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The fruit morphology of some *Xanthium* species

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Abstract

Xanthium species are widespread weed species, in many countries characterized as invasive. Due to the tendency for occasional cross-pollination (less than 12%) among subspecies, many variations arise, which often result in small, local, but unstable taxa so identification could be very problematic. In this research, based on morphological characteristics, two species of the genus *Xanthium* were identified, in Bosnia and Herzegovina: *X. orientale* L. and *X. spinosum* L. Within the species *X. orientale* L., two subspecies were identified: *X. orientale* L. subsp. *italicum* (Moretti) Greuter and *X. orientale* L. subsp. *riparium* (Čelak.) Greuter. Morphological characteristics of the fruit as size, weight, and morphology of the fruit/seed coat can be very helpful for the identification of species or lower taxonomic categories. Morphological characteristics of the fruit (fruit length, length of apical beaks, fruit width with and without lateral beaks and mass of fruit) were recorded. Among the observed subspecies *X. orientale* L. subsp. *italicum* and *X. orientale* L. subsp. *riparium*, morphological differences are noticeable primarily in the appearance of the lateral and the apical beaks of the fruit.

***Key words*:** weed, *Xanthium*, identification, morphological characteristics of fruit

INTRODUCTION

Species of the *Xanthium* genus (family Asteraceae) are significant weed species with a broad distribution, both globally and in Bosnia and Herzegovina (BiH). In many countries, they are economically important weed species and, in some cases, classified as invasive species (Sudhakar et al., 2007; Anastasiu et al., 2007; Khuroo et al., 2007; Galanos, 2015, Vrbničanin et al., 2020). *Xanthium* species are present as weeds in maize (Hussain et al., 2014), wheat (Novák et al., 2009; Pascu, 2012), sunflower (Manilov & Zhalnov, 2018), potato (Ilić & Nikolić, 2011), tomato (Monaco et al., 1981), cotton (Economou et al., 2005), soybean (Sikkema et al., 2008), orchards and vineyards (Kelečević et al., 2020), and ruderal areas (Seifu et al., 2017). At a density of 16 plants per m^2, this species caused up to 54% reduction in maize grain number per ear, whereas grain weight was reduced by 33-40% (Karimmojeni et al., 2009). *X. spinosum* is a weed of vineyard, ruderal area as a pastures, croplands, waterways, grasslands, open woodlands, floodplains, waste areas, roadsides and disturbed sites in sub-tropical and sometimes also tropical, semi-arid, arid environments and temperate areas (Pasiecznik, 2022).

Species of this genus are divided into two sections: *Xanthium* (*Euxanthium* DC.) and *Acanthoxanthium* DC. Within the territory of BiH, one species, *X. spinosum*, is described within the *Acanthoxanthium* section. While a larger number of species and subspecies are described within the *Xanthium* section, with a significant number of synonyms (Greuter, 2006+). Due to the tendency for self-pollination with occasional cross-pollination (less than 12%), many variations arise, often resulting in small, local, but unstable taxa (Love & Dansereau, 1959). Consequently, the determination of species within this genus

is very complex and debatable, ultimately leading to the existence of numerous taxa with a large number of synonyms (Greuter, 2006+). Apart from the morphological characteristics of the plant, some authors have collected and described the fruits in an attempt to determine the precise systematization, especially of the lower systematic categories (Shull, 1915). Seed characteristics such as size, weight, and morphology of the seed coat can be very helpful for the identification of species or genera (Davitashvili & Karrerse, 2010; Gabr, 2014).

The fruit of *Xanthium* species is achene, with a distinctive appearance featuring highly developed apical beaks and a large number of lateral beaks, which are bent at the top, allowing for efficient dispersion. Additionally, the fruit and projections are covered with trichomes and glandular hairs, giving the fruit a characteristic odor. The fruit is divided into upper and lower parts, each containing one seed. The lowerseed (larger) is non-dormant and has a very high germination percentage, unlike the upper seed (smaller), which does not germinate even at optimal temperatures and delays germination for a longer period (Shitaka & Hirose, 1993). Although this fruit structure is characteristic of all *Xanthium* species, biotypes of *X. strumarium* have been recorded to form multi-seeded fruits, with up to 25 seeds in one fruit (Abbas et al., 1999).

As seed characteristics such as size, weight, and morphology of the seed coat can be very helpful for the identification of species or genera, the objective of this research was to morphologically describe the fruit (fruit length, length of apical beaks, fruit width with and without lateral beaks and mass of fruit) of recorded *Xanthium* species, as well as to identify differences between measured parameters of fruit recorded species.

MATERIAL AND METHODS

The fruits were collected from 20 populations across the entire territory of Bosnia and Herzegovina (Table 1).

Table 1. Localities collected *Xanthium* populations

Population	Latitude (N)	Longitude (E)	Elevation (m a.s.l.)	Habitat
		X. orientale		
Agrofin	42°40'14,3"	18°19'38,0"	272	vineyard
Aleksandrovac 1	44°58'09,7"	17°18'25,9"	120	roadside
Aleksandrovac 2	44°58'09,7"	17°18'25,9"	120	ruderal area
Balatun	44°52'57,1"	19°19'56,9"	81	roadside
Bardača	45°05'22,5"	17°26'32,4"	81	ditch
Berek	45°02'35,5"	17°14'30,6"	104	maize field
Cerovljani	45°02'29,4"	17°15'24,1"	103	maize field
Dolgodi 1	43°52'03,3"	18°17'01,5"	486	wasteland
Dolgodi 2	43°51'41,5"	18°17'41,4"	483	maize field
Domanovići	43°08'13,2"	17°47'01,5"	144	vineyard
Gorica	42°42'54,8"	18°21'05,6"	285	riverside
Lončari	44°56'44,7"	18°39'50,7"	85	maize field
Lukavac	45°04'19,1"	17°12'44,0"	104	maize field
Mašići	45°01'43,6	17°15'57,6"	103	maize field
Popovo polje	42°39'54,2"	18°19'33,0"	270	vineyard
Seferovci	44°59'52,9"	17°20'59,9"	106	ruderall area
Velino Selo	44°53'17,6"	19°19'28,2"	82	maize field
Vilusi	45°00'30,0"	17°16'53,0"	108	maize field
Volujac	42°41'06,9"	18°19'25,0"	269	vineyard
		X. spinosum		
Trebinje	42°41'06,9"	18°19'25,0"	269	vineyard

The nomenclature and determination of plant material were conducted using modern systematic principles according to Király (2009) and Greuter (2006+). Determination was based on the morphological characteristics of the collected plant material, as well as the appearance of the fruit (arrangement and shape of lateral and apical protrusions on the fruit). Since fruit mass better reflects the development (or general condition) of plants than the number of fruits per plant, the average fruit mass was measured as a biometric indicator of plant development. Morphological characteristics of the fruit were monitored through the following parameters: fruit length (with apical beak) (cm), length of apical beaks (cm), fruit width with and without lateral beaks (cm) and fruit mass (g). Within each population, 20 fruits were measured in four repetitions.

The statistical analysis of the obtained results was conducted using the statistical software Statistica 10.0. For all data, the mean value with the corresponding standard deviation was calculated. Determination of statistically significant differences between the observed characteristics was performed using analysis of variance (ANOVA). The significance of the observed differences in the observed characteristics was tested using the LSD test.

RESULTS AND DISCUSSION

Based on morphological characteristics, by the descriptions in regional floras and contemporary systematic principles on the investigated territory, two species of the genus *Xanthium* were identified*: X. orientale* L. and *X. spinosum* L. Within the species *X. orientale* L., two subspecies were identified: *X. orientale* L. subsp. *italicum* (Moretti) Greuter and *X. orientale* L. subsp. *riparium* (Čelak.) Greuter. A notable morphological characteristic, commonly used for the determination of lower systematic categories (subspecies) by numerous authors, is the morphological characteristics of the fruit (Shull, 1915; Beck et al., 1983; Király (ed.), 2009).

Description and morphological parameters of fruits for *X. orientale* L**.**

The seed is tightly enveloped by a fused involucre with a characteristic appearance, forming a fruit that, in botanical classification, represents an achene. The fruit contains two seeds that are surrounded by a robust covering, forming a compact unit (Figure 1), which is difficult to separate from the covering.

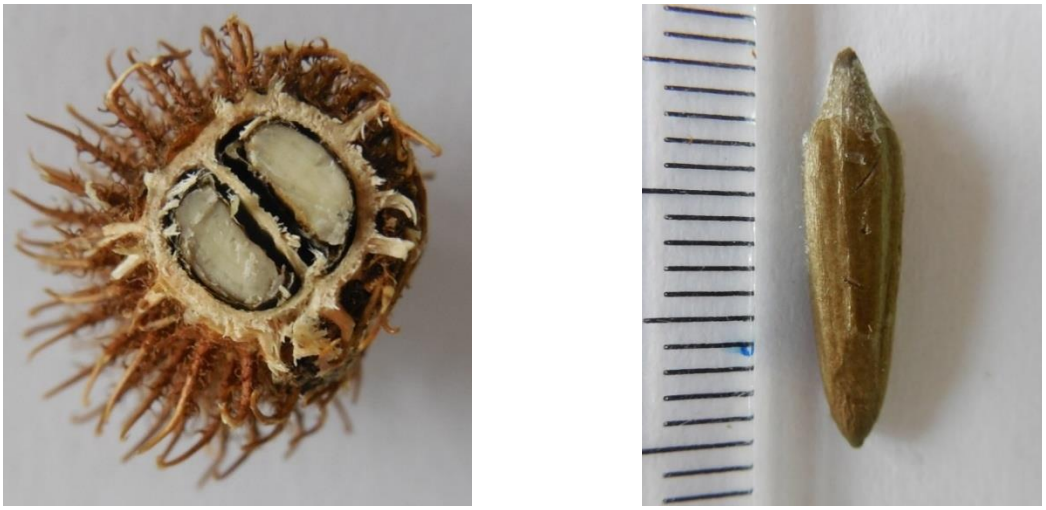

Figure 1. Fruit section and seed of *X. orientale*

The fruit of *Xanthium orientale* is ellipsoidal, with two straight or slightly inclined beaks converging towards each other. The entire fruit is moderately covered with spines that are hooked at the top. The unripe fruit is green with green or red-purple beaks (Figure 2), which is not a determining characteristic of the subspecies. In the mature state, the fruit covering is brownish-red to dark brown (Figure 3).

Figure 2. The unripe fruit of *X. orientale* L. subsp. *italicum*

The fruit, as well as the lower two-thirds of the spines, are covered with glandular hairs, giving the fruit an aromatic scent. According to Gajić (1975), glandular hairs were described as a determinant of the fruit of *X. strumarium* L. Still, they are not mentioned on the fruit of the species *X. italicum* Moretti (syn.) (accepted name - *X. orientale* subsp. *italicum* (Moretti) Greuter). In this study, glandular hairs were observed on the fruits of all identified taxons of the genus *Xanthium*, at *X. orientale* L. subsp. *italicum* (Moretti) Greuter, *X. orientale* L. subsp. *riparium* (Čelak.) Greuter. as at the *X. spinosum* L.

The difference between the subspecies is evident at the level of fruit morphology. The fruit of *X. orientale* subsp. *italicum* has denser lateral beaks, while the apical beaks are arched and bent towards each other (Figure 3a). The lateral beaks of *X. orientale* subsp. *riparium* is less frequent, and the apical beaks are straight and hooked at the top (Figure 3b).

a  b 

Figure 3. The mature fruit of *X. orientale* subsp. *italicum* (a) and *X. orientale* subsp. *riparium* (b)

The mean values of measured morphological fruit characteristics are provided in Table 2. For the *X. orientale* subsp. *italicum*, the fruit length ranged from 1.93 cm to 2.75 cm, the apical beaks length from 0.45 cm to 0.69 cm, the fruit width with beaks from 1.27 cm to 1.76 cm, and the fruit width without beaks from 0.61 cm to 0.96 cm. The fruit mass ranged from 0.20 g to 0.58 g. For the *X. orientale* subsp. *riparium*, the fruit length ranged from 2.47 cm to 2.67 cm, the apical beaks length from 0.60 cm to 0.67 cm, the fruit width with beaks from 1.69 cm to 1.81 cm, and the fruit width without beaks from 0.90 cm to 0.96 cm. The fruit mass ranged from 0.49 g to 0.72 g.

Table 2. Morphological parameters of the fruit for *X. orientale* subsp. *italicum* and *X. orientale* subsp. *riparium*

Population	Fruit length	Length of apical beaks	Fruit width with lateral beaks	Fruit width without lateral beaks	Fruit mass
	$\bar{X} \pm$ SD (cm)	$\bar{X} \pm$ SD (cm)	$\bar{X} \pm$ SD (cm)	$\bar{X} \pm$ SD (cm)	$\bar{X} \pm$ SD (g)
			X. orientale subsp. *Italicum*		
Agrofin	$2.36^{c,d,e,f} \pm 0.08$	$0.58^{c,d} \pm 0.06$	$1.48^{f,g} \pm 0.06$	$0.74^{e} \pm 0.04$	$0.27^{f,g,h,i,j} \pm 0.04$
Aleksandrovac 1	$2.26^{e,f\ g} \pm 0.20$	$0.57^{c,d} \pm 0.02$	$1.55^{d,e,f} \pm 0.06$	$0.69^{f,g,h} \pm 0.02$	$0.33^{e,f,g} \pm 0.03$
Aleksandrovac 2	$2.26^{e,f\ g} \pm 0.21$	$0.58^{c,d} \pm 0.06$	$1.55^{e,f} \pm 0.08$	$0.68^{g,h} \pm 0.03$	$0.42^{b,c} \pm 0.08$
Balatun	$2.27^{e,f\ g} \pm 0.05$	$0.60^{b,c} \pm 0.05$	$1.58^{c,d,e} \pm 0.09$	$0.77^{\ d,e} \pm 0.02$	$0.29^{f,g,h,i} \pm 0.06$
Bardača	$2.63^{a} \pm 0.05$	$0.56^{d} \pm 0.02$	$1.68^{a,b} \pm 0.02$	$0.85^{b,c} \pm 0.04$	$0.41^{b,c} \pm 0.07$
Berek	$2.06^{h} \pm 0.10$	$0.46^{g} \pm 0.02$	$1.30^{i} \pm 0.07$	$0.67^{h} \pm 0.06$	$0.24^{h,i,j} \pm 0.02$
Cerovljani	$2.22^{f,g} \pm 0.08$	$0.51^{e,f} \pm 0.03$	$1.38^{h,i} \pm 0.04$	$0.63^{h} \pm 0.02$	$0.22^{j} \pm 0.02$
Dolgodi 2	$2.22^{f,g} \pm 0.06$	$0.62^{a,b} \pm 0.02$	$1.33^{h,i} \pm 0.06$	$0.57^{i} \pm 0.03$	$0.23^{i,j} \pm 0.02$
Domanovići	$2.35^{c,d,e,f} \pm 0.04$	$0.51^{e,f} \pm 0.03$	$1.72^{a} \pm 0.02$	$0.85^{b,c} \pm 0.03$	$0.44^{b} \pm 0.08$
Gorica	$2.19^{g,h} \pm 0.10$	$0.51^{e,f} \pm 0.03$	$1.39^{g,h} \pm 0.07$	$0.74^{e} \pm 0.04$	$0.29^{f,g,h} \pm 0.01$
Lončari	$2.29^{d,e,f,\ g} \pm 0.07$	$0.60^{b,c} \pm 0.02$	$1.61^{b,c,d} \pm 0.06$	$0.80^{c,d} \pm 0.06$	$0.34^{d,e,f} \pm 0.05$
Lukavac	$2.38^{c,d,e} \pm 0.09$	$0.63^{a,b} \pm 0.01$	$1.69^{a,b} \pm 0.10$	$0.86^{b} \pm 0.06$	$0.39^{b,c,d} \pm 0.04$
Mašići	$2.46^{b,c} \pm 0.04$	$0.47^{f,g} \pm 0.02$	$1.48^{f} \pm 0.02$	$0.74^{e,f} \pm 0.02$	$0.32^{e,f,g} \pm 0.04$
Petrovo polje	$2.25^{e,f\ g} \pm 0.05$	$0.57^{c,d} \pm 0.03$	$1.62^{b,c} \pm 0.05$	$0.75^{d,e} \pm 0.03$	$0.28^{f,g,h,i} \pm 0.03$
Seferovci	$2.70^{a} \pm 0.08$	$0.63^{a,b} \pm 0.03$	$1.69^{a,b} \pm 0.07$	$0.88^{b} \pm 0.06$	$0.54^{a} \pm 0.03$
Velino Selo	$2,.43^{c,d} \pm 0.11$	$0.66^{a} \pm 0.02$	$1.67^{b,c} \pm 0.03$	$0.73^{e,f,g} \pm 0.02$	$0.37^{c,d,e} \pm 0.02$
Vilusi	$2.32^{c,d,e,f,\ g} \pm 0.04$	$0.58^{d} \pm 0.02$	$1.51^{e,f} \pm 0.02$	$0.73^{e,f} \pm 0.03$	$0.33^{e,f,g} \pm 0.02$
Volujac	$2.34^{c,d,e,f,\ g} \pm 0.16$	$0.54^{d,e} \pm 0.02$	$1.47^{f,g} \pm 0.05$	$0.76^{d,e} \pm 0.03$	$0.27^{g,h,i,j} \pm 0.04$
			X. orientale subsp. *riparium*		
Dolgodi 1	$2.59^{a,b} \pm 0.08$	$0.63^{a,b} \pm 0.03$	$1.73^{a} \pm 0.05$	$0.93^{a} \pm 0.03$	$0.61^{a} \pm 0.10$

Based on the statistical analysis of the measured data, the fruits of *X. orientale* subsp. *riparium* differed from those of *X. orientale* subsp. *italicum* only in fruit width with beaks, while there were no statistically significant differences for the other measured traits.

A significant morphological difference between the observed subspecies is noted in the appearance of the fruit covering, specifically the beaks on the fruit. In the fruit of *X. orientale* subsp. *italicum*, lateral beaks are denser, while the apical beaks are arched towards each other. In contrast, the lateral beaks of the fruit of *X. orientale* subsp. *riparium* is less frequent, and the apical beaks are straight with a hooked tip. The fruit length, as a morphological characteristic, is relatively consistent between subspecies in these studies. The measured morphological parameter values are higher compared to the literature data. The mean value of fruit length for *X. orientale* subsp. *italicum* was 2.33 cm, while in the study by Moran et al. (1981), the fruit length was significantly smaller, measuring 2.02 cm. Also, in this study, the fruit length of other species within the *Xanthium* section varied, ranging from 1.87 cm for *X. chinense*, 2.11 cm for *X. pennsylvanicum*, to 2.25 cm for *X. cavanillesii*.

The mean fruit mass was 0.45 g for *X. orientale* subsp. *italicum* and 0.67 g for *X. orientale* subsp. *riparium* (Table 2). In the study Moran et al. (1981), the fruit mass for *X. italicum* was significantly smaller, measuring 0.19 g, while for other species within this section, the fruit mass ranged from 0.16 g for *X. chinense*, 0.29 g for *X. pennsylvanicum*, to 0.42 g for *X. cavanillesii*. Since fruit mass is largely influenced by external environmental factors (Baskin & Baskin, 1998) and competitive relationships between plants (Senseman & Oliver, 1993), it follows that the number of formed fruits per plant is a highly variable characteristic and is not suitable for defining species or as a determinant of lower taxa

(subspecies, varieties) (Lutman, 2002). According to Gajić (1975), glandular hairs were described as a determinant of the fruit of *X. strumarium* L. Still, they are not mentioned on the fruit of the species *X. italicum* Moretti (syn.) (accepted name - *X. orientale* subsp. *italicum* (Moretti) Greuter). In this study, glandular hairs were observed on the fruits of all identified species of the genus *Xanthium*, confirming that all analyzed individuals belong to the species *X. orientale* L. (syn. *X. italicum* subsp. *italicum* (Moretti) D. Löve), which is in accordance with Flora Europaea (Love, 1976).

Description and morphological parameters of fruits for *X. spinosum* L.

Like the species *X. orientale*, the seeds of *X. spinosum* are tightly enveloped by fused involucral bracts, forming an achene. Each fruit contains two seeds. The fruit covering is brownish-yellow to dark brown, moderately covered with hooked, strongly curved brownish-yellow beaks at the top (Figure 4). The entire fruit is sparsely covered with white hairs and golden-yellow, small, glandular discs. Hairs and glandular discs are absent on the hooked spines but have been observed on the fruit covering. The fruit is ellipsoidal with two unequal, short, upright beaks at the top.

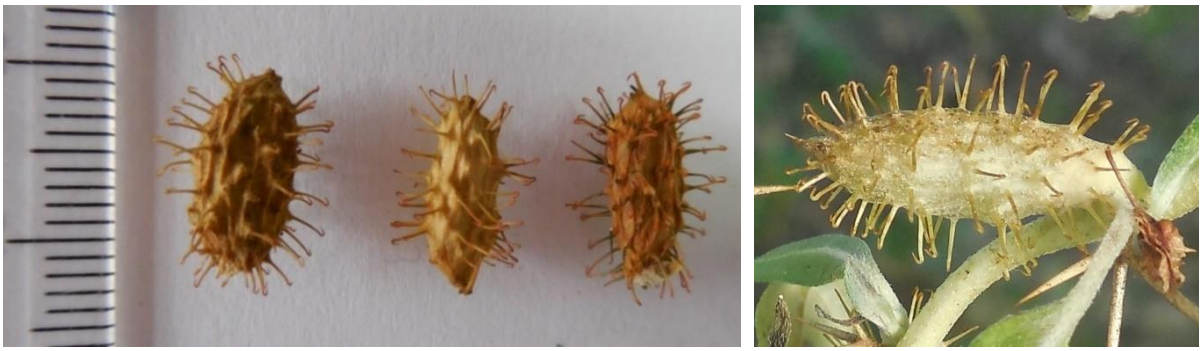

Figure 4. The fruit of *X. spinosum*

The length of the *X. spinosum* fruit ranged from 1.01 to 1.14 cm, the length of the apical beak from 0.20 to 0.24 cm, the width of the fruit with lateral beak from 0.69 to 0.81 cm, and the width without beaks from 0.46 to 0.50 cm. The fruits of this species are much smaller than those of *X. orientale*, with a fruit mass ranging from 0.01 to 0.02 g. The mean values of the morphometric characteristics of the fruit are presented in Table 3.

Table 3. Morphological parameters of the fruit for *X. spinosum* L.

Population	Fruit length	Length of apical beak	Fruit width with lateral beaks	Fruit width without lateral beaks	Fruit mass
	$\bar{X} \pm$ SD (cm)	$\bar{X} \pm$ SD (cm)	$\bar{X} \pm$ SD (cm)	$\bar{X} \pm$ SD (cm)	$\bar{X} \pm$ SD (g)
			X. spinosum L.		
Trebinje	1.07±0.05	0.24±0.04	0.76±0.05	0.48±0.01	0.02±0.00

CONCLUSION

Morphological differences between species belonging to the *Xanthium* and *Acanthoxanthium* sections are clear. However, among the observed subspecies (section *Xanthium*), morphological differences are noticeable primarily in the appearance of the lateral and the apical beaks of the fruit and it is very important characteristic for the determination of individuals, at the subspecies level. *X. orientale* subsp. *italicum* has denser lateral beaks, while the apical beaks are arching towards each other. The lateral beaks of *X. orientale* subsp. *riparium* is sparser, and the apical beaks are straight and strongly curved at the top. Glandular hairs, as well as trichomes, are present on the fruits of the identified species of the genus *Xanthium*. In species of the *Xanthium* section, glandular hairs are present on the fruit covering and the lower part of the lateral beaks, while in *X. spinosum*, they are only present on the fruit covering. The length of the fruit of the *X. orientale* subsp. *italicum* subspecies was 2.33 cm, the length of the apical beaks was 0.57 cm, the width of the fruit with beaks was 1.54 cm, the width of the fruit without beaks

was 0.74 cm, and the mass of the fruit was 0.45 g. The length of the fruit of the *X. orientale* subsp. *riparium* subspecies was 2.59 cm, the length of the apical beaks was 0.63 cm, the width of the fruit with beaks was 1.73 cm, the width of the fruit without beaks was 0.93 cm, and the mass of the fruit was 0.67 g. Statistical analysis of the measured morphological parameters of the fruit reveals a statistical difference between subspecies only for the width of the fruit width without lateral beaks. The average length of *X. spinosum* fruit was 1.07 cm, the length of the apical beaks was 0.24 cm, the width of the fruit with lateral beaks was 0.76 cm, and the width without beaks was 0.48 cm. The average mass of the fruit was 0.02 g.

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