Foliar and pollen grain micromorphology of some species of *Astragalus* sections *Microphysa* and *Campylanthus*

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Abstract

This study focused on identifying the differences between the closely related sections *Microphysa* and *Campylanthus* in the genus *Astragalus* using micromorphological characteristics. The study investigated the morphological characteristics of leaf epidermal cells and stomata as well as pollen grains. The results obtained from LM and SEM indicates that stomatal type is typically anemocytic in most species, while the anisocytic type is found only in two species, namely, *A. callistachys* and *A. microphysa*. The epidermal cells in most species were observed to have a polygonal shape with straight anticlinal walls. However, in *A. cephalanthus*, both polygonal and irregular cell shapes were observed, and sinuate anticlinal walls were also present. All pollen grains are shed as monad, isopolar, small in size, have tricolporate aperture type, and rounded or elongated pores. In general, the micromorphological data alone are not enough to separate these two sections and determine the limits of the species.

Keywords: *Fabaceae*, epidermal cells, microscopy, stomata, pollen grain

ریزریخت‌شناسی برگ و دانه گرده برخی گونه‌های بخش‌های *Microphysa* و *Campylanthus* از جنس *Astragalus* *

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خلاصه

این مطالعه بر شناسایی تفاوت‌های بین بخش‌های نزدیک *Microphysa* و *Campylanthus* در جنس *Astragalus* (باقالاییان) با استفاده از ویژگی‌های ریزریخت‌شناسی متمرکز است. در این تحقیق، ریخت‌شناسی سلول‌های اپیدرمی و روزنه‌های برگ و دانه‌های گرده در هفت گونه بررسی شد. نتایج حاصل از مطالعات میکروسکوپ نوری و الکترونی نشان داد شد که نوع روزنه در بیشتر گونه‌ها به طور مشخص آنموسیتیک است، در حالی که آنیزوسیتیک تنها در دو گونه *A. callistachys* و *A. microphysa* یافت شد. سلول‌های اپیدرمی در بیشتر گونه‌ها به شکل چندضلعی منظم با دیواره صاف مشاهده شدند. اما در گونه *A. cephalanthus* سلول‌های اپیدرمی به هر دو شکل چندضلعی و نامنظم دیده شد. همچنین، دیواره عمودی به شکل سینوسی نیز در این گونه وجود داشت. براساس یافته‌های این مطالعه، تمام دانه‌های گرده بطور منفرد پخش می‌شوند، کوچک، سه شیار منفذدار، جور قطب و متقارن و دارای منفذ گرد یا کشیده بودند. به طور کلی، داده‌های ریز ریخت‌شناسی به تنهایی برای جداسازی این دو بخش و تعیین حدود گونه‌ها کافی نیستند.

واژه‌های کلیدی: دانه گرده، روزنه، سلول‌های اپیدرمی، مطالعه میکروسکوپی، *Fabaceae*

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Introduction

Astragalus L. (*Fabaceae*) is the largest genus of flowering plants with approximately 3,156 species classified into 255 sections (Maassoumi 2020). *Astragalus* is known for its adaptation to various ecological conditions and habitat. The plant can be found in cool to warm climates, semi-arid to arid regions, and mountainous areas, which has resulted in a wide range of forms such as annual, perennial, herbaceous thorn cushions form) Castillon *et al*. 2020). The genus *Astragalus* displays a wide range of morphological diversity, such as differences in vegetative form and habitat, plant size, types of hairs (Ghahremaninejad 2004), inflorescence type, length of inflorescence peduncle and raceme, petal length, and other characteristics (Podlech & Zarre 2013). The diversity of ecological factors and changes in habitat can also result in variation among *Astragalus* species. These variations include preferences for different growing environments such as mountain slopes, flatlands, riverbanks, agricultural fields with dry soil, and barren lands with hard, stony or sandy soils. *Astragalus* spp. exhibit a high level of sensitivity to regional and microclimatic conditions (Maassoumi 2020). Although, *Astragalus* has a long biogeographical history, our understanding of its regional connections and distribution in the Old World is still limited. To date, there is no comprehensive, up-to-date, and integrated information available on diverse spatial distribution patterns in a broad continental-scale framework (Zarre & Azani 2013). With more than 848 species in 70 sections, Iran is a major center of diversity for the *Astragalus* genus, representing more than 10% of the country's plant species. (Ghahremaninejad *et al*. 2022, Maassoumi 2005, Bagheri *et al*. 2014). Of these species, over 570 are endemic to the flora of Iran (Maassoumi 2005). The presence of endemic species with a wide distribution suggests that, their center of origin is mainly in the Zagros Mountains and to a lesser extent in the Alborz Mountains or scattered throughout other high-altitude areas in the central plateau (Ghahremaninejad *et al*. 2016, Mahmoodi *et al*. 2022, Maassoumi 2020, 2005, Noroozi *et al*. 2020).

Modern taxonomists often use palynology as a method to distinguish closely related species (Amina *et al*. 2020, Khan *et al*. 2018, Ullah *et al*. 2018). For micromorphological studies, the Scanning Electron Microscope (SEM) is an advanced approach that enables the examination of pollen grain structures in exceptional detail (Bagheri *et al*. 2019, Majeed *et al*. 2020, Umber *et al*. 2022). SEM has been employed in many plant families, including *Fabaceae* (Ekici *et al*. 2005, Lashin, 2006, Büyükkartal *et al*. 2012, Bagheri *et al*. 2019), *Asteraceae* (Karbalaei *et al*. 2021, Khan *et al*. 2021, Nabila *et al*. 2022, Umbe *et al*. 2022), *Caryophyllaceae* (Ullah *et al*. 2019), *Acanthaceae* (Raza *et al*. 2020), *Cactaceae* (Majeed *et al*. 2020), and *Lamiaceae* (Bahadur *et al*. 2022) due to its high resolution and ability to accurately display the surface of pollen grains.

Despite numerous studies that have attempted to classify the diverse and polymorphic genus *Astragalus*, some classification issues remain unresolved (Khan *et al*. 2022). To address this, micromorphological characteristics of pollen grains have been introduced as a valuable tool in the taxonomic classification of the genus (Yasmin *et al*. 2010, Khan *et al*. 2022). However, only few sections of *Astragalus* species have been studied using pollen analysis, including sect. *Hololeuce* Bunge (Ceter *et al*. 2013, Uzun *et al*. 2021), sect. *Onobrychoidei* DC. (Pinar *et al*. 2009), sect. *Malacothrix* Bunge (Oskouian *et al*. 2007), sect. *Hymenostegis* Bunge (Bagheri *et al*. 2019) and sect. *Macrophyllium* Boiss. (Ranjbar *et al*. 2012). The use of pollen grains in systematics has proven to be valuable due to the information that can be obtained from their characteristics such as their shape, size, pores, modes of attachment, and symmetry. These characteristics aid in distinguishing and classifying closely related species within a genus. *Papilionoideae*, a subfamily of the *Fabaceae* family, displays a great degree of variability in the size, arrangement of pores, and surface decorations of its pollen grains, which is referred to as Eurypalynous. The shape of pollen grains is usually radially symmetrical and free, with a tricolporate arrangement that is occasionally colpate or porate. The shape of the grains is typically prolate or almost subprolate, and the tectum is mostly reticulate, with some species displaying fossulate, foveolate, or fossulate-

rugulate tectum. However, more than 70% of the studied taxa exhibit tricolporate pollen grains with reticulate tectum (Perveen & Qaiser 1998). Changes in the exine decorations, grooves, and pattern of pollen grains are significant and contribute to their differentiation and classification in the subfamily.

The micro-morphological diversity of epidermal cells is one of the main sources of data in taxonomy and plays a fundamental role in systematics (Metcalfe 1988). The foliar epidermal cells of *Astragalus* are remarkable characters for the distinction of different species (Hayat *et al*. 2009). These characters include the size and shape of epidermal cells, distribution of stomata, size of guard cells, and number of subsidiary cells. The use of foliar anatomical attributes in plant systematics has been over the last 100 years (Bahadur *et al*. 2019). Therefore, due to the widespread distribution of this huge genus and for its complex taxonomy, the micro-morphological characteristics of foliar epidermal in some selected species of *Astragalus* were monitored with scanning electron microscopy (SEM) techniques to better understanding the taxonomic value of micro-morphological traits among different species of *Astragalus* and determine the influence these traits to resolving taxonomic conflicts of this genus.

This study focuses on, *Astragalus* sect. *Microphysa* and sect. *Campylanthus*. Different analyses of molecular phylogenetic data have shown that these sections are not monophyletic; instead, their species are intermixed. Additionally, these two sections are identified as sister taxa and are nested within a polytomy clade (Kazempour Osaloo et al., 2003; 2005; Bagheri et al., 2017). In this study, seven species of *Astragalus* from sections *Microphysa* and *Campylanthus* were investigated in terms of micromorphology of foliar epidermal cells and pollen grain. The aim of this study was to examine the micromorphology of reproductive and vegetative parts in seven species of *Astragalus* belonging to the *Microphysa* and *Campylanthus* sections. The study aimed to investigate whether the micromorphological characteristics could be used to distinguish between different species and sections.

Materials and Methods

- Sampling

All leaf and pollen samples were obtained from 24 specimens belonging to seven species (at least 3 specimens per species were studied) from *Microphysa* and *Campylanthus* sections deposited at the herbarium of the Isfahan Agricultural and Natural Resources Research Center (SFAHAN), Isfahan (Iran), listed in table 1.

Table 1. Information of examined *Astragalus* taxa from *Microphysa* and *Campylanthus* sections.

Species	Locality	Altitude (m)	Collector	Voucher No.
A. argyrostachys	Isfahan prov.: Semirom, Garmook	2350	Feyzi	13543■
	Isfahan prov.: Fereydan, Aghche	2300	Feyzi	12267■♦●
	Isfahan prov.: Tiran to Damaneh, Tange kolang	2500	Feyzi- Shams	12514■♦●
A. callistachys	Isfahan prov.: 20 km Shahreza to Semirom	2100	Nowroozi-Etemadi	526■♦●
	Isfahan prov.: Tiran, Ghameshloo	2100	Nowroozi- Feyzi	5336■♦

	Isfahan prov.: 5 km Delijan to Tehran	1450	Nowroozi	4618■♦
	Isfahan prov.: Najaf abad, Jannat abad	1600	Nowroozi	492■
A. campylanthus	Isfahan prov.: Semirom, Vanak, Dalan kooh	2200	Nowroozi-Bozorgi	3597■♦●
	Isfahan prov.: Meymeh	2400	Feyzi	13193■
	Isfahan prov.: Dehaghan, Kheir abad	2200	Feyzi	6755■●
	Isfahan prov.: Fereydan, Aghcheh	2300	Feyzi	12269■♦
A. cephalanthus	Isfahan prov.: Semirom, Hanna	2250	Nowroozi	4005■♦●
	Isfahan prov.: Chale siah to Hosein abab	1850	Nowroozi- Feyzi	6056■♦
	Isfahan prov.: Meymeh	2400	Nowroozi	13191■
	Isfahan prov.: Borojen to Mobarake	2100	Nowroozi- Feyzi	8026■♦
	Isfahan prov.: Semirom to Padena	2600	Mohajeri	7887■
	Isfahan prov.: Chadegan to Bidak	2500	Nowroozi-Etemadi	1059■
	Isfahan prov.: Fereydan	2400	Feyzi- Shams	12466■
	Isfahan prov.: Chadegan, Zayandeh rood	2200	Nowroozi-Matin	6147●
A. microphysa	Isfahan prov.: Chale siah	1850	Nowroozi	6055■♦
	Isfahan prov.: Kashan to Ghamsar	1250	Nowroozi	13565■♦●
	Isfahan prov.: Buein, Tange doozan	2800	Nowroozi-Etemadi	1305■♦
	Isfahan prov.: Meymeh to Muteh	1920	Feyzi	13108♦
	Isfahan prov.: Najaf abad, Dehagh	2100	Feyzi- Shams	12582●
	Isfahan prov.: Zarrin shahr	1750	Feyzi	14644●
A. susianus	Isfahan prov.: Fereydoon shahr, Sang baran	2400	Feyzi-Asfa	10404■
	Isfahan prov.: Khansar	2700	Feyzi	8539■
	Isfahan prov.: Buein ghale	2550	Feyzi-Saeidfar	6045■♦
	Isfahan prov.: Semirom, Hanna	2400	Feyzi- Shams	14370♦

	Isfahan prov.: Daran	2300	Feyzi-Saeidfar	6415●
	Isfahan prov.: Fereydan, Dalan kooh	2400	Feyzi- Eftekhari	11158●
A. ruterianus	Isfahan prov.: Fereydan, Ghale Shahrokh	2500	Nowroozi-Yazdani	13323♦●

■: Specimens used for LM; ♦: Specimens used for SEM from epidermis of the leaf; ●: Specimens used for SEM from Pollen grains

Light Microscopy (LM)

The dried samples were fixed in a solution of ethanol and acetic acid (70:30) for 24–48 hours. They were then washed with distilled water and immersed in a solution of hydrogen peroxide and acetic acid (1:1) for 40–45 minutes at 50–60 °C. The samples were washed with distilled water again and stained with Carmine for 2–3 minutes if necessary. The epidermis was separated and examined under a light microscope, and various photographs were taken using slides.

- Scanning Electron Microscopy (SEM)

Scanning Electron Microscope (SEM) is widely used to observe surface structures. Micro morphological studies were conducted using a scanning electron microscope to investigate the upper epidermis of the leaf and pollen grains (Table 1) from the studied species. The specifications of the SEM device used in the laboratory of Isfahan University of Technology Faculty of Materials are AIS2300C SERON with a maximum length and width of 2.5 cm and a maximum height of 3 cm. To prepare the sample, a 10 nm layer of gold was coated on the surface of the samples, and then the samples were transferred to the SEM device for imaging. After many samples of leaf epidermis and stomata were photographed to record the results, the best pictures were selected, and the parameters were measured and recorded using Digimizer 5.4.9 software.

To prepare the pollen sample, the anther is first separated from inside the flower. The terminal anther is then slowly crushed on a plate with a needle to create a powder. Some of the powdered anthers are carefully and delicately placed on the electron microscope plates and observed after completing the necessary preparation steps. After taking multiple pictures of pollen grain samples with an electron microscope (SEM), the best pictures were selected and the parameters were measured and recorded using Digimizer 5.4.9 software, as shown in Table 5. The micro morphological analysis involved the measurement of 26 characters for each species. Principal Coordinate Analysis (PCoA) was conducted using GenAlex.

Results

- Epidermal cells and stomata

Different foliar epidermal features and the results of qualitative and quantitative analysis of the studied species under LM and SEM are summarized in tables 2–4. Table 2 pertains to light microscope (LM) images, while tables 3 and 4 pertain to electron microscope (SEM) images. The shape of epidermal cells was more specifically investigated, and the type of stomata was determined using LM, while SEM was more suitable for determining quantitative traits, such as measuring the length and width of epidermal cells and stomata. The results obtained from LM and SEM indicates that stomatal type is typically anemocytic in most species, while the anisocytic type is found only in two species, namely *A. callistachys* and *A. microphysa*.

Table 2. Measurement of quantitative traits of epidermis and stomata with light microscope (LM).

No.	Taxon	Herbarium No.	average epidermal cell (μm)		average stomata (μm)		average stomatal guard cell (μm)		average stomatal aperture (μm)	
			length	width	length	width	length	width	length	width
1	*A. argyrostachys*	13543	43	23.6	22	21	20	6.8	11.9	3.2
2		12267	40	18.6	22	21	20.8	7.6	11	4.7
3		12514	39.2	18	21.9	17.2	20.3	4.6	12	4
4	*A. callistachys*	526	34.1	19.5	20	20.5	17.5	6.9	8	4.5
5		5336	43	25.5	24.2	25.3	24.7	9.5	11	5
6		4618	38	20	24	21	20.5	7.2	10.5	4.2
7		492	39	18.7	19.2	17.5	16.5	6.8	10	4.1
8	*A. campylanthus*	3597	32.9	18	17.2	17	17.6	4.4	11.3	3.9
9		13193	34.9	20.1	23	20.6	19.4	4.6	9.2	3.4
10		6755	35.4	17.7	22.7	18.8	20	7.8	9.7	3.5
11		12269	32.8	18.4	18.4	18.8	17.5	7.9	8.9	3.7
12	*A. cephalanthus*	4005	54.6	27.8	24	17	21	6	12.2	5.1
13		6056	50.4	22.1	24.3	19	20	5.4	9.8	5
14		13191	51.4	23.7	22.4	19	22	7.5	11	5
15		8026	55	28	25.4	18	21.7	7.8	12.8	4.8
16		7887	49	21.8	24	17	21.6	7	12	4.9
17		1059	58	34.6	22.5	18	21.2	7.7	11	3.8
18		12466	47	23.8	18	15.5	18	6.5	10.5	3.8
19	*A. microphysa*	12582	45	12	13	12	14.5	7	12.5	2.2
20		6055	25	9.7	10	8.4	10	3.2	6	2.1
21		14219	42	16	23	22	20	7.5	8	4.3
22		13565	34	20	20	13	18.2	5.4	8.8	3.7
23	*A. susianus*	10404	40	14	22	21	18	5.8	12	5
24		6405	37	13	21.7	18	17	4.2	10.5	3.5
25		8539	37	15.3	20	18	18	4.7	10	3.5

Table 3. Quantitative and qualitative characteristics of epidermis with electron microscope (SM).

-			Mean quantitative characteristics of epidermis		qualitative characteristics of epidermis	
No.	**Taxon**	**Herbarium No.**	**epidermal cell length (µm)**	**epidermal cell width (µm)**	**epidermal cell shape**	**Margin**
1	*A. argyrostachys*	12267	54	22	polygonal	straight
2		12514	49	22.8	polygonal	straight
3	*A. callistachys*	526	35.5	17.1	polygonal	straight
4		4618	35.8	15.3	polygonal	straight
5		5336	39.3	20.3	polygonal	straight
6	*A. campylanthus*	3597	24.7	9.5	polygonal	straight
7		12269	28.7	10.8	polygonal	straight
8	*A. cephalanthus*	4005	45.4	22.5	irregular	sinoulate
9		6056	35	8.5	polygonal	undulate
10		8026	38.4	14.1	polygonal	undulate
11	*A. microphysa*	1305	37.6	13.9	polygonal	straight
12		6055	40	16	polygonal	straight
13		13108	50	25.5	polygonal	straight
14		13565	42.5	20.6	polygonal	straight
15	*A. ruterianus*	13323	40.7	10.5	polygonal	undulate
16		14370	30.5	9.5	polygonal	undulate
17	*A. susianus*	6405	38.2	9.2	polygonal	straight

Table 4. Quantitative and qualitative traits of stomata with electron microscope.

-			Mean quantitative traits of stomata							qualitative traits of stomata
No.	**Taxon**	**Herbarium No.**	**stomata length (µm)**	**stomata width (µm)**	**ambiance of stomata**	**stomatal guard cell length (µm)**	**stomatal guard cell width (µm)**	**stomatal pore length (µm)**	**stomatal pore width (µm)**	**Type of stomata**
1	*A. argyrostachys*	12267	22.1	17.8	60.6	23	5.4	12	5.6	anemocytic
2		12514	20.1	13.78	52.5	20.2	4.8	11.5	5	anemocytic
3	*A. callistachys*	526	22.8	13	56.3	23	4	9.5	4.5	Anisocytic
4		4618	18.3	10.5	46	18.8	3.9	10.7	4.4	Anisocytic
5		5336	23.5	16	61	15	2.9	11.3	5.3	Anisocytic
6	*A. campylanthus*	3597	20	17.4	51	17.5	3.5	10.4	4.1	anemocytic
7		12269	17.1	11.5	47.1	14	3.5	9.4	4.4	anemocytic
8	*A. cephalanthus*	4005	25.8	13	61.8	19.3	4.7	12.5	5.1	anemocytic
9		6056	23.1	15	60.6	20	4.7	13.5	5.5	anemocytic
10		8026	25.8	15.9	65	23	5.1	12.3	5	anemocytic
11	*A. microphysa*	1305	20.1	15.2	56.3	16.5	4.1	7.4	3.5	anemocytic
12		6055	18.5	13.2	49.2	15	4	9	3.1	Anisocytic
13		13108	24.7	11.2	59	19.5	3.8	10.3	5.3	anemocytic
14		13565	20.4	13.1	52.9	19	5	9.1	3.2	anemocytic
15	*A. ruterianus*	13323	15.7	7.5	37.9	14.5	2.8	7.5	3.1	anemocytic
16		14370	16	8.4	39.3	15	2.5	9.3	3	anemocytic
17	*A. susianus*	6405	21	17	64	16	3.8	10.7	4.2	anemocytic

Table 5. Data related to the measured characteristics of pollen grains in the studied species.

	-		Mean quantitative traits			Mean qualitative traits		
No.	**Taxon**	**Herbarium No.**	**polar axis (P) (µm)**	**equatorial axis (E) (µm)**	**(P/E)**	**Shape**	**type and number of pore/ furrow**	**type of decoration**
1	*A. argyrostachys*	12267	29	25	1.16	subprolate	tricolporate	rugulate
2		12514	22.5	12	1.87			
3	*A. callistachys*	526	27.5	13.2	2.08	prolate	tricolporate	rugulate
4	*A. campylanthus*	3597	27	13	2.07	prolate	tricolporate	rugulate
5		6755	25.1	11.1	2.26			
6	*A. cephalanthus*	6147	21.6	21.2	1.01	spheridal to prolate	tricolporate	reticulate
7		4005	26.3	13.4	1.96			rugulate
8	*A. microphysa*	12582	28.5	24.5	1.16	subprolate	tricolporate	reticulate
9		13565	33	23.5	1.4			
10		13644	26.1	23.2	1.12			
11	*A. ruterianus*	13323	19.5	16.1	1.21	subprolate	tricolporate	reticulate
12	*A. susianus*	6405	24	20.1	1.19	subprolate	tricolporate	reticulate
13		11158	23.4	20.2	1.16			

The shape of epidermal cells can be divided into two main categories: regular (polygonal) and irregular. Additionally, based on the shape of the anticlinal wall, the cells of the epidermis can be classified into three forms: sinuate, undulate, and straight. In the images obtained from both light and electron microscopes, the epidermal cells in most species were observed to have a polygonal shape with straight anticlinal walls. However, in *A. cephalanthus*, both polygonal and irregular cell shapes were observed, and sinuate anticlinal walls were also present in this species. Based on these observations, the studied species can be divided into three general groups based on the shape of the anticlinal wall and epidermal cells. 1. Polygonal cells with straight walls: *A. argyrostachys* (Figs 1A, 2A), *A. callistachys* (Figs 1D, 2B), *A. campylanthus* (Figs 1B, 2C), *A. susianus* (Figs 1C, 3C). 2. Polygonal cells with undulate walls: *A.*

reurianus: (Fig. 3B), *A. microphysa* (Figs 1F, 3A), *A. cephalanthus* (Figs 1E, 2D). 3. Irregular cells with sinoulate walls: *A. cephalanthus* (Figs 1E, 2D).

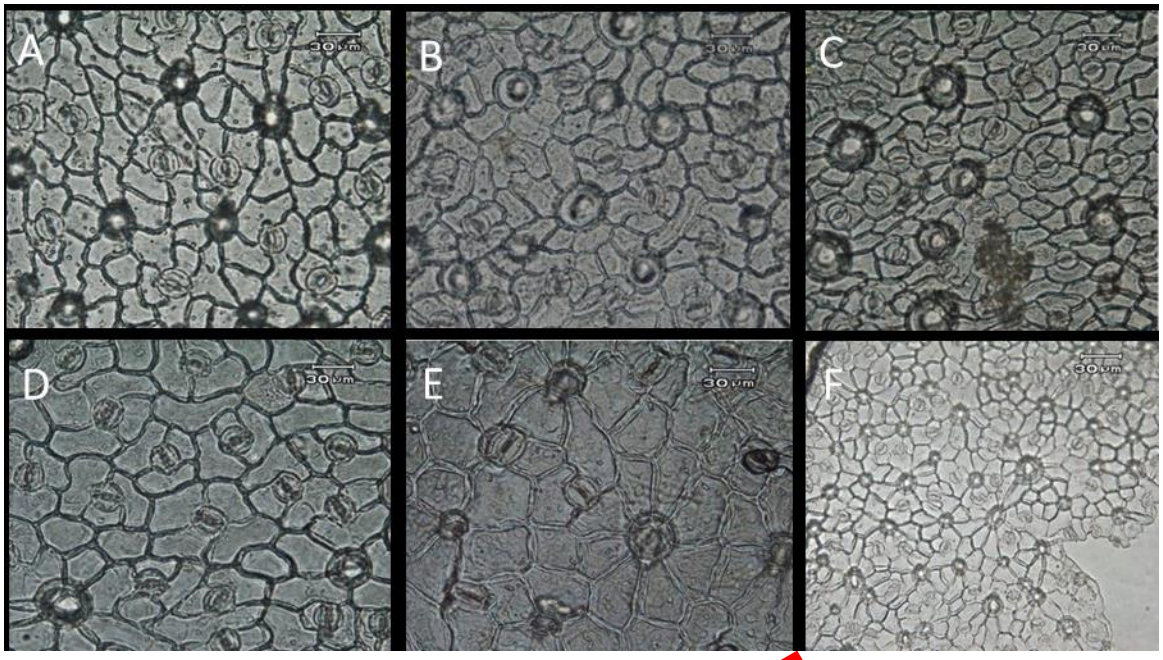

Fig. 1. LM of foliar epidermal cells and stomata of different *Astragalus* species: A. *A. argyrostachyes*, B. *A. campylanthus*, C. *A. susianus*, D. *A. callistachys*, E. *A. cephalanthus*, F. *A. microphysa*.

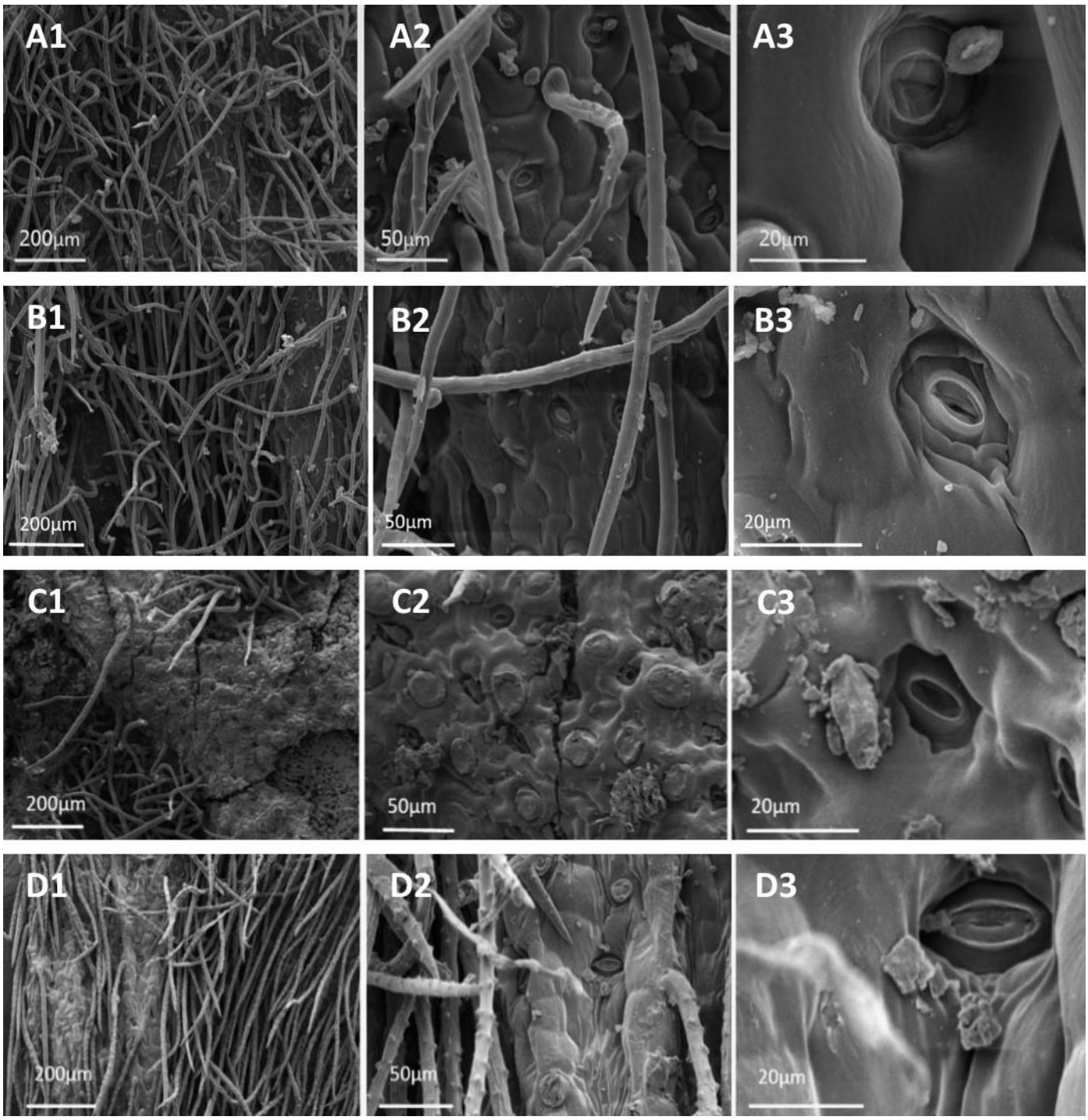

Fig. 2. SEM of foliar epidermal cells and stomata of different *Astragalus* species: A1-A3. *A. argyrostachyes*, B1-B3. *A. callistachys*, C1-C3. *A. campylanthus*, D1-D3. *A. cephalanthus.*

The electron microscope measurements showed that, the longest stomatal length was found in the *A. cephalanthus* species with an average of 25 µm, while the shortest was observed in the *A. callistachys* species with an average of 16 µm. Based on the measurements from electron microscope images, it was found that, the species *A. cephalanthus* has the widest stomatal aperture with an average of 14.1 µm, while the species *A. callistachys* has the narrowest stomatal aperture with an average of 8–10 µm. The longest stomatal guard cell length was observed in the species *A. cephalanthus* with an average of 21 µm, while the shortest length was found in the species *A. microphysa* and *A. campylanthus* with approximately 16 µm. Among the examined taxa, *A. cephalanthus* had the widest stomatal guard

cell, measuring 13 µm, while *A. campylanthus* and *A. callistachys* had the narrowest, measuring around 3.5 µm. The stomatal pores of *A. cephalanthus* were also the longest, with a mean length of 13 µm, whereas those of *A. microphysa* were the shortest, with a mean length of 9 µm. When it comes to stomatal pore width, *A. cephalanthus* had the largest, measuring 3.5 µm, whereas *A. microphysa* had the smallest, also measuring 3.5 µm.

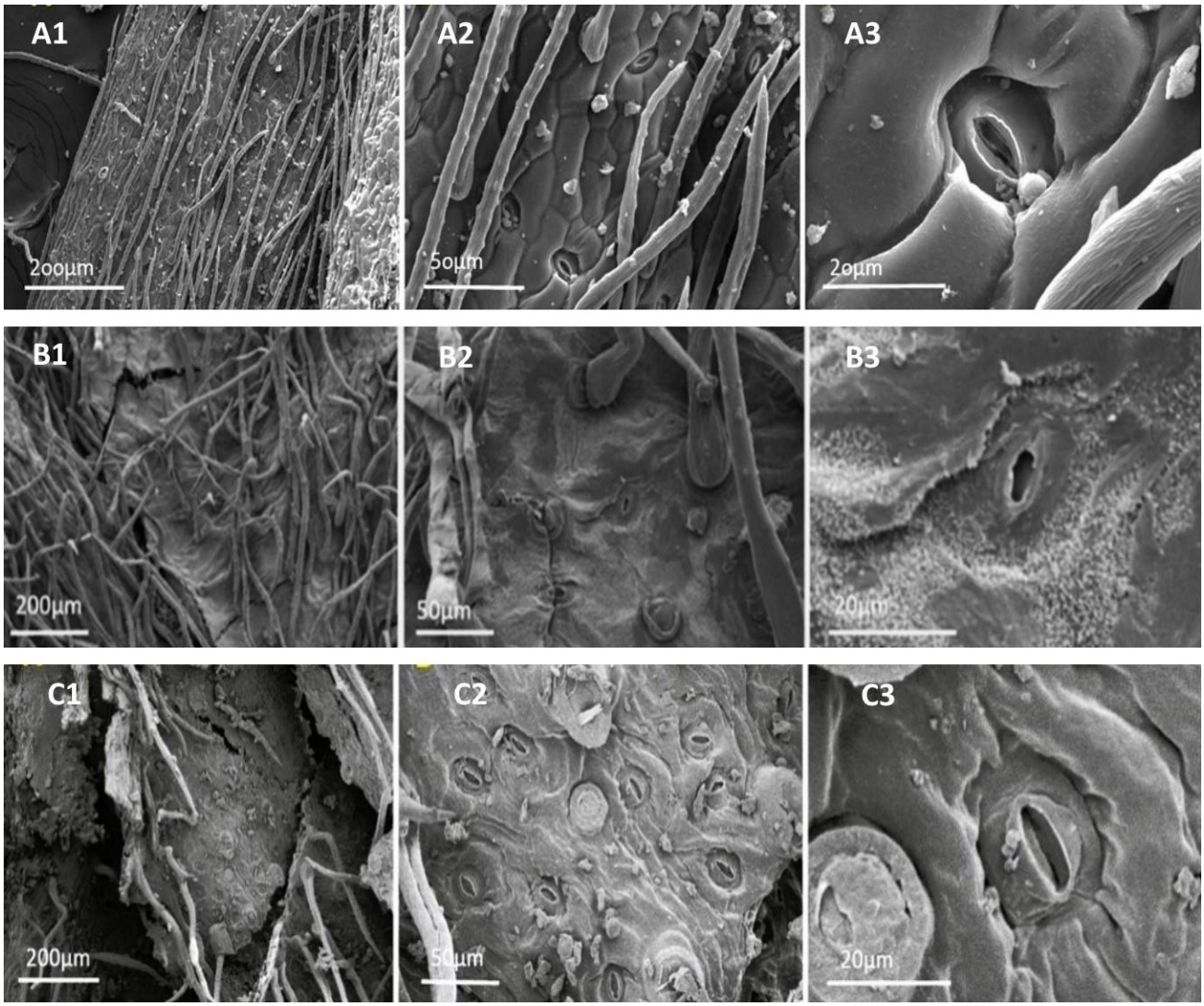

Fig. 3. SEM of foliar epidermal cells and stomata of different *Astragalus* species: A1-A3. *A. microphysa*, B1-B3. *A. ruterianus*, C1-C3. *A. susianus.*

- Pollen grains with SEM

In this research, pollen grains shed from the anthers were examined using an electron microscope. Ideally, the pollen grains that were individually placed were photographed with higher magnification. Based on observations, all pollen grains exhibited small size, single shape, tricolporate aperture type, isopolarity and symmetry, and had round or elongated pores (Figs 4–5). Based on the calculations, the ratio of the polar axis (P) to the equatorial axis (E) was greater than one and ranged from 1.01 in *A. cephalanthus* from the section *Campylanthus* to 2.26 in the species *A. campylanthus* from the section *Microphysa*.

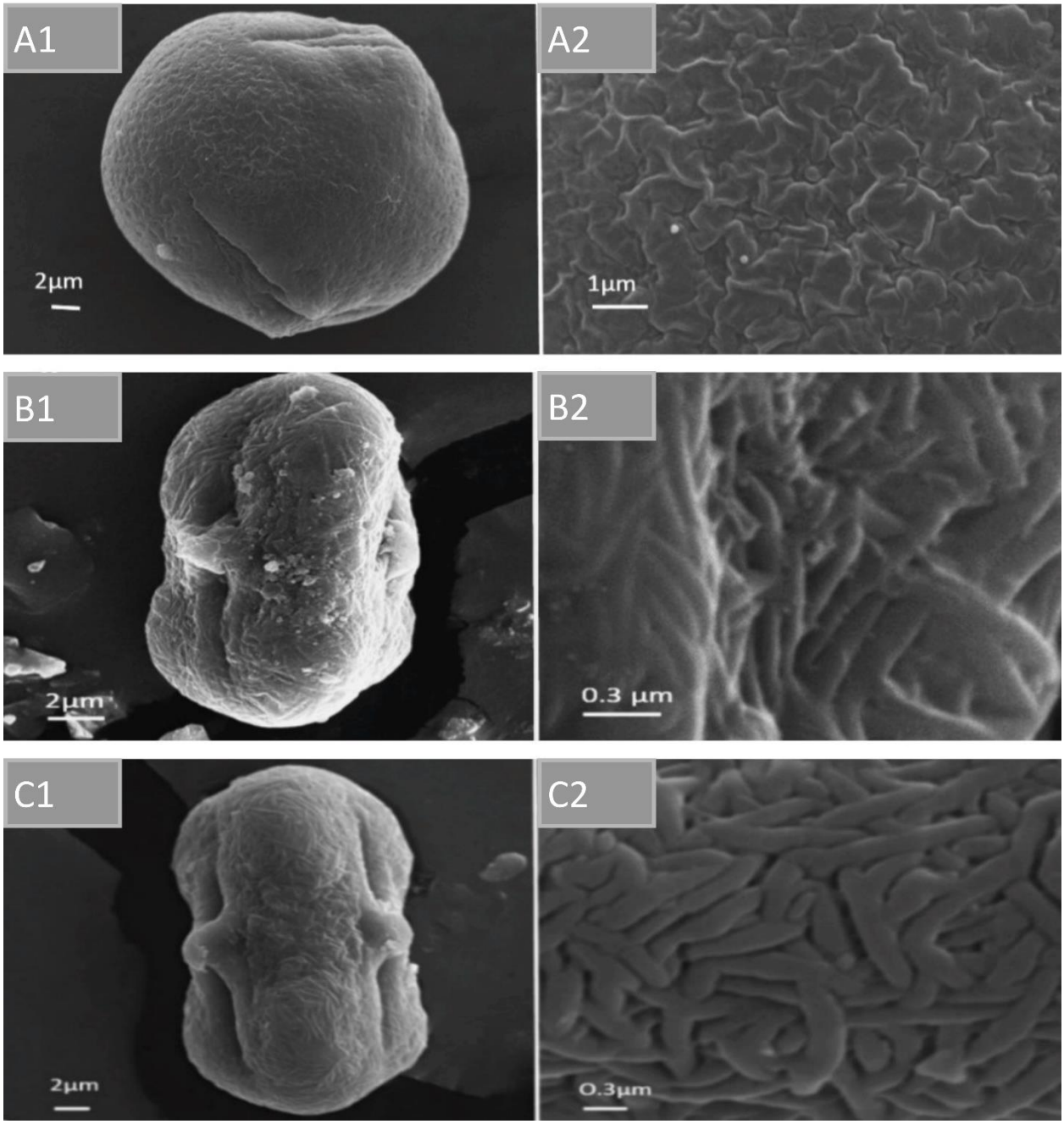

Fig. 4. SEM of pollen grain of different *Astragalus* species: A1-A2: *A. argyrostachyes;* B1-B2: *A. callistachys*; C1-C2: *A. campylanthus.*

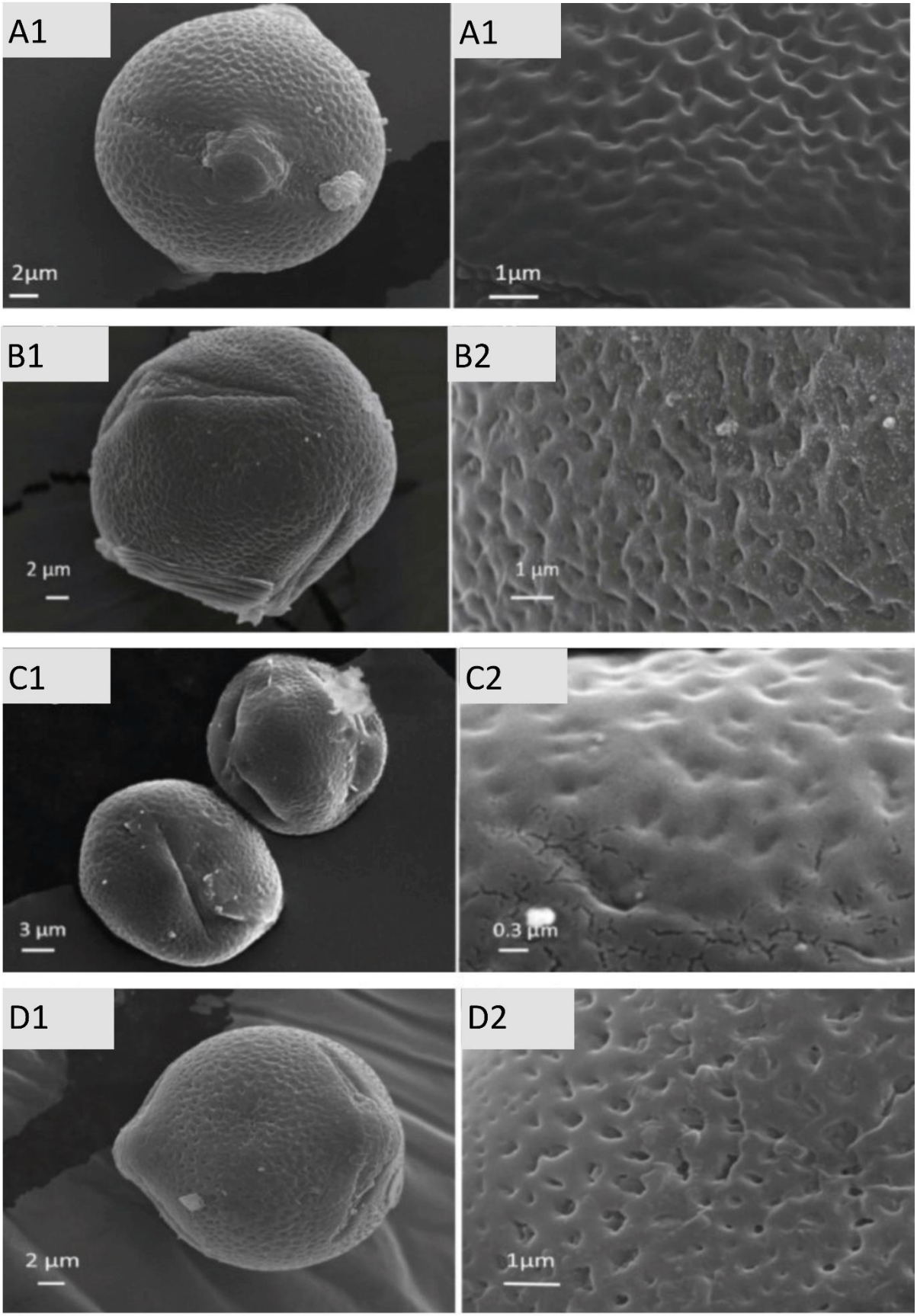

Fig. 5. SEM of pollen grain of different *Astragalus* species: A1-A2: *A. cephalanthus*; B1-B2: *A. microphysa*; C1-C2: *A. ruterianus*; D1-D2: *A. susianus.*

Discussion

Upon investigation of the quantitatively defined traits, no significant difference was observed between the species of the two sections, *Microphysa* and *Campylanthus*. However, among the species studied, *A. cephalanthus* was found to be significantly larger than other species in terms of stomatal length and width, stomatal pore, and guard cells. The minimum and maximum length and width of epidermal cells were found in *A. campylanthus* and *A. agrostachys*, respectively, both of which belong to sect. *Campylanthus*. Therefore, it can be concluded that, the size of epidermal cells is highly variable among different species and cannot be used to differentiate between the species of the two mentioned sections. Based on observations, stomata in the *Microphysa* section were predominantly anemocytic, with some instances of anisocytic types, while all stomata in the *Campylanthus* section were anemocytic.

In terms of the anticlinal wall shape, species in the *Microphysa* section exhibited straight, undulate, and occasionally sinoulate shapes, while species in the *Campylanthus* section exhibited straight and occasionally undulate shapes. The type of pollen grain surface decoration observed in the samples can be broadly categorized as rugulate, mostly observed in species belonging to the *Campylanthus* section, and reticulate, observed in species of the *Microphysa* section. This suggests that pollen grain surface decoration can be an effective taxonomic trait for differentiating between the species of the two sections. Pollen grain shape was found to be broadly categorized into three groups: almost spheroidal in *A. cepalanthus*, subprolate in *A. ruterianus*, *A. susianus*, *A. agrostachys* and *A. microphysa* species, and prolate in the species *A. callistachys* and *A. campylanthus*. In general, species belonging to the *Campylanthus* section exhibited subprolate and prolate shapes, while those in the *Microphysa* section exhibited prolate, subprolate, and spheroidal shapes. This is in congruence to other pollen studies in Astragalus species, which found the pollen in their study groups to be mainly prolate and subprolate (Osman *et al.*, 2014; Pinar *et al.*, 2009).

As mentioned earlier, the shape of the pollen grain is more or less variable, especially in the sect. *Microphysa*. Therefore, it cannot be considered as a significant characteristic in separating the species of the two sections. Other characteristics, such as stomatal features and epidermal cell size, seem to be more effective in distinguishing between the species of these sections. These results are also depicted in Fig. 6 as part of the PCoA analysis. It should be mentioned that the results obtained from the set of micro morphological characters of pollen and epidermis are not consistent with the morphological characteristics of the two studied sections. The findings indicate that these characters were not efficient enough to distinguish between the two sections and their respective species. This suggests that, these characteristics may not be particularly useful in distinguishing between different species within the genus *Astragalus*. Other morphological or genetic traits may need to be considered in order to accurately differentiate between closely related species within the genus.

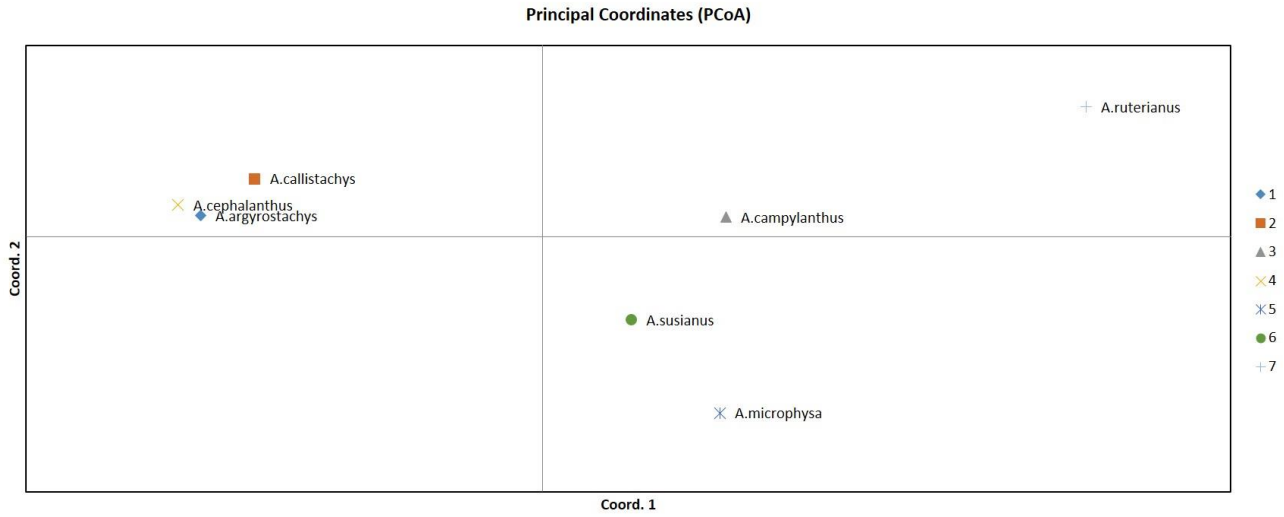

Fig. 6. Principal coordinate analysis (PCoA) score plot expressing the micro morphological variation (epidermis & pollen) of 7 species of *Astragalus* sect. *Microphysa* and sect. *Campylanthus*.

Conclusion

The micromorphological traits of pollen grain, epidermal cells and stomata were studied to distinguish between the closely related sections *Microphysa* and *Campylanthus* in the genus *Astragalus*. The results showed that, these traits were not efficient enough to separate the two sections and their species. Therefore, it is necessary to use a combination of micromorphological and molecular approaches for accurate classification of the genus *Astragalus*. Micromorphological studies can save time and budget in the first step, but genetic studies are required for validation and completion of the results.

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