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مطالعه ریخت‌شناسی، آناتومی و گرده‌شناسی سرده خورشید صبح (تیره کاسنیان) در ایران

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چکیده. سرده *Zoegea* L. از تیره کاسنیان، به‌طور تقریبی دارای ۱۰ گونه در جهان است. این سرده به عنوان یک عنصر ایرانی-تورانی و مدیترانه‌ای شناخته می‌شود که در جنوب غربی و مرکز آسیا و در مرکز، جنوب، شمال غرب و جنوب غربی ایران گسترش یافته است. طبقه‌بندی زیرگونه‌های این سرده در منابع مختلف آرایه‌شناسی متفاوت است. در این پژوهش تفاوت‌های گونه‌های نواحی مختلف ایران مورد مطالعه قرار گرفته است. به‌علاوه ویژگی‌های آناتومی و گرده‌شناسی نیز برای انجام آنالیز خوشه‌ای و تعیین حدود آرایه‌ها مورد استفاده قرار گرفتند. درنهایت این مطالعه نشان داد که *Z. baldschuanica* و *Z. glabricaulis* گونه‌های مستقلی هستند.

واژه‌های کلیدی. مرکبان، پالینولوژی، تشریح، مورفولوژی، میکروسکوپ الکترونی نگاره

Morphological, anatomical and palynological studies of the genus *Zoegea* L. (Asteraceae) in Iran

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Abstract. The genus *Zoegea* L. belongs to Asteraceae family and has about 10 species in the world. This genus is considered to be an Irano-Turanian and Mediterranean element and is distributed in south-western and central Asia and in the central, southern, north-western and south-western parts of Iran as well. The subspecies classification of the genus is not consensus and various classifications could be found in different taxonomy resources. In this study various specimens from different regions of Iran were studied. In addition, anatomical and palynological characters were used to perform a cluster analysis in order to determine species groups. In the end, our results confirmed that Z. *baldschuanica* and Z. *glabricaulis* were distinct species.

Keywords. anatomy, compositae, middle east, morphology, palynology, SEM

INTRODUCTION

The genus *Zoegea* L., also known as Khorshid-e Sobh in Persian, belongs to the Asteraceae family and is classified in Cardueae tribe and Centaurinea subtribe. In the Centaureinae subtribe, the high variation of morphological characters makes the taxonomy of the genus highly problematic. *Zoegea* has unusual combination of plesiomorphic morphological characteristics and apomorphic pollen types (Wgenitz & Hellwig, 1996). Involucral bracts characters and basic chromosome number (x=14 and x=15) are plesiomorph. Therefore, it was regarded as an isolated genus in Centaureinae.

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Later palynological studies showed that *Zoegea* has serratula type pollen (Martin & GaciaGarcia-Jacea 2000). Therefore, both morphological and palynological characteristics had confirmed that *Zoegea* has a basic status in phylogenetic tree. Based on different phylogenetic studies (Gacia-Jacea *et al*., 2002; Gacia-Jacea *et al*., 2001) *Zoegea* is considered to be a monophyletic genus, but there isn't a consensus idea on the situation of *Zoegea* in the subtribe Centaureinae (Funk *et al*., 2005).

Three species of *Zoegea* grow in Iran, Turkey, and Egypt and generally in the central and western zonesof Asia (Funk *et al*., 2005; Kubitzki, 2007; Mabberley, 2008). There are 7 taxa in the area of Flora Iranica: Z. *purpurea* Fres., *Z.crinita* subsp. *crinita* Boiss., Z. *crinita* subsp. *baldschuanica* (C.Winkl.) Rech.f., Z. *crinita* subsp. *glabricaulis* (Czerep.) Rech.f., Z. *leptaurea* L. subsp. *leptaurea*, Z. *leptaurea* subsp. *mesopotamica* (Czerep.) Rech.f., Z. *leptaurea* subsp. *mianensis* (Boiss.) Rech.f. All of these taxa, except *Z. leptaurea* subsp. *leptaurea*, were also reported from Iran, although its presence in the whole area of Flora Iranica is doubtful (Wagenitz, 1980). In this treatment four species were reduced to subspecies rank.

Except some palynological studies (Wagenitz, 1955; Wagenitz & Hellwig, 1996; Garcia-Jacas et al., 2002) there are no significant anatomical and morphological studies in the genus *Zoegea*. In this study, various anatomical and morphological features of Iranian members of the genus *Zoegea* were investigated for the first time.

MATERIALS AND METHODS

Taxonomy and morphology

In addition to our own collections (ALUH) from different provinces of Iran (i.e., Fars, East Azarbayejan, Lorestan, Khuzestan and Bushehr), specimens of TARI, TUH, KAR and RNAK herbaria were studied. The specimens that have been used in palynological and anatomical studies are listed in Table 1. In this study, 21 qualitative and 17 quantitative characters of more than 115 plant samples were measured and used for the morphological studies (Table 2). SPSS software (ver. 18) and Ward's and Canonical Variate Analysis (CVA) methods were used for statistical analysis.

Table 1. The list of specimens used in palynological and anatomical studies.

Species	Examined specimens
Zoegea crinita subsp. *baldschuanica*	Khorassan province: Sarakhs, Cheshmeshuran, 490 m a.s.l., 21 May 1972, H. Foroughi 4266 (TARI); Kermanshah province: Bistun, Kamijeh, 1600 m a.s.l., 3 June 1997, M. Nemati & A. Qaderi 6599 (RANK).
Z. crinita subsp. *crinita*	Hormozgan province: Ca. 170 km from Bandar-Abbas to Sirjan Hajiabad, 900 m a.s.l., 5 January 1995, V. Mozaffarian 74249 (TARI); Kermanshah province: Paveh, the hill above sarab houli, 1500-1800 m a.s.l., 18 June 1987, Hamzehee 1232 (TARI); Kermanshah province: Bistun mountain, 1340 m a.s.l., 3 June 1997, Nemati & Qaderi 5528 (RANK); Fars province: Bamu protected region, Tange Chah Mahaki, 1800-2000 m a.s.l., 1 June 1975, P. Wendelbo & H. Foroughi 17731 (TARI).
Z. crinita subsp. *glabricaulis*	Fars province: Near Khonj, 15 April 1983, M. Assadi & Bazgosha 41581 (TARI); Fars province: 36 km from Khonj to Lar, 700 m a.s.l., 15 April 1983, M. Assadi & Bazgosha 41669 (TARI); Kermanshah province: Qasr-e shirin, Imam Hasan, 600 m a.s.l., 1 June 1996, M. Nemati 6394 (RANK).
Z. leptaurea subsp. *mianensis*	Khuzestanprovince: Sardasht to Behbahan, 20 km to behbahan, 700 m a.s.l., F. Attar 11004 (ALUH); Kordestan province: 101 km from Marivan on road to Paveh between Nowsud and Paveh, 1000 m a.s.l., 5 Mey 1978, H. Runemark & V. Mozaffarian 27435 (TARI); Lorestan province: Khoramabad, 33°42′51″N, 48°26′30″E, 1676 m a.s.l., 31 March 2013, Kh. Mahmoodi 11002 (ALUH).
Z. purpurea	Hormozgan province: 15 km from diviation of Minab, Rudan road to Rudan, 500 m a.s.l., 5 June 1982, V. Mozaffarian, Banihashemi & Shahinzadeh 39470 (TARI); Hormozgan province: West slope of Kuh-e Genu, N. of Tazian, 500-900 m a.s.l., 24 April 1985, V. Mozaffarian 49549 (TARI); Esfahan province: Ghameshloo protected area; Koohe Arre Khar, 2200 m a.s.l., 6 June 1996, M. Uosofi 1455-1 (TARI): Khorassan province: Esferayen, Shah Jahan Mts. Region Rocky, soily Mt., Tourkan from deep gorge close to Noushirvan village, 1400-2500 m a.s.l., 8 June 1984, V. Mozaffarian 48569 (TARI): Yazd province: 14 km to Anarak on the road, from Chupanan, 1450 m a.s.l., 6 June 1986, M. Assadi & Bazgosha 56545 (TARI).

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Table2. Characters used in morphological study

Symbol	Qualitative characteristics	Symbol	Quantitative characteristics
IT	Indumentums Type	LH	Herb Length
EP	Embranchment place	PedL	Peduncle Length
LIT	Leaf Indumentums Type	EPhR	External Phyllary Row
PBL	Petiole in Basal Leaf	AL	Awns Length
PCL	Petiole in Cauline Leaf	PhAL	Phyllary Appendix Length
BLT	Basal Leaf Type	NPhAC	Number of Phyllary Appendix Cilium
CLT	Cauline leaf Type	IL	Inflorescence Length
CLA	Cauline leaf Apex	RL	Radiate floret Length
PH	Pedicle of Head	AcL	Achene Length
IPH	Indumentums in Pedicle of Head	PL	Pappus Length
ESh	Envelope Shape	PR	Pappus Row
IPhA	Internal Phyllary Apex	IPhL	Internal Phyllary Length
IPhT	Internal Phyllary Type	EPhL	External Phyllary Length
TA	Tooth in base of Awns	MLL	Medial Leaf Length
LBL	Lacinia in Base of Limbus	MLW	Medial Leaf Width
IF	Indumentums in Filament	EPhR	External Phyllary Row
BF	Bulge in base of Filament	IPhR	Internal Phyllary Row
FC	Flower Color	-	-
AC	Achene Color	-	-
AI	Achene Indumentums	-	-
IPC	Internal Pappus Color	-	-

Anatomy

This study was conducted on 25 populations of 5 taxa of *Zoegea* including Z. *purpurea*, *Z.crinita* subsp. *crinita*, Z. *crinita* subsp. *baldschuanica*, Z. *crinita* subsp. *glabricaulis*, and Z. *Leptaurea* subsp. *Mianensis* (Table 1). No specimen of *Leptaurea* subsp. *mesopotamica* was available. Leaf and stem anatomy, leaf epidermis, achene and pollen morphology were examined in anatomical study. Leaves were fixed in 70% ethanol and stems in alcohol/glycerine (1:1). Cross sections of the middle part of blade and the third internode of the stem were used and double stained by methyl green and Carmine. Appropriate samples were photographed by means of an Olympus BX51 light microscope. The epidermis was prepared using by the mixture of H_2O_2 and 5% sodium hydroxyl. The following equation was used to calculate the stomatal index: SI= S/E+ S (SI: Stomatal index; S: guard cells; E: epidermal cells).

Palynology

In order to study the pollen grains, flowers were kept in a mixture of absolute acetic acid and ethyl alcohol (96%) 1:1 for 24 hours. The pollen was stained with Carmin and observed by means of an Olympus BX51 light microscope. In order toperform scanning electron microscopy, pollens were coated with a thin layer of gold and observed by means of a SEMTESCAN model VEGA3 Company. About 10-20 pollens were used in measuring polar axis, colpus length, equatorialaxis, pore diameter, exin thickness, number and length of spines. Terminology follows Erdtman (1943). Herbarium codes used follows Thiers (2014).

RESULTS AND DISCUSSION

Morphology

In the dendrogram drawn by Ward's method (Fig. 1), based on leaf character at 25 level, two clusters were separated including Z. *crinita* subsp. *baldschuanica* and Z. *crinia* subsp. *glabricaulis* in one cluster and the rest taxa in the other. Another notable point is that the Z. *crinita* subsp. *crinita* has more affinity with other species of this genus in comparison with the two other subspecies of *Z. crinita*. Results of CVA analysis based on quantitative and qualitative traits have been shown in Fig. 2 and Fig. 3, respectively. This analysis also determined most important quantitative characters (Table 3). Among them, radiate floret length, inflorescence length, pappus row and number of phyllary appendix cilium showed the highest correlation in the first function. Medial leaf width and pappus length showed largest absolute correlation with the second discriminant function. In the third function external phyllary row, medial leaf length and herb length showed the largest absolute correlation.

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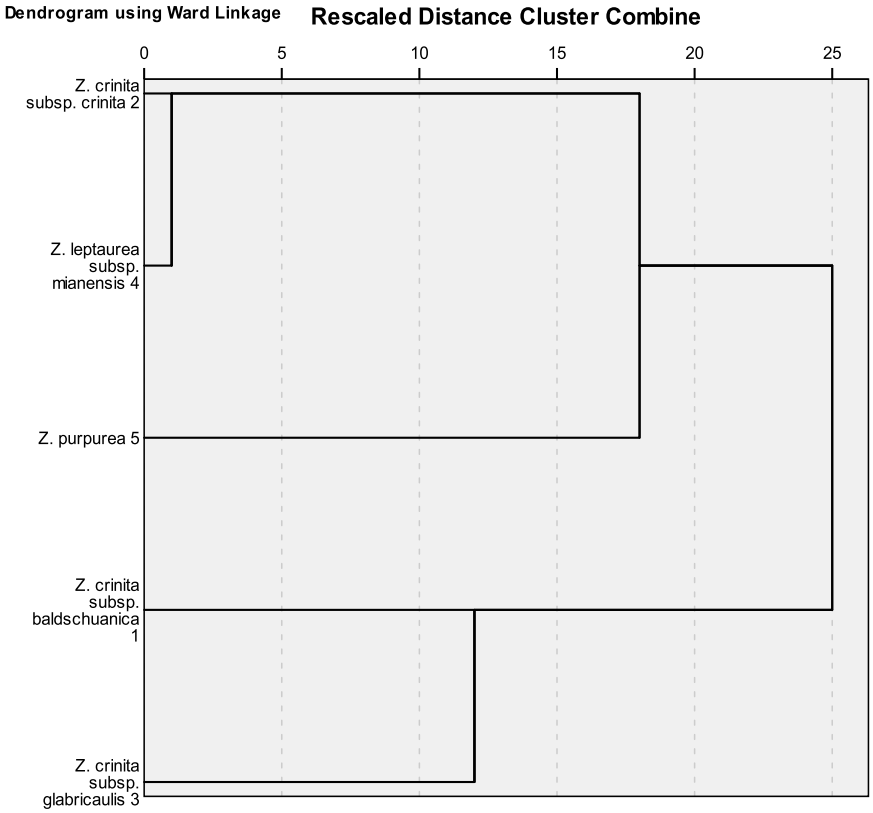

Fig. 1. The dendrogram of cluster analysis by Ward method on the quantitative morphological characters.

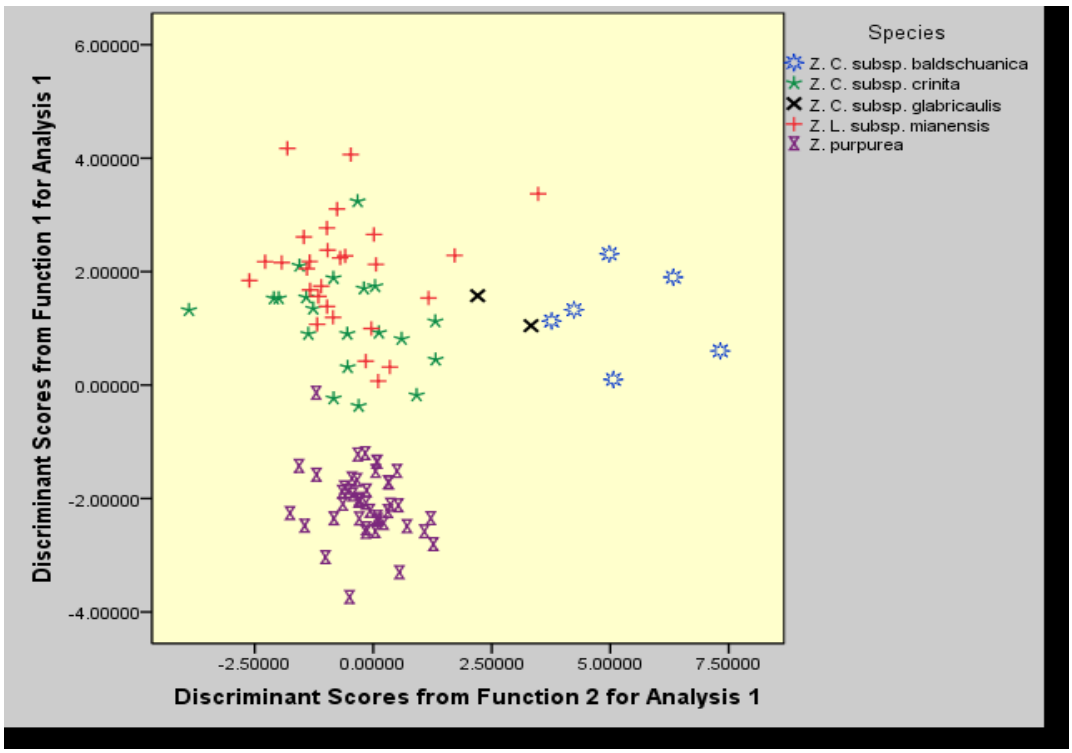

Fig. 2. CVA analysis based on main quantitative morphological characters.

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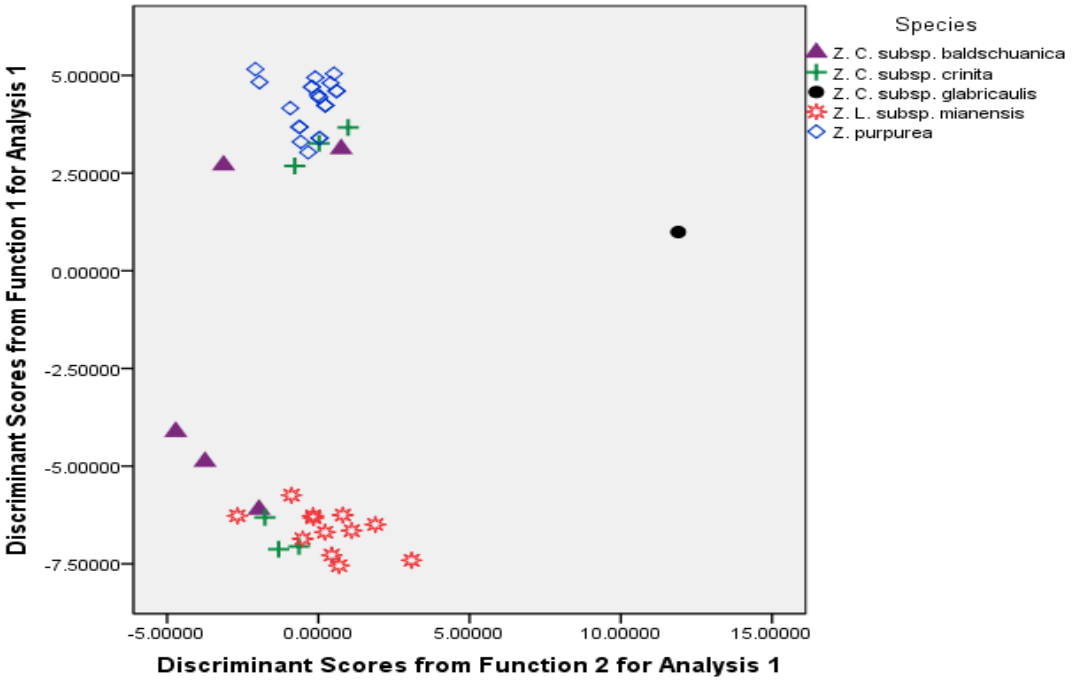

Fig. 3. CVA analysis based on main qualitative Morphological characters.

In the fourth function external phyllary length, internal phyllary length and phyllary appendix length also showed the largest absolute correlation. Also, CVA analysis determined the most important qualitative characteristics (Fig. 3). The first function did not show any correlated characters. In the second function character of leaf indumentums type showed correlation between species. Indumentums type, flower color, internal pappus color, achene indumentums, indumentums in pedicle of head, internal phyllary type, internal phyllary apex and achene color showed the largest absolute correlation in the third function. In addition, in the fourth function embranchment place, petiole in basal leaf, envelope shape and cauline leaf apex showed absolute correlation (Table 4).

Table 3. The list of quantitative morphologic characters ranked by CVA analysis.

Structure Matrix				
Characters	**Function**			
	1	2	3	4
Radiate floret length	.725*	.021	.082	-.003
Inflorescence length	.574*	.038	.310	.117
Pappus row	.520*	-.078	.115	.224
Number of phyllary appendix cilium	.404*	-.035	-.075	.376
Achene length	-.024*	.011	.005	.005
Pappus length	.216	.540*	.094	.242
Medial leaf width	.078	.321*	.122	-.140
External phyllary row	.216	.223	.599*	.008
Medial leaf length	-.099	-.112	.475*	-.012
Herb length	-.074	.118	.391*	-.203
External phyllary length	-.087	.220	.422	.592*
Internal phyllary length	.354	.006	.132	.512*
Internal phyllary rowa	.354	.006	.132	.512*
Phyllary appendix length	.354	.334	.315	.415*
Pooled within-groups correlations between discriminating variables and standardized canonical discriminant functions Variables ordered by absolute size of correlation within function.				
* Largest absolute correlation between each variable and any discriminant function				
a. This variable not used in the analysis.				

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Table 4. The list of qualitative morphologic characters ranked by CVA analysis.

Structure Matrix				
Characters	**Function**			
	1	2	3	4
Leaf indumentums type	-.067	.561*	.291	.080
Indumentums type	.025	-.256	.731*	.203
Flower color	.188	-.075	-.435*	-.032
Internal pappus color	-.175	.041	.366*	-.255
Achene indumentums	.302	-.076	-.349*	-.026
Indumentums in pedicle of head	.040	.143	.343*	-.021
Internal phyllary type	.117	-.026	-.323*	.299
Internal phyllary apex	.178	-.045	-.206*	-.016
Achene color	-.001	-.014	-.168*	.076
Basal leaf type	-.030	-.006	-.127*	.075
Embranchment place	.018	-.051	-.171	.551*
Petiole in basal leaf	.035	-.006	-.201	.262*
Envelope shape	-.167	.061	.211	.219*
Cauline leaf apex	-.041	-.013	-.073	-.105*
Pooled within-groups correlations between discriminating variables and standardized canonical discriminant functions Variables ordered by absolute size of correlation within function.				
*. Largest absolute correlation between each variable and any discriminant function				

Anatomy

Anatomical results of the leaf showed that qualitative characters including cuticle, secretory, transport tissue, number of layers of ladder parenchyma, type of ladder parenchyma, sclerenchyma, collenchymas, central vascular bundle sheath, fiber and trichome were similar in all *Zoegea* species. Also, stem characters couldnot show the separation between taxa. Clustering resulting from anatomy was not the same as morphological analysis and showed the close relationship between the *Z. crinita* subsp. *crinita* and *Z. leptaurea* subsp. *mianensis*in comparison with the two other subspecies of Z. *crinita* (Fig. 7). Stoma type in all taxa was Anomocytic, however, at the ventral surface of *Z.crinita* subsp. *baldschuanica* and *Z.crinita* subsp. *Glabricaulis* the anisocytic type and paracytic type were also observed, respectively (Figs. 4, 5 & 6 and Tables 5 & 6).

Table 5. Results obtained from qualitative and quantitative measurements of leaf (µm).

Species	Cuticle	Secretory	Transport Tissue	number of layers of ladder parenchyma	Type of ladder parenchyma	Sclerenchyma	Collenchyma	Central vascular bundle sheath	Fiber	Trichome
Z. *crinita* subsp. *crinita*	+	+	+	2-3	Bilateral	+	+	+	+	+
Z. *crinita* subsp. *baldschuanica*	+	+	+	2	Bilateral	+	+	+	+	+
Z. *crinita* subsp. *glabricaulis*	+	+	+	2	Bilateral	+	+	+	+	+
Z. *Leptaurea* L. subsp. *mianensis*	+	+	+	2-3	Bilateral	+	+	+	+	+
Z. *purpurea*	+	+	+	2	Bilateral	+	+	+	+	+

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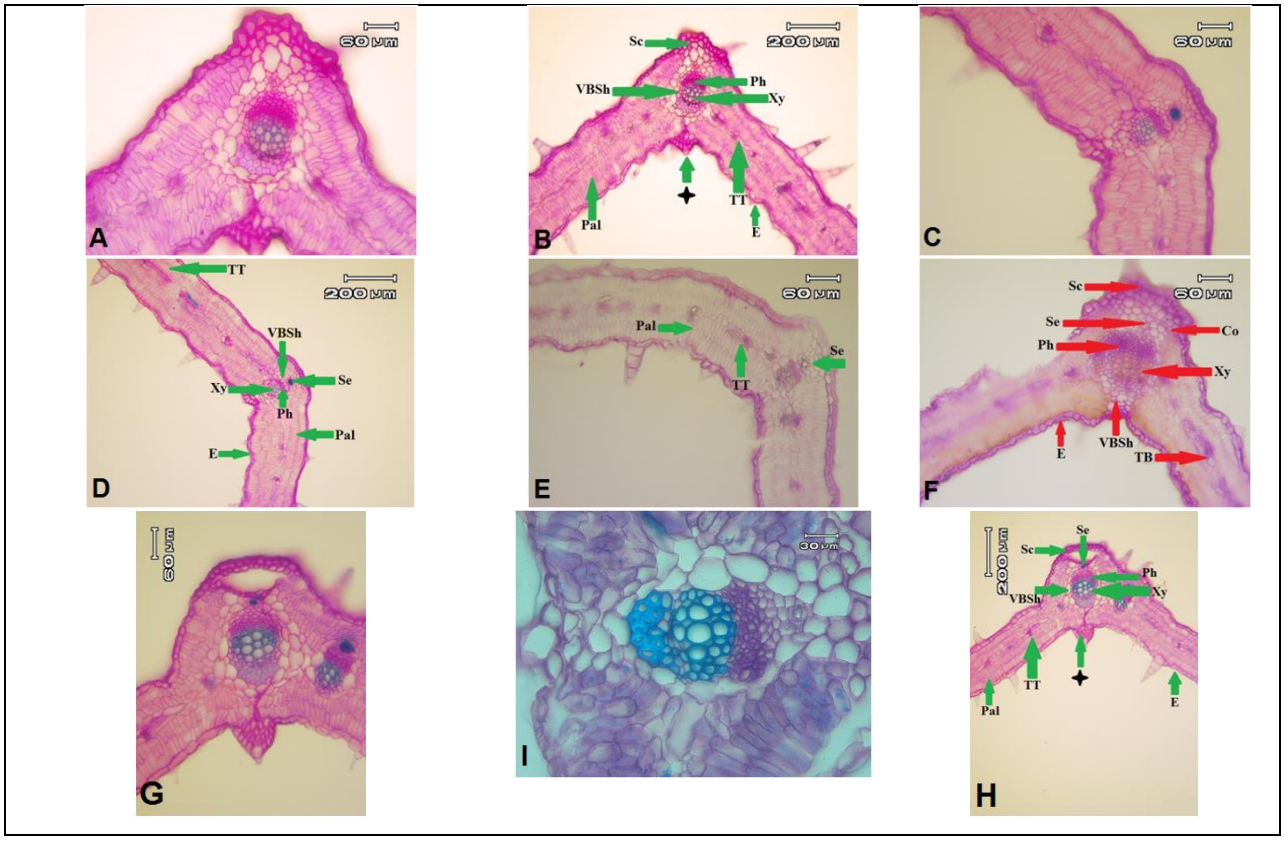

Fig. 4. Cross section of leaf in species studied: **A, B:** *Zoegealeptaurea* subsp. *Mianensis;* **C, D:** Z. *crinita* subsp. *crinita*; **E, F:** Z. *crinita* subsp. *Baldschuanica;* **G, H:** Z. *crinita* subsp. *glabricaulis* (I, J, K) Z. *purpurea*. (E) Epidermis, (Ph) phloem, (Xy) Xylem, (Parenchyma ladder), (Se) Secretory, (VBSh) Vascular Bundle Sheath, (TT) Transport Tissue, (Sc) Sclerenchyma, (Co) Collenchyma, (F) Fiber.

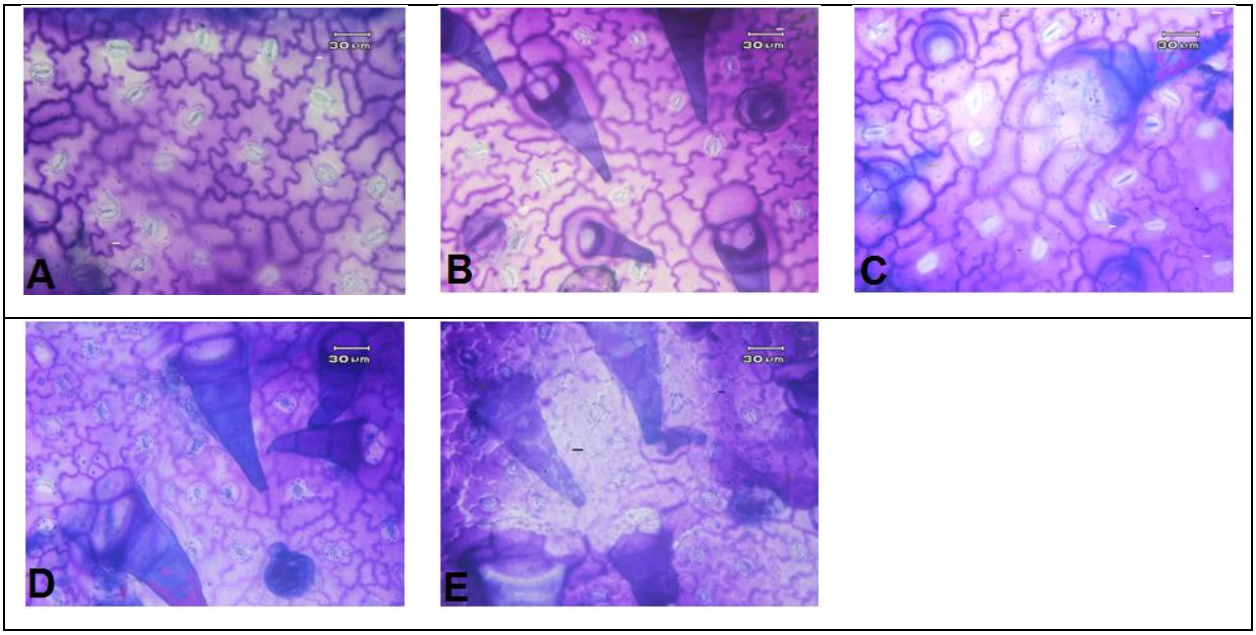

Fig. 5. Upper epidermis of leaf in species studied: **A:** *Zoegeacrinita* subsp. *baldschuanica;* **B:** Z. *crinita* subsp. *crinita*; **C:** Z. *crinita* subsp. *glabricaulis*; **D:** Z. *Leptaurea* subsp. *mianensis*; **E:** Z. *purpurea*.

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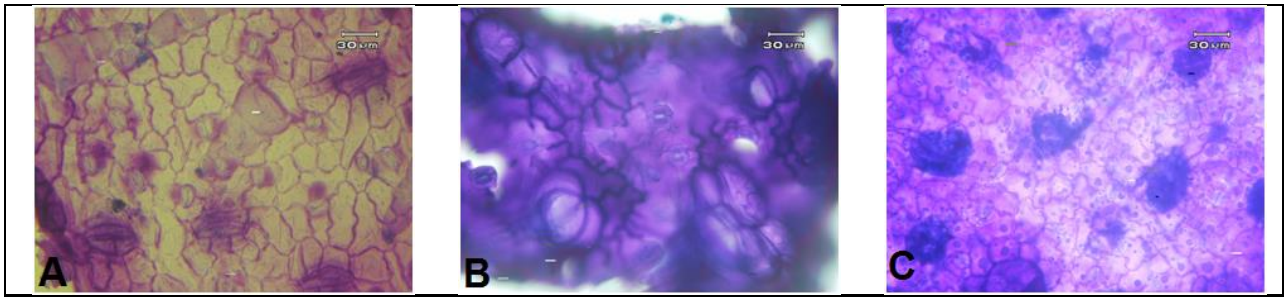

Fig. 6. Lower epidermis of leaf in some studied species: **A:** *Zoegeacrinita* subsp. *baldschuanica*; **B:** *Z.crinita* subsp. *glabricaulis*; **C:***Z.leptaurea* subsp. *mianensis.*

Table 6. Results of the study on features of leaf epidermis, stoma and trichome.

Features / Species	Type of Epidermis	Type of epidermal cell walls	Type of stoma	Type of trichome	Stomatal Index
Z.crinita subsp. *crinita*	Dorsal	Polygonal	Anomocytic	Simple	15
	Ventral	-	-	-	-
Z.crinita subsp. *baldschuanica*	Dorsal	Polygonal	Anomocytic	Simple	16
	Ventral	Polygonal - Flexuose	Anomocytic+Anisocytic	Simple	10
Z.crinita subsp. *glabricaulis*	Dorsal	Polygonal	Anomocytic	Simple	16
	Ventral	Polygonal	Anomocytic+Paracytic	Simple	11
Z.leptaurea subsp. *mianensis*	Dorsal	Polygonal	Anomocytic	Simple	24
	Ventral	Polygonal - Flexuose	Anomocytic	Simple	8
Z.purpurea	Dorsal	Polygonal	Anomocytic	Simple	17
	Ventral	-	-	-	-

Table 7. The results of the cross section of cypsela measured by Digimizer software (Measurements are in (µm).

Species	Cuticle	Secretory channel	Length of Epicarp	Length of Mesocarp	Length of Testa	Length of middle Testa	Length of inner Endosperm
Z. crinita subsp. *crinita*	+	+	12.58	81.49	45.79	67.34	15.89
Z.crinita subsp. *baldschuanica*	+	-	11.06	0	76.83	116.20	13.69
Z.crinita subsp. *glabricaulis*	-	+	15.75	120.40	65.07	47.61	18.10
Z.leptaurea subsp. *mianensis*	-	+	11.29	55.08	27.07	49.93	7.32
Z.purpurea	-+	-+	6.65	30.28	23.09	39.72	5.29

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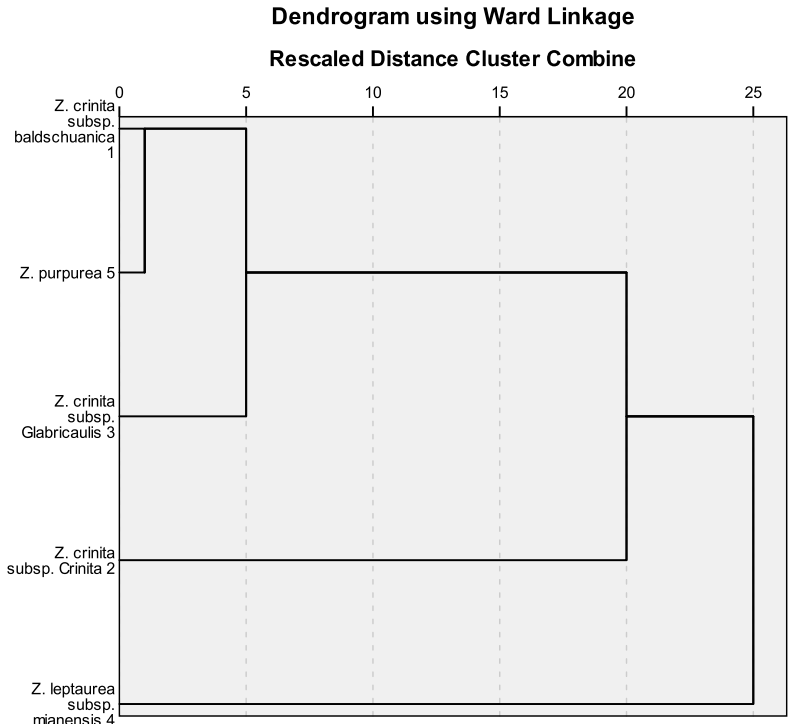

Fig. 7. Cluster analysis by Ward's method on leaf quantitative anatomical characters in some taxa.

Anatomical results of cypsela showed that the testa was made of three layers: epidermis layer, collenchyma layers under the epidermis, and sclerenchyma layers in the middle. Inner layers made of parenchyma and one vascular bundle could be find in each of them (Fig. 8). Analysis of anatomical characters measured in cypsela provided useful information. Chart of PCA (principal component analysis) obtained from quantitative characters of cypsela showed in Fig. 9. Traits of the first factor (i.e., the thickness of the epicarp layers, testa and the inner endosperm) with high correlation (>0.8) with a share of 77/59% of the total variance could separate *Z. purpurea* isolated from other species in the vertical axis. Characters of the second factor (i.e., the thickness of the middle testa layer and testa) with a correlation coefficient (>0.3) with a share of 9/29% of the total variance separated the subspecies of Z. *crinita* in the horizontal axis. In all taxa prismatic crystals were found in the testa layer and the vascular tissue in the middle testa (Table 7 and Fig. 9).

Palynology

Pollen in all specimens of *Zoegea* which were studied was tricolporate and three pore were seen in polar view as bulge. Exine structure was covered with spines in all taxa that were somewhat different in length, but covered the entire pollen surface uniformly (Table 8 and Fig. 10). The longest length of the polar axis was observed in *Z. crinita* subsp. *baldschuanica* and the shortest length was observed in *Z. purpurea*. Also, the longest equatorial axis length was observed in *Z. crinita* subsp. *baldschuanica* and the shortest axis of tropical was observed in *Z. leptaurea* subsp. *mianensis*. The length of spine of the pollen in *Z. leptaurea* subsp. *mianensis* was more than the other taxa.

Morphological characters showed that *Z. purpurea* was well-separated from other species. Results of CVA analysis based on quantitative characters showed that *Z. crinita* subsp. *baldschuanica*wasa completely distinct taxon that could easily be differentiated from Z. *crinita*. But in this analysis based on qualitative characters some specimens of *Z. crinita* subsp. *baldschuanica* occurred near other members of Z. *crinita*.

Also, morphological characters (i.e., leaf indumentums type, indumentums type, flower color, embranchment place, radiate floret length, pappus length, external phyllary length, etc) (Tables 3 & 4) showed that *Z. crinita* subsp. *glabricaulis* was separate from Z. *crinita*. Results of cypsela anatomical studies confirmed morphological results. Morphological characters could not separate *Z. crinita* and *Z. leptaurea* subsp. *mianensis* from each other. However, the cluster analysis by ward's method based on quantitative morphological characters could separate *Z. crinita* and *Z. leptaurea* subsp. *mianensis.*

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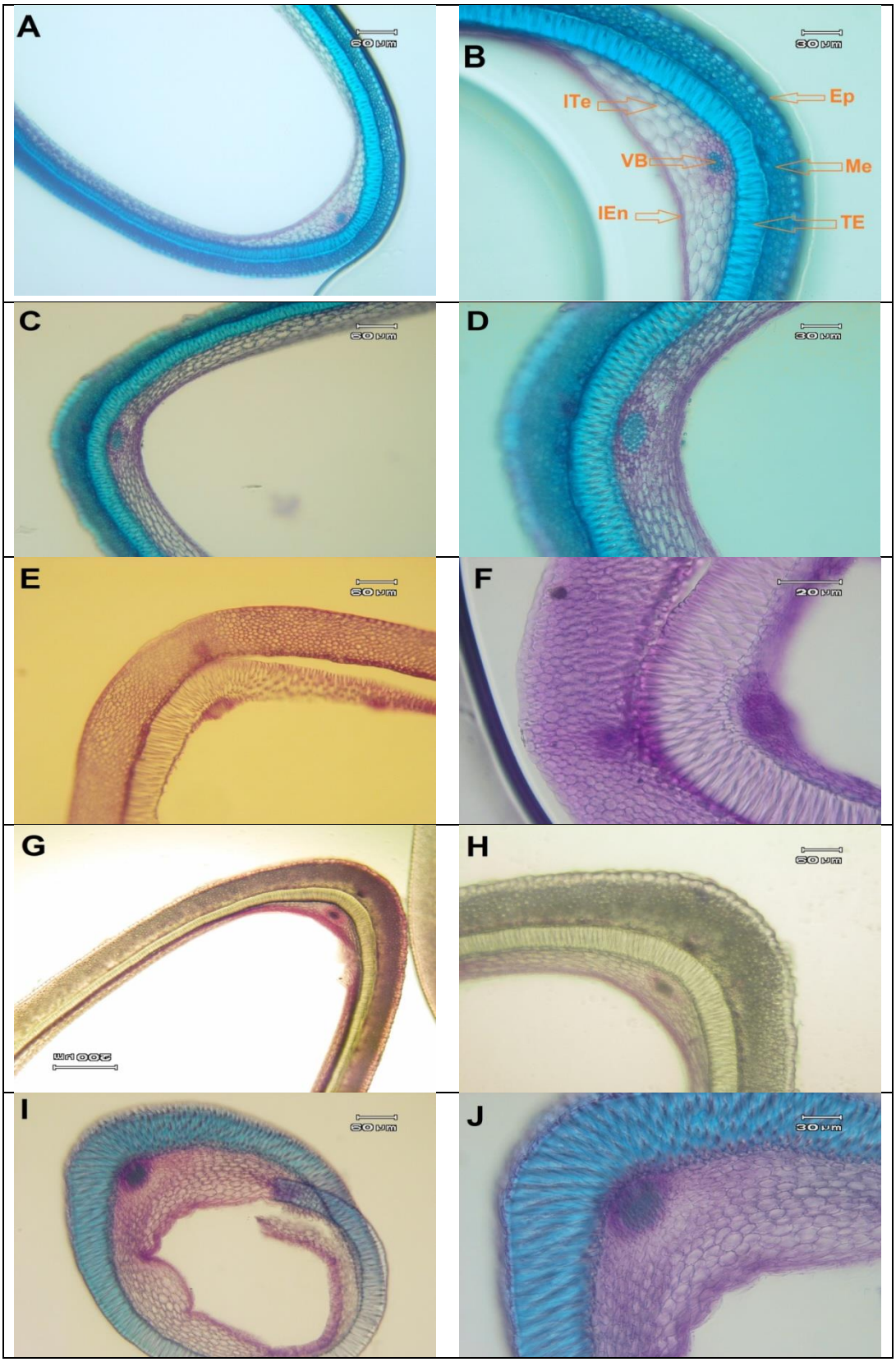

Fig. 8. Cross section of cypsela: **A, B**: *Zoegeapurpurea;* **C, D**: Z. *mianensis;* **E, F**: Z. *glabricaulis;* **G, H**: Z. *crinita;* **I, J**: *Z.baldschuanica*. (Ep) Epicarp, (Me) Mesocarp, (Te) Testa, (ITe) Innermost testa, (IEn) Inner endosperm, (VB) Vascular bundle.

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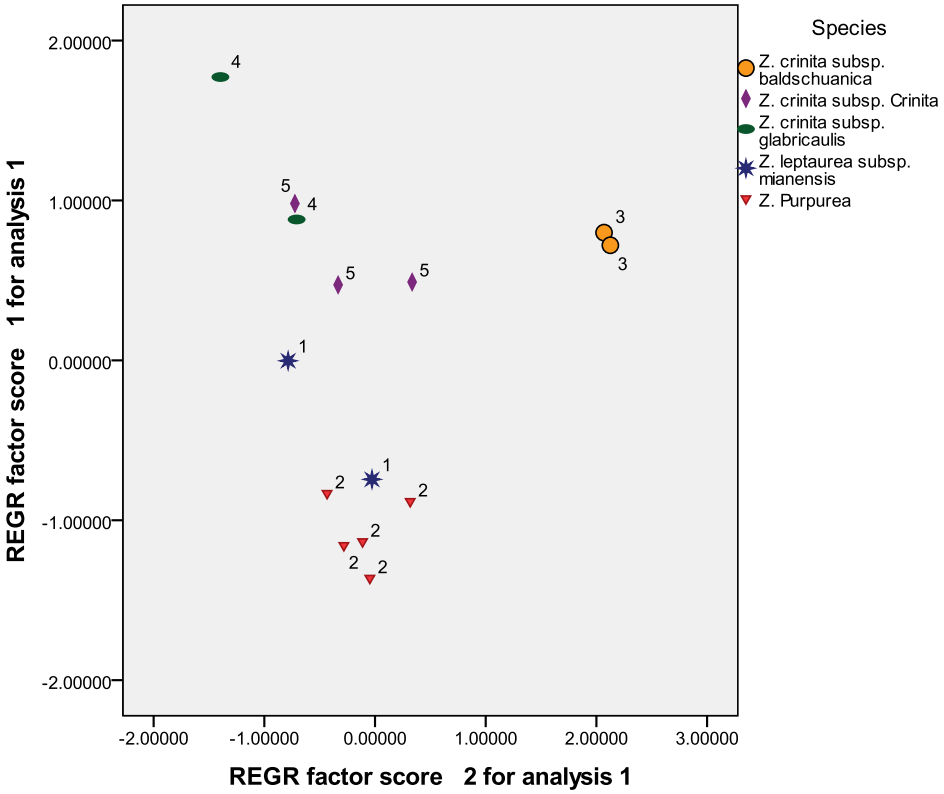

Fig. 9. PCA of the first and second factors based on Morphological quantitative cypsela features.

Since the morphological characters used in identification keys of taxonomic literatures are not suitable to distinguish these taxa. Therefore, we restablished the following taxa on the basis of the result of this study.

Zoegea baldschuanica C.Winkl.
Syn.: *Zoegea crinita* Boiss. subsp. *baldschuanica* (C.Winkl.) Rech.f.

Zoegea glabricaulis Czerep.
Syn.: *Zoegea crinita* Boiss. subsp. *glabricaulis* (Czerep.) Rech.f.

Table 8. Results obtained from evaluation of quantitative and qualitative features of pollen grains (size= µm).

Species	Polar axis Length (P)			Euatorial axis length (E)			P/E	Form	Length of spine
	Minimum	Maximum	Average	Minimum	Maximum	Average			
Z. *crinita* subsp. *crinita*	30.79	31.04	30.92	30.12	35.00	32.56	0.94	OS	1.36
Z. *crinita* subsp. *baldschuanica*	31.61	36.75	34.18	31.42	37.62	34.52	0.99	OS	1.49
Z. *crinita* subsp. *glabricaulis*	26.82	28.75	27.78	25.89	28.88	27.38	1.01	PS	1.85
Z. *leptaurea* subsp. *mianensis*	26.48	26.76	26.62	25.58	27.29	26.44	1.006	PS	3.36
Z. *purpurea*	22.93	24.70	23.81	29.00	29.37	29.19	0.81	SO	0.91
P: Polar axis, E: Equatorial axis, Cl: Colpus Length,OS: Oblate Spheroidal, SO: Suboblate									

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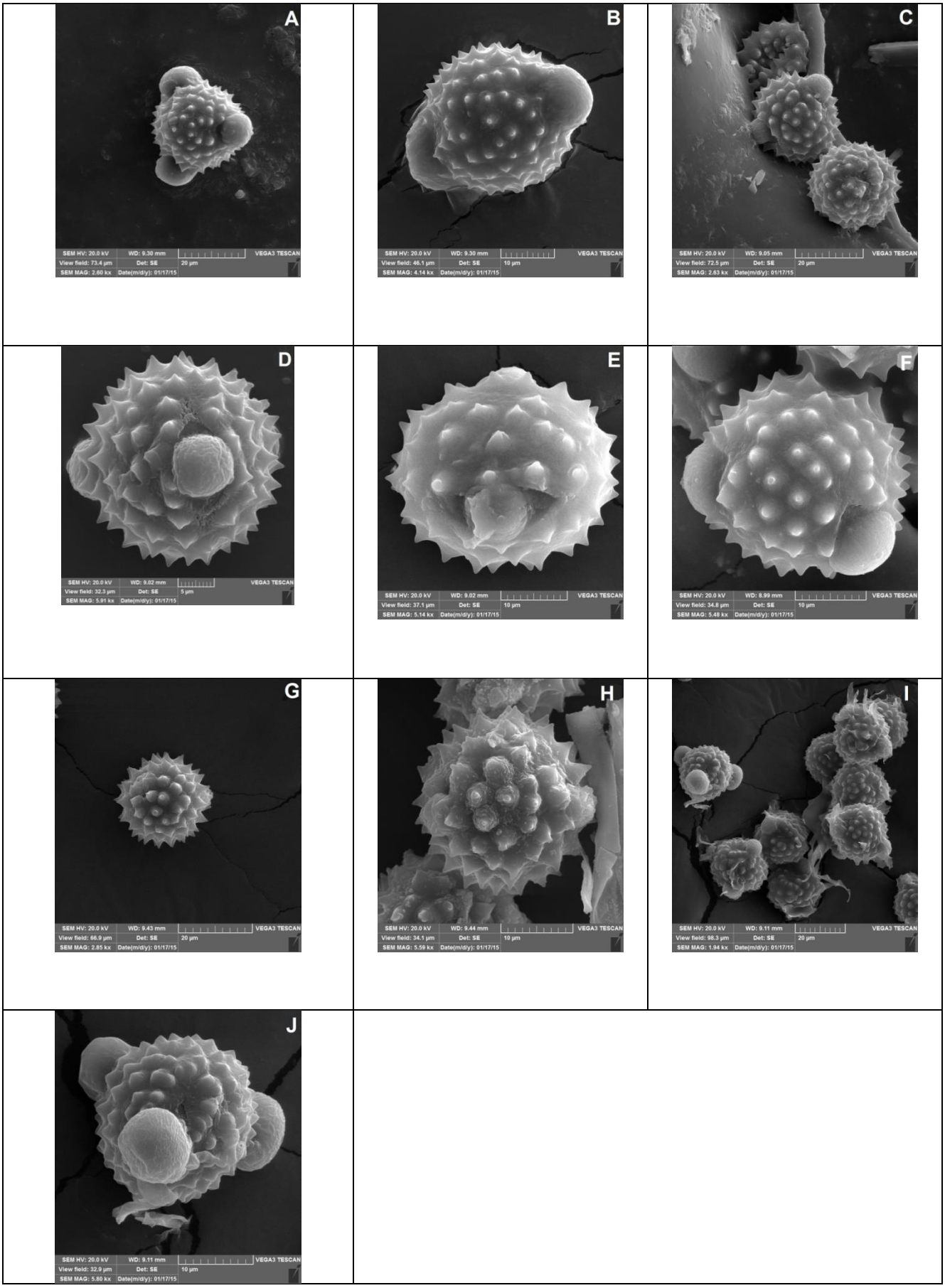

Fig. 10. Pollen micrographs of the studied taxa: **A, B:** *Zoegea crinita* subsp. *baldschuanica;***C, D:***Z. crinita* subsp. *glabricaulis;***E, F:** *Z. crinita* subsp. *crinita;***G, H:** *Z. leptaurea* subsp. *mianensis*; **I, J:** Z. *purpurea*.

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CONCLUSION

We concluded that *Zoegea baldschuanica* and *Z. glabricaulis* were independent species. Also, it was showed that anatomical characters such as stomatal index, existence of trichome, number of ladder parenchyma layers, thickness of epicarp layers, testa layers and inner endosperm and existence of secretary channel were diagnostic characters in distinguishing the species of *Zoegea*.

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