

ULTRASTRUCTURE AND POLLEN MICROMORPHOLOGY OF THREE ENDEMIC *BELLEVALIA* (ASPARAGACEAE) SPECIES IN TURKEY

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ABSTRACT

The pollen morphology of *Bellevalia kurdica* Firat, *B. siirtensis* Firat, *B. hakkariensis* Firat species (Asparagaceae) was examined using Light Microscopy (LM) and Scanning Electron Microscopy (SEM). The taxa are similar in some aspects, such as pollen size and reticulate exine sculpturing. Pollen grains are shed as monads. They are monosulcate, ellipsoidal, isopolar and bilateral symmetric. The exine is semitectate and the tectum is perforate. Sulcus extends from distal to proximal in some pollen of this genus. Besides, this study is the first report dealing with the palynological features of three endemic *Bellevalia* Lapeyr. species.

KEYWORDS:

Asparagaceae, *Bellevalia*, pollen morphology, endemic, Turkey

INTRODUCTION

The Asparagaceae family has 143 genera and 3632 species which are distributed naturally in temperate, sub-tropical and tropical regions. The family is represented by 19 genera and 182 species in the flora of Turkey [1, 2, 3]. *Bellevalia* Lapeyr. is, according to recent literature, a bulbous plant belonging to Asparagaceae Chase et al. [4]. The Irano-Turanian phytogeographical region is a very important region due to the high distribution of the species [5]. Pehlivan and Ozler [6] examined in detail the following pollen grains of 14 Turkish *Muscari* species. Ozler and Pehlivan [7] examined in detail the following pollen grains of 20 taxa from *Asparagus* L. and *Fritillaria* L. of the Liliaceae. Lopez et al. [8] investigated the pollen morphology and wall structure of *Clara* and *Herreria* in order to clarify their taxonomy. The morphological, anatomical and palynological characteristics of *Heldreichia rotundifolia* Boiss. were investigated by Kizilpinar et al. [9].

As far as we know, there is no comprehensive study to be found according to this genus of pollen

(has not been a comprehensive study of pollen). Hence, it was examined in detail in the following pollen grains of three endemic *Bellevalia* species. Therefore, the aim of the present study was to examine the pollen morphology of almost three recognised *Bellevalia* species and to evaluate the significance of pollen characteristics for the infrageneric taxonomy of the genus.

MATERIALS AND METHODS

The pollen grains were obtained from herbarium samples. For light microscopy (LM) study, the pollen slides were prepared according to Wodehouse [10] technique. The following parameters were measured: pollen size (long axis (LA) and short axis (SA)), sulcus width, pore width, exine thickness, and intine thickness. The reported pollen size represents the mean of about 30 pollen grains. Photomicrographs were made with an Olympus BX31 binocular light microscope. The Scanning Electron Microscopy (SEM) was also used to investigate exine patterning in detail. The pollen was mounted on stubs with platinum after treatment with 70% alcohol and air-drying, and photomicrographs were taken with a ZEISS supra 55 electron microscope.

The pollen terminology was used to describe all shapes and sizes, the morphologies and ornamentation were based on Punt et al. [11]. The classification used to describe the aperture size was established by Faegri and Iversen [12].

Specimens investigated. *Bellevalia kurdica* Firat: Turkey. C9 Siirt: Şirvan province, Herzane hill, Kuska region, Humid steppe, 1098 m, coll. 30.04.2014, *M. Firat 30540* (holotype VANF); *Bellevalia siirtensis* Firat: Turkey. C9 Siirt: Eruh province, 12 km from Eruh to Siirt, neer road and steppe, 806 m, coll. 30.04. 2014, *M. Firat 30541* (holotype VANF); *Bellevalia hakkariensis* Firat: Turkey. C10 Hakkari: Şemdinli, Derecik province, Merga nergisa, steppe, 715 m, coll. 30.04.2014, *M. Firat 28760* (holotype VANF).

RESULTS AND DISCUSSIONS

Pollen characteristics of endemic *Bellevalia* species are summarised in Table 1 and are shown in Figs 1 - 2. The sulcus is usually very wide (8.40-15.75 μm). Sulcus length is 42.00-55.65 μm , sulcus width is 15.75-9.45 μm based on LM. The sulcus membrane is rugulate. While the sulcus is sharp at apex in *B. siirtensis* and *B. hakkariensis*, it is round at apex in *B. kurdica* (Figure 1). The exine is tectate and 0.79-1.31 μm thick. Intine thickness ranges from 0.53 to 0.79 μm (Table 1).

B. kurdica; the number of the lumina in 1 μm^2 is 6-9, the diameter of lumina is approximately 0.10-0.55 μm , the number of perforation in 1 μm^2 is 2-5 at the sulcus side or at the lateral surface. The diameter of a perforation is approximately 0.05-

0.13 μm and the thickness of a muri is on average 0.08-0.26 μm (Figure 1-c).

B. siirtensis; the number of the lumina in 1 μm^2 is 6-9, the diameter of lumina is approximately 0.18-0.60 μm , the number of perforation in 1 μm^2 is 4-7 at the sulcus side or at the lateral surface. The diameter of a perforation is approximately 0.04-0.15 μm and the thickness of a muri is on average 0.06-0.18 μm (Figure 1-f).

B. hakkariensis; the number of the lumina in 1 μm^2 is 1-6, the diameter of lumina is approximately 0.14-0.70 μm , the number of perforation in 1 μm^2 is 2-8 at the sulcus side or at the lateral surface. The diameter of a perforation is approximately 0.03-0.10 μm and the thickness of a murus is on average 0.12-0.30 μm (Figure 1-k).

TABLE 1
Pollen morphological parameters of the investigated *Bellevalia* species

Taxa	A(long axis) (μm)	B(short axis) (μm)	A/B	Length of the sulcus (Slg) (μm)	Width of the sulcus (Slw) (μm)	Exine (μm)	Intine (μm)	Ornamentation
	Min-max	Min-max		Min-max	Min-max			
<i>B. hakkariensis</i>	49.77 \pm 3.07	32.52 \pm 2.21	Prolate	60.52 \pm 3.08	12.98 \pm 1.90	1.07 \pm 0.18	0.68 \pm 0.13	Reticulate
	44.10-53.55	27.30-36.75		50.95-65.65	9.45-15.75			
<i>B. siirtensis</i>	43.12 \pm 2.42	30.38 \pm 1.74	Prolate	55.25 \pm 2.16	11.97 \pm 1.65	1.13 \pm 0.12	0.59 \pm 0.10	Reticulate
	39.90-47.25	28.35-33.60		40.95-55.65	9.45-13.65			
<i>B. kurdica</i>	41.27 \pm 2.29	26.36 \pm 1.90	Prolate	52.00 \pm 4.38	10.40 \pm 1.88	0.99 \pm 0.21	0.56 \pm 0.08	Reticulate
	35.70-44.10	24.15-30.45		41.59-51.34	8.40-15.75			

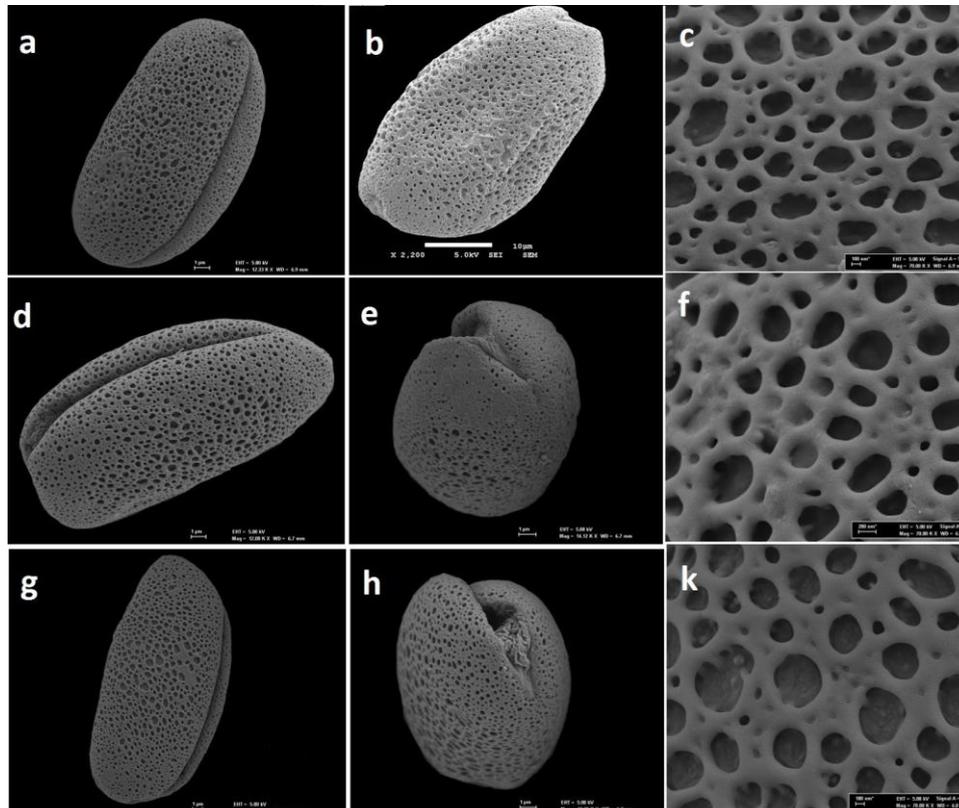


FIGURE 1
SEM microphotography of *B. kurdica* (a-c); *B. siirtensis* (d-f); *B. hakkariensis* (g-k).

B. kurdica and *B. siirtensis* are narrow endemics in the eastern Anatolian region. This species, which is seen in the steppe areas, also spreads in the openings of *Quercus* forests. *B. hakkariensis* is a narrow point endemic and distributed in areas close to the Iraqi border region of the town of Derecik, in the Şemdinli district of Hakkâri province. [13].

The Asparagaceae family is a difficult group among monocotyledonous plants for taxonomical and systematic reasons. In order to supply more morphological data to solve some of these problems, a detailed pollen morphological study of three species of the genus *Bellevalia* has been undertaken. Several of them are endemic for Turkey, and all of them are entomogamous plants.

The common characteristics of the pollen grains of the investigated species are that their pollen apertures are monosulcate. The aperture type is predominantly monosulcate in monocotyledons, as in Asparagales and Liliales, as emphasized by Harley and Zavada [15]. The Asparagaceae have monosulcate pollens. The monosulcate aperture might be a strong palynological evidence for the common origin of monocotyledons and dicotyledons. Sulcate, colpate, colpate pollen apertures are the most common in biotically pollinated families and the sulcate pollen has a much older pollen record than those of both porate and colpate pollen [7, 14, 15]. This is the most common aperture type among the monocots, and is derived from several different developmental pathways, leading thus to homoplasy in the interpretation of monosulcate character state [16]. Monocots generally have monosulcate or monoporate pollen grains, although there are numerous exceptions [17]. Sometimes no aperture is detected, and thus inaperturate pollen

grains are not rare among the Monocots [18]. Within Asparagales clade, most of the families produce monosulcate pollen [16].

We have found that the *Bellevalia* pollen is monosulcate and sulcus extends from distal to proximal. These results are similar to former studies [6, 9, 19-22]. During the present study, some remarkable differences in the measurement of dimensions were observed between taxa. Among the examined genera, the biggest pollen size was found in *B. hakkariensis* (Table 1). No data about sulcus extension to proximal and sulcus ends have been observed in any studies other than those by [6, 21-24]. Accordingly *Bellevalia* species, there has been a transition from a monosulcate to extended sulcate. At the same time, these results are similar to [21, 22]. The widest sulcus was measured in *B. hakkariensis* (Table 1, Figure 1), which agrees with the statement of [21-25]. Extended sulcate pollen grains, found in the *Bellevalia*, may have evolved by extended by joining of the extremities of the aperture. Some researchers have shown that the sulcus features may be a taxonomic characteristic in some families [6, 21-24].

No other previous studies have been done related to these genera provided data about the sulcus membrane characteristics except for the research on *Fritillaria* species by [7, 23, 25]. According to Kosenko [23], sulcus membrane surface were good characteristics for the classification genus. Sulcus membrane of *Bellevalia* pollen grains are rugulate. The edges of the sulcus are irregular in investigated taxa (Figure 1 a-d-g). Semitectate, tectum perforate exine and thin exine in the examined taxa are primitive characteristics in monocotyledones and are

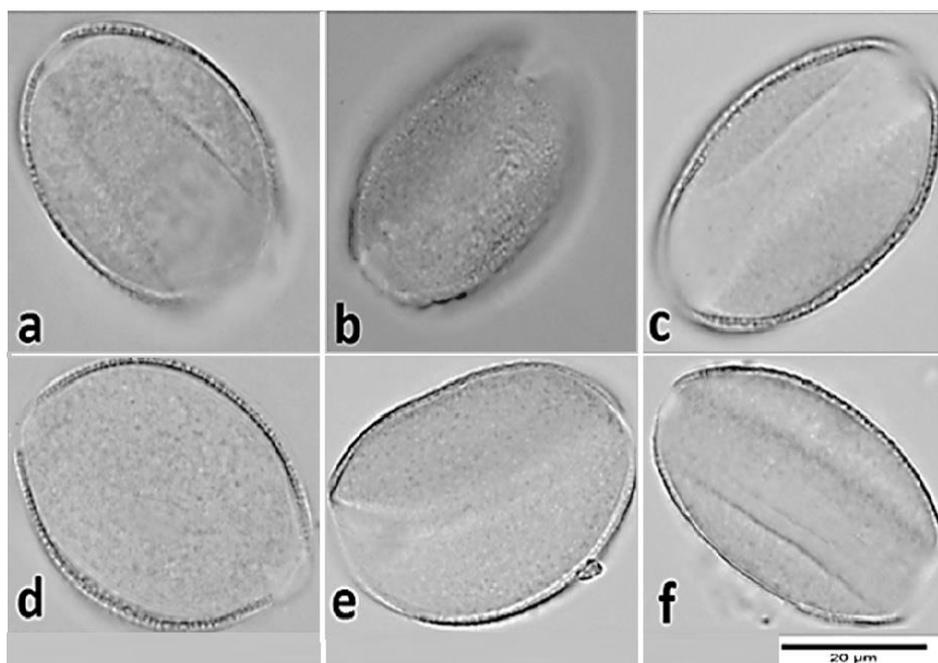


FIGURE 2

LM Photographs of *B. kurdica* (a, b); *B. siirtensis* (c, d);- *B. hakkariensis* (e, f), (scale 20 µm).

observed in the early stages of angiosperm evolution [6, 7, 17, 21, 23, 25-27]. These results showed that monosulcate grains were usually prolate, heteropolar, bilateral and monosulcate-operculate in the *Bellevalia* genus and at the same time, sulcus extends from distal to proximal in all of the species in *Bellevalia* genus (Figure 1 b-e-h; Figure 2 b-d).

Bellevalia colpus extends from distal to proximal. Investigations in other genera and species of the Liliaaceae give comparable information for the genus *Fritillaria*. In the investigated *Bellevalia* species reticulate (columellae form a reticulate pattern beneath the tectum: Faegri & Iversen [12]) or suprareticulate (reticulation only on the outside of the tectum) ornamentations can be distinguished.

CONCLUSIONS

We obtained a correlation between our results and the classification of three species, that is, pollen features, especially pollen size and sulcus extends from distal to proximal, proved to be the most useful characters for the systematics of three endemic *Bellevalia* species.

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