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Study of changes in anatomical structures of young needles *Picea schrenkiana* Fisch et C.A. Mey and shoots of *Haloxylon* bunge species in Kazakhstan

Abstract. The work is devoted to the anatomical study of five populations of the forest-forming species *Picea schrenkiana*, which grows in the Trans Ili Alatau range of Northern Tian Shan Mountains, and three species of the desert tree *Haloxylon*, which grows in three regions of Kazakhstan. As the study was taken young needles of *P. schrenkiana* and shoots of *Haloxylon* species. In this study, anatomical analyses of young needles from five populations of *P. schrenkiana* and shoots from three *Haloxylon* species were performed to identify and describe anatomical features in search of distinctive features that could be used to determine the characteristics of these species. In the cross-section of *P. schrenkiana* needles, all five populations have a rectangular shape, and the tissue types and their location are similar in all populations. Study of the young needles of *P. schrenkiana* revealed the presence of the epidermis, hypodermis, stomata, mesophyll, resin ducts, endodermis and vascular bundles all of the five populations. The area of the vascular bundles varies from 141.4 ± 1.9 to 182.7 ± 1.4 μm². Comparatively, all indicators were the same, which means that the origins of all five populations are similar to each other. As a result, the anatomical structure of *P. schrenkiana* needles corresponds to the structure of coniferous plants. In the study of three species of *Haloxylon*, more precisely *Haloxylon aphyllum, Haloxylon persicum, Haloxylon ammodendron*, differences were found in the section of each species. This is possible due to the fact that they grow in three different regions and different environmental conditions. And it proves that the environmental conditions of the place where these species grow affects the anatomy and structure of plants.

Key words: *Picea schrenkiana, Haloxylon aphyllum*, *Haloxylon persicum, Haloxylon ammodendron,* scanning electron microscopy, anatomical signs, vegetative organs of plants.

Introduction

Despite the fact that the territory of Kazakhstan is vast, it has little forest resources. Forests cover only 4-6% (12.4 million hectares) of the total area. The country has the third largest forest area in Eastern Europe and Central Asia. Forests are unevenly distributed across the country: forest cover is generally 4.57 %, and in some administrative regions 16% in Eastern Kazakhstan prevails compared to 0.1% in Western Kazakhstan, in particular in Almaty, coniferous plantations range from 10-13 %, and deciduous – 12 % and are located in tracts on the inclination of the southern and Eastern mountains. Also, the forests are dominated by saxaul forests, which occupy 49.8 % of the area, and shrub plantations – 24.1%, located mainly in the desert and steppe zones.

The problem of preserving forest genetic resources for current and future generations is becoming increasingly important. Due to the rapid development of scientific and technological progress, the threat of extinction of plant diversity and, above all, forests has increased dramatically. The role of woody vegetation in maintaining the quality of the environment is well known, but even more so in its utilitarian value.

The distribution of ecosystems is subject to the law of latitudinal zoning on the plains and high-altitude zoning in the mountains. On the plains, forest-steppe, steppe and desert ecosystems are major zonal associations.

Mountain ecosystems are much more complex and diverse than on the plain. In the Jungar and Trans-Ili Alatau, most of the forest area is covered with forests of Shrenk spruce, or Tianshan spruce with the inclusion of Siberian fir and Semyonov fir. Desert ecosystems are formed by rare-coniferous forests of arid regions and consist of white saxaul, black saxaul, and desert shrubs on sandy and takyrovid habitats [1]. The flora of Kazakhstan includes 68 species of tree species, 266 species of shrubs, 433 species of semi-shrubs and semi-herbs, 2598 species of perennial grasses, 849 species of annual grasses [2].

Pinaceae include overall 225 species in 11 common genera (*Abies*, *Cathaya*, *Cedrus*, *Keteleeria*, *Nothotsuga*, *Picea*, *Pinus*, *Pseudolarix*, *Pseudotsuga* and *Tsuga*) distributed worldwide [3]. Hart [4] divided the family *Pinaceae* into two groups: first group with the presence of resin canals in the seeds and cleavage polyembryony supported the monophyly of *Abies*, *Cedrus*, *Keteleeria*, *Pseudolarix* and *Tsuga* and second group which the presence of resin canals in the secondary xylem and leaves having an endodermis with thickened Casparian strips supported the monophyly of *Cathaya*, *Larix*, *Picea*, *Pinus* and *Pseudotsuga* [5]. Francis [6] identified four subsystems from the pine family in the generally accepted classification [7], based on the morphological structure of the vegetative and reproductive organs: *Pinoideae (Pinus), Piceoideae (Picea), Laricoideae (Cathaya, Larix and Pseudotsuga),* and *Abietoideae (Abies, Cedrus, Keteleeria, Nothotsuga, Pseudolarix* and *Tsuga)*. In Kazakhstan there are 4 genera and 7 species of *Pinaceae* [8].

One of the most common coniferous genus in this family is *Picea* A. Dietr. Farjon [3] identified 34 species of *Picea* in its list of conifers, of which 24 grow in Asia, eight in North America and two in Europe. It is a very homogeneous genus, and therefore it is difficult to work with it from a taxonomic point of view because species have a relatively narrow range of morphological features and ecological preferences [5; 9-11].

In Kazakhstan, 2 species from the genus are present: *P. obovata* Ledeb. and *P. schrenkiana* Fisch et C. A. Mey [8; 12]. The object of our study is the Shrenk's spruce (*Picea schrenkiana*), which occupies relatively small areas in the mountainous regions of Kyrgyzstan and Kazakhstan, but nevertheless plays a huge water-regulating, soil -, water- and avalanche-protective role [13; 14]. The dwarf form of the Schrenk's spruce (*P. schrenkiana* Fisch et C. A. Mey. f. prostrata K. Isakov) is included in the Red book of Kazakhstan [15] and the world IUCN [16], as it is rare. Outwardly, *P. schrenkiana* resembles *P. obovata,* from which it differs by a longer needling with bare young shoots and larger cones [12].

P. schrenkiana occurs between 1300 and 3000 (-3600) m a.s.l., especially on Northern slopes and in cool ravines. It grows on various mountain soils, usually in stony places with seeping water from snowmelt (eternal snow at high altitudes). The climate is cold continental. It forms usually pure forests, but is sometimes mixed with *Abies sibirica* (*A. sibirica* subsp. semenovii with *Picea schrenkiana* subspecies. tianschanica), on lower elevations with *Ulmus* and *Populus* along streams [16].

P. schrenkiana is a perennial tree up to 40 m high with a narrow cylindrical crown. The bark is dark grey, the branches are often hanging, the young branches are bare. Leaves (needles) are 4-sided, sharp, 2-3.5 cm long. The leaves of many coniferous plants live for several years. For example, *P. schrenkiana* has a leaf life span of 26-28 years. They are adapted to insufficient water supply, especially in winter, and sharp fluctuations in summer and winter temperatures. Therefore, the leaves of most conifers have a xeromorphic structure: they are hard, small, with a small evaporating surface [17]. We looked at young needles anatomy from 5 populations of *Picea schrenkiana.*

Chenopodiaceae Vent. family of more than 100 related and about 1600 species of large family. The family includes herbaceous, shrubby, and even woody plants. A representative of the main tree belonging to the *Chenopodiaceae* family *Haloxylon* Bunge. There are 5 species of saxaul growing all over the world [2]. In Kazakhstan, you can find 3 species of saxaul, this is *Haloxylon aphyllum* (Minkw.) Iljin, *Haloxylon persicum* Bunge ex Boiss. et Buhse and *Haloxylon ammodendron* (C.A. Mey.) [8].

The main forest-forming representative of the desert and semi-desert zones of Kazakhstan is the saxaul tree. Especially in the Ili-Balkhash region of the country, the number of saxaul forests prevails. There is also a shrub 1.5 m high or a woody species that reaches up to 12 m. Branch consists of young fragile shoots. The leaves of the saxaul are reduced, formed by a small colorless film, and the functions of photosynthesis are performed by green shoots consisting of chlorophyll [18].

Desert plants that grow in arid conditions for many years usually have a high convergent adaptation over time and have similar internal structure, morphology, and physiological characteristics. The relative *Haloxylon* has many similarities in the morphological structure of leaves, stems, and fruits.

There is a difference in the types of life forms and environmental conditions of growth. 3 species found in Kazakhstan are halophyte and psammophyte adapted to salty and alkaline soil cover [19].

According to scientists, biomorphology are megaphanerophytes desert with differentiated shoots, deprived of saxaul. The main morphological differences are their leaves, shoots fall in the summer. Fallen plant parts form the main layer of soil cover. The loss of leaves and branches is characteristic of the black saxaul, so it is characterized as a portable ecological plant. Characteristic features of xerophyte, halophyte and mesophyte black saxaul, growing in semi-desert grove and sandstones. Black saxaul, which are halophytes, growing in the old river valleys, takyric soils in reinforced sands. The psammophyte white saxaul, which grows in the sandy deserts and sandstones. In turn, the Zaisan saxaul grows in Eastern Kazakhstan, on eroded soils, clay, and saline soils.

As you know, the generally recognized feature that arose in the course of an evolutionary process adapted to the dry state of the environment is the reduction of the sheet board. The mesophyll layer of vegetation increases, especially stem tissues develop strongly, so the soil resists drought. With increasing density and layers of columnar tissues, the area of photosynthetic organs is significantly reduced. On the other hand, in a small area of a flat surface, photosynthetic plants must provide themselves with plastic substances. This is influenced by the colonial parenchyma developing on both sides of the escape. Since columnar tissue is the most productive type of tissue and the tissue that makes the main contribution to the process of photosynthesis [18; 20].

Thus, the aim of our research is to study the anatomical structure of the young needles of *P. schrenkiana* and green shoots of *Haloxylon* species in Kazakhstan.

Materials and methods

Plant material of Picea schrenkiana Fisch et C.A. Mey. The object of the study is five populations of *Picea schrenkiana* Fisch et C.A. Mey., including the dwarf form (*P. schrenkiana* Fisch et C. A. Mey. f. prostrata K. Isakov) were collected to study the anatomical structure of needles in Trans Ili Alatau range of Northern Tian Shan Mountains (Figure 1).

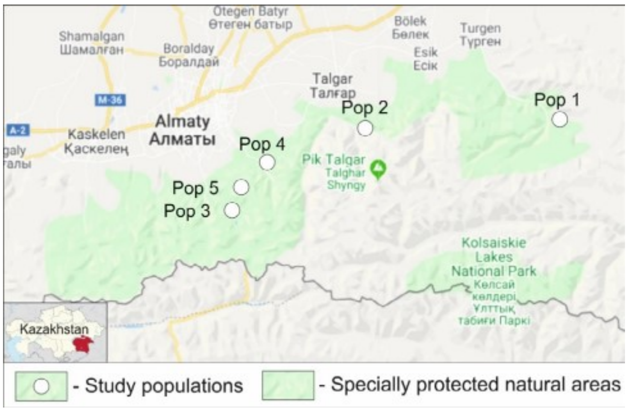

Figure 1 – Location of the collected *Picea schrenkiana* populations

Young needles of *P. schrenkiana* were collected randomly (Figure 2), with the distance between plants of not less than 100 meters.

Figure 2 – Young needles of collected *Picea schrenkiana*. Note: A – general view, B, C – young needles of *P. schrenkiana*

GPS coordinates of the collected populations are given in Table 1. For the study, 3 samples were taken from each population.

Plant material of Haloxylon species. The second object of the study is representatives of the genus *Haloxylon* from the family *Chenopodiaceae* – *Haloxylon aphyllum*, *Haloxylon persicum* and *Haloxylon ammodendron*, living on the territory of Kazakhstan, collected from three regions of Kazakhstan. *Haloxylon aphyllum* was collected from the Western Kazakhstan, *Haloxylon persicum* was collected from the South-East of Kazakhstan and *Haloxylon ammodendron* was collected from the East of Kazakhstan.

Table 1 – The information on five populations of *Picea schrenkiana* collected in Northern Tian Shan Mountains in South-East of Kazakhstan

Species	Population	Geographic coordinates	Altitude (m a.s.l)	Place of collection
P. schrenkiana Fisch. et C. A. Mey.	Pop 1	N 43.23639 E 77.76528	1694	Turgen gorge
	Pop 2	N 43.2215 E 77.30733	1730	Talgar gorge
	Pop 3	N 43.08083 E 76.99314	2160	Big Almaty gorge
	Pop 4	N 43.16275 E 77.07678	1919	Kim Asar gorge
P. schrenkiana Fisch et C. A. Mey. f. prostrata K. Isakov	Pop 5	N 43.12857 E 77.01357	2830	Big Almaty gorge, Tri brata rocks

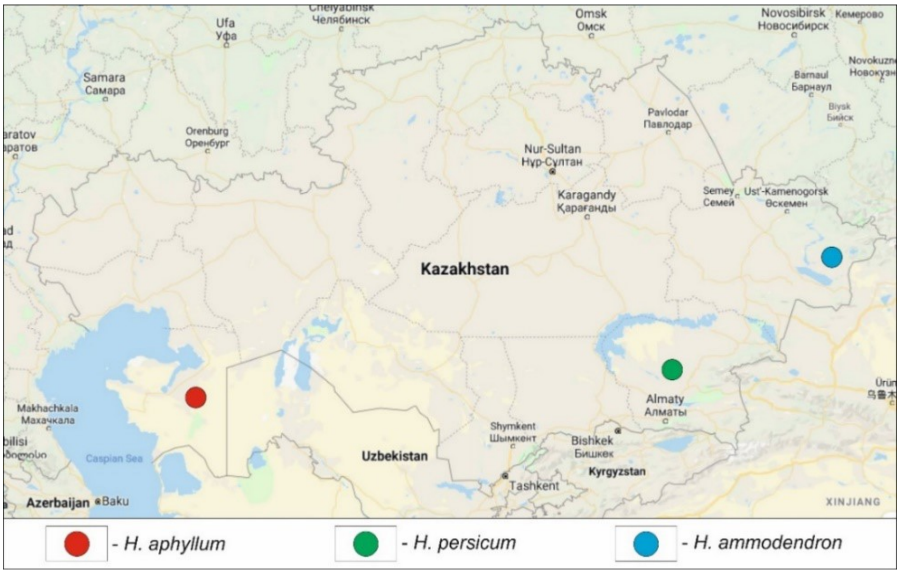

Figure 3 – Map of *Haloxylon,* collected from three regions

Young, annual, green shoots of three species of saxaul were collected from three regions of Kazakhstan.

Methods of research. The route reconnaissance surveys in the mountains of the Northern Tien Shan of the Almaty region, five populations of *P. schrenkiana* were found: Pop 1 – Turgen gorge, Pop 2 – Talgar gorge, Pop 3 – Big Almaty gorge, Pop 4 – Kim Asar gorge, Pop 5 – Big Almaty gorge, Tri brata rocks. Also, as a result of route – reconnaissance surveys, three *Haloxylon* species were collected from three regions of Kazakhstan. *Haloxylon aphyllum* was collected from the Ustyurt plateau, *Haloxylon persicum* was collected from the

Balkhash area, *Haloxylon ammodendron* was collected from the Zaisan basin. For further morpho-anatomical studies, herbarium and fixed specimens were collected. Preservation of plants was carried out according to the method of Strasburger-Fleming [21]. Anatomical preparations are made in accordance with generally accepted methods Prozina M.N. (1960), Permyakov A.I. (1988), Barykina R.P. (2004) [21-23]. Anatomical sections of the needles of *P. schrenkiana* and young green shoots of *Haloxylon* were made using the microtome MZP-01 "Technom". The thickness of the anatomical sections was 10-15 µm. Temporary preparations were enclosed in glycerin. Microphotographs of the anatomical sections were taken on an MC 300 microscope with a CAM V400/1.3M video camera. Measurements of micropreparations were carried out on an MCX100 microscope with a 519CU 5.OM CMOS Camera. The statistical processing of morphometric indicators was carried out using the Microsoft Office Excel 2003 program based on the G.F. Lakin (1990) [24].

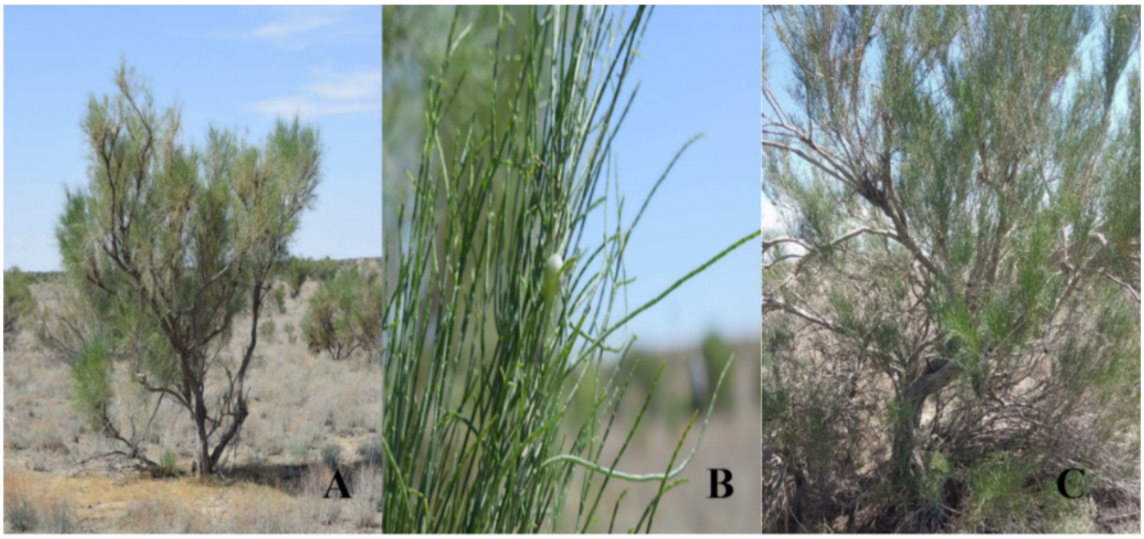

Figure 4 – Photos of *Haloxylon*. Note: A-*Haloxylon persicum*, B-young shoots of *Haloxylon persicum*, C-*Haloxylon ammodendron*

Table 2 – The information of *Haloxylon* collected in three region of Kazakhstan.

Genus	Names of species	Geographic coordinates	Altitude (m a.s.l.)	Place of collection
Haloxylon Bunge	*Haloxylon aphyllum* (Minkw.) Iljin	N 43.31897 E 54.48114	163	West Kazakhstan, The Ustyurt plateau
	Haloxylon persicum Bunge ex Boiss. et Buhse	N 44.41077 E 76.45582	410	South-East Kazakhstan, The Balkhash area
	H.ammodendron (C.A. Mey.) Bunge	N 48.13066 E 84.30375	444	East Kazakhstan, Zaisan basin

Results and discussion

Results of anatomical studies of P. schrenkiana. The results of anatomical studies, cross sections were made from each population with a thickness of 10-15 µm. In the cross-section of *P. schrenkiana* needles, all five populations have a rectangular shape, and the tissue types and their location are similar in all populations.

In the next stage of research, the internal structure of needles from each population was studied. The needles are covered with primary integumentary tissue – the epidermis, the cells of which are tightly closed together and arranged in a row, without intercellular spaces (Figure 5). The width of the epidermal cells significantly exceeds the height. The outer wall of epidermal cells kutinized. There is a well-developed hypodermic layer under the epidermis. Under the hypodermis is a mesophyll consisting of homogeneous cells. The cell walls sometimes grow into the cell cavity, forming folds (folded parenchyma). This significantly increases the area of the cytoplasm layer adjacent to the wall with chloroplasts, and, consequently, the assimilating surface. Stomata are located on the entire surface of the leaf. Their closing cells are located at the hypodermic level, under the nearly stomato cells. Stomata cells are very large, with strongly thickened outer walls. The stomatal apertures leads to a sub-stomatal air cavity surrounded by mesophyll cells. The mesophyll is homogeneous, folded. Folds occur due to the ingrowth of the inner layers of the shell into the cell cavity, which thus acquires a lobed shape. Due to the folds, the surface of the wall layer of the cytoplasm containing chloroplasts increases. Mesophyll cells are tightly connected, the intercellular spaces between them are very small.

Characteristic of coniferous plants in needles, the vascular bundle is surrounded by an endodermis. On the radial walls of the endodermis cells, there are lignified thickenings – Caspari spots. Along the centers are vascular bundles consisting of xylem and phloem. Between the endodermis and the vascular bundles is a transfusion tissue consisting partly of dead cells of irregular shape with bordered pores that transmit water from the xylem of the conducting bundle to the folded parenchyma, and partly of living parenchymal cells that transmit organic substances produced by the chlorenchyma to the phloem of the vascular bundle.

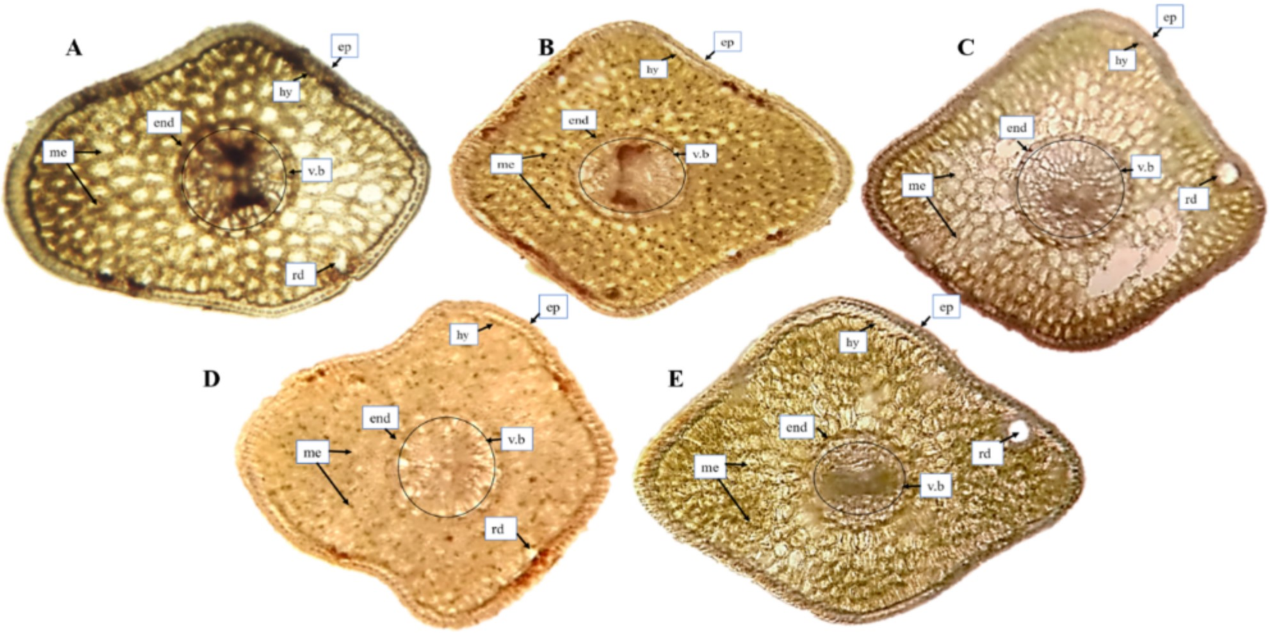

Figure 5 – Cross section (CS) of 5 population of *P. schrenkiana* (A – Pop 1, B – Pop 2, C – Pop3, D – Pop 4, E – Pop 5).
Note: ep – epidermis, hy – hypodermis, end – endodermis, v.b – vascular bundle, rd – resin duct, me – mesophyll

All conifers in the folded parenchyma have a large resin ducts running along the needles and covered with a cover of mechanical fibers (sclerenchyma). The number and location of resin ducts play an important diagnostic role in determining species based on the anatomical structure of needles. In our case, all populations of *P. schrenkiana* had resin ducts. Resin ducts that penetrate the folded parenchyma are lined inside with thin-walled cells that secrete resin inside, and outside they have a lining of thick-walled cells (Figure 5).

As a result of statistical processing of morphometric indicators, the thickness of the epidermis varied from 15.7 ± 2.2 μm (Pop 1) to 34.8 ± 0.9 μm (Pop 3). The epidermis is well protected from the effects of adverse environmental factors by a thick layer of wax coating. Moreover, in many species, the raid is so thick that the needles acquire a blue hue. The thickness of the hypodermis ranges from 7.8 ± 1.7 μm to 22.9 ± 4.4 μm. The area of the vascular bundles varies from 141.4 ± 1.9 to 182.7 ± 1.4 μm². And shows that the area of the vascular bundles in population 3 is greater than 20-40 µm² compared to other populations. The thickness of the endodermis ranged from 11.7 ± 2.2 µm to 14.4 ± 1.1 µm. When studying the anatomical structure of needles, it was found that needles of the Pop 1 have a less thick thickness of the epidermis. The size of the vascular bundles in individuals in Pop 3 is larger than in other populations (Figure 6).

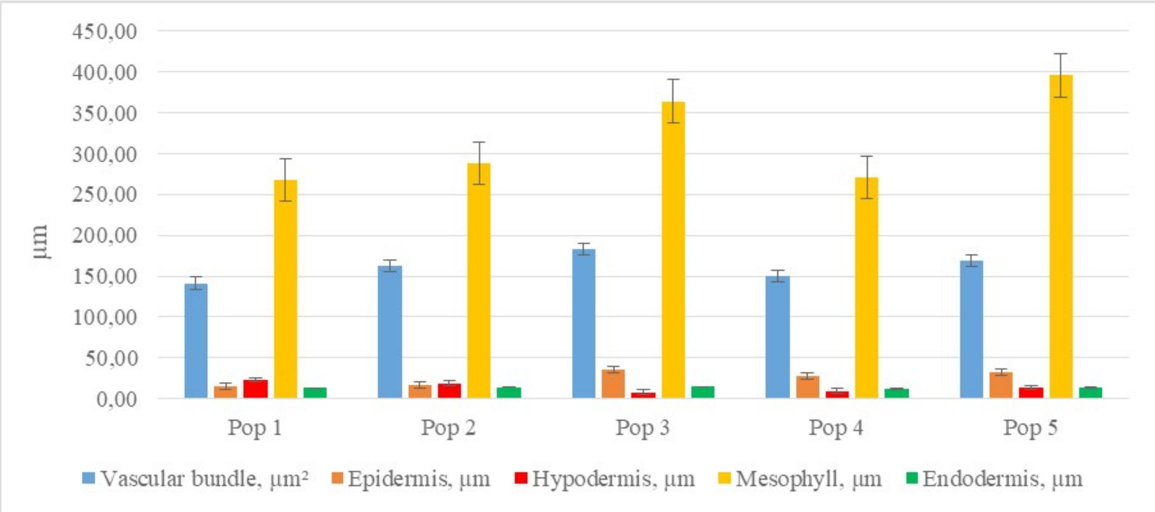

Figure 6 – The data of anatomical structure of needles in 5 populations of *Picea schrenkiana*

The anatomical structure of *P. schrenkiana* needles corresponds to the structure of coniferous plants. It should be noted that the anatomy of the leaves of all populations is clearly traced by the characteristic coniferous and xerophytic features. Some anatomical features, such as the shape of the cross section, the thickness of the epidermis, the location, nature and number of resin ducts, and the distribution of stomata can be key features for distinguishing species.

Results of anatomical studies of Haloxylon species. To identify anatomical similarities and differences between the black saxaul, white saxaul, and Zaisan saxaul, a horizontal segment was developed from the young green shoots of the saxaul. According to the obtained anatomical analyses, the cross section of young saxaul consists of the cuticle, the epidermis layer, calcium oxalate salts,the sclerenchyma, the cambium layer, the conducting layer, the primary shell, the central circle, and the conducting bundles. In the context of each type of saxaul, there is a difference. The diagram shows the average value of 3 segments. We measured the diameter of the shoots, the thickness of cuticle, thickness of epidermis, length of the primary shell, the diameter of the central circle and the volume of the vascular bundle.

The average diameter of the young shoot of *Haloxylon ammodendron* and *Haloxylon aphyllum* exceeds 600 µm, and the diameter of the shoot of *Haloxylon persicum* is 515.48±23.4 µm. In the Balkhash district of Almaty region, there is a high temperature compared to other areas of the environmental survey. Due to the lack of moisture in the white saxaul (*Haloxylon persicum*), the greatest narrowing of shoots was observed. *Haloxylon ammodendron* and *Haloxylon aphyllum* are succulent, succulents. Since the cuticle does not give the plant excessive evaporation, its thickness is important, the study found that the cuticle thickness of *Haloxylon persicum* was 6 mm thicker than *Haloxylon ammodendron* and *Haloxylon aphyllum*. Therefore, because of the thin layer of the cuticle of the white saxaul, the transpiration process is intense and indicates the cause of the thin escape. The thickness of the epidermis also has the lowest index in the white saxaul, where 18.14±3.5µm is respectively the highest index in the black saxaul 27.56±3.5 µm. The thickness of the primary cortex, on the contrary, in the Zaisan saxaul is thick 114.19±2.6 µm, in the white saxaul 107.40±6.7 µm, in the black saxaul 103.65±15.5 µm. The average cylinder diameter in the black saxaul and Zaisan saxaul was 300 µm, and in the white saxaul 215.33±23.3 µm. The volume of the conducting beam located in the phloem and xylem was about 40% in the black saxaul and Zaisan saxaul, and in the white saxaul the values are 2 times less.

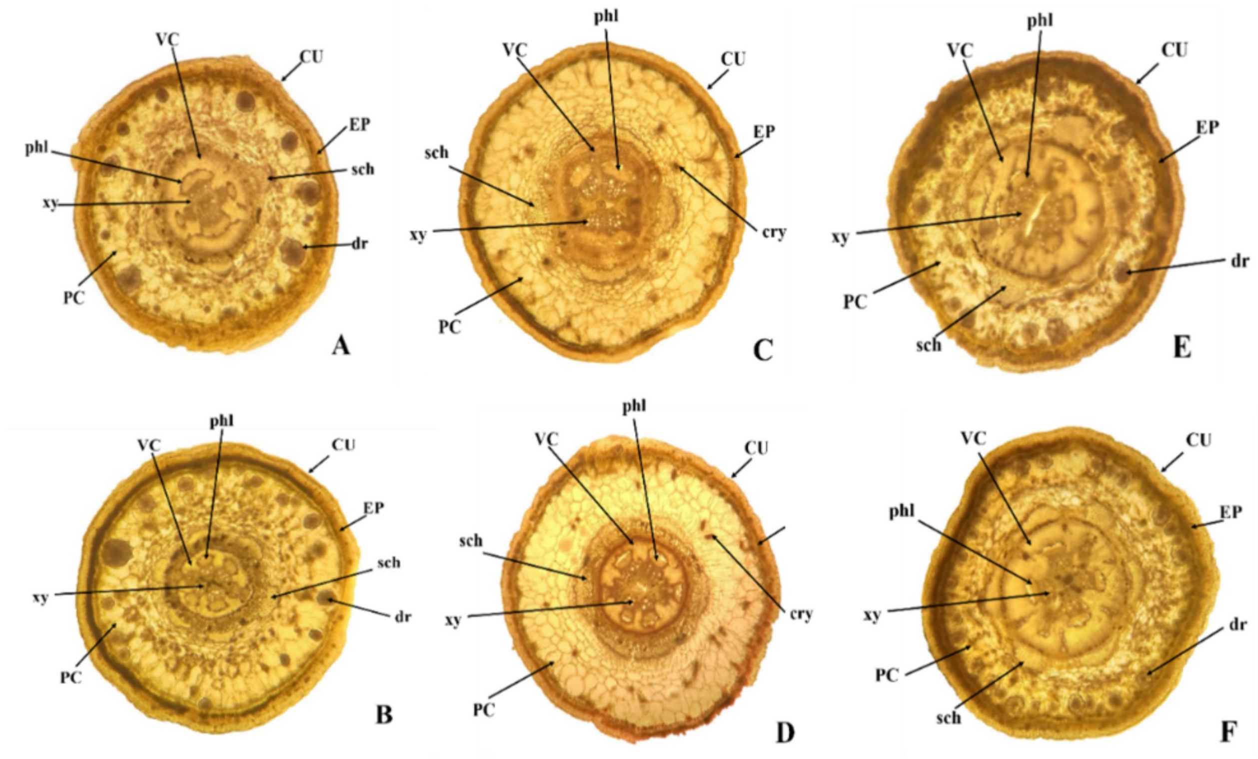

Figure 7 – *Haloxylon persicum (A,B), H.ammodendron (C,D)* және *Haloxylon aphyllum (E,F)* comparative anatomy of annual shoots. CU-cuticle, EP-epidermis, VC-conducting cylinder, PC-primary cortex, xy-xylem, phl-phloem, sch-sclerenchyma, dr-druses of salts, cry-calcium oxalate crystals

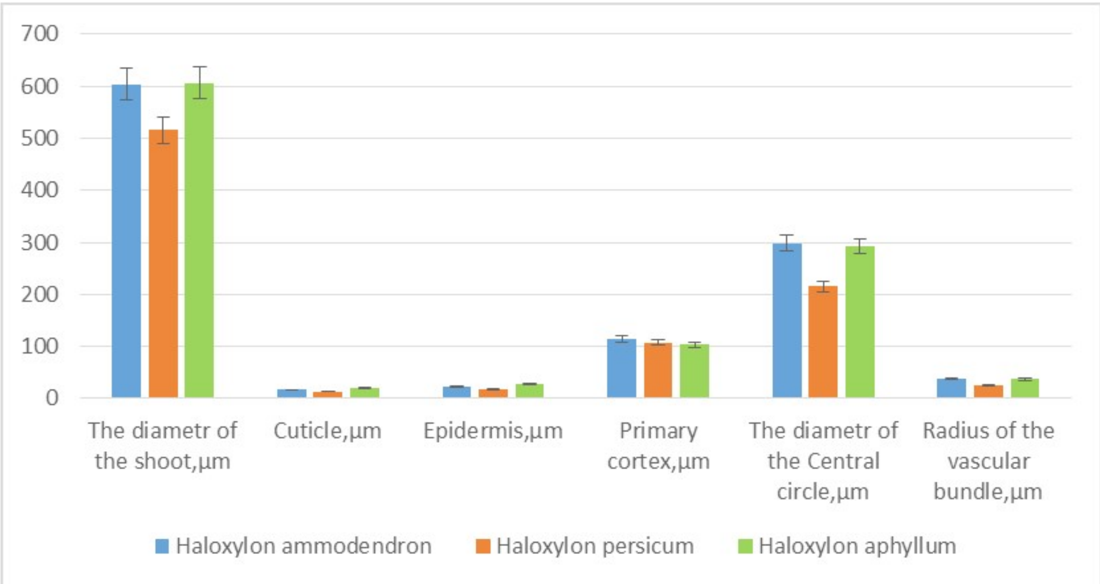

Figure 8 – Data from anatomical analysis of three species of saxaul

In the black saxaul, the crystals are located in the lower part of the epidermis. But the crystal concentrates are crushed in comparison with the crystals of white saxaul. Scientists have developed the concept that these crystals are crystals of calcium oxalate salts [25].

In the anatomical structure of saxaul shoots, a large number of crystals indicates that it grows in a salty area. The mineralization of groundwater can withstand up to 40 g/L and since sodium belongs to the group of alkaline plants [26], the crystals contain more black saxaul than white saxaul.

Conclusion

In conclusion, it should be noted that the anatomy of the needles of all populations is clearly traced by the characteristic coniferous and xerophytic features. Certain anatomical features, such as the shape of the cross section, the location of the subcutaneous tissue, the location, nature, and number of resin ducts, and the distribution of stomata may be key features for distinguishing species. Anatomical data is useful for the systematic study of conifers, and this study has provided potential results for systematic and phylogenetic studies of *Picea*. Similar studies on other species will help clarify the phylogenetic relationships between populations in each species and between species [27].

The following conclusions were made during the analysis of the anatomical structure of the saxaul. The morphometric index of the *Haloxylon ammodendron* and *Haloxylon aphyllum* plants is much higher than that of *Haloxylon persicum*. The anatomical structure of the saxaul species, the location of the phloem and xylem are similar. The location of the crystal salts can be clearly seen from the horizontal section. In the Zaisan saxaul, salt crystals are located in the center of the shell. The shapes are round, small crystals in the form of eggs. In the white saxaul, the crystals are large, several crystals form a druze. The crystals are located in the lower part of the epidermis. Thus, according to the results of studies, it is possible to determine the type of plants by their anatomical sections, by the presence and location of crystals of calcium oxalate and other salts. These results can be used to find out under what environmental conditions this plant grew.

Acknowledgements

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