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Leaf Anatomy of Some Members of Rhizophoracae (Mangroves) In Port Blair, Andaman and Nicobar Islands

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ABSTRACT

Knowledge of the leaf anatomy of the mangrove taxa is crucial for understanding how these plants adapt to the environment. *Rhizophoracae* family is known to be of true mangroves from the dominating flora of Andaman and Nicobar Islands. Few comparative studies concerning the leaf anatomy of Port Blair region have been conducted. We examined the mangrove taxa namely *Rhizophora apiculata* Blume*, Rhizophora mucronata* Lamk.*, Rhizophora stylosa* Griff.*, Bruguiera cylindrica* (L.) Blume*, Bruguiera gymnorhiza* (L.) Lamk. and *Ceriops tagal* (Perr.) C.B. Robinson. Results have shown that apart from the morphological characters of the hybrid *Rhizophora stylosa* there is very closer resemblance to *Rhizophora apiculata* in the laminar characters. Calcium oxalate crystals, thick cuticle, large water storage cells, branched sclerieds and the salt glands were found to be adapted features of mangroves. Employing the leaf anatomical data and petiole anatomical features, artificial dichotomous keys for diagnosis of the species has been provided.

Keywords: Leaf anatomy, Mangroves, Rhizophoraceae, stomatal index

INTRODUCTION

Mangroves are a specialized group of trees and shrubs that reside in the intertidal areas on the edge of the land and sea. Mangrove forests are regarded as the most productive and biodiverse wetlands on earth, as an important natural reserve of biological diversity. The mangrove ecosystem constitutes a bridge between terrestrial and marine ecosystem. This ecosystem serve as excellent reservoirs of nutrients and feeding and nursery grounds for a wide variety of organisms such as crabs, oysters, prawns and fishes. The mangrove vegetation stabilizes the shoreline by checking the erosion of land by the sea. This characteristic anchoring of aerial network of stilt roots and pneumatophores trap sediments and help in building up of land, thus mangroves act as land markers. (Singh and Odaki, 2004).

Andaman and Nicobar Islands situated in the Bay of Bengal off the eastern coast of India are gifted with very dense forest exhibiting rich diversity of plant species. Luxuriant mangroves constitute one of the most important forest types that exist in these islands. The mangrove ecosystem are protected, in the Andaman Islands. Mangroves cover an area of 929 km² and in the Nicobar the extent is 37 km² (Balakrishnan, 1989; Andrews & Sankaran, 2002).

Rhizophoraceae is well known as the richest mangrove family, having four exclusively mangrove genera (*Bruguiera*, *Ceriops*, *Kandelia* and *Rhizophora*) (Tomlinson, 1986). All members of this pantropical genus are considered true mangroves and are prevalent in distribution to the intertidal zone. Red mangroves (*Rhizophora mangle*) are found closest to the water and are the most recognizable mangrove trees due to their "walking" prop roots. These prop roots not only provide support and stability for the tree, but they also have pores (called lenticels) which supply oxygen to the buried roots.

The Present investigation was intended to study the leaf anatomical structures of some members of Rhizophoracae. It includes three genera (*Rhizophora, Bruguiera*, and *ceriops)* and six species (*Rhizophora apiculata, R. mucronata, R. stylosa, Bruguiera cylindrica, B. gymnorhiza,* and *Ceriops tagal)*. The anatomical features of the leaves of the selected taxa, will offer simple means for identification and it delimit correct species identification. Identification of fragmentary leaf samples has vital role in the pharmacognostic area of studies.

MATERIALS AND METHODS

Selection and sampling of leaves

The material for present investigation was collected from different localities of Port Blair and identification of mangroves was done by the Botanical survey of India (BSI) Port Blair. Mature healthy leaves with petiole of six species belonging to 3 genera were collected.

Preparation of Permanent Slides

Johansen's method (Johansen, 1940) was used to prepare permanent slides, Formaldehyde based fixative containing 95% ethanol, 5 ml glacial acetic acid, 10 ml formaldehyde (37%) and 35 ml distilled water were used to kill and fix leaves as soon as they were collected. After 12 hr. in water, lamina strips from the middle part of the leaves consisting of the mid-vein, inter-coastal area and the margin were excised from the leaves, dehydrated in a graded ethanol series, infiltered, embedded in paraffin wax and sectioned by rotary microtom. The transverse sections thus obtained were stained with safranin and toluidine blue O. All the leaves collected were treated on the transverse section in this manner and were permanently mounted on DPX. Then slides were observed under light microscope. From the prepared slide measurements were made by the ocular micrometer for each leaf sample which included measurements of total leaf cross-sectional thickness, palisade and spongy mesophyll cell layer thicknesses and ratios of palisade and spongy layer, upper and lower epidermis thickness, upper and lower cuticle thickness.

Determination of stomatal number and stomatal index

For studying the stomatal morphology, stomata, epidermal cells, and other inclusions, the Book Opening Method has been applied. It is a rapid method for obtaining epidermal peels in plant on treatment with Cupric sulphate and Hydrochloric acid. And stained with safranin. And mounted in glycerine to make temporary slides. The Stomatal Index (SI) was determined according to using the formula:

$$S.I = \frac{SI}{E+S} X \frac{100}{1}$$

Where S= number of stomata per unit area.

E= number of epidermal cells in the same area.

Photomicrographs

Microscopic descriptions of tissues were supplemented with micrograph wherever necessary. Photomicrographs were obtained using a Vision 2000 of different magnifications for showing crystals, lignified cells and other inclusions.

RESULTS

Leaf dorsiventral, petiolate, and hypostomatic in all the species of *rhizophoracae*. In all the three of *rhizophora* species *Rhizophora apiculata*, *R. mucronata* and *R. stylosa* having black spots on the abaxial surface of the lamina known as cork warts which is the key character of the *rhizophora* to differentiate the leaves with in the *rhizophoracae* family. As this species is succulent in nature the highest lamina thickness recorded in the present study were *ceriops tagal* (720µm) and the lowest in *Bruguiera cylindrica* (435 µm).

Table 1: Laminar anatomical characters

S. No	Species	Thickness of the Lamina (in µm)	Thickness of the Cuticle		Thickness of the Epidermis (in µm)		Thickness of the Mesophll Tissue (in µm)		Thickness of the Palisade Cell (in µm) L x B	Thickness of the Spongy Cell (in µm) L x B
			ad	ab	ad	ab	Palisade layer	Spongy layer		
1.	*Rhizophora apiculata*	480	3.8	3.8	15.2	7.6	129.2	136.8	19 x 7.6	19 x 15.2
2.	*Rhizophora mucronata*	525	3.8	3.8	11.4	7.6	135	225	22.8 x 7.6	22.8 x 19
3.	*Rhizophora stylosa*	690	11.4	7.6	15.2	11.4	195	315	45.6 x30.4	22.8 x 19
4.	*Bruguiera cylindrica*	435	3.8	3.8	22.8	11.4	135	150	41.8 x15.2	26.6 x 19
5.	*Bruguiera gymnorhiza*	510	7.6	11.4	15.2	11.4	120	405	34.2 x15.2	26.6 x 19
6.	*Ceriops tagal*	720	11.4	15.2	19	15.2	117.8	480	38 x 19	19 x 22.8

Where : Ad - Adaxial Surface (upper), Ab - Abaxial Surface (lower), L – Length, B – Breadth,

The cuticle is generally smooth in both adaxial and abaxial surfaces in all species studied. The highest cuticle thickness measured in *ceriops tagal* (11.4µm) and lowest in *Bruguiera cylindrica* (3.8 µm). (Table 1)

The cross section of lamina has revealed that mesophyll was composed of palisade and spongy tissues but the palisade ratio is varying, there is 3-4 layers of palisade tissue in all the species except in *ceriops tagal* where it is having only one layer. The hypodermis towards the adaxial surface was 3-4 layered in *Rhizophora* or in *Brugiera* and *Ceriops* it was one layered. This hypodermis region in lamina is also known as water storage tissue. In *Rhizophora*, mucilage cells were present beneath the upper epidermis and it is absent in *Bruguiera* and *Ceriops*. It has been observed that different crystals like druces and raphides were deposited in mesophyll which might be helping for any mechanical injured. Branched sclereids were only seen in the *Rhizophora*. A series of tannin cells in the upper epidermis were only examined in *ceriops* and *Bruguiera* and sparsely in *Rhizophora*. The Spongy tissues possess large intercellular spaces in all the taxa.

In the midrib section all species investigated exhibited conjoint, collateral and closed bundles surrounded by clorenchymic tissue interrelated, extended from the adaxial to abaxial

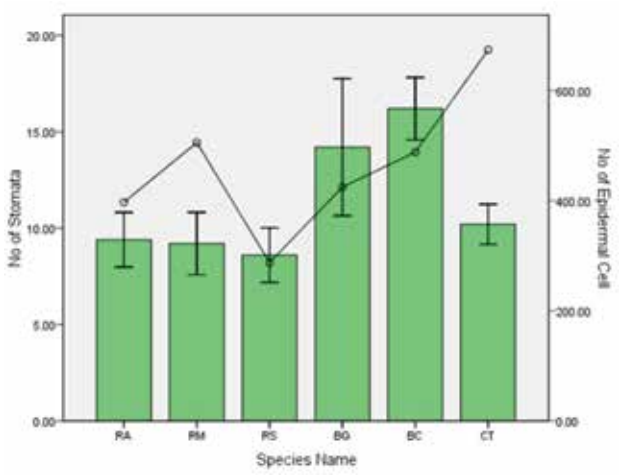


Fig 1: Columns showing number of stomata and line showing number of epidermal cells

surfaces of leaf epidermis but separated by the presence of vascular bundle. Collenchymatic tissue was frequently associated to the mid-veins of *Bruguiera gymnorhiza*.

Different types of stomata has been recorded in this study. The stomata are of anomocytic in *Rhizophora apiculata, Rhizophora stylosa* and *Bruguiera gymnorhiza. Cyclocytic* in *Rhizophora mucronata* and *Ceriops tagal.* Two different stomata found in *Bruguiera cylindrica* i.e., anisocytic and paracytic. (Fig: 2) The stomata were irregularly distributed and their axes are oriented in different direction observed in all the species. The epidermal cells of all taxa have regular or irregular shapes with either uniform or varying sizes on both the surfaces. The highest stomatal index was found in *Bruguiera gymnorhiza* (3.23) and the lowest in *Ceriops tagal* (1.49). (Fig: 1). *Bruguiera gymnorhiza* has the smallest stomata while the largest stomata were found on *ceriops tagal*. (Table 2)

The transverse section of the petiole exhibited various shapes in different taxa. The epidermis of the petiole resembles that of the corresponding surface of the lamina except that there are no glands or stomata on the underside. The epidermis is single layered in all the species and covered by thick waxy cuticle. A few layered collenchymatous hypodermises follows epidermis. It was 3-4 layered deep. Loosely arranged parenchyma followed collenchymatous hypodermis and was a few cells deep. The cells were spherical to oval. The sclerenchymatous patches were found in ground tissue. Crystalliferous cells like raphides and druces were sparsely seen. Sieve plates were observed in the *Bruguiera cylindrica.* The vascular bundle shape is found to be varied, medullated in *Rhizophora apiculata, R. stylosa, Bruguiera gymnorhiza,* and in *Ceriops tagal.* Deep arc in *Bruguiera cylindrica* and in *Rhizophora mucronata.*

Table 1: Quantitative and qualitative of epidermal characteristics

S.No	Species	Stomatal type	Epidermal shape		Epidermal cell wall		Stomatal size L X B (in µm)	Epidermal cell size		SI
			Ad	Ab	Ad	Ab		Ad (in µm)	Ab (in µm)	
1.	*Rhizophora apiculata*	Anomocytic	Polygonal	Polygonal	Straight to arcuate	Straight	41.8 x22.8	19	15.2	2.31
2.	*Rhizophora mucronata*	Cyclocytic	Polygonal	Polygonal	Straight	Straight to arcuate	34.2 x26.6	15.2	11.4	1.78
3.	*Rhizophora stylosa*	Anomocytic	Polygonal to Rectangular	Polygonal	Straight	Straight	38 x 26.6	15.2	15.2	2.90
4.	*Bruguiera cylindrica*	Anisocytic and Paracytic	Polygonal to Rectangular	Polygonal	Straight	Straight to arched	26.6 x30.4	19	22.8	3.21
5.	*Bruguiera gymnorhiza*	Anomocytic	Polygonal	Polygonal	Straight	Straight	22.8 x 19	19	15.2	3.23
6.	*Ceriops tagal*	Cyclocytic	Polygonal	Polygonal to rectangular	Straight to arcuate	Straight to arched	41.8 x 38	15.2	11.4	1.49

Where : Ad - Adaxial Surface (upper), Ab - Abaxial Surface (lower), L – Length, B – Breadth, SI-Stomatal Index.

An Artificial Key Based On the Microscopic Features

1. Lamina with cork warts; Mucilage cells present :

    2. Stomata Anomocytic type:

    3. Cuticle is uneven both adaxial and abaxial epidermal

    surface**…….....……......***Rhizophora apiculata*

    3. Cuticle is even both adaxial and abaxial epidermal

    surface **……......***Rhizophora stylosa*

2. Stomata cyclocytic type……..... *Rhizophora mucronata*

1. Lamina without cork warts; Mucilage cells absent:

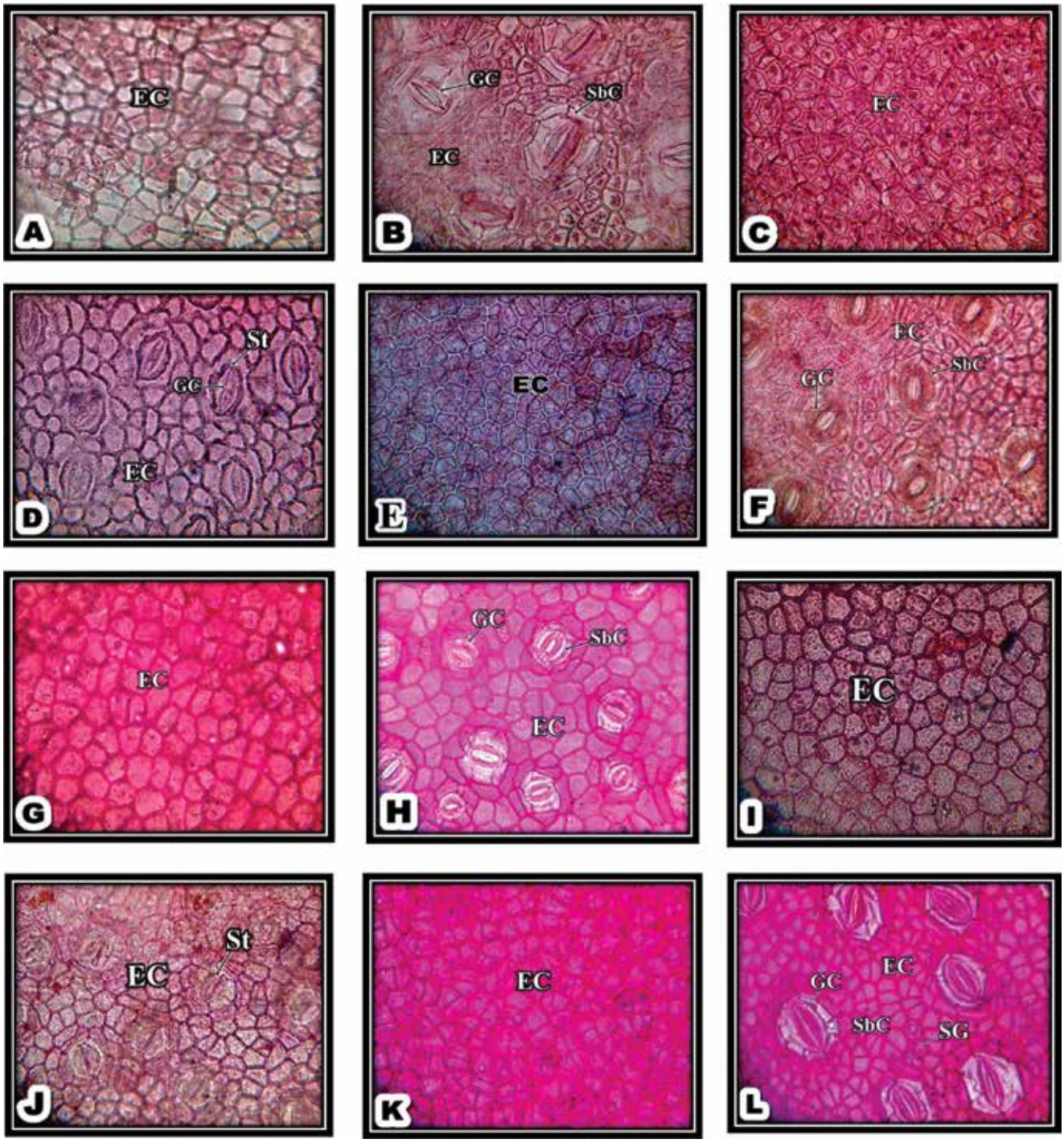
4. Hypodermal single layer; 3-4 palisade layer:

    5. The stomata is Anisocytic and Paracytic**……..***Bruguiera cylindrica*

    5. The stomata is Anomocytic only**…….....** .*Bruguiera gymnorhiza*

4. Hypodermal double layer; Single palisade layer**……..***Ceriops tagal*

Fig. 2: Photographs of leaf epidermal characters: *Rhizophora apiculata* 40X (A-Adaxial surface, B-abaxial surface)*, Rhizophora mucronata* 40X (C-Adaxial surface, D-abaxial surface), *Rhizophora stylosa* 40X *(*E-Adaxial surface, F-abaxial surface), *Bruguiera cylindrica* 40X (G-Adaxial surface, H-abaxial surface), *Bruguiera gymnorhiza* 40X (I-Adaxial surface, J-abaxial surface)*, Ceriops tagal* 40X (K-Adaxial surface, L-abaxial surface) (EC- Epidermal Cell; GC- Guard Cell; SbC- Subsidiary Cell, SG- Salt Gland, St- Stomata)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DISCUSSION

Our observations have indicated vast distribution of oxalate calcium crystals in most of the studied except in the *Ceriops tagal.* Presence of salt glands in *Rhizophora apiculata, Rhizophora mucronata* and *Rhizophora stylosa* on the abaxial surfaces are related to salt excretion of these plants. Large amount of water storage tissue occur in the hypodermal region in all the species, reflecting the adaptive nature of mangroves in their stressful habitat. The tissue increases succulence of leaf. Das and Ghose (1996) believed this is a common feature of the mangrove species.

According to Das and Ghose (1996) mangrove leaves possessed thick cuticle. Colourless water storage tissue is hypodermal in dorsiventral leaves but is deep seated in the mesophyll region of isobilateral leaves. Terminal tracheids at vein endings are commonly found in many species. The presence of water storage tissue and terminal tracheids causes leaf succulence with high water content. Our result are in concordance with his findings. They also pointed out the Branched sclereids present in some species. In this investigation we have observed only in the lamina of *Rhizophora* i.e., Astro, osteo and brachy sclereids.

Zimmermann(1983) reported that both sclereids and tracheids are involved in capillary water storage. Tomlinson (1986) suggested that in addition to water storage, sclereids might also provide mechanical support to leaves with diminished turgor or discourage herbivores. The coriaceous nature of mangrove leaves is due to the presence of sclereids.

Sauren Das and Monoranjan Ghose (1993) revealed that Cyclocytic stomata were found in the *Rhizophora mucronata,* where the two guard cells are encircled by a ring 6-8 subsidiary cells. Our results also confirm his findings. We have observed the Cyclocytic stomata in *Rhizophora mucronata* and in *Ceriops tagal.* The cell walls of the subsidiary cells are very thin, which results in the difficulty to examine the stomata type.

Das and Ghose, 1996 reported that there are two species in *Ceriops*, namely *Ceriops decandra* and *C. tagal*. In *Ceriops tagal* the subsidiary slightly overarching the guard cells than in *Ceriops decandra.* Except this character, there is no character was found to distinguish these two species. But we observed that columnar palisade is single layered which was not mentioned earlier to distinguish Ceriops.

Das and Ghose also had pointed that stomata is paracytic in *Bruguiera cylindrica* but in this study we found paracytic as well as anisocytic where the stoma remain surrounded by three accessory or subsidiary cells of which one is distinctly smaller than the other two. The anomocytic type of the *Bruguiera gymnorhiza*, which were reported by Das and Ghose (1993) and Naskar and Mandal (1999), may attribute to such difficulty. We also considered this findings. Additionally, it was noted that all members of the mangrove *Rhizophoraceae* have cyclocytic stomata (Hsiao and Chen, 1988; Keating and Randrianasolo, 1988; Sheue et al., 2000).

The occurrence of salt gland in mangroves is an adaptation for marshy habitat, because salt cannot accumulate in plant tissues beyond a limit. Salt glands are found abundantly on leaves, though their number is lesser than that of stomata in the lower epidermis. Gland cells differ from normal mesophyll cells in shape and arrangement. Cells are without chloroplast. The salt glands are meant for excreting excess salts accumulated in mangroves, thus maintaining a salt balance to the plants.

Mangroves are important due to their strange morphological and anatomical adaptations and special physiology like osmotic potential of cell sap, reaction to salinity and vivipary. Mangrove ecosystem is commercially very significant and provide many direct and indirect services to man. So mangrove ecosystem must be conserved.

CONCLUSION

In the present study a total of six species were studied which were distributed in three genera of *Rhizophoracae* family. These microscopical characters of leaf and petiole could serve useful in the identification of this plant species. Results revealed that presence of large water storage cells, mucilaginous idioblast cells, thick waxy

cuticle, and crystalliferous cells are similar in all species studied. Three to four layer of palisade layer is present in all species studied although one layer in *Ceriops*. Presence of branched sclereid i.e., osteo, astro, and brachy sclereid cells or secretory cells in the lamina of *Rhizophora* represents an important diagnostic character for this genus. This leaf anatomical characteristics also revealed that apart from morphological characters, the hybrid *Rhizophora stylosa* exhibits a very closer resemblance to *Rhizophora apiculata* through its epidermal features like stomata, epidermal sizes, anticlinal walls, stomatal frequency, position of sclereid cells and vascular bundles of petiole and midrib. As a conclusion, this anatomical study has shown some peculiar characters of mangrove *Rhizophoraceae* as an adaptation to the extreme environment and also have taxonomic value in genus differentiation.

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