

Morphological and Ultrastructural Diversity of Orbicules in Gentianaceae

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Minute granules of sporopollenin, called orbicules, can be observed on the innermost tangential and/or radial walls of secretory tapetum cells. Orbicules were investigated in 53 species of 34 Gentianaceae genera using light microscopy, scanning electron microscopy and transmission electron microscopy. This selection covered all different tribes and subtribes recognized in Gentianaceae (87 genera, ± 1650 species). Orbicules were found in 38 species (23 genera) distributed among the six tribes recognized in Gentianaceae. The orbicule typology is based on those described previously in Rubiaceae. Of the six orbicule types described previously, Type II orbicules are lacking. Type III orbicules are most common (17 species). *Hockinia* Gardner is the only representative with Type I orbicules. The number of representatives with orbicules belonging to the other orbicule types are equally distributed among the species studied: seven species possess Type IV orbicules, six species Type V and six species Type VI. The systematic usefulness of this typology is discussed in comparison with the latest systematic insights within the family, and palynological trends in Gentianaceae. Orbicule data have proven to be useful for evaluating tribal delimitation within Rubiaceae and Loganiaceae *s.l.*; however, they seem not to be useful for tribal delimitation in Gentianaceae. In the tribes Potalieae and Gentianeae orbicule data may be useful at subtribal level.

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Key words: Orbicules, Ubisch bodies, pollen, Gentianaceae, SEM, TEM, morphology, ultrastructure, typology, tapetum, systematics.

INTRODUCTION

In gymnosperms, seed ferns, and anthers of flowering plants, tiny ($<4 \mu\text{m}$) granules may occur on the radial and innermost 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 (Kosmath, 1927). Orbicules were observed for the first time by Rosanoff (1865). Despite their early discovery, little attention was paid to these structures and in many palynological studies they have been overlooked.

Orbicules develop simultaneously with the growing pollen exine and are composed of sporopollenin, similar to the pollen exine. Orbicules may thus serve as a model for the study of sporopollenin biosynthesis and polymerization in comparison with the exine, since orbicules are acellular structures independent of cytoplasmic control (Clément and Audran, 1993a, b, c). Pro-orbicules, which are produced by the endoplasmic reticulum of secretory tapetal cells, are the progenitors of orbicules (Echlin and Godwin, 1968). The pro-orbicules are extruded through the radial and innermost tangential walls of the secretory tapetum cells, at the beginning of the tetrad stage (Christensen *et al.*, 1972; El-Ghazaly and Jensen, 1986; Clément and Audran, 1993a, b, c; Vijayaraghavan and Chaudry, 1993). Pro-orbicules are spherical structures, which are composed of a complex organic mixture. They are surrounded by a glycocalyx. The

deposition of sporopollenin precursors, produced by the protoplast of the secretory tapetum cells on the glycoprotein filaments of the glycocalyx and on the growing pollen exine, starts at the late tetrad stage. The mature orbicule wall is composed of structural and filling elements (Clément and Audran, 1993a, b, c). Proteins, pectins, polysaccharides and glycolipids, organized in a three-dimensional polygonal frame (Rowley, 1990) are part of the structural elements. The filling elements consist of unsaturated lipids and polyphenols (Clément and Audran, 1993a, b, c). At the ultrastructural level, Clément and Audran (1993a, b, c) could distinguish four different zones of electron density in mature orbicules of *Lilium* L. 'Enchantment': (1) an electron-translucent orbicule cavity; (2) a thin and very electron-dense orbicule cavity–wall interface; (3) a homogeneous electron-dense orbicule wall; and (4) a thin, very electron-dense peripheral layer.

Orbicules are considered to be a general feature of species characterized by a secretory tapetum. Huysmans *et al.* (1998) reported, however, that orbicules are lacking in several taxa with such a tapetum. The predominance of the secretory tapetum among land plants was demonstrated by Furness and Rudall (2001). This observation supports the hypothesis of Pacini *et al.* (1985) that the secretory type is plesiomorphic in angiosperms. Species with an amoeboid tapetum, which is believed to be apomorphic, characteristically do not produce orbicules (Sporne, 1973; Pacini *et al.*, 1985), although there are exceptions such as *Gentiana acaulis* L. (Lombardo and Carraro, 1976; Vinckier and

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Smets, 2002a, c). Tiwari and Gunning (1986) reported the presence of sporopollenin granules on the tapetal remnants in species with amoeboid tapeta, generally smaller in size than the orbicules produced by secretory tapeta. Orbicules have been recorded in some Cretaceous (Mesozoic) (Taylor and Alvin, 1984; Osborn *et al.*, 1991; Serbet and Stockey, 1991; Archangelsky and Taylor, 1993) and Miocene (Cenozoic) (Zetter *et al.*, 2002) seed plants which may indicate, along with the proposed plesiomorphic nature of the secretory tapetum (Pacini *et al.*, 1985), that the presence of orbicules is a plesiomorphic feature of seed plants.

During the past 20 years different hypotheses have been suggested to attribute a function to orbicules. However, none of the proposed functions are satisfactorily proven. For a summary of some of the proposed functions see Huysmans *et al.* (1998).

In angiosperms, the ornamentation of the pollen exine and that of the orbicule wall often show striking analogies (Nilsson and Robyns, 1974; El-Ghazaly and Jensen, 1986; Hesse, 1986). These parallels are explained by Hesse (1985) as rooted in the homology of tapetum and sporogeneous tissue. Since ornamentation of the pollen exine offers useful characters for systematics, orbicules might also have taxonomic value. In the subfamily Cinchonoideae of Rubiaceae (Huysmans *et al.*, 1997) an orbicule typology has been worked out based on the morphological and ultrastructural orbicule diversity. It was concluded that orbicule characteristics can be useful for systematics at the generic and tribal level. Apart from the study on Cinchonoideae taxa (Huysmans *et al.*, 1997), the taxonomic usefulness of orbicule characters has so far only been studied in four angiosperm taxa: the genera *Dioscorea* L. (Schols *et al.*, 2001) and *Euphorbia* L. (El-Ghazaly, 1989; El-Ghazaly and Chaudhary, 1993), the Chloanthaceae, now included in Lamiaceae (Raj and El-Ghazaly, 1987), and the order Gentianales (Vinckier *et al.*, 2000; Vinckier and Smets, 2002a, b, c; Vinckier, 2003).

Recently a phylogenetic, genus-level classification of the Gentianaceae, based on cladistic analyses of the *trnL* intron, *matK* and internal transcribed spacer sequence (ITS) data, was presented by Struwe *et al.* (2002). In this new classification, the family Gentianaceae consists of approx. 87 genera with approx. 1615–1688 species distributed among six tribes: Saccifolieae, Exaceae, Chironieae, Helieae, Potalieae and Gentianae (Struwe *et al.*, 2002). It is the third largest family in Gentianales, with Rubiaceae and Apocynaceae *s.l.* being distinctly larger. They have a cosmopolitan distribution from temperate to tropical regions. The representatives of this family are trees, shrubs, vines, or perennial and annual herbs. Flowers are usually bisexual (unisexual in *Veratril* Baill. ex Franch.), hypogynous, 4- or 5-merous, or rarely 3- or 6–16-merous. The calyx is actinomorphic, rarely zygomorphic (*Exacum* L.). Inflorescences are terminal or axillary, and are usually cymose, but less often racemose, capitate, clustered or spicate; the flowers are seldom solitary. Members of the family do not produce latex and stipules are absent (Struwe *et al.*, 2002). Interesting to the scope of the present paper is the highly variable tapetum; both secretory and amoeboid tapetum types occur, as well as very variable pollen exine

characteristics (Nilsson, 1967; Sankara Rao and Chinnappa, 1983). Pollen grains may be released as monads, tetrads or polyads (Nilsson, 2002).

The present study has five major aims: (1) to investigate the presence of orbicules in Gentianaceae; (2) to provide detailed descriptions of the morphology and ultrastructure of orbicules present; (3) to construct an orbicule-typology; (4) to investigate pollen exine characters by examining the possible parallels between pollen exine and orbicule wall ornamentation; and (5) to discuss the systematic usefulness of the orbicule typology in comparison with the latest systematic insights within the family Gentianaceae.

MATERIALS AND METHODS

This study is based on herbarium material: 53 species of 34 Gentianaceae genera were selected for examination (Table 1; Appendix). This selection covered all different tribes and subtribes recognized in Gentianaceae (Struwe *et al.*, 2002).

For scanning electron microscopy (SEM) of orbicules and pollen, dried flowers or buds were rehydrated in the wetting agent Agepon® (Agfa Gevaert, Leverkusen, Germany) for 1–2 h. The anthers were picked out from the flowers and the tips of the anthers were removed with a razor blade to facilitate rehydration of the locules. After dissection, the anthers remained for another hour in the wetting agent. Following dehydration in a graded acetone series, the material was critical point dried (CPD 030, Balzers) and mounted on stubs with double-sided adhesive tape before further preparation. The pollen grains were slightly removed from the opened locules with a fine cactus spine to facilitate observation of the locule surface. The removed pollen grains were collected on the same stubs for further observations. The stubs were sputter coated with gold (SPI-MODULE™ Sputter Coater; SPI Supplies, West Chester, PA, USA). We used a Jeol JSM-6400 microscope, at 10–20 kV, for morphological observations. Comparative size measurements of orbicules were ascertained from SEM micrographs using Carnoy 2-0 (Schols *et al.*, 2002). From each anther investigated, 100 orbicules were measured, and mean values and standard deviations were calculated (Table 1).

For observations using transmission electron microscopy (TEM) anthers of selected flowers were fixed with 2 % glutaraldehyde at pH 7.4, buffered with 0.05 M sodium cacodylate. Selected anthers are embedded in LR-White Resin (Polysciences Inc., Warrington, PA, USA). Prior to embedding in LR-White Resin the material was dehydrated in a graded ethanol series. Semi-thin ($\pm 1 \mu\text{m}$) sections were cut with a Reichert Jung Ultracut E microtome and stained with 0.1 % thionin/0.1 % methylene blue. The semi-thin sections were observed with a Leica DM LB light microscope. The ultra-thin ($\pm 70 \text{ nm}$) sections, on copper grids, were stained with uranyl acetate and lead citrate in a LKB 2168 Ultrastainer and were observed in a Zeiss EM 900 transmission electron microscope at 50–80 kV.

TABLE 1. *Orbicule morphology and ultrastructure of all species studied (classification is according to Struwe et al., 2002)*

Tribe (genera/species)	Subtribe (genera/species)	Species	Size (µm)	Shape	Inden- tations	Aggre- gates	Density	Orbicule ultra-structure						Type	Pollen exine	Figures
								ETC	EDC	EDW	EDP	EDW	EDP			
Saccifoliaceae (5/16–20)		<i>Curtia tenuifolia</i>	–	–	–	–	–	–	–	–	–	–	–	–	mret-perf	6G and H
	Exaceae (6/144–184)	<i>Hockinia montana</i>	0.652 ± 0.179	sp	+	+	a	+	+	+	+	+	+	+	msp-mret-perf	1A and B
		<i>Exacum gracilipes</i>	0.975 ± 0.206	ir	+	+	a	–	–	–	–	–	–	–	stri-perf	
Chironieae (23/159)	Chironiinae (12/106)	<i>Exacum macranthum</i>	1.240 ± 0.244	ir-sgr	+	+	a	+	+	+	+	+	+	+	rug	5E–F
		<i>Exacum oldenlandioides</i>	1.080 ± 0.268	ir-sgr	+	+	a	+	+	+	+	+	+	+	stri-perf	5D
		<i>Sebaea albidiflora</i>	0.451 ± 0.119	aif	+	+	va	–	–	–	–	–	–	–	stri-mret	4C–D
		<i>Sebaea ovata</i>	0.958 ± 0.282	aif	+	+	a	–	–	–	–	–	–	–	stri-mret	4A and B
		<i>Blackstonia perfoliata</i>	0.307 ± 0.163	ir-ro	–	–	a	–	–	–	–	–	–	–	stri-perf	2F–H
		<i>Centaurium serpentinicola</i>	1.151 ± 0.272	(em) ro	–	–	a	–	–	–	–	–	–	–	stri-perf	
	Canscorinae (6/24)	<i>Centaurium spicatum</i>	1.300 ± 0.377	(em) ro	–	–	a	–	–	–	–	–	–	–	stri-perf	5G
		<i>Centaurium tenuiflorum</i>	0.467 ± 0.076	(em) ro	–	–	a	–	–	–	–	–	–	–	stri-perf	
		<i>Chironia pegleriae</i>	0.201 ± 0.064	(em) ro	–	–	a	–	–	–	–	–	–	–	stri-perf	
		<i>Chironia purpurascens</i>	0.210 ± 0.051	(em) ro	–	–	a	–	–	–	–	–	–	–	stri-perf	2A–D
		<i>Eustoma grandiflorum</i>	0.280 ± 0.081	ro	–	–	a	–	–	–	–	–	–	–	stri-perf-mret	2E
		<i>Ixanthus viscosus</i>	0.516 ± 0.126	ro	–	–	va	–	–	–	–	–	–	–	stri-perf-mret	1G and H
Coutoubeinae (5/29)	<i>Sabatia barramii</i>	–	–	–	–	–	–	–	–	–	–	–	–	stri-perf		
	<i>Sabatia brachiata</i>	–	–	–	–	–	–	–	–	–	–	–	–	stri-perf		
	<i>Sabatia quadrangula</i>	–	–	–	–	–	–	–	–	–	–	–	–	stri-perf		
	<i>Canscora decussata</i>	0.189 ± 0.068	(em) ro	–	–	la	–	–	–	–	–	–	–	stri-perf		
Helieae (22/184)	Coutoubeinae (5/29)	<i>Canscora heteroclita</i>	0.208 ± 0.045	(em) ro	–	–	la	–	–	–	–	–	–	–	stri-perf	
		<i>Hoppea fastigiata</i>	0.309 ± 0.083	(em) ro	–	–	va	–	–	–	–	–	–	–	perf-mret	5H
		<i>Coutoubea spicata</i>	–	–	–	–	–	–	–	–	–	–	–	–	tetr: ret-hbr	7A and B
Potalieae (13/154)	Faroinae (9/31)	<i>Schultesia guianensis</i>	–	–	–	–	–	–	–	–	–	–	–	–	tetr: ret	6D
		<i>Schultesia pachyphylla</i>	–	–	–	–	–	–	–	–	–	–	–	–	tetr: stri-ret	
		<i>Calolisianthus pedunculatus</i>	1.229 ± 0.227	ir	+	+	a	–	–	–	–	–	–	–	tetr: ret isl.	4G and H
		<i>Irlbachia purpurascens</i>	–	–	–	–	–	–	–	–	–	–	–	–	hbr, loop-like	
	Lisianthiinae (1/30)	<i>Macroparapea rubra</i>	– (c TEM)	–	–	–	–	–	–	–	–	–	–	–	pol: polar loops	6E and F
		<i>Prepusa montana</i>	2.985 ± 0.772	ro	–	–	la	–	–	–	–	–	–	–	ret-mret	
		<i>Tachia guianensis</i>	0.384 ± 0.100	(em) ro	–	–	a	–	–	–	–	–	–	–	tetr: gr	3C and D
	Lisianthiinae (1/30)	<i>Enicostema verticillatum</i>	–	–	–	–	–	–	–	–	–	–	–	–	psil-gr	3A and B
		<i>Faroa affinis</i>	–	–	–	–	–	–	–	–	–	–	–	–	perf-mret	
		<i>Lisianthus areopolis</i>	0.227 ± 0.048	(em) ir	–	–	a	–	–	–	–	–	–	–	stri-perf-mret	
Potaliinae (3/93)	<i>Lisianthus nigrescens</i>	0.439 ± 0.097	(em) ir	–	–	a	–	–	–	–	–	–	–	ret	6A and B	
	<i>Anthocleista amplexicaulis</i>	0.573 ± 0.144	ir	+	+	a	–	–	–	–	–	–	–	psil-perf	5B	
		<i>Anthocleista scandens</i>	0.873 ± 0.288	ir	+	+	a	–	–	–	–	–	–	perf	5A	

TABLE 1. *Continued*

Tribe (genera/species)	Subtribe (genera/species)	Species	Size (μm)	Shape	Indent- ations	Aggre- gates	Density	Orbicule ultra-structure				Type	Pollen exine	Figures
								ETC	EDC	EDW	EDP			
Gentianeae (17/939–968)	Gentianinae (3/400–403)	<i>Fragaria racemosa</i>	1.512 \pm 0.366	ir	+	+	+	–	–	+	+	V	stri-ret-hbr	5C
		<i>Potalia amara</i>	0.719 \pm 0.323	ir	–	+	a	–	–	–	–	VI	psil-perf	
		<i>Gentiana acaulis</i>	0.376 \pm 0.127	aif	–	+	va	–	–	–	–	IV	stri-mret	
	Swertiae (14/539–565)	<i>Gentiana algida</i>	0.384 \pm 0.140	aif	–	+	va	–	–	–	–	IV	stri-mret	4E–F
		<i>Gentiana alpina</i>	0.487 \pm 0.166	aif	–	+	va	–	–	–	–	IV	stri-mret	
		<i>Tripterospermum fasciculatum</i>	0.285 \pm 0.086	aif	–	+	a	–	–	–	–	IV	stri	
		<i>Frasera albicaulis</i>	0.229 \pm 0.056	ir-ro	–	–	la	–	–	–	–	IIIa	stri-perf	
		<i>Frasera parryi</i>	0.247 \pm 0.069	ir-ro	–	–	la	–	–	–	–	IIIa	stri-perf	
		<i>Gentianella amarella</i>	0.373 \pm 0.180	ro	–	–	la	–	–	–	–	IIIa	perf-mret	
	Genus <i>incertae sedis</i>	<i>Gentianella bellidifolia</i>	0.409 \pm 0.200	ro	–	–	la	–	–	–	–	IIIa	stri-mret	IF
		<i>Gentianopsis barbata</i>	0.168 \pm 0.053	ro	–	+	a	–	–	–	–	IIIa	mret	
		<i>Gentianopsis procera</i>	0.249 \pm 0.066	ro	–	+	a	–	–	–	–	IIIa	mret	1C–E 7C
		<i>Halenia corniculata</i>	– (c TEM)	–	–	–	–	–	–	–	–	–	psil-perf	
<i>Lomatogonium rotatum</i>		–	–	–	–	–	–	–	–	–	–	stri-perf		
<i>Lomatogonium carinthiacum</i>		– (c TEM)	–	–	–	–	–	–	–	–	–	stri-perf		
<i>Obolaria virginica</i>		– (c TEM)	–	–	–	–	–	–	–	–	–	perf-mret	6C 3H	
<i>Swertia bimaculata</i>	0.199 \pm 0.082	ro	–	–	la	–	–	–	–	IIIa	stri-perf			
<i>Swertia perennis</i>	0.145 \pm 0.032	ro	–	–	la	–	–	–	–	IIIa	stri-perf-mret	7D		
<i>Voyria caerulea</i>	–	–	–	–	–	–	–	–	–	–	psil			

Species in bold possess orbicules.

Tribe and subtribe, *sensu* Struwe *et al.* (2002); Size, mean \pm standard deviation; Type, orbicule type *sensu* Huysmans *et al.* (1997) and Vinckier *et al.* (2000).

a, abundant, aif, angular irregular folded; c TEM, confirmed by TEM observation; EDC, electron-dense cavity wall interface; EDP, very electron-dense peripheral layer; EDW, homogeneous electron-dense orbicule wall; em, embedded in tapetal membrane; ETC, electron translucent cavity; gr, granulate exine; hbr, heterobrochate; ir, irregular; isl, islands; la, only few orbicules are present on locule-surface; mret, microreticulate; msp, microspinulose; perf, perforate; pol, polyad; psil, psilate; r, regular; ret, reticulate; ro, rounded oblate; rug, rugulate; sp, spiny; stri, striate; sgr, sporopollenin granulae present on the orbicule surface; tetr, tetrad; va, very abundant.

RESULTS

Orbicule characters

Orbicules were found in 38 species (23 genera) distributed among the six tribes recognized in *Gentianaceae* (Struwe *et al.*, 2002).

The size and shape of the orbicules, presence of indentations in the orbicule wall, occurrence of aggregated orbicules, their distribution density, and ultrastructure are summarized in Table 1.

Size

In *Gentianaceae* orbicules are often very small, the smallest (0–145 μm) being observed in *Swertia perennis* L. The largest orbicules (2.985 μm) occur in *Prepusa montana* Mart. (Table 1).

Shape

There are three distinct shapes: (1) more or less rounded oblate (Figs 1C and G, 2C, E and F and 3B, F and H); (2) irregular-shaped granular orbicules (Fig. 5B–F); and (3) irregular angular and ‘folded’ orbicules (Fig. 4B, C, E and H; Table 1). Apart from these clearly definable shapes, the orbicular elements were generally amorphous.

Indentations and other surface characters

In the majority of species the orbicules have a smooth surface. However, in some species the orbicule wall is indented (Figs 4B and C and 5B and C) or covered by sporopollenin granules (Fig. 5D and F) or microspinulae (Fig. 1A). Thin threads may occur, connecting the orbicules with each other, or between the tapetal membrane and the orbicules (Fig. 1G).

Aggregations

The apparently large orbicules are often aggregations of smaller orbicules. Irregular layers of small to large aggregations of orbicules embedded in residual tapetal material occur in some species (Figs 1G and 5C).

Distribution density and location of orbicules

When present, orbicules are dispersed over the entire locule surface. Orbicules may be attached to the exine of the pollen grains (Fig. 5A). The position of the orbicules relative to the tapetal membrane varies. They may be completely embedded (Fig. 5G and H), or lightly attached to the surface of the tapetal membrane (Figs 1C, 2E and 5F). A continuum between both extremes may occur within one species (Fig. 3B).

Orbicules are always irregularly arranged. The number of orbicules present on the locule surface is well in excess to the number of pollen grains within the locule. Three terms are used (Table 1) to describe the distribution density: ‘very abundant’ denotes that the locule surface is obscured by the orbicule density (Fig. 4C), while ‘abundant’ denotes that the

orbicules are not in direct contact, so that the locule surface is visible between the orbicules (Fig. 1C). In *Gentianaceae*, it was observed (for the first time in *Gentianales*) that small orbicules may be dispersed over the locule surface in very low distribution densities (Fig. 3F and H).

Ultrastructure

Thirteen species (13 genera) were investigated at the ultrastructural level (TEM, Table 1). These species covered all the tribes (excluding *Saccifolieae*) defined recently in *Gentianaceae* (Struwe *et al.*, 2002). The selection was based on the observation of variations in morphological characters of the orbicules. In the descriptions of the ultrastructure (Table 1) much attention is paid to the occurrence of the different zones of electron density, as defined by Clément and Audran (1993a, b, c) (see Introduction).

A shape variation in the cross-sections of orbicules was observed: spherical (Figs 1D, 2D and H and 3G), irregular (Fig. 4D; Figs 46 and 48 of Vinckier and Smets, 2002a), and many-sided appearances occur (Fig. 4F). In many species the orbicules lack an electron-translucent orbicule cavity (Figs 1D, 2D and H, 3G and 4F). When an electron-translucent orbicule cavity is present it might have an elongated (Fig. 4D) or a rounded oblate appearance (Fig. 48 of Vinckier and Smets, 2002a). In some species, the electron-dense orbicule wall possesses a very intense, homogeneous staining (Figs 2D and H and 3G). The only species in which a thin electron-dense orbicule cavity–wall interface is observed, between the orbicule cavity and the homogeneous electron-dense orbicule wall, was *Exacum oldenlandioides* (S. Moore) Klack (Fig. 48 of Vinckier and Smets, 2002a). The presence of a very electron-dense peripheral layer, delimiting the homogeneous electron-dense orbicule wall, is a general feature in the species studied. However, orbicules with very intense, homogeneous staining (e.g. Fig. 2D and H) do not show this electron-dense peripheral layer. This electron-dense peripheral layer sometimes has a granular appearance (Fig. 1D).

Orbicule types

The typology (Table 1) follows the treatment of orbicule types described for the rubiaceous subfamilies, *Cinchonoideae* (Huysmans *et al.*, 1997) and *Ixoroideae* (Vinckier *et al.*, 2000). In the *Cinchonoideae*, Huysmans *et al.* (1997) defined four orbicule types: spiny orbicules (Type I), microrugulate orbicules (Type II), smooth and rounded oblate orbicules (Type III), and irregular and folded orbicules (Type IV). Two additional orbicule types were defined by Vinckier *et al.* (2000) in the *Ixoroideae*: large flattened and irregular orbicules (Type V), and distinctly embedded orbicules (Type VI). In *Ixoroideae* (Vinckier *et al.*, 2000) Type III orbicules were split up into two subtypes: rounded oblate orbicules (Subtype IIIa), and doughnut-shaped orbicules (Subtype IIIb) which are characterized by a central indentation in the orbicule wall. For a more extensive description of the different orbicule types, refer to Huysmans *et al.* (1997), Vinckier *et al.* (2000), and Vinckier and Smets (2002a).

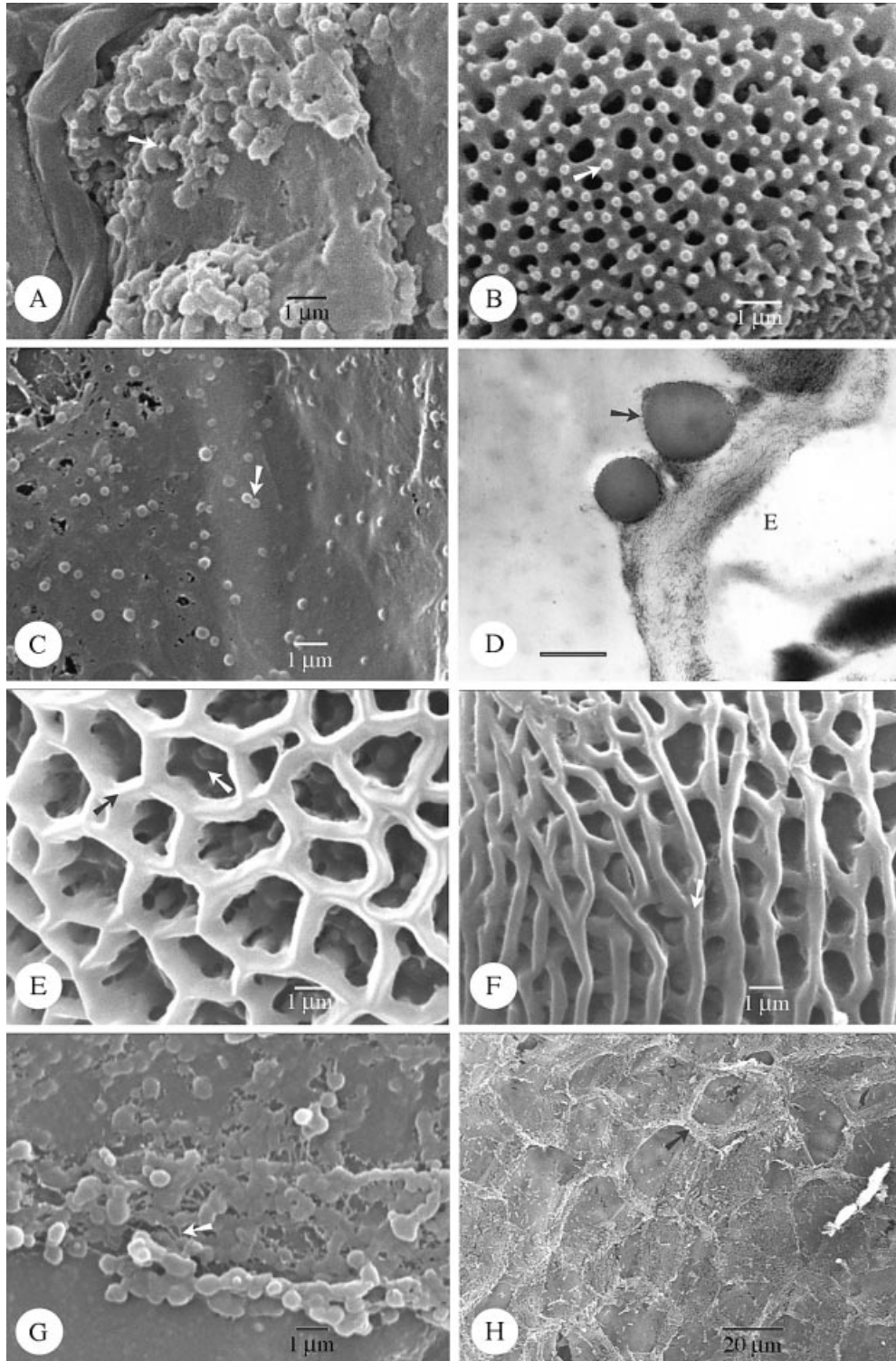


FIG. 1. SEM of species with Type I orbicules (A and B) and SEM and TEM of Type IIIa (C–H) orbicules. Abbreviations used in figures: E, endothecium; ED, electron dense; ET, electron translucent; PE, pollen exine; SEM, scanning electron microscopy; TEM, transmission electron microscopy. A and B, *Hockinia montana*. A, SEM observation of the spiny orbicules (arrow), covering the locule wall surface. B, Detail of the exine ornamentation. Microspines (arrow) are present upon the tectum. C–E, *Gentianopsis procera*. C, SEM observation of the small spherical orbicules (arrow). D, TEM observation of the orbicules. An electron-transparent orbicule cavity is absent. The orbicule wall is delimited by a very electron-dense and granular peripheral layer (arrow). Bar = 0.6 μm . E, Detail of the reticulate exine. The lumina are beset with granules (white arrow). The muri are sharply crested (black arrow). F, *Gentianella bellidifolia*. SEM observation of the striato-reticulate exine. The muri are oriented parallel and criss-crossed with smaller muri. The muri are rounded (arrow). G and H, *Ixanthus viscosus*. G, SEM observation of single or compound orbicules. Sporopollinous threads occur between the orbicules (arrow). H, General view of the locule wall surface, imprints of pollen grains are striking. Orbicules (black arrow) are present on the edges between the imprints.

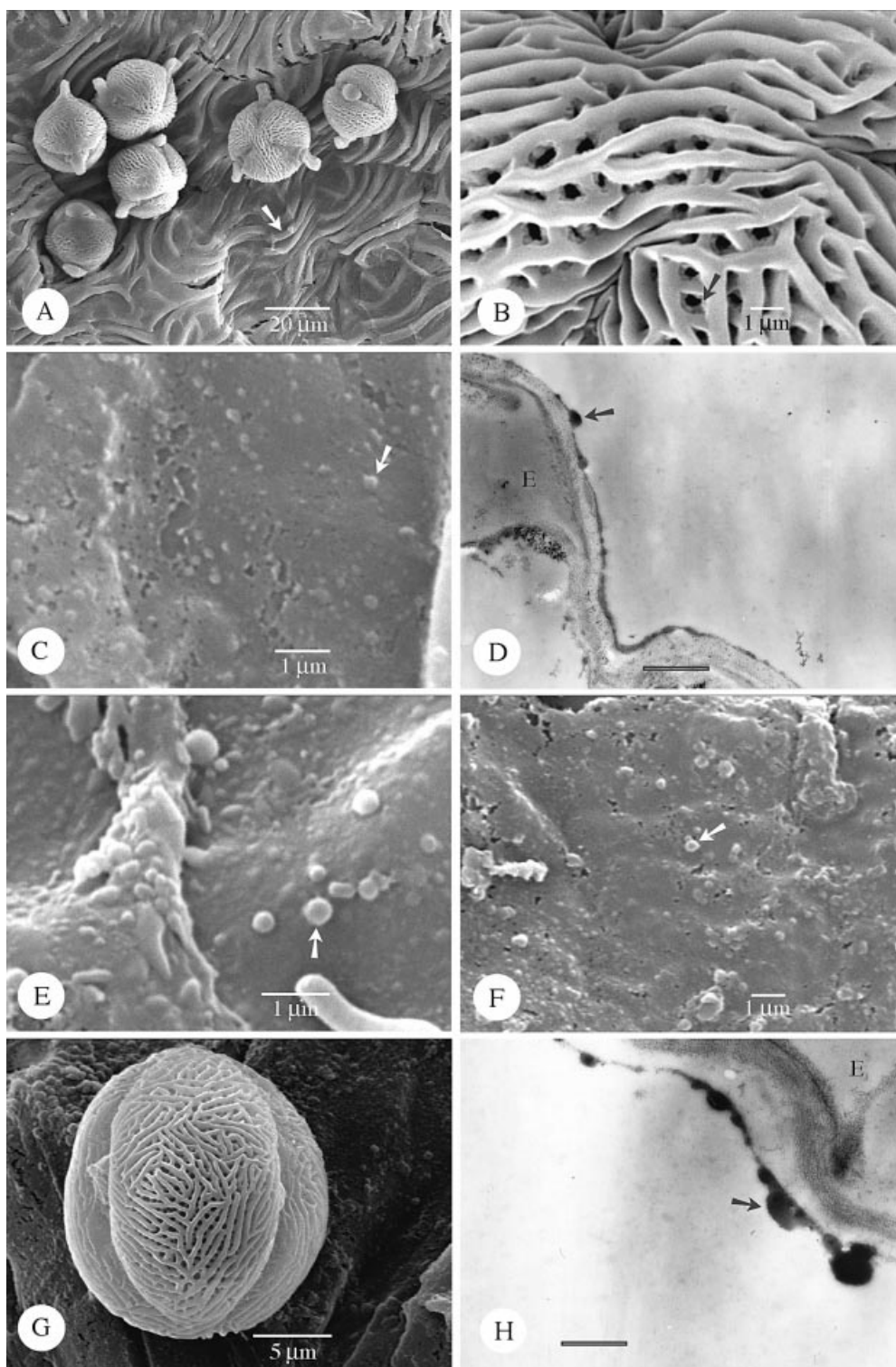


FIG. 2. SEM and TEM of species with Type IIIa orbicules. A–D, *Chironia purpurascens*. A, General observation of six pollen grains lying on the locule wall surface, which consists of wavy ridges (arrow). B, Detail of the striate exine ornamentation in the polar region. The muri possess granules (arrow) on their side walls. C, SEM observation of the small spherical orbicules (arrow) covering the locule wall surface. D, TEM observation of the small orbicules (arrow). An electron-transparent orbicule cavity is lacking. Bar = 0.6 μm . E, *Eustoma grandiflorum*. SEM observation of the small spherical orbicules (arrow) present on the locule wall surface. F–H, *Blackstonia perfoliata*. F, SEM observation of small orbicules (arrow) present on the locule wall surface. G, Equatorial view of a striate pollen grain lying on the locule wall surface. The muri are oriented in various directions. H, TEM observation of the orbicules, lacking an electron-transparent orbicule cavity. Bar = 0.6 μm .

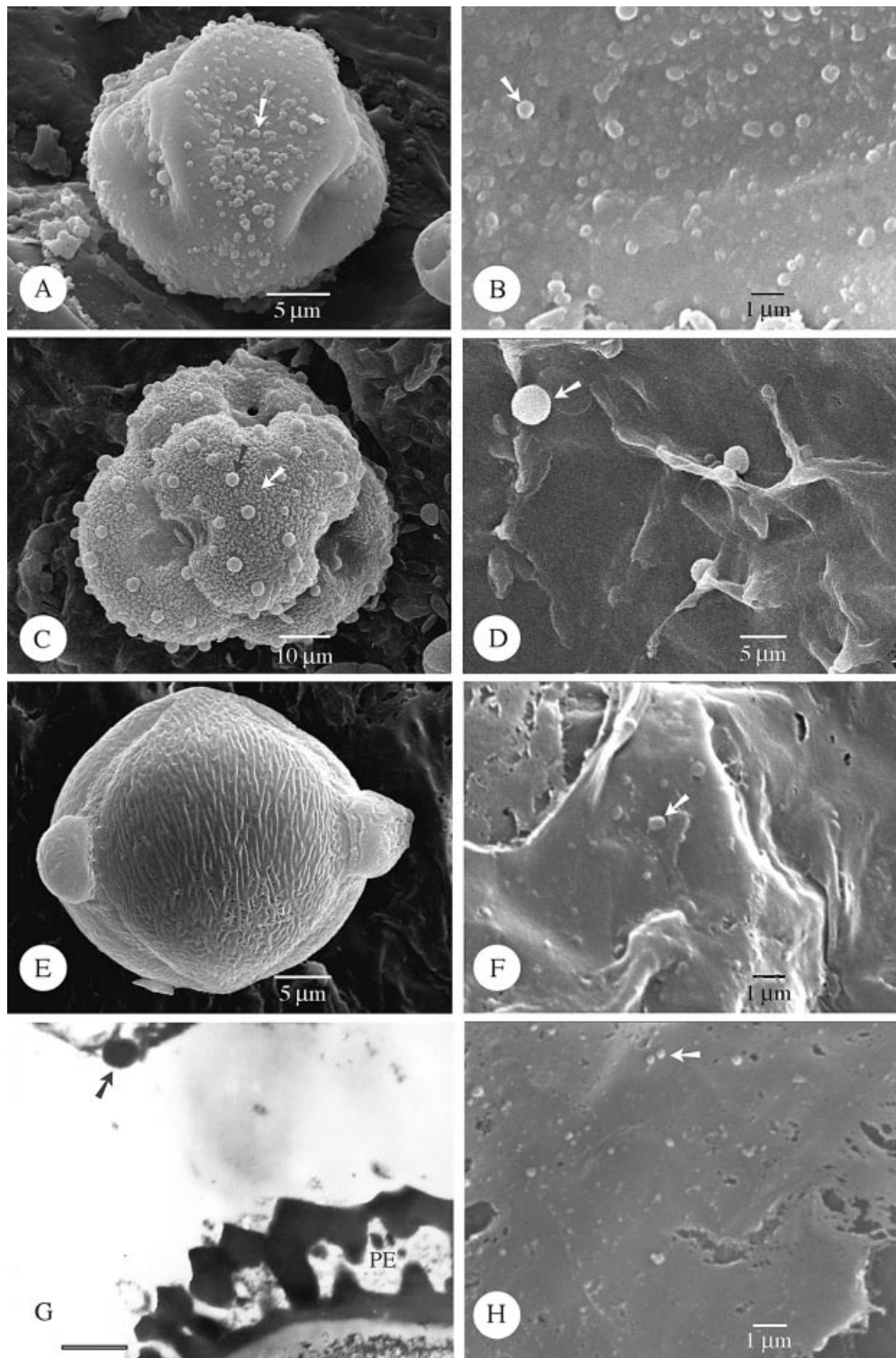


FIG. 3. SEM and TEM of species with Type IIIa orbicules. A and B, *Tachia guianensis*. A, Equatorial view of a granulate pollen grain lying on the locule wall surface. The granulate exine consist of numerous granules (arrow) on the smooth sexine. B, Detail of the spherical orbicules (arrow), resembling the granules present on the exine. C and D, *Prepusa montana*. C, SEM observation of a granulate tetrad lying on the locule wall surface. The granulate exine consist of numerous macroprocesses (black arrow) intermingled with microprocesses (white arrow). D, Detailed observation of the spherical orbicules (arrow), resembling the macroprocesses present on the exine. E–G, *Frasera parryi*. E, Equatorial view of a striate pollen grain lying on the locule wall surface. F, Observation of the small orbicules (arrow) present on the locule wall surface at a very low distribution density. G, TEM observation of a part of a pollen grain, close to the tapetal remnants on which orbicules (arrow) are present. Bar = 0.6 μm. H, *Swertia bimaculata*. SEM observation of the small orbicules (arrow), distributed upon the locule wall surface at a very low distribution density.

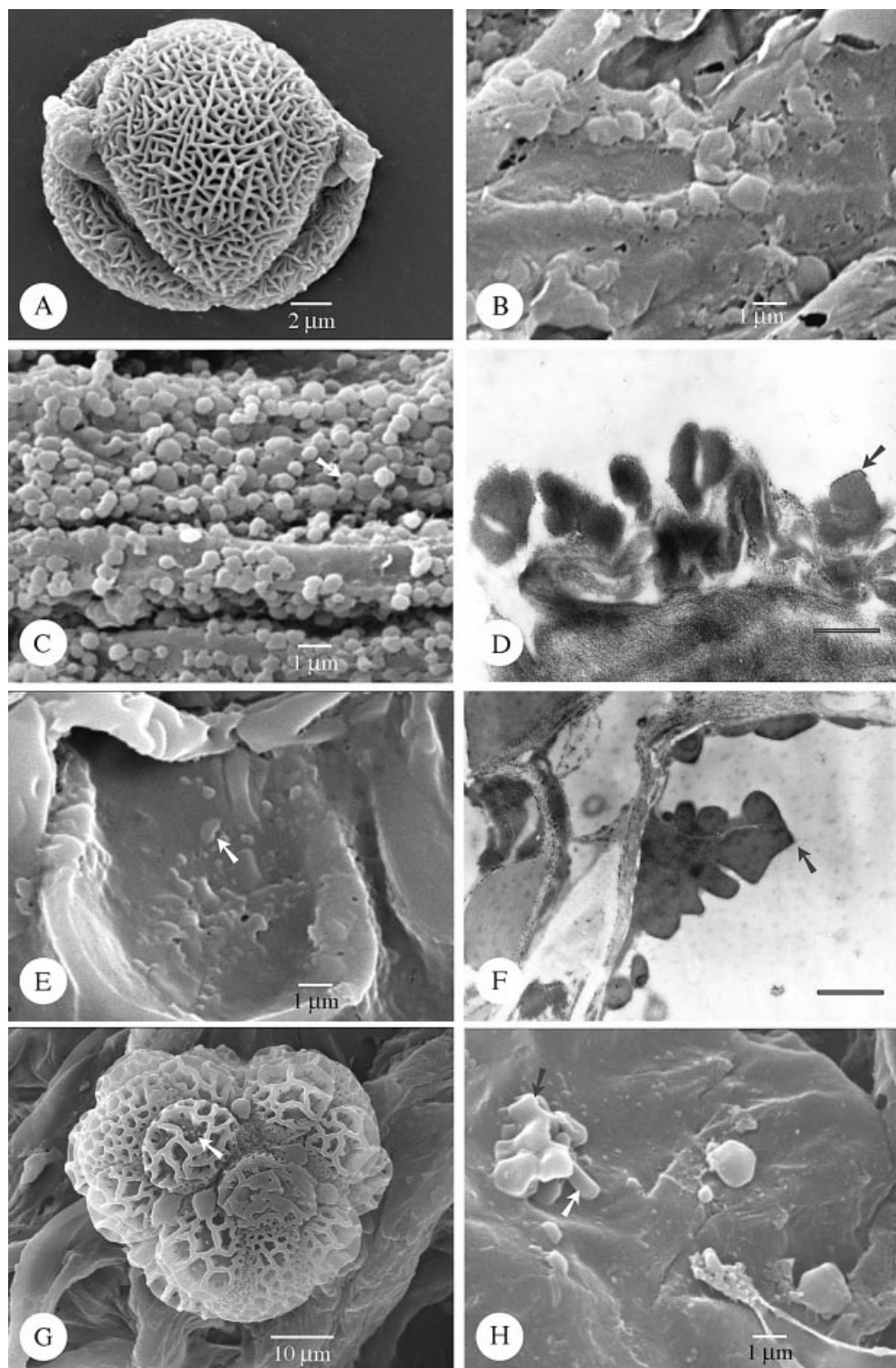


FIG. 4. SEM and TEM of species with irregular and folded Type IV orbicules. A and B, *Sebaea ovata*. A, Equatorial view of a striato-micro-reticulate pollen grain. B, Detail of the irregular and folded orbicules (arrow), resembling parts of the muri building up the exine. C and D, *Sebaea albidiflora*. C, SEM observation of the irregular and folded orbicules (arrow). D, TEM observation of the irregular orbicules positioned on the remnants of the tapetum cells. The orbicules possess an elongated electron-transparent cavity and the homogeneous ED orbicule wall is delimited by an electron-dense peripheral layer (arrow). Bar = 0.6 μm . E and F, *Triptospermum fasciculatum*. E, SEM observation of the irregular and folded orbicules (arrow) covering the locule wall surface. F, TEM observation of the irregular orbicules, lacking an electron-transparent cavity. The homogeneous ED orbicule wall is delimited by an ED peripheral layer (arrow). Bar = 1 μm . G and H, *Calolisianthus pedunculatus*. G, SEM observation of a tetrad lying on the locule wall surface. The muri of the reticulate pattern are organized in heterobrochate reticulate islands. The lumina are beset with granules (arrow). H, SEM observation of the orbicules on the locule wall surface. The orbicules consist of columellae-like particles (white arrow) which support the muri-like part (black arrow) of the orbicules.

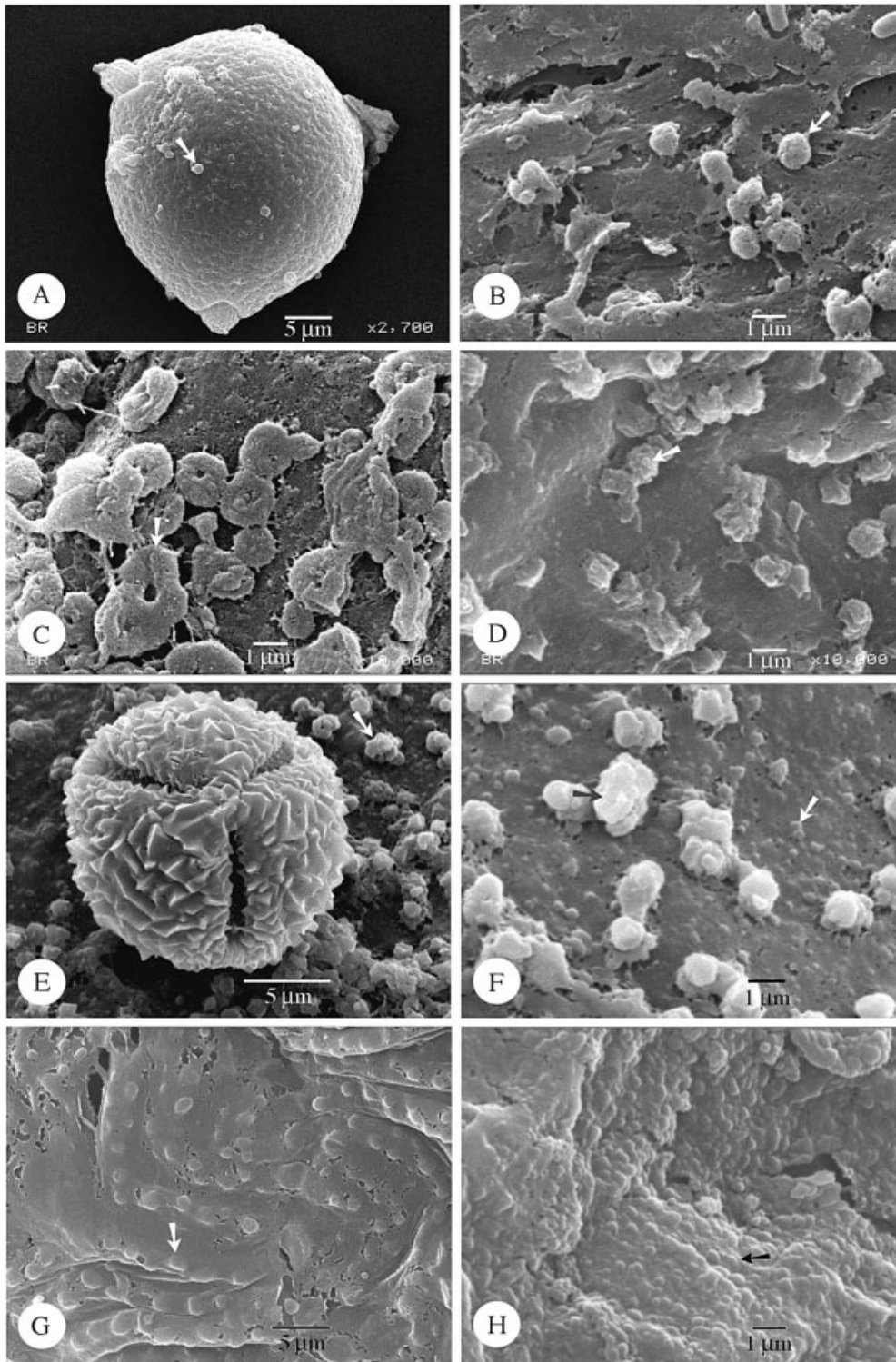


FIG. 5. SEM of species with irregular Type V (A–F) and strongly embedded Type VI (G–H) orbicules. A, *Anthocleista scandens*. Polar view of a tri-porate perforate pollen grain. Orbicules (arrow) are attached to the exine. B, *Anthocleista amplexicaulis*. Detail of the irregular orbicules (arrow) covering the locule wall surface. C, *Fagraea racemosa*. SEM observation of the irregular and compound orbicules (arrow). D, *Exacum oldenlandioides*. SEM observation of the irregular orbicules covering the locule wall surface. On the orbicule wall small sporopollinous granulae are present (arrow). E and F, *Exacum macranthum*. E, SEM observation of a rugulate pollen grain lying on the locule wall surface which is covered by many orbicules (arrow). F, Detailed SEM observation of the orbicules (black arrow) covering the locule wall surface. Intermingled with the orbicules small granules of sporopollenin (white arrow) may cover the locule wall surface. G, *Centaurium spicatum*. SEM observation of the distinctly embedded orbicules (arrow). H, *Hoppea fastigiata*. SEM observation of the distinctly embedded orbicules (arrow).

Orbicules in *Gentianaceae* fit well into the types described previously in *Rubiaceae*, and hence these are used in the present paper (Table 1). Orbicule aggregation does not affect their placement into types, due to the fact that single orbicules or aggregations may occur within one species (Fig. 1G). Of the six orbicule types, Type II orbicules are lacking in the study group. In the majority of species (17 species) Type III orbicules are recorded (Figs 1C–H, 2 and 3; Table 1). *Hockinia* Gardner is the only representative with Type I orbicules (Figs 1A and B). The number of representatives with orbicules belonging to the other orbicule types are equally distributed among the species studied: seven species possess Type IV orbicules (Fig. 4A–H), six species Type V (Fig. 5A–F) and seven species Type VI (Figs 5G and H and 6A and B; Table 1).

Pollen characters

The pollen grains are released as monads (Figs 2A and G, 3A and E, 4A, 5A and E, 6B, C and H and 7D), tetrads (Figs 3C, 4G, 6D and 7A) or polyads (Fig. 6E and F). Pollen grains are mostly 3-colporate (Figs 2A and G, 3A and E, 4A, 5E and 6B, C and H) to 3-porate (Fig. 5A), but 2-porate pollen (Fig. 7D) also occurs in the *Gentianaceae*. The colpi or pores are bordered with a distinct margo or annulus. The characteristics of the pollen sexine are summarized in Table 1, according to the terminology of Punt *et al.* (1994). In the palynologically diverse *Gentianaceae* a considerable variation in pollen ornamentation types is observed among the species studied. Most species possess a striate (Fig. 3E), striato-reticulate (Figs 1F, 2B and G and 4A) to reticulate exine pattern (Figs 1E, 4G, 6B and D and 7A). Apart from these ornamentation types microreticulate (Fig. 6F), microspinulose (Fig. 1B), perforate (Figs 5A and 6H), psilate (Fig. 7D), rugulate (Fig. 5E) and granulate (Figs 3A and C) exine types are present. Of the *Gentianaceae* species studied by Sankara Rao and Chinnappa (1983), *Hoppea fastigata* (Griseb.) Clarke and *Frasera albicaulis* Douglas ex Griseb were also investigated in the present study. For both species the exine data substantiate the results of Sankara Rao and Chinnappa (1983). The granulate sexines may consist of numerous macroprocesses intermingled with microprocesses (Fig. 3C), or only of granules present on a psilate exine (Fig. 3A). The reticulate patterns can be heterobrochate (Figs 4G, 6F and 7A) or, as in most cases, homobrochate. The columellae of the reticulate patterns may be of variable length and the sexine organized in heterobrochate reticulate islands (Fig. 4G). In *Irlbachia purpurascens* (Aubl.) Maas (Fig. 6E and F) polar loops of reticulum that is raised from the general surface of the pollen grain are present. Lumina can be beset with granules (Figs 1E and 4G). Muri are either sharply crested (Fig. 1E) or rounded (Fig. 1F). The muri are oriented parallel (Fig. 3E) or in various directions (Fig. 2G), or criss-crossed and interlaced (Figs 1F, 2B and 4A), in a few cases with granules present on the sidewalls of the muri (Fig. 2B).

Locule surface characters

The appearance of the locule surface is often affected by dehydration. Consequently any definition of the surface morphology must be approached with caution. A ribbed surface, with the ribs parallel to the long axis of the anther, is a common feature in the group studied (Figs 4C and 5G). In some species the locule surface consists of wavy ridges (Figs 2A and 6G), in other species the ridges on the locule surface form a distinct reticulum (Fig. 7C). Sometimes smooth to deep grooves in the locule surface reveal the position of the underlying endothelial cells (Figs 6C and 7B) and the endothelial thickenings (Fig. 6C). Intermingled with the orbicules, small granules of sporopollenin may cover the locule wall surface (Fig. 5F). Imprints of pollen grains in the locule wall surface are also observed (Fig. 1H).

DISCUSSION

The 53 species studied out of approx. 1650 in *Gentianaceae* represent only 3.2 % of the species in this family. Despite the limited sample size, a good representation of the family is presented due to the fact that the selected species cover all the different tribes and subtribes recognized recently in the family (Struwe *et al.*, 2002).

Correlations between pollen exine and orbicule surface ornamentation within the same species

As mentioned in the Introduction, a notable correlation in ornamentation between the pollen exine and the orbicule wall can be present. In the *Gentianaceae* species studied several examples of such a correlation were observed.

Species characterized by a granulate exine possess orbicules that resemble those granules. This correlation was observed in *Tachia guianensis* Aubl. and *Prepusa montana* Mart. (Fig. 3A–D). The orbicules present in *Hockinia montana* Gardner possess similar microspinulae on their orbicule wall to those that occur on the microspinulose pollen tectum (Fig. 1A and B). The characteristics of the rugulate exine pattern in *Exacum macranthum* Arn. are reflected in the morphology of the orbicules present, which have also a rugulate appearance (Fig. 5E and F).

The irregularly folded orbicules (Type IV), present in species with striate, striato-microreticulate or reticulate pollen grains, resemble the morphology of the elements comprising the muri of the pollen exine (Fig. 4A–H). In *Calolisianthus pedunculatus* (Cham. & Schltld.) Gilg an extreme form of this correlation is observed: the orbicules consist of columellae-like particles which support muri-like parts (Fig. 4G and H). The surface of the orbicules is usually smooth. The species where this correlation occurs are *Calolisianthus pedunculatus*, *Gentiana acaulis*, *G. algida* Pall., *G. alpina* Adam, *Sebaea albidiflora* F Muell., *S. ovata* (Labill.) R. Br. and *Tripterospermum fasciculatum* (Wall.) A.O. Chater. In many *Gentianaceae* species with a striate or striato-microreticulate exine pattern no correlation between the orbicule surface ornamentation and the pollen exine surface has been observed. The orbicules in these species can be regular (Figs 1C–H, 2 and 3E–H) or irregular in

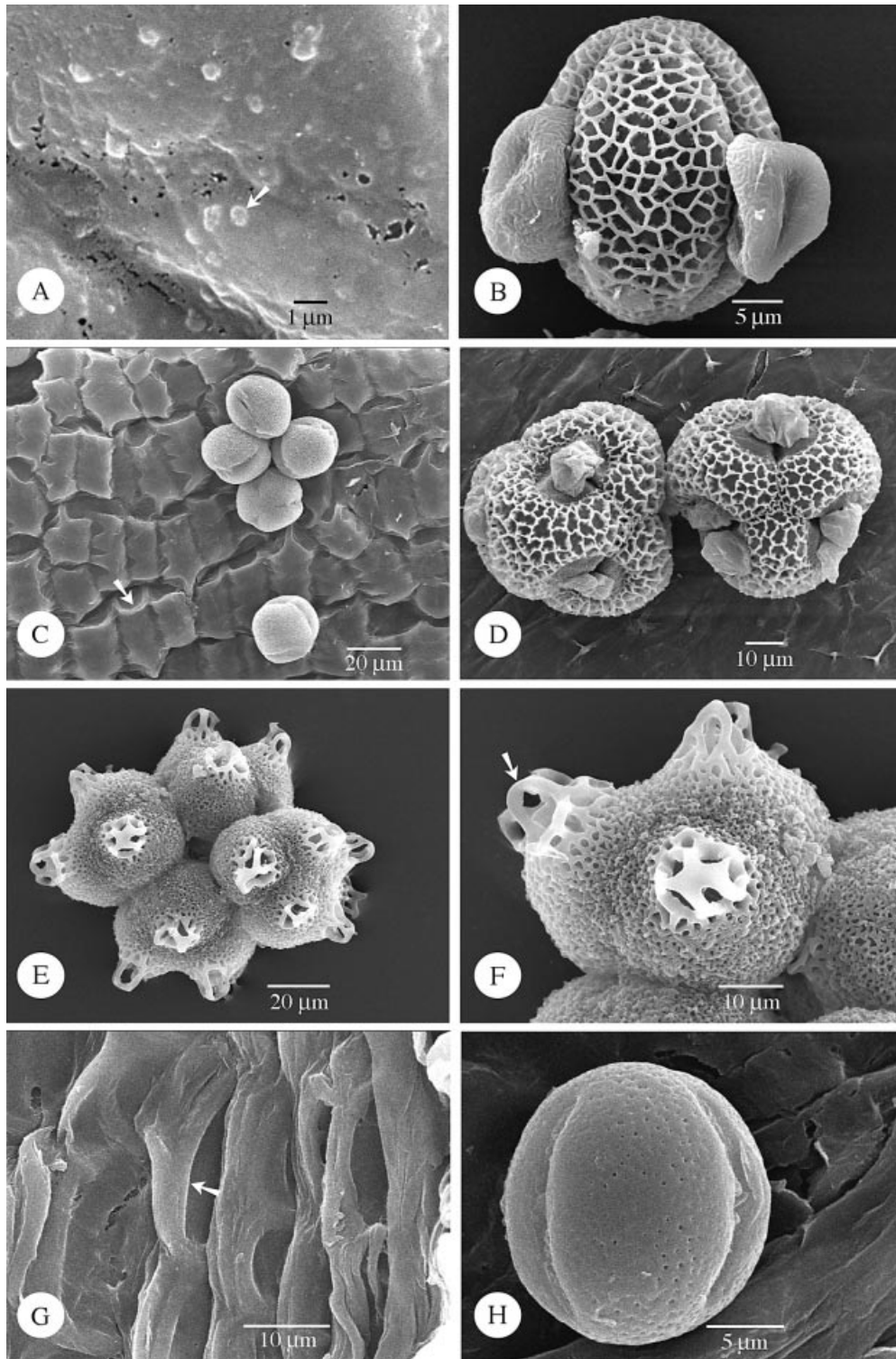


FIG. 6. SEM of species with strongly embedded Type VI (A and B) orbicules, and species lacking orbicules (C–H). A and B, *Lisianthus nigrescens*. A, General observation of the strongly embedded orbicules (arrow). B, Equatorial view of a reticulate pollen grain. C, *Lomatogonium carinthiacum*. General view of five pollen grains lying on the locule wall surface, which lacks orbicules. Deep grooves in the locule surface reveal the position of the underlying endothelial cells, the U-shaped endothelial thickenings (arrow) can be distinguished. D, *Schultesia guianensis*. SEM observation of two reticulate tetrads lying on the smooth locule wall surface. E and F, *Irlbachia purpurascens*. E, General view of a micro-reticulate polyad. F, Detailed observation of the muri of the reticulate pattern, at the distal regions, which are loop-like. The loops of reticulum (arrow) are raised from the general surface of the pollen grain. G and H, *Curtia tenuifolia*. G, SEM observation of the locule surface consisting of wavy ridges (arrow). H, Equatorial view of a perforate pollen grain lying on the locule wall surface.

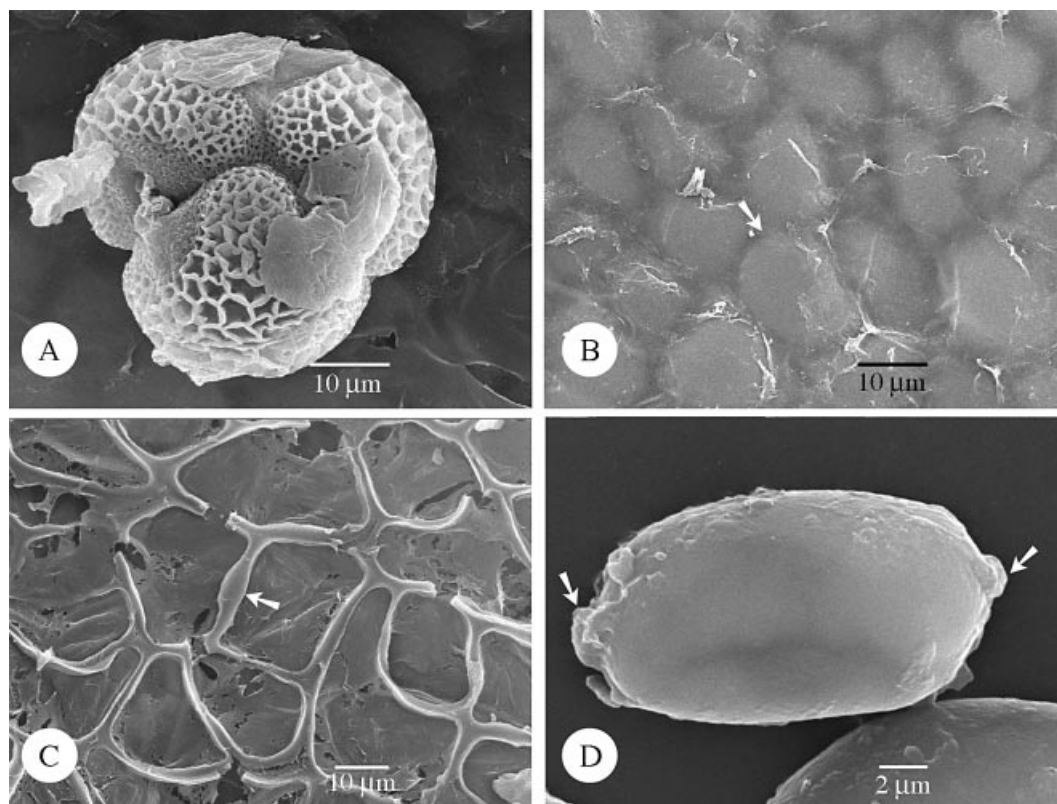


FIG. 7. SEM of species lacking orbicules. A and B, *Coutoubea spicata*. A, General observation of a heterobrochate reticulate tetrad. B, General observation of the locule wall surface lacking orbicules. Smooth grooves (arrow) in the locule surface reveal the position of the underlying endothelial cells. C, *Halenia corniculata*. The locule surface possesses ridges (arrow) forming more or less a reticulum. D, *Voyria caerulea*. General view of a psilate 2-porate (arrows) pollen grain.

shape, and may possess sporopollenin granules on their orbicule wall surface (Fig. 5C and D). Spiny orbicules correlated with a striate exine, as reported in *Dioscorea* (Schols *et al.*, 2001), do not occur in Gentianaceae.

Correlation of orbicule typology with trends in Gentianaceae palynology and systematic usefulness of orbicules

All specimens investigated within one species are characterized by the same orbicule type. No intraspecific variation has been observed.

No Gentianaceae tribe is characterized by a distinctive orbicule type: absence and different orbicule types occur in each tribe (Table 1), which reflects the observed palynological diversity in Gentianaceae. Tribe Saccifolieae is regarded as the most basal clade, followed by the clade Exaceae which is sister to the rest of Gentianaceae (Struwe *et al.*, 2002). Both tribes are characterized by the presence of orbicules [excluding *Curtia tenuifolia* (Aubl.) Knobl., Fig. 6G and H; Table 1]. In Exaceae Type V (Fig. 5E and F) and Type IV (Fig. 4A–D) orbicules are recorded, in Saccifolieae Type I orbicules are present (Fig. 1A; Table 1). The tribe Chironieae is a more apomorphic clade, followed by the crown clade comprising the monophyletic tribes Helieae, Potalieae and Gentianeae. In this clade no particular sister-group relationships are well supported (Struwe *et al.*, 2002).

In tribe Chironieae, subtribe Chironiinae species possess orbicules of Type III, Type VI, or lack orbicules. In Canscorinae Type III and VI orbicules are present, and all Coutoubeinae species lack orbicules (Table 1).

In the majority of Gentianaceae genera monads are present, but tetrads have evolved twice, in Chironieae–Coutoubeinae (*Coutoubea* Aubl. and *Schultesia* Mart.) and in the tribe Helieae (*Calolisianthus* Gilg) (Struwe *et al.*, 2002). The Chironieae–Coutoubeinae genera with tetrads are characterized by the absence of orbicules (see above) (Figs 6D, 7A and B). However, *Calolisianthus* (Helieae), which has tetrads, possesses orbicules (Fig. 4G and H). In Helieae polyads occur in *Irlbachia* Mart. (Fig. 6E and F). These polyads are regarded as being derived from tetrads (Struwe *et al.*, 2002). *Irlbachia* is characterized by the absence of orbicules. Thus, comparable with the palynological trend observed in Apocynaceae *s.l.* (Vinckier and Smets, 2002b), a similar trend is observed in Helieae palynology from the genera with monads and tetrads possessing orbicules (Types III and IV), to the genus *Irlbachia* with polyads which lacks orbicules (Table 1). However, no comparable trend in orbicule typology, as recorded in Apocynaceae *s.l.* (Type III → embedded Type VI orbicules → orbicules absent) (Vinckier and Smets, 2002b), was present in the family Gentianaceae.

As a result of increasing molecular evidence (Struwe *et al.*, 1994, 2002) the three genera *Anthocleista* Afzel. ex R.

Br., *Fagraea* Thunb. and *Potalia* Aubl. have been included in Gentianaceae. However, this grouping seems unnatural from a palynological point of view (Nilsson, 2002), as *Anthocleista* (Fig. 5A) and *Potalia* possess 3(-4)-porate pollen grains, which is a pollen type not found in the rest of the Gentianaceae, although this type has arisen several times in Gentianales (*Geniostoma* J.R. Forst. & G. Forst., *Rauvolfia* L. (Fig. 1E of Vinckier and Smets, 2002b), *Baiassa* A. DC.). On the other hand, *Fagraea* is characterized by 3-colporate pollen, resembling gentianaceous pollen. In both genera *Anthocleista* and *Fagraea* the same orbicule type (Type V, Fig. 5A–D) is present, but in *Potalia* Type VI orbicules are observed (Fig. 52 of Vinckier and Smets, 2002a). The subtribes recognized in the tribe Potalieae (Struwe *et al.*, 2002) are consistent with the orbicule typology: subtribe Faroinae lacks orbicules, Lisianthiinae possess Type VI orbicules (Fig. 6A and B), and Potaliinae are characterized by Type V orbicules (excluding *Potalia*) (Table 1).

In Gentianeae, the subdivision into subtribes Gentianinae and Swertiinae is strongly supported by molecular data (*trnL* intron, *matK*, ITS) (Struwe *et al.*, 2002). Both subtribes are also characterized by the presence of distinct orbicule types: all Gentianinae species studied possess Type IV orbicules (Fig. 4E and F), and species of Swertiinae studied possess Type IIIa orbicules (Figs 1C–F and 3E–H), or lack orbicules (Figs 6C and 7C; Table 1).

Recognizing the systematic significance of the individual orbicule characters, the following points can be revealed. In Exaceae, the orbicules of each species have indentations and are aggregated. In the majority of Chironieae species indentations or aggregated orbicules are lacking and the orbicules possess a rounded oblate shape, similar to the orbicules of subtribe Swertiinae. In subtribe Gentianinae, aggregated angular irregular folded orbicules are present which lack indentations.

Swertia perennis possesses the smallest orbicules in the study group, and this species is known to have an amoeboid tapetum (Sankara Rao and Chinnappa, 1983). Also *Canscora decussata* (Roxb.) Schult. & Schult. f., characterized by an amoeboid tapetum (Maheswari Devi, 1962), produces very small orbicules (0.189 μm). It was observed that, in both species, the very small orbicules were distributed in a very low density on the locule wall surface. Thus it is suggested that presence of very small orbicules (mean 0.2 μm), along with a very low distribution density (mean 2 orbicules 25 μm^{-2}), may give an indication of the presence of an amoeboid tapetum in the species, since sporopollenin granules are reported in taxa with an amoeboid tapetum (Tiwari and Gunning, 1986). Ontogenetical research may elucidate whether the small orbicules present in *Canscora decussata* and *Swertia perennis* are homologous with the sporopollenin granules produced by the amoeboid tapetum in *Tradescantia* (Tiwari and Gunning, 1986) or can be considered as orbicules. Additional information on the tapetum type of species lacking orbicules is required to answer the question as to whether orbicules are lacking in species with amoeboid tapeta.

CONCLUSIONS

Orbicule typology has proved to be useful for evaluating tribal delimitation within Rubiaceae (Huysmans *et al.*, 1997; Vinckier *et al.*, 2000) and Loganiaceae *s.l.* (Vinckier and Smets, 2002a); however, they seem not to be useful for tribal delimitation in Gentianaceae. In the tribes Potalieae and Gentianeae, orbicule typology may be useful at the subtribal level.

In the crown clade comprising Helieae, Potalieae and Gentianeae, no particular sister-group relationships is well supported in the three-gene analysis of Struwe *et al.* (2002). Further data are thus required to resolve these relationships. Despite the lack of congruence between orbicule and molecular data, individual orbicule characters along with pollen and other morphological characters can be used in a future combined analysis. Orbicule types should not be used in a cladistic analysis because they are aggregations of characters.

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APPENDIX

Alphabetical list of Gentianaceae specimens giving species, country, collector, specimen number and herbarium.

- Anthocleista amplexicaulis* Baker, Madagascar, A.J.M. Leeuwenberg & G.R. Rafamantanantsoa 14437 (BR)
- Anthocleista scandens* Hook. f., Madagascar, Bioko Fernando-Po (s.n.) (BR)
- Blackstonia perfoliata* (L.) Huds., Algeria, D. Podlech 37064 (BR)
- Calolisianthus pedunculatus* (Cham. & Schltdl.) Gilg, Brazil, G. Hatschbach 47336 (BR)
- Canscora decussata* (Roxb.) Schult. & Schult. f., India, H.B. Naithouri 3954 (BR)
- Canscora heteroclita* (L.) Gilg, Sri Lanka, A.G. Robyns 7206 (BR)
- Centaurium serpentinicola* Carlström, Turkey, M. Nydegger 44139 (BR)

Centaurium spicatum (L.) Fernald, France, Berger 2310 (BR)

Centaurium tenuiflorum (Hoffm. & Link) Fritsch, France, C. Van den Berghen (s.n.) (BR); France, P. Bamps 7463 (BR); Portugal, J. Bouharmont 27871 (BR)

Chironia pegleriae Prain, South Africa, J. Lambinon & M. Reekmans 82/301 (BR)

Chironia purpurascens (E. Meyer) Benth. & Hook. f., South-Africa, E.S. Kemp 818 (BR)

Coutoubea spicata Aubl., French Guyana, F. Billiet & B. Jadin 1145 (BR)

Curtia tenuifolia (Aubl.) Knobl., Brazil, G. Hatschbach 48278 (BR)

Enicostema verticillatum (L.) Engl. ex Gilg, Sri Lanka, N. Balakrishnan 53619 (BR)

Eustoma grandiflorum (Raf.) Shinnars, USA, Geerinck-Coutrez 4373 (BR)

- Exacum gracilipes* Balf. f., Oman, S.A. Ghazanfar 2337 (BR)
- Exacum macranthum* Arn., Sri Lanka, P.L. Comanor 419 (BR)
- Exacum oldenlandioides* (S. Moore) Klack, Guinea-Bissau, Centro de botanico Lisboa 3761 (BR)
- Fagraea racemosa* Jack, Borneo, Veldkamp 8516 (BR)
- Faroea affinis* De Wild., Democratic Republic of Congo, Dikumbwa, Kisimba & Muzinga 3591 (BR)
- Frasera albicaulis* Douglas ex Griseb., USA, E.B. Babcock 1790 (BR)
- Frasera parryi* Torr., USA, V. Durand 3488 (BR)
- Gentiana acaulis* L., Switzerland, C. Vanden Berghen 50 (BR); France, G.F. De Witte 16457 (BR)
- Gentiana algida* Pall., Russia, Kharkevich (s.n.) (BR)
- Gentiana alpina* Adam, Spain, J. Auoyo, I. Fernandez & J. Pastor 11518 (BR)
- Gentianella amarella* (L.) Borner, Poland, S. Lisowski 57556 (BR)
- Gentianella bellidifolia* (Hook.f.) Holub, New Zealand, M.T. Mangaweka, P.D. Buckley & K.W. Julley 15/2/77 (BR)
- Gentianopsis barbata* (Froel.) Ma, Russia, Kharkevich & Buch (s.n.) (BR)
- Gentianopsis procera* (Th. Holm) Ma, Canada, F. Marie-Victorin & F. Rolland-Germain 49464 (BR)
- Halenia corniculata* (L.) Cornaz, Russia, Primorskiy, Kharkevich & Buch (s.n.) (BR)
- Hockinia montana* Gardner, Brazil, unknown 17150 (BR)
- Hoppea fastigiata* (Griseb.) Clarke, Sri Lanka, A.G. Robyns 7206bis (BR)
- Irlbachia purpurascens* (Aubl.) Maas, French Guyana, F. Billiet & B. Jadin 1550 (BR)
- Ixanthus viscosus* Griseb., Canary Islands, J. Bouharmont 25535 (BR)
- Lisianthus areopolus* B.L. Rob., Mexico, R.L. Wilbur 35600 (BR)
- Lisianthus nigrescens* Schldtl. & Cham., Mexico, Arnold arboretum, Universidad Nacional Autonoma de Mexico 446 (BR)
- Lomatogonium carinthiacum* (Wulfen) Rechb., Kazakhstan, V. Zuev 6649 (BR)
- Lomatogonium rotatum* (L.) Fries ex Fern., Russia, S. Kharkevich & T. Buch (s.n.) (BR)
- Macrocarpaea rubra* Malme, Brazil, R. Kummrow 2385 (BR)
- Obolaria virginica* L., USA, H.E. Ahles & J. Haesloop 53200 (BR)
- Potalia amara* Aubl., French Guyana, collector of the Orstom centre 1188 (BR); French Guyana, F. Billiet & B. Jadin 1821 (BR); F. Billiet & B. Jadin 1900 (BR)
- Prepusa montana* Mart., Brazil, G. Hatschbach 47930 (BR)
- Sabatia bartramii* Wilbur, USA, S. Mc Daniel 6455 (BR)
- Sabatia brachiata* Elliott, USA, A.E. Radford 45410 (BR)
- Sabatia quadrangula* Wilbur, USA, J.F. Matthews, L. Kesler, P. Campbell & W. Weston (s.n.) (BR)
- Schultesia guianensis* (Aubl.) Malme, French Guyana, F. Billiet & B. Jadin 1157 (BR)
- Schultesia pachyphylla* Grisrb., Brazil, G. Hatschbach 47936 (BR)
- Sebaea albidiflora* F. Muell., Australia, A. Mubold 7276 (BR)
- Sebaea ovata* (Labill.) R. Br., D.J.E. Whibley 8862 (BR)
- Swertia bimaculata* (Siebold & Zucc.) Hook. f. & Thomson ex C.B. Clarke, China, Sino-American Guizhou Botanical Expedition 402 (BR)
- Swertia perennis* L., France, J. Bouharmont 7718 (BR)
- Tachia guianensis* Aubl., Brazil, P. Bamps 5416 (BR); Peru, F. Woytkowski 5348 (BR); Peru, S. Mc Daniel & M. Rimachi 16483 (BR)
- Tripterospermum fasciculatum* (Wall.) A.O. Chater, India, G. Panigrahi 19807 (BR)
- Voyria caerulea* Aubl., French Guyana, F. Billiet & B. Jadin 4381 (BR)