Acta Societatis Botanicorum Poloniae

INVITED REVIEW

Acacia (wattle) and Cananga (ylang-ylang): from spiral to whorled and irregular (chaotic) phyllotactic patterns – a pictorial report

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Abstract

Phyllotaxis, i.e., the arrangement of leaves around the stem and leaf-like organs inside flowers is regular in most vascular plants. Thus, developmental models usually explain regular phyllotactic patterns such as Fibonacci spirals and decussate/whorled patterns that obey Hofmeister's rule: primordia form as far away as possible from previously initiated primordia. However, flowering plants showing at first Fibonacci spirals or whorled phyllotaxes may switch to other patterns that lack an obvious order and thus may be called irregular or even chaotic. Vegetative shoot tips of various Australian wattles (Acacia spp., Leguminosae in eudicots) and flower buds of ylang-ylang (Cananga odorata) and other Annonaceae (basal angiosperms) provide examples of irregular patterning. This pictorial report provides food for thought for scientists interested in phyllotaxis patterns beyond the usual spiral and whorled patterns. Emphasis is given on irregular phyllotaxes that occur in wild-type plants, mainly correlated with geometrical parameters such as leaf and stamen primordia that are very small as compared to the size of their apical meristems. They call for additional explanatory models, combining auxin-driven development with geometrical constraints and biophysical processes.

Keywords

irregular phyllotaxis; Acacia; Leguminosae – Mimosoideae; phyllodes; stipules; Cananga; Annonaceae; flower development

Introduction

This pictorial report gives emphasis on badly known or misunderstood phenomena in phyllotaxis of vascular plants using the author's own collection of scanning electron micrographs (most of them unpublished so far). Vegetative shoot apices of seven Acacia species (Leguminosae) from Australia will be presented, as well as floral development in Cananga odorata (ylang-ylang, Annonaceae). The unusual phyllotactic patterns presented in this report do not seem to be explained by developmental and genetic models, including the auxin-based phytohormone models [1,2].

What is due to self-organization and what may be due to genetic interaction? Irregular (chaotic, disrupted, aberrant) phyllotaxes in model organisms such as Arabidopsis and maize are often found as result of unbalanced phytohormone interactions in mutants [3,4]. What are the boundary conditions for the generation of specific phyllotactic patterns? Quantitative analyses emphasized size and shape of incipient leaf primordia as compared to the remaining shoot apical meristem (SAM) as boundary conditions for certain phyllotactic patterns [5–7]. The present paper gives emphasis

on irregular phyllotaxes that occur in wild-type plants, due to geometrical parameters such as leaf or stamen primordia that are very small as compared to the size of the SAM or floral apex [8–10].

There is no need to discuss shortcomings of the used morphological terminology in this paper again. If readers are interested in fuzziness of structural categories, continuum approaches, and complementary perspectives to describe plant architecture they are referred to earlier publications of the Sattler school [11–15].

Material and methods

The following species are studied (alphabetic order, for details of the used *Acacia* specimens see also [13,16]). Voucher specimens for each species are deposited at Z/ZT.

Eudicots – Fabidae – Fabales: Leguminosae – Mimosoideae

- *Acacia adoxa* Pedley. Specimen: T. Willing 160
- *Acacia amblygona* A. Cunn. ex Benth. Specimen: J. von Wartburg P29
- *Acacia baueri* Benth. subspecies *aspera* (Maiden & Betche) Pedley. Specimen: C. U. Kramer 9995
- *Acacia conferta* A.Cunn. ex Benth. Specimen: V. Boughton s.n. (April 25, 1985)
- *Acacia rossei* F. Muell. Specimen: J. von Wartburg P111
- *Acacia sphacelata* Benth. subsp. *verticillata* Maslin. Specimen: J. von Wartburg P6
- *Acacia verticillata* (L'Héritier) Willdenow. Specimen: A. Drinnan s.n. (September 15, 1985)

Basal angiosperms – Magnoliidae – Magnoliales: Annonaceae

- *Cananga odorata* (Lam.) Hook.f. & Thompson. Specimen: R. Rutishauser s.n. (September 17, 1988)

Results and discussion

From spiral to whorled and irregular (chaotic) phyllotaxes in Australian acacias (Leguminosae, subfamily Mimosoideae)

The large genus *Acacia* (Leguminosae – subfamily Mimosoideae) felt apart into few distinct genera due to molecular data [17]. The slightly more than 1000 species of mainly Australian "wattles" (former subgenus *Phyllodineae = Heterophyllum*) remained in the genus *Acacia* whereas most of the species outside Australia became members of other genera (especially *Senegalia*, *Vachellia*). Molecular data help to better understand the infrageneric *Acacia* classification although it is far from being completely resolved [18] (also B. Maslin and D. Murphy, personal communication, 2016).

Most Australian acacias are phyllodineous. They show heteroblasty with two leaf types, somewhat correlated with an absolute size increase of the SAM while the plant changes from the juvenile to its adult stage [19,20]. They start with compound (pinnate to bipinnate) leaves along the seedling axis but later they switch to undivided leaves, so-called phyllodes. These phyllodes are terete and needle-like, resembling leaf stalks (petioles) that lack the pinnae, or they are laterally flattened [19,21–23].

Most phyllodineous acacias reveal helical phyllotaxis along the seedling axis as well as in the adult stage (Fig. 1, Fig. 2). In this pictorial report, however, emphasis is given on those phyllodineous acacias that have spiral phyllotaxis only as long as compound seedling leaves are produced. With the onset of phyllode production they switch to
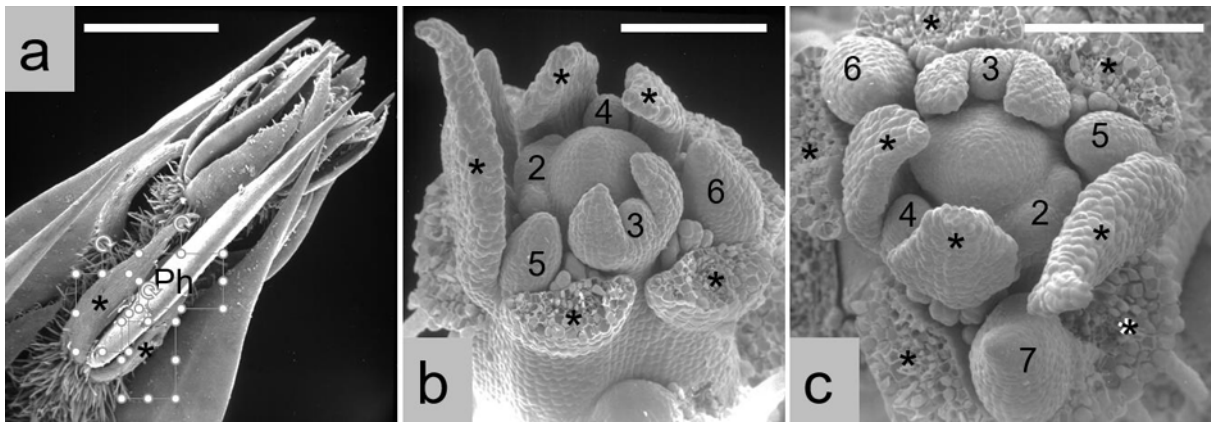
Fig. 1 *Acacia amblygona* (eudicots: Leguminosae – Mimosoideae). Vegetative shoot tips showing spiral phyllotaxis (Fibonacci), each phyllode is provided with two lateral stipules. Vegetative shoot tip before (**a**) and after removal of outer phyllodes (**b,c**). Each phyllode (Ph) is provided with two lateral stipules (*) that show precursive development: in early stages the stipules grow faster than their phyllodes (some stipules have been removed). In **b,c** the young phyllodes (2–7) are labeled according to the ontogenetic spiral as Fibonacci system. Scale bars: **a** 0.5 mm; **b,c** 150 μm.
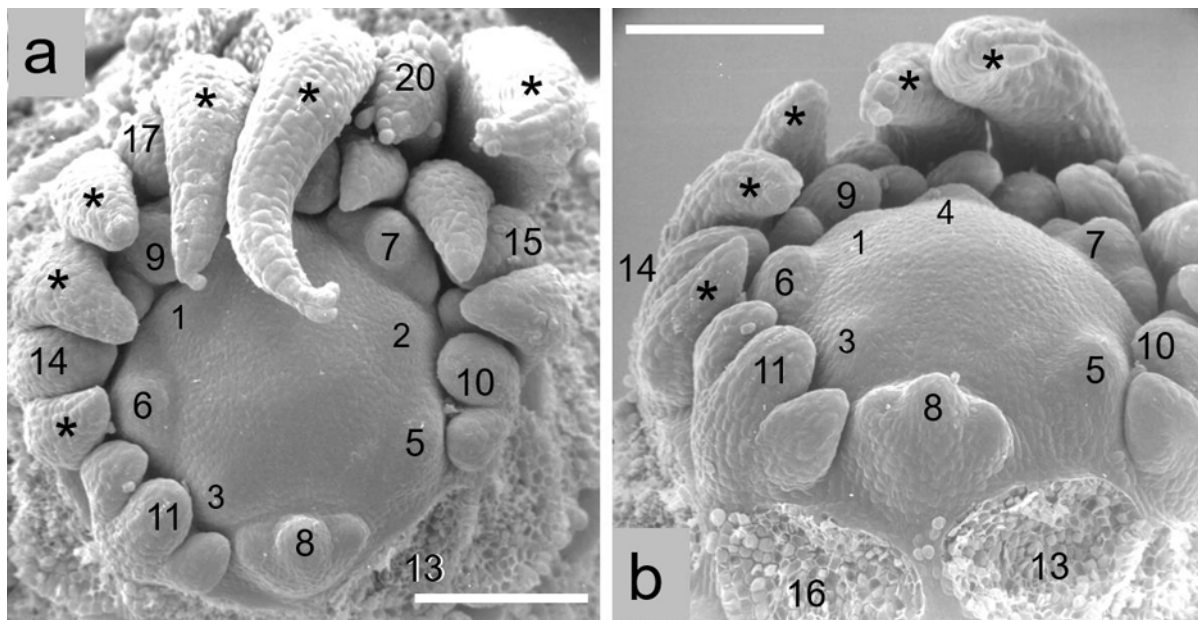

Fig. 2 *Acacia rossei* (eudicots: Leguminosae – Mimosoideae). Two views of vegetative shoot apical meristem (SAM) showing spiral phyllotaxis (Fibonacci), older phyllode primordia are provided with two lateral stipules (not yet visible in very young phyllode primordia). The phyllode primordia (1–11) are labeled according to the ontogenetic spiral as Fibonacci system. Scale bars: **a,b** 150 μm.

other kinds of phyllotactic patterns: either complex whorls or even irregular (i.e., somewhat chaotic) phyllotaxis. Five examples of "unusual phyllotaxes" will be described below (Fig. 3–Fig. 8). The author has published on various aspects of unusual phyllotaxes in acacias already before [11,13,16]. Stipules, as typical for Leguminosae, are present in the genus *Acacia*. Most often there are two lateral stipules per leaf or phyllode (see Fig. 37 in [14]). However, stipules in acacias with whorled or clustered phyllodes may appear as interpetiolar ones, with one stipule filling the gap between two adjacent phyllodes (leaves), similar to what is known in many Cunoniaceae and Rubiaceae.

Morphological descriptions and illustrations to all *Acacia* ("wattle") species are available online [24].

Regular spiral pattern of phyllodes provided with two lateral stipules as found in many acacias: e.g., *Acacia amblygona*, *Acacia rossei*. The phyllotaxes in *Acacia*

amblygona (Fig. 1a–c) and *A. rossei* (Fig. 2a,b) are the usual spiral (helical) patterns as observable in most phyllodineous acacias (wattles) from Australia: phyllotaxis in *A. amblygona* and *A. rossei* stays spiral (equalling a Fibonacci system) also when leaf morphology switches from compound seedling leaves to phyllodes. The phyllodes of *A. amblygona* are laterally flattened blades tapering into a sharp tip (Fig. 1a) whereas those of *A. rossei* are linear needles with a quadrangular cross-section. The leaf bases of both *A. amblygona* and *A. rossei* are provided with two lateral early-outgrowing appendages, called stipules (Fig. 1a–c, Fig. 2a,b). They show precursive development inside the bud, i.e., in early stages they grow faster than the phyllode to which they belong, but then they stop much earlier whereas the phyllodes continue to grow and become the main organs of photosynthesis. The stipules in *A. amblygona* end up as usually persistent rigid appendages accompanying the phyllode bases (Fig. 1a).

The shoot apical meristems (SAMs), as shown for *A. amblygona* (Fig. 1b,c) and *A. rossei* (Fig. 2a,b), have diameters of ca. 100 μm and ca. 190 μm, respectively. Thus, the relative size of the SAM as compared to the youngest phyllode primordia is small in *A. amblygona* whereas it is large in *A. rossei*. As a result of this relative size difference, young phyllodes in shoot tips of *A. amblygona* show a (2+3) contact parastichy pattern whereas young phyllodes in *A. rossei* contact each other according to a (5+8) parastichy pattern, similar to what is known from other acacias [19,20].

Complex whorls consisting of phyllodes and stipules, supplied by a girdling vascular bundle inside the stem node: *Acacia adoxa* (*Lycopodiifoliae* group). This species belongs to section *Lycopodiifoliae*, a species group which is characterized by more or less regular phyllode whorls [13,25]. It appears as monophyletic in molecular trees [18]. In *Acacia adoxa*, there are 9- to 10-merous phyllode whorls with about as many interpetiolar stipules filling the gaps in between.

WHORL DEVELOPMENT IN *ACACIA ADOXA*. A ring meristem with indistinct bumps is the first sign of a new whorl around the SAM (Fig. 3a,b). The arrangement of these bumps as early stages of phyllode primordia is not always regular, as shown in the next stage of whorl formation (Fig. 3c,d). Some bumps may be inserted higher up along the ring-like platform surrounding the SAM (Fig. 3e,f). Phyllodes of adjacent whorls often violate Hofmeister's rule, i.e., they may be superposed (Fig. 3e) rather than alternate [26]. The stipules arise as additional bumps in the next older whorls (i.e., whorl plastochrone 2), they appear as interpetiolar appendages outside and between the phyllode primordia (see asterisks in Fig. 3c,d). These outer set of whorl primordia (prospective stipules) accelerate their growth in length and soon overarch their associated phyllodes (Fig. 3c,f).

MERISTIC VARIATION OF THE WHORLS IN THE *LYCOPODIIFOLIAE* GROUP. In *Acacia adoxa*, there are 6- to 10-merous phyllode whorls whereas in other members of the *Lycopodiifoliae* group the whorls may consist of up to 27 phyllodes and as many interpetiolar stipules in between, e.g., 10–19 phyllodes per whorl in *A. hippuroides*, 15–27 ones in *A. longipedunculata* [11–13]. There are never less than five to nine phyllodes per whorl in the *Lycopodiifoliae* group, as observable in *A. galioides* [25]. Seedlings do not have complete whorls yet. After the two cotyledons, they start with compound seedling leaves, which are arranged along a helix, as shown in *A. longipedunculata* [11]. With the switch to phyllode morphology, phyllotaxis changes from spiral Fibonacci systems via incomplete whorls (i.e., "clusters") to the typical whorls as described above. For more information on "clusters" (fascicles), see paragraph below on *Acacia sphacelata* subsp. *verticillata* (Fig. 4a–c).

UNIQUE VASCULAR SUPPLY OF THE PHYLLODE WHORLS IN THE *LYCOPODIIFOLIAE* GROUP. The whorls of *Acacia adoxa* and other members of the *Lycopodiifoliae* group (e.g., *A. hippuroides*, *A. longipedunculata*) possess a unique vascular supply inside the stem nodes. The number of vascular traces (trunk bundles) leaving the stele is always lower than the number of phyllodes within a whorl. In other words, not all leaves (phyllodes) get their own vascular trace (bundle) from the stele. In *A. hippuroides* and *A. longipedunculata* there are four to eight trunk bundles per whorl. Branches of the trunk bundles form a girdling bundle (i.e., vascular ring) and supply all phyllodes and stipules of a single whorl [11–13]. In *Acacia adoxa*, there are four or five trunk bundles. A reduction of the number of vascular traces inside the stem nodes is not known from other species groups (sections) in *Acacia* although similar
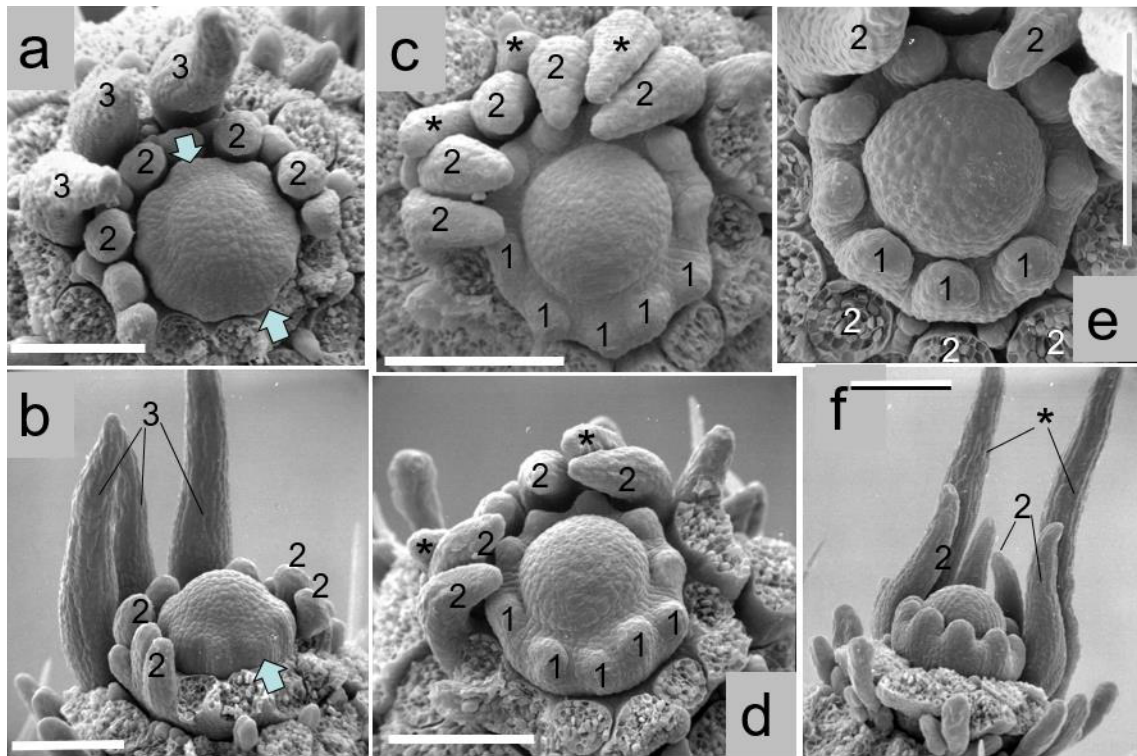
Fig. 3 *Acacia adoxa* (eudicots: Leguminosae – Mimosoideae). Vegetative SAM showing whorled phyllotaxis. Three consecutive developmental stages of phyllode whorl formation, as shown from above (top row) and from lateral side (bottom row). Most members of the outer phyllodes (indicated by numerals) and stipules (indicated by asterisks) have been removed. **a,b** Phyllode whorl development starts with ring meristem (arrows) that soon forms bumps as very early stages of phyllode primordia. The youngest whorl is surrounded by the phyllode primordia ("2") of the preceding whorl, with few stipule primordia (*) already initiated in the gaps between (behind) adjacent phyllodes. Members of the preceding older whorl ("3") have been removed except for few phyllodes. **c,d** The youngest whorl closest to the SAM consists of 10 bumps as early stage of a 10-merous phyllode whorl (associated stipule primordia not yet visible). The preceding whorl already consists of phyllodes ("2") and interpetiolar stipules (*) of about the same size. **e,f** The youngest whorl closest to the SAM is 9-merous composed of clearly distinguishable phyllode primordia ("1"). Most members of the preceding whorl have been removed except for a few phyllodes ("2") overarched by their associated stipules (*). Note the hemispherical SAM ready to form another whorl. Scale bars: **a–f** 100 μm.

phenomena (i.e., less traces than leaves) exist in other angiosperms with whorled phyllotaxis [13].

Irregular phyllotaxis: phyllode arrangement in clusters or without any obvious order in *Acacia sphacelata* subsp. *verticillata* and *Acacia conferta*. Unlike the phyllotactic patterns described above (Fig. 1–Fig. 3), the vegetative apices of the two species presented here do not reveal any kind of order, except for the acropetal (i.e., centripetal) direction of phyllode inception, with the phyllode primordia next to the SAM being younger than the adjacent ones positioned further away from the SAM (= "acropetal-initiation" rule, see below). Irregular or chaotic phyllotaxis patterns are somewhat difficult to be precisely defined. They do show neither spiral nor whorled arrangement of organs when studied along the elongated stem. In *A. sphacelata*, three subspecies can be distinguished with a variable tendency towards phyllode clusters and incomplete phyllode whorls, a situation already found along seedling axes of members of the *Lycopodiifoliae* group such as *A. longipedunculata* [16,24]. The phyllodes in *A. sphacelata* are scattered, vertillate, or subverticillate. Especially *A. sphacelata* subsp. *verticillata* has its phyllodes "in regular or sometimes irregular whorls, a few solitary or clustered (2 or 3 per node)", as mentioned by Maslin in [24]. In *Acacia conferta*, helical leaf arrangement is restricted to the seedling axis (Fig. 4A,B in [16]). The ontogenetic spiral starts to disappear completely with the switch from compound seedling leaves to phyllodes. Phyllode arrangement looks crowded and irregular (chaotic) along adult stem zones, i.e., phyllotaxis no longer has any predictable order (Fig. 4C,D in [16]). In *Acacia conferta*, only few phyllodes are subtending an axillary

bud (Fig. 6c). Occasionally, there are phyllodes that appear as twins, inserted next to each other and sharing one and the same axillary bud (Fig. 4E–G in [16]), or three or more phyllodes may form clusters (fascicles) or even oblique whorls [24,25].

INITIATION PATTERNS OF PHYLLODES AND STIPULES IN ACACIAS WITH IRREGULAR PHYLLOTAXES. *Acacia sphacelata* and *A. conferta* lack ordinary phyllotaxis patterns (i.e., spiral and whorled) when shoot tips with their phyllode-forming SAMs are studied. In *Acacia sphacelata*, the phyllode primordia next to the SAM appear as clusters, i.e., as common primordia for what finally becomes a group of phyllodes. The phyllode primordia around the SAM (Fig. 4a–c) do not reveal any regular order. The primordia on one side of the SAM appear to be clustered, perhaps as first stage of what finally turns out as an incomplete whorl along the mature stem. The stipular primordia (indicated by asterisks in Fig. 4a–c) again do not show any clear order. They arise as lateral or interpetiolar outgrowths and soon start to overarch the associated phyllode primordia as slightly flattened and curved scales. In the shoot tips analyzed for *Acacia conferta* (Fig. 5a–e, Fig. 6a–c) obvious common primordia (i.e., phyllodial clusters) are lacking. All the phyllodes arise as distinct primordia around the SAM. It is not possible to draw parastichies (i.e., curved lines) along adjacent phyllodes as possible hints for any kind of spiral phyllotaxis. Stipule primordia are lacking in the two shoot tips of *A. conferta*. They arise delayed and further down along the shoot tips. Stipules in *A. conferta* end up as inconspicuous and caducous appendages less than 1 mm long. Their presence along fully-grown twigs is not even mentioned in the species description provided by [24]. According to Goebel [27], there are various other Australian species (e.g., *Acacia juniperina*, *A. vomeriformis*) with phyllodes having very inconspicuous stipules. In *Acacia baueri* and *A. verticillata*, there are two types of phyllodes, one provided with prominent stipules and one lacking them completely (see below).

Shoots with two waves of phyllode inception, a first set initiated near the summit of the SAM in a spiral sequence, a second set intercalated lower down the periphery of the apex without obvious order: *Acacia baueri* subsp. *aspera* and *Acacia verticillata*. Biologists usually accept the "acropetal-initiation" rule for most phyllotactic patterns outside flowers: the youngest leaf primordia in a shoot tip are always found next to the SAM. The acropetal-initiation rule is valid for all *Acacia* species presented above (Fig. 1–Fig. 5). However, there are at least two exceptions known with the inception of additional leaves between (and below) a first set of already present leaf primordia: *Acacia baueri* and *A. verticillata*. Developmental aspects of both species were already presented by Rutishauser [13,16].

ACACIA BAUERI consists of two subspecies: *A. baueri* subsp. *baueri* (phyllodes usually in regular whorls) and *A. baueri* subsp. *aspera* (phyllodes usually scattered or in irregular whorls). In *A. baueri* (both subspecies), two types of phyllodes occur interspersed along the same twig, a situation that may be called anisophylly. The first set of phyllodes is provided with an axillary bud and usually two lateral stipules each. These primary, stipulate phyllodes (P-phyllodes) are inserted along a helix with divergence angles between 135–180° (Fig. 7c). *Acacia baueri* contains an additional "secondary" phyllode set that usually lacks both axillary buds as well as stipules. Therefore, these additional stem outgrowths are called "exstipulate" phyllodes (E-phyllodes). Except for stipules and axillary buds, both types of phyllodes look the same at maturity (including extrafloral nectaries) in *A. baueri*. All E-phyllodes are inserted at and between the levels of the first set of stipulate phyllodes. The E-phyllodes of *A. baueri* outnumber the P-phyllodes by a factor 3 to 9. For drawings of elongated stem portions showing the arrangement of both P-phyllodes and E-phyllodes in *Acacia baueri* subsp. *aspera* see Fig. 2A–F in [16].

PHYLLODE INITIATION IN SHOOT TIPS OF *ACACIA BAUERI* SUBSP. *ASPERA*. There are two waves of phyllode formation at the SAM: a first set of P-phyllodes (labeled as "1" to "3" in Fig. 7a–c), then a second set of E-phyllodes. The P-phyllodes arise as prominent hemispherical primordia earlier and higher up at the SAM, soon accompanied by two lateral stipules. The P-phyllodes show helical to (spiro-)distichous arrangement (Fig. 7c) [13,16]. E-phyllodes are initiated later and lower down at the periphery of the apex as slightly smaller primordia, i.e., at and between the insertion levels of the three first P-phyllodes (Fig. 7a–c).
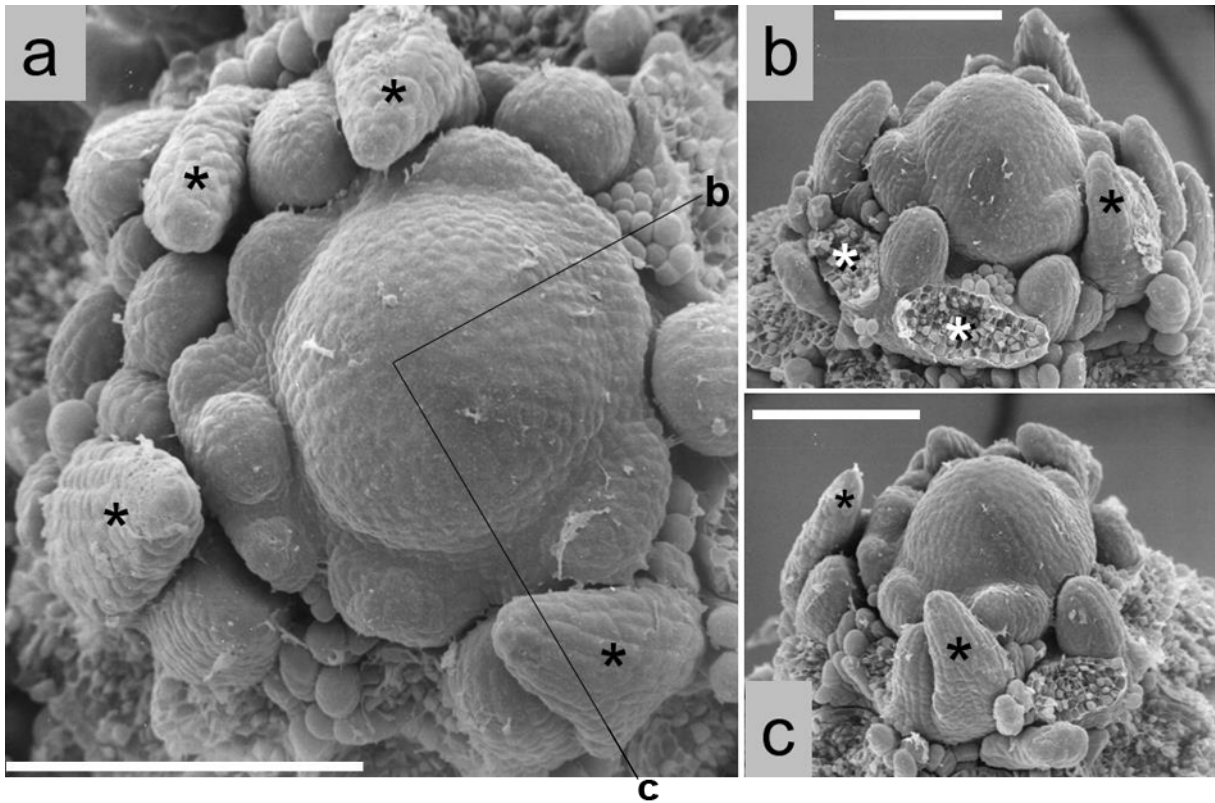
Fig. 4 *Acacia sphacelata* ssp. *verticillata* (eudicots: Leguminosae – Mimosoideae). Vegetative shoot tip, seen from above (**a**) and from two different lateral sides (**b,c**). The radii inserted in **a** indicate the positions of the lateral views. Vegetative SAM showing irregular phyllotaxis with clustered phyllode primordia. Neither spiral nor whorled pattern can be detected. Scale bars: **a–c** 150 μm.
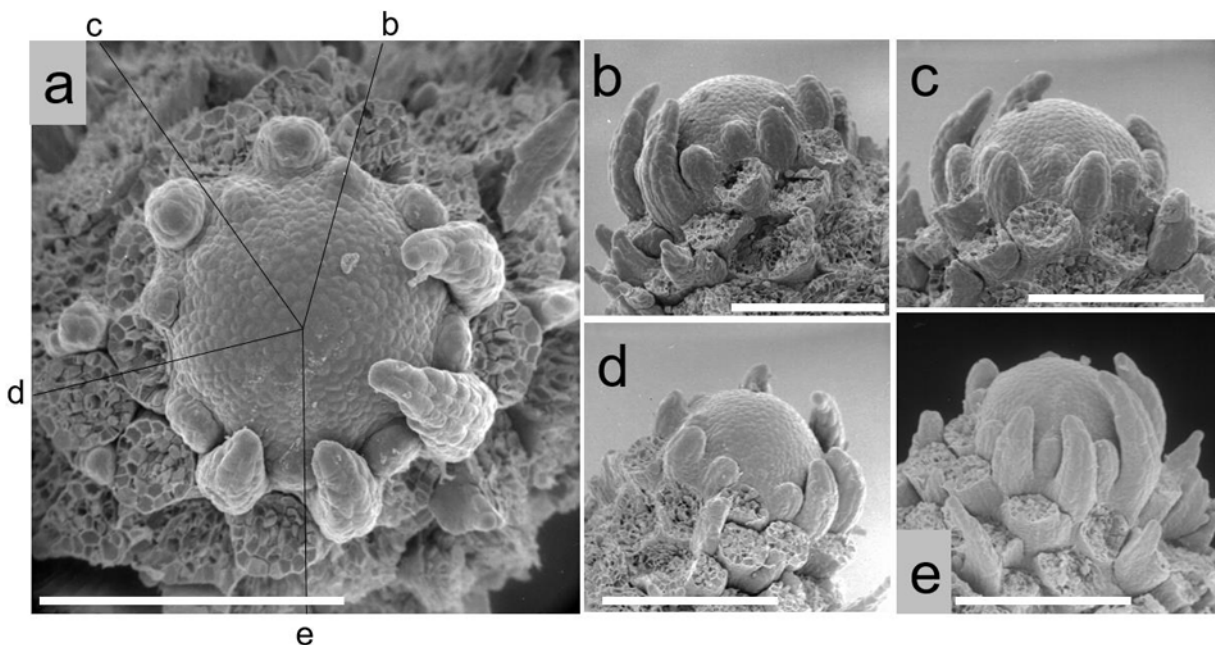

Fig. 5 *Acacia conferta* (eudicots: Leguminosae – Mimosoideae). Vegetative shoot tip, as seen from above (**a**) and from four different lateral sides (**b–e**). The radii inserted in **a** indicate the positions of the lateral views. Vegetative SAM showing irregular phyllotaxis. Neither spiral nor whorled pattern can be detected. The three youngermost primordia seem to be clustered near the radii **b** and **c**. Scale bars: **a–e** 150 μm.
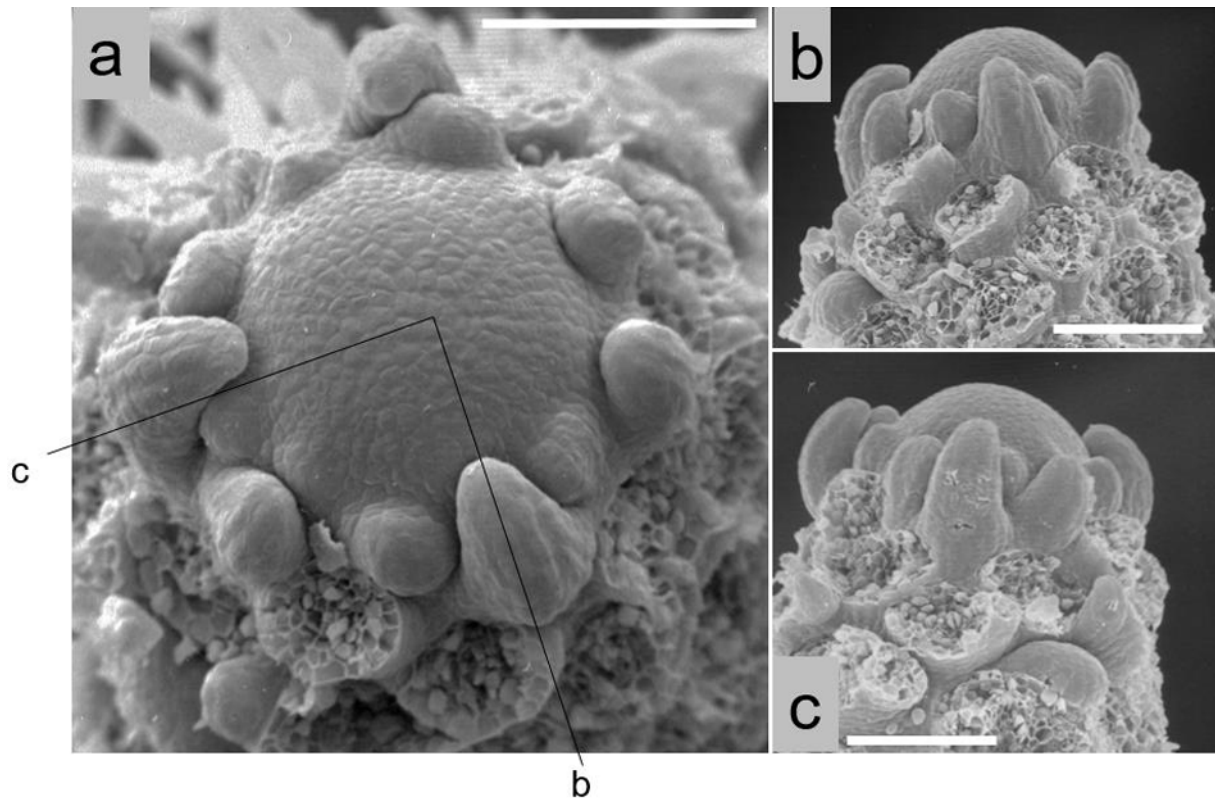
Fig. 6 *Acacia conferta* (eudicots: Leguminosae – Mimosoideae). Another vegetative shoot tip, as seen from above (**a**) and from two different lateral sides (**b,c**). The radii inserted in **a** indicate the positions of the lateral views. Neither spiral nor whorled pattern can be detected. This vegetative SAM again with irregular phyllotaxis. Note presence of an axillary bud in **c**. Scale bars: **a–c** 100 μm.
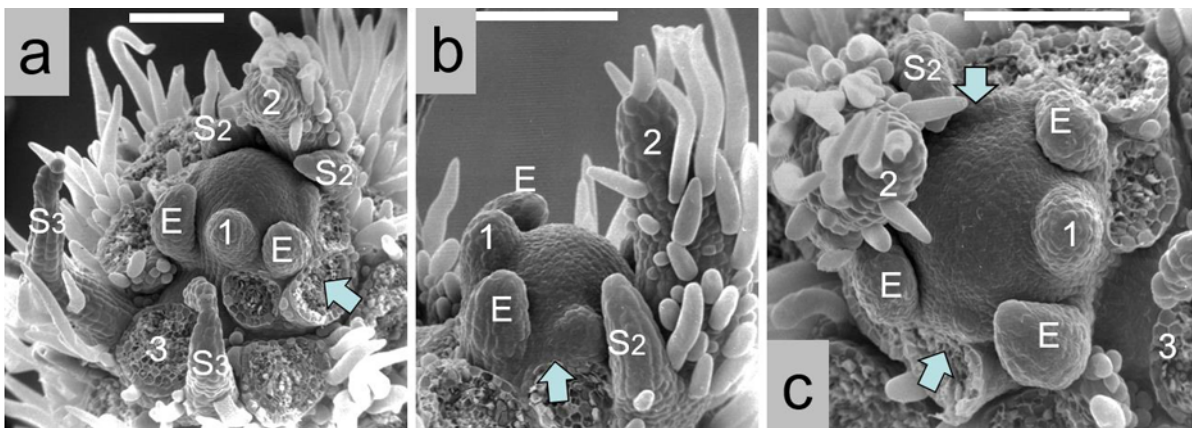

Fig. 7 *Acacia baueri* subsp. *aspera* (eudicots: Leguminosae – Mimosoideae). Vegetative shoot tip seen from three different sides after removal of older phyllodes and stipules. Two lateral views (**a,b**) and one top view (**c**). Vegetative SAM reveals intercalary inception of exstipulate phyllode primordia (E) after formation of first spiral set of stipulate P-phyllodes ("1" to "3"). They are soon provided with two lateral stipules each (S2, S3). A secondary set of E-phyllodes arises later and lower down on the flank of the SAM (E in **a–c**). They are devoid of stipules. Arrows point to youngest primordia of E-phyllodia. Scale bars: **a–c** 100 μm.

ACACIA VERTICILLATA. Similar phyllotaxis patterns with two types of phyllodes (i.e., E-phyllodes and P-phyllodes) as described above for *Acacia baueri* are also known from *Acacia verticillata*, as first described by Braun [28] and Hofmeister [29]. The phyllodes in *Acacia verticillata* are commonly whorled (with 6–12 phyllodes per whorl), but sometimes fascicled or scattered (Fig. 8a,b) [13,16,24]. There are 6–26 times as many E-phyllodes as P-phyllodes in *A. verticillata*. All P-phyllodes in this species tend to be arranged in a spiral Fibonacci pattern (with divergence angles near 137.5°), continuing the helical phyllotaxis of the (compound) seedling leaves of young plants [16,19,21]. Along elongated stem portions there may be 1–3 whorls consisting of E-phyllodes only between two consecutive whorls with a bud-subtending P-phyllode each, or all E-phyllodes tend to be irregularly scattered.

PHYLLODE INITIATION IN SHOOT TIPS OF *ACACIA VERTICILLATA*. The peculiar phyllotaxis pattern with the initiation of phyllodes in two waves at and below the proper SAM, as described above for *Acacia baueri*, also occurs in *Acacia verticillata* (Fig. 8a,b). The P-phyllodes (having stipules) are initiated as rather large primordia near the summit of the SAM. The E- phyllodes (lacking stipules) arise later as considerably smaller primordia lower down the periphery of the apex, i.e., in still meristematic regions below and between the already present P-phyllodes "1" to "4" (Fig. 8a,b). The P-phyllodes of *A. verticillata* usually approach the Fibonacci angle (137.5°) quite nicely. This is unlike *A. baueri*, where the divergence angles of the P-phyllodes vary considerably (often tending towards orthodistichy, see above). Additional vegetative SAMs of *Acacia verticillata* with phyllode inception in two waves are illustrated by [13,16,19,21,27].

VASCULAR SUPPLY OF PHYLLODES IN *ACACIA BAUERI* AND *A. VERTICILLATA*. Each phyllode in both species is supplied by its own trace(s) from the stele. The P-phyllodes are provided with three traces each because they also supply the two lateral stipules. The E-phyllodes have only one trace each, correlated with the lack of stipules [19,30].

MORPHOLOGICAL SIGNIFICANCE OF PHYLLODES IN *ACACIA BAUERI* AND *A. VERTICILLATA*. Since Braun [28] and Hofmeister [29], there is an ongoing debate about the morphological significance of the E-phyllodes as compared to the P-phyllodes in *Acacia verticillata* and *A. baueri*, as summarized in [13,21]. (Hypothesis 1) The perhaps best hypothesis accepts both E-phyllodes and P-phyllodes as leaf homologues. This view was already proposed by, e.g., Goebel [27]. The two types of phyllodes (i.e., leaves) differ only in their initiation time and growth rate next to the
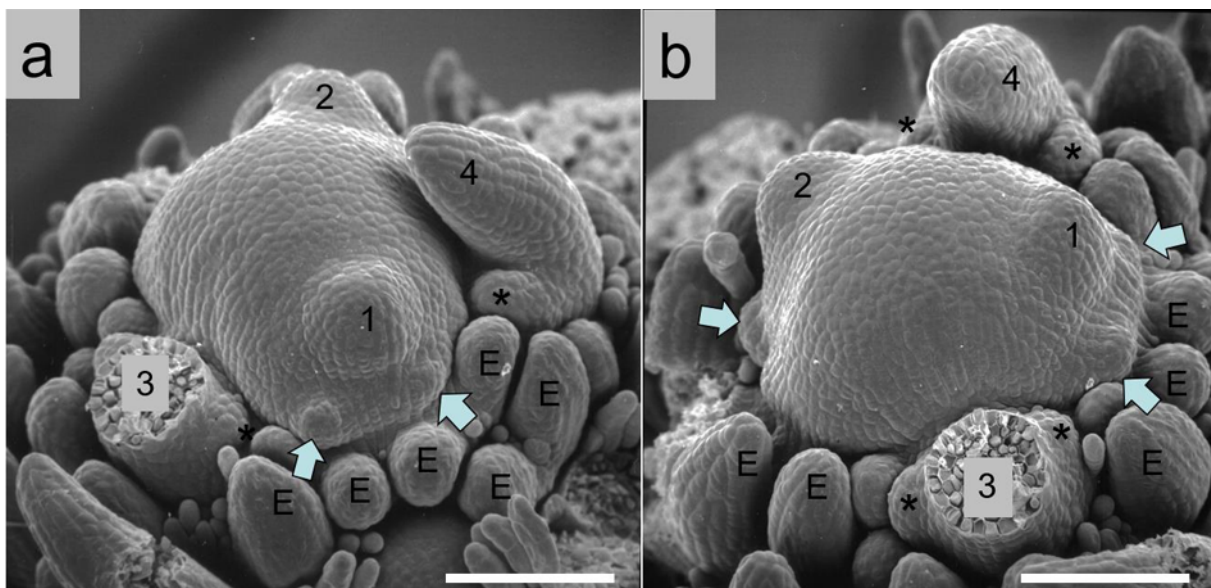

Fig. 8 *Acacia verticillata* (eudicots: Leguminosae – Mimosoideae). Same vegetative shoot tip seen from two different sides. Vegetative SAM reveals intercalary inception of secondary E-phyllode primordia (exstipulate) after formation of a spirally arranged first set of stipulate P-phyllodes ("1" to "4"). Arrows point to youngest primordia of E-phyllodia. Asterisks (*) indicate lateral stipules associated with phyllodes "3" and "4". Scale bars: **a,b** 100 µm.

SAM. (Hypothesis 2) The perhaps second-best hypothesis accepts the E-phyllodes as supernumerary stipules. They are positionally released from the P-phyllodes, which are considered as the only "true" leaves. This view was supported by Hofmeister [29] and Kaplan [21]. Both hypotheses may be seen as complementary, when we accept in eudicots a conceptual continuum from leaves (as major category of photosynthetic stem appendages) to stipules (as a somewhat subaltern category of stem appendages). The two hypotheses may even fuse into one when we accept stipule–leaf indistinction, i.e., fuzziness in the distinction of ("true") leaves and stipules in Leguminosae, as already proposed by Rutishauser et al. [15].

SHOOT APICES WITH TWO WAVES OF LEAF INITIATION MAY HAVE ARISEN TWICE AS EVOLUTIONARY NOVELTY IN AUSTRALIAN ACACIAS. *Acacia baueri* with its globular inflorescences is considered to belong to section *Phyllodineae* or section *Plurinerves* rather than section *Lycopodiifoliae* [24,25,31–33] (also Murphy, personal communication, 2016). Its position in molecular trees, however, is not yet resolved. *Acacia verticillata* belongs to section *Juliflorae*, due to the presence of spicate rather than globular inflorescences [25,31,32]. However, the position of *A. verticillata* in molecular *Acacia* trees is unresolved because it appears only distantly related to other *Juliflorae* members [18] (Maslin, personal communication, 2016). Until better molecular trees are available for *Acacia*, we can only suppose that the strange phyllotaxis type as found in *Acacia baueri* and *A. verticillata* has arisen twice as evolutionary novelty in different groups of Australian wattles. Similar phyllotaxes with two waves of leaf initiation are also known from other Australian acacias such as *Acacia riceana*, a close relative of *A. verticillata*. There are additional *Acacia* species showing axillary buds restricted to (few) stipulate phyllodes, e.g., *Acacia gordonii*, syn. *A. brunioides* ssp. *gordonii* [22].

SHOOT APICES WITH TWO WAVES OF LEAF INITIATION ARE ALSO KNOWN FROM OTHER ANGIOSPERMS. Besides acacias, there are a few other angiosperms with two kinds of leaves interspersed along one and the same shoot axis. For example, *Inversodicraea* (syn. *Ledermanniella – Phyllosoma*) is a genus (ca. 25 spp.) in African riverweeds (Podostemaceae). Its stems are provided with many scale-like leaves (called *feuillettes* in French) which are inserted between the much larger compound leaves. The scale-like leaves outnumber the compound leaves considerably. Only the compound leaves show helical or distichous phyllotaxis whereas the additional *feuillettes* are scattered irregularly (see [34] and literature cited therein). Other examples of angiosperms with two waves of leaf initiation along the same shoot axis are found in *Gunnera tinctoria* and allies (Gunneraceae within eudicots), and *Hydrothrix gardneri* (Pontederiaceae within monocots) [13].

Floral phyllotaxes in *Cananga odorata* and other polyandrous Annonaceae (chirimoya family): whorled and irregular patterns

Annonaceae (123 genera, 2100 spp.) is the largest family of the order Magnoliales within basal angiosperms. Most of the annonaceous members are characterized by a perianth of three trimerous whorls, an androecium of many stamens, and a gynoecium of few to many usually free carpels [35]. The trimerous outer perianth whorl in adult flowers is often labeled as calyx because its members ("sepals") are smaller and less conspicuous than the six members ("petals") of the corolla-like middle and inner perianth whorls. In *Cananga odorata*, the 2×3 petals are yellow ribbons (length up to 8 cm). *Cananga* flowers contain more than 200 stamens which form a firmly packed hemispherical androecium, showing the tips of up to 15 free carpels in its center [35].

Floral development in *Cananga odorata*. After perianth initiation, the floral apex (diameter ca. 450 µm) has a pronounced hexagonal shape, with the sides bordering the three inner petals (Pi) about double as long as those bordering the three outer petals (Po). Stamen inception starts in the six corners (gaps) between the insertion areas of the six petals (Fig. 9a,b). Stamen initiation continues centripetally (acropetally) in these six sectors until the peripheral zone of the floral apex is completely occupied by stamen primordia (Fig. 9c,d). After the formation of more than 200 stamens in
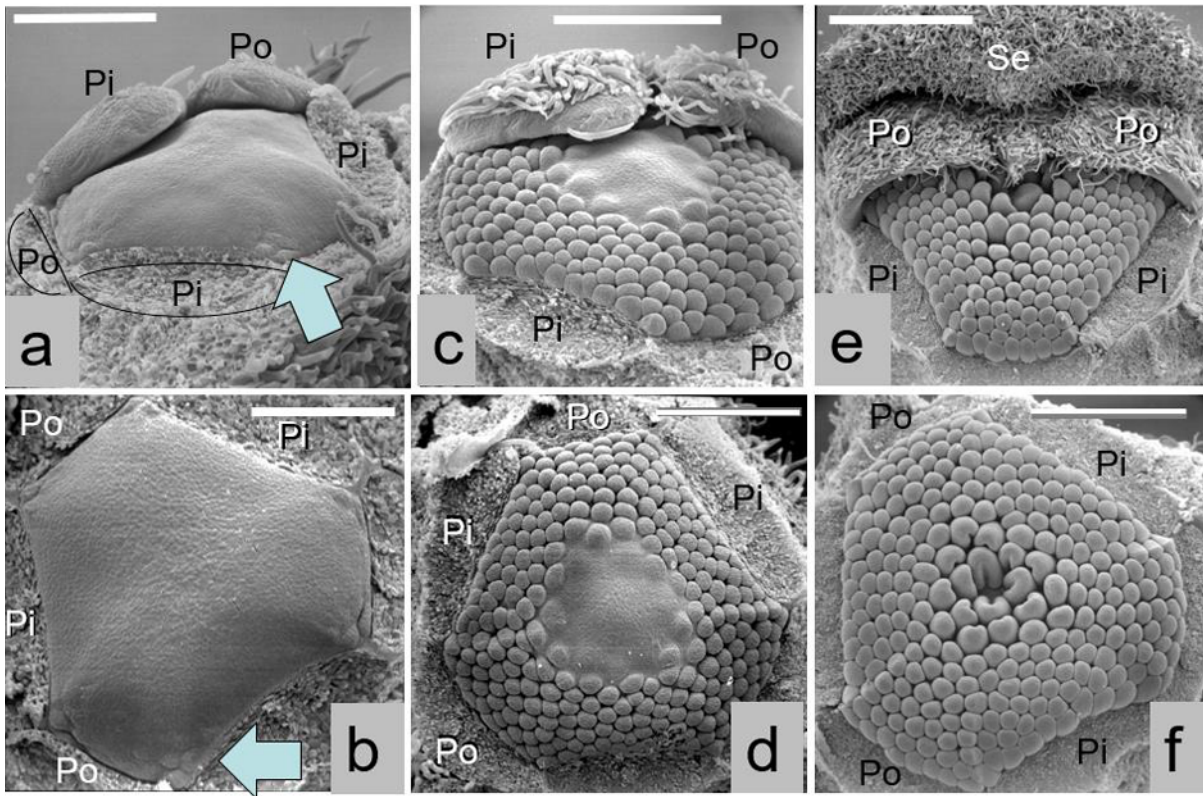
Fig. 9 *Cananga odorata* (basal angiosperms: Magnoliales – Annonaceae). Floral SAM showing irregular inception of a high number of stamen and carpel primordia. Three consecutive developmental patterns of flower buds are shown from the side (top row) and from above (bottom row). Most members of the three trimerous perianth whorls removed (Pi – inner petals; Po – outer petals; Se – sepal). **a,b** Young developmental stage after formation of trimerous calyx (removed) and hexamerous corolla (removed except two). Note hexagonal shape of floral apex, starting with stamen inception in the edges (arrow). **c,d** Developmental stage after initiation of most of the more than 200 stamens, probably in transition to the inception of free carpels. **e,f** Final stage after initiation of all stamens and ca. 14 free carpel primordia (the latter provided with ventral cleft each). Both stamens and carpels with irregular arrangement. Scale bars: **a,b** 200 μm; **c,d** 300 μm; **e,f** 0.5 mm.

Cananga flowers, there is a continuous transition to the inception of several (ca. 15) free carpels in the center of the floral apex (Fig. 9e,f). The stamens of young *Cananga* flowers obviously lack a spiral initiation pattern (Fig. 9d). Thus, this stamen arrangement may be called irregular or chaotic. The same seems to be true also for the carpels (Fig. 9f).

Geometrical constraints favoring irregular floral phyllotaxis in *Cananga* and other Annonaceae. Polyandrous species of *Annona*, *Artabotrys*, *Monodora*, and *Polyalthia* show a floral development similar to *Cananga*: the floral apex after perianth inception has a hexagonal shape and the first six stamens appear in the corners, i.e., alternating with the seemingly hexamerous corolla. It is obvious that the irregular (non-spiral) stamen arrangement of these polyandrous Annonaceae happens inside a whorled perianth that does not allow the floral apex to be circular in outline. For example, in flower buds of *Annona montana* ca. 2000 stamens are initiated on the enlarging floral apex after the inception of the first androecial organs in the six corners [35,36]. Leins and Erbar [35] described stamen initiation in this species as "acropetal, but somewhat chaotic". Irregular stamen and carpel inception appears to be the rule rather than the exception in Annonaceae (Magnoliales). According to Endress and Armstrong [10], "no single documented case of regular spiral floral phyllotaxis is known in the family" although Eupomatiaceae as sister family of Annonaceae (within Magnoliales) have spiral phyllotaxis [37].

Irregular polymerous androecia in Annonaceae are derived phylogenetically from whorled oligomerous androecia. A completely whorled phyllotaxis pattern with several hexamerous androecial whorls is found in the flowers of *Anaxagorea* (especially

A. crassipetala), which is known as basalmost genus in Annonaceae [10]. Inside the three trimerous perianth whorls there are 10 alternating hexamerous whorls: seven androecial ones consist of stamens or staminodes; the innermost three whorls are carpels. Thus, whorled phyllotaxis seems to be the basal (plesiomorphic) character state in Annonaceae whereas in more derived Annonaceae with numerous stamens and carpels (including *Cananga* and other genera listed above) phyllotaxis becomes irregular in androecium and gynoecium, but never spiral. In *Monanthotaxis* (syn. *Popowia*), another annonaceous genus with a clearly whorled androecium, the floral apex has a hexagonal shape prior to androecium development. It starts with the formation of six "corner" primordia and ends up with a total of 18 or 24 stamens/staminodes [38–40].

Floral phyllotaxis in other basal angiosperms beyond Annonaceae, especially polyandrous Magnoliales and Laurales (Magnoliidae). Annonaceae belong to the order Magnoliales within the basal angiosperms (magnoliids). There is a considerable variability of floral organ patterns in this group. The evolution of phyllotactic patterns in polyandrous Magnoliales flowers seems to be somewhat de-coupled [9]. In Magnoliaceae and Annonaceae, the perianth is basically whorled. While the magnoliaceous androecia are usually spiral, the annonaceous ones often turn out as nonspiral, or even irregular (chaotic). *Magnolia* species with polymerous androecia and gynoecia may show a considerable variability in mainly spiral phyllotaxis patterns [41–43].

In Laurales (another magnoliid order), the basal state of floral phyllotaxis is spiral. Calycanthaceae as sister to all other Laurales, have spiral flowers consistently [9,44,45]. Irregular (chaotic) phyllotaxis, however, is found in the pluricarpellate female flowers of Monimiaceae (Laurales) with, e.g., *Tambourissa ficus* containing up to ca. 2000 carpels per flower [45]. While pointing to *Tambourissa* and other somewhat chaotic floral phyllotaxes in Laurales, Staedler and Endress [44] guessed: "These irregularities may be due to the fact that at the time of initiation, the floral apex is large in size compared to primordium size, and that organ identity would depend more on the immediate sectorial neighborhood than the position on the ontogenetic spiral in spiral flowers…". Their conclusion seems to fit also for *Cananga* (Fig. 9) and other polyandrous Annonaceae, starting stamen initiation in the six corners of a rather large hexagonal floral apex! Summarizing various studies in polyandrous angiosperms Endress and Doyle [9: p. 54] concluded: "Chaotic patterns tend to occur when floral organs are numerous and their primordia are small". Or (in other words), as mentioned by Endress and Armstrong [10: p. 842]: "The more numerous the stamens (& carpels) become, the smaller are their primordia with respect to the floral apex and therefore they become more prone to positional irregularities. Thus, the clear phyllotaxis pattern decays.".

Irregular ("chaotic") stamen initiation occurs also in polyandrous angiosperms outside Magnoliales: e.g., *Zygogynum* (syn. *Exospermum*) *stipitatum* (Winteraceae, Winterales) and *Achlys triphylla* (Berberidaceae, Ranunculales), the latter with unequal size of the 3–12 stamen primordia per flower [46,47].

Conclusion and outlook

This pictorial report provides food for thought for scientists interested in phyllotaxis patterns beyond the usual spiral and whorled patterns. The present paper gives emphasis on irregular phyllotaxes that occur in wild-type plants, mainly correlated with geometrical parameters such as leaf and stamen primordia that are very small as compared to the size of their apical meristems.

Two factors (space constraints) may considerably force an apical meristem (vegetative SAM, floral apex) to get rid of any order, i.e., becoming irregular or chaotic:

- Non-circular shape of apical meristem prior to inception of a multitude of organs: e.g., hexagonal shape of floral apices in Annonaceae prior to initiation of polymerous androecia.

- Absolute or relative size of leaf primordia as compared to the size of apical meristems: the smaller their leaf primordia the higher becomes the probability that irregular patterns occur in addition to Fibonacci-type spiral patterns and whorled phyllotaxes.

More quantitative developmental studies need to be done in order to figure out if abrupt changes in primordial size and the switch of organ identity (e.g., from compound leaves to phyllodes in Australian acacias, from petals to stamens in Annonaceae flowers) are additional factors involved in the origin of irregular patterning.

Irregular (chaotic, disrupted, aberrant) phyllotaxes in model organisms such as *Arabidopsis* and maize often result from unbalanced phytohormone interactions in mutants [3,4,48]. Developmental geneticists, biophysicists, and specialists in computer simulation already started to better understand the transition from regular to irregular phyllotactic patterns in model organisms combining molecular (e.g., auxin, cytokinin) drivers with biophysical processes [2–4,48–50]. There is some hope that they will soon explain the various kinds of irregular patterning in shoot tips of Australian acacias and in floral apices of basal angiosperms!

The morphogenetic region of a shoot apical meristem (SAM) is often regarded as a fairly narrow annular zone ("active ring") [2,49]. In *Acacia baueri* and *A. verticillata*, however, "the leaf-forming area of the apical meristem looks like a star (or octopus) with morphogenetic arms as residual meristems that initiate secondary leaves between and below already present primary leaves." [13].

References

1. Reinhardt D, Pesce ER, Stieger P, Mandel T, Baltensperger K, Bennett M, et al. Regulation of phyllotaxis by polar auxin transport. Nature. 2003;426:255–260. https://doi.org/10.1038/nature02081

2. Smith RS, Kuhlemeier C, Prusinkiewicz P. Inhibition fields for phyllotactic pattern formation: a simulation study. Can J Bot. 2006;84:1635–1649. https://doi.org/10.1139/b06-133

3. Lee B, Johnston R, Yang Y, Gallavotti A, Kojima M, Travençolo BAN, et al. Studies of *aberrant phyllotaxy1* mutants of maize indicate complex interactions between auxin and cytokinin signaling in the shoot apical meristem. Plant Physiol. 2009;150:205–216. https://doi.org/10.1104/pp.109.137034

4. Bainbridge K, Guyomarc'h S, Bayer E, Swarup R, Bennett M, Mandel T, et al. Auxin influx carriers stabilize phyllotactic patterning. Genes Dev. 2008;22:810–823. https://doi.org/10.1101/gad.462608

5. Richards FJ. Phyllotaxis: its quantitative expression and relation to growth

in the apex. Philos Trans R Soc Lond B Biol Sci. 1951;235:509–563. https://doi.org/10.1098/rstb.1951.0007

6. Williams RF. The shoot apex and leaf growth: a study in quantitative biology. Cambridge: Cambridge University Press; 1975. https://doi.org/10.1017/CBO9780511753404

7. Rutishauser R. Plastochrone ratio and leaf arc as parameters of a quantitative phyllotaxis analysis in vascular plants. In: Jean RV, Barabé D, editors. Symmetry in plants. Singapore: World Scientific Press; 1998. p. 171–212. https://doi.org/10.1142/9789814261074_0008

8. Endress PK. Angiosperm floral evolution: morphological developmental framework. In: Soltis DE, Leebens-Mack JH, Soltis PS, editors. Developmental genetics of the flower. Amsterdam: Elsevier; 2006. p. 2–62. (Advances in Botanical Research; vol 44). https://doi.org/10.1016/s0065-2296(06)44001-5

9. Endress PK, Doyle JA. Floral phyllotaxis in basal angiosperms: development and evolution. Curr Opin Plant Biol. 2007;10:52–57. https://doi.org/10.1016/j.pbi.2006.11.007

10. Endress PK, Armstrong JE. Floral development and floral phyllotaxis in *Anaxagorea* (Annonaceae). Ann Bot. 2011;108:835–845. https://doi.org/10.1093/aob/mcr201

11. Rutishauser R, Sattler R. Architecture and development of the phyllode-stipule whorls in *Acacia longipedunculata*: controversial interpretations and continuum approach. Can J Bot. 1986;64:1987–2019. https://doi.org/10.1139/b86-263

12. Sattler R, Luckert D, Rutishauser R. Symmetry in plants: phyllode and stipule development in *Acacia longipedunculata*. Can J Bot. 1988;66:1270–1284. https://doi.org/10.1139/b88-182

13. Rutishauser R. Polymerous leaf whorls in vascular plants: developmental morphology and fuzziness or organ identities. Int J Plant Sci. 1999;160(6 suppl):S81–S103. https://doi.org/10.1086/314221

14. Rutishauser R, Isler B. Fuzzy Arberian morphology: *Utricularia*, developmental mosaics, partial shoot hypothesis of the leaf and other FAMous ideas of Agnes Arber (1879–1960) on vascular plant bauplans. Ann Bot. 2001;88:1173–1202. https://doi.org/10.1006/anbo.2001.1498

15. Rutishauser R, Grob V, Pfeifer E. Plants are used to having identity crises. In: Minelli A, Fusco G, editors. Evolving pathways: key themes in evolutionary developmental biology. Cambridge: Cambridge University Press; 2008. p. 194–213. https://doi.org/10.1017/CBO9780511541582.015

16. Rutishauser R. Phyllotactic patterns in phyllodinous acacias (*Acacia* subg. *Heterophyllum*): promising aspects for systematics. Bulletin of the International Group for the Study of Mimosoideae. 1986;14:77–108.

17. Maslin BR. Synoptic overview of *Acacia* sensu lato (Leguminosae: Mimosoideae) in East and Southeast Asia. Gardens' Bulletin Singapore. 2015;67:231–250. https://doi.org/10.3850/S2382581215000186

18. Murphy DJ, Brown GK, Miller JT, Ladiges PY. Molecular phylogeny of *Acacia* Mill. (Mimosoideae: Leguminosae): evidence for major clades and informal classification. Taxon. 2010;59(1):7–19.

19. Kaplan DR. Heteroblastic leaf development in *Acacia*: morphological and morphogenetic implications. Cellule. 1980;73:135–203.

20. Hatt C, Mankessi F, Durand JB, Boudon F, Montes F, Lartaud M, et al. Characteristics of *Acacia mangium* shoot apical meristems in natural and in vitro conditions in relation to heteroblasty. Trees. 2012;26(3):1031–1044. https://doi.org/10.1007/s00468-012-0680-0

21. Kaplan DR. The concept of homology and its central role in the elucidation of plant systematic relationships. In: Duncan T, Stuessy TF, editors. Cladistics: perspectives on the reconstruction of evolutionary history. New York, NY: Columbia University Press; 1984. p. 51–70.

22. Simmons M. Acacias of Australia. Melbourne: Nelson; 1981.

23. Gardner S, Drinnan A, Newbigin E, Ladiges P. Leaf ontogeny and morphology in *Acacia* Mill. Muelleria. 2008;26:43–50.

24. World Wide Wattle. Species gallery [Internet]. 2016 [cited 2016 Dec 26]. Available from: http://worldwidewattle.com/speciesgallery

25. Pedley L. A revision of *Acacia lycopodiifolia* A. Cunn. ex Hook. and its allies. Contributions from the Queensland Herbarium. 1972;11:1–23.

26. Kirchoff BK. Shape matters: Hofmeister's rule, primordium shape, and flower orientation. Int J Plant Sci. 2003;164(4):505–517. https://doi.org/10.1086/375421

27. Goebel K. Organographie der Pflanzen, insbesondere der Archegoniaten und Samenpflanzen. Vol. 1. 3rd ed. Jena: Fischer; 1928.

28. Braun A. Vergleichende Untersuchung über die Ordnung der Schuppen an den Tannenzapfen. Nova acta Academiae Caesareae Leopoldino-Carolinae. 1831;15:195–402. https://doi.org/10.5962/bhl.title.69200

29. Hofmeister W. Allgemeine Morphologie der Gewächse. Leipzig: W. Engelmann; 1868.

30. Dormer KJ. Some examples of correlation between stipules and lateral leaf traces. New Phytol. 1944;43:151–153. https://doi.org/10.1111/j.1469-8137.1944.tb05010.x

31. Pedley L. A revision of *Acacia* Mill. in Queensland. Austrobaileya. 1978;1(2):75–234.

32. Pedley L. A revision of *Acacia* Mill. in Queensland. Austrobaileya 1979;1(3):235–337.

33. Mishler BD, Knerr N, González-Orozco CE, Thornhill AH, Laffan SW, Miller JT. Phylogenetic measures of biodiversity and neo- and paleo-endemism in Australian *Acacia*. Nature Commun. 2014;5:4473. https://doi.org/10.1038/ncomms5473

34. Thiv M, Ghogue JP, Grob V, Huber K, Pfeifer E, Rutishauser R. How to get off the mismatch at the generic rank in African Podostemaceae? Plant Syst Evol. 2009;283:57–77. https://doi.org/10.1007/s00606-009-0214-4

35. Leins P, Erbar C. Early floral developmental studies in Annonaceae. Biosystematics and Ecology Series. 1996;10:1–27.

36. Erbar C, Leins P. Flowers in Magnoliidae and the origin of flowers in other subclasses of the angiosperms. I. The relationships between flowers of Magnoliidae and Alismatidae. Plant Syst Evol. 1994;8(suppl):193–208. https://doi.org/10.1007/978-3-7091-6910-0_12

37. Endress PK. The flowers in extant basal angiosperms and inferences on ancestral flowers. Int J Plant Sci. 2001;162(5):1111–1140. https://doi.org/10.1086/321919

38. Ronse Decraene LP, Smets E. The floral development of *Popowia whitei* (Annonaceae). Nord J Bot. 1990;10:411–420. https://doi.org/10.1111/j.1756-1051.1990.tb01781.x

39. Ronse Decraene LP, Smets E. Correction. Nord J Bot. 1991;11:420. https://doi.org/10.1111/j.1756-1051.1991.tb01238.x

40. Ronse Decraene LP, Smets E. The distribution and systematic relevance of the androecial character polymery. Bot J Linn Soc. 1993;113:285–350. https://doi.org/10.1111/j.1095-8339.1993.tb00341.x

41. Zagórska-Marek B. Phyllotaxic diversity in *Magnolia* flowers. Acta Soc Bot Pol. 1994;63:117–137. https://doi.org/10.5586/asbp.1994.017

42. Zagórska-Marek B, Szpak M. Virtual phyllotaxis and real plant model cases. Funct Plant Biol. 2008;35(10):1025–1033. https://doi.org/10.1071/FP08076

43. Wiss D, Zagórska-Marek B. Geometric parameters of the apical meristem and the quality of phyllotactic patterns in *Magnolia* flowers. Acta Soc Bot Pol. 2012;81:203–216. https://doi.org/10.5586/asbp.2012.029

44. Staedler YM, Endress PK. Diversity and lability of floral phyllotaxis in the pluricarpellate families of core Laurales (Gomortegaceae, Atherospermataceae, Siparunaceae, Monimiaceae). Int J Plant Sci. 2009;170:522–550. https://doi.org/10.1086/597272

45. Staedler YM, Weston PH, Endress PK. Floral phyllotaxis and floral architecture in Calycanthaceae. Int J Plant Sci. 2007;168(3):285–306. https://doi.org/10.1086/510417

46. Endress PK. Floral phyllotaxis and floral evolution. Botanische Jahrbücher für Systematik. 1987;108:417–438.

47. Endress PK. Chaotic floral phyllotaxis and reduced perianth in *Achlys* (Berberidaceae). Bot Acta. 1989;102:159–163. https://doi.org/10.1111/j.1438-8677.1989.tb00085.x

48. Besnard F, Refahi Y, Morin V, Marteaux B, Brunoud G, Chambrier P. et al. Cytokinin signalling inhibitory fields provide robustness to phyllotaxis. Nature. 2014;505:417–421. https://doi.org/10.1038/nature12791

49. Newell AC, Shipman PD, Sun Z. Phyllotaxis as an example of the symbiosis of mechanical forces and biochemical processes in living tissue. Plant Signal Behav. 2008;3(8):586–589. https://doi.org/10.4161/psb.3.8.6223

50. Runions A, Smith RS, Prusinkiewicz P. Computational models of auxin-driven development [Internet]. 2014 [cited 2016 Dec 26]. Available from: http://algorithmicbotany.org/papers/auxin.ARPD2014.pdf