UNIVERSIDADE DE BRASÍLIA
Instituto de Ciências Biológicas
Departamento de Botânica
Programa de Pós-Graduação em Botânica

Anatomia caulinar comparada de subgêneros de *Cereus* Mill. (Cactaceae)

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Dedico essa dissertação a todas as mães que possuo...

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SUMÁRIO

RESUMO

Cactaceae é a principal família de plantas que ocupa os ambientes áridos e com o terceiro maior centro de diversidade localizado no nordeste e sudeste brasileiros. Esta família botânica apresenta conflitos taxonômicos, sendo *Cereus* Mill. talvez o gênero menos compreendido. O objetivo deste trabalho foi analisar a anatomia caulinar de espécies deste gênero, visando identificar caracteres que possam subsidiar sua taxonomia, bem como investigar a morfologia da cera epicuticular de espécies selecionadas. No Capítulo 1, amostras caulinares foram submetidas às técnicas usuais para análise estrutural e histoquímica de 16 espécies dos três subgêneros de *Cereus*, sendo uma espécie nova, além de *Praecereus saxicola* (Morong) N.P. Taylor. Os resultados foram fotografados e os dados compilados em tabela comparativa. Constatou-se que o caule em todas as espécies examinadas é revestido por epiderme unisseriada, com estômatos paracíticos e células-guarda reniformes. As camadas de colênquima são aclorofiladas e espessadas, seguidas por parênquima clorofiliano, parênquima aquífero e numerosos idioblastos mucilaginosos. Nas regiões mais jovens, os feixes vasculares são colaterais; já nas regiões maduras, há um cilindro vascular com calotas externas de fibras no floema. Estas características são semelhantes em todas as espécies do gênero. Os seguintes caracteres se mostraram úteis para a taxonomia do grupo: espessura da cutícula, presença de células papilosas, tamanho relativo das células epidérmicas comuns e sinuosidade de suas paredes anticlinais em vista paradérmica; largura das células-guarda e número de camadas do colênquima. Foi confeccionada uma chave de identificação para as espécies examinadas, acompanhadas de descrições anatômicas e pranchas ilustrativas. A nota científica (Capítulo 2), buscou explicar a variegação do caule de *Cereus spegazzinii* através da comparação de sua epiderme com outras espécies do gênero. A análise foi realizada sob microscopias ótica e eletrônica de varredura. Constatou-se que a variegação nos caules desta espécie se deve à descontinuidade das ceras epicuticulares na epiderme. Os dados contribuem para o conhecimento sobre estas importantes plantas da flora brasileira, possibilitando inclusive o entendimento do aspecto marmorizado do caule em *C. spegazzinii*, que é um estado de caráter único na família dos cactos.

Palavras-chave: Caracteres anatômicos – Cera epicuticular – Taxonomia – Xeromorfismo

ABSTRACT

Cactaceae is the main family of plants that inhabits arid environments, and its third largest center of diversity is in Northeastern and Southeastern Brazil. This botanical family presents taxonomic conflicts, with *Cereus* Mill. perhaps being the least understood genus. The objective of this work was to analyze the stem anatomy of species of this genus, identifying characters that can support its taxonomy, as well as to investigate the morphology of the epicuticular wax of selected species. In Chapter 1, stem samples were subjected to the usual techniques for structural and histochemical analysis of 16 species of the three subgenera of *Cereus*, including one new species, in addition to *Praecereus saxicola* (Morong) N.P. Taylor. The results were photographed and the data compiled in a comparative table. The stem of all studied species was covered by uniseriate epidermis with paracytic stomata and reniform guard cells. The collenchyma cells are achlorophyllous and thickened, followed by chlorophylous and aquiferous parenchymas and numerous mucilaginous idioblasts. The youngest, distal region has collateral vascular bundles, and the older, proximal regions have a vascular cylinder with external caps of phloem fibers. Such characteristics are similar in all species of the genus. The following characters are useful for the taxonomy of the group: thickness of the cuticle, presence of papillose cells, relative size of the common epidermal cells and sinuosity of their anticlinal walls in paradermal view; width of the guard cells and number of collenchyma layers. An identification key for the studied species was prepared, accompanied by anatomical descriptions and illustrative plates. The scientific note (Chapter 2) aimed to explain the variegation of the stem of *Cereus spegazzinii* by comparing its epidermis with other species of the genus. The analysis was performed using optical and scanning electron microscopy. It was found that the variegation in the stems of this species is due to the discontinuity of the epicuticular waxes in the epidermis. The data presented contribute to the knowledge about these important plants of the Brazilian flora, including the understanding of the marbling of the stem in *C. spegazzinii*, which is a unique character state in the cactus family.

Keywords: Anatomic characters – Epicuticular wax – Taxonomy – Xeromorphism

1.INTRODUÇÃO GERAL

Cactaceae é uma das principais famílias de plantas que ocupam ambientes áridos, totalizando cerca de 1435 espécies distribuídas em 127 gêneros (Hunt *et al.*, 2006; Barthlott *et al.*, 2015). Este grupo é quase totalmente exclusivo do Novo Mundo, ocorrendo desde o Canadá até o sul da América do Sul, com exceção de *Rhipsalis baccifera* (J.M. Muell.) Stearn que atinge a África, Sri Lanka e Madagascar, (Anderson, 2001; Carneiro *et al.*, 2016).

No Brasil, dos 38 gêneros e 276 espécies registrados, 15 gêneros e 206 espécies são endêmicas (Zappi & Taylor, 2020). O leste do Brasil representa o terceiro maior centro de diversidade dessas espécies no planeta (Zappi *et al.*, 2011; Cavalcante *et al.*, 2013), após o México e os Andes do norte da Argentina, Bolívia e Peru, que possuem as maiores taxas de riqueza e endemismo do mundo (Guerrero *et al.*, 2019).

De acordo com o APG (2016), as Cactaceae estão inseridas dentro das Superasterídeas, na ordem das Caryophyllales, representando plantas halófitas, xerófitas com metabolismo C4 ou CAM (metabolismo ácido das crassuláceas) (Guerrero *et al.*, 2019). De acordo com as pesquisas de Hernández *et al.* (2011) e Guerrero e*t al.* (2019), é um grupo monofilético fundamentado tanto pela morfologia quanto pelos dados moleculares e divido em 4 subfamílias: Pereskioideae, Opuntioideae, Cactoideae e Mahiuenoideae, que com base em análises filogenéticas e amostragem dos táxons, foi acrescida recentemente sendo um grupo irmão das Cactoideae.

A maioria das espécies de Cactaceae estão distribuídas na subfamília Cactoideae (Eggli, 1984; Hernandez *et al.*, 2011) e o gênero *Cereus* Mill. está localizado dento da tribo Cereeae subtribo Cereinae (Romeiro-Brito *et al*., 2023; Taylor *et al*., 2023), com representantes de espécies colunares neotropicais (Franco *et al.*, 2017). *Cereus* possui cerca de 20 espécies, com 8 endêmicas (Zappi & Taylor, 2020) distribuídas em 4 subgêneros: *Mirabella* (F. Ritter) N.P. Taylor, *Ebneria* (Backeb.) D.R. Hunt, *Cereus* e *Oblongicarpi* (Croizat) D.R. Hunt & N.P. Taylor (Hunt *et al.*, 2006; Franco *et al.*, 2017).

Mauseth (1996) procurou reavaliar as relações filogenéticas de membros da subfamília Cactoideae por meio da anatomia e identificou a existência de caracteres que reorganizaram a taxonomia do gênero de *Monvillea* Britton & Rose, no qual as três espécies estudadas (*Monvillea difusa* Britton & Rose, *Monvillea maritima* Britton & Rose e *Monvillea smithiana* (Britton & Rose) Backeb.) apresentaram estruturas anatômicas similares. Os dados obtidos levaram Mauseth (1996) a manter as espécies no gênero de *Monvillea*, apesar da semelhança com a anatomia de *Cereus hexagonus* (L.) Mill. principalmente devido à presença de grandes

drusas na hipoderme externa e cristais no córtex de *Monvillea*. Apesar de sinonimizado ao gênero *Cereus* (Hunt, 1988; Taylor & Zappi 2004; Hunt *et al.*, 2006) asconclusões de Mauseth (1996) contribuíram para a segregação dessas espécies sob *Cereus* subgênero *Ebneria* (Backeb.) D.R. Hunt.

Apesar das pesquisas sobre a filogenia da tribo Cereeae (Guerrero *et al.*, 2019; Romeiro-Brito, 2023; Taylor *et al*., 2023), e questões evolutivas terem sido esclarecidas, Arruda *et al.* (2005), abordam que as aplicações taxinômicas das estruturas externas e internas ainda são limitadas, principalmente para as espécies presentes no Brasil.

Wallace & Gibson (2002) enfatizaram que pesquisas direcionadas para o entendimento dos padrões de evolução das Cactaceae são necessárias, principalmente para elucidar a delimitação dos gêneros e circunscrição das espécies, associando os dados moleculares aos caracteres morfológicos. Para Terrazas & Mauseth (2002), essa família apresenta potencial a ser estudado, justamente por possuir caracteres morfológicos e anatômicos significativos para distinção de seus táxons, complementando ainda que existem muitas estruturas anatômicas que não são compreendidas e necessitam de investigações mais aprofundadas.

Para Terrazas & Mauseth (2002) os caules das Cactaceae possuem epiderme unisseriada, composta por cutícula hidrofóbica espessada, parênquima clorofiliano e fotossintetizante, com drusas, feixes corticais e medulares. Arruda & Melo-de-Pinna (2015) listaram os caracteres mais relevantes para a família, como a presença de várias camadas de células hipodérmicas com a função de proteção e que representam caracteres diagnósticos para a família, assim como outras estruturas associadas à epiderme, como a cutícula, tipos de cristais, células comuns e complexos estomáticos. A análise das estruturas anatômicas é um eficiente meio de estabelecer caracteres que possam diagnosticar as espécies dentro dos grupos, como o espessamento das paredes da hipoderme, tipos de espinhos, presença ou ausência de feixes vasculares corticais ou medulares e de estruturas secretoras (Arruda *et al.*, 2005). Terrazas & Arias (2003); Calvente e*t al.* (2008) salientaram principalmente a importância dos caracteres anatômicos da região da epiderme para o estudo das Cactoideae.

Judd *et al.* (2009) destacam que a família detém conflitos relacionados à taxonomia dos gêneros e espécies. Em justificativa, Terrazas & Arias (2003) apontam que as características anatômicas das Cactaceae são úteis para a delimitação dos gêneros dentro de Cacteae e reconhecem que a associação de caracteres morfoanatômicos é importante para sustentar análises filogenéticas, podendo auxiliar na taxonomia e compressão dos processos evolutivos, bem como na conservação das Cactoideae.

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CAPÍTULO I – STEM ANATOMY OF *CEREUS* (CACTACEAE, CEREEAE) AS A CONTRIBUTION TO TAXONOMY AND SYSTEMATICS

To be submitted to Acta Botanica Brasilica

RESUMO (não será inserido no artigo)

Cereus é um gênero de Cactaceae com 31 espécies, tradicionalmente subdivididas em quatro subgêneros (*C.* subg. *Cereus*, *C.* subg. *Ebneria*, *C.* subg. *Mirabella* e *C.* subg. *Oblongicarpi*). Dada a necessidade de levantar dados morfológicos que suportem os estudos filogenéticos recentes, o presente trabalho teve como objetivo investigar e comparar anatomicamente os caules de representantes dos subgêneros de *Cereus*, buscando contribuir para a taxonomia do grupo. A anatomia e histoquímica caulinar foram analisadas para 16 espécies representantes distribuídas em três dos subgêneros mencionados acima, além de uma espécie de *Praecereus*. Foram preparadas uma tabela comparativa, uma chave de identificação e descrições anatômicas detalhadas das espécies, acompanhadas de pranchas ilustrativas. Os seguintes caráteres são distintivos para as espécies, relativos à epiderme: espessura da cutícula, formato das paredes anticlinais, espessura relativa das células comuns, relação comprimento/largura das células-guarda, séries e número de células auxiliares estomáticas; no córtex: número de camadas colenquimáticas e tipo de reforço; presença de idioblastos mucilaginosos gigantes, presença e tipo de cristais (prismáticos ou drusas); na vascularização: presença de xilema e floema secundários. Os caráteres analisados contribuem para a robustez da taxonomia de *Cereus* e coadunam com outros estudos sobre a família. Apesar de dados filogenéticos recentes não terem dado suporte aos subgêneros tradicionais, a abordagem morfológica apoia a delimitação dos subgêneros. Devido ao ambiente árido, as adaptações para armazenar e minimizar a perda de água são marcantes, tais como estômatos deprimidos, com cristas nas células-guarda, cutícula espessa e parênquima aquífero; todas estas características foram constatadas nas espécies analisadas.

Palavras-chave: adaptações anatômicas, cactos, diagonal seca, floresta estacional decidual, semiárido brasileiro.

ABSTRACT

Cereus is a genus of Cactaceae comprising 31 species, traditionally seggregated into four subgenera (*C.* subg. *Cereus*, *C.* subg. *Ebneria*, *C.* subg. *Mirabella* and *C.* subg. *Oblongicarpi*). Due to the need to find morphological characters that may support recente phylogenecti studies, the objective of the present work was to investigate and compare the stem anatomy of representatives of the subgenera of *Cereus*, aiming to contribute towards the taxonomy of this group. Analyses of the anatomical and histochemical characters of 16 species distributed in the three of the subgenera above mentioned, as well as a species of *Praecereus* were carried out. A comparative table, an identification key, detailed anatomical descriptions of the species stems, and illustrative plates were prepared. The following characters were found to be useful to distinguish species: for the epidermis, cuticle thickness, anticlinal cell wall shape, relative thickness of common cells, relation between length-width of guard cells, series and number of auxiliar stomatic cells; cortical characters such as the number of collenchyma cells and type of reinforcement, presence of giant mucilaginous idioblasts, presence and type of crystals (prismatic or druses); for the vascularization, the presence of secondary xylem and phloem. The characters studied contribute to the taxonomy of *Cereus* and correlate with other studies regarding the family. Despite the lack of support for the traditional subgenera in the recent phylogenies, the present morphological approach supports their delimitation.

Due to the arid environment, the adaptations to store and minimize water loss are remarkable, such as depressed stomata, with ridges on the guard cells, thick cuticle, and aquiferous parenchyma, and all these characteristics were observed in the analysed species.

Keywords: anatomical adaptations, Brazilian semiarid, cacti, deciduous seasonal forest, dry diagonal.

INTRODUCTION

While cactus taxonomy (Berger, 1905; Britton & Rose, 1909; Hunt *et al.*, 2006) was aptly followed by phylogenetic studies (Wallace & Gibson, 2002; Edwards, 2006; Majure *et al.*, 2019; Romeiro-Brito *et al.*, 2023), comprehensive work regarding their micromorphology has been less expressive (Boke, 1944; Mauseth, 1996; Barthlott & Hunt, 1993; Hernández-Hernández *et al.*, 2011; Arruda *et al.*, 2005). Wallace & Gibson (2002) stated that the understanding of Cactaceae evolution patterns depends on the association of molecular and morphological data, to better circumscribe and understand tribal, generic, and specific delimitation. The genus *Cereus* Mill. comprises 31 species distributed in four subgenera: *C.* subg. *Cereus*, *C.* subg. *Mirabella* (F. Ritter) N.P. Taylor, *C.* subg. *Ebneria* (Backeb.) D.R. Hunt, and *C.* subg. *Oblongicarpi* (Croizat) D.R. Hunt & N.P. Taylor (Hunt *et al.*, 2006; Franco *et al.*, 2017).

Species of *Cereus* share macromorphological characters such as elongated, few ribbed stems, absence of woolly floriferous regions, freshly-scented hawkmoth-pollinated flowers with long, narrow tubes and versatile stamens, smooth, rarely areolate flower tube, oblong or ovoid fruits with solid, white pulp (Berger, 1905; Britton & Rose, 1909; Taylor & Zappi, 2004). Species are cohesive and difficult to differentiate (Franco *et al.*, 2022). Despite the potential of phylogenies to support phylogenetic relationships within the family at several levels (Martínez-Quezada *et al.*, 2020), recent phylogenetic analyses failed to retrieve the abovementioned four groups (Taylor *et al*., 2023).

At the end of last century there were still few anatomical studies to support the systematics of Cactaceae (Nyffeler & Eggli, 1997). The stem structure of these plants is equivalent to that of eudicots, counting with epidermis, cortex, medulla, and vascular bundles (Anderson, 2001). Metcalfe & Chalk (1979) highlighted anatomical characters of the family such as the orientation of stomatic pores in relation to the plant axis that could help in species differentiation, using also the epidermic cells, stomata, and parenchyma for the same purpose.

The integration of anatomical characters with physiology, morphology and phylogenies may be fundamental to distinguish cactus genera and species, especially when investigating the fundamental and vascular stem tissues (Nyffeler & Eggli, 1997; Terrazas & Mauseth, 2002; Soffiatti & Angyalossy, 2007; Arruda *et al.*, 2005, 2015, 2016). According with Mauseth (1996) and Hunt *et al.* (2006), *Cereus* is perhaps one of the least known genera of cacti, however recent phylogenies of tribe Cereeae (Romeiro-Brito *et al.*, 2023) and focussing specifically on the genus (Taylor *et al.*, 2024) retrieve it as a more or less cohesive group of species, however we

still lack distinguishing characters to circumscribe species and species groups within *Cereus*. The aim of this research was to perform a comparative analysis of *Cereus* stems, looking for characters that aid in the distinction of the species. We expect to contribute towards cactus taxonomy, adding diagnostic characters and discussing their relevance for the evolution and conservation of the group.

MATERIAL AND METHODS

Stem samples of 13 to 23 cm long were obtained from the apex of adult, healthy individuals belonging to 17 species (with one representative of each) of Cactaceae subtribe Cereineae from the Cerrado and Caatinga biomes (Table 1), collected with vouchers and determined by Daniela Zappi and Gerardus Olsthoorn.

Table 1. Cactaceae species analysed according to the subgenera and voucher numbers (GO = G. Olsthoorn; Z = D.C. Zappi).

Species	**Subgenera**	**Voucher**	**Herbarium**	**Herbarium number**
Cereus bicolor Rizzini & Mattos	*Cereus*	GO 541	SORO	SORO008173
Cereus fernambucensis Lem.	*Cereus*	GO 196a	SORO	n.a.
Cereus fernambucensis subsp. *sericifer* (F. Ritter) N.P. Taylor & Zappi	*Cereus*	GO 460	SORO	n.a.
Cereus gerardii N.P. Taylor	*Cereus*	GO 836	SORO	SORO007989
Cereus hexagonus (L.) Mill.	*Cereus*	GO 835	SORO	SORO002773
Cereus hildmannianus K. Schum.	*Cereus*	GO 190	SORO	
Cereus insularis Hemsl.	*Cereus*	s/n	SORO	SORO002677
Cereus jamacaru DC.	*Cereus*	GO 477	SORO	SORO007967
Cereus jamacaru subsp. *calcirupicola* (F. Ritter) N.P. Taylor & Zappi	*Cereus*	GO 111	SORO	n.a.
Cereus pierrebraunianus Esteves Pereira	*Cereus*	GO 273	SORO	SORO008155
Cereus stenogonus K. Schum.	*Cereus*	GO 587a	SORO	SORO005736

Cereus phatnospermus K. Schum.	*Ebneria*	GO 568	SORO	SORO007969
Cereus saddianus (Rizzini & A. Mattos) P.J. Braun	*Ebneria*	GO 547a	CGMS	CGMS55528
Cereus spegazzinii F.A.C. Weber	*Ebneria*	Z5135	UB	UB1162419
Cereus albicaulis (Britton & Rose) Luetzelb.	*Mirabella*	Z5187	UB	UB1044449
Cereus mirabella N.P. Taylor	*Mirabella*	Z5137	UB	UB1162417
Praecereus saxicola (Morong) N.P. Taylor	*Praecereus* Buxb.	GO 588	SORO	SORO005703

The anatomical research was carried out at the Plant Anatomy Laboratory of the University of Brasília (UnB). Part of the samples were fixed in FAA 50 (formaldehyde, acetic acid, ethanol 50%) in the proportion of 2:1:17 (v/v; Johansen, 1940) for structural analysis and the other part was used directly in histochemical tests. Histological samples used 1 x 1 cm samples obtained from the epidermis, cortex and vascular cylinder, medulla, and areolar region. The process of pre-infiltration, infiltration, and polymerization with historesin followed the protocol of the Leica Historesin Embedding Kit. Histological slides were prepared using 12 µm thick transversal sections made in Leica RM 2145 rotative microtome coloured with toluidine blue 0.05% (Sakai, 1973). Paradermal slides used dissociated specimens following Franklin (1945), coloured with safranin 1% in water (Bukatsch, 1972).

Following colouring, the transversal sections and paradermal preparations were dehydrated in ethanol series, diaphanized in butyl series and mounted as permanent slides using colourless vitral varnish (Paiva *et al.*, 2006). Histochemical tests were carried out using live tissues to detect a) total lipids using Sudan IV 2% in ethanol 92% (Gerlach, 1984); b) mucilage, with Ruthenium red 0.02% (Johansen, 1940); c) starch, with Lugol (Johansen, 1940); d) lignin, with acidified Phloroglucinol 2% (Johansen, 1940), performed only in the areolar region. Images were made using Olympus BX40 photomicroscope attached to a computer with Olympus U-TV0.5XC-3 image capture system. Plates were produced for each studied species.

For Scanning Electron Microscope (SEM), the samples were fixed in FAA 50 (Johansen, 1940) and stored in 50% ethanol. They were dehydrated in an ethanol series under vacuum, critical point dried, and mounted on stubs. The stubs were gold-coated (Leica Em SCD 500), and the analyses were performed using a SEM (Jeol JSM-700IF).

The analysis of anatomical characters used terminologies used by Boke (1944), Anderson (2001), Terrazas & Mauseth (2002), Calvente *et al.* (2008), Dettke & Milaneze-Gutierre (2008), Faigón *et al.* (2011) and Arruda & Melo-de-Pinna (2015) organized in Table 2. Anatomical descriptions followed taxonomic pattern (no verbs, general description of genus and only distinctive characters mentioned at species level). Parameters for cuticle thickness followed Morris *et al.* (1996) (< 3 μm = thin, 3-10 μm = moderately thick, > 10 μm = thick, while hypodermis thickness followed Nyffeler & Eggli, (1997) (30–50 μm = thin; 60–110 μm = moderately thick and 140–350 μm = thick) and were measured using the ImageJ/Fiji 1.46 software program.

RESULTS

The histology and histochemical results of the 17 studied species are presented in Tables 2–3 and Figures 1–19. Among the species analysed, 16 are placed in the following subgenera: *C.* subg. *Cereus*, *C.* subg. *Ebneria* and *C.* subg. *Mirabella*, alongside a species of *Praecereus* (Table 1).

In frontal view, common epidermic cells have straight anticlinal walls in *C. fernambucensis* (Fig. 3H–I), *C. gerardii* (Fig. 5G–H), *C. hildmannianus* (Fig. 7H–I) and *C. jamacaru* subsp. *calcirupicola* (Fig. 10G–H), curved in *C. insularis* (Fig. 8H–I) and *C. stenogonus* (Fig. 12G–H); and sinuous in all remaining species. All analysed samples have unisseriate simple epidermis and, in transversal sections, it was possible to observe epidermic isodiametric, non-striate papillae (Fig. 19) only in *C. pierrebraunianus* (Fig. 11B), and epidermic crystals only in *P. saxicola* (Fig. 18C). Transversal sections of epidermic cells of *C. stenogonus* (Fig. 12B) show these are taller than wider when compared with other samples.

The hypodermis has collenchymatic walls with thick reinforcements and very reduced lumen, with the exception of *C. insularis* (Fig. 8A) which has thin collenchyma walls. In this tissue the histochemical test with acidified floroglucinol performed in representatives of each subgenus has not indicated the presence of lignin while the Ruthenium red test revealed walls rich in pectic compounds in all studied species. The collenchyma layers varied between 2 – 4 layers, while in *C. jamacaru* (Fig. 9A) 5–6 layers were found.

The chlorophyll parenchyma has numerous intercellular spaces and occupies the largest volume of the stem, presenting primary cells with thin walls and chloroplasts. The stem of *C. spegazzinii* (Fig. 15D) has palisade parenchyma with smaller cells than the remaining studied species, while the largest cells appeared in *C. fernambucensis* subsp. s*ericifer* (Fig. 4A). Except

for *C. bicolor* (Fig. 2C), *C. fernambucensis* (Fig. 3D), *P. saxicola* (Fig. 18C), the studied species have numerous idioblasts containing mucilage, c. 4 times more voluminous than other cells, found among the aquiferous parenchyma.

In the primary stage of growth, all species have dispersed cortical vascular bundles. In the youngest part of the stem, the bundles of the vascular cylinder are collateral (Fig. 2D, 3E, 4D, 5D, 6D, 7E, 8E, 9D, 10D, 11D, 12D, 13D, 14E, 15D, 16D, 17D, 18E). In the secondary growth stage, mature regions have a continuous central cylinder with external caps of primary phloem fibres. All representatives of *Cereus* subgenus *Ebneria* (*C. phatnospermus*, *C. saddianus*, *C. spegazzinii*) and *C.* subg. *Mirabella* (*C. albicaulis*, *C. mirabella*) have mineral cortical inclusions while such structures were seen only in a few species of *C*. subg. *Cereus* (*C. fernambucensis* (Fig. 3C), *C. hildmannianus* (Fig. 7C), *C. insularis* (Fig. 8C))

The hystochemical test using Sudan IV has shown that *C. bicolor* (Fig. 2F), *C. insularis* (Fig. 8G), *C. spegazzinii* (Fig. 15G), and *P. saxicola* (Fig. 18G) have thin cuticle and epicuticular wax was not detected in *C. fernambucensis* subsp. *sericifer* (Fig. 5F), *C. hexagonus* (Fig. 6F), *C. phatnospermus* (Fig. 13F), *C. spegazzinii* (Fig. 15G), and *P. saxicola* (Fig. 18G).

The pith displayed starch deposits identified through lugol tests in *C. fernambucensis subsp. sericifer*, *C. jamacaru.*, *C. phatnospermus*, *C. saddianus*, *C. spegazzinii*, *C. albicaulis*, *C. mirabella*, *C. gerardii* and *P. saxicola.* The histochemical test with acidified floroglucionl indicated the presence of a lignified apex in the areolar region in all species.

The stem has paracytic stomata with reniform guard cells in all examined species. The relation between length and width of guard cells is higher in *C. jamacaru* (Table 3). The stomata are levelled with the epidermis in most species excepting *C. hildmannianus* (Fig. 7A), *C. jamacaru* (Fig. 9F) and *C. pierrebraunianus* (Fig. 11F), where we observed slightly depressed substomatal chambers, and in *C. jamacaru* subsp. *calcirupicola*, where stomata appear in deep depressions (Fig. 10A). The orientation of the stomatic pores is perpendicular to the axis of the plant, and the pores appear randomly or parallel with each other. Substomatal chambers often pass through all hypodermic layers, even the thickest ones, such as seen in *C. jamacaru* (Fig. 9A). The number of stomatic adjacent cells varies between 2–5 common epidermic cells.

Stem anatomy description

***Cereus* Mill.**

Stem photosynthetic and aphyllous; **epidermis** uniseriate; epidermal crystals absent; common epidermic cells in paradermal view 1–2 times longer than wide and axial side smaller than the

tangential side, anticlinal walls straight, curved or sinuous; epidermic papillae absent or present and isodiametric, not striated; cuticle-periclinal wall complex thin to thick; epicuticular wax absent to present, thick and fragile; **stomatic complex** paracytic, with convex subsidiary cells that are less, equally or larger in width than the guard cells; **stomata** at the same level or in depressions; stomatic pores parallel or randomly disposed and in tangential position; **substomatal chambers** present; **guard cells** reniform, 4 times longer than wide; 2–5 cells adjacent to stomata; **trichomes** restricted to the areoles, pluricellular; **hypodermis** collenchymatic with 2–6 layers of cells with reduced lumen and inconspicuous intercellular spaces; chlorophyl parenchyma with numerous intercellular spaces; **cortex** with palisade parenchyma as tall as wide; **mineral inclusions** absent or present in the cortical region; **latex ducts** absent from the cortex; **mucilaginous cells** absent or present in the cortex and medulla (pith); **vascular bundles** libero lignified and collateral, with cortical bundles dispersed in the vascular cylinder; phloem fiber caps present or absent from the vascular cylinder, with wide lumen and thick cell walls; vascular bundles commissural in the areolar region; **starch storage** absent or concentrated in the medulla (pith).

Identification key for the analysed species using the stem anatomy characters described:

1. Epidermic crystals present ..... *Praecereus saxicola*
1'. Epidermic crystals absent 2
2. Epidermic papillae present ..... *C. pierrebrauniannus*
2'. Epidermic papillae absent 3
3. Epicuticular wax conspicuous 8
3'. Epicuticular wax inconspicuous 4
4. Mineral inclusions present in the cortex ..... *C. spegazzinii*
4'. Mineral inclusions absent from the cortex 5
5. Pith with giant mucilaginous cells 6
5'. Pith lacking mucilaginous cells ..... *C. fernambucensis* subsp. *sericifer*
6. Starch storage in the pith ..... *C. kroenlinii*
6'. Starch storage absent from the pith 7
7. Stomata located in depressions ..... *C. jamacaru* subsp. *calcirupicola*
7'. Stomata levelled with the epidermis ..... *C. hexagonus*
8. Anticlinal walls curved 9
8' Anticlinal walls straight 10

8''. Anticlinal walls sinuous 12
9. Collenchymatic hypodermis thin, with 2–3 layers ..... *C. insularis*
9'. Collenchymatic hypodermis thick, with 4–5 layers..... *C. stenogonus*
10. Mineral inclusions in the cortex present..... 11
10'. Mineral inclusions in the cortex absent ..... *C. gerardii*
11. Stomata slightly depressed .....*C. hildmannianus*
11'. Stomata levelled with epidermis..... *C. fernambucensis*
12. Stomata slightly depressed .....*C. jamacaru*
12'. Stomata levelled with epidermis..... 13
13. Mineral inclusions present in the cortex 14
13'. Mineral inclusions absent from the cortex.....*C. bicolor*
14. Epicuticular wax > 20 µm 15
14'. Epicuticular wax < 20 µm..... *C. saddianus*
15. Cuticle relatively thicker (> 12 µm) .....*C. mirabella*
15'. Cuticle relatively thinner (< 12 µm) .....*C. albicaulis*

Species descriptions

1. Cereus bicolor

Primary stem of Cactaceae, **epidermis** without crystals, common cells sinuous, 1 × longer than wide (83.7±54 × 57.6±27.8 µm); procumbent cells predominant; epidermic papillae absent; periclinal **cuticle-cell wall** complex moderately thick (5.9±2.9 µm thick); **epicuticular wax** conspicuous (9.7±4.6 µm thick); **stomata** levelled; **guard cells** reniform 4 × longer than wide (39.2±30.8 × 9.6±4.6 µm); 2–3 stomatic adjacent cells; **hypodermis** collenchymatic, 3–4 layers; **cortex** with palisade parenchyma cells 1 × taller than wide (187.4±104.7 × 101.5±42.8 µm), **mineral inclusions** absent, **mucilaginous cells** absent; primary phloem with fibrous caps; **medulla** without starch reserve or mucilaginous cells.

2. Cereus fernambucensis

Primary stem of Cactaceae, **epidermis** without crystals, common cells straight, 1 × longer than wide (130.4±77.6 × 82.4±42.2 µm); procumbent cells predominant; epidermic papillae absent; periclinal **cuticle-cell wall** complex moderately thick (9.8±4.9 µm thick); **epicuticular wax** conspicuous (16.5±9 µm thick); **stomata** levelled; stomatic pores parallel to the plant axis; **guard cells** reniform 4 × longer than wide (49±38.5 × 11.6±6.4 µm); 2–3

stomatic adjacent cells; **hypodermis** collenchymatic, 2–3 layers; **cortex** with palisade parenchyma cells 1 × taller than wide (161.9±74.6 × 77.9±42.9 μm), **mineral inclusions** present; **mucilaginous cells** absent; primary phloem without fibrous caps; **medulla** without starch reserve or mucilaginous cells.

3. Cereus fernambucensis subsp. *sericifer*

Primary stem of Cactaceae, **epidermis** without crystals, common cells sinuous, 2 × longer than wide (172.9±124.5 × 85.1±39.1 μm); procumbent cells predominant; epidermic papillae absent; periclinal **cuticle-cell wall** complex moderately thick (8.7±4.5 μm thick); **epicuticular wax** inconspicuous; **stomata** levelled; stomatic pores parallel to the plant axis; **guard cells** reniform 4 × longer than wide (54.5±43.9 × 13.2±10.3 μm); 2 stomatic adjacent cells; **hypodermis** collenchymatic, 3–4 layers; **cortex** with palisade parenchyma cells 1 × taller than wide (244.4±97.2 × 127.6±82.2 μm); **mineral inclusions** absent, **mucilaginous cells** present; primary phloem without fibrous caps; **starch reserve** in the medulla present; **medulla** without mucilaginous cells.

4. Cereus gerardii

Primary stem of Cactaceae, **epidermis** without crystals, common cells straight, 2 × longer than wide (142±53.5 × 59.3±33.5 μm); procumbent cells predominant; epidermic papillae absent; periclinal **cuticle-cell wall** complex moderately thick (9.3±5.3 μm thick); **epicuticular wax** conspicuous (20.7±10.7 μm thick); **stomata** levelled; stomatic pores parallel to the plant axis; **guard cells** reniform 3 × longer than wide (47.3±31 × 12±5.3 μm); 2 stomatic adjacent cells; **hypodermis** collenchymatic, 3–4 layers; **cortex** with palisade parenchyma cells 1 × taller than wide (168.2±96.8 × 104.9±49.2 μm); **mineral inclusions** absent, **mucilaginous cells** present; **starch reserve** in the medulla present; **medulla** with mucilaginous cells.

5. Cereus hexagonus

Primary stem of Cactaceae, **epidermis** without crystals, common cells sinuous, 2 × longer than wide (97.8±53 × 50.5±35.4 μm); erect cells predominant; epidermic papillae absent; periclinal **cuticle-cell wall** complex moderately thick (11.8±8 μm thick); **epicuticular wax** inconspicuous; **stomata** levelled; stomatic pores parallel to the plant axis; **guard cells** reniform 3 × longer than wide (44.7±34.1 × 13.3±8.2 μm); 2–3 stomatic adjacent cells; **hypodermis** collenchymatic, 2-3 layers; **cortex** with palisade parenchyma cells 1 × taller than wide (125.4±69.8 × 85.7±61.9 μm); **mineral inclusions** absent, **mucilaginous cells** present; primary phloem without fibrous caps; **starch reserve** in the medulla absent; **medulla** with mucilaginous cells.

6. Cereus hildmannianus

Primary stem of Cactaceae, **epidermis** without crystals, common cells straight, 1 × longer than wide (47.9±33.7 × 41.3±25.3 μm); procumbent cells predominant; epidermic papillae absent; periclinal **cuticle-cell wall** complex thick (22±16.2 μm thick); **epicuticular wax** conspicuous (58.3±20.1 μm thick); slightly depressed **stomata**; stomatic pores parallel to the plant axis; **guard cells** reniform 3 × longer than wide (53±43.1 × 17.7±11 μm); 2 stomatic adjacent cells; **hypodermis** collenchymatic, 3–4 layers; **cortex** with palisade parenchyma cells 1 × taller than wide (225.5±114.2 × 130.3±66.7 μm); **mineral inclusions** present; **mucilaginous cells** present; primary phloem with fibrous caps; **starch reserve** in the medulla absent; **medulla** with mucilaginous cells.

7. Cereus insularis

Primary stem of Cactaceae, **epidermis** without crystals, common cells curves, 2 × longer than wide (121.7±74.9 × 77.5±37.5 μm); procumbent cells predominant; epidermic papillae absent; periclinal **cuticle-cell wall** complex thin (3,4±2,1 μm thick); **epicuticular wax** conspicuous (11,3±6,9 μm thick); **stomata** levelled; stomatic pores parallel to the plant axis; **guard cells** reniform 3 × longer than wide (44.1±34.3 × 12.7±7.1 μm); 2–3 stomatic adjacent cells; **hypodermis** collenchymatic, 2–3 layers; **cortex** with palisade parenchyma as high as it is wide (146±61.9 ×119.3±73.8 μm); **mineral inclusions** present; **mucilaginous cells** present; primary phloem without fibrous caps; **starch reserve** in the medulla absent; **medulla** with mucilaginous cells.

8. Cereus jamacaru

Primary stem of Cactaceae, **epidermis** without crystals, common cells sinuous 2 × longer than wide (146.1±84.8 × 77.1±42.7 μm); erect cells predominant; epidermic papillae absent; periclinal **cuticle-cell wall** complex moderately thick (11±7.3 μm thick); **epicuticular wax** conspicuous (24.6±16.5 μm thick); slightly depressed **stomata**; stomatic pores parallel to the plant axis; **guard cells** reniform 4 × longer than wide (58±44.3 × 13±9.3 μm); 5 stomatic adjacent cells; **hypodermis** collenchymatic, 5–6 layers; **cortex** with palisade parenchyma cells 1 × taller than wide (121.4±77.2 × 84.1±50.4 μm); **mineral inclusions** absent; **mucilaginous cells** present; primary phloem without fibrous caps; **starch reserve** in the medulla present; **medulla** with mucilaginous cells.

9. Cereus jamacaru subsp. *calcirupicola*

Primary stem of Cactaceae, **epidermis** without crystals, common cells straight, 2 × longer than wide (98.2±56.4 × 60±28.9 μm); erect cells predominant; epidermic papillae absent; periclinal **cuticle-cell wall** complex moderately thick (7.2±5.1 μm thick); **epicuticular wax** inconspicuous; depressed **stomata** stomatic pores parallel to the plant axis; **guard cells** reniform 3 × longer than wide (49.8±40.7 × 15.9±10.5 μm); 2 stomatic adjacent cells; **hypodermis** collenchymatic, 3–5 layers; **cortex** with palisade parenchyma cells 1 × taller than wide (107.5±62.7 × 98.1±44.6 μm); **mineral inclusions** absent; **mucilaginous cells** present; primary phloem without fibrous caps; **starch reserve** in the medulla absent; **medulla** with mucilaginous cells.

10. Cereus pierrebrauniannus

Primary stem of Cactaceae, **epidermis** without crystals, common cells sinuous, 1 × longer than wide (91.8±20.7 × 45±24.6 μm); procumbent cells predominant; isodiametric epidermal papillae present, with cells wider than tall (60.3±45.1 × 78.7±56.4 μm); striae on the papillae absent; periclinal **cuticle-cell wall** complex moderately thick (11.6 ±4.5 μm thick); **epicuticular wax** conspicuous (56.3±26.9 μm thick); slightly depressed **stomata**; stomatic pores parallel to the plant axis; **guard cells** reniform 5 × longer than wide (51.4±39.5 × 13.2±6.6 μm); 2 stomatic adjacent cells; **hypodermis** collenchymatic, 4–5 layers; **cortex** with palisade parenchyma cells 1 × taller than wide (128.8±69.7 × 76.2±49.2 μm); **mineral inclusions** absent, **mucilaginous cells** present; primary phloem with fibrous caps; **starch reserve** in the medulla absent; **medulla** with mucilaginous cells.

11. Cereus stenogonus

Primary stem of Cactaceae, **epidermis** without crystals, common cells curves 1 × longer than wide (42.4±20.1 × 33.3±13.5 μm); erect cells predominant; epidermic papillae absent; periclinal **cuticle-cell wall** complex moderately thick (6.9±4.6 μm thick); **epicuticular wax** inconspicuous; **stomata** levelled; stomatic pores parallel to the plant axis; **guard cells** reniform 3 × longer than wide (44.3±35.3 × 14.2±9.2 μm); 2 stomatic adjacent cells; **hypodermis** collenchymatic, 4–5 layers; **cortex** with palisade parenchyma cells 1 × taller than wide (112.8±54.3 × 82.3±50.8 μm); **mineral inclusions** absent, **mucilaginous cells** present; primary phloem without fibrous caps; **starch reserve** in the medulla absent; **medulla** with mucilaginous cells.

12. Cereus phatnospermus

Primary stem of Cactaceae, **epidermis** without crystals, common cells sinuous, 1 × longer than wide (59.5±37.3 × 36.4±2.,4 μm); procumbent cells predominant; epidermic papillae absent; periclinal **cuticle-cell wall** complex thick (12.3±8.4 μm thick); **epicuticular wax** inconspicuous; **stomata** levelled; stomatic pores parallel to the plant axis; **guard cells** reniform 4 × longer than wide (46.5±35.8 × 11.2±6.5 μm); 3 stomatic adjacent cells; **hypodermis** collenchymatic, 3–4 layers; **cortex** with palisade parenchyma cells 1 × taller than wide (207.1±140 × 144.7±85.7 μm); **mineral inclusions** absent, **mucilaginous cells** present; primary phloem with fibrous caps**; starch reserve** in the medulla present; **medulla** with mucilaginous cells.

13. Cereus saddianus

Primary stem of Cactaceae, **epidermis** without crystals, common cells sinuous, 1 × longer than wide (126±68.9 × 71.8±42.6 μm); procumbent cells predominant; epidermic papillae absent; periclinal **cuticle-cell wall** complex thick (13.6±10.6 μm thick); **epicuticular wax** conspicuous (14.5±8.4 μm thick); **stomata** levelled; stomatic pores parallel to the plant axis; **guard cells** reniform 3 × longer than wide (40.6±32.2 × 13.3±6.6 μm); 2 stomatic adjacent cells; **hypodermis** collenchymatic, 2–3 layers; **cortex** with palisade parenchyma cells 1 × taller than wide (118.8±44.2 × 78±54.6 μm); **mineral inclusions** present; **mucilaginous cells** present; primary phloem with fibrous caps; **starch reserve** in the medulla present; **medulla** with mucilaginous cells.

14. Cereus spegazzinii

Primary stem of Cactaceae, **epidermis** without crystals, common cells sinuous, 1 × longer than wide (157.4±99.1 × 119.9±46.5 μm); procumbent cells predominant; epidermic papillae absent; periclinal **cuticle-cell wall** complex moderately thick (4±2.4 μm thick); **epicuticular wax** inconspicuous; **stomata** levelled; stomatic pores parallel to the plant axis; **guard cells** reniform 4 × longer than wide (41.5±29.9 × 9.6±7.7 μm); 2–3 stomatic adjacent cells; **hypodermis** collenchymatic, 2–3 layers; cortex with palisade parenchyma wider than tall (74.9±35.9 × 97.1±51.6 μm); **mineral inclusions** present; **mucilaginous cells** present; primary phloem with fibrous caps; **medulla** without starch reserve or mucilaginous cells.

15. Cereus albicaulis

Secondary stem of Cactaceae, **epidermis** without crystals, common cells sinuous, 2 × longer than wide (122.3±73.1 × 64.2±28.7 μm); procumbent cells predominant; epidermic papillae absent; periclinal **cuticle-cell wall** complex thick (11.9±7.7 μm thick); **epicuticular wax** conspicuous (22.6±14.5 μm thick); **stomata** levelled; stomatic pores parallel to the plant axis; **guard cells** reniform 4 × longer than wide (38.2±33 × 9.2±5.8 μm); 2 stomatic adjacent cells; **hypodermis** collenchymatic, 2–3 layers; **cortex** with palisade parenchyma cells 1 × taller than wide (102.5±61.9 × 104.7±52.6 μm); **mineral inclusions** present; **mucilaginous cells** present; primary phloem with fibrous caps; **starch reserve** in the medulla present; **medulla** with mucilaginous cells.

16. Cereus mirabella

Secondary stem of Cactaceae, **epidermis** without crystals, common cells sinuous, 2 × longer than wide (188.1±74.8 × 71.6±49.1 μm); procumbent cells predominant; epidermic papillae absent; periclinal **cuticle-cell wall** complex thick (15.8±9 μm thick); **epicuticular wax** conspicuous (20.3±13 μm thick); **stomata** levelled; stomatic pores parallel to the plant axis; **guard cells** reniform 4 × longer than wide (38.3±31.7 × 9.6±5.8 μm); 2 stomatic adjacent cells; **hypodermis** collenchymatic, 3 layers; **cortex** with palisade parenchyma cells 1 × taller than wide (131,8±66,9 × 102,8±40,4 μm); **mineral inclusions** present; **mucilaginous cells** present; primary phloem with fibrous caps; **starch reserve** in the medulla present; **medulla** with mucilaginous cells.

17. Praecereus saxicola

Primary stem of Cactaceae, **epidermis** with crystals, common cells straight, 1 × longer than wide (91.6±49.6 × 44.7±29.2 μm); erect cells predominant; epidermic papillae absent; periclinal **cuticle-cell wall** complex moderately thick (5.5±3.8 μm thick); **epicuticular wax** inconspicuous; **stomata** levelled stomatic pores parallel to the plant axis; **guard cells** reniform 4 × longer than wide (30.1±22.1 × 8.7±5.5 μm); 2 stomatic adjacent cells; **hypodermis** collenchymatic, 2 layers; **cortex** with palisade parenchyma cells 1 × taller than wide (202.6±107.9 × 92.1±51 μm); **mineral inclusions** present; **mucilaginous cells** absent; primary phloem without fibrous caps; **starch reserve** in the medulla present; **medulla** without mucilaginous cells.

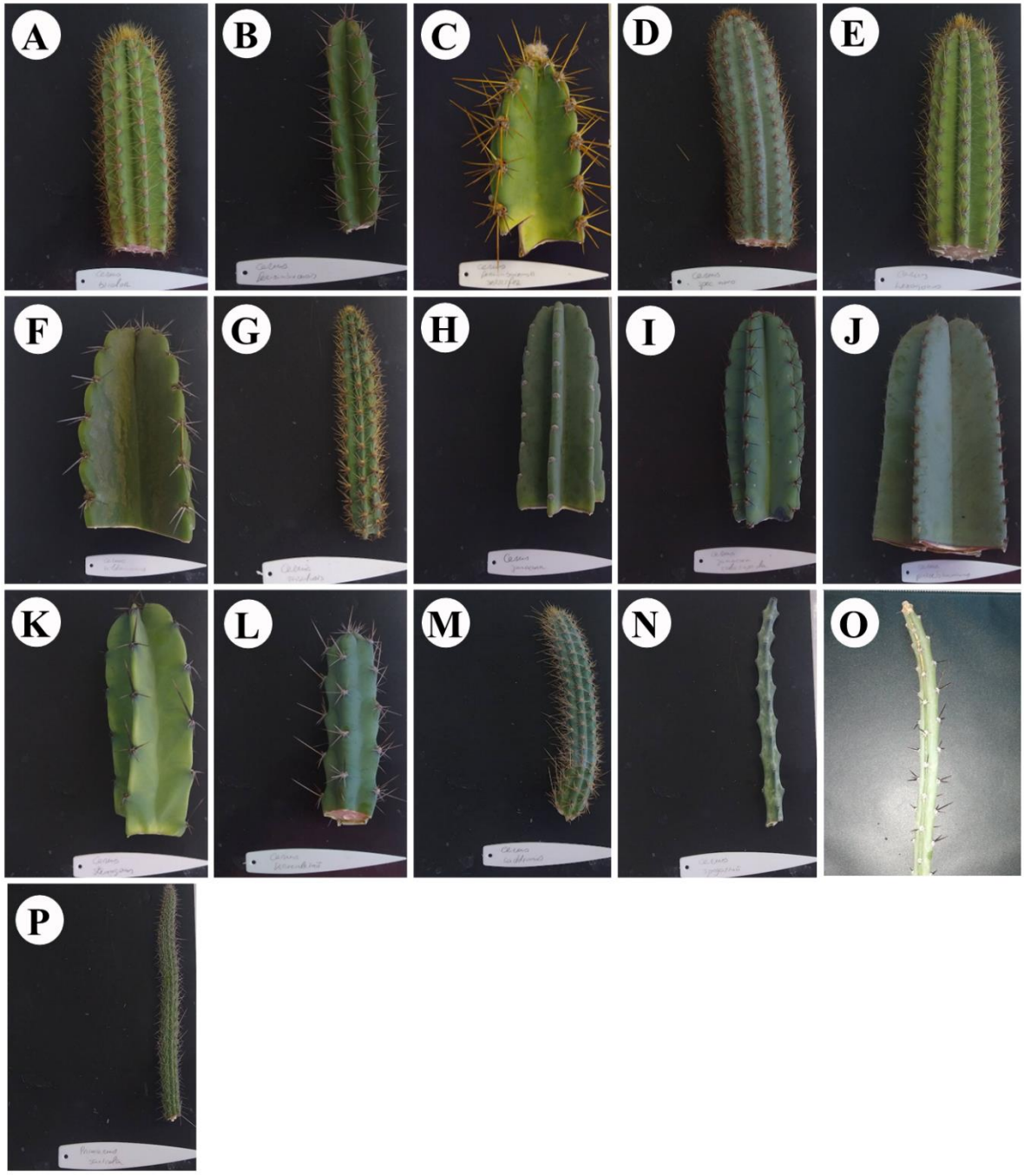

Figure 1. Stem of *Cereus* species before fixation: A: *Cereus bicolor*; B: *Cereus fernambucensis*; C: *Cereus fernambucensis* subsp. *sericifer*; D: *Cereus gerardii*; E: *Cereus hexagonus*; F: *Cereus hildmannianus*; G: *Cereus insularis*; H: *Cereus jamacaru* I: *Cereus jamacaru* subsp. *calcirupicola*; J: *Cereus pierrebraunianus*; K: *Cereus stenogonus*; L: *Cereus phatnospermus:* M: *Cereus saddianus*; N: *Cereus spegazzinii*; O: *Cereus albicaulis*; P: *Praecereus saxicola*.

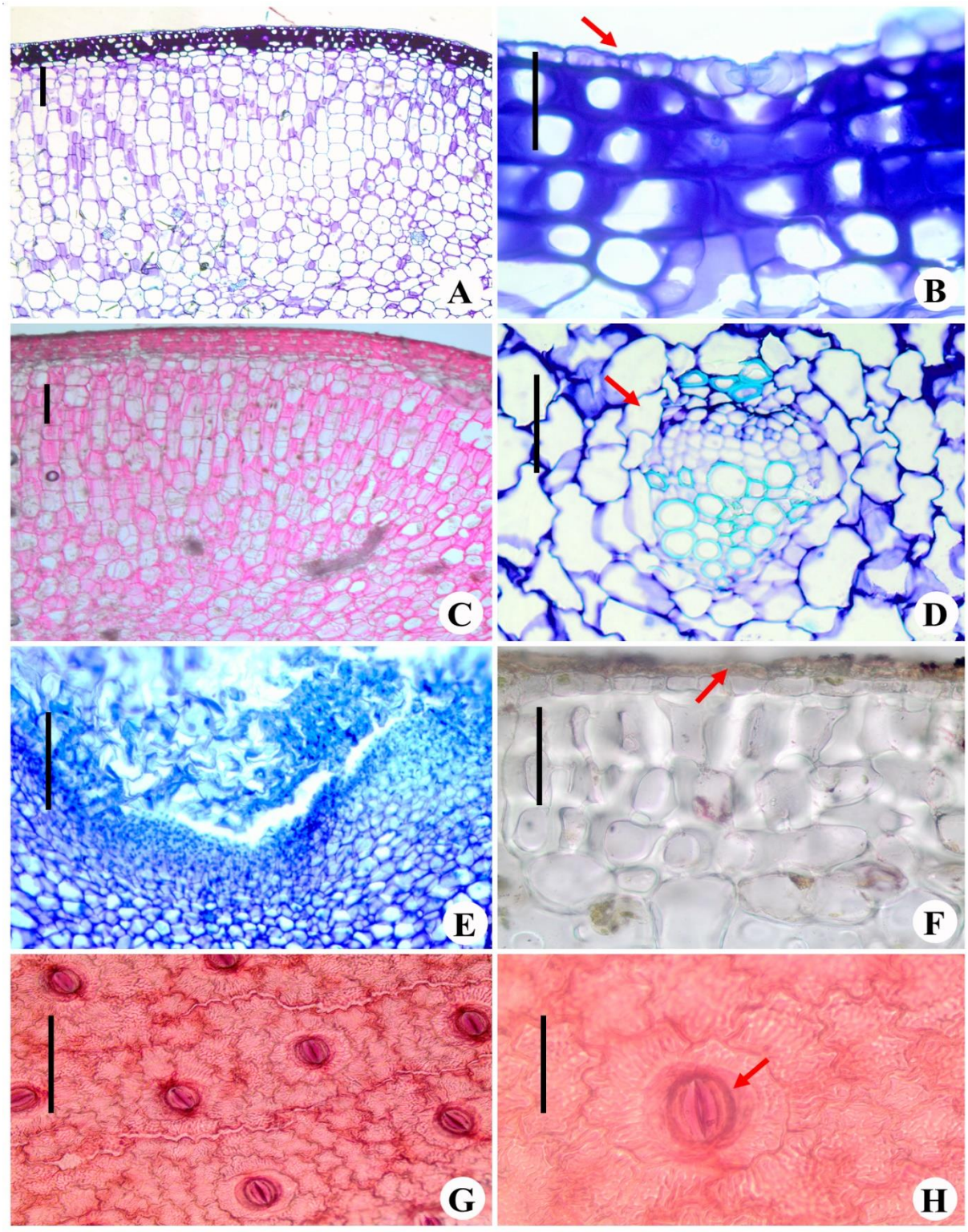

Figure 2. *Cereus bicolor* stem: A: General view of the cortex; B: TS of unisseriate epidermis; C: Histochemical test with Ruthenium red showing absence of mucilaginous cells; D: Vascular bundle; E: TS of the areolar region; F: Histochemical test with Sudan IV showing cuticle; G: General vision of paradermal section; H: Paradermal section with stomata. Scales: A-C: 200µm; B-F-H: 50µm; D-E-G: 100µm.

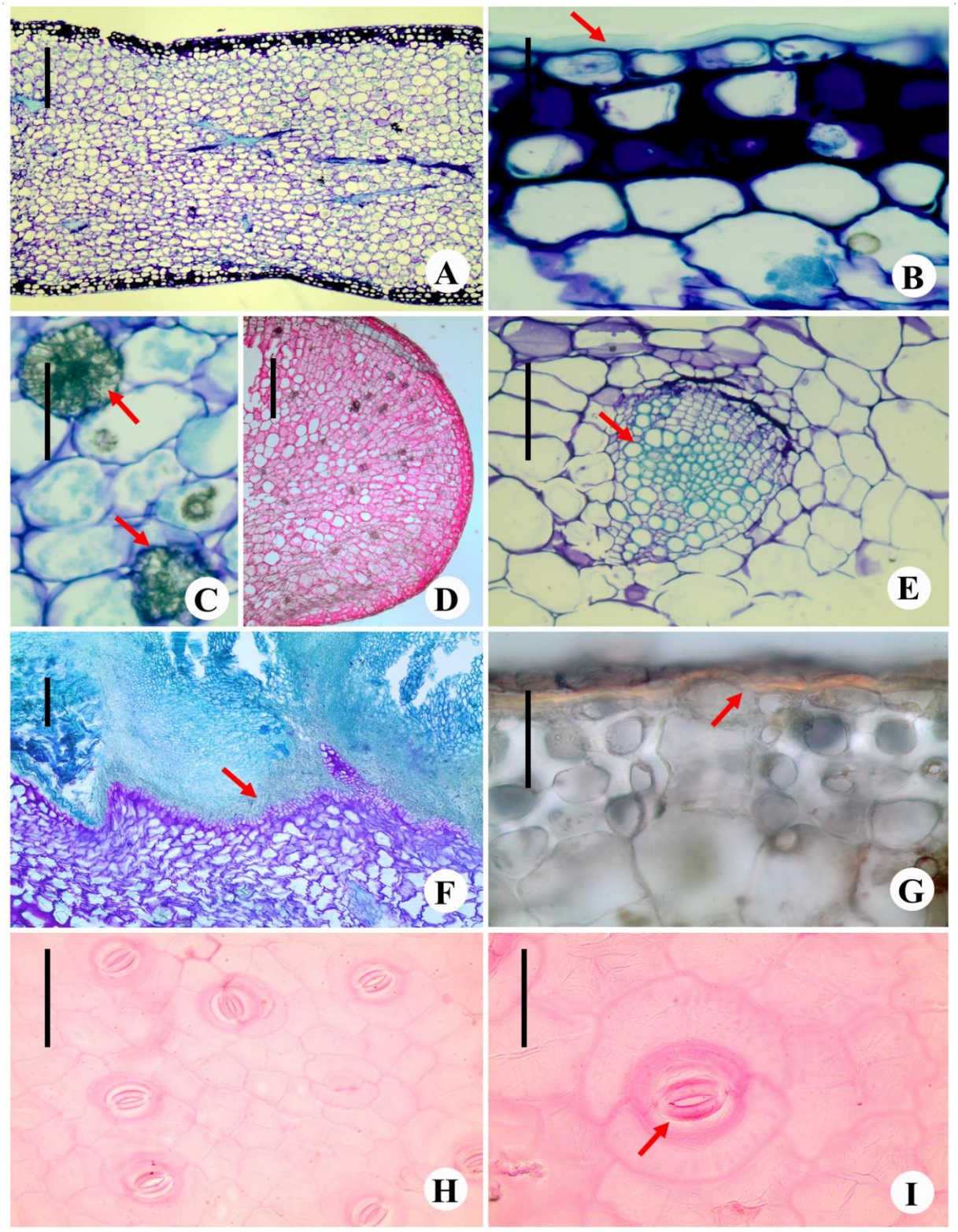

Figure 3. *Cereus fernambucensis* stem: A: General view of cortex; B: TS of simple unisseriate epidermis; C: TS with crystals dispersed in the cortex; D: Histochemical test with Ruthenium red showing the absence of mucilaginous cells; E: Vascular bundle; F: TS of the areolar region; G: Histochemical test with Sudan IV showing cuticle; H: General view of paradermal section; I: Paradermal section with stomata. Scales: A-D: 300µm; B-C-G-I: 50µm; E-F-H: 100µm.

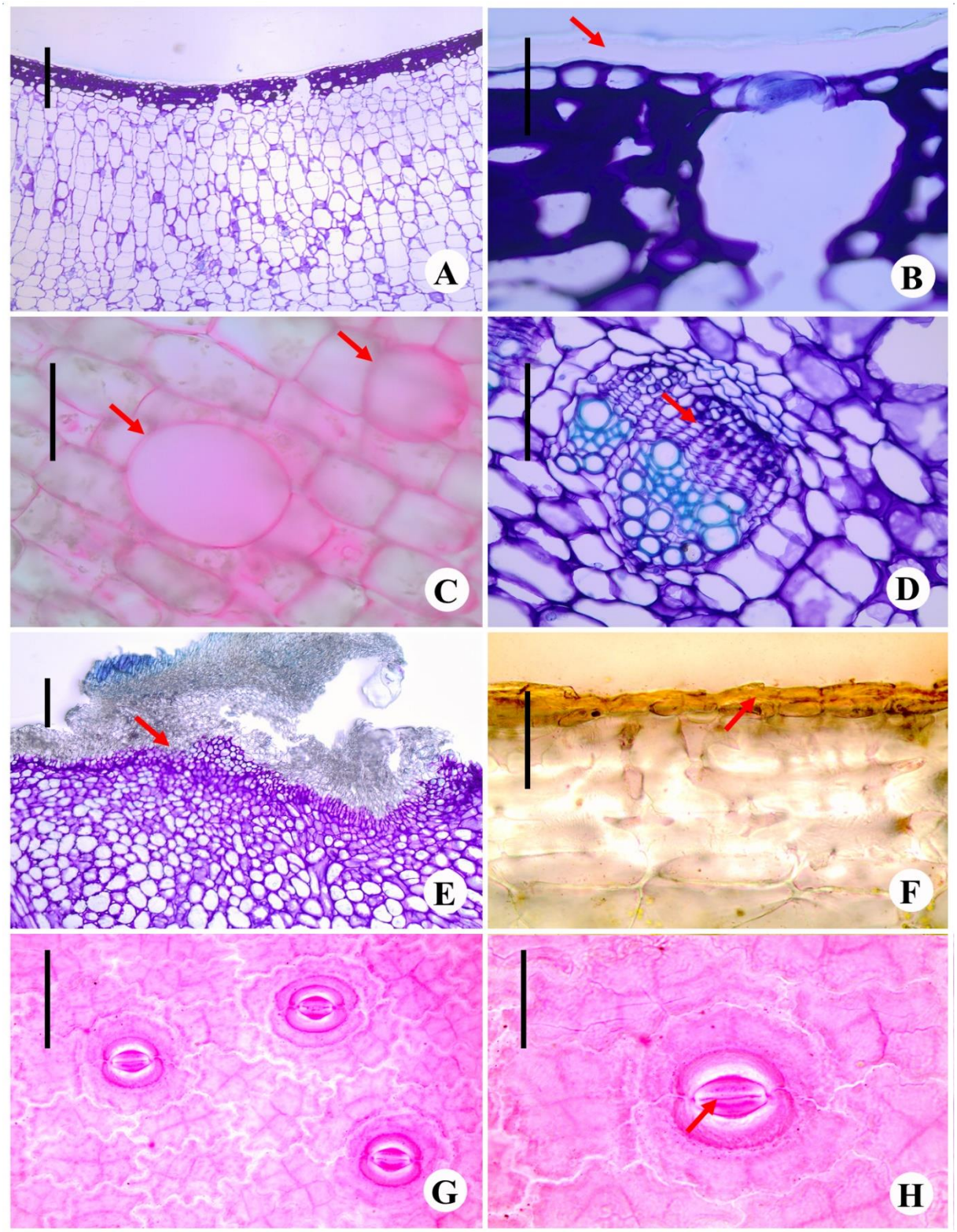

Figure 4. *Cereus fernambucensis* subsp. *sericifer* stem: A: General vision of cortex; B: TS of simple, unisseriate epidermis; C: Histochemical test with Ruthenium red showing mucilaginous cells; D: Vascular bundle; E: TS of the areolar region; F: Histochemical test with Sudan IV showing cuticle; G: General vision of paradermal section; H: Paradermal section showing stomata. Scales: A: 300µm; B-F-H: 50µm; C-D-E-G: 100µm.

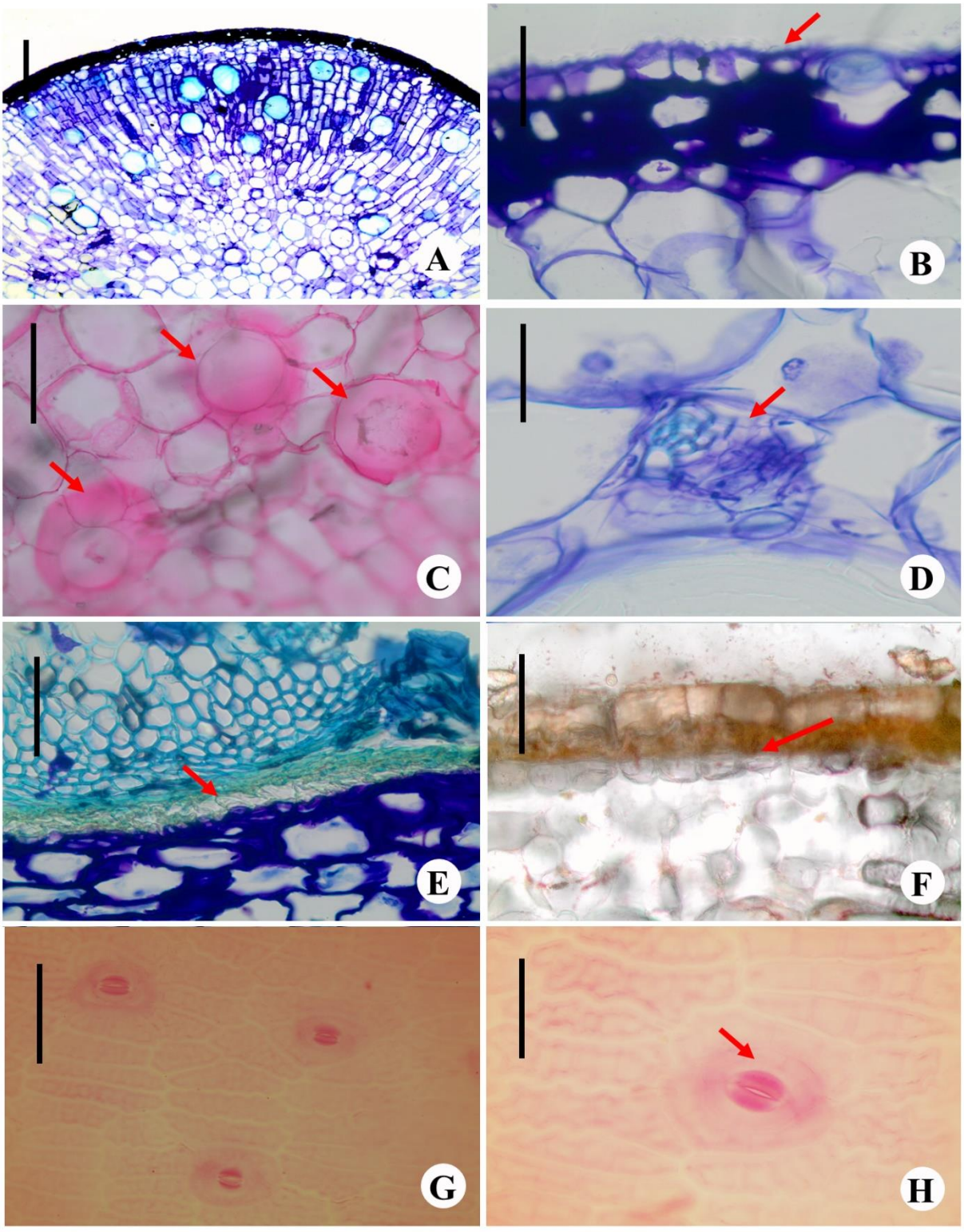

Figure 5. *Cereus gerardii* stem: A: General view; B: TS of the unisseriate epidermis; C: Histochemical test with Ruthenium red showing mucilaginous cells; D: Vascular bundles in the cortex; E: TS of the areolar region; F: Histochemical test with Sudan IV showing cuticle; G: General view of paradermal section; H: Paradermal section with stomata. Scales: A: 300µm, B-E-F-H: 50µm, C: 200µm, D-G: 100µm.

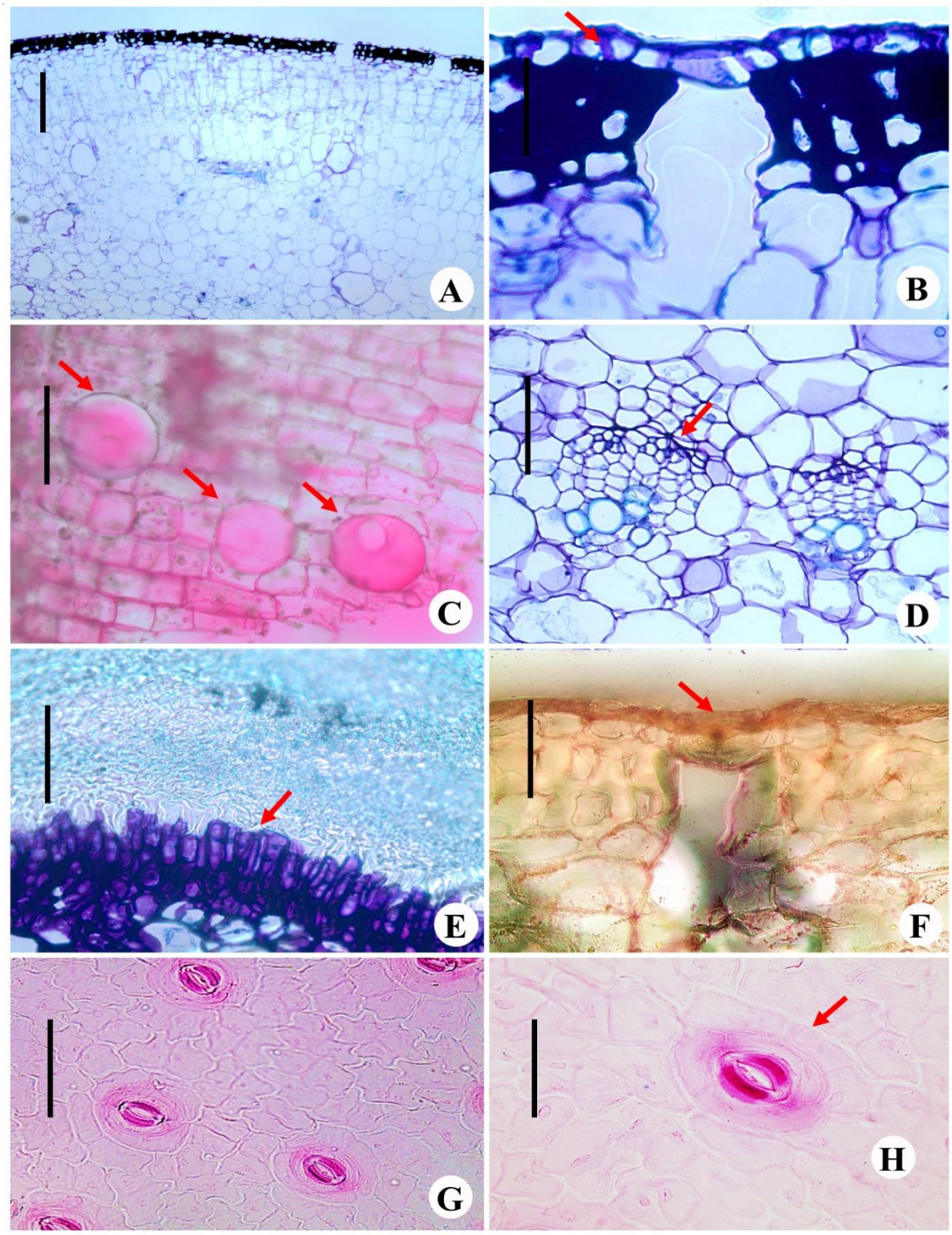

Figure 6. *Cereus hexagonus* stem: A: General view of cortex; B: TS of unisseriate epidermis; C: Histochemical test with Ruthenium red showing mucilaginous cells; D: Vascular bundle; E: TS of the areolar region; F: Histochemical test with IV showing cuticle; G: General view of paradermal section; H: Paradermal section with stomata. Scales: A: 300µm; B-E-F-H: 50µm; C: 200µm; D-G: 100µm.

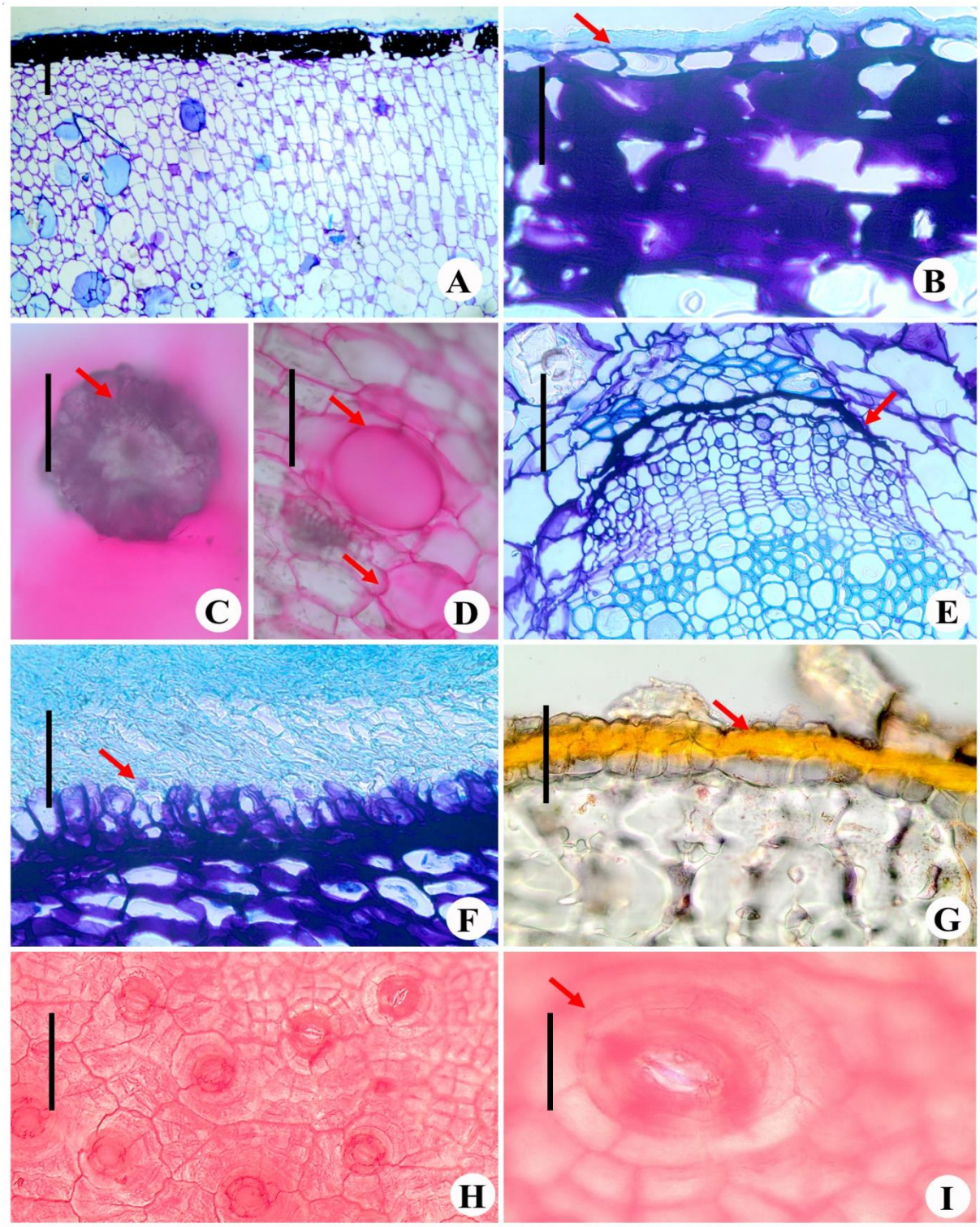

Figure 7. *Cereus hildmannianus* stem: A: General view of the cortex; B: TS of unisseriate epidermis; C: TS with dispersed crystals in the cortex; D: Histochemical tests with Ruthenium red showing mucilaginous cells; E: Vascular bundle; F: TS of the areolar region; G: Histochemical test with Sudan IV showing cuticle; H: General vision of paradermal section; I: Paradermal section with stomata. Scales: A: 300µm; B-F-G-I: 50µm; C-D-E: 100µm; H: 200µm.

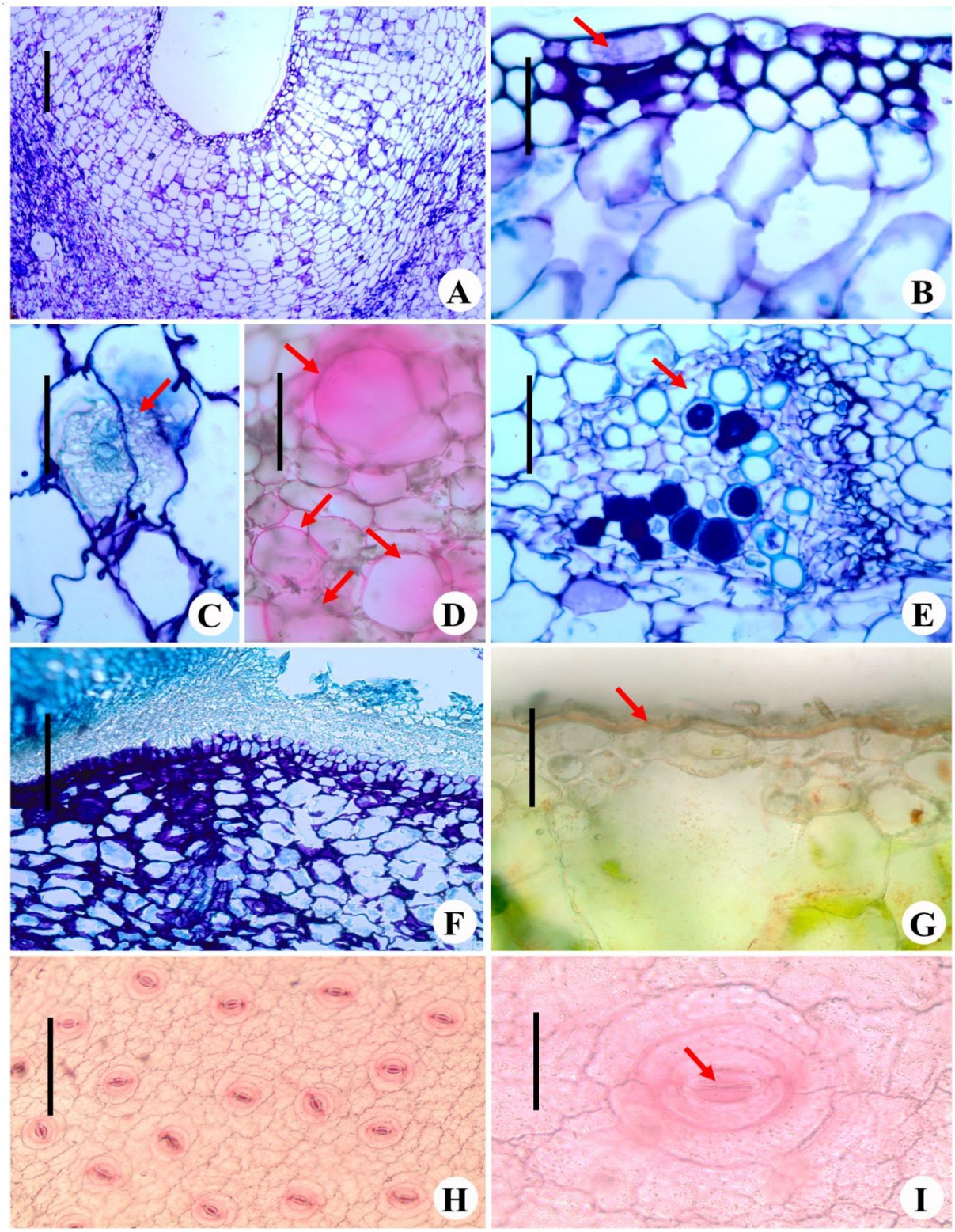

Figure 8. *Cereus insularis* stem: A: General view of the cortex; B: TS of unisseriate epidermis; C: TS with crystal dispersed in the cortex; D: Histochemical test with Ruthenium red showing mucilaginous cells; E: Vascular bundle; F: TS of the areolar region; G: Histochemical test with Sudan IV showing cuticle; H: General vision of paradermal section; I: Paradermal section with stomata. Scales: A: 300µm; B-C-E-G-I: 50µm; D-F: 100µm; H: 200µm.

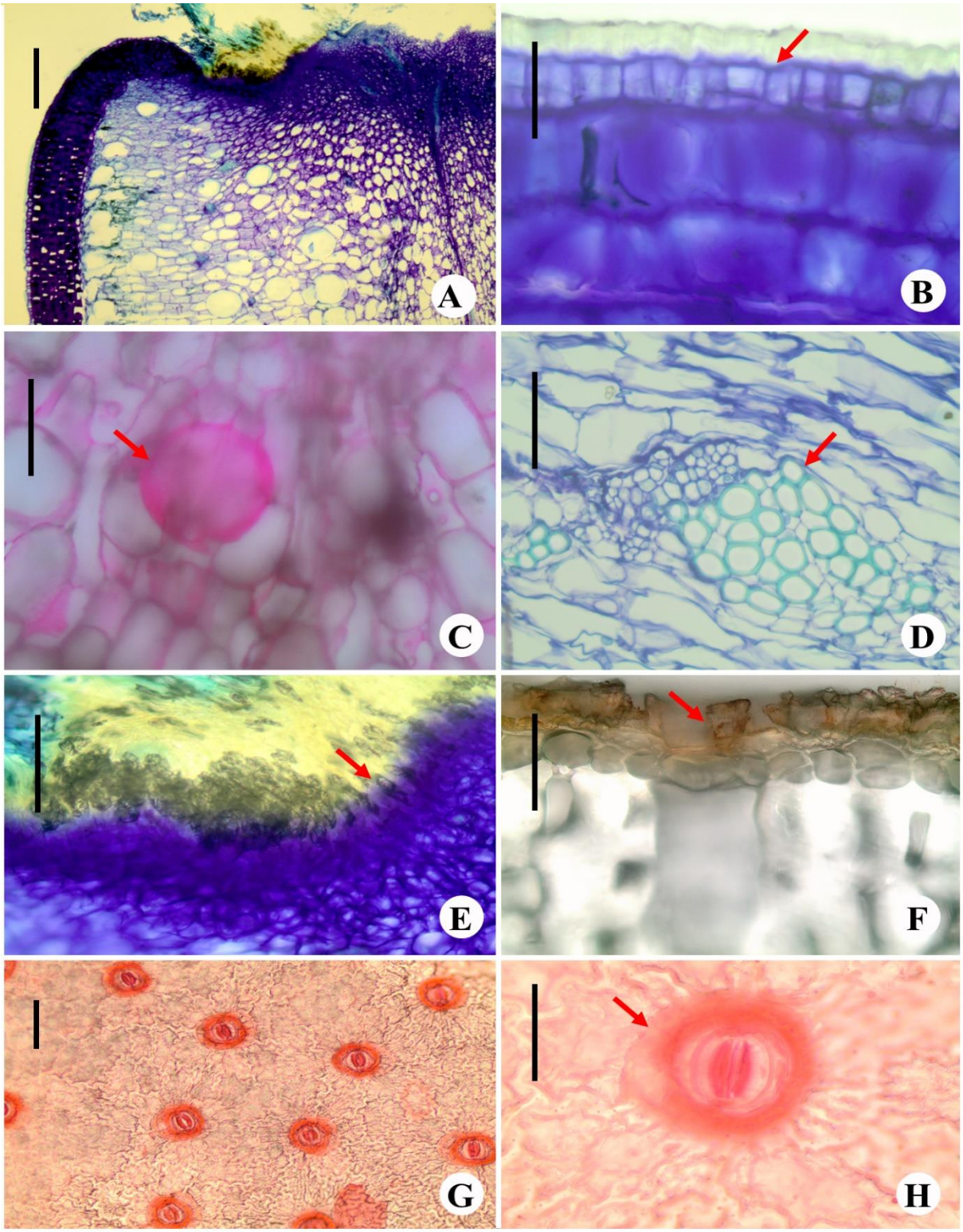

Figure 9. *Cereus jamacaru* stem: A: General view of teh areole; B: TS of simple unisseriate epidermis; C: TS of the cortex with mucilaginous cell; D: TS showing cortex dispersed vascular bundle; E: TS of the areolar region; F: Histochemical test with Sudan IV showing cuticle; G: General view of paradermal section; H: Paradermal section with stomata. Scales: A: 300µm; B-D-E-F-H: 50µm; C-G: 100µm.

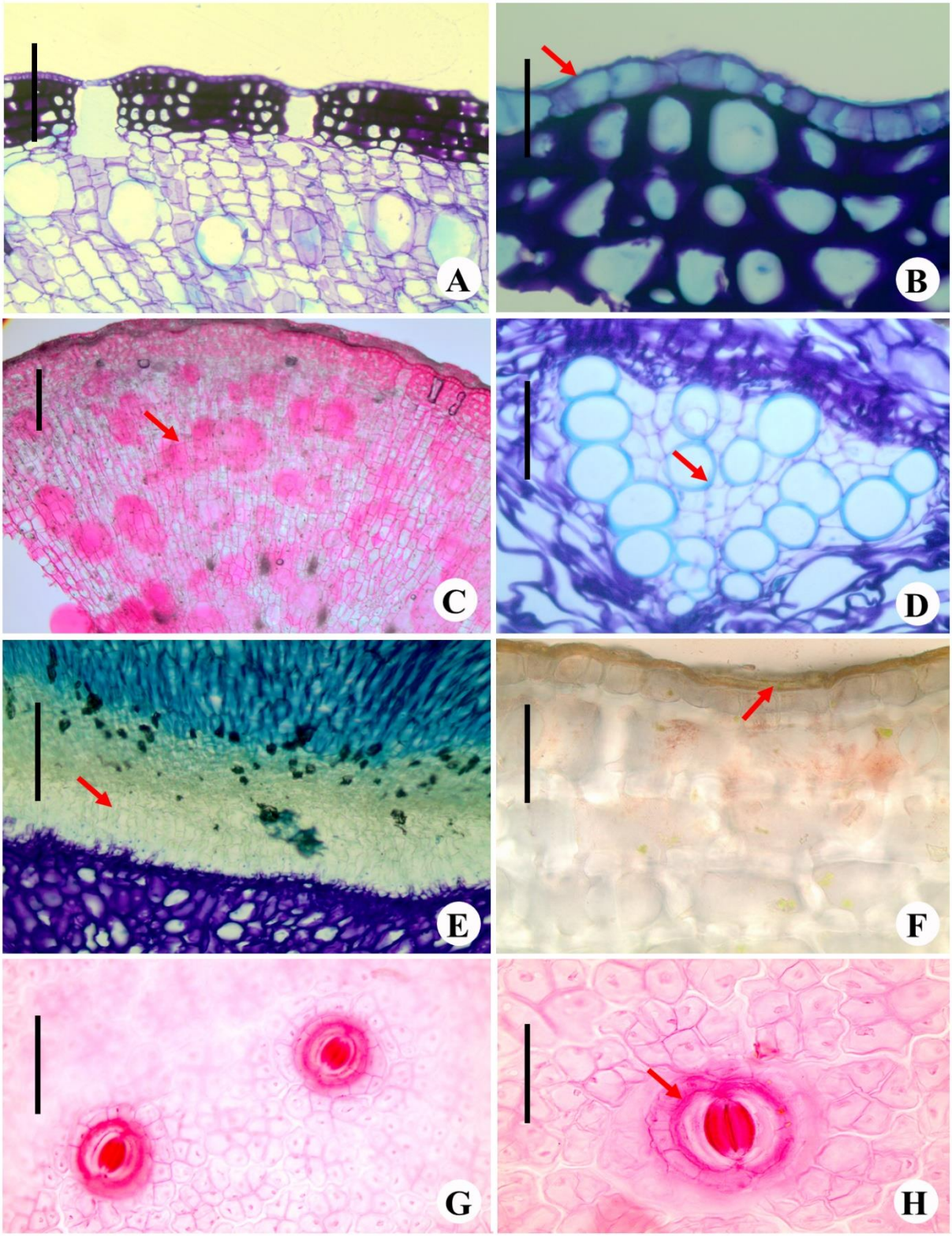

Figure 10. *Cereus jamacaru s*ubs. *calcirupicola* stem*:* A: General view of cortex; B: TS of simple unisseriate epidermis; C: TS of the cortex with mucilaginous cells; D: TS showing cortex dispersed vascular bundle; E: TS of the areolar region; F: Histochemical test with Sudan IV showing cuticle; G: General view of paradermal section; H: Paradermal section with stomata. Scales: A-C-D-E-G: 100µm; B-D-F-H: 50µm; C: 300µm.

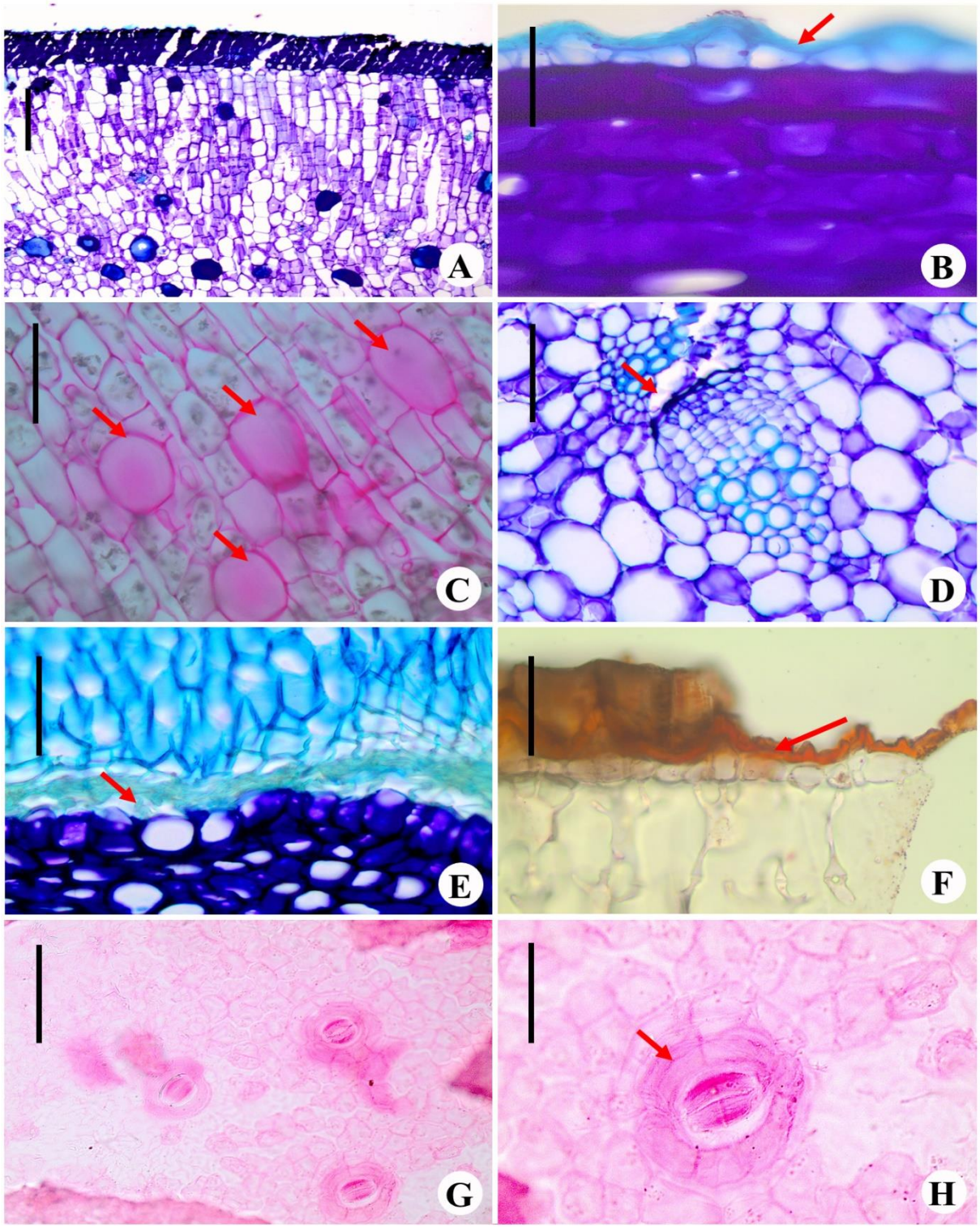

Figure 11. *Cereus pierrebraunianus* stem: A: General view of cortex; B: TS of papillose epidermis; C: Histochemical test with Ruthenium red showing mucilaginous cells; D: Vascular bundle; E: TS of the areolar region; F: Histochemical test with Sudan IV showing cuticle and epicuticular wax; G: General view of paradermal section; H: Paradermal section with stomata. Scales: A-C-D-G: 100µm, B-E-F-H: 50µm.

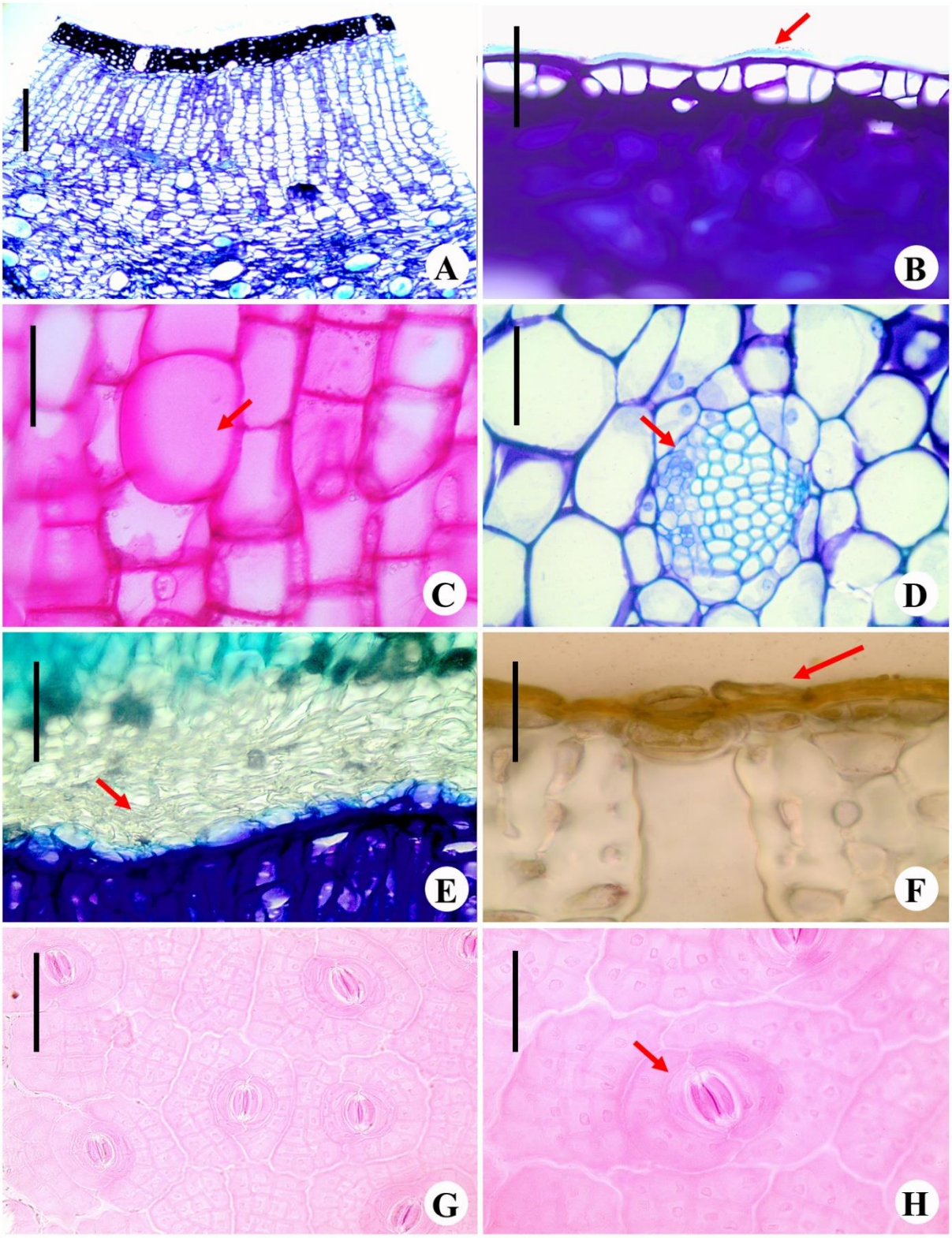

Figure 12. *Cereus stenogonus* stem: A: General view of areole; B: TS of uniseriate epidermis; C: Histochemical test with ruthenium red showing mucilaginous cell; D: Vascular bundle; E: TS of the areolar region; F: Histochemical test with Sudan IV showing cuticle; G: General view of paradermal section; H: Paradermal section with stomata. Scales: A: 300µm; B-D-E-F-H: 50µm, C-G: 100µm.

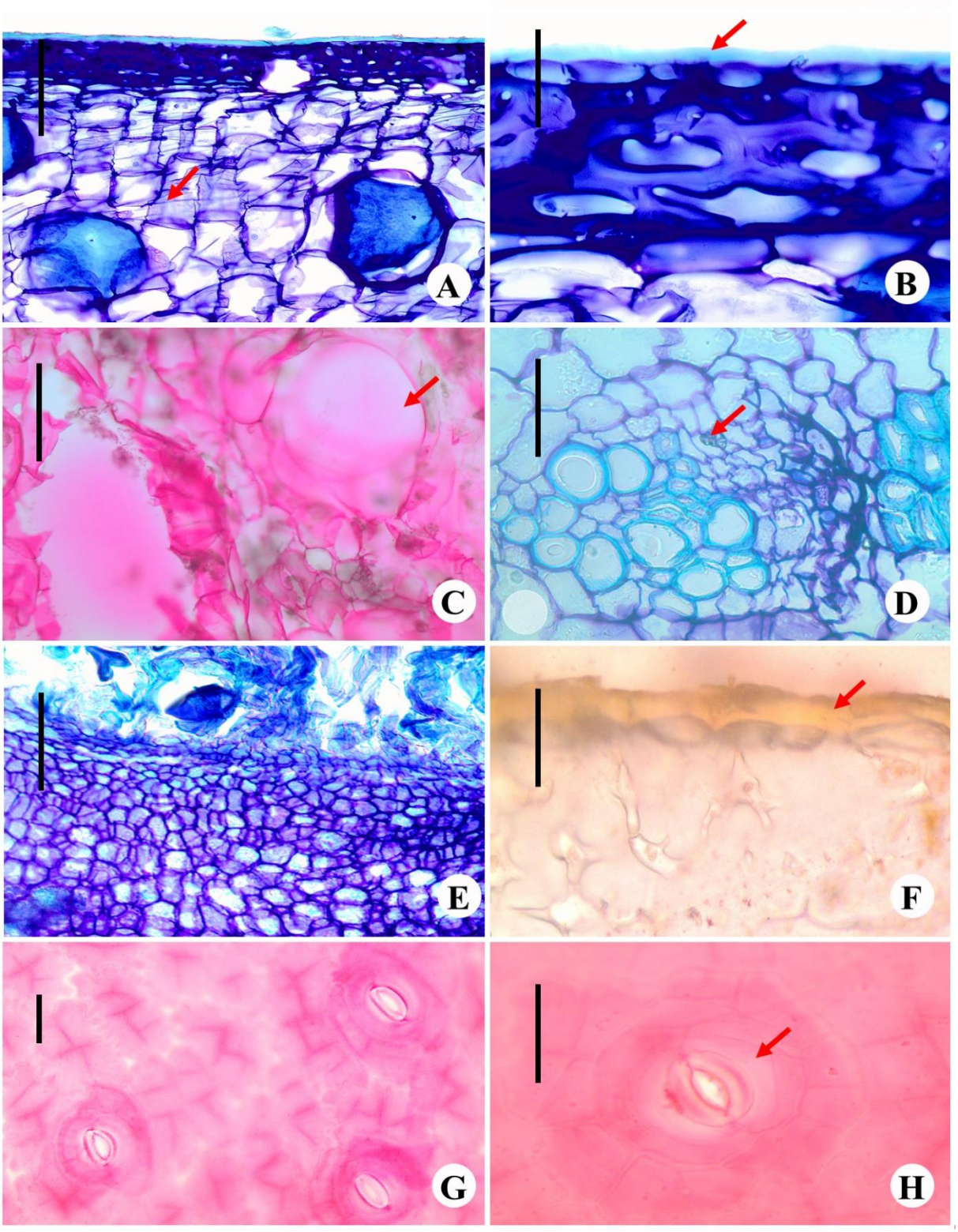

Figure 13. *Cereus phatnospermus* stem: A: General view of the cortex; B: TS of unisseriate epidermis; C: Histochemical test with ruthenium red showing mucilaginous cell; D: TS of vascular bundle; E: TS of the areolar region; F: Histochemical test with Sudan IV showing cuticle; G: General view of paradermal section; H: Paradermal section with stomata. Scales: A: 200µm; B-C-D-E-F-G-H: 50µm.

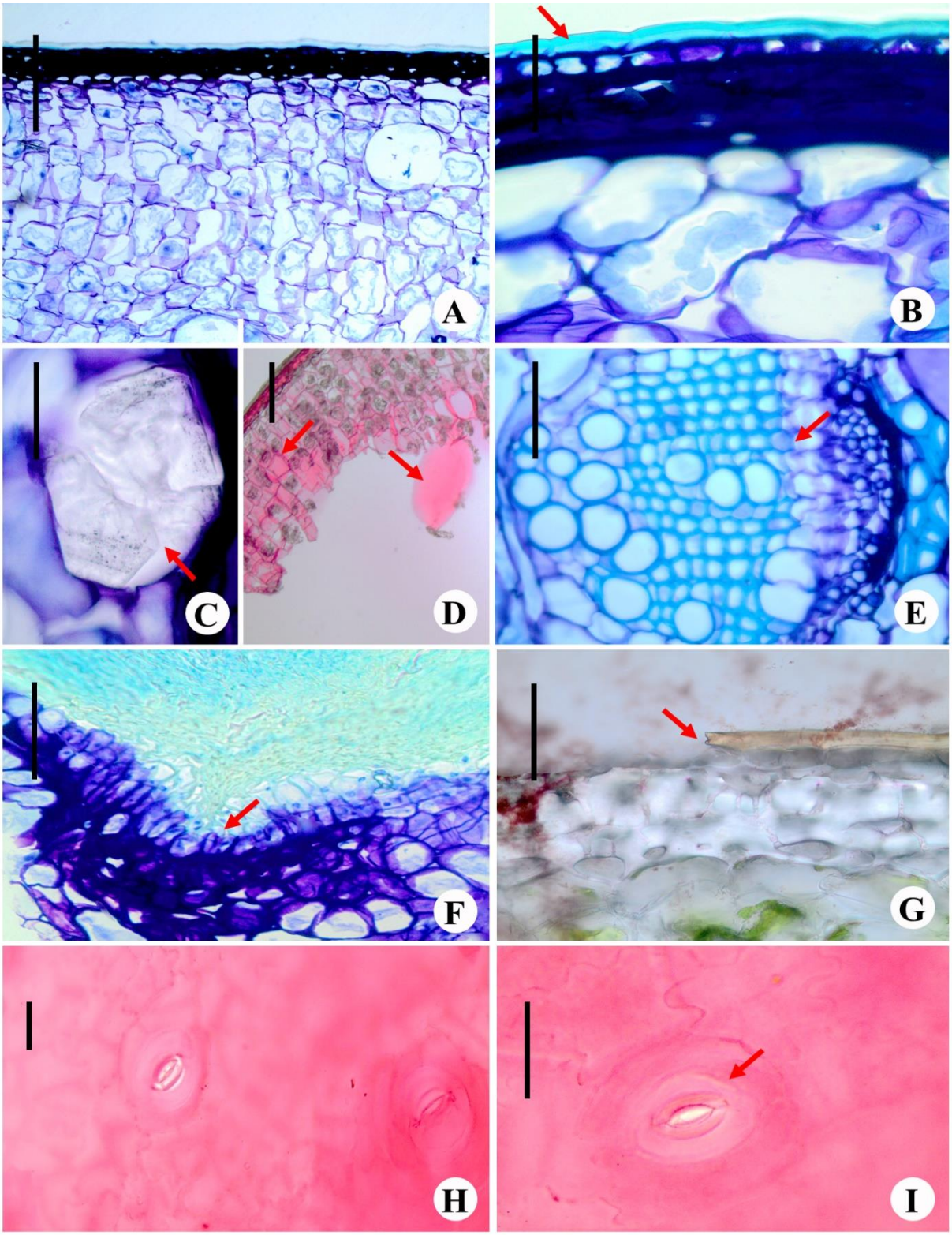

Figure 14. *Cereus saddianus* stem: A: General view of the cortex; B: TS of unisseriate epidermis; C: TS with crystal dispersed in the cortex; D: Histochemical test with Ruthenium red showing mucilaginous cells; E: Vascular bundle; F: TS of the areolar region; G: Histochemical test with Sudan IV showing cuticle; H: General view of paradermal section; I: Paradermal section with stomata. Scales: A: 200µm; B-C-E-F-G-H-I: 50µm; D: 300µm.

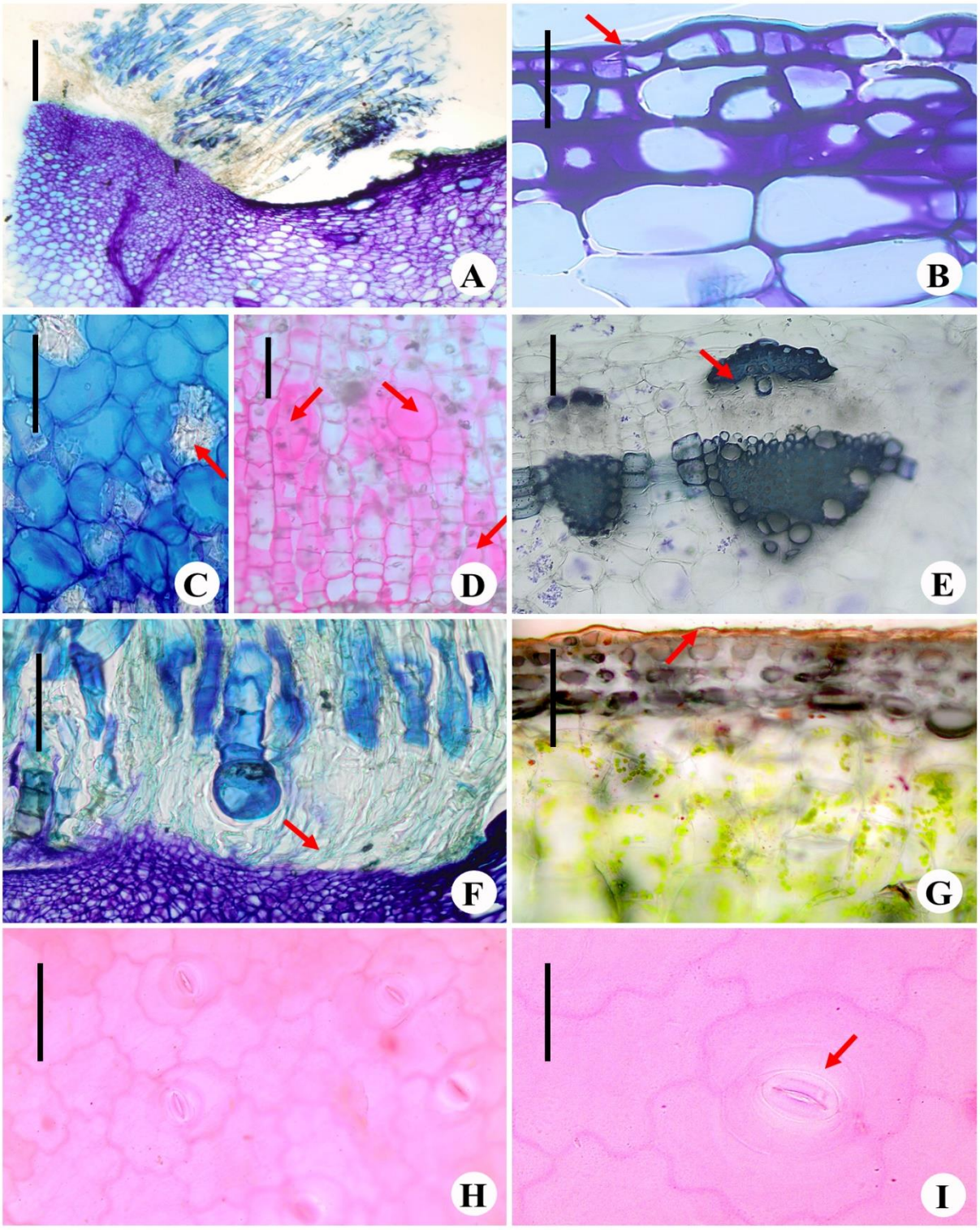

Figure 15. *Cereus spegazzinii* stem: A: General view of the areole; B: TS of the unisseriate epidermis; C: Crystals dispersed in the cortex; D: Histochemical test with Ruthenium red showing mucilaginous cells; E: Freehand cut and stained with Toluidine Blue from the vascular tissue; F: TS of areolar region; G: Histochemical test with Sudan IV showing cuticle; H: General view of paradermal section; I: Paradermal section with stomata. Scales: A: 300µm; B-C-I: 50µm; D-E: 200µm; F-G-H: 100µm.

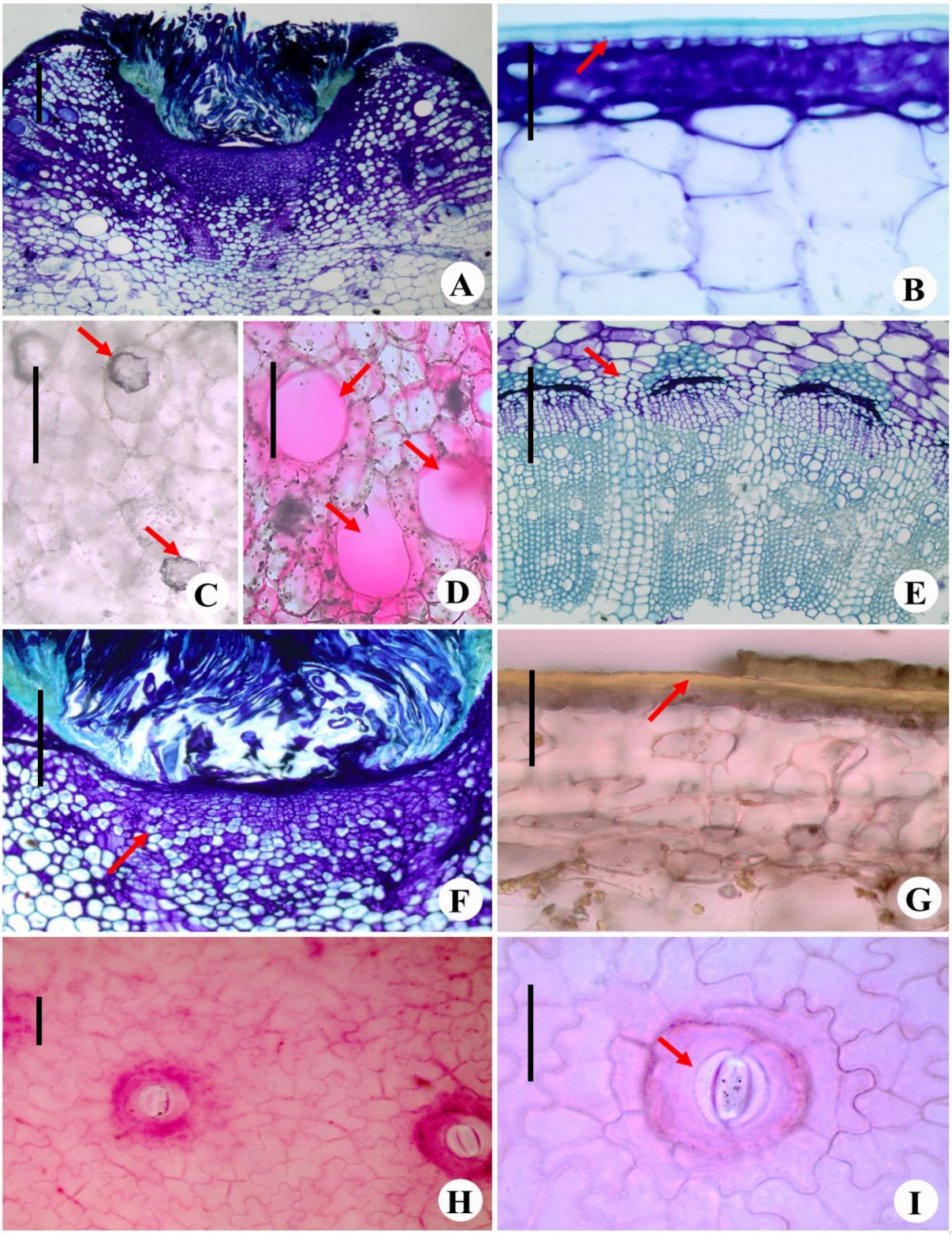

Figure 16. *Cereus albicaulis* stem: A: General view of the cortex; B: TS of the unisseriate epidermis; C: TS with crystals dispersed in the cortex; D: Histochemical test with Ruthenium red showing mucilaginous cells; E: Vascular bundle; F: TS of areolar region; G: Histochemical test with Sudan IV showing cuticle; H: General veiw of paradermal section; I: Paradermal section with stomata. Scales: A: 300µm; B-G-I-H: 50µm; C-D-E-F: 200µm.

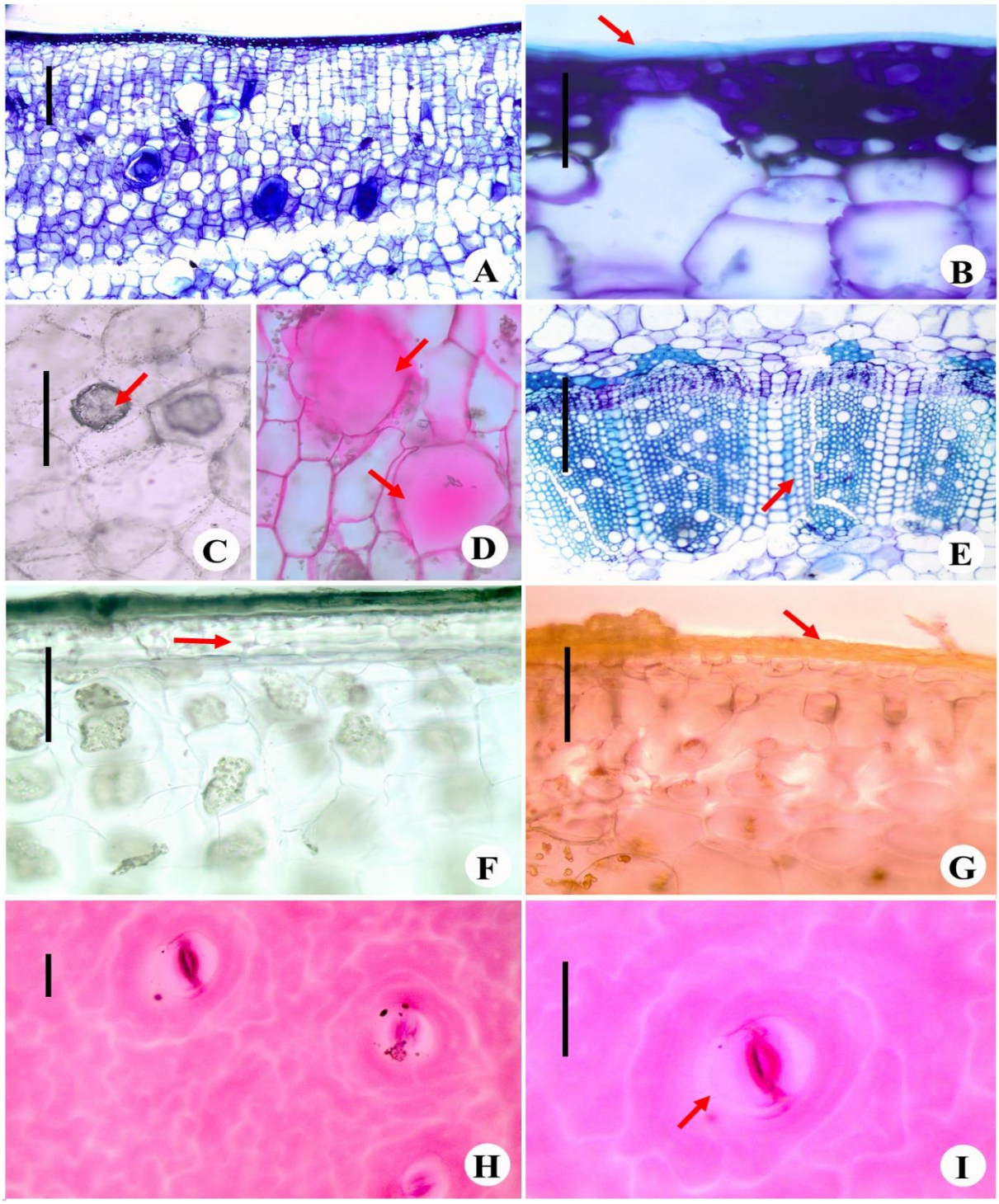

Figure 17. *Cereus mirabella* stem: A: General view of the cortex; B: TS of the unisseriate epidermis; C: TS with crystals dispersed in the cortex; D: Histochemical test with Ruthenium red showing mucilaginous cells; E: Vascular bundle; F: Histochemical test with floroglucinol showing the absence of lignine in the collenchyma region of the hypodermis; G: Histochemical test with com Sudan IV showing cuticle; H: General veiw of the paradermal section; I: Paradermal section with stomata. Scales: A: 300µm; B-G-H-I: 50µm; D-F-H: 100µm, E-C: 200µm.

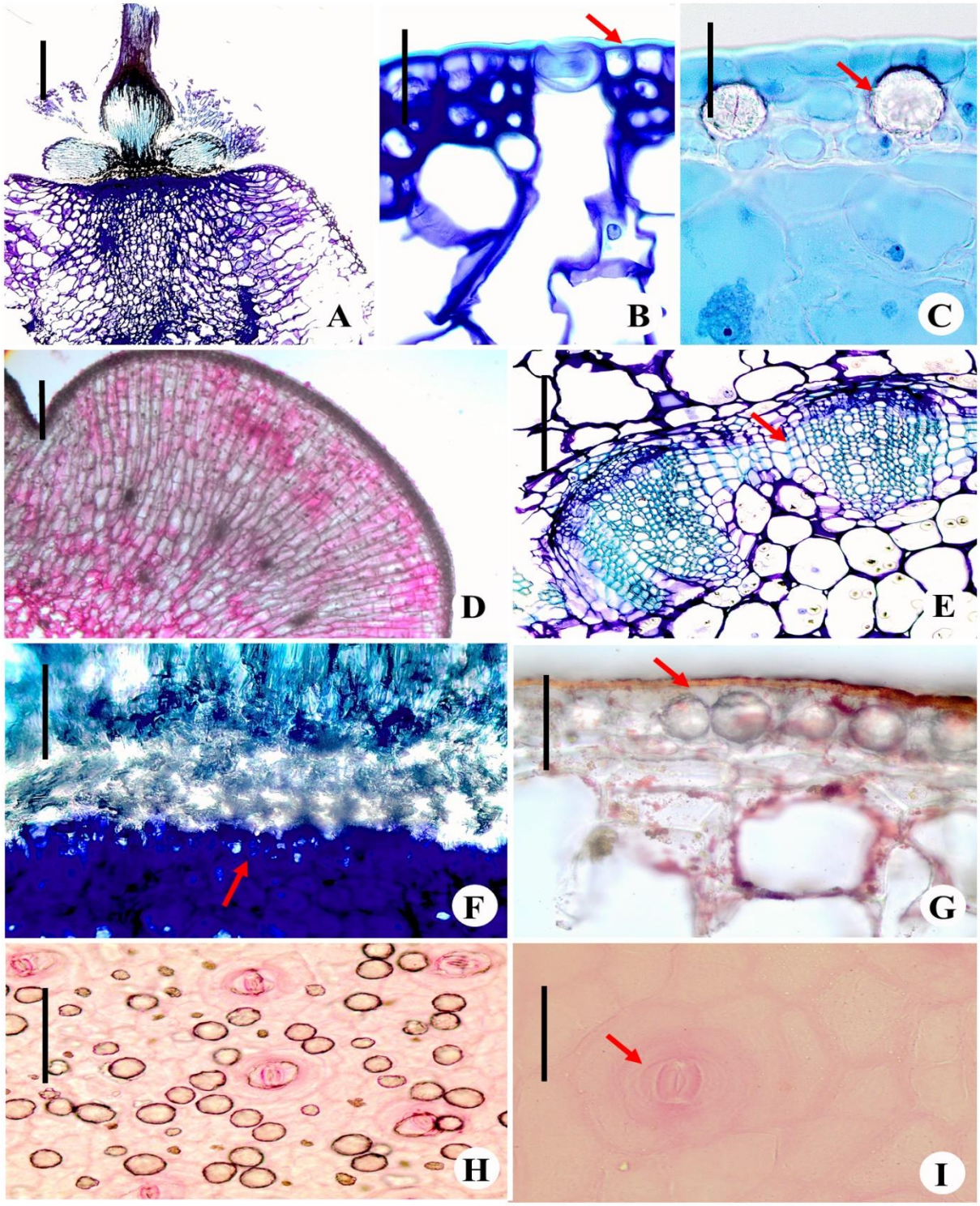

Figure 18. *Praecereus saxicola* stem: A: General view of the areole; B: TS of unisseriate epidermis; C: TS of the epidermis showing mineral inclusion in the hipodermis; D: Histochemical test with Ruthenium red showing absence of mucilaginous cell; E: Vascular bundles; F: TS of the areolar region; G: Histochemical test with Sudan IV showing cuticle; H: General view of paradermal section; I: Paradermal section with stomata. Scales: A-D: 300µm; B-C-F-G-I: 50µm, E: 200µm H: 100µm.

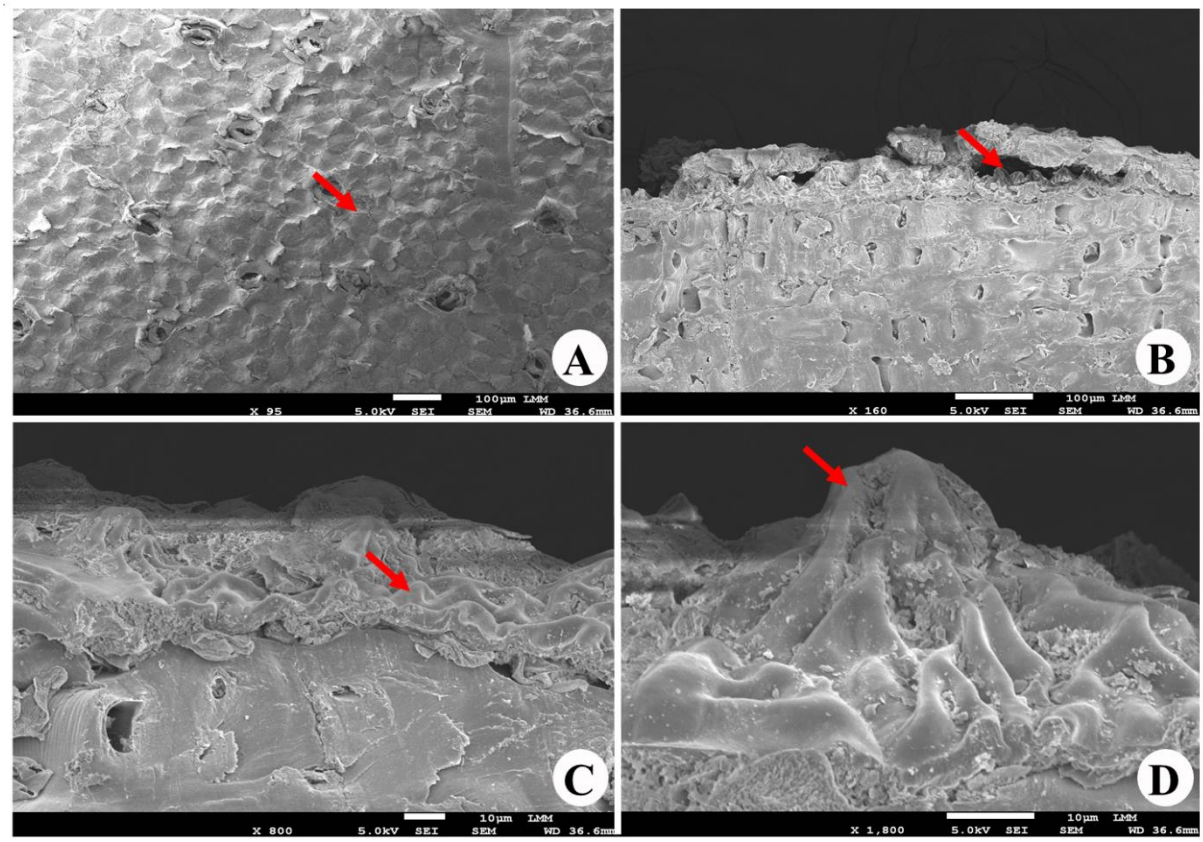

Figure 19. Scanning Electron Microscope of *Cereus pierrebraunianus*: A: General view; B-C-D: TS of the epidermis showing epidermic papillae.

DISCUSSION

Plant anatomy has been used as a taxonomic tool for hundreds of years. Eventual doubts to identify a succulent cactus or euphorbia can be easily dispelled by verifying the presence of secretory structures (laticiferous) in the latter, which are largely absent from the first one (Cutler *et al.*, 2008). Research showing the use of anatomical characters in grouping and diagnosing genera and species of Cactaceae (Solereder, 1908; Gibson *et al.*, 1978; Mauseth, 1996; Calvente *et al.*, 2008; De la Rosa-Tilapa *et al.*, 2019) demonstrates that it is possible to differentiate even seedlings of different species of this family (Kalashnyk *et al.*, 2016). Herewith the use of anatomical charactes to distinguish between 16 studied species of *Cereus* and one species of *Praecereus*, although it was not possible to identify exclusive anatomical characters that seggregate the subgenera of *Cereus*.

Epidermis characters have proven very useful when it comes to differentiating taxa (Dettke & Milaneze-Gutierre, 2008). This tissue also presented the highest number of characters (30 factors) considered for the present study of Cactaceae, being true also for other plant groups,

such as Arecaceae (Pinedo *et al.*, 2016); Lauraceae (Gomes-Bezerra *et al.*, 2018) and Myrtaceae (Gomes *et al.*, 2009), with Poaceae accumulating the largest number of epidermic characters useful for taxonomy (*e.g.* Ellis 1976, 1979; Oliveira *et al.*, 2019). This tissue is in direct contact with the environment, being the first to interact with natural selective pressures, while the remaining tissues are more internal and remain protected by it.

Stem epidermis in Cactaceae may be smooth or papillose (Dettke & Milaneze-Gutierre, 2008), or with convexed cells that produce a microscopically bullate surface (Terrazas & Arias, 2003). The presence of papillae distinguished *Cereus pierrebraunianus* from the remaining analysed species, representing a good taxonomic character. Such structures have been associated with mechanisms that minimize water loss through light reflection and protecting the mesophyll from overheating (Metcalfe & Chalk, 1979). Eventhough such papillae are restricted to a single studied species, more complex analysis of these structures may indicate relevant characters for the family taxonomy (Loza-Cornejo & Terrazas, 2003).

The unisseriate epidermis is common in Cactaceae, as seen here for *Cereus*, with the exceptions being *Astrophytum* Lem., *Eriosyce* Phil., *Eulychnia* Phil., *Pachycereus* (A. Berger) Britton & Rose and *Stenocereus* Riccob. (Gasson, 1981; Terrazas & Mauseth, 2002; Terrazas *et al.*, 2005; Calvente *et al.*, 2008).

Epidermic cells have geen described as square vs rectangular in transversal view, separating taxa (Gasson, 1981; Terrazas & Mauseth, 2002; Calvente *et al.*, 2008), however the overall shape of the cells is different and these often have irregular limits. Equivalent terms used in this study are erect vs procumbent cells, to describe cells that are taller than wide vs other sthat are wider than tall. Most of the species analysed presented procumbent cells, while erect cells were found in the epidermis of *C. hexagonus*, *C. jamacaru*, *C. jamacaru* subsp. *calcirupicola*, *C. stenogonus* and *P. saxicola*.

The stem surface may vary from smooth to rugose in Cactaceae (Anderson, 2001; Terrazas & Mauseth, 2002), reflecting a wax covered cuticle that may present ornamentations (Fahn, 1982). Wax may also mask the cuticle surface and cover stomata (Cutler *et al.*, 2008).

Both cuticle and epicuticular wax have lipidic and hydrophobic nature, and these substances are deposited on the external periclinal cell wall (Cutler *et al.*, 2008). The Sudan test distinguished the cuticle and wax layers (Johansen, 1940), enabling their independent visualization, enabling for measurement of each layer. Some authors did not use this test (*e.g.* Loza-Cornejo & Terrazas, 2003; Terrazas & Arias, 2003), therefore their measurements of cuticle thickness generate uncertainty. The cell wall is normally thinner than the cuticle,

however seedlings of *Melocactus curvispinus* Pfeiff. have thicker cell wall than cuticle, enabling to distinguish this species among other then Cactoideae (Kalashnyk *et al.*, 2016).

In young epidermic cells the cuticle is thin, however it becomes thicker as the cells mature (Darling, 1989); the thickness is also determined by environmental factors (Terrazas & Arias, 2003), such as light, temperature and humidity (Metcalfe & Chalk, 1979). Such factors certainly contributed to the high standard deviation found in our measurements of this structure.

Even though used more often as habitat indicators, cuticle characters may present taxonomic value (Metcalfe & Chalk, 1979), as they are genetically fixed (Martínez-Quezada *et al.*, 2020). Cuticle thickness has been classified from thin to very thick, distinguishing Cactaceae taxa (Conde, 1975; Anderson 1987; Terrazas *et al.*, 2005; Martínez-Quezada *et al.*, 2020). Such classification has proven to be useful in the present work, with extremes of thinnest cuticle in *C. insularis* (3,4±2,1 μm) and thickest in *C. hildmannianus* (22±16,2 μm). This variation is within the amplitude 1–25 μm reported for Cactoideae (Terrazas & Arias 2003), but there are cases of 225 μm thickness in *Ariocarpus fissuratus* (Engelm.) K. Schum., from the same subfamily as *Cereus* (Loza-Cornejo & Terrazas, 2003).

Cactus stomata are found mainly in stem depressions or grooves in Cactaceae (Solereder, 1908) and their frequency has been considered unstable in these plants (Conde, 1975; Terrazas & Mauseth, 2002). The majority of the 150 anatomically documented cactus species has stomata levelled with the epidermis layer (Eggli, 1984), as does the majority of the 21 North American genera of Cactaceae, except *Pachycereus pecten-aboriginum* (Engelm. ex S. Watson) Britton & Rose and *P. tepamo* Gama & S. Arias, with stomata in depressions (Loza-Cornejo & Terrazas, 2003). The stomata depressions may reach the cortical parenchyma, as seen in globular cacti (Gasson, 1981) and in *Rhipsalis grandiflora* Haw., *R. paradoxa* (Salm-Dyck ex Pfeiff.) Salm-Dyck and *R. pentaptera* Pfeiff. ex A. Dietr. (Calvente *et al.*, 2008). Here we report stomata in slight depressions in *C. hildmannianus*, *C. jamacaru* and *C. pierrebraunianus*, while only *C. jamacaru* subsp. *calcirupicola* had stomata in deep caves. The remaining species and *Praecereus euchlorus* have stomata levelled with the epidermis as predominates in the family.

Initially only paracytic stomata were reported for cacti (Metcalfe & Chalk, 1979), and this was confirmed in 80 genera and 350 species (Terrazas & Arias, 2003) and in 21 genera and 70 species of Cactoideae (Loza-Cornejo & Terrazas, 2003). This stomatic type was also reported in 22 species of *Stenocereus* (Terrazas *et al.*, 2005), as well as in 69 species of Hylocereeae and six species of Echinocereeae (Martínez-Quezada *et al.*, 2020), all belonging

to the subfamily Cactoideae. This type of stomata also predominates in Opuntioideae species; however, this subfamily has hexacytic stomata hexacytic (Loza-Cornejo & Terrazas, 2003; Arruda *et al.*, 2005; Arruda & Melo-de-Pinna, 2015). Other stomata types have been reported, including intraspecific variation in *Opuntia ficus-indica* with ciclocytic, tetracytic or opuntioid stomata (Herrera-Martinez *et al.*, 2015).

Stomatic classification is a difficult task especially for *Cereus* species, as the visualization of cells is made harder by the thick layer of epicuticular wax and sometimes by the positioning of stomata in depressions. Careful focal analysis fo the paradermal preparations is essential for interpreting the stomatic complex and is not always possible to record this in the images. On the other hand, Scanning Electron Microscopy is not the most adequate technique to reach this objective (*e.g.* in Loza-Cornejo & Terrazas, 1996; Herrera-Martinez *et al.*, 2015; Martínez-Quezada *et al.*, 2020) as it does not reveal the limits between cells but only the topography of the organ studied.

As well as the methodologic limitations, there is no unanimity in stomata classification. Baranova (1987) points to two classification types, one focusing the ontogeny of the subsidiary cells, the other based on morphology, including cell number and position. Classifying stomata as anomocytic, anisocytic, paracytic and diacytic (Metcalfe & Chalk, 1950) is widely accepted and was followed here, where all analysed species were paracytic.

Baranova (1987) also explains that the terminology grows, including cases of stomata surrounded by epidermic cells, as well as subsidiary cells. Complex terminologies are of difficult application as their subtleties are not always easily distinguished. Herewith we propose counting the adjacent cells to the paracytic stomata, which appears to be a good taxonomic character. Adjacent cells are different from ordinary epidermic cells in size and/or shape; however they are not in contact with the guard cells, thus differing from subsidiary cells. The highest number of adjacent cells (five) has distinguished *C. jamacaru* from all other species, where two to three adjacent cells were found. Ontogeny studies for stomata in Cactaceae are still absent, and only those may help to clarify if all adjacent cells are really subsidiary cells, i.e. originated from the division of the same mother cell.

The orientation of the ostiole is a noteworthy character in Cactaceae and may discriminate taxonomic groups (Solereder, 1908; Metcalfe & Chalk, 1950, 1979), however it is not always considered. The ostiole may appear horizontally or vertical (perpendicular) in relation to the longitudinal stem axis or be distributed randomly in certain groups within the family (Solereder, 1908).

The relation between length and width of the guard cells reveals whether these are relatively wide or thin. These values have never been considered in Cactaceae, however they discriminate *C. pierrebrauniannus* in relation to the other studied species, as these cells are five times longer than wide in this species. In absolute terms, the guard cells are wider in *C. hildmannianus* (17,7±11 μm), being approximately twice as wide as the narrowest ones found in *C. albicaulis* (9,2±5,8 μm).

In cacti the epidermis is typically followed by hypodermic layers that are involved in the survival physiology in xeric environments (Loza-Cornejo & Terrazas, 2003). Substomatal chambers go beyond the hypodermis, a universal character in the Cactaceae (Darling, 1989; Loza-Cornejo & Terrazas, 2003; Soffiatti & Angyalossy, 2007).

The hypodermis in cacti is collenchymatich, with cells bearing thick primary walls, normally appearing in 1–6 layers that may, as the epidermis, contain mineral inclusions (Gibson & Nobel, 1986; Nyffeler & Eggli, 1997; Loza-Cornejo & Terrazas, 2003; Terrazas & Arias, 2003; Soffiatti & Angyalossy, 2007; Calvente et *al.*, 2008; Arruda & Melo-de-Pinna, 2015). The presence of extensive layers of collenchymatic hypodermis is widely documented within the family (Darling, 1989) and was corroborated herewith.

The cortex and pith are regions where the main water storage tissues of cacti are located. These are composed by parenchymatic cells with thin walls that remain alive in the pith even considering the age of the stem in Cactoideae and Opuntioideae, being responsible for the mucilage secretory cells that may be observed in species of *Arrojadoa*, as well as vascular bundles and starch grains (Terrazas & Mauseth, 2002; Soffiatti & Angyalossy, 2007).

Chlorenchyma is found in the external cortical region, consisting in a palisade parenchyma (Terrazas & Mauseth, 2002). Mucilage cells are abundant in cacti and reflect an influence in the family metabolism (Gibson & Nobel, 1986). In Opuntioideae mucilaginous cells correspond to c. 3% of the total volume of the stem, accumulating functions of calcium deposit, water storage and thermic protection to survive elevated temperatures (Gibson & Nobel, 1986). In Cactoideae only mucilage cells are found while species from other subfamilies may have both cells and mucilaginous channels (Arruda *et al.*, 2005). Calvente e*t al.* (2008) point out that, despite the presence and frequency of this structure being different in different groups of Cactaceae, these are inconstant within species and make the character inconclusive for taxonomic delimitation.

Vascular bundles both in the pith and cortex are found in Cactaceae, with secondary phloem characterized by the presence of sieve tube elements that may be solitary or grouped

with companion cells, secondary xylem with solitary or multiple tube elements and simple perforation plates (Soffiatti & Angyalossy, 2007). The cortical vascular and pith bundles identified in histological analyses are correlated with important physiological adaptations to avoid embolism, as well as to influence the transport of products synthetized during photosynthesis in the chlorenchyma to the vascular cylinder (Arruda *et al.*, 2005). Starch deposits are also more frequently found in the pith than in the cortex, (Dettke & Milaneze-Gutierre, 2008) as was also observed in the analysed species.

Considered as synapomorphies for the family, the areoles correspond to axillary buds in other angiosperms, and contain a meristematic region, with spines characterized as foliar structures (Arruda & Melo-de-Pinna, 2015; Carneiro *et al.*, 2016). Mauseth (2007) highlights the difference between cactus spines and the anatomy of leaves of Eudicotiledoneae, therefore the spines are developed from a basal meristem.

The presence of lignin at the apex of the areolar region is due to the spines reaching maturity through cell death and presenting basically lignified cells (Mauseth, 2007), and this was seen in the transversal sections of areoles examined both in the historesin inclusion as in histochemical tests to detect lignin, showing that the studied species already had a dormant meristematic region. Arruda & Melo-de-Pinna (2015) describe for Opuntioideae that spines indicated as persistent do not present vascular tissues in the meristematic region and that, after cell division, these die and become sclerified.

The trichomes found in the areoles are described by Arruda & Melo-de-Pinna (2016) as uniseriate and developed in the region known as tunic, that originates the protodermis through anticlinal divisions. In the tests performed, the trichomes also indicated presence of lignin in their composition, however the presence of pluricellular trichomes was observed. However, other characteristics need to be better examined in order to correlate with the explanations of the authors.

Darling (1989) states that, due to the arid environment, exceptional epidermis adaptations to avoid water loss, such as the presence of trichomes, thick cuticle and with epicuticular wax, as well as substomatal cavities are found in the family, all these are related to the arid environment, adaptations in the epidermis are exceptional to avoid water loss, such as the presence of trichomes and spines, thick cuticle and epicuticular wax, all reported in the studied species.

It is possible to conclude that the characters analysed strengthened the understanding of the taxonomy and anatomy of the species of *Cereus*. No exclusive characters were found for

the genus in comparison to the whole family, however among the new characters tested, the following were considered useful: number of epidermic layers, epidermic crystals, shape of common cells, presence of epicuticular wax, presence of papillae, stomata position, number of hypodermic layers and position of cortical vascular bundles. The following characters may have been useful at generic level but were not useful for distinguishing species: cuticle thickness, type of stomata, stomatic pore orientation in relation to the plant axis, thickening of the hypodermic walls, hypodermic crystals, palisade parenchyma, mucilaginous cavities, starch reserve, pluricellular tector trichomes and absence of sharp cells in the areole. The number of cells adjacent to the stomata, substomatal chambers, collenchyma in cortical cells, latex ducts and lignified apex of the areole were not found to be useful.

It is important to highlight that the coronavirus pandemic, which began in 2020, imposed significant difficulties, limiting the collection and receipt of the necessary sample material for the research, in addition to preventing the implementation of essential plant anatomy protocols for the Cactaceae family. However, it is observed that new avenues are being opened for studies that relate the described evolutionary characters to new phylogenies, as evidenced by Taylor *et al.* (2024). Furthermore, future studies involving populations and a larger number of samples may validate the observed characters and their respective environmental specificities, as samples from the Caatinga and Cerrado were analyzed.

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APPENDIX

Table 2. Anatomic characters of Cactaceae stem (adapted from 1. Arruda & Melo-de-Pinna, 2015; 2. Dettke & Milaneze-Gutierre, 2008; 3. Calvente *et al.*, 2008; 4. Terrazas & Mauseth, 2002; 5. Anderson, 2001; 6. Faigón *et al.*, 2011).

	Characters	**Source**
Number	**Epidermis**	
1	Crystals (presence/absence)	4
2	Crystal type	
3	Common cells: anticlinal cell walls	
4	Ratio thickness: width (ST)	
5	Predominant shape (ST)	
6	Ratio lenght: width (paradermal)	
7	Cuticle + periclinal external wall	2
8	Strip thickness	
9	Epicuticular wax	2
10	Thickness of the epicuticular wax strip	
11	Papillae	4
12	Papillae shape	6
13	Ratio height-width	
14	Striations in papillae	6
15	Stomata type	1
16	Ratio lenght : width of guard cell	
17	Number of stomata adjacent cells	
18	Stomata position	5
19	Orientation of stomatic pores relative to the plant axis	2
	Cortex	
20	Number of hypodermis layers	4
21	Thickening of hypodermis layer	4
22	Hypodermic crystals	
23	Chlorenchyma of cortical cells	5
24	Primary phloem bundle caps	
25	Ratio height: width of palisade parenchyma cells	
26	Cellular inclusions	2
27	Mucilaginous cavities	3
	Medulla (pith)	
28	Mucilaginous cavities	3
29	Starch reserve	2
30	Pith vascular bundles	3

Table 3. Analysis of the anatomical characters of Cactaceae stems.

	Characters	Character states	Source	*C. bicolor*	*C. fernambucensis*	*C. fernambucensis* subsp. *sericifer*	*C. gerardii*	*C. hexagonus*	*C. hildmannianus*	*C. insularis*	*C. jamacaru*	*C. jamacaru subsp. calcirupicola*	*C. pierrebrauniannus*	*C. stenogonus*	*C. phatnospermus*	*C. saddianus*	*C. spegazzinii*	*C. albicaulis*	*C. mirabella*	*P. saxicola*
Nº caráter	**Epidermis**																			
1	Crystals	Presence/Absence	4	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Present
2	Crystal type	Druse, raphid, cube, two pyramids, polyedric		N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	Druse
3	Common cells: anticlinal cell walls	Straight, curved, sinuous		Sinuous	Straight	Sinuous	Straight	Sinuous	Straight	Curve	Sinuous	Straight	Sinuous	Curve	Sinuous	Sinuous	Sinuous	Sinuous	Sinuous	Sinuous	Curve
4	Ratio thickness : width (ST)			18.4±11.3 µm × 40.5±19.7 µm	21±13,6 µm × 37,6±20,7 µm	26,3±11,2 µm × 35,4±15,7 µm	31±18,8 µm × 43,3±19,1 µm	27,1±15,7 µm × 41,8±15,3 µm	29±15,5 µm × 43,4±14,2 µm	37,6±14,9 µm × 42,1±24,5 µm	36,4±21,7 µm × 37,5±20,5 µm	26,1±14,4 µm × 24±10,8 µm	50,5±20,7 µm × 34,9±24,6 µm	25,8±16,2 µm × 30,4±12,3 µm	16,3±8,6 µm × 52,2±20 µm	12,5±9 µm × 29,5±13,2 µm	21±15,5 µm × 32±19,4 µm	11±5,9 µm × 31±16 µm	14,8±8 µm × 21,9±9,7 µm	26,8±9,8 µm × 40,1±9,7 µm
5	Predominant shape (ST)			Procumbent	Procumbent	Procumbent	Procumbent	Erect	Procumbent	Procumbent	Erect	Erect	Procumbent	Erect	Procumbent	Procumbent	Procumbent	Procumbent	Procumbent	Erect
6	Ratio lenght: width (paradermal)			83,7±54 µm × 57,6±27,8 µm	130,4±77,6 µm × 82,4±42,2 µm	172,9±124,5 µm × 85,1±39,1 µm	142±53,5 µm × 59,3±33,5 µm	97,8±53 µm × 50,5±35,4 µm	47,9±33,7 µm × 41,3±25,3 µm	121,7±74, 9 µm × 77,5±37,5 µm	146,1±84, 8 µm × 77,1±42,7 µm	98,2±56,4 µm × 60±28,9 µm	91,8±20,7 µm × 45±24,6 µm	42,4±20,1 µm × 33,3±13,5 µm	59,5±37,3 µm × 36,4±24,4 µm	126±68,9 µm × 71,8±42,6 µm	157,4±99, 1 µm × 119,9±46, 5 µm	122,3±73, 1 µm × 64,2±28,7 µm	188,1±74, 8 µm × 71,6±49,1 µm	91,6±49,6 µm × 44,7±29,2 µm
7	Cuticle + periclinal external wall	Thick, moderately thick, thick	2	Moderately thick	Moderately thick	Moderately thick	Moderately thick	Moderately thick	Thin	Thin	Moderately thick	Moderately thick	Moderately thick	Moderately thick	Thick	Thick	Moderately thick	Thick	Thick	Moderately thick
8	Strip thickness			5,9±2,9 µm	9,8±4,9 µm	8,7±4,5 µm	9,3±5,3 µm	11,8±8 µm	22±16,2 µm	3,4±2,1 µm	11±7,3 µm	7,2±5,1 µm	11,6 ±4,5 µm	6,9±4,6 µm	12,3±8,4 µm	13,6±10,6 µm	4±2,4 µm	11,9±7,7 µm	15,8±9 µm	5,5±3,8 µm
9	Epicuticular wax	Presence/Absence	2	Present	Present	Absent	Present	Absent	Present	Present	Present	Absent	Present	Present	Absent	Present	Absent	Present	Present	Absent
10	Thickness of the epicuticular wax strip			9,7±4,6 µm	16,5±9 µm	N.a.	N.a.	N.a.	58,3±20,1 µm	11,3±6,9 µm	24,6±16,5 µm	N.a.	56,3±26,9 µm	9±4,8 µm	N.a.	14,5±8,4 µm	N.a.	22,6±14,5 µm	20,3±13 µm	N.a.
11	Papillae	Presence/Absence	4	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Present	Absent	Absent	Absent	Absent	Absent	Absent	Absent
12	Papillae shape	Polygonal or Isodiametric	7	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	Isodiametric	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.
13	Ratio height-width			N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	60,3±45,1 µm × 78,7±56,4 µm	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.
14	Striations in papillae	Presence/Absence	7	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.
15	Stomata type	Hexacytic, paracyti or absent	1	Paracytic	Paracytic	Paracytic	Paracytic	Paracytic	Paracytic	Paracytic	Paracytic	Paracytic	Paracytic	Paracytic	Paracytic	Paracytic	Paracytic	Paracytic	Paracytic	Paracytic
16	Ratio lenght : width of guard cell			39,2±30,8 µm × 9,6±4,6	49±38,5 µm × 11,6±6,4 µm	54,5±43,9 µm × 13,2±10,3 µm	47,3±31 µm×12±5,3 µm	44,7±34,1µm × 13,3±8,2 µm	53±43,1 µm × 17,7±11 µm	44,1±34,3 µm × 12,7±7,1 µm	58±44,3 µm × 13±9,3 µm	49,8±40,7 µm × 15,9±10,5 µm	51,4±39,5 µm × 13,2±6,6 µm	44,3±35,3 µm × 14,2±9,2 µm	46,5±35,8 µm × 11,2±6,5 µm	40,6±32,2 µm × 13,3±6,6 µm	41,5±29,9 µm × 9,6±7,7 µm	38,2±33 µm × 9,2±5,8 µm	38,3±31,7 µm × 9,6±5,8 µm	30,1±22,1 µm × 8,7±5,5 µm

17	Number of stomata adjacent cells			2	2	2	2	3	2	2	5	2	2	2	3	2	2	2	2	2
18	Stomata position	Depressed, raised, levelled	5	Levelled	Levelled	Levelled	Levelled	Levelled	Slightly depressed	Levelled	Slightly depressed	Depressed	Slightly depressed	Levelled	Levelled	Levelled	Levelled	Levelled	Levelled	Levelled
19	Orientation of stomatic pores relative to the plant axis	Random or Parallel	2	Random	Random	Random	Random	Parallel	Random	Random	Parallel	Random	Random	Random	Random	Random	Parallel	Random	Random	Random
	Cortex																			
20	Number of hypodermis layers		4	03-04 camadas	02-03 layers	03-04 layers	03-04 layers	02-03 layers	03-04 layers	02-03 layers	05-06 layers	03-05 layers	04-05 layers	04-05 layers	03-04 layers	02-03 layers	02-03 layers	02-03 layers	03 layers	02 layers
21	Thickening of hypodermis layer	Thin, moderately thick, thick	4	Thick	Thick	Thick	Thick	Thick	Thick	Thin	Thick	Thick	Thick	Thick	Thick	Thick	Moderately thick	Thick	Thick	Thick
22	Hypodermic crystals	Presence/Absence		Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
23	Chlorenchyma of cortical cells	Presence/Absence	5	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present
24	Primary phloem bundle caps	Presence/Absence		Present	Absent	Absent	N.a.	Absent	Present	Absent	Absent	Absent	Present	Absent	Present	Present	Present	Present	Present	Present
25	Ratio height: width of palisade parenchyma cells			187,4±104,7µm × 101,5±42,8 µm	161,9±74,6 µm × 77,9±42,9 µm	244,4±97,2 µm × 127,6±82,2 µm	168,2±96,8 µm ×104,9±49,2 µm	125,4±69,8 µm × 85,7±61,9 µm	225,5±114,2 µm × 130,3±66,7 µm	146±61,9 µm × 119,3±73,8 µm	121,4±77,2 µm × 84,1±50,4 µm	107,5±62,7 µm × 98,1±44,6 µm	128,8±69,7 µm × 76,2±49,2 µm	112,8±54,3 µm × 82,3±50,8 µm	207,1±140 µm × 144,7±85,7 µm	118,8±44,2 µm × 78±54,6 µm	74,9±35,9 µm × 97,1±51,6 µm	102,5±61,9 µm × 104,7±52,6 µm	131,8±66,9 µm × 102,8±40,4 µm	202,6±107,9 µm × 92,1±51 µm
26	Cellular inclusions	Presence/Absence	2	Absent	Absent	Absent	Absent	Absent	Present	Present	Absent	Absent	Absent	Absent	Absent	Present	Present	Present	Present	Present
27	Mucilaginous cavities	Present, Absent or Scarce	3	Absent	Absent	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Absent
	Medulla (pith)																			
28	Mucilaginous cavities	Presence/Absence	3	Absent	Absent	Absent	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Absent	Present	Present	Absent
29	Starch reserve	Presence/Absence	2	Absent	Absent	Starchy	Starchy	Absent	Absent	Absent	Starchy	Absent	Absent	Absent	Starchy	Starchy	Absent	Starchy	Starchy	Starchy
30	Pith vascular bundles	Present, Absent or Scarce	3	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Scarce

Nota Científica / Scientific Note

To be submitted to Bradleya

Why is the stem of *Cereus spegazzinii* F.A.C. Weber marbled? A micromorphological investigation of the epidermis of selected *Cereus* species

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ABSTRACT – (**Why is the stem of *Cereus spegazzinii* F.A.C.Weber marbled? A micromorphological investigation of the epidermis of selected *Cereus* species**). *Summary*: The present scientific note aims to explain the variegation of the epidermis of *Cereus spegazzinii* through the comparison of its epidermis with other species of the genus *Cereus*. The results were observed and recorded under optical and scanning electron microscopes. It was concluded that the epidermis variegation is caused by a discontinuous layer of epicuticular wax over a smooth epidermis, and differing from other species that present regular, smooth or papillose epicuticular wax that varies in thickness, but is not discontinuous, adding to the understanding of this unique character state in the cactus family.

Key-words: Stem surface – Cuticle – Epidermis – Ornamental – Pigmentation

RESUMO – (Por que o caule de *Cereus spegazzinii* F.A.C.Weber é marmorizado? Uma investigação micromorfológica da epiderme de espécies selecionadas de *Cereus*). A cera epicuticular pode ser classificada em diversas tipologias, como placas, túbulos, filmes e bastonetes. A presente nota científica tem como objetivo explicar a variegação da epiderme de Cereus spegazzinii por meio da comparação de sua epiderme com outras espécies do gênero Cereus. Os resultados foram observados e registrados em Microscópio Eletrônico de Varredura (MEV). Concluiu-se que a variegação da epiderme é causada por uma camada descontínua de epiderme sobre epiderme lisa, diferentemente de outras espécies que apresentam cera epicuticular regular, lisa ou papilosa, que varia em espessura, mas não é descontínua, contribuindo para o entendimento desse estado de caráter único na família dos cactos.

Palavras-chave: Superfície caulinar – Cutícula – Epiderme – Ornamental – Pigmentação

Easily recognized among higher plants, Cactaceae are mainly native to the Americas, being subjects of horticulture either at a domestic or commercial scale, comprising over 120 genera and more than 1500 species (Hunt et al., 2006). Widespread from the Southern Caribbean to Argentina, *Cereus* is one of the most iconic genera in the Brazilian semiarid region (Albuquerque-Lima et al., 2023). However, this genus is notorious for its difficult taxonomy, with many taxa hard to circumscribe, leading to the publication of a profusion of synonyms both at generic and specific levels. There are around 31 species of *Cereus*, of which 8 are Brazilian endemics (Hunt et al., 2006; Franco et al., 2017; Taylor & Zappi 2019; Zappi & Taylor 2020; Taylor et al., 2023).

Cereus spegazzinii F.A.C.Weber has horticultural value due to its natural variegation, and is cultivated worldwide for its snake-like, marbled stems and fantastic night flowering blooms (Figure 1). Highlighting the adaptations that different species present to survive in specific, narrowly defined habitats (Dettke & Milaneze-Gutierre, 2008), our investigation of *C. spegazzinii* aims to correlate the external morphology with anatomical characters.

The cactus epidermis is a waterproof barrier between the plant and its environment, with important value for the taxonomy of the group (Gasson, 1981). Despite this, there are few studies delimiting the epidermal and hypodermal characters of cacti (Loza-Cornejo & Terrazas, 2003). Epicuticular wax also adds considerable structural and chemical diversity to the family, and its nomenclature has been long established by Barthlott et al. (1998).

The composition of epicuticular wax can be summarized in different types: (1) film, or a thin layer associated with the surface of the cuticle; (2) overlapping, in plants that accumulate waxy particles, known as crystalloids, including platelets, tubes, grains and rods; and (3) crusts and layers, in species that present a regular simple or fissured layer (Barthlott et al., 1998; Ensikat et al., 2006). The morphological analysis of epicuticular wax, as well as the distribution of wax particles in plant species might contribute significantly to the diagnosis and identification of species, thus also aiding more applied research (Metcalfe & Chalk, 1979).

Material and methods

We aimed to investigate what causes the marbled appearance of *C. spegazzinii* F.A.C.Weber (Figure 1) by comparing its epidermis and epicuticular wax with four other species of the genus, namely *C. albicaulis* (Britton & Rose) Luetzelb. (Figure 2), *C. hildmannianus* K.Schum. (Figure 3), *C. jamacaru* DC. (Figure 4) and *C. pierrebraunianus* Esteves-Pereira (Figure 5).

Samples of these five species were analysed at the Plant Anatomy Laboratory of the Universidade de Brasília, Brazil.

Freehand sections were performed with fresh samples for the histochemical test using 2% Sudan IV in 92% ethanol, following Gerlach's (1984) protocol for the identification of lipidic substances. The results were recorded using an Olympus BX40 photomicroscope with image capture system Olympus U-TV0.5XC-3.

For the micromorphological approach, the samples were fixed in FAA 50 (formaldehyde, acetic acid, ethanol 50%) in the proportion 2:1:17 (Feder and O'Brien, 1968) and stored in 50% ethanol. The samples were dehydrated in a progressive ethanolic series up to ethanol 100%, dried to critical point in a Balzers evaporator and fixed on stubs. They were gold sputtered in a Leica Em SCD 500 system and recorded under a Jeol JSM-700IF scanning electron microscope (SEM) to prepare comparative plates.

The classification of the morphology of the epicuticular wax was carried out using the terminology developed by Barthlott et al. (1998).

Results and discussion

The histochemical tests revealed the presence of lipid compounds over the outer periclinal walls of the epidermis, forming a thicker layer in *C. hildmannianus* (Figure 6B) and *C. pierrebraunianus* (Figure 6D). Moreover, a simple manipulation of the samples causes the shedding of a significant portion of this layer, which was demonstrated to be composed of epicuticular waxes, easily removed with minimal friction.

Under SEM, it can be observed that a smooth or regular epicuticular wax, forming a continuous layer is found on the stems of *C. albicaulis* (Figure 7A), *C. hildmannianus* (Figure 7B), *C. jamacaru* (Figure 7C) and *C. pierrebraunianus* (Figure 7D), while in *C. spegazzinii* (Figure 7E) the epidermis appears to be of the overlapping type, showing groups of wax platelets or scales superposed onto the epidermis and constituting a discontinuous layer. This was reinforced by the thickness of epicuticular wax featured in Figure 7, where most species present thick epicuticular wax, while *C. spegazzinii* (Fig. 8E) presents an inconspicuous wax layer.

It is interesting to notice that, despite the fact that sometimes the epicuticular wax obscures the stomata in some plants (Ferreira et al., 2005), in *Cereus* it was possible to observe that the stomata are not obstructed, even in those placed in slight depressions, as in *C. hildmannianus*, *C. jamacaru* and *C. pierrebraunianus*.

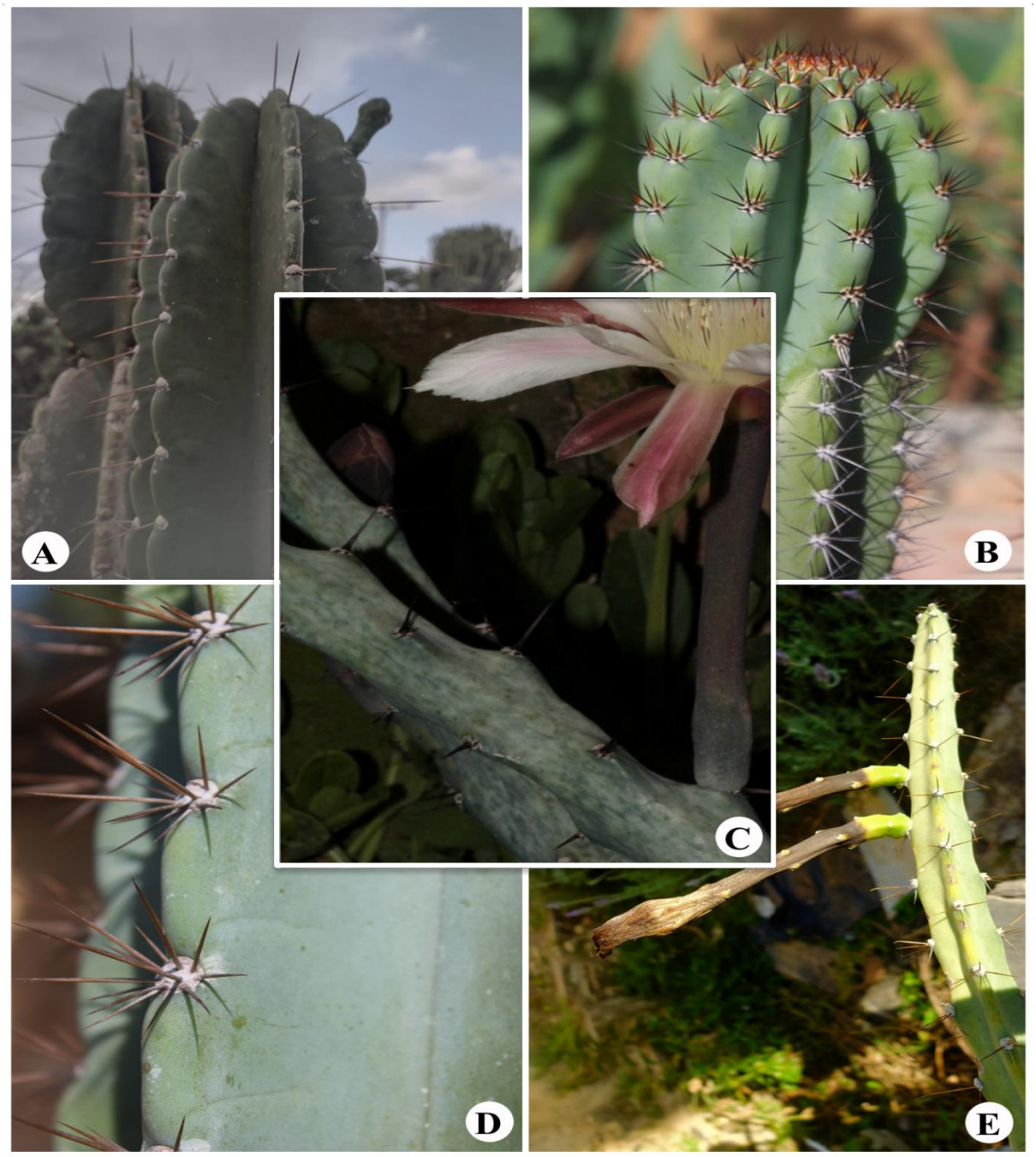

Figure 1. Habit of five studied species, highlighting the epidermis. A: *Cereus hildmannianus* B: *Cereus pierrebraunianus*; C: *Cereus spegazzinii* displaying marbled epidermis; D: *Cereus jamacaru*; E: *Cereus albicaulis.*

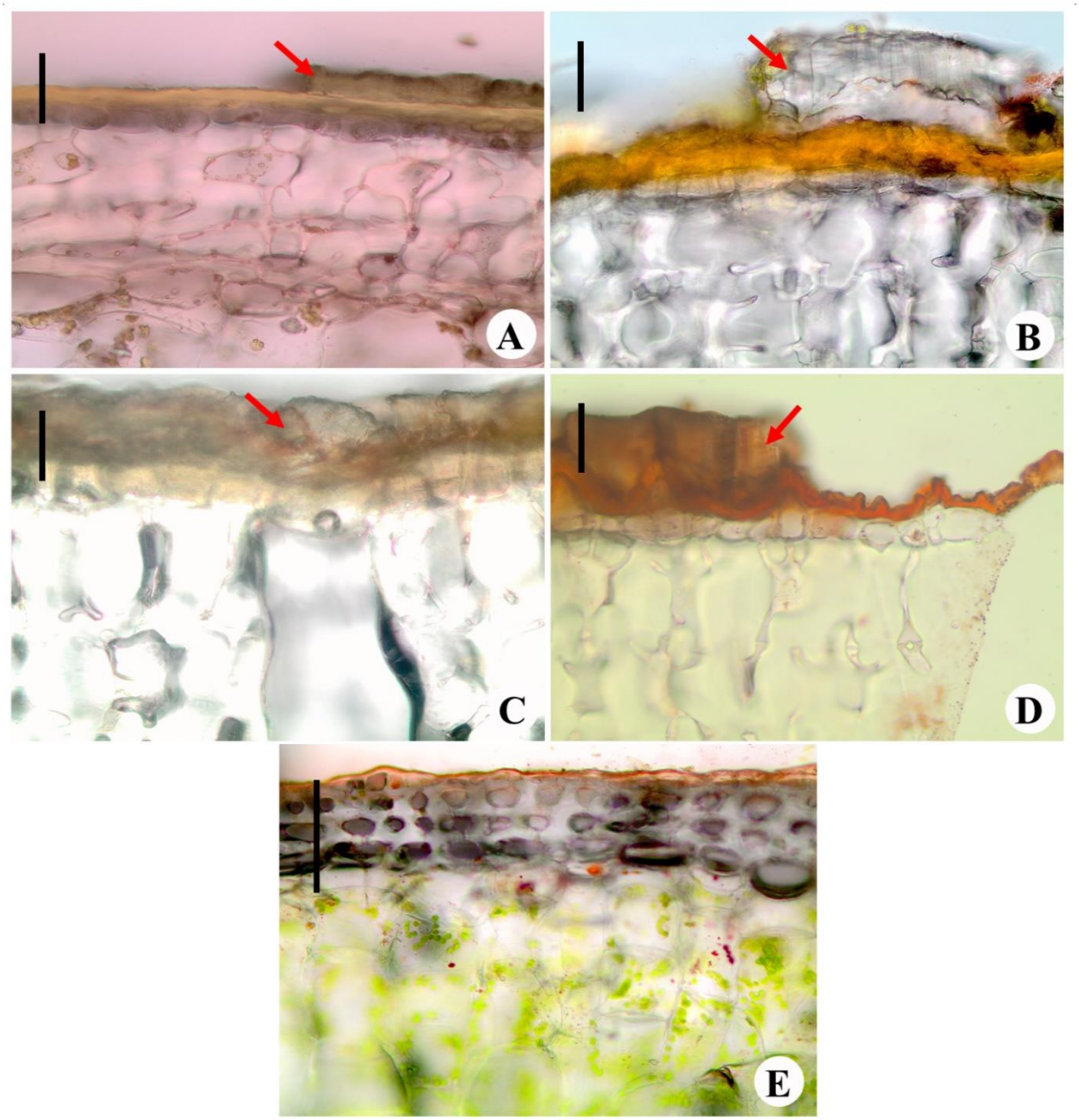

Figure 2. Cross sections of *Cereus* epidermis, stained with Sudan IV, with red arrows highlighting the epicuticular wax: A: *C. albicaulis* with epicuticular wax; B: *C. hildmannianus* with thick layer of epicuticular wax; C: *C. jamacaru* with epicuticular wax; D: *C. pierrebraunianus* with outsandingly thick epicuticular wax; E: *C. spegazzinii* without visible epicuticular wax. Scales: A-B-C-D: 300µm; E:100µm.

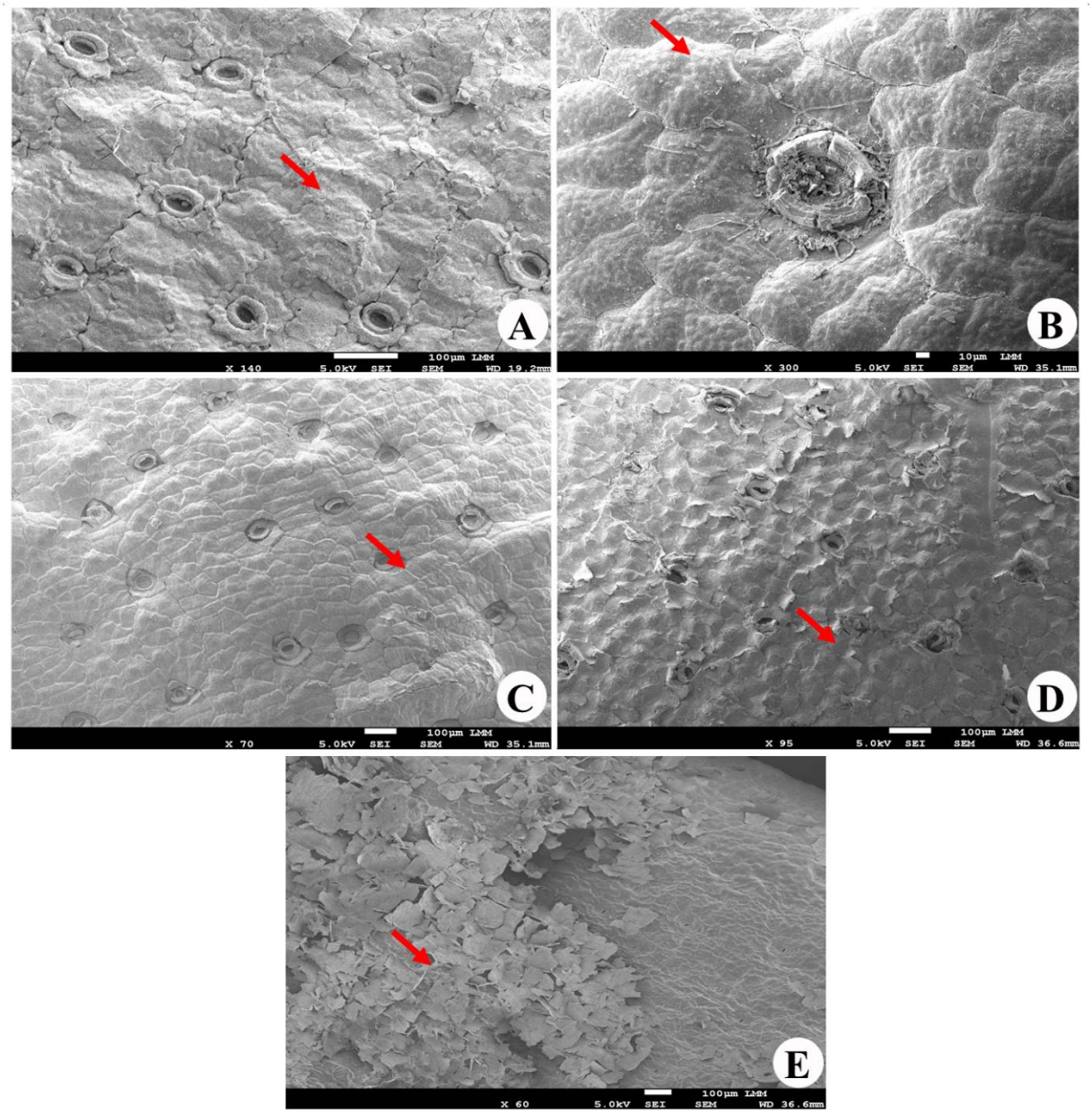

Figure 3. SEM images of *Cereus* epidermis in frontal view. A: *C. albicaulis* (Zappi 5187 – UB) B: *C. hildmannianus* (GO 190 - SORO); C: *C. jamacaru* (GO 477 - SORO); D: *C. pierrebraunianus* (GO 273 - SORO); E: *C. spegazzinii* (Zappi 5135 - UB). Red arrows point at epicuticular wax.

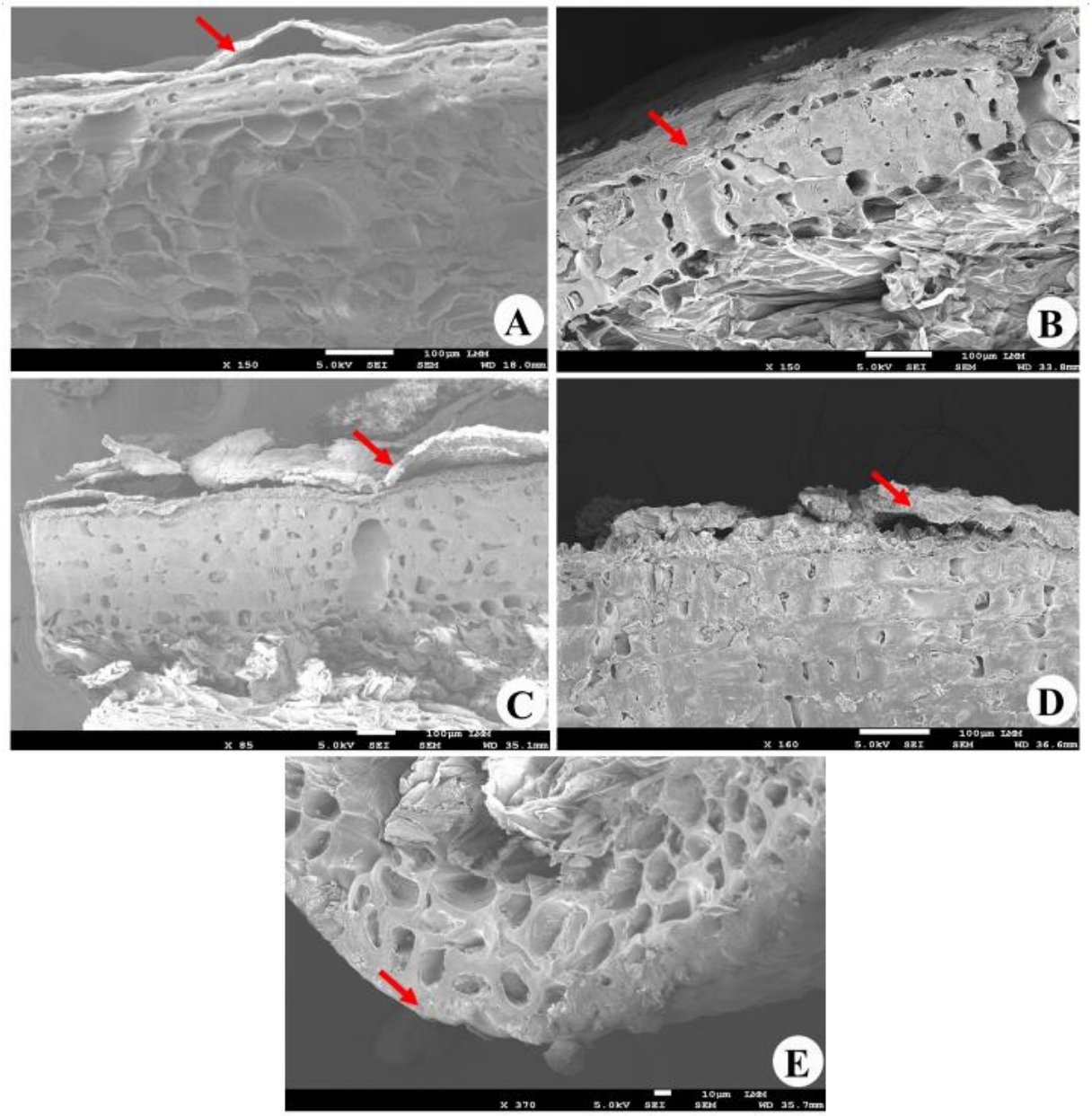

Figure 4. SEM of transverse sections of *Cereus* epidermis: A: *C. albicaulis* with epicuticular wax (red arrow); B: *C. hildmannianus* with thick layer of epicuticular wax (red arrow); C: *C. jamacaru* with fragile epicuticular wax (red arrow); D: *C. pierrebraunianus* with outsandingly thick epicuticular wax (red arrrow); E: *C. spegazzinii* without visible epicuticular wax.

Cactaceae epicuticular wax is described as a product of the protoplasm, where fatty acids migrate to the surface of epidermal cell walls and accumulate over the cuticle (Terrazas & Mauseth, 2002). Also, the wax beneath the cuticle, i.e. intracuticular wax (Koch & Ensikat, 2008) can be associated to stem colour. Zappi (1994) explains the blue colour of the stems of some species of *Pilosocereus* as being a result of dense epicuticular wax. It is easy to remove

such fragile wax by handling the specimens, and it has been pointed out that waxes composed of layers and crusts may be exceptional in nature (Barthlott et al., 1998; Ensikat et al., 2006). The groups of scales or platelets observed in *C. spegazzinii* are probably the reason why the epidermis appears marbled to cactus growers.

Conclusion

We concluded that the epidermis variegation is correlated to the discontinuity of the epicuticular wax layer over the epidermis in *C. spegazzinii* stem, adding to the understanding of this unique character state in the cactus family.

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