

Palynological diversity and major evolutionary trends in Cyperaceae

Anne Nagels · A. Muthama Muasya ·
Suzy Huysmans · Alex Vrijdaghs · Erik Smets ·
Stefan Vinckier

Received: 4 April 2008 / Accepted: 23 September 2008 / Published online: 14 November 2008
© Springer-Verlag 2008

Abstract Pollen and orbicule morphology of 84 species, representing 52 genera from all tribes and subfamilies are investigated, in order to assess the systematic value of palynological data and to determine palynological evolutionary trends in Cyperaceae. A total of 90% of the species are examined for the first time with scanning electron microscopy. Pollen grains of Cyperaceae are oblate spheroidal to prolate in shape, inaperturate to polyporate with opercula or pontopercula on pori or colpi. We distinguished seven different sexine ornamentation patterns. Orbicules occur in all species investigated. Pollen morphological variation within Cyperaceae is considerable and includes dispersal unit; number, location and degree of differentiation of apertural zones; and sexine ornamentation patterns. In subfamily Mapanioideae both tribes can be characterized by palynological synapomorphies. However, in subfamily Cyperoideae, the observed pattern of variation does not fit the most recent molecular phylogeny due to

high levels of homoplasy and polymorphism in major pollen characters.

Keywords Cyperaceae · Cyperoideae · Mapanioideae · Orbicules · Palynology · Pollen · Pseudomonads · SEM

Introduction

Cyperaceae are the third largest family in the monocotyledons consisting of 109 genera and approximately 5,500 species (Govaerts et al. 2007). Recent phylogenetic studies based on molecular data have suggested to maintain only two subfamilies within Cyperaceae: Mapanioideae and Cyperoideae (Simpson et al. 2008; Muasya et al. 2008). In this new delimitation Mapanioideae comprise two tribes: Hypolytreae and Chrysitricheae, while the circumscription of Cyperoideae changed considerably to include taxa previously placed in Caricoideae and Sclerioideae (sensu Goetghebeur 1998) (Table 1).

The palynology of Cyperaceae attracted quite some attention in the past, mainly because of the occurrence of an unusual type of simultaneous microsporogenesis, which leads to the formation of pseudomonads (Selling 1947; Davis 1966) or kryptotetrads (Erdtman 1952). After meiosis of the microspore mother cell, one of the four nuclei enlarges and occupies the centre of the coenocytic cell, while the other three nuclei migrate to the narrow apex where they are separated by septa and subsequently degenerate (Shah 1962; Dunbar 1973; Strandhede 1973; Furness and Rudall 1999; Brown and Lemmon 2000; Simpson et al. 2003). This unusual pattern of microsporogenesis is only known in one other unrelated group: tribe Styphelieae in Ericaceae (Smith-White 1959).

A. Nagels (✉) · S. Huysmans · A. Vrijdaghs · E. Smets
Laboratory of Plant Systematics, Institute of Botany
and Microbiology, Kasteelpark Arenberg 31,
P.O. Box 2437, 3001 Louvain, Belgium
e-mail: annenagels@gmail.com

A. M. Muasya
Botany Department, University of Cape Town,
Private Bag X3, Rondebosch 7701, South Africa

E. Smets
National Herbarium of the Netherlands, Leiden University
Branch, P.O. Box 9514, 2300 RA Leiden, The Netherlands

S. Vinckier
Center for Transgene Technology and Gene Therapy,
Flanders Institute for Biotechnology, K.U. Leuven,
Campus Gasthuisberg, Herestraat 49, 3000 Louvain, Belgium

Table 1 Comparison of Cyperaceae classifications according to Goetghebeur (1998) and Simpson et al. (2008) and Muasya et al. (2008)

Goetghebeur (1998)	Simpson et al. (2008) Muasya et al. (2008)
Subfamily Mapanioideae	
1. Hypolytreae (+ <i>Exocarya</i> , <i>Capitularina</i>)	1. Hypolytreae
2. Chrysitricheae (+ <i>Hellmuthia</i>)	2. Chrysitricheae (+ <i>Exocarya</i> , <i>Capitularina</i>)
Subfamily Cyperoideae	
1. Scirpeae	1. Scirpeae
2. Fuireneae	2. Fuireneae
3. Eleocharideae	3. Eleocharideae
4. Abildgaardieae	4. Abildgaardieae (+ <i>Arthrostylis</i> , <i>Actinoschoenus</i> , <i>Trachystylis</i>)
5. Cypereae	5. Cypereae (+ <i>Hellmuthia</i>)
6. Dulichieae	6. Dulichieae
7. Schoeneae (+ <i>Arthrostylis</i> , <i>Actinoschoenus</i> , <i>Trachystylis</i> , <i>Rhynchospora</i>)	7. Schoeneae
	8. Rhynchosporeae
Subfamily Sclerioideae	
1. Cryptangieae (– <i>Exochogyne</i>)	9. Cryptangieae (+ <i>Exochogyne</i>)
2. Trilepideae	10. Trilepideae
3. Sclerieae	11. Sclerieae
4. Bisboeckelereae	12. Bisboeckelereae
Subfamily Caricoideae	
1. Cariceae	13. Cariceae

Mapania-type pollen (sensu Simpson et al. 2003) differs in several aspects from the common wind pollinated, pyriform pseudomonads with one to six apertures (Furness and Rudall 1999; Van Wichelen et al. 1999). *Mapania*-type pollen, recorded in five genera of the mapanioid tribe Hypolytreae (Simpson et al. 2003), is spheroidal with only one distal aperture and the pollen grains appear to be sticky, suggesting animal pollination. Erdtman (1952) and Haines and Lye (1983) suggested that mapanioid pollen grains are not pseudomonads, and Koyama (1961) considered them as tetrads. Moreover, in contrast to the peripheral pollen arrangement in Cyperaceae, *Mapania*-type pollen grains are centrally arranged in the locules (Kirpes et al. 1996; Simpson et al. 2003).

In the anthers of seed plants, tiny ($\pm 1 \mu\text{m}$) sporopollenin granules might occur on the radial and inner tangential walls of secretory tapetum cells. These granules are sometimes in close contact with the pollen grains and are called orbicules (Erdtman et al. 1961) or Ubisch bodies (Rowley 1962, probably in reminiscence and in honour to Kosmath 1927 and von Ubisch 1927). The distribution, morphology and function of orbicules in angiosperms have been reviewed by Huysmans et al. (1998, 2000). In Poales,

orbicules are reported in the Centrolepidaceae (Rowley and Dunbar 1996) and the Poaceae (e.g. El-Ghazaly and Jensen 1987; Vinckier and Smets 2001). In Cyperaceae, orbicules have been reported in *Carex wallichiana* Presc. (Shah 1962), *Eleocharis palustris* (L.) Roem. and Schult. (Carniel, 1971; Dunbar 1973), *Hellmuthia* Steud., *Mapania* Aubl., *Capitularina* Kern, *Exocarya* Benth. and *Diplasia* Rich. (Simpson et al. 2003; Vrijdaghs et al. 2006).

Several authors have recognized different pollen types in Cyperaceae mainly based on pollen shape, pollen size, and number and type of apertures (see Table 2 for an overview). However, the number of species investigated with scanning electron microscope (SEM) is very limited and the recent availability of a Cyperaceae phylogeny offers great potential for evolutionary interpretations of the data. The major aim of the present study is to provide a palynological overview at family level in order to document with scanning electron microscopy, the pollen and orbicule morphology for a relevant sampling of all major clades. Our data are then used to assess the taxonomically useful characters, and to determine palynological evolutionary trends in Cyperaceae sensu Muasya et al. (2008).

Materials and methods

Sampling

We sampled among all major clades that represent recently recognized taxonomic groups in Cyperaceae (Muasya et al. 2008). A total of 84 species representing 46 genera in Cyperoideae and six genera in Mapanioideae were investigated (see Appendix 1). We mainly used stamens from flowers fixed in FAA (70% ethanol, acetic acid, 40% formaldehyde, 90:5:5), which were collected in the field or in botanical gardens. We supplemented our sampling with herbarium material from the collections in BR and GENT, and living material.

Preparation techniques were optimized for each category of sample: fixed, herbarium or fresh material, respectively.

SEM observations

Stamens of fixed flowers were dissected in 70% ethanol under a Wild M3 stereo microscope (Leica Microsystems AG, Wetzlar, Germany) and preserved in 70% ethanol. The dissected anthers were rinsed twice in 70% ethanol for 5 min and in a mixture (1:1) of 70% ethanol and DMM (dimethoxymethane) for 5 min. Subsequently the anthers were transferred to 100% DMM for 20 min, prior to critical-point drying (Balzers CPD 030).

Table 2 Literature overview of previous palynological studies in Cyperaceae

Reference	Typology	Diagnostic characters	LM/SEM/TEM	Sampling
Wodehouse (1935)	Basic type	Ovoid to pyriform, monoaperturate, granulate sexine	LM	3 species, 2 genera
Erdtman (1952, 1966, 1971)	<i>Carex</i> -type	Elongate, one distal porus + three lateral pori/colpi (majority of Cyperaceae)	LM	65 species, 35 genera
	Type two	Spheroidal, monoaperturate (<i>Mapania</i> and related genera)		
Cranwell (1953)	Two pollen types	Pollen type identification key to native New Zealand Cyperaceae with two pollen types based on pollen shape (pyriform or paraboloid and spheroidal)	LM	26 species, 13 genera
Shah (1962)		Monocolpate, smooth exine	LM	7 species, 5 genera
Huang and Chung (1971)		Identification key to 12 genera	LM	102 species, 12 genera
Padhye and Makde (1980)	<i>Cyperus</i> -type	Ellipsoid, one distal colpus, granulate/foveolate sexine (most frequent)	LM	31 species, 12 genera
	<i>Carex</i> -type	As defined by Erdtman (1966)		
Haines and Lye (1983)	<i>Mapania</i> -type	Spheroidal, monoporate (members of Hypolytreae)	LM/SEM	?
	<i>Carex</i> -type	One distal aperture + three lateral apertures, psilate sexine		
Fernandez (1987)	<i>Cyperus longus</i> -type	Pantoaperturate with one distal pore and 4–6 colpi	LM/SEM	19 species
	<i>Cyperus michelianus</i> -type	Pantoaperturate with several pores		
	<i>Cladium mariscus</i> -type	Inaperturate		
	<i>Schoenus nigricans</i> -type	Pantoaperturate with one distal pore and 4–6 colpi, but dimensions different from first type		
	<i>Carex flacca</i> -type	Pantoaperturate with one distal pore and 4–5 colpi		
	<i>Carex hallerana</i> -type	Pantoaperturate with one distal pore and 4–5 equatorial pores		
Bruhl (1995)	Type one	1–6 apertures (most frequent)	LM	122 genera (according to Bruhl 1995)
	Type two	>6 apertures (genera <i>Baumea</i> , <i>Machaerina</i> and <i>Tricostularia</i>)		
Van Wichelen et al. (1999)	Type one	Spheroidal/oblong ovoid, one distal ulcus (Mapanioideae)	LM/SEM	30 species, 27 genera
	Type two	Broadly obovoid, one distal ulcus + three lateral pori (Sclerioideae and Caricoideae)		
	Different types	Cyeroideae		
Moar and Wilmshurst (2003)	12 groups	Pollen identification key to 15 genera of New Zealand Cyperaceae, mainly based on grain shape and aperture type	LM	38 species, 15 genera
Simpson et al. (2003)	<i>Mapania</i> -type	Pollen development, shape	LM/SEM/TEM	9 species, 7 genera
	Pseudomonad-type	Pollen development, shape		

Herbarium material was treated with a modified enzyme-based method according to Schols et al. (2004a) slightly adjusted to the fragile nature of Cyperaceae pollen. The stamens were rehydrated in Na–cacodylate buffer (pH 7.3, 0.05 M) for ca. 36 h. Subsequently, the anthers were fixed in 2.5% glutaraldehyde in Na–cacodylate buffer for minimum 8 h and rinsed two times

during 30 min in Na–cacodylate buffer. Next, the anthers were transferred to a solution of 8 ml Na–cacodylate-buffer, 0.03 g cellulase (Fluka Biochemica, cellulase of *Trichoderma viride*) and 0.1 g pectinase (Fluka Biochemica, pectinase of *Aspergillus niger*) for 24 h. The test tubes with the material were shaken with a shaking device (VEL GFL 3015). The material was then

dehydrated in a graded ethanol–DMM series and critical-point dried.

Fresh material was immediately fixed in glutaraldehyde (2.5% in Na–cacodylate buffer) for minimum 8 h. Next, it was rinsed in buffer (2×30 min) and transferred to a graded buffer–ethanol series (30% ethanol, 50% ethanol, 70% ethanol). Subsequently, the anthers were transferred to a 1:1 mixture of ethanol and DMM for 5 min. Prior to critical-point drying, the material was moved to 100% DMM for 20 min. The dried anthers were mounted on stubs with double-sided adhesive carbon tape followed by the dissection of the anthers to make the pollen grains visible. The stubs were coated with gold with a SPI-MODULE™ Sputter Coater (SPI Supplies, West Chester, PA, USA). Images were obtained with a SEM (JEOL JSM-6360). Comparative size measurements of orbicules and pollen were made on SEM-micrographs using Carnoy 2.0 (Schols et al. 2002). Only completely rehydrated pollen grains were measured. For each species investigated, 50 orbicules were measured and mean values and standard deviations were calculated. Terminology follows Punt et al. (2007) unless stated otherwise.

Coding

In total 11 characters were scored for three outgroups and 52 Cyperaceae genera (Appendix 2 and C). Three quantitative and continuous characters (polar axis, P/E ratio and orbicule size) were coded using Thiele's gap weighting method as implemented by MorphoCode (Schols et al. 2004b), with $n = 5$, where n is the number of allowed character states in the Thiele formula (Thiele 1993). Non-continuous characters were treated as unordered multistate characters (pollen shape, distinctness of apertures, presence or absence of distal aperture, lateral aperture number, lateral aperture shape, sexine ornamentation, orbicule shape and orbicule ornamentation) and were assigned the default weight of 1.

Character optimization

Characters were optimized on a strict consensus tree obtained from a parsimony analysis of *rbcL* and *trnL-F* sequence data of all genera investigated (data from Muasya et al. 2008) using MacClade 4 (Maddison and Maddison 2001). This particular analysis was selected since it represents the most thoroughly sampled molecular analysis of the entire family available today. Adding the palynological characters (Appendix 3: Table 5) to the *rbcL* + *trnL-F* data matrix produced a strict consensus tree with a slightly less resolved topology compared to the strict consensus tree based on molecular data only (Muasya et al. 2008). The impact on support values by adding the palynological

characters was negligible, so we decided not to show these results here and to use the molecular tree to optimize the pollen characters.

Results

The 84 species studied show considerable variation in both pollen and orbicule morphology. The palynological characters of the species studied are described below and summarized in Table 3 (pollen characteristics) and Table 4 (orbicule characteristics).

Polarity and symmetry

Pyriform, subprolate, prolate or perprolate pollen grains are heteropolar in shape and apertural system. These pollen grains have a broad distal pole and a narrower proximal one (e.g. Fig. 1a). At the distal pole an aperture is present whereas no aperture is observed at the proximal pole (e.g. Fig. 1B). Spheroidal pollen grains are isopolar in shape (Fig. 1c–g) but heteropolar in apertural system, with only a single aperture at one of the poles (e.g. Fig. 1c). The heteropolarity in pollen shape and apertural system of the pollen grains studied, is linked to the typical microsporogenesis of the pseudomonads and the arrangement of the pollen grains during development in the anther locules (Fig. 1v).

Pollen size

Pollen size varies significantly within many species investigated (Table 3). The equatorial diameter (*E*) ranges from 12.9 μm in *Kyllinga* sp. (Fig. 1d) to 30.72 μm in *Cladium mariscus* (Fig. 1a) and the polar axis (*P*) ranges from 12.89 μm in *Lipocarpha nana* (Fig. 1e) to 52.69 μm in *Chrysitrix dodii* (Fig. 1b). The majority of the species investigated have small to medium sized pollen with an equatorial diameter in the 15–30 μm range and a polar axis in the 20–40 μm range (Table 3).

Pollen shape

Pollen shape in equatorial view (*P/E*) ranges from suboblate (*Capeobolus brevicaulis*; Fig. 1f) to perprolate (*Ficinia zeyherii*; Fig. 1j) but is in general spheroidal (Fig. 1c–g) or (sub)prolate (Fig. 1h–i). Spheroidal pollen grains are thought to be dominant in, and restricted to, subfamily Mapanioideae. However, we also observed spheroidal pollen in 21 Cyperoideae species belonging to 16 genera (Table 3). On the other hand, (sub)prolate pollen grains are found in three mapanoid species (Figs. 1b, 2j–k). Pollen shape based on the *P/E* ratio is inadequate to describe the pear-shaped pollen grains in some genera (*Becquerelia*,

Table 3 Summary of major pollen morphological characters for all species studied

Species	P ± SD (µm)	E ± SD (µm)	P/E	Shape	Apertures # + Shape	Ornamentation		Distinctness	Sexine Ornamentation	Figure
						Ornamentation	Distinctness			
Subfamily Cyperoidae										
Abildgaardieae										
<i>Arthrostylis aplylla</i>	21.46 ± 1.38	19.26 ± 2.10	1.12 ± 0.14	Spheroidal	1 distal ulcer, 6 lateral pori	Granules, perf, me	PO	PO	Microechinate, perf	1p-q, 2n
<i>Bulbostylis hispidula</i>	31.14 ± 0.76	25.38 ± 0.62	1.27 ± 0.12	Subprolate	1 distal ulcer, 5 lateral colpi/pori	Fossulate/neg. microreticulate	PO	PO	Granulate, perf	1h
<i>Fimbristylis complanata</i>	17.64 ± 3.10	15.15 ± 1.00	1.16 ± 0.12	Subprolate	No apertures observed	-	-	-	Neg. microreticulate, granulate	2a
<i>Fimbristylis xyridis</i>	25.95	19.74	1.32	Subprolate	No apertures observed	-	-	-	Neg. microreticulate, granulate	2b
Bisboeckeleereae										
<i>Becquerella cymosa*</i>	26.22 ± 0.21	18.70 ± 0.38	1.43 ± 0.01	Prolate	1 distal ulcer, 3 lateral pori	Neg. microreticulate, mgr, perf	PO	PO	Granulate, perf	3k
Cariceae										
<i>Carex capitata</i>	28.94 ± 0.72	25.43 ± 1.09	1.15 ± 0.16	Subprolate	1 distal ulcer, 4 lateral pori	Fossulate	PO	PO	Granulate, perf	
<i>Carex elata</i>	35.93 ± 0.63	22.58 ± 1.09	1.59 ± 0.05	Prolate	1 distal ulcer, 4 lateral pori	Neg. microreticulate	PO	PO	Fossulate granulate, perf	1i
<i>Carex monostachya*</i>	Collapsed	-	-	-	4 ap zones (pori) observed	Neg. microreticulate, mgr, perf	?	?	Granulate, perf	-
<i>Kobresia myosuroides</i>	27.76 ± 1.53	27.01 ± 0.82	1.03 ± 0.05	Spheroidal	1 distal ulcer, 4 lateral ap zones (pori)	Granules, mgr, perf	PO	PO	Granulate, perf	3b
<i>Schoenoxiphium lehmannii</i>	Collapsed	-	-	-	-	-	-	-	Granulate, perf	-
<i>Schoenoxiphium sparteum</i>	-	20.51	-	-	1 distal ulcer, 4 lateral ap zones (pori)	Neg. microreticulate mgr, perf	?	?	Neg.-microreticulaat/ fossulate, mgr, perf	-
<i>Uncinia rubra</i>	Collapsed	-	-	-	-	Fossulate,	PO	PO	Granulate, perf	-
Cryptangieae										
<i>Everardia montana*</i>	Collapsed	-	-	-	1 distal ulcer, 4 lateral pori	Neg. microreticulate, mgr, perf	PO	PO	Granulate, perf	-
<i>Exochogyne amazonica*</i>	23.77 ± 0.96	23.62 ± 0.41	1.03 ± 0.06	Spheroidal	Polyporate ?	Fossulate	PO	PO	Metareticulate ?, granulate, perf	3i
<i>Lagenocarpus rigidus</i> subsp. <i>rigidus</i> *	-	-	-	-	3 pori observed	Granules, mgr, perf	PO	PO	Granulate, perf	-

Table 3 continued

Species	P ± SD (µm)	E ± SD (µm)	P/E	Shape	Apertures # + Shape	Ornamentation		Distinctness	Sexine Ornamentation	Figure
						Ornamentation	Ornamentation			
Cyperaceae										
<i>Courtoisina assimilis</i>	Collapsed	–	–	–	1 distal ulcer, 4 lateral pori/colpi ?	Neg. microreticulate, perf, mgr	PO	Fossulate/granulate, perf	–	
<i>Cyperus alternifolius</i> **	22.17 ± 1.63	20.03 ± 1.54	1.11 ± 0.00	Spheroidal	1 distal ulcer, 4 lateral pori	Granules	O	Granulate, perf	–	
<i>Cyperus articulatus</i>	22.71 ± 1.66	16.07 ± 1.55	1.42 ± 0.14	Prolate	1 distal ulcer, 4–5 lateral pori/colpi	Granules, perf	O	Granulate, perf	–	
<i>Cyperus dubius</i>	18.51 ± 1.30	18.68 ± 0.54	0.99 ± 0.09	Spheroidal	1 distal ulcer 4–6 lateral pori/colpi	Granules, me	O	Psilate, rugulate/ granulate	2s, 3f	
<i>Cyperus hemisphaericus</i> *	22.57 ± 2.39	17.94 ± 1.74	1.27 ± 0.24	Prolate	1 distal ulcer, 5 lateral colpi	Granules, me	O	Granulate/ microechinate, perf	–	
<i>Cyperus laevigatus</i>	27.04 ± 0.61	20.77 ± 1.33	1.30 ± 0.05	Subprolate	1 distal ulcer 4–5 lateral colpi	Granules, me	O	Granulate, perf (f)	–	
<i>Cyperus rotundus</i>	28.09 ± 1.76	21.49 ± 1.01	1.31 ± 0.09	Subprolate	1 distal ulcer	Fossulate, mgr	O	Granulate, perf (f)	1s, 2u	
<i>Ficinia brevifolia</i>	27.11 ± 3	17.35 ± 0.58	1.57 ± 0.21	Prolate	4–5 lateral pori/colpi	Granules, perf	O	Granulate, perf	2r	
<i>Ficinia bulbosa</i> *	30.57 ± 2.85	21.89 ± 1.25	1.40 ± 0.10	Prolate	1 distal ulcer, 5 lateral pori	Granules, perf	O	Granulate, perf	–	
<i>Ficinia capitellum</i>	Collapsed	–	–	–	1 distal ulcer, 4–5 lateral colpi	Doughnut –shaped granules, perf	O	Granulate, perf	–	
<i>Ficinia dunensis</i>	Collapsed	–	–	–	1 distal ulcer, 4 lateral colpi	Granules	O	Granulate, perf	–	
<i>Ficinia gracilis</i>	26.41	20.68	1.28	Subprolate	1 distal ulcer, 3–4 lateral pori	Granules	PO	Granulate, perf	–	
<i>Ficinia minutiflora</i>	19.37 ± 0.28	18.40 ± 0.66	1.05 ± 0.03	Spheroidal	1 distal ulcer, 4 lateral pori	Doughnut-shaped granules	O	Microreticulate, mgr	3g	
<i>Ficinia polystachya</i>	41.93 ± 0.48	22.66 ± 1.37	1.85 ± 0.10	Prolate	1 distal ulcer, 6 lateral colpi	Doughnut-shaped granules	O	Granulate, perf	–	
<i>Ficinia radiata</i>	23.19 ± 0.70	22.29 ± 1.81	1.04 ± 0.05	Spheroidal	1 distal ulcer, 5 lateral pori	Granules, perf, mgr	PO	Granulate, perf	–	
<i>Ficinia tristachya</i>	Collapsed	–	–	–	–	Granules, perf, mgr	O	Granulate, perf	–	
<i>Ficinia zeyheri</i>	33.81 ± 1.90	18.27 ± 1.13	2.3 ± 0.10	Perprolate	1 distal ulcer, 6 lateral colpi	Doughnut-shaped granules	PO	Granulate, perf	1j	
<i>Helminthia membranacea</i>	33.66 ± 1.54	28.08 ± 0.61	1.62 ± 0.11	Prolate	1 distal ulcer, 6 lateral pori	Granules, me, perf	O	Fossulate/granulate, perf	2q	
<i>Isolepis antarctica</i>	23.83 ± 0.85	17.91 ± 0.72	1.33 ± 0.08	(Sub)prolate	1 distal sulcus, 5–6 lateral pori/colpi	Granules, mgr, perf	O	Granulate, perf	2t	

Table 3 continued

Species	P ± SD (µm)	E ± SD (µm)	P/E	Shape	Apertures # + Shape	Sexine Ornementation		Figure	
						Ornementation	Distinctness		
<i>Isolepis digitata</i>	21.47	17.40	1.23	Subprolate	1 distal ulcus, 4 lateral pori	Granules, perf	O	Granulate, perf	1v
<i>Isolepis prolifera</i>	25.79	21.77	1.18	Subprolate	1 distal ulcus, 4–5 lateral pori	Granules, mgr, perf	O	Granulate, perf	–
<i>Isolepis setacea</i>	27.81	22.64	1.23	Subprolate	1 distal ap zone, 5 lateral ap zones (pori)	Fossulate, mgr, perf	PO	Fossulate/granulate, perf (f)	1l
<i>Kyllinga eximia</i>	21.00 ± 1.99	20.44 ± 1.44	1.03 ± 0.06	Spheroidal	5 pori/colpi	Granules, me	O	Microechinate, perf	1g
<i>Kyllinga flava</i>	21.82 ± 0.82	16.39 ± 0.71	1.33 ± 0.04	(Sub)prolate	1 distal (s)ulcus, 4–5 lateral pori/colpi	Granules, me	O	Microechinate, perf (f)	1m, r, 3a
<i>Kyllinga polyphylla</i> **	22.44 ± 0.29	19.19 ± 0.07	1.17 ± 0.02	Subprolate	1 distal ulcus, 4 lateral colpi	Granules, me	O	Microechinate	–
<i>Kyllinga sp.</i>	17.34 ± 1.28	12.93 ± 0.39	1.41 ± 0.15	Prolate	1 distal ulcus, 4 lateral colpi	Granules, me	O	Microechinate, perf	1d
<i>Kyllingiella polyphylla</i>	17.47 ± 1.51	15.96 ± 1.83	1.10 ± 0.06	Spheroidal	1 distal ulcus, 5–6–7 pori	Granules, me, perf	O	Microechinate, perf	–
<i>Lipocarpha nana</i>	12.89 ± 0.47	13.24 ± 3.48	0.98 ± 0.13	Spheroidal	1 distal ulcus, 4 lateral pori	Granules, me, perf	O	Microechinate, perf	1e, 2m
<i>Lipocarpha rehmannii</i>	17.19 ± 1.18	25.06 ± .95	1.04 ± 0.14	Spheroidal	1 distal ulcus, 4–5 lateral pori	Granules, me, perf	O	Microechinate, perf	–
<i>Oxycaryum cubense</i>	23.79 ± 2.51	20.73 ± 2.20	1.15 ± 0.10	Subprolate	1 distal ulcus, 5 lateral colpi	Granules, mgr, perf	PO	Granulate, perf	–
<i>Pycreus flavescens</i> **	26.58	21.83	1.22	Subprolate	1 distal ulcus, 5 lateral colpi	Granules, mgr (f)	O	Granulate, perf	–
<i>Pycreus mundtii</i>	18.27	16.79	1.09	Spheroidal	1 distal ulcus, 5 lateral colpi	Granules, mgr	O	Fossulate/granulate, perf	–
<i>Pycreus sanguinolentus</i>	20.07 ± 1.83	19.90 ± 1.68	1.02 ± 0.18	Spheroidal	1 distal sulcus, 5 lateral pori	Granules	O	Granulate, perf	4b
<i>Scirpoides holoschoenus</i> **	Collapsed	–	–	–	1 distal ulcus, min. 1 lateral porus	Fossulate, mgr, perf	PO	Granulate, perf	–
Dulichieae									
<i>Dulichium arundinaceum</i>	26.15 ± 3.47	20.17 ± 0.46	1.29 ± 0.14	Subprolate	1 distal sulcus, 4 lateral ap zones ?	Neg. microreticulate, perf, mgr	PO	Fossulate, granulate, perf (f)	–
Eleocharideae									
<i>Eleocharis acutangula</i>	35.25 ± 3.26	23.62 ± 3.80	1.52 ± 0.27	Prolate	No apertures observed	–	–	Neg. microreticulate, granulate, perf	2p, 3d
<i>Eleocharis palustris</i>	37.54 ± 3.80	28.40 ± 0.59	1.32 ± 0.11	Subprolate	1 distal ulcus, 5 lateral colpi	Fossulate, perf, mgr	PO	Granulate, perf	2o

Table 3 continued

Species	P ± SD (µm)	E ± SD (µm)	P/E	Shape	Apertures # + Shape	Ornamentation		Distinctness	Sexine Ornamentation	Figure
						Ornamentation	Distinctness			
Fuireneae										
<i>Fuirena abnormalis</i>	30.52 ± 3.79	25.49 ± 3.07	1.21 ± 0.21	Subprolate	1 distal ulcus, 4 lateral pori	Neg. microreticulate, mgr, perf	PO	Granulate, perf	–	
<i>Fuirena leptostachya</i>	23.64	20.96	1.13	Spheroidal	1 distal ulcus, 4 lateral pori	Fossulate, mgr, perf	PO	Fossulate/granulate, perf	3c	
<i>Pseudoschoenus inanis</i>	30.85 ± 5.06	23.70 ± 3.87	1.33 ± 0.33	(Sub)prolate	1 distal ulcus, 5 lateral pori/colpi	Neg. microreticulate, granules, mgr, perf	PO	Granulate, perf	–	
<i>Schoenoplectus senegalensis</i>	29.22 ± 5.43	26.99 ± 3.75	1.08 ± 0.06	Spheroidal	1 distal ulcus; 4 lateral pori	Fossulate, mgr, perf	PO	Granulate, perf	1n, o	
Rhynchosporaeae										
<i>Rhynchospora sp.</i>	22.52 ± 2.68	15.56 ± 0.68	1.45 ± 0.20	Prolate	No apertures observed	–	–	Neg. microreticulate	2c	
Schoeneae										
<i>Baumea rubiginosa</i>	21.50 ± 0.95	22.35 ± 0.88	0.96 ± 0.04	Spheroidal	Polyporate	Granules, perf, mgr	PO	Fossulate/granulate, perf	2d	
<i>Capeobolus brevicaulis</i>	20.75	23.69	0.88	Suboblate/ spheroidal	Polyporate	Granules, perf, mgr	PO	Fossulate/granulate, perf	1f	
<i>Causitis flexuosa*</i>	Collapsed	–	–	–	–	–	–	Granulate, perf	–	
<i>Causitis recurvata*</i>	Collapsed	–	–	–	1 distal ulcus, 4 lateral ap zones observed	Neg. microreticulate, mgr, perf	PO	Neg. microreticulate, mgr, perf	–	
<i>Cladium mariscus</i>	42.00 ± 4.62	30.72 ± 2.15	1.38 ± 0.10	Prolate	0–1 distal (s)ulcus, 0–4 lateral pori/colpi	Fossulate, perf	PO	Granulate, perf	1a, 2f, g	
<i>Costularia humbertii*</i>	33.11 ± 0.38	26.35 ± 1.14	1.26 ± 0.04	Subprolate	1 distal ulcus, min. 5 lateral colpi	Fossulate, mgr, perf	O	Granulate, mgr	–	
<i>Gahnia lanigera*</i>	26.83 ± 3.84	25.03 ± 1.42	1.08 ± 0.21	Spheroidal	Polyaperturate	Granules, mgr, perf	PO	Fossulate/granulate, perf	–	
<i>Machaerina flexuosa*</i>	–	23.29	–	–	3 pori observed	Granules, perf	PO	Granulate, perf	–	
<i>Schoenus nigricans*</i>	Collapsed	–	–	–	1 distal ulcus, 4 lateral colpi	Neg. microreticulate, mgr, perf	PO	Granulate, perf	1t	
<i>Tetraria compar*</i>	45.85 ± 5.96	25.90 ± 0.91	1.77 ± 0.21	Prolate	1 distal ulcus,	Fossulate, mgr, perf	PO	Granulate, perf	–	
<i>Trianoptiles solitaria</i>	21.6	20.39	1.06	Spheroidal	1 distal ulcus, min. 3 lateral pori/colpi	Fossulate, mgr, perf	PO	Granulate, perf	–	
Scirpeae										
<i>Amphiscirpus nevadensis*</i>	34.89 ± 1.92	24.96 ± 2.01	1.40 ± 0.08	Prolate	1 distal ulcus, 4 lateral ap zones (pori)	Fossulate, mgr, perf	PO	Granulate, perf	–	
<i>Eriophorum latifolium</i>	28.05 ± 0.44	19.99 ± 1.97	1.41 ± 0.14	Prolate	1 distal ulcus, 4 lateral ap zones (colpi)	Fossulate, mgr, perf	PO	Granulate, perf	2i	

Table 3 continued

Species	P ± SD (µm)	E ± SD (µm)	P/E	Shape	Apertures # + Shape	Sexine		Figure	
						Ornamentation	Distinctness		
<i>Phylloscirpus acaulis</i> *	Collapsed	-	-	-	1 distal ulcus, 4 lateral ap zones (pori)	Fossulate, mgr, perf	PO	Neg.-microreticulate/granulate, perf	-
<i>Scirpus sylvaticus</i> *	24.80 ± 1.94	21.72 ± 1.74	1.15 ± 0.09	Subprolate	1 distal ulcus, 4 lateral ap zones (pori)	Fossulate, mgr, perf	PO	Neg.-microreticulate/fossulate, mgr, perf	-
<i>Trichophorum alpinum</i> *	Collapsed	-	-	-	1 distal (s)ulcus, 4 pori	Granules, mgr, perf	PO	Granulate, perf	-
Sclerieae									
<i>Scleria rugosa</i>	24.32	21.26	1.14	Spheroidal/Subprolate	1 distal ulcus, 4 poorly visible apertures	Fossulate, mgr, perf	PO	Granulate, perf	2e
<i>Scleria terrestris</i> **	28.35 ± 0.40	25.70 ± 1.44	1.13 ± 0.11	Spheroidal	1 distal ulcus, 3-4 lateral ap zones (pori)	Neg. microreticulate, perf (f)	PO	Granulate, perf	-
Trilepideae									
<i>Afrotrilepis pilosa</i> *	Collapsed	-	-	-	-	-	-	Granulate, perf	-
<i>Coleochloa setifera</i>	25.64 ± 1.63	21.24 ± 1.41	1.22 ± 0.15	Subprolate	1 distal ulcus	Granules, me, perf	O	Fossulate/rugulate, perf, mgr	2h, 3e
Subfamily Mapanioideae									
Chrysitricheae									
<i>Chorizandra cymbaria</i> *	Few material	-	-	-	1 distal ulcus, no lateral apertures observed	Granules, me, perf	PO	Rugulate, perf, mgr, me	-
<i>Chorizandra enodis</i> *	46.12 ± 5.46	28.79 ± 2.80	1.60 ± 0.14	Prolate	1 distal ulcus, 4 lateral pori	Fossulate/granules, mgr, perf	PO	Granulate, perf	2j
<i>Chrysitrix dodii</i>	52.69 ± 1.05	29.08 ± 1.59	1.82 ± 0.13	Prolate	1 distal ulcus	Granules, mgr	O	Granulate, perf	1b
<i>Lepironia articulata</i> *	28.29 ± 2.86	23.18 ± 0.96	1.23 ± 0.17	Subprolate	1 distal ulcus, 0-4 lateral pori observed	Granules	PO	Granulate, perf	2k
Hypolytreae									
<i>Diplasia karatfolia</i> *	18.08 ± 1.19	18.01 ± 2.09	1.01 ± 0.14	Spheroidal	1 distal ulcus	-	-	Microreticulate, mgr	1k
<i>Hypolytrum jennmanii</i> subsp. <i>jennmanii</i> *	17.35 ± 1.92	15.85 ± 1.48	1.10 ± 0.14	Spheroidal	1 distal ulcus	Granules	-	Microreticulate	3h
<i>Mapania cuatrecasarii</i> *	18.56 ± 1.13	18.32 ± 0.69	1.01 ± 0.06	Spheroidal	1 distal ulcus	Granules	O	Fossulate, perforate	2i
<i>Mapania linderi</i> *	16.02 ± 1.35	15.26 ± 0.99	1.05 ± 0.11	Spheroidal	1 distal ulcus	Granules, me	-	Microreticulate/microechinate	1c, 3j

Species are listed alphabetically; fixed material (without asterisk), herbarium material is indicated with an asterisk and living material with double asterisks. # aperture number, ap apertural, E equatorial axis, f few, mgr microgranules, me microechinae, neg. negative, O operculum, P polar axis, perf perforations, PO pontopericulum

Table 4 Summary of major morphological orbicule characters for all species studied

Species	Diameter (μm) \pm SD	Shape	Ornamentation	Remark	Figure
Subfamily Cyperoideae					
Abildgaardieae					
<i>Arthrostylis aphylla</i>	0.59 \pm 0.11	Ang	Sm	–	4g
<i>Bulbostylis hispidula</i>	0.40 \pm 0.07	Irr	Mgr	–	–
<i>Fimbristylis complanata</i>	0.29 \pm 0.05	Irr	Mgr	–	–
<i>Fimbristylis xyridis</i>	0.56 \pm 0.07	Irr	Me	–	–
Bisboeckelereae					
<i>Becquerelia cymosa</i> *	0.43 \pm 0.06	Sph	Mgr	–	–
Cariceae					
<i>Carex capitata</i>	0.45 \pm 0.09	Irr	Mgr	–	–
<i>Carex elata</i>	0.56 \pm 0.09	Sph	Sm	–	–
<i>Carex monostachya</i> *	0.47 \pm 0.10	Sph	Mgr	–	–
<i>Kobresia myosuroides</i>	0.41 \pm 0.09	Ang	Mgr	–	–
<i>Schoenoxiphium lehmannii</i>	0.30 \pm 0.06	Irr	Mgr	–	–
<i>Schoenoxiphium sparteum</i>	0.35 \pm 0.07	Sph	Mgr	Em, aggr	–
<i>Uncinia rubra</i>	0.39 \pm 0.10	Irr	Mgr	Aggr	–
Cryptangieae					
<i>Exochogyne amazonica</i> *	0.46 \pm 0.07	Irr	Mgr, me	–	–
<i>Lagenocarpus rigidus</i> subsp. <i>rigidus</i> *	0.60 \pm 0.13	Sph	Me	–	–
Cypereae					
<i>Courtoisina assimilis</i>	0.41 \pm 0.08	Irr	Me	Aggr	–
<i>Cyperus alternifolius</i> **	0.52 \pm 0.10	Sph	Mgr (f)	–	–
<i>Cyperus articulatus</i>	0.44 \pm 0.08	Sph	Me, mgr	–	–
<i>Cyperus dubius</i>	0.62 \pm 0.12	Irr	Mgr	–	–
<i>Cyperus haspan</i>	0.86 \pm 0.18	Sph	Me	Em	–
<i>Cyperus hemispaericus</i> *	0.46 \pm 0.09	Ang	Mgr, perf (f)	–	–
<i>Cyperus laevigatus</i>	0.72 \pm 0.15	Sph	Me	–	–
<i>Cyperus rotundus</i>	0.73 \pm 0.16	Irr	Me	Aggr	4a
<i>Ficinia brevifolia</i>	0.58 \pm 0.09	Sph	–	–	–
<i>Ficinia bulbosa</i> *	0.56 \pm 0.10	Irr	Mgr	Thr	–
<i>Ficinia capitellum</i>	0.44 \pm 0.07	Sph	Sm	–	–
<i>Ficinia dunensis</i>	0.59 \pm 0.11	Sph	Mgr	–	–
<i>Ficinia gracilis</i>	0.69 \pm 0.10	Sph	Sm	Aggr	–
<i>Ficinia minutiflora</i>	0.74 \pm 0.14	Sph	Mgr	–	–
<i>Ficinia polystachya</i>	0.53 \pm 0.07	Ang	Mgr	–	–
<i>Ficinia radiata</i>	0.64 \pm 0.14	Sph	Sm, perf (f)	Aggr	4c
<i>Ficinia tristachya</i>	0.32 \pm 0.06	Irr	Mgr	–	–
<i>Ficinia zeyheri</i>	0.66 \pm 0.10	Sph	Mgr (f)	Aggr	–
<i>Hellmuthia membranacea</i>	0.53 \pm 0.08	Irr	Mgr	–	–
<i>Isolepis antarctica</i>	0.42 \pm 0.09	Irr	Me	–	–
<i>Isolepis digitata</i>	0.46 \pm 0.08	Sph	Mgr	–	–
<i>Isolepis prolifera</i>	0.47 \pm 0.07	Irr	Mgr	–	–
<i>Isolepis setaceae</i>	0.46 \pm 0.09	Irr	Me	–	–
<i>Kyllinga eximia</i>	1.28 \pm 0.25	Irr	Me	Aggr	–
<i>Kyllinga flava</i>	0.65 \pm 0.13	Irr	Me	Spheres	4l
<i>Kyllinga polyphylla</i> **	0.69 \pm 0.13	Irr	Me	Aggr	–
<i>Kyllinga sp.</i>	0.76 \pm 0.15	Irr	Me	–	4i
<i>Kyllingiella polyphylla</i>	0.75 \pm 0.15	Irr	Me	–	–

Table 4 continued

Species	Diameter (μm) \pm SD	Shape	Ornamentation	Remark	Figure
<i>Lipocarpa nana</i>	0.64 \pm 0.12	Irr	Me	–	–
<i>Lipocarpa rehmannii</i>	0.58 \pm 0.09	Irr	Me	–	–
<i>Oxycarium cubense</i>	0.37 \pm 0.08	Irr	Me	–	–
<i>Pycreus flavescens</i> **	0.85 \pm 0.15	Irr	Me	–	–
<i>Pycreus mundtii</i>	0.20 \pm 0.09	Irr	Mgr, me	–	4f
<i>Pycreus sanguinolentus</i>	0.53 \pm 0.10	Irr	Me	–	4b
<i>Scirpoides holoschoenus</i> **	0.45 \pm 0.08	Irr	Mgr	Aggr	–
Dulichieae					
<i>Dulichium arundinaceum</i>	–	Irr	–	Aggr	–
Eleocharideae					
<i>Eleocharis acutangula</i>	0.36 \pm 0.07	Irr	–	Ret aggr	4d, e
<i>Eleocharis palustris</i>	0.24 \pm 0.04	Irr	Mgr, perf	Aggr	–
Fuireneae					
<i>Fuirena abnormalis</i>	0.44 \pm 0.05	Irr	Mgr	–	–
<i>Fuirena leptostachya</i>	0.33 \pm 0.08	Irr	Mgr	Aggr	–
<i>Pseudoschoenus inanis</i>	0.49 \pm 0.09	Irr	Mgr	–	–
<i>Schoenoplectus senegalensis</i>	0.46 \pm 0.08	Ang	Sm	–	–
Rhynchosporae					
<i>Rhynchospora sp.</i>	0.37 \pm 0.09	Irr	Mgr	–	–
Schoeneae					
<i>Baumea rubiginosa</i>	0.97 \pm 0.21	Sph	Sm	–	–
<i>Capeobolus brevicaulis</i>	0.62 \pm 0.12	Irr	Mgr	Em	–
<i>Caustis flexuosa</i> *	0.57 \pm 0.12	Irr	Mgr	Aggr	–
<i>Caustis recurvata</i> *	0.53 \pm 0.10	Irr	Me	Thr	–
<i>Cladium mariscus</i>	0.58 \pm 0.10	Irr	Mgr	–	–
<i>Costularia humbertii</i> *	0.47 \pm 0.07	Irr	Mgr	Thr	–
<i>Gahnia lanigera</i> *	0.50 \pm 0.07	Sph	Mgr, me	–	–
<i>Machaerina flexuosa</i> *	0.49 \pm 0.08	Sph	Mgr (f)	–	–
<i>Schoenus nigricans</i> *	0.47 \pm 0.09	Irr	Mgr	–	–
<i>Tetaria compar</i> *	0.40 \pm 0.06	Sph	Sm	–	–
<i>Trianoptiles solitaria</i>	–	Irr	Sm	Aggr	–
Scirpeae					
<i>Amphiscirpus nevadensis</i> *	0.48 \pm 0.07	Irr	Mgr	–	–
<i>Eriophorum latifolium</i>	0.51 \pm 0.07	Irr	Mgr	–	–
<i>Phylloscirpus acaulis</i> *	0.53 \pm 0.11	Irr	Mgr	–	–
<i>Scirpus sylvaticus</i> *	0.43 \pm 0.07	Irr	Mgr	Aggr	–
<i>Trichophorum alpinum</i> *	0.43 \pm 0.09	Irr	Mgr	Aggr	–
Sclerieae					
<i>Scleria rugosa</i>	0.42 \pm 0.06	Irr	Mgr	Em	–
<i>Scleria terrestris</i> **	0.51 \pm 0.02	Irr	Sm	Aggr	–
Trilepideae					
<i>Afrotrilepis pilosa</i> *	0.51 \pm 0.11	Irr	Mgr	Thr	–
<i>Coleochloa setifera</i>	0.41 \pm 0.07	Irr	Mgr, str	–	4k
Subfamily Mapanioideae					
Chrysitricheae					
<i>Chorizandra cymbaria</i> *	0.49 \pm 0.09	Irr	Mgr	Thr	–
<i>Chorizandra enodis</i> *	0.50 \pm 0.08	Sph	Mgr (f), perf (f)	Conn	–
<i>Chrysitrix dodii</i>	0.70 \pm 0.12	Irr	Mgr	–	–

Table 4 continued

Species	Diameter (μm) \pm SD	Shape	Ornamentation	Remark	Figure
<i>Lepironia articulata</i> *	0.42 \pm 0.07	Irr	Mgr	–	–
Hypolytreae					
<i>Diplasia karatifolia</i> *	0.51 \pm 0.11	Sph	Mgr	–	4h
<i>Hypolytrum jenmanii</i> subsp. <i>jenmanii</i> *	0.25 \pm 0.10	Sph	Sm	Em	–
<i>Mapania cuatrecasasii</i> *	0.82 \pm 0.19	Do	Sm	–	4j
<i>Mapania linderi</i> *	No orbicules observed	–	–	–	–

Aggr Aggregates, *ang* angular, *conn* connected, *do* doughnut-shaped, *em* embedded, *f* few, *irr* irregular, *mgr* microgranules, *me* microechinae, *perf* perforations, *sm* smooth, *sph* spherical, *str* striae, *thr* threads

Species are listed alphabetically; fixed material (without asterisk), herbarium material is indicated with an asterisk and living material with double asterisks

Carex, *Eleocharis*, *Fuirena*, *Hellmuthia*, *Isolepis*, *Lepironia* and *Kyllinga*; Figs. 1i, r, 2k, o–q). We use the term pyriform to describe this characteristic pollen shape.

The shape in polar view (amb), is circular (Fig. 1k), angular (Fig. 1l) or irregular (Fig. 1m).

The peripheral arrangement of the pollen grains in the locules also has a major impact on the shape of mature pollen grains (Fig. 1v). Pollen is closely packed in a uniseriate layer which often results in more or less angular grains.

Apertures

The number of apertures ranges from zero (*Eleocharis acutangula*, *Fimbristylis complanata*, *F. xyridis* and *Rhynchospora* sp.; Fig. 2a–c, p) to more than eight (*Baumea rubiginosa*; Fig. 2d). In cases where the sexine ornamentation is not differentiated in the apertural region, the number of apertures is difficult to determine even on SEM observations (e.g. *Scleria rugosa*; Fig. 2e). Therefore, we judged it more appropriate to use the term ‘apertural zones’. Pentaaperturate pollen grains are most common in Cyperaceae (Table 3). These pollen grains usually have one distal aperture (often an ulcer) (e.g. *Eriophorum latiolium*; Fig. 2l) and four lateral apertures or apertural zones (e.g. *Lipocarpa nana*; Fig. 2m). In the species studied, the number of lateral apertures varies from three (e.g. *Becquerelia cymosa*; Table 3) to six (*Arthrostylis aphylla*; Fig. 2n), even within a genus, (e.g. *Eleocharis*; Fig. 2o–p). In *Cladium mariscus*, we observed some pollen grains lacking visible apertures and others with apertures within the same specimen (Fig. 2f–g). Monoaperturate pollen grains were found in only one cyperoid genus *Coleochloa* (Fig. 2h) and in four of the six mapanioid genera studied, *Chrysitrix* (Fig. 1b), *Diplasia* (Fig. 1k), *Hypolytrum*, and *Mapania* (Fig. 1c). We observed lateral apertures in the two other mapanioid genera *Chorizandra* (Fig. 2j) and *Lepironia* (Fig. 2k).

In Cyperaceae apertures are pori (e.g. *Baumea rubiginosa*; Fig. 2d) or colpi (e.g. *Kyllinga* sp.; Fig. 1d) and the

shape of apertures can vary within the same species (e.g. *Isolepis antarctica*; Fig. 2t). In *Cyperus rotundus* we observed irregularly shaped apertures (Fig. 2u). In *Hellmuthia membranacea* (Fig. 2q) and *Ficinia brevifolia* (Fig. 2r), the pollen grains display depressions above and below the lateral apertures. Pollen grains of *Carex elata* (Fig. 1i) and *Coleochloa setifera* (Fig. 2h) possess an elevated thickened ring around the aperture (aspis). In a few species we observed papillae (e.g. *Cyperus dubius*; Fig. 2s). Our observations demonstrate that number and shape of apertures can vary within a genus and even within a single species (heteromorphic pollen). The plasticity in the development of apertural zones in Cyperaceae pollen considerably reduces the systematic value of characters such as the number and position of apertures.

Sexine ornamentation

We observed seven different sexine ornamentation patterns with occasionally intermediate forms, including microechinate (e.g. *Kyllinga flava*; Fig. 3a), granulate–perforate (e.g. *Kobresia myosuroides*; Fig. 3b), fossulate (e.g. *Fuirena leptostachya*; Fig. 3c), negative microreticulate (e.g. *Eleocharis acutangula*; Fig. 3d), fossulate–rugulate (*Coleochloa setifera*; Fig. 3e), psilate–rugulate (*Cyperus dubius*; Fig. 3f) and microreticulate (e.g. *Ficinia minutiflora* and *Hypolytrum jenmanii* subsp. *jenmanii*; Fig. 3g–h). However, the majority of species are granulate–perforate. *Exochogyne amazonica* shows a rather exceptional sexine with narrow, elevated ridges (Fig. 3i) that may suggest a metareticulum. Apertures are always covered with an operculum (e.g. *Ficinia minutiflora* and *Kyllinga flava*; Figs. 1m, 3a, g) or a pontoperculum (e.g. *Bulbostylis hispidula* and *Becquerelia cymosa*; Figs. 1h, 3k), except in *Diplasia karatifolia* (Fig. 1k). These opercula or pontopercula consist of several sporopollenin elements, which also show variation in ornamentation patterns: elements with microgranules and microechinae (Fig. 3a, b), elements with perforations

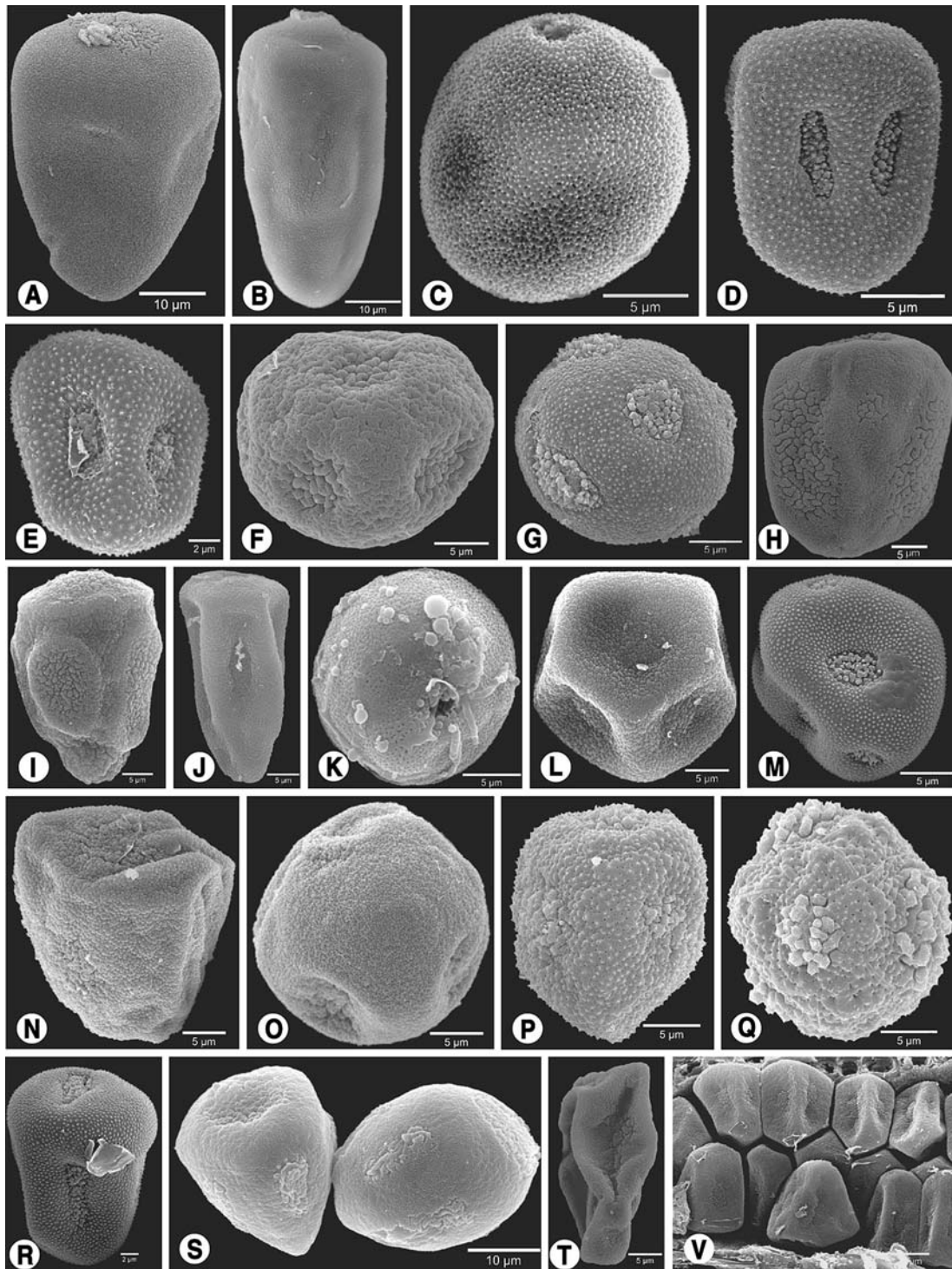
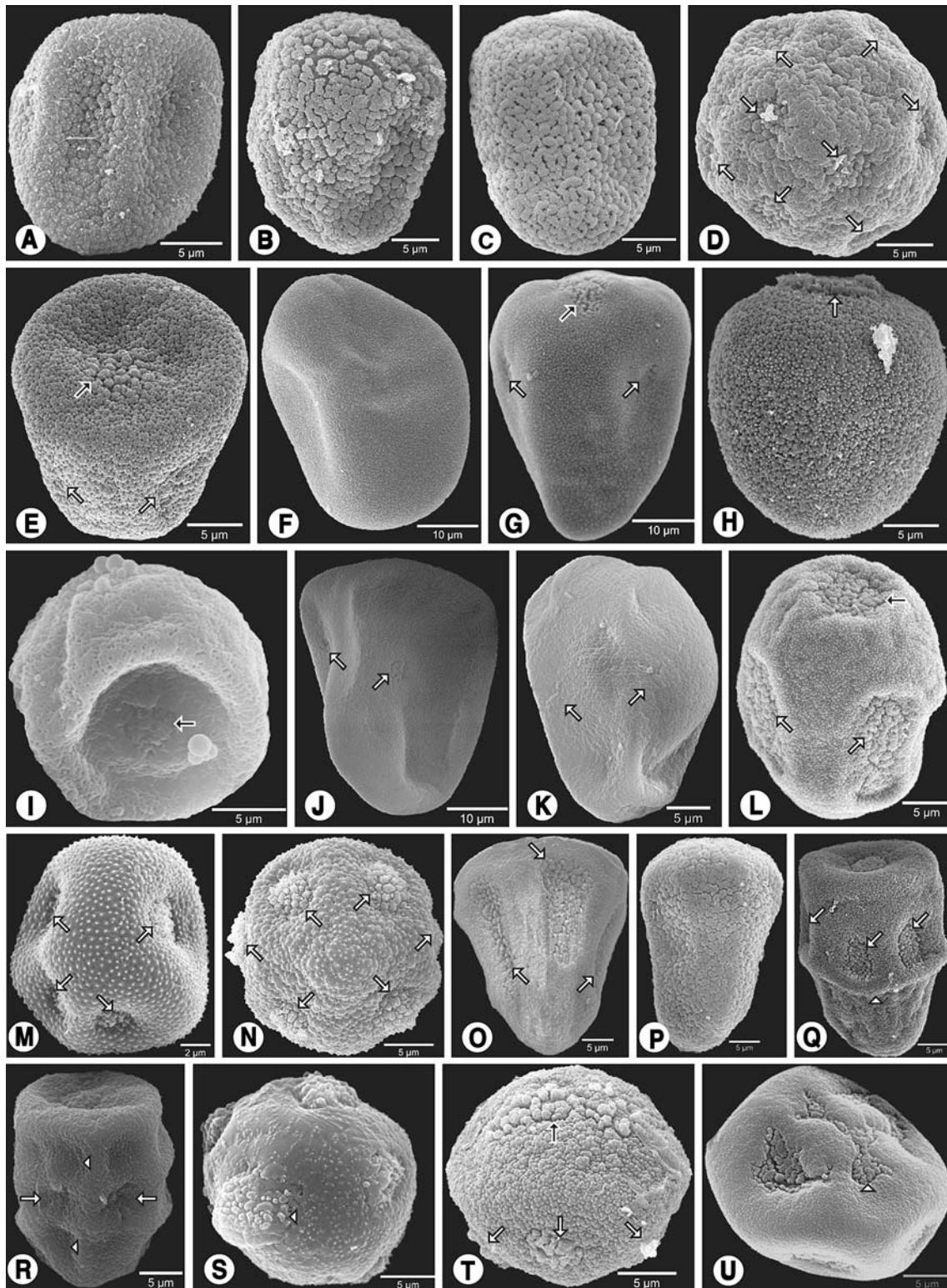


Fig. 1 Shape in equatorial or polar view (SEM). **a** *Cladium mariscus*. **b** *Chrysitrix dodii*. **c** *Mapania linderi**, spheroidal pollen grain in equatorial view. **d** *Kyllinga* sp. **e** *Lipocarpa nana*. **f** *Capeobolus brevicaulis*, suboblate/spheroidal pollen grain in equatorial view. **g** *Kyllinga eximia*, spheroidal pollen grain. **h** *Bulbostylis hispidula*, subprolate pollen grain in equatorial view. **i** *Carex elata*, prolate pollen grain in equatorial view. **j** *Ficinia zeyherii*, perprolate pollen grain in equatorial view. **k** *Diplasia karatifolia**, circular amb (polar view). **l** *Isolepis setacea*, angular amb (polar view). **m** *Kyllinga flava*,

irregular amb (polar view). **n, o** *Schoenoplectus senegalensis*. **p, q** *Arthrostylis aphylla*. **n, p** semi-collapsed pollen grain (equatorial view). **o, q** rehydrated pollen grain. **r** *Kyllinga flava*, pyriform pollen grain in equatorial view. **s** *Cyperus rotundus*, semi-rehydrated (left) and fully rehydrated (right) pollen grain. **t** *Schoenus nigricans*, collapsed pollen grain in equatorial view. **v** *Isolepis digitata*, overview of the pollen grain arrangement in the locule. Scale bar 10 μm . Species without asterisk fixed material, asterisk herbarium material, double asterisks living material



(Fig. 3b), fossulate (e.g. Fig. 2u), negative microreticulate (Fig. 3k), and doughnut-shaped granules (Fig. 3g). The ornamentation of these elements displays often similarities with the mesocolpial or mesoporial sexine ornamentation (e.g. Fig. 3a).

Orbicules

We observed orbicules in all species studied, except in *Mapania linderi* and *Everardia montana*. Size and shape of orbicules, and the ornamentation of the orbicular wall at

◀ **Fig. 2** Apertural conditions (SEM). **a–c** Equatorial view of pollen grains without visible apertures. **a** *Fimbristylis complanata*. **b** *Fimbristylis xyridis*. **c** *Rhynchospora* sp. **d** *Baumea rubiginosa*, polyporate pollen grain (white arrows). **e** *Scleria rugosa*, poorly visible ulcus (black arrow) and lateral apertures (white arrows). **f, g** *Cladium mariscus*. **f** Pollen grain without visible apertures. **g** Equatorial view of pollen grain with ulcus (black arrow) and lateral apertures (white arrows). **h** *Coleochloa setifera*, monoaperturate pollen grain with aspis (white arrow). **i** *Mapania cuatrecasii**, polar view of collapsed pollen grain with a single ulcus (black arrow). **j** *Chorizandra enodis**, equatorial view of pollen grain with two visible lateral apertures (white arrows). **k** *Lepironia articulata**, lateral apertures (white arrows). **l** *Eriophorum latifolium*, pentaaperturate pollen grain, ulcus (black arrow) and two lateral apertural zones (white arrows) visible. **m** *Lipocarpa nana*, view at proximal pole, four lateral apertures (white arrows). **n** *Arthrostylis aphylla*, view at proximal pole, six lateral apertures (white arrows). **o** *Eleocharis palustris*, three lateral apertures visible (white arrows). **p** *Eleocharis acutangula*, equatorial view of pollen grain without visible apertures. **q** *Hellmuthia membranacea*, ring (white arrowhead) below lateral apertures (white arrows). **r** *Ficinia brevifolia*, depressions (white arrowheads) below and above lateral apertures (white arrows). **s** *Cyperus dubius*, pollen grain with papilla (white arrowhead). **t** *Isolepis antarctica*, equatorial view of pollen grain with distal sulcus (black arrow) and lateral pori (white arrows). **u** *Cyperus rotundus*, pollen grain with irregular shaped aperture (white arrowhead). Species without asterisk fixed material, asterisk herbarium material, double asterisks living material

species level are summarized in Table 4. Orbicules often occur in high densities and are dispersed over the entire inner locule wall (Fig. 4a). Occasionally they are attached to the pollen grain wall (e.g. *Pycneus sanguinoletus*; Fig. 4b). In some species, aggregations of single orbicules occur (e.g. *Ficinia radiata*; Fig. 4c). The morphology of the locule wall reveals the position of underlying endothelial cells and thickenings (Fig. 4d). In *Eleocharis acutangula* (Fig. 4e), the orbicules form a reticulate pattern over the entire locule wall or the orbicules may be absent at rounded spots on the locule wall (Fig. 4d). Orbicules of *Pycneus mundtii* (Fig. 4f) are the smallest (ca. 0.20 μm), whereas the largest orbicules (ca. 1.28 μm) occur in *Kyllinga eximia*. Four shapes were distinguished: (1) angular as found in *Arthrostylis aphylla* (Fig. 4g); (2) more or less spherical as observed in *Diplasia karatifolia* (Fig. 4h); (3) doughnut-shaped as present in *Mapania cuatrecasii* (Fig. 4j) and (4) irregular as occurring in *Kyllinga* sp. (Fig. 4i).

In some species, the orbicules are embedded in the locule wall (e.g. *Hypolytrum jenmanii* subsp. *jenmanii*). In *Kyllinga flava* we found spherical bodies, with a similar ornamentation as the orbicular wall, but much larger in size ($\pm 2.26 \mu\text{m}$). These spherical bodies are scattered all over the locule wall but less dense than the orbicules (Fig. 4l).

The majority of species have orbicules with a microgranulate (e.g. *Kyllinga* sp.; Fig. 4i) or a microechinate (e.g. *Kyllinga flava*; Fig. 4l) wall ornamentation. However, in some species the orbicular wall is smooth (*Mapania cuatrecasii*; Fig. 4j). In *Coleochloa setifera*, striae were

observed (Fig. 4k) on the orbicular wall. Occasionally, perforations were present (*Kyllinga* sp.; Fig. 4i). We often observed similarities between the sexine ornamentation (or the ornamentation and shape of the sporopollenin granules on the opercula or pontopercula) and the ornamentation and shape of the orbicules (e.g. *Kyllinga flava*; Figs. 3a, 4l and *Coleochloa setifera*; Figs. 3e, 4k).

Discussion

Palynological data in a phylogenetic framework

In order to determine possible synapomorphies and evolutionary trends in the Cyperaceae, we optimized the coded palynological characters on a phylogenetic hypothesis based on parsimony analysis of *rbcL* and *trnL-F* sequence data of the genera investigated (Muasya et al. 2008, modified). Three pollen characters (number of lateral, apertures distinctness of apertures and sexine ornamentation) show some congruence with the molecular phylogeny and are illustrated (Fig. 5). The other eight palynological characters were also optimized but were incongruent with the phylogeny (not shown). There is a transition in the lateral aperture number from zero (outgroup and subfamily Mapanioideae) to polyporate (*Baumea*-clade) or most commonly to four (clade Cariceae–Dulichieae–Scirpeae and tribe Fuireneae) with in some cases an increase to five or even six lateral apertures (tribe Cyperae) (Fig. 5a). Evolution from a low to a higher aperture number has been suggested for several groups, for example, Alismatales (Chanda et al. 1988) and *Dioscorea* (Schols et al. 2005). An increased number of apertures may offer a potential selective advantage because it increases the number of prospective germination sites, thus facilitating contact between at least one aperture and the stigmatic surface (Furness and Rudall 2004). Occasionally a reversal from five to zero was identified, for instance in *Fimbristylis*. We observed also a shift from operculate pollen grains in the outgroup and the subfamily Mapanioideae to pontoperculate pollen in Cyperoideae with a reversal to operculate pollen in tribe Cyperae (Fig. 5b). It has been proposed that pollen aperture number and pattern is related to microsporogenesis (e.g. Blackmore and Crane 1998; Rudall and Bateman 2007) and that simultaneous cytokinesis allows variation in the interaction between nuclei, which is impossible in successive microsporogenesis, thus leading to heteromorphic pollen (Ressayre et al. 2002). Changes in aperture number are thus potentially easy to achieve (Furness and Rudall 2004). Granulate–perforate sexine occurs throughout the outgroup and the majority of Cyperaceae species sampled and can be considered as the plesiomorphic

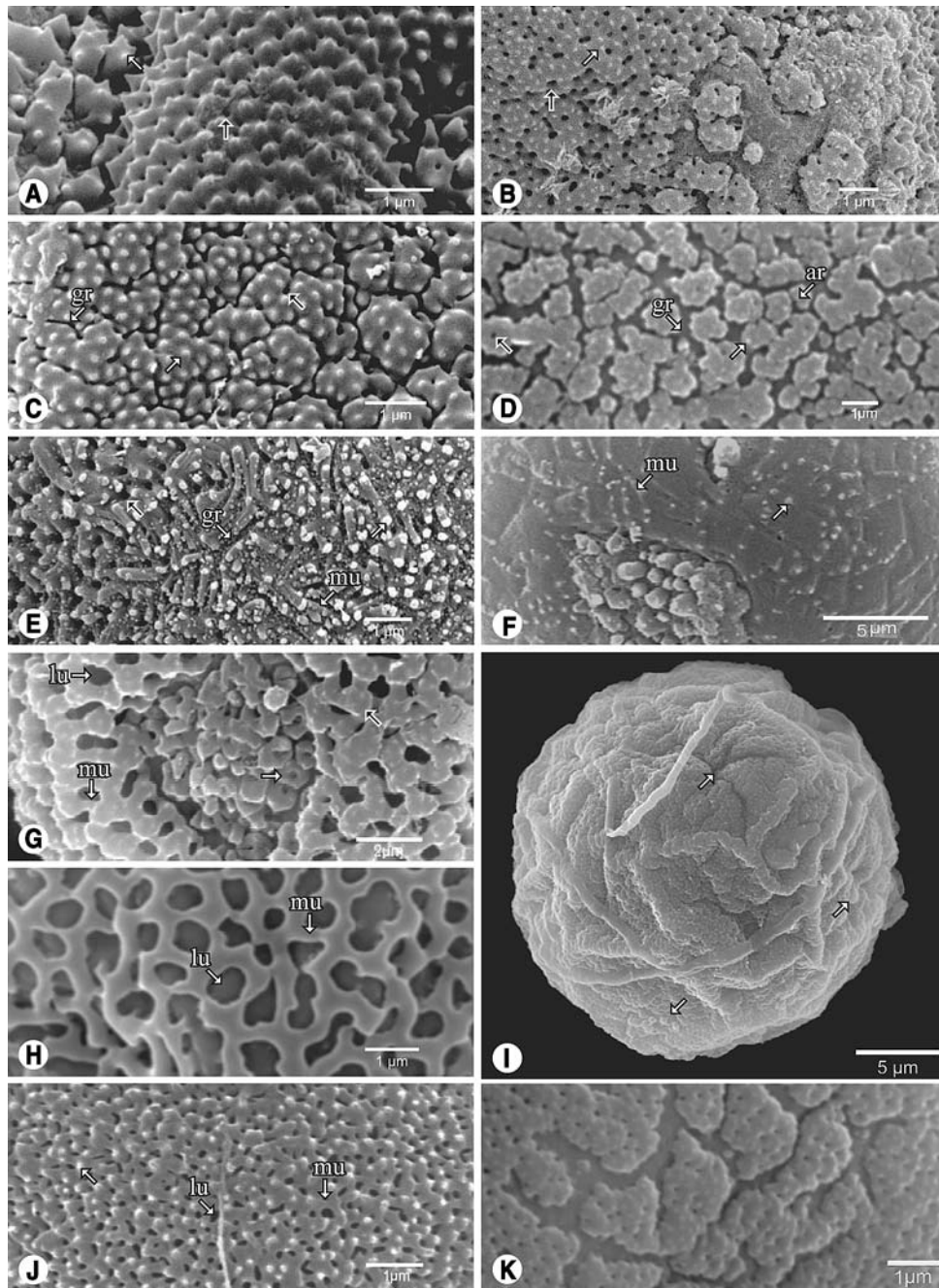
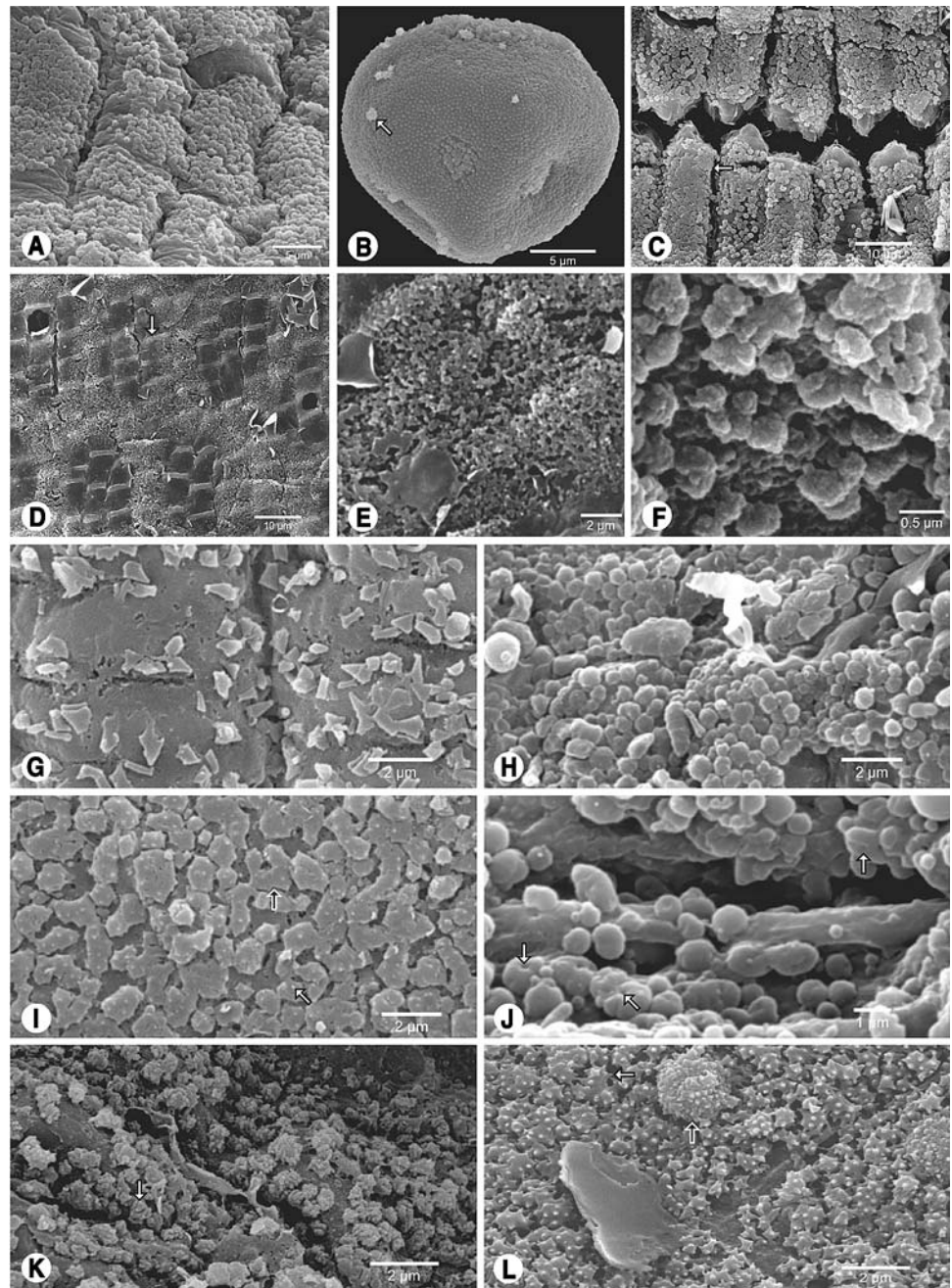


Fig. 3 Sexine and aperture ornamentation (SEM). **a** *Kyllinga flava*, microechinate sexine with perforations (black arrow), operculate aperture with microechinate sporopollenin granules (white arrow). **b** *Kobresia myosuroides*, granulate (white arrow) sexine with perforations (black arrow), in the right angle is the apertural zone with pontopericulum visible. **c** *Fuirena leptostachya*, fossulate sexine with microgranules (white arrow) and perforations (black arrow). **d** *Eleocharis acutangula*, negative microreticulate sexine with perforations (black arrow) and microgranules (white arrow). **e** *Coleochloa setifera*, fossulate/rugulate sexine with perforations (black arrow) and microgranules (white arrow). Scale bar 1 μm . **f** *Cyperus dubius*, psilate/rugulate sexine with microgranules (white arrow).

g *Ficinia minutiflora*, microreticulate sexine with thickenings on the crossings of the muri (black arrow), which resemble the orbicules; operculate aperture with doughnut-shaped sporopollenin granules (white arrow). Scale bar 2 μm . **h** *Hypolytrum jenmanii* subsp. *jenmanii**, microreticulate sexine. **i** *Exochogyne amazonica**, pollen grain with metareticulum, arrows indicate assumed apertures. **j** *Mapania linderi**, microreticulate sexine with echinae (white arrow). **k** *Becquerelia cymosa**, pontoperculate aperture with negative microreticulate ornamentation. *gr* Groove, *ar* areola, *lu* lumen, *mu* muri). *Species without asterisk* fixed material, *asterisk* herbarium material, *double asterisks* living material

Fig. 4 Orbicules (SEM). **a** *Cyperus rotundus*, overview locule wall with orbicules. Scale bar 5 μm . **b** *Pycurus sanguinoletus*, orbicules attached to pollen grain wall (arrow). **c** *Ficinia radiata*, aggregations of orbicules (arrow). Scale bar 10 μm . **d** *Eleocharis acutangula*, overview locule wall with blank spots where pollen grains used to be; endothelial thickening (arrow). **e** *Eleocharis acutangula*, orbicules arranged in reticulate pattern. **f** *Pycurus mundtii*, smallest orbicules (0.20 μm). **g** *Arthrostylis aphylla*, angular orbicules. Scale bar 2 μm . **h** *Diplasia karatifolia**, spherical orbicules. **i** *Kyllinga* sp., irregular orbicules (black arrow) with microgranules (white arrow). **j** *Mapania cuatrecasii**, doughnut-shaped orbicules (arrows). Scale bar 1 μm . **k** *Coleochloa setifera*, orbicules with striae (arrow). **l** *Kyllinga flava*, orbicules with microechinae (white arrow), spherical bodies with microechinae (black arrow). Species without asterisk fixed material, asterisk herbarium material, double asterisks living material



condition in the group. Most other sexine ornamentation patterns have evolved more than once. Microechinate sexines are notably present in the *Kyllinga-Oxycaryum* clade (Fig. 5c) and could be a highly informative character to split certain genera that are most likely non-monophyletic, such as *Cyperus* (Muasya et al. 2002).

Mapanioideae

In Mapanioideae, several pollen characters have a distinct systematic value at tribal level: Hypolytreae is characterized by spheroidal, monad-like pollen grains (Figs. 1c, k, 2i)

while Chrystricheae have (sub)prolate pollen grains that are most likely pseudomonads (Simpson et al. 2003; Figs. 1b, 2j–k). Moreover, Chrystricheae display a peripheral pollen arrangement within the locule and the Hypolytreae a central pollen arrangement (Kirpes et al. 1996; Simpson et al. 2003). Members of Hypolytreae have monoaperturate pollen grains (Figs. 1c, k, 2i), in contrast to Chrystricheae which possess besides monoaperturate pollen grains with a distal ulcus also pollen with several lateral apertures (Fig. 2j–k), a feature that is common in the Cyperoideae. Mapanioid pollen grains have always been reported as being monoaperturate (Haines and Lye 1983;

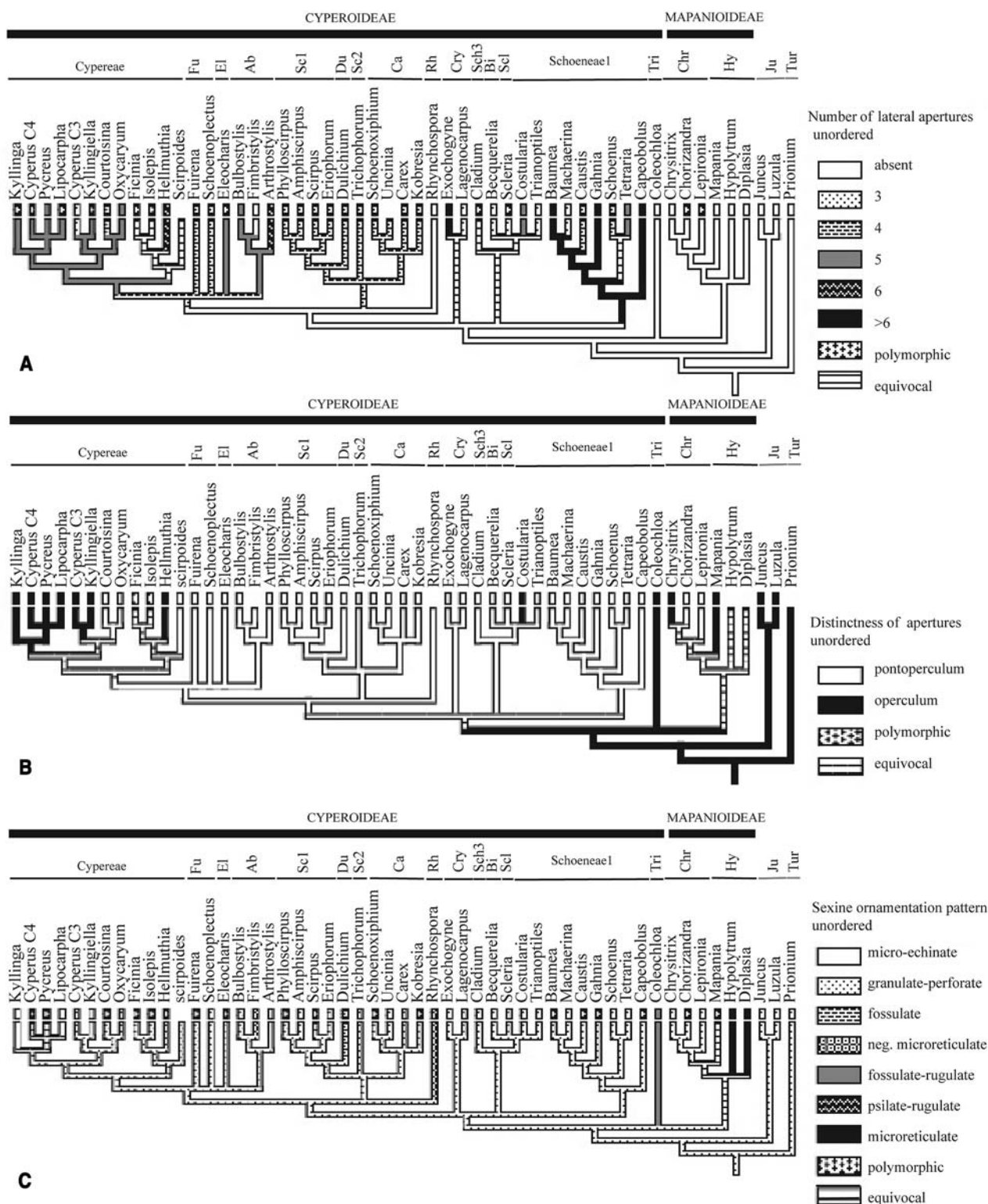


Fig. 5 Optimization of pollen characters on a plastid phylogeny of the Cyperaceae. **a** Number of lateral apertures. **b** Distinctness of apertures. **c** Sexine ornamentation. Strict consensus tree obtained from a parsimony analysis of *rbcL* and *trnL-F* sequence data (Muasya et al. 2008). Optimization was carried out using MacClade 4.04

(Maddison and Maddison 2001). *Ab* Abildgaardieae, *Bi* Bisboeckele-reae, *Ca* Cariceae, *Chr* Chrysitricheae, *Cry* Cryptangieae, *Du* Dulicheae, *El* Eleocharideae, *Fu* Fuirenae, *Hy* Hypolytreae, *Ju* Juncaceae, *Rh* Rhynchosporae, *Sc* Scirpeae, *Sch* Schoeneae, *ScL* Scleriae, *Tri* Trilepideae, *Tur* Thurniaceae

Van Wichelen et al. 1999; Simpson et al. 2003) except for the observation of lateral apertures in *Lepironia mucronata* by Erdtman (1966, 1971).

In literature, the sexine ornamentation of pollen grains in Mapanioideae has been described as (sub)reticulate (Simpson et al. 2003). This is only partially confirmed by our results (Fig. 3h, j), because we also observed granulate and rugulate pollen grains in Mapanioideae. Moreover, Hypolytreae and Chrysitricheae can be readily distinguished by their sexine pattern. Hypolytreae pollen grains are microreticulate (Fig. 3h, j), except those of *Mapania cuatrecasii* (fossulate–perforate), while Chrysitricheae pollen grains are granulate or rugulate–granulate. We observed orbicules in all species studied, with exception of *Mapania linderi*. The systematic value of orbicules in Mapanioideae is rather low, rendering no support for the two tribes based on orbicular morphology. Based on non-molecular data, Bruhl (1995) suggested that Mapanioideae comprise only one tribe (Hypolytreae), since Chrysitricheae were usually nested in other mapanioids. However, our data (Fig. 5) strongly supports the recognition of both tribes Hypolytreae and Chrysitricheae as inferred by Simpson et al. (2003, 2008) and Muasya et al. (2008).

Tribe Trilepideae

The position of this tribe is unresolved in the phylogenetic hypothesis in Fig. 5. In the recent molecular phylogenies of Simpson et al. (2008) and Muasya et al. (2008), however, this clade is sister to the rest of Cyperoideae although with weak support only. The monoporate pollen grains of *Coleochloa setifera* (Trilepideae) (Fig. 2h) offer support for Trilepideae as an early diverging lineage in Cyperoideae. This feature occurs in only one other cyperoid genus (*Cladium*) and in the closely related Mapanioideae. Pollen grains of *Coleochloa setifera* also have a rugulate sexine pattern (Fig. 3e) in common with *Chorizandra cymbaria* (Mapanioideae). The other species of Trilepideae studied have pollen with the plesiomorphic granulate–perforate sexine ornamentation. Further observations in Trilepideae are needed to search for useful systematic pollen characters, which can help to clarify the position of this tribe.

Cyperoideae

Pollen morphological variation within subfamily Cyperoideae is considerable. The different pollen shapes that occur in Cyperoideae have arisen several times in the phylogeny. Even within a single genus, for example *Cyperus*, we observed different pollen shapes (Table 3). This variability is probably caused by harmomegathic effects on the fragile Cyperaceae pollen grains. Furthermore, we conclude that the peripheral arrangement of the pollen grains in the

locules affects their shape at maturity. Species with pollen grains without visible apertures (Fig. 2a–c, f, p) are scattered over the Cyperoideae according to the most recent molecular phylogenies. *Baumea rubiginosa* (Fig. 2d), *Gahnia lanigera* and *Capeobolus brevicaulis* (Fig. 1f) have polyporate pollen grains similar to those of *Machaerina* sp. (Van Wichelen et al. 1999) (Fig. 5a). *Baumea* and *Machaerina* are sister genera in the tribe Schoeneae (Fig. 5), to which also the genus *Gahnia* belongs. A broader sampling of *Lepidosperma* and *Neesenbeckia*, the closest relatives of *Baumea* and *Machaerina*, is needed to determine if the polyporate condition is also present in other genera of this small clade. The number of apertures in the remainder of tribe Schoeneae varies between zero and six. The clade with tribes Scirpeae 1 and 2, Dulichieae and Cariceae, possess pollen grains with one distal aperture and four lateral apertures (Fig. 5a). This apertural system is also present in pollen grains of tribe Fuireneae (Fig. 5a). The rest of Cyperoideae has an aperture number varying between three and six, with most species having pentaaperturate pollen grains (Fig. 2m). Consequently, our observations oppose Shah (1962) and Padhye and Makde (1980), who stated that monocolpate pollen grains are the dominant type in Cyperaceae. Cyperaceae generally possess pollen grains with one distal aperture and five lateral apertures (Fig. 5a), but the number of apertures can vary within a genus and even in representatives of a single species, indicating a high level of plasticity in this clade. The presence of pontopercula is plesiomorphic in Cyperoideae and there is a reversal to opercula in tribe Cypereae (Fig. 5b).

The sexine ornamentation in species of Cyperoideae is mostly described as granulate (Wodehouse 1935; Huang and Chung 1971; Padhye and Makde 1980), perforate (Moar and Wilmschurst 2003), psilate (Haines and Lye 1983) or foveolate (Padhye and Makde 1980). These sexine ornamentation descriptions should be treated with caution since most are based on LM observations only, in contrast with our more detailed SEM observations. Our results indicate that the granulate–perforate sexine ornamentation pattern (e.g. Fig. 3b) is most common in the Cyperaceae species examined and also in Juncaceae (Fig. 5c). We therefore conclude that the granulate–perforate sexine ornamentation is primitive in Cyperaceae with transitions to the other sexine ornamentations recognized. The six other sexine ornamentation patterns occur throughout the phylogeny. Microechinate pollen grains are present in tribe Cypereae (*Cyperus hemisphaericus*, *Kyllinga*, *Lipocarpha*, *Kyllingiella*) and in *Arthrostylis aphylla* (Abildgaardieae, sensu Muasya et al. 2008) and *Mapania linderi* (Hypolytreae, sensu Muasya et al. 2008) (Fig. 5c). Pollen grains of *Ficinia minutiflora* show a microreticulate sexine pattern similar to the pattern in some mapanioid genera (Fig. 3g). We conclude that at generic level sexine ornamentation

shows a high degree of plasticity which prevents the detection of a clear pattern. Although orbicule data have proven to be useful for evaluating systematic relationships in certain groups (Raj and El-Ghazaly 1987; Huysmans et al. 1997; Vinckier et al. 2000; Vinckier and Smets 2002), they only have minor taxonomic importance in Cyperoideae due to lack of variation. Most species possess spherical to irregularly-shaped orbicules with microgranules on the orbicule wall. We found some striking parallelisms between the shape and ornamentation of orbicules and sexine ornamentation or the ornamentation of the sporopollenin granules on the opercula and pontopercula (e.g. Hesse 1986; Huysmans et al. 1997; Vinckier et al. 2000). The systematic value of palynological data at tribal level in Cyperoideae is rather low as a consequence of high levels of homoplasy and polymorphism in most palynological characters.

Sexine ornamentation patterns and pollination

The majority of Cyperaceae are thought to be anemophilous. Entomophily in Cyperaceae is associated with taxa that are fragrant (e.g. *Mapania*) or that have coloured inflorescences (e.g. *Ascolepis*, *Cyperus*, *Rhynchospora*) (Goetghebeur 1998). It has been linked to forest habitats (Goetghebeur 1998), but the taxa with microechinate pollen occur in savannah or open woodland. Sexine projections are often associated with entomophily (e.g. Tanaka et al. 2004); taxa with microechinate pollen in our study (e.g. *Kyllinga*, *Lipocarpha*) may be entomophilous according to this hypothesis. However, some Betulaceae, Asteraceae and grasses with microechinate pollen grains are wind pollinated (Vinckier and Smets 2001). The presence of brightly coloured inflorescences (white, orange, brown) in *Kyllinga* could offer additional evidence for entomophily. However, the brightly coloured *Ficinia radiata* flowers produce granulate pollen grains, suggesting that alternative adaptations to promote entomophily may exist in different taxa or habitats. This shows that ecological studies are needed to conclude with certainty. The forest-dwelling genera of Hypolytreae with microreticulate pollen grains (e.g. *Mapania* and *Diplasia*), are thought to be adapted to invertebrate pollination by having sticky pollen due to the presence of lipids (Simpson et al. 2003). The differences in sexine ornamentation of the pollen grains observed in Hypolytreae and Chrysitricheae pollen do not reject the hypothesis that the groupings within subfamily Mapanioideae are based on the specialization of some taxa in terms of their pollination biology (Simpson et al. 2003).

Pollen morphological similarities with other Poales

In Juncaceae and Thurniaceae, the sister groups of the Cyperaceae (Muasya et al. 1998, 2000), the four meiotic

products become viable, fertile pollen grains, which are dispersed as tetrads (e.g. Erdtman 1952, 1971; Furness and Rudall 1999). The pollen grains are ulcerate in both families (Dahlgren et al. 1985), which is also the case in basal and some other members of the Cyperaceae (Fig. 5a). Pollen tetrads in Juncaceae are operculate, and are described as granulate (Buchner and Weber 2000), a sexine ornamentation pattern which is also present in the Cyperaceae (Fig. 3b). Unlike in most Cyperaceae, pollen arrangement in the locule of the anther in Juncaceae is central (Kirpes et al. 1996). Sexine ornamentation, distinctness of apertures and pollen arrangement in Thurniaceae is not documented.

Restionaceae have pollen grains dispersed as monads with a scrobiculate (perforate) sexine (Dahlgren et al. 1985). The latter is also reported in our study group (Table 3). Monoaperturate pollen grains occur in both families (Chanda 1966; Dahlgren et al. 1985). Like Juncaceae, the pollen grains of Restionaceae are centrally arranged in the locule in contrast to the peripheral pollen arrangement in most Cyperaceae.

Some palynological similarities between Cyperaceae and Poaceae are observed. In both groups orbicules are present in huge numbers (Vinckier and Smets 2001). In several species of both families, orbicules are fused or connected with each other by sporopollinose threads forming a network of orbicules covering the locule wall (Vinckier and Smets 2001). Similar sexine ornamentations are observed in Cyperaceae and Poaceae species (e.g. negative microreticulate with microgranules/echinae and perforations) (Vinckier and Smets 2001; Datta and Chaturvedi 2004). Both groups possess species with spheroidal, monoaperturate pollen grains with an annulus (Skvarla et al. 2003; Datta and Chaturvedi 2004; Perveen 2006); and the peripheral arrangement of the pollen grains in the locule is dominant in Poaceae and Cyperaceae (Kirpes et al. 1996).

Conclusions

The pollen morphological variation encountered is far greater than was conceived so far. Our data support the delimitation of only two subfamilies. In Mapanioideae pollen polarity, pollen shape, aperture number and sexine ornamentation provide additional support for the recognition of two tribes (Hypolytreae and Chrysitricheae). Hypolytreae are characterized by monoaperturate, spheroidal pollen grains while Chrysitricheae have pear-shaped pseudomonads.

In Cyperoideae we could identify an evolutionary trend concerning the number and localization of apertures. Early diversified groups lack lateral apertures, and polyporate pollen grains are an intermediate form towards the presence of four lateral apertures to five lateral apertures in the

derived Cyperae. A second evolutionary trend concerns the shift from the plesiomorphic granulate–perforate sexine to the other sexine patterns. No other distinct trends in character evolution could be discerned in subfamily Cyperoideae because of the high degree of homoplasy in some major pollen morphological features, thus restricting the taxonomic value of those palynological characters. Therefore, the use of pollen types in Cyperaceae is an oversimplification of the palynological variation present.

In the literature there is controversy about the number of apertures in Cyperoideae pollen. This can be explained by (1) observation of collapsed or insufficient hydrated pollen grains, (2) apertural zones that are difficult to distinguish due to only slightly differentiated sexine ornamentation in the apertural regions, and (3) intraspecific variation in aperture number. Ultrastructural observations of living material are indispensable to provide evidence on the exact number of apertures and to investigate whether grains without externally visible apertures are inaperturate or omniaperturate.

Further developmental studies on the pseudomonad or monad (*Mapania*-type pollen) nature of pollen grains in Cyperaceae are needed to answer phylogenetic questions such as, are there intermediate forms between monad-central pollen arrangement and pseudomonad-peripheral pollen arrangement?

Acknowledgments This research was financially supported by the research council of K. U. Leuven (OT/05/35) and the Fund for Scientific Research—Flanders (Belgium) (F.W. O.—G.0268.04). S. Vinckier was a postdoctoral fellow of F.W. O. in the course of this study. A. M. Muasya acknowledges the Norwegian Council for Higher Education Programme for Development Research & Education (NUFU project 53/03) for funding and Flora Research Permit from CapeNature (AAA005-00054-0028). We thank Prof. P. Goetghebeur from the University of Ghent and Prof. J. Rammeloo, Director of the National Botanic Garden of Belgium (BR) for the supply of living material and herbarium specimens. We are grateful to Anja Vandepierre for technical assistance. Dr. Ochoterena and Steven Janssens are acknowledged for their assistance with the character optimization.

Appendix 1: Species studied are listed alphabetically; fixed material (without asterisk), herbarium specimens are indicated with an asterisks and living material with double asterisks

Afrotrilepis pilosa (Ridl.) Gilly (*), Cameroon, P. Goetghebeur, 5182 (GENT).
Amphiscirpus nevadensis S. Watson (*), USA, J. Bouharmont, 19926, BR-S.P. 917869 (BR).
Arthrostylis aphylla R. Br., Australia, R.K. Harwood, RKH 1161.
Baumea rubiginosa Boeck., Australia, J.J. Bruhl and Hodges, JH 792.

Becquerelia cymosa Brogniart (*), Brazil, M. Luceno, 33B (GENT).
Bulbostylis hispidula (Vahl) R.W. Haines, Kenya, A.M. Muasya, AM 2466.
Capeobolus brevicaulis (C.B. Clarke), J. Browning, South Africa, A.M. Muasya, AM 2203.
Carex capitata L., Botanical Garden University Ghent, Belgium, P. Goetghebeur, PG 10466.
Carex elata Lowe, cultivated in the Botanical Garden of the Institute of Botany and Microbiology (K.U.Leuven) Belgium, A. Vrijdaghs, AV11.
Carex monostachya, A. Rich. (*), Senegal, C. Vanden Berghen, 8701 (BR)
Caustis flexuosa, R. Br. (*), USA, E.F. Constable, 26663 (BR).
Caustis recurvata Spreng. (*), Australia, P.K. Endress, 4381 (GENT).
Chorizandra cymbaria R. Br. (*), Australia, K.L. Wilson and K. Frank, 8954 (GENT).
Chorizandra enodis Nees, Australia, K.L. Wilson and K. Frank, 8922 (GENT).
Chrysitrix dodii C.B. Clarke, South Africa, A.M. Muasya, AM 2797.
Cladium mariscus (L.) Pohl, National Botanical Garden of Belgium, A. Vrijdaghs, AV06.
Coleochloa setifera (Ridl.) Gilly, Kenya, A.M. Muasya, AM 2464.
Costularia humbertii Bosser (*), Madagascar, J.S. Miller and P.P. Lowry, II4175 (GENT).
Courtoisina assimilis (Steud.) P. Maquet, Kenya, A.M. Muasya, AM 2124.
Cyperus alternifolius L. (**), Botanical Garden University Ghent Belgium, A. Vrijdaghs, 2001/1114.
Cyperus articulatus L., Kenya, A.M. Muasya, AM 2168.
Cyperus dubius Rottb., Kenya, A.M. Muasya, AM 2188.
Cyperus haspan L., Kenya, A.M. Muasya, AM 2135 (EA).
Cyperus hemisphaericus Boeckeler (*), Tanzania, E. Milne-Redhead and P. Taylor, 8053A (BR).
Cyperus laevigatus L., Botanical Garden University Ghent, Belgium, P. Goetghebeur, PG 10202.
Cyperus rotundus L., Kenya, A.M. Muasya, AM 2164.
Diplasia karatifolia L.C. Rich (*), Bolivia, R. Rueda, 921 (GENT).
Dulichium arundinaceum Britton, Botanical Garden University Ghent, Belgium, P. Goetghebeur, PG 9914.
Eleocharis acutangula (Roxb.) Schult., Kenya, A.M. Muasya, AM 2437.
Eleocharis palustris R. Br., Hortus Botanicus Lovaniensis Belgium, A. Vrijdaghs, AV07b.
Eriophorum latifolium Hoppe, Hortus Botanicus Lovaniensis Belgium, A. Vrijdaghs, AV04.
Everardia montana Ridley (*), Venezuela, P.E. Berry, O. Huber and J. Rosales, 4912 (GENT).

- Exochogyne amazonica* C.B. Clarke (*), Brasil, M. Aparecida da Silva, C. Proença, E. Cardoso and J.P. Paixão, 23.5.1994 (GENT).
- Ficinia brevifolia* Nees, South Africa, A.M. Muasya, AM 2205 (BOL, EA, K).
- Ficinia capitellum* Nees, South Africa, A.M. Muasya, AM 2206 (BOL, EA, K).
- Ficinia dunensis* Levyns, South Africa, A.M. Muasya, AM 2242.
- Ficinia gracilis* Schrad., Kenya, A.M. Muasya, AM 2571.
- Ficinia minutiflora* C.B. Clarke, South Africa, A.M. Muasya, AM 2257 (BOL, EA, K).
- Ficinia polystachya* Levyns, South Africa, A.M. Muasya, AM 2320.
- Ficinia radiata* Kunth, South Africa, A.M. Muasya, AM 2262 (BOL, EA, K).
- Ficinia tristachya* (Vahl) Nees, South Africa, A.M. Muasya, AM 2255.
- Ficinia zeyheri* Boeckeler, South Africa, A.M. Muasya, AM 2209 (BOL, EA, K).
- Fimbristylis complanata* (Retz.) Link, Kenya, A.M. Muasya, AM 2147.
- Fimbristylis xyridis* R. Br., Australia, R.K. Harwood, RKH 1162.
- Fuirena abnormalis* C.B. Clarke, Kenya, A.M. Muasya, AM 2192.
- Fuirena leptostachya* Oliver, Kenya, A.M. Muasya, AM 2136.
- Gahnia lanigera* (R. Br.) Benth. (*), Australia, B.J. Blaylock, 1227 (GENT).
- Hellmuthia membranacea* (Thunb.) R.W. Haines and Lye, South Africa, A.M. Muasya, AM 2792 (KUL).
- Hypolytrum jenmanii* C.B. Clarke subsp. *jenmanii* (*), Guyane, J.J. de Granville, F. Crozier, 13652 (GENT).
- Isolepis antarctica* (Willd.) Roem. & Schult., South Africa, A.M. Muasya, AM 2247 (BOL, EA, K).
- Isolepis digitata* Nees ex Schrad., South Africa, A.M. Muasya, AM 2258.
- Isolepis prolifera* (Rottb.) R. Br., South Africa, A.M. Muasya, AM 2265.
- Isolepis setacea* (L.) R. Br., Kenya, A.M. Muasya, AM 2547.
- Kobresia myosuroides* Fiori & Paoletti, Botanical Garden University Ghent, Belgium, P. Goetghebeur, PG 10009.
- Kyllinga eximia* C.B. Clarke, Kenya, A.M. Muasya, AM 2137.
- Kyllinga flava* C.B. Clarke, Kenya, Musili, MM 009.
- Kyllinga polyphylla* Thou. ex Link (**), Botanical Garden University Ghent, Belgium, A. Vrijdaghs, 2004/21768.
- Kyllinga* sp. Rottb., Kenya, A.M. Muasya, AM 2658.
- Kyllingiella polyphylla*, Kenya, A.M. Muasya, AM 2435.
- Lagenocarpus rigidus* (Kunth) Nees subsp. *rigidus* (*), French Guiana, D. Torida-Marbot, 329 (GENT).
- Lepironia articulata* (Retzius) Domin (*), Papua New Guinea, P. Goetghebeur and W. Vyverman, 6673 (GENT).
- Lipocarpha nana* (A. Rich.) Cherm., Kenya, A.M. Muasya, AM 2194.
- Lipocarpha rehmannii* (Ridl.) Goetgh., Kenya, A.M. Muasya, AM 3132.
- Machaerina flexuosa* (Böckeler) Kern (*), Madagascar, J.S. Miller and A. Randrianasdo, 4382 (GENT).
- Mapania cutatrecasasii* T. Koyama (*), Costa Rica, G. Herrera, 3282 (GENT).
- Mapania linderi* Hutchinson (*), Ivory Coast, C.C.H. Jongkind, 4435 (GENT).
- Oxycarium cubense* (Poepp. & Kunth) Palla, Kenya, Mwachala, M340.
- Phylloscirpus acaulis* (Philippi) Goetghebeur and D.A. Simpson subsp. *pachycaulis* (*), Equador, S. Laegaard, S. Dhooge and E. Jones, 21519 (GENT).
- Pseudoschoenus inanis* (Thunb.) Oteng- Yeboah, South Africa, A.M. Muasya, AM 3061.
- Pycreus flavescens* Beauv. ex Rchb. (**), Botanical Garden University Ghent, Belgium, A. Vrijdaghs, 2005/0401.
- Pycreus mundtii* Nees, Kenya, A.M. Muasya, AM 2156.
- Pycreus sanguinolentus* (Vahl) Nees, Kenya, A.M. Muasya, AM 2157.
- Rhynchospora* sp. Vahl, Australia, R.K. Harwood, RKH 1127.
- Schoenoplectus senegalensis* (Hochst. ex Steud.) Palla, Kenya, Malombe 40.
- Schoenoxiphium lehmannii* Kunth ex Steud., Kenya, Malombe, KG 96.
- Schoenoxiphium sparteum* C.B. Clarke, Kenya, A.M. Muasya, AM 2566.
- Schoenus nigricans* L., UK, K. De Wale, 1239 (GENT).
- Scirpoides holoschoenus* (L.) Sojak (**), Botanical Garden University Ghent, Belgium, A. Vrijdaghs, 2003/1536.
- Scirpus sylvaticus* L. (*), Botanical Garden University Ghent, Belgium, P. Goetghebeur, 5382 (GENT).
- Scleria rugosa* R. Br., Australia, R.K. Harwood, RKH 1143.
- Scleria terrestris* (L.) Fassett (**), Botanical Garden University Ghent, Belgium, A. Vrijdaghs, 21768.
- Tetraria compar* H.C. Taylor (*), South africa, H.C. Taylor, 9996 (GENT).
- Trianoptiles solitaria* (C.B. Clarke) Levyns, South Africa, A.M. Muasya, AM 3024
- Trichophorum alpinum* Pers. (*), USA, D. Collet, 670 (BR).

Uncinia rubra Colenso ex Boott, Botanical Garden University Ghent, Belgium, P. Goetghebeur, PG 9727.

Appendix 2: A detailed list of the characters and their states as defined for the optimization and the combined analysis. Characters 1, 2 and 9 are quantitative and continuous characters

1. Polar axis

- (0) 0 (<18)
- (1) 1 (<28)
- (2) 2 (<41)
- (3) 3 (<52)
- (4) 4 (>52)

2. P/E

- (0) 0 (<1)
- (1) 1 (<1.4)
- (2) 2 (<1.7)
- (3) 3 (<2.3)
- (4) 4 (>2.3)

3. Pollen shape

- (0) suboblate (0.75–0.88)
- (1) spheroidal (0.88–1.14)
- (2) subprolate (1.14–1.33)
- (3) prolate (1.33–2.00)
- (4) perprolate (>2.00)

4. Distal aperture

- (0) absent
- (1) 1 ulcus
- (2) 1 sulcus

5. Number lateral apertures

- (0) absent
- (1) 3
- (2) 4
- (3) 5
- (4) 6
- (5) >6

6. Shape lateral apertures

- (0) colpi
- (1) pori
- (2) variable

7. Distinctness of apertures

- (0) pontopericulum
- (1) operculum

8. Sexine ornamentation

- (0) microechinate
- (1) granulate–perforate
- (2) fossulate
- (3) negative microreticulate
- (4) fossulate–rugulate
- (5) psilate–rugulate
- (6) microreticulate

9. Orbicule diameter

- (0) 0 (<0.3)
- (1) 1 (<0.6)
- (2) 2 (<0.9)
- (3) 3 (<1.2)
- (4) 4 (>1.2)

10. Orbicule shape

- (0) angular
- (1) irregular
- (2) spherical
- (3) doughnut-shaped

11. Orbicule ornamentation

- (0) smooth
- (1) microgranules
- (2) microechinae
- (3) striae

Appendix 3

Table 5.

Table 5 Data matrix with coded characters

	1	2	3	4	5	6	7	8	9	10	11
<i>Kyllinga</i>	0,1	0,1	1,2,3	1,2	2,3	0	1	2	2,4	1	0
<i>CyperusC4</i>	1,2	0,1,2	1,2,3	1	2,3,4	0,1,2	0,1,2	1,2	1,2	1	0,1,5
<i>Pycreus</i>	1	0,1	1,2	1,2	3	0,1	1	1,2	0,1,2	1	1,2
<i>Lipocarpha</i>	0	0	1	1	2,3	1	1	2	1,2	1	0
<i>CyperusC3</i>	1	1	1	1	1	1	1,2	1,2	1,2	1	1

Table 5 continued

	1	2	3	4	5	6	7	8	9	10	11
<i>Kyllingiella</i>	0	1	1	1	3,4,5	1	1	?	2	1	0
<i>Courtoisina</i>	?	?	?	1	2	2	1	2	1	0	1,2
<i>Oxycaryum</i>	1	1	2	1	3	0	1	2	?	0	1
<i>Ficinia</i>	1,2,3	0,1,2,3,4	1,2,3,4	1	1,2,3,4	2	0,1,2	0,1	0,1,2	0,1	1,6
<i>Isolepis</i>	1	1	2,3	1,2	2,3,4	2	1,2	1,2	1	0,1	1,2
<i>Hellmuthia</i>	2	2	3	1	4	1	1	1	?	1	1,2
<i>Scirpoides</i>	?	?	?	?	?	?	1	1	1	0	?
<i>Fuirena</i>	1,2	1	1,2	1	2	1	1	1	0,1	0	1,2
<i>Schoenoplectus</i>	2	1	1	1	2	1	0	0	1	0	1
<i>Eleocharis</i>	2	1,2	2,3	0,1	0,3	0	1	1	1,2	0	1,3
<i>Bulbostylis</i>	2	1	2	1	3	2	1	1	1	0	1
<i>Fimbristylis</i>	0,1	1	2	0	0	?	1	1,2	0,1	?	3
<i>Arthrostylis</i>	1	1	1	1	4	1	0	0	1	0	0
<i>Phylloscirpus</i>	?	?	?	1	2	1	1	1	1	0	1,3
<i>Amphiscirpus</i>	2	1	3	1	2	1	1	1	1	0	1
<i>Scirpus</i>	1	1	2	1	2	1	1	1	1	0	2,3
<i>Eriophorum</i>	2	1	3	1	2	0	1	1	1	0	1
<i>Dulichium</i>	1	1	2	2	2	?	1	?	?	0	2
<i>Trichophorum</i>	?	?	?	1,2	2	1	1	1	1	0	1
<i>Schoenoxiphium</i>	?	?	?	1	2	?	1,2	1	0,1	0	1,2,3
<i>Uncinia</i>	?	?	?	?	?	?	1	1	1	0	1
<i>Carex</i>	2	1,2	2,3	1	2	1	1,2	0,1	1	0	1,2
<i>Kobresia</i>	1	1	1	1	2	1	0	1	1	0	1
<i>Rhynchospora</i>	1	2	3	0	0	?	1	1	1	?	3
<i>Exochogyne</i>	1	1	1	?	5	1	1	1,2	1	0	1
<i>Lagenocarpus</i>	?	?	?	1	1	1	2	2	1	0	1
<i>Cladium</i>	3	1	3	0,1,2	0,1,2	2	1	1	1	0	1
<i>Becquerelia</i>	1	2	3	1	1	1	2	1	1	0	1
<i>Scleria</i>	1,2	1	1,2	1	1,2	1	1	0,1	1	0	1
<i>Costularia</i>	2	1	2	1	3	0	1	1	1	1	1
<i>Trianoptiles</i>	1	1	1	1	1	2	1	0	?	0	1
<i>Baumea</i>	1	1	1	1	5	1	2	0	3	0	1,2
<i>Machaerina</i>	?	?	?	?	1	1	2	1	1	0	1
<i>Caustis</i>	?	?	?	1	2	?	1	1,2	1	0	1,3
<i>Gahnia</i>	1	1	1	2	5	2	2	1,2	1	0	1,2
<i>Schoenus</i>	?	?	?	1	2	0	1	1	1	0	1
<i>Tetraria</i>	3	3	3	1	3	0	2	0	1	0	1
<i>Capeobolus</i>	1	0	0,1	1	5	1	1	1	2	0	1,2
<i>Coleochloa</i>	1	1	2	1	0	?	1	1,3	1	1	4
<i>Chrysitrix</i>	4	3	3	1	0	?	1	1	2	1	1
<i>Chorizandra</i>	3	2	3	1	0,2	1	1,2	1	1	0	1,4
<i>Lepironia</i>	2	1	2	1	0,2	1	1	1	1	0	1
<i>Mapania</i>	0,1	0	1	1	0	?	3	0	2	1	0,2,6
<i>Hypolytrum</i>	0	1	1	1	0	?	2	0	0	?	6
<i>Diplasia</i>	1	0	1	1	0	?	2	1	1	?	6
<i>Juncus</i>	?	?	?	1	0	?	?	?	?	1	1
<i>Luzula</i>	?	?	?	1	0	?	?	?	?	1	1
<i>Prionium</i>	?	?	?	1	0	?	?	?	?	?	1

References

- Blackmore S, Crane PR (1998) The evolution of apertures in the spores and pollen grains of embryophytes. In: Owens SJ, Rudall P (eds) Reproductive biology in systematics, conservation and economic botany. Royal Botanic Gardens, Kew, pp 159–182
- Brown RC, Lemmon BE (2000) The cytoskeleton and polarization during pollen development in *Carex blanda* (Cyperaceae). *Amer J Bot* 87:1–11
- Bruhl JJ (1995) Sedge genera of the world—relationships and a new classification of the Cyperaceae. *Austral Syst Bot* 8:125–305
- Buchner R, Weber M (2000) PalDat—a palynological database: descriptions, illustrations, identification, and information retrieval. <http://www.paldat.org/>
- Carniel K (1971) Über die lamelläre Struktur und die Herkunft des Pollenkittes bei *Heleocharis palustris*. *Pl Syst Evol* 119:464–474
- Chanda S (1966) On the pollen morphology of the Centrolepidaceae, Restionaceae and Flagellariaceae, with special reference to taxonomy. *Grana* 6:355–415
- Chanda S, Nilsson S, Blackmore S (1988) Phylogenetic trends in the Alismatales with reference to pollen grains. *Grana* 27:257–272
- Cranwell LM (1953) Cyperales. *New Zealand pollen studies: The monocotyledons*. Bulletin of the Auckland Institute and Museum, vol 3. Auckland Institute and Museum, Auckland, pp 42–47
- Dahlgren RMT, Clifford HT, Yeo PF (1985) The families of the monocotyledons. Academic Press, London
- Datta K, Chaturvedi M (2004) Pollen morphology of Basmati cultivars (*Oryza sativa* race Indica)—exine surface ultrastructure. *Grana* 43:89–93
- Davis GL (1966) Systematic embryology of the angiosperms. Wiley, New York
- Dunbar A (1973) Pollen development in *Eleocharis Palustris* Group (Cyperaceae). 1. Ultrastructure and ontogeny. *Bot Not* 126:197–254
- El-Ghazaly G, Jensen WA (1987) Development of wheat (*Triticum aestivum*) pollen. II. Histochemical differentiation of wall and Ubisch bodies during development. *Amer J Bot* 74:1396–1418
- Erdtman G (1952) Pollen morphology and plant taxonomy. Almqvist & Wiksell, Stockholm
- Erdtman G (1966) Pollen morphology and plant taxonomy. Angiosperms. Hafner, New York
- Erdtman G (1971) Pollen morphology and plant taxonomy. Angiosperms, 2nd edn. Hafner, New York
- Erdtman G, Berglund B, Pragłowski J (1961) An introduction to a Scandinavian pollen flora. *Grana Pal* 2:3–92
- Fernandez I (1987) Contribution al conocimiento palinológico de Cyperaceae. *Acta Bot Malacitana* 12:173–182
- Furness CA, Rudall PJ (1999) Microsporogenesis in monocotyledons. *Ann Bot* 84:475–499
- Furness CA, Rudall PJ (2004) Pollen and aperture evolution—a crucial factor for eudicot success? *Trends Pl Sci* 9:154–158
- Goetghebeur P (1998) Cyperaceae. In: Kubitzki K (ed) The families and genera of vascular plants. Flowering plants-monocotyledons, vol 4. Springer, Berlin, pp 141–190
- Govaerts R, Simpson DA, Goetghebeur P, Wilson K, Egorova T, Bruhl JJ (2007) World checklist of Cyperaceae. The Board of Trustees of the Royal Botanic Gardens, Kew. Available at <http://www.kew.org/wcsp/monocots/>, Accessed on 1 October 2007
- Haines RW, Lye KA (1983) The sedges and rushes of East Africa. African Natural History Society, Nairobi
- Hesse M (1986) Orbicules and the ektexine are homologous sporopollenin concretions in Spermatophyta. *Pl Syst Evol* 153:37–48
- Huang TC, Chung TF (1971) Pollen grains of formosan plants (7). *Taiwania* 16:85–104
- Huysmans S, El-Ghazaly G, Nilsson S, Smets E (1997) Systematic value of tapetal orbicules: a preliminary survey of the Cinchonoideae (Rubiaceae). *Canad J Bot* 75:815–826
- Huysmans S, El-Ghazaly G, Smets E (1998) Orbicules in angiosperms: morphology, function, distribution, and relation with tapetum types. *Bot Rev* 64:240–272
- Huysmans S, El-Ghazaly G, Smets E (2000) Orbicules: still a well hidden secret of the anther. In: Nordenstam B, El-Ghazaly G, Kassar M (eds) Plant Systematics for the 21st Century. Wenner-Gren International Series, vol 77. Portland Press, London, pp 201–212
- Kirpes CC, Clark LG, Lersten NR (1996) Systematic significance of pollen arrangement in microsporangia of Poaceae and Cyperaceae: review and observations on representative taxa. *Amer J Bot* 83:1609–1622
- Kosmath L (1927) Studien über das Antherentapetum. *Österr Bot Z* 76:235–241
- Koyama T (1961) Classification of the family Cyperaceae (1). *J Fac Sci, University of Tokyo* 8:1609–1622
- Maddison DR, Maddison WP (2001) MacClade 4: analysis of phylogeny and character evolution, Version 4.04. Sinauer Associates, Sunderland, Massachusetts
- Moar NT, Wilmshurst JM (2003) A key to the pollen of New Zealand Cyperaceae. *New Zealand J Bot* 41:325–334
- Muasya AM, Simpson DA, Chase MW, Culham A (1998) An assessment of suprageneric phylogeny in Cyperaceae using *rbcl* DNA sequences. *Pl Syst Evol* 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 II. Monocots: systematics and evolution. CSIRO, Melbourne, pp 593–601
- Muasya AM, Simpson DA, Chase MW (2002) Phylogenetic relationships in *Cyperus* L. s.l. (Cyperaceae) inferred from plastid DNA sequence data. *Bot J Linn Soc* 138(2):145–153
- Muasya AM, Simpson DA, Verboom GA, Goetghebeur P, Naczi RFC, Chase MW, Smets E (2008) Phylogeny of cyperaceae based on DNA sequence data: current progress and future prospects. *Bot Rev* (in press)
- Padhye MD, Makde KH (1980) Pollen morphology of Cyperaceae. *J Palynol* 16:71–81
- Perveen A (2006) A contribution to the pollen morphology of family Gramineae. *World Appl Sci J* 1(2):60–65
- Punt W, Hoen PP, Blackmore S, Nilsson S, Le Thomas A (2007) Glossary of pollen and spore terminology. *Rev Palaeobot Palynol* 143: 1–81. <http://www.bio.uu.nl/~palaeo/glossary/glos-int.htm>
- Raj B, El-Ghazaly G (1987) Morphology and taxonomic application of orbicules (Ubisch bodies) in Chloanthaceae. *Pollen Spores* 29:151–166
- Ressayre A, Raquin C, Mignot A, Godelle B, Gouyon PH (2002) Correlated variation in microtubule distribution, callose deposition during male, post-meiotic cytokinesis, and pollen aperture number across *Nicotiana* species (Solanaceae). *Amer J Bot* 89:393–400
- Rowley JR (1962) Stranded arrangement of sporopollenin in the exine of microspores in *Poa annua*. *Sci* 137(3529):526–528
- Rowley JR, Dunbar A (1996) Pollen development in *Centrolepis aristata* (Centrolepidaceae). *Grana* 35(1):1–15
- Rudall PJ, Bateman RM (2007) Developmental bases for key innovations in the seed-plant microgametophyte. *Trends Pl Sci* 12(7):317–326
- Schols P, Dessein S, D'hondt C, Huysmans S, Smets E (2002) Carnoy: a new digital measurement tool for palynology. *Grana* 41:124–126

- Schols P, Es K, D'hondt C, Merckx V, Smets E, Huysmans S (2004a) A new enzyme-based method for the treatment of fragile pollen grains collected from herbarium material. *Taxon* 53:777–782
- Schols P, D'hondt C, Geuten K, Merckx V, Janssens S, Smets E (2004b) Morphocode: coding quantitative data for phylogenetic analysis. *PhyloInformatics* 4:1–4
- Schols P, Wilkin P, Furness CA, Huysmans S, Smets E (2005) Pollen evolution in Yams (*Dioscorea*: Dioscoreaceae). *Syst Bot* 30(4):750–758
- Selling OH (1947) Studies in the Hawaiian pollen statistics, Part II. The pollens of the Hawaiian phanerogams. *Bulletin of the Bishop Museum, Honolulu* 38:1–360
- Shah CK (1962) Pollen development in some members of the Cyperaceae. *Plant embryology—a symposium*. CSIR, New Delhi, pp 81–93
- 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. *Amer J Bot* 90:1071–1086
- Simpson DA, Muasya AM, Alves M, Bruhl JJ, Dhooge S, Chase MW, Furness CA, Ghamkhar K, Goetghebeur P, Hodkinson TR, Marchant AD, Nieuborg R, Reznicek AA, Roalson EH, Smets E, Starr JR, Thomas WW, Wilson KL, Zhang X (2008) Phylogeny of Cyperaceae based on DNA sequence data—a new *rbcL* analysis. In: *MonocotsIII/Grasses IV*. Claremont, CA: Aliso 23: 72–83
- Skvarla JJ, Rowley JR, Hollowell VC, Chissoe WF (2003) Annulus-pore relationship in Gramineae (Poaceae) pollen: the pore margin of *Pariana*. *Amer J Bot* 90:924–930
- Smith-White S (1959) Pollen development patterns in the Epacridaceae. *Proc Linn Soc NSW* 84:8–35
- Strandhede SO (1973) Pollen development in *Eleocharis Palustris* Group (Cyperaceae). 2. Cytokinesis and microspore degeneration. *Bot Not* 126:255–265
- Tanaka N, Uehara K, Murata J (2004) Correlation between pollen morphology and pollination mechanisms in the Hydrocharitaceae. *J Pl Res* 117:265–276
- Thiele K (1993) The holy grail of the perfect character: the cladistic treatment of morphometric data. *Cladistics* 9:275–304
- von Ubisch G (1927) *Entwicklungsgeschichte der Antheren*. *Planta* 3:490–495
- Van Wichelen J, Camelbeke K, Chaerle P, Goetghebeur P, Huysmans S (1999) Comparison of different treatments for LM and SEM studies and systematic value of pollen grains in Cyperaceae. *Grana* 38:50–58
- Vinckier S, Huysmans S, Smets E (2000) Morphology and ultrastructure of orbicules in the subfamily Ixoroideae (Rubiaceae). *Rev Palaeobot Palynol* 108:151–174
- Vinckier S, Smets E (2001) The potential role of orbicules as a vector of allergens. *Allergy* 56:1129–1136
- Vinckier S, Smets E (2002) Morphological and ultrastructural diversity of orbicules in relation to evolutionary tendencies in Apocynaceae s.l. *Ann Bot* 90:647–662
- Vrijdaghs A, Goetghebeur P, Smets E, Muasya AM (2006) The floral scales in *Hellmuthia* (Cyperaceae, Cyperoideae) and *Paramapania* (Cyperaceae, Mapanioideae), an ontogenetic study. *Ann Bot* 98:619–630
- Wodehouse RP (1935) *Pollen grains. Their structure identification and significance in science and medicine*. Hafner, New York