

Comparative anatomical study on leaves of three *Euphorbia* L. species

Rodica Bercu & Dan Răzvan Popoviciu

Summary: The paper presents a comparative study on the leaf structure of three species belonging to Euphorbiaceae: *Euphorbia myrsinites*, *E. nicaeensis* subsp. *dobrogeensis* and *E. seguieriana*. Anatomically, the leaves of the three species are relatively similar in their basic structure. However, differences appear regarding epidermal cells and cuticular papillae, mesophyll, density of stomata and type of stomatal complex, laticifers and development of the vascular system.

Keywords: comparative anatomy, laticifers, leaves, stomata, *Euphorbia*

Euphorbiaceae is the sixth largest plant family comprising 322 genera and about 8910 species, most common in the humid tropical and subtropical regions of both hemispheres (WEBSTER 1987). Many species are commonly known as spurges (RADCLIFFE-SMITH 1987).

Euphorbia myrsinites L., also known as myrtle spurge, creeping spurge or donkey tail, is a perennial, herbaceous plant with sprawling stems growing up to 20–40 cm. The leaves are spirally arranged, fleshy, pale, glaucous, blueish-green, 1–2 cm long. The flowers are inconspicuous, but surrounded by bright sulphurous-yellow bracts (tinged red). They are flowering in spring. In the Romanian flora, *Euphorbia myrsinites* is considered as an endangered species (DIHORU & NEGREAN 2009).

Euphorbia nicaeensis subsp. *dobrogeensis* (Prodán) Kuzmanov is a herbaceous perennial, pale green, with hairless stems growing up to 40 cm, densely-foliated and branched at the top. The leaves are spirally arranged, 30–60 mm long and 15–20 mm wide, elliptical in shape, obtuse on top and shortly mucronated. The flowers are grouped in inflorescences with yellow ovate-cordate bracts. It is a synonym of *E. glareosa* Pall. ex Bieb., but CIOCÂRLAN (2000) considered this species in the Romanian Flora to be synonymous with *E. pannonica* Host. Nevertheless, PRODÁN (1953) considered *E. glareosa* and *E. pannonica* as different species. According to the Bulgarian Flora, *E. nicaeensis* subsp. *dobrogeensis* is a synonym of *E. dobrogeensis* Prodán (KUZMANOV & ANTONOV 1979; GALEȘ & TOMA 2006, 2007).

Euphorbia seguieriana Neck. is a herbaceous perennial. The tall, erect, hairless stems growing up to 60 cm are yellowish-green. Leaves are hairless, glaucous, spirally arranged, 1–2 cm long and 4–5 mm wide, linear or linear-lanceolate, mucronate-acuminate on top. The inflorescences have cordate, yellowish bracts that become green later on (CIOCÂRLAN 2000; PRODÁN 1953).

The purpose of this paper is to highlight the similarities and differences in leaf anatomy of the three species. In literature, we did not find any comparable study. Only few studies concern leaf anatomy of Euphorbiaceae (GAUCHER 1902; METCALFE & CHALK 1950; SEHGAL & PALIWAL 1974; KAKKAR & PALIWAL 1973; FAHN 1990; BATANOUNY 1992; JAFARI & NASSEH 2009; LUDOVIĆ et al. 2009; ESSIETT et al. 2012).

In Romanian literature, very few data on the structure of *Euphorbia* species in connection with laticifers were found in textbooks dealing with histology, morphology and anatomy of plants

textbooks (ȘERBĂNESCU-JITARIU & TOMA 1980; GRINȚESCU 1985; TOMA & GOSTIN 2000; BAVARU & BERCU 2002). Relatively recent, comprehensive information concerning origin, development and distribution of laticifers and anatomy of the vegetative organs of a large number of *Euphorbia* species (*E. seguieriana* excluded) including the leaf, have been found in GALEȘ & TOMA (2006, 2007).

Materials and methods

Mature leaves were collected from the arid SW coastlines of Conacu, Constanța, Romania (*E. nicaeensis* subsp. *dobrogeensis* and *E. seguieriana*) and outside from Yailata Reservation, Bulgaria (*E. myrsinites*) in June 2012. Small pieces of mature leaves were fixed in FAA (formalin : glacial acetic acid : alcohol = 5 : 5 : 90). Cross sections of the vegetative organs were performed manually. The samples were stained with alum carmine, iodine green and safranin 1% (the paradermal sections; ANDREI & PREDAN 2001; BERCU & JIANU 2003). The samples were embedded in glycerol gelatine. Anatomical observations and micrographs were performed with a Biorom-T brightfield microscope, equipped with a Topica 6001A video camera.

Results and discussion

Figure 1 shows the leaf blade structure of all three species in cross section. In Fig. 2 details of the mesophyll revealing the structure of the assimilatory parenchyma can be seen. The structure of midrib veins is presented in Fig. 3. Epidermises with papillae and stomata are shown in Fig. 4.

All species investigated have sessile, bifacial leaves. Leaf cross-sections exhibit an upper and a lower epidermis and a mesophyll with vascular bundles embedded (Fig. 1). The epidermal cells are rectangular (*E. nicaeensis* subsp. *dobrogeensis* and *E. seguieriana*) or tabular-shaped (*E. myrsinites*) with thick periclinal walls and covered by a variably thick, papillose cuticle in *E. myrsinites* and *E. nicaeensis* subsp. *dobrogeensis* and a non-papillose cuticle in *E. seguieriana*. The continuity of both epidermises (abaxial and adaxial) is interrupted by numerous stomata with large substomatal cavities in *E. myrsinites* and *E. nicaeensis* subsp. *dobrogeensis* and with smaller substomatal cavities in *E. seguieriana* (Fig. 2). Our findings in *E. myrsinites* differ from those reported by GALEȘ & TOMA (2007), i.e. shallow substomatal cavities. In the two species with papillose epidermises, each hair-like papilla is placed in the middle of external epidermal cell walls (Figs 2A, B; 4A, B), just like GALEȘ & TOMA (2007) reported for other *Euphorbia* species. They are shorter and thicker in *E. myrsinites*, confirming the findings of KAKKAR & PALIWAL (1973), and thinner and longer in *E. nicaeensis* subsp. *dobrogeensis*, respectively.

The mesophyll is heterogenous and the palisade tissue occurs adaxially and abaxially. The palisade tissue has two layers just below the upper epidermis in all studied species. Below the lower epidermis, one layer of palisade tissue occurs in *E. nicaeensis* subsp. *dobrogeensis* and *E. seguieriana*, whereas in *E. myrsinites* there are two layers of palisade cells. The spongy tissue between the palisade tissue layers is represented by three rows of cells in all species. The spongy tissue is relatively compact in *E. seguieriana* (Fig. 2).

The vascular bundles are embedded in the mesophyll. Especially the phloem of the main vein is protected by a group of collenchyma cells, which are more developed in *E. myrsinites* and less in the two other species. The xylem placed towards the upper epidermis, has a more or less radial

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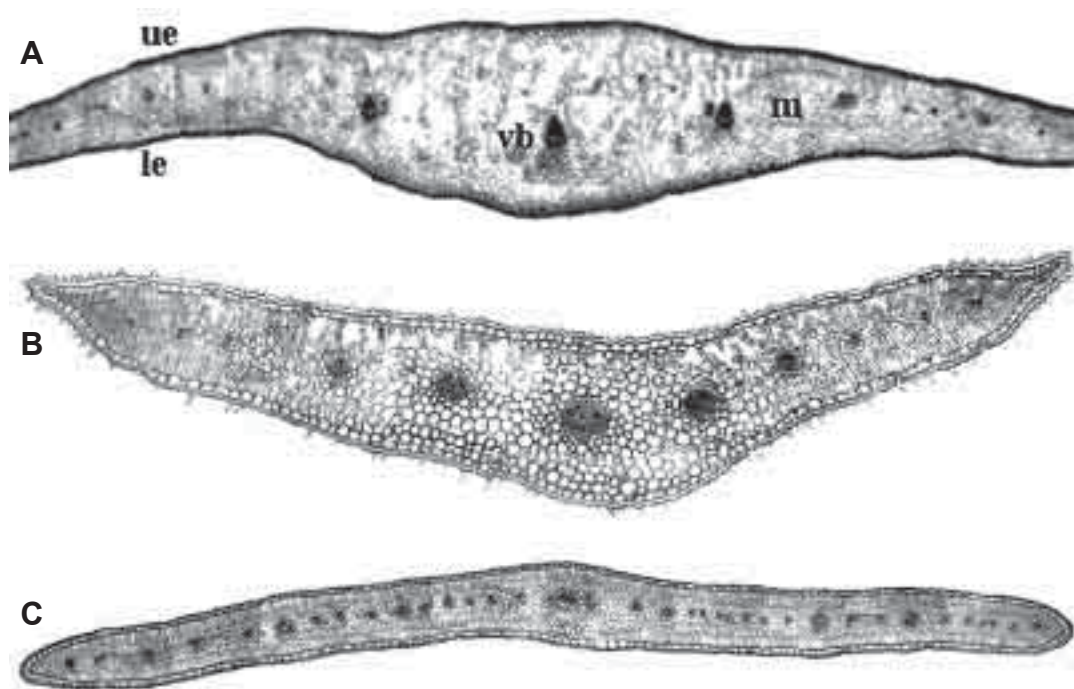


Figure 1. Cross section of the leaf blade: A – *Euphorbia myrsinites* (30×); B – *Euphorbia nicaeensis* subsp. *dobrozensis* (30×); C – *Euphorbia seguieriana* (40×). le – lower epidermis, m – mesophyll, ue – upper epidermis, vb – vascular bundles.

arrangement with few parenchymatous cells between vessels. The phloem is less developed than the xylem. Laticifers are present in all species, isolated or in groups of 2–3 (Fig. 3).

Just like in other studied *Euphorbia* species (GALEŠ & TOMA 2007), laticifers are mainly present in the phloem of the bundles but also around the vascular bundles. These more or less round-shaped secretory elements are non-articulated and non-branched and possess relatively thick cellulose walls (Figs 3; 4A, B).

Foliar epidermis is one of the most noteworthy taxonomic characters from the bio-systematic point of view. Therefore, taxonomic studies on Euphorbiaceae have been made based on leaf epidermises (STACE 1984).

Paradermal sections of the blade disclose for all three investigated species that the surface of the foliar blade has polygonal, generally straight-walled epidermal cells with stomata. The stomata cells are irregularly and evenly distributed on both surfaces in some cases and their axes are oriented in different directions (DILCHER 1974) (Fig. 4C).

According to METCALFE & CHALK (1950), stomata of European *Euphorbia* species are mostly of ranunculaceous (anomocytic) type. In our findings, anomocytic stomata are dominant with few anisocytic ones in *E. myrsinites* (Fig. 4A), whereas in *E. nicaeensis* subsp. *dobrozensis* more anisocytic and less anomocytic stomata (Fig. 4B) occur. In *E. seguieriana*, the epidermis has anisocytic and few anomocytic as well as staurocytic stomata, just as ESSİETT et al. (2012) reported for *E. hirta* and *E. heterophylla*.

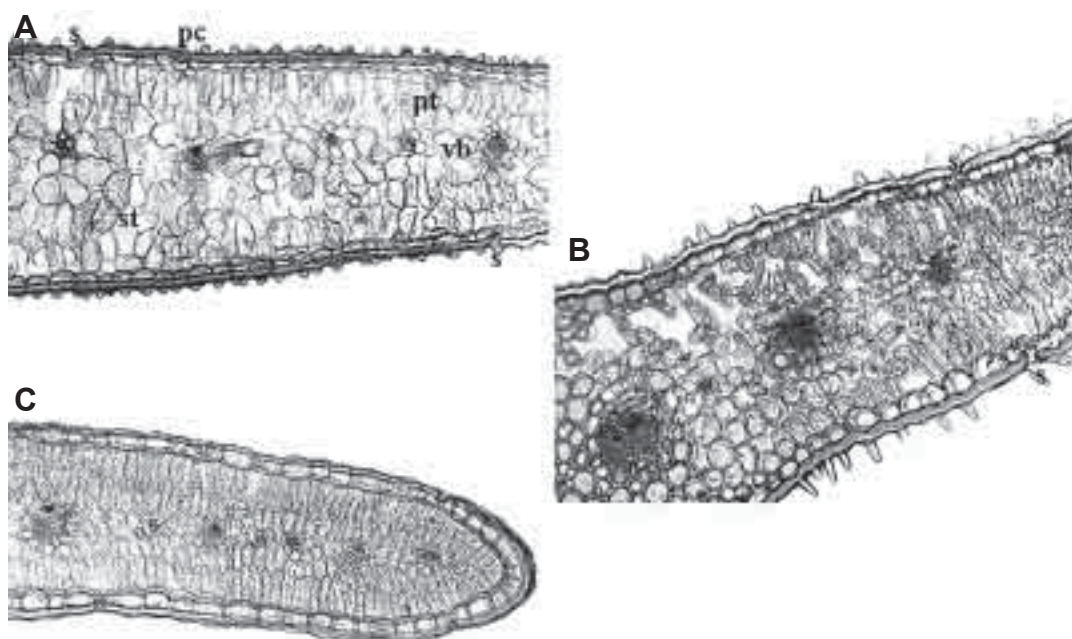


Figure 2. Parts of the leaf blade with mesophyll (detail): A – *Euphorbia myrsinites* (140×); B – *Euphorbia nicaeensis* subsp. *dobrozensis* (90×); C – *Euphorbia seguieriana* (200×). pc – cuticle papillae, pt – palisade tissue, s – stoma, st – spongy tissue, vb – vascular bundle.

Conclusions

The *Euphorbia* species studied show both similarities and differences concerning their anatomical structure. The blade of all three species exhibits a cuticle covering both epidermises, papillose in *E. nicaeensis* subsp. *dobrozensis* and *E. myrsinites* and non-papillose in *E. seguieriana*. Both epidermises possess stomata more abundant in the lower epidermis. The mesophyll is isobilateral and heterogeneous.

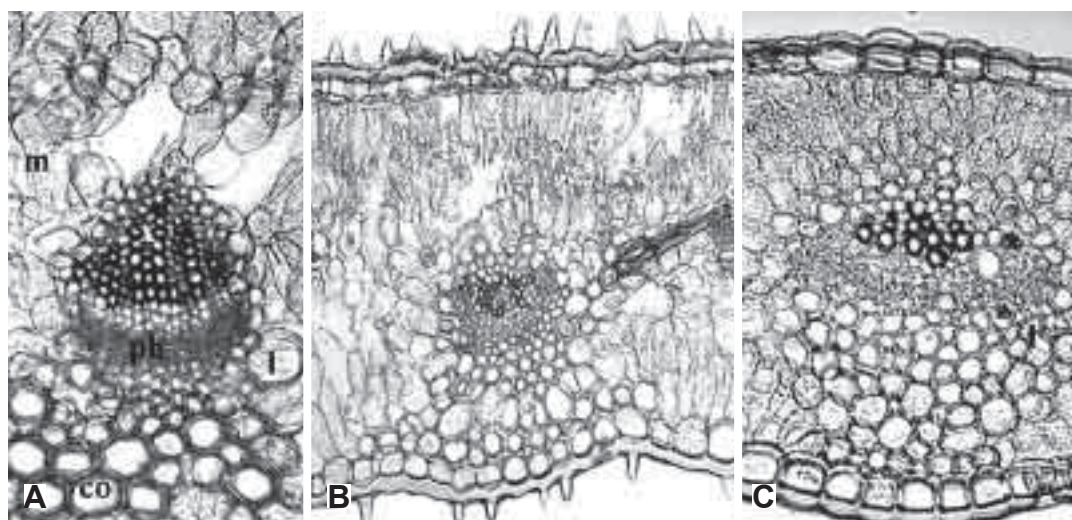


Figure 3. Cross section of midrib vein: A – *Euphorbia nicaeensis* subsp. *dobrozensis* (140×); B – *Euphorbia myrsinites* (300×); C – *Euphorbia seguieriana* (200×). co – collenchyma, l – laticifers, m – mesophyll, ph – phloem, x – xylem.

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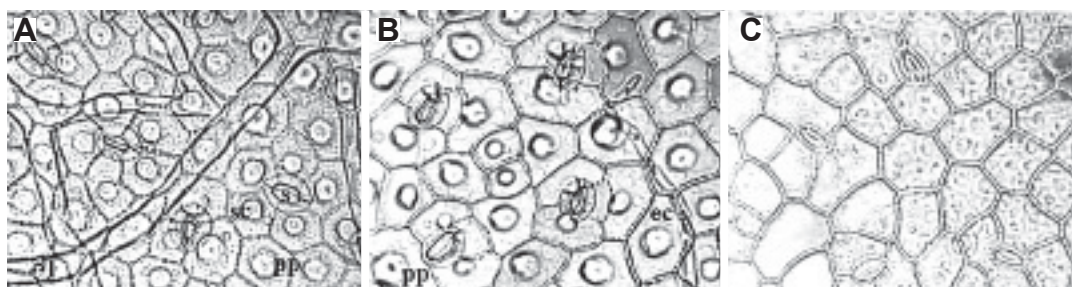


Figure 4. Paradermal sections of the lower epidermis: A – *Euphorbia myrsinites* (300×); B – *Euphorbia nicaeensis* subsp. *dobrozensis* (400×); C – *Euphorbia seguieriana* (480×). ec – epidermal cells, l – laticifers, s – stoma, sc – subsidiary cell, pp – prominent papillae.

The non-articulated laticifers are placed mainly in the main vein area in all studied species, but they are more abundant in *E. myrsinites*. The vascular system represented by many collateral bundles is more developed in *E. myrsinites*, followed by *E. nicaeensis* subsp. *dobrozensis*.

The mechanical tissue occurs only in the vascular bundles just below the lower epidermis in all studied species and is represented by groups of collenchyma cells.

Paradermal sections of the blade disclose straight-walled epidermal cells with different types of stomata.

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