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MORPHOLOGICAL, ANATOMICAL STRUCTURE AND MOLECULAR PHYLOGENETICS OF *ANTHEMIS TROTZKIANA* CLAUS

K. IZBASTINA[1], M. KURMANBAYEVA[1], A. BAZARGALIYEVA[2], N. ABLAIKHANOVA[1], Z. INELOVA[1], A. MOLDAKARYZOVA[3], S. MUKHTUBAEVA[4], Y.TURUSPEKOV[1]

[1]Department of Biodiversity and Bioresources, Faculty Biology and Biotechnology, Al-Farabi Kazakh National University, Almaty, Kazakhstan
[2]Department of Biology, Natural Sciences Faculty, K. Zhubanov Aktobe Regional State University Aktobe, Kazakhstan
[3]Asfendiyarov Kazakh National Medical University, Kazakhstan, Almaty
[4]Institute of Botany and Phytointroduction, Astana, Kazakhstan
Corresponding author's email zhanat_2006@mail.ru

Abstract

In this study, morphological and anatomical properties of a rare species *Anthemis trotzkiana* Claus were investigated. Morphology structure of flower, seed, leaf, root and anatomical structure of root, stem, leaves and molecular phylogenetics *Anthemis trotzkiana* from Aktobe region of the Kazakhstan are also studied. *Anthemis trotzkiana* Claus (*Asteraceae*) is a rare and an endemic species of the Volga region and the Western Kazakhstan. The species is calcefite, occurs on sediments of cretaceous rocks and for research features substratum were studied regarding chemical structure of soil from different horizon. The anatomical results showed that the roots have tetrachium xylem rays and schizogenic channels. When comparing the anatomical structure of virginal roots in three populations, it was found that the morphometric parameters of plants in the 1-2nd populations were high, while the data of the 3rd population were lower. The epidermis of the leaf is strongly cutinized and leaves are isolateral, the palisade mesophyll is found on both sides of the leaf. This is peculiar to xerophilous plants. The abundance of essential oils clearly indicates the healing characteristics of the plant and is the basis for studying of essential oils of the leaf.

In the paper, also were determined unique molecular markers of the species and used for the creation of a phylogenetic tree.To clarify the taxonomic provision of rare *A. trotzkiana* phylogenetic analysis based on the change of the sequence ITS nrDNA of *Anthemideae* representatives was conducted. For molecular resear chDNA analysis on phylogeny of *A. trotzkiana* was conducted based on ITS (internal transcribed spacers) markers. Alignment of *Anthemis*sequences was performed using nucleotide sequences available at the NCBI and MEGA 6 package. The Neighbor Joining phylogenetic tree suggested that *A. trotzkiana* along with *A. marschalliana, A. futiculosa,* and *A. calcarea* form a single cluster within *Tanacetum* clade, while other *Anthemis* species formed a separate *Anthemis* clade.

Key words: Aktobe region, Rare soil, Morphology, Anatomy, Flowers, Phylogenetic tree.

Introduction

The *Asteraceae* is one of the morphologically most advanced families of the dicotyledons. The family is one of the largest families of flowering plants with close to comprising of 43 tribes, 1600-1700 genera and some 24000 species. The members of the family are easily recognized by the presence of some characteristic features, namely capitulum with a specialized mechanism of pollen presentation and pappus structure, nature of fruit and features of seeds. Due to the great variability of morphology and anatomy of tissue composition of vegetative and reproductive structures, they are pertinently fascinating to the taxonomist(Paul, 2017; Bremer, 1994; Stevens, 2001; Donoghue *et al.*, 1998; Spjut, 1994).

The family *Asteraceae* has a fairly wide range of distribution, which includes all 6 inhabited continents; they are cosmopolitan. Most of the family species are herbs, a significant number is also shrubs, vines or trees (Panero and Crozier, 2012; Shahid, 2014).

Nevertheless, the detailed analysis of the molecular networks involved in formation of complexes of inflorescences of *Asteraceae*, initiation of floral rudiments and coordination of their different growth which are still not solved substantially. An example of big difference in flower and inflorescences are the *Asteraceae* family which bears difficult inflorescences which of structurally and functionally different species of flowers (Broholm *et al.*, 2014).

Taxonomic and genetical (Karanovic *et al.*, 2015; Hoda´Lova´*et al.*, 2015; Andre´S-Sa´Nchez *et al.*, 2015; Ruvimbo & Glynis., 2016) studies have been conducted to identify relationships among *Asteraceae* taxa.

The *Asteraceae* family is divided into 2 subfamilies: *Asteroideae*, which integrates the vast majority of family and includes up to 12 species (Funk *et al.*, 2009). With the last general abstract of the tribe of Anthemideae (Asteraceae), the tribe consists of 111 generas and 1800 species worldwide. The majority of species of *Anthemideae* are concentrated in Central Asia, the Mediterranean region and the southern regions of Africa species of a tribe are well-known as aromatic plants, and some are used for pharmaceutical or have pesticidal value (Oberprieler *et al.*, 2007) withal, classification of sub tribe has led to considerable difficulties in all taxonomical history of a tribe.

Many morphological, anatomical studies (Karaismailoğlu, 2015; Özcan *et al.*, 2015; Candan *et al.*, 2016; Norouzı *et al.*, 2016; Ivashchenko & Ivarenko, 2017), palynological (Türkmen *et al.*, 2010; Akyalcın *et al.*, 2011; De Abreu *et al.*, 2015) were carried out in recent years.

Due to numerous attempts, developments of satisfactory taxonomy for tribes have been made. In this, the most important source of information is phylogenetic researches. Despite some taxonomical questions in Anthemideae, J. Brik uses symbols of a pericarp for the description of the Mediterranean genera in a tribe (Kim & Jansen, 1995).

Molecular phylogenetic researches on the basis of ITS for the species *Anthemis* (Oberprieler, 2004, 2005, Oberprieler *et al.*, 2006) have shown that *Anthemis subg.*

is more closely connected with *Tripleurospermum* and *unspecific Nananthea*, than the species which are earlier processed in *A. subg. Cota*. It has led to recognition of *Cota* as an independent species and a reproduction of number of species *Anthemis* in this species. The joint analysis of all these aspects has revealed important and various mechanisms participating in formation of distribution of the species *Anthemis* (Greuter *et al.,* 2003).

To define relative geographical and ecologic-climatological differentiation of the species of *Anthemis* (*Compositae, Anthemideae*) to the Mediterranean region, genealogy of the species of *Anthemis* has been received with use of a method with data internal transcribed spacers (ITS) of ribosomal DNA (nrDNA) and chronology of diversification**(**Lo Presti & Oberprieler, 2009).

The general limits of Anthemis were disputable and barely understood for a long time. Several previous sub childbirth (for example, *Chamaemelum* and *Cota*) has received the general rank now. The present report corresponds to the last molecular phylogenetic researches and *Anthemis* s.str follows clearly ribbed seed. *Anthemis* and *Cota* are only morphologically various, there are also phytochemical, karyological and cytogenetic species. Thus, *Anthemis* still contains 175 species, the most widespread are in Europe, Southwest Asia and North Africa. In Belgium two species are a family (perhaps, archeophytes): *Anthemis arvensis* L. and *A. cotula* L. (Lambinon *et al.,* 2004). Both are originally confined to several fields and became much rarer in the past decades (Lo Presti *et al.,* 2010). Unique molecular markers among the three *Anthemis* species collected from different locations in Saudi Arabia, was creation of a phylogenetic tree was determined by the method of random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR).

Also was analyzed genetic diversity among 15 populations three species of *Anthemis* (*A. melampodina, A. pseudocotula* and *A. bornmuelleri*) collected from different locations in Saudi Arabia using RAPD primers. It is shown that the RAPD-PCR method is an accurate tool, including for establishing plant relations between Anthemis species (Hasan, 2017).

Many species among *Anthemis* are characterized as rare, endemic or being under the threat of disappearance (Cigdem, 2016). Species of *Anthemis* are widely used in pharmaceutics, cosmetics and food industry. Flowers of the species have good antiseptic properties, the main components are natural flavonoids and essential oils (Vaverkovaet *et al.,* 2007).

Genus *Anthemis* L. (Asteraceae) is presented in flora of Turkey by 81 taxa belonging to 51 species 29 of which are endemics for Turkey (Albayrak & Aksoy, 2013), most of them are applied to folk and traditional medicine **(**Başer *et al.,* 1997). In Iran 39 species are included to the species *Anthemis* from which 15 endemic species showing about 38% of an endemism are found (Shokoohinia *et al.,* 2015).

In the check list of the Italian vascular flora of *Anthemis* L. is presented 25 specific and inside the specific taxa located in different sections (Conti *et al.,* 2005). Near 35 species are widespread in Greece from which 14 are endemic (Grierson, 1975). In flora of Europe there are 61 species (Fernandes *et al.,* 1976), corresponds to ser. *Rumata* Fed. 1961, which includes other perennial species endemic to *Caucasus* (A. *marschalliana* and A. *futiculosa*) or with a west- Asian distribution (A. *calcarea*) **(**Flora Kazahstan, 1966) *while at Serbia and Montenegro nine species* (Gajic & Josifovic, 1975) are present. Each has appearance peculiar only to its morphological features in which it differs from other species. A. *chrysantha* (with yellow flowers on a rather convex disc of 12–25mm in diameter), have peduncles up to 6cm in length. The receptacle is hemispherical to oblong– ovoid, and rounded at the apex(Tutin *et al.,* 1980). Morphological characteristics of some species *Anthemis chrysantha* J. Gay and *A. samariensis* of genera *Anthemis* were studied (Aguado, 2012).

In the territory of the CIS there are 51 species of *Anthemis* L. and in the ninth volume of Flora of Kazakhstanis described 6 species of Kazakhstan: - *A. trotzkiana* Claus, *A. candidissima* Willd. ex Spreng. A. *tinctoria* L., A. *cotula* L., *A. microcephala* (Schrenk) B. Fedtsch., *A. deserticola* Krasch. ex M. Pop. (Flora of the SSSR, 1961).

The species of *Anthemis* L. (Asteraceae) are stored in some herbaria of Kazakhstan, 5 species from six have been found in flora of Kazakhstan, the abstract on these found species is also for the first time described (*A. trotzkiana* Claus., *A. candidissima* Willd., *A. tinctoria* L., *A. cotula* L., *A. microcephala* (Schrenk) B. Fedtsch.), it should be noted that the species of *Anthemis deserticola* Krasch.& M. Pop., has not been found by anybody and demands carrying out further researches on identification of locations of the type (Izbastina *et al*., 2017).

A. trotzkiana Claus (Asteraceae) is a rare species meeting on cretaceous breaks and limestones. *Anthemts trotzkiana* is distributed in the middle part of Volga, the southern part of Urals, and the northwestern part of Kazakhstan (Red List of Kazahstana, 2014). The plant investigated showed the chromosome number of 2n= 18. The chromosome number and the karyotype of this species are reported for the first time in the present study. The karyotype consisted of 16m and two sm chromosomes, A satellite was located on the terminal of the short arm of two m chromosomes (Kokubugatai *et al.,* 2002).

The analysis of the current state of populations of rare plant species of the natural monument Trinity Chalk Mountains (Orenburg Region)made by researchers of Orenburg Botanical Garden. According to the research on the ecological and biological characteristics of four rare calciphyte species including *Anthemis trotzkiana* the current state of the populations was analyzed for the first time on the territory of a natural monument (Karimova *et al.,* 2017). *A. trotzkiana* Claus (Asteraceae) - refers to species that are threatened with extinction and needs to monitor the abundance and rate of reproduction, as well as in measures that contribute to the conservation of their habitat. That's why to identify the cause of the disappearance of this species it is necessary to conduct detailed study of morphological and anatomical structures of the plants.

Our research focuses on the study of endangered species on the territory of the Chalk Mountains: *Anthemis trotzkiana* Claus. The biology, structure, and status of the "red list" populations of plant species that are extremely rare in Kazakhstan and worldwide have not been studied and research in this area is therefore relevant.

The aim was to study the morphology and anatomical characteristics and the status of rare species populations from the Chalk Mountains. One task was to compare indicators and the distribution of three populations by the types of developmental spectra and to study the morphometric parameters morphology and anatomical structure, molecular phylogenetics of the rare and endangered endemic species *A. trotzkiana* (Asteraceae) of the Aktobe region (Kazakhstan).

Materials and Methods

Route-reconnoitering method has been used in the process of field research. Collection of material was made on cretaceous breaks and limestones in August, 2016-2018 years in the Aktobe region.

In case of the species determination there were considered the main morphological features in a blossoming phase. During the expedition for the purpose of a further morphologic-anatomic research of *A. trotzkiana* plants were collected in a herbarium.

During the research of the morphological structure of the object, stereoscopic binocular "MBS-10" was used, researches were conducted at department of the Biodiversity and bioresources, al-Farabi Kazakh National University. For anatomical studies conservation of plants was carried out by Strasburger-Flemming. Anatomical preparations were made according to standard techniques (Prozina, 1960, Permjakov, 1988, Barykina, 2004). An anatomical structure of plants were studied in the national nanotechnological laboratory in al-Farabi Kazakh National University with use of optic microscope Leica DM 6000 Mwith the high-allowing digital cameras and the software for the analysis and saving images. Statistical processing of morphometric indicators was carried out by Lakin, 1990.

Elemental analysis of the above ground and under ground parts of the plant was carried out on the FOCUS - 2M X-ray fluorescence spectrometer.

Direct sequencing of two samples from *A. trotzkiana* was carried out using of the nuclear ribosomal DNA internal transcribed spacer region (ITS) and Genetic Analyzer 3130 (Applied Bio systems, USA). Genetic distances between genotypes by analyzing genes were calculated using Neighbor-Joining method (Saitou & Nei, 1987) based on nucleotide sequences alignment of 23 species of the genus *Anthemis* L., including 21 species downloaded from the NCBI database (https://www.ncbi.nlm.nih.gov). Phylogenetic tree was produced using Neighbor-Joining method and MEGA, version 5 (Tamura *et al.*, 2011). This work was supported byNTP 0237/PTF-14 granted by the Ministry of Education and Sciences of the Republic of Kazakhstan.

Results

Three populations of a rare, endemic and endangered plant species *Anthemis trotzkiana* Claus (Asteraceae) from the territory of the Aktobe region were found. The first population is Akshatau, the second population is Bestau and the third population is the boric mountains of Ishkaragantau. The geographical location of the populations found was revealed (Fig. 1. A, D, G).

Morphological characters of the *Anthemis trotzkiana* Claus (virginal plants): Signs of maturity of the plant *Anthemis trotzkiana* Claus appeared at the virginal stage, and no generative organs were formed. The height of the plant is 4-7 cm. The plant leaf is petiolate, length is 3.4-4.77 cm and width is 0.2 cm. The pair feathery leaf is fleecy, located along the radius, and the color is whitish (Table 1; Fig. 1B, E, H).

Morphological characters of the *Anthemis trotzkiana* Claus (generative plants): *Anthemis trotzkiana*Claus is a perennial subshrub, up to 27.2-49.3 cm in height and diameter 18.3-35.3 cm. As typical for Asteraceae the main root is thick, and lateral roots are thin. However, they are good enough to survive on chalky limestone slopes. The woody root produces numerous shortened erect stems. The lower part of the stem is dark brown. On the bases of the stem there is rosette of the leaves (Fig. 1C, F, I).Stems branched and slightly woody at base, leafless or with a few small leaves, sometimes glabrous or sub glabrous. The erect stems of the plant are made up of small leafless thin (0,2 cm) and fragile branches, each carrying one basket. The number of generative shoots ranges from 3 up to 6. The number of leaves per plant ranges in the interval of 21-53. The leaves are twice pinnately divided, petiole bases are attenuate. Mean values of the length of the leaves per populations are 3.4-4.7 cm, while width of the leaves ranges in the interval 1-1.9 cm. Lamina of the leaf is not fleshy or if slightly fleshy, covered by hairs. The young leaves of this species are pale green and the older leaves are green (Table 2; Fig. 1C, F, I).

Table 1. Morphological characteristics of the*Anthemis trotzkiana* Claus in Aktobe region (virginal plants).

Character	Populations					
	1-Akshatau		2-Bestau		3- Ishkarahantau	
	Mean	CI	Mean	CI	Mean	CI
Plant						
Height, cm	5.83	0.7	7.07	0.5	4	2.1
Diameter, cm	5.19	0.7	35.3	3.9	4.48	4.5
Leaves						
Number, pcs.	36.6	3.5	53.3	9.7	21.3	2.9
Length, cm	4.77	0.8	4.7	0.3	3.4	0.5
Width, cm	0.2	0.1	1.9	0.2	1	0.2

Table 2. Morphological characters of the *Anthemis trotzkiana* Claus in Aktobe region (generative plants).

Character	Populations					
	1- Akshatau		2- Bestau		3- Ishkarahantau	
	Mean	CI	Mean	CI	Mean	CI
Plant						
Height, cm	32.2	1.1	49.3	4.1	27.2	2.1
Diameter, cm	18.3	2.7	35.3	3.9	29.3	4.5
Shoots						
Number, pcs.	3.4	0.7	6.1	1.3	2.7	0.5
Height, cm	31.0	1.1	48.2	4.2	26.2	2.1
Thickness, cm	0.2	0.05	0.3	0.05	0.2	0.05
Leaves						
Number, pcs.	27.3	5.1	53.3	9.7	21.3	2.9
Length, cm	3.8	0.4	4.7	0.3	3.4	0.5
Width, cm	1	0.1	1.9	0.2	1	0.2
Flowers						
Number, pcs.	3.6	0.9	17.9	4.8	3.1	0.9
Inflorescence length, mm	1.2	0.09	1.2	0.08	1.1	0.09
Diameter, mm	1.9	0.1	2.2	0.1	1.3	0.1
Number ray florets, pcs.	10.8	0.7	11.3	1.4	9	0.6
Number disc florets, pcs.	52.6	2.1	63.9	3.0	44.2	3.4
Seeds						
Number of fruits of a capitula, pcs	43.6	3.7	45.7	4.0	33.62	3.6
Length, mm	1.9	0.09	2.1	0.1	0.9	0.1
Thickness, mm	0.99	0.11	1.04	0.09	0.55	0.11
Mass of 100 pcs, d.m., g	0.037	0.005	0.047	0.004	0.033	0.005

Disk flowers are bisexual, on the apex denticles of the crown are blunt, the form of achenes is primal, oblong, four-folded. To the base few narrowed, smooth, truncated achenes, which on the upper edge have a short, finely toothed margin (Table 2; Fig. 1).

Anthemis fruit achene, unicellular, extended, not cracking nutlet with a ligneous cover. Length is 2, 31 mm, width of the top part of a fruit varies from 695,57, the middle part 576,84, part 403,79 down narrowed, and the most lower part makes 202,80 μm. For studying phylogenetics type, flowers of *A. trotzkiana* collected in the Aktobe region flower spike is long with single calathids, one plant has 4-5 calathids of average size (Table 2; Fig. 2).

Anatomical structure of the *Anthemis trotzkiana* Claus (generative plants)

Root

1 – The cross section of the root of the Akshatau population consists of the rhizodermis, the primary cortex and the central cylinder, consisting of conducting tissue. In the anatomical structure of the root of an annual virginal tree of the 1st Akshatau population, the rhizodermal cells are quadrangular, large and homogeneous, the rhizodermis consists of a single layer of cells. The outer surface of the rhizodermal cells' membranes is cutined. The exodermal cells consist of a layer of cells of various shapes and sizes. Colorless parenchymal cells, located evenly in the primary cortex, form 9-10 layers. The primary cortex ends with an endoderm consisting of a single layer of small cells. The central cylinder starts with a pericycle. The xylem in the middle of the central cylinder is a three-comb triarchal. The xylem elements are composed of large xylem vessels. Surrounding the xylem, a cambium is formed. The phloem elements located between the pericycle and cambium are three-frame, sclerenchyma is developed in the lower part of the phloem (Table 3; Fig. 3.A).

2 – In the cross section of the root of the Bestau population's virginal plant, the rhizodermis consists of a layer of densely located cells of medium size and similar forms. The exodermal cells are slightly different. Unevenly located 7-8 layers of the primary cortex's parenchyma. The primary cortex is completed by an endoderm consisting of small round-shaped cells. The central cylinder begins with a pericycle, consisting of homogeneous distinctly distinguishable cells. It is clearly seen that the xylem rays originate from the three-comb triarchal xylem. In the recess of the three combs there is a phloem. In the phloem sclerenchyma is found (Table 3; Fig. 3B).

3 – In the anatomical structure of the root of the Ishkaragantau population's virginal tree, the rhizodermal cells are reduced. Exodermal cells differ in a large, multifaceted and diverse form. 6-7 layers of parenchymal cells form the primary cortex. The parenchyma of the primary cortex is egg-shaped. The endoderm and primary cortex are complete. The pericycle layer is clearly distinguished. In the central cylinder the xylem is a two-frame. The phloem is developed on both sides from the cambium (Table 3; Fig. 3C).

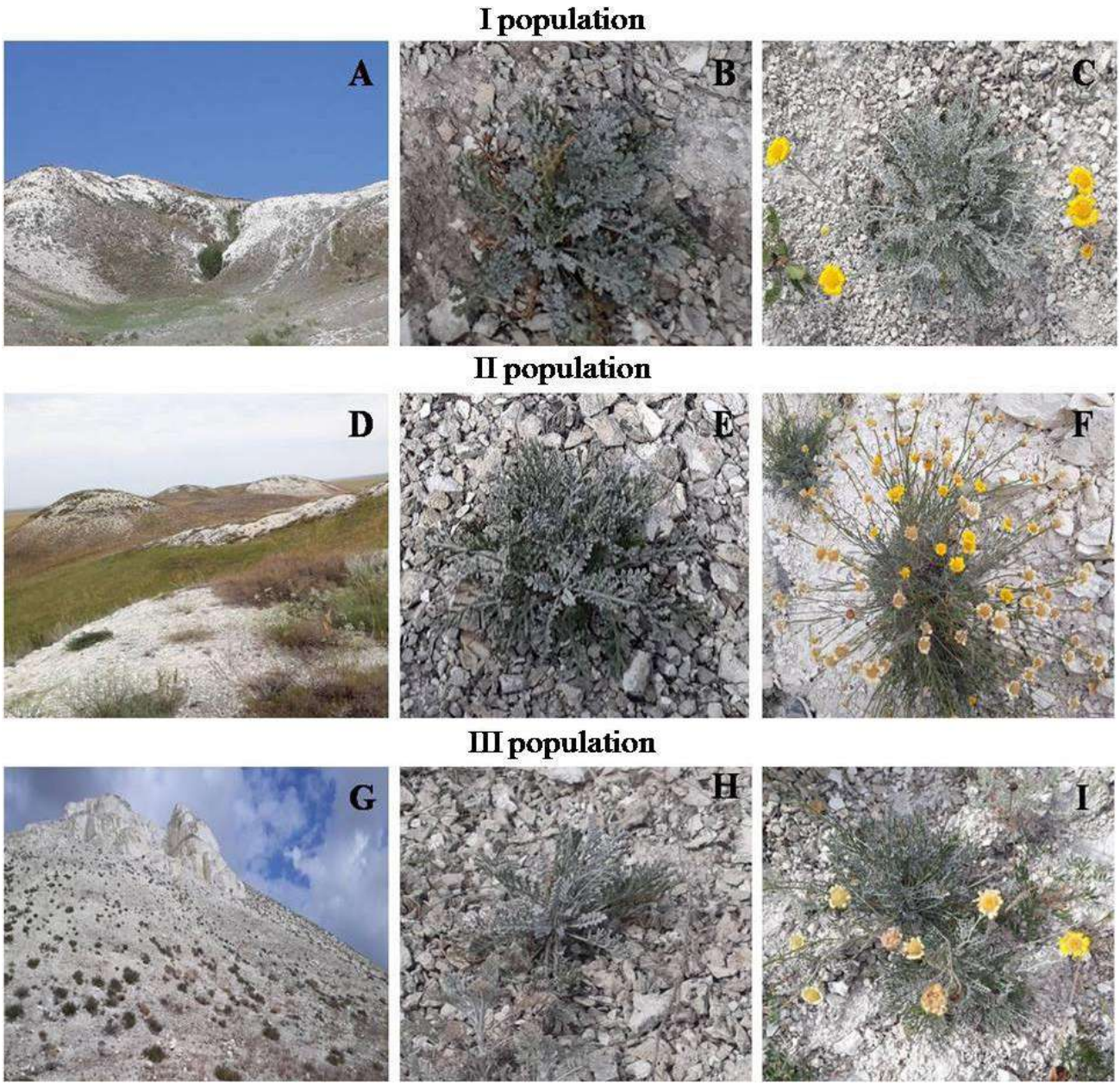

Fig. 1. General view of the *Anthemis trotzkiana* Claus individuals in the chalky mountains Akshatau (A), Bestau (D), Ishkarahantau (G) of Aktobe region.

Table 3. Anatomicalcharactersof the*Anthemis trotzkiana* Claus in Aktobe region (virginal plants).

Characteristics	Populations		
	1-Akshatau	2-Bestau	3-Ishkaragantau
Root			
Rootdiameter	697.11 ± 13.17	700.2 ± 37.01	617.12 ± 11.21
Rhizodermisthickness	27.02 ± 4.02	35.01 ± 6.21	37.31 ± 8.24
Thickness of the primary cortex	176.01 ± 26.13	192.3 ± 21.11	154.0 ± 6.79
Diameter of the central cylinder	301.51 ± 18.01	265.08 ± 26.08	205.03 ± 4.18
Thickness of the xylem vessels	17.98 ± 2.02	17.52 ± 1.78	14.12 ± 5.13
Leaf			
Thickness of the upper epidermis	23.12 ± 2.14	32.03 ± 6.21	15.8 ± 3.52
Thickness of the lower epidermis	24.07 ± 15.11	34.11 ± 4.58	27.66 ± 2.87
Thickness of the upper palisade mesophyll	130.0 ± 4.94	172.0 ± 4.10	157.12 ± 3.41
Thickness of the lower palisade mesophyll	225.2 ± 65.01	148.1 ± 36.12	147.0 ± 65.22
Thickness of the spongy mesophyll	311.2 ± 11.74	270.1 ± 18.23	226.21 ± 10.13
Diameter of the main vascular bundles	109.69 ± 42.31	140.8 ± 11.12	58.31 ± 7.59
Thickness of the central part of the leaf	734.07 ± 20.06	723.42 ± 2.74	624.09 ± 14.21

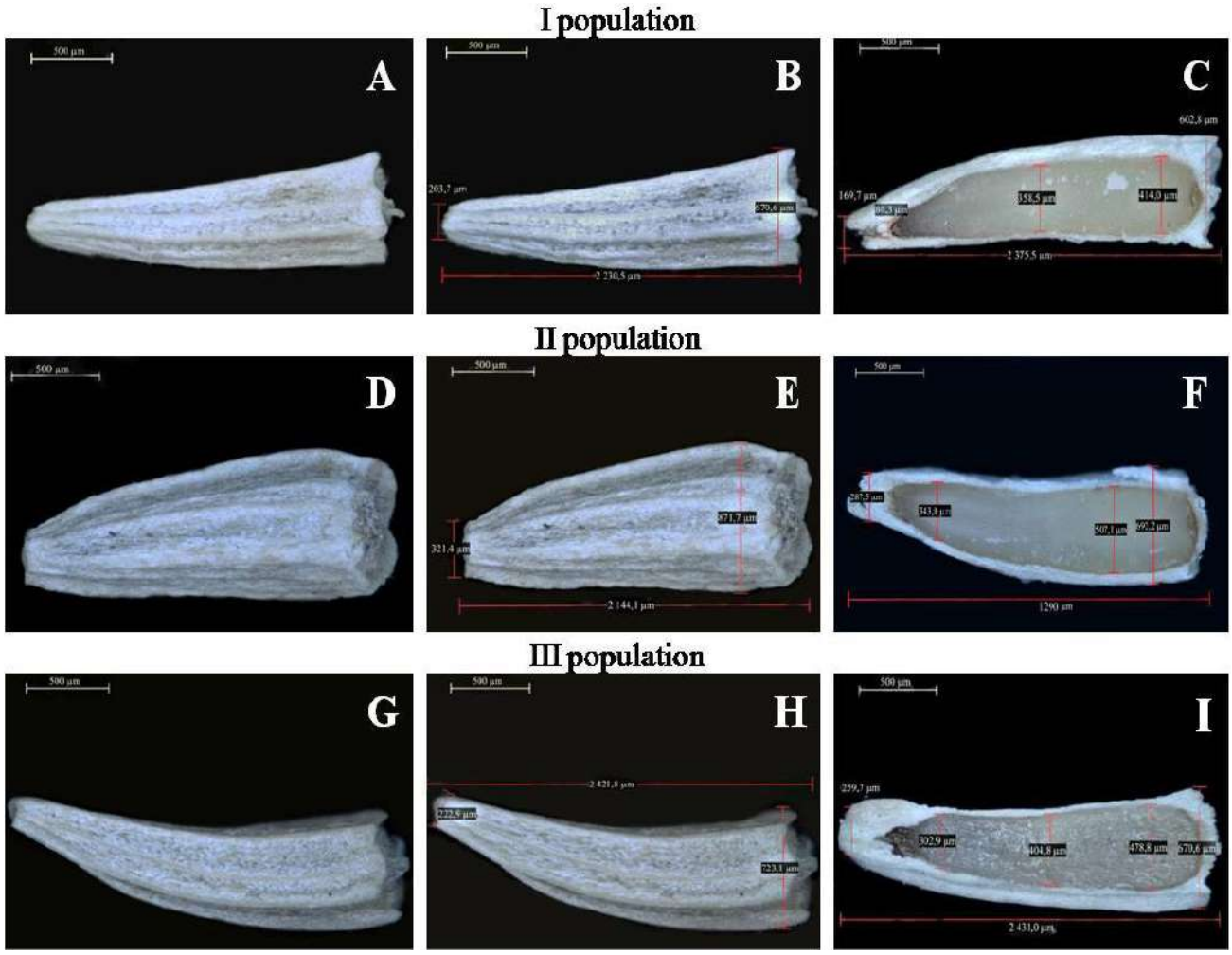

Fig. 2. Seed structure of the *Anthemis trotzkiana* Claus
Akshatau (A-C), Bestau (D-F), Ishkarahantau (G-I) of Aktobe region.

Leaf

1 – The lamina of *Anthemis trotzkiana* Claus in the Akshatau population in the virginal state is heart-shaped, with its lower part convex. The epidermal cells are small, single-row, densely located, cuticular. In the upper epidermis, the vast majority are trichomes. The leaf is isolateral, i.e., the palisade mesophyll is found on both sides of the leaf. This is peculiar to xerophilous plants. In the palisade mesophyll, colorless round lipid droplets are clearly visible. Palisade mesophyll is characterized by intercellular cavities. Vascular bundles are located in the central part of the leaf. The main central vascular bundle is surrounded by special cells. The xylem is directed to the upper epidermis, the phloem is directed to the lower epidermis. The vascular bundles are collaterally closed, the cambium is absent. In addition, in the central part of the leaf, the ethereal brown passageways are marked (Table 3; Fig. 3J).

2 – In the cross section of the virginal leaf of*Anthemis trotzkiana* Claus in the Bestau population, a solid cuticle, surrounding, covers the epidermal cells. The cells of the epidermis are small, form a homogeneous layer consisting of one row. In the lower part of the upper epidermis and in the upper part of the lower epidermis, there is a bilateral columnar mesophyll, the leaf is isolateral. The columnar mesophyll consists of two rows in each side. Essential oils are clearly visible in the columnar mesophyll. Vascular bundles are located in the central part of the spongy mesophyll. The cells surrounding the large central vascular bundle are well developed. In the main vascular bundle, the distance between the xylem and phloem is the same. Sclerenchyma developed in the lower part of phloem (Table 3; Fig. 3K).

3 – The leaf of the plant *Anthemis trotzkiana* Claus in the Ishkaragantau population has a heart-shaped form in the virginal state, with a thickened sprout in its lower part. The epidermis is covered with a thick layer of cuticle, and has trichomes. Between the cells of the epidermis there are also large cells. The leaf is bilateral columnar mesophilic. Columnar mesophyll consists of two rows. The spongy mesophyll occupies the central part. In the columnar mesophyll, the vascular bundles are arranged in rows. In the middle of the leaf traced an ethereal passageway. The volumes of the vascular bundles are varied(Table 3; Fig. 3L).

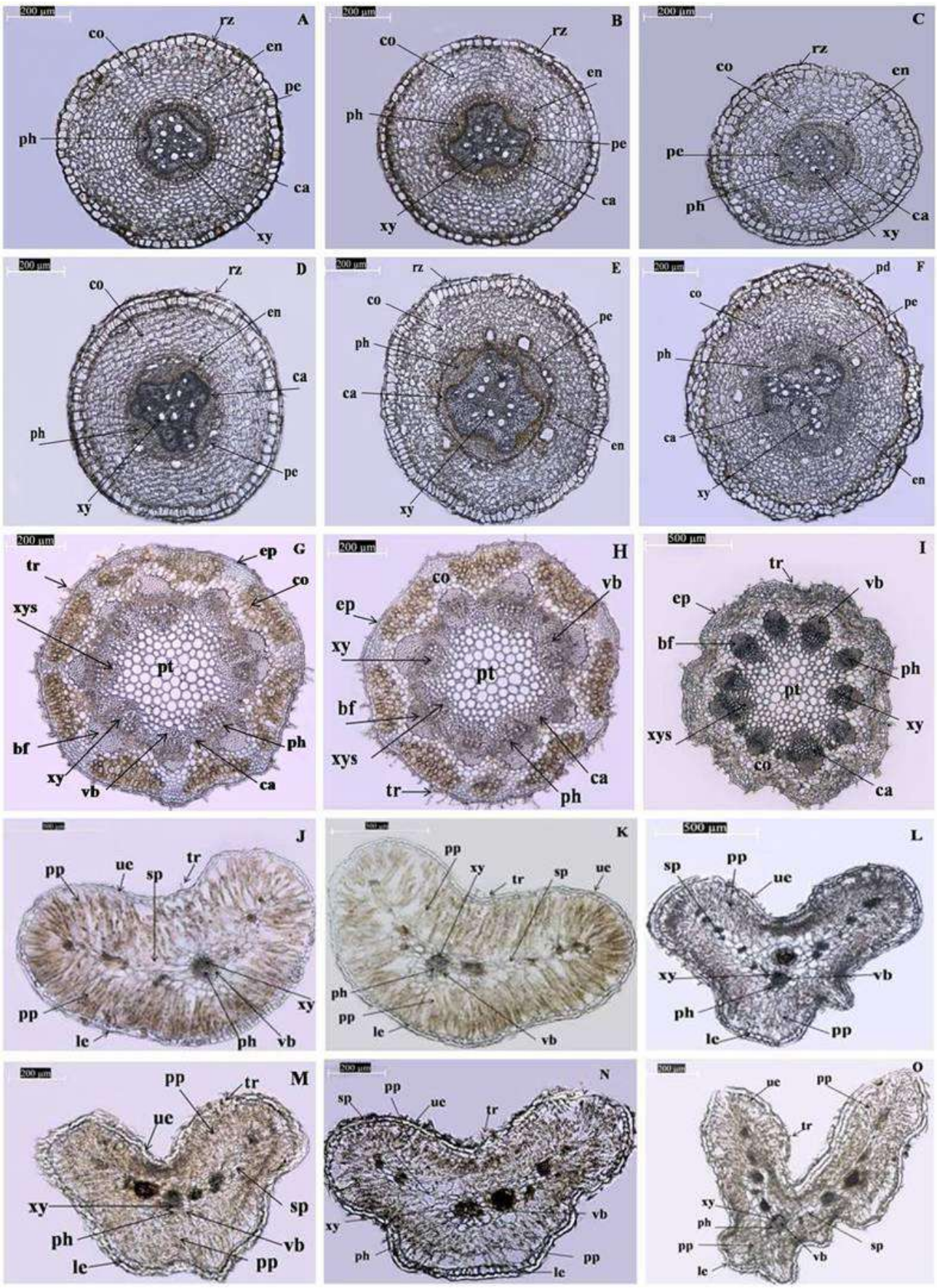

Fig. 3. Anatomical cross sections of *Anthemis trotzkiana* Claus.
root (A-F), stem (G-I), and leaf (J-O).cambium (ca), cortex parenchyma (co), endodermis (en), epidermis (ep), lower epidermis (le), periderm (pd), pericycle (pe), phloem (ph), palisade parenchyma (pp), pith (pt), rhizoderma (rz),spongy parenchyma (sp), trachea (tr), upper epidermis (ue), vascular bundle (vb), xylem (xy), xylem sclerenchyma (xys)

Table 4. Anatomical characters of the *Anthemis trotzkiana* Claus in Aktobe region (generative plants).

Characteristics	Populations		
	1-Akshatau	2-Bestau	3-Ishkaragantau
Root			
Rootdiameter	698.01 ± 1.98	822.11 ± 4.24	859.0 ± 4.69
Peridermthickness	31.1 ± 1.52	42.3 ± 3.21	57.03 ± 2.66
Thickness of the primary cortex	161.0 ± 7.02	189.02 ± 7.11	192.2 ± 2.42
Diameter of the central cylinder	313.0 ± 4.10	359.07 ± 1.63	360.01 ± 2.31
Thickness of the xylem vessels	66.11 ± 3.01	45.15 ± 3.12	16.84 ± 3.27
Leaf			
Thickness of the upper epidermis	21.01 ± 2.02	27.1 ± 6.11	23.02 ± 3.08
Thickness of the lower epidermis	31.02 ± 10.01	29.01 ± 11.00	24.03 ± 6.33
Thickness of the upper palisade mesophyll	124.1 ± 24.10	113.3 ± 22.18	119.2 ± 3.72
Thickness of the lower palisade mesophyll	186.3 ± 6.33	169.7 ± 9.01	116.2 ± 1.89
Thickness of the spongy mesophyll	202.2 ± 24.02	150.4 ± 35.12	2.20 ± 6.24
Diameter of the main vascular bundles	65.02 ± 2.17	70.01 ± 2.00	58.99 ± 0.66
Thickness of the central part of the leaf	505.44 ± 8.42	470.2 ± 3.74	458.58 ± 12.3
Stem			
Stemdiameter	1120.1 ± 37.2	851.01 ± 14.01	401.01 ± 0.13
Epidermisthickness	12.01 ± 2.17	17.19 ± 2.14	30.01 ± 3.01
Thickness of the primary cortex	135.9 ± 14.02	107.9 ± 15.9	89.7 ± 7.08
Diameter of the central cylinder	814.1 ± 4.20	602.03 ± 0.29	161.16 ± 2.86
Length of vascular bundle	217.21 ± 8.02	178 ± 723	161 ± 10.11

Anatomical structure of the *Anthemis trotzkiana* Claus (generative plants)

Root

1 – In the cross section of the average generative tree's root in the Akshatau population, a decrease in the number of rhizodermal cells was noted, while the exodermal cells increased in size. After the rhizodermis disappeared completely, since the exoderm performs the covering function, its cells became homogeneous and densely located. The number of rows of cells of the primary parenchyma decreased, and cavities with a special form appeared in the endomerm. The central cylinder has expanded, and the frames in a three-frame xylem divided in two. In the phloem of the recesses of the frames, the number of sclerenchyma increased. In the middle of the root, xylem vessels increased in size (Table 4; Fig. 3D).

2 – In the cross section of the average generative plant's root in the Bestau population, the rhizodermis is noted only in the residual form. The cells of the exoderm are folded in the form of bricks. Primary bark decreased, and the central root increased. Large cavities were found on the border of the primary cortex and central cylinder. In the central part the xylem is polyarchal, six frames are found. Between xylem and phloem, the cambium layer is clearly visible. Phloem volumes increased. Sclerenchyma well developed. The number of large xylems' vessels increased (Table 4; Fig. 3E).

3 – In the anatomical structure of the root of individuals in the average generative stage of the Ishkagantau population, the external root bark is transformed into periderm. The cross section is not rounded, sinuous. The volume of parenchymal cells of the cortex is reduced. Large cavities were formed in the completed layer of the primary cortex. In the central cylinder the phloem completely surrounds the cambium. In the central cylinder is clearly traced three-frame xylem (Table 4; Fig. 3F).

Stem

Since the stem of the plant *Anthemis trotzkiana* Claus is undeveloped in the virginal state, for comparison purposes, the stems were examined in an average generative state.

In the anatomical cross section of the stem of *Anthemis trotzkiana* Claus in the average generative (g2) state of life, three anatomical and topographic zones were noted: the epidermis, the primary cortex and the central cylinder.

1 – The trichome of the epidermis is well developed in the internal structure of the stem in the Akshatau population. The epidermal cells are densely arranged in one row, the epidermis consists of highly cutinned cells. The stem in the cross section has a rounded shape, but slightly sinuous. In the primary cortex in the lower part of the epidermis, the collenchyme and sclerenchyma are located as a bundle, in a row. It is noticed that the stem in the collenchyma area goes deep, while in the sclerenchyma area it protrudes slightly. The parenchyma forms a winding ring. 3-4 row sclerenchyma occupies a small part of the stem, and collenchyme – a larger part. The primary cortex ends with endoderm. In the central cylinder, 9 large vascular bundles are located in the region of a single cylinder, surrounding it. The vascular bundle is collaterally open; a cambium is formed between the xylem and the phloem. In the vascular bundle in the

upper part of the phloem, the bast layer is very well developed. Sclerenchyma is located in the upper part of the bast layer, while collenchyme is well developed in the upper part of two vascular bundles. The sclerenchymal cells surrounding the xylem are clearly visible. The xylem is four- or five-beam, each beam consists of 5-6 xylem vessels. The core consists of parenchymal cells of various sizes (Table 4; Fig. 3G).

2 – The anatomical structure of the stem in the Bestau population is rounded, the edges are curved. Epidermal cells that have cuticle are heterogeneous; trichome is well developed. In the primary cortex, elements of the mechanical tissue are clearly visible: they are sclerenchyma and collenchyma, parenchyma and endoderm. In the lower part of the sclerenchyma, located as beams in the lower part of the epidermis, 2-3 rows of parenchyma, 1 row of collenchyma are developed. The volume of collenchyma compared with sclerenchyma is several times more developed. The parenchyma forms a continuous ring. The endoderm cells are egg-shaped. The layer of bast is developed in the upper part of the phloem; there is a cambium between the phloem and the xylem. Surrounding the xylem, sclerenchyma is formed. In the middle part of the core, the parenchyma is very voluminous (Table 4; Fig. 3H).

3 – The cross section of the stem of the average generative tree in the Ishkaragantau population is rounded and deeply curved. The epidermis has even, densely located small cells. Trichomes are well distinguished. The primary cortex, located below the epidermis, consists of layers of sclerenchyma, collenchyma and parenchyma. The parenchyma of the primary cortex is well developed, forms several rows. The central cylinder consists of 10 vascular bundles located at a distance from each other. In the vascular bundle, the beamy- and inter-beamy cambium located between two beams are well distinguished. The core consists of large parenchymal cells (Table 4; Fig. 3I).

Leaf

In the anatomical structure of the leaf of *Anthemis trotzkiana* Claus, the lamina is composed of the upper and lower epidermis, palisade and spongy mesophyll, and vascular bundles.

1 – The palisade and spongy mesophyll between the upper and lower epidermis of the lamina of the average generative state of *Anthemis trotzkiana* Claus in the Akshatau population is noted. Densely located epidermis is covered with a thick layer of cuticle. 2 layers of spongy mesophyll are located on both sides of the leaf. Vascular bundles are located close to the upper epidemic. The spongy mesophyll in the lower part of the vascular bundleoccupies the most part. The ethereal passageway, located near the main vascular bundle, is colored dark brown. Drops of essential oil are preserved (Table 4; Fig. 3M).

2 – In the cross section of the lamina of *Anthemis trotzkiana* Claus in the Bestau population, the trichome in the upper epidemic is well developed. The cells of the epidermis are covered with a thick layer of cuticle. The cells of the epidermis have become larger. The cells of the palisade mesophyll form 2 rows, cover the leaf, and have an isolateral structure. The spongy mesophyll occupies the middle part of the leaf. The intercellular regions are clearly visible in the spongy mesophyll. The specialized cells surrounding the main vascular bundle are well developed. The size of the phloem, compared with the xylem, is larger; sclerenchyma is formed under the phloem. Essential oils are preserved in the spongy mesophyll (Table 4; Fig. 3N).

3 – The leaf form of the average generative plant *Anthemis trotzkiana* Claus in the Ishkaragantau population is special. Cells of the lower epidermis form a curved line. Trichoma is well developed in the upper epidermis, compared with the lower one, strongly cutinned. Lamina is thin. The leaf is completely covered with a palisade mesophyll, only in the middle small part there is a spongy mesophyll. Spongy mesophyll is located only between the vascular bundles. The sclerenchyma is well developed in the main vascular bundle in the lower part of the phloem. Essential oils and cavities are preserved (Table 4; Fig. 3O).

Table 5. Physical and chemical characteristics of soil Aktobe region.

Population	Humus, %	Hygroscopic water, %	The amount of salts, %	pH
		0-30		
Akshatau	3.40	2.3	1.49	7.79
Bestau	3.15	2.4	0.14	8.41
Ishkaragantau	4.90	2.5	1.13	8.19
		30-60		
Akshatau	-	2.6	2.33	7.49
Bestau	-	2.7	0.63	8.21
Ishkaragantau	-	2.7	1.97	7.75
		60-90		
Akshatau	-	2.8	1.95	7.67
Bestau	-	2.9	0.34	8.48
Ishkaragantau	-	3.0	1.72	8.28

The main reason that the *A. Trotzkiana* in the Bestau population has a complex morphological structure is that the soil pH of the plant growth is higher compared to other populations (Table 5; Fig. 1).

Phylogenetic assessment of the molecular taxonomy of *A. trotzkiana*: The Neighbor Joining phylogenetic tree based on using 23 sequences of ITS is presented in Fig. 17. The *Artemisia vulgaris* was selected as an out group for the analysis. The dendrogramm is clearly separated all studied samples in two distinct clades where both locally collected samples of the *Anhtemis*, *A. trotzkiana* and *Tanacetum ulutavicum*, were placed in the bottom groups of the tree (Fig. 4).

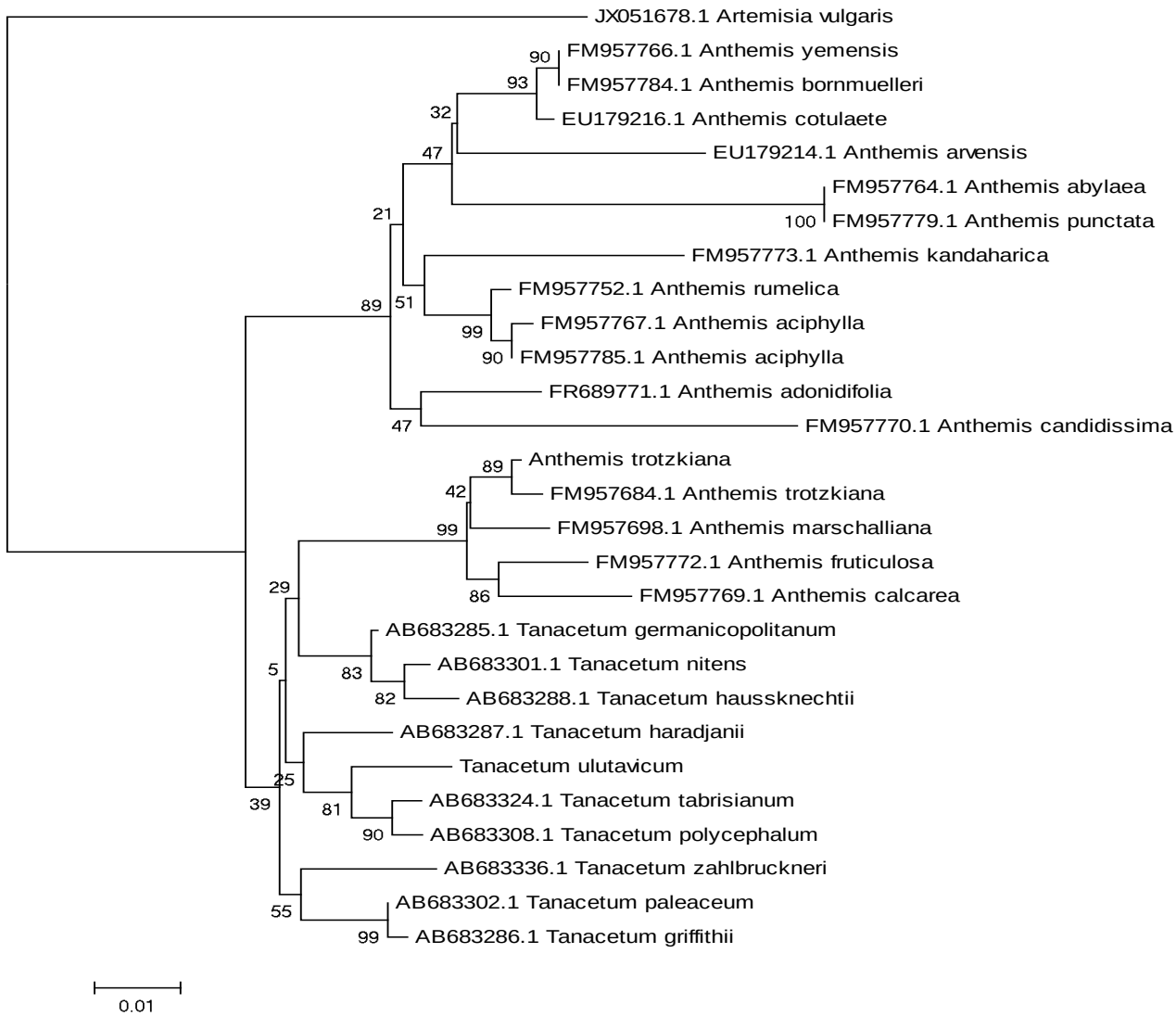

Fig. 4. Comparison of a phylogenetic tree of a tribe of *Anthemideae* (camomile) on ITS.

There clasterizations suggested that *A. trotzkiana* a long with *A. marschalliana, A. futiculosa,* and *A. calcarea* form a single cluster within *Tanacetum* clade, while other *Anthemis* species formed a separate *Anthemis* clade. The comparative analysis of the ITS nucleotide sequences between collected samples of *A. trotzkiana* analyzed in this study and NCBI sample of *A. trotzkiana* (FM957684.1) revealed two nucleotide differences confirming the identity of local sample (4e-125, 99% of identity).

Discussion

The comparative characteristics of morphological features and individual variability of flowers give the chance to draw a conclusion that the types of a camomile met in the territory Kazakhstan are polymorphic.

Object of the research *Anthemis trotzkiana* Claus grows exactly on slopes in cretaceous limestones, the location is separated from other plants of population, flowers are brightly expressed, which instantly catch the eye, and capitula of *Anthemis trotzkiana* of juicy-yellow color make it easier to find the plants making accurate outlines, (Fig. 1).

Anthropogenic factors influence on disappearance of a rare species, first of all, on these cretaceous slopes chalk is obtained from close settlements and secondly a cattle pasture, and also there is the trampleness of cattle, in this regard plants are not in time before complete maturing of seeds.

It is necessary to take measures for saving a biodiversity and also to conduct complex researches of this rare species, in the solution of problems considering that population number depends on natural germinating ability of seeds. In numerous researches of rare endangered species of a plant it was revealed that germinating ability of seeds of such species of a plant is small.

Were study the flower and fruits of *Anthemis trotzkiana,* according to the results, there was revealed large number of seeds. To an increase in population, abiotic factors as the terrain fire influence and also wind carries away many seeds since limestone is very hard, after rain it hardens even more, it explains its narrow distribution and confusedness to certain types of soils. Studying the flower structure and seed forms allowed to establish the reason of decreasing species number, main reason is dependence on climate and relief factors,

especially chalk deposits; the second reason on grazing animals and mowing during florescence period and seed maturation, seed production is not appearing, also by using the chalk in domestic goals.

Complex research implies profound study of morphological and anatomical structures of plants. In the anatomical structures of the root, schizogenic channels were found and in the leaves were numerous drops of essential oils.

The results suggested that *A. trotzkiana* along with *A. marschalliana, A. futiculosa*, and *A. calcarea* form a single cluster within *Tanacetum* clade, while other *Anthemis* species formed a separate Anthemis clade.

In Popov P.P.(1967) handbook "Systematics and origin of (*Asteraceae*)" where were investigated the structure and origin of *Asteraceae* plants flowers inhabited Middle Asia and Kazakhstan; more accurate research gave style structure features of *A. trotzkiana,* differs from other species of genus*Anthemis.* Characterization of researched species reproductive organs structure was studied in detail, that ray corolla is oval-shaped at the top rounded with barely expressed 3-5 veins.

The genus, in its traditional circumscription, is not monophyletic due to the consistent placement of a member of *Tripleurospermum* amidst the *Anthemis* representatives. Subdivision of *Anthemis* into two subgenera *(A. subg. Anthemis, A. subg. Cota)* is supported, with the exception of the placement of *A. sect. Chiae* and sect. *Odontostephanae* which fall outside *A. subg. Anthemis*. Within *A. subg. Anthemis*, life form dependent delimitation of *A. sect. Anthemis* (annuals) against *A. sect. Hiorthia* (perennials) is not supported, whereas the monophyly of other sections (*A. sect. Maruta, A. sect. Rascheyanae*) is confirmed including the placement of *Ammanthus Boiss.* &*Heldr.* into *Anthemissubg. Anthemis* **(**Oberprieler 2001).

The literature survey suggested that both cpDNA and ITS datasets do not support the monophyly of the genus *Anthemis.* There are in particular four species - the *perennials A. trotzkiana, A. calcarea, A. marschalliana* and *A. futiculosa, endemic* to the Caucasian region that are more basal than representatives of the genus *Cota* or the outgroup taxa *Tanacetum* and *Gonospermum* and that form a well supported clade in the BI analysis of the ITS dataset **(**Lo Presti, 2010).

For confirmation of the studied species there was defined and constructed a phylogenetic tree of *A. trotzkiana* based on the ITS sequence alignment. The NJ phylogenetic tree revealed that DNA sample of the *A. trotzkiana* collected in Aktobe region is highly significantly matched to the NCBI sample FM957684.1, confirming the correct identity of the studied species. Moreover, the ITS phylogenetic tree over 23 *Anthemis* species indicated polyphyletic.

The topology of the ITS phylogenetic tree over 23 studied species confirmed the polyphyletic origin of *Anthemis*, as A. trotzkiana clustered together with *A. marshalliana, A. futiculosa* and *A. calcarea* within of *Tanacetum* clade while all other species formed a separate clade of *Anthemis* (Fig. 4). Morphological features of a tansy differ in some criteria, in particular a flower as *Anthemis* is in capitula have disk and ray flowers, and for a tansy, generally only disk, but false-ray flowers are sometimes met.

Conclusion

1. When studying the morphological structure of *Anthemis trotzkiana* Claus of three populations growing in the Aktobe region, during their virginal and generative life cycles, it was found that the highest parameters were characteristic of 2-Bestau population plants. And it has been determined that the 3- Ishkaragantau population had the lowest parameter. A large number of leaves in virginal state have been marked. In the virginal state of the plant, the stem has not developed.
2. When comparing the anatomical structure of virginal roots in three populations, it was found that the morphometric parameters of plants in the 1-2nd populations were high, while the data of the 3rd population were lower. The rhizodermis in the virginal root of the 1-2nd populations is preserved; in the 3rd population, its transformation into periderm is seen. Architectural features of the 3rd population are as well more. When considering 3 populations, it has been found that the growth factor has a positive effect on the root of 2 populations. When studying the causes of low morphometric indicators of the 3rd population, the following evidence – the result of the study of the soil chemical composition – was obtained. The study results of the chemical composition of the soil showed that in the 3rd population, the acidity is higher than normal, and the salinity is lower. A high concentration of acidity led to poor root development, i.e. correlation has been marked. The peculiarity of the generative root was noted – in all 3 populations different air cavities have been formed. Biometric indicators of the root of the 3rd population have relatively high rates.
3. In the result of a study of the anatomical structure of 3 populations' stem it has been determined that the morphometric data of the 2-Bestau population, compared with other populations, have a high rate. The results of the study showed that the 1-Akshatau population has the lowest. In the vascular bundles of the 1st and 2nd populations, the xylem sclerenchyma is very well developed, and in the 3rd population, on the contrary, it has not been developed.
4. The feature of the leaf is in its isolaterality, i.e., palisade mesophyll occurs on both sides of the leaf. The characteristic feature of the leaf of this plant is the abundance of ethereal granules in the palisade mesophyll. In the generative stage, in comparison with the virginal one, an increase in the number of ethereal passageways around the vascular bundles in all 3 populations has been found. The abundance of essential oils clearly indicates the healing characteristics of the plant and is the basis for studying of essential oils of leaf.
5. The results suggested that *A. trotzkiana* along with *A. marschalliana, A. futiculosa*, and *A. calcarea* form a single cluster within *Tanacetum* clade, while other *Anthemis*species formed a separate *Anthemis* clade.

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