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Floral structure and development in the monoecious palm *Gaussia attenuata* (Arecaceae; Arecoideae)

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- **Background and Aims** Sexual dimorphism, at both the flower and plant level, is widespread in the palm family (Arecaceae), in contrast to the situation in angiosperms as a whole. The tribe Chamaedoreeae is of special interest for studies of the evolution of sexual expression since dioecy appears to have evolved independently twice in this group from a monoecious ancestor. In order to understand the underlying evolutionary pathways, it is important to obtain detailed information on flower structure and development in each of the main clades.
- **Methods** Dissection and light and scanning electron microscopy were performed on developing flowers of *Gaussia attenuata*, a neotropical species belonging to one of the three monoecious genera of the tribe.
- **Key Results** Like species of the other monoecious genera of the Chamaedoreeae (namely *Hyophorbe* and *Synechanthus*), *G. attenuata* produces a bisexual flower cluster known as an acervulus, consisting of a row of male flowers with a basal female flower. Whereas the sterile androecium of female flowers terminated its development at an early stage of floral ontogeny, the pistillode of male flowers was large in size but with no recognizable ovule, developing for a longer period of time. Conspicuous nectary differentiation in the pistillode suggested a possible role in pollinator attraction.
- **Conclusions** *Gaussia attenuata* displays a number of floral characters that are likely to be ancestral to the tribe, notably the acervulus flower cluster, which is conserved in the other monoecious genera and also (albeit in a unisexual male form) in the dioecious genera (*Wendlandiella* and a few species of *Chamaedorea*). Comparison with earlier data from other genera suggests that large nectariferous pistillodes and early arrest in staminode development might also be regarded as ancestral characters in this tribe.

Key words: Flower development, floral structure, *Gaussia attenuata*, Arecaceae, Arecoideae, Chamaedoreeae, monoecy, sexual expression.

INTRODUCTION

Flower structure is a key morphological character that governs the success of flowering plants. Many floral characters display complex evolutionary patterns that mirror the great diversity of angiosperms, a notable example being sexual expression and its diverse forms. Although it is considered likely that the ancestral angiosperm flower was bisexual, some room for doubt remains (Endress and Doyle, 2009). Hermaphroditism (the production of a single type of flower bearing functional male and female reproductive organs) is thought to account for 85–90 % of all angiosperms (Endress and Doyle, 2009; Diggle *et al.*, 2011). The remaining minority of species display either monoecy (separate male and female flowers on the same plant), dioecy (separate sex plants) or intermediate forms often referred to as polygamy. Sexual dimorphism is thought to provide a means to optimize resource allocation for reproduction in the plant (Sánchez and Pannell, 2011) and to promote

outbreeding and heterosis (Freeman *et al.*, 1997). The existence of separate male and female flowers may be a key element in plant–pollinator interactions (Barrett, 2013).

Given the wide variety of forms of sexual dimorphism that occur in angiosperms and the complex phylogenetic patterns underlying their distribution, there is considerable interest in focusing on specific clades that can serve as evolutionary case studies. A particularly relevant group in this context is the palm family (Arecaceae). This family contains 185 genera and ~2500 species (Govaerts and Dransfield, 2005; Dransfield *et al.*, 2008), confined mostly to the tropics. Floral sexual dimorphism is particularly prevalent in palms, in which it is the rule rather than the exception; in contrast with angiosperms as a whole, roughly four-fifths of palm genera are dioecious, monoecious or polygamous (Dransfield *et al.*, 2008). The most widespread type of sexual system is monoecy, which occurs in around 65 % of palm genera (Dransfield *et al.*, 2008) and which dominates in the largest subfamily, Arecoideae, within which only one of the
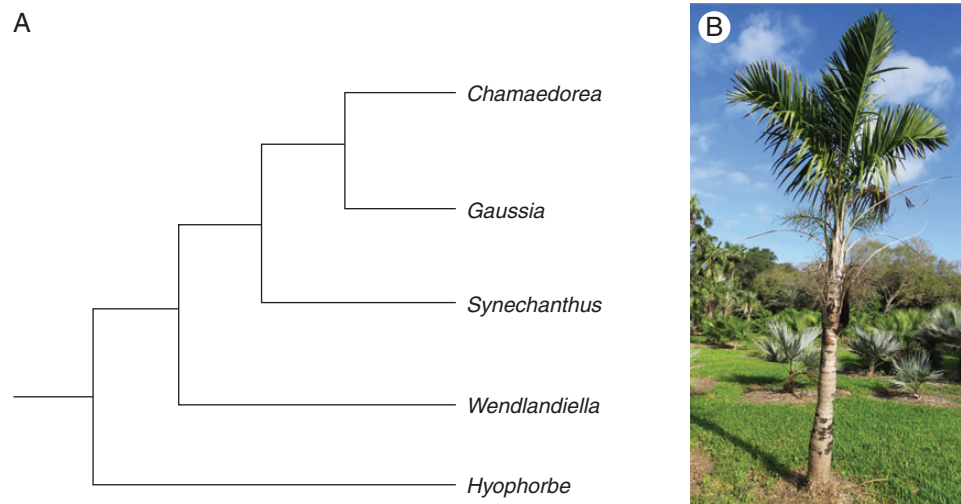
A

B

F_{IG}. 1. Schematic phylogeny of the tribe Chamaedoreeae and an adult specimen of *G. attenuata*. (A) Possible phylogenetic relationships between genera of the tribe Chamaedoreeae based on the studies of Cuenca *et al.* (2008). (B) Adult specimen of *Gaussia attenuata* (photo courtesy of L. Noblick, Montgomery Botanical Center).

14 tribes is not uniformly monoecious. This exception is the tribe Chamaedoreeae, which forms a monophyletic group of five genera, two of which are dioecious (*Chamaedorea* and *Wendlandiella*) and the remainder monoecious (*Gaussia*, *Hyophorbe* and *Synechanthus*). The tribe includes the most species-rich palm genus in the neotropics, *Chamaedorea*, which displays high variability in its vegetative and reproductive morphology. Moreover, the tribe exhibits one of the most extraordinary disjunctions in the palm family, with *Hyophorbe* endemic to the Mascarene Islands and the other genera distributed in northern South America, Central America and the Caribbean (Cuenca *et al.*, 2008; Dransfield *et al.*, 2008). The tribe Chamaedoreeae has been resolved as monophyletic with high support in several phylogenetic studies based on morphological and molecular data (Uhl *et al.*, 1995; Hahn, 2002; Asmussen *et al.*, 2006; Cuenca and Asmussen, 2007; Cuenca *et al.*, 2008, 2009; Baker *et al.*, 2009, 2011). The possible phylogenetic relationships of the component genera, as deduced from the aforementioned studies, are represented schematically in Fig. 1A. The inclusion of Chamaedoreeae within the subfamily Arecoideae (Dransfield *et al.*, 2005; Asmussen *et al.*, 2006), as opposed to earlier classification within the Ceroxyloideae (i.e. Uhl and Dransfield, 1987; Dransfield and Uhl, 1998) raises evolutionary questions concerning sexual expression and the arrangement of flowers within the inflorescence. In the vast majority of palm genera, flowers are grouped in sympodial clusters or cincinni. The majority of species in the subfamily Arecoideae produce flowers clustered in triads or groups reduced from this presumed ancestral form (Moore, 1973; Uhl and Dransfield, 1987; Dransfield and Uhl, 1998; Dransfield *et al.*, 2008). The triad is a sympodial flower cluster consisting of two lateral staminate flowers and a central pistillate flower with three associated bracteoles (Uhl, 1976; Ortega and Stauffer, 2011). Chamaedoreeae is the only tribe in the Arecoideae that deviates from this general pattern, producing flowers arranged in a distinctive structure known as the acervulus (Uhl and Moore, 1978; Uhl and Dransfield, 1987; Ortega and Stauffer, 2011). The acervulus consists, in the case of the monoecious genera, of a variable number of male flowers arranged in a row that terminates with a basal female flower. In the dioecious

genera (*Chamaedorea* and *Wendlandiella*), acervuli consisting of male flowers only are observed in some species, others producing solitary male flowers. Although sexual system and flower cluster features have been considered important when inferring the evolutionary relationships among genera in the Chamaedoreeae, recent studies have suggested that multiple transitions occurred within the tribe between the presumed ancestral characters of monoecy and acervulus production on one hand and the derived states of dioecy and solitary flowers on the other (Thomas *et al.*, 2006; Cuenca and Asmussen, 2007; Cuenca *et al.*, 2009).

Developmental studies on a range of palm species have led to an understanding of the ontogeny and diversity of flower clusters (Uhl and Dransfield, 1984). The cincinnus structure mentioned above is a monochasium in which flowers follow a zigzag course along one side of the axis (Rickett, 1955). This type of growth produces floral clusters of various forms. The acervulus produced by members of the tribe Chamaedoreeae is currently considered as a reversed cincinnus composed of distinct lines of closely two-ranked unisexual, sessile flowers which are adnate to the rachillae (Uhl and Moore, 1978; Uhl and Dransfield, 1987; Dransfield *et al.*, 2008; Ortega and Stauffer, 2011). The proximal flower is usually female and the distal flowers male, with bracteoles that are reduced or not evident at anthesis. Acervuli display an almost exclusively protandrous floral maturation pattern. In some monoecious species, the occasional occurrence, towards the tips of rachillae, of acervuli composed of only male flowers, or even the production of solitary male flowers, has been reported (Quero, 1982; Quero and Read, 1986; Ortega and Stauffer, 2011).

As regards the evolution of sexual expression, monoecy and dioecy appear to have evolved independently on numerous occasions in palms (Weiblen *et al.*, 2000). It is estimated that dioecy appeared at least nine times during their evolution, from either monoecious or hermaphrodite ancestors. Very little is known of the mechanisms by which palms and their flowers acquire their male or female identity, yet there is great interest in studying this phenomenon as it would improve our understanding of the evolution of the palm flower and provide knowledge to improve

the productivity of cultivated palms and obtain new insights into the population dynamics of threatened species. In this context, the remarkable features of the tribe Chamaedoreeae with respect to sexual expression patterns and other floral characters have led us to investigate developmental and structural traits in one of its constituent genera, so as to improve our knowledge of sexuality in the palm family as a whole. If we are to understand how floral characters have evolved within and beyond the Chamaedoreeae, it is first necessary to obtain detailed knowledge of floral structure and development in all the main clades. Earlier studies have provided floral morphology and anatomy data for the genera *Hyophorbe* and *Chamaedorea* (Uhl and Moore, 1971, 1978; Uhl, 1978; Askgaard *et al.*, 2008; Ortega and Stauffer, 2011), with little information as yet available for *Synechanthus*, *Wendlandiella* and *Gaussia*. We describe here studies of flower and acervulus development in the species *Gaussia attenuata*, a monoecious, solitary, medium-size palm (Fig. 1B), endemic to Puerto Rico (Quero and Read, 1986; Henderson *et al.*, 1995) and known under the common name of Llume palm. *Gaussia* has proved to be monophyletic with high support (Cuenca and Asmussen, 2007), the genus consisting of five recognized species (Quero and Read, 1986; Moya *et al.*, 1991). Based on similarities in the vegetative condition with *Pseudophoenix* (a member of the sister subfamily Ceroxyloideae), Uhl and Dransfield (1987) considered *Gaussia* as the least specialized genus of the tribe and pointed out shared characters with *Hyophorbe* and *Synechanthus*. The most recent phylogenetic analyses have not produced unequivocal clarification of the relationships between the five genera of the Chamaedoreeae (Fig. 1A; Thomas *et al.*, 2006; Cuenca and Asmussen, 2007; Cuenca *et al.*, 2009; Baker *et al.*, 2011). Nevertheless, *Gaussia* has been recovered as sister to *Chamaedorea* with moderate to high support in analyses based on morphological or molecular data (Thomas *et al.*, 2006; Cuenca and Asmussen, 2007; Cuenca *et al.*, 2008, 2009; Baker *et al.*, 2011). Irrespective of the exact phylogenetic topology of the tribe, the genus *Gaussia* constitutes an important part of the jigsaw that needs to be assembled in order to understand how floral characters evolved in Chamaedoreeae and related clades. The data presented here were obtained with the aim of filling this notable gap in current knowledge.

MATERIALS AND METHODS

Plant material

Morpho-anatomical and ontogenetic studies of floral structure in *Gaussia attenuata* were performed on flowers at different developmental stages from early floral buds to flowers at or close to anthesis. Flowers were harvested from the living collection of the Montgomery Botanical Center (MBC) in Miami (USA). The study was limited by the number of available specimens, the availability of inflorescences at a suitable developmental stage and the need to obtain flowers without lethal consequences for the individual, which may result when a palm is extensively dissected. Fixation was carried out as previously described (Adam *et al*., 2005). Sepals and petals were removed from some flowers in order to facilitate observation of the inner organs.

Morphological analysis

Flowers were dissected for morphological study under a stereomicroscope (Wild M3B). Some parts of the dissected specimens were chosen for scanning electron microscopy (SEM). They were dehydrated, critical-point-dried and sputter-coated with gold at the Muséum d'Histoire Naturelle de la Ville de Genève (Geneva, Switzerland) and the Centre de Ressources en Imagerie Cellulaire (CRIC, Montpellier, France). Micrographs were obtained using a JEOL (JSM-6510LV) scanning electron microscope at the Bioimaging Center at the Faculty of Sciences (University of Geneva) and a Hitachi S4000 scanning electron microscope at the CRIC laboratory.

Anatomical analysis

The detailed anatomical and ontogenetic characterization of the *G. attenuata* inflorescence and flowers was carried out using paraffin embedding, followed by staining of sections with astra blue and fuchsin and their mounting with Eukitt medium in the Laboratory of Histotechnology, Biology Department at the Universidad Nacional de Colombia. Photo-micrographs of the anatomical sections were obtained using a Leica DMIRE2 microscope and a Leica DC 300F camera.

RESULTS

Development of the acervulus

The inflorescence of *G. attenuata* was interfoliar (Fig. 1B). It was ∼1 m in total length, about half of which corresponded to the peduncle. It was characterized by the presence of four to seven persistent tubular peduncular bracts. Basally, it was branched to two orders, though only first-order branches were seen to be produced at mid-length and distally. Each rachilla bore numerous spirally arranged acervuli (Fig. 2A shows a group of immature acervuli), typically composed of four unisexual flowers, although in some cases five flowers were observed. Only one of these flowers was female (see later). Each acervulus was subtended by a very short bract and it was in the axil of this bract that all flowers were initiated (Fig. 2B). The male flowers were initiated sequentially in a basipetal pattern that concluded with the initiation of the female flower (Fig. 2C). In each acervulus, the flowers were arranged in a zigzag pattern, although prior to anthesis they had the superficial appearance of being linearly disposed (Fig. 2A, C).

Once the male flowers reached anthesis, they were shed consecutively, so that the last flower to reach anthesis was the female one, located proximally with respect to the rachilla. The proximal acervuli were observed to display a more advanced stage of development than the distal ones. Thus, within a given inflorescence a range of flower developmental stages was seen, depending on flower position in the acervulus and on the position of this acervulus within the inflorescence. Six key stages of development were observed in the acervuli of the *G. attenuata* inflorescence (Table 1). Stage 1 corresponded to the earliest stage of development observed, all flowers of the acervulus being in bud (Fig. 2A, C). In the stage 2 acervulus, the distalmost male flower had reached anthesis, the second male flower being at late bud stage or only just starting to open, accompanied by a third immature male flower and one immature female flower
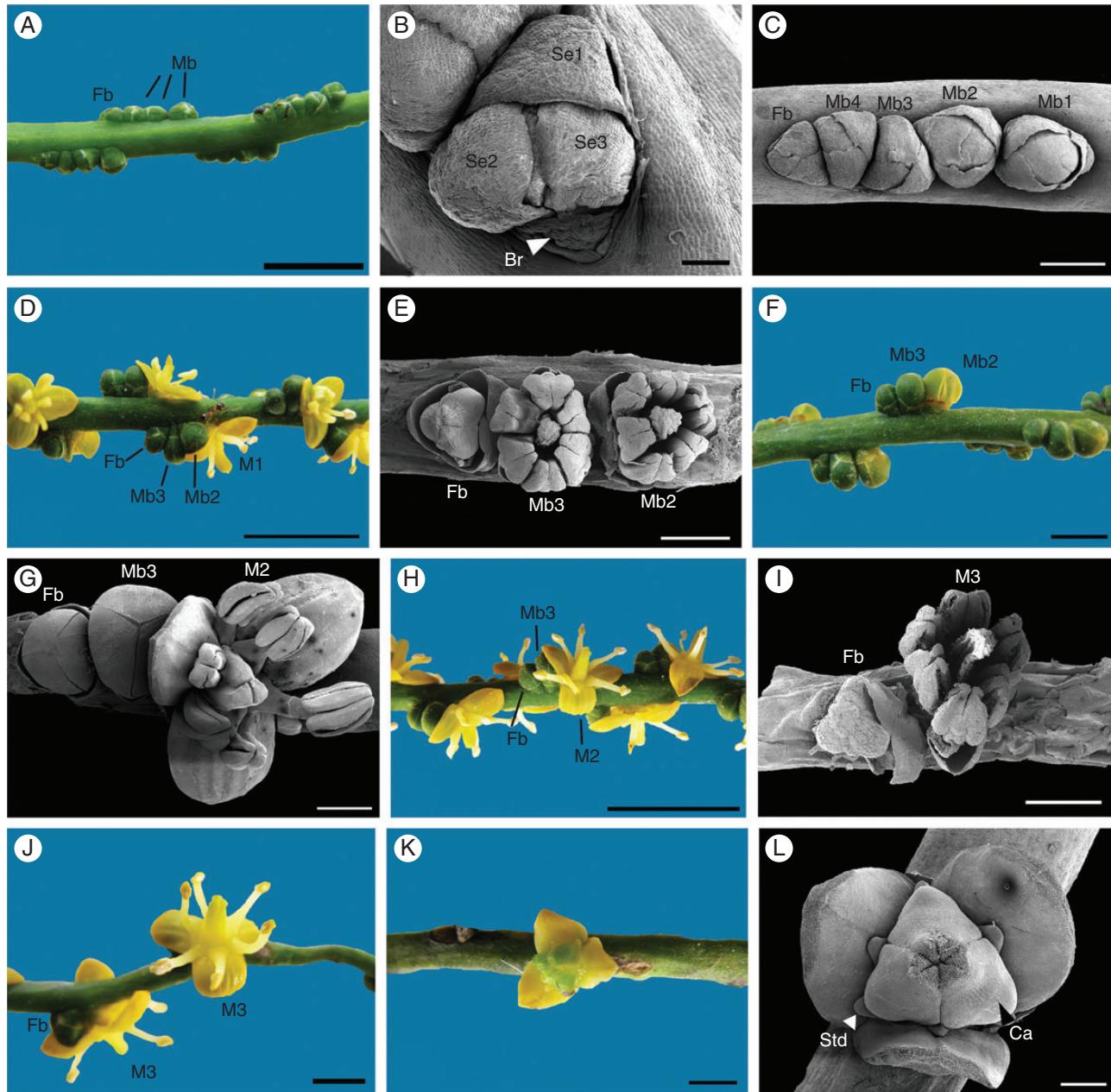
FIG. 2. SEM and macroscopic views of the developing acervulus in *Gaussia attenuata*. (A) Portion of rachilla showing acervuli with all flowers in bud (stage 1; Table 1). (B) Female floral bud and subtending bract. (C) Acervulus with all flowers in bud (stage 1; Table 1). (D) Portion of rachilla showing acervuli with one male flower at anthesis and two male flowers plus the female flower in bud (stage 2; Table 1). (E, F) Portion of rachilla showing acervulus with two male flowers plus the female flower in bud (stage 3; Table 1). Note that the petals have been removed in (E) and that one male flower has already been shed. (G, H) Portion of rachilla showing acervulus with one male flower at anthesis and one male flower plus the female flower in bud (stage 4; Table 1). (I, J) Portion of rachilla showing acervulus with one male flower at anthesis and the female flower in bud (stage 5; Table 1). Note that the petals were removed in I. (K, L) Portion of rachilla showing the female flower at anthesis. Scale bars: (A) = 5 mm; (B) = 200 μm; (C) = 1 mm; (D) = 5 mm; (E) = 1 mm; (F) = 2 mm; (G) = 1 mm; (H) = 5 mm; (I) = 1 mm; (J) = 1 mm; (K) = 1 mm; (L) = 500 μm. Br, bract; Ca, carpel; Fb, female floral bud; M1, first male flower at anthesis; M2, second male flower at anthesis; M3, third male flower at anthesis; Mb1, first male flower at bud stage; Mb2, second male flower at bud stage; Mb3, third male flower at bud stage; Mb4, fourth male flower at bud stage; Se1, first sepal; Se2, second sepal; Se3, third sepal; Std, staminode.

(Fig. 2D). At stage 3, one male flower had already been shed, the remaining two male flowers were still at bud stage and the female flower was still closed (Fig. 2E, F). The acervulus defined as stage 4 contained one male flower at anthesis and another male flower at late bud stage with the female flower in bud (Fig. 2G, H). At stage 5, a single male flower at anthesis was present, accompanied by one late bud stage female flower, the other male flowers having been shed (Fig. 2I, J). At stage 6, all the male flowers had reached anthesis and had been shed, only the mature female flower remaining (Fig. 2K, L).

Characters shared between male and female flowers

Figures 3–7 show the different developmental stages identified for male flowers (Figs 3 and 4) and female flowers (Figs 5–7) of *G. attenuata*. A number of characters were

TABLE 1. *Summary of developmental stages of the acervulus in* G. attenuata

Stage	Male flowers in bud	Male flowers open	Male flowers shed	Female flowers in bud	Female flowers open
1	3	–	–	1	–
2	2	1	–	1	–
3	2	–	1	1	–
4	1	1	1	1	–
5	–	1	2	1	–
6	–	–	3	–	1

observed to be common to male and female flowers. In transverse section, both types of flower were conspicuously triangular shortly after initiation, a characteristic particularly marked in female flowers (Figs 2B, C, 3E and 5B), although they became mostly rounded in late development. Although the pedicel was extremely short and the flowers were slightly sunken in the rachillae, a conspicuous floral base, 0·5–1 mm in height, could be observed (Figs 3D and 5A). Tissue organization at the base of the flowers was similar for both sexes at the different developmental stages observed. Once the sepals had separated from each other in this region and the petals had started separating, vascularization was observed in the form of an external ring composed of six vascular bundles and a central vascular complex formed by procambial strands (Figs 3E and 6C). The external ring clearly corresponded to the basal vascularization of the sterile or fertile androecium. The central complex integrated three already differentiated vascular bundles to serve the three lobes of the fertile or sterile gynoecium. The abaxial epidermis
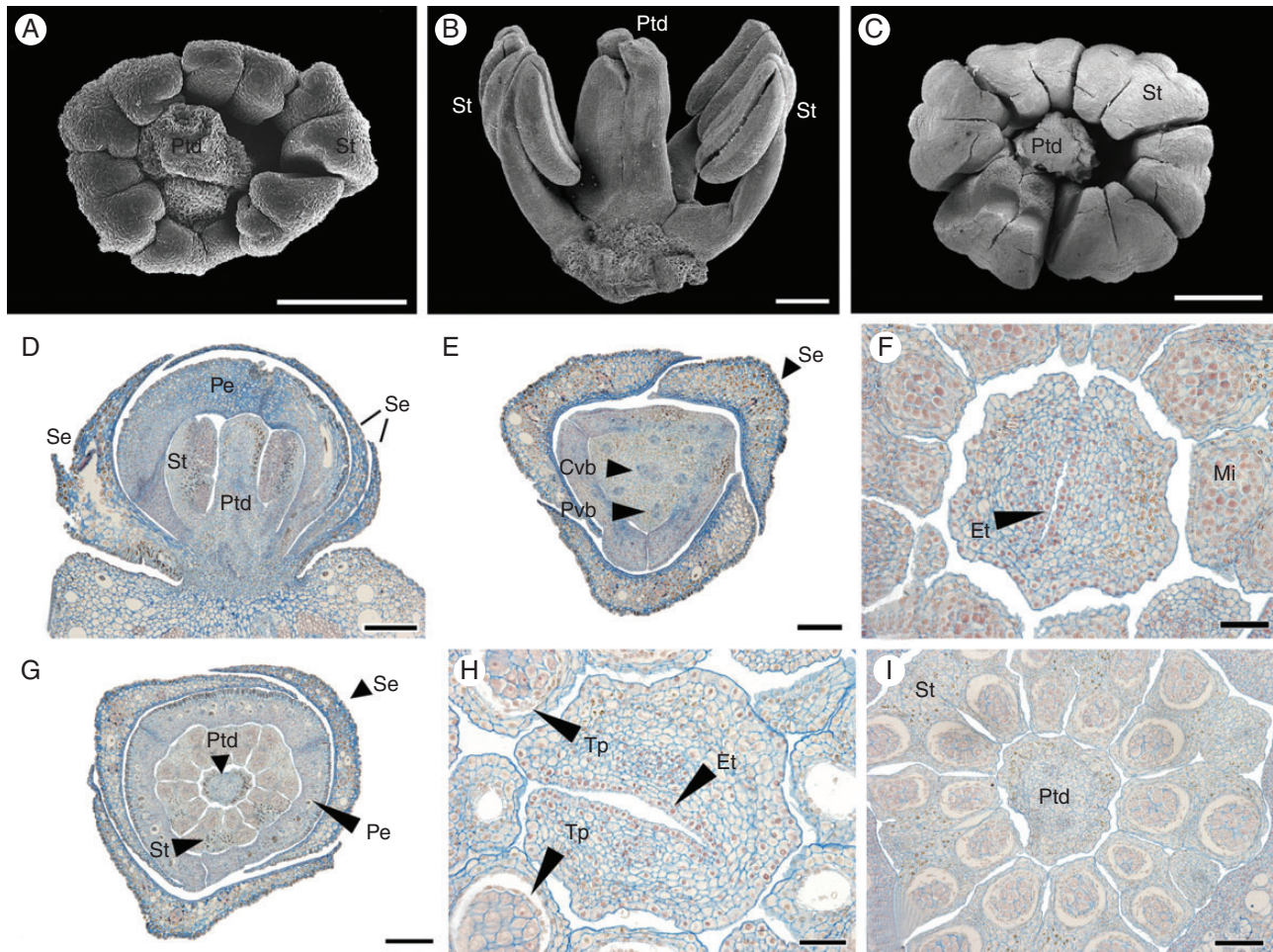

FIG. 3. Developmental stages of the male flower of *Gaussia attenuata*, I. (A) Male flower in early bud (SEM upper view, perianth removed). (B) Male flower in late bud (SEM lateral view, perianth and two stamens removed). (C) Male flower in late bud (SEM upper view, perianth removed). (D) Longitudinal section of the male flower in bud. (E) Transverse section of the male flower in early bud towards the basal region. (F) Transverse section of the male flower in early bud at mid-height level of the pistillode, revealing the early developing nectary and microspore mother cells. (G) Transverse section of the male flower in late bud towards the basal region of the pistillode. (H) Transverse section of the male flower at late bud stage towards pistillode mid-height, revealing tapetal degradation and a later stage of development of the nectary. (I) Transverse section revealing detail of the androecium and the pistillode of a late bud stage male flower. Scale bars: (A) = 250 μm; (B) = 500 μm; (C) = 500 μm; (D) = 200 μm; (E) = 200 μm; (F) = 50 μm; (G) = 200 μm; (H) = 50 μm; (I) = 100 μm. Cvb, central vascular bundle; Et, epithelial tissue; Mi, microspore mother cells; Pe, petal; Ptd, pistillode; Pvb, peripheral vascular bundle; Se, sepal; St, stamen; Tp, tapetum.
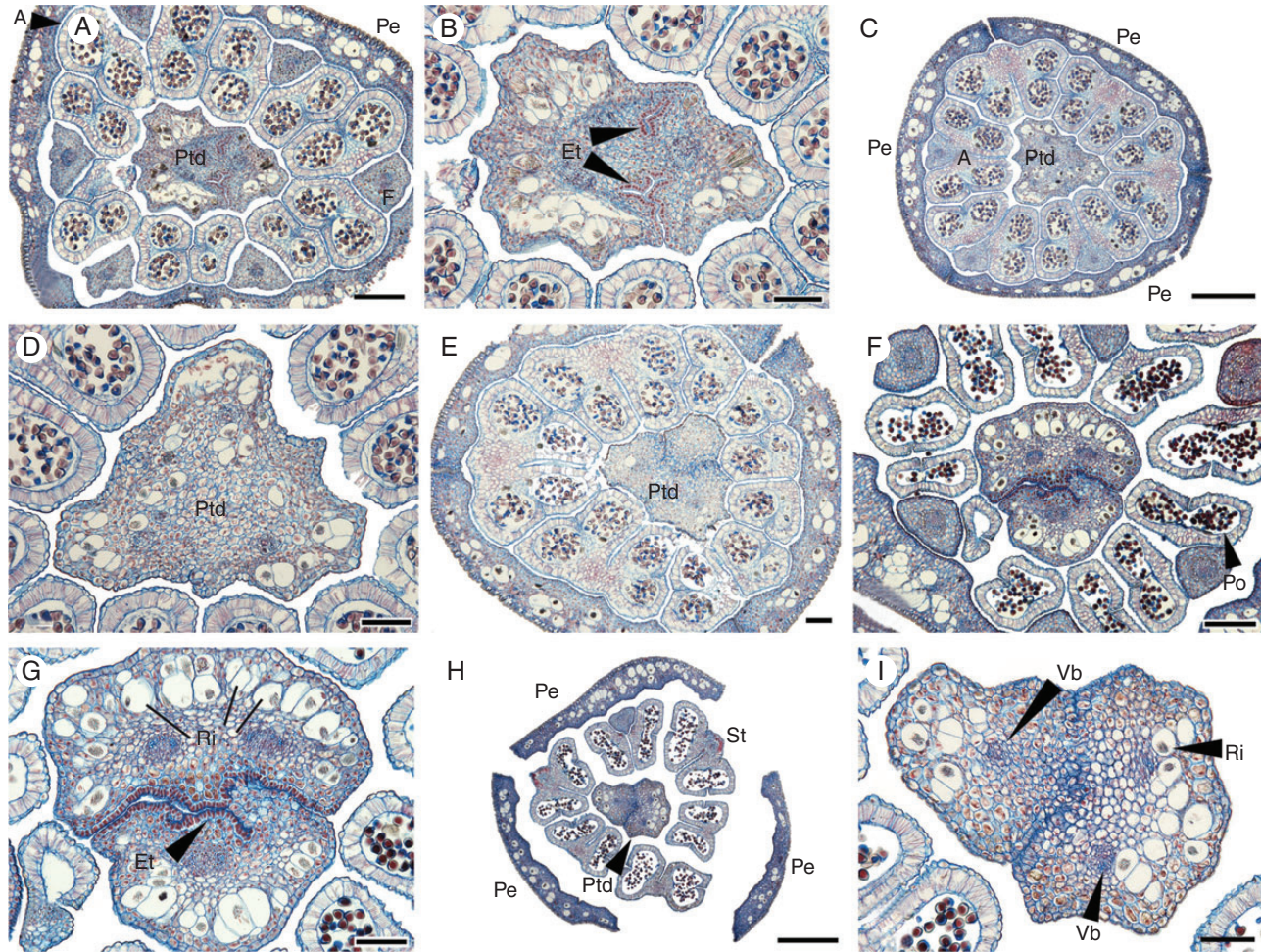
FIG. 4. Developmental stages of the male flower of *Gaussia attenuata*, II. (A) Transverse section of the male flower in late bud, through the basal region of the pistillode. (B) Transverse section showing detail of the pistillode at the level of the nectary, revealing the epithelial tissue (flower in late bud). (C) Transverse section of the male flower in late bud showing valvate aestivation (pistillode mid-height). (D) Transverse section showing detail of the pistillode at mid-height (flower in late bud). (E) Transverse section at the upper level of the male flower in late bud, revealing the apical lobes of the pistillode. (F) Transverse section of the male flower at anthesis, showing the basal region of the pistillode in the centre and the mature pollen grains. (G) Transverse section of the male flower at anthesis showing detail of the pistillode revealing a late stage of development of the nectary and numerous raphide idioblasts. (H) Transverse section of the male flower at anthesis at a level slightly below the pistillode apex. (I) Detail of the pistillode showing the vasculature and raphide idioblasts. Scale bars: (A) = 200 μm; (B) = 100 μm; (C) = 500 μm; (D) = 100 μm; (E) = 100 μm; (F) = 200 μm; (G) = 100 μm; (H) = 500 μm; (I) = 100 μm. A, anther; Et, epithelial tissue; Pe, petal; Po, pollen grains; Ptd, pistillode; Ri, raphide idioblast, St, stamen; Vb, vascular bundle.

of all perianth organs was characterized by the presence of a continuous layer of papillae that became especially conspicuous with development (Figs 3G, 5B and 7G).

Structure and development of the male flower

Morphology. The calyx was formed of three free, imbricate sepals (Fig. 3E, G). They were concave, orbicular and notably fleshy from the base to mid-height, whereas towards the apex and the margins they were slim (Fig. 3D). In the early bud they were ∼0·5–1 mm long and 1 mm wide and completely enveloped the inner organs (Fig. 2C). However, as the inner organs grew, the sepals started to open gradually, becoming cuneate and slightly thinner, reaching ∼2 mm in width and 1–1·5 mm in length at maturity (Fig. 2E, F).

The corolla was composed of three valvate petals (Fig. 2F, G). They were concave, fleshy throughout their length and to a limited extent adnate to the androecium at the base (Fig. 3D). In early stages of development, after the sepals had already separated and the petals were visible, the latter were ∼1–1·5 mm long and 1 mm in diameter (Fig. 2C, F). The petals tightly enclosed the reproductive organs, aided by the epidermal papillae, which in the lateral margins acted as a zipper (Fig. 2G). Once the corolla had opened, allowing the inner organs to expand, the petals started to become reflexed, reaching ∼2–2·5 mm in length and 1·5–2 mm in width at anthesis (Fig. 2G, J).

The androecium comprised six stamens, organized into two whorls of three stamens, one opposite to the sepals and the other to the petals (Fig. 3A–C, G). They were all fertile, attached at the same level and slightly adnate to the corolla at their base (Fig. 3D). At the earliest developmental stage studied (Fig. 3A, D), anthers were seen to be already bilobed and each lobe was starting to split into two different locules. By this time, anthers were ∼0·2 mm wide and 0·3–0·4 mm long, and their shape was
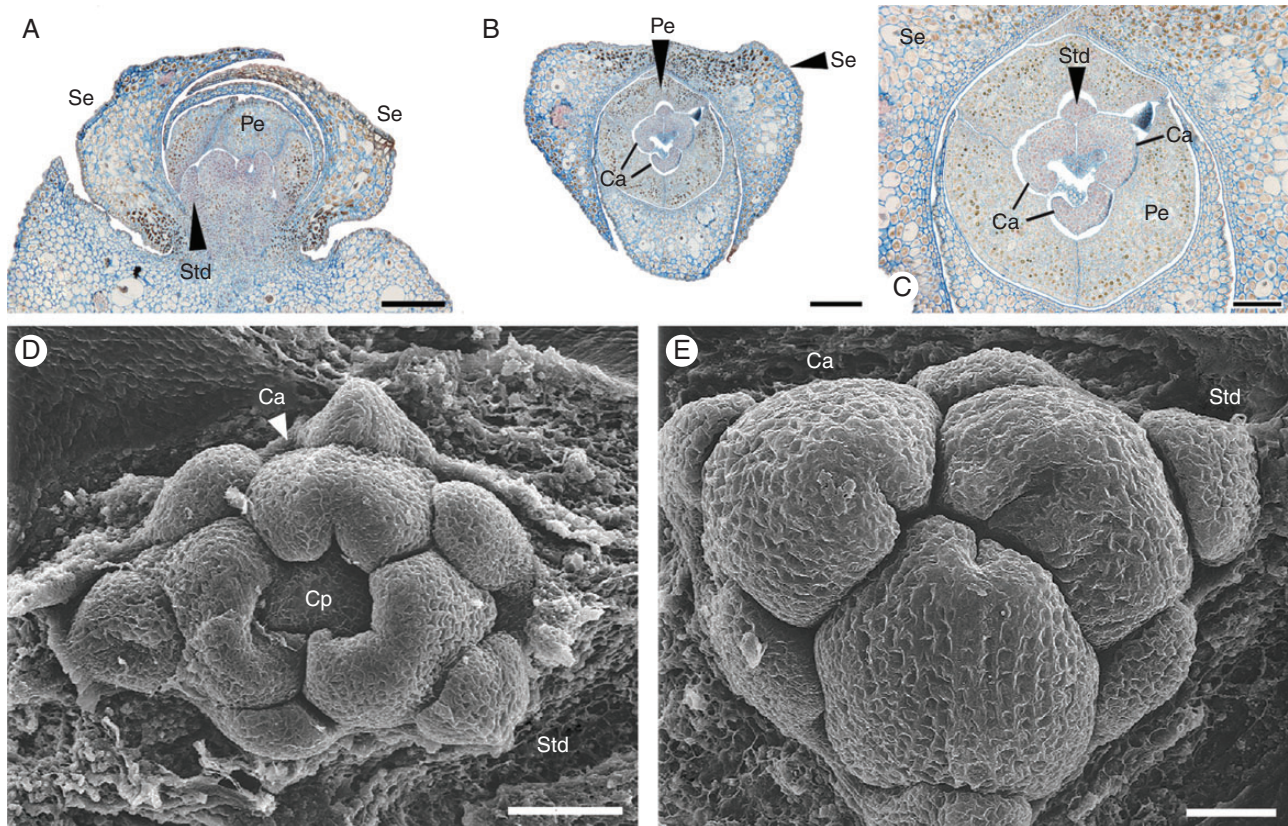
FIG. 5. Developmental stages of the female flower of *Gaussia attenuata*, I. (A) Longitudinal section of the female flower in early bud showing a developing staminode. (B) Transverse section of the female flower in early bud at mid-height. (C) Detail of the gynoecium in transverse section at the early floral bud stage, revealing oil bodies in the corolla. (D) SEM of the gynoecium and sterile androecium of an early floral bud (perianth removed). (E) SEM of the gynoecium and sterile androecium at a slightly later stage (perianth removed). Scale bars: (A) = 200 μm; (B) = 200 μm; (C) = 100 μm; (D) = 100 μm; (E) = 50 μm. Ca, carpel; Cp, central protrusion; Pe, petal; Se, sepal; Std, staminode.

partially defined by interaction with the other closely appressed anthers and the pistillode. The filaments were distinguishable, although some of them had not yet started elongating; most were very short with a maximum length of 0·1 mm (Fig. 3D). Later in development both anthers and filaments elongated simultaneously and separated from the sterile gynoecium, attaining 1–1·5 mm in length just before anthesis and the opening of the corolla (Fig. 3B, C). At this stage both the androecium and the pistillode attained the same height. Around the time of anthesis, the petals separated along their lateral margins, allowing the filaments to elongate until they reached ~1·5–2 mm long (Fig. 2G–J). In mature flowers, the filaments displayed a cylindrical and recurved shape. The anthers were by this stage dorsifixed and displayed extrorse dehiscence through a longitudinal stomium, attaining ~2 mm in length (Figs 2G–J and 4H).

A very conspicuous pistillode could be observed in the centre of the flower. The earliest developmental stages studied showed the pistillode as a compact, more or less trigonous column with two apical lobes (Fig. 3A–D), which in some cases became apically trifid as anthesis approached (Figs 2J and 3B). Nevertheless, in most flowers observed in late bud or at anthesis, an apically bifid pistillode could be seen (Fig. 4H, I). The external surface shape was defined by interaction with the inner regions of the closely disposed anthers (Fig. 4A–E). At early developmental stages it was 0·5 mm long, attaining ~2–2·5 mm in length at maturity.

Anatomy. Three vascular bundles, two lateral and one central, were observed at the base of each sepal, orientated towards the adaxial face (Fig. 3E, G). The lateral bundles disappeared at the mid-length of the sepal blade and only the central bundle extended slightly further upwards, being absent towards the apex (Fig. 3G). In the petals there were five to nine vascular bundles in the basal region and three to five towards the apex (Figs 3G and 4C, E, H). The pattern of vascularization of the perianth remained constant with development.

One vascular bundle irrigated each filament throughout its length, reaching the connective region (Figs 3I and 4A, E, H). This bundle was observed from the earliest stages of development studied (Table 1), when the sporogenic tissue was already differentiated (Fig. 3F). At this stage, we observed the epidermis of the anther, the endothecium, a single middle layer and the tapetum. Later in development, the tapetum was observed to disintegrate in parallel with tetrad formation (Fig. 3H, I). At the latest stages observed, the epidermis of the anther was still present, the endothecium had developed its fibrous wall thickenings and the pollen grains were mature (Fig. 4F, H).

Apart from the fact that vascular strands were better defined in mature flowers, the vasculature of the pistillode was not observed to display significant changes during its development. Three vascular bundles were seen to run through the centre of the pistillode from the basal level through to mid-height, extending to slightly
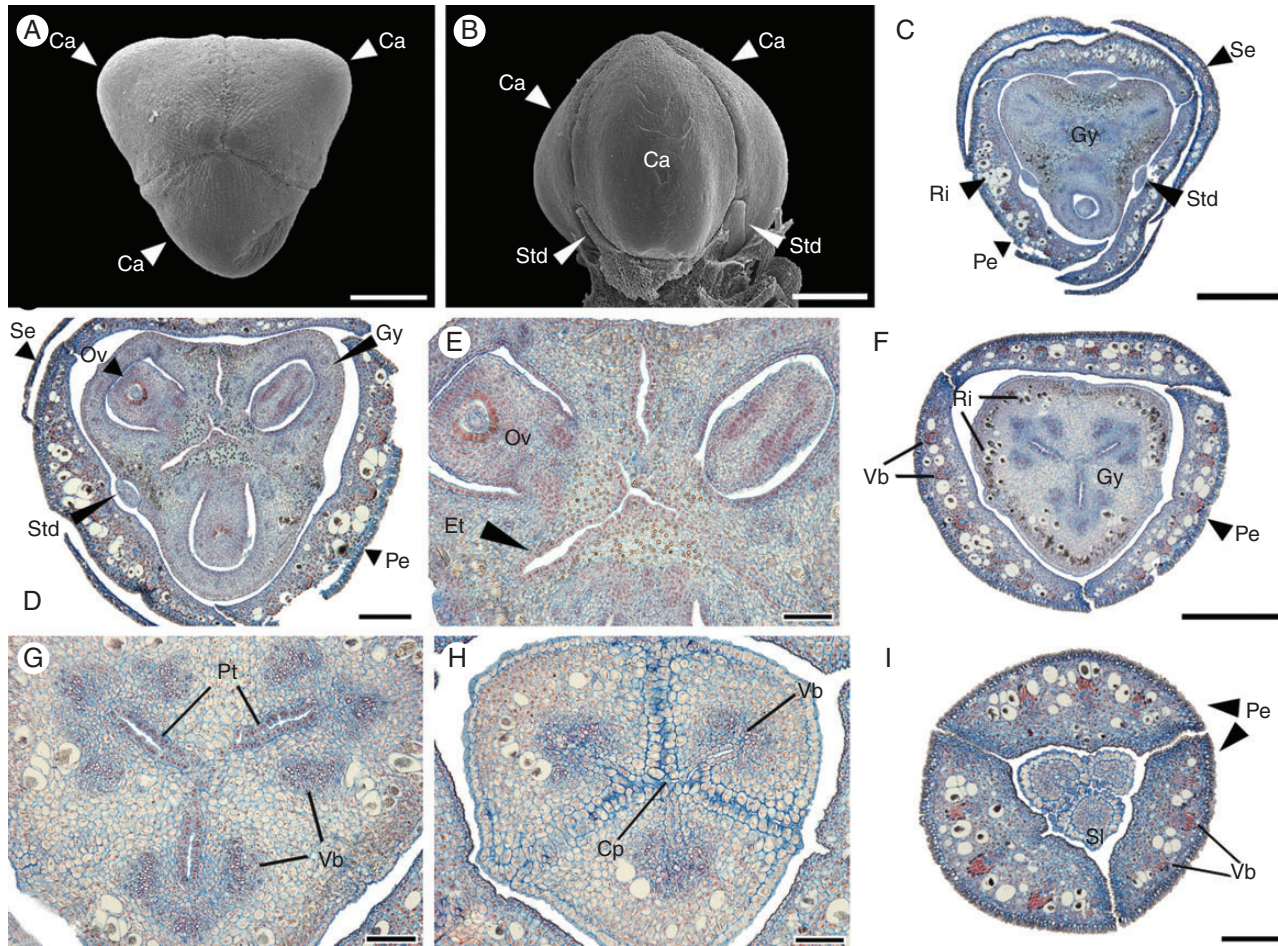
Fɪɢ. 6. Developmental stages of the female flower of *Gaussia attenuata*, II. (A) SEM upper view of the gynoecium (perianth removed). (B) SEM lateral view of the gynoecium (perianth removed). (C) Transverse section at the basal level of the flower. (D) Transverse section of the flower at the level of the locules. (E) Detail of the locular region showing developing septal nectary. (F) Transverse section of the flower at style mid-height, revealing raphide idioblasts accumulated in the carpel walls. (G) Transverse section at style mid-height showing the vascularization of the carpels and the pollen tube transmitting tract. (H) Transverse section at the upper level of the style showing the compitum. (I) Transverse section at the upper level of the flower revealing the stigmatic lobes. Scale bars: (A) = 250 μm; (B) = 250 μm; (C) = 500 μm; (D) = 200 μm; (E) = 100 μm; (F) = 500 μm; (G) = 100 μm; (H) = 100 μm; (I) = 200 μm. Ca, carpel; Cp, compitum; Et, epithelial tissue; Gy, gynoecium; Ov, ovule; Pe, petal; Pt, pollen tube transmitting tract; Ri, raphide idioblast; Se, sepal; Sl, stigmatic lobe; Std, staminode; Vb, vascular bundle.

below the apex (Fig. 4D, G, I), although they were not observed at the apex itself (not shown). In the bilobed pistillode, the larger lobe was usually seen to be served by two vascular bundles (Fig. 4G, I). At mid-height, the inner regions of the pistillode displayed epithelial cells that formed a nectary-like structure consisting of independent canals (Fig. 4G). Nectary development was seen to have been initiated in early floral buds, in the interstices formed between the lobes of the pistillode (Fig. 3F, H). However, as development progressed, the epithelial cells were seen to differentiate into a well-defined convoluted secretory tissue which communicated with the external surface through lateral openings (Fig. 4B, G).

Tanniniferous idioblasts in the floral base were only observed near the epidermis (Fig. 3E). In the perianth organs, they were seen to be scattered throughout the mesophyll, mostly towards the abaxial epidermis, being more numerous towards the base (Fig. 3E), a pattern that remained constant with development.

Putative oil bodies of different sizes were observed mostly in the reproductive organs. Within the androecium, they were densely grouped in the endothecium (mostly towards the mid-height of the anther), the epidermal cells of the anther, the connective tissues and near the stomium (Fig. 3I). They formed a conspicuous ring surrounding the vascular bundle of each filament. This ring could reach three or four cell layers in thickness towards mid-height and in the base of the filaments. Oil bodies were also present in the pistillode, associated with the epithelial tissues of the nectary (Figs 3F and 4B), whereas they became scarce towards the base or at the distal end. The abundance of oil bodies was significantly higher in flowers at early stages of development than in flowers in late bud or at anthesis, at which stage they were rarely observed.

Raphide-containing idioblasts were not observed in the basal region of the flower. However, they were seen to be profusely distributed in the mesophyll, mainly in the lower central portion of the perianth, throughout its development (Figs 3G and 4A, E). Raphide idioblasts were also observed in the androecium, restricted to the tapetum of flowers near anthesis (Fig. 4A, C, E). They were also seen in the pistillode, becoming more
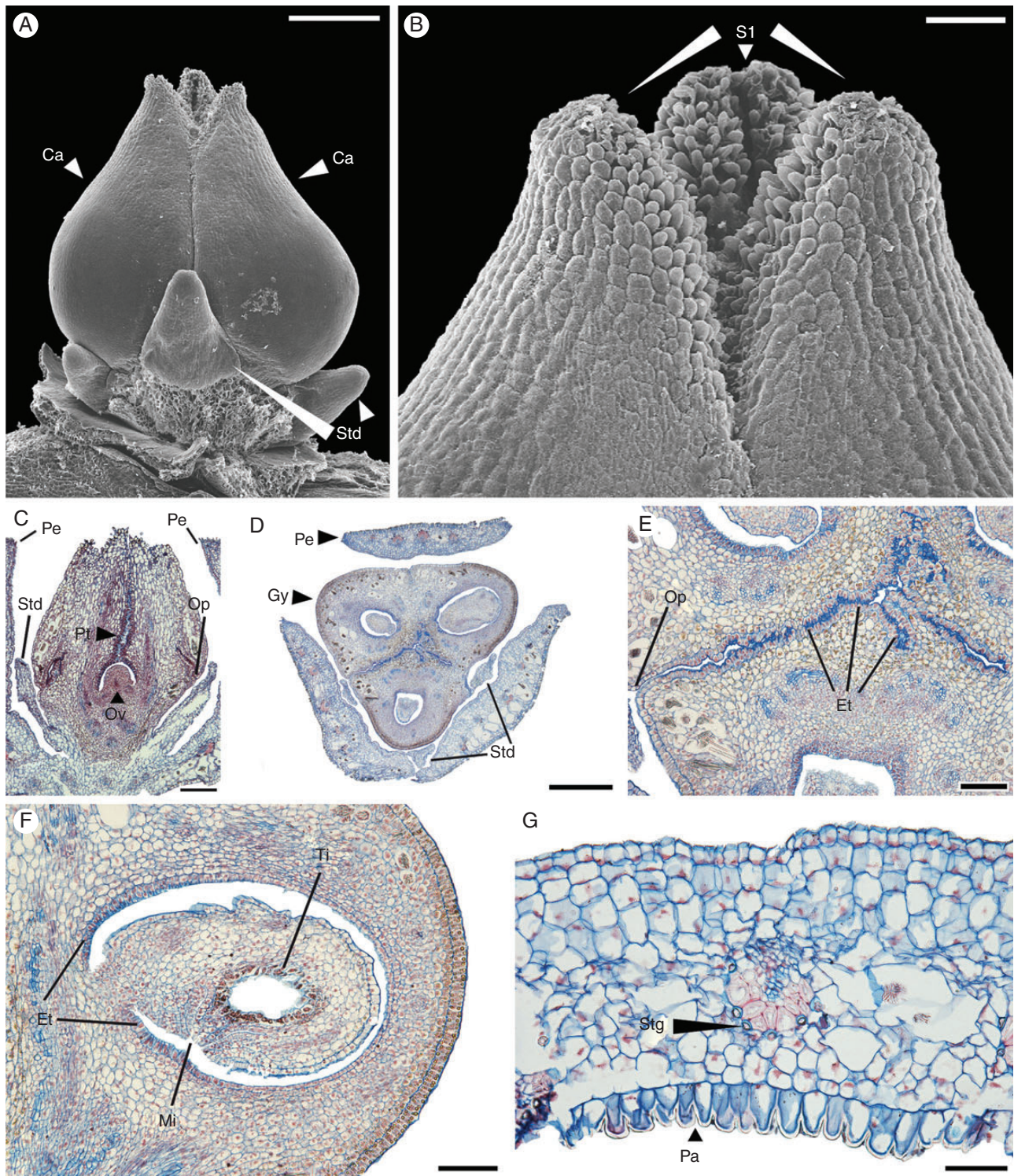
FIG. 7. Developmental stages of the female flower of *Gaussia attenuata*, III (anthesis). (A) SEM lateral view of the gynoecium (perianth removed). (B) SEM lateral view of the stigma showing papillate inner surface. (C) Longitudinal section of the flower towards the centre of the gynoecium revealing the nectary openings. (D) Transverse section of the flower at the upper level of the locules. (E) Detail of the septal nectary in transverse section showing the conspicuous epithelial tissue and nectary opening. (F) Longitudinal section of a locule showing the epithelial tissue adjacent to the funicle, the micropyle and the layer of tannin idioblasts surrounding the nucellus. (G) Transverse section of a petal revealing stegmata associated with the vascular tissue and the papillate abaxial epidermis. Scale bars: (A) = 500 μm; (B) = 100 μm; (C) = 200 μm; (D) = 500 μm; (E) = 100 μm; (F) = 100 μm; (G) = 50 μm. Ca, carpel; Std, staminode; Sl, stigmatic lobe; Pe, petal; Pt, pollen tube transmitting tract; Ov, ovule; Op, nectary opening; Gy, gynoecium; Et, epithelial tissue; Mi, micropyle; Ti, tanniniferous idioblast; Stg, stegmata; Pa, papilla.

conspicuous during the progression towards anthesis, although they first appeared in the earlier stage floral buds, always restricted to the periphery (Fig. 4B, D, G, I).

Structure and development of the female flower

Morphology. The calyx of the female flower was formed by three imbricate, fleshy sepals, which were thicker than those of the male flower (Fig. 5A, B). Early in development, the overlapping sepals entirely enclosed the inner floral organs, giving the flower a characteristic triangular shape (Figs 2B, C and 5B). At this stage, sepals were strongly concave, attaining ∼0·5 mm in length and ∼1 mm in width, whereas towards anthesis their size doubled to ∼1 mm in length and ∼2 mm in width, their margins becoming slightly revolute towards the apex (Fig. 6C, D).

The corolla of female flowers was composed of three petals, almost entirely valvate but slightly imbricate towards the base (Fig. 6C). In the early floral buds, they were more or less triangular and concave, ∼1–1·5 mm in length and ∼1 mm in width, enclosing the inner whorls hermetically (Fig. 2F, G). Nevertheless, at anthesis the corolla opened and the petal blades became strongly reflexed, reaching 2 mm in length and 2 mm in width towards the base (Fig. 2K, L).

The sterile androecium was composed of six staminodes. The initiation of the development of these organs took place in two stages, each generating a different whorl (Fig. 5D, E). The first whorl to be formed (the external whorl) was composed of three antesepalous staminodes (Fig. 7A). The second (inner) whorl was composed of three antepetalous staminodes, which were also basally adnate to the carpels (Figs 6C and 7A, C). At the earliest stages observed (Fig. 5D, E), the organs of the external whorl were slightly larger than those of the inner whorl. At this time, staminodes appeared as very short, concave flattened protuberances. Subsequently, the staminodes were seen to continue their elongation, displaying a cylindrical, subulate, filament-like shape as anthesis was reached (Figs 6B and 7A, C). In spite of the fact that the staminodes attained almost 1 mm in length at anthesis, the formation of a differentiated anther was not observed (Figs 2L and 7A).

The gynoecium was formed of three carpels, all of which were fertile and opposite to the sepals. The earliest stage of carpel development observed was in the form of three free, very short cuneate knobs forming a symmetrical triangle with a conspicuous central protrusion (Fig. 5C, D). Subsequently, the carpels were seen to undergo postgenital fusion through their lateral edges, starting from the base with the concomitant appearance of ventral slits (Fig. 5E). Later in development, connation between the lateral flanks of adjacent carpels took place as they elongated, allowing the carpels to assume their characteristic urceolate shape as anthesis approached (Figs 6A, B and 7A, C). At maturity, each carpel was ∼1·5 mm long, ascidiate at the base and plicate towards the distal end. Together, the three carpels constituted a gynoecium displaying a basal synascidiate region (Figs 6D and 7D) and a distal symplicate region (Fig. 6F, G), the carpels being free only towards the stigmatic end (Figs 6I and 7A, B). The three independent plicate lobes observed in the stigmatic region, which were densely covered with unicellular papillae, were consolidated only just before anthesis, when they also became reflexed (Fig. 7A, B).

Anatomy. Basally, the sepals were seen to be vascularized from the early stages of development by one to three vascular bundles (Fig. 5B), which were also observed at mid-length and at late stages. However, the sepals were not vascularized in their apical region at any developmental stage. In the petals, a smaller number of strands was observed early in development: three were seen at the floral base (Fig. 5C), one reaching the apex. Later in development, six or seven vascular bundles were observed at the basal level, five at mid-height and two or three in the apical region (Fig. 6D, F, I). One vascular bundle could be distinguished at the base of each staminode throughout floral development. In the carpels, striking changes in vascularization were observed as development progressed. At early stages only one vascular strand was seen to feed into the base of each carpel (Fig. 5A). In parallel with the differentiation of the carpels, vascular strands were observed to become branched to produce a complex of at least one dorsal and two lateral vascular bundles irrigating each carpel from the basal region, through the middle region of the locules (Fig. 6D) and all along the style (Fig. 6G). Nevertheless, no vascularization was observed to feed into the stigmatic region.

The upper region of the style was characterized by a compitum formed between the partially fused dorsal faces of the carpels and their confluent ventral slits (Fig. 6H). The middle region of the style displayed a triangular shape in cross-section, the three carpels being completely fused and the three ventral slits remaining independent until the point where they reached the locules (Fig. 6F, G). The pollen tube transmitting tract (PTTT) was observed to be superficial, formed by a one-cell-thick layer (Fig. 6G) lining the apical plicate zone of the carpels and the ascidiate zone, where the ventral slits were independent. The PTTT was seen to be secretory along all its length (Fig. 7C), including the locule, where an epithelial cell layer could be observed contiguously to the funicle (Fig. 7F). A septal nectary was observed to be formed in the synascidiate region of the gynoecium, extending upwards throughout the length of the locules. It was observed to develop from open channels formed between adjacent carpels in the locular region, as a consequence of their differential postgenital fusion (Figs 6D, E and 7E). These channels were observed to be confluent towards the middle of the ovary and to have opening ducts towards the external epidermis of the carpels, from the mid-height region of the locules to their base (Fig. 7C–E). The nectary was seen to be composed of epithelial secretory tissue one or possibly two cell layers thick, forming a continuous layer from the central channels to the three external openings (Fig. 7E). It could be considered as an intermediate between a confluent-simple and a confluent-convolute type of nectary. Each carpel was observed to possess an anatropous ovule, attached to the locule at mid-length. The ovule was crassinucellate, filling ∼80 % of the locular cavity (Fig. 7F). Papillate tissue was observed, restricted to a zone adjacent to the funicle, but mostly associated with the locular wall, representing a continuation of the PTTT. The bitegmic ovule possessed an outer integument that was ten cell layers thick at mid-length and about five cell layers thick towards the micropyle end. The inner integument was two cell layers thick throughout its length. The micropyle was exclusively formed by the inner integument, which was in close contact with the papillate zone of the locular cavity at this point (Fig. 7F).

Tanniniferous idioblasts could be observed scattered in the mesophyll of the sepals throughout their development, mostly associated with the abaxial face (Fig. 5A). A continuous two-cell-thick layer containing tannins was also seen to surround the nucellus (Fig. 7F). Oil bodies were observed in all floral organs, apart from staminodes, from the early bud stage until anthesis. They were seen to accumulate basally in the sepals at all developmental stages, but were distributed throughout the petals at very early stages of development (Fig. 5A–C). As development progressed, they became progressively more abundant in the gynoecium, especially in the basal region, towards the external surface of the carpels, and in the nectary and stigmatic papillae (Figs 6E and 7E, F).

In early floral buds, some small raphide idioblasts were observed exclusively in the mesophyll of perianth organs (Fig. 5A–C). The raphides became larger and particularly abundant towards the abaxial face of the sepals and petals as anthesis approached (Figs. 6D, F and 7D). During the intervening developmental stages, small raphide idioblasts also appeared in the gynoecium and staminodes, becoming larger towards anthesis. A few scattered raphides were seen to be distributed in the basal region of the ovary and in the locules at mid-height, restricted to the lateral carpel walls (Fig. 6D, E). Moreover, the presence of raphides became more marked in the upper region of the locules and in the style, mostly in the peripheral cell layers of the carpels (Figs 6F–H and 7C).

DISCUSSION

Acervulus development in Gaussia attenuata

Our observations of the acervulus in *G. attenuata* revealed the presence of at least one associated bract, a structure unobserved in previous studies of the genus (Quero, 1982; Quero and Read, 1986; Uhl and Dransfield, 1987; Moya *et al.*, 1991; Dransfield and Uhl, 1998; Dransfield *et al.*, 2008). This bract subtends the acervulus and can presumably protect the floral meristems in their early stages of differentiation, as already proposed by Ortega and Stauffer (2011) for *Hyophorbe lagenicaulis.* The existence of more than one subtending bract in the acervulus of *G. attenuata* (observed for one acervulus in the present study) was previously suggested by Uhl and Moore (1978), but further studies will be needed in order to better understand this character in the floral cluster of Chamaedoreeae.

The developmental sequence of the *G. attenuata* acervulus reveals that, as in the majority of arecoid palms, this species is protandrous. Dichogamy has been interpreted as a mechanism to reduce self-fertilization (Bertin and Newman, 1993) and to promote outcrossing (Henderson, 2002) and therefore heterosis or hybrid vigour (Freeman *et al.*, 1997). Although dichogamy has been shown to have evolved in a complex pattern within the arecoid tribe Areceae (in the subtribe Arecinae), with a number of distinct protogynous and protandrous lineages (Loo *et al.*, 2006), the protandrous character of *G. attenuata* conforms to the predominant condition in the palm family. It is interesting to note that this tendency is reversed in monocots as a whole, in which protogyny is more widespread (Bertin and Newman, 1993).

Morpho-anatomical features of the male and female flowers

Although the functions in angiosperms of silica bodies and raphide and tanniniferous idioblasts are still not clear, some studies suggest a role as depositories for metabolic wastes or in the prevention of herbivory (Uhl and Moore, 1973, 1977; Prychid and Rudall, 1999; Dransfield *et al.*, 2008). Floral tissues in *G. attenuata* were frequently seen to contain tannins and raphides. In addition, we observed the presence of stegmata and oil bodies. Stegmata or silica cells are common in palms, and have previously been observed to be associated with vascular bundles in Chamaedoreeae (Tomlinson, 1961; Tomlinson *et al.*, 2011), although they have never been reported in reproductive organs. In the case of oil bodies, these structures are rarely observed in monocot flowers and to date have never been reported in this context in palms (Lersten *et al.*, 2006). Further research needs to be carried out in order to characterize the precise composition and distribution of these cellular components in the family. As far as we could note, the different types of cellular inclusion observed appeared early in development but showed contrasting distributions in time and in space. Oil bodies were conspicuously present at early stages of development and raphides/stegmata at later stages. This pattern could constitute an effective mechanism to counter herbivory of the flowers, corroborating the observations of Uhl and Moore (1977), who suggested that tannins deter feeding and that raphides together with fibrous bundles constitute a physical barrier. It will also be of interest to investigate the importance of cellular inclusions in the different members of the tribe with regard to their insect visitors.

Septal nectaries are exclusive to the monocotyledons and have been reported in numerous palm taxa. However, little is known about their morphology and anatomy and structural and developmental studies have been conducted in only a few taxa of the Arecaceae (Stauffer *et al.*, 2009). Moreover, nectaries in the pistillode of male palm flowers have been reported for only a few species, such as *Euterpe precatoria* (Küchmeister *et al.*, 1997) and *Hyophorbe lagenicaulis* (Uhl, 1978). The presence of a conspicuously differentiated nectary in both male and female flowers suggests an affinity for insect pollination. In the case of *Synechanthus warscewiczianus*, Siefke and Bernal (2004) inferred from observations of nectar production, inflorescence appearance and numerous insect visitors that this species was entomophilous. Similarly, the presence of a septal nectary and basipetal maturation of the acervulus was reported for *Hyophorbe indica* (Uhl, 1978). The previously mentioned traits (basipetal floral development, protandry, cellular inclusions and nectar secretion) are all suggestive of interactions between the flowers of *G. attenuata* and its putative pollinators.

Sexual differentiation and its evolutionary significance

The fact that all 107 genera of the palm subfamily Arecoideae are monoecious, with the exception of the two dioecious genera, *Chamaedorea* and *Wendlandiella*, suggests strongly that the common ancestor of this group was also monoecious. Moreover, given that the two aforementioned genera probably represent independent evolutionary transitions from monoecy to dioecy within the same tribe, it seems likely that Chamaedoreeae are more predisposed than other arecoid palms

towards the evolution of unisexual plants (Cuenca *et al.*, 2009). It is important to note that the molecular mechanisms governing flower unisexuality almost certainly originated in a common monoecious ancestor of the five extant genera. Thus, even though genomic rearrangements will have occurred in the dioecious lineages to produce a genetic system with separate male and female plants, it is likely that the same sex determination genes will operate in these clades as in the monoecious ones. At the morpho-anatomical level, comparisons between *Gaussia*, *Hyophorbe* and *Chamaedorea* (i.e. the three genera of the tribe for which data are now available) reveal generally strong similarities in sexual differentiation patterns. Perhaps the most notable divergence that can be seen between the genera is in the degree of differentiation of the staminodes: studies performed by Askgaard *et al.* (2008) revealed that in certain (but not all) species of *Chamaedorea* female flowers are produced that lack any traces of a sterile androecium. This situation contrasts with that of *Hyophorbe* and *Gaussia*, in which staminodes are always clearly identifiable, despite showing relatively early arrested development. Differences between *Chamaedorea* species in terms of staminode presence or absence may reflect genetic changes that have occurred since the appearance of the *Chamaedorea* lineage. The fact that sexual dimorphism has evolved on numerous occasions in angiosperms and a number of times in palms implies that different genetic determinants will probably apply in each lineage (Diggle *et al.*, 2011). To date, the only two angiosperm species for which the functional identification of sex determination genes has been performed are maize (DeLong *et al.*, 1993; Chuck *et al.*, 2007; Acosta *et al.*, 2009) and melon (Boualem *et al.*, 2008; Martin *et al.*, 2009). In the case of the Arecaceae, evidence has been obtained for the conservation of at least some gene functions associated with the floral ABC model (Coen and Meyerowitz, 1991; Pelaz *et al.*, 2000) in the African oil palm *Elaeis guineensis* (Adam *et al.*, 2007). However, no direct link has been established to date between genes determining the homeotic identities of floral whorls and the process of sex determination *per se*. In the case of the Chamaedoreeae, further studies aimed at characterizing the cellular changes associated with sterile organ arrest should help to shed light on the sexual differentiation process. An approach of this type has already been used with date palm (*Phoenix dactylifera*) to demonstrate that cell division ceases in the sterile androecium and gynoecium of female and male flowers respectively in the absence of any loss of nuclear integrity (Daher *et al.*, 2010). In *G. attenuata*, the developmental arrest appears to take place at a relatively early stage after initiation in the case of the staminode and at a much later stage for the pistillode; however, in the latter case ovule development is lacking and replaced instead by the appearance of vegetative tissues enclosing a nectary.

In conclusion, our data provide useful new information on floral development in a previously unstudied genus of the Chamaedoreeae, which will help to improve our understanding of the evolutionary changes that have accompanied sexual system transitions in this tribe. Further studies using complementary molecular histological approaches should provide information as to how sterile reproductive organ arrest is achieved and the development of genomic resources will subsequently help to provide insights into the underlying molecular events.

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