

Gynoecial anatomy and development in Cyperoideae (Cyperaceae, Poales): congenital fusion of carpels facilitates evolutionary modifications in pistil structure

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Background and aims – In Cyperaceae, the single-ovuled, usually triangular gynoecia are widely considered to have a basic number of three carpels, often reduced to two, resulting in dimerous pistils. However, laterally flattened dimerous pistils cannot be explained by any existing carpel reduction theories, because a single stigma in median position replaces the two adaxial stigmata. This paper presents a comparative study of the ontogenetic and anatomical adaptations facilitating the origin of new pistil forms in Cyperoideae, focusing on modified gynoecia. It includes a re-evaluation of Blaser's (1941) anatomical studies in Cyperaceae. We aim to test Blaser's hypothesis that is based on an acropetal developmental model of the floral vasculature and the general Cyperoid ontogenetic model of Vrijdaghs et al. (2009), which states that cyperoid ovaries originate from an annular primordium.

Methods – SEM, dark field and phase contrast microscopy.

Key Results – All cyperoid pistils studied develop according to a cyperoid floral ontogenetic pattern, in which carpel primordia are congenitally fused. In *Pycneus sanguinolentus* (and other species), separate procambial initiation zones were observed in both the flower receptacle and separate floral primordia, which connect (or not) at later developmental stages.

Conclusions – The presence of an annular ovary primordium instead of individual carpel primordia, combined with the bidirectional development of the pistil vasculature liberate the developing gynoecium from the structural constraints proper to a typical carpellate organisation. Procambial initiation zones in the receptacular vascular plexus and in individual floral primordia constitute the basis for the formation of a flexible vascular system in cyperoid flowers. Moreover the development of the ovary and ovule are decoupled. Consequently, in Cyperoideae the acquired developmental freedom of the pistil resulted in various adaptations.

Key words – Congenital fusion, Cyperoideae, floral anatomy, floral ontogeny, gynoecium, laterally compressed pistil, procambial initiation, ovule, ring primordium, vascular bundles.

INTRODUCTION

Cyperoideae and their flowers

Cyperaceae, the third largest family in monocots, are characterised by their small, easily dispersible nutlets with a single ovule, which greatly contributed to their worldwide success. Whereas previously four subfamilies were considered (Muasya et al. 1998, Simpson et al. 2007), currently two

main clades have been recognised as the only subfamilies of Cyperaceae, namely Cyperoideae and Mapanioideae (fig. 1). At tribal and generic levels, the classification of Goetghebeur (1998) is still in use (Govaerts et al. 2007).

In Mapanioideae, the reproduction units (flowers or synanthia?) are enveloped by two lateral scales (e.g. Kern 1974). The reproductive units are controversial because of the presence of scales in between a terminal gynoecium and

the more proximally situated stamens. Until now, only one complete floral ontogeny has been done, on *Exocarya* Benth. (Richards et al. 2006), unfortunately without much attention to the development of the gynoecium. From our current knowledge it becomes more and more clear that the whole floral organisation in mapanioids is fundamentally different from the one in cyperoids (Richards et al. 2006, Simpson et al. 2003, 2007, Vrijdaghs 2006). Therefore a thorough comparative study in Cyperoidae stands on its own. In Cyperoidae, flowers are typically monocotyledonous (e.g. Rudall & Bateman 2004) with a trimerous perianth, a trimerous androecium and a trimerous, superior gynoecium, or they are considered to be derived from this pattern (e.g. Goetghebeur 1998, Vrijdaghs et al. 2009). A cyperoid flower usually originates in the axil of a subtending bract, called glume, with the glumes and their flowers being organized in spikelets (e.g. Haines & Lye 1983, Goetghebeur 1998, Vrijdaghs et al. 2009, 2010).

Aims of this study

This paper presents an overview, strictly limited to the Cyperoidae, of existing anatomical and ontogenetic data of the gynoecium, complemented with new anatomical and floral ontogenetic evidence. For the first time, old and new anatomical and floral ontogenetic observations are integrated in

order to test two existing hypotheses outlined in the following paragraphs: (1) Blaser's (1941a, 1941b) anatomy-based interpretation of vascularisation in Cyperoidae, especially for the gynoecium wall in *Pycneus*, where he assumed lateral bundles took over the function of the dorsal bundles (see chapter on pistil vascularisation for more details), and (2) the scirpoid floral ontogenetic model of Vrijdaghs et al. (2009) in which the pistil of all Cyperoidae is explained to originate from a ring primordium. In the anatomical study, special attention was given to procambial initiation patterns, which have not yet been studied in cyperoid flowers, and to evaluate Blaser's conclusions in the context of recent molecular phylogenetic hypotheses (Muasya et al. 2002, Larridon et al. 2011a) especially concerning dimerous flattened pistils.

Moreover, to illustrate two fundamentally different developmental patterns that result in coenocarpous gynoecia (i.e. gynoecia with fused carpels, Weberling 1992), cyperoid gynoecium development is compared with gynoecial development in a relatively distantly related monocot, *Sansevieria trifasciata* Prain (included in *Dracaena*, Ruscaceae, Asparagales, Stevens 2001 onwards). *Sansevieria* was chosen as a highly illustrative monocotyledonous species with an ovary developing from individual carpel primordia that fuse post-genitally.

Finally, our conclusions are presented as a developmental model for the gynoecium and its vascularisation, giving a possible explanation for the variation of pistils found in Cyperoidae.

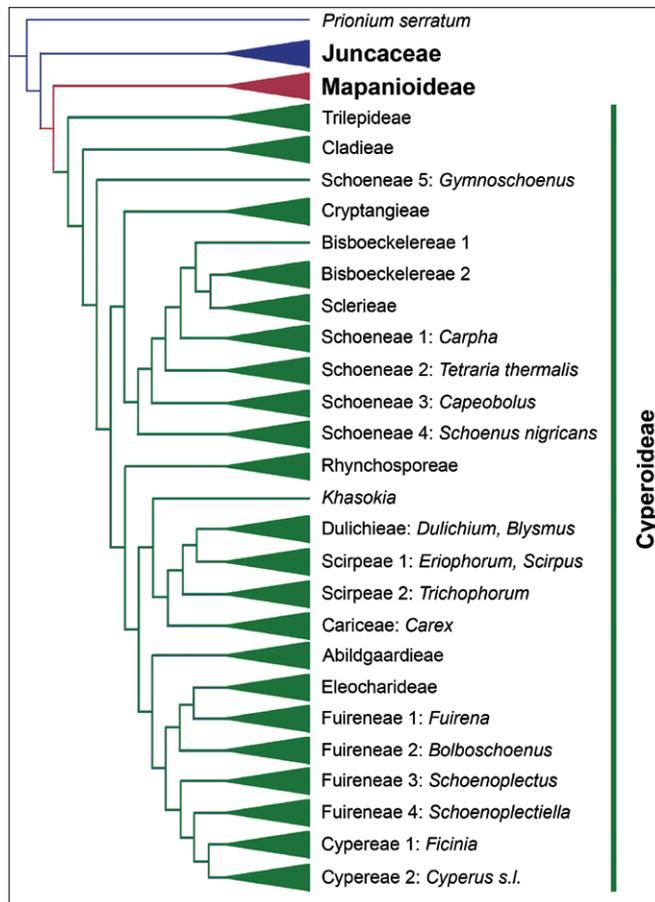


Figure 1 – Simplified cladogram of Cyperaceae, adapted from a strict consensus tree from Muasya et al. (2009a).

Pistil modifications in Cyperoidae

As in other monocots, in Cyperaceae, a trimerous gynoecium can be considered as the plesiomorphic condition (Endress 1995). Usually, species of Cyperoidae have two lateral stigma branches at the adaxial side and one at the abaxial side (fig. 2A & B). While tetramerous, hexamerous and octamerous pistils occur in some tribes, dimerisation of pistils is more widespread within the family (Goetghebeur 1998). In sedges, derived pistils have often been used for specific and generic delimitations, but their systematic value is controversial (e.g. Goetghebeur 1998, Muasya et al. 2009b).

In Cyperoidae, two main types of dimerous pistils occur: either dorsiventrally (fig. 2C) or laterally flattened pistils (fig. 2D), causing bilateral floral symmetry. A dorsiventrally flattened pistil has laterally positioned stigma branches (fig. 2C). This type occurs in almost all cyperoid tribes sensu Goetghebeur (see table 1). In Mapanioideae, this pistil type is also common (Kern 1974). Based on early anatomical and ontogenetic studies, many authors suggested that dorsiventrally flattened pistils could be explained by a reduction of the abaxial carpel (e.g. Baillon 1893, Schumann 1890, Snell 1936, Goetghebeur 1986). Although often used in generic circumscriptions, the taxonomic value of the dorsiventrally compressed dimerous pistil should be handled with caution (Goetghebeur 1986). This is illustrated by some species such as *Cyperus alopecuroides* Rottb., *Kyllingiella polyphylla* (A.Rich.) Lye, *Eleocharis variegata* (Poir.) C.Presl. and *Schoenoplectus corymbosus* (Roth ex Roem. & Schult.) J.Raynal, which can have both trimerous and dorsiventrally flattened dimerous pistils within a single spikelet (Haines & Lye 1983, Larridon

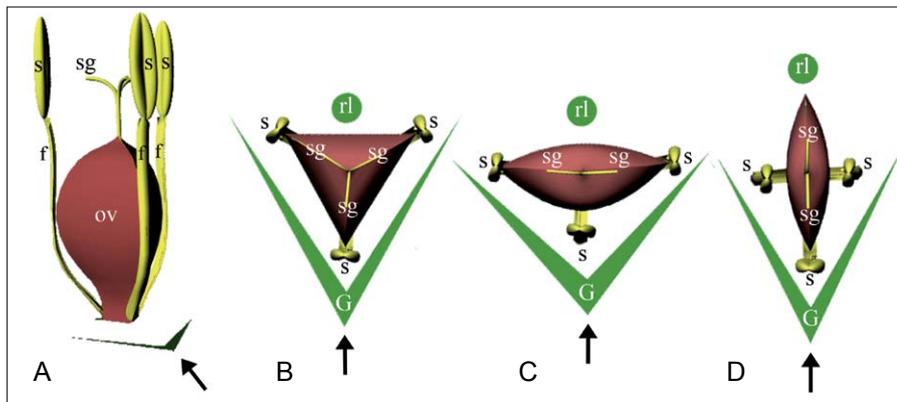


Figure 2 – Overview of the pistil types present in Cyperoideae, which are studied in this paper. A, lateral-abaxial view of a typical flower in Cyperoideae (without perianth); B, apical view of a flower with a trimerous gynoeceium; C, apical view of a flower with a dorsiventrally flattened dimerous gynoeceium; D, apical view of flower with a laterally flattened dimerous gynoeceium. Abbreviations: f, filament; G, glume; RL, rachilla; s, stamen; sg, stigma. A black arrow indicates the abaxial side of each flower.

et al. 2011b). In other taxa, the dimerous, dorsiventrally flattened pistil state is constant such as in *Cyperus laevigatus* and *Eleocharis minuta* (Haines & Lye 1983).

Laterally flattened pistils are less common and their origin has been more controversial. In this pistil type, the two stigma branches have median positions (fig. 2D). According to Goetghebeur (1986), reduction of one of the (two adaxial) carpels is not likely since there is no trace of the then expected asymmetry. Laterally flattened pistils have only been recorded in three cyperoid tribes: Cyperaceae (*Pycreus*, ± 120 spp.; *Kyllinga*, ± 80 spp.; *Queenslandiella*, 1 sp.), Cryptangiaceae (*Exochogyne Clarke* [accepted name = *Lagenocarpus amazonicus*]) and Rhynchosporaceae (*Rhynchospora rubra* subsp. *rubra* (Lour.) Makino) (Goetghebeur 1998). Goetghebeur (1986) postulated a single origin of laterally flattened pistils for *Kyllinga*, *Pycreus* and *Queenslandiella* (Cyperaceae). However, a putative homology of the laterally flattened dimerous pistils in *Kyllinga*, *Pycreus* and *Queenslandiella* has not been confirmed by molecular phylogenetic hypotheses (Muasya et al. 2001, 2002, 2009a). Moreover, the recognition of these three taxa as genera (e.g. Goetghebeur 1998) is controversial (e.g. Haines & Lye 1983, Tucker et al. 2002, Muasya et al. 2009b). They are nested in a paraphyletic *Cyperus* (together with several other segregate ‘genera’), in a polytomy of the clade of the *Cyperus* s.l. species using C_4 photosynthesis (C_4 *Cyperus* clade) (Muasya et al. 2002, 2009a, Besnard et al. 2009, Larridon et al. 2011a). Despite their obvious close relationships with *Cyperus*, these three taxa are generally considered as well circumscribed entities (whether considered as separate genera or at subgeneric level in *Cyperus*), based on, among others, the presence of laterally flattened pistils in each of them. In expectance of a more elaborate molecular study on C_4 *Cyperus* and its segregate genera, we chose here to follow the classification of Goetghebeur (1998) and Govaerts et al. (2007) for the currently accepted names of the taxa. To be complete, it must be mentioned that some authors were not convinced of the systematic value of laterally flattened pistils for generic delimitation (e.g. Koyama 1961).

Finally, in several Cyperoideae taxa, apparent monomerous pistils can be found. In most of these cases the style is strongly elongated bearing very short stigma branches at its top (e.g. *Rhynchospora* sect. *Haplostylis* and *Cyperus* sect. *Anosporum*, Haines & Lye 1983). In *Cyperus meeboldii* and

relatives (e.g. *C. holostigma*, *C. clavinox*), an unbranched style has also been observed (Raynal 1966).

The carpel concept and its application to Cyperoideae

Goetghebeur (1986) pointed out that laterally flattened pistils in Cyperoideae are difficult to explain as resulting from the reduction of one of the two adaxial carpels. Instead, the two adaxial carpels seem to be replaced by a single carpel in median position. This novel position is remarkable since in most angiosperm groups, carpel positions appear to be highly conserved (Endress 1995). In addition, Vrijdaghs et al. (e.g. 2005a, 2005b) showed that the cyperoid ovary originates from an annular ovary primordium. In this context, we think it useful to recapitulate the carpel concept and its application in Cyperoideae.

von Goethe (1790, in Miller 1949: 256) was the first to see the carpel as a reproductive phyllome: “Earlier I tried to make as clear as possible that the various plant parts developed in sequence are intrinsically identical despite their manifold differences in outer form. It should come as no surprise that I also intend to explain the structure of the female parts in the same way.” Goethe did not suggest carpels to be derived from leaves, but rather that leaves and all floral parts are lateral appendages of the stem, reflecting an archetypical leaf-like structure, called ‘Blatt’ by Goethe and from the end of the 19th century called ‘phyllome’ (Arber 1937). The word ‘carpellum’ dates from the early 19th century, from the same period in which de Candolle (1827) hypothesised carpels to be structures derived from sporangium-bearing leaves. Goebel (1888) described a gynoeceium as being formed by macrosporophylls, bearing ovules at the inner or ventral side of the leaf margins. This view became ‘classic’ with Engler (1900–1968), who adopted it for all angiosperms. In the course of the 19th and 20th centuries, several alternative floral hypotheses arose, such as the pseudanthium hypothesis (e.g. Wettstein 1935) or the gonophyll hypothesis (Melville 1969), consequently suggesting other interpretations of carpels. As a summary, carpels can be seen as either (1) homologous with leaves (phyllomes) and thus formed laterally on an axis and the ovules formed upon them (phyllospory) or (2) leaf-axis structures, with the ovules being formed on an axis subtended by a carpel (stachyospor). Endress (2001) added that a carpel can also be a compound organ with an ovule producing part and a leaf component, or a totally new

Table 1 – List of cyperoid genera that include species with derived pistils based on Goetghebeur (1998).

Abbreviations: D, distichous; dors., dorsally compressed; lat., laterally compressed; S, spiral; X, the most common situation; x, the less common situation; (x), observed as a rare variation within species that normally have another type of pistils.

Clade (Muasya et al. 2009a)	Taxonomy Tribe and genus (Goetghebeur 1998)	Gynoecium: number of stigmata							Glume placement
		2 lat.	2 dors.	3	4	6	8	9	
Abildgaardieae	Abildgaardieae Lye								
	<i>Bulbostylis</i> Kunth		x	X					S
	<i>Fimbristylis</i> Vahl		X	X					S
Bisboeckelereae 1	<i>Nemum</i> Desv. ex Ham.		X	x					S
	Bisboeckelereae Pax ex L. T. Eiten								
Cariceae	<i>Calyptracarya</i> Nees		X						D
	Cariceae Kunth ex Dumort.								
	<i>Carex</i> L.		x	X	x	(x)			S
Cryptangieae	<i>Kobresia</i> Willd.		x	X					S
	Cryptangieae Benth.								
	<i>Exochogyne</i> C.B. Clarke	X							D
Cypereae	Cypereae Dumort.								
	<i>Ascolepis</i> Nees ex. Steud.		x	X	(x)				S
	<i>Cyperus</i> L. s.s.		x	X					D/s
	<i>Ficinia</i> Schrad.		x	X					S
	<i>Isolepis</i> R.Br.		x	X					S
	<i>Kyllinga</i> Rottb.	X							D
	<i>Lipocarpa</i> R.Br.		x	X					S
	<i>Oxycaryum</i> Nees		X						S
	<i>Pycreus</i> P.Beauv.	X							D
Dulichieae	<i>Queenslandiella</i> Domin	X							D
	Dulichieae Rchb. ex J. Schultze-Motel								
	<i>Blysmus</i> Panz. ex Schult.		X						D
?	<i>Dulichium</i> Pers.		X						D
	<i>Sumatrosclirpus</i> Oteng-Yeb.		X	(x)					S
Eleocharideae	Eleocharideae Goetgh.								
	<i>Eleocharis</i> R.Br.		x	X					S
	<i>Websteria</i> S.H.Wright		X						D
Fuireneae 2	Fuireneae Rchb. ex Fenzl								
	<i>Bolboschoenus</i> (Asch.) Palla		x	X					S
Fuireneae 3 & 4	<i>Schoenoplectus</i> (Rchb.) Palla		x	X					S
Rhynchosporeae	Schoeneae Dumort								
	<i>Pleurostachys</i> Brongn.		X						S/d
	<i>Rhynchospora</i> Vahl	x	X						D
Schoeneae 3	<i>Cladium</i> P.		(x)	X					D
	<i>Cyathochaeta</i> Nees		X						S/d
	<i>Evandra</i> R.Br.						X		S
Schoeneae 1	<i>Gahnia</i> J.R.Forst. & G.Forst.			X	(x)				S
	<i>Lepidosperma</i> Labill.			X	(x)				S
	<i>Neesenbeckia</i> Levyns					X	(x)		D
	<i>Schoenus</i> L.		(x)	X					D
	<i>Tetaria</i> P.Beauv.			X	x			(x)	D
?	<i>Trachystylis</i> S.T.Blake		X						S
Scirpeae 1	Scirpeae Kunth ex Dumort.								
	<i>Amphiscirpus</i> Oteng-Yeb.		X						S
	<i>Scirpus</i> L.		x	X					S

organ. Leins & Erbar (2011) consider carpels to be megasporophylls and describe them as comparable with a hollow cylinder, through which an inclined section is made above the base of the cylinder and consequently defining a circular basal zone or ascidiate zone, and distally an open zone called plicate. In ovaries consisting of several carpels, the dorsal side of it always forms the ovary wall, whereas placentas/ovules are formed at the ventral side of the carpels, usually at the margins (axile placentation) or situated more centrally at the ventral side of the carpels and hence at the inner side of the ovary wall (laminal placentation).

In Cyperaceae, Payer (1857: 698) described the origin of the gynoeceum “dans les *Scirpus* et les *Eriophorum*” as carpellate: “trois autres bourrelets qui leur [stamen primordia] sont superposés et qui sont les rudiments du pistil”, with postgenital fusion of these carpel primordia: “...ils sont promptement réunis à leur base, de façon à produire un sac ovarien” (Payer 1857: 699). Until the last decade, Payer was the only author who did a thorough and complete floral ontogenetic investigation in Cyperoideae. In the course of the 20th century, floral ontogenetic data were only sporadically published (e.g. Schumann 1890, Barnard 1957, Schulze-Motel 1959, Mora 1960, Mora-Osejo 1987, Bruhl 1991). Since Payer (1857), in Cyperaceae-Cyperoideae, the ovary has always been described as tricarpellate, unilocular with one basal, anatropous and bitegmic crassinucellar ovule (e.g. Goetghebeur 1998). However, according to Vrijdaghs et al. (e.g. 2009), in Cyperoideae, the ovary rises from an annular ovary primordium, enveloping the single, central ovule.

Recent insights in the development of (floral) primordia

Gould (2002) linked classic morphology with evolutionary developmental biology (evo-devo), suggesting that members of the floral whorls, including the gynoeceum, originate from phyllome primordia that are ‘empty’, undetermined structures, getting identity through the functioning of developmental regulator genes/programs, such as the ABC model of Coen & Meyerowitz (1991). According to Endress (2006), annular primordia often result in a decoupling of the development of the concerned organ type from the neighboring floral organs, with alterations in time (sequence of floral organ appearance) and number. In *Eriophorum*, Vrijdaghs et al. (2005a) observed that congenital fusion of the perianth primordia into a massive perigonial primordium resulted in a new kind of perianth consisting of many perianth hairs. The development of a pappus in some Asteraceae from fused sepal primordia is a similar situation (see Harris 1995).

Pistil vascularisation in Cyperoideae

In angiosperms, a carpel is most often vascularised by three vascular bundles: a midvein (or dorsal carpellary bundle), running towards the stigma and two marginal veins (or ventral carpellary bundles), which connect to the placentae and ovules (e.g. Dickison 2000). In Cyperoideae, only the dorsal bundles run within the ridges of the gynoeceum wall towards the stigmata (fig. 3). Saunders (1937) and Snell (1936) both remarked that the vascular system within the receptacle is highly disorganised. However, since this vascular plexus connects towards the ovule, its bundles were interpreted as ventral bundles by

Table 2 – List of genera studied by Blaser (1941a, 1941b) with the reference of the corresponding models of the floral vasculature shown in fig. 3.

Genus	Model (fig. 3)
<i>Bolboschoenus</i> (Asch.) Palla (as <i>Scirpus</i> L.)	A1/B1
<i>Carex</i> L.	A2/B2
<i>Cyperus</i> L.	A2
<i>Dulichium</i> Pers.	C1
<i>Eleocharis</i> R.Br. (as <i>Heleocharis</i> T.Lestib.)	A2/C2
<i>Eriophorum</i> L.	A1
<i>Fimbristylis</i> Vahl	A2/B2
<i>Lipocarpa</i> R.Br.	A2/B2
<i>Pycreus</i> P. Beauv. (as <i>Cyperus</i> L.)	D1
<i>Rhynchospora</i> Vahl	B1/B2/C1/C2
<i>Schoenoplectus</i> (Rchb.) Palla (as <i>Scirpus</i> L.)	A1/B1

all previous authors. Therefore, the ventral bundles of each carpel have been described to branch off already below the gynoeceum and fuse in the centre of the receptacle to supply the single basal ovule (indicated as central bundle) (Saunders 1937, Snell 1936, Blaser 1941a, 1941b) (fig. 3). Sometimes, adjacent ventral bundles fuse and continue shortly in the sides of the gynoeceum wall (indicated as lateral bundles) (Blaser 1941a, 1941b) (fig. 3, orange bundles). In an elaborate study of a wide spectrum of genera in Cyperaceae, Blaser (1941a, 1941b) used the vascularisation of *Scirpus* as a prototype from which all other patterns were derived (for details see fig. 3 and table 2). Based on the frequent presence of vestigial ventral and dorsal vascular bundles belonging to the hypothetical abaxial carpel (fig. 3B), Blaser (1941a, 1941b) concluded that dorsiventrally flattened pistils originated by reduction of the abaxial carpel.

Concerning the laterally flattened type, Blaser (1941a) observed that vascularisation patterns in pistils of *Pycreus bipartitus* (Torr.) C.B. Clarke, *P. lanceolatus* (Poir.) C.B. Clarke and *P. diander* (Torr.) C.B. Clarke include bundles towards the stigma branches, which connect to the ventral branches of the two adaxial main bundles entering the flower (fig. 3D1). He interpreted these bundles as lateral bundles, stating: “In this species (*Pycreus bipartitus*), the dorsal bundles, which are characteristically midribs of foliar carpels, form none of the vascular supply of the pistil. The ventrals, in forming the ovule supply, are shortened as usual and the supply to the styles is assumed by the lateral bundles. The laterals are seen as vestiges in many Cyperaceae but are well developed here” (Blaser 1941a: 547; figs 3D1 & 19C). In contrast, the vascularisation patterns in flowers of *Kyllinga* and *Queenslandiella* were not yet clear. Blaser (1941a) also included *Kyllinga pumila* Michx. in his study but surprisingly concluded that its vascular pattern is similar to the vascularisation of trimerous *Cyperus* pistils.

Based on his anatomical studies in mature plants, Blaser (1941a, 1941b) assumed the vascular bundles running in the gynoeceal wall of *Pycreus* up to the stigma branches to be homologues of the lateral branches found in scirpoid taxa. In *Cyperus*, lateral branches are absent and as a consequence

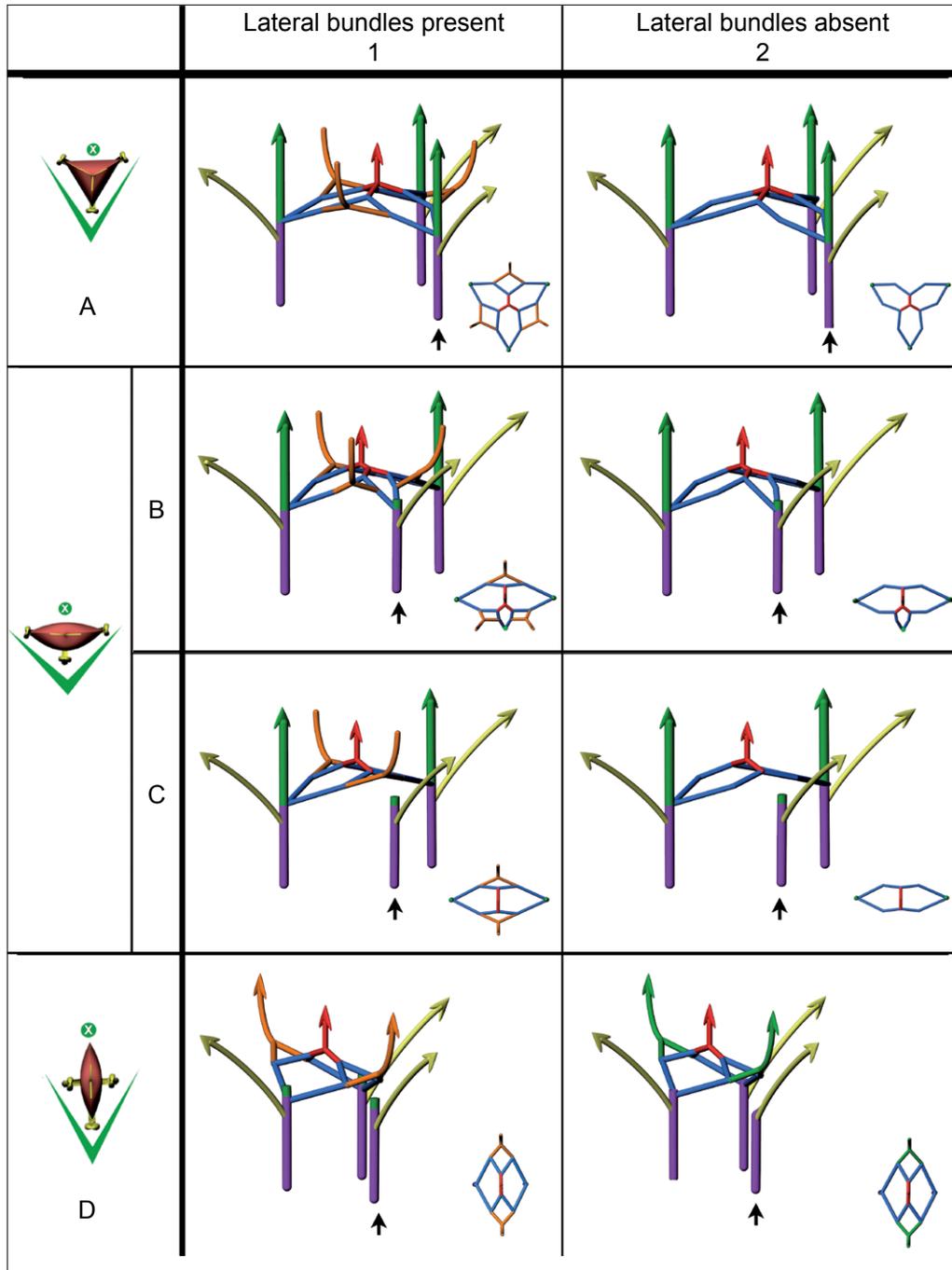


Figure 3 – 3D reconstructions of the vascular bundle patterns within the base of different Cyperaceae flowers as interpreted by Blaser (1941a, 1941b). Vascular patterns are grouped according to the pistil types (A–D) and the presence or absence of lateral bundles within the gynoecium wall (1–2). Vascular traces towards perianth parts are omitted. Bundles, which continue into the different floral organs, are arrowed, rudimentary bundles are not arrowed. The larger models are shown from a lateral-abaxial position and the abaxial position is always indicated with a small black arrow. A smaller inlay adds an apical view of each separate model (with staminal traces removed). Colour codes and circumscription for the different vascular bundles: purple, receptacular bundles, these bundles connect the vascular tissue of the flower with the stele; yellow, staminal bundles; green, dorsal bundles, running in the carpels and continuing in the stigmata; blue, ventral bundles, usually two bundles for each carpel that run towards the placentation of the ovule; orange, lateral bundles, running in the wall of the gynoecium in some taxa; red, central and ovular bundles connecting the ventral bundles to the ovule. A, trimerous pistil type; B, dorsiventrally flattened dimerous pistil with the abaxial receptacular bundles still contributing to the vascularisation of the pistil, however the abaxial dorsal bundle is reduced; C, dorsiventrally flattened dimerous pistil, the abaxial receptacular bundle does not contribute to the vascularisation of the pistil; D, the situation in the laterally compressed pistils of *Cyperus rivularis* (= *Pycneus bipartitus*) where Blaser (1941a) interpreted the bundles within the gynoecium wall to be lateral bundles (D1). D2 shows a hypothetical, alternate interpretation of these bundles to be interpreted as dorsals. See table 1 for a list of taxa and their vascularisation types as observed by Blaser (1941 a, 1941b).

Table 3 – Species studied and voucher data.

Abbreviations: HBUG, Ghent University Botanical Garden, Belgium; KDTN-Leuven, Kruidtuin, botanical garden of the town of Leuven, Belgium; Ptk-K.U.Leuven, botanical garden of the Institute of Botany of the K.U.Leuven, Belgium.

Taxa	Collected by	Origin	Voucher
<i>Cyperus haspan</i> L.	Reynders M.	HBUG 2006-1243 (wild origin Philippines)	20061243 (GENT)
<i>Cyperus laevigatus</i> L.	Reynders M.	HBUG 2002-0878 (wild origin Zimbabwe)	20020878 (GENT)
<i>Dulichium arundinaceum</i> (L.) Britton	Goetghebeur P.	HBUG 2002-1303 (P)	PG 9914 (GENT)
<i>Eriophorum latifolium</i> Hoppe	Vrijdaghs A.	KDTN-Leuven	AV 04
<i>Lagenocarpus amazonicus</i> (C.B.Clarke) H. Pfeiff.	Aparecida da Silva M., Proença C., Cardoso E. & Paixao J.P.	Brazil	1986 (GENT)
<i>Kyllinga microbulbosa</i> Lye	Muasya A.M.	Kenya	AM 2658 (EA)
<i>Kyllinga nemoralis</i> (J.R.Forst. & G.Forst.) Dandy ex Hutch. & Dalziel	Reynders M.	HBUG 2006-1238 (wild origin Philippines)	20061238 (GENT)
<i>Pycreus bipartitus</i> (Torr.) C.B.Clarke	Reynders M.	HBUG 2005-0801 (S)	20050801 (GENT)
<i>Pycreus flavescens</i> (L.) P.Beauv. ex Rchb.	Reynders M.	HBUG 2005-0401 (S)	20050401 (GENT)
<i>Pycreus sanguinolentus</i> (Vahl) Nees	Reynders M.	HBUG 2007-1753 (wild origin China)	20071753 (GENT)
<i>Queenslandiella hyalina</i> (Vahl) Ballard	Muasya A.M.	Mombassa, Kenya	AM 2189 (EA)
<i>Rhynchospora corymbosa</i> (L.) Britton	Reynders M.	HBUG 2007-1418 (wild origin Cameroon)	20071418 (GENT)
<i>Sansevieria trifasciata</i> Prain.	Goetghebeur P.	HBUG 1900-1241	19001241 (GENT)
<i>Scirpus sylvaticus</i> L.	Vrijdaghs A.	Ptk-K.U.Leuven	AV 02

Blaser postulated a separate origin of *Pycreus* from an ancestor with lateral bundles.

In order to correctly address homology questions and evolutionary interpretations of vascularisation patterns it is necessary to have an idea of how these vascular strands develop (Pizzolato 2000). In Cyperaceae, procambial initiation has so far only been studied in the leaves and culms of *Cladium mariscus* (L.) Pohl (Fisher 1971). The vascular ontogeny in this species concurs with patterns found in other monocots, such as *Tradescantia zebrina* Bosse (Commelinaceae, Pizzolato 2006), in which differentiation of veins within the leaves is bidirectional and starting from several separate procambial initiation points, in a later stage these merge with each other and with the older vascular bundles of the culms (e.g. Dickison 2000). Similar patterns have also been observed in the formation of reticulations between the main veins within the leaves of *Arabidopsis* Heyhn. (Scarpella et al. 2006). Merging of remote procambial strands from different plant organs seems to be the basic pattern in the development of the floral vascularisation found in angiosperms (e.g. Endress 1994). According to Aloni (2004), auxin plays a leading role in procambial initiation and polar auxin transport from primordia is a controlling factor in both phyllo-tactic (Reinhardt et al. 2003) and leaf venation patterning (Scarpella & Meijer 2004, Scarpella et al. 2006), resulting in self-regulated development of vascular bundles where needed. Before these modern insights were generally accepted, vascularisation development in angiosperm flowers had been assumed to be acropetal (e.g. Grégoire 1938).

The ovule

Cyperaceae are characterised by unilocular ovaries with a single basal ovule (e.g. Goetghebeur 1998). According to Snell (1936), the presence of a single basal ovule could be interpreted as a final reduction state of a free central placentation. Van der Veken (1965) was the first to show in a dorsiventrally oriented longitudinal section through a semi-mature gynoecium, that hairs occur within the locule, around the micropylar zone of the ovule, which grow into the micropyle. He suggested that these hairs have a pollen tube guiding function and called them therefore ‘obturator hairs’. Several authors reported outgrowth of funicular cells into a ‘funicular obturator’ (e.g. Coan et al. 2008). Based on its ‘glandular nature’ observed in *Bulbostylis*, Gonzalez & López (2010) suggested an integumentary origin of the obturator. While Bouman (1984) considers the obturator to be degenerating after fertilisation, Gonzalez & López (2010) observed lignification of the obturator after fertilisation and persistence in the mature fruit.

MATERIALS AND METHODS

Plant material

Spikelets and flowers of 108 species from 34 cyperoid genera (fig. 1) were examined at early and mature stages (see appendix 1 of Vrijdaghs et al. 2010), of which only a representative selection of illustrative examples is presented here (table 3). Numbering of glumes and subtended flowers was done from most recently originated (1) to oldest (n), in order to avoid abstract numbers in spikelets with many and/or a

variable number of (flower subtending) glumes. Partial inflorescences were collected in the field or in botanical gardens (electronic appendix 1) and immediately fixed in FAA (70% ethanol, acetic acid, 40% formaldehyde, 90 : 5 : 5). Spikelets were dissected in 70% ethanol under a Wild M3 stereo microscope (Leica Microsystems AG, Wetzlar, Germany) equipped with a cold-light source (Schott KL1500, Schott-Fostec LLC, Auburn, NY, USA).

Scanning electron microscopy

To prepare the material for critical-point drying, it was washed twice with 70% ethanol for 5 min. Next it was placed in a mixture (1:1) of 70% ethanol and DMM (dimethoxymethane) for 5 min. The material was then transferred for 20 min to pure DMM. Critical-point drying was done using liquid CO₂ with a CPD 030 critical-point dryer (BAL-TEC AG, Balzers, Liechtenstein). The dried samples were mounted on aluminium stubs using Leit-C. For SEM observation, the material was coated with gold via a SPI-Module™ Sputter Coater (SPI Supplies, West-Chester, PA, USA). SEM images were obtained with a JEOL JSM-6360 (JEOL Ltd, Tokyo, Japan) at the Laboratory of Plant Systematics (K.U.Leuven), or with a JEOL JSM-5800 LV scanning electron microscope at the National Botanical Garden of Belgium in Meise.

Bleaching

Flowers and spikelet tips were dissected and bleached with a 5% sodium hypochlorite (NaClO) solution for 15–60 min. Subsequently, samples were washed for the same period in distilled water and mounted on slides. Cleared up samples were studied under dark field, a technique using indirect light which is scattered by the object, enhancing contrast between different tissues in the sample and makes the xylem vessels to light up. Dark field images were recorded with a Nikon Eclipse E600 microscope, equipped with a Nikon digital camera DXM1200. To enhance sharpness in depth, optical section images were taken from the samples and manually combined in Adobe Photoshop® CS 8.0 (Adobe Systems Inc., San Jose, USA).

Anatomy

Entire spikelets were fixed overnight in FAA (50% ethanol, 5% acetic acid and 5% commercial formalin in distilled water). Dehydration was performed using a 50%, 70%, 85% and 94% ethanol series. After the last alcohol step, the tissue was infiltrated using a mixture of Technovit® 7100 liquid (Heraeus, Kulzer, Wehrheim, Germany) (2-hydroxymethylmethacrylate) and Hardner I (dibenzoylperoxide), which was diluted to 30%, 50% and 70% with ethanol 94%. To enhance infiltration, samples were placed under vacuum during 24 h. The infiltrated samples were transferred to a 100% infiltration liquid for 48 h. Next, the samples were embedded according to Leroux et al. (2007) to obtain an optimal orientation of the samples within the resin. Transverse and longitudinal sections were cut at 5 µm with a rotation microtome (Minot, 1212, Leitz Wetzlar, Germany), equipped with a holder for disposable Superlap Knives (Adamas Instrumenten, Nether-

lands). The sections were collected on water drops on slides, which were subsequently dried on a hot plate at 40°C and stained with a 0.05% [w/v] aqueous solution of toluidine blue O (Meck, Darmstadt, Germany, C.I. No. 52040) and 0.1% [w/v] Na₂B₄O₇, and subsequently mounted with DePeX (Gurr, BDH Laboratory, Poole, U.K.).

LM images were made with a Nikon Eclipse E600 microscope, equipped with a Nikon digital camera DXM1200 (Nikon, Tokyo, Japan). All anatomical images were taken using the phase contrast II position of the microscope, which

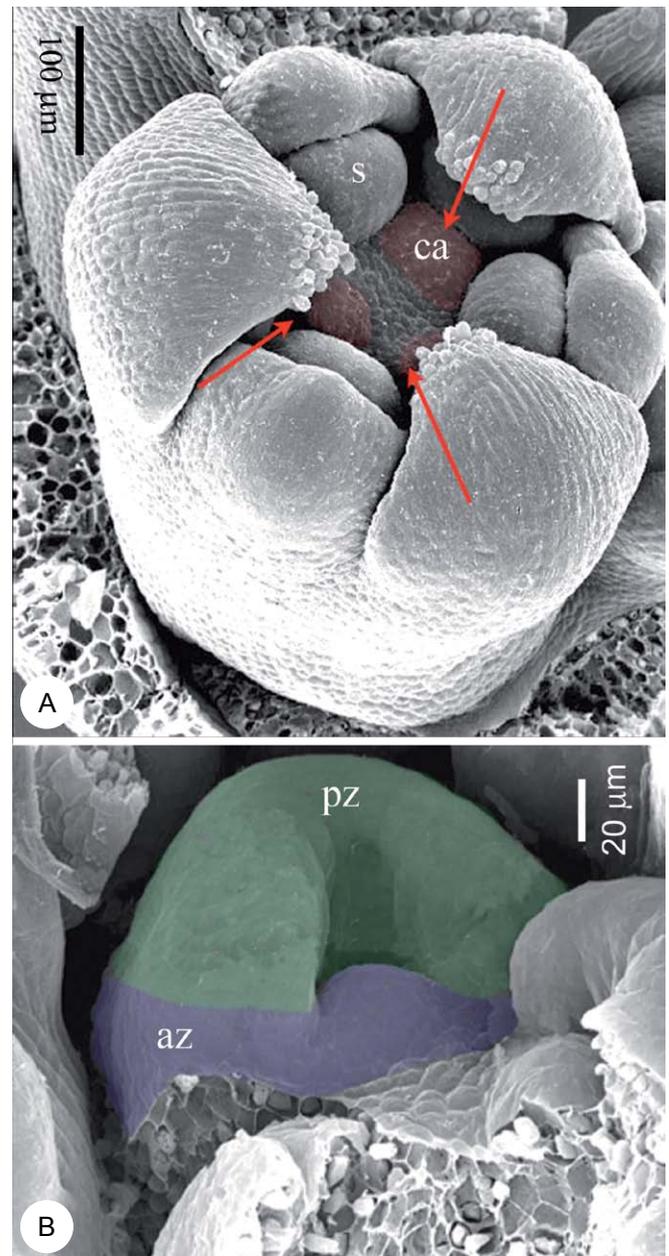


Figure 4 – SEM images of developing gynoecia in *Sansevieria trifasciata*. A, flower at early developmental stage in *S. trifasciata*. In red and arrowed, carpel primordia alternating with the stamen primordia; B, detail of a developing carpel in *S. trifasciata*, with a plicate zone (purple) and an ascidiate zone (green). Abbreviations: az, ascidiate zone; ca, carpel primordium; pz, plicate zone; s, stamen primordium.

creates a dark field effect under a magnification of 200x. Phase contrast adds contrast between the different stained tissues and makes xylem bundles to light up in bright blue when coloured with a toluidine blue O solution. Slices are shown for the first distal flower with a fully developed vascular system.

3D diagrams

The 3D diagrams as shown in figs 2, 3, 18, 19 & 20 were manually drawn in Rhinoceros 3D® (Mc Neel, Seattle, USA) and were based on the data of Blaser (1941a, 1941b) and our new data.

RESULTS

An illustration of individual carpel formation in flowers of *Sansevieria*

At early developmental stages, *Sansevieria trifasciata* has three individual carpel primordia. These grow out into carpels in which a plicate zone and an ascidiate zone can be distinguished (fig. 4A & B).

Cyperoid gynoecium development

In *Scirpus sylvaticus*, an undifferentiated flower primordium is positioned in the axil of a glume (fig. 5A). The different floral whorls appear with apically an annular ovary wall primordium surrounding a central ovule primordium (fig. 5B). The ovary wall grows up from the ring primordium, forming a single style and three stigma branches developing from three stigma primordia on the top of the ovary wall, two lateral-adaxial and a single abaxial one. In some individuals, four stigma branches are formed like in this example of *Scirpoides holoschoenus* (fig. 5D). A detailed observation of the differentiation of the ovary wall primordium and ovule primordium at the floral apex in *Scirpus sylvaticus* shows that both primordia originate simultaneously, after the formation of the stamen primordia, and also simultaneously with the formation of the perianth primordia (fig. 6A–D). In species with dimerous, dorsiventrally flattened gynoecia, such as *Dulichium arundinaceum*, an annular ovary wall primordium surrounding a central ovule primordium is formed in the same way as in *S. sylvaticus* (fig. 7A & B). Two lateral stigma primordia appear on the top of the ovary wall, growing out into two stigma branches (fig. 7C & D). The development of dimerous, laterally flattened gynoecia only differs in the dorsiventral position of the two stigma primordia on the top of the ovary wall as illustrated in *Pycneus bipartitus* (fig. 8A & B), *Kyllinga microbulbosa* (fig. 8C & D) and *Queenslandiella hyalina* (fig. 8E & F). In *Pycneus flavescens* and *P. sanguinolentus* a delay in the development of the adaxial stigma branch was observed with respect to the abaxial stigma branch (figs 15A & 16A). In *Lagenocarpus amazonicus*, the ribs of the mature nutlets are in the symmetry plane formed by the spikelet bract, prophyll and glumes of the spikelet for which we can confirm these to be laterally compressed dimerous pistils (fig. 9A). Several nutlets were observed with three in stead of two ribs (fig. 9B–D), in such

case, the second adaxial rib has sometimes only partially developed.

Development of the vascular system within spikelets and flowers

In cleared up spikelets and flowers, the annular xylem vessels are visible using a dark field light microscope (figs 10–12, electronic appendix 1). The combination of bleaching and dark field microscopy forms a fast and cheap technique enabling the study of vascular developmental patterns in flowers and spikelets of Cyperoideae. However, the quality of the images obtained by this technique was variable. Species with flattened spikelets, which are continuously producing new flowers and species with strongly reduced floral parts, such as *Pycneus* sp., were the most convenient to interpret.

For spikelets of *Pycneus sanguinolentus*, *P. flavescens* and *Queenslandiella hyalina*, xylem vascular elements appear first within the rachilla and glumes (figs 10A & 15A, electronic appendix 1A). The vascular bundles within the rachilla of the *Pycneus* species studied run in two opposite laterally positioned vascular zones (figs 10A & 13B, electronic appendices 2–3). In contrast, the rachilla in *Cyperus laevigatus* contains three groups of vascular bundles (fig. 13C, electronic appendix 6B) and a single group is present in *Kyllinga nemoralis* (fig. 13A, electronic appendices 4–5).

In the flowers, vascular bundles start to develop only after all floral organs have been initiated (figs 10A & 11B, electronic appendix 1). At this stage, the stigma primordia are developing on the top of the ovary wall and the developing ovule starts to turn towards the abaxial base. The development of the xylem vessels is initiated within the receptacle of the flowers (fig. 10A). From there, connections to the vascular bundles of the rachilla (receptacular bundles) and to the different floral organs are formed (fig. 10A, electronic appendix 1C). In the *Pycneus* and *Queenslandiella* species studied, only two receptacular bundles are present (figs 10A & 13B, electronic appendices 1A & 4C), whereas in the receptacle of the *Cyperus* species studied, three bundles are observed (figs 11B & 13C).

Stamens are the first floral organs in which vascular bundles originate (staminal bundles, figs 10A & 11B, electronic appendix 1A) and these subsequently connect to the developing bundles in the receptacle, which are at this stage not yet fully connected with the vascular traces of the rachilla. Within the receptacle of *Cyperus laevigatus* and *C. haspan*, a single trace branches off from each main receptacular bundle towards the stamen in the corresponding position. In *Pycneus* and *Queenslandiella*, there is no abaxial receptacular bundle and the vascular bundle of the abaxial stamen connects to both adaxial bundles (figs 10A & 13B, electronic appendices 1C & 2–3). Subsequently and only after the stamens are fully vascularised and connections between vascular bundles in the rachilla and receptacle are made, vascularisation appears in the ovary wall and ovule (figs 10A, 11B & 15A). Meanwhile, the receptacular vascular network becomes denser towards adaxial and abaxial positions and towards the centre (figs 10, 11C & 13B, electronic appendices 2–3). In *C. laevigatus*, there are no connections between the abaxial receptacular bundle and the receptacular plexus (fig. 11C). In all species

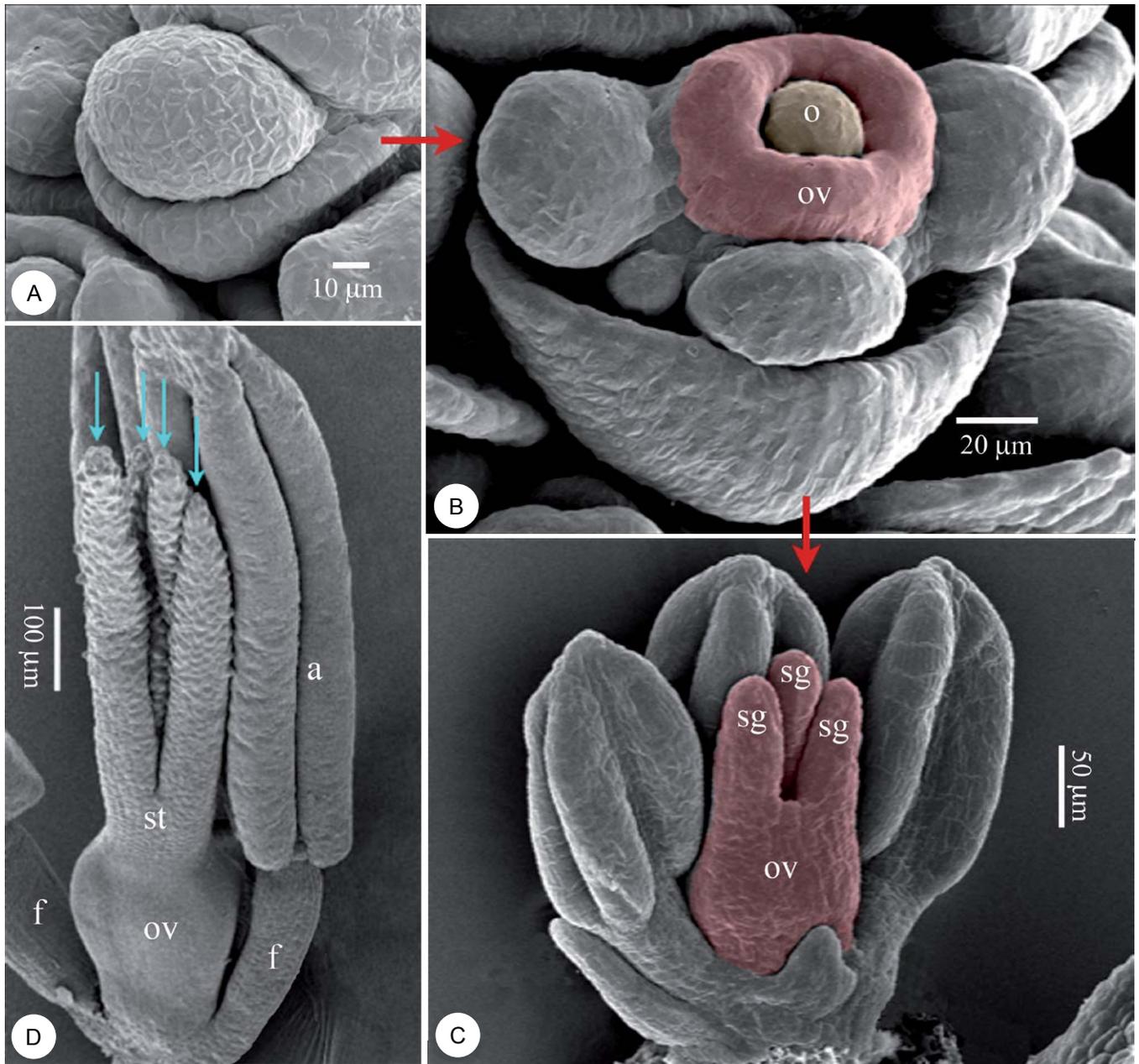


Figure 5 – SEM images of the early development of the trimerous gynoecium in *Scirpus sylvaticus* (A–C) and of a developing gynoecium in *Scirpoides holoschoenus* (D). A, flower primordium in the axil of a developing glume; B, early stage in gynoecium development with an annular ovary wall primordium (red) around a central ovule primordium (amber); C, developing gynoecium with three growing stigma branches; D, developing gynoecium with four stigma branches (arrowed).

Abbreviations: a, anther; f, filament; o, ovule primordium; ov, ovary wall primordium; sg, stigma primordium; st, style.

studied, the positions of the dorsal vascular bundles within the ovary wall are in line with the positions of the stigma primordia. Consequently, in *Cyperus haspan*, the ovary wall has two lateral and one abaxial dorsal bundles (fig. 11D), while in *C. laevigatus*, only the two lateral ones are present (figs 11B–C & 13C, electronic appendix 6B), in both species dorsal bundles run in the prolongation of the main receptacular bundles. Both in *Cyperus haspan* and *C. laevigatus* (figs 11 & 13C, electronic appendix 6) lateral bundles seem to be absent. *Rhynchospora corymbosa* is characterised by a very long style (fig. 12A), bearing two very short stigma

branches at its top (fig. 12B). Within the whole gynoecium and style two dorsal vascular bundles run in lateral positions (fig. 12C), revealing the dorsiventrally flattened, dimerous nature of these pistils. In *Pycurus* and *Kyllinga*, a single adaxial and a single abaxial bundle are observed (figs 10A–B & 13A–B, electronic appendices 3 & 5D), which do not run in the prolongation of the two main receptacular bundles but merge with the vascular plexus in the receptacle. The ventral bundles that run towards the centre fuse to form the central bundles, eventually connecting to the developing ovule vascular bundle (figs 10B, 11C & 13B, electronic appendix 3B–D).

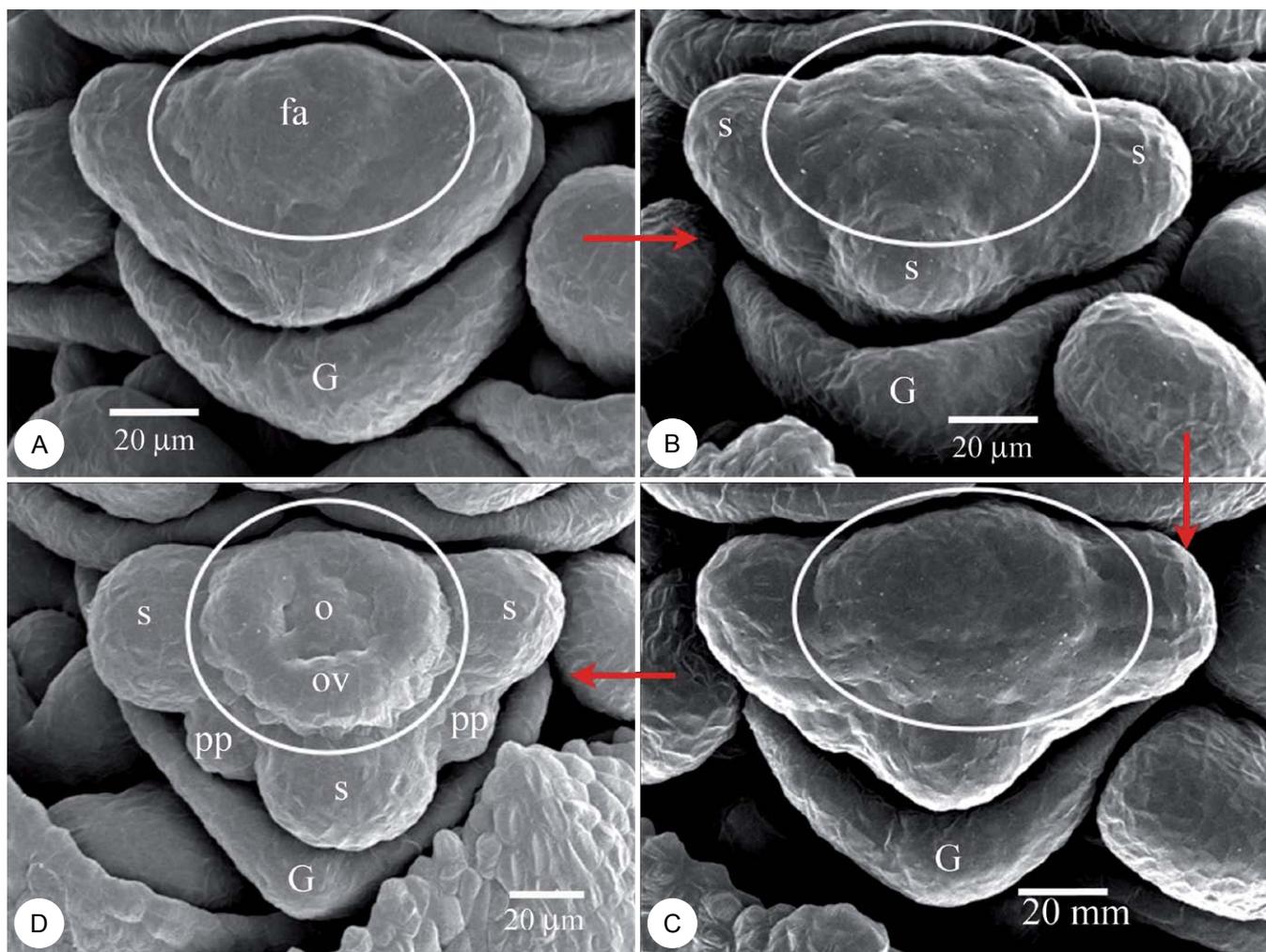


Figure 6 – SEM images of the earliest differentiation steps of the floral apex in *Scirpus sylvaticus* into annular ovary wall primordium and ovule primordium. A, flower primordium with perianth and stamen primordia and yet undifferentiated floral apex (encircled); B–C, an annular ovary wall primordium is being formed (encircled). B is a lateral-abaxial view, and C is an apical view on the same developmental stage; D, a central ovule primordium becomes visible, surrounded by the annular ovary wall primordium (encircled). The primordia of the other floral whorls also become more prominent.

Abbreviations: fa, floral apex; G, glume subtending flower; o, ovule primordium; ov, ovary wall primordium; pp, perianth part primordium; s, stamen primordium.

In *C. laevigatus*, this connection appears to be eccentric (fig. 11C). The vascular bundles of the ovary wall (dorsal bundles) and ovule (ovule bundles) independently connect to the vascular bundles of the receptacle (figs 10B, 11C–D & 13B, electronic appendix 3C–D). Meanwhile, the xylem vessel elements of the vascular bundles within the rachilla, glume and receptacle are become denser (figs 10A–B & 11C, electronic appendix 1B).

In *Kyllinga nemoralis*, the vascular bundles are concentrated into two laterally situated zones as they enter in the receptacle of the flower (fig. 13A, electronic appendix 4B). These soon split into two sets of three separate centres (fig. 13A, electronic appendix 4C). These centres expand abaxially and adaxially (fig. 13A, electronic appendix 4D–F) until eventually a ring of vascular bundles is formed (fig. 13A, electronic appendix 4G). Subsequently the staminal traces and the adaxial dorsal bundle are branching off from the bundle ring (fig. 13A, electronic appendix 4G) followed by the abax-

ial dorsal bundle and several traces, which eventually fuse in the centre to form the ovule vascular bundle (fig. 13A, electronic appendix 5B–C). The position of the abaxial ovary wall bundle is not on the symmetry plane formed by the rachilla and glume (fig. 13A, electronic appendix 5D–E).

Xylem elements differ in length and width among different organs. Rachilla and glume xylem bundles consist of long elements and the vascular tissue of the receptacle consists of a dense network of many short vessels, which results in a vascular plexus. The xylem bundles within the floral organs consist of only one or few long and narrow annular vessels (figs 10A–B & 11C, electronic appendix 1A).

Ovule development

The ovule primordium is formed from the apex of the flower primordium, simultaneously with the annular ovary wall primordium (figs 5–8 & 14A). Once the developing ovary wall

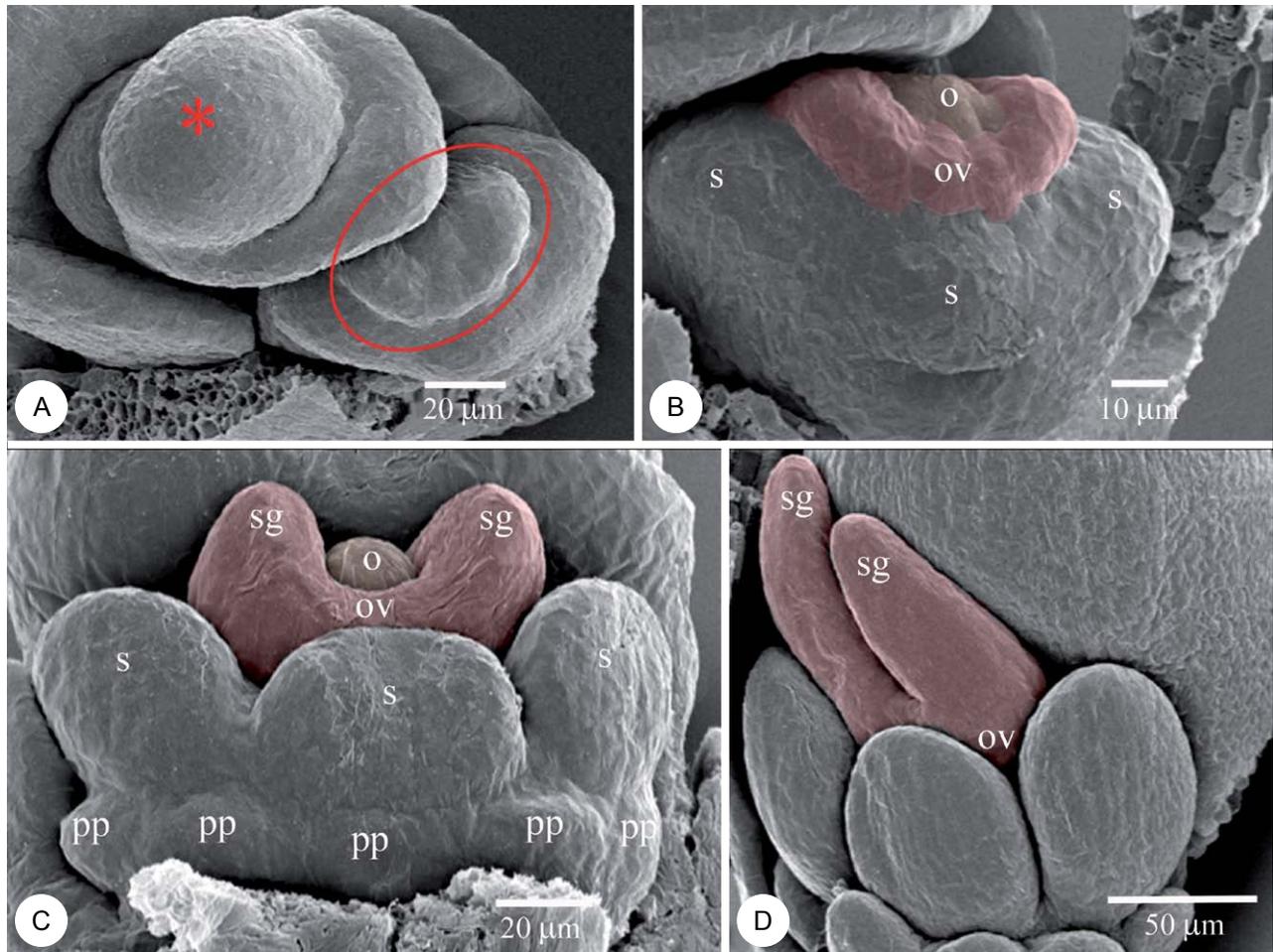


Figure 7 – SEM images of the development of the gynoecium in *Dulichium arundinaceum*, a species with a dorsiventrally flattened dimerous gynoecium. A, distal part of a spikelet with a developing flower with floral apex differentiating into annular ovary wall primordium surrounding a central ovule primordium (encircled in red); B, the annular ovary wall primordium (in red) surrounding a central ovule primordium (in amber). The stamen primordia are also visible; C, rising ovary wall with two laterally oriented stigma primordia (in red) surrounding the central ovule primordium (amber). All primordia of the other floral whorls are visible; D, the ovary wall encloses the ovule, and the stigma branches grow out. Abbreviations: o, ovule; ov, ovary wall (primordium); pp, perianth part primordium; s, stamen primordium; sg, stigma primordium; asterisk (*), apex of the rachilla.

encloses it, the ovule primordium starts to differentiate, (figs 10A, 14B & C). Subsequently and in successive order the interior and exterior teguments are formed, defining the micropylar zone (figs 14B, 15A & 16A). At this stage, three layers in the ovary wall start to differentiate (figs 14B & 15A). Meanwhile the ovule primordium grows out cylindrically, bending so that the micropylar zone forms an angle of 90° , directed to the abaxial side of the flower (figs 14B & 16C). When reaching this stage, the xylem of the vascular traces of pistil and ovule becomes visible (fig. 15A). The bending concurs with an elongation, continuing until the micropyle is turned over 180° and positioned against the basal-abaxial part of the funiculus (figs 14C & 16D). Meanwhile, at the basal and ventral (abaxial) part of the funiculus, numerous ‘obstructor hair’ primordia originate (figs 14C & 16B–D). They develop fast in the direction of the micropyle, often sticking together to form a kind of cover, which closes the micropyle (figs 13A–C, 14C, 16C & D, electronic appendices 2A, 4A & 6A).

DISCUSSION

Gynoecial ontogeny: congenitally fused carpels

According to Payer (1857) and never previously tested (Vrijdaghs et al. 2009), a cyperoid gynoecium originates from individual carpel primordia, which fuse postgenitally (as can be observed in e.g. *Sansevieria*, fig. 4). However, our results show that in the earliest developmental stages of the gynoecium in all species studied, no individual carpel primordia are present (e.g. fig. 5–8). Instead, the ovary wall originates as a ring primordium surrounding the central ovule primordium. This annular ovary wall primordium grows up as a bag-like structure, as also observed by Payer (1857: 699), who called it a ‘sac ovarien’. We agree with Payer (same page) that the development of the stigma branches originates from “deux ou trois bourrelets primitifs”, two or three primitive bulges on the top of the rising ovary wall, which we call stigma primordia since they are distinct meristematic

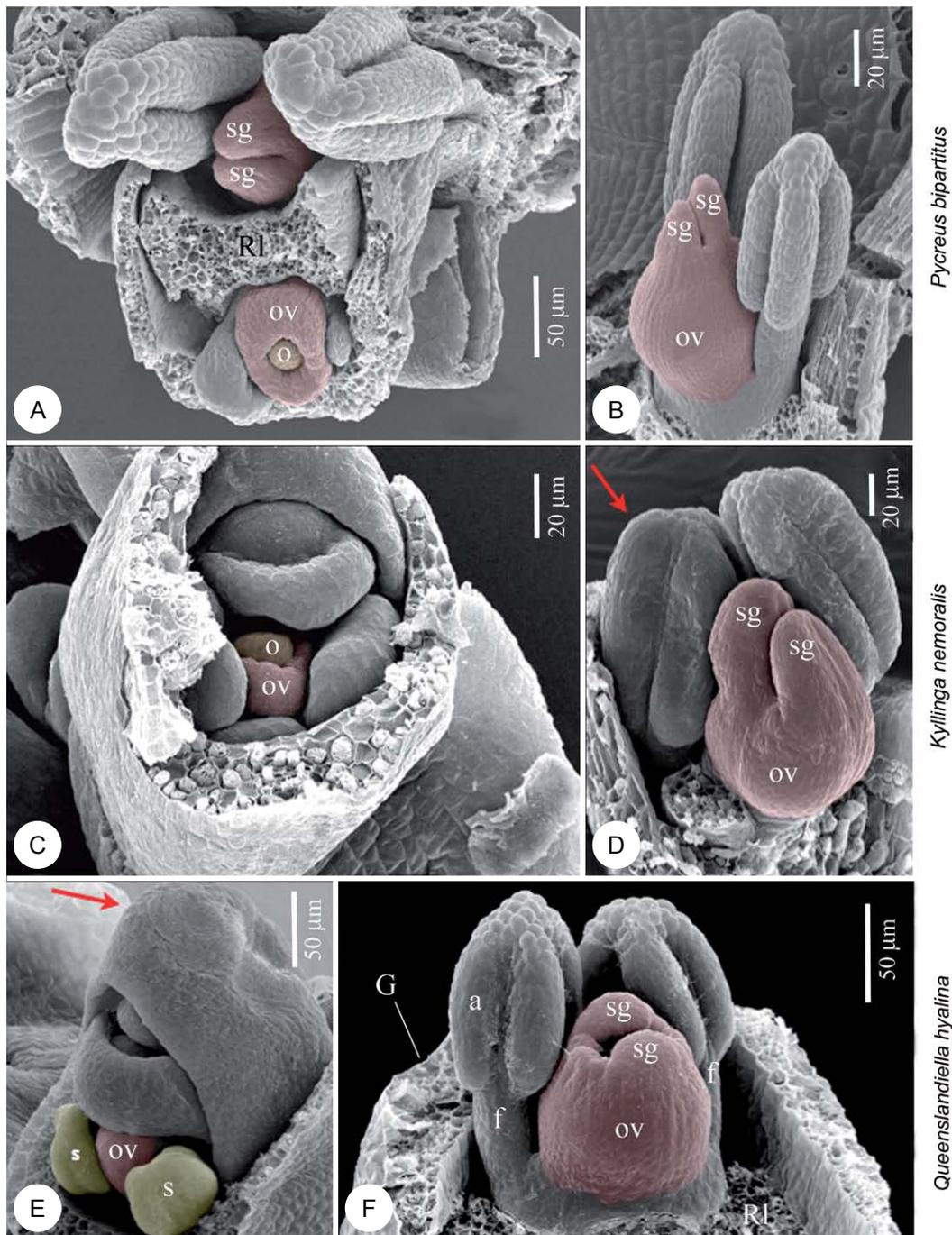


Figure 8 – SEM images of the development of the gynoecium in *Pycreus bipartitus* (A–B), *Kyllinga microbulbosa* (C–D) and *Queenslandiella hyalina* (E–F), species with laterally flattened gynoecia. A, apical view of a middle part of a spikelet in *P. bipartitus* with the distal part of it removed. Two alternate flowers at different developmental stages are visible. The in the image lower (youngest) flower has a still open, developing gynoecium with annular ovary wall (red) surrounding a central ovule primordium (amber). In the upper (oldest) flower, the ovary wall (red) envelops totally the ovule, and two dorsiventrally situated stigma branch primordia are growing out; B, developing flower in *P. bipartitus* with laterally flattened, developing pistil (red); C, developing flower with annular ovary wall primordium (red) surrounding a central ovule primordium (amber) in *K. microbulbosa*; D, developing gynoecium in *K. microbulbosa* with two dorsiventrally placed stigma branches. The red arrow indicates the abaxial stamen. The left lateral stamen is removed; E, lateral view of the distal part of a developing spikelet in *Q. hyalina*. Proximally, a developing flower is visible with two lateral stamens (yellow) and an ovary (red). The rachilla apex is hidden by older bonnet-shaped glume (arrowed); F, adaxial view of a developing flower in *Q. hyalina*. The ovary wall (red) is rising from the base and enveloping the central ovule. Two dorsiventrally positioned stigma primordia are visible on the top of the ovary wall. The stamen primordia have developed into anther and filament.

Abbreviations: a, anther; f, filament; G, glume; o, ovule; ov, ovary wall (primordium); RL, rachilla; s, stamen primordium; sg, stigma primordium.

zones positioned upon a structure with different nature (the ovary wall) and determined to grow out as stigma branches. In summary, in all cyperoid species studied, the floral apex consists of a fusion of the floral axis and the (three) carpels. From this tissue, both the annular ovary wall primordium, and the single, centrally positioned ovule primordium originate. The carpel tips (stigma primordia), from which the stigma branches develop subsequently can be considered as ontogenetic ‘witnesses’ of the carpellary origin of the ovary wall. Only when the stigma primordia originated on the top of the rising ovary wall, the positions of the original carpels become clear. Subsequently, vascular traces are initiated in these primordia, which will connect with the stele and give form to the ribs of the gynoecium/fruit. As carpel positions are

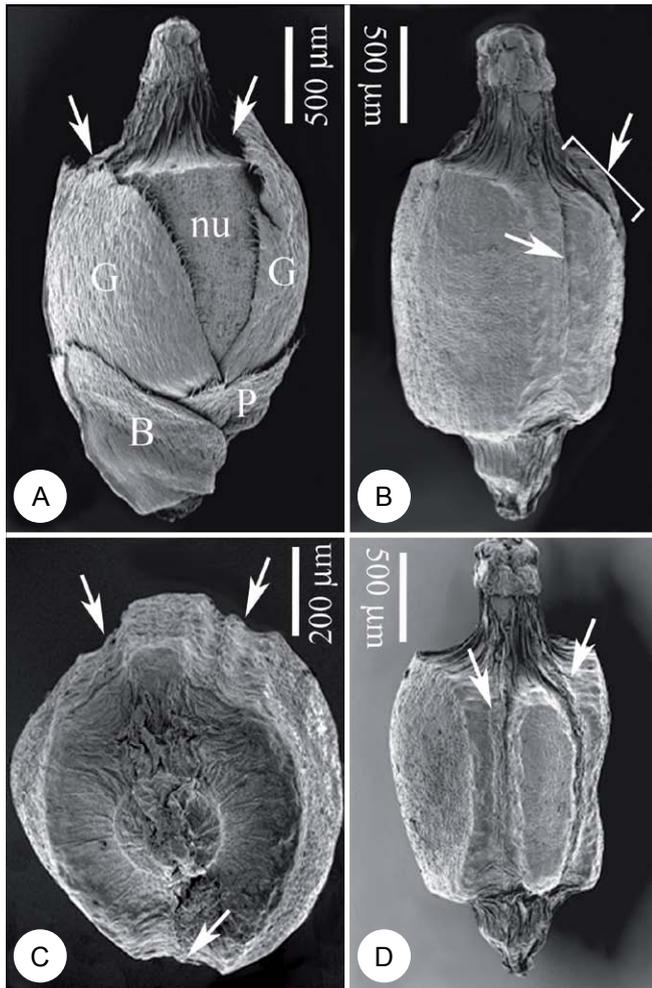


Figure 9 – SEM pictures of mature spikelets and nutlets in *Lagenocarpus amazonicus*, a species with laterally compressed dimerous nutlets. A, lateral view of a spikelet showing the ridges of a typical nutlet to lie in the symmetry plane formed by the bract, prophyll and glumes; B, lateral adaxial view on an atypical nutlet showing one fully developed ridge and one partially developed ridge at the adaxial side of the nutlet. The abaxial ridge is not visible; C, apical view of an atypical nutlet with one abaxial and two adaxial ridges; D, adaxial view of the same nutlet as in C showing two fully developed adaxial ridges.

Abbreviations: B, spikelet bract; G, glume; nu, nutlet; P, prophyll. Arrows indicate the positions of the ridges of the different nutlets.

quite well conserved (Endress 1995, 2001), it is not surprising that in the majority of Cyperoidae, the pistil is triangular with three stigma branches. However, the annular origin of the ovary wall gives it *de facto* new freedom of organisation since the stigma primordia are no longer linked to the rigid positions of individually developing carpels as found in most other monocots (fig. 17). We hypothesise that in Cyperoidae, the acquired organisational freedom might be reflected in the derived, dimerous pistils in at least some of the most recent, rapidly radiated taxa in the C₄ clade of the giant genus *Cyperus* with laterally flattened gynoecia, such as *Pycneus*, *Kyllinga* and *Queenslandiella* (fig. 8) (Larridon et al. 2011b).

Consequences of the presence of an annular ovary primordium

In our opinion, the observations of Endress (2006) and Vrijdaghs et al. (2005a) are examples of how congenital fusion of individual primordia into an annular primordium creates the condition to develop something totally new. Also for the Cyperoid pistils, several consequences of the presence of the annular pistil primordium can be identified especially involving alterations in the amount and positions of stigma primordia.

Decoupling of gynoecium wall and ovule – Firstly, in Cyperoidae, the development of ovary and ovule seem to be decoupled. This is already visible in the earliest stages of pistil, where the ovule starts developing already before the ovary has closed above it (fig. 18). Ovules, which appeared in the evolution much earlier than carpels, are to be considered as individual organs (Endress 2006). While in most angiosperms ovules are superimposed on carpels, the development of carpels and ovules appear to be decoupled in Cyperoidae. Similar patterns in early ovary development have been reported for other angiosperm families with syncarpous fruit types and basal uniovulate placentation such as Asteraceae (Harris 1995), Chenopodiaceae (Flores Olvera et al. 2008, 2011) and advanced Poaceae (Philipson 1985). Vascular evidence and other developmental characteristics will be discussed in a separate chapter on ovules.

Dedoublements and polymerisations – As the stigma branches are supposed to grow from meristematic zones in the carpel tips (stigma primordia), their number (in Cyperaceae usually three) reflects the number of original carpels. However, due to the congenital fusion of the carpel primordia, the number of stigma branches does not necessarily reflect anymore the original number of carpels. Moreover, splitting of a given primordium (dedoublement) is a common phenomenon, which can also result in deriving numbers of stigma branches. E.g. during our studies, we observed specimens with four stigma branches instead of three in *Cyperus capitatus* Vand. (Vrijdaghs et al. 2011) and *Scirpoides holshoenus* (fig. 5D). In a similar way, in taxa with laterally flattened dimerous pistils, we frequently found flowers with three instead of two stigma branches (e.g. in *Pycneus bipartitus* and *P. flavescens*, Vrijdaghs et al. 2011).

In *Lagenocarpus amazonicus* (fig. 9B–D), we observed nutlets with three instead of two ribs, in that case one of the adaxial ribs sometimes only partially developed. In our opin-

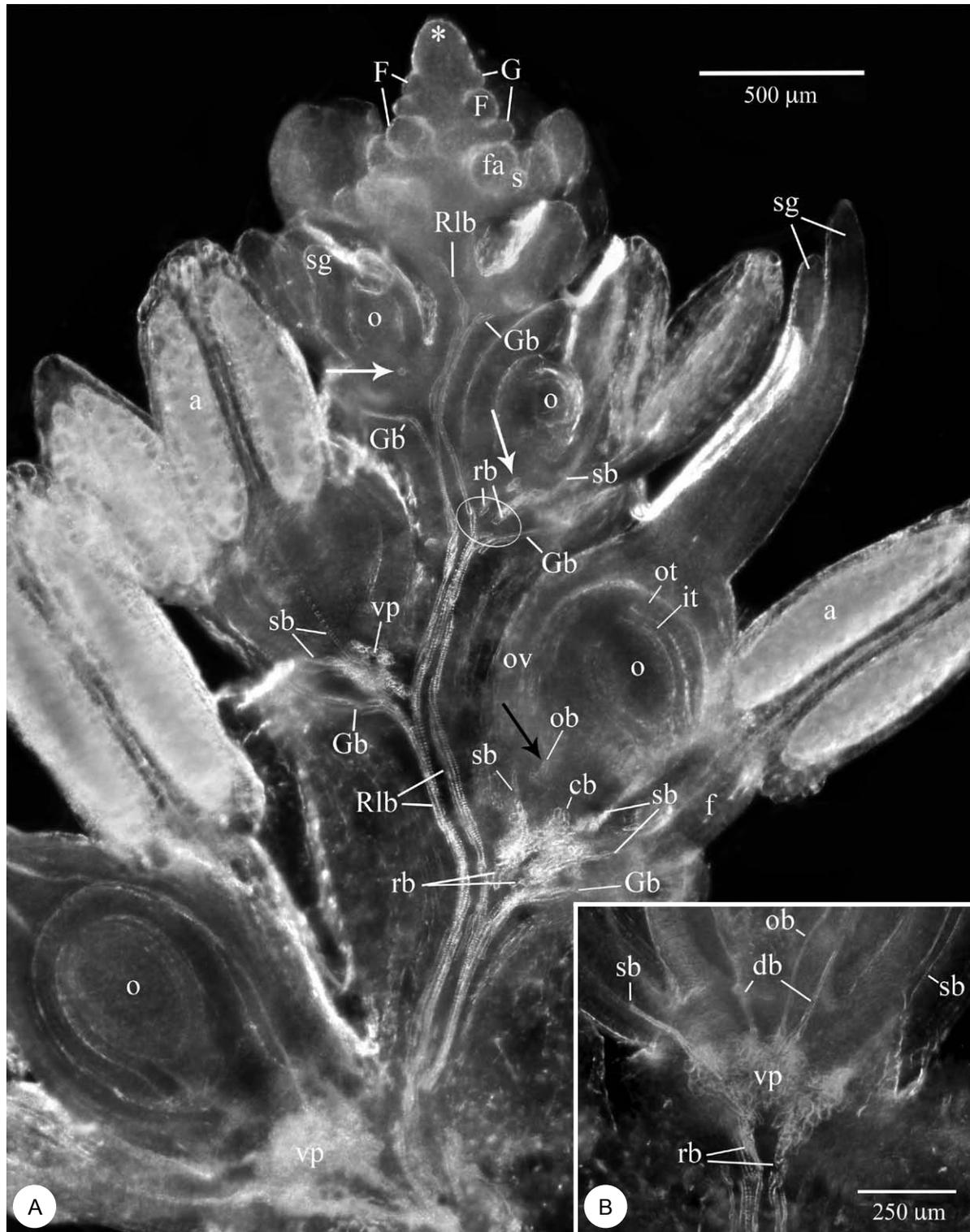


Figure 10 – Dark field images of a cleared up spikelet in *Pycneus sanguinolentus*, with laterally compressed dimerous gynoecia. Proximate glumes are removed. A, lateral view of a spikelet showing the gradual development of the vascular system within consecutive flowers; B, adaxial view of the base of a flower with all vascular traces developed.

Abbreviations: a, anther; cb, central vascular bundle; db, dorsal vascular bundle; F, flower (primordium); f, filament; fa, flower apex; G, glume (primordium); Gb, glume vascular bundle; it, inner tegument; o, ovule (primordium); ob, ovule vascular bundle; ot, outer tegument; ov, ovary wall; rb, main receptacular vascular bundle; Rlb, rachilla vascular bundle; s, stamen (primordium); sb, staminal vascular bundle; sg, stigma primordium; vp, vascular plexus (ventral bundles); asterisk (*), apex of the rachilla. White arrows indicate procambial initiation points within the receptacle. The black arrow indicates developing vascular connections of the ovule. A white ellipse indicates the developing vascular bundles within the base of the receptacle, which are still unconnected with the rachilla vascular bundles.

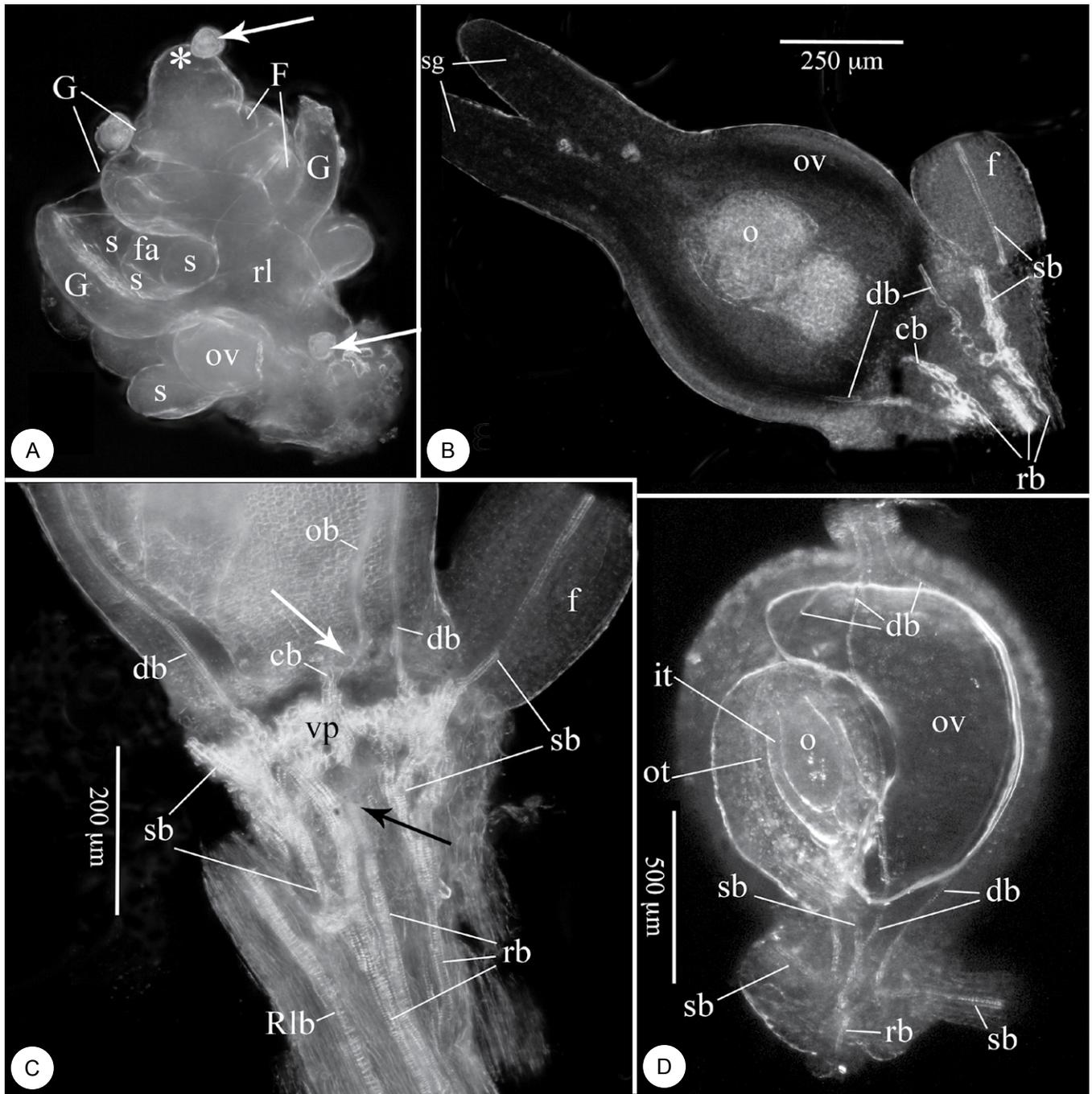


Figure 11 – Dark field images of cleared up spikelets and flowers in *Cyperus laevigatus* (A–C) that has dorsiventrally flattened dimerous gynoecia and *Cyperus haspan* (D), with a trimerous gynoecium. A, lateral view of a spikelet tip of *C. laevigatus* showing consecutive developing flowers. Vascular bundles have not yet originated. White arrows indicate pollen grains; B, adaxial view of an immature flower of *C. laevigatus* showing development of the vascular bundles towards the ovary wall, ovule and stamens; C, adaxial view of a mature flower of *C. laevigatus* with densification of vascular bundles within the receptacle. Vascular bundles of all floral organs have differentiated. The white arrow shows the eccentric connection of the ovular vascular bundle with the central vascular bundle. A black arrow indicates the unconnected abaxial receptacular bundle; D, young fruit of *C. haspan* in lateral view showing the three dorsal bundles within the ovary wall. Abbreviations: cb, central vascular bundle; db, dorsal vascular bundle; F, flower primordium; f, filament; fa, flower apex; G, glume (primordium); it, inner tegument; o, ovule (primordium); ob, ovule vascular bundle; ot, outer tegument; ov, ovary wall; ovb, ovary wall vascular bundle; rb, main receptacular vascular bundle; rl, rachilla; Rlb, rachilla vascular bundle; s, stamen (primordium); sb, staminal vascular bundle; sg, stigma primordium; vb, ventral bundle; asterisk (*), apex of the rachilla.

ion this is rather a reversal to the plesiomorphic trimerous state.

In several Cyperoid species (see table 2), such as *Carex dolichostachya* Hayata (described by Hayata (1921) as a separate genus *Diplocarex* Hayata) and *C. concinnoides* Mack. (Snell 1936), populations with four to six stigma branches were reported. In addition many rhynchosporoid species show a constant number of four (*Tetraria* p.p., *Tetrariopsis*), six (*Neesenbeckia*) or even eight (*Evandra*) to nine (*Tetraria* p.p.) stigma branches (Goetghebeur 1998). Also in Mapanioideae, Kern (1974) reported that six stigma branches occur often in *Chrysitrix* L and in *Paramapania* Uittien (Mapanioideae) he observed tetramerous pistils, most often in species which have normal trimerous pistils. In *Paramapania gracillima* (Kük. & Merr.) Uittien, tetramerous pistils are the dominant type (Kern 1974). The multiplication of the number of stigmas has not yet been clarified and may be part of a larger polymerisation phenomenon in these taxa. This ‘polymerisation phenomenon’ is reflected in the entire floral organisation, including perianth and androecium as well as the culm. It seems logical that the tetra- and octamerous pistils in the taxa mentioned above, are most probably polymerizations of dimerous pistils. Nevertheless we believe both dedoublements and polymerizations to be facilitated by congenitally fused carpels.

Facilitation of pistil dimerisations – Reductions in carpel numbers are common in Poales and usually, these are explained by reduction or fusion of carpels (e.g. Philipson 1985). Where reduction tendencies occur, a carpel (predestined to be reduced) first becomes sterile (e.g. in Eriocaulaceae, Ronse

Decraene et al. 2002) and due to the rigid position of the carpel primordia, reduction of an adaxial carpel mostly results in an asymmetric gynoeceium. Dimerisation may also result from a fusion between two of the three carpels, of which at least one is mostly sterile. The presences of multiple dorsal bundles are generally considered as proof of such a fusion product (e.g. Linder 1992). This widely adapted classic carpel theory is difficult to apply for dimerous Cyperoid genera since all carpels are congenitally fused. We believe the ring primordium facilitates dimerisations since these can be established by a simple loss of one of the stigma primordia together with all vascular traces at this side of the gynoeceium wall, instead of a gradual reduction of a complete carpel. Especially dorsiventrally flattened dimerous pistils can be explained by the loss of the abaxial stigma primordium (e.g. *C. laevigatus*, figs 11 & 13C). The multiple origins of this pistil type within many different genera, representing almost all Cyperoid tribes (table 2), corroborates the relative ease in which this pistil type can be derived from a trimerous pistil due to the presence of congenitally fused carpels (fig. 17D). The situation in laterally flattened pistils seems to be more complex since it involves stigmata in novel positions (fig. 17C). Therefore, Goetghebeur (1986) remarked that it was not possible to explain the origin of laterally compressed pistils as a result from simple carpel reductions. However, now we understand the laterally compressed pistil as a result of newly acquainted organisational freedom due to (1) the invention of annular ovary primordia and (2) the initiation of floral vessels in the different floral primordia. Subsequently, the initiated vessels grow to and connect with the stele. Probably, spacial pressure in compact inflorescences/spikelets triggers in *Pycreus* the gynoeceium adaptation from trimerous to dimerous (reduction in number of stigma branches) and laterally compressed.

Alterations in stigma positions – In most monocots, carpels develop as independent units that may (or not) fuse postgenitally with adjacent carpels (fig. 4). Consequently, at later developmental stages, their positions remain strongly conserved and this is even still the case for all trimerous Cyperaceae. However, in the laterally flattened dimerous pistils, this pattern is lost since they have two carpels in median positions (fig. 17C). Remarkably, both adaxial stigmata encountered in a regular cyperoid pistil are now replaced by a single stigma in an intermediate position. These pistils should therefore be considered as an exception of Endress’ (1995) observation concerning well-conserved carpel positions in angiosperms.

Blaser (1941a) explained the nature of this pistil type by reorganisation of the vasculature. However, our observations (summarised in fig. 19A, B & C) falsifies Blaser’s theory, which is further explained in the chapter on anatomical evidence. We believe that due to the organisational freedom in a congenitally fused carpel complex, after loss of one of the adaxial stigma primordia, the remaining stigma primordium develops in a more optimal position concerning the available space on the ring primordium with respect to the other stigma primordia, which is in the case of *Pycreus*, *Kyllinga* and *Queenslandiella*, opposite to the abaxial carpel (figs 8, 13A–B & 17C). This is the organisational freedom that we assume to be a result from congenital fusion of carpels. In cases where an additional adaxial stigma primordium is formed in a few individual flowers as a developmental er-

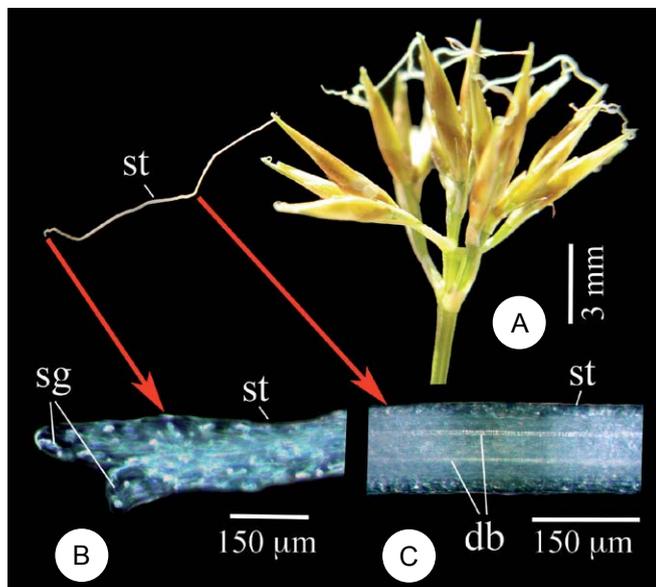


Figure 12 – Pseudomonomery in *Rhynchospora corymbosa*, which has dorsiventrally flattened dimerous gynoeccia. A, part of an inflorescence showing the long and single styles emerging from the tips of the spikelets. Red arrows indicate the parts of the style from which details are shown in B & C; B, dark field microscopy picture of a cleared up tip of the style showing the presence of two stigma rudiments; C, dark field microscopy picture of a cleared up middle part of the style showing two dorsal vascular bundles. Abbreviations: db, dorsal bundle; sg, stigma; st, style.

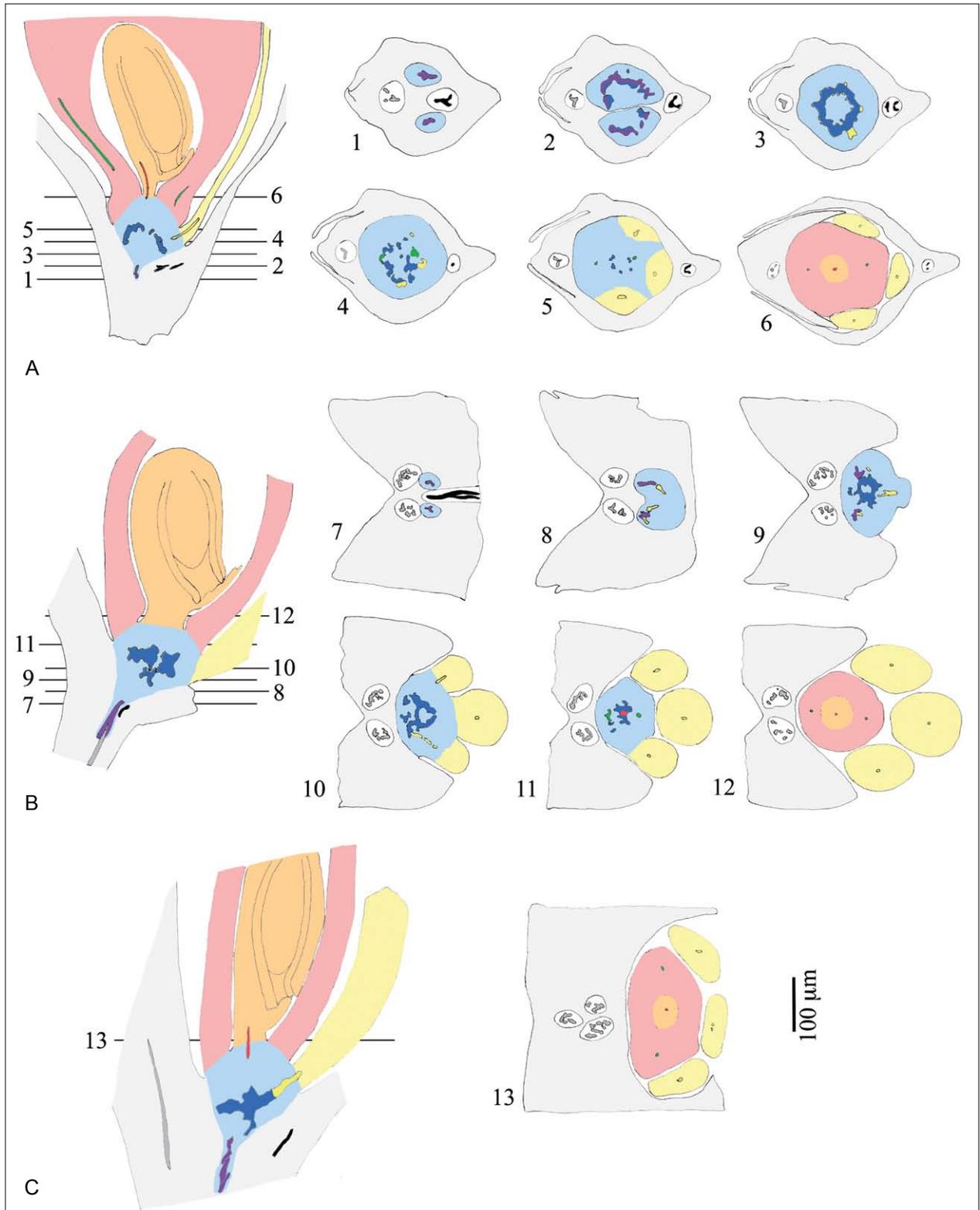


Figure 13 – Diagrams of slices through some Cyperoid flowers based on phase contrast microscopy pictures (original pictures in electronic appendices 2–6). A, *Kyllinga nemoralis*, with laterally compressed dimerous pistils. B, *Pycurus flavescens*, with laterally compressed dimerous pistils. C, *Cyperus laevigatus*, with dorsiventrally compressed pistils.

The first diagram of each series shows a longitudinal slice, subsequent transversal slices are indicated with black lines and numbered on the diagrams. Colour codes floral organs: pale blue, receptacle; pale green, gynoecium wall; pale red, ovule; pale yellow, stamens; grey, rachilla and glume. Colour codes vascular bundles: black, glume bundle; blue, receptacular plexus (ventral bundles); green, dorsal bundles; grey, receptacular bundle; red, ovule bundle; purple, main receptacular bundle; yellow, stamen bundle.

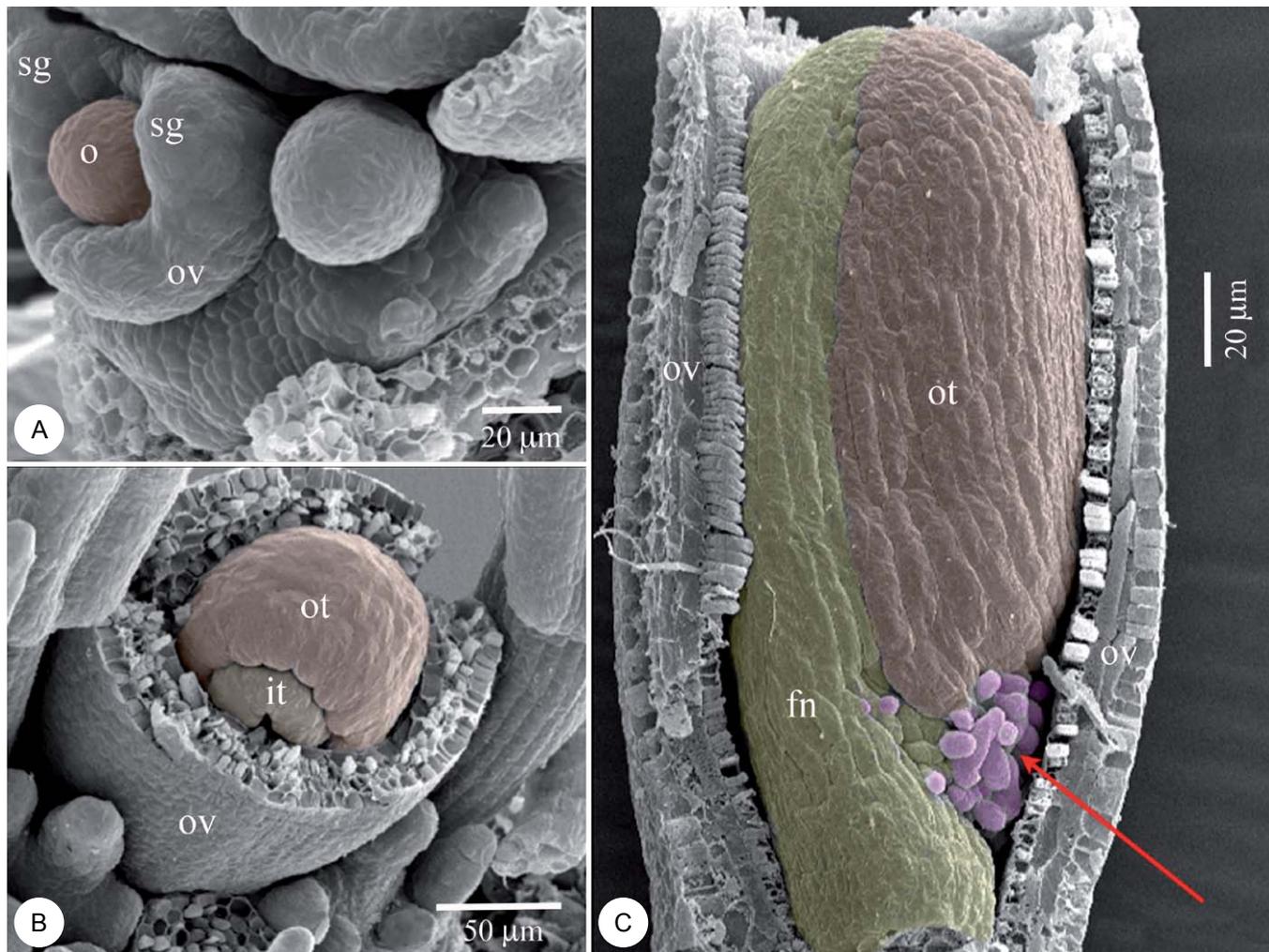


Figure 14 – SEM images of the development of the ovule in *Eriophorum latifolium* (A–B) and *Dulichium arundinaceum* (C). A, ovule primordium surrounded by the annular ovary wall primordium; B, developing ovule with outer integument (red) and inner integument (amber); C, longitudinal view of a mature, anatropous ovule with the funiculus (yellow), outer integument (red) and obturator hairs (purple) growing upon the funiculus and covering the micropylar zone (arrowed). Abbreviations: fn, funiculus; it, inner integument; o, ovule; ot, outer integument; ov, ovary wall (primordium); sg, stigma primordium.

ror, these shift again out of the intermediate position (e.g. *Pycneus*, Vrijdaghs et al. 2011, *Lagenocarpus amazonicus*, fig. 9B–D) into a spatially more optimal position. A similar situation in which a single carpel in an intermediate position where originally two carpels were present has also been described in Eriocaulaceae and hypothesised to have resulted from the fusion of sterile carpels and their dorsal bundles (Ronse Decraene et al. 2002). We can assume congenitally fused carpels of Cyperoideae (which are decoupled from the ovule) can behave in a similar way as fused sterile carpels in other taxa. However, our findings on vascular development (see chapter on anatomical evidence) do not support the idea of fusion of two dorsal bundles to a single bundle in an intermediate position but support our theory that stigma primordia can shift to more optimal positions in some complexes of fused carpels after loss of additional stigma primordia.

All dimerous pistils found in Cyperoideae can thus be explained by a combination of a fusion step (congenital fusion of all the carpels, resulting in a new kind of primordium,

the annular ovary primordium) and a reduction in number of stigma primordia, which implies a corresponding reduction in number of dorsal bundles. While theoretically a ring primordium would allow for any novel position of the stigmata, in the case of dimerous pistils only two types can be distinguished in which the pistils are either in lateral (dorsiventrally flattened type) or median (laterally flattened type) positions, which are the only positions congruent with the symmetry plane of the flowers and their subtending bract (figs 2 & 17). In the following chapter we discuss some underlying mechanisms that could help to explain these patterns of pistil dimerisations in Cyperoideae.

Underlying mechanisms for pistil dimerisations in Cyperoideae

Spatial pressures – In Poales, reduction of the number of carpels has often been interpreted to be a consequence of spatial pressures that the pistil suffers during its development, especially in taxa that bear dense spikelets such as Restionaceae

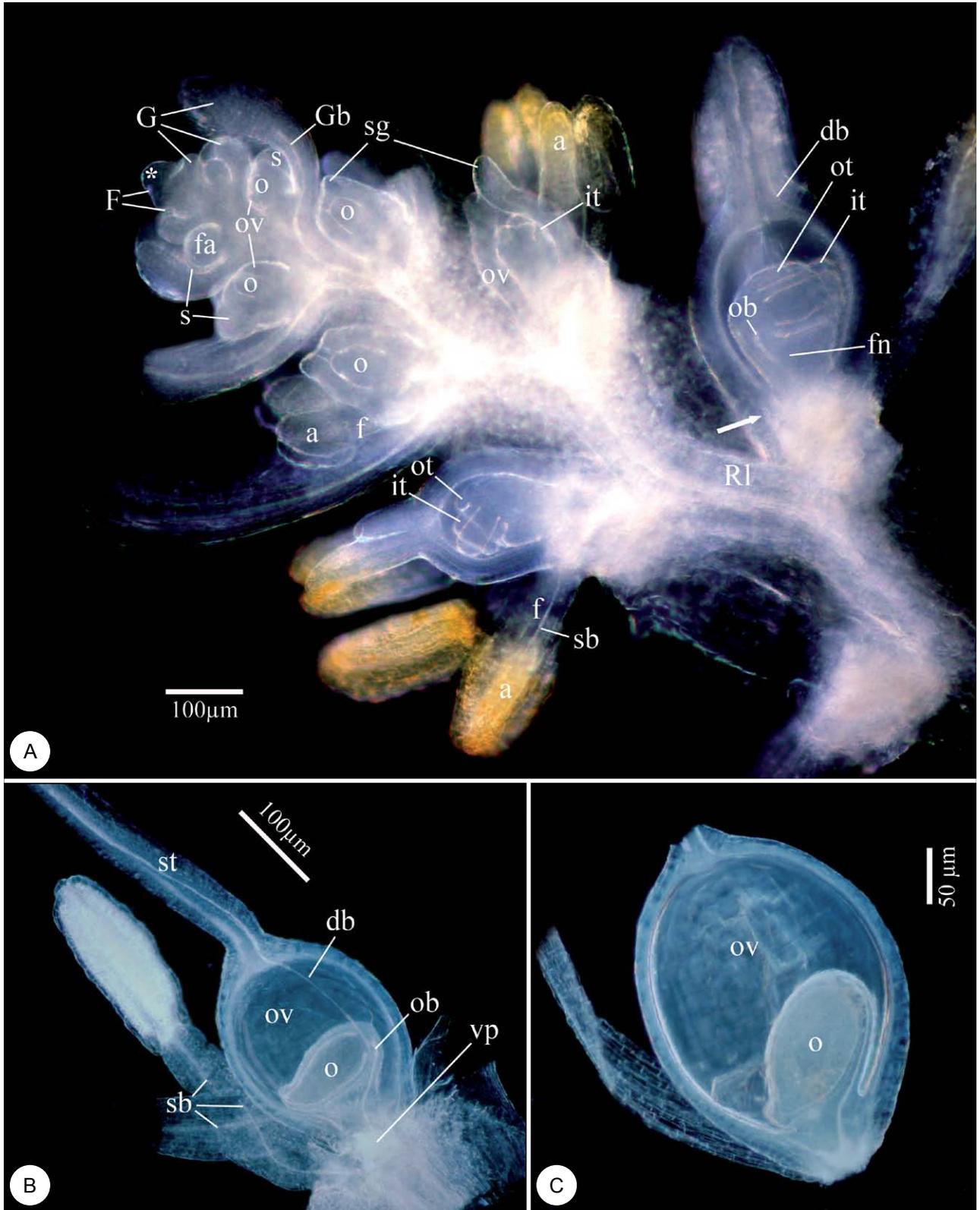


Figure 15 – Dark field microscopy image of cleared up spikelets and flowers in *Pycreus flavescens*. A, lateral view of a spikelet with proximal glumes removed, showing the early development of the gynoecium and ovule in the subsequent flowers. A white arrow indicates a developing dorsal bundle; B, lateral view of a flower just before anthesis with fully developed vascular system; C, lateral view of a flower after anthesis. Abbreviations: a, anther; db, dorsal vascular bundle; f, filament; F, flower (primordium); fa, flower apex; fn, funiculus; G, glume (primordium); Gb, glume vascular bundle; it, inner tegument; o, ovule (primordium); ob, ovule bundle; ot, outer tegument; ov, ovary wall (primordium); RI, rachilla; sb, staminal vascular bundle; st, style; vp, vascular plexus; asterisk (*), apex of the rachilla.

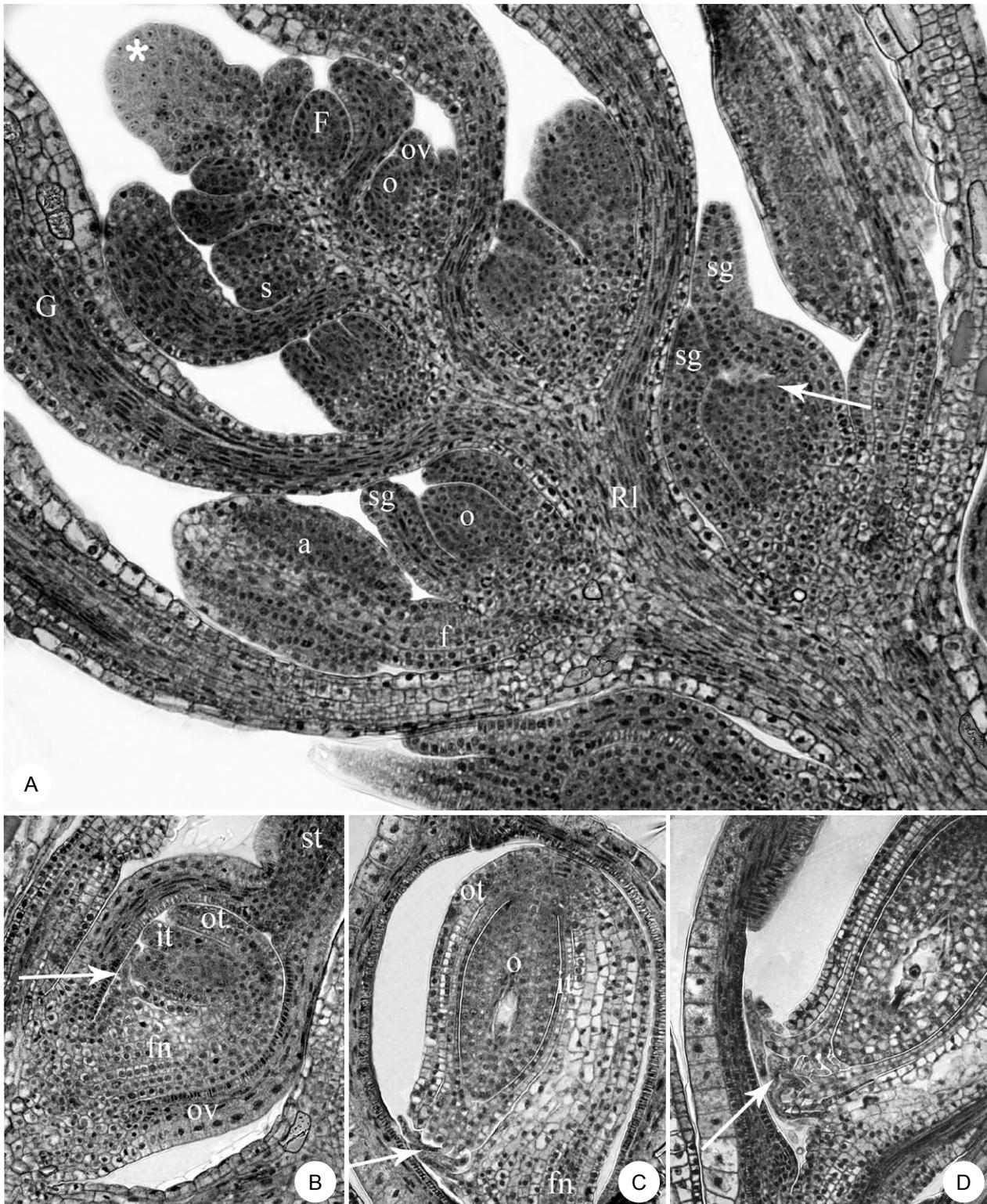


Figure 16 – Light microscopic images of sections through spikelets and gynoecia of *Pycneus sanguinolentus*. A, section through the central part of a spikelet showing a lateral view of the early development of the gynoecium and ovule in the subsequent flowers. A white arrow indicates the development of the inner tegument when the ovule tip starts to bend towards the abaxial side; B, older developmental stage showing a bending ovule with obturator hairs growing from the abaxial side of the funiculus (arrowed); C, lateral view of a mature ovule, fully bended ovule in which the obturator covers the micropylar zone; D, detail of the obturator of a mature ovule (arrowed) showing the obturator hairs growing into the micropyle.

Abbreviations: a, anther; f, filament; F, flower (primordium); fn, funiculus; G, glume (primordium); it, inner tegument; o, ovule (primordium); ot, outer tegument; ov, ovary wall (primordium); Rl, rachilla; sg, stigma primordium; st, style; asterisk (*), apex of the rachilla.

(Ronse Decraene et al. 2002) and Poaceae (Philipson 1985). In Cyperoideae, only part of the variation can be explained in this way. Dorsiventrally flattened pistils are quite common in Cyperaceae (table 1) and often appear to be correlated with taxa bearing dense spikelets with restricted developmental space for the flowers in the abaxial direction, e.g. *Mapania* Aubl., *Nemum* (Larridon et al. 2008). One could also try to understand dimerous, laterally flattened pistils as a result of lateral pressures caused by the two lateral stamens, forcing the adaxial carpel that remains after reduction into a median position. However, we would then expect the connection of the dorsal bundles to the ventral plexus to reflect the original position of the remaining carpel and therefore to be asymmetric, which is not the case (fig. 13A–B, electronic appendices 2–5). Moreover, at early developmental stages of the flower, there is an equally strong spatial pressure on the median adaxial part of the ring primordium by the rachilla and the higher glume. In the species of *Pycreus* and *Queenslandiella* studied, this spatial limitation even results in a delay of the development of the adaxial stigma branch with respect to the abaxial stigma (figs 8B, 15A & 16A) (Vrijdaghs et al. 2011), even before the developing stamens become large enough to interfere with the development of the stigma branches. In addition, the wings of the alternating glume, which are attached to the rachilla by epicaulescent growth (Vrijdaghs et al. 2009), push the developing stamens towards the abaxial position (fig. 8E). In *Kyllinga*, *Lagenocarpus amazonicus* and *Rhynchospora rubra* subsp. *rubra* spikelets only bear a single flower and are aggregated in very dense florescences. Possibly spatial limitations played a more important role in these taxa than in *Pycreus* and *Queenslandiella*. From a spatial point of view trimerous pistils still seem to be the most advantageous in most Cyperoid spikelets since stigma primordia are in optimal positions to grow up easily in the spaces between the different overlapping glumes of the spikelet.

Zygomorphy of the spikelet – All cases of laterally compressed dimerous pistils seem to be linked with distichously arranged spikelets (table 1). In other taxa, such as *Rhynchospora*, *Dulichium*, *Websteria* (now in *Eleocharis*) and *Cyperus*,

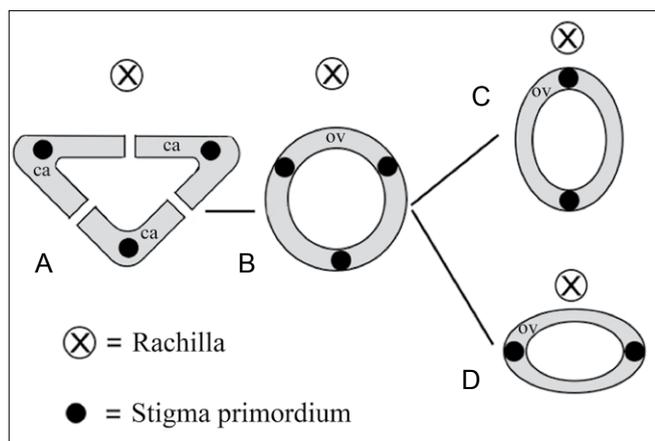


Figure 17 – Schematic representation of: A, a tricarpellate gynoecium; B, a trimerous gynoecium developing from an annular ovary wall primordium and gynoecia derived from B; C, laterally flattened with dorsiventrally positioned stigma primordia; D, dorsiventrally flattened with laterally positioned stigma primordia.

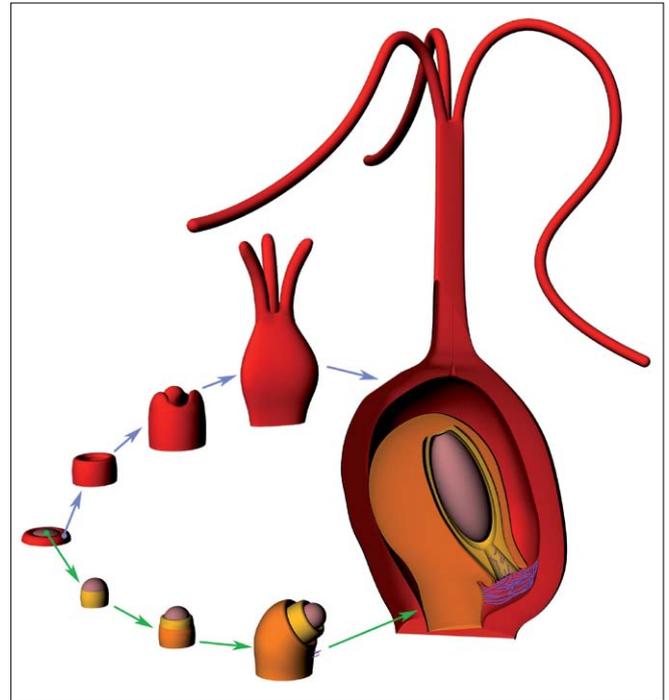


Figure 18 – Series of models representing the decoupling in the development of the ovary wall (upper series) and of the ovule (lower series) in Cyperoideae.

Colour codes: red, ovary wall; reddish brown, ovule tip; yellow, inner tegument; orange, outer tegument and funiculus; purple, obturator hairs.

there may be a link of dorsiventrally compressed pistils with the presence of distichously placed glumes. This apparent correlation of floral zygomorphy with the presence of distichously organised spikelets may be part of an underlying pattern of bilateral symmetry of the complete spikelet as a functional flowering unit. Cyperoid spikelets (and sometimes whole inflorescences) tend to take over floral function (e.g. Vrijdaghs et al. 2009). In wind pollinated grasses and sedges, this often goes together with a reduction of floral parts (Rudall & Bateman 2004). As an example, the loss of abaxial stamens occurs frequently with bilateral floral symmetry (Rudall & Bateman 2004). Vrijdaghs et al. (2011) observed that in most Cyperoideae with a reduction of the number of stamens, the abaxial stamen disappears first. This is particularly the case in *Pycreus*, which has zygomorphic gynoecia (Kükenthal 1936). Pressures from surrounding organs fail as an explanation for the frequent loss of the abaxial stamen in *Pycreus*. Possibly, in *Pycreus*, there is a connection with the absence of abaxial main bundles within rachilla and receptacle (fig. 13B, electronic appendices 2–3). In addition, we also observed a dimerisation of the vascular system at the level of the rachilla in all *Pycreus* and *Queenslandiella* species studied (figs 10 & 13B, electronic appendices 1–3). However, the stigma primordia, and hence also the pistil vascularisation develop only after the formation of the vascular system within the rachilla (fig. 10). The number of dorsal bundles entering a flower equals the number of bundles within the rachilla. This can be explained by procambial initiation occurring in the centre of the receptacle. Subsequently, connections with

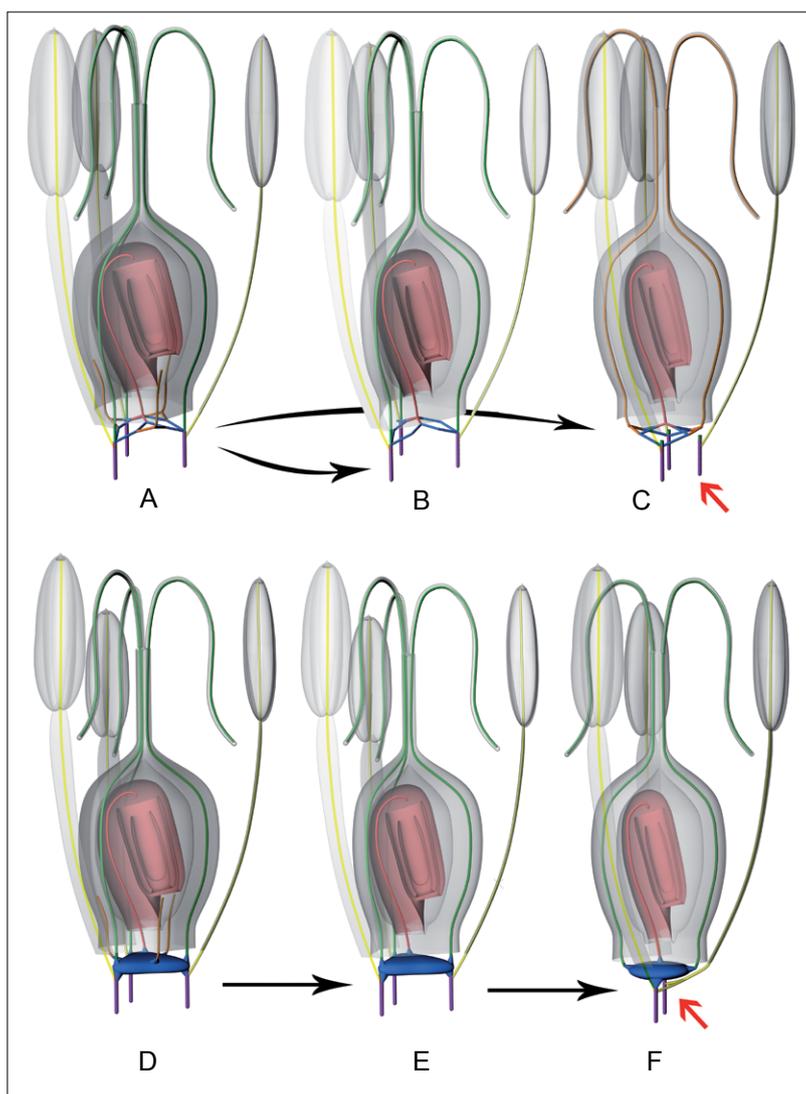


Figure 19 – Series of diagrams contrasting Blaser’s (1941a, 1941b) model (A–C) with our current interpretation (D–F) of the vasculature in laterally compressed dimerous pistils. A, Blaser’s (1941a, 1941b) model of the vasculature in a primitive sedge (e.g. *Scirpus*) in which lateral bundles are present in the sides of the gynoeceum wall, which connect to the ventral bundles; B, Blaser’s (1941a, 1941b) model of a *Cyperus* flower, where lateral bundles are absent; C, Blaser’s (1941a, 1941b) interpretation of a *Pycreus* flower, which has laterally flattened dimerous pistils. Since the vascular bundles that run within the gynoeceum wall towards the stigmata connect with the ventral bundles, Blaser (1941a) interpreted these as lateral bundles, which took over the function of the dorsals. Subsequently he postulated separate origins of models B and C from A (black arrows). A red arrow indicates an abaxial receptacular bundle connecting towards the abaxial anther, which Blaser (1941a) reported in his study; D, this model corresponds to model A. However, our current study shows the vasculature within the receptacle is highly disorganised and is here shown as a vascular plexus, which corresponds to what Blaser (1941a, 1941b) indicated as ventral bundles; E, same as model B with ventral bundles shown as a vascular plexus; F, same as model C with ventral bundles shown as a vascular plexus. Vascular traces within the ovary wall are interpreted as dorsal bundles and thus as homologous to these in models D & E. This corroborates with the origin of *Pycreus* from a *Cyperus* ancestor (black arrows). A red arrow indicates the absence of an abaxial dorsal bundle in *Pycreus* and connection of the vascular bundle of the abaxial stamen to both adaxial receptacular bundles, as observed in this study.

Colour codes: purple, receptacular bundles; yellow, staminal bundles; green, dorsal bundles; blue, ventral bundles; red, central and ovule bundles; orange, lateral bundles.

the rachillar bundles are made (fig. 20). In their study of the wandering carpel mutant (*wcr*) of *Zea mays* (Poaceae), Irish et al. (2003) hypothesise that both spikelet polarisation/orientation and floral symmetry are regulated by changes in a same factor. Several other authors assumed that zygomorphic development in flowers and leaves is the result of the inhibition of growth in the adaxial part of the floral meristem, which is induced by the shoot meristem (e.g. Wardlaw 1949, Luo et al. 1996). These hypotheses may help to understand the apparently higher frequency of pistil and other dimerisations in taxa with distichously organised spikelets in Cyperaceae and Poaceae.

We can conclude that congenital fusion of carpels allowed several separate origins of laterally flattened pistils within Cyperoidae. Moreover, our study shows this pistil type originated much easier than previously assumed when using classic carpel reduction theories. Therefore we might have to face the possibility of multiple origins of laterally flattened pistils even within Cyperaceae, which is however still waiting for molecular confirmation.

Anatomical argumentations, integration and comparison of our data with Blaser’s (1941a, 1941b)

Bidirectional development of the floral vascular system

– At early developmental stages of flowers of *Pycreus sanguinolentus* and *Queenslandiella hyalina*, we found vessel initiations at several separate procambial zones within the rachilla and the base of the different floral organ primordia (fig. 10, electronic appendix 1). The development of vascular bundles in the species studied concurs with the basic pattern found in culms and leaves of *Cladium* (Fisher 1971) and in angiosperms in general (Endress 1994, Dickison 2000), therefore we think that the formation of a vascular system by the merging of remote procambial strands from different organs in the plant may be the general pattern in all Cyperaceae. The formation of vascular connections with nearby main bundles seems to be regulated by signals from developing primordia resulting in the formation of vascular bundles where necessary. This is reflected in the sequence of initiation of the vascular tissue of the floral organs and their connections to the receptacular bundles (ventral bundles/receptacular plexus), which follow the same order as the sequence

of appearance of the floral primordia, starting with the stamens (figs 10–11 & 20, electronic appendix 1).

The presence of procambial initiation points within the receptacle and the formation of connections induced by the primordia of the different floral organs explains the connection of the gynoecial wall vascular traces in all pistil types to the central plexus within the receptacle. It also explains that in species, which lack the abaxial receptacular bundle, the abaxial staminal bundle connects to both adaxial bundles (e.g. *Pycreus sanguinolentus*, fig. 10A, *Pycreus flavescens*, figs 13B & 19F, electronic appendix 2D–G, *Rhynchospora macrostachya* Torr. ex A.Gray, Blaser 1941b). Concerning this case we should remark that Blaser (1941a) described an abaxial receptacular bundle in *Cyperus rivularis* (= *Pycreus bipartitus*), which branches towards the abaxial stamen and subsequently disappears. In *P. diandrus*, a species without abaxial stamen, he also observed only two receptacular bundles entering the flowers. Both *P. bipartitus* and *P. diandrus* are closely related to *P. sanguinolentus* (Clarke 1908, Kükenthal 1936), which is included in this study. Next, our model predicts the amount and positions of dorsal bundles of the flower, which connect to the corresponding bundles within the rachilla, and it explains the existence of a dense network of rather disorganized and short vessels within the floral receptacles (e.g. figs 10B, 11C & 20F). Moreover, this concurs with the dissimilarities in xylem vessels we observed within the receptacle and floral organs. Vascular traces within the receptacle consist of a large amount of rather disorganised short vessels in comparison with the traces within the floral organs, which mostly consist of few, long and narrow annular vessels (e.g. fig. 10A). Finally, pistil and ovular vascular bundles in all samples studied branch off within the receptacle to form independent bundles before entering the gynoecia, which corroborates the presence of separate primordia for the ovule and ovary wall from the start of the differentiation of the floral apex (figs 18 & 20).

In the receptacle of the Cyperoideae studied we observed a strong density of vascularisation (figs 10–11, electronic appendices 1–5), which confirm the observations of Saunders (1937) and Snell (1936). In contrast with Blaser (1941a, 1941b), these authors considered the vascularisation within the receptacle to be disorganised. In our opinion, the dense and disorganised nature of the vascularisation within the receptacles of Cyperoideae reflects the presence of the annular primordium. This vascular plexus of the receptacle might be interpreted as an adaptation to fruit dispersal, since it position concurs with the abscission zone of the mature nutlets.

To conclude, our observations suggest an ontogenetic pattern for the vascular system in Cyperoideae, which appears to be formed from different initiation zones from which the growing vessels find each other (fig. 20). In contrast, Blaser (1941a) followed an acropetal model (e.g. Grégoire 1938) to understand the development of a vascular system in Cyperaceae, in despite of the fact that he reported unconnected vascular supply in rudimentary abaxial style branches of dimerous *Schoenoplectus* species and that he logically suggested a bidirectional development of the vascular system within the receptacle, rather than acropetal development.

Dorsiventrally flattened dimerous pistils – As in most taxa with dorsiventrally compressed pistils, we observed that in *C. laevigatus* the abaxial stigma branch disappears together with all vascular traces at this side of the ovary wall (figs 11 & 13C, electronic appendix 6). Blaser (1941a), reasoning from an acropetal vascularisation model, supported the idea of the loss of the abaxial carpel in *Schoenoplectus*, based on the presence of unconnected bundles within rudimentary abaxial style branches. However, traces originate in the floral organ primordia to subsequently connect with the stele. In this case, the connection with the stele was not made, but the presence of unconnected bundles can indeed be interpreted as an indication of an original third carpel. In many taxa with dorsiventrally compressed pistils, the ovule is still vascularised with bundles from the abaxial receptacular trace, which were interpreted by Blaser (1941a) as vestigial bundles of the abaxial carpel (see fig. 3B). However, in our opinion, the ovule is connected to the rachillar plexus, independently from the dorsal bundles of the carpels (figs 10–11, 13 & 20). In the species studied, the number and positions of the main receptacular bundles reflect the number and positions of vascular bundles within the rachilla of the spikelet, rather than the number and positions of the carpels (fig. 13). As a conclusion, general patterns of vascularisations of unrelated taxa with dorsiventrally flattened pistils can be similar (see table 2) since they all reflect the same underlying general pattern of pistil ontogeny.

Laterally flattened pistils – The origin of laterally compressed pistils required developmental freedom of the pistil, which is present as an annular primordium in several recently evolved lineages of Cyperoideae. Moreover, floral vascular patterns are a reflection of the floral ontogeny (fig. 20). In the next paragraphs, we discuss the vascular evidence supporting our theory on alterations of stigma positions but falsifying alternative hypotheses on homology (Blaser 1941a) or fusion (Ronse Decraene et al. 2002) of vascular bundles. In addition, a discussion of some specific observations in taxa with laterally compressed pistils is presented here.

The observed vascular ontogeny and recent phylogenetic studies (Muasya et al. 2002, Larridon et al. 2011a) imply the homology of the bundles within the ovary wall of *Cyperus* and *Pycreus* (fig. 19D–F). This is in contrast with Blaser (1941a), who, based on the connections of the bundles, interpreted the pistil bundles in *Cyperus* as dorsal bundles and the ones in *Pycreus* as lateral bundles (fig. 19A–C). In Blaser's (1941a) view, the dorsal bundles within the ribs of the ovary are a continuation of the main vascular bundles within the receptacle after all bundles towards other floral organs and the ventral bundles have branched off (figs 3A–C & 19A & B). However, in *Pycreus*, *Kyllinga*, and *Queenslandiella*, stigma branches connect to the vascular plexus (ventral bundles) within the receptacle (figs 3D, 10, 13A–B & 19C, electronic appendices 2–5) and do not form a continuation of the main vascular bundles in the receptacle. Therefore, Blaser (1941a) saw the vascular bundles in the ovary wall of *Pycreus* as lateral bundles (figs 3D1 & 19C), comparable with those he found in the scirpoid taxa (figs 3A1 & 19A). As these bundles continue to the style branches, Blaser assumed that they took over the function of the dorsals. In contrast, in the scirpoid taxa, the lateral bundles usually run within the sides of the triangular ovary wall and end below the style (fig. 19A).

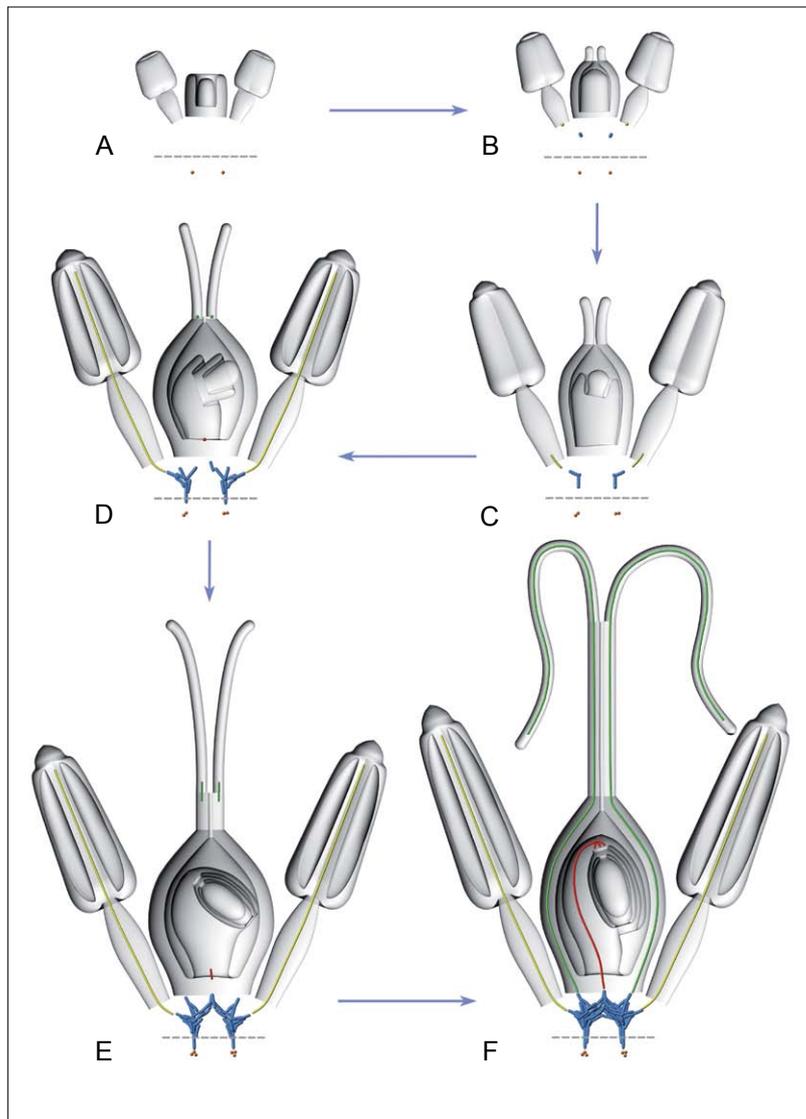


Figure 20 – Series of diagrams showing the development of organs and vasculature of a hypothetical cyperoid flower with a dorsiventrally flattened dimerous pistil and two stamens in adaxial positions. A, at the stage of the annular gynoeceium wall primordium surrounding an ovule primordium the vascular traces of the rachilla start to differentiate; B, once all floral organs are initiated (including the stigma primordia) several procambial initiation points originate within the receptacle and from the base of the stamens; C, in this stage the stamens are strongly developing. Connections are made between the procambial initiation points within receptacle with those of the stamens and towards the vascular traces of the rachilla. Meanwhile the stigma primordia are elongating and the first integument is formed on the ovule primordium that starts to bend; D, stamens and their vascular system are fully differentiated. On the tip of the ovule primordium the second integument is formed. Additional procambial initiation points are formed from the base of the ovule and the stigma branches. Meanwhile, the vascular traces within the receptacle become denser and branch towards the different primordia of the gynoeceium; E, procambial initiation points from the base of the stigma branches differentiate further within the elongating pistil and stigmata. The ovule vascular traces also start differentiating in this stage; F, all floral organs and their vascular traces are completed and these all have formed connections with the now very dense vascular plexus within the receptacle.

Diagrams are shown from an abaxial viewpoint and a black dotted line indicates the hypothetical border between the rachilla and the flower. Colour codes: grey, floral organs; orange, vascular bundles within the rachilla, here seen in transversal section; blue, vascular traces within the rachilla (= receptacular, ventral and central bundles); green, vascular traces within the gynoeceium wall (= dorsal bundles); red, vascular traces within the ovule.

However, the time gap between the connecting of the receptacular plexus with the rachillar bundles and the connecting of the receptacular plexus with the vascular bundles of the pistil, suggests that both vascular systems are independent and thus do not necessarily need to converge. Also the vascular bundles from the different organs connect independently to the receptacular vascular plexus. Since lateral bundles are lacking in all *Cyperus* species studied by Blaser and the authors of the current study (figs 3A1 & 11) and taking the most recent phylogenetic hypothesis about *Cyperus* into consideration, with *Pycneus*, *Kyllinga* and *Queenslandiella* nested within the C_4 *Cyperus* clade (Muasya et al. 2002, 2009a, Besnard et al. 2009, Larridon et al. 2011b), there are no reasons to assume homology of the ovary wall bundles in *Pycneus* and the lateral bundles in scirpoid taxa. The development of the vascularisation in angiosperms tends to allow the formation of vessels ‘wherever needed’, as the main vascularisation systems are determined by the positions of organ primordia, from where newly initiated bundles connect with existing vascular bundles (Endress 1994). Consequently, the vascular bundles in the ovary in e.g. *Pycneus*, *Kyllinga* and

Queenslandiella are therefore to be regarded as dorsal bundles similar to those found in ovaries of other Cyperoidae.

Secondly, although all carpels are congenitally fused, we found no evidence in *Pycneus* and related taxa for a further fusion of the two adaxial stigmata and their dorsal bundles to form a single stigma and a dorsal bundle in intermediate position. The latter theory was applied by some authors to explain similar cases in African Restionaceae, where transitional series are known in which two dorsal bundles are present in a pair of fused sterile carpels, while in the more derived situation only a single dorsal bundle is present in an intermediate position (Linder 1992, Ronse Decraene et al. 2002). It is more parsimonious to assume a developmental reorganisation of the fused carpel complex resulting in an ovary with a single, intermediately positioned stigma primordium from which a corresponding dorsal bundle is initiated.

Next, Blaser (1941a) included *Kyllinga pumila* in his study, concluding that its vascular pattern is similar to the vascularisation in trimerous *Cyperus* flowers. However, *Kyllinga* pistils are dimerous and logically our observations do not con-

firm those of Blaser (1941a). Possibly the latter were based on misidentified material. In the rachilla of *Kyllinga nemoralis*, we observed that the vascularisation is concentrated into a single V-shaped bundle at a level above the branching of the first flower (fig. 13A). This can be explained by the strong reduction of the spikelet in most *Kyllinga* species, in which only one flower is functional. Vascular bundles entering the flowers of *K. nemoralis* run into two laterally positioned centres, comparable with the two bundles entering the flowers in the *Pycreus* species studied. However, in *Kyllinga nemoralis* these two bundles expand and split into three smaller bundles each, which then fuse again into an annular bundle before splitting off staminal and gynoecial traces (fig. 13A, electronic appendices 3–4). These observations for *K. nemoralis* concur with the observations of Blaser (1941a) for *Cyperus retrorsus* Champ. in which he observed six separate bundles entering the flowers. These six bundles fuse two by two to form the dorsal bundles of the flower. Possibly, this is due to the position of the flowers studied within the spikelet. In *K. nemoralis* as well as in *C. retrorsus*, only the most proximal flowers of the deciduous spikelets are functional. Also in other groups with strongly reduced or condensed spikelets, vascular connections of different structure within the spikelets tend to interfere, making correct interpretations of the original vascularisation almost impossible, e.g. *Carex* (Snell 1936), *Scleria* P.J. Bergius (Blaser 1941b). This adaptive nature of vascular development makes vascular patterns in our opinion less reliable for the study of evolutionary relationships between different taxa in Cyperoidae.

Pseudomonomerous pistils – Finally, in both *Rhynchospora* and *Cyperus*, lineages arose in which the style is elongated with reduced the stigma branches. Such pistils are often called pseudomonomerous gynoecia (Dickison 2000). In these cases, remnants of the stigma primordia are still visible on the top of the style and two or three dorsal bundles can be observed within the style (fig. 12).

Vascular connections of the ovule – The vascularisation of the ovule connects to the central vascular plexus within the receptacle independently from the dorsal bundles of the gynoecial wall (fig. 20). This also reflects the developmental reorganisation of the gynoecium. The central position of the ovule results in an equal contribution of vascular connections (ventral bundles) from all vascular poles within the receptacle instead of a polarisation towards a single carpel. As discussed by Snell (1936) and Blaser (1941b), this reflects a vascularisation supply typical of axile or free central placentae from which the situation in Cyperoidae can be interpreted as a final reduction stage. Blaser (1941b) observed in some species that the ovule is asymmetrically positioned, with the funiculus inserted at the adaxial side of the locule and the micropyle bent back at the abaxial side. In *Cyperus laevigatus*, we observed an asymmetric connection of the ovule vascular bundle with the central vascular bundle coming from the receptacle (fig. 11C). However, ontogenetically, in all species studied by us, the ovule primordium is centrally positioned. Therefore, we assume that the asymmetric position of the ovule along the adaxial-abaxial axis is due to the bending of the ovule, which initially is atropous and subsequently turns to become anatropous.

The Cyperoid ovule

Reduced ovule numbers – Apparently, the congenital fusion of the carpel primordia allows a new developmental organisation with as a symptom the central, basal ovule (fig. 18). We can agree with Snell's (1936) interpretation of a central basal ovule as a 'reduction' as far as we can see a reduction trend in Juncaceae where in *Luzula* DC. the ovary wall also originates from an annular primordium (Vrijdaghs et al. 2006, unpubl. res.). In contrast to Cyperaceae, in *Luzula* the originally carpellary structure of the gynoecium is still noticeable in the presence of the three basal ovules. This tendency apparently reaches its maximum in Cyperaceae with the single, basal ovule, which is not longer linked to a carpellary structure (fig. 18). However, in our opinion, 'reduction' here rather means reorganisation of the development the ovary, accompanied by a simplification. According to Linder & Rudall (2005) reduced ovule number is often associated with aggregated inflorescences.

Is there a link with pseudomonad pollen? – Kress (1981) suggested that while it could be advantageous to have simultaneous fertilisation of multiple ovules within the same ovary by a pollen unit (four at once for tetrads), such advantage is lost when only a single ovule is present. McGlone (1978) discussed this hypothesis for Styphelioideae (Ericaceae) where such parallels between ovule number and tetradmonad reductions exist. A similar link might exist between respectively single ovuled pistils and pseudomonad pollen (Cyperaceae), and multi-ovuled pistils and tetrad pollen (Juncaceae, Thurniaceae). However, this comparison does not consider the difference in pollination strategy between sedges (wind) and Styphelioideae (insect), which are known to have different effects on the natural selection of successful floral and pollen morphologies. Char et al. (1973) assumed pseudomonads are advantageous in wind pollination due to the smaller pollen size, which might explain the situation in sedges but not in Styphelioideae (McGlone 1978). In addition, tetrad pollen might still be advantageous for single ovuled gynoecia since it maintains a possibility of selection of the fittest member of the pollen tetrad.

Placentation – Blaser (1941b) also mentioned a distinct spine-like projection along one side of the ovule towards the top of the ovary of *Bolboschoenus robustus* (Pursch) Soják, which he interpreted to be remnants of a placental column. However, since the ovary wall rises from an annular primordium, and since the development of the ovule occurs independently of the development of the ovary wall, we consider it (in contrast to Blaser 1941b) impossible to find remnants of carpellary structures, which could only occur in a developing gynoecium resulting from postgenital fusion of (morphologically reduced) carpels, quod non. The term 'central placenta', however, can be used in the meaning of centrally positioned region of adhesion of the ovule, following Leins (2000: 100), who defined a placenta as: "Im weitesten Sinne ist die Plazenta der Gewebeteil eines Karpells (oder der Blütenachse), der die Samenanlagen hervorbringt [In the widest sense, the placenta is the part of the tissue of a carpel or of the floral axis, which produces the ovules]".

Obturator hairs – The recent observations of Coan et al. (2008) in *Rhynchospora* and *Hypolytrum* Rich. ex Pers., of

Gonzalez & López (2010) in *Bulbostylis*, as well as our observations in 37 mostly African species in Scirpeae, Fuireneae, Eleocharideae, Abildgaardieae, Cypereae, Cariceae, Schoeneae and Trilepideae sensu Goetghebeur (1998) about intralocular hairs confirm the observations of Van der Veken (1965). In all species studied, obturator hairs originate at the basal-abaxial side of the funiculus and subsequently grow towards the micropyle where they stick together, thus closing the micropyle. Observed variation appeared to be totally random and hence of no systematic value.

CONCLUSIONS

Understanding pistil diversification in Cyperoideae, an integrated developmental model

Integrating our anatomical, floral ontogenetic data and recent functional insights makes it possible to present a developmental model for the gynoeceum in Cyperoideae to understand all variations of its essentially trimerous morphological *Bauplan*.

(1) The ovary originates from an annular ovary primordium (Vrijdaghs et al. 2009) – During the earliest ontogenetic stages, the annular ovary primordium grows upwards to form a bag-like structure. At this stage, the primordium can be considered as an ‘empty box’ (Gould 2002), with no other developmental determination than growing upwards. Next, in most cyperoid species, two adaxially positioned and one abaxially positioned stigma primordia (according to the conservative positions of the original carpel tips) are formed on the top of the cylindrical ovary wall. However, other numbers and positions of stigma primordia are possible. We believe that congenital fusion of carpels allows shifts in positions of stigma primordia to novel, more optimal positions with respect to the available space (fig. 17).

(2) Signaling from primordia causes bidirectional origin of the vascular system in the pistil (Endress 1994) – Vessel initiation zones are present in the stigma primordia (fig. 20). From there, the vessels grow to the receptacular plexus, to be connected with the stele. These vessels constitute the ribs of the pistil. Consequently, the number and positions of the stigma primordia determine the future shape of the pistil, which develops initially as a bag-like structure, and subsequently typically assumes a triangular shape, or a derived dimerous, dorsiventrally or laterally flattened shape, or a polymerous shape.

(3) Annular primordia facilitate decoupling of the development of a whorl with respect to the neighboring whorls (Endress 2006) – The annular ovary primordium and central ovule primordium differentiate simultaneously from the floral apex. The development of the ovary wall and ovule appear as two distinct phenomena (fig. 18). Ovary wall and ovule vascular traces show independent connections with the receptacular plexus, thus reflecting the ontogenetic separation of the annular ovary wall primordium and ovule primordium.

SUPPLEMENTARY DATA

Supplementary data are available at *Plant Ecology and Evolution*, Supplementary Data Site (<http://www.ingentaconnect.com/content/bothel/plecevo/supp-data>), and consist of the following: (1) dark field image of a cleared up developing spikelet in *Queenslandiella hyalina* (pdf format); (2) and (3) phase contrast pictures of slices through a flower of *Pycreus flavescens* (pdf format); (4) and (5) phase contrast pictures of slices through a flower of *Kyllinga nemoralis* (pdf format); and (6) phase contrast pictures of slices through a spikelet of *Cyperus laevigatus* (pdf format).

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