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Seed morphology and micromorphology of *Prangos* species in Iran

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Abstract

The seed morphology of 15 species of *Prangos* (*Apiaceae*) from Iran has been examined using light and scanning electron microscopies. Macro- and micromorphological features, including seed shape, color, size, epidermal cell shape, anticlinal boundaries, outer periclinal cell wall, and characteristics of outer cell walls have been investigated. Based on epidermal cell size, cell arrangement, cell anticlinal and periclinal walls; three types of anticlinal cell wall boundaries were recognized. The study showed that, the seed coat ornamentation pattern could be helpful in identification of species. The purpose of this study was to describe and compare external seed morphological characteristics of *Prangos* species and to evaluate their possible use for taxonomic considerations. In addition, based on the seed exomorphic criteria extracted from LM and SEM, an artificial key to the species of the genus is provided.

Keywords: *Apiaceae*, *Apioideae*, micromorphology, seed coat, SEM, *Ulopterae*

بررسی ریخت‌شناسی و ریزریخت‌شناسی بذر گونه‌های جنس *Prangos* در ایران*

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خلاصه

ریخت‌شناسی بذر ۱۵ گونه *Prangos* (چتریان) از ایران با استفاده از میکروسکوپ نوری و الکترونی روبشی مورد بررسی قرار گرفته است. ویژگی‌های ریخت‌شناسی و ریزریخت‌شناسی شامل شکل بذر، رنگ، اندازه، شکل سلول‌های اپیدرمی، مرزهای آنتی‌کلینال، دیواره سلولی بیرونی پریکلینال و ویژگی‌های دیواره‌های سلولی خارجی مورد بررسی قرار گرفت. براساس اندازه سلول‌های اپیدرمی، آرایش سلولی، دیواره‌های آنتی‌کلینال و پری‌کلینال، سه نوع مرز دیواره سلولی شناسایی شد. این مطالعه نشان داد که الگوی تزیینات پوشش دانه می‌تواند در شناسایی گونه‌ها مفید باشد. هدف از این مطالعه، توصیف و مقایسه ویژگی‌های ریخت‌شناسی سطح خارجی بذر گونه‌های *Prangos* و ارزیابی کاربرد احتمالی آن‌ها برای مطالعات رده‌بندی می‌باشد. همچنین، براساس معیارهای شکل خارجی بذر به دست آمده از میکروسکوپ نوری و الکترونی روبشی، یک کلید مصنوعی برای گونه‌های جنس مورد نظر ارایه گردید.

واژه‌های کلیدی: ریزریخت‌شناسی، پوشش دانه، چتریان، SEM، *Apioideae*، *Ulopterae*

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Introduction

Apiaceae (*Umbelliferae*) is a very large and cosmopolitan family consisting of 466 genera and about 3820 species (Plunkett *et al.* 2018) which is mostly distributed in temperate Eurasia and North America (Plunkett *et al.* 2018). In Iran, the family is represented by 124 genera and 375 species (Ghahremaninejad *et al.* 2017). The fruits in the family are typically schizocarps with two-ribbed mericarps.

Based on the shape of the endosperm (Drude 1898), the genus *Prangos* Lindl. has been traditionally placed in the subfamily *Apioideae* Seem. and tribe *Smyrnieae* Spreng. *Prangos* is represented by 45 taxa worldwide (Lyskov *et al.* 2017b) while most species are found in Asia (Pimenov & Leonov 1993). The center of diversity of the genus is the Irano-Turanian region (Senol *et al*. 2011). Iran and Turkey are important centers for the genus *Prangos* as half of all species of the genus grow in these areas (Rechinger 1987, Davis *et al*. 1988).

Kuzjmina (1962) conducted the first revision of *Prangos* using carpological characters, in which two sections with two subsections were delimited, whereas Herrnstadt & Heyn (1977) also had great emphasis on carpological characters modified Kuzjmina's classification and divided the genus into three sections [*Prangos* (type: *P. pabularia*), *Intacta* Kuzmina (type*: P. bucharica* B. Fedtsch.), and *Meliocarpoides* Herrnst. & Heyn (type: *P. meliocarpoides* Boiss.)]. According to Flora Iranica, *Prangos* has 16 species in Iran (Rechinger 1987) of which five endemic species including *P. tuberculata* Boiss. & Hausskn. ex Boiss., *P. gaubae* (Bornm.) Herrnst. & Heyn, *P. crossoptera* Herrnst. & Heyn, *P. calligonoides* Rech.f., and *P. cheilanthifolia* Boiss. are distributed in the country (Mozaffarian 1996). *Prangos* is a polymorphic genus and varies considerably in habit, floral as well as fruit morphology that, these features have made some ambiguities in determining the boundaries within the genus (Lyskov *et al*. 2017b). Rechinger (1987) divided the genus into three sections viz. *Intacta*, *Prangos*, and *Meliocarpoides* Herrnst. & Heyn. Section *Intacta* includes *P. asperula* subsp. *haussknechtii* (Boiss.) Herrnst. & Heyn, *P. corymbosa* Boiss., *P. serpentinica* (Rech.f., Aellen & Esfand.) Herrnst. & Heyn, *P. crossoptera*, *P. gaubae*, *P. ferulacea* Lindl., *P. acaulis* (DC.) Bornm., *P. calligonoides*, *P. tuberculata*, and *P. longistylis* (Boiss.) Pimenov & Kljuykov; section *Prangos* includes *P. uloptera* DC., *P. pabularia* subsp. *pabularia* Lindl., and *P. latiloba* Korovin, and section *Meliocarpoides* includes *P. cheilanthifolia* (Mozaffarian 2007) only. *Prangos* is also a monophyletic genus closely related to monophyletic genus *Cachrys* (Lyskov *et al*. 2017a,b). Many species of *Cachrys* were transferred to *Prangos* and *Bilacunaria* (Pimenov & Tikhomirov 1983) but according to a molecular study carried out by Downie *et al.* (2010), *Prangos* should be placed in the *Cachrys* clade.

Seed morphological characters (length, width, shape, and color) contributed useful data and are frequently used to discriminate the taxa in different taxonomic ranks. In general, the studied species of *Prangos* have close morphological characteristics and is sometimes difficult to differentiate them from each other (Lyskov *et al*. 2017b). Different researchers have performed seed morphological studies emphasizing the taxonomic value of several *Umbelliferae* taxa (Fedoronchuk 1983, Duran *et al*. 2010, 2015, Ostroumova *et al*. 2016), but seed morphology of *Prangos* is poorly known so that only a few occurrences of its seed is available in published work (Pimenov & Tikhomirov 1983, Lyskov *et al*. 2017a,b). The coat surface of seeds (such as epidermal cell size, cell arrangement, cell outlines, anticlinal and periclinal walls) are valuable features for taxonomic studies that used in the species level (Fukuhara *et al*. 1999, Menemen & Jury 2001, Ghimire *et al*. 2016, Ostroumova 2018). Several recent phylogenetic studies have helped the systematics of genus *Prangos* (Downie *et al*. 2000, Valiejo-Roman *et al*. 2006, Ajani *et al*. 2008, Downie *et al*. 2010, Banasiak *et al*. 2013, Lyskov *et al*. 2015, Lyskov *et al*. 2017a,b, Lyskov & erSamigullin 2017c). Recently, Lyskov *et al*. (2017b) divided the genus into two subgenera viz. *Prangos* and *Koelzella* (M.Hiroe) Lyskov & Pimenov. Heywood (1971) suggested the importance and effectiveness of scanning electron microscopy (SEM) in solving systematic problems. SEM studies showed that, seed has useful taxonomic characteristics for different families and genera and plays an important role in the study of plant systematic (Akçin *et al*. 2013), hence, has some taxonomic significance at the generic and species levels (Brochmann 1992, Koul *et al*. 2000). The

present aim is, therefore, to describe morphological characteristics and ornamentation of the seed of all Iranian members of the genus *Prangos*, with emphasis on their micromorphological characteristics, mostly focusing on the utility of the obtained data (treated separately and combined) for the taxonomy of the genus.

This study is mainly aimed to survey the diversity of seed morphology in the native species of *Prangos* in Iran to find useful seed characteristics for delimitation of their closely related species that may not be clear by morphological and molecular characteristics. Scanning electron microscopy was also used to solve the problems in systematic of the taxa to establish the taxonomic relationship between close species. Most of the examined taxa have been studied for the first time in Iran. Based on seed morphology, a key to the species of the genera is also provided.

Materials and Methods

Fifteen *Prangos* species that had been collected from different localities from west, south and central parts in Iran during 1972–2014 years, were examined here (Figs 1–5, Table 1). Collection details of the selected specimens were shown in Table 1. Seeds of all 15 species of *Prangos* occurring in Iran were studied. The work is based on studying the collections deposited at SARI (Research Institute of Forests and Rangelands Herbarium, Sanandaj), TARI (Research Institute of Forests and Rangelands Herbarium, Tehran), and UUH (University of Urmia Herbarium, Urmia) (Iran). These collections were then closely compared with various collections of E (https://data.rbge.org.uk/search/herbarium), K (https://www.kew.org/science/collections-and-resources/collections/herbarium), and W (https://herbarium.univie.ac.at/database/search.php) herbaria. In some cases, images are accessible from GBIF (https://www.gbif.org). References to the International Code of Nomenclature for algae, fungi and plants IPNI (https://www.ipni.org/?q=*Prangos*) were also provided.

All collections were critically studies for important taxonomic characters in the genus including the shape, color and size, presence or absence winged ribs, epidermal cell shape, characters of anticlinal boundaries, and periclinal cell wall of each taxon seeds (Table 2).

For macromorphological studies, observations were carried out in a Leica WILD M3Z stereomicroscope, and 12 seeds for each taxon were chosen to cover the range of variation. (Table 2). For micromorphological observations of the seeds including the surface ornamentation, anticlinal and periclinal cell walls, and the structure of epidermal cell, the specimens examined with a Hitachi SU3500 scanning electron microscope. For scanning electron microscopy (SEM), the seeds were mounted onto a metallic stub with a double-sided adhesive tape. Gold coating of few nanometers was applied using sputter coating machine (Pvd.ir-Dedktop magnetron sputtring) to avoid charging and capture high quality images. The stubs were sputter-coated with gold-palladium for 5 min (seed in whole mount with X = 10, 11, 13, 16, 18, and seed scan with X = 100, 400, 450, 500). The values of the length and width seeds were calculated by Simpson & Roe graphical test (Van der Pluym & Hideux 1997). The terminology of morphological characteristics was carried out in accordance with Corner (1976), Stearn (1985), and Barthlott (1981). Five seed micromorphological characters were chosen to separate the 15 taxa of the *Prangos.* The characters and states (such as: epidermal cells size, cell arrangement, cell outlines, anticlinal and periclinal walls; characters states with coded: small: 0, large: 1; random: 0, in rows: 1; isodiametric: 0, oblong: 1; raised: 0, slightly raised:1, depressed: 2; flat: 0, convex: 1, concave: 2, with small acute projection: 3, with small compressed: 4) have been subjected to numerical analysis under a program using similarity and dissimilarity assessment percentage method (Kovach 1999). The taxa were grouped according to the variation of selected characters by use of the clustering analysis method (unweighted pair group method with arithmetic mean [(UPGMA); Fig. 5 B]. The MVSP software Ver. 3.2 (Kovach 1999) was used to calculate Jaccard's (1908) similarity coefficients among the taxa. A dendrogram was constructed using UPGMA (unweighted pair-group method with arithmetic mean).

Table 1. Species of *Prangos* examined in this study

Taxa	Voucher specimen	Location	Coordinates	Altitude (m)	Date	Collector
P. acaulis (DC.) Bornm.	93429 TARI	E. Azarbaijan prov.: Miyaneh Bozgoush mountain region, Varankesh village	37°39'26" N 47°27'0.5" E	1909	2.9.2007	Mozaffarian
P. asperula subsp. *haussknechtii* (Boiss.) Herrnst. & Heyn	3012 UUH	W. Azarbaijan prov.: 20 km Sardasht to Baneh road	36°10'58" N 45°41'40" E	1500	9.7.1974	Siami & Zehzad
P. calligonoides Rech.f.	37022 TARI	Lorestan prov.: ca. 20 km SW of Doroud, Bisheh	33°19'43" N 48°53'23" E	1250–1600	11.7.1981	Assadi & Mozaffarian
P. cheilanthifolia Boiss.	79243 TARI	Esfahan prov.: 15 km from Naein to Yazd	32°46'42" N 53°13'40" E	1400	15.5.1999	Mozaffarian
P. corymbosa Boiss.	23810 TARI	E. Azarbaijan prov.: Miyaneh, 30 km north of Miyaneh road to Khalkhal, Neshagh village	37°41'04" N 47°40'39" E	1500	24.5.1974	Babakhanlou
P. crossoptera Herrnst. & Heyn	788 SARI	Kordestan prov.: Sanandaj, Narran village, 38 km from Sanandaj, Sanandaj-Kamyaran	35°07'57" N 46°59'04" E	1500–2400	15.6.1986	Fattahi, Tavakoly & Khaledian
P. eriantha (DC.) Lyskov & Pimenov	71622 TARI	W. Azarbaijan prov.: Urumieh, Salmas to Tasuj, after Sadeghian	38°16'02" N 45°01'00" E	1480	25.10.1991	Mozaffarian
P. ferulacea Lindl.	29301 TARI	Kordestan prov.: 32 km from Baneh, on road to Marivan	35°43'02" N 46°03'25" E	1640	30.5.1978	Runemark & Mozaffarian
P. gaubae (Bornm.) Herrnst. & Heyn	105328 TARI	Zanjan prov.: Zanjan to Dandi, 3 km after Gharaei village, rocky slope	36°32'27" N 47°55'37" E	1860	29.5.2014	Mahmoodi
P. latiloba Korovin	35966 TARI	Khorassan prov.: Between Mashhad to Torbat-e Heydarieh, Robat-Sefid	35°46'26" N 59°22'37" E	1700–1900	16.6.1972	Assadi & Mozaffarian
P. longistylis (Boiss.) Pimenov & Kljuykov	30709 TARI	E. Azarbaijan prov.: Kuh-e Sahand	37°48'39" N 46°17'34" E	2200–2900	3.7.1978	Assadi & Mozaffarian
P. pabularia subsp. *pabularia* Lindl.	87681 TARI	Kermanshah prov.: Kermanshah to Kamyaran, Varmangeh, Padegan-e Shahid Rajaie	34°38'23" N 46°56'37" E	1915	8.7.2003	Hamzehee & Asri
P. serpentinica (Rech.f., K. Rasbach, Reichst. & Bennert) Herrnst. & Heyn	48436 TARI	Khorasan prov.: Esferayen, N slope of Kuh-e Shah-Jahan from Darparchin-e Bala village	37°06'21" N 57°43'25" E	1700–2500	6.6.1984	Mozaffarian
P. tuberculata Boiss. & Hausskn. ex Boiss.	46677 TARI	Fars prov.: Shiraz, Hossein-abad Protected Area	29°38'15" N 52°11'50" E	1850	3.6.1983	Mozaffarian
P. uloptera DC.	32622 TARI	Tehran prov.: W Tehran, Suleghun valley	35°48'44" N 51°15'48" E	1500–2000	31.6.1979	Assadi & Mozaffarian

Results

In the classification of *Apiaceae* taxa, seed features are very valuable taxonomically, that represent distinct differences in shape and size. In this study, the seed features of 15 *Prangos* species were determined by light and scanning microscopes. The micrograph images of seeds of *Prangos* species are represented in figures 1–4. The ten quantitative characters were measured in seeds of 15 species. The morphological characters of the seeds including shape, size, color, and length/width ratio for each taxon were presented in Table 2. The shape of seeds showed variation. Seeds are cylindrical, ovate, ovate to elliptic, oblong, oblong to linear, elliptic or elliptic to ovate in shape. The size of seeds in the studied species are different from 6.37 × 2.32 mm (in *P. latiloba*) to 13.45 × 2.87 mm (in *P. ferulacea*). The seed length and width varies greatly among the examined species (Fig. 5 B). The color of fruits varies from green in *P. acaulis* and *P. gaubae*, gray in *P. latiloba*, light brown in *P. pabularia* subsp. *pabularia*, *P. serpentinica*, and *P. uloptera*, brown to black in *P. corymbosa*, and dark brown in the rest of the taxa (Fig. 2, Table 2). The micromorphological features, including seed epidermal cells size, cell arrangement, cell outlines, anticlinal and periclinal walls have been investigated.

Micromorphological characters

- Epidermal cells

The epidermal cell size showed considerable variation among the studied species (Table 2). Small epidermal cells observed in *Prangos corymbosa*, *P. acaulis*, *P. calligonoides*, *P. tuberculata*, *P. longistylis*, and *P. pabularia* subsp. *pabularia* (Figs 1 D-F, 2 A-C, 1 D-F, 1 G-I, 1 M-O, 3 G-I). The large epidermal cells observed (seed scan with X=100, 400, 450, 500) in *P. asperula* subsp. *haussknechtii*, *P. serpentinica*, *P. crossoptera*, *P. gaubae*, *P. ferulacea*, *P. cheilanthifolia*, *P. uloptera*, *P. latiloba*, and *P. eriantha* (Figs 1 A-C, 1 G-I, 1 J-L, 1 M-O, 2 J-L, 3 A-C, 3 D-F, 3 J-L, 3 M-O).

- Anticlinal cell wall boundaries

The cell outlines varied from isodiametric to oblong in shape. The sculpture features of the seed surface are shown in figures 1–3. The cell arrangement varied from random to in rows (Table 2). Based on seed characters, our cluster analyses separated the taxa into two major clusters, 1 (Group I) and 2 (Group II). Group I comprises the sculpture ornamentation with cell outlines isodiametric and random. Based on anticlinal wall, the group I, was further divided into two clusters (subgroup). Cluster 1: raised-subgroup and cluster 2: slightly raised-subgroup (Fig. 5 B). Group II comprises the sculpture ornamentation with cell outlines oblong and in rows. Based on anticlinal wall, this group was further divided into two clusters (subgroup). Cluster I: raised-subgroup and cluster 2: depressed-subgroup (Fig. 5 B). Two groups of ornamentation patterns were observed:

Group I (Isodiametric and random): the sculpture ornamentation of group I is formed by cell outlines isodiametric and random (with 10 species). This group included *Prangos asperula* subsp. *haussknechtii*, *P. corymbosa*, *P. serpentinica*, *P. crossoptera*, *P. gaubae*, *P. acaulis*, *P. calligonoides*, *P. tuberculata*, *P. longistylis*, and *P. cheilanthifolia* (Figs 1 A-C, 1 D-F, 1 G-I, 1 J-L, 1 M-O, 2 A-C, 2 D-F, 2 G-I, 2 M-O, 3 A-C; Table 2). Based on anticlinal wall, this seed group was further divided into two subgroups (Fig. 5 B). The raised-subgroup is easily recognized by having anticlinal wall raised seed [with six species of *P. asperula* subsp. *haussknechtii*, *P. corymbosa*, *P. crossoptera*, *P. calligonoides*, *P. tuberculata*, and *P. cheilanthifolia* (Figs 1 A-C, 1 D-F, 1 J-L, 2 D-F, 2 G-I, 3 A-C)]. The slightly raised-subgroup is easily recognized by having anticlinal wall slightly raised seed [in four species of *P. serpentinica*, *P. gaubae*, *P. acaulis*, and *P. longistylis* (Figs 1 G-I, 1 M-O, 2 A-C, 2 M-O)].

Group II (oblong and in rows): the seed-coat surface of group II is formed by cell outlines oblong and in rows, included *Prangos ferulacea*, *P. uloptera*, *P. pabularia* subsp. *pabularia*, *P. latiloba*, and *P. eriantha* (Figs 2 J-L, 3 D-F, 3 G-I, 3 J-L, 3 M-O, Table 2). Based on anticlinal wall, this seed group was further divided into two subgroups. The raised-subgroup is easily recognized by having anticlinal wall raised seed (in *P. ferulacea*, *P. uloptera*, *P. pabularia* subsp. *pabularia*, and *P. latiloba* (Figs 2 J-L, 3 D-F, 3 G-I, 3 J-L). The depressed-subgroup is easily recognized by having anticlinal wall depressed seed with only species *P. eriantha* (Fig. 3 M-O).

Table 2. Morphological and micromorphological data obtained from *Prangos* seeds

Taxa	Length (mm)	Width (mm)	Length/width ratio (mm)	Winged rib	Shape	Color	Epidermal cell size	Cell arrangement	Cell outline	Anticlinal wall	Periclinal wall
P. acaulis	10.86(10.41-11.32)	3.03(2.11-3.95)	3.58	Presence	Ovate to elliptic	Green	Small	Random	Isodiametric	Slightly raised	Convex
P. asperula subsp. *haussknechtii*	13.39(12.93-13.85)	3.41(3.14-3.68)	3.92	Presence	Oblong	Dark brown	Large	Random	Isodiametric	Raised	Flat
P. calligonoides	7.41(6.26-8.56)	2.16(1.64-2.64)	3.43	Presence	Ovate	Dark brown	Small	Random	Isodiametric	Raised	With small acute projection
P. cheilanthifolia	6.65(6.39-6.95)	2.53(2.17-2.89)	2.62	Absence	Elliptic	Dark brown	Large	Random	Isodiametric	Raised	Convex
P. corymbosa	8.45(7.89-9.01)	1.53(1.13-1.93)	5.52	Presence	Oblong	Brown to black	Small	Random	Isodiametric	Raised	With small compressed
P. crossoptera	9.45(8.8-10.10	3.52(3.03-4.01)	3.95	Presence	Ovate	Dark brown	Large	Random	Isodiametric	Raised	Concave
P. eriantha	11.02(10.06-11.98	2(1.89-2.11)	4.50	Presence	Elliptic to oblong	Dark brown	Large	In rows	Oblong	Depressed	Concave
P. ferulacea	13.45(12.96-13.94)	2.87(2.72-3.02)	4.59	Presence	Elliptic to ovate	Dark brown	Large	In rows	Oblong	Raised	Flat
P. gaubae	6.75(6.49-7.01)	2.39(1.93-2.85)	2.82	Absence	Ovate	Green	Large	Random	Isodiametric	Slightly raised	With small acute projection
P. latiloba	6.37(5.8-6.94)	2.32(1.88-2.76)	2.74	Presence	Cylindrical	Gray	Large	In rows	Oblong	Raised	Flat
P. longistylis	13.18(12.59-13.78)	3.03(2.58-3.48)	4.34	Presence	Ovate to elliptic	Dark brown	Small	Random	Isodiametric	Slightly raised	With small acute projection
P. pabularia subsp. *pabularia*	10.66(9.94-11.38)	2.09(1.35-2.83	5.10	Presence	Oblong to linear	Light brown	Small	In rows	Oblong	Raised	Concave
P. serpentinica	9.29(8.69-9.89)	2.94(2.68-3.20)	3.15	Absence	Ovate	Light brown	Large	Random	Isodiametric	Slightly raised	Concave
P. tuberculata	7.74(7.55-7.93)	2.74(2.38-3.1)	2.82	Presence	Ovate	Dark brown	Small	Random	Isodiametric	Raised	Concave
P. uloptera	10.67(10.31-11.03)	2.08(1.21-2.95)	5.12	Presence	Oblong to linear	Light brown	Large	In rows	Oblong	Raised	With small acute projection

- Periclinal cell walls

The periclinal walls of the seed in *Prangos* species were flat [*P. asperula* subsp. *haussknechtii*, *P. ferulacea*, *P. latiloba* (Figs 1 A-C, 2 J-L, 3 J-L)], concave [*P. serpentinica*, *P. crossoptera*, *P. tuberculata*, *P. pabularia* subsp. *pabularia*, and *P. eriantha* (Figs 1 G-I, J-L, 2 G-I, 3 G-I, M-O)], convex [*P. acaulis* and *P. cheilanthifolia* (Figs 2 A-C, 3 A-C)], with small acute projection [*P. gaubae*, *P. calligonoides*, *P. longistylis*, and *P. uloptera* (Figs 1 M-O, 2 D-F, M-O, 3 D-F)], minutely compressed [*P. corymbosa* (Fig. 1 D-F)].

Morphological characters

The seed morphology is very important to separate species in *Apiaceae*. In this study, it was found that, the morphological characters are taxonomically valuable: for example: shape, size, color, and seed coat and texture within each taxon (15 species) were given in Table 2 and the image by light microscope represented in figure 4.

- Seed shape, size, and color

The seeds of *Prangos* species are cylindrical, ovate, ovate to elliptic, oblong, oblong to linear, and elliptic or elliptic to ovate. The elliptic or elliptic to ovate seeds were found in *P. cheilanthifolia* and *P. ferulacea* (Fig 4 N, E). The oblong or oblong to linear seeds were characterized in *P. corymbosa*, *P. asperula* subsp. *haussknechtii*, *P. pabularia* subsp. *pabularia* and *P. uloptera* (Fig. 4 A, J, K, L). Seeds of *P. eriantha* were elliptic to oblong (Fig. 4 O). The ovate or ovate to elliptic seeds were found in *P. gaubae*, *P. serpentinica*, *P. calligonoides*, *P. tuberculata*, *P. crossoptera*, *P. acaulis*, and *P. longistylis* (Fig. 4 B, C, D, F, G, H, I). Seeds of *P. latiloba* were cylindrical (Fig. 4 M).

The length and the width of the seeds were almost unequal in the investigated species and ranged from 6.37–13.45 × 1.53–3.52 mm. The smallest seeds found in *Prangos latiloba* (6.37 × 2.3 mm) and the largest observed in *P. ferulacea* (13.45 × 2.87 mm). Seeds of *P. ferulacea* were distinguished from other seeds of *Prangos* in being larger (above 13 mm in length) (Table 2). In addition, based on the seed width, three distinct seed groups were recognized viz. narrow seed (1.5 ≤ mm), medium-width seed (1.5–2.5 mm), and wide seed (2.5 ≥ mm). The narrow seed was observed in *P. corymbosa*; the medium-width seeds founded in *P. calligonoides*, *P. corymbosa*, *P. gaubae*, *P. latiloba*, *P. pabularia* subsp. *pabularia* and *P. uloptera*; and the wide seed noticed in *P. acaulis*, *P. asperula* subsp. *haussknechtii*, *P. cheilanthifolia*, *P. crossoptera*, *P. ferulacea*, *P. longistylis*, *P. serpentinica*, and *P. tuberculata*.

The seed color in *Prangos* species were green, light brown, dark brown, brown to black, gray and dark brown to black. Green seeds were found in *P. gaubae* and *P. acaulis* (Fig. 4 G, H). Gray seeds were found in *P. latiloba* (Fig. 4 M). Brown to black seeds found in *P. corymbosa* (Fig. 4 A). The seeds were dark brown or light brown observed in other species (*P. cheilanthifolia*, *P. longistylis*, *P. uloptera*, *P. serpentinic*, *P. pabularia* subsp. *pabularia*, *P. crossoptera*, *P. ferulacea*, *P. asperula* subsp. *haussknechtii*, *P. calligonoides*, *P. tuberculata*, and *P. eriantha*) (Fig. 4 B, C, D, E, F, J, I, J, K, L, N, O). Different specimens of *Prangos* were characterized by seed color varying from green to dark brown to black.

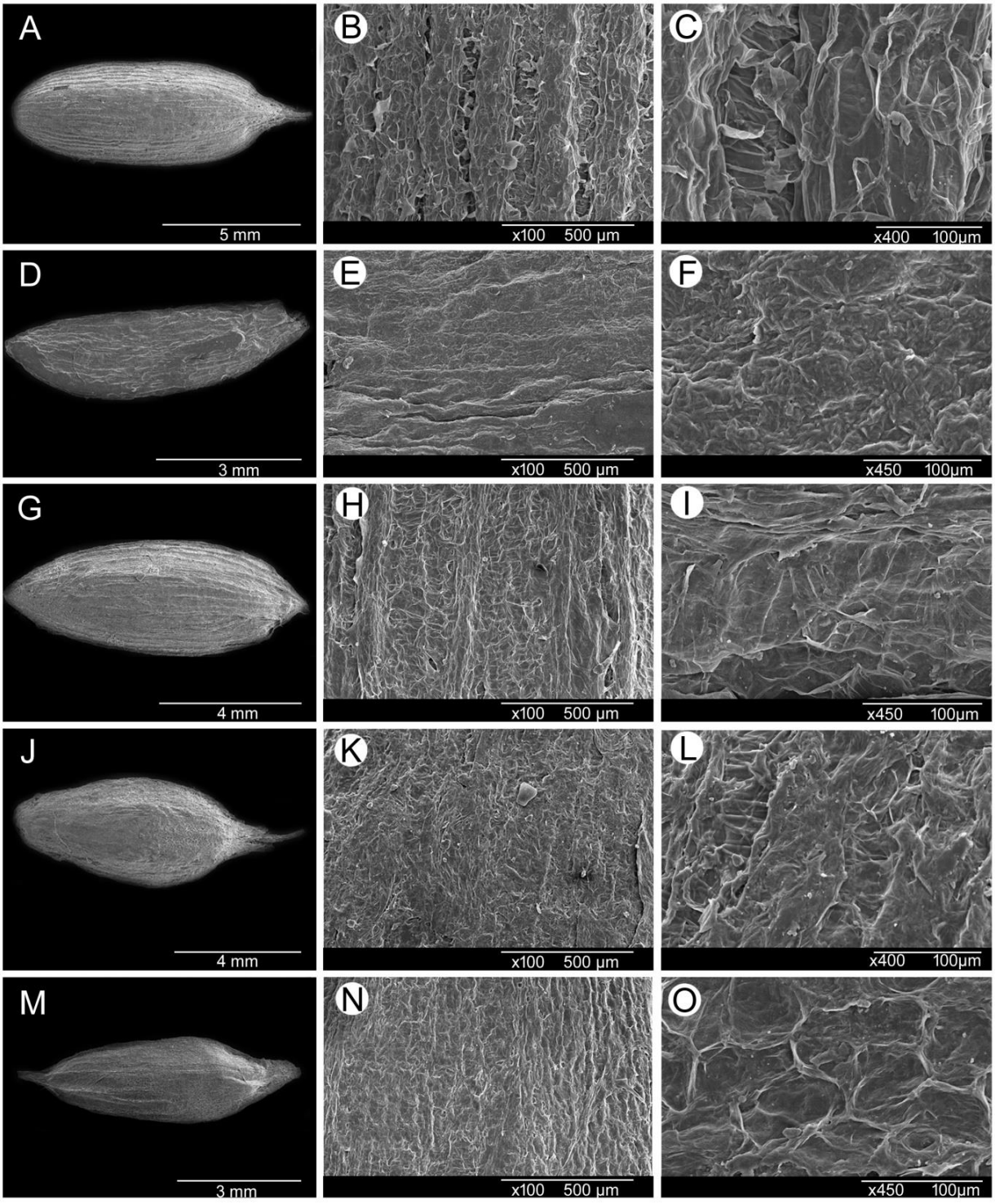

Fig. 1. Scanning Electron Micrographs of *Prangos* seeds and details of seed coat surface: A-C. *P. asperula* subsp. *haussknechtii*, D-F. *P. corymbosa*, G-I. *P. serpentinica*, J-L. *P. crossoptera*, M-O. *P. gaubae*.

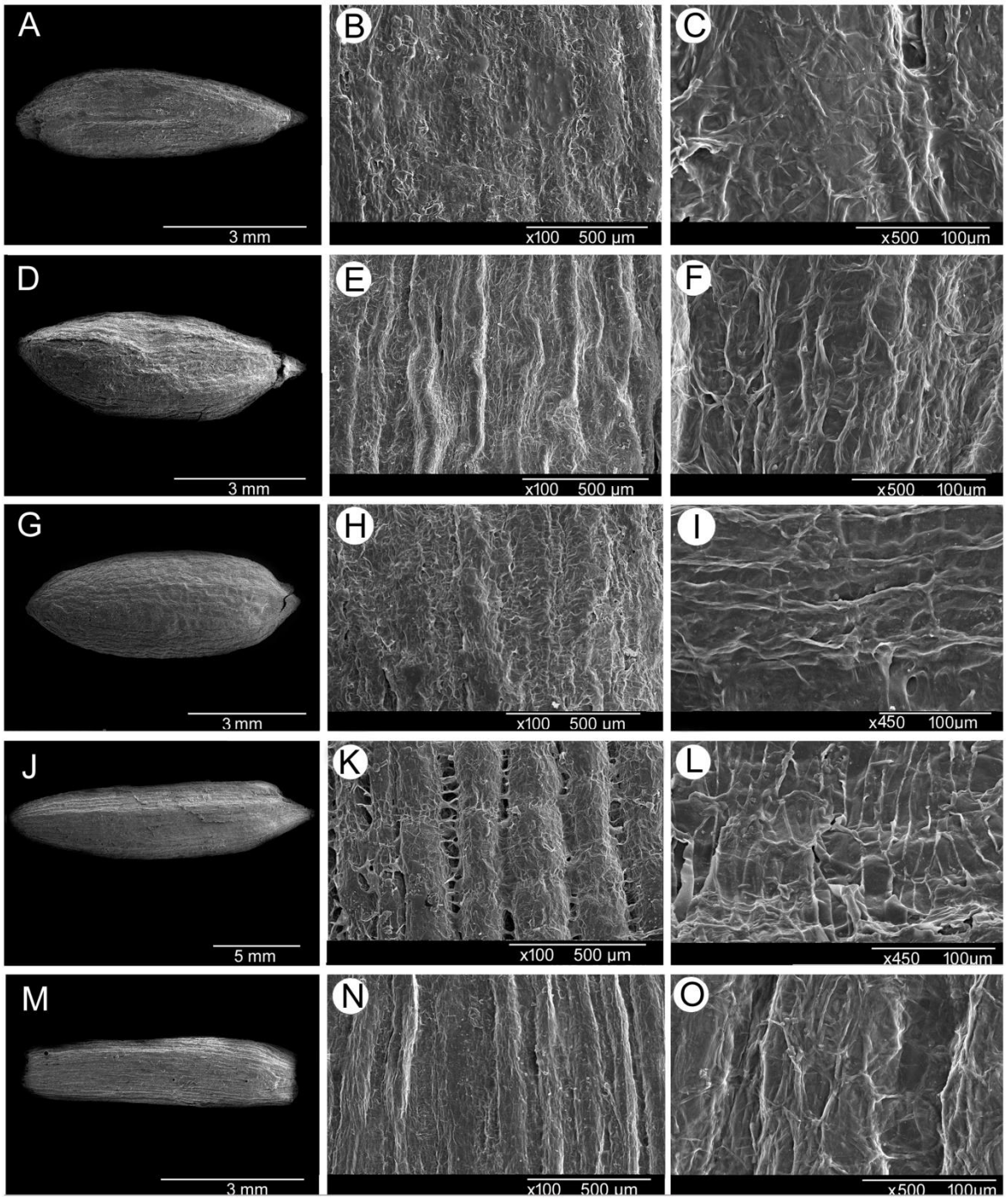

Fig. 2. Scanning Electron Micrographs of *Prangos* seeds and details of seed coat surface: A-C. *P. acaulis*, D-F. *P. calligonoides*, G-I. *P. tuberculata*, J-L. *P. ferulacea*, M-O. *P. longistylis.*

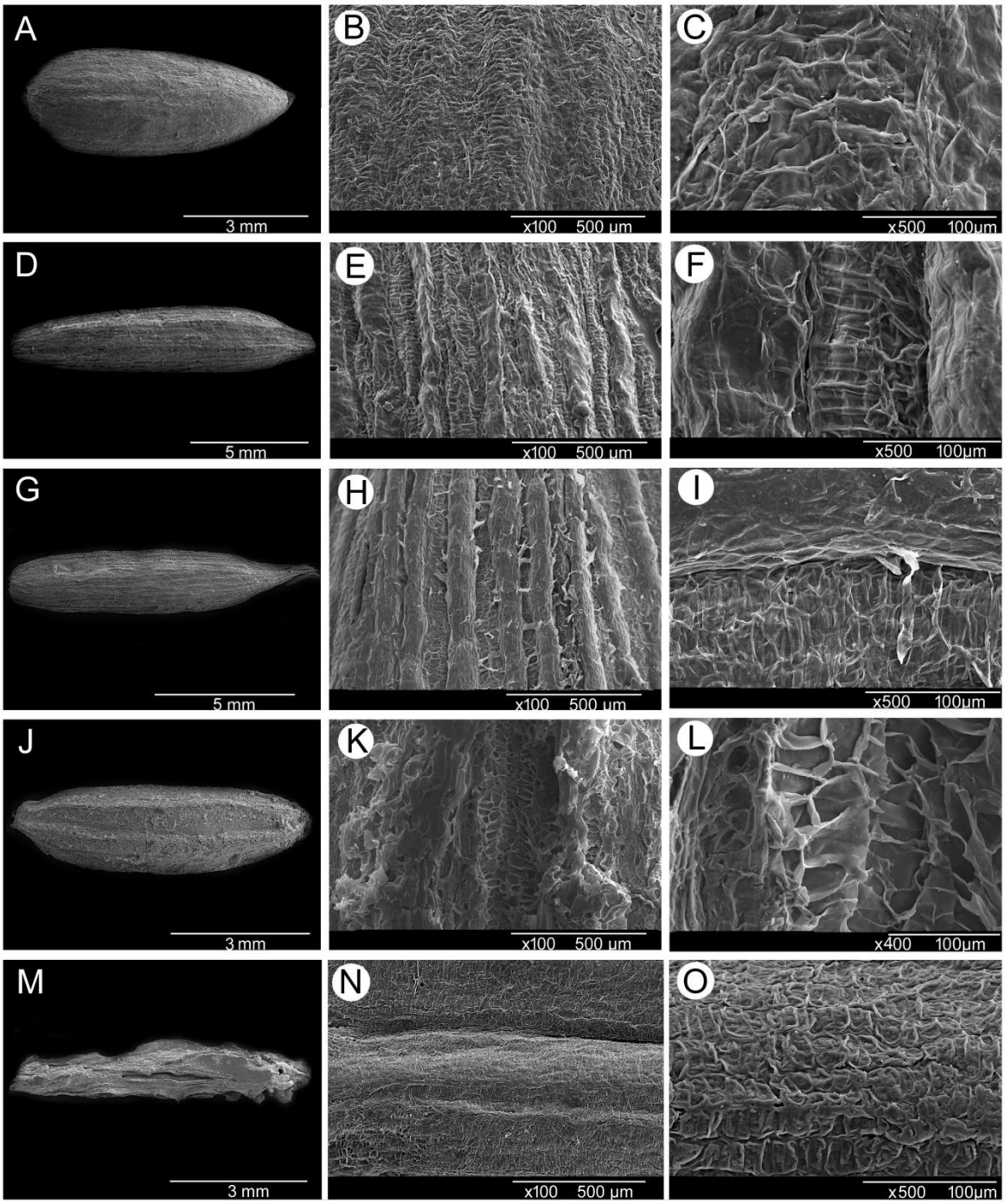

Fig. 3. Scanning Electron Micrographs of *Prangos* seeds and details of seed coat surface: A-C. *P. cheilanthifolia*, D-F. *P. uloptera*, G-I. *P. pabularia* subsp. *pabularia*, J-L. *P. latiloba*, M-O. *P. eriantha*.

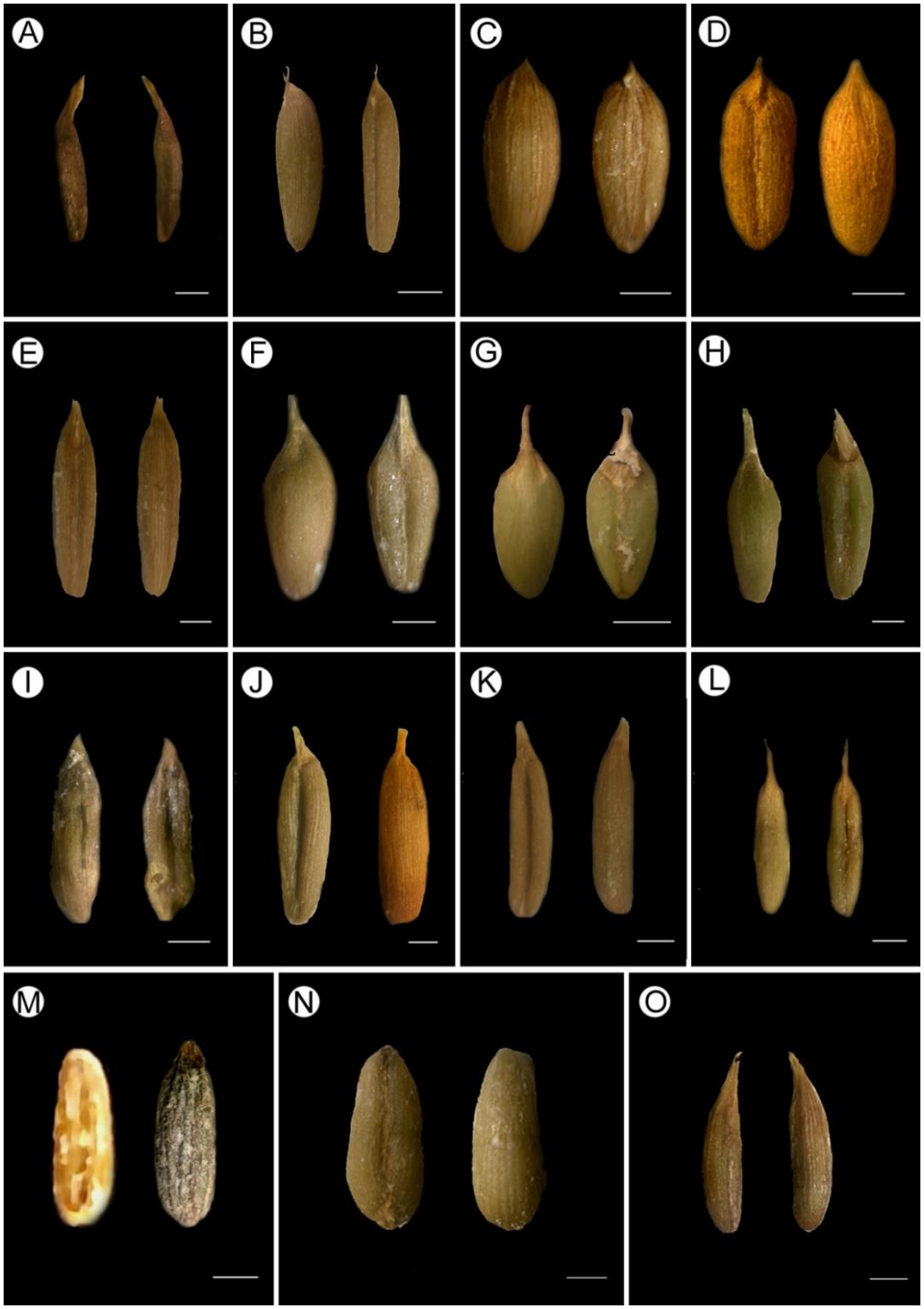

Fig. 4. Light Micrographs of *Prangos* seeds: A. *P. corymbosa*, B. *P. longistylis*, C. *P. serpentinica*, D. *P. tuberculata*, E. *P. ferulacea*, F. *P. crossoptera*, G. *P. gaubae*, H. *P. acaulis*, I. *P. calligonoides*, J. *P. asperula* subsp. *haussknechtii*, K. *P. pabularia* subsp. *pabularia*, L. *P. uloptera*, M. *P. latiloba*, N. *P. cheilanthifolia*, O. *P. eriantha* (Bars = 2 mm).

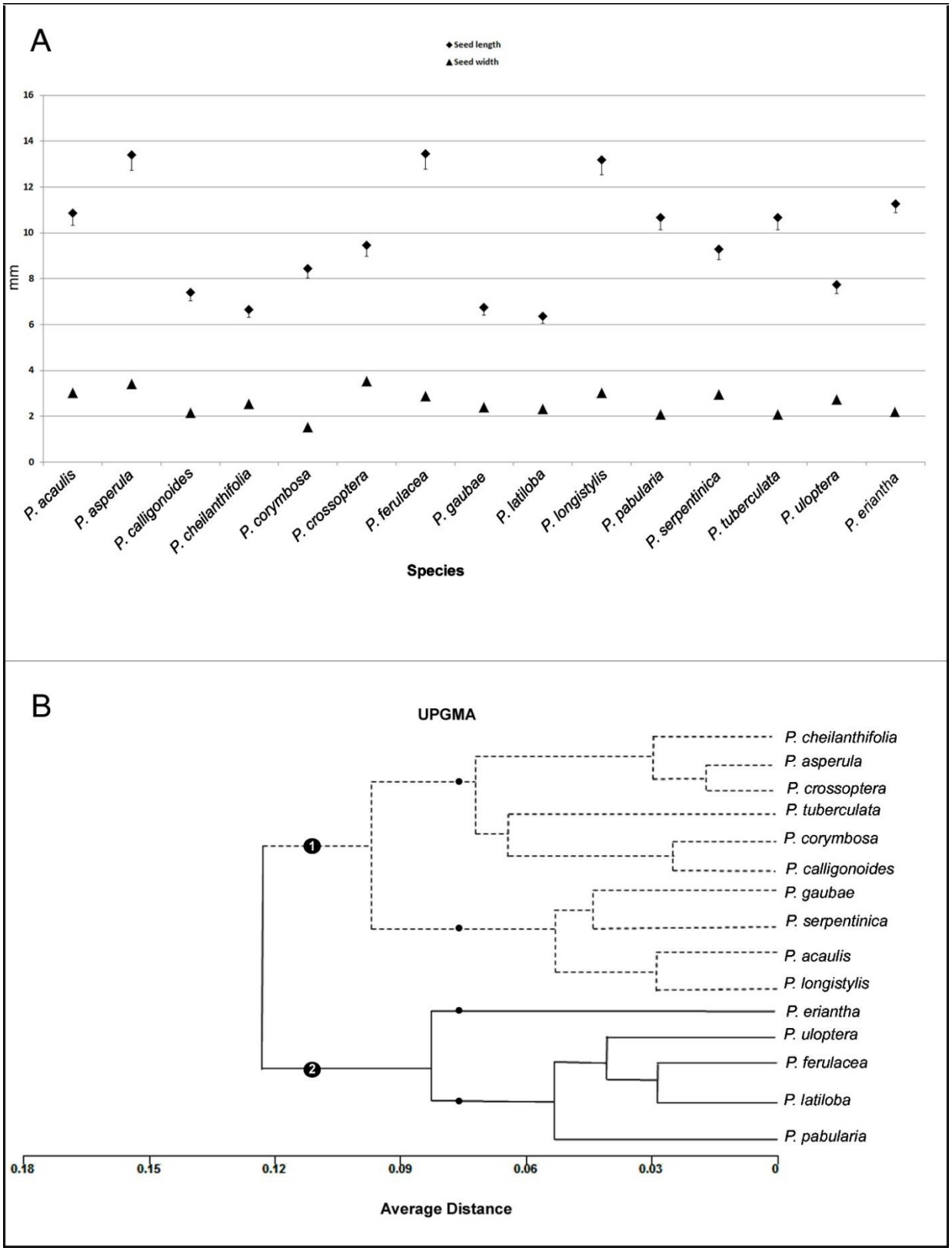

Fig. 5. A. Simpson & Roe test for *Prangos* seed length and width, B. UPGMA clustering of the examined taxa based on seed characters.

Discussion

The micromorphological and morphological characteristics of the seeds provide precise information about the closely related species of flowering plants (Corner 1976, Barthlott 1981). The morphological variation, such as the surface ornamentation, shape, color, and size of the seeds of the *Apiaceae* are valuable characters in discriminating the taxa (Sun *et al.* 2012). The micromorphological characteristics of seeds of the *Prangos* taxa vary among the species. The species examined in our study were very diverse in terms of seed color, for example, light brown; dark brown to black dominates the species, whereas a small number of taxa had green and gray seeds. The color is effective in separating some of the closely related species. Our study of the seed provided some important new data concerning macro- and micromorphology.

A detailed analysis of the morphological features of seeds greatly broadens our knowledge of individual taxa and may be helpful in providing more insight into the phylogeny of the taxa. Moreover, combined micromorphological and macromorphological characteristics of the seeds can be used as an important tool for species classification of *Prangos*. In this study, it was observed that, seed surfaces of the examined taxa were in various forms: isodiametric and oblong. According to the results of the seeds surface ornamentation, the Iranian members of the genus *Prangos* can be included within two different groups (*P. corymbosa*-group and *P. ferulacea*-group), which described according to ornamentation pattern.

A certain heterogeneity was observed in length and width seeds values (Fig. 5 A). *Prangos ferulacea* and *P. asperula* subsp. *haussknechtii* displayed great variations in the length and width seeds (Van der Pluym & Hideux 1997). SEM examination showed the cell outlines were random or in rows on the surface pattern. However, based on seed ornamentation patterns, two distinct groups were recognized here viz. *Prangos corymbosa*-group and *Prangos ferulacea*-group. *Prangos corymbosa*-group is easily recognized by having the isodiametric and random epidermal cells among all seeds group (with 10 species: *P. asperula* subsp. *haussknechtii*, *P. corymbosa*, *P. serpentinica*, *P. crossoptera*, *P. gaubae*, *P. acaulis*, *P. calligonoides*, *P. tuberculata*, *P. longistylis*, and *P. cheilanthifolia*). *Prangos ferulacea*-group is easily recognized by having the oblong and in rows epidermal cells among all seed group (with 5 species: *P. ferulacea*, *P. uloptera, P. pabularia* subsp. *pabularia*, *P. latiloba*, and *P. eriantha*) (Fig. 5 B). Lyskov *et al*. (2017b) divided the genus into two subgenus: *Prangos* and *Koelzella*. The subgenus *Prangos* is included sections *Prangos* (includes subsection *Prangos*), *Meliocarpoides*, *Apteropleura* (includes subsection *Peucedanifoliae* and *Bucharicae*), *Latilobae*, *Alococarpum* (Riedl & Kuber) Lyskov & Pimenov, *Cryptodiscus*, and *Ulopterae. Prangos pabularia* subsp. *pabularia* is the only species that known from subgenus *Koelzella* in Iran.

The results of the present study indicated that, the cell arrangement varied from random or in rows was observed among all studied species, but they were different in size (Figs 1–3). The seed surface previous studies showed that, the appearances of anticlinal and periclinal cell walls are good diagnostic parameters at the species level within the genus in flowering plants (Barthlott 1981, Tantawy *et al*. 2004). In addition, our results showed that, the anticlinal cell wall (raised, slightly raised, depressed) and the periclinal cell (flat, concave, convex, with small acute projection, with small compressed) varied. The phylogenetic study showed a very close relationship between *Prangos cheilanthifolia* and *P. crossoptera* (Lyskov *et al.* 2017b). The results of the present study indicated that, this species distinguished from *P. crossoptera*, by its the periclinal walls convex (vs. concave). In addition, *P. pabularia* differs from *P. eriantha* by its the anticlinal walls raised (vs. depressed) and small of epidermal cells (vs. large) (Table 2). According to the results

from a molecular phylogeny (Lyskov *et al*. 2017a) the *P. gaubae* is more similar to *P. acaulis*, which is in agreement with the results of this research. Based on the results of this research these species have share in micmorphological characters such as the cell arrangement, cell outlines and anticlinal wall. *P. gaubae* separated from the related species *P. acaulis* in having the periclinal walls with small acute projection (vs. convex) (Table 2).

The *Prangos pabularia* and *P. uloptera* were very similar in plant morphological characters. Kuzjmina (1962) and Herrnstadt & Heyn (1977) placed two species in the sect. *Prangos*. Pimenov & Tikhomirov (1983) showed both *P. pabularia* and *P. uloptera* as closely related species by the morphological studies of subsection *Koelzella* (eight species). In addition, the phylogenetic study showed a very close relationship between *P. pabularia* and *P. uloptera* (Valiejo-Roman *et al*. 2006, Ajani *et al*. 2008, Lyskov *et al*. 2015). Recently, *P. uloptera* has been transferred from the sect. *Prangos* to new section *Ulopterae* (Lyskov *et al*. 2017b). *P. latiloba* was separated from the other *Prangos* species and was placed in a new section *Latilobae* (Pimenov & Tikhomirov 1983), which is in agreement with the results from molecular studies by Lyskov *et al*. (2017b). Moreover, Lyskov *et al*. (2017a) showed both *P. latiloba* and *P. serpentinica* as closely related species by the morphological studies. In the present study, based on the cell arrangement and cell outlines (in rows and oblong vs. random and isodiametric, respectively), *P. latiloba* separated from *P. serpentinica*. In addition, it was found that, *P. uloptera*, *P. latiloba*, and *P. ferulacea* share more micromorphological characteristics of seeds such as epidermal cells size, cell arrangement, cell outlines, and anticlinal wall, which agrees with the results from previous molecular phylogeny studies (Lyskov *et al*. 2015, Lyskov *et al*. 2017a). The based on the results of this research the cell arrangement and cell outlines section *Latilobae* is more similar to section *Ulopterae*. Therefore, Lyskov *et al*. (2015, 2017a) showed that one clade consisted sections *Latilobae*, *Ulopterae*, *Prangos*, and *Alococarpum*, which is in agreement with the results of this research.

According to the results from a molecular phylogeny of *Prangos* (Ajani *et al*. 2008) *Cachrys* group consisted of *P. acaulis, P. uloptera, P. ferulacea*, and *P. pabularia*. Based on the results of this research the seed characteristics these species have different the cell random arrangement and cell outlines; random and isodiametric in *P. acaulis* and in rows and oblong in the rest of the taxa. Thus, the cell arrangement was found to be useful for differentiation of the closely related species such as *P. acaulis* and *P. pabularia*. Our findings support the recently postulated hypothesis on the inclusion of *P. acaulis* in the subgenus *Prangos* (Lyskov *et al*. 2017b).

However, recent phylogenetic study showed a very close relationship among *Prangos calligonoides*, *P. acaulis, P. gaubae, P. tuberculata*, *P. cheilanthifolia*, and *P. crossoptera* within subclade clade (Lyskov *et al*. 2017a). In this study, *P. crossoptera*, *P. acaulis*, *P. gaubae*, *P. tuberculata*, and *P. cheilanthifolia* share some features seed such as cell random arrangement and cell isodiametric outlines, which agrees with the results from previous molecular phylogeny studies (Lyskov *et al*. 2017a). In addition, our results indicated that, *P. calligonoides* and *P. tuberculata* differs from the related species *P. acaulis* and *P. gaubae* in having the anticlinal walls raised (vs. slightly raised), which agrees with the results from previous molecular phylogeny studies (Lyskov *et al*. 2015).

The size of epidermal cells, the anticlinal and periclinal walls can be considerably diagnostic with systematic value and useful for separating the species. The results of this research showed that *Prangos pabularia* is similar to *P. ferulacea, P. latiloba*, and *P. uloptera* species based on the cell arrangement in rows, cell outlines oblong and anticlinal raised wall but *P. pabularia* differs from the related species in some characteristics such as the small

epidermal cells (vs. large) and the periclinal walls concave (vs. flat or with small acute). In addition, *P. calligonoides* is obviously allied to *P. gaubae* based on the cell arrangement random and cell outlines isodiametric but is easily distinguishable by the small epidermal cells and anticlinal raised wall (vs. large and slightly raised). In addition, *P. corymbosa* resembles *P. crossoptera* in having cell arrangement, cell outlines and anticlinal wall but is distinguished by small epidermal cells (vs. large) and periclinal wall small compressed (vs. concave), which this result is supported by molecular phylogenetic studies (Lyskov *et al.* 2017a).

Based on morphological studies, Herrnstadt & Heyn (1977) and Leute (1987) noted that, *Alococarpum* resemble those of *Prangos*. The phylogenetic study showed a very close relationship between *Prangos* species and *Alococarpum erianthum* (DC.) Riedl & Kuber (Pimenov *et al*. 2001, Valiejo-Roman *et al*. 2006). Recently, *A. erianthum* has been transferred from the genus *Alococarpum* to *Prangos* section *Alococarpum* as *P. eriantha* (Lyskov *et al*. 2017a), which is in agreement with the results of this research. In addition, *P. eriantha* differ from the species *P. pabularia* in having the anticlinal walls depressed (vs. raised) and large of epidermal cells (vs. small).

Conclusion

The seed morphology and ornamentation play a significant role in the systematic and taxonomy of *Prangos* groups and the examined characteristics in this study can be successfully added to the future taxonomic revision of the genus. According to the current results, some taxa can be separated viz. *P. ferulacea* and *P. gaubae*. In this study, based on cell outlines and cell arrangement, two groups of seed surface were identified. The first group consists of *P. asperula* subsp. *haussknechtii*, *P. corymbosa*, *P. serpentinica*, *P. crossoptera*, *P. gaubae*, *P. acaulis*, *P. calligonoides*, *P. tuberculata*, *P. longistylis*, and *P. cheilanthifolia* whereas the second group contains *P. ferulacea*, *P. uloptera, P. pabularia, P. latiloba*, and *P. eriantha.* The results of the present study based on the cell arrangement and cell outlines, the first group (*corymbosa*-group) included sections *Meliocarpoides* and *Prangos* of subgenus *Prangos* except *P. ferulacea* (with cell arrangement in rows and oblong), with the section *Meliocarpoides*, shows many micromorphological characters similar to the characters of section *Prangos*. In addition, the second group (*ferulacea*-group) included sections *Latilobae* (*P. latiloba*), *Ulopterae* (*P. uloptera*), *Prangos* (*P. ferulacea)*, and *Alococarpum* (*P. eriantha*) of subgenus *Prangos* are more similar. The present results indicated that, *P. pabularia* of subgenus *Koelzella* more closely related to *P. latiloba* of subgenus *Prangos* have the cell arrangement in rows and oblong. Therefore, seed characteristics support that separating species and sections in the genus *Prangos*, which this result is supported by molecular phylogenetic studies (Lyskov *et al.* 2017a,b). In this study, the seed features, the morphology and the seed surface coating are described as a useful tool for species identification. In general, scanning electron microscope studies showed that, the detailed examination of seed characteristics of the *Prangos* taxa is very useful in separating species and sections from each other.

Key to species of *Prangos* based on seed morphology

1. Cell arrangement in rows, cell outlines oblong ……..2
- Cell arrangement random, cell outlines isodiametric ……..6
2. Anticlinal cell walls depressed ……..……...…… *P. eriantha*
- Anticlinal cell walls raised ……..…… 3
3. Epidermal cells small, periclinal walls concave …….. *P. pabularia* subsp. *pabularia*
- Epidermal cells large, periclinal walls flat or with small acute projection ……..…….……. 4
4. Periclinal cell walls with small acute projection …….……. *P. uloptera*
- Periclinal cell walls flat ……..……..…… 5
5. Seeds longer than 10 mm, elliptic to ovate, seeds dark brown ……..……..…..…..…..…… *P. ferulacea*
- Seeds shorter than 10 mm, cylindrical, gray ……..……..……..…… *P. latiloba*
6. Anticlinal cell walls slightly raised ……...…… 7
- Anticlinal cell walls raised ……..……...10
7. Epidermal cells large ……..……..……..…….. 8
- Epidermal cells small ……..……..……..…… 9
8. Periclinal cell walls with small acute projection, seeds 6.75 × 2.39 mm, green ……..……..…… *P. gaubae*
- Periclinal cell walls concave, seeds 9.29 × 2.94 mm, light brown ……..……....…… *P. serpentinica*
9. Periclinal cell walls with small acute projection, seeds dark brown ……..……....……..… *P. longistylis*
- Periclinal cell walls convex, seeds green ……..……..……. *P. acaulis*
10. Epidermal cells small ……..……..…..…..…….... 11
- Epidermal cells large ……..……..……..... 13
11. Periclinal cell walls concave ……....……..……. *P. tuberculata*
- Periclinal cell walls with small compressed or with small acute projection ……..……..……..……12
12. Periclinal cell walls with small compressed, seeds oblong, brown to black ……..……..…….. *P. corymbosa*
- Periclinal cell walls with small acute projection, seeds ovate, dark brown ……..…… *P. calligonoides*
13. Periclinal cell walls convex, seeds width > 3 mm ……..……..…… *P. cheilanthifolia*
- Periclinal cell walls flat or concave, seeds width < 3 mm ……..……..……14
14. Periclinal cell walls flat, seeds oblong, dark brown ……..……..…..… *P. asperula* subsp. *haussknechtii*
- Periclinal cell walls concave, seeds ovate, 9.45 × 3.52 mm ……...…… *P. crossoptera*

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