

Research note

Cycad collicles (“leaflet nodules”)

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Abstract

The presence of small knobs/nodules found on the adaxial leaflet surface of some *Encephalartos* species was investigated in 134 cycad species of nine genera. The knobs, referred to as collicles, were found to be the result of connecting mucilage canal ‘bridges’ over vascular bundles, connecting mucilage canals situated between adjacent parallel vascular bundles. Collicles were found on some *Encephalartos* species only and not always on all individuals of the same species. The possible taxonomic value of these collicles is discussed.

Highlights

- Small knobs were found on the adaxial leaflet surface of some *Encephalartos* species.
- Their possible presence was investigated in 134 species of nine cycad genera.
- The knobs were referred to as collicles and found on *Encephalartos* species only.
- Collicles are the result of anastomoses between adjacent mucilage canals.

Keywords: Mucilage canals; leaflets; colliculate; leaflet anatomy.

Small knobs/nodules (Fig. 1A) clearly visible to the unaided eye occur on the adaxial leaflet surface of some species of *Encephalartos* Lehm. (Grobbelaar, 2002). Plant surfaces covered by such small, rounded protuberances have traditionally been described as “colliculate” or “colliculose” (e.g. Jackson, 1928; Beentje, 2010). These knobs, as is described below, differ structurally from the macroscopically rather similar-looking bacterial leaf nodules that commonly occur in certain plants, for example some members of *Pavetta* L. and *Psychotria* L. (Bremekamp, 1933, 1934; Lersten & Horner, 1967; Lemaire et al. 2012). Hence we here propose the new term **collicle/collicles** (singular/plural), from the Latin *colliculus* for hillock or mound, to refer to these knobs

on the leaflet surfaces in cycads. No previous or subsequent reference to these structures appears to have been published. Even Koeleman (1978) and Koeleman *et al.* (1981), who made an extensive anatomical study of the petioles and leaflets of 28 South African species of *Encephalartos*, did not mention these knobs. Consequently a survey of the adaxial leaflet surface in living plants of 134 extant cycad species from nine genera encompassing all three extant families of the Cycadophyta was undertaken. These included two species of *Bowenia* Hook ex J.D. Hook. viz. *B. serrulata* (W. Bull.) Chamb. and *B. spectabilis* Hook. ex J.D. Hook.; six of *Ceratozamia* Brongn., viz. *C. hildae* Landry & K.L. Wilson, *C. kuesteriana* Regel, *C. latifolia* Miq., *C. mexicana* Brongn., *C. microstrobila* Vovides & Rees and *C. norstogii* D. Stevenson; 25 of *Cycas* L., viz. *C. angulata* R. Br., *C. armstrongii* Miq., *C. beddomei* Dyer, *C. cairnsiana* Muell., *C. chamberlainii* W. H. Brown & Kienholz, *C. circinalis* L., *C. clivicola* K. Hill, *C. couttsiana* K. Hill, *C. debaoensis* Zhong & Chen, *C. guizhouensis* Lan & R. F. Zhou, *C. hainanensis* C. J. Chen, *C. media* R. Br., *C. nongnoochiae* K. Hill, *C. panzhihuaensis* L. Zhou & S. Y. Yang, *C. pectinata* Buchanan-Hamilton, *C. petraea* A. Lindstrom & K.D. Hill, *C. revolute* Thunb., *C. rumphii* Miq., *C. schumanniana* Lauterbach, *C. seemannii* A. Braun, *C. simplicipinna* (Smitinand) K. D. Hill, *C. taitungensis* C. F. Shen, K.D. Hill, C. H. Tsou & C. J. Chen, *C. tansachana* K. D. Hill & S. L. Yang, *C. thouarsii* R. Br. Ex Gaudich. and *C. wadei* Merr.; ten of *Dioon* Lindl., viz. *D. califanoi* De Luca & Sabato, *D. caputoi* De Luca, Sabato & Vazquez-Torres, *D. edule* Lindley, *D. holmgrenii* De Luca, Sabato & Vazquez-Torres, *D. mejiae* Stanley & L. O. Williams, *D. merolae* De Luca, Sabato & Vazquez-Torres, *D. purpusii* Rose, *D. rzedowski* De Luca, Moretti & Vazquez-Torres, *D. sonorensis* De Luca, Sabato & Vazquez-Torres and *D. spinulosum* Dyer; 61 of *Encephalartos* Lehm. (see Table 1); two of *Lepidozamia* Regel viz. *L. hopei* (W. Hill) Regel and *L. peroffskyana* Regel; 15 of *Macrozamia* Miq., viz. *M. communis* L. Johnson, *M. concinna* D. L. Jones, *M. diplomera* (F. Mueller) L. Johnson, *M. douglasii* W. Hill & Bailey, *M. fawcettii* C. Moore, *M. fearnsidei* D. L. Jones, *M. johnsonii* D. L. Jones & K. Hill, *M. lucida* L. Johnson, *M. macdonnellii* (F. Muelles ex Miquel) A. de Candolle, *M. miquellii* (F. Mueller) A. de Candolle, *M. moorei* F. Mueller, *M. mountperriensis* Bailey, *M. riedlei* (Fish. Ex Gaudich.) C. A. Gardner, *M. secunda* (Bentham) C. Moore and *M. spiralis* (Salisb.) Miq.; one of *Stangeria* T. Moore, viz. *S. eriopus* (Kunze) Baillon; and 12 of *Zamia* L., viz. *Z. amblyphyllidia* D. Stevenson, *Z. encephalartoides* D. Stevenson, *Z. furfuracea* Aiton, *Z. inermis* Vovides, Rees & Vazquez-Torres, *Z. integrifolia* Aiton, *Z. loddigesii* Miq., *Z. paucijuga* Wieland, *Z. portoricensis* Urban, *Z. pumila* L., *Z. spartea* A. De Candolle, *Z. standleyi* Schutzman and *Z. vazquezii* D. Stevenson, Sabato & De Luca. Several leaflets from each plant were examined and in nearly all cases at least five plants of each species were included in the survey. Material for the collicle study was collected from plants growing in three different gardens in Pretoria, each with an extensive cycad collection: a) Garden of the first author, b) Botanic Garden of the

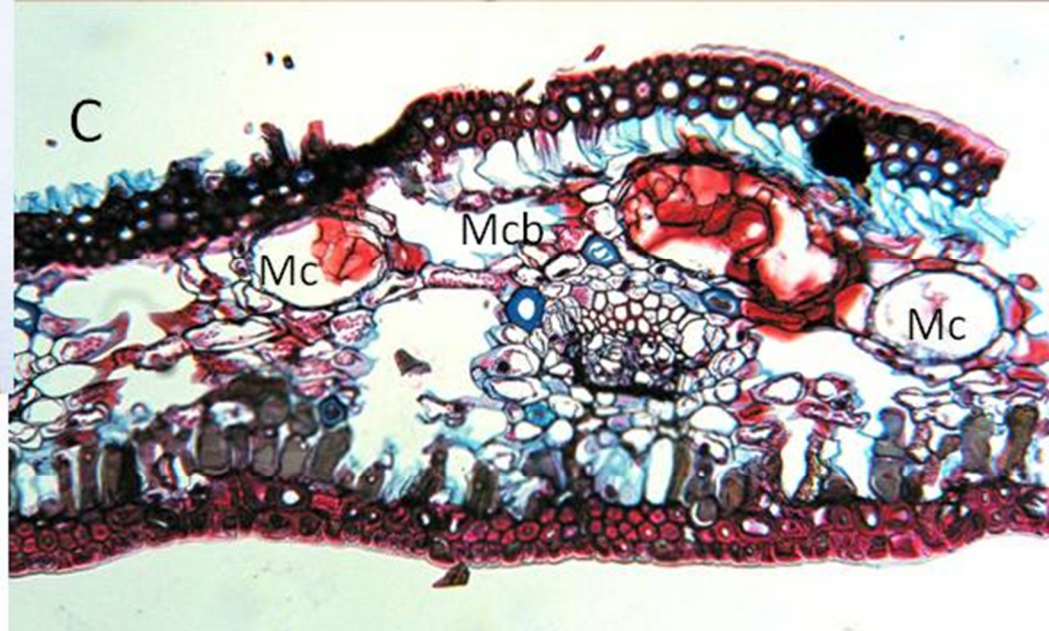
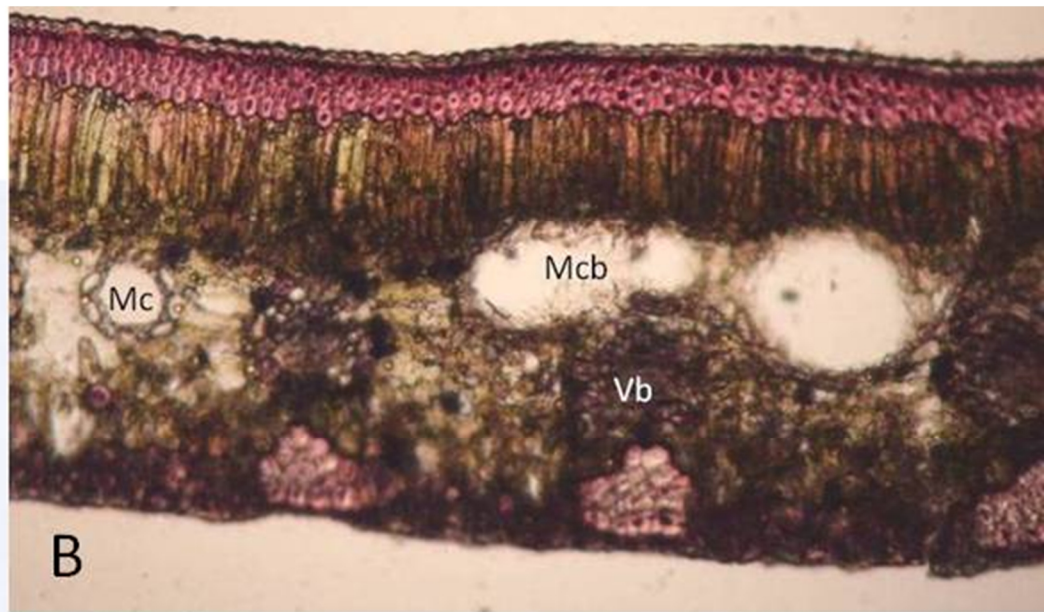
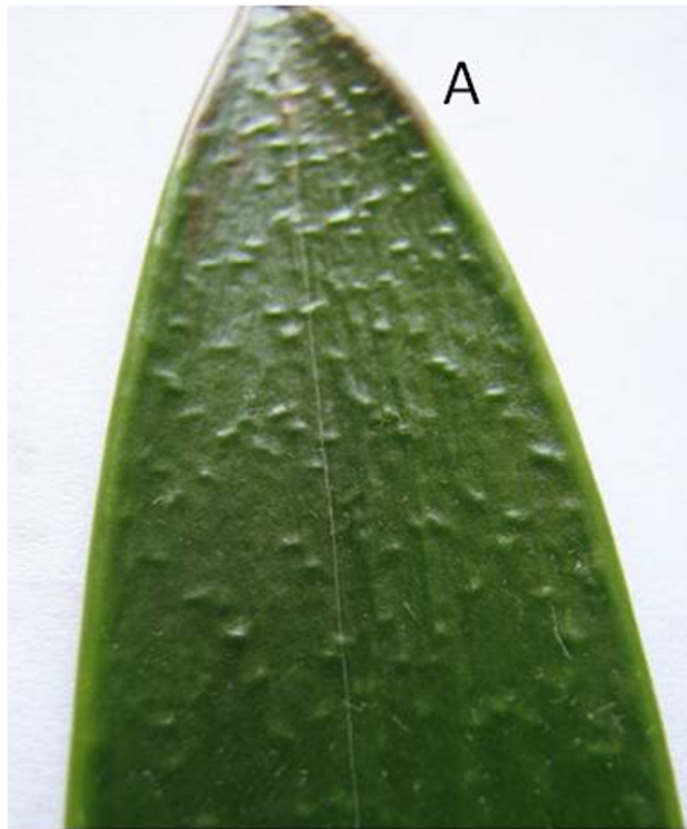


Figure 1. Position and structure of collicles. A - Collicles on apical portion of adaxial leaflet surface of *Encephalartos natalensis*; B - transverse hand section of *Encephalartos concinus* leaflet stained with Safranin, showing mucilage canals (Mc) between neighbouring vascular bundles and a mucilage canal 'bridge' (Mcb) crossing the vascular bundle to form a collicle; C - transverse section of wax-embedded *E. longifolius* leaflet stained with Safranin and Fast Green, also showing a mucilage canal 'bridge'.

Table 1. *Encephalartos* leaflet characteristics from Koeleman (1978;

columns 2 and 3) and present authors (column 4). Cells left open indicate data not available.

Species investigated ¹	Palisade position ²	Presence /absence of mucilage canals	Number of collicles per leaflet ³
<i>E. aemulans</i> ^{ac} Vorster,Robbertse & S. van der Westhuizen			M
<i>E. altensteinii</i> ^{abc} Lehm.	Ad	+	F - M
<i>E. aplanatus</i> ^{ab} Vorster			0
<i>E. arenarius</i> ^{abc} R. A. Dyer	Ad	+	0
<i>E. barteri subsp. barteri</i> ^a Carath. ex Miq.			0
<i>E. bubalinus</i> ^a Melville			M
<i>E. caffer</i> ^{ac} (Thunb.) Lehm.	Ad	+	0
<i>E. cerinus</i> ^{ac} Lavranos & Goode			0
<i>E. concinnus</i> ^a R. A. Dyer			M
<i>E. cupidus</i> ^{abc} R. A. Dyer	Ad + Ab	+	0
<i>E. cycadifolius</i> ^{abc} (Jacq.) Lehm.	Ad + spAb	+	0
<i>E. delucanus</i> ^a Malaisse, Sclavo & Crossiers			0
<i>E. dolomiticus</i> ^{ac} Lavranos & Goode			0
<i>E. dyerianus</i> ^{ac} Lavranos & Goode			0
<i>E. equatorialis</i> ^a P. J. H. Hurter & Glen			0
<i>E. eugene-maraisii</i> ^{abc} I. Verd.	Ad + Ab	+	0
<i>E. ferox</i> ^{abc} G. Bertol	Ad	+	0 - rF
<i>E. friderici-guilielmi</i> ^{abc} Lehm.	Ad+spAb	+	0
<i>E. ghellinckii</i> ^{abc} Lehm.	Ad	-	0
<i>E. gratus</i> ^a Prain			0
<i>E. heenanii</i> ^{ac} R. A. Dyer	Ad+spAb	+	M
<i>E. hildebrandtii</i> ^a A. Br. & Bouche			0 - F
<i>E. hirsutus</i> ^{ac} P. J. H. Hurter & Glen			0 - M
<i>E. horridus</i> ^{abc} (Jacq.) Lehm.	Ad+spAb	+	0 - rM
<i>E. humilis</i> ^a I. Verd.	Ad+spAb	-	0
<i>E. inopinus</i> ^{abc} R. A. Dyer	Ad+spAb	+	0
<i>E. ituriensis</i> ^a Bamps & Lisowski			0
<i>E. kisambo</i> ^a Faden & Beentje			0 - M
<i>E. laevifolius</i> ^{abc} Stapf & Burtt-Davy	Ad+spAb	-	0
<i>E. lanatus</i> ^{abc} Stapf & Burtt-Davy	Ad+spAb	-	0
<i>E. latifrons</i> ^{ac} Lehm.	Ad+spAb	+	0
<i>E. laurentianus</i> ^a De Wild.			0

<i>E. lebomboensis</i> ^{abc} I. Verd.	Ad	+	0 - M
<i>E. lehmannii</i> ^{abc} Lehm.	Ad+spAb	+	r0 - M
<i>E. longifolius</i> ^{abc} (Jacq.) Lehm.	Ad+spAb	+	0 - M
<i>E. mackenziet</i> ^a Newton			0 - M
<i>E. macrostrobilus</i> ^a S. Jones & Wynants			F - M
<i>E. manikensis</i> ^{ac} (Gilliland) Gilliland			0 - M
<i>E. marunguensis</i> ^a Devred			0
<i>E. middelburgensis</i> ^{abc} Vorster, Robbertse & S. van der Westhuizen			0
<i>E. msinganus</i> ^a Vorster			F
<i>E. munchii</i> ^a R. A. Dyer			0
<i>E. natalensis</i> ^{abc} R. A. Dyer	Ad	+	F - M
<i>E. ngoyanus</i> ^{ac} I. Verd.	Ad	+	0
<i>E. nubimontanus</i> ^{ac} P. J. H. Hurter			0
<i>E. paucidentatus</i> ^{abc} Stapf & Burtt-Davy	Ad	+	F - M
<i>E. poggei</i> ^a Asherson			0
<i>E. princeps</i> ^{abc} R. A. Dyer	Ad+spAb	+	0 - rM
<i>E. pterogonus</i> ^a R. A. Dyer			0
<i>E. schmitzii</i> ^a Malaisse			0
<i>E. sclavoi</i> ^a De Luca, D. Stevenson & Moretti			F - M
<i>E. senticosus</i> ^{abc} Vorster			0 - M
<i>E. septentrionalis</i> ^a Schweinf.			0 - F
<i>E. tegulaneus</i> ^a Melville			M
<i>E. transvenosus</i> ^{abc} Stapf & Burtt-Davy	Ad	+	F - M
<i>E. trispinosus</i> ^{abc} (Hook.) R. A. Dyer	Ad+spAb	+	0 - rM
<i>E. turneri</i> ^a Lavranos & Goode			0 - M
<i>E. umbeluziensis</i> ^{ac} R. A. Dyer	Ad	-	0
<i>E. villosus</i> ^{abc} Lem.	Ad	-	0
<i>E. whitelockii</i> ^a P. J. H. Hurter			0
<i>E. woodii</i> ^{abc} Sander	Ad	+	0

¹ At least five plants of each species were examined for collicles, except for *E. woodii* where only three, and *E. delucanus* where only two plants were examined

² Ad = leaflets with adaxial palisade only; Ad + Ab = leaflets with both adaxial and abaxial palisade; Ad + spAb = leaflets with adaxial and sparse abaxial palisade

³ 0 = No collicles per leaflet were observed.

0 - F = No or a few collicles per leaflet.

0 - M = No, a few or many collicles per leaflet.

0 - rF = Collicles usually absent but rarely a few present

0 - rM = Collicles usually absent, rarely a few or many
per leaflet.

r0 - M = Rarely collicles absent. Usually few to many per leaflet

F = Few collicles per leaflet.

F - M = Few to many collicles per leaflet.

M = Many collicles per leaflet.

Superscripts in column 1: Plants collected in ^a garden of N. Grobbelaar; ^b Botanic Garden of the University of Pretoria; ^c garden of Dr. Martin Bruwer (Exclusive Cycads), Pretoria

University of Pretoria, and c) garden of Dr. Martin Bruwer (Exclusive Cycads) as indicated in Table 1.

The leaflets were usually wiped/brushed free of wax and/or hairs before they were examined with the unaided eye under strong light to enhance the detection of the collicles. Of the genera scrutinized, *Encephalartos* was found to be the only one to bear collicles. However, collicles occur in certain species of the genus only. For the species that do form collicles, the number of collicles per leaflet, in the case of some species, varies considerably from plant to plant but not much between the leaflets of a single plant. In a few cases, notably *E. ferox*, *E. horridus*, *E. princeps* and *E. trispinosus* (The author citations for the *Encephalartos* species are given in the table and are not repeated in the text) where considerably more than five plants were included in the survey, almost all the plants appeared to be devoid of collicles although one or a few plants with many collicles per leaflet were nevertheless encountered as exceptions. For *E. lehmannii*, the opposite was found – nearly all the plants examined bore several to many collicles per leaflet with a few that appeared to be devoid of collicles. In the absence of cones, *E. lehmannii* and *E. princeps* are morphologically very similar but by examining the collicle status and the s-angle pattern (Grobbelaar, 2002) of the two species, they can invariably be successfully distinguished. For species such as *E. altensteinii*, *E. ferox*, *E. hildebrandtii*, *E. horridus*, *E. longifolius*, *E. manikensis*, *E. natalensis*, *E. senticosus* r, *E. transvenosus* and *E. turneri*, for which several morphologically forms are known, the variation in the collicle status within a species should probably not be unexpected.

To elucidate the structure of the collicles, hand-cut transverse sections of the leaflets of *E. longifolius* and *E. concinnus*. (Fig. 1B) and wax-imbedded sections of *E. longifolius* (Fig 1C) were examined and compared with the anatomical observations by Koeleman (1978) and Koeleman et al. (1981).

Within the genus *Encephalartos*, apart from *E. cupidus*, *E. dolomiticus*, *E. eugene-maraisii* and *E. middleburgensis* whose leaflets are isobilateral, all the other investigated species, including those with weakly developed abaxial palisade cells (see Table 1), have dorsiventral leaflets with stomata confined to the abaxial epidermis. The adaxial palisade cells are separated from the epidermis by one or more layers of sclerenchyma (Figs. 1B and 1C). The vascular bundles run parallel along the long axis of the leaflets, and are embedded in the mesophyll, more or less midway between the adaxial and abaxial epidermises. A single mucilage canal occurs more or less midway between neighbouring vascular bundles and is either located about midway between the two epidermises or adjacent to the palisade cells in the spongy mesophyll (Figs. 1B, 1C and Koeleman, 1981). Girder sclerenchyma below the vascular bundles and adjacent to the abaxial epidermis was observed by Koeleman (1978) in most of the species he investigated.

Occasionally a somewhat dilated mucilage canal branch connects two mucilage canals to form a ‘bridge’ (anastomosis) overarching the vascular bundle lying between them (Figs. 1B and 1C). These turgid branches (bridges) manifest on the leaf surface as collicles (Fig. 1) due to the space they occupy between a vascular bundle and the overlying palisade, adaxial sclerenchyma and adaxial epidermis.

In the case of *E. ghellinckii*, *E. humilis*, *E. laevifolius*, *E. lanatus*, *E. umbeluziensis* and *E. villosus* which do not contain mucilage canals in their leaflets, it is logical that collicles are absent (see Table 1). However, the reason for the variable collicle status of some species still needs to be resolved. From the results at hand (Table 1), seven of the 15 species with palisade bordering both epidermises are devoid of collicles. It is noteworthy that all the *Encephalartos* species with narrow leaflets and/or subterranean stems, viz. *E. aplanatus*, *E. barteri*, *E. caffer*, *E. cerinus*, *E. cupidus*, *E. cycadifolius*, *E. friderici-guilielmi*, *E. ghellinckii*, *E. humilis*, *E. laevifolius*, *E. lanatus*, *E. ngoyanus*, *E. schmitzii*, *E. umbeluziensis* and *E. villosus*, without exception, are devoid of collicles. However, several arborescent species with wide leaflets such as *E. arenarius*, *E. gratus*, *E. laurentianus* and *E. woodii* are also devoid of collicles. Most of the species with "blue-green" leaflets such as *E. cupidus*, *E. dolomiticus*, *E. dyerianus*, *E. eugene-maraisi*, *E. inopinus*, *E. middelburgensis* and *E. nubimontanus* are also devoid of collicles, but here there are also striking exceptions such as *E. hirsutus* and *E. lehmannii*.

The fact that the potential to form collicles are found in certain species of *Encephalartos* only, raises the question of its taxonomic importance even though not visible in all individuals of a species. It would also be informative to know whether the collicle status of a plant is significantly affected by the environment. The functional significance of the mucilage canals in *Encephalartos* is still the subject of speculation. In animals, for example, anastomoses in the circulatory system is common and serve as backup routes for blood to flow if one link is blocked or otherwise compromised. Anastomoses are also common between parallel veins in the leaves of monocots. It is possible that the links between the mucilage canals in *Encephalartos* may ensure canals stay connected following local damage or necrosis of the leaflets. More research is required to resolve these questions.

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