

Elaborate Petals in Australian *Spermacoce* (Rubiaceae) Species: Morphology, Ontogeny and Function

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- **Background and Aims** Australian *Spermacoce* species display various types of elaborate petals. Their precise morphology, ontogenetic origin, and function are hitherto unknown. The aim of the present paper is to unravel the development and nature of the diverse types of elaborate petals in *Spermacoce* through a floral ontogenetic study.
- **Methods** The floral ontogeny of six species characterized by different types of corolla appendages was studied by scanning electron microscopy and light microscopy. In order to elucidate the possible functions of the elaborate petals, field observations were conducted as well.
- **Key Results** Scanning electronmicrographs show that full-grown petals of *Spermacoce lignosa*, *S. phaeosperma* and *S. redacta* bear appendages on their ventral side. Despite their different appearance at anthesis, the appendages develop very similarly in all three species. They are initiated at the same developmental stage and are first visible as two arcs of primordia converging from the upper margins of the petal towards its midvein and downwards. In *S. brevidens*, *S. caudata* and *S. erectiloba*, the full-grown petals have two long, concave protuberances, which develop from the tissue at both sides of the petal's mid-vein. In these three species, initiation of appendages on the ventral side of the petals is also observed, but they are hardly visible on the mature petals. The two types of elaborate petals tightly enclose the anthers, both in bud and during most of the flowering period.
- **Conclusions** Among Australian *Spermacoce* species, two types of elaborate petals can be distinguished. The former hypothesis that the two types of elaborate petals are essentially homologous is here rejected. Field investigations point out that the elaborate petals might play a role in the pollination biology of the species.

Key words: Australia, corolla appendages, elaborate petals, floral ontogeny, pollination, Rubiaceae, *Spermacoce brevidens*, *S. caudata*, *S. erectiloba*, *S. lignosa*, *S. phaeosperma*, *S. redacta*.

INTRODUCTION

Spermacoce belongs to the family Rubiaceae and comprises about 275 species found in tropical and subtropical America, Africa, Australasia and Australia (Dessein *et al.*, 2006). *Spermacoce* species are herbs or small shrubs, with sessile, decussate leaves, and interpetiolar, fimbriate stipules. The small- to medium-sized, 4-merous flowers are arranged in congested, terminal, pseudoaxillary or axillary, capitate inflorescences, which are subtended by one or more pairs of involucreal leaves. The pseudanthia are sometimes eye-catching as calyx lobes are often coloured, especially among the Australian species. Corolla colour is variable, including white, all tints of blue, pink or maroon (Fig. 1). The fruit usually is a 2-seeded capsule, more rarely a schizocarp or a nut.

Spermacoce belongs to the Spermacoceae *sensu stricto*, a clade comprising 19 genera (Dessein, 2003). *Spermacoce* is the only genus of the clade native to Australia. Dessein (2003) postulated that *Spermacoce* originated in South America and that the genus reached Africa and Australia by long-distance dispersal. On both continents, the genus underwent a second radiation. While this second radiation

in Africa presumably was triggered by tectonic activities, the diverse flower types in Australia may point to co-evolution with pollinators.

In a revision of the Australian *Spermacoce* of the Northern Territory (Dessein *et al.*, 2005; Harwood and Dessein, 2005) 57 species were recognized, 53 of them native. It is estimated that the number of *Spermacoce* species in Australia is approx. 85, including several undescribed taxa from Queensland and Western Australia.

The flowers of many of the Australian *Spermacoce* species are more complex and appear to be more derived than those in America and Africa. In several Australian species elaborate petals, as discussed by Endress and Matthews (2006), are observed. The structure and appearance of the elaborate petals differs strongly among species. Each petal of *S. lignosa* bears two arcs of white, trichome-like outgrowths on its ventral side (Fig. 1A, B). In other species, the appendages consist of a solid base that is crowned with trichome-like outgrowths (Fig. 1D, E). The ratio between the height of the solid base and the length of the trichome-like outgrowths is species dependent. In species presumed to have more derived appendages, the appendages resemble the petals in texture and colour (Fig. 1G, H), and the free trichome-like part is reduced. Harwood and Dessein (2005) refer to this as variation in degree of fusion between trichome-like outgrowths.

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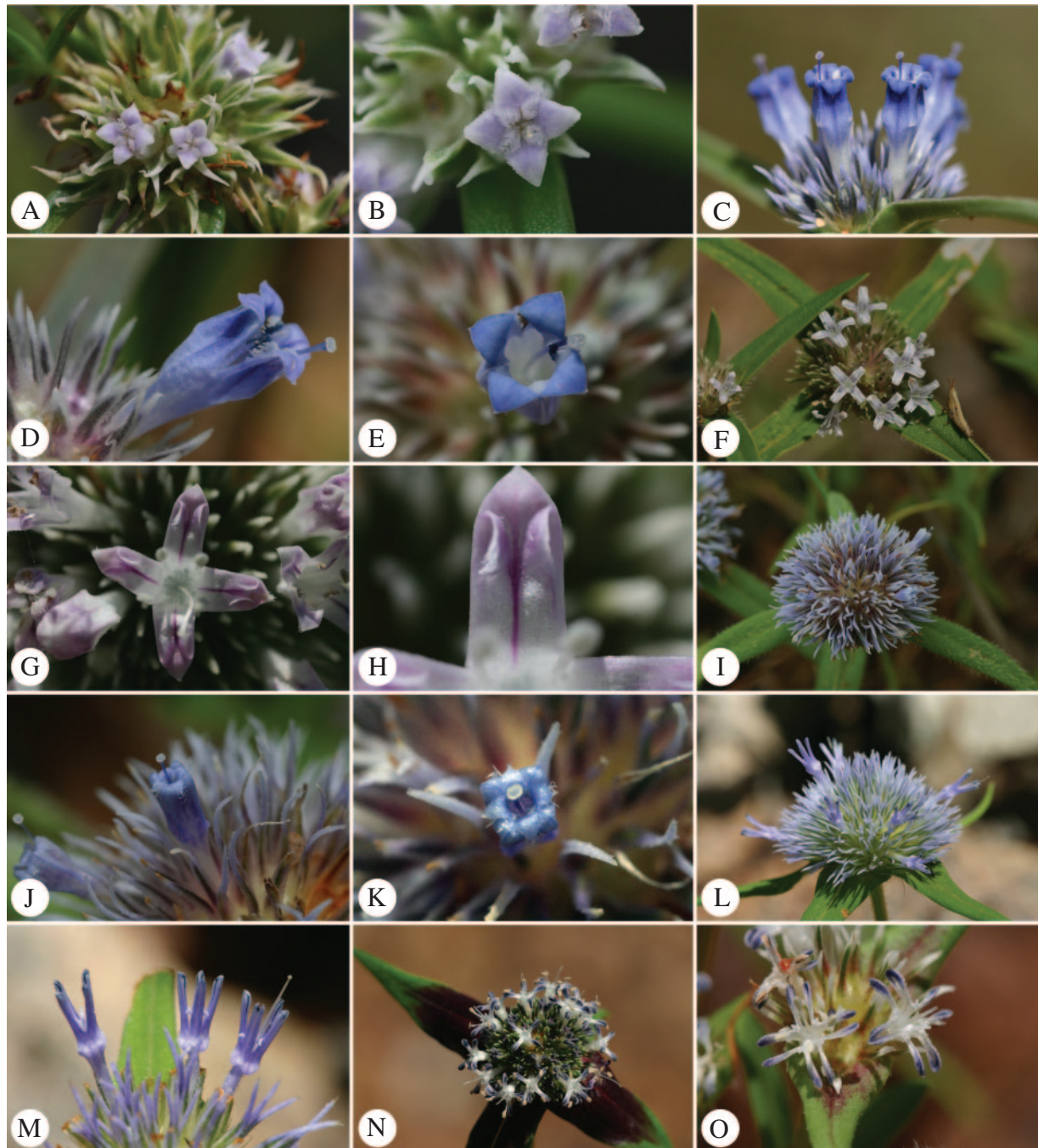


FIG. 1. Floral diversity among Australian *Spermaceo* species with 'appendages'. (A) Top view of flowering head of *S. lignosa*. (B) Flower of *S. lignosa* showing appendages of trichome-like outgrowths. (C) Side view of flowering head of *S. calliantha*. (D) Side view of flower of *S. calliantha* showing the anthers released from the appendages after visit by pollinator. (E) Top view of flower of *S. calliantha* showing the anthers enclosed by the appendages. (F) Top view of flowering head of *S. auriculata*. (G) Top view of flower of *S. auriculata* showing spreading free petal parts with well-developed appendages. (H) Detail of (G) showing appendages similar in texture and colour as petals. (I) Side view of flowering head of *S. redacta*. (J) Side view of flower of *S. redacta* showing the concave petals and reduced petal apices. (K) Top view of flower of *S. redacta*. (L) Side view of flowering head of *S. erectiloba*. (M) Side view of three flowers of *S. erectiloba*: the middle and left flower have the style still included, the right one has the style exerted and the distal-lateral protuberances are separated from each other. (N) Top view of flowering head of *S. brevidens*. (O) Top view of two flowers of *S. breviflora*. All photographs taken by S. Dessein.

In four species (*S. brevidens*, *S. cardiophora*, *S. caudata* and *S. erectiloba*), all restricted to Kakadu National Park and the adjoining western Arnhem Land, each of the petals seems deeply divided, the corolla thus appearing

to be 8-lobed (Fig. 1L–O). Harwood and Dessein (2005) hypothesized that these species have evolved from a species with particularly well-formed appendages as observed in *S. redacta* (Fig. 1I–K), and that the

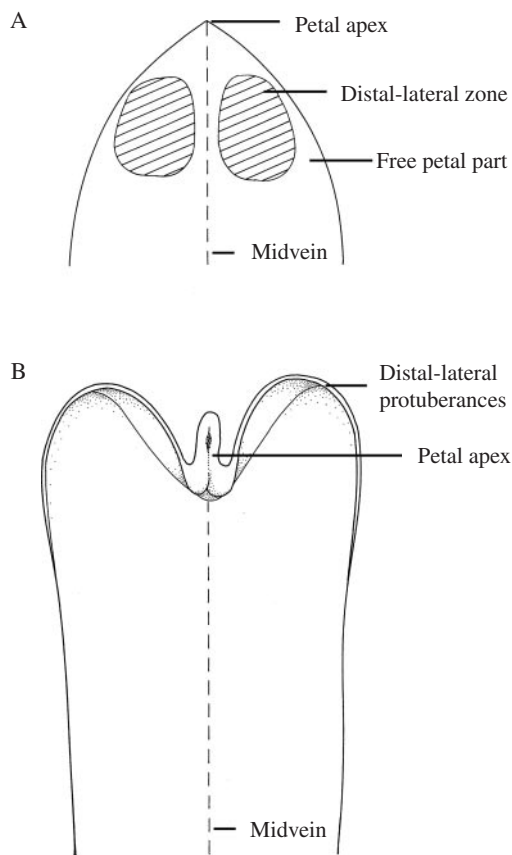


FIG. 2. Terminology applied to elaborate petals in Australian *Spermaceae* species: (A) young petal (free part); (B) mature bilobed petal.

'concave halves of the petal' have ultimately separated from the midvein and each other. In this way, each petal has become distinctly 2-lobed with the remnants of the petal apex forming a short third projection in between the two lobes. The terminology that will be consistently used for describing corolla and appendage morphology throughout this paper is illustrated in Fig. 2.

The main objectives of this study are (a) to reconstruct the overall floral ontogeny of Australian *Spermaceae* species, (b) to determine the homology between the different kinds of elaborate petals observed and (c) to consider the possible functions of the elaborate petals.

MATERIALS AND METHODS

Flowers and floral buds of *Spermaceae brevidens* (Australia, NT, Kakadu National Park; Harwood 1381), *S. caudata* (Australia, NT, south of Nabarlek; Harwood 1160), *S. erectiloba* (Australia, NT, south of Jabiluka lease; Harwood 1149), *S. lignosa* (Australia, NT, beyond Koongarra saddle, Kakadu National Park; Harwood 1151), *S. phaeosperma* (Australia, NT, Timber Creek, 2.6 km past new bridge over Victoria river; Harwood 1168) and *S. redacta* (Australia, NT, Roper highway, east of Mount McMinn station; Harwood 1140) were collected in the field and fixed in 70% ethanol. Voucher specimens are kept at the Department of Natural Resources, Australia (DNA).

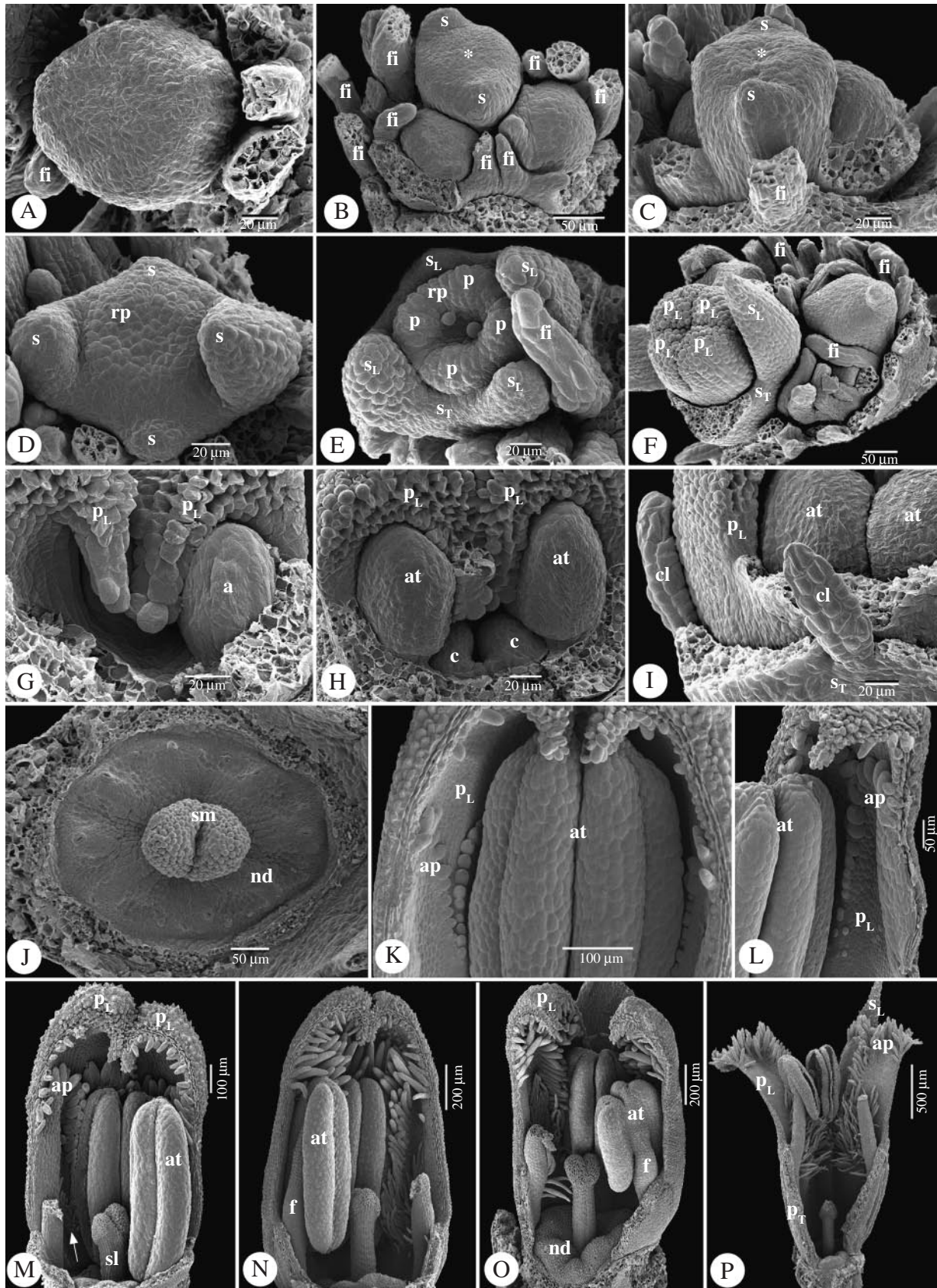
The floral buds were dissected in 70% ethanol under a stereo microscope (Wild M3; Leica Microsystems AG, Wetzlar, Germany), equipped with a cold light source. Subsequently, the buds were rinsed twice for 5 min with 70% ethanol, before being exposed to a mixture (1:1) of 70% ethanol and DMM (dimethoxymethane) for 20 min. The material was then placed in pure DMM for 20 min and critical-point dried using liquid CO₂ with a BAL-TEC CPD030 (BAL-TEC AG, Balzers, Liechtenstein). Finally, the dried material was mounted on stubs using Leit-C and gold-coated with a sputter coater (SPI Supplies, West Chester, PA, USA). Observations were made using a JEOL JSM-5800 LV scanning electron microscope (JEOL, Tokyo, Japan) at the National Botanic Garden of Belgium or a JEOL JSM 6360 scanning electron microscope (JEOL, Tokyo, Japan) at the Laboratory of Plant Systematics.

RESULTS

The overall ontogeny of the Australian *Spermaceae* species examined is nearly identical, whereas the detailed ontogeny of the petal elaborations is highly variable. Therefore, the overall ontogeny will be exemplified by one species, *S. lignosa*. Ontogenetic descriptions for all other species studied will be restricted to the petals and associated structures.

Floral ontogeny of Spermaceae: S. lignosa

The smallest inflorescence unit is a dichasium (Fig. 3B, C). Each three-flowered dichasium is encircled by fimbriae, which are basally connate (Fig. 3B, F). Development of the inflorescence is basipetalous: the top flower is initiated first and differentiates prior to the two younger lateral flowers (Fig. 3B, C). Flower primordia are initially spherical (Fig. 3A). Soon, two sepal primordia develop (Fig. 3B). The pair of sepal primordia, positioned in a plane determined by the dichasium's lateral flower primordia, is delayed in growth. As the latter pair of sepal primordia is initiated, a corolla ring primordium emerges on the floral apex (Fig. 3D). Next, four petal primordia develop on top of the ring primordium alternating with the sepal primordia, and a sepal rim becomes evident (Fig. 3E). The stamen primordia only appear after petals have met centrally in a valvate aestivation (Fig. 3F, G). At this stage, the inner surface of the petals is still glabrous. The free sepal parts have enlarged, while the sepal tube remains short (Fig. 3F). The stamen primordia, which are borne on the short common stamen–corolla tube, subsequently start differentiating into anther and filament (Fig. 3H). They are enclosed by the petals of which the apices fold inwards towards the proximity of the receptacle (Fig. 3G, H). Simultaneously, two carpel primordia appear in the centre (Fig. 3H). The primordia fuse to produce a short style with bilobed, papillose stigma (Fig. 3J). Later, an undulating, epigynous nectary develops surrounding the base of the style. The nectarial disc is covered with a peripheral ring of paracytic nectarostomata (Fig. 3J). Cylindrical collectors arise on the sepal tube in between the free sepal parts (Fig. 3I).



Appendages on the ventral side of the petals become visible after anther thecae have become elongated and bilobed (Fig. 3K). The appendage primordia originate in a pattern of two arcs, converging from the upper margins of the petal towards its midvein and downwards (Fig. 3K, L). The appendage arcs of one petal run along the upper edge of the dorsolateral pollen sacs of the adjoining anthers (Fig. 3K). Together with the appendages, trichomes emerge on the margin of the petals (Fig. 3L, M). The appendages and trichomes elongate simultaneously, eventually covering the whole dorsolateral surface of the anthers (Fig. 3M–O). Meanwhile, longitudinal rows of simple trichomes emerge on the inner, lower half of the free petal part (Fig. 3M, arrow). As anther filaments and free petal parts further elongate, a speckled waxy coating appears on the surface of the dorsifixed anther. The surface of the nectary disc becomes papillose (Fig. 3O) and also shows a conspicuous waxy coating.

At anthesis, the free petal parts including the appendages remain erect, except for the apices. The petal apices are spreading and form a landing platform for the pollinators (Fig. 1B). The mature corolla is actinomorphic, erect and tubular. Each anther is completely enclosed by the ventral appendages of its bordering petals (Fig. 1B) and exceeds the corolla tube. Thecae open introrsely with a longitudinal slit, releasing the pollen. It is only after pollinators have visited the flowers that the free petal parts curl further backwards and that the anthers become free (Fig. 3P). The stigma is situated at the base of the free petal parts.

Ontogeny of corolla appendages

The early developmental pattern of corolla appendages in the other species examined is the same as described for *S. lignosa*, i.e. appendage primordia appear when anthers and filaments are well-differentiated, forming a pattern of two converging arcs on each petal (Figs 4A, 5A, 6A and 7A). Their further specific development is as follows.

Spermaceo phaeosperma. A growth zone at the base of the appendage primordia forms a continuous rim of elongated cells, carrying the individual outgrowths on its upper margin (Fig. 4B–F). Initially, the appendage arcs only envelop the apical part of the anthers (Fig. 4B–D), whereas at anthesis they have enlarged to cover them completely (Fig. 4E). The petal apices are curled back (Fig. 4F). In full-grown appendages, the basal, fused part is longer than the free upper part.

Spermaceo redacta. As the individual outgrowths of each appendage arc enlarge, the distal-lateral zones of the

petals start to grow outward. As a result, this region at either side of the midvein becomes concave (Fig. 5C–E). Simultaneously, each appendage arc grows from its base into a continuous tissue, bearing the enlarged individual outgrowths on its margin (Fig. 5B–E). The individual outgrowths are longest at the basal part of the arc (Fig. 5D). At anthesis, the appendage arcs of neighbouring petals enclose the whole upper half of a stamen (Fig. 5E). The petal apices curl back (Fig. 1J, K).

Spermaceo brevidens and *S. caudata*. As the ontogeny of the elaborate petals is very similar in these two species, they are discussed together. When anthers and filaments start differentiating, the distal-lateral zones of the petals are already slightly concave (Fig. 6A, B). At this stage, the first appendage primordia on the ventral side of the petals become visible on the longitudinal midvein ridge. The distal-lateral zones (dotted ovals) continue to grow outwards, while the petal apex remains short, so that two distal-lateral protuberances are formed. These protuberances are somewhat twisted so that one protuberance from each petal combines with a protuberance of the adjacent petal to enclose the anther (Fig. 6C, G).

Meanwhile, a row of trichomes has developed on the inner (adaxial) margins (Fig. 6C, arrow) as well as on the dorsal surface of the protuberances (Fig. 6C, D). The anthers and protuberances further elongate, while growth of the petal apices has arrested (Fig. 6E). The arc-shaped pattern of appendage outgrowths can now be recognized again, though the upper part of the appendage arcs in these species has moved from the vertical to the horizontal plane, right below the upwardly directed petal apices (Fig. 6F). The appendage outgrowths are tapering and smooth. The trichomes on the adaxial margins and the dorsal surface of the protuberances, in contrast, show a striate cuticular pattern. At this stage, it is also visible that the style has grown faster than the corolla tube, and has become curved (Fig. 6E).

Finally, each petal apex further protrudes upward (Fig. 6F). Below the appendages, a short longitudinal row of capitate trichomes appears in *S. caudata* (Fig. 6F). Further below, additional longitudinal rows of cylindrical trichomes emerge and elongate (Fig. 6I). In *S. brevidens*, however, capitate trichomes are lacking but the number of cylindrical trichomes in the corolla tube is higher (Fig. 6H, J). Before anthesis, the protuberances enclosing the anthers are twisted at a 90° angle with the corolla tube (Figs 1N, O and 6H). Each anther and upper part of the curved filament is still tightly enclosed by the protuberances. Also, a horseshoe-shaped area of swollen, spherical

FIG. 3. Floral development in *Spermaceo lignosa*. (A) Undifferentiated flower primordium: top view. (B and C) Dichasial cyme encircled by fimbriae; inception of first sepal pair. (D) Inception of second sepal pair; development of corolla ring primordium. (E) Origin of petal primordia on the corolla ring primordium; differentiation of sepal tube and free sepal parts. (F) Developing inflorescence: top view; petals of median flower meet centrally. (G) Older stamen primordium. (H) Development of carpel lobes; differentiation of stamens into anther and filament. (I) Colleters borne on sepal tube. (J) Style with bilobed stigma surrounded by nectary disc with nectarostomata: top view. (K) Origin of corolla appendages. (L) Detail of one petal with corolla appendage primordia. (M) Development of outgrowths on distal petal margins; inception of rows of trichomes at bases of free petal part. (N) Elongation of stamen-corolla tube. (O) Flower bud just before anthesis. (P) Mature flower. *, Inflorescence apex; a, stamen (primordium); ap, corolla appendages; at, anther; c, carpel lobe; cl, colleter; f, filament; fi, fimbriae; nd, nectary disc; p, petal primordium; p_L, free petal part; p_T, corolla tube; rp, ring primordium; s, sepal primordium; s_L, free sepal part; sl, style; sm, stigma; s_T, sepal tube.

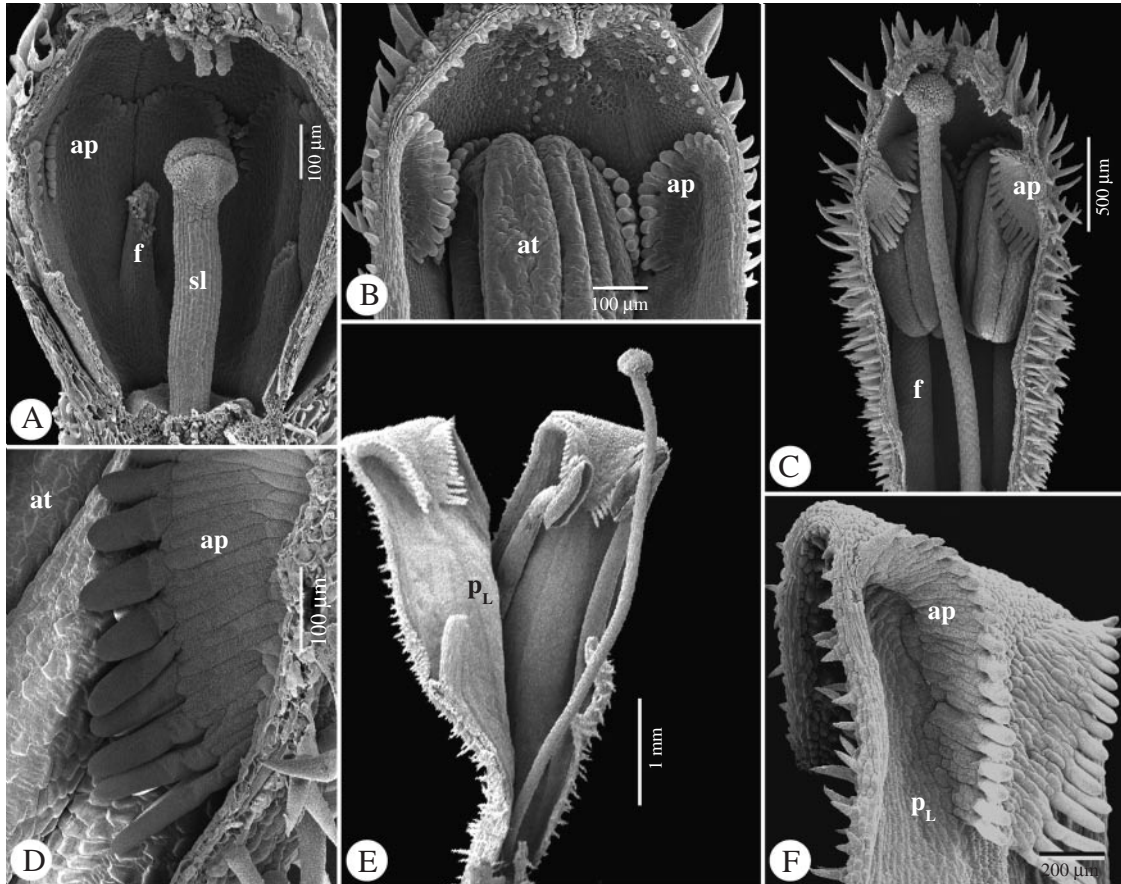


FIG. 4. Appendage development in *Spermacoco phaeosperma*: (A) inception of corolla appendages; (B) apical part of corolla showing the further development of the appendages; (C) floral bud just before anthesis; (D) detail of corolla appendage showing the basal continuous part bearing the trichome-like outgrowths; (E) mature flower at anthesis—notice how the right-hand anther is tightly enclosed by one of its two neighbouring appendages; (F) detail of curled back petal apices with appendages (stamens removed). ap, Corolla appendages (primordia); at, anther; f, filament; p_L, free petal part; sl, style.

cells is visible on the dorsal surface of the corolla surrounding the positions of the filament insertion (Fig. 6I, K). Eventually, both protuberances and petal apices open, the corolla now seemingly being 8-lobed (Figs 10 and 6I). The protuberances are spreading, while petal apices stay more or less upright (10). The filaments also stay upright, at least in cases where the flower has been visited by a pollinator (see Discussion). The style becomes exerted after release of the pollen grains.

Spermacoco erectiloba. As in *S. brevidens* and *S. caudata*, petal apices stay positioned upright immediately above the stigma, as the protuberances and anthers extend (Fig. 7B–D). Both the adaxial margin and dorsal surface of the protuberances are glabrous (Fig. 7C, D). Strong upward growth of the petal apices causes each apex to become linear (Fig. 7B–D), and a hollow cavity is formed in between. The style grows into this cavity (Fig. 7C). As shown in Fig. 7D, hindering further style growth by the petal apices causes it to coil up in the corolla tube. The shape of the appendage outgrowths on the ventral side of the petals is ellipsoid and their cuticle shows tiny knots (Fig. 7E, F). At this stage, there is also a longitudinal row of capitate trichomes beneath the petal

apex (Fig. 7C, E). At anthesis, the petal apices open, allowing the coiled-up style to stretch out. The distal-lateral protuberances, eight in total, stay upright (Figs 1L, M and 7G).

DISCUSSION

Floral ontogeny

Floral ontogenetic studies in the Rubiaceae are few and often concentrated on gynoecial development (e.g. Svoma, 1991; Igersheim, 1993; Igersheim and Rohrhofer, 1993; Igersheim *et al.*, 1994). Complete floral ontogenetic studies are published by Rutishauser *et al.* (1998) on *Theligonum*, and by Ronse Decraene and Smets (2000) on *Galopina*. Erbar and Leins (1996) discussed corolla tube formation and the difference between early and late sympetaly in Rubiaceae and related families.

Our floral ontogenetic results of *Spermacoco* support several earlier observations within Rubiaceae and Spermaceaceae. The congested inflorescences of *Spermacoco* can be derived from a thyrsoid, which is the basic inflorescence type in Rubiaceae. The fimbriae that surround the dichasial inflorescence units are hereby interpreted as the remnants of the prophylls. The reduction of leaf-like prophylls to

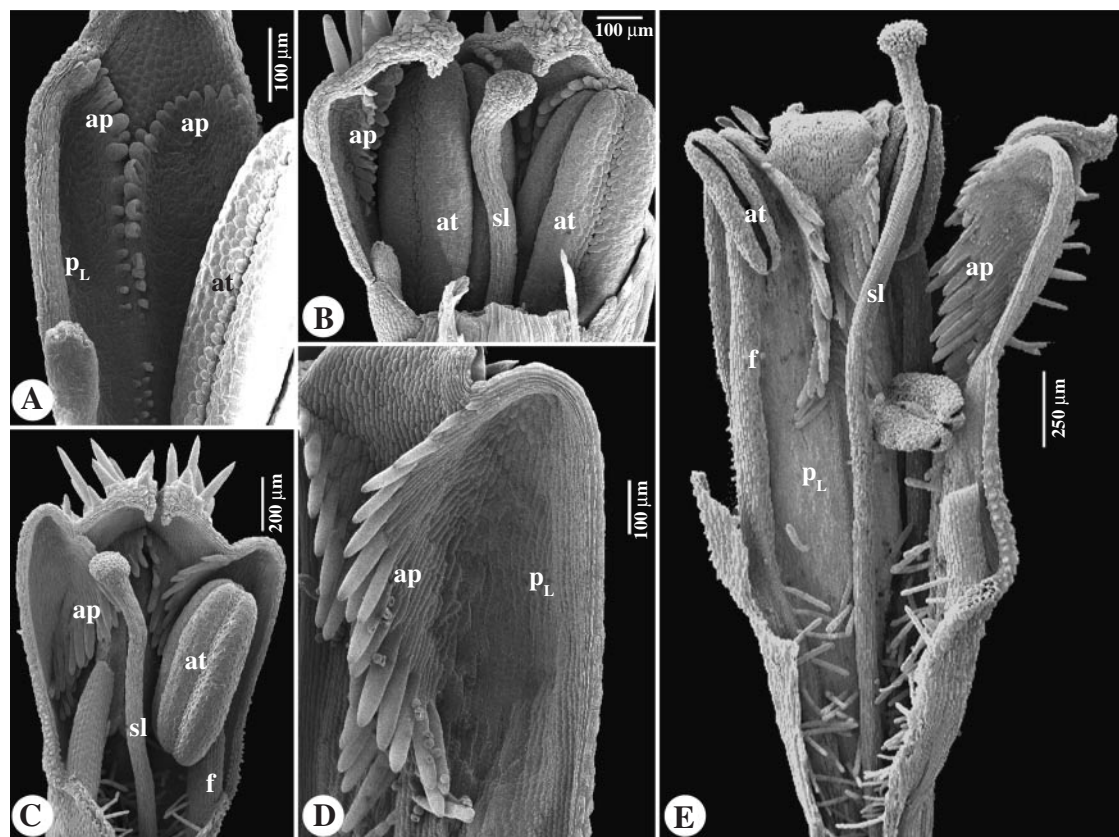


FIG. 5. Appendage development in *Spermacoce redacta*: (A) inception of corolla appendages; (B) further appendage development; outward growth of the distal-lateral zone of the petals resulting in concave regions left and right from the midvein; (C) flower bud just before anthesis; (D) detail of partly fused appendages and concave petal; (E) mature flower—notice how the anthers are enclosed by both the concave petal zone and the appendages. ap, Corolla appendages (primordia); at, anther; f, filament; p_L, free petal part; sl, style.

fimbriate structures has already been described for other Spermacoceae taxa with strongly congested inflorescences (*Crusea*, Anderson, 1972; *Richardia scabra*, Weberling and Troll, 1998). Also, early sympetaly, a feature believed to be constant for Rubiaceae and characterized by a corolla ring primordium (Erbar and Leins, 1996), is confirmed. Initial unequal sepal development, as documented here for *S. lignosa*, was previously demonstrated in *Galopina tomentosa* (Ronse Decraene and Smets, 2000).

Elaborate petals

A study by Endress and Matthews (2006) shows that elaborate petals are found throughout the eudicots and that they are very diverse in appearance, structure, ontogeny and function, and that in asterids, elaborations are often associated with the entire corolla rather than the individual petals. Rubiaceae petals, however, do not show much diversity. Apart from trichomes that are frequently present at the entrance or around the middle of the corolla tube, elaborate petals are reported in a few Rubiaceae genera only. *Corynanthe*, *Paracorynanthe* and *Pausinistalia* have conspicuous linear appendages on the petal apices (Robbrecht, 1988; Stoffelen *et al.*, 1996; Endress and Matthews, 2006; P. De Block, National Botanic Garden of Belgium, personal communication). Trilobed

petals are observed in *Dentella repens* (Schumann, 1891). In *Joosia*, the lobes are \pm verrucate on the adaxial surface and have fleshy, showy appendages on either side of the apex (Andersson, 1997). There is also an irregular, fleshy strip along the edge of the lobe running down from the appendage to the lobe base. According to Andersson (1997), these fleshy structures are elaborations of the lobe margin. The frequent occurrence of elaborate petals among Australian *Spermacoce* species is thus rather exceptional for Rubiaceae.

Ontogeny of the elaborate petals

Ontogeny of the four species with clear appendage arcs on the ventral side of the petals reveals that the appendages become first visible after differentiation of stamen primordia into filament and anther, but well before the elongation of the corolla tube. The arcs of appendage primordia converge from opposite lobe margins towards the midvein. Based on the time and position homology criteria, it is concluded that the appendages in these species are homologous. Although the appendages are initially similar, their morphology is remarkably different in mature flowers. In *S. lignosa* the appendages look like arcs of trichomes (Fig. 3P), whereas in *S. redacta* they strongly resemble parts of the petals (Fig. 5D). The morphology

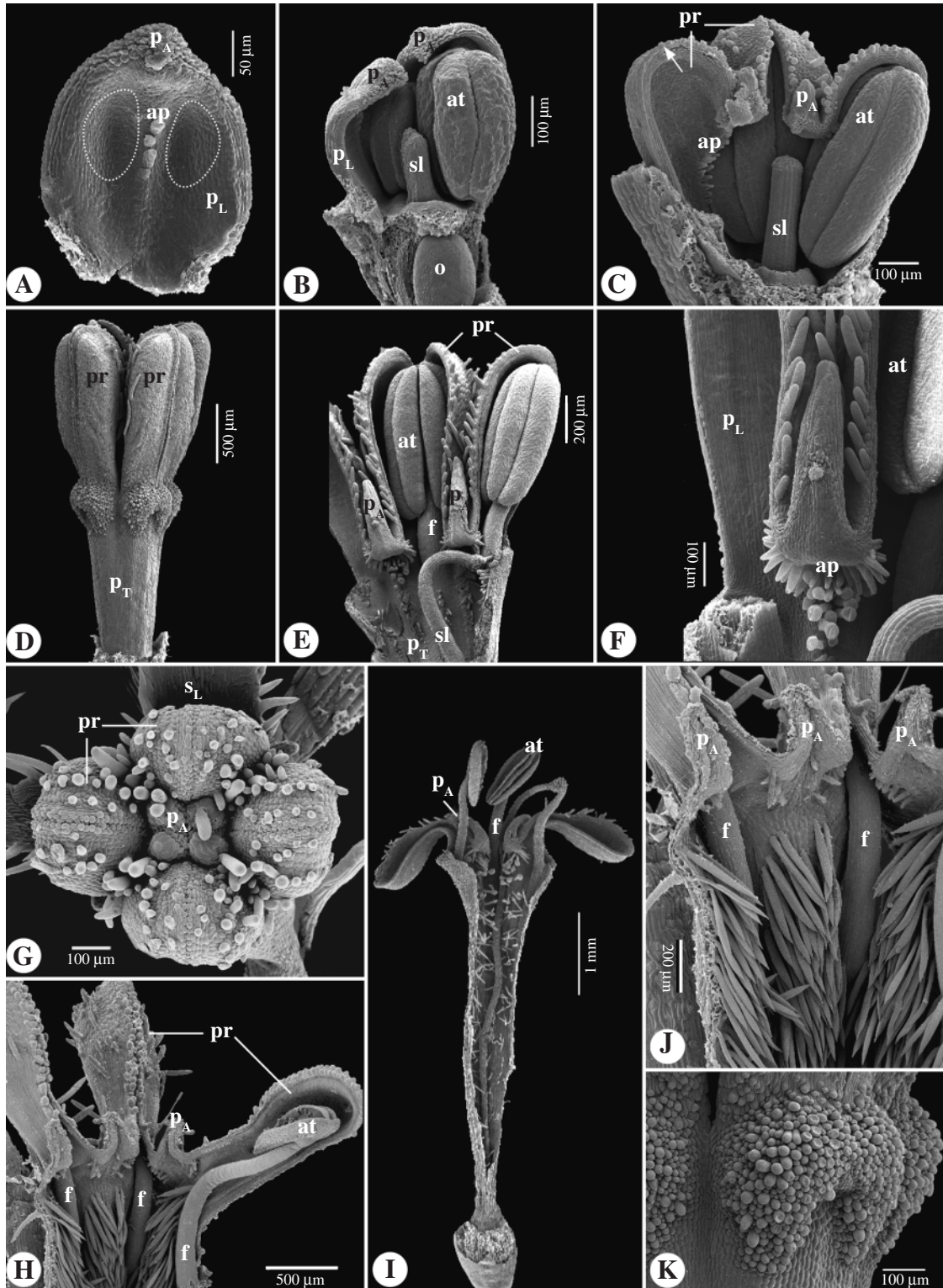


FIG. 6. Appendage development in *Spermacoe caudata* (A–F, I and K) and *S. brevidens* (G, H and J): (A) ventral surface of petal with appendage primordia; (B) young floral bud with appendage primordia; (C) development of distal-lateral protuberances; origin of trichomes on adaxial petal margins; (D) older floral bud with presence of horseshoe-shaped thickening at the base of the free petal parts (E) inside view of older floral bud showing the curved style and the stamens enclosed by the distal-lateral protuberances; (F) detail of petal apex with appendages and outgrowths on adaxial petal margins; (G) top view of floral bud showing the petal apices in the centre encircled by the distal-lateral protuberances; (H) inside view of floral bud just before anthesis; (I) inside view of mature flower; (J) detail of inside view of mature flower; (K) horseshoe-shaped thickening at basis of free petal part. ap, Corolla appendages (primordia); at, anther; f, filament; o, ovule; p_A, petal apex; p_L, free petal part; pr, protuberances; p_T, corolla tube; s_L, sepal.

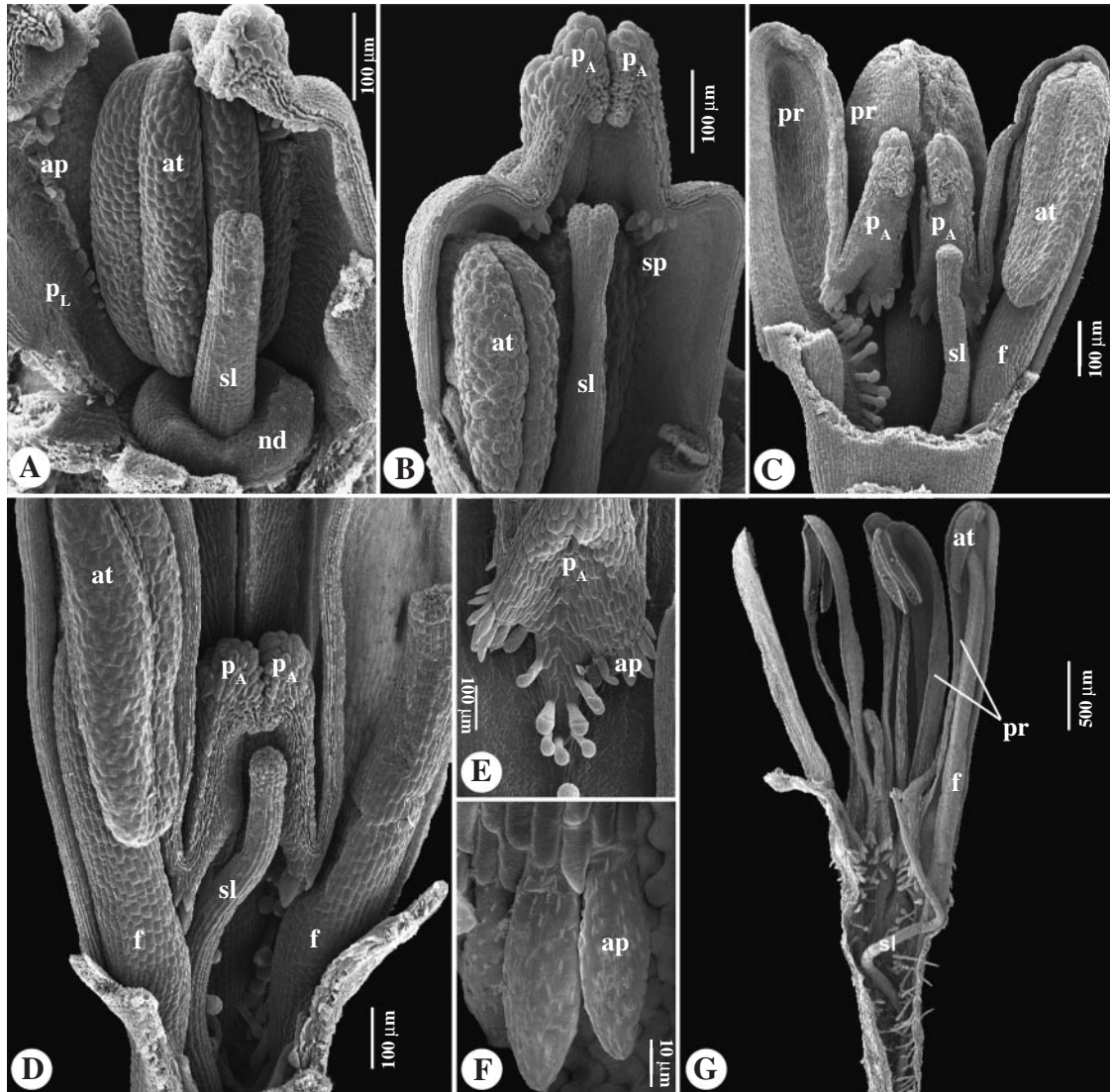


FIG. 7. Appendage development in *Spermacoce erectiloba*: (A) young floral bud showing presence of appendages on the ventral side of the petals; (B) development of distal-lateral protuberances; (C) stamen filament growth; (D) style growth hindering by united petal apices; (E) detail of petal apex with appendages and capitate trichomes; (F) detail of corolla appendages; (G) mature flower in which the style has not yet been released. ap, Corolla appendages (primordia); at, anther; f, filament; nd, nectary disc; p_A, petal apex; p_L, free petal part; pr, protuberances; sl, style.

of the appendages of *S. phaeosperma* is somewhat intermediate (Fig. 4D). The present ontogenetic observations show that this variation can be explained by (a) the degree of elongation of the individual outgrowths of the appendage and (b) the degree of activity of an intercalary growth zone positioned immediately below the outgrowths. In *S. lignosa* the intercalary growth zone is not or only slightly active while the outgrowths are long, resulting in appendages looking very similar to arcs of normal trichomes. In the other two species, on the contrary, the elongation of the outgrowths is limited especially at the top end of the appendages, while the intercalary growth zone produces a lot of petal-like tissue. As a result, the appendages have a petal-like base crowned with (short) trichome-like outgrowths.

In the species with distal-lateral protuberances, appendage primordia are also visible when the anthers

start differentiating. The present results refute, however, that the protuberances in *S. caudata* and *S. erectiloba* are derived from appendages. This is one of the suggestions that Harwood and Dessein (2005) put forward to explain the origin of these structures. This explanation would imply that the row of outgrowths on the adaxial margin of the protuberances in *S. caudata* is the further continuation of the appendage outgrowths below the petal apex (Fig. 8). However, (a) detailed morphology of the outgrowths is different, as the appendage outgrowths do not show the striate cuticular pattern; (b) outgrowths on the adaxial protuberance margins are optically active in contrast to appendage outgrowths, as can be seen by examining the corolla with a light microscope using polarized light. Consequently, the trichomes on the protuberance margins belong to the external indumentum (Robbrecht, 1988); (c) the vascularization pattern also provides evidence that

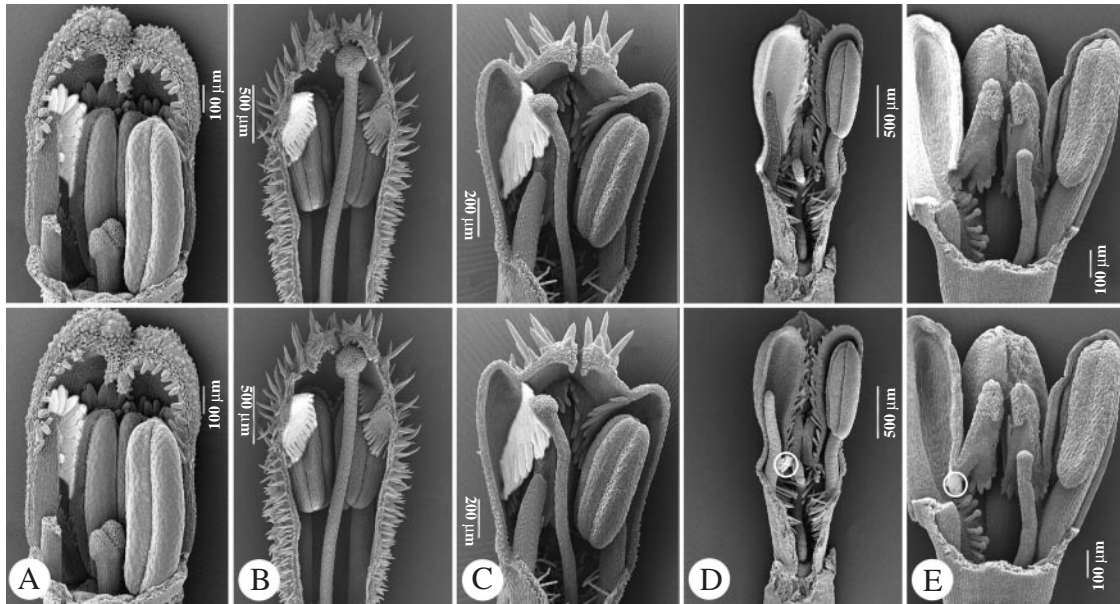


FIG. 8. Homology hypothesis of Australian *Spermacoce* species according to Harwood and Dessein (2005) (upper row) and present work (lower row). Whitened and encircled flower structures are considered to be homologous. (A) *S. lignosa*; (B) *S. phaeosperma*; (C) *S. redacta*; (D) *S. caudata*; (E) *S. erectiloba*.

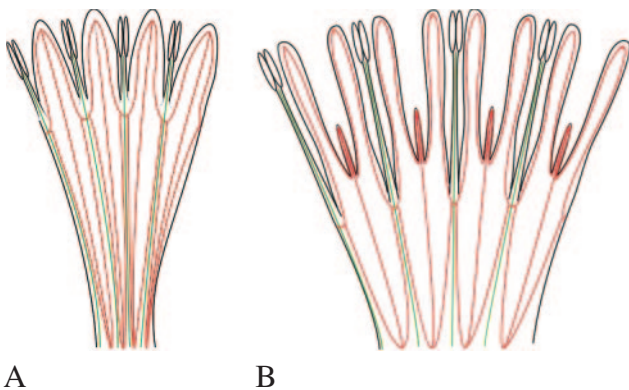


FIG. 9. Corolla vascularization pattern in *Spermacoce* species: (A) *S. lignosa*; (B) *S. caudata*. The vascular bundles of the corolla are shown in red; the staminal vascularization pattern in green.

the protuberances are entirely derived from the petal (Fig. 9). The vascular bundles running along the edge of the protuberances in *S. caudata* are homologous to the vascular bundles that cross the free petal part in species without protuberances.

Harwood and Dessein (2005) hypothesized that there has been (a) an evolution towards increased fusion of appendage outgrowths and (b) an evolution from entire to 'deeply divided' petals (i.e. petals with distal-lateral protuberances). It is confirmed here that these are two morphological lineages but it is demonstrated that they are independent from each other.

Whereas the protuberances in *S. erectiloba* at anthesis stay upright, those of the other three species are reflexed. Harwood and Dessein (2005) explain that this reflexion is due to torsion stress imposed on the lower part of the protuberances, as they have to twist away from each other

to enclose neighbouring anthers. It is possible that torsion stress does contribute to protuberance movements, but the present observations indicate that the horseshoe-shaped zone of thickened cells at the base of the protuberances in *S. caudata* may also play an important role. This zone is absent in *S. erectiloba*. Lobe reflexion could thus be the result of changes in turgor pressure in this zone. In *S. brevidens*, however, the zone of thickened cells is less clear.

Function of the elaborate petals

Little is known about the pollination biology of *Spermacoce* species which makes it difficult to estimate the function of the elaborate petals. Harwood and Dessein (2005) mention that the appendages on the ventral side of the petals form an effective barrier between anthers and stigma and thus might favour cross-pollination. Field observations of *Spermacoce calliantha* (Fig. 1C–E), a species with appendages similar to *S. phaeosperma*, confirm that anthers and style are well separated by the appendages. The appendages, however, might also provide other advantages to the plant. The most plausible ones are listed here. (a) Appendages hide the pollen grains from possible pollen thieves or less good pollinators. In this way, the plant ensures that pollen is not wasted on unwanted visitors. (b) The fact that pollen is enclosed by the appendages ensures that it is not blown away by wind or washed away by mild rainstorms. (c) The fact that the pollen grains are hidden for the pollinator lengthens the time needed by the pollinator to collect the pollen grains and more pollen grains may remain attached to the body of the pollinator. This in turn increases the chance of deposition of pollen grains on the style. The latter hypothesis is reinforced by pollination observations in

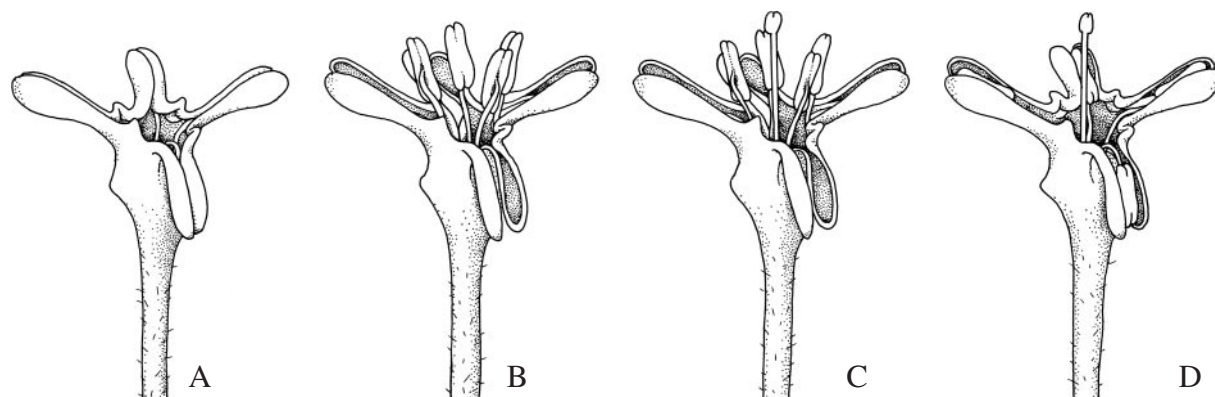


FIG. 10. Different flowering stages observed in the capitula of *Spermacoce brevidens*: (A) mature flower awaiting pollinator; (B) flowering stage after visit of pollinator—the anthers were catapulted towards the centre giving the pollinators a shower of pollen grains; (C) flowering stage following stage depicted in (B) in which the elongated style is exposed; (D) flowering stage without prior visit by pollinator—the anthers are released from the distal-lateral protuberances, but the filaments have already lost the turgor to become erect.

S. calliantha. This species is pollinated by a native bee species. It was observed that pollen is not easily collected by the bees and that the insects are covered with pollen grains. It was also observed that the bees forage between the different individuals of a population, making cross-pollination more plausible.

All these factors might explain why so many Australian *Spermacoce* species are provided with appendages. It is also noteworthy that species, of which appendages are presumed to have been lost during evolution, are invariably narrow endemics specialized to particular growth conditions. In this context, *S. auriculata* is remarkable. This species has appendages, but the free petal parts, including the appendages, are—at least in some populations—spreading at anthesis (Fig. 1F–H). The appendages thus cannot fulfil the same functions as described above. Nevertheless, the species is widespread and common in its native range.

In *S. brevidens*, *S. caudata* and *S. erectiloba*, the barrier function of the appendages has been taken over by the protuberances and it is here hypothesized that the corolla appendages have been secondarily reduced as a result of their decreased functional importance. The pollination strategy in these species is also different. Unlike most species with corolla appendages, the style is not exerted at the moment pollen is released from the anthers. In fact, field experiments with *S. brevidens* have shown that the stamens are catapulted towards the centre of the flower and release their pollen when pressure is exerted on the central part of the corolla, which is provided with many rigid trichomes. At this time, the style is still included in the corolla tube. Only after the pollen has been released, do the protuberances and the petal apices open and the style becomes exerted. In this way, the plant ensures that pollen is transferred to the body of the pollinator. The pollinator of the species with protuberances is yet to be identified. It is presumed that if no pollinator visits the flower, the protuberances open as well, yet the anther filaments have lost the turgor to assume an upright position and consequently remain in a 90° position to the corolla tube and style. As such the different flowering stages (Fig. 10)

observed in the flowering heads of *Spermacoce brevidens* can be explained: (a) stamens enclosed by the distal-lateral protuberances and style included: situation before pollination; (b) stamens released from protuberances and upright, while the style is included: situation after visit by pollinator; (c) stamens upright and style exerted: stage following stage (b) after elongation of the style; (d) stamens released from protuberances but not erect and style exerted: late flowering stage without prior visit of pollinator.

Overall, two phylogenetic lineages can be discerned in the Australian *Spermacoce* species studied, based on the morphology of their elaborate petals. In one lineage the appendage outgrowths are variously fused, whereas in the other lineage they are reduced and functionally replaced by two distal-lateral petal protuberances. Both corolla appendages and protuberances seem to play an important role in the pollination biology of the species. Molecular phylogenetic studies are needed to assess the proper relationships between Australian *Spermacoce* species and to further understand the evolutionary origin of the different floral morphs observed.

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