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Pollen and seed morphology of the genus *Marrubium* (Lamiaceae) in Turkey

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Morphological features of pollen and seeds of 19 Turkish species of the complex genus *Marrubium* were examined using light and scanning electron microscopy. On the basis of exine sculpturing and seed shape, three main types are recognized in *Marrubium*. The study revealed that palynological and seed morphological characters are of taxonomic significance in the genus.

Key words: *Marrubium*, pollen morphology, seed morphology, SEM, taxonomy

Introduction

Marrubium (Lamiaceae) contains herbaceous plants distributed in the Irano–Turanian and Mediterranean phytogeographic regions (Hedge 1992). The total number of taxa is about 40. Twelve are recorded in Europe (Cullen 1972), 14 in the former USSR, (Komarov 1954) and 15 in Iran (Seybold 1978). In Turkey there are 21 species, with one subspecies and six variates (Akgül 2004).

The genus was first revised by Bentham (1834, 1848), who divided it into two sections, *Lagopsis* and *Marrubium*. Later, the taxonomy was treated by several workers and the genus was divided into various sections on the basis of morphological characters: three sections (*Ballatoides*, *Marrubium* and *Lagopsis*) by Briquet (1896), two sections (*Eumarrubium* and *Ballatoides*) by Boisser (1879) and four sections (*Marrubium*, *Afghanica*, *Stellata* and *Microdontha*) by Seybold (1978). On the other hand Grossheim (1967) had only the name section, and Cullen (1982) and more recently Akgül (2004) did not assign the Turkish species to any sections.

The taxonomy of some species, such as *M. astracanicum* and *M. cordatum* is problematic. Because the vegetative characters are very variable, rendering species identification difficult. However, the surface morphological features of pollen and seeds and their taxonomical significance have not been investigated.

Pollen morphology is meaningful regarding the systematic relationships among the genera of the Lamiaceae (Erdtman 1966, Cantino 1992a, 1992b, Harley & Paton 1992, Abu-Asab & Cantino 1994). Erdtman (1945) studied the pollen grains of *Marrubium*. In a recent investigation by Abu-Asab and Cantino (1994), the pollen morphology of many Lamiaceae members, including the Turkish *Marrubium anisodon*, *M. cuneatum*, and *M. heterodon* as well as species from Europe

(*M. incanum* and *M. supinum*) were studied using scanning (SEM) and transmission (TEM) electron microscopy. The systematic implications were also discussed.

Seed surface morphology is also of systematic significance at generic and specific levels (Brochmann 1992, Hedge 1992). The aim of this study is to illustrate the range of variability in seed and pollen characters of the *Marrubium* species found in Turkey, in order to establish their availability for future taxonomic work.

Material and methods

The material was collected from wild populations. The collectors and localities are provided in the "Specimens examined" for each taxon. The specimens are deposited in ANK.

Pollen slides were prepared using the technique of Wodehouse (1935). LM studies were done with a Leitz-Wetzlar microscope. Measurements are based on at least 20 pollen grains for each population. Lengths and widths of 10 seeds from each plant were measured under a stereomicroscope lens to the nearest 0.1 mm. For SEM studies, pollen grains were coated with gold for four minutes in a sputter-coater. Morphological observations were made with a Jeol 100 CXII electron microscope.

The pollen terminology follows Faegri-Iversen (1975) and Brochmann (1992), and the seed terminology follows Murley (1951) and Koul *et al*. (2000). The Simpson and Roe graphical test (*see* Van der Pluym & Hideux 1997) was used for statistical calculations.

Specimens examined

The order of the species was adapted from De Candolle (1948) and Grossheim (1967). All the specimens are deposited in ANK: *M. persicum*, A9 Iğdır, *Akgül 2521*; *M. catariifolium*, A9 Ardahan, *Akgül 2522*; *M. vulcanicum*, A9 Ağrı, *Akgül 2525*; *M. vanense*, B9 Van, *Akgül 2508*; *M. astracanicum*, B5 Aksaray, *A. Düzenli 434*; *M. cordatum*, A9 Ardahan, *Akgül 2523*; *M. trachyticum*, A4 Ankara, *Akman 8774*; *M. globosum*, C4 Konya, *Akman 68-5*; *M. rotundifolium*, C2 Manisa, *Akgül 2536*; *M. bourgaei*, C2 Muğla, *Davis 14018*; *M. heterodon*, C5 Adana, *Akgül 2575*; *M. lutescens*, B4 Konya, *R. Çetik 984*; *M. cephalanthum*, C5 Adana, *Akgül 2579*; *M. peregrinum*, B3 Eskişehir, *Ekim 2385*; *M. depauperatum*, B5 Kayseri, *Akgül 2578*; *M. parviflorum*, A4 Ankara, *Akman 6635*; *M. cuneatum*, B9 Bitlis, *Davis 22259*; *M. anisodon*, B3 Afyon, *Vural 1919*; *M. vulgare*, C5 Hatay, *Davis 26978*.

Results

Pollen morphology

Size, symmetry and shape

The pollen grains are radially symmetrical and isopolar. The shape is prolate-spheroidal and oblate-spheroidal (shape classification follows Erdtman (1969) based on the *P*/*E* ratio in Table 1), with the polar axes 16.6–32.2 μm and the equatorial axes 16.6–34.3 μm (Table 1 and Fig. 1). The outline is elliptic in equatorial optical section and circular in meridional optical section (Figs. 2–4).

Aperture

The pollen grains are inoperculate and usually tricolpate. Some species have heterocolpate characteristics. For example, tetracolpate-tricolpate pollen grains were observed in *M. lutescens* and *M. catariifolium*, and syncolpate-tricolpate in *M. cordatum* (Table 1). The apertural membrane is generally psilate or rarely granulate (Figs. 2–4).

Exine

The exine is tectate and 1.7–3.1 μm in thickness. The ectexine is slightly thicker than endexine. The intine thickness ranges from 0.3 to 1.3 μm (Table 1). Various ornamentation types were observed: psilate-perforate (*M. vulgare*, *M. anisodon*, *M. parviflorum*, *M. cuneatum*, *M. bourgaei*, *M. trachyticum*, *M. peregrinum and M. cordatum*), reticulate (*M. persicum*, *M. astracanicum*, *M. globosum*, *M. rotundifiolium* and

Table 1. Pollen morphology of the examined species of *Marrubium*. Unit is µm.

Taxa	Polar axes (*P*)			Equatorial axes (*E*)			*P*/*E* ratio, shape	Exine		Intine	Aperture type
	min	max	mean	min	max	mean		thickness	ornamentation		
M. persicum	24.7	28.1	26.7	20.2	25.9	22.7	1.18, prolate-spheroidal	2.1	Reticulate	0.5	Tricolpate
M. catariifolium	25	28.1	26.5	21.8	26	23.9	1.1, prolate-spheroidal	1.8	Psilate-perforate	0.3	30% tetracolpate, 70% tricolpate
M. vulcanicum	26	32.2	29.1	22.9	27.1	25.5	1.14, prolate-spheroidal	1.8	Psilate-foveolate	1.3	Tricolpate
M. vanense	25	29.1	27.1	22.9	27.1	25	1.08, prolate-spheroidal	2.1	Rugulate-reticulate	0.3	Tricolpate
M. astracanicum	19.8	27.1	23.9	16.6	27.1	23.9	1, spheroidal	3.1	Reticulate	0.5	Tricolpate
M. cordatum	16.6	31.2	23.2	17.7	27.1	21.8	1.06, prolate-spheroidal	2.1	Psilate-perforate	0.5	4% syncolpate, 90% tricolpate
M. trachyticum	25	29.1	27.1	26	30.2	28.1	0.96, oblate-spheroidal	1.8	Psilate-perforate	0.5	Tricolpate
M. globosum	22.9	30.2	26	22.9	26	24.3	1.07, prolate-spheroidal	2.1	Reticulate	0.5	Tricolpate
M. rotundifolium	20.8	25	22.9	22.9	28.1	30.1	0.8, oblate-spheroidal	1.8	Reticulate	1	Tricolpate
M. bourgaei	20.8	27.1	23.9	23.9	28.1	26	0.92, oblate-spheroidal	2.1	Psilate-perforate	0.8	Tricolpate
M. heterodon	27.9	27.1	24.6	27.1	31.2	29.1	0.93, oblate-spheroidal	1.8	Psilate-foveolate	0.8	Tricolpate
M. lutescens	20.8	26	24.9	25	29.1	27.1	0.91, oblate spheroidal	1.8	Psilate-foveolate	0.5	10% tetracolpate 90% tricolpate
M.cephalanthum	25	31.2	28.1	20	25	23	1.2, prolate spheroidal	2.1	Reticulate	0.5	Tricolpate
M. peregrinum	25	30.2	28.1	20.8	26	23	1.2, prolate spheroidal	2.1	Psilate-perforate	0.3	Tricolpate
M. depauperatum	24.2	30.2	27.2	19.4	27.1	22.5	1.2, prolate spheroidal	2	Granulate-perforate	0.3	Tricolpate
M. parviflorum	25	31.2	28.2	28.1	32.2	30.2	0.9, oblate-spheroidal	2.3	Psilate-perforate	0.5	Tricolpate
M. cuneatum	25	27.1	26	22.9	25	23.9	1.08, prolate-spheroidal	1.8	Psilate-perforate	0.5	Tricolpate
M. anisodon	30.2	32.2	31.2	31.2	34.3	32.5	0.96, oblate-spheroidal	2.1	Psilate-perforate	0.5	Tricolpate
M. vulgare	20	27	24.5	20	26	24.79	0.98, oblate-spheroidal	1.7	Psilate-perforate	0.89	Tricolpate

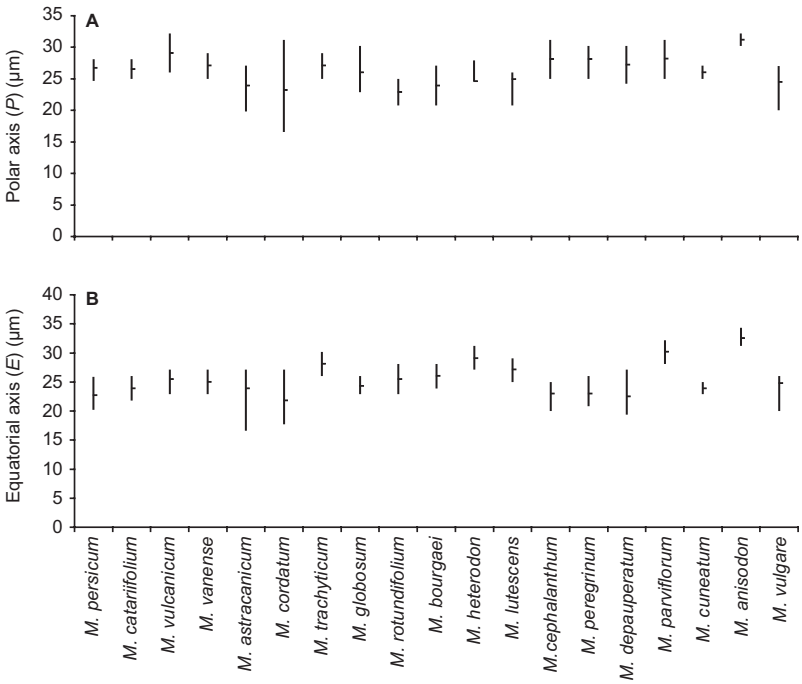

Fig. 1. Measurements of pollen grains in the studied *Marrubium* species— **A**: polar axis (*P*). — **B**: equatorial axis (*E*).

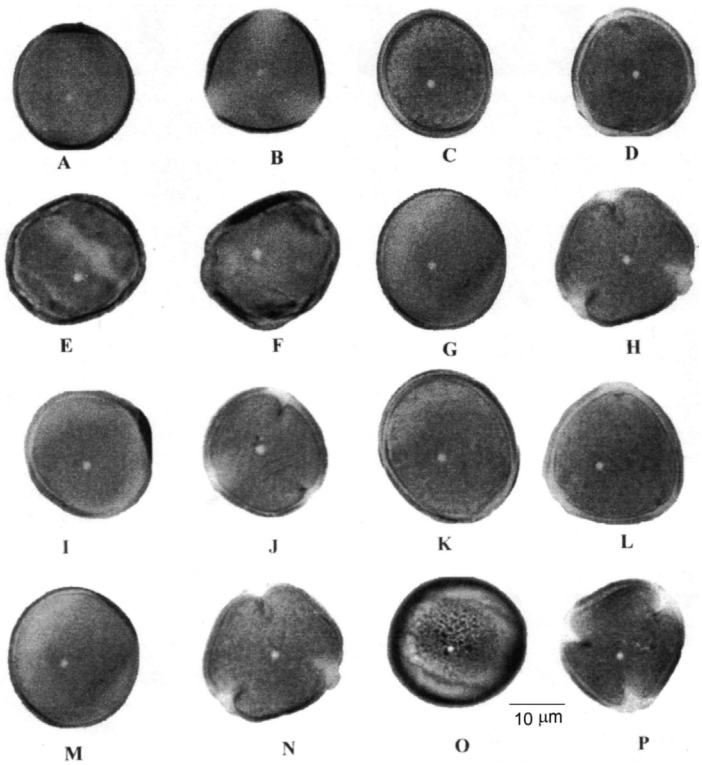

Fig. 2. Pollen grains. — **A** and **B**: *Marrubium persicum*. — **A**: Meridional, optical section, outline. — **B**: Polar view, apertures, optical section. — **C** and **D**: *M. catariifolium*. — **C**: Meridional, optical section, outline. — **D**: Polar view, apertures, optical section. — **E** and **F**: *M. vulcanicum*. — **E**: Meridional, optical section, outline. — **F**: Polar view, apertures, optical section. — **G** and **H**: *M. vanense*. — **G**: Meridional, optical section, outline. — **H**: Polar view, apertures, optical section. — **I** and **J**: *M. astracanicum*. — **I**: Meridional, optical section, outline. **J**: Polar view, apertures, optical section. — **K** and **L**: *M. cordatum*. — **K**: Meridional, optical section, outline. — **L**: Polar view, apertures, optical section. — **M** and **N**: *M. trachyticum*. — **M**: Meridional, optical section, outline. — **N**: Polar view, apertures, optical section. — **O** and **P**: *M. globosum*. — **O**: Meridional, optical section, outline. — **P**: Polar view, apertures, optical section.

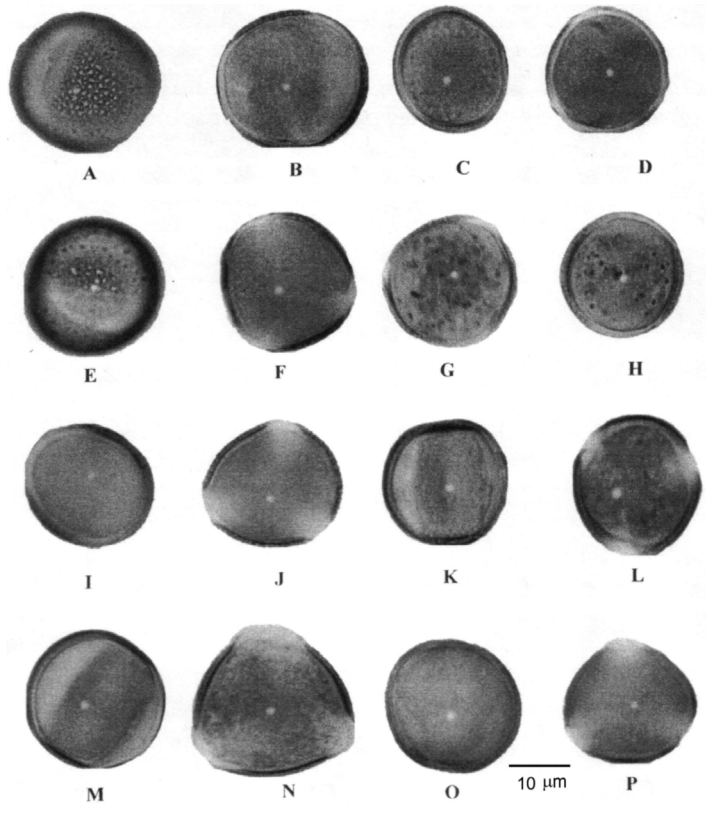

Fig. 3. Pollen grains. — **A** and **B**: *Marrubium rotundifolium*. — **A**: Meridional, optical section, outline. — **B**: Polar view, apertures, optical section. — **C** and **D**: *M. bourgaei*. — **C**: Meridional, optical section, outline. — **D**: Polar view, apertures, optical section. — **E** and **F**: *M. heterodon*. — **E**: Meridional, optical section, outline. — **F**: Polar view, apertures, optical section. — **G** and **H**: *M. lutescens*. — **G**: Meridional, optical section, outline. — **H**: Polar view, apertures, optical section. — **I** and **J**: *M. cephalanthum*. — **I**: Meridional, optical section, outline. — **J**: Polar view, apertures, optical section. — **K** and **L**: *M. peregrinum*. — **K**: Meridional, optical section, outline. — **L**: Polar view, apertures, optical section. — **M** and **N**: *M. depauperatum*. — **M**: Meridional, optical section, outline. — **N**: Polar view, apertures, optical section. — **O** and **P**: *M. parviflorum*. — **O**: Meridional, optical section, outline. — **P**: Polar view, apertures, optical section.

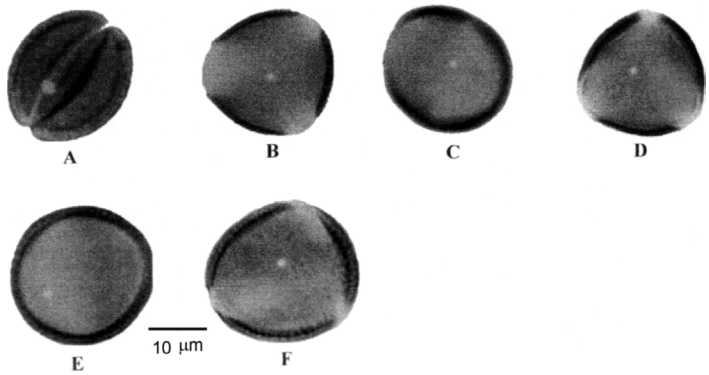

Fig. 4. Pollen grains. — **A** and **B**: *Marrubium cuneatum*. — **A**: Meridional, optical section, outline. — **B**: Polar view, apertures, optical section. — **C** and **D**: *M. anisodon*. — **C**: Meridional, optical section, outline. — **D**: Polar view, apertures, optical section. — **E** and **F**: *M. vulgare*. — **E**: Meridional, optical section, outline. — **F**: Polar view, apertures, optical section.

M. cephalanthum), psilate-foveolate (*M. vulcanicum*, *M. heterodon and M. lutescens*), rugulate-reticulate (*M. catariifolium and M. vanense*) and partially granulate-perforate (*M. depauperatum*). In the species with psilate-perforate ornamentation the size of perforations is ≤ 1 μm diameter, they are distributed regularly over the whole surface and there are 5–10 perforations per 5 μm^2. In the psilate-foveolate species, the foveolae are ≥ 1 μm diameter, are distributed regularly over the whole surface and there are 4–8 foveolae. In the rugulate-reticulate and reticulate species, the tectum is subtectate and the columellae are usually scarcely branched (Fig. 5).

Seed morphology

The largest seeds occur in *M. heterodon* (average 2.75 long, 1.3 mm in wide) and *M. cephalan-*

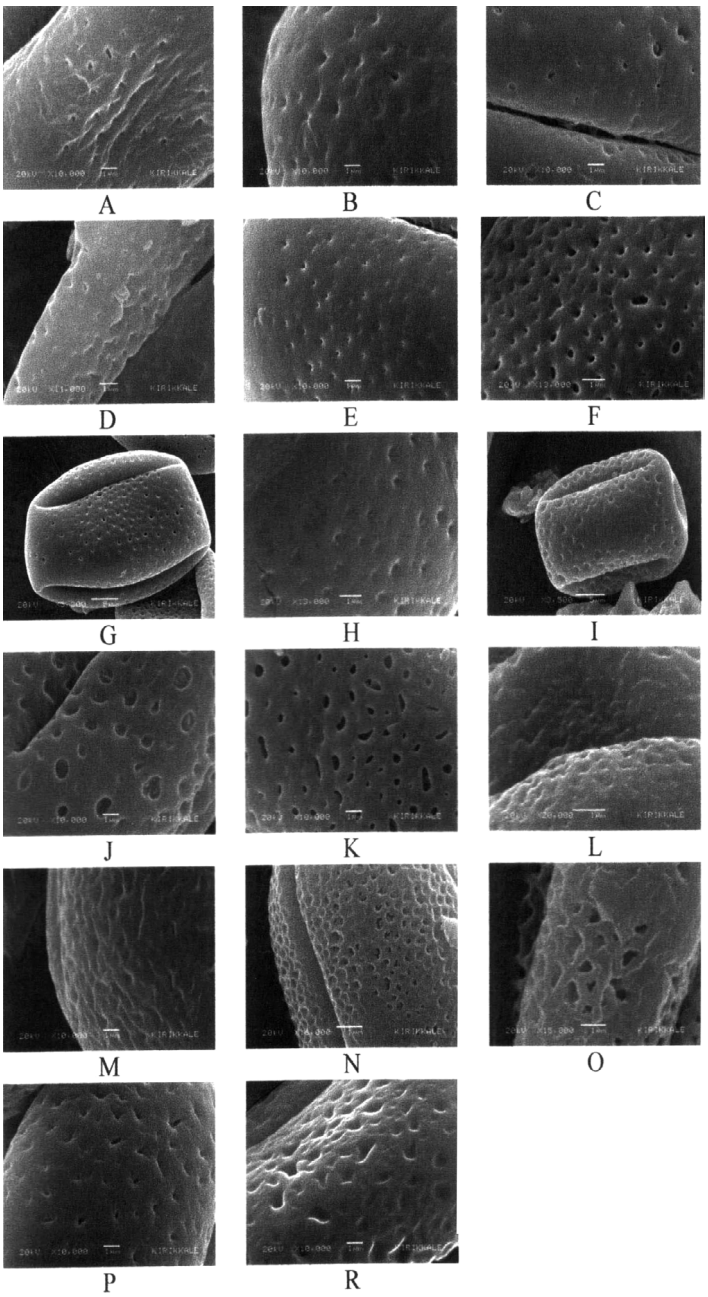

Fig. 5. Pollen grains. SEM photos of Type I, Type II and Type III grains. — **A**–**H**: Type IA. — **A**: *Marrubium vulgare* (SEM × 10000). — **B**: *M. anisodon* (SEM × 10000). — **C**: *M. cuneatum* (SEM × 10000). — **D**: *M. parviflorum* (SEM × 11000). — **E**: *M. peregrinum* (SEM × 10000). — **F**: *M. bourgaei* (SEM × 13000). — **G**: *M. trachyticum* (SEM × 3500). — **H**: *M. cordatum* (SEM × 13000). — **I**–**K**: Type IB. — **I**: *M. vulcanicum* (SEM × 3500). — **J**: *M. heterodon* (SEM × 10000). — **K**: *M. lutescens* (SEM × 10000). — **L**: Type II. *M. depauperatum* (SEM × 10000). — **M**: Type IIIC. *M. vanense* (SEM × 10000). — **N**–**R**: Type IIID. — **N**: *M. persicum* (SEM × 16000). — **O**: *M. astracanicum* (SEM × 15000). — **P**: *M. globosum* (SEM × 10000). — **R**: *M. cephalanthum* (SEM × 10000).

thum (average 2.7 mm long, 1.33 mm wide). The seeds are smallest in *M. vulcanicum* (1.3 mm long, 0.9 mm wide) and *M. peregrinum* (1.3 mm long, 1.1 mm wide). The general shape of the seeds is very similar, being elliptic to oblong and with a visible hilum and a more or less perceptible furrow. Only in *M. trachyticum*, *M. bourgaei* and *M. cephalanthum* the seeds are ovate. The colour of the seeds varies among the species. *Marrubium persicum*, *M. catariifolium*, *M. astracanicum*, *M. cordatum*, *M. trachyticum*, *M. globosum*, *M. heterodon*, *M. lutescens*, *M. peregrinum*, *M. parviflorum*, *M. cuneatum* and *M. vulgare* have dark-brown seeds. On the basis of exine sculpturing and seed shape, three main types are recognized in *Marrubium* (Fig. 6).

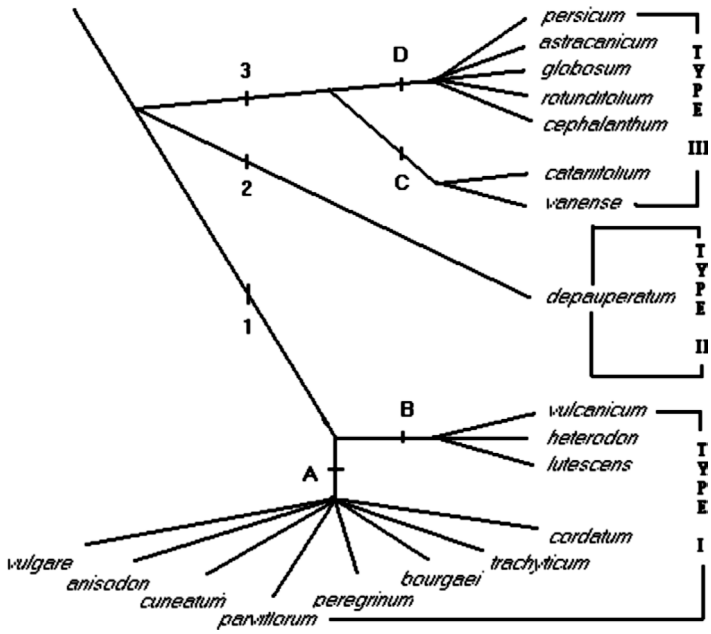

Fig. 6. Phylogenetic hypotheses based on the pollen and seed data of the studied *Marrubium*. — Synopomorphies: 1: Type I (A, psilate-perforate; B, psilate-foveolate). 2: Type II (granulate-perforate). 3: Type III (C, rugulate-reticulate; D, reticulate).

Type I: The exine sculpturing is psilate-perforate or psilate-foveolate. Pollen shape is generally prolate-spheroidal or oblate-spheroidal (Table 1, Figs. 2 and 3). Among the species examined, *M. cordatum*, *M. trachyticum*, *M. bourgaei*, *M. peregrinum*, *M. parviflorum*, *M. cuneatum*, *M. anisodon* and *M. vulgare* have a psilate-perforate sculpturing. Psilate-foveolate sculpturing was observed in *M. vulcanicum*, *M. heterodon* and *M. lutescens* (Figs. 5A–K, and 6). Seeds are generally very small (1.3 × 0.9–2.75 × 1.3 mm), generally elliptic in outline and dark-brown (Table 2 and Fig. 7).

Type II: The exine sculpturing is granulate-perforate. Among the species examined, only *M. depauperatum* has this type (Table 1, Figs. 5L and 6).

Type III: The pollen grains have a reticulate or rugulate-reticulate exine sculpturing. The shape is generally prolate-spheroidal (Table 1). Among the Turkish *Marrubium* species, *M. persicum*, *M. astracanicum*, *M. globosum*, *M. rotundifolium* and *M. cephalanthum* have a reticulate sculpturing. Rugulate-reticulate sculpturing is present in *M. catariifolium* and *M. vanense* (Table 1, Figs. 5M–R and 6). In this type, seeds are larger (1.9 × 1.1–2.7 × 1.3 mm), generally oblong in outline and dark-brown (Table 2 and Fig. 7)

Discussion

The pollen and seed morphology of the Turkish *Marrubium* species have taxonomic significance. We observed variation mainly in pollen and seed shape, size and ornamentation and recognize three main types (Tables 1 and 2), defined by pollen sculpturing, seed size and seed shape.

The sculpturing of the pollen exine is useful for ascertaining relationships among species (Brochman 1992). In the species belonging to type I, the sculpturing is psilate. These species have a scrobiculate exine (for example, perforations and foveolae). The sculpturing is granulate-perforate in type II, while in type III it is reticulate and rugulate-reticulate (Table 1 and Fig. 5).

The pollen morphology of *M. anisodon*, *M. cuneatum*, *M. heterodon*, *M. incanum* and *M. supinum* received little attention by Abu-Asab and Cantino (1992) and Cantino (1994). According to those authors the sculpturing in these species is psilate. It has been suggested that reticulate, rugulate and granulate sculpturing types evolved from psilate ancestors (Abu-Asab & Cantino 1992, Cantino 1992a). We observed that the primitive species have psilate sculpturing, while the advanced species have either reticulate or rugulate sculpturing (Fig. 6). The phyloge-

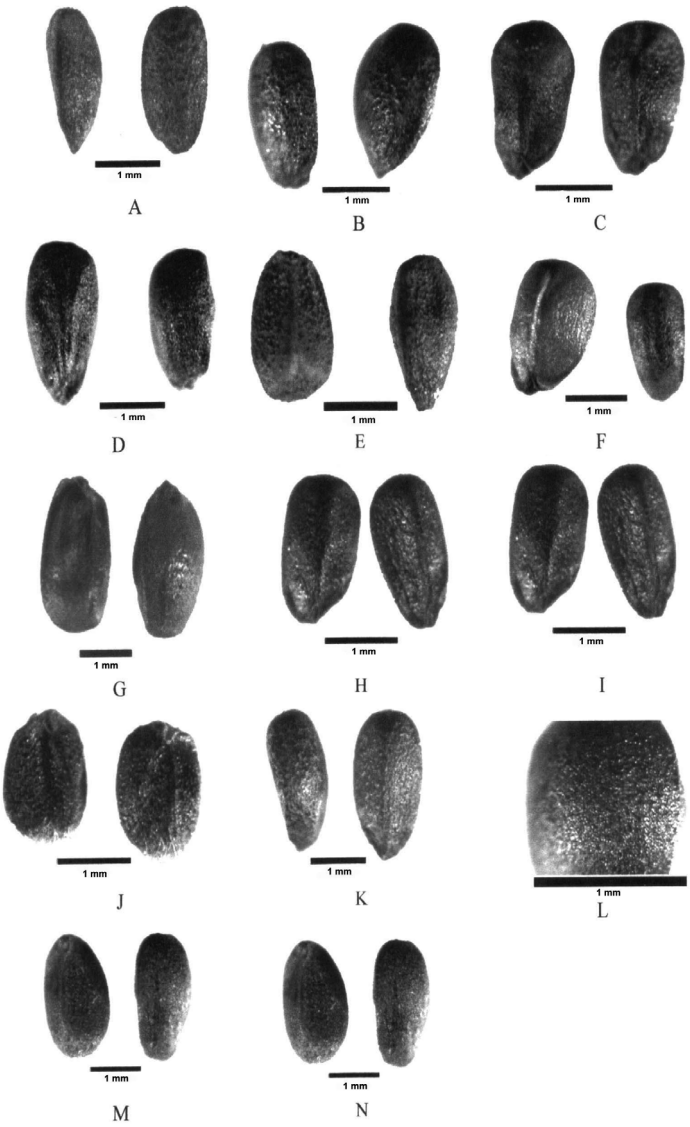

Fig. 7. Seeds. — **A**: *Marrubium astracanicum*. — **B**: *M. cordatum*. — **C**: *M. trachyticum*. — **D**: *M. globosum*. — **E**: *M. rotundifolium*. — **F**: *M. bourgaei*. — **G**: *M. heterodon*. — **H**: *M. lutescens*. — **I**: *M. cephalanthum*. — **J**: *M. peregrinum*. — **K**: *M. parviflorum*. — **L**: *M. cuneatum*. — **M**: *M. anisodon*. — **N**: *M. vulgare*.

netic hypothesis outlined above is summarized in Fig. 6. Our analysis suggests that *Marrubium* is not monophyletic, and because it appears to be paraphyletic, the species groups are defined by the three pollen types and correlated with floral characters.

In the analysis of the mean P and E values, the largest grains were found in *M. anisodon* and the smallest *P* values in *M. cordatum*, while the smallest *E* values were found in *M. astracanicum* (Table 1 and Fig. 1A–B).

Differences in pollen shape and aperture types are not particularly significant (Table 1) as prolate-spheroidal and oblate-spheroidal grains, and tricolpate and rarely tetracolpate, are dominant in all species.

Possible differences in tectum perforation and foveolation density and size were noted by Roca Salinas (1978). We think that is of little significance, since the greater or lesser proportion in any one zone of the grain was not constant.

We have shown that seed shape and size characters are of taxonomic value and can be divided into three main types in *Marrubium*. In type I, the seeds are usually small and generally elliptic in outline. Type II has the largest seeds and they

Table 2. Seed morphology of *Marrubium*. Seed lengths and widths (mm) are mean values.

Taxa	Length	Width	Length/ Width	Outline	Color	Ornamentation
M. persicum	2	1.1	1.81	Oblong	Dark brown	Verrucate
M. catariifolium	1.95	1.4	1.39	Oblong	Dark brown	Verrucate
M. vulcanicum	1.3	0.9	1.44	Elliptic	Black	Verrucate
M. vanense	2	1.3	1.54	Oblong	Brown	Verrucate
M. astracanicum	1.9	1.1	1.73	Oblong	Dark brown	Verrucate
M. cordatum	1.7	1.2	1.42	Widely elliptic	Dark brown	Verrucate
M. trachyticum	2	1	2	Ovate	Dark brown	Verrucate
M. globosum	2.1	0.9	2.3	Narrowly blong	Dark brown	Verrucate
M. rotundifolium	2.1	1.3	1.62	Oblong	Clear brown	Verrucate
M. bourgaei	2.1	1.3	1.62	Ovate	Clear brown	Verrucate
M. heterodon	2.75	1.3	2.11	Narrowly oblong	Dark brown	Verrucate
M. lutescens	2	1.1	1.81	Oblong	Dark brown	Verrucate
M. cephalanthum	2.7	1.3	2.1	Ovate	Brown	Verrucate
M. peregrinum	1.3	1.1	1.18	Widely elliptic	Dark brown	Verrucate
M. parviflorum	1.9	1.2	1.58	Narrowly elliptic	Dark brown	Verrucate
M. cuneatum	1.7	1.1	1.55	Elliptic	Dark brown	Verrucate
M. anisodon	1.9	1.1	1.73	Narrowly elliptic	Brown	Verrucate
M. vulgare	1.9	1.1	1.73	Elliptic	Dark brown	Verrucate

are generally oblong in outline. The seed shape in the Turkish *Marrubium* species seems to be related to their habitat ecology. The species with elliptic seeds grow in ruderal areas; those having ovate seeds grow in montane steppes and those having oblong seeds grow in rocky and stony places.

Acknowledgements

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