### **PLANT SCIENCE**

# Dilovar Khamraeva<sup>1</sup>, Mansur Usmonov<sup>1,2</sup>, Rainer Bussmann<sup>3</sup>, Elvira Khalilova<sup>4</sup>, and Ulugbek Kodirov<sup>1</sup>

<sup>1</sup>Institute of Botany, Academy of Sciences of the Republic of Uzbekistan, ul. Durmon Yuli, 32, Tashkent, 100125, Republic of Uzbekistan
<sup>2</sup>Department of Biology Education, Kongju National University, Gongjudaehak-ro, 56, Gongju, 32582, South Korea
<sup>3</sup>Department of Ethnobotany, Institute of Botany and Bakuriani Alpine Botanical Garden, llia State University, ul. Botanikuri, 1, Tbilisi, 0105, Georgia
<sup>4</sup>S. Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, ul. Mirzo Ulugbek, 77, Tashkent, 100170, Uzbekistan
Address correspondence and requests for materials to Dilovar Khamraeva,

Address correspondence and requests for materials to Dilovar Khamraeva, hamraeva.dilovar@mail.ru

## Abstract

For the first time, the morphological and anatomical structure of vegetative and generative organs, the localization of secretory ducts in the underground and aboveground parts of the species plants were studied. New GIS technologies were used to elucidate the spatial structure of the rare endemic species Komarovia anisosperma of the monotypic genus Komarovia Korovin of the Apiaceae family. The performed analysis showed the most significant diagnostic features of the studied organs, such as a deep taproot system, the presence of a multi-branched caudex, underdeveloped stem leaves or stem leaves reduced to sheaths, the central cylinder of the main root showing separate concentric circles of large and small vascular bundles, the presence of numerous secretory ducts in the secondary root cortex, strongly elongated palisade leaf cells etc. As a result of phytochemical study, extracts of Komarovia anisosperma roots and inflorescences were analyzed. According to our data, the main component of underground organs from terpenes was  $\alpha$ -muurolene, and for inflorescences it was  $\alpha$ -curcumene. Grid mapping showed that the species is mainly concentrated in the Samarkand and Kashkadarya regions, where the most favorable conditions for its growth are found. These areas are the natural area of origin of the species, and the establishment of a protected natural area has led to an increase in the number of natural populations.

**Keywords:** anatomy, coumarins, gas-chromatography, GIS technology, grid mapping, *Komarovia anisosperma*, leaf, root, secretory ducts, terpenes

## Introduction

In the era of a dramatic increase in anthropogenic stress and the threat of an impending ecological catastrophe, a comprehensive and in-depth study of especially valuable plant species, as well as their preservation for the present and future, is especially urgent. Endemic plants often constitute the most vulnerable component of a flora, and the loss of any of them becomes irreparable for biodiversity in general. Among these rare species, endemic or relict plant species only occurring in a narrow territory, are the most important for conservation.

*Komarovia anisosperma* Korovin, one of the poorly studied taxa of the Apiaceae Lindley family, is widespread in the Samarkand and Kashkadarya regions of Uzbekistan, and the existing literature mainly focuses on its taxonomic status.

The genus *Komarovia* was described in 1939 with the only endemic species *Komarovia anisopterum* Korovin with heteromericarp fruits serving as the most

**Citation:** Khamraeva, D., Usmonov, M., Bussmann, R., Khalilova, E., and Kodirov, U. 2023. New data on the morphological and anatomical structure, chemical composition and distribution area of a rare species *Komarovia anisosperma* in Uzbekistan. Bio. Comm. 68(1): 10–20. https://doi. org/10.21638/spbu03.2023.102

Authors' information: Dilovar Khamraeva, Dr. of Sci. in Biology, Leading Researcher, orcid.org/0000-0003-3689-8660; Mansur Usmonov, Junior Researcher, orcid. org/0000-0002-2177-3171; Rainer Bussmann, Dr. of Sci. in Biology, Professor, Head of Department, orcid.org/0000-0002-3524-5273; Elvira Khalilova, Junior Researcher, orcid.org/0000-0002-2244-525X; Ulugbek Kodirov, PhD, Senior Researcher, orcid.org/0000-0003-1346-9636

Manuscript Editor: Anton Nizhnikov, Department of Genetics and Biotechnology, Faculty of Biology, Saint Petersburg State University, Saint Petersburg, Russia

Received: February 14, 2022;

Revised: June 19, 2022;

Accepted: December 12, 2022.

**Copyright:** © 2023 Khamraeva et al. This is an open-access article distributed under the terms of the License Agreement with Saint Petersburg State University, which permits to the authors unrestricted distribution, and self-archiving free of charge.

Funding: No funding information provided.

**Ethics statement:** This paper does not contain any studies involving human participants or animals performed by any of the authors.

**Competing interests:** The authors have declared that no competing interests exist.

important feature (Korovin, 1939). The first vouchers were collected by Zakirov in 1937 in the Agalyk mountains in the outskirts of Samarkand in humid places, near Sai. The genus *Komarovia* is considered a part of the tribe *Peucedaneae* Dumort, close to the genus *Ferula* L., possibly due to the similarity of the leaves and the arrangement of umbels, as in some species of *Ferula* (Shishkin, 1952; Korovin, 1959).

To establish phylogenetic relationships of the genus *Komarovia* with other taxa of the subfamily Apioideae, morphological, serological, and molecular analyses have been used (Pimenov et al., 1999). The results indicated that the genus *Parasilaus* Leute is the closest related to *Komarovia*. They are both similar in the structure of the mesocarp and endosperm, although the secretory ducts of their fruits are different. This confirmed that the inclusion of the monotypic genus *Komarovia* in the tribe *Pyramidoptereae* Boiss. and *Peucedaneae* was erroneous.

Based on the results of the ITS study of the nrDNA region in representatives of the subfamily *Apioideae*, the genus *Komarovia* was assigned to the new clade *Komaroviae* J. Zhou et S. R. Downie (Downie, Spalik, Katz-Downie, and Reduron, 2010).

*Komarovia anisosperma* is distributed in the mountainous territory of the Samarkand and Kashkadarya regions of Uzbekistan, on the northern and southern slopes of the western spurs of the Zarafshan Range. The species is a perennial herbaceous polycarpic plant that grows in shady, humid, narrow gorges, in rocks in cracks filled with fine earth, on steep gravelly slopes at an altitude of 1300–1700 m (Fig. 1A). The species flowers in June-July, and bears fruit in August-September (Pimenov, 2009).

*Komarovia anisosperma* is included in the Red Book of the Republic of Uzbekistan with status 1. It is a relict, rare and endemic species of the Zarafshan Range (Pimenov, 2009). The author, when studying the territory of the distribution of the species, found only few scattered populations with a different number of individuals (from 10 to 100) in six areas.

The chemical composition of the roots was used to elucidate the taxonomic affinity of *Komarovia anisospermum* with the species of the genus *Ferula* L. However, the variety of furocoumarin isolated from the roots did not confirm their relationship (Sokolova, Sklyar, Perelson, and Pimenov, 1976). At the same time, the furocoumarin derivatives isolated from the roots — isoimperatorin, fellopterin and 5-methoxy-8-geranyloxypsoralen, might be potentially biologically active compounds, given that they are psoralen derivatives. In this connection, this plant is of interest for more in-depth phytochemical studies.

Many species of the family Apiaceae, such as *Angelica brevicaulis* (Rupr.) B.Fedtsch., *Bupleurum exaltatum* M.Bieb., *Ferula penninervis* Regel & Schmalh., *F. prangifolia* Korovin, *F. tenuisecta* Korovin and other plants are

widely used in traditional medicine for the treatment of a number of diseases, and there is a need for their chemical study (Tunçtürk and Özgökçe, 2015; Fedoseeva and Bashar, 2016; Bouchra et al., 2017; Mamadalieva, Siddikov and Sagdullaev, 2018; Vieira et al., 2018). In recent years, geoinformation technology has become widely used in floristic studies for a variety of purposes, allowing the authors to analyze the flora of the studied region in detail (Grishutkin, 2013; Abramova et al., 2011; Seregin, 2014; etc.). The use of grid mapping allows to collect and analyze accurate data on the location of populations of a particular species in the study area. For rare species, the conservation status is assessed in accordance with IUCN Red List criteria (Gärdenfors, Hilton-Taylor, Mace, and Rodríguez, 2001; Usmonov, Tojibaev, Jang, and Sennikov, 2021).

Due to the lack of literature data on the detailed morphological and anatomical structure of vegetative and generative organs of *Komarovia anisosperma*, this study was conducted, and the conservation status of the species was assessed.

## Material and methods

Material for studying the morpho-anatomical structure of the species was collected in the Kitab State Geological Reserve in 2018. For anatomical studies, the underground axial organs and the leaves were fixed in 70% ethyl alcohol. The thickened part of the main root and the middle part of the middle segments of the leaves were dissected, cross sections were made by hand, stained with methylene blue, and enclosed in glycerol-gelatin following (Barykina and Chubatova, 2005). Microphotography was performed with a digital camera (Canon A 2300, Canon EOS 600D) and a binocular microscope (Moticam 5 N-300M). Statistic processing of quantitative data (arithmetic mean and standard error of the arithmetic mean) was carried out following Zaytsev (Zaytsev, 1990) using MS Excel.

Five samples were taken for morphological and anatomical analysis and the same number of plants were used for chemical studies.

Benzine extracts were analyzed using an Agilent Technologies 7890A GC gas chromatograph (Agilent Technologies, USA) with an inert MSD 5975 C quadrupole mass spectrometer (Agilent Technologies, USA) as a detector. The identification of compounds was based on a comparison of RI (Retention Index) and the characteristics of the mass spectra with the data of the W8N05ST.L electronic library (Wiley Registry, 8<sup>th</sup> Edition, 2006). The method of the gas chromatographymass spectrometry (GC-MS) makes it possible to determine volatile components, which in turn are extracted (dissolved) in non-polar solvents, such as, for example, extraction benzine. We chose this solvent as available.

For the GC-MS analysis, 10 g of dry crushed underground and above-ground organs of generative plants in



**Fig. 1.** Morphological and anatomical features of *Komarovia anisosperma*:  $\mathbf{A}$  — view of flowering plants;  $\mathbf{B}$  — the part of the main root;  $\mathbf{C}$ ,  $\mathbf{D}$  — view of the flowers;  $\mathbf{E}$  — a cross-section of the leaf main vein;  $\mathbf{F}$  — a cross-section of the leaf edges. Scale ruler:  $\mathbf{B}$ ,  $\mathbf{C}$ ,  $\mathbf{D}$  — 1 mm;  $\mathbf{E}$ ,  $\mathbf{F}$  — 50 µm. Symbols: **vb** — vascular bundle;  $\mathbf{sd}$  — secretory duct.

the initial phase of flowering were taken, extracted with benzine, the extracts were dried using a rotary evaporator. The determination of the qualitative composition of the extracts was carried out according to Khalilova et al. (Khalilova, Bobakulov, Aripova, and Abdullaev, 2013) and Shchipitsyna, Efremov, and Narchuganov (Shchipitsyna, Efremov and Narchuganov, 2013). To obtain ethanol extracts, 10 g of dry ground raw materials were taken, extraction was carried out with 80% ethanol three times, the extracts were combined and evaporated to a dry residue on a rotary evaporator. IR spectra were recorded from KBr (potassium bromide) pellets (Nakanishi, 1962) on a model 2000 FT-IR spectrometer (PerkinElmer, USA).

To study the distribution and determine the conservation status of rare species of all biological organisms (plants, animals, etc.), according to IUCN (IUCN, 2010), it is recommended to use a  $2 \times 2$  km scale grid mapping, in connection with which we have compiled a grid map for *Komarovia anisosperma*. Plant species distribution maps were compiled using ArcGIS 10.5 software based on a  $2 \times 2$  km grid.

For conducting grid mapping data from field expeditions (2013–2020), herbarium collections from 1937– 2010 stored in the funds of the National Herbarium of Uzbekistan (TASH), the TASH electronic database, the Red Book of Uzbekistan (Pimenov, 2009), the two-volume monograph "Flora and Vegetation of the Zarafshan River Basin" (Zakirov, 1955; 1962) and other literary sources were used. The coordinates of the locations of the plants were determined using a GPS navigator. The collection points of historical herbarium specimens were georeferenced using Google Earth.

## Results

#### Morphological structure

The root system is characterized by a deep taproot, with a multi-branching caudex and a cylindrical radish-like thickened base (Fig. 1B). The root bark is dark brown in color, thick, cracking and exfoliating, with horizontal folds wrinkled due to contractility, the root in the basal part is up to 9 cm in diameter. Lateral roots are ephemeral, while small, or scanty skeletal roots are present in the upper part of the main root.

The leaves are predominantly basal, with strong petioles, and with rigid ovate-lanceolate stalk-enclosing sheaths. The blades of basal leaves are triangular, repeatedly pinnate, slightly rigid, 30–70 cm long, and 30–45 cm wide. The segments of leaves are lanceolate, narrowed at both ends, pointed at the top, the basal leaves with petioles, and the terminal ones are sessile. The terminal segments of the leaves are 3–5.5 cm long, up to 1 cm wide. Stem leaves are smaller in size, petioles are greatly shortened, their blades are less dissected, often reduced to lanceolate amplexicaul sheaths.

The flowers are actinomorphic, 5-membered (Fig. 1C, D). The calyx is reduced, fused with the ovary, its teeth are membranous, triangular. Petals are 1.2–1.5 mm long, 1.2–1.3 mm wide, pale yellow or yellow, glabrous, obovate, depressed along the midvein, with a narrowed ligulate, inward curved apex. During flowering, the petals are slightly deflected, and at the end of flowering they are completely bent back (Fig. 1C, D). The stamens are yellow and bent inward before flowering. The ovary is semi-inferior, oblong, 1.8–2 mm long and 1.1–1.3 mm wide. 2 shortened stylodia with obtuse apical stigmas extend from the stylopodium. Stylopodium is conical, yellow, with wavy margin, 1.2–1.4 mm long, 1.5–1.7 mm wide.

#### Anatomical structure of the main root and leaf

The main root is characterized by a very multi-layered cork (Fig. 2A, B). The cork is uneven, the outer layer is dark and peeling, and the inner layer consists of oblong-flattened cells. A single layer phellogen is located under the cork, which lays the cork tissue outward, and phelloderm inward. The phelloderm consists of several (3–5) layers. The secondary cortical parenchyma is multilayered, consisting of strongly flattened or locally compressed cells, forming horizontal or transverse layers (Fig. 2B).

Due to the activity of the contractility of the root, the central cylinder is formed by separate arrays (large and small) of conducting tissue, with scattered or circular arrangement, where the phloem part is larger in volume than the xylem. In each group of vascular bundles, secretory ducts are found in oblique lines (Fig. 2C). Epithelial cells of secretory ducts are 6–8, and cubic in shape (Table 1). Closer to the cork are old, disintegrated, with empty gaps secretory ducts, while the phloem parenchyma shows well-developed secretory ducts, and directly under the cambium are smaller, young secretory ducts. In the secondary phloem, cells are densely filled with starch grains form a sheath around the epithelial

Table 1. Morphometric indicators of secretory ducts in
the vegetative organs of Komarovia anisosperma (n = 30)

Indicat	ors	Root	Leaf
Root diameter, cr	n	9	-
The number of secretory ducts per 1 mm <sup>2</sup>		23.15 ± 0.78	-
Cavity diameter of ducts, µm		92.04 ± 1.25	32.22 ± 0.41
	number	7.23 ± 0.12	7 ± 0.11
Epithelial cells	height, µm	25.6 ± 0.24	13.7 ± 0.20
	width, µm	53.46 ± 0.42	18.56 ± 0.34

± — standard error of the mean.

13



Fig. 2. The anatomical structure of the main root *Komarovia anisosperma*: A — cross-section bark of the main root, B — part of the periderm,
C — part of the section with concentric circle, D — secretory ducts in the bark parenchyma; E — part of the section in the cambia zone. Scale:
A–D — 100 µm, E — 50 µm. Symbols: b — bark; br — bark rays; c — cork; ca — cambium; ph — phloem; phl — phellogen; sd — secretory duct; sg — starch grains; v — vessel.

cells of secretory ducts (Fig. 2D). Numerous biseriate bast rays pass through the phloem, their cells also filled with starch grains. Fascicular cambium contains rectangular 5–11 layers of small, thin-walled cells (Fig. 2E). Small and large vessels form numerous groups, some oblique or sinuous due to root reduction.

On the transverse section, the leaf blade is glabrous, lamellar, in the region of the central bundle with a wellpronounced rounded-triangular projection on the abaxial side, and with the adaxial side in concave shape (Fig. 2E). Abaxial and adaxial epidermis are single-layered. The cells of the abaxial epidermis are 18-28 µm in height, 10–33  $\mu$ m in width, the cells of the adaxial epidermis are 15–26 μm in height, 11–29 μm in width. The mesophyll is dorsiventral. The palisade parenchyma consists of one layer of tightly closed, or sometimes loosely arranged, strongly elongated cells (Table 2). The spongy parenchyma is 5-layered and consists of horizontally elongated flattened cells (Table 2). The central vascular bundle is larger than the lateral bundles, there are 15-18 vessels; under the abaxial epidermis there is a small group of collenchyma cells. The central and lateral vascular bundles are of the collateral type. The phloem of the central bundle is narrow. The spongy parenchyma is interrupted in the region of the central bundle by parenchymal cells. On the abaxial side, under the central bundle and at the ends of the leaf blade under the lateral bundles, there is one secretory duct each with 6–8 epithelial cells (Fig. 2E, F; Table 1).

Table 2. Quantitative indicators of the anatomica
structure of the leaf, μm ( <i>n</i> = 30)

Indicato	Komarovia anisosperma	
The thickness of the outer	abaxial	6.24 ± 0.09
wall of the epidermis	adaxial	6.18 ± 0.1
Coll boight	abaxial	22.18 ± 0.55
	adaxial	21.1 ± 0.66
Epidormal call width	abaxial	26.14 ± 0.94
	adaxial	22.6 ± 0.78
The number of epidermal	abaxial	128.9 ± 0.86
cells per 1 mm <sup>2</sup>	adaxial	145.57 ± 0.94
% of the outer wall of (abax./ada	28.13 / 29.28	
Thicknoss	spongy parenchyma	171.4 ± 2.1
	palisade parenchyma	
Palicado caro	height	115.24 ± 1.33
	width	19.4 ± 0.32
Central vascular bundle	number of vessels	16.66 ± 0.19
	vessel diameter	16.68 ± 0.46

± — standard error of the mean.

#### Phytochemical research

The presence of coumarin derivatives in the underground and aboveground parts was confirmed by IR spectroscopy. In the IR spectrum of the alcoholic extracts of the roots and inflorescences of *Komarovia anisosperma*, absorption bands characteristic of coumarin derivatives were observed — 1704, 1629, 1580 cm<sup>-1</sup> and 1733, 1605, 1540 cm<sup>-1</sup>, respectively. The data of GC-MS analysis of benzine extracts are presented in Tables 3–5.

The results of the analysis show that the predominant compounds in the aerial part (inflorescences) were cis-ocymene,  $\beta$ -bisabolene,  $\alpha$ -curcumene, and in the roots —  $\alpha$ -copaene and  $\alpha$ -muurolene.

Table 3. GC-MS analysis of the benzine extract of the roots of *Komarovia anisosperma* 

N⁰	Compound name	RI	Content, %
1	β-Myrcene	1151	4.26
2	dl-Limonene	1181	7.37
3	(+)-Cycloisosative	1455	4.12
4	α-Copaen	1468	12.49
5	(-)-1,2,2-α,3,3,4,6,7,8,8α-dekahydro- 2α,7,8- trimethylacenaphthyl	1586	11.11
6	y-Muurolene	1656	5.24
7	α-Muurolene	1693	50.80
8	Naphtalen, 1,2,3,5,6,8a-hexahydro-4,7- dimethyl-1-(1-metylethyl)-, (1 <i>S-cis</i> )-	1725	3.21
	Total		98.60
	Unidentified		1.40

Table 4. GC-MS analysis of the benzine extract of the leaves of *Komarovia anisosperma* 

N₂	Compound name	RI	Content, %
1	<i>tran</i> s-α-Bisabolene	1680	1.80
2	ar-Curcumene	1717	4.34
3	Kamphene	1762	1.40
	Total		7.54
	Unidentified		92.46

Highly volatile components were not found in the stems, and their insignificant amount was found in the leaves (trans- $\alpha$ -bisabolene, ar-curcumene and camphene). It should be noted that  $\alpha$ -curcumene has an antiinflammatory effect (Podlogar and Verspohl, 2012). The identification of compounds was carried out according to the obtained GC-MS data, for which the percentage of probability of coincidence of the mass spectrum of the

Nº	Compound name	RI	Content, %
1	1,4- Dimethylbenzene	1124	0.05
2	β-Pinene	1149	0.12
3	D-Limonene	1181	0.54
4	1,8-Cineol	1191	0.07
5	β-Ocimene	1209	0.97
6	<i>cis</i> -Ocimene	1217	6.92
7	1-Methyl-3-(1-Methylethylene) Benzene	1223	0.04
8	γ-Terpinene	1267	0.03
9	trans-Ocimene	1288	0.04
10	β-Curcumene	1514	0.42
11	Caryophyllene	1565	0.69
12	1.4-Dihydro-3,5-dimetoxy-2- Methylnaphthalene	1628	0.08
13	Di-epi-α-cedrene	1639	0.44
14	<i>cis</i> -β-Farnesan	1649	1.02
15	β-Bisabolene	1705	4.73
16	α-Curcumene	1749	41.30
	Total		57.49
	Unidentified		42.51

Table 5. GC-MS analysis of the benzine extract of the inflorescences of *Komarovia anisosperma* 

substance is not lower than 75%. We did not identify substances that did not meet these requirements.

The purpose of the analysis of the volatile compounds of *Komarovia anisosperma* was to compare their content in different organs of the plant. *K. anisosperma* is a monotypic species of the genus *Komarovia*. Therefore, there is nothing to compare it with, and of several genera which are closely related, only *Sphaerosciadium denaense* grows on the territory of Uzbekistan. The chemical composition of this species has not been studied, it is also a rare and endemic plant. In this connection, the identified substances in any case serve as species-specific signs for the species *K. anisosperma*.

#### Distribution area

According to the botanical and geographical zoning (Tojibaev, Beshko, and Popov, 2016), the distribution area of the studied species is a part of the Kuhistan district, which includes the Zarafshan Range. Based on the results of the analysis of the available data on the distribution of *Komarovia anisosperma* on the western spurs of the Zarafshan Range, grid mapping was conducted, dividing the study area into cells, in which only

15 cells were found occupied by *Komarovia anisosperma* (Fig. 3–4). According to the seven-point system of Seregin (2014), *Komarovia anisosperma* belongs to the rare species of the Zarafshan Range. The map showed that the species is concentrated in a narrowly-localized area, and that its range covers two neighboring regions (4 points in Samarkand region and 11 points in Kashkadarya region).

# **Discussion and conclusions**

The morphological and anatomical structure of the vegetative organs of some representatives of the Apiaceae family was studied with the aim of gaining a deeper understanding of their ecological significance and changes in the internal organization of plants that arose under the influence of various growing conditions. Different life forms have been described in Apiaceae species growing in central Russia. Based on characteristics like life expectancy, the number of fruits and the structure of underground organs, 14 biomorphological types have been identified. In this region Apiaceae are dominated by short-rhizome polycarpic or monocarpic species with taproots or adventitious roots. The data obtained made it possible to develop biomorphological characters, i. e., systematic characters that can be applied in the study of taxonomy, ecology, or the economic use of taxa (Petrova, 2015). As a result of our earlier comparative study of the morphological and anatomical structure of underground axial organs in the generative phase of the development of Kamelinia tianschanica F.O. Khass. et I. I. Malzev and Korshinskya olgae (Regel & Schmalh) Lipsky we found species-specific characters for these structures (Khamraeva, 2019). According to our data, the first species is a polycarpic plant with a vertical epigeogenic short rhizome with numerous adventitious roots, and the second species is a monocarpic plant with a shortened main root and lateral roots. Komarovia anisosperma is a polycarpous species with underground organs represented by a multi-branched caudex, deeply penetrating the main root, and, in its upper part, by scanty skeletal lateral roots. According to the classification of Krasilnikov (1968), Apiaceae species form nine main types of root systems, depending on the growing conditions. The author believes that in drier conditions, plants with a deep taproot system predominate, and that most of these species are perennial polycarpics. It should be noted that the ecological and biological characteristics of species affect the formation of their root system. Komarovia anisosperma, due to the duration of its growing season, and growing in narrow gorges, cracks in rocks, on steep gravelly slopes in the middle belt of mountains, forms a deep tap-root system, serving as a storage site for organic matter and water, allowing the species to thrive in harsh conditions. As a result of



Fig. 3. Distribution grid map of Komarovia anisosperma.



**Fig. 4.** Extent of Occurrence (EOO, shows one possible boundary to the extent of occurrence, which is the measured area within this boundary) And AOO, shows one measure of the area of occupancy which can be achieved by the sum of the occupied grid squares) analysis; red squares — an appropriate scale of 2 km<sup>2</sup> cells (a cell area) as the reference scale recommended by IUCN. (EOO / AOO) Extent of Occurrence-686.908 km<sup>2</sup>, Area of Occupancy-56.000 km<sup>2</sup> (EN-Endangered). Evaluation by criteria IUCN Red List VU B1B2ab (ii).

our study of the anatomical structure of the main root in *Komarovia anisosperma*, the presence of numerous secretory ducts in the bark and starch grains in the cells of the parenchyma, the absence of mechanical tissue in the xylem, the formation of cavities, cracks and breaks in the secondary cortex (crustal parenchyma) and the central cylinder from for contractility were found. In this polycarpous species terpenoid substances are intensively stored annually in the root.

Safina and Isaeva (Safina and Isaeva, 1981a) found in Ferula iliensis Krasn. ex Korovin a deep-taproot type of root system, multi-layered cork, strong parenchyma of the thickened part of the root with almost all cells filled with starch grains, splitting of the central cylinder into numerous centroxylem conducting bundles, the presence of numerous secretory ducts in the phloem part of the bundles, etc. Komarovia anisosperma shows similarity in the structure of underground organs, although Ferula iliensis is classified as a perennial monocarpic species but grows in mountainous conditions. Consequently, it appears that environmental conditions are the most important determining factor in the structural features of underground organs. In addition, the root is an important organ for the accumulation of biologically active substances. According to the available literature data, these biologically active substances are enclosed in secretory ducts, which have a protective role (against herbivorous insects, evaporation, solar radiation, pathogenic effects of microorganisms, etc.) mainly due to ether compounds, which are often complex resins (Vasiliev, 1977; Khalilova et al., 2013; Tkachenko, 2013; Parisa, 2014; Amiri and Joharchi, 2016). Thus, for the first time, benzine extracts of the underground and aboveground parts of the Komarovia anisosperma were analyzed using chromato-mass spectrometric analysis. It was shown that the main component of the roots is α-muurolene, and the main component of inflorescences is α-curcumene.

Studies on leaf anatomy of Apiaceae species were carried out in order to determine the systematic position or adaptability to natural or introduced conditions (Nesmeyanova, 1960; Tyurina, Guskova, and Valutskaya, 1976; Safina and Isaeva, 1981b; 1985; 1988; Imanbaeva, Sarsenbaev, and Sagyndykova, 2015; Khamraeva, 2018; Idman, Ulusoy, Karakaya, and Bani, 2019; Bani and Idman, 2020; etc.). According to these sources, characteristic mesophyll types of Apiaceae are dorsiventral, isolateral-palisade isopalisade (sometimes centric) and rarely isolateral-spongy, i.e., homogeneous. According to some researchers (Tyurina, Guskova, and Valutskaya, 1976), the dorsiventral mesophyll and wide-lobed leaves in the species of the genus Ferula reveal a genetic relationship with the mesomorphic structure and their origin from mesophilic ancestors. More complex rearrangements in leaves with an isolateral-palisade or centric type of mesophyll are associated with the effect of elevated air temperature and insolation, leading to plants acquiring xeromorphic features during evolution.

According to the modern systematic interpretations based on the results of the ITS analysis of the nrDNA locus, Komarovia anisosperma and Sphaerosciadium denaense (Schischk.) Pimenov et Kljuykov are assigned to the clade Komarovieae (Downie, Spalik, Katz-Downie, and Reduron, 2010). When studying the leaf structure of Sphaerosciadium denaense, we noted a dorsiventral type of mesophyll with a single-layered palisade parenchyma (Khamraeva, 2018). In contrast, the leaf of Komarovia anisosperma is characterized by a thickened leaf blade, taller palisade cells, small cells, and less thickened outer walls of the abaxial and adaxial epidermis, as well as a very small number of secretory ducts (only 3 throughout the blade). Thus, Komarovia anisosperma shows large basal complex leaves with a lanceolate plate, and a dorsiventral type of mesophyll indicates the mesomorphic origin of the species, but due to the length of the growing season, xeromorphic characters (thickened leaf blade, high palisade cells, small epidermal cells stem leaves) were acquired in the course of evolution to mountain habitat conditions.

Pimenov and Kluykov (Pimenov and Kluykov, 1981) critically examined the systematic position of some genera in the Apiaceae using morphological and anatomical features of generative organs, including those of Sphaerosciadium denaense. According to these authors, Sphaerosciadium denaense has large diamond-shaped, completely flat, white petals that are not bent at the apex, but pointed at the end. However, the flower structure in Komarovia anisosperma is completely different from this closely related species. Komarovia anisosperma petals are yellow, obovate, with a narrowed ligulate, inward curved apex. At the beginning of flowering, the petals are slightly deflected, and at the end of flowering they are completely bent back. The structural features of the flower are characterized by the shape of petals and the changes in their position before and after flowering.

The results of our grid mapping data made it possible to reveal the pattern of distribution of the rare and endemic species Komarovia anisosperma in the natural habitats of the floras of the Samarkand and Kashkadarya regions. Tojibaev et al. (Tojibaev, Beshko, Qodirov, and Akbarov, 2019) in their work revised the status of a number of rare species of the Zarafshan Range, including our object of study. During 2010-2019, the authors obtained new data on the number of individuals in populations, clarified the ranges, identified new places of growth of rare and endemic species of the Zarafshan Range and compiled maps according to IUCN. According to their data and our observations in the protected area of the Kitab State Geological Reserve, the number of individuals of Komarovia anisosperma has increased and there is no acute picture of the disappearance of the species.

Field studies conducted over the past decades (Tojibaev et al., 2019) provide a reason to lower the threat status of Komarovia anisosperma to 2, for a new edition of the Red Book of the Republic of Uzbekistan. This reflects the positive trend in the number of populations and individuals of the species, especially in the protected natural area of the Kitab State Geological Reserve, where, without the influence of anthropogenic factors, the vegetation cover is being stabilized. In addition, the authors assessed Komarovia anisosperma based on the IUCN Red List (IUCN, 2010) criteria, according to which the species can be classified as VU B1B2ab (a species included in the national Red Book in need of protection). The grid map of Komarovia anisosperma yielded 7 localities in the western part of the Zarafshan Range. As mentioned above, we identified 15 distribution points for Komarovia anisosperma. The species mainly concentrates in areas where the most favorable conditions for dispersal are found.

Based on the results of the morphological (underground and aboveground organs) and anatomical (structure of the main root and leaves) study of mature generative specimens of *Komarovia anisosperma*, the following most taxonomically significant characters were identified:

- the root system is of the deep-stem type with a multi-branched caudex, the main root is radishlike thickened at the base, cylindrical in the rest; the central cylinder of the main root shows separate arrays (large and small) of conducting tissue, in a scattered or circular arrangement, and with a large volume phloem;
- it features basal complex large, multiply pinnately dissected leaves with lanceolate entire segments, and stem leaves are poorly developed or reduced to lanceolate stalk-enclosing sheaths; the mesophyll is dorsiventral, consists of strongly elongated palisade cells, the secretory ducts are located on the abaxial side under the central and at the ends of the leaf blade under the lateral vascular bundles;
- the teeth of the calyx are triangular; the narrowed top of the ligulate petals, curved inward, during flowering they are slightly deflected, and at the end of flowering they are completely bent back.

#### References

- Abramova, L. A., Volkova, P. A., Borisova, P. B., and Mitireva, E. A. 2011. Preliminary results of grid mapping of the flora of the Udomelsky district of the Tver region. *Tver State University Bulletin. Series: Biology and Ecology* 24:127–143. (In Russian)
- Amiri, M. S. and Joharchi, M. R. 2016. Ethnobotanical knowledge of Apiaceae family in Iran: A review. *Avicenna Journal of Phytomedicine* 6(6):621–635.
- Bani, B. and Idman, D. 2020. New morphological and anatomical data on the genus *Fuernrohria* (Apiaceae). *Phy-*

*totaxa* 429 (1):048–056. https://doi.org/10.11646/phytotaxa.429.1.3

- Barykina, R. P. and Chubatova, N. V. 2005. Great workshop on botany (Ecological anatomy of flowering plants.) 77 pp. T-vo nauchnykh izdanii Publ. Moscow. (In Russian)
- Bouchra, S.-A., Talou, T., Saad, Z., Hijazi, A., and Merah, O. 2017. The Apiaceae: Ethnomedicinal family as source for industrial uses. *Industrial Crops and Products* 109:661– 671. https://doi.org/10.1016/j.indcrop.2017.09.027
- Downie, S. R., Spalik, K., Katz-Downie, D. S., and Reduron, J. P. 2010. Major clades within Apiaceae subfamily *Apioideae* as inferred by phylogenetic analysis of nrDNA ITS sequences. *Plant Diversity and Evolution* 128(1–2):111–136. https://doi.org/10.1127/1869-6155/2010/0128-0005
- Fedoseeva, L. M. and Bashar, D. B. 2016. The study of saponins in the underground organs of *Ferula hermonis*. *Chemistry of Plant Raw Materials* 1:181–184.
- Gärdenfors, U., Hilton-Taylor, C., Mace, G. M., and Rodríguez, J. P. 2001. The Application of IUCN Red List Criteria at Regional Levels. *Conservation Biology* 15(5):1206–1212. https://doi.org/10.1111/j.1523-1739.2001.00112.x
- Grishutkin, O. G. 2013. Possibilities of applying GIS technology in botanical research. *Bulletin of the Mordovian University* 3–4:16–20. (In Russian)
- Idman, M., Ulusoy, F., Karakaya, M., and Bani, B. 2019. Comparative vegetative anatomy of the genera *Grammosciadium, Caropodium* and *Vinogradovia* (Apiaceae) in Turkey. *Phytotaxa* 427(1):9–21. https://doi.org/10.11646/ phytotaxa.427.1.2
- Imanbaeva, A. A., Sarsenbaev, K. N., and Sagyndykova, M. S. 2015. Anatomical structure of the aboveground and underground organs of *Ferula foetida* (Bunge) Regel in natural populations of Mangystau. *Siberian Ecological Journal* 6:899–908. (In Russian)
- IUCN. 2010. Guidelines for Application of IUCN Red List Criteria at Regional Levels: Version 4.0. IUCN Species Survival Commission. IUCN, Gland, Switzerland and Cambridge, UK. https://www.iucnredlist.org/technical-documents/ categories-andcriteria
- Khalilova, E. Kh., Bobakulov, H. M., Aripova, F. S., and Abdullaev, N. D. 2013. Secondary metabolites of *Ferula foetida*. *Chemistry of Natural Compounds* 49(1):141–142. https:// doi.org/10.1007/s10600-013-0535-y
- Khamraeva, D. T. 2018. Structure of leaves and localization of secretory ducts in *Sphaerosciadium denaense* (Apiaceae). *Turczaninowia* 21(2):5–12. https://doi.org/10.14258/turczaninowia.21.2.1 (In Russian)
- Khamraeva, D. T. 2019. Comparative study of underground organs of *Kamelinia tianschanica* and *Korshinskya olgae* (Apiaceae). *Turczaninowia* 22(4):57–69. https://doi. org/10.14258/turczaninowia.22.4.7 (In Russian)
- Korovin, E. P. 1939. *Komarovia* Eug. Kor. A new Asian genus family Umbelliferae. In the book: to the President of the USSR Academy of Sciences (Academician Vladimir Leontyevich Komarov in connection with his 70<sup>th</sup> birthday) Leningrad, pp. 427–428. (In Russian)
- Korovin, E. P. 1959. *Umbelliferae*; pp. 257–470 in Flora of Uzbekistan. Vol. 4. Fan. Tashkent. (In Russian)
- Krasilnikov, P. K. 1968. The subterranean organs of Angelica descending Ledeb. Proceedings of the Botanical Institute Academy of Sciences of the USSR, Leningrad 5(15):36–106. (In Russian)
- Mamadalieva, N. Z., Siddikov, D. R., and Sagdullaev, Sh. S. 2018. Medicinal plants of the Apiaceae and Rutaceae families from the Chimgan mountains (Uzbekistan): Ethnopharmacology, Chemical Composition and Biological Activities. *Current Traditional Medicine* 4(3). https://doi.or g/10.2174/2215083804666180416125733

- Nakanishi, K. 1962. Infrared Absorption Spectroscopy. 233 pp. Tokyo Kyoiku University. Tokyo.
- Nesmeyanova, A. D. 1960. Comparative anatomical study of leaves of two *Ferula* species. *Botanical Journal* V(XLV):1542–1546. (In Russian)
- Parisa, S. 2014. Traditional uses, phytochemistry and pharmacological properties of the genus *Peucedanum*: A review. *Journal of Ethnopharmacology* 156:235–270. https://doi. org/10.1016/j.jep.2014.08.034
- Petrova, S. E. 2015. Life forms of Apiaceae in central Russia. *Nordic Journal of Botany* 33(6):747–753. https://doi. org/10.1111/njb.00808
- Pimenov, M. G. 2009. *Komarovia anisosperma* Korovin; pp. 116–117 in Red Book of the Republic of Uzbekistan: Rare and endangered species of plants and animals (Plants and mushrooms). Vol. 1. Chinor ENK Publ. Tashkent. (In Russian)
- Pimenov, M. G. and Kluykov, E. V. 1981. Materials for the taxonomy of *Korshinskya*, *Physospermum*, *Astomatopsis* and related genera *Umbelliferae* — *Smyrnieae* s. str. *Botanical Journal* 66(4):465–482. (In Russian)
- Pimenov, M. G., Shneyer, V. S., Valiejo-Roman, K. M., Terentieva, E. I., and Troitsky, A. V. 1999. *Komarovia* Korovin: a multidisciplinary study of a genus of uncertain position. *Komarovia* 1:61–73.
- Podlogar, J. A. and Verspohl, E. J. 2012. Antiinflammatory effects of ginger and some of its components in human bronchial epithelial (BEAS-2B) cells. *Phytotherapy Research* 26(3):333–336. https://doi.org/10.1002/ptr.3558
- Safina, L. K. and Isaeva, S. N. 1981a. To the anatomy of *Ferula iliensis. Tidings of the Academy of Sciences of the Kazakh SSR* 2:85–86. (In Russian)
- Safina, L. K. and Isaeva, S. N. 1981b. Anatomical analysis of the vegetative organs of two endemic species of *Ferula*. *Tidings of the Academy of Sciences of the Kazakh SSR* 6:8– 16. (In Russian)
- Safina, L. K. and Isaeva, S. N. 1985. About the plasticity of *Ferula* leaf. *Tidings of the Academy of Sciences of the Kazakh* SSR 4:91–93. (In Russian)
- Safina, L. K., and Isaeva, S. N. 1988. Features of the anatomical structure of *Ferula* leaf; pp. 142–152 in Study of medical plants in Kazakhstan. Alma-Ata. (In Russian)
- Seregin, A. P. 2014. The spatial structure of the flora of the Vladimir region. Dr. Sci. in Biological Sciences thesis, Moscow, 518 pp. (In Russian)
- Shipitsyna, O. S., Efremov, A. A., and Narchuganov, A. N. 2013. Determination of coumarins and furocoumarins in various vegetative organs of *Angelica decurrens* (Ledeb.) Fedtsch. by gas chromatography-mass spectrometry. *Sorption and Chromatographic Processes* 13(6):928–938. (In Russian)

- Shishkin, B. K. 1952. *Umbelliferae* Umbel; pp. 223–225 in Flora of the USSR. Vol. 16. Academy of Sciences of the USSR Press. Moscow; Leningrad. (In Russian)
- Sokolova, A. I., Sklyar, Yu. E., Perelson, M. E., and Pimenov, M. G. 1976. Furocoumarins *Komarovia anisospermum. Chemistry of Natural Compounds* 1:166–169. (In Russian)
- Tkachenko, K. G. 2013. Essential oil plants of the Apiaceae, Asteraceae and Lamiaceae families in the North-West of Russia (biological features, composition and prospects of using essential oils). Dr. Sci. in Biological Sciences thesis abstract, St. Petersburg, 40 pp. (In Russian)
- Tojibaev, K. Sh., Beshko, N. Yu., and Popov, N. Yu. 2016. Botanical-geographical regionalization of Uzbekistan. *Botanical Journal* 101(10):1105–1132. (In Russian)
- Tojibaev, K. Sh., Beshko, N. Yu., Qodirov, U. X., and Akbarov, F. I. 2019. Endemic and rare species of the flora and identification of key botanical territories in the western part of Zerafshan range. *Bulletin of Gulistan State University, Series Natural and Agricultural Sciences* 4:9–20. (In Russian)
- Tunçtürk, M. and Özgökçe, F. 2015. Chemical composition of some Apiaceae plants commonly used in herby cheese in Eastern Anatolia. *Turkish Journal of Agriculture and Forestry* 39:55–62. https://doi.org/10.3906/tar-1406-153
- Tuyrina, E. V., Guskova, I. N., and Valutskaya, A. G. 1976. Apiaceae of Southern Siberia as a material for introduction. 256 pp. Nauka Publ. Novosibirsk. (In Russian)
- Usmonov, M. X., Tojibaev, K. Sh., Jang, C-G., and Sennikov, A. N. 2021. Species conservation profile and amended distribution of *Cousinia knorringiae* (Asteraceae), a narrow endemic of the Western Tian-Shan. *Biodiversity Data Journal* 9:e64115. https://doi.org/10.3897/BDJ.9.e64115
- Vasilev, A. E. 1977. Functional morphology of plant secretory cells. 206 pp. Nauka Publ. Leningrad. (In Russian)
- Vieira, J. N., Gonçalves, C. L., Villarreal, J. P. V., Gonçalves, V. M., Lund, R. G., Freitag, R. A., Silva, A. F., and Nascente, P. S. 2018. Chemical composition of essential oils from the Apiaceae family, cytotoxicity, and their antifungal activity in vitro against candida species from oral cavity. *Brazilian Journal of Biology* 1–6. https://doi.org/10.1590/1519-6984.182206
- Wiley Registry, 8<sup>th</sup> Edition / NIST. 2005 Mass spectral library. 2006.
- Zakirov, K.Z. 1955. Flora and vegetation of the Zeravshan river basin. Vol. 1. 205 pp. Academy of Sciences of the Uzbek SSR Press. Tashkent. (In Russian)
- Zakirov, K.Z. 1962. Flora and vegetation of the Zeravshan river basin. Vol. 2. 446 pp. Academy of Sciences of the Uzbek SSR Press. Tashkent. (In Russian)
- Zaytsev, G. N. 1990. Mathematics in experimental botany. 296 pp. Nauka Publ. Moscow. (In Russian)