



Research Article

Palynological Investigation of *Echium italicum*, *E. plantagineum* and *E. glomeratum* Species Distributed in Türkiye

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Abstract: In this study, genera of *Echium* from the family Boraginaceae, *Echium italicum*, *Echium plantagineum* and *Echium glomeratum* taxa the morphologies of the pollen were revealed using light microscopy (LM) and scanning electron microscopy (SEM). According to the research results, the symmetry, aperture, and ornamentation in the pollens of the species generally show similarities. All the pollens examined were heteropolar symmetry and aperture type tricolpate, while in two of the three species investigated, the pollen grains were subprolate, whereas in the remaining species they were prolate. In polar view, the broader pole exhibits a rounded-triangular outline, while the narrower pole is characterized by a more deeply incised, three-lobed configuration. In equatorial view, pollen shape ranges from ovoid to ovoid-triangular. Exine ornamentation is generally reticulate the pollen surface, but appears distinctly granulate within the colpus region.

Keywords: *Echium*, Boraginaceae, LM, Pollen morphology, SEM.

1. Introduction

The Boraginaceae family is represented by 154 genera and approximately 2,500 species worldwide, and is distributed in the temperate and subtropical areas of the Northern and Southern Hemispheres (Akçin 2009; Kapp, 1969). In Türkiye, it includes a total of 357 taxa, consisting of 34 genera, 325 species, 16 subspecies, and 16 varieties. Most of the plants belonging to the family are used in the production of used for ornamental, culinary, and dye purposes (Heywood 1978). The genus *Echium*, comprising approximately 60 species, represents one of the largest genera in the Lithospermeae subfamily of the Boraginaceae family, which includes about 25 genera and 460 species found across Europe, Africa, Asia, and the Americas. The principal diagnostic features of *Echium* include a distinctly zygomorphic corolla, usually bearing long, exserted stamens of unequal length, and a style that is more or less deeply divided into two stigmatic branches (Gibbs 1972; Johnston 1924).

There are nine *Echium* species (Boraginaceae) in Türkiye (*Echium italicum* L., *Echium vulgare* L., *Echium angustifolium* Miller, *Echium parviflorum* Moench, *Echium plantagineum* L., *Echium orientale* L.,



Echium russicum J.F. Gmel., *Echium glomeratum* Poir. and *Echium arenarium* Guss). However, according to the DNA sequencing studies published by Hilger and Böhle (2000), since the species *E. russicum* is included in a different genus (*Pontechium*), the genus *Echium* is represented by eight species of which *E. orientale* has been reported as endemic (Davis, 1978; Güner and Ekim, 2014). Additionally, *E. arenarium* is known to be widespread in the Greek islands, but it has not yet been detected in our country.

Echium (Turkish name of Engerek otu) is known as a honey plant due to its high nectar content. However, thanks to its high content of unsaturated fatty acids (ALA, GLA, SDA), it can be offered as an alternative to fish oil, especially for vegetarians, and is already used in food, health, and cosmetics. Pharmacologic studies have demonstrated that the species of *Echium* possesses antioxidant, anti-inflammatory, antibacterial, antiviral, antiproliferative and antidepressant activities (Tabata et al., 1994; Mehrabani et al., 2005; Al-Snafi 2017; Turker et al., 2018). The therapeutic effect of this genus may be due to the presence of phenolic compounds like rosmarinic acid and shikonin. A survey of the literature indicated that *Echium* species have been used in folk medicine as diuretic, diaphoretic, febrifuge, expectorant, analgesic, vulnerary, sedative, anxiolytic and in the treatment of upper respiratory tract infections (Ahvazi et al., 2012; Çakılçioğlu and Türkoğlu, 2010; Chevallier 1996; Grieve 1982). There are more ethnopharmacological reports on the species of *E. italicum* and *E. vulgare* than on other species. This may be related to their widespread distribution in Turkey. *E. italicum* has been reported as diuretic, sweet and sedative in the form of tea (Çakılçioğlu et al., 2007).

Boraginaceae family has been subdivided into different taxonomic groupings by various authors. Accordingly, De Candolle (1845) recognized six subtribes within the tribe Borageae (subfamily Boraginoideae), whereas Glirke (1897) distinguished seven tribes within Boraginoideae. In contrast, Johnston (1924) adopted a more conservative approach, recognizing only four tribes, while Riedl (in Rechinger, 1967) proposed a classification comprising three subfamilies and seven tribes within the Boraginoideae subfamily. With respect to the palynology of the group, Erdtman et al. (1961) characterized Boraginaceae as a stenopalynous family. Subsequent investigations, however, including those of Avertisian (1956), Clarke (1977), Sahay (1979), Diez (1984, 1991, 1994), Ahn and Lee (1986), Popova (1995), Bigazzi (1998), and others, have clearly demonstrated that the family exhibits a high degree of palynological diversity. These studies indicate that Boraginaceae is among the most eurypalynous plant families, with a substantial proportion of its species being distinguishable on the basis of their palynological characteristics.

The aim of this study is to contribute to the understanding of pollen morphology of the genus *Echium* (Boraginaceae) using LM and SEM observations, and to clarify interspecific relationships that may help resolve existing taxonomic problems.

2. Materials and Methods

Light microscopy (LM) and scanning electron microscopy (SEM) were used to examine the palynological characteristics of three species belonging to the genus *Echium*. Reference samples and relevant location data are provided in Table 1. All reference samples are stored at the Fırat University Herbarium (FUH).

For LM observations, pollen grains were prepared according to standard acetolysis-free procedures described by Wodehouse (1935). For light microscopy examination, pollen grains were placed in stained glycerol gel and observations were made using an Olympus binocular light microscope at 1000 \times magnification. To ensure statistical reliability, at least 30 well-developed pollen grains were measured for each sample. The measured parameters included the polar axis (P), equatorial diameter (E), P/E ratio, exine thickness (ex), intine thickness (i), colpus long axis (Clg), and colpus short axis (Clt). The quantitative data obtained are summarised in Table 2.

For SEM analysis, pollen grains were mounted on gold-coated aluminium pins and examined using a JEOL JSM-6510 scanning electron microscope located at the Bingöl University Central Research Laboratory. The palynological terminology and descriptive criteria used in this study follow the standard nomenclature system proposed by Erdtman (1952), Faegri and Iversen (1989), Moore et al. (1991), and Punt et al. (2007).

Table 1. Voucher specimens and they locality.

Taxa	Province	Locality
<i>E. italicum</i>	B7: Elazığ	Harput, roadside, 1400 m
<i>E. glomeratum</i>	C5: Adana	Adana-Kadirli, roadside, 100 m
<i>E. plantagineum</i>	C5: Adana	Karataş-Tuzla Lake, 20 m

3. Results

Three taxa belonging to the *Echium* genus in Turkey were studied palynologically. As a result, all the pollens examined were heteropolar pollen grains and aperture type tricolpate, while in two of the three species investigated, the pollen grains were subprolate, whereas in the remaining species they were prolate. In polar view, the broader pole exhibits a rounded-triangular outline, while the narrower pole is characterized by a more deeply incised, three-lobed configuration. In equatorial view, pollen shape ranges from ovoid to ovoid-triangular. Exine ornamentation is generally reticulate on the pollen surface, but appears distinctly granulate within the colpus region.

It was observed that the polar axis varied between 15.70 µm and 19.33 µm, and the equatorial axis varied between 12.17 µm and 14.47 µm. The largest pollen grains were observed *E. glomeratum* with an average polar axis of 19.33 µm, and with an approximate equatorial axis of 14.47 µm. The smallest pollen grains were recorded *E. italicum* with an average polar axis of 15.70 µm, and with an approximate equatorial axis of 12.17 µm. The colpus length (Clg) of the pollen grains ranges from 12.06 to 15.46 µm, and the colpus width (Clt) ranges from 2.10 to 3.32 µm. The species with the highest approximate Clg and Clt values is *E. glomeratum*, while the lowest is *E. italicum*. The exine thickness of the pollen grains examined is between 0.38 and 1.00 µm (Table 2).

***Echium italicum* L.**; The pollen shape was subprolate (P/E= 1.29) with a polar axis of 15.70 ± 1.35 µm and an equatorial diameter of 12.17 ± 0.20 µm. The pollen aperture type was tricolpate. The P/E ratio of 1.29 indicates that the pollen grains are in the subprolate shape. This ratio reveals that the pollen grains have a slightly elongated structure along the polar axis and is consistent with the typical pollen morphology of the genus *Echium* genus. The colpus length was 12.06 µm and width of 2.10 µm. Exine thickness was 0.38 ± 0.01 µm. Ornamentation was generally reticulate however granulate within the colpus region (Table 2, Figure 1A, 2A, 3A).

***Echium glomeratum* Poir.**; The pollen shape was prolate (P/E: 1.34) with a polar axis of 19.33 ± 1.45 µm and an equatorial diameter of 14.47 ± 0.99 µm. The pollen aperture type was tricolpate. The P/E ratio of 1.34 indicates that the pollen grains are in the prolate shape. The colpus length of was 15.46 µm and width of 3.32 µm. Exine thickness was 0.44 ± 0.08 µm. Ornamentation was generally reticulate however, granulate within the colpus region (Table 2, Figure 1B, 2B, 3B).

***Echium plantagineum* L.**; The pollen shape was prolate (P/E: 1.25) with a polar axis of 17.45 ± 1.83 µm and an equatorial diameter of 13.95 ± 1.79 µm. The pollen aperture type was tricolpate. The colpus length of 12.36 µm and width of 2.98 µm. Exine thickness was 1.00 ± 0.03 µm. Ornamentation was generally reticulate however granulate within the colpus region (Table 2, Figure 1C, 2C, 3C).

Table 2. Quantitative pollen morphological parameters of *Echium* taxa.

Taxa	P (µm)	E (µm)	P/E	Pollen Shape	Ex (µm)	Clt (µm)	Clg (µm)
<i>E. italicum</i>	15.70 ± 1.35	12.17 ± 0.20	1.29	subprolate	0.38 ± 0.01	2.10 ± 0.10	12.06 ± 0.20
<i>E. glomeratum</i>	19.33 ± 1.45	14.47 ± 0.99	1.34	prolate	0.44 ± 0.08	3.32 ± 0.40	15.46 ± 0.20
<i>E. plantagineum</i>	17.45 ± 1.83	13.95 ± 1.79	1.25	subprolate	1.00 ± 0.03	2.98 ± 0.30	12.36 ± 0.20

P: Polar axis, E: Equatorial diameter, Clg: Colpus length, Clt: Coplus width, Ex: Exine thickness.

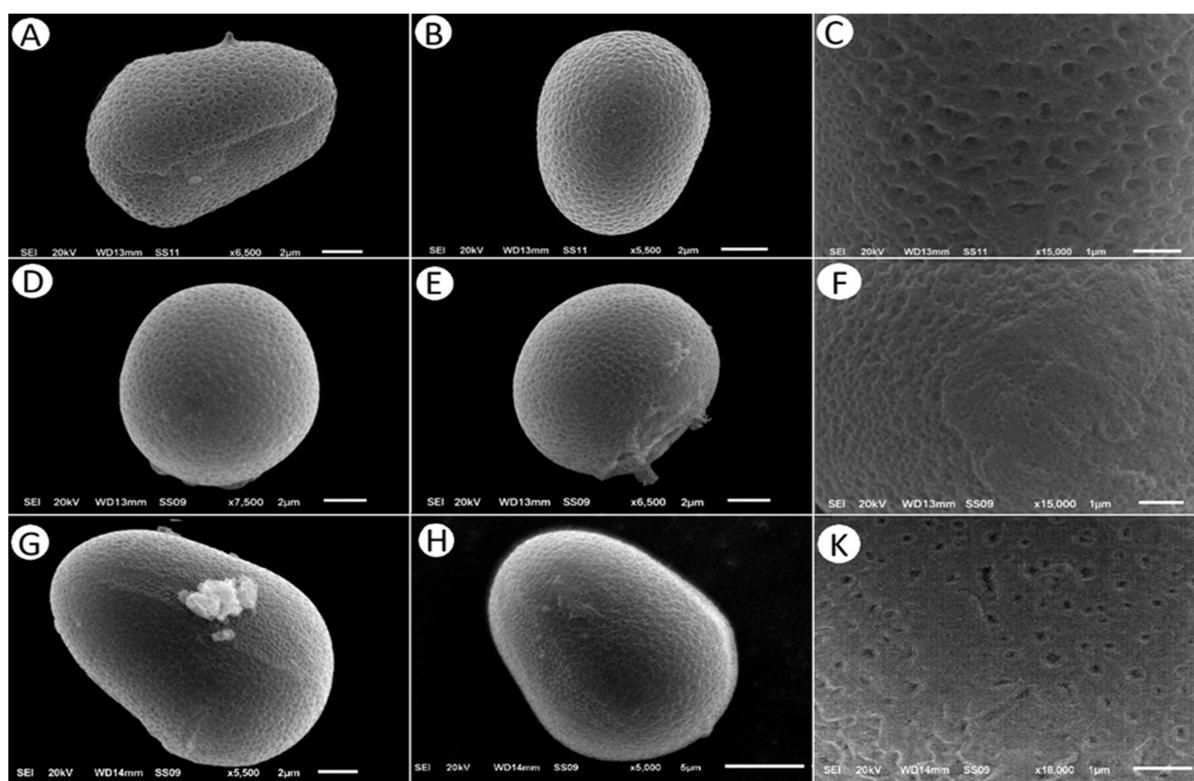


Figure 1. General and ornamentation view SEM micrograph of studied taxa **A-C**: *E. italicum*, **D-F**: *E. glomeratum*, **G-K**: *E. plantagineum*.

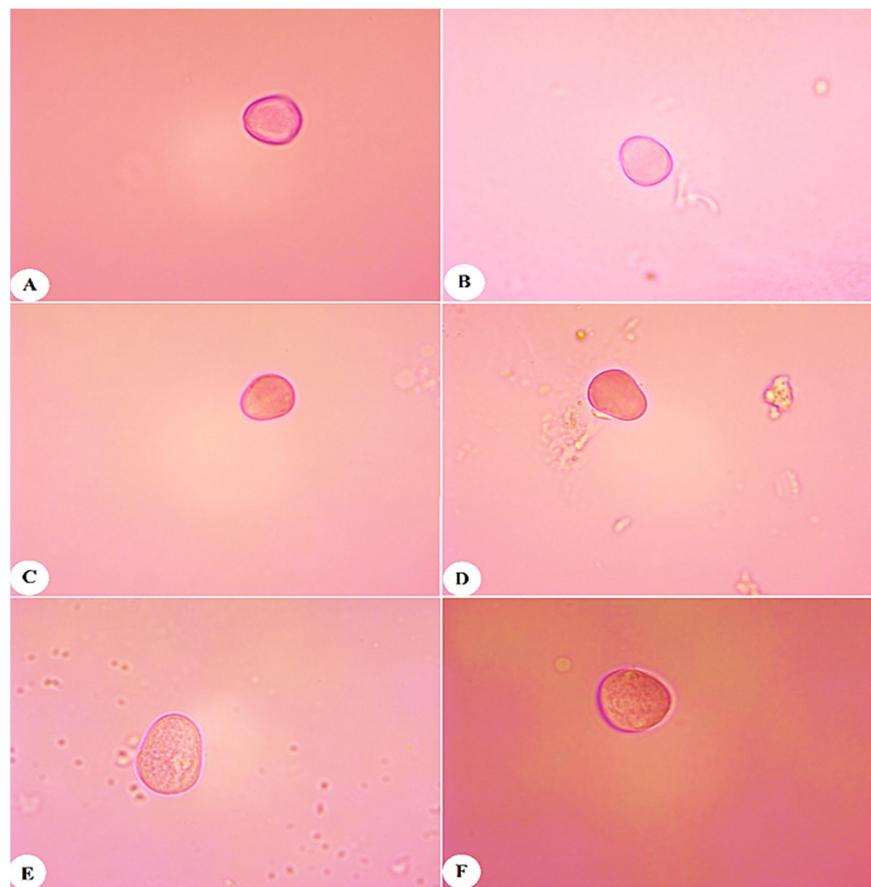


Figure 2. LM micrograph of studied taxa. **A, B**: *E. italicum* **C, D**: *E. glomeratum* **E, F**: *E. plantagineum*.

4. Discussion

Pollen morphological features of three taxa belonging to the *Echium* genus of the Boraginaceae family were examined using light microscopy and SEM. According to Clarke (1977), Boraginaceae is considered a euryhaline family. He also reported that the Boraginaceae family pollen grains display a broad spectrum of morphological forms, which are grouped into colporate and heterocolporate major types (Clarke, 1977).

In 2001, the pollen morphology of 42 species belonging to 32 genera of the Boraginaceae family in Iran was examined using SEM and LM. The results confirmed the wide pollen diversity of this family, where numerous species can be distinguished based on pollen characteristics. Palynological results revealed that the pollen grains of the family have a tricolporate opening and other types derived from it, and are mainly divided into two groups: those with and without pseudocolpys (Khatamsaz, 2001).

In a study on the palynology of members of the genera *Lobostemon*, *Echiostachys*, and *Echium* belonging to the Boraginaceae family in South Africa, pollen grains from all these genera were examined, and a single pollen type was identified. The grains are tricolporate, heteropolar, and triangular in equatorial view, with the ora being more or less circular and positioned toward the broader pole. The tectum is reticulate with densely spaced lumina, and suprarectal nodules occur along the margins of the lumina. The P/E ratio ranges from 1.08 to 1.63. The presence of zygomorphic flowers and the absence of calcium carbonate in the fruit wall justify the inclusion of *Lobostemon*, *Echiostachys*, and *Echium* within the Echieae Tribe. Additionally, the pollen grain morphology of the South African Echieae indicates a close relationship between these three genera, supporting the taxonomic grouping of *Lobostemon* and *Echiostachys* with *Echium* (Retief and Van Wyk, 1997).

According to Sahay (1977), the pollen grain structure of *Lobostemon*, *Echiostachys*, and *Echium* is very similar, suggesting a very close relationship between the genera. The association of *Lobostemon* and *Echiostachys* with *Echium* is supported by pollen morphology, with both having 3-colporate, anisopolar grains (Sahay, 1977).

Illustrations by Reille (1992, 1995) show that the morphology of pollen from the genera *Onosma* and *Alkanna* in the family Boraginaceae is generally similar to that of *Lobostemon*, *Echiostachys*, and *Echium*. In all these genera, pollen grains are heteropolar and characterized by three apertures, although differences in the configuration of the apertures are noticeable. Specifically, some species of *Onosma* exhibit syncolpate apertures at one pole, while in *Echium*, *Lobostemon*, and *Echiostachys*, the apertures remain continuously free at their distal ends. These differences may reflect variations in pollen aperture morphology and should be considered when examining closely related genera.

In his 1954 study, Johnston noted that pollen grains of *Echium* and *Lobostemon*, as well as *Alkanna*, *Onosma*, and several smaller genera are characterized by the presence of only three apertures, with the grains generally exhibiting a distinctly triangular outline in polar view (Johnston, 1954).

According to literature data, *Echium* pollen grains are generally isopolar to weakly heteropolar in structure. Pollen shape is mostly prolate or subprolate, with perprolate forms rarely observed. Pollen size varies from medium to large. Pollen grains are typically tricolporate or, in some species, tricolpate in structure. Apertures are free-ended, and syncolpate (fusion at the ends) is not observed. Colpus are generally long and narrow, with pointed or slightly rounded tips. The exine structure is mostly reticulate or reticulate-perforate type. Ornamentation in the colpus region often shows a granular or weaker reticulate structure (Sahay, 1977; Reille, 1992; 1995; Retief and Van Wyk, 1997).

In her 2015 master's thesis, Zağyapan examined the pollen morphologies of four species of the genus *Echium* (*E. orientale* L., *E. vulgare* L., *E. angustifolium* Miller, *E. parviflorum* Moench) in Türkiye using light microscopy (LM) and scanning electron microscopy (SEM). The results indicated that the pollen ornamentation of the four species was generally similar. Based on pollen morphological characteristics, the species were divided into two groups. The first group included *E. parviflorum*, which exhibited an oblate-spheroidal pollen shape, whereas the second group comprised *E. orientale*, *E. vulgare*, and *E. angustifolium*, all of which displayed subprolate pollen grains. In general, the pollen grains were heteropolar, tricolporate, and granulate. In addition, in his palynological study on the species *Echium amoenum* in 2001, Khatamsaz stated that the pollen grains were heteropolar, triangular in shape, blunt-ended, 3-colporate, with a gemmate tectum and a colpus of 9.29 μm in length (Khatamsaz, 2001).

5. Conclusions

In all three taxa used in our study, general genus characteristics such as prolate or subprolate pollen shape, tricolpate pollen structure, and predominantly reticulate ornamentation were confirmed by both LM and SEM data. In conclusion, this study characterized the morphological characteristics of pollen grains of three taxa belonging to the *Echium* genus of the Boraginaceae family. We believe that the data obtained here will be useful in many fields, especially plant taxonomy.

Conflicts of Interests

Authors declare that there is no conflict of interests

Ethical Statement

I testify on behalf of all co-authors that our article submitted followed ethical principles in publishing.

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Statement contribution of the authors

This study's experimentation, analysis and writing, etc. all steps were made by the authors.

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