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Pollen morphological support for the Catesbaeeae-Chiococceae-Exostema-complex (Rubiaceae)

SUZY HUYSMANS, ELMAR ROBBRECHT, PIERO DELPRETE and ERIK SMETS

The systematic value of pollen morphological characters in the mostly tropical family Rubiaceae has been convincingly demonstrated (Robbrecht 1988 for general information, and inter alia Johansson 1987, Andersson 1993, Persson 1993, Huysmans et al. 1998, Delprete 1999a). Pollen data are now commonly incorporated in cladistic analyses designed to investigate the phylogenetic relationships within Rubiaceae (Rova & Andersson 1995, Andersson 1996, Andreasen & Bremer 1996). Unfortunately, phylogenetic studies are rarely complemented by a careful palynological investigation. The characters used are often ‘classics’ taken from the literature (as number of apertures, pollen type, sexine pattern), and not necessarily those that show a cladistically useful variation within the study group. The fact that most Rubiaceae pollen have interesting and systematically significant patterns at the inner nexine was first recognised by Van Campo (1978: 306): “...chez les Rubiaceae, les endosculptures sont particulièrement nettes, même en Mph dans la région aperturale où elles peuvent d’ailleurs fournir des caractères de diagnose.” Lobreau-Callen (1978) discussed the complex structure of the mostly compound apertures in Rubiaceae. Since these works, the variation and taxonomic value of nexine ornamentation as well as endoapertures have been assessed for several tribes: Anthospermeae and Paederieae (Robbrecht 1982, 1985), Gardenieae (Keddam-Malplanche 1985), Coptosapelteae (Huysmans et al. 1993), Psychotrieae (Jansen et al. 1996), Isertieae (Bosser & Lobreau-Callen 1998). In the palynological study of Huysmans et al. (1998) of the tribe Isertieae nexine characters were included in the cladistic analysis.

Many Rubiaceae groups are palynologically poorly known; one of them is the group dealt with in the present paper. In the traditional tribal concepts used in the Rubiaceae (Hooker 1873, Schumann 1891, Bremekamp 1952, 1966; Verdcourt 1958, Robbrecht 1988) the core of the genera studied here were placed in the Condamineae, a tribe with many-seeded capsular fruits. Molecular data (Bremer & Jansen 1991, Bremer 1992) indicated that a part of these genera (Condamineae subtribe Portlandiinae) are related to the Chiococceae, with fleshy fruits and one-seeded locules. This relationship was supported by floral features, among them filaments fused into a tube adnate to the base of the corolla-tube (but several genera of the Portlandiinae have free filaments attached to the base of the corolla-tube) and some genera of Chiococceae have filaments inserted on top of the ovary). Bremer (1992), based on the results of the analysis, proposed a wide concept for the Chiococceae, including the Portlandiinae.

Delprete (1996) re-evaluated the two tribes by means of cladistic analyses using morphological features. One pollen character only (pollen exine in surface view) was included in his data matrix. He concluded that the Catesbaeeae (an uncertain tribe in Robbrecht’s classifications of the Rubiaceae in: 1988, 1993b) belong to the same alliance. He...
reduced the Condamineae sensu stricto to the Rondeletieae and included its subtribe Portlandiinae in the Catesbaeeae, wherein two informal groups were recognized (Catesbaea-group and Portlandia-group). The results of the analysis did not support the wide delimitation of the Chiococceae by Bremer. *Exostema*, transferred from Cinchoneae to Chiococceae sensu lato in the above cited works of Bremer, was treated by Delprete as an informal group closely related to these two tribes. In the ‘Exostema-group’, Delprete also included *Badusa* and *Morierina*. This entire alliance is the subject of the present paper and is here called the Catesbaeeae-Chiococceae-Exostema complex, further abbreviated as the CCE-complex. It is interesting to note that in Delprete’s study (1996), in the two cladistic analyses with *Joosia* and *Cinchona* as outgroup, the synapomorphic character that supported the CCE-complex as a monophyletic group was echinate pollen.

In this study we also included *Molopanthera*, because it was considered as a member of the *Portlandia*-group sensu lato by Robbrecht (1993). Delprete (1996), however, excluded it from the Catesbaeeae on the basis of flower structure, dorsal anther attachment, and peltate seeds with fringed margins.

For a more extensive review of the CCE-complex, morphological descriptions of tribes and groups, and genera included, we refer to and follow Delprete (1996).

Pollen of few genera of the study group was investigated previously by Darwin (1977; *Masistiodendron*-SEM) and Aiello (1979; *Portlandia* and *Isidorea*-SEM, related genera only descriptive). McDowell (1996) illustrated his treatment on the taxonomy of *Exostema* with SEM micrographs of pollen of two species. One SEM micrograph of *Coutarea* pollen was published by Rova & Andersson (1995). General palynological studies were performed by Delprete in order to code the nexine ornamentation for his phylogenetic analysis, but the SEM micrographs were not published.

In the present paper we aim to give an account of the pollen morphology in the CCE-complex, using LM and SEM, focusing primarily on the nexine ornamentation and secondarily on the tapetal orbicules. The systematic value of pollen characters in the circumscription of this complex, together with the intergeneric variation was evaluated.

**MATERIAL AND METHODS**

This study is based on 45 specimens from 33 species of 20 genera. Observations were made on dried material from the following herbaria: BM, BR, L, S, and US (see appendix Specimens Investigated). For details on treatment and equipment used, see Huysmans et al. (1998). Because the pollen apertures of certain taxa appeared damaged after standard chemical treatment, acetolysis at room temperature during four and six minutes was tried on pollen of *Bikkiia retusiflora* in order to evaluate the effect of acetolysis compared to non-acetolysed grains. The damage to the pollen aperture increased proportionally with the increasing length of the treatment. All observations and illustrations are of standard acetolysed grains unless stated otherwise.

The ornamentation of the inner surface of the pollen exine was studied systematically with LM. Acetolysed pollen of selected specimens was cut with a freezing microtome (CryoStat) at −20°C. The thickness of the sections was adjusted to ± half of the pollen diameter. Sections were collected with a brush and transferred to a stub.

Length of polar axis (P) and length of equatorial diameter (E) were measured on ten grains in equatorial view with LM using a camera lucida. Other measurements were made on SEM micrographs. Terminology follows Punt et al. (1994). Shape classes in equatorial view are according to Nilsson & Pragowski (1992: 40).

We have used the term ‘nexine ornamentation’ for structures that partly may also be called ‘endoapertures’. According to the glossary of Punt et al. (1994), an endoaperture is “an aperture in the inner layer of the sporoderm, often the inner aperture of a compound aperture”, and an aperture is defined as “a specialized region of the sporoderm, that is thinner than the remainder of the sporoderm and generally differs in ornamentation and/or structure”. To enable descriptions of the complete inside ornamentation of the pollen grains, we have chosen the more general term ‘nexine ornamentation’ instead of ‘endoapertures’.

**RESULTS**

**General pollen descriptions**

The Catesbaeeae-Chiococceae-Exostema complex is a remarkably stenopalous group. The pollen morphological variation within the complex is very limited at first external sight, and its pollen is also highly characteristic and differs from other Rubiaceae. Palynological descriptions are given for the tribes, and detailed information on generic level and reference to figures are presented in Table I. The heading x/y (studied/total number of genera) informs on the relative number of genera studied for each group. Nexine ornamentation and orbicules are described separately.

**Catesbaeeae** (Catesbaea-group: 113; Portlandia-group: 712; Figs. 1–12)

The pollen grains of members of Catesbaeeae studied were always radially symmetrical, isopolar and medium-sized (P 17–48 μm, E 20–50 μm). Grains were 3-colpate with spiny colpus membranes. If an opening in the middle of the colpus membrane was present, it was a probable artefact caused by too aggressive acetolysis. All species investigated had a perforate tectum with microspines.

In *Catesbaea*, the only genus investigated of the “Catesbaea-group” (Figs. 1–4), four out of five species studied show a tectum densely beset with more or less blunt and short spines; the minute perforations in the tectum were much less numerous than the echinate processes. Pollen of *Catesbaea spinosa* (Figs. 1, 2), however, is easy recognisable by fewer, longer spines, and irregularly spaced perforations with a protruding rim (crateriform perforations).

In the “Portlandia-group”, two genera differ slightly from the general pollen type: *Bikkiia* pollen has a subtriangular amb and relatively short ectocolpi (Fig. 6), and in *Coutarea* pollen the ectocolpus margins are only vaguely defined.

**Chiococceae** (814; Figs. 13–17)

Pollen of Chiococceae was also medium-sized (P 15–29 μm, E 18–30 μm) and 3-colpate with spiny colpus membranes. It had a sexine ornamentation similar to that of the
Table 1. Some variable pollen morphological characters for each genus studied in Catesbaeeae, Chiococceae and Exostema-group.

<table>
<thead>
<tr>
<th>Tribe</th>
<th>Genus</th>
<th>Size</th>
<th>Shape</th>
<th>Pollen apertures</th>
<th>Wall ornamentation</th>
<th>Fig.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>P (µm)</td>
<td>E (µm)</td>
<td>P/E</td>
<td>Eq. view</td>
<td></td>
</tr>
<tr>
<td>CAT.C</td>
<td>Catesbaea (5/15)</td>
<td>17-(20.9)-27</td>
<td>20-(23.5)-28</td>
<td>0.75-(0.90)-1.00</td>
<td>SO-S</td>
<td>3-colpate perforate, microechinate</td>
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<td></td>
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<td></td>
<td>1-4, 37, 38</td>
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<tr>
<td>CAT.P</td>
<td>Bikkie (3/20)</td>
<td>20-(27.8)-35</td>
<td>24-(28.5)-34</td>
<td>0.74-(0.97)-1.21</td>
<td>O-SO-S-SP</td>
<td>3-colpate perforate, microechinate</td>
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<td></td>
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<td>5, 6, 40, 41</td>
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<tr>
<td></td>
<td>Coutaportla (1/3)</td>
<td>26-(28.0)-30</td>
<td>24-(29.0)-33</td>
<td>0.93-(1.03)-1.17</td>
<td>S-SP</td>
<td>3-colpate perforate, microechinate</td>
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<td>9, 10, 28</td>
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<td></td>
<td>Coutarea (1/7)</td>
<td>32-(37.9)-48</td>
<td>30-(36.9)-50</td>
<td>0.85-(1.20)-1.30</td>
<td>S-SP</td>
<td>3-colpate perforate, microechinate</td>
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<td>11, 12</td>
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<td></td>
<td>Hirtonia (3/4)</td>
<td>21-(25.5)-30</td>
<td>23-(28.0)-32</td>
<td>0.81-(0.91)-1.0</td>
<td>SO-S</td>
<td>3-colpate perforate, microechinate</td>
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<td></td>
<td>7, 8, 35</td>
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<tr>
<td></td>
<td>Isidorea (3/12)</td>
<td>18-(22.9)-28</td>
<td>22-(26.7)-33</td>
<td>0.74-(0.87)-1.05</td>
<td>O-SO-S</td>
<td>3-colpate perforate, microechinate</td>
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<td>36</td>
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<tr>
<td></td>
<td>Nernstia (1/1)</td>
<td>20-(20.7)-22</td>
<td>25-(25.4)-26</td>
<td>0.80-(0.81)-0.88</td>
<td>SO-S</td>
<td>3-colpate perforate, microechinate</td>
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<tr>
<td></td>
<td>Portlandia (1/5)</td>
<td>29-(30.0)-33</td>
<td>26-(28.9)-32</td>
<td>1.00-(1.04)-1.18</td>
<td>S-SP</td>
<td>3-colpate perforate, microechinate</td>
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<tr>
<td>CHI</td>
<td>Asemmantha (1/1)</td>
<td>24-(26.0)-29</td>
<td>23-(25.7)-28</td>
<td>0.93-(1.01)-1.12</td>
<td>S</td>
<td>3-colpate perforate, microechinate</td>
</tr>
<tr>
<td></td>
<td>Ceratopyxis (1/1)</td>
<td>23-(24.6)-27</td>
<td>25-(26.3)-28</td>
<td>0.88-(0.94)-0.96</td>
<td>S</td>
<td>3-colpate perforate, microechinate</td>
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<td>31, 32</td>
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<tr>
<td></td>
<td>Chiococca (2/20)</td>
<td>18-(22.4)-27</td>
<td>20-(24.9)-30</td>
<td>0.85-(0.90)-1.00</td>
<td>SO-S</td>
<td>3-colpate perforate, microechinate</td>
</tr>
<tr>
<td></td>
<td>Erithalis (2/10)</td>
<td>18-(20.0)-23</td>
<td>22-(25.3)-30</td>
<td>0.70-(0.80)-0.88</td>
<td>O-SO-S</td>
<td>3-colpate perforate, microechinate</td>
</tr>
<tr>
<td></td>
<td>Placocarpa (1/1)</td>
<td>20-(22.0)-24</td>
<td>25-(26.0)-27</td>
<td>0.80-(0.83)-0.92</td>
<td>SO-S</td>
<td>3-colpate perforate, microechinate</td>
</tr>
<tr>
<td></td>
<td>Salzmannia (1/1)</td>
<td>15-(16.3)-18</td>
<td>18-(19.0)-21</td>
<td>0.80-(0.86)-0.90</td>
<td>SO-S</td>
<td>3-colpate perforate, microechinate</td>
</tr>
<tr>
<td></td>
<td>Scolosanthus (1/1)</td>
<td>18-(19.4)-20</td>
<td>22-(23.3)-24</td>
<td>0.78-(0.83)-0.87</td>
<td>SO</td>
<td>3-colpate perforate, microechinate</td>
</tr>
<tr>
<td>CHI?</td>
<td>Mastixiodendron (2/7)</td>
<td>20-(25.5)-30</td>
<td>24-(28.3)-33</td>
<td>0.79-(0.90)-1.04</td>
<td>SO-S</td>
<td>3-colporate perforate, microechinate</td>
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<td>17, 30, 42</td>
</tr>
<tr>
<td>EXO</td>
<td>Badusa (1/3)</td>
<td>26-(26.9)-29</td>
<td>23-(24.6)-26</td>
<td>1.04-(1.06)-1.13</td>
<td>S</td>
<td>3-colpate perforate, microechinate</td>
</tr>
<tr>
<td></td>
<td>Exostema (2/40)</td>
<td>33-(36.8)-40</td>
<td>30-(35.4)-39</td>
<td>1.00-(1.04)-1.10</td>
<td>S</td>
<td>3-(4)-colpate perforate, microechinate</td>
</tr>
<tr>
<td></td>
<td>Morierina (1/2)</td>
<td>24-(25.4)-27</td>
<td>18-(20.4)-22</td>
<td>1.19-(1.25)-1.33</td>
<td>SP-P</td>
<td>3-colporate perforate, microechinate</td>
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<td></td>
<td></td>
<td>19, 20, 39</td>
</tr>
<tr>
<td>RON</td>
<td>Molopanthera (1/1)</td>
<td>14-(14.7)-16</td>
<td>14-(15.6)-17</td>
<td>0.88-(0.95)-1.00</td>
<td>S</td>
<td>3-colporate perforate, microechinate</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>23, 24</td>
</tr>
</tbody>
</table>
Figs. 1–6. Pollen grains of Catesbaeeae (SEM). 1–2. *Catesbaea spinosa*. (1) Polar view of tricolpate grain; (2) Mesocolpial view, note the spiny colpus membranes. 3–4. *Catesbaea glabra*. (3) Polar view of subtriangular, tricolpate grain; (4) Detail of tectum in mesocolpium with small perforations and short, blunt microspines. 5–6. *Bikkia retusiflora*. (5) Damaged apertures on standard acetolysed grains; (6) Grain of same specimen after 4 min. acetolysis on room temperature, intact apertures and spiny orbicules on its surface at the left. Scale bars: 5 µm in Figs. 1, 2 & 6; 10 µm in Fig. 5.
Catesbaeeae, that is perforate and microechinate. Nevertheless, two of the genera of this group deviate considerably from this general description both as internal pollen characters:

- *Placocarpa* (Figs. 16, 43) has reticulate pollen grains with muri bordered by granules and supported by long columellae; the three ectoapertures are narrow colpi, and in the granular nexine endocolpi with small extensions are situated.

- *Mastixiodendron* (Figs. 17, 42), which was only tentatively included in the tribe (Delprete 1996), has pollen grains with a tectum perforatum without any supratectal elements. The three compound apertures are composed of a narrow acute ectocolpus, a large lolongate mesosporus surrounded by an annulus, and an endocingulum with small, acute extensions.

**Exostema-group** (3/3; Figs. 18–23)

Pollen of this group is very similar to those of Catesbaeeae and most of the Chiococceae in having 3-colpate pollen with spiny colpus membranes and with a perforate and microechinate sexine (P 24–40 μm, E 18–31 μm).

**Molopanthera** (Figs. 23, 24)

Pollen morphology of this genus provides further evidence to justify its transfer from the *Portlandia*-group sensu Robbrecht (1993b) to the Rondeletieae (Delprete 1996, 1999b): the 3-colporate pollen grains are microreticulate without any supratectal processes. The mesosporus is as broad as the ectocolpus and has slightly protruding margins. Pollen grains are also smaller (14–17 μm) than the average within the study group and have characteristic H-shaped endostructures (Fig. 18).

**Nexine ornamentation**

Extra attention was paid to the morphological structures at the inside of the pollen grains. Their variation was studied systematically by LM (Figs. 31–43) and by additional SEM observations of freeze sectioned pollen grains (Figs. 24–30). The endopatterns were often very distinct at LM, but less so in e.g. *Catesbea* and *Coutarea*.

**Ornamentation types**

Seven types of nexine ornamentation were identified for all species studied. Four types were recognized in the CCE-complex sensu stricto (excl. *Placocarpa, Mastixiodendron* and *Molopanthera*).

(1) *Endocingulum* with extensions fused at poles (Figs. 25, 31–34). – This type is the most pronounced one in the group. In the granular inner layer, wide and straight cut-aways occur in a fairly constant pattern. This pattern can be described as an endocingulum with a single, broad extension in each mesocolpium. These extensions meet at the poles and form a star-shaped thinning that is well visible at LM. Near each ectoaperture, four smaller extensions are present, two at each side of the endocingulum, which fuse most commonly with the mesocolpial bands near the poles. In the cut-aways a smooth, perforated layer becomes visible (in *Coutarea* small granules occur). In the star-shaped thinning at the poles, small patches of the granular layer may be present (e.g. *Ceratopyxis*, Fig. 31).

Observed in: Catesbaeeae “*Portlandia*-group” (Hintonia latiflora, *Neristania*);

- *Chiococcaeae* (*Ceratopyxis, Chiococca nitida*); *Exostema-group* (*Badusa, Exostema caribaenum*).

(2) Endocolpi with diverging ends, star at poles and endocracks in mesocolpium (Figs. 26, 35–36). – There are three endocolpi perpendicular to the ectocolpi, with diverging, often branching ends. On the latitudinal sides of the endocolpi band-like cut-aways in the granular layer occur; these bands fuse at the poles appearing at the LM as a star-shaped thinning (Fig. 36). In the mesocolpia, short endocracks are situated at random. Where the granular inner layer is missing, a smooth and perforated layer is visible.

Observed in: Catesbaeeae “*Portlandia*-group” (Coutarea, Isodorea, Hintonia standleyana and *H. octomera, Portlandia*);

- *Chiococcaeae* (*Asemantha, Chiococca phaenostemon, Erithalis, Sclosanthus*)

(3) Endocolpi, each with four extensions (Figs. 27, 37–39). – Three endocolpi occur perpendicular to the ectocolpi and each with four acute extensions, two at each side pointing towards the poles. The extensions do not fuse at the poles, so there is no star-shaped thinning at the poles. The structure as seen in equatorial view often resembles an animal hide with four strong paws and a small head and tail. In *Salzmannia*, however, we observed endocolpi with diverging ends but without the four clear extensions pointing towards the poles. Endocracks occur in the mesocolpia. The cut-aways in the granular layer show a coarse lower layer without perforations.

Observed in: Catesbaeeae “*Catesbea*-group” (*Catesbea*);

- *Chiococcaeae* (*Salzmannia*); *Exostema-group* (*Exostema elegans, Morierina*).

Although all species investigated of *Catesbea* were classified under type 3, the nexine ornamentation is quite variable even within a single specimen. ‘Animal hide’-like endoapertures (type 3) are obviously most common but endocolpi with acute or diverging ends occur as well, mostly in combination with endocracks in the mesocolpia and at the poles. A clear star-shaped thinning at the poles as in our type 1 and 2 is lacking.

(4) Endocracks (Figs. 28, 40–41). – All over the inside of the grain, irregular endocracks occur in the granular layer, revealing a perforated lower layer. At LM no star-shaped structures were seen at the poles. A zone without endocracks below the ectocolpi is often present, which gives the impression of costae ectocolpi at LM. In a revision of *Bikkia* of New Caledonia, Jérémie & Hallé (1976) mentioned the occurrence of endocracks in their concise pollen descriptions (not illustrated). The endocracks can form an endopectification, especially in *Bikkia*.

Observed in: Catesbaeeae “*Portlandia*-group” (*Bikkia, Coutaportila*).

The three remaining types were each observed in one
genus only. The external pollen morphology of the genera concerned, indicated already that they do not fit within the main pollen type of the CCE-complex (see above).

(5) Endocolpus with short extremities (Figs. 29, 43). – Endocolpi occur in the granular inner layer, perpendicular to the ectocolpi. Short extremities are common on their
Figs. 31–43. Types of inside ornamentation in CCE-complex (LM). All pictures are taken with an oil immersion lens (100x) and relative proportions were respected. 31–34. Type 1. 31–32. Ceratopyxis verbenacea. (31) Polar view showing fusion at pole of three mesocolpial extensions of endocingulum with triangular patch of granular inner wall layer in centre; (32) Equatorial view on mesocolpium showing endocingulum with two extensions running perpendicular toward poles. Note small patch of granular inner layer where mesocolpial extensions meet the endocingulum. 33–34. Budusa corymbifera. (33) Polar view showing fusion at pole of three mesocolpial extensions of endocingulum; (34) Two pollen grains showing endostructures beneath the ectocolpus at different angles. In grain to the
Orbicules

No special preparations for observation of orbicules were made for this study; however, orbicules were often abundant, so that they were observed between the pollen grains and were observed with LM and SEM. Orbicules were observed in all genera studied of the Catesbaeeae; for Nernstia and Portlandia confirmation of orbicule presence is desirable. For the genera studied of the Chiococceae, orbicules were observed only in Salzmannia. In the Exostema-group orbicules were numerous in all taxa investigated.

Orbicules in the study group are relatively large (1–4 μm); they lie free on the tapetal membrane and are characteristically spiny, often with small perforations in their wall. According to the orbicule typology for the Cinchonoideae (Rubiaceae) presented by Huysmans et al. (1997), the orbicules of the CCE-complex belong to Type I (spiny orbicules). Orbicules of three species, which are relevant here, were illustrated: Bikkia retusiflora (their Figs. 4, 31), Catesbaea glabra (their Figs. 5, 6), and Hintonia standleyana (their Figs. 7, 8).

DISCUSSION

Pollen morphological features

The inner surface of the nexine is hardly ever completely smooth in Rubiaceae (e.g. Van Campo 1978, Keddam-Malplancha 1980, 1985; Huysmans et al. 1993, 1998, Jansen et al. 1996). In the CCE-complex, there is generally a granular inner layer with a smooth, often perforated layer underneath. TEM-observations are needed to decide if these layers correspond with the exine and the foot layer respectively (in progress). In an ontogenetic investigation of the pollen wall of Rondeletia odorata (Rubiaceae; El-Ghazaly et al. 2000) it is shown that the granular inside of the mature, acetylated exine most likely corresponds to the “membranous granular layer”, a wall layer that is formed proximally to the endexine, prior to intine formation. If this is also true for the CCE-complex, the smooth layer may correspond to the endexine. Our current knowledge of pollen wall ultrastructure in Rubiaceae is insufficient to draw any conclusions on the nature of the morphologically different layers of the sporoderm. TEM-images of pollen exines were published for few genera only, mainly in systematic papers (Abadie & Keddam-Malplancha 1975, Johansson 1987, Igersheim & Weber 1993, Weber & Igersheim 1994, Endress et al. 1996, Tilney & van Wyk 1997).

El-Ghazali (1990) provided a typology of endoapertures based on a LM study of 350 species of angiosperms. Twenty-four types were recognized, but these are difficult to correlate with the ornamentation patterns described in the present study.

The nexine ornamentation in Rubiaceae is mostly constant at generic level (e.g. Huysmans et al. 1993, 1998; Persson 1993; Delprete 1999 a, b). This seems to be the case in the study group as well, and we believe that the systematic value of these structures is underrated. In the CCE-complex exceptions are limited to Exostema where type 1 and 3 co-occur, and to Hintonia and Chiococca where both type 1 and 2 were found. Catesbaea which shows an intrageneric and also an intraspecific variation in nexine ornamentation was mentioned above.

Orbicules

The study group is a good example of the much cited parallelism between pollen and orbicule morphology within the same species (e.g. El-Ghazaly & Jensen 1986, El-Ghazaly 1989, Hesse 1986, Clément & Audran 1993). It is striking that spiny orbicules were only found in species with (micro)echinate pollen (see also Huysmans et al. 1997). For Rubiaceae this means that so far spiny orbicules were only found in the CCE-complex. Because of the strong parallelism in ornamentation between pollen sexine and orbicular wall as observed in this study and their common presence in the CCE-complex, orbicule morphology might offer additional morphological characters to distinguish the CCE-complex as a monophyletic group.

right, the endocolpus with four smaller extensions can be seen. The grain to the left is slightly tilted toward the pole where fusion of the mesocolpial bands can be seen, the smaller extensions fuse with these mesocolpial bands close to the pole. 35–36. Type 2. (35) Hintonia standleyana. Equatorial view on ectocolpus. The endocolpus having diverging, branching ends. (36) Isidorea pungens. Polar view showing star-shaped thinning. 37–39. Type 3. (37) Catesbaea grayi. Two grains in equatorial view, slightly tilted toward pole. Endocolpi perpendicular to ectocolpi with two out of four extensions visible; (38) Catesbaea spinosa. Equatorial view on ectocolpus, showing endocolpus with diverging, branching ends; (39) Morierina montana. Equatorial view on ectoaperture, showing endocolpus with acute ends and with four extensions running toward poles. 40–41. Type 4. Bikkia retusiflora. (40) Polar view, endocracks in inner granular layer; (41) Endocracks in mesocolpium. 42. Mastixiodendron plectocarpum. Endocolpus. 43. Placocarpa mexicana. Endocolpus.
Systematic relationships

Several molecular studies within the family Rubiaceae have recently been undertaken, among them those using trnL-F (Rova et al. 1997) and rps16 (L. Andersson, in progress) sequence data. In addition, Delprete et al. (in progress) started a multidisciplinary study that focuses on the phylogeny of the Catesbaeeae-Chiococceae-Exostemata complex, using trnL-F, ITS1&2, and 5S-NTS sequences, and morphological, anatomical and palynological studies. The molecular phylogenies obtained in the studies above indicate are in strong overall agreement in recognizing the CCE-complex as a monophyletic group.

Pollen morphology proved to be a powerful, additional tool in delimiting the CCE-complex. The characteristic pollen features of the complex that are not found so far in any other group of the Rubiaceae, permit pollen morphology to be a test for any hypothesis on phylogeny and its implementation in a classification. Pollen features apparently represent several synapomorphies supporting the complex as a monophyletic group: simple colpate apertures, perforate and microechinate tectum, and a smooth, often perforated layer under the granular inner nexine. However, a cladistic analysis is necessary to evaluate the pollen characters in this respect.

Our results clearly demonstrate that Mastixiodendron and Placocarpa could be excluded from the CCE-complex based on their pollen morphology.

The anomalous genus Mastixiodendron, originally assigned to Cornaceae (Melchior 1925), has semi-superior ovaries and is probably the only genus of the Rubiaceae assigned to Cornaceae (Melchior 1925), has semi-superior ovaries and is probably the only genus of the Rubiaceae

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SPECIMENS INVESTIGATED

Specimens of which pollen grains were sectioned with a freezing microtome for SEM observations are indicated by an asterisk.

Catesbaeeae “Catesbaea-group”

Catesbaea glabra Urb.: DOMINICAN REPUBLIC, Liogier 11607* (US).
C. gravi Griseb.: CUBA, Wright 371 (BR).
C. melanocarpa Urb.: ANTIGUÁ, Box 776 (US).

Catesbaeeae “Portlandia-group”

Bikkia campanulata Schltr.: NEW CALEDONIA, Fallen, Suprin & Favier 120 (L).
B. grandiflora Reinw. ex Blume: IRIAN JAYA, Versteegh BW4989 (L).
B. retusiiflora Schltr.: NEW CALEDONIA, Mac Kee 21703 (US).
Bikkia sp.: NEW CALEDONIA, Deplanche 850 (BR).
Coutaporta guatemalenis (Standl.) Lorence: MEXICO, Alush Méndez 6078* (BR).
Couturea hexandra (Jacq.) Schum.: NIGERIA, Stevens 3361 (BM); PANAMA, de Nevers, Herrera, Charnley & Diaz 5746 (BM); BRAZIL, Silva 59730 (S); Smith & Klein 14076 (S).
BELIZE, Dwyer 10960* (L).
Hintonia latiflora (Sessé & Moc.) Bullock: MEXICO, Hinton et al. 8123 (BR), Hinton et al. 15948* (US), Hinton et al. 15960 (US).
H. octomera (Hems.) Bullock: MEXICO, Gaumer 682 (BR).
H. standleyana Bullock: MEXICO, Hinton et al. 7676 (US); GUATEMALA, Contreras 7996 (US).
Isidorea lepantha Urb.: DOMINICAN REPUBLIC, Pater Fuertes 634 (BR).
L. pedicellaris Urb. & Ekem.: HAITI, Ekman 8251* (S).
L. pungetus (Lam.) B.L. Robins.: HAITI, Ekman 2413* (S); DOMINICAN REPUBLIC, Pater Fuertes 665 (BR).
Nernstia mexicana (DC.) Urb.: MEXICO, Carranza 737 (BR).
Portlandia gypsofila Macfadyen ex Griseb.: CUBA, Wright 259 (BR).

Chiococceae

Asennantha pubescens Hook.f.: GUATEMALA, Contras 1365 (S).
Ceratopyxis venenacea (Griseb.) Hook.f.: CUBA, Ekman 16553; Shafer 13517* (BM).
Chiococca nitida Benth.: FRENCH GUYANA, Billiet & Jadin 2015 (BR).
C. phaenostemon Schlecht.: COSTA RICA, Wilbur 27233 (BR).
Eritalis fraticosa L.: GAUELOUPE, Billiet & Jadin 5104 (BR).
E. odorifera Jacq.: GAUELOUPE, Billiet & Jadin 5048* (BR).
Placocarpa mexicana Hook.f.: MEXICO, Botteri 913 (BM).
Salzmannia nitida DC.: BRAZIL, Bamps 5062 (BR).
Scolosanthes versicolor Vahl: VIRGIN ISLANDS, St. Thomas, Eggers 191 (BR).
Mastixiodendron pachyclados (K.Schum.) Melch. var. tomentosum S. Darwin: IRIAN JAYA, Schram BW1929* (BR).
M. plectocarpum S. Darwin: IRIAN JAYA, Kalkman BW8516 (L).
**Exostema-group**

Badesa corymbifera A.Gray: TONGA, Yuncker 16090* (BM);
Fiji, Smith 4738* (L).

Exostema carubeum (Jacq.) Roem. & Schult.: DOMINICA, Ernst 1561* (BM).

E elegans Krug & Urb.: DOMINICAN REPUBLIC, Miguel Fuertes 1940* (L).

Morerita montana Vieill.: NEW CALEDONIA, Veillon 4804 (L).

**Rondeletieae**

Melopanthera paniculata Turcz.: BRAZIL, Loino 75* (US), Williams & Assis 6861 (BR).

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