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

PhD thesis

Anatomical and ontogenetic variations among palms (Arecaceae)

Variações anatômicas e ontogenéticas entre palmeiras (Arecaceae)

PhD student: André Silva Pinedo
Advisor: Professor PhD Sueli Maria Gomes

Brasília
August 2021

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PhD Thesis submitted to the Postgraduate Program in Botany of the Universidade de Brasília, as a requirement for obtaining the title of Doctor in Botany.

PhD student: MSc. André Silva Pinedo
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[Deus é] Aquele que é capaz de fazer infinitamente mais do que tudo o que pedimos ou pensamos, de acordo com o seu poder que atua em nós.

Efésios 3:20

Tudo que a mente do homem pode conceber e acreditar ela pode realizar.

Napoleon Hill

Agradecimentos

Ao concluir esta etapa em minha vida acadêmica, sinto-me profundamente grato pelo tanto que já consegui percorrer e o tanto que pude amadurecer ao longo de meus 31 anos... Considero uma grande vitória ter alcançado o título de doutor em meio a um período de tantas incertezas no mundo, com o COVID-19 se alastrando por vários países.

Primeiramente, sou extremamente grato a Deus, que desde o início dessa minha jornada como acadêmico foi me orientando sobre como proceder em cada momento, não me deixando desamparado e me incentivando a perseverar. Agradeço a Ele pela capacitação, conselhos, oportunidades, saúde e disposição proporcionadas ao longo de toda essa pós-graduação, principalmente nessa última etapa, em meio a pandemia.

Sou grato a minha esposa, Elizangela, por seu incentivo e apoio para que eu concluísse esta importante etapa de minha vida. Graças a ela tenho sido motivado a me tornar, a cada dia, a melhor versão de mim mesmo. Sou igualmente grato a meus sogros, Eliseu e Raimunda, e a meu cunhado, Eldo.

Minha profunda gratidão à minha família, especialmente aos meus pais, Luiz e Lúcia, sem os quais não teria sequer chegado até o início desse doutorado. Minha irmã, Carolina, bem como meu cunhado Ricardo e minhas sobrinhas Rafaela e Gabriela, também me acompanharam e incentivaram ao longo dessa jornada, por isso meu muito obrigado a eles. Meus tios, Tom, Cristina, Moacyr e Olinda, muito me ensinaram e ajudaram nesses últimos quatro anos, de modo que meus agradecimentos não estão completos sem incluí-los nessa lista. Agradeço aos meus avós, Helion, Laura, Alberto e Walkyria, todos já falecidos (os dois primeiros partiram recentemente, em meio à pandemia), mas que deixaram uma herança que vai além de riquezas materiais, um legado permanente em meu coração. Incluo também nessa seção meus primos, Mariana, Gaia, Luna, Murilo, Lílian e Raphaello, que, muito embora estejam distantes fisicamente, são considerados meus amigos próximos.

Minha gratidão à minha orientadora, Sueli, pelas ideias, sugestões e constantes críticas construtivas, que fizeram desse doutorado uma oportunidade para meu crescimento como pessoa e como profissional.

Sou grato aos membros da banca avaliadora, por terem disposto de tempo para tecer críticas construtivas a este estudo, permitindo que eu me torne um profissional qualificado.

Minha gratidão à Carolina Vasconcelos e ao Vinícius Falcão, por promoverem o bem-estar físico do meu corpo por meio de exercícios e dieta e da minha mente por meio da amizade, garantindo a disposição necessária para que essa tese pudesse ser concluída.

Tenho muita consideração também pelos pastores e por todos os meus irmãos em Cristo das igrejas Atos de Vida, Encontro Vinho Novo e Presença de Deus. Tenho todos em alta estima, pois me deram suporte espiritual ao longo de toda a minha jornada como profissional.

Agradeço aos técnicos da UnB (Thiago, Flávia, Josemília, Valéria, Daniela, Jéssika, Fábio, Ingrid), que sempre foram bastante solícitos em me dar apoio durante minha trajetória acadêmica como pós-graduando.

Faz-se necessário o reconhecimento dos professores de pós-graduação da UnB, que acompanharam meu desenvolvimento profissional, portanto meu muito obrigado a Augusto, Cristiane, Cássia, Lúcio, Júlia, Sarah, Regina, Eneida, Cristina, Luiz, Michelle e Renata Martins.

Não posso deixar de mencionar meus colegas de pós-graduação (Francivaldo, Giuliano, Laísa, Babeky, Carol, Vinícius, Felipe, Hérika) pelo apoio com palavras e sugestões de como desenvolver esta tese.

Sou grato à Fundação Capes (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Ministério da Educação do Brasil), pelo auxílio financeiro proporcionado por meio da bolsa de doutorado, bem como expresso minha gratidão à FAPDF (Fundação de Apoio à Pesquisa do Distrito Federal, Governo do Distrito Federal) e ao DPG (Decanato de Pós-Graduação) da UnB, pelo financiamento proporcionado por meio de editais de participação em eventos e publicação de artigos.

Em meio a este doutorado, foram necessárias viagens e contatos com pessoas de outros locais, por isso incluo aqui minha gratidão àqueles de outros estados e países que fui conhecendo ao longo dessa jornada e que me aconselharam para que eu pudesse realizar este meu sonho de vida. Incluo aqui Juliano (Inhotim), Rodrigo Singer (UFRS), Rosana Farias Singer (SEMA-RS), André (UFU), Jefferson (UFAM), Lázaro (UFBA), Julia Nowak (UNBC, Canada) e Larry Noblick (Montgomery, EUA).

Gratidão à Nayra e Paulo, que foram instrumentos de Deus, me auxiliando em momentos em que eu estava perdido e necessitando de orientação e apoio.

Sou agradecido à Martha Briski, minha psicóloga ao longo dos primeiros anos desse doutorado, que me fez acreditar na minha capacidade de desenvolver um trabalho de excelência.

Minha gratidão a meus colegas de infância, Pedro, Philipp, Douglas, Rodrigo e Alex, que até hoje se fazem presentes em minha vida.

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GENERAL ABSTRACT

Palms are widely distributed and well represented in Brazil, having great relevance to national economics and ecosystems. Nevertheless, the leaf anatomy and ontogeny for their Neotropical species are poorly known. In this sense, this work aimed to investigate the process of leaf development in *Euterpe oleracea* and *Mauritia flexuosa*, and we also compared populations of the latter morphoanatomically. Seed germination and seedling anatomy of *Phoenix roebelenii* were studied as well. More than fifty buds of *E. oleracea* and *M. flexuosa* were dissected in the present work. The methodology comprised the exam of external morphology, the analysis under scanning electron microscopy, and the anatomical approach with paraffin inclusion and serial sections for light microscopy. The fan leaf development of *M. flexuosa* resembles, mainly at the earliest stages, that observed for feather-leaved palms, such as the ones of *E. oleracea*. Plications first appear on the leaf primordia at different stages of development for the studied species and change their orientation along the ontogenetic process in *E. oleracea*. The leaf developmental processes in *E. oleracea* and *M. flexuosa* have some similarities with what is observed in other palm species. Strong evidence was found to sustain that palm leaves are simple along the leaf development process. It is thus recommended that adult leaf blade divisions are called 'segments' instead of 'leaflets' or 'pinnae', terminologies usually applied to eudicot compound leaves. This statement also implies in the substitution of the term 'rachis' for 'midrib'. The work contributed to the understanding of histological changes that occur during palm leaf development, being a contemporary report on both histological and micromorphological study on palm leaf ontogeny. Concerning the comparison among populations of *M. flexuosa*, Amazonian and Cerrado populations presented very similar values for epidermis and hypodermis thickness. Nevertheless, anatomically, Amazonian specimens present leaves with larger stomata, abaxial epidermal cells with more sinuous anticlinal walls, thinner mesophyll, palisade parenchyma with taller cells, vascular bundles closer to each other and parenchymatic bundle sheath more developed than in individuals from the Cerrado. Cuticle and epidermal thickness are similar on plants from both biomes, and hypodermis is normally inconspicuous. There is a clear morphoanatomical distinction among the buriti palm populations studied and these populations are also distinct taxonomically. A remote germination in *P. roebelenii* was registered, and the anatomy of the apocole draws our attention. The bundles organization within this structure resemble the pattern observed for eudicots, suggesting that this same feature evolved independently in these plants and in palms. The apocole has a very distinct anatomy from the petiole in adult leaves. In this way, we do not recommend the alternative terminology 'cotyledonary petiole' since its structure and anatomy are distinct from an adult petiole. This observation seems to be applicable to other palms with remote germination. This thesis represents an important contribution for the developmental process of simple fan- and feather-shaped leaves, for the divergence among distinct populations of the same species which occur in distinct biomes, and for the understanding of the eustelic structure of the apocole.

Keywords: leaf development; taxonomy; seedlings; *Euterpe*; *Mauritia*; *Phoenix*.

RESUMO GERAL

As palmeiras são amplamente distribuídas e bem representadas no Brasil, tendo uma grande relevância para a economia e para os ecossistemas nacionais. Entretanto, a anatomia e a ontogenia foliar ainda são pouco conhecidas para as espécies Neotropicais. Neste sentido, este trabalho visou investigar o processo de desenvolvimento foliar em *Euterpe oleracea* e *Mauritia flexuosa*, e nós também comparamos as populações desta última espécie morfoanatomicamente. A germinação da semente e a anatomia da plântula de *Phoenix roebelenii* também foram estudadas. Mais de cinquenta gemas de *E. oleracea* e *M. flexuosa* foram dissecadas no presente estudo. A metodologia compreendeu o exame da morfologia externa, a análise sob microscopia eletrônica de varredura e a abordagem anatômica com inclusão em parafina e secções seriadas para microscopia de luz. O desenvolvimento da folha em forma de leque de *M. flexuosa* lembra, especialmente nos estágios mais jovens, aquele observado para folhas em forma de pena, tais como as de *E. oleracea*. Forte evidência foi encontrada para sustentar que folhas de palmeiras são simples durante todo o processo de desenvolvimento foliar. É, portanto, recomendado que as divisões na lâmina foliar adulta sejam chamados "segmentos" em vez de "folíolos" ou "pinas", terminologias geralmente aplicadas a folhas compostas de eudicotiledôneas. Esta constatação também implica na substituição do termo "raque" por "nervura mediana". O trabalho contribuiu para o entendimento das mudanças hsitológicas que ocorrem durante o desenvolvimento foliar das palmeiras, sendo um relato contemporâneo tanto no estudo histológico quanto micromorfológico da ontogenia foliar das palmeiras. Com relação a comparação entre populações de *M. flexuosa*, as populações Amazônica e do Cerrado apresentaram valores muito similares para a espessura da epiderme e da hipoderme. Entretanto, anatomicamente, os espécimes da Amazônia apresentaram folhas com estômatos maiores, células epidérmicas abaxiais com paredes anticlinais mais sinuosas, mesofilo mais delgado, parênquima paliçádico com células mais altas, feixes vasculares mais próximos entre si e bainha parenquimática do feixe mais desenvolvida do que nos indivíduos do Cerrado. A espessura da cutícula e da epiderme são similares nas plantas de ambos os biomas, e a hipoderme é normalmente inconspícua. Há uma clara distinção morfoanatômica entre as populações de buriti estudadas, e estas populações também são distintas taxonomicamente. Uma germinação remota em *P. roebelenii* foi registrada, e a anatomia da apocole chama a nossa atenção. A organização dos feixes dentro desta estrutura lembra o padrão observado para eudicotiledôneas, sugerindo que esta mesma característica evoluiu independentemente nessas plantas e em palmeiras. A apocole tem uma anatomia muito distinta do pecíolo nas folhas adultas. Nesse sentido, nós não recomendamos a terminologia alternativa “pecíolo cotiledonar”, uma vez que sua estrutura e anatomia são distintas de um pecíolo adulto. Esta observação parece ser aplicável a outras palmeiras com germinação remota. Esta tese representa uma importante contribuição para o processo de desenvolvimento de folhas simples em forma de leque e em forma de pena, para a divergência entre populações distintas da mesma espécie que ocorrem em biomas distintos, e para o entendimento da estrutura eustélica do apocole.

Palavras-chave: desenvolvimento foliar; taxonomia; plântulas; *Euterpe*; *Mauritia*; *Phoenix*.

GENERAL INTRODUCTION

Arecaceae Bercht. & J. Presl (Arecales) is a family of monocots widely used and explored, mainly for ornamental, food, and medicinal purposes (Lorenzi *et al.* 2010, Balslev *et al.* 2016). Although Arecales is a well-supported monophyletic order (Judd *et al.* 2009), the relationship with other monocots is still unclear, and most authors place the group in the Commelinids Takht., together with Poales Small, Commelinales Mirb. ex Bercht. & J. Presl, and Zingiberales Griseb. (Graham *et al.* 2006, Barret *et al.* 2016). According to Dransfield *et al.* (2008), Arecaceae comprises five subfamilies, and their phylogenetic relationships are shown in Fig. 1.

Fig. 1. Phylogenetic relationships among palm subfamilies and tribes (Baker and Dransfield 2016).

Calamoideae Beilschm., Coryphoideae Burnett, and Arecoideae Burnett are the largest palm subfamilies, comprising 95% of the total number of palm genera. Although subfamily Calamoideae includes only 21 genera, it represents almost a quarter of all known palms due to the size of genus *Calamus* L. (Dransfield *et al.* 2008). This subfamily is easily identified by the overlapping scales that cover ovaries and fruits. Coryphoideae is a large subfamily, almost always palmate-leaved (Dransfield *et al.* 2008), and includes two main clades, the CSPT clade and the syncarpous clade (Baker and Dransfield 2016), each one with four tribes. Arecoideae, a monophyletic group, contains many popularly known palm genera, such as *Cocos* L., *Elaeis* Jacq., and *Bactris* Jacq. ex Scop. (Meerow *et al.* 2015) and is characterized by unisexual flowers normally disposed in triads (Dransfield *et al.* 2008).

The Brazilian flora of palms is very rich, and species like the macaw palm (*Acrocomia aculeata* (Jacq.) Lodd. ex Mart.), the carnauba wax palm (*Copernicia prunifera* (Mill.) H.E. Moore), the buriti (*Mauritia flexuosa* L.f.), the açaí (*Euterpe oleracea* Mart.), and the oil palm (*Elaeis guineensis* Jacq.) are widely known for their great economic importance (Lorenzi *et al.* 2010). Besides their relevance to the Brazilian flora and culture, some of these genera are representative of the variety of leaf forms observed in palms in general (Table 1). In this sense, Brazil is an outstanding region to investigate the differences and similarities among distinct palms.

Table 1. Leaf types represented in palm (Arecaceae) species of Brazil.

Leaves	**Reduplicate (Λ-shaped)**	**Induplicate (V-shaped)**
Palmate	Calamoideae: *Lepidocaryum* Mart., *Mauritia* L.f., and *Mauritiella* Burret	Coryphoideae: *Chelyocarpus* Dammer, *Copernicia* Mart. Ex Endl., *Itaya* H.E. Moore and *Trithrinax* Mart.
Pinnate	Arecoideae (all 28 Brazilian genera) Ceroxyloideae Drude: *Aphandra* Barfod, *Phytelephas* Ruiz & Pav. Calamoideae: *Raphia* P. Beauv.	None (species restricted to Africa and Southern Asia)

Leaves are highly adapted organs for photosynthesis, respiration, transpiration, and resource distribution functions, with many shape and size variations in angiosperms (Evert and Eichhorn 2013). Despite this diversity, all leaves undergo the same basic stages of development: initiation, morphogenesis, expansion, and histogenesis (Thomas 2016). Leaves are formed in the shoot apical meristem by the action of hormones, such as auxin and cytokinin (Poethig 1997).

In Chapter 1 of this thesis, I present a study concerning the micromorphological and anatomical changes that occur along the process of leaf development in the açaí palm (*Euterpe oleracea*). In chapter 2 I compare the buriti palm (*Mauritia flexuosa*) populations from two contrasting biomes (Cerrado and the Amazon), both morphologically and anatomically. Chapter 3 uses the developmental approach employed in Chapter 1, applying it to *M. flexuosa*, which has a leaf that is completely distinct from *E. oleracea*. Finally, in Chapter 4, I present the phenological changes that occur during the germination process and the seedling anatomy of *Phoenix roebelenii*.

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Chapter I

LEAF DEVELOPMENT IN *EUTERPE OLERACEA* MART. (ARECACEAE): LEAFLETS, PINNAE OR SEGMENTS?

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Submitted to AoB Plants on July 2nd, 2021

ABSTRACT

Euterpe oleracea (açaí palm) presents long leaves with divisions in the blade frequently referred to as leaflets or pinnae, as if they were compound. The purpose of this paper is to present the first leaf ontogeny study for this species. Buds of dissected açaí palms were classified according to their size and morphology and examined under scanning electron and light microscopy. The youngest leaf primordia are conical. The first plications in the leaf blade appear in primordia ca. 0.8mm in length and already have procambium. When young leaves reach 1.5mm long, these plications separate from one another. Segments change their orientation throughout leaf development. Leaf blade in *E. oleracea* is initially entire (non-plicate), becoming plicate at a later stage, and plications separate from each other as a final step. This developmental process has some similarities with what is observed in other palm species. According to early stages of the leaf ontogeny, these plants have simple feather-shaped leaves, and not compound pinnate leaves. The use of terminology such as 'leaflets' or 'pinnae' is incongruent with simple leaves and we recommend that adult leaf blade subdivisions of Arecaceae should be called 'leaf segments' instead. This work contributed to the understanding of histological changes during palm leaf development, being a contemporary report on both histological and micromorphological study on palm leaf ontogeny.

Keywords: apical meristem, simple leaves, compound leaves, ontogeny, plications.

Euterpe oleracea (açaizeiro) apresenta folhas longas com divisões na lâmina frequentemente denominadas folíolos ou pinas, como se elas fossem compostas. O propósito deste estudo é apresentar o primeiro estudo ontogenético foliar para esta espécie. Gemas de açaizeiros dissecados foram classificadas de acordo com seus tamanhos e morfologias, e analisadas sob microscopia eletrônica de varredura e microscopia ótica. Os primórdios foliares mais jovens são cônicos. As primeiras plicações na lâmina foliar aparecem em primórdios com cerca de 0,8mm de comprimento e já possuem procâmbio. Quando as folhas jovens alcançam 1,5mm de comprimento, essas plicações se separam umas das outras. Os segmentos mudam de orientação ao longo do desenvolvimento. A lâmina foliar em *E. oleracea* é inicialmente inteira (não plicada), tornando-se plicada em um estágio mais tardio e as plicações se separam umas das outras em uma etapa final. Este processo de desenvolvimento possui algumas similaridades com o que é observado para outras espécies de palmeiras. De acordo com os estágios primitivos da ontogênese foliar, estas plantas possuem folhas simples e em formato de pena, e não folhas compostas pinadas. O uso de terminologias como "folíolos" ou "pinas" é incongruente com folhas simples, e nós recomendamos que as subdivisões da lâmina foliar adulta de Arecaceae sejam chamados "segmentos foliares". Este trabalho contribuiu para o entendimento das mudanças histológicas durante o desenvolvimento da folha das palmeiras, sendo um relato contemporâneo de abordagem tanto histológica quanto micromorfológica da ontogenia foliar das palmeiras.

Palavras-chave: meristema apical, folhas simples, folhas compostas, ontogenia, plicações.

INTRODUCTION

The tribe Euterpeae (Arecoideae) is monophyletic (Pichardo-Marcano *et al.* 2019) comprising five genera which occur primarily at low altitudes in humid rainforests in Central and South America, including the Caribbean (Dransfield *et al.* 2008). *Euterpe* encompasses seven species and is easily differentiated from other palms in this tribe by its pendulous leaf segments (Henderson 1999).

Euterpe oleracea inhabits lowland and flooded forest habitats in the Amazon region, occurring from Ecuador to the Guyanas (Smith 2014). Different from *E. precatoria* and *E. edulis*, *E. oleracea* is sympodial, making extraction of palm hearts possible without killing the individual (Smith 2014). Moreover, the fruits of this species, popularly known as *açaí* in Brazil, have high energetic and nutritional content (Ribeiro *et al.* 2012) and are one of the Amazonian products that stand out in the international market (Smith 2014).

Leaves are organs formed on the shoot apical meristem by the action of hormones, such as auxin and cytokinin (Shani *et al.* 2010). An interesting question is how development differs between compound and simple leaves. Some recent works were carried out approaching these two leaf types (Efroni *et al.* 2010; Conklin *et al.* 2019), but their main focus is on the genes that determine leaf complexity, and we still lack studies about the meristematic activities that occur during leaf ontogenesis. It is important to understand the process of cell proliferation activity during leaf morphogenesis, once this mechanism may be responsible for controlling leaf complexity (Kang and Sinha 2010). A typical palm leaf primordium emerges in the shoot apical meristem with a hood-shaped apex in their earliest stages, when the stem-encircling leaf base is formed, distinguishing the primordium regionally into a distal hooded blade and a proximal tubular base (*et al.* 1982a).

Concerning the ontogeny of *Euterpe* species, researchers have focused on seeds (Panza *et al.* 2004; Neto *et al.* 2010) and fruits (Ribeiro *et al.* 2012). Studies of *Euterpe* leaves focus on its ecology (Gatti *et al.* 2011), leaf epidermis in seedlings (Ceolin *et al.* 2007), or the developmental process that affects its populations (Carvalho *et al.* 1999; Portela and Santos 2011). Barabé *et al.* (2010) analyzed the shoot apical meristem of *E. oleracea* only in the first developmental stage, when plications are still not present as the work aimed to understand the phyllotaxis. Thus, there is no research about the micromorphological and anatomical changes that occur along the process of leaf development in this species.

This research aimed to investigate the açaí palm (*E. oleracea*) leaf micromorphology and anatomy at distinct stages of development, focusing on the meristematic activity during ontogeny. Moreover, we aim to question some terminology frequently employed for palm leaves, since incongruences were detected.

MATERIALS AND METHODS

Seedlings of *E. oleracea* at about 100–150 cm in height (Fig. 1A) were purchased from nurseries. The vouchers *Pinedo 35*, *46*, *47*, *48*, *49,* and *50* were stored at the University of Brasilia Herbarium (UB, Thiers 2021) under register numbers 217308, 217309, 217310, 217311, 217312, and 217313, respectively. Shoot apices dissection was made carefully with the naked eye. The axillary buds were delicately removed from the main axis and dissected separately under a stereomicroscope. The developmental stages were isolated in distilled water based on their size and morphology.

A total of 60 dissected buds 0.2–200mm in length were employed in this work. Stages ranging in size from 0.2 to 50mm were examined under scanning electron microscopy, according to the protocol presented by Kuo (2007). Light microscopy was performed with stages between 2–200mm, according to the methodology employed by Kraus and Arduin (1997), with adaptations tested by Paiva *et al.* (2006). The detailed methodology can be found in Supporting Information 1.

RESULTS

Fully developed leaf of an *E. oleracea* seedling (Fig. 1B) contains about 8-14 vegetative units. During its development, all these segments are vertically-oriented and remain closely appressed to the midrib (Fig. 1C). A leaf cross section at this stage reveals the segments well fitted in a pattern, with all main vascular bundles aligned in the middle of the structure, making a distinct separation between leaf sides (Fig. 1D). A more detailed report on morphological and anatomical observations of *E. oleracea* leaves can be found in Supporting Information 2.

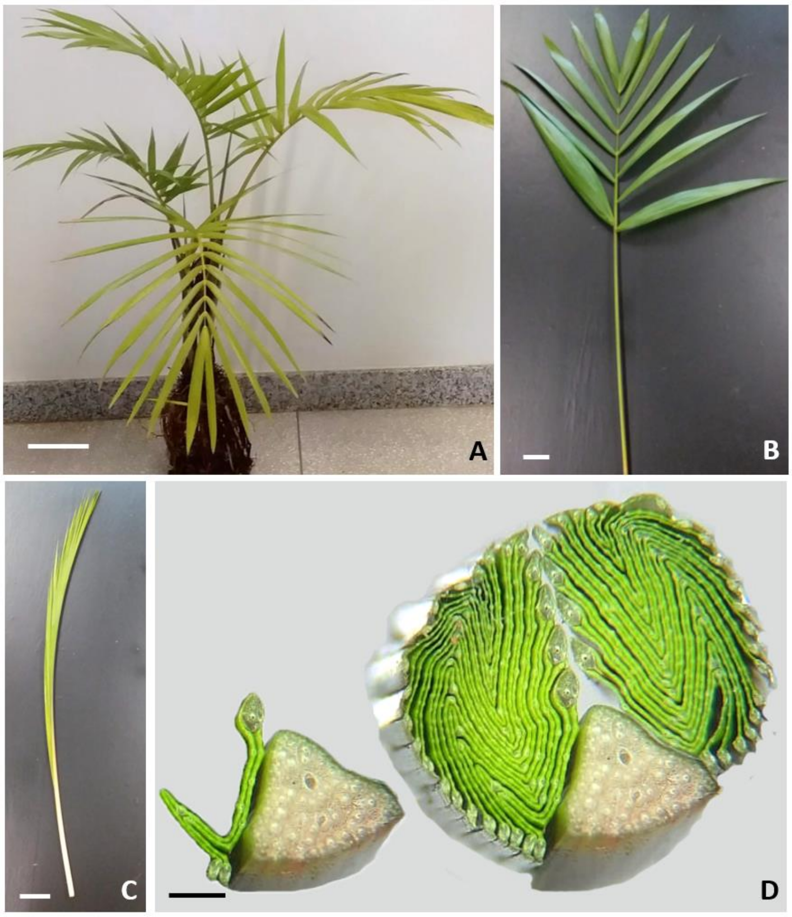

Figure 1. *Euterpe oleracea* segments organization along development. (**A**) Plant seedling. (**B**) Completely developed leaf. (**C**) Leaf expanding. (**D**) A single segment (left) and the organization of the whole group of segments (right) around the midrib. **Scale bars:** A: 10cm; B–C: 5cm; D: 1mm.

Concerning leaf ontogenesis, *E. oleracea* leaf primordium is initiated as a horn near the dome of the shoot apical meristem. The group of meristematic cells of this horn is the first visible step in the leaf ontogenesis (Fig. 2B). By intercalary growth, a tubular base of the horn develops and embraces the shoot apical meristem (Fig. 2A), forming the young leaf sheath. Until now, the leaf blade has no plications, and the apical region of the primordium is pro-meristematic (Figs. 2B, D–E).

A larger number of cells is soon added by anticlinal and periclinal divisions, and the horn grows, being more evident when buds are more than 200µm in length (Fig. 2D). The leaf blade primordium maintains its characteristic conical shape at the distal region, and a longitudinal plication develops from apex to base of this apical horn (Figs. 2C, 2E, arrow). This longitudinal region will originate the leaf midvein and both sides will form the blade wings.

The first lateral plications normally emerge when the bud is as small as 700µm, but it can vary among individuals of *E. oleracea*. These plications initially resemble a series of very slight mounds or undulations on each wing of primordium (Fig. 2F), and first appear and are more developed on the adaxial side. First plications are vertically oriented and are added basipetally, and newly added plications assume a horizontal orientation.

There are about 6–8 plications in a 1mm primordium (Fig. 3A). The developmental size distinction among young leaf wings, more evident in previous stages (Figs. 2C–E), becomes inconspicuous, while the longitudinal plication that delimits the sides deepens and becomes more evident. A smooth (non-plicate) margin strip, which divides abaxial from adaxial side plications (Fig. 3A), grows less and becomes narrower than the rest of the blade (compare Figs. 3A with 3F) and forms the ramenta in the adult leaf. Anatomically, the plicate pattern along the blade is in an advanced stage, with deep furrows and ridges (Fig. 3B).

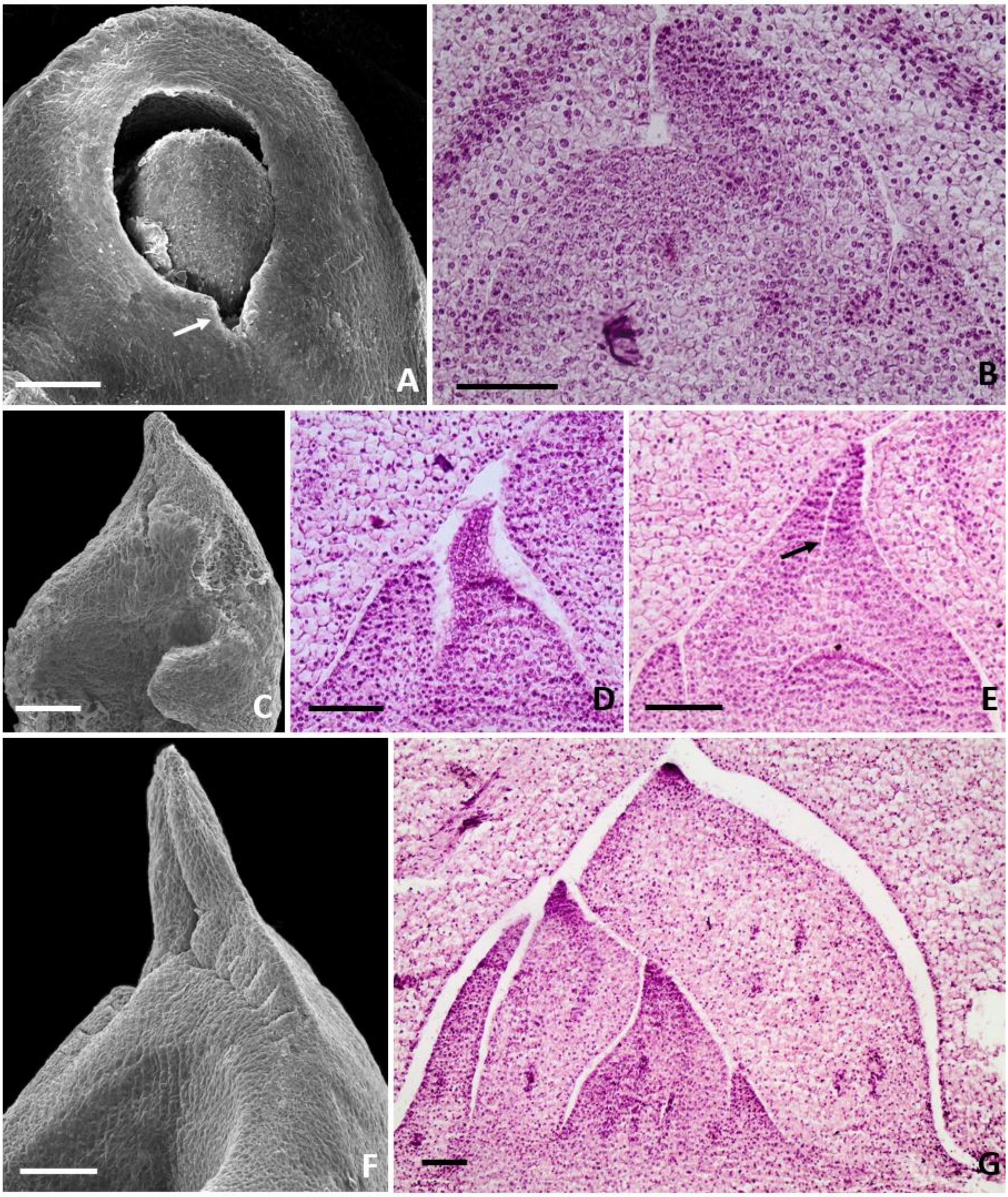

Figure 2. Leaf ontogenesis of *Euterpe oleracea* I: primordia 0.2–1.0mm long. (**A**) Shoot apical meristem with first leaf primordium emerging. (**B**) Leaf primordium inside an older leaf sheath. (**C**) Apical horn. (**D–E**) Larger and smaller sides of the primordium and the apical furrow which delimitates them (E, arrow). (**F**) Leaf segments in vertical orientation. (**G**) Smaller stages of development inside later stages. A,C,F: SEM; B,D–E,G: light microscopy**. Scale bars:** A–G: 100μm.

On a later stage, plications have already grown to 100μm width, and are more distant apart (Fig. 3C), contrasting with their closely appressed organization during the initial stages (Figs. 2F and 3A). However, as development advances, plications seem to be again more closely appressed (Figs. 3F–G). The apical horn elongates more, and plications on both adaxial and abaxial sides are completely formed at this stage (Fig. 3C). The midvein region elongates while the plications change from horizontal (Fig. 3C) to vertical position (Fig. 3G). The difference in size of the two sides virtually does not exist at this stage. Anatomically, procambium bundles are evident along the leaf blade (Fig. 3D), and they also start to form in each plication.

As leaf primordia approach 4mm, plications are more vertically oriented (Figs. 3E–G). The apical horn has less growth and maintains a reduced size in comparison with the rest of the leaf blade (Figs. 3F–G). From this point on, the young leaf is almost entirely constituted by the plicate zone of the blade on both sides of the conical midvein (Figs. 3F–H).

The anatomical sections allow us to observe again that many younger primordia are housed inside older ones (Fig. 3I). In some samples, the most basal segments are oriented horizontally, and the most apical ones are oriented vertically (Fig. 3I). Moreover, in Fig. 3I it can also be observed that most basal plications separate from one another at first, while the ones situated at the apex of the primordia are connected for a longer period. Procambium bundles are observed on the ridges of the developed plications (Fig. 3J), being distinguished by the long and narrow cells.

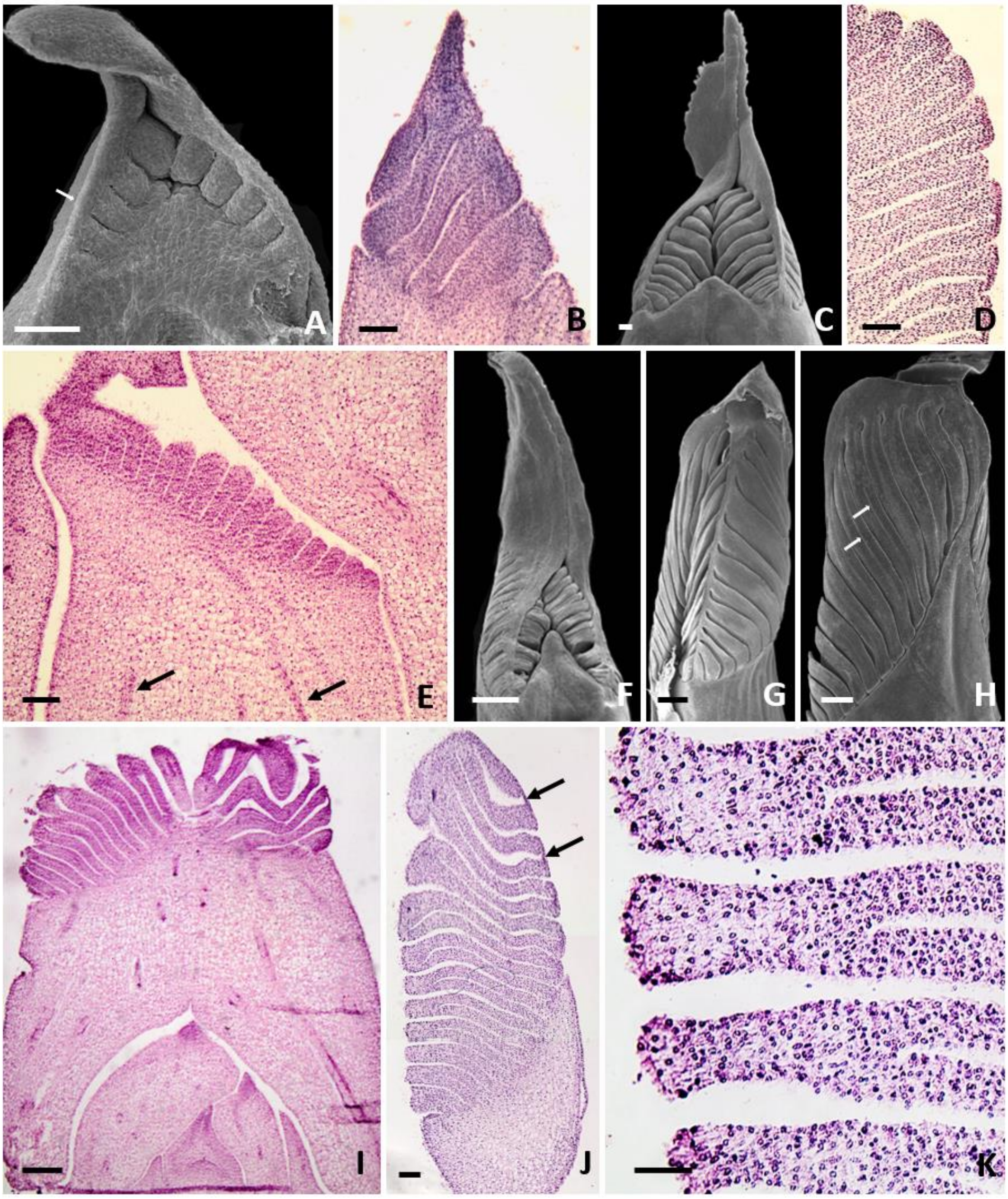

Figure 3. Leaf ontogenesis of *Euterpe oleracea* II: primordia 1.0–5.0mm long. (**A**) Horizontal plications and non-plicated marginal strip (arrow). (**B**) Plicated pattern in the blade. (**C-D**) Plications enlarge and become more distant apart. (**E**) Plications in a vertical orientation in primordium with procambium bundles (arrows). (**F–G**) Apical horn present (F) and missing (G). (**H**) Projection at the middle of plications (arrows), indicating future ripping zone. (**I–J**) Vertical and horizontal segments in the same leaf. (**K**) Procambium bundles in ridges of plications. A, C, F–H: SEM; B, D–E, I–K: light microscopy. **Scale bars:** A–E, J: 100μm; F–H: 500μm; I: 200μm; K: 50μm.

CROSS-SECTIONS

Figure 4 shows serial cross-sections of a 2mm bud, in which there is an external young leaf (Figs. 4A–E), embracing two leaf primordia (Fig. 4J), and the shoot apical meristem (Figs. 4J–K). The external young leaf has two wings of the blade united by a reduced midvein at the apex (Figs. 4A–C), while the midvein is well-developed below (Figs. 4D–E), followed by the petiole with two series of vascular regions (Figs. 4F–H), and the sheath with only one vascular series (Figs. 4I–K).

The two leaf wings are different in size, revealing more development on one wing than in the other (Figs. 4A–D), being twice larger than the other at the leaf apex (Fig. 4A, 40µm below the tip). In a lower level, the bigger ala presents three well-developed plications and the smaller one has none (Fig. 4B, 170µm below the tip). Below, there are four plications with different sizes versus two smaller plications with similar sizes (Fig. 4C, 300µm below the apex).

The midvein region is much more developed at the leaf base (Figs. 4D–E) than at the apex (Figs. 4A–C). The connection of the innermost plication to the midvein at the leaf apex is shown in Figs. 4A–C. Below, the connection of other plications to the midvein is presented (Fig. 4D, 440µm below the apex) up to the union of the most external plications to the midvein, just at the base of the leaf lamina (Fig. 4E, 580µm below the apex).

The petiole region has an adaxial depression distally and embraces the next primordium (Fig. 4F, star, 690µm below the apex). This depression is deeper in the middle of the petiole (Fig. 4G, 820µm and Fig. 4H, 940µm sections below the apex), and the sulcate petiole accommodates the youngest leaf primordia (Fig. 4G–H). Beneath, the petiole margin is very narrow (Fig. 3I, 1070µm below the apex), transitioning to the sheath region (Fig. 4J, 1220µm, Fig. 4K, 1440µm, and Fig. 4L, 1570µm below the apex). The sheath encircles the stem and has a thinner region than the other one (Figs. 4J–L).

Analyzing the vascularization from the base to the distal region, procambium bundles form a circle in the leaf sheath (Figs. 4J–K), two levels of a semicircle in the petiole (Figs. 4G–I), and a scattered pattern in the midvein (Figs. 4D–E). In the leaf margin, there is a vascular bundle from the leaf base to its apex (Figs. 4A–E), and it is more developed than those of the adaxial plications. There is no vascularization in the abaxial plications, which have fewer cell layers than the adaxial ones.

The two youngest primordia embraced by the external young leaf also exhibit one side larger than the other (Figs. 4F–I).

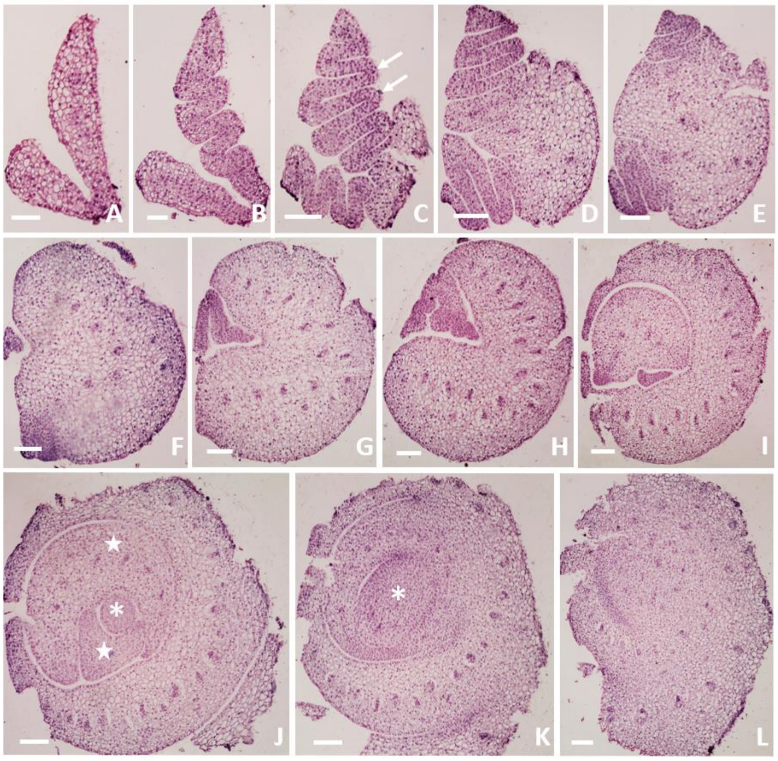

Figure 4. Leaf ontogenesis of *Euterpe oleracea* III: primordium 2.0mm long. Cross sections at distinct levels below the apical tip: (**A**) 40µm, (**B**) 170µm, (**C**) 300µm, (**D**) 440µm, (**E**) 580µm, (**F**) 690µm, (**G**) 820µm, (**H**) 940µm, (**I**) 1070µm, (**J**) 1220µm, (**K**) 1440µm and (**L**) 1570µm. Arrows: plications; stars: leaf primordia; asterisk: shoot apical meristem. **Scale bars:** A–B: 50µm; C–L: 100µm.

DISCUSSION

Here we report for the first time the leaf development process in *E. oleracea*. Previous studies on leaf ontogenesis in palm species with pinnate leaves (Table 1) give support to our understanding of the events observed here.

More than two decades separate the most recent studies on leaf ontogeny (Nowak *et al.* 2007, 2008) from the older ones (Table 1). The present work comprises a contemporary and detailed report of a palm leaf ontogenesis and combines both micromorphological and histological illustration of the events.

Table 1. Studies that approach the ontogeny of pinnate palm leaves.

Species	**References**
Chamaedorea elegans	Nowak *et al.* (2007)
Chamaedorea seifrizii	Kaplan *et al.* (1982a) Nowak *et al.* (2008)
Chrysalidocarpus lutescens (now *Dypsis lutescens*)	Kaplan *et al.* (1982b) Dengler *et al.* (1982)
Cocos nucifera	Venkatanarayana (1957)
Elaeis guineensis	Yampolsky (1922)
Jubaeopsis caffra	Robertson (1983)
Phoenix dactylifera	Naumann (1887) Deinega (1898)
Phoenix reclinata	Goebel (1884)
Phoenix sylvestris	Periasamy (1962) Padmanabhan (1967)
Roystonea	Eames (1953)

Palms have complete amplexicaule leaves, and the origin of the distal lamina and tubular sheath is well documented. The leaf primordium emerges in the shoot apical meristem with a hood-shaped apex in their earliest stages, when the stem-encircling leaf base is formed, distinguishing the primordium regionally into a distal hooded lamina and a proximal tubular base (Kaplan *et al.* 1982a; Figs. 2A–B, F). Here, a split was observed marking the end of the tubular sheath and the beginning of the petiole (Fig. 2A, arrow).

The bud is organized with several stages of development within a larger structure. An asymmetry was observed between both sides of the primordium mainly on the first stages of the palm leaf ontogeny, being called 'cathodic' and 'anodic' sides by some authors (Kaplan *et al.* 1982a; Nowak *et al.* 2007). This asymmetry is also well documented in the present work (Fig. 3C). According to Kaplan *et al.* (1982a), since the smallest side of the leaf is located within the leaf base of an older stage, it is subjected to compression, and it is reasonable to consider that many processes that occur during leaf development may be due to a reduced space.

In the earliest stages of development, all cells present about the same size, but cells of the apical dome are the smallest of all and are present in greater number than the others (Figs. 2B, 2D). According to Periasamy (1965), primordia first grow by perpetuative growth (growth due to an increase in the number of cells of nearly the same volume), but a differentiative growth (growth due to an increase in the volume of units of constant number) takes place in their regions that will not form plications. These processes seem to be occurring in the early stages of *E. oleracea* leaf development (Figs. 2D, 2E).

The differences between the two leaf sides could result in asymmetrical leaves. This asymmetry is observable mainly on the first stages of leaf development in *Chamaedorea* and *Dypsis* (Kaplan *et al.* 1982a, 1982b). However, such asymmetry is not so evident when we analyze cross-sections of *Syagrus inajai* embryos (Genovese-Marcomini *et al.* 2014). In our observations, cross-sections through the primordia also revealed a clear asymmetry between the sides of the young leaf of *E. oleracea* (Fig. 4).

The presence of procambium on a still non-plicated primordium was observed in the classical works of Kaplan *et al.* (1982a, 1982b) and Dengler *et al.* (1982). Indeed, these authors state that there is a correlation among plication formation and procambium emergence since high cell proliferation within the original procambial strand causes provascular tissue to increase in coordination with the uprise of the adaxial ridge. A later vascular differentiation is present in *E. oleracea*, since procambium appears in the primordium from about 2mm, when virtually all plications are well developed, a result that matches with the studies of four genera by Periasamy (1966).

According to Nowak *et al.* (2008), segments in *Chamaedorea* are initially disposed in a vertical orientation, then they become oriented horizontally and assume again a vertical disposition in later stages of development, as we observed for *E. oleracea* in the present study (Figs. 3A, 3C, 3G). This change in plication orientation seems to be related to the amount of space available for the primordium, but it may also have a genetic influence. It was curious to observe some segments oriented horizontally and others vertically on the same primordium (Fig. 3I), indicating that leaf primordium sides do not change their orientation simultaneously, presenting a delay among them.

The lines observed along the segments of primordia about 4mm long (Figs. 3F, 3G) represent the first step of the stage of individual segments separation. This process is not exactly an abscission; it is rather an abscission-like mechanism, since abscission requires that a given plant part becomes detached (Nowak *et al.* 2007). In reduplicate palms, a zone of schizogeny develops between major vascular bundles and involves epidermal and ground tissues only (Gunawardena and Dengler 2006), and this statement also seems to be true for *E. oleracea* (Fig. 3G, arrows).

The mechanism of leaf folding is not exclusive of palms. Other groups of monocots, such as Cyclanthaceae, also present plicated leaves in their ontogenetic process (Wilder 1976). However, despite their resemblance, Cyclanthaceae leaves evolved independently from palm leaves, since there are significant differences among them, such as initiation site, sheath and petiole morphology, number of costae, and direction of newly formed plications (Wilder 1976). Since Arecaceae and Cyclanthaceae are not closely related phylogenetically, this feature seems to have evolved independently in the two groups.

The very tip of primordia, which is formed at the initial stage of plant leaf development (Melo-de-Pinna and Cruz 2020), forms two longitudinal projections. Plications arise only below these projections, in the part that will constitute the adult leaf lamina, so leaves in palms are simple and dissected, and not compound, just as pointed by De Candolle (1827) – possibly the first to make the observation that palms have simple leaves.

In this sense, it is necessary to review the terminology to describe palm leaves in respect to their segmentation. For instance, Dransfield *et al.* (2008) consider 'segments' the palm leaf units in palmatisect leaves, and 'pinnae' or 'leaflets' in the pinnatisect ones. Nevertheless, since palm leaves are simple, the terms employed for pinnate leaves are questionable once they only apply for plants with compound leaves.

In some studies, the individual subunit of a mature palm leaf is sometimes referred to as 'pinna' (*e. g.* Defaveri *et al.* 2015; Simozrag *et al.* 2016; Salem and Ali 2020) or 'leaflet' (*e. g.* Kaplan *et al.* 1982a; Nowak *et al.* 2009; Noblick 2013; Vianna *et al.* 2017), while some works even employ both terms apparently as synonyms (Dransfield 1986; Kaplan 2001; Horn *et al.* 2009). Although Glassman (1972) uses only the term 'pinna' in his work, his successor Noblick (2017) uses both terminologies, with the term ‘leaflet’ most frequently employed throughout his paper. Nevertheless, since both terms are used for eudicot compound leaves (Harris and Harris 1994; Vidal and Vidal 2000), we consider they are inadequate to be employed for the subunits of the palm leaves.

According to Bell and Bryan (2008), leaflets of a pinnate-leaved plant develop from an isolated patch of marginal meristem, and hence each unit will be organized in a similar manner to a whole simple leaf. The authors also state that each leaflet has at its base a small petiole, named petiolule. This is clearly not the case for palms with simple pinnatisect leaves.

On the other hand, for palms with palmate leaves, the terminology ‘segment’ is commonly used by Dransfield *et al.* (2008) to designate the blade subunits. ‘Segment’ is employed in Plant Sciences with a much wider meaning, to refer to the sections or divisions of a plant organ (Harris and Harris 1994). Thus, we propose that the term segment be used to name all units of palm leaves – regardless of being pinnatisect or palmatisect. In a previous work, we already used the terminology ‘segment’ instead of ‘pinna’ and ‘leaflet’ precisely thinking about the consistency of this hypothesis (Pinedo *et al.* 2016).

The term ‘rachis’ is also inadequate since it is employed to designate the midvein of compound leaves (Font Quer 1953). We propose to renounce the term ‘rachis’ and standardize the terminology ‘midrib’ for palm leaves, as is seen for the simple leaves of other plants. This can also be applied to other plants with simple sectioned leaves, such as Cyclanthaceae.

The monitoring of the distinct stages of development of the açaí palm revealed many similarities with previous works for pinnate palms, highlighting that the ontogeny of palm leaves tends to follow a general pattern, with some few peculiarities for each species. The review of terminologies proposed in the present study, despite being used for a long time by researchers, allows an appropriate description of the palm leaves. Moreover, *E. oleracea* ontogeny on later stages of development is still unknown, and the process of segment separation needs to be analyzed.

ACKNOWLEDGEMENTS

The authors acknowledge Capes for the doctoral grant for the first author, and the Microscopy and Microanalyses Laboratory of Universidade de Brasília for support in preparing the SEM images.

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SUPPORTING INFORMATION

1. SEM ANALYSES AND HISTOLOGICAL STUDIES DETAILED METHODOLOGY

For SEM analyses, samples were fixed in Karnovsky solution at 4°C for at least 1h (Kuo 2007), and then sodium phosphate buffer 0.1M (pH 6.8) ratio 1:1 (v:v) was added. The material stayed for 24–72h in this solution (Kuo 2007), and was washed three times in the same buffer solution. Leaf primordia were post-fixed in 2% osmium tetroxide for 2h and washed three more times in phosphate buffer. Samples were dehydrated in an ethanol series (10, 20, 30, 40, 50, 60, 70, 80, 90, and 100%) for at least 20min in each solution, twice in each concentration higher than 60%. The primordia were dried in the critical point using liquid CO_2 (Kuo 2007) and assembled on copper stubs using black double-sided tape. The metallization was performed with 8nm thick of gold. Images were taken in the scanning electron microscope at the Microscopy and Microanalysis Laboratory of the Universidade de Brasilia, Brazil.

For the histological studies, samples were fixed at room temperature (ca. 24°C) in FAA50 (Johansen 1940) for at least 12h. The primordia were dehydrated in an ethanol gradient (50, 60, 70, 80, 90, and 100%). The larger structures were soaked longer in each concentration up to 4h. Thereafter, samples passed through a series of ethanol butyl acetate solutions (25, 50, 75, and 100%) for at least 1h in each concentration.

The specimens were infiltrated with melted paraffin 3:1 butyl acetate and were maintained overnight at 60°C for 24h. The samples were replaced in pure paraffin for more two times. Paraffin blocks were mounted, positioning the primordia in a such way that plications appear in the sections, with the aid of a stereomicroscope.

Serial sections were obtained on a rotary microtome according to the size of the sample: 2.0–3.0mm, 10µm thickness; more than 3.0mm, 15µm thickness. Longitudinal sections were made with buds of 2.0–20mm and cross sections in a 2mm primordium.

The sections were stained with safranin and alcian blue, according to Kraus and Arduin (1997). Colourless vitral varnish sealed up the slides with coverslips (Paiva *et al.* 2006). Observations were made and images were captured with the program LAS ES on a photomicroscope Olympus associated to the computer.

2. OBSERVATIONS UNDER STEREOMICROSCOPE AND NAKED EYE

First leaves of *E. oleracea* are bifid (Fig. S1A). When the plant reaches about 0,7m, two new segments are added basally to the bifid vegetative structure (Fig. S1B). As the leaf develops, more segments are added (Fig. S1C-F). The two segments that are located on the leaf tip of different stages of açaí palm resemble the bifid structure from its earliest stages (compare Fig. S1A with smaller boxes in Figs. S1E-F).

First primordia develop from the shoot apical meristem, illustrated in Fig. S2A. These primordia soon develop an apical horn (Fig. S2B). When this structure is developed, plications are already visible on the adaxial side of the outer primordium. Its apex is thin and very tender, and plications apparently do not occur at this apical region. A posterior stage reveals that little changes happened, being the most noticeable an increase in the number of plications (Fig. S2C).

Açaí leaf primordia with 3,0 mm long are also similar to the previous stages (Fig. S2D). A slight change on the orientation of plications occurs. Leaves with 4.0 mm long present some distinct characteristics (Figs. S2E–F). The apical horn elongates more, resembling a string, that may have the same size of the primordium. In some individuals, this elongation is subtler (Fig. S2G).

As well as on earlier stages of development, plications do not occur on this apical part (Figs. S2G–I). Plications on both adaxial and abaxial sides are completely formed in a 5.0 mm long primordium, and the difference between the sides of the leaf is no more present (Fig. S2G). In a posterior stage, all plications are horizontally oriented (Fig. S2H). The primordium presents two bands of well-developed plications (Fig. S2H), and from this point the young leaf grows more in height, generally assuming a more uniform shape (Fig. S2I).

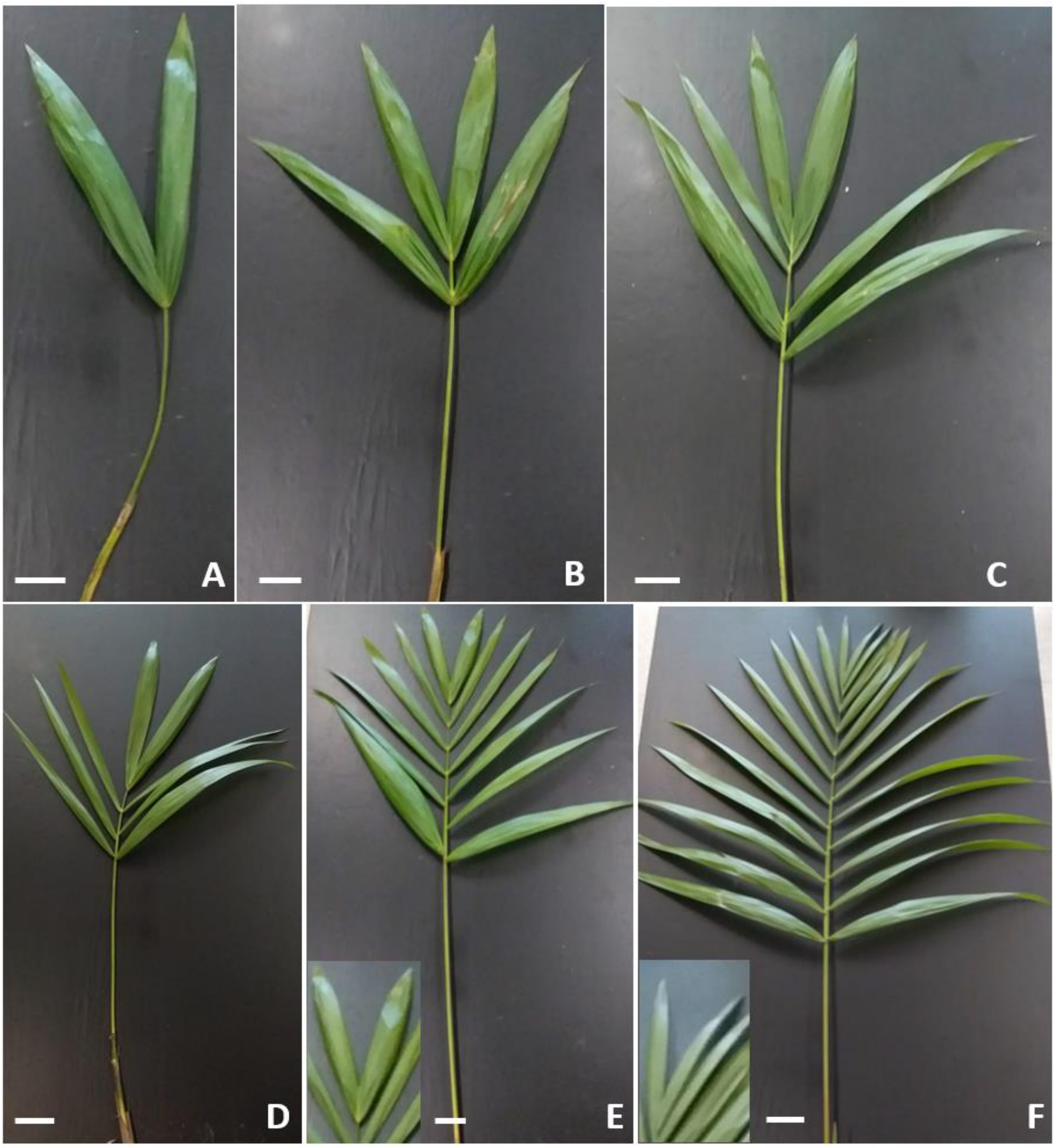

Figure S1. Morphological observations concerning plant development in *Euterpe oleracea*. (**A**) Bifid leaf. (**B**) Two new segments added basally to the bifid leaf. (**C–F**) More segments added along with plant development. The two segments located on the leaf tip of later stages resemble the bifid structure from its earliest stages (compare A with smaller boxes in E-F). **Scale bars:** A–F: 5,0cm.

Apparently, when the leaf reaches 5,0 cm long, a petiole is already formed (Fig. S2J). However, a closer look reveals that this structure is only constituted by plications (Fig. S2K). In this sense, new plications were added in relation to the previous stage and the plicate portion elongated more, representing more than 85% of the total size of the young vegetative organ (Fig. S2K). The apical flat portion (Fig. S2J) resembles the apical horn observed on earliest stages (compare with Figs. S2B–C), since it is also marked by the absence of plications. At this stage, plications are not anymore horizontally oriented, but start to assume again a vertical orientation. It seems that compression caused by lack of space may change the orientation of plications. As development occurs, the short portion of the petiole elongates, and the tip portion of the leaf assumes a greenish color, when it is about 12,0 cm long. Leaves with more than 15,0 cm are morphologically similar to those with 5,0 cm, with a flat portion on the top (compare Figs. S2J and S2L). They have, however, an important distinction, since the basal part of a 15,0 cm leaf is mostly constituted by petiole, and not by plications anymore. By the moment when leaves start to emerge, they have a green color at least in half of its size (Fig. S2M). Plications are still compressed in the vegetative organ, and they start to open only when the whole leaf is already exposed to the environment (Fig. S2N). When this occurs, all young segments are oriented vertically, parallel to the midrib, but they soon are reoriented horizontally and perpendicular to it.

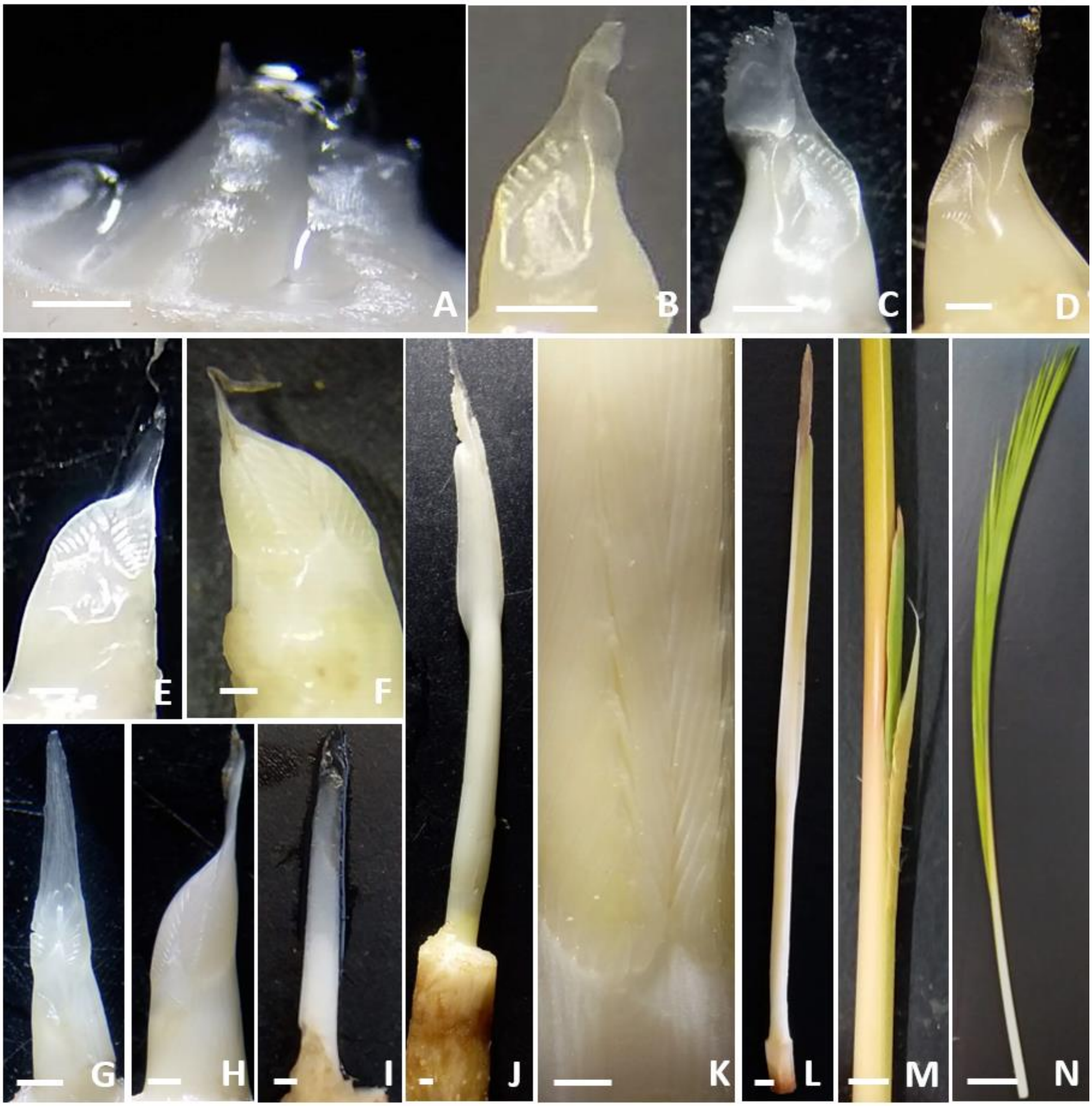

Figure S2. *Euterpe oleracea* morphogenesis under a stereomicroscope. (**A**) Shoot apical meristem. (**B**) Leaf primordium with apical horn and plications. (**C**) The number of plications increases. (**D**) A slight change in the orientation of plications. (**E–F**) Apical horn elongates and resembles a string in some individuals. (**G**) Difference between leaf sides no more evident. (**H**) Plications horizontally oriented. (**I**) Young leaves with a more uniform shape. (**J–K**) Petiole apparently being formed, but the structure is constituted by vertical-oriented plications. (**L**) Real petiole being developed. (**M**) Leaf acquiring the typical green color. (**N**) Plications starting to open. **Scale bars:** A–F: 500μm; G–K: 1mm; L: 5mm; M: 1cm; N: 5cm.

3. HISTORICAL BACKGROUND OF PALM LEAF DEVELOPMENT

Since the beginning of the 19th century, palm leaves have raised interest of researchers. De Candolle (1827) was one of the first scientists to postulate that the blade in Arecaceae is entire, assuming a dissected form during the developmental stages. Nonetheless he admitted being hard to explain the mechanism by which a simple leaf changes into a fan or feather-shaped blade. In his work, de Candolle also proposed a phyllode theory, according to which the unifacial leaves of monocots leaves are a result of the loss of the distal lamina and a compensatory expansion of the petiole observed in eudicots. Von Mohl (1845) and Trécul (1853) were the first ones to analyse the structure of a dissected palm leaf in different stages of development. Later, Hofmeister (1868) pointed that segments on palm leaves are formed because the meristem is firmly compressed inside the sheath of the older leaf, which causes its folding. These pioneer works raised an important question, which remained unanswered for more than a century: are segments in palm leaves formed by a meristem folding or are they the result of the leaf lamina splitting? Unfortunately, the works performed until then did not present concrete evidence about this.

The next two works (Goebel 1884, Eichler 1885) supported the theory of meristem folding, but they also did not provide strong evidence to support this view. Moreover, the samples studied by Eichler were from later stages of development, when the foldings were already completely formed in the leaf meristem.

At the end of the 19th century, two relevant papers were published concerning palm leaf development. Based on the observations performed for 12 palm species, Naumann (1887) concluded that, taken separately, neither meristem folding neither tissue separation could explain the plications on palm leaves, being their final shape a combination of these two processes. However, Kaplan *et al.* (1982a) pointed that despite the significance of Naumann's work, his cross sections were obtained from this single plan, and not from the plane of the plications themselves. Deinega (1898) was aware of this failure, and the leaf sections of the nine palm species studied by him were performed in the plane of the plications. His conclusions favored the meristem folding hypothesis, and unlike Naumann, he argued that the lack of space imposed for the young leaf to develop is relevant to understand leaf morphogenesis. Nevertheless, Deinega could not prove that the palm leaf shape is a result of small space inside the older leaf sheath. Moreover, there were large gaps among the stages of leaf development studied by him. Even so, these two authors provided substantial data concerning palm leaf ontogeny, and their results were not questioned for almost a century.

In the first half of 20th century, little was added to the understanding of leaf development in palms. Hirmer (1919) discussed more about the previous authors controversial points related to this issue than presented his own conclusions, being his article considered as a review paper. Arber (1918) reinforced the phyllode theory of de Candolle and raised interest to the development of palm leaves. However, although she had performed cross sections in the leaves of different palm genera in a posterior work (Arber 1922), she did not analyze different stages of development, and hence her results concerning leaf development are questionable. An ontogenetic study concerning *Elaeis guineensis* (Yampolsky 1922) repeated the same mistake of not sectioning the primordia in the plane of the plications previously made by Naumann (1887), wrongly characterizing young leaves as sac-like structures. Goebel (1926) criticized Yampolsky affirming that his results presented nothing new. However, although Goebel supports the meristem folding process, his work's images may be interpreted as a result of tissue splitting. Consequently, the discussion about the origin of plications originally raised more than fifty years before remained unanswered. In the 1940's, an interesting work compared palm meristems of different genera (Ball 1941), but its considerations concerning the origin of plications in palms were few.

As well as Yampolsky (1922), Eames (1953) also agreed with the questionable results of Naumann. With few images and without any strong evidence to support his hypothesis, Eames work counted as one more that did not answer the original question. A later study (Venkatanarayana 1957) supported Eames findings, but since only older stages of development were analyzed, none was added concerning the origin of plications in the younger leaf primordia.

Studying palm seedlings, Tomlinson (1960) elucidated that the process of leaf dissection occurs in two independent stages. In the first one, called plication formation, corrugations are formed in the leaf lamina and an abscission-like process occurs along these folds, and in the second one, named individual leaflets separation, the outer edges of the leaf are separated. Later, a series of deeper studies were conducted by Periasamy (1962, 1965, 1966a, 1966b, 1967, 1977), Padmanabhan (1963, 1967a, 1967b, 1967c, 1969a, 1969b) and Padmanabhan and Veerasamy (1973, 1974). These papers were conducted with more palm species and aimed specifically to better understand the origin of plications. However, while Periasamy (1962) concluded that there is no evidence of tissue splitting in palms, Padmanabhan (1969a) showed that tissue splitting is the responsible for the corrugate shape of a palm leaf. Unfortunately, both researchers had their limitations: Periasamy's images apparently support tissue splitting instead of meristem folding, and careful should be taken when one analyses Padmanabhan's images, as they seem to have many artifacts.

The whole process of leaf development in *Carludovica palmata* (Cyclanthaceae) was elucidated by Wilder (1976). Since the leaves of Cyclanthaceae resemble palm leaves, some aspects of this work may be applied to palms. An important contribution was the observation that the origin of plications in *Carludovica* is associated with the procambium development, which also seems to occur in palm leaves (Dengler *et al.* 1982). Also at this time, other works concerning the ontogeny of palm leaves were published. Bugnon (1980) studied the ontogenetic process in *Washingtonia filifera* Wendl., while Robertson (1983) focused on *Jubaeopsis caffra*.

Nevertheless, one of the most important contributions to the developmental process in monocots, particularly in palms, came from Kaplan. This scientist started his career objecting the phyllode theory proposed by de Candolle (Kaplan 1970). According to his observations in *Acorus calamus*, the unifacial leaves of monocots are homologous to the bifacial leaves of eudicots, and not merely a modified petiole (Kaplan 1970, 1973).

Raising interest about how palm leaves develop, Kaplan and his team deeply investigated this issue both in palmate and pinnate leaves. Although *Phoenix* seeds germinate faster and easier than other palm seeds, they did not use this genus in the analysis, since it presents many peculiarities that are not observed in other palm seedlings, and these particularities were possibly the reason why so many controversies occurred (Kaplan *et al.* 1982a). In a series of well performed papers, Kaplan and his group were able to solve the long-lasting question whether plications are originated through meristem folding or tissue splitting (Kaplan *et al.* 1982a, 1982b; Dengler *et al.* 1982). Working with *Chrysalidocarpus* (*Dypsis* nowadays; pinnate reduplicate) and *Rhapis* (palmate induplicate), they found no evidence of splitting were observed during their devoted analysis in TEM and SEM (Dengler *et al.* 1982, Kaplan 1983, Kaplan 1984). Hence, the meristem folding model was favoured, being to this day the most accepted theory to explain the plications origin in palms. Indeed, Kaplan excelled not only as a developmental researcher, but he also was able to explain in a simple form the complex process of leaf ontogeny, leaving a legacy that lasts until today (Demason and Hirsch 2006).

After Kaplan's robust work, most of the studies concerning leaf development focused on other groups of monocots, such as Periasamy and Muruganathan (1986), who analyzed this process in the genus *Arisaema* (Araceae). The mode of leaf development of monocots was compared by Bharathan (1996), who gathered many papers in this theme and presented their conclusions. However, very little attention is given to palms, that are only mentioned in his study. Actually, from the 90's onwards a molecular approach was added to the works of plant leaf development.

A team directed by Gunawardena was interested in the process of programmed cell death in monocots. Although she started studying this process in roots of maize (Gunawardena *et al.* 2001), she was soon attracted to understand this process in the intriguing leaf shapes of Aponogetonaceae (Gunawardena *et al.* 2004) and Araceae (Gunawardena *et al.* 2005). In a comprehensive review, she described the three distinct modes of leaf dissection in monocots, mainly observed in the orders Dioscoreales, Pandanales, Alismatales and Arecales (Gunawardena and Dengler 2006). Nevertheless, this review showed that the model of leaf dissection in palms is unique among the monocots since they don't undergo programmed cell death neither perforation formation.

Given that species from the genus *Chamaedorea* have both bifid and pinnate leaves, they draw the attention of Nowak et al. (2007). This group of researchers argue that, although the process of plication formation in palms had already been elucidated by Kaplan and his team (1982a, 1982b), little is known concerning the process of individual leaflets separation in this group of plants. Analysing the different developmental stages of *Chamaedorea elegans*, they noted the formation of an abscission zone in the young leaves. Studying how this process of abscission occurs, they concluded that what happens in *Chamaedorea* – and possibly in other palms – is an abscission-like process, and not properly an abscission, "since nothing falls from the plant in the process". This same process was then studied in *Chamaedorea seifrizii* (Nowak *et al.* 2008), and later three species of *Chamaedorea* with different leaf types were compared concerning their development (Nowak *et al.* 2009). A couple of years later, Nowak *et al.* (2011) found that leaf development in *Chamaedorea elegans* Mart. is non-dependable from *KNOX* genes, complementing the work of Jouannic *et al.* (2007).

4. DETAILED INFORMATION ON *EUTERPE* AND *EUTERPE OLERACEA*

EUTERPE MART.

Name origin: *Euterpe* is a reference to a Greek goddess of the song and poetry, but also considered a goddess of the waters.

Plant description: Monoecious, unarmed, solitary or clustered palms, small to medium sized. Stems erect or bent, medium to large, usually obscurely ringed and slender with an enlarged base. Leaves pinnate reduplicate, 5-18 in number, dead leaves falling immediately from stem; sheath elongate and tubular, forming a green to bright orange crownshaft; petiole short or absent, sulcate adaxially and rounded abaxially; midrib flattened adaxially and rounded abaxially; segments pendulous, long, and numerous, disposed on a single plane along the midrib, with an evident midrib. Inflorescence axillary, infrafoliar at anthesis; peduncle short, covered with indumenta; prophyll tubular, elongate and chartaceous; peduncular bract longer than prophyll, tubular and chartaceous; midrib longer than peduncle, normally densely covered with hairs; rachillae also covered with indumenta, subtending a triad of unisexual flowers. Staminate flowers elongate, with 3 distinct sepals and 3 distinct petals; 6 stamens with short filaments. Pistillate flowers ovoid, with 3 distinct and imbricate sepals and 3 distinct and imbricate petals; gynoecium unilocular, with 3 stigmas. Fruit globose or sub-globose, rarely ellipsoid, single-seeded, purple-black colored (Henderson *et al.* 1995, Dransfield *et al.* 2008, Lorenzi *et al.* 2010).

Distribution: Central America, Caribbean, Trinidad, Lesser Antilles, and South America (Venezuela, Colombia, Ecuador, Peru, Bolivia, Brazil, Suriname, and the Guianas).

Habitat: Lowland rainforests and montane forests and swamps, often along rivers, but sometimes at higher elevations.

Relationships: Monophyletic genus (Henderson 1999), considered as sister to a clade of *Neonicholsonia*, *Oenocarpus* and *Prestoea* with moderate support (Asmussen *et al.* 2006), or as sister to all Euterpeae (Henderson 1999).

Uses: The genus is one of the most important concerning palm hearts, which are marketed in many countries. Some species produce fruits explored to produce reserves and beverages. Even inflorescences, when unopened, can be turned into pickles. Due to its pendulous segments, the genus is also used for ornamental purposes.

EUTERPE OLERACEA MART.

Common names: Açaí, açaí branco, açaizeiro, jussara, juçara (Brazil), ka-be-re (Apinajé); chapil, maquenque, murrapo, naidí, palmicha (Colombia); bambil, palmiche (Ecuador); pinot (French Guiana); baboenpina, kiskis pina, manaka, pina, prasara, wapoe, wapu, wasei (Suriname); manac (Trinidad).

Plant description: Palms clustered, with up to 25 stems per clump. Stems 3-20 m tall, 7-18 cm diameter, grayish, rounded, and smooth, covered with scars of senescent leaves. Leaves 2-3 m tall, pinnate, arched, spirally arranged, 8-14 per plant; sheath 60-150 cm; petiole shorter than sheath (about 50 cm), covered with scales; midrib up to 3,5 m, with scales similar to the petiole; segments 20-50 cm long, 40-80 per leaf, acuminate, pendulous or rarely slightly horizontal (especially in young individuals), regularly spaced, organized in the same plane, with midrib adaxially prominent and abaxially with few ramenta. Inflorescences infrafoliar, horizontally disposed, quickly developing after leaf abscission; peduncle 5-15 cm long; prophyll nearly 50 cm long; peduncular bract 70-100 cm long; midrib 35-70 cm long, densely covered with whitish-brown hairs; rachillae 80-160 per midrib; flowers in triads, pistillate flowers surrounded by two staminate flowers, except on the upper part of the rachillae, which usually has staminate flowers only; staminate and pistillate flowers about 0,3 cm long. Fruits 1,5 cm, globose drupes, violet to deep purple colored, containing a single and large seed (0,7-1 cm) (Henderson *et al.* 1995, Lorenzi *et al.* 2010).

Distribution: Pacific coast of Colombia, Ecuador, coastal regions of Venezuela, Trinidad, the Guianas, Suriname and north of Brazil (Amapá, Maranhão, Pará and Tocantins).

Habitat: Large stands of high density in low-lying, often near the sea in tidal areas, but also in wet places near rivers far from the sea.

Uses: The palm heart is eaten by local people or is canned to be exported. Macerated fruits are an important component of the diet of people in Belém, being used as an accompaniment to the meal together with fish, meat, or prawns, and have been more recently used to produce an 'ice cream', popular in Brazil. It is widely used as an ornamental tree due to its graceful stems and elegant pendulous segments.

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Chapter II

BURITI (*MAURITIA FLEXUOSA* L.F., ARECACEAE) ACROSS TWO BRAZILIAN BIOMES: WHAT DO WE LEARN FROM A MORPHOANATOMICAL COMPARISON?

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Text formatted according to International Journal of Plant Sciences guidelines

The buriti palm (*Mauritia flexuosa*, Arecaceae) is a key species of two Brazilian ecosystems, maintaining biodiversity in humid areas. Bearing in mind that buriti palm populations are genetically distinct, it was questioned if leaf anatomy may help to distinguish them. This study aimed to compare leaf morphology and anatomy of populations from the Amazon and Cerrado biomes to investigate if their differences granted taxonomic recognition. As well as leaf material, literature descriptions were also scrutinized. Morphologically, buritis from Cerrado are smaller, probably as a response to the higher levels of aluminum in the soil, making it more acidic and less favorable to the species. Anatomically, Amazonian specimens present leaves with larger stomata, abaxial epidermal cells with more sinuous anticlinal walls, thinner mesophyll, palisade parenchyma with taller cells, vascular bundles closer to each other and parenchymatic bundle sheath more developed than in individuals from the Cerrado. Cuticle and epidermal thickness are similar on plants from both biomes, and hypodermis is normally inconspicuous. There is a clear morphoanatomical distinction among the buriti palm populations studied and it may be possible to distinguish them taxonomically.

Keywords: seasonality; speciation; morphological variation; taxonomy.

O buritizeiro (*Mauritia flexuosa*, Arecaceae) é uma espécie chave para os ecossistemas brasileiros, mantendo a biodiversidade em áreas úmidas. Sabendo que populações de buriti são geneticamente distintas, foi questionado se a anatomia foliar pode ajudar a diferenciá-las. Este estudo visou comparar morfológica e anatomicamente folhas de populações dos biomas da Amazônia e do Cerrado para investigar se suas diferenças outorgam reconhecimento taxonômico. Assim como material foliar, descrições da literatura também foram examinadas. Morfologicamente, os buritis do Cerrado são menores, provavelmente como uma resposta aos mais elevados níveis de alumínio no solo, tornando-o mais ácido e menos favorável à espécie. Anatomicamente, espécimes da Amazônia apresentam folhas com estômatos maiores, células epidérmicas abaxiais com paredes anticlinais mais sinuosas, mesofilo mais delgado, parênquima paliçádico com células mais altas, feixes vasculares mais próximos entre si e bainha parenquimática dos feixes mais desenvolvida que nos indivíduos do Cerrado. As espessuras da cutícula e da epiderme são semelhantes nas plantas de ambos os biomas, e a hipoderme é geralmente inconspícua. Há uma clara distinção morfoanatômica entre populações de buritizeiro estudadas, e pode ser possível distingui-las taxonomicamente.

Palavras-chave: sazonalidade; especiação; variação morfológica; taxonomia.

INTRODUCTION

Amazon and Cerrado (the Brazilian savanna) are the largest South American domains, and together they cover about 60% of the continent – more than 2/3 of the Brazilian national territory (Silva et al. 2008). Both biomes have poor soils, but nutrients cycling in the Amazon is so high that the soil is constantly being enriched (Grau et al. 2017). Another striking difference is the constant rainfall of the Amazon, which contrasts with the semi-humid climate of Cerrado, where the dry season may last for five months (Oliveira-Filho and Ratter 2002). Indeed, rainfall frequency is the main characteristic that distinguishes these two biomes. In this sense, even though some physiognomic types of Cerrado occur near water courses (such as *veredas* and gallery forests), overall, this biome has lower atmospheric humidity and is prone to natural and man-made fires that impose structural and physiological adaptations (Amaral et al. 2013).

Calamoideae Beilschm. is a monophyletic and pantropical palm subfamily composed by 21 genera and about 650 species (Baker et al. 2000). The genus *Mauritia* L.f. comprises two species: *M. flexuosa* L.f. and *M. carana* Wallace, both occurring in South America (Lorenzi et al. 2010). In Brazil, the most widespread species is the buriti palm (*M. flexuosa*), occurring on the Amazon, Cerrado and part of the Caatinga and Pantanal biomes (Flora do Brasil website 2020). *Mauritia flexuosa* is one of the most important plants for the local communities, due to its multiplicity of uses (Henderson et al. 1995).

The buriti palm typically occurs on permanently flooded soils of non-coastal areas of northern South America (Lorenzi et al. 2010). Both in the Amazon and in the Cerrado, the species occurs in forested and open areas where herbaceous vegetation is abundant (Ribeiro and Walter 2008; Junk et al. 2014), and variations are expected according to the place where this palm grows. At least six domesticated varieties are found only in Peru considering fruit color and shape (Delgado et al. 2007). In this sense, we wonder if this same diversity is also found among natural populations from distinct climates.

Research concerning the intra-specific variation of buriti palm focused mostly on their genetic diversity. Natural populations of *M. flexuosa* present 77,18% genetic variation within and 22,82% genetic variation among populations along the Solimões river (Gomes et al. 2011). The rivers have a crucial role in shaping this palm genetic structure, and the distribution of these rivers has a direct influence in determining buriti population structure (Sander et al. 2018). More encompassing research observed high genetic differentiation among the populations of buriti from the Cerrado and Amazon (Melo et al. 2018).

Habitat affects plant height, diameter at breast height, leaves and seed number, and fruit and seed mass, with forest populations taller and with more leaves than those native of savanna (Rosa et al. 2014). Fruits from Cerrado plants have more antioxidant activity and less total carotenoids than those from Amazonia (Cândido et al. 2015). Moreover, physical characteristics of the fruits also vary according to the biome (Cândido and Silva 2017).

Leaves of plants from the savanna present hairs and a thicker cuticle than those from forested environments (Menezes et al. 2009). Moreover, morphoanatomical features have served as an important tool to the taxonomy of distinct plants (Gomes et al. 2005), aiding in the identification of groups (Leandro et al. 2016). Considering the buriti palm, Viana et al. (2018) pointed out that differences that local communities observed among male and female individuals were observed in the leaf anatomy, thus proving that this technique is useful to analyze divergences among populations.

Considering that *M. flexuosa* populations are genetically distinct (Melo et al. 2018) and bearing in mind that populations from open areas in Roraima (Northern Brazil) have some morphological features that distinguish them from populations from open areas in Goiás (Central Western Brazil; compare morphological descriptions from Ribeiro (2010) and Martins (2012)), it is appropriate to further investigate these morphological differences. It is also worthy to question if there are anatomical divergences between the populations, and if these differences are consistent enough to justify recognition at taxonomic level within this species. In this sense, the aim of the present work was to compare *M. flexuosa* populations from Amazon and Cerrado both morphologically and anatomically to check whether there are divergences among them, and, if applicable, to delineate distinct intraspecific groups.

MATERIAL AND METHODS

Two populations of *Mauritia flexuosa* from the Amazon and two populations from Cerrado were sampled. In the Amazon region, samples were gathered in the cities of Manaus (Amazonas state, 3°07'38"S 60°01'41"W) and Tiradentes (Pará state, 2°36'24"S 56°43'16"W), and in the Cerrado region, in the cities of Brasília (Distrito Federal, 15°56'24"S 47°52'00"W) and Uberlândia (Minas Gerais state, 18°54'45"S 48°16'33"W). To assure a better representativeness, herbarium exsiccates gathered in Xapuri (Acre state, Amazon region) and in Edeia (Goiás state, Cerrado region) were also analyzed (CEN 12975, Pinard 855; UB 213050, VEFCBF 41, shown in Fig. 1).

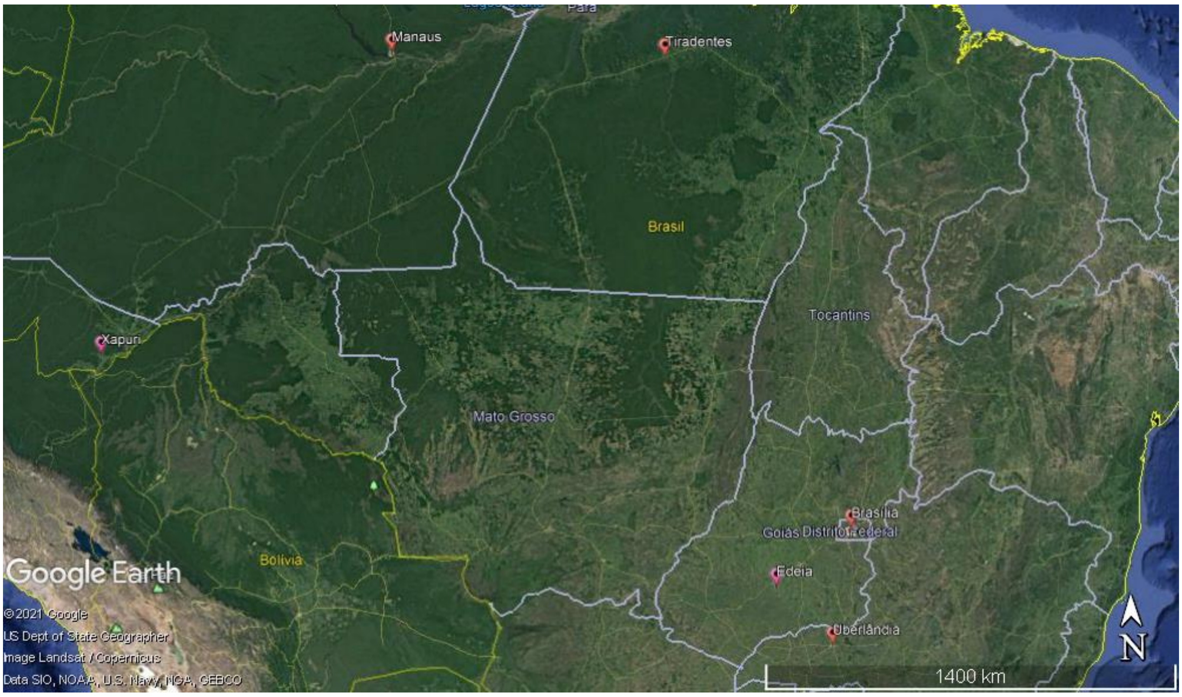

Figure 1. Populations of *Mauritia flexuosa* L.f. that were analyzed in this study.

Four individuals from each population were analyzed, to ensure populational representativeness. The methodology for palm analysis and gathering proposed by Martins and Filgueiras (2010) was followed. The gathered samples were stored in the following herbaria: Universidade de Brasília (UB), Herbário da Universidade Federal do Amazonas (HUAM) (abbreviations according to Thiers, 2021).

PLANT MORPHOLOGY

The morphological aspects of *M. flexuosa* were studied based on the field observations, herbarium collections analysis, and literature (i.e., Lorenzi et al. 2010; Martins 2012; Martins et al. 2018; Pimenta et al. 2018). In the last case, care was taken to observe the biome where the individual described in the work was analyzed, since it was presumed that morphological and anatomical descriptions of the populations may diverge according to their location. A comparison among different vegetative and reproductive organs of individuals from the Amazon and Cerrado regions was made. Whenever numerical data was present (for instance, number of leaves, stem diameter, fruit length), mean and standard deviation of the samples was calculated.

Information concerning exsiccates of *M. flexuosa* was required from Species Link website (www.splink.cria.org.br), obtaining a total of 223 samples of which 61 were from Amazon region (samples collected in Amazonas, Pará, Rondônia, Acre, and Amapá states), and 14 from Cerrado region (samples collected in Goiás, Distrito Federal, São Paulo, Minas Gerais, Mato Grosso, and Tocantins states).

LEAF ANATOMY

Segments of adult leaves were collected from each of the morphologically analyzed individuals. The collected material was fixed directly in ethanol 70% (Silva 2012). Samples were removed from the median third of the leaf segments, situated in the leaf lamina median region.

Small samples were cross sectioned on a table microtome (type Ranvier). The sections were placed on a Petri dish with distilled water and were clarified with sodium hypochlorite 2,5%. They were washed five times with distilled water and received double aqueous staining with Safranin and Alcian blue 4:1 for about 1 min. The staining excess was removed with ethanol 50%. The histological sections were mounted in permanent slides according to Paiva et al. (2006).

For the paradermal preparations, leaf samples with about 1cm² were immersed in Franklin solution 30% (hydrogen peroxide with acetic acid 1:1) overnight at 60°C (Kraus and Arduin 1997), or until the dissociation of the epidermis, which normally occurred when the structure lost its natural color, becoming transparent. Franklin solution was discarded, and the samples were washed in distilled water three times to remove the excess of the solution. The mesophyll was carefully removed with brushes, obtaining the paradermal preparations, which undergo staining with safranin for around 1 min. The same procedure was carried out for the cross sections to obtain the permanent slides.

All images were recorded using a photomicroscope Leica associated with the image capture system LAS EZ. Measurements of plant morphology were made by taking plant biometrics and calculating the mean of our results and the biometrics available both in herbaria and in other works. Leaf anatomy measurements were performed in the software Image Pro-Plus, taking a mean of three measurements of the same structure for three different individuals. A t-test was performed both for morphological and anatomical measurements to analyze whether the divergences among populations were significant or not.

RESULTS

MORPHOLOGY

The external morphology of the buriti palm is similar among the analyzed populations, specially concerning floral characteristics. In a general way, Amazonian populations are taller and presents a higher number of leaves and segments than the individuals from the Cerrado populations (Table 1). This divergence is significant (18.1608; $p \geq 0.05$). Moreover, the average size of *M. flexuosa* palms was larger in the studied Amazonian samples (13.89 m, compared to 10.50 m in Cerrado), which hence presented larger stems.

Table 1. External morphological characters of populations of *Mauritia flexuosa* L.f. from Amazon and Cerrado, with mean and standard deviation.

	Amazon	**Cerrado**
Plant height (m)	13.89 ± 4.9357	10.50 ± 5.5168
Leaf length (m)	4.60 ± 1.0304	3.92 ± 1.2908
Number of leaves	22.10 ± 2.6726	12.90 ± 2.3813
Number of segments	220.86 ± 18.8894	120.22 ± 62.9711

Concerning leaf length, buriti palm populations from the Amazon presented the mean of leaf length 71cm longer than the ones observed in the Cerrado populations. The number of leaves was also higher in the Amazonian populations, with a mean of 22.1 leaves per individual, in contrast to 12.9 leaves in the Cerrado samples. There was also a difference concerning the number of segments: Amazonian populations had about 100 leaf segments more than the Cerrado ones (220.86 segments for the Amazonian individuals and 120.22 for the Cerrado ones). This difference was significant (84.2068; $p \geq 0.05$).

LEAF ANATOMY

Although leaf anatomy of buriti populations is similar, they also present clear divergences (Table 2, Supplementary Data 1). Concerning statistical analyses, mesophyll thickness (5.0455; $p \geq 0.05$) and interveinal distance (9.0613; $p \geq 0.05$) were significant.

Table 2. Anatomical characters of leaf lamina of populations of *Mauritia flexuosa* L.f. from Amazon and Cerrado, with mean and standard deviation. CT – cuticle; PW – periclinal wall.

	Amazon	**Cerrado**
Adaxial CT + PW thickness (µm)	4.699 ± 0.742	3.197 ± 0.470
Abaxial CT + PW thickness (µm)	2.183 ± 0.443	2.310 ± 0.468
Adaxial epidermis thickness (µm)	11.066 ± 1.155	10.457 ± 1.224
Abaxial epidermis thickness (µm)	8.360 ± 1.074	8.300 ± 1.147
Adaxial hypodermis thickness (µm)	18.259 ± 1.952	18.327 ± 1.750
Abaxial hypodermis thickness (µm)	14.937 ± 2.719	14.883 ± 2.061
Mesophyll thickness (µm)	213.544 ± 11.882	237.574 ± 10.718
Interveinal distance (µm)	184.081 ± 34.034	225.267 ± 24.994
Stomata equatorial length (µm)	13.740 ± 1.044	13.971 ± 1.630
Stomata polar length (µm)	34.641 ± 1.847	33.600 ± 1.963

The complex cuticle–periclinal wall from both populations had about the same thickness, although adaxial complex from Amazonian plants was slightly thicker than on the abaxial surface. The epidermis of buriti palm from both biomes had about the same biometrics.

Stomata were commonly observed on both leaf epidermis surfaces of both Amazon and Cerrado populations. However, they were more frequent on the epidermis abaxial surface. Stomata were larger on plants from the Amazon, but stomatal density was lower on these plants. Conversely, individuals from Cerrado had more stomata per area, which were smaller in size.

Bulliform cells were inconspicuous on both samples. Moreover, the paradermal sections showed that anticlinal cell walls of the leaves from Amazon populations were more sinuous than those of the Cerrado populations, which were straighter. Hypodermis was inconspicuous on populations from both biomes, and even absent in some individuals.

Both populations have dorsiventral mesophyll with 5-7 cell layers and some intercellular space among cells. Mesophyll was about 25% thicker in individuals from the Amazon Forest. Moreover, palisade parenchyma had taller cells for this biome, characterizing a typical dorsiventral mesophyll. On the other hand, the distinction between palisade and spongy parenchyma was more subtle in Cerrado populations.

Plants from the Amazon and Cerrado had collateral vascular bundles, with a sclerenchymatic inner sheath and a parenchymatic outer sheath. Larger bundles were connected to adaxial and abaxial epidermis by a single layer of cells (semi-locked), while smaller bundles were dispersed in the mesophyll, with no connections with the epidermis (free). Interveinal distance was larger on the Cerrado samples analyzed. Smaller bundles had a more developed parenchymatic sheath in the populations from the Amazon Forest. The shape of the midrib and of the vascular bundles in general is rounded on Amazonian individuals and elliptical on those native from Cerrado. Midrib was more prominent on the Cerrado specimens.

DISCUSSION

The smaller size of stems and leaves in Cerrado populations observed in this work may be a consequence of the higher levels of aluminum in the biome. This finding was already pointed by Rosa et al. (2014), who also proposed that soil conditions are the main responsible for most of the significative lower vegetative biometrics observed for savanna populations of buriti. Also, according to them, these divergences among forest and savanna habitats have a crucial role on *M. flexuosa* fruit and seed production, which is higher on disturbed savanna, a relevant information to the agroindustry. Another aspect that must be considered is the fact that plants in forested regions develop longer internodes than plants in more open areas, such as the Cerrado region (Salati and Marques 1984).

It was also observed that fruits biometrics, composition and colors vary among biomes. According to Cândido and Silva (2017), *M. flexuosa* fruits from the Amazon tend to be smaller and lighter than those from Cerrado, although Amazonian fruits present higher contents of proteins, lipids, carbohydrates, monosaturated fatty acids and iron.

Concerning leaf anatomy, the irregular shape observed for buriti palms from Cerrado seems to be the result of the low air humidity conditions. In these conditions, parenchymatic cells collapse and shrink, assuming an irregular shape, as observed for the grass *Arundo donax* (Obataya et al. 2004). The thin cuticle observed both on Amazon and Cerrado populations was an intriguing feature, since for dryer environments this structure was expected to be thicker to avoid water loss (Menezes et al. 2009). Working with three stages of development of the species on the Amazon Forest, Passos and Mendonça (2006) observed a thin cuticle for buriti. For the Cerrado, a thin cuticle was also registered for the species in Jalapão, Tocantins state (Eichemberg and Scatena 2011). It seems that the species thin cuticle observed on both biomes is related with the available water supply of the flooded soils where it is most found. If this hypothesis is correct, then the environment air humidity plays a secondary role in this feature, having soil moisture the greatest influence on this plant structure. Another possibility is that this feature is well conserved within the species, not subjected to strict environmental modulation.

Studying leaf anatomy in the genus *Allagoptera* Nees., Pinedo et al. (2016) concluded that dorsiventral mesophyll can be classified in two types: classical and gradual. Amazonian buriti palms tend to present classical dorsiventral mesophyll, with a clear distinction between palisade and spongy parenchyma, while Cerrado plants, with a subtle distinction between these parenchyma, have gradual dorsiventral mesophyll.

Somavilla and Ribeiro (2011), comparing the leaf anatomy of three Melastomataceae species in Cerrado *sensu stricto* and in palm swamp (Vereda) habitats, concluded that individuals growing in the latter environment have thicker mesophyll and chlorophyllian parenchyma due to higher radiation and lower shading levels. Our results do not match these, possibly indicating that air humidity plays a more important role on mesophyll thickness than solar radiation and shading.

As well as for epidermis and hypodermis, water supply and sunlight intensity are also the main responsible environmental conditions involved on interveinal distance. Indeed, environmental factors have a great influence, affecting the dimension and the arrangement of a plant vascular system (Ariano and Silva 2016). In general, the shorter the distance among vascular bundles, the less water plants will require (Kawamitsu et al. 1985) and the more tolerant plants will be to shade (Ogle 2003). The shorter interveinal distance observed for individuals sampled on the Amazon seem to be more a result of light conditions than of water supply, since the Amazonian buriti palms normally grow on more shaded areas, compared to those from Cerrado.

Several divergences were observed among the populations analyzed in this work. There is a clear morphoanatomical distinction among buriti palm populations that grow in the two biomes studied. Our data suggests that there are differences among *M. flexuosa* populations from the Amazon and from the Cerrado, and even the last ones diverge according to the latitude where they grow. The hypothesis that populations belong to distinct species is plausible, and the exam of the typus and taxonomic literature is necessary to define it or to subdivide the species in different subspecies and varieties.

ACKNOWLEDGEMENTS

The authors acknowledge Capes for the doctoral grant for the first author.

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SUPPLEMENTARY DATA

1. AMAZONIAN AND CERRADO *MAURITIA FLEXUOSA* L.F. LEAF ANATOMY

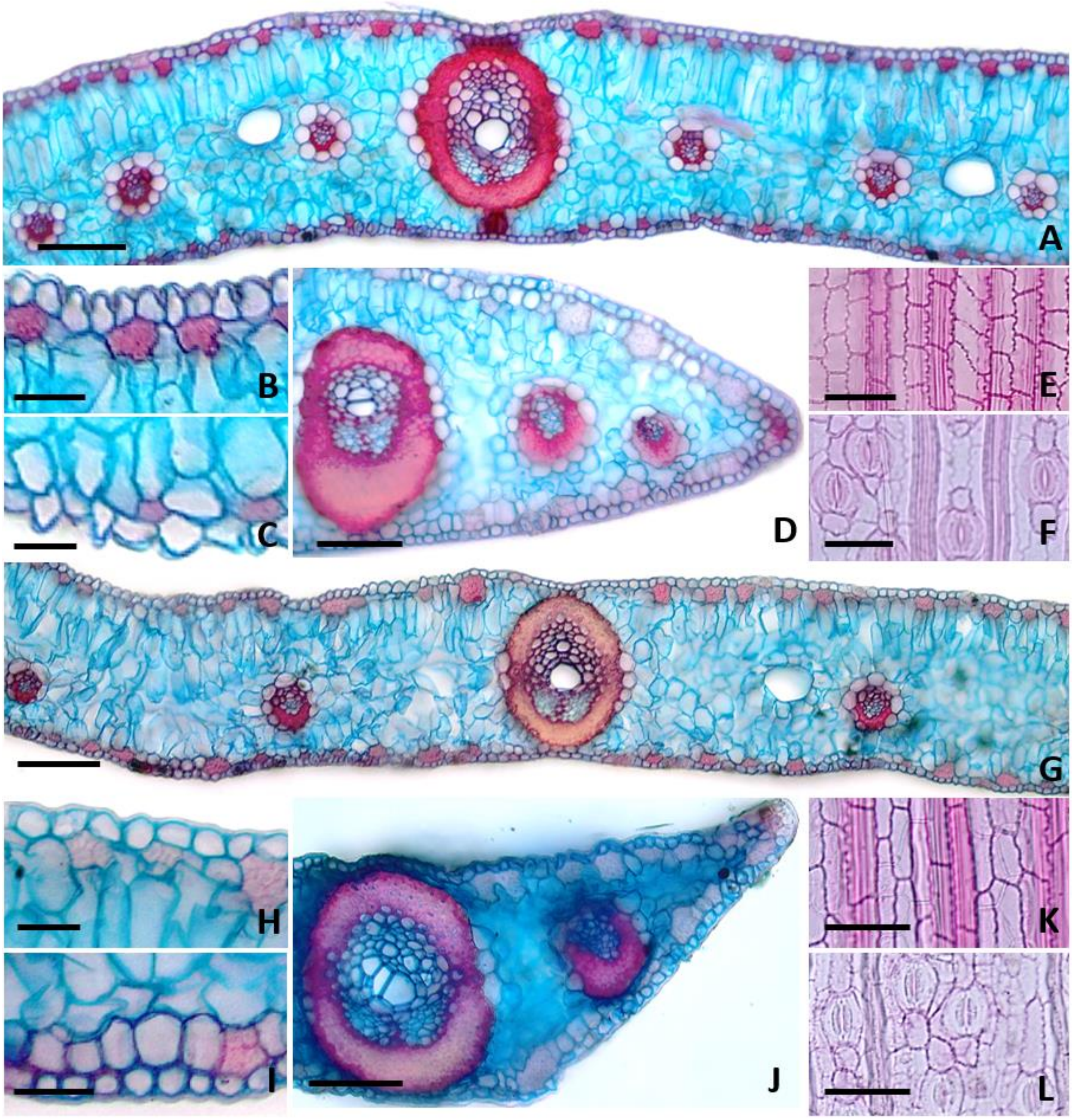

Figure S1. Leaf anatomy of the buriti palm (*Mauritia flexuosa*) from Xapuri (state of Acre, Amazon) and Edeia (state of Goiás, Cerrado). **A,G.** Leaf lamina overview. **B,H.** Leaf lamina adaxial surface. **C,I.** Leaf lamina abaxial surface. **D,J.** Leaf segment margin. **E,K.** Paradermal section of adaxial surface. **F,L.** Paradermal section of abaxial surface. **Scale bars: A,D,G,J:** 100µm; **B,C,H,I:** 25µm; **E,F,K,L:** 50µm.

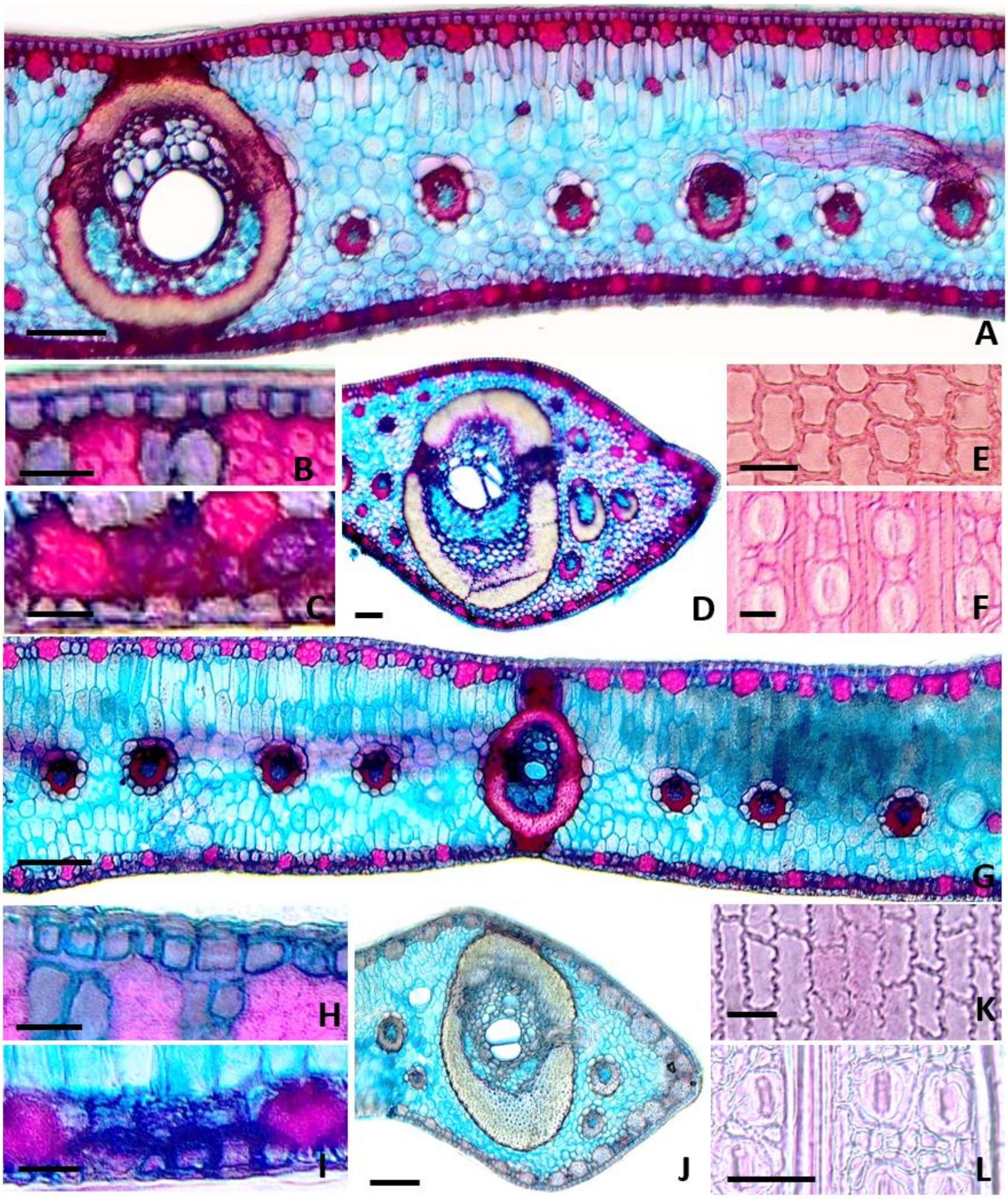

Figure S2. Leaf anatomy of the buriti palm (*Mauritia flexuosa*) from Manaus (state of Amazonas, Amazon) and Uberlândia (state of Minas Gerais, Cerrado). **A,G.** Leaf lamina overview. **B,H.** Leaf lamina adaxial surface. **C,I.** Leaf lamina abaxial surface. **D,J.** Leaf segment margin. **E,K.** Paradermal section of adaxial surface. **F,L.** Paradermal section of abaxial surface. **Scale bars: A,D,G,J:** 100µm; **B,C,H,I:** 25µm; **E,F,K,L:** 50µm.

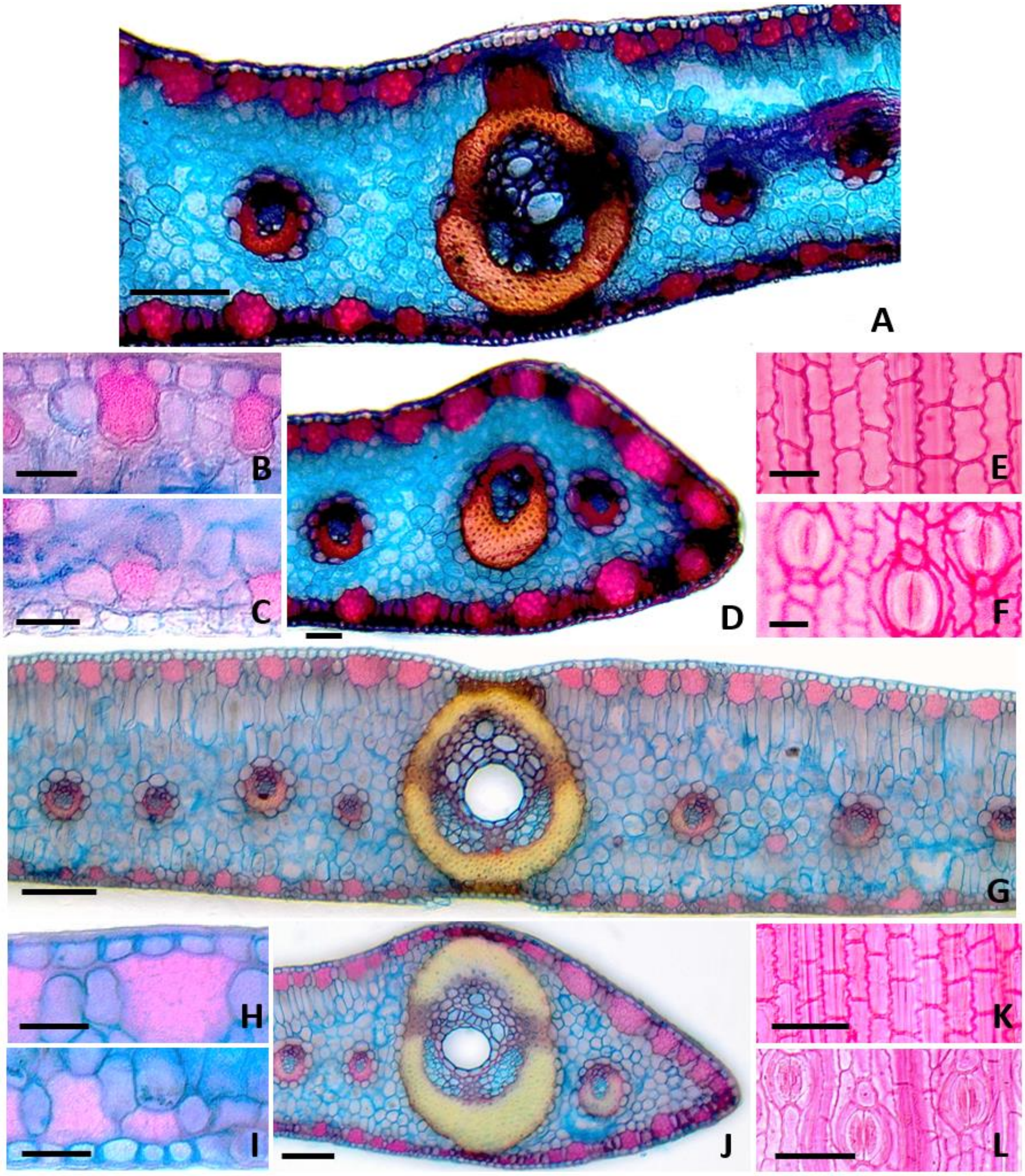

Figure S3. Leaf anatomy of the buriti palm (*Mauritia flexuosa*) from Tiradentes (state of Pará, Amazon) and Brasília (Distrito Federal, Cerrado). **A,G.** Leaf lamina overview. **B,H.** Leaf lamina adaxial surface. **C,I.** Leaf lamina abaxial surface. **D,J.** Leaf lamina overview. **E,K.** Paradermal section of adaxial surface. **F,L.** Paradermal section of abaxial surface. **Scale bars: A,D,G,J:** 100µm; **B,C,H,I:** 25µm; **E,F,K,L:** 50µm.

Chapter III

THE SIMPLE LEAF DEVELOPMENT IN *MAURITIA FLEXUOSA* L.F. (ARECACEAE)

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Text formatted according to Botanical Journal of the Linnean Society guidelines

The buriti palm (*Mauritia flexuosa*) is an important palm of the South American flora, being widely distributed throughout the country. Since there is no research approaching this species leaf development, this study aimed to investigate the buriti palm leaf anatomy during different stages of development, comparing it with the leaf ontogeny of other palms. Dissected *M. flexuosa* buds were imbedded in paraffin and sliced as serial sections for the anatomical analyses. Leaf development of the buriti palm is similar to the process that occurs in feather-leaved palms, mainly at the earliest stages. Plications separate into individual segments at a relatively young stage of development. A constriction separating petiole from leaf blade is strong evidence that the leaf type is simple. Thus, a review on the terminologies 'pinna', 'leaflets' and 'rachis' is proposed, since they apply to eudicot compound leaves. This is the first report concerning leaf ontogeny on a fan-shaped palm reduplicate leaf.

Keywords: *buriti palm, leaf ontogeny, constriction.*

O buritizeiro (*Mauritia flexuosa*) é uma palmeira importante da flora Sul Americana, estando amplamente distribuída pelo país. Uma vez que ainda não existe pesquisa abordando o desenvolvimento foliar dessa espécie, este estudo visou investigar a anatomia foliar do buritizeiro em diferentes estágios de desenvolvimento, comparando com a ontogenia foliar de outras palmeiras. Gemas de *M. flexuosa* dissecadas foram incluídas em parafina e cortadas em secções seriadas para as análises anatômicas. O desenvolvimento foliar do buriti é semelhante ao processo que ocorre em palmeiras com folhas em forma de pena, principalmente nos estágios mais jovens. As plicações se separam em segmentos individuais em um estágio de desenvolvimento relativamente jovem. Uma constricção separando o pecíolo da lâmina foliar é uma forte evidência de que o tipo foliar é simples. Dessa forma, uma revisão das terminologias "pina", "folíolos" e "raque" é proposta, uma vez que elas se aplicam às folhas compostas de eudicotiledôneas. Este é o primeiro relato abordando a ontogenia foliar de uma folha de palmeira reduplicada em forma de leque.

Palavras-chave: *buriti, ontogenia foliar, constricção.*

INTRODUCTION

Palm leaves have raised the interest of researchers since the beginning of the 19th century (De Candolle, 1827). After an intense discussion concerning palm development, carried out for more than 100 years, the meristem folding process proved to be the most faithful to what occurs in the first stages [see Kaplan *et al.* (1982a) and Pinedo and Gomes (chapter 1 in this thesis) for a more detailed historical background]. The focus of discussion nowadays is on later stages of development, aiming on the answer about which process is involved on individual segment separation (Nowak *et al.*, 2009). An intriguing observation that was made in some works was that leaf early ontogeny processes are identical in both palmate and pinnate leaves (Periasamy, 1962). Thus, even though the diversity of leaf forms in palms, the same processes occur during the first stages (Kaplan *et al.*, 1982b), turning an initially simple and entire leaf into a deeply dissected leaf (Gunawardena and Dengler, 2006).

The subfamily Calamoideae is easily identified by its overlapping scales that cover ovaries and fruits (Dransfield *et al.*, 2008). Almost all genera in Calamoideae have palmate reduplicate leaves, a feature that draws our attention since reduplicate leaves are mainly pinnate (Dransfield *et al.*, 2008). The subfamily was already studied concerning its phylogeny (Baker *et al.*, 2000; Asmussen *et al.*, 2006), morphology (Baker *et al.*, 1999) and anatomy (Seubert, 1996; Matthew and Bhat, 1997), but there are still no studies on leaf development for this group.

Mauritia flexuosa L.f., commonly known as buriti palm, is a widely distributed palm in Brazil and considered a keystone species to the local fauna since it only grows in waterlogged areas, such as the *veredas* or the *buritizais* (Ribeiro and Walter, 2008), where many species establish. This surely makes the buriti palm a priority in actions of conservation (van der Hoek *et al.*, 2019). In this sense, *Mauritia flexuosa* is a central palm in Brazilian flora (Lorenzi *et al.*, 2010), and knowing its biology is surely useful for the implementation of such conservation policies. The differences between leaf epidermis of three distinct stages of development (young vegetative, intermediate vegetative and adult reproductive stage) of this species had already been observed (Passos and Mendonça, 2006), but works concerning the process of leaf formation are lacking.

In this sense, the present work aimed to investigate the buriti palm (*M. flexuosa*) leaf anatomy on the first stages of development, comparing it with the ontogeny of other palms with contrasting leaf types (such as the pinnate reduplicate leaf).

MATERIAL AND METHODS

MATERIAL PREPARATION

Seedlings of *Mauritia flexuosa* with around 50-150cm were purchased from nurseries. Leaves and sheaths were removed about 10cm above the bulb, and used to make voucher specimens, which were deposited in University of Brasilia Herbarium (acronym UB, according to Thiers 2021; vouchers *Pinedo 41*, *42*, *43* and *44*, under register numbers 217414, 217415, 217416 and 217417, respectively). The most outer sheaths were removed and the most internal ones were separated under a stereomicroscope according to their sizes. Distinct stages of development were transferred to Eppendorfs, where they were fixed for the anatomical observations. A total of 6 leaf buds with 3-15mm were dissected and employed.

ANATOMICAL STUDY

Samples were fixed at room temperature (ca. 24°C) in FAA50 (Johansen, 1940) for at least 12h. The meristems passed then through an ethanolic gradient (50, 60, 70, 80, 90 and 100%). Thereafter, samples passed through a series of ethanolic butyl acetate solutions (25, 50, 75 and 100%) for at least 1h in each concentration. The specimens received melted paraffin 3:1 butyl acetate and were maintained at 60°C for 24h. Samples were replaced in pure paraffin for more two times. Paraffin blocks were mounted, positioning buds in a such way that plications appear in the sections, and were sectioned serially on a rotary microtome at variable thickness, according to the size of the primordium (0,5–1mm: 8µm thickness; 1–3mm: 12µm thickness; more than 3mm: 15µm thickness). Paraffin strips were distended, and the staining with safranin and Alcian blue was performed according to Kraus and Arduin (1997). Vitral varnish was used to seal up the slides with coverslips as the procedure of Paiva *et al.* (2006). Observations were made and images were captured with the program LAS ES in a photomicroscope OLYMPUS associated to the computer.

RESULTS

LONGITUDINAL SECTIONS

A group of cells situated inside the youngest primordia sheath start to divide by anticlinal divisions, forming a dome, which is the shoot apical meristem (Fig. 1A). Two leaf primordia develop and arise from it (Fig. 1B). More cells are added by anticlinal divisions, and periclinal divisions also take place, so the young leaves grow in length (Fig. 1C). This growth is accompanied by an elongation of the primordium, and when it reaches more than 200µm an apical horn begins to emerge from it (compare Fig. 1B with Fig. 1C). Primordia are still as small as 300µm when they start to present some dark stained structures above them, which correspond to hairs when we analyze the species morphologically. These hairs appear along all process of *M. flexuosa* development (Figs. 1C-F), even though they are rarer on later stages. In general, there is little difference regarding the size of both sides of the primordia.

When primordia are more than 500µm, a younger stage of development normally starts to grow (Fig. 1D). Older stages function henceforward as protective structures to younger and newly developed stages. Although in this stage plications are not formed yet, it is possible to observe in some samples a rudimentary vascular bundle system. In primordia with more than 950µm, both plications and vascular system are evident and easily identifiable (Fig. 1E). Leaf primordia continue to grow apically, and when they reach about 2mm, plications remain vertically oriented, just as observed on younger stages (see older stage of development in Fig. 1E). It is also noticeable in this stage how the basal part of the leaf had expanded vertically, lifting the plicate lamina, and making it develop on a higher level than the tip of the younger stage, a feature that is always observed from this stage on.

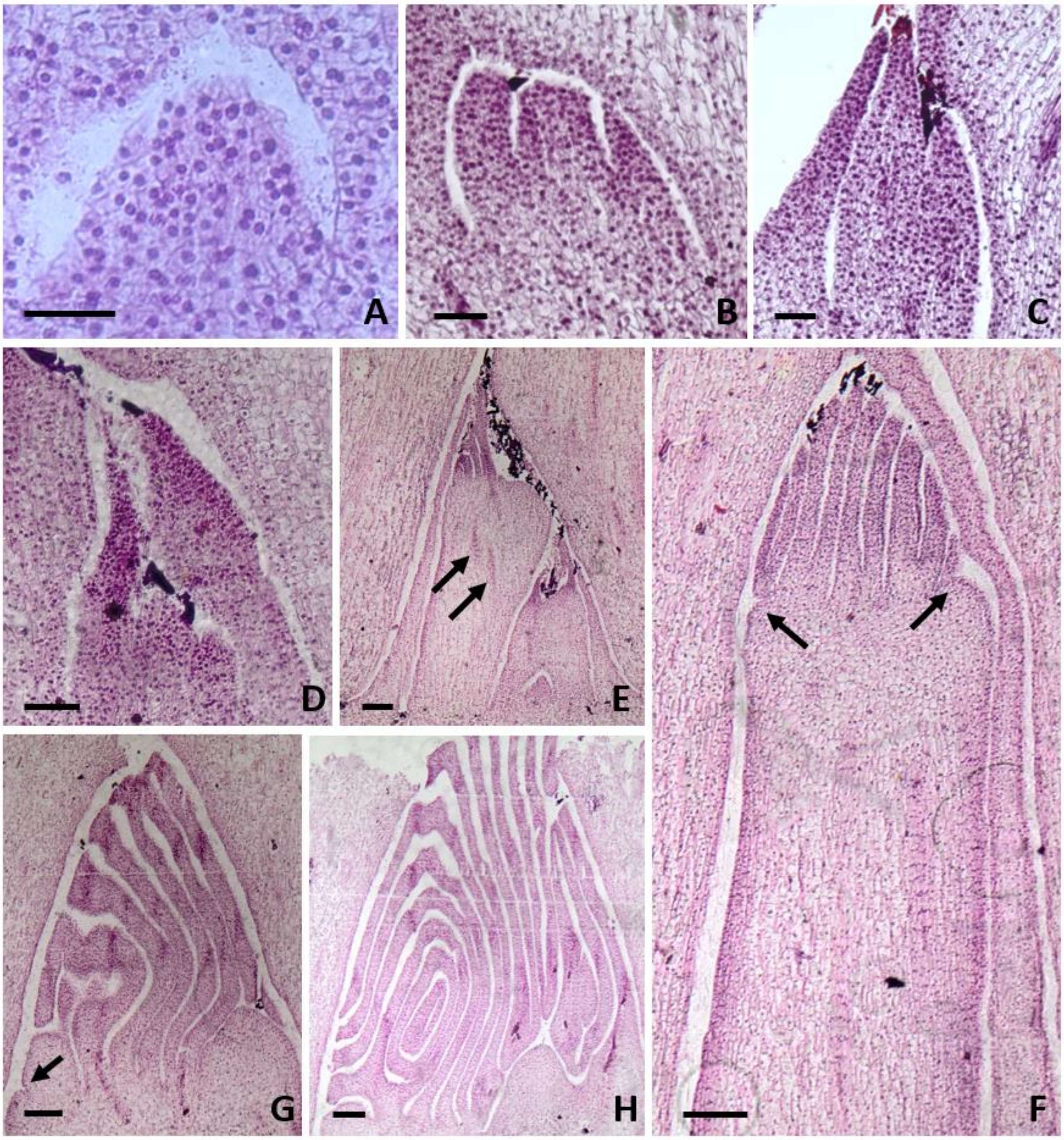

Figure 1. *Mauritia flexuosa* L.f. histogenesis (longitudinal sections). (**A**) Stem apical meristem. (**B**) Leaf primordia. (**C**) Leaf elongated, forming an apical horn. (**D**) Younger stage of development growing inside an older primordium. (**E**) Procambium bundles (arrows) in a larger primordium, which also present hairs (black dots above primordium). (**F**) Plicated lamina and petiole, separated by constriction (arrows). (**G**) Plications bend and become packed (arrow: constriction). (**H**) Plications in a 4mm length primordium. **Scales: A-C:** 50µm; **D:** 100µm; **E-H:** 200µm.

Primordia with about 2,5mm present a very tall and elongated base (Fig. 1F). Some hairs are observed on the apex of the plications. From this point on, a constriction is observed, separating the plicate lamina from the unplicated petiole (see arrows in Figs. 1F and 1G). When young leaves reach 3mm, plications grow more in length, but since the space inside the older stage sheath is limited, they bend and become packed (Fig. 1G). An interesting feature is that plications emerge only from the most central part of the petiole apex, even though they spread laterally once they have emerged. Primordia with about 4mm length present their most central plications vertical-oriented and the lateral ones packed (Fig. 1H).

CROSS SECTIONS

When cross sections are performed on the very tip of a 5,2mm primordium (about 340µm below the tip), it is possible to see that individual segments are already distant apart from one another (Fig. 2A). Virtually all segments, from both sides of the primordium, have their midribs facing the most inner part of it. Differences in size and shape among leaf sides are few, although it seems that one side segments are slightly larger than the other side ones (compare segments from the right and from the left in Fig. 2A). Hairs that appeared near the tip of the primordia in longitudinal sections are also evident and frequent in cross sections performed at this level.

A section at about 730µm below the tip reveals that most segments tear in a slightly asymmetric way, producing individual structures that are larger and sometimes thicker in one side than in the other (Fig. 2B). Nevertheless, in some cases this tearing goes straightly through all leaf segment extension, as dividing it in two equal halves (see segment located on the bottom left of Fig. 2B). At this level, some few hairs are also present, although they are rarer than in cross sections performed nearer to the apex (compare the number of hairs in Figs. 2A and 2B).

In a 5,2mm primordium, segments are seen linked with the midrib protrusion region from 1200µm below the tip downwards, since in a cross section made about 1280µm below the tip most of them are already merged (Fig. 2C). This union of segments with midrib protrusion happens abruptly and suddenly in *Mauritia flexuosa*. At 1450µm below the tip, all segments have already merged with the midrib (Fig. 2D). It is not possible to see at this level younger stages of development within the midrib protrusion, since they are in a lower level in the primordium.

Cross sections in young buriti palm leaves also show some starch grains that are located inside them, which are translucent under light microscopy (Fig. 2E). When procambium starts to be differentiated, it consists of a group of small sized cells packed together in a circular region of the primordium. This regions expands and develops, generating xylem and phloem that typically compose a developed vascular bundle (Fig. 2F).

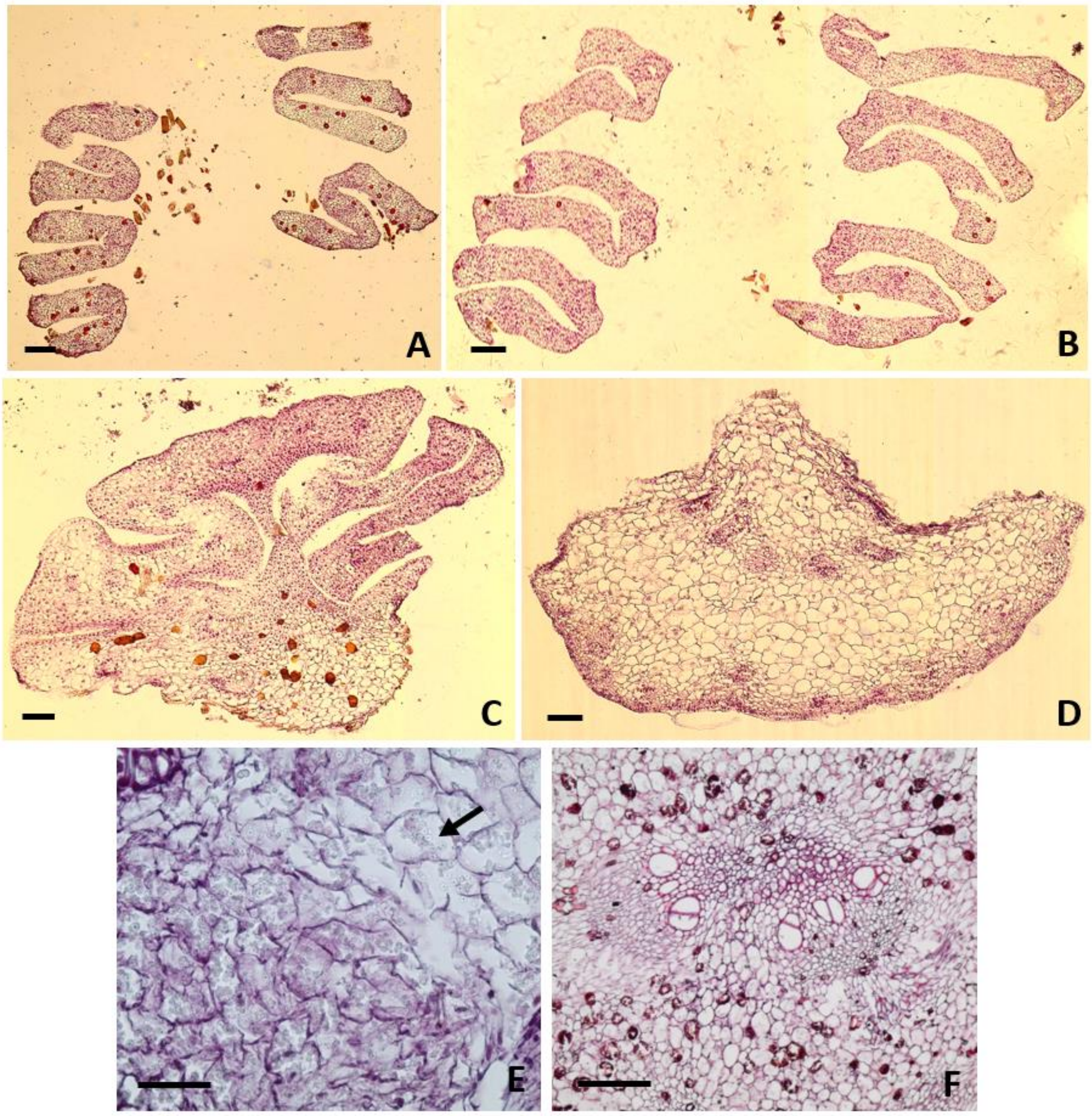

Figure 2. Cross sections on a 5,2 mm primordium of *Mauritia flexuosa* L.f. seen under light microscope. Sections were performed at distinct levels below the apical tip: (**A**) 340μm, (**B**) 730μm, (**C**) 1280μm and (**D**) 1450μm. (**E**) Starch grains located inside the cells (arrow). (**F**) Procambium being differentiated. **Scales: A-B, E-F:** 100μm; **C-D:** 200μm.

DISCUSSION

From the works concerning palm leaf ontogeny, virtually all of them were performed with the subfamily Arecoideae (e.g., Venkatanarayana, 1957; Dengler *et al.*, 1982; Nowak *et al.*, 2007) or with the genus *Phoenix* (e.g. Naumann, 1887; Periasamy, 1962). Moreover, although some works on palm leaf ontogeny have focused on palmate leaf development (Ball, 1941; Periasamy, 1965; Periasamy, 1966; Periasamy, 1967; Bugnon, 1980; Kaplan *et al.*, 1982b), there are no ontogenetic studies concerning palmate reduplicate leaves, being all focused on palmate induplicate leaves (see Table 1). Since palmate reduplicate leaves are rare in nature, only occurring in subtribe Mauritiinae Meisn. (Dransfield *et al.*, 2008), it is not surprising that their process of leaf development has not been studied yet.

Table 1. A review of the studied palm genera with palmate leaves, concerning leaf ontogeny.

Species / Genera	References
Chamaerops L.	Deinega, 1898 Arber, 1922
Rhapis L.f. ex Aiton	Deinega, 1898 Periasamy, 1967 Kaplan *et al.*, 1982b
Pritchardia Seem. & H. Wendl. ex H. Wendl	Arber, 1922
Thrinax Sw.	Arber, 1922
Trachycarpus H. Wendl.	Arber, 1922 Ball, 1941
Washingtonia H. Wendl.	Ball, 1941 Bugnon, 1980
Borassus L.	Periasamy, 1965 Periasamy, 1966

Some palm genera, such as *Chamaedorea* (Nowak *et al.*, 2009) and *Dypsis* (Dengler *et al.*, 1982, at that time *Chrysalidocarpus*), present a distinction in size and morphology of their leaf sides, being one more developed than the other. However, in *Mauritia flexuosa* this divergence was not so sharp as the one observed for *Chamaedorea fragrans*, for instance (Nowak *et al.*, 2009).

In contrast to palms with pinnate leaves, which present their plications horizontally oriented along most part of leaf development, palmate leaves have their plications oriented vertically. Once we know how plications are disposed in primordia, it is possible to analyze sections perpendicular and parallel to them more clearly, which is an important point, since many misunderstandings concerning leaf development were made in the past (Kaplan *et al.*, 1982b).

The most important finding of this study was the constriction separating the petiole from the plicated lamina (see arrows in Figs. 2F-G). This structure shows that plications, which will further become leaf segments in palms, only occur in the foliar lamina. When we contrast this with the compound leaf development of legumes (Fabaceae Lindl.), such as *Medicago truncatula* Gaertn. (Ge *et al.*, 2014) and *Vigna radiata* (L.) R. Wilczek (Jiao *et al.*, 2019), the pattern observed for palms is different, since in these species the segments develop along the petiole (named 'rachis' in plants with compound leaves). Although it is already known that palm leaves are simple, dissecting along development (Gunawardena and Dengler, 2006), few images prove this fact in such a didactic way.

In this sense, since the terminologies 'pinna' and 'leaflet' are applied to eudicot compound leaves (Harris and Harris, 1994; Vidal and Vidal, 2000), they seem inappropriate for designating the foliar units of palms. In a typical pinnate-leaved plant, each leaflet unit is organized in a similar way to a whole simple leaf, and has at its base a small petiole, named petiolule (Bell and Bryan, 2008) This clearly does not apply to palms. Thus, it is here proposed that the term segment be used to name all units of palm leaves – both pinnatisect and palmatisect. Pinedo *et al.* (2016) had used the terminology ‘segments’ instead of ‘pinna’ and ‘leaflet’ precisely thinking about the consistency of this hypothesis.

The term ‘rachis’ is also inadequate since this is employed to designate the midvein of compound leaves (Font Quer 1953). We propose this structure to be named ‘midrib’, just as in simple leaves. These terminologies can also be applied to other plants with simple sectioned leaves, such as Cyclanthaceae.

The findings of this study reinforce and present more evidence that leaf type in palms is simple dissected. Nevertheless, the question concerning why reduplicate leaves of *Mauritia flexuosa* have a palmate morphology, contradicting the most observed trend in nature, is still unknown. We strongly believe that future developmental works can shed light on this important question.

ACKNOWLEDGEMENTS

The authors acknowledge Capes for the doctoral grant for the first author.

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Chapter IV

ANATOMICAL AND ONTOGENETIC STUDIES ON *PHOENIX ROEBELENII* O'BRIEN (ARECACEAE) SEEDLINGS

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Phoenix roebelenii is a palm species with remote germination that sprouts more rapidly than the average palms. The apocole, structure that connects the seed to the seedling in remote germination palms, has been few explored anatomically, and the process of eophyll development has not been the focus of most ontogenetic works, so this was our purpose. Seeds of *Phoenix roebelenii* were germinated and 5 cm tall seedlings were dissected. Serial sections of the eophyll were obtained with help of a rotary microtome, while cross sections of the apocole, first emerging root and eophyll were performed under a stereomicroscope. Concerning seedling anatomy, our data agree with other information already present in the literature for the species. The anatomy of the apocole draws our attention, since bundles organization within this structure resemble the pattern observed for eudicots, suggesting that this same feature evolved independently in these plants and in palms. This observation seems to be applicable to other palms with remote germination. Seedling leaf development revealed characteristics similar to what is found in adult individuals.

Keywords: *apocole, development, eustele.*

Phoenix roebelenii é uma espécie de palmeira de germinação remota que brota mais rapidamente que a média das palmeiras. A apocole, estrutura que conecta a semente à plântula em palmeiras com germinação remota, tem sido pouco explorada anatomicamente, e o processo de desenvolvimento do eófilo não tem sido o foco da maioria dos estudos ontogenéticos, então este foi o nosso objetivo. Sementes de *Phoenix roebelenii* foram germinadas, e plântulas de 5 cm de altura foram dissecadas. Secções seriadas do eófilo foram obtidas com o auxílio de um micrótomo rotativo, enquanto secções transversais da apocole, da primeira raiz emergente e do eófilo foram realizadas sob lupa. Com relação a anatomia da plântula, nossos dados concordam com outras informações já presentes na literatura para a espécie. A anatomia do apocole chama a nossa atenção, uma vez que a organização dos feixes dentro dessa estrutura lembra o padrão observado para eudicotiledôneas, sugerindo que esta mesma característica evoluiu independentemente nessas plantas e em palmeiras. Essa observação parece ser aplicável a outras palmeiras com germinação remota. O desenvolvimento foliar das plântulas revelou características similares às dos indivíduos adultos.

Palavras-chave: *apócole, desenvolvimento, eustelo.*

1. INTRODUCTION

Phoenix L. is a palm genus from the Old World composed by 15 species distributed through the Canary Islands, tropical Africa and southern Asia (Dransfield et al., 2008). The group is easily distinguished from other palm genera due to their pinnate induplicate leaves and spines covering the petiole (Dransfield et al., 2008). The genus was the first and the most employed in studies concerning palm leaf development (Naumann, 1887; Deinega, 1898), being also studied by Ball (1941), Padmanabhan (1967) and Padmanabhan and Veerasamy (1974) under optical microscopy.

Since the genus presents a relatively quick germination when compared to other palms, *Phoenix* has also been the focus of palm germination studies (Emerson et al., 2003; Iossi et al., 2006; Sghaier-Hammami et al., 2009). The pigmy date palm (*Phoenix roebelenii* O'Brien) originated from southeast Asia is widely used for tropical landscaping purposes worldwide due to its easy adaptation to the most diverse climatic conditions (Iossi et al., 2007).

According to Meerow (1999), the germination type in palms such as *Phoenix* spp. is classified as remote germination, where the first structure to emerge from the seed is the apocole, structure that connects it to the seedling. From it, the first root and the eophyll emerge. Few studies were carried out to understand the real nature of the apocole in palms (Corrêa et al., 2019).

The purpose of this work was to understand the process of germination from the histogenetic point of view and the seedling anatomy in *P. roebelenii*, comparing with literature information. Moreover, the anatomy of the newly germinated apocole was also analyzed and compared to the anatomy of mature petioles of *P. roebelenii* adult samples.

2. MATERIAL AND METHODS

2.1. ONTOGENETIC STUDIES

Seeds of *Phoenix roebelenii*, obtained from adult individuals cultivated in the Instituto de Biologia da UnB (IB), Brasília, were germinated. Leaves of the mother plants were used to make voucher specimens, which were deposited in University of Brasilia Herbarium (acronym UB, according to Thiers 2021; vouchers *Pinedo 45* and *52*). Phenological observations made during the period of seeds and seedlings development can be found in Supplementary Data.

When seedling eophylls reached the size of 5 cm tall, the outer sheath was dissected under a stereomicroscope, and the inner primordium was fixed at ca. 24°C in FAA50 (Johansen, 1940) for at least 12h. The complete methodology employed henceforward is the same already described in Chapter I of this thesis.

2.2. CROSS SECTIONS

Cross sections were performed at the apocole, first emerging root and eophyll of *Phoenix roebelenii* seedlings (Fig. 2A). Thin sections were obtained under a stereomicroscope by using stainless blades, with the aid of a brush and a needle. Permanent slides were assembled, employing glass varnish and coverslips to seal them. Moreover, a mature and completely developed leaf of the species was gathered and its petiole was also cross sectioned in a Ranvier table microtome. Permanent slides were assembled in the same way described for seedlings. The cross-section analyses and the image capture process were made in the program LAS ES on a photomicroscope Olympus associated to the computer.

3. RESULTS

3.1. ONTOGENETIC OBSERVATIONS

The first evidence of the leaf primordium is a small lateral hump developing from the shoot apex (Fig. 1A). This primordium soon grows by both anticlinal and periclinal divisions, and when it reaches 200μm its uppermost cells are smaller than the ones that occur in other regions (Fig. 1B). Plications are formed in *Phoenix roebelenii* seedlings during an early stage of development, when leaves are even smaller than 500μm (see older stage in Fig. 1C).

When we analyze cross sections, three to four plications are being formed in the eophyll, a number that is maintained in the morphology of this first leaf (Fig. 1D). These plications were observed in leaf primordia with only 400μm length. These plications apparently are originated between cells of the protoderm and first appear as alternated lines that are formed at the edges of the organ, deepening inside it until near the opposite edge, but not reaching it.

A cross section performed at the base of the eophyll revealed a leaf with three plications (Fig. 1E). This organ is constituted of recently differentiated protoderm, parenchyma, and vascular bundles, from the protoderm, fundamental tissue and procambium, respectively. The vascular bundles in this young leaf are in divergent regions: in the middle part of the outermost fold, in the middle part of the leaf and in both median folds. A closer look at the plications indicates that in some cases the protoderm has smaller cells (Fig. 1F) and in other cases it presented larger cells (Fig. 1G). These are found more at the apex of the eophyll, while smaller cells are more found at its apex.

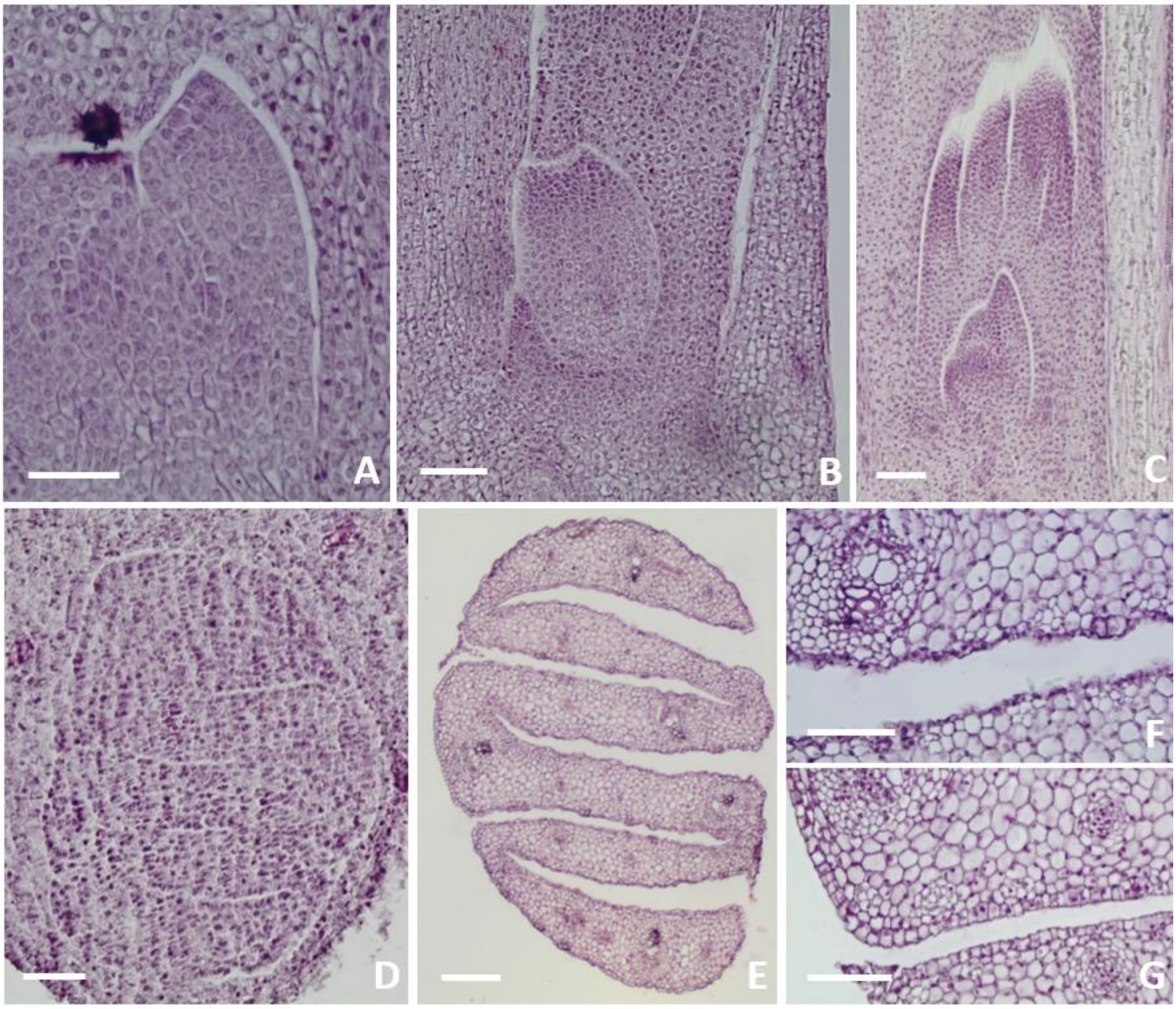

Figure 1. Longitudinal and cross sections of *Phoenix roebelenii* O'Brien seedlings. (**A-C**) Prophyll and leaf primordia. (**D**) Plications being formed. (**E**) Plicated pattern. (**F**) Protoderm near the base of the eophyll. (**G**) Protoderm near the apex of the eophyll. **Scale bars: A, D, F-G:** 50μm; **B-C, E:** 100μm.

3.2. SEEDLING LEAF ANATOMY OBSERVATIONS

The regions and planes of sectioning where young seedlings of *Phoenix roebelenii* were cross sectioned are depicted in Fig. 2A.

The eophyll (Fig 2B; I in Fig. 2A) is composed of a sheath that surrounds the young developing leaf inside it. This leaf is like the one illustrated in Fig. 1E, with three plications.

Vascular bundles are already well developed in this leaf primordium, occurring mainly at the middle and the extremities of each plication. Concerning the older leaf sheath, vascular bundles are mainly at its extremities. The first root of this species (Fig. 2C; II in Fig. 2A) is hexarch and has the anatomical characteristics of a typical monocot root.

An anatomical observation of the apocole (Fig. 2D; III in Fig. 2A) revealed a structure with six vascular bundles disposed in a circle around the medulla, resembling an eustele. Cross sections in the petiole of a mature individual of *Phoenix roebelenii*, on the other hand, reveals scattered vascular bundles (Fig. 2E), resembling an atactostele of a monocot stem.

Figure 2. Cross sections obtained in the sectioning of *Phoenix roebelenii* O'Brien. (**A**) Regions of sectioning: I – eophyll; II – first root; III – apocole. (**B**) Eophyll cross section. (**C**) First root cross section. (**D**) Apocole cross section, evidencing the eustele organization of the bundles. (**E**) Plant mature petiole cross section, evidencing the atactostele organization of the bundles.

4. DISCUSSION

Palm seedling leaves were studied by Tomlinson (1960). Henderson (2006) published a comprehensive work concerning morphological and anatomical aspects of palm seedlings. Nevertheless, in the last-mentioned study there are no images to illustrate the internal structure of *Phoenix roebelenii*, but only a description approaching its anatomy. More detailed information and images of this species were provided by Iossi et al. (2006), and our results match with the observations made by these authors. Even though, we consider it is important to take a closer look on the apocole.

Sometimes named 'cotyledonary petiole' or 'hyperphyll' (Henderson, 2006), the apocole presents an intriguing vascular bundle organization, which resembles the eustelic bundle disposition of eudicot stems. The petiole of an adult leaf of *Phoenix roebelenii*, in contrast, shows the typical atactostele bundle organization found in monocots stems and petioles. Since there are so many divergences concerning this primary structure and the mature petiole, we find it inappropriate to call it 'cotyledonary petiole', being the terminologies 'hyperphyll' and 'apocole' more recommended.

Concerning leaf developmental processes, Padmanabhan and Veerasamy (1974) analyzed the ontogeny of *Phoenix dactylifera* L. eophylls. Their results were similar to the ones obtained in this study.

The eustelic disposition of the bundles in the apocole resembles a eudicot. This character can indicate that palms and eudicots both present this feature, in distinct stages of development. According to APG IV (2016), the Commelinids (group that encompasses Arecaceae) is a clade resultant of many divergences within the monocots, and this also suggest that this eustelic organization of the bundles was acquired independently both in some Commelinales and in eudicots.

5. CONCLUSION

Our contributions lead to a more accurate understanding of the apocole and shed light on the fact that palms have an eustelic bundle organization in this structure. More studies are needed to confirm whether this same bundle organization is present in other palms with remote germination.

6. ACKNOWLEDGEMENTS

We acknowledge Capes for the doctoral grant for the first author and Paulo Linhares for the seeds provided.

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SUPPLEMENTARY DATA

1. PHENOLOGICAL OBSERVATIONS ON *PHOENIX ROEBELENII* O'BRIEN GERMINATION

1.1. MATERIAL AND METHODS

Germination was carried out both on Petri dishes and on disposable coffee cups. For each treatment, a total of 100 seeds were employed.

For the Petri dishes treatment, all material was firstly sterilized. The seeds were immersed in ethanol 70% for 1min and sodium hypochlorite (NaClO) 2% for 2min and were then washed in distilled water. In a sterilized chapel, five seeds were disposed on each Petri dish over a filter paper. The Petri dishes were sealed with Parafilm and were put in a BOD incubator greenhouse. Every 3 days the samples were watered with 1ml of distilled water, for a total of 90 days.

Seeds were also planted in the sand within disposable coffee cups. For this proceeding, seeds were disinfected only with ethanol 70% for 1min. PVC was used to cover the top of the disposable cups, to avoid contamination. Samples were put in a germination chamber and remained there for 120 days, being watered every 5 days.

1.2. RESULTS

Phoenix roebelenii seeds are elliptic and small, with 10-12mm length and 4-5mm width, presenting a brownish tegument. A deep furrow that occurs along the whole length of the seed divides it in two parts (Fig. S1A). A cross section performed along the seed illustrates that the integument is thin, but in the region that corresponds the furrow this structure goes inwards, occupying the whole central part of the seed in some cases (Fig. S1B). The endosperm is the white and starchy tissue that occupies the rest of the seed, and on the opposite side of the deep furrow there is a cavity where the hilum is located (Fig. S1B). Nevertheless, in some individuals the furrow does not occupy such a wide space inside the seed (Fig. S1C). When seeds are not viable, the endosperm shows a dark color instead of white (Fig. S1D).

Germination rate was of 60% for the seeds cultivated in petri dishes (Fig. S1E). Nevertheless, many of the germinated apocole did not even form the first root, once some seeds were found dry a few times during the experimental observations, while others were found with fungus, which means that watering frequency was not sufficient or sterilization was not efficient enough. After 40 days, only 28,3% of the total seedlings formed the first roots, and after 60 days, only 3,3% of the total seedlings formed prophylls.

The first seedlings of *Phoenix roebelenii* germinated 48 days after they were disposed in the petri dishes. By this time, the apocole was visible as a small spot (Fig. S1F). After 62 days, a peak was observed, with 43 of the 60 seeds emitting apocole. The rate of seedlings death raised afterwards due to dryness or contamination by fungi. Indeed, as development occurred, seeds were found every time drier, even though they were watered with the same amount of water. It seems the seedlings need higher levels of moisture as development occurs. The apocole grew and bend in a 90° angle, and from it the first root emerged 61 days after the beginning of the experiment, and the first prophyll about 70 days after it (Figs. S1G-I). Some seeds grew to the point of emitting the second cataphyll (Fig. S1J).

Figure S1. Process of germination in *Phoenix roebelenii* O'Brien. (**A**) *Phoenix roebelenii* seed. (**B**) Longitudinal section of a viable seed. (**C**) Cross section of a viable seed, evidencing the embryo. (**D**) Longitudinal section of a non-viable seed. (**E**) Seeds disposed in a Petri dish. (**F**) Apocole germinating from the seed. (**G**) Apocole growing and bending. (**H**) First root being formed in the apex of the apocole. (**I**) Prophyll emerging from the apocole. (**J**) Germinated seedling. **Scale bars: A-D:** 2,0mm; **E:** 1,0cm; **F-J:** 2,0mm.

For seeds planted in the sand, germination rate was of 42%, considering the time when the first prophylls emerged from the soil (Fig. S2A). These first prophylls appeared 44d after seeds were put to germinate. Seedlings continued to grow (Fig. S2B), and in average with about 72d the aerial part reached 5cm tall (Fig. S2C), size from which they started to be removed from the sand for desiccation. However, germination was very heterogeneous (Fig. S2D), and some seedlings emerged only 23d after the first ones had emerged from the soil.

Once seedlings were removed from the sand, it was observed that the underground part was as long as or even longer than the aerial part (Fig. S2E), even though in many cases roots were confined to the reduced space of the disposable coffee cups. The apocole is connected to the ventral side of the seed in one of its sides and to a sheath that surrounds the seedling base on the other side. First root emerges from the lowest part of this sheath, growing downwards (Fig. S2F).

Plicate leaves started to dissect only after the plant has reached more than eleven months. By this time, a plicate leaf emerged with two leaflets at its base. The first completely dissected leaf appeared two months later (Fig. S2G).

Figure S2. Germination of *Phoenix roebelenii* O'Brien in the sand. Prophyll emerging from the sand after germination. (**A**) Seed with 44 days, (**B**) 51 days and (**C**) 72 days. (**D**) Heterogeneity of sizes among seedlings. (**E**). Seedling germinating. (**F**) Seed connected to the seedling sheath by apocole. (**G**) First dissected leaf of the sample.