

The Floral Scales in *Hellmuthia* (Cyperaceae, Cyperoidae) and *Paramapania* (Cyperaceae, Mapanioideae): An Ontogenetic Study

A. VRIJDAGHS^{1,*}, P. GOETGHEBEUR², E. SMETS³ and A. M. MUASYA⁴

¹Laboratory of Plant Systematics, Institute of Botany and Microbiology, K.U.Leuven, Kasteelpark Arenberg 31, B-3001 Leuven, Belgium, ²Research Group Spermatophytes, Department of Biology, Ghent University, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium, ³National Herbarium of The Netherlands, Leiden University Branch, PO Box 9514, NL-2300 RA Leiden, The Netherlands and ⁴Botany Department, University of Cape Town, 7701 Rondebosch, South Africa

Received: 12 April 2006 Returned for revision: 9 May 2006 Accepted: 19 May 2006 Published electronically: 28 June 2006

• **Background and Aims** In 1976 the monotypic genus *Hellmuthia* was placed in the Hypolytreae *s.l.*, but was subsequently ascribed to the Mapanioideae, tribe Chrysitricheae, mainly because of the presence in *Hellmuthia* of two lateral, mapanioid-like floral scales with ciliated keels, the anatomy of the nutlet, the embryo and the inflorescence. Recently, based on cladistic analyses and supported by pollen ontogenetic evidence, *Hellmuthia* was transferred to a Cyperaceae, tribe Cyperae, clade mainly consisting of *Ficinia* and *Isolepis*. In this study, the floral ontogeny in *Hellmuthia* was investigated and compared with the floral ontogeny in *Paramapania*, with special attention for the floral scales.

• **Methods** Freshly collected inflorescences of *Hellmuthia membranacea* and *Paramapania parvibractea* were investigated using scanning electron and light microscopy.

• **Key Results** In the conical ‘spikelet’ in *Hellmuthia*, proximal bracts occur, each axillating an axis with empty glume-like structures, or a reduced spikelet. Hence, it is a reduced partial inflorescence. In *Hellmuthia*, the stamen primordia originate before the primordia of the perianth–gynoecium appear. Moreover, a third adaxially positioned ‘floral scale’ was observed for the first time. The position and relative time of appearance of the floral scales in *Hellmuthia* are typical for perianth parts in Cyperoidae. The basal position of *Hellmuthia* within a clade of floral scales with usually perianthless flowers, allows the presence of rudiments of a perianth in *Hellmuthia* to be interpreted as a primitive state. Development of the lateral ‘scales’ in *Paramapania* follows a different pattern. Therefore, it was decided that the lateral ‘scales’ in *Paramapania* are different from the lateral perianth parts in *Hellmuthia*. The pollen grains in *Hellmuthia* are cyperoid, with one polar and five lateral apertures, of which the membrane is covered with sexinous bodies. The pollen surface is granulate and perforate with microspines.

• **Conclusions** The floral ontogeny in *Hellmuthia* occurs according to the general cyperoid pattern. The lateral scales in *Hellmuthia* are perianth parts, and they are not homologous to the lateral ‘scales’ in *Paramapania*.

Key words: Floral scales, *Paramapania*, floral ontogeny, Cyperaceae, *Hellmuthia*, SEM, homology.

INTRODUCTION

According to the molecular phylogenetic studies of Simpson *et al.* (2006; see Table 1), the Cyperaceae can be subdivided into two subfamilies, the Mapanioideae and the Cyperoidae (Table 1). Morphologically, the Mapanioideae are characterized by a particular ‘flower’, also called ‘spicoids’. The term ‘spicoid’ refers to a synanthial interpretation of the inflorescence unit, opposite to the use of the term ‘flower’. A typical ‘spicoid’ (e.g. Kukkonen, 1984) or ‘flower’ (e.g. Goetghebeur, 1998) is subtended by a large glume-like bract, which envelops most of the reproductive unit. The ‘flower’ consists of a very short and contracted central axis, several spirally placed scales subtending a stamen (or not), and a terminal pistil. The two proximal scales are keeled, opposite to each other and larger than the other scales, each subtending or opposite to a stamen (or not) (Kukkonen, 1984; Goetghebeur, 1986). In the Cyperoidae, a flower is trimerously built, tetracyclic with two whorls of each three perianth parts, one whorl of three

stamens, and a trimerous gynoecium. In many genera, there is a tendency to reduce the number of floral parts or modify them (Vrijdaghs *et al.*, 2004, 2005a, b).

The taxon, initially described as *Scirpus membranaceus* by Thunberg (1794), has been treated as a member of a number of several distinct genera depending on the interpretation of the floral characters. The species was transferred to *Isolepis* (Nees von Esenbeck, 1835), *Ficinia* (Kunth, 1837), and independently, the same plant was placed by Steudel (1855) in a new genus named after his son, *Hellmuthia*: ‘genus in nomine filii Hellmuth Steudel dixi’. The genus *Hellmuthia* consisted of one species *H. restoides*, because of its resemblance with the Restionaceae (Steudel, 1855).

In 1898, Clarke used the name *Scirpus membranaceus* Thunb. for the current *Hellmuthia membranacea*, arguing that it belongs to *Scirpus*: ‘I regard the squamellae here present as analogous to the broad scale-like hypogynous setae sometimes developed in *Scirpus littoralis* Schrad ... the species seems to me a *Eu-Scirpus*, near *S. littoralis* Schrad., rather than an *Isolepis*.’ (see Fig. 8A, after Clarke, 1909). Schönland (1922) mentioned that the

* For correspondence. E-mail alexander.vrijdaghs@bio.kuleuven.be

TABLE 1. *Suprageneric classification of Cyperaceae*

Goetghebeur (1998)	Simpson <i>et al.</i> (2006)
Subfamily Mapanioideae	Subfamily Mapanioideae
1. Hypolytreae	1. Hypolytreae
2. Chrysitricheae (+ <i>Hellmuthia</i>)	2. Chrysitricheae
Subfamily Cyperioideae	Subfamily Cyperioideae
1. Cypereae	1. Cypereae (+ <i>Hellmuthia</i>)
2. Fuireneae	2. Fuireneae
3. Eleocharideae	3. Eleocharideae
4. Abildgaardieae	4. Abildgaardieae
5. Scirpeae	5. Scirpeae
6. Dulichieae	6. Dulichieae
7. Schoeneae	7. Schoeneae
Subfamily Sclerioideae	8. Cryptangieae
1. Cryptangieae	9. Trilepideae
2. Trilepideae	10. Sclerieae
3. Sclerieae	11. Bisboeckelerae
4. Bisboeckelerae	12. Cariceae
Subfamily Caricoideae	
1. Cariceae	

lateral scales (Clarke's squamellae) were often called 'prophylls', though not fully approving this view: '...the view is gaining ground that all lateral cyperaceous flowers are without prophylls, whereas these are a constant feature in Gramineae. Structures which most botanists would consider as prophylls, ... are also occasionally found in *Scirpus membranaceus* Thunb. and *Ficinia ixioides* Nees.' (Schönland, 1922, p. 12).

In 1976, Haines and Lye changed *Scirpus membranaceus* into *Hellmuthia membranacea*, belonging to the monotypic genus *Hellmuthia*, and they transferred *Hellmuthia* to the Hypolytreae *s.l.*, tribe Mapanioideae. The spikelet and flower in *Hellmuthia* was described as a cone with a wide prophyll at its base and numerous imbricate membranous scales, each subtending a single, bisexual flower. Distinction was made between 'lower' and 'upper flowers', the lower ones having a membranous 'perianth'. Haines and Lye (1976) did not describe a perianth, but a drawing of a flower and a floral diagram showed two lateral scales enclosing the two lateral-axial stamens (Haines and Lye, 1976, fig. 1G and I, p. 62). In the captions of the figure, the 'flower' is called spikelet, though in the description the term 'flower' is used. Goetghebeur (1998) used unambiguously the term 'flower', and 'floral scales', and he classified *Hellmuthia* within his subfamily Mapanioideae, tribe Chrysitricheae (Table 1), mentioning that the taxonomic position of the genus is controversial. About the lateral scales, Haines and Lye (1976, p. 66) wrote: '... They bear no resemblance to the true perianth members found in other species of *Scirpus s. lat.* They can, however, be matched precisely, particularly as regards the ciliation of the keels, in the inflorescences of the Mapanioideae.' According to Haines and Lye (1976), as well as Goetghebeur (1986), only the more distally positioned flowers without lateral scales form fruits. The lateral scales with ciliated keels within the flower, as well as the anatomy of the fruit, were for Haines and Lye (1976) decisive argu-

ments to revalidate the genus *Hellmuthia* and classify it in the Mapanioideae. *Hellmuthia* was used by Holtum (1948) and Schultze-Motel (1959) as an argument in favour of their synanthial hypothesis. More recently, however, based on a combined morphological and molecular analysis, Muasya *et al.* (2000) suggested *Hellmuthia* to be phylogenetically closer to *Ficinia*, *Isolepis* and *Scirpoides*. Eventually, *Hellmuthia* has been classified in the Cyperioideae tribe Cypereae, in the *Ficinia-Isolepis* clade (Simpson *et al.*, 2003, 2006; Muasya *et al.*, 2006). Table 1 summarizes the suprageneric phylogenetic hypotheses of Goetghebeur (1998) and Simpson *et al.* (in press), which are used in this study. The global floral ontogenetic pattern in *Hellmuthia* was examined, comparing it with the ontogenetic pattern in the reproductive unit of *Paramapania*, and with the recently published (Richards *et al.*, 2006) floral ontogenetic data in *Exocarya* (Mapanioideae), with special attention for the two lateral 'floral scales'. In addition, some pollen morphological data in *Hellmuthia* are presented.

MATERIALS AND METHODS

Partial inflorescences of *Hellmuthia membranacea* were collected in South Africa by A. M. Muasya (voucher 2792, KUL) and Byetebier (voucher 2645, STELL), in June–July 2005 and fixed in FAA (70 % ethanol, acetic acid, 40 % formaldehyde; 90 : 5 : 5). Partial inflorescences of *Paramapania parvibractea* (Clarke) Uittien were collected by Reynders and Sabulao (voucher 32, UG) on Samar Island, Philippines in January 2006. Floral buds 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).

The material was washed twice with 70 % ethanol for 5 min and then placed in a mixture (1 : 1) of 70 % ethanol and DMM (dimethoxymethane) for 5 min. Subsequently, the material was transferred to 100 % DMM for 20 min, before it was critical point dried 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 and coated with gold with a SPI-Module™ Sputter Coater (SPI Supplies, West-Chester, PA, USA). Scanning electron microscope (SEM) images were obtained with a JEOL JSM-6360 (JEOL Ltd, Tokyo) at the Laboratory of Plant Systematics (K.U.Leuven).

For light microscopic (LM) observations, semi-mature spikelets of *Hellmuthia membranacea* were dehydrated through a *t*-butyl alcohol series to be embedded in paraffin. Transverse and longitudinal serial sections (Microm HM360) were cut at 12 µm and stained with saffranin in 70 % ethanol and anilin blue in an automatic staining machine Varistain 24-3 (Shandon, Runcorn, UK) and were mounted with Eukitt. LM images were observed with a Leitz Dialux 20 microscope (Germany) and digital photographs were made with an Olympus DP50 camera (Germany).

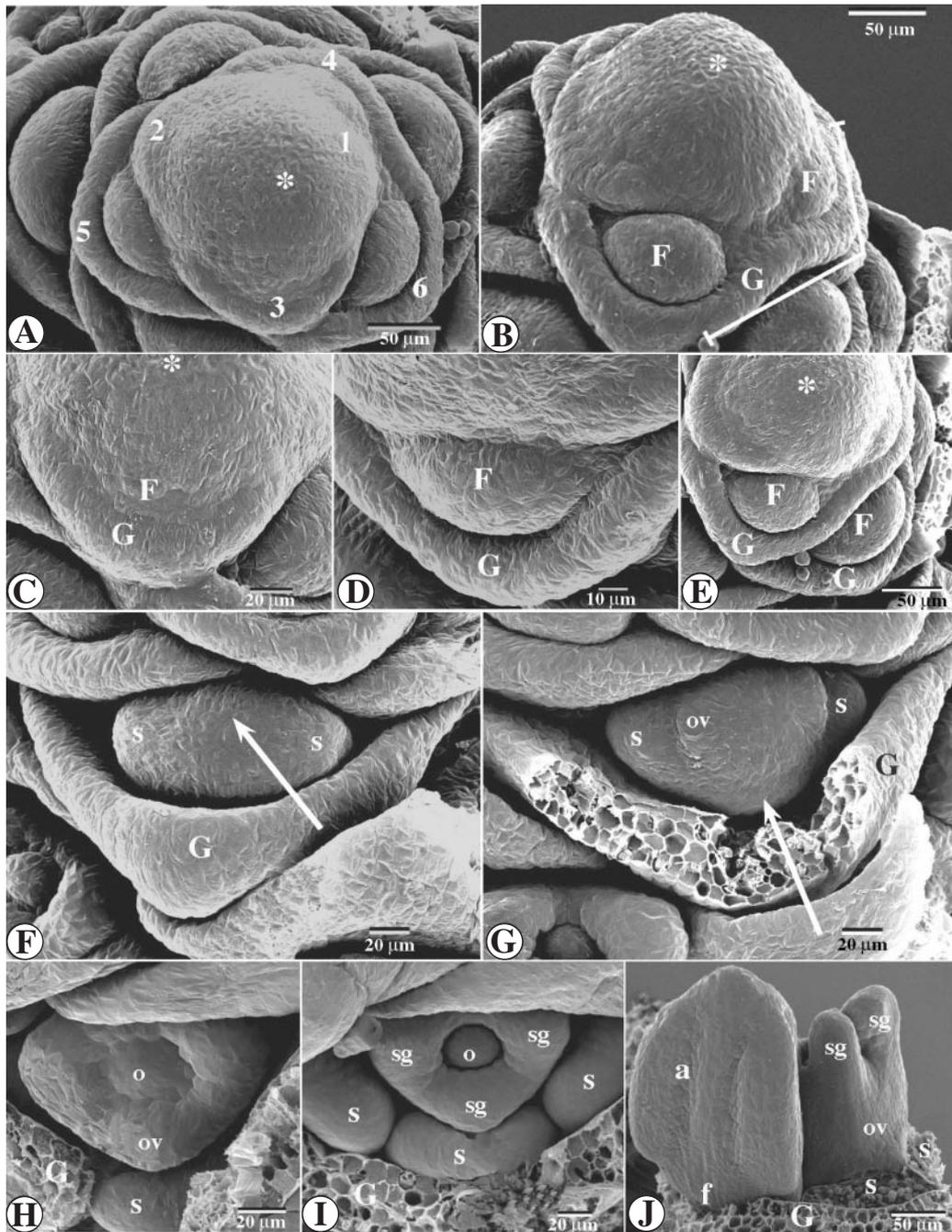


FIG. 1. Floral ontogeny in *Hellmuthia membranacea*. (A) Apical view of the rachilla apex and flower primordia in successive stages of development (1–6). The glumes of stages 4 and 6 are fused. (B) Lateral view of the rachilla apex with two flower primordia with fused glumes (marked). (C) Glume primordium with the floral primordium appearing in its axil. (D) Early flower primordium. (E) Two floral primordia at successive stages of development. The oldest flower primordium (right-hand side) expands laterally. (F) Flower primordium with two lateral stamen primordia and developing flower apex (arrowed). (G, H) The flower apex differentiates into an ovary primordium surrounding a central ovule. In (G), the abaxial stamen primordium is appearing (arrowed). (I) Two lateral and one abaxial stigma primordia originate on the top of the ovary wall. (J) Lateral-abaxial view of a developing flower. The abaxial and opposite lateral stamen have been removed. Anther and filament become visible. The ovary wall encloses the central ovule, and the stigma primordia are growing out. *, rachilla apex. Abbreviations: a, anther; f, filament; F, flower primordium; G, glume (primordium); o, ovule (primordium); ov, ovary (primordium); s, stamen (primordium); sg, stigma.

RESULTS

The conical ‘spikelet’ in *Hellmuthia* is indeterminate, with spirally placed glumes, each subtending a bisexual flower (Fig. 1A). Proximally, bracts axillating lateral axes with

empty glume-like structures, or rudimentary spikelets with glumes axillating floral primordia (Fig. 4E), occur. At early stages, glumes subtending neighbouring flowers may be fused (Fig. 1B). In the axil of the glume a floral

primordium originates (Fig. 1C, D), which expands laterally, forming two stamen primordia (Fig. 1E–G). Meanwhile, a floral apex becomes apparent as a bulge. Next, the floral apex differentiates into an annular ovary primordium surrounding a central ovule primordium (Fig. 1H). Simultaneously, the abaxial stamen primordium originates (Fig. 1H). The ovary primordium rises, and on the top of it two lateral and one abaxial stigma primordium are formed (Fig. 1I). Subsequently, the ovary wall envelops the central ovule, and the stigma primordia grow out (Figs 1J and 2A, B). Simultaneously, each stamen primordium develops into a filament and a basifixed and introrse anther (Figs 1J, 2A–E and 5A). In proximal flowers, opposite the lateral stamens, a scale-like structure is formed (Fig. 2C–G), which appears only after the formation of the stamens (Fig. 2C). At later stages, the scale-like structures obtain ciliated margins and keels, each one enveloping a filament (Fig. 2F, G). Meanwhile, a short single style and three papillose stigma branches are formed (Figs 2H and 3C, D, G, H). At semi-maturity, the relatively short style shows spiny protuberances, consisting of four cells, one more elongated cell, two spherical cells, and an antrorsely protruding spine (Fig. 3I). In the stamens, first the anthers elongate. Antrorse spine-like cells protrude from the distal part of the anther and a spiny apiculus has been formed (Figs 2H and 4B). Next, the filaments elongate and become flattened (Fig. 3E–G), continuing in a broad connective (Figs 3E and 4A). The base of each pollen sac is prolonged proximally (Fig. 4A), and on the top of each anther a spiny apiculus is formed (Figs 2D and 4B). In the proximal half of the spikelet, in between the larger glumes, small, membranous glumes are present (Fig. 3A), subtending (or not) each a reduced flower (Figs 3B and 4C). The margin of such a small glume is partially ciliated (Figs 3A, B and 4C). The large glumes are very wide (Figs 3C, D and 4D) with lateral wings that at later stages fold back (Figs 3C and 4D). At maturity, the lateral wings might separate from the main body of the glume (Fig. 3G). The margins of the wings and the apex of the mature glume are ciliated (Fig. 4D). In many proximally situated, (semi-)mature flowers, the ovary appears to protrude out of the conical ‘rachilla’, having a curved shape (Figs 2F–H and 5D). Lateral scales do not occur in more distally situated flowers (Fig. 5A, B), whereas in the proximal part of the spikelet flowers with lateral scales are present (Fig. 5C, D). In a more basal transverse section of flowers with lateral scales, a third, adaxial scale can also be present (Fig. 5E, F). *Hellmuthia* pollen grains have six apertures, five equatorial, and a polar one (Fig. 6A–D). Critical point-dried (partially collapsed) pollen grains are bullet-shaped with an average length of 37 µm and diameter of 20 µm (Fig. 6C). The surface is granulate with microspines, and perforate (Fig. 6E). Each aperture membrane is covered with sexinous bodies (Fig. 5D, G) showing the same ornamentation and microspines as the sexine. The tapetum wall is covered with irregularly shaped aggregated (or free) orbicules with connecting threads (Fig. 6F). The

orbicules tend to have the same ornamentation as the sexinous bodies (Fig. 6E–G).

In *Paramapania parvibractea*, the two lateral scales originate and develop before other parts of the reproduction unit become visible (Fig. 7A). The scales elongate, enveloping the rest of the floral unit (Fig. 7B, C), and obtain the typical margins with spiny outgrowths. At maturity, the lateral scales envelop the filaments of the stamens, and protect the inner part of the floral unit which consists of three inner scales and the gynoeceum (Fig. 7D). Only the style and three short stigma branches protrude above the inner scales (Fig. 7D). The lateral scales are fixed at the floral apex neatly below the point of attachment of the filaments (Fig. 7E). The nutlet is terete, and projected on a plane, it shows an obovate shape. The surface is smooth to slightly wrinkled. The style base is persistent with the fruit (Fig. 7F).

DISCUSSION

The conical ‘spikelet’ in *Hellmuthia* is considered to be a reduced partial inflorescence, because of the presence of proximal bracts subtending reduced spikelets. These spikelets are sterile, or glumes subtending floral primordia (Fig. 4E) do occur. The floral ontogeny in *Hellmuthia* (Figs 1–5) follows the general floral ontogenetic pattern as it was observed in all Cyperoideae studied (Vrijdaghs *et al.*, 2004, 2005a–c). There are, however, some particular features in its floral development and morphology, such as (a) fused glumes (Fig. 1A, B) at early developmental stages, (b) the membranous and partially ciliated small glumes subtending reduced flowers (Figs 3A, B and 4C), and (c) the extremely wide glumes with folding, ciliated wings at (semi-)mature stage (Figs 3C, D, G and 4D). These glume features might originate from initially fused glumes of which one part develops into a usual wide, winged glume subtending a well-developing flower, and the other part into a small membranous glume subtending a underdeveloped flower.

Most controversy on *Hellmuthia* was related to the presence of two lateral floral scales, which occur only in the proximal flowers (Figs 2F, G and 5C–F). Haines and Lye (1976) mentioned that the lateral scales might join adaxially below. The present observations show the existence of a third adaxial scale (Fig. 5F), which is basally connate with the two lateral scales (Fig. 5D, E). Scale-like perianth parts also occur in *Fuirena*, a genus in which a wide diversity of scale morphology and several reduction trends in the number of perianth parts can be observed (Muasya, 1998; Vrijdaghs *et al.*, 2004; Muasya *et al.*, 2006). In *Fuirena*, however, laminar perianth parts always belong to the inner whorl of the perianth. The lateral scale-like structures observed in *Hellmuthia* are positioned exactly where an outer perianth part could be expected (Fig. 2C, E), at the base of the filaments. In some flowers, situated in the proximal zone of the conical ‘spikelet’, a third, adaxial (and inner) perianth part occurs (Fig. 5F). Schönland (1922, p. 44)

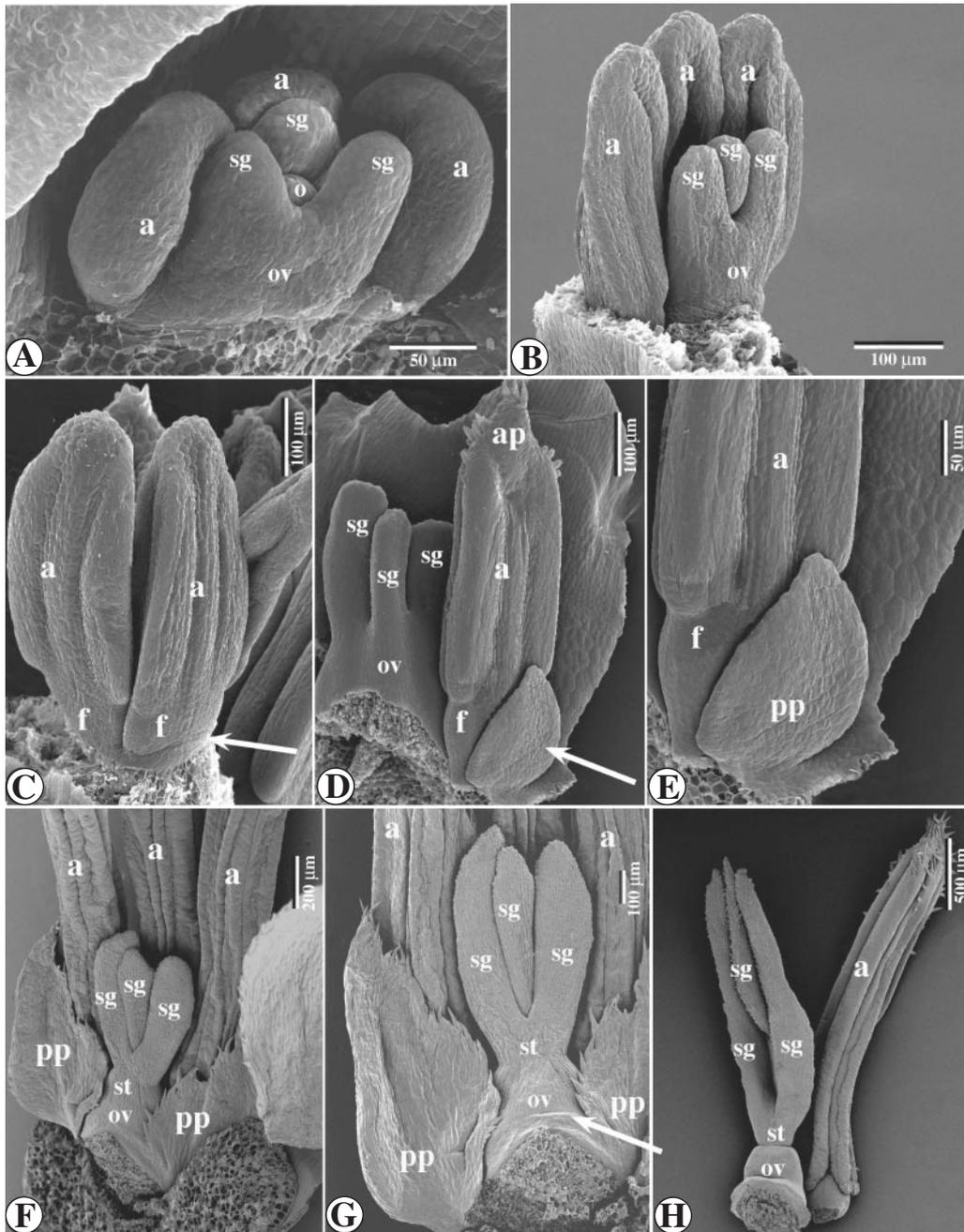


FIG. 2. Floral ontogeny in *Hellmuthia membranacea*. (A) Adaxial view of a developing (distally positioned) flower, with the anthers appearing, the ovary wall enveloping the central ovule, and the stigma primordia starting to grow out. (B) Adaxial view of a successive developmental stage of a (distally positioned in the spikelet) flower. (C) Lateral-abaxial view of a developing (proximally positioned in the 'spikelet') flower. Connective crests appear on the tops of the anthers. At the base of the lateral stamen, a primordium of a lateral perianth part originates (arrowed). (D) Abaxial-lateral view of a developing flower with a lateral stamen. Opposite it, a scale-shaped perianth part is developing (arrowed). (E) Detail of a lateral perianth part. (F) Adaxial view of a developing flower. The anthers rise high above the still underdeveloped gynoecium. The lateral perianth parts ('scales') show their typical morphology with ciliated keels. (G) Adaxial view of a developing flower with well-developed lateral perianth parts. At the adaxial base of the gynoecium, a rudiment of an adaxial perianth part is visible (arrowed). (H) Semi-mature gynoecium and stamen. The stigma branches are becoming papillose. Antrorse spine-like cells protrude at the distal part of the anther. Abbreviations: a, anther; ap, apiculus; f, filament; o, ovule (primordium); ov, ovary; pp, perianth part; sg, stigma; st, style.

described a similar adaxial structure in *Ficinia ixoides* Nees: 'Its most extraordinary character is the association with each flower of an adaxial, linear, obtuse, (often obtusely red) scale'. The adaxial scale-like structure, in *H. membranacea* (as well as in *F. ixoides* as illustrated by

Schönland; Fig. 8B) has the position of an adaxial, inner perianth part (Fig. 5F). Basal fusion of perianth parts is not uncommon (Vrijdaghs *et al.*, 2004, 2005a). The position and the relative time of appearance of the scale-like structures in *Hellmuthia*, after the formation and

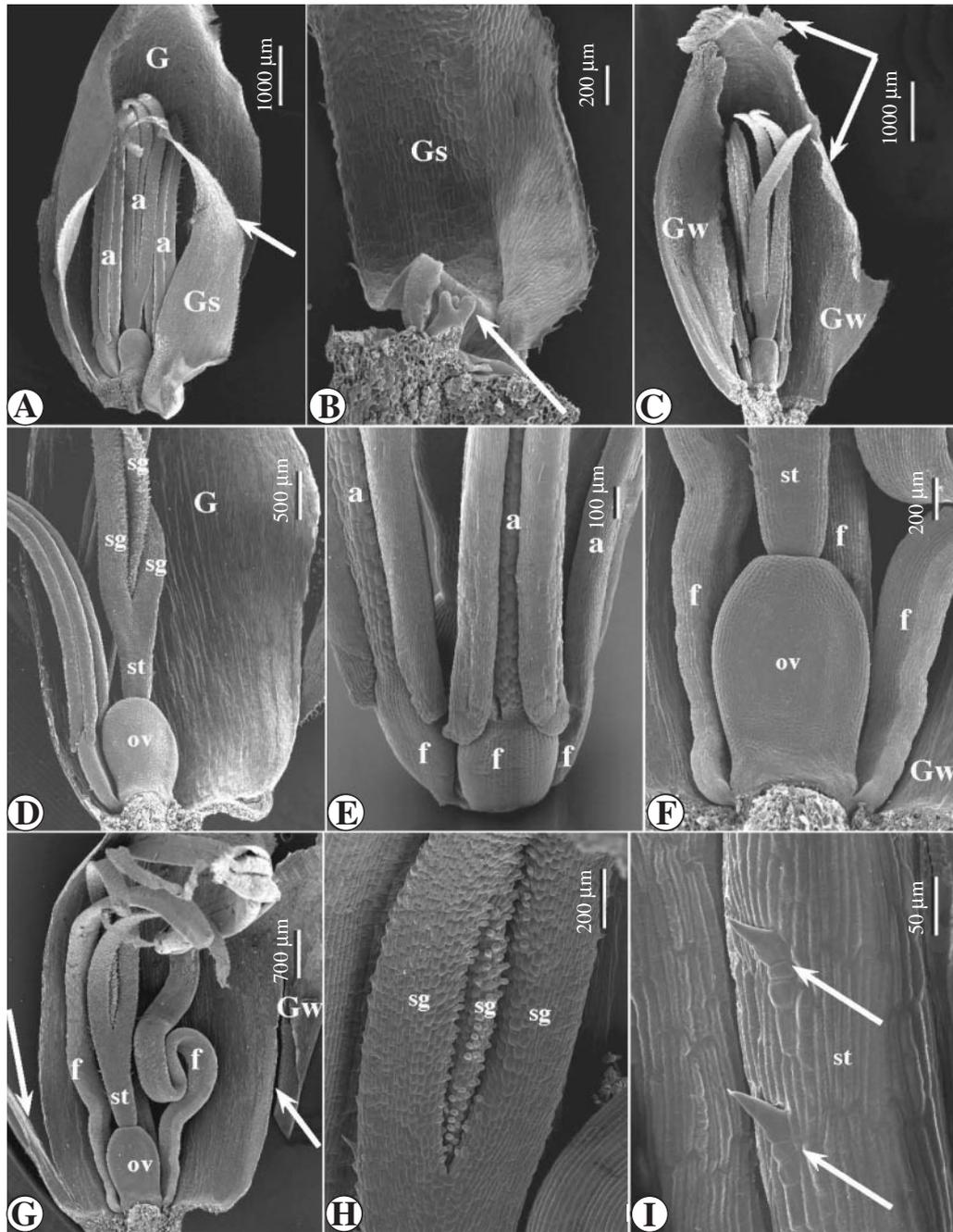


FIG. 3. Floral ontogeny in *Hellmuthia membranacea*. (A) Adaxial view of a nearly mature flower and a small glume (Gs) with ciliated margin (arrowed). (B) Small glume with a reduced flower (arrowed). (C) Adaxial view of a semi-mature flower and its subtending glume, with two lateral wings (Gw) and ciliated distal margins and keel (arrowed). (D) Adaxial view of a nearly mature flower. A ribbon-shaped single style with three ribbon-shaped, papillose stigmatic branches are formed. Anthers and filament are elongating. The ovary wall shows a typical texture. (E) Abaxial view of a semi-mature flower. (F) Detail of a mature ovary and style (with spiny antorse epidermal protuberances) from the adaxial side. The filaments are elongated. At the base of the ovary, there is a rudimentary hypogynous stalk. (G) Mature flower, adaxial side. The wings of the glume tend to tear off (arrowed). (H) Detail of the spiny epidermal protuberances of the style (arrowed). (I) Detail of the spiny epidermal protuberances of the style (arrowed). Abbreviations: a, anther; f, filament; G, glume; Gs, small glume; Gw, wing of a glume; ov, ovary; sg, stigma; st, style.

differentiation of the staminal primordia into filament and anther, which is later than in most other Cyperoideae observed by us (Vrijdaghs *et al.*, 2006), supports the conclusion that they are homologous to perianth parts. Clarke (1897–98) had already suggested that the lateral

scales are perianth members, a suggestion that is supported by the present results. The abaxial outer perianth part, as well as the two abaxial inner ones, are suppressed, which might be explained by the dorsoventral compression of the flowers due to the conical form of the

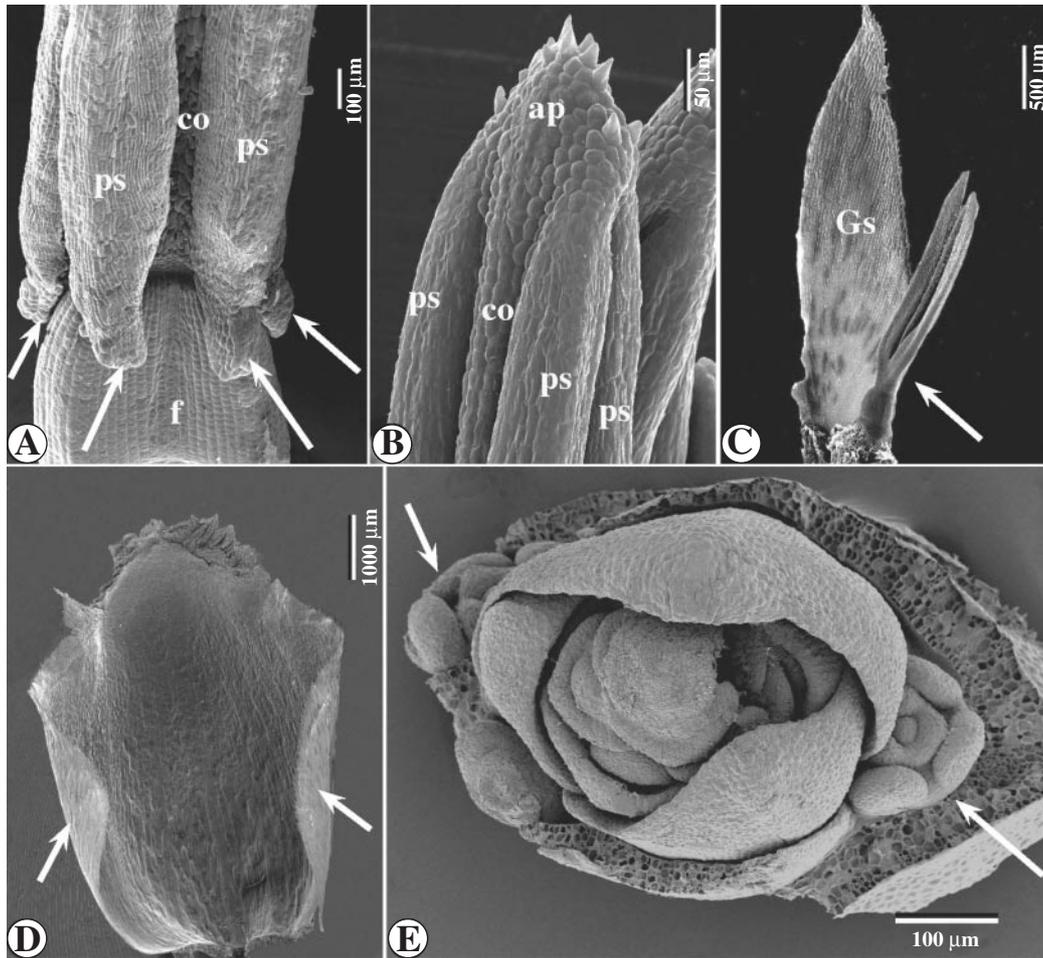


FIG. 4. Floral ontogeny in *Hellmuthia membranacea*. (A) Detail of the base of an anther in a developing stamen. The base of each pollen sac is prolonged proximally (arrowed). A broad connective can be observed in between the pollen sacs. The filament is wide and ribbon-shaped. (B) Detail of the connective crest (apiculus) on the top of a developing anther. (C) Small glume (Gs) with ciliated margin, subtending a reduced flower (arrowed). (D) Flower subtending glume with ciliated distal parts of the margins and glume apex, and wings that fold back (arrowed). (E) 'Spikelet' with developing early floral primordia (arrowed), removed from its subtending glume-like bract in the proximal part of a conical 'spikelet'. Abbreviations: Ap, piculus; co, connective; f, filament; Gs, small glume; ps, pollen sac.

'spikelet' axis. The early development of the lateral scales resembles the ontogeny of the inner perianth parts in *Fuirena*.

The detailed floral ontogenetic results in *Exocarya* published by Richards *et al.* (2006), show a similar developmental sequence for the lateral scales as in *Paramapania*. Figures 14–21 of this publication show that the lateral scales in *Exocarya* are actually the very first 'floral' primordia to appear, followed by the lateral stamen primordia. The point of attachment of the lateral scales in *Paramapania*, neatly below the point of attachment of the filaments, suggests that the lateral scales do not belong to the reproductive unit. The developing lateral scales seem to envelop and protect the 'floral apex', without being part of it. The abaxial and adaxial scales in *Exocarya*, as shown by Richards *et al.* (2006), originate apparently at the same positions and relative time as perianth parts in a cyperoid flower. In *Paramapania*, the lateral scales also originate and develop

before the appearance of any other structure on the 'floral apex'. The two lateral scale primordia develop fast, enveloping the 'floral apex' (floral primordium?), on which the first (lateral) stamen primordia appear only when the lateral scales have already been well developed. This is a different floral ontogenetic sequence from the general scirpoid floral ontogenetic pattern observed by Vrijdaghs *et al.* 2006. Therefore, the lateral scales in *Paramapania* are not considered to be homologous with the lateral perianth parts in *Hellmuthia*.

The presence of antrorse, spiny cells on the style (Fig. 3F, H, I) and anthers (Fig. 2H) is peculiar. Since the style is deciduous (Haines and Lye, 1976, fig. 3A, p. 64; Goetghebeur, 1998), these hook-shaped epidermal protuberances directed distally cannot play a role in the distribution of the fruit. They might protect the plant inflorescence against herbivores. Pollen grains in *Hellmuthia* are medium sized compared with other Cyperoidae (Fig. 6A–D). They have a typical cyperoid

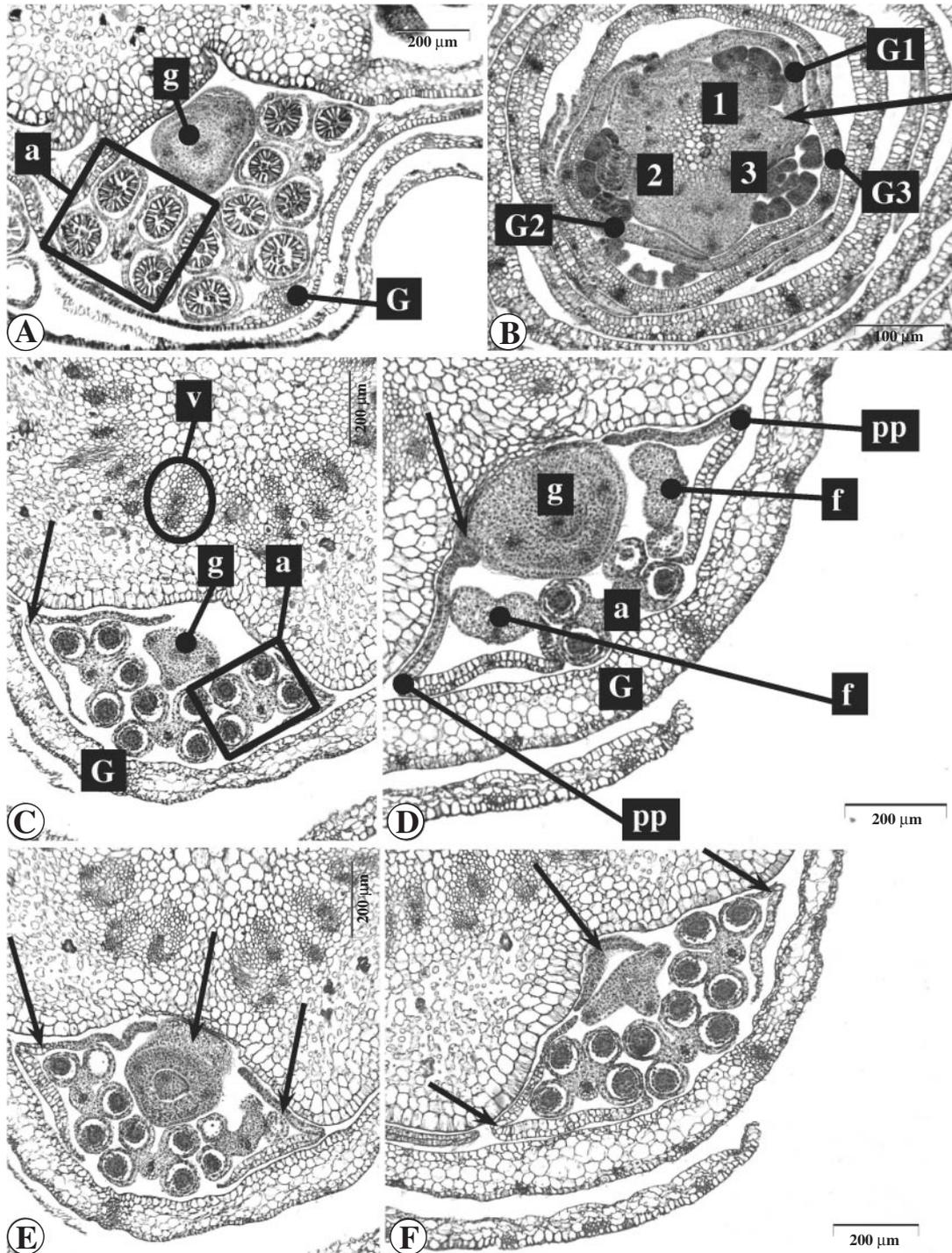


FIG. 5. Images of transverse LM sections through the 'spikelet' of *Hellmuthia membranacea*. (A) Section through a flower at the more distal part of a 'spikelet'. (B) Section through the distal part of a 'spikelet'. In '1' and '2' a transverse section through the basal part of the floral primordium shows three stamen primordia around the floral axis. Floral primordium '2' is older and the section through it is higher. The transverse section through flower '3' is made at the transition between the just formed filament and anther. In the centre of the flower the ovary wall surrounding the central ovule primordium is visible. The glumes (G1–G3) are imbricate. Glume 'G1' encloses a developing flower primordium as well as an undifferentiated primordial zone (arrowed). (C) Section at the height of the style of a developing flower and through the central part of the rachilla, with numerous, circular positioned vessels (v). Opposite each lateral stamen there is a flat and plied perianth part (arrowed). (D) Section through the central part of the ovary of a developing flower, and through the filaments (f) of the lateral stamens and the anther of the abaxial stamen (a). The perianth part (pp) at the left-hand side is fused with a tissue (primordial adaxial perianth part) in between the adaxial side of the ovary and rachilla (arrowed). (E) Section through a developing flower, with two free lateral perianth parts and an adaxial perianth part fused with the more basal central ovary wall (arrowed). (F) Section at the height of the transition between style and stigma branches through a developing flower. Two lateral and a free adaxial perianth part are visible (arrowed). Abbreviations: a, anther; g, gynoecium; f, filament; G, glume; pp, perianth part; v, vessel.

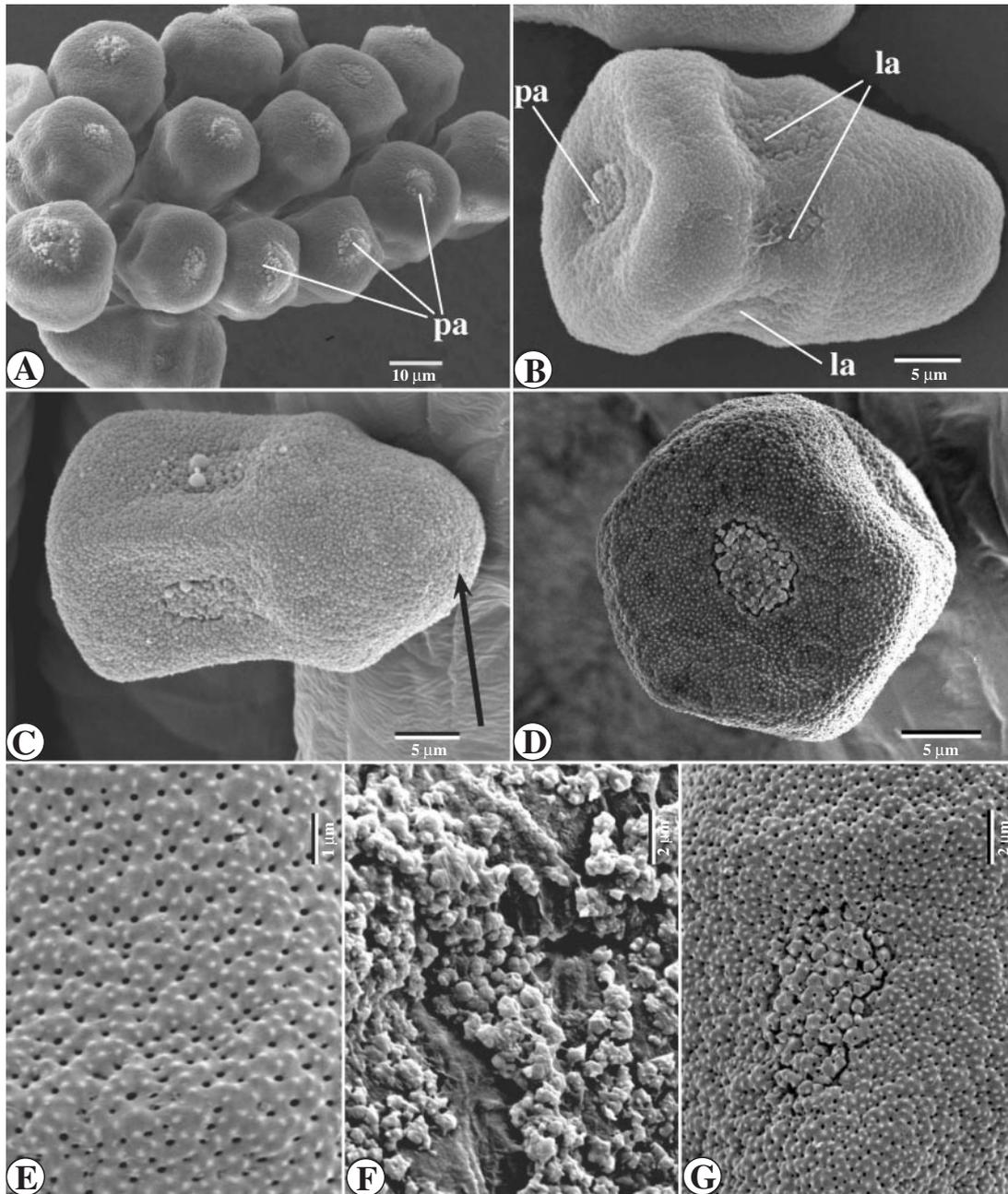


FIG. 6. SEM images of critical point-dried pollen grains in *Hellmuthia membranacea*. (A) View of a group of pollen grains, seen from the polar side, each with a polar aperture (pa). (B) Lateral view of a pollen grain, with three lateral (la) and the polar apertures visible. (C) Lateral view of a pollen grain with two lateral apertures and the zone of the previous adhesion (arrowed) with the three deteriorated pollen grains. (D) Polar view of a pollen grain. (E) Detail of the granulate, perforate pollen surface with microspines. (F) Tapetum covered with orbicules. (G) Detail of an aperture with sexinous bodies with microspines on its membrane. Abbreviations: pa, polar aperture; la, lateral (equatorial) aperture.

morphology, mostly described as triangular to pear-shaped (e.g. Haines and Lye, 1983) or cuneiform (e.g. Goetghebeur, 1998; Wichelen *et al.*, 1999) with six apertures. Many authors mention four apertures for pollen grains within the Cyperoideae and monoporate pollen within the Hypolytreae tribe (e.g. the *Carex*-like type and the *Mapania* type pollen in Haines and Lye, 1983). Pollen grains, however, differ in number of apertures as well as morphologically within the Cyperoideae and within the

Mapanioideae sensu Simpson (2006) (e.g. Erdtman, 1966; Koyama, 1969; Fernandez, 1987; Bruhl, 1995). The authors' observations (unpublished) confirm that, within Cyperoideae, the number of apertures varies. In Haines and Lye (1983, p. 20), an SEM photograph of pollen in *Fuirena* is shown, with four equatorial and one polar aperture out of probably six apertures. The pollen surface in *Hellmuthia* is similar to a 'Carex-type' pollen surface. Simpson *et al.* (2003) showed that pollen grains in

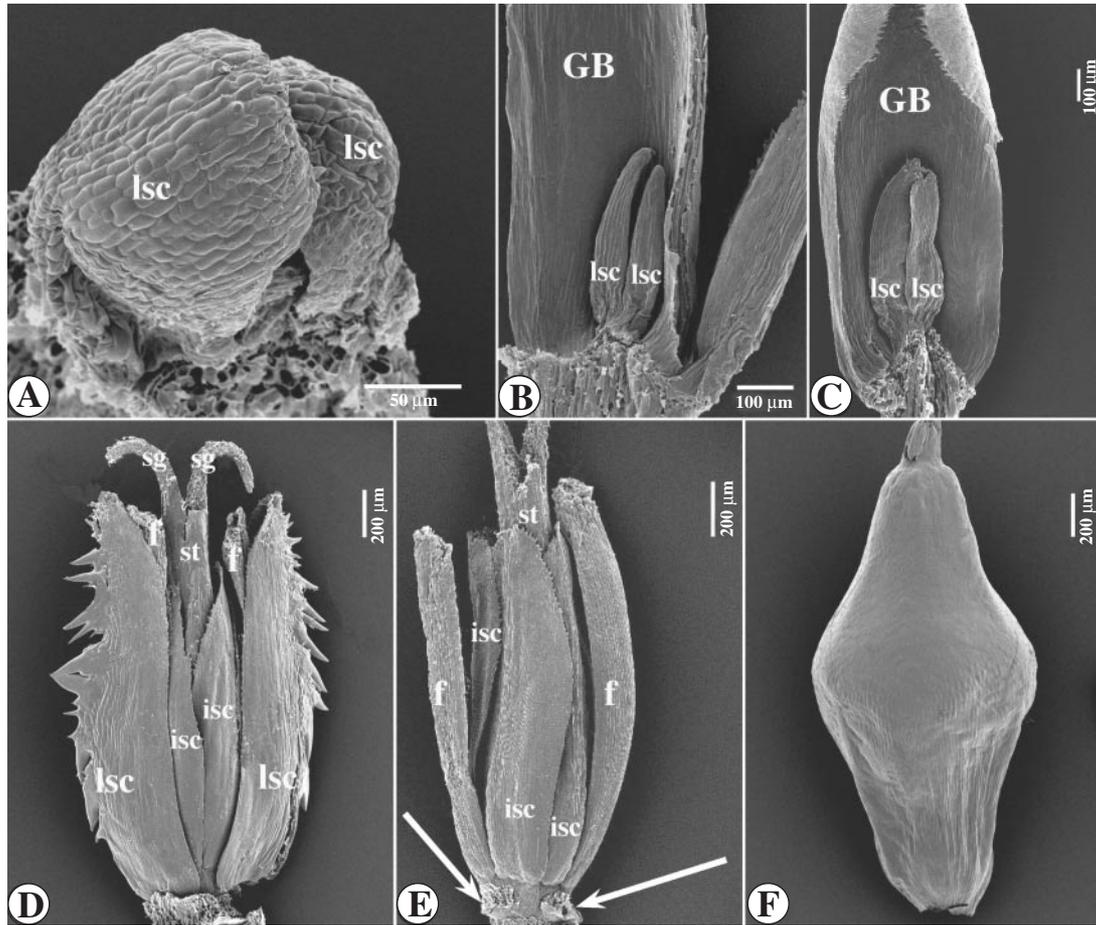


FIG. 7. Floral ontogenetic stages in *Paramapania parvibractea*. (A) Apical view of a reproductive unit hidden by two lateral scales at an early developmental stage. (B) Adaxial view of a subtending glume-like bract with developing lateral scales, which, at this stage, envelop the entire reproductive unit. (C) Successive developmental stage. The distal part of the margins of the lateral scales becomes ciliated. (D) Mature flower with the two massive lateral scales, abaxial view. (E) Mature flower, the lateral scales have been removed. The lateral scales are attached below the point of attachment of the filaments (arrowed). (F) Beaked nutlet. Abbreviations: f, filament; GB, glume-like bract; isc, inner scale; lsc, lateral scale; sg, stigma; st, style.

Hellmuthia are pseudomonads, like most pollen grains in Cyperoideae. Opposite the polar aperture (Fig. 6A) is the zone (Fig. 6B, C) where the three deteriorated cells of the former tetrad are present (Simpson, 2003). Following Simpson *et al.* (2003), the present observations confirm that the pollen morphology in *Hellmuthia* is cyperoid (Wichelen *et al.*, 1999) and hence the conclusion is corroborated that *Hellmuthia* is better placed away from Mapanioideae, Chrysitricheae.

Muasya *et al.* (1998, 2000) suggested that *Hellmuthia* should not be maintained in the Chrysitricheae *sensu* Goetghebeur (1998) and that it should be placed closer to *Ficinia*, *Isolepis* and *Scirpoides*. The mature nutlet of *Hellmuthia membranacea*, as illustrated with a SEM image by Haines and Lye (1976, p. 64, fig. 3A), has a hypogynous stalk and its morphology resembles the typical *Isolepis*-type nutlet (Muasya and Simpson, 2002; Vrijdaghs *et al.*, 2005c). Eventually, as a result of a cladistic analysis based on plastid DNA sequences, and pollen developmental evidence, *Hellmuthia membranacea* was placed in a Cyperaceae clade, sister to *Ficinia gracilis* (Muasya *et al.*, 2006; Simpson *et al.*, 2006).

Cyperaceae have always been defined based on, among others, perianthless flowers (e.g. Goetghebeur, 1998). There are, however, indications that flowers with perianth parts do occur within the Cyperaceae (Muasya *et al.*, 2006). The basal position of *Hellmuthia* in the *Ficinia*–*Isolepis* complex suggests that the tendency in this clade to reduce the number of perianth parts to zero has not yet been fully established. As has already been illustrated in *Fuirena* (Vrijdaghs *et al.*, 2004), the absence or presence of perianth parts can no longer be used as a decisive character for the delimitation of genera or tribes.

It is concluded that (a) the ‘spikelet’ in *Hellmuthia* is actually a reduced partial inflorescence, (b) the third, adaxial ‘floral scale’ and the two lateral ones in some proximal flowers in ‘spikelets’ in *Hellmuthia* are perianth members, (c) the overall floral ontogeny in *Hellmuthia* occurs according to the cyperoid general floral ontogenetic model, (d) the lateral ‘floral scales’ in *Hellmuthia* are not homologous to the lateral ‘floral scales’ in *Paramapania*, (e) the present morphological results support the transfer, based on molecular data, of *Hellmuthia* to a Cyperaceae clade.

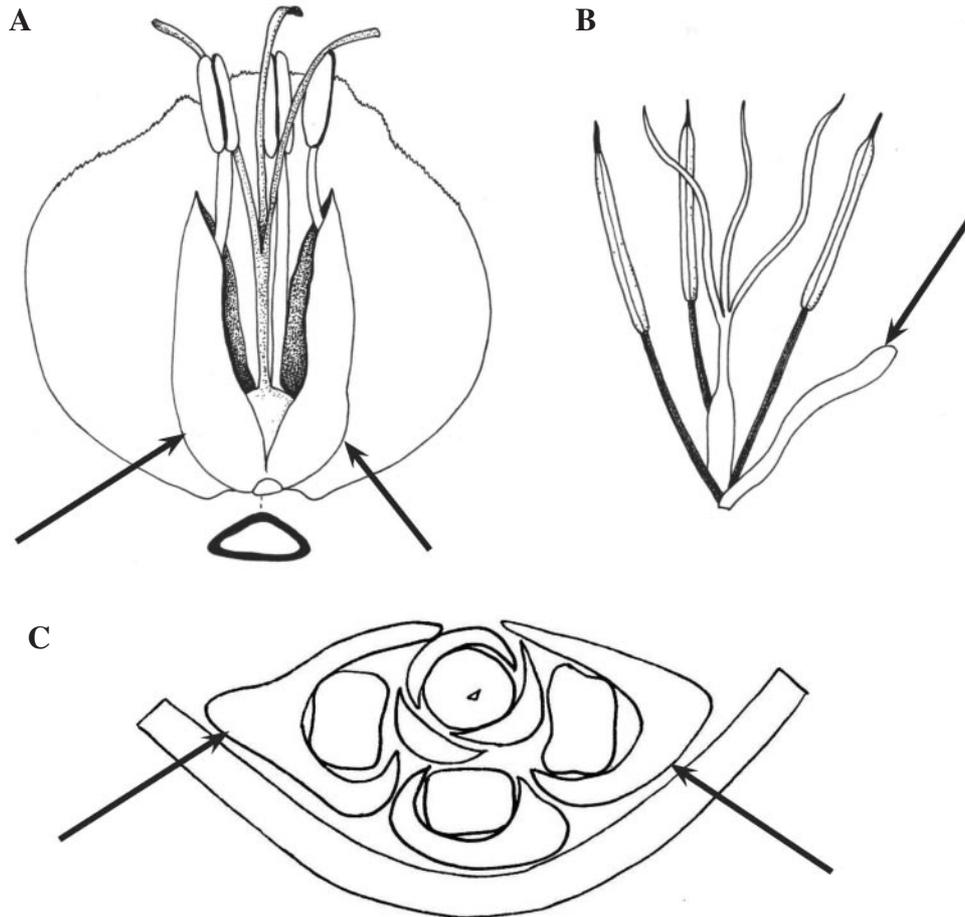


FIG. 8. Reproductions of diagrams. (A) Drawing of a flower (adaxial view) of *Hellmuthia membranacea*, reproduced from Clarke (1909), plate XLVII, 12. The lateral perianth parts are arrowed. (B) Drawing of a flower of *Ficinia ixoides*, reproduced from Schönland (1922), plate 44). The adaxial perianth part is arrowed. (C) Floral diagram of *Mapania*, reproduced from Goebel (1888), plate XIV, 14. The lateral 'scales' are arrowed.

ACKNOWLEDGEMENTS

We thank Marc Reynders (Research Group Spermatophytes, University of Gent) for kindly providing *Paramapania* material and DENR-PAWB region 8, Tacloban city, Philippines for providing collection permits, Benny Bytebier (Stellenbosch University) for collecting *Hellmuthia* material, Anja Vandepierre for her most professional help with the LM preparations and drawings, and palynologists Dr Stefan Vinckier and Dr Suzy Huysmans (Laboratory of Plant Systematics, K.U.Leuven). This work was supported financially by research grants of the K.U.Leuven (OT/05/35) and the Fund for Scientific Research-Flanders (FWO-Vlaanderen, Belgium, G.0268-04). A. Muthama Muasya was a visiting postdoctoral fellow of the Fund for Scientific Research-Flanders (FWO-Vlaanderen, Belgium) and of the Research Fund of the K.U.Leuven.

LITERATURE CITED

- Bruhl JJ. 1995.** Sedge genera of the world: relationships and a new classification of the Cyperaceae. *Australian Journal of Botany* **8**: 125–305.
- Clarke CB. 1897–98.** Cyperaceae. In: Thiselton-Dyer WT, ed. *Flora Capensis*, Vol. 7. London: Reeve, 266–524.
- Clarke CB. 1909.** *Illustrations of Cyperaceae*. London: Williams & Norgate.
- Erdtman G. 1966.** *Pollen morphology and plant taxonomy. Angiosperms*. New York, NY: Hafner.
- Fernandez I. 1987.** Contribucion al conocimiento palinologico de Cyperaceae. *Acta Botànica Malacitana* **12**: 173–182.
- Goebel K. 1888.** Über den Bau der Ärchen und Blüten einiger Javanischer Cyperaceen. *Annales du Jardin Botanique de Buitenzorg* **7**: 120–140.
- Goetghebeur P. 1986.** *Genera Cyperacearum. Een bijdrage tot de kennis van de morfologie, systematiek en fylogeneses van de Cyperaceae-genera*. PhD Thesis, Groep Plantkunde, Rijksuniversiteit Gent, Gent.
- Goetghebeur P. 1998.** Cyperaceae. In: Kubitzki K, ed. *The families and genera of vascular plants*. Vol. 4. Berlin/Heidelberg: Springer-Verlag, 141–190.
- Haines RW, Lye KA. 1976.** Studies in African Cyperaceae. XIV. The genus *Hellmuthia* Steud. *Botaniska Notiser* **129**: 61–68.
- Haines RW, Lye KA. 1983.** *The sedges and rushes of East Africa*. Nairobi, Kenya: East African National History Society.
- Holttum RE. 1948.** The spikelet in Cyperaceae. *Botanical Review* **14**: 525–541.
- Koyama T. 1969.** *Delimitation and classification of the Cyperaceae-Mapanioideae*. In: Gunkel JE, ed. *Current topics in plant science*. New York: Academic Press, 201–228.

- Kukkonen I. 1984.** On the inflorescence structure in the family Cyperaceae. *Annales Botanici Fennici* **21**: 257–264.
- Kunth CS. 1837.** *Enumeratio plantarum. 2. Cyperographia synoptica.* Stuttgart: Colla.
- Muasya MA. 1998.** A synopsis of *Fuirena* (Cyperaceae) for the flora of Tropical East Africa. *Kew Bulletin* **53**: 187–202.
- Muasya AM, Simpson DA. 2002.** A monograph of the genus *Isolepis* R. Br. (Cyperaceae). *Kew Bulletin* **57**: 257–362.
- Muasya AM, Simpson DA, Culham A, Chase MW. 1998.** An assessment of suprageneric phylogeny in Cyperaceae using *rbcL* DNA sequences. *Plant Systematics and Evolution* **211**: 257–271.
- Muasya AM, Bruhl JJ, Simpson DA, Culham A, Chase MW. 2000.** *Suprageneric phylogeny of Cyperaceae: a combined analysis.* In: Wilson KL, Morrison DA, eds. *Monocots: systematics and evolution.* Melbourne, Victoria, Australia: CSIRO, 593–601.
- Muasya AM, Vrijdaghs A, Simpson DA, Chase MW, Goetghebeur P, Smets E. 2006.** What is a genus in Cyperaceae: phylogeny, character homology assessment and generic circumscription. *Botanical Review* (in press).
- Nees von Esenbeck CG. 1835.** Uebersicht der Cyperaceengattungen. *Linnaea* **9**: 273–306.
- Richards JH, Bruhl JJ, Wilson KL. 2006.** Flower or spikelet?—Understanding the morphology and development of reproductive structures in *Exocarya* (Cyperaceae, Mapanioideae, Chrysitricheae). *American Journal of Botany* (in press).
- Schönland S. 1922.** South African Cyperaceae. *Memoirs of the Botanical Survey of South Africa* **3**.
- Schultze-Motel W. 1959.** Entwicklungsgeschichtliche und vergleichend-morphologische Untersuchungen im Blütenbereich der Cyperaceae. *Englers Botanische Jahrbuch der Systematischen Pflanzengeschichte und Pflanzengeographie* **78**: 129–170.
- Simpson DA, Furness CA, Hodkinson TR, Muasya AM, Chase MW. 2003.** Phylogenetic relationships in Cyperaceae subfamily Mapanioideae inferred from pollen and plastid DNA sequence data. *American Journal of Botany* **90**: 1071–1086.
- Simpson DA, Muasya AM, Alves M, Bruhl JJ, Dhooge S, Chase MW, et al. 2006.** *Phylogeny of Cyperaceae based on DNA sequence data—a new rbcL analysis.* In: Monocots III/Grasses IV. Claremont, CA: ALISO, Rancho Santa Ana Botanic Garden (in press).
- Steudel EG. 1855.** *Synopsis plantarum glumacearum 2. Cyperographia synoptica.* Stuttgart: Metzler.
- Thunberg PC. 1794.** *Prodromus Plantarum Capensis.* Uppsala.
- Vrijdaghs A, Goetghebeur P, Muasya AM, Smets E, Caris P. 2004.** The nature of the perianth in *Fuirena* (Cyperaceae). *South African Journal of Botany* **70**: 587–594.
- Vrijdaghs A, Caris P, Goetghebeur P, Smets E. 2005a.** Floral Ontogeny in *Scirpus*, *Eriophorum*, and *Dulichium* (Cyperaceae), with special reference to the perianth. *Annals of Botany* **95**: 1199–1209.
- Vrijdaghs A, Goetghebeur P, Smets E. 2005b.** *Homology problems in cyperoid flowers: a floral ontogenetic approach.* In: XVII International Botanical Congress, Abstracts. Vienna, Austria, 219.
- Vrijdaghs A, Goetghebeur P, Muasya AM, Caris P, Smets E. 2005c.** Floral ontogeny in *Ficinia* Schrad. and *Isolepis* R.Br. (Cyperaceae), with focus on the nature and origin of the gynophore. *Annals of Botany* **96**: 1247–1264.
- Vrijdaghs A, Muasya AM, Goetghebeur P, Caris P, Nagels A, Smets E. 2006.** A floral ontogenetic approach to homology questions within the Cyperoideae (Cyperaceae). *Botanical Review* (in press).
- Wichelen J, Camelbeke K, Chaerle P, Goetghebeur P, Huysmans S. 1999.** Comparison of different treatments of LM and SEM studies and systematic value of pollen grains in Cyperaceae. *Grana* **38**: 50–58.