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Article

MORPHOLOGICAL AND ANATOMICAL STUDIES OF *Hippocrepis* L. GENUS IN TURKEY

*Estudos Morfológicos e Anantômicos do Gênero **Hippocrepis** L. na Turquia*

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ABSTRACT - In this study, the morphological and anatomical features were investigated of three taxon of *Hippocrepis* L. species which spreading naturally in Turkey. In the morphological part of the study, *H. unisiliquosa* subsp. *unisiliquosa*'s stem is erect or decumbent. The species *H. ciliata*'s Willd. stem is erect. *H. multisiliquosa*'s L. stem is decumbent. The fruit of *H. multisiliquosa* is much more convoluted than the other two taxa. There are cilia on the fruit of *H. ciliata*, but there are not found any cilia the other two taxa. The stem anatomy of all the studied taxa is hexagonal. In the stem cross section of *H. ciliata* 12-14 vascular bundle are found, *H. unisiliquosa* subsp. *unisiliquosa* 12-15 and *H. multisiliquosa* 12-13. Leaf vascular bundles are arranged regularly, *H. unisiliquosa* subsp. *unisiliquosa* have 18-20, *H. ciliata* have 6-8, *H. multisiliquosa* have 9-13 vascular bundles.

Keywords: anatomy, Leguminosae, morphology.

*RESUMO - Neste estudo, foram investigadas as características morfológicas e anatômicas de três táxons de espécies de **Hippocrepis** L. que se espalham naturalmente na Turquia. Na parte morfológica do estudo, o tronco de **H. unisiliquosa** subsp. **unisiliquosa** está ereto ou decumbente. A haste de **H. ciliata** é ereta. O tronco de **H. multisiliquosa** é decumbente. O fruto de **H. multisiliquosa** é muito mais enrolado do que o dos outros dois taxa. Há cílios sobre o fruto de **H. ciliata**, mas não foram observados cílios nos outros dois taxa. A anatomia do caule de todos os taxa estudados é hexagonal. Na seção transversal do tronco de **H. ciliata**, 12-14 feixes vasculares são encontrados; em **H. unisiliquosa** subsp. **unisiliquosa**, 12-15; e em **H. multisiliquosa**, 12-13. Os feixes vasculares foliares são dispostos regularmente, com **H. unisiliquosa** subsp. **unisiliquosa** apresentando 18-20 deles; **H. ciliata**, 6-8; e **H. multisiliquosa**, 9-13.*

Palavras-chave: anatomia, Leguminosae, morfologia.

INTRODUCTION

Hippocrepis L. is the genus of Leguminosae family. The genus is distributed in various parts of the World. For example, there are 21 species in Spain, 6 species and 1 variety in Morocco, 5 species in Algeria and 1 species in Caucasia. However, in Turkey *Hippocrepis* is represented by 4 taxa belongs 3 species (Davis, 1984). There are some systematic, chromosomal and enzymatic studies about genus *Hippocrepis* (Fearn, 1972; Lonn et al., 1995; Mansour et al., 1995; Hipkin et al., 1999; Rosselló et al., 2002; Hennenberg and Bruelheide, 2003).

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In this paper, the anatomy and morphology of *Hippocrepis unisiliquosa* subsp. *unisiliquosa* (Forssk.) Bornm., *Hippocrepis ciliat*a Willd. and *Hippocrepis multisiliquosa* L. was studied in order to understand the usefulness of these characteristics for systematic purposes. By examining these three taxa belonging to *Hippocrepis*, it is aimed to find out if there is another differentiating property of these taxa within each other.

MATERIALS AND METHODS

Plant specimens for anatomical studies were collected between 2011 and 2012 during the flowering period from natural populations (Table 1).

Table 1 - The location and collection data of the material studied

Taxa	Specimen location and habitat
H. unisiliquosa subsp. *unisiliquosa*	B1 Manisa: from the fallow land far 200 meters to Turgutlu District Gendarmerie Command (new record) B1 İzmir: Nif mountain at 180 meters altitude, in calcareous rocks and Stones C5 Mersin: Erdemli and Kale 10-14 /05 /2011 and 7-12 /05 / 2012
H. ciliata	C1 Muğla: 40 km from Datça, at Knidos ruins and the limestone area (new record) 20-28 /05 /2011 and 15-18 /05 / 2012
H. multisiliquosa	C5 Mersin: (new record) C5 Adana: close the fire tower of Tuzla Shipyard at inshore sandy soil 04-13 / 04 /2011 and 10- 26 /04 /2012

A total of 30 individuals obtained from subpopulations at different altitudes were used. For anatomical studies, plant specimens were fixed in 70% ethanol. The paraffin method (Algan, 1981) was used for preparing cross-sections of root, stem and leaves of each sample. Roots of the same diameter have been used for cross sections. Transverse sections 15-20 µm were made using a sliding microtome. Slides stained with safranin-fast green. Slides were photographed with motorised Leica DM 300 microscope. The measurements of root, stem and leaf cell size of the species were taken using an ocular micrometer. Minimum, maximum, mean and standard deviation were determined.

RESULTS AND DISCUSSION

Morphological characteristics

All the investigated taxa are annual herbs with a tap-root. The stem of *H. unisiliquosa* subsp. *bisiliqua* is ascending to erect and clearly quadrangular. The stem is approximately 25-105 cm height, branched at and near the base, pubescent with curved hair. *H. unisiliquosa* subsp. *unisiliquosa'*s stem is erect or decumbent. While the stem is erect of *H. ciliat*a species *H. multisiliquosa'*s stem is decumbent. *H. unisiliquosa* subsp. *unisiliquosa's* stem is 17-26 cm, *H. ciliata'*s stem is 16-28 cm, *H. multisiliquosa'*s stem is 10-42 cm (Table 2). The fruits of the taxa are lomentoid and convoluted. The fruit of *H. multisiliquosa* is much more convoluted than the other two taxa. There are cilla on the fruit of *H. ciliata*, but there are not found any cillia the other two taxa. *H. unisiliquosa* subsp. *unisiliquosa'*s fruit has 4-8 seed *H. ciliat*a's fruit has 5-8 seed, *H. multisiliquosa'*s fruit has 7-12 seed (Figures 1 and 2).

Anatomical characteristics

When the root structure was examined in terms of its anatomical characteristics, a single row epidermis consisting of rectangular shaped cells was observed in all three taxa. The cortex layer in the root of *H. unisiliquosa* subsp. *unisiliquosa* is composed of 5-6 cell lines, in *H. ciliata* 9-13 cell lines, in *H. multisiliquosa* 10-12 cell lines. The stem anatomy of all the studied taxa is

Table 2 - Comparison of the morphological measurement values of the taxa we collected with each other

	H. unisiliquosa subsp. *unisiliquosa*						*H. ciliata*						*H. multisiliquosa*					
	Min - Max (cm)			Mean ± SD (cm)			Min - Max (cm)			Mean ± SD (cm)			Mean ± SD (cm)			Mean ± SD (cm)		
Root length	7.00	-	11.5	8.79	±	1.56	5.20	-	11.4	7.69	±	1.71	8.00	-	27.0	15.2	±	3.50
Stem length	16.4	-	25.7	20.4	±	3.78	16.0	-	27.2	19.5	±	3.31	10.0	-	42.0	23.6	±	9.20
Leaf																		
Leaf length	0.70	-	0.90	0.75	±	0.10	0.60	-	1.00	0.79	±	0.11	0.50	-	1.30	0.86	±	0.23
Leaf width	0.20	-	0.50	0.35	±	0.07	0.20	-	0.50	0.32	±	0.07	0.20	-	0.47	0.31	±	0.11
Flower																		
Calix	0.30	-	0.41	0.37	±	0.04	0.25	-	0.27	0.26	±	0.02	0.31	-	0.33	0.32	±	0.03
Corolla	5.00	-	8.00	7.20	±	0.09	3.00	-	5.00	4.40	±	0.07	6.00	-	8.00	7.23	±	0.07
Fruit																		
Fruit length	2.50	-	4.40	3.16	±	0.50	2.70	-	4.70	3.56	±	0.47	1.70	-	4.30	2.90	±	0.34
Fruit width	0.50	-	0.52	0.51	±	0.20	0.25	-	0.50	0.39	±	0.06	0.27	-	0.52	0.32	±	0.15

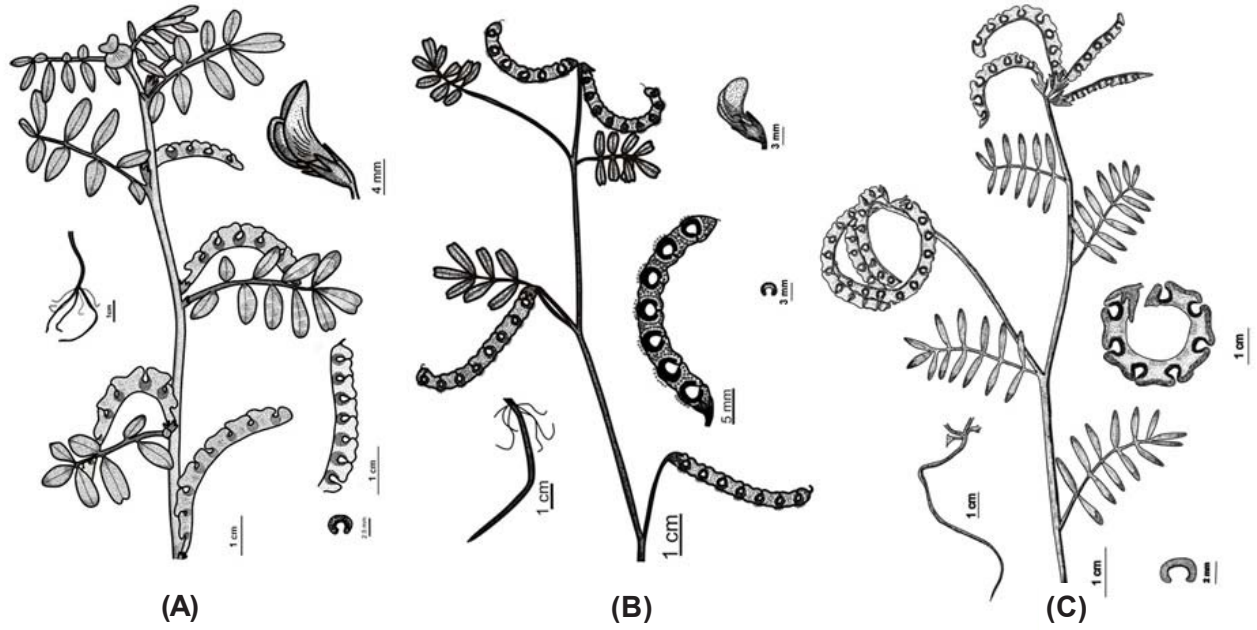

Figure 1 - (A) *H. unisiliquosa* subsp. *unisiliquosa*, (B) *H. ciliata*, (C) *H. multisiliquosa*.

hexagonal. *H. unisiliquosa* subsp.. *unisiliquosa* and *H. ciliata*'s the length of epidermis cells are larger than their widths. In the other species is vice versa. The cortex layer of *H. unisiliquosa* subsp. *unisiliquosa* has 5-6, at *H. ciliata* 5-9, *H. multisiliquosa* 5-11 cell lines. In the stem cross section of *H. ciliata* 12-14 vascular bundle are found, *H. unisiliquosa* subsp. *unisiliquosa* 12-15 and *H. multisiliquosa* 12-13. The stem of all taxa investigated in this paper have large pith which are orbicular or oval parenchymatic cells with intercellular spaces and there is no fragmentation in the pith. When the leaf cross sections were examined, it was observed that all three taxa were of bifacial leaf type. The outer surface of the leaf blade is covered with a cuticle layer in all studied taxa. There is a single row of epidermis cells both upper and the lower face of all leaves. Lower epidermal cells are larger than upper epidermal cells in three taxa. The numbers of layers of palisade parenchyma cells are different in studied taxa, there are 1-2 layers in *H. unisiliquosa* subsp. *unisiliquosa,* there are 2-3 layers in the other two taxa. In taxon *H. ciliata's* palisade parenchyma cells have intercellular space. In studied all taxa, the mesophyll is made up of elongated rectangular palisade parenchyma and isodiametric spongy parenchyma cells which contain very rich chloroplasts. The vascular bundles are arranged regularly, *H. unisiliquosa* subsp. *unisiliquosa* have 18-20, *H. ciliata* have 6-8, *H. multi siliquosa* have 9-13 vascular bundles. Spongy

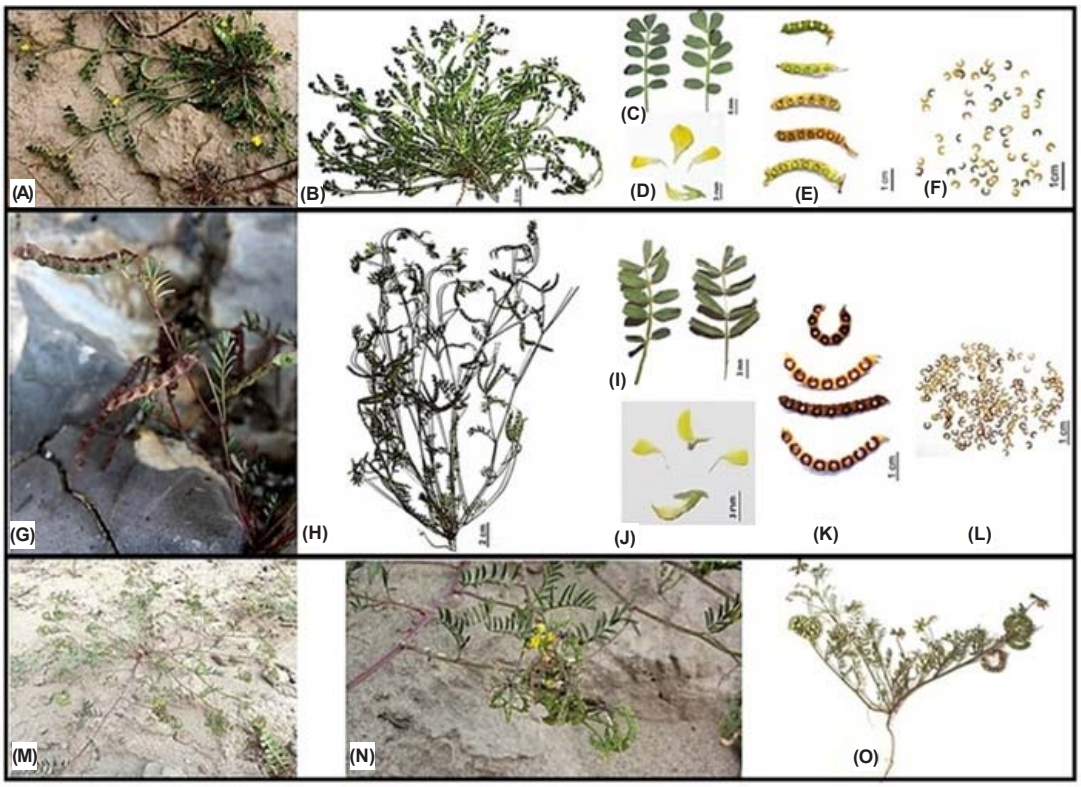

Figure 2 - (A), (B): *H. unisiliquosa* subsp. *unisiliquosa*, (C): leaf of *H. unisiliquosa* subsp. *unisiliquosa*, (D): flower of *H. unisiliquosa* subsp. *unisiliquosa*, (E): fruits of *H. unisiliquosa* subsp. *unisiliquosa*, (F): seeds of *H. unisiliquosa* subsp. *unisiliquosa* (G), (H): *H. ciliata*, (I): leaf of *H. ciliata*, (J): flower of *H. ciliata*, (K): fruits of *H. ciliata*, (L): seeds of *H. ciliata*, (M), (N), (O): *H. multisiliquosa*.

parenchyma cells are 1-2 layer in cross section of leaves of all studied taxa. *H. unisiliquosa* subsp. *unisiliquosa* and *H. ciliata's* spongy parenchyma cells have larger intercellular spaces than *H. multisiliquosa*. All studied taxa are amphistomatic. When classified according to neighbouring epidermis cells, all types of stomata of the taxa are anomocytotic. According to friend cells, they have amaryllis type stomata (Figures 3, 4, and 5).

In the current literature *H. unisiliquosa* subsp. *unisiliquosa* is referred to as *H. unisiliquosa* subsp. *biflora*. *H. unisiliquosa* subsp. *bisiliquosa* that is mentioned in Flora of Turkey was not found in any herbarium or field. The inability to find any permission for this subtype suggests the proposal of combining two subspecies. According to our findings morphological measurement of *H. unisiliquosa* subsp. *unisiliquosa* and *H. unisiliquosa* subsp. *bisiliqua* shows similarities. According to our findings the number of the flower of *H. unisiliquosa* subsp. *unisiliquosa* is 1-2, and their fruits have 4-8 seeds. According to Flora of Turkey the number of the flower of *H. unisiliquosa* subsp. *bisiliqua* 1-3 and their fruits have 6-10 seeds. There will be a definite decision after more detailed taxonomic examinations to be made (Table 3).

Tezcan (2008) studied on morphology, anatomy and karyology of *Thermopsis turcica* (Leguminosae). He found that rizoderma in roots of the species is composed of 6-8 rowed cells. Adjacent to the rizoderma cells, there are 13-19 layered cortex. They also said that there were pith rays from xylem tissue to phloem tissue and cambium rings are visible between xylem and phloem. It is reported that xylem occupied the very large area. The cortex layer at the root of *H. unisiliquosa* subsp. *unisiliquosa* consists of 5-6 cell lines, *H. ciliata* consists of 2-3 cell lines, *H. multisiliquosa* consist of the 10-12 cell lines. In the roots of all studied taxa, primer and secondary pith rays are observed. The very large area of the transverse section occurs with the component of xylem in all investigated taxa. It is stated that there is a regular sequence of epidermis in *Thermopsis turcica*'s stem. It has been reported that under the epidermis, there are single rowed collenchyma cells which are reaching up to ten rows at corners. And below them, there are 6-13 ordered parenchyma cells. It has been reported that there are phloem pith rays from cortex to xylem, cambium visible clearly, and the pith is composed of 7-10 rowed cells. The cortex region

is composed of a 5-6 layer of cells in *H. unisiliquosa* subsp. *unisiliquosa*, 5-9 layer of cells in *H. ciliata*, a 5-11 layer of cells in *H. multisiliquosa*. It has been reported that at the transverse section of the leaf blade, upper and lower epidermis cells are different each other. There is an isolateral structure due to palisade parenchyma on the leaves, the sponge parenchyma cells have different shapes and because of the presence of stomata on both sides, leaf is amphistomatic and anomocytic stoma type was seen (Tezcan, 2008). In our study, it was observed that amphistomatic leaves and anomocytic stoma type in all three taxa.

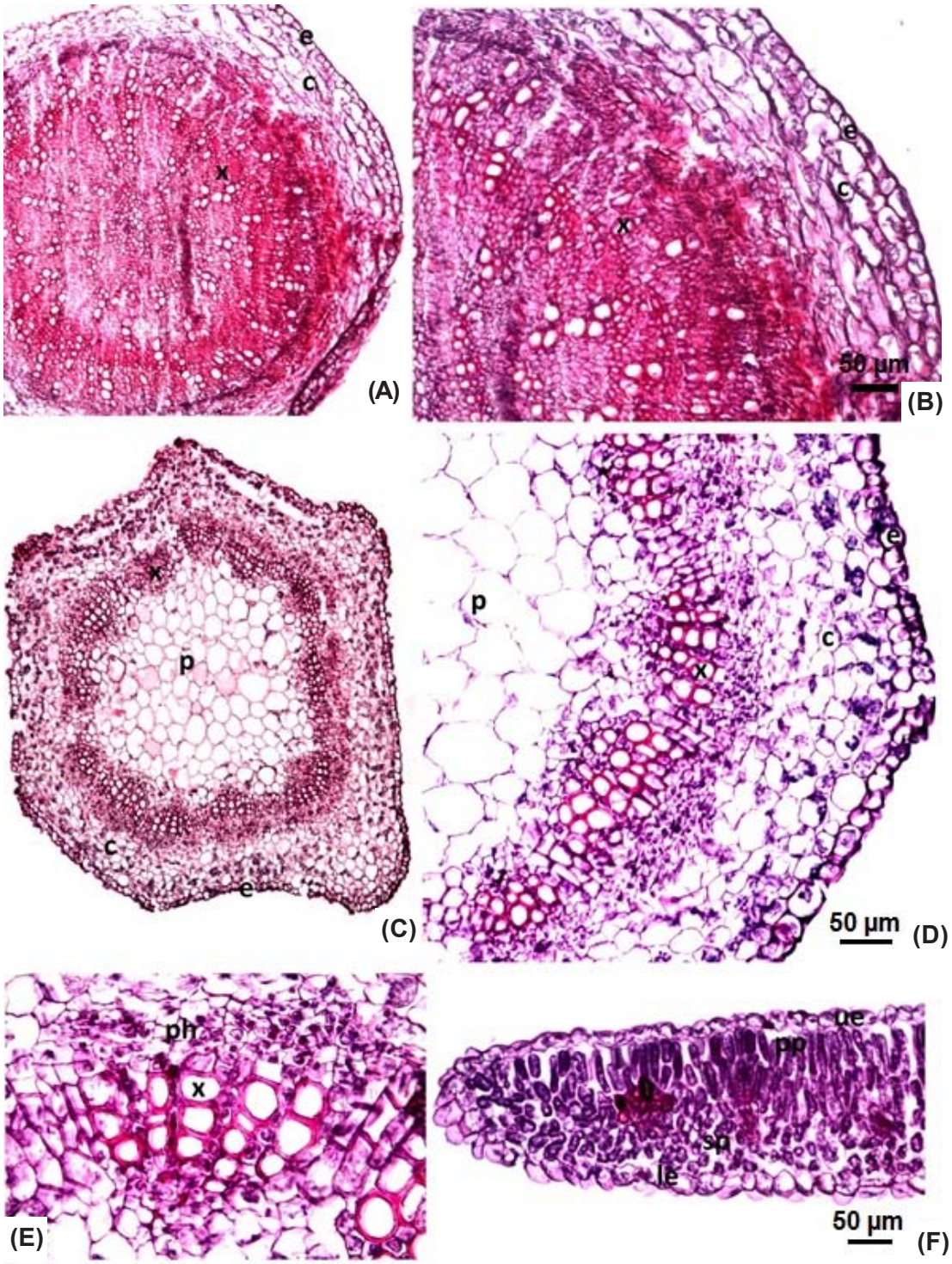

Figure 3 - Anatomical cross sections of *H. unisiliquosa* subsp. *unisiliquosa* (A), (B): root, (C), (D), (E): stem, (F): leaf; c:cortex, e: epidermis, le: lower epidermis, p: pith, ph: phloem, pp: palisade parenchyma, sp: spongy parenchyma, ue: upper epidermis, x: xylem.

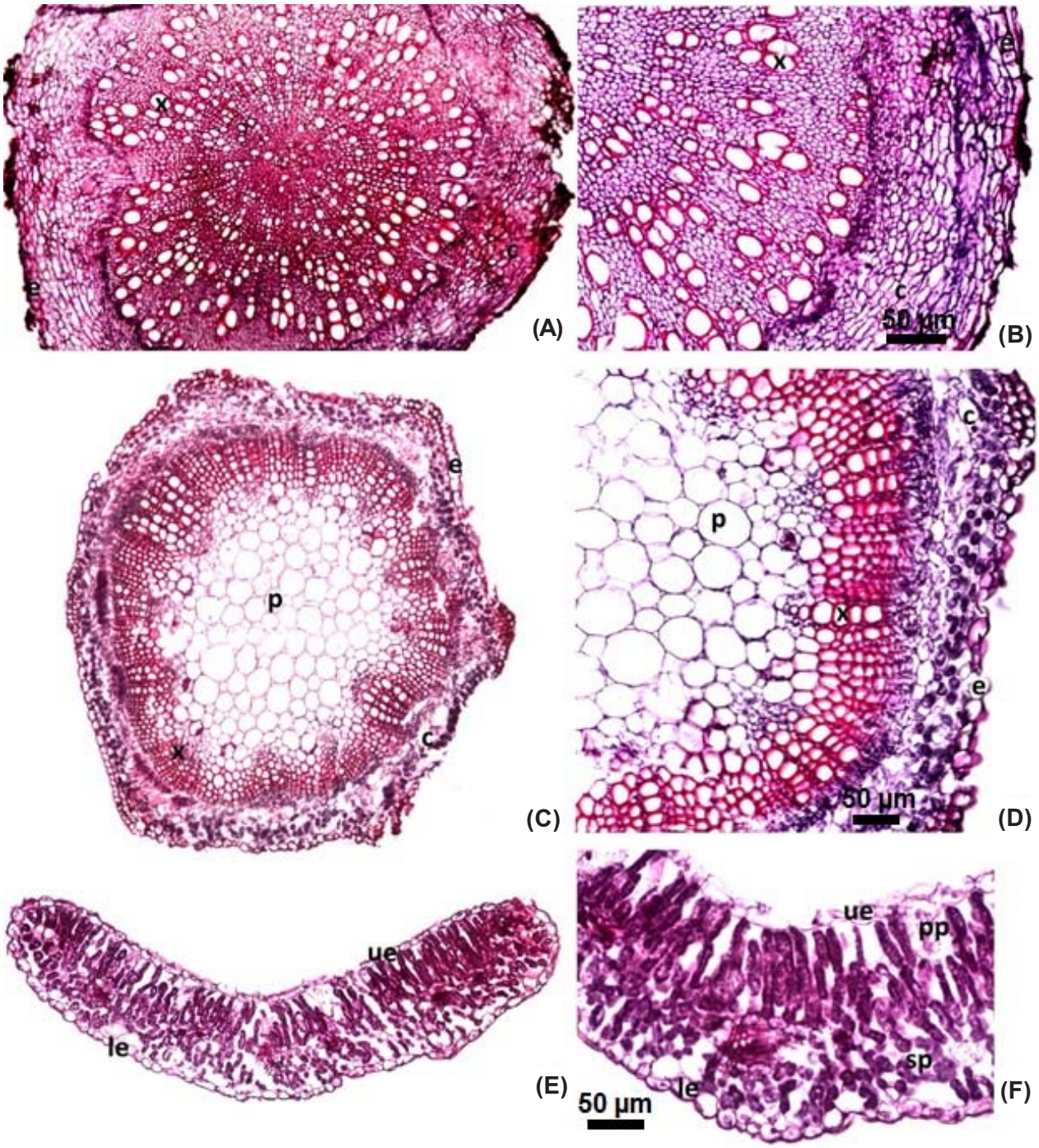

Figure 4 - Anatomical cross sections of *H. ciliata* (A), (B): root, (C), (D): stem, (E), (F): leaf; c: cortex, e: epidermis, le: lower epidermis, p: pith, pp: palisade parenchyma, sp: spongy parenchyma, ue: upper epidermis, x: xylem.

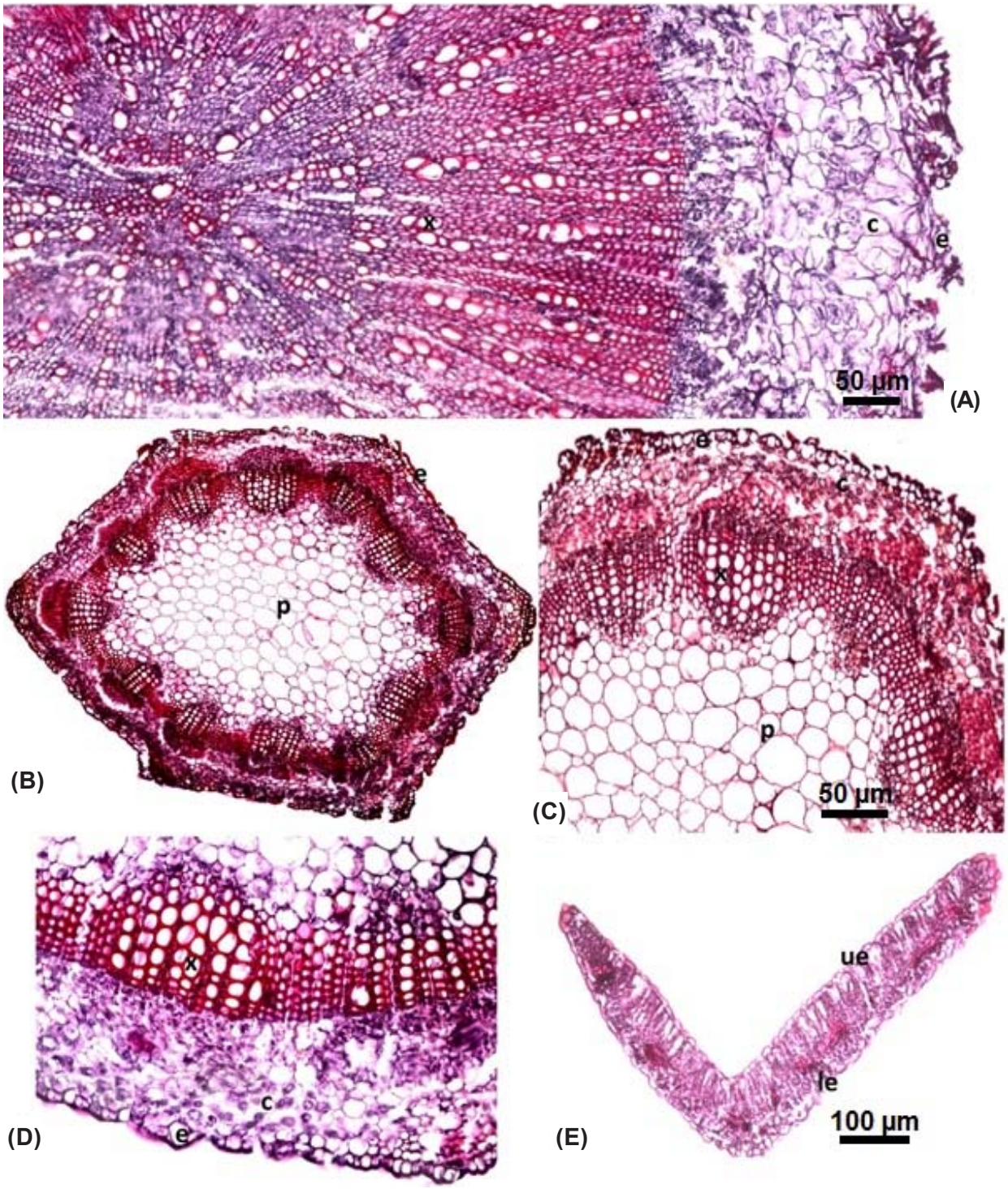

Figure 5 - Anatomical cross sections of *H. multisiliquosa* (A): root, (B), (C), (D): stem, (E), (F): leaf; c: cortex, e: epidermis, le: lower epidermis, p: pith, ue: upper epidermis, x: xylem.

Table 3 - Comparison of the anatomical measurement values of the studied taxa

	H. unisiliquosa subsp. *unisiliquosa*				*H. ciliata*				*H. multisiliquosa*			
	Width		Length		Width		Length		Width		Length	
	Min-Max (µm)	Mean±SD	Min-Max (µm)	Mean±SD	Min-Max (µm)	Mean±SD	Min-Max (µm)	Mean±SD	Min-Max (µm)	Mean±SD	Min-Max (µm)	Mean±SD
Root												
Cuticle (thickness)	10.0-12.5	11.2±1.2			10.0-15.0	12.0±1.5			10.0-15.0	13.1±1.8		
Epidermis	12.0-15.0	13.7±1.2	30.0-35.0	33.0±2.3	10.0-17.5	11.8±3.6	12.5-37.5	21.2±9.7	30.0-37.0	33.0±3.1	7.50-12.5	10.0±1.7
Cortex (diameter)	10.0-22.5	15.2±5.1			11.0-25.0	15.0±5.2			17.5-30.0	23.0±4.7		
Trachea (diameter)	10.0-47.5	27.0±15.			7.50-37.5	23.0±11			12.5-20.0	18.3±2.6		
Stem												
Cuticle (thickness)	5.00-10.0	7.00±1.8			5.00-7.5	5.62±1.0			5.00-7.50	5.23±1.4		
Epidermis	15.0-30.0	22.5±5.4	10.0-30.0	20.0±7.4	17.5-35.0	25.6±7.1	15.0-30.0	23.5±5.4	12.5-27.5	19.0±5.1	15.0-30.0	22.5±5.4
Cortex (diameter)	17.0-40.0	27.0±8.2			22.5-37.5	30.6±4.8			15.0-40.0	18.3±1.8		
Trachea (diameter)	10.0-20.0	14.5±4.8			7.50-25.0	17.0±5.7			12.5-17.5	15.0±1.8		
Pith (diameter)	25.0-112	64.1±28			50.0-360	220±21			27.5-75.0	42.0±10		
Leaf												
Upper epidermis	10.0-22.0	16.0±4.8	20.0-30.0	27.0±4.9	10.0-24.0	15.0±4.1	20.0-30.0	26.0±4.8	20.0-37.5	5.00±5.9	15.0-30.0	18.0±1.8
Palisade cell	10.0-24.0	12.0±4.0	10.0-70.0	48.0±17	15.0-20.0	12.5±4.2	30.0-60.0	44.0±10	7.50-15.0	26.0±2.8	30.0-57.0	53.0±7.5
Spongy cell	10.0-20.0	14.0±4.9	30.0-20.0	18.0±4.0	10.0-22.0	14.0±1.9	10.0-30.0	20.0±6.3	7.50-17.5	13.0±3.2		
Lower epidermis	20.0-30.0	21.0±4.0	20.0-40.0	36.0±4.8	12.5-25.0	17.0±4.0	20.0-30.0	26.0±4.9	15.0-37.5	14.0±8.3	18.0-26.0	19.0±2.0
Petiol												
Epidermis	12.0-20.0	15.3±1.2	14.0-25.0	21.0±2.3	18.0-30.0	24.5±1.0	13.5-22.0	17.5±1.3	20.0-32.0	25.7±1.1	15.6-24.0	19.2±1.2
Chlorenchyma	15.5-20.3	16.8±2.1	25.0-35.0	31.3±2.3	14.0-18.3	15.7±1.2	30.0-42.0	36.4±2.9	12.0-16.5	14.3±0.7	27.0-38.0	35.2±1.0
Parenchyma (diameter)	25.0-48.0	42.3±1.33			16.0-50.0	37.5±1.4			14.0-36.0	29.0±1.2		
Calix												
Upper Epidermis	15.0-32.0	27.0±2.1	10.0-22.0	17.5±1.7	12.0-34.0	25.0±2.5	8.00-18.0	15.2±1.3	15.0-36.0	29.0±2.7	9.25-22.0	17.2±1.4
Lower Epidermis	12.0-28.0	18.9±1.7	7.50-17.0	14.8±1.0	11.0-24.0	17.5±1.4	6.75-14.0	12.6±1.4	13.0-27.0	18.0±1.8	7.00-19.0	16.8±1.2
Parenchyma (diameter)	10.0-18.0	15.3±1.8			12.0-17.0	14.7±1.2			15.0-21.0	16.8±1.6		
Corolla												
Upper Epidermis	8.00-14.0	11.3±1.4	7.00-10.0	8.25±1.2	7.00-12.0	9.7±1.2	8.00-11.0	9.75±1.2	8.50-15.3	11.7±1.0	7.00-17.0	12.8±1.1
Lower Epidermis	6.00-12.0	9.72±0.9	5.00-8.50	7.25±0.8	5.75-13.0	11.0±0.7	6.00-7.50	7.10±0.4	7.50-18.0	14.7±1.8	8.50-11.0	9.20±1.0
Parenchyma (diameter)	16.0-25.0	21.3±1.2			17.0-26.0	19.5±1.1			19.0-30.0	23±1.7		

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