

Early flower and inflorescence development in *Dioscorea tokoro* (Dioscoreales): shoot chirality, handedness of cincinni and common tepal-stamen primordia

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Summary: Inflorescence and early flower development in the East Asian *Dioscorea tokoro* were investigated using scanning electron microscopy (SEM). The synflorescence is typically a raceme of open thyrses. Lateral units of thyrses are cincinni, which in female plants are often replaced by single flowers with a bracteole. Phyllotaxy of thyrse axis follows the Fibonacci pattern. There is a correlation between clockwise or anticlockwise direction of phyllotaxy along the thyrse axis and handedness of lateral cincinni. Two types of this correlation are theoretically possible, and both have been recorded in different angiosperms. Flower orientation in *Dioscorea* is the same as in many other monocots that possess a bracteole, i.e. an outer whorl tepal is inserted opposite the bracteole and an inner whorl tepal is inserted on the same radius as the bracteole. The outer tepal opposite the bracteole is the first floral organ to initiate. In contrast to many other monocots that possess a bracteole, initiation of inner whorl tepals is synchronous in *Dioscorea*. This phenomenon is probably linked with the occurrence of common inner tepal-inner stamen primordia, delayed receptacle expansion and retarded carpel initiation. Common tepal-stamen primordia are reported for the first time for a member of Dioscoreales. Our data support the view that presence/absence of common primordia is homoplastic in lilioid monocots and correlates with timing of receptacle expansion.

Keywords: bract, bracteole, cincinnus, chirality, common primordia, Dioscoreaceae, flower, handedness, inflorescence, monocots, Nartheciaceae, phyllotaxy, scanning electron microscopy

Most recent molecular phylogenetic studies revealed Dioscoreales and Pandanales as two sister clades of monocots (summarized in APG III 2009; see DAVIS et al. 2004 for alternative results). Despite close relationships between these two groups, patterns of flower diversity differ considerably among Pandanales and Dioscoreales (e.g., RUDALL & BATEMAN 2006; RONSE DE CRAENE 2010; REMIZOWA et al. 2010). Flowers of Pandanales are extremely diverse in terms of number and relative arrangement of tepals, stamens and carpels. Among Pandanales, trimerous pentacyclic flowers (that are so typical for monocots in general) are only found in some Velloziaceae. In contrast, flowers of Dioscoreales follow the typical monocot trimerous pentacyclic groundplan, though sometimes the outer or the inner stamen whorl is missing (e.g., CADDICK et al. 2000). While the number and relative position of organs are conserved in Dioscoreales, the most important evolutionary trend is towards increased synorganization of parts and functional specialization of flowers to particular pollination modes (e.g., ENDRESS 1995; CADDICK et al. 2000). There is no obvious explanation of the strongly different patterns of floral diversity in the two orders. Each order comprises a wide range of plants with different ecological adaptations and different pollination biology. A detailed comparative analysis of flower development in Dioscoreales and Pandanales is clearly an important goal, but developmental data are still fragmentary for several important members of each order. This is especially true

for observations of earliest stages of flower development, which are of particular interest for our understanding of the evolution of flower groundplan in this group.

According to molecular phylogenetic data, the family Nartheciaceae is sister to the rest of Dioscoreales (e.g., CADDICK et al. 2002). Flower development has been studied in two genera of Nartheciaceae, *Narthecium* and *Metanarthecium* (REMIZOWA et al. 2006a, b, 2008; REMIZOWA 2008). Flowers of Nartheciaceae are morphologically less specialized than those of most other Dioscoreales. CADDICK et al. (2000) provided a detailed and extensively illustrated account of flower morphology and development in members of Dioscoreales other than Nartheciaceae. This is apparently the only publication where scanning electron microscopy (SEM) was extensively used, though scattered earlier light microscopy observations are also available. For example, *Tamus communis* was studied as far back as by PAYER (1857). The study of CADDICK et al. (2000) is concentrated on relatively late events in flower development. They do not discuss details of flower orientation with respect to the flower subtending bract (and bracteole, if present) and the occurrence of simultaneous or successive organ initiation within a whorl. The present study is aimed in filling this gap by investigating inflorescence and early flower development in *Dioscorea tokoro* Makino (Dioscoreaceae).

Dioscorea is the largest genus of Dioscoreales that comprises more species than all other members of the order taken together (ca. 450; WILKIN et al. 2005). Molecular phylogenetic studies showed that several formerly recognized genera, including *Tamus*, are nested within *Dioscorea* in its traditional circumscription. As a result, a wide concept of *Dioscorea* is adopted (CADDICK et al. 2002; WILKIN et al. 2005). *Dioscorea* s.l. is the only dioecious genus of Dioscoreaceae (WILKIN et al. 2005), though flowers are typically only functionally unisexual, i.e. with either sterile carpels or sterile stamens (PAYER 1857; KNUTH 1924; CADDICK et al. 2002). Species of *Dioscorea* are climbers that are widely distributed in tropics and subtropics. Some of them are economically important as food and medicinal plants.

Material and methods

Plants of *Dioscorea tokoro* Makino were collected in July 2009 in Atsugi Campus of Tokyo University of Agriculture, Japan as well as in several other localities on the Honshu Island in Kanagawa and Shizuoka Prefectures. The material was fixed and stored in 70% ethanol. Flower and inflorescence morphology were studied with an Olympus SZX7 stereomicroscope. For detailed morphological and developmental studies, scanning electron microscope (SEM) was used. Parts of the inflorescences and flowers were dehydrated through 96% ethanol and 100% acetone. Dehydrated material was critical-point dried using a Hitachi HCP-2 critical point dryer, mounted onto the stubs using double sided sticky tape, coated with gold and palladium using a Giko IB-3 ion-coater, and observed using a JSM-6380LA SEM and Camscan 4DV SEM at Moscow University.

Results

Male plants

Phyllotaxy of the main axis of synflorescence follows the Fibonacci pattern. Internodes are elongate. The main axis bears foliage leaves, though the growth of their blade is delayed compared to differentiation of axillary structures. A terminal flower or terminal partial inflorescence is

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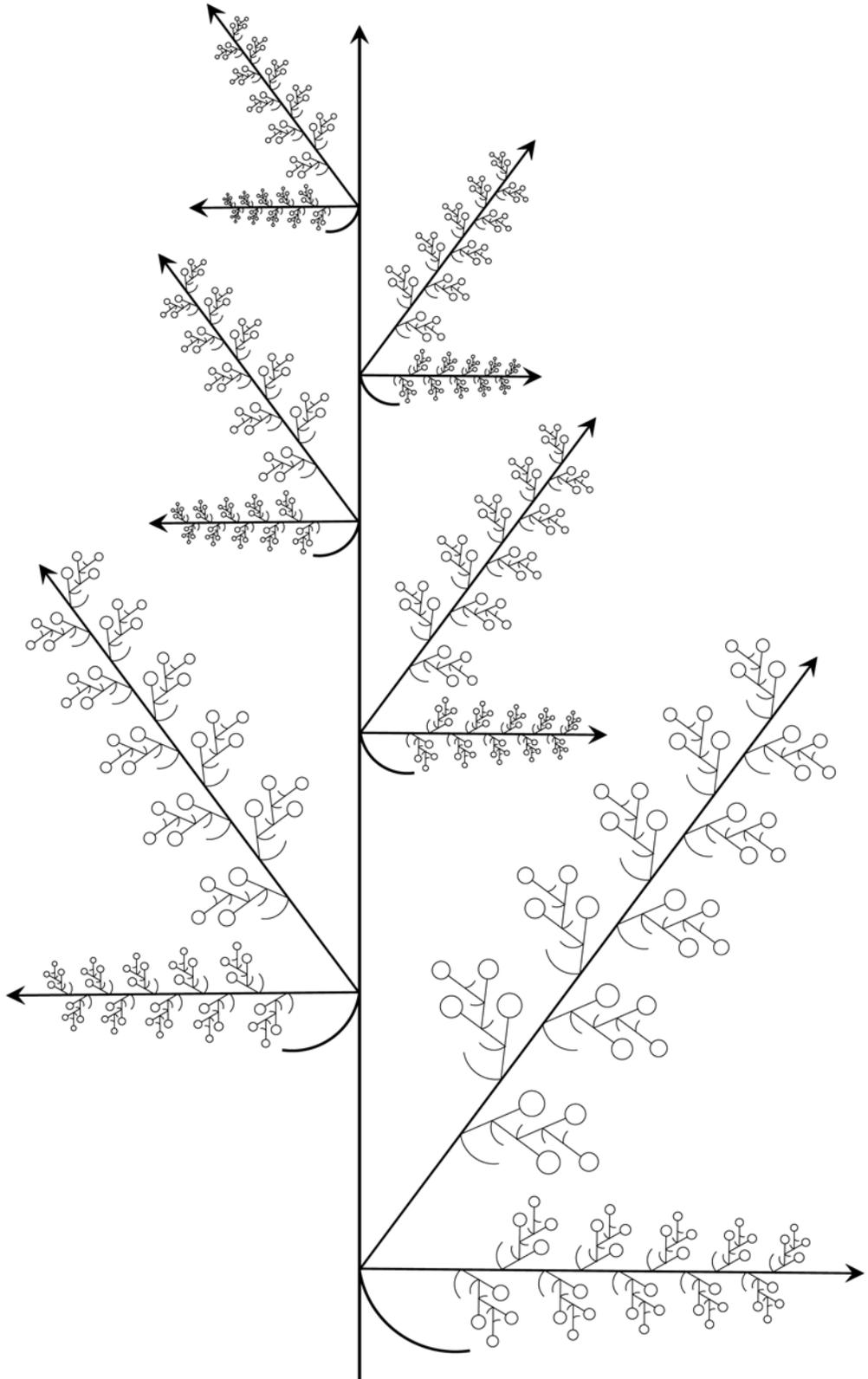


Figure 1. *Dioscorea tokoro*. Upper part of the synflorescence of a male plant.

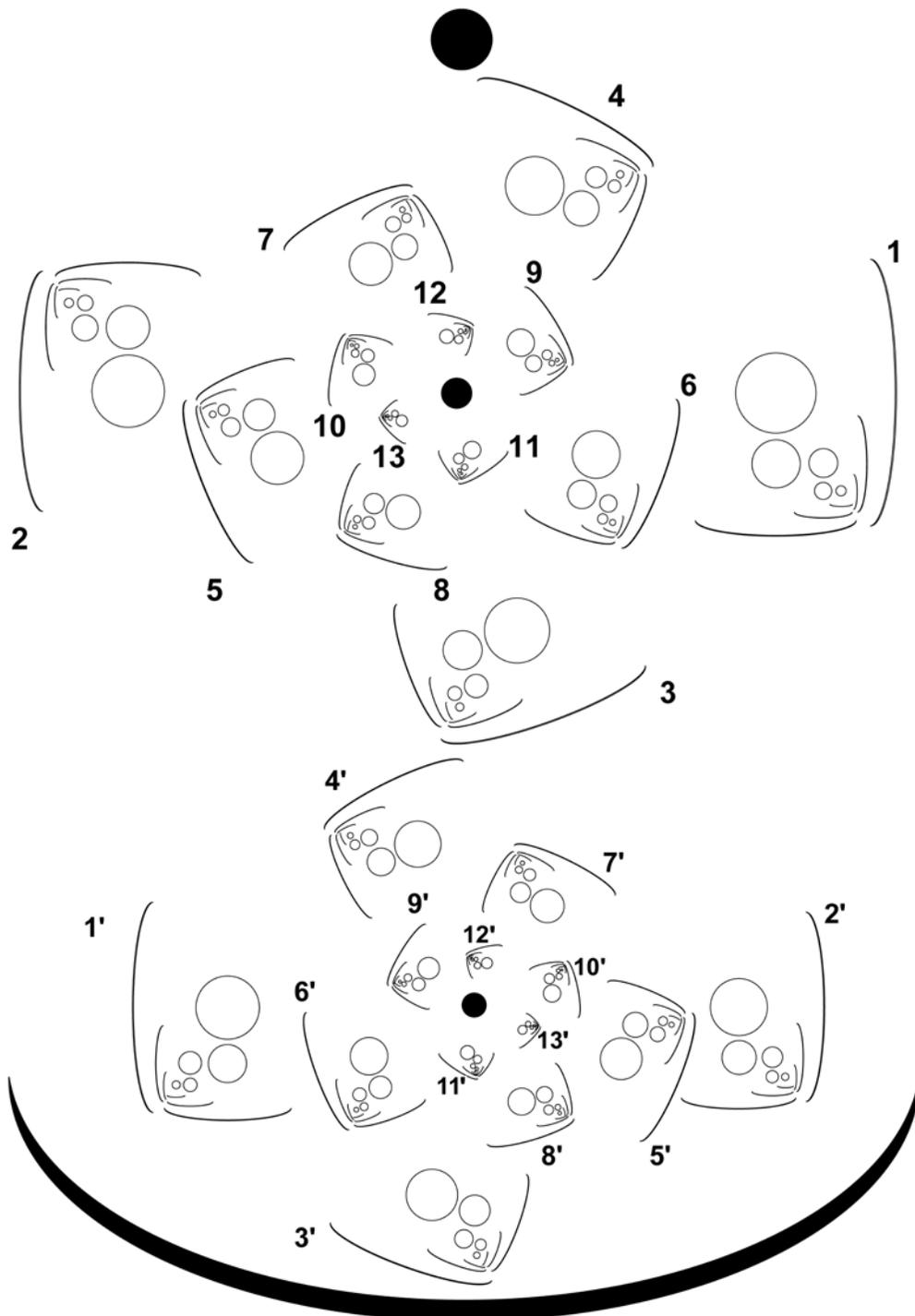


Figure 2. *Dioscorea tokoro*, diagram of two thyrses forming a descending series in axil of a foliage leaf on the main axis of the synflorescence of a male plant. Large black circle = main axis of synflorescence. Small black circles = main axes of thyrses. Open circles = flowers. Large black arc = subtending leaf of the serial complex consisting of two thyrses. Small arcs = flower subtending bracts / bracteoles. Numbers indicate phyllotaxy in thyrses (and the sequence of cincinni). The upper thyrsis has anticlockwise spiral while the lower thyrsis has clockwise spiral. Note that the handedness of a cincinnus numbered 2' in the lower thyrsis differs from the handedness of all other cincinni of the lower thyrsis.

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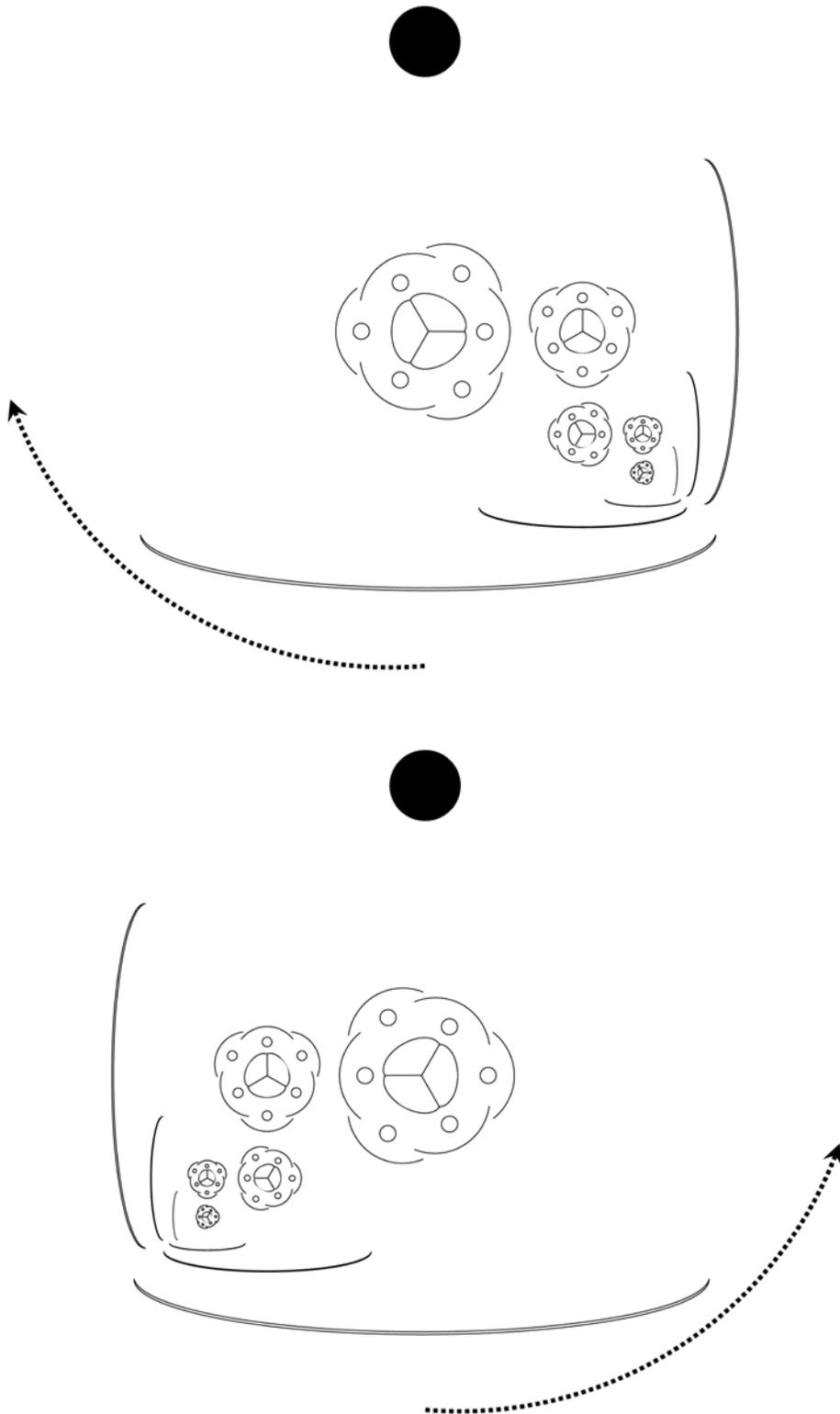


Figure 3. *Dioscorea tokoro*, a correlation between direction of phyllotaxy on the axis of a thyrse and handedness of a cincinnus. Black circle = thyrse axis. Dotted line = direction of phyllotaxy on the thyrse axis.

absent. All leaves on the main inflorescence axis possess axillary complexes that initiate, develop and proceed to flowering in an acropetal sequence. Each axillary complex contains a descending series of buds (occurrence of two buds was most common in our material). In most cases, all buds of a serial complex develop structures of the same type, namely, open thyrses (Fig. 1). Sometimes, more complex structures can be seen. For example, the first bud of a serial complex may develop a shoot with one or two lateral thyrses followed by a terminal thyrse.

Internodes on the main axis of each thyrse (= the second order axes of the synflorescence) are elongate. Phyllotaxy on second order axes of synflorescence starts with two transversal phyllomes (which can be classified as prophylls) and then follows the Fibonacci pattern (Fig. 2). Each node

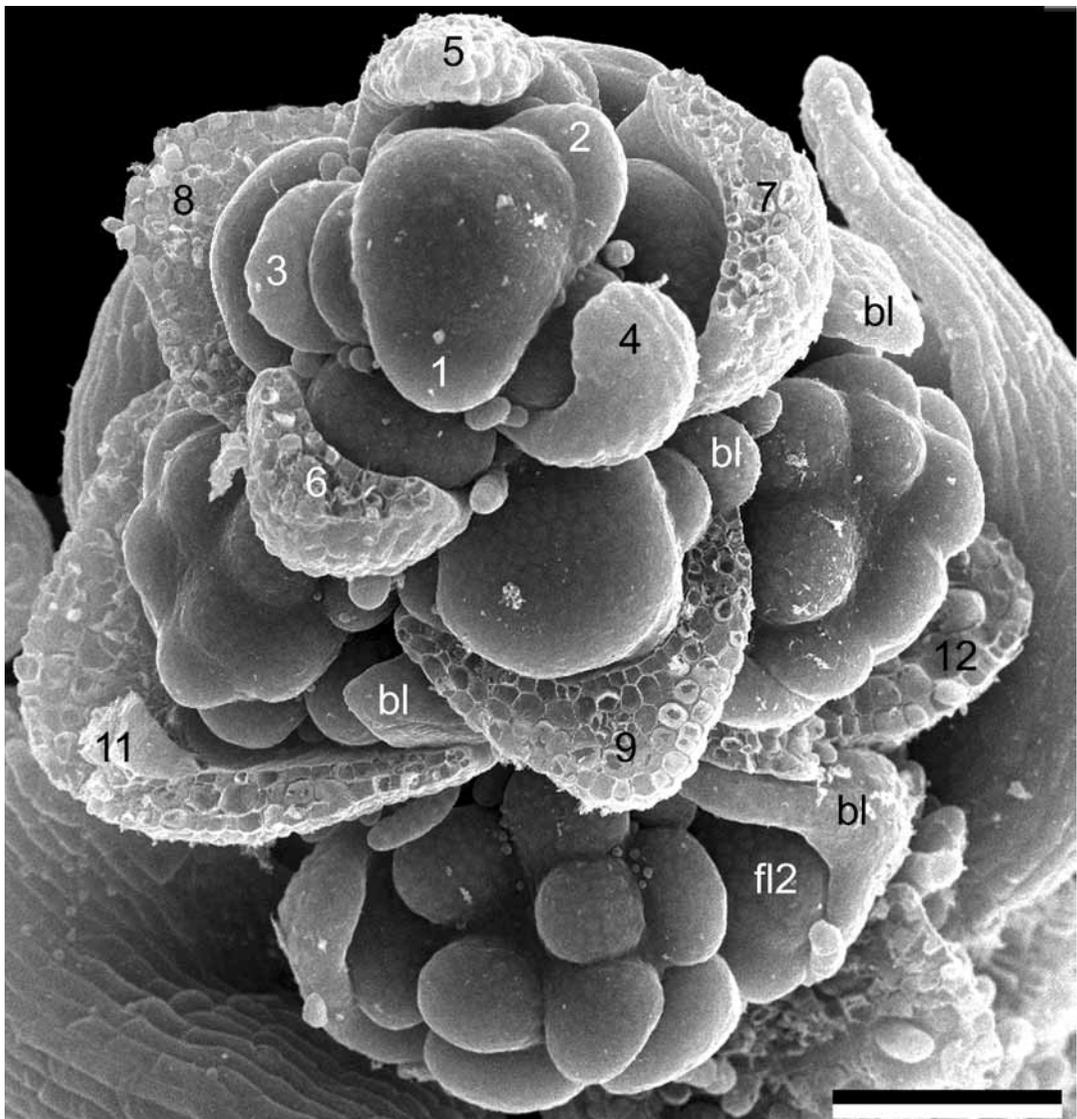


Figure 4. *Dioscorea tokoro*, oblique top view of a young thyrse of a male plant. Phyllotaxy on the thyrse axis is clockwise. Phyllomes of the main axis (i.e., subtending bracts of cincinni) are numbered starting from the youngest one. bl = bracteole of the first flower of a cincinnus, which is here in a right hand position. fl2 = next order flower in the axil of the bracteole. Scale bar = 100 μ m.

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of second order axes of synflorescence possesses a bract that subtends a lateral cyme or, more precisely, a cincinnus. Prophylls on second order axes of synflorescence are morphologically similar to subsequent bracts and in most cases subtend axillary cincinni (rarely, as mentioned above, they subtend lateral thyrses).

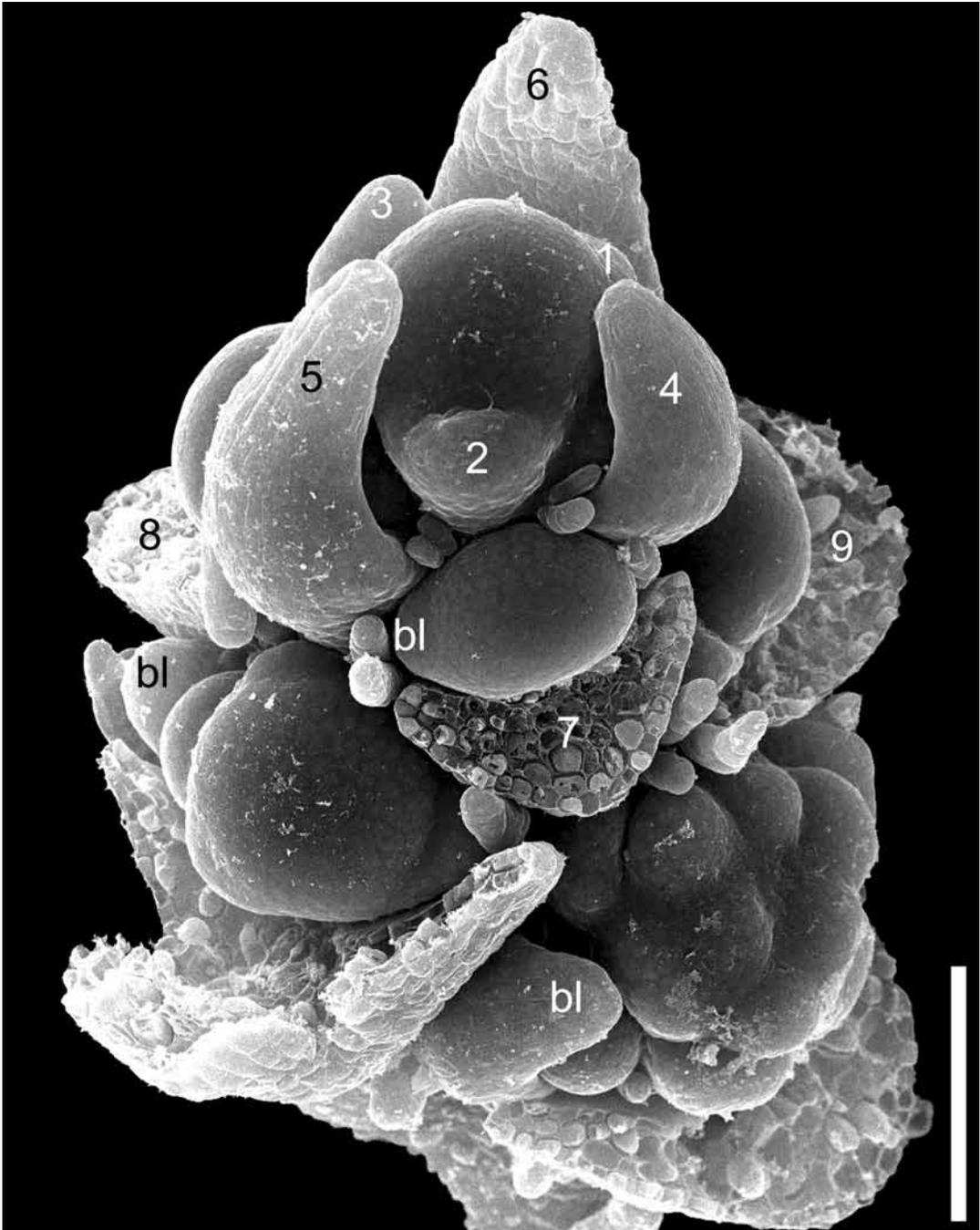


Figure 5. *Dioscorea tokoro*, oblique top view of a young thyrsus of a male plant. Phyllotaxy on the thyrsus axis is anticlockwise. Phyllomes of the main axis (i.e., subtending bracts of cincinni) are numbered starting from the youngest one. bl = bracteole of the first flower of a cincinnus, which is here in a left hand position. Scale bar = 100 μ m.

In our material, thyrses which developed from the first and the second bud of the same serial complex differed in their chirality (Fig. 2). If the thyrses from the first bud had clockwise spiral of phyllotaxy, then the second thyrses had anticlockwise spiral. Vice versa, if the thyrses developed from the first bud had anticlockwise spiral of phyllotaxy, then the second thyrses had clockwise spiral. Only limited material was studied to compare chiralities of first thyrses formed in successive nodes along the same main axis. According to preliminary data, first thyrses in adjacent nodes of the main axis have different chiralities.

The first flower of a cincinnus (that belongs to the third order axis of the synflorescence) has a bracteole that subtends a fourth order flower. The fourth order flower also has a bracteole that subtends a fifth order flower, and so on.

The bracteole of the first flower of a cincinnus always lies in a transversal plane with respect to the axis of the subtending bract. In thyrses with a clockwise direction of the genetic spiral of phyllotaxy, bracteoles lie on the right side (if the flower is seen in a top view, with the subtending bract facing down and the main axis up) (Fig. 3, top; Fig. 4). In thyrses with anticlockwise direction of the genetic spiral, bracteoles lie on the left side (Fig. 3, bottom; Fig. 5). Only rarely we observed exceptions from this general. For example, the second cincinnus of the lower thyrses illustrated in Fig. 2 has 'wrong' handedness.

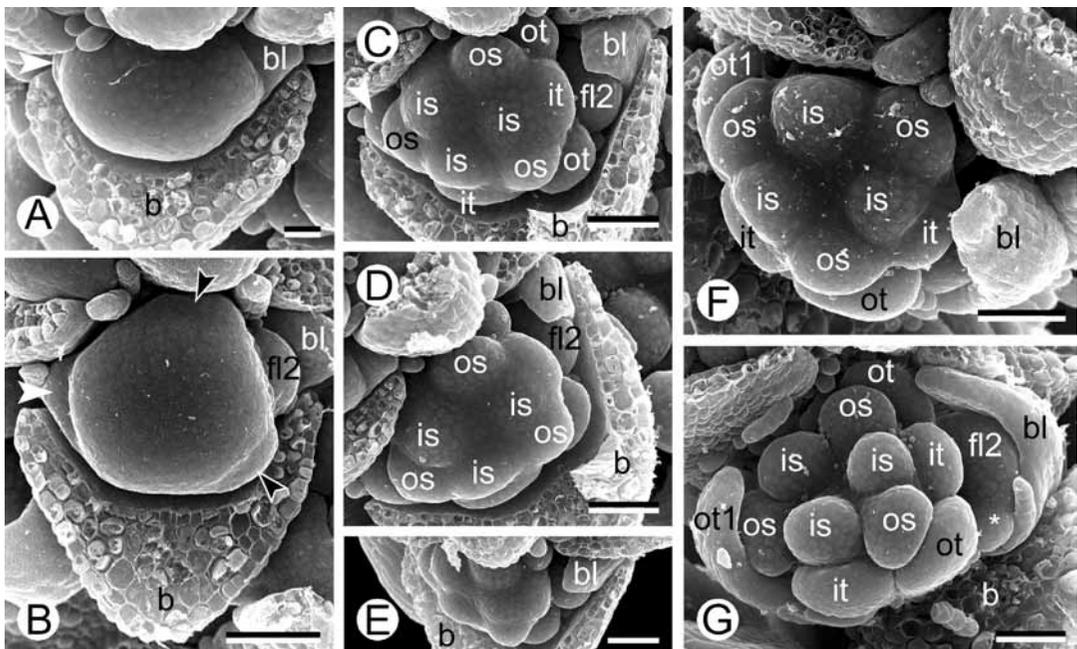


Figure 6. *Dioscorea tokoro*, development of functionally male flowers with right hand position of the bracteole. All images show first flowers of a cincinnus. A, bracteole is initiated, and the first outer whorl tepal is just slightly visible on the opposite radius. B, bracteole and all three outer whorl tepals are initiated; undifferentiated floral meristem is visible in the axil of the bracteole. C–E, the same young flower viewed from different angles. Close association between inner tepal and inner stamen primordia is visible. Consider especially the tepal-stamen primordium inserted on the radius of the bracteole. F, G, subsequent stages of flower development. Note a bracteole of the next order flower visible in G (asterisk). b = flower-subtending bract; bl = bracteole; fl2 = next order flower formed in the axil of the bracteole; is = inner whorl stamen; it = inner whorl tepal; os = outer whorl stamen; ot or black arrowhead = outer whorl tepal; ot1 or white arrowhead = the first formed outer whorl tepal. Scale bars = 20 μ m in A, 50 μ m in B–G.

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A cincinnus initiates in the axil of a subtending bract (which is also the subtending bract of the first flower of the cincinnus) as a primordium that is considerably elongate in the transversal plane. Only a small part of the cincinnus meristem is used to initiate a bracteole of the first flower (Fig. 6A). Soon after initiation of the bracteole, the first outer whorl tepal of the first flower initiates on the opposite radius of the meristem, quickly followed by two other outer whorl tepals (Fig. 6A,B). We were unable to trace a sequence in initiation between the second and the third tepal. At the stage when all three outer whorl tepals are initiated, a lateral meristem can be traced in the axil of the bracteole, which will develop a next order flower (Fig. 6B, 7A). Development of this flower is considerably delayed with respect to the first flower of a cincinnus. When all tepals and stamens of the first flower are already initiated, the lateral axis just initiates a bracteole of the second flower, and tepals of the second order flower are not yet recognizable (Fig. 6G, 7D).

After initiation of the outer whorl tepals, three inner tepals and two whorls of stamens initiate in a very rapid sequence. We did not observe any flower with six tepals initiated and without recognizable stamen primordia. The outer whorl stamens initiate independently from the outer whorl tepals. The outer whorl stamen which is inserted on the radius opposite the bracteole appears to be initiated before the two other outer whorl stamens (consider the difference in sizes of outer whorl stamen primordia in Fig. 7B). The inner whorl stamens are closely associated with the inner whorl tepals. Some images taken in this study (Fig. 6C–E, 7B) suggest the occurrence

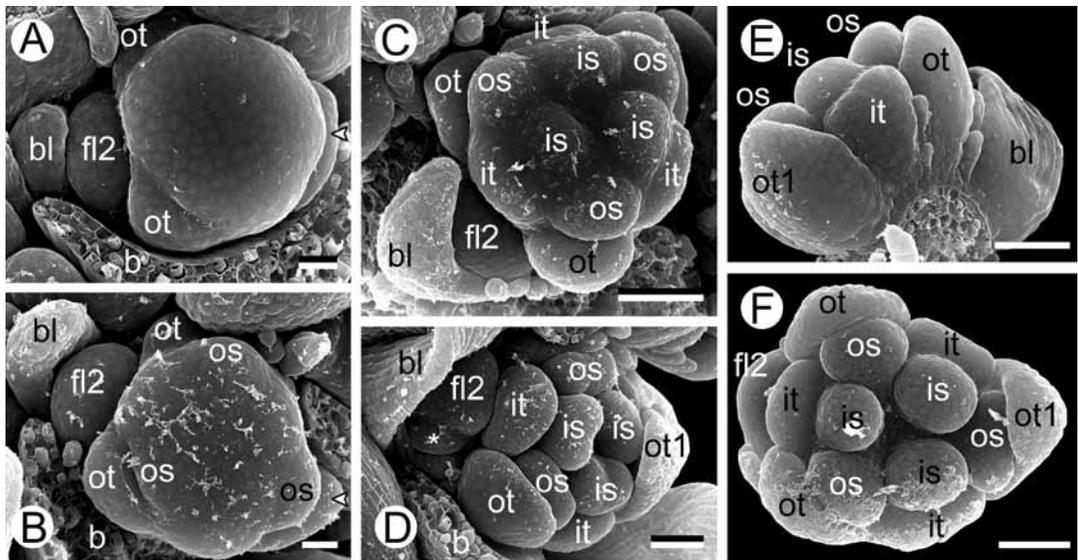


Figure 7. *Dioscorea tokoro*, development of functionally male flowers with left hand position of the bracteole. All images show first flowers of a cincinnus. A, bracteole and three outer whorl tepals are initiated, other floral organs are just starting to initiate; undifferentiated meristem of the next order flower is visible. B, just slightly older stage than in A. Outer whorl stamen primordium on the radius opposite the bracteole appears to be larger than two other outer whorl stamen primordia. Small primordia are visible between the radii of outer whorl tepals/stamens. These can be interpreted either as inner whorl tepal primordia or as inner whorl tepal/stamen common primordia. C, D, subsequent stages of flower development. Note a bracteole of the next order flower visible in D (asterisk). E, a flower seen from the adaxial side (the view is opposite to that in previous images, and the bracteole is seen to the right here). F, top view of a young flower. b = flower-subtending bract; bl = bracteole; fl2 = next order flower formed in the axil of the bracteole; is = inner whorl stamen; it = inner whorl tepal; os = outer whorl stamen; ot = outer whorl tepal; ot1 or white arrowhead = the first formed outer whorl tepal. Scale bars = 20 μ m in A, B, 50 μ m in C–F.

of three common primordia, each developing an inner whorl tepal and a stamen inserted on the same radius.

In flower buds, tepal aestivation is imbricate (Fig. 8A). The first initiated tepal is the outer one and the largest one, though it is smaller than the bracteole. Inner whorl tepals are smaller than outer whorl ones in young flowers. Stamens of the two whorls of preanthetic flowers differ in the positions of their thecae (Fig. 8B). At a very late stage, a sterile gynoecium of three carpels is formed.

Female plants

Synflorescences are similar to those in male plants, but they are generally less branched. The following differences from male synflorescences can be traced. (1) Leaves on the main axis of the synflorescence often (though not always) subtend individual second order axes because additional buds of serial complexes remain dormant. (2) Formation of lateral cymes takes place only in the proximal part of a second order axis, or cannot be traced at all. In the distal part of second order axis (or throughout its full length), single lateral flowers develop instead of cymes. These single flowers always bear a bracteole in a transversal position, like the first flower of a cyme.

The phyllotaxic patterns are the same as in male inflorescences (Fig. 9). In second order axes with a clockwise genetic spiral of phyllotaxy, bracteoles of the first flowers of cincinni (or of solitary flowers replacing cincinni) lie on the right side (Fig. 9C, D). In second order axes with an anticlockwise genetic spiral, the bracteoles lie on the left side (Fig. 9A, B).

Early development of female flowers is very similar to that in male flowers (Figs. 10–12). More precisely, the flowers are functionally female, because they always possess six staminodes in the same positions as stamens of male flowers. Until the stage of gynoecium initiation, the staminodes of functionally female flowers are nearly identical to the stamens of male flowers of the same

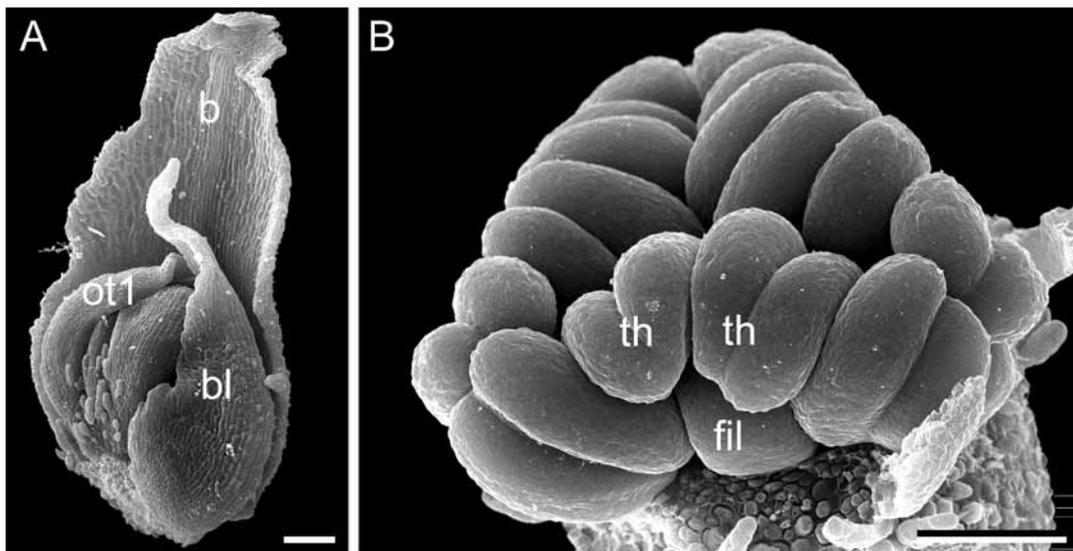


Figure 8. *Dioscorea tokoro*, late developmental stages of functionally male flowers. A, flower bud seen from the adaxial side with bract (b) and bracteole (bl) visible; ot1 = the first formed outer tepal. B, flower bud with perianth removed. Thecae (th) and filament (fil) of an inner whorl stamen are labeled. Scale bars = 100 μ m.

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developmental stage. In later stages, the staminodes develop two small thecae (Figs 13, 14), but do not differentiate microsporangia recognizable in surface view.

Like in male flowers, the first tepal initiates on the radius opposite the bracteole, and two other whorl tepals initiate to the left and to the right of the bracteole (Fig. 10A, B, Fig. 11A–D).

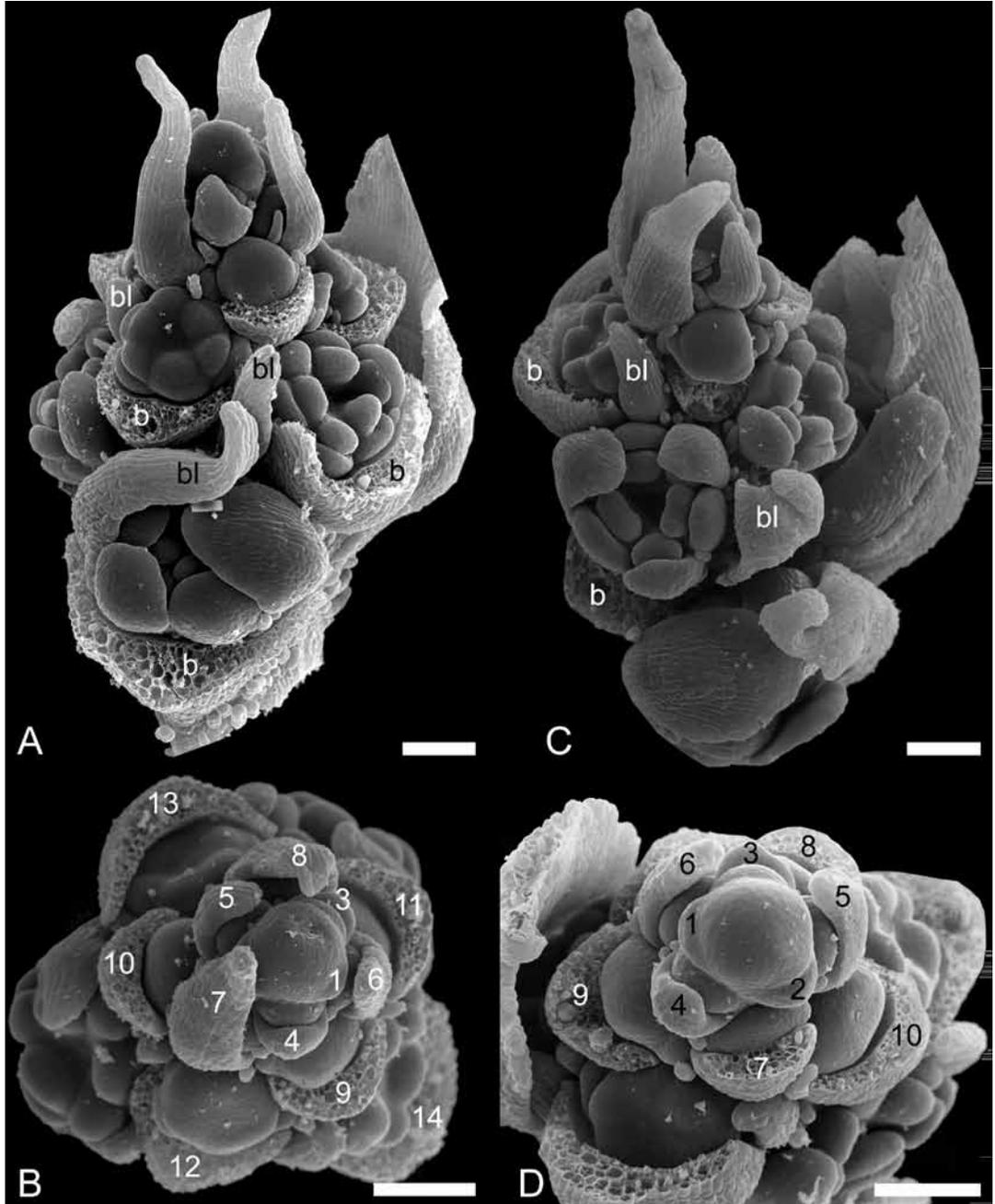


Figure 9. Chiral features of functionally female inflorescences. A,B, distal portions of young second order inflorescence axes with anticlockwise phyllotaxy. C, D, distal portions of young second order inflorescence axes with clockwise phyllotaxy. In B and D, flower-subtending bracts are numbered. b = flower-subtending bract, bl = bracteole. Scale bars = 100 μ m.

The flower meristem is clearly convex at this stage. Close association between the inner whorl stamens and inner whorl tepals of the same radii appears to be even more pronounced than in male flowers (Fig. 10D, Fig. 11G–I). Some images clearly suggest the occurrence of three common primordia, each giving rise to a staminode and a tepal (Fig. 11E, F). On later stages, these two organs are basally united (Fig. 12B).

There is a long plastochron between staminode and carpel initiation. The flower meristem is flat or only slightly concave at the time when staminodes are just initiated, and the space between the inner whorl staminodes is smaller than the size of a staminode at this stage. During next stages, the receptacle between stamens becomes strongly concave. The shape of the concave part is triangular in outline (when the flower is observed in a top view), with angles on the radii of the outer whorl staminodes. In a young gynoeceum (Fig. 13), plicate carpels can be seen on the radii of the outer whorl staminodes. Upper parts of carpels are free from each other but largely congenitally united with the concave receptacle (Fig. 13B, C). The lower part of the young gynoeceum should be probably interpreted as a unilocular symplicate zone. A developed gynoeceum consists of three plicate stigmas, a common style, and an inferior ovary (Fig. 14).

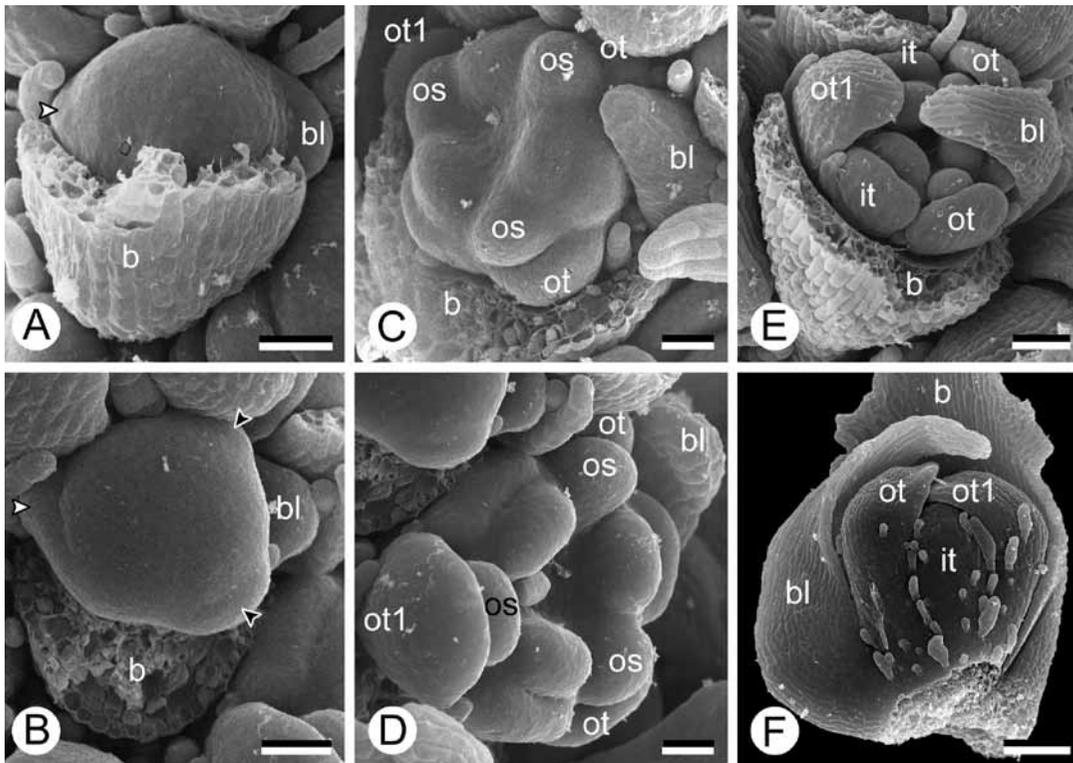


Figure 10. *Dioscorea tokoro*, development of functionally female flowers with right hand position of the bracteole. All images show first flowers of a cincinnus. A, bracteole is initiated, and the first outer whorl tepal is visible on the opposite radius. B, bracteole and all three outer whorl tepals are initiated; primordia of outer whorl staminodes are just slightly visible. C–E, subsequent stages of flower development. F, flower bud seen from adaxial side. b = flower-subtending bract; bl = bracteole; it = inner whorl tepal; os = outer whorl staminode; ot or black arrowhead = outer whorl tepal; ot1 or white arrowhead = the first formed outer whorl tepal. Scale bars = 30 µm in A–E, 100 µm in F.

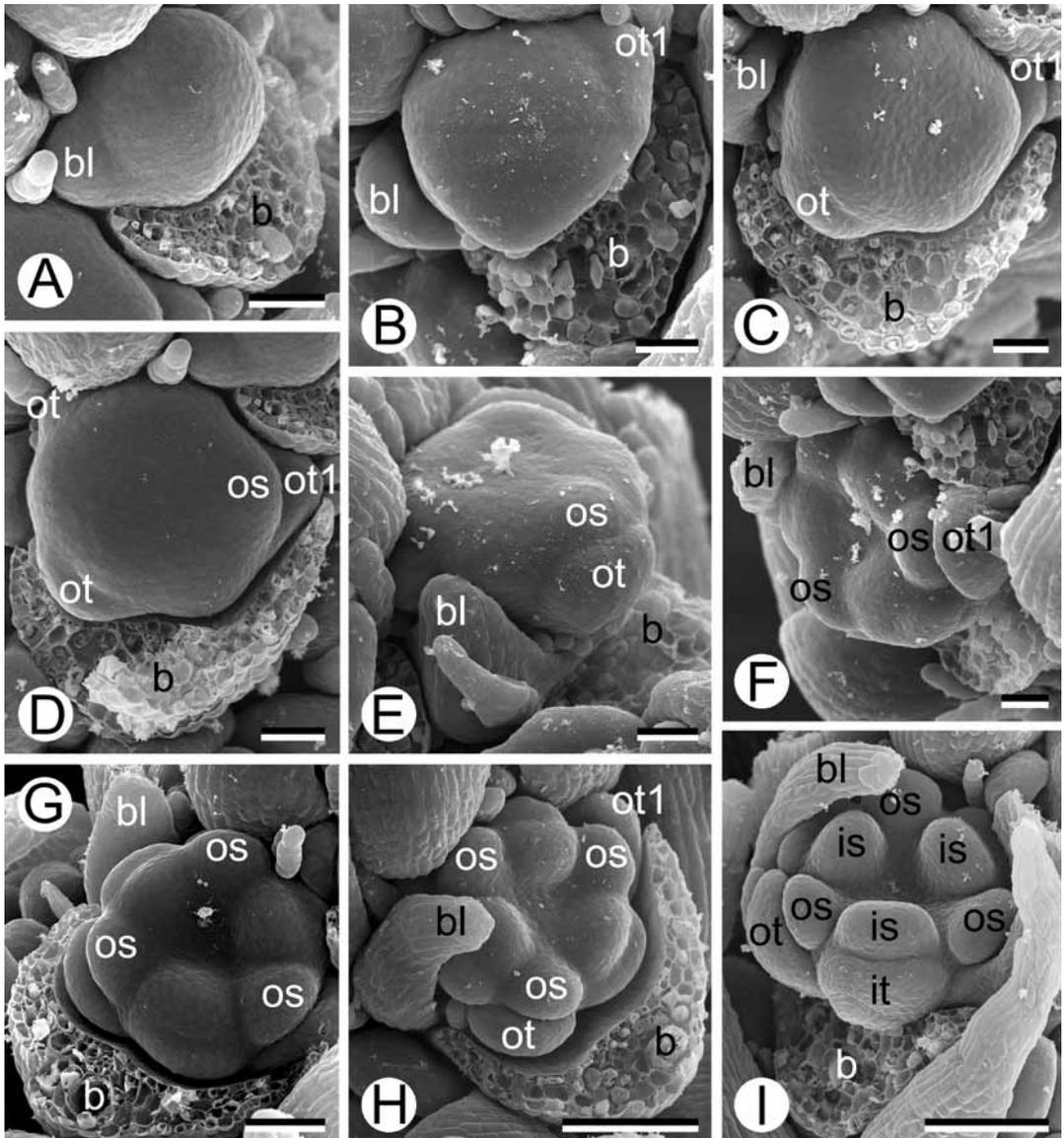
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Figure 11. *Dioscorea tokoro*, development of functionally female flowers with left hand position of the bracteole. A, bracteole is initiated. B, bracteole and outer whorl tepals are initiated; the largest and thus presumably the first formed tepal is on the radius opposite the bracteole. C–E, slightly older stages showing initiation of outer whorl staminodes; the largest and thus possibly the first formed staminode primordium is on the radius opposite the bracteole. Primordia that can be recognized between adjacent outer whorl tepal primordia could be interpreted as common inner whorl tepal-staminode primordia. Their double nature is especially obvious in E. F–I, subsequent stages showing initiation of inner whorl tepals and inner whorl staminodes. b = flower-subtending bract; bl = bracteole; is = inner whorl staminode; it = inner whorl tepal; os = outer whorl staminode; ot = outer whorl tepal; ot1 = the first formed outer whorl tepal. Scale bars = 30 µm in A–F, 50 µm in G–I.

Discussion

Shoot chirality and handedness of cincinni

Our data show a correlation between direction of the phyllotactic spiral on the axis of a thyse and the handedness of lateral cincinni inserted along the thyse axis (Fig. 2). If the phyllotaxy on

a thyse axis is clockwise, the bracteole of the first flower of a cincinnus lies on the right side. If the phyllotaxy on a thyse axis is anticlockwise, the bracteole lies on the left side. When in female inflorescences a solitary flower is developed instead of a cincinnus, the orientation of its bracteole follows the same rule. Exceptions from this general rule were quite rare in our material.

When discussing handedness of lateral structures, the terms ‘cathodic side’ and ‘anodic side’ are useful. The anodic end of a leaf is oriented in the direction up the genetic spiral of phyllotaxy towards the younger end while the cathodic end is oriented towards the beginning of the genetic spiral (KORN 2006). In these terms, the bracteole of the first flower of a cincinnus (or a solitary

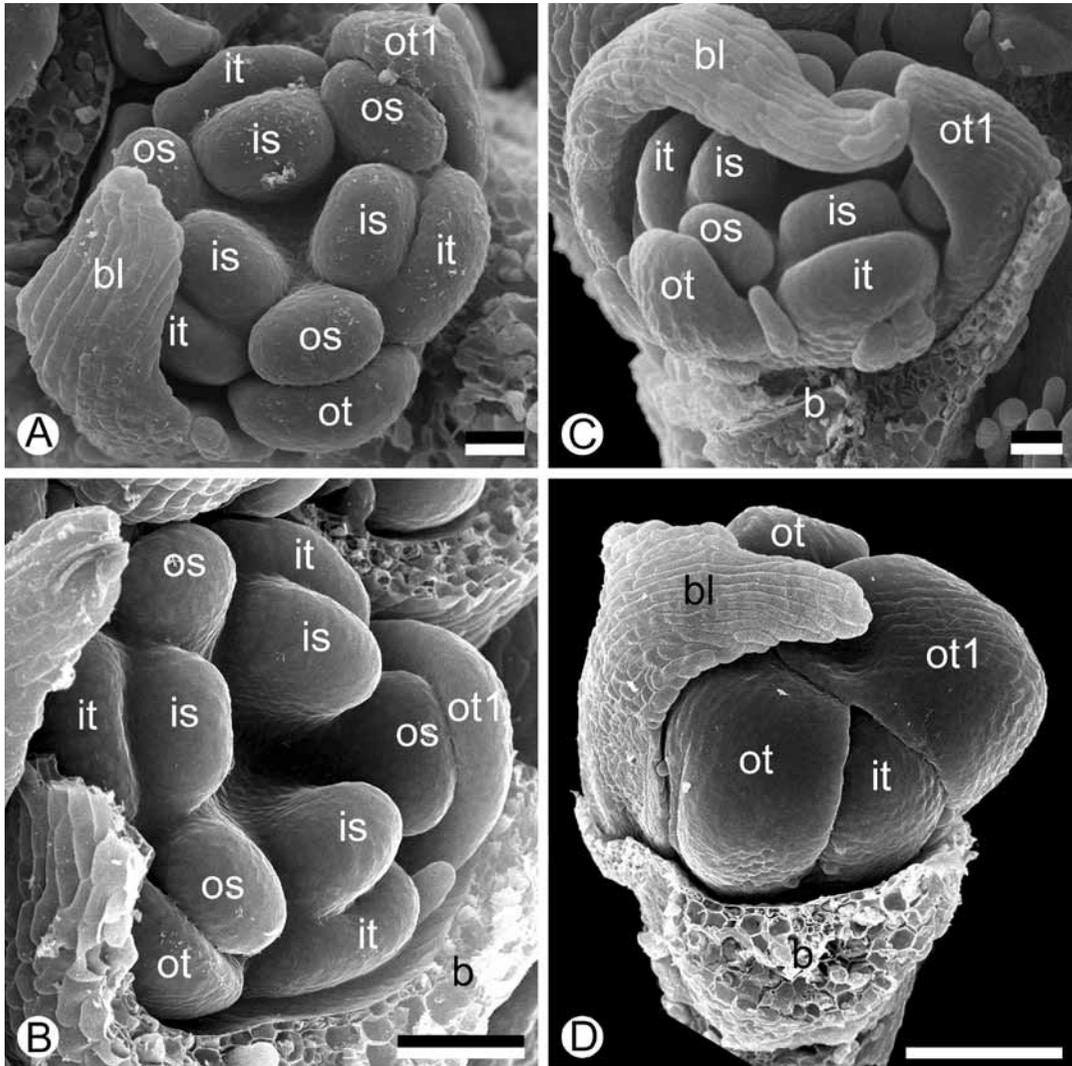


Figure 12. *Dioscorea tokoro*, development of functionally female flowers with left hand position of the bracteole (continued from Fig. 11). A–B, young flowers at stages before elongation of tepals; any signs of gynoecium are yet absent. Note that inner whorl tepals and inner whorl staminodes of the same radii are basally united. C, bracteole is considerably elongated; the first formed outer whorl tepal is the largest one. D, flower bud with tepals and bracteole covering other floral organs. b = flower-subtending bract; bl = bracteole; is = inner whorl staminode; it = inner whorl tepal; os = outer whorl staminode; ot = outer whorl tepal; ot1 = the first formed outer whorl tepal. Scale bars = 30 µm in A, C, 50 µm in B, 100 µm in D.

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female flower) lies on the cathodic side of the axil of its subtending leaf in *Dioscorea tokoro*. KORN (2006) summarized several examples of stabilized anodic/cathodic asymmetry in various angiosperms, which is expressed in a correlation between the direction of the genetic spiral of phyllotaxy and handedness of leaves and/or their axillary structures. He suggested that theories explaining phyllotaxy must include some asymmetric component. Indeed, the asymmetry of a leaf or its axillary structure should be likely a consequence of an asymmetric inception or asymmetric rates of growth between the cathodic and the anodic sides of a young leaf. As KORN (2006) mentioned, at least some cases of anodic/cathodic asymmetry can be explained by the delayed growth of the anodic side of a leaf. However, in some other cases, growth of the cathodic side is delayed. For example, in inflorescences *Theligonum cynocrambe* (Rubiaceae), where phyllotaxy follows the Lucas pattern, anodic stipules are more vigorous than cathodic ones, at least in early

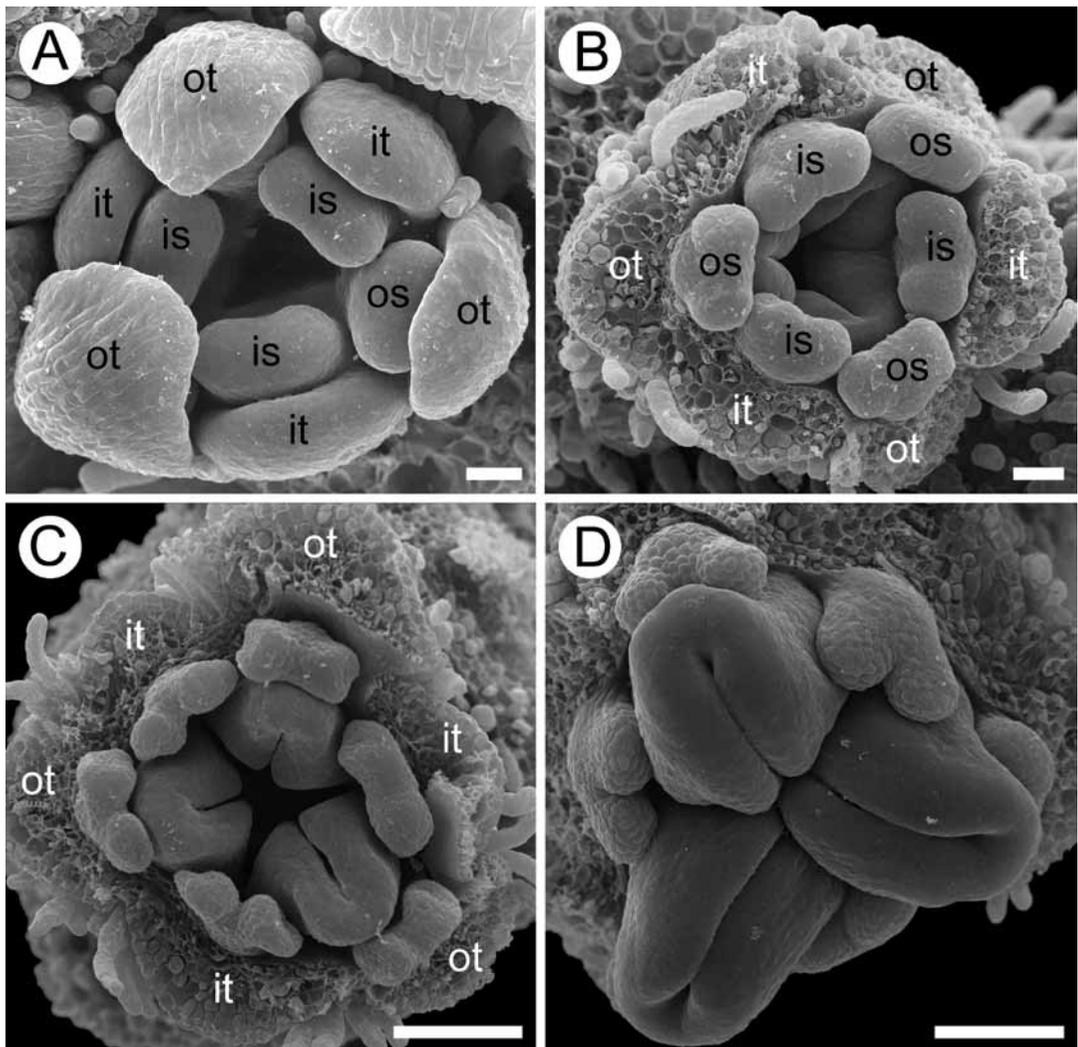


Figure 13. *Dioscorea tokoro*, late stages of development of functionally female flowers. A, the earliest stage of gynoecium initiation. The gynoecium is visible as a triangular structure between the inner whorl staminodes. B–D, development of plicate stigmas; tepals removed. is = inner whorl staminode; it = inner whorl tepal; os = outer whorl staminode; ot = outer whorl tepal. Scale bars = 30 µm in A, B, 100 µm in C, D.

development (RUTISHAUSER et al. 1998)¹. Our observations of young bracts on the thyrses axis of *Dioscorea* revealed only weakly pronounced asymmetry.

When comparing features of stabilized anodic/cathodic asymmetry in different taxa, it is crucial to select plants with similar manifestation of asymmetry. For example, it is interesting to compare *Dioscorea* with other plants possessing lateral cincinni. KIRCHOFF (1988) analyzed chiral features in thyrses of *Costus* (Costaceae, Zingiberales). He confirmed earlier results of Eichler that each cincinnus consists of an axis bearing a terminal flower and a bracteole on the anodic side of the flower (a bud forms in the axil of the bracteole but does not complete development). This is just opposite to what we found in *Dioscorea*, where the bracteole of the first flower of a cincinnus is inserted on the cathodic side. The phyllotaxy of the main axis is unusual in *Costus*, with divergence angles often less than 90 degrees (KIRCHOFF & RUTISHAUSER 1990). In *Canna* (Cannaceae, Zingiberales), where phyllotaxy in inflorescences is close to $\frac{1}{3}$, lateral cincinni have the same chiral features as in *Costus* (see KUNZE 1985).

1) See KONDORSKAYA (1988) for an alternative morphological interpretation of inflorescence structure in *Theligonum cynocrambe*.

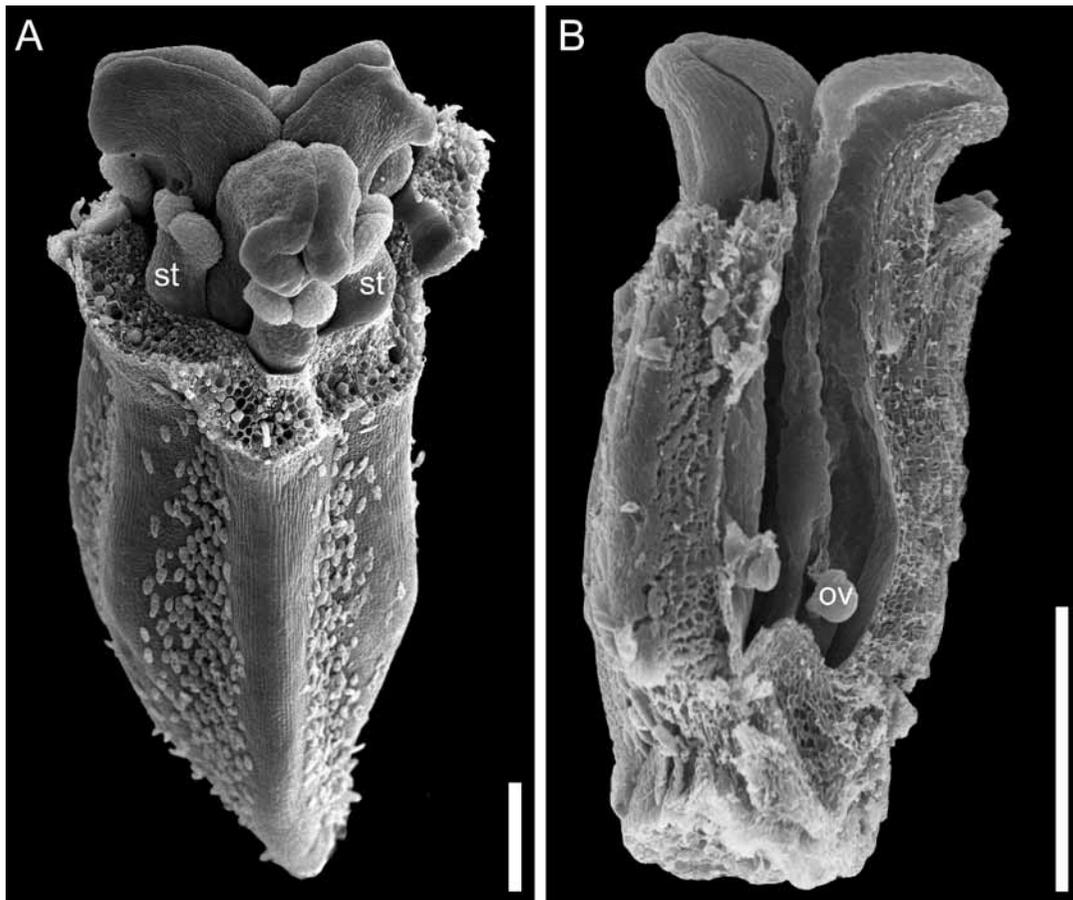


Figure 14. *Dioscorea tokoro*, late stages of development of functionally female flowers (continued from Fig. 13). Pre-anthetic flowers with tepals removed. A, side view of entire flower. B, longitudinally dissected flower. ov = ovule; st = staminode. Scale bars = 200 μ m in A and 300 μ m in B.

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Occurrence of a stabilized anodic/cathodic asymmetry, at least in some cases, might be of a phylogenetic significance. Another member of Dioscoreales, *Metanartheceum luteo-viride* does not possess this feature. Both left and right bracteole position can be found in the same inflorescence (see Fig. 15). In Nymphaeales, stabilized anodic/cathodic asymmetry is found in Nymphaeaceae (e.g., flower insertion is anodic to the putative subtending leaf in *Victoria* and cathodic in *Euryale* – CUTTER 1961; SCHNEIDER et al. 2003), but not in Hydatellaceae, at least in perennial species (SOKOLOFF et al. 2009).

Stabilized anodic/cathodic asymmetry found in plants with spiral phyllotaxy is very similar to pendulum symmetry in distichous shoots of various angiosperms (CHARLTON 1997), where left- and right-handed structures alternate in successive nodes. For example, in inflorescences of Loteae (Leguminosae), sterile bracts (prophylls) of lateral umbels are in either left or right position, and these two types alternate in successive nodes along the main axis of the inflorescence (SOKOLOFF et al. 2007). Leaf primordium insertion with respect to the shoot apex is usually asymmetric in plants with pendulum symmetry (e.g., GOEBEL 1928; CHARLTON 1997; SOKOLOFF et al. 2007), which is similar to the phenomenon discussed by KORN (2006) for his examples of spiral phyllotaxy.

Flower orientation with respect to surrounding phyllomes and tepal initiation

EICHLER (1875) and KIRCHOFF (2003) demonstrated that flower orientation in monocots is highly dependent on the presence and position of a bracteole. REMIZOWA et al. (2006a) further supported this idea and highlighted the phylogenetic and taxonomic significance of presence or

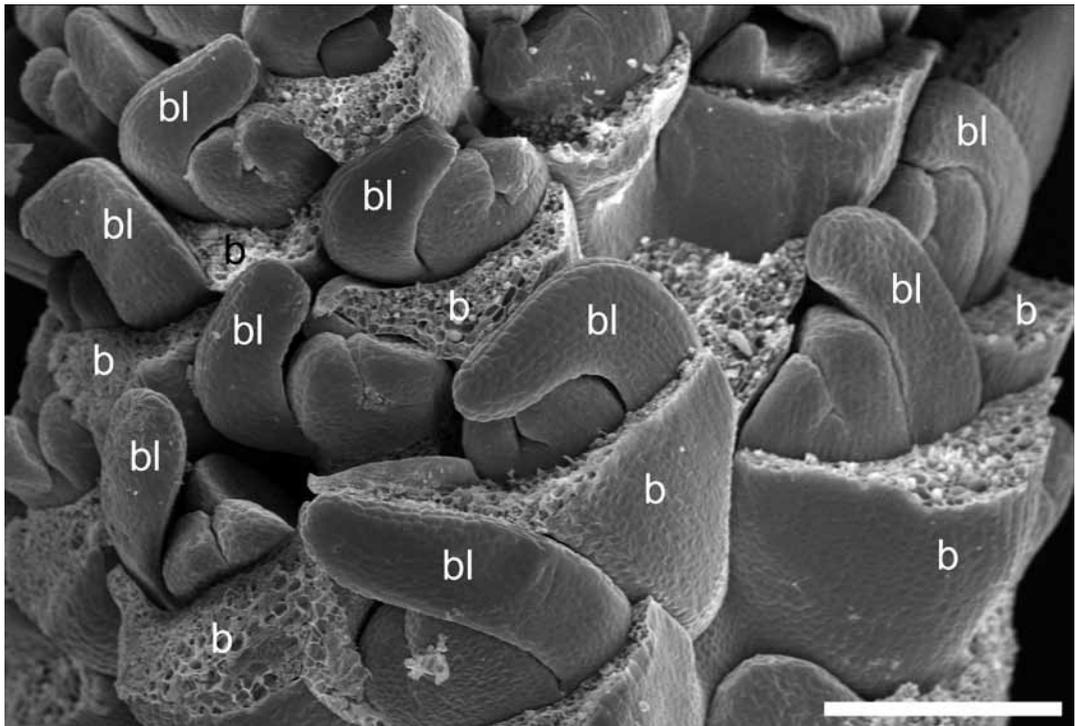


Figure 15. A portion of young inflorescence of *Metanartheceum luteo-viride* (Nartheciaceae, Dioscoreales). Side view; flower subtending bracts (b) removed. Bracteoles (bl) are on either left or right hand side. Scale bar = 200 μ m.

absence of a bracteole in monocots. In trimerous lateral flowers which are subtended by a bract and lacking the bracteole, the median tepal of the outer whorl occupies an abaxial position. If a bracteole is present, the median tepal occurs either in an intermediate (between median adaxial and transverse) or transverse position. The bracteole is always situated on the same radius as one of the inner whorl of tepals. In plants with unstable bracteole position the floral orientation is also unstable.

Trimerous monocot flowers with a bracteole tend to show a spiral sequence of tepal initiation within each whorl. The first outer tepal arises opposite the bracteole, and the second between the first outer tepal and bracteole, close to the bracteole. The direction of initiation of the inner tepals is the reverse of the outer. This scenario was observed in *Allium* (JONES & EMSWELLER 1936), *Lilium* (GRELLER & MATZKE 1970), *Nartheceum* (REMIZOWA 2008) and *Metanartheceum* (REMIZOWA et al. 2008). It seems that the presence of a bracteole is a strong regulatory factor for a spiral tepal (or sepal) initiation (e.g., GRELLER & MATZKE 1970; KIRCHHOFF 2003; REMIZOWA et al. 2006a; see also CHOUB 2002 and PRENNER et al. 2009 on importance of prophylls and bracteoles).

Our data on flower orientation in *Dioscorea tokoro* agree with earlier observations of EICHLER (1875) on *Dioscorea* and with general ideas on flower orientation in monocots. A bracteole is present in an almost transversal position, and one of three outer whorl tepals is inserted on the radius opposite the bracteole. Initiation of outer whorl tepals is not synchronous in *D. tokoro*. Like in the examples outlined above, the first tepal appears opposite the bracteole. However, in contrast to these examples, we did not find robust evidence of successive initiation of the second and third tepals of the outer whorl. Also, initiation of inner whorl tepals was synchronous in our material. This agrees with PAYER's (1857) observations in *Tamus* (successive initiation of outer whorl tepals and simultaneous initiation of the inner whorl tepals). Further studies should clarify whether the differences in tepal initiation sequence found between Nartheciaceae (*Nartheceum* and *Metanartheceum*) and *Dioscorea* s.l. are stable at the family level.

The presence of a bracteole is an important common feature of Nartheciaceae and Dioscoreaceae. Occurrence of a bracteole is much more significant than presence or absence of branching in its axil, i.e. than the difference between flowers arranged in open spikes/racemes or in open thyrses. Both inflorescence types can be found in each family, sometimes in the same species, as in *D. tokoro*.

Common tepal-stamen primordia

ENDRESS (1995) highlighted the occurrence of two principal developmental patterns in flowers of lilioid monocots. In the first type, all tepals and stamens initiate independently and early expansion of the floral apex takes place before the appearance of carpels. The second type is characterized by formation of common (inner) tepal-stamen primordia and further delayed expansion of the apex only after carpel appearance, so that the carpel primordia are more or less hidden between stamens. In Dioscoreales, flowers of *Nartheceum* and *Metanartheceum* clearly belong to the first type, while flower development of *Dioscorea tokoro* follows the second type. These observations support ENDRESS' view (1995) that these two types of flower development are homoplastic in monocot evolution. Indeed, both types occur in Liliales, Asparagales and commelinoid monocots. It seems that the presence of common primordia of inner tepals and inner stamens (which was

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not documented so far in Dioscoreales) is intimately linked with delayed receptacle expansion and delayed carpel initiation. In taxa with delayed receptacle expansion, initiation of (inner) tepals and stamens takes place in a very rapid sequence or almost simultaneously. This is why the spiral sequence of initiation of inner whorl tepals is not recognizable in *Dioscorea*, in contrast to *Nartheceum*, *Metanartheceum* and some other monocots that possess a bracteole.

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