RESEARCH ARTICLE

AMERICAN JOURNAL OF BOTANY

Flowers of the early-branching papilionoid legume *Petaladenium urceoliferum* display unique morphological and ontogenetic features[1]

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PREMISE OF THE STUDY: Floral development can help to shed light on puzzling features across flowering plants. The enigmatic Amazonian monospecific genus *Petaladenium* of the legume family (Leguminosae) had rarely been collected and only recently became available for ontogenetic studies. The fimbriate-glandular wing petals of *P. urceoliferum* are unique among the more than 19 000 legume species. Ontogenetic data illuminate the systematic position of the genus and foster our understanding on floral evolution during the early diversification of the papilionoid legumes.

METHODS: Flower buds were collected in the field, fixed in 70% ethanol, and investigated using scanning electron microscopy (SEM). Results were compared with existing material from early-diverging papilionoid legumes.

KEY RESULTS: Formation of sepals and petals shows bidirectional tendencies. Stamens arise in two whorls, and the single carpel arises concomitantly with the outer stamen whorl. Gland formation starts early on the edges of the wing petals. The carpel reopens for a short time when the initiation of ovules is visible. Stomata at the base of the hypanthium indicate that the flower functions like other standard flag blossoms.

CONCLUSIONS: The floral ontogeny confirms the close affinity of *P. urceoliferum* with the florally heterogeneous, early-diverging papilionoid Amburaneae clade. The results strengthen the theory of a distinct experimental phase among early-branching papilionoid legumes during which a wider range of floral morphologies arose. Polysymmetry, monosymmetry, variable organ numbers, and a wide range of ontogenetic patterns laid the foundation for a successful canalization toward the more restricted but well-adapted dorsiventral papilionoid flag blossom.

KEY WORDS Amburaneae clade; flag blossom; flower development; flower evolution; Leguminosae; Papilionoideae; *Petaladenium urceoliferum*

Molecular phylogenies have largely impacted our understanding of morphological affinity and evolution in flowering plants by revealing unexpected phylogenetic relationship among florally disparate species (e.g., Davis et al., 2007; Saarela et al., 2007; Cardoso et al., 2012). Hidden similarities in morphologically heterogeneous clades are sometimes rooted deep in floral development or ontogeny. However, when set in a phylogenetic context, ontogeny can illuminate enigmatic features that have long puzzled morphologists, such as the very reduced unisexual apetalous flowers of the aquatic Hydatellaceae (Rudall et al., 2007, 2009) or the world's largest flowers of the holoparasitic Rafflesiaceae (Nikolov et al., 2013, 2014).

The more than 13 000 species in the Papilionoideae lineage of the economically important legume family (Leguminosae) are mostly characterized by monosymmetric, papilionate flowers ("flag blossoms") involving strong petal differentiation into an adaxial standard petal or vexillum, two lateral wing petals, and an abaxial keel that is formed of two petals tightly enclosing the gynoecium and androecium (Knuth, 1908; Leppik, 1966; Westerkamp and Weber, 1997; Lewis et al., 2005). However, the early diversification of the papilionoids is noticeable by many floral deviations from the basic papilionate morphology (Pennington et al., 2000; Cardoso et al., 2012). One such unique example is found in the flowers of the monospecific Amazonian genus *Petaladenium* Ducke (Ducke, 1938), which occurs in tropical lowland rain forests at the upper

[1] Manuscript received 28 July 2015; revision accepted 24 September 2015.

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doi:10.3732/ajb.1500348

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Rio Negro basin (Brazil) and which was historically known from the holotype and two subsequent collections only (Cardoso et al., in press). *Petaladenium urceoliferum* Ducke are large trees with imparipinnate leaves and flowers in compound racemes. The most striking character of the species, as highlighted by the genus name, is its fimbriate-glandular wing petals, which are unique among papilionoid legumes (Figs. 1, 2).

Polhill (1981) classified *Petaladenium* as a member of the *Dussia* group (tribe Sophoreae), and Pennington et al. (2005) speculated that the genus might be congeneric with *Clathrotropis* (Benth.) Harms (also in the *Dussia* group sensu Polhill, 1981). However, phylogenetic analyses of chloroplast and nuclear DNA sequences ruled out a close relationship of these two genera (Fig. 1; Cardoso et al., 2012, 2013, 2015). Cardoso et al. (2015) revealed that *Petaladenium* forms a new branch among the early-branching ADA clade (Angylocalyceae, Dipterygeae, and Amburaneae) where it is sister to *Dussia* Krug & Urb. ex Taub. in the Amburaneae clade (Fig. 1). Kite et al. (2015) recently found chemical compounds in *Petaladenium* that are unique among Leguminosae confirming the enigmatic status of this taxon.

In the current study, we present a detailed analysis of the morphology and ontogeny of the flowers and inflorescences with a special focus to the glandular wing petals of *P. urceoliferum*. We discuss our results based on a recent phylogenetic hypothesis (Fig. 1, Cardoso et al., 2015), on ontogenetic data from related taxa (Table 1) (Tucker, 1993; Prenner, 2004a; Leite et al., 2015) and in the light of existing morphological studies on pollen (Ferguson et al., 1994) and on endothecial characters (Manning and Stirton, 1994) of *P. urceoliferum*. We test whether ontogenetic characters are shared among the Amburaneae and to which extent ontogeny matches phylogenetic relationships among early-branching papilionoid legumes. We also present testable hypotheses on the possible nature and function of the unique glandular wing petals.

MATERIALS AND METHODS

Inflorescences, flowers, and flower buds of *Petaladenium urceoliferum* were collected at the type locality (*D. Cardoso et al., 3345*, HUEFS, INPA, K; Cardoso et al., in press) and immediately fixed

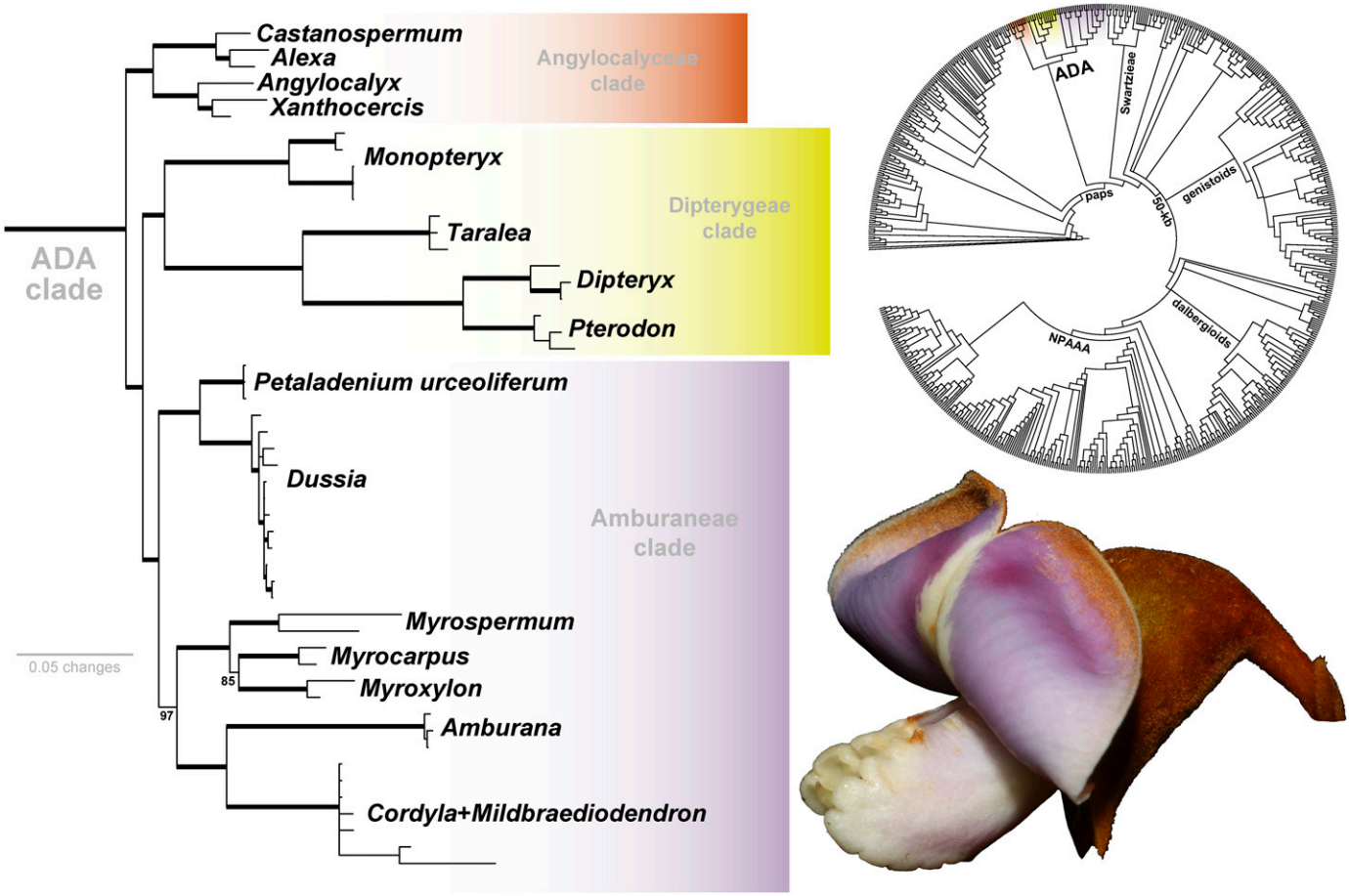

FIGURE 1 Summary of a majority-rule consensus tree of the papilionoid legumes (Papilionoideae) derived from the combined Bayesian analysis of nuclear ITS/5.8S and plastid *matK* and *trnL* intron DNA sequences (after Cardoso et al., 2015). The focus is set on the earliest-branching ADA clade (Angylocalyceae, Dipterygeae, and Amburaneae) outside the large and diverse 50-kb inversion clade to show the phylogenetic placement of the monospecific Amazonian genus *Petaladenium*. Branches in bold are those supported by a posterior probability ≥0.99. Numbers on branches are Bayesian posterior probabilities >0.80. The photo (by Domingos Cardoso) of the flower of *Petaladenium urceoliferum* shows the unique gland structures on the margins of the wing petals.

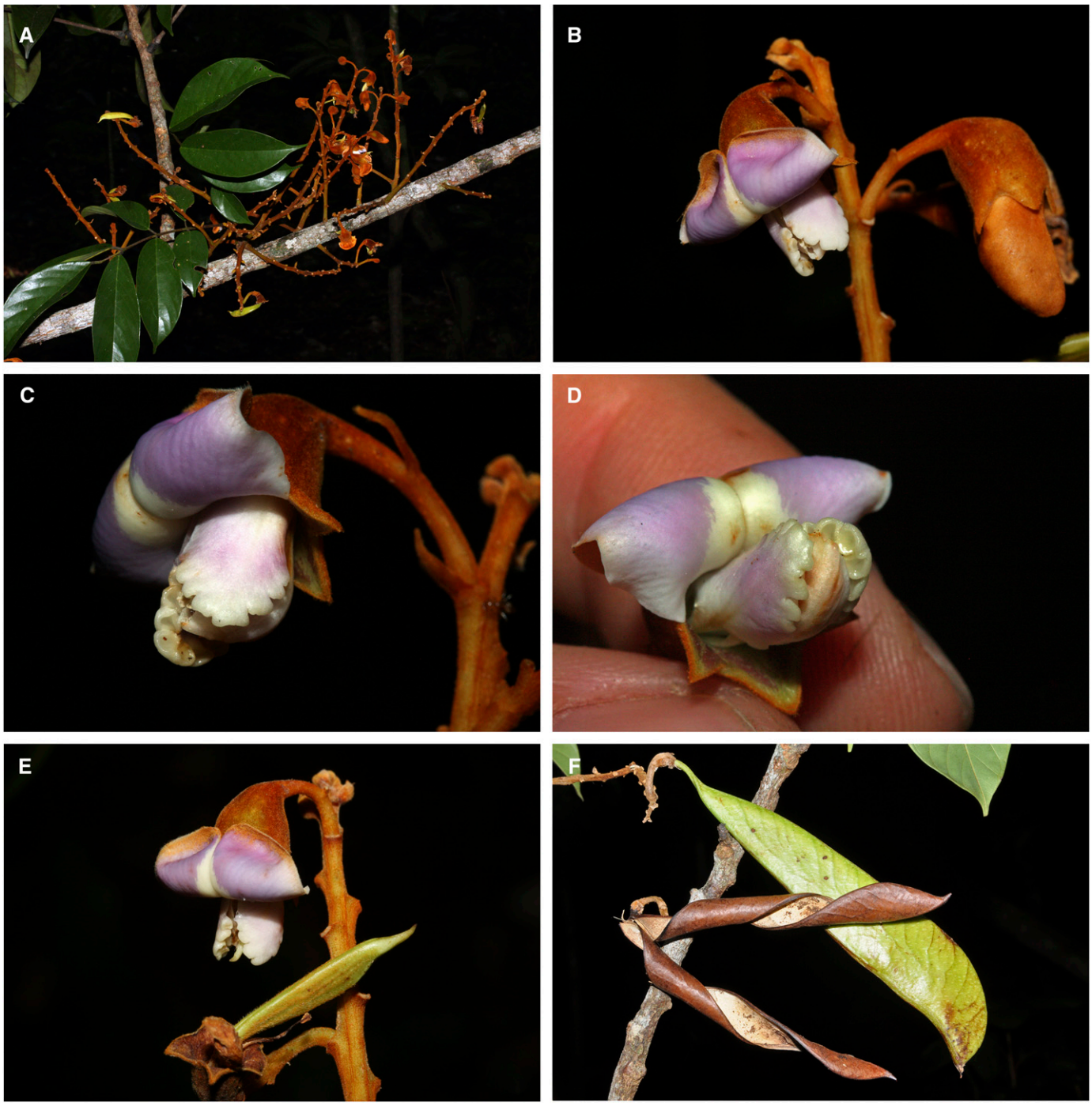

FIGURE 2 Inflorescence, flowers, and fruits of *Petaladenium urceoliferum*. (A) Inflorescence. (B) Opened flower and closed bud (note the densely pubescent adaxial surface of the standard petal that encloses the bud). (C, D) Close up of flowers showing the urceolate-glandular margins of the wing petals. (E) Opened flower and young fruit. (F) Mature fruit and curled valves of a fruit that has already dehisced and released its seeds. Photographs: Domingos Cardoso.

and stored in 70% ethanol. For scanning electron microscopy (SEM), flowers and floral buds were dissected in 70% ethanol, dehydrated to 100% ethanol, and critical point dried using an Autosamdri-815B critical-point dryer (Tousimis, Rockville, Maryland, USA). Dried samples were mounted onto aluminum stubs using clear nail polish and coated with platinum in a Quorum (Laughton, UK) Q150T sputter coater. SEM images were taken with a Hitachi (Tokyo, Japan) S-4700-II cold field emission SEM and images were processed using Adobe (San Jose, California, USA) Photoshop CS5.

TABLE 1. Organ initiation in the early-branching Amburaneae clade of papilionoid legumes.

Species	Sepals	Petals	A	a	G	Reference
Petaladenium urceoliferum	bid	bid	simult	simult	G+A	present study
Amburana cearensis	bid	bid	rev uni	rev uni	G+P	Leite et al. (2015)
Dussia discolor	sequ	—	—	—	—	Prenner (2004a)
Dussia macroprophyllata	uni	simult	uni	uni	G+A	G. Prenner. pers. obs.
Myrocarpus frondosus	uni	—	simult?	simult?	—	G. Prenner, pers. obs.
Myroxylon balsamum	uni (adax sim or in close succession)	simult (very close in time [a])	td simult	td simult	G+P	Tucker (1993)
Cordyla sp.	—	—	—	—	—	no data available
Myrospermum sp.	—	—	—	—	—	no data available
Mildbraediodendron sp.	—	—	—	—	—	no data available

Notes: A = antesepalous stamen whorl; a = antepetalous stamen whorl; bid = bidirectional; G = gynoecium with information about the timing of its initiation; pers. obs. = personal observation; rev uni = reversed unidirectional; sequ = sequential; simult = simultaneous; td simult = tendency toward simultaneous; uni = unidirectional; — = missing data.
[a] Tucker (1993, p. 68).

RESULTS

***Flower morphology*—**Flowers are subtended by a bract and preceded by two lateral lanceolate bracteoles. The pentamerous calyx is synsepalous, 8–11 mm long, and shows five subequal calyx lobes. The papilionate corolla is pinkish to lilac, and the five clawed petals are differentiated into a typical flag blossom consisting of an adaxial standard, which is 16–20 mm long and strongly recurved at anthesis, two lateral wing petals, and two abaxial keel petals. Wing and keel petals are around 14–17 mm long and 6–7 mm wide. The wing petals are unique among the legume family because they bear 5–7 short stipitate, urceolate, whitish glands on their distal margins. The androecium is composed of 10 nearly free stamens with dorsifixed anthers that are about 3 mm long. The gynoecium consists of a single carpel with 4–5 ovules and is terminated by a minute papillate stigma. The carpel matures into a compressed linear-oblong fruit.

***Flower development*—**The inflorescences are racemes with spiral flower formation in acropetal direction (Fig. 3A). Individual flowers are formed in the axils of floral bracts. Floral primordia initially are oval shaped (Fig. 3B). They soon become round, and two bracteoles are formed synchronously to the left and right of the flower primordium (Fig. 3C). Bracteoles enlarge somewhat and form distinct hairs, which arch inward toward the center of the young flower (Fig. 3D, E).

The first sepal is formed in an abaxial position (Fig. 3D). This sepal is soon followed by the remaining four sepals, which arise almost simultaneously (Fig. 3E). In some buds the two adaxial sepals appear slightly older than the lateral ones, indicating a bidirectional initiation (Fig. 3F, G). Sepals enlarge fast, and they soon bend inward to cover and protect the floral apex (Fig. 3G). A dense indumentum is formed early on the individual sepals, which further strengthens bud protection (Fig. 3H, I).

Petal initiation starts after a distinct plastochron during which the sepals enlarge and fully cover the floral bud (Fig. 3H–J). The two abaxial and the adaxial petals arise slightly earlier than the two lateral organs (Figs. 3K, 3L, 4B). The five petals enlarge quickly and ahead of the outer stamen whorl (Fig. 4C–E). The petals cover the inner stamen whorl, which is formed simultaneously (Fig. 4E, F). Petal growth remains relatively fast concurring only with the carpel, which also enlarges and develops fast (Fig. 4G, H). Somewhat later the petals are arranged in descending aestivation in which the adaxial standard is the outermost petal covering the edges of the two lateral wing petals, which cover the edges of the abaxial keel petals (Fig. 4I). Hairs start to form at the tip of the vexillum (Fig. 4J), which enlarges distinctly and which is the largest organ at a mid-developmental stage (Fig. 5C). The indumentum increases successively (Fig. 5C), and the adaxial side of the vexillum is ultimately covered by a dense indumentum. Gland formation starts early at the distal edges of the lateral wing petals (Figs. 5C, 5E, 6A). The glands enlarge successively (Figs. 5G, 6B) and finally approximately seven distinct cup-shaped structures are discernible along the distal rims of the two lateral wing petals (Figs. 2, 6). On the inner surface of these structures, we found some evidence for secretory activity (Fig. 6E–G).

The carpel appears slightly earlier than the stamens of the outer (antesepalous) whorl (Fig. 4C). Carpel development is relatively fast, and soon the cleft starts to form in adaxial position (Fig. 4D–F). The carpel cleft seems to close first (Fig. 4G, H), but it opens again later, clearly showing the ovule initials (Figs. 4I–L, 5 A, B). Eventually, the carpel closes again, and ovule development proceeds inside the closed carpel (Figs. 5C–H, 8A, 8C). Relatively late in development, the proximal end of the carpel starts to bend toward the adaxial side of the flower (Fig. 5G), and a short style and papillate stigma are formed (Figs. 5I, 5J, 7A, 7D, 7E, 8A).

Shortly after the carpel is initiated, the five antesepalous stamens are formed simultaneously (Fig. 4C, D). The five stamens of the inner whorl are also formed simultaneously after a longer plastochron when the stamens of the outer whorl enlarged (Fig. 4F). Stamen development is delayed compared with the quick development of sepals, petals, and the carpel. A slight broadening of the apical part of the outer stamens indicates the onset of anther formation (Fig. 4K, L). Stamen filaments remain relatively short over a longer period (Fig. 5C, D, G, I, M); they have a broad base (Fig. 5D, I, K–M), and filament fusion becomes evident late in floral ontogeny (Fig. 5M). Filaments are shortly connate in mature flowers (Fig. 7A). The anthers are dorsifixed (Fig. 5K, 5L, 7A, 8A), and they produce tricolporate pollen grains with a microreticulate surface (Fig. 7B, C). At the base of the flower, the androecium is positioned on a distinct hypanthium (Fig. 8A), which shows nectariferous stomata at its base (Fig. 8D, E).

DISCUSSION

With the discovery of new ontogenetic data from early-branching papilionoid legumes, a broader picture starts to emerge. We still

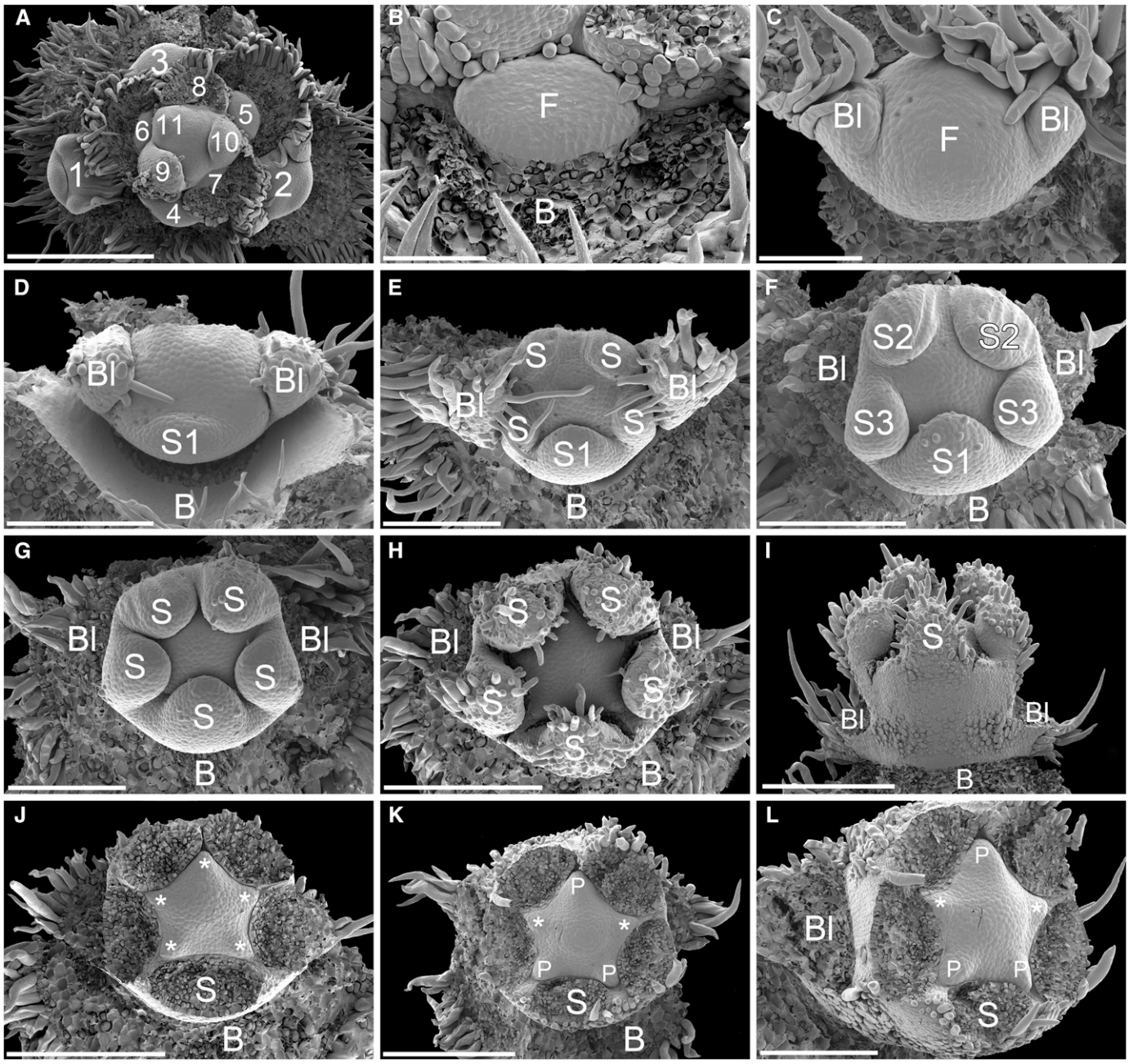

FIGURE 3 *Petaladenium urceoliferum*, inflorescence and early floral development (SEM). (A) Flowers and floral bracts are formed in an anticlockwise spiral along the racemose inflorescence. (B) Floral primordium in the axil of a bract. (C) Floral primordium flanked by two simultaneously formed bracteoles. (D) Formation of first sepal in abaxial position. (E) The remaining four sepals are formed in a rapid succession with some evidence for bidirectional formation found in (F) and (G). Bracteoles enlarge and form long hairs. (F) The oldest sepal in abaxial position bends inward and starts forming hairs. The two adaxial sepals appear slightly larger and older than the two lateral sepals. (G) Similar to (F), formation of hairs starts on the abaxial and one of the two adaxial sepals. (H). Sepals are covered with hairs and enclose the young bud (frontal view). (I) Abaxial view on (H) showing the dense indumentum on the sepals and the position of the two lateral bracteoles. (J) Same as (H and I), sepals removed, showing pentagonal floral apex, which is just about to form petal primordia in its corners (asterisks). (K, L) Slightly older flower showing rapid formation of petal primordia; (L) lateral view, the two abaxial and the adaxial petal appear slightly older than the two lateral petals. B, bract; Bl, bracteole; F, floral primordium; P, petal; S, sepal. Scale bars: A = 500 µm; B, C = 100 µm; D–G, L = 200 µm; H–K = 300 µm.

cannot establish good synapomorphic characters, but our results rather indicate a distinct experimental phase among early-branching papilionoid legumes (cf. Tucker, 1993; Leite et al., 2014, 2015; present study). Such an experimental phase was proposed by Prenner and Klitgaard (2008) based on ontogenetic data of *Duparquetia orchidacea* Baill., which is among the earliest-diverging Leguminosae.

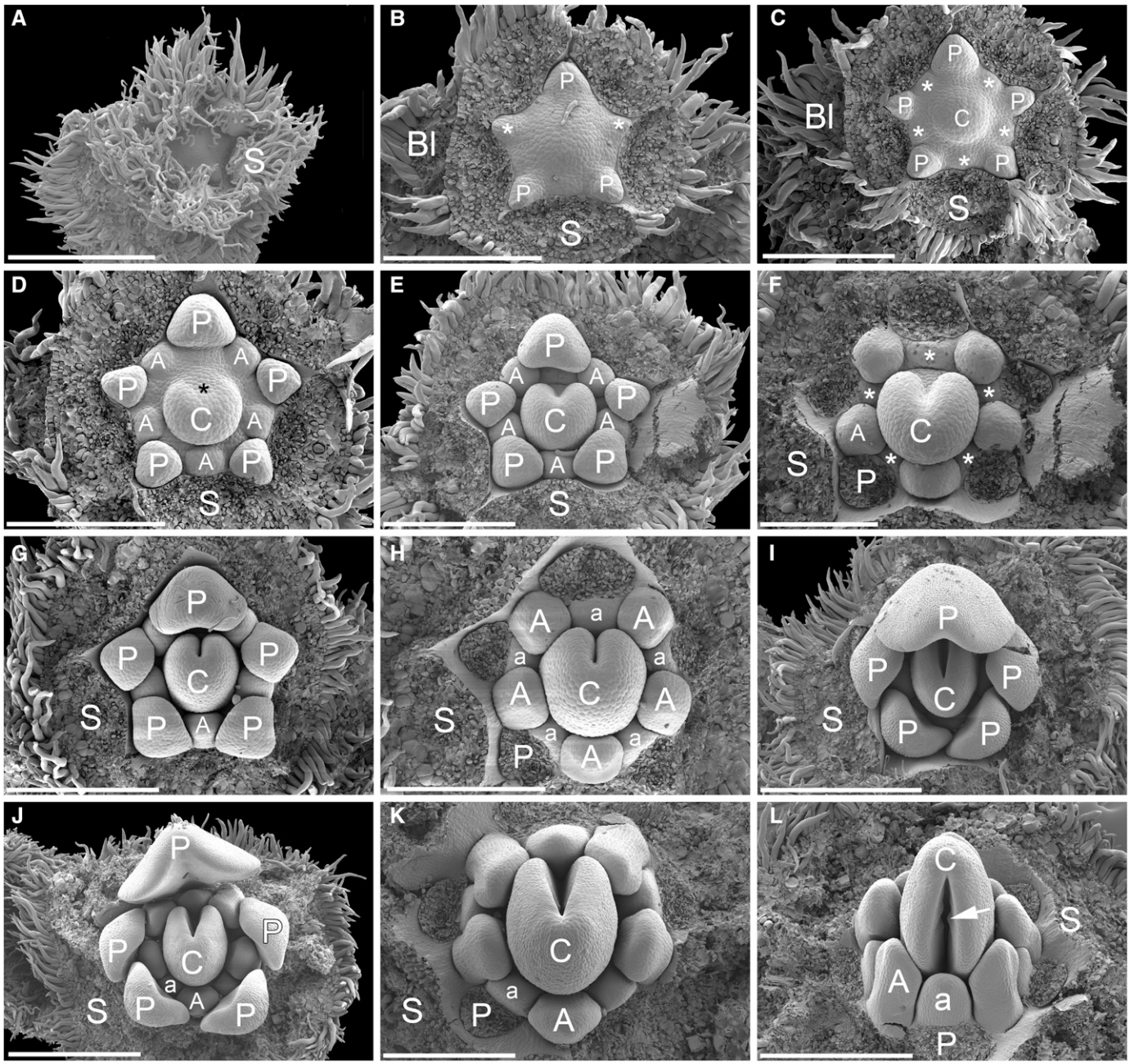

FIGURE 4 *Petaladenium urceoliferum*, formation of petals, stamens, and carpel (SEM). (A) Young flower fully enclosed by sepals, which are hard to recognize individually and which are covered in a dense indumentum. (B) Same as (A), calyx removed, showing young petals. The two abaxial and the adaxial petals are larger than the two laterals (asterisks) of which the left one is still not well developed. (C) All five petals and the carpel primordium are clearly visible. Five antesepalous stamens become just visible (asterisks). (D) Five petals enlarge relatively quickly. Five antesepalous stamens and the carpel, which already has developed an adaxial depression (asterisk), are clearly visible. (E) Petals continue to grow inward, starting to cover the inner floral organs. In the carpel, the adaxial cleft is clearly visible. (F) Same as (E), petals removed to show the simultaneous formation of the inner, antepetalous stamen whorl (asterisks). (G) Petals continue to enlarge and grow inward, toward the floral center. Carpel cleft closed. (H) Same as (G), petals removed to show the inner stamen whorl, which is still relatively small and undifferentiated. Outer stamens enlarged but still undifferentiated. (I) Petals enlarge and showing descending aestivation (adaxial standard covers the lateral wing petals, which are covering the abaxial keel petals). Carpel cleft open. (J) Similar developmental stage, standard petal bent backward to show inner floral organs and the open carpel cleft. Hairs start to form at the tip of the standard petal. (K) Same as (J), petals removed, showing the outer stamen whorl, which starts to differentiate anthers distally, inner stamen whorl still undifferentiated and carpel with widely open cleft. (L) Adaxial view on (K) showing the large carpel, the widely open cleft and three ovule primordia, which are just discernible (one highlighted with arrow). A, antesepalous stamen; a, antepetalous stamen; Bl, bracteole; C, carpel; P, petal; S, sepal. Scale bars: A = 500 µm; B–E, H, K = 300 µm; F = 200 µm; G, L = 400 µm; I, J = 500 µm.

In early-branching Papilionoideae, the pronounced diversity in floral morphology such as variable organ number and variable floral symmetry that are both based on a broad range of ontogenetic patterns suggest an experimental phase and lack of constraints during the early evolutionary history of the papilionoid legumes. Flexibility in morphology and ontogenetic pathways was ultimately canalized among the more derived Papilionoideae into the characteristic zygomorphic flag blossom, which might have evolved as a key innovation for most derived papilionoid legumes. Here we speculate that the experimental phase was an important precondition for the success-story of papilionoid legumes.

Floral ontogeny and morphology are in accordance with new systematic hypotheses—Several characters seem to support recent molecular hypotheses that place *Petaladenium* sister to *Dussia* in the early-branching tribe Amburaneae (Cardoso et al., 2015). Both genera have typical dorsiventral flag blossoms, and the general morphology of wing and keel petals is relatively simple and less complex than in more derived taxa. For example, the keel petals are not fused, and wing and keel petals are not interlocked by specialized structures. The pollination mechanism is a simple valvular mechanism in which the displacement of the keel downward allows the androecium and gynoecium to come into close contact with the floral visitor (Arroyo, 1981). The keel petals subsequently flap back into their original position after the visitor has left, so this reversible mechanism may be repeatedly triggered. Furthermore, in both genera, the stamens are almost entirely free, which again can be seen as less specialized than the various degrees of fusion found in more derived taxa (monadelphous, diadelphous, pseudomonadelphous sensu Tucker, 1987). The few available data on other characters, such as the microreticualte pollen exine of *Petaladenium* (Ferguson et al., 1994) resembling that of *Myroxylon* L.f. (Amburaneae clade sensu Cardoso et al., 2013) and *Dalhousiea bracteata* Graham ex Benth. (Baphieae clade sensu Cardoso et al., 2013) also corroborate the current phylogenetic hypothesis. Furthermore, Manning and Stirton (1994) showed that there are strong similarities of endothecial thickenings between *Petaladenium* and *Dussia*, which reflect the sister relationship of these two genera (Cardoso et al., 2015).

A comparison of ontogenetic characters in tribe Amburaneae—The sequence of organ initiation in *P. urceoliferum* fits relatively well into the broader emerging picture among early-branching Amburaneae clade (Table 1). In *P. urceoliferum* no organ whorl is formed in a strict unidirectional pattern, which is frequently found in more derived papilionoid lineages (cf. Tucker, 1984; Prenner, 2004a). Instead, we found tendencies toward bidirectional initiation in the sepal and petal whorl, drawing links in ontogenetic patterns between *Petaladenium* and *Amburana cearensis* (Allemão) A.C.Sm., which shows bidirectional organ formation in both perianth whorls (Leite et al., 2015). Bidirectional organ formation is relatively rare among Leguminosae, but interestingly it was found in all but one of the taxa studied in the caesalpinioid tribe Dialiinae (cf. Zimmerman et al., 2013; Bruneau et al., 2014). In contrast to this, sepals are formed sequentially in *Dussia discolor* (Benth.) Amshoff (Prenner, 2004a) and unidirectionally in *Myroxylon balsamum* Harms (Tucker, 1993), *Dussia macroprophyllata* Harms, and *Myrocarpus frondosus* Allem. (G. Prenner, personal observation). Petal formation is simultaneous in *Dussia macroprophyllata* (G. Prenner, personal observation) and in *Myroxylon balsamum* (Tucker, 1993). Simultaneous petal formation can also be found in a wider range of Papilionoideae (see Table 1 in Prenner, 2013) and is not restricted to early-branching taxa.

In *Petaladenium urceoliferum* both stamen whorls are formed simultaneously. Almost simultaneous stamen formation was also found in *Myroxylon balsamum* (Tucker, 1993) and possibly in *Myrocarpus frondosus* (G. Prenner, personal observation, based on limited material). However, the closely related *Dussia macroprophyllata* shows unidirectional stamen formation (G. Prenner, personal observation). In *Petaladenium urceoliferum* the adaxial stamen of the inner whorl lies exactly in the median plane (i.e., symmetric androecium sensu Prenner, 2004b). The same pattern is found in *Myroxylon balsamum* (Tucker, 1993) and in *Amburana cearensis* (Leite et al., 2015). In contrast, the androecial symmetry in *Dussia discolor* is not stabilized and both, asymmetric and symmetric androecia were found (Prenner, 2004b). The precocious formation of the carpel (concomitantly with the outer stamen whorl) is a character that occurs in many other papilionoid taxa (see Table 1 in Prenner, 2013).

Evolutionary significance of the uniquely derived glandular wing petals—The most outstanding character of *P. urceoliferum* is its wing petals that are lined with a series of glands. This unique character has not been reported elsewhere in the more than 19 000 species of legumes. Our analysis shows that the formation of these structures starts very early in ontogeny and long before the wing petals differentiate into claw and blade.

Despite the uniqueness of this character in legumes, it is striking that in *Dussia*, which is closely related to *Petaladenium* (Fig. 1), there are similar gland-like structures. However, in *Dussia* these are stalked and are found on the subtending bract and bracteoles. A somewhat similar pattern is found in *Duparquetia orchidacea* in which the abaxial petals are reduced and lined with gland-like globular structures (Prenner and Klitgaard, 2008). However, the morphology of these structures in *Duparquetia* differs considerably from what we found in *Petaladenium*. Whereas the structures in *Duparquetia* were described as shortly stalked gland-like hairs (Prenner and Klitgaard, 2008), the structures in *Petaladenium* are without a stalk and distinctly cup shaped, comparable to ascocarps in Ascomycota.

At the current state of knowledge, it is hard to interpret the function of glandular wing petals in *Petaladenium*; however, a connection with pollination seems plausible. In a typical papilionoid flag blossom, nectar glands are found at the very base of the flower where nectar is only accessible by floral visitors who are able to

just become visible. (I) Young flower showing the androecium with well-developed anthers and different length of filaments in the outer and inner whorl (sepals and petals removed). The carpel is closed and a short style bends in adaxial direction. (J) Detail of (I) showing the young stigma. (K) Dorsal view and (L) side view on young stamen with dorsifixed filament insertion. (M) Adaxial view on young bud (sepals and four petals removed). The broad filaments are elongated and basally fused for a short distance. The anthers are monomorphic (no difference between inner and outer whorl anthers). A, antesepalous stamen; a, antepetalous stamen; an; anther; C, carpel; fi, filament; o, ovule; P, petal. Scale bars: A, D, E, K, L = 500 µm; B = 200 µm; C, G, I, M = 1 mm; F, J = 100 µm; H = 300 µm.

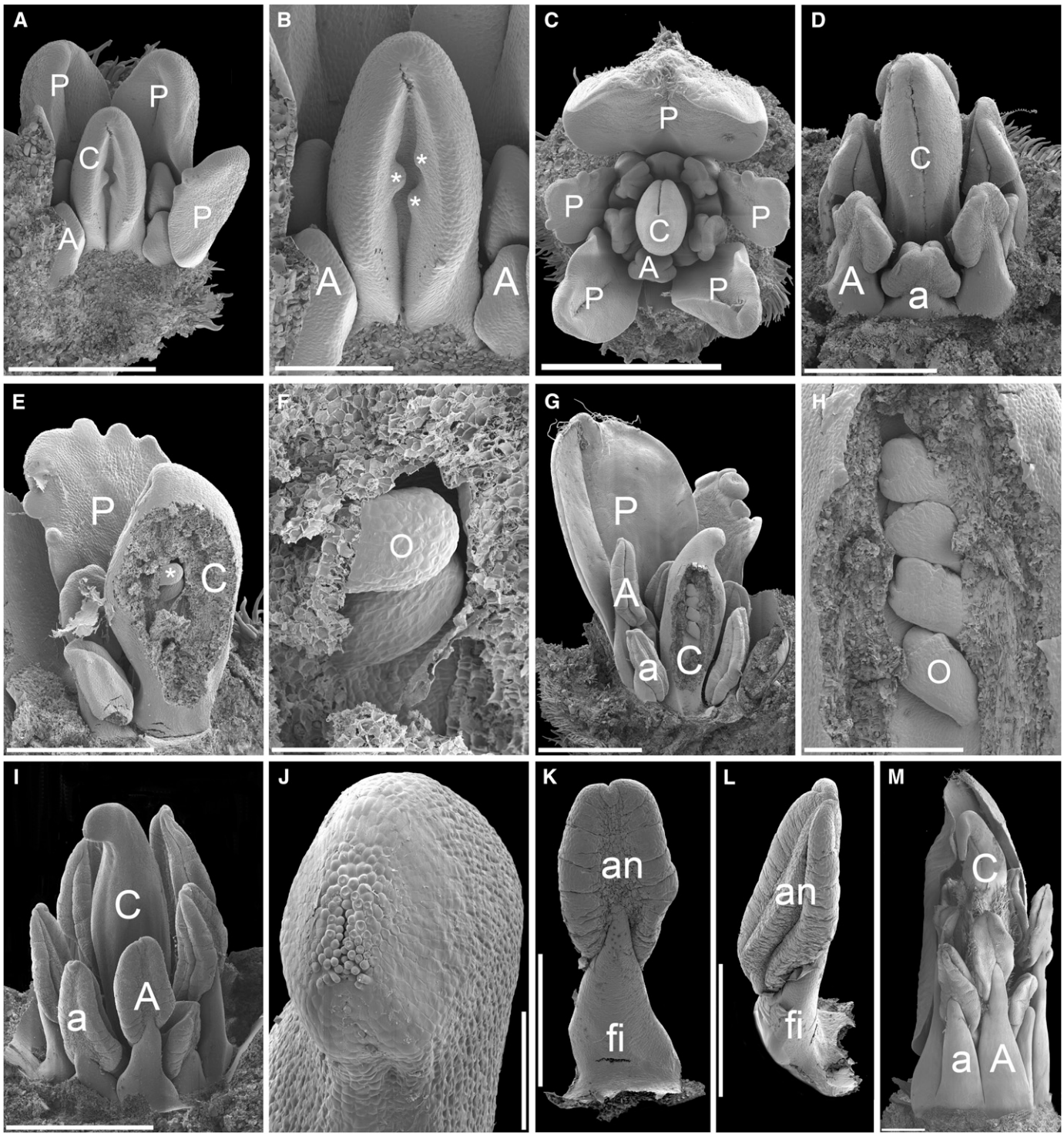

FIGURE 5 *Petaladenium urceoliferum*, carpel and stamen development (SEM). (A) Adaxial view on young flower with widely open carpel. Standard petal, adaxial stamen, and part of lateral wing petal removed. The right wing petal shows already first signs of gland formation. (B) Detail of (A), showing the open carpel with three ovule initials (asterisks). (C) Frontal view on somewhat older flower. The standard petal is largest and already shows some distinct indumentum. The lateral wing petals are smallest and already clearly show signs of gland formation along their edges. Abaxial keel petals with distinct ridges along their center. In the androecium, the anthers are differentiated and the gynoecium is closed and flattened laterally. (D) Adaxial view on (C), petals removed to show the closed carpel cleft and stamen differentiation in both whorls (note the relatively broad filament bases). (E) Similar developmental stage than (D), with opened carpel to show young and still undifferentiated ovules. Wing petal in the background with signs of gland formation. (F) Detail of (E) showing two ovules. (G) Somewhat older stage in which the style is already visible. Standard petal with a tuft of hairs on top and wing petal in the background with young glands. (H) Detail of (G), young ovules in which the nucellus and the integuments

trigger the floral mechanism, i.e., who are able to push the wing and keel petals downward and to force their head and sucking mouthparts deep into the flower where the nectar is located. In such conventional papilionate flowers, the wing and keel petals are important not only as an optical attractant, but they also function as a landing platform and as an aid for the visitor to hold onto the flower. This aid to hold onto the flower is important, because the floral visitor needs some power to press the wing and keel downward, whereas the flag petal functions as an abutment (Westerkamp and Weber, 1997). Furthermore, the flag's claw frequently acts as a guide for the animals tongue toward the nectar at the base of the flower. In this way, by pushing the wing and keel petals downward, the stamens and the gynoecium will be exposed and rubbed against the visitor's abdomen. Visitors who have visited another plant of the same species before, will bring pollen with them and will act as pollinators.

Floral rewards that are produced on the outer surface of the wing petals seem counterproductive because floral visitors would not be guided into the flower. Therefore, there is no incentive to trigger the floral mechanism (i.e., to push the wing and keel petals downward), and flowers will not be pollinated. The occurrence of stomata on the inside of the hypanthium of *P. urceoliferum* is a strong indicator that the flowers produce nectar, which is available at the base of the hypanthium (Prenner, 2003; Paiva and Machado, 2008) and that this species follows the "traditional" way of flag blossoms as described above.

Based on our current knowledge, we propose the following testable hypotheses on possible functions of the petal nectar glands:

(1) Osmophores ("perfume hypothesis"): Production of volatile substances such as perfume for long-distance attraction of potential pollinators. Osmophores are in general inconspicuous (Endress, 1994; Marinho et al., 2014), and during collection, no particular odor of the flowers was noticed (D. Cardoso and C. E. Zartman, personal observation). However odor production could also be restricted to a certain period of the day (e.g., in the morning or evening) and therefore could have been missed.
(2) Nectary ("nectar hypothesis"): As mentioned, this hypothesis seems to us the least plausible because it would be counter productive for the function of the flower.
(3) Production of a sticky secretion ("glue hypothesis"). A sticky secretion close to the anthers could help to "glue" the pollen grains onto a visitor's body (De Frey et al., 1992; Prenner and Teppner, 2005). Because in *Petaladenium* the stamens are nearly free and wing and keel petals are not fused, pollen will be spread over a larger area of the floral visitor and most pollen grains will not end up in the "right" position (i.e., where the stigma will contact the visitor's body). Such a "diffuse" pollen deposition differs from the more elaborate and precise pollen deposition in higher papilionoids in which the joined stamens direct the pollen and stigma to a limited area on the visitor's body. Thus, sticky substances from the wing tips would collect pollen in areas where the stigma will also contact the visitor. The microreticulate exine of the pollen grains indicates that pollen kit might be absent. This theory is only testable in the field via direct observation of the flowers and floral visitors.
(4) Production of a solid secretion such as wax ("lever hypothesis"). Solidifying secretions could increase petal strength and play a mechanical role in pollination. Among Amburaneae, only *Dussia* and *Petaladenium* present papilionate corollas with standard, wing, and keel petals differentiated and with a gamosepalous calyx; but with almost free stamens. The valvular tripping mechanism requires that the floral visitor pushes down wing and keel petals to get access to the nectar and, as a consequence, exposes the stamens and stigma, which touch the visitor's body promoting pollination (Knuth, 1908; Leppik, 1966). The advanced valvular mechanism requires some way to transfer movements from the wing to the keel. In most derived papilionoid legumes, this transfer is achieved through interconnections between wing and keel petals such as auricles, corresponding sockets, wrinkles, etc. In *Petaladenium*, wing and keel petals are loosely arranged, and thus when a pollinator pushes down the wing, this downward movement is not transferred to the keel. The rigid wing margins (assuming a solid secretion) could provide a connection between wing and keel petals and enhance the valvular mechanism of this flower.

   An alternative to the "lever hypothesis" is that the waxy glandular wing margins could provide areas for the floral visitors to hold onto the wings during flower manipulation. In most papilionate flowers, the wing petals have areas of sculptured surface where bees could grab the petal with their claws (cf. Stirton, 1981). But *Petaladenium* lacks such sculptured areas, making it difficult for floral visitors to hold onto the petals. This hypothesis can be tested by observation of the visitor's behavior during flower visitation.
(5) Protective function. The early formation of the glands and the tight closure of the buds especially in the distal region where the glands occur indicate that these structures might have a protective function for the floral bud and the sexual organs. The tight closure of the bud might prevent predators such as bud-mining insects from entering and damaging the bud (cf. Endress, 1994, p. 23, on additional functions of the perianth). Another testable hypothesis is that the glands produce a chemical repellent against florivorous animals and other nonlegitimate (i.e., not pollinating) visitors (e.g., Ballantyne and Willmer, 2012).

Open carpels with developing ovules: A rarely found character in papilionoid legumes—Another unexpected result is that the carpels reopen for a short time after they were already closed. In this phase, the developing ovules are visible. Tucker and Kantz (2001) reported open carpels with developing ovules for 25 caesalpinioid species and only one papilionoid legume, *Aeschynomene americana* L., which is a dalbergioid legume belonging to the broader group of early-branching papilionoid legumes (sensu Cardoso et al., 2013). The observed morphological pattern in *P. urceoliferum* closely resembles those found in caesalpinioid legumes documented by Tucker and Kantz (2001), which raises the question as to why such open carpels evolved infrequently and independently among papilionoid legumes.

However, Endress (2015) was cautious about the examples presented by Tucker and Kantz (2001), and he notes that they may not represent the natural condition and that they rather are drying artifacts because the carpels are relatively long and slender with the flanks not yet fused. Our observations agree with Endress (2015) that the fusion of the carpel flanks occurs late in *P. urceoliferum*. However, our material consistently revealed a relatively wide-open carpel with visible ovule formation so that an artifact is less obvious to us. To us, it seems also surprising that such "artifacts" are mainly restricted to Caesalpinioideae and much rarer in Papilionoideae. Even if the documented open carpels were artifacts they still point toward some structural differences such as late closure of the carpel edges.

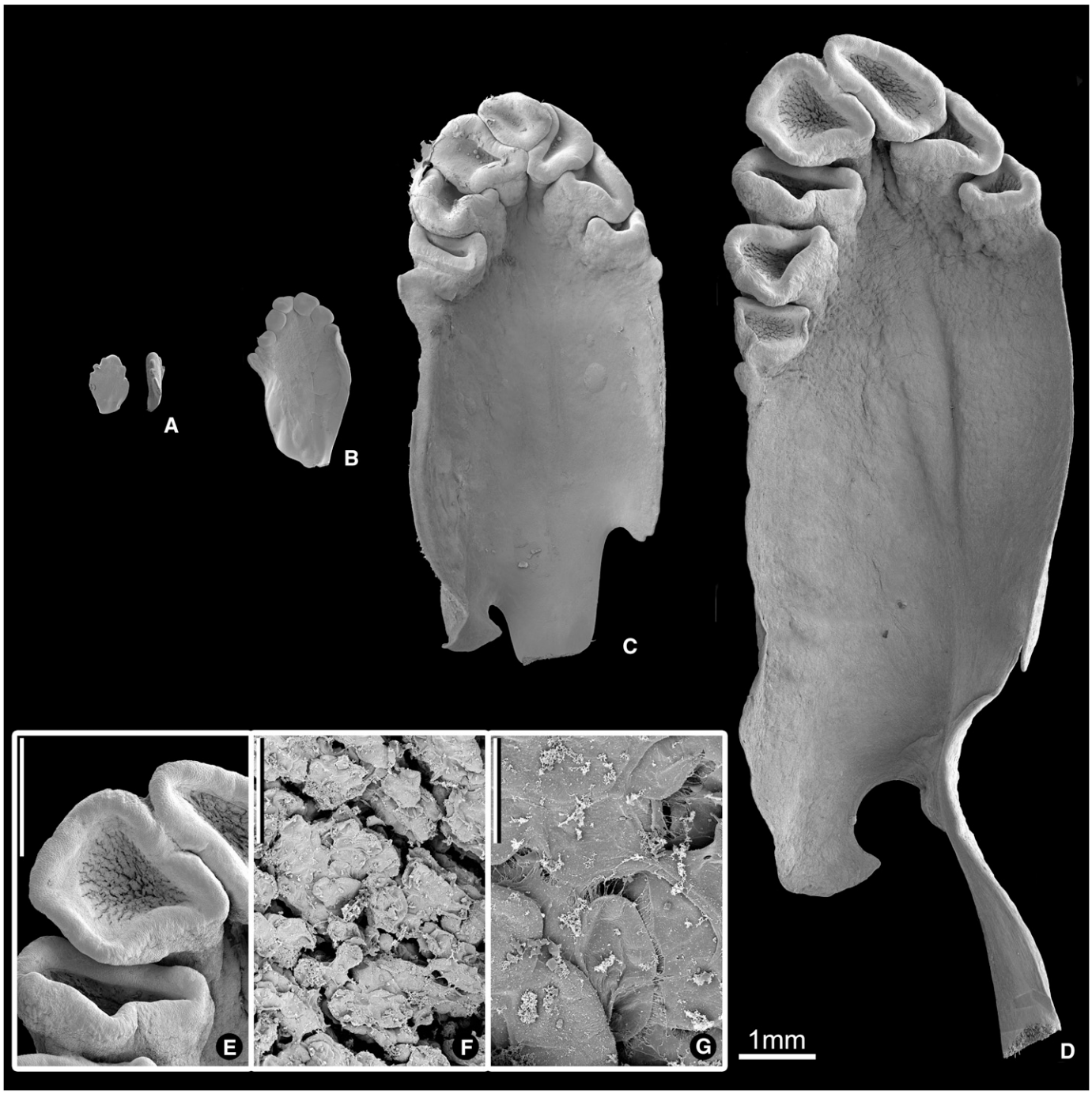

FIGURE 6 *Petaladenium urceoliferum*, wing and gland development (SEM). (A) Frontal and lateral view on young wing petal, which is less than 1 mm long and in which gland formation just sets in. (B) Older wing petal, still undifferentiated with young glands clearly discernible along its rim. (C) The basal claw and basal outgrowths of the blade start to differentiate. Distal glands are formed. (D) Mature wing petal showing long claw, basal outgrowth and seven distal glands along the wing's rim. (E) Detail of (D), showing gland with distinct bulging margin and the central area, which appears reticulate-porose-granulate. (F) Detail of (E) showing the inner part of the gland. (G) Detail of (F), showing the surface of the glandular tissue. Scale bars: A–E = 1 mm; F = 100 µm; G = 20 µm.

***Outlook*—**Among early-branching papilionoid legumes, our knowledge of detailed floral morphology and floral biology is still very patchy. In Amburaneae there are currently distinct gaps in our knowledge of the genus *Myrocarpus* Allem., and no data are available for the genera *Cordyla* Lour., *Myrospermum* Jacq., and *Mildbraediodendron* Harms (Table 1). Other taxa for which no data are available are *Alexa* Moq. and *Xanthocercis* Baill. from the tribe Angylocalyceae and the genus *Monopteryx* Spruce ex Benth. from Dipterygeae (sensu Cardoso et al., 2012, 2013). Adding data from these taxa will not only foster our understanding of the floral

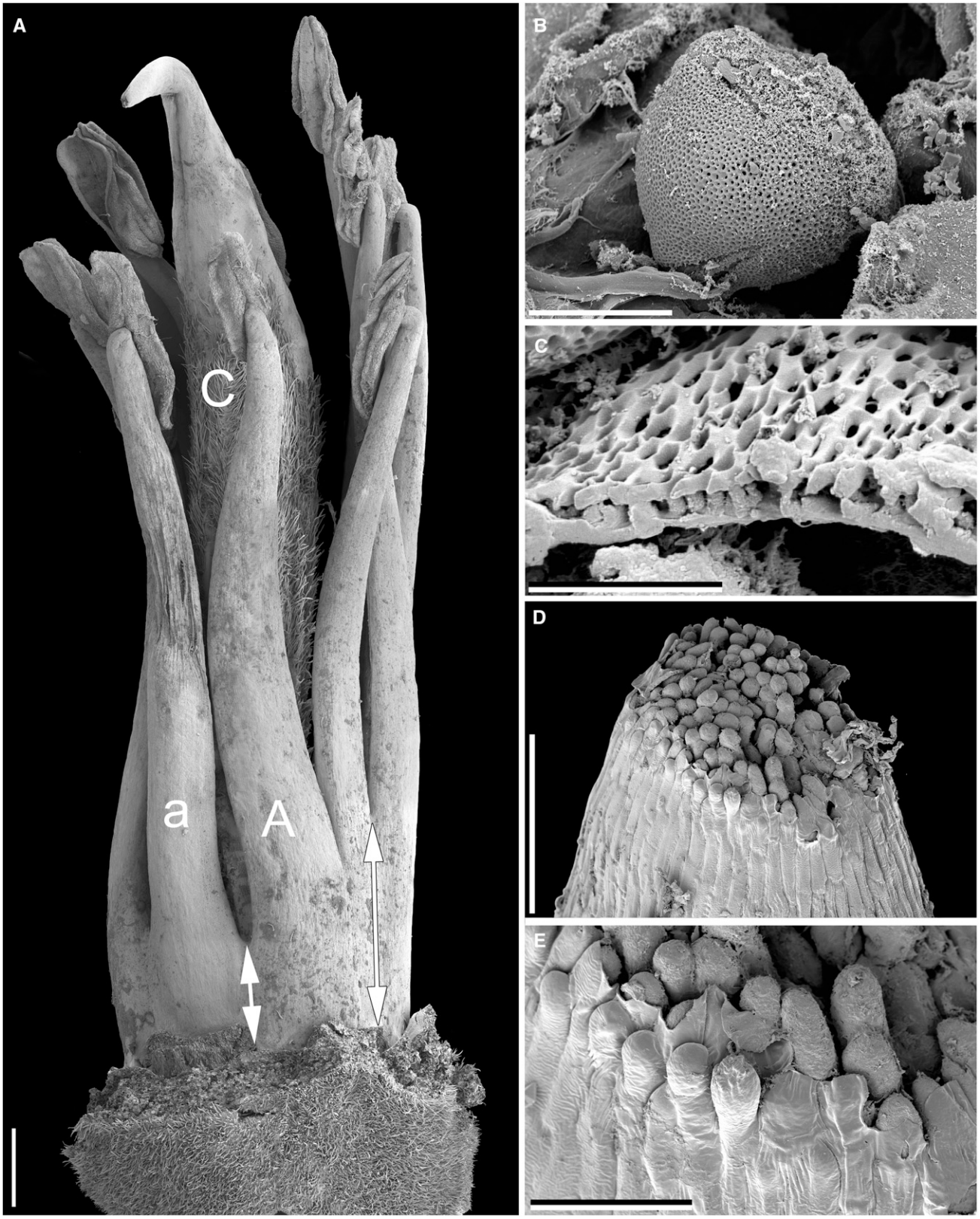

FIGURE 7 *Petaladenium urceoliferum*, mature flower (SEM). (A) Androecium and gynoecium. The filaments of all the stamens are fused at the base (double arrows). The most distal part of the carpel is bent upward (in adaxial direction). Sepals and petals removed. (B) Pollen grain with microreticulate surface and three colpi. (C) Detail of pollen exine from a broken pollen grain. (D) Papillate stigma. (E) Detail of (D) showing papillae at the rim of the stigma. A, antesepalous stamen; a, antepetalous stamen; C, carpel. Scale bars: A = 1 mm; B = 10 µm; C = 3 µm; D = 100 µm; E = 30 µm.

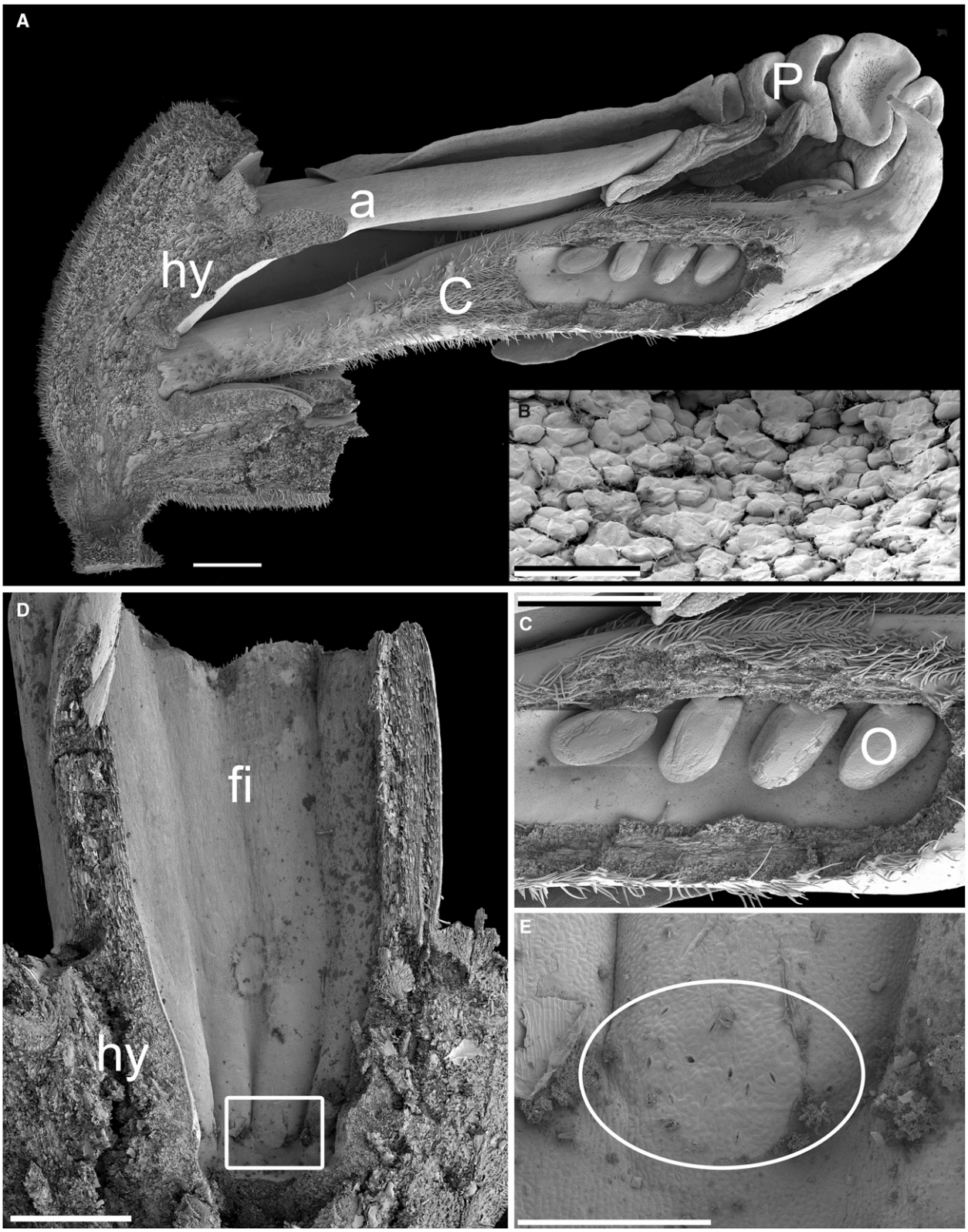

FIGURE 8 *Petaladenium urceoliferum*, mature flower (SEM). (A) Longisection through mature flower showing the opened carpel with four ovules and a distinct hypanthium and a wing petal with distinct glands. (B) Detail of (A) showing the glandular surface. (C) Detail of (A) showing the four ovules. (D) Longisection of the base of a mature flower showing the hypanthium and fused filaments. (E) Detail of region highlighted in (D) showing distinct stomata at the base of the hypanthium. a, antepetalous stamen; C, carpel; fi, filaments (fused); hy, hypanthium; o, ovule; P, petal. Scale bars: A, C, D = 1 mm; B = 100 µm; E = 300 µm.

morphology among early-branching papilionoids, but they will also form the basis for new testable hypotheses regarding how and when the successful evolutionary history of the papilionoids (i.e., the canalization of morphological characters into the papilionoid flag blossom) started.

ACKNOWLEDGMENTS

The authors thank Agapito Bu'u, Adão Mirupu, Dirce Komura, Zé Adailton, and Billy, for help during field expeditions in the remote areas of Brazilian Amazonia, the indigenous community São Jorge at São Gabriel da Cachoeira for logistic support, Haroldo Cavalcante de Lima for details on the localities and habitats where some interesting legumes had been collected in the upper Rio Negro, Instituto Nacional de Pesquisas da Amazônia (INPA) for technical support during fieldwork and kindly making their herbarium collections available during our visits, and Peter Endress and an anonymous reviewer for valuable comments on the manuscript. This work received financial support from FAPESB/CNPq (PNX0014/2009), Sistema Nacional de Pesquisa em Biodiversidade–SISBIOTA (Processes CNPq 563084/2010-3 and FAPESB PES0053/2011), and National Environmental Research Council grant NE/I027797/1. D.C. acknowledges CNPq for supporting his postdoctoral fellowships (Processes 156143/2012-7 and 150129/ 2014-9) at Universidade Estadual de Feira de Santana (UEFS). Another postdoctoral fellowship to D.C. supported by CAPES (Process BEX 2812/13-4) through the Reflora program (Processes CNPq 563546/2010-7 and FAPESB PES0054/2011) made possible the study of important collections of *Petaladenium* and other basal papilionoids at the Royal Botanic Gardens, Kew.

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