Histoanatomy of the leaves of *Trapa natans* (*Trapaceae*)

Rodica Bercu

Department of Vegetal Biology, Faculty of Natural Sciences, Ovidius University, 124, Mamaia St., 900527 Constanza, Romania, e-mail: rodicabercu@yahoo.com

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Abstract. The paper deals with the anatomical and histological features of the leaves of *Trapa natans* – a free-floating hydrophyte, living in the Danube Delta channels. The special characteristics of both surfacing and submerged leaves of this plant are described and disscused.

Key words: anatomy, petiole, submerged leaf, surfacing leaf, *Trapa natans*

Introduction

Trapa natans L. (syn. *Trapa astrachanica* (Flerow) N. A. Winter, *Trapa quadricornis* Stokes, *Trapa quadrispinosa* Roxb.) was introduced from Eurasia into North America in 1874 (Britton & Brown 1913). It is an annual aquatic plant growing in fresh-water lakes and ponds and slow-moving streams and rivers, up to 60 cm deep, requiring a sunny position in slightly acidic water. The plant dislikes calcium-rich water and prefers rich soil (Muhlberg 1982). Initially, *Trapa natans* was cultivated in Asia Gray's Botanical Garden at Harvard University in 1877. By 1879 it had escaped into the local waters (Crow & Hellquist 1983). This plant now occurs over a considerable part of Europe, the Caucasus and Siberia.

Trapa natans is known as water chestnut and has surfacing and submerged leaves. Surfacing leaves are triangular with toothed edges and an inflated petiole and form a rosette on the water surface. Submerged leaves are feather-like; each leaf is divided into segments that are whorled around the leaf stem. White flowers form in the axils of the surfacing leaves in July. Fruit are nut-like and "woody," typically with four sharp, barbed spines, large, fleshy, and edible.

Long, cord-like, rarely branching stems can attain lengths of up to 16 feet. The seed germinates in substrate, firmly anchored by lateral roots. In literature some authors described the morphological characteristics of this plant and the foliar dimorphysm (Ţopa 1957; Andrei 1974; Tarnavschi & al. 1974; Grinţescu 1985; Strasburger 1991; Bavaru & Bercu 2002), but the anatomy of the leaves, especially the petiole, remains almost unknown. Some authors have noticed the anatomical characteristics of the lamina's surfacing leaf (Batanouny 1992).

Material and methods

Cross sections of the leaves (the surfacing leaves – blade and petiole and submersed leaves) were performed using the manual technique. The sections were stained with alum-carmine and iodine green. The samples were embedded in glycerine gelatine. Histological observations and micrographs were performed with a BIOROM –T bright field microscope, equipped with

a TOPICA 601-A video camera. The microphotographs were obtained from the video camera through a computer.

Results and discussion

Cross section of the surfacing leaf lamina reveals the following structures: epidermis, mesophyll and vascular system (midrib). The lower epidermis is differentiated into an upper and a lower epidermis, each consisting of a single layer of compactly arranged thin-walled barrel-shaped cells. Externally, both types of epidermis are covered by a thin cuticle, not only the upper one, as Batanouny (1992) suggested. Numerous stomata occur among the upper epidermal cells in accordance with its free-floating nature (Plate I, Figs 1, 2). Remarkable is the presence of many-celled hairs among the lower epidermal cells (Plate I, Fig. 4).

The heterophylous mesophyll is well developed. It is differentiated into palisade tissue and spongy tissue. The palisade region is composed of two or three layers of radially-elongate cells, compactly arranged and possessing numerous stomata. The continuity of the upper palisade region is broken by the sub-stomatal cavities located at the stomata base (Plate I, Fig. 1).

Batanouny (1992) had noticed that at the base of sub-stomata cavities, certain cells bear druses (calcium oxalate crystals) (Plate I, Fig. 1), but they could be observed in the spongy region too (Plate I, Fig. 1).

The spongy tissue is composed of one or two layers of compactly arranged parenchyma cells, lying just bellow the palisade region. Most of the spongy region is represented by small and large air cavities. The air cavities are separated by a uniseriate partition. Most parenchyma cells bear druses, facing the air chambers (Plate I, Fig. 1). The vascular system is poorly represented by small vascular bundles. Each bundle is composed of phloem and poorly developed xylem represented by a large lacuna.

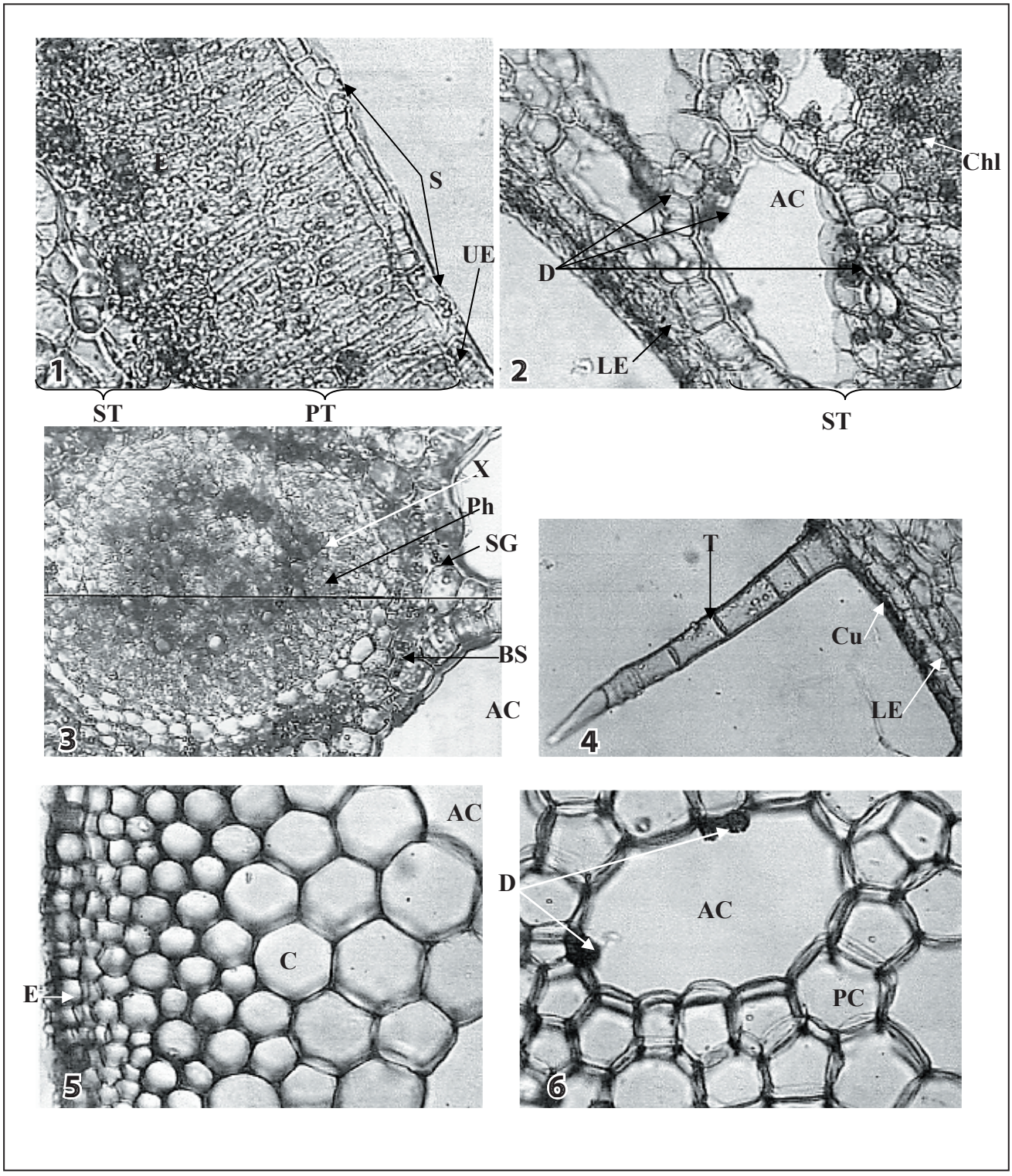
The midrib is enclosed in a parenchyma sheath protected by collenchyma cells. The vascular system is poorly developed and consists of a few xylem vessels towards the centre and more phloem vessels towards the periphery region of the vein. Many starch grains are present around (Plate I, Fig. 3).

Cross section of the thin (Plate I, Figs 5, 6; Plate II, Figs 1, 2) and inflated regions (Plate II, Figs 3-5) of the petiole exhibits almost the same anatomical structure, epidermis, hypodermis, aerenchyma and the vascular system. However, some structural features occur.

The outermost layer of cells, the epidermis, consists of thin-walled, barrel-shaped cells, characterised by the presence of chloroplasts. Hypodermis consists of 5–6 layers of collenchymatous cells in the thin zone of the petiole and 3–4 layers of parenchyma cells in the inflated zone. The hypodermal cells are compactly arranged. Bellow the hypodermis lies the continuous region of the ground tissue (aerenchyma) enclosing irregular air cavities (larger in the inflated region), separated by one-cell-thick partitions. The aerenchyma cells contain starch grains and numerous calcium oxalate crystals (druses), mostly facing the cavities (Plate I, Figs 5, 6). Cross section of the inflated petiole reveals the same structural organization but the collenchymatous region is absent (Plate II, Figs 3, 4). In both regions, the vascular system of the petiole is represent by two types of vascular bundles. Five small vascular bundles are scattered at regular intervals in the ground tissue, mostly in the outer parenchyma region. The small vascular bundles are poorly represent by few phloem and xylem vessels. In the centre a large xylem lacuna occurs (Plate II, Figs 2-5). The kidney-shaped large, centrally located, vascular bundle is composed of xylem and phloem. Xylem shows exarch position, and phloem is well developed and is presented between the xylem groups. The stele is enclosed by a single-layered endodermis and a pericycle. The centre of the stele is fill by compactly arranged parenchyma cells known as pith (Plate II, Fig. 1).

Cross section of the submersed opposite finely dissected leaves, reveals a simple anatomical structure: epidermis, cortex and vascular system of the "vein". The cortex consists of eight or nine layers of parenchyma cells. Small air spaces are present among the cortex cells. They are smaller than those of other aquatic plants such as *Vallisneria spiralis* (Bercu & Făgăraş 2002), *Myriophyllum verticillatum* (Şerbănescu-Jitariu & Toma 1980), etc. The inner layer of the cortex, the endodermis, has a single layer of large parenchyma cells. The vascular system consists of xylem and phloem surrounded by pericycle. The centrally located xylem consists of two metaxylem vessels and four protoxylem vessels. Phloem consists of metaphloem vessels towards the xylem and protophloem vessels towards the periphery (Plate II, Fig. 6).

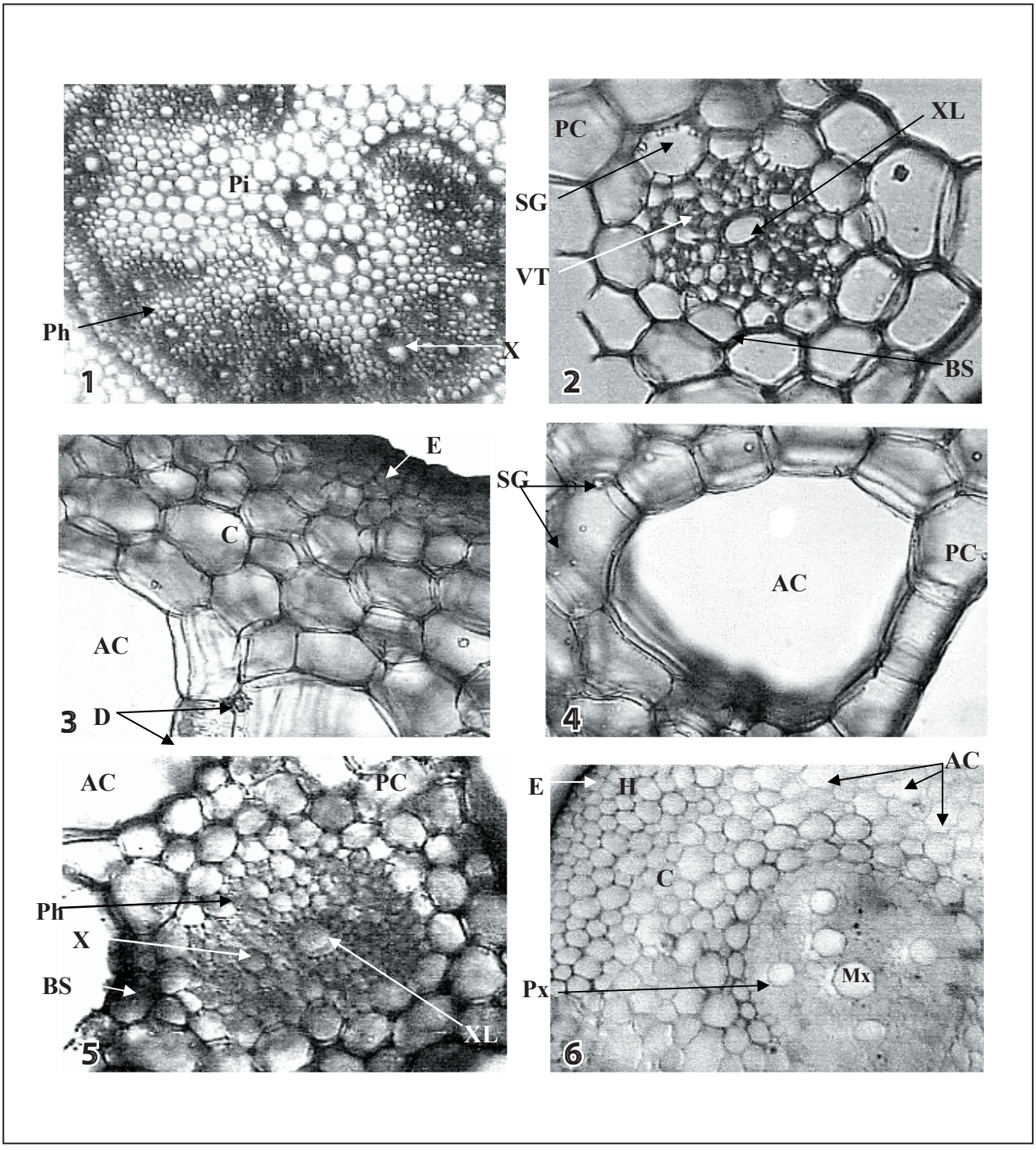
Plate I



Figs 1-4. Cross section of the floating leaf: **1**, portion of the palisade tissue (×180); **2**, portion of the spongy tissue (×190); **3**, the midrib vein (×110); **4**, portion of lower epidermis with trichome (×210): **AC** – air chamber; **BS** – bundle sheath; **C** – cortex; **Ch** l- chlororplasts; **Cu** – cuticle; **D** – druses; **E** – epidermis; **LE** – lower epidermis; **Ph** – phloem; **PC** – parenchyma cell; **PT** – palisade tissue; **S** – stomata; **SG** – starch grains; **ST** – spongy tissue; **UE** – upper epidermis, **T** – trichome. Orig.

Figs 5-6. Cross section of the thin part of the petiole: **5**, portion of epidermis and cortex; **6**, aerenchyma (×200): **AC** – air chamber; **C** – cortex; **D** – druses; **PC** – parenchyma cell. Orig.

Plate II



Figs 1-2. Cross section of the thin part of the floating leaf petiole: **1**, large vascular bundle (×170): **2**, small vascular bundles (×230): **BS** – bundle sheath; **Ph** – phloem; **Pi** – pith; **PC** – parenchyma cell; **SG** – starch grains; **VT** – vascular tissue; **X** – xylem; **XL** – xylem lacuna. Orig.

Figs 3-5. Cross section of the inflated petiole: **3**, portion with epidermis and cortex; **4**, aerenchyma; **5**, small vascular bundle (×190): **AC** – air chamber; **BS** – bundle sheath; **C** – cortex; **D** – druses; **E** – epidermis; **Ph** – phloem; **PC** – parenchyma cell; **SG** – starch grains; **X** – xylem; **XL** – xylem lacuna. Orig.

Fig. 6. Portion of a cross section of the submerged leaf: **AC** – air chamber; **C** -cortex; **E** – epidermis; **Ed** -endodermis; **H** – hypodermis; **Mx** – metaxylem; **Px** – protoxylem (×300). Orig.

Conclusions

Results indicate that the surfacing leaves of *Trapa natans* exhibit special characters that justify its free-floating nature. The upper epidermis such as theand lower one is covered by a thin cuticle. Stomata are presented only on the upper epidermis of the floating leaf. Stomata open into large substomatal cavities. Well-developed air chambers are present in the spongy tissue of the lamina and in the petiole too. Numerous druses are presented in the lamina and petiole, mostly facing the air cavities. The vascular tissue is well developed, represented by two types of vascular bundles. Mention deserves the kidney-shaped, large, centrally located vascular bundle.

Cross section of the dissected leaf discloses a root-like primary structure consisting of epidermis, cortex and vascular system. The cortex is characterized by small air spaces. The xylem elements are reduced in number. Usually, the pericycle has one layer of parenchyma cells.

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