

PALYNOLOGICAL STUDIES IN THE SUB GENUS *COPROSMA* (RUBIACEAE) FROM NEW ZEALAND

K. AZIZ

*Department of Botany,
University of Karachi, Karachi-32, Pakistan.*

Abstract

Ten species of the subgenus *Coprosma* (Rubiaceae) have been studied palynologically. TEM was more informative where different components of the exine provide taxonomic characters. The useful characters for taxa differentiation include pollen size (the section *Coprosma*) and the thickness of exine components.

Introduction

Considerable pollen morphological work has been done in the family Rubiaceae (Erdtman, 1952). Verdcourt (1958) recognised 4 pollen grain types in the family and though pollen variability is common, the tribes (Spermeaceae and Rubieae) mentioned in his fourth group are well differentiated by their pollen and many genera are similarly characterised. Walter (1965), Melhem (1974), Abadie *et al* (1975) and Robbercht (1978), found pollen characters useful in the taxonomy of this family. Walter (1965) working with aperture characters, contributed to the general understanding of the taxonomy and evolution of 31 species belonging to *Hedyotis*. Cranwell (1962) has studied pollen morphology of *Coprosma macrocarpa*. The purpose of present studies was to use palynology in taxonomy of the subgenus *Coprosma*.

Materials and Methods

Both herbarium and fresh material were used. For light microscopy, acetolysed pollen were used (Erdtman, 1952) using conc. H₂SO₄ and acetic acid anhydride in 1:1 ratio. Small samples were acetolysed on the slides. The pollen were mounted in glycerine jelly and lactic acid.

For SEM both treated with 10% KOH (Faegri & Iverson, 1950) and untreated pollen were subjected to gold coating after fixing the material on double sided non sensitive tape on stub. For ultra thin sectioning non-acetolysed dried or fresh anthers were treated with 6% gluteraldehyde in 0.05 M phosphate buffer pH 7.3 at 4°C for 24 h. Post fixation was in 4% buffered OsO₄ (osmium tetroxide) at 4°C overnight. After washing with buffer, dehydration, infiltration and embedding was carried out. Dehydration was carried out in 30, 50, 70, 95% ethanol series and dried in 100% ethanol. Material was kept for 30 min in each grade.

Infiltration was in a propylene-oxide-ethanol series in 1:3, 1:1, 3:1 ratio each for 20 min and 100% propylene-oxide 2 x 20 min. Material was then passed through an embedding mixture, 25, 50, 75 and 100% (2 x 1 h.) consisting of epen in propylene oxide on a rotator. For pollen structure, the material was kept in each grade for ½ day (the grades used were 30, 50, 70, 95 and 100% of epeon mixture in propylene oxide). The embedding was in epeon and capsules were hardened at 60°C for more than 36 h.

Ultra thin sectioning was done with the LKB 4804 A Nife Ultramicrotome, using a glass knife (LKB Bromma). Sections were placed on uncoated size 400 grids in a drop of distilled water and after drying the grids were stained in Uranyl acetate in 50% ethanol for 10 min and 0.4% aqueous solution of Lead citrate for 5 min on parafilm. Each time the grids were washed with distilled water. The microscope used was an EM Philips 200 electron microscope. Microphotographs were generally taken at x 8000 for comparison and exine measurements from non apertural region. Chemicals used were of EM grade.

All species of the subgenus were examined though appropriate details for only two are given in each section depending on the availability of the material. The pollen morphological study is based on observations from 10 species belonging to the subgenus *Coprosma*. For palynological terminology Faegri & Iverson (1950), Erdtman (1952), Kremp (1965) and Walker (1976) were followed.

Results and Conclusions

Mature pollen grains are generally single usually spherical to slightly oblong, usually 3 colporate sometimes 4 and rarely 2-colporate. Colpi are lanceolate and free. The exine is thin and usually finely reticulate. (*C. acutifolia*, Fig. 2b). The pollen wall is well differentiated into endexine, foot layer, columellae, and caputa. The basic structure of the pollen wall is constant within the subgenus (Fig. 1-2).

The caputa (tectum) are usually compact but may be loose. Generally there is an intermixing of smaller and larger caputa in the same pollen grain. The caputa are oblong to spherical in shape. Presence of these two forms is common in the same species. There is a great deal of general variation in pollen characters, and therefore character expression is averaged and is based on the major tendencies. The columellae are mostly simple rod-like structure, however in some cases (e.g. *C. lucida*, Fig. 1d 1, 2) simple and reticulate columellae are present. The columellae are sometimes with spaces between tectum and foot layer.

The endexine is usually thinner than the foot layer. It is sometimes irregularly present. It is thicker near the base of the aperture, where it usually ends abruptly and the aperture seems to have ektexine components (e.g. *C. tenuifolia*). The intine appears to

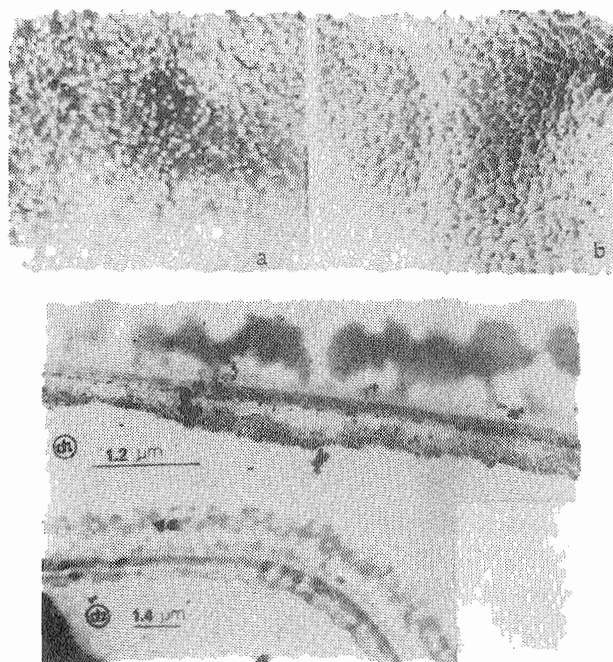


Fig. 1. *Coprosma lucida*. a & b surface pattern (SEM x 15000) d1, d2 exine stratification.

be lamellate and forms a plug-like structure (e.g. *C. tenuifolia*, Fig. 2a, 1, 2) in the apertural region. In some cases the intine is differentiated into outer colourless and inner lamellate parts (e.g. *C. lucida*), Fig. 1d, 1, 2).

Systematic Accounts: The section *Coprosma* is differentiated in having greater length/breadth of pollen grain (38/36.25 μm), intermediate length of columellae (0.175 μm), caputa breadth (0.345 μm), thickness of foot layer (0.26 μm) and endexine (0.14 μm). There is a greater tendency towards circular caputa in sec. *Petiolatae*. Secs. *Petiolltae* and *Australes* show similarities, and the sec. *Coprosma* is intermediate in position (Table 1).

Within the section *Australes* specific differentiation is in the combination of exine characters. Exine is thin (0.98 μm) in *C. tenuifolia* as compared to *C. robusta* (1.07 μm) and *C. macrocarpa* (1.25 μm). The foot-layer is thin in *C. tenuifolia* (0.25 μm) and thicker in *C. robusta* (0.31 μm) and *C. macrocarpa* (0.39 μm). The endexine is thin in *C. macrocarpa* (0.13 μm) whereas it is 0.25 μm in *C. robusta* and 0.16 μm in *C. tenuifolia*.

In *C. macrocarpa* caputa tend to be oblong sometimes circular, and the tectum is generally not compact. Caputa in *C. grandifolia* are oblong or circular. In *C. robusta* is usually compact but sometimes loose.

Table 1. Pollen study based on pollen size, and thickness of various exine components in *Coprosma*.

	Pollen size (equatorial x polar) (μm)	Exine thickness (μm)	Caputa (length x width) (μm)	Columellae length (μm)	Footlayer thickness (μm)	Endexine thickness (μm)
<i>C. acutifolia</i>	32.0 x 30.0	-	-	-	-	-
<i>C. grandifolia</i>	32.0 x 28.0	-	-	-	-	-
<i>C. macrocarpa</i>	32.0 x 31.0	1.25	0.57 x 0.35	0.19	0.39	0.13
<i>C. robusta</i>	34.0 x 33.5	1.07	0.63 x 0.42	0.2	0.31	0.25
<i>C. tenuifolia</i>	33.0 x 31.5	0.98	0.42 x 0.38	0.19	0.25	0.16
<i>C. dodonaefolia</i>	39.0 x 36.5	1.30	0.59 x 0.35	0.20	0.30	0.17
<i>C. lucida</i>	38.0 x 36.0	1.0	0.5 x 0.34	0.15	0.22	0.11
<i>C. chathamica</i>	-	1.07	0.52 x 0.4	0.25	0.34	0.17
<i>C. petiolata</i>	31.0 x 29.5	-	-	-	-	-
<i>C. repens</i>	36.0 x 30.0	1.06	0.52 x 0.46	0.16	0.32	0.19

Section Australes: *C. acutifolia*, *C. grandifolia*, *C. macrocarpa*, *C. robusta*, *C. tenuifolia*. Sec. *Coprosma*. *C. dodonaefolia*, *C. lucida*. Sec. *Petiolatae*. *C. chathamica*, *C. petiolata*, *C. repens*. (Allan, 1961).

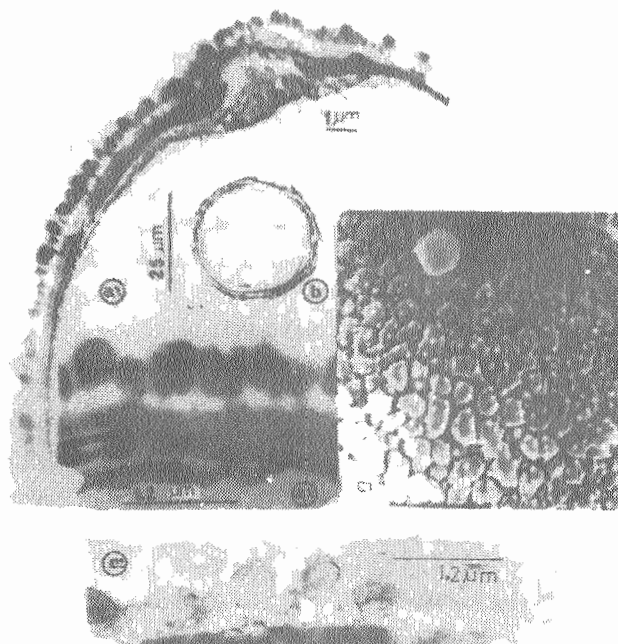


Fig. 2. *Coprosma tenuifolia*, a1 exine under apertural region & a2 under non apertural region. *C. acutifolia*, 2b pollen grain 1 μ m. *C. petiolata*, c1 surface pattern SEM x 15000 & c2 exine stratification.

C. dodonaefolia (Sec. Coprosma), is differentiated from *C. lucida* in having thicker exine and endexine with a relatively greater tendency towards spherical caputa, though oblong caputa are also frequent (Table 1).

The section *Petiolatae* is comparatively homogeneous. Caputa are generally spherical. In *C. chathamica* and *C. repens* sometimes oblong caputa are also found. *Coprosma petiolata* (Fig. 2c, 1, 2) is further differentiated in generally having indistinct Columellae, which are well pronounced in *C. chathamica* and *C. repens*. *Coprosma baueri* is closely similar to *C. repens* in pollen characters.

The basic pollen morphology and pollen surface patterns are fairly consistent throughout the subgenus, regardless of collection site and do not offer major characters of taxonomic value. The main differentiation is in the pollen size in some cases and caputa shape. Other characters are the thickness of various different exine components which are well developed and complex. Thus TEM appears to be more informative than SEM for species distribution.

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