# Notes on floral symmetry in the *Pterocarpus* clade (Leguminosae: Papilionoideae: Dalbergieae)

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*Summary:* Androecium and corolla morphology is characterized in the *Pterocarpus* clade (Leguminosae: Papilionoideae: Dalbergieae) with a special aim to compare genera with monosymmetric (*Arachis*, *Centrolobium*, *Pterocarpus*, *Tipuana*) and polysymmetric (*Etaballia*, *Inocarpus*) flowers. The study on epidermal features of petals indicates that monosymmetry in *Etaballia* (and probably *Inocarpus*) results from insufficient cell expansion in all petals rather than from homeosis. Petals become ribbon-shaped because of deficient (or absent) cellular divisions towards the lateral edges.

*Keywords:* *Etaballia*, evolution, monosymmetry, Papilionoideae, petal epidermis, polysymmetry

The evolutionary success of a certain taxon is generally associated with a high level of both phenotypic plasticity and variability. A wide area of distribution, a large number of subtaxa (species) and a broad range of variation characterize the 'fittest' taxon in Darwinian sense. Studies on genetic control and precise mechanisms of ontogeny together with surveys in phylogenetic history comprise the evolutionary developmental biology, called evo-devo. Classical plant morphology has brought numerous descriptions, which are now subject to identification of causative mechanisms and evaluation, whether certain morphological features comprise ancestral state or arose secondarily, often independently in different taxa.

The third largest angiosperm family, Leguminosae (Fabaceae), exhibits a remarkable variation in floral morphology with a basic plan conserved, i.e. pentamerous and pentacyclic flower with a monomerous gynoecium ($K_5 C_5 A_{5+5} G_1$). In different lineages, this morphology transformed in different ways. While many of legumes (at least in subfamily Papilionoideae) have a monosymmetric flower, there are numerous cases of polysymmetry. These used to be evaluated as an ancestral state, but are now generally reconsidered as apomorphies, i.e. evolutionary novelties (Cardoso et al. 2012; Povydysh et al. 2014).

The pantropical tribe Dalbergieae (or, more strictly, the Dalbergioid clade sensu Lavin et al. 2001), although generally characterized by a 'flag blossom' typical of many papilionoids, includes several genera with polysymmetric flowers. Some of them are nested within the monosymmetric subclades (Klitgård et al. 2013). To date, several ways to establish a secondary polysymmetry are hypothesized in legumes, associated with homeosis and illustrated with cases from both model (Feng et al. 2006; Wang et al. 2008) and non-model (Citerne et al. 2006; Sinjushin et al. 2015) species. It is of special interest to provide detailed morphological descriptions of polysymmetric leguminous flowers in different taxa, as this may give clues to mechanisms of shifts in floral symmetry and, in future, to identification of causatives genetic factors.

The present work focuses on the floral structure of selected representatives of dalbergioid legumes belonging to the so-called *Pterocarpus* clade sensu Klitgård et al. (2013). Most of the examined plant species are tropical trees, so living material is not easy to access for description

**Table 1.** Plant material used for analysis.

| Species | Herbarium: initial identification (if differs). Specimen description. |
|---|---|
| *Acosmium dasycarpum* ssp. *glabratum* (Benth.) Yakovlev = *Leptolobium dasycarpum* Vogel | MHA: *Sweetia elegans* (Vog.) Benth. Paraguay, dept. Paraguarí, Chololó. Alt. 400 m. November 27, 1950. Coll., det. Sparre & Vervoorst 615. |
| *A. subelegans* (Mohlenbr.) Yakovlev = *L. elegans* Vogel | MW: 0581643, 0581644. Paraguay, Canindeyú. Mbaracayú Natural Reserve. Around Lagunita. October 29, 1998. Coll. E.M. Zardini, I. Chaparro 49289. Det. H. Bach. |
| *Centrolobium yavizanum* Pittier | LE: 761. Plants of Panama. Provincia del Darien. Vicinity of El Real, Río Tuira; foothills of Cerro Pirre, S. of El Real. June 30, July 13, 1959. Coll. W.L. Stern, K.L. Chambers, J.D. Dwyer, J.E. Ebinger. Det. J. Dwyer. |
| *Pterocarpus acapulcensis* Rose | LE: 39859. *P. podocarpus* Blake. The New York Botanical Garden Middle Orinoco expedition. Estado Bolivar, Venezuela. Rio Orinoco, alt. 80–90 m. December 13, 1955. Coll. J.J. Wurdack, J.V. Monachino. Det. R.S. Cowan. |
| *P. erinaceus* Poir. | LE: Mali. Near the village of Solo, northern edge of Korofing National Park (formerly Bafing Faunal Reserve). 12°57.808' N, 10°27.215' W. February 1, 2004. Coll., det. C.S. Duvall 517. |
| *P. lucens* Guill. & Perr. | MHA: Mali, Katibougou. November 05, 1966. Coll., det. N.N. Kaden 67163. |
| *P. marsupium* Roxb. | LE: Ceylon Flora Project. Monte Kokegala ad orientem Mahiyangana. November 11, 1975. Coll. L. Bernardi 15708. |
| *P. santalinoides* DC. | LE: 5920. *P. amazonicus* Huber. B.A. Krukoff's 5th expedition to Brazilian Amazonia. Region of the lower Amazon. State of Pará: near Bocca do Paru. On varzea land. August 28–29, 1934. |
| | MW: 0587745, 0587746. *P. santaloides* L. Hér. Mali, road Bamako – Koulikoro. January 24, 1967. Coll., det. N.N. Kaden 34. |
| *Etaballia dubia* (Kunth) Rudd | LE: *E. guianensis* Benth. Guiana angl. Coll. R. Schomburgk 381. |
| | LE: 2925. *E. guianensis* Benth. Herb. Fischer. Origin unclear. Det. G.P. Yakovlev. |
| *Inocarpus fagifer* (Parkinson) Fosberg | LE: *I. fagiferus* (Forster) Fosberg. Herb. Fischer. ?St. Vincents N. India. ?Hooker. Det. G.P. Yakovlev. |
| *Arachis hypogaea* L. | MW: 0897158. Commercially available seed specimen (Uzbekistan) reproduced in a glasshouse. |
| *Tipuana tipu* (Benth.) Kuntze | MW: 0799483. Spain, Barcelona. August 22, 2019. Coll., det. Yu. Trifonova. |

of sequential stages of ontogeny. However, all species of this group follow a similar pentamerous and pentacyclic floral plan, and the resulting floral symmetry is most probably established lately in ontogeny, at stages of perianth differentiation. That is why the principal object of study was a petal epidermal micromorphology, as it is insightful for identification of petal identity in legumes (Ojeda et al. 2009).

## Materials and methods

For analysis of epidermal micromorphology, herbarium specimens were mainly used (Table 1), as they are not less informative than living material (Ojeda et al. 2009). Flowers were soaked in a hot water (ca. 90°C) and then kept in a 70% ethanol solution in a thermostate (60°C) for 12 h and stored in 70% ethanol. After these softening procedures, flowers were dissected under a stereomicroscope. Images of petals were captured from an Olympus SZ61 stereomicroscope

(Olympus Corp., Tokyo, Japan) using a UHCCD05000KPA camera (ToupTek Photonics, Zhejiang, China). All measurements were carried out in digital images using the program ImageJ 1.51k (National Institutes of Health, Bethesda, MD, USA). For measurements of area, 2–4 petals of each type and (with few exceptions) 20 cells were taken.

Ethanol-preserved petals were dehydrated through ethanol and acetone series, and then dried using a HCP-2 (Hitachi, Japan) critical point dryer, mounted onto metal stubs using nail polish and coated with Pd in an Eiko IB-3 (Eiko, Japan) sputter coater. Specimens were visualized under CamScan-S2 (Cambridge Instruments, UK) microscope in Secondary Electron Image (SEI) regime with an accelerating voltage of 20 kV. Only adaxial surfaces were studied in flags, while wings and keel petals were observed mostly from abaxial side. All SEM procedures were performed in the interdepartmental laboratory of electronic microscopy of Biological Faculty of the Lomonosov Moscow State University. Digital images were processed using Corel PHOTO-PAINT 2017 (Corel Corporation, USA) in course of their preparation for publication.

# Results

## Corolla morphology in examined species of the *Pterocarpus* clade

In all cases, the corolla is pentamerous. In *Arachis*, *Centrolobium*, *Pterocarpus* and *Tipuana*, it is monosymmetric, papilionate, with three types of petals more or less clearly recognizable (Fig. 1A–C). Two lateral petals (wings) are longer and broader than abaxial ones (*P. erinaceus*, *P. marsupium*, Fig. 1A, Table 2) or of approximately the same size (*P. santalinoides*, *P. acapulcensis*, Fig. 1C). Two abaxial petals are connate forming a keel. In some species (*A. hypogaea*, *C. yavizanum*, *P. acapulcensis*, *P. santalinoides*, *T. tipu*; Figs 1B, C; 2), the lateral petals have a
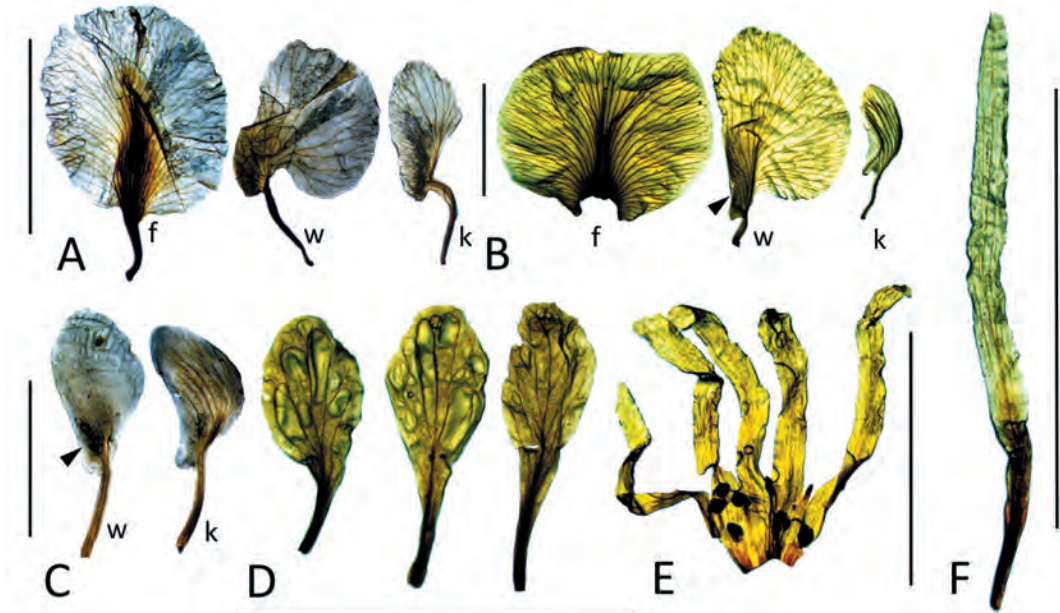


**Figure 1.** Corolla morphology in some species of the *Pterocarpus* clade. A – *Pterocarpus marsupium*; B – *Tipuana tipu*, claw of a flag not visible; C – *P. acapulcensis*; D – *Acosmium subelegans* (= *Leptolobium elegans*), three petals of five; E – *Inocarpus fagifer*, adnate stamens are visible; F – *Etaballia dubia*, one petal of five. f = flag, k = keel petal, w = wing, arrowhead = area of ridge-like sculpture. Scale bars = 1 cm.

ridge-like epidermal sculpture in their proximal parts clearly seen with a stereomicroscope. While the lateral and abaxial petals are asymmetric with respect to the midvein, the adaxial petal (flag) is always symmetric (Fig. 1A, B). In genera with a papilionate corolla, all petals are unguiculate (clawed), although in *Arachis* the claw is very short. Lateral and abaxial petals sometimes bear auriculate appendages on a boarder between claw and wing (Fig. 1A–C).

Both species of *Acosmium* available for investigation, *A. dasycarpum* ssp. *glabratum* and *A. subelegans*, are now placed in the separate genus *Leptolobium* within the Genistoid rather than the Dalbergioid clade (Cardoso et al. 2012). However, the corolla morphology in both *Leptolobium* and *Acosmium* s. str. seems similar (Rodrigues & Tozzi 2009), therefore petals of two *Leptolobium* species have been studied in detail. All five petals are of (sub)equal size, slightly unguiculate (with gradual transition between limb and claw), without auricles, more or less symmetric with respect to the midvein (Fig. 1D).

In *Etaballia*, all five petals of a polysymmetric corolla are of equal size, internally symmetric and ribbon-shaped (Fig. 1F). These petals are not typically unguiculate, with differences between claw and limb visible only on the micromorphological level (see below).

The corolla of *Inocarpus* is remarkable with its five connate petals producing a short corolla tube (Fig. 1E). Shape of petals is similar to those in *Etaballia*, as they are ribbon-shaped and have no clear boarder between claw and limb.

## Micromorphology of petal epidermis

In species with a papilionate corolla, different petals exhibit a somewhat distinct epidermal micromorphology. In *A. hypogaea*, epidermis of flag and wings consists of tabular rugose-striate cells (Fig. 3A, B; here and further terminology of Ojeda et al. (2009) is applied), while keel is covered with tabular flat cells, lacking (in proximal and middle parts, Fig. 3C) or bearing longitudinal striation (in distal part, Fig. 3D). In *T. tipu*, flag and wings have papillose, knobby cells with a rugose striation (Fig. 3E, F). These are more or less isodiametric cells (as seen from the surface) with straight or slightly incurved adjacent (anticlinal) walls. However, the keel petals are covered with tabular rugose cells having a similar mode of striation (Fig. 3G). These cells are clearly oblong with their longer side oriented along a proximodistal axis. A similar pattern is found in *Centrolobium* (Fig. 3L) and *Pterocarpus*. A flag petal typically has the maximum number of epidermal cells as compared to wings and keels (Table 2). Keel epidermis in most cases consists of the largest cells (Table 2). In *Leptolobium*, all petals have a similar epidermal type (Fig. 3H).
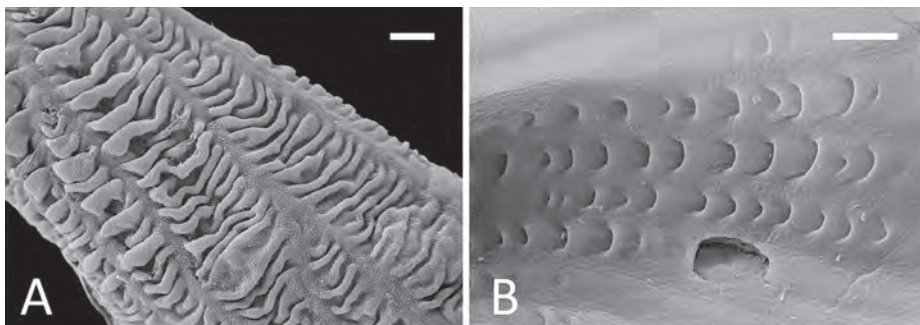


**Figure 2.** Ridge-like sculpture on outer (abaxial) side of wings: A – *Tipuana tipu* and B – *Arachis hypogaea*. Scale bars = 300 µm.

**Table 2.** Features of corolla macro- and micromorphology in studied species.

| Species | Limb area[a], mm² | | | Cell area[b], ×10⁻⁶ mm²/ *Cell number* per limb[c], ×10³ | | |
|---|---|---|---|---|---|---|
| | Flag | Wing | Keel | Flag | Wing | Keel |
| *Arachis hypogaea* | 155.9 ± 8.8 | 40.6 ± 2.4 | 13.7 ± 0.4 | 1089.5 ± 231.3/ *143.1* | 1038.4 ± 160.1/ *39.1* | 509.4 ± 95.0/ *30.0* |
| *Acosmium dasycarpum* | 4.8 ± 0.5 | | | 398.6 ± 74.1/ *12.0* | | |
| *A. subelegans* | 8.0 ± 0.6 | | | 372.4 ± 93.9/ *21.6* | | |
| *Centrolobium yavizanum* | – | 15.2* | 21.1 ± 0.6 | – | 280.2 ± 75.2/ *54.2* | 501.8 ± 130.8/ *42.1* |
| *Etaballia dubia* | 7.0 ± 0.6 | | | 158.4 ± 32.3/ *44.2* | | |
| *Inocarpus fagifer* | 9.6 ± 0.8 | | | 130.0 ± 26.1/ *74.2* | | |
| *Pterocarpus acapulcensis* | 113.4 ± 9.4 | 43.1 ± 1.6 | 36.2 ± 3.5 | 479.5 ± 101.8/ *236.5* | 522.7 ± 127.8/ *82.4* | 579.6 ± 173.5/ *62.5* |
| *P. erinaceus* | 79.0 ± 13.1 | 31.6 ± 3.2 | 11.9 ± 1.5 | 228.8 ± 43.0/ *345.5* | 364.5 ± 81.1/ *86.8* | 596.6 ± 116.4/ *19.9* |
| *P. lucens* | 89.1 ± 10.5 | 36.3 ± 1.6 | 21.1 ± 0.6 | 418.1 ± 72.1/ *213.1* | 397 ± 72.2/ *91.5* | 482.8 ± 117.5/ *43.7* |
| *P. marsupium* | 79.8 ± 5.3 | 44.9 ± 3.9 | 15.7 ± 0.7 | 337.1 ± 66.8/ *236.8* | 358.7 ± 66.6/ *125.3* | 1209.2 ± 145.8/ *13.0* |
| *P. santalinoides* [MW] | 95.6 ± 3.5 | 32.4 ± 1.1 | 26.7 ± 0.8 | 368.9 ± 110.8/ *259.1* | 302.2 ± 81.8/ *107.4* | 640.6 ± 211.9/ *41.7* |
| *Tipuana tipu* | 338.1 ± 75.7 | 127.7 ± 15.5 | 23.1 ± 0.4 | 598.6 ± 126.3/ *564.9* | 777.8 ± 127.5/ *164.2* | 726.4 ± 124.3/ *31.7* |

[a] Average ± stand. dev. [b] Median ± stand. dev., measured as seen from above. [c] Calculated as ratio between limb area and cell area. – Data absent. * Only one petal was available.

All listed characteristics refer to the epidermis of distal part of the petal. In all species, a claw is covered with oblong, flat tabular cells, either striated (Fig. 3I, T) or almost smooth. A similar epidermal type is found along veins in a limb (not shown).

The mutual arrangement of epidermal cells in the distal part of mature petals seems to have no long-range order (Fig. 3A, B, E, F, H, K, R, S).

The striation of a cell surface has a kind of ontogenetic dynamics. On early stages, ridges are oriented along a proximodistal axis of a petal (Fig. 3J, Q). In mature petals, it is difficult to trace the preferential orientation of ridges (Fig. 3A, B, E–G, H, K, L, N, R, S). Sister cells originating from a single mitosis have a similar pattern of striation (Fig. 3K).

Petals of *Etaballia* consist of two types of cells. In their proximal part, they are covered with oblong cells with a minute longitudinal striation, resembling the claw in other species (not shown). The distal petal part bears oblong to isodiametric, often almost quadrangular, cells; their innate convexity is unclear (Fig. 3N). These cells are organized as longitudinal arrays sharing the same orientation of surface striae, especially seen on their borders (Fig. 3M, N).
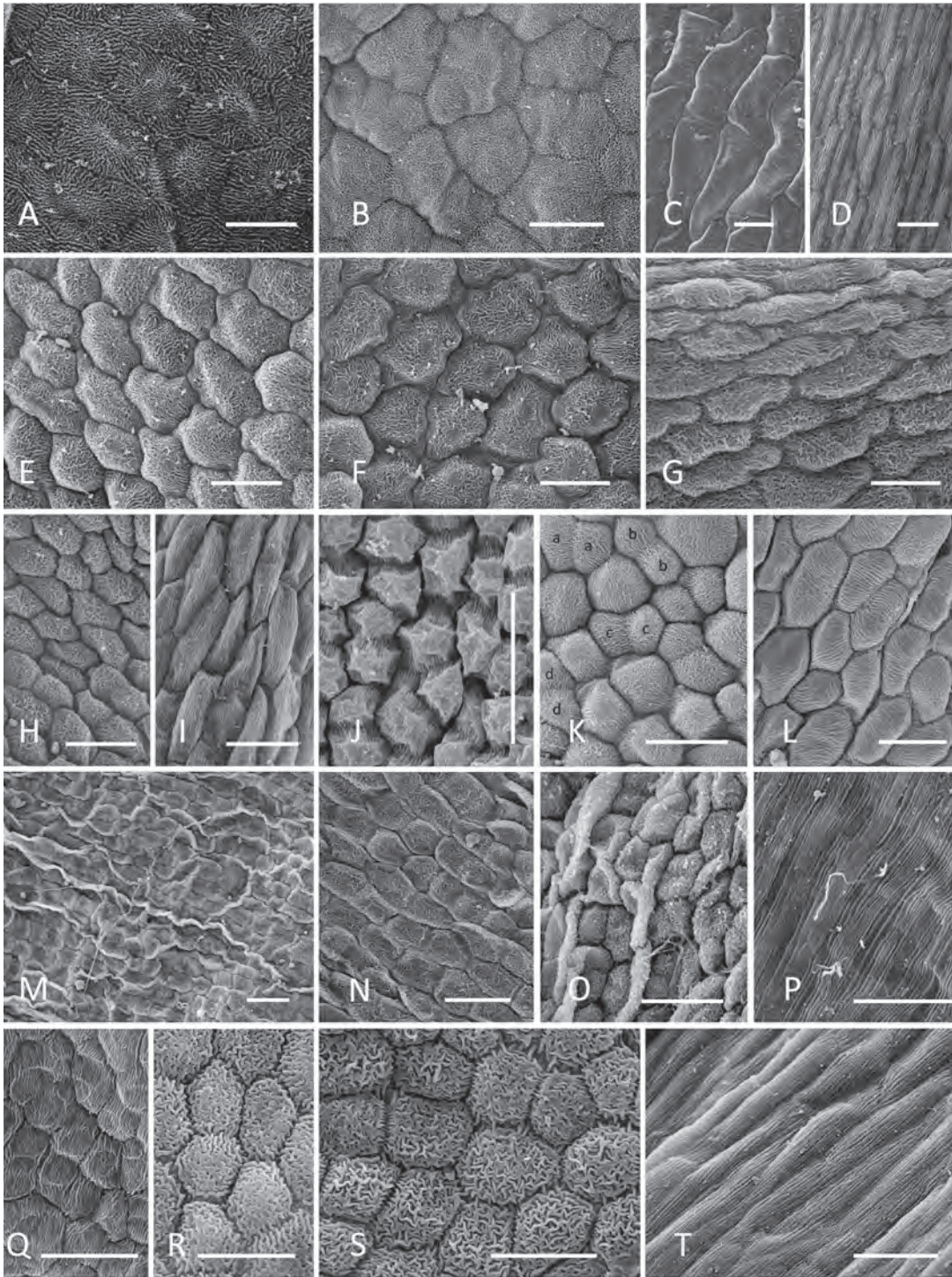
**Figure 3.** Examples of epidermal types in the *Pterocarpus* clade. A–D *Arachis hypogaea*: A – flag, B – wing, C – keel, middle part, D – keel, distal part. E–G *Tipuana tipu*: E – flag, F – wing, G – keel. H, I *Acosmium subelegans* (= *Leptolobium elegans*): H – limb, I – claw. J–L *Centrolobium yavizanum*: J – immature flag, K – wing (identical letters indicate putative sister cells), L – keel. M, N *Etaballia dubia*. O, P *Inocarpus fagifer*: O – middle part of petal, P – corolla tube as viewed from inside. Q–S *Pterocarpus santalinoides* [LE]: Q – immature flag, R – mature flag, S – wing. T – *P. lucens*, flag claw. Scale bars = 30 μm.

Inner surface of the corolla tube of *Inocarpus* has the same epidermal type as petal claws in other species (Fig. 3P), although boarders between individual cells are almost indiscernible. Free parts of petals have small papillose, knobby cells with a rugose striation (Fig. 3O). The available data suggest that these cells lack a preferential orientation of surface ridges as well as clear long-range order.

# Discussion

## Unusual corolla morphology in *Etaballia* originates from atypical cell expansion rather than from homeosis

Detailed analysis of epidermal micromorphology in corolla in the *Pterocarpus* clade provides an insight into the origin of floral monosymmetry in this group. As morphogenesis in higher plants is not associated with cellular migrations, all changes in shape of developing structures can be achieved only through directed growth, often anisotropic (Coen et al. 2004). As demonstrated by Sauret-Güeto et al. (2013), growth of petal cell clones involves different division rates along the major axis of the petal. The anisotropy is most remarkable in distal parts of petals.

Studies on both natural variation and developmental genetics suggest several ways to acquire secondary monosymmetry. Klitgård et al. (2013) proposed that polysymmetry in dalbergioids results from homeotic replacement, with all petals acquiring regulatory (and hence morphological) features of the adaxial one. This scenario has been proved for *Cadia* (core genistoids) by Citerne et al. (2006), thus representing the only successful 'junction' between naturally existing morphology and phenotypes of mutants of model species. In many angiosperm taxa, the *TCP* gene family regulates a floral monosymmetry, with genes *CYCLOIDEA* (*CYC*) and *DICHOTOMA* (*DICH*) being the best known representatives. In genomes of legumes, *CYC*-like genes usually exist in several copies, which regulate different aspects of floral monosymmetry. For example, in a garden pea (*Pisum sativum*: inverted repeat-lacking clade, IRLC), there are two mutations affecting floral symmetry (Wang et al. 2008). One of them, *lobed standard 1* (*lst1*), impairs one of three pea orthologs of *CYC*, *PsCYC2*, hence causing defects in the development of the flag. One more mutation, *keeled wings* (*k*), damages *PsCYC3* and results in wings converted into keel petals. The double mutants *k lst1* have all five petals abaxialized, i.e., keel-like. In addition, mutation *symmetric petals 1* (*syp1*) diminishes the internal asymmetry of petals (Wang et al. 2008). The authors of the cited work hypothesized that adaxialized floral morphology with all five petals being flag-like may also exist. This is exactly the case of *Cadia* (Citerne et al. 2006) and anomalous forms of *Clitoria* (milletioid clade) and *Astragalus* (IRLC) (Sinjushin et al. 2015).

The corolla consisting of five equal petals can be derived from the typically papilionate corolla via the way illustrated by homeotic mutants, i.e. through adaxialization or abaxialization of all petals. The latter scenario would also require additional changes in regulatory network to make petals internally symmetric. However, the homeosis would result in a corolla consisting of five petals similar to the ones normally found in related species. This is possibly the case of *Acosmium* s. l. with five subequal petals roughly resembling flags of papilionate members of the *Pterocarpus* clade (Fig. 1D).

Oppositely, both *Etaballia* and *Inocarpus* possess unusual, ribbon-shaped, clawless petals, which can hardly represent the result of homeosis. These two genera are remarkable regarding their epidermal features, viz. comparatively small number of very petite cells arranged in longitudinal

files (the latter feature is found at least in *Etaballia*; Fig. 3M, N; Table 2). This syndrome indicates that the unusual corolla morphology together with a monosymmetry in these genera emerges from deficient cellular divisions (most probably insufficiency of marginal meristems) and expansion. Floral mutants with a similar phenotype were recorded in different plant species. For example, in anomalous *Ipomoea nil* (Convolvulaceae), all lateral organs such as leaves and floral parts are reduced in width, while epidermal features remain intact, i.e. frequency of cell divisions is reduced (Kᴀᴊɪᴛᴀ & Nɪsʜɪɴo 2009). A similar phenotype characterizes floral mutants of *Rhododendron* spp. (Ericaceae) (Tᴀsᴀᴋɪ et al. 2012a, b). Remarkably, although *Rhododendron* normally has a gamopetalous corolla with slight features of monosymmetry (dissimilar size and shape of petals, their aestivation and pigmentation of the adaxial petal), most or all these characteristics are lost in mutant flowers, also lacking petal fusion. Anomalous flowers have reduced stability of merism and sometimes produce additional stamens/staminodes (Tᴀsᴀᴋɪ et al. 2012a, b), but clearly illustrate how atypical cell expansion may affect corolla morphology and symmetry.

To date, numerous genes have been identified in different species, which, once mutated or subjected to overexpression, result in atypical petal morphology. Some of them regulate preferentially one of the floral domains. For example, gene *LATHYROIDES* in pea controls lateral organ growth, and the effect of its mutation *lath* is mostly visible in the flag (Zʜᴜᴀɴɢ et al. 2012). Oppositely, 'open flower' mutants of *Cajanus cajan* produce narrow, free keel petals (Bᴏʜʀᴀ et al. 2016). In *Arabidopsis* (Brassicaceae), mutants *rabbit ears* (*rbe*) have an ectopic expression of stamen-specific gene *AGAMOUS* in petal primordia and hence develop narrow, malformed petals (e.g. Kʀɪᴢᴇᴋ et al. 2006). As many other aspects of plant development, petal size, shape and epidermal features are dependent on phytohormones and external factors such as light (Zʜᴀɴɢ et al. 2012; ᴠᴀɴ Es et al. 2018). On cellular level, action of genes and other agents is mediated by properties of the cytoskeleton and cell wall (Zʜᴀɴɢ et al. 2012; Fᴜᴊɪᴋᴜʀᴀ et al. 2014). No wonder that cell micromorphology of petals is variable in certain taxa, sometimes even on the intraspecific level (Bᴀɪʟᴇs & Gʟᴏᴠᴇʀ 2018).

With the ongoing progress in genomics, it is doubtless that causative genetic factors underlying an 'anomalous' floral phenotype in *Etaballia* and *Inocarpus* will be eventually identified. However, one may state almost confidently that it results from atypical cell proliferation and expansion in petals rather than from homeotic switch between different petal types. The homeosis scenario is possibly actual for *Acosmium* s. l., which is also remarkable due to a comparatively small number of petal cells (Table 2).

Reversals to polysymmetry are not rare in different lineages of legumes, but, as discussed previously, may have different mechanisms and hence are not identical in distinct clades. In other words, there are not many ways (possibly only one) to establish floral monosymmetry, but there are several ways to disturb it (Oᴊᴇᴅᴀ et al. 2019).

### Plasticity of androecium morphology in the *Pterocarpus* clade and what it may tell about floral evolution

Discussing regulatory and evolutionary aspects of floral symmetry in different taxa, the corolla morphology is usually considered. For comparison, much less attention is paid to the associated morphology of androecium. The *Pterocarpus* clade is remarkable by its variable androecial structure (Fig. 4), often labile within a single species (Fig. 4A, Table 3). As Rᴏᴊᴏ (1972: 12) stated considering *Pterocarpus*, "stamens… connate into a sheath, always widely split above

**Table 3.** Androecium characters in selected genera and species of the *Pterocarpus* clade; phylogeny according to Klitgård et al. (2013).

| Group | | Genus/species | Androecium | Source |
|---|---|---|---|---|
| Pteroarpus clade: Clade A: Clade B | Asian *Pterocarpus* | *P. dalbergioides, P. indicus, P. macrocarpus, P. marsupium, P. santalinus* | $M^S/M^*$, AbS+ | Rojo 1972 |
| | African *Pterocarpus* | *P. angolensis, P. erinaceus, P. mildbraedii* | $M^S/M^*$, AbS± | Rojo 1972 |
| | | ?*P. brenanii, P. lucens, P. officinalis, P. osun, P. rotundifolius, P. soyauxii, P. tessmannii* (= *P. casteelsii*: Evrard 1988; = *P. soyauxii*: Rojo 1972), *P. tinctorius* | $M^S/M^*$ | Rojo 1972 |
| | | ***Etaballia*** | $M^T$ | This work; Fig. 4C |
| | Neotropical *Pterocarpus* | *P. amazonum* | $M^S$ | Rojo 1972 |
| | | *P. amphymenium* (= *P. orbiculatus*), *P. rohrii* (incl. *P. steinbachianus, P. floribundus, P. reticulatus, P. hayesii*), *P. santalinoides, P. villosus* (= *P. rohrii*: Rojo 1972), *P. violaceus* (= *P. rohrii*: Rojo 1972), *P. zehntneri* (= *P. rohrii*: Rojo 1972) | $M^S/M^*$ | Rojo 1972 |
| | | *P. monophyllus* | $M^*$ | Klitgaard et al. 2000 |
| | *Paramachaerium* | | ?$M^S$, AbS± | Rudd 1981 |
| *Pterocarpus* clade: Clade A | *Maraniona* | | $M^S$ | Hughes et al. 2004 |
| | *Tipuana* | | $M^S$ | This work; Fig. 4A |
| | ***Inocarpus*** | | $M^T$, adnate to corolla tube | Adema 2007 |
| | *Centrolobium* | | $M^S$ | Pirie et al. 2009 |
| | *Ramorinoa* | | $M^*$, the most abaxial stamen partly free | Gómez-Sosa 1972 |
| | *Pterocarpus acapulcensis* | | $M^S/M^*$ | Rojo 1972 |
| Selected other genera of the *Pterocarpus* clade | ***Acosmium* s. str.** | | F | Rodrigues & Tozzi 2009 |
| | *Arachis* | | $M^T$ | This work; Fig. 4B |
| | ***Riedeliella*** | | Rarely 9 or ?11, $M^T$, in ⅔ species adnate to petals | Mohlenbrock 1962; Lima & Vaz 1984 |

$M^T$ – monadelphous (tube) with vexillary filament coherent with both adjacent stamens; $M^*$ – monadelphous with vexillary filament free from both adjacent stamens in different degree, sometimes (almost) to the base; $M^S$ – monadelphous (sheath) with a vexillary stamen coherent with only one adjacent stamen; F – free stamens; AbS – abaxial split, either always present (AbS+) or sometimes present (AbS±). Genera with non-papilionate corolla are marked bold. *Pterocarpus brenanii* not sampled by Klitgård et al. (2013) is placed in a subclade according to its habitat and marked with ?.

completely down the base or narrowly split below, thus forming two bundles usually in equal parts, the upper (vexillary) stamen free or partially free or connate".

At the moment, there are seemingly no surveys on whether different intraspecific variants are heritable or not, what their frequencies are and if they can be found in the same plant. The prevailing androecial type is monadelphous exhibiting a tendency towards diadelphy, with the
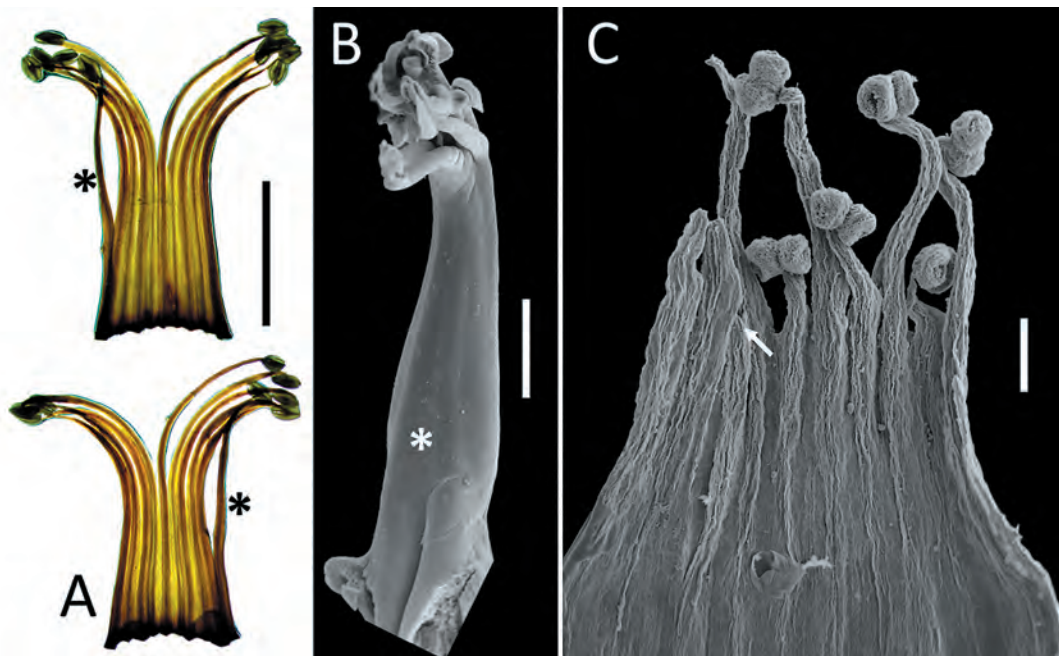
**Figure 4.** Morphology of androecium. A – *Tipuana tipu*, monadelphous androecium (unfolded), with vexillary stamen coherent with either left (upper image) or right (lower image) adjacent stamen; B – *Arachis hypogaea*, monadelphous androecium; C – *Etaballia dubia*, monadelphous androecium (dissected, four anthers are lost). Asterisk = filament of vexillary stamen, arrow = position of inner whorl stamen which is not visible on photo. Scale bars = 5 mm (A), 1 mm (B), 300 μm (C).

adaxial stamen of the inner whorl (vexillary stamen) fusing with the adjacent stamens in different degree (Table 3), so the transition between monadelphy and diadelphy is gradual even within *Pterocarpus* (ROJO 1972). In ten flowers of *T. tipu* examined, a vexillary stamen was connate with one of the adjacent stamens from either left or right side as seen from floral apex, with almost equal frequencies (6:4, respectively; Fig. 4A). True diadelphy with a completely free vexillary stamen is infrequent for the clade. Moreover, some of the species exhibit a so-called 'equal diadelphy' (TUCKER 1987) with ten stamens fusing in two equal groups of five. Such androecial morphology is found in other dalbergioid legumes beyond the *Pterocarpus* clade (e.g. in *Aeschynomene*: SAMPAIO et al. 2013). The variable degree of freedom of vexillary stamen and presence/absence of abaxial split associate in all possible combinations in *Pterocarpus* (Table 3).

In an earlier paper (SINJUSHIN et al. 2015), it was concluded that the monosymmetric leguminous flower might have either free stamens or different forms of staminal fusion. Oppositely, a polysymmetry almost never accompanies fused stamens, so *Etaballia* is remarkable in this regard, possibly unique among Papilionoideae but rarely found among Leguminosae (the *Brownea* clade of the Detarioideae subfamily: REDDEN & HERENDEEN 2006; *Inga* in Caesalpinioideae). Floral mutants and terata of legumes with a polysymmetric corolla also have free stamens (SINJUSHIN et al. 2015). It should be noted, however, that all model legumes (*Pisum*, *Medicago*, *Lotus*, etc.) normally possess a diadelphous androecium. If every petal acquires features of flag, every antepetalous stamen is differentiated as vexillary, i.e. opposed to flag and lacking fusion with adjacent stamens. In the *Pterocarpus* clade, *Acosmium* has five subequal petals and free stamens (unusual for the whole clade), hence suggesting that homeosis might have caused corolla

polysymmetry. This hypothesis is reliable, only if the hypothetical monosymmetric ancestor of *Acosmium* possessed diadelphous (or with another type of incompletely fused) androecium, which is not rare in this group (Table 3). Notably, four of six species of *Pictetia* (the *Dalbergia* clade) combine non-papilionate perianth (equal calyx lobes and nearly rotate corolla) with unstable androecial fusion and almost free filaments, while other species have a papilionate corolla and diadelphous androecium, sometimes with abaxial split (Beyra & Lavin 1999).

Oppositely, the androecium of *Etaballia* is fully monadelphous, also having such unusual features as a notably different length of filaments in different whorls (also found in *Inocarpus*) and short free parts of inner whorl stamens (Fig. 4C). If a hypothetical ancestor of *Etaballia* possessed a monosymmetric flower with more or less free vexillary stamen or adaxial split (common in the African *Pterocarpus* subclade where *Etaballia* is nested), then homeotic dorsalization would result in a more or less free androecium. The extant androecium structure of *Etaballia* also counters the hypothesis of homeosis-derived polysymmetry in this plant.

The androecium of *Inocarpus* is remarkable with its adnation to the corolla tube. Such morphology is rare among Leguminosae, although not unique (found in distinct genera of different subfamilies, such as *Trifolium* or *Gymnocladus*). Fusion between petals and stamens is also found in *Riedeliella* p. p. having a non-papilionate corolla (Lima et al. 1984).

### Morphological novelties lead to the overestimation of taxon status

Studies in model plant species and dissection of more details in plant developmental genetics demonstrate that subtle molecular events may have a striking effect on the phenotype. Numerous monogenic mutations cause developmental anomalies, which do not influence fertility and productivity, but may alter morphology significantly. If the emergence of such mutation(s) is documented, there won't be efforts to assign a special taxonomic status to mutants, although historically such cases had been recorded (e.g. *Pisum umbellatum* for the fasciated form of a garden pea).

When developmental mutations arise spontaneously in non-model species, there is a temptation to consider associated phenotypical novelties as significant enough to recognize a higher taxonomic level. There are several examples of such kind in legume taxonomy and beyond. For example, the aforementioned *Cadia* was separated in a monogeneric tribe Cadieae Baill. due to its polysymmetric flowers, but now it is included into the group of core genistoids. *Thermopsis turcica* with its unusual trimerous gynoecium was excluded in a separate genus *Vuralia*, although this status is debatably confirmed by molecular phylogeny (Uysal et al. 2014). Unusual fasciated flowers of *Tupidanthus* (Araliaceae) served as basis of its generic status, while it is clearly nested within *Schefflera* (Nuraliev et al. 2010). Similarly, *Etaballia* was found morphologically distinct from related *Pterocarpus* and hence received its generic status, later confirmed again due to 'anomalous' morphology (Rudd 1970–1971). After the first description of *Etaballia*, *P. monophyllus* with simple (or probably unifoliolate) leaves and wingless fruits has been described, thus reducing morphological differences between two genera only to floral features (Klitgård et al. 2013). Molecular approaches also confirm a close affinity between *Etaballia* and *Pterocarpus* again suggesting recognition of the former taxon as *P. dubius* (Kunth) Spreng. (Klitgård et al. 2013).

Although reasons to confirm or reject generic status are probably even more debatable than those considering specific level, application of molecular phylogeny helps to avoid overestimation of

morphological novelties. Future researches in plant genetics will probably identify the causative morphological mutations and estimate the age of their emergence.

## Conclusion

The results of this study lead to the conclusion that polysymmetric flowers in the *Pterocarpus* clade appeared as an evolutionary innovation and several times in different subclades. As for *Etaballia* and *Inocarpus*, there is no reason to accept the hypothesis of homeotic adaxialization of the corolla. The most probable mechanism causing polysymmetry in these genera is deficient cell proliferation and expansion, especially critical for lateral growth of petals.

Although no material of *Acosmium* s. str. was available for this study, this genus might have acquired its floral polysymmetry via homeosis, as all five petals roughly resemble flags in related genera with a papilionate corolla.

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