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Comparison of different treatments for LM and SEM studies and systematic value of pollen grains in Cyperaceae

JEROEN VAN WICHELEN, KONRAED CAMELBEKE, PETER CHAERLE, PAUL GOETGHEBEUR and SUZY HUYSMANS

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The pollen grain morphology of 30 species of 27 genera from the four subfamilies of the Cyperaceae have been studied with LM and SEM. Several methods were tested in order to find the optimal treatment for the delicate Cyperaceae pollen grains. For LM, treatment with wetting agent Agepon and KOH yielded the best results, while critical point drying (CPD) after fixation with alcohol proved to be the best method for studying pollen grains of Cyperaceae with SEM.

Our results show that the Mapanioideae have a pollen grain type (only one distal ulcus) clearly different from the other Cyperaceae. Representatives of the examined Sclerioideae and Caricoideae show a similar pollen grain type (mostly one distal ulcus and three lateral pores) while in the Cyperoideae different pollen grain types are found.

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Up till now only fragmentary data on the morphology of pollen grains of Cyperaceae have been published. These data are often based on few genera or species and the resulting typologies are insufficient to answer the question whether pollen grain morphological characters could be of taxonomic value in the Cyperaceae. The aim of this study was (1) to experiment with different techniques in order to find the optimal treatment for morphological investigation of the fragile Cyperaceae pollen grains and (2) to test the systematic value of pollen grain characters in the family and to identify the taxonomic level on which they are useful.

Throughout this article we have consistently used the term “pollen grain”. However, it should be noted that these “pollen grains” are in fact degraded tetrads, in which 3 out of 4 nuclei degenerate and usually they are called pseudomonads (Selling 1947) or cryptotetrads (Erdtman 1952). This formation of pseudomonads is a feature almost unique among angiosperm pollen grain development, but the special ontogeny has no real effect on the nature of the pollen grain wall. Pollen mother cells are enveloped in callose layers and there is a polymerisation of sporopollenin in the wall (see Dunbar 1973). It is therefore, in our opinion (see also Erdtman 1966), correct to use the term aperture, keeping in mind that it concerns pseudomonads. However, as we have done no ontogenetic studies, we here use descriptive, morphological terms for the type of apertures observed, following the terminology of Punt et al. 1994. The terms used here are “aperture”, a region of the sporoderm that is thinner than the remainder of the sporoderm; “pore”, a more or less circular aperture; “colpus”, an elongated aperture; and “ulcus”, a rounded aperture situated at the distal pole of the

pollen grain. For more information on the ontogeny of Cyperaceae pollen grains we refer to Shah (1962, 1967), Dunbar (1973), Strandhede (1973) and Makde (1982).

Several authors tried to classify the pollen grains of the Cyperaceae into different morphological types on the basis of their shape, size and most of all the number and type of apertures.

Shah (1962) stated that pollen grains of Cyperaceae have a single distal ulceroid aperture. Wodehouse (1965) noticed a single, poorly defined, irregularly shaped germ pore at the large end of the pollen grain. Erdtman (1966) pointed out that the aperture type with one ulceroid aperture at the thick end and three lateral, ± faintly marked poroid or elongate apertures, is the most frequent type within the Cyperaceae (the *Carex* – type). He also stated that the genus *Mapania* and several others differ from the rest of the Cyperaceae because mapanioid pollen grains are spheroidal and only have one distinct aperture, an ulcus.

Haines & Lye (1983) translated this distinction into two major types: the *Mapania* – type (found only in tribe Hypolytreae) and the *Carex* – type (found in the vast majority of sedges).

Koyama (1961) distinguished three pollen grain types within the Cyperaceae: (a) apple – shaped, 1+3 or 1+6 aperturate (most of the Cyperaceae); (b) spheroidal, polyforate (representatives of the genus *Machaerina*); (c) spheroidal, inaperturate (representatives of the genus *Hypolytrum*).

Padhye & Makde (1980) distinguished two types: the *Cyperus* – type with one distal colpus and the *Carex* – type as defined by Erdtman (1952). The *Cyperus* – type was found among members of the following genera: *Bulbostylis*,

Courtoisina, *Cyperus* p.p., *Eleocharis*, *Fimbristylis*, *Rhynchospora* and *Scirpus* s.l.(= *Schoenoplectus*). The *Carex* – type was found only in *Cyperus imbricatus* and members of *Pycnus* and *Lipocarpa*.

Dahlgren & Clifford (1982) stated that the majority of the pollen grains of Cyperaceae are ulcerate, with some of them still presenting 3 lateral pores or long thinnings.

Fernandez (1987) distinguished 6 types of pollen grains among the Cyperaceae but her typology is based on a study of 19 species only. The following types were recognised: *Cyperus longus* – type (pantoaperturate with 1 distal pore and 4–6 colpi); *Cyperus michelianus* – type (pantoaperturate with several pores); *Cladium mariscus* – type (inaperturate); *Schoenus nigricans* – type (pantoaperturate with 1 distal pore and 4–6 equatorial colpi but with dimensions different from the first type); *Carex flacca* – type (pantoaperturate with 1 distal pore and 4–5 colpi); *Carex hallerana* – type (pantoaperturate with 1 distal pore and 4–5 equatorial pores).

Bruhl (1995) distinguished two groups of pollen grain types within the Cyperaceae, either with few (1–6) apertures (some 20 genera) or with many (>6) apertures (only *Baumea*, *Machaerina* and *Tricostularia*).

MATERIAL AND METHODS

We have studied the morphology of pollen grains of Cyperaceae by means of light microscopy (LM) and scanning electron microscopy (SEM) from 30 species (see Specimens Investigated) belonging to 27 genera in 16 tribes of the 4 subfamilies of Cyperaceae (classification following Goetghebeur 1986).

Most of the specimens are deposited in the Herbarium of Gent University (GENT) according to Specimens Investigated list. Fertile anthers were selected by means of a stereoscopic binocular, they were separated with a needle and preserved in small paper bags.

For a clear observation of the structures in the pollen grain wall, it is important to remove the cytoplasm. The standard technique to do so is acetolysis as described by Erdtman (1960, 1969). However, according to Erdtman (1969) the pollen grains of some plants are so severely damaged or destroyed by acetolysis that other methods need to be used. Sultan et al. (1994) mentioned that this technique causes the collapse of the pollen grains of Cyperaceae. Padhye & Makde (1980) used Erdtman's acetolysis with a few modifications suggested by Nair (1960). Another technique is the use of KOH (Fernandez 1987).

For the present study, five methods were tested. We examined (1) untreated pollen grains, (2) pollen grains treated with Agepon only, (3) pollen grains acetolysed according to the method of Reitsma (1969), (4) pollen grains treated with KOH and finally (5) critical point drying (CPD) prior to SEM observation.

Untreated

For LM the pollen grains were put on a slide in a drop of distilled water and covered with a cover slip. For SEM, the dry pollen grains were directly placed on a stub.

Treatment with Agepon

In order to restore the original shape of the dehydrated and shrivelled pollen grains, they were rehydrated in an Agepon solution in distilled water (1:200) on a watch glass [Agepon (AGFA 69552) is used as wetting agent in the development of photographic films]. The duration of this treatment is to be determined empirically. We used a standard time of ± 15 minutes.

Acetolysis according to Reitsma (1969)

As acetolysis was likely to be too strong for the pollen grain wall of Cyperaceae, we used two conditions:

- normal procedure with heating for 6 min in a water bath at 95°C (strong reaction)
- without heating, test tubes at room temperature for 6 min (milder reaction)

KOH method

Four conditions were tried out:

- anthers immediately in 10% KOH for ± 10 min
- anthers first in Agepon (1:200) for 15 min, then in 10% KOH for ± 10 min
- anthers first in Agepon (1:200) for 15 min, then in 10% KOH for ± 30 min
- anthers first in Agepon (1:200) for 30 min, then in 10% KOH for ± 30 min

The anthers need to be immersed, otherwise they keep floating on the water surface due to surface tension.

Critical point drying for SEM

For this method we used pollen grains of *Tetraria compar* (Fig. 20). Before the critical point drying, the pollen grains received a number of treatments. Anthers with pollen grains were put in small permeable containers. At the bottom of these small containers, gauze with pores of 5 μ m was fitted to make sure not to lose any pollen grains. For rehydration of the pollen grains, these containers were put in Agepon and then washed with distilled water. Next, the containers were passed through an alcohol series (25–50–75–94–100%) for dehydration. Each step of the treatment took at least 2 hours. The best results were obtained after 4 hours in 25%, overnight in 50%, 4 hours in 75%, 4 hours in 94% and overnight in 100% alcohol in order to complete the dehydration.

Four different conditions were tested before going through the alcohol series:

- anthers not treated
- anthers fixed in formaldehyde 3.5% (overnight), then washed with distilled water
- anthers fixed in glutaraldehyde 2% (overnight), then washed with distilled water
- anthers treated with KOH 10% for 10 min, then washed with distilled water

For LM the pollen grains were mounted in Kaiser's glycerine – gelatine for microscopy (Merck nr 9242).

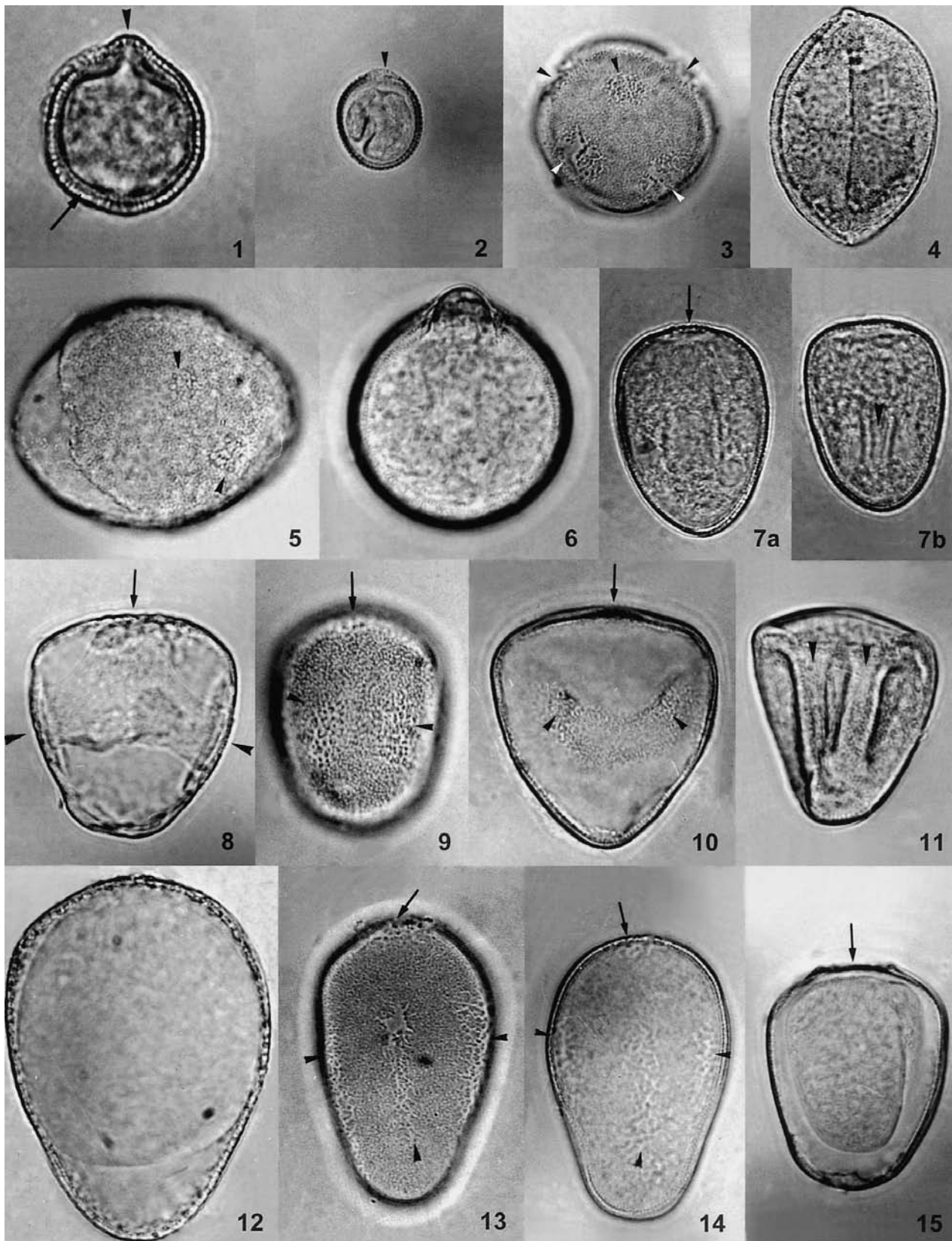
The shape, the dimensions and the number and position of ectoapertures were examined. Length and width dimensions are based on LM measurements of 10 pollen grains for each preparation. In this study, the length is equal to the distance from the distal ulcus up to the proximal pole. As for the width, the largest distance possible was measured.

Light microscopic observations were made with a Zeiss axioskop equipped with a MC 100 photo unit and a Leitz diaphan microscope fitted with a Wild MPS 51 photo camera. Pictures were taken with a 100X oil immersion objective. Scanning electron microscopic observations were executed on a Jeol JSM – 840.

RESULTS

Comparison of methods

The lateral apertures, if present, are difficult to observe in untreated pollen grains, due to the presence of cytoplasm. Very often the pollen grains are shrunken, especially when



herbarium material is used, and do not correspond with their natural shape. Treatment with Agepon was effective in 18 out of 25 cases. The pollen grains of *Lagenocarpus rigidus* (Fig. 4), all of which were still collapsed after 15 min in Agepon, were still collapsed after an hour and a half in Agepon. For *Androtrichum trigynum* (Figs. 7a, 7b) the treatment with Agepon even appeared to be disadvantageous. For this species, the pollen grains treated with distilled water looked better than those treated with Agepon. In 8 cases out of 10 the treatment with KOH yield better results. Only for *Androtrichum trigynum* and *Sphaerocyperus erinaceus* (Fig. 11) did KOH not affect the result positively after 30 min. For *Chrysitrix* cf. *capensis* (Figs. 17, 18) more normal pollen grains were found in the preparations treated only with 10% KOH for 10 min, than in preparations treated with Agepon and then followed by 10% KOH for 10 min or 30 min. Acetolysis at 95°C turned out to be destructive to the pollen grains, which were partly dissolved and collapsed. Also during the “soft” acetolysis, at room temperature, many pollen grains were collapsed, but enough of them with normal shape were left to examine. As for SEM, the acetolysed pollen grains also seemed to be collapsed. Regardless of the pre-treatment the pollen grains received, the results of the critical point drying were more or less the same: the pollen grains of *Tetraria compar* (Fig. 20) seemed to have adopted their natural shape again. As for *Machaerina flexuosa* (Fig. 3), the dimensions appeared to differ very strongly according to the method used. Only the dimensions of pollen grains treated solely with Agepon were determined. Pollen grains of this specimen treated with KOH seemed to be much larger.

Pollen grain morphology

The morphological observations are summarised in Table I. All representatives of the Mapanioideae examined had only one distal ulcus. The representatives of *Hypolytrum* (Figs. 1, 2) and *Mapania* (Fig. 6) have spheroidal pollen grains, while those of *Chrysitrix* cf. *capensis* (Fig. 17) are oblong obovoid.

The pollen grain type of all examined representatives of the Sclerioideae apparently corresponds with the pollen grain type of *Carex serotina* (Fig. 10) of the Caricoideae. This type corresponds with the basic type of Erdtman (1966). Except for *Lagenocarpus rigidus* (Fig. 4) all examined Sclerioideae and Caricoideae show broadly obovoid pollen grains. *Lagenocarpus rigidus* is exceptional because of the deviating elliptic to slightly citriform shape of its pollen grain.

All the pollen grains of the examined species of the Cyperoideae had a distal ulcus and most species had pollen grains with several lateral pores or colpi. The prevailing

shape is broadly obovoid. In *Isolepis fluitans*, *Kyllinga elatior* and *Rhynchospora tenuis*, only one distal ulcus was observed. In this subfamily, *Fuirena hirsuta* (Fig. 5) and *Machaerina flexuosa* (Fig. 3) are also notable because of the many pores and deviating shape, respectively broadly elliptic and circular. During this research, only ulci, colpi and pores were perceived as apertures.

The smallest pollen grains were found in *Hypolytrum poecilolepis* (Fig. 2; average length: 17.8 µm, average width: 16.4 µm), the largest pollen grains were found in *Bolboschoenus nobilis* (Fig. 12; average length: 60.5 µm, average width: 43.4 µm). Furthermore, *Hypolytrum longifolium* ssp. *longifolium* (Fig. 1), *Hypolytrum poecilolepis* (Fig. 2), *Fimbristylis complanata*, *Sphaerocyperus erinaceus* (Fig. 11) and *Kyllinga elatior* were notable because of their relative thick exine. In *Androtrichum trigynum* (Fig. 7 a, b), *Cyperus exaltatus* (Fig. 19) and *Sphaerocyperus erinaceus* (Fig. 11) longitudinal ridges were perceived.

DISCUSSION AND CONCLUSIONS

As the pollen grains of most Cyperaceae have a thin exine, acetolysis is a too drastic method, which results in completely dissolved or collapsed pollen grains. Instead, a treatment with Agepon and KOH seems to be an appropriate method for LM research. For SEM, critical point drying after fixation with alcohol appears to be the most suitable method.

We have studied 30 species belonging to 27 genera in 16 tribes of the 4 subfamilies of Cyperaceae. However, as we have studied only a small number of species, caution is necessary when drawing systematic conclusions. From this study, it appears that the Mapanioideae have a pollen grain type different from the other Cyperaceae, due to the presence of only 1 distal ulcus, the frequently spheroidal shape and the more or less thick wall. This is in agreement with several authors. Erdtman (1966) mentioned *Chrysitrix capensis*, *Hypolytrum schraderianum* and *Mapania amplivaginata*. However, his observation of “normal” pollen grains in *Lepironia* is rather strange and contradicts his assumption of the “different” Mapanioideae, because *Lepironia* is a clear, although specialised, member of the Mapanioideae (Goetghebeur 1986). In spite of the collapsed state of all pollen grains of *Lepironia articulata*, no apertures except the distal ulcus could be observed in the present study. Haines & Lye (1983) mentioned a “*Mapania* – type” of pollen grain found in *Mapania*, *Thoracostachyum* and possibly other members of the tribe Hypolytreae. Bruhl (1995) referred to Guppy (unpublished data) and Raynal (unpublished data), who observed uniaperturate pollen grains in *Hypolytrum* and *Mapania*. Bruhl therefore suggested that uniaperturate pollen

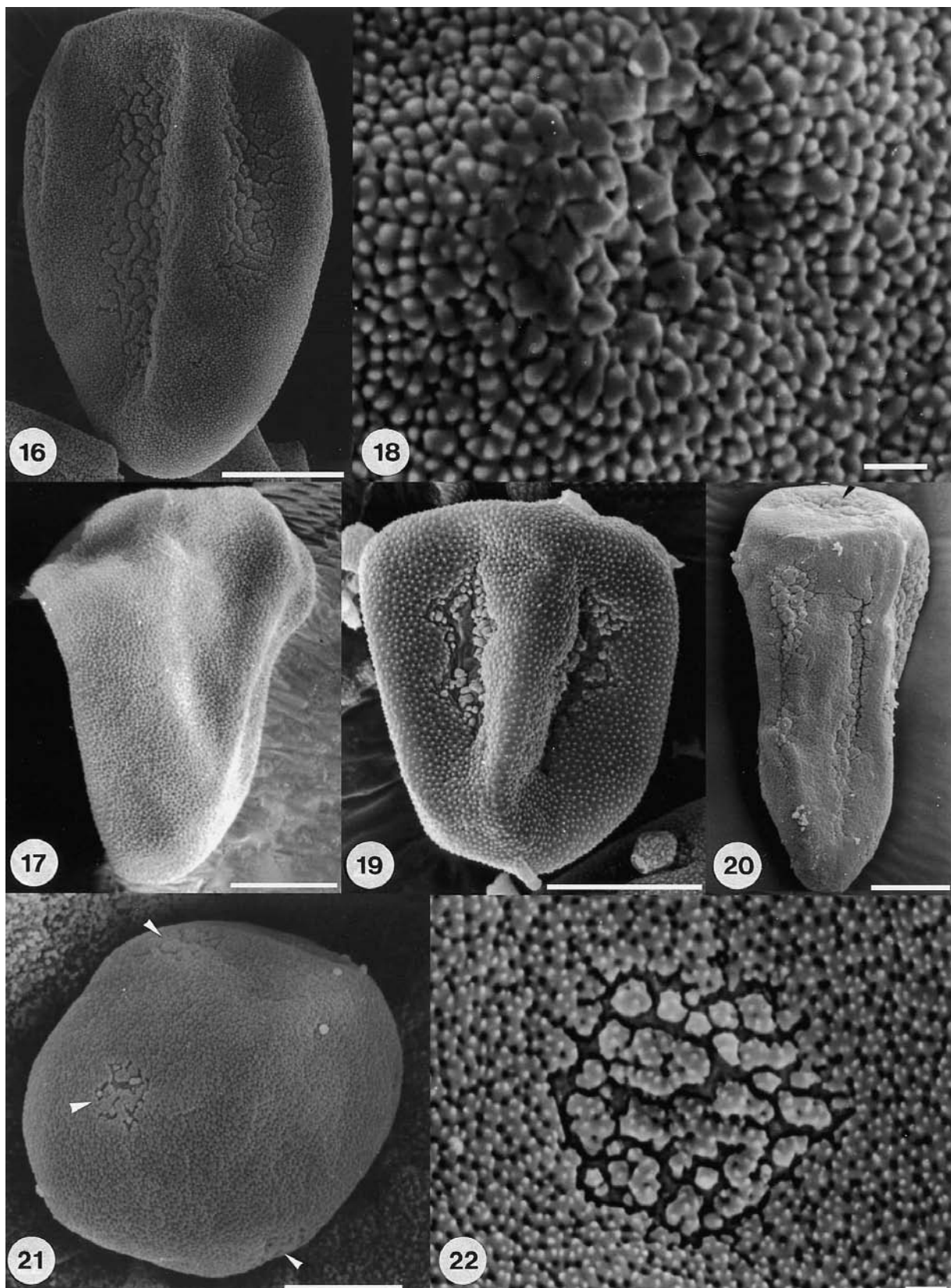
Figs. 1–15. *Hypolytrum longifolium* ssp. *longifolium*: note thick exine (arrow) and distal ulcus (arrowhead). (2) *Hypolytrum poecilolepis*: note distal ulcus (arrowhead). (3) *Machaerina flexuosa*: note 5 pores in focus (arrowheads). This specimen was treated with KOH. (4) *Lagenocarpus rigidus*. (5) *Fuirena hirsuta*: note 2 lateral pores in focus (arrowheads). (6) *Mapania assimilis*. (7a, b) *Androtrichum trigynum*: note distal ulcus (arrow) and the existence of ridges (arrowhead). (8) *Eriophorum spissum*: note distal ulcus (arrow) and lateral pores (arrowheads). (9) *Eleocharis dombeyana*: note distal ulcus (arrow) and 2 colpi (arrowheads). (10) *Carex serotina*: note distal ulcus (arrow) and 2 lateral pores in focus (arrowheads). (11) *Sphaerocyperus erinaceus*: note the existence of ridges (arrowheads). This specimen is a bit collapsed at the left. (12) *Bolboschoenus nobilis*. (13) *Schoenus nigricans*: note distal ulcus (arrow) and 3 colpi (arrowheads). (14) *Bulbostylis megastachys*: note distal ulcus (arrow) and 3 colpi (arrowheads). (15) *Scleria amazonica*: note distal ulcus (arrow). All pictures by a magnification of 1000×.

Table I. Summary of some important pollen morphological characters for the species studied.

In the second column the treatment is specified: **1.** untreated; **2.** treatment with Agepon; **3.** acetolysis according to Reitsma (1969): a) with heating at 95°C and b) without heating (room temperature); **4.** treatment with KOH: a) in KOH 10% for 10 min, b) in Agepon for 15 min + in KOH 10% for 10 min, c) in Agepon for 15 min + in KOH 10% for 30 min and d) in Agepon for 30 min + in KOH 10% for 30 min; **5.** critical point drying: a) without pretreatment, only Agepon, b) Agepon + fixation in formaldehyde 3.5%, c) Agepon + fixation in glutaraldehyde 2% and d) Agepon + KOH 10%.

Species	Treatment (see text)	Shape	Length (µm)	Width (µm)	Apertures number and type	Fig.
Mapanioideae						
<i>Hypolytrum longifolium</i> ssp. <i>longifolium</i>	3b LM + SEM	Circularis Thick exine (± 10 normal shaped)	25.0–(27.2)–31.0	22.0–(26.2)–31.0	Distal ulcus observed only	1
<i>Hypolytrum poecilolepis</i>	2 LM	Circularis thick exine (2/3 collapsed)	16.3–(17.8)–18.8	15.0–(16.4)–17.5	1 distal ulcus	2
<i>Mapania assimilis</i>	2 LM	Circularis	40.0–(42.9)–47.0	38.0–(41.5)–45.0	1 distal ulcus	6
<i>Lepironia articulata</i>	2 LM	All collapsed	–	–	Distal ulcus observed only	–
<i>Chrysitrix</i> cf. <i>capensis</i>	1 SEM 2 LM 4 a,b,c LM	Lengthened obovatus (many collapsed)	33.8–(45.8)–56.3	28.8–(32.0)–37.5	1 distal ulcus	17, 18
Cyperoideae						
<i>Scirpus atrocinctus</i>	1 SEM 2 LM	All collapsed	–	–	1 distal ulcus + lateral colpi	–
<i>Eriophorum spissum</i>	2 LM	Late obovatus	32.5–(37.9)–43.8	30.0–(33.6)–36.3	1 large distal ulcus + 4–5 lateral pores	8
<i>Fuirena hirsuta</i>	2 LM	Late ellipticus	40.0–(45.0)–48.8	38.8–(41.9)–47.5	Pantoaperturate 7–8 pores	5
<i>Bolboschoenus nobilis</i>	2 LM 4c LM	Late obovatus	51.3–(60.5)–67.5	36.3–(43.4)–47.5	1 distal ulcus + at least 3 lateral pores	12
<i>Eleocharis dombeyana</i>	2 LM 4c LM	Late obovatus	31.0–(34.4)–41.0	25.0–(27.2)–28.0	1 distal ulcus + 3–5 lateral colpi	9
<i>Fimbristylis complanata</i>	2 LM	Obovatus thick exine (only few not collapsed)	30.0–(31.3)–33.9	17.5–(19.5)–23.9	Clear apertures not observed	–
<i>Bulbostylis megastachys</i>	1 SEM 2 LM 4c LM	Pyriformis	46.3–(48.9)–51.3	27.5–(31.6)–33.1	1 distal ulcus + 4–6 lateral colpi	14, 16
<i>Ficinia laciniata</i>	2 LM	Obovatus	40.0–(42.4)–43.8	23.8–(26.2)–30.0	1 distal ulcus + 3 (4) lateral pores	–
<i>Isolepis fluitans</i>	2 LM 4d LM	All collapsed	–	–	At least 1 distal ulcus	–
<i>Androtrichum trigynum</i>	1 LM 2 LM 4d LM	Late obovatus Ridges observed (many collapsed)	33.0–(35.8)–39.0	25.0–(28.5)–33.0	Mostly distal ulcus observed only, sometimes 1 distal ulcus + 4 pantoapertures (pores) (but difficult to observe)	7a, 7b
<i>Cyperus exaltatus</i>	3a LM + SEM	Late obovatus Ridges observed (only 2 ± normal shaped)	30.0	24.0	1 distal ulcus + 3–4 lateral colpi	19

Species	Treatment (see text)	Shape	Length (µm)	Width (µm)	Apertures number and type	Fig.
<i>Sphaerocyperus erinaceus</i>	2 LM 4d LM	Late obovatus ridges observed thick exine (only few normal shaped)	33.0–(36.2)–39.0	31.0–(33.2)–35.0	1 distal ulcus+at least 3 (4) colpi observed	11
<i>Kyllinga elatior</i>	2 LM	Late obovatus thick exine (many collapsed)	22.5–(24.6)–27.5	17.5–(20.4)–22.5	Only distal ulcus observed	–
<i>Blysmus rufus</i>	2 LM	Late obovatus (only few normal shaped)	46.6	40.9	1 distal ulcus + 3 lateral colpi	–
<i>Trachystylis stradbrokenensis</i>	2 LM	All collapsed	–	–	–	–
<i>Rhynchospora tenuis</i>	2 LM SEM	Obovatus	24.0–(26.4)–29.0	18.0–(19.9)–21.0	LM: only distal ulcus observed, 1 SEM: most likely few lateral colpi	–
<i>Schoenus nigricans</i>	1 LM 2 LM	Obovatus	43.0–(46.6)–50.0	30.0–(33.4)–40.0	1 distal ulcus + 3–5 colpi	13
<i>Tetraria compar</i>	4c LM	Obovatus	52.4	25.5	1 distal ulcus+at least 3 colpi	20
<i>Machaerina flexuosa</i>	5 a–d SEM 2 LM 4c LM	Circularis	27.0–(29.5)–34.0	25.0–(28.4)–30.0	8–10 pores Pantoperturate	3
Sclerioideae						
<i>Lagenocarpus rigidus</i>	2 LM 4d LM	Ellipticus – weakly citriformis (only few ± normal shaped)	40.6	27.5	Only 1 large pore observed	4
<i>Afrotrilepis pilosa</i>	1 LM 2 LM 4c LM 4d LM	Late obovatus	29.0–(33.9)–37.0	25.0–(27.2)–29.0	1 distal ulcus + 3 lateral pores	–
<i>Scleria amazonica</i>	3a SEM 3a LM 1 SEM 2 LM	Late obovatus	35.0–(37.6)–40.0	30.0–(32.8)–37.5	1 clear distal ulcus+at least 3 poorly visible lateral pores	15
<i>Scleria bracteata</i>		All collapsed	–	–	–	–
<i>Scleria bulbifera</i>		Late obovatus	34.0–(36.7)–39.0	27.0–(31.0)–34.0	1 large distal ulcus + 3 poorly visible lateral pores	21, 22
Caricoideae						
<i>Carex serotina</i>	3b LM + SEM	Late obovatus (many collapsed)	44.0–(47.0)–52.0	33.0–(38.6)–43.0	1 distal ulcus + 3 poorly visible lateral pores	10



Figs. 16–22. *Bulbostylis megastachys*: note 2 clear colpi. (17, 18) *Chrysitrix* cf. *capensis*: note distal ulcer in detail (Fig. 18) and the absence of lateral apertures (Fig. 17). (19) *Cyperus exaltatus*: note ridges and 2 clear colpi. (20) *Tetraria compar*: note distal ulcer (arrowhead) and 2 clear colpi [specimen treated with treatment 5a (see results)]. (21, 22) *Scleria bulbifera*: note 3 lateral pores (Fig. 21, arrowheads) and lateral pore in detail (Fig. 22). Scale bar = 10 μ m, and 1 μ m in Figs. 18, 22.

grains could be recognised as a separate group. Erdtman (1952) suspected that the mono – aperturate mapanioid pollen grains do not belong in the category of pseudomonads, but Koyama (1961) affirmed their tetrad nature. However, there is still no agreement on the nature of mapanioid pollen grains, and ontogenetic studies are awaited.

Pollen grains of representatives of the Sclerioideae resemble those of the representatives of the Caricoideae while pollen grains of the Cyperoideae seem to combine $\pm$ clearly different types. In our opinion, the Cyperoideae can be subdivided based on their pollen grain morphology. For instance, *Fuirena hirsuta* (Fig. 5) and *Machaerina flexuosa* (Fig. 3) can be separated from the rest of the subfamily, because of their spheroidal pollen grains with many pores whereas the rest of the subfamily has either one distal ulcus and mostly 3–6 colpi or has one distal ulcus and 3–5 lateral pores. The pollen grain morphology of these two species corresponds with that of the *Cyperus michelianus* – type from Fernandez (1987). Further research of these genera should show that we are not dealing with exceptions in this case. Anyway, Padhye & Makde (1980) mentioned two *Fuirena* species (*F. ciliaris* and *F. wallichiana*) which are monocolpate. Also Huang & Chung (1971) mentioned the same *F. ciliaris* and *F. umbellata* as monocolpate. Other differences between our observations and literature do apply to *Eleocharis* which has a 1 – aperturate type of pollen grain according to Strandhede (1965 cit. in Shah 1972), Huang & Chung (1971) and Padhye & Makde (1980); *Bulbostylis*, *Rhynchospora*, and *Scirpus* which Huang & Chung (1971) and Padhye & Makde (1980) found to be 1 – aperturate; *Scleria* which Huang & Chung (1971) found to be 1 – aperturate and *Eriophorum* which Padhye & Makde (1980) found to be monocolpate. Data on the pollen grain type of *Eleocharis* are contradictory in the literature: Padhye & Makde (1980) mentioned the monocolpate pollen grain type for *Eleocharis capitata* while Sultan et al. (1994) observed 4 – aperturate pollen grains in this species. Our results do agree most the results of Sultan et al. (1994), while most of the data presented by Padhye and Makde (1980) contradict our results. They state that their monocolpate pollen grain type is the dominant type in Cyperaceae, but this statement is possibly based on the study of collapsed or insufficiently rehydrated pollen grains as we have never encountered this type in our study. *Cyperus* and *Carex* have different types of pollen grain morphology according to the literature.

Perhaps, for the Cyperoideae a distinction can be made between species with lateral pores and these with lateral colpi.

The presence of ridges on the pollen grain wall of *Androtrichum trigynum* (Figs. 7a, b), *Cyperus exaltatus* (Fig. 19) and *Sphaerocyperus erinaceus* (Fig. 11) is exceptional. Possibly the pollen grains from these specimens were not yet mature or they did not rehydrate sufficiently. Makde (1982) namely observed folds during development, which introduced 3–5 concavities over the grain, and after rest they disappeared at a later stage of pollen grain development. Whereas Wodehouse (1965) stated that dehydration and shrinkage of the pollen grains is mostly accompanied by the formation of three or four long lateral concavities and a small distal one.

A species, which is rather exceptional, because of its strongly deviating shape, is *Lagenocarpus rigidus* (Fig. 4).

This observation should be reconfirmed because few pollen grains were rehydrated sufficiently.

Further research is necessary, but it is clear from this study that pollen grain shape, number and type of apertures and pollen grain dimensions (if measured under identical conditions) are useful characters which can have taxonomic value on subfamily level.

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SPECIMENS INVESTIGATED

Mapanioideae

Hypolytreae

Hypolytrum longifolium (Rich.) Nees ssp. *longifolium*; Palacios et al. 9039 (GENT)

Hypolytrum poecilolepis Nelmès; Malaisse 2505 (GENT)

Mapania assimilis T. Koyama; Herrera 2299 (GENT)

Chrysitricheae

Lepironia articulata (Retz.) Domin; Wilson 7697 (GENT)

Chrysitrix cf. *capensis* L.; Hugo 1808 (GENT)

Cyperoideae

Scirpeae

Scirpus atrocinctus Fernald; Brisson 76/498 (GENT)

Eriophorum spissum Fernald; Mc. Neilus 89/222 (GENT)

Fuireneae

Fuirena hirsuta (Berg.) P.L. Forbes; Edwards 1138 (GENT)

Bolboschoenus nobilis (C.B. Clarke) Goetgh. & D.A. Simpson; Hess 52/1958 (GENT)

Eleocharideae

Eleocharis dombeyana Kunth; Goetghebeur 4812 (GENT)

Abildgaardieae

Fimbristylis complanata (Retz.) Link; Hess 52/168 (GENT)

Bulbostylis megastachys (Ridl.) C.B. Clarke; Hess 51/170 (GENT)

Ficinieae

Ficinia laciniata Nees; Edwards 924 (GENT)

Isolepis fluitans (L.) R. Br.; Lægård 16025 (GENT)

Cypereae

Androtrichum trigynum (Spreng.) H. Pfeiff.; Goetghebeur 4764 (GENT)

Cyperus exaltatus Retz.; Kilian 1812 (GENT); Lobin 6666 (GENT)

Sphaerocyperus erinaceus (Ridl.) Lye; Symoens 7202 (GENT)

Kyllinga elatior Kunth; Malaisse & Goetghebeur 190 (GENT)

Dulichieae

Blysmus rufus (Huds.) Link; Pahnsh 356 (GENT)

Arthrostylideae

Trachystylis stradbokensis (Domin) Kük.; Wilson & Sharpe 8175 (GENT)

Rhynchosporeae

Rhynchospora tenuis Link; Viane 1211 (GENT)

Schoeneae

Schoenus nigricans L.; Vanhecke 2213 (GENT)

Tetraria compar (L.) Lestib.; Taylor 9996 (GENT)

Machaerina flexuosa (Boeck.) J. Kern; Miller & Randrianasolo 4382 (GENT)

Sclerioideae

Cryptangieae

Lagenocarpus rigidus (Kunth) Nees; Maas & Westra 4035 (GENT)

Trilepideae

Afrotrilepis pilosa (Ridl.) Gilly; Goetghebeur 5182 (GENT)

Sclerieae

Scleria amazonica Camelbeke, M.T. Strong & Goetgh.; Romero et al. 2076 (MO)

Scleria bracteata Cav.; Steyermark et al. 85134 (VEN)

Scleria bulbifera A. Rich.; Læggaard 15808 (GENT)

Caricoideae

Cariceae

Carex serotina Mérat; Kruyt & van Alphen 6375 (GENT)

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