


Floral structure in the Asian palm *Eugeissona tristis* Griff. (Arecaceae: Calamoideae), and description of a new nectary type in the family

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Abstract The South-East Asian palm genus *Eugeissona* has an unusual flower structure, and it occupies a basal position in the early divergent palm subfamily Calamoideae. This article describes the floral structure of *Eugeissona tristis*, a palm that has been associated with an uncommon pollination syndrome in which non-flying mammals ingest large amounts of fermenting nectar produced by flowers. Our study focuses on the architecture of the nectaries responsible for this nectar production. The nectariferous tissues differentiate in four histological regions along the length of the gynoeceium, and appear to be primarily associated with ventral slits of the carpels, corresponding to pollen tube transmitting tracts (PTTT). Nectariferous tissues also develop along the carpel septa—as is typical for the Monocotyledons—but to a much lesser extent. A comprehensive review of the nectariferous systems diversity in palms confirms that it represents an undescribed type of nectary unique to the family, and demonstrates a topographical shift when compared to the septal or labyrinthine types typical of other palms. The non-flying mammal pollination syndrome observed in *Eugeissona tristis* is described and compared with similar syndromes in other angiosperms.

Keywords Anatomy · Calamoideae · Flowers · Morphology · Nectaries · Non-flying mammals · Palms

Introduction

The genus *Eugeissona* Griff. is the single member of tribe Eugeissoneae Griff. and belongs to Calamoideae, according to the most recent palm phylogenies (Asmussen et al. 2006; Baker et al. 2009; Baker and Couvreur 2012) an early-divergent palm subfamily comprising 21 genera and around 609 species distributed pantropically, but mostly diversified in the Old World (Dransfield et al. 2008). From the six currently recognized species in the group, four are restricted to Borneo (*E. ambigua* Becc., *E. insignis* Becc., *E. minor* Becc., *E. utilis* Becc.), whereas two have been reported in Peninsular Malaysia and Peninsular Thailand (*E. tristis* Griff., *E. brachystachys* Ridl.) (Dransfield et al. 2008; Henderson 2009; Barfod and Dransfield 2013), in general growing in lowland forests under high rainfall conditions in swampy areas, but also reported in hill slopes and ridge tops in hill dipterocarp forest, to 1000 m altitude (Barfod and Dransfield 2013). There is no modern taxonomic revision for the genus *Eugeissona*, and the most comprehensive treatment remains the work of Beccari (1918), who proposed detailed descriptions as well as highly informative plates representing the gross morphology of vegetative and reproductive organs of several species. Important efforts were undertaken by Dransfield (1970), who studied different aspects on the morphology and anatomy of this genus. *Eugeissona* has raised increasing interest from an evolutionary perspective among the palm research community because of its most surprising phylogenetic position. Although only low to moderately supported, *Eugeissona* showed a sister relationship with the rest of the

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Calamoideae, in turn sister of all other palms and thus becoming the earliest divergent palm lineage recovered so far in the family (Asmussen et al. 2006; Dransfield et al. 2008; Baker et al. 2009; Baker and Couvreur 2012).

The relatively large size of vegetative and reproductive organs in *Eugeissona*, with leaves and inflorescences up to 3 m long and flowers up to 9 cm long, in part explains the scant morphological and anatomical knowledge on these palms. Palm flowers are in general small (Dransfield et al. 2008; Nadot et al. 2011), but in the case of *Eugeissona* the flowers are very large and this has strongly hindered the use of traditional anatomical techniques. According to Uhl and Dransfield (1984) members of *Eugeissona* produce the largest staminate and hermaphrodite flowers in the family, only being reported as larger the flowers in *Lodoicea* (Coryphoideae: Borasseae) and members of Phytelphaeae (Phytelphantoideae). Some studies have nevertheless contributed with important data on the genus. Details on the perianth organs and the gynoeceum were first described by Bosch (1947), whereas thorough developmental aspects on the androeceum and the gynoeceum in the group were provided by Uhl and Dransfield (1984). The gynoeceum of this palm was not included in the classical study by Uhl and Moore (1971), but the recent study by Bobrov et al. (2012) provided detailed description of its complex carpel anatomy and fruit development. In this investigation the “incompletely trilocular” condition of the gynoeceum, previously identified by Dransfield and Uhl (1998) and Dransfield et al. (2008) for all Calamoideae, was confirmed.

The ecology of *E. tristis* was initially studied by Wong (1959). Further observations by Wiens et al. (2008) demonstrated that the species displays a remarkable and fascinating non-flying mammal pollination syndrome, until now only scantily reported in palms (Barfod et al. 2011). In the case of *E. tristis*, the most frequent nocturnal visitors were the Penta-tailed treeshrews (*Ptilocercus lowii*) and the Slow loris (*Nycticebus coucang*), the first consuming high

amounts of fermenting nectar produced by the flowers, with alcohol doses that would intoxicate humans. The succession of floral stages, nectar production and fermentation of the floral nectar of *E. tristis* are unusual. Flowering within a population is year-round and flowering of one inflorescence extends to about 3–4 months. The staminate flowers open first followed by the hermaphrodite ones. Nectar production is initiated in both hermaphrodite and staminate flowers during bud stage, before pollen and stigmas are fully exposed, but also both flower types with no nectar production occur. Nectar started fermenting while the flower was still in bud and after some time of fermentation the maximum concentration of alcohol recorded in nectar reached 3.8 %, which ranks among the highest concentrations reported in a natural food (Wiens et al. 2008). The origin of the highly unusually abundant nectar produced by the flowers and the overall architecture of the nectaries responsible of its production were not evaluated in *Eugeissona*; this encouraging us (1) to conduct structural studies of all floral whorls in the hermaphrodite and staminate flowers of *Eugeissona*, emphasising nectary morphology, (2) to compare the aforementioned nectary with septal and labyrinthine nectaries reported in other palms, and (3) to compare the non-flying mammal pollination syndrome currently understood in palms with that already described in other angiosperm families.

Materials and methods

Plant material

Liquid fixed material used for our study was collected as part of the ecological studies undertaken on Malaysian wild populations of *E. tristis* by Frank Wiens during four field trips between 2003 and 2005 (Table 1) in the Segari Melintang Forest Reserve in Manjung District, Perak,

Table 1 Plant material included in this study

| Species | Collector and collection number | Spirit collection | Floral bud type ^a |
|---------------------------------------|---------------------------------|-------------------|------------------------------|
| <i>Eugeissona brachystachys</i> Ridl. | Dransfield 621 | BH, K | – |
| <i>E. tristis</i> Griff. | Moore 9059 | BH | – |
| <i>E. tristis</i> Griff | Moore 9105 | BH | – |
| <i>E. tristis</i> Griff | Gottsberger 11-260196 | G | I, II, III, IV |
| <i>E. tristis</i> Griff | Wiens s.n. | G | I |
| <i>E. tristis</i> Griff | Wiens s.n. | G | II |
| <i>E. tristis</i> Griff | Wiens s.n. | G | III |
| <i>E. tristis</i> Griff | Wiens s.n. | G | IV |
| <i>E. utilis</i> Becc. | Moore 9219 | BH | – |

^a Structural and functional floral bud types identified in *Eugeissona tristis* I: nectariferous male flower bud, II: non-nectariferous male flower buds, III: nectariferous hermaphrodite flower bud, IV: non-nectariferous hermaphrodite flower buds

West-Malaysia (4°18'N, 100°34'E) and by Ilse Silberbauer-Gottsberger in January, 1996 on a field trip in the Ulu Gombak Forest Research area, 30 km from Kuala Lumpur., 3°15'N, 101°40'E. Based on field observations this material was preliminarily classified by Ilse Silberbauer-Gottsberger in four functional flower bud types, depending on the presence or absence of nectar production. Additional flower buds in pre-anthetic stages of *E. brachystachys*, *E. tristis* and *E. utilis* species were sampled from the spirit collection of the Bailey Hortorium Herbarium (BH) (Table 1).

Morphology and anatomy

Hermaphrodite and staminate flower buds (Fig. 1a, b) were dissected in ethanol (70 %) under a Stereomicroscope (Wild M3B) for gross morphological studies. For histological and anatomical studies flower buds were dehydrated and embedded in Kulzer's Technovit 7100 [2-hydroxyethyl methacrylate (HEMA)] as described by Igersheim and Cichoki (1996) and then serial sectioned between 5 and 15 µm on a rotary microtome (Leitz 1512) with a conventional microtome knife type C. Sectioned material was later stained with ruthenium red and toluidine blue and mounted in Histomunt. All permanent slides, including slides produced in the lab of Ilse Silberbauer-Gottsberger [Department of Systematic Botany and

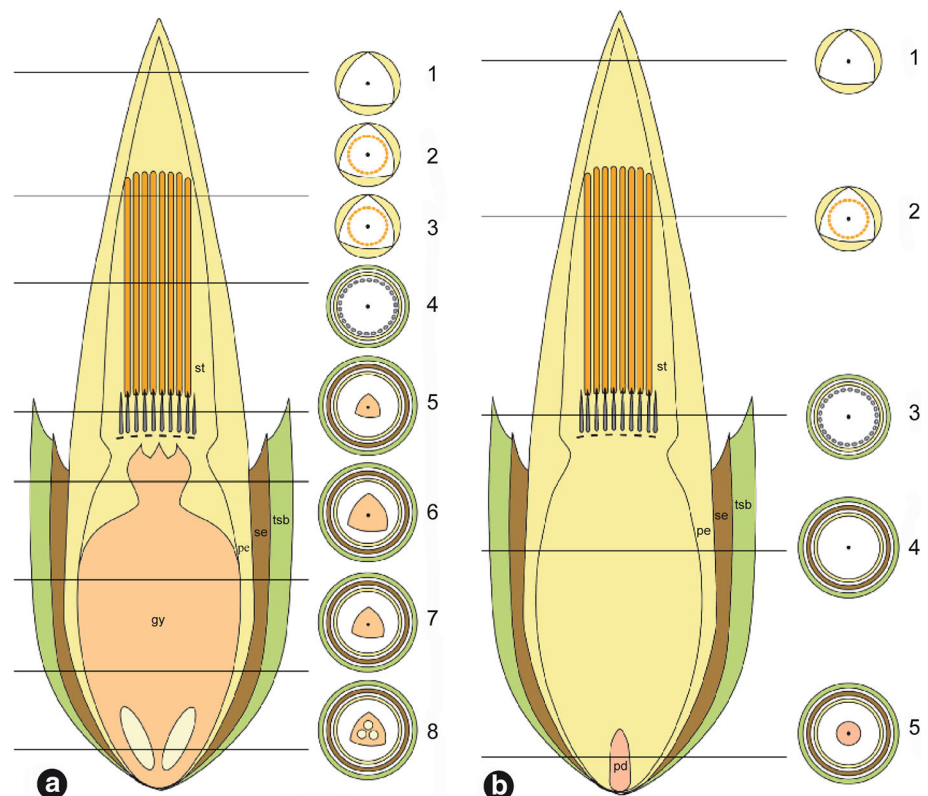
Ecology (University of Ulm)] are deposited in the Laboratory of Micro-morphology of the Conservatory and Botanical Garden of Geneva (CJBG). Images of the anatomical sections were captured with a light microscope (Nikon Elipse80i), using the Java imaging processing software for the microscope NIS-Elements (Nikon), then stitched with the Java imaging processing and analysis software Image J and finally edited in Adobe Photoshop Elements (version 6.0) and Adobe InDesign (version CS5). For scanning electron microscopy (SEM) studies, the dissected specimens were dehydrated in an ethanol series and acetone, and then critical point dried, and sputter-coated with gold. Micrographs were obtained using a Zeiss DSM 940A scanning electron microscope (Orion 6.60 Imaging System) at the Natural History Museum of Geneva.

Results

General structure of the inflorescence, the flower cluster and individual flowers

Inflorescences in *Eugeissona* are terminal, erect, very variable in size (1 to 3 m long), composed by lateral branches. Each inflorescence has two types of flowers, hermaphrodite and staminate (Fig. 1a, b), borne in dyads (Fig. 2a) surrounded by several cupular bracts. The flowers

Fig. 1 Schematic reconstruction of hermaphrodite and staminate flowers in *Eugeissona tristis* (longitudinal view). **a** Hermaphrodite flower; verticilles represented at eight different levels. **b** Staminate flower; verticilles represented at five different levels (note reduced pistillode with respect to the fertile ovary shown in **a**). *gy* gynoeceium, *pd* pistillode, *pe* petals, *se* sepals, *st* stamens, *tsb* tubular subtending bract



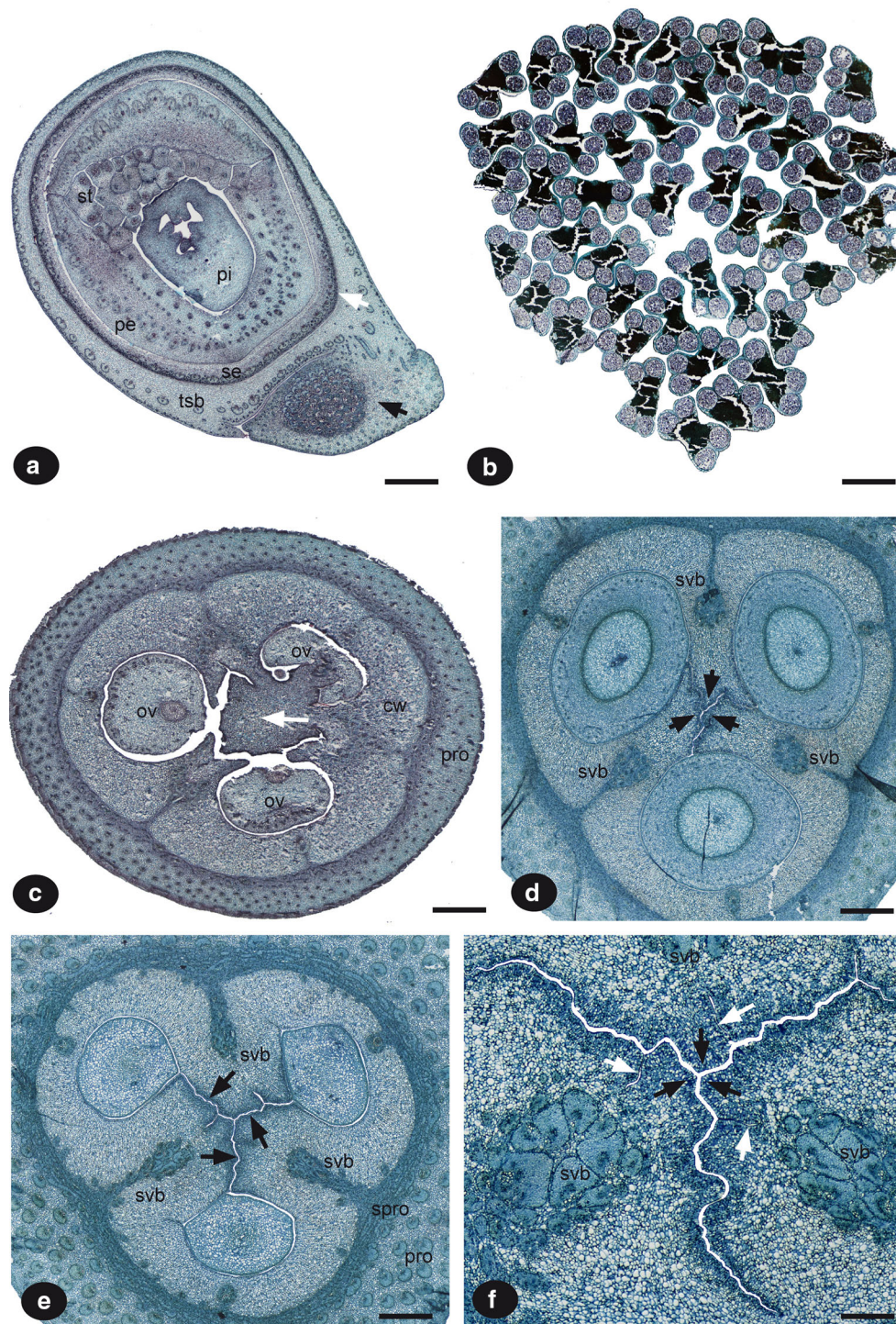


Fig. 2 Flower arrangement and floral structure in *Eugeissona tristis*. **a** Floral pair. Young staminate bud (*white arrow*) sectioned at mid-height and young hermaphroditic bud (*black arrow*) sectioned at basal level of pedicel, *scale bar* 650 μm ; **b** polyandrous androecium (42 stamens) sectioned at level of anthers, *scale bar* 600 μm ; **c** fertile gynoecium showing three equally developed carpels at the level of the ovary, *arrow* points to the central floral axis, *scale bar* 600 μm ; **d** symplicate gynoecium sectioned at mid-height level of the ovary, *arrows* point to nectariferous tissues associated to the ventral slits,

scale bar 600 μm ; **e** symplicate gynoecium sectioned towards the ovary tops, *arrows* point to nectariferous tissues associated to the ventral slits, *scale bar* 600 μm ; **f** detail of nectariferous regions in the ovary showing tissues associated to ventral slits (*black arrows*) and true septal nectaries (*white arrows*), *scale bar* 100 μm . *cw* carpel wall, *ov* ovule, *pe* petal, *pi* pistillode, *pro* peripheral region of the ovary, *se* sepal, *spro* sub-peripheral region of the ovary, *st* fertile stamens, *svb* synlateral vascular bundles, *tsb* tubular subtending bract

display a peculiar fusiform shape, narrowly lanceolate, 6.5–9.0 cm long \times 1.1–1.4 cm diam.; the petals are woody, very hard and spiny towards the top. The staminate flower develops first and is followed by a hermaphroditic one (Fig. 2a); the latter is apparently protandrous. Our observations show that flowers of both types may sometimes produce nectar. In the afternoon, and perhaps also during only one night, the flowers emit a strong vanilla-like perfume. The morphological and anatomical characterization of hermaphrodite and staminate flowers are here presented together due to the slight differences observed between them. Hermaphrodite flowers are similar in size and shape to the staminate flowers, except for the apex of petals, which is asymmetric in hermaphrodite flowers and symmetric in the staminate ones. Additionally, hermaphrodite flowers present a fertile gynoecium (Figs. 1a, 2c–e) whereas staminate flowers present a pistillode reaching different degrees of development (Figs. 1b, 3d, e).

The cupular bract

The flower is at the base surrounded by 9–13, tightly packed and spirally arranged bracteoles; each bracteole is open throughout its length or presents a solid tubular area extending up to mid-height. The bracteoles are 1.1–3.8 cm long \times 2.2–2.6 cm wide, with ciliated margins and sometimes with irregular invaginations towards the apex, apex acuminate or apically split in 2–3 segments, chartaceous and dark brown, being the innermost one frequently slightly membranaceous towards the base, smooth and glabrous. Incompletely developed bracteoles are rarely present; when slightly developed, lanceolate, 1.0–1.8 cm long \times 0.3 cm wide, margins entire, apex irregularly rounded, chartaceous, smooth and glabrous, dark brown. The epidermis of the bracteoles is composed of elongated cells. The mesophyll presents a complex histology; it is densely vascularized towards the dorsal region; it is

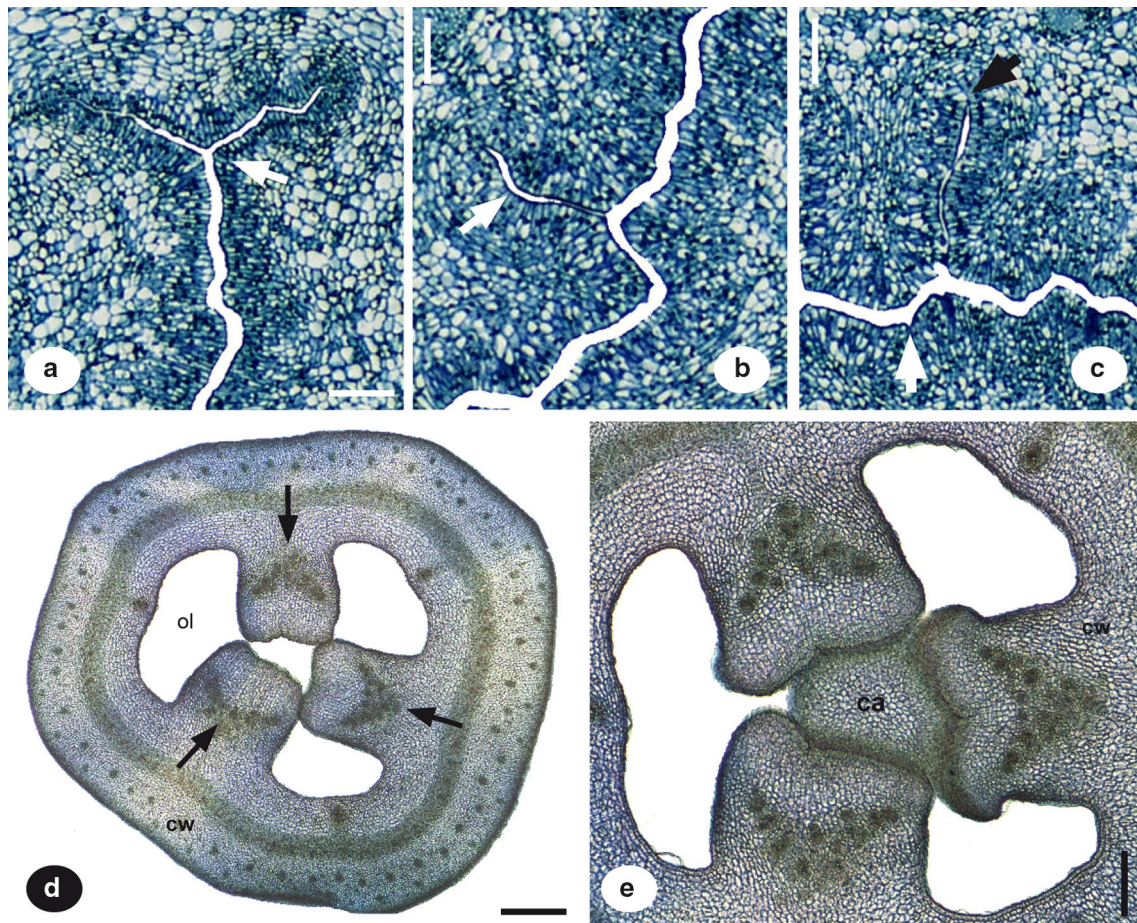


Fig. 3 Nectariferous tissues and pistillodes in *Eugeissona tristis*. **a** Detail of nectariferous epithelium associated to the ovary ventral slits (white arrow), scale bar 150 μ m; **b** detail of nectariferous epithelium associated to the true septal nectary between neighboring carpels (white arrow), scale bar 150 μ m; **c** detail of nectariferous epithelium associated to the ovary ventral slits (white arrow) and true

septal nectary between neighboring carpels (black arrow), scale bar 150 μ m; **d** general view of a non-nectariferous pistillode with arrows pointing to synlateral vascular bundles, scale bar 600 μ m; **e** detail of a non-nectariferous pistillode showing the extended floral axis, scale bar 300 μ m. *ca* central axis of the flower, *cw* carpel wall, *ol* ovary locule

composed of 4–5 layers of elongate parenchymatic cells and is less vascularized towards the ventral region. Vascular bundles are large and organized in a continuous row towards the dorsal region; they alternate with 1–3 adaxial girders of sclerenchymatic fibres. The vascular bundles are characterized by complex strengthening tissues such as thick bundle sheath of sclerenchymatic fibres, adaxial and abaxial bundle caps of sclerenchymatic fibres and abaxial bundle caps of turgid parenchyma.

The perianth

The calyx is composed by three sepals, which are fused (Fig. 2a) up to $\frac{2}{3}$ its length, 2.2–3.5 cm long \times 2.0–3.0 cm wide, with an apex with 3 apical segments, sepal tips emerging 5 mm from the top of uppermost subtending bracteoles; sepals are coriaceous, smooth and glabrous, light brown in the tubular region and dark brown towards the apex. The epidermis of the sepals is composed of elongated cells. The mesophyll presents in general the same complex histology as observed in the bracteoles towards the dorsal region; it is composed of 6–8 cell layers of parenchyma and produces only few smaller vascular bundles towards the ventral region (Fig. 2a). The woody corolla is composed by three petals, which are basally fused (Fig. 2a), apically valvate at bud stage and reflexed at anthesis; they are narrowly lanceolate, fused up to mid-length, valvate in the upper half of the corolla, 6.3–8.0 cm long \times 0.7–0.8 cm wide, margins entire, apex acuminate and asymmetrical, tubular region chartaceous, valvate region woody, brown, inner surface completely glabrous, smooth in the tubular region, slightly channelled due to mechanic pressure of the anthers in the valvate region; outer surface completely smooth, glabrous in the tubular region, covered by stellate trichomes in the valvate region. The epidermis of the petals is composed of elongated cells. The mesophyll resembles that observed in the bracteoles and the sepals and it is densely vascularized (Fig. 2a). Large vascular bundles concentrate towards the dorsal region of the petals; they are organized either in a continuous row at the tubular level of the petals (Fig. 2a) or in two alternating rows at their free level. Marginal sclerenchymatic fibres are well differentiated. Small and abundant girders of sclerenchyma fibres are spread throughout the mesophyll at the tubular region of petals, specially concentrated in the dorsal region, whereas in the free level of the petals they concentrate towards the ventral region.

The androecium

The androecium is composed by 20–26 stamens (Figs. 1a, b, 2b), all inserted at the same level at mid-height of the

corolla tube (Fig. 1a, b), 1.7–3.5 cm long \times ca. 1 mm wide; filaments connate at the base ca. 1 mm, linear to slightly sinuous, 0.5–1.5 cm long \times ca. 1 mm wide, light brown; anthers basifixed (Fig. 1a, b), inflexed in bud and spread at anthesis, linear, 1.7–2.3 cm long \times ca. 1 mm wide, brown, dehiscence extrose, apparently depauperate and sterile in nectariferous flower buds; pollen released through a longitudinal stomium. The epidermis of filaments is composed of isodiametric cells and the mesophyll is composed of undifferentiated parenchyma. It is served by only one central vascular bundle. The connective has a thin epidermis composed of isodiametric cells. The mesophyll is composed of isodiametric cells intermixed with abundant tanniferous idioblasts (Fig. 2b). It is served by one central vascular bundle. The pollen sac has a thin epidermis composed of isodiametric cells; the endothecium is continuous around the pollen sacs and composed by one cell layer; cell walls of the endothecium are clearly thicker towards the inner side; the intermediate layers are discontinuous and compressed. The pollen sacs of the thecae are separated by a septum extending to the stomium region; it is composed of 5–7 elongated parenchymatic cell layers.

The gynoecium and the pistillode

The gynoecium and the pistillode are syncarpous (Figs. 2c, d, 3d, e), 2.3–2.7 cm long \times 0.5–1.0 cm diam. synascidiate at the level of the base of the locules and symplicate from the base of the central protrusion up to the stigmatic region (Figs. 2c, 3d). The ovary is oblong-ovoid, with 3 more or less flattened sides, 2.0–2.3 cm long \times 0.5 cm diam., densely covered by imbricate minute scales developing basipetally. A pyramidal central protrusion differentiates among the three united carpels and carpelodes and it extends up to the mid-height of the ovary (Figs. 2c, 3e); the later presenting ventral slits that are slightly open from the base up to the stigmatic region (Fig. 2d, e). Above the level of the central protrusion they form a 3-radiate canal that alternates with three small differentiated slits corresponding to the carpel flanks (Fig. 2e, f). The mesophyll is at the level of the ovary differentiated in three regions. The peripheral region is formed by 45–50 cell layers (Fig. 2e); the vascular bundles are abundant in this region and present an acropetal-centrifugal direction. The sub-peripheral region is formed by 12–15 cell layers and is characterized by dense and minute procambial strands (Fig. 2e). This region presents two clearly differentiated areas in the gynoecium: the peripheral region with a highly vascularized mesophyll and the inner parenchymatic region composed by isodiametric cells. The vasculature of this region, including the one observed in the pistillode, is supplied by the main synlateral complexes (Figs. 2d–f, 3d, e), and also by the dorsal vascular bundles of the carpel (Fig. 2d, e).

The innermost region is composed by 70–80 cell layers of homogeneous parenchymatic cells. Tanniferous and raphide idioblasts are lacking in the ovary. The vasculature of the gynoeceium is at the base represented by a vascular bundle complex that irrigates the three carpels at the level of the ovary, but some traces branch also towards the dorsal region.

Three well developed, anatropous and crassinucellate ovules differentiate at the base of the ovary; they present an axillary placentation and completely fill the locular cavities (Fig. 2c–e). The funiculus is inserted at the base of the central protrusion and it is very thick (Fig. 2c); towards the ventral region it presents irregular outgrowths, one of them directly pointing to the micropyle and maybe interpreted as an obturator. The ovules have two integuments clearly differentiated. The outer one integument is extremely thick, composed by 50–60 cell layers and the inner one much thinner, composed by 5–10 cell layers. The micropyle is straight, formed by the two integuments and it is oriented towards the center of the gynoeceium. The ovules are vascularised by individual vascular bundles arising from the vascular bundle complex at the base of the ovary. The lateral and ventral vascular bundles of each ovary are in the intercarpellary regions and throughout the whole length of the gynoeceium merged in a synlateral vascular complex. The dorsal vascular bundles of the carpels are from the base of the ovary up to the stigmatic region connected with the sub-peripheral vascularization of the gynoeceium (Fig. 2d, e).

The stigmas are sessile, cylindrical up to mid-length, pyramidal towards the top, ca. 4 mm long × 5 mm diam. The stigmatic branches are connivent at bud and anthetic stages. They have unicellular papillae in the ventral sides and some are exposed to the dorsal areas. The ventral slits corresponding to the inner surfaces of the gynoeceium are branched and show an undulated and convoluted appearance, from the upper level of the ovary up to the stigmatic region. Disperse vascular bundles were observed in the stigmatic branches. Tanniferous and raphides idioblasts are lacking.

The nectary

The nectary differentiates in four major zones that differentiate in the symplicate region of the gynoeceium. It extends from the level of the base of the locules (Fig. 2c) up the stigmatic region and is characterized by a well differentiated epithelium lining the ventral slits of the carpels. The zones identified include: (1) the pyramidal central protrusion that emerges from the main floral axis (Fig. 2c), extending up to the mid-height of the ovary, (2) the ventral and sub-ventral regions of the locule walls, basically represented in the locular level, (3) the ventral

slits of the carpels (Figs. 2d–f, 3a), open from the mid-height of the central protrusion up to the level of the stigmas; it forms a tri-radiate canal above the top of the central protrusion in the apical half of the ovary (Fig. 2f) and it is undulated in the gynoeceium (Fig. 3a) and non-undulated in the pistillode, and (4) the short slit-like carpel flanks representing the true septal nectaries (Fig. 2f); they alternate with the tri-radiate canal formed by the ventral slits in the apical half of the ovary (Figs. 2f, 3b, c). In the fertile gynoeceium the epithelium represents a large surface along the symplicate region of the gynoeceium, extending from the base of the locular region up to the stigmas. In the pistillode, when present, the epithelium of the nectariferous system in the locular region is composed by nectariferous, less papillated cells organized in a 7–10 layers whereas in the supralocular region it is composed by only a one cell layer of elongated papillae. The nectar produced in the four regions of the gynoeceium is released at the level of the stigmatic region.

Discussion

Unique reproductive features in the genus *Eugeissona*

Eugeissona displays an array of reproductive characters unique to the early divergent subfamily Calamoideae and palms in general. Uhl and Dransfield (1984) first highlighted its isolated position in an alliance of its own within the lepyocaryoid palms (now Calamoideae) based on the apparently monopodial flower clusters. *Eugeissona* is also unique among all Calamoideae because of the large number of stamens (up to 70) and being the only genus in the subfamily for which the polyandrous condition (i.e. androecia with more than 6 stamens) is represented in all the species. Polyandry has been reported in about 43 % of all palm genera and in Calamoideae identified in about 30 % of the currently recognized genera, in the tribes Eugeissoneae (*Eugeissona*), Lepidocaryeae (*Raphia*) and Calameae (*Calamus*, *Korthalsia* and *Plectocomia*) (Dransfield et al. 2008). According to Nadot et al. (2011), polyandry is a derived condition in the palm flower, evolving numerous times independently during the diversification of the family.

The present study shows that *Eugeissona* palms possess an unusual gynoeceium structure within Arecaceae, especially with respect to carpel fusion and differentiation of complex nectariferous regions. Our results confirm studies (i.e. Dransfield and Uhl 1998; Dransfield et al. 2008; Bobrov et al. 2012) supporting the syncarpous condition of the gynoeceium in members of Calamoideae. The gynoeceium of *Eugeissona* originates from three individual

carpels arising from the flanks of the floral apex (Uhl and Dransfield 1984), which fuse later in development; it is characterized by three complex synlateral vascular bundles resulting of the fusion of adjacent lateral bundles of neighbouring carpels.

In the syncarpous gynoecium of Angiosperms the carpel plication may range from synascidiate to symplicate (Endress 1994); in the case of the palms, intermediate states of plication may be identified depending on the level of the ovary studied. Although Bobrov et al. (2012) described the gynoecium of *Eugeissona* as completely symplicate, our study shows that the carpel plication is more complex and results in the peculiar nectariferous system. The gynoecium in these palms is clearly synascidiate at the base of the locules whereas it becomes symplicate from this level up to the stigmas. The synascidiate region of the gynoecium is structurally and functionally much simpler than the symplicate region; the latter differentiating in the four topographical regions attributed to the nectary (i.e. the central protrusion, ventral and sub-ventral region of the locule walls, ventral slits and the slit-like carpel flanks representing the true septal nectary). Published data (i.e. Uhl and Moore 1971) show that carpel fusion in palm flowers is strongly diverse and its implication in the evolution, systematics and reproductive biology of the family requires further exploration. The gynoecium is completely syncarpous in several lineages of Calamoideae, as shown in our results on *Eugeissona* and also reported in members of Lepidocarpaceae-Mauritiaceae (Guevara 2010; Guevara et al. 2010), completely apocarpous in Nypoideae (Uhl and Moore 1971; Stauffer et al. unpubl. data.), apocarpous at the level of the ovary and postgenitally syncarpous at the level of the stigmas in some Coryphoideae (Stauffer et al. 2009; Rudall et al. 2011; Guevara et al. 2014), and postgenitally syncarpous at the level of the ovary in Ceroxyloideae (Balhara et al. 2013) and several tribes of Arecoideae (Dransfield et al. 2008; Rudall et al. 2003; Stauffer and Endress 2003; Stauffer et al. 2002, 2004, 2009; Villimova and Stauffer 2013; Castaño et al. 2014).

Diversity of nectariferous systems in the palm family

Septal nectaries are prevalent within the monocots and largely dominant within the palms (Silberbauer-Gottberger 1973, 1990; Schmid 1983; Kuchmeister et al. 1997; Stauffer et al. 2009). They result in an incomplete fusion of the carpel flanks and they are mostly present at the level of the ovary, appearing as a straight cleft of densely stained epidermis in the carpel flanks (Stauffer et al. 2009). In palms labyrinthine nectaries are much more unusual than the septal nectaries; in fact only known for the Asian species *Licuala peltata* (Coryphoideae). Labyrinthine architecture includes carpel flanks in an extremely

undulated and convoluted structure; hence they are derived from septal nectaries (Stauffer et al. 2009). The nectaries in this palm continuously produce large amounts of nectar, making the flowers attractive to bees and other insects with high energy requirements (Barfod et al. 2011).

Our study shows that the complex nectary observed in the gynoecium of *Eugeissona* represents a type of nectary not yet described for the palm family and supports the isolated position of the genus within Calamoideae. The *Eugeissona* type of nectary clearly differs in topography and with respect to what has been described so far for the septal and labyrinthine nectaries. In these two types of nectaries the secretory tissues are always associated to the carpel flanks in a postgenitally syncarpous ovary. The *Eugeissona* type of nectary is mainly associated with the ventral slits of the carpels, corresponding to pollen tube transmitting tracts (PTTT), and representing a topographical shift with respect to what can be observed in the septal or labyrinthine types. The *Eugeissona* type of nectary extends from the level of the base of the locules up to the stigmatic region and is characterized by a well differentiated epithelium lining the ventral slits of the carpels. Although still fragmentary, available data suggests that this type of nectary may be much more represented in Calamoideae than previously understood. Indeed, floral studies in other Calamoid palms confirm the lack of the septal nectaries in the gynoecia of this group, suggesting alternative nectariferous histological sources. A relatively similar type of secretory tissue to that observed in *Eugeissona*, although clearly much less differentiated, was identified by Guevara (2010) in *Mauritia flexuosa* (Lepidocarpaceae: Mauritiaceae), and van Heel (1988) indicated that possibly the PTTT epidermis and the extended floral axis of *Salacca edulis* Reinw. (now *S. zalacca* (Gaertn.) Voss) are secretory.

Non-flying mammal pollination syndromes in *Eugeissona* and other angiosperms

Dransfield (1970) noted a copious yield of fermenting nectar in *Eugeissona* and stated that the plants at anthesis smell alcoholic. His observations indicate trigonid bees visiting flowers to collect pollen during staminate anthesis; however, such visits do not occur during pistillate anthesis. Wiens et al. (2008) observed that several weeks before the exposure of stamens and/or stigmas, staminate and hermaphrodite flowers, still in bud stage produce abundant nectar. The exuding nectar is being incubated by yeasts, which transform the sugars into alcohol. The long-lasting flower buds function as brewing chambers, providing an environment conducive of fermentation by the yeast community. This closed structure also protects the nectar from complete depletion by nectar-licking animals. The maximum alcohol concentration recorded in the bud stage was

3.8 %. Seven species of small tree-climbing non-flying mammals weighing less than 1 kg visited the buds and flowers. During their visits, the mammals move up and down an inflorescence, lick off available nectar and pollen on staminate and hermaphrodite flowers and also move to other inflorescences. As all inflorescence stages of *E. tristis* are available in a population year-round, the alcoholic nectar is a non-seasonal food source for the pollinating mammals. It was calculated for one of the mammals, the Penta-tailed treeshrews (*Ptilocercus lowii*), that it frequently consumed alcohol doses from the inflorescences that would intoxicate humans, yet the flower-visiting mammals showed no signs of intoxication. The authors concluded "...that treeshrews cope with the risk of chronic high oral alcohol intake through an increase in effectiveness of the glucuronidation pathway of alcohol detoxification that keeps concentrations of alcohol in the blood and the brain low. This study has found chronic alcohol consumption by certain mammals within an ecological context suggestive of a beneficial effect of an alcohol rich diet." (Wiens et al. 2008). A study on Neotropical phyllostomid bats also shows broad patterns of apparent ethanol tolerance are, potentially indicating an important early step in the evolution of frugivory and nectarivory in those animals (Orbach et al. 2010). In this context, traits such as woodiness in the flowers of *Eugeissona tristis* are thus far interpreted as having a dual function. All known non-lignified herbaceous nectar producing counterparts cannot structurally support continuous licking and chewing combined with vertical traversing of relatively small mammals. Flowering parts in conjunction with long-lasting woody chewing chambers contribute to a system of enhanced attractiveness for visiting mammalian pollinators.

The mammalian orders and families, which are known to have one or several representatives recognized as non-flying pollinators are Polyprotodonta (Didelphidae), Diprotodonta (Dasyuridae, Phalangeridae), Rodentia (Muridae, Gliridae), Carnivora (Procyonidae, Viverridae), Artiodactyla (Giraffidae), Primates (Lemuridae, Lorisidae, Galagidae, Callithrichidae, Cebidae, Colobidae, Cercopitheidae) (Westerkamp 1999). Mammal-pollination syndromes in the palm family have also been reported for the Neotropical understory species *Calyptrogyne ghiesbreghtiana* (Linden and H. Wendl.) H. Wendl. (Arecaceae). But in fact, the main pollinators of *C. ghiesbreghtiana* are bats (Cunningham 1995; Tschakapa 2003), while the Mexican mouse opossum (*Marmosa mexicana*) is just an additional and less effective pollinator (Sperr et al. 2009). Thus, the first report of exclusively non-flying mammal pollination for the palm family was documented for *Eugeissona* (Wiens et al. 2008). Historically, mammals were regarded as occasional pollinators in palms, reported in only 3 % of studied taxa, whereas increasing evidence suggests that

beetles, followed by bees and flies, constitute the most important group (Barfod et al. 2011).

In addition to Arecaceae, non-flying mammal pollination occurs in several other Angiosperm families. Available studies (i.e. Kress and Beach 1994; Carthew and Coldingay 1997; Sperr et al. 2009) suggest that in the receptacle and all floral whorls, the perianth (tepals, petals), the gynoecium or the androecium (intra and extra staminal regions), are potentially responsible for nectar production. Genera involved in this particular syndrome are among others *Ravenala* (Strelitziaceae), *Ganua* (Sapotaceae), *Parkia* (Mimosaceae), *Freycinetia* (Pandanaeae), *Adansonia* (Malvaceae), *Banksia*, *Dryandra*, *Hakea*, *Protea* (Proteaceae), *Symphonia* (Clusiaceae), *Mucuna*, *Strongylocodon* (Fabaceae), *Mabea* (Euphorbiaceae), *Combretum* (Combretaceae), *Eucalyptus*, *Melaleuca*, *Callistemon* (Myrtaceae), *Marcgravia* (Marcgraviaceae), *Blakea* (Melastomataceae) (e.g. Faegri and van der Pijl 1979; Grünmeier 1992; Kress and Beach 1994; Endress 1994; Yumoto 2005; Gottsberger and Silberbauer-Gottsberger 2006; Leins and Erbar 2008). The aforementioned list includes few species exclusively pollinated by non-flying mammals. The majority of species hosting non-flying mammals as flower visitors and pollinators are indeed mainly pollinated by birds or bats. Often non-flying mammal pollination is derived from bird or bat pollination. On the other hand, it is also believed that certain plant species presently pollinated by bats were originally pollinated by non-flying mammals, thereby inferring that the legacy of non-flying mammalian pollinators was pre-adaptation to bat pollination (Sussman and Raven 1978; Heithaus 1982).

In opposition to most examples mentioned above, the non-flying mammal pollination syndrome of *Eugeissona tristis* and perhaps of other species of the genus is quite a particular case with clear morphological and physiological adaptations. The woody floral tube is a long-lasting, gnawing-resistant brewing chamber. The abundant nectar production, providing sugars, alcohols, yeasts and their by-products, creates optimal conditions attracting the mammal community year-round. It is most remarkable that the alcohol-containing nectar is not at all toxic to the mammals but probably has even a positive health effect. Our results suggest that the relationship of *E. tristis* and its mammals is highly co-adapted; however, further ecologic studies are recommended in order to identify whether these mammals would be also adapted to feed on other fermenting organs (i.e. fruits) produced by the palm. There remains the open question of why and how such a sophisticated pollination system, complex woody flower structure and extreme polyandrous condition has evolved in such an early-divergent palm lineage. One thing is clear, non-flying mammal pollination in *Eugeissona* is neither derived from

bat or bird-pollination and seemingly no continuation or repetition of this special pollination in any other palm group exists. The main question of how this particular syndrome arises from a common ancestor to the Calamoideae remains open, and will be answered in future structural studies on palm flowers and pollinator relations in this important and early unique palm lineage.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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