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ANATOMICAL STUDY OF STEMS FOR SOME SPECIES FROM CARYOPHYLLACEAE FAMILY IN IRAQ

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Abstract

This research is a comparative anatomical study of 8 species belonging to 8 different genera of Caryophyllaceae, which were collected during 2016-2018 from phyto-districts of Iraq. The specimens were identified and conserved in the Herbarium of Anbar University. Cross section of stems of species was studied anatomically. The results showed that the species were varied in their cortex components. The sclerenchyma tissue was varied in a number of rows and thickness; 1 row and 24 micrometers in *Stellaria media* to 4 rows and 93 micrometer thickness in *Minuartia picta*. Species also varied in a number of vascular bundles, which ranged between 6 bundles in *Stellaria media* and *Holosteum umbellatum* to 14 bundles in *Spergula fallex*, the largest one. Furthermore, the thickness of pith and rupture of the cell wall also differed among the species. *Herniaria hirsuta*and *Minuartia picta* were distinguished with druses crystals in their cortex, finally, the anatomical key was prepared for all of the studied taxa.

***Key words*:** Anatomy, stems, caryophylaceae, Iraq.

Introduction

Caryophyllaceae is one of the largest plant families in the temperate regions. It is highly available and planted in the Mediterranean Sea. Caryophyllaceaeincludes 2200 species belonging to 86 Cosmopolitan genus. In Iraq the family composed of 30 genus with147species (Ghazanfar & Edmondson, 2010). Most of the species are wild herbaceous or weeds and very few are cultivated (Al-Musawi, 1987). Many scientists have confirmed the importance of anatomical characters in the taxonomical studies. The anatomical features show many variations in taxonomical categories and can be considered as a diagnostic trait as well as in phylogenetic relationships in plants (Davis & Heywood, 1973). Many researchers considered the anatomical characteristics as evidence in taxonomical studies (Radford et *al*., 1974). Some traits used in isolation of taxonomical categories (genus, species, variety) because they showed significant variations in plant groups (Stace, 1991). Metcalf and Chalk (1950) studied Caryophyllaceae anatomically and Keshavarzi & Bozchaloyi (2014) isolated 8 species belonging to 3 genera of Caryophyllaceae in Iran depending on 12 qualitative characters and 30 quantitative characters. Ataslar (2004) and Schweingruber, (2007) write in their study on some species that vascular cylinder as a continues ring in cross section of stems. The study of Ulukus (2018) reported that the rows of sclerenchyma were stable in the individuals of the same species. There are many studies on Caryophyllaceae like the study of Musa (2007) on the genus *Silene* L. (38 species in Iraq), as well as the study of Al-Saadi (2014) on the genus *Minuartia* L. (8 species), in addition, Zarinkamar (2001) studied stomata characters, trichomes and longitudinal section of leaves in some Caryophyllaceae species in Iran.

The aim of this study are:

1. Using anatomical characters as a taxonomic property for identification and isolation of the species.
2. Preparing anatomical key for isolation the taxa.
3. Giving a clear anatomical photo by microscopic filming.

Materials and Methods

The study depended on the dry specimens which were collected from Iraqi phyto-districts during the period 2016-2018. The identification of species was according to the flora of Iraq (Ghazanfar and Edmondson, 2010). The specimens were preserved in the Herbarium of Anbar University, each specimens label data containing the following information: scientific name, collector name, location, date of collection and altitude (Table 1).

The study depended on hand sectioning (Al-Haji, 1998).

1. Dry stems put in boiling water for 5 minutes.
2. The stems were cut accurately to obtain very thin sections by using a sharp blade.
3. The thin section transferred to the slide and 2 drops of safranin 1% was added for 5 minutes.
4. One drop of Glycerin was added and covered the section, then the cover slid placed over it.

5. The slides were placed on a hot plate for 1 hour to remove the air inside the cells.
6. The slides were examined under a compound microscope (Olympus).

The measurement reported by ocular micrometer, the sections photographed by micro- camera Canon.

Results and Discussion

This study showed the cross-sections of stems of eight species belonging to a different genus from Caryophyllaceae were circular or semicircular except *Cerastium semidecandrum* which was prolate circular because the stem was winged. The average diameter of stems was between 737 µm in *Robbairea polycarpaea*, which was the smallest to 1173 µm in the *Minuartia picta*, which was the largest, while the rest of the species were between these values (Table 2).

The epidermis of species varied in shape, size, and thickness of the cells and the cuticle. In general, the thickness of the epidermis with uniserriate ranged from 16 µm in *Herniaria hirsuta* average, as the smallest and 38 µm in the *Stellaria media,* as the largest species, while the rest species ranged between them. The shape of cells was oblong in *Stellaria media*, isodiametric in *Holosteum umbellatum* and heterogeneous cells in *Arenaria leptoclados*. The thickness of the cuticle ranged between (1.8 µm and 1.1 µm) as a thin layer in Mountain region species (Shaqlawa) while the species grown in the desert region appeared in an uneven layer (3.1 - 4.1 µm). This data was in agreement with both Chalk and Metcalfe (1950) and Evert (2006). The trichomes were glandular and non-glandular, glandular in *Herniaria hirsuta,Cerastiumsemidecandrum,* and *Arenaria leptoclados*, in contrast, allotherspecies were glabrous (Figure 1-4).

The Cortex composed of chlorenchyma and ordinary parenchyma, with 1-5 rows. The species were divided into three groups based on the presence or absent of chlorenchyma and parenchyma:

1. The cortex consisted of chlorenchyma and ordinary parenchyma as in *Spergula fallex, Minurtiapicta,* and *Arenaria leptoclados*.
2. The cortex consisted of ordinary parenchyma as in *Holosteum umbellatum* and *Cerastium semidecandrum*.
3. The cortex consisted of chlorenchyma and ordinary parenchyma as in *Stellaria media, Polycarpaea robbairea* and *Herniaria hirsuta*.

The pericycle founded as a ring of sclerenchyma cells, the outer surface was lignified with thick walls but the inner was not. This result agrees with (Atasagun *et al*., 2016). The researchers referred to the importance of sclerenchyma tissue in Caryophyllaceae, which was stable in the plants of the species (Atasla *et al.*, 2017); Armağan, and Özgökçe, 2018; Malek *et al*., 2018). The thickness of sclerenchyma tissue was 24 µm one row in *Stellaria media* which grown in mountain region with prostrate stems, while the thickness reached to 93 µm 4 rows in *Minurtiapicta*which grown in the desert region, but the rest of the species ranged between them. Vascular cylinder consisted of collateral bundles (Xylem and phloem were on the same diameters), but it varied in nature, size, and number of bundles. Vascular cylinder was appeared as circular ring with a regular thickness in d *Polycarpaea robbairea*, *Minurtia picta,* and *Herniaria hirsuta*, because they grow in the mountain region with rainy weather and moderate winter temperature. *Holosteum umbellatum* and *Stellaria media* were with 6 bundles (regular distribution with small size), but *Spergula fallex*recognized with 14 regular bundles which were closed togetherbecause of the species grown in desert region with little rains and high temperature. Our results were in agreement with results reported by (Fahn, 1983). *Arnenaria leptoclados and Cerastium semidecandrum* dignosed with non-regular thickness of vascular cylinder*,* subring, xylem wider in the first species which grown in desert region, while the second type was with narrow xylem which grown in mountain region. Ploem thickness was 17 µm (rang) in *Spergola fallex,* as the smallest species, while 27 µm in *Arenaria leptoclados and Spergola fallex*as the highest thickness*,* the rest species were between them. The species varied in thickness of xylem; they were 33 µm in *Holosteum umbellatum*,as the smallest, but 110 µm in *Arnenaria leptoclados*as the largest. The pith founded in the center of cross section of the stems consisted of parenchyma with thin and large size walls, especially in the center and the species were varied in the diameter range of (Table 2).

The species were divided into three groups based on pith:

1. Species with broad pith, (313-550) µmin diameter, but the cells ruptured in the center of old stems, so the stems was hollow as in *Arenaria leptoclados Cerastium semidecandrum, Holosteum umbellatum, Stellaria media* and *Spergula fallex*.
2. Species with narrow to medium pith, (231-253) µm in diameter, and their pith consisted of ordinary parenchyma as in *Minurtiapicta*and *Polycarpa robbairea.*
3. Species with narrow pith that consisted of storage parenchyma as in *Herniaria hirsuta.*
4. The study showed that the cortex in *Herniaria hirsuta* and *Minurtiapicta* contained druses crystals existed in the stems and the leaves of Caryophyllaceae (Gangulee *et al.*, 1972).

Anatomical key of Species

1-Pith consisted of storage parenchyma ……..…… ***Herniaria hirsuta***
1-Pith consisted of ordinary parenchyma……..…… 2
2-Vascular cylinder consisted of regular distribution bundles ……..…… 3
2-vascular cylindrical consisted of a continuous ring of xylem and phloem ….....…… 5
3-Number of vascular bundles more than 14……..……***Spergula fallex***
3- Number of vascular bundles 6-7 ……..……..…… 4
4-Sclerenchyma tissue only one row ……..…..……. ***Stellaria media***
4-Sclerenchyma tissue consisted of 3 rows ……..…..…… ***Holosteum umbellatum***
5-Vascular ring of xylem and phloem with regular thickness……..…… 6
5- Vascular ring of xylem and phloem with irregular thickness ……..……..……. 7
6-Cortex consisted of parenchyma ……..……***Minuartia picta***
6- Cortex consisted of parenchyma and chlorenchyma ….....…....***Polycarpaea robbairea***
7- Cortex consisted of parenchyma, cross-section oblong circular ……***Cerstiumsemidecandrum***
7- Cortex consisted of chlorenchyma, cross section circular ….....…. ***Arenaria leptoclados***

Table 1 : Plant species, locations, date of collection, altitude, and samples numbers.

Species	Place of collection	Date of collection	Altitude (m.)	Number of plant sample
Arenaria leptoclados (Reich.) Guss.	DLJ- Rawa	5.4.2017	160	AUH 2217
Cerastium semidecandrum L.	MRO- Shaqlawa	18.5.2016	980	AUH 2989
Herniaria hirsuta L.	DWD- Rutba	15.3.2017	615	AUH 2179
Holosteum umbellatum L.	MRO- Shaqlawa	5.5.2016	985	AUH 2208
Minuartia picta (Sibth. & Sm.) Bornm.	DWD- Haditha	3.4.2018	140	AUH 3382
Polycarpaea robbairea (Kuntze) Greuter & Burdet	DWD- Habbaniya	4.4.2017	44	AUH 3011
Spergula fallex (Lowe) E.H.L.Krause	LCA- Abo-Ghraib	5.4.2017	34	AUH 3079
Stellaria media (L.) Vill.	MRO- Shaqlawa	30.5.2016	955	AUH 2908

Table 2: Anatomical characters of stems in *Caryophyllaceae* species (In micrometer)

Species	Stem diameter	Cuticle thickness	Epidermis thickness	Cortex thickness		pericycle (Sclerenchyma)	Phloem thickness	Xylem thickness	Pith thickness
				Chlorenchyma	Ordinary parenchyma				
Arenaria leptoclados	(805-880) 852	(2.8-3.2) 3.1	(16-19) 17	(18-22) 21	_	(86-89) 88	(25-29) 27	(102-118) 110	(320-350) 332
Cerastium semidecandrum	(920-980) 945	(1.7-1.9) 1.8	(21-25) 22	_	(68-76) 71	(54-70) 64	(21-24) 22	(53-58) 55	(303-325) 313
Herniaria hirsute	(1120-1160) 1135	(3.4-3.7) 3.6	(15-17) 16	(31-38) 36	(55-71) 62	(78-85) 83	(25-29) 26	(98-112) 103	(280-295) 288
Holosteum umbellatum	(975-1100) 1050	(1.8-2.2) 1.9	(23-28) 24	_	(60-75) 68	(47-54) 51	(19-23) 20	(31-38) 33	(573-595) 550
Minuartia picta	(1125-1210) 1173	(3.9-4.2) 4.1	(25-30) 27	(46-55) 51	_	(88-98) 93	(23-28) 24	(40-53) 44	(235-285) 253
Polycarpaea robbairea	(720-800) 773	(2.8-3.3) 3.2	(20-27) 25	(34-50) 44	(27-38) 32	(70-79) 73	(24-30) 27	(82-93) 86	(210-255) 231
Spergula fallex	(855-930) 898	(3.3-3.9) 3.7	(21-24) 23	(32-39) 36	_	(55-63) 58	(15-22) 17	(42-53) 48	(370-420) 406
Stellaria media	(850-910) 885	(2.0-2.2) 2.1	(35-43) 38	(25-33) 31	(29-38) 35	(22-27) 24	(24-29) 26	(40-49) 43	(480-530) 511

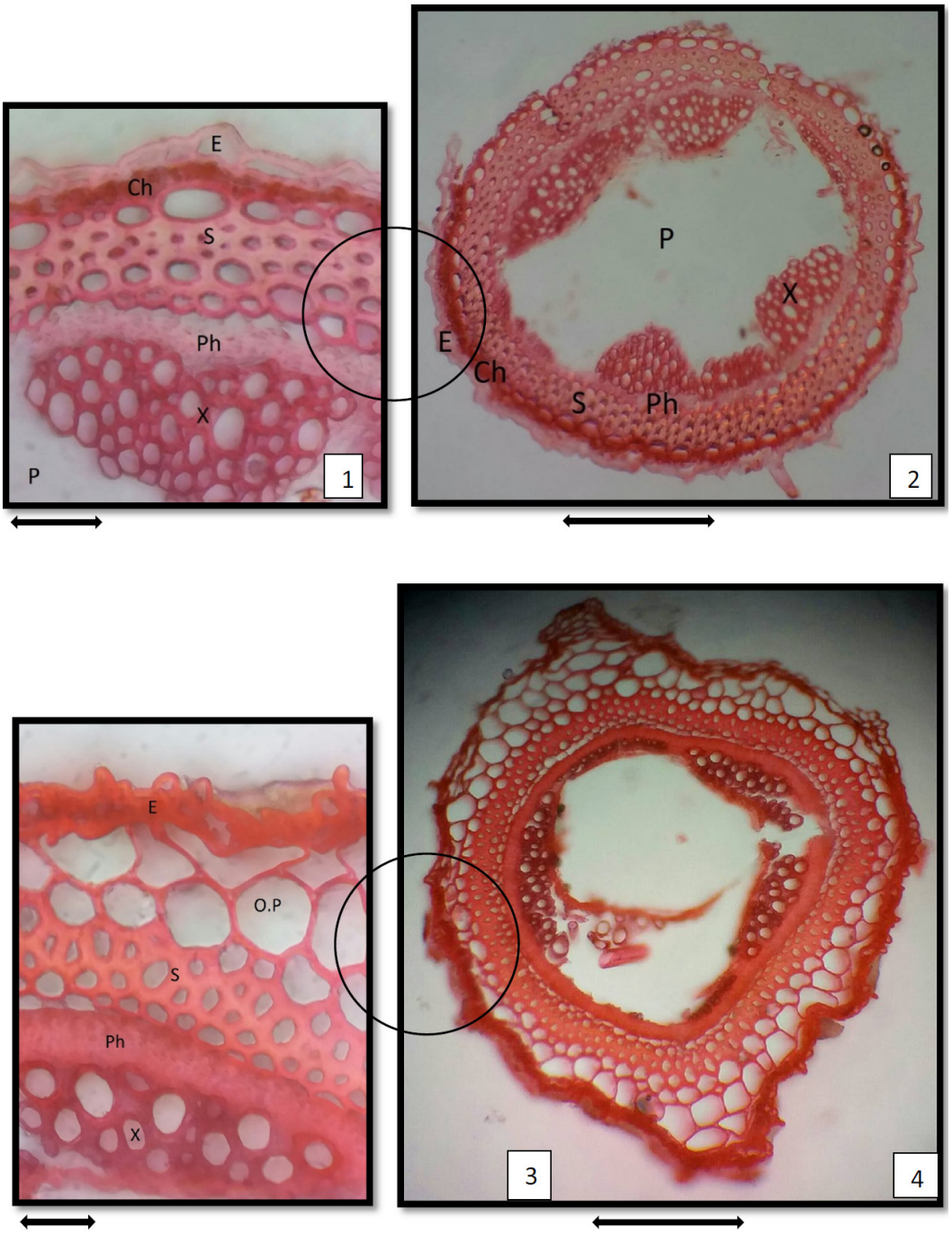

Fig. 1 : Transverse section of stem (scale 100µm); 1, 2 *Arenaria leptoclados;* 3, 4*Cerastium semidecandrum*
Ch: chlorenchyma, D: Druses, E: Epidermis, Op: Ordinary parenchyma, P: Pith, Ph: Phloem, P: Pith, S: Sclerenchyma, X: Xylem.

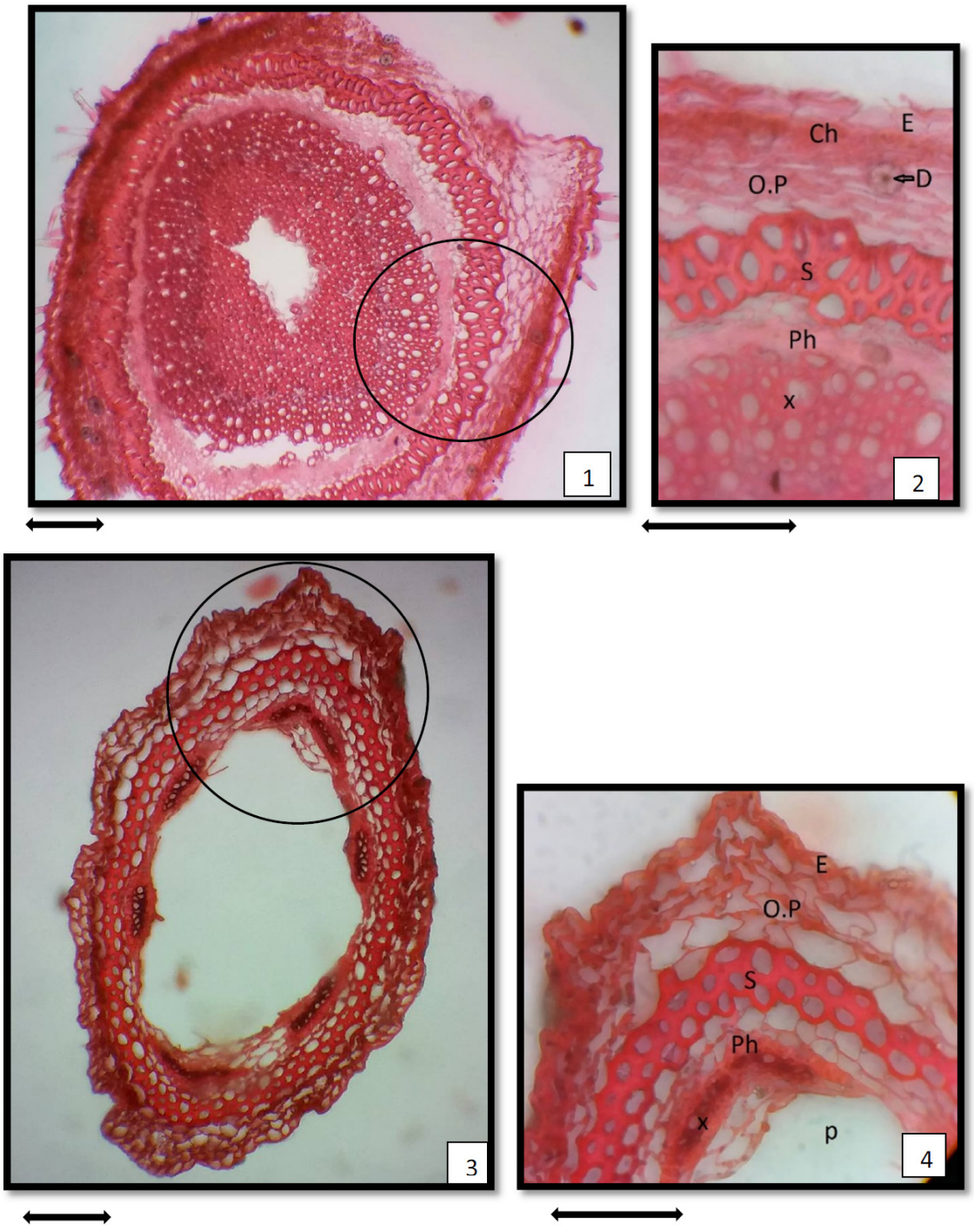

Fig. 2 : Transverse section of stem (scale 100µm)
1,2 *Herniaria hirsuta* 3,4 *Holosteum umbellatum*

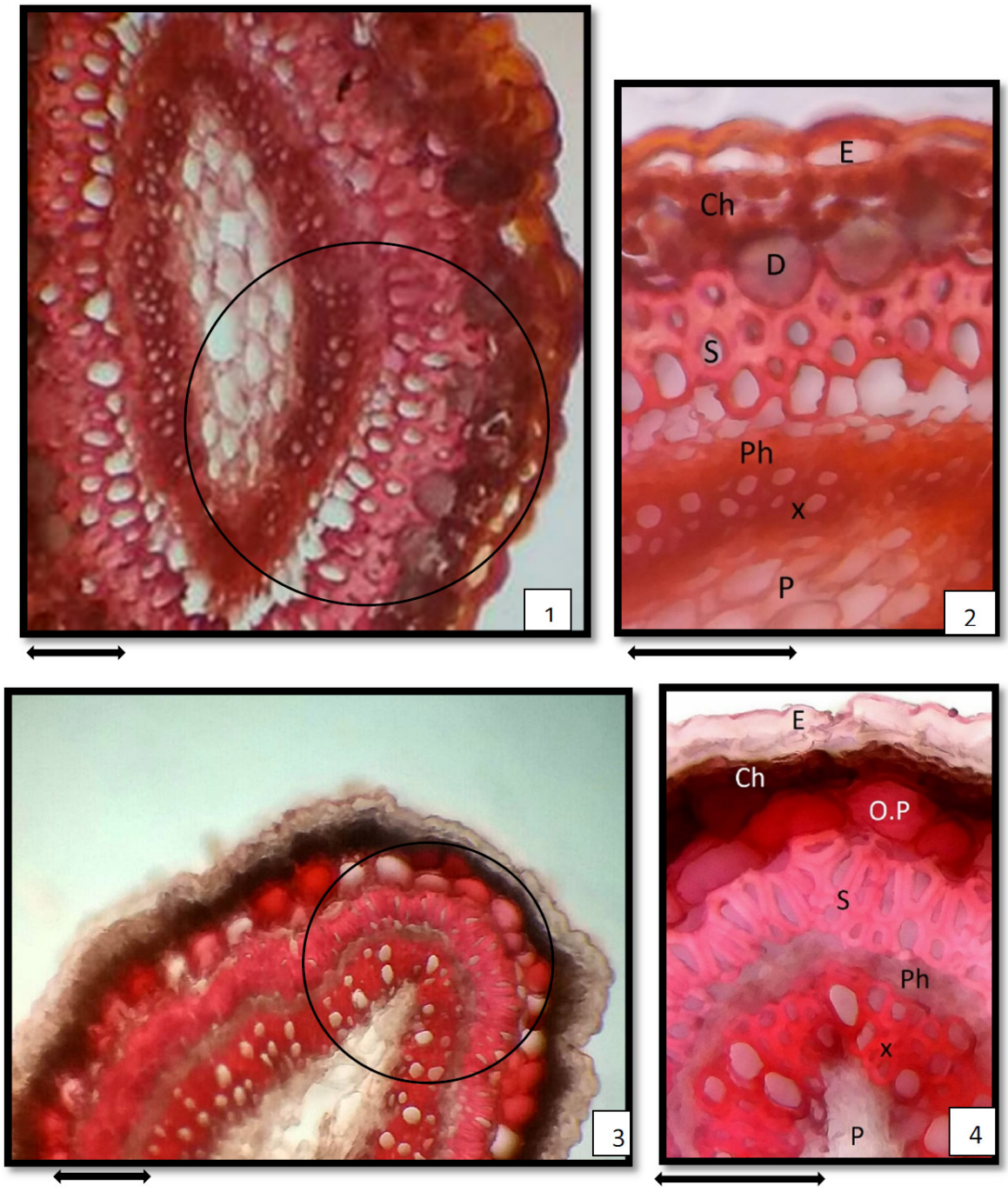

Fig. 3 : Transverse section of stem (scale 100µm)
1,2 *Minuartia picta* 3,4 *Polycarpaea robbairea*

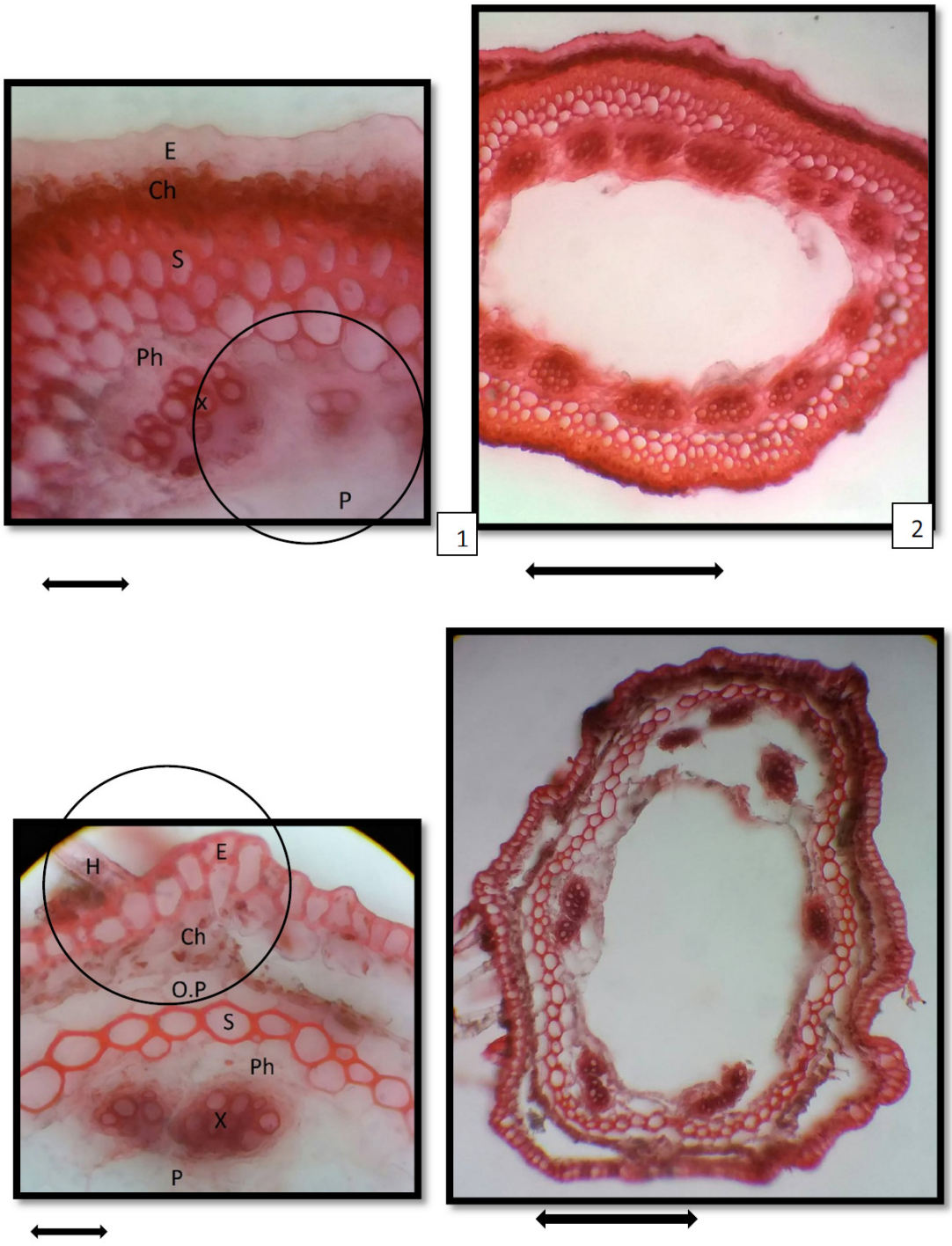

Fig. 4 : Transverse section of stem (scale 100µm)
1,2 *Spergula fallex* 3,4 *Stellaria media*

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