ANATOMICAL CHARACTERISTICS OF *XERANTHEMUM* L. (COMPOSITAE) SPECIES: TAXONOMICAL INSIGHTS AND EVOLUTION OF LIFE FORM

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Abstract

Comparative anatomical and micromorphological analyses of root, stem, peduncle, leaf and inflorescence have been conducted on two *Xeranthemum* species, *X. annuum* and *X. cylindraceum*, by light microscopy (LM) and scanning electron microscopy (SEM). The main goal of the study was to examine the most important anatomical features and to find new valid taxonomic delimiting characters for the first time in both species. Regarding vegetative organs anatomy, the data obtained in this study indicated that both species possessed secondary tissues in the root, although these plants are annual. Also, stem anatomy was a typical of the Compositae family members, and anomocytic stomata type and dorsiventral leaf structure were present. On the involucral bracts surface crystals were noticeable, while highly developed multilayer sclerenchyma was present in the mesophyll. Palea anatomy was very similar to bract anatomy. Some floral features were as follows: lateral anther dehiscence, corolla composed of uniseriate epidermis and with a homogeneous parenchyma in the mesophyll, inferior ovary and anatropous ovule with basal placentation. The specific quantitative characters that were different between the examined species were emphasized. Some selected, well defined qualitative anatomical characters (e.g. shape of the young stem and peduncle cross-sections, type of glandular trichomes and cortical vascular bundles occurrence), on the basis of which the studied species were distinguishable, strengthen the taxonomy of the *Xeranthemum* genus and provide features for better identification of the taxa. This is the first study of the anatomy of *Xeranthemum* species and some of the characters found (secondary growth and dorsiventral leaves) points towards an adaptation of mesophilous ancestors to xeric habitats in *Xeranthemum*.

Key words: *Xeranthemum*, Cardueae, Anatomy, Micromorphology, Taxonomy.

Introduction

For more than a century, comparative anatomy is used as a tool in the plant systematic studies. Anatomical characters are very important in perceiving systematic and phylogenetic relationships of particular plant groups. Plant taxonomic studies traditionally use morphological and karyological (Stebbins, 1953), as well as micromorphological characters (pollen and trichomes) (Hayat *et al*., 2009; Bak & Ozcan, 2018). Indeed, anatomical features can provide useful characters which could help in identification of problematic plant taxa, as well as establishing their taxonomic relationships (Metcalfe & Chalk, 1957; Scatena *et al*., 2005; Makbul *et al*., 2011; Sosa *et al*., 2014; Karanović *et al*., 2015). According to Dengler (2002), anatomical data can also be useful in determination of the systematic status and evolutionary relation among the genera and species.

The large family Compositae counts around 1600 genera (23 000 species) with global distribution (Anderberg *et al*., 2007). Taxa belonging to this family are mostly annual or perennial herbaceous plants. Taxonomic investigations of Compositae based on anatomy are focused on aerial parts (e.g. seeds, pollen) (Wang *et al*., 2009), as well as on rhizome and root (Ginko *et al*., 2016). Some particular anatomical characteristics, which are shown to possess considerable taxonomic value within the family, are: presence of secretory and laticiferous ducts, types of glandular trichomes and non-glandular trichomes, occurrence of medullar and cortical vascular bundles, as well as anomalous secondary thickening (Metcalfe & Chalk, 1957).

The genus *Xeranthemum* L. is a member of the *Xeranthemum* group within subtribe Carduinae of the tribe Cardueae, together with *Amphoricarpos* Vis.*, Chardinia* Desf.*, Siebera* J. Gay (Susanna & Garcia-Jacas, 2009) and the newly described genus *Shangwua* Yu J. Wang, Raab-Straube, Susanna & J. Quan Liu (Wang *et al*., 2013). The genera of the group possess very characteristic pappus of the cypselae and the papery silver-white bracts of the capitulum. The group is relatively well characterized regarding morphological and molecular characters (Susanna & Garcia-Jacas, 2007), and phylogenies based on molecular markers confirm that it is a natural group (e.g., Barres *et al*., 2013).

Xeranthemum comprises five annual taxa which grow in Southern Europe, North Africa and SW Asia (Garnatje & Martín, 2007). We have chosen two species, which grow in Serbia and have different chromosome numbers (Garnatje *et al*., 2004a), *Xeranthemum annuum* L. and *X. cylindraceum* Sm. These taxa grow on similar arid habitats (steppes) and the low mountains in the Eastern Mediterranean and Asia Minor (Gajić, 1975; Garnatje *et al*., 2004a). Morphologically, *X. annuum* and *X. cylindraceum* differ in the size of capitulum (30–50 mm and 8–15 mm in diameter, respectively), outer involucral bracts (mucronate, glabrous and obtuse or emarginate in *X. annuum*; with a whitish patch of appressed hairs in the center of lower surface in *X. cylindraceum*), inner involucral bracts [17–25 mm, oblong, patent, bright pink (rarely white) and 10–13 mm, pink, suberect, respectively], number of fertile florets (70–120 and 10–15, respectively), number of the pappus scales (5 and 10–15, respectively), as well as the length of the pappus scales (about equaling cypsela and very unequal, shorter than cypsela, respectively; cf. Webb, 1976).

Xeranthemum genus shows the great cytogenetic diversity (*X. annuum* with 2n = 12, *X. squarrosum* Boiss. with 2n = 12, *X. longepapposum* Fisch. and C. A. Mey with 2n = 14, *X. cylindraceum* with 2n = 20 and *X. inapertum* Mill. with 2n = 28) (Garnatje *et al.*, 2004a).

There are many papers dealing with phytochemical investigations (Valant-Vetschera & Wollenweber, 2007; Zemtsova & Molchanova, 1979; Samek *et al.*, 1977; Powell *et al.*, 1967; Hîbel *et al.*, 1982; Schwind *et al.*, 1990; Skaltsa *et al.*, 2000; Dekić *et al.*, 2015), biological effect (Stanković *et al.*, 2011), flower ontogeny (Dadpour *et al.*, 2012), palynology (Garnatje & Martín, 2007), involucral bracts and petals micromorphology (Gavrilović *et al.*, 2017) and molecular cytogenetics (Garnatje *et al.*, 2004a) of *Xeranthemum*. Kartal (2016), within the style and ovary cells of *X. annuum*, found styloid crystals. There is no previous report about anatomy of vegetative and reproductive organs of *Xeranthemum* species. Also, remaining genera of the *Xeranthemum* group are almost unexplored from anatomical point of view.

Some particular floral anatomical and morphological characters are useful for the classification of the taxa within the Compositae tribes, e.g. pappus form, branched style, anatomy and morphology of the corolla and anthers (Judd *et al.*, 2002). Historically, Compositae represents difficulties for ontogenetic studies (Dadpour *et al.*, 2012), and the available data regarding floral and inflorescence anatomy is still scarce. Batista & De Souza (2017) studied the floral ontogeny of ten Compositae species from Brazil and showed the usefulness of the flower characters in distinguishing species. Also, Franca *et al.*, (2015) investigated embryology of two *Ageratum* L. species and confirmed heterogeneity of embryological processes within family. Embryological data are constant at the genus level and thus could be useful in perceiving taxonomic relation among families, or between genera and taxa (Palser, 1975; Stuessy, 2009). Dadpour *et al.*, (2012) showed substantial differences in flower morphology and development between female and perfect florets of *X. squarrosum*. Harris (1995) carried out a detailed study on florets and inflorescences development of 39 taxa (representing 12 tribes) of Compositae, including *X. annuum*. A comprehensive work on development of *X. annuum* inflorescence, disk and ray flower is supported with detailed SEM micrographs in Harris (1995).

Anatomical investigations of *X. annuum* and *X. cylindraceum* have not been conducted so far. Therefore, the current study aims to examine vegetative and reproductive organs anatomy of these two species in order to introduce the important anatomical features and to find new valid taxonomic characters, which may strengthen the taxonomy of the genus and provide features for better identification of the taxa. Also, examined features could provide guidelines for possible new delimitating characters for other genera of the *Xeranthemum* group.

Material and Methods

Plant material: Plant material (parts of roots, stems, peduncles, leaves and inflorescences), from 5 individuals per species, was collected during the flowering period (more or less identical ontogenetic stage) in 2016, from the locality Iron Gates (Serbia): *X. annuum* (N 44.65780; E 22.54079) and *X. cylindraceum* (N 44.61238; E 22.50830) and kept in 50% ethanol. Voucher specimens (accession numbers: BEOU 17280 and BEOU 17281) were deposited in the University of Belgrade - Faculty of Biology, Herbarium of the Institute of Botany and Botanical Garden "Jevremovac".

Anatomical methods: Temporary and permanent slides of mature roots, stems, leaves, peduncles and inflorescences of adult plants were prepared. Plants parts were sectioned fresh or fixed (50% ethanol) before preparation for a standard paraffin method (Ruzin, 1999). Handmade cross-sections of fresh plant material were performed using sharp razor blades. Some temporary slides (stem and leaf) were stained with Toluidine blue (0.05% w/v, aqueous) or with Lugol solution. Leaf blade epidermal prints were made using transparent varnish and adhesive tape. Paraffin method was applied for preparing cross-sections (8–10 μm thick) of middle parts of mature roots, stems, leaves, peduncles and inflorescences as well as longitudinal sections of inflorescences. Sections were double stained in Safranin O (1%, w/v, 50% ethanol) and Alcian blue (1% w/v, aqueous) and then were mounted on slides using Canada balsam. The permanent slides were preserved in the Department of Morphology and Systematics of Plants, University of Belgrade - Faculty of Biology. Observations and measurements were taken on a light microscope Leica DM2000 with a digital camera Leica DFC320 and using a computer with the imaging and measurement software Leica IM 1000.

Micromorphological methods: Micromorphological analysis of both species was carried out using scanning electron microscopy (SEM). Small parts of dry leaves were sputter-coated with gold for 180 s at 30 mA (BAL-TEC SCD 005), and observed using a JEOL JSM-6460LV electron microscope at an acceleration voltage of 20 kV.

Data analysis: All measurements data are presented as the means ± standard deviations. Statistical analyses were done by Microsoft Excel software.

Results

Root anatomical characteristics: The cross-sections of the root of both investigated species were shown in Fig. 1. Although these plants are annual, secondary tissues were noticed in the cross-sections of both species [especially a well-developed xylem (Fig. 1C-D)]. A multilayered exodermis was present on the surface of the root of both species (Fig. 1A-B). Below exodermis, a reduced cortex (due to the presence of secondary structure) was present (Fig. 1C-D). Also, phloem and some sclerenchyma fibers were below cortex. The dominant part of the root cross-sections was the secondary xylem composed of vessels and tracheids (Fig. 1C-D).

Important quantitative characters were average thickness of the exodermis and average diameter of the main root and xylem. The exodermis thickness for *X. annuum* was 198.0 μm for the main root and 92.0 μm for the lateral root, while for *X. cylindraceum* these values were 294.9 μm and 131.2 μm, respectively. Main root diameter and xylem diameter also differed among species, 1390.5 μm and 831.7 μm for *X. annuum* and 1930 μm and 1212.1 μm for *X. cylindraceum*, respectively (Table 1).
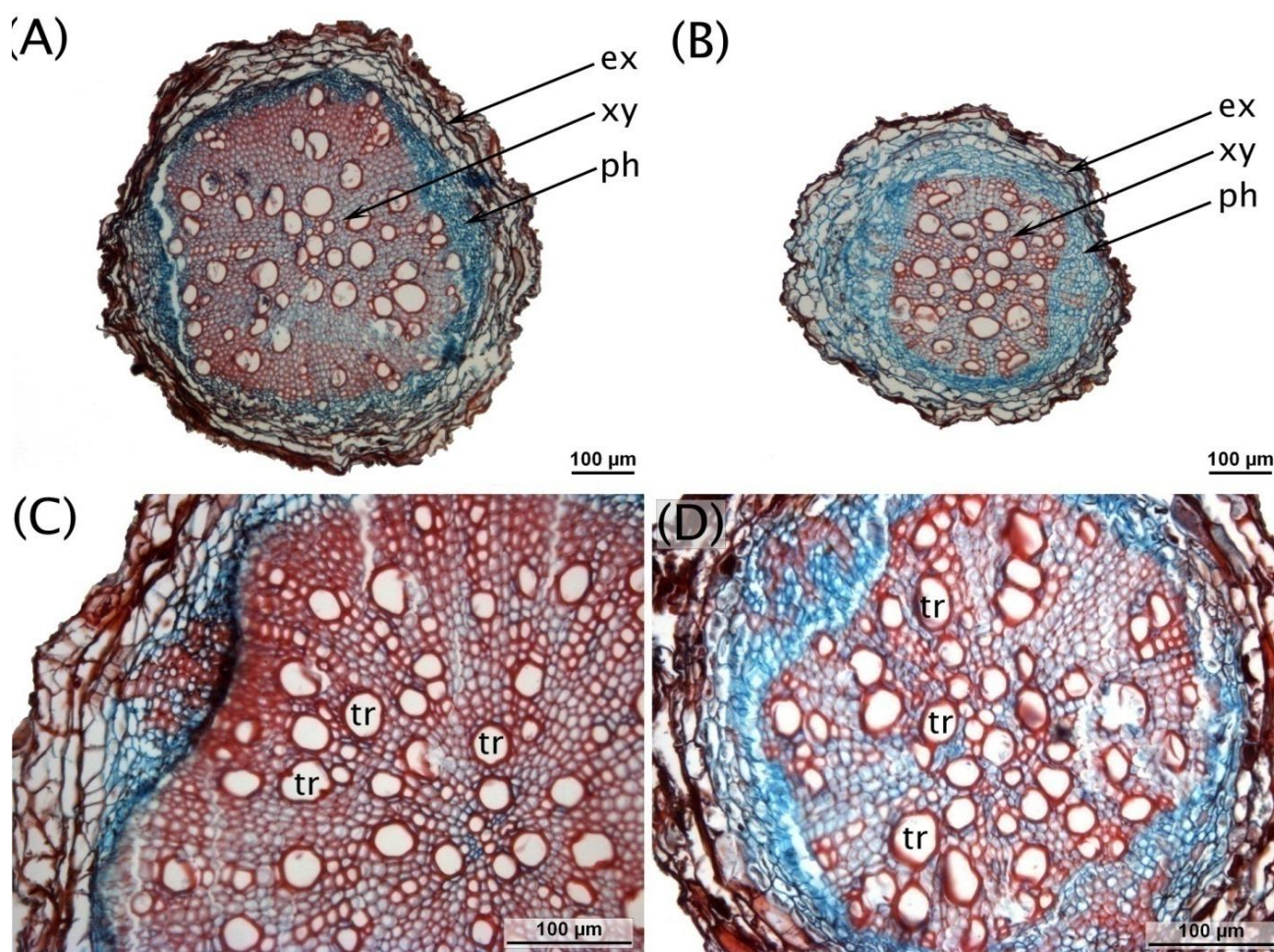
Fig. 1. Light micrographs of the root cross-sections of *Xeranthemum*. Root cross-sections of *X. annuum* (A). Root cross-sections of *X. cylindraceum* (B). Detail of root cross-section of *X. annuum* (C) and *X. cylindraceum* (D) illustrating multilayered exodermis and well developed xylem. Abbreviations: ex = exodermis, ph = phloem, xy = xylem, tr = tracheas. All slides are permanent, stained with Safranin O and Alcian blue.

Stem anatomical characteristics: The cross-sections of the stem of the investigated species were shown in Fig. 2. Young stem cross-sections of *X. annuum* had five pronounced ribs (Fig. 2A). Later, shape of the stem cross-sections became more or less round or polygonal (Fig. 2C). In contrast, young stem cross-sections were pentangular in *X. cylindraceum* (Fig. 2B), which later also became more regular and rounded (Fig. 2D).

One-layered epidermis, composed of oval to isodiametric cells, covered with a thick cuticle, was present on the surfaces of both species (Fig. 2E-F). In both investigated species, there were numerous multicellular non-glandular, uniseriate, curly trichomes, with one or two cells forming a basal part of the trichome, in which cell content could be noticed, and a transparent, elongated, twisted peak cell (Fig. 2G). The glandular trichomes found in *X. annuum* were biseriate (Fig. 2G), whereas *X. cylindraceum* possessed capitate sessile trichomes with secretory reservoir covering almost the entire trichome (Fig. 2H).

The cortex of the stem was composed of collenchyma and chlorenchyma, which were arranged alternately. In *X. annuum*, there were up to 8 layers of cortex, in contrast to 3–4 layers in *X. cylindraceum* (Fig. 2A-B). Prominent ribs

contained collenchyma tissue, whereas chlorenchyma was present between the ribs (Fig. 2A-B, F). Chlorenchyma cells were arranged in 2–4 rows. Only in *X. annuum* we found presence of cortical vascular bundles (Fig. 2A). A clearly visible endodermis layer separated cortex from the central cylinder (Fig. 2F). Medullary vascular bundles were in one circle (Fig. 2C-D). Sclerification of the central cylinder was seen due to sclerenchyma tissue and well developed xylem formed a thick ring (Fig. 2C-D). In the central cylinder 10–12 collateral vascular bundles were observed in *X. annuum* (Fig. 2C) and 10–15 in *X. cylindraceum* (Fig. 2D). Each vascular bundle consisted of a less developed phloem and well developed xylem. Well lignified sclerenchyma was above the phloem and in some cases sclerenchyma almost completely surrounded bundles (Fig. 2D-E). Moreover, parenchyma cells, in the perimedullar zone, between the bundles, were with thickened walls (Fig. 2D-F). A large pith parenchyma cells was present in the central region (Fig. 2D-E).

Selected above-mentioned qualitative characters and their states of both examined species were shown in Table 4. Among important results of measurements was average stem diameter, which was 1709.6 μm for *X. annuum* and 1316.9 μm for *X. cylindraceum* (Table 2).
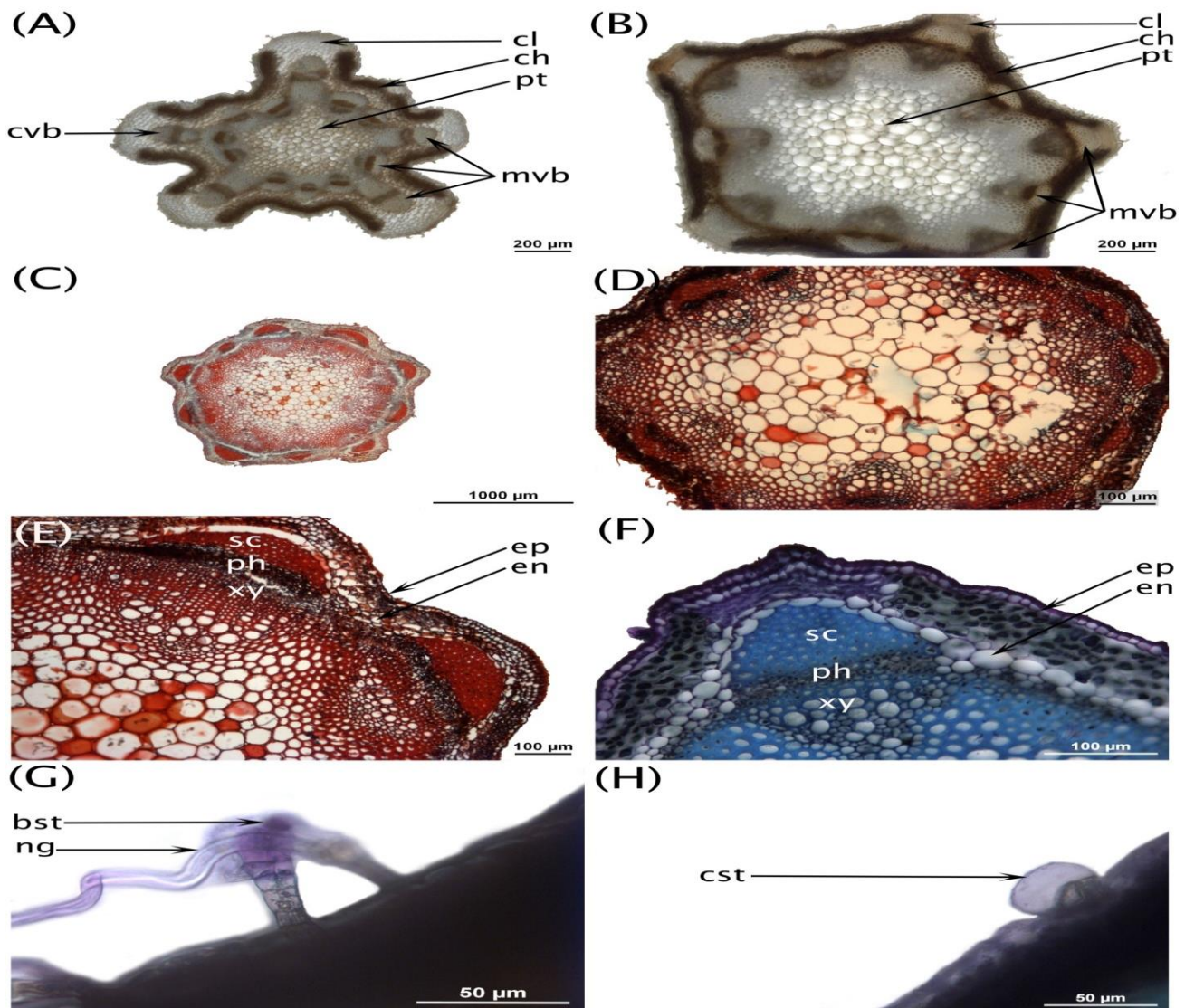
Fig. 2. Light micrographs of the stem cross-sections of *Xeranthemum*. Young stem cross-sections of *X. annuum* with five pronounced ribs (A). Young stem cross-sections of *X. cylindraceum* - pentangular shape (B). Polygonal old stem cross-sections of *X. annuum* and *X. cylindraceum*, respectively (C), (D). Detail of stem cross-sections showing collenchyma alternates with chlorenchyma of *X. annuum* and *X. cylindraceum*, respectively (E), (F). Non-glandular and biseriate glandular trichomes of *X. annuum* (G) and capitate sessile glandular trichome of *X. cylindraceum* (H). Abbreviations: cl = collenchyma, ch = chlorenchyma, pt = pith, cvb = cortical vascular bundle, mvb = medullary vascular bundles, ep = epidermis, en = endodermis, sc = sclerenchyma, ph = phloem, xy = xylem, ng = non-glandular trichome, bst = biseriate glandular trichome, cst = capitate sessile glandular trichome. (A), (B) temporary noncoloured slides, (C), (D), (E) permanent slides stained with Safranin O and Alcian blue, (F), (G), (H) temporary slides stained with Toluidine blue.

Peduncle anatomical characteristics: The cross-sections of the peduncle of the investigated species were shown in Fig. 3. Anatomy of the peduncle was similar to the stem anatomy. The peduncle cross-section was polygonal in shape, with more pronounced ribs in *X. annuum* (Fig. 3A), and pentangular in *X. cylindraceum* (Fig. 3B). Few small cortical vascular bundles were present only in *X. annuum* cortex (Fig. 3A). A variable and different number of medullar vascular bundles, arranged in a circle, were recorded: 15–30 in *X. annuum* and 10–12 in *X. cylindraceum* (Table 2).

Selected above-mentioned qualitative characters and their states of both examined species were shown in Table 4. The average value of peduncle diameter for *X. annuum* was almost twice as for *X. cylindraceum* (1813.3 μm and 913.9 μm, respectively) (Table 2). The average thickness of cortex between ribs was important difference between species, 130.5 μm and 71.5 μm, as well as beneath ribs, 106.2 and 58.3 μm, respectively (Table 2).

Leaf anatomical characteristics: The cross-sections of the leaf, leaf blade epidermal prints and SEM micrographs were shown in Figs. 4, 5 and 6, respectively. Epidermal cells of both studied taxa were polygonal and with irregular shape with sinuous anticlinal walls and ribbed thickenings (Fig. 5C-D). In both studied species, the cells of upper epidermis were visibly larger compared to the cells of lower epidermis (Fig. 4C-D). Thus the average thickness of the adaxial epidermis in *X. annuum* was 17.8 μm and 12.6 μm in *X. cylindraceum*, compared with the average thickness of the abaxial epidermis, 10.3 μm and 8.0 μm, respectively. The abaxial epidermis was also one-layered and covered with a thinner cuticle comparing to adaxial epidermis.
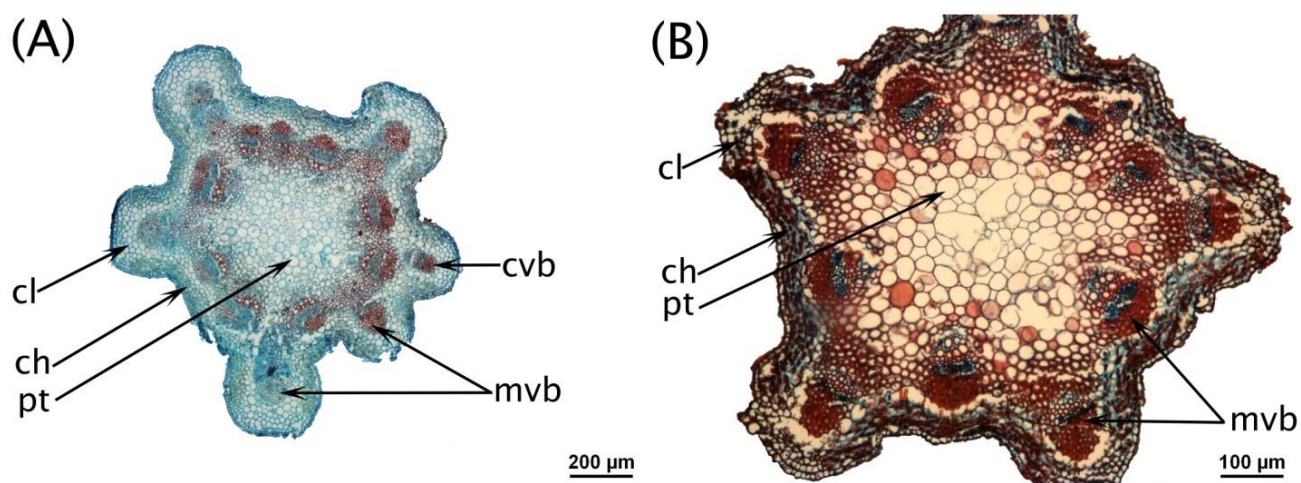
Fig. 3. Light micrographs of the peduncle cross-sections of *Xeranthemum*. *X. annuum*, showing presence of cortical vascular bundle (A) and *X. cylindraceum* (B). Abbreviations: cl = collenchyma, ch = chlorenchyma, pt = pith, cvb = cortical vascular bundle, mvb = medullary vascular bundles. All slides are permanent, stained with Safranin O and Alcian blue.

Table 1. Root anatomical characteristics of *Xeranthemum*. Data displayed as mean values ± standard deviations.

	X. annuum	*X. cylindraceum*
Main root		
Exodermis thickness (μm)	198.0 ± 64.5	294.9 ± 57.5
Root diameter (μm)	1390.5 ± 477.6	1930.1 ± 673.8
Xylem diameter (μm)	831.7 ± 320.6	1212.1 ± 346.2
Single vessel diameter (μm)*	41.6 ± 4.6	43.1 ± 6.9
Lateral root		
Exodermis thickness (μm)	92.0 ± 28.5	131.2 ± 26.0
Root diameter (μm)	526.1 ± 180.1	570.0 ± 216.9
Xylem diameter (μm)	342.2 ± 143.4	341.1 ± 185.3
Single vessel diameter (μm)*	33.30 ± 9.5	28.50 ± 6.2

*Five largest vessels are measured

Table 2. Stem and peduncle anatomical characteristics of *Xeranthemum*. Data displayed as mean values ± standard deviations.

	X. annuum	*X. cylindraceum*
Stem		
No. of vascular bundles	10–12	10–15
Single vessel diameter (μm)*	31.70 ± 4.3	22.60 ± 4.1
Stem diameter (μm)	1709.6 ± 162.0	1316.9 ± 289.9
Cortex thickness beneath ribs (μm)	56.5 ± 14.80	46.7 ± 19.0
Cortex thickness between ribs (μm)	70.7 ± 14.20	64.6 ± 17.1
Peduncle		
No. of vascular bundles	15–30	10–12
Single vessel diameter (μm)*	14.0 ± 4.1	10.6 ± 1.2
Peduncle diameter (μm)	1813.3 ± 513.5	913.9 ± 133.0
Cortex thickness beneath ribs (μm)	106.2 ± 38.7	58.3 ± 13.2
Cortex thickness between ribs (μm)	130.5 ± 64.0	71.5 ± 18.9

* Five largest vessels are measured

Table 3. Leaf anatomical characteristics of *Xeranthemum*. Data displayed as mean values ± standard deviations.

	X. annuum	*X. cylindraceum*
Younger leaf thickness (μm)		
Leaf blade between veins	137.7 ± 60.1	119.1 ± 11.8
Leaf blade in the zone in the midvein	299.0 ± 21.6	297.5 ± 55.0
Adaxial epidermis	17.8 ± 8.0	12.6 ± 3.0
Palisade tissue	42.7 ± 18.5	40.6 ± 5.5
Spongy tissue	66.8 ± 35.0	57.9 ± 11.3
Abaxial epidermis	10.3 ± 4.5	8.0 ± 2.4
Older leaf thickness (μm)		
Leaf blade between veins	126.9 ± 24.4	124.5 ± 14.0
Leaf blade in the zone in the midvein	317.7 ± 115.5	380.0 ± 78.8
Adaxial epidermis	17.3 ± 4.7	12.7 ± 2.8
Palisade tissue	41.9 ± 9.9	43.9 ± 7.2
Spongy tissue	57.9 ± 16.0	60.2 ± 13.5
Abaxial epidermis	9.7 ± 3.2	7.7 ± 2.0

Table 4. Qualitative characters of leaf, stem and peduncle of *Xeranthemum*.

		X. annuum	*X. cylindraceum*
Leaf			
Leaf structure	Dorsiventral	+	+
Anticlinal epidermal cells walls	Sinuous	+	+
Anomocytic stomata		+	+
Non-glandular trichomes	Curly	+	+
Glandular trichomes	Biseriate	+	−
	Capitate sessile	−	+
Palisade tissue	Compact	+	+
Bundle sheath expanding to epidermis		+	+
Arrangement of xylem tissue in the main vein	Regular	+	+
Prominent main vein		+	+
Stem and peduncle			
Shape of young stem and peduncle	Pentangular	−	+
	With five ribs	+	−
Shape of old stem and peduncle	Polygonal	+	+
Epidermal cells	Isodiametric	+	+
Non-glandular trichomes	Curly	+	+
Glandular trichomes	Biseriate	+	−
	Capitate sessile	−	+
Collenchyma	In the ribs	+	+
Chlorenchyma	Compact	+	+
Presence of cortical bundles		+	−

+ = Present, − = Absent

The leaf blades were amphistomatous, with anomocytic type of stomata (Fig. 5A-D). Epidermal cells around stomata were 4–6 (Fig. 5C-D). Densely distributed non-glandular trichomes, described earlier for the stem, were noticed at both sides on epidermis of both species, but more on adaxial side (Fig. 4E). Rare glandular trichomes were found on the surface of both species, but with different morphology, like on the stem surface. The glandular trihomes found in *X. annuum* were biseriate (Fig. 4G), compared to the capitate sessile trichomes in *X. cylindraceum*, located in epidermal depressions (Fig. 4H). SEM analysis of investigated species confirmed the presence of multicellular, uniseriate, non-glandular hairs, as well as two types of multicellular glandular trichomes (Fig. 6A-D).

The leaf blade of both species had dorsiventral structure (Fig. 4C-D). Below adaxial epidermis, the palisade tissue consisted of large rich in chloroplasts cells, arranged in one layer and spongy tissue, composed of several layers of polygonal cells, which possessed less chloroplasts and large intercellular spaces. In the central leaf blade plane collateral closed vascular bundles could be noticed in a row, surrounded by parenchyma tissue (Fig. 4C-D). On the cross-sections the main vein of both studied species had a heart shape with two ribs (Fig. 4A-B). One vascular bundle was seen in the main vein, with a surrounding parenchyma sheath which extended to both epidermises (Fig. 4A-B). Subepidermal collenchyma tissue could be seen on the adaxial side, while on the abaxial side it alternated with chlorenchyma (Fig. 4A-B). Well developed sclerenchyma surrounded vascular bundles.

Selected above-mentioned qualitative characters and their states of both examined species were shown in Table 4. The average thickness of the younger leaf blade between veins was 137.7 μm in *X. annuum* and 119.1 μm in *X. cylindraceum*, while the average thickness of the older leaf blade in the zone in the midvein was 317.7 μm in *X. annuum* and 380.0 μm in *X. cylindraceum* (Table 3).

Inflorescence anatomical characteristics: The cross and longitudinal sections of the inflorescence were shown in Fig. 7. On the surface of the involucral bracts of both examined species, crystals were noticeable (Fig. 7A-B). On the central part of the *X. cylindraceum* involucral bracts indumentum could be seen, while crystals were present outside of the epidermal cells, on the periphery (Fig. 6B). Below one-layer epidermis, a one-layer hypodermis and a highly developed multilayer sclerenchyma were observed (Fig 7A-B). Below sclerenchyma, a loose parenchyma tissue with vascular bundles was present.

On the inflorescence cross-sections, numerous florets could be seen at different stages of development subtended by a bract, called palea. Palea anatomy was similar to involucral leaf anatomy (Fig. 7C-D). In the early stages of development, only the meristematic tissue could be observed, and through the various stages of the development of individual flowers, the formation of stamens and pollen were noticeable (Fig. 7D). Anther dehiscence were considered to be lateral (Fig. 7D). The corolla was composed of uniseriate epidermis and the mesophyll consisted of parenchyma with collateral vascular bundles. Longitudinal sections of both examined species showed an inferior ovary. The ovule was anatropous with basal placentation (Fig. 7E-F). Embryo and pericarp wall as well as pollen grains in the stamens were clearly seen (Fig. 7E-F).

Discussion

The occurrence of the root secondary tissues of both studied species seems to be an unusual, since *Xeranthemum* taxa are annual and secondary growth in an annual taxon is an exception (Sidhu & Saini, 2011). Fritz & Saukel (2011) also investigated anatomy of underground plant parts of some medicinally important species and noticed the secondary phloem. The exact reason why *Xeranthemum* species form secondary tissues in the root is not clear. The initiation of root secondary growth could be considered as a change of life habit from annual to perennial, because when the roots gained viability, it could give rise to new shoots in the upcoming season (Sidhu & Saini, 2011). However, secondary growth was not observed in the stem of both investigated taxa. Thus, only root showed the secondary tissues. However, there is an alternate hypothesis: the change may have been in the opposite direction, from perennial to annual. In the *Xeranthemum* group, *Amphoricarpos* and *Shangwua* species grow in the Caucasus and East-Mediterranean mountains (Susanna & Garcia-Jacas, 2009) and the Qinghai-Tibetan Plateau and Himalayas (Wang *et al*., 2013), respectively, while the taxa from the other annual genera, *Xeranthemum*, *Chardinia* and *Siebera*, grow in open steppes in the Near and Middle East. Previous works have suggested that the ancestors of *Xeranthemum* and related

genera are *Shangwua* and *Amphoricarpos*, both of them are perennials from conservative habitats (mountains). Our favored hypothesis is that the annual habit in *Xeranthemum*, *Chardinia* and *Siebera* is a secondary adaptation to arid climates from mesophilous perennial ancestors. Adaptation is not complete and secondary growth is still present in the roots despite the old date of the separation of *Xeranthemum* from *Amphoricarpos* in the Oligocene (ca. 27 million years ago) according to Barres *et al*., (2013). Also, this hypothesis is supported by Garnatje *et al*., (2004b) which stated that, based on morphological and other characters, *Xeranthemum* is closely related to the: *Amphoricarpos*, *Chardinia* and *Siebera*. The genus *Amphoricarpos* based on molecular phylogeny, is considered as basal genus to *Chardinia*, while *Chardinia* is considered as basal to the other genera of the group (Garnatje *et al*., 2004a).

Stem anatomy of the examined species was typical one described for the Compositae (Metcalfe & Chalk, 1957). Gajić (1975) stated that the stem of *X. annuum* was triangular, while we showed that young stem had typically five clearly pronounced ribs. In addition, young stem of *X. cylindraceum* was pentangular. This character (shape of the young stem cross-section) could be important for taxonomy. The anatomical characters of the peduncle could also be of taxonomic importance. In *X. annuum*, only, we observed several cortical vascular bundles in the cortex parenchyma (Fig. 2A). Occurrence of cortical and medullar vascular bundles in the stems and peduncules, were also recorded as one of the main anatomical characteristics for the *Centaurea* spp. (Metcalfe & Chalk, 1957), as documented in *Centaurea sadleriana* Janka (Luković *et al*., 2013). Collateral cortical vascular bundles were also found in the stem of *Ianthopappus corymbosus* (Less.) Roque & D.J.N. Hind from the Compostae tribe Mutisieae (Melo-de-Pinna and Menezes, 2002). According to Metcalfe and Chalk (1950) the occurrence of medullar and cortical bundles is of significant taxonomic value.

Leaf blade characters are highly significant for taxonomy (Cilliers & Kruger 1993; Milan *et al*., 2006), especially leaf blade epidermis characters (Adedeji & Jewoola, 2008; Karanović *et al*., 2015). According to Pătrut *et al*., (2005), cuticle ribbed thickenings, which we documented on the upper epidermis surfaces of both studied species, were an adaptation for reducing evapotranspiration. Indumentum features (morphology, distribution and density of glandular and non-glandular trichomes and stomata characteristics) represent valuable characters in taxonomy (Karanović *et al*., 2015). Most members of the Carduinae have a woolly indumentum on both leaf surfaces or on the abaxial side (Häffner, 2000), which were also the case in our studied species. Four stomata types were reported in the Compositae: anomocytic, brachyparacytic, anisocytic and diacytic (Adedeji & Jewoola, 2008), which are surrounded by typical epidermal cells or by subsidiary cells. Freire *et al*., (2007) investigated epidermal characters of the genus *Baccharis* L. and found six types of stomata: anomocytic, anisocytic, cyclocytic, actinocytic, tetracytic and staurocytic. The data obtained in our study indicated that both *Xeranthemum* species possessed the same stomata type - anomocytic (Fig. 5A-D).
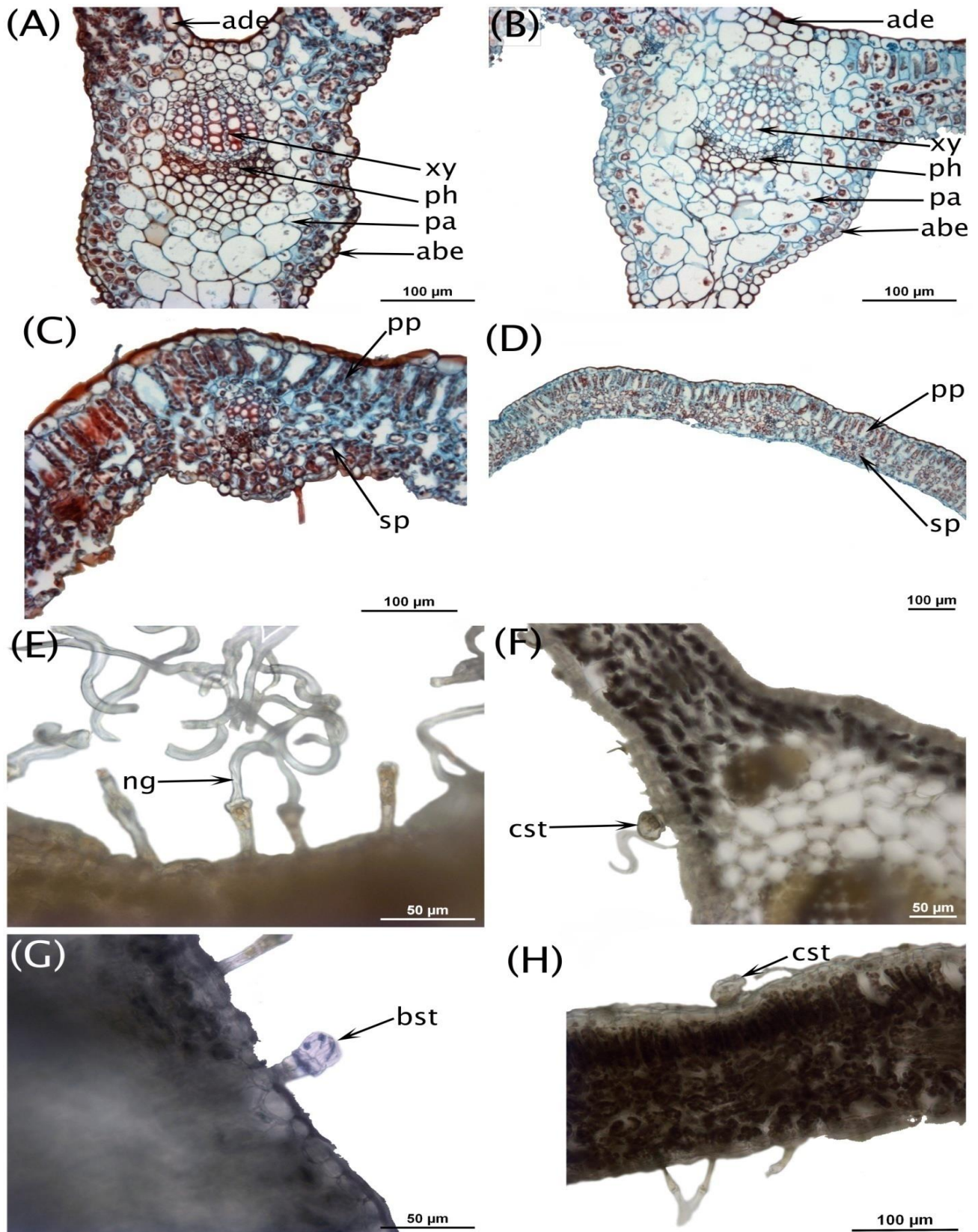
Fig. 4. Light micrographs of the leaf cross-sections of *Xeranthemum*. *X. annuum* (A) and *X. cylindraceum* (B) cross-sections in the main vein region showing one vascular bundle. Lateral side of leaf of *X. annuum* (C) and *X. cylindraceum* (D), showing dorsiventral leaf structure. Numerous non-glandular trichomes of *X. annuum* (E). Non-glandular and capitate sessile glandular trichomes of *X. cylindraceum* (F). Biseriate glandular trichome of *X. annuum* (G) and capitate sessile glandular trichomes of *X. cylindraceum* (H) . Abbreviations: ade = adaxial epidermis, ph = phloem, xy = xylem, pa = parenchyma, abe = abaxial epidermis, pp = palisade parenchyma, sp = spongy parenchyma, ng = non-glandular trichome, bst = biseriate glandular trichome, cst = capitate sessile glandular trichome. (A), (B), (C), (D) permanent slides stained with Safranin O and Alcian blue, (E), (F), (H) temporary slides stained with Lugol solution, (G) temporary slide stained with Toluidine blue.
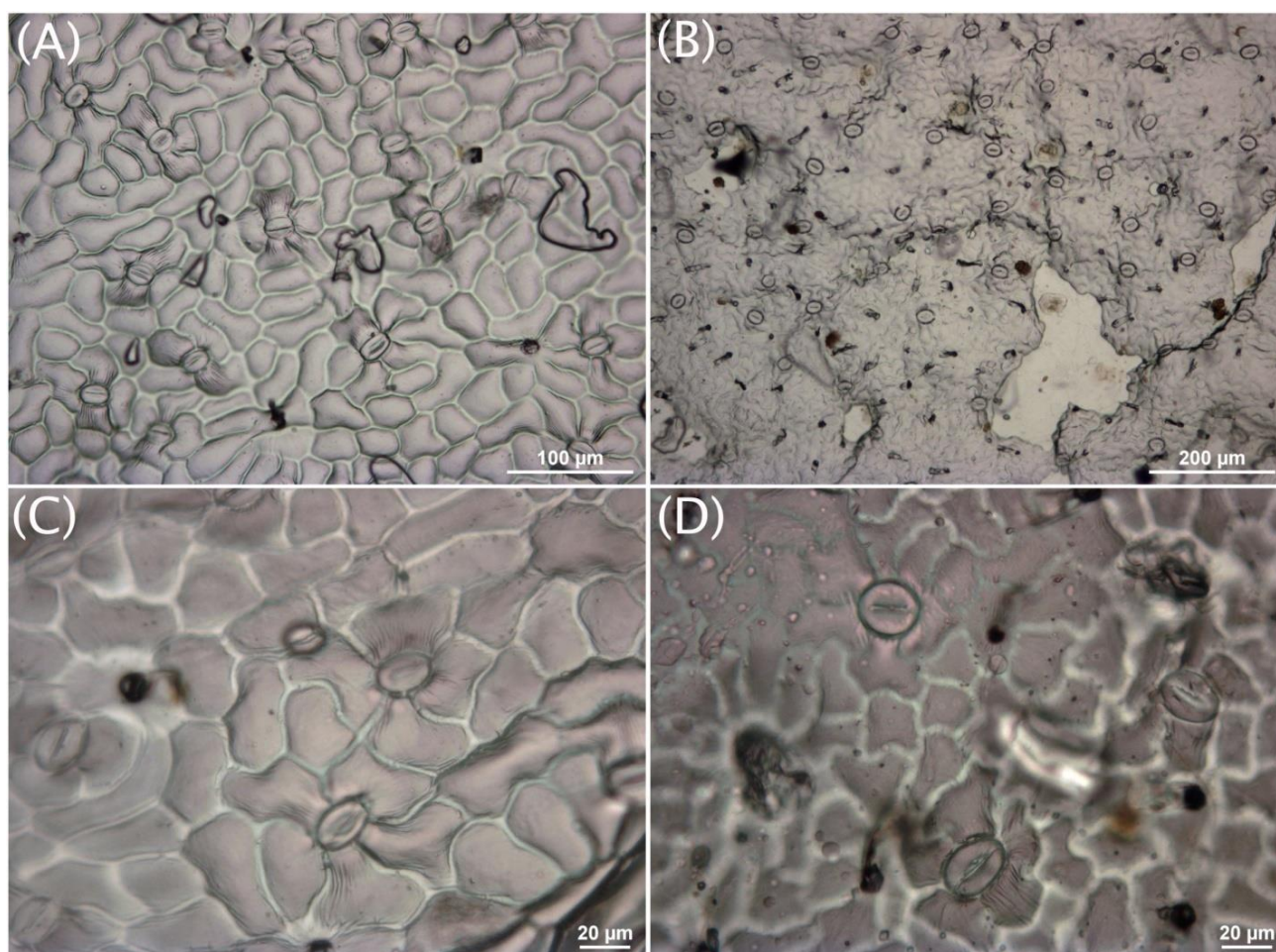
Fig. 5. Light micrographs of adaxial epidermis of *Xeranthemum*. Frontal view of adaxial epidermis, showing anomocytic stomata type, ribbed thickening and sinuous anticlinal walls in *X. annuum* (A), (C) and in *X. cylindraceum* (B), (D)

Cavities, ducts, idioblasts, as well as glandular trichomes were observed in and on the leaves of many Compositae members (Milan *et al*., 2006; Duarte *et al*., 2011; Camilotti *et al*., 2014). Essential oils, resins, lipids, alkaloids, sesquiterpene lactones, tannins, pectin-like substances and flavonoids are the dominant products of glands and ducts (Bartoli *et al*., 2011). Most Cardueae taxa have only laticifers in the aerial parts or have no secretory organs, e.g. *Xeranthemum*, *Siebera*, *Chardinia*, *Cardopatium* Juss. etc. (Dittrich, 1997). Morphology and distribution of secretory structures are of high taxonomic importance.

Classification of trichomes is very difficult due to high morphological diversity and microstructure, different origin and location, as well as capability and different mode of secretion (Werker *et al*., 1985). Trichomes could differ in cell numbers, arrangement, shape and length (Werker, 2000). Thus, more than 300 types of trichomes were described (Spring, 2000). Despite this enormous heterogeneity in morphology, often a indumentum similarity observed between related genera also resembles these genera in other characters (Stebbins, 1953). Types of trichomes are important characters, particularly at lower taxonomic levels (Stebbins, 1953, Faust & Jones, 1973; Sahu, 1982; Korolyuk, 1997; Krak & Mráz, 2008). The results obtained in this study indicated that leaf and

stem micromorphological characteristics were useful in the delimitation of the examined species. These two related species differed in the type of glandular trichomes, *X. annuum* has biseriate, while *X. cylindraceum* possesses capitate sessile type (Fig. 4G-H).

Leaf anatomy often reflects environmental condition. Thick cuticle, notably developed mechanical tissue, hydrenchyma, as well as small surface-to-volume ratio indicate xeromorphy (Anderson & Creech, 1975). Plants with isolateral leaves usually grow on habitats with intense solar radiation. Species belonging to the genera *Aster* L., *Galatella* Cass. and *Tripolium* Nees (Karanović *et al*., 2015), as well as most of *Centaurea* species, have an isolateral leaf structure, which is characteristic for plant species which grow on dry habitats (Fahn & Cutler, 1992; Luković *et al*., 2013). Although *Xeranthemum* taxa inhabit open, insolated, arid habitats, both studied species have dorsiventral leaf structure (Fig. 4C-D). This finding is another indicator that the genus may have originated from mesophylous ancestors. In Compositae, leaf mesophyll typically contains palisade tissue and spongy tissue (Jane *et al*., 2011, Duarte *et al*., 2011), as we also found in *Xeranthemum* taxa. Although leaf anatomical characters are often related with the environment but they are genetically controlled, and thus could have taxonomic value (Anderson & Creech, 1975).
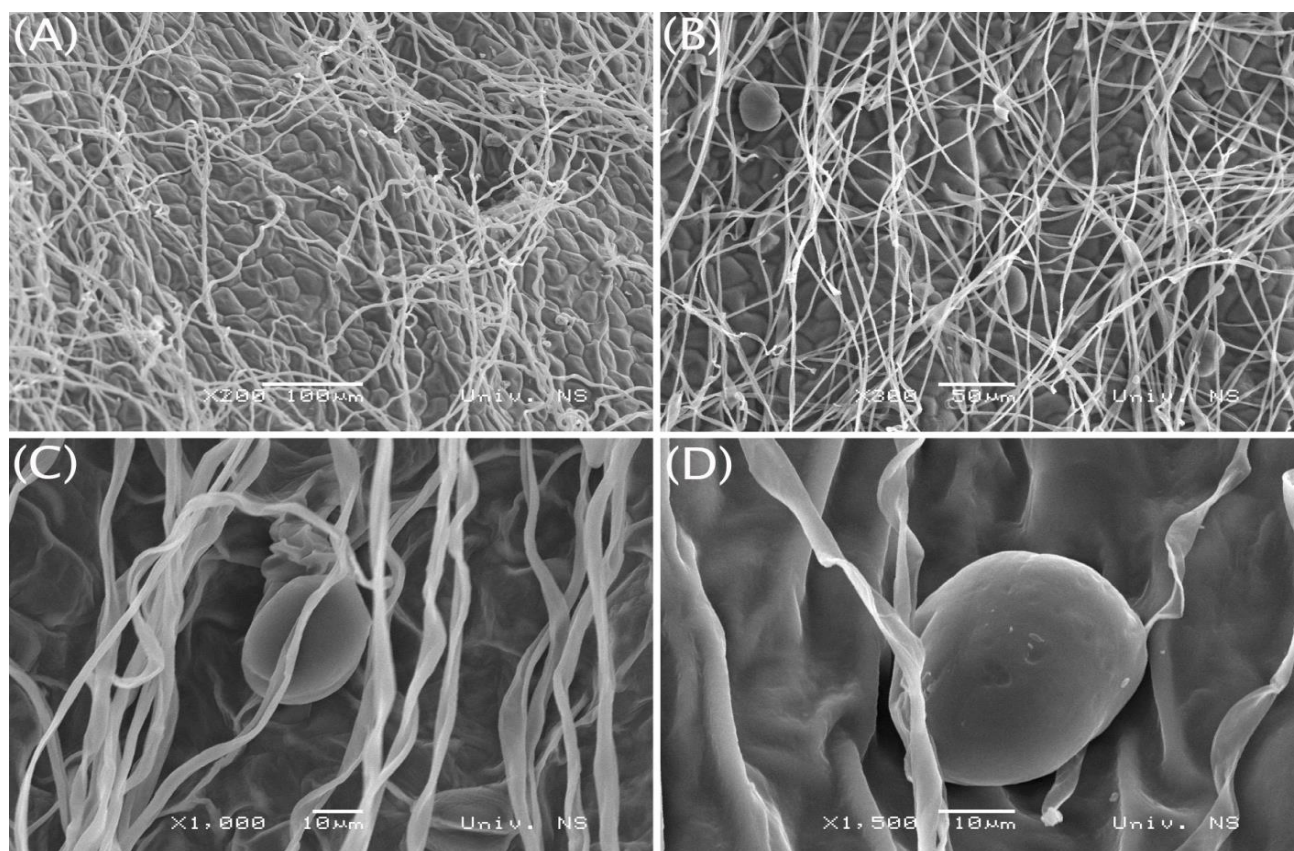
Fig. 6. Scanning electron micrographs of leaf blade epidermis of *Xeranthemum*. Non-glandular curly trichomes on the adaxial epidermis in *X. annuum* (A). Non-glandular curly trichomes and multicellular capitate sessile glandular trichomes on the adaxial epidermis in *X. cylindraceum* (B). Non-glandular curly trichomes and multicellular biseriate glandular trichome on the abaxial epidermis in *X. annuum* (C) and capitate sessile glandular trichome in *X. cylindraceum* (D).

In a previous work, weddellite crystals were reported for the first time on the involucral bracts of these two species (Gavrilović *et al*., 2017). Crystals of weddellite occured as a tetragonal bipyramid (hhl), or rarely in combination of a bipyramid and tetragonal prism (h00). Radiate inner involucral bracts are characteristic of *Xeranthemum* (Bremer, 1994). A highly developed multilayer sclerenchyma, which was present in bracts of both examined species, could be considered as a good protection layer for the inflorescence. As we have shown, palea anatomy is very similar to involucral bract anatomy, because they are homologous structures which differ only in the position on the capitulum (Harris, 1995). The paleae could be considered either rudimentary involucral bracts which support individual florets or involucral bracts which are located among the florets (Keil & Stuessy, 1981; Bremer, 1987; Robinson & Funk, 1987). In the Cardueae the receptacle is usually bristly, and bristles are more numerous than florets and scattered over the receptacle (Bremer, 1994). Regarding ovary, as earlier documented for the family (Davis, 1966; Johri *et al*., 1992), both examined species possess an anatropous ovule with basal placentation (Fig. 7E-F). Lateral anther dehiscence was observed, although in Compositae introrse anthers are common (Katinas *et al*., 2016).

Conclusion

The obtained results on anatomical and micromorphological characteristics of *X. annuum* and *X. cylindraceum*, revealed many quantitative and qualitative characters which could have taxonomic importance. There are some selected quantitative and qualitative characters of the stem, leaf and peduncle (shape, type of glandular trichomes, cortical vascular bundles presence), on the basis of which the studied species are anatomically and micromorphologically distinguishable from each other, thus provide valuable features for better identification of the taxa and strengthen the taxonomy of the genus. In addition, these characters may be considered as additional characters which could help in the delimitation of other Compositae taxa. A comprehensive anatomical and micromorphological analysis of the remaining taxa from the *Xeranthemum* group will certainly help in resolving their taxonomic relationships.

Further investigations of related species from the *Xeranthemum* group or the tribe Cardueae may put light to the process of formation of secondary tissues in the root and may help to understand why it is happening only in the root. Extracellular crystal formation is still an ambiguous question. Thus, further inflorescences anatomical and embryological study of related taxa are needed to truly understand the extracellular crystal deposition, as well as to explore inflorescence characteristics as anther dehiscence, corolla, stigma and ovary anatomy, etc. Finally, anatomical features, combining with morphological, phytochemical and molecular data certainly will give significant contribution in resolving phylogeny of the *Xeranthemum* group.
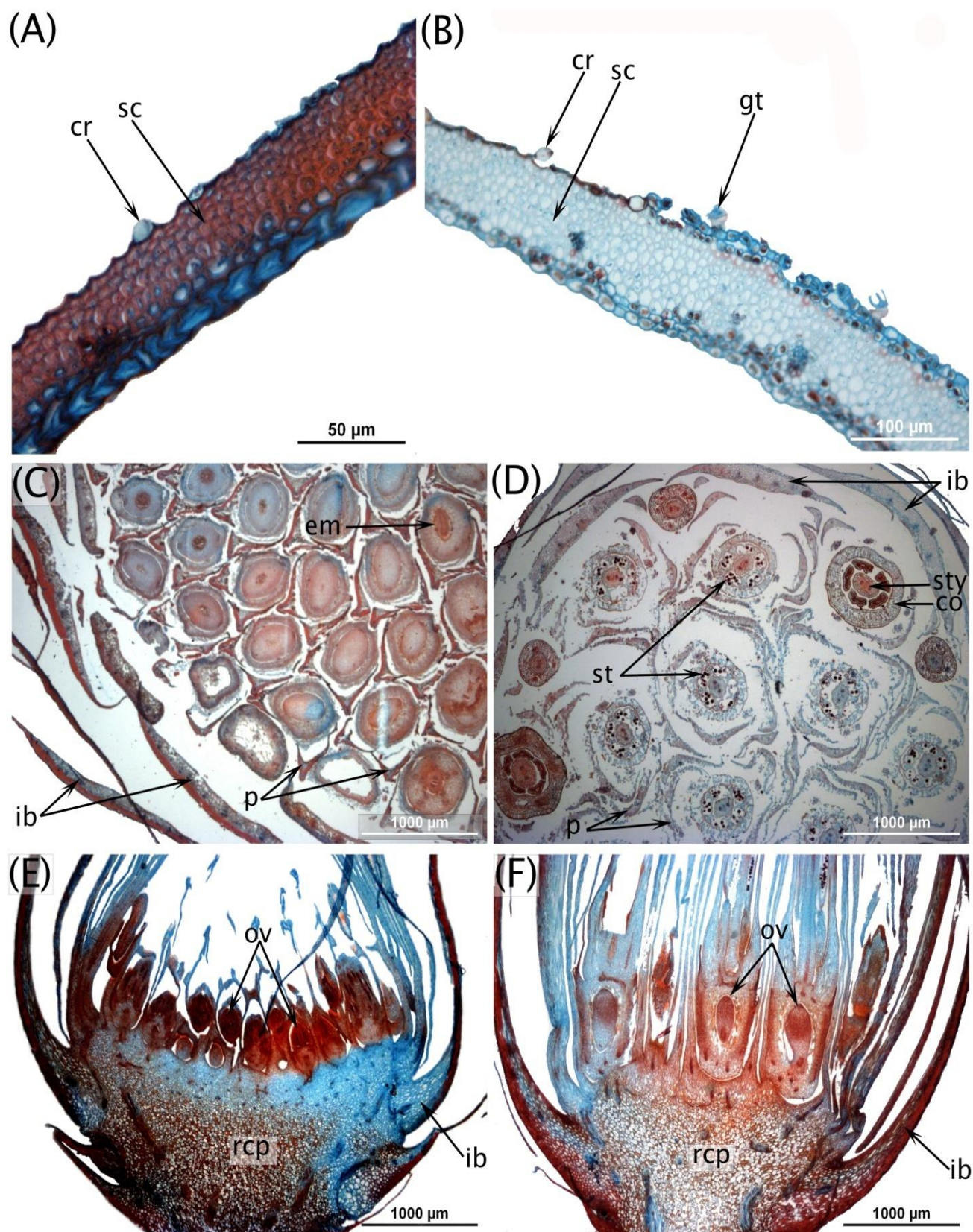
Fig. 7. Light micrographs of involucral bracts and inflorescence cross and longitudinal sections of *Xeranthemum*. Involucral bract cross-section of *X. annuum*, showing presence of crystals on the surface and highly developed multilayer sclerenchyma (A). Involucral bract cross-section of *X. cylindraceum*, chowing indumentum, crystals on the surface and highly developed multilayer sclerenchyma (B). Inflorescence cross-section of *X. annuum* at ovary level showing embryo development (C). Inflorescence cross-section of *X. cylindraceum* at stamens level showing pollen grains (D). Inflorescence longitudinal section of *X. annuum* (E) and *X. cylindraceum* (F), showing inferior ovary, anatropous ovule with basal placentation. Abbreviations: cr = crystal, sc = sclerenchyma, gt = glandular trichome, ib = involucral bracts, p = paleae, em = embryo, st = stames, sty = stylus, co = corolla, ov = ovule, rcp = receptacle. All slides are permanent, stained with Safranin O and Alcian blue.

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