

Full Length Research Paper

A palynological study of the genus *Smyrnum* (Umbelliferae) from Turkey

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In this study, the pollen morphology and exine structure of six taxa of the genus *Smyrnum* L. (Umbelliferae) were investigated using light microscopy and scanning electron microscopy (SEM). Eight micromorphological characters (pollen shape, apocolpium, mesocolpium, polar length, equatorial width, exine thickness, colpus length and colpus width) of pollen grains of *Smyrnum* have been identified. The palynological observations revealed that pollen grains of all studied taxa of *Smyrnum* were prolate in shape and possess tricolporate aperture. Tectal surface sculpture was not a good criterion to identify particular taxa from *Smyrnum*. The pollen of which is characterized by rugulate, the pollen ornamentation was similar in all studied taxa. In addition the taxa could be included in sub-rectangular pollen type as classified by Cerceau – Larrival.

Key words: *Smyrnum*, Umbelliferae, pollen, scanning electron microscopy.

INTRODUCTION

Umbelliferae Juss. comprise approximately 450 genera and 3700 species, chiefly in north temperate regions (Pimenov and Leonov, 1993). The genus *Smyrnum* L. as a member of family Umbelliferae is a biennial plant growing in western and southern Europe, especially in coastal areas of the British Islands and the Mediterranean region (Bertoli et al., 2004).

The most comprehensive information on the distribution of *Smyrnum* in Turkey is in the Flora of Turkey and East Aegean Islands (Stevens, 1972). *Smyrnum* includes 6 taxa in the Turkish flora; *Smyrnum olusatrum* L., *Smyrnum perfoliatum* L. subsp. *perfoliatum*, *Smyrnum perfoliatum* subsp. *rotundifolium* (Miller) Hartwig, *Smyrnum cordifolium* Boiss., *Smyrnum connatum* Boiss. and Kotschy and *Smyrnum creticum* Miller. The all studied taxa are biennials with a fusiform tap root not crowned by a fibrous collar. Basal leaves are 2 to 4 ternate-pinnate. Upper leaves are alternate or opposite.

Inflorescence is much branched; bracts and bracteoles usually absent. Their flowers are hermaphrodite; sepals obsolete, petals yellow. Fruit is ovoid.

Smyrnum taxa are constantly considerable as a plant with diuretic, depurative and aperient properties, particularly through its root. However, their most outstanding quality is perhaps as an antiscorbutic because of its high vitamin C content. The fruit has carminative and stomachic properties (Bermejo and Leon, 1994). Some *Smyrnum* taxa were cultivated as an edible vegetable in ancient times (Tutin et al., 1968; Jafri and El-Gadi, 2001). Their commonest use has been as a fresh vegetable, with a preference being shown for its leaves, young shoots and leaf stalks, which impart a pleasant flavour similar to celery, although somewhat sharper (Bermejo and Leon, 1994).

In *Smyrnum*, histological and physiological studies (Weber, 1991, 1994; Lux et al., 1995; Poli et al., 1995), taxonomical studies (Tutin et al., 1968; Stevens, 1972; Hartvig, 1986; Randall, 2003) and phytochemical studies (Gören and Ulubelen, 1987; Bertoli et al., 2004; Amiri et al., 2006; Khanahmadi et al., 2010) have been conducted.

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Table 1. The location and habitats of studied specimens in *Smyrniium*.

Taxa	Specimen location and habitat
<i>S. olusatrum</i>	Izmir: Central exit of Izmir-Cesme highway, inside fence area, 50 m, 38°16'366"N, 26°22'298"E, 12.03.2010
<i>S. perfoliatum</i> subsp. <i>perfoliatum</i>	Bolu: Bolu towards Abant Lake, Akcaalan village , 1000 m, 40°39'335"N, 31°24'028"E, 22.04.2010
<i>S. perfoliatum</i> subsp. <i>rotundifolium</i>	Izmir: Kemalpassa, Bağyurdu town, 190 m, 38°24'588"N, 27°38'472"E, 11.03.2010
<i>S. cordifolium</i>	Ankara: Northeast of Hasanoğlan district, Hasanoğlan stream, 1350 m, 36°36'039"N, 33°03'195"E, 15.06.2010
<i>S. connatum</i>	Denizli: Honaz district towards Honaz Mountain, 1000 m, 37°44'331"N, 29°14'487"E, 09.04.2010
<i>S. creticum</i>	Manisa: Between Muradiye and Emiralem (Menemen), 70 m, 38°36'561"N, 27°09'069"E, 21.05.2010

Pollen morphology of various members of the family Umbelliferae has been studied by different workers. The common stenopalynous type of pollen has been reported for the Umbelliferae (Erdtman, 1952). Ting et al. (1964) examined pollen morphology of the subfamily Hydrocotyloideae. Pollen morphology of North European Flora of the family Umbelliferae has been examined by Punt (1984). However, the most comprehensive study on pollen morphology of Umbelliferae is that of Cerceau-Larrival (1962, 1971 and 1981). Pollen morphology of the family has also been studied by Erdtman et al. (1961), Nilsson et al. (1977) and Moore and Webb (1978), but only a few studies have been conducted on the genus *Smyrniium*. Hesse et al. (2009) investigated the pollen morphology of *S. perfoliatum*. In addition, pollen grains of *S. perfoliatum* appear on the web pages of Paldat (a palynological database) (Halbritter and Weber, 2000).

Except for *S. perfoliatum*, the literature survey revealed that the genus *Smyrniium* has not been considered for previous investigation on account of its pollen morphology. The purpose of the present paper was to provide a detailed account of the pollen morphology of Turkish *Smyrniium* taxa as a whole by light microscopy (LM) and scanning electron microscopy (SEM), and to determine the extent to which these palynological data can be used as a taxonomic character in the genus.

MATERIALS AND METHODS

The pollen grains of six *Smyrniium* taxa examined consisted of fresh specimens collected in their natural habitats (Table 1). The voucher specimens of the samples were deposited at the Celal Bayar University herbarium. Stevens (1972) and Hartvig (1986) are consulted in the identification of the studied taxa.

All the pollen grains for LM examination were prepared following the standard procedure of Wodehouse (1935). They were observed

in glycerin-water using a standard Olympus CX21FS1 microscope with D plan 1.00–1.25 160/0.17 oil immersion objective and NFKx3.3 LD 125 lens. Fifty pollen grains per specimen were regarded as sufficient for the palynological analysis.

For SEM, pollens were directly mounted on stubs using double-sided adhesive tape. Samples were coated with gold POLARON SC7620 ion-sputter and then observed by standard techniques using a LEO 440 SEM. The measurements are based on 15 to 20 readings from each specimen. Pollen shape, apocolpium, mesocolpium, polar length, equatorial width, exine thickness, colpus length and colpus width were measured suggested by Perveen and Qaiser (2006) and De Leonardis et al. (2009).

Erdtman (1969, 1971), Moore and Webb (1978) and Punt (1984) were consulted for the palynological terminology. In particular, the polar perimeter has been defined according to the terminology used by Cerceau-Larrival and Roland-Heydacher (1976).

RESULTS

Except for *S. perfoliatum*, the pollen properties of the taxa studied are here described for the first time. The pollen grain characters of the taxa studied are presented in Table 2.

Smyrniium pollen grains are characterized by radiosymmetric monads with an internal sub-rhomboidal nexine outline. All studied taxa are prolate in shape; the polar perimeter is triangular, tricolporate whereas the equatorial perimeter is elliptic. The three longitudinal equatorial colpi are narrow with acute apices. The exine sculpturing of all studied taxa are rugulate (Figure 1).

The average size of pollen grains was from 20.8 to 26.2 µm polar length and 14.1 to 17.5 µm in equatorial width (Table 2). The apocolpium (A), mesocolpium (M) and exine thickness mean value of pollen grains were determined very close to each other. Colpus length and colpus width mean values were measured 17.3 to 22.1 µm and 0.28 to 0.63 µm, respectively. There was no

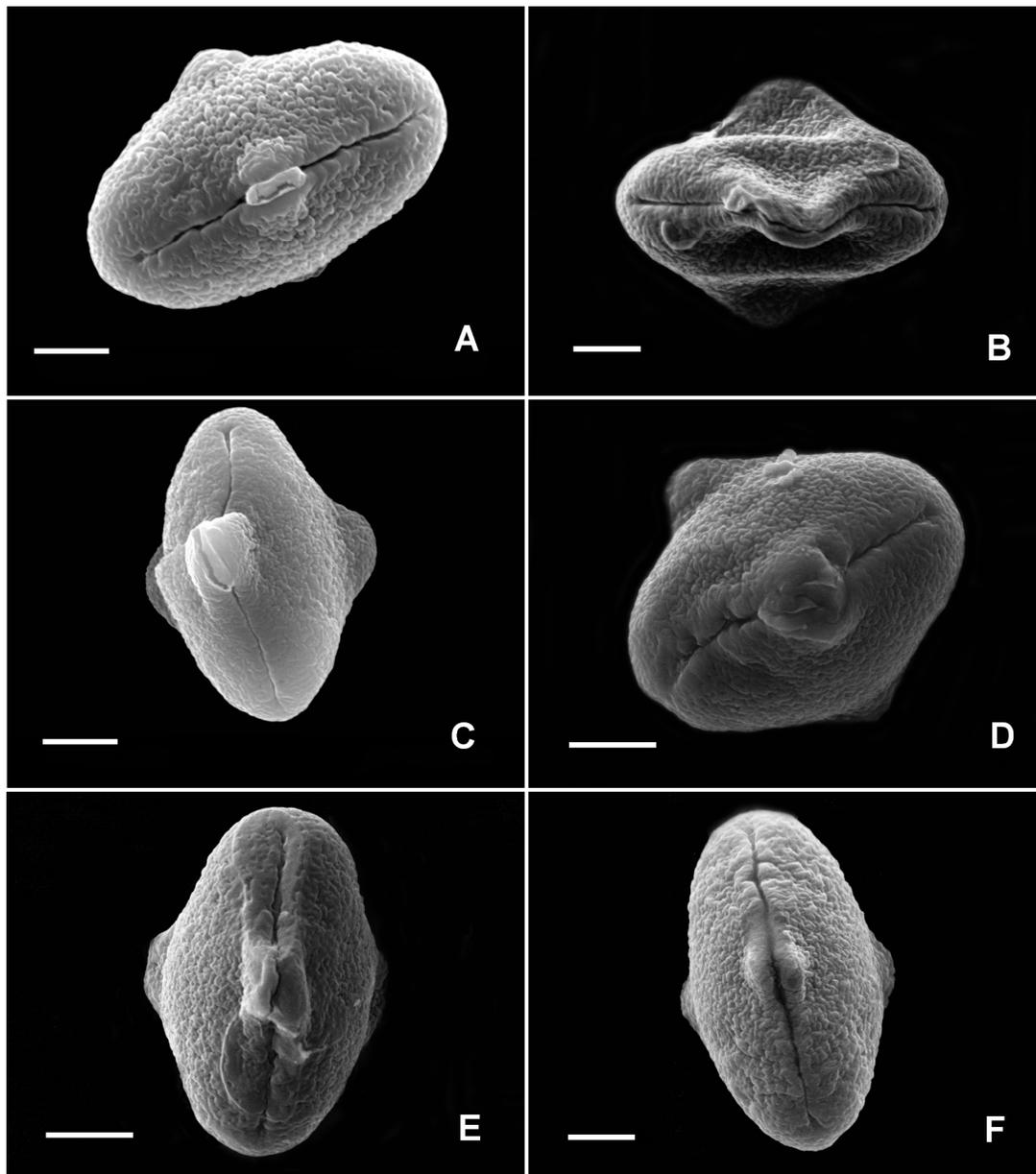


Figure 1. Scanning electron micrographs showing the equatorial view of pollens of studied taxa: (A) *S. olusatrum*, (B) *S. perfoliatum* subsp. *perfoliatum*, (C) *S. perfoliatum* subsp. *rotundifolium*, (D) *S. cordifolium*, (E) *S. connatum*, (F) *S. creticum*. Scale bar = 5 μ m.

significant difference in the surface of pollen grains of the examined taxa (Figure 2).

DISCUSSION

Umbelliferae is a stenopalynous family (Erdtman, 1952). Pollen morphology of six taxa viz., *S. olusatrum*, *S. perfoliatum* subsp. *perfoliatum*, *S. perfoliatum* subsp.

rotundifolium, *S. cordifolium*, *S. connatum* and *S. creticum* has been examined by light and scanning microscope. Pollen grains were remarkably uniform in their pollen characters. Pollen grains of *Smyrniium* taxa were generally tricolporate, shape of the grains was prolate. Tectum was uniformly rugulate. P/E ratio ranged from 1.38 to 1.49. On the basis tectum, distinct pollen types were not recognized.

Despite the fact that the *Smyrniium* genus has been

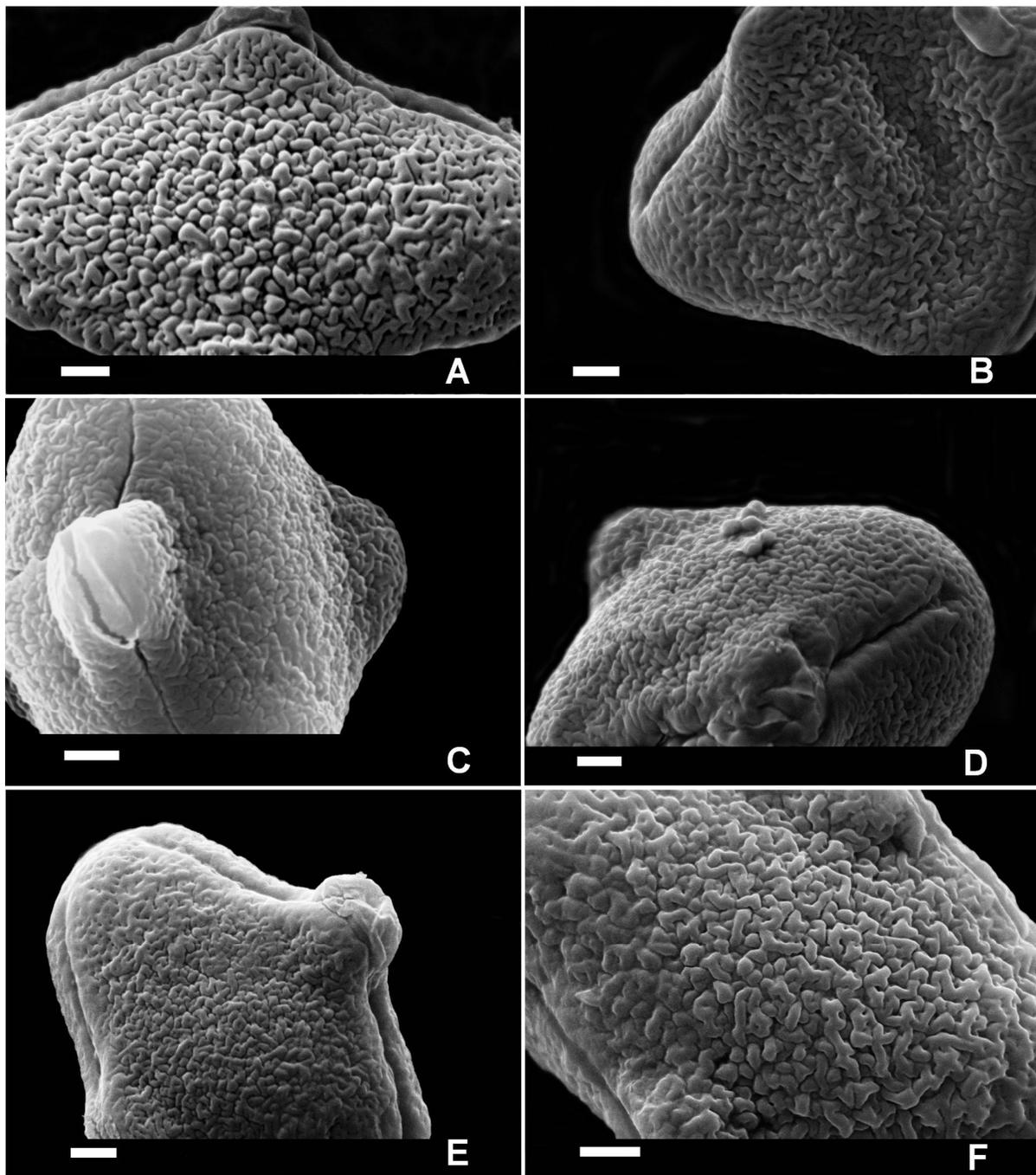


Figure 2. Scanning electron micrographs showing the exine sculpture of studied taxa: (A) *S. olusatrum*, (B) *S. perfoliatum* subsp. *perfoliatum*, (C) *S. perfoliatum* subsp. *rotundifolium*, (D) *S. cordifolium*, (E) *S. connatum*, (F) *S. creticum*. Scale bar = 2 μ m.

traditionally placed in the Apioideae sub-family (Heywood, 1971), later studies in several scientific fields (Arenas and Garcia, 1993; Plunkett and Downie, 1999; Neves and Watson, 2004) have pointed out its monophyletic origin and the divergence from the other

genera of the family. Palynological contributions on the family Umbelliferae are numerous but often fragmentary and concern few species (Anefred, 1960; Jacques-Felin, 1970; Erdtman, 1971; Moore and Webb, 1978; Punt, 1984; Faegri and Iversen, 1989). The most complete and

interdisciplinary studies are by Cerceau-Larrival (1962, 1963, 1965, 1967, 1968, 1971, 1974, 1981), Cerceau-Larrival and Deroquet (1975) and De Leonardis et al. (2009) which not only allowed us to correlate the shape of fruits with the symmetry of radiosymmetric pollen, the pollen shape with the size of cotyledonous leaves, the value of the P/E ratio with the phenotype stability of the belonging tribe, but also to further deepen the knowledge of the shape of the pollen grains, the variability of the sporodermic wall and the presence of columellar hypertrophy as adaptation to environmental conditions.

De Leonardis et al. (2009) worked the morphobiometric characters of pollen grains of six *Bupleurum* L. (Umbelliferae) taxa. They reported palynological properties of *Bupleurum* taxa as follows; radiosymmetric monads, sub-rhomboidal, the polar perimeter was sub-triangular, the equatorial perimeter and shape were elliptical and longiassic respectively, tricolporate, exine was sub-tectate and rugulate, size: P 18 to 32 and E 13 to 19 μm . We have the very similar results about these palynological properties. Our results were presented in Table 2. De Leonardis et al. (1997) have highlighted a close palynological affinity between taxa from genus *Bupleurum* with those from genus *Smyrniium* L. for the presence in both genera of "subrhomboidal-shaped" grains.

The presence of a sub-rhomboidal nexine outline in the Eocene, related to the genus *Smyrniium*, has strengthened the hypothesis of the old origin of this genus and the diversification in the Umbelliferae family, of the sub-rhomboidal outline in the phyletic series subrhomboidal>sub-

circular>oval>subrectangular>equatorially-constricted (Gruas-Cavagnetto and Cerceau-Larrival, 1978). In fact, this subrhomboidal type can only be found in five or six genera of Umbelliferae, are almost certainly very ancient (Cerceau-Larrival and Roland-Heydacker, 1976). The ancestors of *Smyrniium* genus must have been connected with the Tertiary arctic and Antarctic paleoflora (Cerceau-Larrival, 1968, 1974).

Cerceau-Larrival (1962) divided the pollen of Umbelliferae into 4 distinct types based on P/E ration viz., Subrhomboidal type-I (P/E = 1-1.5), Subcircular type-II (P/E ratio 1-1.5), Oval type-III (P/E = 1.5-2), Subrectangular type-IV (P/E = 2), Equatorially constricted type-V (P/E > 2). Van Zeist et al. (1977) also divided the pollen grains of Umbelliferae into nine pollen types that is *Anisoscidium*-type, *Bunium*-type, *Bupleurum*-type, *Eryngium*-type, *Ferula*-type, *Malabaila*-type, *Pimpinella*-type, *Sium*-type, *Erectum*-type and *Thurgenia*-type. Pollen morphology of 50 species representing 27 genera of the family Umbelliferae from Pakistan was examined by Perveen and Qaiser (2006). They determined that the pollen grains of Umbelliferae were generally tricolporate, shape of the grains varied from prolate-perprolate, P/E ratio ranged from 1.2 to 2.6. In their study all the taxa

examined belong to all the pollen types of Cerceau-Larrival that is, subrhomboidal type to equatorially constricted type. In the present study, all the taxa examined belong to subrhomboidal type. According to Perveen and Qaiser (2006), other pollen characters such as exine thickness, colpal membrane and amb of the grains are of little taxonomic value and can rarely be useful at specific level.

Two *Tetrataenium* (DC.) Madnen species were investigated by Yousefzadi et al. (2006). They determined that the pollen grains of two studied species were prolate, tricolporate; exine sculpturing was rugulate; size medium and P/E ratio 1.97 to 2.53 (subrectangular shape). Their results compared with our study, other pollen characteristics except for P/E ratio are mostly similar. Also, pollen shapes of the *Psammogeton* Edgew., *Selinum* L. and *Ammi* L. were determined sub-rectangular (P/E=2) by Perveen and Qaiser (2006). Sub-rectangular pollen shape is more advanced characters than sub-rhomboidal type. Accurately, *Tetrataenium*, *Psammogeton*, *Selinum*, and *Ammi* were reported more advanced genera than *Smyrniium* taxonomically (Pimenov and Leonov, 1993).

Halbritter and Weber (2000) reported the palynological properties of *S. perfoliatum* subsp. *perfoliatum* as follows; monad, small, prolate, triangular, tricolporate and rugulate. Their findings are parallel to our study results (Table 2).

In addition, pollen characteristics of the taxa were determined mostly similar each other in our study. There were no significant differences among the palynological properties of the studied taxa. The pollen morphology does not appear to be useful as a taxonomic technique in the identification of *Smyrniium* species. We believe that the results of the present study are important for taxonomically and evolution in/inter the group concerned. Such results will be the base for future biosystematic studies in *Smyrniium*.

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