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COMPARATIVE ANATOMY OF LEAF LAMINA OF TWENTY SIX WOODY SPECIES OF *TAMAULIPAN THORNSCRUB* FROM NORTHEASTERN MEXICO AND ITS SIGNIFICANCE IN TAXONOMIC DELIMITATION AND ADAPTATION OF THE SPECIES TO XERIC ENVIRONMENTS

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Abstract

The present study was undertaken to determine the variability in leaf anatomical traits and its relation to taxonomic delimitation and adaptation of the woody species in xeric environments. The results show large variability in anatomical traits with respect to cuticle thickness, presence and absence of trichomes, length of palisade cells and thickness of spongy tissue. Species have been classified on the basis of the length and compactness of palisade cells to determine its relation to the taxonomic delimitation and the adaptation of the species to drought condition. The species *viz., Karwinskia humboldtiana, Lantana macropoda, Prosopis laevigata, Zanthoxylum fagara, Helietta parvifolia, Acacia berlandieri* and *Acacia wrightii* having long and compact palisade cells are expected to be efficient in photosynthetic function and adaptation to drought. Future research needs to be directed in this direction.

Key words*:* Leaf anatomy, Woody species, Anatomical traits, Taxonomy, Adaptation.

Introduction

Leaves contributes to the productivity of plants in a forest ecosystem through photosynthesis, gas exchange (CO_2 and O_2), and transpiration. Leaves possess some structural mechanisms for variations to environments. Leaf anatomy plays an important role in the taxomic determination and adaptation of trees to drought. A comparative study on leaf surface anatomy of few trees and shrubs revealed that there exist a large variabilitywith respect to type of stomata, their size and abundance. Several species possess the absence of stoma and sunken which serve as water budget and impart drought esistance (Maiti *et al*., 2016).

Some studies have been directed on the use of anatomical traits of leaf lamina in the taxonomic delimitation of various species; for example, revealing anatomical similarities among three Brazilian species *Peperomia dahlstedtii* CDC. *Ottonia martiana* Miq. and *Piper diospyrifolium* Kunth (Souza *et al*., 2004); variation in 293 trees of *Pseudotsuga* in (Reyes Hernández *et al*., 2005); twelve species of *Populus* (Afas *et al*., 2007); 35 species of the genus *Kalanchoë* Adans (Crassulaceae DC.) (Chernetskyy, 2012); different species of *Chamaecrista* (L.) Moench (Leguminosae-Caesalpinioideae) (Coutinho *et al*., 2016). In other study, 25 *Ficus* species were characterized effectively on anatomical basis revealing that multilayerd hypodermis, one to three layers palisade parenchyma are the characteristic features to distinguish species (Sonibare *et al*., 2016).

Ten species of *Crocus* have been investigated numerically (analysis of variance and correlation) revealing that palisade cell height and spongy cell width are best parameter to distinguish species (Ozdemir & Ozdemir, 2010). On the other hand, few studies have demonstrated the use of anatomical traits to determine the adaptation of the species to different habitats. A study has shown variability in leaf anatomical characteristics of *Solanum nigrum* collected from different habitats in Europe and Yugoslavia. The species showed variations with respect to stomata number, number of hairs, thickness of lamina, palisade and spongy parenchyma, the size of mesophyll cells thereby showing variability in adaptation to xeric conditions (Krstic *et al*., 2002). The study undertaken on the anatomical basis of resistance on plant species to a typical arid Mediterranean ecosystem revealed that most of the species studied showed the presence in their internal leaf tissues of ergastic substances, such as tannins and calcium oxalate, which act with defensive functions and adaptive resistance of plants to water stress. Nearly all the species showed adaptations for protection against the photo damage possibly induced from the strong UV-B solar irradiance in the summer. The more significant anatomical features were the trichomes, covering the abaxial surfaces of leaves which maintained water budget of the plants both by influencing the diffusion boundary layer of the leaf surface and by regulating leaf optical parameters and, leaf temperature. In many species, the presence of wax layers help in reducing radiation absorbance, besides the presence of two or three layers of palisade parenchyma presumably provide a better efficiency in utilizing the photosynthetic light. In almost every plant examined, stomata were sunken or well protected, thereby helping in preventing loss of water by transpiration (Rotondi *et al*., 2003).

A comparative study of leaf epidermis of orchid, *Cattleya jenmanii, in vitro* conditions demonstrated that compared to normal ones, the epidermal cells were larger in size, lower anticlinal cell wall, small size of stomata for adaptation of orchid, the leaves showed changes in cells with an increase of mechanical resistance and rigidity (Torres *et al*., 2006).

An ecophysiological investigation in two urban forestry species (*Azadirachta indica* and *Millettia thonningii*) revealed that the pattern of transpiration in *M. thonningii* was low in the morning, high in the noon and low in the afternoon. Leaf anatomical study revealed the presence of thick cuticle and high stomatal frequency in *A. indica* and a low stomatal frequency in *M. thonningii* (Dzomeku & Enu-Kwes, 2006).

Significant variability was observed in anatomical characters among cultivars of *Hibiscus* indicating cultivars were well adapted in variable environment (Noman *et al*., 2014).

Anatomical studies of the leaves of 15 species of vascular plants occurring in coastal zones of the Falcon State (Venezuela) revealed that the development of water storing tissue in the mesophyll and/or epidermal cells was the main characteristic associated with the saline habitat in those species. Besides, the presence of trichomes, stomata protected by papillae, crystals in mesophyll cells, secretory tissues, and Kranz anatomy other characteristics were also of potential adaptive value in saline habitat (García *et al*., 2008).

A study on the foliar anatomy of shrubs in the semiarid region in Argentina, revealed that different species possess different types of trichomes which could be related to the adaptation to environmental stresses (Arambarri *et al*., 2011). Similarly, an investigation was carried out on the use of leaf anatomical traits for better understanding of the plant-environment relationships and to the development of technologies for a sustainable use and conservation of Chacoan forests in Argentina (Arambarri *et al*., 2012).

Subsequently, a study was undertaken on morpho-anatomical characteristics of *Celtis ehrenbergiana* showed that its shade leaf structure possessed bifacial, epidermis with wavy-sinuous anticlinal cell walls, mesophyll formed by palisade and spongy parenchyma, and angular-lacunar collenchyma. The sun leaf type had a thick leaf-limb which was leathery and dark green and its anatomical structure was equifacial, epidermis with straight anticlinal cell walls, mesophyll formed by homogeneous palisade parenchyma, and angular-massive collenchyma. Stomata were absent or scarce on adaxial surface in shade type leaf, however there were numerous in the sun type leaf. *Celtis ehrenbergiana* exhibited phenotypic plasticity showing an adaptation to survive in different climatic conditions and has an advantage over other species (Nughes *et al*. 2013). In the context of the literature survey, the present study was undertaken to determine the variability in leaf anatomical traits of few woody species adapted to semiarid condition in northeastern Mexico.

Materials and Methods

Five leaves of each woody species were collected from the Tamaulipan thornscrub forest, northeastern Mexico. Transverse sections of leaves were cut manually with the help of fine razor blades, then passed through different grade of alcohol from 50% to 100% alcohol, stained with safranin and finally made permanent slides and taken photograph with the help of digital camera fixed in a microscope. We cut transverse sections of 26 species to evaluate variations in leaf anatomical characters such as thickness of cuticle, epidermal cell layers, length, compactness of palisade layers and so on.

Results

A leaf in a transverse section consists of the upper and lower epidermis and mesophyll tissue consisting of palisade cells below the upper epidermis followed by loose spongy tissue containing air spaces. The upper epidermis consists of epidermal cells with cuticle varying in thickness among species. The epidermis may possess trichomes or glands of varying shapes or forms depending on species. Thick cuticle prevent loss of water by transpiration. The epidermal cells vary in size, shapes, layers. Below the upper epidermis a layer of palisade cells is present containing dense chloroplasts varying in length and compactness. The presence of compact palisade cells and its length is expected to prevent loss of water by transpiration which could impart drought resistance under arid environments. The thickness of spongy tissue and leaf thickness vary from species to species.

It is observed that there exists a large variability in anatomical traits of leaf lamina with respect to presence of trichomes, thickness of cuticle, epidermal cells, length of palisade cells and spongy parenchyma (Figs. 1-4). Each species has specific characteristics which help in the taxonomic delimitation of the species. Few species possess two layers of compact palisade cell viz., *Karwinskia humboldtiana, Lantana macropoda, Celtis laevigata, Havardia pallens*, and *Prosopis laevigata*, and *Acacia wrightii* has palisade cells both below adaxial and abaxial surface. Several species have hypostomatic cavity to maintain microclima and reduce loss in transpiration.

The intensity and length of palisade tissue reflects the photosynthetic capacity of the species. On the other hand, compact palisade tissue function as impervious layer impeding the loss of water by transpiration.

It may be observed (Figs. 1-3) the species show large variations in leaf anatomical characteristics with respect to the presence or absence of trichomes, cuticle thickness both in the upper and lower epidermis, shape and size or epidermal cells, thickness of palisade tissue, compactness or looseness of spongy tissue. We mention here few of the characteristics.

1. Trichome. Only few species possess trichomes *viz*., *Condalia hookeri, Sargentia greggii, Cordia boissieri, Celtis pallida, Diospyros texana*, and *Bernardia myricaefolia.*
2. Cuticle. Many species possess thick cuticle *viz*., *Leucophyllum frutescens, Condalia hookeri, Caesalpinia mexicana, Cordia boissieri, Celtis pallida, Celtis laevigata, Diospyros texana, Karwinskia humboldtiana, Ebenopsis ebano, Lantana macropoda, Prosopis laevigata, Bernardia myricaefolia, Forestiera angustifolia, Acacia berlandieri, Guaiacum angustifolium,* and *Helietta parvifolia*.

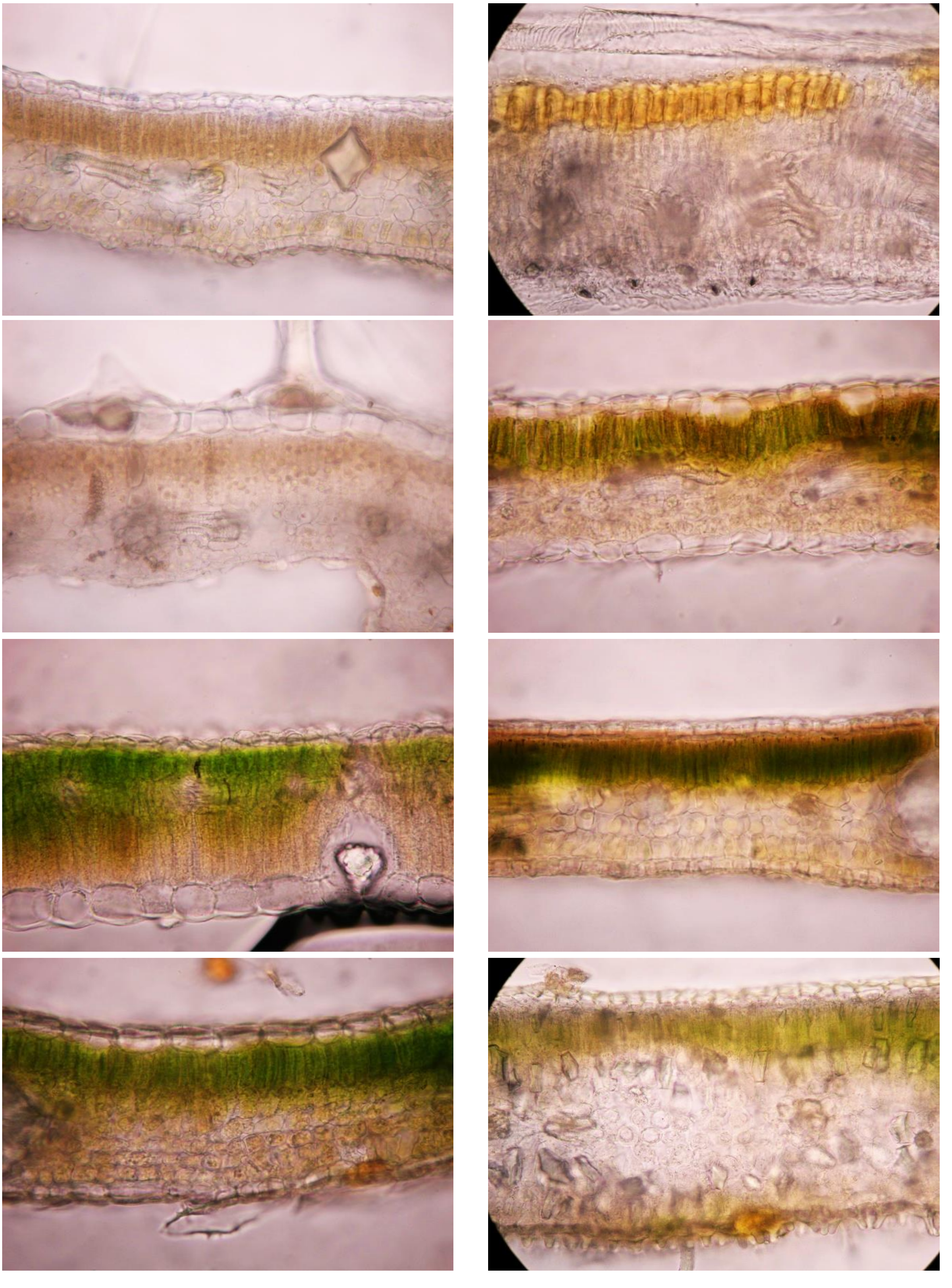


Fig. 1. Small and compact palisade cell
a. *Leucophyllum frutescens* (40x), b. *Condalia hookeri* M.C. Johnst. (40x), c. *Celtis laevigata* Willd., d. *Ebenopsis ebano* (Berland.) Barneby & J. W. Grimes, e. *Sideroxylon celastrinum* (Kunth) T.D. Penn, f. *Cercidium macrum* I.M. Johnst, g. *Diospyros palmeri* Eastw., h. *Amyris madrensis* S. Watson.

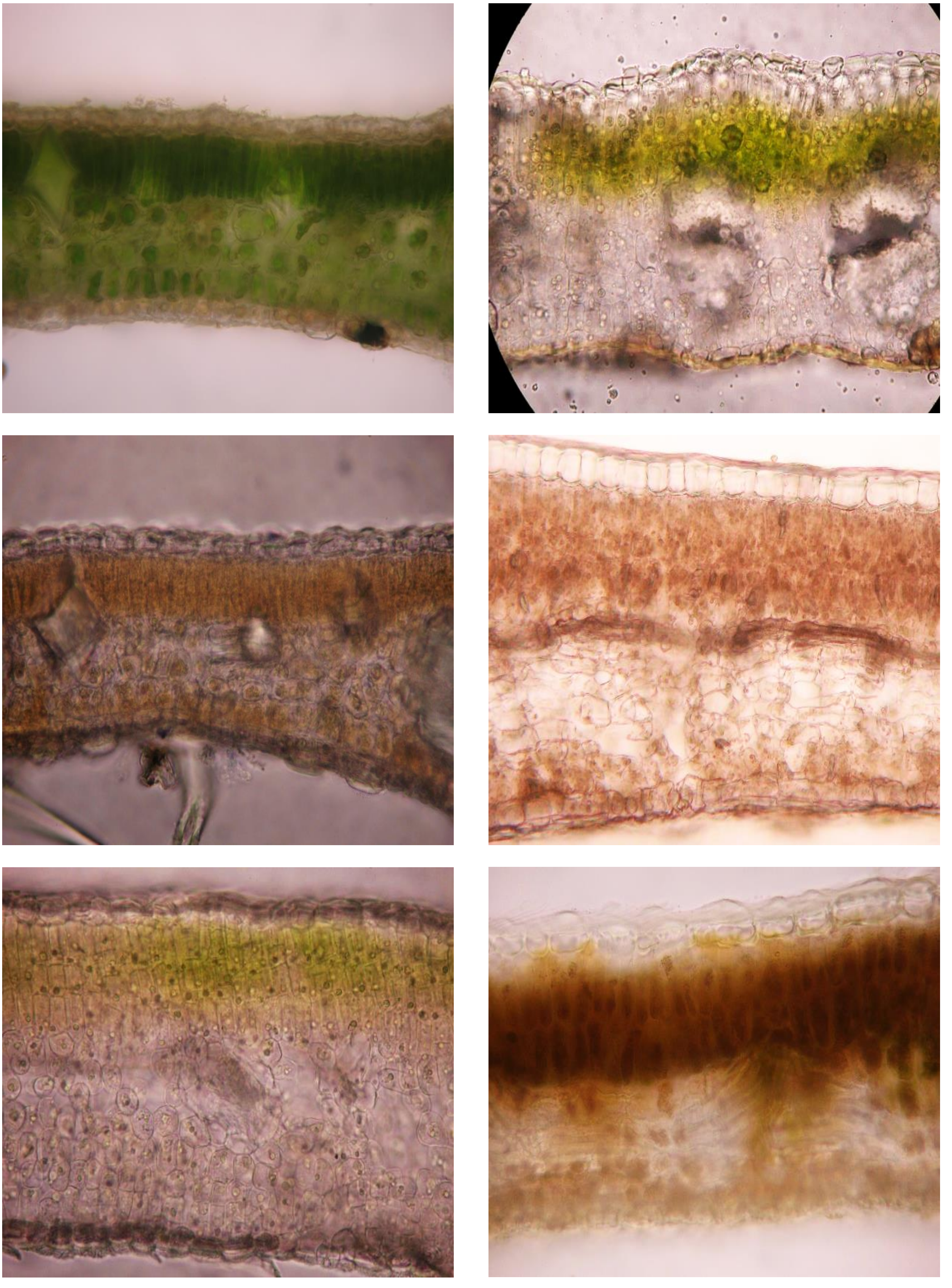
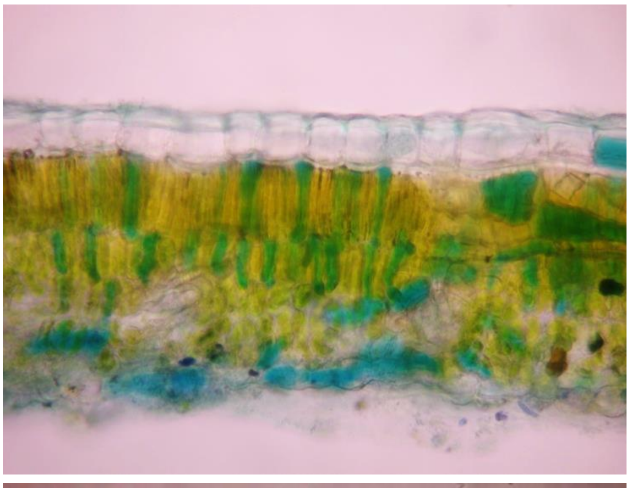


Fig. 2. Medium and compact palisade cell
a. *Sargentia greggii* S. Watson. (10x), b. *Diospyros texana* Scheele. (40x), c. *Forestiera angustifolia* Torr, d. *Guaiacum angustifolium* Engelm., e. *Havardia pallens* (Benth.) Britton & Rose, f. *Berberis chococo* Schltdl.



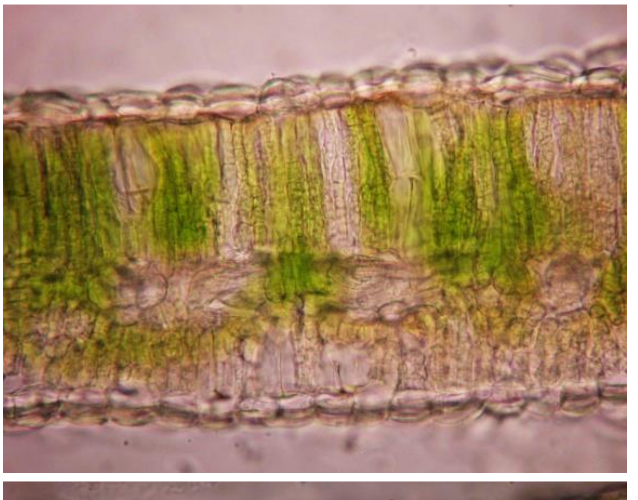
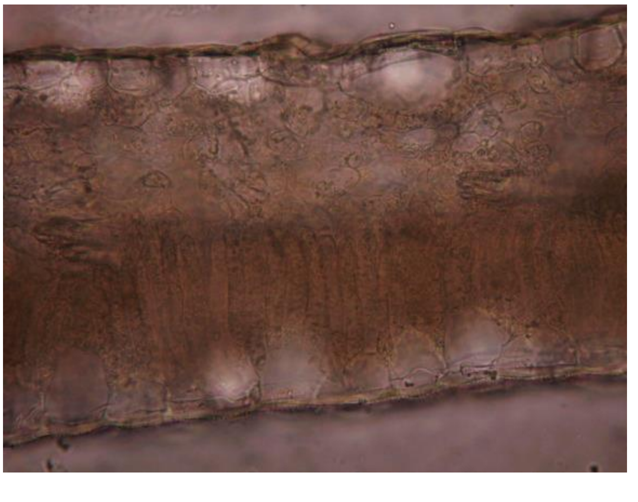
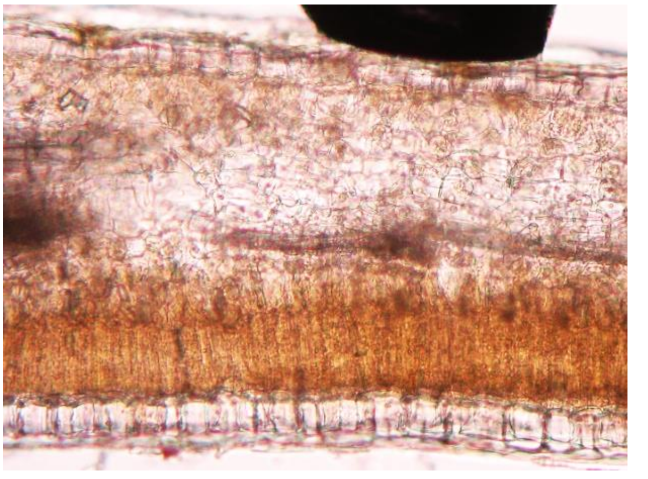
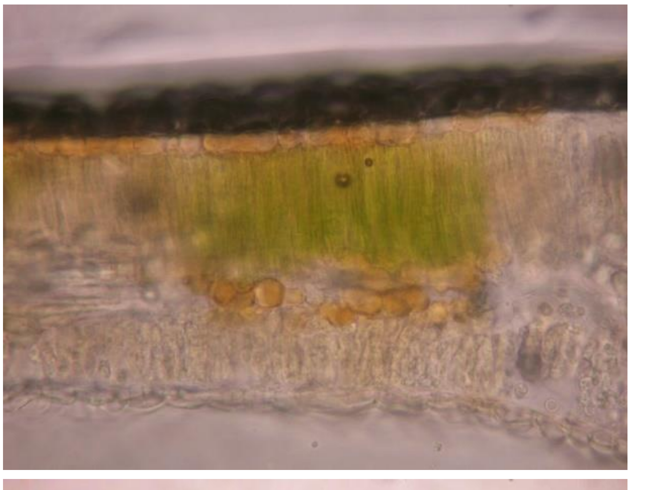
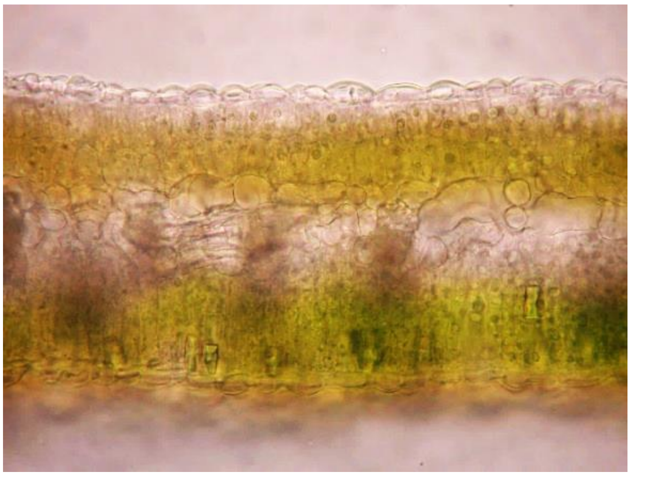
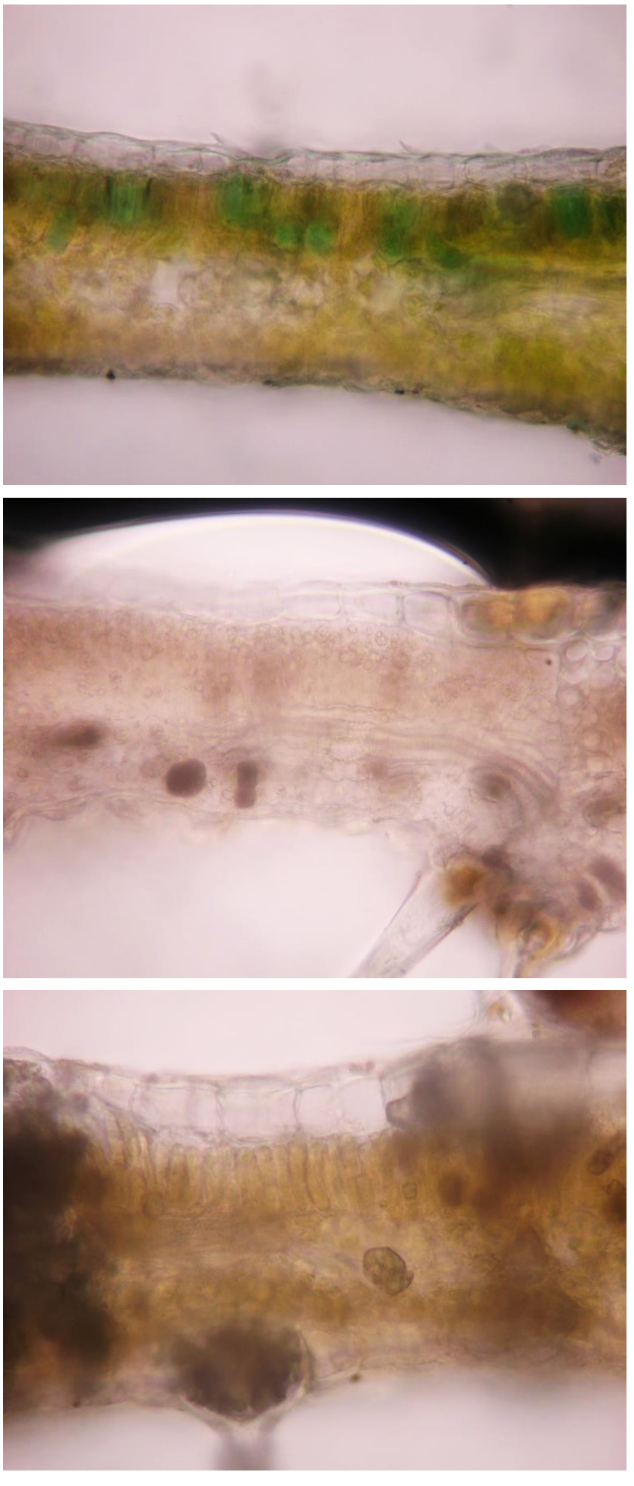






Fig. 3. Long and compact palisade cell
a. *Karwinskia humboldtiana* (Schult.) Zucc. (40x)
b. *Lantana macropoda* Torrey.
c. *Prosopis laevigata* (H. & B.) Jonhst
d. *Zanthoxylum fagara* (L.) Sarg.
e. *Helietta parvifolia* (Gray) Benth
f. *Acacia berlandieri* Benth.
g. *Acacia wrightii* Benth. ex A.Gray.



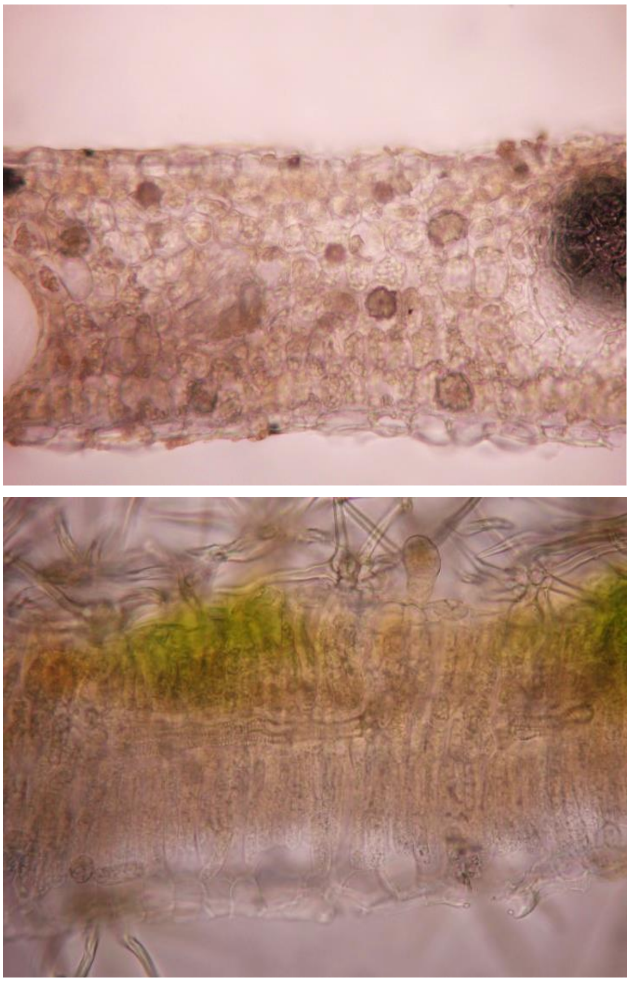


Fig. 4. Palisade cell loose
a. *Caesalpinia mexicana* A. Gray (40x)
b. *Cordia boissieri* A. DC. (40x)
c. *Celtis pallida* Torrey (40x)
d. *Amyris texana* (Buckley) P. Wilson. (40x)
e. *Bernardia myricaefolia* (Sheele) Benth. & Hook. F.

3. Epidermal cells mostly rectangular in shape, mostly small in size. Few species possess large epidermal cells *viz.*, *Condalia hookeri*, *Caesalpina mexicana*, *Cordia boissieri*, *Celtis pallida*, *Karwinskia humboldtiana*, and *Helietta parvifolia.*
4. Substomatal cavity. Few species possess substomatal cavity *viz.*, *Zanthoxylum fagara*, *Ceridium macrum*, and *Celtis laevigata*.
5. Spongy tissue. In general, spongy tissue is loose and greater in thickness compared to palisade tissue. Species having compact spongy tissue *viz*., *Ebenopsis ebano*, *Cercidium macrum*, *Forestiera angustifolia*, *Prosopis laevigata,* and *Lantana macropoda*.

On the basis of palisade cell length and compactness the woody species are classified into four categories.

1. Figure 1: Small and compact palisade cell (8): *Leucophyllum frutescens*, *Condalia hookeri*, *Celtis laevigata*, *Ebenopsis ebano*, *Sideroxylon celastrinum*, *Cercidium macrum*, *Diospyros palmeri*, and *Amyris madrensis.*
2. Figure 2. Medium and compact palisade cell (6): *Sargentia greggii*, *Diospyros texana*, *Forestiera angustifolia*, *Guaiacum angustifolium*, *Havardia pallens*, and *Berberis chococo.*
3. Figure 3. Long and compact palisade cell (7): *Karwinskia humboldtiana*, *Lantana macropoda*,

Prosopis laevigata, Zanthoxylum fagara, Helietta parvifolia, Acacia berlandieri and *Acacia wrightii.*

4. Figure 4. Palisade cell and loose (5): *Caesalpinia mexicana, Cordia boissieri, Celtis pallida, Amyris texana* and *Bernardia myricaefolia.*

Discussion

The results of the present study coincide with the results of various authors who used leaf anatomy in the taxonomic classification and possible adaptations to environmental stress as discussed below.

The species can be distinguished taxonomically in various characters such as the presence or absence of trichomes, cuticle thickness, size and shape of epidermal cells and compactness of spongy tissue. The results of the present study on the importance of stomata and epidermal cell characteristics in taxonomic delimitation of the species are in agreement with the findings of other researchers (Souza *et al*., 2004; Lin & Tan, 2015) used in the delimitation of species in Piperaceae, taxonomy of *Crocus* (Ozdemir & Ozdemir, 2010), in the genus *Kalanchoë* (Chernetskyy, 2012) and *Ficus* spp. (Sonibare *et al*., 2016). Few anatomical characters such as stellate trichomes, cystolitic trichomes, papilate epidermis, cystolitic trichomes (Arambarri *et al*., 2011; Veeramohan & Haron, 2015) are utilized to distinguish different species. The species studied in the Tamaulipan thornscrub are adapted in the semiarid environments in northeastern Mexico, although varying in its adaptability depending on the species (Stienen *et al*., 1989; González Rodríguez *et al*., 2011). Some of the species possess special traits related to adaption in the environments such as waxy leaf surface, sunken stomata, the absence of stomata on the adaxial and or abaxial surface, length and palisade cells thereby reducing loss in transpiration mentioned before. The presence of waxy leaf surface in most of the species such as *Amyris texana, Celtis pallida, Guaiacum angustifolium, Acacia berlandieri*, and *Karwinskia humboldtiana* could help in the reflectance of solar radiation, thereby keeping leaf temperature cooler. In addition, the presence of sunken stomata in many species *viz. Amyris texana, Celtis pallida, Guaiacum angustifolium, Forestiera angustifolia, Eysenhardtia texana, Parkinsonia texana, Gymnosperma glutinosum, Prosopis laevigata, Condalia hookeri, Diospyros palmeri, Karwinskia humboldtiana, Leucaena leucocephala, Forestiera angustifolia,* and *Ehretia anacua* helps to maintain microclimate in the stomatal cavity, thereby reduce transpiration in the semiarid environment. This hypothesis needs to be confirmed in future studies. In this respect, the variability in epicuticular wax has been observed in 37 woody species in Tamaulipan thorn scrub (Maiti *et al*., 2015). The species viz. *Karwinskia humboldtiana, Lantana macropoda, Prosopis laevigata, Zanthoxylum fagara, Helietta parvifolia, Acacia berlandieri* and *Acacia wrightii* having long and compact palisade cells are expected to be efficient in photosynthetic function and adaptation to drought. Future research needs to be directed in this direction.

Conclusions

In the context of the present results, there exists a large variability in anatomical traits of twenty six woody species which may be effectively used in the taxonomic delimitation of the species and capacity of adaptation to xeric conditions. It may be expected that the woody species with thick cuticle and compact palisade cells as in *Karwinskia humboldtiana* having two-layered palisade cells, thick cuticle and compact palisade cells in *Sargentia greggii, Celtis pallida* and *Diospyros texana* could prevent loss of water by transpiration, thereby imparting drought resistance. On the other hand, the species with loose palisade cells in *Caesalpinia mexicana* and *Amyris texana*, to some extent *Leucophyllum frutescens* could not impede loss of water by transpiration, thereby imparting susceptibility to drought. To confirm this hypothesis a concerted research is needed to relate the quantification of cuticular and palisade layer length to water relation of the species concerned.

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