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ANATOMICAL-HISTOLOGICAL OBSERVATIONS CONDUCTED ON AQUATIC FERNS IN THE DANUBE DELTA

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Abstract: This paper analyses aquatic ferns from the genera *Azolla* Lam., *Marsilea* L. and *Salvinia* Séguier, which occur in the Danube Delta, Romania, and comprises a series of anatomical and histological observations of taxonomical, chorological and eco-morphological importance. The research conducted on specimens collected between 2005-2013 from the natural habitats of the Danube Delta, but also from the extra-deltaic artificial habitats have enabled: i) a reconsideration of some chorological aspects regarding the species of the genus *Azolla* in Romania; ii) a greater understanding of the adaptive plasticity relative to the factor water for the taxon *Marsilea quadrifolia* L. collected from natural and artificial habitats; iii) the enrichment of the data regarding the structural characteristics of the taxon *Salvinia natans* (L.) All., particularly around the adaptive elements associated with living on the surface of the water.

Keywords: adaptability, anatomy, aquatic ferns, chorology, taxonomy.

Introduction

This paper discusses the aquatic ferns in the Danube Delta, namely the species of the genera *Azolla* Lam., *Salvinia natans* (L.) All. and *Marsilea quadrifolia* L.

The representatives of the genus *Azolla* in Romania are *Azolla filiculoides* Lam., *A. caroliniana* Willd. and *A. mexicana* C. Presl., aquatic-natant adventive hydrophytes [SÂRBU & al. 2013]. As regards the presence and the distribution of these taxa in the flora of Romania, numerous bibliographical references have been made over time. Initially, only *Azolla filiculoides* and *A. caroliniana* were mentioned in the flora of Romania [ANTONESCU, 1951; ŢOPA, 1952; CIOCÂRLAN, 1994]. In the year 2000, in the paper The Illustrated Flora of Romania *Pteridophyta* et *Spermatophyta* [CIOCÂRLAN, 2000] the species *Azolla filiculoides* and *A. mexicana* are noted, the latter with unconfirmed presence. In subsequent papers [OPREA, 2005; SÎRBU & OPREA, 2011; SÂRBU & al. 2013] all three species of the genus *Azolla* (*filiculoides*, *caroliniana*, *mexicana*) are mentioned in Romania, but for the Danube Delta only the presence of the taxon *Azolla filiculoides* is recognised [OPREA, 2005; CIOCÂRLAN, 2011; SÂRBU & al. 2013].

Salvinia natans, an aquatic-natant, annual hydrophyte, protected at European level [Council Directive 92/43/EEC, 1992 – Habitat Directive] is mentioned at being presented in

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the Danube Delta [ŢOPA, 1952; CIOCÂRLAN, 1994; OPREA, 2005, SÂRBU & al. 2005; SÂRBU & al. 2011; SÂRBU & al. 2013], particularly in the habitat type 3150 – Natural eutrophic lakes with vegetation of *Magnopotamion* or *Hydrocharition* [GAFTA & MOUNTFORD, 2005], in lakes, ponds, moors, shallow closed canals, spread across the deltaic complex [SÂRBU, 2003; SÂRBU, 2006; SÂRBU & al. 2013].

Marsilea quadrifolia, a perennial hydro-hygrophyte plant, vulnerable in Romania [OLTEAN & al. 1994] and protected at European level [Council Directive 92/43/EEC, 1992 – Habitat Directive], is present in the Danube Delta through two forms of growth, namely f. *terrestris* and f. *natans* [ŢOPA, 1952; CIOCÂRLAN, 1994; SÂRBU, 2002; SÂRBU, 2015]. The species is mentioned in the Natura 2000 habitats in the Danube Delta, belonging to the habitat type 3150 - Natural eutrophic lakes with vegetation of *Magnopotamion* or *Hydrocharition*, in shallow stagnant waters and swampy areas, which offers them an enhanced conservation value [SÂRBU & al. 2013]. In 2013, the presence of this taxon was reported in the specialty literature also for the artificial aquatic habitats in Romania [SÂRBU & al. 2014]. The plant was identified in a pre-decanter of the Arcuda Station for Treating and Drinking Water Production.

This paper presents a series of anatomical and histological observations regarding the taxa mentioned above. These observations are of taxonomical and chorological importance in the case of the *Azolla* species, while new structural information of eco-morphological importance is provided for *Marsilea quadrifolia.* Using optical microscopy images, the paper also documents a series of data from the literature regarding *Salvinia natans.*

Materials and methods

For the representatives of the genus *Azolla*, vegetal material was collected during the period 2005-2010 from several aquatic ecosystems of the Danube Delta: Mila 23 Zone, Şontea Canal and Înfundata Canal (Fig. 1). The specimens of *Salvinia natans* were collected during the period 2005-2008, from shallow waters connected to the Magearu Canal (Fig. 2). *Marsilea quadrifolia* was collected from two types of habitat: i) natural habitats from the Danube Delta, where specimens of f. *natans* and f. *terrestris* were collected between 2004 and 2005 (Fig. 3, Fig. 4) and ii) an artificial habitat represented by the pre-decanter of the Arcuda Station for Treating and Drinking Water Production, where three samples of the f. *natans* were collected in 2013 (Fig. 5, Fig. 6).

The vegetative organs were analysed morphologically, anatomically and histologically: i) the leaf of the specimens of *Azolla*, with an emphasis on the characteristics of the epidermal trichomes which are important in the identification of the species, ii) the floating leaf, the rhizophyll (root-like leaf submerged) and the stem of *Salvinia natans,* and iii) the rhizome, petiole and lamina of the two forms of growth of the taxon *Marsilea quadrifolia.* The vegetal material was analysed as required, eitherun-sectioned and observed in apical image (e.g. *Azolla*), or sectioned (cross-section, paradermal section), processed according to the double coloration technique (Carmine alum and Iodine green) or treated with specific identification substances such as IIK and Sudan III [ŞERBĂNESCU-JITARIU & al. 1983]. The cross sections were carried out, through the median area of the vegetative organs.

The dimensional measurements were made with the micrometer kit and the microphotographs were taken using the optic microscope DOCUVAL, equipped with a photographic camera NIKON 90. All the optical microscopy images are original: Anca Sârbu (Fig. 1-25, 27-30, 32-54), Daniela Smarandache (Fig. 26, 31, 54 – the schema).

Fig. 1. *Azolla filiculoides*, Danube Delta.

Fig. 2. *Salvinia natans*, Danube Delta.

Fig. 3. *Marsilea quadrifolia*, f. *natans*, Danube Delta.

Fig. 4. *Marsilea quadrifolia*, f. *terrestris*, Danube Delta.

Fig. 5. *Marsilea quadrifolia*, f. *natans*, Arcuda Station.

Fig. 6. *Marsilea quadrifolia*, f. *natans*, Arcuda Station.

Results and discussion

***Azolla* species.** According to the data in the literature [LUMPKIN, 1993; SÂRBU & al. 2013] the three species of the genus *Azolla* (*filiculoides*, *caroliniana*, *mexicana*) mentioned in the flora of Romania are thermophile plants, alien to Romania and native to North America. Of these three species, only *Azolla filiculoides* has been confirmed for the Danube Delta.

These taxa differ among themselves through several types of characteristics such as the size of the plants and the leaves, the morphology of the megaspores and the aspect of the glochidia [TRYON & TRYON, 1982; CODY & BRITTON, 1989; STRASBURGER & al. 1990], but also through the characteristics of the epidermal trichomes [LUMPKIN, 1993; SÂRBU & al. 2013]. The species of the genus *Azolla* are often much more difficult to differentiate on the basis of the morphology of the vegetative body or the characteristics of the megaspores, because often the sporocarps are absent.

Given these difficulties, this paper aims to highlight the characteristics of the epidermal trichomes using microscopy images, and to use these images as an initial tool for the differentiation of the *Azolla* species recorded in Romania.

According to the description provided in the Flora of the North America [LUMPKIN, 1993], the characteristics of the epidermal trichomes are the following:

- *Azolla filiculoides* has strictly only unicellular trichomes, located on the superior lobes of the leaves;
- *Azolla caroliniana* has the longest trichomes, formed of two or more cells, located on the superior lobe of the leaf, close to the stem; the apical cell is often curved;
- *Azolla mexicana* has the longest trichomes formed of 2(-3) cells, located on the superior lobe of the leaf, close to the stem; the apical cell is often curved.

Based on the characteristics of the epidermal trichomes, the *Azolla* plants collected in the Danube Delta can be grouped in three categories: *Azolla filiculoides* – collected on the Şontea Canal, *Azolla caroliniana* – collected in the Mila 23 area, and probably *Azolla mexicana* – collected on the Înfundata Canal. All the taxa have small, sessile, bi-lobed, imbricate leaves with a membranous margin. In 1972, OGURA defined these lobes as 'natant' – for the superior lobe, and 'submersed' – for the inferior lobe. Subsequently KAUL (1976) introduced the terms 'aerial lobe' for the superior lobe, and 'submersed lobe' for the inferior one.

Regardless of their position, all the foliar lobes have a membranous margin, which becomes narrower towards the top. The term 'membranous margin' is somewhat inadequate because this part of the leaf is in fact a multi-cellular structure, unistratified, formed by hetero-dimensional cells, which contain chloroplasts. The remaining part of the leaf, has a pluristratified structure, with prominent and ramified nervures. The two epidermises, superior and inferior, are organised differently and they differentiate stomata. Below the superior epidermis there is a layer of assimilating cells, rich in chloroplasts, which offers the dark green colour to the foliar lobes.

At *Azolla filiculoides* (Fig. 7, Fig. 8) the superior epidermis of the foliar lobes features stomata and strictly unicellular trichomes, displayed relatively uniformly (Fig. 9). These have a papilliform appearance (Fig. 10) and are 50-60 µm long. The papilliform formations that are differentiated by the superior epidermis of the foliar lobes were described by OGURA as early as 1972. Subsequently, in the identifying keys these have been considered unicellular trichomes. The stomatic cells have a relatively triangular form (Fig. 11). The inferior epidermis has elongated cells with a sinuous outline in apical image. These cells contain chloroplasts (Fig. 12).

At *Azolla caroliniana* (Fig. 13) the superior epidermis of the foliar lobes differentiates unicellular, papilliform trichomes (Fig. 14), ~50 µm long, similar to the ones observed at *Azolla filiculoides*. The epidermises also differentiate stomata, with relatively triangular shaped stomatic cells, which in some cases coalesce, forming a circular stomatic cell approximately 100 µm long (Fig. 15). The inferior epidermis of the foliar lobes is formed of cells with a sinuous outline (~80-100 µm long; ~30-50 µm wide), rich in chloroplasts (Fig. 16) and differentiate long, uniseriate, multicellular trichomes of 3-12 cells, with a long basal cell and the apical cell acuminate curved (Fig. 17, Fig. 18).

Azolla mexicana differs from the previous two taxa through the characteristics of the epidermal trichomes. At this species, the superior epidermis of the foliar lobe differentiates papilliform unicellular trichomes (~60 µm long), but also bi-cellular trichomes (Fig. 19, Fig. 20).

The three groups of plants included in the genus *Azolla* and collected in the Danube Delta, differ among themselves through the characteristics of the epidermal trichomes, as suggested by the specialty literature [LUMPKIN, 1993].

The epidermal trichomes can be considered an initial anatomical clue towards the differentiation of the three species of *Azolla* recorded in Romania. According to this criterion, it can be argued that in the Danube Delta there exist not only *Azolla filiculoides* and *A. caroliniana*, but most likely also *Azolla mexicana*. Further morphological and anatomical research on the latter taxon is required.

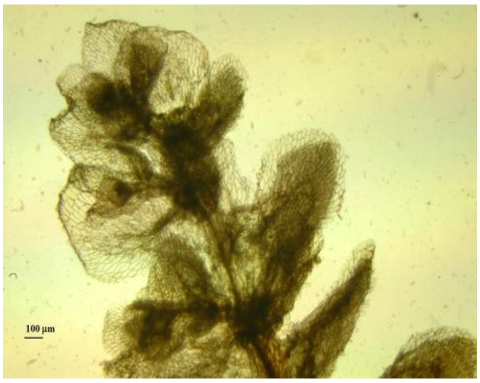

Fig. 7. *Azolla filiculoides* – a fragment.

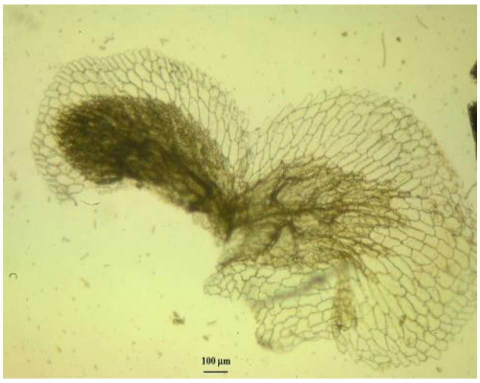

Fig. 8. *Azolla filiculoides* – foliar lobes.

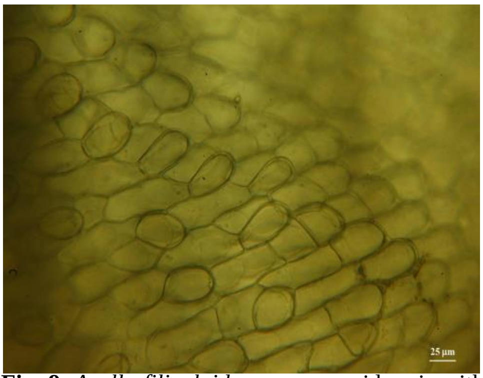

Fig. 9. *Azolla filiculoides* – upper epidermis with unicellular, papiliform trichomes (in front side view).

Fig. 10. *Azolla filiculoides* – upper epidermis with unicellular, papiliform trichomes (lateral view).

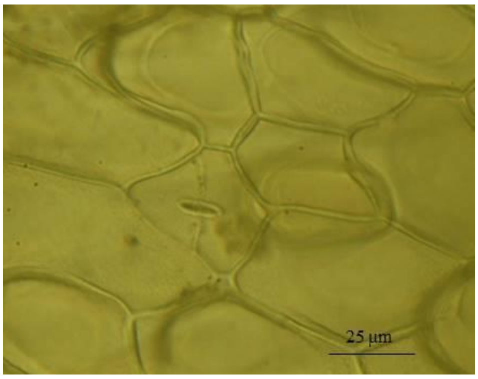

Fig. 11. *Azolla filiculoides* – upper epidermis with stomata (in front side view).

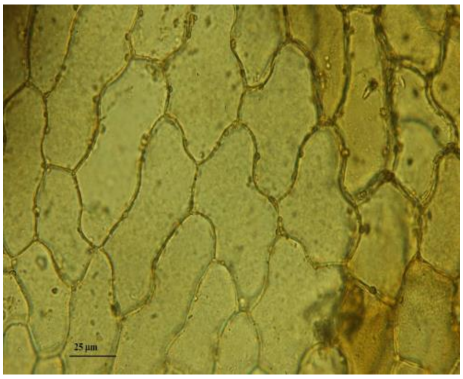

Fig. 12. *Azolla filiculoides* – lower epidermis (in front side view).

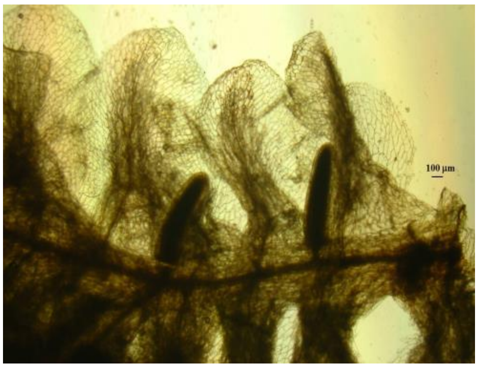

Fig. 13. *Azolla caroliniana* – a fragment.

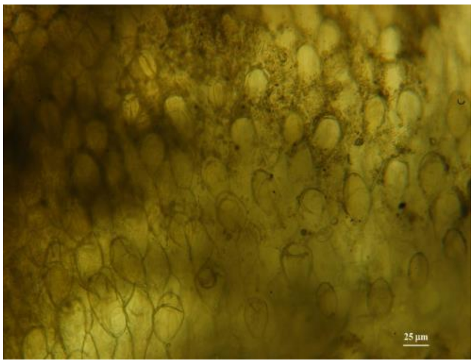

Fig. 14. *Azolla caroliniana* – upper epidermis with unicellular, papiliform trichomes (in front side view).

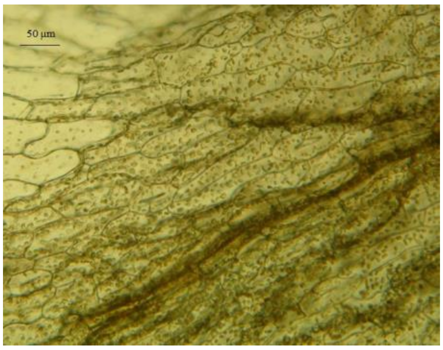

Fig. 15. *Azolla caroliniana* – lower epidermis with stomata (in front side view).

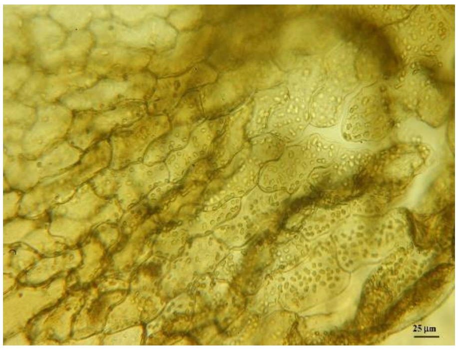

Fig. 16. *Azolla caroliniana* – lower epidermis (in front side view).

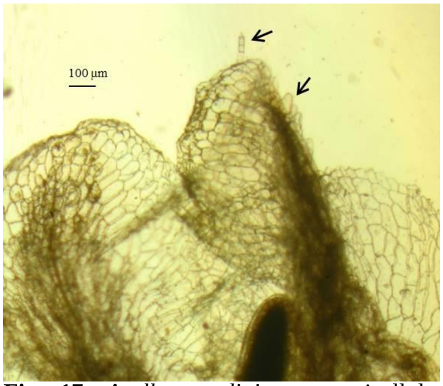

Fig. 17. *Azolla caroliniana* – unicellular papiliform on the upper epidermis and multicellular trichomes on the lower leaf epidermis.

Fig. 18. *Azolla caroliniana* – multicellular trichomes, on the lower leaf epidermis.

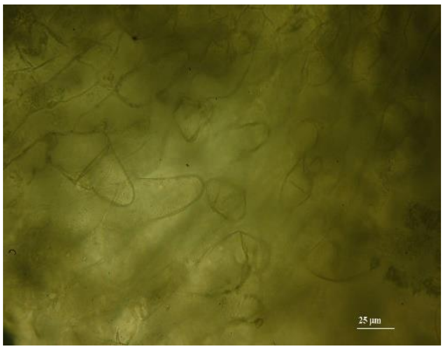

Fig. 19. *Azolla mexicana* – upper epidermis with unicellular and bicellular trichomes (in front side view).

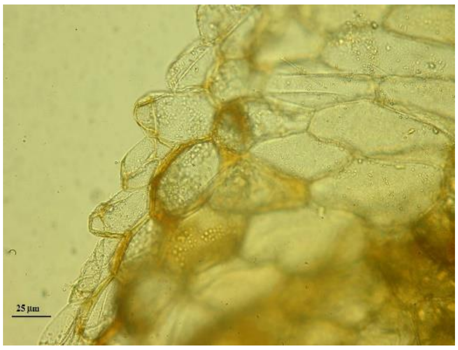

Fig. 20. *Azolla mexicana* – detail of bicellular trichomes.

The observations conducted on the specimen of *Azolla* collected in the Danube Delta highlighted the following aspects:

i. *Azolla filiculoides* features only papilliform unicellular trichomes, which are only differentiated at the level of the superior epidermis, while the inferior epidermis does not differentiate any trichomes,
ii. *Azolla caroliniana* features at the level of the superior epidermis papilliform unicellular trichomes and at the level of the inferior epidermis long and rare multicellular trichomes, with an acicular-curved apical cell,
iii. for *Azolla mexicana* at the level of the superior epidermis there have been observed both papilliform unicellular epidermal trichomes and bi-cellular trichomes, and
iv. at all the taxa analysed, the membranous margin of the foliar lobes is represented by a unistratified and photosynthesising multicellular structure.

Salvinia natans. This fern, which floats on the surface of the water, has a vegetative body formed of a stem and leaves. The stem is short and ramified. The leaves are dimorphic, displayed in trimerous whorls. Two leaves are floating, whole, oval and oval-elliptical, papillated, pubescent and greenish. The third leaf in the whorl is submerged metamorphosed, finely divided and root-like, multi-trichomic and pendent in the water.

The floating leaf

The floating leaf is differentiated in the lamina and the short petiole. The lamina is bifacial, uni-nerve, with a mesophyll of 0.7-0.8 mm width, partitioned, formed of two overlaid layers of polygonal chambers (Fig. 21). The adaxial side of the lamina is concave and papillate. At the level of the papillae, the superior epidermis differentiates moniliform trichomes (shaped like a string of beads), up to 0.7mm long, grouped in bunches of 3-4. The abaxial side of the lamina, which is in contact with the water, is convex and differentiates multicellular trichomes (4-5 cells), uniseriate, ~0.5 mm long, with an acicular apical cell (Fig. 21, Fig. 22).

The two epidermises of the lamina have irregularly-shaped cells. The epidermises are kept distanced from each other by a network of polygonal spaces delimited by, elongated parenchymatic cells arranged uniseriat (Fig. 23). From this structure there start uni-stratified multicellular trabeculae which partition the mesophyll into two levels of hexagonal chambers (Fig. 24). The chambers of the mesophyll host septate, assimilating cells (Fig. 25). In the adaxial zone of the mesophyll, the assimilating cells are smaller (~120 µm long) and rich in chloroplasts. In the abaxial zone, the assimilating cells are bigger (~250 µm long) and have less chloroplasts. As KAUL mentioned in 1976, all the parenchymatic cells of the floating leaf contain chloroplasts, with the exception of the trichomes. It should be added here that these cells with a role in photosynthesis also accumulate starch (Fig. 24, Fig. 25).

The nervure, with a median position, is delimitated by a uni-stratified endodermis with cells that are 30-50 µm long. The cells of the endodermis have thickened and suberified walls (Sudan III). The pericycle is uni-stratified, and the vascular bundle from the structure of the nervure is hadrocentric (Fig. 26).

The root-like leaf (rhizophyll)

The rhizophylles are filamentous, cylindrical, with a circular outline in cross section (Fig. 27). The epidermis differentiates numerous multicellular trichomes (10-15 cells), uniseriated, with a sharp apical cell. The apical cells of the rhizophyllic trichomes often appear pluri-nucleate (Fig 28). The cortex is a well-represented aerenchym, formed of a ring of eight aeriferous canals. The endodermis is present. The stele, delimitated by the pericycle, contains a hadrocentric vascular bundle.

The stem

The stem is short, cylindrical, with a diameter of 1.5-2 mm, with a circular outline in cross section (Fig. 29). The epidermis differentiates numerous trichomes 0.5-2.0 mm long, multicellular (up to 15 cells), uniseriated, with a sharp apical cell. The cortex is an aerenchym with a single ring of large aeriferous canals (8-9 canals), delimitated by uni-stratified multicellular walls (Fig. 30). The uni-stratified endodermis feature unequal cells (~25-60 µm long) with thickened and suberified cellular walls (Sudan III). The stele (~150 µm diameter) can be considered a protostele, with few phloem elements (Fig. 31). The disorganisation of the xylem from the central zone of the stele can often be observed, with a forming of lacuna (Fig. 32).

As regards the structure of the floating leaf, the rhizophyll and the stem of *Salvinia natans*, there are numerous relevant data in the specialty literature [OGURA, 1972; KAUL, 1976; CROXDALE, 1981; SEO & KIM, 2008; JAMPEETONG & BRIX, 2009]. In this context, our observations completed the existing data with optical microscopy images, highlighting the architecture of the mesophyll of the floating leave, the particularities of the endodermis, the formation of the medullary lacune, and the characteristics of trichomes, structures which ensure the efficient floating of the plant on the surface of the water and prevent the plant from sinking.

On the two types of leaves at *Salvinia natans* there are three types of trichomes: i) groups of moniliform trichomes at the level of the superior epidermis of the floating leaf, which due to their relatively spherical cells prevent the adaxial zone of the lamina from becoming wet, ii) solitary, uniseriated, multicellular trichomes, with a sharp apical cell, differentiated at the level of the inferior epidermis of the floating leaf, which contribute alongside the cameral mesophyll to the stability of the floating leaf on the surface of the water, and iii) submersed multicellular rhizophyllic trichomes, long and numerous, involved in absorption.

The presence of moniliform trichomes on the adaxial side of the lamina provides to it hydrophobe properties (water repellent surface). Drops of water remain on the top of the trichomes, while the stomata can continue their exchange of respiratory gases [CERMAN & al. 2009]. This characteristic offers the plant remarkable ecological advantages.

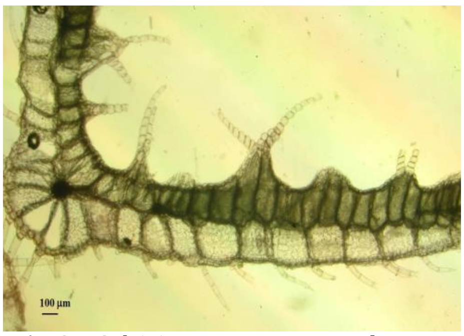

Fig. 21. *Salvinia natans* – transversal section throught the floating leaf (Iodine green, Carmine alum).

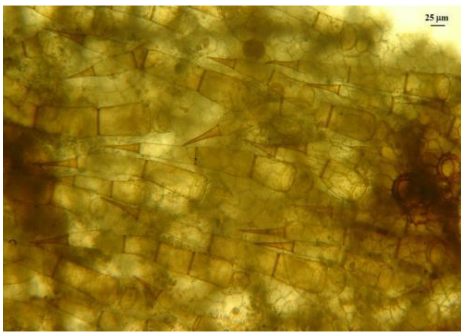

Fig. 22. *Salvinia natans* –multicellular trichomes on the lower epidermis (in front side view).

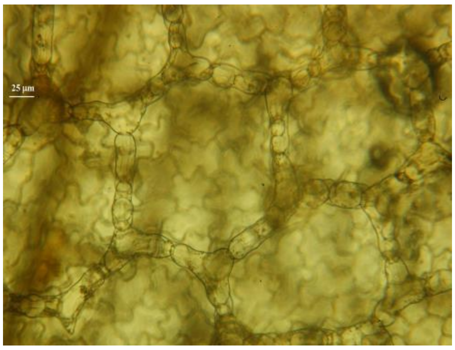

Fig. 23. *Salvinia natans* – subepidermal frame of the floating leaf (in front side view).

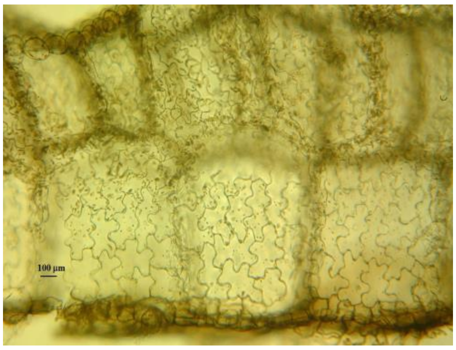

Fig. 24. *Salvinia natans* – pluricameral mesophyll of the floating leaf, in transversal section (IIK).

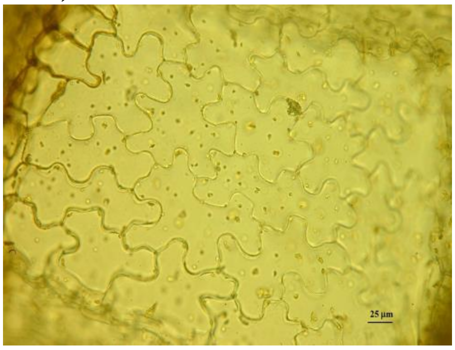

Fig. 25. *Salvinia natans* – cells from the adaxial chamber floor of the mesophyll (IIK).

Fig. 26. *Salvinia natans* – transversal section through the floating leaf (Sudan III).

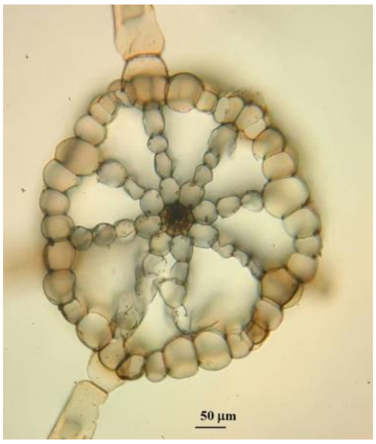

Fig. 27. *Salvinia natans* – transversal section through the rizophylle (Iodine green, Carmine alum).

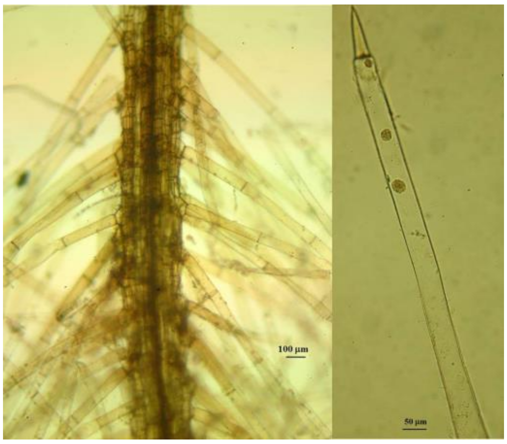

Fig. 28. *Salvinia natans* – the rizophylle and the rizophylle trichomes.

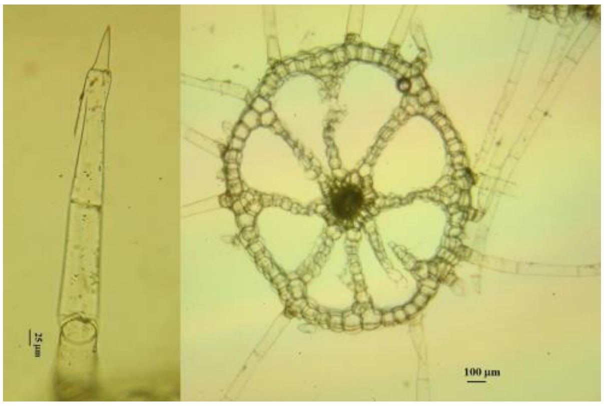

Fig. 29. *Salvinia natans* – transversal section through the stem, emphasizing structure and trichomes.

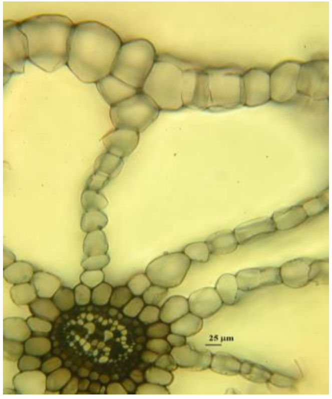

Fig. 30. *Salvinia natans* – transversal section through the stem (Iodine green, Carmine alum).

Fig. 31. *Salvinia natans* – the stele structure, in transversal section through the stem (Sudan III).

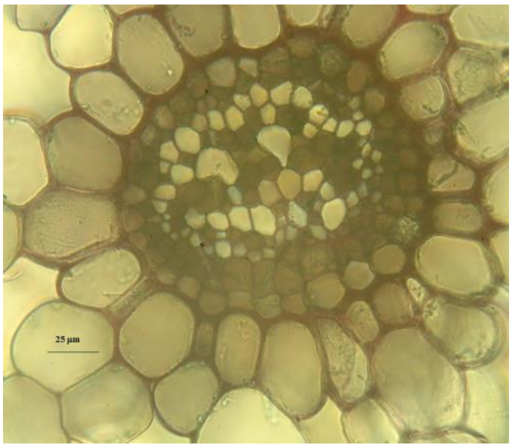

Fig. 32. *Salvinia natans* – transversal section through the stem highlighting the stele's gaps (Iodine green, Carmine alum).

Marsilea quadrifolia. This perennial hygro-hydrophyte, has a long rhizome (0.5-1 m), thin, crawling, on which there grow fixating, adventive roots and petiolate leaves with 4 obovate leaflets, with a whole edge.

The rhizome

For all the growth forms analysed, the rhizome has a circular outline in cross section (Fig. 33, Fig. 34, Fig. 35). The uni-stratified epidermis is formed of small cells, relatively isodiametrical.

The cortex is voluminous, reaching up to 70-80% of the diameter of the rhizome. The external cortex is represented by an aerenchym, with 20-22 large aeriferous canals, separated by uni-stratified trabeculae and containing diaphragmatic tissue (Fig. 36). The internal cortex, peristelic, is formed of 6-7 layers of parenchymatic cells at f. *terrestris* and f. *natans* collected in the Danube Delta, and 4-5 layers of cells with slightly and uniformly thickened walls at f. *natans* collected in Arcuda (Fig. 37). The last layer of the cortex is a uni-stratified endodermis, formed of small cells (~10-12 µm long; ~6-10 µm wide). Caspary thickenings are small and lenticular in cross section. The cortical cells, except for the endodermis, accumulate starch (Fig. 38).

The stele is an amphiphloic siphonostele, with a dense parenchymatic pith at f. *terrestris* and meatic at f. *natans* (Fig. 39, Fig. 40, Fig. 41). At f. *terrestris* the stele has the largest diameter. This is about 20% smaller at the f. *natans* from the Danube Delta and about 50% smaller at f. *natans* from Arcuda, for which a reduction of 60% in the number of xylem vessels has been observed as compared to f. *terrestris*. Only at the f. *natans* from the Danube Delta a pith lacuna was observed (Fig. 40).

The petiole

The petiole is cylindrical, more or less long, depending on the form of growth and the type of habitat (Tab. 1).

At f. *terrestris,* the petiole is short and thick.

At f. *natans* the petiole is longer, but its diameter is smaller. The longest (35-40 cm) and thinnest (1.0 mm diameter) petiole was observed at f. *natans,* collected from Arcuda (Fig. 42).

Tab. 1. *Marsilea quadrifolia* – petiole, morphology data.

Growth form	Petiole length (cm)	Petiole diameter (mm)
f. *terrestris* – Danube Delta	8-10	2.5
f. *natans* – Danube Delta	20-25	1.3
f. *natans* – Arcuda Station	35-40	1.0

In the cross sections conducted through the median zone of the petiole, this features a circular outline (Fig. 43, Fig. 44, Fig. 45). The epidermis is uni-stratified. At f. *terrestris* it is formed of relatively isodiametrical cells in cross section and it differentiated stomata (Fig. 46). At f. *natans* from the Danube Delta, the epidermal cells are heterogeneous, small, and the stomata are absent (Fig. 47). At f. *natans* collected from Arcuda the epidermal cells are rectangular and have uniformly thickened walls (Fig. 47).

The cortex is voluminous, representing 80% of the diameter of the petiole at f. *terestris* and 85% at f. *natans*. The external cortex is an aerenchym with 10-16 large aeriferous canals, separated from the uni-stratified trabeculae and containing a diaphragmatic tissue (Fig. 43, Fig. 44, Fig. 45). The internal cortex is parenchymatic and meatic at f. *natans* collected in the Danube Delta and at Arcuda (Fig. 44, Fig. 45).

At f. *terrestris* there is a median zone of the cortex formed of 1-2 layers of cells with slightly and uniformly thickened walls (sclerenchymatous cells) and a parenchymatic slightly meatic internal zone (3-4 layers of cells) (Fig. 43). The last layer of the cortex is an endodermis, uni-stratified, of primary type.

The stele has a roughly semi-circular outline in cross section. It is delimitated by a uni-stratified pericycle and has a protostelic structure. The xylem is well represented and is surrounded by phloem. The protoxylem has an exarch disposition, and the metaxylem formed of several large vessels is endarch. The protoxylematic lacuna is only present at f. *natans* (Fig. 48, Fig. 49, Fig. 50).

The lamina

The diameter and thickness of the lamina vary according to the form of growth and the type of habitat (Tab. 2). The natant forms have a wider and thinner lamina than f.

terrestris. At f. *natans* from Arcuda the lamina is ~40% wider and ~35% thinner than f. *terrestris*.

Tab. 2. *Marsilea quadrifolia* – lamina, morphology data.

Growth form	Lamina diameter (cm)	Lamina thickness (mm)
f. *terrestris* – Danube Delta	2.5	0.24
f. *natans* – Danube Delta	3.2	0.21
f. *natans* – Arcuda Station	4.0	0.16

The lamina is epistomatic at the floating forms and amphistomatic at f. *terrestris*. Analysed at the level of the leaflets, the lamina features at all three types of plants a dorsi-ventral heterogeneous structure, characterised by the presence of a zone of palisadic tissue, positioned adaxially, and a zone of lacunous tissue, displayed abaxially (Fig. 51, Fig. 52, Fig. 53). The nervures each contain a vascular bundle of hadrocentric concentric type, surrounded by a uni-stratified endodermis.

At the floating forms, the palisadic tissue is form of a single layer of long palisadic cells (~50-80 µm), and at f. *terrestris* from 1-2 layers of shorter palisadic cells (~30-40 µm long), but very rich in chloroplasts (Fig. 51, Fig. 52, Fig. 53). In this latter case, as the specialty literature mentions [ESAU, 1965], there is a transition zone between the palisadic tissue and the lacunous tissue. The lacunous tissue is more developed at the natant forms, where it forms aeriferous canals (Fig. 52, Fig. 53). At f. *terrestris*, the chloroplasts are abundant both in the palisadic cells and in those of the lacunous tissue (Fig. 51).

At f. *natans* collected from the artificial habitat at Arcuda (Fig. 54), where the lamina is wide (~4 cm) and thin (~0.16 cm), between the level of palisadic cells and the zone of lacunous tissue there is a continuous layer of short mechanical cells, represented by isodiametrical and slightly elongated sclereids, which contribute to the rigidity of the lamina.

The general organisation of the vegetative body of the species *Marsilea quadrifolia* analysed from a morphological and anatomical point of view generally fits in with the characteristics mentioned for this plant in the literature [OGURA, 1938; TRYON & TRYON, 1982; GRINŢESCU, 1985; LERSTEN, 1997; TOMA & GOSTIN, 2000; BERCU, 2004].

However, differences have been noted as regards the forms of growth, reflecting the adaptation of the species to life in palustre habitats with reduced humidity, but also in natural and artificial aquatic habitats of different depths. As such, the transition from the aquatic environment to the terrestrial one is associated with a series of morphological and structural changes:

i. changes which enhance the resistance of the vegetative body, such as the reduction in the dimensions of the petiole and the lamina; the increase in the share of the xylem and the mechanical elements in the structure of the vegetative organs; the reduction of the intercellular spaces, and
ii. changes which support the respiratory and photosynthesis processes, such as the transition from epistomatic to amphistomatic leaves, the organisation of the mesophyll and the increase in the number of chloroplasts in the cells.

At *Marsilea quadrifolia* f. *natans* collected from Arcuda, the greater depth of the water accentuated some of the morphological changes, such as the elongation of the petiole, the enhancement of the foliar surface and the reduction of the thickness of the lamina, generating at the same time associated structural changes which help improve the resistance of the plant, such as the formation of sclereids in mesophyll, the thickening of the cellular walls of the epidermis and cortical cells from the structure of the petiole, the lack of the pith lacuna in the rhizome.

In the literature, the maximum length of the petiole of the f. *natans* is 20-25 cm [ŢOPA, 1952; CODY & BRITTON, 1989]. Our observations have highlighted a greater adaptive plasticity of this parameter, and respectively this plant, in relation to the depth of the water, which points to the ability of this plant to populate a wider range of aquatic habitats.

Phytohormones are thought to be some of the most important factors responsible for the morphological and structural adaptive changes that occur in plants with the transition from one life environment to the other.

For example, the literature highlights that for *Marsilea quadrifolia* the transitioning from an aquatic environment to a terrestrial one involved the abscisic acid phytohormone (ABA) which, as it has been demonstrated, influences the length of the petiole, the morphology of the lamina and the structure of the internodes [LIN & al. 2005].

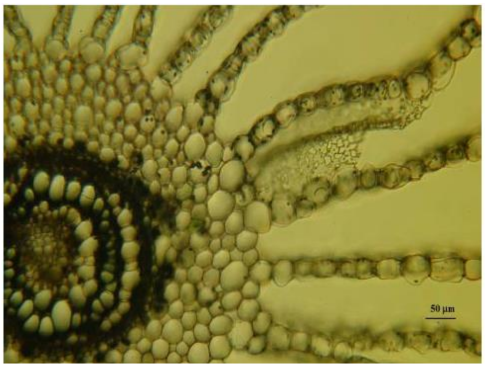

Fig. 33. *Marsilea quadrifolia*, f. *terrestris* – transversal section throught the rhizome (Iodine green, Carmine alum, IIK).

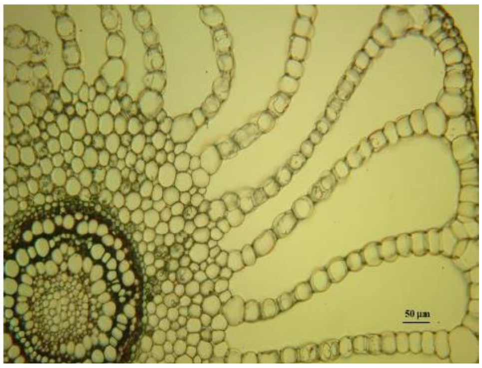

Fig. 34. *Marsilea quadrifolia*, f. *natans* (Danube Delta) – transversal section through the rhizome (Iodine green, Carmine alum).

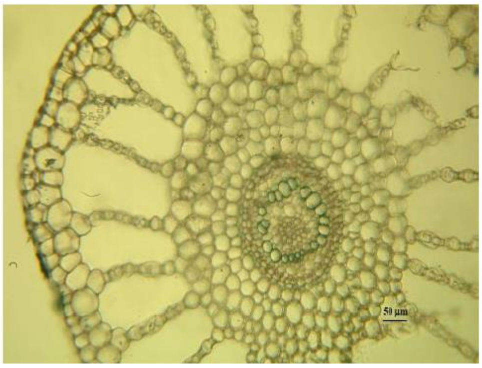

Fig. 35. *Marsilea quadrifolia*, f. *natans* (Arcuda Station) – transversal section through the rhizome (Iodine green, Carmine alum).

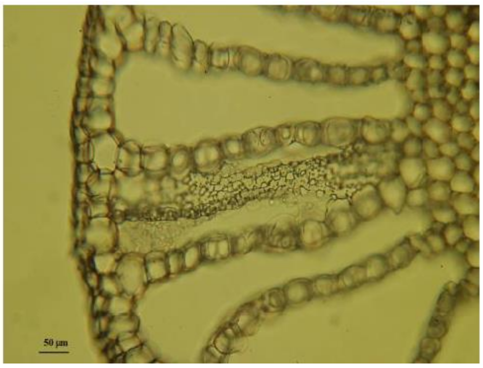

Fig. 36. *Marsilea quadrifolia*, f. *terrestris* – rhizome in transversal section; diaphragmatic tissue (Iodine green, Carmine alum).

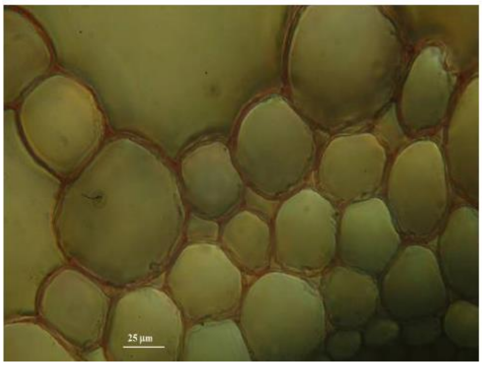

Fig. 37. *Marsilea quadrifolia*, f. *natans* (Arcuda Station) – rhizome in transversal section; peristelic area (Iodine green, Carmine alum).

Fig. 38. *Marsilea quadrifolia*, f. *natans* (Arcuda Station) – rhizome in transversal section; starch granules (IIK).

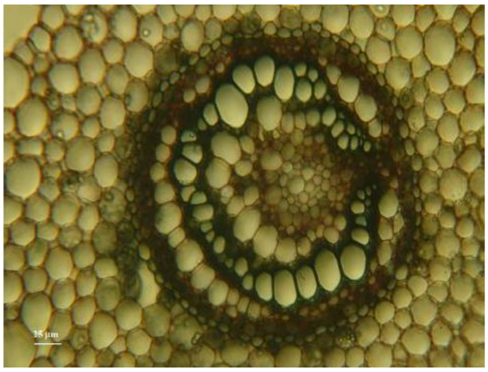

Fig. 39. *Marsilea quadrifolia*, f. *terrestris* – rhizome in transversal section; the stele (Iodine green, Carmine alum).

Fig. 40. *Marsilea quadrifolia*, f. *natans* (Danube Delta) – rhizome in transversal section; the stele (Iodine green, Carmine alum).

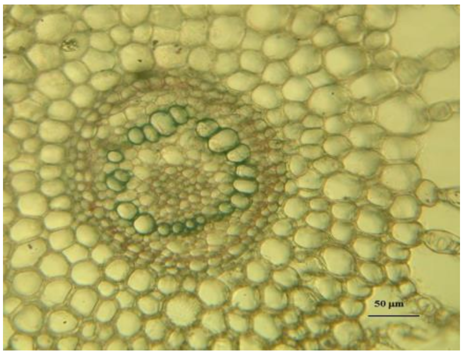

Fig. 41. *Marsilea quadrifolia*, f. *natans* (Arcuda Station) – rhizome in transversal section; the stele (Iodine green, Carmine alum).

Fig. 42. *Marsilea quadrifolia*, f. *natans* (Arcuda Station) – habitus.

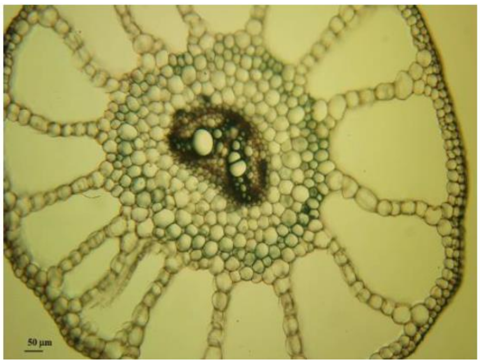

Fig. 43. *Marsilea quadrifolia*, f. *terrestris* – petiole in transversal section (Iodine green, Carmine alum).

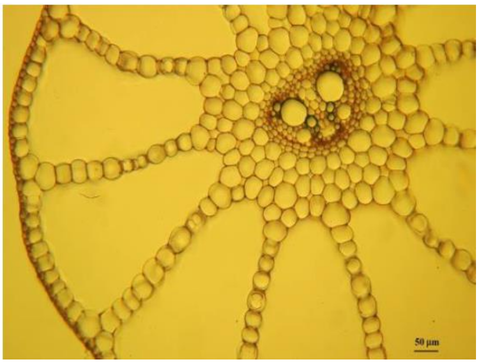

Fig. 44. *Marsilea quadrifolia*, f. *natans* (Danube Delta) – petiole in transversal section (Iodine green, Carmine alum).

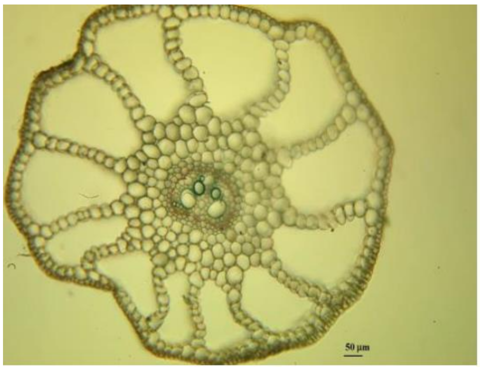

Fig. 45. *Marsilea quadrifolia*, f. *natans* (Arcuda Station) – petiole in transversal section (Iodine green, Carmine alum).

Fig. 46. *Marsilea quadrifolia*, f. *terrestris* – petiole in transversal section; the epidermis (Iodine green, Carmine alum).

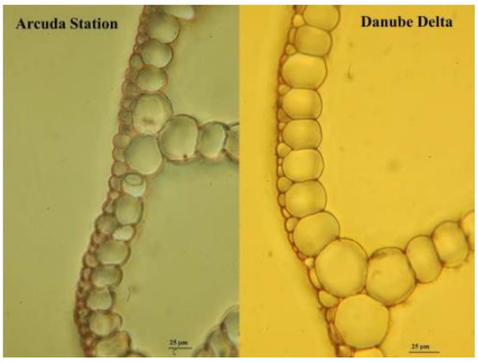

Fig. 47. *Marsilea quadrifolia*, f. *natans* – petiole in transversal section; the epidermis (Iodine green, Carmine alum).

Fig. 48. *Marsilea quadrifolia*, f. *terrestris* – petiole in transversal section; the stele (Iodine green, Carmine alum).

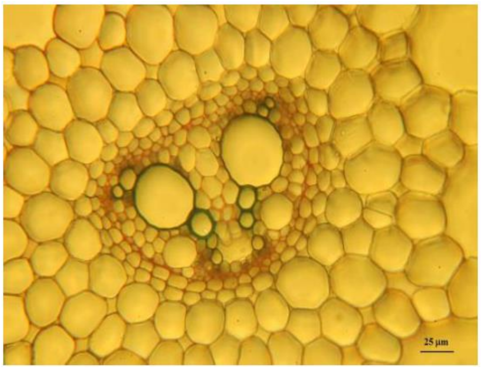

Fig. 49. *Marsilea quadrifolia*, f. *natans* (Danube Delta) – petiole in transversal section; the stele (Iodine green, Carmine alum).

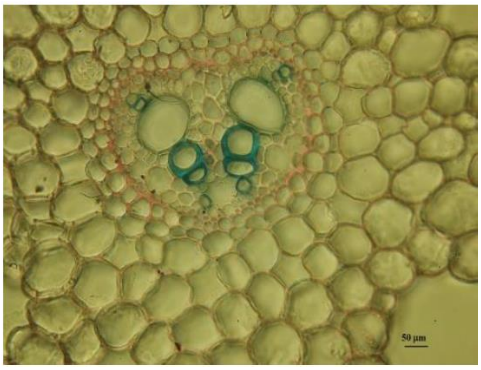

Fig. 50. *Marsilea quadrifolia*, f. *natans* (Arcuda Station) – petiole in transversal section; the stele (Iodine green, Carmine alum).

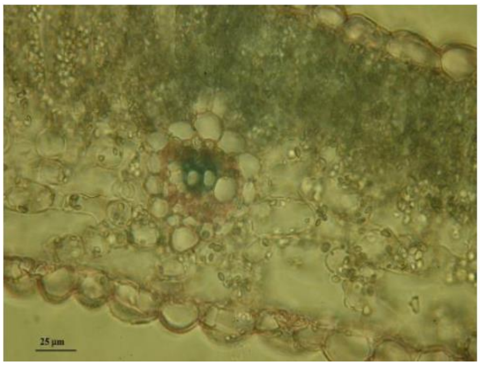

Fig. 51. *Marsilea quadrifolia*, f. *terrestris* – transversal section through the foliole (Iodine green, Carmine alum).

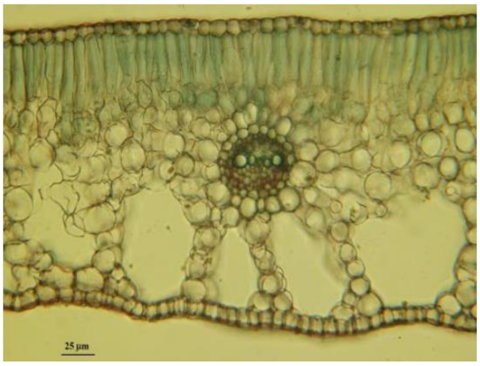

Fig. 52. *Marsilea quadrifolia*, f. *natans* (Danube Delta) - transversal section through the foliole (Iodine green, Carmine alum).

Fig. 53. *Marsilea quadrifolia*, f. *natans* (Arcuda Station) – transversal section through the foliole (Iodine green, Carmine alum).

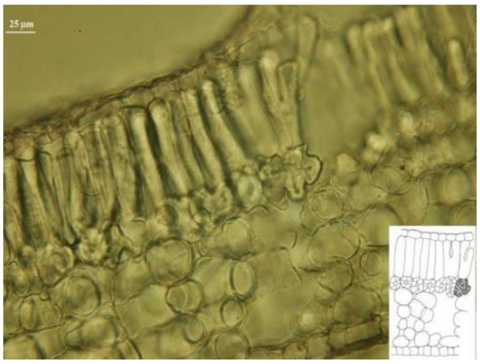

Fig. 54. *Marsilea quadrifolia*, f. *natans* (Arcuda Station) – foliole in transversal section; the sclereides (Iodine green, Carmine alum).

Conclusions

The species of the genus *Azolla* are difficult to identify based on the appearance of the vegetative body such as the shape, dimension and colour of leaves, and the sporocarps are not always present. Nonetheless they can be differentiated through the micro-morphological characteristics of the epidermal trichomes, which can be utilized as an initial, more accessible criterion for identifying these taxa. Based on this type of character, we can demonstrate the presence of the taxon *Azolla caroliniana* in the Danube Delta, while the presence of the taxon *Azolla mexicana* can be considered likely in the habitats of the Danube Delta.

Salvinia natans, a natant plant, shows numerous adaptations to life on the surface of the water. Among these adaptations it is worth noting the architecture of the mesophyll and the presence of the two types of epidermal trichomes. These adaptive elements contribute to enabling the plant to float and maintain its balance on the surface of the water, thus preventing it from sinking under the direct impact of the water. The hydrophobe structure of the foliar surface ensures an unhindered functioning of the stomata.

At *Marsilea quadrifolia*, which was analyzed both in natural and artificial habitats, the organization of the vegetative body reveals morpho-structural adaptations induced by the life in aquatic habitats of varying shallows and by the transition from an aquatic to a terrestrial environment. These observations support the adaptive plasticity of this protected species.

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References

SEO A. R. & KIM I. S. 2008. Development of the trichomes in floating leaves of *Salvinia* species. *Korean J. Microscopy*. **38**(2): 117-124.

ANTONESCU C. 1951. *Plante de apă şi de mlaştină*. Bucureşti: Edit. de stat pentru literatură ştiinţifică şi didactică, 223 pp.

BERCU R. 2004. Anatomical features of the vegetative organs of *Marsilea quadrifolia* L. *Contribuţii Botanice*. **39**: 149-154.

CERMAN Z., STRIFFLER B. F. & BARTHLOTT W. 2009. *Dry in the Water: The Super hydrophobic Water Fern Salvinia – a Model for Bio-mimetic Surfaces*: 97-111. In: GORB S. N. (ed.) *Functional Surfaces in Biology. Little Structures with Big Effects*. Vol. **1**. Springer Science + Business Media B.V., 383 pp.

CIOCÂRLAN V. 1994. *Flora Deltei Dunării*. Bucureşti: Edit. Ceres, 115 pp.

CIOCÂRLAN V. 2000. *Flora ilustrată a României. Peteridophyta et Spermatophyta*. (ed a 2-a). Bucureşti: Edit. Ceres, 1141 pp.

CIOCÂRLAN V. 2011. Vascular flora of the Danube Delta. *An. Şt. Univ. Iaşi, s. II.a, Biol. Veget.* 57(**1**): 41-64.

CODY W. J. & BRITTON D. M. 1989. *Ferns and fern allies of Canada*. Reprint Edition 2016. Ottawa: Canadian Government Publishing Centre, 440 pp. (amazon.com/Ferns-Fern-Allies-Canada-William/dp/15233081184) (accessed 15.12.2016).

COUNCIL OF EUROPE 1992, Council Directive 92/43/EEC on the Conservation of natural habitats and of wild fauna and flora (Habitat Directive). http://ec.europa.eu/environment/nature/legislation/habitatsdirective /index_en.htm (accessed 31 October 2015).

CROXDALE J. G. 1981. *Salvinia* leaves. III. Morphogenesis of the submerged leaf. *Canadian Journal of Botany* 59(**11**): 2065-2072.

ESAU K. 1965. *Plant anatomy*. (2nd edition). Wiley J. & Sons, Inc. (eds.), New York, London, Sydney, 767 pp.

GAFTA D. & MOUNTFORD O. (eds.) 2008. *Manualul de interpretare a habitatelor Natura 2000 din România*. Cluj-Napoca: Edit. Risoprint, 101 pp.

GRINȚESCU I. 1985. *Botanica* (ed. a 2-a). București: Edit. Științifică și Enciclopedică, 477 pp.

JAMPEETONG A. & BRIX H. 2009. Oxygen stress in *Salvinia natans*: Interactive effects of oxygen availability and nitrogen source. *Environmental and Experimental Botany*. **66**: 153-159.

KAUL R. B. 1976. Anatomical observations on floating leaves. *Aquatic Botany*. **2**: 215-234.

LERSTEN N. R. 1997. Occurrence of endodermis with a casparian strip in stem and leaf. *Bot. Rev.* **63**: 265-272.

LIN B. L., WANG H. J., WANG J. S., ZAHARIA L. I. & ABRAMS S. R. 2005. Abscisic acid regulation of heterophylly in *Marsilea quadrifolia* L.: effects of R-(-) and S-(-) isomers. *Journal of Experimental Botany*. **56**(421): 2935-2948. [HTML] oxfordjournals.org (accessed 14.12. 2016).

LUMPKIN Th. A. 1993. *Azollaceae* Wettstein *Azolla* Family. In: Flora of North America Editorial Committee, eds, 1993 + Flora of North of Mexico. 20 + Vols. New York and Oxford. Vol. **2**: 338-342.

OGURA Y. 1938. *Anatomie der Vegetationsorgane der Pteridophyten*: 128-140. *In*: Handbuch der Pflanzenanatomie, VII, T. 2. Berlin: Gebrüder Borntraeger, 476 pp.

OGURA Y. 1972. *Comparative anatomy of vegetative organs of the Pteridophytes*. 2nd revised edition. Handbuck der Pflanzenanatomie VII, T. 3, Berlin: Gebrüder Borntraeger, 502 pp.

OLTEAN M., NEGREAN G., POPESCU A., ROMAN N., DIHORU G., SANDA V. & MIHĂILESCU S. 1994. *Lista roşie a plantelor superioare din România*. *In*: M. OLTEAN (ed.), *Studii, sinteze, documentaţii de ecologie*. **1**: 1-52. Bucureşti: Academia Română, Institutul de Biologie.

OPREA A. 2005. *Lista critică a plantelor vasculare din România*. Iaşi: Edit. Universităţii „Al. I. Cuza", 668 pp.

SÂRBU A. 2002. *Ecomorfologia plantelor*. Bucureşti: Edit. Alo, Bucureşti!, 109 pp.

SÂRBU A. 2003. Inventory of aquatic plants in the Danube Delta, a pilot study in Romania. *Arch. Hydrobiol. Suppl.* **147**(1-2): 205-216.

SÂRBU A., SMARANDACHE D., JANAUER G. & PASCALE G. 2005. *Plante acvatice şi palustre din sectorul românesc al Dunării*. Bucureşti: Edit. Universităţii din Bucureşti, 178 pp.

SÂRBU A. 2006. *Aquatic macrophytes:* 133-175. In: TUDORANCEA C. & TUDORANCEA M. (eds.) *Danube Delta Genesis and Biodiversity*. Leiden: Backhuys Publishers, The Netherlands, 444 pp.

SÂRBU A., JANAUER G., SCHMIDT-MUMM U., FILZMOSER P., SMARANDACHE D. & PASCALE G. 2011. Characterization of the potamal Danube River and the Delta: connectivity determines indicative Macrophytes assemblages. *Hydrobiologia, The International Journal of Aquatic Science*. **671**(1): 75-93.

SÂRBU A., ANASTASIU P. & SMARANDACHE D. 2013. *Habitate acvatice din Delta Dunării – Repere în evaluare şi monitorizare*. pp. 45-88. In: M. DOROFTEI & S. COVALIOV (eds.) *„Manual de ... Delta Dunării"*. Tulcea: Edit. Centrului de Informare Tehnologică Delta Dunării, 482 pp.

SÂRBU A., SÂRBU I., GACHE C., STAICU C., PARASCHIV A. M. & MIHAI D. C. 2014. *Diversity of flora, vegetation and fauna in the Arcuda Station area*. Bucureşti: Edit. Ceres, 192 pp.

SÂRBU A. 2015. *Delta Dunării. Plante acvatice şi palustre*. Bucureşti: Edit. Ceres, 302 pp.

SÎRBU C. (coord.) & OPREA A. 2011. *Plante adventive în flora României*. Iași: Edit. Ion Ionescu de la Brad, 733 pp.

SÂRBU I., ŞTEFAN N. & OPREA A. 2013. *Plante vasculare din România. Determinator de teren*. Bucureşti: Edit. Victor B Victor, 1317 pp.

STRASBURGER E., NOLL F., SCHENCK H. & SCHIMPER A. F. W. 1990. *Lehrbuch der Botanik fur Hochschulen*. Berlin: Spektrum Akademischer Verlag Heidelberg, 1007 pp.

ŞERBĂNESCU-JITARIU G., ANDREI M., RĂDULESCU-MITROI N. & PETRIA E. 1983. *Practicum de biologie vegetală*. Bucureşti: Edit. Ceres, 296 pp.

TRYON R. M. & TRYON A. F. 1982. *Ferns and allied plants with special reference to Tropical America*. Springer-Verlag New York, 858 pp.

TOMA C & GOSTIN I. 2000. *Histologie vegetală*. Iași: Edit. Junimea, 214 pp.

ŢOPA E. 1952. Fam. *Marsileaceae*. pp. 150. In: SĂVULESCU T. (ed.). *Flora României*, Vol. **1**. Bucureşti: Edit. Academiei Române, 657 pp.

ŢOPA E. 1952. Fam. *Salviniaceae*. pp. 152-153. In: SĂVULESCU T. (ed.). *Flora României*, Vol. **1**. Bucureşti: Edit. Academiei Române, 657 pp.

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