



Floral ontogeny in Dipterygeae (Fabaceae) reveals new insights into one of the earliest branching tribes in papilionoid legumes

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Flowers of Dipterygeae (Fabaceae, Papilionoideae) exhibit an unusual petaloid calyx. The two adaxial sepals are large and petaloid, and the three abaxial sepals form a three-toothed lobe. The goal of this study was to elucidate the ontogenetic pathways of this peculiar calyx in light of the floral development of the three genera that comprise the tribe. Floral buds of *Dipteryx alata*, *Pterodon pubescens* and *Taralea oppositifolia* were analysed using scanning electron microscopy and light microscopy. The order of bracteole and sepal initiation varies among the species. The androecium is asymmetric. The carpel cleft is positioned to the right or to the left, and is opposite the adaxial antepetalous stamen. The peculiarity of the calyx becomes noticeable in the intermediate stages of floral development. It results from the differential growth of the sepal primordia, in which the abaxial and lateral primordia remain diminutive during floral development, compared with the adaxial ones that enlarge and elongate. Bracteoles, abaxial sepals, petals and anthers are appendiculate, except in *T. oppositifolia*, in which the appendices were not found in bracteoles or anthers. These appendices comprise secretory canals or cavities. Considering that the ontogenetic pathway for the formation of the petaloid calyx is similar and exclusive for Dipterygeae, it might be a potential synapomorphy for the group, with the presence of secretory canals in the appendices of abaxial and lateral sepals and petals. © 2014 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2014, **174**, 529–550.

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INTRODUCTION

The tribe Dipterygeae belongs to Fabaceae subfamily Papilionoideae. The members of this subfamily have flowers with five sepals, generally equal to subequal in size and shape. They are typically united to a large extent of their length, resulting in a gamosepalous calyx with five lobes (Polhill, 1981). Such union, in the species studied so far, occurs on the margins of the sepals during intermediate stages of floral development (Mansano, Tucker & Tozzi, 2002; Tucker, 2003a;

Teixeira, Ranga & Tucker, 2009; Paulino, Groppo & Teixeira, 2011). Different conditions rarely occur in Papilionoideae, tribe Dipterygeae being among the exceptions. The name of the tribe originates from the flowers with a two-lipped calyx, one of which is composed of two enlarged and petaloid sepals and the other of an abaxial and two lateral, reduced and united sepals. In *Monopteryx* Spruce ex Benth., the genus that is sister to Dipterygeae (see Francisco, 2010; Cardoso *et al.*, 2012), the two adaxial sepals are almost completely united and cover the floral bud (Polhill, 1981).

Dipterygeae is monophyletic (Pennington *et al.*, 2001; Wojciechowski, Lavin & Sanderson, 2004;

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Barham, 2005; Cardoso *et al.*, 2012) and, according to Cardoso *et al.* (2012), forms a clade with *Monopteryx*, sister to the so-called *Amburana* clade composed of the genera *Amburana* Schwacke & Taub., *Dussia* Krug & Urb. ex Taub., *Myroxylon* L.f., *Myrospermum* Jacq. and *Myrocarpus* Allemão (Cardoso *et al.*, 2012; see also Pennington *et al.*, 2001 and Wojciechowski *et al.*, 2004). Dipterygeae comprises about 22 species in three genera, *Dipteryx* Schreb., *Pterodon* Vogel and *Taralea* Aubl., with *Taralea* considered as the sister group to *Pterodon* + *Dipteryx* (Pennington *et al.*, 2001). The species are distributed in Central and South America (Polhill, 1981) and are bee pollinated (Afonso, 1997; Oliveira & Sigrist, 2008). A remarkable feature of the tribe and the *Amburana* clade is the absence of symbiotic association with root-nodulating bacteria (Sylvester-Bradley *et al.*, 1980; Faria *et al.*, 1989; Moreira, Silva & Faria, 1992; Sprent, 2001).

Dipteryx, the largest genus, consists of 12 species distributed in the Amazonian region, Panama, Honduras and dry areas of central Brazil (Barham, 2005). *Dipteryx alata* Vogel is popularly known in Brazil as 'baru' or 'cumbaru', and is utilized as food, fodder, ornamentation, wood, syrup, oilseed and a tanniferous agent (Almeida *et al.*, 1998). The oil is used as an anti-rheumatic (Barros, 1982), the fruits are edible (Lorenzi, 1992; Togashi & Sgarbieri, 1995) and the seeds are high in protein (Cruz *et al.*, 2011).

Pterodon is the genus with the smallest number of species, about three, distributed in dry areas of central western Brazil and eastern Bolivia (Barham, 2005). There are various infrageneric delimitation problems (Lewis, 1987; Rocha, 2006), despite the fact that it is the genus of the tribe on which the most morphological and structural studies have been conducted. Among these, we can mention the study of secretory structures by Rodrigues, Santos & Machado (2011) and Paiva *et al.* (2001), fruit anatomy by Paiva, Oliveira & Machado (2008), seed anatomy by Oliveira & Paiva (2005), pulvinus, petiole and rachis anatomy by Rodrigues & Machado (2004) and Machado & Rodrigues (2004), and wood anatomy by Paula & Cardoso (1995). *Pterodon pubescens* (Benth.) Benth. is commonly known as 'sucupira-branca'. The oil extracted from its fruits has been traditionally used for arthritis (Coelho *et al.*, 2001), rheumatism (Correa, 1984) and schistosomiasis (Santos-Filho *et al.*, 1987; Katz *et al.*, 1993), and as an anti-inflammatory (Nunan *et al.*, 1982; Carvalho *et al.*, 1999), antinociceptive (Duarte *et al.*, 1996; Coelho *et al.*, 2005) and antiulcerogenic (Dutra *et al.*, 2009).

Taralea comprises about seven species in the Amazonian region of the Guianas, Brazil, Colombia and Peru. Its wood is used for construction and firewood. *Taralea oppositifolia* Aubl. is commonly known as

'cumaru-da-praia' and contains an unscented oil extracted for industrial use (Barham, 2005).

The developmental stages of the organs that constitute the flower of *Dipteryx*, *Pterodon* and *Taralea* are unknown. Thus, it is not yet understood how and in which stages of development the changes in the sepal primordia that culminate in a petaloid calyx occur. As Dipterygeae is monophyletic, it is important to verify whether the ontogenetic pathways involved in the formation of this peculiar calyx in the different genera are homologous, and how the ontogeny can explain the differences in the morphology of the flowers.

Thus, the goal of this study is to compare the floral development and morphology of one species of each of the three genera of Dipterygeae (*D. alata*, *P. pubescens* and *T. oppositifolia*), with an emphasis on the calyx, to understand how and when the changes resulting in the petaloid calyx in Dipterygeae occur. Other data collected in the study of the floral development and morphology are discussed to establish diagnostic characters and potential synapomorphies for the tribe, as already documented in studies of floral development for other legumes (see Tucker, 1993, 1997, 2003a; Mansano *et al.*, 2002; Prenner, 2004a; Mansano & Teixeira, 2008; Paulino *et al.*, 2011).

MATERIAL AND METHODS

Floral buds at different stages of development and flowers of at least two individuals of *D. alata*, *P. pubescens* and *T. oppositifolia* were collected in September, October and November of 2009 and 2010. Vouchers specimens were deposited in SPFR (Teixeira *et al.* 37, 38 and 39), INPA (Holanda & Pereira 480) and IAN (Freitas 233) herbaria. Samples were dissected and prepared for surface observation under scanning electron microscopy and anatomical analyses under light microscopy.

For the surface analyses of floral organs, samples were fixed in 5 parts formalin : 5 parts acetic acid : 90 parts 50% ethanol (FAA 50) (Johansen, 1940), dissected in 70% alcohol under a stereomicroscope, critical point dried in a Balzers CPD 030 dryer, mounted on aluminium stubs with colloidal carbon, coated with gold in a Bal Tec SCD 050 sputter coater and observed with Jeol JSM 5200, Zeiss EVO 50 and Zeiss EVO 40 scanning electron microscopes.

For anatomical analyses, samples were fixed in FAA 50 or Karnovsky's solution (0.075 M in phosphate buffer, pH 7.2–7.4) (Karnovsky, 1965) for 24 h, dehydrated gradually in an ethanol series, embedded in historesin (Gerrits & Horobin, 1991) and transversely and longitudinally sectioned (2–3 µm thick) on a Leica RM 2245 rotary microtome. Sections were stained with 0.05% toluidine blue (O'Brien, Feder & McCully, 1964), mounted in synthetic resin (Gerlach, 1969) and

observed under a Leica DM 500 light microscope. For the detection of compounds in tissues, we used Sudan black B for total lipids (Jensen, 1962), xyloidine de Ponceau for proteins (Vidal, 1970) and periodic acid/Schiff reagent (PAS) for polysaccharides (O'Brien & McCully, 1981). Standard control materials were obtained simultaneously. Photomicrographs were taken with a Leica DM 500 B microscope coupled to a Leica DFC 295 digital camera.

The terminology used to describe the observed floral ontogeny followed Tucker (1987, 1997, 2003a), Klitgaard (1999) and Prenner (2004a, b). The adaxial side of the flower is the upper one, which is closest to the inflorescence axis; the abaxial side of the flower is the lower one, which is opposite the inflorescence axis and closest to the bract (Tucker, 1984). The floral development stages are divided here into early development (organ initiation), mid-development (early enlargement and differentiation of organ form) and late development (late elongation of organs and tissue and cell differentiation). The ovule type terminology follows Prakash (1997).

RESULTS

The organography, developmental stages and secretory structures of the flowers are presented in a comparative fashion for *D. alata*, *P. pubescens* and *T. oppositifolia*. The differences between species are highlighted in Table 1.

ORGANOGRAPHY

The flowers are hermaphrodite, zygomorphic, small and have coloured nectar-guides (Fig. 1A–I). Bracts and bracteoles are early deciduous.

The calyx is greenish in *D. alata*, pink and basally green in *P. pubescens* and purple in *T. oppositifolia*. All abaxial lobes are appendiculate.

The corolla is typically papilionoid, formed by the standard, two wings and two keels, varying from pink to white in *D. alata* (Fig. 1A), pink in *P. pubescens* (Fig. 1D) and purple in *T. oppositifolia* (Fig. 1G). In the distal portion of all five petals, there are appendices in which a secretory canal is found.

The androecium is heterodynamous and monadelphous, comprising ten stamens with dorsifixed, longitudinally dehiscent anthers. Anthers are appendiculate only in *D. alata* and *P. pubescens*.

The gynoecium is formed of a single carpel and a single hemitropous ovule. There is a short hypanthium, c. 1.0 mm long in *P. pubescens* and 2.0 mm long in *D. alata* and *T. oppositifolia*. It is formed by the union of the receptacle and the base of the sepals, petals and filaments; the petal claw is attached to the hypanthium margins. The ovary is more than two

times longer than wide and glabrous in *D. alata* and *P. pubescens*, and almost as long as wide and covered by non-secretory trichomes in *T. oppositifolia*. The stigma is terminal and is truncate in *D. alata*, capitate in *P. pubescens* and punctiform in *T. oppositifolia*, the style is hollow in all species.

ORGANOGENY

The floral apex is elliptical (Fig. 2A–C). The order of initiation of the different floral whorls is similar: bracteoles, sepals, petals, carpel + the antesealous stamen primordia and, finally, the antepetalous stamens.

Two bracteole primordia are initiated asynchronously in *D. alata* (Fig. 2D) and simultaneously in *P. pubescens* (Fig. 2E) and *T. oppositifolia* (Fig. 2F); these bracteoles cover the floral apex (Fig. 2G–I).

The order of sepal initiation is modified unidirectional in *D. alata* (Fig. 3A–C): the abaxial sepal primordium is initiated first, followed by two lateral sepal primordia. The last two arise on the adaxial side at different times. In *P. pubescens*, the order of sepal initiation is helical (Fig. 3E–G): the abaxial sepal primordium is initiated first, followed by two adaxial sepal primordia. The last two arise laterally. In *T. oppositifolia*, the order of sepal initiation is sequential or modified sequential (Fig. 3I–K): the abaxial sepal primordium is initiated first, followed by two lateral sepal primordia. The last two are the adaxial sepal primordia. This pattern can differ, with the adaxial sepals initiated in a reversed sequence. Initiation of the five petals is simultaneous in all three species (Fig. 3D, H, L) after sepal elongation. The carpel primordium arises as a central protuberance after petal initiation and concurrently with the initiation of the abaxial and lateral antesealous stamen primordia (Fig. 4A, E, I).

The order of initiation of antesealous stamens is modified unidirectional (Fig. 4B, F, J), in which the abaxial and lateral antesealous stamens are formed simultaneously, and the two adaxial antesealous stamens arise later. The five antepetalous stamen primordia are the last to be initiated, the adaxial one being the last of these (Fig. 4C, G, K). The adaxial antepetalous stamen is not fully median, but is placed either to the left (Fig. 4H) or to the right (Fig. 4D, L) of the vertical plane of the flower (Fig. 4D, H, L), characterizing it as an asymmetric androecium.

MID- AND LATE DEVELOPMENTAL STAGES

The carpel cleft is formed when all organs are initiated. It is positioned to the right or the left, opposite the adaxial antepetalous stamen primordium (Fig. 5A–F). The ovules are initiated after closure of the carpel cleft.

Table 1. Comparative characters of *Dipteryx alata*, *Pterodon pubescens* and *Taralea oppositifolia*

Character	<i>Dipteryx alata</i>	<i>Pterodon pubescens</i>	<i>Taralea oppositifolia</i>	Reference
Flower				
Order of bracteole initiation	Asynchronous	Simultaneous	Simultaneous	Present study
Order of sepal initiation	Modified unidirectional	Helical	Sequential or modified sequential	Present study
Type of wing sculpture	Lamellate	Lunate	Deep lamellate	Present study; Oliveira & Sigrist (2008)
Stigma shape	Truncate	Capitate	Punctiform	Present study
Style curvature	Curved	Curved	Erect	Present study; Francisco (2010)
Stipe length	Long	Long	Short	Present study; Francisco (2010)
Ovary surface	Glabrous	Glabrous	Pilose	Present study; Francisco (2010)
Occurrence of anther appendix	Present (discrete)	Present (conspicuous)	Absent	Present study
Occurrence of bracteole appendix	Present	Present	Absent	Present study
Fruit	Drupe	Cryptosamara	Legume	Francisco (2010); Barroso <i>et al.</i> (1999); Polhill (1981); Pinto <i>et al.</i> (2014)
Seed				
Seed shape	Elliptic to oblong or linear, slightly compressed	Elliptic to oblong, slightly compressed	Circular to ovate, compressed	Francisco (2010); Pinto <i>et al.</i> (2014)
Plumule evidence	Conspicuous	Conspicuous	Inconspicuous	Francisco (2010); Pinto <i>et al.</i> (2014)
Leaf				
Rachis shape	Winged	Margined	Winged	Francisco (2010); Pinto <i>et al.</i> (2014)
Position of central vein	Eccentric	Central	Central	Francisco (2010); Pinto <i>et al.</i> (2014)
Shape of foliole apex	Cuspidate to obtuse	Retuse to marginate	Cuspidate to acute	Francisco (2010); Pinto <i>et al.</i> (2014)

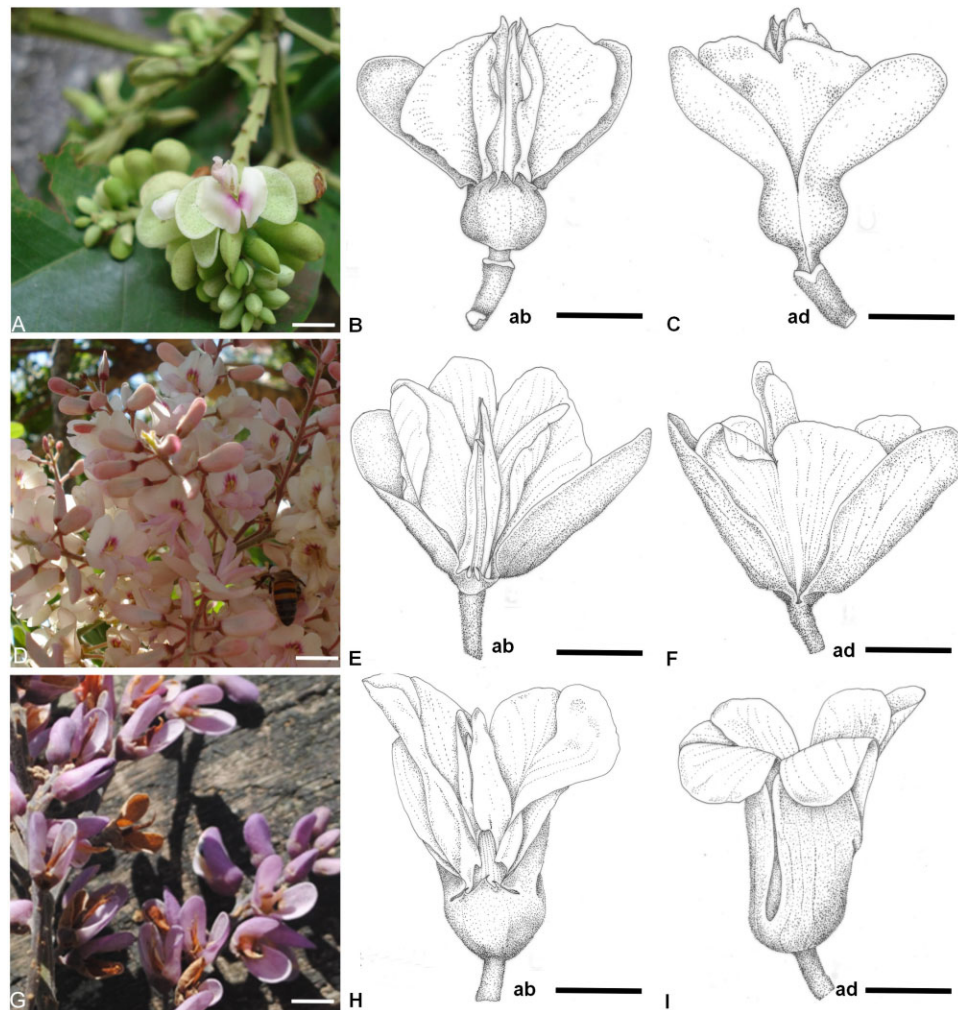


Figure 1. Flowers and inflorescences of *Dipteryx alata* (A–C), *Pterodon pubescens* (D–F) and *Taralea oppositifolia* (G–I). A, D, G, Front view of a flower. B, E, H, Abaxial side of a flower. Note that the three abaxial sepals unite, forming teeth-like lobes. C, F, J, Adaxial side of a flower. Note the two large adaxial sepals. ab, abaxial side; ad, adaxial side. Scale bars: A, D, G, 1 cm; B, C, E, F, H, I, 2.5 mm.

The adaxial sepals enlarge and elongate, resulting in two-winged large organs, whereas the abaxial and lateral sepals do not elongate, remaining reduced in size. These three abaxial sepals unite resulting in a three-lobed lip (Fig. 6A–C). During the closure of the carpel cleft, the margin of the adaxial sepals thickens as a result of an increase in the number of layers and volume of the phenolic cells, located in epidermal and subepidermal layers (Fig. 6D–F).

After elongation and enlargement of the adaxial sepals, other organs start to elongate. In the corolla, the standard elongates first, becoming more evident than the other petals. During the elongation, the margins of the keels unite. The petal appendices are already visible in the intermediate stages of development, whereas the wing sculpturing becomes evident in the late stages. The sculptures are observed on the

abaxial side of the wings near the margins and consist of epidermal indentations (Fig. 7A–F): the indentations are deeper in *D. alata* (Fig. 7A, B) and *T. oppositifolia* (Fig. 7E, F), characterizing the lamellate type, whereas, in *P. pubescens*, they are lunate (Fig. 7C, D). The epidermis is uniseriate and papillose, with densely stained cells and a striated cuticle. The mesophyll contains wide intercellular spaces in the area of sculptures (Fig. 7D, F). In *D. alata* and *P. pubescens*, the intercellular spaces are in the upper basal and central region, whereas, in *T. oppositifolia*, they are in the basal and central parts of the upper and lower region.

The ten stamens reach two different lengths, the antesealous stamens being larger than the antepetalous ones. The antesealous and antepetalous stamens are interleaved (Fig. 8A–C, E, F, H, I).

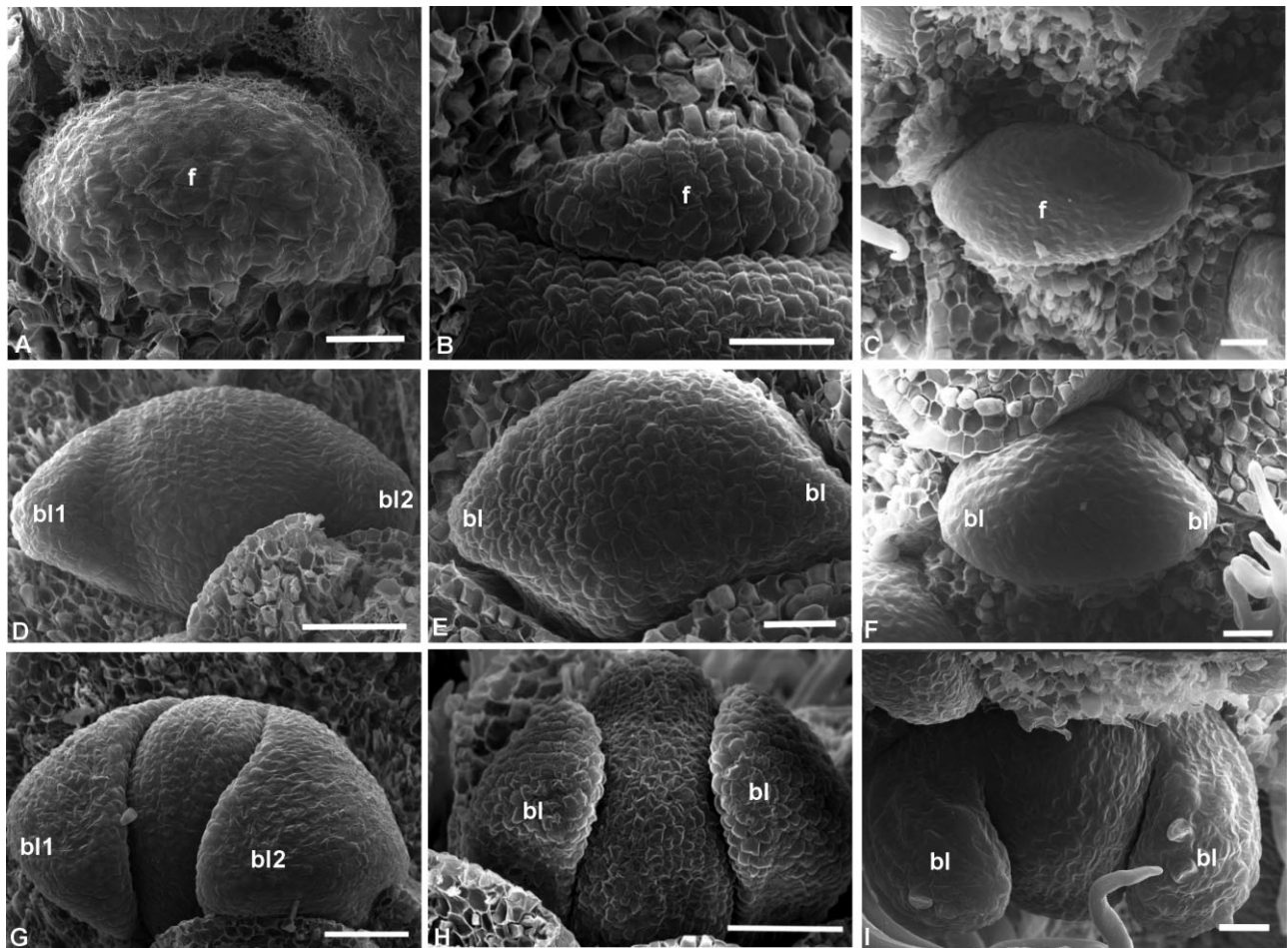


Figure 2. Initiation and elongation of bracteoles in *Dipteryx alata* (A, D, G), *Pterodon pubescens* (B, E, H) and *Taralea oppositifolia* (C, F, I) (scanning electron microscopy, SEM). A–C, Floral meristem. D, F, Asynchronous initiation of two bracteoles. E, Simultaneous initiation of two bracteoles. G–I, Floral meristem covered by two bracteoles. bl1 and bl2, bracteole primordia; f, floral meristem. Scale bars: A–C, E, F, I, 20 µm; D, G, H, 50 µm.

Figure 3. Initiation of sepals and petals of *Dipteryx alata* (A–D), *Pterodon pubescens* (E–H) and *Taralea oppositifolia* (I–L) (scanning electron microscopy, SEM). A–C, Modified unidirectional sepal initiation. D, Simultaneous petal initiation. E–G, Helical sepal initiation. H, Simultaneous petal initiation. I–K, Sequential/sequential modified sepal initiation. L, Simultaneous petal initiation. p, petal primordia; s1, abaxial sepal primordium; s2, s3, s4, s5, lateral sepal primordia (s3 and s4, adaxial sepals in *Dipteryx alata*; s4 and s5, adaxial sepals in *Taralea oppositifolia*). Scale bars: A–D, F–H, 50 µm; E, I–L, 20 µm.

Initially, the filaments are free, but later, when the stigmatic region starts to differentiate, their bases unite (Fig. 8A, B, E, H), resulting in an adaxially open sheath. This sheath is constricted at *c.* 1 mm from the base of the flower where a nectary is formed (Fig. 8C, F, I). The filaments unite as a result of the intercalary meristem activity, through periclinal divisions. This meristem originates from the differentiation of the parenchymatic cells.

The carpel is elevated by a stipe, which is shorter in *T. oppositifolia* than in the other species. Elongation

of the carpel is concurrent with elongation and union of the stamens (Fig. 8B, E, H). The style bends towards the adaxial side in *D. alata* and *P. pubescens* (Fig. 8C, F) and remains erect in *T. oppositifolia* (Fig. 8I); the stigma is totally differentiated in pre-anthetic buds and its surface is composed of papillose cells (Fig. 8D, G, J).

SECRETORY STRUCTURES IN THE FLOWER

Secretory canals are found in the appendices of the bracteoles in *D. alata* and *P. pubescens* and in the

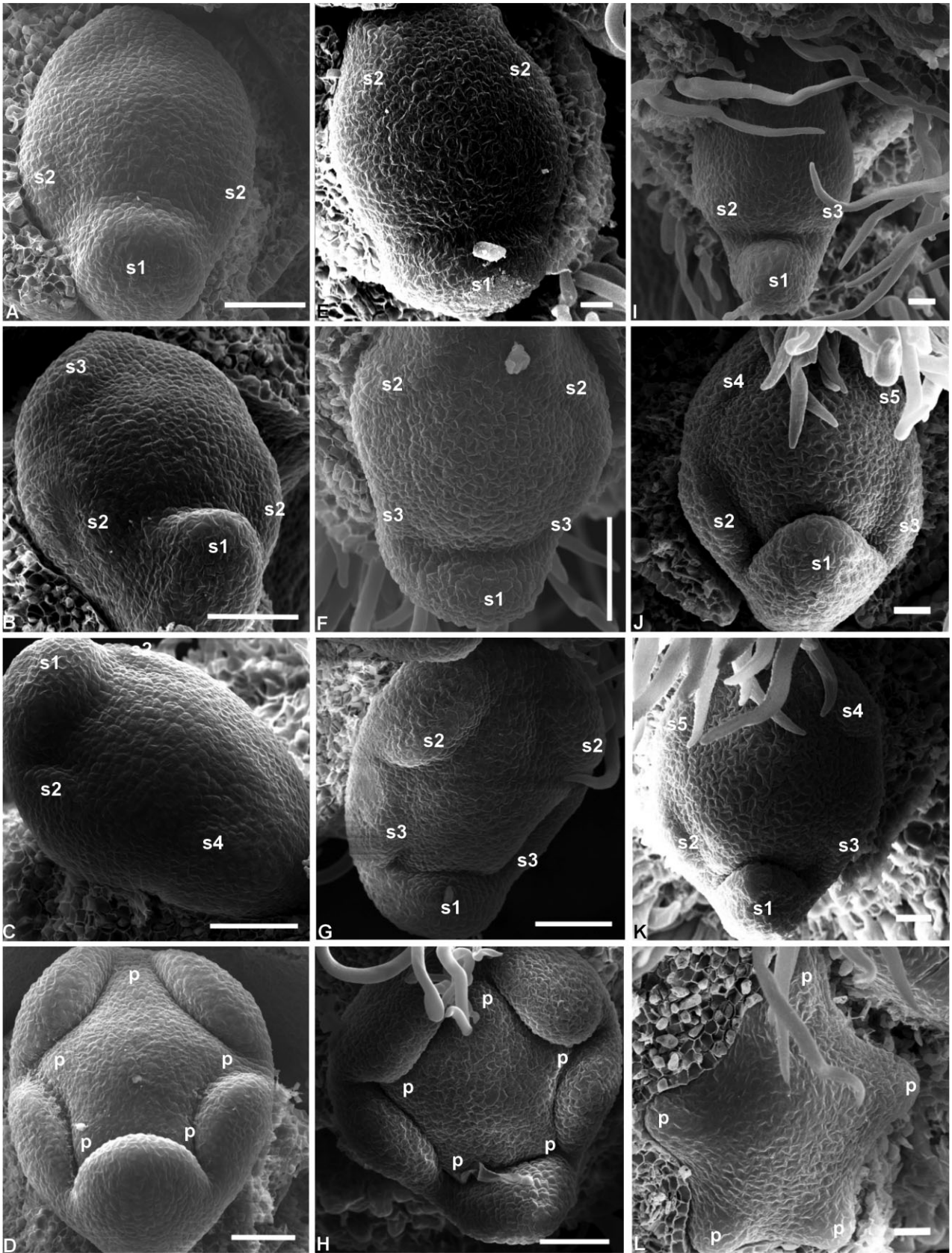


Figure 3. See caption on previous page.

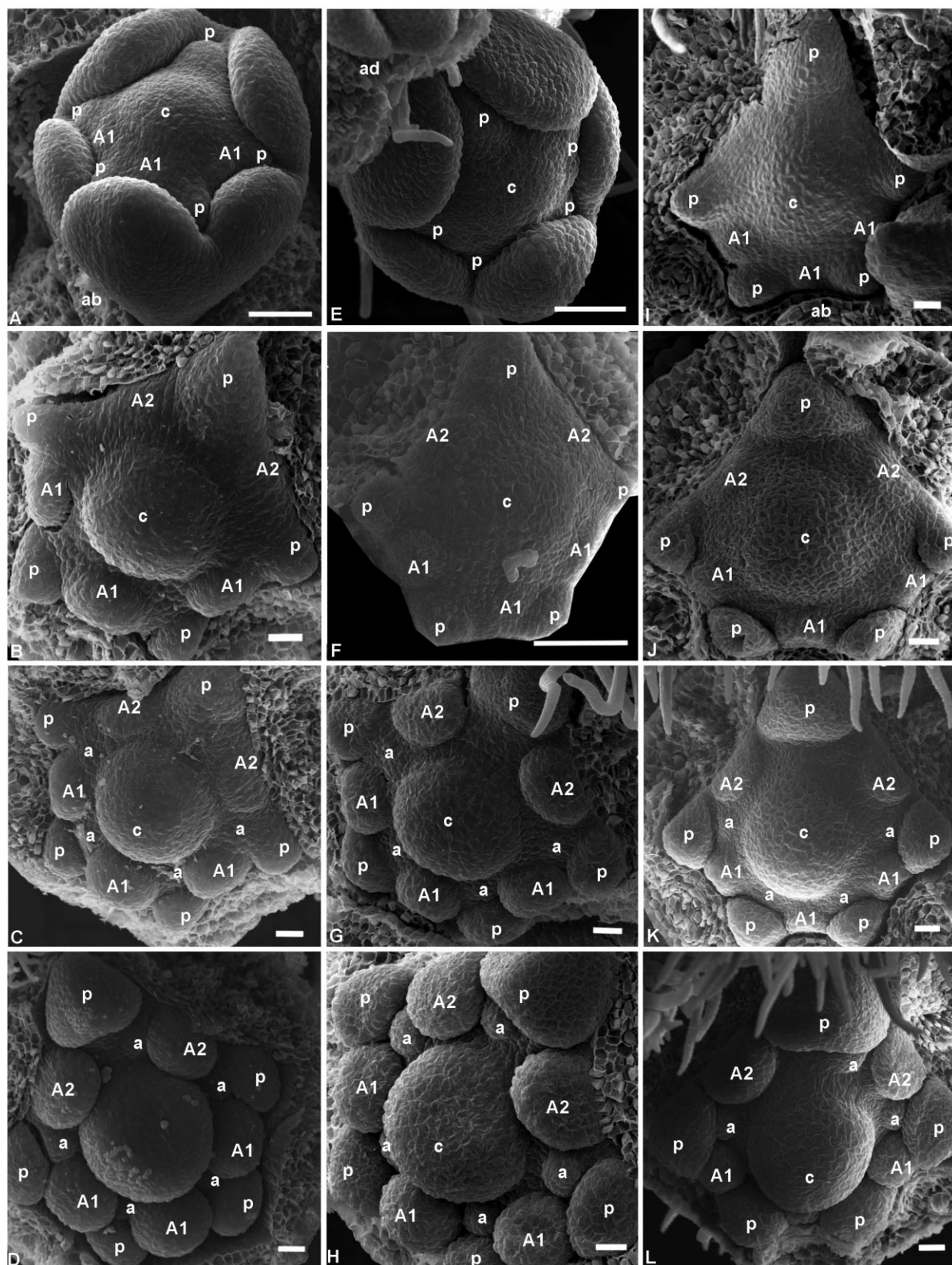


Figure 4. See caption on next page.

Figure 4. Initiation of carpel and stamens in *Dipteryx alata* (A–D), *Pterodon pubescens* (E–H) and *Taralea oppositifolia* (I–L) (scanning electron microscopy, SEM). A, Initiation of carpel primordium concomitant with the first antesepalous stamen primordia. B, Remaining two adaxial antesepalous stamens initiated. C, Initiation of abaxial antepetalous stamen primordia. Note that the adaxial stamen primordium is not yet formed. D, Adaxial antepetalous stamen formed to the right of the median plane. E, Initiation of the carpel primordium concomitant with the first antesepalous stamen primordium. F, Modified unidirectional antesepalous stamen initiation. G, Initiation of abaxial antepetalous stamens. Note that the adaxial stamen primordium is not yet formed. H, Formation of the last antepetalous stamen to the left of the median plane. I, Initiation of carpel primordium concomitant with the first antesepalous stamens. J, Modified unidirectional antesepalous stamen initiation. K, Initiation of abaxial and lateral antepetalous stamens. Note that the adaxial stamen primordium is not yet formed. L, Initiation of the last antepetalous stamen in an adaxial position to the right of the median plane. A1, lateral and abaxial antesepalous stamen primordia; A2, adaxial antesepalous stamen primordia; a, antepetalous stamen primordia; ab, abaxial side; ad, adaxial side; c, carpel primordium; p, petal primordia. Scale bars: A, E, F, 50 μm ; B–D, G, I–L, 20 μm ; H, 10 μm .

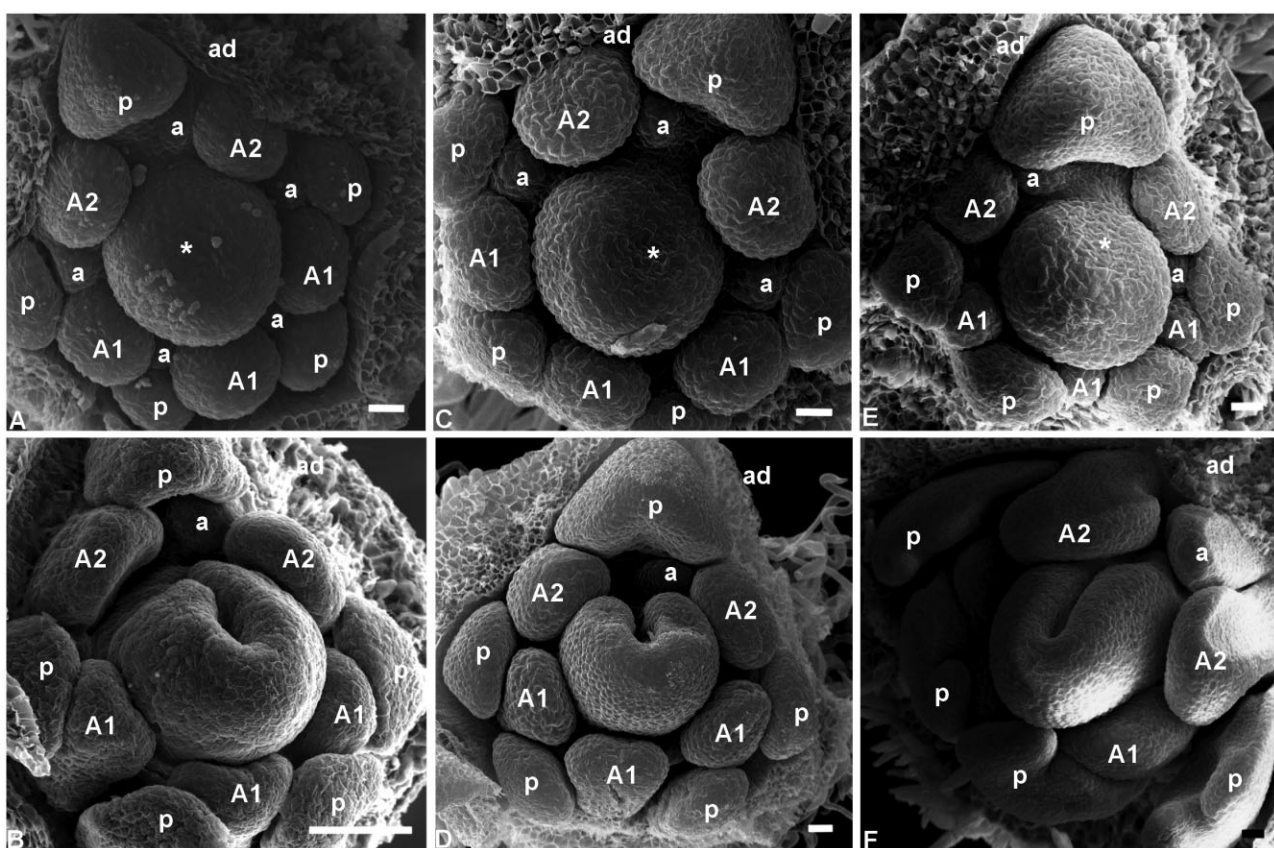


Figure 5. Carpel development in *Dipteryx alata* (A, B), *Pterodon pubescens* (C, D) and *Taralea oppositifolia* (E, F) (scanning electron microscopy, SEM). A, B, Carpel cleft positioned to the left and adaxial stamen to the right. C, Carpel cleft positioned to the right and adaxial stamen to the left. D, Carpel cleft positioned to the left and adaxial stamen to the right. E, Carpel cleft positioned to the right and adaxial stamen to the left. F, Carpel cleft positioned to the left and adaxial stamen to the right. Note (asterisk) that the carpel cleft position depends on the adaxial antepetalous stamen position. A1, lateral and abaxial antesepalous stamens; A2, adaxial antesepalous stamens; a, antepetalous stamens; ad, adaxial side; p, petal primordia. Scale bars: 20 μm .

petals and abaxial and lateral sepals in *D. alata*, *P. pubescens* and *T. oppositifolia* (Fig. 9A–H). They are characterized by an elongated lumen delimited by a secretory epithelial cell layer and two or three layers of parenchymatic cells (Fig. 9C, H).

Secretory cavities are present in the anther appendices in *D. alata* and *P. pubescens* (Fig. 10A–D) and absent in *T. oppositifolia* (Fig. 10E, F). These cavities are also present in the bracteole mesophyll and on the adaxial side of sepal margins in all three species

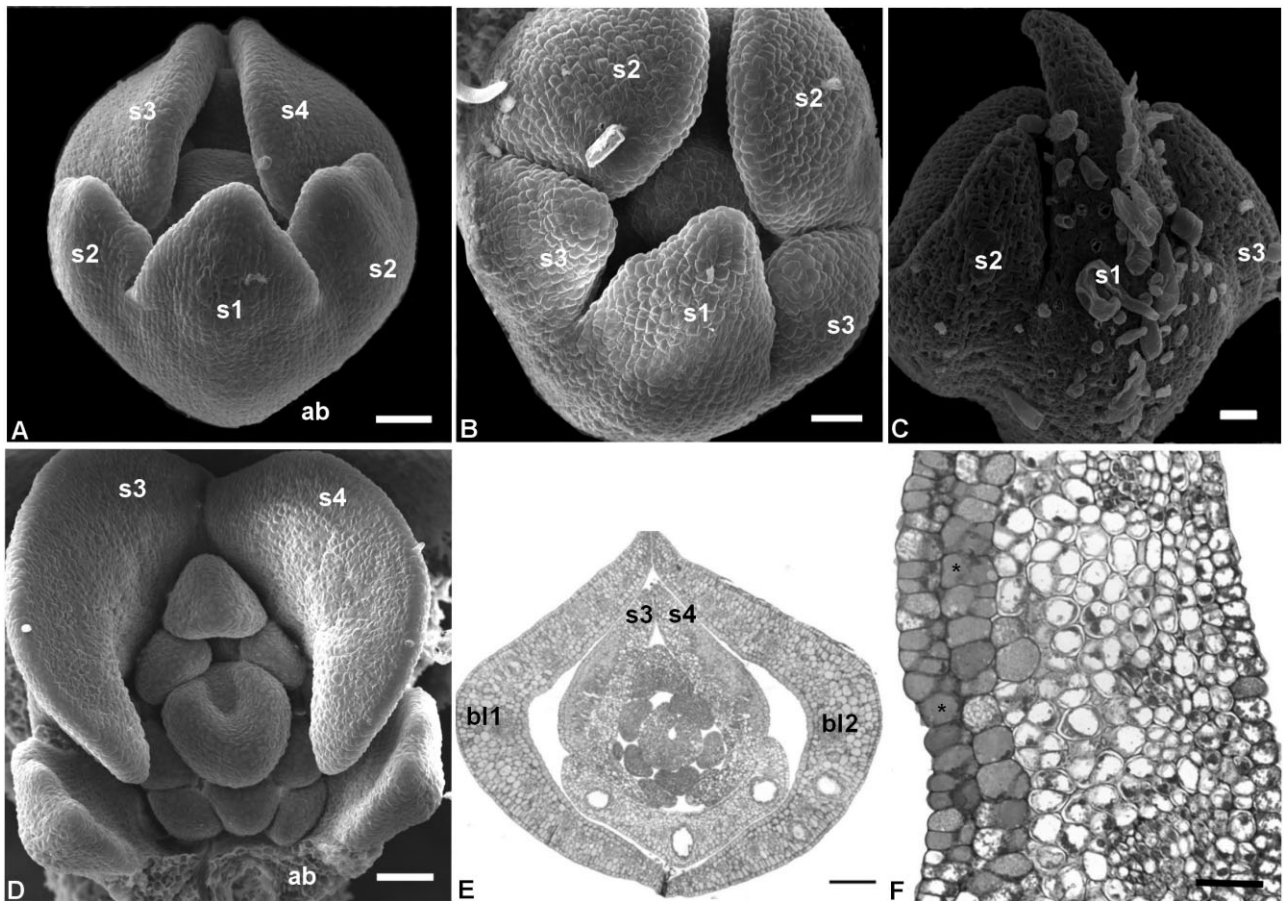


Figure 6. Late stages of sepal development in *Dipteryx alata* (A, D, E, J), *Pterodon pubescens* (B, F) and *Taralea oppositifolia* (C). A–D, Differential growth of the sepal primordia. Note that the adaxial primordia enlarge and the abaxial primordium remains small (scanning electron microscopy, SEM). E, Thickened margin of adaxial sepals (light microscopy, LM). F, Margin of adaxial sepal. Note the increased number of layers and volume of phenolic cells compared with that of the abaxial and lateral sepals (asterisk) (LM). ab, abaxial side, bl1 and bl2, bracteole primordia; s1, abaxial sepal primordium; s2, s3 and s4, lateral and adaxial sepal primordia. Scale bars: 50 µm.

(Fig. 9I). They are characterized by an isodiametric lumen delimited by a secretory uniseriate epithelium. Filled secretory cavities are found in the anther appendices during microsporogenesis (Fig. 10B, D).

A non-structural nectary is located on the inner face of the hypanthium (Fig. 11A–F). It is formed late in floral ontogeny and is confined to the lateral side of the receptacle, where modified stomata are distributed symmetrically (Fig. 11B, D, F).

DISCUSSION

THE FORMATION OF THE PETALOID CALYX

The atypical petaloid calyx of these members of Dipterygeae results from differential growth of the sepal primordia, in which the abaxial primordium, despite being the first to initiate as in other papilionoids, remains diminutive during floral development, com-

pared with the adaxial ones, which enlarge and elongate extensively giving the biallate aspect that characterizes the group. The aberrant enlargement of the two adaxial sepals occurs during the intermediate stages of floral development. This is in accordance with Tucker's (1997) hypothesis, in which characteristics linked to lower taxonomic levels generally arise later in the floral ontogenetic process. In *D. alata* and *T. oppositifolia*, the adaxial and petaloid sepals are the last to arise, but, in *P. pubescens*, they initiate immediately after the abaxial one, a distinctive characteristic of *Pterodon*. Considering that this ontogenetic route is similar and exclusive to Dipterygeae, it might be a synapomorphy for the group, with the presence of secretory canals in the appendices of abaxial and lateral sepals and petals.

The fact that the two adaxial sepals are showy and petaloid might also be involved in an additional attrac-

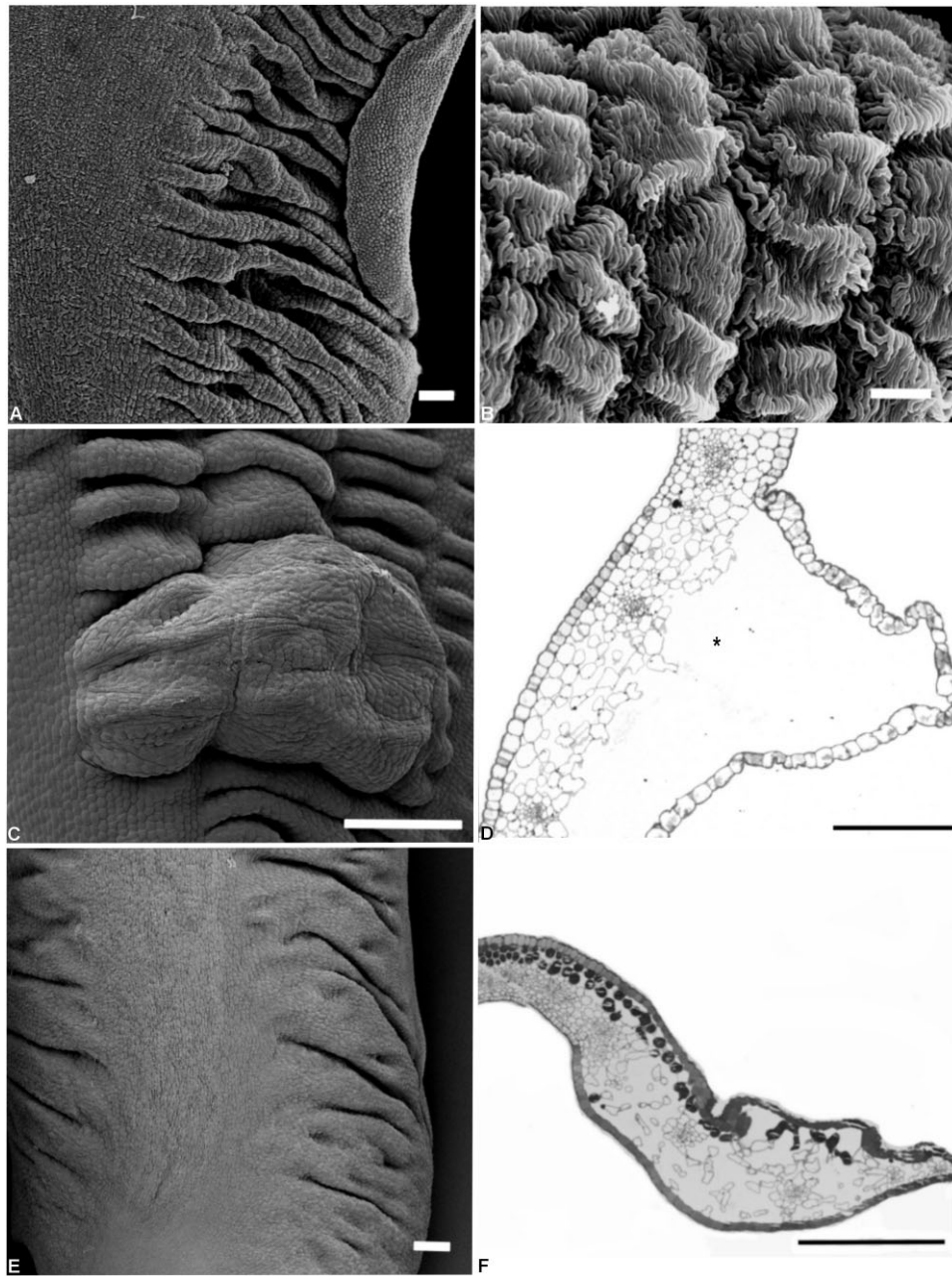


Figure 7. Scultured wings of *Dipteryx alata* (A, B), *Pterodon pubescens* (C, D) and *Taralea oppositifolia* (E, F). A, B, Wing abaxial surface with lamellate sculptures, upper basal and central region (scanning electron microscopy, SEM). C, Wing abaxial surface with lunate sculptures, upper basal and central region (scanning electron microscopy, SEM). D, Transverse section of a wing showing a large subepidermal space (asterisk) (light microscopy, LM). E, Wing petal abaxial surface with lamellate sculptures, basal and centre of upper and lower region (SEM). F, Transverse section of a wing showing the epidermal indentation and aerenchyma on the abaxial surface (LM). Scale bars: A, F, 100 μm ; B, 10 μm ; C, 300 μm ; D, E, 200 μm .

tion to floral visitors (the petals of all Dipterygeae also showy). However, the presence of secretory canals from the early stages of development in the abaxial and lateral sepals seems to be involved in the protection of young buds. The petaloid adaxial sepals are also

found in *Monopteryx* (Polhill, 1981; Pennington, Stirton & Schire, 2005), a genus that might be closely related to Dipterygeae (see Cardoso *et al.*, 2012).

Petaloid sepals are also present in Polygalaceae, but Prenner (2004c) showed that, despite the super-

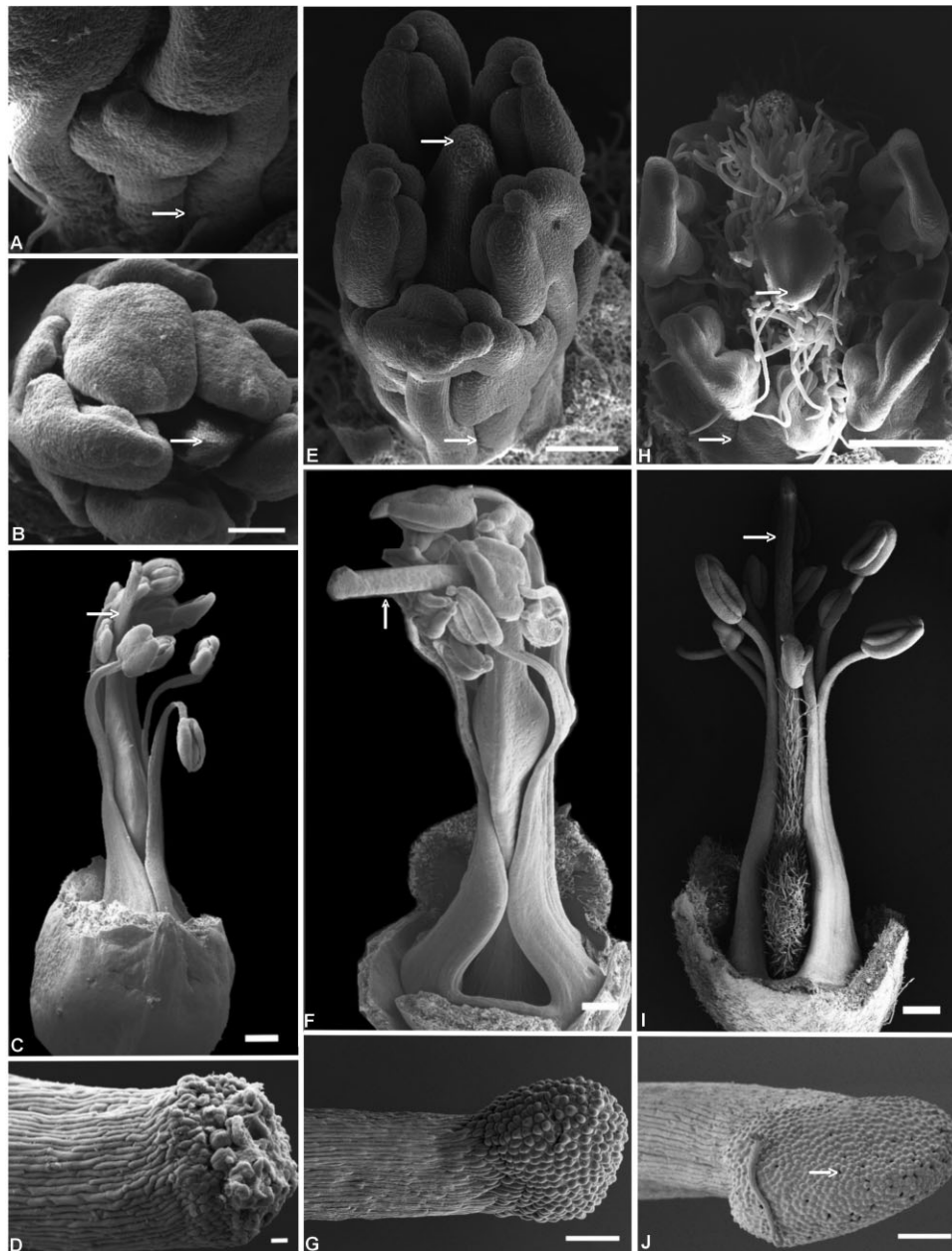


Figure 8. Androecium and gynoecium in *Dipteryx alata* (A–D), *Pterodon pubescens* (E–G) and *Taralea oppositifolia* (H–J) (scanning electron microscopy, SEM). A, B, Position of stamens and stigmatic region in differentiation (arrow). Note that filament union begins at the base (arrow). C, Androecium and gynoecium from mature flower. The filaments are united to an adaxially open sheath. Note the curved style (arrow). D, Truncate stigma. E, Position of stamens and stigmatic region in young flower (arrow). Note that the filament union begins at the base (arrow). F, Androecium and gynoecium from mature flower. The filaments are united to an adaxially open sheath. It is constricted c. 1 mm above the floral base, forming a single nectar window. Note the curved style (arrow). G, Capitulate stigma. H, Position of stamens and stigmatic region in differentiation (arrow). Note that filament union begins at the base (arrow). I, Androecium and gynoecium from a mature flower. The filaments are united to an adaxially open sheath. It is constricted c. 1 mm above the floral base, forming a single nectar window. Note the simple trichomes on the ovary surface and the erect style (arrow). J, Punctiform stigma. Note the cuticle with pores (arrow). Scale bars: A, B, E, G, J, 100 μm ; C, F, H, I, 20 μm ; D, 200 μm .

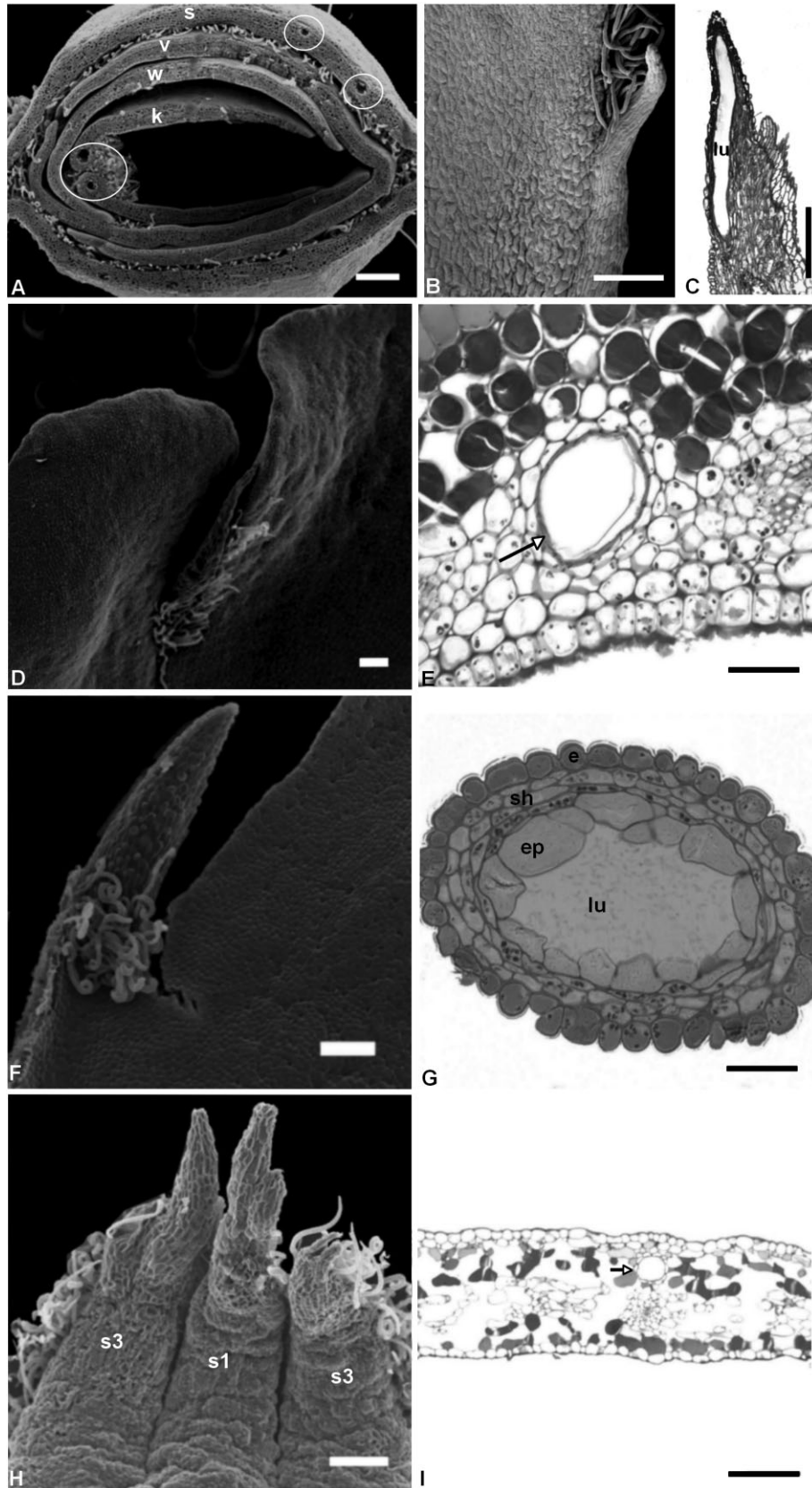


Figure 9. See caption on next page.

Figure 9. Distribution of secretory canals and cavities in floral organs of *Pterodon pubescens* (A, F–H), *Dipteryx alata* (D) and *Taralea oppositifolia* (B, C, E, I). A, Transverse section of the floral bud showing secretory canals and cavities in the keel and sepals (scanning electron microscopy, SEM). B, Appendix of the keel covered by simple trichomes (SEM). C, Longitudinal section of a keel appendix showing a secretory canal. D, Appendix of a wing (SEM). E, Transverse section of a wing appendix showing a secretory canal (light microscopy, LM). F, Vexillum appendix (SEM). G, Transverse section of a vexillum appendix showing a secretory canal with polysaccharides stored in the lumen. H, Abaxial and lateral sepals with appendices (SEM). I, Transverse section of the adaxial sepal showing marginal secretory cavities. e, epidermis; ep, epithelial cells; k, keel petals; lu, lumen; s1, abaxial sepal; s3, lateral sepal; s, sepal; sh, sheath cells; v, vexillum; w, wing. Scale bars: A–C, E–I, 50 μ m; D, 100 μ m.

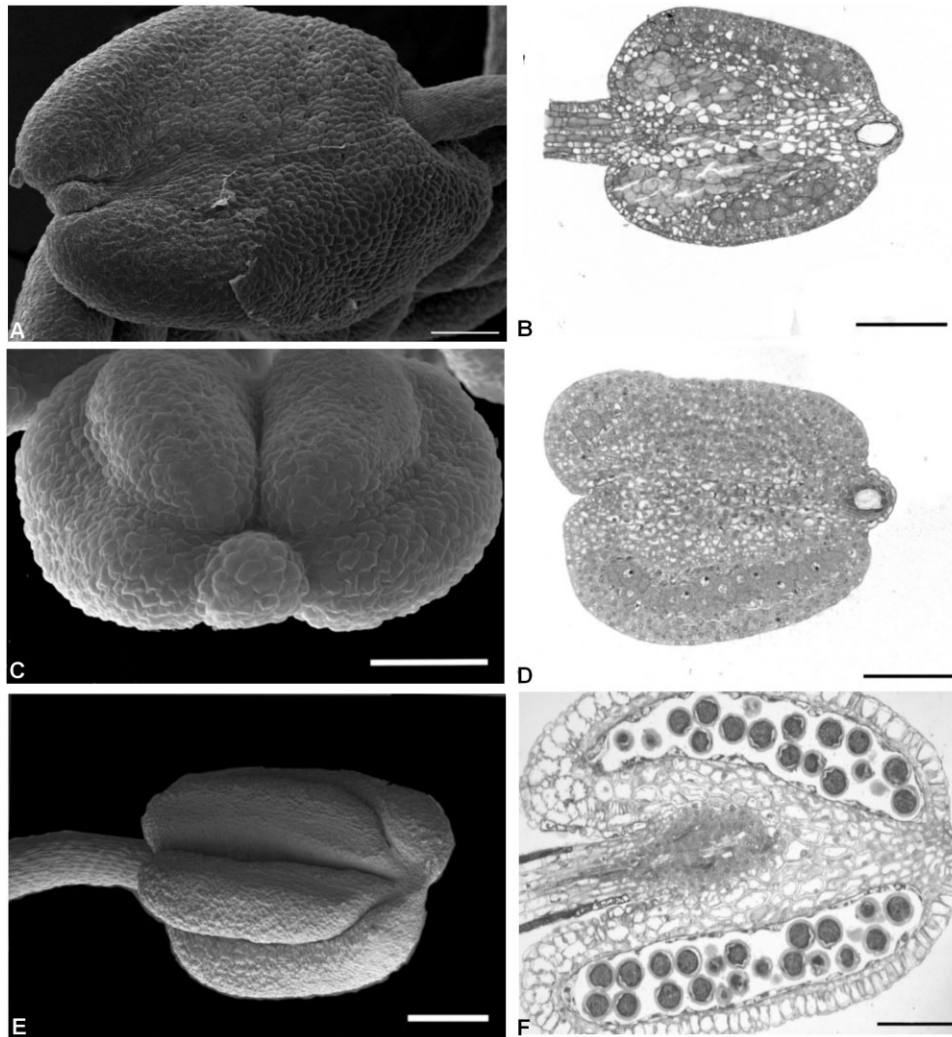


Figure 10. Anther of *Dipteryx alata* (A, B), *Pterodon pubescens* (C, D) and *Taralea oppositifolia* (E, F). A, Anther with an inconspicuous appendix (scanning electron microscopy, SEM). B, Longitudinal section of the anther showing the appendix containing a secretory cavity. Note that the secretory cavity is active at the same time as microsporogenesis is ongoing (light microscopy, LM). C, Anther with a conspicuous appendix (SEM). D, Longitudinal section of the anther showing the appendix containing a secretory cavity. Note that the secretory cavity is active at the same time as microsporogenic cells are formed (LM). E, Anther without an appendix (SEM). F, Longitudinal section of the anther showing a broad and uniform connective without any sign of a secretory cavity or appendix. Scale bars: A, B, D, F, 100 μ m; C, E, 200 μ m.

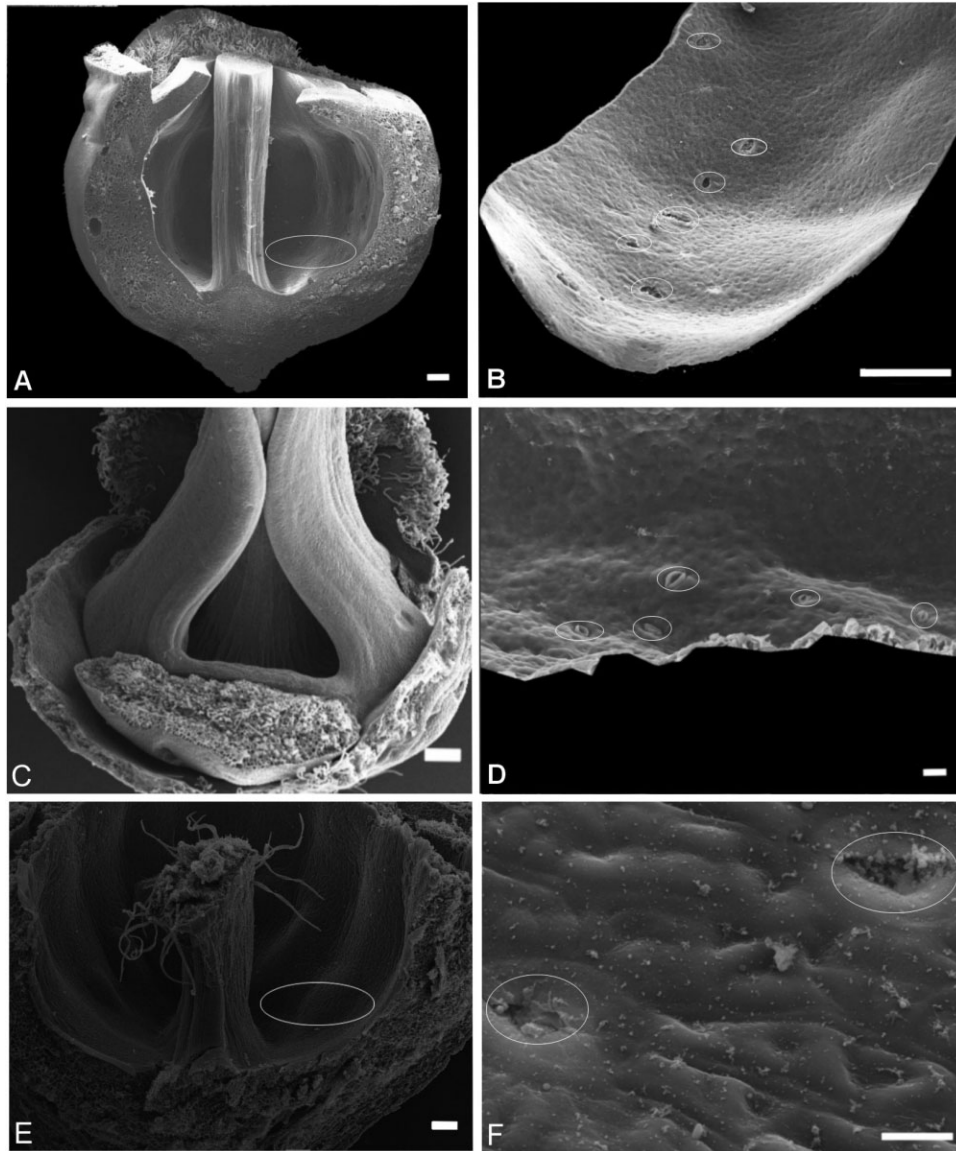


Figure 11. Receptacle and nectary of an anthetic flower of *Dipteryx alata* (A, B), *Pterodon pubescens* (C, D) and *Taralea oppositifolia* (E, F). A, Overview of the receptacle, marked ellipse out of B, showing the field of nectar stomata in a lateral position. C, Overview of the nectar window (fenestra) at the base of the flower, marked ellipse out of D, showing the field of modified stomata in a lateral position. E, Overview of the receptacle, marked ellipse out of E, showing the field of modified stomata in a lateral position. Scale bars: A, 200 µm; B, C, E, F, 100 µm; D, 20 µm.

ficial similarity of the flower of Polygalaceae to that of Papilionoideae, they show distinctive ontogenies. In Polygalaceae, the calyx is composed of two abaxial (versus one in Papilionoideae), one adaxial (versus two in Papilionoideae) and two lateral sepals. Also, the lateral sepals are the ones that constitute the petaloid calyx in Polygalaceae, not the adaxial ones as in Dipterygeae. Thus, the flowers of Dipterygeae are quite different from those of Polygalaceae.

Apparently the two adaxial sepals in *D. alata*, *P. pubescens* and *T. oppositifolia* perform two functions: protection during the early ontogenetic stages, and pollinator attraction when the flower is open. Because the petals are also showy in Dipterygeae, we do not interpret the petaloid calyx as a transference of the function of attraction to the sepals, but the sepals are indeed additional attractive floral organs.

The presence of a petaloid calyx is often correlated with a reduction in size or variability in the presence or absence of the petals, but this was not found in Dipterygeae. This phenomenon might be an indication that sepals and petals are functionally homologous structures, but it affects flowers at maturity, whereas sepals and petals maintain their distinct morphologies in earlier developmental stages (Craene, 2007). This correlation can be seen in the tribe Detarieae (Caesalpinioideae *s.l.*), where a petaloid calyx has been reported in *Amherstia*, *Brownea* (Tucker, 2000c), *Crudia* (Tucker, 2001), *Saraca* (Tucker, 2000b) and *Tamarindus* (Tucker, 2000c). In all these genera, there is abortion of petals, whereas, in tribe Liparieae, a member of subfamily Papilionoideae, a petaloid calyx has been reported in *Liparia* and *Priestleya* (Schutte & van Wyk, 1994), but without abortion or absence of organs. This may be because organ abortion or variation in the number of floral organs is not common in Papilionoideae (Klitgaard, 1999; Mansano *et al.*, 2002; Paulino, Mansano & Teixeira, 2013).

The highly characteristic petaloid lateral sepals of Polygalaceae involve integration of the bracteoles to the flower and a reduction or loss of the lateral petals (Gutierrez, 2008). This result corroborates the findings of lateral petal abortion by Prenner (2004c) for *Polygala myrtifolia* L. In Dipterygeae, all petals are fully formed and showy. The enlarged lateral sepals are petaloid in behaviour, but sepals by position (Gutierrez, 2008).

SHARED AND DIFFERENT FEATURES AMONG THE GENERA OF DIPTERYGEAE

The monophyly of Dipterygeae has been verified in different studies (Pennington *et al.*, 2001; Wojciechowski *et al.*, 2004; Cardoso *et al.*, 2012), and our results found the following potential ontogenetic synapomorphies: (1) simultaneous order of petal initiation; (2) carpel initiation concurrent with the first antesealous stamen initiation; (3) modified unidirectional antesealous stamen initiation; (4) thickened adaxial sepals caused by an increase in the number of layers and volume of the phenolic cells; (5) asymmetrical androecium; (6) monadelphous androecium forming an adaxial open sheath; and (7) a non-structural nectary.

Floral characters differing between the three genera can be found in early and late developmental stages (see Table 1). During the early developmental stages, we can mention the order of initiation of the bracteoles and sepals. In later developmental stages, the wing sculptures, shape of the stigma, curvature of the style, surface of the ovary, length of the stipe, prominence of the anther appendices and pigmenta-

tion of the epidermal cells are noticeable differences between the species studied.

THE SHORT HYPANTHIUM, PECULIAR ANDROECIUM AND SECRETORY STRUCTURES

The short hypanthium is another shared characteristic between members of Dipterygeae. It has been described in some other groups of Papilionoideae, including the tribes Amorpheae (McMahon, 2005; McMahon & Hufford, 2005), Loteae (Prenner, 2003a), Dalbergieae (Costa *et al.*, 2007; Filardi, Garcia & Carvalho-Okano, 2007), Sophoreae (Tucker, 1993, 1994, 2002a; Sartori & Tozzi, 2004; Filardi *et al.*, 2007) and Phaseoleae (Tucker, 2006) and the *Lecointea* clade (Mansano *et al.*, 2002). In Caesalpinioideae *s.l.*, it has been reported in tribes Cercideae (Tucker, 2002b) and Detarieae (Tucker, 2000a, 2003c). The hypanthium in Dipterygeae is characterized by the direct insertion of the petal claw in the hypanthium margin. A similar condition has been reported for Amorpheae (McMahon & Hufford, 2002, 2005; McMahon, 2005). Anatomical comparative studies of the vascularization would be valuable to confirm how the hypanthium is formed in different groups of Fabaceae.

The basal opening in the monadelphous androecium has been interpreted as a window for nectar (Klitgaard, 1999) and, according to Tucker (1989), it is a strong adaptive advantage among Papilionoideae. This type of window, as found in Dipterygeae (in which the filaments are united to an adaxially open sheath), is not well reported in the literature. There are only three reports in Amorpheae (McMahon & Hufford, 2002; Prenner, 2004a) and five in Dalbergieae (Klitgaard, 1999). However, filaments united to a closed tube with only a single window are reported more frequently, with one report in tribe Abreae (Prenner, 2004a), one in Aeschynomeneae (Tucker, 1987), three in Bossiaeeae (Prenner, 2004a), one in Crotalarieae (Prenner, 2004a), four in Genisteeae (Prenner, 2004a) and three in Podalyrieae (Prenner, 2004a). The single window from a monadelphous androecium is less common than the presence of two nectar windows from a diadelphous androecium (9 + 1). It is found, for instance, in other tribes, with one report in Carmichaelieae (Prenner, 2004a), one in Cicereae (Prenner, 2004a), 23 in Phaseoleae (Tozzi, Agostini & Sazima, 2005; Moura, 2013), two in Galegeae (Prenner, 2004a), four in Indigofereae (Prenner, 2004a; Paulino *et al.*, 2011), three in Liparieae (Prenner, 2004a), four in Loteae (Tucker, 1987; Prenner, 2003a, 2004a), one in Millettieae (Tucker, 1987), six in Phaseoleae (Tucker, 1987; Prenner, 2004a), one in Podalyrieae (Prenner, 2004a), two in Robinieae (Prenner, 2004a) and two in Vicieae (Tucker, 1989; Prenner, 2003b). Unlike Dalbergieae

and other Papilionoideae, in which stamen union is common and occurs by intercalary growth (Tucker, 1987), some members of the so-called ADA clade (see Cardoso *et al.*, 2012), which includes Dipterygeae, do not normally exhibit stamen union or the stamens are only united basally.

The non-structural nectary in *D. alata*, *P. pubescens* and *T. oppositifolia*, in the basal portion of the hypanthium, is a rare condition in Fabaceae. The position of the non-structural nectary on the inner face of the hypanthium is also unusual, as studies have shown that nectaries in species with a monadelphous androecium are located in the filament column (Bernardello, 2007). Non-structural nectaries are rarely described in Papilionoideae, with a single report in tribe Vicieae (Prenner, 2003b), two in Dalbergieae (Klitgaard, 1999), one in Loteae (Prenner, 2003a) and 13 in Genisteae (Vogel, 1997). Studies of non-structural nectaries have been neglected, probably because they are not easily noticeable (Fahn, 1979; Bernardello, 2007).

It is not clear in the literature whether the wing sculptures are indeed osmophores, for the emission of compounds for pollinator attraction, or aerenchyma, involved in the reduction of the weight of the petal, without any evidence of secretory activity. According to Oliveira & Sigrist (2008), these wing sculptures act as osmophores and landing platforms for pollinating bees in *D. alata*. Studies of *Dalbergia brasiliensis* Vogel and *Platymiscium floribundum* Vogel (Stirton, 1981; Klitgaard, 1999) have also shown that these sculptures are used as a landing platform for pollinating bees. However, these authors did not verify whether the sculptures are osmophores. Our anatomical data showed the presence of aerenchyma inside these sculptures, which could be involved in reduction in petal weight, without any evidence of secretory activity. It is possible that the petal epidermis is secretory and is involved in pollinator attraction.

The presence of an asymmetric androecium in the species analysed here has already been reported for some species of tribes Dalbergieae (Klitgaard, 1999; Prenner, 2004a) and Sophoreae (Tucker, 1987, 1993, 1994, 2002a; Prenner, 2004a, b). Their occurrence, independent of the level of stamen union is more frequent in groups with a monadelphous androecium (Table 2). Our results reinforce the hypothesis of Prenner (2004a), postulating that androecium asymmetry appeared repeatedly in early branching lineages of Papilionoideae.

COMPARISON OF ONTOGENETIC DATA WITH OTHER PAPILIONOID GENERA

Ontogenetic studies for the genera belonging to the *Amburana* clade (sister to Dipterygeae according to

Cardoso *et al.*, 2012) are scarce. *Dussia* is poorly studied in terms of floral development. There is only one report for the sequential order of sepal initiation [*Dussia discolor* (Benth.) Amshoff.; Prenner, 2004b]. Another taxon belonging to this clade with some ontogenetic data is *Myroxylon balsamum* Druce; this species shows unidirectional and precocious carpel initiation (Tucker, 1993). For other papilionoids, there are reports of precocious initiation of the carpel (concurrently with the initiation of the first abaxial antesealous stamen) in *Sophora japonica* L. (Tucker, 1994) and *Machaerium villosum* Vogel (Klitgaard, 1999), an asymmetrical androecium (Tucker, 1987, 1994, 2002a; Klitgaard, 1999; Prenner, 2004a) and also the variable order of sepal initiation: modified unidirectional (Tucker, 1994; Prenner, 2004b), unidirectional (Tucker, 1990, 1993, 1994, 2002a, 2003b; Klitgaard, 1999; Mansano *et al.*, 2002; Paulino *et al.*, 2011, 2013), modified helical (Klitgaard, 1999), sequential (Prenner, 2004b) and modified sequential (Tucker, 1994) (see Table 2).

The helical order of sepal initiation reported here for *P. pubescens* is rarely found in Papilionoideae, with records only for *Dalbergia brasiliensis* (Klitgaard, 1999) and *Psoralea pinnata* Andrews (Tucker & Stirton, 1991). This condition is most commonly found in species of Caesalpinioideae *s.l.*, as in tribes Detarieae (Tucker, 2000b, c, 2002c, 2003a, c; Pedersoli *et al.*, 2010), Caesalpinieae (Tucker, 2003a; Prenner, 2004a), Cassieae (Tucker, 2003a) and Cercideae (Tucker, 1984, 2003a). In Polygalaceae, there is a report for *Polygala myrtifolia* (Prenner, 2004c).

DIAGNOSTIC CHARACTERISTICS AMONG THE THREE DIPTERYGEAE GENERA

Diagnostic characters for each genus of Dipterygeae have been investigated by various authors (Polhill, 1981; Barroso *et al.*, 1999; Oliveira & Paiva, 2005; Francisco, 2010; Pinto *et al.*, 2014). Representatives of *Dipteryx* are characterized by asymmetric leaflets as a result of the eccentric primary vein (Francisco, 2010), a drupaceous fruit (Barroso *et al.*, 1999; Francisco, 2010; Pinto *et al.*, 2014), seeds with a coriaceous testa, a hilum in lateral or subapical position and a rugose embryo with conspicuous plumule (Francisco, 2010; Pinto *et al.*, 2014). In *Pterodon*, the leaf rachis is exalate (Francisco, 2010), the fruit is a cryptosamarra with oil glands in the epicarp (Polhill, 1981; Barroso *et al.*, 1999; Francisco, 2010; Pinto *et al.*, 2014), the seed testa is smooth and the raphe is apparent, with the hilum in a lateral position covered by an aril and a smooth embryo (Francisco, 2010; Pinto *et al.*, 2014). Representatives of *Taralea* have a black and rugose petiolule, an elliptical, hairy ovary (Francisco, 2010), a legume with elastic dehiscence

Table 2. Characters of floral development of the species studied here and available in the literature for other species of 'basal' (early branching) tribes (*sensu* Polhill, 1981) in Papilionoideae (according to Cardoso *et al.*, 2012). Symbols: A, asymmetric; B, bidirectional; H, helical; HM, modified helical; S, sequential; SI, simultaneous; SM, modified sequential; SU, successive; SY, symmetric; U, unidirectional; UM, modified unidirectional. Empty cells mean missing information

Tribe	Species	Order of sepal initiation	Order of petal initiation	Androecium symmetry	Stamen connation	Source
Dalbergiae	<i>Dalbergia brasiliensis</i> Vogel.	HM	U	A	Monadelphous (sheath)	Klitgaard (1999)
	<i>Machaerium villosum</i> Vogel.	U	U	A	Monadelphous (sheath)	Klitgaard (1999)
	<i>Platymiscium floribundum</i> Vogel.	U	U	A	Monadelphous (sheath)	Klitgaard (1999)
	<i>Pterocarpus rotundifolius</i> (Sond.) Druce.	U		A	Monadelphous (sheath)	Klitgaard (1999)
	<i>Machaerium arboreum</i> (Jacq.) Vogel.	S		A	Monadelphous (sheath)	Prenner (2004a)
Dipterygeae	<i>Dipteryx alata</i> Vogel.	UM	SI	A	Monadelphous (open sheath)	Present study
	<i>Pterodon pubescens</i> (Benth.) Benth.	H	SI	A	Monadelphous (open sheath)	Present study
	<i>Taralea oppositifolia</i> Aubl.	S or SM	SI	A	Monadelphous (open sheath)	Present study
Sophoreae	<i>Ammodendron lehmannii</i> Bunge ex Boiss.			A	Free	Prenner (2004a)
	<i>Baphia polyantha</i> Harms.			SY	Free	Prenner (2004a)
	<i>Cadia purpurea</i> (G.Piccioli) Aiton	U	U	A	Free	Tucker (1987, 2002a)
	<i>Cladrastis tinctoria</i> Raf.			A	Shortly connate	Prenner (2004a)
	<i>Cladrastis sinensis</i> S.Y.Hu			A	Shortly connate	Prenner (2004a)
	<i>Castanospermum australe</i> A.Cunn. & C.Fraser	U	SI	SY	Free	Tucker (1993)
	<i>Dussia discolor</i> (Benth.) Amshoff	S		A/SY	Free	Prenner (2004a, b)
	<i>Maackia amurensis</i> Rupr.			A	Free	Prenner (2004a)
	<i>Myroxylon balsamum</i> Druce.	U	SI	SY	Free	Tucker (1993)
	<i>Sophora alopecuroides</i> L.			SY	Shortly connate	Prenner (2004a)
	<i>Sophora davidii</i> (Franch.) Skeels.	UM		A	Shortly connate	Prenner (2004a, b)
	<i>Sophora flavescens</i> Aiton.	UM		A	Shortly connate	Tucker (1994); Prenner (2004a)
	<i>Sophora japonica</i> L.	U				Tucker (1994)
	<i>Sophora pachycarpa</i> Schrenk ex C.A.Mey.			A	Free	Prenner (2004a)
<i>Sophora tomentosa</i> hort. ex Dippel			A	Shortly connate	Tucker (1994)	
<i>Styphnolobium japonicum</i> (L.) Schott	SM			A/SY	Shortly connate	Tucker (1994); Prenner (2004a)
Swartzieae	<i>Ateleia herbert-smithii</i> Pittier	U	Single adaxial primordium	SY	Free	Tucker (1990)
	<i>Exostyles venusta</i> Schott ex Spreng.	U	SI	SY	Free	Mansano <i>et al.</i> (2002)
	<i>Harleyodendron unifoliolatum</i> R.S.Cowan.	U	SI	SY	Free	Mansano <i>et al.</i> (2002)
	<i>Lecointea hatschbachii</i> Barneby	U	SI	SY	Free	Mansano <i>et al.</i> (2002)
	<i>Zollernia ilicifolia</i> (Brongn.) Tul.	U	SI	SY	Free	Mansano <i>et al.</i> (2002)
	<i>Swartzia aureosericea</i> R.S.Cowan.	B (not confirmed)	Single adaxial primordium (ring meristem)			Tucker (2003b)
	<i>Swartzia laurifolia</i> Benth.	U	Single petal primordium (median position on the adaxial side)			Tucker (2003b)
	<i>Swartzia dipetala</i> Willd. ex Vogel.	U	Single adaxial primordium		Free	Paulino <i>et al.</i> (2013)
	<i>Swartzia madagascariensis</i> Desv.	SI or SU	Single adaxial primordium			Tucker (2003b)

(Polhill, 1981; Barroso *et al.*, 1999; Francisco, 2010; Pinto *et al.*, 2014) and a circular, oval, compressed seed with a basal hilum; the embryo displays a cleft below the radical–hypocotyl axis and an inconspicuous plumule (Barroso *et al.*, 1999; Francisco, 2010; Pinto *et al.*, 2014) (see Table 1).

The absence of appendices in anthers and bracteoles in *Taralea* and their presence in the sister clade, *Dipteryx* + *Pterodon*, constitutes a potential synapomorphy for this clade. In addition, the secretory canals observed in all studied species constitutes a synapomorphy for the tribe. Bearing in mind the phylogenetic framework of the group (Pennington *et al.*, 2001; Wojciechowski, Lavin & Sanderson, 2004; Francisco, 2010; Cardoso *et al.*, 2012), it is possible to infer that the presence of appendices in anthers and bracteoles is a synapomorphy for *D. alata* and *P. pubescens* (as it is lacking in *T. oppositifolia*). However, the noticeable similarity of the calyx in late developmental stages in *Dipteryx* and *Taralea* is a consequence of the similar order of initiation of the sepal primordia among all members of Dipterygeae, in which the adaxial primordia are the last ones to be initiated, except in *P. pubescens*, in which the adaxial sepals arise immediately after the abaxial sepal. This difference may reflect the floral morphology, as the adaxial sepals are more open in *P. pubescens* than in *D. alata* and *T. oppositifolia*.

We found seven ontogenetic similarities among the genera of Dipterygeae and three ontogenetic and other morphological characters distinguishing them. It was also verified that Dipterygeae constitutes a morphologically atypical group of Papilionoideae, with some distinctive ontogenetic pathways. This study shows that floral ontogeny is an important tool in understanding how mature flowers, such as the members of Dipterygeae, develop their atypical morphology.

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