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Morphological and anatomical adaptations of selected *Juniperus* L. species to arid environments in Azerbaijan

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Abstract: The article presents a morphological and anatomical analysis of leaves and shoots of *Juniperus excelsa* subsp. *polycarpos* (syn. *Juniperus polycarpos*) and *Juniperus oxycedrus*. The conditions for these species to grow in the territory of the republic are quite limited, therefore morphological growth of species was valued normal under arid and semi-arid zone. Samples of leaves and young shoots of both species have been collected in their natural habitats in the Turyanchay State Nature Reserve, in arid sparse forest conditions. The characteristics of their structure reflecting adaptation to xerophytic conditions have been established. Both species are characterized by a single-layer epidermis with a thickened cuticle, submerged stomata, developed mechanical tissue and the presence of resin ducts. The species *J. polycarpos* has been found to have two- and three-lobed shoots with varying degrees of xylem development and central cylinder shapes, while *J. oxycedrus* is characterized by the presence of triangular shoots and well-defined resin ducts in the corners. In contrast to *J. oxycedrus*, the shoots of *J. excelsa* subsp. *polycarpos* are distinguished by their two- and three-lobed nature, reflecting the opposite and whorled arrangement of leaves. The data obtained confirm the similarity of the main anatomical features of both species and their functional role in increasing plant resistance to moisture deficiency and extreme temperatures. The results of the research are important for understanding the mechanisms of adaptation of these species to different climatic conditions.

Keywords: *adaptation, anatomy, Juniperus excelsa subsp. polycarpos, Juniperus oxycedrus, needles, stem, Turyanchay*

INTRODUCTION

The genus *Juniperus* L. comprises over 70 species, distributed widely in temperate and subtropical regions [Adams, 2014]. There are eight species of *Juniperus* growing in Azerbaijan [Asgarov, 2016]. Within the republic, the occurrence of *Juniperus* species is predominantly observed in the lower, middle and upper mountain belts, manifesting as discrete groups within the context of arid, sparse forest vegetation [Gurbanov, Rzayeva, 2019]. A more extensive population of representatives of the genus was observed in the vicinity of Bozdag Ridge, particularly within the confines of the Turyanchay State Nature Reserve (TSNR).

Life forms are represented by shrubs or low trees with evergreen leaves. The leaves of *J. oxycedrus* L. are characterized by their dense and linear morphology, with a pointed tip that is characterized by a sharp apex. The dimensions of these leaves range from 15-20 mm in length and 1,3-2 mm in width. A distinctive feature is the presence of two white longitudinal stripes. The underside of the needles is shiny green with a prominent longitudinal keel, without resin glands. The leaves of *J. excelsa* subsp. *polycarpos* (K. Koch) Takht. are trifoliate on the branches, long-pointed, pressed on secondary twigs, convex on the back, slightly keeled, ovate or ovate-rhombic, bright green in color. The shoots of this species of *Juniperus* are characterized by opposite and whorled leaf arrangement, which appears as a two- or three-lobed shape on a cross-section of the stem [Flora of Azerbaijan, 1950].

The preservation and restoration of unique juniper-pistachio forests and other natural resources, as well as the prevention and localization of erosion hotspots in the foothills, are priority tasks for this reserve. Three species of juniper grow on its territory, which are important components of the reserve's ecosystem biotopes: prickly juniper (*J. oxycedrus*), stinking juniper (*J. foetidissima* Willd.) and Persian juniper (*J. excelsa* subsp. *polycarpos*). The species composition and structure of juniper plant communities, such as *Junipero-Elaeagnosum*, *Juniperotum polycarpotum-Juniperus foetidisosum*, *Juniperotum foetidisosum-*

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Rubosum were analysed in the study area [Gurbanov and Rzaeva, 2017].

In Azerbaijan, the anatomical structure of the leaves of *J. excelsa* M. Bieb., *J. foetidissima* and *J. oblonga* M. Bieb., as well as the anatomical structure of the stems of *J. excelsa* subsp. *polycarpos* and *J. foetidissima*, collected in the TSNR, was studied [Farzaliyev, 2021]. It has been established that in each species of the genus *Juniperus*, resin canals are surrounded by two or more layers of secretory cells. The number of secretory cells in proximity to the resin canals serves as the primary diagnostic indicator. Furthermore, the structure of the stem cross-section and the type of mesophyll are also key distinguishing features.

The genus *Juniperus* is characterized by a high concentration of biologically active substances, which have been demonstrated to possess a range of pharmacological effects, including antibacterial, anti-inflammatory, antimicrobial, and antioxidant properties [Yujia et al., 2020; Zouaoui et al., 2024]. Biologically active components accumulate in the plant in special reservoirs [Kuang et al., 2019].

Scientists from many countries have also studied the anatomical features of various species of juniper [Vasić, Dubak, 2012; Radoukova et al., 2024]. Some scientists conducted a study devoted to the description and quantitative assessment of the anatomical features of *J. excelsa* subsp. *polycarpos* shoots from the foothills of Dagestan, identified characteristics related to their age and shape, and found that the presence of two types of shoots (two- and three-lobed), a significant number of stony cells in the parenchyma of leaf cushions in two-lobed shoots, the specific structure of the central cylinder, and the presence and number of oil cells are characteristic features of these species [Asadulaev, 2020].

Furthermore, an analysis of the morphological characteristics of *J. communis* and *J. oxycedrus* leaves was conducted, which demonstrated their dependence on the altitude gradient [Vasić, Dubak, 2012]. As the altitude at which the plants grow increases, the length of the leaves of both species decreases, reflecting their adaptation to the less favorable environmental conditions of mountainous areas. These changes are indicative of the high plasticity and adaptability of the species to different habitats.

The primary objective of morphological and anatomical studies is to identify indications of adaptation of *Juniperus* species to arid ecosystems, as well as to ascertain any differences or similarities in anatomical features.

MATERIAL AND METHODS

Samples of leaves and young shoots of both species, such as prickly juniper (*J. oxycedrus* L.) and Persian juniper (*J. excelsa* subsp. *polycarpos* (K. Koch) Takht.) were collected in their natural habitats in the TSNR, at an altitude of 400-650 m above sea level, where they grow as part of the *Caraganetum-Pistaciae-Juniperosum*, *Astragaletum-Acantholimonae-Juniperosum* and *Paliurusetum-Pistaciae-Juniperosum* associations in arid sparse forest conditions. A proportion of the samples were utilized in the creation of a herbarium. Samples of leaves and annual shoots were collected from different layers of the crown (upper, middle and lower) in order to assess anatomical heterogeneity. In order to preserve the plant material, a solution of 70–75% ethanol was used. Cross-sections and longitudinal sections were manually prepared from the fixed samples using a sharp blade [Humbatov, 2017]. The sections were stained with a 1% safranin solution (Sigma-Aldrich, USA) to visualize cellular structures, and phloroglucinol (Merck, Germany) was used in combination with concentrated hydrochloric acid (Honeywell, USA) to reveal lignified tissues. Starch was determined by reaction with iodine solution in potassium iodide, lipid inclusions and essential oils using Sudan III (Sigma-Aldrich, USA). The diagnosis of tannins was facilitated by the application of ferric chloride solution ($FeCl_3$, Merck, Germany). The thickness of the cuticle, the number of mesophyll layers, the characteristics of conductive tissues and mechanical elements were evaluated.

The preparations were fixed in a glycerol-gelatin medium. Microscopic observations were performed using Biolam (LOMO, Russia) and Zeiss light microscopes (Carl Zeiss, Germany). Morphometric analysis was performed using an eyepiece micrometer. Photographic fixation of anatomical structures was performed using an NLCD-307B digital microscope (Novel Optics, China). The anatomical structure was studied using the generally accepted methodology of R.P. Barykin et al. [2004], and the terminology of R.F. Evert [2015], L.I. Lotova [2007] was used in the description of tissues.

RESULTS AND DISCUSSION

The presented study describes and provides morpho-anatomical characteristics of two species of juniper (*J. excelsa* subsp. *polycarpos* (K. Koch) Takht. and *J. oxycedrus* L.), as the morpho-anatomical structure of the vegetative organs of these plants is of particular interest, reflecting their adaptation to environmental conditions

and the pharmacological action of biologically active substances found in special compartments.

Morphological and anatomical research led to the following conclusions:

Leaves (*J. oxycedrus*). Anatomical examination revealed that the cross-section of the specimen exhibited a triangular leaf shape, as well as the presence of a thick layer of cuticle on the surface of the epidermal cells. The epidermis is constituted by a single layer of cells that are small and tightly fitting. The stomata are embedded in the epidermis, as is characteristic of all gymnosperms, and are also surrounded by a substomal chamber to reduce transpiration. Subsequent to the epidermis is the hypodermis layer, which exhibits a thickness of two or occasionally three layers at the leaf's corners, accompanied by thickened cell walls (Fig. 1a).

On the adaxial side, the mesophyll of the leaf consists of a single layer of palisade cells, while on the abaxial side there are areas with two rows of palisade parenchyma, represented by densely packed cylindrical cells with a large amount of chlorophyll. This is a characteristic feature of plants growing in arid conditions. The spongy parenchyma is distinguished by its loose texture and small intercellular spaces. The clear distinction between the palisade and spongy tissue is moderately pronounced (Fig. 1b).

In the centre of the leaf is a vascular-fibrous bundle, with the xylem directed towards the centre and the phloem towards the periphery. The bundle is surrounded by a layer of mechanical tissue – sclerenchyma – with thickened cell walls that provide protection and strength. It is evident that beneath the accumulation of conductive tissues, a schizogenous resin canal can be discerned, surrounded by secretory cells and mechanical tissue (sclerenchyma). This serves to separate the canal from the cells of the primary tissue (Fig. 1c).

The combination of thickened epidermis, a powerful cuticle, dense mesophyll, and hypodermis with resin ducts indicate pronounced xeromorphic adaptations to the arid conditions of the habitat. The anatomical structure of juniper leaves is characterized by a single-layered epidermis with a thick cuticle, a hypodermis, mainly palisade mesophyll, a central vascular bundle with a mechanical zone, and resin ducts. This configuration optimizes the performance of barrier, photosynthetic, conductive, and protective functions.

Stem (*J. oxycedrus*). The stem's cross-section exhibits a triangular configuration, with three distinctly delineated

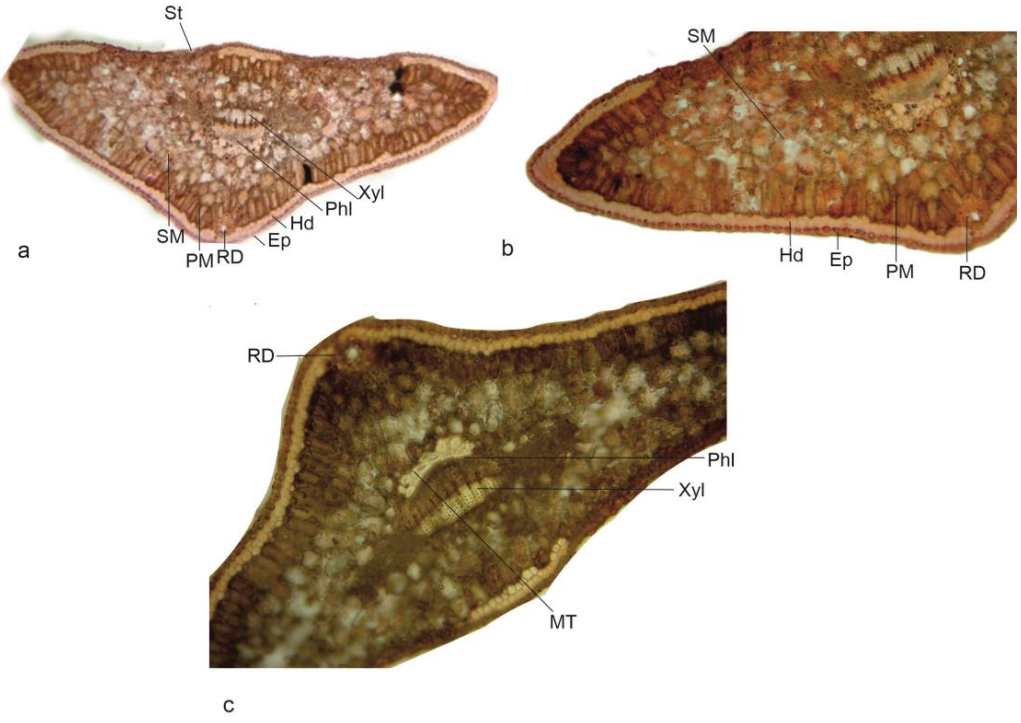

Figure 1. Anatomical structure of *Juniperus oxycedrus* leaf: a. general view; b. leaf mesophyll; c. conducting vascular bundle: Ep - epidermis with cuticle layer, Hd - hypodermis, RD -resin ducts (schizogenous cavity), PM - palisade mesophyll, SM - spongy mesophyll, Phl - phloem, Xyl - xylem, St - stomata immersed in the epidermis.

resin canals positioned at the corners. Microscopic analysis of the anatomical structure of the cross-section of the stem revealed the primary and secondary structure characteristic of conifers with a pronounced non-bundled stem type. The figure illustrating the primary structure of the stem clearly reveals the remains of the epidermis, with a thick layer of cuticle forming a continuous protective barrier. This configuration serves to minimise moisture evaporation and prevent mechanical damage.

The next layer is a well-developed periderm, consisting of lignified cells of the phelloderm and the phellogen. The cortical parenchyma consists of multiple rows of parenchymal cells, interspersed with mechanical tissue. In some regions, the presence of resin ducts is discernible, accompanied by the presence of crystalline inclusions characteristic of coniferous trees. The cambium, a layer of cells found between the bark and the wood, plays a key role in the formation of new layers of secondary xylem and phloem, which are essential for the structural integrity and function of the tree. Secondary xylem constitutes the main part of the stem and is characterised by radially oriented tracheids and wood rays. These rays form radial rows, thereby facilitating the transportation of substances and providing mechanical support. The xylem is distinguished by its compact and lignified structure, a typical feature of dense wood, such as that found in junipers. The heartwood consists of larger parenchyma cells. Moreover, the presence of resin deposits within the heartwood (pith) has been observed in the specimen (Fig. 2a).

The secondary structure of the stem exhibits more clearly defined layers of the aforementioned tissue types. The secondary structure is characterised by the presence of elongated cells of cambium, mechanical tissue, and xylem vessels with medullary rays. It is evident that the secondary tissue of the stem shows a conspicuous absence of additional schizogenous cavities. These cavities, which are characteristic of the primary bark of plants, are now located in the secondary tissues (Fig. 2b).

Anatomical features of pronounced xeromorphism, such as a thickened epidermis, cuticle, compact palisade mesophyll, and hypodermis, as well as the presence of resin ducts, provide the juniper with resistance to sudden temperature fluctuations and lack of moisture,

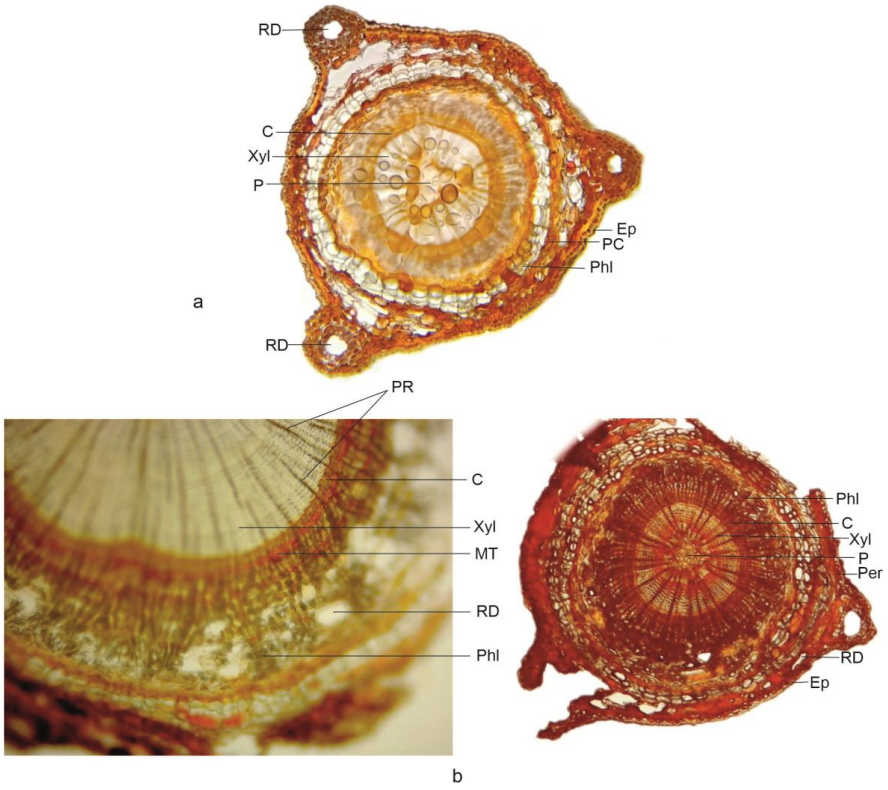

Figure 2. *Juniperus oxycedrus*: a. the anatomical structure of a one-year-old stem (EP – epidermis with a layer of cuticle, PC – parenchyma cells of the primary cortex, RD – resin ducts (schizogenous cavities), Phl – phloem, C – cambium, Xyl – xylem, P – pith); b. enlarged image of the secondary structure of the stem (EP - epidermis with a layer of cuticle, Per - periderm cells, MT - mechanical tissue, Phl - phloem, C - cambium, Xyl - xylem (wood), P - pith, PR - pith rays, and RD – resin ducts (schizogenous cavities)).

especially during winter, when roots cannot absorb water from frozen soil.

Leaves (*J. excelsa* subsp. *polycarpos*). This type of *Juniper* L. has two- and three-lobed shoots, characterized by the presence of two or three leaf cushions. Externally, the skin is covered by a single layer of epidermis, which is characterized by thickened outer walls and a clearly visible cuticle layer. The stomata are clearly visible, embedded in the epidermis. Beneath the epidermis, a layer of hypodermis is often present, providing mechanical protection and is typically represented by collenchyma tissue. Collenchyma is distinguished by the presence of two to three rows of cells, characterised by unevenly thickened cell walls. The main part of the leaf cushion is occupied by mesophyll, with a slight differentiation into palisade and spongy tissue. The mesophyll borders the primary bark of the shoot. Palisade parenchyma consists of two or three rows of elongated cells rich in chlorophyll. The spongy tissue is loose, with a large number of intercellular spaces. In the centre of each leaf cushion, resinous schizogenous passages are clearly visible, surrounded by several rows of lining cells. The leaves of *Juniperus excelsa* subsp. *polycarpos* have a slightly differentiated mesophyll, but, like the leaves of *J. oxycedrus*, they have thickened epidermal cells with a clearly visible cuticle layer.

Stem (*J. excelsa* subsp. *polycarpos*). The central part of the shoot exhibits a structure that is characteristic of gymnosperms (Fig. 3 a-b). Following the deposition of phellogen under the leaf cushions, periderm cell development begins. The central part of the shoot is represented by the primary cortex, which consists of the periderm and parenchymal cells of the phelloderm (Fig. 3c). These cells are known as cortical parenchymatous cells. The tissue has a mechanical nature, as it consists of cells with thickened cell walls. The primary cortex contains phloem elements. Phloem forms a continuous ring around the periphery of the central part of the shoot and consists of sieve tubes and parenchyma cells, which may also have separate resin canals. The

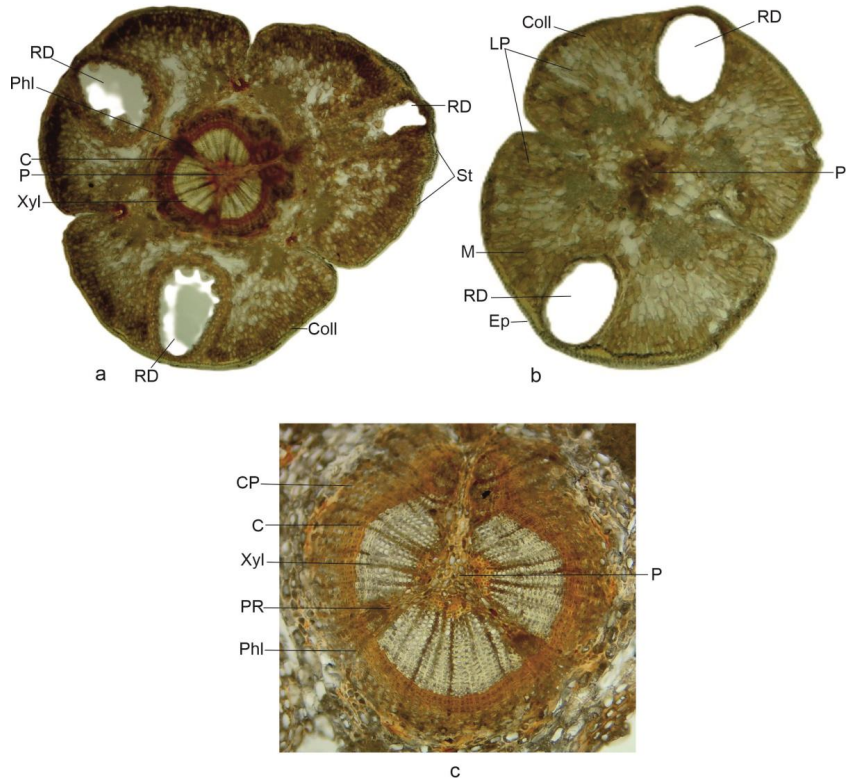

Figure 3. *Juniperus excelsa* subsp. *polycarpos*: a. Three-lobed shoot of (EP – epidermis with cuticle layer, M – mesophyll, RD – resin ducts, Xyl – xylem, C – cambium, P – pith, Phl – phloem, St – stomata, Coll – collenchyma, Vb – vascular bundle, LP – leaf pads); b. Two-lobed shoot (EP – epidermis with cuticle layer, M – mesophyll, RD – resin ducts, Xyl – xylem, C – cambium, P – pith, Phl – phloem, St – stomata, Coll – collenchyma, Vb – vascular bundle, LP – leaf pads); c. Anatomical structure of the central part of the shoot (stem) (CP – cortical parenchyma, Phl – phloem, C – cambium, Xyl – xylem vessels, P – pith cells containing essential oils, PR – pith rays).

layer of meristematic tissue, known as cambium, is located between the phloem and xylem rings. Cambium is responsible for the formation of xylem elements within the central region of the shoot. The xylem is composed of tracheids that are arranged radially and form clearly defined annual layers. Pith is comprised of parenchyma cells that are capable of storing various nutrients. Between the rows of xylem, medullary rays are clearly visible, consisting of one or two rows of cells that facilitate radial transport of water and nutrients. The thickness of the medullar rays varies depending on the age of the shoot. The central cylinder in two- and three-lobed shoots differs in shape and the degree of development of woody elements.

CONCLUSION

An anatomical analysis of *Juniperus oxycedrus* L. leaves revealed a triangular morphology characterised by a single-layer epidermis with thickened cell walls and a pronounced cuticle, which offers significant protection against excessive transpiration. The presence of two- and three-layer hypodermis, developed palisade mesophyll and sclerenchyma ring around the conducting bundle reflects pronounced xeromorphic adaptations of the plant to arid conditions. The resin canals located beneath the vascular bundle perform a protective function and increase the resistance of shoots to environmental stress factors.

The stem of *J. oxycedrus* is characterized by a triangular cross-section with three resin canals, well-developed periderm, and pronounced secondary xylem. This provides mechanical strength and durability to the shoots, while the cambium and medullary rays actively promote secondary thickening. The compact structure of xylem efficiently transports water. The combination of these characteristics – thickened cuticle, dense mesophyll, hypodermis, and resin ducts – indicates the plant's high resistance to sudden temperature fluctuations and lack of moisture in winter.

Double-lobed shoots have two well-defined growths on a cross-section. The bark consists of large-celled parenchyma with sparse intercellular spaces. The central cylinder has a ring-like structure with clearly distinct phloem and xylem, and the pith is dense without cavities. It is evident that three-lobed shoots are distinguished by a more symmetrical radial organisation, in addition to the presence of three protrusions corresponding to the location of the leaves. The epidermis and collenchyma exhibit a comparable structural configuration; however, the cortical parenchyma demonstrates enhanced development. The central cylinder is closed, the conducting elements are distributed uniformly around the circumference, and the pith is comparatively large.

It was thus determined that the shoots of *J. oxycedrus* L. and *J. excelsa* subsp. *polycarpos* (K. Koch) Takht. exhibit only divergent morphological structures, while no substantial disparities were identified in their anatomical structures.

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Juniperus L. cinsinin bəzi növlərinin Azərbaycanın quraq şəraitinə morfo-anatomik uyğunlaşmaları

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Məqalədə *Juniperus excelsa* subsp. *polycarpos* (syn. *Juniperus polycarpos*) və *Juniperus oxycedrus* növlərinin yarpaq və zoğlarının morfoloji və anatomik analizi təqdim olunur. Bu növlərin respublika ərazisində bitmə şəraitinin kifayət qədər məhdud olmasına baxmayaraq, arid və yarım-arid (quraq və yarımquraq) zonalarda növlərin morfoloji inkişafı normal qiymətləndirilmişdir. Hər iki növün yarpaq və gənc zoğlarının nümunələri onların Türyançay Dövlət Təbiət Qoruğundakı təbii areallarından, arid seyrək meşə şəraitindən toplanmışdır. Onların kserofit şəraitə uyğunlaşmasını əks etdirən quruluş xüsusiyyətləri müəyyən edilmişdir. Hər iki növ qalınlaşmış kutikulaya malik birqatlı epidermis, batıq ağızcıqlar, inkişaf etmiş mexaniki toxuma və qətran kanallarının mövcudluğu ilə xarakterizə olunur. *J. oxycedrus* növü üçün üçbucaqlı zoğların və küncləndə aydın ifadə olunmuş qətran kanallarının olması xarakterik olduğu halda, *J. polycarpos* növündə müxtəlif inkişaf dərəcəsinə malik ksilema və fərqli formalı mərkəzi silindrlə seçilən iki və üçpərli zoğlar aşkar edilmişdir. *J. oxycedrus* növündən fərqli olaraq, *J. excelsa* subsp. *polycarpos* növünün zoğları iki və üçpərli forması ilə fərqlənir ki, bu da yarpaqların qarşı-qarşıya və topaşəkilli düzülüşünü əks etdirir. Əldə edilmiş məlumatlar hər iki növün əsas anatomik xüsusiyyətlərinin oxşarlığını və bitkilərin rütubət qıtlığına, həmçinin ekstremal temperaturlara qarşı davamlılığının artırılmasında onların funksional rolunu təsdiqləyir. Tədqiqatın nəticələri bu növlərin müxtəlif iqlim şəraitinə adaptasiya mexanizmlərinin dərk edilməsi baxımından əhəmiyyətlidir.

Açar sözlər: *adaptasiya, anatomiya, Juniperus excelsa subsp. polycarpos, Juniperus oxycedrus, iynəyarpaq, gövdə, Türyançay*

Морфологические и анатомические адаптации некоторых видов рода *Juniperus* L. к аридным условиям Азербайджана

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В статье представлен морфологический и анатомический анализ листьев и побегов видов *Juniperus excelsa* subsp. *polycarpos* (син. *Juniperus polycarpos*) и *Juniperus oxycedrus*. Условия для произрастания этих видов на территории республики весьма ограничены, поэтому морфологическое развитие видов оценивалось как нормальное для засушливой и полузасушливой зоны. Образцы листьев и молодых побегов обоих видов были собраны в их естественных местах обитания в государственном природном заповеднике Турьянчай, в условиях засушливого редколесья. Установлены особенности их строения,

отражающие приспособленность к ксерофитным условиям. Оба вида характеризуются однослойным эпидермисом с утолщённой кутикулой, погружёнными устьицами, развитой механической тканью и наличием смоляных канальцев. У вида *J. polycarpos* обнаружены двух- и трёхлопастные побеги с различной степенью развития ксилемы и разной формой центрального цилиндра, тогда как для *J. oxycedrus* характерно наличие треугольных побегов и чётко выраженных смоляных канальцев в углах. В отличие от *J. oxycedrus*, побеги *J. excelsa* subsp. *polycarpos* отличаются двух- и трёхлопастной формой, что отражает супротивное и мутовчатое расположение листьев. Полученные данные подтверждают сходство основных анатомических особенностей обоих видов и их функциональную роль в повышении устойчивости растений к дефициту влаги и экстремальным температурам. Результаты исследования важны для понимания механизмов адаптации этих видов к различным климатическим условиям.

Ключевые слова: *адаптация, анатомия, Juniperus excelsa subsp. polycarpos, Juniperus oxycedrus, хвоя, стебель, Турьянчай*