

An Anatomical Character to Support the Cohesive Unit of *Butia* Species

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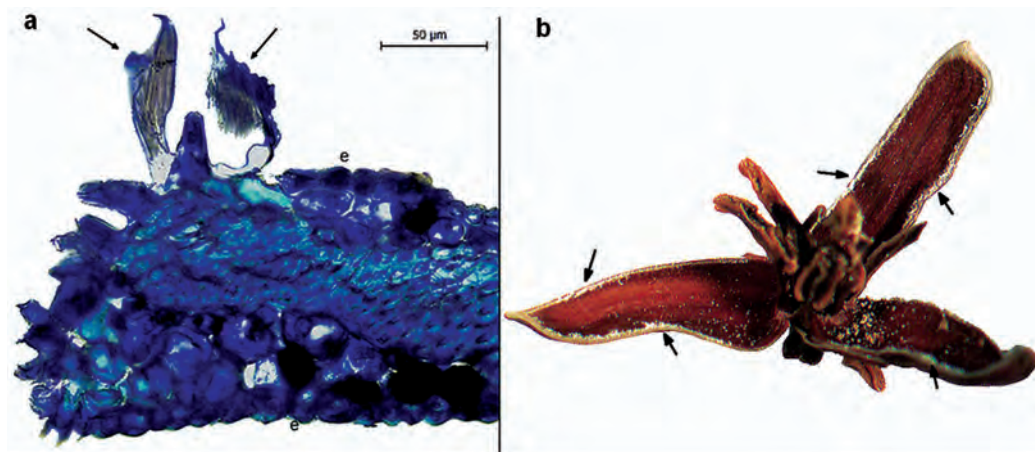
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Raphides are common cell inclusions in vegetative and reproductive palm organs. However, epidermal raphide-containing idioblasts are quite unusual and have been recorded only a few times on flower petals in palms. Here we describe their presence in all *Butia* staminate flowers sampled, and we discuss their taxonomic and biological significance.

Raphides are widespread cell inclusions in angiosperms. They are linked to a wide range of functions in plants, including storage forms of calcium and oxalic acid (Arnott & Pautard 1970, Sunell & Healey, 1979), as simple depositories for metabolic wastes or promoting air space formation (Prychid & Rudall 1999), as defense device against herbivores (Sakai et al. 1972, Perera et al., 1990, Ward et al. 1997)

or even as a reward for bee pollinators (D'Arcy et al. 1996). They have been described by Tomlinson et al. (2011) to be intrinsic to palm anatomy, commonly occurring in diverse organs (Uhl & Dransfield 1987, Tomlinson 1961, 1990, Tomlinson et al. 2011). In palm flowers, they have been recorded in almost all parts of the flower (Castaño et al. 2009). Thus, they have been reported in the base of the



1. Staminate flowers of *Butia*. A. Anatomical micrograph of *B. paraguayensis* (transversal view) showing epidermal raphide idioblasts. B. Dried staminate flower of *B. purpurascens* (at anthesis) 60x. Arrows indicate raphide bundles on petal margins. e, epidermis.

flower (Castaño et al. 2011), in the mesophyll of petals and sepals (Uhl 1972, Askgaard et al. 2008), on petals as trichome “sacs” (Robertson 1978), scattered throughout the pistil (Uhl & Moore 1971), in the mesophyll of the ovary (Uhl & Moore 1971, Barfod & Uhl 2001, Giddey et al. 2008), inside the pollen sacs (Henderson & Rodriguez 1999) and embedded in the inner tissue of the embryos (Zona 2004).

Butia (Becc.) Becc. is a medium size palm genus that includes 20 species (Govaerts et al. 2012, Noblick 2010). It is endemic to subtropical southern South America and occurs in Brazil, Paraguay, Uruguay and Argentina, growing in open dry areas, grasslands and *cerrado* formations (Henderson et al. 1995). *Butia* species form a highly diverse group due to the fact that they have probably undergone a rapid adaptive radiation (Pintaud et al. 2008).

During a recent survey on scent-producing organs in palm flowers, we identified characteristic structures on the corolla margins in staminate flowers of *B. paraguayensis* (Barb. Rodr.) L.H. Bailey (Fig 1a). Crystals on these areas were clearly identified by stereomicroscopy and this prompted us to undertake an in-depth study on their presence in other species within the genus. In this contribution we discuss the taxonomic and biological implications of these epidermal raphide-containing idioblasts in *Butia*.

Materials and methods

The study was carried out from February to June 2012 in the Montgomery Botanical Center (MBC) and the Fairchild Tropical Botanic Garden’s Herbarium (FTG). We

analyzed 13 out of 20 recognized *Butia* species. Fresh staminate flowers were sampled from live individuals at MBC: *B. marmorii* Noblick, *B. paraguayensis* (Barb. Rodr.) L.H. Bailey, *B. yatay* (Mart.) Becc., and *Butia* sp. Buds and open dried staminate flowers were sampled from specimens deposited at FTG: *B. archeri* (Glassman) Glassman, *B. capitata* (Mart.) Becc., *B. catarinensis* Noblick & Lorenzi, *B. eriospatha* (Mart. ex Drude) Becc., *B. exospadix* Noblick, *B. lallemantii* Deble & Marchiori, *B. marmorii* Noblick, *B. matogrossensis* Noblick & Lorenzi, *B. microspadix* Burret, *B. odorata* (Barb. Rodr.) Noblick, *B. paraguayensis* (Barb. Rodr.) L.H. Bailey, *B. purpurascens* Glassman, *B. yatay* (Mart.) Becc. Furthermore, we analyzed staminate flowers of related taxa, such as *Jubaea chilensis* (Molina) Baill. sampled from Los Angeles County (University of California) and Lotusland; *Syagrus coronata* (Mart.) Becc., *S. kellyana* Noblick & Lorenzi, *S. romanzoffiana* (Cham.) Glassman, all from MBC.

Flowers were studied under a light stereomicroscope searching for exposed raphide crystals and trichomes on the petal margins. Anatomical slides were prepared through free-hand sections or embedded in Technovit 7100 (2-hydro-xyethyl methacrylate). Serial sectioning was carried out using a rotary microtome (Sorvall Porter-Blum MT-1) at 5 µm. The sections were stained with toluidine blue. Observations were made using a Leica microscope (DM-500), micrographs were taken with a Leica ICC-50 and the images were edited using Leica Application System-LAS EZ (Leica Microsystems Framework).

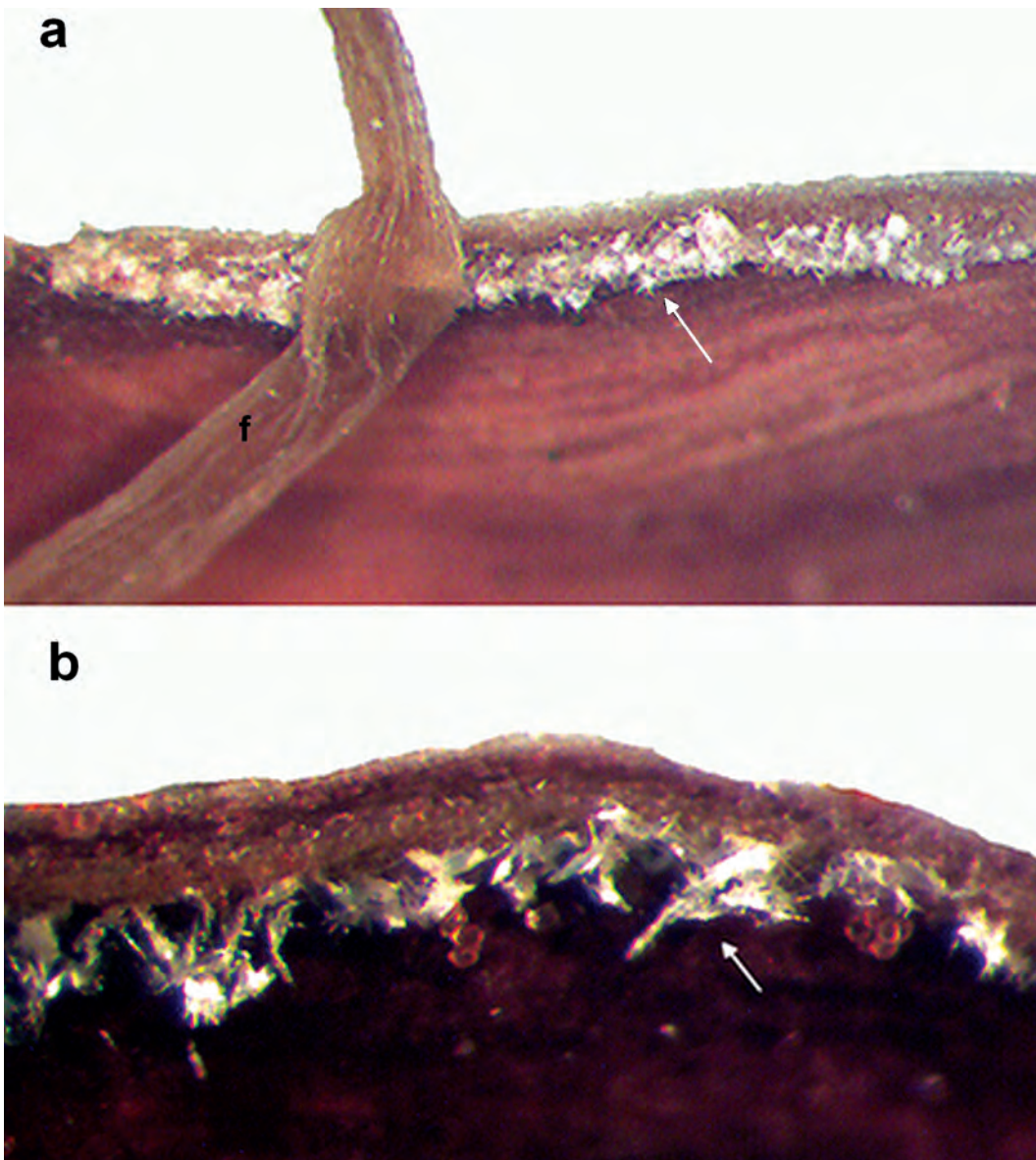
Results and Discussion

Exposed raphide bundles on the corolla were identified in all *Butia* species sampled. They were easily recognizable under a stereomicroscope (Figs. 1b & 2). High densities of exposed raphide crystals were commonly observed in almost all taxa studied. They could not be easily identified in a few samples of *B. marmorii* flowers as they were remarkably smaller than in the other species studied; however, their presence was confirmed by optical microscopy. These idioblasts are globe-shaped and are dispersed in the epidermis of

the petal (Fig. 1a). The raphide containing idioblasts, either of epidermal or subepidermal origin, are easily broken due the presence of thin cell walls. Therefore raphide crystals are commonly exposed in open staminate flowers (Fig. 1b). Raphide bundles and raphide containing idioblasts were not detected on the petal margins of the staminate flowers of the *Jubaea* and *Syagrus* species that were studied.

For many years, *Jubaeopsis caffra* has been reported as the only palm with raphide containing idioblasts ("sacs") on its corolla epidermis (Robertson 1978). However, similar

2. Raphide bundles on the inner zone of the petal margins of *Butia* species. A. Petal of *B. yatay* at 250x. B. Petal of *B. matogrossensis* at 250x. Arrows indicate the presence of raphides. f, stamen filament.



idioblasts have been recorded on the staminate flowers of *Aphandra natalia* (Barfod & Uhl 2001) and also on floral organs outside the palm family in the asparagoid family Tecophleaceae (Prychid & Rudall 1999). Furthermore, Cocucci (1964) probably referred to these kind of idioblasts when he recorded the presence of raphide containing idioblasts (described by him as hypertrophied cells) on the "tepals" of *B. paraguayensis* flowers; however, no further details were provided. Raphide containing idioblasts on the corolla could be present in other palm taxa, but a much wider research effort would be required to confirm this fact.

Past studies have suggested a close relationship between *Butia* and *Syagrus* (Henderson et al. 1995). Indeed, some currently recognized *Butia* species were actually placed for a long time in *Syagrus*, such as *B. campicola* (as *S. campicola*; Noblick 2004) and *B. leptospatha* (as *S. leptospatha*; Noblick 2006). However, cladistic analysis of morphological and anatomical characters (Noblick et al. in press) and DNA analysis based on PKR and seven WRKY gene markers showed that *Butia* is more closely related to *Jubaea* (Gunn 2004; Meerow et al. 2009). Both *Butia* and *Jubaea* share some morphological characteristics (Seubert 1998a & b, Dransfield et al. 2008, Noblick et al. 2012) and floral features, such as papillate petal margins. Our study shows that *Jubaea chilensis* does not present raphide-containing idioblasts on the petal margins. This supports *Jubaea* as a genus clearly distinct from *Butia*. Raphide-containing idioblasts could be proposed as a new synapomorphic character for *Butia*, further supporting its cohesive monophyly. Raphides have been used to support the establishment of the "*Chelyocarpus* alliance" (Moore 1972, Uhl 1972) and the relationships within other taxa such as Rubiaceae (Lersten 1974) and *Prunus* (Lersten & Horner 2000). Their absence represents a synapomorphic character in some Monocots (Prychid & Rudall 1999).

Raphide-containing idioblasts can be explained as a defensive device against florivory. In palms, they protect the flower against deleterious fauna (Uhl & Moore 1973) and form a physical barrier to keep insects away (Uhl & Moore 1977). High densities of raphide crystals could represent an efficient physical barrier to pollen-eating insects (Askgaard et al. 2008). Barfod et al. (1999) hypothesized that pollen-like raphide idioblasts are responsible for thwarting pollen-feeding insects such as

Derelomini and *Mystrops* beetles in *Aphandra natalia*. The same authors indicate that the presence of raphide blisters could explain (among others) the absence of egg chambers in flowers of *Ammandra* species. Moreover, exposed raphide crystals could also be related to a pollination shift from predominately beetle-pollinated species, as observed for *Syagrus* spp., to bee-pollinated, as reported for *Butia* spp. (see Henderson 1986, Silberbauer-Gottsberger 1990, Gottsberger and Silberbauer-Gottsberger 2006 for pollination reports on *Butia* and *Syagrus*). Due to the fact that raphides do not damage the alimentary canals of bees (Roubik 1989), they could play an as yet underestimated role as a pollinator reward for bees (D'Arcy et al. 1996). Further studies are needed in order to understand the reproductive significance of raphide bundles in palms and, specifically, the significance of the raphide-containing idioblasts on the petals.

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