PLANT
ECOLOGY
EVOLUTION

Morphology and development of spikelets and flowers in *Cyperus* and *Pycreus* (Cyperaceae)

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Background – *Pycreus*, *Kyllinga*, and *Queenslandiella* cluster together with *Cyperus* within the *Cyperus* s. lat. clade, one of the two large clades in Cypereae. However, in contrast with *Cyperus*, they have laterally flattened pistils/nutlets. *Pycreus, Kyllinga* and *Queenslandiella* form morphologically well circumscribed independent genera. In the context of a broader systematic project to work out a well supported, evolution based taxonomy for *Cyperus* s. lat., we present in this paper general morphological and developmental data of species of *Pycreus* in comparison with three species of *Cyperus*, including *C. laevigatus* with dorsiventrally flattened nutlets.

Approach – Freshly collected material was investigated using scanning electron microscopy (SEM) and light microscopy (LM). Special attention was given to spikelet and gynoecial development.

Results – SE micrographs of all species studied show an indeterminate rachilla with distichously arranged glumes, each subtending a bisexual flower. In spikelets of *C. capitatus* and *P. pumilus*, the proximal glume sometimes subtends a lateral spikelet instead of a flower. In the species of *Pycreus* studied, each flower sits in a cavity formed by the growth of the rachilla, which is congenitally fused with the wings of the glume of the higher, alternate flower. Glumes appear successively, each soon forming a flower primordium in its axil, which develops according to a general cyperoid ontogenetic pattern. In *Pycreus*, the stigma branches grow out from dorsiventrally positioned primordia. During gynoecium development, a hypogynous stalklet (gynophore) appears in all species studied.

Conclusion – In spikelets of *Pycreus*, the rachilla and wings of the glumes are congenitally fused and consequently develop with epicaulescent displacements of the glumes resulting in typical spikelets with flowers in cavities. In spikelets of *Cyperus*, a similar though less pronounced development results in spikelets with zigzagging rachilla. The particular positions of the stigma branches in *C. laevigatus* and *Pycreus* are explained by the development of the gynoecium from an annular primordium, which facilitates shifts in localisation of the stigma primordia. Though we consider the combination of the typical spikelet ontogeny and the independently originated laterally flattened nutlets to be strong arguments in favour of a genus *Pycreus*, a phylogenetic confirmation that the taxon is monophyletic is an absolute, until now unfulfilled, condition. Moreover, the consequences for the giant genus *Cyperus* must be taken in consideration.

Key words – Cypereae, *Cyperus*, *Cyperus* s. lat., laterally flattened dimerous gynoecium, floral ontogeny, *Pycreus*, scanning electron microscopy, spikelet.

INTRODUCTION

Taxonomical data of *Cyperus* s. lat.

According to molecular phylogenetic studies in Cyperaceae (Muasya et al. 2009a), the subfamily Cyperoideae comprises most of the cyperaceous genera, including the derived Cypereae clade (corresponding to Cypereae sensu Goetghebeur 1998). Within this clade, *Cyperus* and allied genera, called *Cyperus* s. lat., form a subclade that is sister to a *Hellmuthia-Scirpoides-Isolepis-Ficinia* clade (Muasya et al. 1998, 2001b, 2009a, Simpson et al. 2007, fig. 1). Based on the em-

bryological study of Van der Veken (1964) and corroborated by more recent molecular phylogenetic studies (Muasya et al. 2002, 2009a, 2009b, Simpson et al. 2007), several smaller satellite genera appear to be nested *within* the *Cyperus* s. lat. clade, such as among others, *Kyllinga*, *Queenslandiella* and *Pycreus*. Each of these is characterised by specialised inflorescence and flower morphologies. *Kyllinga* can be distinguished by its reduced spikelets and flowers with laterally flattened ovaries, *Pycreus* by flattened spikelets and flowers with laterally flattened ovaries, and *Queenslandiella* by dehiscent spikelets (formerly placed in *Mariscus*) and flowers with laterally flattened ovaries (Goetghebeur 1986). In 1998, Goetghebeur wrote: "*Pycreus* and *Kyllinga*, plus some highly specialized smaller taxa are often excluded [from *Cyperus* s. lat.] and recognized at the generic level. Authors who include these taxa into *Cyperus* s. lat. mostly maintain them on the subgeneric level." Moreover, the more derived part of *Cyperus* s. lat., including *C. capitatus*, *C. laevigatus*, and *Pycreus*,

consists of genera with C_4 photosynthesis and Kranz anatomy (fig. 1). Since *Kyllinga*, *Pycreus* and *Queenslandiella* are not sister taxa, we hypothesize independent and multiple origin of the laterally flattened pistil.

Inflorescence morphology in *Cyperus* s. lat. and *Pycreus*

The inflorescence in Cyperoideae is a compound inflorescence, essentially a panicle of spikelets with the main axis called a culm. The ultimate branch in a cyperoid inforescence is always a lateral spikelet, consisting of a rachilla and spirally to distichously placed glumes, each subtending (or not) a bisexual (most Cyperoideae) or unisexual (Cariceae) flower. Lateral spikelets are subtended by a bract and have a prophyll (Goetghebeur 1998). Terminal spikelets end the culm or a branch of it as a (co)florescence sensu Troll (1964; see Weberling 1992), and as a consequence it is separated from its prophyll by the length of the culm/lateral branch, which
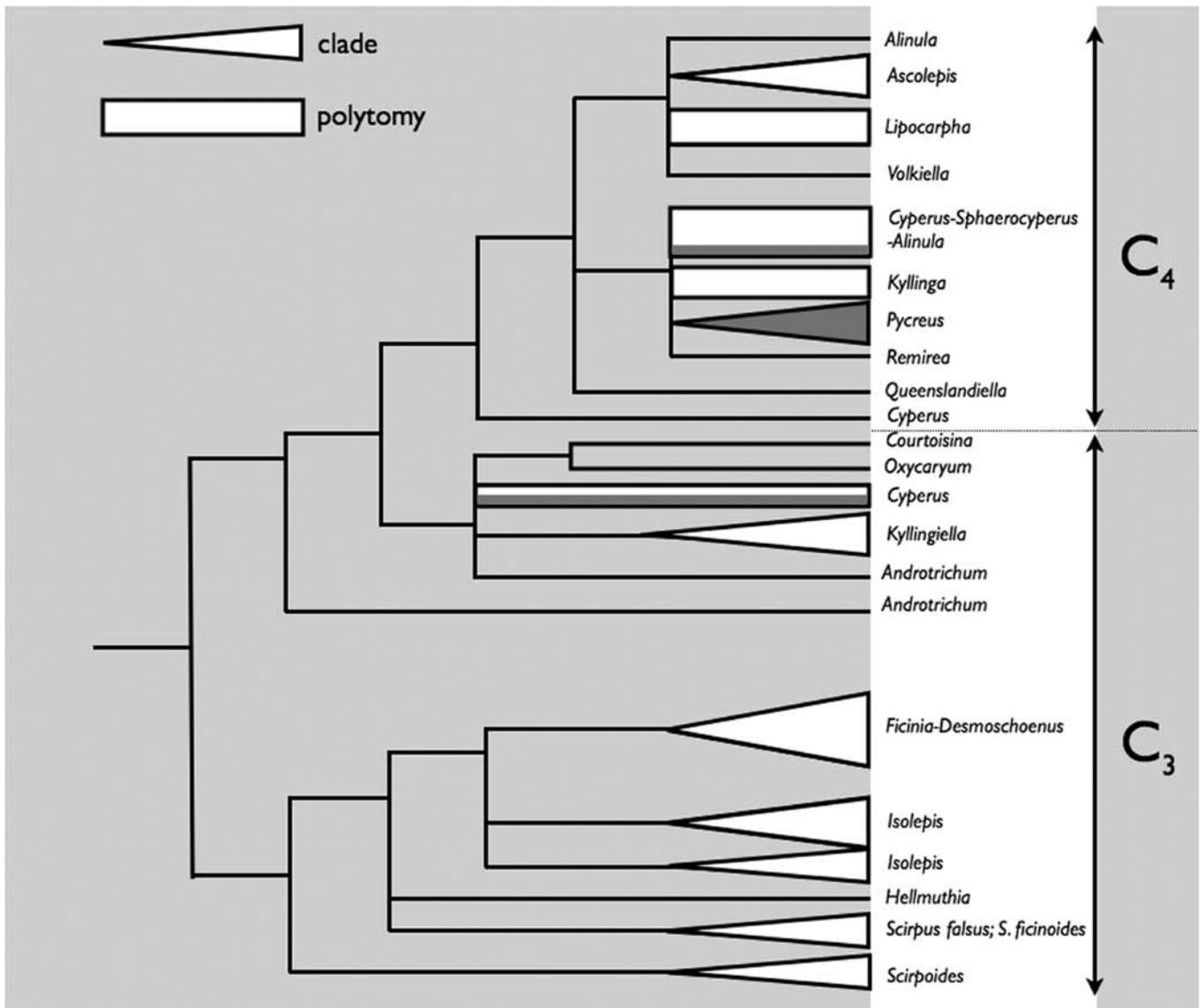

Figure 1 – Simplified cladogram of Cypereae based on Muasya et al. (2009a). In dark grey, taxa of which species were used in this study. *Cyperus luzulae* is a C_3 species, whereas *C. capitatus* and *C. laevigatus* are C_4 species.

constitutes also the axis of the terminal spikelet (Haines & Lye 1983, Goetghebeur 1998). In *Cyperus* s. lat., spikelets are distichously organised, which can be considered as a synapomorphy though a number of reversals to terete spikelets are recorded (Muasya et al. 2001b). In *Pycreus*, the inflorescence is antheloid with as well the culm as lateral branches ending with a terminal spike of flattened spikelets. In *Pycreus*, the inflorescence is antheloid with as well the culm as lateral branches ending with a terminal spike of flattened spikelets.

Vrijdaghs et al. (2010) showed that cyperoid spikelets, including several, mostly distichously organised controversial ones that by some authors were interpreted as sympodial (e.g. Celakovsky 1887, Kern 1962, Zhang et al. 2004), have an indeterminate rachilla and can be considered to be an open spike as cited by Weberling (1992). Guarise & Vegetti (2008: 41) reported that in *Cyperus*, section *Luzuloidei*, fascicles of spikelets occur, "which can be serial, prophyllar, or mixed". Serial fascicles of spikelets are mainly found in the distal part of the florescence and paraclades, the latter being a repetition of the main inflorescence's structure. A serial fascicle of spikelets is subtended by a single bract. Guarise & Vegetti (2008: 55) also mentioned a 'torsion' within the spikelets: "some spikelets appear with the glumes in the same plane as the pherophyll and prophyll, or in an intermediate position". Several species in *Cyperus* s. lat., formerly grouped together in *Mariscus*, have dehiscent spikelets. Haines (1967: 57) reported a 'pulvinus' or swelling body at the base of lateral spikelets in *Cyperus tenuis* Sw., stating "But at the attachment of the prophyll, and probably a part of the prophyll, is a pulvinus which adjusts the position of both the branch and the umbel bract that subtends it". Haines & Lye (1983: 17) mentioned "a callus is developed at the prophyll base, swelling of this callus causing divergence of the shoot".

**Floral morphology and development in *Cyperus* s. lat. and *Pycreus***

Flowers in Cyperoideae either have a perianth (3 + 3 parts or less) of varying size and shape or lack a perianth as observed in most species of Cypereae (e.g. Goetghebeur 1998, Muasya et al. 2009b). The androecium in most Cyperoideae is haplostemonous with usually three stamens with basifix and introrse anthers (Bruhl 1991, Vrijdaghs et al. 2005a), resulting from the reduction of the inner staminal whorl (Takh-

tajan 1997). However, particularly in *Cyperus* s. lat., the number of stamens can be reduced to two or one (Haines & Lye 1983). In the first developmental stages in cyperoid flowers, the stamens grow faster than the gynoecium (Vrijdaghs et al. 2005a), but at maturity of the flower, the stigma branches usually are functionally active before the pollen grains are released (Goetghebeur 1998). In many species of *Cyperus*, an apiculus or connective crest is formed on the top of the anthers (Haines & Lye 1983).

The pistils in flowers within the *Cyperus* s. lat. clade vary from triangular with three stigma branches to dorsiventrally or laterally compressed with only two stigma branches. Raynal (1966) studied some African *Cyperus* species (e.g. *C. meeboldii* Kük., *C. clavinux* C.B.Clarke, *C. laterinticus* Raynal) with triangular nutlets and a single stigma branch. Most species with a dorsiventrally flattened pistil were often classified in a separate taxon (*Juncellus*) by several authors (e.g. Clarke 1893, Kükenthal 1936, Podlech 1960). Already Clarke expressed some doubt: "This species [*Juncellus pustulatus*] has differentiated itself into *Juncellus*, but has not broken its connection with *Cyperus* entirely yet." (Clarke 1901: 308). In some former *Juncellus* species, even within single specimens, the flowers can have both trigonous and dorsiventrally compressed nutlets (e.g. *Cyperus alopecuroides* Rottb., *C. pustulatus* Vahl, *C. pygmaeus* Rottb.). The polyphyletic dispersion of the *Juncellus* species was confirmed by many other authors (e.g. Goetghebeur 1986, Muasya et al. 2002). Therefore, a separate genus *Juncellus* is no longer recognised. Moreover, dorsiventrally flattened pistils can also be found in diverse other cyperoid genera such as *Dulichium arundinaceum* (L.) Britton, *Eleocharis*, *Fimbristylis*, *Nemum*, and *Carex*.

On the other hand, laterally compressed pistils are restricted to three genera, *Pycreus, Kyllinga* and *Queenslandiella*. Blaser (1941) showed that the laterally flattened pistil in *Pycreus* concurs with new vascular patterns. Several authors based the subdivisional classification within *Pycreus* among others on the morphology of the fruit wall epiderm cells (e.g. Clarke 1897, Chermezon 1919, Kükenthal 1936). Clarke (1897: 155) described the epiderm cells of nutlets in his "Zonatae" as follows: "Superficial cells of the nut longitudinally oblong; nut often appearing zonate by reason of the narrow ends of the cells running into an undulating or broken horizontal line." In several species, these cells contain silica bodies, though

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◄ **Figure 2** – *Cyperus luzulae*, SE micrographs of floral ontogeny. A, lateral view of the rachilla apex, with six distichously placed glumes at successive developmental stages (numbered 1–6 from young to older; '1', '3' and '5' show the wings of alternately positioned glumes); B, lateral view of glume 2 and a flower primordium in its axil; C, apical view of developing ovary wall surrounding a central ovule primordium with two adaxial and one abaxial stigma primordium; D, lateral view of a developing bractless spikelet belonging to a spikelet fascicle subtended by a common bract (not visible here) with flowers at successive developmental stages (encircled) and numbered from 1 (distal flower) to 7 (proximal flower). Arrows shows the wings of glume 4, which is also visible as the glume protecting the rachilla apex. The main axis, indicated as rachis, actually belongs to another, older spikelet in the fascicle; E, lateral view of a developing gynoecium. A single style appears (arrowed); F, lateral view of a developing ovary, with three stigma branches becoming papillose (encircled); G, lateral view of a part of a spikelet. Proximally, a developing flower with elongating stamen, and a stigma branch protruding above it (arrowed). At right hand side a glume with a wing enveloping the rachilla and a part of the stamen of the alternate flower; H, apical view of a part of a spikelet with removed glumes and stamens (arrowed); I, apical view of the distal part of a spikelet, with some glumes removed (arrows indicate the wings of the glumes); J, adaxial view of a developing flower; K, detail of developing style and stigma branches; L, nutlet with gynophore (arrowed); M, detail of apical part of a glume, with numerous stomata (encircled) and prickles (arrowed); N, detail of prickles.
Abbreviations: a, anther; F, flower primordium; f, filament; fa, floral apex; G, glume; nu, nutlet; o, ovule primordium; ov, ovary wall (primordium); R1, rachilla; Ra, rachis; s, stamen primordium; sg, stigma (primordium); st, style; *, rachilla apex.
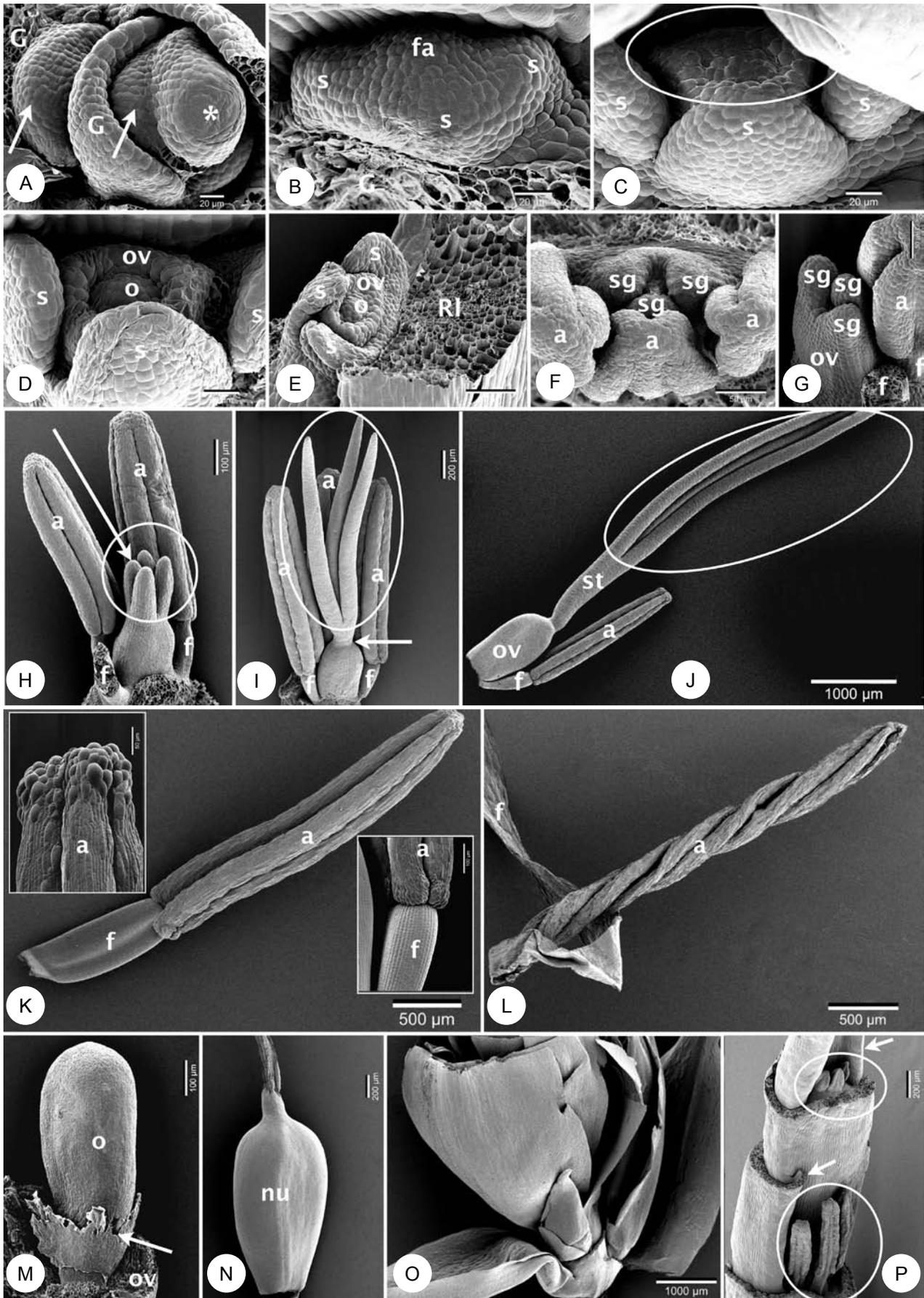
according to Metcalfe (1971), they have little taxonomical value.

Aims

This study represents the first results in a broader project in which the *Cyperus* s. lat. clade is investigated in analogy with our earlier study of the *Hellmuthia-Scirpoides-Isolepis-Ficinia* clade, which resulted in several publications (Muasya et al. 2009a, 2009b, Vrijdaghs et al. 2005b, 2006a, 2006b, 2009). By combining molecular phylogenetic data, anatomy, morphology and spikelet/floral ontogeny, our goal is to clarify the evolution of *Cyperus* s. lat. and the position of the so-called 'satellite genera' within it. In this paper, we present and discuss original SEM and LM images of the morphology and spikelet/floral development in species of *Pycreus*, which were selected based on our preliminary phylogenetic data and compared with two *Cyperus* C$_4$ and one *Cyperus* C$_3$ species (respectively *C. laevigatus, C. capitatus*, and *C. luzulae)*, starting from the hypothesis that *Pycreus* can be considered to be a genus of its own. Of the three genera with laterally compressed pistils, the mainly African genus *Pycreus* (±120) was chosen to be examined first, because it is the largest one.

MATERIALS AND METHODS

Plant material

Inflorescences of the species studied were collected in the field and at the Ghent University botanical garden (table 1) and subsequently fixed in FAA (70% ethanol, acetic acid, 40% formaldehyde, 90/5/5). Spikelets and floral buds were dissected in 70% ethanol under a Wild M3 (Leica Microsystems AG, Wetzlar, Germany) stereo microscope equipped with a cold-light source (Schott KL1500; Schott-Fostec LLC, Auburn, NY, USA).

Since in *Cyperus* s. lat. most spikelets have many and a variable amount of flowers, and consequently in order to avoid the use of abstract numbers, (flower subtending) glumes are numbered from young (1) to old (x).

Scanning electron microscopy (SEM)

Table 1 – Species of Cypereae (Cyperaceae) studied and voucher data.

taxa	collector and origin	voucher
Cyperus capitatus Poir.	Goetghebeur, Sep. 2004, HBUG 2003-1782 (w)	PG10744
Cyperus laevigatus L.	Goetghebeur, Sep. 2004, HBUG1997-1237 Reynders, Nov. 2007, HBUG2003-1192	PG10202
Cyperus luzulae Rottb.	Vrijdaghs, HBUG1900-3306	AV05
Pycreus bipartitus C.B.Clarke	Reynders, Nov. 2004, HBUG 2003-0327 (s)	
idem (fig. 12)	Laegadr, Ecuador	GENT101015
Pycreus flavescens (L.) P.Beauv. ex Rchb.	Reynders, Jul. 2007, HBUG2005-0401 (s)	
idem (fig. 12)	Muasya, 2005, Kenya	AM2585
Pycreus pelophilus (Ridl.) C.B.Clarke	Musili, 2005, Kenya	PM029
idem (fig. 12)	Reekmans, Burundi	GENT2547
Pycreus polystachyos subsp. *holocericeus* (Rottb.) P.Beauv.	Reynders, Jul. 2007, HBUG 2006-1258 (w)	
idem (fig. 12)	Lewalle, Burundi	GENT6290
Pycreus pumilus (L.) Nees	Muasya, 2005, Kenya	AM2150
idem (fig. 12)	Reekmans, Burundi	GENT5795
Pycreus sanguinolentus (Vahl) Nees	Reynders, Jul. 2007, HBUG2006-1753 (w)	

The prepared material was washed twice with 70% ethanol for 5 minutes and then placed in a mixture (1/1) of 70% ethanol and DMM (dimethoxymethane) for 5 minutes. Subsequently, the material was transferred to 100% DMM for 20 min, before it was CO$_2$ critical point dried using a CPD 030 critical point dryer (BAL-TEC AG, Balzers, Liechtenstein). The dried samples were mounted on aluminium stubs using
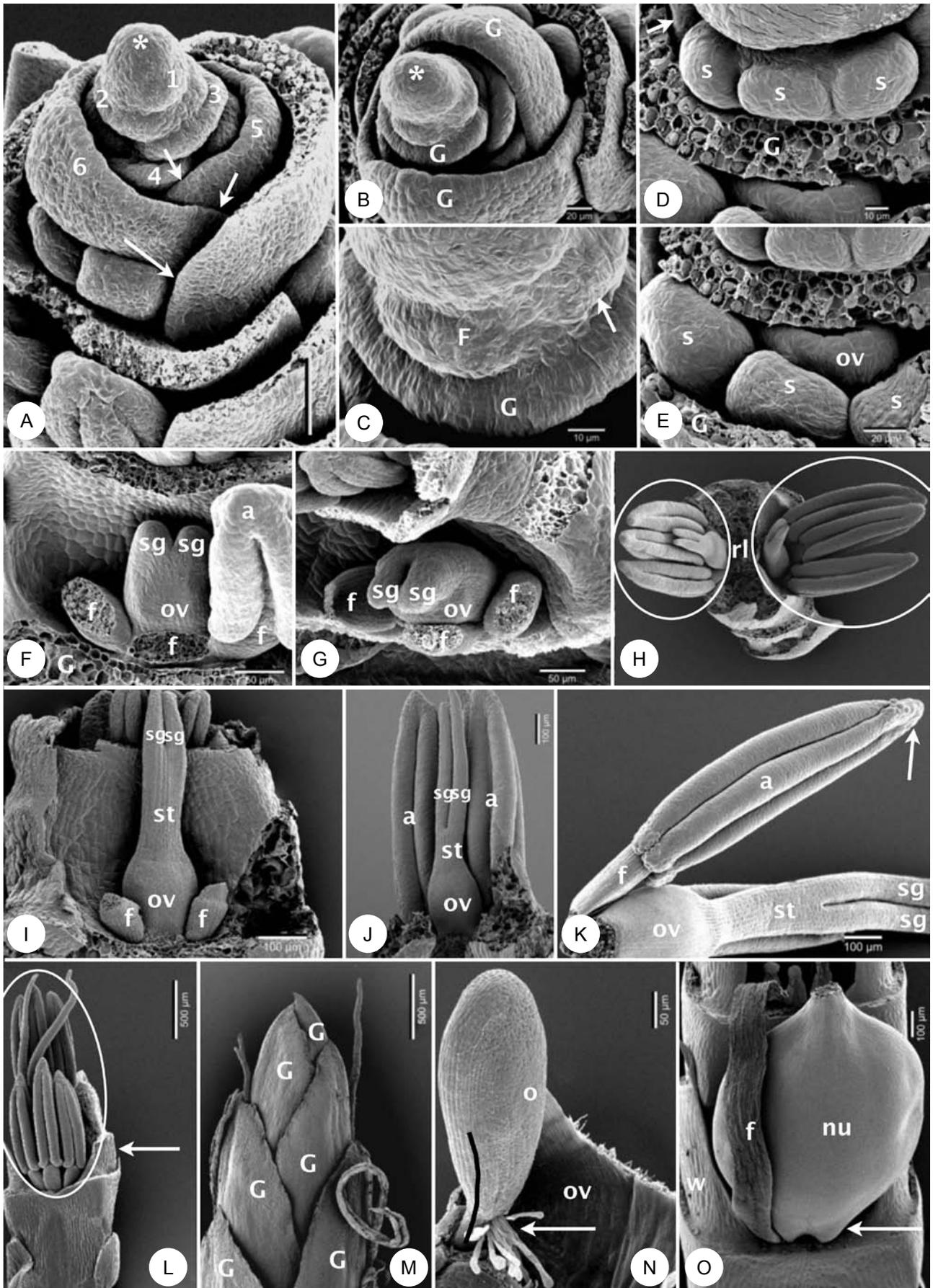
◄ **Figure 3** – *Cyperus capitatus*, SE micrographs of floral ontogeny. A, apical view of the rachilla apex, and two flower primordia (arrowed) at early stages of development; B, differentiating flower primordium with three stamen primordia and a floral apex; C, annular ovary primordium surrounding a central ovule primordium (encircled), and three stamen primordia; D, idem as in 'C', with the ovary wall growing up from the base; E, position of a flower at early developmental stage with respect to the rachilla; F, apical view of developing flower, with the ovary wall enveloping the ovule (two adaxial stigma primordia and an abaxial one appear); G, lateral-adaxial view of developing flower (lateral stamen is removed); H, adaxial view of a developing flower, with one lateral stamen removed. The four (!) stigma primordia are growing out (encircled); I, adaxial view of a developing flower (arrow indicates single style); J, developing gynoecium and a single stamen, with stigma branches protruding high above the stamen (encircled); K, developing stamen before the elongation starts, with apiculus (left upper corner inset) and papillose cells at the bases of the pollen sacs (right hand side inset); L, elongated, withered stamen, with spiralised anther; M, ovule with obturator hairs covering the micropyle (arrowed); N, nutlet, with withered style still present; O, distal part of a culm, in the transition zone between florescence and lateral branches. These are spiro-tristichously positioned and each subtended by a bract, whereas in the terminal spikelet (florescence), the glumes are distichously arranged. This explains the position of the proximal glume-like bracts subtending a rudimentary spikelet; P, middle-apical part of spikelet with two developing flowers (encircled) and the wings of the glumes of the higher, opposite flower (arrows).

Abbreviations: a, anther; f, filament; fa, floral apex; G, glume; nu, nutlet; o, ovule (primordium); ov, ovary wall (primordium); Rl, rachilla; s, stamen primordium; sg, stigma (primordium); st, style; *, rachilla apex.

Leit-C and coated with gold with a SPI-ModuleTM Sputter Coater (SPI Supplies, West-Chester, PA, USA). Images were obtained on a Jeol JSM-6360 (Jeol, Tokyo) at the Laboratory of Plant Systematics (K.U. Leuven).

Light microscopy (LM)

Samples were prepared in ethanol 70% and subsequently gradually transferred to ethanol 100%. Then, the samples were transferred to LR White Resin, hard grade (London Resin Company Ltd, Reading, England) in a graded LR White Resin/ethanol series using solutions of 25/75, 50/50, 75/25, 100/0 resin/ethanol 100% for at least 5 h each. Next, the samples were placed in a closed capsule filled with fresh resin, and hardened at 60°C during 48 h. Sections of 2 μm were made with a rotation microtome (Microm HM360 Waldorf, Germany) and subsequently stained with 0,1% toluidine blue. The stained sections were fixed on microscopy slides using Eukitt© quick hardening mounting medium (Fluka Chemie GmbH, Switzerland). Observations were done with a light microscope (Leitz Dialux 20, Van Hopplynus, Brussels, Belgium) equipped with a camera (PixeLINK PL-B622CF, Ottawa, Canada) with specially developed software (Microscopica v1.3, Orbicule, Leuven, Belgium).

RESULTS

The development and morphology of spikelet and flower in *Cyperus* and *Pycreus* are described below.

Cyperus: spikelet structure

In all species studied, the spikelet consists of a open axis (rachilla) and many distichously arranged glumes, each subtending a bisexual flower (figs 2A, 3A, 4A & B). Glumes develop fast, the older glumes not only protecting the flower they subtend, but also the apical part of the spikelet (figs 2A, B & D, 3A, 4A, B & M). Mature glumes have lateral wings, which partially envelop the rachilla and alternate, lower flower (figs 2D & I, 3P, 4A, C & L, fig. 13). The basal part of glume and wings is congenitally fused with the rachilla (fig. 13B–E). In *C. luzulae*, mature glumes have conspicuous prickles at the distal side, as well as high numbers of stomata (fig. 2M & N).

Cyperus: floral ontogeny

A new glume originates below the rachilla apex, forming a rim-like primordium (figs 2A & D, 3A, 4A–C). Soon, a flower primordium appears in the axil of the glume. The flower primordium expands laterally, forming a stamen primordium at each side, followed by a third abaxial one (figs 2A, B & D, 3A & B, 4B–D). In *C. luzulae*, usually there is a single, lateral stamen primordium (fig. 2A, B & D). Simultaneously, the floral apex becomes convex (figs 2B & D, 3B, 4D) and starts differentiating into an annular ovary primordium surrounding a central ovule primordium (figs 3C–E, 4E). Subsequently, the ovary wall grows up from the base, enveloping the ovule (figs 2C & D, 3C–E, 4E). On its top, one abaxial and two adaxial stigma primordia appear (figs 2C & D, 3F & G). The stigma primordia grow out into three papillose stigma branches (figs 2D–F & K, 3G–J). In *C. laevigatus*, only two laterally positioned stigma primordia appear, which results in a dorsiventrally flattened ovary (fig. 4F–L & O). In *C. capitatus*, samples with four stigma branches occur (fig. 3H). Meanwhile, the ovary wall continues its growth, forming a single style (figs 2E & F, 3I & J, 4I–K). Simultaneously with the development of the ovary, the stamen primordia differentiate into filament and anther (figs 2G, 3F–J, 3P, 4F–H). Until this stage, the development of the stamens is as fast as or faster than the development of the pistil (figs 2G, 3G–I, 3P, 4E–H). However, at the later floral developmental stages style and stigma branches elongate faster, so that eventually they protrude above the stamens and even the glume (figs 2J, 3J, 4J–M). Meanwhile, the base of each pollen sac becomes papillose (figs 2J, 3K, 4K), and on the top of the anther an apiculus is formed (figs 3K, 4K). The ovule primordium develops into an anatropous bitegmic ovule, and within the locule, in a zone around the micropyle, hairs appear (figs 3M, 4N). In *C. capitatus*, the anther of the mature stamen becomes spiralised (fig. 3L). The nutlets of *C. laevigatus* and *C. luzulae* have a hypogynous stalklet, also called gynophore (figs 2L, 4O). The nutlet in *C. laevigatus* is dorsiventrally flattened (figs 4O, 12I).

Pycreus: spikelet structure

The spikelet in all species studied consists of an indeterminate rachilla and many distichously arranged glumes, each subtending a bisexual flower (figs 5A & B, 7A, 8A & B, 9A &

◄ **Figure 4** – *Cyperus laevigatus*, SE micrographs of floral ontogeny. A, lateral view of a spikelet apex with flower subtending glumes at successive stages of development, numbered '1' (youngest) to '6' (oldest). The wings of each glume envelop partially the alternate, lower flower (arrowed); B, apical-abaxial view of spikelet apex with developing glumes; C, detail of very young glume subtending a flower primordium, and a wing of the alternate, higher glume (arrowed); D, differentiating flower primordium with three stamen primordia, and a part of the floral apex; E, developing flower with ovary wall growing up, and three stamen primordia beginning to differentiate; F, apical-abaxial view of a developing flower. Two laterally positioned stigma primordia are growing out on the top of the ovary wall, which envelops the ovule. Filaments and anthers are well developed; G, apical view of developing flower. The two stigma primordia are growing out; H, apical view of a transversally cut spikelet, with two alternating flowers at intermediary developmental stages (encircled); I, abaxial view of a developing flower; J, adaxial view of a developing flower; K, detail of a developing stamen, with apiculus (arrowed); L, abaxial view of a developing flower (encircled) in a tranversely cut spikelet. The subtending glume is removed. The wings of the higher, opposite flower can be seen (arrowed); M, apical part of a spikelet, with several, distichously placed glumes, and protruding style branches; N, lateral view of an ovule, with funiculus (black line) and obturator hairs covering the micropyle (arrowed); O, dorsiventrally flattened nutlet with a hypogynous stalklet or gynophore (arrowed).

Abbreviations: a, anther; F, flower primordium; f, filament; G, glume; nu, nutlet; o, ovule primordium; ov, ovary wall (primordium); Rl, rachilla; s, stamen primordium; sg, stigma (primordium); st, style; *, rachilla apex.
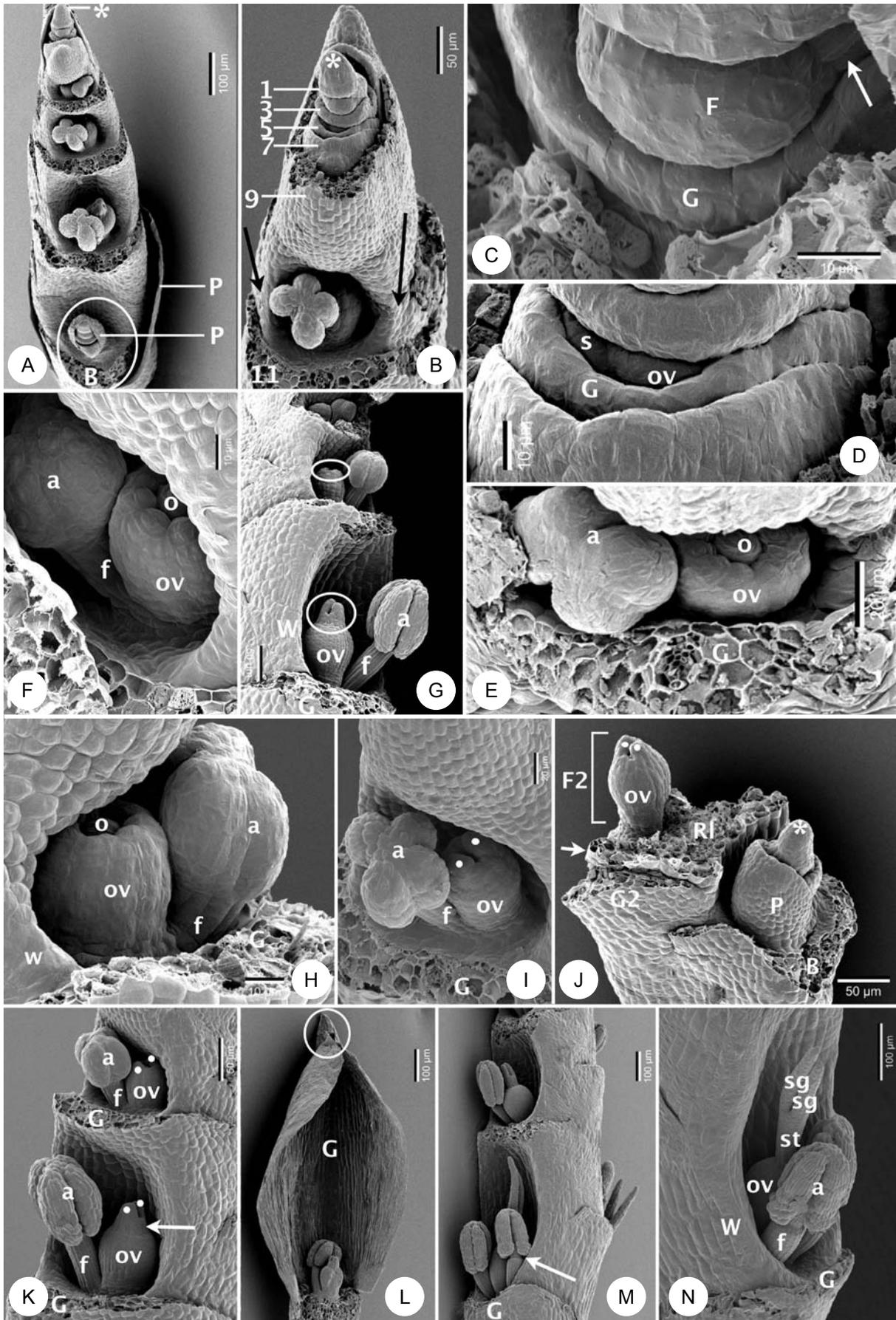
B, 10A & B). The basal part of glume and wings is fused with the rachilla (fig. 13F–I). At later stages, each flower stands in an alcove-like cavity (figs 5B, 5J, K & M, 6A, C & E, 7B & C, 8A & B, 9B & C, 10B & C, fig.13). In *P. pumilus*, a glume-like bract in proximal position alternating with the prophyll subtends a lateral axis, which develops into a spikelet (fig. 5A & J), instead of a flower. In *P. pelophilus, P. polystachyos* and *P. sanguinolentus*, an adaxial swelling body can be seen at the base of the spikelet (figs 6G, 7H, 9L). The spikelet of *P. polystachyos* has a long first internode or epipodium, which is enveloped by the tubular sheath of the spikelet prophyll (fig. 7G & H). The developing glumes at the apical part of the spikelet envelop the apex of the rachilla with a bonnet-like mucro (figs 5B, 7A, 9A).

Pycreus: floral ontogeny

Glumes originate below the indeterminate spikelet apex (figs 5A & B, 7A, 8A & B, 9A & B, 10A & B), forming a rim-like primordium, of which the edges partially envelop the alternate, lower flower primordium (figs 5C, 5H & J, 7B, 8B). Soon after the formation of a new glume primordium, a flower primordium appears in its axil (figs 5B & C, 7B, 8B, 9B & C, 10B). With the glume developing, the flower primordium expands laterally, forming two lateral stamen primordia, followed with some delay by a third abaxial one (*P. flavescens, P. sanguinolentus*; figs 9C–E, 10B & C). In species with flowers with two stamens, no abaxial stamen primordium is formed (*P. bipartitus, P. pelophilus, P. polystachyos*; figs 7B, 8B–D). In flowers with only one stamen, the flower primordium expands laterally, forming only one stamen primordium (*P. pumilus*; fig. 5B–D). Simultaneously with the formation of the stamen primordia, a floral apex appears (figs 8C, 9D). Next, the floral apex differentiates into an annular ovary primordium, surrounding a central ovule primordium (figs 5E, 6A, 8D, 9E & F, 10C & D). The ovary wall primordium grows up from the base, gradually enveloping the central ovule (figs 5F & H, 6A & B, 8D & E, 9F, 10E). At this stage, on the top of the ovary wall two dorsiventrally positioned stigma primordia appear (figs 5G, I & J, 6C, 7C, 8E, 9G, 10F). The development of the adaxial stigma primordium is sometimes slightly delayed with respect to the abaxial one (figs 8E & F, 9G–I, 10F–H, 11A & B). In *P. bipartitus*, the early adaxial stigma primordium sometimes splits (figs 8G & H). Subsequently, the growing ovary wall develops a single style without distinct style base, while the stigma primordia grow out into two papillose stigma branches (figs 5G & K, 6D–F, 7D & E, 8G & H, 9I, 10G & H, 11A & B). In *P. sanguinolentus*, at this stage, an annular constriction appears in the apical part of the ovary (fig. 9K). Meanwhile, the stamen primordia have developed into introrse stamens with basifixed anthers with longitudinal slits (figs 5G, H, I & K, 6C–F, 7C–F, 8E–G, H & J, 9G & H–J, 10E & F, 11A & B). In semi-mature flowers of *P. flavescens*, and *P. sanguinolentus*, the anthers are as long as or longer than the filaments (figs 9J, 11A & B), whereas in *P. bipartitus, P. pelophilus, P. polystachyos* and *P. pumilus* the anthers are relatively short with respect to the filaments (figs 5K–N, 6D–G, 7E & F, 8J & K). In *P. pelophilus* and *P. pumilus*, a short connective stalklet appears between filament and anther (figs 5M, 6G & H). In all species studied, the cells at the base of the pollen sacs in developing anthers become more or less papillose (figs 5M & N, 6H, 7F, 8G & K, 9J, 10A & B). An apiculus is absent or remains rudimentary, with the apical cells becoming papillose (e.g. in *P. bipartitus*. fig. 8H). Maturing gynoecia and nutlets have a hypogynous stalklet or gynophore (e.g. figs 7I, 8K, 11C & D, 12A, C, E & G–I). In *P. polystachyos* and *P. pumilus*, the cells of the nutlet wall each contain a conspicuous tabular silica body (figs 7I & J, 12A–F), in *P. polystachyos* often with microsatellites around its top. In *P. pelophilus*, similar cells only occur in the center of each lateral side (fig. 12C & D). In *P. flavescens*, the epidermal cells of the mature nutlet become longitudinally elongated (zonate cells) pushing up the transverse cell walls, which gives the nutlet its typical wrinkled appearance (fig. 11D & E, 12H).

◄ **Figure 5** – *Pycreus pumilus*, SE micrographs of floral ontogeny. A, lateral view of a branched spikelet with proximally a glume-like bract subtending a secondary spikelet (encircled). The prophylls (P) of the main and secondary spikelet are parallel to each other. All visible flowers have a single stamen; B, detail of a spikelet apex with 11 glumes, each subtending a flower (primordium), numbered 1–11 from young '1' to older '11'. In flower 11, the wings (arrowed) of the opposite, higher glume (number 10, only partially visible) form the walls of an alcove-like cavity in which the flower develops; C, detail of a young glume with flower primordium. At the right hand side, the wing of the alternate, superior glume is visible (arrowed); D, differentiating flower with primordia of stamen and ovary wall; E, flower with developing stamen and early gynoecium. The ring primordium of the ovary wall surrounds the central ovule primordium; F, developing flower with ovary wall growing up from the base, and stamen with distinct filament and anther; G, lateral-abaxial view of part of a spikelet with three flowers at different developmental stages. In the middle flower, two dorsiventrally oriented stigma primordia appear on the top of the ovary wall (encircled). In the lower flower, the ovary wall entirely envelops the ovule, a single style appears, and the stigma primordia are growing (encircled); H & I, detail of the development of the ovary and appearance of the dorsiventrally positioned stigma primordia, and simultaneously the development of the stamen; J, transversely cut proximal part of a main spikelet with proximally a glume-like bract, subtending a lateral spikelet. Alternately of it, the second glume can be seen, subtending a flower of which only the developing gynoecium is visible. This is partially enveloped by the wing (arrowed) of the third glume (removed together with the flower it subtends). This wing is fused with the rachilla of the main spikelet; K, lateral-abaxial view of a part of a spikelet with two flowers at developmental stages following on the developmental stage at 'I'. In the lowest flower, a single style appears (arrowed); L, adaxial view of a developing gynoecium and a glume with a conspicuous mucro (encircled) subtending a flower; M, lateral view of a part of a spikelet. In the lowest flower, consisting of a gynoecium and two stamens, a 'connective stalklet' can be observed between filament and anther (arrowed); N, lateral view of semi-mature flower with two stamens, protected by the wings of the alternate, higher glume.

Abbreviations: a, anther; B, bract; F, flower primordium; f, filament; G, glume; o, ovule primordium; ov, ovary wall (primordium); P, prophyll; Rl, rachilla; s, stamen primordium; sg or white dot, stigma (primordium); st, style; W, wing of glume; *, rachilla apex.
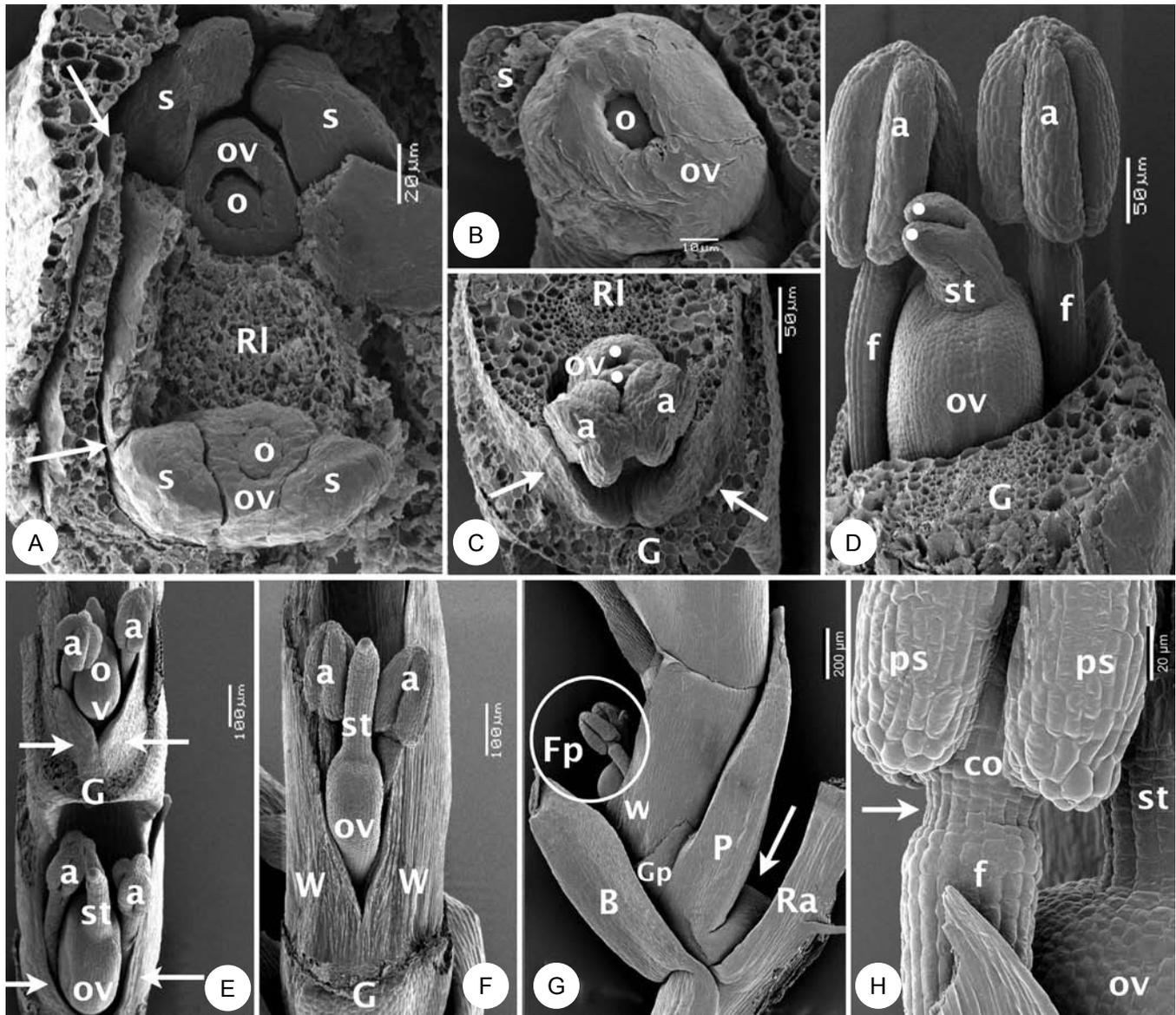
Figure 6 – *Pycreus pelophilus*, SE micrographs of floral ontogeny. A, transverse section in the distal part of a spikelet, with two flowers at early developmental stages. In the flower below, two stamen primordia and an annular ovary primordium surrounding a central ovule primordium are visible. In the upper flower, the stamen primordia start differentiating into anther and filament (not visible), and the annular ovary primordium grows up from the base; B, growing ovary wall enclosing the central ovule; C, apical-abaxial view of a developing flower and part of a tranverse section through the distal part of the rachilla. Two dorsiventrally positioned stigma primordia appear on the top of the ovary wall. In between the glume and the flower it subtends, two wings of the alternate, higher flower (removed) partially envelop the flower (arrowed). The wings are fused with the rachilla; D, abaxial view of a developing flower. A single style appears. The anthers are shorter than the filaments; E, lateral view of the middle part of a spikelet with removed glumes. Two developing flowers are visible, each partially envelopped by the wings of the higher, opposite glume (arrowed); F, abaxial view of a semi-mature flower; G, lateral view of the proximal part of a spikelet, with spikelet subtending bract, prophyll of the spikelet, proximal glume and proximal flower (encircled), partially hidden by the wing of the next glume. At the base of the prophyll, a swelling body or pulvinus is visible (arrowed); H, detail of the connective stalklet (arrowed) in between filament and anther.

Abbreviations: a, anther; B, bract; co, connective; f, filament; Fp, proximal flower primordium; G, glume; Gp, proximal glume; o, ovule primordium; ov, ovary wall (primordium); P, prophyll; ps, pollen sac; Ra, rachis; Rl, rachilla; s, stamen primordium; sg or white dot, stigma primordium; st, style; W, wing; *, rachilla apex.

Anatomical data

Cross sections were made at different levels through developing spikelets of *Cyperus laevigatus* (fig. 13A–E) and *Pycreus flavescens* (fig. 13F–I). Figure 13A serves as a key to symbols for 13B–I. Cross sections at the basal part of a flower in *C. laevigatus* (fig. 13B–D) and *P. flavescens* (fig. 13F–H) reveal that glume and rachilla are fused below the level where the filaments are clearly distinguishable. Cross sections at anther
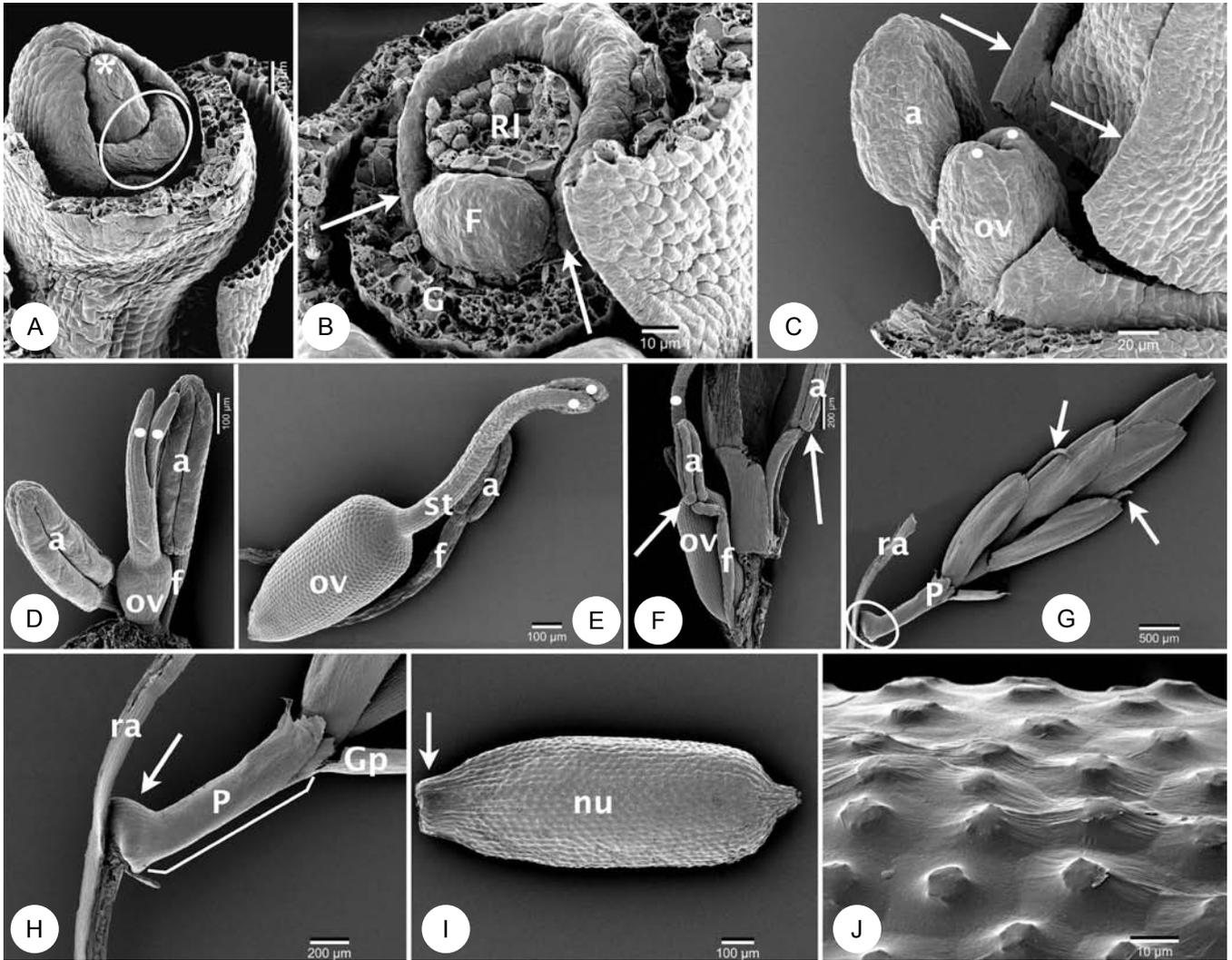
Figure 7 – *Pycreus polystachyos*, SE micrographs of floral ontogeny. A, rachilla apex and first glume (encircled); B, transverse section in the apical part of the spikelet, showing a newly formed glume with wings (arrowed) partially enveloping the alternate, lower flower primordium. The glume subtending this flower primordium is removed; C, lateral view of a developing flower. The wings (arrowed) of the alternate, higher flower contribute to its protection. The ovary wall is enveloping the ovule, and two dorsiventrally positioned stigma primordia appear. The two stamen primordia are differentiating into filament and anther; D, developing flower. A single style is formed, and the stigma primordia grow out into stigma branches; E, semi-mature flower. The anther becomes shorter than the filament; F, lateral view of a part of a spikelet with two semi-mature flowers. The cells at the bases of the pollen sacs become papillose; G, entire spikelet, with a long first internode enveloped by a sheath-like prophyll. At the base of the prophyll, a swelling body is present (encircled). Stigma branches protrude above the glumes (arrowed); H, detail of the first internode (white bar) and spikelet prophyll, with a conspicuous swelling body. The spikelet subtending bract is removed. (I) Nutlet with hypogynous stalklet or gynophore; J, detail of the surface of the nutlet, with tabular silica-bodies.
Abbreviations: a, anther; F, flower primordium; f, filament; G, glume; Gp, proximal glume; nu, nutlet; ov, ovary wall (primordium); P, prophyll; Ra, rachis; Rl, rachilla; st, style; white dot, stigma (primordium); *, rachilla apex.

level show a separate glume and rachilla (fig. 13E & I). In both species, a cross section through the rachilla at internode level is butterfly-shaped (fig. 13E & I).

<div align="center">DISCUSSION</div>

Spikelet development and morphology

In all our observations presented here on *Cyperus* and *Pycreus*, the spikelet consists of an indeterminate rachilla and numerous, acropetally developing glumes, each subtending a single flower. This concurs with our earlier observa-

tions in a wide range of cyperoid species (Vrijdaghs et al. 2006a, 2007, 2010). In Cypereae, a lateral spikelet (which is defined as 'ultimate branch' and hence should not have any ramification within it) is not always clearly distinguishable from a branched partial inflorescence; in some species, a secondary spikelet instead of a flower is formed in the axil of a glume (e.g. in *Hellmuthia*; Vrijdaghs et al. 2006b). This was also observed in *Ficinia* (Muasya, unpubl. res.), *Cyperus* (figs 2D, 3E), and *Pycreus* (fig. 5A & J). Therefore, in strict sense, in such cases a glume subtending a secondary spikelet should be called 'glume-like bract', and the rachilla of the
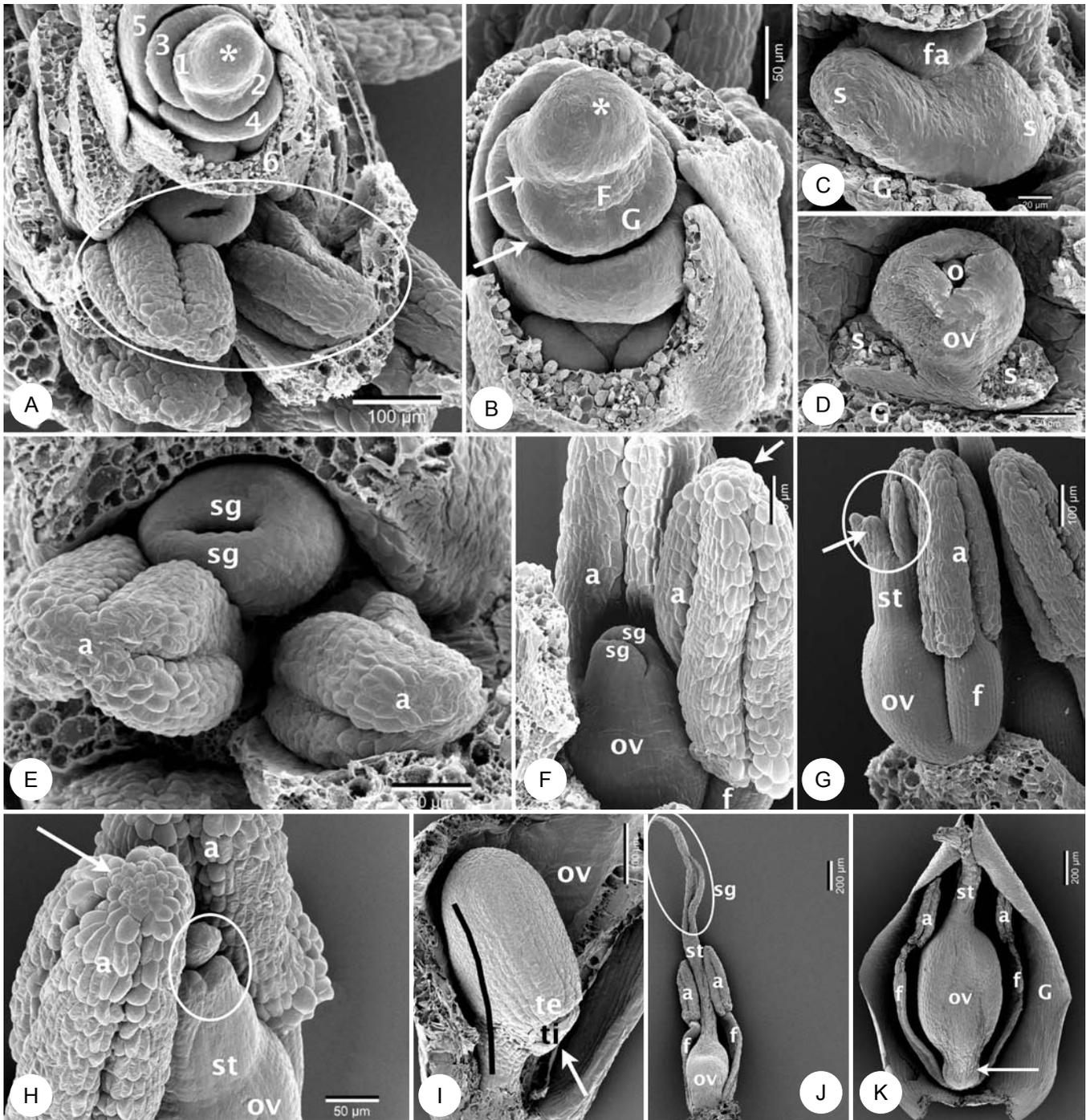
Figure 8 – *Pycreus bipartitus*, SE micrographs of floral ontogeny. A, apical view of a spikelet apex with glumes/flowers at different developmental stages, numbered from young '1' to older '5'. Encircled is a proximal developing flower, with the ovary wall enclosing the ovule, and two developing stamens; B, spikelet apex with very young glume subtending a yet undifferentiated flower primordium. Arrows indicate wings of two superposed glumes at the other side of the spikelet; C, differentiating flower primordium with two lateral stamen primordia and a conspicuous floral apex; D, developing flower with the two stamens removed. The ovary wall is enveloping the central ovule; E, apical view of a developing flower. Two dorsiventrally oriented stigma primordia originate on the top of the ovary wall; F, lateral view of a developing flower. On the top of the anthers, an apiculus appears (arrowed); G, lateral view of a developing flower. A single style appears, with the stigma primordia growing out into stigma branches (encircled). The adaxial stigma primordium is split into two (arrowed); H, detail of stigma primordia with splitted adaxial one (encircled), and apiculus (arrowed); I, developing ovule with the micropyle nearly bent back over 180° (arrowed). The funiculus is indicated with a black line; J, mature flower. Stigma branches are encircled; K, lateral view of mature flower. The gynoecium/nutlet has a hypogynous stalklet or gynophore (arrowed).

Abbreviations: a, anther; F, flower primordium; f, filament; G, glume; o, ovule primordium; ov, ovary wall (primordium); s, stamen primordium; sg, stigma (primordium); st, style; te, outer tegument; ti, inner tegument; *, rachilla apex.

main spikelet 'rachis'. In *C. luzulae*, spikelets belong to a serial fascicle of spikelets (fig. 2D), in which several spikelets originate in the axil of a common bract, the one above the other, as described by Guarise & Vegetti (2008: fig. 8). We also observed similar spikelet clusters in *C. eragrostis* Lam. (both belonging to the section *Luzuloidei*; Denton 1978). In all C_3 species studied, the position of the spikelet prophyll of rather distally on the rachis positioned spikelets is shifted in comparison with the plane determined by the distichous arrangement of the spikelet's other glumes. In C_4 species, such torsion has not been observed. Similar observations are reported by Guarise & Vegetti (2008).

In *C. capitatus*, the culm ends in a terminal spikelet (florescence) in which the glumes are distichously placed. Below the florescence, lateral branches, each subtended by a bract, are spiro-tristichously positioned (fig. 3O). This allows us to interpret the proximal glume-like bracts as bracts subtending a lateral spikelet, positioned out of the plane determined by the higher distichously placed glumes of the terminal spikelet. In the transition zone between florescence and the lower part of the culm with lateral branches, primordia in the axil of a bract have a high flexibility to become flower or lateral axis. This flexibility to determine a given, yet undetermined primordium in the axil of a glume(-like bract) also explains the presence of secondary spikelets in spikelets of e.g. *Pycreus pumilus* (fig. 5A & J; Vrijdaghs et al. 2010).

The glumes in all species studied are winged, with the wings of one glume partially enveloping the opposite, lower flower. In both *Cyperus* and *Pycreus*, the basal part of the glume including (part of) the wings is congenitally fused with the rachilla (figs 6C, 7B, 13) and grows up with it. This is most obvious in *P. pumilus*, whereas in *P. pelophilus* (fig. 6) and *P. flavescens* (fig. 10), a large part of the wings grows free from the rachilla. Consequently, the main part of the glume and the flower primordium it subtends are epicaulescently displaced to a more apical position (actually, until the next node) on the rachilla. As a result, the rachilla itself is winged along the common growth zone (Vrijdaghs et al. 2010). In *Pycreus*, this epicaulescent metatopic displacement is more pronounced than in species of *Cyperus* or other Cyperoideae, resulting in the typical alcove-like cavities along the rachilla, of which the lateral walls consist mainly of the wings of the opposite, higher glume (fig. 13).

In *Pycreus*, the glumes often have a prolonged midvein or mucro, which becomes cap-shaped, protecting the rachilla apex (e.g. figs 5B, 7A, 9A). At the adaxial lower part of prophylls of both inflorescence branches and spikelets in *P. pelophilus, P. polystachyos* and *P. sanguinolentus*, an adaxial swelling body can be seen (figs 6G, 7H, 9L). We also observed it in other Cypereae, such as *C. luzulae* (Reynders, unpubl. res.) and *Kyllinga* Rottb. (Huygh, University of Ghent, Belgium, and Vrijdaghs, unpubl. res.). These observations allow confirming Haines' (1967) suggestion that the swelling body or pulvinus is part of the prophyll.

Floral ontogeny and fruit morphology

The floral ontogenetic pattern in *Cyperus* and *Pycreus* is similar to the pattern observed by us in many other Cyperoideae (e.g. Vrijdaghs et al. 2005, 2009). However, there is no formation of perianth primordia, which is a common feature for *Cyperus* s. lat. and Cypereae. However, in the *Ficinia-Isolepis* clade, two southern African species previously named as *Scirpus* (*S. falsus* and *S. ficinioides*) were added, as well as the formerly mapanioid *Hellmuthia*. These three species are the only recorded Cypereae with remnants of a perianth (Simpson et al. 2003, Vrijdaghs et al. 2006, Muasya et al. 2009a, 2009b).

In *Pycreus*, the number of stamens is highly variable, with basic number three as in most other Cyperoideae (e.g. figs 9D, E, G & H, 10C, E & F). Kükenthal (1936) reported that nearly half of the 72 species he recognized in *Pycreus* have a constant number of two stamens instead of three. In these cases it is the abaxial stamen that does not develop (e.g. figs 6, 8A–G). In some species, the number of stamens can also vary within the species, and even within a single plant (e.g. *P. pumilus*, fig. 5M). We observed a tendency to delay the formation of the abaxial stamen or to reduce it completely in various other cyperoid genera, such as *Eriophorum, Scirpoides* (Vrijdaghs et al. 2005a), *Fuirena* (Vrijdaghs et al. 2004), *Ficinia* and *Isolepis* (Vrijdaghs et al. 2005b). From these observations, we may deduce that the reduction of the abaxial stamen occurred independently in different cyperoid clades. In all cases, this tendency can probably be explained by a limited spacial freedom to develop the three stamens. *Pycreus pumilus*, with its highly compacted spikelets and flowers with usually one, sometimes two stamens, clearly illustrates this. Moreover, in stamens of flowers of *P. pelophilus* and *P. pumilus*, a 'connective stalklet' appears in semi-mature stamens. Similar observations were made in other Cypereae (e.g. *Kyllinga* and *Oxycaryum*; Vrijdaghs, unpubl. res.). We admit that this 'connective stalklet' acts as an articulation allowing the anther to bend over for better pollen dispersal by the wind.

As in all other Cyperoideae studied by us, the gynoecium in the species of *Cyperus* and *Pycreus* presented here are formed from an annular ovary primordium surrounding a central ovule primordium. Since the ovary wall in Cyperoideae is not resulting from a postgenital fusion of three distinct carpel primordia but growing up from an annular ovary primordium, new possibilities arise in organizing the vascularisation of the gynoecium and consequently also for its morphology, such as the positions and number of the stigmas. In *Pycreus*, only two stigma branches are formed, positioned dorsiventrally, which results in laterally flattened gynoecia/nutlets (figs 5–11). Similar pistils also occur in *Kyllinga* and *Queenslandiella*. However, molecular phylogenetic data (Muasya et al. 2009a) show that these genera form different clades within *Cyperus* s. lat., which suggests that this feature evolved independently in each of the three genera characterized by it. Also in *C. laevigatus*, only two, though laterally positioned stigma primordia appear, resulting in a dorsiventrally flattened pistil/nutlet (fig. 4F–H). Goetghebeur (1986) suggested that such a pistil, wich also occurs in other *Cyperus* species and other cyperoid genera such as *Blysmus, Dulichium, Eleocharis* and *Fimbristylis*, results from the reduction of the abaxial carpel and a fusion of the two remaining adaxial carpels. However, each attempt to explain the *Pycreus* type pistil using the carpel concept fails. Moreover, in strict sense, carpels are not present in cyperoid Cyperaceae since the ovary originates
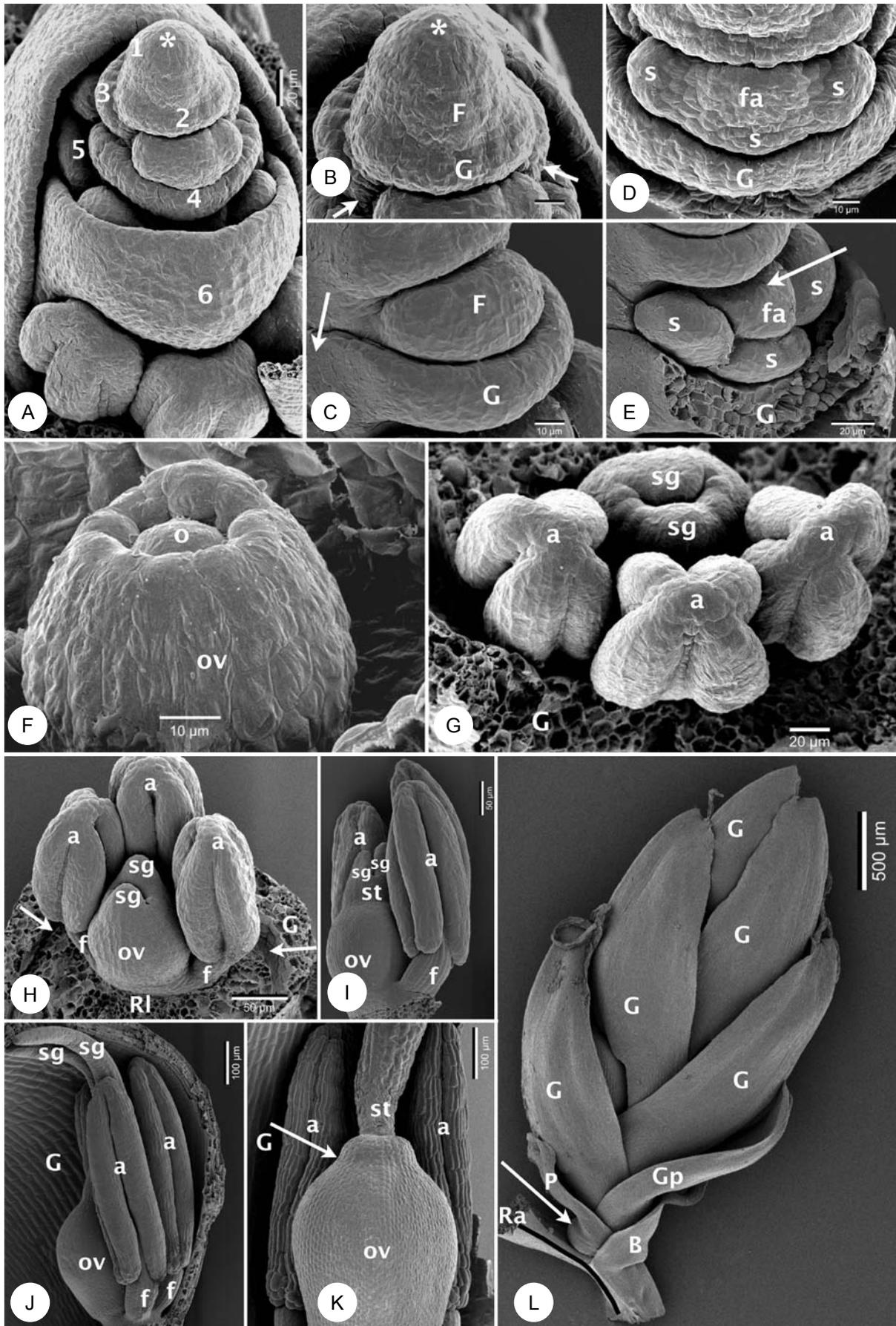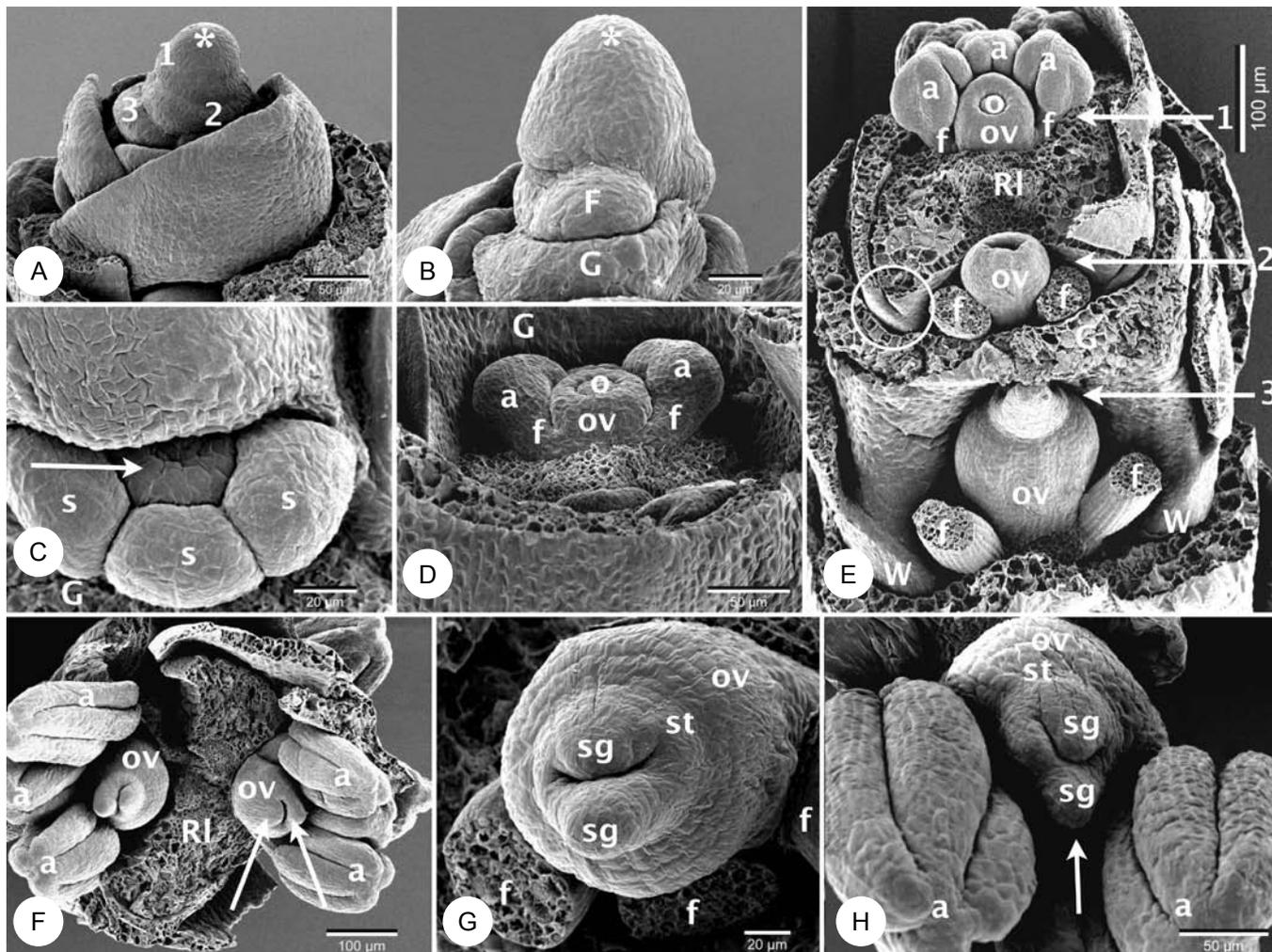
Figure 10 – *Pycreus flavescens*, SE micrographs of floral ontogeny. A, lateral view of a spikelet apex, with glumes/flowers at successive developmental stages numbered from young to older 1–3; B, rachilla apex with young glume subtending a flower primordium; C–D, differentiating flower primordium with one abaxial and two adaxial stamen primordia, and with the floral apex differentiating into an annular ovary primordium (arrowed) surrounding a central ovule primordium. In 'D', the stamen primordia start differentiating into filament and anther; E, transverse section through the rachilla, with three flowers (1, youngest; 3, oldest) at different developmental stages. Flower '1' is shown from an adaxial viewpoint, flowers '2' and '3' from an abaxial viewpoint, each with removed stamens. In flower '2', the adhesion of the wings of the subtending glume of flower '1' to the rachilla can be seen (encircled); F, apical view of a transverse section through the rachilla, with two flowers. The right hand one is less developed, with two dorsiventrally stigma primordia appearing on the top of the ovary wall (arrowed); G–H, apical view of a developing gyncoecium. A single style appears, and the stigma primordia grow out, the adaxial one (arrowed) delayed with respect to the abaxial stigma primordium.

Abbreviations: a, anther; F, flower primordium; f, filament; G, glume; o, ovule primordium; ov, ovary wall (primordium); s, stamen primordium; sg, stigma (primordium); W, wing; *, rachilla apex.

◄ **Figure 9** – *Pycreus sanguinolentus*, SE micrographs of floral ontogeny. A, apical part of a spikelet, with glumes/flowers at successive developmental stages, numbered from young '1' to older '6'; B, detail of rachilla apex with a young glume primordium with undifferentiated flower primordium. The wings of the alternate glume reach the underlaying flower (arrowed); C, detail of a glume and flower primordium, which is expanding laterally. The wings of the glume envelop partially the rachilla (arrowed); D, differentiating flower primordium, with two lateral and a slightly delayed abaxial stamen primordium, and a floral apex. E, developing flower. The floral apex is starting to form an annular ovary primordium (arrowed); F, developing ovary, with ovary wall enveloping the central ovule; G, apical view of a developing flower. Two dorsiventrally positioned stigma primordia are visible on the top of the ovary wall; H, apical-adaxial view of a developing flower. The wings (arrowed) of the opposite, higher flower (not in the image) envelop partially the lateral stamens; I, lateral view of a developing flower. A single style appears; J, lateral view of a developing flower. The stigma branches are growing out; K, lateral view of semi-mature flower, one lateral stamen is removed. An annular constriction around the apical part of the ovary is formed (arrowed); L, entire spikelet. At the base of the prophyll, a conspicuous swelling body or pulvinus is visible (arrowed).

Abbreviations: a, anther; B, bract; f, filament; fa, floral apex; G, glume; Gp, proximal glume; o, ovule primordium; ov, ovary wall (primordium); P, prophyll; Ra, rachis; Rl, rachilla; s, stamen primordium; sg, stigma (primordium); st, style; *, rachilla apex.
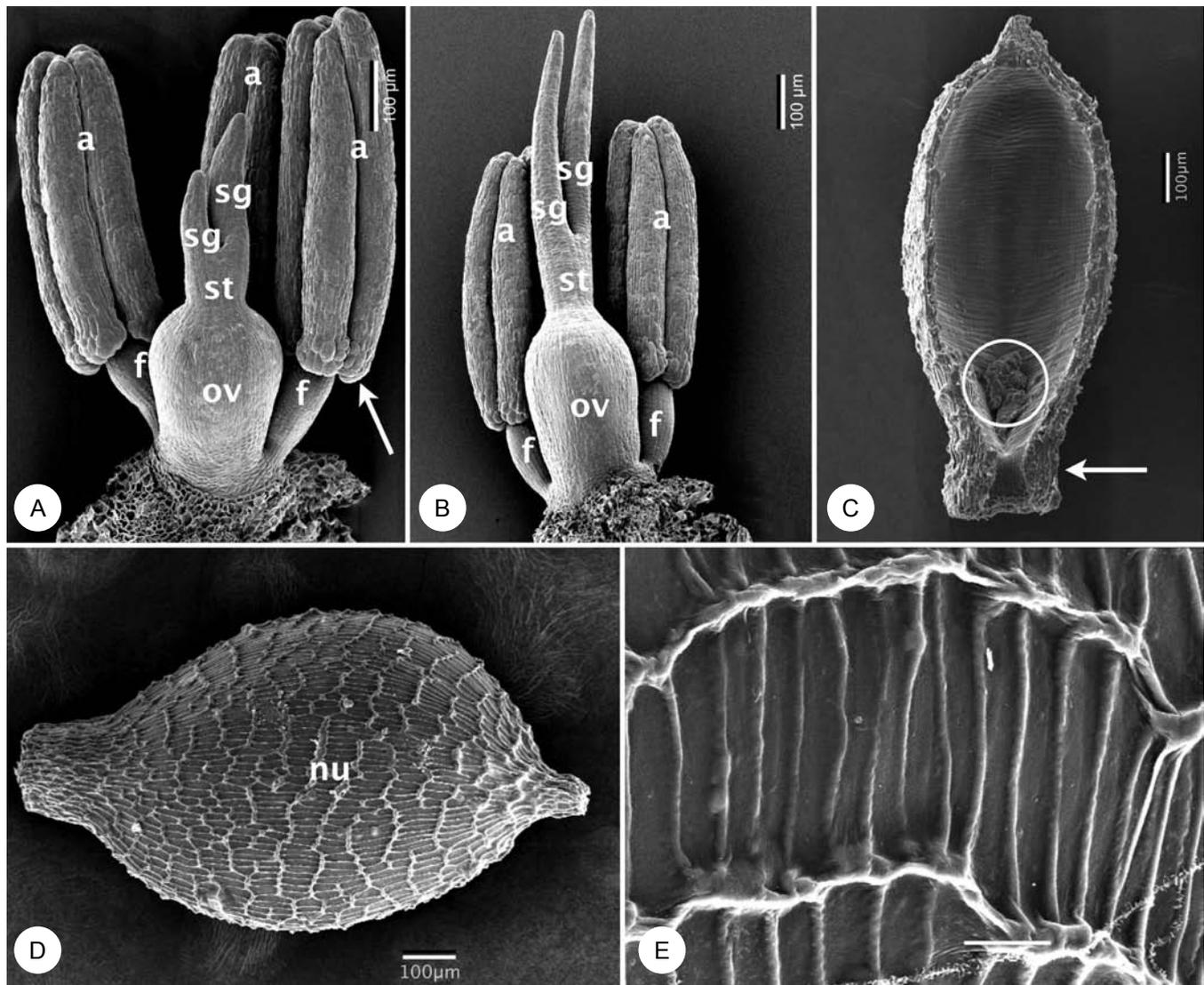
Figure 11 – *Pycreus flavescens*, SE micrographs of floral ontogeny. A–B, lateral-adaxial view of developing flower. In 'B', the delay of the development of the adaxial stigma branch diminishes; C, longitudinal section of a fruit wall with rests of the obturator hairs, and a hypogynous stalklet or gynophore (arrowed); D, nutlet; E, detail of the fruit wall.

Abbreviations: a, anther; f, filament; nu, nutlet; ov, ovary wall; sg, stigma (primordium); st, style.

from an annular ovary primordium. We believe that the organizational freedom resulting from the congenital fusion of the carpels into an annular ovary primordium made laterally flattened nutlets like in *Pycreus*, as well as dorsiventrally flattened nutlets like in *C. laevigatus*, possible.

In *P. bipartitus*, at early developmental stages, two adaxial stigma branches can occur (fig. 8G). Haines & Lye (1983) also reported the presence of three stigma branches in some specimens of *P. nigricans*. It is tempting to interpret these observations as an argument to state that the adaxial stigma branch in *Pycreus* resulted from the fusion of the two ancestral lateral ones. However, how to explain the presence of four stigma branches in *C. capitatus* (fig. 3H)? Therefore, we consider these particular structures rather as developmental accidents; the meristematic zones from which the stigma branches originate (we call them stigma primordia because they are not carpel tips, though we do not exclude that they are homologous with carpel tips) can be splitted (dédoublement).

In *P. flavescens*, the development of the adaxial stigma branch at early developmental stages is slightly delayed with respect to the abaxial one (figs 10, 11A). This too might be explained by a temporary lack of space. In *P. sanguinolentus*, an apical constriction of the ovary appears at semi-mature stage. We observed a similar phenomenon in *Fuirena abnormalis* C.B.Clarke (Vrijdaghs et al. 2004). In maturing flowers of several *Cyperus* and *Pycreus* species, a rudimentary hypogynous stalklet or gynophore appears (e.g. figs 2E
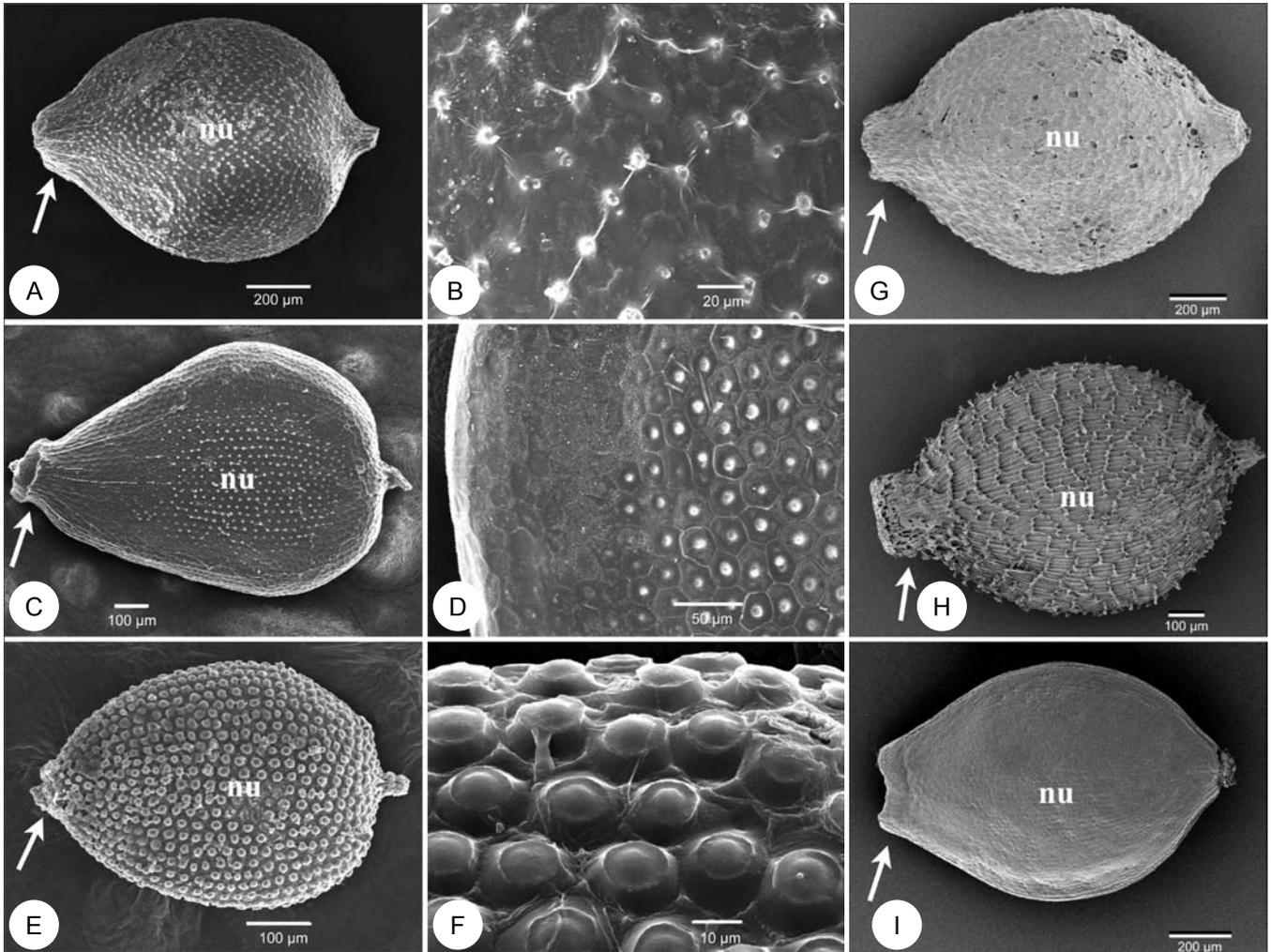
Figure 12 – SE micrographs of mature nutlets in *Pycreus* (A–H) and *Cyperus* (I). A, *P. bipartitus*, lateral view of a nutlet with a gynophore (arrowed); B, *P. bipartitus*. Detail of the fruit wall epidermis with cells with small conical silica bodies; C, *P. pelophilus*, lateral view of a nutlet with a gynophore (arrowed); D, *P. pelophilus*. Detail of the fruit wall epidermis with cells with each a tabular silica body; E, *P. pumilus*, lateral view of a nutlet with a gynophore (arrowed); F, *P. pumilus*. Detail of the fruit wall epidermis with small cells, each filled with a tabular silica body; G, *P. sanguinolentus*, lateral view of a nutlet with a hypogynous stalklet or gynophore (arrowed); H, *P. flavescens*, lateral view of a nutlet with a gynophore (arrowed). The epidermis consists of zonate (longitudinally elongated) cells; I, *Cyperus laevigatus*. Dorsiventral view of a nutlet with a gynophore (arrowed).
Abbreviation: nu, nutlet.

& L, 4O, 7I, 8K). This also occurs in other genera in Cypereae, such as *Ficinia*, *Isolepis*, and *Scirpoides* (Vrijdaghs et al. 2005a, 2006b).

Fruit wall epiderm cells in *Pycreus pelophilus*, *P. pumilus* and *P. polystachyos* have (at least partially) similar, tabular silica-bodies (fig. 12C–F). The fruit wall epiderm cells in *P. flavescens* are zonate and do not have silica-bodies (fig. 11D & E, 12H). *Pycreus bipartitus* has fruit walls with isodiametric epiderm cells with small conical silica bodies (fig. 12A & B), which is also reported in *P. sanguinolentus*, though we did not observe this in nutlets from herbarium specimens (fig. 12G). According to Metcalfe (1971), only the few neither conical nor tabular silica-bodies found in some species might have systematic value.

CONCLUSIONS

The spikelet ontogeny and morphology in the *Cyperus* and *Pycreus* species studied concurs with our observations in many other Cyperoideae that cyperoid spikelets consist of an indeterminate rachilla and many glumes which usually subtend (or not) a bisexual flower (Cariceae and sclerioid Cyperaceae not included). However, in Cypereae, proximal bracts of the spikelet may axillate a secondary spikelet. We consider this phenomenon to be a result of the flexibility plants have to activate different developmental patterns (to become a flower, a spikelet or a vegetative axis) in yet undetermined primordia. Spikelets in *Cyperus* s. lat. have a typical zigzagging morphology, resulting from a congenital fusion of the rachilla and the wings of the glumes, which causes epicaulescent growth
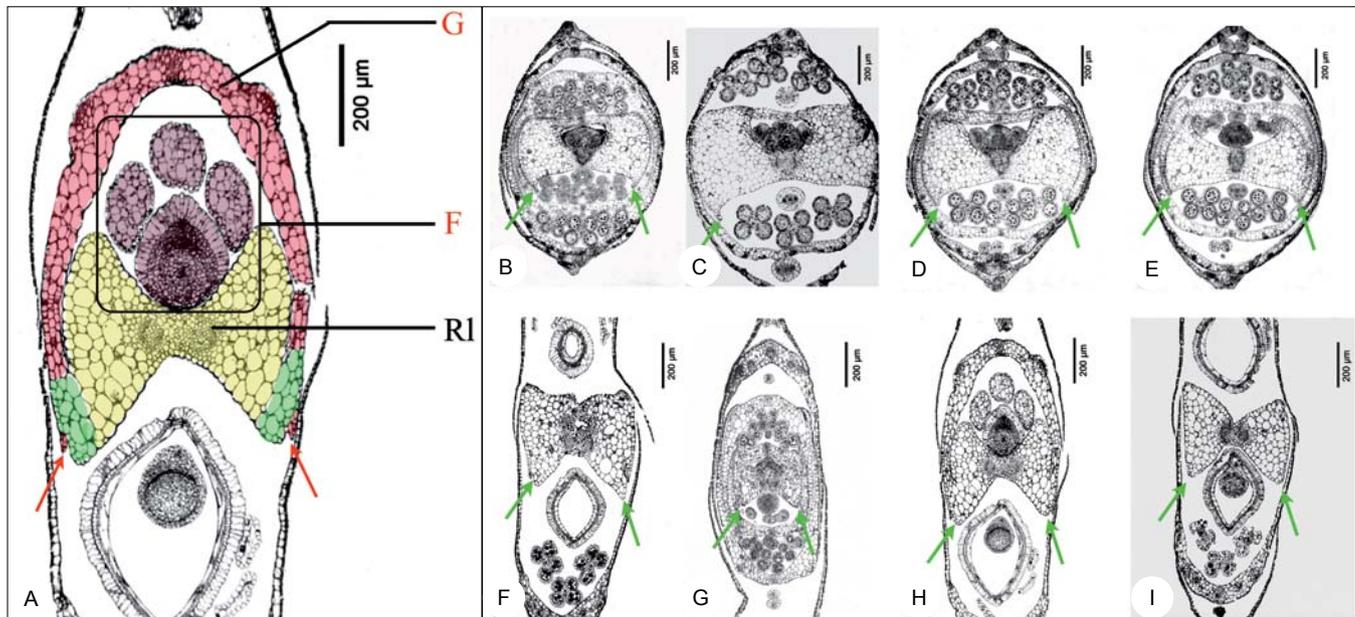
Figure 13 – A, Key for B–I; LM image of a cross section through a spikelet of *Pycreus flavescens*, at the height of the filaments. The green coloured areas are the fusion zones between rachilla and wings of a glume. The section of this glume and the flower in its axil (three filaments and centrally the gynoecium) are coloured in red. The wing tips are also coloured in red, and arrowed. The rachilla is coloured in yellow. In each section shown in figure B–I a similar glume with the flower it subtends can be observed, as well as a fusion zone of wings and rachilla; B–I, LM images of cross sections through the spikelet at different levels in *Cyperus laevigatus* (B–E) and *Pycreus flavescens* (F–I); B & F, cross sections through the basal level of a glume and its flower. In *P. flavescens*, the fusion of glume and rachilla (green arrow) is less complete than in *C. laevigatus*; C,D, G & H, cross sections at filament level; E & I, cross sections at anther level (or internode). Here, the glume is free from the rachilla.
Abbreviations: F, flower; G, glume; Rl, rachilla.

of the glumes with the rachilla. The particular morphology of a spikelet in *Pycreus* results from a pronounced epicaulescent growth of the glumes with the rachilla. The floral ontogeny in all species studied occurs according to the general cyperoid floral ontogenetic pattern, though no perianth primordia are formed. The pistil, as it originates from an annular primordium, gets more organisational freedom, which is illustrated by the two dorsiventrally positioned stigma branches in *Pycreus*, as well as the two laterally positioned stigma branches in species with dorsiventrally flattened nutlets, such as *C. laevigatus*. Only on condition that in cladistic analysis *Pycreus* would appear as a monophyletic taxon, we think that the combination of 1) its particular spikelet ontogeny resulting in a '*Pycreus*-type' spikelet, 2) the laterally flattened ovaries/nutlets which originated independently in the evolution from other taxa with similar ovaries, are strong arguments to consider this taxon to be a genus on its own. However, we also realise that this would make *Cyperus* paraphyletic.

ACKNOWLEDGEMENTS

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