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Original Article

Identity Characteristics of Three Guatemalan Edible and Medicinal Species

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Abstract

The diagnostic characteristics for the correct identification of *Fernaldia pandurata*, *Cucurbita argyrosperma* and *Solanum nigrescens*, popularly used as food and medicine, were established in the present study. The macroscopical and microscopical description, histochemical screening of secondary metabolites, organoleptical analysis of fresh and dry drug, acute toxicity, determination of total and acid ashes, and moisture percent for each species, were carried out. *F. pandurata* flowers, strongly aromatic and greenish-white in fresh plant material, become brown in the dry drug, which include some leaves and stem parts. Microscopically, large epidermal cells, evident cuticle, lacunar collenchyma, paracytic type stomata, and different varieties of hairs, appear. Nonlandular hairs show multicellular, verrucous, or acicular shape, and diversity of bases; glandular ones with unicellular blunt heads, and unicellular short stalks; sclereids, prismatic calcium oxalate crystals and pollen of circular shape with size ranges from 60-80 microns and pore-like opening were also found. *C. argyrosperma* seed with green color, microscopically exhibits reticulated cover and undulations; the leaves and stem, like other Cucurbitaceae members, show angular collenchyma, anomocytic type stomata, bicollateral vascular bounds surrounded by sclerenchyma, abundant nonglandular multicellular large and short elevated hairs, multicellular glandular ones with unicellular foot. *S. nigrescens* flowers are pale purple or white and exhibit a dark zone near de base; microscopically, leaves and stem shows nonglandular hairs, multicellular, verrucous with 4 and 6 cells in the base, plains and unicellular with blunt end with diversity of bases, glandular ones with unicellular foot and multicellular blunt heads and calcium oxalate druses. None of the species showed significant toxicity.

Keywords: *F. pandurata, C. argyrosperma, S. nigrescens,* Endomorphology, Exodomorphology

Introduction

In human history, plants had always been around and is well known that the same species can be used for different purposes, so, if we consider what Hippocrates said many centuries ago: "Let food be your medicine and medicine be your food," it is no surprising that many Mesoamerican edible plants are also used in Guatemalan traditional medicine.[1]

The species included in this survey were chosen because they are native of the region, free commercialized and commonly used with different edible and medicinal purposes. Those were *Fernaldia pandurata* (A. DC.) Woodson (flower), *Cucurbita argyrosperma* Huber (seed) and *Solanum nigrescens* Mart & Gal (leaf).

The flowers of *F. pandurata*, shows galactogogue properties.[2] The root is considered highly toxic, because it's pyrrolizidine alkaloids.[3] The flowers can be eaten cooked with cheese, eggs, rice, creamy chicken and tamales.[4]

C. argyrosperma seeds, shows antihelmintic and galactogogue properties.[1,5] Fruits, as well as the seeds, can be found in popular markets and supermarkets[2].

Antibacterial activity against *Pseudomona aeruginosa*, *Staphylococcus aureus*, and *Streptococcus pyogenes*, as well as antimicotic activity against *Candida albicans*, had been confirmed for the leaves of *S. nigrescens*, which are usually eat in broth or fried with eggs. They are commonly found in popular markets and supermarkets.[6]

This study was carried out to contribute to the knowledge of *Fernaldia pandurata* (A. DC.) Woodson, *Cucurbita argyrosperma* Huber and *Solanum nigrescens* Mart & Gal, popularly used as food and medicine.

Considering that plants can display variations in their morphology, due to differences in the climatic conditions of their habitat, our research group has undertaken this study in order to define identity characteristics, useful not only for the adulterants detection, but also for assuring the raw material quality control of native species.

Materials and Methods

From collected plant material, herbarium specimens, ac-

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cording to Solís *et al*,[7] were done. The identification and deposit of the specimens take place at the "Herbario de Biología de Guatemala (BIGU)"; dry drug samples were also made and deposited in the Citohistology Department, both from the Faculty of Chemical & Pharmaceutical Sciences of the San Carlos of Guatemala University.

Identity Tests

Fresh and dried plant materials were used for the macroscopic, organoleptic, micro-morphologic and quantitative studies. For microscopical analysis, handmade transverse sections from aerial parts of the three species were performed and stained with Safranin according to Solís *et al*.[7]; Gattuso & Gattuso[8] and Soria,[9] said sections were mounted with gelatin-glycerin[8] and observed with optical microscope Leica® CME.

Steam and seeds were dissociated, leaves were cleared and all stained and mounted by conventional methods according to Solis et al[7], Gattuso & Gattuso[8] and observed with the Micromaster® microscope, visualized and photographed using a Westover™ camera, and digitalized with Micron (USB) program. Photomicrographical charts for each species were done.

Histochemical screenings of secondary metabolites were performed according to Gattuso and Gattuso methods.[8]

Purity Tests

Total ashes percent was performed by quintuplicated, using the weight differences before and after drying it for one hour. Necessary acid ashes were establish by addition of 3N chlorhydric acid and incineration.[7]

Humidity percent was carried out by quintuplicated, using the weight differences of 1 g of material, after one hour of drying in a 105°C oven.

Toxicity Tests

Acute toxicity bioassay using *Artemia nauplii*, was performed by triplicate according to Michael et al[10] and Wah,[11] using ethanolic extracts of the three plant materials; extracts were obtained from Citohistology extract bank. CL_{50} was determined using the Statgraphics Plus 5.1 program PROBIT.

Results

Plant materials were collected in different geographical regions of Guatemala, been those: for *G. sepium*, ecoparcela El kakawatal, cantón Chiguaxte, Samayac, Suchitepéquez (14°33'56.66" N, 91°27'53.19" O); *F. pandurata*, Sanarate, El Progreso (14°47'8.55" N, 90°12'0.1" O); *C. argyrosperma*, Salamá, Baja Verapaz (15°06'12" N, 90°16'0" O); *S. nigrescens*, San Bartolomé Milpas Altas, Sacatepéquez (14°36'22.36" N, 90°40'19.86" O). The collected and herborized materials were entered to BIGU herbarium collection with vaucher 64284 for *F. pandurata*; 64492 for *C. argyrosperma*; and 64285 for *S. nigrescens*.

Organoleptic Analysis of Fresh and Dry Drug

Drug of F. *pandurata* constituted by flowers, alone or in small groups with a few leaves and part of the stem. Flowers are strongly aromatics with sweet odor but better taste, greenish white in fresh plant material, but brown and hard in the dry drug.

C. argyrosperma drug constituted by seeds, which show an elliptical to lanceolate shape, slightly inflated. The seed has a smooth, rigid, thin and white outer integument with thickened margins, and an olive-green and thinnest inner integument. Dry drug fragmented by physical action, with pleasant and slightly acid smell and palatable and salty flavor.

S. nigrescens drug, constituted by petiolate leaves, green in fresh drug and dark green or brown color in dried drug, with soft and fragmental consistence, pleasant and heavenly scent but better and unpleasant taste.

Diagnostic Micromorphological Characteristics

Fernaldia pandurata. Stem transverse section show a 2 layered epidermis, evident cuticle, small clusters of lacunar collenchyma, layered phellodermic tissue formed by chlorenchymatic parenchymal cells, a discontinuous ring of sclerenchyma surround a bicollateral vascular continuous cylinder, internal phloem with additional bundles of phloem more deeply seated in the pith. Solitary prismatic crystals are seen in parenchymal cells and abundant elevated non-glandular hairs (Figure 1A).

Petiole transverse section shows a concave shape at the upper side, which confer to it, a bilobate shape. An unstratified epidermis with round cells and a thin cuticle form a smooth margin with abundant multicellular, unicellular, acicular, verrucous and conical trichomes. Bellow the epidermal cells, a 2-4 layered lacunar collenchyma, can be observed, parenchyma shows secretory channels and an arch shaped bicollateral vascular boundless opened to the upper side, with to accessory boundless, one on each lobe (Figure 1B).

Cross section of the blade shows an unstratified epidermis with a thick cuticle; adaxial epidermal cells are rectangular and 40-60 micrometers high. Mesophyll is dorsiventral, with an unlayered and irregular palisade parenchyma, and a 3-5 layered spongy parenchyma. The midrib consists of bicollateral vascular bundles, arranged in a flat arch, with 2-3 cellular lacunar collenchyma under the epidermal cells and abundant unicellular and multicellular hairs, parenchymal cells shown solitary prismatic crystals (Figure 1C, E). The surface view shows abaxial epidermal cells with thin and sinuous anticlinal walls, a hypostomatic leaf with paracytic stomata (Figure 1D). Cross section and surface view of both epidermises shows variety of hairs, nonglandular ones: multicellular with blunt end, multicellular with pointed end, conical and verrucous and unicellular with thin walls, glandular ones with short unicellular stalk and globular head (Figure 1C, E).

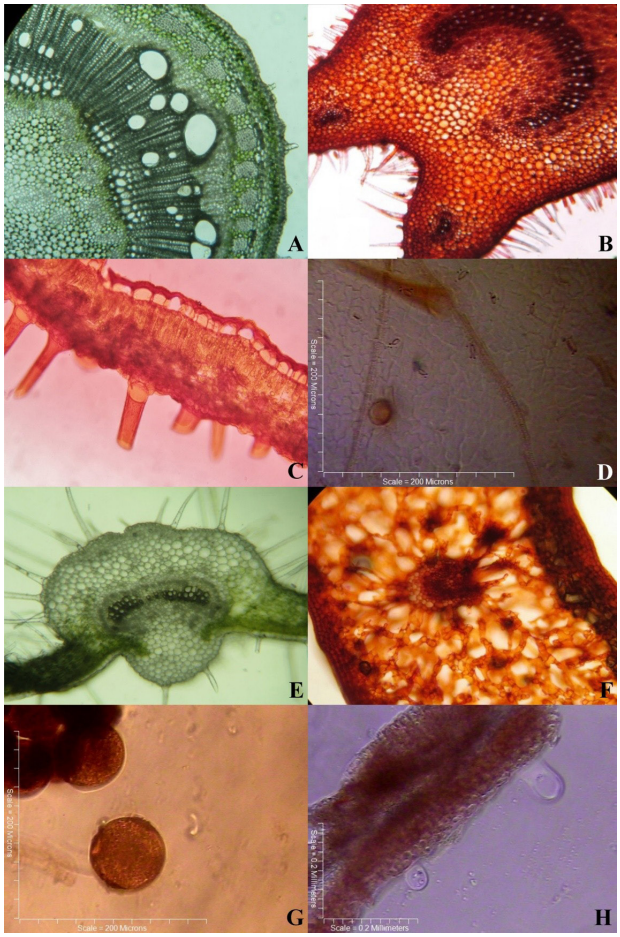

Figure 1. Micromorphological characteristics of *F. pandurata*. A: Stem transverse section. B: Petiole transverse section. C: Foliar Limb transverse section. D: Superficial view of the Foliar Limb. E: Leaf midrib level transverse section. F: Petal transverse section. G-H: Dissociated flowers.

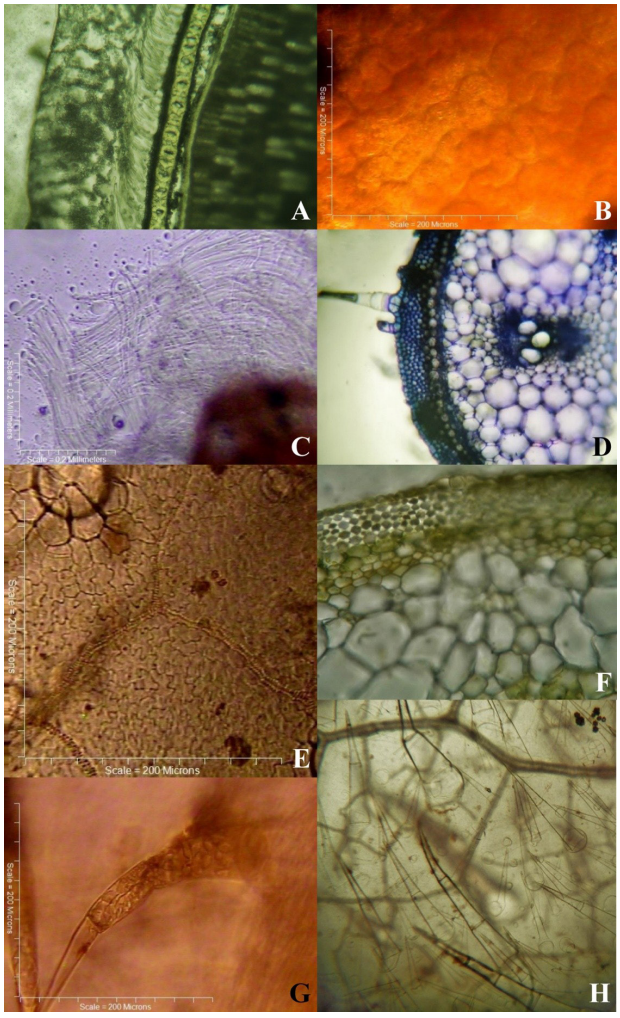

Figure 2. Morphological characteristics of *C. argyrosperma.* A: Seed longitudinal section. B: Exotesta superficial view. C: Dissociated seed, Mesotesta Macrosclereids. D: Stem transversal section. E: Leaf superficial view. F: Stem transversal section, angular Collenchyma. G-H: Leaf superficial view.

Petal cross section exhibits triseriate epidermis, bicollateral vascular bundles surrounded by large parenchyma cells and spongy mesophyll with large intercellular spaces. The macerated flowers shows anther cells, papilla like secretory hairs, 4 aperturate, porate pollen grains, brachysclereids, helical xylem and parenchymal cells (Figure 1G-H).

Cucurbita argyrosperma. The longitudinal section of the dicotyledon seed shows an outer tegument composed by sclerenchymatic cells with three well differentiated strata (Figure 2A). The sclerenchymatic cells in the first strata, surrounded by mucilage, shows an irregular shape, their surface in the macerate appear reticulated and wavy (Figure 2B). The middle strata is formed by a compact columnar macrosclereid layer (Figure 2A), in the macerate they appear thick walled and elongated (Figure 2C). The third strata, exhibits a unique layer of large quadrangular sclereids. The inner tegument shows a thin layer of very close sclerenchymatous and ovoid cells, wider than high attached to de embryo constituted by parenchymatic cells (Figure 2A).

Leaf, stem and petiole in transversal section show: angular collenchyma, chlorophyllic parenchyma, bicollateral vascular bounds surrounded by sclerenchyma, abundant nonglandular multicellular large and short elevated hairs, multicellular glandular ones with unicellular foot (Figure 2D, F). Leaf upper view shows a hypostomatic blade, with anomocytic and elevated stomata. Adaxial epidermis with anticlinal thin and flat walls and abaxial epidermis with thin and sinuous walls. Abundant nonglandular multicellular large and short elevated hairs, arising from a rosette of cells at the base, calcium oxalate crystals within non-glandular conical hairs and multicellular glandular ones with unicellular foot (Figure 2E, G-H).

Solanum nigrescens. Stem transversal section shows unstratified epidermis with round and irregular cells. Three layer angular collenchyma, and a concentric ring of bicollateral vascular bundles reinforced by an irregular ring of sclerenchyma. Dissociated shows sclereids, collenchyma cells, parenchyma cells and variety of

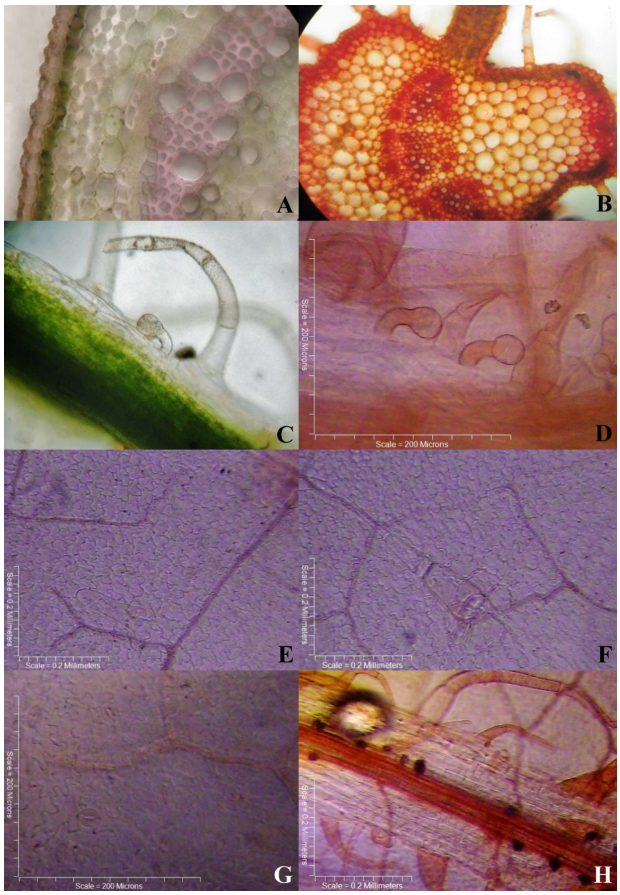

Figure 3. Micromorphological characteristics of *S. nigrescens*. A: Stem transverse section. B-C: Leaf transverse section. D-F: Superficial view of Leaf's Adaxial Epidermis. G-H: Superficial view of Leaf's Abaxial Epidermis.

trichomes (Figure 3A).

Transverse section of the leaf shows a bifacial mesophyll, an unstratified epidermal tissue with round cells at midrib, but rectangular ones in the blade with a thin cuticle, the vascular tissue is bicollateral and arranged in a flat arch. The blade shows short unlayered palisade parenchyma and 3-4 layer spongy parenchyma. 1-3 layer angular collenchyma in the midrib (Figure 3B). In the transverse section as well as in the surface view of epidermis, variety of hairs can be seen on both sides of the leaf surfaces, non-glandular trichomes: conical unicellular with four cells under, short and bicellular, verrucose multicellular with a 4-cell rosette in the base, thin walled multicellular. Glandular trichomes: with unicellular stalk (large or short) and unicellular or multicellular blunt heads, verrucous with tiny blunt end (Figures 3B-H).The surface view shows an hypostomatic leaf with anomocytic stomata and calcium oxalate sandy druses. Both epidermises, shows sinuously walled cells (Figure 3E-G).

Histochemical Screening

Table 1 shows a synthesis of the histochemical screening for secondary metabolites on the different structures analyzed for each species, being alkaloids and essential oils the most prominent in the three species and in all the organs analyzed.

Purity Tests

In the Tables 2 and 3, the results of moisture and total acid ashes can be observed. All the measures were made by quintuplicate, and the results demonstrated de quality of the plant materials used in this study, considering that all were between the OMS standards.

Toxicity Test

From all the results, only *S. nigrescens* shows some activity to *Artemia nauplii* after 4 dilutions (1:2, 1:4, 1:8, 1:16), with 0.35 mg/mL of median lethal dose (LD_{50}), for a 95% confidence interval of 0.30 to 0.35 mg/mL.

Discussion

This study was conducted with the purpose of establishing identity characteristics, useful for assuring the raw material quality control, of three edible and medicinal native species, popularly known as loroco (*F. pandurata*), pepitoria (*C. argyrosperma*) and macuy (*S. nigrescens*).

Regarding to *F. pandurata* drug constituted by flowers, the only report found commented about its bitterness, which increases with maturation.[12] The observed macroscopic characteristics matches those reported for the specie, by Morales[13] and by Rzedowski and Calderón.[14]

Table 1. Histochemical Screening and Metabolites Localization on Plant Structures

Metabolite	Reagent	*F. pandurata*				*C. argyrosperma*			*S. nigrescens*		
		Stem	Petiole	Leaf	Flower	Stem	Petiole	Leaf	Stem	Peteiole	Leaf
Alkaloids	Dragendorff	+	+	+	+	+	+	+	+	-	+
Saponins	Concentrated sulfuric acid	-	+	+	-	-	-	-	+	+	+
Tannins	Ferric sulfate	-	-	-	-	-	-	-	-	-	-
Starch (Amylum)	Lugol's Iodine	+	-	+	-	-	+	-	+	+	-
Fats y Oils	Sudan IV	+	+	+	+	+	+	+	+	+	+
Mucilages	Cresyl blue 1%	+	+	+	-	+	-	-	+	+	+

(+): Positive; (-): Negative.
(n = 5 for every plant and organ).

Table 2. Statistical Value of the Moisture Percentage of the Study Plants

Plant Material	Average	Standard Deviation	Median	Range
F. pandurata	5.52	0.13	5.47	5.40-5.66
C. argyrosperma S[a]	2.43	0.34	2.36	1.97-2.87
C. argyrosperma T[b]	7.50	0.83	7.32	6.61-8.83
C. argyrosperma SST[c]	4.87	0.27	4.89	4.50-5.12
S. nigrescens	5.20	0.67	5.37	4.30-6.08

[a] Seed; [b] Tegument; [c] Seed without tegument.
(n= 5 for each plant).

Table 3. Statistical Value of the Total and Acid Ashes Percentage of the Study Plants

Type of Ashes, Calculated Values	*F. pandurata*	*C. argyrosperma*			*S. nigrescens*
		S[a]	T[b]	SST[c]	
Total Ashes					
Average	10.82	4.15	1.62	4.58	11.37
Standard Deviation	0.35	0.13	0.12	0.06	0.22
Median	10.70	4.15	1.63	4.58	11.33
Range	10.44-11.39	4.00-4.31	1.45-1.74	4.50-4.68	11.11-11.67
Acid Ashes					
Average	1.28				1.55
Standard Deviation	0.10	NA[d]	NA	NA	0.10
Median	1.30				1.58
Range	1.11-1.36				1.44-1.69

[a] Seed; [b] Tegument; [c] Seed without tegument; [d] Does not apply.
(n= 5 for each plant).

Microscopically, variety of trichomes were observed, that were also been reported in other members of these family,[15] and the calcium oxalate crystals were reported, but like druses.

The macroscopic characters find for *C. argyrosperma* seeds and aerial parts, are consist with those found in literature.[16] The microscopic characteristics of stem petiole and leaf correspond with descriptions of Agbagwa and Ndukwu,[17] for some species of *cucurbita*. The bicollateral vascular bundles are typical of Cucurbitaceae family.[19]

The sinuous walls of abaxial epidermis and flat walls of adaxial epidermis, was mentioned for several species from the Cucurbitaceae family.[19]

Anomocytic and elevated stomata on abaxial epidermis were found for this specie and according with other authors, this is a variable character for Cucurbitaceae, because a variety of stomata had been reported for this family.[19]

The presence of glandular and non glandular trichomes matches descriptions made by Kolb and Müller[20], who report these as a common characteristic of *Cucurbita* genus. The glabrous and reticulate seed outer integument surface, was previously described by Ajmal et al.[21] The description of the sclerenchymatous tissue strata, observed in cross and transverse sections, matches other author's reports for Cucurbitaceae family.[17, 22]

The macroscopic features found for S. nigrescens, are similar to those described for other members of *Solanum* genus.[23] Microscopically, the presence of bicollateral bundles are typical for Species of the *Solanum* genus. The presence of multilayered angular collenchyma and the sclerenchymatic tissue around vascular bundles in the stem is a common feature of this genus.[24]

The anomocytic type stomata found in this survey, according to literature, are the most common in this genus.[25]

The cumulated sandy druses found in the mid rib, were previously reported in other species of *Solanum*.[26] The variety of glandular and nonglandular trichomes are very similar to those described for several species of this genus by Figueroa et al[24] and Cosa et al.[27]

Even when ethanolic extracts of *S. nigrescens* demonstrate a DL_{50} = 0.35 mg/mL (3500 µg/mL), it can be consider as toxic, because an extract is toxic when DL_{50} is less than 1000 µg/mL.[28] According to Silva et al,[29] the biocide activity of this genus is attributed to the presence of alkaloids and saponins, which matches with the study findings.

The histochemical screening results together with those reported by Cáceres[30] explain some of the medicinal properties popularly attributed to this species. It is therefore important to continue with ongoing efforts that allow to establish those properties.

According to World Health Organization (WHO), herbal materials are categorized according to sensory, macroscopic and microscopic characteristics,[31] hence the used protocols accomplished the establishment of some useful characters to the identity assurance of each species such as the shape and size of the pollen, and the strongly aroma of *F. pandurata* fresh and dry drug. The shape and type of the seed cover of *C. argyrosperma* and the variety of trichomes and the presence of alkaloids and saponins of *S. nigrescens*. In addition, to know the microscopic characteristics of the whole plant, allowed to establish adulterants from the same plant as well as identify the presence of different plant materials; taking into account the incidence of such adulterants in the quality of herbal drugs and into their medicinal activity.

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