

Title: An ultrasound absorbing inflorescence zone enhances echo-acoustic contrast of bat-pollinated cactus flowers

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Running title: ultrasound absorbing inflorescence zones in a bat-pollinated cactus

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Abstract

1 Flowering plants have evolved an extraordinary variety of signaling traits to attract and
2 guide their pollinators. Most flowers rely on visual and chemical signals, but some bat-
3 pollinated plants have evolved reflective surfaces to acoustically guide echolocating bats.
4 All known echo-acoustic flower signals rely on the same basic acoustic principles of
5 increased sonar reflectivity. Here we reveal a novel mechanism through which plants
6 acoustically communicate with bats, a principle that relies on increased absorption of the
7 area surrounding the target flower, thereby enhancing echo-acoustic contrast. In a bat-
8 pollinated columnar cactus (*Espostoa frutescens*) from the Ecuadorian Andes we found a
9 hairy inflorescence zone, a so called lateral cephalium. Flowers of this cactus solely
10 emerge out of this hairy zone. We measured the ultrasound echoes of the hairy zones, the
11 flowers and unspecialized column surfaces with a biomimetic sonar head and recorded
12 echolocation calls of approaching bats. We found that the hairy inflorescence zones act as
13 strong ultrasound absorber, attenuating the sound by -14 dB compared to other parts of
14 the column. The absorption was highest around the echolocation call frequencies of
15 approaching bats. Our results indicate that, instead of making flowers more reflective,
16 plants can also evolve specific structures to attenuate the background echo, thereby
17 enhancing the acoustic contrast with the target. Similar sound absorbing mechanisms
18 may be found in other species that interact with bats across a wide range of ecological
19 contexts.

20 Introduction

21 Flowering plants rely on a wide variety of communication strategies to attract their
22 pollinators. Conspicuous visual flower signals are in particular useful to guide receivers,
23 as they are easy to locate and the use of colours makes flowers stand out against the
24 vegetation background [1, 2]. Nocturnally flowering bat-pollinated plants however are
25 limited in the use of visual signals to attract their pollinators. Several plants have
26 therefore independently evolved echo-reflective structures to acoustically guide these
27 nocturnal pollinators [3-6]. Echo-acoustic signalling plants all use concave shapes with
28 either triple mirror, bell- or dish-like structures. These concave shaped structures share
29 the same basic acoustic principle of focusing returning echoes to an approaching bat,
30 thereby increasing the range over which they can be detected. Some flower signals use
31 additional spectral-temporal signatures increasing conspicuousness [4]. Reflective
32 structures also evolved in bat-plant-interactions even outside a pollination context. The
33 carnivorous pitcher plant *Nepenthes hemsleyana*, for example, has a highly reflective
34 prolonged pitcher backwall to advertise their pitcher-leaves as roosts [6]. Bats roosting
35 inside the pitcher provide additional nitrogen intake through their droppings[7].

36 Here we assess an evolutionary novel adaptation that enhances acoustic communication
37 between plants and pollinating bats. Interestingly, some cacti species exhibit at a certain
38 age inflorescence zones that are particularly hairy, the so-called *cephalium*. There are
39 several different morphologies of cacti described as cephalia, and we refer here to what
40 is described as a lateral cephalium by Mauseth (2006) [8]. Several functions of these
41 *cephalia* zones, have been proposed. The hairy structure may shield buds from UV
42 radiation at high altitudes, or protect against nectar robbers and herbivores [8-10]. Here
43 we test a hypothesis by von Helversen et al. (2003) [5], which states that such hairy zones
44 may have been co-opted to serve in bat-pollinated cacti as sound-absorbing structures
45 that support detection and localization of sound-reflecting flowers by pollinating bats.

46 Using a bat-mimetic sonar-head we carried out ensonification experiments with different
47 parts of the cactus *Espositoa frutescens* [5] from the Andes. Specifically, we ensonified the
48 cactus' column, flowers as well as the hairy *cephalium* zone. Additionally, we recorded the
49 echolocation calls of its main pollinator, the nectar-feeding bat *Anoura geoffroyi*
50 (Phyllostomidae) and assessed whether the cephalium was especially absorbent in the
51 ultrasonic frequency range of the calls.

52 **Material and Methods**

53 We studied *Espositoa frutescens* and its pollinator, *Anoura geoffroyi*. The study was carried
54 out in a dry valley of the Ecuadorian Andes, close to the city Oña in the province of Azuay.
55 As it was not possible to conduct the echo measurements in the field – the cacti are
56 growing in rocky and steep habitat - we cut the columns and conducted the measurements
57 indoors at a nearby farm. All experiments were approved by the local authorities
58 (Ministeria del Ambiente, Cuenca, Ecuador, autorizacion para investigación científica N.
59 035-DPA-MA-2012). A specimen is deposited at Herbario Azuay (Cuenca, Ecuador) with
60 the number HA 7814.

61 To measure the reflectance of the different parts of the cacti we mounted the columns on
62 tripods and used a custom-built biomimetic sonar head to ensonify them. The sonar head
63 consisted of a 1/4" condenser microphone (40BF; preamplifier 26AB; power module
64 12AA; G.R.A.S. Sound & Vibration, Holte, Denmark) and a custom-made EMFi (Electro
65 Mechanical Film) loudspeaker (sound pressure levels at 1 m distance: 92 dB ± 8 dB,
66 frequency range: 30-160 kHz; Department of Sensor Technology, University of Erlangen-
67 Nuremberg, Erlangen, Germany). The speaker and the microphone were embedded in an
68 aluminium body and placed next to each other as they would be located on the head of a
69 bat. We ensonified cacti from a distance of 15 cm with a continuously replayed MLS Signal
70 (Maximum Length Sequence) of 16383 samples length. We recorded the reflected sound
71 and obtained the impulse responses (IR) by deconvolution of the reflected echo and the
72 original MLS [5]. The spectral target strength was obtained by windowing the IRs (1024
73 samples) and calculating the power spectral density (PSD). To obtain spectral target
74 strength (TS), independent of the frequency response of the loudspeaker, we calculated
75 the difference between PSD from the reflector and the PSD of an acrylic glass plate
76 oriented perpendicular to the direction of sound propagation at exactly the same position
77 as the column/flower (For more information on the setup see also [4, 5, 11]).

78 Using our ensonification setup we measured the acoustic properties of six freshly cut
79 columns of *E. frutescens*, focusing on the hairy cephalium zone and the unspecialized parts
80 (backside) of the column. For both measurements we scanned the columns by moving the
81 sonarhead upwards along its vertical axis and made 10 measurements at different heights
82 of the column. We also measured the reflectance of six isolated flowers, which were

83 mounted on a stepping motor. We rotated the flower in 3° steps and measured 20 echoes
84 around the opening (0°) of the flowers from -30° to 30°.

85 To understand how the echo of a flower would be received if it would grow on an
86 unspecialized part of the column we manipulated one column. We first scanned the hairy
87 cephalium with an open flower by moving the sonar head upwards along the vertical axis
88 of the column over an area of 30 cm. The flower was located central on this area and we
89 measured in 1 cm steps. After the measurements we cut out the flower from the
90 cephalium and fixed it on the hairless backside of the column (Fig. 2B). For this
91 experimentally manipulated column we made the same detailed vertical scan (30 cm, 1
92 cm steps).

93 We also recorded echolocation calls of two male Geoffroy's tailless bats (*Anoura geoffroyi*)
94 approaching an *Espostoa* column with an open flower. The microphone (1/4" condenser
95 microphone 40BF; preamplifier 26AB; power module 12AA; G.R.A.S. Sound & Vibration,
96 Holte, Denmark) was placed next to the flower and we recorded with a sampling rate of
97 500 ks/s. We obtained 45 manually triggered recordings, each with a length of 2 s, during
98 the approaches of the bats. To ensure a good signal-to-noise-ratio for the call analysis we
99 selected 21 approach sequences where at least two calls had an amplitude of more than
100 6% full scale. We analysed the calls using the program Avisoft-SASLab Pro (Avisoft
101 Bioacoustics, Glienicke, Germany).

102 We tested for significant effects of plant structure on echo-acoustic target strength using
103 the lmer package in R (version 3.5.3). We constructed linear mixed models and checked
104 model assumptions by visual inspection of the residuals. Target strength was averaged
105 over the 10 measurements per plant individual and structure and modelled as dependent
106 variable. Plant structure (column, flower or cephalium) was added as fixed factor and
107 plant individual as a random intercept term. For the different frequency ranges we
108 modelled the interaction between plant part and frequency band. We tested for
109 significance of main effect of plant structure on target strength and for significance of the
110 interaction between structure and frequency band by comparing models with and
111 without terms using likelihood ratio tests.

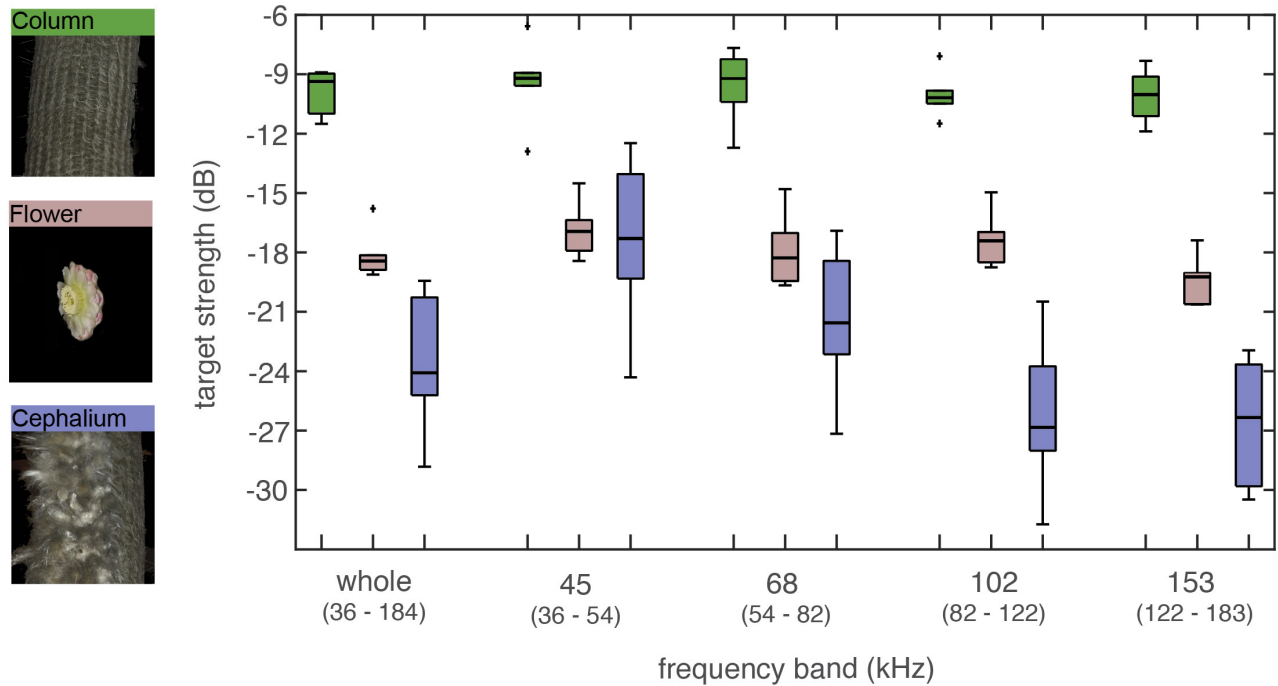


Figure 1. Spectral target strength (TS) of different morphological structures of *Espostoa frutescens* for different frequency bands. The spectral target strength was obtained from ensonification measurements at a distance of 15cm. We measured unspecialized parts of the cactus column (green boxplots; n = 6 columns, 10 measurements per column), isolated flowers (rose boxplots; n = 6 columns, 20 measurements per flower from different angles) and the hairy cephalium zone (purple boxplots; n = 6 columns, 10 measurements per column).

112 Results

113 We found a significant effect of plant structure on overall target strength (LMM, n = 18
114 plant structures, n = 180 measurements, d.f. = 2, X² = 39.31, P < 0.001). Furthermore,
115 target strength depended on the interaction between frequency range and plant structure
116 (LMM, d.f. = 8, X² = 37.51, P < 0.001). Overall, the plain column surface of *E. frutescens*
117 reflected the strongest echoes. We measured a high target strength (average TS -9.8 dB)
118 for these unspecialized surfaces of the cactus across a wide range of frequencies (Fig. 1).
119 The overall average TS of the flower was much lower compared to the column (-18.1 dB)
120 but also remained similar across all measured frequency bands (Fig 1). The hairy
121 cephalium zone on the other hand showed differences in TS for the different frequency
122 bands (Fig 1). For the lower frequency band (45 kHz) the TS was about the same level as
123 the flower (-17.5 dB) but for higher frequency bands it was much lower, down to -26.3 dB
124 for the 102 kHz frequency band. Overall, the cephalium zone had an average target

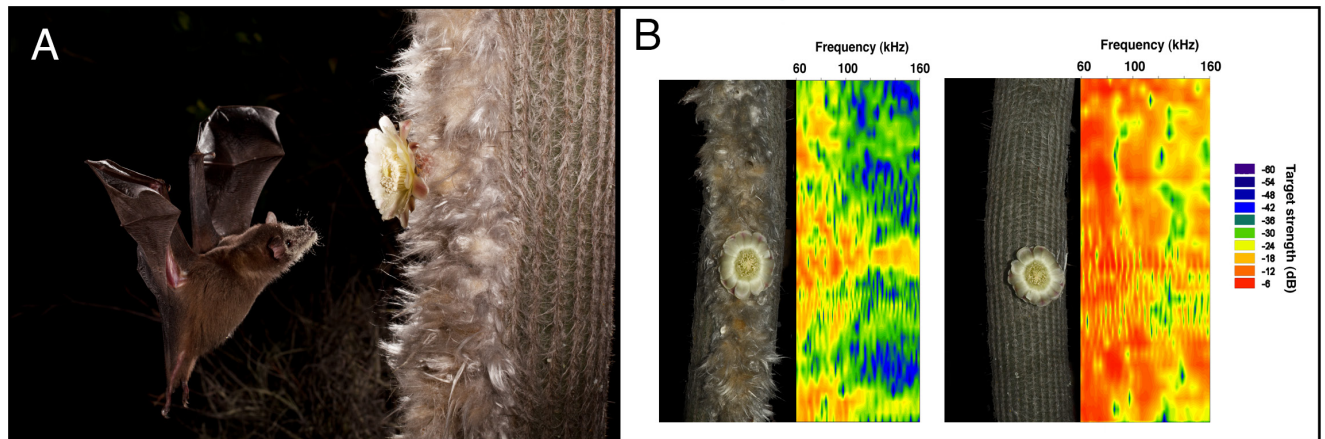


Figure 2. Nectar-feeding bat approaching a flower and echo fingerprints of different cactus surfaces with flowers. **(A)** Image of a Geoffroy's tailless bat (*Anoura geoffroy*) approaching a flower of *Espostoa frutescens*, which is embedded in the hairy cephalium zone (photo credit: Merlin Tuttle's Bat Conservation). **(B)** Echo fingerprints of acoustic scans along the cactus column. The left column is a natural column with cephalium and flower, for the right measurement we experimentally manipulated the column. The flower was cut out of the hairy zone and fixed on an unspecialized part of the column. The intensity (spectral target strength in dB) of the echo is given in colour gradation (red indicates high intensities, blue low intensities).

125 strength of -23.7 dB, which is around 14 dB lower than the unspecialized parts of the
126 column.

127 A qualitative analysis of the echo-acoustic fingerprint of specialized versus unspecialized
128 parts of the column revealed more detailed insight into the effect of the background on
129 detectability of flower targets (Fig 2B). The unspecialized column reflects high TS echoes
130 for almost the entire bandwidth, which are only sometimes interrupted by some
131 frequency notches. The specialized cephalium side of the column reflects much less sound
132 energy, especially for frequencies above 90 kHz. When scanning the column at the
133 position of the flower, the unmanipulated flower stands out from the less-reflecting
134 background, in particular at frequencies above 90 kHz. When we placed a flower on the
135 unspecialized part of the column the flower echoes almost completely disappear within
136 the loud background echoes, although there might be some additional interference
137 patterns affecting the TS.

138 In total we analysed 279 echolocation calls of two individuals of *A. geoffroyi*, see Fig. 3 for
139 an example of an echolocation call sequence during an approach to an *E. frutescens*

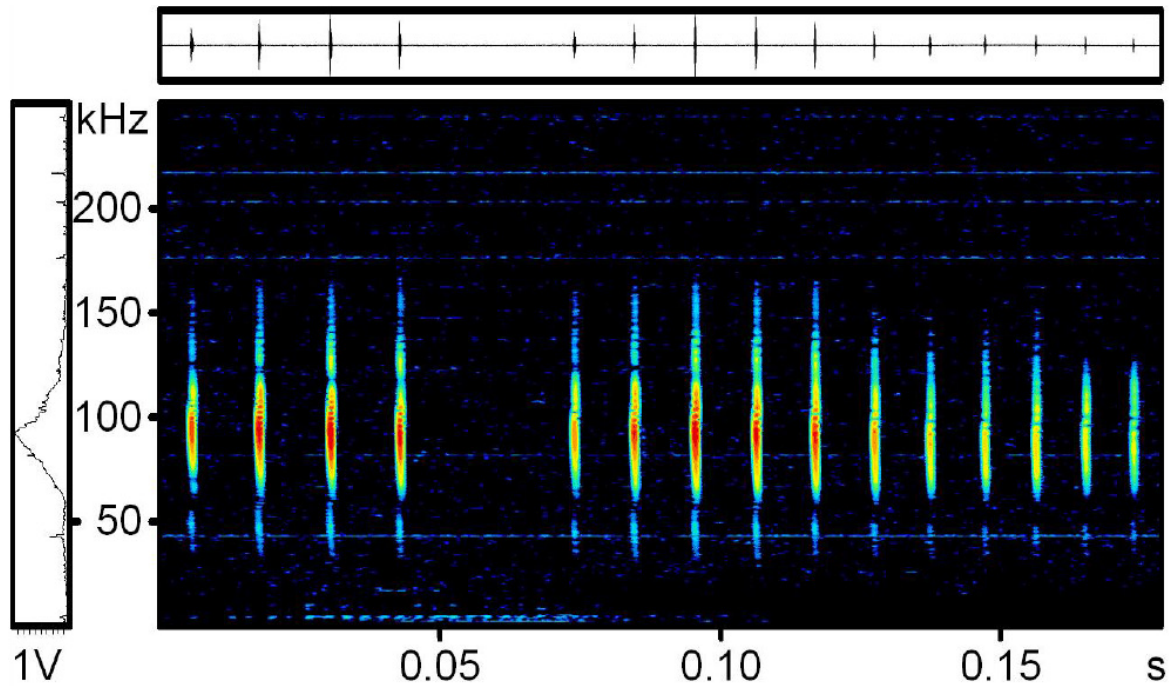


Figure 3. Typical series of calls of a Geoffroy's tailless bat (*Anoura geoffroyi*) while approaching an *Espostoa frutescens* flower. The microphone (1/4" G.R.A.S. free field microphone) was placed next to the flower.

140 flower. The calls were short, having a duration of only $0.47 \text{ ms} \pm 0.18 \text{ ms}$ (mean \pm SD)
141 and they were step frequency modulated starting at $132.7 \text{ kHz} \pm 8.1 \text{ kHz}$ and ending at
142 $59.8 \text{ kHz} \pm 10.0 \text{ kHz}$. The peak frequency of the calls was at $92.5 \text{ kHz} \pm 4.4 \text{ kHz}$, which falls
143 into the frequency band where sound absorption of the cephalium was highest.

144 Discussion

145 Our ensoufication experiments revealed distinct and frequency-dependent differences in
146 echo-acoustic reflectance of different cacti parts. We found that the plain column of
147 *Espostoa* acts as a strong reflecting surface as it is cylindrical, providing reflective surfaces
148 from all directions, and also because the surface has ridges, which may additionally act as
149 small retroreflectors. The flowers of *Espostoa* reflect much less energy compared to the
150 column, mainly due to the facts that the reflecting surface is smaller and that flowers have
151 a lot of anthers, which scatter the sound energy. The specialized cephalium surrounding
152 the flowers reflected the least energy, in particular in the echolocation call frequency
153 range of the plant's main pollinator, *A. geoffroyi*. These results strongly suggest that the
154 cephalium of *Espostoa* functions as a sound-absorbing structure and thus enhances the

155 echo-acoustic contrast between the flower and the vegetative part of the plant for an
156 approaching bat. While scanning cacti columns for flowers along the cephalium the bats
157 will receive faint echoes unless their call hits a flower, which increases the echo response
158 by around 10 dB. In contrast, flowers growing on the unspecialized parts of the column
159 would be much more difficult to recognize in front of the highly reflective background.
160 Bats might be able to pick up on the interference patterns caused by the flowers, however,
161 this would require much more processing than a salient flower echo in front of an
162 absorbing surface.

163 Such a simple yet efficient mechanism of dampening the background of the flowers thus
164 may help the bats to save on foraging time – nectar feeding bats have to visit or revisit
165 several hundred flowers each night to cover their nightly energy expenditure – and thus
166 increase foraging efficiency [12]. The plant on the other hand will benefit from a higher
167 cross pollination rate - bats are very efficient pollinators that carry a lot of pollen in their
168 fur (see Fig 2A) and have a huge home range so they can pollinate plants growing far apart
169 [13].

170 The absorption of the *cephalium* is most efficient for the 102 kHz frequency band (82 kHz
171 – 122 kHz), which translates to a wavelength of around 3.4 mm (4.2 mm – 2.8 mm). The
172 microstructure of the *cephalium* apparently favours absorption of sound around this
173 wavelength, while larger wavelengths (e.g., 7.6 mm for the 45 kHz band) are around 10
174 dB less attenuated. The hairs are much smaller in diameter than the wavelengths of sound
175 they absorb best and therefore probably do not scatter the incoming sound waves. An
176 alternative explanation could be that the hairs create a layer of air with different
177 temperature that reflects the sounds in a frequency-dependent manner.

178 As other species of *Espostoa* show the same hairy cephalium zone this floral acoustic
179 adaptation might not only be limited to this species and even other genera have similar
180 hairy cephalium zones e.g. *Microanthocereus* [8]. Interestingly, bird pollinated species of
181 the genus *Microanthocereus* have also cephalium zones, however the fur is much less
182 dense. We argue that cephalium-like structures originally evolved for protection of floral
183 structures, but was co-opted at some point in time to serve an additional or new
184 functional role in pollinator attraction. Once co-opted, the cephalium of bat-pollinated
185 flowers got optimized for this new function through selection by the echolocating bat
186 pollinators.

187 Our study reveals that bat-pollinated flowers can also rely on absorption in addition to
188 reflectance as an acoustic adaptation towards their pollinators. Echoacoustic absorption
189 likely plays a much larger role across a wide range of ecological contexts than so far has
190 been appreciated. Sound absorbent structures have already been described for moth
191 scales [14] as well as for thoracic moth fur [15]. Whether absorption has adapted in the
192 context of predator-prey arms races remains however to be tested, ideally in a
193 comparative phylogenetic framework.

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199 identification and assistance with the *E. frutescens* specimen.

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Supplementary Figure

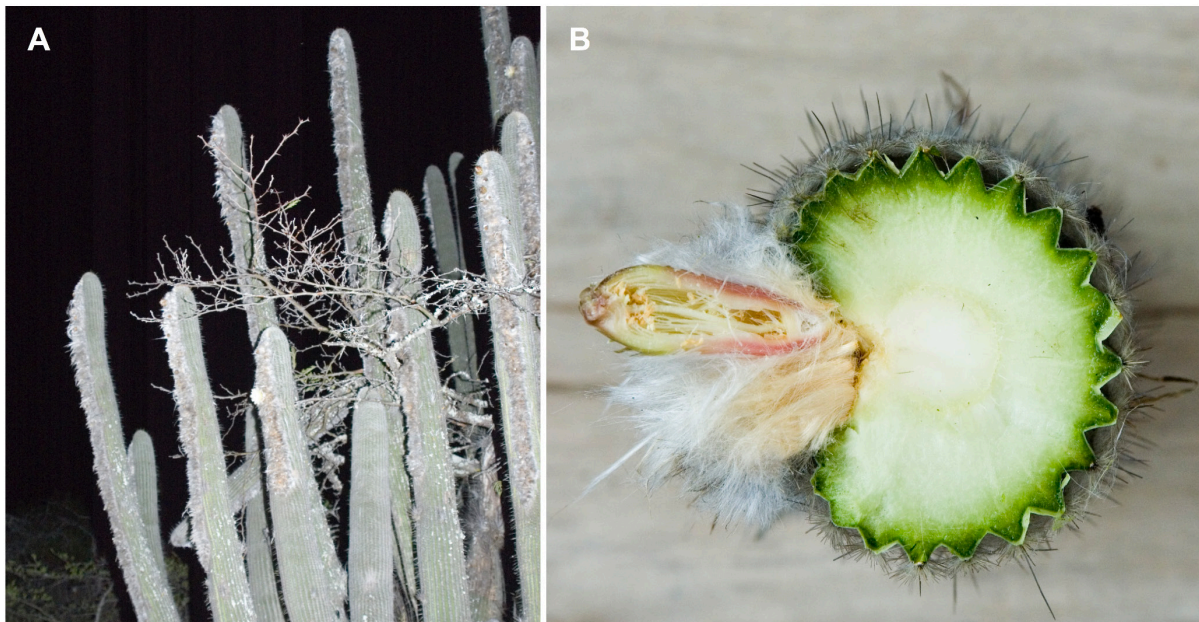


Figure S1. (A) Habitus of an *Espostoa frutescens* plant and (B) cross section of a column with the hairy cephalium and a closed flower.