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How and why does the areole meristem move in *Echinocereus* (Cactaceae)?

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● **Background and Aims** In Cactaceae, the areole is the organ that forms the leaves, spines and buds. Apparently, the genus *Echinocereus* develops enclosed buds that break through the epidermis of the stem adjacent to the areole; this trait most likely represents a synapomorphy of *Echinocereus.* The development of the areole is investigated here in order to understand the anatomical modifications that lead to internal bud development and to supplement anatomical knowledge of plants that do not behave according to classical shoot theory.
● **Methods** The external morphology of the areole was documented and the anatomy was studied using tissue clearing, scanning electron microscopy and light microscopy for 50 species that represent the recognized clades and sections of the traditional classification of the genus, including *Morangaya pensilis* (*Echinocereus pensilis*).
● **Key Results** In *Echinocereus*, the areole is sealed by the periderm, and the areole meristem is moved and enclosed by the differential growth of the epidermis and surrounding cortex. The enclosed areole meristem is differentiated in a vegetative or floral bud, which develops internally and breaks through the epidermis of the stem. In *Morangaya pensilis*, the areole is not sealed by the periderm and the areole meristem is not enclosed.
● **Conclusions** The enclosed areole meristem and internal bud development are understood to be an adaptation to protect the meristem and the bud from low temperatures. The anatomical evidence supports the hypothesis that the enclosed bud represents one synapomorphy for *Echinocereus* and also supports the exclusion of *Morangaya* from *Echinocereus*.

Key words: Areole meristem, Cactaceae, enclosed meristem, *Echinocereus*, enclosed bud, erumpent bud, sealed areole, periderm.

INTRODUCTION

The shoot apical meristem in angiosperms shows active cell division and differentiation, resulting in stem elongation and the formation of leaves and buds. The buds are immature shoots located in the axils of the leaves, which may grow to form vegetative or reproductive branches (Simpson, 2006). In Cactaceae, the photosynthetic body or stem is represented by a long shoot (Mauseth, 2006) that bears a large quantity of areoles. The areole is the most distinctive morphological trait and is recognized as a Cactaceae synapomorphy. Traditionally, the areole has been recognized as a structure homologous to the axillary bud (Buxbaum, 1951; Bravo-Hollis, 1978; Gibson and Nobel, 1986). However, the areole is recognized as a short shoot covered (Mauseth, 2006) with trichomes and dynamically produces stems (long shoots), leaves, spines and/or flowers. The study of areole morphology and ontogeny has revealed the dynamics of the areole meristem in Cactaceae (Troll, 1937). Of the 1438 species accepted in the most recent taxonomic synthesis of the Cactaceae family (Hunt *et al.*, 2006), ~85 % have monomorphic areoles; trichomes, stems or branches, leaves, spines and/or flowers are produced on the areole surface. The remaining 15 % of the species, included in the subfamily Cactoideae, show certain modifications in the morphology and anatomy of their areoles, which are expressed in the placement of their organs. For example, *Coryphantha*, *Escobaria* and *Neolloydia* have pseudo-dimorphic areoles (Boke, 1952, 1961)

that are elongated over the tubercle and produce spines on the proximal region and flowers or stems on the distal end. By contrast, *Mammillaria* has dimorphic areoles (Boke, 1953, 1955, 1958) in which a spiny areole appears on the apex of the tubercle and a flower-bearing areole appears in the axil. *Echinocereus* presents monomorphic areoles, and according to Britton and Rose (1922) 'the flower-buds as well as the young shoots are deep-seated in their origin and do not appear just at the areoles as in most cacti and hence must break through the epidermis when they develop'. Taylor (1985) called this trait an erumpent bud; however, he argued that it is not present in all members of *Echinocereus*. Nevertheless, the erumpent bud has been recognized as an identifying trait (Moran, 1977) and is one of the synapomorphies (Sánchez *et al.*, 2014) of the genus. Despite the unusual origin of the vegetative and floral buds in *Echinocereus*, the morphology and development of the buds have not received much attention in anatomical research. Therefore, this work aims to describe the morphology and anatomy of the erumpent buds in *Echinocereus* and postulates an adaptive scenario for this trait.

MATERIALS AND METHODS

Sampling

We collected 50 taxa that represent the recovered clades within *Echinocereus* (Sánchez *et al.*, 2014), as well as the sections

recognized by Hunt *et al.* (2006). In particular, we included *Morangaya pensilis*, *E. brandegeei*, *E. maritimus* and *E. ferre- irianus*; according to Taylor (1985), these species represent members of *Echinocereus* that diverge from the pattern de- scribed by Britton and Rose (1922). The majority of the sam- ples were collected in Mexico between 2007 and 2013; most of these are maintained by cultivation in the Botanical Garden of Instituto de Biología at UNAM, and the additional specimens were deposited in the MEXU herbarium (Appendix 1). For the 50 taxa, a database including some climate information (CENAPRED, 2013) and geographical references has been gen- erated as part of the systematic study of the genus (available by request from the first author).

Morphological and anatomical observations

Based on field and greenhouse observations, we documented the origin of the floral and vegetative buds for the majority of the species. Several areoles were sampled in the sub-apical re- gion of the stem 2 months before the floral buds began to sprout (January and February). The areoles were fixed in 52 % etha- nol, 10 % formalin and 5 % acetic acid (Johansen, 1940). If necessary, spines were removed from the samples to facilitate sectioning. For light microscopy analysis, the areoles were gradually dehydrated in *tert*-butyl alcohol (TBA) and included in paraffin in a tissue processor (Leica TP1020) with 24-h im- mersions starting at 50 % TBA. The samples were placed in Histowax (Leica) paraffin with a melting point of 56–58 °C. Longitudinal sections of 12 µm were obtained with a rotary mi- crotome (Leica RM2125). The sections were stained with safra- nin and fast green (Johansen, 1940) and mounted in synthetic resin. Using scanning electron microscopy, the areoles were dehydrated in graded ethanol and critical point dried with an Emitech K850 drier. They were mounted on aluminium stubs with double-sided carbon conductive tape and coated with gold using a Hitachi-S-2460N sputter coater. The samples were viewed and photographed using a Hitachi-S-2460N scanning electron microscope at 15 kV. Lastly, another portion of the sample was cleared with a modified Debenham's (1939) treat- ment. For clearing, the areoles were submerged in 70 % lactic acid for 5 d and were washed with a 70 % glycerine solution, which was replaced every 24 h for 3 d and changed every 24 h to solutions of 50 % ethanol, 30 % ethanol and distilled water. We obtained images during this phase of the treatment. Afterwards, the areoles were bleached with a 50 % solution of commercial NaClO for 30 min on a hotplate at 40 °C and washed with three changes of distilled water for 24 h. Lastly, the areoles were stained with a solution of 0.03 % basic fuchsin in ethanol and washed in 70 % ethanol until the desired contrast was achieved.

RESULTS

Morphological observations of the areoles and buds in Echinocereus *and* Morangaya

Echinocereus areoles were monomorphic and circular to oval in shape (Fig. 1A). The immature areoles, which were very close to the apex, showed a high density of long trichomes with short and soft spines, whereas the mature areoles in the sub-apical region presented short trichomes and sclerified spines (Fig. 1A–C). All of the *Echinocereus* species that were reviewed had erumpent buds, including the *Echinocereus* species from the Baja California Peninsula, which have been reported as taxa with non-erumpent buds (*E. brandegeei*, *E. fer- reirianus* and *E. maritimus*) (Figs 1–3). In *E. maritimus*, a small bud arose on the edge of the areole, and it was not clearly rec- ognized as an erumpent bud when we employed only external observations (Fig. 1J). The presence of erumpent buds may be corroborated directly or indirectly in the species of the genus throughout the three stages of development. During the first stage, when approaching the flowering period, a protuberance developed above the areole, corresponding to the 'hidden' floral bud (Fig. 1B, C; arrows); the areoles associated with these pro- tuberances did not show any modification on the surface or in their spine organization (Fig. 1B–D). During the second stage, the floral bud broke through the stem epidermis (Fig. 1E–G; ar- rows). During the third stage, upon removal of the branches, flowers or stem fruit, a large scar (4–5 mm) could be observed outside the areole, without any modification to the areole; the scar had a diameter of 1.5 mm (Fig. 1H). In addition, the devel- opment of new branches occurred from the vegetative bud; this action also broke the epidermis adjacent to the areole, which upon emergence had its own areoles (Fig. 1I). By con- trast, *Morangaya pensilis* (*E. pensilis*) showed circular mono- morphic areoles, and both the immature and mature areoles were densely covered by trichomes. This species presented non-erumpent buds, and the floral bud developed within the boundary of the areole on the surface; the floral bud did not break through the epidermis (Fig. 1K, L). The scanning electron photomicrograph of the *M. pensilis* areole showed the tepals (Fig. 1L; red arrows) of the floral bud embedded in the tri- chomes of the areole.

Anatomical development of the areoles and buds in Echinocereus

In the longitudinal sections of immature areoles, we observed a superficial areole meristem surrounded by trichomes on the distal end of the areole (Fig. 2B). As the areole matured, the spines lignified and the base of the areole was sealed by a peri- derm with six to ten strata (Fig. 2E, arrow). In general, the are- oles in the sub-apical region of the stem were already sealed by the periderm. After sealing the areole, significant growth oc- curred in the epidermis and the cortex located above the are- ole's meristem (Fig. 2B, C, arrows). At the same time, the meristem moved and was wrapped in these tissues (Fig. 2B, C); therefore, the areole meristem was enclosed in the outer cortical region and differentiated into floral or vegetative buds, which grew diagonal to the areole axis (Fig. 2C, E). The cleared areole corroborated the enclosing of the areole meristem (Fig. 2A, ar- row) as well as the later development of the bud into the outer cortical region and measuresd ~2 mm, which could not be ob- served externally (Fig. 2A, D, G; arrows). The vascular trace branches formed two groups of vascular bundles, one directed towards the areole and another towards the bud. As the bud in- creased in size, its vascular tissue became more evident (Fig. 2E, G). In slender stems, such as those of *E. poselgeri* (<2 cm), the vascular traces were directly connected to the stem's vascular cylinder (not shown).
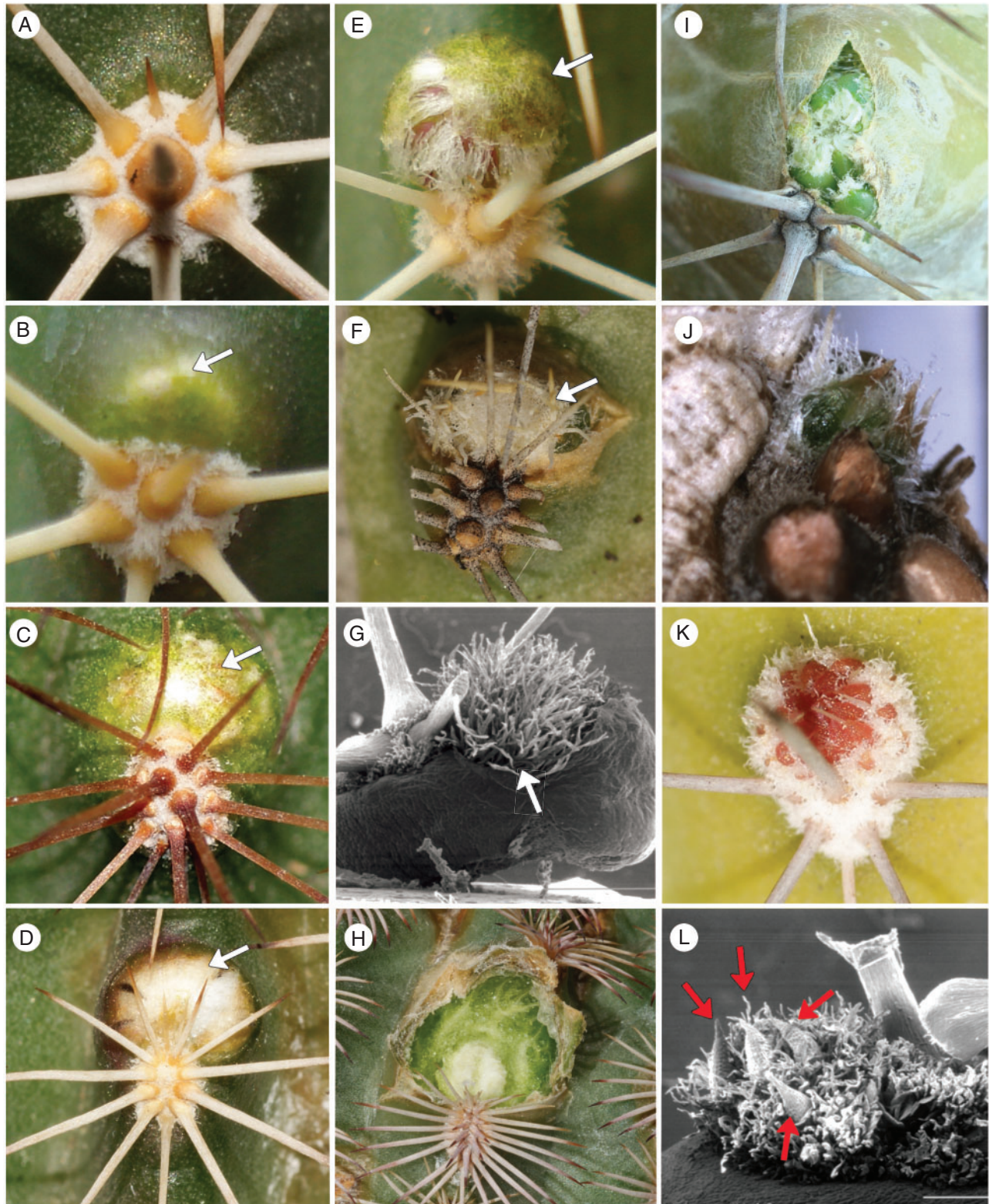
FIG. 1. Areole and bud morphology. (A) Monomorphic areole in *Echinocereus* (*E. scheeri Sánchez 36*). (B) Areole with protuberance in *E. pentalophus* (*Arias 1740*). (C) Areole with larger protuberance in *E. spinigemmatus* (*Arias 1874*). (D) Areole with the floral bud showing (*E. fendleri, Arias 2031*). (E) Areole with erumpent floral bud (*E. pentalophus, Arias 1740*). (F) Areole with erumpent vegetative bud in a species with putative non-erumpent buds (*E. ferreirianus, Sánchez 95*). (G) Scanning electron micrograph of erumpent bud (*E. pentalophus, Arias 1740*). (H) Scar formed after removing a flower (*E. pectinatus, Terrazas 906*). (I) Vegetative bud breaking through stem epidermis (*E. enneacanthus, Arias 1427*). (J) Small floral bud in a species with putative non-erumpent buds (*E. maritimus, Sánchez 99*). (K) Non-erumpent floral bud in *Morangaya pensilis* (*Arias 1295*). (L) Scanning electron micrograph of non-erumpent bud in *M. pensilis* (*Arias 1295*). Scale bar (L) = 1 mm.

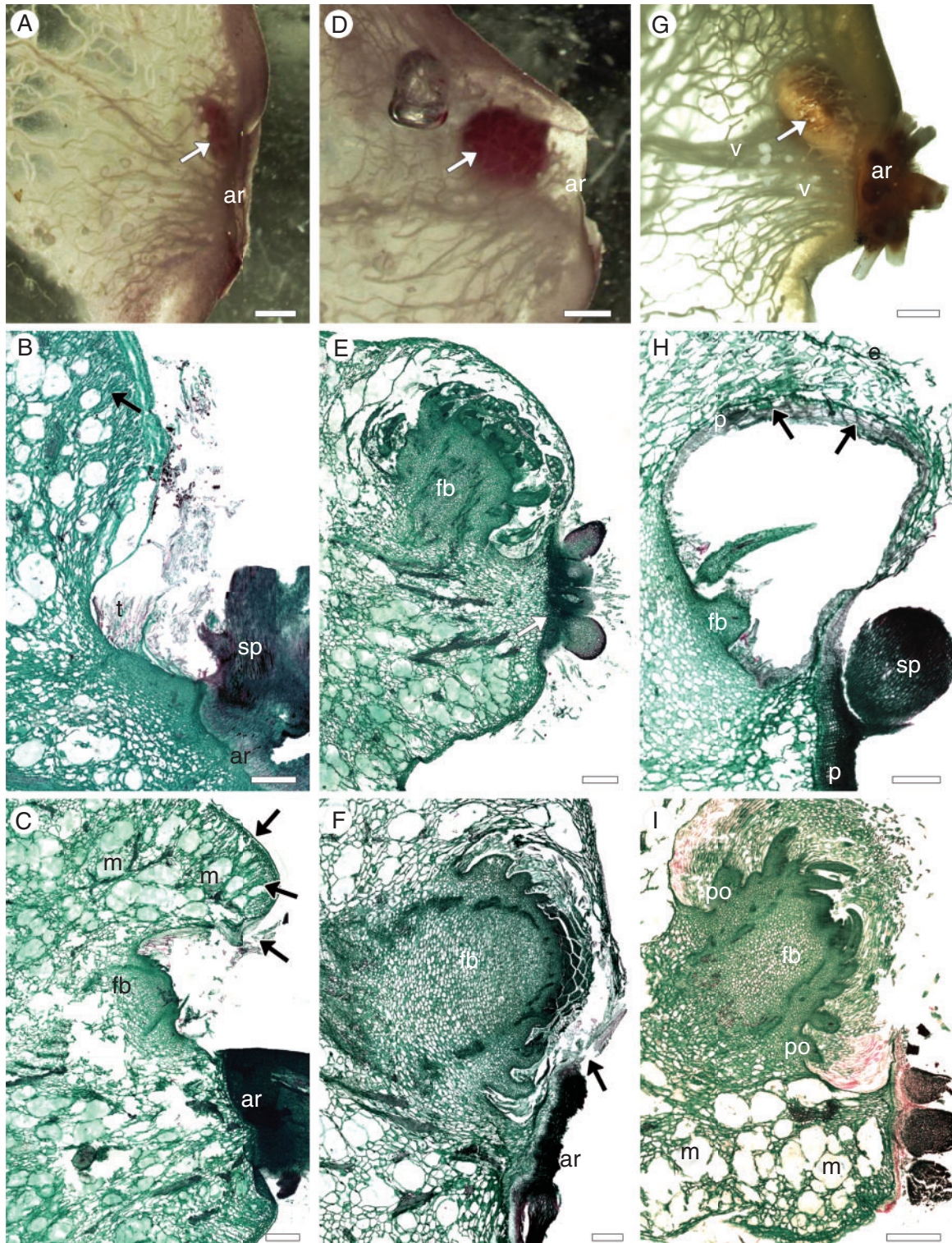Fɪɢ. 2. Areole and bud anatomical development. (A) Mature areole clearing (*Echinocereus fendleri, Arias 2031*). (B) Immature areole, longitudinal section (*E. ferreirianus* subsp. *lindsayi, Sánchez 89*). (C) Mature areole, longitudinal section (*E. fendleri, Arias 2031*). (D) Mature areole clearing with an enclosed bud (*E. fendleri, Arias 2031*). (E) Areole with an enclosed bud, longitudinal section (*E. fendleri, Arias 2031*). (F) Enclosed bud, longitudinal section (*E. scheeri, Sánchez 36*). (G) Areole clearing with an enclosed floral bud (*E. fendleri, Arias 2031*). (H) Areole, tangential section (*E. pentalophus, Arias 1740*). (I) Areole with an erumpent bud, longitudinal section (*E. pamanesiorum, Arias 1879*). Scale bar (A, D, G) = 1 mm; (B, C, E, F, I) = 500 μm; (H) = 250 μm. Abbreviations*:* am, areole meristem; ar, areole; e, epidermis; fb, floral bud; m, mucilage; p, periderm; po, podarium; sp, spine; t, trichomes; v, vascular trace.

The areole's longitudinal sections confirmed that the protuberance above the areole corresponded to the floral bud that was developing internally. During this stage, when the protuberance was visible on the surface, the floral bud had initiated perianth differentiation and was immersed in the outer cortical tissue of the stem, surrounded by abundant mucilage cells (Fig. 2E, F, I). As the floral bud increased in size, it caused thinning of the rib cortex and epidermis. The epidermis became translucent (Fig. 1C, D; arrows) and the floral bud became evident underneath the epidermis (Figs 1C, D and 2E, F). Afterwards, the epidermis covering the floral bud became opaque (Fig. 1D) because the cells of the cortical tissue that were in contact with the floral bud differentiated into a wound periderm (Fig. 2H, arrows). The floral bud broke through the epidermis and a large quantity of trichomes could be observed through the opening (Figs 1G and 2F, I). For those species that are endemic to Baja California, the areole was sealed by the periderm; the internally developing buds broke the epidermis or stem periderm to reach the surface, as exemplified by *E. brandegeei* (Fig. 3A, B). Another case is *E. maritimus*, a species with small buds (1 mm) in the distal edge of the areole (Fig. 3C); in this species, the small floral bud showed several periderm strata at the base coming from the stem (Fig. 3D). This observation was a result of the growth of the enclosed bud breaking through the stem periderm (Fig. 3D).

Anatomical development of the areoles and buds in Morangaya pensilis

The longitudinal areole sections indicated that the areole spines became lignified along with stem growth. The areole base remained covered by trichomes and normal epidermal cells; thus, the periderm did not develop and the tissue
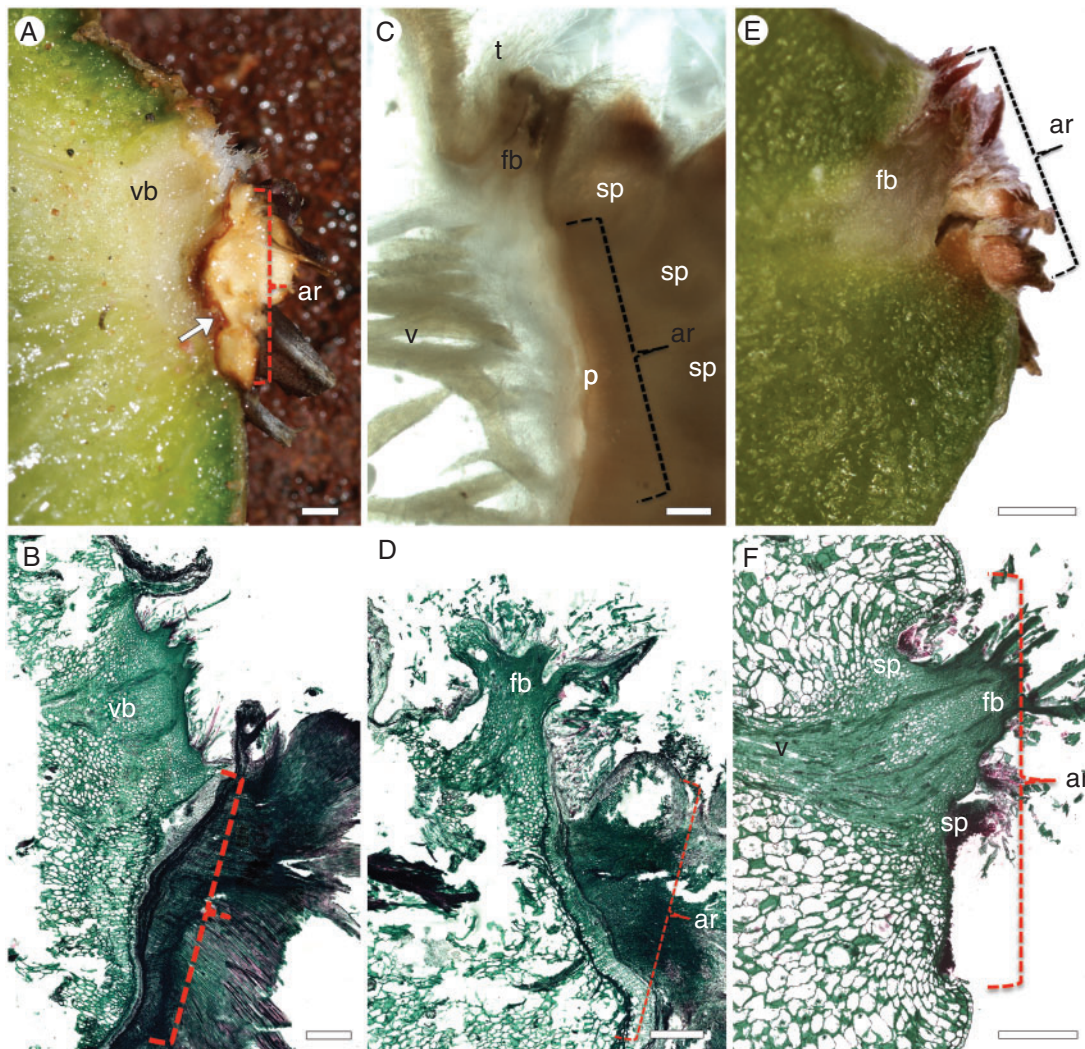

FIG. 3. Areole and bud anatomical development (continuation). (A) Fresh longitudinal section of an areole of *Echinocereus brandegeei* (*Sánchez 90*). (B) Areole of *E. brandegeei* with an erumpent vegetative bud, longitudinal section (*Sánchez 90*). (C) Areole clearing of *E. maritimus*, longitudinal section (*Sánchez 99*). (D) Areole of *E. maritimus* with an erumpent floral bud, longitudinal section (*Sánchez 99*). (E) Fresh longitudinal section of an areole of *Morangaya pensilis* (*Arias 1295*). (F) Areole of *M. pensilis* with non-erumpent floral bud, longitudinal section (*Arias 1295*). Scale bar (A, E) = 1 mm; (B, C, D) = 500 μm. Abbreviations: ar, areole; fb, floral bud; p, periderm; sp, spine; t, trichomes; v, vascular trace; vb, vegetative bud.

remained alive. The areole's meristem was found in the distal region of the areole but was not enclosed and remained on the surface; thus, there was no internal bud development (Fig. 3E, F). Floral bud development was superficial and never broke through the stem epidermis adjacent to the areole. A single vascular trace irrigated the areole, including the floral bud, although greater development of vascular tissue was observed in areoles with a floral bud (Fig. 3F).

DISCUSSION

Areole meristem movement and bud development

The early development of the areole in *E. reichenbachii* (Boke, 1951) supports our observations for the 50 *Echinocereus* species in this study. The areole meristem is located on the distal end, and the various periderm strata at the areole base developed after growth of the spine primordia. However, Boke (1951) did not describe the cause of areole meristem movement in areole development. The growth of the epidermis and cortex located above and adjacent to the sealed areole may not be compensated because the periderm seals and disrupts its growth. This differential growth causes the tissue to curl and the areole meristem located at the distal end to begin to move. These tissues curl and form a loop; they fuse and the areole meristem thus becomes enclosed. This process has been discussed by other authors; e.g. Dickinson (1978) proposed that zonal growth and postgenital fusion are ontogenetic mechanisms that provoke the displacement of axillary buds and modify the classic shoot organization, as observed in several epiphyllous shoots (e.g. *Turnera*). Similarly, Kerstetter and Hake (1997) noted that in certain cases differential growth alters the position of the meristem in relation to other identifiable parts of the plant. The movement of the areole's meristem as a result of differential growth has been observed in other Cactaceae, such as *Coryphantha* and *Mammillaria* (Boke, 1952, 1953, 1955, 1958, 1961), although even in these genera the enclosed meristem is non-existent. The enclosed areole meristem in *Echinocereus* is demonstrated for the first time in the Cactaceae family in this study. Although Ross (1982) proposed, without showing evidence, that the areole meristem moves during the ontogeny of the rib and that an endogenous origin does not exist, the early development of the bud is clearly internal due to the moving and enclosing of the meristem. The enclosed areole meristem remains latent until it is defined as a floral bud or a vegetative bud depending on the interaction of the homeotic genes that are implicated in the identity of the meristem (Molinero-Rosales *et al.*, 1999). This phenomenon is also observed in *Coryphantha* and *Mammillaria*, in which the areole meristem and the flower-bearing meristem may develop stems and flowers, respectively. Therefore, both vegetative and floral buds in *Echinocereus* show internal development, as suggested by Britton and Rose (1922). The ontogeny of the areole's meristem in *Echinocereus* may be summarized in four stages after the formation of the spine primordia: (1) location of the meristem on the distal end of the areole; (2) development of the periderm at the base of the areole; (3) moving and enclosure of the meristem due to differential growth; and (4) definition of the meristem as a floral bud or a vegetative bud with internal development.

Certain species of *Echinocereus* show modifications in the areole and bud development patterns described above. For example, in the *Echinocereus* group from Baja California, particularly in *E. maritimus*, the areole is also sealed by the periderm and the meristem also moves, but the internal development of the bud never achieves a diameter greater than 1 mm. Therefore, the breaking through of the stem's epidermis or periderm is not as conspicuous as in the other species of the genus, giving the impression that the *Echinocereus* group from Baja California do not have erumpent buds, as proposed by Taylor (1985). However, our evidence is convincing and shows that all *Echinocereus* species develop erumpent buds.

In the case of *E. knippelianus* subsp. *reyesii*, the flowers emerge in the areoles closer to the apex (Blum *et al.*, 1998) and do not emerge by breaking through the epidermis. For this species, the determination of the areole's meristem as a floral bud occurs when the meristem is still superficial. While the development of new branches always occurs in areoles that are close to the base of the stem where the meristem is enclosed and the development of the vegetative bud is internal, the long shoot branches emerge by breaking the epidermis adjacent to the areole. Therefore, in *E. knippelianus* subsp. *reyesii* the development of the flowers only on the apex may be a result of the globose-depressed growth form and basitonic branching. Therefore, flowers must be formed quickly in the apex of the stem to ensure that the delicate floral buds in the areoles do not emerge close to the ground; this type of development prevents damage due to friction with the substrate.

Adaptive significance of the movement of the areole meristem and enclosed bud

Echinocereus is primarily distributed in the mountainous areas of northwestern Mexico and the southwestern USA (Taylor, 1985; Blum *et al.*, 1998), where snowfall and frosts are common during the winter (CENAPRED, 2013). In this context, the enclosed floral buds in *Echinocereus* begin to differentiate and grow during the winter months; therefore, the internal bud development and the sealing of the areole by the periderm represent traits that protect the bud against possible damage from low temperatures. In addition, the enclosed bud is surrounded by a large quantity of mucilage, which allows a gradual flow of water from the cell towards the extracellular ice crystals under freezing temperatures, and is thus less harmful to cells (Goldstein and Nobel, 1991).

Moreover, Gibson and Nobel (1986) suggested that in many members of Cactaceae, once the areole forms a flower and the fruit has matured, the periderm develops to seal the stem areole, thus preventing water loss and infection. In *Echinocereus*, this process occurs prematurely before the areole develops a flower or a stem and provides the same advantages. Even when the new shoot emerges and breaks the epidermis, the exposed tissues develop a wound periderm to seal and protect the stem (Nawrath, 2002). As a consequence, the *Echinocereus* areoles do not form new spines and only have a single flowering or branching event upon sealing the areole, as has been reported in the cephalia of certain cacti (e.g. Mauseth, 1989, 1999; Vázquez-Sánchez *et al.*, 2005, 2007). In contrast, other related genera may generate new spines (e.g. *Cephalocereus*;

Bárcenas-Argüello *et al.*, 2014) or produce various flowering events in a single areole (e.g. *Lophocereus*, *Myrtillocactus* and *Stenocereus*; Mauseth, 2006).

Systematic implications of enclosed buds

Taylor (1985) suggests that the erumpent bud is a specialized trait that is not found in all *Echinocereus* species, which is why *Morangaya pensilis* was included in the genus. However, this study revealed that the buds of *Echinocereus* are enclosed and consequently erumpent and that the areole is sealed by the periderm; however, in *M. pensilis* the buds are external and non-erumpent, and the areole is not sealed by the periderm. The anatomical mechanism of floral and vegetative bud development in *Morangaya* is typical of what has been observed in the majority of the Cactaceae. These results support the proposal to exclude *M. pensilis* from *Echinocereus* (Moran, 1977; Sánchez *et al.*, 2014). Taylor (1985) also suggests that, in addition to *Morangaya*, various *Echinocereus* taxa (e.g. *Erecti* section) show non-erumpent buds. Our results refute this proposal because *E. brandegeei*, *E. maritimus* and *E. ferreirianus* show enclosed buds that are erumpent. Therefore, our results support the proposal of Sánchez *et al.* (2014), who recognize the erumpent bud trait as a synapomorphy of the genus.

In a broader context, the most recent phylogenies (Bárcenas *et al.*, 2011; Sánchez *et al.*, 2014) suggest that *Echinocereus* and the *Stenocereus* group (*Escontria*, *Morangaya*, *Myrtillocactus*, *Polaskia* and *Stenocereus*; Sánchez *et al.*, 2014) are sister groups. The innovation of the enclosed meristems and the consequent development of enclosed buds in *Echinocereus* had a determining role in the evolutionary process of this lineage, which originated $4{\cdot}6 \pm 1{\cdot}7$ m.y.a. (Arakaki *et al.*, 2011) and has diversified extensively in specific regions of central northern Mexico and the southern USA (64 species; Hunt *et al.*, 2006). However, the *Stenocereinae* lineage did not diversify. Thus, we conclude that in *Echinocereus* the sealing of the areole by the periderm, the moving of the areole meristem and the development of enclosed buds is a well-coordinated anatomical process that represents a synapomorphic trait of the genus. These modifications constitute an adaptation to protect the areole meristem or bud from the low temperatures that prevail in its habitat and promote the diversification of this lineage. Although the anatomical process is described, it will be necessary to investigate the molecular mechanism of the areole tissues that causes the movement of the meristem and promotes the diagonal growth of the bud to break the stem epidermis and emerge.

ACKNOWLEDGEMENTS

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APPENDIX

List of species sampled in this study, presented in alphabetical order, and following this format: Species, Voucher (HERBARIUM ACRONYM), Provenance and Observations: 1: External morphology of the areole examined; 2: Anatomical slides of the areole reviewed; *Examined or reviewed but not shown in figures.

Echinocereus acifer (Otto ex Salm-Dyck) Jacobi, *Sánchez 21* (MEXU), MEX, Zacatecas: 1*, 2*. *Echinocereus adustus* Engelm., *Sánchez 23* (MEXU), MEX, Chihuahua: 1*. *Echinocereus arizonicus* Rose ex Orcutt, *Sánchez 44* (MEXU), MEX, Chihuahua: 1*. *Echinocereus berlandieri* (Engelm.) Haage, *Arias 1454* (MEXU), MEX, Nuevo León: 1*. *Echinocereus brandegeei* (J. M. Coult.) K. Schum., *Sánchez 90* (MEXU), MEX, Baja California Sur.: 1, 2. *Echinocereus chisosensis* W. T. Marshall, *Sánchez 87* (MEXU), MEX, Durango: 1*. *Echinocereus cinerascens* (D. C.) Lem., *Arias 1732* (MEXU), MEX, Hidalgo: 1*. *Echinocereus coccineus* Engelm., *Sánchez 64* (MEXU), MEX, Chihuahua: 1*. *Echinocereus dasyacanthus* Engelm., *Sánchez 63* (MEXU), MEX, Chihuahua: 1*. *Echinocereus engelmannii* (Parry ex Engelm.) Lem., *Sánchez 104* (MEXU), MEX, Baja California: 1*. *Echinocereus enneacanthus* Engelm., *Arias 1427* (MEXU), MEX, Durango: 1, 2*. *Echinocereus fendleri* (Engelm.) Rümpler, *Arias 2031* (MEXU), MEX, Sonora: 1, 2. *Echinocereus ferreirianus* H. E. Gates, *Sánchez 95* (MEXU), MEX, Baja California: 1, 2. *Echinocereus ferreirianus* subsp. *lindsayi* (J. Meyrán) N. P. Taylor, *Sánchez 89* (MEXU), MEX, Baja California: 1*, 2. *Echinocereus knippelianus* Liebner, *Arias 2127* (MEXU), MEX, Nuevo León: 1*. *Echinocereus knippelianus* subsp. *reyesii* Lau, *Arias 1939* (MEXU), MEX, Nuevo León: 1*. *Echinocereus koehresianus* (G. Frank) W. Rischer, *Sánchez 14* (MEXU), MEX, Sinaloa: 1*. *Echinocereus laui* G. Frank, *Sánchez 33* (MEXU), MEX, Sonora: 1*. *Echinocereus leucanthus* N. P. Taylor, *Arias 1845* (MEXU), MEX, Sonora: 1*. *Echinocereus longisetus* (Engelm.) Lem., *Guzmán 1501* (MEXU), MEX, Coahuila: 1*. *Echinocereus mapimiensis* Anderson, *Sánchez 84* (MEXU), MEX, Durango: 1*. *Echinocereus maritimus* (M. E. Jones) K. Schum., *Sánchez 99* (MEXU), MEX, Baja California: 1, 2. *Echinocereus metornii* G. Frank, *Sánchez 83* (MEXU), MEX, Coahuila: 1*. *Echinocereus nicholii* (L. D. Benson) B. D. Parfitt, *Arias 2029* (MEXU), MEX, Sonora: 1*. *Echinocereus nivosus* Glass & R. A. Foster, *Sánchez 88* (MEXU), MEX, Coahuila: 1*. *Echinocereus palmeri* Britton & Rose, *Sánchez 66* (MEXU), MEX, Chihuahua: 1*, 2. *Echinocereus pamanesiorum* A. B. Lau, *Arias 1879* (MEXU), MEX, Zacatecas: 1*, 2. *Echinocereus parkeri* N. P. Taylor, *Arias 2122* (MEXU), MEX, San Luis Potosí: 1*, 2*. *Echinocereus pectinatus* (Scheidw.) Engelm., *Terrazas 906* (MEXU), MEX, San Luis Potosí: 1. *Echinocereus pentalophus* (DC) Lem., *Arias 1740* (MEXU), MEX, San Luis Potosí: 1, 2. *Echinocereus polyacanthus* Engelm., *Sánchez 24* (MEXU), MEX, Chihuahua: 1*, 2*. *Echinocereus poselgeri* Lem., *Arias 1452* (MEXU), MEX, Nuevo León: 1*, 2*. *Echinocereus primolanatus* Fritz Shwarz ex N. P. Taylor, *Arias 1959* (MEXU), MEX, Coahuila: 1*. *Echinocereus pulchellus* (Mart.) C. F. Först ex F. Seitz, *Sánchez 08* (MEXU), MEX, Zacatecas: 1*. *Echinocereus rayonesensis* N. P. Taylor, *Arias 1947* (MEXU), MEX, Nuevo León: 1*. *Echinocereus reichenbachii* (Terscheck ex Walp.) Haage, *Arias 1956* (MEXU), MEX, Coahuila: 1*. *Echinocereus rigidissimus* (Engelm.) Haage, *Arias 2019* (MEXU), MEX, Sonora: 1*, *Echinocereus scheeri* (Salm-Dyck) Scheer, *Sánchez 36* (MEXU), MEX, Chihuahua: 1, 2. *Echinocereus schereri* G. Frank, *Sánchez 72* (MEXU), MEX, Durango: 1*. *Echinocereus schmollii* (Weing.) N. P. Taylor, *Arias 91* (MEXU), MEX, Querétaro: 1*. *Echinocereus sciurus* (K. Brandegee) Dams, *Arias 1836* (MEXU), MEX, Sinaloa: 1*. *Echinocereus scopulorum* Britton & Rose, *Arias 2017* (MEXU), MEX, Sonora: 1*. *Echinocereus spinigemmatus* A. B. Lau, *Arias 1874* (MEXU), MEX, Jalisco: 1*. *Echinocereus stoloniferus* W. T. Marshall, *Sánchez 32* (MEXU), MEX, Chihuahua: 1*. *Echinocereus stramineus* (Engelm.) Engelm. ex F. Seitz, *Sánchez 77* (MEXU), MEX, Coahuila: 1*. *Echinocereus subinermis* (Salm-Dyck) Scheer, *Guzmán 1312* (MEXU), MEX, Sinaloa: 1*. *Echinocereus viereckii* Werderm., *Arias 1996* (MEXU), MEX, Tamaulipas: 1*. *Echinocereus viridiflorus* Engelm., *Sánchez 80* (MEXU), MEX, Coahuila: 1*. *Echinocereus waldeisii* Haugg, *Arias 2002* (MEXU), MEX, San Luis Potosí: 1*. *Morangaya pensilis* (K. Brandegee) G. D. Rowley, *Arias 1295* (MEXU), MEX, Baja California Sur: 1, 2.