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Comparative anatomy of the vegetative organs from two species of the genus *Ruscus* L.

C. Timuc, I. N. Gostin*

"Alexandru Ioan Cuza" University of Iași, Iasi, Romania

**Corresponding author: irinagostin@yahoo.com*

Abstract. In this paper, the anatomy of normal and metamorphosed stems from two species of the Ruscus genus (R. *hypoglossum* L. and R. *aculeatus* L.) were analyzed using light microscopy. The structural characteristics of the two species were presented, with emphasis on the adaptations they present to environmental conditions. The normal strain from the species R. *hypoglossum* shows a greater amount of sclerenchyma fibers in the central cylinder compared to R. *aculeatus*; vascular bundles are more numerous in R. *aculeatus*, but smaller, with few elements of sclerenchyma, especially near the phloem. The metamorphosed stem (phyllocladia) shows a similar structural plan to the two species, with assimilating tissue under the epidermis and colorless tissue in the central part. The number of vascular bundles differs, they being more numerous, arranged in a circle, in R. *hypoglossum*. The anatomical peculiarities of the investigated species help us to understand their adaptation to the living environment, and also can help to identify the species when we only have fragments of the plant's body.

Key words: R. *hypoglossum*, R. *aculeatus,* stem, phyllocladia, anatomy.

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Introduction

The genus *Ruscus* L. includes 6 currently accepted species, two of which (R. *hypoglossum* L. and R. *aculeatus* L.) are found in the flora of Romania (POWO, 2024) [1]. The representatives of the genus *Ruscus* attracted the attention of researchers due to their botanical, medicinal and ornamental characteristics. Studies by various research teams, starting with plant anatomy and histology and continuing with investigations into medicinal properties, have revealed interesting details about this genus.

The anatomical studies carried out by Bălică and collaborators [2] in 2005 highlighted the complex structure of the vegetative organs of R. *aculeatus*, compared with those obtained from in vitro cultures. Root analysis demonstrated the presence of a thick, compact cortical parenchyma and a central cylinder with numerous simple vascular bundles of phloem and xylem. The normal stem shows chlorenchyma in the external area; in phyllocladia (modified leaf-like stems), the researchers observed a homogeneous assimilative parenchyma and leptocentric concentric vascular bundles.

Guvenc et al., in 2011 [3], performed comparative studies on the phyllocladia of five different species of Ruscus and found a consistent structure among them. Collateral-type vascular bundles vary in number between species, with R. *hypoglossum* and R. *colchicus* having more bundles than *Ruscus* aculeatus species.

Phenological studies on the species R. *aculeatus* have shown that this plant has a low rate of sexual reproduction. Martinez-Palle and Aronne [4] investigated these issues, suggesting that the lack of pollinators is the main cause of the decline in fruit set. However, research has shown that plants can form viable fruit when artificially pollinated, eliminating the possibility of internal physiological deficiencies.

The genus *Ruscus* is known for its bioactive compounds, especially steroidal saponins, such as ruscogenin and neoruscogenin, which are used in the treatment of venous insufficiency, varicose veins, and hemorrhoids. Chemical and pharmacological studies by Mascullo et al (2016) [5] have deepened the knowledge of these compounds and their therapeutic applicability.

R. *aculeatus* has also been investigated for its anti-inflammatory potential. Bălică et al. (2013) [6] demonstrated the beneficial effect of steroid saponins in acute inflammation models in rats, by inhibiting the activity of prostaglandins.

Another direction of research is related to the conservation of the R. *aculeatus* species through in vitro propagation techniques. The studies carried out by Banciu and Aiftimie-Păunescu (2012) [6] showed that plants can be effectively regenerated in controlled environments, contributing to the repopulation of affected natural habitats and the conservation of genetic material.

The genus *Ruscus* is a model of interest to botanists and pharmaceutical and ecological researchers due to its unique characteristics and wide medical applicability.

In this paper we have carried out a comparative study of the aerial vegetative organs from two species of the genus Ruscus, with the aim of highlighting the particular features related to the adaptations of the species to the living environment, with emphasis on the phyllocladia, particular structures present in the species of this genus.

Material and Methods

For the histo-anatomical analyses, the plant material, represented by fragments of stems and phyllocladia of *Ruscus aculeatus* L. and R. *hypoglossum* L. were preserved in 70% ethyl alcohol. Cross sections were performed using a manual microtome and a razor blade. The sections were stained either with ruthenium red (0.05%) and iodine green [7-8]. Microphotographs were taken using an Olympus C 330 digital camera (Olympus Corporation, Tokyo, Japan), after observations with an Olympus BX41 (Olympus Corporation, Tokyo, Japan) research microscope.

Results and discussions

The structure of the normal stem of *Ruscus* hypoglossum shows quantitative variations between its base, middle and top, reflecting adaptations of the stem according to position and function.

At the base of the stem (Figure 1a -d), the cross-section has a rounded, irregular shape. The epidermis is single-layered, consisting of square, isodiametric cells, with straight side walls and slightly thickened on the outside. From place to place, the epidermis is interrupted by stomata, consisting of two guard cells and two subsidiary cells, without suprastomatal chambers, indicating that the plant developed in a high-humidity environment. Beneath the epidermis, the cortex is made up of parenchyma cells. The endodermis, the last layer of the cortex, contains simple cells, with uniformly thin walls. Inside the central cylinder is the pericycle, consisting of 6-8 layers of sclerenchyma cells. The vascular bundles are closed, by collateral type, with phloem oriented outward and xylem inward, the phloem being surrounded by a sheath of sclerenchyma cells. Some smaller vascular bundles are completely embedded in the pericycle.

In the middle of the stem (Figure 1 e-h), the section is circular, slightly elongated, with a single-layered epidermis, consisting of small, square cells, covered by a thick cuticle. Stomata are rare, with the guard cells at the same level as epidermal cells. The cortex is thinner than at the base of the stem, contains parenchyma cells (with a role in photosynthesis), those near the epidermis being smaller, and those near the endodermis being larger and thinner-walled. The central cylinder contains fewer layers of sclerenchyma than at the base, and the vascular bundles of closed collateral type are protected by a thick layer of sclerenchyma, which surrounds the phloem.

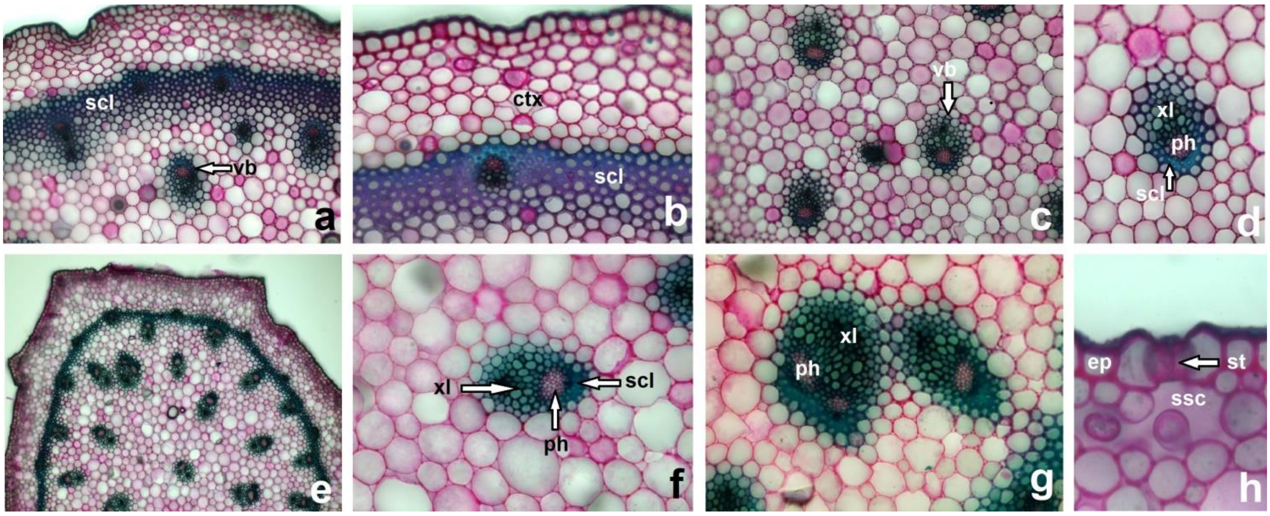

Figure 1 - Cross sections through the mature normal stem of *Ruscus hypoglossum* - the order of magnification is mentioned in round brackets: a - d: base of the stem (a -x100, b, d - x400, c - x200), e - h: middle of the stem (e - x100, f,g - x400, h - x1000), : ctx- cortical parenchyma, ep - epidermis, ph - phloem, scl - sclerenchyma, st - stomata, ssc - substomatic chamber, vb - vascular bundle, xl - xylem

At the top of the stem, the cross-section is circular and elongated, with an extension to one side. The epidermis is single-layered and covered by a thick cuticle, the epidermal cells being square shaped. Stomata are rarer than in the lower sections. The cortex consists of rounded cells, with small air spaces between them; next to the ribs, larger parenchymal cells with thin walls can be observed. The endoderm marks the boundary of the cortex and the central cylinder, consists of 2-4 sclerenchyma cells layers. Vascular bundles are similar in structure to those described at the other levels of the stem; they have an important role in supporting the stem.

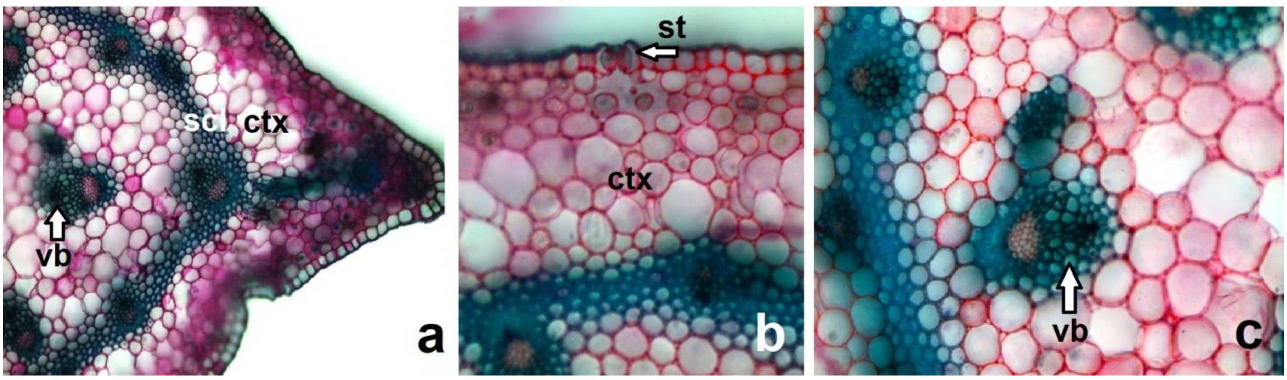

Figure 2 - Cross sections through the mature normal stem of Ruscus hypoglossum - the order of magnification is mentioned in round brackets: a - c: top of the stem of the stem (a -x200, b, c- x400), : ctx- cortical parenchyma, scl - sclerenchyma , st - stomata, vb - vascular bundle

The structure of the metamorphosed stem in *Ruscus hypoglossum* shows several distinct features that differentiate it from ordinary stems (Figure 3). The shape of the cross section is similar to that of a normal leaf, corresponding to the morphological appearance of the phyllocladia. The epidermis consists of small square cells with straight side walls and slightly thickened upper ones, covered with a thin cuticle. Both the upper and lower epidermis has a similar structure, and the stomata are few and are located at the same level as the epidermal cells.

Beneath the epidermis an assimilatory tissue can be observed; it consists of cells small and circular towards the center of the section and rectangular or elongated tangentially towards the edges and veins. Between the upper and lower assimilatory tissues is the lacunar parenchyma, consisting of colorless large cells arranged in 2-3 layers, interrupted by veins.

The central cylinder consists of 3-4 layers of sclerenchyma cells and fundamental parenchyma in its center. The sclerenchyma cells are small, thick-walled and circular, while those in the underlying parenchyma are larger, thin-walled. The central cylinder contains 3-4 large and 2-3 small vascular bundles, of the collateral type, with phloem facing the center of the central cylinder.

In the mesophyll, vascular bundles are found that form the parallel veins, each surrounded by a parenchymatous sheath. This sheath is made up of small cells, lacking chloroplasts, and has a role similar to the endoderm. The conducting fascicles inside the sheath are of the collateral type.

This structure reflects the morphological and functional adaptations of the metamorphosed stem, contributing to the conduction of sap, as well as to the process of photosynthesis.

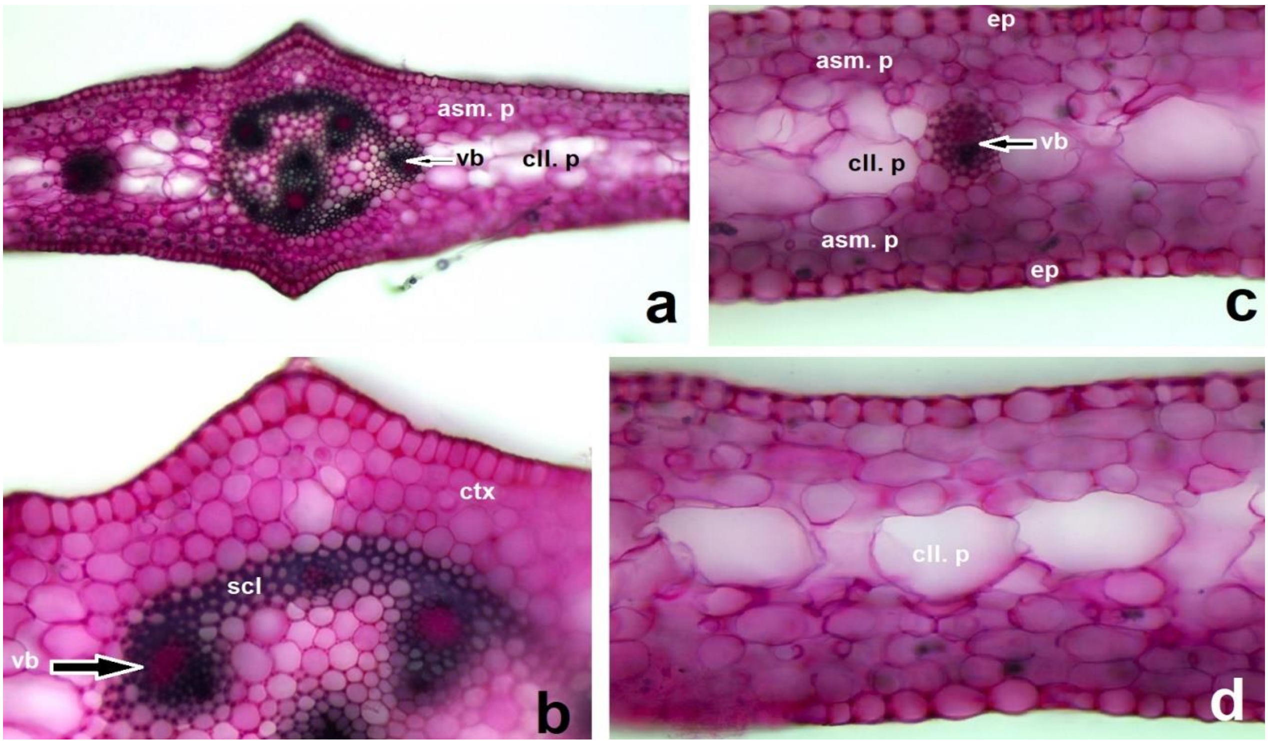

Figure 3 - Cross sections through the metamorphosed stem of *Ruscus hypoglossum* - the order of magnification is mentioned in round brackets: a - overall image (x100), b - detail from the central area with vascular tissues (x200), c, d - details from the area lateral (x400): asm. p - assimilatory parenchyma, cll. p - colorless parenchyma, ctx- cortical parenchyma, scl - sclerenchyma, vb - vascular bundle.

In the normal stem of *Ruscus aculeatus,* observations were made in three distinct sections: base, middle and tip, emphasizing the anatomical differences of each area.

At the base of the stem (Figure 4 a-d), the section is circular, with irregular ridges on the outline. The epidermis is unilayered (consisting of a single layer of square cells), tightly joined together, with thin side walls and thickened upper/lower ones. This epidermis is covered by a thick cuticle. The cortex, immediately below the epidermis, consists of 3-4 layers of assimilatory tissue. Below it is the cortical parenchyma, consisting of large, thin-walled cells. The fundamental parenchyma from central cylinder, in which vascular bundles are dispersed, consists of large, round cells, with slightly thickened and lignified walls, especially in the external area, with spaces between them (meats). Compared to Ruscus hypoglossum, in Ruscus aculeatus there are more vascular bundles, but also more sclerenchyma. The xylem vessels are smaller in size, similar to those of the phloem.

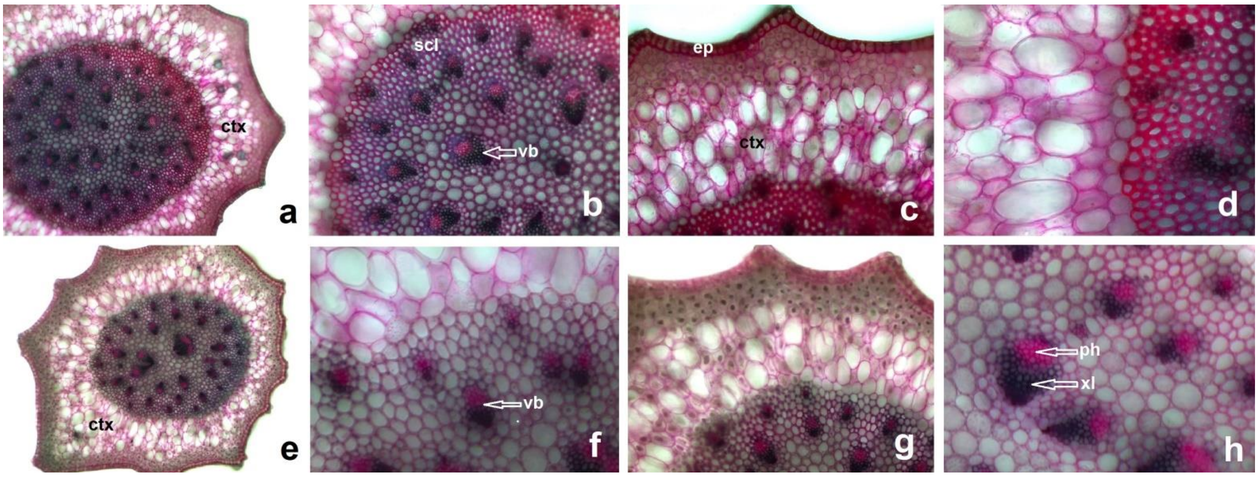

Figure 4 - Cross sections through the mature normal stem of *Ruscus aculeatus* - the order of magnification is mentioned in round brackets: a - d: base of the stem (a -x100, b, c – x200, d – x400), e - h: middle of the stem (e - x100, f-h - x400), : ctx- cortical parenchyma, ep - epidermis, ph - phloem, scl - sclerenchyma, vb - vascular bundle, xl - xylem.

In the middle of the stem (Figure 4 e-h), the epidermis is still unilayered, with small and square cells, covered by a thick cuticle. Under the epidermis, there are 2-3 layers of assimilatory tissue, with cells containing chloroplasts. The rest of the cortex is made up of large, thin-walled cortical parenchyma cells. Fundamental parenchyma has large cells, between which are observed closed collateral conducting bundles, formed by xylem and phloem. The parenchyma cells are slightly sclerified in the external area. The more developed vascular bundles, located deep in the central cylinder, have phloem protected by few sclerenchyma fibers.

At the top of the stem (Figure 5), the epidermis is similar, being thin, single-layered, with small, equal cells, covered by a thick cuticle. The cortex consists of external parenchyma, with smaller, rounded cells near the epidermis, but the rest of the cells are large, thin-walled. In the central cylinder there are numerous closed collateral vascular bundles. The fundamental parenchyma has large, thin-walled cells. In the thickness of the pericycle, conductive bundles are observed in formation, in which the phloem is more developed than the xylem.

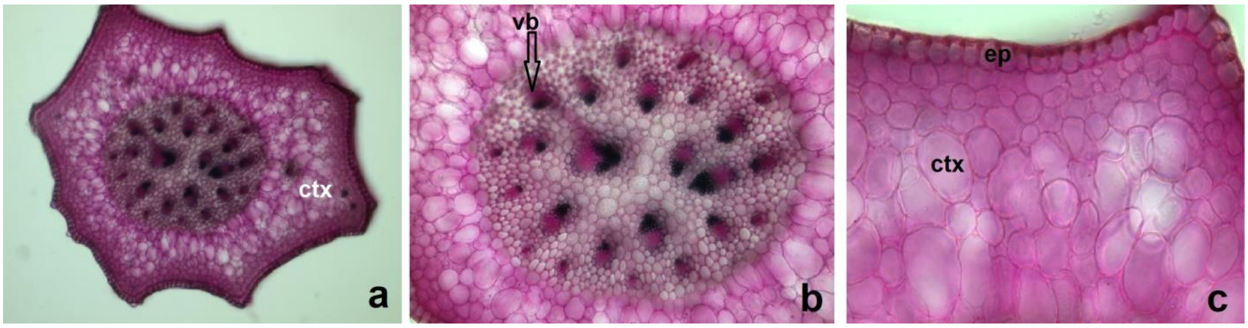

Figure 5 - Cross sections through the mature normal stem of *Ruscus aculeatus* - the order of magnification is mentioned in round brackets: a - c: top of the stem of the stem (a -x200, b, c- x400): ctx- cortical parenchyma, ep – epidermis, vb - vascular bundle.

The structure of the metamorphosed stem in R. *aculeatus* (Figure 6): the epidermis is single-layered, the epidermal cells are square shaped, approximately equal in size, with thin side walls and the upper ones covered with a thick layer of cuticle. A few stomata can be observed on both the upper and lower epidermis which cannot be differentiated.

The mesophyll is made up of 4 – 5 layers of assimilating parenchyma cells, with an irregular shape, predominantly circular, slightly larger in size compared to the epidermal cells and with meats between them. Layers of similar cells can be observed under both epidermises, constituting another factor that does not allow their differentiation.

Between the assimilatory tissues, there is the lacunar parenchyma made up of similarly sized and thin-walled, colorless cells.

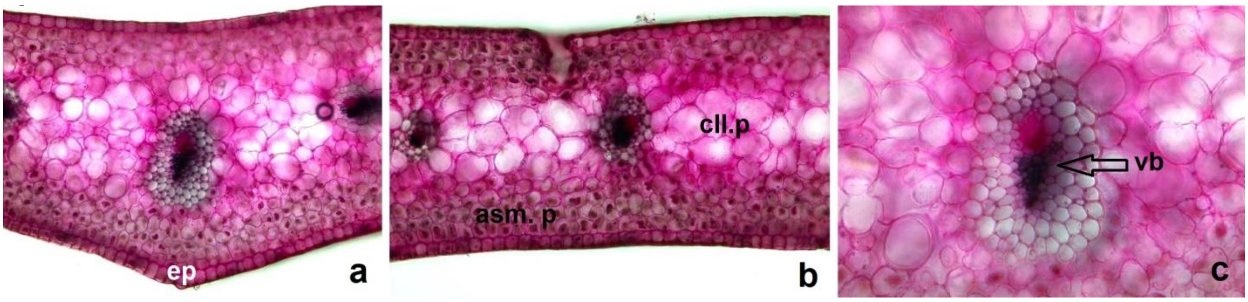

Figure 6 - Cross sections through the metamorphosed stem of Ruscus aculeatus - the order of magnification is mentioned in round brackets: a - overall image of the central area (x200), b - overall image of the lateral area (x200), c - details from the lateral area (x400): asm. p - assimilatory parenchyma, cll. p - colorless parenchyma, ep – epidermis, vb - vascular bundle.

Veins are represented by conducting bundles of closed collateral type, surrounded by a relatively fascicular sheath, consisting of 3-4 layers of parenchymal cells closely united with each other, with the role of support and protection. At the level of the vascular bundles, both the phloem vessels arranged towards the lower epidermis and the xylem vessels, directed towards the upper one, are well developed. Our observations differ from those of Bălică et al., 2005 [2], who described vascular bundles of leptocentric type at this level.

Being found in areas with limited nutritional and water resources (at least part of the growing season), species of the genus *Ruscus* exhibit several anatomical adaptations in their stems that help them thrive in their specific environments. One of the most notable adaptations in *Ruscus* species is the presence of cladodes. Cladodes are flattened, leaf-like structures that are actually modified stems. In *Ruscus* species, the leaves on the stem are reduced to small, scalelike, and membranous structures due to the transformation of the stem into photosynthetic cladodes [3]. Since they have reduced or absent true leaves, the cladodes take over the function of photosynthesis [9-10]. This adaptation reduces water loss and makes the plant more efficient in arid environments. The flat structure also maximizes the surface area for light absorption while minimizing water loss, which is crucial in dry and shaded habitats [11-12].

The normal and metamorphosed stems (cladodes) of *Ruscus* species are covered with a thick cuticle, an important adaptation to prevent water loss. A cuticle is a waxy layer that acts as a barrier against desiccation, helping the plant conserves moisture in environments where water is scarce [13-14].

Stomata are often reduced in density or absent on the stem surfaces of Ruscus species. This reduction is another way the plant conserves water, as fewer stomata mean less opportunity for water vapor to escape, a vital adaptation for surviving in dry conditions [9-14].

The sclerenchyma presents in the stems of analyzed *Ruscus* species, particularly around the vascular bundles (xylem and phloem), provide mechanical support and protect the vascular tissues. This adaptation ensures structural stability and enhances the plant's ability to transport water and nutrients efficiently, even under conditions of water stress (Raven et al., 2005).

At the same time, the vascular bundles in *Ruscus* species are adapted to efficiently transport water and nutrients in environments with irregular water availability. The presence of well-differentiated xylem (water-conducting tissue) and phloem (nutrient-conducting tissue) ensures that the plant can survive periods of drought and recover when water becomes available [15].

Many *Ruscus* species have a compact and bushy growth habit. This minimizes the surface area exposed to the sun and wind, further reducing water loss through evaporation [13]. The dense arrangement of stems and cladodes also helps the plant capture and retain moisture, essential in drier environments.

Conclusions

This study examines the anatomy of normal and metamorphosed stems (phyllocladia) in Ruscus hypoglossum and *Ruscus* aculeatus using light microscopy, focusing on structural adaptations to environmental conditions. In R. *hypoglossum*, the central cylinder contains more sclerenchyma fibers compared to R. *aculeatus,* which has more, but smaller, vascular bundles with fewer sclerenchyma elements. The metamorphosed stems of both species share a similar structure, with assimilating tissue beneath the epidermis and colorless tissue in the central part. However, R. *hypoglossum* has fewer, larger cells in the central tissue, while R. *aculeatus* has more, but smaller, cells. R. *hypoglossum* also has more vascular bundles (3-4 large and 2-3 small), arranged in a circle and surrounded by a common sclerenchyma sheath.

In both species, the true leaves are reduced to small, scalelike structures, while the photosynthetic function is taken over by cladodes. These adaptations minimize water loss and maximize light absorption, making *Ruscus* species highly efficient in dry, shaded habitats. The anatomical features discussed provide insight into their environmental adaptations and could help in species identification from plant fragments.

Authors' contribution:

Gostin Irina Neta: Principal author of the manuscript. She participated in collecting the material, performing laboratory investigations and writing the manuscript.

Ţimuc Casiana: made microscopic slides for analysis, participated in writing the manuscript. The results from the manuscript are included in her Bachelor Thesis presented at the Faculty of Biology, Alexandru Ioan Cuza" University of Iași, in 2024.

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С. Тимук, Н. Гостин*
А.И. Куза атындағы Ясса университеті, Яссы, Румыния

Ruscus L екі тұқымдасының вегетативті мүшелердің салыстырмалы анатомиясы

Аңдатпа. Бұл жұмыста *Ruscus* тұқымдасының екі түрінің (R. *hypoglossum* L. және R. *aculeatus* L.) қалыпты және метаморфозға ұшыраған сабақтарының анатомиясы жарық микроскопиясының көмегімен талданды. Екі түрдің құрылымдық сипаттамалары, олардың қоршаған орта жағдайларына бейімделуіне баса назар аударылды. R. *hypoglossum* түрінен қалыпты штамм R. *aculeatus*-пен салыстырғанда орталық цилиндрдегі склеренхима талшықтарының көп мөлшерін көрсетеді; тамыр шоғырлары R. *aculeatus*-да көп, бірақ кішірек, склеренхима элементтері әсіресе флоэма жанында аз. Метаморфозға ұшыраған өзек (филлокладиа) екі түрге ұқсас құрылымдық жоспарды көрсетеді, эпидермис астында ассимиляциялық ұлпа және орталық бөлігінде түссіз ұлпа бар. Қан тамырларының шоғырларының саны әртүрлі, олар көп, шеңбер түрінде орналасқан, R. *hypoglossum.* Зерттелетін түрлердің анатомиялық ерекшеліктері олардың тіршілік ету ортасына бейімделуін түсінуге көмектеседі, сонымен қатар бізде тек өсімдік денесінің фрагменттері болған кезде түрді анықтауға көмектеседі.

Түйінді сөздер: R. *hypoglossum,* R. *aculeatus,* өзек, филлокладия, анатомия.

К. Тимук, И.Н. Гостин
Ясский университет имени А. И. Куза, Яссы, Румыния

Сравнительная анатомия вегетативных органов двух видов растении рода _Ruscus_ L.

Аннотация. В данной работе методами световой микроскопии проанализирована анатомия нормальных и метаморфизированных стеблей двух видов растении рода Ruscus (R. *hypoglossum* L. и R. *aculeatus* L.). Представлены их структурные характеристики с учетом их адаптации к условиям окружающей среды. Показано, что растения R. *hypoglossum* имеют большее количество волокон склеренхимы в центральном цилиндре по сравнению с R. *Aculeatus.* Однако сосудистые пучки у растении R. *aculeatu*s более многочисленные и более мелкие, с небольшим количеством элементов склеренхимы, особенно вблизи флоэмы. Метаморфизованный стебель (филлокладия) имеет у обоих видов растении схож и характеризуется ассимилирующей тканью под эпидермисом и бесцветной тканью в центральной части. Число сосудистых пучков различается, у R. *hypoglossum* они более многочисленны и расположены по кругу. Таким образом, анатомические особенности исследованных видов помогают нам понять механизмы их адаптацию к среде обитания, а также могут помочь в идентификации вида по отдельным фрагментам растения.

Ключевые слова: R. *hypoglossum,* R. *aculeatus*, стебель, филлокладия, анатомия.

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Information about authors:

***Gostin I. N* –** She is Associate Professor PhD in Plant Science, "Alexandru Ioan Cuza" University of Iași, Faculty of Biology, Bdul Carol I, no. 11, 700506 Iasi, Romania

***Timuc C.* –** She is master's student, in Medical Laboratory, first year, "Alexandru Ioan Cuza" University of Iași, Faculty of Biology, Bdul Carol I, no. 11, 700506 Iasi, Romania

Сведения об авторах:

***Гостин И.Н.* –** А.И. Куза атындағы Ясса университетінің биология факультетінің доценті өсімдіктану ғылымдары бойынша PhD, Бдул Кэрол I, №. 11, 700506 Яссы, Румыния

***Тимук К.* –** А.И. Куза атындағы Ясса университетінің медициналық зертханасының, бірінші курс магистранты, Бдул Кэрол I, №. 11, 700506 Яссы, Румыния