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CONTRIBUTIONS TO THE ANATOMY OF *ASPLENIUM RUTA-MURARIA*

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The paper provides new data regarding the anatomical structure of the adventitious root, the rhizome and the leaf (petiole, rachis and pinnulae) of *Asplenium ruta-muraria*. The petiole and rachis structure were analysed by serial cross sections, distributed from the base up to the rachis tip, with special reference to the variation in the vascular system organisation. With 8 figures and detailed illustrations.

Key words: anatomy, adventitious root, *Asplenium ruta-muraria*, pinnae, rhizome

INTRODUCTION

Asplenium ruta-muraria L. (Aspleniaceae), known as wall rue, is a very common fern often found growing on old stone or brick walls and in the wild on stony outcrops, cliffs across the Carpathians. *Asplenium ruta-muraria* currently can be found in the Eastern Carpathians (Rodna Mountains (Pietrosu peak), Ceahlau and Piatra Mare Mountains, in Southern Carpathians, especially in Bucegi Mountains (Ciubotea, Babele, Ialomița Cave), Saint Ana lake, Sibiu, Fagaraş and Retezat Mountains. It is common throughout the Western Carpathians in the Banat Mountains region (Herculane, Great Valley, Cernei Valley, Domoglod Mountain) and Apuseni Mountains (Piatra Cetei, Trascau Mountains) and Turzii Gorge and in Bihor mountains (Ponorului Fortresses) as well. It can be found sporadically in Dobrudja Plateau hills in Măcin Mountains (Consul and Suluc peaks), which is a solitary mountain isolated from the Carpathians by the Danube river. In natural habitats it occurs on limestone or sometimes basalt bedrock. Following spring growth, it ripens in the summer and has wintergreen leaves. Its axis and stalk are green, and brown at the very base. Fronds are evergreen, with a length of 1–6.5 cm. Pinnulae leathery, ovate shaped and toothed. It is a tufted perennial but often many dead stalks remain attached around the plant. It is sporulating from June to September (SĂVULESCU 1952). In general, knowledge of the fern structure, especially those concerning the variation in the vascular system organisation of the vegetative organs, are quite limited, especially in the case of *Asplenium ruta-muraria* L.

MATERIAL AND METHODS

Cross sections of the root, rhizome and leaf were made with a rotary microtome. The samples were stained with alum-carmine and iodine green, and were embedded in Canada balsam. The observations were made with a BIOROM-T bright field microscope, equipped with a TOPICA-1006A video camera. The microphotographs were obtained from the video camera through a computer.

RESULTS AND DISCUSSIONS

Cross sections of the adventitious root revealed that the cortex is composed of 3–4 layers of large parenchyma cells. The inner layers of cortex cells are modified and have thick walls. KROEMER (1903) suggested that this wall thickening was the result of cutinised blade superpositions. Noticed by early authors, the presence of such "curious cells" around the stele has led to the recognition of this tissue belonging to the stele and naming it "sclerenchymatous mass" (RUSSOW 1872, BIERHORST 1971) or a "stereomic sheath" (DE BARY 1877, OGURA 1938, BERCU 1998*a*, BERCU *et al.* 2000) (Figs 1A, B). This configuration has led SCHNEIDER (1996) to include this type of root to that of *Asplenium*.

The stele consists of xylem and phloem and is surrounded by the pericycle. The xylem elements are joined together towards the centre by their metaxylem vessels (two for each bundle). The protoxylem vessels (four for each bundle) are in an exarch position and face the pericycle. The phloem sieve cells lacking companion cells are located on either side of the xylem string (Fig. 1B). This attributes to the adventitious root a diarch structure.

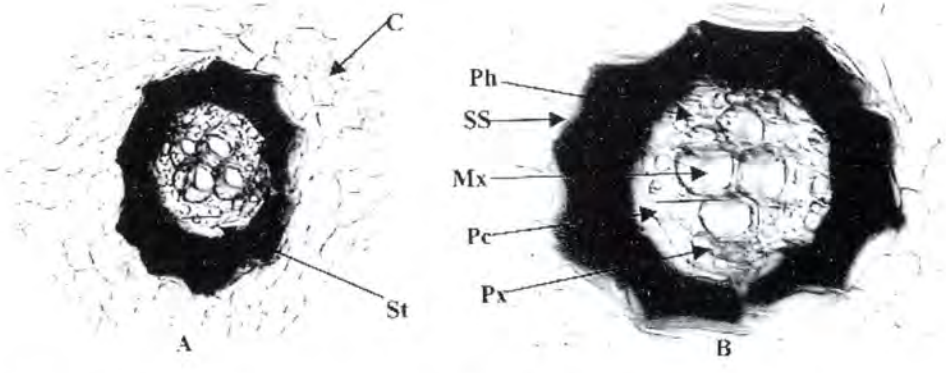


Fig. 1. Cross section of the adventitious root. – **A**: General view of the root (×269). – **B**: Detail of the stele (×330): C = cortex, Mx = metaxylem, Pc = pericycle, Ph = phloem, Px = protoxylem, St = stele, SS = sclerenchyma sheath. Orig.

Cross sections of the rhizome revealed that the epidermis consists of a single layer of cells uncovered by cuticle (Fig. 2B). Below the epidermis is the cortex consisting of large parenchyma cells. Remarkable is the abundance of starch grains in the cortical cells (Figs 2A, B).

As OGURA (1938) and BIR (1957) have reported for Aspleniaceae species, the stele is a dictyostele composed of a variable number of meristeles (3–4) (in accordance with the number of foliar traces) (Fig. 2A), each surrounded by its own endodermis (starch sheath) and pericycle. The latter is composed of parenchyma cells regularly arranged in one row, (locally in two or even three rows). Each meristele is hadrocentric with a binary structure (metaxylem vessels towards the center, and protoxylem elements facing the pericycle) (Fig. 2C). The pith occupies the central area of the rhizome. Each meristele possesses a large packet of sclerenchyma cells facing the pith, with mechanical role (Fig. 2A).

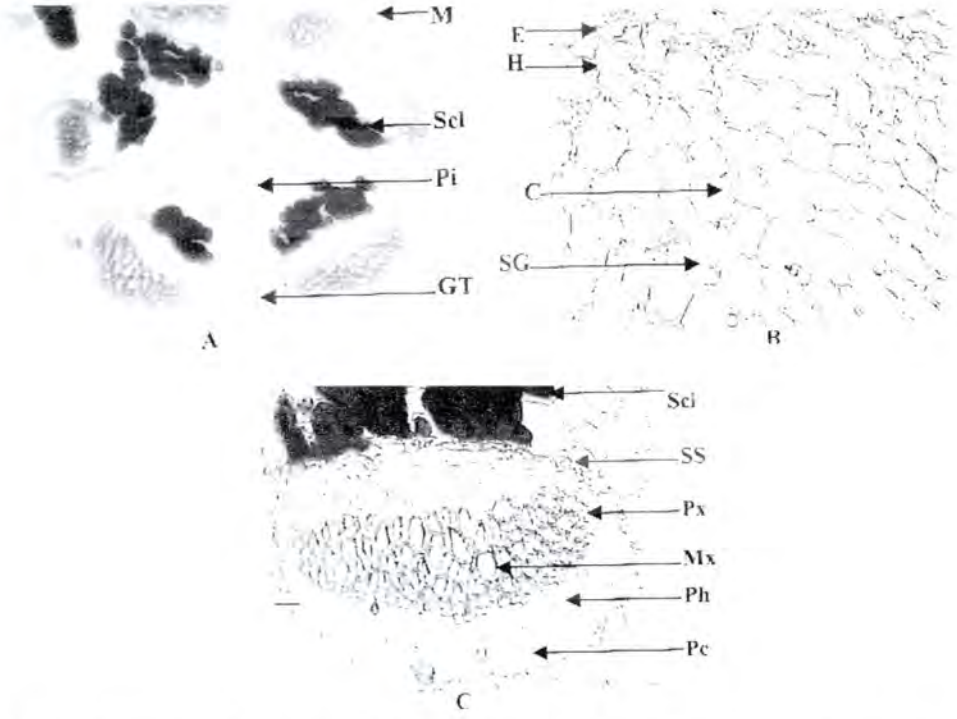


Fig. 2. Cross sections of the rhizome. – **A**: The stele (×47). – **B**: Portion with epidermis and cortex. – **C**: A meristele stele (×180): C = cortex, E = epidermis, GT = ground tissue, H = hypodermis, M = meristele, Mx = metaxylem, Pc = pericycle, Ph = phloem, Pi = pith, Px = protoxylem, Scl = sclerenchyma, SG = starch grains, SS = starch sheath. Orig.

Transections of the petiole and rachis cut from the base up to 1.5 cm disclose almost the same succession of tissues, that is a unistratose epidermis covered by cuticle, a cortex and a stele. Below the epidermis there is the hypodermis, consisting of a few layers of compactly arranged sclerenchyma cells. The hypodermis is internally followed by a region of ground tissue (Fig. 3B). Remarkable is the abundance of starch grains in the cortex and the presence of a single packet of sclerenchymatous cells (Figs 3A, B). The sclerenchyma packet of cells from 2 cm up to the tip of the base is absent (Figs 5–7). The stele is monofascicular, composed of two centrally located metaxylem strings. The phloem surrounds the xylem elements. That attributes to the vascular bundle a hadrocentric structure (Fig. 3C). The two xylem curved strings configuration reveals that initially two vascular bundles from the rhizome join together to form a foliar trace (Figs 2A, 3C).

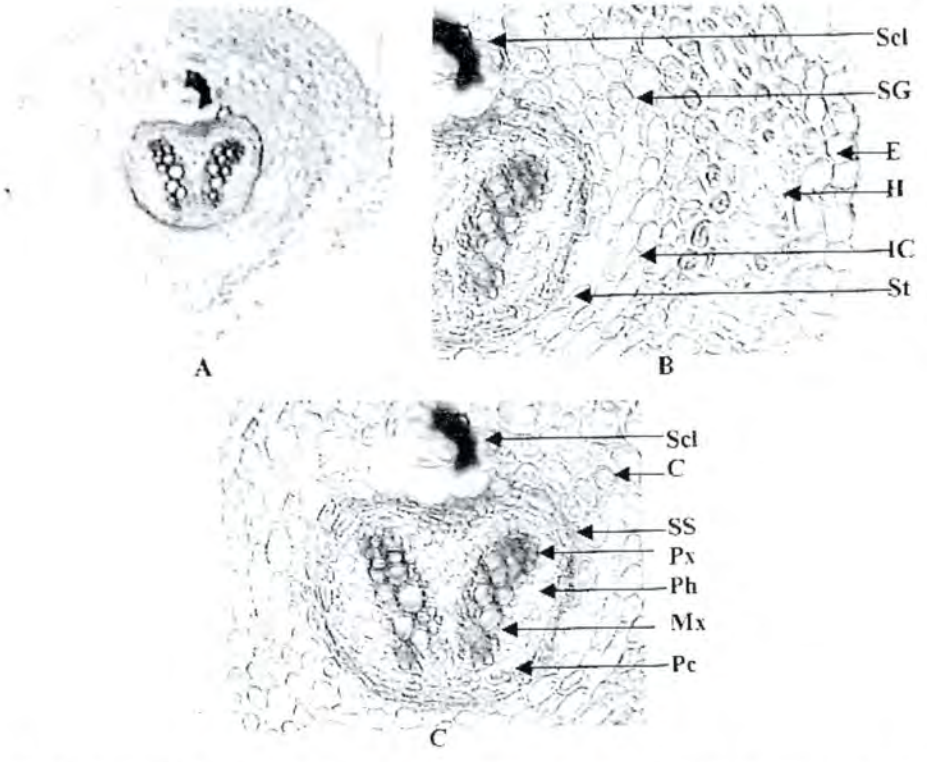


Fig. 3. Cross section of the petiole base. – **A**: General view (×115). – **B**: Portion of the epidermis and cortex. – **C**: The stele (×150): C = cortex, E = epidermis, H = hypodermis, IC = inner cortex, Ph = phloem, Mx = metaxylem, Pc = pericycle, Px = protoxylem Scl = sclerenchyma, St = stele, SG = starch grains, SS = starch sheath. Orig.

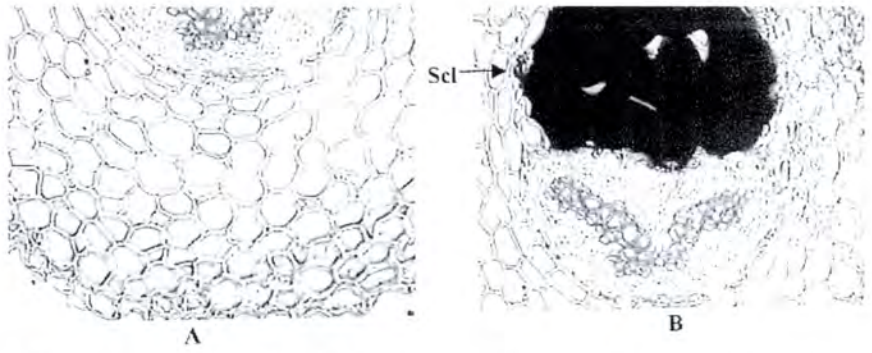


Fig. 4. Cross section of the petiole (1.5 cm from the petiole base). – **A**: Portion of the epidermis and cortex. – **B**: The stele (×170): Scl = sclerenchyma. Orig.

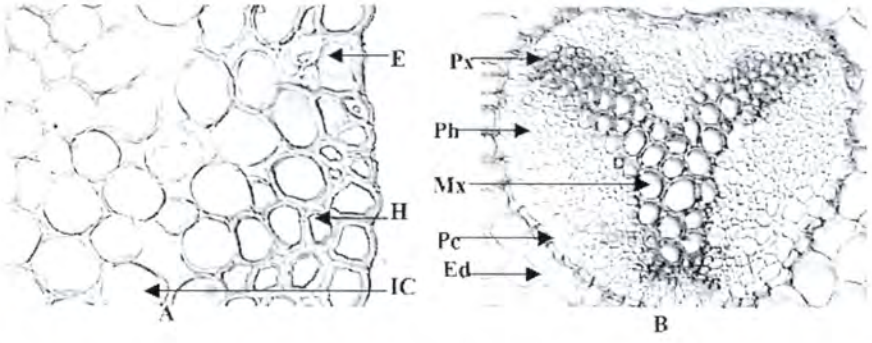


Fig. 5. Cross section of the petiole (2 cm from the petiole base). – **A**: Portion of the epidermis and cortex . – **B**: The stele (×240): E = epidermis, Ed = endodermis, H = hypodermis, IC = inner cortex, Mx = metaxylem, Pc = pericycle, Ph = phloem, Px = protoxylem. Orig.

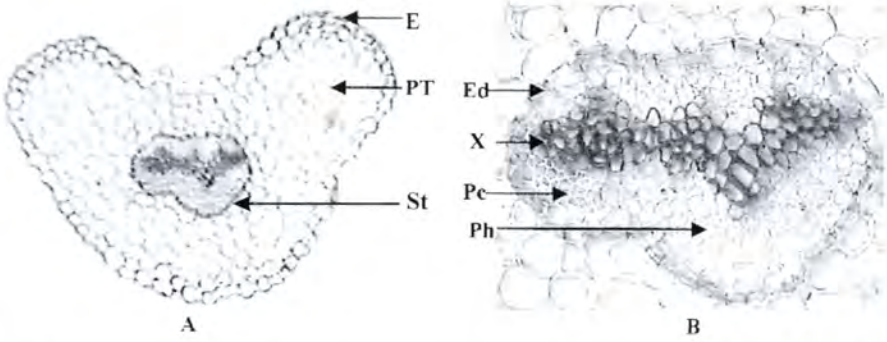


Fig. 6. Cross section of the subterminal rachis. – **A**: General view (×80). – **B**: The stele (×180): E = epidermis, Ed = endodermis, Pc = pericycle, Ph = phloem, PT = parenchyma tissue, St = stele, X = xylem. Orig.

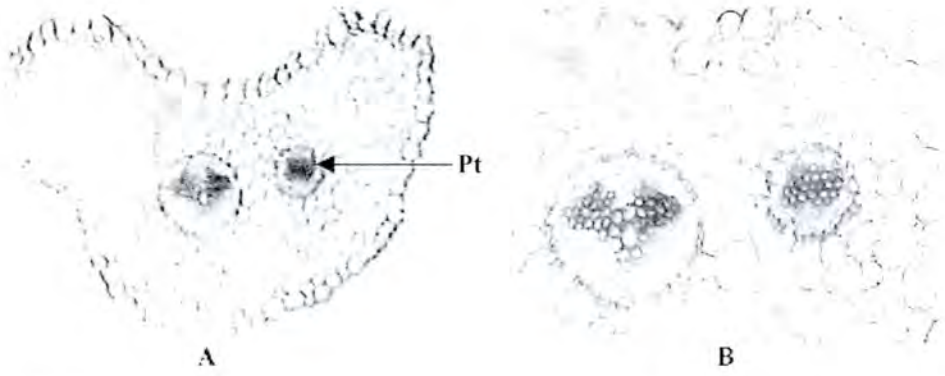


Fig. 7. Cross section of the rachis tip (6 cm from the petiole base). – **A**: General view (×115). – **B**: Portion of the stele with a pinnulae trace (×266): Pt = pinnulae trace. Orig.

The cross section, performed at 1.5 cm from the petiole base, exhibits almost the same structure but the xylem strings of the monostele are joined, towards the centre, by their abaxial protoxylem vessels (Figs 4A, B).

The cross section cut at 2 cm from the petiole base shows a monofascicular stele, composed of metaxylem vessels arranged in an X-shape, characteristic to the Aspleniaceae (OGURA 1972, BIR 1957, BERCU 1998*a*, BERCU *et al.* 2000). The phloem surrounds the xylem elements (Figs 4A, B). The transection of the rachis from 2 cm up to 6 cm (from the petiole base) exhibits the same succession of tissues but the xylem vessels are in a T-shape arrangement (Figs 5A, B).

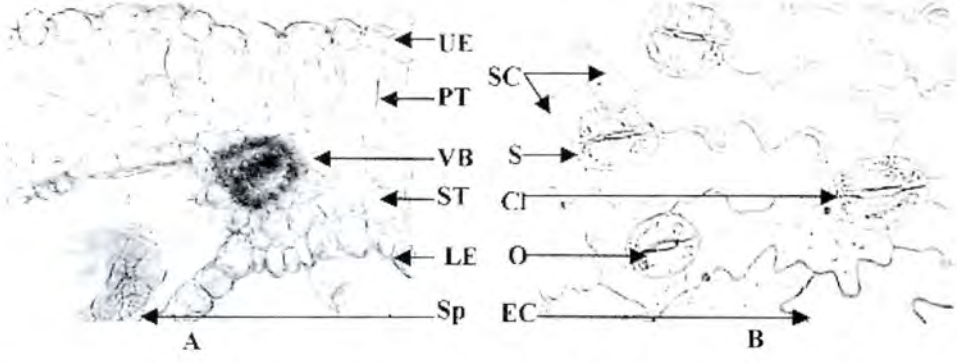


Fig. 8. Cross section of pinnulae. – **A**: Portion of the mesophyll and midrib (×205). – **B**: Portion of the lower epidermis (×250): Cl = chloroplasts, EC = epidermal cell, LE = lower epidermis, O = ostiole, PT = parenchyma tissue, S = stoma, Sp = sporangium, SC = subsidiary cell, ST = spongy tissue, UE = upper epidermis, VB = vascular bundle. Orig.

The transection of the rachis tip (6 cm from the petiole base) shows that the centrally located xylem of the stele has a slightly excavated V-shape, surrounded by phloem, the pericycle and endodermis, the latter with parenchymatous sheath value (Figs 6A, B) (BERCU 1998*b*). In the rachis tip two vascular bundles appear, the smaller derived from the rachis stele to form the pinnulae vein (Figs 7A, B). Remarkable is the reduced number of the vascular elements because of the marginal formation of the pinnulae veins. A typical hypodermis is absent (Figs 6A, 7A).

The pinnulae appear in cross section to consist of a single layer of irregularly arranged epidermal cells, covered by a thin cuticle (Fig. 8A). On the lower epidermis numerous polycytic and anomocytic stomata occur (VAN COTHEM 1970, DILCHER 1974). The epidermal cells possess wrinkled anticlinal walls (Fig. 8B).

The mesophyll is differentiated into palisade tissue (arranged into one row) and spongy tissue. The latter consists of a number of branched and lobed cells arranged into 2–3 rows, forming a system of intercellular spaces (POIRAULT 1893). The poorly developed vascular system of the midrib is represented by a hadrocentric vascular bundle with xylem in a centre position. The bundle is surrounded by a bundle sheath (Fig. 8A).

CONCLUSIONS

The root of *Asplenium ruta-muraria* L. has a diarch structure with modified layers of cortex around the stele and a monolayered pericycle. The rhizome (analysed by serial transections) consists of an epidermis, a parenchymatous cortex and a dictyostele, containing a variable number of meristeles. The leaf petiole and rachis exhibit a differential cortex (a sclerenchymatous hypodermis and an inner parenchyma). Close to the rachis tip, the sclerenchymatous hypodermis disappears. Remarkable are the variations in the organisation of the vascular system. The vascular system of the leaf petiole base is monostelic possessing two xylem strings surrounded by phloem elements, while up to the subterminal rachis the monofascicular stele is X-, and than T-shaped. The pinnulae have a heterogeneous mesophyll composed of palisade and spongy tissue, the latter consisting branched and lobed cells. The midrib has a hadrocentric structure. The lower epidermis continuity is broken by a number of stomata of the polycytic and anomocytic type.

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