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Seasonal variation of the agar quality and chemical composition of *Gracilaria veleroae* and *Gracilaria vermiculophylla* (Rhodophyceae, Gracilariaceae) from Baja California Sur, Mexico

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SUMMARY

Yield and physico-chemical properties of agar from *Gracilaria veleroae* E.Y. Dawson and *Gracilaria vermiculophylla* (Ohmi) Papenfuss were studied and the chemical composition of the two seaweeds was determined. Samples were collected seasonally from summer 2003 to spring 2005. The agar yield did not vary significantly between seasons for both species. The lowest agar gel strength was obtained from *G. veleroae* (207.5 g cm^{-2}) in summer 2003 and the highest from *G. vermiculophylla* (793.1 g cm^{-2}) in winter 2004. Melting temperatures and hysteresis were higher in *G. vermiculophylla*, whereas gelling temperatures and 3,6-anhydrogalactose content were higher for *G. veleroae*. Moisture, ash, crude fiber, and ether extract showed no significant seasonal variation for *G. veleroae*. The chemical composition of *G. vermiculophylla* showed significant seasonal variation. *G. vermiculophylla* possesses a better agar quality than *G. veleroae* and is a species that could be considered as a source of agar for commercial use.

Key words: agar, ash, crude fiber, gel strength, seasonal variation

INTRODUCTION

The exploitation of agarophytes represents an important commercial interest. In Mexico, the agar industry has depended mainly on *Gelidium*, however, 19 species of *Gracilaria* exist along the west coast of Mexico (Lobo-Niembro & Marcos-Ramírez 1988), whose chemical composition has not been studied. Of the 16 species of *Gracilaria* in the Gulf of California, seven are endemic (Norris 1985). Most research on *Gracilaria* from the Baja California Peninsula coast consists of taxonomic, descriptive, and prospective studies (Lobo-Niembro & Marcos-Ramírez 1988) and particularly for *G. veleroae* E.Y. Dawson in the Gulf of California, knowledge is limited to floristic (Mateo-Cid *et al*. 1993, 2000), taxonomic (Dawson 1946), and some general biological observations (Riosmena-Rodríguez, pers. comm., 2007). No reports exist either concerning the physical and chemical characteristics of the agar from this species, or the chemical composition of this alga.

Studies of the biology, ecology, and chemistry of *G. vermiculophylla* (Ohmi) Papenfuss (formerly *G. pacifica*) began in 1986 in the estuary of Punta Banda in the State of Baja California. In this area, Lobo-Niembro and Marcos-Ramírez (1988) reported the content of agar (15–25%), 3,6-anhydrogalactose (25–30%), and sulfate (<3%). In Laguna San Ignacio, the *Gracilaria* beds cover 60 ha, with a biomass of 5–15 kg m^{-2} (wet weight). The beds recover rapidly after harvest, with an estimated standing stock of 900 tons dry weight $year^{-1}$ (Zertuche-González 1993). Vergara-Rodarte (2009) estimated a total of 100 tons dry weight of *G. vermiculophylla* in spring in the same area, with an agar content of 12–17%.

Agar is a complex polysaccharide composed of repeating agarobiose units of alternating 1,3-linked D-galactose and 1,4-linked 3,6-anhydro-L-galactose residues (Lahaye & Yaphe 1988). The yield and quality of agar vary according to species and environmental factors related to algal growth, such as temperature, light, and nutrients (Armisén 1995). Numerous reports show the effects of season on agar properties of *Gracilaria*; however, results are contradictory (Lahaye & Yaphe 1988), partly due to different methods being used for extracting agar and also because the nature of seasonal changes varies geographically.

This study compared the seasonal differences in agar characteristics from *G. veleroae* from the Gulf of California and *G. vermiculophylla* from the Pacific coast. We measured the yield and physico-chemical

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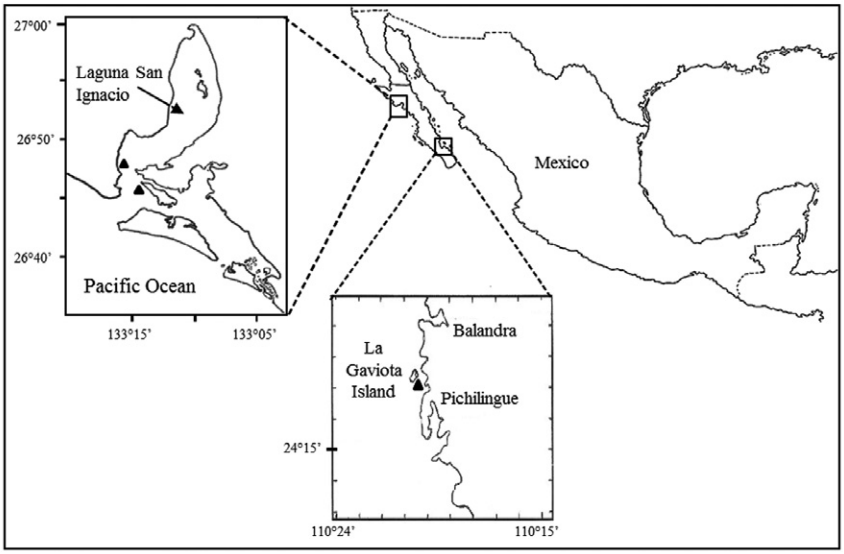

Fig. 1. Location of the sampling areas (▲). *Gracilaria vermiculophylla* collected in Laguna San Ignacio and *Gracilaria veleroae* collected in La Gaviota Island.

properties, as well as the chemical composition of both species, to determine whether they offered potential to become a commercial source of agar.

MATERIALS AND METHODS

Study areas

La Gaviota Island is located to the southeast of La Paz Bay in Baja California Sur (24°17′20″ N, 110°20′30″ W). The island is an exposed site with a pronounced slope and is characterized by steep crags and rocky platforms that extend almost to 50 m. The bottom thereafter, is sandy (Cruz-Ayala *et al*. 1998). The relative humidity is 50% during the day, causing a semi-desert climate; the mean annual rainfall is 180 mm and mean annual evaporation is 300 mm. There is only one rainy season from August to February; however, a second rainy season sometimes occurs in winter. The dominant winds are from the northwest in November to March and from the southeast in April to October (Jiménez-Illescas *et al*. 1997). The Laguna San Ignacio is located on the northwest coast of Baja California Sur (26°46′30″ N, 113°14′50″ W) and has an approximate area of 150 km^2. The length is 30 km and the width varies from 2 to 6 km. It is a shallow site, with a depth of only 2–4 m in most of the area, reaching 20 m in the channels that communicate with the ocean. The coast is composed of sandy beaches and rock-shell clusters. The climate is warm and very dry, with a mean annual sea water temperature between 18 and 22°C. The rainy season is mainly in winter, with a mean annual rainfall of 56 mm (Nuñez-López *et al*. 1998).

Sampling

Gracilaria veleroae was collected seasonally from summer 2003 to summer 2004, by scuba diving at a depth of 15 m at La Gaviota Island in Bahía de La Paz and similarly, *G. vermiculophylla* was collected from summer 2004 to spring 2005 from a depth of 3–5 m in the Laguna San Ignacio (Fig. 1). The algae of both species were sun-dried on the beach and transported to the laboratory where they were ground, passed through a 0.8 mm screen mesh and stored in plastic bags at room temperature for further analysis.

Agar extraction

Sample extraction was performed in triplicate basically according to the method of Freile-Pelegrín and Robledo (1997), with some modifications. In a preliminary study, the effect of sodium hydroxide on the yield and quality of agar in *G. veleroae* and *G. vermiculophylla* was tested and best results were obtained with concentrations of 3% and 7% sodium hydroxide, respectively, thus, these concentrations were used for the experiments described below. Each 25 g (dry weight) sample of *G. veleroae* and *G. vermiculophylla* was soaked overnight in 500 mL 3% or 7% NaOH solution, respectively, at room temperature (22°C). The algae were then heated in a water bath at 85°C for 3 h under constant agitation and the excess NaOH was removed by washing the algae three times with 500 mL distilled water for 5 min. Samples were then washed with 500 mL 0.025% (v/v) H_2SO_4 solution for 2 h under constant agitation and were again washed with 500 mL distilled water for 5 min. The agar was extracted in 900 mL hot distilled water under constant agitation. When the solution reached 80°C, the pH was adjusted to 6.2–6.5 with 10% phosphoric acid, and it was boiled for 1.5 h. The extract was mixed with diatomaceous earth and filtered under pressure, the filtrate was then allowed to gel at room temperature on plastic trays, then frozen overnight, and subsequently thawed to room temperature. Finally, the agar was dehydrated with ethanol, dried in an oven at 55°C for 24 h, cooled to room temperature, and weighed to calculate the percentage yield of agar.

Physical properties

To measure physical properties (gel strength and melting and gelling temperature), 1.5% of each agar solution (w/v) was prepared. Agar solutions were allowed to gel at room temperature (22°C) overnight in plastic containers (9 × 3 × 2.5 cm). Gel strength was measured using a modified Nikansui Shiki gel strength meter (Arvizu-Higuera *et al*. 2008). The melting temperature was measured by preparing the agar gel in a test tube (1.7 cm diameter, 15 cm height) and placing an iron ball (7 mm diameter) on the gel surface. The test tube was clamped in a water bath and the temperature gradually increased. The melting temperature was recorded with a precision thermocouple thermometer when the gel started to melt and the ball sank into the solution (Armisén & Galatas 1987). The gelling temperature was measured using this same solution, which was allowed to cool down while continuously moving the tube vertically and horizontally. The probe of the thermocouple thermometer was inserted into the solution when it ceased flowing, and the gelling temperature was recorded. Hysteresis was calculated as the difference between the melting and gelling temperatures (Armisén & Galatas 1987).

Chemical properties

The concentration of 3,6-anhydrogalactose (3,6-AG) was determined by the resorcinol-acetal method (Yaphe & Arsenault 1965) using D-fructose as the standard. Absorbance was measured at 555 nm.

Chemical composition

Moisture content was measured using the thermo balance method (AOAC 1990). Ashes were obtained after heating the algae in an electric furnace (AOAC 1990). Crude fiber was measured by acid and alkaline hydrolysis and ether extraction (Larsen 1978).

Statistical analyses

All extractions and analyses were performed in triplicate. Mean data were compared using analysis of variance (Kruskal–Wallis ANOVA) and standard error (SE) at the 95% significance level. Spearman rank correlation was also calculated for all measurements ($P < 0.05$). Calculations were performed using Statistica 7.0 software (StatSoft, Tulsa, OK, USA).

RESULTS

Agar

The lowest and highest yields for *G. veleroae* occurred in summer 2004 (9%) and autumn 2003 (12.1%), respectively. For *G. vermiculophylla*, the lowest yield also occurred in summer (9%) and the highest in winter (13.5%). For both species, yields did not vary significantly between seasons ($P > 0.05$) (Table 1). The physical properties of the two species varied significantly ($P < 0.05$). Agar gel strength was lowest in summer 2004 (524 g cm^{-2}) for *G. vermiculophylla* and in summer 2003 (207 g cm^{-2}) for *G. veleroae*. The highest gel strength occurred in winter for *G. vermiculophylla* (793 g cm^{-2}) and in spring for *G. veleroae* (343 g cm^{-2}) (Table 1). A low positive correlation was found between gel strength and agar yield ($r = 0.47$; $P < 0.05$) for *G. vermiculophylla*, whereas the correlation for *G. veleroae* was very close to zero ($r = -0.03$; $P < 0.05$) and therefore not significant (Table 2). The agar melting temperature for *G. vermiculophylla* ranged from 96.9°C (autumn) to 99.7°C (winter and spring) and for *G. veleroae* from 83.7°C (summer 2003) to 86°C (spring). For both species, the correlations between melting temperature and season, and between melting temperature and gel strength was significant (*G. veleroae* $r = 0.62$, $r = 0.53$; $P < 0.05$) (*G. vermiculophylla* $r = 0.79$, $r = 0.8$; $P < 0.05$). Gelling temperature of agar from *G. vermiculophylla* ranged from 35.2°C (summer) to 38.3°C (autumn and spring) and for *G. veleroae* from 41.5°C (summer 2003) to 46.3°C (summer 2004). Similarly, a significant positive correlation was found between gelling temperature and gel strength for *G. veleroae* ($r = 0.42$; $P < 0.05$) and *G. vermiculophylla* ($r = 0.23$; $P < 0.05$) (Table 2). Hysteresis was higher for *G. vermiculophylla* (58–62°C) than for *G. veleroae* (38–42°C) (Table 1).

Seasonal differences in the content of 3,6-anhydrogalactose in *G. vermiculophylla* were significantly different ($P < 0.05$) and were lowest in autumn (35.5%) and highest in winter (39.4%). In *G. veleroae*, the content was similar in all seasons (40.8–41.6%) (Table 1). There was no correlation between 3,6-anhydrogalactose content and gel strength for *G. veleroae* ($r = -0.01$; $P < 0.05$), whereas that for *G. vermiculophylla* was significant ($r = 0.79$; $P < 0.05$) (Table 2).

Chemical composition

The chemical composition of the two species of *Gracilaria* is shown in Table 3. Significant differences were found in moisture and ash content for both species ($P < 0.05$). A significant correlation between moisture and season was observed for *G. veleroae*

Table 1. Seasonal variation of physical and chemical properties of *Gracilaria veleroae* and *Gracilaria vermiculophylla* collected in Baja California Sur, Mexico. Values are the mean ± SE ($P < 0.05$) and Kruskal–Wallis ANOVA (H and probability)

Season	Yield (%)	Gel Strength (g cm^{-2})	Melting temperature (°C)	Gelling temperature (°C)	Hysteresis (°C)	3,6-AG (%)
Gracilaria veleroae						
Summer 03	11.0 ± 0.5	207.5 ± 11.3	83.7 ± 0.8	41.5 ± 1.3	42.2 ± 1.5	41.5 ± 2.4
Autumn 03	12.1 ± 0.6	255.7 ± 0.2	84.2 ± 0.5	44.0 ± 0.7	40.2 ± 0.8	41.4 ± 1.3
Winter 03	11.8 ± 0.5	297.1 ± 2.1	84.9 ± 0.8	45.4 ± 0.5	39.5 ± 1.3	40.8 ± 1.1
Spring 04	11.8 ± 0.5	343.5 ± 1.4	86.0 ± 0.9	43.9 ± 0.3	42.1 ± 0.8	41.6 ± 0.3
Summer 04	9.0 ± 0.5	292.1 ± 0.6	84.7 ± 0.1	46.3 ± 0.6	38.4 ± 0.6	ND
ANOVA						
H	7.911	21.746	11.602	14.678	17.946	1.126
Probability	0.094	0.000	0.020	0.005	0.001	0.770
Gracilaria vermiculophylla						
Summer 04	9.0 ± 0.5	524.0 ± 1.8	97.2 ± 0.3	35.2 ± 0.3	62.0 ± 0.4	36.3 ± 0.6
Autumn 04	9.8 ± 0.2	540.5 ± 3.0	96.9 ± 0.4	38.3 ± 0.4	58.6 ± 1.0	35.5 ± 0.4
Winter 04	13.5 ± 0.5	793.1 ± 7.2	99.7 ± 0.4	37.0 ± 0.2	62.7 ± 0.6	39.4 ± 1.8
Spring 05	11.4 ± 0.4	644.0 ± 49.0	99.7 ± 0.3	38.3 ± 0.6	61.4 ± 0.4	38.9 ± 0.7
ANOVA						
H	7.607	14.046	12.714	13.539	14.376	13.043
Probability	0.054	0.002	0.005	0.003	0.002	0.0045

Table 2. Correlation coefficients ($n = 3$) between yield, physical and chemical properties of agar from *Gracilaria veleroae* and *Gracilaria vermiculophylla*

	Yield	Gel Strength	Melting temperature	Gelling temperature	Hysteresis	3,6-AG
Gracilaria veleroae						
Season	0.11	0.79*	0.62*	0.52*	0.03	–0.04
Yield		–0.03	–0.15	–0.05	–0.15	0.12
Gel Strength			0.53*	0.58*	–0.19	–0.02
Melting temperature				0.70*	0.14	–0.16
Gelling temperature					–0.51*	–0.29
Hysteresis						0.21
Gracilaria vermiculophylla						
Season	0.34	0.75*	0.79*	0.56*	0.09	0.77*
Yield		0.47	0.47*	0.33	0.16	0.20
Gel Strength			0.80*	0.19	0.52*	0.79*
Melting temperature				0.33	0.49*	0.69*
Gelling temperature					–0.62*	0.08
Hysteresis						0.41

*Significant ($P < 0.05$).

(r = 0.86; $P < 0.05$) and a significant correlation between season and ash content was found for *G. vermiculophylla* (r = –0.92; $P < 0.05$) (Table 4). The mean crude fiber content for *G. veleroae* was higher than for *G. vermiculophylla* and for *G. vermiculophylla*, was significantly different ($P < 0.05$) between seasons, but not for *G. veleroae* ($P > 0.05$). The lowest value was 2.5% (autumn) for *G. vermiculophylla*, and the highest was 7.5% (spring) for *G. veleroae*. There was a significant correlation between ash and crude fiber content for both species (*G. vermiculophylla* r = –0.73; *G. veleroae* r = –0.65; $P < 0.05$) (Table 4). Ether extract in both species was significantly different between seasons ($P < 0.05$) (*G. vermiculophylla* 2.9–4.2%; *G. veleroae* 3.1–5%). For *G. vermiculophylla*, a significant correlation between ether extract and crude fiber (r = 0.78, $P < 0.05$) was found.

DISCUSSION

This is the first report concerning agar content from *G. veleroae*, a species that has received little attention to date. Agar yield obtained from *G. vermiculophylla* was lower than reported for this species in other

Table 3. Seasonal variation of chemical composition of *Gracilaria veleroae* and *Gracilaria vermiculophylla* collected in Baja California Sur, Mexico. Values are the mean ± SE ($P < 0.05$) and Kruskal–Wallis ANOVA (H and probability)

Season	Ash (%)	Moisture (%)	Crude fiber (%)	Ether extract (%)
Gracilaria veleroae				
Summer 03	35.17 ± 4.2	7.63 ± 0.4	6.49 ± 0.2	5.05 ± 1.4
Autumn 03	29.43 ± 3.2	6.77 ± 0.5	7.24 ± 0.7	3.22 ± 1.2
Winter 03	32.73 ± 1.1	6.64 ± 0.5	6.68 ± 0.2	3.06 ± 1.7
Spring 04	38.10 ± 2.3	5.79 ± 0.3	7.50 ± 2.2	3.71 ± 0.3
ANOVA				
H	20.082	10.323	5.152	8.550
Probability	0.0002	0.016	0.161	0.035
Gracilaria vermiculophylla				
Summer 04	37.97 ± 0.4	7.76 ± 0.5	3.42 ± 0.4	3.75 ± 0.1
Autumn 04	31.11 ± 5.0	11.41 ± 0.5	2.50 ± 0.8	2.91 ± 0.7
Winter 04	8.23 ± 0.0	7.88 ± 0.1	4.81 ± 0.0	4.25 ± 0.1
ANOVA				
H	8.336	9.378	6.291	9.346
Probability	0.015	0.009	0.043	0.009

Table 4. Correlation coefficients ($n = 3$) of the chemical composition from *Gracilaria veleroae* and *Gracilaria vermiculophylla*

	Moisture	Ash	Crude fiber	Ether extract
Gracilaria veleroae				
Season	–0.86*	–0.12	0.25	–0.40
Moisture		0.09	–0.31	0.55*
Ash			–0.65*	0.01
Crude fiber				–0.34
Gracilaria vermiculophylla				
Season	0.00	–0.92*	0.58*	0.38
Moisture		–0.07	–0.58*	–0.67*
Ash			–0.73*	–0.50
Crude fiber				0.78*

*Significant ($P < 0.05$).

localities (Orduña-Rojas *et al.*, 2008); Villanueva *et al.* 2010), but similar to that reported by other authors (Arvizu-Higuera *et al.* 2008; Sousa *et al.* 2010; Vergara-Rodarte *et al.* 2009).

In winter, the gel strength of *G. vermiculophylla* agar (793 g cm^{-2}), was similar to the standard (700 g cm^{-2}) for some agar producers (AgarGel 2011) and indicates its potential for use in the food industry, whereas agar from *G. veleroae* had a lower strength. In general, the gel strength of *G. vermiculophylla* was greater than values reported by Orduña-Rojas *et al.* (2008) and Mollet *et al.* (1998), and similar to, or lower than that in other reports (Arvizu-Higuera *et al.* 2008; Sousa *et al.* 2010; Villanueva *et al.* 2010). Gel strength in *G. veleroae* was comparable to those reported for other agarophytes, such as *Gracilaria verrucosa* and *G. salicornia* (Oyieke 1993), *G. gracilis* (Mollet *et al.* 1998), *G. arcuata* (Montaño *et al.* 1999), *G. eucheumatoides* (Romero *et al.* 2008), and *Gracilariopsis longissima* (Orduña-Rojas *et al.* 2008). A significant correlation was observed for both species between the gel strength and season: the highest gel strength occurred in winter for *G. vermiculophylla*, in contrast to that for *G. veleroae*, which occurred in spring, possibly in response to environmental conditions (Christiaen *et al.* 1987). Gel strength is species-specific, irrespective of whether the alga is collected from different sites, as in this study, or from the same site, as reported by Marinho-Soriano and Bourret (2003).

Melting temperature, gelling temperature and hysteresis in both species were greater than for commercial agar (Pereira-Pacheco *et al.* 2007). For *G. vermiculophylla*, there was a significant seasonal variation in melting temperature, gelling temperature, and hysteresis, but there were no significant changes during the year for *G. veleroae*. An inverse relationship between gel strength and gelling temperature was also shown by Pondevida and Hurtado-Ponce (1996). The differences in the molecular structure and molecular weight of agar in agarophytes leads to diversity in gelling properties, even under the same extraction conditions (Murano 1995).

For *G. vermiculophylla*, a significant correlation existed between 3,6-anhydrogalactose and the season, gel strength or melting temperature, which agrees with results for *Gracilaria* species from different geographical origins (Rebello *et al*. 1997).

The chemical composition of *Gracilaria* species in many countries has been studied; however, in Mexico, only Caribbean species have been investigated (Robledo & Freile-Pelegrín 1997; Orduña-Rojas *et al*. 2002) and no information is available regarding the chemical composition of *G. veleroae*.

The moisture content is dependent on the drying method used, which is important, because a significant loss of nutritional value occurs if the alga is not properly dried (<20%) (Armisén 1995). In this study, moisture content was always lower than 12%.

Ash content in *Gracilaria* species ranges from 3.6% to 53.4% (Robledo & Freile-Pelegrín 1997; Norziah & Ching 2000; Orduña-Rojas *et al*. 2002; Marinho-Soriano *et al*. 2006; Renaud & Luong-Van 2006; Wen *et al*. 2006; Gressler *et al*. 2010; Rohani-Ghadikolaei *et al*. 2011). The variation observed in ash content of both species in this study might be influenced by geographic origin and season (Ferreira-Patarra *et al*. 2011); however, values were within the published range.

The crude fiber content in both species was similar to that of *Gracilaria cornea* (5.21%) (Robledo & Freile-Pelegrín 1997) and *Gracilaria cervicornis* (5.65%) (Marinho-Soriano *et al*. 2006), but much lower than that of *Gracilaria changgi* (24.7%) (Norziah & Ching 2000). In general, the amount of ether extract in seaweeds is low; as meaningful comparisons can only be made with studies that use similar procedures to obtain the extract (McDermid & Struercke 2003), our results were compared with those obtained by similar methods in other *Gracilaria* species, where the ether extract content ranged from 0.26–3.30% (Robledo & Freile-Pelegrín 1997; Norziah & Ching 2000; Marinho-Soriano *et al*. 2006; Valente *et al*. 2006).

In summary, our findings suggest that agar content and physico-chemical characteristics of *G. vermiculophylla* can be used to produce food-grade agar (Grade 3) (Marine Chemicals 2011). This species is abundant and can be harvested mainly in spring when highest biomass is reached (100 tons dry weight) (Vergara-Rodarte 2009), and high gel strength and agar yield are shown. In Baja California Sur, *G. vermiculophylla* is a further agarophyte suitable for exploitation, after *Gelidium robustum*, with a sustainable approach and conservation strategy.

For *G. veleroae*, the gel strength is lower than commercial standards for agar; however, many food-grade agars are produced with seaweeds of the genus *Gracilaria* or *Gelidiella* with gel strength between 300 and 500 g cm^{-2} (Myco Supply 2011).

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