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ORIGINAL ARTICLE

Spicoid ontogeny in *Diplasia* (Mapanioideae, Cyperaceae): an approach on the developmental processes operating in Mapanioideae spicoids

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Abstract

Mapanioideae (besides Cyperoideae the other subfamily of Cyperaceae) is divided in two tribes (Hypolytreae and Chrysitricheae) in which the reproductive units (spicoids) are interpreted either as flowers or as inflorescences. Little is known about the ontogenetic factors operating during the spicoid development and how they may contribute to the spicoid final morphology. In this context, we studied the spicoid development in the monotypic genus *Diplasia* and compared it with other developmental studies in Mapanioideae. Our results show that the main developmental variations are related to the sequence of organ formation, the size and shape of the spicoid primordium and the influence of the subtending bract constraints in different sides of such primordium. Our hypotheses to explain the spicoid's morphological variation in Mapanioideae are: that size and shape of the spicoid primordium contribute to changes in the number and position of organs to be formed, and spatial constraints of the subtending bracts influence the reduction of adaxial parts. Developmental features of the spicoids showed to be stable within the tribe. Our observations of the nature of spicoids in *Diplasia* give arguments for both, floral and inflorescence interpretations, sustaining the uncertainty towards a satisfactory definition. The difficulty in distinguishing the boundaries of flower and inflorescence nature in the spicoids raised a third hypothesis where the spicoid could be interpreted as a de novo structure, due to a reorganisation of regulatory genes resulting in a single unit with flower and inflorescence features combined, considered a synapomorphy of Mapanioideae.

Keywords *Diplasia karatifolia* · Mapaniid · Mapanioideae · Pseudanthium · Spatial constraints · Spicoid

Introduction

Floral traits are important to establish relationships among angiosperm taxa and concerning ontogenetic methods, they allow the test of molecular phylogenetic hypotheses as a solid method to determine characters and character states (c.f. Ronse De Craene et al. 2003; Endress 2006; Vrijdaghs et al. 2006, 2010; Chartier et al. 2014; Sauquet et al. 2017; Reyes et al. 2018). In Cyperaceae, in the subfamily Cyperoideae, the spikelets are formed of true flowers with a typical trimerous tetracyclic bauplan (Bruhl 1995; Goetghebeur 1998; Vrijdaghs et al. 2009), whereas in Mapanioideae, the nature of the spicoids has been a matter of debate for a long time and authors have described the spicoids as flower or as inflorescence (see Koyama 1971; Kern 1974; Simpson 1992; Bruhl 1995; Goetghebeur 1998; Richards et al. 2006; Prychid and Bruhl 2013; Monteiro et al. 2016).

Mapanioideae comprise 11 genera distributed in two tribes (Hypolytreae and Chrysitricheae) (Simpson et al. 2007; Muasya et al. 2009, Hinchliff and Roalson 2013; Semmouri et al. 2019). The reproductive units of the subfamily (spicoids) are described as consisting of two large lateral scales enclosing two lateral stamens and a central gynoecium. Between the lateral stamens and the central

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gynoecium, a variable number of additional stamens may be found which may be intervened by inner scales (Simpson 1992; Bruhl 1995; Goetghebeur 1998; Richards et al. 2006; Prychid and Bruhl 2013; Monteiro et al. 2016). This peculiar morphology raised the debate about the euanthial versus pseudanthial hypothesis in this subfamily (Koyama 1971; Kern 1974; Eiten 1976; Dahlgren et al. 1985; Bruhl 1995; Goetghebeur 1998; Richards et al. 2006; Vrijdaghs et al. 2006; Prychid and Bruhl 2013; Monteiro et al. 2016). The most common interpretations of the spicoids in Mapanioideae are: (1) The unit is a flower, where the lateral and inner scales are considered perianth parts, which were relocated between androecium and gynoecium in some species (Kern 1974; Eiten 1976; Goetghebeur 1998); (2) The reproductive unit is a reduced inflorescence of male flowers consisting of single stamens surrounding a central female flower (gynoecium) and lateral and inner scales (Dahlgren et al. 1985; Simpson 1992; Bruhl 1995).

The morphology of the mature spicoids hampers a satisfactory interpretation, and therefore developmental studies have been carried out focusing on the debated nature of these reproductive units (Richards et al. 2006; Prychid and Bruhl 2013; Monteiro et al. 2016). Richards et al. (2006) studied the development of spicoids in the monotypic genus *Exocarya* Benth. (tribe Chrysitricheae) and proposed that the reproductive unit of *Exocarya sclerioides* Benth. is rather a spikelet than a flower. The spicoid in this species is subtended by a bract and composed of two lateral scales (lateral leaf-like structure) enveloping two lateral stamens, two ad- and abaxial inner scales, the latter enveloping an inner stamen, and a dimerous dorsiventrally flattened gynoecium (Fig. 1a). Prychid and Bruhl (2013) studied the monotypic genus *Lepironia* Pers. (tribe Chrysitricheae) and showed the reproductive unit of *Lepironia articulata* Domin arising in the axil of a subtending bract and composed of two lateral scales (prophyll-like units) enveloping two lateral stamens, and two whorls of inner scales (leaf-like structures)—the scales of the outer whorl enveloping 12-15 inner stamens (five adaxial and a variable number abaxially), and the scales of the inner whorl surrounding a dimerous dorsiventrally flattened gynoecium (Fig. 1b). The ontogeny and the localization of floral gene proteins in the spicoid structures of this species favoured the inflorescence interpretation (Prychid and Bruhl 2013). Monteiro et al. (2016) studied the spicoid development of *Mapania pycnostachya* (Benth.) T. Koyama and *Hypolytrum schraderianum* Nees (tribe Hypolytreae), the most diverse genera of Mapanioideae in number of species. The authors showed that *Mapania pycnostachya* and *Hypolytrum schraderianum* share the most reduced spicoids in number of structures, with *Mapania pycnostachya* composed of two lateral scales (prophyll-like units) enveloping two lateral stamens, and two inner scales (leaf-like structures) positioned between the lateral stamens and the dimerous dorsiventrally flattened gynoecium (Fig. 1c). *Hypolytrum schraderianum* differs from *Mapania pycnostachya* only by the absence of inner scales (Fig. 1d). Based on anatomical and developmental differences of these reproductive units compared to Cyperoideae flowers, the results by Monteiro et al. (2016) favoured the inflorescence interpretation.

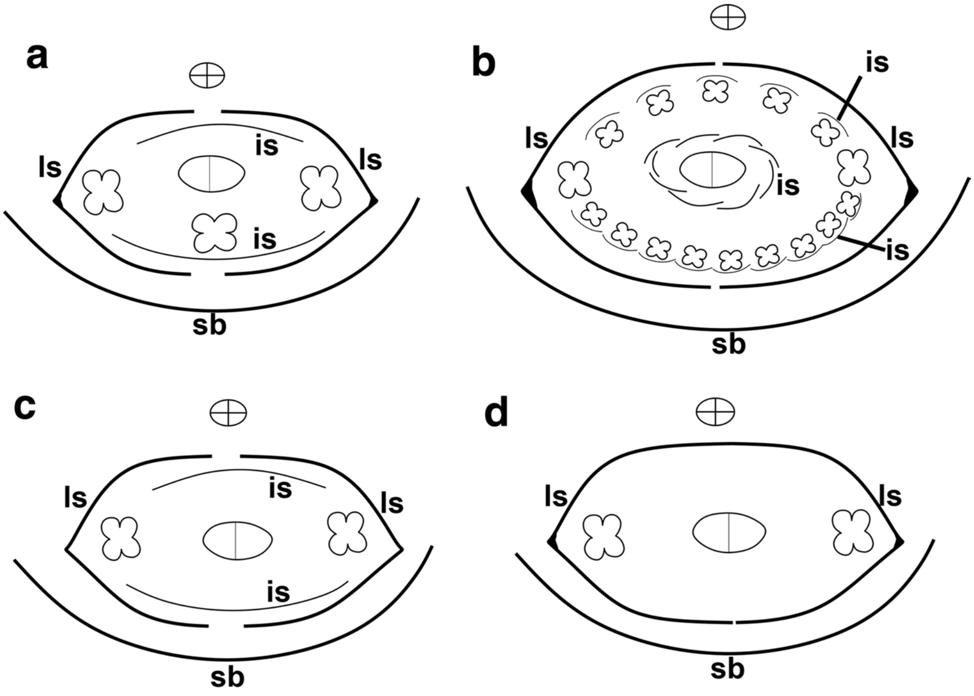


Fig. 1 Simplified spicoid diagrams of Mapanioideae species based on previous developmental studies. Tribe Chrysitricheae **a** *Exocarya sclerioides* (Richards et al. 2006). **b** *Lepironia articulata* (Prychid and Bruhl 2013). **c** *Mapania pycnostachya* and **d** *Hypolytrum schraderianum* (Monteiro et al. 2016). The circle filled with a cross indicate the position of the spike axis. *is* inner scale; *ls* lateral scale; *sb* subtending bract

Although the previous studies are consistent towards the inflorescence interpretation, none of them are conclusive in excluding the possibility of a flower interpretation (Richards et al. 2006; Prychid and Bruhl 2013; Monteiro et al. 2016). Furthermore, little is known about the factors operating during spicoid development in most of the genera and how they may affect the spicoid's final morphology, as well as how stable they are within and among genera. From the 11 genera of Mapanioideae, five are monotypic and for some of them information is restricted to the taxonomic descriptions. This is the case of *Diplasia* Rich. (Bentham 1877; Clarke 1909; Simpson 2006), a genus restricted to tropical South America (Simpson 2006). The phylogenetic placement of *Diplasia* has been controversial. It emerged within the tribe Hypolytreae (Muasya et al. 2009), in an unresolved placement (Hinchliff and Roalson 2013), as a sister-group to Mapanioideae (Spalink et al. 2016), and most recently, within the tribe Chrysitricheae (Semmouri et al. 2019). The complexity in understanding the position and origin of organs in the mature spicoids, in addition to the unresolved phylogenetic position of the genus highlights the importance of morphological and developmental studies to provide informative data for comparative analyses and to better understand the phylogenetic intergeneric relationships.

Here, we aim to present a complete ontogenetic analysis of the spicoid of *Diplasia karatifolia* Rich. and compare these data with other developmental studies for Mapanioideae (Richards et al. 2006; Prychid and Bruhl 2013; Monteiro et al. 2016) in order to answer the following questions: (1) What are the main developmental features that vary among *Diplasia*, *Exocarya*, *Lepironia*, *Mapania* and *Hypolytrum* species? (2) How may such features influence the final spicoid morphology? (3) How stable and informative are the developmental features among Mapanioideae genera? Based on our results and a comparison with other Mapanioideae genera, we finally discuss the hypotheses to interpret *Diplasia* spicoid as a flower or as an inflorescence.

Materials and methods

Morphological sampling

Spikes of *Diplasia karatifolia* were collected in the Reserva Florestal Adolpho Ducke (Manaus, Amazonas, Brazil), in the moist understory of the Amazon rainforest. Vouchers (AG 1658) were deposited in the herbarium SPF (University of São Paulo – Brazil).

Light and scanning electron microscopy

Spikes at different developmental stages were fixed in FAA (formaldehyde, glacial acetic acid, 50% ethanol, 1:1:18 v/v) (Johansen 1940) and stored in 70% ethanol for morphological and anatomical studies.

For scanning electron microscopy, fixed spikes were dissected under a Leica Wild M8 stereomicroscope to expose the spicoids at different developmental stages. The samples were then dehydrated, and critical-point dried using CO_2 in an Autosamdri-815B (Tousimis Research, Rockville, Maryland, USA). Dried material was mounted onto aluminium stubs using nail polish, coated with platinum using a Quorum Q-150-T sputter coater (Quorum Technologies, East Grinstead, UK) and examined with a Hitachi S-4700-II cold field emission scanning electron microscope (Hitachi High Technologies, Tokyo, Japan). To measure the spicoid primordia, and for a standard evaluation, we measured the spicoid primordia in the earliest recognizable developmental stage, before the differentiation of any organs and no visual sign of the lateral scales (Table 1).

Terminology

Mapanioid spike: A partial or entire inflorescence consisting of an indeterminate axis and laterally, spirally arranged bracts, each subtending a spicoid or reproductive unit.

Spicoid: The reproductive unit of Mapanioideae consisting of an axis terminated by a gynoecium, which is surrounded by stamens and inner scales. The spicoid is enveloped by two lateral scales.

Subtending bract: a large bract that subtends the entire spicoid (lateral scales + stamens + inner scales + gynoecium).

Lateral scales: a pair of lateral scales, each enveloping a lateral stamen. Usually the first organ to appear during development, also named prophyll-like units (Prychid and Bruhl 2013) and lateral leaf-like structures (Richards et al. 2006)

Inner scales: scales placed within the spicoid, between the outer and inner whorls of stamens, or between the androecium and gynoecium, also named leaf-like structures (Richards et al. 2006; Prychid and Bruhl 2013).

Results

The spicoids of *Diplasia* arise in the axil of a subtending bract and consist of two lateral scales enveloping two lateral stamens, two inner scales enveloping three stamens each and a central dimerous dorsiventrally flattened gynoecium. The subtending bracts arise in a spiral phyllotaxis on the indeterminate spike axis (Fig. 2a). Each spicoid primordium is flattened adaxially, expanded in the laterals and rounded abaxially (Fig. 2a; the subtending bract was removed in Fig. 2b (asterisk) onwards). The development of each spicoid starts with the appearance of two triangular lateral scales (Fig. 2b—dark green), which enlarge, leaving a central

Table 1 Developmental and morphological traits of spicoids of *Diplasia karatifolia* and other Mapanioideae species. References: *Lepironia articulata* (Prychid and Bruhl 2013); *Exocarya scleriodes* (Richards et al. 2006); *Mapania pycnostachya* and *Hypolytrum schraderianum* (Monteiro et al. 2016)

Features	Species				
	Diplasia karatifolia Rich.	*Lepironia articulata* Domin	*Exocarya scleriodes* Benth	*Mapania pycnostachya* (Benth.) T.Koyama	*Hypolytrum schraderianum* Nees
Width (µm) of spicoid primordium	128.3	221.3	78	95.8	72.6
Height (µm) of spicoid primordium	98.9	87.6	41.1	56.1	36.8
Sequence of organ appearance	Centrifugal	Centrifugal	Centrifugal	Centripetal	Centripetal
Spicoid primordium shape	Flattened adaxially and rounded abaxially	Flattened adaxially and rounded abaxially	Flattened adaxially and rounded abaxially	Flattened adaxially and rounded abaxially	Rounded adaxially and abaxially
Number of lateral scales	2	2	2	2	2
Number of lateral stamens	2	2	2	2	2
Number of inner scales	2	20–28	2	2	0
Number of inner stamens	6	12–15	1	0	0

oval apex (Fig. 2c). Subsequently, the apex expands laterally acquiring an elliptical shape and two stamen primordia appear in the lateral position, simultaneously with the early appearance of a dome-shaped gynoecium primordium in the centre of the meristem (Fig. 2d). The appearance of the lateral stamens and the gynoecium is followed by the formation of two additional inner scales in adaxial and abaxial positions (Fig. 2e—light green). The former seems to be slightly delayed compared to the abaxial one (Fig. 2e, f—light green).

Shortly after the emergence of the inner scales, three inner stamen primordia arise in abaxial position (Fig. 2f—arrowheads) concomitantly with the three adaxial inner stamen primordia (Figs. 2f; 3a–c—arrows). The adaxial inner stamen primordia appear slightly delayed (Fig. 3d—arrows) when compared to the abaxial ones (Fig. 3d, e—arrowheads). This slight difference disappears soon and no distinction between the six inner stamens is discernible in later developmental stages (Fig. 3f–h). While the lateral scales are keeled and eventually fully enclose the lateral stamens (Fig. 3d–g—dark green), the abaxial and adaxial inner scales are flat, membranaceous, enveloping the inner stamens and partially the lateral ones as well (Fig. 3h—lateral scales removed). Later in development, the spicoid is completely covered by scales (Fig. 4a—inner scales marked in light green; the lateral scales were removed—yellow arrow).

Although the gynoecium primordium arises before the inner scales (Fig. 2d), its development is delayed until the appearance of all stamens (Fig. 3a–c). Only then an annular ovary wall primordium develops surrounding a central depression, and the two stigmas arise as two lateral projections (Fig. 3d–f). The projections elongate upward (Fig. 3g) and the developing ovary wall progressively enclose the central ovule (Fig. 3h), forming a dimerous dorsiventrally flattened gynoecium. In older spicoids, the lateral stamens are about the same length as the gynoecium, while the six inner stamens are shorter (Fig. 4b–d). Each stamen differentiates into a basifixed anther and a filament (Fig. 4c, d). The ovary and style elongate by intercalary growth and papillose protuberances appear along the stigmas (Fig. 4d).

Table 1 shows our results for *Diplasia* compared to the developmental data obtained for other species of Mapanioideae.

Discussion

What are the main developmental variations in Mapanioideae spicoids and how are they influencing the spicoid morphology?

A comparison of our results for *Diplasia karatifolia* with other developmental studies for Mapanioideae (including *Exocarya sclerioides* (Richards et al. 2006), *Lepironia articulata* (Prychid and Bruhl 2013), *Mapania pycnostachya* and *Hypolytrum schraderianum* (Monteiro et al. 2016), shows that the main developmental variations

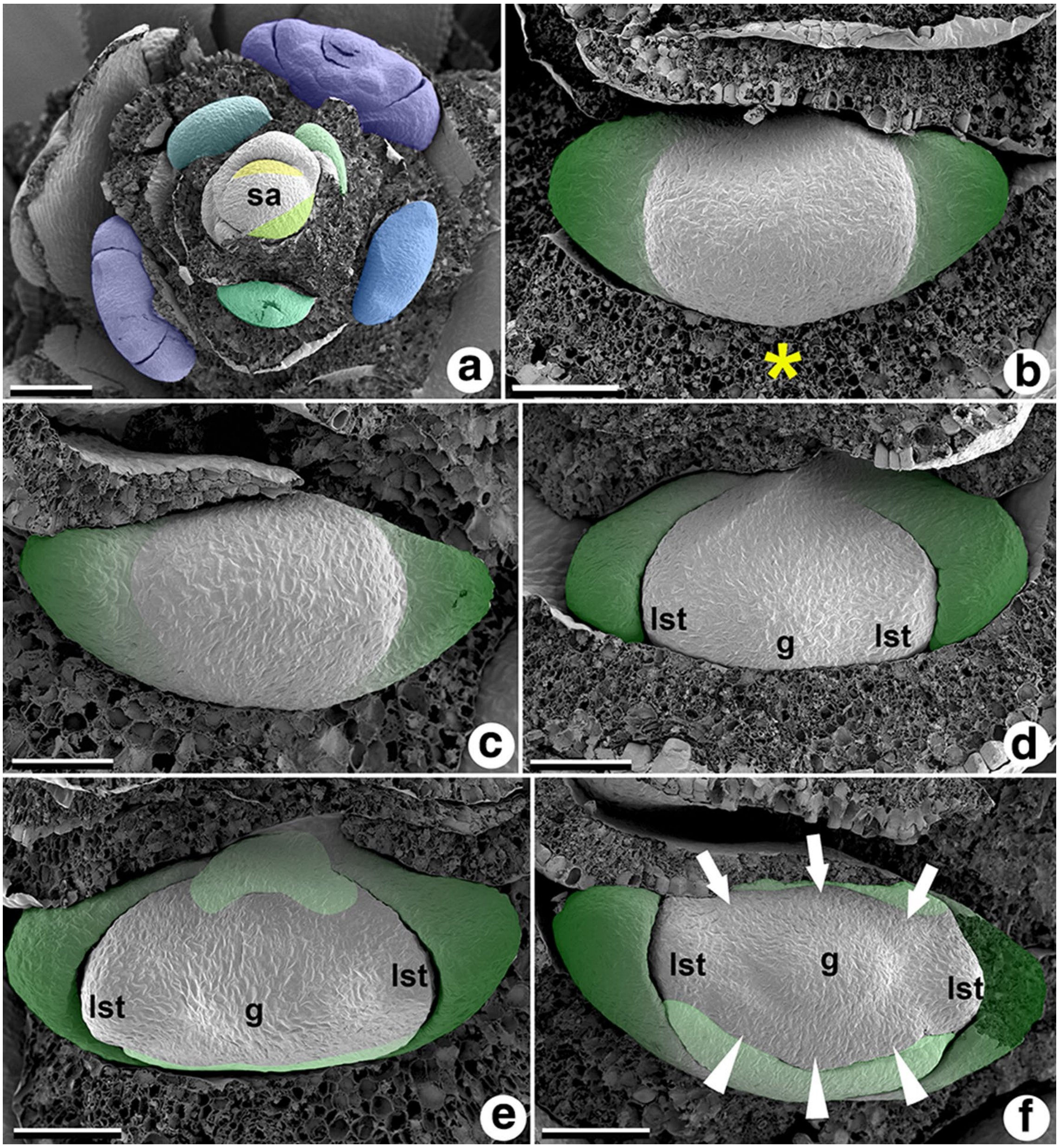


Fig. 2 Scanning electron micrographs of the spicoid of *Diplasia karatifolia* at successive developmental stages. **a** Spike showing the spiral arrangement of the spicoids (spicoids coloured in a gradient sequence from blue (older) to yellow (younger)). **b**,**c** Formation of the two lateral scales (dark green); the subtending bract was removed (asterisk). **d** Emergence of two lateral stamens and a central rounded gynoecium primordium. **e** Formation of the two inner scales (light green). **f** Three inner stamen primordia initiate in abaxial position (arrowheads) concomitantly with the adaxial ones (arrows). *g* gynoecium; *lst* lateral stamen; *sa* spike axis. Bars = 100 µm (**a**); 50 µm (**b**–**f**)

among the species are related to the sequence of organ appearance, the size and shape of the spicoid primordium and the way the subtending bract constraints are distributed in such primordium. Such variations are possibly some of the factors leading to changes in the number and position of organs to be formed. The influence of such variations in the final spicoid morphology will be discussed below.

Temporal sequence of organ formation

Concerning the sequence of organ appearance, it is important to highlight that in *Diplasia* the spicoids show centrifugal/basipetal tendencies, because of the early appearance of the gynoecium, simultaneously with the two lateral stamens and before the appearance of the inner scales and the six inner stamens in ab- and adaxial positions. The centrifugal sequence was also found in the spicoid

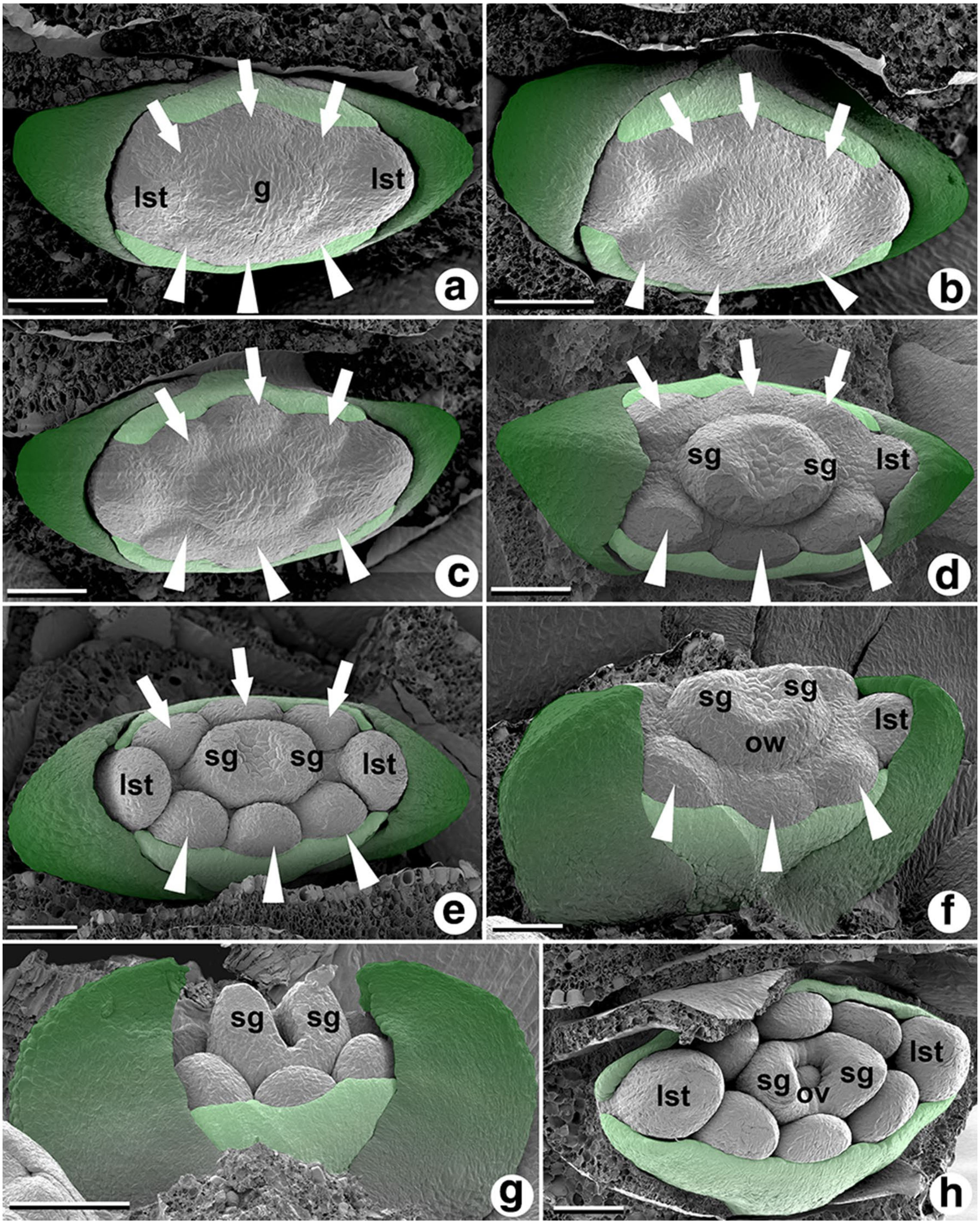


Fig. 3 Scanning electron micrographs of the spicoid of *Diplasia karatifolia* at successive developmental stages. **a**–**c** Ongoing growth of the stamen primordia in adaxial position (arrows), as well as the abaxial ones (arrowheads). **d**,**e** Elongation of the lateral scales (dark green) that partially enclose the lateral stamens. The gynoecium development continues with the emergence of two lateral stigma primordia. **f**,**g** The lateral scales (dark green) completely cover the lateral stamens. The young stigmas grow upward. **h** The inner scales (light green) elongate to the sides and envelop the inner stamens and partially the lateral stamens. The ovary starts to close over the central ovule. *g* gynoecium; *lst* lateral stamen; *ov* ovule; *ow* ovary wall; *sg* stigma. Bars = 50 µm (**a**–**f; h**); 100 µm (**g**)

development of *Exocarya* (Richards et al. 2006) where the lateral stamens and gynoecium develop before the inner scales and inner stamen. For *Lepironia articulata*, the lateral and inner stamens emerge simultaneously (Prychid and Bruhl 2013), and a distinction in their timing of appearance is not as clear as in *Diplasia* and *Exocarya*.

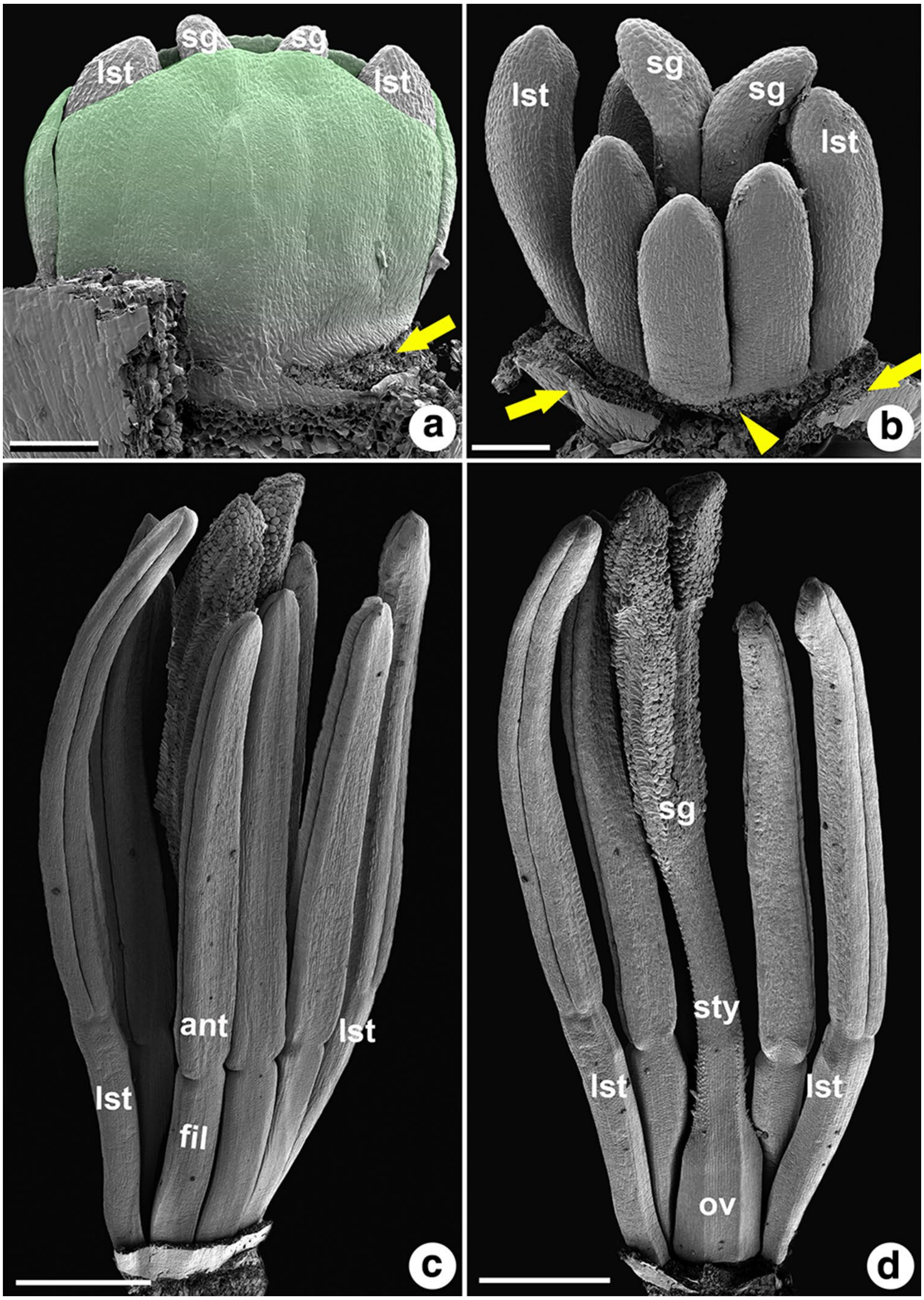


Fig. 4 Scanning electron micrographs of the spicoid of *Diplasia karatifolia* at successive developmental stages. (**a**, **b**) Lateral views of the spicoid. **a** The lateral scales were removed (yellow arrow) to show the inner scales (light green) elongated and enclosing the spicoid. **b** The lateral (yellow arrows) and inner (yellow arrowheads) scales were removed to show the distinction in the height of the lateral stamens and gynoecium, compared to the inner stamens. **c** The mature stamens are differentiated into a basifixed anther and a filament. **d** Mature spicoid with the scales and three abaxial inner stamens removed to show the dimerous dorsiventrally flattened gynoecium with papillose stigmas. *ant* anther; *fil* filament; *lst* lateral stamen; *ov* ovary; *sg* stigma; *sty* style. Bars = 100 µm (**a**, **b**); 0,5 mm (**c**, **d**)

However, in *Lepironia*, a primordial gynoecium becomes apparent before all other organs, remaining undeveloped until all stamens and scales start to develop (Prychid and Bruhl 2013). Later in the development, the lateral stamens show a distinct bigger size than the inner ones (Prychid and Bruhl 2013). Therefore, although the extremely short timing interval of organs emerging may hamper the clear understanding towards the sequence that they appear, the early development of the gynoecium primordium before the remaining organs in *Diplasia*, *Exocarya* and *Lepironia* suggests that organs are formed in a centrifugal sequence (Table 1).

Such sequence is quite distinct when comparing the spicoid development of the above cited genera with *Hypolytrum* and *Mapania* spicoids (tribe Hypolytreae). For the latter genera, the spicoid development follows a centripetal/acropetal sequence of organ formation (Monteiro et al. 2016), where the lateral scales are the first to appear, followed by the stamens, inner scales and at last, the gynoecium. For such species, no sign of gynoecium primordium is seen before the emergence of stamens and inner scales (Monteiro et al. 2016). Such sequence has also been observed in Cyperoideae flowers (Vrijdaghs et al. 2009, 2010).

Despite the differences, for all species the lateral scales are the first organs to appear, with the longest timing of emergence between the organs, which was interpreted as an evidence for the hypothesis that they are foliar structures connected to an axis (Richards et al. 2006; Prychid and Bruhl 2013; Monteiro et al. 2016). Richards et al. (2006) reported the similarity of the lateral scales with a two-keeled prophyll and the interpretation of an extra-floral nature of the lateral scales was reinforced by the absence of floral proteins in such structures in *Lepironia* (Prychid and Bruhl 2013).

Moreover, we can verify that the inner scales in *Diplasia* are two solid structures enveloping the inner stamens, as stated by Clarke (1909). *Diplasia* spicoids were described either as having two inner scales (Clarke 1909) or as having six inner scales that fuse three by three and give only the impression of being two scales in the mature spicoid (cf. Bentham 1877; Simpson 2006). The hypothesis of such scales being a result of a fusion of initially free scales (Bentham 1877; Simpson 2006) was not supported by our results, since only two inner scale primordia were observed with no fusion of parts during development.

Size and shape of the spicoid primordium

For Mapanioideae, we observed that the size and shape of the spicoid primordium vary among the genera (Table 1). *Diplasia*, *Lepironia*, *Exocarya* and *Mapania* show primordia that are laterally expanded, adaxially flattened and abaxially rounded, which means more available space for organs to initiate on median size (Richards et al. 2006; Prychid and Bruhl 2013, Monteiro et al. 2016). This is distinct from the rounded shaped primordium of *Hypolytrum* (Table 1) (Monteiro et al. 2016). From the above cited genera, *Diplasia* and *Lepironia* show a larger primordium in size when compared to the other genera (Table 1). Remarkably, the spicoid in *Diplasia* possesses eight stamens, and in *Lepironia* (Prychid and Bruhl 2013) spicoids are composed of 12-15 stamens and two whorls of 20-28 inner scales (Table 1). Alternatively, in *Exocarya* and *Mapania* the spicoid primordium is considerably smaller (Table 1), as well as in *Hypolytrum*, exhibiting spicoids with lower number of organs when compared to *Diplasia* and *Lepironia* (Table 1). We observed that the larger and elliptical spicoid primordia in *Diplasia* and *Lepironia* exhibit more space on the median size and an increase in the number of median stamens when compared to the smaller spicoid primordia of *Exocarya*, *Mapania* and *Hypolytrum*. Since the number of organs to be formed is known to be influenced by the existing space on the primordium (Claßen-Bockhoff and Meyer 2016) and based on the notable relation of spicoid primordium size and shape with the number of organs in the genera mentioned above, we considered the hypothesis that the variation in the number of inner stamens and scales in Mapanioideae spicoids is possibly regulated by the available space in the primordium. Although available space is not the only process regulating the number of organs in a reproductive unit (Chandler 2014; Claßen-Bockhoff and Meyer 2016), previous studies has shown the correlation between an increase in the number of median organs with the expansion of the primordium size (Doust 2001; Doust and Drinnan 2004; Ronse De Craene 2013; Dos Santos and Ronse De Craene 2016). Such correlation was here observed and should be tested in further Mapanioideae genera.

Spatial constraints applied by the subtending bract

It is known that the shape of flower primordium is affected by gene interactions regulating the identity of floral organs as well as by the spatial constraints applied by surrounding organs such as bracts, contributing to the primordium geometry and possibly leading to new morphs (Chandler 2014; Ronse De Craene 2018). In Cyperaceae, inflorescences are commonly composed of several reproductive units arising in the axil of bract-like structures, which grow to envelop the whole unit (Bruhl 1995; Goetghebeur 1998; Vrijdaghs et al. 2009). The position of such bracts affects not only the morphology of the inflorescence itself, but it also seems to influence the morphology of floral organs as well (Vrijdaghs and Smets 2017).

Although Cyperoideae flowers and Mapanioideae spicoids are not homologous units, in both cases the reproductive organs arise as a primordium in the axil of a bract-like structure, which is in contact with the abaxial side of the primordium and opposite to the main axis (Bruhl 1995; Goetghebeur 1998; Vrijdaghs et al. 2009). In Cyperoideae, the reduction of floral parts commonly occurs on the abaxial side, which may be caused by spatial constraints by the glume (i.e. Vrijdaghs et al. 2009; Lucero et al. 2014; Monteiro et al. 2017). In Mapanioideae, it is notable that median organs (inner scales and inner stamens) are positioned exclusively or in a higher number on the abaxial side and reductions seem to occur on the adaxial side (Richards et al. 2006; Prychid and Bruhl 2013). In *Exocarya*, the only inner stamen present is the abaxial one (Richards et al. 2006) and for *Lepironia*, a higher number of inner scales and inner stamens is reported on the abaxial side, while the adaxial side always shows only five stamens each with its own scale (Prychid and Bruhl 2013). The latter authors considered that in the beginning of spicoid development in *Lepironia*, the primordium expands laterally, and a restriction of growth is observed in the adaxial side (Prychid and Bruhl 2013). Such restriction is possibly due to the pressure of the subtending bract on the growing primordium, which results in an asymmetrical shape with a flat adaxial side in contact with the axis, expanded in the laterals and a convex abaxial side. The convex abaxial side provides more available space in the primordium and coincide with the increase in the number of stamens in this region as observed for *Lepironia* (Prychid and Bruhl 2013) and *Exocarya* (Richards et al. 2006). In *Diplasia* a site of reduction in the developing spicoid is not clear, although during some stages of the development we found that the inner adaxial stamens develops slightly slower when compared to the abaxial ones. For *Mapania* and *Hypolytrum*, the spicoids commonly lack median positioned organs (inner scales and inner stamens), however when present, such organs are positioned at the abaxial side, such as the abaxially positioned inner stamen of, e.g. *Mapania holttumi* J. Kern, *Mapania bancana* (Kurz) T.Koyama, *Mapania graminea* Uittien, *Mapania macrocephala* K. Schum. ex Warb. (Simpson 1992) and *Hypolytrum pulchrum* H.Pfeiff. (Kern 1974). Moreover, changes in the number of scales are also observed, where species with larger elliptical primordia exhibit a higher number of scales, such as in *Lepironia* (Prychid and Bruhl 2013) when compared to species with smaller primordia that exhibit few simple scales, such as *Exocarya* (Richards et al. 2006) and *Mapania* (Monteiro et al. 2016).

It is notable that in addition to genetic factors regulating the organ position, spatial constraints caused by the bracts may influence different sides of the primordium and affect its geometry (Ronse De Craene et al. 2002; Ronse De Craene 2010, 2018; Chandler 2014). Changes in organ number and arrangement were previous correlated with variation in the size and symmetry of floral primordium (Doust 2001; Doust and Drinnan 2004). In *Drimys Winteri* J.R. and G. Forst. (Winteraceae), the elliptical primordium of lateral flowers is affected by the constraints of the subtending bract and the development of organs initiate towards the poles of the primordium, where more space is available (Doust 2001). The author highlights the strong influence of development constraints in number and arrangement of floral organs. In species of *Lewisia* Pursh (Montiaceae), the elliptical shape of the primordium caused by the pressure of the bract was linked with an increase of available space on median size allowing an increase in the number of median stamens (Dos Santos and Ronse De Craene 2016). Here, we hypothesize that the constraints applied by the subtending bract in Mapanioideae spicoids may influence the shape of the primordium, restricting the growth of parts on the flat adaxial side and allowing the variability on the number of stamens and scales in lateral and abaxial sides of the spicoids in the subfamily.

How stable and informative are the developmental features among Mapanioideae genera?

Combining our results for *Diplasia* with developmental studies of spicoids in other genera (Richards et al. 2006; Prychid and Bruhl 2013; Monteiro et al. 2016), and the phylogenetic relationships among these genera (Semmouri et al. 2019), some features appear to be consistent among genera of the same tribe (Fig. 5).

In Chrysitricheae, *Diplasia*, *Lepironia* and *Exocarya* show spicoids with organs emerging first in the centre and laterals simultaneously and before the remaining median positioned organs. Furthermore, *Diplasia* and *Lepironia* have a larger spicoid primordium and a higher number of organs in such spicoids, compared to other Mapanioideae. The presence of spicoids with a higher number of stamens and inner scales is a notable feature in Chrysitricheae genera, with genera such as *Chrysitrix* L. exhibiting spicoids with ca. 150 stamens (Clarke 1909). Considering the developmental similarities of *Diplasia* with *Exocarya* and *Lepironia*, our results support the systematic placement of *Diplasia* close to both genera, in Chrysitricheae (Semmouri et al. 2019).

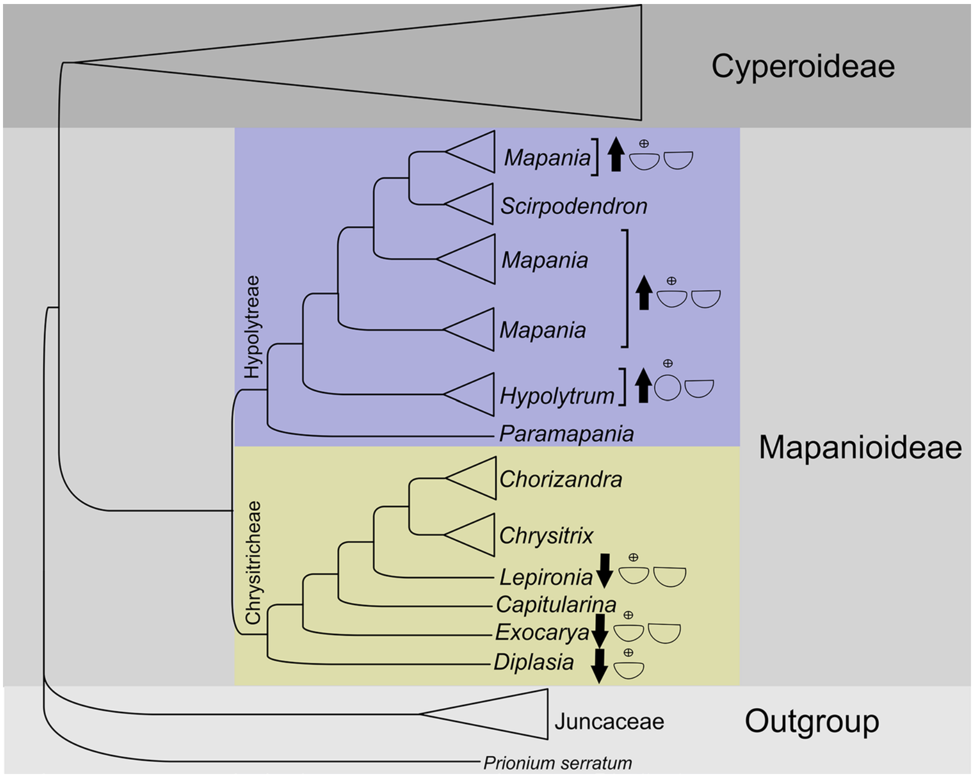
In Hypolytreae, *Hypolytrum* and *Mapania* exhibit spicoids with organs appearing in an acropetal sequence in the developing spicoid and a lower number of parts (Monteiro et al. 2016) when compared to Chrysitricheae genera. Spicoids with lower number of parts appear as a common feature in Hypolytreae genera, as for instance in *Paramapania* Uittien with spicoids consisting of the spicoid axis, two lateral scales, two lateral stamens, three inner scales, a single inner stamen, and a centrally trimerous gynoecium (Vrijdaghs et al. 2006), as well as in *Principina* Uittien with spicoids consisting of the spicoid axis, two lateral scales, two lateral stamens, one inner scale, one inner stamen, and a trimerous gynoecium (Mesterhazy and Browning 2014).

While the genera of each tribe seem to share some developmental features, the presence of inner scales and inner stamens exclusively or in a higher number on the abaxial side of the spicoid seems to be a common feature for Mapanioideae species. Furthermore, variation is found within the tribes, as for instance *Mapania pycnostachya* that shares developmental features with *Hypolytrum schraderianum* (Monteiro et al. 2016), as well as with *Exocarya* (Richards et al. 2006). Therefore, more data on other Mapanioideae species are necessary to obtain a more comprehensive picture of the developmental features that are informative for the phylogenetic relationships in the subfamily.

Interpretations of the nature of the *Diplasia* spicoid

The spicoid in *Diplasia* can be interpreted as a dimerous flower (Fig. 6a). Alternatively, it can be interpreted as a reduced inflorescence with four male flowers and a central

Fig. 5 Simplified cladogram of Cyperaceae, adapted from Semmouri et al. (2019), showing the relationships among Mapanioideae genera. The genera highlighted in blue correspond to tribe Hypolytreae and the genera highlighted in yellow correspond to tribe Chrysitricheae. The arrow downwards represents the presence of spicoids with organs appearing first at central + lateral position (centrifugal) and the upwards arrow represents the spicoids with organs appearing first at lateral and median position (centripetal). The shape of the spicoid primordium is represented by a rounded ab- and adaxially shape and a flattened adaxially and rounded abaxially shape, the circle filled with a cross indicate the position of the axis. The reduction and/or loss of organs in the adaxial side is represented by a circle without the upper half



female flower (Fig. 6b). Here, we will discuss the arguments pro and contra of both hypotheses (flower and inflorescence).

Diplasia *spicoid as a flower*

Regarding the floral interpretation, the spicoid can be interpreted as a dimerous flower with four perianth parts (two outer – dark green and two inner parts – light green), eight stamens and one dimerous dorsiventrally flattened gynoecium (Fig. 6a). The interpretation of a dimerous flower is based in the decussate arrangement of the dimerous whorls (Ronse De Craene and Smets 1994; Ronse De Craene 2010, 2016). Although Cyperoideae usually exhibit trimerous flowers (Fig. 6c) (Vrijdaghs et al. 2009, 2010, 2011), in Mapanioideae this pattern is often altered and dimerous arrangement is reported in the reproductive units of the subfamily (Goetghebeur 1998), as well as for other Poales (Remizowa et al. 2010).

In favour of the floral interpretation we may point out the central position and early development of the gynoecium simultaneous with the lateral stamens, the absence of scales between the stamens and the gynoecium, and the absence of vestiges of a rachilla. However, a few aspects raise questions towards the interpretation of the *Diplasia* spicoid as a flower: the nature of the lateral scales and the ontogenetic sequence and position of the inner scales (both interpreted as perianth parts).

Considering the spicoid as a flower hypothesis, the lateral scales are interpreted as outer perianth parts, however during development such scales show a relative long difference in timing of appearance when compared to the other organs. Although plastochrons are quite variable and challenging to interpret, it is noteworthy that in *Diplasia* and other Mapanioideae genera (Richards et al. 2006; Prychid and Bruhl 2013; Monteiro et al. 2016) such long difference in timing of appearance of the lateral scales compared to the remaining organs is a stable feature, resembling the development of an extra-floral structure, as observed for the glumes of Cyperoideae flowers, which appear first and develop earlier than the remaining floral organs (Vrijdaghs et al. 2005, 2007, 2009, 2011). The extra-floral nature of the lateral scales is reinforced by the absence of floral identity in such organs as reported by Prychid and Bruhl (2013) for *Lepironia*, different from the inner scales that showed floral protein localization. Thus, the results support the interpretation of the lateral scales as involucral bracts and not perianth parts.

Furthermore, the sequence of development of stamens and inner scales (interpreted as perianth parts) in *Diplasia* is quite different from perianth development in Cyperoideae flowers. In the *Diplasia* spicoid, the emergence of organs follows the sequence: first the lateral stamens + gynoecium, followed by inner scales and at last, inner stamens. Thus, the emergence of the inner scales alternate with the emergence of lateral and inner stamens. When comparing to flowers in Cyperoideae, although the perianth parts appear after the

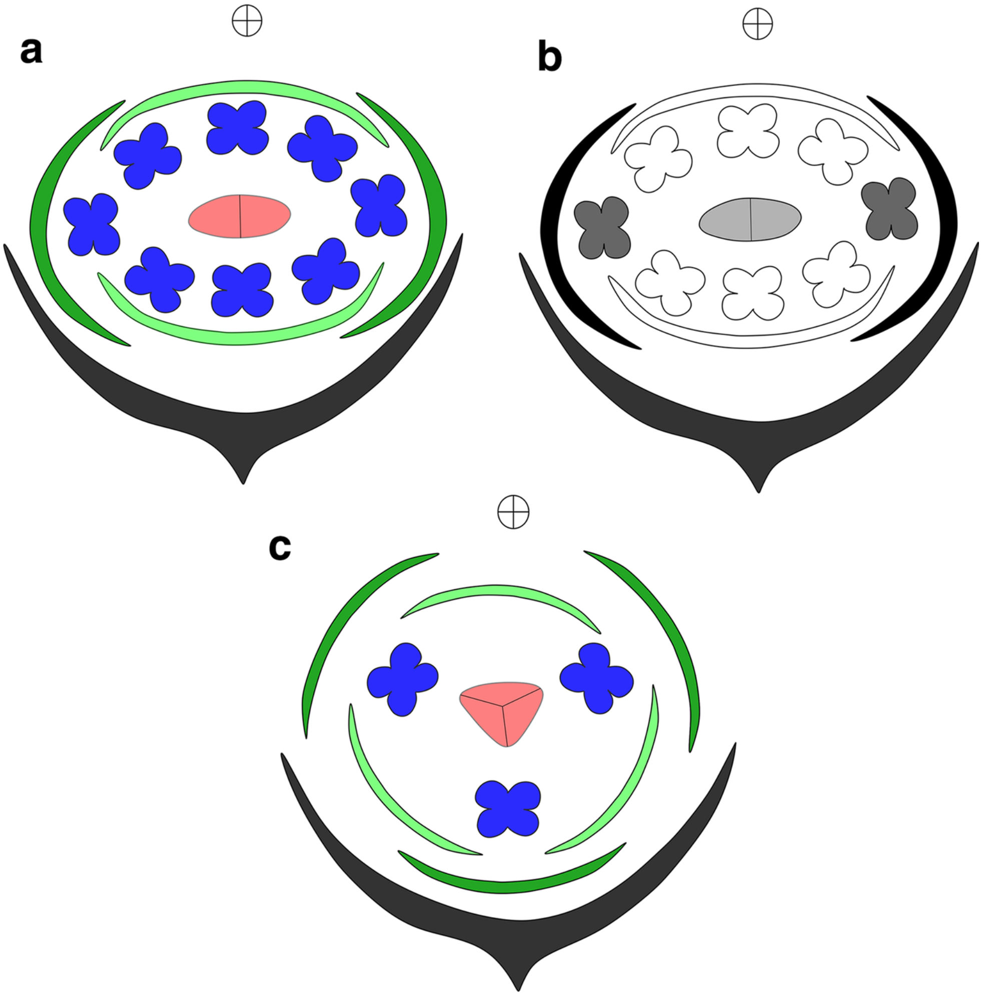


Fig. 6 The distinct hypothesis of interpretation of *Diplasia karatifolia* spicoid as flower or inflorescence. Floral interpretation **a** A dimerous flower with four perianth parts (two outer parts—dark green and two inner parts—light green), eight stamens (blue) and a dimerous dorsiventrally flattened gynoecium (red). Inflorescence interpretation **b** A reduced inflorescence consisting of two involucral bracts (lateral scales—black), two lateral male flowers (composed of one lateral stamen each—dark grey), two male flowers in median position (each with three stamens—white) subtended by a bract (inner scales—white) and a central female flower (gynoecium—light grey). **c** A general Cyperoideae flower, based on Vrijdaghs et al. (2009), showing the usual morphology with a glume (grey) enveloping a flower with three outer perianth parts (dark green), three inner perianth parts (light green), one whorl of three stamens (blue) and a trimerous gynoecium (red)

emergence of the stamens (Vrijdaghs et al. 2009), such parts emerge and develop always positioned extrastaminally with no report of perianth parts developing alternate with stamens (Mora-Osejo 1967; Vrijdaghs et al. 2005a, 2006, 2009). Although plastochrons between organs are short, it is noteworthy that the emergence of the inner scales in between the stamen whorls in *Diplasia* hampers to compare such scales with the development of perianth parts.

Diplasia *spicoid as a reduced inflorescence*

If the spicoid is interpreted as a reduced inflorescence, it consists of two involucral bracts (lateral scales—black), two lateral male flowers (composed of one lateral stamen each—dark grey), two male flowers in median position (each with three stamens—white) each subtended by a bract (inner scales—white) and a central female flower (gynoecium—light grey) (Fig. 6b).

The features mentioned above such as the extra-floral nature of the lateral bracts and development of inner scales alternating with stamens favour the interpretation of the spicoid as a reduced inflorescence. The central position of the female flower suggests that the spicoid is a determinate inflorescence, different from the rest of Cyperaceae, which exhibit indeterminate inflorescences (Goetghebeur 1998; Vegetti 2003; Guarise and Vegetti 2008; Vrijdaghs et al. 2010). Monteiro et al. 2016 reported for *Hypolytrum* and *Mapania* spicoids, despite the central position of the gynoecium suggesting the interpretation of a determinate inflorescence, the acropetal development of organs resembles the sequence of an indeterminate inflorescences. Furthermore, the terminal position of the female flower may be only apparent, in which a displacement of the lateral pedicel of the flower to an erect position may simulate the presence of a terminal flower (Monteiro et al. 2016), as observed in flower-like terminal structures in racemose inflorescence (Sokoloff et al. 2006). In *Diplasia* spicoids, the central position of the gynoecium in addition to its early development before the remaining organs support the interpretation of a determinate inflorescence. The question relies on the fact that although the central gynoecium has an early development, it appears simultaneously with the lateral stamens, hampering a clear definition of the inflorescence interpretation.

Concerning other Mapanioideae genera, the interpretation of spicoids as inflorescences is favoured in spicoids with a high number of stamens, as in *Lepironia, Scirpodendron* Zipp. ex Kurz, *Capitularina* J. Kern and *Chrysitrix*, the latter exhibiting ca. 150 stamens per spicoid, each stamen subtended by its own scale (Clarke 1909). This differs from the typical Cyperoideae morphology where flowers commonly have zero to four stamens (Vrijdaghs et al. 2009, 2010, 2011). Even for genera with reduced spicoids, which resemble a single flower with two stamens and a gynoecium, as in *Hypolytrum* and *Mapania*, features as stamens developing at different levels, disposition of the spicoid organs and organization of vascular tissues resembling an inflorescence axis favour the inflorescence interpretation of such units, which possibly have undergone reduction and loss of structures during evolution (Monteiro et al. 2016). Differences in the developmental sequence of scales in *Exocarya* and perianth parts in Cyperoideae flowers favoured the interpretation of *Exocarya* spicoids as reduced inflorescences (Richards et al. 2006). Based on the same developmental differences, the absence of floral protein in the lateral scales of *Lepironia*, Prychid and Bruhl (2013) favoured the inflorescence interpretation as well.

Diplasia *spicoid as a* de novo *reproductive unit*

Despite the effort of ontogenetic studies to determine the nature of the spicoids as flower or as inflorescence (i.e. Richards et al. 2006; Prychid and Bruhl 2013; Monteiro et al. 2016), arguments pro and contra to the many possibilities in both interpretations sustain the uncertainty towards a satisfactory definition. Such instability highlights the difficulty in distinguishing the boundaries of flower and inflorescence nature in the reproductive units in Mapanioideae. Our observations showed that spicoids in *Diplasia* exhibit features such as simultaneous appearance of the gynoecium and lateral stamens before the remaining organs and the absence of scales between stamens and gynoecium that suggest the floral nature of spicoids. Alternatively, the nature of the lateral scales as extra-floral structures and the emergence of inner scales in between the stamen whorls favour the inflorescence interpretation. The difficulty in distinguishing the limits of flower and inflorescence nature of the spicoids in *Diplasia* raises the possibility of a third hypothesis that the spicoid could be interpreted as a de novo reproductive structure with flower and inflorescence features combined in a single unit. Such possibility may be linked with strongly reduced flowers, consisting of a single organ, reorganised in a pseudanthial unit hampering the clear distinction of the boundaries between flower and inflorescence.

The difficulty in establishing boundaries of flower and inflorescences features in the same unit were previously reported for Malpighiales genera (Gagliardi et al. 2018), as for the cyathium in *Euphorbia* L., possibly due to an overlap of the expression of genes that regulate the flower and inflorescence development, hampering the clear distinction between organs (Prenner and Rudall 2007; Prenner et al. 2009, 2011). This indistinct nature is notable in extremely reduced reproductive units (Sokoloff et al. 2006; Rudall and Bateman 2010; Prenner et al. 2011; Claßen-Bockhoff and Frankenhäuser 2020) where the balance of morphological changes and regulatory genes is reorganised (Rosin and Kramer 2009; de Bruijn et al. 2012; Claßen-Bockhoff and Frankenhäuser 2020). Further studies on Mapanioideae species related to flower and inflorescence gene expression during spicoid development could improve the understanding towards such distinctive reproductive unit, considered a synapomorphy of Mapanioideae.

Conclusions

Our results on *Diplasia* spicoids, compared to studies on other Mapanioideae (*Lepironia, Exocarya, Hypolytrum* and *Mapania*), show that the main developmental variations among the species are related to the sequence of organ appearance, the size and shape of the spicoid primordium and the influence of the subtending bract constraints on such primordium. The difference in size and shape of the spicoid primordium among species contribute to the available space for organs to be formed, influencing their number and position, as well as the spatial constraint of the subtending bract in such primordium may influence reduction of adaxial parts. Both statements are here reported as possible hypotheses to explain the spicoid's morphological variation in Mapanioideae, in addition to genetic regulations. The spicoid developmental patterns support the phylogenetic placement of *Diplasia* in the tribe Chrysitricheae, as it shares more developmental features with *Lepironia* and *Exocarya* than with Hypolytreae species. Although the developmental features of the spicoids appear to be consistent among genera of the same tribe, ongoing research aims to provide more details on the morphology of spicoids among Mapanioideae in an evolutionary framework.

Concerning the nature of the *Diplasia* spicoids, our observations showed that spicoids exhibit features that are in favour of both floral, as well as the inflorescence interpretation. Arguments pro and contra to the many possibilities in both interpretations sustain the uncertainty towards a satisfactory definition, and highlight the difficulty in distinguishing the boundaries of flower and inflorescence nature in the reproductive units of Mapanioideae. Such indistinct nature raises a third hypothesis that the spicoid could be interpreted as a de novo structure with an unclear morphological boundary between flower and inflorescence features, possibly due to a reorganisation of regulatory genes, resulting in a single unit with mixed identity considered a synapomorphy of Mapanioideae.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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