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POLLEN MORPHOLOGY OF SOME *FRITILLARIA* L. SPECIES (LILIACEAE) FROM IRAN

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Abstract

Pollen grains of 5 taxa from the genus *Fritillaria* L. in Iran were studied by scanning electron microscopy. Detailed pollen morphological features are given for these taxa. Pollens were monosulcate and ellipsoidal. Sulcus extends from distal to proximal in all studied taxa. Results shows that the sculpturing of the exine, pollen membrane ornamentation and lumina shape provides valuable characters for separating species. Based on these characters, 3 main pollen types were determined with three different exine sculpturing: reticulate, reticulate-perforate and suprareticulate.

Key words: *Fritillaria*, Liliaceae, Pollen morphology, SEM.

Introduction

Genus *Fritillaria* L. (Liliaceae) comprises of approximately 170 taxa (130-140 species) which are distributed through the temperate regions of the northern hemisphere (Day *et al*., 2014; Metin *et al*., 2013). Most of the species in this genus are belong to the main subgenus, *Fritillaria* (Rix *et al.,* 2001). The Mediterranean region is the center of genetic diversity of *Fritillaria* species, with most of the taxa described from Turkey (Rix, 1984 and Ozhatay, 2000) and the Zagros mountains of Iran as the center of diversity above the species level (Rix, 1997).

They are characterized by bisexual nodding flowers, campanulate to cupulate perianth of six tepals marked with light/dark colored squares or with longitudinal stripes or fascia, and with nectaries at the base, or at the inflection (Rechinger, 1990; Rix, 1997). These genera have economic and pharmaceutical importance (Guner *et al.,* 2000). In order to obtain more morphological data to solve taxonomical problems, detail study of pollen morphology in Liliaceae family always has been suggested and pollen structure of some species of the genus *Fritillaria* has been subjected to earlier investigations (Schulze, 1980; Kosenko, 1992, 1999; Özler & Pehlivan, 2007; Tekşen & Aytaç, 2004, 2008; Tekşen *et al*., 2010). So the purpose of this study was to determine pollen characteristics of 5 Iranian *Fritillaria* species and to estimate the importance of pollen features for the infrageneric taxonomy of this genus.

Materials and Methods

The samples of *Fritillaria* species were collected from Kurdistan Research Center of Agriculture and Natural Resources. Information about localities of investigated specimens have been provided in table 1. For SEM after acetolysis, pollen grains were soaked in absolute ethanol, and were pipetted directly onto 12.5 mm diameter stubs, air-dried at room temperature, then coated in a sputter coater with approximately 25 nm of Gold Palladium. The specimens were examined and photographed with a TESCAN MIRA 3 scanning electron microscope. Dimensions of pollens were based on 30 or more pollen grains per sample. The pollen expressions are based on Faegri & Iversen (1975), Erdtman (1969), Punt *et al*., (2007) and Pinar & Oymak Dönmez (2000) literatures.

Results

The main characters of the investigated pollen are summarized in Tables 2-4. The general report can be given as follows:

Size and shape: The shape of pollen grains in *Fritillaria* are typically symmetrical, heteropolar, prolate, or sometimes subprolate. In this study, all five species were prolate. Classification of pollen size and shape based on Pınar and Oymak Dönmez (2000) was presented in Table 2. Based on SEM result average of long and short axis were between, LA: 44.79-57.51 µm and SA: 22.54-31.91 µm respectively (Table 2, Fig. 1).

Aperture: The pollen grains were monosulcate. Sulcus length and sulcus width were 25.88-43.43 µm and 10.79-22.87 µm respectively (Table 3). The sulcus membrane was verrucate (*F. avramanica*, *F. fleischeriana* and F. sp) and psilate (*F. poluninii* and *F. assyriaca*).

Table 1. Origin of the material.

Species	Location	Altitude (m)	Herbarium voucher
F. poluninii	Sarvabad, Seline, bandol Village	2550	sku-0205
F. avramanica	Maryvan to Paveh, Seline Village	1800	sku -1251
F. assyriaca	Hamedan to Sanandaj road, salvat-abad Village	1865	sku-1236
F. fleischeriana	Sanandaj, Abidar mountain	1700	HOK- 1220
F. sp.	Maryvan to Paveh, Seline Village	2000	HOK- 1212

Table 2. Pollen morphological parameters of *Fritillaria* taxa (values in µm).

Taxa	Long axis (LA)			Short axis (SA)			LA/SA ratio	Shape	Exin ornamentation
	Min.	Max.	Mean	Min.	Max.	Mean			
F. poluninii	40. 32	49.26	44.79	20.52	24.57	22.54	1.98	Prolate	Reticulate- perforate
F. avramanica	43.27	46.64	44.95	21.35	23.86	22.6	1.98	Prolate	Suprareticulate
F. assyriaca	51.8	63.22	57.51	26.64	37.19	31.91	1.80	Prolate	Reticulate- perforate
F. fleischeriana	43.29	46.1	44.69	24.13	27.64	25.88	1.73	Prolate	Reticulate
F. sp	44.25	48.95	46.6	32.21	24.32	28.26	1.64	Prolate	Suprareticulate

Table 3. The pollen morphological characters of *Fritillaria* taxa on SEM (values in µm).

Taxa	Aperture type	Sulcus length			Sulcus width			Sulcus membrane ornamentation
		Min.	Max.	Mean	Min.	Max.	Mean	
F. poluninii	Monosulcate	30.27	35.12	32.69	17.24	19.38	18.31	Psilate
F. avramanica	Monosulcate	23.67	28.10	25.88	9.13	11.45	14.85	Verrucate
F. assyriaca	Monosulcate	40.80	46.87	43.43	20.10	25.64	22.87	Psilate
F. fleischeriana	Monosulcate	40.14	43.29	41.71	9.68	11.9	10.79	Verrucate
F. sp.	Monosulcate	40.15	43.96	42.05	16.34	18.06	17.2	Verrucate

Table 4. Microsculpturing features of reticulate pollen ornamentation (values in µm).

Taxa	The number of lumen in 5 µm 2	Lumen			Murus			Lumen shape	Murus shape	
		Min.	Max.	Mean	Min.	Max.	Mean		Smooth	Undulate
F. poluninii	27	0.23	0.43	0.33	0.31	0.46	0.38	Irregular amorphous	+	-
F. avramanica	17	0.47	1.27	0.87	0.28	0.46	0.37	Regular polygonal	+	-
F. assyriaca	14	0.20	0.68	0.44	0.51	1.44	0.97	Irregular amorphous	+	-
F. fleischeriana	13	0.18	0.88	0.53	0.29	0.57	0.43	Irregular amorphous	+	-
F. sp.	15	0.42	1.72	1.07	0.21	0.61	0.41	Regular polygonal	+	-

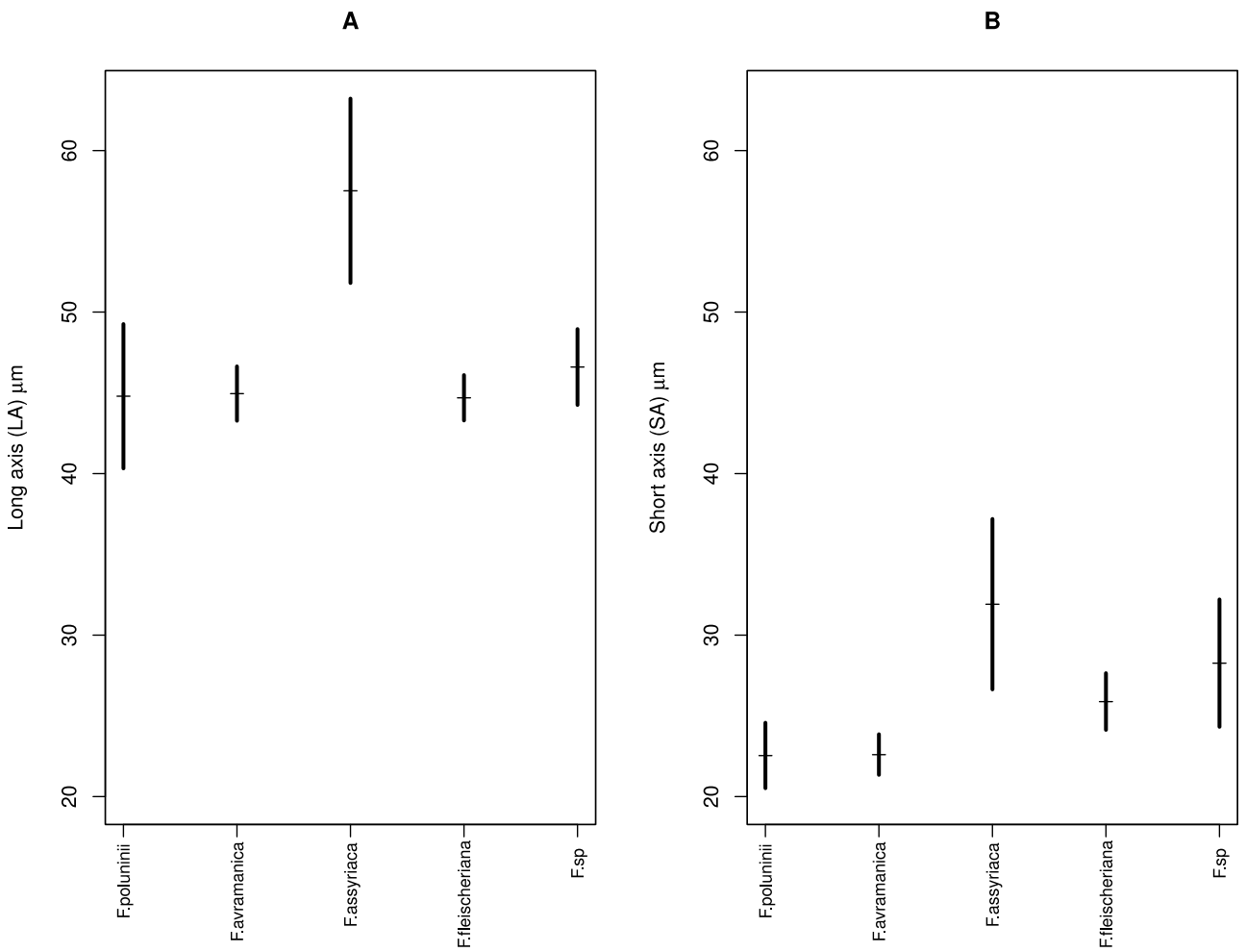

Fig. 1. Measurements of pollen grains in *Fritillaria* based on SEM. A: Long axis (LA), B: short axis (SA).

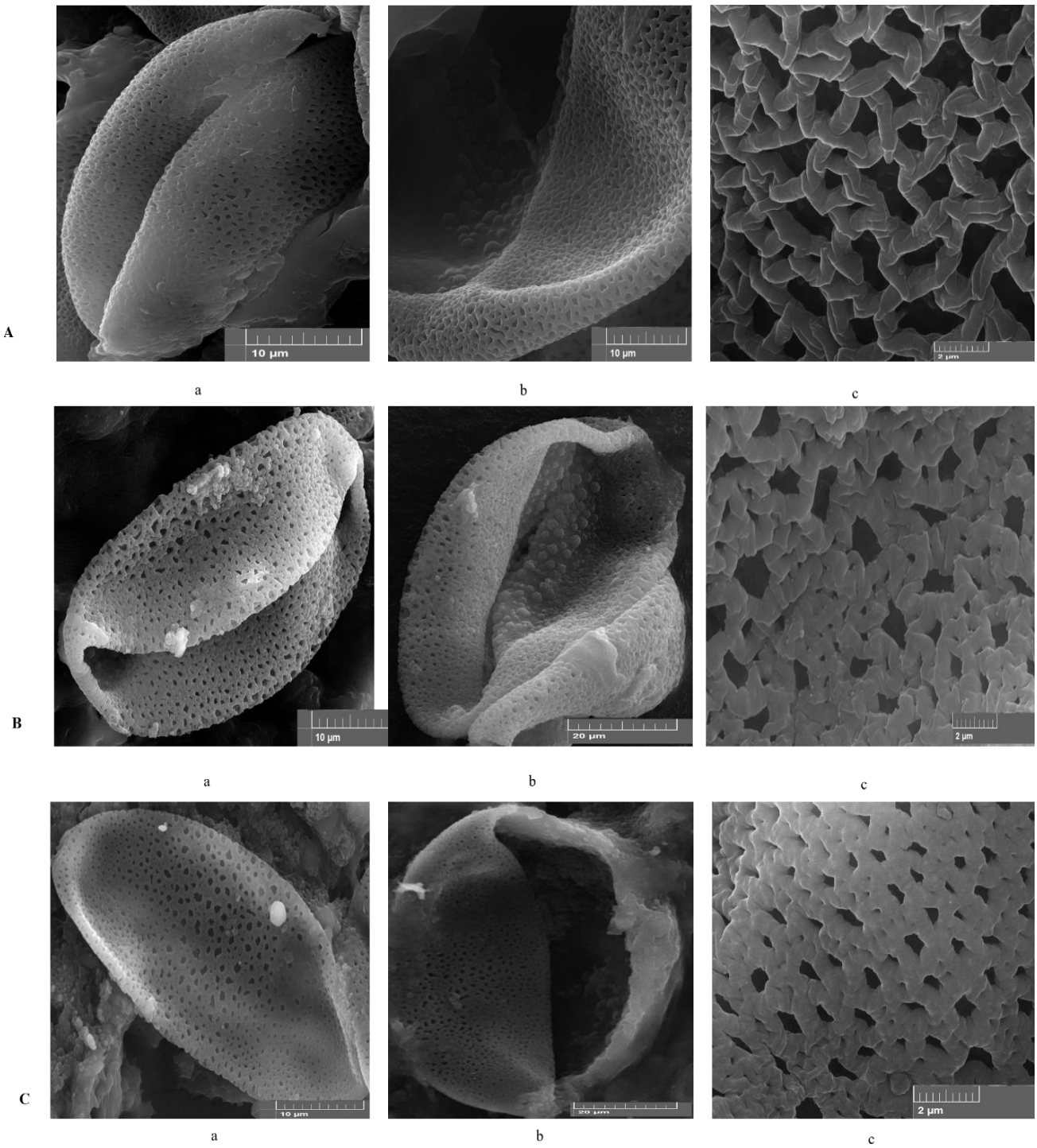

Fig. 2. SEM micrographs of pollen grains. A: *Fritillaria avramanica*. a. equitorial view, b. sulcus membrane, c. exine surface. B*:* *Fritillaria fleischeriana*. a. lateral view, b. sulcus membrane, c. exine surface. C: *Fritillaria assyriaca*. a. lateral view, b. sulcus membrane, c. exine surface.

Exine ornamentation: The exine was tectate. The ornamentation was reticulate (*F. fleischeriana*); suprareticulate (*F. avramanica* and *F.* sp.); reticulate-perforate (*F. poluninii* and *F. assyriaca*). The lumen width being 0.33-1.07 µm. The number of lumina across 5 μm^2 was 13-27 (Table 4). The pollen grains lumina shape was irregular amorphous but in *F. avramanica* and F. sp, it was regular-polygonal. Murus width was 0.37-0.97 µm. Murus shape in all studied species were smooth (Table 3).

Based on exine structure, 3 main pollen types are recognized in studied *Fritillaria* species: Type I: The exine sculpturing is reticulate- perforate. Lumen shape was Irregular amorphous and Sulcus membrane was psilate (*F. poluninii* and *F. assyriaca*). Type II: The pollen ornamentation was suprareticulate. Lumen shape was Regular polygonal. Sulcus membrane was verrucate (*F. avramanica* and F.sp). Type III: The pollen grain had reticulate exine sculpturing. Sulcus membrane ornamentation was verrucate and lumen shape was irregular amorphous with smooth murus shape (*F. fleischeriana*).

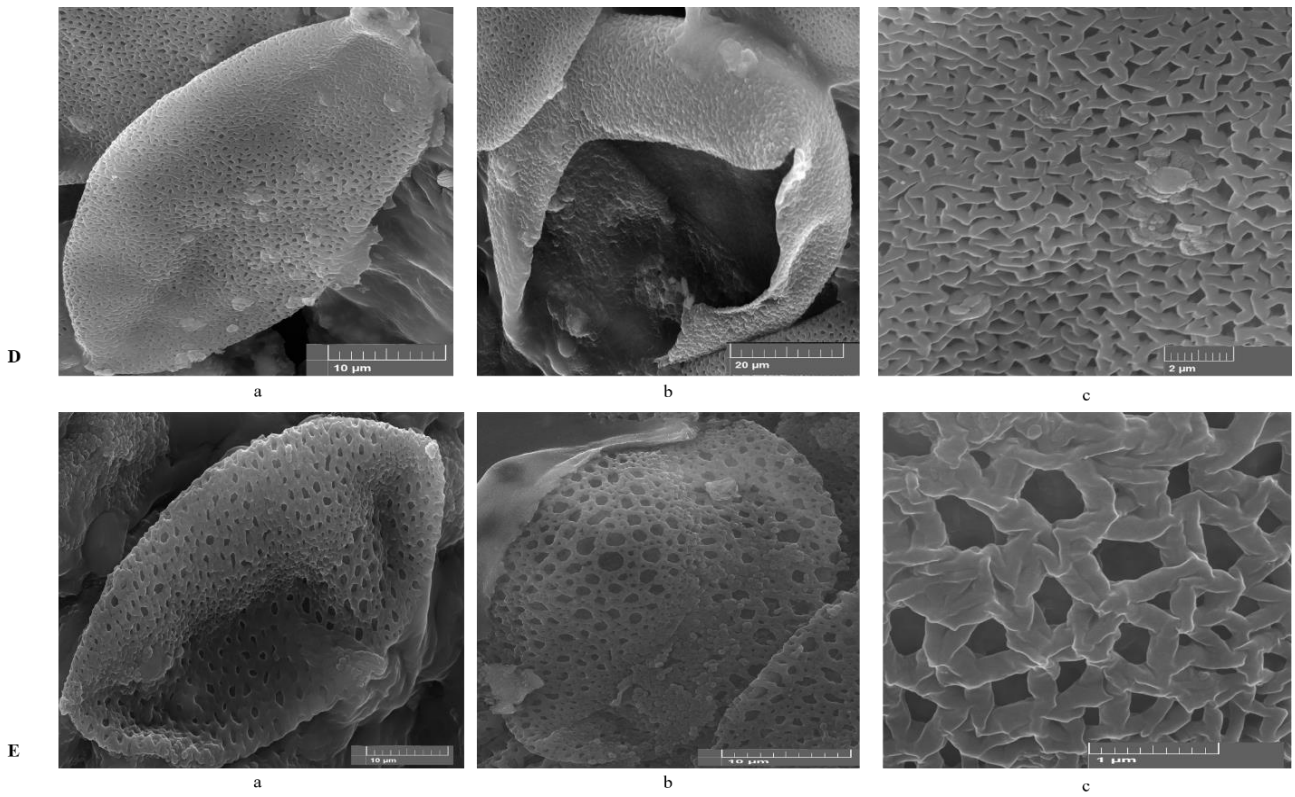

Fig. 3. SEM micrographs of pollen grains. D: *Fritillaria poluninii*. a. equatorial view, b. sulcus membrane, c. exine surface. E: *Fritillaria sp*. a. lateral view, b. equatorial view, c. exine surface.

Discussion

This study shows that pollen characters have potential to being significant in classification of *Fritillaria*. By using pollen morphology the unknown species of this study is evaluated as a close species to *F. avramanica* within *Fritillaria* subgenus. Differences are faced in pollen shape, size, exine sculpturing, ornamentation of sulcus membrane and lumen shape, size and murus size. We recognized 3 main types, distinct by pollen sculpturing, sulcus membrane ornamentation and Lumina shape. *Fritillaria* species are separated into 4 pollen types by Özler & Pehlivan (2007) and to 5 type by Tekşen *et al.*, (2010). All of these features are significant for interspecies classification of *Fritillaria* (Schulze, 1980; Kosenko1992, 1999; Özler & Pehlivan, 2007; Tekşen *et al.*, 2010) (Tables 2-4, Figs. 2 and 3).

The sculpturing of the pollen exine is valuable for founding relationships among species. We recognized various types of exine sculpturing. In the species belonging to type I, the sculpturing is reticulate perforate. The sculpturing suprareticulate is in type II. In type III, it is reticulate (Table 1, Figs. 2 and 3). Özler & Pehlivan (2007) and Kosenko (1999) have indicated that the most diverse genus in Liliaceae is *Fritillaria* where several types of exine arrangements (reticulate, suprareticulate, rugulatereticulate, striate-reticulate, microreticulate, scabrate, and macroreticulate).

Sulcus long, getting the ends of the pollen grain or spreading to the proximal side. Kosenko (1992) and Özler & Pehlivan (2007) have shown that sulcus features are valuable characters in terms of taxonomy among *Fritillaria* pollens. Sulcus apex of studied species was rounded. Özler & Pehlivan (2007) and Tekşen *et al.*, 2010 indicated that the sulcus apex is rounded in *Fritillaria* pollen grains except for *F. aurea* and *F. bithynica*. Sulcus membrane sculpturing was a suitable characteristic for classification of *Fritillaria* (Kosenko, 1992; Özler & Pehlivan, 2007).

Among *Fritillaria* species a plicate-granulate, gemmate, and granulate sulcus membrane is distinctive. In this study, *Fritillaria* species divided into 2 pollen types according to sulcus membrane sculpturing: psilate amd verrucate. In studied taxa lumen with reticulate ornamentation, shape is regular-polygonal or irregular-amorphous. Muri are smooth. Schulze (1980) and Pınar *et al.*, (2009) have shown that muri and lumen shapes of the pollen are taxonomically important characters. Analysis of the mean long axis (LA) and short axis (SA) was indicated, the largest grains in *F. assyriaca* (51.8-63.22 µm) and the smallest LA values in *F. fleischeriana* (43.29-46.1 µm), while the smallest SA value was found in *F. poluninii* (22.54 µm).

Palynological data related to exine ornamentation indicate the heterogeneous characters of this genus. In this study we have determined that there are intraspecific variations among studied species as well which are based on exine sculpturing, and sulcus ornamentation.

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