



### Full Length Article

## Investigation of Palinological Features of Taxa Belonging to the Genus *Cuscuta* Distributed in Turkey

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### Abstract

In this study the species belonging to *Cuscuta* L. genus showing the distribution of agricultural and non-agricultural lands in Turkey was examined palynological perspective. This study was carried out between the years 2013–2015. The pollen morphology of 15 taxa of *Cuscuta*, including *C. campestris* Yunck., *C. hyalina* Roth., *C. lupuliformis* Krock., *C. monogyna* Vahl subsp. *monogyna*, *C. monogyna* Vahl subsp. *esquмата* (Englm.) Plitm., *C. kotschyana* Boiss. subsp. *caudata* Bornm & Schwarz, *C. babylonica* Aucher ex Choisy var. *babylonica*, *C. babylonica* Aucher ex Choisy var. *elegans* (Boiss. & Bal.) Englm., *C. europaea* L., *C. kurdica* Engelm., *C. epithymum* L., *C. brevistyla* A.Braun ex A.Rich., *C. planiflora* Ten., *C. approximata* Bab. var. *approximata*, and *C. approximata* Bab. var. *macranthera* (Boiss.) Feinbr. & Greuter was investigated by using scanning electron microscope (SEM). *C. campestris* and *C. brevistyla* species were found 3 (-4) zonocolpate, *C. planiflora* 3 (-6) zonocolpate. Pollen structure of taxa belonging to *Cuscuta* L. genus were determined prolate, subprolate, perprolate and prolate-spheroidal. The apertures of the pollens of these taxa were found scabrate, scabrate-perforate, oscabrate-perforate, reticulate and ekinate-reticulate. It was concluded that the pollen characteristics are convenient and a reliable method in identifying species. © 2017 Friends Science Publishers

**Keywords:** *Cuscuta*; Pollen; SEM; Turkey

### Introduction

*Cuscuta* (dodder) is nearly cosmopolitan in distribution comprising some 200 currently described species (Yuncker, 1932; Hunziker, 1950; Mabberley, 1997; Stefanovic *et al.*, 2007; Garcia *et al.*, 2014). According to Stefanovic and Olmsted (2004, 2005) *Cuscuta* L. has evolved within Convolvulaceae. "In the Flora of Turkey", *Cuscuta* L. has been reviewed in the genus Cuscutaceae (Davis, 1978). Members of the genus *Cuscuta* are stem parasites, mostly annual, pale stems, slender, herbaceous vines with twining, with very little or no chlorophyll, and no roots (Kuijt, 1969; Cronquist, 1981; Stewart and Press, 1990; Garcia *et al.*, 2014). These stem parasites are attached to the various hosts via haustoria and depend entirely on their hosts to supply nutrients and water (Kuijt, 1969; Cronquist, 1981; Dawson *et al.*, 1994). Approximately 15–20 *Cuscuta* sp. worldwide are agricultural and horticultural pests (Dawson *et al.*, 1994; Costea and Tardif, 2006).

According to Kuijt (1969); in diagnosis of the species, the floral characters in parasitic plants are inadequate in some cases. Therefore, for the identification of species belonging to the genus *Cuscuta* L. some other methods must be used. The morphological characteristics of pollen are used as an

important character in systematic botanicals (Hsiao and Kouh, 1995; Perween and Qaiser, 1998; Nemli *et al.*, 2015).

Today, little is known about the pollen morphology of *Cuscuta* species. More importantly, pollen morphology of this genus has not been extensively analyzed (Welsh *et al.*, 2010). At the same time, there are no morphological studies on the pollen of *Cuscuta* species in Turkey (Nemli *et al.*, 2015).

Pollen morphology is very important in the systematic diagnosis of plants belonging to the family Convolvulaceae. For example, Hallier (1893) assigned the genera within this family to two major groups, "Echinoconieae" and "Psiloconieae" based on their echinate or psilate exine, respectively.

According to Welsh *et al.* (2010), pollen morphology is used to determine the kinship relationships of members of the Convolvulaceae family. Sengupta (1972) studied 21 *Cuscuta* species and according to pollen structures these species as tricolpate and penta-hexa-colpate classified two groups. In addition, Van Campo (1976) has collected the Convolvulaceae family under two major groups, "tricolpat" and "pentaporat", according to their pollen properties.

The genus *Cuscuta* is represented in the flora of Turkey

by 16 species (Davis, 1978). A large majority of the species belonging to *Cuscuta* L. genus attacks more than a host and cause significant yield losses. Identification at morphological level of these species are very important in terms of their control. The aim of this study is to illustrate the range of variability in pollen characters in some taxa of *Cuscuta* found in Turkey in order to establish their availability for future taxonomic works.

## Materials and Methods

### Field Surveys and the Collecting the Samples

This study was carried out between the years 2013 to 2015 and pollen morphology of 15 taxa belonging to the genus *Cuscuta* L. were identified. The samples of *Cuscuta* spp. were collected from different geographical regions in Turkey as indicated in the Flora of Turkey (Davis, 1978) (Table 1).

The pollen ornamentation of this taxa was analyzed. Using Scanning Electron Microscopy (SEM) were analyzed pollen ornamentation of this taxa. Pollen slides were prepared using the technique of Jones and Pucci (2012), Pinar and Inceoglu (1999) and Ridgway and Skyvarla (1969). For SEM, scanning electron microscope stubs were covered with double-stick tape. Dry pollen grains were placed onto the stubs and samples were stored for 24 hours in the hot room (28–30°C) conditions. After all the samples were prepared, the stubs were coated with a thin layer of gold by a Polaron SC 502 sputter coater. They were examined under the scanning electron microscope (Jeol JSM-840-A, Japan).

The size of the pollen was measured by polar axis and equatorial axis when detected from the equatorial view, and 30 pollen grains were measured per sample. Studied pollen was characterized in size as small (10–25 µm) or medium (25–50 µm) according to Erdtman (1952). The terminology used is mainly that Punt *et al.* (2007). Shape classification follows Erdtman (1969), based on P/E ratios in the Table. The minimum and maximum range values and standard deviations (STD) of the measured grains were determined.

## Results

Species of *Cuscuta* L. distributed in agricultural and non-agricultural areas in Turkey were studied palynological. Based on our findings the pollen of taxa of this genus are generally 3-zonocolpate. Pollen shape of this genus taxa are generally prolate, subprolate, prolate-spheroidal, oblate-spheroidal and peripolar. Among the examined taxa, the smallest pollen (14.9–26.1 × 12.7–16.1 µm) belongs to the *C. epithymum* L. while the largest pollen (32.3–37.6 × 26.8–32.7 µm) belongs to the *C. monogyna* Vahl. subsp. *esquamata* (Engelm.) Plitm. (Fig. 1).

### *C. campestris* Yunck

Pollen shape of this species; prolate, subprolate (P/E 1.19–

1.60), size; (24.7–28.1 × 17.220.8 µm), small. Colpus; 3 (-4) zonocolpate, colpus measurements; 17.5–24.8 × 1.83–3.9 µm, space between the colpus is granulosa. Exine; scabrate-perforate (Table 2; Fig. 1).

### *C. hyalina* Roth

Pollen shape of *C. hyalina*; prolate, (P/E 1.38–1.67), size; (27.6–29.4 × 17.2–20.4 µm). Colpus; 3-zonocolpate, colpus measurements; 22.8–25.7 × 2.11–2.79 µm. Exine; scabrate (Table 2; Fig. 1).

### *C. lupuliformis* Krock

Pollen form of this taxon; subprolate, prolate, oblate-spheroidal, (P/E 0.98–1.44), size; (28.5–37.6 × 24.7–28.9 µm) medium. Colpus; 3-zonocolpate, colpus measurements; 20.122.8 × 4.15–5.34 µm. Exine; reticulate (Table 2; Fig. 1).

### *C. monogyna* Vahl subsp. *monogyna*

Pollen shape of this taxon; prolate, (P/E 1.44), size; (32.8 × 22.7 µm) medium. Colpus; 3-zonocolpate, colpus measurements; 18.7–19.1 × 2.25–3.74 µm. Exine; echinate-reticulate (Table 2; Fig. 1).

### *C. monogyna* Vahl subsp. *esquamata* (Engelm.) Plitm

Pollen shape of this taxon; prolate, oblate-spheroidal, (P/E 0.99–1.40), size; (32.3–37.6 × 26.8–32.7 µm) medium. Colpus; 3-zonocolpate, colpus measurements; 24.3–25.2 × 1.66–6.90 µm. Exine; echinate-reticulate (Table 2; Fig. 1).

### *C. kotschyana* Boiss. subsp. *caudata* Bornm and Schwarz

Pollen shape of this taxon; prolate, (P/E 1.46–1.86), size; (24.8–30.3 × 17.1–18.4 µm). Colpus; 3-zonocolpate, colpus measurements; 25.1–28.1 × 1.59–2.27 µm. Exine; markedly scabrate (Table 2; Fig. 1).

### *C. babylonica* Aucher ex Choisy var. *babylonica*

Pollen shape of *C. babylonica* Aucher ex Choisy var. *babylonica*; prolate, (P/E 1.371.62), size; (19.1–26.2 × 12.8–16.6 µm) small. Colpus; 3-zonocolpate, colpus measurements; 14.6–21.4 × 1.41–2.06 µm, singlet. Exine; very distinctly scabrate (Table 2; Fig. 1).

### *C. babylonica* Aucher ex Choisy var. *elegans* (Boiss. and Bal.) Englm

Pollen shape of this taxon; prolate, (P/E 1.37–1.62), size; (19.1–26.2 × 12.8–16.6 µm) small. Colpus; 3-zonocolpate, colpus measurements; 14.6–21.4 × 1.41–2.06 µm. Exine; scabrate (Table 2; Fig. 1).

**Table 1:** Geographic distribution of 15 taxa belonging to the genus *Cuscuta* L., and the person who added a specimen into the collection

Taxon	Collection Site	Collector
<i>Cuscuta campestris</i>	Van/Gevaş	Ibrahim Demir
<i>C. hyalina</i>	Bitlis/Çeltiklisu	Ibrahim Demir
<i>C. lupuliformis</i>	Hakkari/Zap Valley	Ibrahim Demir
<i>C. monogyna</i> subsp. <i>monogyna</i>	Bitlis/Hizan	Ibrahim Demir
<i>C. monogyna</i> subsp. <i>esquamata</i>	Erzurum/Uzundere	Muzaffer Mükemre
<i>C. kurdica</i>	Bitlis/Nemrut Mountain	Murat Ünal
<i>C. kotschyana</i> subsp. <i>caudate</i>	Kayseri/Hınzır Dağı	N.Çelik & B.Yıldız
<i>C. babylonica</i> var. <i>babylonica</i>	Van/Gürpınar	Murat Unal
<i>C. babylonica</i> var. <i>elegans</i>	Gümüşhane	Murat Unal
<i>C. europaea</i>	Bitlis/Hizan	Ibrahim Demir
<i>C. epithymum</i>	Izmir/Kemalpaşa	Ilhan Kaya & Ibrahim; Demir
<i>C. brevistyla</i>	Bitlis/Hizan	Ibrahim Demir
<i>C. planiflora</i>	Mardin/Yeşilli	Ibrahim Demir
<i>C. approximata</i> var. <i>approximata</i>	Van/Hoşap	Ibrahim Demir
<i>C. approximata</i> var. <i>macranthera</i>	Izmir/Seyirtepe	Ilhan Kaya & Ibrahim; Demir

**Table 2:** Pollen morphological characteristics of the examined taxa

Taxa	Polar lenght in $\mu\text{m}$ $\pm\text{STD}$	Equatorial lenght in $\mu\text{m}$ $\pm\text{STD}$	P/E ratio	Colpus length in $\mu\text{m}$ $\pm\text{STD}$	Number of Colpat	Ornamentation
<i>Cuscuta campestris</i>	26.2(24.7-28.1) $\pm$ 1.7	19.0(17.2-20.8) $\pm$ 1.5	1.37	21.0(17.5-24.8) $\pm$ 2.5	3 (-4)	Scabrate-Perforate
<i>C. hyaline</i>	28.5(27.6-29.4) $\pm$ 0.7	18.8(17.2-20.4) $\pm$ 1.3	1.51	24.2(22.8-25.7) $\pm$ 1.4	3	Scabrate
<i>C. kotschyana</i> subsp. <i>caudate</i>	28.8(24.8-30.3) $\pm$ 2.5	17.9(17.1-18.4) $\pm$ 0.5	1.59	26.5(25.1-28.1) $\pm$ 1.5	3	Scabrate
<i>C. babylonica</i> var. <i>babylonica</i>	29.2(19.1-26.2) $\pm$ 6.0	17.7(12.8-16.6) $\pm$ 2.5	1.65	18.6(14.6-21.4) $\pm$ 2.8	3	Scabrate
<i>C. babylonica</i> var. <i>elegans</i>	20.8(19.7-21.2) $\pm$ 1.0	15.2(12.7-17.7) $\pm$ 3.5	1.34	20.8(18.7-22.9) $\pm$ 2.9	3	Scabrate
<i>C. europaea</i>	23.2(21.6-26.3) $\pm$ 2.7	17.5(16.9-18.4) $\pm$ 0.7	1.32	19.7(16.2-21.2) $\pm$ 2.0	3	Scabrate
<i>C. kurdica</i>	27.5(21.4-30.7) $\pm$ 3.6	17.6(17.1-18.4) $\pm$ 0.6	1.56	21.7(15.7-23.9) $\pm$ 3.5	3	Scabrate
<i>C. epithymum</i>	20.1(14.9-26.1) $\pm$ 5.6	14.0(12.7-16.1) $\pm$ 5.6	1.43	16.0(10.4-22.2) $\pm$ 5.9	3	Scabrate
<i>C. brevistyla</i>	22.4(21.7-23.2) $\pm$ 1.06	17.7(17.5-18.0) $\pm$ 0.3	1.26	15.8(11.2-20.5) $\pm$ 6.5	3 (-4)	Scabrate
<i>C. planiflora</i>	23.6(22.6-27.5) $\pm$ 4.1	15.3(12.3-19.4) $\pm$ 3.6	1.54	15.7(13.8-17.6) $\pm$ 2.6	3 (-6)	Scabrate
<i>C. approximata</i> var. <i>approximate</i>	22.4(19.8-24.5) $\pm$ 1.6	13.4(11.6-7.5) $\pm$ 1.8	1.66	16.9(13.4-20.7) $\pm$ 2.4	3	Scabrate
<i>C. approximata</i> var. <i>macranthera</i>	25.0(21.1-27.4) $\pm$ 2.7	18.1(15.7-20.4) $\pm$ 2.1	1.37	20.7(18.1-21.9) $\pm$ 1.5	3	Scabrate-Perforate
<i>C. lupuliformis</i>	32.7(28.5-37.6) $\pm$ 4.1	26.1(24.7-28.9) $\pm$ 2.0	1.25	21.5(20.1-22.8) $\pm$ 1.3	3	Reticulate
<i>C. monogyna</i> subsp. <i>monogyna</i>	32.8(31.8-34.2) $\pm$ 1.2	22.7(21.9-33.8) $\pm$ 0.9	1.44	19.3(18.7-19.1) $\pm$ 0.7	3	Echinate-Reticulate
<i>C. monogyna</i> subsp. <i>esquamata</i>	34.9(32.3-37.6) $\pm$ 3.7	29.7(26.8-32.7) $\pm$ 4.1	1.17	24.7(24.3-25.2) $\pm$ 0.6	3	Echinate-Reticulate

***C. europaea* L**

Pollen form of *C. europaea*; prolate, subprolate, (P/E 1.18–1.55), size; (21.6–26.3  $\times$  16.9  $\mu\text{m}$ ) small. Colpus; 3-zonocolpate, colpus measurements; 16.2–21.2  $\times$  2.65–2.75  $\mu\text{m}$ . Exine; scabrate (Table 2; Fig. 1).

***C. kurdica* Engelm**

Pollen shape of this species; prolate, subprolate, (P/E 1.26–1.79), size; (21.4–30.7  $\times$  17.1  $\mu\text{m}$ ). Colpus; 3-zonocolpate, colpus measurements; 15.7–23.9  $\times$  1.55–3.05  $\mu\text{m}$ , singlet. Exine; markedly scabrate (Table 2; Fig. 1).

***C. epithymum* L**

Pollen shape of *C. epithymum* pollen; subprolate, perprolate, prolate-spheroidal (P/E 1.12–1.04), size; (14.9–26.1  $\times$  12.7–16.1  $\mu\text{m}$ ). Colpus; 3-zonocolpate, colpus measurements; 10.4–22.2  $\times$  1.21–3.37  $\mu\text{m}$ . The exine is scabrate (Table 2, Fig. 1).

***C. brevistyla* A. Braun ex A. Rich**

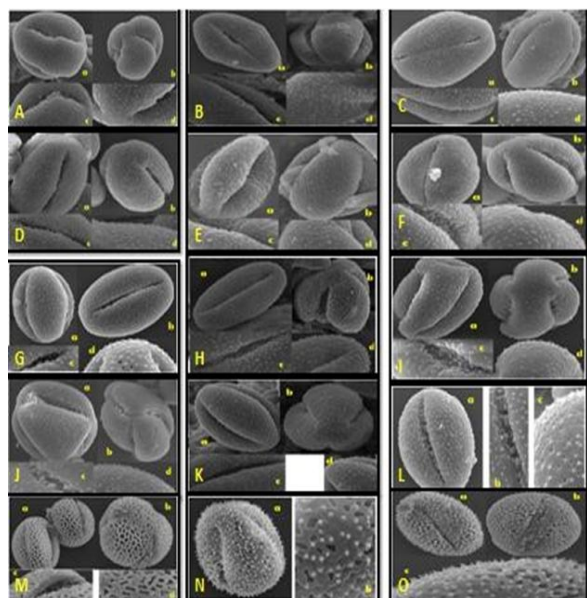
Pollen shape of this taxon; subprolate, (P/E 1.26), size; (22.4  $\times$  17.7  $\mu\text{m}$ ). Colpus; 3 (-4) zonocolpate, colpus measurements; 11.2–20.5  $\times$  1.12–2.56  $\mu\text{m}$ . Exine; scabrate (Table 2; Fig. 1).

***C. planiflora* Ten**

Pollen shape of this taxon; prolate, prolate-spheroidal, (P/E 1.54), size; (23.6  $\times$  15.3  $\mu\text{m}$ ) small, Colpus; 3 (-6) zonocolpate, colpus measurements; 13.8–17.6  $\times$  1.19–1.41  $\mu\text{m}$ . Exine; scabrate (Table 2; Fig. 1).

***C. approximata* Bab. var. *approximata***

Pollen form of *C. approximata* Bab. var. *approximata* taxon; subprolate, prolate (P/E 1.32–1.94), size; (19.8–24.5  $\times$  11.6–17.5  $\mu\text{m}$ ). Colpus; 3-zonocolpate, colpus measurements; 13.4–20.7  $\times$  1.13–2.28  $\mu\text{m}$ . Exine; scabrate (Table 2; Fig. 1).



**Fig. 1:** SEM pollen images of *Cuscuta* taxa a: equatorial view, b: polar view, c: colpus, d: ornamentation A. *Cuscuta campestris*, B. *C. hyalina*, C. *C. kotschyana* subsp. *caudata*, D. *C. babylonica* var. *babylonica*, E. *C. babylonica* var. *elegans*, F. *C. europaea*, G. *C. kurdica*, H. *C. epithymum*, I. *C. brevistyla*, J. *C. planiflora*, K. *C. approximata* var. *approximata*, L. *C. approximata* var. *macranthera*, M. *C. lupuliformis*, N. *C. monogyna* subsp. *monogyna*, O. *C. monogyna* subsp. *Esquamata*

#### ***C. approximata* Bab. var. *macranthera* (Boiss.)**

Feinbr. & Greuter Pollen shape of this taxon; subprolate, prolate (P/E 1.29–1.56), size; (21.1–27.4 × 15.7 pm). Colpus; 3-zonocolpate, colpus measurements; 18.1–21.9 × 1.75–2.72 pm. Exine; scabrate-perforate (Table 2; Fig. 1).

#### **Discussion**

In the present study, the pollen of 15 taxa belonging to the genus *Cuscuta* L. were examined using a scanning electron microscope. The morphological pollen structures were found different from each other and these properties were found to be important for taxonomy of *Cuscuta* L. (Table 2). The genus is extremely variable in their pollen characters (Perveen and Qaiser, 2004). Twelve (80%) of the examined taxa's pollen were 3-zonocolpate. *C. campestris* and *C. brevistyla* species were found 3 (-4) zonocolpate, *C. planiflora* 3 (-6) zonocolpate. Welsh *et al.* (2010) found that the pollen structures of *Cuscuta* genus were heteromorphic and almost 95% of the studied species were 3-zonocolpate. But they have indicated that this prevalent apertural type may be accompanied in the same anther by a small portion of 4-, 5- or even 6-zonocolpate grains, and extremely rarely by pantocolpate grains. Sengupta (1972), in the study of

Convolvulaceae family on pollen morphology, determined that 19 pollen grains belonging to the genus *Cuscuta* L. were generally 3-zonocolpate. Liao *et al.* (2005) were found that pollen of *Cuscuta* in Taiwan colpate, zonocolpate or pantocolpate using electron microscopy. *C. campestris* is a dodder common worldwide (Parker and Riches, 1993). The characteristics of pollen of *C. campestris* can be easily distinguished from those of the pollen of other species in *Cuscuta*. The pollen of *C. campestris* are pantocolpate, the other pollen are zonocolpate (Liao *et al.*, 2005).

Yuncker (1932) separated the genus *Cuscuta* into three subgenera; *Grammica*, *Cuscuta* and *Monogyna*. According to the results of this study, it was determined that pollen ornamentation of *Grammica* and *Cuscuta* subgenus were generally scabrate, and pollen ornamentation of *Monogyna* subgenus were reticulate, reticulate-echinate. In their study by Perveen and Qaiser (2004), it was determined that the ornamentation of 11 species of *Cuscuta* L. genus is reticulate, scabrate or punctate-scabrate. Our results support this hypothesis, because apertures of the pollens of these taxa were found scabrate, scabrate-perforate, oscarate-perforate, reticulate and echinate-reticulate. Welsh *et al.* (2010), stated that pollen with reticulate tectum is unknown in other Convolvulaceae and has evolved in *Cuscuta* only some species of subgenus *Monogyna* and *Grammica*.

A majority of the examined samples were characterized as prolate, prolate-subprolate but also there were oblate, perprolate, prolate-spheroidal and oblate-spheroidal shapes. Welsh *et al.* (2010) found that pollen forms of *Cuscuta* L. genus were polymorphic. Some studies have shown that pollen shapes of *Cuscuta* species are oblate, suboblate, subprolate, spheroidal, blate-spheroidal, and prolate-spheroidal (Liao *et al.*, 2000; Perveen and Qaiser, 2004; Welsh *et al.*, 2010).

In conclusion, the pollen morphology can help distinguishing the taxons, which have morphologically similar taxonomic characters as *C. brevistyla* A. Braun ex A. Rich and *C. planiflora* Ten. It was shown that two species can easily be separated from one another due to the differences of pollen properties (Table 2, Fig. 1). The characters used in the morphological differentiation of *C. lupuliformis* and *C. monogyna* species are very close to each other. The most important morphological features used in the separation of these two species are the staminal bracts (flakes). These structures are often not visible, or even if these structures are visible, it is often difficult to distinguish, which one they belong to. However, it is seen that exine ornamentation of these two species which is pollen morphological features is very different from each other (Fig. 1).

Pollen morphologic characters may show differences in species differentiation, as well as in sub specific taxa (subspecies, varieties). For example, it was determined in this study that pollen structures of subspecies *C. babylonica* Aucher ex Choisy var. *babylonica* and *C. babylonica* Aucher ex Choisy var. *elegans* (Boiss. & Bal.) Englm. were at least different (Table 2; Fig. 1).

Studies on the identification of pollen morphology of the genus *Cuscuta* in the world are very limited. In Turkey, there aren't any studies related to the subject. We believe that pollen characters of species belonging to the genus *Cuscuta* in future species-level taxonomic revisions will benefit enormously.

## Conclusion

Pollen characteristics of 15 taxa from genus *Cuscuta* were analyzed by scanning electron microscope (SEM). The size of the pollen was measured by polar axis and equatorial axis when detected from the equatorial view. The pollens of these taxa are generally identified as 3-zonocolpate. Pollen shape of this genus are generally prolate, subprolate, prolate-spheroidal, oblate-spheroidal and periporate. Among the examined taxa, the smallest pollen belonged to *C. epithymum* while the largest pollen belongs to *C. monogyna* subsp. *esquamata* (Engelm.) Plitm. Some of the pollen morphological parameters such as size and exine can be used to distinguish different *Cuscuta* taxa.

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