

**POLLEN MORPHOLOGY OF 10 TAXA BELONGING TO *PRANGOS* LINDL.
AND *EKIMIA* H. DUMAN & M.F. WATSON (UMBELLIFERAE) FROM
TURKEY AND ITS TAXONOMIC SIGNIFICANCE**

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Keywords: Ekimia; Pollen morphology; Prangos; SEM; Taxonomy; TEM.

Abstract

Pollen grains of nine taxa of *Prangos* Lindl. and one of *Ekimia* H. Duman & M.F. Watson (Umbelliferae) were examined with LM (light microscope) and SEM (scanning electron microscope), and of them four with TEM (transmission electron microscope). The quantitative data were also subjected to cluster analysis. The obtained phenogram revealed that *Ekimia bornmuelleri* (Hub.-Mor. & Reese) H. Duman & M.F. Watson is strictly different from the taxa of *Prangos* regarding their quantitative pollen profile; except *P. ferulacea* Lindl., all taxa included in section *Intactae* formed a cluster together; members of section *Meliocarpoides* and section *Prangos* show a closer relationship regarding their pollen features. Exine ornamentations of *Prangos* and *Ekimia* are rugulate-striate and are of no value for identification purposes.

Introduction

The genus *Prangos* Lindl. has around 28 species worldwide (Herrnstadt and Heyn, 1972; 1977). The Anatolian part of Turkey is considered as one of the primary centers of the genus (Duran *et al.*, 2005). In the Flora of Turkey, Herrnstadt and Heyn (1972) recognized 10 species of *Prangos*. In addition, they mentioned about two incompletely known species, namely *Cachyrys papillaris* Boiss. and *P. bornmuelleri* Hub.-Mor. & Reese. Later on, Duman and Watson (1999) transferred *P. bornmuelleri* to the monotypic genus *Ekimia* H. Duman & M.F. Watson as *E. bornmuelleri* (Hub.-Mor. & Reese) H. Duman & M.F. Watson. Since the publication of the Flora of Turkey, three more species of *Prangos* were described from Turkey, making the total species number 13 (Davis *et al.*, 1988; Duman and Watson, 1999; Duman, 2000; Duran *et al.*, 2005).

There are plenty of studies concerning the pollen morphological features of the family Umbelliferae (Erdtman, 1952; Cerceau-Larrival, 1962, 1963, 1965; Aytuğ *et al.*, 1971; Cerceau-Larrival, 1971; Ferreira and Purper, 1972; Cerceau-Larrival and Roland Heydacker, 1976; Herrnstadt and Heyn, 1977; Moore and Webb, 1978; Punt, 1984). Pollen grains of Umbelliferae are usually very distinctive with their inner and outer outlines and very characteristic 'bone' shape. These distinctive palynological features have also been used by several authors in general pollen keys (Erdtman, 1952; Aytuğ *et al.*, 1971; Moore and Webb, 1978).

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The previous study on pollen morphology of *Prangos* was limited and conducted using only light microscope (Herrnstadt and Heyn, 1977). Therefore, an attempt has been made to describe the palynological features of the genera *Prangos* (nine taxa) and *Ekimia* (one species) to validate the utility of these data to provide additional support to sectional groups currently recognized within *Prangos* and also to provide new insights on the justification of transfer of *P. bornmuelleri* to *Ekimia* using numerical approach.

Materials and Methods

The pollen characteristics of nine *Prangos* taxa belonging to three sections, namely sect. *Meliocarpoides*, sect. *Prangos* and sect. *Intactae*, and one taxon of *Ekimia* were studied (Table 1). Seven of these taxa are endemic to Turkey. The studied plant materials were collected from different populations in Turkey. A list of specimens examined is given in Table 1.

Table 1. Voucher specimens of 10 taxa of *Ekimia* and *Prangos*.

Taxon	Details of voucher specimens**
<i>E. bornmuelleri</i> (Hub.-Mor. & Reese) H. Duman & M.F. Watson *	C2 Burdur: Yeşilova Salda lake side, 1150 m, F.A. Kravelioğulları 3237 (GAZI).
Section <i>Intectae</i>	
<i>P. denticulata</i> Fisch. & Mey.*	A4 Ankara: Hüseyin Gazi Mountain, 1150 m, H. Duman 8770 (GAZI).
<i>P. ferulaceae</i> Lindl.	C5 Konya: Ereğli, Aydos Mountain, Delimahmutlu, edge of Kapız stream, calcerous mainrock, 1600 m, S. Erik 2277 (HUB).
<i>P. heyniae</i> H. Duman & M.F. Watson *	C4 Konya: 13 km from Bozkır to Hadim, slopes, 1330 m, B. Başer 1002 (GAZI).
<i>P. peucedanifolia</i> Fenzl	B6 Malatya: Malatya-Pötürge Gündüz Village Darı Mektep Vicinity Quercus community, 1250 m, Y. Altan 1411 (FUH).
<i>P. platychoena</i> subsp. <i>platychoena</i> Boiss. ex Tchihat *	B7 Erzincan: Kemaliye Sırakoruklar Village up Sarıçiçek Plato 1500 m, M. Vural ve M. Koyuncu 8402 (GAZI).
<i>P. platychoena</i> Boiss ex Tchihat subsp. <i>engizekensis</i> H. Duman & M.F. Watson. *	C6 Kahramanmaraş: Engizek Mountain southwestern of Küçükyesil Plato 2100-2300m, rocky areas, H. Duman 3622 (GAZI).
<i>P. uechtrizii</i> Boiss. & Hausskn. *	B5 Kayseri: Kayseri-Yahyalı 1 km Eşkidut Vicinity river side 1350 m, B. Başer 1001 (GAZI).
Section <i>Meliocarpoides</i>	
<i>P. meliocarpoides</i> Boiss. *	B5 Kayseri: Bünyan-Korumaz mountain rocky slopes, 1850-1900 m, M.E. Uzunhisarcıklı 1619 (GAZI).
Section <i>Prangos</i>	
<i>P. pabularia</i> Lindl.	B9 Bitlis: Tatvan-Nemrut Mountain upper sections of Şahmıran Village, 1700 m, M. Ekici <i>et al.</i> 2242 (GAZI).

*Endemic to Turkey. **ANK (Ankara University), FUH (Firat University), GAZI (Gazi University) and HUB (Hacettepe University).

For light microscope (LM) investigations, pollen grains were taken from the herbarium materials and prepared according to the methods of Wodehouse (1935) and Erdtman (1960). Twenty (20) intact pollen grains from each taxon were scored under LM

(Leica DM 1000) for the following parameters: P, polar axis; E, equatorial diameter; P/E, ratio of polar axis and equatorial diameter; Clg, colpus length; Clt, colpus diameter; Plg, pore length; Plt, pore width; I, thickness of intine; Coswid, costae width.

Cluster analysis (CA) was performed to determine the pattern of grouping of the taxa based on these nine quantitative pollen characters. In CA, the data matrix were used to produce the distance matrix based on Gower General Similarity Coefficient by the group average method as suggested by Gower (1971) using statistic package MVSP version 3.1 (Kovach, 1999).

For scanning electron microscope (SEM) investigations, the pollen grains were put on stubs, sputter-coated with gold plate, and examined under a Jeol JSM-6060 scanning electron microscope. For transmission electron microscope (TEM) studies, acetolysed pollen grains were stained with 2% OsO₄ and with uranyl acetate, dehydrated and embedded in epon-araldite (Skvarla and Turner, 1966). Ultrathin sections of the pollen grains were obtained with a glass knife in a Reichert Supernova microtome. Post-staining was done with lead citrate for 5 minutes (Reynold, 1963), and the sections were examined under a Jeol 1220 TEM. The terminologies for pollen morphology proposed by Punt (1984) and Faegri and Iversen (1989) were followed.

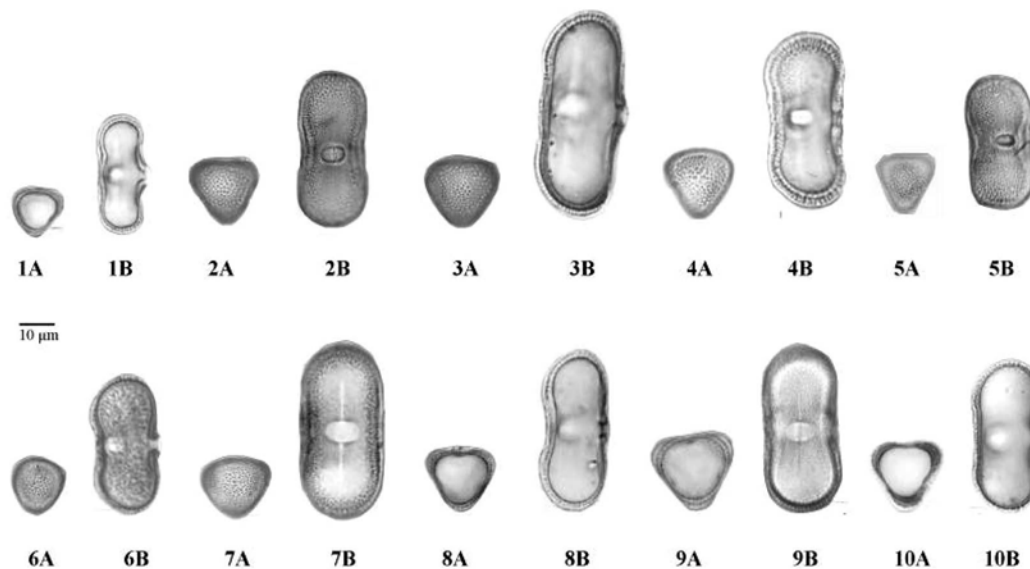
Results and Discussion

According to LM and SEM investigations, the pollen grains of the taxa belonging to *Prangos* and *Ekimia*, are perprolate, trizonocolporate, operculate, isopolar and bilaterally symmetrical, subrectangular in equatorial view, triangular in polar view (Figs 1-10), with a rugulate-striate exine sculpture (Figs 11-20). There is a thickening around the aperture of exine (costae) with a decreasing diameter towards the poles. Apertures are on the same plane in the equatorial region (Figs 1-10).

Pollen grains of the Umbelliferae show uniformity to some extent. Detailed palynological investigations have been carried out by Cerceau-Larrival (e.g. 1962, 1963, 1965, 1971). As a result of these investigations, five basic types, namely subrhomboidal (Rh), subcircular (C), ovoid (O), subrectangular (Rg) and equatorially constricted (E), were defined according to inner outline of the endexine (nexine). Two species were examined from the genus *Prangos* "*Cachrys alpina*" (= *Prangos trifida* (Miller) I. Herrnstadt & Heyn) and "*Cachrys goniocarpa*" (= *P. ferulacea*). On the basis of Cerceau-Larrival's classification, all studied pollen grains of the present study were consistent with subrectangular pollen type.

Herrnstadt and Heyn (1977) studied acetolyzed pollen of eight species of *Prangos* and six of them are included in our study. They found the range of pollen grains from 39 to 65 µm in size. In our study, we found that *E. bornmuelleri* (29.24±1.17 (A)) has the shortest pollen grains relative to the taxa of *Prangos* (32.49±2.32 - 48.23±2.59 (A)) (Table 2).

SEM investigations revealed that all investigated taxa have rugulate-striate ornamentation. Punt (1984) stated that ornamentation is of little value as a discriminating factor for identification of pollen grains in Umbelliferae. Three ornamentation patterns have been determined in his studies: irregularly regulate, irregularly striate or cerebroid. Punt, however, did not examine any species belonging to the genus *Prangos*. Aytuğ *et al.* (1971) also studied the pollen grains of species of *Ferula* (Tourn.) L. and *Oenante* (Tourn.) L. belonging to Umbelliferae. He noted that these pollen grains have exine sculptures in the form of simple columella.



Figs 1-10. Light photomicrographs of acetolyzed pollen grains of *Ekimia* and *Prangos* taxa. A, polar view; B, equatorial view. 1. *E. bornmuelleri*; 2. *P. denticulata*; 3. *P. ferulacea*; 4. *P. heyniae*; 5. *P. meliocarpoides*; 6. *P. pabularia*; 7. *P. peucedanifolia*; 8. *P. platychloena* subsp. *platychloena*; 9. *P. platychloena* subsp. *engizekensis*; 10. *P. uechtrizii*.

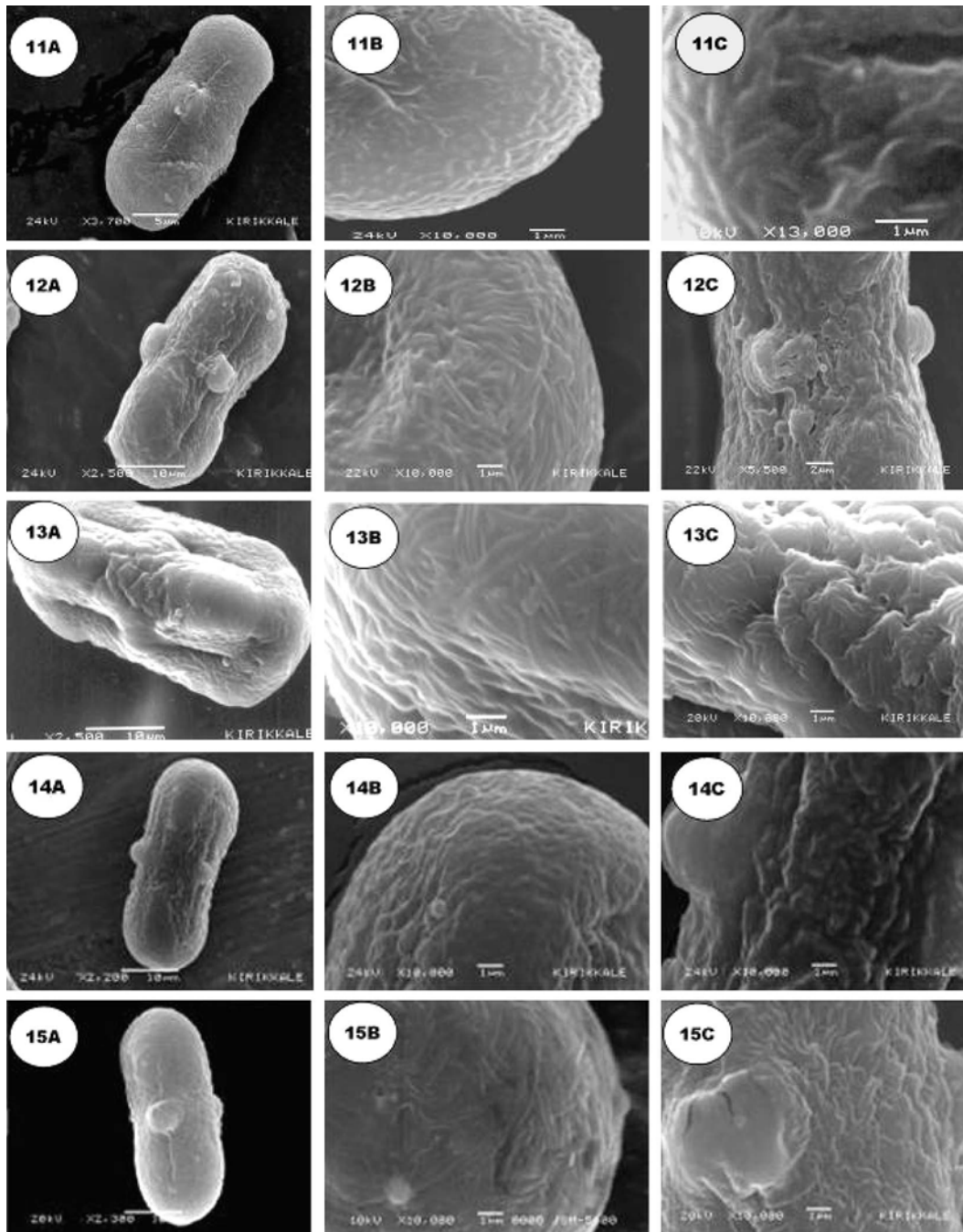
The present TEM examinations revealed that endexine is very thin in all studied taxa. Endexine is thinner in *Ekimia* pollen grain in comparison with *Prangos* pollen grains. Ectexine is observed to be thinnest in *P. meliocarpoides* (Table 3, Fig. 21).

The results of the present study indicate that the most variable pollen characteristics among the investigated taxa are the polar axis, equatorial axis and colpus length. The relative size of pollen grains was determined to be effective in separating the genera *Ekimia* and *Prangos*. The polar and equatorial axes are smaller in *Ekimia* than the members of *Prangos*. The genus *Prangos*, especially section *Intactae* shows variability regarding their pollen sizes. Section *Meliocarpoides* and section *Prangos* have similar pollen characteristics thus formed a tight cluster on the obtained phenogram (Table 2, Fig. 22).

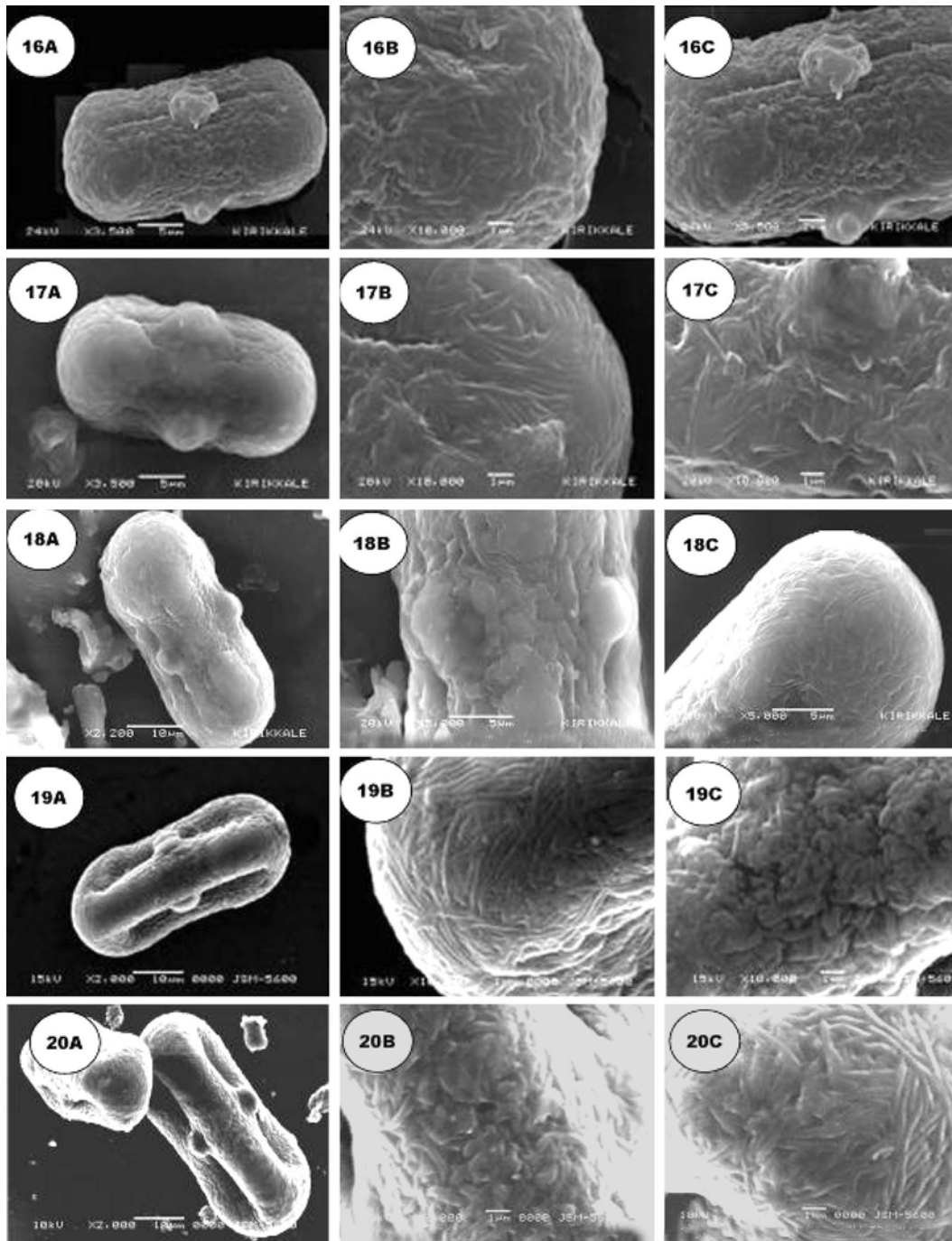
Table 2. Pollen morphological parameters of 10 *Ekimia* and *Prangos* taxa (mean±standard deviation), n = 30.

Taxon	P (µm)	E (µm)	P/E	Clg (µm)	Clt (µm)	Plg (µm)	Plt (µm)	I (µm)	Coswid (µm)	Om
<i>E. bornmuelleri</i>	28.54 ± 1.64 (N) 29.24 ± 1.17 (A)	12.67 ± 1.07 (N) 12.94 ± 0.77 (A)	2.25 (N) 2.26 (A)	17.43 ± 1.42 (N) 17.43 ± 1.02 (A)	0.88 ± 0.15 (N) 0.25 ± 0.08 (A)	4.41 ± 0.60 (N) 3.22 ± 0.49 (A)	5.40 ± 0.73 (N) 4.07 ± 0.62 (A)	0.32 ± 0.11 (N) Absent (A)	0.65 ± 0.12 (N) 1.03 ± 0.15 (A)	RG-S
Section <i>Intectae</i>										
<i>P. denticulata</i>	39.32 ± 2.56 (N) 41.92 ± 3.64 (A)	17.07 ± 1.13 (N) 18.09 ± 1.66 (A)	2.30 (N) 2.32 (A)	24.74 ± 2.99 (N) 26.67 ± 2.81 (A)	0.57 ± 0.29 (N) 0.48 ± 0.13 (A)	3.86 ± 0.80 (N) 3.42 ± 0.47 (A)	5.72 ± 1.02 (N) 6.22 ± 0.96 (A)	0.48 ± 0.14 (N) Absent (A)	1.12 ± 0.20 (N) 2.38 ± 0.35 (A)	RG-S
<i>P. ferulacea</i>	38.63 ± 2.05 (N) 48.23 ± 2.59 (A)	16.34 ± 1.76 (N) 21.26 ± 1.64 (A)	2.20 (N) 2.31 (A)	21.32 ± 1.76 (N) 29.22 ± 2.31 (A)	0.48 ± 0.16 (N) 0.52 ± 0.52 (A)	3.98 ± 0.68 (N) 4.09 ± 0.64 (A)	5.80 ± 1.12 (N) 7.35 ± 1.11 (A)	0.47 ± 0.14 (N) Absent (A)	1.05 ± 0.18 (N) 1.91 ± 0.49 (A)	RG-S
<i>P. heymiae</i>	36.25 ± 1.77 (N) 41.25 ± 4.18 (A)	16.60 ± 0.97 (N) 17.83 ± 2.06 (A)	2.18 (N) 2.31 (A)	24.27 ± 1.88 (N) 24.08 ± 2.77 (A)	0.67 ± 0.23 (N) 1.08 ± 0.40 (A)	4.65 ± 0.72 (N) 3.99 ± 0.69 (A)	7.18 ± 1.12 (N) 6.78 ± 1.32 (A)	0.34 ± 0.18 (N) Absent (A)	1.08 ± 0.30 (N) 1.67 ± 0.20 (A)	RG-S
<i>P. platychoena</i> subsp. <i>platychoena</i>	40.88 ± 2.75 (N) 40.44 ± 1.95 (A)	18.56 ± 1.48 (N) 17.41 ± 1.20 (A)	2.20 (N) 2.32 (A)	26.00 ± 2.12 (N) 27.66 ± 2.14 (A)	0.43 ± 0.08 (N) 0.81 ± 0.22 (A)	4.15 ± 0.63 (N) 3.47 ± 0.58 (A)	6.55 ± 1.01 (N) 5.67 ± 0.79 (A)	0.47 ± 0.01 (N) Absent (A)	1.01 ± 0.23 (N) 1.58 ± 0.19 (A)	RG-S
<i>P. platychoena</i> subsp. <i>engizekensis</i>	40.84 ± 2.83 (N) 39.55 ± 1.52 (A)	17.90 ± 1.39 (N) 16.24 ± 0.85 (A)	2.28 (N) 2.44 (A)	25.44 ± 3.16 (N) 24.48 ± 2.62 (A)	0.65 ± 0.22 (N) 0.90 ± 0.23 (A)	4.54 ± 0.97 (N) 4.20 ± 0.49 (A)	6.67 ± 1.24 (N) 5.72 ± 0.80 (A)	0.51 ± 0.15 (N) Absent (A)	1.04 ± 0.19 (N) 1.51 ± 0.31 (A)	RG-S
<i>P. peucedanifolia</i>	33.84 ± 1.76 (N) 41.62 ± 2.56 (A)	15.72 ± 1.08 (N) 18.01 ± 1.28 (A)	2.15 (N) 2.30 (A)	20.11 ± 1.55 (N) 26.23 ± 2.30 (A)	0.85 ± 0.17 (N) 0.64 ± 0.20 (A)	4.57 ± 0.74 (N) 4.17 ± 0.63 (A)	6.01 ± 1.01 (N) 6.44 ± 0.90 (A)	0.57 ± 0.16 (N) Absent (A)	0.72 ± 0.19 (N) 1.55 ± 0.35 (A)	RG-S
<i>P. uehrizii</i>	42.66 ± 2.32 (N) 38.33 ± 1.87 (A)	19.30 ± 1.57 (N) 16.05 ± 1.00 (A)	2.21 (N) 2.39 (A)	29.84 ± 2.62 (N) 26.23 ± 1.86 (A)	0.54 ± 0.22 (N) 0.83 ± 0.19 (A)	5.01 ± 0.80 (N) 3.50 ± 0.62 (A)	7.40 ± 1.21 (N) 5.24 ± 0.75 (A)	0.43 ± 0.21 (N) Absent (A)	1.07 ± 0.23 (N) 1.71 ± 0.28 (A)	RG-S
Section <i>Mellocarpoides</i>										
<i>P. mellocarpoides</i>	34.92 ± 2.42 (N) 32.49 ± 2.32 (A)	15.59 ± 1.44 (N) 13.53 ± 1.56 (A)	2.24 (N) 2.40 (A)	21.76 ± 2.33 (N) 19.96 ± 2.38 (A)	0.67 ± 0.24 (N) 0.76 ± 0.18 (A)	4.38 ± 0.85 (N) 3.74 ± 0.58 (A)	6.34 ± 0.90 (N) 4.67 ± 0.91 (A)	0.53 ± 0.28 (N) Absent (A)	1.26 ± 0.29 (N) 1.52 ± 0.31 (A)	RG-S
Section <i>Prangos</i>										
<i>P. pabularia</i>	33.27 ± 2.36 (N) 33.88 ± 1.67 (A)	15.37 ± 1.22 (N) 14.11 ± 1.25 (A)	2.16 (N) 2.40 (A)	22.44 ± 1.90 (N) 22.30 ± 1.30 (A)	0.64 ± 0.17 (N) 0.56 ± 0.19 (A)	4.60 ± 0.73 (N) 3.56 ± 0.66 (A)	5.57 ± 0.95 (N) 5.52 ± 0.88 (A)	0.52 ± 0.14 (N) Absent (A)	0.94 ± 0.18 (N) 1.49 ± 0.29 (A)	RG-S

N, Non-acetolyzed pollen grains; A, Acetolyzed pollen grains; P, Polar axis; E, Equatorial diameter; P/E, Ratio of polar axis and equatorial diameter; Clg, Colpus length; Clt, Colpus diameter; Plg, Pore length; Plt, Pore width; I, Thickness of intine; Coswid, Costae width; Om, Ornamentation; RG-S, Rugulate-striate.



Figs 11-15. SEM microphotographs of pollen grains of *Ekimia* and *Prangos* taxa. A, general view; B-C, exine ornamentations. 11. *E. bornmuelleri*; 12. *P. denticulata*; 13. *P. ferulacea*; 14. *P. heyniae*; 15. *P. meliocarpoides*.



Figs 16-20. SEM microphotographs of pollen grains of *Prangos* taxa. A, general view; B-C, exine ornamentations. 16. *P. pabularia*; 17. *P. peucedanifolia*; 18. *P. platychloena* subsp. *platychloena*; 19. *P. platychloena* subsp. *engizekensis*; 20. *P. uechtrizii*.

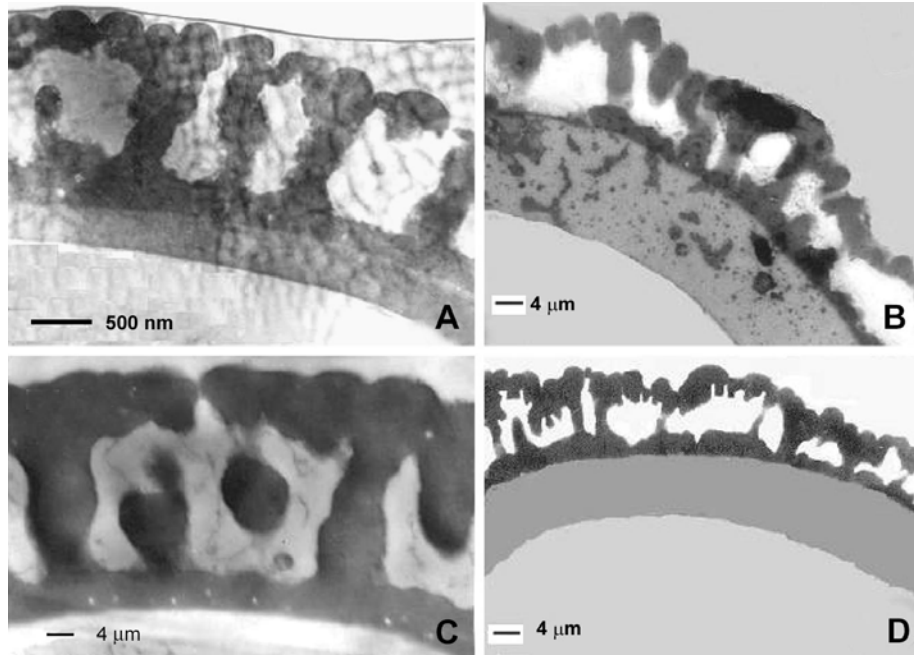


Fig. 21. TEM microphotographs of exine structure of pollen grains of *Ekimia* and *Prangos* taxa. A. *E. bornmuelleri*; B. *P. heyntia*; C. *P. meliocarpoides*; D. *P. pabularia*.

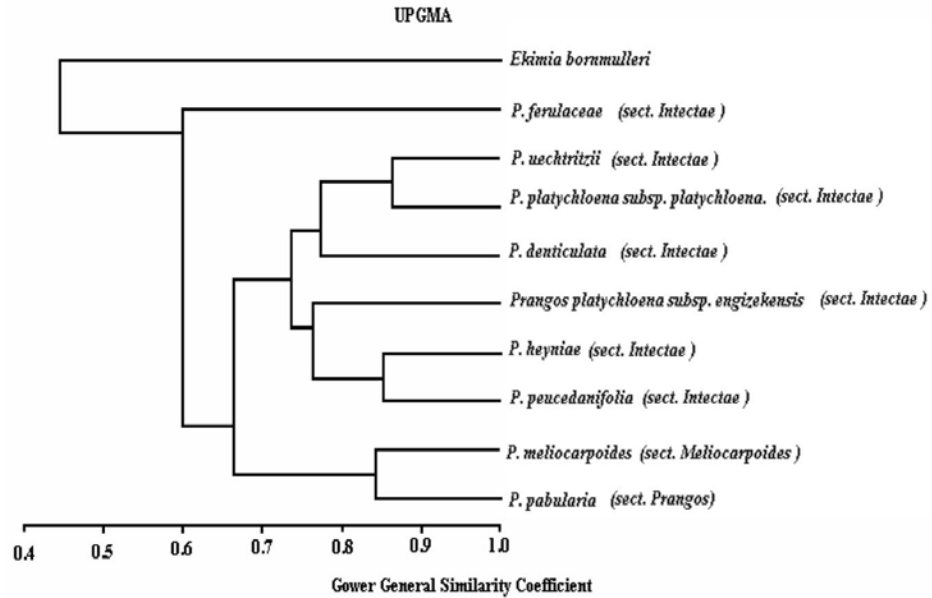


Fig. 22. UPGMA phenogram of the investigated *Ekimia* and *Prangos* taxa based on Gower General Similarity Coefficient.

Table 3. Transmission electron microscope (TEM) characteristics of four taxa of *Ekimia* and *Prangos*.

Taxon	Ectexine (μm)			Endexine (μm)
	Tectum	Columella	Food layer	
<i>E. bornmuelleri</i>	0.28	0.75	0.30	0.33
<i>P. heyniae</i>	0.23	0.80	0.38	0.55
<i>P. meliocarpoides</i>	0.25	0.50	0.27	0.46
<i>P. pabularia</i>	0.35	1.05	0.40	0.48

The obtained UPGMA phenogram (Fig. 22) based on Gower General Similarity Index supplements that the genus *Ekimia* (*E. bornmuelleri*) is distinctly different from the members of the *Prangos* concerning the quantitative pollen data. Section *Intactae* members form a cluster except *P. ferulacea* taxa.

The application of statistical methods, in this study the cluster analysis, proved the viability of using quantitative pollen data as taxonomic characters for effective discrimination, especially among genera.

Acknowledgements

The authors thank to the curators of four herbaria, namely ANK (Ankara University), FUH (Firat University), GAZI (Gazi University) and HUB (Hacettepe University), who allowed formers to study their *Prangos* specimens; Yrd. Doç. Dr. Hakan Güngüneş who helped in taking electron photographs of pollen surface; and Prof. Hayri Duman for providing some samples. The authors also appreciate the valuable efforts of Dr. Hülya Özler and Mr. Umut Toprak for editing an early version of the manuscript. This study was supported by Gazi University under grant 05/2001-37.

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(Manuscript received on 8 January 2009; revised on 15 July 2009)