Floral developmental and anatomical evidence clarify the causes for the intrinsic floral morphological variation in Cyperoideae (Cyperaceae): some case studies

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Abstract

Cyperaceae is a widespread family composed of two subfamilies, Mapanioideae and Cyperoideae. As opposed to the dimerous Mapanioideae spicoid inflorescences, Cyperoideae flowers are known for their typical trimerous Bauplan, however, morphological variation is reported in each floral whorl. This wide range of variation is challenging, mainly regarding taxonomic descriptions and accessing morphological homologies to understand Cyperoideae evolutionary relationships. Therefore, it is compelling to investigate floral morphology variation in the subfamily, particularly in species belonging to the most diverse genera, *Carex*, *Cyperus* and *Rhynchospora*, as potential models to explore the main floral variations found among Cyperoideae. Thus, we aimed to investigate the floral anatomy and development of species belonging to these genera to clarify following questions: 1) the ontogenetic sequence of the *Rhynchospora* perianth, 2) floral sexuality within *Cyperus* spikelets, 3) the increase on the number of stamens in *Carex* male flowers and 4) the pistil morphological variation in Cyperoideae flowers. We reported a distinct ontogenetic sequence for the perianth formation in *Rhynchospora*, highlighting a different developmental pattern for the Cyperoideae perianth. The presence of bisexual and unisexual flowers within the same spikelet in *Cyperus* was confirmed and needs to be considered in further taxonomic descriptions. The increase on the number of stamens in *Carex* male flowers is caused by the fusion of primordia, corroborated by the presence of several vascular bundles supplying a single filament. Additionally, the presence of male flowers with a dome-shape primordium and a plexus of receptacular bundles were related to features commonly found in monocots inflorescences. This raises questions whether *Carex* male flowers could be interpreted as reduced inflorescences. In general, all species exhibited restrictions on the development of abaxial structures, mainly due to the mechanical constraints applied by the glume, which may be influencing the position of the dimerous dorsiventrally flattened pistil in Cyperoideae flowers.

Introduction

Cyperaceae, the third largest monocot family, is known for its wide distribution, occurring in a variety of environments with the highest concentration in the tropics (Govaerts et al. 2020). This places the family in a pivotal position regarding the understanding and conservation of biodiversity. Added to its wide distribution, the wide range of morphological variation, mainly concerning floral morphology, makes Cyperaceae a remarkable model for ecological, physiological, morphological and molecular studies, in order to understand macroevolutionary events (i.e.; Demeda et al. 2018; Larridon et al. 2021; Monteiro et al. 2022; Semmouri et al. 2019; Spalink et al. 2016 a,b). The family comprises ca. 5600 species in 90 genera distributed in two subfamilies (Hinchliff and Roalson 2013; Larridon et al. 2021; Muasya et al. 2009; Simpson et al. 2007) from which 79 belongs to Cyperoideae and 11 genera belongs to Mapanioideae (Govaerts et al. 2020). Whilst Cyperoideae possess flowers with a typical Bauplan of two trimerous perianth whorls, one whorl of stamens and a trimerous gynoecium (Vrijdaghs et al. 2009), Mapanioideae exhibits a particular reproductive unit named spicoid (Monteiro et al. 2016, 2020, 2022; Prychid and Bruhl 2013; Richards et al. 2006; Simpson 1992).

The variation in floral features of Cyperoideae affects the morphology and number of organs in each floral whorl (Lucero et al. 2014; Reutemann et al. 2015; Vrijdaghs et al. 2009, 2010, 2011). Such variation occurs not only at species level, but also in the same individual, which often hampers the taxon limits and descriptions based exclusively on the mature flowers. This is the case of the most species-rich genera, *Cyperus* L. and *Carex* L., which often exhibit floral morphological variation different from the taxonomic descriptions. *Cyperus* flowers are reported as bisexual, commonly exhibiting a single stamen (Vrijdaghs et al. 2011) and the presence of unisexual flowers is associated to the late detachment of this single mature stamen (i.e. Araujo and Longhi-Wagner 1996; Chen et al. 2009; Ribeiro et al. 2015). However, considering that taxonomic descriptions are based on mature flowers, questions remain whether unisexual flowers are, in fact, strictly unisexual or a result of the late detachment of stamens. Another variation occurs in several species of *Carex*, with flowers exhibiting an increase in the number of stamens within the same species, varying from the typical trimerous Bauplan (Smith 1966; Smith and Faulkner 1976).

Concerning variations in each floral whorl of Cyperoideae, the perianth exhibits a wide range of modifications from foliar to reduced bristle-like structures, ranging from three to eight parts (Bruhl 1995; Goetghebeur 1998; Vrijdaghs et al. 2009, 2010). However, when it comes to the developmental sequence, a stable ontogenetic pattern is reported, where perianth whorls are formed after the stamens and simultaneously with the appearance of the ovary primordium (Vrijdaghs et al., 2009, 2010, 2011). The androecium usually consists of two latero-adaxial and one abaxial stamen, the latter may be reduced or absent (Bruhl 1995; Goetghebeur 1998; Vrijdaghs et al. 2009, 2010) and although reduction is a common tendency, an increase on the number of stamens is also reported (Smith 1966; Smith and Faulkner 1976). The gynoecium varies in shape from trimerous to dimerous dorsiventrally flattened or laterally flattened pistils (Reynders et al. 2012; Vrijdaghs et al. 2009, 2010).

Therefore, in order to assess morphological homologies and understanding the evolutionary relationships in Cyperoideae, it is compelling to investigate the floral morphological variation in the subfamily. Considering that the studied species belong to some of the most diverse genera of Cyperaceae, they are potential models to explore the main variations found among Cyperoideae flowers. Therefore, combining anatomical and developmental analyses of flowers of three species we aim to answer following questions: (1) Does the perianth of *Rhynchospora* follow the typical developmental pattern described for Cyperoideae flowers? (2) Are the unisexual flowers in *Cyperus* strictly unisexual? (3) What are the processes involved in the increase of the number of stamens in *Carex* male flowers? (4) What is the morphological evidence behind the pistil variation in Cyperoideae flowers?

Material and methods

Morphological sampling - Spikelets of *Rhynchospora sparsiflora* (Kunth) L.B. Sm. were collected in the Reserva Florestal Adolpho Ducke (Manaus, Amazonas, Brazil), in the humid understorey of the Amazon rainforest. Spikelets of *Carex brasiliensis* A. St.-Hil. were collected in altitude fields (with grassland vegetation) in the Parque Nacional do Itatiaia (Itatiaia, Rio de Janeiro, Brazil). Spikelets of *Cyperus surinamensis* Rottb. were collected in a secondary forest at the University of São Paulo campus (São

Paulo, SP, Brazil). The spikelets of *Cyperus* are grouped in fascicles, which are of three types: prophyllar, serial and mixed (Guarise and Vegetti 2008) and in this study, we used spikelets from prophyllar fascicles (axillary spikelets with a prophyll at their base). For each species, we sampled at least five individuals, from at least two populations. Vouchers were deposited in the herbarium SPF (University of São Paulo – Brazil) (*Rhynchospora sparsiflora* – MM/AG/JE 248; *Carex brasiliensis* – MM/LL 257; *Cyperus surinamensis* – MM 259).

Light and Scanning Electron Microscopy - Inflorescences and flowers 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. Inflorescences with mature flowers were dissected under a Leica EZ4 stereomiscroscope (Leica Microsystems, Wetzlar, Germany) and photographs were taken using a Leica DFC 320 camera device coupled to stereomicroscope Leica MZ8, using Scan System Images (IM50).

Fixed spikelets at different developmental stages were dissected under a Zeiss Stemi SV6 stereomicroscope (Zeiss, Oberkochen, Germany). The material was dehydrated through an ethanol-acetone series, critical-point dried using CO_2 in a k850 KPD critical-point drier (Quorum Technologies, Kent, UK), coated with platinum using an Emitech k575x Sputter Coater (Quorum Technologies, Kent, UK) and examined with a LEO Supra 55VP scanning electron microscope (Zeiss, Oberkochen, Germany). In addition, transverse anatomical sections of the flowers were made; the samples were subjected to a *tert*-butyl alcohol dehydration series and embedded in Paraplast® (Leica Microsystems Inc., Heidelberg, Germany). The embedded material was sectioned at 5–7 μm on a Leica RM2145 rotary microtome (Leica Microsystems, Wetzlar, Germany), stained with astra blue and safranin (Gerlach 1984) and mounted on slides with Permount resin (Fisher Scientific, Pittsburgh, United States). Light microscope micrographs were taken using a Leica DFC 320 camera coupled to a Leica DMLB microscope, using Scan System Images (IM50).

The diagrams illustrating the spikelet architecture of each species were made using the program Corel Draw Graphics Suite X7.

Results

Rhynchospora sparsiflora - Lateral spikelets in this species are composed of an indeterminate rachilla in the axil of a subtending glume, where the most proximal phyllome of the axis is a sterile prophyll followed by five to nine sterile glumes and further distal glumes with flowers arising in their axils (Fig. 1a, b). The flowers are bisexual and possess a perianth consisting of two whorls of three bristles each, one whorl of three stamens and a dimerous dorsiventrally flattened pistil (Fig. 1c). The glumes emerge in a spirodistichous sequence on the rachilla (Fig. 2a). The flower primordium arises in the axil of a glume and has an elliptical shape with flattened adaxial and lateral sides and rounded abaxial side, acquiring a triangular shape (Fig. 2a – coloured).

Two latero-adaxial perianth primordia appear followed by the third one in abaxial position on the early developing flower that keeps a triangular shape (Fig. 2b − arrows). The abaxial one remains undeveloped during the appearance of subsequent organs (Fig. 2c, d, f − arrow). The outer perianth is followed by the emergence of a larger adaxial inner perianth primordium (Fig. 2b −arrowhead), followed by two less developed abaxial ones (Fig. 2c-e − arrowhead). Two adaxial stamen primordia appear slightly before the abaxial stamen and after the emergence of the outer perianth whorl (Fig. 2b-d). The development of the inner perianth whorl is delayed compared to the stamens (Fig. 2e, f), and the perianth parts are visible as small floral appendages. The abaxial perianth parts and the abaxial stamen also have a delayed development as compared to the adaxial ones (Fig. 2g). Each stamen differentiates into a basifixed anther and a filament (Fig. 2h). The development of the abaxial outer perianth part is arrested and visible as a small appendage at maturity (Fig. 2i − asterisk). After the initiation of perianth and stamens, the floral apex differentiates into an elliptical ovary wall primordium surrounding a central depression from which a central ovule develops, and two stigmas appear as two lateral projections (Fig. 2e, f, g). The gynoecium assumes a dorsiventrally flattened shape during development. in later development, the perianth parts develop into papillose bristles (Fig. 2h, i).

Cyperus surinamensis - The spikelets of *Cyperus surinamensis* are composed of a hyaline prophyll, smaller and thinner than the distal fertile glumes (Fig. 1d, e). Most of the flowers are female, composed of one pistil (Fig. 1f, g); bisexual flowers, composed of one stamen and one pistil, occur randomly in the spikelet (Fig. 1d - arrow). The glumes are distributed in a distichous arrangement on the rachilla (Fig. 3a). The flower primordium arises in the axil of a glume in which the lateral sides expand at the base and not only envelop but also fuse to the rachilla (Figs. 1f − asterisks; 3a, b − arrows). During development, the glumes elongate and partially envelop the alternate new formed glumes (Fig. 3c − arrow). In the female flower, the floral development starts with the appearance of an elliptical flower primordium, flattened adaxially and rounded laterally and abaxially (Fig. 3c - coloured). In the bisexual flower, a lateral stamen develops concomitantly with the gynoecium (Fig. 3d). The gynoecium primordium differentiates into a circular ovary wall primordium surrounding a central depression from which a central ovule develops (Fig. 3e). On this ring the stigmas appear as three projections, the abaxial one seems to be a little less developed than the latero-adaxial ones (Fig. 3e, f - triangle). The stigmas are lifted upwards by the growth of the ovary wall, progressively enclosing the central ovule (Fig. 3f). Papillose protuberances appear along the developed stigmas (Fig. 3g).

Carex brasiliensis - Two to three female inflorescences surround a terminal male spikelet (Fig. 4a). The male flower is subtended by a glume (Fig. 4b) while the female flower is surrounded by a glume and a modified prophyll (Fig. 4c − dotted circle), thus interpreted as a spikelet. In this case, each female flower constitutes a female spikelet itself. The whole architecture represents a spike of spikelets (Fig. 4c). In both cases, the glumes are arranged spirally (Fig. 4d, e). The male flowers exhibit a variation on the number of stamens between four (Fig. 5a), five (Fig. 5b) and six stamens (Fig. 5c), which may be free (Fig. 5a) or fused by their filaments (Fig. 5b, c - arrow). The male spikelet exhibits a rachilla with flowers developing in the axil of glumes, which exhibit a narrowing apex (Fig. 6a, b - arrows). The flower primordium is dome-shaped, rounded abaxially and adaxially, from which two lateral primordia appear

first (Fig. 6b), followed by an abaxial and an adaxial primordium (Fig. 6c). All observed male flowers start the development with four stamen primordia (Fig. 6b, c) that will develop and may (or not) split at the level of the anthers resulting in flowers with four or more stamens.

The female spikelets exhibit a dome-shaped spikelet primordium, rounded abaxially and adaxially, arising in the axil of a glume (Fig. 6d - arrow). A prophyll primordium appears as a broken ring expanding around the dome-shaped spikelet primordium (Fig. 6E – coloured yellow). The two margins of the prophyll connect on the adaxial side (Fig. 6f) and expand as a tube (Fig. 6g, h, j). The floral primordium apex differentiates into an annular ovary wall and the stigmas appear as two latero-adaxial and one abaxial projections (Fig. 6f). These projections surround a central ovule (Fig. 6f) that develops before being completely enclosed by the ovary wall (Fig. 6g). The prophyll develops as a tubular structure, enclosing the entire spikelet, except for the tips of stigmas (Fig. 6h). When removing the prophyll, it is possible to observe a reduced rachilla that never exceeds the prophyll height (Fig. 6i). Later in development, the style and stigmas emerge beyond the tubular prophyll (Fig. 6j).

Vacularization of *Carex brasiliensis* – Regarding the variation on the number of stamens in *Carex brasiliensis* male flowers, a survey of the vascularization was carried out in order to assess additional observations to aid on the interpretation of such structural variation. Independently of the number of stamens, their vascular traces diverge initially from a central plexus of receptacular bundles (Fig. 5d – circle). In flowers with four stamens (Fig. 5a), the anatomy shows the presence of four independent vascular traces, the two lateral diverges first, followed by the abaxial and adaxial vascular traces that remain in the center (Fig. 5e), each observed in separated filaments (Fig. 5f) that will supply an anther (Fig. 5g). In flowers with a higher number of stamens (i.e., five stamens), the divergence of the vascular traces follows the same initial pattern, diverging first to the lateral stamens, leaving a central structure with one abaxial and two adaxial vascular traces (Fig. 5h). At a higher level four filaments are observed, the adaxial one with two vascular bundles (Fig. 5i - arrowhead). This filament with two vascular traces divides into two separated anthers (Fig. 5j – arrowhead, k).

Discussion

The anatomical and developmental evidence support the morphological floral variation in all studied species. For *Rhynchospora sparsiflora*, a distinct ontogenetic sequence for the perianth formation was reported, highlighting a different developmental pattern for the perianth in Cyperoideae (Vrijdaghs et al. 2009). For *Cyperus surinamensis*, we reported the presence of bisexual and unisexual flowers within the same spikelet, differing from previous taxonomic descriptions (i.e. Araujo and Longhi-Wagner 1996; Chen et al. 2009; Ribeiro et al. 2015). The variation in the number of stamens of *Carex* male flowers was notable and caused by the fusion of primordia, corroborated by the staminal vasculature. Floral variation is supported by the anatomical and developmental evidence in each floral whorl, which is detailed discussed below.

Perianth – The presence/absence and variation on the number of perianth parts is commonly a stable character state for Cyperoideae tribes. For Cypereae (Bauters et al. 2014; Reutemann et al. 2014; Vrijdaghs et al. 2009, 2011), Cariceae (Gehrke et al. 2012; Smith 1966; Smith and Faulkner 1976) and Abildgaardieae (Reutemann et al. 2015) the absence of a perianth is a stable character state, whilst in Eleocharideae, a perianth is always present (San Martin 2014; Vrijdaghs et al. 2009). However, the stability on the presence/absence of perianth within tribe Rhynchosporeae is not a rule where this character state is remarkably labile (Lucero et al. 2014; Monteiro et al. 2017; Vrijdaghs et al. 2009). Moreover, the tribe also exhibits a notable lability on the number of perianth parts. This is the case of *Rhynchospora sparsiflora*, previously placed in *Pleurostachys* and described as exhibiting five perianth parts (Alves and Thomas 2015; Thomas and Alves 2008; Thomas et al. 2013). However, in the present study we observed the development of six perianth bristles, although the outer abaxial bristle is weakly developed. The absence or non-development of the outer abaxial perianth part may be related to the pressure applied by the glume subtending the developing meristem, as previously reported by Lucero et al. (2014). This observation highlights the relevance of developmental studies for taxonomic description, mainly in families with reduced structures such as Cyperaceae.

Whilst the presence and number of perianth parts can be quite labile in Cyperoideae flowers, the sequence of development is reported as a stable pattern, where the formation of perianth parts seems to start after the formation of the stamens and simultaneously with the appearance of the ovary primordium (Vrijdaghs et al. 2009, 2010, 2011). Based on our results, the late formation of a perianth was not observed in *Rhynchospora sparsiflora*, on the contrary, the outer perianth parts starts slightly before all stamens are formed, and before any sign of the ovary primordium. The same pattern was reported for flowers in *Oreobolus* R. Br. (Mora-Osejo 1967), which corroborates a distinct ontogenetic sequence in the perianth from the general pattern described to Cyperoideae (Vrijdaghs et al. 2009, 2010). These previous observations raise the question about the variation in the developmental patterns of the perianth in Cyperoideae, which may be explained by an initial development of perianth primordia, followed by an interruption of such development, and then a regain in growth after the appearance of the stamens. Further investigations are needed in Cyperoideae on this matter.

Androecium – The variation in the number of stamens in Cyperoideae flowers ranges commonly from zero to six stamens per flower (Vrijdaghs et al. 2009, 2010, 2011). Such observation is reinforced here by the variation among studied species, from *Rhynchospora sparsiflora* maintaining the trimerous condition in the androecium, with occasionally bisexual flowers in *Cyperus surinamensis* with a single stamen, and the extreme variation on *Carex brasiliensis* ranging from four to six stamens in the male flowers. The instability of the number of stamens within species is remarkable, which in terms, may hamper the taxonomic description of some species. This is the case of *Cyperus surinamensis*, which is described in taxonomic studies as having bisexual flowers with one stamen (i.e. Araujo and Longhi-Wagner 1996; Chen et al. 2009; Ribeiro et al. 2015). However, most of the flowers of *C. surinamensis* analysed are unisexual, consisting of a single gynoecium, with no vestige of a stamen primordium, confirming that they are strictly female. This is a pattern previously reported for *Cyperus eragrostis* Lam., with no correlation between sex and position of flowers in the spikelet (Barnard 1957). Furthermore, in *Carex*

brasiliensis, male flowers exhibiting four to six stamens differ from the typical flowers of *Carex* which usually exhibit three stamens (Gehrke et al. 2012; Smith 1966; Smith and Faulkner 1976) and seems to be a derived condition, since Cyperaceae flowers ancestral condition is reported as exhibiting a single whorl of three stamens, opposite to the outer perianth whorl (Remizowa et al. 2010; Vrijdaghs et al. 2009).

The reduction of floral parts is a common tendency among Cyperoideae flowers. However, the increase on the number of stamens in male flowers of *Carex brasiliensis* is notable. Considering the development of such flowers, although two or more anthers share the same filament, we did not observe separate primordia fusing later in development. Moreover, we did not report a single primordium splitting into two equal daughter primordia. Therefore, we believe this increase of the number of stamens is not a case of an equal division of a primordium, known as *dédoublement* (Ronse De Craene and Smets 1993). However, the anatomical analyses shed light on such increase. Regarding the stamen vascularization, two patterns are found: 1) For flowers with four stamens, a single vascular bundle supplies each stamen; 2) For flowers with more than four stamens, two or three vascular bundles run into a single filament and supply separate anthers. This latter observation suggests that two or more stamen primordia are congenitally fused, since stamens are usually vascularized by a single vascular bundle (Puri 1951). The same pattern of vasculature was reported previously for male flowers of other species of *Carex* (*Carex flacca* Schreb., *Carex nigra* All. and *Carex panicea* L.) (Smith 1966; Smith and Faulkner 1976).

Although the anatomy clarifies the fused nature of stamens in the male flowers of *Carex brasiliensis*, the reasons concerning such random and extreme variation remain unclear. Here we highlight two distinct interpretations for the increase on the number of stamens in male flowers of *Carex brasiliensis*, 1) the higher number of stamens is due to a secondary splitting of initial primordia; 2) the higher number of stamens is explained by the nature of the male flower of *Carex brasiliensis* as an extremely reduced and condensed spikelet, bearing several male flowers. The first interpretation is based on previous report in other angiosperms exhibiting an increase in the number of stamens, which are commonly basally fused in the mature flower, due to a secondary division of the initial primordia, known as secondary polyandry (Endress 1994; Ronse De Craene 2022). However, in this current study we did not observe any evidence of a secondary splitting of the initial primordia in male flowers primordium. For the second interpretation, three main features here observed is noteworthy: 1) the flowers exhibit a dome-shape primordium, commonly observed in spikelets and differing from the elliptical floral primordium in Cyperoideae (Vrijdaghs et al. 2009, 2011); 2) a plexus with numerous vascular bundles is present in the receptacle, resembling the typical vascular pattern in racemose inflorescence in monocots with spiral phyllotaxis (Remizowa et al. 2013). This pattern differs from the consistent feature observed in Cyperoideae flowers, which is the presence of three main bundles in the receptacle that fuse to form a central vascular plexus before the divergence of floral parts (Blaser 1941a, b; Monteiro et al. 2017; Reynders et al. 2012). Therefore, we hypothesize that such features suggest the nature of the male flower of *Carex brasiliensis* as an extremely reduced and condensed spikelet, bearing four male flowers, each one composed of only one or more (two or three) stamens.

Gynoecium – The gynoecium in Cyperoideae flowers develops from a ring primordium surrounding a central meristematic zone (corresponding to the floral apex) as a result of the congenital fusion of carpels (Reynders et al. 2012). The annular ovary wall primordium was interpreted as causing a break in the strict positional pattern of the stigma lobes and allowing shifts in the number and position of stigmas responsible for the variation in pistil types in Cyperoideae (trimerous, dorsiventrally or laterally flattened dimerous) (Reynders et al. 2012). Considering that the position of styles and stigmas is strongly fixed and predictable in the floral meristem (Ronse De Craene et al. 2002; Ronse De Craene 2022), we believe that new gynoecium morphs are more likely to be a matter of mechanical constraint of the glume, which is in direct contact with the abaxial side of the primordium. In *Cyperus surinamensis* we observed that a ring is formed from which three lobes appear and the abaxial one is slightly less developed than the adaxial ones, probably due to the pressure applied on the gynoecium by the glume. This is also observed in *Rhynchospora sparsiflora*, where spatial constraints seem to be restricting the floral development on the abaxial side (in contact with the glume). Evidence for this are: 1) the undeveloped abaxial perianth part; 2) the delayed development of the abaxial stamen; 3) the presence of only two latero-adaxial stigmas. Such observations reinforce the hypothesis that one of the factors modulating the dimerous dorsiventrally flattened pistil in *Rhynchospora sparsiflora* is the mechanical constraint of the glume.

Conclusions

The present study reveals that the perianth formation starts slightly before all other whorls in *Rhynchospora sparsiflora*, being a distinct ontogenetic sequence from the general pattern described for Cyperoideae. This divergent perianth ontogenetic pattern raises questions whether this feature could be informative for the phylogenetic relationships in Cyperoideae. Our results show that unisexual flowers of *Cyperus surinamensis* are strictly female, with a single gynoecium and no vestige of a stamen primordium. This finding confirms that this species exhibits bisexual and unisexual flowers within the same spikelet and could be used to clarify further taxonomic descriptions.

Although reduction of floral parts is a common tendency among Cyperoideae flowers, the increase in the number of stamens in male flowers of *Carex brasiliensis* is notable. The presence of two or three anthers sharing a single filament is possibly due to the congenital fusion of stamen primordia, corroborated by the presence of more than one vascular bundle in the shared filament. Furthermore, the presence of male flowers with a dome-shape primordium and a plexus with numerous receptacular bundles, resembles the typical pattern in racemose inflorescences in monocots. These observations suggest the nature of male flowers of *Carex brasiliensis* as being an extremely reduced and condensed spikelet bearing four male flowers, each with one or two to three fused stamens. The development of all species investigated showed that mechanical constraints, applied by the glume, restrict the floral development on the abaxial side, which may also modulate the dimerous dorsiventrally flattened pistil in the Cyperoideae flowers.

Declarations

Acknowledgments

Statements and Declarations

Author Contribution Statement

MMM, AO, LRD and DD designed the research, MMM carried out the lab work. All authors integrated and interpreted the results and contributed with further discussion, suggestions, and writing of the manuscript.

Conflict of interest

The authors declare that they have no confict of interest.

Ethical approval

The authors guarantee compliance with ethical standards.

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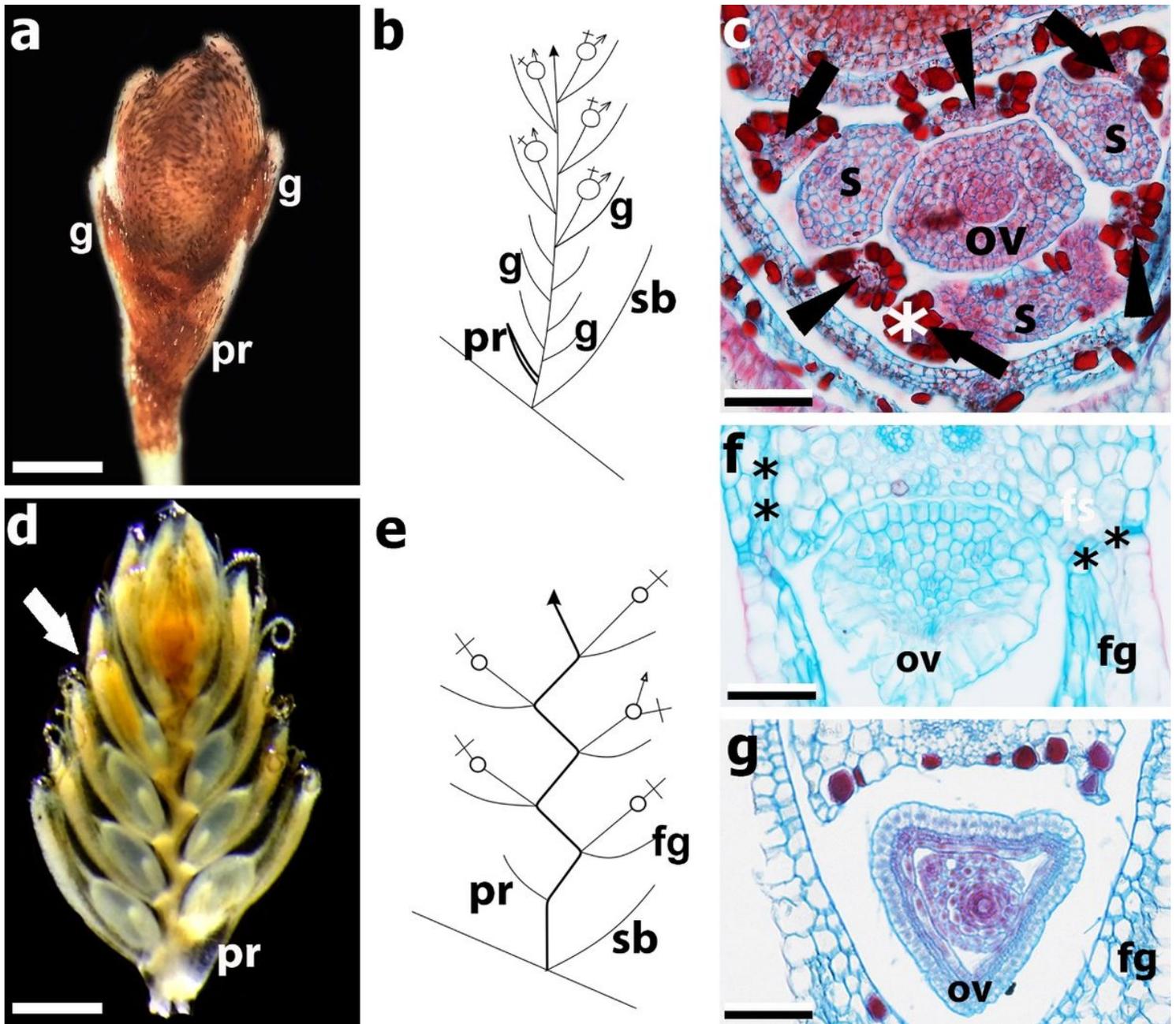
Figures

Figure 1

Structure of spikelets and cross-section of flowers of cyperoid species. **a-c** *Rhynchospora sparsiflora*. **a**, Lateral view of spikelet with glumes showing the presence of a prophyll and glumes. **b**, Lateral presentation of a spikelet showing the position of subtending bract, prophyll and glume. **c**, TS of bisexual flower with outer (arrow) and inner (arrowhead) perianth parts, the abaxial outer perianth part (asterisk) displaced towards the abaxial inner one, three stamens and a gynoecium. **d-g** *Cyperus surinamensis*. **d**, Lateral view of spikelet with glumes showing the presence of a prophyll (pr), the arrow points to a single bisexual flower among the female flowers. **e**, Lateral presentation of a spikelet showing the subtending bract, the prophyll and fertile glumes. **f**, Female flower; note the region of fusion of glume lobes with the rachilla (asterisks). **g**, Female flower subtended by a fertile glume. fg, fertile glume; g, glume; ov, ovary; pr, prophyll, s, stamen; sb, subtending bract. Scale bars: 1mm (**a, d**); 100µm (**c, f, g**)
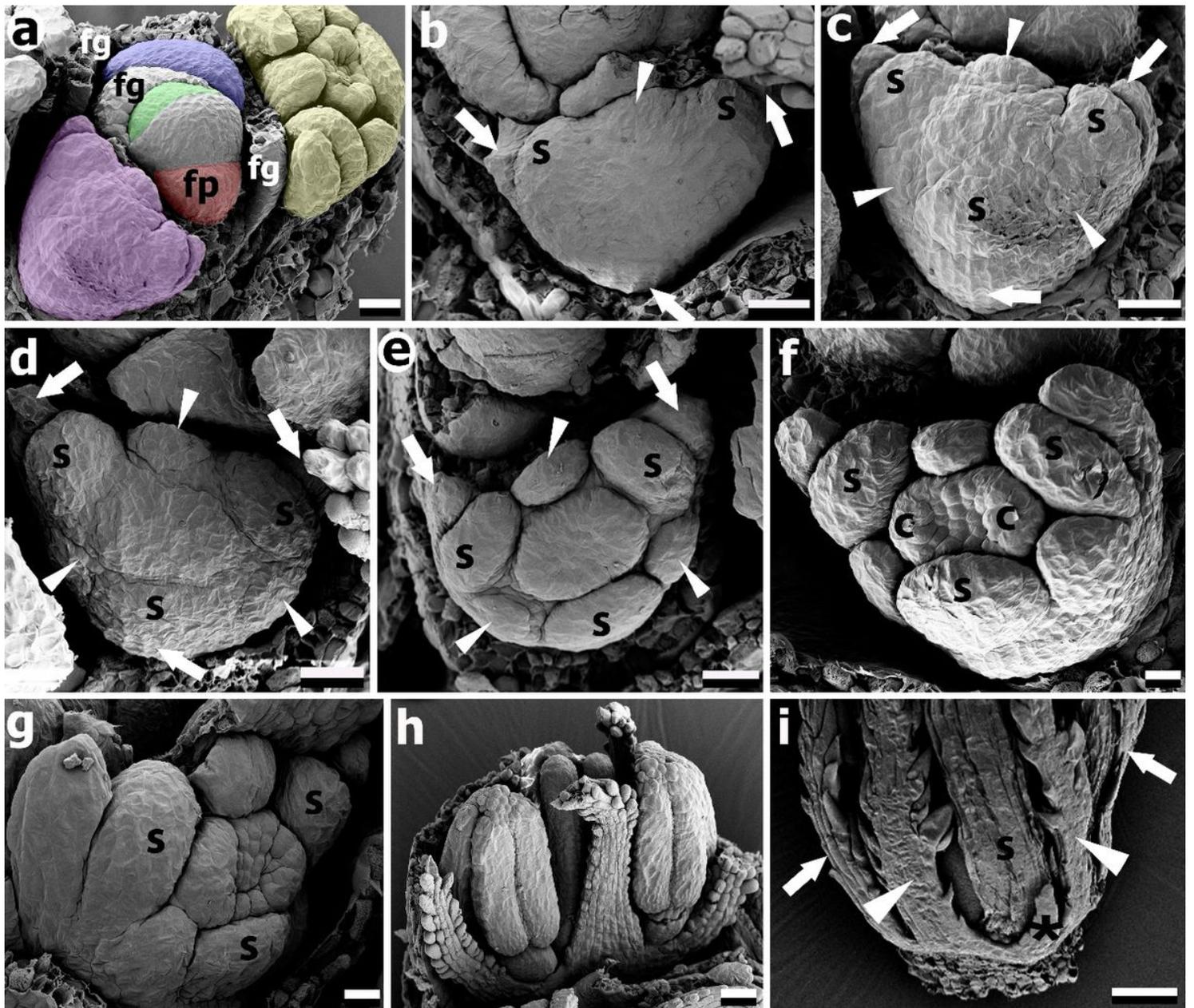

Figure 2

Scanning electron micrographs of the bisexual flower of *Rhynchospora sparsiflora* at different developmental stages. **a**, Spikelet axis with spirodistichous fertile glumes, each subtending a floral primordium (different colours). **b**, Initiation of the outer perianth parts (arrows), the adaxial inner perianth part (arrowhead) and the adaxial stamen primordia. **c-d**, Initiation of the abaxial inner perianth parts (arrowheads) and simultaneous inception of the abaxial stamen primordium. The outer perianth parts are formed (arrows); note that the abaxial outer perianth part is delayed relative to the adaxial ones. **e**, Stamen primordia surrounding a central raised area. **f-g**, Development of the ovary wall primordium surrounding the central ovule. **h**, Mature flower without glume showing stamens with basifixed anthers and papillose structures on the perianth parts. **i**, Mature flower showing the outer (arrows) and inner (arrowheads) perianth parts and a weakly developed abaxial outer perianth part (asterisk). c, carpel lobe; fg, fertile glume; fp, flower primordium; s, stamen primordium. Scale bars: 20µm (**a**); 10µm (**b-h**); 100 µm (**i**)
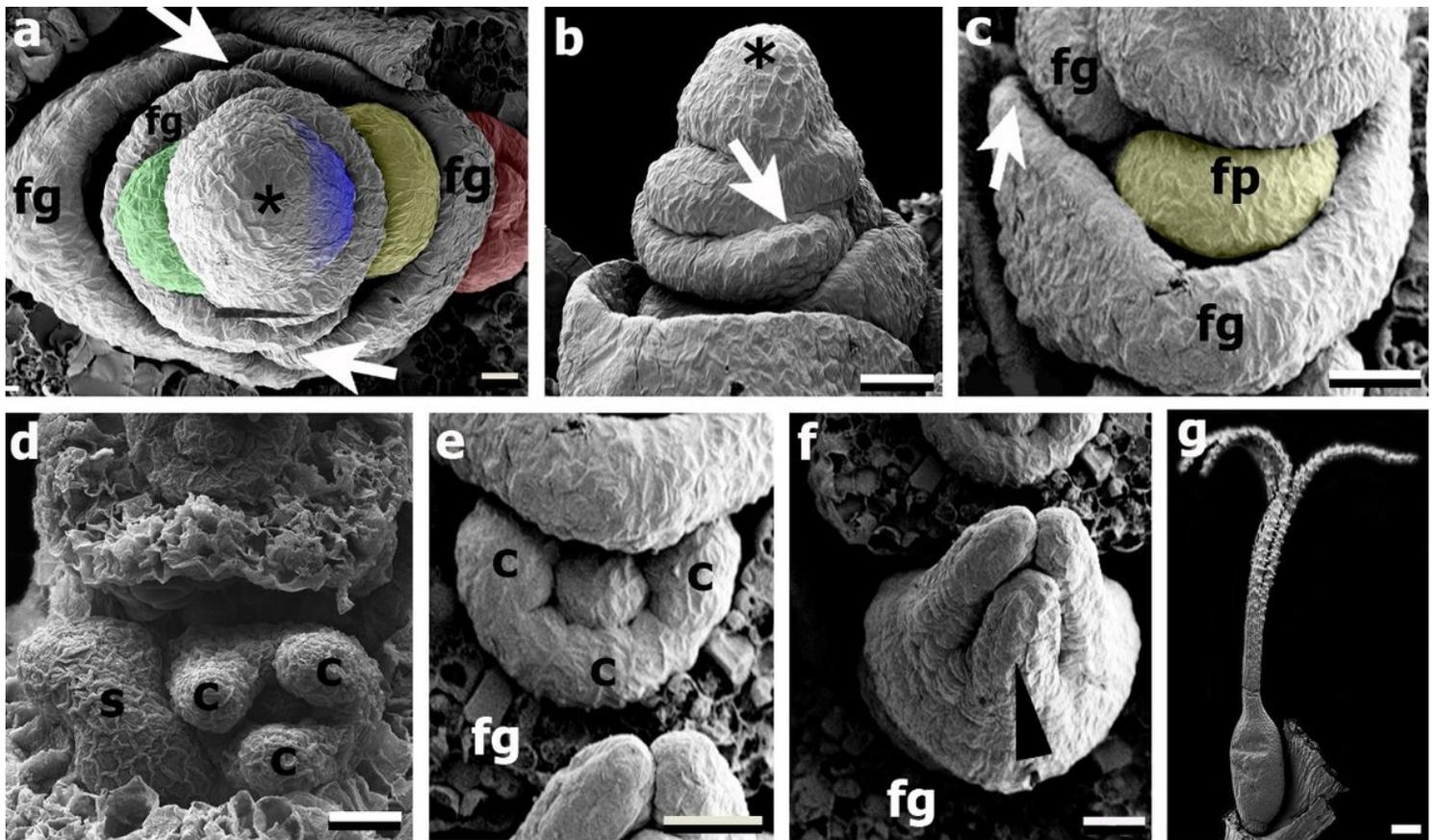

Figure 3

Scanning electron micrographs of the female flower of *Cyperus surinamensis* at different developmental stages. **a**, Spikelet axis with distichously arranged fertile glumes enveloping the flower primordia (different colours) and rachilla (asterisk). The glumes exhibit expanded margins enveloping each other (arrow). **b**, Lateral view of the spikelet, showing the base of the glume fused to the rachilla and expanding towards the lower flower primordium (arrow). **c**, Flower primordium subtended by a fertile glume showing the expanded margins of the opposite new glume formed in contact with the laterals of the flower

primordium (arrow). **d**, Bisexual flower showing a single lateral stamen and the trimerous pistil. **e**, Carpel lobes developing in the ovary wall surrounding the central ovule, note the subtending glume. **f**, Development of the ovary wall and stigmas. Note the less developed abaxial stigmatic lobe (arrowhead) towards the glume. **g**, Mature flower with glume removed, showing papillose structures on the stigmas. c, carpel lobe; fg, fertile glume; fp, flower primordium; s, stamen. Scale bars: 10μm (**a, c-f**); 20 μm (**b**); 100μm (**g**)
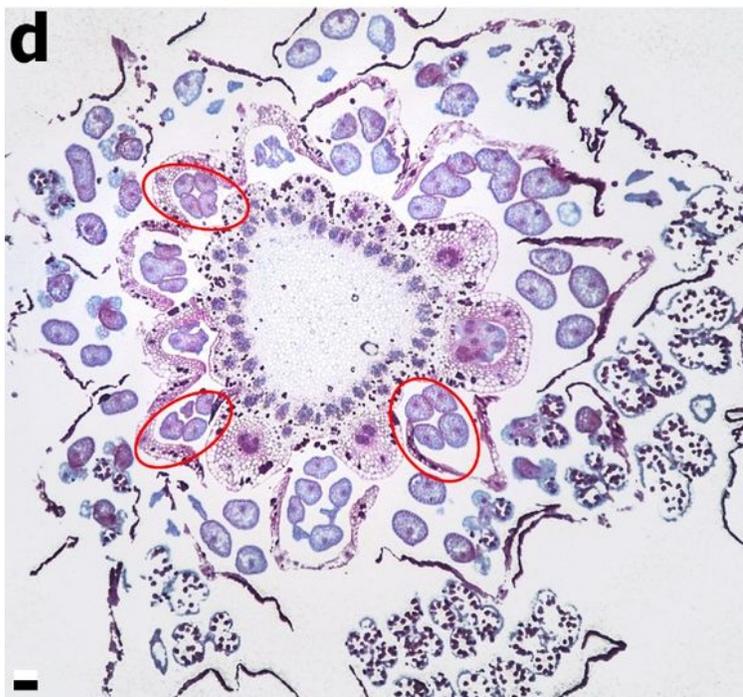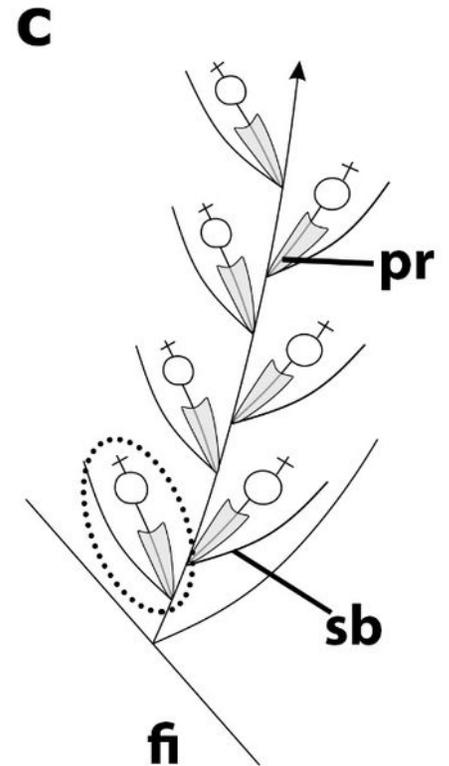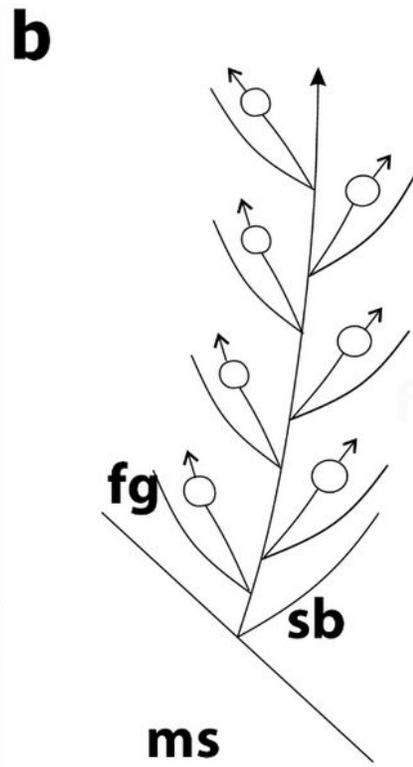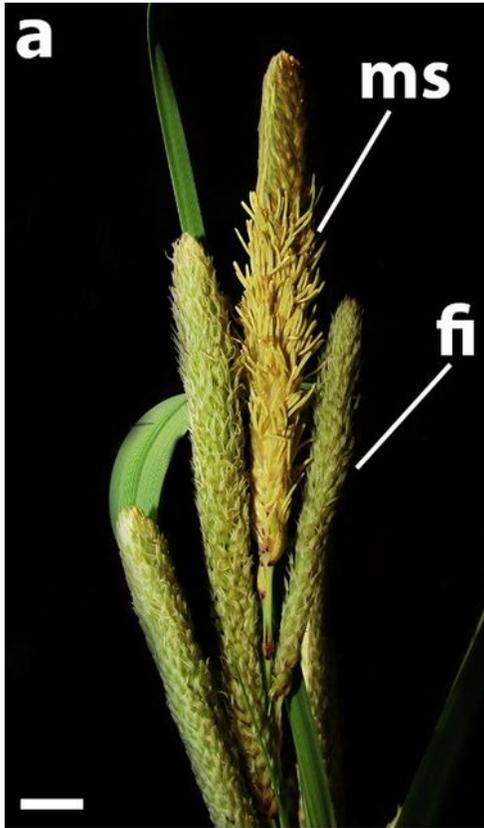
Figure 4

Morphology, diagram and cross section of the spikelet and mature flowers of *Carex brasiliensis*. **a**, Lateral view of inflorescence showing the male spikelet in the center surrounded by female inflorescences. **b**, Lateral overview of a male spikelet showing the fertile glumes subtending the flowers. **c**, Lateral overview of a female inflorescences showing the individual spikelets (dotted circle) with their prophylls enveloping each flower and the subtending bracts. **d**, Male spikelet with flowers spirally arranged. Note the presence of a single vascular bundle per stamen in flowers with four stamens (red circles). **e**, Female inflorescence with spikelets spirally arranged. fg, floral glume; fi, female inflorescence; ms, male spikelet; pr, prophyll; sb, subtending bract. Scale bars: 1mm (**a**); 200 μm (**d, e**)
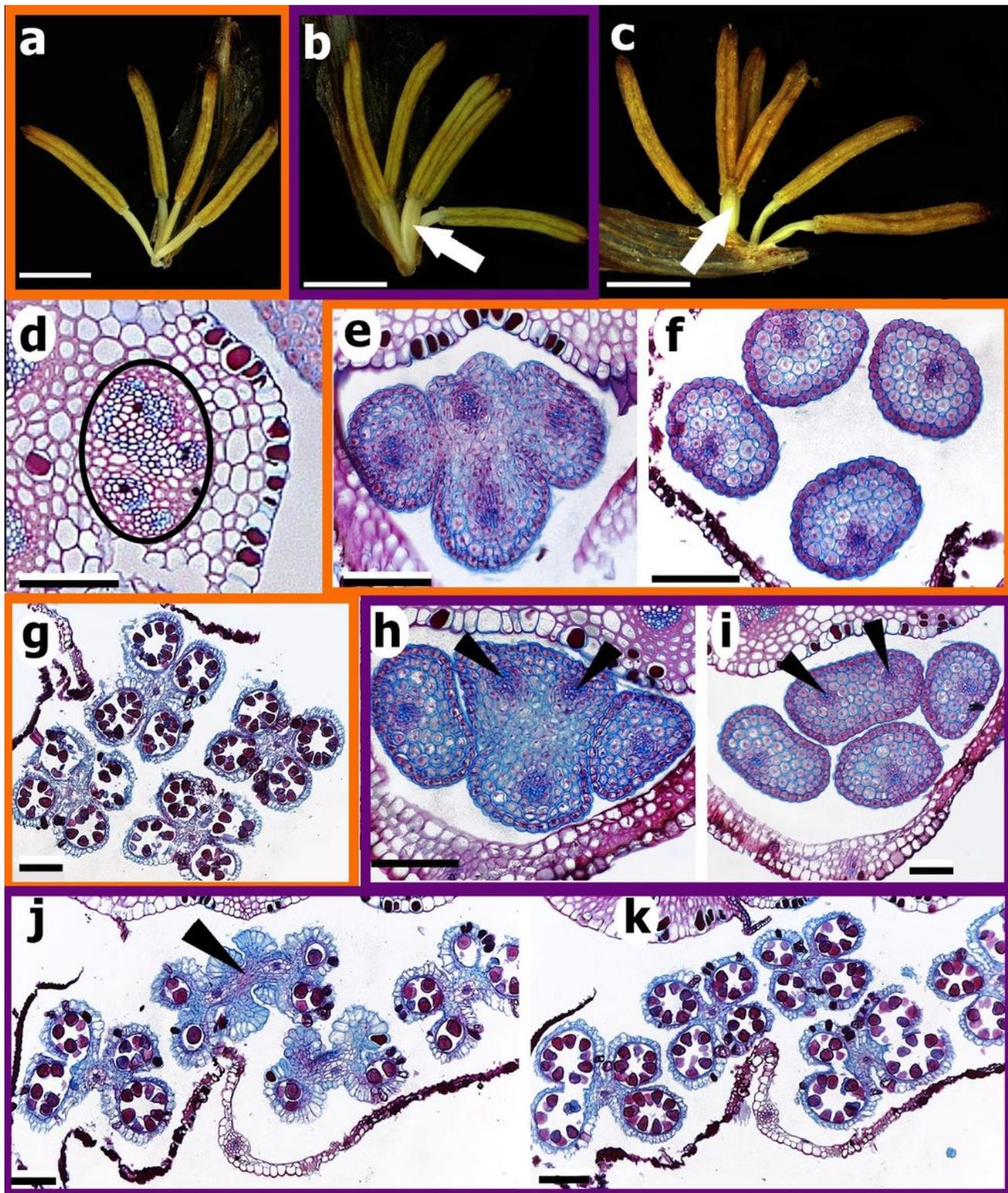
Figure 5

Morphology and cross section of mature male flowers of *Carex brasiliensis*. **a**, Mature male flower with four stamens. **b-c**, Mature male flower with five (b) and six (c) stamens; note the fused filaments (arrow). **d**, Cross section of a mature male flower showing that the initial divergence of the vascular traces starts with a vascular complex in the receptacle (circle). **e-g**, Male flower with four stamens showing the floral receptacle splitting first to the lateral stamens, leaving an abaxial and adaxial vascular trace (**e**), which

will split in four filaments, each with a single vascular bundle (**f**) that will supply each anther (**g**). **h-k,** Cross section of a mature male flower with five stamens showing the lateral stamens separating first, leaving an abaxial and two adaxial vascular traces (arrowhead) (**h**), which will split in four filaments, one of them with two vascular bundles (arrowhead) (**i**); filaments remain attached up to the level of the anthers (**j**), before completely separating in five stamens (**k**). Scale bars: 1mm (**a-c**); 200μm (**d, i-k**); 100μm (**e-h**)
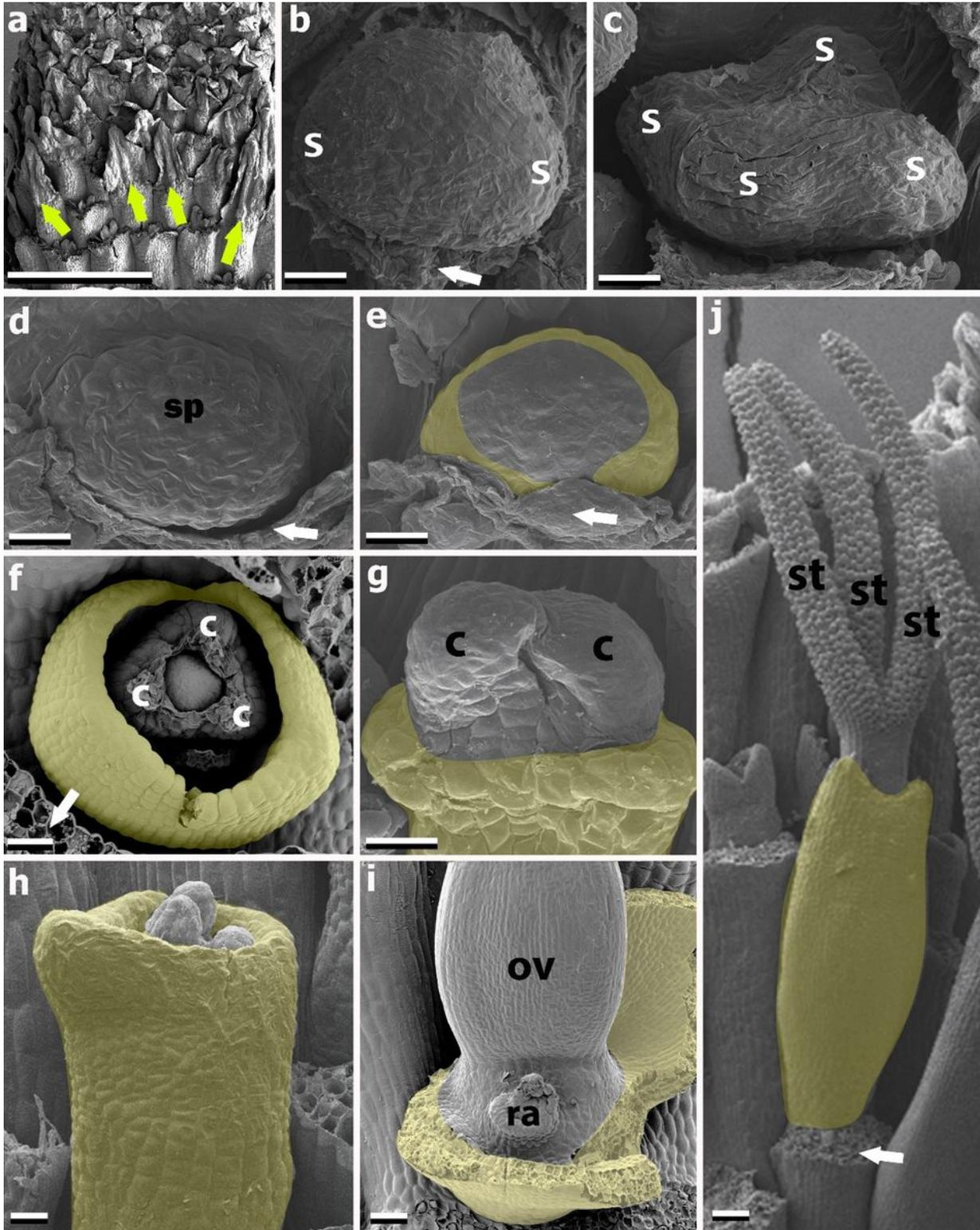
Figure 6

Scanning electron micrographs of the male flower and female spikelet of *Carex brasiliensis* at different developmental stages. **a-c**, Male Flower. **a**, Spikelet axis with glumes subtending the stamens, not the narrow tip of the glumes (arrow). **b**, Initiation of two staminal primordia from the flower primordium subtended by a glume (arrow). **c**, Flower primordium with four staminal primordia. **d-j**, Female spikelet. **d**, Spikelet primordium in the axil of a subtending bract (arrow). **e**, Initiation of the prophyll or utricle (yellow) as a ring around the primordium, subtended by a bract (arrow). **f**, Gynoecium developing the ovary wall surrounding the central ovule; the prophyll is highlighted in yellow. **g-h**, Development of the carpel and the prophyll (yellow). **i**, Prophyll removed showing an undeveloped rachilla and the ovary wall. **j**, Mature female spikelet with enclosing prophyll (yellow) and the subtended bract removed (arrow). c, carpel; ov, ovary wall; s, stamen primordium; sp, spikelet primordium; st, stigmatic lobe; ra, undeveloped rachilla. Scale bars: 1mm (**a**); 20µm (**b-i**); 200µm (**j**)