Contribuţii Botanice, *XXXIX*, **2004**
Grădina Botanică "Alexandru Borza"
Cluj-Napoca

ANATOMICAL FEATURES OF THE VEGETATIVE ORGANS OF *MARSILEA QUADRIFOLIA* L.

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Abstract: The article comprises the investigation on the structure vegetative organs of an aquatic fern, namely *Marsilea qudrifolia* L. The specific fern characters such as the stele type in the root, rhizome, petiole and the vaiations of vascular system from the base towards the apex are described and discussed. The paper deals also with such hydrophytic features, such as the presence of aerenchyma in the root and stem.

Introduction

Marsilea qudrifolia L. (fam. *Marsileaceae*) – water clover - is an aquatic perennial fern living in Romania occurring in large aquatic areas (lakes, chanels etc.). It bears 4-parted leaf resembling "4-leaf clover" (*Trifolium*). Leaves are floating in deep waters, but erect in shallow water or on land. Leaflets ob-deltoid, glaucous, petioles to 5-8 cm long; sporocarps ellipsoid, on stalks, attached to the base of petioles Rhizome is to 0.5-1 m long [13, 15]. It is known that the juice obtained from the leaves is diuretic and febrifuge. It is also used to treat snakebite, abscesses etc. The plant is anti-inflammatory, diuretic, depurative, febrifuge and refrigerant [5].

The knowledge on the anatomical features of the aquatic ferns, in accordance with their floating nature in Romania is quite limited and those of *Marsilea qudrifolia* L. almost lack.

Material and Methods

The plant was collected in august, 2003 from the lake "Dunărică", Zimnicea (Teleorman) and fixed with A.F.A. Cross sections of the root, rhizome and surface leaves (blade and petiole) were performed using manual techniques. The samples were stained with alum-carmine and iodine-green. The samples were embedded in glicerine jelly. The observations were performed with BIOROM-T bright field microscope, equipped with TOPICA-1006A video camera. The micrographs were obtained from the video camera through a computer.

Results and Discussions

Cross-sections of the root revealed a primary structure consisting of epidermis, cortex and stele. The rhizodermis and exodermis often become destroyed by the development of the inner cortex and by the friction with the soil particles.

The root cortex is distinguished into an external region of basic parenchyma (rests of parenchyma cells) and an internal one. The latter consists of five layers of thick-walled cells, surrounding the stele. The innermost layer of cortex is the endodermis, protecting the stele. It forms a kind of "stereomic sheath" (de Bary, 1877) or "sclerenchymatous mass" (Rumpf 1904). This tissue appears in many autochthonous *Polypodidae* species too [2].

Under the endodermis is the pericycle. It consists of a single layer of slightly flattened parenchymatous cells, surrounding the vascular system. The vascular system consists of two xylem and two phloem bundles. The xylem bundles (one meta- and one protophloem vessel for each bundle) join by their metaxylem elements, to the center. The protoxylem vessels are exarch, facing the pericycle. The xylem string is located between the phloem bundles (two phloem

bundles). That attributes to the root a diarch structure. The phloem bundles consist of sieve cells, which are not associated with companion cells (Fig. 1b).

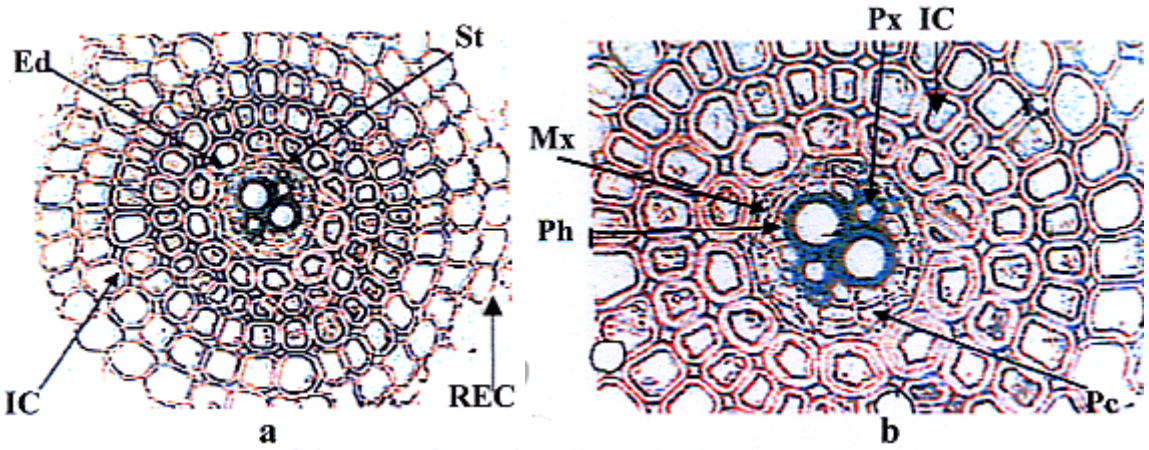


Fig. 1: Cross section of the root. General view (A). X- 190. The stele – detail (B). X 240: Ed- endodermis; IC- inner cortex; Mx- metaxylem; Pc- pericycle; Ph- phloem; Px- protoxylem; REC- rests of the external cortex; St- stele (Orig.).

Cross-sections of the rhizome disclose that the outer layer is epidermis. It consists of simple barrel-shaped cells. Under the epidermis is the cortex, lying between the epidermis and stele. The cortex is well-developed, being differentiated into three distinct regions. The outer cortex is composed of 28 conspicuous air chambers, which are separated from one another by one-seriate partitions – trabeculae [1]. The middle cortex covers a small portion of the root. It is composed of two layers of sclerenchyma cells with mechanical role (Fig. 2b). The inner cortex is well-developed and consists of 3-4 layers of loosly arranged parenchymatous cells, enclosing intercellular spaces. It cells contain starch grains (Fig. 2a, b).

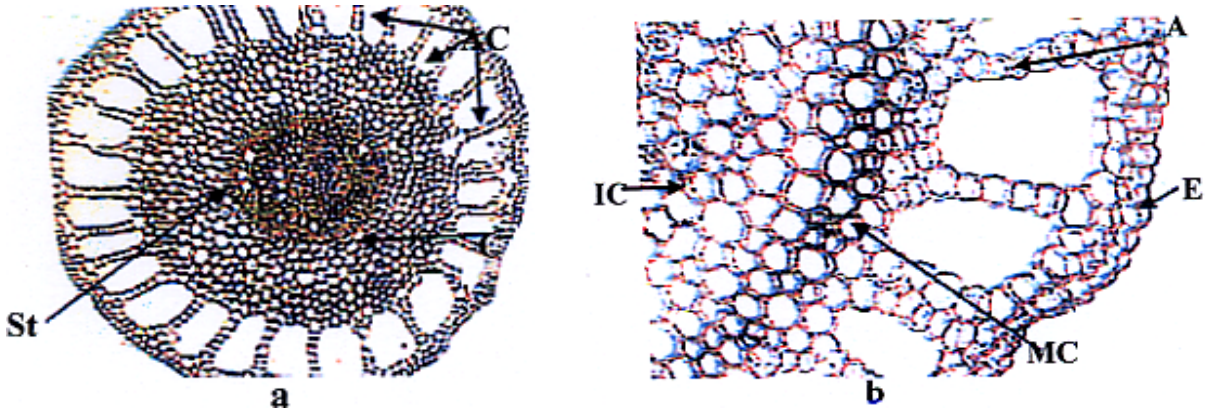


Fig. 2: Cross section of the rhizome. General view (a). X 64. Portion with epidermis and cortex (b). X 285: A- aerenchyma; AC- air chambers; C- cortex; E- epidermis; IC- inner cortex; MC- middle cortex; St- stele (orig.).

The cells are nearly spherical in shape and irregularly arranged around the stele. The stele is enclosed by a single-layered endodermis and a pericycle (Fig. 3a). The endodermis exhibits the usual casparian strips. The stele is an amphiphloic siphonostele (solenostele) [6, 7, 9]. The cylinder of solenostele is interrupted by a leaf gap, formed by the leaf trace (Fig. 3b) as Ogura (1938), Launert (1968) and Sporne (1975) reported to other solenostelic ferns.

The xylem string consists of meta- and protoxylem vessels and is located between the phloem bundles. Metaphloem cells face the xylem elements. The central portion of the stem is occupied by compactly arranged parenchyma cells and it is known as pith.

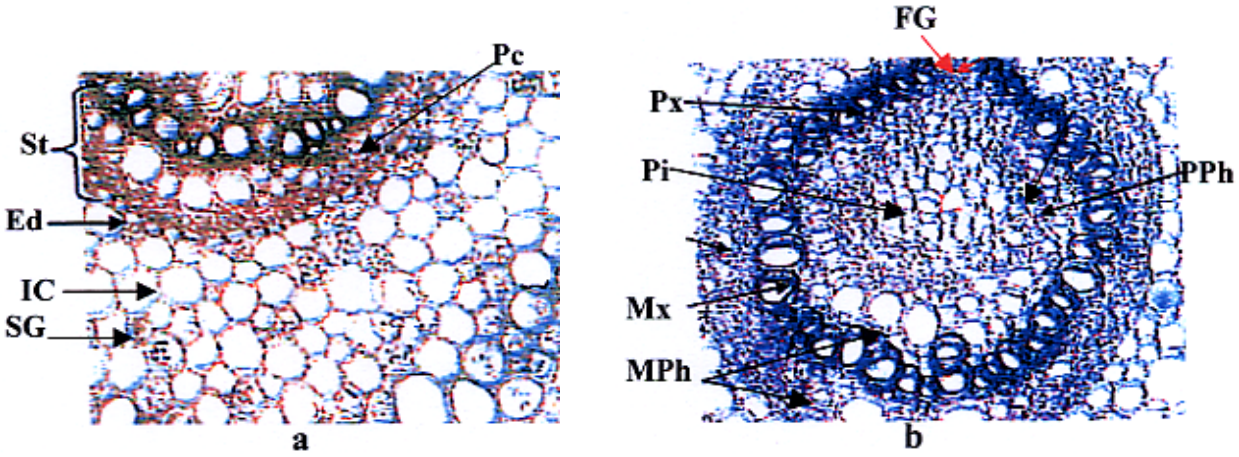


Fig. 3: Cross sections of the rhizome. Portion with inner cortex and stele (a). The stele (b). X 285: Ed- endodermis; IC- innercortex; Mx- metaxylem; MPh- metaphloem; Pc- pericycle; Pi- pith; Px- protoxylem; PPh- protophloem; St- stele; SG- starch grains; UF- foliar gap (orig.).

The petiole (5 cm in length), analyzed on serial cross sections from the base to the apex, reveals almost the same succession of structures: epidermis, cortex and stele.

Cross-sections of the petiole base (3 mm) exhibits that the single-layered epidermis consists of compactly arranged small barrel-shaped, cuticularized cells (Fig. 4a, b) As Ogura (1938) reported its continuity is broken by the presence of stomata and hairs as well. Stomata open into large cavities. The cortex is differentiated into three zones. The external region is an aerenchyma, composed of 16 large air chambers, which are separated from one another by thin-walled uniseriate partitions (Fig. 4a). The middle cortex consists of two sclerenchymatous cell layers (Fig. 4b). The inner multi-layered cortex consists of densely arranged parenchymatous cells, surrounding the stele (Fig. 4a).

The stele of *Marsilea quadrifolia* L. petiole is the so called *Marsilea* type [3, 10, 11], and descend from the modification of *Gramites* type [8]. It consists of two curved xylem strings. Protoxylem elements are exarch, facing towards the periphery; metaxylem ones are located in the center. Phloem is well-developed and arranged between the xylem strings. It consists of sieve cells (meta- and protophloem cells), lacking companion cells. Towards the apex, the petiole has the same stele structure (Fig. 4c).

Cross section of the middle portion of the stele (3.5 cm from the base) reveals the usually succession of tissues. The cortex is distinguished into two regions, an external zone of aerenchyma tissue with a reduced numbers of air cavities (13 air chambers) and an internal one. The latter is composed of basic parenchyma (Fig. 6a).

Cross-section of the subterminal region of the petiole exhibits the approaching tendency of the xylem strings, towards the center of the stele (Fig. 6a, b).

The terminal portion of the petiole reveals almost the same structure, but has only 9 air cavities (air chambers).

Transversal sections of the leaf blade reveal that the upper epidermis, as well as the lower one, consists of simple slightly flattened cells. Cuticle is absent. Stomata are present only on the upper epidermis.

The spongy tissue lies between the palisade region and the lower epidermis. It consists of several layers of simple or branched parenchymatous cells, forming a system of intercellular spaces. Small vascular bundles, protected by parenchymatous sheaths, are embedded in the mesophyll (Fig. 7).

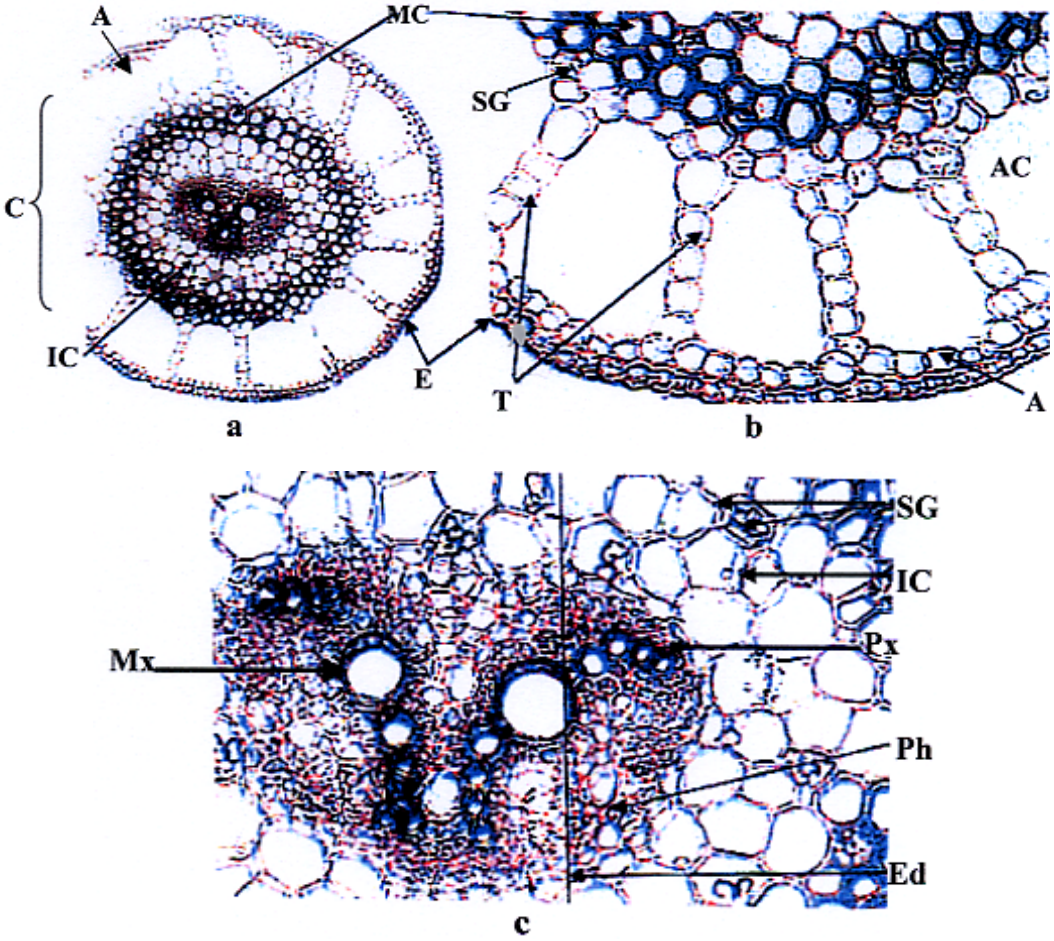


Fig. 4: Cross sections of the petiole (3 mm from the base). General view (a). X 109. Portion with epidermis and cortex (b). X 325. The stele (c). X 279: A- aerenchyma; AC_ air chambers; C- cortex; E- epidermis; Ed- endodermis; IC- inner cortex; Mx- metaxylem; MC- middle cortex; Ph- phloem; Px- protoxylem; T- trabeculae (orig.).

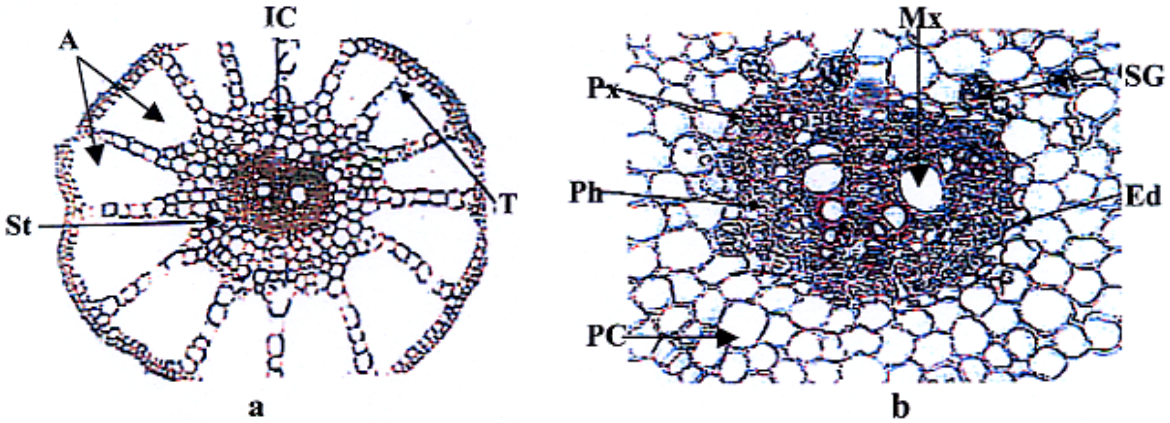


Fig. 5: Cross sections of the petiole (3.5 cm from the base). General view (a). X 109. The stele (b). X 325. A- aerenchyma; Ed- endodermis; IC- inner cortex; Mx- metaxylem; Ph- phloem; Px- protoxylem; PC- parenchyma cortex; St- stele; SG- starch grains; T- trabeculae orig.).

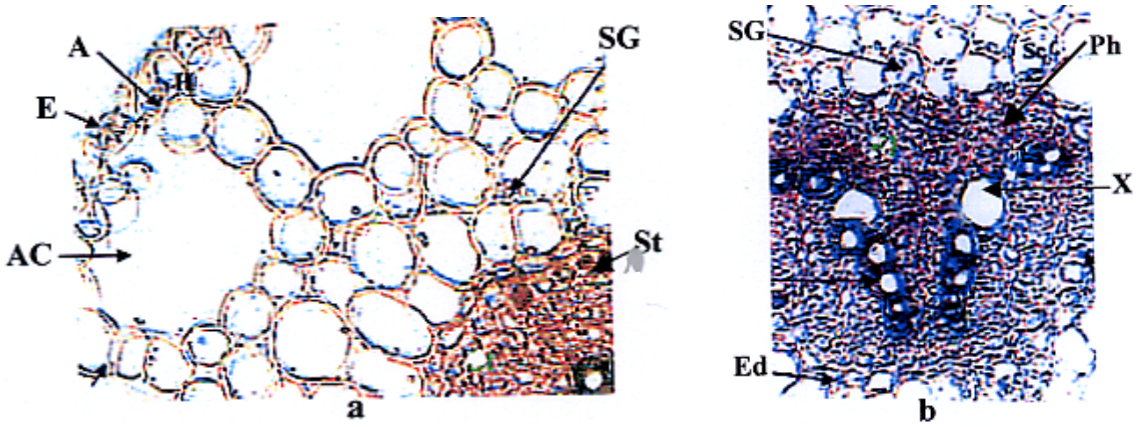


Fig. 6: Cross section of the petiole (sub-terminal region). Portion with epidermis and cortex (a). The stele (b). X 300: A- aerenchyma; AC- air chamber; E- epidermis; Ed- endodermis; Ph- phloem; St- stele; SG- starch grains; X- xylem (orig.)

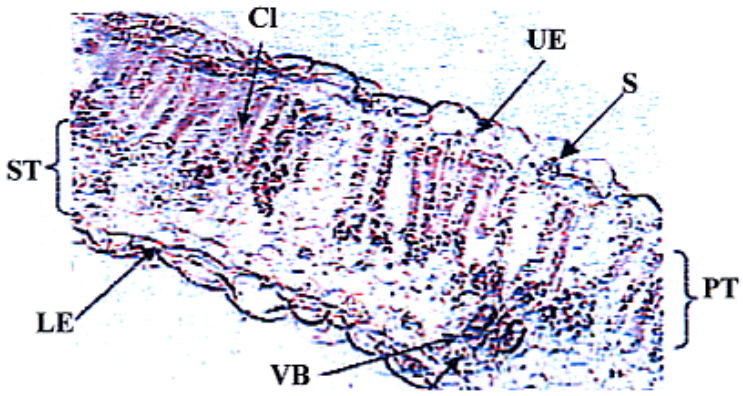


Fig. 7: Cross section of the blade. X 216: Cl- cloroplasts; LE- lower epidermis; PT- palisade tissue; S- stoma; ST- spongy tissue; UE- upper epidermis; VB- vascular bundle (orig.).

Conclusions

The present results indicate that the small floating hydrophytes fern *Marsilea quadrifolia* exhibits anatomical features in accordance with its aquatic habit. The root is of diarch type. Characteristically, the root possesses a thick stereomatic sheath around the stele. The external cortx of rhizome is built up of large aerenchyma, enclosing regular air chambers, separated form one another by one-celled partitions. The stele is a amphiphloemic solenostele. A thick cuticle covers the epidermal cells of the rhizome. The cortex from the base (3 mm) to 3.5 cm (from the base), is differentiated into three regions such as those of the rhizome, but the air chambers of the external cortex are reduced in number (from 16 to 9) to the apex. The stele of petiole of the water clover is the so called *Marsilea* type. Its xylem elements conjoin towards the apex of the petiole. The mesophyll of lamina is heterogenous, differentiated into palisade and spongy tissues. The vascular bundles of the veins are poorly developed being embedded in the mesophyll. The mechanical tissue of the rhizome and petiole (till 3.5 cm from the base) consists of two layers of sclerenchymatous cells. From 3.5 cm from base towards the apex of the petiole the mechanical tissue is absent.

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PARTICULARITĂȚILE ANATOMICE ALE ORGANELOR VEGETATIVE LA *MARSILEA QUADRIFOLIA* L.

(Rezumat)

În lucrarea de față este prezentată structura organelor vegetative (rădăcina adventivă, rizomul şi frunza) ale ferigii *Marsilea quadrifolia* L., având în vedere faptul că referiri asupra histo-anatomiei organelor unor ferigi acvatice sunt sporadice în țara noastră, lipsind cele referitoare la această specie.

Rizoderma şi exoderma rădăcinii nu apar în secțiuni ele fiind exfoliate în întregime, ca şi cea mai mare parte de cortex (Fig. 1a). Cortexul este diferențiat în două zone, una externă, de natură parenchimatică (aproape în întregime exfoliată) iar cea de a doua zonă, internă, formată din cinci straturi de celule cu pereții îngroşați formând aşa numita teacă de stereom, caracteristică şi multor specii de polipodiate [2, 4, 12]. Stelul prin distribuția elementelor sale imprimă rădăcinii un caracter diarh (Fig. 1b).

Rizomul prezintă la exterior epiderma sub care se găseşte scoarța, diferențiată în trei zone. Scoarța externă este reprezentată printr-un aerenchim alcătuit din 28 de camele aerifere separate de trabecule [1]. Porțiunea mijlocie de scoarță este formată din două straturi de sclerenchim, iar regiunea internă este de natură parenchimatică (Fig. 2a, b). Pericicluul protejează stelul, format din xilem şi floem (Fig. 3a). Aşa cum au arătat Ogura (1938), Launert (1968) şi Sporne (1975), stelul este un solenostel dorsi-ventral amfifloemic (după şcoala clujană) [3, 6, 10]. Cercul solenostelului este întrerupt de lacuna foliară lăsată de urma foliară [7, 9] (Fig. 3b). Peţiolul a fost analizat pe secțiuni transversale seriale, pornind de la bază la vârf. Sub epidermă şi hipodermă se găseşte cortexul reprezentat prin canale aerifere al căror număr descreşte spre de vărf. Se remarcă variații ale sistemului vascular la 3 mm, 3.5 cm de la baza peţiolului, bandeletele de xilem finnd depărtate între ele (Fig. 4, 5). La nivelul rahisului subterminal se constată o reducere a distanței dintre bandeletele de xilem, cu tendință de unire în X, unire care se realizează în vârf (Fig. 6). Stelul acestei specii este de tip *Marsilea* [8, 11, 12, 14]. Straturile de sclerenchim, observate în cortexul rizomului, se mențin şi în peţiol numai până la 3.5 cm de la baza sa. Spre vârf acestea dispar (Fig. 5a, 6a). Limbul frunzei de *Marsilea quadrifolia* prezintă succesiunea cunoscută de țesuturi.

Între cele două epiderme, unistratificate, acoperite de o cuticulă fină, se găseşte mezofilul heterogen. Continuitatea celulelor țesutului palisadic este întreruptă, din loc în loc, de camere largi substomatice. Fasciculele vasculare ale nervurilor frunzei sunt slab dezvoltate, reprezentate prin câteva vase de xilem şi floem, protejate la exterior de o teacă parenchimatică perifasciculară (Fig. 7).