POLLEN MORPHOLOGY AND TAXONOMY OF

CLUTIA L. (EUPHORBIACEAE)

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Abstract

The pollen morphlogy of 34 species of *Clutia* L. (Euphorbiaceae) has been studied by light and scanning electron microscopy. The grains are medium sized, prolate to subprolate and rarely prolate spheroidal, tricolporate and distinctly tectate. The tectum is reticulate to punctate and the lumina are variable in size and shape. Pollen dimensions were found to be of no significance in defining infrageneric relationships while reticulation pattern, pitting density and roughnes of the exine distinguished several pollen groups when analysed by multivariate methods. The three large groups maintained their integrity regardless of method of multivariate analysis employed. A further comparison with the sections of *Clutia* suggested by Pax (1911) and Prain (1913) gave substantial support for some of these sections.Type ED 1 is characterised by irregular exine pits and rough tecta and is correlated to the section *C. alaternoideae* recognized by both workers in earlier sectioning of *Clutia*. Type RT 1 corresponds to *C. abyssinica* and *C. pulchallae* of Prain and the sections of Pax to a large extent. In general several associations of species based on pollen morphology are found in the recognised sections of *Clutia.* Implications of pollen morphology in the taxonomy of *Clutia* are discussed.

INTRODUCTION

General

Clutia L. (Clutieae: Crotonoideae: Euphorbiaceae) is a genus of small trees, shrubs, subshrubs or woody herbs which have simple hairs. Leaves are simple, entire and mainly alternate except for the South African species which are ericoid. They are monoecious plants, though some species are rarely dioecious, bearing distinctive yellowish-green flowers in clusters at the leaf axils. The fruit is a more or less spherical capsule which encloses glossy black ovoid shaped seeds.

Taxonomic history

When Linnaeus erected the genus in 1753, he included 5 species: *C. alaternoides* L., *C. pulchella* L., *C. bridelia* L, *C. eluteria* L. and *C. cascarilla* L., C. *cascarilla* and *C. eluteria,* but of these only the first two remain today. The rest were transferred to *Bridelia* (*B. retusa*) and Croton (*C. cascarilla* and *C. eluteria*). The first major treatment was that of Mueller Argorviensis in 1866, who recognised 29 species from the Arabia, NE Tropical Africa and the majority from the Cape. Pax (1911) added a further 48 species to Mueller's treatment from E & S tropical Africa and Prain (1920) described more species in South Africa. Prain's work was the last comprehensive revision of the genus and it includes 70 recognized species which are distributed along the Afromontane escarpments from the Cape to NE Tropical Africa with two of these occurring in tropical Arabia (Thulin, 1993). The genus is well represented in South Africa where 40 species occur and most of these are found in the Western Cape. Thus the last comprehensive account was by Pax.

The affinities of the genus

The placement of *Clutia* within Euphorbiaceae is somewhat problematic (Radcliffe-Smith, 1992). *Clutia* was originally placed by Mueller in the tribe *Hippomaneae* which included most of the non-involucrate uniovulate platylobes with an imbricate calyx, but the genus was kept in a subtribe of its own because of its distinctive male flowers especially the column on which stamens are borne. Bentham (1880) retained only 4 of Mueller's 16 subtribes in the tribe *Hippomaneae* in his revision of the tribe *Crotoneae* and placed *Clutia* in his subtribe *Chrozophoreae* together with 23 other genera which have petaliferous male flowers, though their inflorenscences were variable. Pax (1890) on the other hand raised Mueller's subtribe to tribal status and associated with *Clutia* the genera with a valvate calyx whilst Hutchinson (1969) pruned down Pax's tribe by taking out subtribes *Codiaeinae* and *Ricinodendedrine* which he elevated to tribal status. The last treatment is by Webster (1989) who removed the two other tribes in *Clutia*e, *Moultonianthus* and *Syndryophyllum*, and maintains that *Clutia* is distinct enough to be in its own tribe.

Relationships among species within *Clutia*

Two major works in establishing an infra-generic taxonomy in *Clutia* are by Pax (1911) and Prain (1913). Using leaf characters and number of glands in male flowers (Table 1) Pax (1911) divided the genus into eight sections (Table 2). Prain (1913) who studied mostly Southern African species and recognised only two large sections sections (Table 1). In both of these treatments the sections appear to be artificial as number of glands on the petals of male flowers was used and the unreliability of the this character was later demonstrated by Taylor (1965) who found it to be inconsistent because some species in the section *Pauciglandulosae* develop subsidiary glands in their flowers (Taylor, 1965). The utility of this character thus proves to be of limited value due to the fact that a considerable number of flowers require have to be examined even before a specimen can be placed in the primary subdivision of the genus. There is therefore no modern treatment of the infra-generic relationships in *Clutia.*

Table 1. Key characters used by Pax (1911) to divide *Clutia* into sections

A. Leaves not ericoid	
a. Leaves large for the genus, membranous, usually bearing all the stomata on the undersurface.	
α Stems branching	
I. Male petals with one basal gland , fundus of calyx of the other aglandular	1. *PAUCIGLANDULOSAE*
II. Fundus of calyx of male multi glandular	2. *MULTIGLANDULOSAE*
β Stems (from rhizome) more than one , simple	3. *SIMPLICES*
b. Leaves medium or small, usually firm coriaceous, not sericio-pilose	
α Glabrous. Stomata on both surfaces	4. *ALATERNOIDAE*
β Hairy. No stomata on upper surface	5. *DAPHNOIDAE*
c. Leaves small, densely sericeo-pilose	6. *TOMENTOSAE*
B. Leaves ericoid	
a. Leaves revolute, all stomata on lower surface	7. *REVOLUTE*
b. Leaves involute, stomata on upper surface and lower surfaces	8. *INVOLUTE*

Table 2. The relationship between the sections of genus *Clutia* given by Pax (1911) and Prain (1913), modified from Taylor, 1965.

PRAIN	PAX
I. *PAUCIGLANDULOSAE*	***PAUCIGLANDULOSAE***
1. *Involutae*	*8. **INVOLUTAE***
2. *Tomentosae*	6. **TOMENTOSAE** emend. et *C. thunbergii excl.*
3. *Alaternoidae*	4. **ALATERNOIDAE** ampl. et emend. et *C. crassifolia* exclus.
4. *Impeditae (C. impedita* Pr.)	
5. *Alpinae* (*C. alpina* Prain)	
6. *Pulchellae*	1. ***PAUCIGLANDULOSAE*** sensu strictiore
II. ***MULTIGLANDULOSAE***	2. ***MULTIGLANDULOSAE*** ampl.
7. *Myricoideae*	2. ***MULTIGLANDULOSAE*** sensu strictiore
8. *Disceptatae*	3. ***SIMPLICES*** necnon 5. ***DAPHNOIDEAE*** pro parte maxima sed *C. daphnoides*, Lamk., exclus.
	5. ***DAPHNOIDAE*** pro parte maxima et quoad *C. daphnoides*, Lamk., tantum
9. *Daphnoidae*	6. ***TOMENTOSAE*** pro parte minima et quoada *C. thunbergii*, Sond. , tantum
	7. ***ALATERNOIDAE*** pro parte minima et quoada *C. crassifolium*, Pax, tantum
10. *Polygonoideae*	7. ***REVOLUTAE*** pro parte minima et quoada *C. polygonoides*

Delimitation among some of the difficult species

The difficulty of defining species in some of the sections is by illustrated Taylor (1965) who did a critical evaluation of key and descriptive characters of species in the section *Alaternoideae*. Within the section he chose five species which are difficult to separate and examined the leaf and flowers characters used in Prains key. His work revealed that more *Alaternoidae* reliable characters are needed to separate species within the complex.

Character studies in *Clutia*

Morphological

Taylor (1965) investigated characters used by Prain to separate five difficult within the *Alaternoidae* species *C. africana, C . alaternoides, C. laxa, C. rubricaulis.* and *C. virgata.* He plotted leaf lengths against widths and found leaf shapes to be in adequate in separating species. He further examined the extent of variation of leaf denticulation and revoluteness in the group and concluded that it is too wide for these characters to form a sound basis for separating species. The only leaf character he found to be useful is the nature of the midrib when considered together the shape of the apex. Floral morphology, including the number of glands in the petals were found to be the same in the group. No other morphological studies in the genus were found in literature search.

Palynology

The only two accounts of pollen based studies in *Clutia* are in the works of Punt (1962) and Mtotomwema and Mahunnah (1985). Punt (1962) presented a survey of pollen morphological features of Euphorbiaceae which included *Clutia* and his work revealed that pollen grains of species within one genus in the family generally fit to the same pollen type although dimensions differ. More recently, Mtotomwema and Mahunnah (1985) studied exine patterning in six *Clutia* species of Tanzania and distinguished several pollen types which they used to describe the realtionships of the species.

Problem setting

Literature search has shown no account of anatomy and other organ based studies which have been done in *Clutia*. The taxonomy of *Clutia* is clearly inadequate. The need to locate new characters to indicate relationships among species is obvious. This is of crucial importance because species limits (i.e. the alpha taxonomy) can only be resolved in the context of a good sectional taxonomy and to achieve that task good characters are needed. Palynology has proven to be useful in the revision of genera (Stuessy, 1989) thus the project aims to use pollen morphology the determine the infra-generic realtionships within *Clutia.*

MATERIALS AND METHODS

Sampling

Thirty-four southern African and six tropical species, representing all the sections of Pax and Prain in *Clutia*, were sampled for their pollen (Table 3). Flower material for the preparation of acetolysed pollen slides and scanning electron micrographs was obtained from specimens housed in the Bolus herbarium at the University of Cape Town. As far as possible, two [plants or specimens] were sampled per species, but in some cases only one specimen was available. As the plants are dioecious there is often little male material available. A pair of forceps was used to remove 3-6 ripe flower buds / undehisced young male flowers from the plants. This ensured that the likelihood of obtaining pollen remained high, as older flowers tend to lose pollen easily (Forman and Bridson, 1989) and chances of using flowers which are contaminated by foreign pollen grains remained minimal.

Acetolysis

For both light microscopy and scanning electron microscopy the pollen grains were acetolysed according to a method modified from Erdtman (1952). Anthers are removed from the flowers, and soaked in a wetting agent (liquid dish-washing soap as used in this study) To release pollen grains from the remaining fragments of anthers, a glass stirring rod was used to crush the debris against the bottom of the centrifuge tubes and the mixture was centrifuged at 1000 RPM for 5 minutes. The wetting agent is then removed and washing with Acetic anhydride (Glacial Acetic Acid) is done before the anthers are digested using an acetolysis mixture of 9:1 ratio of Acetic Anhydride and Sulphuric Acid. Acetic Anhydride serves as a suitable medium between water and acid in the Acetolysis mixture and also stains the pollen grains. The anthers were then digested by adding about 2 ml of the acetolysis mixture and heating gently for 10 minutes in a boiling water bath placed inside a fume cupboard.

The loose pollen grains were washed with acetic anhydride and then in distilled water before examination by LM and SEM. At this stage, half portions (0.5-1.0 ml) of the acetolysed material were removed from the centrifuge tubes and transferred to small glass vials. To each of these glass vials, ca. 1 ml 90 % ethanol was added to preserve the pollen as it was examined using SEM at least three weeks later.

Preparation of LM slides

A 1:1 glycerol-water mixture was added to the remaining half portions of pollen in the centrifuge tube and left to stand for 1 hour. Afterwards this was centrifuged and decanted then test tubes were left upside down for another hour which left a deposit of pollen grains at base of the centrifuge tubes. Permanent slides were prepared by extracting pollen grains from the deposit by touching it with 0.5 mm diameter glycerine blocks. Each , with its pollen grains, was placed in the middle of a microscope slide between two plastic strips to prevent the cover slips from crushing the pollen grains. The slides were then heated gently to melt the gelatine block and sealed with paraffin wax. Procedures for preparing the glycerine-gelatine are described in the appendix.

Measurements using LM

A compound microscope (Zeiss Axioskop) fitted with a measuring graticule was used to make 10 measurements, at 1000 X magnification, per slide for the Polar axis (P) and equatorial axis (E). The measurements were converted from graticule units to micrometers. The ranges, standard errors (S.E.) and the P/E ratios were calculated to estimate the size and shapes of pollen (Erdtman, 1952). A scatter diagram has been made where P axis has been plotted against E axis to correlate these variables and to find out any possible separation among sections.

Since observation of the tectum at 1000 X magnification showed differences in sizes of the lumina between different taxa, counts of the number of pores per 5 graticule units were made to quantify these differences. These were later confirmed by an estimation of Pit density (μm^{-2}) using electron micrographs. Further detailed studies of the exine stratification are based on SEM.

Scanning Electron Microscopy (SEM)

Acetolysed pollen grains which were suspended in ethanol and stored in glass vials were mounted on metal stubs. The stubs were cleaned and marked on the bottom before being coated with a water-insoluble glue and Carbon. The glue serves to stick the pollen grains in the stub whereas the Carbon is for conducting the electrons discharged in the beam within the microscope. Using a pipet, a drop was transferred from the vial to the metal stubs and the ethanol was allowed to evaporate at room temperature. The samples were then sputter-coated with gold and stored in a desiccator before examination to keep them clean and dry. Observations were then made using a Cambridge 400 electron microscope set at a low acceleration voltage (10 KV) to avoid damage of samples. Pictures of whole grains and detailed surfaces were taken and the micrographs were later used to describe the tectum and appetural system and to calculate pore densities by counting the number of pores The terminology used in the description and measurement of pollen grains follows that of Erdtman (1952) and Faegri and Iversen (1964). For description of the exine patterns the recommendations of Praglowski and Punt (1973) and Nilsson and Muller(1978) are followed.

Numerical analysis

Eight characters (Table 4) were used in the numerical analysis. The items in the matrix are species, and where two or more specimens were available, data collected from only one specimen was entered, since in most cases there were no significant differences observed between the two specimens observed. In order to reduce the effects of different scales of measurement in pollen characters, the data were standardized by subtracting the mean of each variable and dividing the result difference by the standard deviation. Pair-wise comparisons of species were made and then two different coefficients were used to calculate a similarity matrix. Since most of the data are quantitative, the first coefficient used was Rogers & Tanimoto which measures similarity or the number of actual agreement between the items as compared with the number of theoretically possible ones (Sneath and Sokal, 1973). The level of agreement between pairs of OTUs is estimated over an array of two state and multi-state characters which are coded (e.g. 0=present, 1=absent) to make them suitable for the computation of association coefficient (Table 2). To take into account the two continuous characters included in the data matrix, P/E ratios and pitting density, a second method that calculates average taxonomic distances was applied. This method differs from the first in that the coefficient it uses aims to compute average taxonomic distances between OTUs, thus it is best suited for continous data whereas the former works well with categorical data. These distances are calculated between any two OTUs in a multi-dimensional hyperspace corresponding to the number of characters and are called Euclidean distances (Abbott *et. al.*, 1985). In each case, data in the resulting similarity matrix was subsequently used to calculate a phenogram by unweighted pair-group arithmetic average (UPGMA) clustering. The computation for multivariate analysis was done using the NTSYS-pc (Numerical Taxonomy and Multivariate Analysis System) package, version 1.8 (Rohlf, 1989).

OBSERVATIONS

The pollen grains of *Clutia* are single, isopolar and tricolporate. The pollen is medium sized ranging from 21-50 μm on the polar axis. The smallest pollen in the genus is found in *C. laxa*, while *C. pulchella* has the greatest polar length (Table 1). The majority of the species have pollen which is subprolate (P:E = 1.14-1.33) to prolate (P:E = 1.33-2.00) in shape, while a few species in the studied taxa including *C. ericoides*, *C. laxa*, *C. impedita and C. marginata* have prolate spheroidal (P:E=1.00 -1.14) grains. The exine surface of grains appears smooth in some species whereas for others it has a bumpy roughness. The ectexine is distinctly tectate with a reticulate surface of two different types; a coarse reticulation pattern with well expressed and warted supratectral reticulation and larger lumina e.g. *C. impedita* (Plate3A-B) and a perforate or punctate type with less developed supratectral reticulation (e.g. *C. ericoides*)(Plate 2C). There are two different types of coarsely reticulate grains: one type is characterised by ridges which occur between the pores and in other grains there were no ridges observed (e.g. *C. disceptata*)(Plate 2B). The lumina generally tends to be irregular in shape and size but few species which belong to the coarsely reticulate type deviate from this trend. These are marked with ridges around the lumina which form a network that is shaped in the fashion of regular polygons. The pits in the exine are uniformly distributed over different parts of pollen in the genus. Some differences were observed in *C. abyssinica*, *C. africana*, *C. brevifolia* and *C. virgata*, where the lumina of the poles are smaller than in the mesocolpium. The size of the pores ranges from 0.125 μm to 0.25 μm in the perforate grains whereas the size range in the coarsely reticulate type is 1.2 - 2.5 μm. In all species observed, wall stratification is characterised by a prominent foot layer which is thicker in areas surrounding the endoaperture. The colpi are long and extend to over 80% of the whole length of the grain along the polar axis in all species with large and well defined endoapetures varying in shape between ±circular, square, rectangular and saddle.

Table 3: Mean values of *Clutia* pollen measurements for polar and equatorial axis (μm), P/E ratios and shapes. n = 10.

		EQUATORIAL AXIS			POLAR AXIS				
TAXA	VOUCHER	MIN	MEAN ±S.E	MAX	MIN	MEAN ±S.E	MAX	P/E	Remarks
C. abyssinica	Msiska 74	25	(27.3 ±0.24)	27.5	30	(32.0 ±0.47)	35	1.17	subprolate
C. affinis	Fourcade 439	17.5	(23.3 ±0.87)	27.5	25	(28.8 ±0.53)	30	1.24	subprolate
	Levyns	17.5	(22.5 ±0.87)	27.5	27.5	(31.5 ±0.88)	35	1.4	prolate
C. africana	Flanagan	25	(28.8 ±0.53)	30	35	(35.5 ±0.32)	37.5	1.23	subprolate
	Pillans8863	27.5	(30.0 ±0.50)	32.5	37.5	(38.5 ±0.52)	42.5	1.28	subprolate
C. alaternoides	Esterhuysen	25	(27.5 ±0.35)	30	32.5	(34.5 ±0.47)	37.5	1.25	subprolate
	Pillans4039	25	(26.0 ±0.39)	27.5	32.5	(33.3 ±0.56)	37.5	1.28	subprolate
	Kensit1913	27.5	(30.3 ±0.55)	32.5	32.5	(34.3 ±0.36)	35	1.14	subprolate
C. alpina	Prain 6827	20	(23.5 ±0.88)	27.5	27.5	(30.3 ±0.83)	35	1.29	subprolate
C. brevifolia	Compton 4256	22.5	(24.0 ±0.39	25	25	(27.5 ±0.61)	30	1.15	subprolate
	Mauch 1220	25	(27.0 ±0.59)	30	32.5	(35.5 ±0.77)	40	1.31	subprolate
C. daphnoides	Fries 513	15.6	(22.0 ±1.29)	27.5	22.5	(27.3 ±0.9)	32.5	1.24	subprolate
	Flanagan 5105	17.5	(19.5 ±0.47)	22.5	23.8	(29.3 ±1.73)	31.3	1.5	prolate
C. disceptata	Tyson 1113	25	(26.5 ±0.39)	27.5	35	(36.8 ±0.36)	37.5	1.39	prolate
	Tyson 1234	25	(26.8 ±0.36)	27.5	32.5	(36.3 ±0.53)	37.5	1.36	prolate
C. dregeana	Rogers 27506	25	(26.0 ±0.39)	27.5	30	(33.0 ±0.47)	35	1.27	subprolate
C. ericoides	Wally-Dodd 1109	20	(27.3 ±0.83)	30	35	(37.5 ±0.35)	40	1.38	prolate
	Prain 1365	25	(29.8 ±0.90)	35	25	(29.3 ±1)	32.5	0.98	prolate spheroidal
C.galpinii	Galpin	18.8	(25.0 ±0.88)	31.3	27.5	(30.0 ±0.35)	32.5	1.2	subprolate
C. heterophylla	Galpin	23.8	(26.4 ±0.58)	30	27.5	(30.5 ±0.47)	32.5	1.15	subprolate
C. hirsuta	Haptron 840	25	(26.8 ±0.36)	27.5	30	(33.0 ±0.59)	35	1.23	subprolate
	Prain 899	12.5	(23.9 ±2.13)	30	20	(29.3 ±1.73)	32.5	1.22	subprolate
	Acock 840	22.5	(26.8 ±0.71)	30	35	(37.3 ±0.43)	40	1.39	prolate
C. imbricata	Hutchinson 1053	20	(21.9 ±0.54)	22.5	25	(26.5 ±0.39)	27.5	1.21	subprolate
	341	20	(23.0 ±0.59)	25	27.5	(29.4 ±0.54)	30	1.28	subprolate
C. impedita	Prain 1899	22.5	(25.5 ±0.77)	30	27.5	(29.0 ±0.39)	30	1.14	subprolate
C. inyangensis	Fries 3860	22.5	(24.0 ±0.39)	25	27.5	(31.0 ±0.72)	35	1.29	subprolate
C. katharine	Esterhuysen 12862	20	(24.8 ±0.66)	27.5	32.5	(34.8 ±0.75)	40	1.4	prolate
	Esterhuysen 10198	20	(25.0 ±0.94)	30	30	(33.0 ±0.69)	37.5	1.32	subprolate

C. laxa	Flanagan 4063	22.5	(25.5 ±0.77)	30	22.5	(25.5 ±0.77)	30	1	prolate spheroidal
C. marginata	Steyn 233	20	(31.1 ±1.95)	37.5	30	(34.0 ±0.63)	37.5	1.09	prolate spheroidal
	Butt-Daury 2049	20	(24.8 ±0.97)	27.5	32.5	(42.9 ±1.78)	47.5	1.73	prolate
C. monticola	Sankey 236	25	(28.5 ±0.52)	30	32.5	(35.8 ±0.71)	40	1.25	subprolate
C. nana	Prain 2870	17.5	(23.6 ±1.50)	27.5	22.5	(27.1 ±1.18)	30	1.15	subprolate
C. natalensis	Galpini 78	22.5	(25.3 ±0.43)	27.5	27.5	(29.8 ±0.24)	30	1.18	subprolate
	Wylie 1935	22.5	(25.3 ±0.43)	27.5	27.5	(28.5 ±0.39)	30	1.13	subprolate
C. paxii	Stolz 2132	25	(27.0 ±0.47)	30	30	(33.3 ±0.71)	37.5	1.23	subprolate
C. polifolia	Esterhuysen 5498	22.5	(24.8 ±0.55)	27.5	27.5	(29.3 ±0.51)	32.5	1.18	subprolate
	Theorow 2068	22.5	(24.3 ±0.51)	27.5	27.5	(29.3 ±0.36)	30	1.21	subprolate
C. polygonoides	Pillans8871	30	(32.9 ±0.79)	35	35	(36.8 ±0.51)	40	1.12	prolate spheroidal
C. pterogona	Wally-Dodd	22.5	(24.0 ±0.39)	25	30	(32.8 ±0.82)	37.5	1.37	prolate
	Koutnik	20	(23.5 ±0.63)	27.5	27.5	(29.0 ±0.81)	35	1.23	subprolate
C. pubescence	Esterhuysen 27746	20	(21.5 ±0.39)	22.5	32.5	(34.0 ±0.39)	35	1.58	prolate
	Prain	20	(21.8 ±0.62)	25	25	(30.0 ±0.79)	35	1.38	prolate
C. pulchella	Rodin 1960A	25	(26.3 ±0.4)	27.5	30	(31.5 ±0.52)	32.5	1.2	subprolate
	Compton 26858	27.5	(29.3 ±0.62)	32.5	35	(36.8 ±0.36)	37.5	1.26	subprolate
	Levyns 3980	30	(32.5 ±0.54)	35.5	42.5	(44.0 ±0.52)	47.5	1.3	suprolate
C. rubricaulis	D'Urban 916B	20	(23.0 ±0.69)	27.5	25	(26.5 ±0.39)	27.5	1.15	subprolate
C. swynnertonii	Moore 3936	22.5	(26.8 ±0.79)	30	27.5	(33.0 ±0.99)	37.5	1.23	prolate
C. thungergii	Bruyns 4778	20	(21.9 ±0.54)	22.5	25	(28.3 ±0.51)	30	1.29	subprolate
C. tomentosa	Esterhuysen 3048	20	(25.0 ±1.06)	32.5	35	(41.8 ±1.00)	45	1.67	prolate
	Salter 4795	32.5	(35.3 ±0.66)	37.5	32.5	(37.3 ±1.6)	50	1.06	prolate spheroidal
C. virgata	Rogers 14864	20	(23.3 ±0.62)	27.5	30	(31.5 ±0.39)	32.5	1.35	prolate
	Hutchinson 2418	20	(22.8 ±0.55)	25	27.5	(29.0 ±0.39)	30	1.27	subprolate

Pollen descriptions

C. abyssinica **Jaub. & Spach. (Plate 1A, 4 A&B)**

Msiska 74

Size: P(30-)32(-35) µm, E(25-)27(-28), P/E 1.18, pollen grains subprolate. Tectum bumpy and coarsely reticulate, lumina generally variable in size and shape, with prominent ridges in between. Smaller pores concentrated around mesocolpi, ± circular in shape, up to 1.5 µm in diameter. Pit density 0.32 pores per μm^2

C. affinis **Sond. (Plate 1B & C)**

Levyns s.n.

Size: P (25-)29(30) µm, E (18-)23(-28) µm, P/E = 1.25 grains subprolate. SEM data not available.

Fourcade 439

Size: (28-)32(-35) µm, E (18-)23(-28) µm, P/E = 1.41 grains prolate.

Tectum bumpy, reticulate. Lumina variable in shape and size, without ridges longest axis up to 1µm. Wall perforations sometimes clogged with lid-like substances. No ridges in between lumina. Endoaperture shape saddle, longer axis up to 5 µm long. Pit density 1.80 pores per μm^2.

C. africana **Poir. (Plate 1D)**

Pillans 8863, Flanagan s.n. .

Size: P (38-)39(-43), E (28-) 30(-33) µm. P/E = 1.23-1.28, pollen grains subprolate. Tectum smooth, punctate, lumina less than 1µm in diameter, with no ridges in between. Pit density 1.96 per μm^2. Endoaperture ± circular, ca. 2 µm in diameter.

No significant differences have been observed between the two specimens examined.

C. alaternoides **L. (Plate 1E -G)**

Esterhuysen s.n., Pillans 4039

Size: P(25-)28(-30) µm, E(33-)35(-38). P/E = 1.25-1.28. Colpi run almost the entire length of grain. Columella shallow exposing layer underneath exine. Lumina hexagonal shaped, 0.8 µm in diameter. Pit density 0.4 per μm^2.

No significant differences have been observed between the two specimens examined

Kensit 191

Size: P(33-)34(-35) µm, E(28-)30(-32). P/E = 1.14, pollen grains subprolate. Tectum reticulate and bumpy with tongue-like substances hanging out of lumen. Lumina hexagonal in shape, 0.75 µm in diameter and ridged. Pit density 0.46 per μm^2.

C. alpina **Prain**

Prain 6862

Size: P(28 -)30(-35) µm, E(20-)24(-28). P/E = 1.29, pollen grains subprolate. Tectum smooth, reticulate. Lumina ±circular to hexagonal, up to 0.8 µm in diameter, not ridged. Pit density 0.36 per μm^2.

C. brevifolia **Sond (*Plate* H)**

Compton 4256, Mauch 1220

Size: P(25-)32(-40) µm, E(23-)26(-30). P/E = 1.15 - 1.31, pollen grains subprolate. Tectum with small, round and bubble-like substances on otherwise smooth surface, reticulate and ridged. Lumina ca. 1µm wide, hexagonal shaped. Endoaperture ±square in shape. Pit density 0.64 pores per μm^2.

No significant differences have been observed between the two specimens examined.

C. daphnoides

Fries 513

Size: E(16-)22(-28) µm, P(23-)27(-33). P/E = 1.24, pollen grains subprolate.

Flanagan 5105

Size: E(18-)20(-23) µm, P(24-)29(-29). P/E = 1.5, pollen grains prolate.

SEM data not available.

***C. disceptata* Prain (Plate 1I, 2A, 4C)**

Tyson 1113, 1234

Size: P(33-)37(-38) µm, E(25-)27(-28). P/E = 1.36-1.39, pollen grains prolate. Tectum smooth, lumina vary in shape between circular, saddle and triangular. Columella shallow. Endoaperture shape saddle shaped. Pit density 0.96 per μm^2.

No significant differences between specimens observed.

***C. dregeana* Scheele (Plate 4C)**

Rogers 27506

Size: P(30-)33(-35) µm, E(25-)26(-28)., P/E =1.27, pollen grains subprolate. Tectum rough, covered with small bubbly objects and some lid-like structures protruding out of lumina of ca. 0.8 µm diameter which form a regular network pattern of reticulation. Endoaperture ± circular, 2.5µm in diameter. Pit density 0.60 per μm^2.

***C. ericoides* Thunb (Plate 2C, 7D)**

Wolley-Dodd 1109

Size: P(35-)38(-40) µm, E(20-)27(-30). P/E = 1.38, pollen grains prolate. Tectum rough and punctate. Ridges form around 2-4 lumina of less than 0.05 diameter and circular shape. Columella shallow. Pit density 2.68 per μm^2

Prain 136

Size: P(25-)29(-33) μm, E(25-)30(-35). P/E = 0.98, grains subspheroidal. Tectum smooth and punctate. Lumina shape varies between circular, hexagonal, elongate and elliptic. Pores density 2.65 per μm^2.

***C. galpinii* Pax (Plate 2E&F)**

Galpins.n.

Size: P(28-)31(-38) μm, E(19-)25(-31), P/E = 1.21, pollen grains subprolate. Tectum distinctly reticulate, exposing undersurface of exine. Lumina irregular in shape and size, ridged. Endoaperture circular to elliptic elongate. Pore density 0.48 per μm^2.

***C. heterophylla* Thunb.**

Galpin s.n.

Size: P(28-)31(-33) μm, E(24-)26(-30). P/E = 1.15, pollen grains subprolate.
Tectum rough, reticulate. Lumina circular to hexagonal in shape, 0.2 μm in diameter.
Endoaperture circular, 1.6 μm in diameter. Pit density 2.88 per μm^2.

***C. hirsuta* E. Mey ex Sond. (Plate 2G)**

Haptron 840, Prain 899

Size: P(30-)33(-35) μm, E(25-)27(-28). P/E = 1.22-1.23, pollen grains subprolate.
Tectum spotted with small bubble-like spheres, reticulate (plate). Lumina circular to elliptic, up to 0.5 and 1 μm diameter and length of longest axis respectively. Endoaperture ± circular.
Pit density 0.52 per μm^2.

No significant differences between the two specimens observed.

***C. imbricata* Emey ex Sond. (Plate I&J)**

Hutchinson 1053

Size: P(25-)27(-28) µm, E(20-)22(- 23). P/E = 1.21-1.28, pollen grains prolate.

Tectum rough, reticulate, with lid-like structures covering lumina which form a regular network of hexagons of up to 1.5 µm diameter. Ridges found around lumina. Endoaperture ± circular, 2 µm diameter. Pit density 0.60 per μm^2.

***C. impedita* Prain (Plate 3 A&B)**

Prain 1899

Pollen grains subprolate, P/E = 1.15. Size: P(28-)29(-30) µm, E(23-)26(-30). Tectum smooth, distinctly reticulate. Lumina shape ranges from circular to saddle, triangular and irregular, varying in size within range of 0.2-1.4 µm, with ridges around. Endoaperture ± rectangular ca. 3µm in length. Pit density 0.40 per μm^2.

***C. inyangensis* (Plate 3C)**

Fries 3860

Pollen grains subprolate, P/E = 1.29. Size: P(28-)31(-35) µm. E(23-)24(-25) µm. Tectum smooth, reticulate, lumina variable in shape, including ± circular, triangular to irregular types, 0.1 to 0.8 µm in diameter. No ridges in between lumina. Endoapertures ± rectangular ca. 3µm in length. Pit density 0.56 per μm^2.

***C. katharine* Pax**

Esterhuysen 10198

Size: P(30-) 33(-38) µm, E(20-)25 (-30). P/E = 1.32, pollen grains subprolate. Tectum reticulate, rough, with shallow columella. Lumina hexagonal shaped, ridged, up to 1.5 µm in diameter. Pit density 0.64 per μm^2.

Esterhuysen 12 862

Size: P(33-)35(-40) µm, E(20-)25(-28), P/E = 1.4, pollen grains prolate.

SEM data not available

***C. laxa* Eckl. ex Sond (Plate 4H)**

Flanagan 4603

Size: P(23-)26(-30) μm, E(23-)26(-30) μm. P/E = 1.00, pollen grains prolate spheroidal. Tectum bumpy, covered with small sphere and rods resembling bacterial colonies, distinctly reticulate, with a regular honeycomb-like structure. Lumina ca. 1.5 μm in diameter. Endoapertures almost square shaped, ca. 2.5 μm in length. Pit density 0.36 per μm^2.

***C. marginata* E. Mey ex Sond**

Stein 233

Size: P(30-)34(-38) μm, E(20-)28(-33). P/E = 1.09, pollen grains prolate spheroidal. Tectum reticulate, ridged, with hexagonal lumina of ca. 1 μm diameter. Columella deep. Endoaperture rectangular shaped, up to 2.5 μm long. Pit density 0.68 per μm^2.

Burtt-Davy s.n.

Size: P(33-)43(-48) μm, E(20-)25(-28). P/E = 1.73, pollen grains prolate. Tectum reticulate, without prominent ridges. Lumina hexagonal shaped, up to 1.2 μm in diameter. Endoaperture ±circular, 2 μm in diameter. Pit density 1.88 per μm^2.

***C. monticola* S. Moore (Plate 3D)**

Burtt-Davy 2049

Pollen grains subprolate P/E = 1.26. Size: P(33-)36(-40) μm, E(25-)29(-30). Tectum smooth, punctate or not distinctly reticulate with ± circular shaped lumina within diameter range of 0.2 -1.0 μm. Endoaperture ± rectangular shaped up to 4 μm in length. Pit density 0.60 per μm^2.

***C. nana* Prain**

Sankey 236

Pollen grains subprolate P/E = 1.17. Size: P(23-)27(-30) µm, E(18-)24(-28). Tectum smooth, reticulate. Endoaperture saddle shaped ca. 3 µm long and 1.25 µm wide. Pit density 1.08 per μm^2.

***C. natalensis* Bernh. ex Krauss (Plate 3E)**

Galpin 78, Wylie s.n.

Size: P(28-)29(-30) µm, E(23-)25(-28). P/E = 1.13-1.18, pollen grains prolate spheroidal to subprolate. Lumina hexagonal in shape. Pit density 0.76 per μm^2.

No significant differences between two specimens observed.

***C. paxii* Knauf**

Stolz 2132

Size: P(30-)33(-38) µm, E(25-)27(-30) P/E = 1.23, pollen grains subprolate. Tectum smooth, reticulate, lumina irregular in shaped, rarely circular, c. 1.2 µm long or 0.8 µm in diameter, with deep columella and no ridges in between. Pit density 0.56 per μm^2.

***C. polifolia* Jacq.**

Esterhuysen 5498, Theorow 2068

Pollen grains subprolate P/E = 1.18-1.21. Size: P(28-)29(-33) µm, E(23-)25(-28). Tectum smooth, with hexagonal shaped lumina of ca. 1-2 µm diameter separated by conspicuous ridges. Pores density 0.32 per μm^2.

No significant differences between the two specimens observed.

C. polygonoides **L. (Plate 3F &G)**

Pillans 887

Size: P(35-)37(-40) μm, E(30-)33(-35). P/E = 1.12, pollen grains prolate spheroidal. Tectum distinctly reticulate, Pollen grains prolate spheroidal, ridged. Lumina hexagonal in shape, ca.1 μm in diameter. Pit density 0.68 per μm^2.

C. pterogona **Mull. Arg.**

Wolley-Dodd s.n.

Size: P(30-)33(-38) μm, E(23-)24(-25), P/E = 1.37, pollen grains prolate.

No SEM data.

Koutnik s.n.

Size: P(28-)29(-35) μm, E(20-)24(-28), P/E = 1.23, pollen grains subprolate. Tectum smooth, reticulate without ridges. Lumina irregular in size and shape. Columella shallow, exposing base of the endexine, with lid-like substances sticking out. Pit density 0.44 per μm^2.

C. pubescence **Thunb.**

Prain s.n.

Size: P(25-)30(-35) μm, E(20-)22(-25). P/E = 1.38, pollen grains prolate.

Tectum rough, punctate to reticulate. Pit density 2.76 per μm^2.

Esterhuysen 27746

Size: P(32-)34(-35) μm, E(20-)22(-23). P/E = 1.58, pollen grains prolate.

No significant differences between two specimens observed.

C. pulchella **L. (Plate 3H)**

Rodin 1960A

Size: P(30-)32(-33) μm, E(25-)26(-28). P/E = pollen grains subprolate. Tectum smooth, reticulate, ridged. Lumina irregular in size and shape. Pits enclosed in ones, twos threes and fours (two to four contoured). Columella deep. Pit density 0.32 per μm^2.

Levyns 3980

Size: P(43-)44(-48) μm, E(30-)33(-36). P/E = 1.3 pollen grains. Tectum smooth, reticulate, with distinct ridges. Lumen varies in shape between circular, triangular, saddle, hexagonal and irregular. Columella deep. Pit density 0.48 per μm^2.

Compton 26858

Size: P(35-)37(-38) μm, E(28-)29(-33). P/E = 1.26, pollen grains subprolate. No SEM data

C. rubricaulis **Eckl. ex Sond.**

D'Urman 916 B

Pollen grains subprolate, 1.16-1.28. Size: P(25-)27(-33) μm, E(20-)23(-28). Tectum is coarsely reticulate. Columella forming honeycomb-like structures of more or less hexagonal shaped pores, with prominent ridges, shallow, exposing endexine at the base of pores. Endoaperture ± circular c. 1 μm in diameter. Pit density 0.68 per μm^2.

C. swynnertonnii

Moore 3936

Size: P(25-)28(-30) μm, E(23-)27(-30). P/E = 1.24, pollen grains subprolate. Reticulation of tectum less expressed, lumina circular or convex to irregular in shape, size variable within each shape. Endoaperture rectangular shaped c. 2 μm long. Endexine floor exposed under shallow columella. Pit density 0.88 per μm^2.

C. thunbergii **Sond. (Plate 4I-K)**

Bruyns 4778

Size: P(23-)29(-38) µm, E(20-)24(-28), P/E = 1.26, pollen grains subprolate. Tectum rough, with well expressed course reticulation, lumina hexagonal shaped up to 1.2 µm wide. Pit density 1.24 per μm^2.

C. tomentosa **L. (Plate 4L)**

Salter 479

Size: P(35-)42(-45) µm, E(20-)25(-33). P/E = 1.06, pollen grains prolate spheroidal. Tectum smooth, reticulate, not ridged, shiny. Lumina circular to hexagonal in shape, 0.7-1.5 µm in diameter. Pit density 0.76 per μm^2.

Esterhuysen 3048

Size: P(33-)38(-50) µm, E(33-)35(-38). P/E = 1.67, pollen grains prolate.

No SEM data.

C. virgata **Pax & K. Hoffm. (Plate 3I)**

Hutchinson 2418, Rogers 14

Size: P(28-)29(-30) µm, E(20-)22(-25) µm. P/E = 1.27-1.35, pollen grains subprolate to prolate. Tectum smooth, reticulate, ridged. Lumina generally hexagonal, 1 µm wide, smaller and circular around mesocolpi (diameter less than 0.5µm). Pore diameter 0.56 per μm^2.

No significant differences between the two specimens observed.

C. volubilis

Fries 2484

Size: P(28-)29(-30) µm, E(20-)23(-25). P/E = 1.38. pollen grains are prolate

SEM data not available.

Multivariate analysis

Phenetic analysis using the two different methods (Rogers & Tanimoto (R&T) and average taxonomic distance (ED)) produced somewhat different results (Figs 1 and 2). By drawing phenon lines at 0.43 and 1.15 more or less similar groups can be delimited: these are discussed below.

Groups retrieved by Rogers & Tanimoto dissimilarity.

Rogers &Tanimoto dissimilarity retrieved five pollen types and distance methods of analysis retrieved nine groups. The groups are defined by several pollen characters shared by at least one species shown in the dendrograms (Figs. 1 & 2) formed by cluster analysis.

Type RT 1: **The *C. abyssinica type*** (includes the *C. affinis* type i.e. Type ED 2)
This group includes *C. abyssinica, C. affinis, C. africana, C. disceptata, C. hirsuta and C. swynnertonnii.* Characters which appear constantly in this group include a rough exine surface, lumen irregularity in size and shape and a shallow columella which exposes the base of the endexine. All the species in the group have subprolate shaped grains, P/E = 1.18-1.24, except for *C. disceptata* which has a P/E ratio of 1.36 and a prolate shape. The presence or absence of ridges is not an informative character as it is variable within the group. Likewise, reticulation patterns in the group does not define the group since there is an equal number of reticulate and punctate species; consequently there is also a broad range of variation in Pit density (0.22-1.96 pores per μm^2).

Type RT 2: The *C. alpina* type
Thirteen species fall into this group and they are: C. *alpina, C. ericoides, C. imbricata, C. impedita, C. brevifolia, C. dregeana, C. heterophylla, C. katharine, C. laxa, C. marginata, C. polygonoides, C. rubricaulis, C. thunbergii* and *C. virgata*. This group is defined by rough exine surfaces, a reticulate tectum with regular lumina and a consistent occurrence of ridges around the lumina.

The different shapes found within the group are subprolate, prolate and prolate spherical. Density of pores ranges between 0.36 and 2.88 pores per μm^2. Height of the columella varies among taxa between shallow and deep. The shape of the endoaperture does not separate the group from others.

Type RT 3: The *C. inyangensis* type

The group is composed of *C. inyangensis, C. paxii, C. natalensis* and *C. tomentosa.*
It is characterised by smooth exines, a reticulate structure which does not have ridges between lumina. The lumina are irregular in shape and size (except in *C. tomentosa*) and the columella are deep. Pit density varies within a narrow range of 0.56 - 0.76 pores per μm^2.
All species in the group are subprolate (P/E = 1.18-1.29)

Type RT 4: The *C. alartenoides* type

This group includes *C. alaternoides, C.galpinii, C. monticola, C. nana, C. polifolia, C. pterogona* and *C. pulchella.* These species share a smooth surface, a reticulate pattern of the tectum and a subprolate shape (P/E = 1.17-1.26). Pit density varies between 0.32 and 1.08.

Type RT 5: The *C. pubescence type*

C. pubescence is the only species found in this group. The pollen grains have a rough tectum which is punctate to reticulate and a Pit density of 2.76 per μm^2.

Euclidean distance methods retrieved the following groups:

Type ED 1 : The *C. abyssinica* type

This group is similar in composition to the *C. abyssinica* type except for a minor difference in that it excludes *C. disceptata* and includes *C. pulchella.*
This group is marked by pollen which is reticulate and has regular lumina which is ridged. The pollen grains are subprolate (P/E = 1.18-1.23) and Pit density ranges between 0.22 and 0.88 pores per μm^2.

Type ED 2: The *C. affinis* type

There are two species in this group, *C. affinis* and *C. africana*. They have reticulate grains which have ridges and a shallow columella in common. The two species have an equal P/E ratio of 1.24 and their pore densities are 1.8 and 1.96 pores per μm^2, respectively.

Type ED 3: The *C. alaternoides* type

This group includes 13 species ranging from a P/E ratio of 1.00 in *C. laxa* to 1.29 in *C. thunbergii* (Fig). The main characteristics defining the group are reticulate tecta, regular lumina with ridges on the surface and shallow columella.. The roughness of their surface is variable between bumpy and smooth and their pit density is between 0.32 and 1.08 pores per μm^2.

Type ED 4: The *C. disceptata* type

Pollen grain are smooth, reticulate, lack ridges and posses a shallow columella.
Their shapes are subprolate (*C. hirsuta* and *C. thurnbergii*) to subprolate (*C. disceptata*).

Type ED 5: The *C. inyangensis* type

This group includes *C. inyangensis*, *C. paxii*, *C. natalensis* and *C. tomentosa* and is marked by smooth reticulate surfaces which have shallow columella, lacks ridges and pore densities ranging between 0.56 and 0.76 pores per μm^2. Their grains are mainly subprolate (P/E =1.18-1.29) in shape except in *C. tomentosa* where grains are prolate spherical (P/E= 1.06).

Type ED 6: The *C. dregeana* type

The two species found in this group are *C. dregeana* and *C. rubricaulis* sharing rough, punctate and ridged lumina which are almost regular in shape and size.
There is no correlation between the groups retrieved by pollen morphology and the sections of Prain

Type ED 7: The *C. ericoides* type

The group includes *C. ericoides*, *C. heterophylla* and *C. marginata*. These species have a smooth surface, lumina which are regular in shape and size and a pattern marked by the occurrence of ridges in common.

Type ED 8: The *C. marginata type*

C. marginata is the only group species in this group.

Plate 1 SEM **A** *C. abyssinica* **B-C** *C. affinis* **D** *C. africana* **E-G** *C. alaternoides* **H** *C. brevifolia.* **A,H** equatorial view showing detail of colpus **B** detailed view of endoaperture **E** equatorial view showing two colpi. **C,D-G, I** detail of tectum structure X 10000.

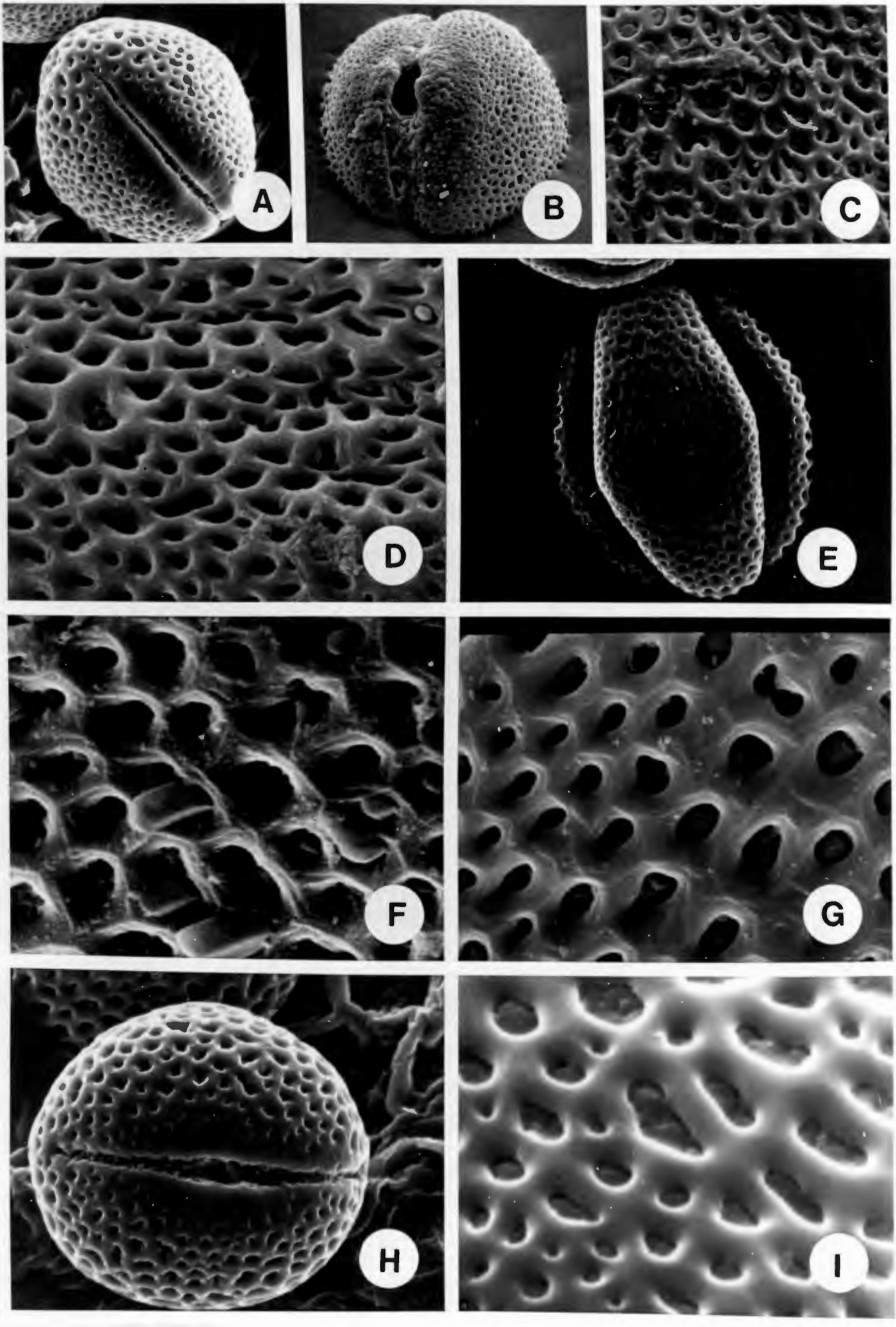
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Plate 2 SEM **A-B** *C. disceptata* . **C-D** *C. ericoides*. **E-F** *C. galpinii* **G** *C. hirsuta* **H-I** *C. imbricata.* **A,C, H** equatorial view showing length of colpus detail of colpus 3000, **B,D,F,G,I** detail of tectum structure X 10000 **E** detailed view of colpus X 14000.

Plate 3 SEM **A-B** *C. impedita*. **C** *C. inyangensis* **D** *C. monticola* **E** *C. natalensis* **F-G** *C. polygonoides* **H** *C. pulchella* **I** *C. virgata*. **A, H** detail of colpi in semi-equatorial and polar views, respectively. **B,E,G,H** detail of tectum structure X 10000. **C,F, I** equatorial view .

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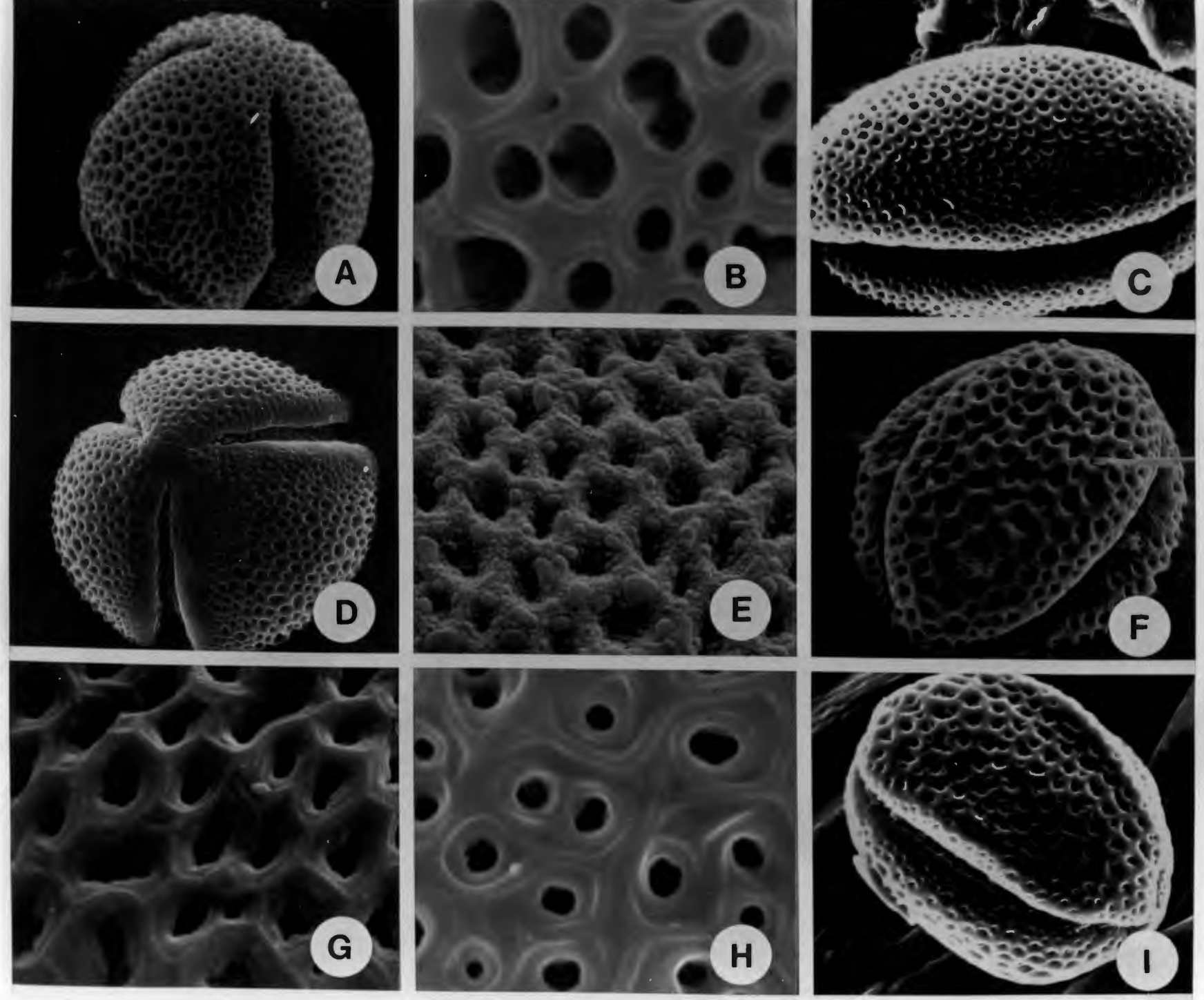
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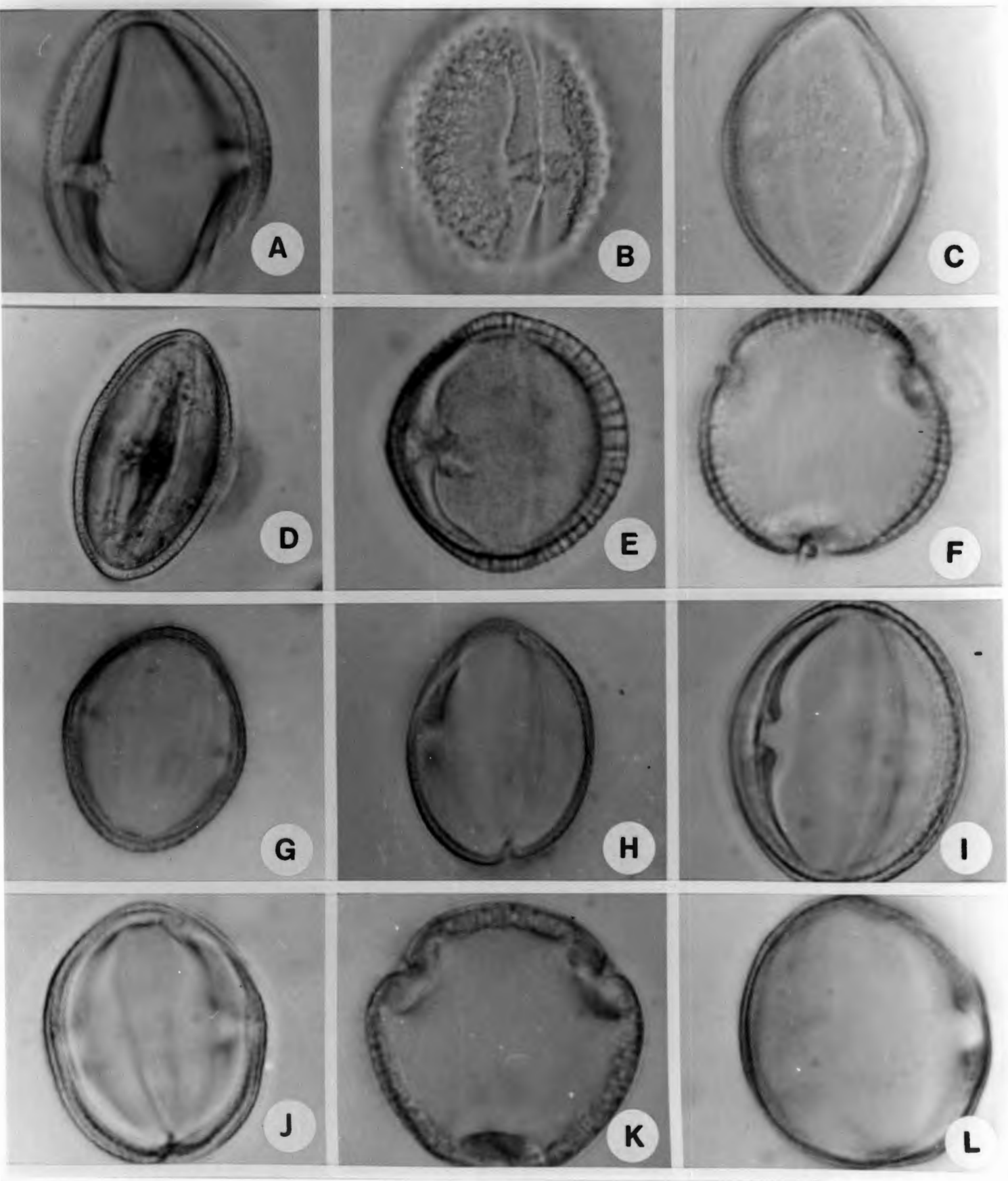
Plate 4 A-B *C. abyssinica*. **C** *C. disceptata*, **D** *C. dregeana*. **E-F** *C. impedita*. **G-H** *C. laxa*. **I-K** *C. thunbergii*. **L** *C. tomentosa*. LM **A-C** X 1800, **D,E,G, H** X 2000, **F,I-L** X 2200. Equatorial view **A-E,G-J, L. F, K** Polar view .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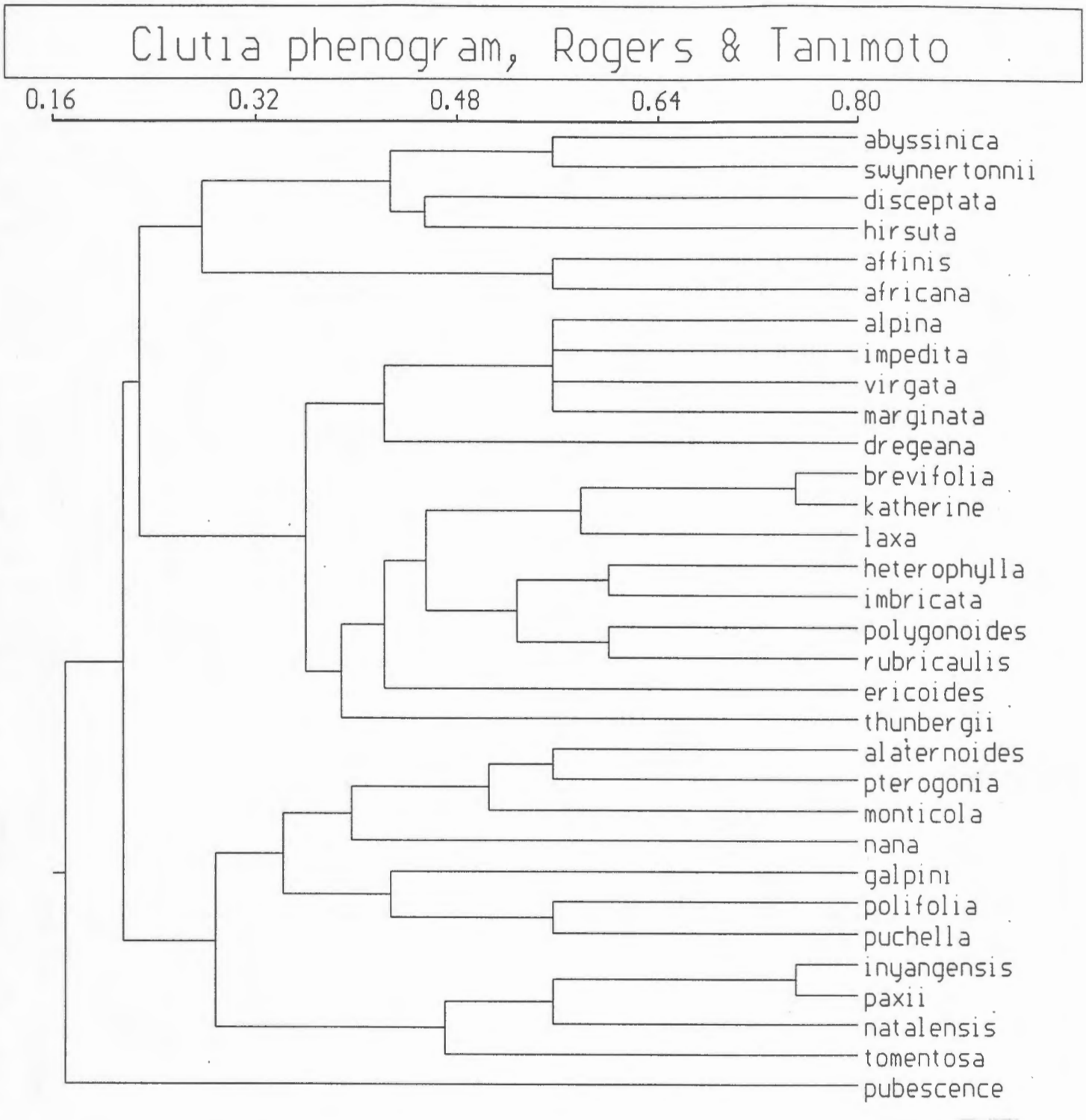

Fig. 1 Unweighted pair group method phenogram representing the relationships among species of *Clutia* based on Rodgers and Tanimoto coefficient from eight pollen morphological characters.

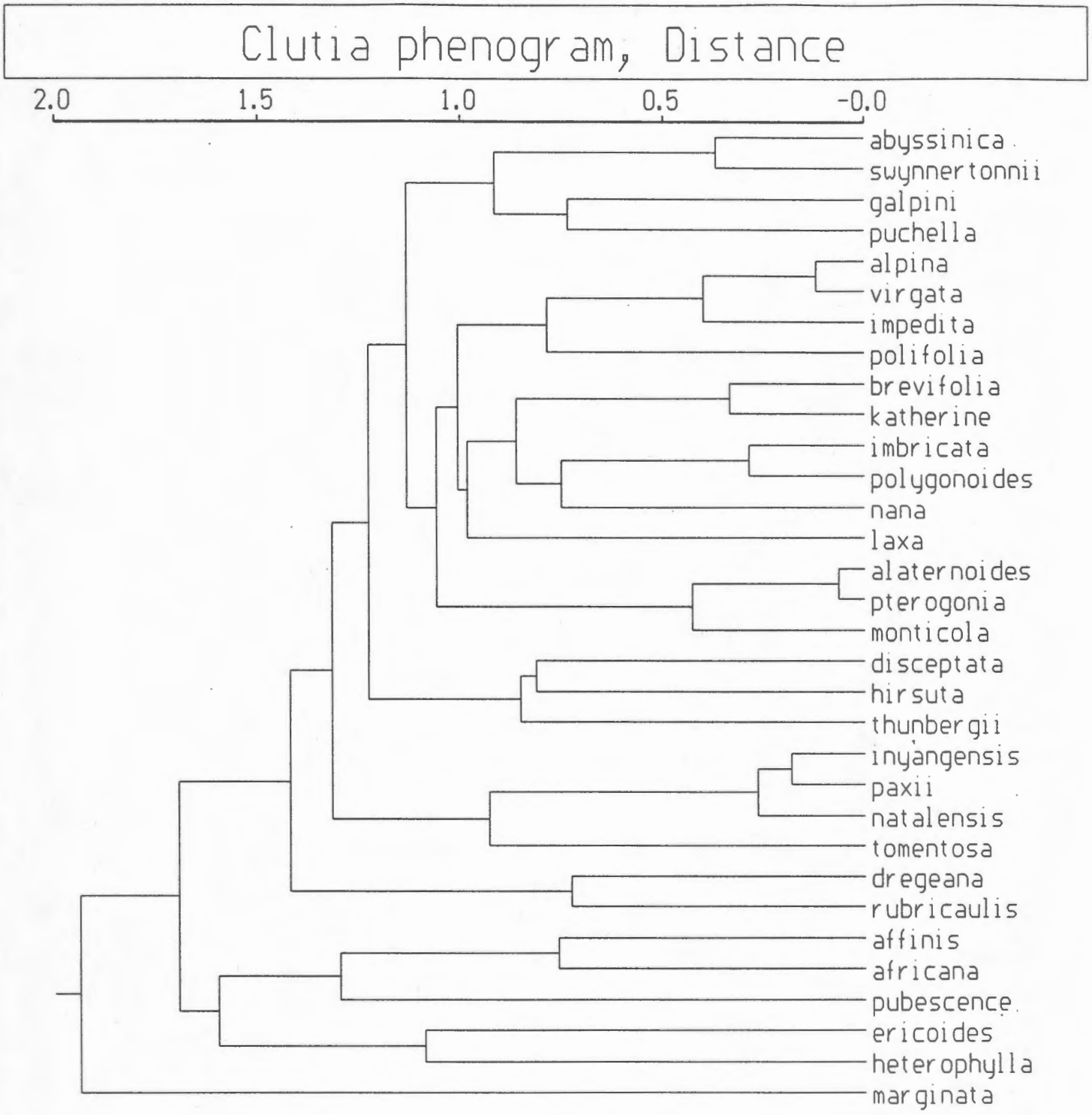

Fig. 2 Unweighted pair group method phenogram representing the relationships among species of *Clutia* based on Taxonomic distance coefficient from eight pollen morphological characters.

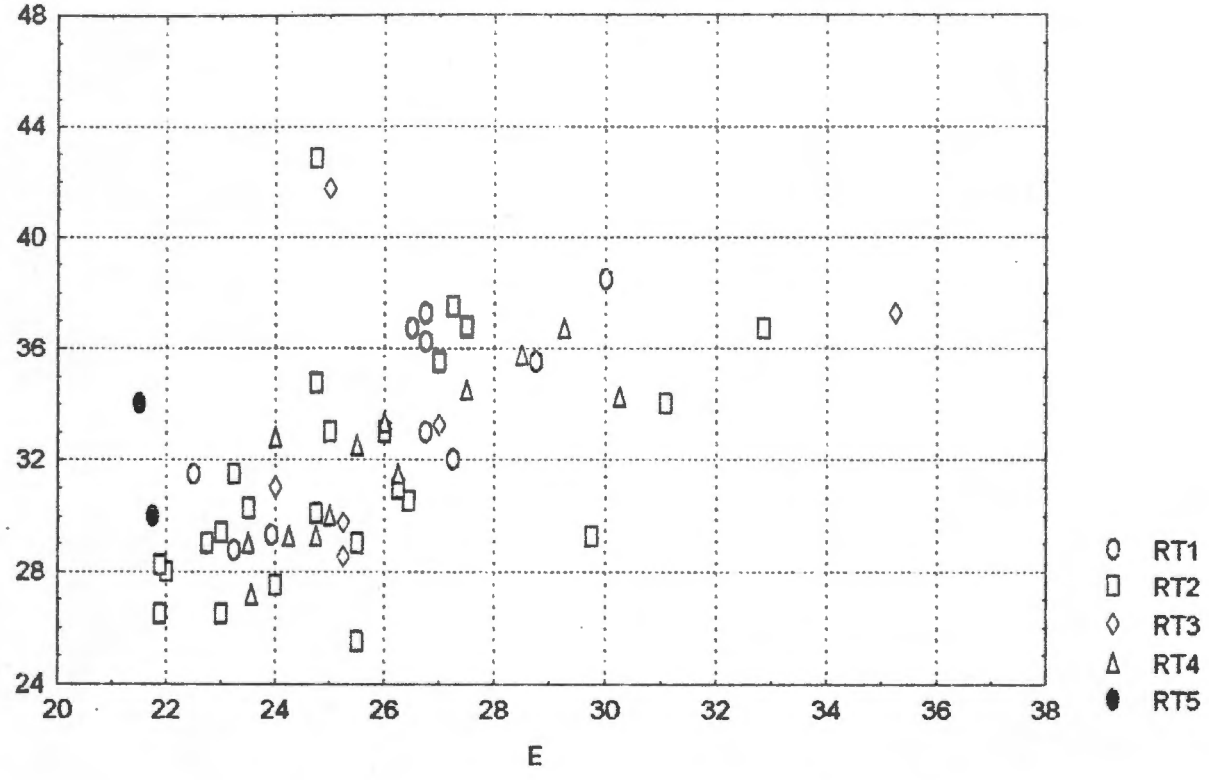

Fig. 3 A scatter diagram for Polar and Equatorial measurements in the sections retrieved by R&T dissimilarity coefficient. Each different symbol represents a different pollen group.

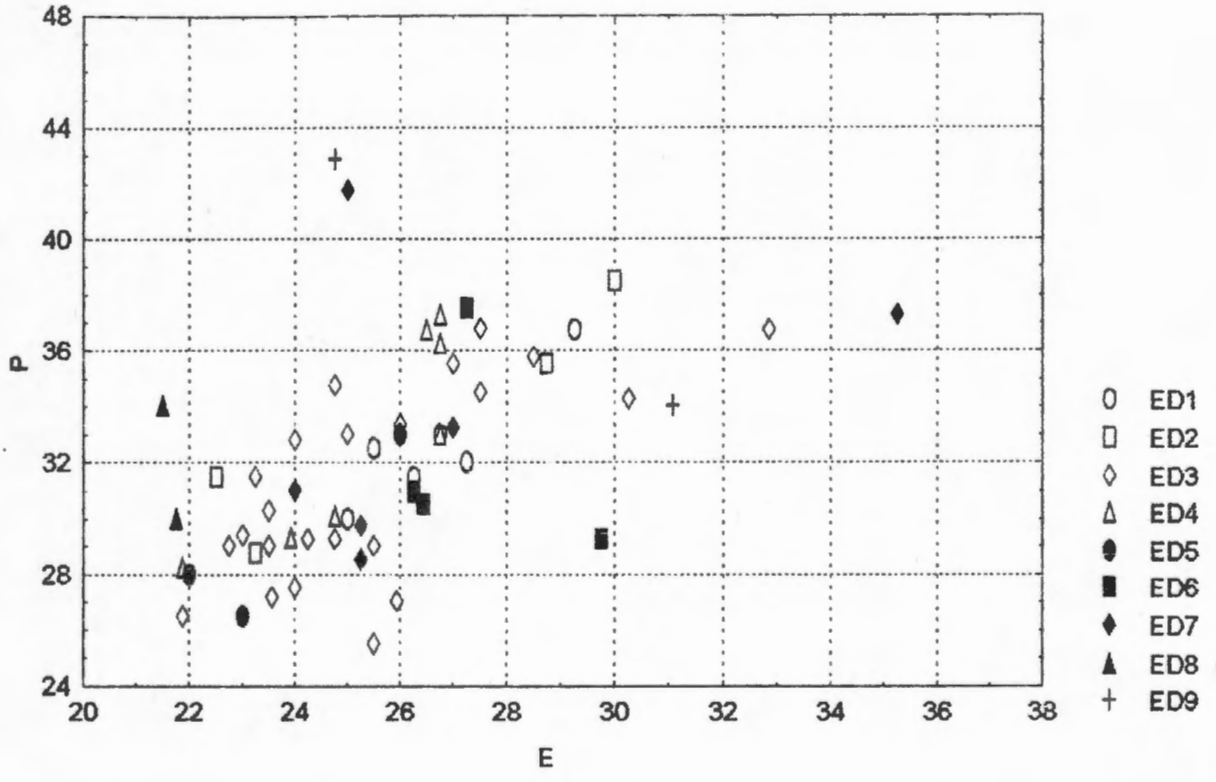

Fig. 4 A scatter diagram for Polar and Equatorial measurements in the sections retrieved by Taxonomic distance coefficient. Each different symbol represents a different pollen group.

DISCUSSION

The pollen morphology of the *Clutia* species of South Africa is in general similar to that described by Punt (1962) and by Mtotomwena and Mahunnah (1985), as being tricolporate, subprolate to prolate in shape with perforate or reticulate surface sculpturing. The variety of shapes described for simple pits in the six Tanzanian species of *Clutia* studied by Mtotomwena and Mahunnah (1985) was seen in most of the species with irregular lumina. However, a notable feature of the South African *Clutia* is the complete absence of the compound pits in most of their species except for *C. pulchella* (Plate 3H), *C . impedita* (Plate 3B) which have three to four contoured pits. Thus in as much as these several features define pollen as relatively homogenous, the exine surface, including texture, reticulation pattern, pitting density and the height of the columella show that the genus is eurypalynous because they differ from one species to the other and separate species into groups discussed above.

Significance of pollen dimensions and shapes in separating groups

Pollen dimensions do not separate groups within the genus as shown by the scatter plots of correlation between equatorial and polar axis (Fig s 3&4). Punt (1962) suggested pollen dimensions of different species within *Clutia* may differ but the results here do not support that. However, extensive sampling within the species complexes can better explain the extent of utility of pollen dimensions in separating different species within the genus since there is a general consensus among taxonomists that biased interpretation may of qualitative data is minimised when the sample is large (Sokal and Sneath, 1973).

The significance of other characters

Shape of the endoaperture proved to be of little significance in separating taxa belonging to different groups. The utility of pore shape as a differentiating character must be assessed more closely by examining the degree to which it can be altered with varying osmotic changes taking place between the pollen grains and their surroundings during acetolysis. Harmomegathy is a term associated with these changes is a phenomenon that deals . Another character which can be viewed with some degree of sceptism is the roughness of the surface of the exine since it can be argued that the bumpy surface observed in more than half the number of species studied may have been induced by acetolysis. For example in the cases where two specimens were sampled it is worrying to note that in four species *C. alaternoides, C. brevifolia, C. disceptata and C. natalensis* one specimen appeared smooth whereas the other has a bumpy surface. Admittedly, this could illustrate variation within the species or indicate that the two specimens observed in each case belong to different species, but there is yet another suspicious aspect with the character in question. Some of the species which were scored as rough in nature of the tectum had a smooth appearance except for what appeared to be sitting on the surface rather than being part of the exine per se. However, due to the limited nature of infra-specific sampling in this study it is not possible to finalise the issue here, hence extensive sampling within individual species would have to be carried out in order to substantiate these observations on pore shape and roughness. The limited sampling within species further showed that there was no account where on specimen was reticulate and the other was punctate. Ferguson and Harley, (1982) suggested that the punctate tectum is more is a more advanced character than the reticulate form and if their evidence is accepted, it is not possible to tell which group is more advanced than others because the few species which are punctate are found in different groups.

A comparison of groups retrieved by the two methods and their relations to the sections of *Clutia.*

Pollen characters which are of great taxonomic significance in separating groups in the genus are reticulation of the tectum, depth of columella, exine roughness and occurrence of ridges. The different pollen groups defined by these characters support the notion that species form complexes within the genus. Some species in RT groups remained associated with one another when distance methods of analysis were used, while others were removed and associated into new groups. It is interesting to note that some species found in four of the RT groups, the *C. abyssinica, C. alaternoides, C. inyangensis* and the *C. pubescence* type were not affected by methods of data analysis and are also found in the original sections of the genus (Prain's *Pulchellae* and *Alaternoidae* sections respectively). When the data was analyzed using the R&T dissimilarity coefficient, the *C. abyssinica* type included *C. affinis* and *C. africana* which were retrieved as an association of their own by distance methods probably due to their dissimilarity from the rest of the group in roughness of their surface and a denser aggregation of pores which gives a punctate surface whereas the rest of the group are reticulate. All the species retrieved by distance methods in this group are in section *Pulchellae*. A close resemblance between *C. pulchella* and *C.galpinii* which has often led to the former being confused for the latter in the past as matched by similarity of pollen is also shown. R&T method placed these two species together in pollen type IV whereas the distance method included both species in Type RT 1 together with *C. abyssinica* and *C. swynnertonni*. This association is a result of the fact that the two share in common all the characters used in the analysis except for slight differences in the height of the columella and the absence of contoured pits in *C. galpinii* hence it is no surprise that the two are placed together in Prain's *Pulchallae* group. *C. galpinii* was not included in the 48 species treated by Pax and was later identified among species of the Cape by Prain. The pollen data supports the idea that *C. galpnii* is a synonym of *C. pulchella* (Arnold and De Wett, 1993).

Pollen type RT 2 includes more species which had the associations illustrated above since eight of the 14 species retrieved by R&T method were also put together in type ED 3 by distance methods. This is by far the largest group retrieved by both methods and it corresponds to Prain's *Alaternoideae* section which included 11 species and is consequently the largest group in his treatment. Only four of the type RT 2 species and as many as seven of type ED 3 species are found in Prain's section . Two of the four species put by Pax in his group of *Alaternoideae* were studied and are found in both type RT 2 and type ED 3. The elusive nature of species similarities and the task of making delimitation within this group poses a number of difficulties, however, it is beyond the aims of this project to resolve those questions as sampling of specimens done here is rather limited.

The other two groups which proved to be robust when analyzed by both methods are the *C. inyangensis* group and the *C. disceptata* group. The *C. inyangensis* group had all four of its members retained as a group in each case and two of these species *C. natalensis* and *C. paxii* were placed in Pax's group the *MULTIGLANDULOSAE* because they have more than one gland in the fundus of their calyx. Lastly, the *Diceptatae* section in Prain's treatment contains six species including *C. disceptata* and *C. hirsuta* which form their own association that is found in pollen types R1 and ED 4 as retrieved by R&T method and distance methods respectively. Of the remaining species in the section *C. dregeana* and *C. heterophylla* are grouped into pollen type RT 2 together with other species of the *Alaternoideae* and the rest are distributed between other groups.

Phenetic analysis

It is somehow worrying to note that the R&T dissimilarity and average taxonomic distance coefficients retrieved slightly different groups because one would expect that the two methods should give similar results since only two of the eight characters used in the data matrix (pitting density and P/E ratios) are continuous, the rest being qualitative data. However, the following consistent patterns point to some level of similarity between results of the two methods and the characters which define them are worth discussing. Both methods isolate *C. pubescence* into a group of its own and all the other four groups spelled out by R&T dissimilarity have species which occur in similar associations in the Euclidean distance classification as discussed above. In other words all groups of the first method maintain their integrity when subjected to the second method except for the fact that some of their components are stripped and associated into additional groups. In a way the second method recognises the five groups of the first plus four additional groups. When one considers the fact that some of these additional groups are subsets of groups defined by the first method, similarity of results in the two methods becomes less further. For example, pollen type ED 2 is a subset of pollen type RT 1 because of several characters these two groups share including regularity of lumina and shallowness of columella. It is likely that the average taxonomic distance method separate *C. affinis* and *C. africana* from the rest of type RT 1 members on the basis of that they differ by being not reticulate and having denser pits in their surfaces.

While it seems the level of disagreement between the groups retrieved by the two methods is negligible, it remains uncertain as to whether taxonomic bias in the interpretation of observed trends is completely out of the picture or not. The importance of collecting large amounts of data, in order to minimize the effects of a taxonomists bias and for general purpose of classification, have long been emphasized in systematics (Sokal and Sneath, 1973; Stevens, 1991). Characters used in multivariate analysis in this study are largely based on observations made from one specimens since only one voucher provided material for a large number of taxa. In cases where more than one specimen was observed characters scored in the data matrix are based on one specimen when ideally they should be representations of averaged data of observations made.

Rather than allowing these implications to cast a dark shadow over the validity of the results, they should be viewed as proper ground set for future work. It should be borne in mind that interspecific relations can be resolved with better understanding of species complexes in the genus, thus extensive sampling which is demanded at that level can best account to the question posed above.

Limitations of this study

The pollen information supplied in this project is by no means presented as a complete study or final documentation as other useful pollen characters which require transmission electron microscopy techniques such as exine thickness as well as sexine and endexine patterning were not explored. However, it can be appreciated that the LM and SEM results have shed light on infra-generic relationships in *Clutia* to a certain extent. The support for sections established by previous workers is interesting and its implications need to be explored in a broader context by examining more characters. In the same logic, as far as resolution of the phylogeny of the genus is concerned, the results of this project are not enough but more organ based studies like wood anatomy and embryology still need to be done.

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