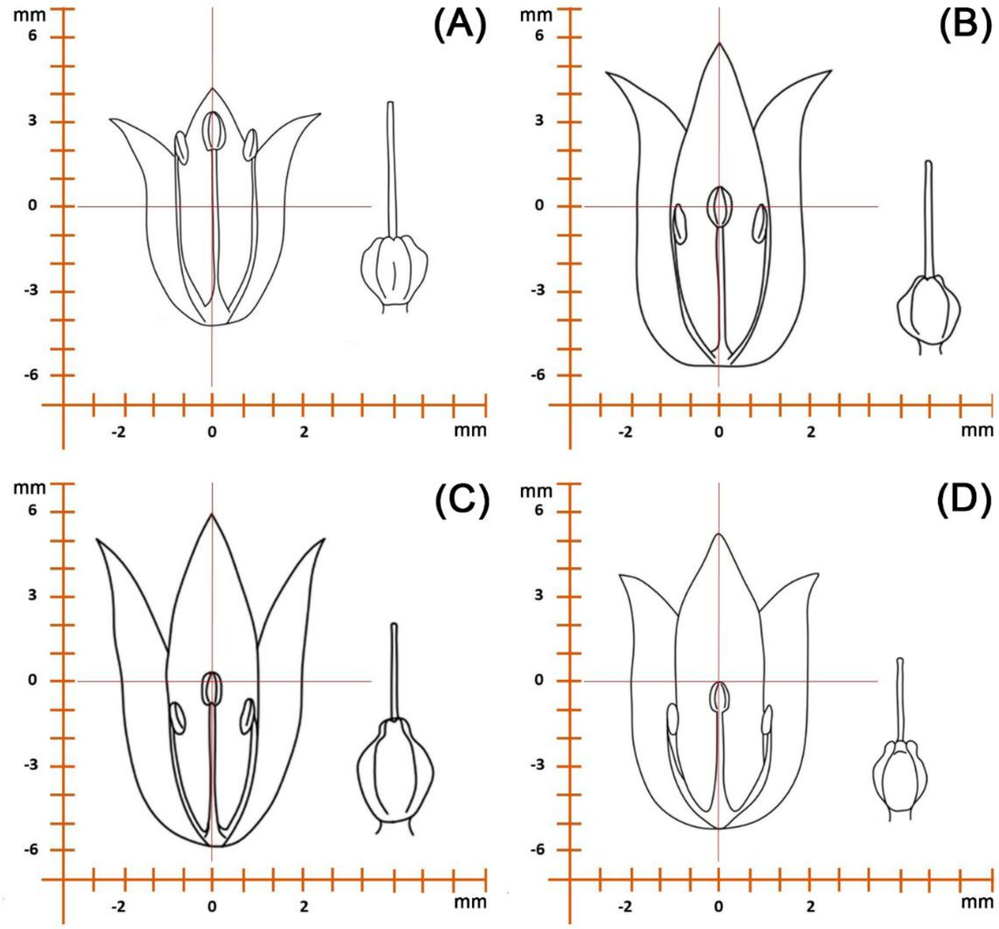

Figure 5. Diagrams of standardized structure dimensions of flowers (on the left) and ovaries (on the right) of four *Schoenoprasum* species from the Kazakhstan Altai. *A. ledebourianum* (A), *A. ivasczenkoae* (B), *A. ubinicum* (C), and *A. schoenoprasum* (D).

To determine the underlying relationships among floral architecture traits and their potential response to environmental pressures, Pearson's correlation analysis was performed on the morphometric parameters for each species, as well as on the mean values of the four *Allium* species against the relevant ecological indicator values (EIVs) (Figure 6).

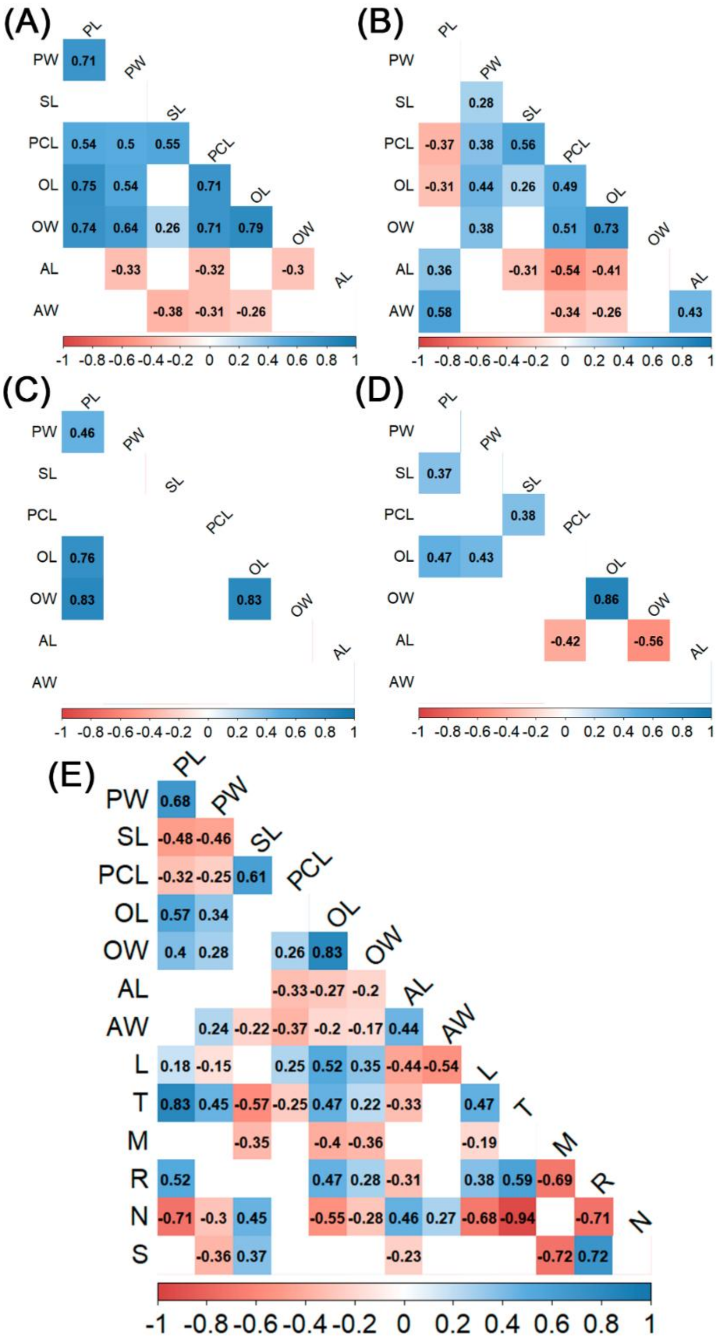

Figure 6. Correlations among morphometric parameters of *Allium* flowers and environmental factors. *A. ledebourianum* (A), *A. ivasczenkoae* (B), *A. ubinicum* (C), and *A. schoenoprasum* (D), mean morphometric parameters of four *Allium* species and EIVs (E). Positive correlations are indicated by blue, while negative correlations are indicated by red. The intensity of the color corresponds to the strength of the correlation coefficient ($P < 0.05$), with *r*-values provided. PL – petal length, PW – petal width, SL – stamen length, PCL – pistil column length, OL – ovary length, OW – ovary width, AL – anther length, AW – anther width, L – light, T – temperature, M – moisture, R – soil reaction, N – nutrient availability, and S – salinity.

In *A. ledebourianum*, strong positive correlations were observed between PL and PW, and between SL and SW (Figure 6A). Petal width also showed a strong positive correlation with OL. Conversely, significant negative correlations were noted between AL and both OW and AW. A moderate negative correlation was also found between AL and OL. In *A. ivasczenkoae*, PL exhibited a moderate positive correlation with PW (Figure 6B). SL showed positive correlations with SW and OL. Petal width also correlated positively with OL. AL displayed a notable positive correlation with AW.

Similar to *A. ledebourianum*, negative correlations were present between AL and both OW and AW. *A. ubinicum* displayed fewer significant correlations among the morphometric parameters (Figure 6C). A strong positive correlation was observed between PL and PW. A very strong positive correlation was found between OL and OW, and also between SL and PCL. For *A. schoenoprasum*, a moderate positive correlation was found between PL and PW (Figure 6D). SL also showed a positive correlation with SW. A strong positive correlation was observed between OL and OW. Distinctly, AL demonstrated moderate negative correlations with OW and AW.

As for the environmental factors, PW showed a strong positive correlation with L. PL exhibited strong positive correlations with EIVs for M and N (Figure 6E). Conversely, PL was negatively correlated with R and S. AL showed strong negative correlations with L, M, and N, while correlating positively with S and R. EIVs for T were moderately correlated with SL and negatively correlated with PCL.

To assess the multivariate patterns and relationships among the four Allium species based on their morphometric parameters, PCA and hierarchical clustering were performed (Figure 7).

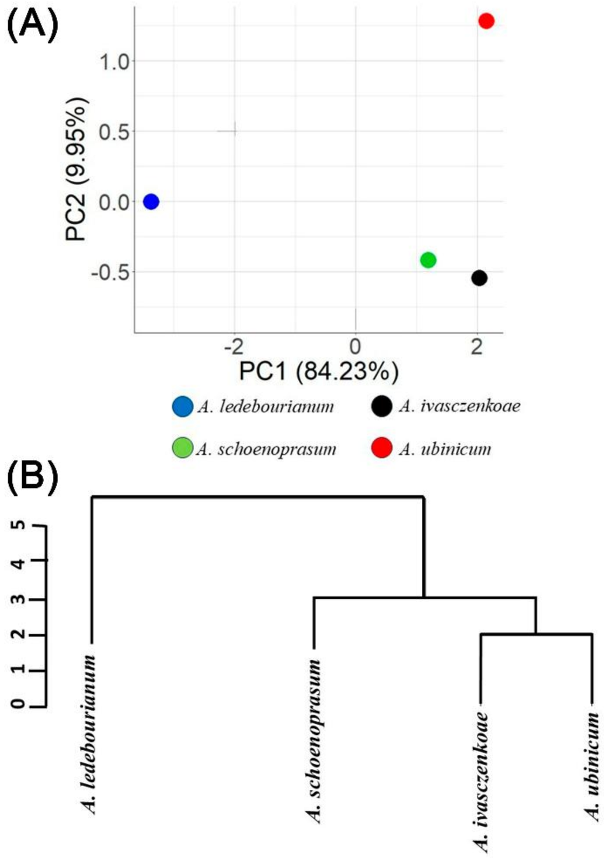

Figure 7. Principal Component Analysis (PCA) (A) and hierarchical clustering (B) of four *Allium* species based on flower morphometric parameters.

The PCA biplot (Figure 7A) illustrates the distribution of the four *Allium* species in a two-dimensional space defined by the first two principal components, which together accounted for 94.18% of the total variance in the dataset. The plot reveals a clear separation among the species, with *A. ledebourianum* and *A. ivasczenkoae* clustering closely together, indicating a high degree of similarity in their floral morphology. *A. schoenoprasum* occupies an intermediate position but is positioned closer to the *A. ledebourianum* / *A. ivasczenkoae* cluster than to *A. ubinicum*, which is distinctly separated from the others, suggesting unique morphometric characteristics. The dendrogram (Figure 7B), on the opposite, grouping *A. ubinicum* and *A. ivasczenkoae* into a single, tightly knit cluster, with *A. schoenoprasum* joining this cluster at a moderate distance. In contrast, *A. ledebourianum* remains an outlier, joining the main cluster only at the highest linkage distance, thus confirming its morphological distinctiveness.

The ANOVA results (Table S4) revealed that temperature was the predominant environmental driver influencing floral morphology in *Allium* species, showing the strongest effects across several traits, particularly petal length (PL) and petal width (PW) ($P < 0.001$). Light intensity and soil reaction also exerted significant but more selective influences, especially on ovary and anther dimensions. Notably, salinity had a pronounced effect on anther traits – both anther length (AL) and anther width (AW) – indicating high environmental sensitivity of reproductive structures to soil salinity levels. In contrast, factors such as nutrient availability and moisture showed trait-specific effects, contributing moderately to the variation in petal, style, and ovary traits. Overall, these results highlight that temperature and salinity are key determinants shaping interpopulation differences in floral morphology.

3.3. Cytotoxic and Antioxidant Activity of Ethanolic Extracts

The study demonstrated that extracts from four *Allium* species collected in the Kazakhstan Altai exhibited different levels of cytotoxic activity against *Artemia salina* (Table 5).

Table 5. Cytotoxic activity of extracts from four *Allium* species section *Schoenoprasum* of the Kazakhstan Altai.

Species	Concentration (µg/mL)	Dead/alive			Mean Mortality ± SD (%)	LC$_{50}$ (µg/mL) (95% CI)
		Rep 1	Rep 2	Rep 3		
Control (0.5% EtOH)	–	0/20	0/20	0/20	0.0 ± 0.0	–
A. schoenoprasum	1	1/20	2/20	1/20	6.7 ± 2.3	9.6 (7.8–11.5)
	5	6/20	5/20	7/20	30.0 ± 4.4	
	10	11/20	12/20	10/20	55.0 ± 4.3	
A. ivasczenkoae	1	2/20	1/20	2/20	8.3 ± 2.3	7.8 (6.4–9.7)
	5	7/20	6/20	8/20	35.0 ± 5.0	
	10	13/20	12/20	12/20	62.0 ± 3.6	
A. ledebourianum	1	0/20	1/20	1/20	3.3 ± 1.9	10.9 (8.9–11.9)
	5	3/20	4/20	3/20	16.7 ± 2.9	
	10	6/20	7/20	6/20	31.7 ± 2.3	
A. ubinicum	1	3/20	2/20	3/20	13.3 ± 2.3	5.9 (4.7–7.3)
	5	9/20	8/20	9/20	42.0 ± 3.5	
	10	15/20	14/20	13/20	70.0 ± 3.5	

The brine shrimp lethality assay revealed that ethanolic extracts of the four *Allium* species exhibited dose-dependent cytotoxic effects (Table 5). Mortality remained at 0% in the solvent control (0.5% EtOH), confirming that the ethanol fraction (≤ 0.5% v/v) did not contribute to toxicity. Among the tested species, *A. ubinicum* showed the highest cytotoxic activity, with an LC$_{50}$ of 5.9 µg/mL, followed by *A. ivasczenkoae* (7.8 µg/mL) and *A. schoenoprasum* (9.6 µg/mL). *A. ledebourianum* displayed the lowest toxicity, with an LC$_{50}$ of 10.9 µg/mL. No neurotoxic symptoms (spasmodic movements or paralysis) were observed in nauplii for any extract. The order of cytotoxic potency was therefore: *A. ubinicum* > *A. ivasczenkoae* > *A. schoenoprasum* > *A. ledebourianum*. These results indicate that ethanolic extracts of *A. ubinicum* and *A. ivasczenkoae* possess relatively higher cytotoxic potential, warranting further bioactivity-guided fractionation and compound identification studies.

To quantify the antioxidant activity of the samples, the IC$_{50}$ value was determined, representing the concentration of the extract required to reduce free radical levels by 50% (Table 6).

Table 6. IC$_{50}$ values of the studied extracts.

#	Species	IC$_{50}$, µL
1	*A. schoenoprasum*	245 ± 4.2

2	*A. ivasczenkoae*	319 ± 7.6
3	*A. ledebourianum*	-
4	*A. ubinicum*	88 ± 1.4

The results (Figure 8) show that *A. ledebourianum* exhibited no detectable antioxidant activity, as its optical density remained unchanged over time across all tested volumes, indicating the absence of radical scavenging.

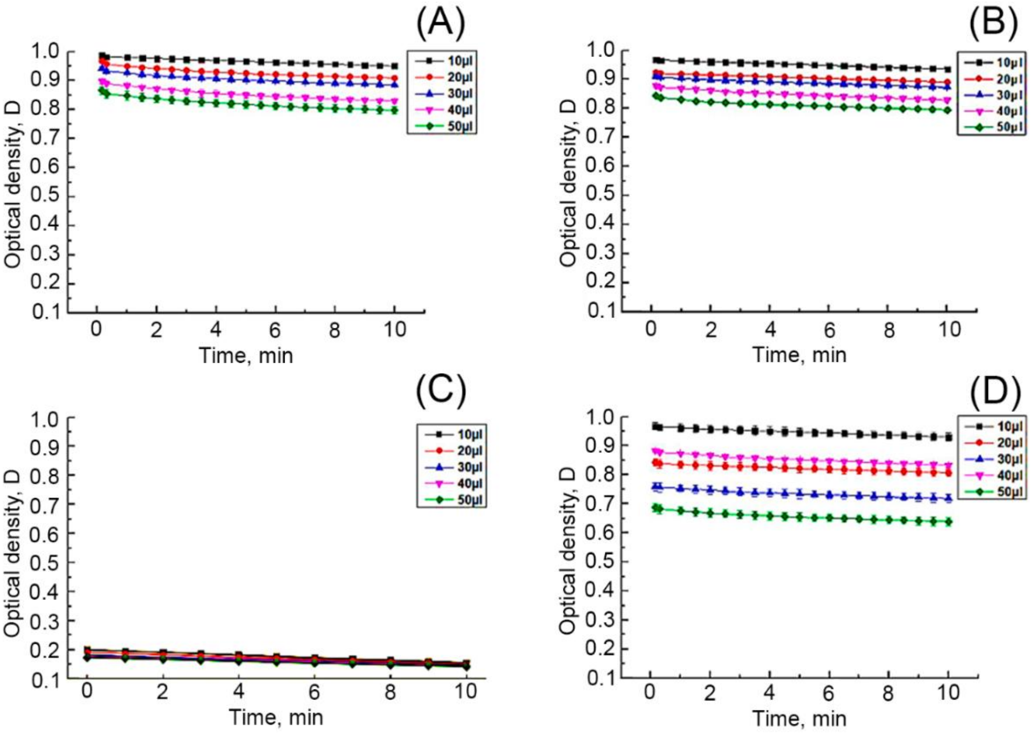

Figure 8. Antioxidant activity of *A. schoenoprasum* (A), *A. ivasczenkoae* (B), *A. ledebourianum* (C), *A. ubinicum* (D).

Among the studied species, *A. ubinicum* exhibited the strongest antioxidant activity, as indicated by its lowest IC50 value, followed by *A. schoenoprasum* and *A. ivasczenkoae*. In contrast, *A. ledebourianum* did not show detectable antioxidant activity. The decline in optical density over time was most pronounced at higher extract volumes, particularly for *A. ubinicum*, confirming its superior radical scavenging potential. Thus, the antioxidant activity of the studied *Allium* species decreases in the order *A. ubinicum* > *A. schoenoprasum* > *A. ivasczenkoae*.

3.4. Genetic Analysis

In this study, a comparative analysis of three chloroplast markers – *matK*, *psbA–trnH*, and *rbcL* – was conducted for four *Allium* species. Raw sequences are provided in Table S5. For each marker, the total aligned sequence length and nucleotide composition (A, T(U), G, C) were determined, followed by phylogenetic assessment using the Neighbor-Joining (NJ) method. The sequence statistics are summarized in Table 7.

Table 7. Nucleotide composition of three chloroplast DNA regions (*MatK*, *PsbA-TrnH*, and *RbcL*) across four *Allium* species.

Base pairs	*A. ivasczenkoae*, %	*A. ledebourianum*, %	*A. schoenoprasum*, %	*A. ubinicum*, %	Mean, %
			MatK		
T(U)	32.0	32.2	31.9	32.2	32.1
C	14.0	14.0	14.3	14.0	14.1
A	39.5	39.5	39.2	39.5	39.4

G	14.5	14.3	14.6	14.3	14.4
Total	684	100	100	100	100
			PsbA-TrnH		
T(U)	34.1	34.1	34.3	34.1	34.1
C	17.4	17.1	17.3	17.3	17.3
A	29.5	29.8	29.5	29.6	29.6
G	19.0	19.0	19.0	19.0	19.0
Total	648	100	100	100	100
			RbcL		
T(U)	28.3	28.4	28.7	28.9	28.6
C	21.7	21.6	21.3	21.3	21.5
A	28.3	28.1	27.9	27.7	28.0
G	21.7	21.9	22.1	22.1	22.0
Total	538	100	100	100	100

The *matK* region (684 bp) showed an AT-rich composition (A 39.4%, T 32.1%) typical of chloroplast coding sequences, with minimal interspecific variation (±0.3%), confirming its high conservation. The *psbA–trnH* spacer (648 bp) exhibited higher T content (34.1%) and slightly greater variability due to its noncoding nature, while *rbcL* (538 bp) was the most compositionally balanced (A 28.0%, T 28.6%, G 22.0%, C 21.5%), consistent with strong functional constraints on this gene.

Based on the aligned sequences, dendrograms were constructed for each marker using the NJ method (Figure 9).

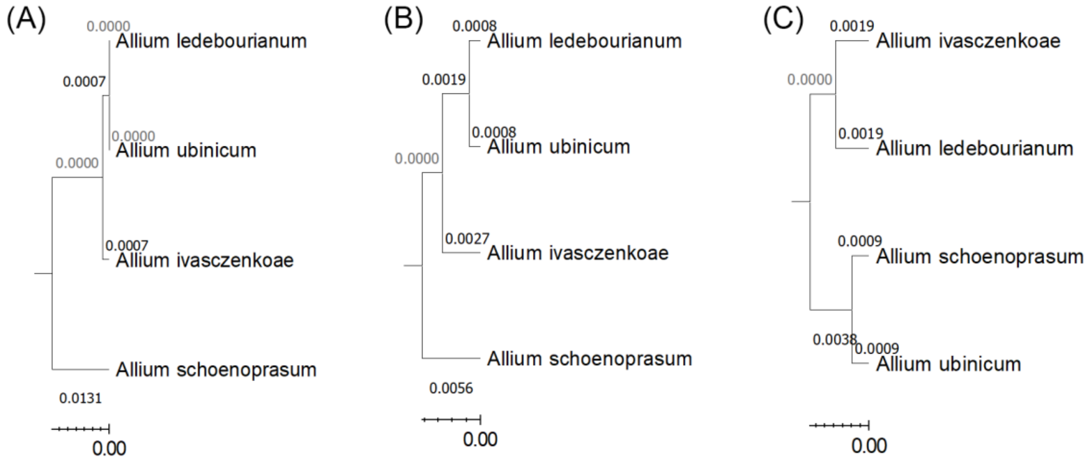

Figure 9. Phylogenetic relationships among four *Allium* species (*A. ledebourianum*, *A. ubinicum*, *A. iyasczenkoae*, and *A. schoenoprasum*) based on *MatK* (A), *PsbA−TrnH* (B), and *RbcL* (C). The horizontal scale bar represents the genetic distance, while the numbers displayed on the nodes are branch lengths between the species.

The *matK* and *psbA–trnH* phylograms revealed a consistent topology, showing *A. ledebourianum* and *A. ubinicum* as the most closely related species (pairwise distance 0.000–0.001). *A. ivasczenkoae* formed a sister clade to this pair, while *A. schoenoprasum* was the most genetically distinct, branching basally to the others. In contrast, the *rbcL* tree displayed a different topology: *A. ledebourianum* clustered with *A. ivasczenkoae* (distance 0.0019), whereas *A. ubinicum* appeared as the most divergent taxon. Thus, while *matK* and *psbA–trnH* support a close relationship between *A. ledebourianum* and *A. ubinicum*, *rbcL* indicates affinity between *A. ledebourianum* and *A. ivasczenkoae*. Such topological incongruence among chloroplast markers is not uncommon in *Allium* and may reflect heterogeneous evolutionary histories—for instance, incomplete lineage sorting, hybridization, or chloroplast capture among sympatric taxa of the section *Schoenoprasum*. Consequently, the present data should be interpreted as showing two alternative but related hypotheses: (1) *A. ledebourianum* is central to both clusters, sharing plastid similarity with *A. ubinicum* (*matK*, *psbA–trnH*) and with *A. ivasczenkoae* (*rbcL*); and (2) *A. schoenoprasum* remains consistently divergent across all markers. Further multilocus or genomic analyses (e.g., complete plastome sequencing or nuclear ITS/GBS datasets) are required to resolve these interspecific relationships more conclusively.

4. Discussion

4.1. Demographic Structure, Ecological Niche and Morphometric Differentiation of the Allium Section Schoenoprasum in Kazakhstan Altai

The ontogenetic spectra of *A. ledebourianum*, *A. ivasczenkoae*, *A. ubinicum*, and *A. schoenoprasum* are dominated by early stages (young, juvenile, immature) with few virginile and generative individuals (Figure 3). Such regeneration-biased distributions are typical for perennial herbs in montane environments and likely reflect high recruitment with slow or uncertain transition to reproductive maturity rather than decline from biotic damage – an interpretation supported by the absence of pest or disease impacts [48].

Floristically, Allium-bearing communities represent a broad meadow–steppe assemblage (124 associated vascular species) dominated by Poaceae, Asteraceae, and Cyperaceae (Table 1), consistent with regional West and Kazakhstan Altai vegetation patterns. This indicates that *Allium* populations occur within common regional matrices rather than isolated, taxonomically unique assemblages. Ecological indicator values (EIVs) further define their niches: all four taxa are heliophilous to semi-shade tolerant (L = 7–8.5), inhabit fresh to moist soils (M = 7–9), prefer neutral to slightly acidic substrates (R ≈ 5–5.5), require moderate–high nutrients (N = 4.5–8), and exhibit low salinity tolerance (S = 0–4) (Table S2). Within-section differences – *A. ledebourianum*’s higher nutrient demand (N = 8) and *A. schoenoprasum*’s preference for wetter microsites (M = 9) – reflect microtopographic specialization. The use of EIVs as ecological proxies is well established in regional floristics and provides a reliable framework for interpreting species distributions where detailed environmental data are lacking [49,50]. Comparable ecological patterns have been reported in other Central Asian endemics such as *Allium karataviense*, *A. oreoprasoides*, and *A. fedtschenkoanum*, which also occupy narrow alpine–subalpine niches and display strong specialization to light and moisture gradients in the Tien Shan and Pamir–Alai regions [51,52]. This similarity suggests that adaptive responses to steep ecological gradients are a common feature driving diversification of endemic *Allium* taxa throughout Central Asia

These demographic and ecological traits align with broader *Allium* patterns, as many taxa show strong phenotypic plasticity to light, moisture, and nutrient gradients that structure niches in montane habitats [52]. Juvenile-dominated spectra and narrow habitat preferences likely reflect life-history strategies and environmental filtering. Petal and anther traits vary with ecological factors (Figure 6E), indicating environment-driven allocation shifts – larger petals in moist, shaded sites for pollinator attraction, versus greater investment in male traits in open, nutrient-poor areas. Similar plastic floral and foliar responses are reported in *A. prattii* and related taxa along elevation and moisture gradients [53].

Multivariate analyses (PCA, clustering) separate *A. ledebourianum* and *A. ubinicum* from the remaining taxa, consistent with seed-morphology data showing *A. ubinicum*’s distinct propagule traits (larger seed mass), suggesting divergent life-history strategies within the section (Figure 7). The congruence of floral, vegetative, and seed traits supports integrated adaptive divergence or strong phenotypic plasticity across environmental gradients in the Kazakhstan Altai [54]. Overall, despite co-occurrence in humid, nutrient-rich microsites, the four species exhibit distinct demographic patterns and coordinated morphological responses to environmental factors. These findings align with broader literature on *Allium* ecological and morphological variation [50], emphasizing the combined roles of life-history strategy, environmental filtering, and plasticity in shaping species differentiation within montane meadow–steppe systems.

Vegetative and reproductive morphometrics reveal species-specific profiles: *A. ledebourianum* reaches the greatest stature and leaf number, while *A. ubinicum* is smallest but bears relatively larger individual flowers – indicating a trade-off between vigor and flower size (Tables 2 and 4). Inflorescence traits (length, diameter, flower number) vary interspecifically, reflecting divergent allocation strategies (many small vs. few large flowers) (Table 3). Correlation matrices reveal

doi:10.20944/preprints202511.1040.v1

integrated floral modules (petal and ovary dimensions) and, in some taxa, trade-offs between male and female organs (negative anther–ovary correlations in *A. ledebourianum*) (Figure 6).

The demographic and ecological features observed in the Kazakhstan Altai *Allium* taxa carry important conservation implications. The dominance of early ontogenetic stages and narrow ecological amplitudes indicate limited reproductive turnover and potential sensitivity to habitat alteration. Endemic species such as *A. ivasczenkoae* and *A. ubinicum*, confined to specific alpine and subalpine niches, may be especially vulnerable to anthropogenic pressures and climate-induced shifts in moisture regimes. Conservation measures should therefore prioritize the protection of moist meadow–steppe habitats and maintenance of population connectivity to support natural regeneration. Integrating demographic monitoring with molecular data could help identify genetically distinct or isolated populations that warrant targeted in situ and ex situ conservation efforts.

4.2. Bioactivity in Four Allium Species from the Kazakhstan Altai

The differential cytotoxic and antioxidant responses of the *Allium* section *Schoenoprasum* extracts from the Kazakhstan Altai parallel patterns observed in other *Allium* species, yet with distinctive traits. The *A. ubinicum* extract showed the strongest toxicity (LC_{50} of 5.9 μg/mL), exceeding the relatively weak effects seen in *A. ledebourianum*, which aligns with prior reports of low cytotoxicity in *A. cepa* and *A. sativum* extracts [29]. In antioxidant assays, *A. ubinicum* again performed best (IC_{50} = 88 μL), whereas *A. ledebourianum* lacked detectable radical scavenging activity. Comparable IC_{50} ranges in other *Allium* taxa (e.g. *A. rotundum* with IC_{50} ≈ 260 μg/mL) support the view that antioxidant potency varies widely even within the genus [18]. Together, these results suggest that *A. ubinicum* may harbor enriched bioactive compounds – likely phenolics or sulfur-containing molecules – that confer both moderate cytotoxicity and strong antioxidant potential, making it a promising candidate for deeper pharmacological investigation.

4.3. Phylogenetic Implications and Evolutionary Insights of Chloroplast Markers

Molecular phylogenetics is essential for clarifying evolutionary relationships within *Allium*, one of the most taxonomically complex monocot genera due to its diversity, hybridization, and polyploidy [55,56]. Using three chloroplast markers (*matK*, *rbcL*, and *psbA–trnH*), we examined four species of section *Schoenoprasum* from the Kazakhstan Altai. The *matK* and *psbA–trnH* trees each recovered two main clusters: one grouping *A. ivasczenkoae* with *A. ledebourianum*, and another uniting *A. schoenoprasum* and *A. ubinicum*. In contrast, the *rbcL* tree showed a slightly different topology, with *A. schoenoprasum* occupying an intermediate position. Although the topologies varied among markers, all analyses consistently indicated a close relationship between *A. ivasczenkoae* and *A. ledebourianum*.

The chloroplast markers differed in their resolving capacity. *MatK* produced well-supported clades, reflecting its relatively high substitution rate and established value as a core plant barcode [57]. *psbA–trnH* yielded a similar overall topology, though with lower statistical support, which is consistent with its higher variability and potential homoplasy. The congruence of these two markers supports the recognition of two main clusters among the studied taxa. In contrast, *rbcL*, a locus generally more informative at higher taxonomic levels, showed lower variability and a slightly different topology, in agreement with previous findings of limited resolution in *Allium* [58,59].

Our phylogenetic results partially align with earlier *Allium* studies based on ITS and rDNA [35,54], which also recognized *Schoenoprasum* as a distinct lineage but reported unresolved relationships among Eurasian taxa. The close association between *A. ivasczenkoae* and *A. ledebourianum* in *matK* and *psbA–trnH* trees corresponds with their morphological similarity in scape and floral traits [60]. The intermediate position of *A. schoenoprasum* in the *rbcL* tree likely reflects its wide ecological amplitude and genetic variability across its extensive Eurasian–North American range rather than evidence of recent introgression.

Incongruence among chloroplast markers is a common feature in *Allium* phylogenetics, often resulting from lineage sorting or historical hybridization events at deeper evolutionary scales [20,61]. Given the maternal inheritance of plastid DNA, our results are interpreted as reflecting maternal lineage relationships rather than direct evidence of hybridization.

The Kazakhstan Altai represents an important center of *Allium* diversity in Central Asia [60]. The close affinity between the endemic *A. ivasczenkoae* and the more widespread *A. ledebourianum* may indicate recent local divergence, possibly driven by ecological specialization of the former in alpine habitats compared with the broader distribution of the latter. Similar patterns of ecological differentiation have been documented in other *Allium* complexes [58].

Phylogenetic clustering among the Altai taxa likely results from historical isolation, post-glacial recolonization, and habitat-driven divergence. The region's complex topography and Pleistocene climatic oscillations fragmented montane flora, promoting allopatric differentiation and endemism. The relationship between *A. ivasczenkoae* and *A. ledebourianum* may represent a recent diversification event maintained by ecological separation, whereas *A. schoenoprasum*, with its broad range and heterogeneity, may have acted as a widespread ancestor or genetic bridge facilitating secondary introgression among regional lineages. Similar east–west diversification patterns across the Altai occur in other steppe–alpine taxa, reflecting shared biogeographic processes shaping Central Asian *Allium*.

Comparable evolutionary trends occur in other Central Asian endemics, such as *A. carolinianum*, *A. oschaninii*, and *A. oreoprasum* from the Tien Shan and Pamir–Alai, which also exhibit strong regional structuring and hybridization between high- and mid-altitude lineages [36]. Like our findings, these taxa show chloroplast haplotype sharing and weak interspecific barriers, indicating that introgression and ecological adaptation shaped diversification across Central Asian *Allium*. The Kazakhstan Altai thus represents part of a broader evolutionary continuum linking the mountain systems of Central Asia, where repeated glacial–interglacial cycles promoted lineage exchange, divergence, and persistence in microrefugia.

Taxonomically, our results support the distinctiveness of all four species, each forming monophyletic groups in the combined tree. The close clustering of *A. ivasczenkoae* with *A. ledebourianum* raises the question of rank, but consistent molecular, morphological, and ecological divergence supports their recognition as separate species [58]. The heterogeneity of *A. schoenoprasum* echoes ongoing debates over its intraspecific taxonomy.

While plastid markers provided valuable insights, they represent only maternal inheritance and may obscure reticulate evolution [62]. Nuclear loci such as ITS and low-copy genes, and genomic approaches like RAD-seq and genome skimming, are increasingly resolving complex groups. Future studies integrating nuclear and plastid data with morphology and ecology will be essential for clarifying diversification and species boundaries in the Altai *Allium*

5. Conclusions

All investigated *Allium* populations occurred in humid, nutrient-rich habitats on gentle slopes (<10°) with mountain chernozems. Age structure analysis showed dominance of young individuals and few mature plants, suggesting stable regeneration. The associated flora comprised 124 vascular species, mainly Poaceae (14.5%), Asteraceae (9.6%), and Cyperaceae (8.8%), typical of Altai meadow–steppe communities. Morphometric assessment revealed clear interspecific variation: *A. ledebourianum* reached up to 110 cm with large umbels (49 mm, 74 flowers), whereas *A. ubinicum* was smaller (30–50 cm) but had proportionally larger petals and robust ovaries. Correlation and PCA analyses distinguished species clusters, with *A. ledebourianum* and *A. ivasczenkoae* closely related, while *A. ubinicum* and *A. schoenoprasum* were distinct, explaining 94.2% of total variance. Floral traits were significantly influenced by temperature, moisture, light, and salinity, indicating strong morphological plasticity and adaptation to local environments. Although this study focused on phenotypic differentiation, future work combining molecular and metabolomic profiling with broader geographic sampling will better resolve evolutionary relationships, adaptive mechanisms, and chemical diversity. Overall, Altai *Allium* species demonstrate pronounced ecological specialization and adaptive morphological diversity shaped by the region's environmental heterogeneity.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Table S1: Associated species in communities involving species of the genus *Allium*, section *Schoenoprasum*; Table S2: Description of population locations of 4 *Allium* species of the section *Schoenoprasum* found in Kazakhstan Altai; Table S3: Morphometric parameters of flowers. Table S4: ANOVA of flower morphometric parameters and environmental factors. Table S5: Nucleotide sequences of *MatK*, *rbcL*, and *psbA-trnH*.

Author Contributions: Conceptualization, A.S. and A.D.; methodology A.S., Z.A., and A.D.; software, Z.A. and O.L.; validation, Z.A., A.D. and O.L.; formal analysis, A.D., O.L., Z.T., and A.O.; investigation, O.L.; resources, A.D.; data curation, O.L. and Z.T.; writing—original draft preparation, A.S., Z.A. and Z.T.; writing—review and editing, A.D., A.O. and O.L.; visualization, Z.A., A.O. and O.L.; supervision, A.D.; project administration, A.S.; funding acquisition, A.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan, scientific project AP19674778 "Study of distribution and current state of rare species (genus *Allium*) in Eastern Kazakhstan and introduction in Altai Botanical Garden" for 2023-2025.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: All data and materials supporting the findings of this study are fully available within the main text and the supplementary materials accompanying this article.

Acknowledgments: None.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

AL	Anther length
ANOVA	Analysis of variance
AW	Anther width
L	Light
M	Moisture
N	Nutrient availability
OL	Ovary length
OW	Ovary width
PCA	Principal component analysis
PCL	Pistil column length
PL	Petal length
PW	Petal width
R	Soil reaction (ph)
S	Salinity
SL	Stamen length
T	Temperature

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