Areolar structure in some Opuntioideae: occurrence of mucilage cells in the leaf-glochid transition forms in *Opuntia microdasys* (Lhem.) Pfeiff.

Emilia Cristina Pereira de ARRUDA
Departamento de Botânica, Centro de Biociências,
Universidade Federal de Pernambuco,
50670-901, Recife, Pernambuco (Brazil)
emilia.arruda@bol.com.br

Gladys Flavia MELO-DE-PINNA
Departamento de Botânica, Instituto de Biociências,
Universidade de São Paulo, CP 11461, 05422-970, São Paulo (Brazil)
gfmpinna@usp.br

Published on 30 December 2016

Arruda E. C. P. de & Melo-De-Pinna G. F. 2016. — Areolar structure in some Opuntioideae: occurrence of mucilage cells in the leaf-glochid transition forms in *Opuntia microdasys* (Lhem.) Pfeiff. *Adansonia*, sér. 3, 38 (2): 267-274. https://doi.org/10.5252/a2016n2a10

ABSTRACT
One of the most remarkable features of Cactaceae are the areoles, axillary outgrowths, which produce trichomes, spines and leaves. The subfamily Opuntioideae K. Schum. shows the widest diversity of transition forms between leaves and spines, which represents anatomical evidence that spines and glochids are modified leaves. The purpose of this paper is to provide an anatomical description of the areolar structure in four species of Opuntioideae, in order that new anatomical homology between spines/glochids and leaves may be clarified. Different patterns of areole morphology are observed: 1) *Austrocylindropuntia subulata* (Muehl.) Backeb. showing terete and persistent leaves; areoles with persistent spines, glochids and trichomes; 2) *Opuntia monacantha* (Willd.) Haw. with terete caducous leaves and persistent spines, glochids and trichomes; 3) *Opuntia rufida* Engelm. with terete deciduous leaves and areoles with glochids and trichomes; and 4) *Opuntia microdasys* (Lhem.) Pfeiff. with early caducous leaves and areoles showing trichomes and leaf-spine and leaf-glochid transition forms. The development of the areolar structures is very similar in all species, following the description of the literature for other Cactaceae Juss. In *O. microdasys* is described a new anatomical character in the family: mucilage cells in the leaf-glochid transition forms, which may have functional importance for water storage in species devoid of persistent leaves.

KEY WORDS
Cactaceae,
modified leaves,
anatomical homology.

RÉSUMÉ
Structure aréolaire de quelques Opuntioideae: mise en évidence de cellules à mucilage dans les pièces intermédiaires entre les feuilles et les glochides chez Opuntia microdasys *(Lhem.) Pfeiff.*
L'une des caractéristiques les plus remarquables des Cactaceae sont les aréoles, excroissances axillaires produisant des trichomes, des épines et des feuilles. La sous-famille des Opuntioideae K. Schum. présente la plus grande diversité de formes de passage entre les feuilles et les épines, ce qui prouve anatomiquement que les épines et les glochides sont des feuilles modifiées. Le but de ce travail est de décrire en détail la structure aréolaire dans quatre espèces d'Opuntioideae, afin de préciser les homologies. Différentes organisations sont observées : des aréoles à épines, glochides et trichomes persistants, associées à des feuilles étroites persistantes chez *Austrocylindropuntia subulata* (Muehl.) Backeb., ou caduques chez *Opuntia monacantha* (Willd.) Haw. ; des aréoles à glochides et trichomes, mais sans épines, associées à des feuilles étroites décidues chez *Opuntia rufida* Engelm. ; enfin des aréoles à trichomes accompagnés de formes intermédiaires (épines et glochides foliacés), aréoles associées à des feuilles rapidement caduques chez *Opuntia microdasys* (Lhem.) Pfeiff. Le développement des structures aréolaires est très semblable dans toutes ces espèces et confirme la littérature existante. Un nouveau caractère anatomique est décrit chez *O. microdasys* : la présence de cellules à mucilage dans les formes de transition entre feuilles et glochides, qui pourraient jouer un rôle important de stockage de l'eau dans une espèce dépourvue de feuilles persistantes.

MOTS CLÉS
Cactaceae,
feuilles modifiées,
homologie anatomique.

INTRODUCTION

Opuntioideae is the second largest of three traditionally recognized subfamilies of Cactaceae with approximately 350 species, and show a great diversity of areole morphology and leaf characters, including non-persistent or early deciduous terete leaves and persistent leaves (Barthlott & Hunt 1993; Anderson 2001; Griffith 2009).

Areoles represent a morphological synapomorphy of the cactus family defined as axillary outgrowths, which produce spines instead of foliage leaves and trichomes (Boke 1944), as well as roots, flowers, fruits and branches or stem segments (Anderson 2001; Nyffeler 2002).

In Opuntioideae two types of spines can occur in areoles: 1) persistent spines firmly attached to areole; and 2) deciduous tiny spines, termed glochids (Gibson & Nobel 1986). In general, glochids differ from spines in size (smaller than spines) and for the barbed cells along of the structure (Boke 1941, 1944). However, Norman H. Boke was one of the most important researchers in areoles, who describes transition forms between spines and glochids in *Opuntia cylindrica* (Lam.) DC. (Boke 1944); areole development in *Trichocereus spachianus* (Lem.) Riccob. (Cactoideae) (Boke 1941) and four *Echinocereus* (Cactoideae) species (Boke 1951). According to Boke (1944) *Opuntia cylindrica* has transition forms, which show features leaf-like as constriction at the base; reduced lignified region at the apex and vascular tissue.

The purpose of the present paper is to provide an anatomical description of areole structure of four Opuntioideae species, showing new characters what we believe to be important contribution to understanding of the evolutionary aspects involving leaves and spines/glochids in this subfamily.

MATERIALS AND METHODS

Adult individuals of four species were collected and the material was sent to specialists for identification and subsequently deposited in the herbarium of the Departamento de Botânica da Universidade de São Paulo (SPF). Were analyzed samples of three individuals of *Austrocylindropuntia subulata* (Muehl.) Backeb., *Opuntia monacantha* (Willd.) Haw., *Opuntia microdasys* (Lhem.) Pfeiff. and *Opuntia rufida* Engelm.

Samples of the areole were fixed in FAA (formalin/acetic acid/alcohol 50%) for 48h and stored in 70% ethanol. The material was dehydrated through an ethanol/ tertiary butanol series and embedded in paraffin (Ruzin 1999). Sections were cut on a rotary microtome (10-20 µm) and then double stained in astra blue-safranin 9:1 (modified by Bukatsch 1972) and mounted in Entellan. Mucilage cells were detected by ruthenium red staining according to Gregory & Baas (1989).

Stem samples fixed in Karnovsky (Karnovsky 1965) were dehydrated through an ethanol series and dried to the critical point with CO_2 (CPD 030, Balzer), mounted on stubs with double-sided carbon adhesive tape and gold-coated (Ruzin 1999). The samples were analyzed using a Zeiss DSM 940 scanning electron microscope.

RESULTS

The different morphological types of areole and leaves are shown in the Figure 1A-D. In *Austrocylindropuntia subulata* the persistent leaves are terete and the areoles show persistent spines, glochids and trichomes; *Opuntia monacantha* has terete deciduous leaves and persistent spines, glochids and trichomes; *Opuntia rufida* with terete

FIG. 1. — Morphology of the areoles and leaves: **A**, *Austrocylindropuntia subulata* (Muehl.) Backeb. with persistent leaves; areoles with persistent spines; **B**, young segment of the cladodes in *Opuntia monacantha* (Willd.) Haw. showing the terete deciduous leaves and areoles with persistent spines. Detail of the adult segment stem showing the persistent spines and the absence of leaves; **C**, *Opuntia rufida* Engelm. with terete deciduous leaves (**arrow**) in young stem segment; **D**, *Opuntia microdasys* (Lhem.) Pfeiff., showing absence of leaves and areoles with many glochids, but no persistent spines. Photos by Emilia Cristina Pereira de Arruda. Scale bars: 1 cm.

deciduous leaves and areoles with glochids and trichomes, and *Opuntia microdasys* with early caducous leaves and areoles with trichomes, glochids, leaf-spine and leaf-glochid transition forms.

In the early development the areole meristem produces multicellular trichomes in adaxial and abaxial sides of the areole (Fig. 2A), following by glochids, persistent spines and transition forms. The trichomes develops from the protodermis, with a multicellular base and lignified apex (Fig. 2B). In the species with leaves, the development of the other structures begins by a central structure, following by two lateral structures, as shown in Figure 2C.

Persistent long spines were observed only in *Austrocylindropuntia subulata* and *Opuntia monacantha* (Fig. 2D), which are multiseriate structures. All primordia of spines, glochids and transition forms show a basal meristematic zone and elongation zone at the apex (Fig. 2E) and a centrifugal differentiation (Fig. 2F, G). The mature structure is different between spines/glochids and transition forms. The persistent spines show completely lignified (apex to base), and without barbed apex (Fig. 3A, B). The glochids are smaller than persistent spines, and the mature glochids show lignified barbs cells (Fig. 3C, D).

Transition forms (leaf-spine and leaf-glochid) were observed only in *O. microdasys* (Fig. 4A-E), which are mainly

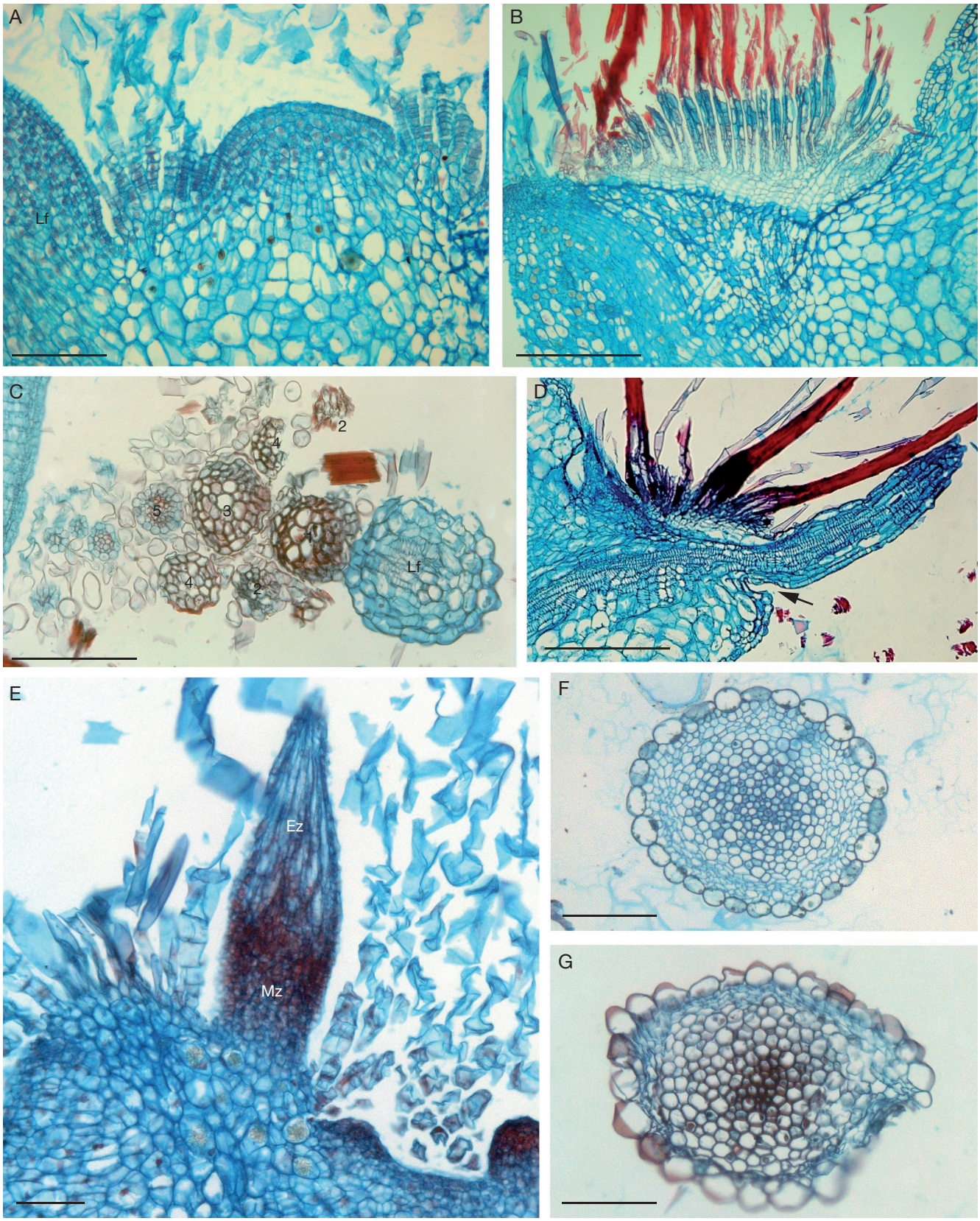

Fig. 2. — Longitudinal sections of the areoles; **A**, longitudinal section of the areole meristem of *Austrocylindropuntia subulata* (Muehl.) Backeb., showing the trichomes in adaxial and abaxial sides; **B**-**D**, areole of *Opuntia rufida* Engelm.; **B**, longitudinal section showing multicellular trichomes with lignified apex; **C**, numbers of the structures produced from areole meristem, sequence of first to last glochid (1 to 5); **D**, mature areole with persistent spines and trichomes. Note constriction at the leaf base (**arrow**); **E**, longitudinal section of persistent spine primordia of *O. monacantha* (Willd.) Haw. showing basal meristematic zone (**Mz**) and elongation zone (**Ez**) at the apex; **F**, **G**, transverse sections of the persistent spine in middle (**F**) and basal region (**G**) of *A. subulata* with the centrifugal differentiation. Abbreviation: **Lf**, leaf. Scale bars: A-E, 40 µm; F, G, 80 µm.

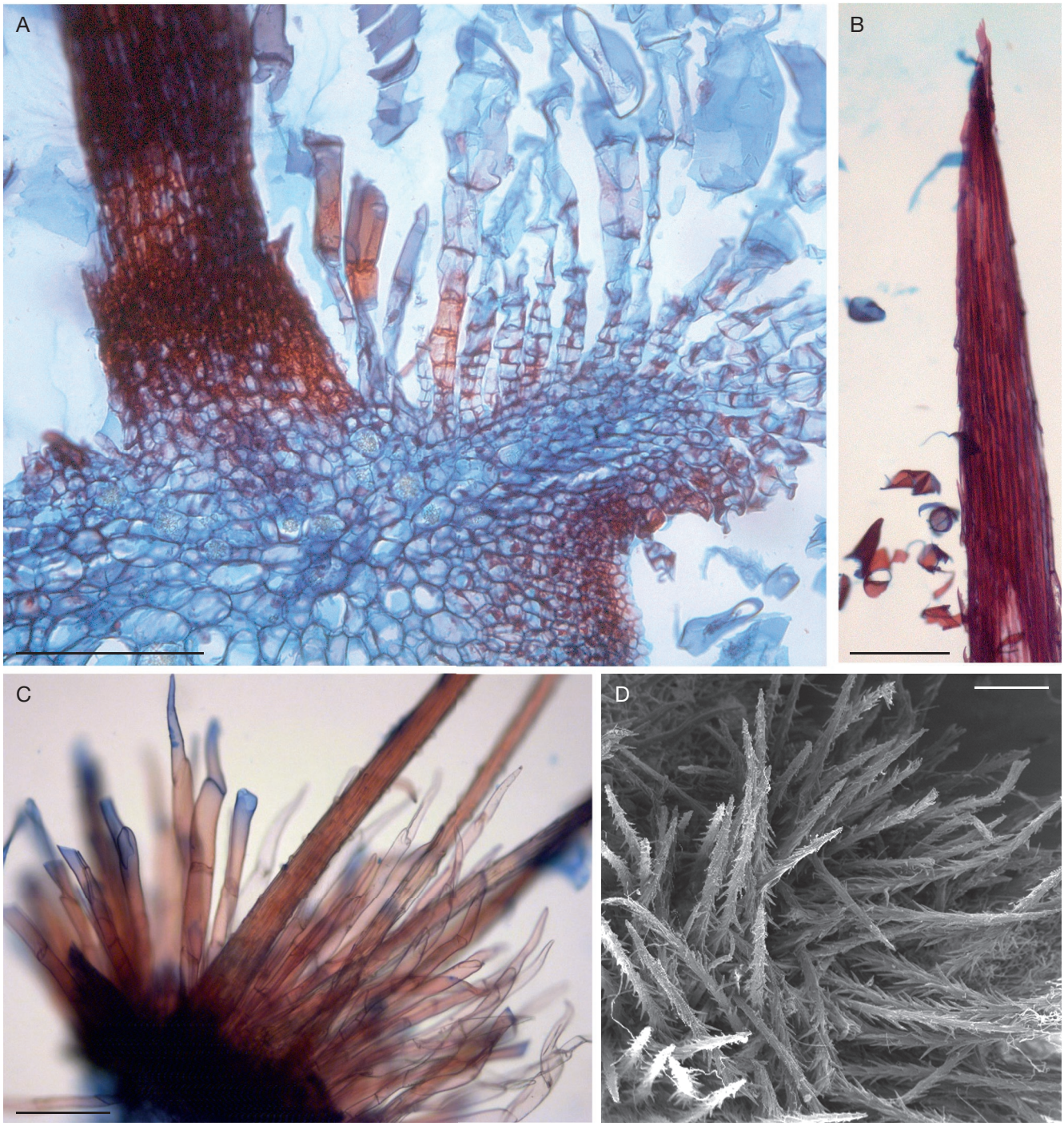

FIG. 3. — Longitudinal sections of the areoles: **A**, **B**, areole of *Opuntia monacantha* (Willd.) Haw., showing mature persistent spines, with lignified base (**A**) and apex (**B**); **C**, areole of *Opuntia microdasys* (Lhem.) Pfeiff. with abundance of trichomes and glochids; **D**, scanning electron micrograph of the areole of *O. rufida* Engelm. Note barbed cells along of the glochids. Scale bars: A, B, 40 µm; C, 80 µm; D, 40 µm.

characterized by the enlarged base with a constriction (Fig. 4A, E) and vascular tissue (Fig. 4A, B). However, these structures differ by presence of lignified barb-like cells at the apex (Fig. 4A) and mucilage cells at the middle region (Fig. 4B-D) only in the leaf-glochid transition forms. In the leaf-spine transition, the structure is less lignified than leaf-glochid transition forms, limited only to the apical region (Fig. 4E).

DISCUSSION

The areole is an axillary bud situated on leaf base, whose the meristem produces trichomes, persistent spines, glochids and leaf-spines transition forms instead of foliage leaves (Boke 1944; Gibson & Nobel 1986). In the present studies, we demonstrate that the trichomes originate in a different way than spine, glochids and transition forms, as

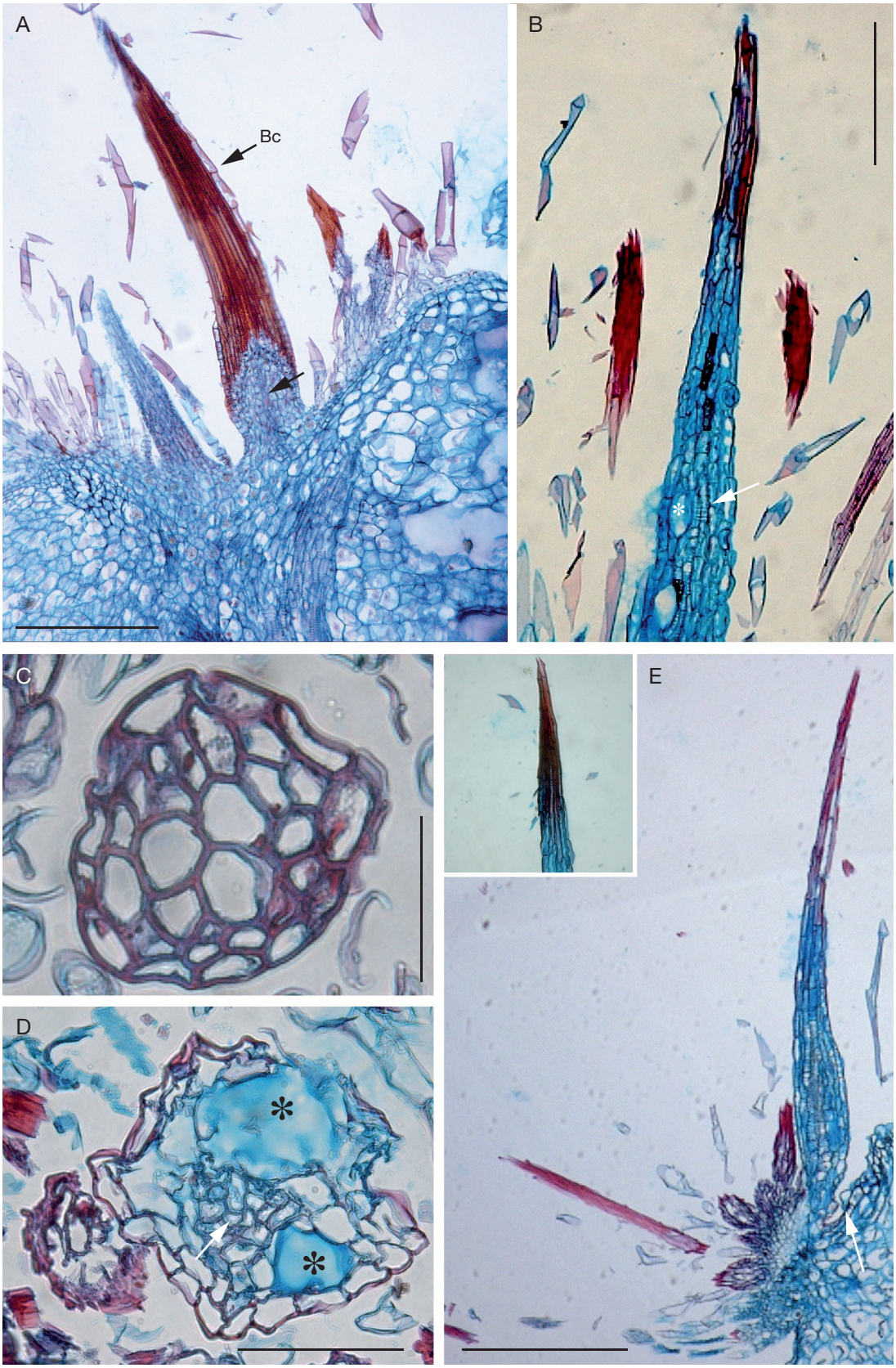

Fig. 4. — Transition forms on the areoles of *Opuntia microdasys*: **A**, longitudinal section of the leaf-glochid transition forms showing vascular tissue (**arrow**) and constriction at the base. Note barbs cells (**Bc**) at the apex; **B**-**D**, leaf-glochid transition forms; **B**, longitudinal section showing mucilage cell (*) and vascular tissue (**arrow**) at the middle region; **C**, **D**, sequence of different regions in transverse section; **C**, apex region; **D**, middle region showing mucilage cells (*) and vascular tissue (**arrow**); **E**, longitudinal section of the leaf-spine transition forms showing constriction at the base (**arrow**). Detail of the lignified cell at the apex (left corner) without barbs cells. Scale bars: A, B, 80 µm; C, D, 40 µm; E, 150 µm.

observed by Boke (1944) and Gibson & Nobel (1986) in other Cactaceae species. The uniseriate trichomes arise from a protodermis cell (tunica), which remains meristematic with periclinal divisions, producing cells that are pushed outward and have basipetal differentiation. However, all persistent spines, glochids and transition forms are products of the areolar meristem from the primordia.

The primordia of the spines, glochids and transition forms primordia have basal meristematic zone and elongation cell zone. According to Boke (1944) and Gibson & Nobel (1986) all spines (persistent spines and glochids) and leaf-spines transition arise from the apical growth for a very short period and, subsequently, the primordium growth by cell divisions at its base, which region is called the basal meristematic zone or an intercalary meristem. These growth regions are important anatomical aspects of the leaf development (Esau 1965), showing an evidence of the leaf origin of these structures in Cactaceae. Anatomically, the leaf development shows apical growth, with anticlinal and periclinal divisions in the outer layers at the tip of the primordium, and intercalary growth, with length increase of the leaf axis (Foster 1936; Eames & MacDaniels 1947; Esau 1965). Other phases during the leaf development, as adaxial growth (increase in width on the adaxial side of the leaf primordium) and marginal growth (development into the lamina), are not observed in the spines/glochids and transition forms development. However, Boke (1944) described a peripheral meristem during development of the large spines, as homologous to the peripheral meristem of the leaf and leaf base. Peripheral meristem is described in the development of terete leaves, i.e., leaves lacking a flattened blade and no obvious dorsiventrality (Boke 1944; Freeman 1970; Gibson 1977).

As described by Boke (1944) and Gibson & Nobel (1986), we also reported persistent spines and glochids showing basipetal differentiation, whose cells became completely lignified, except at the base of glochids and transition forms. According Gibson & Nobel (1986), this process could be related to the evolutionary loss of the signal that initiates vascular system differentiation, which comes from the apex in the leaf primordia, and in the apex of persistent spines and glochids the cells are sclerified and dead. In persistent spines the vascular tissue does not penetrate the basal meristematic zone, where cells are actively dividing. After division, the cells are dead and sclerified, as on the apex. However, in the glochids and transition forms, as observed in *Opuntia microdasys*, there are vascular tissues at the middle region, although these structures show lignified cells at the apex. In this case, it is possible that the lignification of the cells occurs after the signal that initiates vascular system differentiation or the apical cells are not dead, allowing the signaling from the apex.

We described a first report of mucilage cells along of the leaf-glochid transition forms in *O. microdasys*. Mucilage structures are reported in many tissues of Cactaceae and are related to protection, storage and taxonomy (Gibson & Nobel 1986; Loza-Cornejo & Terrazas 2003). The mucilage is usually related to water storage and is considered a major source of calcium and sugars, acting in defense against high temperatures (Gibson & Nobel 1986). Therefore, the presence of mucilage cells and vascular tissue in the persistent leaf-glochid transition forms of *O. microdasys* can be related to the supply of sugars, calcium and water, as well as protection against the incidence of high temperatures on the surface of the areole. In conclusion, our results show that *Opuntia microdasys* represents a very important model to prove evidences for evolutionary studies in cactus areoles.

Acknowledgements

The authors thank Marlon Machado of the Universidade Estadual de Feira de Santana-Bahia for help in identification of the botanical material, and CNPq – Conselho Nacional de Desenvolvimento Científico e Tecnológico (Process 308070/2012-7) and FAPESP – Fundação de Amparo à Pesquisa do Estado de São Paulo (Process 06/58023-2, 09/14708-0) for research grants and financial support. We also thank the reviewers, who helped to improve an earlier version of the manuscript.

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Submitted on 8 January 2015;
accepted on 17 March 2016;
published on 30 December 2016.