

Anatomical Study and Pollen Micromorphology of *Onopordum* L. in Iran

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Abstract

In this study, pollen micromorphological structure, leaf and stem anatomy structure of six species of *Onopordum* (*O. acanthium* L., *O. armenum* Grossh., *O. carduchorum* Bornm. & Beauv., *O. carmanicum* (Bornm.) Bornm., *O. heteracanthum* C. A. Mey and *O. leptolepis* DC) were examined with different repetitions. About 17 qualitative anatomical features were statistically analyzed. Glandular trichome, cortex fiber tissue, embowed in subsidiary vascular, its fiber tissue and vascular sheath extensions, adaxial collenchyma tissue and types of adaxial and abaxial stomata were distinct characters in separating of the species. In palynologic studies based on P/E ratio, 2 types of pollen grains were observed: oblate- spheroidal and suboblate. SEM study of the exine showed echinate, reticulate and perforate sculpturing in all six species.

Keywords: Asteraceae, Iran, palynology, SEM, taxonomy

Introduction

The genus *Onopordum* L. belongs to *Cardueae* tribe of Asteraceae family, native to Europe (mainly the Mediterranean region), northern Africa, the Canary Islands, the Caucasus, and southwest and central Asia (Keleonikos, 2006). It has about 40 species in world which seven species has recorded from Iran (Rechinger, 1991; Aghababaeayan 2012), one including species native (Mozaffarian, 1996). This genus is resistant to hot weather and can grow in desert regions of Iran (Karimi, 2007). Fægri *et al.* (1989) have studied the pollen grain of *Onopordum* and combined pollen of *Onopordum* with those of the genera *Saussurea*, *Arctium*, *Carlina* and *Carthamus* in their *Saussurea* type on the features of blunt spines which the high are as long as broad. Moore *et al.* (1991) included the pollen type of *Onopordum* in the *Serratula* type. Valdés *et al.* (1987) placed the pollen of *Onopordum* in *Senecio vulgaris* type, a type containing many genera like *Arctium*, *Carlina*, *Stachaelina*. But Beug (2004) considered *Cirsium*, *Doronicum*, *Senecio*, *O. acanthium* and many others in the large *Saussurea*-Typ. The *O. acanthium* type is characterized by its broad, dome-shaped echinae with small, subobtusate top and high digitate columellae of sexine which are distinctly higher in the equatorial plane than at the poles (Punt & Hoen, 2009). Osman (2009) considered *Onopordum* and *Atractylis* in *Onopordum* pollen type.

The present study considers anatomy and micromorphological study of pollen in six *Onopordum*

species occurring in Iran with the aim of providing some basic anatomical and palynological data and also to obtain the relationships between taxonomy in the one hand and anatomy and palynology in the other hand.

Materials and methods

The plant materials were collected from the natural habitats in Iran (Tab. 1).

This study have performed on 40 populations of six *Onopordum* species, namely *O. acanthium* L., *O. armenum* Grossh., *O. carduchorum* Bornm. & Beauv., *O. carmanicum* (Bornm.) Bornm., *O. heteracanthum* C. A. Mey. and *O. leptolepis* DC. (Tab.1). Anatomy of the leaf and stems, leaf epidermis and pollen morphology were examined. Leaves were fixed in 70% ethanol and stems in alcohol/glycerin (1:1). Cross sections of 1/3 of the middle part of blade and third inter nod of the stem were used, double stained by methyl green and Carmine. Appropriate samples were photographed by light photomicroscope model B×51. The epidermis was prepared using mixture of H₂O₂ and 5% sodium hydroxyl. The following equation was used to calculate the stomatal index (Cutter and Arnold, 1973):

$SI = \frac{S}{E + S}$ (SI :Stomatal index; S: guard cells; E: epidermal cells)

For studding the morphology of pollen, flowers were kept in a mixture of acetic acid 1:1 for 24 hours. The pollen were stained with Carmin and observed with lighth microscope. For scanning electron microscopy pollen were coated with a thin layer of gold. About 20-30 pollens were used in measuring polar axis, equatorial axis, colpus length,

Tab. 1: Voucher specimen of *Onopordum* specimens. Abbreviations: ALUH: Alzahra University Herbarium.

Species	Herbarium number	Address	Collector
<i>O. leptolepis</i>	ALUH, 11000	Tehran, Gisha	Aghababaeian
	ALUH, 11001	Tehran, Hesarak	Ghahremaninejad
	ALUH, 11002	Alborz, Golshahr	Aghababaeian
	ALUH, 11003	Isfahan, Semirom	Aghababaeian
	ALUH, 11004	Isfahan, 30km Shahreza	Aghababaeian
	ALUH, 11005	Isfahan, 10km Shahreza	Aghababaeian
	ALUH, 11006	Isfahan, Shahreza	Aghababaeian
	ALUH, 11007	Isfahan, Chadegan	Aghababaeian
	ALUH, 11008	Isfahan, Najafabad	Aghababaeian
	ALUH, 11009	Kohkiluyeh vabuyerahmad, Sisakht	Aghababaeian
	ALUH, 11010	Fars, Dasht arjan	Aghakuchaki
<i>O. armenum</i>	ALUH, 11011	Fars, Maharlu	Aghakuchaki
	ALUH, 11012	Karaj-Ghazvin road, 10km Ghazvin	Aghababaeian
	ALUH, 11013	Gilvan-Zanjan road, 22km Zanjan, Zaker	Aghababaeian
	ALUH, 11014	Zanjan-Miane road, 6km Miane	Aghababaeian
	ALUH, 11015	Zanjan-Ghazvin, 10km Ghazvin	Aghababaeian
	ALUH, 11016	Zanjan	Aghababaeian
	ALUH, 11017	Azarbayejan sharghi, Ahar	Aghakuchaki
	ALUH, 11018	Azarbayejan sharghi, 8km Kalibar	Aghakuchaki
	ALUH, 11020	Isfahan, Najafabad, Azad university	Aghababaeian
	ALUH, 11021	Isfahan, Vilashahr	Aghababaeian
	ALUH, 11022	Isfahan, Aliabad	Aghababaeian
<i>O. heteracanthum</i>	ALUH, 11023	Isfahan, 40km Chadegan	Aghababaeian
	ALUH, 11024	Karaj-Ghazvin road, 70km Ghazvin	Aghababaeian
	ALUH, 11025	Karaj-Ghazvin road, 10km Ghazvin	Aghababaeian
	ALUH, 11026	Kermanshah, Gilane gharb, Latechegha	Najafian
	ALUH, 11027	Azarbayejan saharghi, Arasbaran	Pakravanfard
	ALUH, 11028	Markazi, Saveh	Ghahremaninejad
	ALUH, 11029	Isfahan, 60km Naiin	Aghababaeian
	ALUH, 11030	Isfahan, 10km Naiin	Aghababaeian
	ALUH, 11031	Yazd, Taft	Aghababaeian
	ALUH, 11033	Zanjan	Aghababaeian
	ALUH, 11034	Azarbayejan sharghi, 8km Kalibar	Aghakuchaki
<i>O. carmanicum</i>	ALUH, 11035	Ghazvin, Takestan	Tayebnejad
	ALUH, 11032	Gilvan-Zanjan, 50km Zanjan	Aghababaeian
	ALUH, 11036	Azarbayejan sharghi, Bostanabad, Shebli	Tayebnejad
	ALUH, 11037	Gilan, Gilvan	Aghababaeian
<i>O. acanthium</i>	ALUH, 11038	Lorestan, 25km Poldokhtar	Aghababaeian
	ALUH, 11039	Lorestan, 15km Khoramabad	Aghababaeian
	ALUH, 11040	Lorestan, Khoramabad	Aghababaeian

pore diameter, exin thickness, number and length of spines. Spine numbers of the pollen were determined using the following equation (Christensen, 1986):

$$N = (P / \text{spines distance}) 2\pi$$

Results and discussion

Leaf and Stem: In transverse section of the leaf the midrib and upper surface was flat and the lower surface was convex (Fig. 2, 3).

A thick cuticle layer was observed on the outer surface of the stem epidermis. Several laminar layers of collenchymas were recognized under the epidermis. Under the collenchymas, parenchyma layers and in the central part,

vascular bundles could be seen which were surrounded by sclerenchyma layers. Some trichomes were observed on epidermis which some of them were simple and others were glandular, which glandular trichomes in all taxa were sessile but in *O. carmanicum* were stalked (Fig. 4). The shape of cross section of the leaves in all species except *O. armenum* were U shape while in this taxon lower surface in subsidiary vascular bundles was flat (Fig. 3- I). *O. acanthium*, *O. carduchorum* and *O. carmanicum* have dorsiventral mesophyll and *O. leptolepis*, *O. heteracanthum* and *O. armenum* have isobilateral mesophyll (Fig. 3, 4).

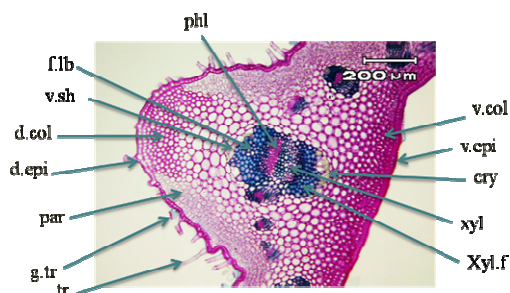


Fig. 2. Midrib of *O. leptolepis* in T.S. Tr: Trichome, G. Tr: Glandular Trichome, D. Epi: Dorsal Epidermis, D. Col: Dorsal Collenchyma, Par: Parenchyma, V. Sh: Vascular Sheath, F. Lb; Phloem Fiber, Phl: Phloem, Xyl: Xylem, Xyl. F: Xylem Fiber, Cry: Crystal, V. Col: Ventral Collenchyma, V. Epi: Ventral Epidermis. (X=60)

Tab. 2. Anatomical qualitative characters of leaf in studied species.

Species	<i>O. acanthium</i>		<i>O. carduchorum</i>	<i>O. armenum</i>	<i>O. leptolepis</i>	<i>O. heteracanthum</i>	<i>O. carmanicum</i>
Characters	<i>O. acanthium</i>	<i>O. acanthium</i> subsp.					
Transverse section shape	U shape	U shape	U shape	—	U shape	U shape	U shape
convex in subsidiary vascular bundle	*	—	*	—	*	*	*
Mesophyll	Dorsiventral	dorsiventral	Dorsiventral	isobilateral	isobilateral	Isobilateral	dorsiventral
Glandular trichome	sessile	sessile	Sessile	sessile	sessile	Sessile	stalked
Chrystal in vascular bundle	*	*	*	*	*	*	—
Chrystal in mesophyll	*	*	—	*	*	—	—
Subsidiary vascular bundle fiber	Xylem & phloem	phloem	Xylem & phloem	—	Xylem & phloem	Xylem & phloem	Xylem & phloem
Subsidiary vascular sheath extension	Upper & lower	upper	Upper & lower	upper	Upper & lower	Upper & lower	Upper & lower
Cortex fiber	—	disport	Disport	continual	disport	Disport	disport

In *O. carmanicum* vascular fiber tissue completely surrounded the vascular bundle (Fig. 4-P) but in *O. carduchorum* these fibers were located in the upper and lower bundles (Fig. 3-D) and in *O. armenum*, *O. leptolepis*, *O. heteracanthum* and *O. acanthium* were in the upper and lower bundles or completely surrounded the vascular bundles (Fig. 3, 4). Xylem parenchyma was observed in all taxa. Vascular bundle sheath layer and its extensions into the upper and lower side were similar in all taxa. Crystal was observed in vascular bundle sheath of all species except *O. carmanicum* (Fig. 4-P). Crystal in mesophyll cells was observed in *O. acanthium*, *O. armenum* and *O. leptolepis* (Fig. 3). Cortex fiber tissue continued in abaxial side in *O. armenum* (Fig. 3- I), but in *O. acanthium* was not observed (Fig. 3-B) and were disport in other species. In *O. armenum* subsidiary vascular bundles were without fiber (Fig. 3-C), this specie

and *O. acanthium* subsp. *araneosotomentosum* had vascular sheath extensions in the upper side (Fig. 3-I). The quantitative traits: thickness of cuticle, epidermis, collenchymas, xylem and phloem fiber in central vascular bundle, xylem and phloem tissues, palisade and spongy parenchyma were measured (Tab. 5).

In a ventral and dorsal view the epidermal cells had flat walls. The anemocytic stomata occurred more frequently in the lower and upper epidermis. In addition the actinocytic stomata were observed on the lower epidermis in *O. heteracanthum* (Fig. 6-F) and upper epidermis in *O. armenum* and *O. carmanicum* (Fig. 5-B, D). The anisocytic stomata were observed on the upper epidermis in *O. carduchorum* and *O. heteracanthum* (Fig. 5-D, F).

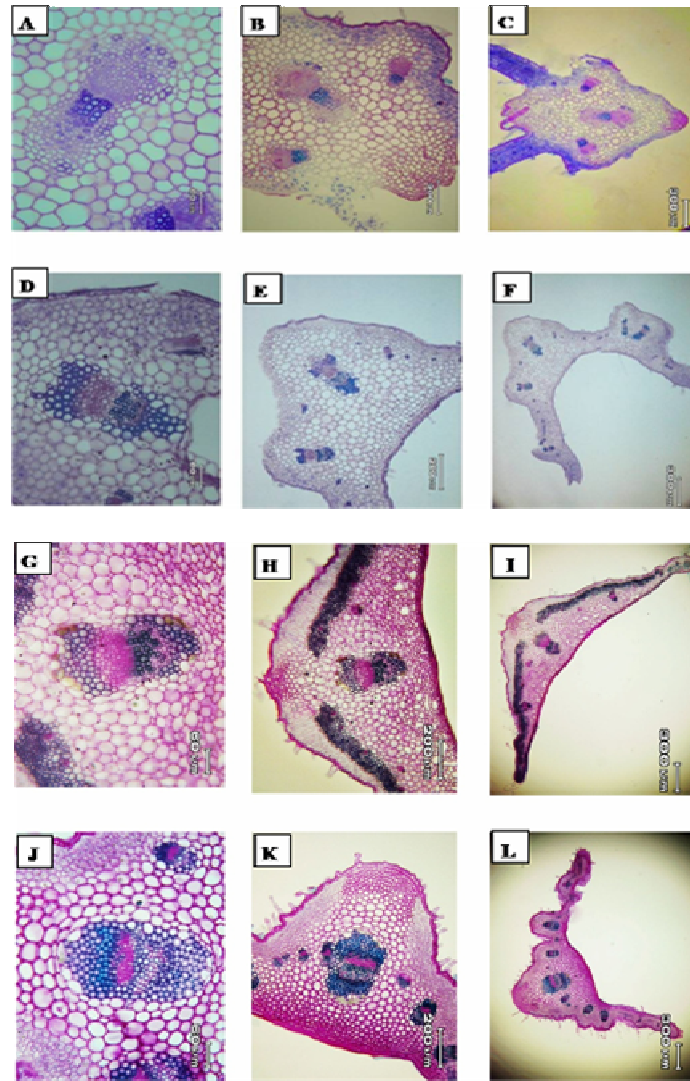


Fig. 3. Cross section of leaf in *Onopordum* species: (A-C): *O. acanthium*, (D-F): *O. carduchorum*, (G-I): *O. armenum*. (Scale bar=200μm in B, E, K, H, X= 40; Scale bar=60 μm in A, G, D, J, X= 100; Scale bar=300 μm in C, F, I, L, X=20)

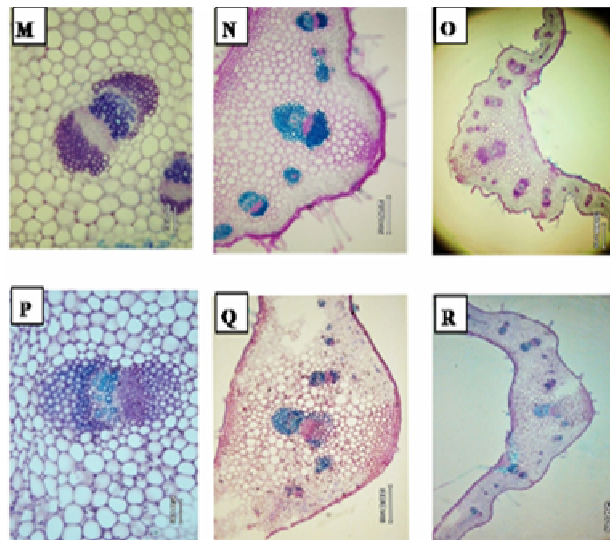


Fig. 4. Cross section of leaf in *Onopordum* species: (M-O): *O. heteracanthum*, (P-R): *O. carmanicum*. (Scale bar=60 μm μm in M, P, X= 100; Scale bar=200 μm in N, Q, X= 40; Scale bar=300 μm in O, R, X=20)

Tab. 3. Anatomical quantitative characters of leaf in studied species (The unit is μm). Le/Wid: Length of stem section/Width of stem section, Ad. Cu: Adaxial Cuticle's Thickness, Ab. Cu: Abaxial Cuticle's Thickness, Ad. Epi: Adaxial Epidermis Length, Ab. Epi: Abaxial Epidermis Length, Ad. Col. N: Adaxial Collenchyma Layer Number, Ab. Col. N: Abaxial Collenchyma Layer Number, Ad. Col. T: Adaxial Collenchymas Thickness, Ab. Col. T: Abaxial Collenchymas Thickness, Pal. Ad. N: Adaxial Palisade Layer Number, Pal. Ad. Le: Adaxial Palisade Cell Length, Pal. Ad. T: Adaxial Palisade Cell Thickness, Pal. Ab. N: Abaxial Palisade Layer Number, Spa. N: Spongy Layer Number, Spa. T: Spongy Thickness, Phl. F. N: Phloem Fiber Layer Number, Xyl. F. N: Xylem Fiber Layer Number, Phl. F. T: Phloem Fiber Thickness, Phl. T: Phloem Thickness, Xyl. T: Xylem Thickness.

Species Characters	<i>O. acanthium</i>	<i>O. acanthium</i> subsp.	<i>O. carduchorum</i>	<i>O. armenum</i>	<i>O. leptolepis</i>	<i>O. heteracanthum</i>	<i>O. carmanicum</i>
Le/wid	4.1	2.7	4.2	3.7	3.6	6.3	4.5
Ad.cu	6.37	14.02	5.1	7.1	8.9	7.9	10.3
Ab.cu	5.92	10.57	4.6	7.3	7.5	7.3	7.4
Ad.epi	14.99	16.62	11	13.5	11.2	12.8	16.2
Ab.epi	10.74	7.96	12.8	10.9	13.8	13.5	16.9
Ad.col.n	1	1	1	1	1	2	1
Ab.col.n	6	6	5	7	7	6	5
Ad.col.T	19.66	17.82	17.3	16.6	21.7	28.6	23.9
Ab.col.T	99.44	96.72	88.34	112.32	156.2	118.25	108.69
Pal.Ad.n	2-3	2_3	2	2-3	2-3	2-3	3
Pal.Ad.le	33	—	41.8	31.35	27.8	27.3	29.1
Pal.Ad.T	89.7	—	79.7	77.55	65.3	79.3	94.3
Pal.Ab.n	—	—	—	1_3	1_3	2_3	—
Spa.n	4-7	3	4-5	2-4	2-5	4-5	3-5
Spa.T	72.91	59.98	88.9	63.8	75.6	50.5	73.2
Phl.F.n	6-9	6	5	4-7	4-10	6-12	5-12
Xyl.F.n	6	4	6	3-5	3-10	5-13	5-9
Phl.F.T	93.04	73.8	78.9	69.5	91	129.8	134.2
Xyl.F.T	83.94	41.3	81.5	58	92.4	113.3	111.4
Phl.F/Xyl.F	1.17	1.7	0.9	1.3	1	1.1	1.1
Phl.T	73.8	35.95	94.9	51.4	50.3	54.2	58.5
Xyl.T	100.61	51.98	88.8	58.2	66.3	78	53.5
Phl/Xyl	0.73	0.69	1	0.9	0.7	0.5	0.7

Cross section of the stem included cuticle layer, trichomes, epidermal cells, parenchyma, collenchymas, phloem and xylem fiber and vascular bundles (Fig.7). The shape of cross section of the stem was angular in *O. acanthium* while were circular in other taxa (Fig. 5-A). Simple and glandular trichomes were observed in all taxa,

but glandular trichomes with stalk were just observed in *O. carmanicum* and *O. carduchorum* (Fig. 5-B, D). Crystals observed in vascular sheath of all the species except for *O. carmanicum*. Measured characters are shown in the Tab. 5.

Tab. 4. Stomatal character of leaf In Studied Species. LL: Lower Layer, LU: Upper Layer, SI: Stomata Index.

Species	epidermis	Stomatal type	SI
<i>O. acanthium</i>	LL	Anemocytic	6.7
	LU	Anemocytic	9.13
<i>O. carduchorum</i>	LL	Anemocytic	4.12
	LU	Anemocytic, Anizocytic	4
<i>O. armenum</i>	LL	Anemocytic	7.1
	LU	Anemocytic, Actinocytic	8.4
<i>O. leptolepis</i>	LL	Anemocytic	13
	LU	Anemocytic	2.5
<i>O. heteracanthum</i>	LL	Anemocytic, Actinocytic	4.5
	LU	Anemocytic, Anizocytic	6.2
<i>O. carmanicum</i>	LL	Anemocytic	7
	LU	Anemocytic, Actinocytic	7

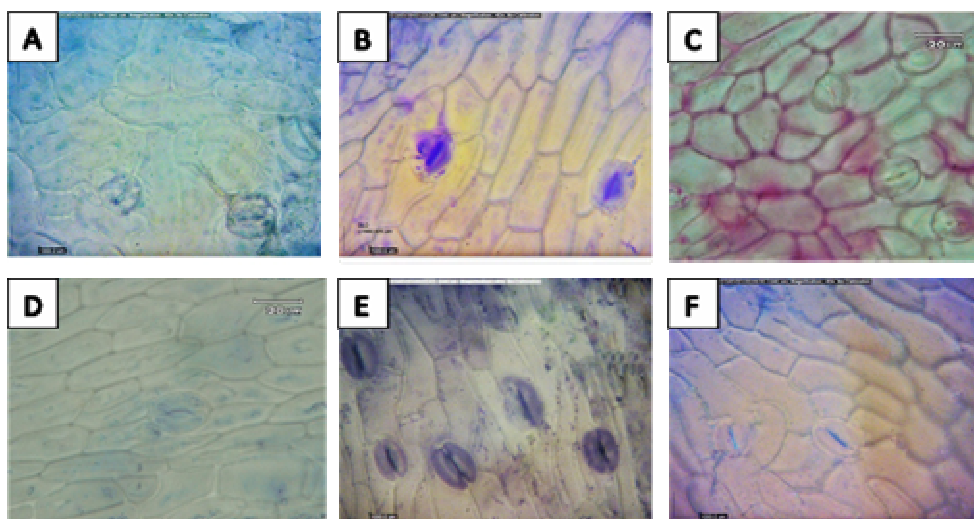


Fig. 5. Upper epidermis in : (A): *O. acanthium*; (B) : *O. armenum*; (C) : *O. carmanicum*; (D) : *O. carduchorum*; (E) : *O. leptolepis*; (F) : *O. heteracanthum*. (Scale bar=20 μ m; X= 400)

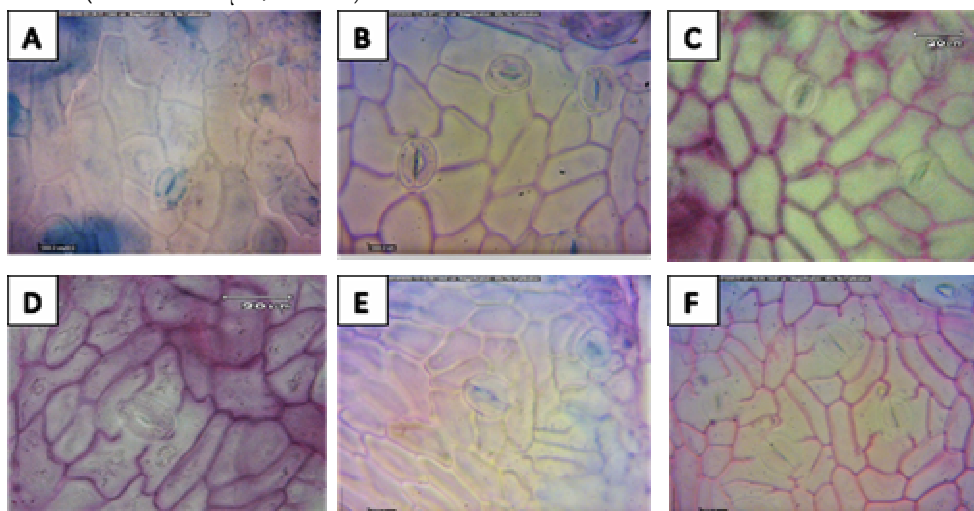


Fig. 6. Lower epidermis in : (A): *O. acanthium*; (B) : *O. armenum*; (C) : *O. carmanicum*; (D) : *O. carduchorum*; (E) : *O. leptolepis*; (F) : *O. heteracanthum*. (Scale bar=20 μ m; X= 400)

Tab. 5. Anatomical quantitative characters of stem in studied species (The unit is micrometer). Cu. T: Cuticle Thickness, Epi. T: Epidermis Thickness, Epi. Le: Epidermis Cell Length, Col. T: Collenchymas Thickness, P. C. T: Parenchyma Thickness, Ecl. Sh. T: Sclerenchymatous Sheaths Thickness, F. Lb. T: Phloem Fiber Thickness, Xyl. F. T: Xylem Fiber Thickness, Phl. T: Phloem Thickness, Xyl. T: Xylem Thickness.

Species	<i>O. acanthium</i>	<i>O. acanthium</i> subsp.	<i>O. carduchorum</i>	<i>O. armenum</i>	<i>O. leptolepis</i>	<i>O. heteracanthum</i>	<i>O. carmanicum</i>
Characters							
Cu.T	3.03-4.33 3.87	4.02	1.96-2.57 2.35	3.12-6.59 4.52	3.06-7.11 4.84	3.69-8.88 5.57	6.26-7.45 7.06
Epi.T	13.55-15.69 14.9	15.33	10.81-15.7 13.61	9.6-17.25 12.76	10.41-18.12 14.37	11.16-23.39 17.09	15.43-18.04 17.22
Epi.le	9.03-12.87 10.64	6.83	11.03-15.12 12.67	5.18-11.06 8.5	6.8-13.73 9.33	8.08-12.99 10.66	14.69-20.98 17.2
Col.T	122.93-253.72 188.92	73.77	108.45-180.97 146.67	72.16-252.75 164.4	107.97-369.31 201.44	89.54-332.18 204.98	222.68- 373.09 268.37
p.c.T	139.46-308.48 188.52	130	343.22-451.92 575.79	53.8-289.48 169.17	107.02-263.26 204.89	116.61-400.99 233.52	217.26- 494.36 358.66

Ecl.sh.T	446.72-692.12 557.75	570.1	483.31-699.54 575.79	413.12-830.97 593.73	218.31- 1010.67 764.22	432.77- 1059.59 661.73	472.64-698.29 607.82
f.lb.T	135.19-236.21 191.13	182	165.62-298.7 229.95	53.91-298.83 177.88	157.91-283.68 221.07	120.49-317.65 206.05	102.71-234.3 170.09
Xyl.f.T	249-339.88 233.84	172.8	120.56-254.85 220	128.23-229.87 173.41	87.89-282.64 217.77	127.59-373.28 228.1	67.74-145.53 110.01
f.lb.T/xyl.f.T	0.5-1.2 0.77	1	0.7-1.3 1.02	0.4-1.3 0.96	0.77-1.7 1.01	0.6-1.3 0.92	1.4-1.7 1.5
Phl.T	57.78-96.72 76.71	57.78	64.26-111.37 84.41	43.17-129.94 74.91	44.89-103.07 66.28	40.52-82.19 62.05	60.66-94.87 82.39
Xyl.T	100.49-266.65 226.46	100.4	109.26-243.75 179.5	98.16-176.21 133.38	102.85-339.65 187.95	99.79-210.09 202.26	111.02-192.76 145.74
Phl.T/xyl.T	0.2-0.57 0.33	0.57	0.32-0.58 0.47	0.31-0.9 0.53	0.2-0.55 0.35	0.27-0.65 0.42	0.5-0.6 0.53

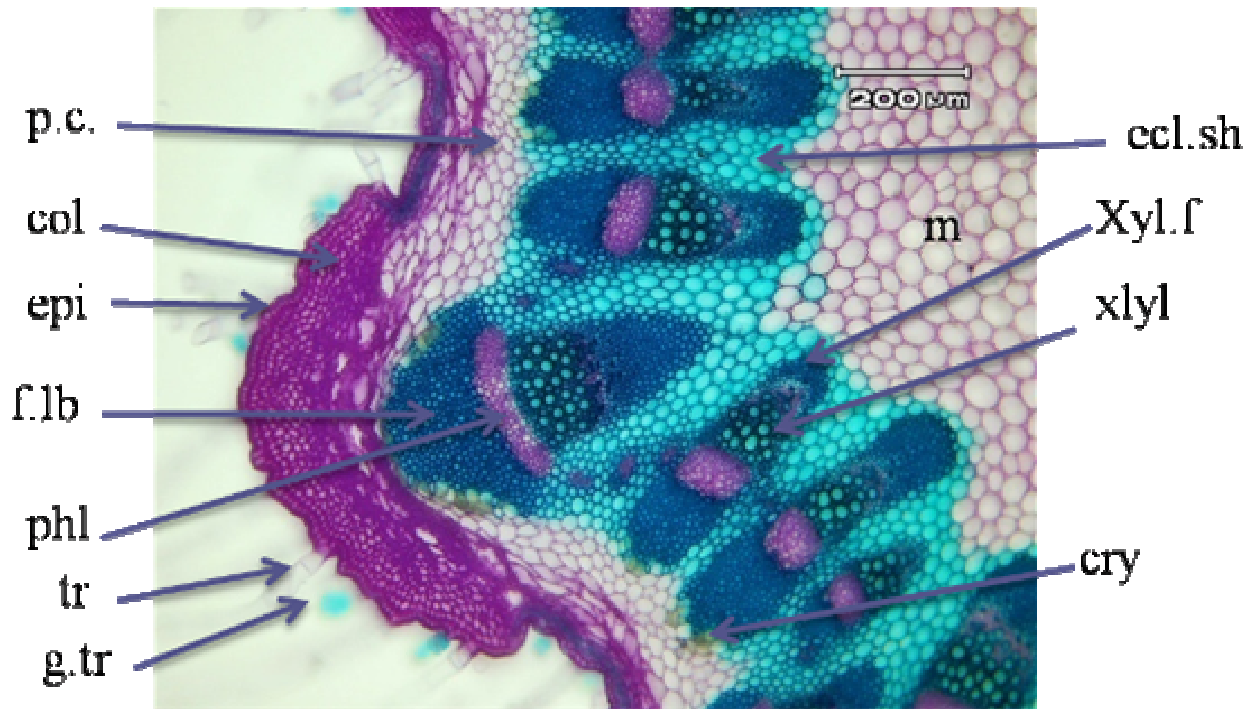


Fig. 7. The cross section of stem, in *O. armenum*. Tr: Trichome, G. Tr: Glandular Trichome, Epi: Epidermis, Col: Collenchyma, P. C: Parenchyma, Ecl. Sh: Sclerenchyma, F. Lb: Phloem Fiber, Phl: Phloem, Xyl: Xylem, Xyl. F: Xylem Fiber, Cry: Crystal, M: Medulla. 00μm in B, E, K, H; Scale bar=200 μm; X= 50)

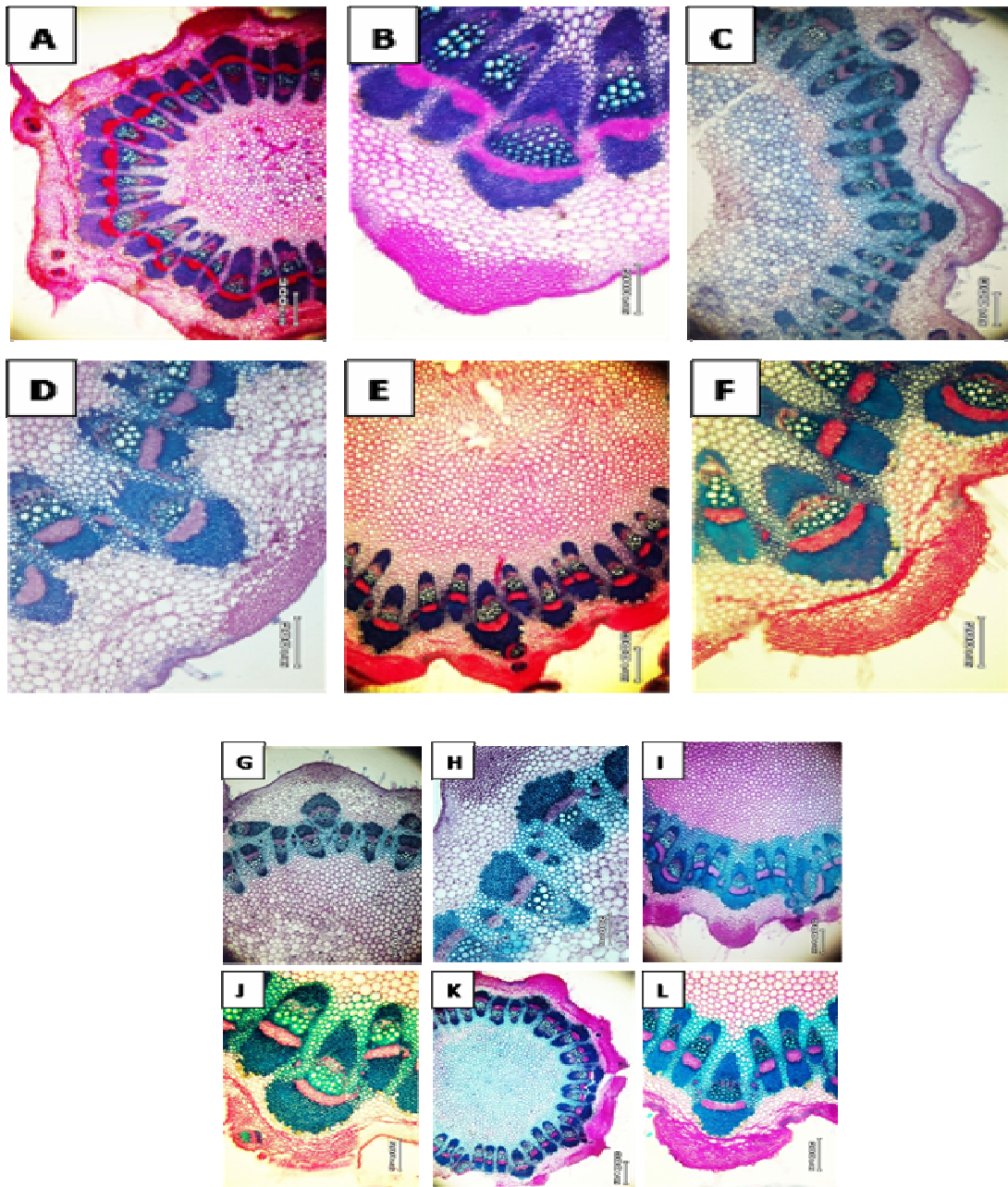


Fig. 8. Cross section of stem in *Onopordum* species: (A-B): *O. acanthium*; (C-D): *O. carduchorum*; (E-F): *O. leptolepis*; (G-H): *O. carmanicum*; (I-J): *O. heteracanthum*; (K-L): *O. armenum*. (Scale bar=200 μm ; B, , F, H, J, L; X= 40; (A, C, E, G, I, K: X= 20)

Pollen characters: All taxa had 3-zonocolporate pollen grains and Amb were rounded triangular or 3-lobate and elliptic equatorial view. According to P/E ratio; *O. acanthium* and *O. leptolepis* were oblate-spheroidal and other suboblate (Tab. 6). Exin had echinate sculpture with the different length and number of spine in different species (Fig. 9). Heterobrochate sculpture in *O. carduchorum* and *O. heteracanthum*, microreticulate

sculpture in *O. acanthium* and perforate sculpture in *O. carmanicum* and *O. leptolepis* were observed (Fig. 8). Lumen with 0.2-1.26 μm diameter in *O. carduchorum*, with 0.44-1.155 μm diameter in *O. heteracanthum* and with in 0.3-0.53 μm *O. acanthium* were observed. Perforate diameter in *O. leptolepis* were 0.2-0.76 μm and in *O. carmanicum* 0.26-0.46 μm (Tab. 6).

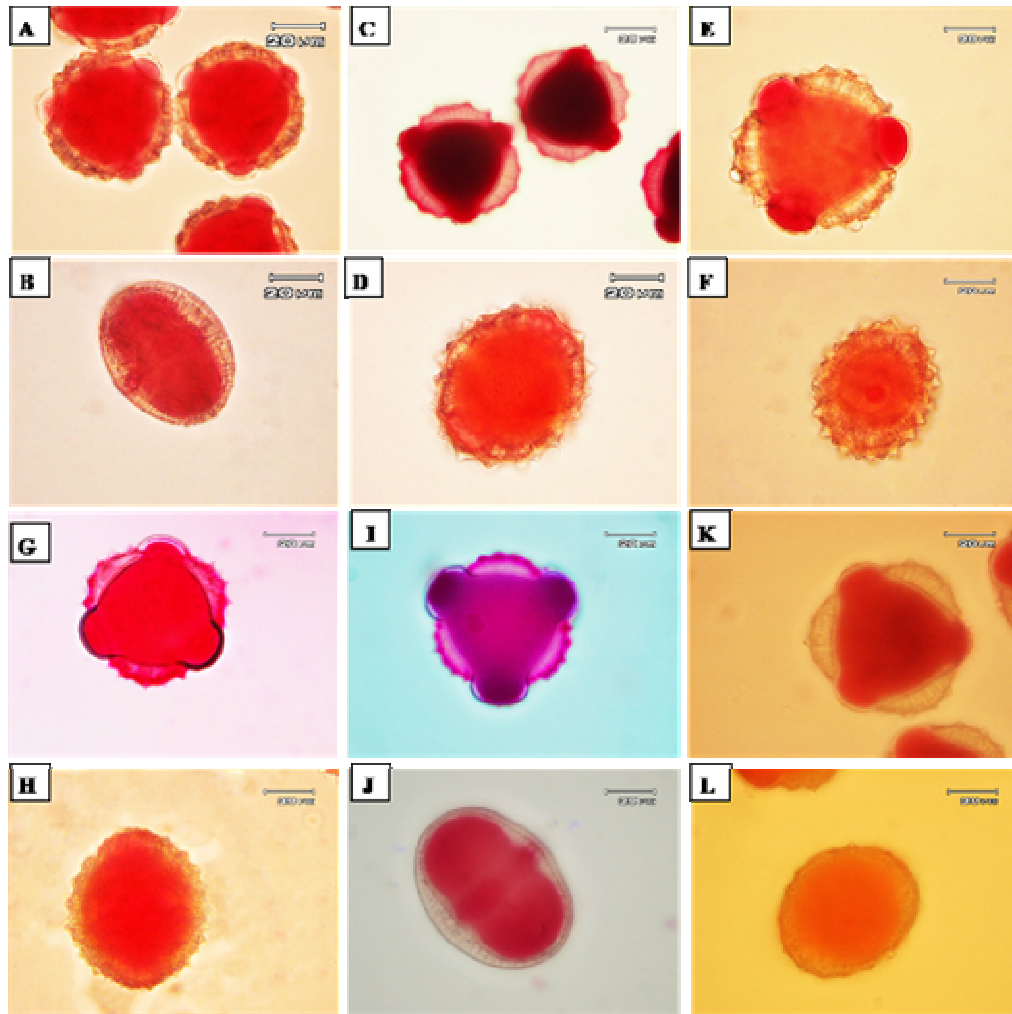


Fig. 8. Pollen grains in *Onopordum* species by light microscope. (A, B): *O. acanthium*, (C, D): *O. carduchorum*, (E, F): *O. armenum*, (G, H): *O. leptolepis*, (I, J): *O. heteracanthum*, (K, L): *O. carmanicum*. (Scale bar=20 μ m, X= 400)

Tab. 6. Tabular summary showing the pollen grains dimensions (The unit is micrometer). P: Polar axis, E: Equatorial axis, Cl: Colpus Length, Po. Di: Pore Diameter, Et: Exin Thickness, N. Sp: Number of Spines, Le. Sp: Length of Spines, OS: Oblate Spheroidal, SO: Suboblate.

Species		<i>O. acanthium</i>	<i>O. carduchorum</i>	<i>O. armenum</i>	<i>O. leptolepis</i>	<i>O. heteracanthum</i>	<i>O. carmanicum</i>
Characters	Min	43.57	41.27	48.1	49.31	51.68	45.29
	Mean	45.93	51.62	55.05	55.99	52.63	49.62
P	Max	47.84	63.53	51.19	59	53.59	59.52
	Min	47.3	56.43	59.32	58.18	59.3	56.47
E	Mean	50.37	66.41	63.54	62.28	63.03	58.11
	Max	53.85	71.12	66.66	66.55	66.71	60.74
P/E	0.91	0.77	0.86	0.89	0.83	0.85	
shape	OS	So	So	OS	So	So	
CL	52.50	66.68	39.93	50	54.19	53.76	
Po.di	15.88	23.36	19.02	22.28	20.33	20	
ET	6.8	10.89	11.86	11.21	10.25	10.58	
N.Sp	100	135	148	153	104	99	
Le.Sp	1.7	1.44	4.18	3.99	3.13	2.5	

Phenetic analysis: In order to find most variable traits, principal component analysis was implemented. Primitive analysis showed that three first factors were responsible for the 80% of total studied variation in taxa. In the first factor with almost 35% of the total variation, leaf cross section shape, lower surface in subsidiary vascular bundles and vascular sheath extensions had the highest positive correlations. In the second factor with nearly 24% of observed variation, cortex fiber and subsidiary vascular bundles fiber had the highest positive correlation. In the third factor with 20% of total variation, upper leaf collenchymas and leaf stomata had the highest positive correlations.

By studding cluster analysis using anatomical characters (Ward method) showed the clear separation of the species studied (Fig.1). Two clusters were observed. The first cluster was composed of *O. acanthium*, *O. leptolepis*, *O. heteracanthum* and *O. armenum*. The species of *O.*

carduchorum and *O. carmanicum*, were positioned in the second cluster. *O. acanthium* and *O. leptolepis* had similar stomata on their ventral epidermis and crystals in their mesophyll cells, they had also oblate-spheroidal pollens. *O. acanthium* had minimum number of pollen grain, pore diameter and exin thickness, this species was the only one with angular shape of transverse section of the stem. Results revealed that *O. acanthium* and *O. leptolepis* were closely related, they have similar stomata on ventral epidermis. Dendrogram showed the close relationships between *O. carmanicum* and *O. carduchorum*, both having glandular trichomes with stalk on their stems. *O. armenum* was located in a separate cluster by itself, because of cortex fiber tissue, cross section shape, lower surface in subsidiary vascular bundles and subsidiary vascular bundles fiber.

Dendrogram using Ward Method

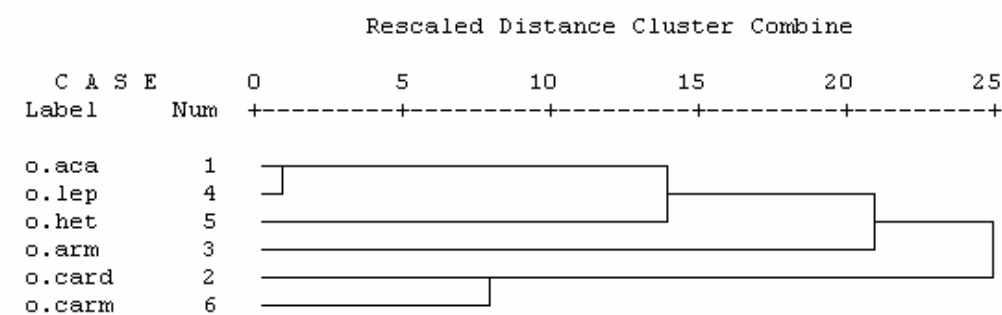


Fig.1: Dendrogram by Ward method for *Onopordum* species studied. 1: *O. acanthium*, 2: *O. carduchorum*, 3: *O. armenum*, 4: *O. leptolepis*, 5: *O. heteracanthum*, 6: *O. carmanicum*.

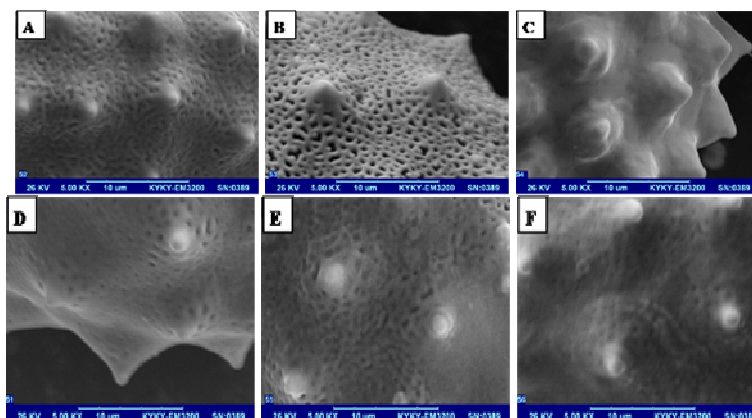


Fig. 9. SEM picture of exin in species of *Onopordum*: (A): *O. acanthium*, (B): *O. carduchorum*, (C): *O. armenum*, (D): *O. leptolepis*, (E): *O. heteracanthum*, (F): *O. carmanicum*. (X= 3600)

Our results showed that in anatomical study, *O. armenum* could be easily identified because of certain characters that has mentioned above. *O. armenum* comprised a separate cluster by itself, more over this species was the only species without microreticulate or perforate sculpture of the pollens, also it had the maximum length of spines. *O. carduchorum* and *O.*

carmanicum had glandular trichomes with stalk, similar number of abaxial collenchymas layers, and convex midrib in abaxial surface, phloem thickness and xylem thickness. Pollens were suboblate in both species and the exine thickness was similar. They could be distinguished from each other by maximum polar axis, maximum equatorial axis, colpus diameter and pore diameter (Table 6).

We concluded that *O. acanthium* and *O. leptolepis* are closely related species and *O. armenum* is an independent species that is separated from other species.

Also it has shown that anatomical characters such as leaf shape in cross section, subsidiary vascular bundles in leaf, vascular sheath, presence of fiber and subsidiary vascular bundles fiber in cortex, stomata type and index, presence of crystal, mesophyll type, trichome type in leaf and stem were diagnostic characters in the genus *Onopordum*. More over P/E ratio, lumen diameter and number of spine were useful characters in distinguishing the species.

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