CYTOLOGICAL AND PALYNOLOGICAL STUDIES ON THE FAMILY APOCYNACEAE

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BY

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CERTIFICATE

This is to certify that this thesis entitled **"CYTOLOGICAL AND PALYNOLOGICAL STUDIES ON THE FAMILY APOCYNACEAE"** is an authentic record of research work carried out by **Sri. B.SANTHOSH** under my supervision and guidance and that no part of the thesis has previously formed the basis for the award of any Degree, Diploma, Fellowship or any other similar titles of this, or any other University or Society.

Sri. B.SANTHOSH has passed the M.Sc. Degree Examination (Genetics and Plant Breeding (First Class)) of the University of Kerala.

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DECLARATION

*I do hereby declare that this thesis entitled "**CYTOLOGICAL AND PALYNOLOGICAL STUDIES ON THE FAMILY APOCYNACEAE** is a bonafide record of research work done by me, and that no part of the thesis has been presented earlier for any Degree, Diploma or similar title or recognition.*

Thiruvananthapuram,
19-04-1999.

B. Santhosh

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LIST OF PUBLICATIONS

1. B. Santhosh and N. Omanakumari, 1995. Cytological studies in *Tabernaemontana divaricata. J. Cytol. Genet.* **30** (1) : 79-84.

2. B. Santhosh and N. Omanakumari, 1996. Cytomixis in *Plumeria rubra. J. Cytol. Genet.* **31** (1) : 73-77.

3. B. Santhosh and N. Omanakumari, 1997. Karyomorphological studies on two varieties of *Thevetia peruviana. J. Cytol. Genet.* **32** (2) : 95-98.

Part - I

CYTOLOGICAL STUDIES

INTRODUCTION

The Apocynaceae, otherwise known as the 'dogbane' family consists of 300 genera with more than 1400 species (Huber, 1983). Members of this family occur mostly in the tropical, subtropical regions and poorly represented in the temperate regions. Plants of the Apocynaceae are often poisonous and are rich in alkaloids or glycosides, especially in the seeds and latex. Some species are valuable sources of medicine (*Rauvolfia serpentina* Benth. ex Kurz., *Catharanthus roseus* (L.) G. Don., *Holarrhena antidysenterica* Wall., *Tabernaemontana divaricata* (L.) R. Br. ex Roem. and Schult., *Alstonia scholaris* R. Br., *A. venenata* R. Br.). Some species are garden plants (*Nerium oleander* Linn., *Kopsia fruticosa* A. DC., *Tabernaemontana divaricata* (L.) R. Br. ex Roem. and Schult., *Vallaris solanacea* (Roth.) Kuntze., *Strophanthus gratus* (Wall. and Hook.) Baill., *Odontadenia grandiflora* Schum., *Plumeria alba* Linn., *P. rubra* Linn.).

The family is characterised by erect or climbing woody plants or perennial, very rarely annual herbs, with latex or rarely watery juice. Leaves simple, opposite, rarely whorled or alternate. Flowers are bisexual actinomorphic and pentamerous. Corolla usually salver shaped or funnel form, lobes overlapping to right or left, rarely valvate. Stamens basically 5 in number, filaments short, anthers mostly sagittate. Ovary is superior with one or two to numerous ovules. Fruit is usually a berry or drupe, dehiscent or indehiscent.

Previously a number of investigators like Chatterjee *et al.* (1978), Akinloye and Court (1980), Amer and Court (1980), Mital *et al.* (1980), Rastogi *et al.* (1980),

Banerjee (1983), Nasser and Court (1984), Fujimoto *et al.* (1998) and Pereza-sanches *et al.* (1998) have studied the biochemical aspects in different species of the family Apocynaceae. Gibbs (1974), Parvathi and Santhakumari (1984), Daniel and Sabnis (1985) have studied the chemical phylogeny of the family. Sevenet *et al.* (1994) has reported chemotaxonomy of the genus *Kopsia.*

A number of investigators such as Rau (1940), Davis (1966), Bhasin (1971), Maheswari Devi (1970, 1971, 1974, 1975), Lattoo (1974), Corner (1976), Chauhan (1979) and Sud (1985) have studied the embryological and anatomical aspects of the members of Apocynaceae.

The pollination mechanism of *Adenium* in this family was done by Rowley (1980). Phytochemical investigation of *Catharanthus roseus* was done by Madati *et al.* (1979). Peerzada and Khan (1989) studied the development and structure of seeds of *Vallaris solanacea.* New species of *Hunteria* (*H. ghanensis*) from Ghana reported by Hall and Leeuwenberg (1979). Kochummen and Wong (1984) reported a new species of *Alstonia* (*A. undulifolia*) from the Malay Peninsula. Bahadur and Bennet (1978) reported a new species of *Wrightia* (*W. dolichocarpa*) from India. Van der Ploeg (1984) reported notes on the African species of the genus *Malouetia.* Series of revisions of Apocynaceae was done by Leeuwenberg and Berndsen (1988) and Leeuwenberg and Dilst (1989). Sidiyasa (1996) reported a new species of *Alstonia* (*A. beatricis*) from Irian Jaya, Indonesia. An intensive investigation on individual genus basis on this family was done by Witkus (1951), Veyret (1974), Beentje (1982), Zhu *et al.* (1990).

Bulk of the cytological reports on Indian members of the family Apocynaceae, are from the North, North east and Central India (Raghavan 1957, 1959; Bhatacharjee and Bahaduri, 1959; Tapadar and Sen, 1960; Singh, 1961; Gajapathy, 1962; Janaki Ammal, 1962, 1963; Raman and Kesavan, 1963; Tapadar, 1964; Dnyansagar and Sudhakaran, 1966, 1969a, b, 1970; Datta and Maiti, 1972; Raghuvanshi and Chauhan, 1969a, b, c, 1970, 1974; Sharma, 1970; Chauhan and Raghuvanshi, 1971, 1977; Sarkar *et al.* 1975; Bir and Neelam, 1980; Bedi *et al.* 1980; Balamani and Rao, 1981; Sanjappa and Dasgupta, 1981 and Singh *et al.*, 1982). Cytotaxonomical studies of the family was done by Tapadar (1964), from North India and Laan and Arends (1985) from Netherland.

The present work has been undertaken with a view to study the evolution of the members of the family on cytological evidences from South India. The present investigation involves cytological studies on 40 taxa coming under 20 genera and 3 tribes of the family. The present data, along with previous results are used to discuss aspects such as basic chromosome number, polyploidy, karyotype evolution and their role in speciation and evolution in the family.

MATERIALS AND METHODS

Materials for the present investigation were collected from different places of South India, which lies in the monsoon belt between the North latitudes 8° and 18° and East longitudes 73° and 85°, enjoys a tropical climate. Most of the materials investigated during the present study were collected from different low and high altitude regions of the Western Ghats which lies in the Kerala, Tamil Nadu and Karnataka section of South India such as Kallar (300 m), Ponmudi (450-950 m), Bonacaud (400-850 m), Kulathupuzha (200 m), Aryankavu (200 m), Courtallum (450m), Silent Valley (1300 m), Munnar (1050-2000 m), Peermade (900-1200 m), Kodaikanal (1800-2100 m), Ootaccamund (2500 m), Yercaud (1600 m). Materials from a few coastal regions and plains such as Thiruvananthapuram, Kollam, Kottayam, Konni, Ranni, Pambavalli, Pathanamthitta (Kerala), Coimbatore, Maruthumalai, Mettupalayam (Tamil Nadu), Mysore, Bangalore (Karnataka) were also included in this study.

Chromosome studies were made from PMCs at meiosis and/or root tips. For meiotic studies, materials were mostly taken from plants growing in the wild conditions, and this was supplemented with materials procured from plants maintained under green house conditions in the Botanic Garden, Department of Botany, University of Kerala. Young flower buds of suitable stage were fixed in a modified Carnoy's fluid (1 glacial acetic acid, 1 chloroform and 3 absolute ethyl alcohol), and

kept in the refrigerator at 10°C. Frequent change of fixative is given to get clear preparations. Somatic chromosome studies from root-tip cells were possible only in cases of plants which thrived under green house conditions. Root tips were collected between 12 and 12.30 AM, and then pretreated with 0.002 M solution of 8-Hydroxy quinoline. After 2 hrs pretreatment, materials were washed thoroughly in distilled water and fixed in Carnoy's fluid (1 glacial acetic acid and 3 absolute ethyl alcohol). A fixation period of 24 hrs was found to yield best results.

For staining meiotic chromosomes acetocarmine (2%) was used. For studying meiosis the stamens were smeared in a drop of acetocarmine, and coverglass put on it after removing the debris. The slide was then gently heated and pressed uniformly under the folds of a blotting paper. For mitotic studies also acetocarmine was tried. Root tips, after 24 hrs fixation in Carnoy's fluid, were gently heated in 2 drops of acetocarmine. The heated root tips were then smeared in a drop of acetocarmine stain, and put a cover glass on it, after removing the debris. Similar to meiotic preparation, here also the slide was gently heated and pressed uniformly under the folds of blotting paper. Observations were made from temporary slides, and then made permanent following Mc Clintock's (1929) method.

Photographs of PMCs and root tip cells were taken at magnification of x1500. For preparing photographic plates, the photographs of chromosome preparations were also taken in the same magnification.

Karyotype analysis was made based on average measurements from a minimum of 10 cells at metaphase in each species. Values of measurements such as

long arm (L), short arm (S), and total length of chromosomes were tabulated and arm ratios (r) for each of the homologous pairs were calculated (L/S). Classification of chromosomes were made following the system proposed by Levan *et al.* (1964) in which chromosome with absolute median position of centromere (r = 1) and 1.7 as m-type; arm ratio between 1.7 and 3.00 as sm-type; arm ratios between 3 and 7 as st-type and those with arm ratios exceeding 7 as t-type. Relative chromosome length is abbreviated as RCL; total length as TCL; average chromosome length as ACL. Categorization of karyotype asymmetry has been made according to the classification proposed by Stebbins (1958), who has recognized 12 degrees of karyotype asymmetry (1A – 4C) taking into account the position of the centromere and the ratio between the longest and smallest chromosomes of the complement.

TF% was calculated by using the formula proposed by Huziwara (1962) as

$$\frac{\text{Total short arm length}}{\text{Total long arm + short arm}} \times 100$$

Idiograms were provided for all the species whose karyomorphology was studied. Idiograms were suitably reduced. Appropriate scales were given on the plates which carry the idiograms.

Pollen fertility was determined by scoring the stained and unstained pollen grains in 1:1 mixture of 1% acetocarmine and glycerine. The stained ones being considered as fertile and the unstained as sterile. Percentage of fertility was estimated from the data on about 1000 pollen grains taken from 10 random fields in 5 slides for each species.

Herbarium sheets of the presently studied taxa were prepared and the voucher specimens were deposited in the Central Herbarium of the University, Department of Botany, Kerala University.

For arranging tribes and genera of the family included in the study, Bentham and Hooker's (1876) classification was followed. The localities from where the plants were collected during the present study were listed in Appendix 1.

OBSERVATIONS

TRIBE I CARISSEAE

This tribe includes 18 genera of which 2 genera (*Allamanda* Linn. and *Carissa* Linn.) are represented in this study.

Allamanda Linn.

The genus *Allamanda* consists of 3 species (Bailey, 1960). Gamble (1923) reported only one species, *A. cathartica* from South India. The members of the genus are straggling climbers, sparsely puberulous or almost glabrous woody plants. It is a native of tropical America, largely grown for its beautiful showy flowers, and naturalized in moist zones. During the present study cytology of 4 species has been carried out.

A. cathartica Linn.

This is an ornamental evergreen climber with whorled leaves. Flowers are large and yellow in colour. Stamens are inserted at the mouth of the narrow corolla tube. Milky latex is present in the plant. Materials for the present investigation were procured from Botanic Garden, Department of Botany, Kariavattom; Palode, and Kottayam.

Pollen mother cells showed 9 bivalents at metaphase I (Fig. 1) and meiosis was normal with regular segregation of chromosomes at anaphase I (Fig. 2). Secondary association of bivalents was noticed in 40% of PMC's (Fig. 3). Previous investigations such as Sugiura (1936); Pathak *et al.* (1949); Sen and Tapadar (1956);

Balamani and Rao (1981); Datta and Bhattacharya (1981) have also reported the same gametic number for this species.

Root tip cells showed 18 chromosomes (Fig. 4) which ranged in length from 2.50 μm to 4.50 μm. The TCL and ACL were calculated as 28.75 μm and 3.20 μm respectively. The karyotype consisted of 4 pairs of m-type, 2 pairs of sm-type and 3 of st-type chromosomes (Table 1, Text Fig. 1). The karyotype was moderately symmetrical (TF% = 40.86) and belonged to 2A category.

Previous workers (Sugiura, 1936; Kumar *et al.* 1952; Sen and Tapadar, 1956; Tapadar and Sen, 1960; Tapadar, 1964; and Datta and Bhattacharya, 1981) have reported 2n = 18 in this material from North India. This species is found to be diploid one based on the basic chromosome number x = 9.

Table 1. Details of the karyotype of *A. cathartica* (2n=18)

Chromosome No.	Chromosome length (μm)			RCL	Arm ratio (r)	Position of centromere
	Long arm	Short arm	Total			
1	2.50	2.00	4.50	15.65	1.25	m
2	2.00	1.75	3.75	13.04	1.14	m
3	2.25	1.25	3.50	12.17	1.80	st
4	2.25	1.00	3.25	11.30	2.25	st
5	2.00	1.00	3.00	10.43	2.00	st
6	1.50	1.50	3.00	10.43	1.00	m
7	1.50	1.25	2.75	9.56	1.20	m
8	1.50	1.00	2.50	8.69	1.50	sm
9	1.50	1.00	2.50	8.69	1.50	sm

TCL = 28.75 μm

ACL = 3.20 μm

Karyotype = 2A

TF% = 40.86

***A. schottii* Pohl.**

This is a beautiful climbing shrub similar to *A. cathartica*, except large yellow flowers, and is commonly grown as an ornamental. Milky latex is present in the plant. The materials for the present investigation were collected from the Botanic Garden, Department of Botany, Kariavattom; Palode, and Kottayam.

Pollen mother cells showed 9 bivalents at metaphase I (Fig. 5). Subsequent stages of meiosis were normal and there was 80% pollen fertility. The present count of n = 9 is in conformity with the previous reports (Tapadar and Sen, 1960; Tapadar 1964; Laan and Arends 1985). However, Bir and Neelam (1980) reported n = 10 in this material from North India.

Root tip cells showed 18 chromosomes at metaphase (Fig. 6) which ranged in length from 1.75 μm to 3.00 μm, with TCL 21.99 μm and ACL 2.44 μm. Of the 18 somatic chromosomes 2 pairs were m-type, 4 pairs sm-type and 3 pairs st-type (Table 2, Text Fig. 2). The TF% was 40.35. The karyotype belonged to 2A category.

Table 2. Details of the karyotype of *A. schottii* (2n = 18)

Chromosome Number	Chromosome length (μm)			RCL	Arm ratio (r)	Position of centromere
	Long arm	Short arm	Total			
1	2.00	1.00	3.00	13.64	2.00	st
2	1.75	1.00	2.75	12.51	1.75	st
3	1.75	1.00	2.75	12.51	1.75	st
4	1.50	1.25	2.75	12.51	1.20	m
5	1.50	1.00	2.50	11.36	1.50	sm
6	1.37	1.00	2.37	10.77	1.37	sm
7	1.25	1.00	2.25	10.23	1.25	sm
8	1.00	0.88	1.88	8.50	1.13	m
9	1.00	0.75	1.75	7.95	1.33	sm

TCL = 21.99 μm, Karyotype = 2A,

ACL = 2.44 μm TF% = 40.35

***A. neriifolia* Hook.**

This plant is a climber with milky latex. The leaves are thick and narrow lanceolate in shape. Flowers are yellow in colour and medium as that of *A. schottii.* During the present study materials were collected from different localities of Thiruvananthapuram and Wayanadu districts.

Flower buds collected from different localities consistently showed 9 regular bivalents at metaphase I (Fig. 7). Subsequent stages of meiosis were normal with 80 percent pollen fertility.

Meiotic count was confirmed by mitotic studies, which showed 2n = 18 chromosomes at metaphase (Fig. 8). Tapadar and Sen (1960) have reported the same number from North India. This is the first report from South India for this species.

The mitotic chromosomes ranged in length from 1.37 μm to 4.25 μm, with TCL 26.74 μm and ACL 3.00 μm. The karyotype consisted of 3 pairs of m-chromosomes, 2 pairs of sm-chromosomes and 4 pairs of st-chromosomes. (Table 3, Text Fig. 3). It belonged to 2B karyotype category. The TF% was 35.97.

Table 3. Details of the karyotype of *A. neriifolia* (2n = 18)

Chromosome Number	Chromosome length (μm)			RCL	Arm ratio (r)	Position of centromere
	Long arm	Short arm	Total			
1	3.25	1.00	4.25	15.89	3.25	st
2	2.25	1.25	3.50	13.08	1.80	st
3	2.00	1.25	3.25	12.15	1.60	sm
4	1.75	1.50	3.25	12.15	1.16	m
5	2.37	0.75	3.12	11.66	3.16	st
6	1.50	1.50	3.00	11.22	1.00	m
7	1.75	1.00	2.75	10.28	1.75	sm
8	1.50	0.75	2.25	8.41	2.00	st
9	0.75	0.62	1.37	5.12	1.20	m

TCL = 26.74 μm Karyotype = 2B

ACL = 3.00 μm TF% = 35.97

1. *violacea* Gardn. and Field.

This is a small ornamental shrub with thick leaves and wine purple-coloured flowers. Stem and leaf surfaces are rough due to the presence of hairs. Latex is present in the plant. Materials for the present investigation were collected from Botanic Garden, Department of Botany, Kariavattom; Palode, Nagercoil, and Bangalore.

The pollen mother cells showed 18 bivalents at metaphase I (Fig. 9). Subsequent stages of meiosis were normal with appreciable pollen fertility (88%). Kumar *et al.* (1952); and Sen and Tapadar (1956) have reported the same gametic chromosome number.

The present study showed 36 chromosomes in the root tip cells (Fig. 10). This species is a tetraploid taxon based on x = 9.

***Carissa* Linn.**

A genus of 30 species possessing densely branched spinous, erect or climbing shrubs and small trees. They are distributed mostly in the warmer parts of Africa, Australia and Asia. Hooker (1882) reported five species, but Gamble (1923) reported eight species from South India. During the present investigation cytology of two species has been studied.

***C. spinarum* Linn.**

This is a small shrubby, glabrous plant, with small thorns. The leaves are small, and flowers are white in colour. They are distributed throughout the tropics.

Milky latex is present in the plant. Materials for the present study were collected from Veli near Thiruvananthapuram, Kollam, Ponmudi and Thirunelveli.

Root tip cells showed 2n = 22 chromosomes at metaphse (Fig. 11). Previous reports on this species by Singh (1951); Chauhan and Raghuvanshi (1977); Balamani and Rao (1981) and Bir *et al.* (1984a) have showed n = 11 and 2n = 22. This species could be a diploid taxon on x = 11.

***C. carandas* Linn.**

This plant is dichotomously branched large shrub or small tree with strong, simple thorns in pairs. The plant is seen throughout India, Burma, Ceylon and Malasia. Milky latex present in the plant. The flowers are white in colour. The fruit is small and ellipsoid in shaped, turning from green to red and finally to black and shiny when ripe. The unripe fruit is sour and astringent, and it is used for making pickles. Medicinally it is used for hyperdipsia, diarrhoea, and intermittent fever (Varier, 1994). The materials for the present investigation were collected from Kottayam, Kariavattom and Nagercoil.

Mitotic studies showed 22 chromosomes in root tip cells at metaphase (Fig. 12). The chromosomes ranged from 1.50 μm to 2.50 in length. The TCL and ACL were estimated as 21.55 μm and 2.40 μm respectively. The TF% was 43.52. The karyotype included 3 pairs of m-type chromosomes and 8 pairs of sm-type chromosomes (Table 4, Text Fig. 4), and it belonged to IB category.

Tapadar and Sen (1960), Tapadar (1964), Chauhan and Raghuvanshi (1977) have reported 2n = 22 in the materials of this species studied by them from North India.

Table 4. Details of the karyotype of *C. carandas* (2n = 22)

Chromosome Number	Chromosome length (μm)			RCL	Arm ratio (r)	Position of centromere
	Long arm	Short arm	Total			
1	1.50	1.00	2.50	11.76	1.50	sm
2	1.25	1.00	2.25	10.58	1.25	sm
3	1.00	1.00	2.00	9.41	1.00	m
4	1.00	1.00	2.00	9.41	1.00	m
5	1.25	0.75	2.00	9.41	1.66	sm
6	1.25	0.75	2.00	9.41	1.66	sm
7	1.00	0.75	1.75	8.24	1.33	sm
8	1.00	0.75	1.75	8.24	1.33	sm
9	1.00	0.75	1.75	8.24	1.33	sm
10	1.00	0.75	1.75	8.24	1.33	sm
11	0.75	0.75	1.50	7.05	1.00	m

TCL = 21.55 μm Karyotype = 1B

ACL = 2.40 μm TF% = 43.52

Previous reports on the materials of this species by Chauhan and Raghuvanshi (1977), Balamani and Rao (1981) and Sanjappa and Dasgupta (1981) showed n = 11. This species is a diploid taxon based on x = 11.

TRIBE II PLUMERIEAE

This tribe includes 33 genera of which 9 have been cytologically worked out during the present investigation.

Sub tribe 1 – Rauvolfieae

***Rauvolfia* Plum. ex Linn.**

This genus comprises 40 species which are chiefly tropical American in distribution (Bailey, 1960). Hooker (1882) had recorded 7 species from the Indian subcontinent, and Gamble (1923) reported four species from South India. During the present investigation cytology of four species has been worked out.

***R. serpentina* Benth. ex Kurz.**

This is a small herbaceous undershrub with red pedicels and calyx, white corolla and purplish black fruits. Leaves are thin glabrous and bright green in colour and seen in whorled arrangement. Milky latex present in the plant. Alkaloid 'reserpine' extracted from the plant is medicinally very important for hypertension. Materials for the present investigation were collected from the forest ranges of Kulathupuzha and Aryankavu.

The pollen mother cells of this species showed 11 regular bivalents at metaphase I (Fig. 13). Subsequent stages of meiosis were normal with appreciable percentage of pollen fertility (80%). The previous cytological reports by Raghavan (1957), Milovidov and Storchova (1958), Tapadar (1964), Sharma and De (1976), De (1979), Bedi and Gill (1982) and Laan and Arends (1985) showed n = 11. While a different chromosome number (n = 12) was reported by Chandra (1957).

The root tip cells showed 2n = 22 (Fig. 14). Previous reports on the material of this species by Raghavan (1957), Milovidov and Storchova (1958), Tapadar (1964), Datta and Maiti (1972), Sharma and De (1976), and De (1979) showed 2n = 22. However, Chandra (1957) reported 2n = 24 and Singh (1961) reported 2n = 20.

***R. tetraphylla* Linn.** (= ***R. canescens* Linn.**

= ***R. tomentosa* Jacq.**

= ***R. nitida* Jacq.**)

The plants are erect, glabrous tracelets or subshrubs with little latex. Three obovate – lanceolate to broadly lanceolate leaves are seen in whorled arrangements. Flowers are very small with cream colour. Corolla tube is slightly swollen at the top with small lobes. The fruits are purplish black in colour. The plant for the present study were collected from Kulathupuzha forest ranges, Kariavattom, and Tropical Botanical Garden and Research Institute, Palode.

The present study showed 55 chromosomes in the root tip cells (Fig. 15). The same somatic count was reported by Sharma and Sharma (1957). However, previous reports on the material of this species by Raghavan (1957), Tapadar and Sen (1960), Tapadar (1964), Datta and Maiti (1972), Sharma and De (1976), Gadella (1978), De (1979) and Laan and Arends (1985) have showed 2n = 66.

***R. beddomei* Hook. f.**

Plants are undershrubs. Leaves are oblanceolate and accuminate in shape. Inflorescence is a receme. Flowers are small and white in colour. Corolla small,

tubular slightly swollen at the top with very small lobes. The materials for the present study were collected from Palode and Kariavattom.

The pollen mother cells showed n = 66 (Fig. 16) at metaphase I. Subsequent stages during the course of meiosis were normal with very high pollen fertility (80%).

***R. densiflora* Benth. ex Hook. f.**

This plant is a large shrub. Leaves are oblanceolate and arranged in a whorl. Milky latex present in the plant body. The flowers are small and white in colour. The materials for the present study were collected from Palode and Aryankavu.

Root tip cells showed 2n = 44 (Fig. 17). This is the first chromosome number report for this species from South India.

Sub tribe 2. Cerbereae

***Thevetia* Linn.**

A small genus comprising seven species, is chiefly tropical American in distribution. The members of this genus are glabrous shrub or small trees, now naturalised throughout India. Corolla is funnel shaped with scales at the throat. Milky latex is present in the plant body. Only one species (*T. peruviana*) has been studied in the present investigation. Different varieties of this species occur with white, yellow and pink flowers.

***T. peruviana* (Pers.) K. Schum.**

(= *T. nereifolia* Juss. ex Stend.) (variety 1)

This is the most common species of the genus. The plants are large shrubs. Leaves are narrow lanceolate. Thick cuticle is present in the upper side of the leaves.

Milky latex is present in the plant body. Flowers are large and yellow in colour. Corolla is funnel shaped. The alkaloid 'peruvin' extracted from the plant is useful in the treatment of cardiac diseases. Materials for the present investigation were collected from different localities of Thiruvananthapuram and Kollam districts.

Mitosis in root tip cells of this variety showed 20 chromosomes at metaphase (Fig. 18). The chromosomes are small sized and ranged in length from 1.4 μm to 3.0μm. The TCL and ACL were calculated to be 18.9 μm and 2.00 μm respectively (Table 5, Text Fig. 5). The TF% was 44.9. The second and the eighth chromosome pairs of the complement were M-type, while the remaining chromosomes were m-type. The karyotype belonged to 1B category.

Table 5. Details of the karyotype of *T. peruviana* (2n = 20)

(= *T. nereifolia*) (variety 1)

Chromosome Number	Chromosome length (μm)			RCL	Arm ratio (r)	Position of centromere
	Long arm	Short arm	Total			
1	1.6	1.4	3.0	15.87	1.14	m
2	1.2	1.2	2.4	12.69	1.00	M
3	1.2	1.1	2.3	12.16	1.09	m
4	1.2	0.8	2.0	10.58	1.50	m
5	1.1	0.7	1.8	9.52	1.57	m
6	0.9	0.7	1.6	8.46	1.28	m
7	0.8	0.8	1.6	8.46	1.00	m
8	0.8	0.6	1.4	7.40	1.33	M
9	0.8	0.6	1.4	7.40	1.33	m
10	0.8	0.6	1.4	7.40	1.33	m

TCL = 18.9 μm Karyotype = 1B

ACL = 2.00 μm TF% = 44.9

The same somatic chromosome number (2n = 20) has been reported earlier in this species by Tapadar and Sen (1960), Tapadar (1964), Datta and Maiti (1972), Chauhan and Raghuvanshi (1977), Gadella (1977), Ugborogho (1983), and Laan and Arends (1985). While Pathak *et al.* (1949) have reported the haploid number as n = 9, and Nanda (1962) reported n = 11 in the material studied by them.

***T. peruviana* (Pers.) K. Schum. (= *T. nereifolia* Juss. ex stend.)**

(variety 2)

This plant is very similar to *T. peruviana*, variety 1. The only difference is the presence of white flowers. The materials for the present investigation were collected from Kariavattom, Ponmudi, Pathanamthitta and Nagercoil.

Somatic chromosome determination in the root tip cells of this variety showed 20 chromosomes at metaphase (Fig. 19). The chromosome of this varieties are small in size and their length varied from 1.4 μm to 3.0 μm. The TCL and ACL were calculated to be 21.8 μm and 2.18 μm respectively. The TF% was 43.57. There was only one pair of M-type chromosome (fourth pair) and the remaining chromosomes were of m-type (Table 6, Text Fig. 6). The karyotype belonged to 1B category.

Table 6. Details of the karyotype of *T. peruviana* (2n = 20)

(= *T. nereifolia*) (variety 2)

Chromosome Number	Chromosome length (μm)			RCL	Arm ratio (r)	Position of centromere
	Long arm	Short arm	Total			
1	1.6	1.4	3.0	13.76	1.14	m
2	1.6	1.2	2.8	12.84	1.33	m
3	1.4	1.0	2.4	11.00	1.40	m
4	1.2	1.2	2.4	11.00	1.00	M
5	1.2	0.8	2.4	9.17	1.50	m
6	1.2	0.8	2.0	9.17	1.50	m
7	1.2	0.8	2.0	9.17	1.50	m
8	1.2	0.8	2.0	9.17	1.50	m
9	1.0	0.8	1.8	8.25	1.25	m
10	0.8	0.6	1.4	6.42	1.33	m

TCL = 21.8 μm Karyotye = 1B

ACL = 2.18 μm TF% = 43.57

***Cerbera* Linn.**

A genus with eight species distributed in Deccan Peninsula, Ceylon and Malaysia. One species is reported to occur in Madagascar and Ceylon (Huber, 1983). Hooker (1882) recorded one species from Peninsular India. Gamble (1923) reported only one species (*C. manghas* L.) from South India. In South India this plant occurs

in marshy or back water areas. The members are small glabrous trees with white large flowers in terminal cymes.

***C. odollam* Gaertn. (= *C. manghas* Linn.)**

A moderate sized evergreen maritime tree with acrid milky poisonous juice. Leaves are bright green in colour, lanceolate or oblanceolate in shape and glabrous, tapering sharply to the base. Flowers are large, white in colour with a yellow throat born in terminal cymes. Fruits are large, green drups with fibrous pericarp.

The bark, leaves and milky juice are purgative and emetic. The bark is used for ring worm infestation. The fruits are poisonous and are used in hydrophobia (Varier, 1994). The materials for the present study were collected from Kollam, Paravoor and Sasthamkottah.

Root tip cells of the species showed 2n = 40 (Fig. 20). Previous reports on the material of this species by Rau (1940) showed n = 20 and Laan and Arends (1985) showed 2n = 40. The present species is found to be a tetraploid taxon based on $x = 10$ condition.

***Kopsia* Blume**

This is a small genus with three species distributed chiefly in tropical Asia (Hooker, 1882). The plants are glabrous trees or shrubs. Leaves are seen in opposite arrangement and narrowed into very short petioles. Flowers born in terminal cymes. Corolla tube very slender with hairy throat and the lobes overlapping to the right. Stamens arranged near the top of the corolla tube.

***K. fruticosa* A. DC.**

This is a large handsome evergreen shrub. Leaves are glossy and shiny above and paler beneath. Flowers are large pretty pink, which appears nearly throughout the year. It is a native of Burma, but often cultivated in Indian gardens. The material for the present study were collected from Palode, Thiruvananthapuram, Bonacaud, Nagercoil and Mysore.

Root tip cells showed 2n = 36 (Fig. 21). Previously Tapadar and Sen (1960), Tapadar (1964) had reported n = 18 and 2n = 36 in this species. The same somatic count was reported by Laan and Arends (1985). The present species could be a tetraploid one based on x = 9.

Sub tribe 3. Euplumerieae

***Catharanthus* (L.) G. Don.**

A small genus of six species, all except one are natives of Madagascar (Huber, 1983). Members are erect or procumbent herbs or shrubs. Gamble (1923) reported one species *Lochnera rosea* (= *Catharanthus roseus*) from South India.

***C. roseus* (L.) G. Don. (= *Lochnera rosea* (L.) Reichb.**

= *Vinca rosea* (L.) G. Don.

This plant grows profusely in sandy beaches and deserted places and hence known by the common name 'the deadman's flower' (Janaki Ammal and Bezbarah, 1963). This plant is medicinally very important. The alkaloids 'Vincaleukoblastine' (VLB) and 'Vincristine', present in this species are useful in the treatment of

leukemia. Two varieties of this species have been studies during the present investigation.

variety 1

The plant is a perennial herb with pink flowers. This variety is credited with 74 different kinds of alkaloid of which 'Vinblastine' and 'leurocristine' are used effectively in the treatment of cancer (Svoboda, 1969; Sur, 1985). Thus besides being ornamental, the plant is medicinally very important. In the present study the materials were collected from Veli, Kariavattom, Aryankavu and Kollam.

Meiotic studies have shown the presence of 8 bivalents at metaphase I (Fig.22). The course of division during meiosis was normal. Pollen mother cells showed equal distribution of chromosomes at anaphase I (Fig.23) and there was appreciable pollen fertility (90%). Sugiura (1931), Pannochia – Laj (1938), Bowden (1940, 1945), Tapadar (1964), Gill and Abubakar (1975), Sarkar *et al.* (1975), Koul *et al.* (1976) and Balamani and Rao (1981) have recorded the same gametic number (n=8) in the material studied by them.

variety 2

This plant is very similar to *C. roseus*, variety 1. It differs from the former in having white flowers. The materials for the present study were collected from Thiruvananthapuram, Kollam, Aryankavu and Kottayam.

A haploid chromosome number of n=8 is observed during metaphase I of meiosis (Fig.24). Subsequent stages of meiosis were normal with 88% of pollen fertility. Tapadar (1964) reported n=8 in this variety.

***Vinca major* Linn.**

This is a perennial herbaceous ornamental plant. Flowers are blue in colour. The materials for the present study were collected from the Botanic Garden Ootacamand, Nilgiris.

The pollen mother cells showed 23 bivalents at metaphase I (Fig.25). Subsequent stages of meiosis were normal with appreciable degree of pollen fertility (85%). This is the first report from South India.

***Plumeria* Tourn. ex. Linn.**

The genus represented by 50 species, is a native of tropical America (Bailey, 1960). Hooker (1882) has described only one species from the Indian subcontinent. Gamble (1923) and Huber (1983) have reported two species in this genus. The genus is now naturalised throughout India and the members are cultivated for their fragrant flowers. Two species, *P. alba* and *P. rubra* were studied during the present investigation.

***P. alba* Linn.**

This is a small glabrous tree with thick fleshy branches marked with prominent scars left by fallen leaves. Flowers are large white with sweet fragrance. Milky latex is produced from the plant body when it exudes. In the present study, materials were collected from Kariavattom, Thiruvananthapuram, Kottayam, Munnar, Ranni and Madurai.

Pollen mother cells showed 18 bivalents at diakinesis (Fig.26). Precocious separation of bivalents was often observed in 70-80 percent of PMCs. Subsequent

stages were normal and pollen fertility was calculated as 73 per cent. The previous workers such as Singh, (1951), Kumar *et al.* (1952), Tapadar, (1964) and Banerjee (1974) had reported the same haploid chromosome number n=18.

Root tip cells showed 2n=36 (Fig.27). The same somatic chromosome number was reported earlier by Banerjee (1974), Kumar *et al.* (1952), Tapadar (1964) and Sharma (1970). While Banerjee (1974), Datta and Maiti (1972) Raghuvanshi and Chauhan (1974), Tapadar and Sen (1960) and Sharma (1970) showed the somatic chromosome number 2n=54.

***P. rubra* Linn. (= *P. lutea* Ruiz. and Pav.; *P. bicolour* Ruiz. and Pav.**

= *P. rubra L.* var. *acutifolia* Poir.

= *P. tricolor* Ruiz. and Pav.)

A small ornamental tree with thick fleshy and rather smooth branches. Leaves are large and pointed at the ends and arranged spirally. The flowers are white in colour with pale yellow centre or white with reddish colour, sweet scented and used for garlands in temples (Gamble, 1923). Milky latex is exuded from the plant parts when an injury occurs. The materials for the present study were collected from Thiruvananthapuram, Kollam, Kottayam, Konni, Ranni and Madurai.

Cytomixis was noticed in this species. Meiocytes were seen mostly connected in a series of 3-5 cells (Fig.28). The chromatin materials in a condensed stage move from one PMC to the other through cytoplasmic connection (Fig.29). Chromosome clumping at metaphase I, sticky bridges and lagging chromosomes at anaphase I were also more frequent during summer season (Figs.30,31,32).

About 20% PMCs showed 18 bivalents at metaphase I (Fig.33). The previous workers such as Singh (1951), Kumar *et al.* (1952), Tapadar and Sen (1960), Tapadar (1964) and Bawa (1973) had reported the same gametic number n=18 in this species. Subsequent stages of meiosis were normal with about 35-40 per cent pollen fertility. There was high incidence of pollen sterility (60%).

Root tip cells showed 2n=36 (Fig.34). Previous workers like Kumar *et al.* (1952), Tapadar and Sen (1960), Tapadar (1964), Nanda (1962), Sharma (1970), Datta and Maiti (1972), Bawa (1973), Raghuvanshi and Chauhan (1974) Banerjee (1974) and Renard *et al.* (1983) and Laan and Arends (1985) have also reported the same somatic number.

***Alstonia* R. Br.**

This genus comprises 34 species distributed throughout the old world tropics, mostly in the West Pacific region (Huber, 1983). Hooker (1882) had described eight species from Peninsular India. Gamble (1923) recorded two species in South India. During the present investigation two species have been cytologically studied.

***A. scholaris* R. Br.**

This is a large evergreen tree, bark greenish brown in colour, and exudes milky juice when injured. Leaves are arranged in whorls of five to ten and coriaceous, elliptic – oblong in shape. Flowers are small, greenish white in colour with short corolla tube. In indigenous medicine, the bark is used as a bitter tonic and is popularly used in the treatment of malaria. It is also stated to be useful in diarrhoea and dysentery. The milky exudate is bitter and is good for ulcers (Varier, 1994). The

materials for the present investigation were collected from Kulathupuzha, Palaruvi and Thiruvananthapuram.

During meiotic studies the pollen mother cells showed 22 bivalents at metaphase I (Fig.35). Subsequent stages of meiosis were normal with appreciable pollen fertility (85%). The previous cytological reports by Tapadar and Sen (1960) showed the haploid chromosome number as n=22, while Mehra (1976) reported n=20 in this species from North India.

Root tip cells showed 44 chromosomes at metaphase (Fig.36). The somatic chromosome number as 2n=44 had also been reported by Tapadar and Sen (1960) and Chauhan and Raghuvanshi (1977) in the material from North India.

***A. venenata* R. Br.**

This is a small tree with greyish brown bark, and bright yellow hard and woody root. Leaves are arranged in whorls of 3-6, and lanceolate in shape with wavy margin. Flowers are small and white in colour. The roots are bitter and astringent. They are useful in skin diseases, leprosy, cobra bite, and other venomous bites (Varier, 1994). The materials for the present study were collected from Kallar, Palaruvi and Aryankavu.

Pollen mother cells showed n=11 at metaphase I (Fig.37). Subsequent stages of meiosis were normal with high pollen fertility (90%). Previous cytological investigations on this species by Singh *et al.* (1982) have shown the same haploid chromosome number.

Root tip cells showed the somatic chromosome number as 2n=22 (Fig.38).

Sub tribe 4. *Tabernaemontaneae*

***Tabernaemontana* Plum. ex. Linn.**

This is a large genus with 160 species distributed in the tropical and sub tropical regions of the world (Bailey, 1960). Members of the genus are famous for their indol alkaloid contents. Hooker (1882) has described 14 species from the Indian subcontinent and Gamble (1923) recorded only one species from South India. *T. dichotoma* and five varieties of *T. divaricata* were studied here.

***T. dichotoma* Roxb.**

This is a large shrub or small tree with stout woody resinous branches. The leaves are broad and coriaceous. Flowers are large, white in colour with a yellow corolla tube. Materials for the present study were collected from Kottayam, Thiruvananthapuram, Palode, Pambavalli, Maruthumalai and Mettupalayam.

The pollen mother cells at diakinesis revealed 11 bivalents at first metaphase (Fig.39). Subsequent stages of meiosis appeared as normal with appreciable pollen fertility (88%).

The somatic complement of this materials showed 2n=22 (Fig.40). Previous workers like Kumar *et al.* (1952), Tapadar and Sen (1960), Tapadar (1964) have reported n =11 and 2n = 22 in the materials studied by them. The present material is found to be a diploid based on x=11.

***T. divaricata* (L.) R. Br. ex Roem. and Schult.**

(= *T. coronaria* Willd.

= *Ervatamia divaricata* (L.) Alston.

= *E. divaricata* (L.) (Burkill)

This plant is an evergreen ornamental hedge distributed throughout India (Bailey, 1960), Duthie (1960). The leaves are dark in colour and glacious in texture. Flowers are white in colour. Milky juice is present in the plant body. Five varieties of this species have been studied during the present investigation.

variety 1

This variety is an evergreen shrub with glossy leaves. Flowers are small, white in colour with five petals and single-whorled with pointed lobes. The materials for the present study were collected from Thiruvananthapuram, Courtallum and Peermade.

The pollen mother cells showed varying frequencies of univalents and bivalents at diakinesis (Fig.41). Very rarely in about 10-12 per cent of PMCs a ring of four chromosomes and 9 bivalents were noticed at metaphase I (Fig.42). During anaphase I and II lagging chromosomes were noticed in appreciable frequency (80-90%) (Fig.43), and hence there was very high pollen sterility (96%).

Root tip preparations revealed 22 chromosomes at metaphase (Fig.44). The same somatic count was reported by Tapadar and Sen (1960), Tapadar (1964), Raghuvanshi and Chauhan (1970), Datta and Maiti (1972) and Sharma and De (1976). Karyomorphological studies revealed that the chromosomes ranged from 2.75 μm to 3.75μm in length with TCL of 33.97μm and ACL 3.10μm. The TF% was 34.53. All

chromosomes were sm-types except the eighth pair which was m-type (Table 7, Text Fig.7). The karyotype belonged to 2A category.

Table 7. Details of the karyotype of *T. divericata* (variety 1) (2n=22)

Chromosome number	Chromosome length (μm)			RCL	Arm ratio (r)	Position of centromere
	Long arm	Short arm	Total			
1	2.75	1.00	3.75	11.03	2.75	sm
2	2.25	1.25	3.50	10.30	1.80	sm
3	2.12	1.12	3.24	9.53	1.89	sm
4	2.12	1.25	3.37	9.92	1.79	sm
5	1.75	1.00	2.75	8.09	1.75	sm
6	2.25	0.88	3.13	9.18	2.57	sm
7	2.00	1.12	3.12	9.18	1.78	sm
8	1.75	1.12	2.87	8.44	1.56	m
9	1.75	1.00	2.75	8.09	1.75	sm
10	1.75	1.00	2.75	8.09	1.75	sm
11	1.75	1.00	2.75	8.09	1.75	sm

TCL = 33.97 μm Karyotype = 2A

ACL = 3.10 μm TF% = 34.53

variety 2

This plant is similar to variety 1. Flowers are white in colour and possesses five large round corolla lobes. The materials for the present study were collected from Kottayam, Thiruvananthapuram, Munnar, Coimbatore, and Kodaikanal.

The pollen mother cells showed irregular meiotic divisions due to the occurrence of high frequency of univalents (3-5 in each PMC) at diakinesis and metaphase I (Figs. 45,46). Subsequent stages of meiosis were highly irregular, and there was high pollen sterility (95%).

Root tip cells showed 22 chromosomes at metaphase (Fig.47). The chromosomes ranged from 2.75μm to 4.25 μm in length. The TCL and ACL were calculated to be 32.23 μm and 3.00 μm respectively. The TF% was 39.03. The analysis of centromeric position of chromosomes revealed the occurrence of one pair of m-type chromosome and 10 pairs of sm-type chromosomes (Table 8, Text Fig.8). The karyotype belonged to 2A category.

Table 8. Details of the karyotype of *T. divaricata* (variety 2) (2n=22)

Chromosome number	Chromosome length (μm)			RCL	Arm ratio (r)	Position of centromere
	Long arm	Short arm	Total			
1	2.75	1.50	4.25	13.18	1.83	sm
2	2.25	1.25	3.50	10.85	1.80	sm
3	2.25	1.00	3.25	10.08	2.25	sm
4	2.00	1.00	3.00	9.30	2.00	sm
5	1.75	1.00	2.75	8.53	1.75	sm
6	1.75	1.00	2.75	8.53	1.75	sm
7	1.62	0.88	2.50	7.72	1.85	sm
8	1.25	1.00	2.25	6.98	1.25	m
9	1.62	0.88	2.50	7.72	1.85	sm
10	1.75	1.00	2.75	8.53	1.75	sm
11	1.75	1.00	2.75	8.53	1.75	sm

TCL = 32.23 μm

ACL = 3.00 μm

Karyotype = 2A

TF% = 39.03

variety 3

The plants are more stout and leaves are larger than the varieties 1 and 2. Flowers are white in colour. Petals are arranged in two whorls, each with five lobes. The materials for the present study were collected from Thiruvananthapuram, Palode, and Coimbatore.

The pollen mother cells showed varying number of univalents (5-10 in each PMC) and bivalents at diakinesis (Fig.48). Subsequent stages of meiosis were highly irregular, and there was very high degree of pollen sterility (90%).

Root tip cells showed 22 chromosomes at metaphase (Fig.49) which ranged from 2.75μm to 4.74μm in length. The TCL and ACL were calculated to be 37.49μm and 3.41 μm respectively. The TF% was 43.47. Karyotype consisted of one pair of m-type chromosome and 10 pairs of sm-type chromosomes (Table 9, Text Fig.9). The karyotype belonged to 2A category.

Table 9. Details of the karyotype of *T. divaricata* (variety 3) (2n = 22)

Chromosome number	Chromosome length (μm)			RCL	Arm ratio (r)	Position of centromere
	Long arm	Short arm	Total			
1	3.08	1.66	4.74	12.64	1.85	sm
2	2.50	1.25	3.75	10.00	2.00	sm
3	2.50	1.25	3.75	10.00	2.00	sm
4	2.25	1.25	3.50	9.33	1.80	sm
5	2.25	1.25	3.50	9.33	1.80	sm
6	2.25	1.25	3.50	9.33	1.80	sm
7	2.00	1.00	3.00	8.00	2.00	sm
8	2.00	1.50	3.50	9.33	1.33	m
9	1.75	1.00	2.75	7.33	1.75	sm
10	1.75	1.00	2.75	7.33	1.75	sm
11	1.75	1.00	2.75	7.33	1.75	sm

TCL = 37.49 μm Karyotype = 2A

ACL = 3.41μm TF% = 43.47

variety 4

In general appearance this variety is almost similar to variety 3 except in size of the leaves and number of petals. Flowers white in colour with petals more than 10 (11-13), arranged concentrically. The materials for the present study were collected from Kariavattom, Palode, Kottayam, Mettupalayam and Bangalore.

The pollen mother cells showed varying number of univalents (25-29) and bivalents (1-2) in each PMC at metaphase I (Fig. 50). Irregular separation of univalents resulted in unequal segregation of chromosomes at anaphase I and II (Figs. 51, 52), and hence there was high incidence of pollen sterility (95%).

The materials showed 33 chromosomes in root tip cells (Fig. 53). The same somatic count was reported by Kumar *et al.* (1952), Tapadar and Sen (1960), Tapadar (1964), Raghuvanshi and Chauhan (1969b, 1974), De (1978) and Laan and Arends (1985). The somatic chromosome ranged from 2.75 to 3.75 μm in length. The TCL and ACL were calculated to be 34.86 μm and 3.20 μm respectively (Table 10). The TF% was 34.39. The karyotype consisted of 3 m-type and 30 sm-type chromosomes (Table 10, Text Fig. 10). The karyotype belonged to 2A category.

Table 10. Details of the karyotype of *T. divaricata* (variety 4) (2n = 33)

Chromosome Number	Chromosome length (μm)			RCL	Arm ratio (r)	Position of centromere
	Long arm	Short arm	Total			
1	2.50	1.25	3.75	10.75	2.00	sm
2	2.25	1.25	3.50	10.04	1.80	sm
3	2.75	1.00	3.75	10.75	2.75	sm
4	2.25	1.25	3.50	10.04	1.80	sm
5	2.00	1.00	3.00	8.60	2.00	sm
6	2.12	1.12	3.24	9.29	1.89	sm
7	2.00	1.00	3.00	8.60	2.00	sm
8	1.75	1.12	2.87	8.23	1.56	m
9	1.75	1.00	2.75	7.88	1.75	sm
10	1.75	1.00	2.75	7.88	1.75	sm
11	1.75	1.00	2.75	7.88	1.75	sm

TCL = 34.86 μm
ACL = 3.20 μm

Karyotype = 2A
TF% = 34.39

Previous reports on this species by Kumar *et al.* (1952); Tapadar and Sen (1960); Tapadar (1964); Raghuvanshi and Chauhan (1969b, 1974); De (1978); and Laan and Arends (1985) have also reported 2n = 22.

variety 5

This plant is similar to variety 1, but leaves are variegated. The materials were collected from Thiruvananthapuram, Nagercoil Mysore and Bangalore.

During meiosis the pollen mother cells showed varying frequencies of univalents (1-7) (Fig. 54). Subsequent stages of meiosis were highly irregular with high incidence of pollen sterility (85%). Very rarely 11 regular bivalents were noticed in 5-10 percent of pollen mother cells (Fig. 55).

Root tip cells showed 2n = 22 in this materials (Fig. 56). This is a new report from South India.

***Holarrhena* R. Br.**

This is a small genus distributed in tropical Asia and Africa. Hooker (1882) had reported two species from Peninsular India, and Gamble (1923) described one species (*H. antidysenterica*) from South India.

***H. antidysenterica* Wall. (= *H. pubescens* (Bunch-Ham.) Wall. ex. G. Don.**

This is a small deciduous tree with woody branches. The bark is thick and brown in colour with abundant milky white latex. Leaves are simple and arranged oppositely. Flowers white in colour and born in terminal corymbose cymes. The bark and seeds are bitter and constipating. They are useful in amoebic dysentery and

diarrhoea. The materials for the present investigation were collected from Kariavattom, Ponmudi, Yercaud and Silent Valley.

The pollen mother cells showed 11 bivalents at metaphase I (Fig. 57). Subsequent stages of meiosis were normal and there was appreciable degree of pollen fertility (90%). Chromosome reports on this species by Raghavan (1959), Tapadar and Sen (1960), Sarkar *et al.* (1975), Mehra (1976), Sharma and De (1976), De (1978) and Bir *et al.* (1984a) have showed n = 11. Sen and Tapadar (1957), Datta and Maiti (1972), Sharma and De (1976), Mehra (1976), Chauhan and Raghuvanshi (1977) and De (1978) have reported somatic chromosome number as 2n = 22.

TRIBE III ECHITIDEAE

This tribe includes 52 genera, of which 8 genera have been cytologically worked out during the present investigation.

Sub tribe 1. Parsonsieae

***Vallaris* Burm.**

This is a small genus with four species grown in India, Ceylon, Southeast Asia and Malaysia (Huber, 1983). Hooker (1882) reported four species distributed in Asiatic and Malayan region. Gamble (1923) reported only one species (*V. solanacea)* from South India. Six species have been reported in Tropical Asia and Malaya (Bailey, 1960). In the present study two species were cytologically studied.

***V. solanacea* (Roth.) Kuntze. (= *V. heynei* Spreng.)**

This is a tall climbing shrub. Leaves are oppositely arranged. Flowers are small with creamy white in colour. Milky latex is exuded when injured. The plant has

a characteristic 'goat's smell'. In the present study the materials were collected from Thiruvananthapuram, Palaruvi, Thenmala and Lalbagh Gardens, Bangalore.

Pollen mother cells at metaphase I revealed the gametic number as n = 10 (Fig. 58). The present finding is in conformity with the reports of Rau (1940) and Tapadar (1964). But Bir *et al.* (1980, 1984a), Bedi *et al.* (1980) had reported n = 11.

***V. lancifolia* Hook. f.**

This is a twinning shrub. Leaves are small and narrowly lanceolate. Flowers are small and creamy white in colour like that of *V. solanacea.* The materials for present investigation were collected from Wayanadu, Kulathupuzha and Mysore.

The pollen mother cells at metaphase I revealed the gametic number as n = 10 (Fig. 59). Subsequent stages of meiosis were normal with appreciable degree of pollen fertility (88%). The chromosome number of this species is reported for the first time from South India.

***Parsonsia* R. Br.**

This genus consists of 80 species chiefly distributed in India, Ceylon, Southeast Asia, and Oceania (Huber, 1983). Bailey (1960) had reported 20 species in Tropical Asia, Australia and New Zealand. Hooker (1882) and Gamble (1923) reported only one species from South India.

***P. spiralis* Wall.**

This is a twinning shrub. Leaves are opposite in arrangement, and ovate oblong or oblong lanceolate in shape. The upper side is dark green and glabrous, and the lower side is pale green in colour. The flowers are small and white. Milky juice

exudes when injured. In the present study materials were collected from Palaruvi, Kallar, Kulathupuzha and Silent Valley.

Pollen mother cells showed n = 9 at diakinesis (Fig. 60). Subsequent stages of meiosis were normal with high degree of pollen fertility (82%).

The root tip cells showed 2n = 18 (Fig. 61). The chromosome number of this species is reported for the first time from South India. Since somatic chromosomes are very small in size, karyotype analysis was not carried out.

***Wrightia* R. Br.**

This is a small genus distributed in tropical Asia. Hooker (1882) reported six species and Gamble (1923) reported two species from South India. The plants are shrubs or small trees. Only one species was studied here.

***W. tinctoria* R. Br.**

This is a small deciduous tree with white flowers. Leaves are elliptic ovate or oblong in shape. Materials for the present investigation were collected from Wayanadu, Kallar and Coimbatore.

The present study revealed the somatic chromosome number as 2n = 22 (Fig. 62). Tapadar (1964) and Bir *et al.* (1984b) had reported the same somatic chromosome number (2n = 22). However, Gajapathy (1962) and Raman and Kesavan (1963) had reported a different somatic number (2n = 20).

Sub tribe 2 Nerieae

***Strophanthus* DC.**

This is a small genus with 40 species distributed in Tropical Asia, and Africa (Bailey, 1960). Hooker (1882) had reported five species from the Indian subcontinent and Gamble (1923) reported two species from South India. In the present study two species have been cytologically studied.

***S. gratus* (Wall. and Hook.) Baill.**

This is an erect shrub with stout branches and dark bark possessing lenticels. Leaves are coriaceous and glossy above. Flowers are large and whitish with purple colour. Usually cultivated in gardens as an ornamental. In the present investigation the materials were collected from Wayanadu, Thiruvananthapuram and Kodaikanal.

The pollen mother cells showed 9 bivalents at metaphase I (Fig. 63), and equal chromosome distribution at anaphase I (Fig. 64). However, during anaphase II about 10-12% of the PMCs showed multipolar chromosome distribution. There was 89 percentage of pollen fertility. Tapadar and Sen (1960), Tapadar (1964) had reported the haploid chromosome number as n = 10.

In the present study the somatic chromosome complement showed 2n = 18 (Fig. 65). Arends and Laan (1982) and Beentje (1982) reported the same somatic chromosome number 2n = 18.

***S. wightianus* Wall.**

This is a climber with shining leaves. Flowers are yellow with faint reddish stripes. Anther possessed very long and slender pointed tip. In the present study

materials were collected form Wayanadu, Kottayam, Thiruvananthapuram, Thirunelveli and Silent Valley.

The pollen mother cells showed n = 9 at diakinesis (Fig. 66). Subsequent stages of meiosis were normal with 90 percentage of pollen fertility.

The root tip cells showed 2n = 18 (Fig. 67). Previously a different somatic chromosome number was reported in this species by Witkus (1951) as 2n = 20.

Sub tribe 4. Ichnocarpeae

***Ichnocarpus* R. Br.**

This genus consists of 10 species ranging from the Western Himalayas to Ceylon, South China, the Phillippines and Queensland (Huber, 1983). Hooker (1882) had reported only one species in this genus from the Indian subcontinent. Gamble (1923) reported only one species (*I. frutescens*) from South India. Members of this genus are woody twiners. Milky juice is present in the plant body.

***I. frutescens* (L.) R. Br. (= *Apocynum frutescens* L.**

(= *Echites frutescens* (L.) Roxb.)

This plant is a climber. Leaves are variable in size and shape and dark green colour in the upper side. Flowers are very small with pale yellow colour. The materials for the present investigation were collected from Kallar, Palaruvi, Kayamkulam and Thekkady.

The pollen mother cells of this species showed 10 bivalents at metaphase I (Fig. 68). The course of division during meiosis was regular and showed 85

percentage of pollen fertility. The present count n = 10 forms a new report for the species from South India.

***I. ovatifolius* A. DC.**

This plant is very closely allied to *I. frutescens* but the leaves are much larger and broader. In the present investigation materials were collected from Palaruvi, Kulathupuzha and Munnar.

The root tip cells showed 2n = 40 (Fig. 69). The present material is found to be a tetraploid taxon based on x = 10. The chromosome number of this species is reported for the first time. Since the somatic chromosomes are very small, karyotype analysis was not tried.

Sub tribe 5. Euechitideae

***Chonemorpha* G. Don.**

This genus consists of 12 species ranging from India and Ceylon to South China and the Philippines (Huber, 1983). Members are large woody twiners with pubescent to almost tomentose branches. Leaves are broad and opposite in arrangement. Flowers are large, and white tinged with yellow colour. Hooker (1882) had reported only two species from India and Malaya. Gamble (1923) reported only one species (*C. macrophylla*) from South India. In the present study two species were cytologically investigated.

***C. fragrans* (Moon.) Alston. (= *C. macrophylla* (Roxb.) G. Don.**

= *Echites fragrans* Moon.

= *E. macrophylla*, Roxb.

This is a large climber with milky juice and large nearly orbicular leaves. Flowers are large and sweet scented. The plant is medicinally very important. The root is used as a laxative and carminative. They are also used in skin diseases like leprosy and scabies (Varier, 1994). The materials for the present study were collected from Kallar, Kottayam, Ponmudi and Bonacaudu.

The pollen mother cells of this taxon showed 10 regular bivalents at metaphase I (Fig. 70). Subsequent stages of meiosis were normal with 90 percentage pollen fertility. Gametic number in this species is reported for the first time from South India.

The root tip cells showed 2n = 20 (Fig. 71). Tapadar (1964) had reported the same chromosome number (2n = 20) from North India.

***C. griffithii* Hook.**

This is a climber with milky juice. Leaves are smaller in size than in *C. fragrans*. The materials for the present study were collected from Palaruvi and Kallar.

Due to the nonavailability of flower buds, meiosis was not carried out. In the present study the somatic chromosome number showed 2n = 30 (Fig. 72). This is the first chromosome report of this species.

***Adenium* Roem. and Schult.**

This is a small genus with four species (Bentham and Hooker, 1876). The members are succulent shrubs or under shrubs, often with swollen stems and fleshy branches. Leaves are rather fleshy and spirally arranged. Flowers are pink or purple in colour. During the present investigation only one species (*A. obesum*) has been studied.

***A. obesum* Roem. and Schult.**

This is a small xerophytic plant with succulent stem and small fleshy leaves. The flowers are very attractive. The materials for the present study were procured from Veli, Coimbatore, Courttalam and Ootacamund.

The pollen mother cells at metaphase I revealed gametic chromosome number as n = 11 (Fig. 73). Subsequent stages of meiosis were normal with 85% of pollen fertility. The gametic number in this species is reported for the first time.

The root tip cells showed 2n = 22 (Fig. 74). The present findings is in confirmity with the reports of Tapadar (1964), and Laan and Arends (1985).

***Aganosma* G. Don.**

This is a small genus with about 12 species, ranging from the Himalayas to Ceylon and eastwards to the Philippines (Huber, 1983). Hooker (1882) had reported five species and four varieties in this genera. Gamble (1923) reported two species from South India. In the present study cytology of one species was carried out.

***A. caryophyllata* G. Don. (= *A. dichotoma* K. Shum**

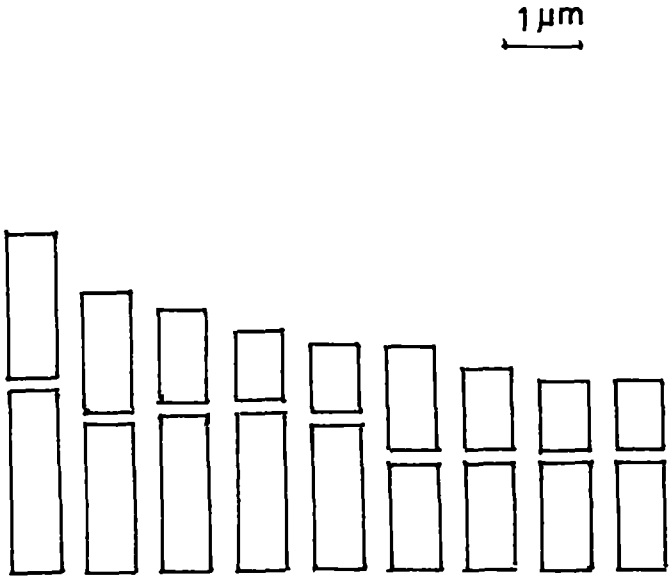
(= *Echites caryophyllata* Waliich Wall.

(= *E. dichotoma* Roth.)

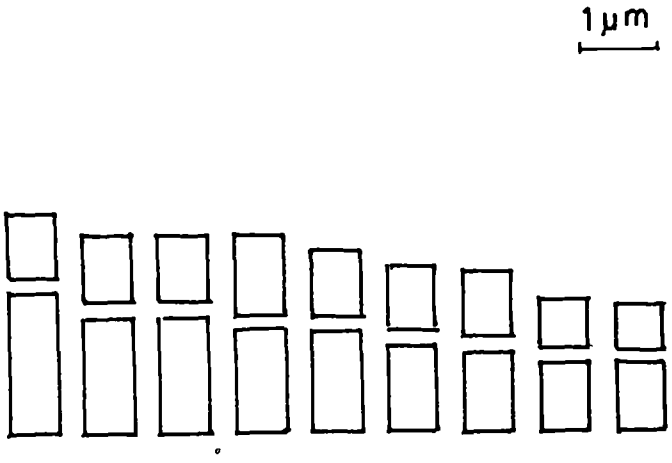
This is a large climber with stout stem. Leaves are coriaceous. Flowers are white in colour. The decoction of its root is taken for urinary troubles, as a tonic for fever, and as an emmenagogue.

The material for the present investigation were collected from Wayanadu, Thiruvananthapuram, and Thenmala.

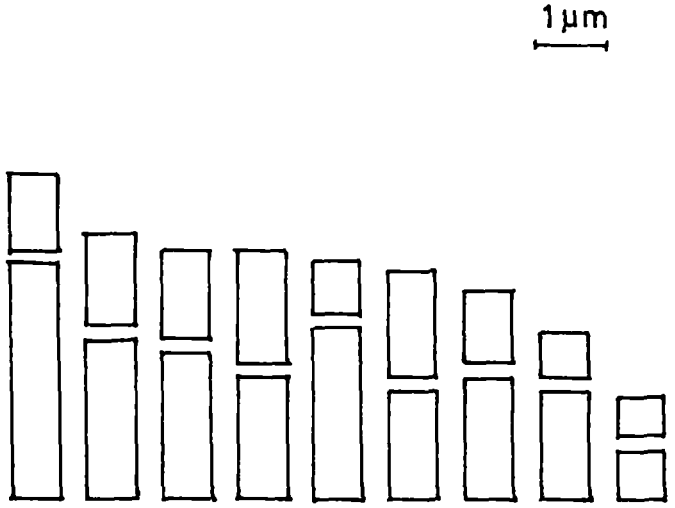
In the present investigation the root tip cells showed 2n = 22 (Fig. 75). The same number (2n = 22) was reported by Tapadar and Sen (1960).



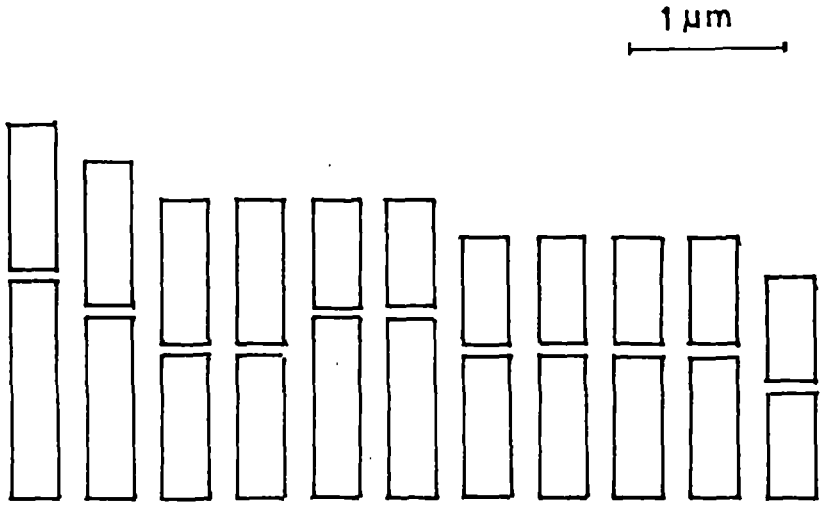
Text Fig.1. Allamanda cathartica 2n = 18



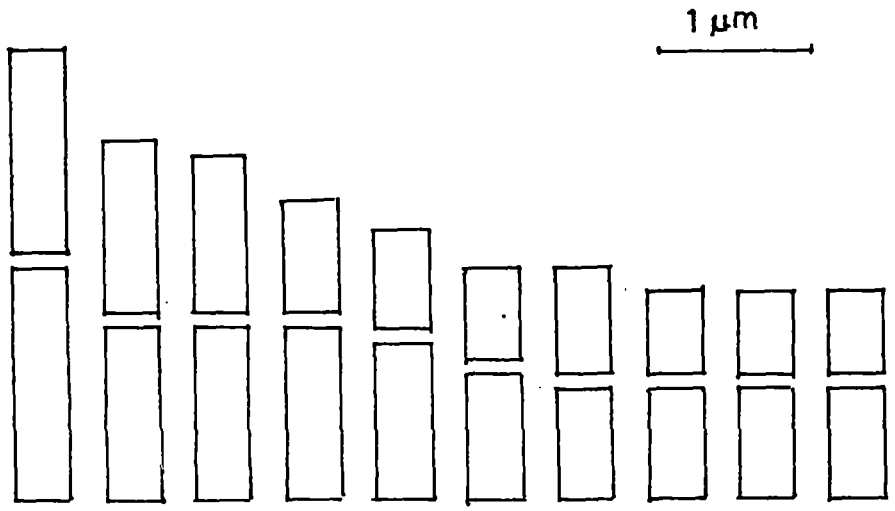
Text Fig. 2. Allamanda schottii 2n = 18



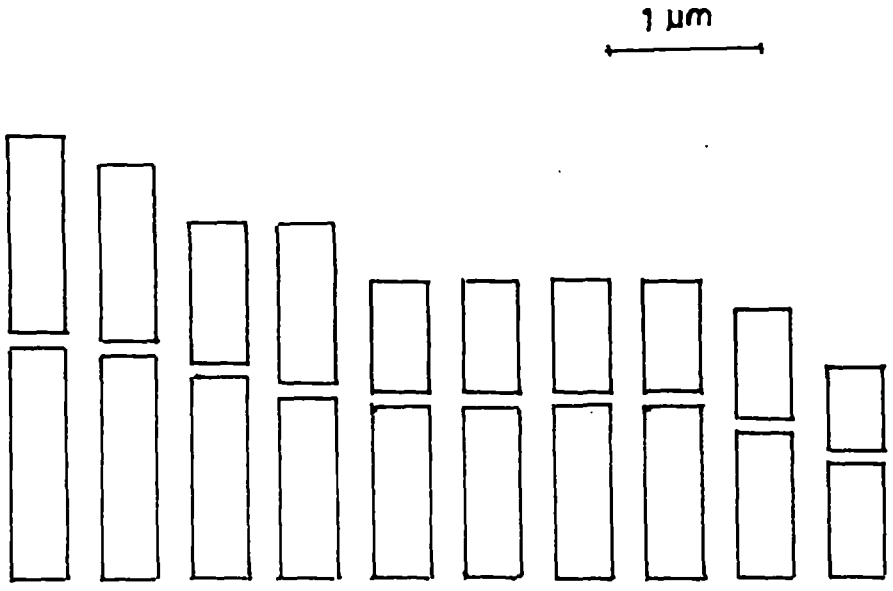
Text Fig. 3. Allamanda neriifolia 2n = 18



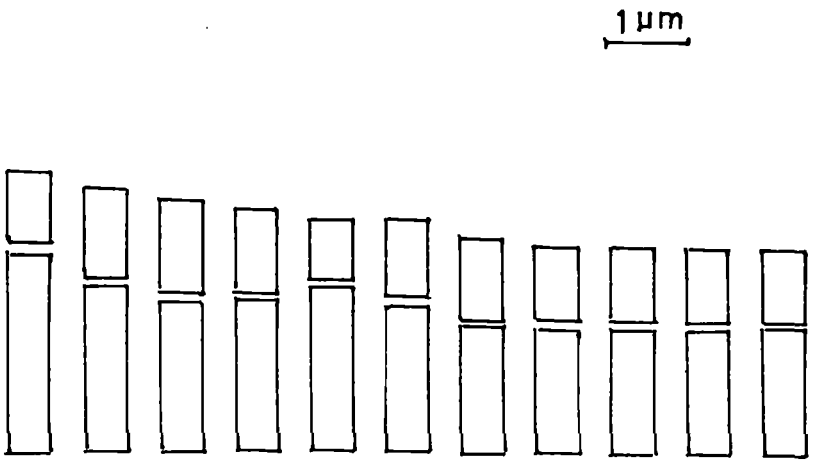
Text Fig. 4. Carissa carandas 2n = 22



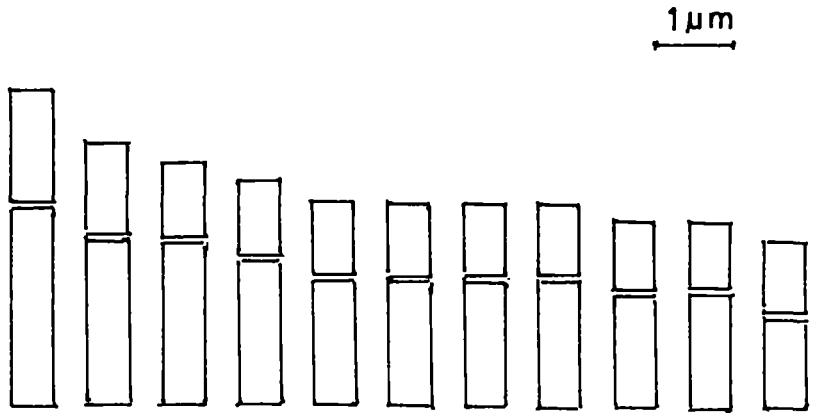
TextFig.5. Thevetia peruviana (var.1)2n = 20



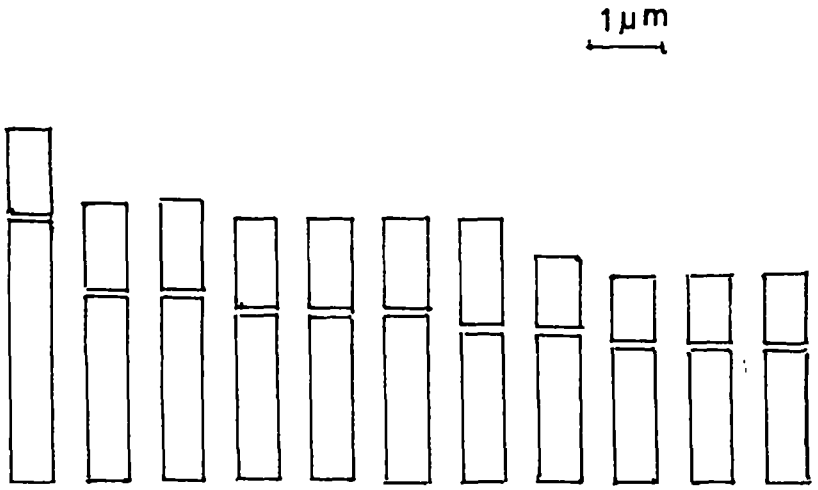
Text Fig.6. Thevetia peruviana (var.2)2n = 20



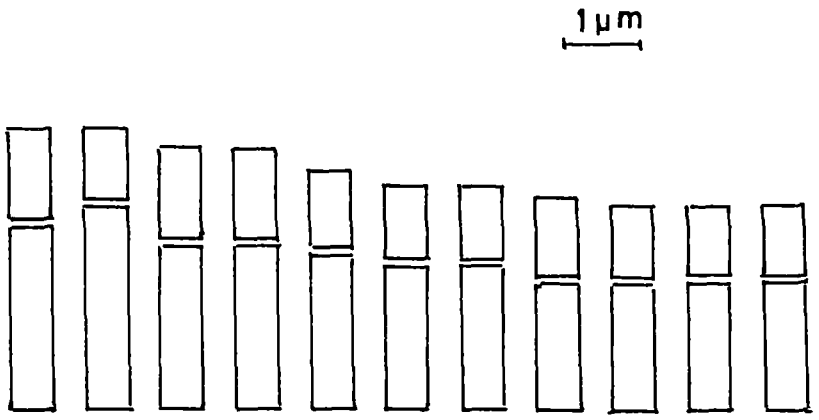
Text Fig.7. Tabernaemontana divaricata (var.1) 2n=22



Text Fig.8. Tabernaemontana divaricata (var.2) 2n=22



Text Fig.9. Tabernaemontana divaricata (var.3) 2n=22



Text Fig.10. Tabernaemontana divaricata (var.4) 2n=3

DISCUSSION

Cytological evolution

Cytological investigations have been carried out in 40 taxa under 20 genera and 3 tribes of the family Apocynaceae from South India, and the results are summarised in Table 11. The present data, coupled with the previous reports from various geographical regions is used here to discuss the chromosome evolution in the group in terms of the prevailing basic chromosome numbers and their evolution, and the occurrence of polyploids, aneuploids, and their role in speciation, in the family. An attempt is also made to evaluate the nature and trend of chromosome structural evolution in a few genera based on their karyotype data.

Basic chromosome number

The basic chromosome number has been one of the widely used characters in formulating the phylogenetic speculations (Jones, 1970), and has been considered as a dependable and stable marker of the direction of evolution. The concept of basic chromosome number at the level of genus and family has played a prominent role in shaping the prevailing concept of evolution. The basic number is defined as the primitive or original number from which polyploid numbers have been derived (Swanson, 1958). Changes in basic chromosome numbers have occurred in the evolution of plants, and this constitutes a major class of changes, which has played important role in speciation in many plant groups. Stebbins (1950) has envisaged diminution of the base number as playing a significant role in the process of evolution.

Polyploidy has influenced and played a prominent role in the higher order, usually by "polyploid drop" (Darlington, 1956) and occassionally by "polyploid lift" (Jones, 1970). This 'drop' and 'lift' by disploidy might produce new unrelated basic numbers in a group. As Raven (1975) has pointed out, it is of utmost importance in the application of chromosomes on phylogenetic consideration to first deduce the original basic chromosome number of the group in question. In the light of present findings along with the previous chromosome number reports, the probable basic chromosome constitutions in different genera and tribes included in the present study are outlined as below.

Tribe I: CARISSEAE

In the tribe Carisseae two genera such as *Allamanda* and *Carissa* have been cytologically investigated. The cytological data so far available on the genus *Allamanda* (Table 11) appear to be suggestive that this is a monobasic (x = 9) genus (Sugiura, 1936; Pathak *et al.* 1949; Kumar *et al.* 1952, Sen and Tapadar 1956; Tapadar and Sen, 1960; Tapadar, 1964; Datta and Bhattacharya, 1981; Balamani and Rao, 1981; and Laan and Arends, 1985). Barring a stray report of n = 10 (Bir and Neelam, 1980) the entire reports in species of *Allamanda* are consistently x = 9 based on n = 9 and 2n = 18, or n = 18 and 2n = 36. *Allamanda violacea* with gametic number n = 18 and somatic number 2n = 36 could be a tetraploid based on x = 9. The n = 10 reported by Bir and Neelam (1980) on *A. schottii* could be an aneuploid (ascending) derivation from n = 9. The present findings of x = 9 in all the three species from South India appear to confirm this as the established basic constitution of the genus.

Carissa is a monobasic genus with n = 11 and 2n = 22 in all cytologically known seven species. A perusal of the cytological data (Table 11) showed that a host of authors have considered x = 11 as the primary basic number of the genus (Singh, 1951; Tapadar and Sen, 1960; Tapadar, 1964; Chauhan and Raghuvanshi, 1977; Sanjappa and Dasgupta, 1981; Balamani and Rao, 1981; and Bir *et al.* 1984a). The present finding supports this contention.

Tribe II: PLUMERIEAE

In the tribe plumerieae cytological studies have been carried out on fifteen species and six varieties belonging to nine genera.

During the present cytological analysis, four species have been studied in the genus *Rauvolfia.* It has been noticed that *R. serpentina* is diploid (2n = 22); *R. densiflora* tetraploid (2n = 44); *R. tetraphylla* pentaploid (2n = 55) and *R. beddomei* (n=66) hexaploid taxa, all based on x = 11. A perusal of the previous chromosome reports (Raghavan, 1957; Milovidov and Storchova, 1958; Tapadar, 1964; De, 1979; Sharma and De, 1976; Bedi and Gill, 1982; and Laan and Arends, 1985) and the present findings show that *Rauvolfia* is a monobasic genus based on x = 11.

Two varieties of the species *Thevetia peruviana* studied presently in the genus *Thevetia* showed that both are diploids (2n = 20) on the basic number x = 10. Hardas and Joshi (1954); Tapadar and Sen (1960); Tapadar (1964); Datta and Maiti (1972); Chauhan and Raghuvanshi (1977); Gadella, (1977); Ugborogho (1983); and Laan and Arends (1985) have also suggested x = 10 as the basic number in the genus. However, Pathak *et al.* (1949) and Nanda (1962) had reported the gametic number as n = 9, and

n = 11 respectively in the material of *T. peruviana* studied by them. These two sporadic numbers could have arisen from n = 10 situation by descending and ascending aneuploidy.

Analysis of the available cytological data including the present study (Table 11) showed that the genus *Catharanthus* is monobasic based on x = 8. Of the cytologically known six species, four are diploids (n = 8, 2n = 16), one triploid (2n = 24), and the other tetraploid (2n = 32) both based on x = 8. Almost all the earlier reports are also in line with the present finding (Sugiura, 1931; Pannochia – Laj, 1938; Bowden, 1940, 1945; Tapadar, 1964; Gill and Abubakar, 1975; Sarkar *et al.* 1975; Koul *et al.* 1976 and Balamani and Rao, 1981).

Out of the six species in the genus *Cerbera* studied, five showed 2n = 40. Only one species, (*C. venenifera*) showed 2n = 44 and this could be an ascending aneuploid derivation from the x = 10 situation. *C. odollam* (2n = 40), which is studied during the present investigation could be a tetraploid based on x = 10. The predominant occurrence of taxa with 2n = 40 in *Cerbera* is suggestive of x = 10 being the established basic constitution in the genus.

Cytological data including the present study (Table 11) revealed that *Kopsia* is a monotypic genus with x = 9 constitution. This chromosome situation was reported earlier by Tapadar and Sen (1960); Tapadar (1964) and Laan and Arends (1985). The present material with 2n = 36 could be a tetraploid taxon on x = 9.

The genus *Alstonia* showed three different gametic numbers such as n = 11, 20 and 22, of which n = 11 is the predominant one. Species with n = 22 is a polyploid

derivation from n = 11 while n = 20 could be the product of doubling from n = 11 followed by loss of two chromosomes. Materials of *A. scholaris* studied have shown n=22 and 2n = 44. This could be a tetraploid taxon based on x = 11. Tapadar and Sen (1960) and Singh *et al.* (1982) have also reported the same basic chromosome number for this genus (Table 11). The overall data show that x = 11 is the established basic constitution of the genus *Alstonia.*

Available cytological information including the present findings (Table 11) showed that *Tabernaemontana* is a monobasic genus based on x = 11. Out of the cytologically known 46 taxa, 35 are diploids with n = 11, and 2n = 22 and the others either triploids (2n = 33) or hexaploids (2n = 66). Tapadar (1964); and Laan and Arends (1985) have reported the same basic chromosome constitution.

In the genus *Holarrhena,* all the cytologically known species except *H. floribunda* have shown n = 11 and 2n = 22 (Table 11) and this is suggestive of x = 11 as the basic chromosome constitution of the genus. *H. floribunda* showed 2n = 20 which might be a descending aneuploid derivative from x = 11 (10 ← x = 11).

The chromosome data in genus *Plumeria* showed that this is a monobasic genus with x = 9. Great majority of the cytologically known species (23 out of 29) are diploids with n = 18 and 2n = 36 (Table 11). One accession showed 2n = 45 which could be a pentaploid, and the remaining ones with n = 27, and 2n = 54 could be a triploid and hexaploid respectively based on x = 9. The deviant somatic numbers such as 2n = 44 and 46 could be aneuploid derivatives from 2n = 45, the former descending and the latter ascending.

The phenomenon of cytomixis was defined by Gates (1911) as the passage of chromatin materials from one pollen mother cell to the adjacent one through cytoplasmic connections. Since then, a number of investigators have reported this phenomenon both in mitotic (Sarvella, 1958, Bowes, 1973) and meiotic cells of different plant species (Lakshmi and Raghavaiah, 1981; Datta, 1982; Bahl and Tyagi, 1988; Lakshmi *et al.* 1989; Koul and Kuldeep 1990; Soman and Bhavanandan, 1993; Consolaro and Pagliarini, 1995; de Souza and Pagliarini, 1997). Gottschalk (1970) has pointed out its occurrence limited to genetically unbalanced types such as haploids, triploids and other genetically disturbed plants. Certain other investigators reported that this process could be induced by the use of mutagens, clastogens and carcinogens (Morisset, 1978; Sasikumar and Susan Abraham, 1993).

The materials from different populations of *Plumeria rubra* showed normal meiosis at normal temperature. However, extrusion of chromatin material from one PMC to the adjacent PMC was observed in materials collected from different localities in Thiruvananthapuram district. As the atmospheric temperature increases a corresponding increase in the frequency of cytomixis was also noticed. The relationship between cytomixis and fluctuating atmospheric temperature has been reported earlier in different plant species like *Urochloa panicoides* (Basavaiah and Murthy, 1987), *Jasminum* (George and Geethamma, 1983), *Capsicum annum* (Lakshmi *et al.* 1989), *Helicanthus elastica* (Soman and Bhavanandan, 1993) and *Brassica napus* and *B. campestris* (de Souza and Pagliarini, 1997).

Cytomixis is usually presumed to be the result of unknown physiological disturbances, which may themselves be associated with other meiotic irregularities or with hybridization (Bell, 1964). However, based on the occurrence of cytomixis in meiotically normal plants of *Clitoria ternatea*, Banerjee and Sharma (1988) have suggested that meiotic irregularities might not be the sole criteria of cytomixis.

There exists differences of opinion among cytologists regarding the origin and significance of cytomixis. Woodworth (1931) and Sarvella (1958) explained cytomixis as due to mechanical injury. Several other reasons are also there like faulty fixation and handling (Linnert, 1955; Kamara, 1960) and nutritional deficiency (Milajajev, 1967). Some others have the opinion that cytomixis is due to pathological phenomenon (Maheshwari, 1950; Morisset, 1978) or genetic mechanism (Brown and Bertke, 1974; Omara, 1976). de Souza and Pagliarini (1997) suggested that high temperature causes the appearance of cytomixis between microsporocytes of *Brassica napus* and *B. campestris*. Koul and Kuldeep (1990) suggested that some changes in the biochemical process are responsible for cytomixis. Soman and Bhavanandan (1993) have suggested that cytomixis occurs as a natural phenomenon. They further suggested that some bio-chemical changes might have initiated at high atmospheric temperature which ultimately culminate in a deviation in the physiological process.

Contradicting views were put forward by different authors regarding the role of cytomixis in evolution. Sarvella (1958) suggested that aneuploid plants could be originated by cytomixis. Soman and Bhavanandan (1993) have suggested that cytomixis may result in hyperploid plants. It may be noted that all the species of

Plumeria so far reported are diploids with 2n = 36 on the basic chromosome number x = 18. However, there is every possibility of the origin of aneuploids due to cytomixis. Repeated observation in two successive years by the present author led to suggest that transmigration of chromatin could be attributed to some physiological changes in *P. rubra* to initiate cytomixis, and the frequency is enhanced by increased atmospheric temperature.

A perusal of chromosome number in the tribe Plumerieae showed that chromosomally it is a heterogenous group (x = 8, 9, 10, 11, 20, 22 and 23) (Table 11). Of the nine cytologically known genera, four have predominantly x = 8 and x = 23. Of these basic chromosome numbers x = 11 predominates among the cytologically studied diploid species. Most of the workers agreed on the possibility of x = 11 being the earlier evolved condition in the tribe. A high incidence of diploidy on this number and also infraspecific polyploidy known in the genera *Alstonia* (2n = 22, 44, 88) and *Tabernaemontana* (2n = 22, 33, 66) appears to strengthen this possibility. The lower numbers such as x = 10, 9 and 8 might have evolved from x = 11 condition by aneuploid reduction (8 ← 9 ← 10 ← x = 11). The other higher number x = 23 could be from the basic number x = 11 by chromosome doubling followed by ascending aneuploidy, or it could be the product of fusion of n = 12 and 11 gamates. Thus in the genus, x = 23 could be a secondarily evolved condition.

Tribe III: ECHITIDEAE

In the tribe Echitideae 10 genera have been cytologically known including the one presently studied (Table 11). Chromosome numbers showed considerable variation among the various genera.

In the genus *vallaris* three accessions of the species, *V. solanacea* have been cytologically reported by previous authors (Rau, 1940; Tapadar, 1964; Bedi *et al.* 1980), of which two showed n = 10 and one showed n = 11. During the present investigation, two species (*V. solanacea* and *V. lancifolia*) have been studied, and both showed n = 10. The predominant occurrence of n = 10 is suggestive of x = 10 as the basic chromosome constitution for the genus. The accession with n = 11 could be an ascending aneuploid derivative from x = 10.

In the genus *Parsonsia,* only one species (*P. spiralis*) has been cytologically studied which showed n = 9 and 2n = 18. The same number was previously reported by Beizenberg and Hair (1983) on the materials (*P. capsularis* and *P. heterophylla*) studied by them, and no other cytological information is available in the genus. Hence, with this scanty data a valid suggestion on the basic chromosome situation of the genus is not possible.

In the genus *Wrightia,* four species have been cytologically reported, of which three are diploids based on x = 11. The remaining one, *W. tinctoria* showed n = 10 (Gajapathy, 1962 and Raman and Kesavan, 1963). In the presently studied material, the root tip cells showed 2n = 22. Tapadar (1964), and Bir *et al.* (1984b) have reported n = 11 and 2n = 22 in this species from North India. Based on the available

chromosome reports x = 11 may be considered as the basic chromosome constitution for this genus. The other number (n = 10) could be a derived one from x = 11 by aneuploid reduction.

A perusal of the previous chromosome number reports on the genus *Strophanthus* showed that out of the 19 cytologically known species, 14 are diploids with n = 10 and 2n = 20. The remaining five species are diploids based on x = 9. The predominant occurrence of 2n = 20 could be an indication of x = 10 as the basic situation in this genus. The chromosome numbers n = 9 and 2n = 18 might have originated by descending aneuploidy from the n = 10 condition.

Available previous and the present chromosome reports in the genus *Ichnocarpus* showed that this is a monobasic genus with x = 10 (Table 11). In the presently studied species *I. frutescense* showed n = 10 and *I. ovatifolius* showed 2n = 40. The latter species could be a tetraploid taxon based on the basic chromosome number x = 10.

Cytologically only two species are known in this genus *Chonemorpha* (*C. fragrans* 2n = 20, and *C. griffithii* 2n = 30). Tapadar (1964); and Laan and Arends (1985) have reported x = 10 as the basic chromosome constitution in the genus. The present findings also support this view.

So far only two species have been cytologically known in the genus *Adenium* including the present material (*A. obsesum*) from South India with n = 11, and 2n = 22. Tapadar (1964); and Laan and Arends (1985) have reported the same number, which is suggestive of x = 11 as the basic chromosome constitution.

Cytology of only one species of *Aganosma* (*Aganosma caryophyllata*, 2n = 22) has been presently studied. Tapadar and Sen (1960), Tapadar (1964) had reported the same number in this genus from North India. Based on this scanty data it is not possible to make a valid conclusion on the basic chromosome constitution of the genus.

Of the cytologically known genera in the tribe Echitideae three genera showed x = 11, three showed x = 10, two showed x = 9, and two showed x = 6 as the basic chromosome situations. Though x = 11 predominates among the woody members of the tribe, the possibility of x = 6 situation can not be ruled out, since the lowest somatic number reported in the tribe Echitideae (In *Echites umbellata* and *Urechites lutea* by Fritsch, 1970; 1972) is 2n = 12. The basic number x = 11 could have been evolved by doubling of x = 6 followed by dropping of one chromosome within a pair.

Table 11 Chromosome numbers in the family Apocynaceae

Taxa	n	2n	Authority
Tribe : CARRISEAE			
Allamanda cathartica	9	18	Sugiura, 1936
	9		Pathak *et al.* 1949
	9	18	Kumar *et al.* 1952
	9	18	Sen and Tapadar, 1956
	9		Balamani and Rao, 1981
	9	18	Datta and Bhattacharya, 1981
	9	18	Present study
A. schottii	9	18	Tapadar and Sen, 1960
			Tapadar, 1964
	10		Bir and Neelam, 1980

		18	Laan and Arends, 1985
	9	18	Present study
A. neriifolia	9	18	Present study
A. violacea	18	36	Kumar *et al.* 1952
		36	Sen and Tapadar, 1956
	18	36	Present study
Carissa spinuram	11	22	Singh, 1951
	11		Chauhan and Raghuvanshi, 1977
	11		Balamani and Rao, 1981
	11		Bir *et al.* 1984a
		22	Present study
C. carandas	11		Singh, 1951
		22	Tapadar and Sen, 1960
			Tapadar, 1964
	11	22	Chauhan and Raghuvanshi, 1977
	11		Balamani and Rao, 1981
	11		Sanjappa and Dasgupta, 1981
		22	Present study
Tribe : PLUMERIEAE			
Rauvolfia serpentina	11	22	Raghavan, 1957
	12	24	Chandra, 1957
	11	22	Milovidov and Storchova, 1958
		20	Singh, 1961
	11	22	Tapadar, 1964
		22	Datta and Maiti, 1972
	11	22	Sharma and De, 1976
	11	22	De, 1979
	11		Bedi and Gill, 1982
	11		Laan and Arends, 1985

	11	22	Present study
R. tetraphylla		66	Raghavan, 1957
		88	Sharma and Sharma, 1957
		55, 68	Sharma and Sharma,1957
	33	66	Tapadar and Sen, 1960,
			Tapadar, 1964
		66	Datta and Maiti, 1972
	33	66	Sharma and De, 1976
		66	Gadella, 1978
	33	66	De, 1979
		66	Laan and Arends, 1985
		55	Present study
R. beddomei	66		Present study
R. densiflora		44	Present study
Thevetia peruviana	9		Pathak *et al.* 1949
	10		Hardas and Joshi, 1954
	10	20	Tapadar and Sen, 1960
	11		Nanda, 1962
	10		Tapadar, 1964
	10	20	Tapadar 1964
		20	Datta and Maiti, 1972
		20	Chauhan and Raghuvanshi, 1977
		20	Gadella, 1977
		20	Ugborogho, 1983
		20	Laan and Arends, 1985
		20	Present study
Cerbera odollam	20		Rau, 1940
		40	Laan and Arends, 1985
		40	Present study

Kopsia fruticosa	18		Tapadar, 1964
		36	Tapadar and Sen, 1960
		36	Laan and Arends, 1985
		36	Present study
Catharanthus roseus	8	16	Sugiura, 1931
	8	16	Pannochia-Laj, 1938
	8	16	Bowden, 1940, 1945
		16, 32	Janaki Ammal *et al.* 1963
	8	16	Tapadar, 1964
		24	Dnyansagar and Sudhakaran, 1970
		32	Datta and Maiti, 1972
	8		Gill and Abubakar, 1975
	8		Sarkar *et al.* 1975
	8		Koul *et al.* 1976
	8		Balamani and Rao,1981
C. roseus (variety 1)	8		Present study
C. roseus (variety 2)	8		Present study
Vinca major	23		Present study
Plumeria alba	18	36	Singh, 1951
	18	36	Kumar *et al.* 1952
	27	54	Tapadar and Sen, 1960
	18	36	Tapadar, 1964
		36, 54	Sharma, 1970
		44, 46	Datta and Maiti, 1972
		54	Datta and Maiti, 1972
	18	36, 54	Banerjee, 1974
		54	Raghuvanshi and Chauhan, 1974
	18	36	Present study
P. rubra	18		Singh, 1951

	18	36	Kumar *et al.* 1952
	18	36	Tapadar and Sen, 1960,
			Tapadar, 1964
		36	Sharma, 1970
		36	Datta and Maiti, 1972
	18		Bawa, 1973
		36	Banerjee, 1974
		36	Raghuvanshi and Chauhan, 1974
		36	Renard *et al.* 1983
		36, 45	Laan and Arends, 1985
	18	36	Present study
Alstonia scholaris	22	44	Tapadar and Sen, 1960
	20		Mehra, 1976
		44	Chauhan and Raghuvanshi, 1977
	11		Singh *et al.* 1982
	22	44	Present study
A. venenata	11		Singh *et al.* 1982
	11	22	Present study
Tabernaemontana dichotoma	11	22	Kumar *et al.* 1952
	11	22	Tapadar and Sen, 1960
			Tapadar, 1964
	11	22	Present study
T. divaricata		33	Kumar *et al.* 1952
	11	22, 33	Tapadar and Sen, 1960
			Tapadar, 1964
		22, 33	Raghuvanshi and Chauhan, 1969
		22	Raghuvanshi and Chauhan, 1970
		22	Datta and Maiti, 1972
		22, 33	Raghuvanshi and Chauhan, 1974

	11	22, 33	Sharma and De, 1976
	11	22, 33	De, 1978
		22, 33	Laan and Arends, 1985
		22, 33	Present study
T. divaricata (var. variegata)	11	22	Present study
Holarrhena antidysenterica	11	22	Sen and Tapadar, 1957
	11		Raghavan, 1959
	11		Tapadar and Sen,1960
		22	Datta and Maiti, 1972
	11		Sarkar *et al.* 1975
	11	22	Mehra, 1976
	11	22	Sharma and De, 1976
		22	Chauhan and Raghuvanshi, 1977
	11	22	De, 1978
	11		Bir *et al.* 1984a
	11		Present study
Vallaris solanacea	10		Rau, 1940
	10	20	Tapadar, 1964
	11		Bedi *et al.* 1980
	10		Present study
V. lancifolia	10		Beizenberg and Hair, 1983
	10		Present study
Parsonsia spiralis	9	18	Present study
Wrightia finctoria		20	Gajapathy, 1962
		20	Raman and Kesavan, 1963
	11	22	Tapadar, 1964
		22	Bir *et al.* 1984 b
		22	Present study
Strophanthus gratus		18	Beentje, 1982

		18	Arends and Laan, 1982
	9	18	Present study
S. wightianus		20	Wiktus, 1951
	9	18	Present study
Ichnocarpus frutescens		20	Gajapathy, 1962
	10		Present study
I. ovatifolius		40	Present study
Chonemorpha fragrans		20	Tapadar, 1964
	10	20	Present study
C. griffithii		30	Present study
Adenium obesum		22	Tapadar, 1964
		22	Laan and Arends, 1985
	11	22	Present study
Aganosma caryophyllata		22	Tapadar and Sen, 1960
		22	Present study

Primary basic number in Apocynaceae

Cytological data on different genera of the family Apocynaceae showed an array of basic chromosome numbers such as 6, 7, 8, 9, 10, 11, 12, 16, 18, 20, and 23, of which x = 11 is the most predominant number being present in 37 of the known genera (Table 12). Among the South Indian taxa reported here x = 11 is the most predominant one, and the other numbers exist singly or in combination with x = 11. This situation appears to be very much in favour of considering x = 11 as the most established constitution in the entire Apocynaceae. It is to be noted that such a higher number could hardly be the primary basic number for a large taxonomic group. Stebbins (1950) had suggested that only x = 10 or lower numbers are of primary origin, and all the others of secondary origin. Raven and Kyhos (1965), Ehrendorfer

et al. (1968) and Walker (1972) have suggested that angiosperms had monophyletic origin from progenitors with x = 7. Raven's (1975) suggestion regarding the origin of x = 11 is in accordance with Stebbin's (1971) hypothesis that the relatively higher gametic numbers or basic numbers of the woody dicotyledons are old polyploid numbers. Goldblatt (1980) has also suggested that almost all angiosperms with gametic numbers above 9 probably had polyploidy in the evolutionary history. Similarly Ehrendorfer *et al.* (1968) considered the basic number of 12, 13 and 14 to be monobasic or dibasic combination of the original x = 7, and its aneuploid derivative x = 6. Fritsch (1970, 1972) suggested that x = 6 could be the original number in the Apocynaceae, since the genera like *Echitinae* and *Urechitinae* possessed 2n = 12 in some members. But these two genera are more advanced than the primitive Carisseae, and Tabernaemontanae with x = 11. Grant (1982) has questioned this hypothesis by pointing out that x = 6 is rare in woody dicotyledons, while x = 7 as the original basic number is also less probable. As an alternative, he has proposed a long aneuploid series of higher basic numbers up to 14 or 15, which have evolved from some low original number lying possibly between x = 7 and 9.

Laan and Arends (1985) supported Raven's (1975) hypothesis of x = 7 as the ancestral basic numbers in the Apocynaceae, since this situation is predominant in most dicotyledonous families.

However, the occurrence of 2n = 12 in the *Echites* and *Urechites* discussed elsewhere appears to shed light on the possibility of x = 6 as the original basic number

from which x = 11 might have arisen by polyploidy followed by dropping of one chromosome.

The number x = 11 might have been evolved by doubling of x = 6 followed by the loss of one chromosome. The number 12 is either a multiple of 6 or an aneuploid derivative of 11. The number x = 16 which occurs very rarely in the family (*Montoniella*, 2n = 32) appears to be the product of union of gametes with n = 8, which occurs in the Plumerieae and also in the Apocyneae. In the related genera *Catharanthus* also the basic number is x = 8. It is postulated that x = 16, could have evolved by doubling of an ancestral x = 8 which in turn derived from x = 9 by dropping of one chromosome. The number x = 18 occurs in the four genera (*Allamanda, Himantanths, Pachypodium, Prestonia*) could be of polyploid origin from x = 9 which occurs in the related tribes Plumerieae. It is also possible to envisage x = 18 as a derived one from x = 6 also by polyploidy. The number x = 19 could be an aneuploid derivative of x = 18 as in *Stamandenia.* The number x = 20 in the case of *Cerbera* obviously could be a double of x = 10. The same gametic number x = 20 could be an aneuploid derivative of the polyploid condition based on x = 11. The number x = 23 is found exclusively in the genus *Vinca*, whose occurrence can be explained by considering it as an ascending aneuploid of x = 11, or in otherwords it can be considered as a derived number by the fusion of gametic numbers such as n = 11 and n = 12.

The general trend of descending aneuploidy from x = 11 in the Apocynaceae implies a reversal with respect to the evolutionary mechanism of aneuploidy. Thus the

number x = 10 might have been derived from x = 11 condition by descending aneuploidy. Likewise x = 9 and 8 might have been derived from the corresponding higher gametic numbers through descending aneuploidy and x = 12 might be due to ascending aneuploidy (9 ← 10 ← x = 11 → 12). The number n = 7 in the species *Stephanostema stenocarpum* (Rao and Mwasumbi, 1981) might be a derivation from x = 6 by ascending aneuploidy.

Table 12 Basic chromosome number of the family Apocynaceae

Genera	Basic chromosome number
Vinca	23
Cerbera	20
Alyxia	18
Mortoniella	16
Beaumontia	12
Odontadenia	12
Acokanthera	11
Adenium	11
Aganosma	11
Apocynum	11
Ancylobotrys	11
Anthoclitandra	11
Aphanostylis	11
Alstonia	11
Baissea	11
Callichilia	11
Dictyophleba	11
Diplorhynchus	11
Carissa	11

Farguharia	11
Funtumia	11
Holarrhena	11
Hunteria	11
Isonema	11
Landolphia	11
Mascarenhasia	11
Melodinus	11
Malouetia	11
Nerium	11
Ochrosia	11
Oncinotis	11
Orthopichonia	11
Picralima	11
Pleiocarpa	11
Pleioceras	11
Rauvolfia	11
Rhazya	11
Schizozygia	11
Stemmadenia	11
Stephanostema	11
Tabernaemontana	11
Tabernanthe	11
Voacanga	11
Vahadenia	11
Wrightia	11
Ichnocarpus	10
Ecdysanthera	10
Vallaris	10

Chonemorpha	10
Strophanthus	10
Thevetia	10
Trachaelospermum	10
Allamanda	9
Plumeria	9
Kopsia	9
Pachypodium	9
Parsonsia	9
Prestonia	9
Apocynum	8
Catharanthus	8
Stephanostema	7
Echites	6
Urechites	6

Polyploidy

Polyploidy is known to be the most wide spread cytogenetic process which has greatly contributed to species formation and evolution in higher plants (Stebbins, 1971). It is a dramatic mutational event in the process of evolution, has wide implications in nature and for the generation of new and improved types (Lewis, 1980a).It is a common phenomenon in plants and is of wide occurrence in some plant groups, and rare or absent in others.

According to Jones and Smith (1967) polyploidy clearly plays a part in initiating discontinuity both within and between species. The 'rhythmic highs and lows' in the frequency of angiosperm chromosome numbers are generally attributed to

polyploidy (Grant, 1982). Various aspects of this phenomenon such as the modes of origin and development of different types of polyploids, their cytogenetic behaviour and significance in speciation and evolution have been discussed and reviewed by many leading cytologists (Clausen *et al.* 1945; Darlington, 1937; 1956; 1963; Stebbins, 1947; 1950; 1971; Harlan and de Wet, 1975; Love, 1963; Gottschalk, 1978; de Wet, 1980; Jackson and Casey, 1980; Goldblatt, 1980; Tal, 1980; Ehrendorfer, 1980).

Various investigators have estimated the frequency of polyploids in flowering plants. According to the estimate made by Love (1963) there are about 30% polyploids in dicots, 50% in monocots and still higher percentage in pteridophytes. Subsequently Grant (1971) reported a higher estimate of 43% in dicots, and 58% in monocots. Stebbins (1971) has reported that about 30-35% of the flowering plants are straight forward polyploids fitting into polyploid series. Gustaffson (1948) pointed out that occurrence of polyploidy is higher in plants with efficient means of vegetative reproduction. Many genera and families of higher plants have had a polyploidy origin. Gottschalk (1985) has also estimated the incidence of polyploidy among the higher plants as 70%. The importance of polyploidy in evolution and speciation of plants has greatly been emphasized by Muntzing, 1936; Stebbins, 1940; 1950; 1971; 1980; de Wet, 1971; Jackson, 1976; Goldblatt, 1980 and Lewis, 1980a,b. Goldblatt (1980) was of the view that species with n = 11 or above probably many of those with n = 9 and 10 have polyploidy in their evolutionary history, and he estimated that 70-80 percent of the species of monocotyledons are thus polyploids. Lewis (1980a), while

considering x = 7 as the primary basic number for a majority of the dicotyledonae assumed that about 70% of the species are polyploids.

In the family Apocynaceae the incidence of polyploidy is relatively less frequent, being prevalent only in 9 out of the 60 cytologically known genera. This low incidence of polyploidy apparently gives an impression that this phenomenon has played relatively very little role in speciation and evolution in the family. Although the cytological data on the family Apocynaceae presented here is not comprehensive enough, the available information compares well with the general polyploidy situation in the family. Out of the 40 taxa studied from South India, 9 are polyploids (Table 13) at different levels (two triploids, five tetraploids, one pentaploid and one hexaploid). All the polyploid numbers noticed during the present investigation are met within woody climbers and shrubs. But Muntzing (1936), Stebbins (1938; 1950; 1971), Fagerlind (1944), Gustaffson (1947; 1948), Favarger (1967), and de Wet (1980) have concluded that polyploidy is comparatively more frequent in perennial herbs than in the annual and woody species. Kumari and Bir (1987) on the other hand indicated that the frequency of polyploidy is higher in trees, moderate in shrubs and very low in herbs. Stebbins (1971) estimated the frequency of polyploids to be highest in herbaceous perennials, lowest in annuals and intermediate in woody plants. The exact reason for the low frequency of polyploids in woody angiosperms is not clearly understood. Darlington (1965) and others have formerly advanced the hypothesis that polyploids are uncommon in woody plants, because increase in cell size associated with polyploidy decreases the efficiency of their cambial tissues. Stebbins (1971), has

discussed the various probable factors responsible for the lower incidence of polyploids in woody angiosperms, and has pointed out that the difference between chromosome number in woody and herbaceous genera is due to the ecological and historical factors rather than physiological or developmental.

As already pointed out, x = 11 could be the possible primary basic number in the Apocynaceae from which the other lines have originated mostly through polyploidy and aneuploidy at different polyploid levels. Stebbins (1950,. 1971) has suggested that polyploidy in woody angiosperms is related to the incidence of newly opened habitats, and therefore has occurred sporadically since the cretaceous period.

Speciation through polyploidy has been observed in 9 out of 40 taxa studied during this investigation. Polyploid numbers in the presently studied genera (*Allamanda, Rauvolfia, Kopsia, Alstonia, Tabernaemontana, Ichnocarpus* and *Chonemorpha*) suggest that polyploidy has been a factor in the origin of new species and varieties in this genera.

The role of polyploidy in the origin of new types are seen in few genera. Among the species of *Allamanda, A. violacea* with 36 chromosomes in the root tips is a tetraploid while all the others studied so far have 18 chromosomes. *A. violacea* can be readily distinguished from the rest of the species in having smaller but thick leaves, larger, but fewer stomata per unit area, hairiness and wine-coloured flowers.

In the genus *Rauvolfia*, a series of polyploids were investigated, such as *R. serpentina* (2n = 22), *R. densiflora* (2n = 44), and *R. tetraphylla* (2n = 55), R. canescens (2n=88), Sharma and sharma (1957) also detected the same polyploid in

the genus *Rauvolfia.* Tapadar and Sen (1960); Tapadar (1964), Datta and Maiti (1972), De (1979); and Laan and Arends (1985) detected a hexaploid taxon *R. tetraphylla,* 2n = 66, from North India.

Kopsia fruticosa is a tetraploid taxon with 36 chromosomes in its somatic complement. Cytological reports on another species *K. arborea* showed 2n=72. Tapadar and Sen (1960) and Laan and Arends (1985) have reported tetraploid species from different geographical regions.

In the genus *Tabernaemontana,* a triploid species (*T. divaricata,* 2n = 33) was observed. It is considered to be a polyploid when compared with *T. divaricata* having 22 chromosomes. Compared to its diploid species studied, the leaves are wider, thicker, and dark green in colour. The flowers are much larger with 2-3 whorls of petals. The scanty pollen grains available are small in size and all sterile. The gigas character associated with the triplication of chromosome set may be an indication of the active role of polyploidy in this genus. A perusal of the literature also revels the occurrence of triploid species (2n = 33) in this genus from other geographical regions Tapadar and Sen, 1960; Tapadar, 1964; Raghuvanshi and Chauhan, 1974; De, 1978; and Laan and Arends, 1985).

Alstonia scholaris is a tetraploid taxon with 44 chromosomes in its somatic complement. The leaves are smaller, and 5-7 leaves per whorl in comparison with diploid species *A. macrophylla* (2n = 22). Tapadar and Sen, (1960), Chauhan and Raghuvanshi, (1977) and Laan and Arends (1985) also reported the tetraploid species from different geographical regions.

As regards the genus *Ichnocarpus* the chromosome data show a striking instance of very little polyploidy. In the present study one triploid species, *I. ovatifolius* (2n = 30) was observed. Compared to its diploid species (n = 10) studied the leaves are wider, thicker and dark green in colour in the triploids.

Available cytological data on the genus *Chonemorpha* appears to show that polyploidy has played very little role in the evolution of the genus. One species *C. griffithii* (2n = 30) studied during the present investigation is a triploid taxon. However, due to the lack of evidence from their meiotic behaviours and karyomorphology no suggestion can be made regarding the genome.

Of the three cytologically known tribes from South India, polyploids are met within five species belonging to the tribes Plumerieae (*Alstonia scholaris* (2n = 44), *Tabernaemontana divaricata* (2n = 33), *Rauvolfia densiflora* (2n = 44), *R. tetraphylla* (2n = 55), *R. beddomei*, (n = 66); and *Kopsia fruticosa*, two species in Echitideae (*Ichnocarpus ovatifolius* 2n = 30 and *Chonemorpha griffithii*, 2n = 30); and only one species in Carisseae (*Allamanda violacea*, 2n = 36). In this respect the tribe Plumerieae can be considered as more advanced from evolutionary point of view than the other tribe, Echitideae and Carisseae.

The frequency of polyploid species and varieties in the family Apocynaceae has been estimated to be only 16% among 188 species investigated so far from different geographical regions. Tapadar (1964) estimated the frequency of polyploid species and varieties of Apocynaceae from North India as 12.5%. Laan and Arends (1985) also estimated very low percent of polyploids. Out of the 40 taxa cytologically

studied 18 percent of them are polyploids. Thus, it appears that in the family Apocynaceae, polyploidy has played some role in speciation during the course of evolution in this geographical region also.

Table 13 Incidence of polyploidy in different taxa of the Apocynaceae

No.	Name of taxa	Basic number	n	2n	Level of ploidy
1	*Allamanda violacea*	9		36	4x
2	*Rauvolfia tetraphylla*	11		55	5x
3	*Rauvolfia beddomei*		66		6x
4	*Rauvolfia densiflora*	11		44	4x
5	*Alstonia scholaris*	11		44	4x
6	*Tabernaemontana divaricata*	11		33	3x
7	*Ichnocarpus ovalifolius*	10		40	4x
8	*Chonemorpha fragrans*	10		30	3x
9	*Kopsia fruticosa*	9		36	4x

Infrageneric and infraspecific polyploidy

The occurrence of more than one euploid type with in a taxonomic species has been reported in many plant groups. Such differences in chromosome number with notable differences in morphology (Kliphius, 1967; Vijayavalli and Mathew, 1986), and when they do so, the phenomenon can be considered to bring about speciation. Chromosome data on the family Apocynaceae (Table. 11) show that a number of species complexes occur in the family. Although most of the species of this family reported here are studied from different localities, infraspecific polyploidy was observed only in one species (*Tabernaemontana divaricata*). However, in the case of many other species, chromosome data from different geographical regions show that many of them exist in two or more cytotypic forms (Table 14). As regards

Tabernaemontana divaricata, which is widely distributed in the different geographical regions, there exists two cytotypic form (diploid 2n = 22) and triploid (2n = 33) in South India and elsewhere in the Indian sub-continent. The triploids differ recognizably from the diploids in morphological features. They have larger and thicker leaves, and the flowers are larger with many whorls of petals (Table 15). This low figure corroborates with the conclusion of Stebbins (1971) that cytotypes (chromosomal races) within taxonomic species of the tropics are rare. Polyploid cytotypes of the above mentioned species is of an autopolid nature.

Table 14 Incidence of infrageneric polyploidy in different genera of Apocynaceae

No.	Genera	Chromosome number
1	*Allamanda*	2n = 18, 36
2	*Rauvolfia*	2n = 22, 44, 55
3	*Tabernaemontana*	2n = 22, 33

Table 15 Incidence of infraspecific polyploidy in varieties of Tabernaemontana

Varieties	Chromosome number	Leaves		Flower	Sterility (%)
		Length(cm)	Width(cm)	No. of petals	
Variety 1	2n = 22	11 – 12	3.5-4.5	5	96
Variety 2	2n = 22	12 – 13	4.0-4.5	5	95
Variety 3	2n = 22	14 – 14.5	5.0-5.5	10	90
Variety 4	2n = 22, 33	17 – 17.5	6.0-6.5	11-13	95

Karyotype evolution

The study of the morphology of chromosomes has gained a new sphere of usefulness as a tool in taxonomy. Karyotype studies have been useful in addressing systematic and evolutionary issues in diverse genera of flowering plants. Several authors (Jakson, 1971; Moscone, 1989; Bernardello and Anderson, 1990; Ruas *et al.* 1991; Bernadello *et al.* 1994) have shown the importance of both chromosome numbers and karyotype analysis in plant systematics. The karyotype may undergo evolutionary changes and that the trend of karyotype evolution may be towards increasing symmetry or asymmetry. The evolution of karyotype is also a continuous process and as Jones (1978) has pointed out its trend is difficult to discern by observing the process from any one point of view or at any point in time. Trends in karyotype evolution according to him can be brought out only by synthetic approaches taking into consideration all the factors involved along with morphology of very closely related species or taxa. When the plant materials are suitable for detailed karyotype analysis, the data can render in formation of considerable evolutionary and taxonomic significance (Love and Love, 1975). Karyotype characteristics can be a useful guide to assessing taxonomic relationships and evolutionary trends. Variation in the chromosome number and form in closely related groups are of great interest and important in the karyotype evolution. Karyotype is characterised in general by the number and size of the chromosomes, position of centromere, secondary constriction, NOR, the distribution of euchromatin and heterochromatin and DNA content.

Detailed karyomorphological studies have been made in six species and four varieties under two tribes from South India. The karyotype details are summarised in

table 16. Obviously this data is not large enough to be used for a critical discussion of karyotype evolution of the tribes. However, this information imparts a general idea about the pattern of karyotype evolution in the group, and also particularly the possible trend of karyotype variability between species of some of the genera are reported here.

Table 16 Karyotype formulae in the members of Apocynaceae from South India

Sl. No.	Taxa	Karyotype formula	Karyotype category
	Tribe 1 CARISSEAE		
1	*Allamanda cathartica*	2n = 18 = 8m + 4sm + 6st	2A
2	*Allamanda schottii*	2n = 18 = 4m + 8sm + 6st	2A
3	*Allamanda neriifolia*	2n = 18 = 6m + 4sm + 8st	2B
4	*Carissa carandas*	2n = 22 = 6m + 16sm	1B
	Tribe 2 PLUMERIEAE		
	Sub tribe 2 Cerbereae		
5	*Thevetia peruviana* (var. 1)	2n = 20 = 4m + 16m	1B
6	*Thevetia peruviana* (var. 2)	2n = 20 = 2M + 18m	1B
	Sub tribe 4 Tabernaemontanae		
7	*Tabernaemontana divaricata* (var.1)	2n = 22 = 2m + 20sm	2A
8	*Tabernaemontana divaricata* (var.2)	2n = 22 = 2m + 20sm	2A
9	*Tabernaemontana divaricata* (var.3)	2n = 22 = 2m + 20sm	2A
10	*Tabernaemontana divaricata* (var.4)	2n = 33 = 3m + 30sm	2A

1. Chromosome size

In flowering plants, phylogenic decrease or increase in chromosome size are equally frequent and the process probably reversible (Stebbins, 1950). It is generally

considered that in angiosperms, with respect to growth habit, evolutionary advancement has proceeded from woody to herbaceous annual habit through shrubs and herbaceous perannials (Stebbins, 1950; Cronquist, 1968). Babcock and Cameron (1934) have reported association of reduction in chromosome size with annual growth habit and reduction in size of all parts of the involucre and flowers in some genera of the Compositae. Subsequently a few others (Mathew and Mathew, 1976; Mathew and Mathew, 1983) have reported genera of this family in which woody taxa had larger, annual herbs smaller, and herbaceous perennials intermediate chromosome size.

Variations in chromosome size was noticed within and between the genera of the presently studied taxa. In the tribe Carisseae two genera (*Allamanda* and *Carissa*) are subjected to karyotype analysis. From the genus *Allamanda,* three species such as *A. cathartica, A. schottii* and *A. neriifolia* have been studied. These species possess chromosomes of intermediate size. Among these three species *A. cathartica* shows somewhat large chromosome size (2.50μm – 4.50μm) (Table 17). The chromosome size of *A. schottii* ranged from 1.75μm – 3.00μm and *A. neriifolia* from 1.37μm – 4.25μm (Table 17). *Carissa carandas*, the only one species in the genus *Carissa* showed intermediate chromosome size (1.50μm – 2.50μm)(Table 17). Their absolute chromosome size also varied (Text Fig.11).

Four varieties of the genus *Tabernaemontana divaricata* have been studied. All varieties showed medium size chromosomes. The chromosome size ranged from 2.75μm – 3.75μm in variety 1 from 2.75μm – 4.25μm, in variety 2 from 2.75μm –

4.74μm, in variety 3, and 2.75μm – 3.75μm in variety 4 (Table 17). Absolute chromosome size also varied significantly (Text Fig.11) in them.

Being a family of mostly woody climbers, shrubs or trees, the chromosomes are relatively small which vary in length from about 1.37μm to 4.75μm. Tapadar (1964) also noticed that the chromosome in this family show relatively small size in the materials from North India. Stebbins (1938) pointed out the correlation between chromosome size and characteristic of external morphology. Here the karyomorphologically studied species belong to shrubs. Among the six species and four varieties studied the longest chromosome has shown only 4.74μm in length. Thus, it appears that in Apocynaceae – a family of mostly woody climbers, shrubs or trees, the chromosomes are relatively small in size (Table 17; Text Fig.11).

During the present study it has been noticed that same chromosome number exists in different species under the same genus, with varying chromosome size and karyotype categories. Three species of *Allamanda* showed varying chromosome size. Their ACL and TCL values were also different (Table 17). Similarly four varieties of *Tabernaemontana divaricata* showed the same chromosome number (var. 1, 2 and 3) but they slightly differ in their chromosome size (Table 17).

Tapadar (1964) reported that in several genera with constancy in chromosome number, karyotype vary from species to species in the family Apocynaceae. Sharma and Sharma (1959) while analysing the chromosome morphology in different species and varieties of plants, observed that often they differ from each other in minute

details of their karyotype. The chromosomes in the presently studied members are provided with mostly median to submedian primary constriction.

Table 17 Chromosome size in members of the tribe Carisseae and Plumerieae of Apocynaceae

Sl. No.	Taxa	Basic number	Chromosome size range (μm)	Chromosome size range (μm)	
				ACL (μm)	TCL (μm)
	Tribe 1 CARISSEAE				
1	*Allamanda cathartica*	9	2.50 – 4.50	3.20	28.75
2	*A. schotti*	9	1.75 – 3.00	2.44	21.99
3	*A. neriifolia*	9	1.37 – 4.25	3.00	26.74
4	*Carissa carandas*	11	1.50 – 2.50	2.40	21.25
	Tribe 2 PLUMERIEAE				
	Sub tribe 2 Cerbereae				
5	*Thevetia peruviana* (var. Yellow)	10	1.40 – 3.00	2.00	18.90
6	*T. peruviana* (var. White)	10	1.40 – 3.00	2.18	21.80
	Sub tribe 4 Tabernaemontaneae				
7	*Tabernaemontana divaricata* (var. 1)		2.75 – 3.75	3.10	33.97
8	*T. divaricata* (var. 2)	11	2.75 – 4.25	3.00	32.23
9	*T. divaricata* (var. 3)	11	2.75 – 4.74	3.20	34.86
10	*T. divaricata* (var. 4)	11	2.75 – 3.75	3.20	34.86

2. Karyotype asymmetry

The information about karyotypes have been known to be useful for assessing the relationship in many plant taxa, where association occurs between increasing karyotype asymmetry and their characteristics such as plant morphology and plant habits. In order to facilitate an effective evaluation of such association, Stebbins (1958) have classified the karyotype asymmetry into 12 categories (1A – 4C). It may be seen that out of the ten taxa, whose karyomorphology has been analysed here six showed 2A type, one 2B type and three 1B type chromosomes.

Levitzky (1931) suggested that the chromosomes become more asymmetrical as evolution progressed. Stebbins (1950; 1971) supported the above hypothesis and suggested that in flowering plants symmetrical karyotypes are primitive, and the trend in karyotype evolution is towards increasing asymmetry. According to Jones (1970; 1978; 1984) the direction of chromosomal evolution can be from symmetry to asymmetry or vice-versa depending on the state of karyotype in the evolving taxon. Stebbins (1974) and Moore (1978) had reported that the karyotype is varied in the degree of symmetry among the plant genera and species. Symmetrical karyotypes are considered more primitive than asymmetrical ones. The evolution of latter from the former has been recorded in several genera, for example *Crepis* (Babcock 1947), *Clarkia* (Lewis and Lewis 1955), *Leontodon* (Rousi 1973), *Crotalaria* (Gupta and Gupta 1978), *Phaseolus* (Sarbhoy 1980).

According to Stebbins (1971), karyotypes with chromosomes of similar size and possessing median to sub median centromeres are considered symmetrical. Asymmetrical karyotype consists of many chromosomes with sub terminal centromeres and great size difference between the chromosomes. During the present study most of the taxa showed chromosomes with not much difference in size, and possessing median and sub median centromeres. According to Rees (1984), the chromosome complements become more symmetrical with increasing nuclear DNA content. It has also been shown that taxa with higher DNA content are usually more advanced than their relatives.

Karyotype with less asymmetry (2A) has been observed in 2 taxa of *Allamanda* and 4 taxa of *Tabernaemontana.* One species of *Allamanda,* (*A. neriifolia*) showed relatively more asymmetrical (2B) karyotype. Thus it may be more advanced species in the genus *Allamanda.*

3. Infrageneric karyotype variation

In the genus *Allamanda,* the three taxa studied like *A. cathartica, A. schottii,* and *A. neriifolia* possess specific karyotype variations, which indicates the importance of chromosome repatterning mechanism in the genus. *A. neriifolia* with 2B karyotype category is more asymmetrical (Text Fig.12) than the other two species (*A. cathartica* and *A. schottii*) which belong to less asymmetrical karyotype category (2A). The ACL value (Text Fig.11) and TF% of *A. neriifolia* (35.97) also varied from *A. cathartica* (40.86) and *A. schottii* (40.35).

Here *A. cathartica* possess 8m-type, 4sm-type and 6st-type chromosomes, while *A. schottii* possess 4m-type, 8sm-type and 6st-type chromosomes. But *A. nerifolia* showed 6m-type, 4sm-type, and 8st-type chromosomes. This indicates that recognizable morphological changes of chromosomes involving shift in centromere positions have occurred during species differentiation in this genus (Table 18).

Table 18 Comparison of karyotype features of three taxa of the genus *Allamanda*

Taxa	Chromosome number (2n)	Frequency of chromosome types					ACL	TF%	Karyotype category
		M	m	sm	st	t			
Allamanda cathartica	18	-	8	4	6	-	3.20	40.86	2A
A. schottii	18	-	4	8	6	-	2.44	40.35	2A
A. neriifolia	18	-	6	8	8	-	3.00	35.97	2B

Infraspecific karyotype variation

Infraspecific karyotype variation is known to be an important evolutionary mechanism as it can lead to species differentiation (Stebbins, 1950). Instances of such recognizable variations, mostly due to deletion in chromosome arms resulting in chromosome size heteromorphism in the species such as *Thevetia peruviana* and *Tabernaemontana divaricata* have been noticed during the present study.

Thevetia peruviana : In variety 1 (2n = 20) the karyotype formula is 4M + 16m,but in variety 2 (2n=20) it is 2M + 18m. M-type and m-type chromosomes occur in both varieties, but M-type is predominant in variety 1. Similarly ACL (Text Fig. 11) and TF% values also vary in the two varieties (Table 19; Text fig.13).

In four varieties of *Tabernaemontana divaricata,* the karyotype details disclose the same type of chromosomes in all the varieties, except the triploid one. Based on the morphological differences the four varieties are named as variety 1, 2, 3 and 4 respectively. These varieties showed considerable variations in their flower characters. The varieties 1, 2 and 3 were true diploids (2n = 22), and the fourth variety is a triploid taxon based on the basic chromosome number x = 11 (Table 20). In all the four varieties karyotype formula is same (Table 16; Text Fig.14).

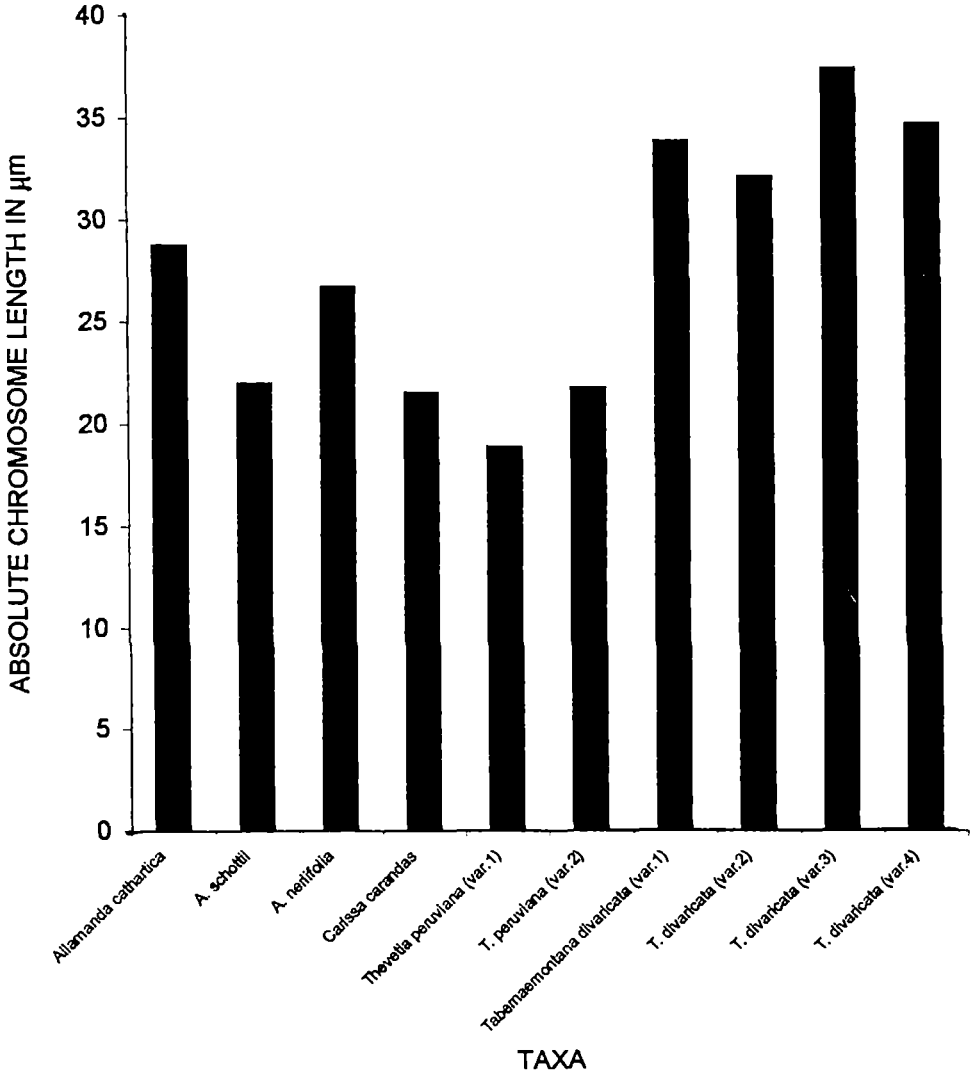
Table 19 Comparison of karyotype features of two varieties of *Thevetia peruviana* (2n = 20)

Taxa	Chromosome number (2n)	Frequency of chromosome types					ACL	TF%	Karyotype category
		M	m	Sm	St	t			
Thevetia peruviana (var. 1)	20	4	16	-	-	-	2.00	44.9	1B
Thevetia peruviana (var. 2)	20	2	18	-	-	-	2.18	43.57	1B

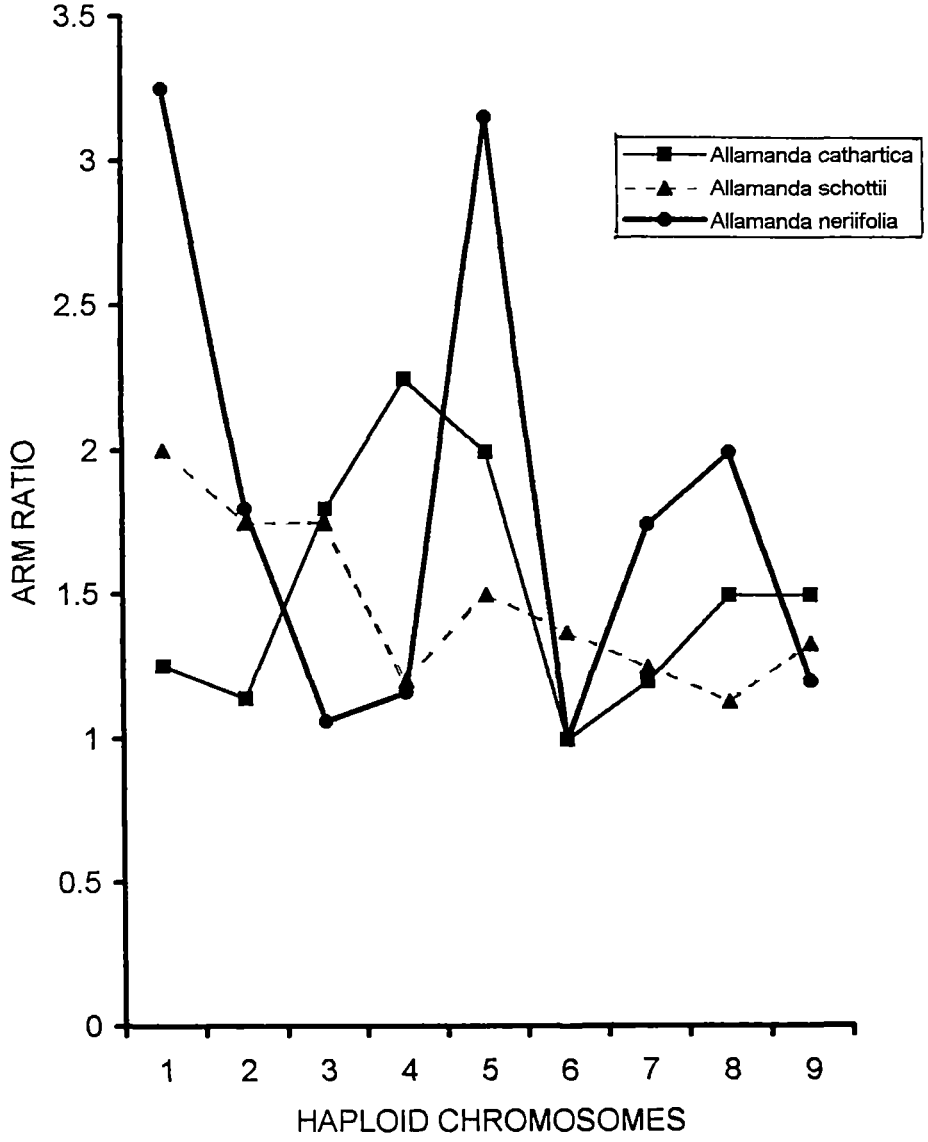
Table 20 Comparison of karyotype features of four varieties of *Tabernaemontana divaricata*

Taxa	Chromosome number (2n)	Frequency of chromosome types					ACL	TF%	Karyotype category
		M	m	Sm	St	t			
Tabernaemontana divaricata (var. 1)	22	-	2	20	-	-	3.10	34.53	2A
T. divaricata (var. 2)	22	-	2	20	-	-	3.00	39.03	2A
T. divaricata (var. 3)	22	-	2	20	-	-	3.41	43.47	2A
T. divaricata (var. 4)	33	-	3	30	-	-	3.16	34.39	2A

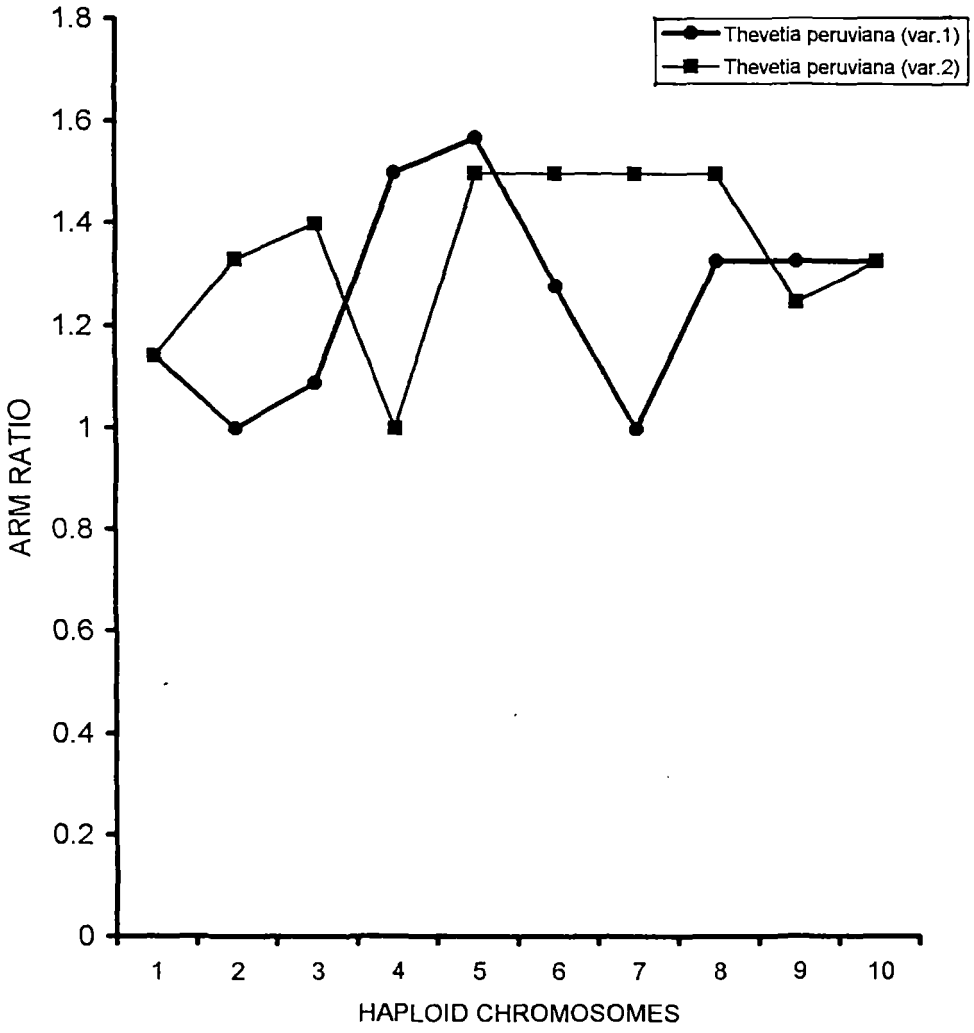
TEXT FIG.11. HISTOGRAM SHOWING THE ABSOLUTE CHROMOSOME LENGTH OF THE PRESENTLY STUDIED TEN TAXA



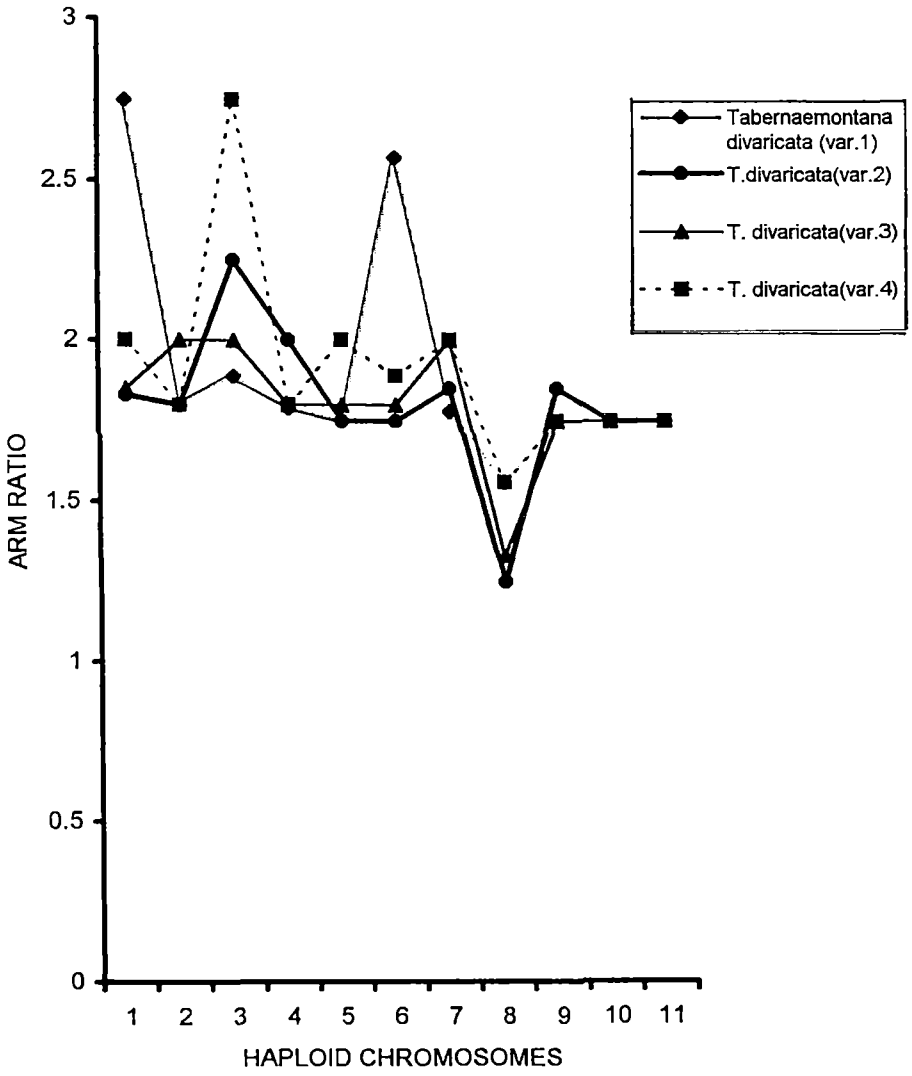
TEXT FIG. 12. KARYOMORPHOLOGY OF THREE DIPLOID SPECIES OF *ALLAMANDA* SHOWING ARM RATIO (LONG ARM/ SHORT ARM)



TEXT FIG. 13. KARYOMORPHOLOGY OF TWO VARIETIES OF *THEVETIA PERUVIANA* SHOWING ARM RATIO (LONG ARM/SHORT ARM)



TEXT FIG. 14. KARYOMORPHOLOGY OF FOUR VARIETIES OF *TABERNAEMONTANA DIVARICATA* SHOWING ARM RATIO



Part – II

PALYNOLOGICAL STUDIES

INTRODUCTION

The term 'pollen' was first introduced by the great Swedish Botanist Linnaeus (cf. Knox 1979), it forms a vital unit in the biology of reproduction in plants. They are having characteristic structural organisation and functional behaviour. During the eighteenth and nineteenth centuries a large volume of scattered information on pollen characters have accumulated. But the publication of book 'Pollen grains' by Wodehouse (1935) may be considered to mark beginning of the contemporary history of pollen study as a Science. Following this Erdtman (1952) laid a strong foundation for the pollen studies with the publication of his book 'Pollen Morphology and Plant Taxonomy\Angiosperms' which is an authentic record, give the importance of pollen morphology in plant taxonomy. Hyde and Williams (1945) coined the term, palynology to cover all aspects of pollen grains and spores. The word palynology is derived from a Greek word 'palynein' which means to scatter meal or powder. Palynology is now a powerful tool that can be applied to issues of global concern, such as the modelling of climatic change (Van Campo *et al.* 1990). The significance of the study of pollen morphological features in oil exploration, apiculture, archaeology, medical science, paleogeography, criminology etc. has received wide attention.

Major palynological characters in the order of their importance in taxonomic and phylogenetic considerations are the germinal aperture (its form, number, position and distribution), exine ornamentation, pollen shape and size (Nair 1970a) Of these, the aperture characteristics are considered to be of prime importance because of its

evolutionarily conservative nature, which helps to the identification and delimitation of taxa, especially at the generic and lower levels.

The introduction of Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) were proved to be useful tools for palynological studies with increased accuracy and precision. SEM has helped to unravel the subtle details of the exine surface and TEM helps, the minute details of exine stratification. This has opened up possibilities for better understanding of the exine ornamentation pattern and enabled application of subtle exine features in studies involving systematic relationships of microtaxa, particularly, sub species, varieties, cultivars, cytotypes, bioforms etc. (Ravikumar and Nair 1985). A few authors have discussed the potential of palynological data to contribute to higher-level systematics (Nowicke and Skvarla, 1980), most taxonomic application have focussed relationships below the family level, between species or genera.

Pioneering studies on pollen morphology of the Apocynaceae was done by Nilsson (1986, 1990). Nilsson *et al.* (1993) suggested the relationship of the Apocynaceae and Periplocaceae. Cousin (1979) studied the tapetum and pollen grains of *Vinca rosea*, El-Ghazali and Nilsson (1990) reported development of tapetum and orbicules in this species. Palynotaxonomic studies in *Tabernaemontana* was studied by Van Campo *et al.* (1979). Pollen grains of *Parsonsia* was done by Sampson and Anusarnsunthom (1990). Pollen grains of *Rauvolfia* was studied by Nair and Koul (1965), Dnyansagar (1965) and Pasha and Roy (1980). Nayar (1990) constructed a key to this family on the basis of pollen characters. Thanikaimoni

(1962), Marques and Melbem (1966) Huang (1972, 1986) Allorge (1975) were studied the morphological features of pollen grains in this family. Kuijt and Vander Ham (1997) studied the pollen morphology of *Alstonia*.

The present study covers pollen morphology of 37 taxa belonging in 19 genera of 3 tribes. Data presented here are mostly based on light microscopic studies. SEM observations have been made in 16 taxa. The available palynological data on the group has been used to discuss the palynological evolution in the family. A pollen key of the taxa studied here is also proposed based on the aperture morphoforms, pollen shape and size. A discussion is presented in which the systematic relationships of the family are considered in the light of evidences from palynological study.

MATERIALS AND METHODS

Mature pollen-bearing fresh flowers were collected at the time of anthesis. Pollen grains were then preserved immediately in vials containing glacial acetic acid or 70% alcohol. Each vial was numbered and proper records were maintained.

Pollen samples for the study were prepared according to the modification suggested by Nair (1970a) to the acetolysis method proposed by Erdtman (1952). The main schedule is given below.

1. The preserved pollen materials in 70% alcohol was transferred to plastic centrifuge tube and to release the pollen from the mature buds, it was crushed using a glass rod. The dispersion was passed through a mesh having 40 divisions per sq.cm. and the pollen containing alcohol was collected in glass centrifuge tube and centrifuged alcohol decanted and about 5 ml of glacial acetic acid added to the sediment and centrifuged.
2. The supernatant solution has been decanted and the pollen sediment has been covered with the acetolysis mixture, freshly prepared every time, consisting of nine parts of acetic anhydride and one part of concentrated sulphuric acid, in order to maintain a starting temperature of 70°C.
3. To disperse the pollen sediments in the acetolysis mixture, it has been stirred well at intervals using glass rod in each centrifuge tube. These tubes were transferred to a water bath and heated from 70°C to boiling point of water and then the flame was put off leaving the tubes in the hot water for three to five minutes till a brown

colour was attained by the acetolysis mixture, thus ensuring total dissolution of the pollen protoplasm.

4. The dispersion was centrifuged and the supernatant mixture was decanted. The pollen sediment was washed with glacial acetic acid followed by decantation of the supernatant acid. The pollen sediment was washed with distilled water two or three times followed by decantation each time and finally the sediment was brought into 50% glycerine.
5. After about 10-15 minutes, the glycerine containing pollen was centrifuged, glycerine decanted, and the tubes containing the sediments were kept upside down on a filter paper to drain of excess glycerine.
6. For slide preparation, a clear slide was placed on the table, followed by catching a bit of pollen sediment from the centrifuge tube held upside down, on a pellet of glycerine jelly carried on a needle, and transferred to the slide.
7. The slide was warmed on a hot plate of melted jelly, followed by mounting with a cover glass and sealed by running molten paraffin wax in the space around the glycerine jelly. The excess was removed by cleaning the slide with xylol. A label with relevant information such as slide number, name of the plant and family was pasted on the slide.
8. These permanent slides prepared were used for the Light Microscopic (LM) studies. Olympus BH-2 Research Microscope was used for Light Microscope (LM) analysis and almost all measurements were made under oil immersion

(100x) using an ocular (16) of which one division, after calibration, amounted to 1μm.

For Scanning Electron Microscopic Studies (SEM), the acetolysed grains preserved in 70% alcohol were washed, dried and mounted on specimen tubes. They were vacuum coated with carbon, followed by gold-palladium (Walker 1974).

The description of exine ornamentation patterns, Nair's (1970a) terminology was used. The terminology proposed by Walker and Doyle (1975) was used relating to pollen-unit, aperture type, size and shape of pollen grains.

Average pollen size was measured from a random sample of 50 grains in each taxon. Mean ($\bar{X}$) of pollen size, (for both polar (P), and equatorial (E) views) were calculated using the formula

$$\bar{X} = \frac{\Sigma f_i x_i}{n}$$

where f_i = frequency of pollen size

x_i = size of the pollen grains in μ for the ith size

n = total no. of grains = Σf_i

In the case of spherical grains only the radial diameter (E) was taken. The size classes of pollen have been determined following the classification proposed by Walker and Doyle (1975) as shown below.

Pollen size classes according to Walker and Doyle (1975)

Size class	Longest axis
1. Minute grains	< 10 μm
2. Small grains	10 – 24 μm
3. Medium sized grains	25 – 49 μm
4. Large grains	50 – 99 μm
5. Very large grains	100 – 199 μm
6. Gigantic grains	> 200 μm

Walker and Doyle (1975) recognized the shape classes based on the P/E ratio as below:

Shape class	Ratio P/E
Prolate	
Perprolate	≥ 2.00
Euprolate	1.34 – 1.99
Subprolate	1.15 – 1.33
Prolate-spheroidal	1.01 – 1.14
Spheroidal	1.00
Oblate	
Oblate-spheroidal	0.88 – 0.99
Suboblate	0.76 – 0.87
Euoblate	0.51 – 0.75
Peroblate	≤ 0.50

In the present study shape classes of pollen have been determined by the above classification.

Morphological features analysed here include pollen aperture, exine ornamentation, pollen size and shape using Light Microscope (LM) and in some of the taxa (16 taxa) with Scanning Electron Microscope (SEM). Photomicrographs of pollen grains of all the taxa studied have been provided. The slides of pollen of all the taxa are kept in the Department of Botany, Kerala University. Scanning Electron Microscopic pictures were taken from the Life Science Department, Kamaraj University, Madurai.

The different pollen morphological characters analysed are presented in the following sequence.

Aperture

Exine ornamentation

Exine thickness

Pollen shape

Pollen size class

Pollen size measurements (range, mean ($\bar{X}$))

OBSERVATIONS

TRIBE I CARISSEAE

Allamanda Linn.

A. cathartica Linn.

Grains 3-zonocolporate, ora circular to lalongate. Exine surface rugulate, rugula with tip nodules (LM). Exine thickness 2μm. Grains large, subprolate. Grain size (range 64-112μm x 64-96μm), $\bar{X}$ = (90.88μm x 72.96μm).

(Figs. 76 – 79)

A. schottii Pohl.

Grains 3-zonocolporate, ora circular to lalongate. Exine surface faintly foveolate (LM). Exine thickness 2μm. Grains large, subprolate. Grain size (range 80-96μm x 80μm), $\bar{X}$ = (93.44μm x 80μm).

(Fig. 80)

A. neriifolia Hook.

Grains 3-zonocolporate, ora circular to lalongate. Exine surface rugulate, rugula with tip nodules (LM). Exine thickness 2μm. Grains large, subprolate. Grain size (range 48-96μm x 48-80μm), $\bar{X}$ = (77.44μm x 64.64μm).

(Figs. 81 – 83)

***A. violacea* Gardn. and Field.**

Grains 3-zonocolporate, ora circular to lalongate. Exine surface rugulate-nodulate (LM). Exine thickness 2μm. Grains large, prolate-spheroidal. Grain size (range 64-80μm x 64μm), $\bar{X}$ = (71.04μm x 64μm).

(Figs. 84 – 85)

***Carissa* Linn.**

***C. spinarum* Linn.**

Grains 3-zonocolporate with tapering ends, aperture floor with ornamentation, ora circular to lalongate. Exine surface granulate-perforate or punctate (SEM). Exine thickness 1μm. Grains medium sized, prolate-spheroidal. Grain size (range 32-40μm x 24-32μm), $\bar{X}$ = (32.64μm x 30.08μm).

(Figs. 86 – 87)

***C. carandas* Linn.**

Grains 3-zonocolporate with tapering ends, aperture floor with ornamentation, ora circular to lalongate. Exine surface scrobiculate (LM). Exine thickness 1μm. Grains medium sized, prolate-spheroidal. Grain size (range 32-48μm x 32-48μm), $\bar{X}$ = (47.36μm x 41.60μm).

(Figs. 88 – 89)

TRIBE II PLUMERIEAE

Subtribe 1 Rauvolfieae

***Rauvolfia* Plum. ex. Linn.**

***R. serpentina* Benth. ex. Kurz.**

Grains 3-zonocolporate, endoexine as nodules, thickening (kidney shaped) at the colpus region, behind the pseudocolpi, ora lalongate. Exine surface reticulate (LM). Exine thickness 1μm. Grains large, prolate-spheroidal. Grain size (range 80-96μm x 64-80μm), $\bar{X}$ = (84.48μm x 77.44μm).

(Figs. 90 – 94)

***R. tetraphylla* Linn.**

Grains 3-zonocolporate, ora circular to lalongate. Exine surface reticulate (LM). Exine thickness 1μm. Grains medium sized, euprolate. Grain size (range 32-48μm x 32μm), $\bar{X}$ = (46.08μm x 32μm).

(Figs. 95 – 98)

***R. beddomei* Hook. f.**

Grains 3-zonocolporate, ora circular to lalongate. Exine surface reticulate (LM). Exine thickness 1.5μm. Grains medium sized, prolate-spheroidal. Grain size (range 24-32μm x 24-32μm), $\bar{X}$ = (31.68μm x 28.48μm).

(Figs. 99 – 100)

***R. densiflora* Benth. ex Hook. f.**

Grains 3-zonocolporate, endoexine as nodules, thickening at the colpus region, ora lalongate. Exine surface reticulate (LM). Exine thickness 2.5μm. Grains very

large, prolate-spheroidal. Grain size (range 96-128μm x 96-112μm), $\bar{X}$ = (109.44μm x 106.24μm).

(Figs. 101 – 102)

Subtribe 2 Cerbereae

***Thevetia* Linn.**

***T. peruviana* (Pers.) K. Schum.**

(= *T. neriifolia* Juss. ex. Stend.) (variety 1)

Grains 3-zonocolporate, ora lalongate. Exine surface microreticulate (LM). Exine thickness 1.5μm. Grains large, prolate-spheroidal. Grain size (range 80-112μm x 64-96μm), $\bar{X}$ = (83.84μm x 76.80μm).

(Figs. 103 – 105)

***Cerbera* Linn.**

***C. odollam* Gaertn.**

Grains 3-zonocolporate, ora lalongate. Exine surface densely punctate with a depression in the apocolpate region, which is highly ornamented (SEM), grains lophate with 2 apocolpial and 3 equatorial lacunae. Exine thickness 2μm. Grains large, subprolate. Grain size (range 80-96μm x 64-80μm), $\bar{X}$ = 90.24μm x 78.08μm).

(Figs. 106 – 109)

***Kopsia* Blume**

***K. fruticosa* A. DC.**

Grains 3-zonocolporate, ora lalongate. Exine surface reticulate (LM). Exine thickness 2μm. Grains large, prolate-spheroidal. Grain size (range 80-96μm x 64-80μm), $\bar{X}$ = (89.60μm x 78.72μm).

(Figs. 110 – 113)

Subtribe 3 Euplumerieae

***Catharanthus* (L.) G. Don.**

***C. roseus* (L.) G. Don. (variety 1)**

Grains 3-zonocolporate, ora faint with subcolpal exinous bands (margo) on either side, lolongate. Exine surface reticulate heterobrochate (LM). Exine thickness 1μm. Grains large, subprolate. Grain size (range 48-64μm x 48-64μm), $\bar{X}$ = (62.08μm x 53.12μm).

(Figs. 114 – 116)

***C. roseus* (L.) G. Don. (variety 2)**

Grains 3-zonocolporate, ora faint with subcolpal bands (margo) on either side, lolongate. Exine surface reticulate heterobrochate (LM). Exine thickness 1μm. Grains large, subprolate. Grain size (range 48-64μm x 48-64μm), $\bar{X}$ = (62.08μm x 53.12μm).

(Figs. 117 – 119)

***Plumeria* Tourn. ex. Linn.**

***P. alba* Linn.**

Grains 3-zonocolporate, ora circular to lalongate. Exine surface microreticulate (LM). Exine thickness 1.2μm. Grains medium sized, subprolate. Grain size (range 32μm x 24-32μm), $\bar{X}$ = (32μm x 24.96μm).

(Figs. 120 – 122)

***P. rubra* Linn.**

Grains 3-zonocolporate, ora circular to lalongate. Exine surface reticulate heterobrochate (LM). Exine thickness 1.2μm. Grains medium sized, subprolate. Grain size (range 32μm x 24-32μm), $\bar{X}$ = (32μm x 25.60μm).

(Figs. 123 – 124)

***P. rubra* Linn. (variety 1)**

Grains 3-zonocolporate, ora circular to lalongate. Exine surface reticulate heterobrochate (LM). Exine thickness 1.2μm, Grains medium sized, prolate-spheroidal. Grain size (range 32μm x 24-32μm), $\bar{X}$ = (32μm x 30.08μm).

(Fig. 125)

***Alstonia* R. Br.**

***A. scholaris* R. Br.**

Grains 3-zonocolporate, ora circular to lalongate. Exine surface reticulate (LM). Exine thickness 1μm. Grains medium sized, spheroidal. Grain size (range 32μm x 32μm), $\bar{X}$ = (32μm x 32μm).

(Figs.126-127)

***A. venenata* R. Br.**

Grains 3-zonocolporate, thickening at the colpus region, behind the pseudocolpi, ora circular to lalongate. Exine surface reticulate heterobrochate (LM). Exine thickness 2μm. Grains large sized, subprolate. Grain size (range 64-96μm x 48-80μm), $\bar{X}$ = (73.60μm x 63.36μm).

(Figs. 128 – 130)

Sub tribe 4 Tabernaemontaneae

***Tabernaemontana* L.**

***T. dichotoma* Roxb.**

Grains 3(4-) zonocolporate, ora lalongate. Exine surface reticulate (LM). Exine thickness 1.5μm. Grains medium sized, subprolate. Grain size (range 32-48μm x 32-48μm), $\bar{X}$ = (38.40μm x 33.28μm).

(Figs. 131 – 134)

***T. divaricata* (L.) R. Br. ex. Roem. and Schult. (variety 1)**

Grains 4-parasyncolporate, ora lalongate. Exine surface reticulate heterobrochate (LM). Exine thickness 1.5μm. Grains medium sized, spheroidal. Grain size (range 32μm x 32μm), $\bar{X}$ = (32μm x 32μm).

(Figs. 135 – 136)

***T. divaricata* (L.) R. Br. ex. Roem. and Schult. (variety 2)**

Grains 4-parasyncolporate, ora lalongate. Exine surface reticulate heterobrochate (LM). Exine thickness 1.5μm. Grain medium sized, spheroidal. Grain size (range 32μm x 32μm), $\bar{X}$ = (32μm x 32μm).

(Figs. 137 – 138)

***T. divaricata* (L.) R. Br. ex. Roem. and Schult. (variety 3)**

Grains 4-parasyncolporate, ora lalongate. Exine surface reticulate heterobrochate (LM). Exine thickness 1.5μm. Grains medium sized, spheroidal. Grain size (range 32μm x 32μm), $\bar{X}$ = (32μm x 32μm).

(Figs. 139 – 140)

***T. divaricata* (L.) R. Br. ex. Roem. and Schult. (variety 4)**

Grains 4-parasyncolporate, ora lalongate. Exine surface reticulate heterobrochate (LM). Exine thickness 1.5μm. Grains medium sized, spheroidal. Grain size (range 32μm x 32μm), $\bar{X}$ = (32μm x 32μm).

(Fig. 141)

***Holarrhena* R. Br.**

***H. antidysenterica* Wall.**

Grains 3-porate, pore circular. Exine surface foveolate (LM). Exine thickness 1.5μm. Grains medium sized, subprolate. Grain size (range 24-48μm x 24-48μm), $\bar{X}$ = (41.91μm x 34.56μm).

(Figs. 142 – 144)

TRIBE III ECHITIDEAE

Sub tribe 1 Parsonsieae

***Vallaris* Burm.**

***V. solanacea* (Roth.) Kuntze.**

Grains 3-porate, pore circular. Exine surface microreticulate (LM). Exine thickness 2μm. Grains medium sized, subprolate. Grain size (range 48μm x 32-48μm), $\bar{X}$ = (48μm x 37.76μm).

(Fig. 145)

Sub tribe 2 Nerieae

***Strophanthus* DC.**

***S. gratus* (Wall. and Hook.) Baill.**

Grains 3-porate, pore circular. Exine surface reticulate heterobrochate (LM). Exine thickness 1.5μm. Grains large sized, subprolate. Grain size (range 48-64μm x 48μm), $\bar{X}$ = (62.72μm x 48μm).

(Figs. 146 – 150)

***S. wightianus* Wall.**

Grains 3-zonocolporate, ora lolongate. Exine surface microreticulate (LM). Exine thickness 1.5μm. Grains medium sized, euprolate. Grain size (range 32-48μm x 32μm), $\bar{X}$ = (46.72μm x 32μm).

(Figs. 151 – 152)

Sub tribe 3 Ichnocarpeae

***Ichnocarpus* R. Br.**

***I. frutescens* (L.) R. Br.**

Grains 3-zonocolporate, ora lalongate. Exine surface reticulate heterobrochate (LM). Exine thickness 1μm. Grains medium sized, prolate-spheroidal. Grain size (range 32-48μm x 32-48μm), $\bar{X}$ = (43.52μm x 41.60μm).

(Fig. 153)

Sub tribe 4 Euechitideae

***Chonemorpha* G. Don.**

***C. fragrans* (Moon) Alston.**

Grains 3-zonocolporate, ora lalongate. Exine surface reticulate (LM). Exine thickness 1.5μm. Grains large, subprolate. Grain size (range 48-64μm x 32-48μm), $\bar{X}$ = (56.32μm x 42.88μm).

(Fig. 154)

***Aganosma* G. Don.**

***A. caryophyllata* G. Don.**

Grains 3-zonocolporate, ora lalongate. Exine surface reticulate (LM). Exine thickness 1μm. Grains medium sized, euprolate. Grain size (range 32μm x 48μm), $\bar{X}$ = (48μm x 32μm).

(Figs. 155 – 156)

***Adenium* Roem. and Schult.**

***A. obesum* Roem. and Schult.**

Grains 3-porate, pore circular. Exine surface reticulate (LM). Exine thickness 1μm. Grains medium sized, prolate-spheroidal. Grain size (range 24-32μm x 24-32μm), $\bar{X}$ = (31.68μm x 28.48μm).

(Fig. 157)

***Odontadenia* Benth.**

***O. grandiflora* Schum.**

Grains 3-4-porate, pore circular. Exine surface reticulate heterobrochate (LM). Exine 1μm thickness. Grains large, subprolate. Grain size (range 48-64μm x 48μm), $\bar{X}$ = (60.80μm x 48μm).

(Figs. 158 – 159)

***Nerium* Linn.**

***N. oleander* Linn. (variety 1)**

Grains 3(4)-porate, pore irregular. Exine surface reticulate heterobrochate (LM). Exine thickness 1μm. Grains medium, subprolate. Grain size (range 48-56μm x 32-48μm), $\bar{X}$ = (48.64μm x 40.64μm).

(Figs. 160 – 161)

***N. oleander* Linn. (variety 2)**

Grains 3(4)-porate, pore circular. Exine surface reticulate (LM). Exine thickness 1μm. Medium sized grains, prolate spheoridal. Grain size (range 48μm x 32-48μm), $\bar{X}$ = (48μm x 44μm).

(Figs. 162 – 163)

***N. oleander* Linn. (variety 3)**

Grains 3(4)-porate, pore circular. Exine surface reticulate heterobrochate (LM). Exine thickness 1μm. Grain medium sized, subprolate. Grain size (range 32-48μm x 24-32μm), $\bar{X}$ = (35.84μm x 30.40μm).

(Fig. 164)

DISCUSSION

In terms of taxonomic-phylogenetic usefulness, pollen aperture type, their numbers, position, structure and the internal structure of exine are the most important characters. The taxonomic and evolutionary importance of pollen morphology may be at specific, generic or higher levels. In many cases the types of pollen of a taxon is characteristic and constant. But the types of pollen may vary considerably in size, aperture and stratification of the exine within natural groups. As an organ which is considered to be less influenced by changing ecological conditions, the characters of pollen grains are regarded as a more dependable and useful tool in studies of comparative morphology that leads to conclusions in plant taxonomy, phylogeny and evolution than those of any other morphostructures (Saad, 1972; Nair, 1974). Of these above mentioned characters, the germinal aperture character forms the stable fundamental basis, and hence given primary importance in the interpretation of interrelationships, while exine surface pattern as secondary and the other characters such as pollen size and shape are tertiary in the degree of significance (Erdtman, 1952; Vishnu-Mittre, 1964; Nair, 1965a; Walker, 1974; Walker and Doyle, 1975). Chaloner (1976) has remarked that a great body of information have bearing on the process of evolution is seen in the exine. According to him, exine is the most important or informative part of the pollen (spore) in terms of its biology. A host of palynologists have focussed their attention on the pollen aperture in phylogenetic considerations

(Kuprianova, 1948; Nair, 1970a; 1974; Walker, 1976). The apertural characteristics based on their number, position and character show great diversity so as to be used in the identification of genera, species and even varieties, and also to assess taxonomic kinships.

The exine surface patterns serve as supplementary characters in relating taxonomic and phylogenetic conclusions (Erdtman, 1952; Nair, 1970b; Walker, 1974). The other characters such as pollen shape and size are considered to be of only tertiary importance, and are of very little value in applied taxonomy due to their inconsistancy (Erdtman, 1952; Vishnu-Mittre, 1964; Nair, 1970a; Walker, 1974; Walker and Doyle, 1975), but often have useful supplementary value.

1. Analysis of palynological characters

During the present investigation pollen aperture, exine ornamentation and pollen size and shape in 37 taxa of 19 genera are taken into consideration (Table 21).

(i) Pollen aperture

Apertures are well defined weak areas of the exine through which the pollen tube emerges during germination. According to Muller (1979) apertures are local modifications of the exine structure. Functionally they act as regulatory harmomegathic structure as constant points for the exchange of intine-bound recognition substancnes, and as exits for pollen tube (Wodehouse, 1935; Heslop-Harisson, 1971). They constitute the main characteristic features, which permit the determination of the taxon to which the pollen grains or spores belong (Kuprianova, 1967). The aperture morphoform provides one of the best taxonomic characters,

especially at the higher levels of taxonomy. Their number, position and character (NPC) have provided valuable data to the phylogeneticists and systematists in the past.

The pollen of angiosperms may or may not have apertures. In those with aperturate pollen, apertures may be simple or complex. The apertures may be categorised largely on the basis of their number, position and form. The number of apertures in angiosperms show notable variation from 0 to over 30, of which the 3-aperturate condition is the most common in dicots. Only one aperture is generally needed for the exit of pollen tube. The extra apertures are considered to perform certain other roles including regulation of movement of water into and cut of the grain (Wodehouse, 1935), and as exit sites for the proteins which function in the recognition reaction between pollen grains and stigma (Heslop-Harisson, 1971). As regards the position of the pollen characters, the proximal position is considered to be the most primitive, while the others such as distal, zonal and global are derived and advanced conditions. Proximal, distal, zonal and global are considered to be the heirarchial order of evolutionary progress with regard to the position of the aperture (Nair, 1970a; 1985). The proximal aperture (fissurate) are commonly found in bryophytes and pteridophytes. The distal (sulcate) type is predominant in gimnosperms, and monocotyledons and primitive dicotyledons with one colpate pollen, while the zonal and global (complex) apertures are found only in angiosperms. According to Erdtman's (1964) scheme of evolution, pollen grains having pore-like or ill-defined proximal aperture have given rise to those with three equidistant equatorial apertures

(3-zonocolpate), and are the most common type in dicots. The form of apertures in angiosperms is either elongate (colpate) or circular (porate) along with intermediate types. Phylogenetically the colpate condition is primitive in angiosperms.

Table 21 Pollen morphological features in the family Apocynaceae (Present study)

Tribe	Taxon	Aperture morphotype	Exine ornamentation	$\bar{X}$ size (μm)	Shape
Carisseae	*Allamanda cathartica* Linn.	3-zonocolporate	Rugulate	90.88 x 72.96	Sub prolate
	Allamanda schottii Pohl.	3-zonocolporate	Faintly foveolate	93.44 x 80	Sub prolate
	Allamanda neriifolia Hook.	3-zonocolporate	Rugulate	77.44x64.64	Sub prolate
	Allamanda violacea Gardn. and Field.	3-zonocolporate	Rugulate-nodulate	71.04 x 64	Prolate spheroidal
	Carissa spinarum Linn.	3-zonocolporate	Granulate-perforate or punctate	32.64 x 30.08	Prolate spheroidal
	Carissa carandas Linn.	3-zonocolporate	Scrobiculate	47.36 x 41.60	Prolate spheroidal
Plumerieae	*Rauvolfia serpentina* Benth. ex Kurz.	3-zonocolporate	Reticulate	84.48 x 77.44	Prolate spheroidal
	Rauvolfia tetraphylla Linn.	3-zonocolporate	Reticulate	46.08 x 32	Euprolate
	Rauvolfia beddomei Hook. f.	3-zonocolporate	Reticulate	31.68 x 28.48	Prolate spheroidal
	Rauvolfia densiflora Benth. ex Hook. f.	3-zonocolporate	Reticulate	109.44 x 106.24	Prolate spheroidal
	Thevetia peruviana (Pers.) K. Schum.	3-zonocolporate	Microreticulate	83.84 x 76.80	Prolate spheroidal
	Cerbera odollam Gaertn.	3-zonocolporate	Densely punctate	90.24 x 78.08	Sub prolate
	Kopsia fruticosa A. DC.	3-zonocolporate	Reticulate	89.60 x 78.72	Prolate spheroidal
	Catharanthus roseus (L.) G. Don. (var. 1)	3-zonocolporate	Reticulate heterobrochate	62.08 x 53.12	Sub prolate
	Catharanthus roseus (L.) G. Don. (var. 2)	3-zonocolporate	Reticulate heterobrochate	62.08 x 53.12	Sub prolate

	Plumeria alba Linn.	3-zonocolporate	Microreticulate	32 x 24.96	Sub prolate
	Plumeria rubra Linn.	3-zonocolporate	Reticulate heterobrochate	32 x 25.60	Sub prolate
	Plumeria rubra Linn. (var. 1)	3-zonocolporate	Reticulate	32 x 30.08	Prolate spheroidal
	Alstonia scholaris R. Br.	3-zonocolporate	Reticulate	32 x 32	Spheroidal
	Alstonia venenata R. Br.	3-zonocolporate	Reticulate heterobrochate	73.60 x 63.36	Sub prolate
	Tabernaemontana dichotoma Roxb.	3-(4-) zonocolporate	Reticulate	38.40 x 33.28	Sub prolate
	Tabernaemontana divaricata (L.) R. Br. ex Roem. and Schult.(var.1)	4-parasyncol-porate	Reticulate heterobrochate	32 x 32	Spheroidal
	Tabernaemontana divaricata (L.) R. Br. ex Roem. and Schult.(var.2)	4-parasyncol-porate	Reticulate heterobrochate	32 x 32	Spheroidal
	Tabernaemontana divaricata (L.) R. Br. ex Roem. and Schult.(var.3)	4-parasyncol-porate	Reticulate heterobrochate	32 x 32	Spheroidal
	Tabernaemontana divaricata (L.) R. Br. ex Roem. and Schult.(var.4)	4-parasyncol-porate	Reticulate heterobrochate	32 x 32	Spheroidal
	Holarrhena antidysenterica Wall.	3-porate	Foveolate	41.91 x 34.56	Sub prolate
Echitideae	*Vallaris solanacea* (Roth.) Kuntze.	4-porate	Microreticulate	48 x 37.76	Sub prolate
	Strophanthus gratus (Wall. and Hook.) Baill.	3-porate	Reticulate heterobrochate	62.72 x 48	Sub prolate
	Strophanthus wightianus Wall. ex Wright.	3-zonocolporate	Microreticulate	46.72 x 32	Euprolate
	Ichnocarpus frutescens (L.) R. Br.	3-zonocolporate	Reticulate	43.52 x 41.60	Prolate spheroidal
	Chonemorpha fragrans (Moon.) Alston.	3-zonocolporate	Reticulate	56.32 x 42.88	Sub prolate
	Aganosma caryophyllata G. Don.	3-zonocolporate	Reticulate	48 x 32	Euprolate
	Odontadenia grandiflora Schum.	3-porate	Reticulate heterobrochate	60.80 x 48	Sub prolate
	Nerium oleander Linn. (var. 1)	4-porate	Reticulate heterobrochate	48.64 x 40.64	Sub prolate

	Nerium oleander Linn. (var. 2)	4-porate	Reticulate heterobrochate	48x44	Prolate spheroidal
	Nerium oleander Linn. (var. 3))	4-porate	Reticulate heterobrochate	35.84 x 30.40	Sub prolate
	Adenium obesum Roem. and Shult.	3-porate	Reticulate	31.68 x 28.48	Prolate spheroidal

In the present analysis (Table 22) two main type of aperture forms, such as colporate and porate is observed among the three tribes viz., Carisseae, Plumerieae and Echitideae.

Table 22 The distribution of aperture types in different tribes (Present study)

Tribes	Aperture morphotypes
1. Carisseae	3-zonocolporate
2. Plumerieae	3-zonocolporate, 3-(4-) zonocolporate
	4-parasyncolporate, 3-porate
3. Echitideae	3-zonocolporate, 3-4-porate

In the tribe Carisseae two genera such as *Allamanda* and *Carissa*, all the species studied here showed 3-zonocolporate pollen grains (Table 21). Nilsson (1986, 1990) reported 3-colporate and 3-4 colporate pollen grains in the species of *Allamanda* and *Carissa* respectively.

In the tribe Plumerieae nine genera viz., *Rauvolfia, Thevetia, Cerbera, Kopsia, Catharanthus, Plumeria, Alstonia, Tabernaemontana* and *Holarrhena* were studied. 3-zonocolporate pollen grains were predominant in this tribe (14 out of 20 taxa) (Table 21). Four species of *Rauvolfia* (*R. serpentina, R. tetraphylla, R. beddomei* and *R. densiflora*) showed 3-zonocolporate pollen grains (Table 21). However, Dnyansagar

(1965) has reported 3-zonocolporate pollen grains in *R. serpentina.* Thanikaimoni (1962) has reported tricolpore pollen grains in *R. canescens* and Huang (1972) reported 3-4-colporate pollen in *R. verticillata.* In the present study *Thevetia peruviana, Cerbera odollam, Kopsia fruticosa,* and *Alstonia scholaris* were showed 3-zonocolporate pollen grains (Table 21). Nilsson (1986) reported 3-zonocolporate pollen in *Thevetia peruviana.* In *Cerbera manghas,* 3-colporate pollen grains were reported by Huang (1972) and Nilsson (1990). Nilsson (1990) also reported 3-colporate pollen grains in *Kopsia fruticosa.* Thanikaimoni (1962) reported 2-3-colporate pollen grains in *Alstonia scholaris.* Nair (1965b) reported 3-aperturate pollen grains in this species from Western Himalaya. Kuijt and van der Ham (1997) reported 3-aperturate pollen grains in *Alstonia* species. The presence of both, 2 and 3-aperpturate species may be regarded as a further radiation within this group. As far as is known, 2-colporate pollen does not occur in any other Apocynaceae. Therefore, 2-colporate pollen probably represents the apomorphic condition within *Alstonia.* Of interest is the observation by Guinet (1962) of more than 50% 2-aperturate grains in a sample of *A. scholaris* from India. This species is 100% 3-aperturate pollen grains in the present study. Kuijt and van der Ham (1997) suggested that the 2-aperturate type originated from 3-aperturate type by stabilisation of the 2-aperturate condition in a mixed population. In the present study, 3-4-zonocolporate pollen grains in *Tabernaemontana dichotoma* were noticed. In *Tabernaemontana undulata* and *T. ventricosa,* Nilsson (1990) were reported 3-4-zonocolporate pollen grains. Four varieties of *Tabernaemontana divaricata* showed 4-parasyncolporate pollen grains

(Table 21). But Nilsson (1990) had reported 3-4-colporate pollen grains in this species. Only one species *Holarrhena antidysenterica* in the tribe Plumerieae showed 3-porate pollen grains (Table 21). Huang (1986) reported pantoporate pollen grains in this species. Nilsson (1990) had reported 3-porate pollen grains in the species *Holarrhena floribunda* and *H. pubescens.*

In the tribe Echitideae eight genera viz., *Vallaris, Strophanthus, Ichnocarpus, Chonemorpha, Aganosma, Odontadenia, Nerium* and *Adenium* were studied. The predominant occurrence of 3-4-porate pollen grains were found in this tribe (7 out of 11 taxa), (Table 21). The species of *Ichnocarpus, Chonemorpha* and *Aganosma* have showed 3-zonocolporate pollen grains. The species of *Vallaris, Strophanthus, Odontadenia, Nerium* and *Adenium* showed 3-4-porate pollen grains. Nilsson (1990-1991) had reported 3-porate pollen grains in *Nerium oleander* and *Strophanthus gratus.* Huang (1972) had reported 4-6-porate pollen in *N. indicum.* In *Adenium obesum,* 3-porate pollen grains was reported by Nilsson (1986). The frequency of taxa with various aperture forms (Table 21, Text Fig. 15) shows that 3-zonocolporate forms are dominant followed by 4 porate and other forms.

The colpus margin (margo) is either thick or thin which should be considered to have functional significance in harmomegathy (Wodehouse, 1935) and it may also be considered to control the germination process. The endocolpium is mostly lalongate and the other forms include, lolongate and circular. The frequency of taxa with various endocolpium forms are listed in the table 23 and text fig. 16.

Table 23 Distribution of different endocolpium forms in the presently studied tribes.

Tribe	Lalongate	Lolongate	Circular
Carisseae	6	-	-
Plumerieae	17	2	1
Echitideae	3	1	7

(ii) Exine ornamentation

The surface ornamentation (sculpturing) of exine is considered to be a significant morphological character helping a great deal in the categorisation of various genera and species. Palynologists regard these characters as of secondary taxonomic value. Moore and Webb (1978) have reported that the structure and sculpturing of pollen make it a highly recognizable object and identification can often be taken to the species level, though sometimes it is only possible to deduce the genus or family from which it comes. A perusal of the literature clearly reveals the significance of this character in taxonomic and phylogenetic discussions (Erdtman, 1952; Ferguson and Webb, 1970; Chambers and Godwin, 1971; Hebda and Lott, 1973; Skvarla *et al.* 1976; Tewari and Nair, 1979; Graham and Barker, 1981; Vezey, 1990; Vezey and Skvarla, 1990; Vezey *et al.* 1991, 1992; Klitgaard, 1991; Weirmann and Gubalz, 1992).

Twelve major sculpturing patterns have been recognised in angiosperm families by Walker and Doyle (1975), such as psilate, foveolate, fossulate, scabrate, verrucate, baculate, pilate, gemmate, echinate (spiny), rugulate, striate and reticulate.

Nair (1970b) has classified the exine surface patterns under two major categories (i) depression type (foveolate, striate, reticulate, scrobiculate, fossulate, rugulate, vermiculate and lophate) and (ii) excrescence types (spinulate, baculate, clavate, gemmate, verrucate and granulate), of which the depression type may be considered less advanced than the excrescence type. In the present investigation the exine sculpturing types recognised from the LM observation reveal that a majority of the pollen grains has the depression type of ornamentation. The most predominantly occurring types in the family Apocynaceae is the reticulate and reticulate heterobrochate form (Table 24, Text Fig. 17).

In *Allamanda cathartica the* exine surface is rugulate, while Nilsson (1990-1991) has reported it as densely perforated exine. But Huang (1972) has reported as granulate exine form. The exine surface pattern of *Carissa spinarum* is granulate or punctate. However, Thanikaimoni (1962) has reported foveolate exine pattern in this species. The exine surface of *Carissa carandas* is scrobiculate, although Nilsson (1986) has reported perforated exine. In *Catharanthus roseus* exine ornamentation is reticulate heterobrochate form, while Nilsson (1990) has reported perforate exine. In *Thevetia peruviana* exine ornamentation is microreticulate. However, Nilsson (1990) has reported exine pattern as perforate to microreticulate in this species. In *Alstonia scholaris* exine ornamentation is reticulate, while Kuijt and Van der Ham (1997) reported microfossulate to perforate to psilate exine ornamentation patter in this species.

Table 24 Distribution of exine ornamentation patterns in different genera of three tribes

Tribe	Genera	No. of taxa examined	Depression type					Excrescence type
			Foveo-late	Reti-culate	Scrobi-culate	Rugulate	Micro-reticulate	Granulate-perforate or punctate
Carisseae	*Allamanda*	4	1	-	-	3	-	-
	Carissa	2	-	-	1	-	-	1
Plumerieae	*Rauvolfia*	4	-	4	-	-	-	-
	Thevetia	1	-	-	-	-	1	-
	Cerbera	1	-	-	-	-	-	1
	Kopsia	1	-	1	-	-	-	-
	Catharanthus	2	-	2	-	-	-	-
	Plumeria	3	-	2	-	-	1	-
	Alstonia	2	-	2	-	-	-	-
	Tabernaemontana	5	-	5	-	-	-	-
	Holarrhena	1	1	-	-	-	-	-
Echitideae	*Vallaris*	1	-	-	-	-	1	-
	Strophanthus	2	-	1	-	-	1	-
	Ichnocarpus	1	-	1	-	-	-	-
	Chonemorpha	1	-	1	-	-	-	-
	Aganosma	1	-	1	-	-	-	-
	Odontadenia	1	-	1	-	-	-	-
	Nerium	3	-	3	-	-	-	-
	Adenium	1	-	1	-	-	-	-

(iii) Pollen size and shape

Pollen size and shape are highly unfixed characters and have less phylogenetic value (Saad, 1972). A wide range of grain size and shape may occur in the same

taxon. Moreover, the method of pollen preparation also affects considerably the pollen size and shape (Walker and Doyle, 1975). The effects of acetolysis (Nair, 1979; Ravikumar and Nair, 1985; Hao and Zhang, 1988), glycerol and glycerine jelly (Rull and Rinaldi, 1988) on grain size and shape well reveal their unstable nature. However, these characters when considered statistically, possess some diagnostic value as in taxonomy (Nair, 1980). Maurizio (1956) and Ravikumar and Nair (1985) have reported that polyploidy is known to produce considerable differences in pollen size. Gould (1957) has reported that in grasses pollen size is associated with polyploidy. While Bir and Kumari (1981) have revealed that polyploidy has no significant effect on the size of the pollen grains. Lesins and Lesins (1963) had reported that there is no correlations between pollen size and chromosome number within the genus *Medicago* (Fabaceae). But Brochman (1992) has shown that in *Draba* (*Brassicaceae*) the pollen size was strongly correlated with chromosome numbers. Dnyansagar (1965) has reported that the doubling of chromosomes has resulted in the increase in the number of pores from 3 to 4 in *Rauvolfia serpentina* and *R. canescens,* in which the grains tetra colporate. This may indicate a tendency to change the pattern from trimerous to tetramerous as a result of polyploidy. Subsequently Dnyansagar and Sudhakaran (1972) had reported that doubling of chromosome number in tetraploid *Vinca rosea* (2n=32) also showed an increase in size of pollen grains followed by an increase in size of the pores.

According to the size classes by Walker and Doyle (1975), the pollen size of the species studied here fall under three classes such as medium (25-49μm), large (50-

99μm), and very large (100-199μm). Medium grains occur in two species of *Carissa* (*C. spinarum* and *C. carandas*), two species of *Rauvolfia* (*R. tetraphylla* and *R. beddomei)*, three taxa of *Plumeria*, five taxa of *Tabernaemontana* (*T. dichotoma, T. divaricata* – 4 varieties), three varieties of *Nerium oleander*, one species each of *Alstonia* (*A. scholaris*), *Holarrhena* (*H. antidysenterica*), *Vallaris* (*V. solanacea*), *Strophanthus*, (*S. wightianus*), *Ichnocarpus* (*I. frutescens*) and *Aganosma* (*A. caryophyllata*) and *Adenium* (*A. obesum*). Large grains occur in four species of *Allamanda*, (*A. cathartica, A. schottii, A. neriifolia* and *A. violacea*), two varieties of the species *Catharanthus roseus*, one species each of the genera *Rauvolfia* (*R. serpentina*), *Strophanthus* (*S. gratus*), *Thevetia* (*T. peruviana*), *Cerbera* (*C. odollam*), *Kopsia* (*K. fruticosa*), *Alstonia* (*A. venenata*), *Odontadenia* (*O. grandiflora*), and *Chonemorpha* (*C. fragrans*). Grains of very large size occurred only in *Rauvolfia densiflora* (Table 25, Text Fig. 18).

Table 25 List of pollen grain size of various taxa in the presently studied genera

Genera	Number of taxa examined	Size of pollen grains		
		Medium	Large	Very large
Allamanda	4	-	4	-
Carissa	2	2	-	-
Rauvolfia	4	2	1	1
Thevetia	1	-	1	-
Cerbera	1	-	1	-
Kopsia	1	-	1	-
Catharanthus	2	-	2	-
Plumeria	3	3	-	-
Alstonia	2	1	1	-

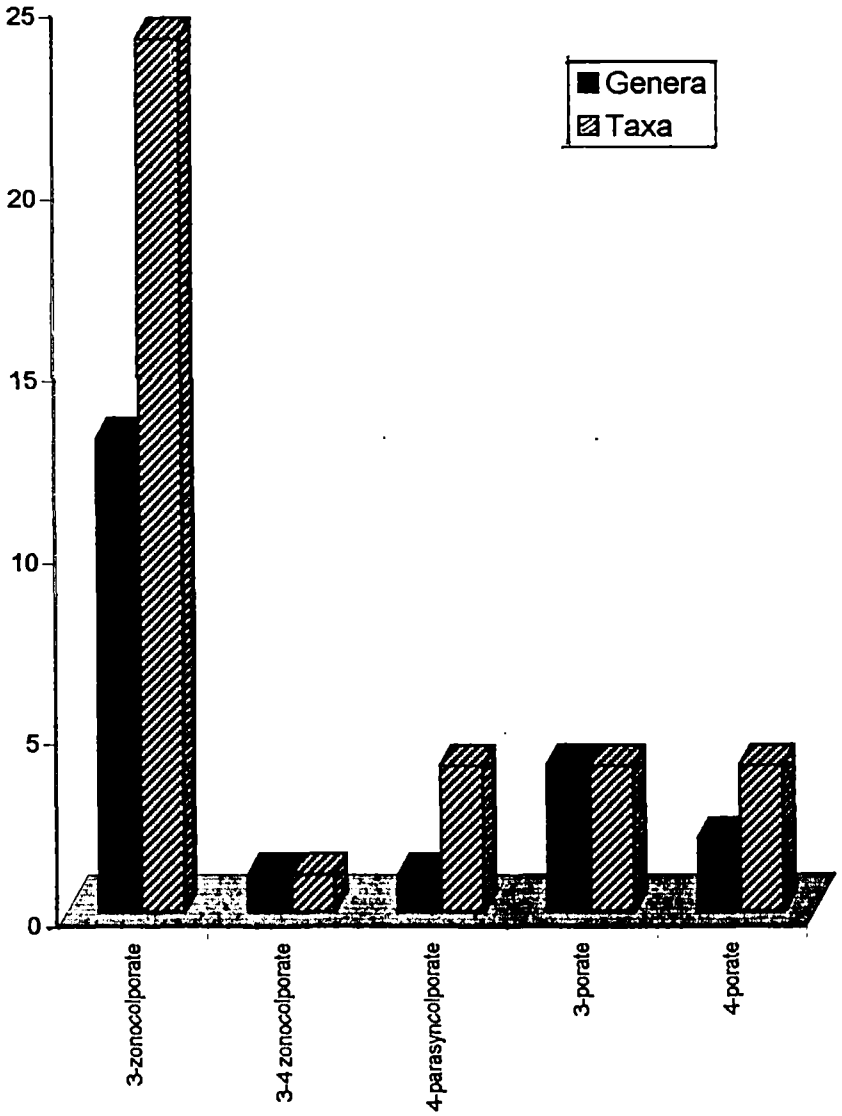
Tabernaemontana	5	5	-	-
Holarrhena	1	1	-	-
Vallaris	1	1	-	-
Strophanthus	2	1	1	-
Ichnocarpus	1	1	-	-
Chonemorpha	1	-	1	-
Aganosma	1	1	-	-
Odontadenia	1	-	1	-
Nerium	3	3	-	-
Adenium	1	1	-	-

The shape of the grain is unfixed and hence this character is not considered as a reliable character in morphological analysis (Nair, 1970a). Based on shape the pollen grains can be grouped into different classes such as perprolate, euprolate, subprolate, prolate-spheroidal, spheroidal, oblate-spheroidal, suboblate, euoblate and peroblate. In the present study the pollen grains exhibit most of these shape classes such as euprolate (3 taxa), subprolate (17 taxa), prolate-spheroidal (12 taxa) and spheroidal (5 taxa). The frequency of various shapes of pollen grains of different tribes presently studied is furnished in table 26, text fig. 19.

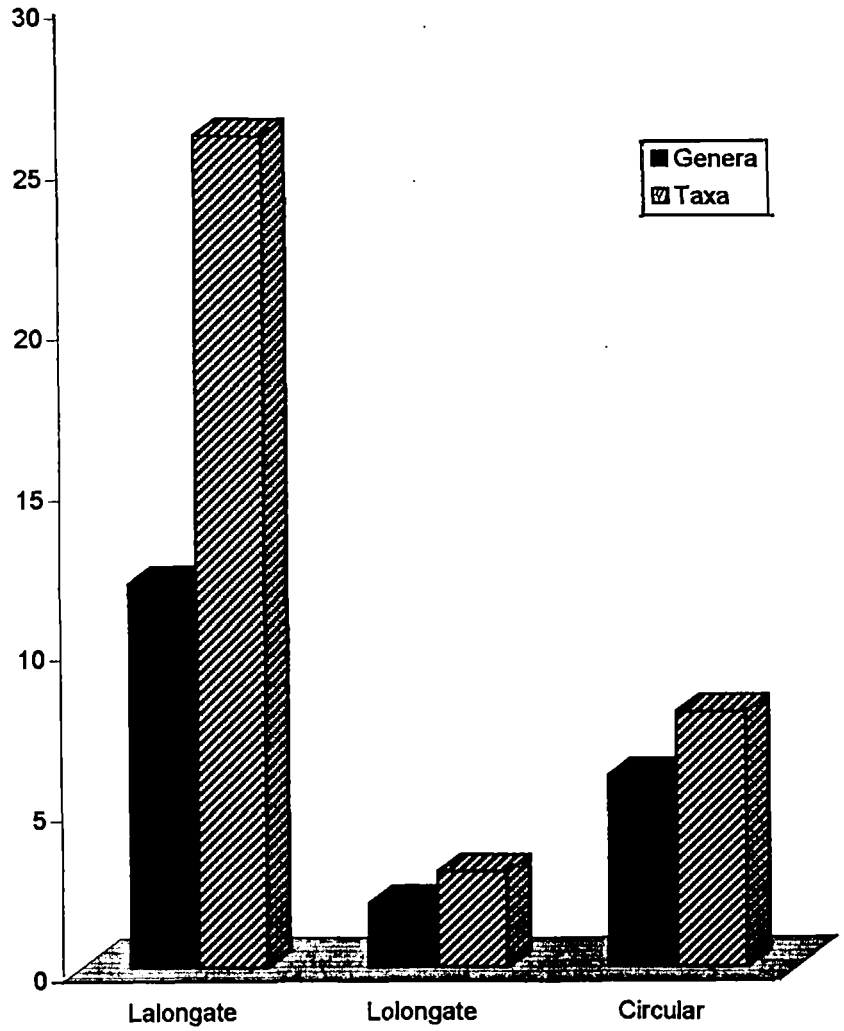
Table 26 Frequency of different shape classes of pollen grains in the presently studied taxa

Tribe	Euprolate	Subprolate	Prolate spheroidal	Spheroidal
Carisseae	-	3	3	-
Plumerieae	1	8	6	5
Echitideae	2	6	3	-

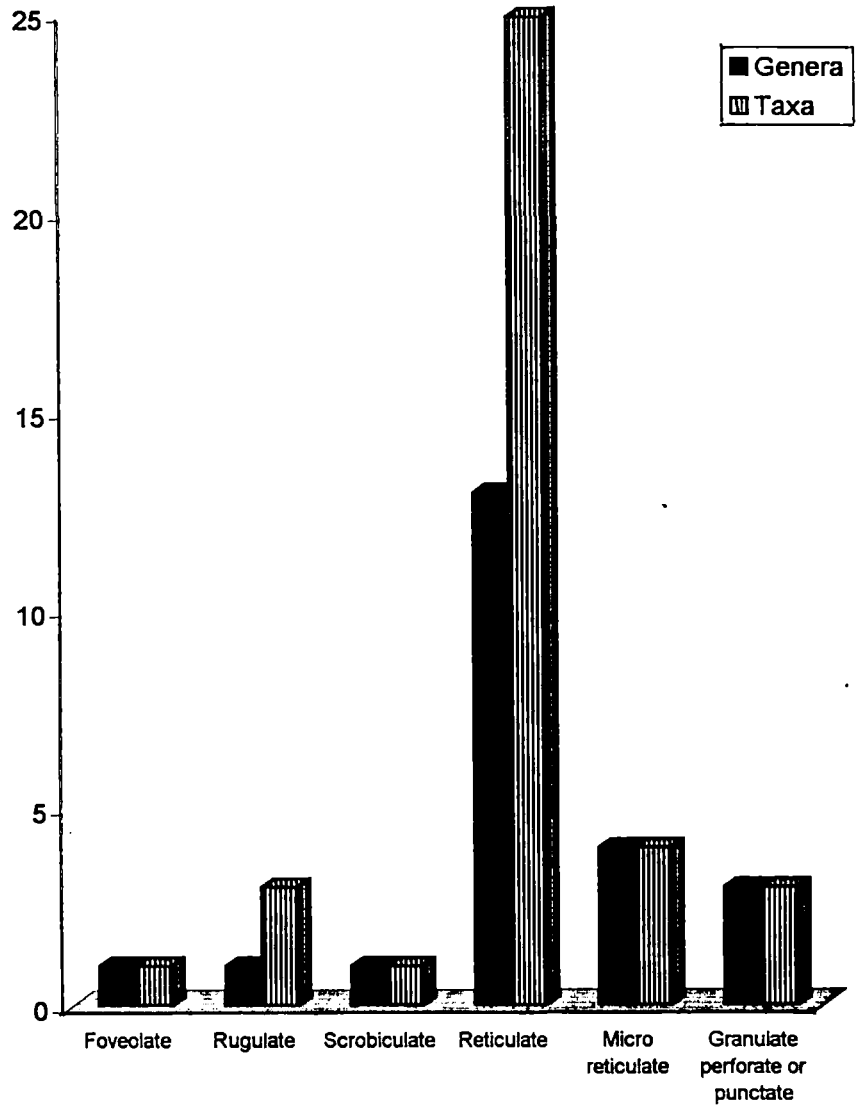
TEXT FIG.15. FREQUENCY DISTRIBUTION OF POLLEN APERATURE MORPHOTYPES IN THE FAMILY APOCYNACEAE (PRESENT STUDY)



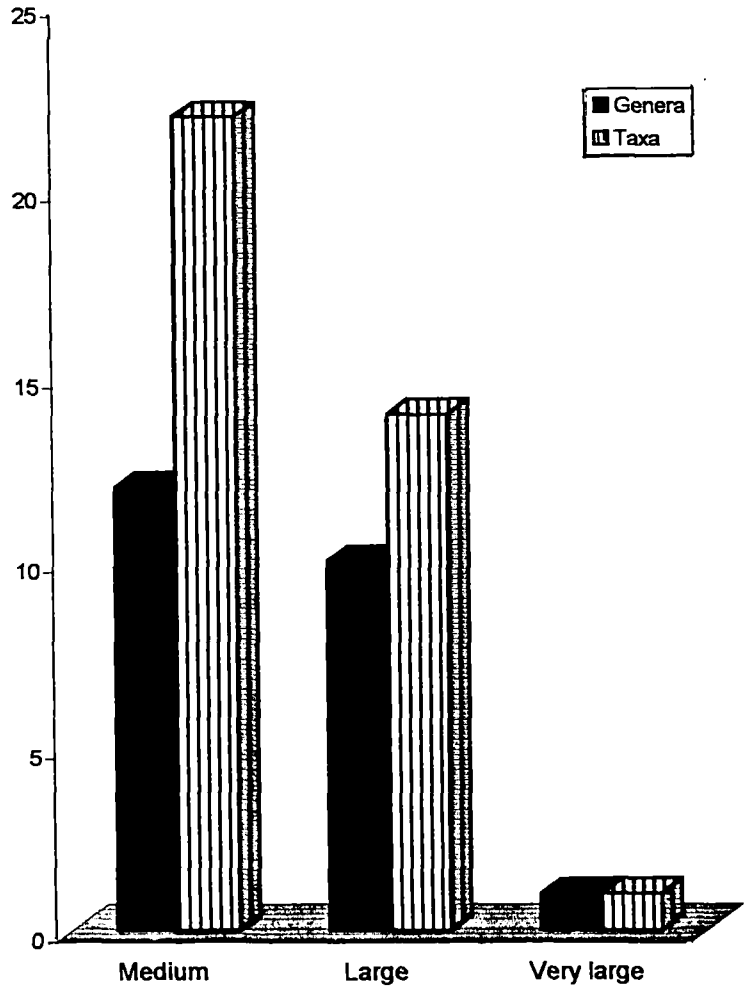
TEXT FIG. 16. FREQUENCY DISTRIBUTION OF ENDOCOLPIUM PATTERN IN THE FAMILY APOCYNACEAE (PRESENT STUDY)



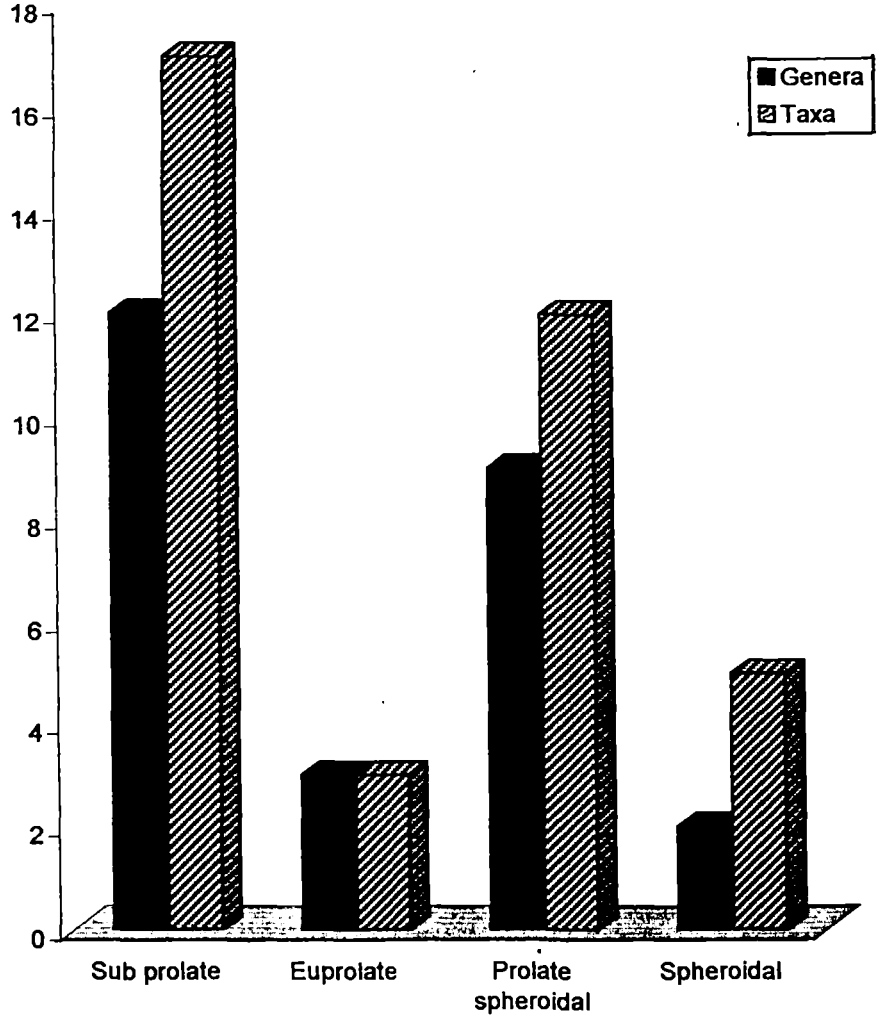
TEXT FIG.17. FREQUENCY DISTRIBUTION OF POLLEN EXINE ORNAMENTATIONS IN THE FAMILY APOCYNACEAE (PRESENT STUDY)



TEXT FIG. 18. FREQUENCY DISTRIBUTION OF POLLEN SIZE CATEGORIES IN THE FAMILY APOCYNACEAE (PRESENT STUDY)



TEXT FIG.19. FREQUENCY DISTRIBUTION OF POLLEN SHAPES IN THE FAMILY APOCYNACEAE (PRESENT STUDY)



2. Pollen morphology and taxonomy

Pollen morphology as a useful tool in solving problems of taxonomy and also in the recognition, identification and interpretation of relationships of plants at various taxonomic levels have been stressed by Erdtman (1952) and Nair (1964), and its application in plant taxonomy is amply evidenced in many angiosperm families (Nair and Rehman, 1962;Nair and Rastogi, 1966,1967; Chanda and Ghosh, 1976; Mathew and Philip, 1983; Valsaladevi, 1987; Yunus and Nair, 1989; and Graham *et al.* 1990). Pollen morphological investigations have served to group plant families as eurypalynous (multipalynous) and stenopalynous (unipalynous). In the eurypalynous group, there are different morphological types which form a basis to delimit the taxa comprising the family, while in the stenopalynous families a single morphological type typifies the whole family and hence in such cases the demarcation of different taxa on the basis of pollen morphology may be difficult (Erdtman, 1952). In the former case, apertures are considered to be of primary importance, ornamentation secondary and size and shape tertiary, whereas in the latter size and shape alone are significant.

Several workers have attempted to classify the clones and species of fruit trees based on the aperture, size, shape and exine characteristics of pollen with SEM (Foggle, 1977; Mass, 1977; Nema and Sharma, 1981; Ahmedullah, 1983; and Marcucci *et al.* 1984). Grant (1971) however, was unable to distinguish between sub species of *Zea mays* on the basis of sculpturing of pollen grains. Rao and Shukla

(1975) found that different species of *Zizyphus* could be differentiated based on size, shape and pore structure of the pollen grain. Varietal differences in shape and size of the pollen grain were also reported by Singh and Misra (1979) and Nehra *et al.* (1984). Foggle (1977), Mass (1977), Nema and Sharma (1981) and Ahemedullah (1983) reported that pollen shape, exine pattern and size can be useful for taxonomic purposes.

The present data apparently make the family heterogenous with colporate, parasyncolporate and porate pollen grains with a wide range of ornamentation pattern which are distinctly peculiar in many taxa of the family. Based on the palynological data of the present investigation a pollen key for the family is presented.

Based on the nature of occurrence of pollen, the family can be brought under single group: Monads. Of the three tribes studied two are eurypalynous and one stenopalynous.

Construction of key is mainly based on aperture morphotypes and exine pattern of sculpturing, except in few where the tertiary characters were taken into consideration.

Key to the family Apocynaceae on account of the present data

Stenopalynous tribe	
Carisseae	3-zonocolporate
Eurypalynous tribes	
Plumerieae	3-zonocolporate, 3-4-zonocolporate 4-parasyncolporate
Echitideae	3-zonocolporate, 3-4-porate.

In the stenopalynous and eurypalynous tribes, further grouping at generic level may be possible based on aperture character and the following grouping are proposed.

Tribe Carisseae		
	Grains 3-zonoclporate	*Allamanda*
		Carissa
Tribe Plumerieae		
	Grains 3-zonocolporate	*Rauvolfia*
		Alstonia
		Thevetia
		Cerbera
		Kopsia
		Catharanthus
		Plumeria
	Grains 4-parasyncolporate	*Tabernaemontana*
	Grains 3-porate	*Holarrhena*
Tribe Echitideae		
	Grains 3-zonocolporate	*Ichnocarpus*
		Aganosma
		Chonemorpha
	Grains 3-4-porate	*Strophanthus*
		Odontadenia
		Nerium
		Vallaris
		Adenium

In the case of a few genera such as *Allamanda*, *Rauvolfia*, *Plumeria*, *Alstonia*, *Tabernaemontana* and *Nerium*, different species within a genus were found to possess

different pollen morphoforms with respect to primary and or secondary and tertiary characters and the following grouping are proposed in them:

Allamanda

1. Grains 3-zonocolporate
   2. Exine surface rugulate — *A. cathartica*
   2. Exine surface faintly foveolate — *A. schottii*
   2. Exine surface rugulate nodulate — *A. violacea*

Rauvolfia

1. Grains 3-zonocolporate
   Endoexine as nodules, thickening (kidney shaped) at the colpus region
   2. Exine surface reticulate
      3. Shape prolate-spheroidal
         4. Size large ($\bar{X}$ = 84.48µm x 77.44µm) — *R. serpentina*
         4. Size very large ($\bar{X}$ = 109.44µm x 106.24µm) — *R. densiflora*

1. Grains 3-zonocolporate
   2. Exine surface reticulate
      3. Shape prolate
         4. Size medium ($\bar{X}$ = 46.08µmx32µm) — *R. tetraphylla*
      3. Shape prolate spheroidal
         4. Size medium ($\bar{X}$ = 31.68µmx28.48µm) — *R. beddomei*

Plumeria

1. Grains 3-zonocolporate
   2. Exine surface microreticulate
      3. Shape subprolate — *P. alba*

2. Exine surface reticulate heterobrochate
   3. Shape subprolate spheroidal — *P. rubra*
2. Exine surface reticulate
   3. Shape prolate-spheroidal — *P. rubra* (var. 1)

Alstonia

1. Grains 3-zonocolporate
   2. Exine surface reticulate
      3. Shape oblate spheroidal
         4. Size medium ($\bar{X}$ = 32µm x 32µm) — *A. scholaris*

1. Grains 3-zonocolporate
   Thickening at the colpus region behind the psuedocolpi
   2. Exine surface reticulate
      3. Shape subprolate
         4. Size large ($\bar{X}$ = 73.60µm x 63.36µm) — *A. venenata*

Tabernaemontana

1. Grains 3 (4-)zonocolporate
   2. Exine surface reticulate
      3. Shape sub oblate
         4. Size medium ($\bar{X}$ = 38.40µm x 33.28µm) — *T. dichotoma*

1. Grains 4-parasyncolporate
   2. Exine surface reticulate heterobrochate
      3. Shape oblate spheroidal
         4. Size medium ($\bar{X}$ = 32µm x 32µm) — *T. divaricata*

Nerium

1. Grains 3-4-porate
   2. Exine surface reticulate
      3. Shape prolate-spheroidal — *N. oleander* (var. 1)
      3. Shape sub oblate — *N. oleander* (var. 2)

Strophanthus

1. Grains 3-zonocolporate
   2. Ora lolongate
      3. Shape euprolate
         4. Size medium ($\bar{X}$ = 46.72µm x 32µm) — *S. wightianus*
1. Grains 3-porate
   2. Pore circular
      3. Shape subprolate
         4. Size large ($\bar{X}$ = 62.72µm x 48µm) — *S. gratus*

Special morphology

Certain types which specialized by virtue of the pseudocolpium, that met in *Alstonia venenata, Rauvolfia serpentina, R. densiflora.* And further in *Cerbera odollam,* the occurrence of cavity like depression (lacuna) number in five (two in apocolpium and three in mesocolpium). In *Rauvolfia Serpentina,* the endo exine thickening (kidney shaped) in the colpus region behind the pseudocolpi. Other species of *Rauvolfia* like *R. tetraphylla, R. beddomei* and *R. densiflora* endo exine thickening at the colpus region.

Pollen morphological evolution

The pollen grains being the most vital unit in plants, the morphological evolution reflected in them is considered to be indicative of the course of evolution of plant forms, which they represent (Nair, 1970a). According to Chanda and Ghosh (1976) the principles of morphological evolution of pollen grains, the most conservative or primary character are the germinal aperture, less conservative or secondary is exine ornamentation and exine stratification, and least conservative or tertiary characters are size and shape. The evolution of aperture is one of the major advancement of seed plants and it obviously reflects in their phylogenetic trends (Walker and Doyle, 1975). As such the aperture evolution has been given prime importance over other pollen characters by various pollen phylogenists. The first true aperture, the distal furrow – like aperture (sulcus), evolved in the pollen of primitive gymnosperms (Walker 1976). Wodehouse (1936) considered the monocolpate, trilete and 3-zonocolpate forms to be of basic significance and the major evolutionary changes in aperture have started from the one furrowed form of the primitive gymnosperms by the process of modification, protection or elimination. According to Erdtman (1952) pollen grains with pore like (ill-defined) proximal aperture have given rise to those with zonal, distal or global apertures. According to Vishnu-Mittre (1964) the most primitive form is the trilete type from which has evolved the inaperturate type followed by the aperturate ones. Muller (1970) argued that the inaperturate form could be transitional stage between the primitive monocolpate and the advanced tricolpate. Chaloner (1970) reported the possibility of origin of tricolpate apertures via

trichotomosulcate pollen which is occassionally seen mid Cretaceous taxa (Hedlund and Norris, 1968). Walker (1974) has considered the inaperturate pollen grain as primitive from which evolved the aperturate ones, of which the tricolpate type is the progenitor of all the other aperture forms. Evidences from extant primitive angiosperm suggest that the inaperturate and not trichotomosulcate pollen to be the ancestral pollen type which gave rise to the uniquely angiospermous (docotyledonous) colpate pollen charactersr (Walker and Doyle, 1975). Chanda *et al.* (1979) has suggested that with regard to the trend of apertural evolution, it is often thought that the sulcate condition was derived from the primitive monosulcate stage. Takhtajan (1980) was of the view that the main trend of evolution of the sporoderm aperture in dicotyledonous is from monocolpate to tricolpate and from tricolpate to tricolporate. From the basic tricolpate form, a diversity of non-magnolioid aperture types such as 5-colpate, 6-colpate, colporate, porate, pororate, rugate, forate appear to have evolved (Walker and Doyle, 1975). Nair (1965a, 1968, 1970a, 1979) proposed that the evolution of the dicotyledons itself has taken place along the line of Magnoliaceae and the other along the line of Ranunculaceae, represented by the fundamentally monocolpate form and the tricolpate form respectively; while the monocots with predominantly monocolpate morphoform evolved along an independent line. According to him the "Magno-Ranalian Complex" has originated from the trilete or trichotomocolpate form of progenitor characteristic of the preangiosperms, trilete apertures being considered to be the most primitive type and the other apertural forms and the inaperturate advanced types in the scale of morphological evolution of pollen

grains. Forms such as colporate, porate, pororate and spiraperturate are considered to have been evolved from the colpate type by the reduction and specialisation of the apertures. Tewari and Nair (1979) have suggested that 3-zonocolpate pollen morphoform is the fundamental type in the scheme of evolution of Ranalean dicotyledons. According to Jonker (1974) colpate and porate grains have evolved from the sulcate type through the intermediate trichotomosulcate type. The apertural evolution seems to have originated from a trilete (trichotomous) form. Subsequent evolution proceeded in two main directions to result (i) in the formation of the monocolpate form, by the widening and elongation of the trichotomous aperture and (ii) the trizonocolpate form, by the separation of the three arms of the trilete and their subsequent zonation. From these two main forms, all others evolved by the process of secondary development, reduction or degeneration (Nair, 1965a).

Puri (1990) has suggested that the basic difference between monocots and dicots is seen in connection with the number and location of the aperture in the pollen grain. In monocots, Cycads, Pteridosperms and Bennettiales the pollen is uniaperturate (monocolpate or monosulcate) and the pore is located at the distal end in dicots. It is essentially triaperturate (tricolpate) and the furrows containing the apertures radiate from the proximal or inner end. Nair (1991) has suggested that at the angiosperm level, the trimorphous situation suddenly changed with the origin of several new apertural morphoforms starting with the tricolpate followed by the tricolporoidate – tricolporate and other forms, the highest level of which is the monoporate characteristic of grasses.

From the palynological data gathered during the preset study, a brief consideration of the morphological evolution of the pollen grains in the Apocynaceae is made. It is found that the family is fairly eurypalynous with different aperture morphoforms occurring among the various tribes and genera. The different aperture morphotypes recognised are colporate, parasyncolporate and porate. The distribution of the various tribes which possess the different apertural morphoforms according to the order of aperture evolution proposed is given below.

Aperture type		Tribe
	3-zonocolporate	Carisseae, Plumerieae, Echitideae
Colporate	3-4 zonocolporate	Plumerieae
	4-parasyncolporate	Plumerieae
Porate	3-porate	Plumerieae, Echitideae
	4-porate	

A majority of genera exhibited tricolporate grains with circular/lalongate ora and shape subprolate/prolate spheroidal as the basic type in the family. Generally speaking the tribe Carisseae have 3-zonocolporate pollen grains only, while the tribe Plumerieae has 3-zonocolporate, 3-4 zonocolporate, 4-parasyncolporate, and 3-porate pollen grains. The tribe Echitideae has 3-zonocolporate and 3-4 porate pollen grains. From the palynological studies it is readily apparent that considerable pollen diversity occurs in the different members of the family. The four different aperture types

noticed are 3-zonocolporate, 3-4-zonocolporate, 4-parasyncolporate and 3-4 porate forms.

The distribution of aperture types within the tribes is shown in table 21 and a tentative scheme of aperture evolution in the family Apocynaceae is pictured (Chart 1). The colporate condition is considered as the most primitive followed by parasyncolporate and porate. The derived forms such as 4-parasyncolporate and 3-(4-) zonocolporate forms have been considered as evolved from parallel lines. The most advanced porate condition is evolved along the colpate forms.

Although the germinal aperture is of primary importance in the morphology of pollen and spores, there are also other morphological characters in which the evolutionary phenomena are expressed. Walker and Doyle (1975) have suggested that the evolutionary trends in pollen wall architecture offer great potential as sources of phylogenetic information of major importance. Nair (1974) has proposed that a character that would serve to increase the protection of pollen protoplasm may be considered primitive and so exine with 'excrescences' types (spinulate, baculate, verrucate etc) are primitive to the 'depression' types (reticulate, foveolate, fossulate, rugulate, striate). In tracing the evolution of exine ornamentation, such characters which increases the protective value of exine, are considered to be primitive. In this respect the excrescences types are considered to be more primitive than the depression types.

In Polygonaceae pollen type with thick and heavily ornamented exine have been considered to be primitive, while those with this and unornamented or lightly

ornamented exine advanced (Wodehose, 1936). Wodehouse (1935) had observed a gradual reduction of exinous process in the family Compositae. Praveen and Bhandari (1981) have reported the evolutionary reduction of spine size in the Asteraceae. A similar trend of reduction in the complexity of exine sculpturing has been shown in Convolvulaceae (Nair and Rehman, 1962). Meerow and Dehgan (1988) have concluded that the commonly occurring coarsely reticulate exine is primitive to the rarely observed finely reticulated ones in the Eucharideae of Amaryllideaceae. In the tribe Antirrhineae of Scrophulariaceae, microreticulate exine has been reported to be primitive to reticulate or perforate exine (Elisens, 1986).

In the results gathered from the present data, the exine ornamentation appeared highly mixed due to the occurrence of variously combined exine patterns with in the genus. Considering the phylogenetic pattern of exine ornamentation, it is suggested that the excrescence forms (which increase the protective aspect of exine) are more primitive than the depression types. The evolutionary trend in the depression type is from reticulate to the foveolate. The other forms such as rugulate may have evolved from foveolate in one line and scrobiculate and punctate in another. The comparitive primitiveness is difficult to settle because of the mixed occurrence in related taxa, but almost indicate a possible transitional stage of evolution.

The present palynological studies show that 3-colporte pollen grains with reticulate exine predominate in the family Apocynaceae. Of the three presently studied tribes viz., Carisseae, Plumerieae and Echitideae, the frequent occurrence of 3-colporate and very rarely 4-parazyncolporate aperture morphoforms have been noticed

in Carisseae and Plumerieae. Only one genus in the tribe Plumerieae (*Holarrhena*) shows 3-porate condition, which is considered to be an advanced character.

In the tribe Echitideae all genera except *Ichnocarpus, Chonemorpha* and *Aganosma* show porate condition which is the most advanced pollen aperture character. Hence, based on palynological studies it can be concluded that the tribe Echitidieae as the most advanced one in the family Apocynaceae.

Pollen and spore shapes do not apparently possess much phylogenetic significance. Nair (1965a), Walker (1976), Le Thomas (1980, 1981) and Walker and Walker (1984) have stated that large, boat shaped, granular and monosulcate pollen is the primitive type in angiosperm, while Punt (1990-1991) has considered small size as primitive. With regard to shape of the pollen grains, Clarke (1980) has reported that in related species of dicotyledons, oblate grains are more advancned than the prolate ones.

Among the data received here subprolate grains predominate followed by prolate-spheroidal and spheroidal, where taxonomically evolved tribe showed sub prolate/prolate-spheroidal grains. Spheroidal grains are seen in a very few taxa (*Alstonia scholaris,* four varieties of *Tabernaemontana divaricata*).

SYSTEMATIC CONSIDERATION

a. Interfamiliar relationship

The family Apocynaceae belongs to the order Gentianales (Bentham and Hooker 1876) along with other families namely Asclepiadaceae, Longaniaceae, Oleaceae, Gentianaceae and Salvadoraceae. Rendle (1959) considered these families under the order Contortae. Woodson and Moore (1938) considered the vestigial calycine scales, gynoecial nectories, corolline scales of the Apocynaceae bearing a possible relationship with Rosales. Benson (1957) included Apocynaceae in the order Apocynales. According to Takhtajan (1966), the family Apocynaceae comes under the order Gentianales along with Loganiaceae, Rubiaceae, Thaligonaceae, Apocynaceae, Asclepiadaceae, Gentianaceae, Menyanthaceae and Dialypetalanthaceae; while Cronquist (1968) included only four families (Loganiaceae, Gentianaceae, Apocynaceae and Asclepiadaceae) in his Gentianales and excluded Buddlejaceae, Menyanthaceae and Oleaceae from this order. Later on Hutchinson (1973) has grouped Apocynaceae, Periplocaceae (derived from Asclepiadaceae) and Asclepiadaceae in the order Apocynales. Cronquist (1968) has stated the distinction between the Apocynaceae and Asclepiadaceae based on androecial characters. Androecium is free from stigma and without translators, pollen granular, not forming pollininia, carpels often united by part or all of the style as well as by the stigma, or even wholly united, corona mostly poorly developed in the Apocynaceae.

Androecium concrescent to the stigma and provided with translators, the pollen grains united (pollinia), carpels united only by the stigma, corona more or less well developed in the Asclepiadaceae.

Hallier (1905, 1912) had reported the close relationship of Apocynaceae to the Periplocoideae – subfamily of Asclepiadaceae. Takhtajan (1966), Thorne (1968, 1976), and Stebbins (1974) have also suggested a very close relationship between Apocynaceae and Asclepiadaceae particularly through Periplocoideae.

According to Rendle (1959) members of the Asclepiadaceae are closely related to Apocynaceae except the great specialization in the stamens and pistil to ensure transference of pollen in the Asclepiads. Metcalfe and Chalk (1950) reported the similarity between Asoclepiadaceae and Apocynaceae. In correlation with their close relationship Asclepiadaceae and Apocynaceae have many features in common. Of these the most important is the universal occurrence of laticiferous tubes in both stem and leaves, together with the presence of intraxylery phloem. In *Apocynum* (Apocynaceae) the pollen is released as tetrads as it is in the Periplocaceae, whereas in all other Apocynoid members the pollen grains are released as monads. In addition, *Apocynum* has simple translators that are homologous to those of the Periplocaceae (Schick, 1982). *Apocynum*, then would seem to be the closest extent link between the Apocynaceae and Asclepiadaceae. Nilsson *et al.*(1993) reported that there is a trend towards increasing synorganization of the gynoecium and androecium from Plumerioideae to the Apocynoideae and Periplocaceae.

Gibbs (1974) recorded that the Apocynaceae resembles Asclepiadaceae in the absence of raphides, aluminium accumilates, myrecitin, delphinidiam, ellagic acid and presence of coumarins, cardenolides, latex, quercetin, kaempferol, caffeic acid and differ in the absence of acubin type glycosides.

Parvathi and Santhakumari (1984) have revealed the similarities as well as differences in these two families (Apocynaceae and Asclepiadaceae) based on chemotaxonomy. Chemotaxonomically both these families resemble each other in the presence of carbohydrates, flavanoides, phenols, alkaloids, lignins and in the absences of indols, sapanins, juglones, hydroxyquinones, aurones, syringin and HCN. However, in the presence of tritetraphenols, steroids, lignins, leucoanthocyanins, catechol, tannin accubin type of glucosides, Apocynaceae differs from Asclepiadaceae.

According to Huber (1983), Apocynaceae is related to Loganiaceae (particularly to the tribe Potalieae), Periplocaceae and Asclepiadaceae. From the Loganiaceae, Apocynaceae is distinguished by the occurrence of lateciferous tubes in the vegetative organs, whereas the latter families deviate in their highly specialized pollen carriers and pollination mechanism. Danniel and Sabnis (1985) revealaed that the family Periplocaceae comprising the subfamily Periplocoideae of family Asclepiadaceae are chemically indistinguishable from the subfamily Echitoideae of Apocynaceae. Recent morphological, palynologial and molecular studies by Judd *et al.*(1994), Nilsson *et al.*(1993) and Struwe *et al.*(1994) have shown that the Asclepiads form a well-knit assemblage, and they can be grouped as a subfamily of the Apocynaceae.

According to Good (1997) these two families (Apocynaceae and Asclepiadaceae) showed generalized characters and mutual resemblance in their tribes (Plumerioidaeae, Echitoideae, and Periplocoideae, Cynanchoideae respectively). Genera such as *Allamanda, Tabernaemontana, Thevetia, Nerium, Ichnocarpus, Strophanthus, Wrightia, Parsonsia* possess glands inside the calyx, a common feature in Asclepiads. In Plumerioideae stamens free, or loosely connected with the apical parts of the style, anther lobes full of pollen, pollen granular, no translators, fruits often a berry, seeds often hairless. In Echitoideae stamens join to the apical parts of the styles, anther lobes empty at the base, pollen granular, no translators, fruit follicular, seeds hairy. In Periplocoideae stamens not actually joined to the apical part of the styles (stigma-head) anther lobes full of pollen, pollen in tetrads which fall into or on to translators, fruit follicular, seeds hairy. In Cynanchoideae stamens joined to the apical part of the styles, (stigma-head), anther lobes empty either above or below, pollen in pollinia which are united in pairs by yoke like translators, fruit follicular, and seeds hairy.

Historically the Periplocaceae were classified in the Asclepiadaceae (Bentham and Hooker, 1876; Schumann, 1895). In the recent years number of botanists like Kunze (1990, 1993), Venter *et al.* (1990), Dave and Kuriachen (1991), Nilsson *et al.* (1993) and Swarupanadan *et al.* (1996) have reported that the Periplocoideae is related to the Apocynaceae and Asclepiadaceae as a connecting link. Recent morphological and molecular studies do not support the relationship of the Asclepiadaceae and Periplocaceae, but some recent workers (Kunze, 1990, 1996; Judd et al. 1994;

Endress, 1997 and Sennblad, 1997) have suggested a close relationship between the Apocynaceae and Periplocacaeae.

Cytologically known genera in the family Asclepiadaceae have shown that majority of them possess x = 11 condition. A perusal of cytological data on the family Apocynaceae also revealed the predominant occurrence of x = 11 situation. Hence, cytologically also these two families showed close relationship. Palynologically Apocynaceae members showed monad type of pollen grains, while Asclepiadaceae members showed pollinium except in members of Periplocoideae, where pollen grains are seen in tetrad form, a common feature in the genus *Apocynium* of the family Apocynaceae.

The family Apocynaceae has very less affinity to other families such as Oleaceae, Gentianaceae and Salvadoraceae except their perennial habit.

b. Intrafamilial relationship

The family Apocynaceae has been classified by various taxonomists. Bentham and Hooker (1876) had divided this family into three major tribes viz., Carisseae, Plumerieae and Echitideae. Based on the relationship between the androecium and gynoecium, Miers (1878), Schumann (1895) and Macfarlane (1933) have divided the family into two sub families as Plumieroideae and Echitoideae. The Plumieroideae consists of free anthers from the gynoecium apex and the Echitoideae consists of postgenitally united anthers with the style-head forming gynostegium. Differences in the position and structure of stamen in these two sub families have been studied and reported by Pichon (1948), Schick (1980, 1982) and Fallen (1983, 1986).

Chemotaxonomic analysis on Apocynaceae has been done by Guggisberg and Hesse (1983), Kisakurek *et al.*(1983), Zhu *et al.*(1987) and Zhu (1988). These authors have noticed the presence of indol alkaloids in Plumerioideae,where as it is absent in the Apocynoideae (Echitoideae). Leeuwenberg (1986) subdivided the family Apocynaceae into three sub families viz., Plumerioideae, Cerberoideae and Apocynoideae.

Tribe 1. Carisseae

In the treatment of Bentham and Hooker (1876), the tribe Carisseae comprises two genera, *Carissa* and *Allamanda*. But Schumann (1895) placed *Carissa* in the sub tribe Melodininae and *Allamanda* in the sub tribe Landolphiinae of the tribe Arduineae under the subfamily Plumieroideae. Pichon (1948), Engler (1964) and Leeuwenberg (1986) have placed these two genera on two separate tribes, namely Carisseae and Allamandeae.

The genus *Allamanda* is distinctly different from the genus *Carissa* in having plants with trailing habit, whorled leaves, funnel shaped corolla, and the stamens inserted below the mouth of the corolla-tube. The species of *Carissa* are dichotomously branched shrubs, with strong and simple thorns. Cytologically the genus *Allamanda* showed larger but lesser number of chromosomes (2n = 18) and their length ranged from 1.37μm to 4.50μm. The chromosome studies in three species of *Allamanda* showed relatively asymmetrical karyotype category (2A, 2B). Species of *Carissa* showed 2n = 22 with smaller chromosomes which ranged in their length from 1.50μm to 2.50μm and possess 1B karyotype category. Palynological data also

showed distinct features such as large sized pollen in the genus *Allamanda* (64μm – 93.44μm), while medium sized pollen (30.08μm – 47.36μm) in the genus *Carissa*. The exine ornamentation of *Allamanda* species showed rugulate and foveolate conditions, while *Carissa* species showed granulate-perforate or scrobiculate forms. Species of *Allamanda* showed sub prolate pollen shape, but *Carissa* species showed prolate-spheroidal shape. Thus, differences in the morphological, cytological and palynological data supports their placement in two separate tribes (Allamandeae, Carisseae) as suggested by Pichon (1948), Engler (1964), Leeuwenberg (1986) and Nilsson (1986) (Table 27).

Tribe II Plumerieae

Bentham and Hooker (1876) considered it as the second major tribe with four sub tribes viz., Rauvolfieae, Cerbereae, Euplumerieae and Tabernaemontaneae. Schumann (1895) had considered this tribe as a subfamily Plumieroideae and divided into six subtribes viz., Melodininae, Landolphiinae, Rauwolfiinae, Cerberinae, Alstoniinae and Tabernaemontaninae on the basis of morphological characters. However, Pichon (1948), Engler (1964) and Leeuwenberg (1986) placed this tribe under the subfamily Plumerioideae by detailed phylogenetic studies.

Bentham and Hooker (1876) had placed the genera *Rauvolfia* and *Kopsia* in the same tribe Plumerieae, but in different subtribes namely Rauvolfieae and Cerbereae. However, Leeuwenberg (1986) has placed these two genera under the same tribe Rauvolfieae. With respect to chromosome constitution the genus *Rauvolfia* showed x = 11, while the basic chromosome number of *Kopsia* is x = 9. Based on the

palynological data pollen grains of these two genera showed similarities in pollen aperture morphoform (3-zonocolporate) and exine ornamentation (reticulate). In the genus *Rauvolfia,* the presently studied species showed endoexine thickening in the colpus region behind the pseudocolpium, but such a special feature is absent in *Kopsia.* So the placement of these two genera in the two subtribes (Rauvolfieae and Cerbereae) under the tribe Plumerieae is more suitable.

Bentham and Hooker (1876) had placed the genera *Thevetia* and *Cerbera* in the subtribe Cerbereae under the tribe Plumerieae and Schumann (1895) had placed these two genera in the subtribe Cerberinae under the subfamily Plumieroideae. Pichon (1948) and Leeuwenberg (1986) had included these two genera in the same tribe Cerbereae under the subfamily Cerberoideae. Nilsson (1986) has included these two genera in the tribe Cerbereae based on palynological studies. Cytological data revealed same basic chromosome number (x = 10) in these two genera. Palynological characters also showed similarities among these genera on their aperture morphoform (3-zonocolporate), but vary in their exine ornamentation (microreticulate in *Thevetia* and densely punctate in *Cerbera*). So the placement of these two genera in the same tribe as suggested by Pichon (1948), Leeuwenberg (1986) and Nilsson (1986) is found correct (Table 27).

According to Nilsson (1986) the taxonomic position of *Allamanda* (under the tribe Allamandeae) stands doubtful. Allorge (1975) suggested its position either under Plumerioideae or Cerberoideae based on 3-colporate pollen grains. Fallen (1986) suggested the placement of *Allamanda* to a tribe of its own. However, in a previous

work, he has reported a possible relationship between *Allamanda* and the tribe Cerberoideae based on exine ultrastructure. Nilsson (1990) has also reported that the exine ultrastructure of *Allamanda cathartica* is very similar to *Thevetia amazonica*, therefore, he supported Fallen's (1986) assumption of relationship between the tribe Allamandeae and the tribe Cerbereae based on a number of other common features. *Thevetia* resembles *Allamanda* in funnel-shaped corolla, large flower, corona-like structures and syncarpous pistil, though they are diverse in habits, fruit characters, chromosome morphology and chromosome number (*Thevetia* x = 10, *Allamanda* x = 9). Thus phylogenetic relationship of *Thevetia* needs a critical review.

Bentham and Hooker (1876) included *Lochnera* Reich and *Vinca* under the same group. Bowden (1945) had already showed that *Lochnera rosea* with n = 9 is cytologically different from the other *Vinca* species (n = 23). Differences in morphological features corroborated with presently studied chromosome numbers justify the separation of *Catharanthus* (Syn = *Lochnera*) and *Vinca* into two distinct genera as was done by Leeuwenberg (1986) and Nilsson (1986). Nilsson (1990) has supported this suggestion from pollen morphological studies and gave them genus status as *Catharanthus* and *Vinca* in the tribe Plumerieae. The present observation supports Leeuwenberg's (1986) and Nilsson's (1986) classifications.

The two genera *Plumeria* and *Alstonia* have been placed in the subtribe Euplumerieae of the tribe Plumerieae by Bentham and Hooker (1876). While Schumann (1895) has included the genera *Plumeria* and *Alstonia* in the subtribe Alstoniinae of the subfamily Plumieroideae. But Pichon (1948), Engler (1964), and

Leeuwenberg (1986) have comprised these genera in the tribe Plumerieae under the subfamily Plumerioideae. Cytologically these two genera (*Plumeria* x = 9, and *Alstonia* x = 11) have shown different basic chromosome numbers. However, x = 9 might be a derived number from the original stock, x = 11 situation. Palynologically both these genera showed 3-zonocolporate aperture morphoform and reticulate ornamentation. Hence, their placement in the same tribe Plumerieae under the sub family Plumerioideae as suggested by Leeuwenberg (1986) is acceptable.

Bentham and Hooker (1876) included the genus *Tabernaemontana* under the tribe Plumerieae, and Schumann (1895) placed it under the subtribe Tabernaemontaninae. The genus *Tabernaemontana* was raised to the level of a tribe Tabernaemontaneae, under the subfamily Plumerioideae by Pichon (1948), Engler (1964), Leeuwenberg (1986) and Nilsson (1986). Cytologically all the species in the genus showed the chromosome number n = 11 and 2n = 22. The somatic chromosome length ranged from 2.75μm – 4.74μm, and karyotype category is 2A. Nilsson (1990) has reported that the pollen grains of *Tabernaemontana* have distinct features and are fairly uniform (3-colporate aperture with reticulate ornamentation). Hence, his suggestion of Tabernaemontanae as a tribe could be well accepted along with other tribes.

The genus *Holarrhena* was included in the major tribe Plumerieae by Bentham and Hooker (1876). However, Pichon (1948) and Leeuwenberg (1986) have placed this genus in the tribe Plumerieae under the subfamily Plumerioideae. Cytological data revealed that the basic chromosome number of the genus *Holarrhena* is x = 11.

Based on palynological characters, Nilsson (1990) has reported that the sub families Plumerioideae and the Cerberoideae have 3-colporate pollen grains, while the sub family Apocynoideae has 3-porate ones. The pollen grains of *Holarrhena* species have much resemblance to members of the Apocynoideae (*Pleioceras* and *Alafia*). De Kruif (1981) also has reported that there is similarity between *Holarrhena* and some Apocynoid members in their pollen characters, seed morphology and aestivation. In view of pollen characters and seed morphology, he urged the need for the placement of *Holarrhena* in the subfamily Apocynoideae. Endress *et al*. (1990) have also suggested the inclusion of the genus *Holarrhena* to the subfamily Apocynoideae under the tribe Nerieae, based on its special floral structure and pollen morphological features. The presence of 3-porate aperture condition and reticulate ornamentation observed in the pollen grains of the presently studied taxa of *Holarrhena* also give evidences to support the suggestions of the above mentioned authors.

Tribe III Echitideae

Bentham and Hooker (1876) treated it as a major tribe, with five subtribes viz., Parsonsieae, Nerieae, Ecdysanthereae, Ichnocarpeae and Euechitideae. Hooker (1882) had considered it as a major tribe Echitideae, with three subtribes viz., Parsonsieae, Nerieae and Euechitideae. Schumann (1895) had considered this tribe as a subfamily Echitoideae. Pichon (1948) and Leeuwenberg (1986) have considered it as a sub family Apocynoideae. They divided this sub family into four tribes viz., Parsonsieae (Echitheae), Nerieae, Apocyneae and Ichnocarpeae.

Members of the tribe Echitideae are mostly woody climbers and shrubs, but *Adenium* is a fleshy xerophyte. Bentham and Hooker (1876) placed the genera *Parsonsia, Vallaris* and *Wrightia* in the subtribe parsonsieae under the tribe Echitideae. But Pichon (1948) and Leeuwenberg (1986) have placed these genera in the tribe Nerieae under the subfamily Apocynoideae. Cytologically these genera showed different basic numbers (x = 9, 10 and 11). The genus *Vallaris* of the subtribe Parsonsieae under the tribe Echitideae (Bentham and Hooker, 1876) has several common morphological features with that of *Chonemorpha* of the subtribe Euechitideae under the tribe Echitideae. The similarities among them are climbing habit, salver-shaped corolla, follicular fruits and chromosome numbers. Therefore, the transference of the genus *Vallaris* from the subtribe Parsonsieae to subtribe Euechitideae may be more acceptable. But palynologically these two genus showed much differences in their pollen characters. In *Vallaris solanacea,* the pollen grains showed 4-porate aperture form and exine ornamentation micro reticulate. Their size ranged from 37.76µm to 48µm and shape is sub prolate, while *Chonemorpha fragrans* has 3-zonocolporate aperture form with reticulate exine ornamentation. Their size ranged from 41.60µm – 43.52µm and prolate spheroidal in shape. Studies on other aspects may give additional evidences to clarify their relationship.

The genera *Nerium* (x = 11) and *Strophanthus* (x = 9) were included in the sub tribe Nerieae by Bentham and Hooker (1876) and in the tribe Echitideae by Schumann (1895). Leeuwenberg (1986) placed *Nerium* in the new tribe Nerieae of the sub family Apocynoideae. Palynologically *Nerium* and *Strophanthus* (*S. gratus*) showed porate

pollen grains and reticulate heterobrochate exine ornamentation. But one species of *Strophanthus* (*S. wightianus*) showed 3-zonocolporate pollen grains. Morphologically members of the two genera are shrubs (*Nerium oleander and Strophanthus gratus*) having watery latex. So the placement of these two genera in the same tribe is more suitable.

Bentham and Hooker (1876) had considered the placement of the genus *Ichnocarpus* in a separate sub tribe Ichnocarpeae under the major tribe Echitideae. But on taxonomic evidences the genus *Ichnocarpus* was raised to the level of a tribe Ichnocarpeae, under the subfamily Apocynoideae by Pichon (1948) and Leeuwenberg (1986). Cytologically the genus showed the basic number x = 10. Palynological data showed that they have 3-zonocolporate aperture form and reticulate heterobrochate ornamentation. This distinct nature of cytological and palynological characters support the elevation of the genus as a tribe Ichnocarpeae under the subfamily Apocynoideae.

Cytologically the genus *Adenium* and *Nerium* showed similarity (x = 11). The palynological data showed that they have 3-porate pollen grains. Hence, the placement of the genus *Adenium* along with *Nerium* in the same tribe Nerieae under the subfamily Apocynoideae is acceptable (Table 27).

The genera *Aganosma* and *Odontadenia* are climbing shrubs. Cytological data showed that in *Aganosma* x = 11,whereas in *Odontadenia* x = 12. Schumann (1895) included *Aganosma* under the tribe Echitideae, while Pichon (1948) and Leeuwenberg (1986) have considered the genus under the tribe Parsonsieae and Echitheae respectively

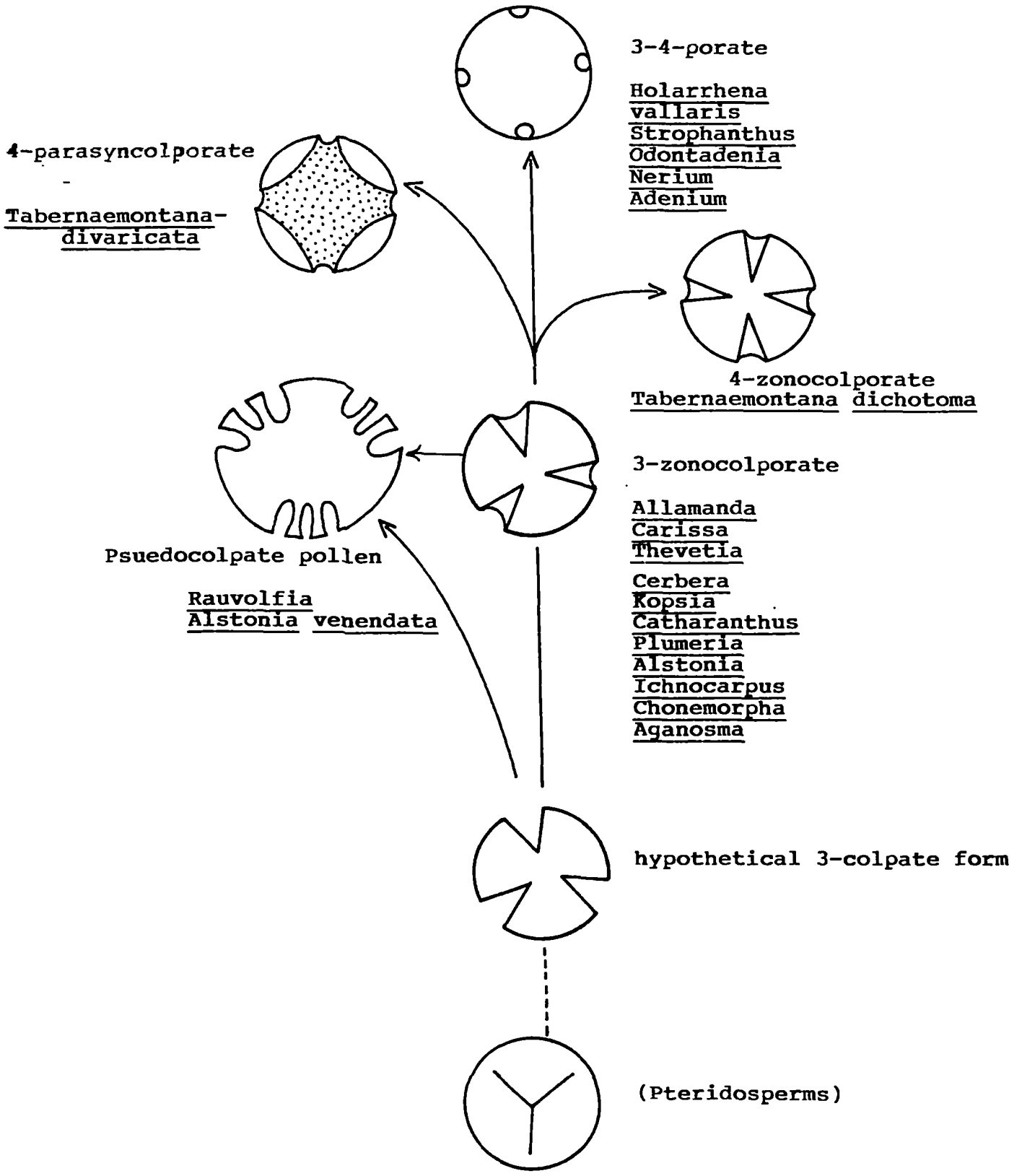
under the subfamily Apocynoideae. Palynological data also showed dissimilarities like 3-porate pollen grains with reticulate heterobrochate exine ornamentation and sub prolate shape in *Odontadena*, while *Aganosma* species showed 3-zonocolporatet aperture form with reticulate exine ornamentation and euprolate shape. So the placement of these two genera in separate tribes under the subfamily Apocynoideae is found more justifiable (Table 27).

Table 27. Systematic positions of the presently studied general according to the classification of Bentham and Hooker (1876), Schumann (1895), Pichon (1948) and Leeuwenberg (1986) and Nilsson (1986)

Genera	Bentham and Hooker (1876)	Schumann (1895)	Pichon (1948)	Leeuwenberg (1986)	Nilsson (1986)
Carissa	Tribe I. Carisseae	Subfamily I. Plumieroideae Tribe 1.1. Auduineae Subtribe 1.1a. Melodininae	Subfamily I. Plumerioideae Tribe 1. Carisseae	Subfamily I. Plumerioideae Tribe 1. Carisseae	Subfamily I. Plumerioideae Tribe 1. Carisseae
Allamanda	Tribe I. Carisseae	Subfamily I. Plumieroideae Tribe 1.1. Arduineae Subtribe 1.1b.Landolphiinae	Subfamily I. Plumerioideae Tribe 5. Allamandeae	Subfamily I. Plumerioideae Tribe 8. Allamandeae	Subfamily I. Plumerioideae Tribe 8. Allamandeae
Rauvolfia	Tribe II. Plumerieae Subtribe 1. Rauvolfieae	Subfamily I. Plumieroideae Tribe 1.3. Plumiereae Subtribe 1.3c. Rauwolfiinae	Subfamily I. Plumerioideae Tribe 4. Rauvolfieae	Subfamily I. Plumerioideae Tribe 7. Rauvolfieae	Subfamily I. Plumerioideae Tribe 7. Rauvolfieae
Thevetia	Tribe II. Plumerieae Subtribe 2. Cerbereae	Subfamily I. Plumieroideae Tribe 1.3. Plumiereae Subtribe 1.3d. Cerberinae	Subfamily II. Cerberoideae Tribe 6. Cerbereae	Subfamily II. Cerberoideae Tribe 9. Cerbereae	Subfamily II. Cerberoideae Tribe 9. Cerbereae
Cerbera	Tribe II. Plumerieae Subtribe 2. Cerbereae	Subfamily I. Plumieroideae Tribe 1.3. Plumiereae Subtribe 1.3d. Cerberinae	Subfamily II. Cerberoideae Tribe 6. Cerbereae	Subfamily II. Cerberoideae Tribe 9. Cerbereae	Subfamily II. Cerberoideae Tribe 9. Cerbereae
Kopsia	Tribe II. Plumerieae Subtribe 2. Cerbereae	Subfamily I. Plumieroideae Tribe 1.3. Plumiereae Subtribe 1.3d. Cerberinae		Subfamily I. Plumerioideae Tribe 3. Rauvolfieae	Subfamily I. Plumerioideae Tribe 3. Rauvolfieae
Vinca (Lochnera)	Tribe II. Plumerieae Subtribe 3. Euplumerieae	Subfamily I. Plumieroideae Tribe 1.3. Plumiereae Subtribe 1.3a. Alstoniinae		Subfamily I. Plumerioideae Tribe 6. Plumerieae	Subfamily I. Plumerioideae Tribe 6. Plumerieae
Catharanthus			Subfamily I. Plumerioideae Tribe 3. Plumerieae	Subfamily I. Plumerioideae Tribe 6. Plumerieae	Subfamily I. Plumerioideae Tribe 6. Plumerieae
Plumeria	Tribe II. Plumerieae Subtribe 3. Euplumerieae	Subfamily I. Plumieroideae Tribe 1.3. Plumiereae Subtribe 1.3a. Alstoniinae	Subfamily I. Plumerioideae Tribe 3. Plumerieae	Subfamily I. Plumerioideae Tribe 6. Plumerieae	Subfamily I. Plumerioideae Tribe 6. Plumerieae
Alstonia	Tribe II. Plumerieae Subtribe 3. Euplumerieae	Subfamily I. Plumieroideae Tribe 1.3. Plumiereae Subtribe 1.3a. Alstoniinae	Subfamily I. Plumerioideae Tribe 3. Plumerieae	Subfamily I. Plumerioideae Tribe 6. Plumerieae	Subfamily I. Plumerioideae Tribe 6. Plumerieae

Tabernae-montana	Tribe II. Plumerieae Subtribe 4. Tabernae-montaneae	Subfamily I. Plumiroideae Tribe 1.3. Plumiereae Subtribe 1.3b.Tabernae-montaninae	Subfamily I. Plumerioideae Tribe 2. Tabernaemon-taneae	Subfamily I. Plumerioideae Tribe 5. Tabernaemon-taneae	Subfamily I. Plumerioideae Tribe 5. Tabernaemon-taneae
Holarrhena	Tribe II. Plumerieae Subtribe 4. Tabernae-montaneae		Subfamily I. Plumerioideae Tribe 3. Plumerieae	Subfamily I. Plumerioideae Tribe 6. Plumerieae	Subfamily I. Plumerioideae Tribe 6. Plumericae
Vallaris	Tribe III. Echitideae Subtribe 1. Parsonsieae	Subfamily III. Echitoideae Tribe II.5. Parsonsieae	Subfamily III. Apocynoi-deae Tribe 8. Nerieae	Subfamily III. Apocynoi-deae Tribe II. Nerieae	Subfamily III. Apocynoi-deae Tribe II. Nerieae
Parsonsia	Tribe III. Echitideae Subtribe 1. Parsonsieae	Subfamily III.Apocynoideae Tribe II. Parsonsieae	Subfamily III. Apocynoi-deae Tribe 7. Parsonsieae		
Wrightia	Tribe III. Echitideae Subtribe 1. Parsonsieae	Subfamily II. Echitoideae Tribe II.5. Parsonsieae	Subfamily III. Apocynoi-deae Tribe 10. Nerieae	Subfamily III. Apocynoi-deae Tribe 8. Nerieae	Subfamily III. Apocynoi-deae Tribe II. Nerieae
Nerium	Tribe III. Echitideae Subtribe 2. Nerieae	Subfamily II. Echitoideae Tribe II.4. Echitideae	Subfamily III. Apocynoi-deae Tribe 8. Nerieae	Subfamily III. Apocynoi-deae Tribe II. Nerieae	Subfamily III. Apocynoi-deae Tribe II. Nerieae
Strophan-thus	Tribe III. Echitideae Subtribe 2. Nerieae	Subfamily II. Echitoideae Tribe II.4. Echitideae		Subfamily III. Apocynoi-deae Tribe II. Nerieae	Subfamily III. Apocynoi-deae Tribe II. Nerieae
Ichnocar-pus	Tribe III. Echitideae Subtribe 4.Ichnocarpeae		Subfamily III. Apocynoi-deae Tribe 10. Ichnocarpeae	Subfamily III. Apocynoi-deae Tribe 10. Ichnocarpeae	Subfamily III. Apocynoi-deae Tribe 10. Ichnocarpeae
Chone-morpha	Tribe III. Echitideae Subtribe 5.Euechitideae	Subfamily II. Echitoideae Tribe II.4. Echitideae	Subfamily III. Apocynoi-deae Tribe 7. Parsonsieae	Subfamily III. Apocynoi-deae Tribe 10. Echitheae	Subfamily III. Apocynoi-deae Tribe 10. Echitheae
Adenium	Tribe III. Echitideae Subtribe 5. Euechitideae	Subfamily II. Echitoideae Tribe II.4. Echitideae		Subfamily III. Apocynoi-deae Tribe II. Nerieae	Subfamily III. Apocynoi-deae Tribe II. Nerieae
Odonta-denia	Tribe III. Echitideae Subtribe 5.Euechitideae	Subfamily II. Echitoideae Tribe II.4. Echitideae			
Aganosma		Subfamily II. Echitoideae Tribe II.4. Echitideae	Subfamily III. Apocynoi-deae Tribe 7. Parsonsieae	Subfamily III. Apocynoi-deae Tribe 10. Echitheae	Subfamily III. Apocynoi-deae Tribe 10. Echitheae

Chart 1. A tentative scheme of evolution of pollen aperture in the family Apocynaceae (Present Study)



SUMMARY

This thesis includes the cytological and palynological studies on South Indian members of the family Apocynaceae and the results are presented in two parts, Part-I cytological studies and Part-II palynological studies.

Part – I Cytological studies

1. This part comprises the results of cytological studies on 40 taxa under 20 genera (*Allamanda, Carissa, Rauvolfia, Thevetia, Cerbera, Kopsia, Catharanthus, Vinca, Plumeria, Alstonia, Tabernaemontana, Holarrhena, Vallaris, Parsonsia, Wrightia, Strophanthus, Ichnocarpus, Chonemorpha, Aganosma* and *Adenium*) under 3 tribes viz., Carisseae, Plumerieae and Echitideae of the family.
2. On the basis of the present findings, together with available chromosome data, cytological evolution of the group is discussed in terms of basic chromosome numbers and polyploidy in speciation.
3. The gametic chromosome numbers recorded in the present study are n = 8, 9, 10, 11, 18, 22 and 23, of which n = 11 is the most frequent one.
4. It has been noticed that x = 11 in the Apocynaceae is the established basic constitution, which could be a secondarily evolved number by doubling of the primary basic number x = 6 followed by the loss of one chromosome through descending aneuploidy.

5. Among the 20 genera, polyploidy was relatively less frequent. Out of the 40 taxa, 9 were polyploids. The highest level of ploidy noticed in the present study was hexaploid.
6. Overall chromosome data showed that many species are species complexes with chromosome numbers existing in different ploidy levels. Such intraspecific polyploidy was seen in varieties of *Tabernaemontana divaricata* (2x, 3x) during the present study.
7. Karyotype analysis has been done on ten taxa belonging to four genera (*Allamanda, Carissa, Thevetia* and *Tabernaemontana*). Out of the three species of *Allamanda*, two (*A. cathartica, A. schottii*) showed 2A karyotype category and TF% 40.86 and 40.35 respectively. *A. neriifolia* showed 2B category, and TF% 35.97 which indicates a relatively more advanced condition than the other two species. *Carissa carandas* showed 1B karyotype category and TF% was 43.52. Two varieties of *Thevetia peruviana* showed 1B karyotype category and their TF% were 44.90 and 43.50 respectively. Four varieties of *Tabernaemontana divaricata* showed 2A karyotype category and their TF% varies as 34.53, 39.03, 43.47 and 34.39.

Part – II Palynological studies

1. Palynological studies have been carried out in 37 taxa coming under 19 genera (*Allamanda, Carissa, Rauvolfia, Thevetia, Cerbera, Kopsia, Catharanthus, Plumeria, Alstonia, Tabernaemontana, Holarrhena, Vallaris, Strophanthus, Ichnocarpus, Chonemorpha, Aganosma, Adenium, Odontadenia* and *Nerium*)

belonging to 3 tribes (Carisseae, Plumerieae and Echitideae). All the taxa included here were studied for the first time from South India.

2. Pollen grains were seen in monad form.
3. Palynological observations were made from LM (37 taxa) as well as SEM (16 taxa). The pollen morphological characters studied includes the number of apertures, exine sculpturing, exine thickness, pollen size and shape.
4. Palynological observations showed that the Apocynaceae is eurypalynous with different apertural types such as 3-zonocolporate, 4-zonocolporate, 4-parasyncolporate, 3-porate and 4-porate. However, the data of their distribution revealed that the 3-zonocolporate pollen type predominates in this family.
5. The exine sculpturing showed considerable diversity. Majority of the pollen grains have the depression type of ornamentation (foveolate, reticulate, reticulate heterobrochate, microreticulate). The reticulate and reticulate heterobrochate forms are predominated among the presently studied taxa, and were seen in almost same frequency. Thus the basic pollen type in Apocynaceae is tricolporate-reticulate or reticulate heterobrochate.
6. The size and shape of the pollen vary considerably. The pollen grains showed different shapes such as subprolate, prolate-spheroidal, euprolate and spheroidal. Regarding the size of the pollen, medium, large and very large pollen grains were observed.
7. The tribe Carisseae is stenopalynous. Four species of *Allamanda* (*A. cathartica*, *A. schottii*, *A. neriifolia*, *A. violacea*) showed 3-zonocolporate aperture, ora circular to

lalongate and large grains. Exine surface is rugulate in *A. violacea, A. cathartica* and *A. neriifolia* whereas it is foveolate in *A. schottii*. Shape showed subprolate condition except in *A. violacea* (prolate-spheroidal).

8. Two species of the genus *Carissa* (*C. carandas* and *C. spinarum*) showed 3-zonocolporate aperture, ora lalongate and prolate-spheroidal shape category and grains are medium sized. Exine surface varied in nature, granulate perforate or punctate in *C. spinarum* whereas scrobiculate in *C. carandas*.
9. Nine genera belonging to the tribe Plumerieae have been studied. Four species of *Rauvolfia* (*R. serpentina, R. tetraphylla, R. beddomei* and *R. densiflora*) showed 3-zonocolporate grains with reticulate exine pattern. Ora circular to lalongate in all the species. A special feature, endoexine thickening in the colpus margin (kidney shaped) behind the psuedocolpi was noticed in *R. serpentina*. The thickening in the colpus region was also seen in other species of *Rauvolfia* (*R. tetraphylla, R. beddomei* and *R. densiflora*).
10. *Thevetia peruviana* showed 3-zonocolporate, prolate-spheroidal pollen grains, lalongate ora, micro reticulate exine surface.
11. In *Cerbera odollam* and *Kopsia fruticosa* the pollen grains are 3-zonocolporate and ora lalongate. In the former exine pattern is densily punctate, while it is reticulate in the latter. A depression was noticed in the apocolpate and mesocolpate regions in *C. odollam*.
12. Two varieties of *Catharanthus roseus* showed 3-zonocolporate aperture, lolongate ora and reticulate heterobrochate exine ornamentation.

13. Two species of *Plumeria* (*P. alba* and *P. rubra*) showed 3-zonocolporate grains, ora circular to lalongate. But their exine surface varied. Reticulate/reticulate heterobrochate in two varieties of *P. rubra*, but microreticulate in *P. alba*.

14. In the genus *Alstonia*, two species (*A. scholaris* and *A. venenata*) have been studied. *A. scholaris* showed 3-zonocolporate aperture with reticulate ornamentation and spheroidal shape. While *A. venenata* showed 3-zonocolporate aperture form with thickening in the colpus region, and reticulare heterobrochate exine ornamentation.

15. In *Tabernaemontana*, four varieties of *T. divaricata* showed 4-parasyncolporate aperture, reticulate heterobrochate exine surface, but in *T. dichotoma* the grains showed 3(4-) zonocolporate aperture and reticulate exine pattern.

16. *Holarrhena antidysenterica* showed 3-porate and medium sized grains with foveolate exine ornamentation. This is a deviation in pollen characters from the rest of the genera, in the tribe Plumerieae, where the grains possessed 3-zonocolporate aperture, and reticulate/reticulate heterobrochate ornamentation.

17. *Vallaris solanacea* showed 4-porate and subprolate, medium sized grains with micro reticulate exine ornamentation.

18. Two species of *Strophanthus* (*S. gratus, S. wightianus*) have been studied. *S. gratus* showed 3-porate grains with reticulate heterobrochate exine, while *S. wightianus* showed 3-zonocolporate with microreticulate exine pattern.

19. *Ichnocarpus frutescens* possessed 3-zonocolporate and medium sized prolate-sperhoidal grains with reticulate heterobrochate exine ornamentation and ora lalongate.
20. *Chonemorpha fragrans* showed 3-zonocolporate, large pollen grains with lalongate ora, reticulate ornamentation and subprolate shape.
21. *Aganosma caryophyllata* showed 3-zonocolporate grains with lalongate ora, reticulate exine surface and euprolate shape.
22. *Adenium obesum* possessed 3-porate, medium sized pollen grains with reticulate exine pattern and prolate-spheroidal shape.
23. *Odontadenia grandiflora* showed 3-porate grarins with reticulate heterobrochate exine ornamentation and subprolate shape.
24. Two varieties of *Nerium oleander* showed 3(4-)porate medium sized pollen grains with irregular pore. The exine surface in variety 1 has reticulate heterobrochate pattern and subprolate shape, while variety 2 showed reticulate exine surface and prolate-spheroidal shape. Third variety showed 3(4-)porate aperture, circular pore, reticulate heterobrochate exine thickness and subprolate shaped pollen grains.
25. In the tribe Plumerieae, four types could be recognized in terms of aperture characters such as 3-zonocolporate, 4-zonocolporate, 4-parasyncolporate and 3-porate. It is assumed that 3-zonocolporate condition is the primitive type and 3-4 porate is the advanced type in the family.

Systematic consideration

1. Intrafamilial relationship of the Apocynaceae, according to the classification of Bentham and Hooker (1876), Schumann (1895), Pichon (1948), Engler (1964), Leeuwenberg (1986) and Nilsson (1986) were discussed in the light of available cytological and palynological data. It has been noticed from the reported descriptions that species of *Allamanda* are more related to *Thevetia* based on morphological and palynological characters. Cytologically also *Allamanda* (x = 9) is more related to *Thevetia* (x = 10) where x = 9 situation might be derived from x = 10 by descending aneuploidy.
2. Differences in morphological characters corroborated with presently studied chromosome numbers justify the separation of *Catharanthus* and *Vinca* into two distinct genera under the tribe Plumerieae as was done by Leenwenberg (1986) and Nilsson (1986).
3. The unique nature in cytology (x = 11, 2A karyotype category) and palynology (3-zonocolporate and 4-parasyncolporate aperture with reticulate ornamentation) support the placement of *Tabernaemontana* in a higher status as Tabernaemontaneae.
4. The genus *Holarrhena* was included in the major tribe Plumerieae by Bentham and Hooker (1876). But its palynological characters (3-porate condition) shed light on evidences to show more relationships to Echitideae.
5. Though *Vallaris* and *Chonemorpha* were included under the subtribe Euechitideae of the tribe Echitideae by Bentham and Hooker (1876), they are similar in

morphological characters such as climbing habit, salver shaped corolla and follicular fruits. But palynologically they showed differences in their aperture form. Hence further studies may be needed to prove their position.

6. The distinct cytological and palynological characters of the genus *Ichnocarpus* supported its elevation to the status of a tribe as suggested by Leeuwenberg (1986) and Nilsson (1986).
7. *Aganosma* and *Odontadenia* are climbing shrubs, but cytologically they showed different basic chromosome constitutions (*Aganosma* x = 11, *Odontadenia* x = 12). Palynologically also they are dissimilar, where members of *Aganosma* possessed 3-zonocolporate aperture with reticulate exine ornamentation, and *Odontadenia* possessed 3-porate pollen grains with reticulate heterobrochate exine ornamentation. Hence, their placement in separate tribes under the subfamily Apocynoideae is more justifiable.
8. Interfamilial relationship of the family Apocynaceae was also considered. It has been noticed that the family shows affinity towards Asclepiadaceae, particularly to the subfamily Periplocoideae based on basic chromosome numebr (x=11) and pollen characters.

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* Original not seen.

Appendix 1. Sources of Plant Materials Collected

Name of Taxa	Locality	Voucher Number (KUBOTH Number)
Allamanda cathartica Linn.	Palode, Kariavattom, Kottayam	4900
Allamanda schottii Pohl.	Palode, Kariavattom, Kottayam	4901
Allamanda neriifolia Hook.	Thiruvananthapuram, Wayanadu	4902
Allamanda violacea Gardn. and Field.	Palode, Kariavattom, Nagercoil, Bangalore	4903
Carissa carandas Linn.	Kottayam, Kariavattom, Nagercoil	4904
Carissa spinarum Linn.	Veli, Kollam, Ponmudi, Thirunelveli	4905
Rauvolfia serpentina Benth. ex Kurz.	Kulathupuzha, Aryankavu	4906
Rauvolfia tetraphylla Linn.	Kulathupuzha, Kariavattom, Palode	4907
Rauvolfia beddomei Hook. f.	Palode, Kariavattom	4908
Rauvolfia densiflora Benth. ex Hook. f.	Palode, Aryankavu	4909
Thevetia peruviana (Pers.) K. Schum. (variety 1)	Thiruvananthapuram, Kollam	4910
Thevetia peruviana (Pers.) K. Schum. (variety 2)	Kariavattom, Ponmudi, Pathanamthitta, Nagercoil	4911
Cerbera odollam Gaertn.	Kollam, Paravoor, Sasthamkottah	4912
Copsia fruticosa A. DC.	Palode, Thiruvananthapuram, Bonacaud, Nagercoil, Mysore	4913
Catharanthus roseus (L.) G. Don. variety 1)	Veli, Kariavattom, Aryankavu, Kollam	4914
Catharanthus roseus (L.) G. Don. variety 2)	Thiruvananthapuram, Kollam, Aryankavu, Kottayam	4915
Vinca major Linn.	Botanic Garden, Ootacamand, Nilgiris	4916

Plumeria alba Linn.	Kariavattom, Thiruvananthapuram, Kottayam, Munnar, Ranni, Madurai	4917
Plumeria rubra Linn.	Thiruvananthapuram, Kollam, Kottayam, Konni, Ranni, Madurai	4918
Alstonia scholaris R. Br.	Kulathupuzha, Palaruvi and Thiruvananthapuram	4919
Alstonia venenata R. Br.	Kallar, Palaruvi, Aryankavu	4920
Tabernaemontana dichotoma Roxb.	Kottayam, Thiruvananthapuram, Palode, Pambavalli, Maruthamalai, Mettupalayam	4921
Tabernaemontana divaricata R.Br. ex Roem. and Schult (var.1)	Thiruvananthapuram, Courtallum, Peermade	4922
Tabernaemontana divaricata R.Br.ex.Roem. and Schult. (var.2)	Kottayam, Thiruvananthapuram, Munnar, Kodaikanal, Coimbatore	4923
Tabernaemontana divaricata R.Br.ex.Roem. and Schult. (var.3)	Thiruvananthapuram, Palode, Coimbatore	4924
Tabernaemontana divaricata R.Br. ex.Roem. and Schult.(var.4)	Kariavattom, Palode, Kottayam, Mettupalayam, Bangalore	4925
Tabernaemontana divaricata R.Br.ex.Roem. and Schult. (var.5)	Thiruvananthapuram, Nagercoil, Mysore, Bangalore	4926
Holarrhena antidysenterica Wall.	Kariavattom, Ponmudi, Yercaud, Silent Valley	4927
Vallaris solanacea (Roth.)Kuntze.	Thiruvananthapuram, Palaruvi, Thenmala, Bangalore	4928
Vallaris lancifolia Hook. f.	Wayanadu, Kulathupuzha, Mysore	4929
Parsonsia spiralis Wall.	Palaruvi, Kallar, Kulathupuzha, Silent Valley	4930
Wrightia tinctoria R. Br.	Wayanadu, Kallar, Coimbatore	4931
Strophanthus gratus (Wall. and Hook.)	Wayanadu, Thiruvananthapuram, Kodaikanal	4932

Strophanthus wightianus Wall.	Wayanadu, Kottayam, Thiruvananthapuram, Thirunelveli, Silent Valley	4933
Ichnocarpus frutescens (L.) R. Br.	Kallar, Palaruvi, Kayamkulam, Thekkady	4934
Ichnocarpus ovatifolius A.DC.	Palaruvi, Kulathupuzha, Munnar	4935
Chonemorpha fragrans (Moon.) Alston.	Kallar, Kottayam, Ponmudi, Bonacaud	4936
Chonemorpha griffithii Hook.	Palaruvi, Kallar	4937
Adenium obesum Roem. and Schult.	Veli, Coimbatore, Courttalam, Ootacamund	4938
Aganosma caryophyllata G. Don.	Wayanadu, Thiruvananthapuram, Thenmala	4939

CYTOLOGY

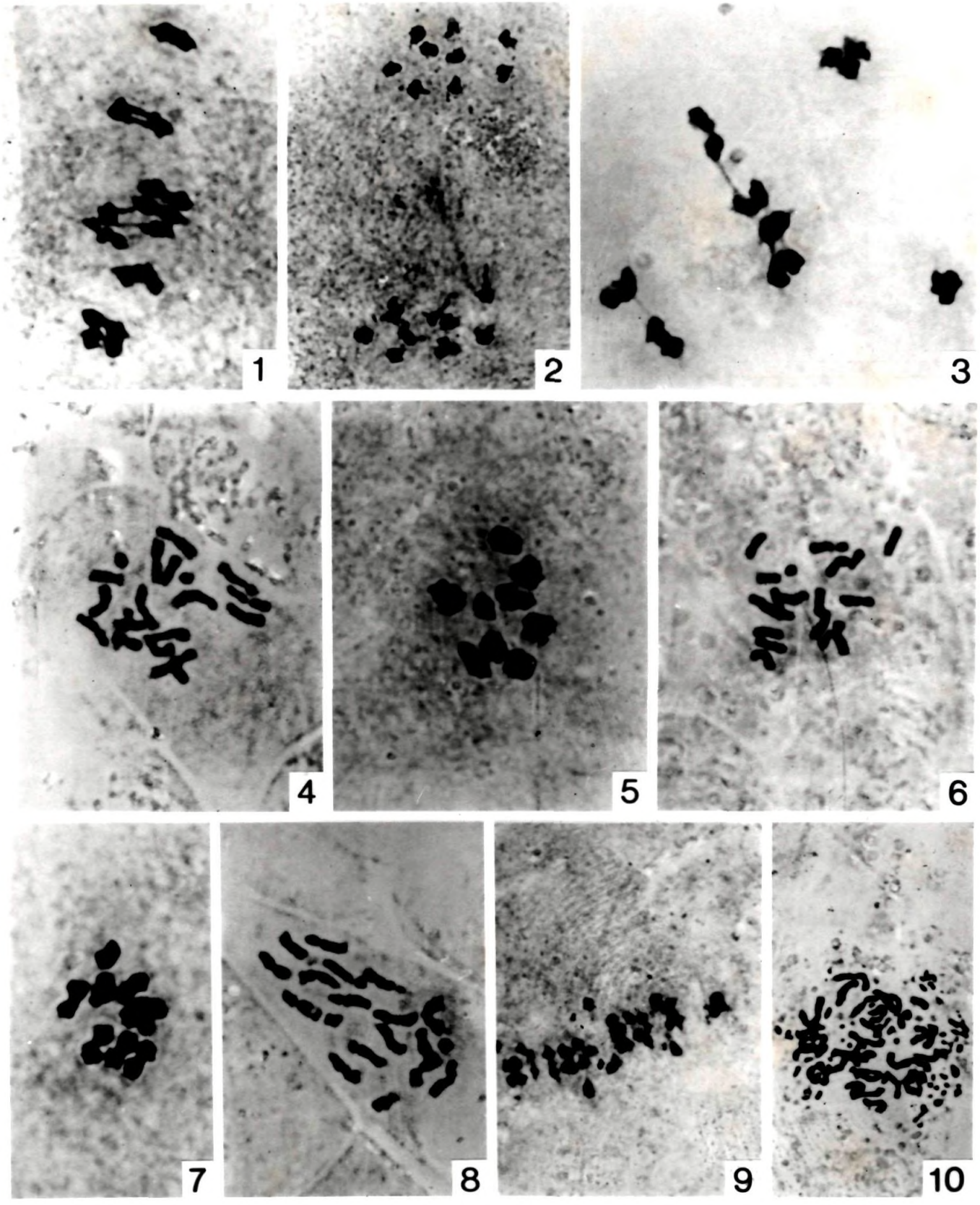
Explanation of Figures

(PMC = Pollen Mother Cells)

(All Figures x 1500)

(Figs. 1 – 10)

Figs.1 – 4	*Allamanda cathartica*
Fig. 1	PMC showing 9 bivalents at metaphase I
Fig. 2	PMC showing regular segregation of chromosomes at anaphase I
Fig. 3	PMC showing secondary association of chromosomes
Fig. 4	Root tip cell with 18 chromosomes at metaphase
Figs. 5 – 6	*Allamanda schottii*
Fig. 5	PMC showing 9 bivalents at metaphase I
Fig. 6	Root tip cell with 18 chromosomes at metaphase
Figs. 7 – 8	*Allamanda neriifolia*
Fig. 7	PMC showing 9 bivalents at metaphase I
Fig. 8	Root tip cells showing 18 chromosomes at metaphase
Figs. 9 – 10	*Allamanda violacea*
Fig. 9	PMC showing 18 bivalents at metaphase I
Fig. 10	Root tip cell showing 36 chromosomes at metaphase.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Explanation of Figures

(PMC = Pollen Mother Cells)

(All Figures x 1500)

(Figs. 11 – 19)

Fig. 11 *Carissa spinarum*, root tip cell showing 22 chromosomes at metaphase

Fig. 12 *Carissa carandas*, root tip cell showing 22 chromosomes at metaphase

Fig. 13 - 14 *Rauvolfia serpentina*

Fig. 13 PMC showing 11 bivalents at metaphase I

Fig. 14 Root tip cell showing 22 chromosome at metaphase

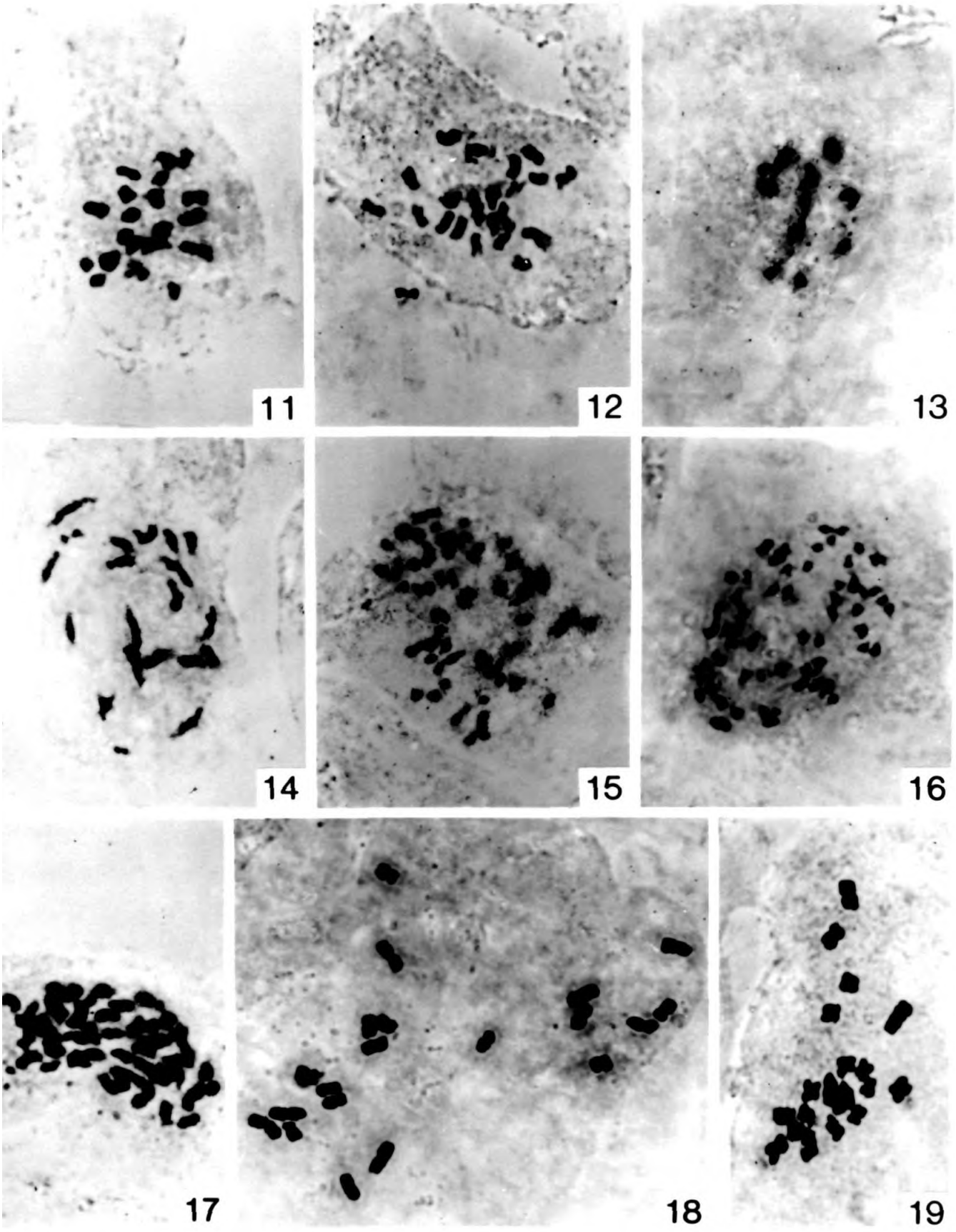
Fig. 15 *Rauvolfia tetraphylla*, root tip cell showing 55 chromosomes

Fig. 16 *Rauvolfia beddomei*, PMC showing 66 bivalents at metaphase I

Fig. 17 *Rauvolfia densiflora*, root tip cell showing 44 chromosomes at metaphase

Fig. 18 *Thevetia peruviana*, (var. 1), root tip cell showing 20 chromosomes at metaphase

Fig. 19 *Thevetia peruviana*, (var. 2), root tip cell showing 20 chromosomes at metaphase.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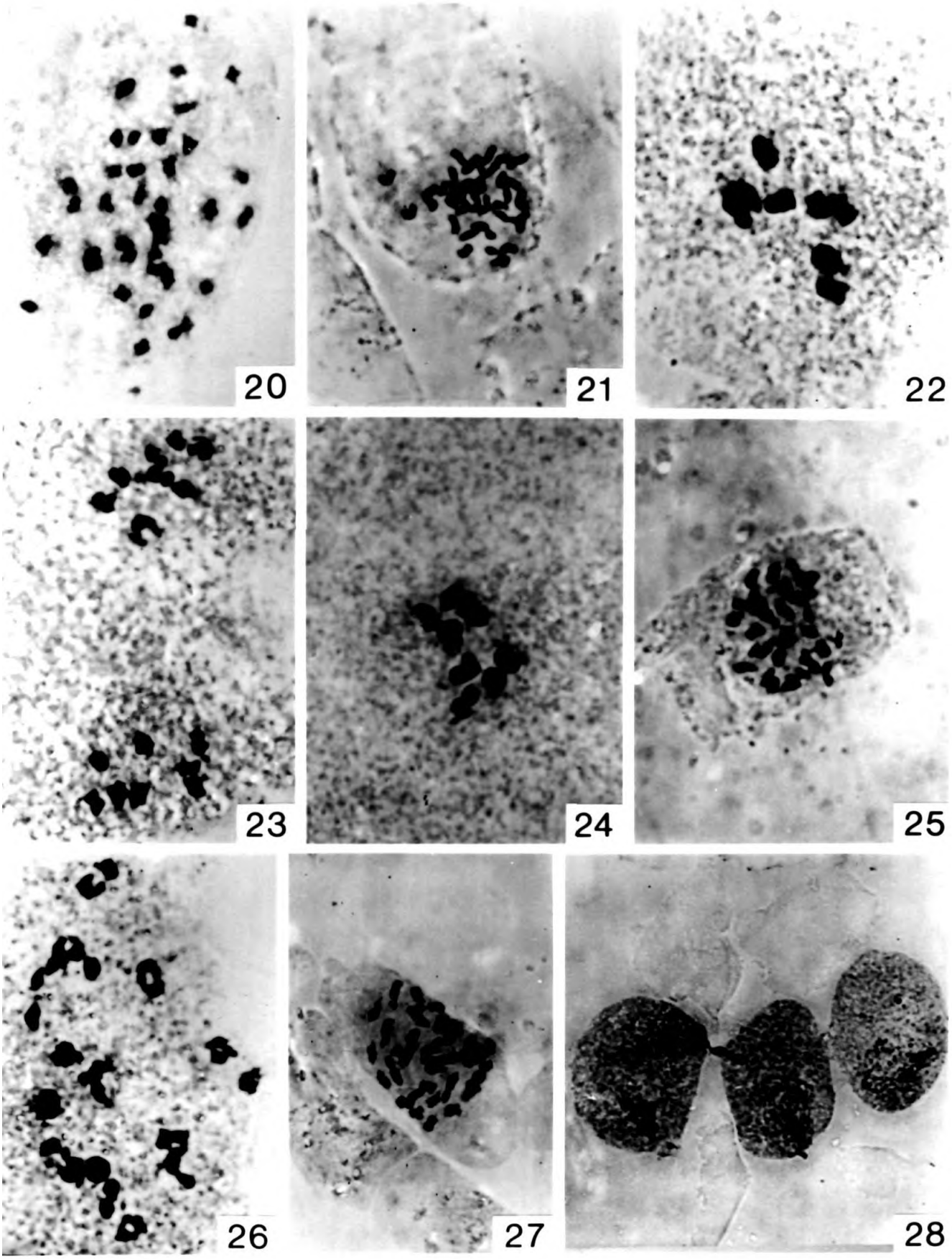
Explanation of Figures

(PMC = Pollen Mother Cells)

(All Figures x 1500)

(Figs. 20 – 28)

Fig. 20	*Cerbera odollam*, root tip cell showing 40 chromosomes at metaphase
Fig. 21	*Kopsia fruticosa*, root tip cell showing 36 chromosome at metaphase
Fig. 22 – 23	*Catharanthus roseus* (variety 1))
Fig. 22	PMC showing 8 bivalents at metaphase I
Fig. 23	PMC showing equal distribution of chromosome at anaphase I
Fig. 24	*Catharanthus roseus* (variety 2), PMC showing 8 bivalents at metaphase I
Fig. 25	*Vinca major*, PMC showing 23 bivalents at metaphase I
Figs. 26 – 27	*Plumeria alba*
Fig. 26	PMC showing 18 bivalents at diakinesis
Fig. 27	Root tip cell showing 36 chromosomes at metaphase
Figs. 28 – 34	*Plumeria rubra*
Fig. 28	Meiocytes showing cytomixis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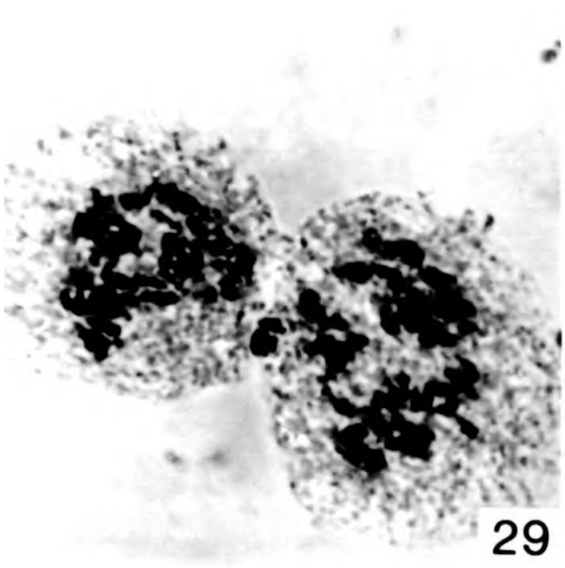
Explanation of Figures

(PMC = Pollen Mother Cells)

(All Figures x 1500)

(Figs. 29 – 38)

Fig. 29	PMCs showing the movement of chromatin material through cytoplasmic connection
Fig. 30	PMC showing chromosome clumping at metaphase I
Fig. 31	PMC showing sticky bridges at anaphase I
Fig. 32	PMC showing lagging chromosomes at anaphase I
Fig. 33	PMC showing 18 bivalents at metaphase I
Fig. 34	Root tip cell showing 36 chromosomes at metaphase
Figs. 35 – 36	*Alstonia scholaris*
Fig. 35	PMC showing 22 bivalents at metaphase I
Fig. 36	Root tip cells showing 44 chromosomes at metaphase
Figs. 37 – 38	*Alstonia venenata*
Fig. 37	PMC showing 11 bivalents at metaphase I
Fig. 38	Root tip cell showing 22 chromosomes at metaphase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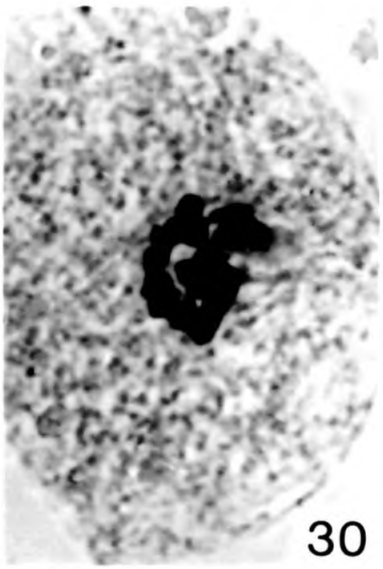
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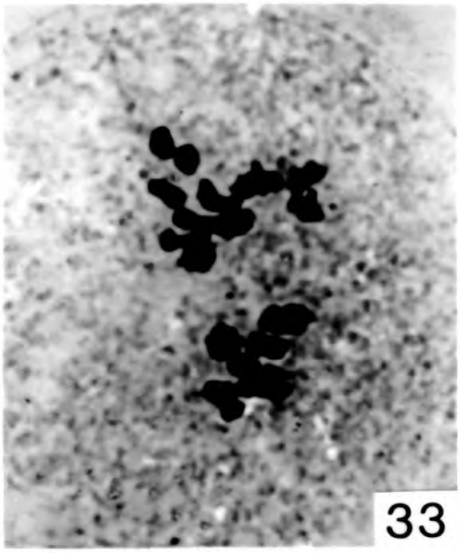
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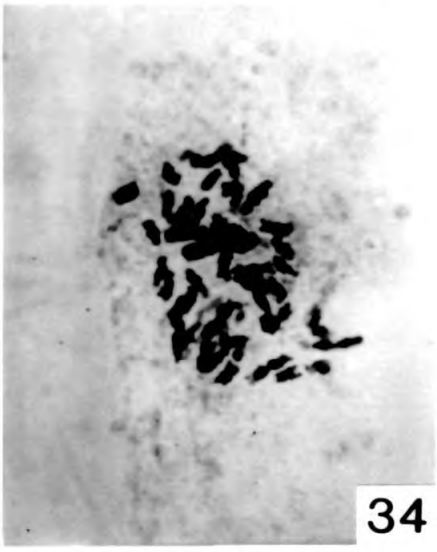
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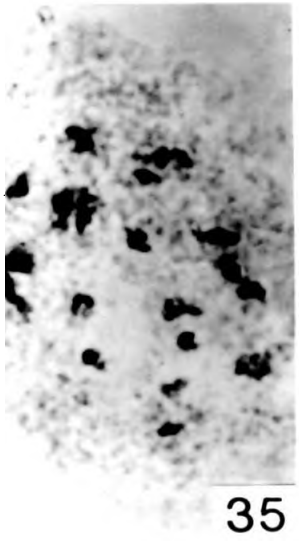
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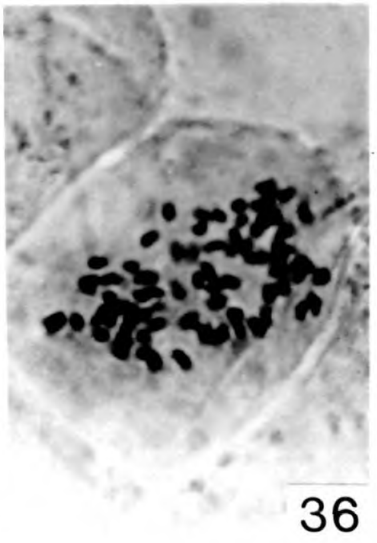
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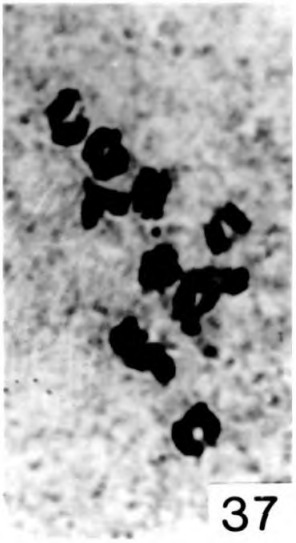
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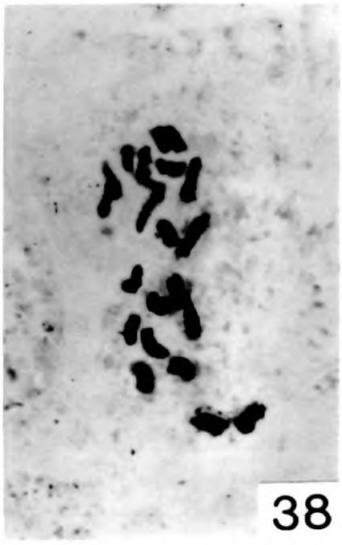
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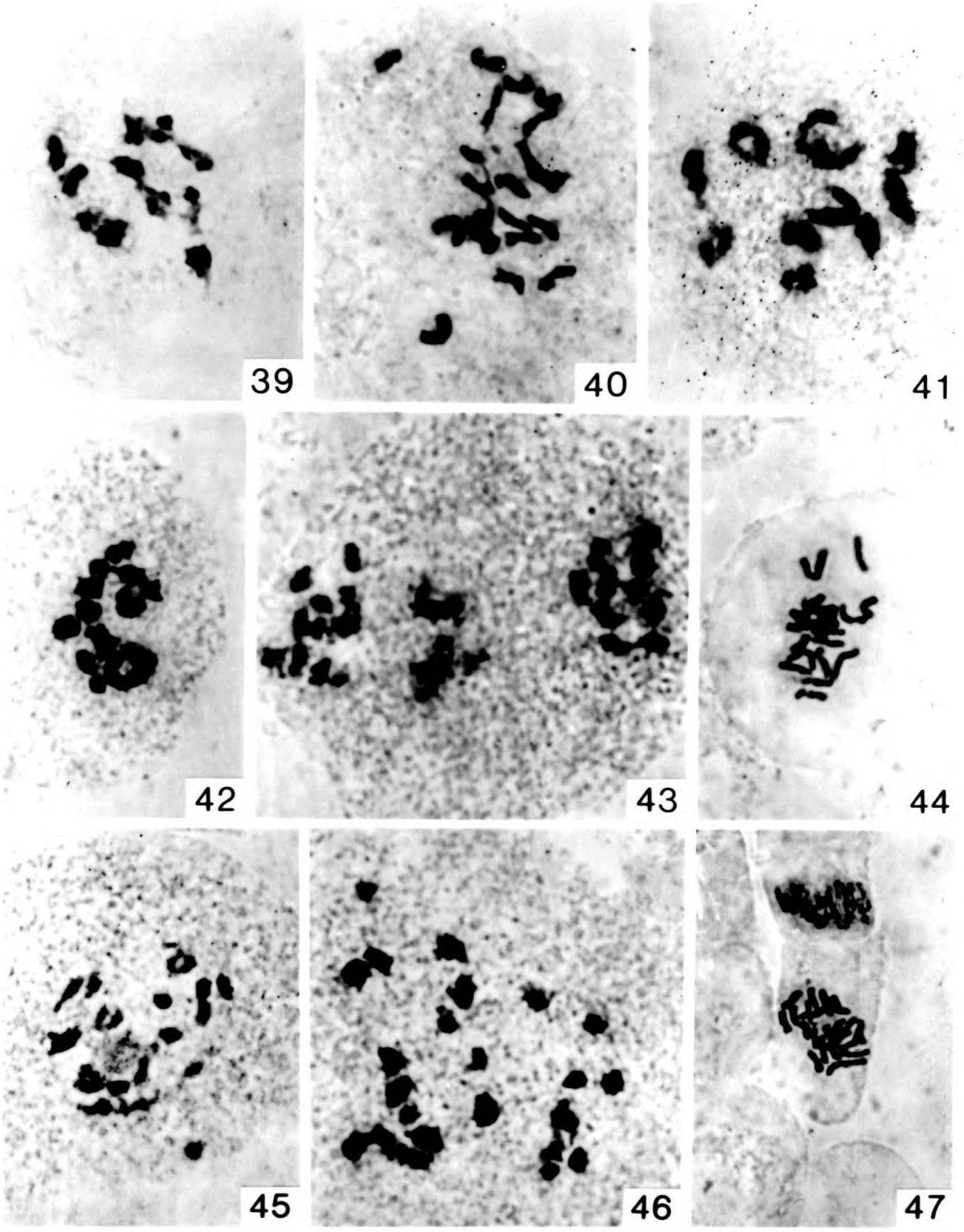
Explanation of Figures

(PMC = Pollen Mother Cells)

(All Figures x 1500)

(Figs. 39 – 47)

Figs. 39 – 40	*Tabernaemontana dichotoma*
Fig. 39	PMC showing 11 bivalents at diakinesis
Fig. 40	Root tip cell showing 22 chromosomes at metaphase
Figs. 41-56	*Tabernaemontana divaricata*
Fig. 41	PMC of variety 1 showing varying numbers of unvalents and bivalents
Fig. 42	PMC of variety 1 showing a ring of 4 chromosomes and 9 bivalents
Fig. 43	PMC of variety 1 showing lagging chromosomes at anaphase I
Fig. 44	Root tip cell of variety 1 showing 22 chromosome at metaphase
Fig. 45	PMC of variety 2 showing univalents at diakinesis
Fig. 46	PMC of variety 2 showing univalents at metaphase I
Fig. 47	Root tip cell of variety 2 showing 22 chromosomes at metaphase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Explanation of Figures

(PMC = Pollen Mother Cells)

(All Figures x 1500)

(Figs. 48 – 56)

Fig. 48	PMC of variety 3 showing univalents and bivalents at diakinesis
Fig. 49	Root tip cells of variety 3 showing 22 chromosomes at metaphase
Fig. 50	PMC of variety 4 showing univalents and bivalents at metaphase I
Fig. 51	PMC of variety 4 showing unequal segregation of chromosome at anaphase I
Fig. 52	PMC of variety 4 showing unequal segregation of chromosome at anaphase II
Fig. 53	Root tip cell of variety 4 showing 33 chromosomes at metaphase
Fig. 54	PMC of variety 5 showing univalents
Fig. 55	PMC of variety 5 showing 11 bivalents
Fig. 56	Root tip cell of variety 5 showing 22 chromosomes


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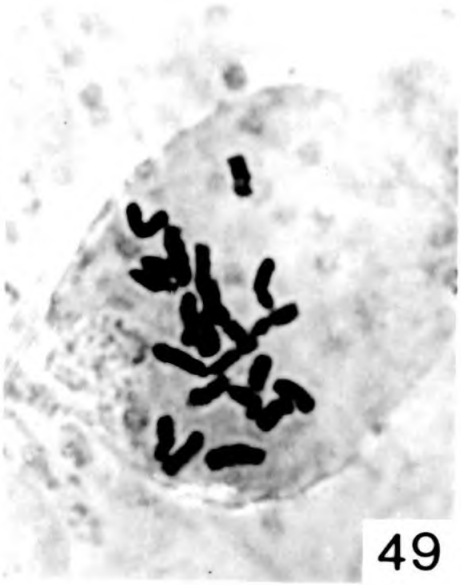
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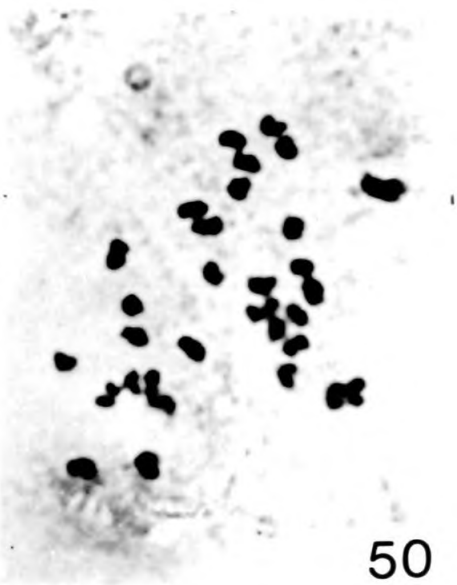
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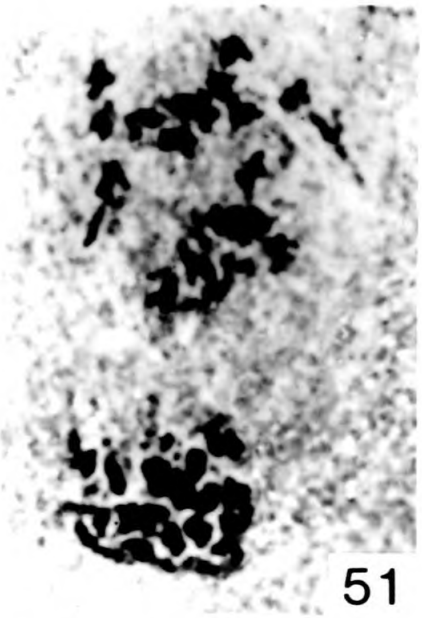
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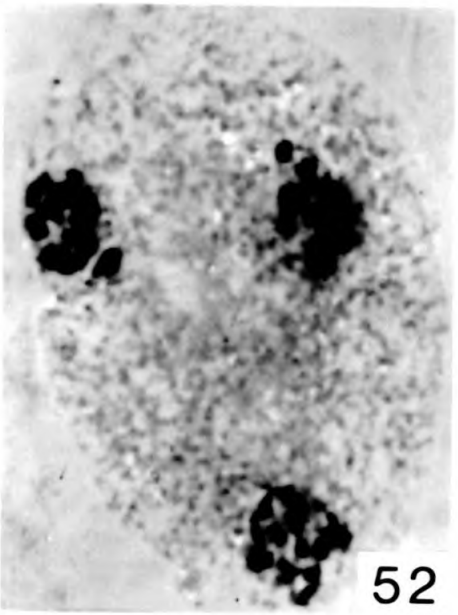
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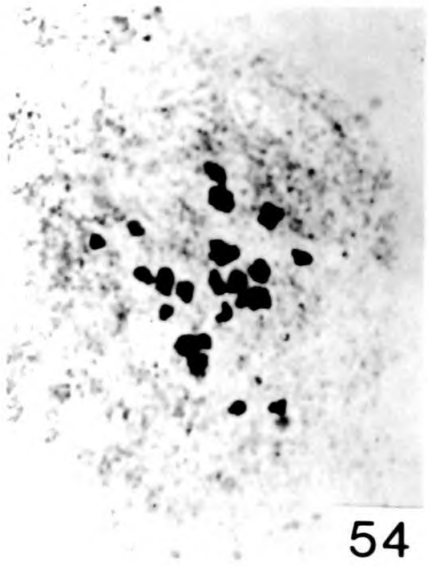
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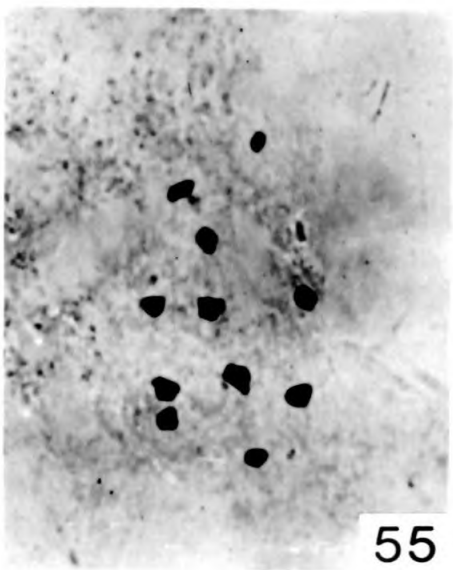
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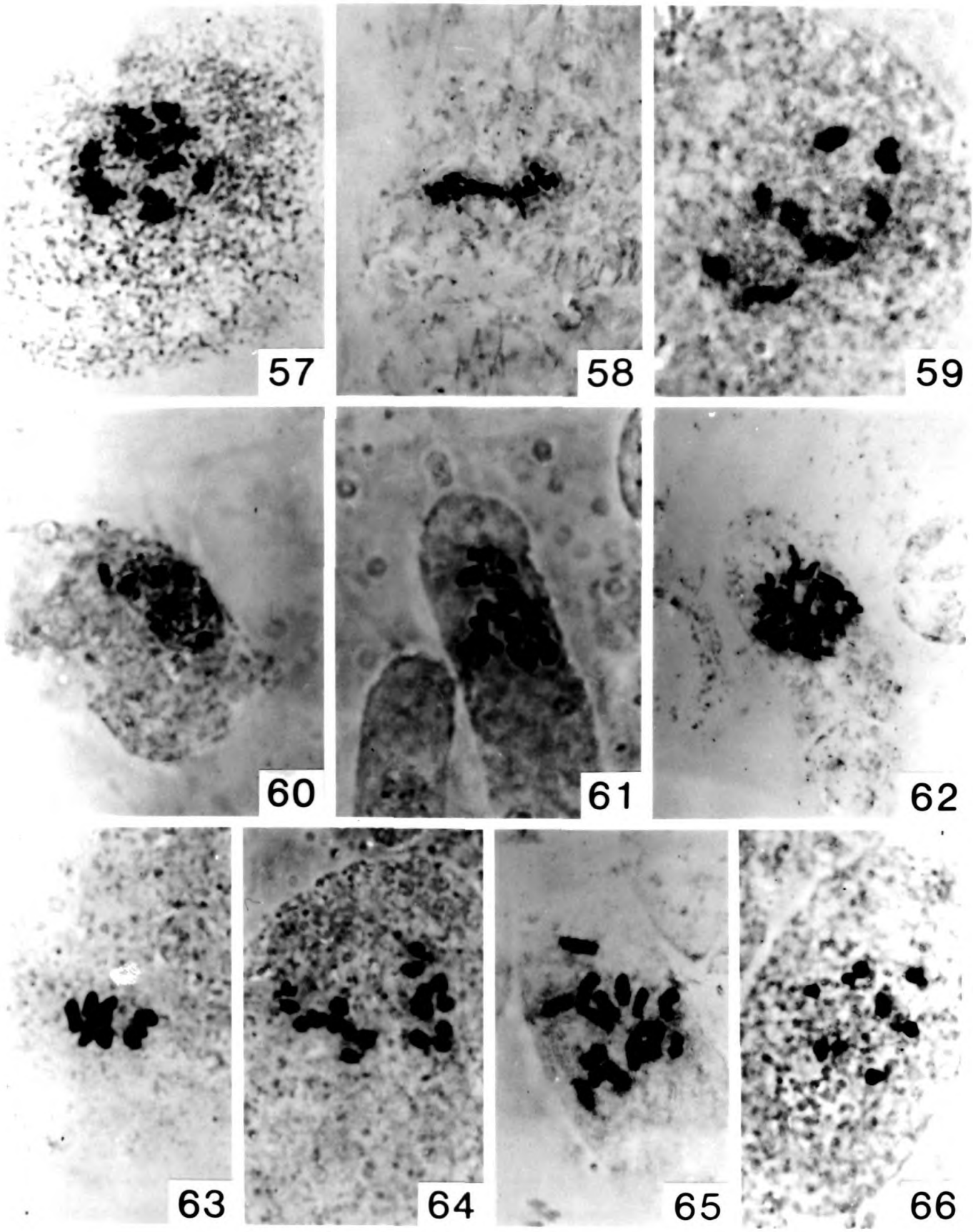
Explanation of Figures

(PMC = Pollen Mother Cells)

(All Figures x 1500)

(Figs. 57 – 66)

Fig. 57	PMC of *Holarrhena antidysenterica* showing 11 bivalents at metaphase I
Fig. 58	PMC of *Vallaris solanacea* showing 10 bivalents at meteaphase I
Fig. 59	PMC of *Vallaris lancifolia* showing 10 bivalents at metaphase I
Fig. 60 – 61	*Parsonsia spiralis*
Fig. 60	PMC showing 9 bivalents at diakinesis
Fig. 61	Root tip cell showing 18 chromosomes
Fig. 62	Root tip cell of *Wrightia tinctoria* showing 22 chromosomes at metaphase
Figs. 63 – 65	*Strophanthus gratus*
Fig. 63	PMC showing 9 bivalents at metaphase I
Fig. 64	PMC showing equal chromosome distribution at anaphase I
Fig. 65	Root tip cell showing 18 chromosome at metaphase.
Figs. 66 – 67	*Strophanthus wightianus*
Fig. 66	PMC showing 9 bivalents at diakinesis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Explanation of Figures

(PMC = Pollen Mother Cells)

(All Figures x 1500)

(Figs. 67 – 75)

Fig. 67 Root tip cell showing 18 chromosomes at metaphase

Fig. 68 PMC of *Ichnocarpus frutescens* showing 10 bivalents

Fig. 69 Root tip cell of *Ichnocarpus ovatifolius* showing 40 chromosomes

Fig. 70 – 71 *Chonemorpha fragrans*

Fig. 70 PMC showing 10 bivalents at metaphase I

Fig. 71 Root tip cell showing 20 chromosomes at metaphase

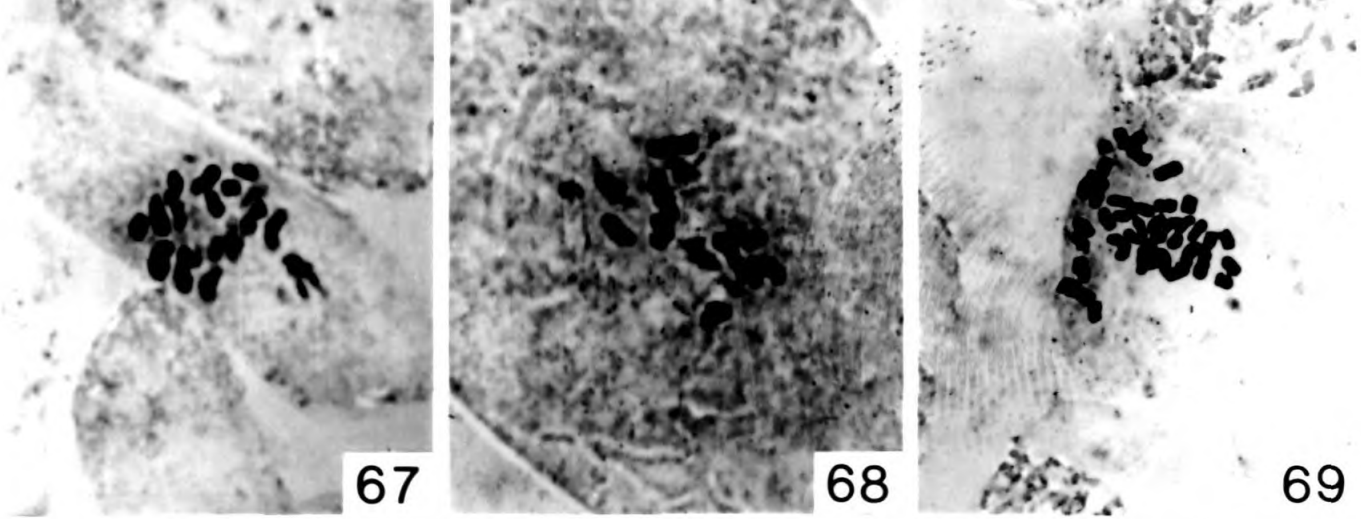
Fig. 72 Root tip cell of *Chonemorpha griffithii* showing 30 chromosomes

Fig. 73 – 74 *Adenium obesum*

Fig. 73 PMC showing 11 bivalents of metaphase I

Fig. 74 Root tip cell showing 22 chromosomes at metaphase

Fig. 75 Root tip cell of *Aganosma caryophyllata* showing 22 chromosomes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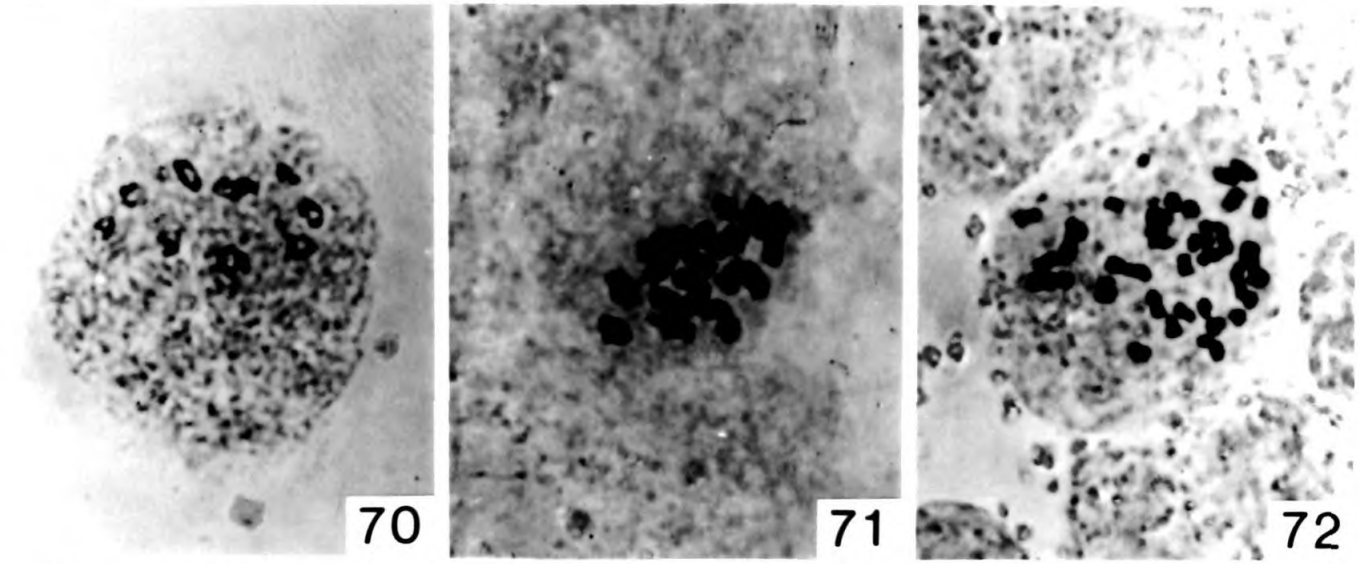
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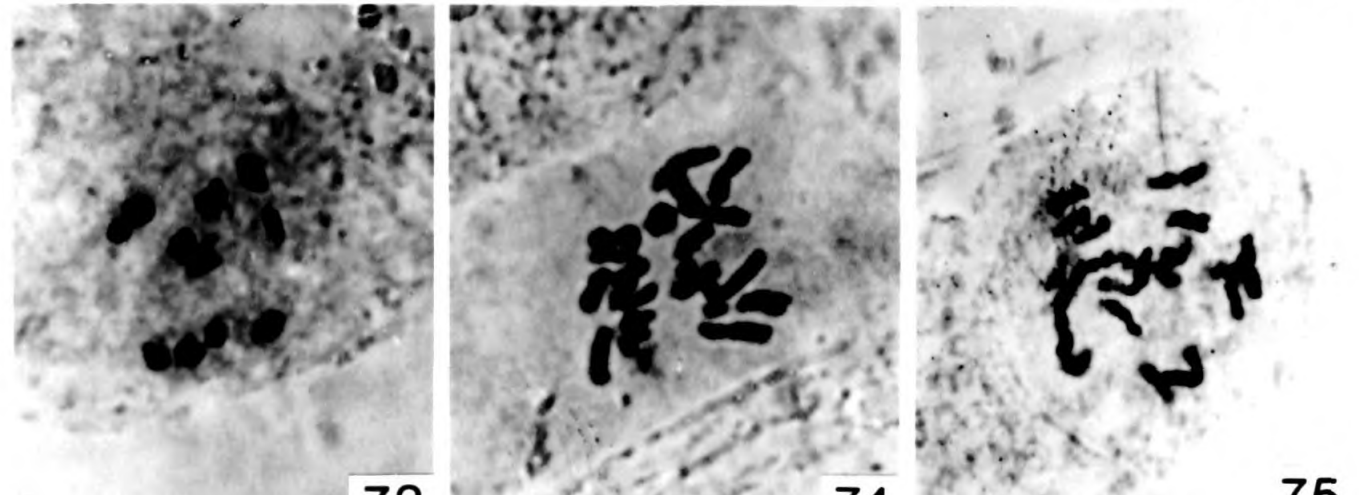
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PALYNOLOGY

Explanation of Figures

(LM = Light Microscopic photograph; SEM = Scanning Electron microscopic photomicrograph)
(Figs. 76 – 87)

Fig. 76 *Allamanda cathartica*, 3-zonocolporate grain, polar view (SEM, x 2000)

Fig. 77 *Allamanda cathartica*, ora circular to lalongate (LM, x 1166)

Fig. 78 *Allamanda cathartica*, ora in equatorial view (LM, x 1166)

Fig. 79 *Allamanda cathartica*, exine ornamentation rugulate, rugula with tip nodules (LM, x 1166)

Fig. 80 *Allamanda schottii*, faintly foveolate exine ornamentation (LM, x 1166)

Fig. 81 *Allamanda neriifolia*, 3-zonocolporate grain, polar view (LM, x 1166)

Fig. 82 *Allamanda neriifolia*, exine ornamentation rugulate, rugula with tip nodules (LM, x 1166)

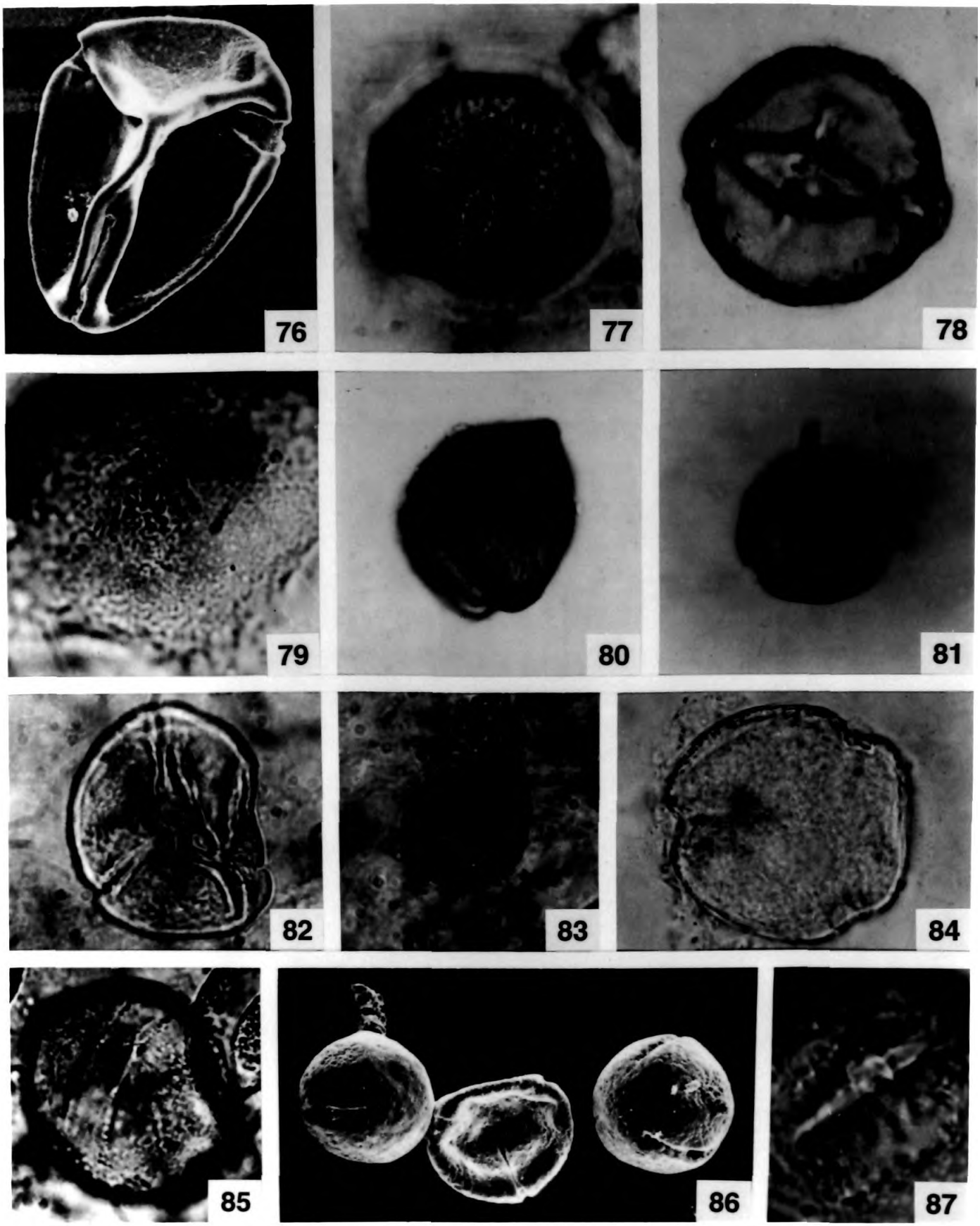
Fig. 83 *Allamanda neriifolia*, equatorial view (LM, x 1166)

Fig. 84 *Allamanda violacea*, 3-zonocolporate grain, polar view (LM, x 1166)

Fig. 85 *Allamanda violacea*, ora lalongate, exine showing rugulate-nodulate ornamentation (LM, x 1166)

Fig. 86 *Carissa spinarum*, 3-zonocolporate grains, equatorial view, polar view, punctate ornamentation (SEM, x 2000)

Fig. 87 *Carissa spinarum*, exine showing granulate-perforate or ora lalongate (LM, x 1166)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Explanation of Figures

(LM = Light Microscopic photograph; SEM = Scanning Electron microscopic photomicrograph)
(Figs. 88 – 96)

Fig. 88 *Carissa carandas*, 3-zonocolporate grains, ora circular to lalongate (SEM, x 2000)

Fig. 89 *Carissa carandas*, exine ornamentation scrobiculate (LM, x 1166)

Fig. 90 *Rauvolfia serpentina*, 3-zonocolporate grain, oblique polar view (SEM, x 2000)

Fig. 91 *Rauvolfia serpentina*, a portion showing the endoexine thickening (kidney shaped) at the colpus margin between pseudocolpium (SEM, x 2300)

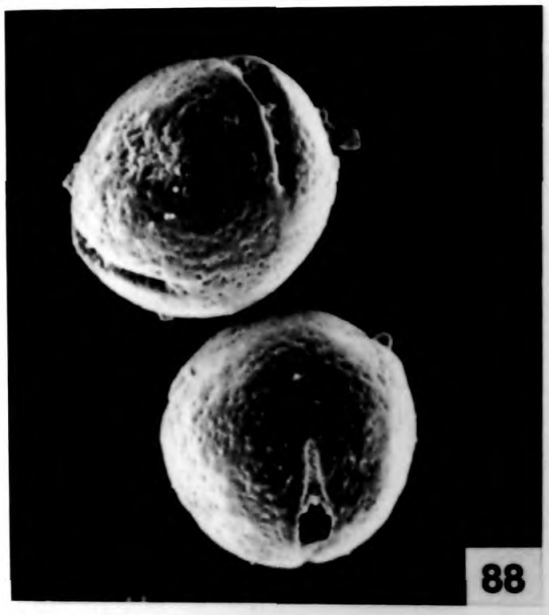
Fig. 92 *Rauvolfia serpentina*, 3-zonocolporate grain, polar view (LM, x 1166)

Fig. 93 *Rauvolfia serpentina*, 3-zonocolporate grain, oblique equatorial view (LM, x 1166)

Fig. 94 *Rauvolfia serpentina*, exine ornamentation reticulate (LM, x 1166)

Fig. 95 *Rauvolfia tetraphylla*, 3-zonocolporate grain, polar view (SEM, x 2000)

Fig. 96 *Rauvolfia tetraphylla*, 3-zonocolporate grain, ora circular to lalongate, polar view (SEM, x 2000)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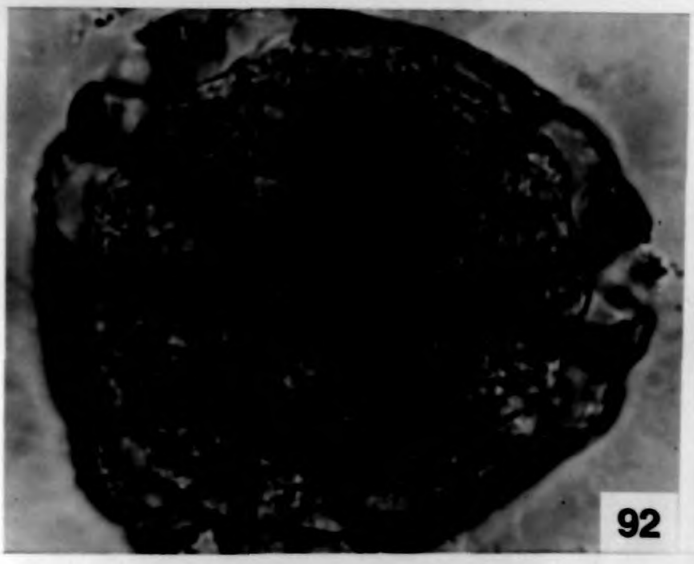
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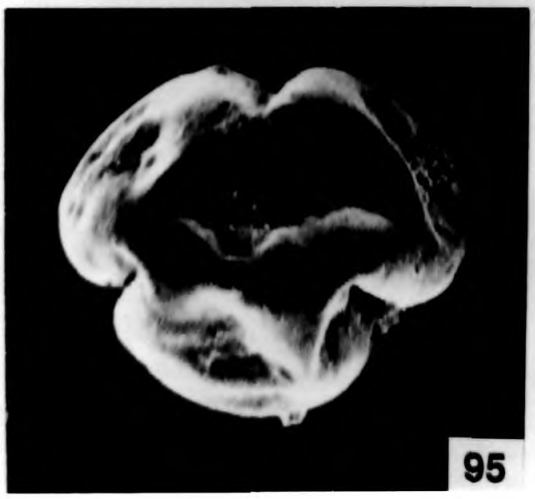
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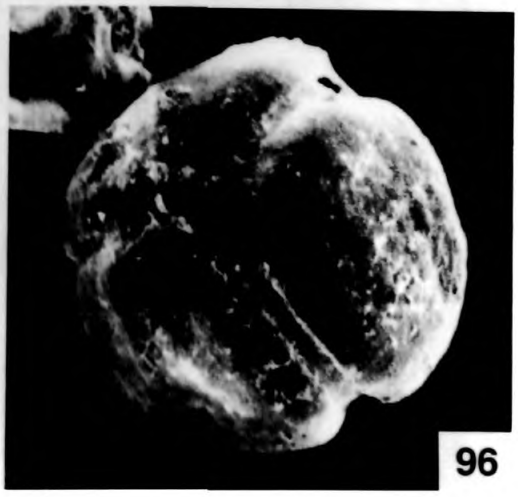
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Explanation of Figures

(LM = Light Microscopic photograph; SEM = Scanning Electron microscopic photomicrograph)
(Figs. 97 – 107)

Fig. 97 *Rauvolfia tetraphylla*, ora circular to lalongate, a portion enlarged (SEM, x 2300)

Fig. 98 *Rauvolfia tetraphylla*, 3-zonocolporate grain, polar view (LM, x 1166)

Fig. 99 *Rauvolfia beddomei*, 3-zonocolporate grain, polar view (LM, x 1166)

Fig. 100 *Rauvolfia beddomei*, ora lalongate, oblique equatorial view (LM, x 1166)

Fig. 101 *Rauvolfia densiflora*, 3-zonocolporate grain, polar view (LM, x 1166)

Fig. 102 *Rauvolfia densiflora*, oblique equatorial view showing the endoexine thickening at the colpus margin between the pseudocolpium (LM, x 1166)

Fig. 103 *Thevetia peruviana*, 3-zonocolporate grain, polar view (SEM, x 1166)

Fig. 104 *Thevetia peruviana*, ora lalongate, a portion enlarged (SEM, x 2300)

Fig. 105 *Thevetia peruviana*, exine ornamentation microreticulate (LM, x 1166)

Fig. 106 *Cerbera odollam*, 3-zonocolporate grain, polar view (SEM, x 2100)

Fig. 107 *Cerbera odollam*, a depression in the apocolpate region and three depression in mesocolpium, polar view (SEM, x 2100).


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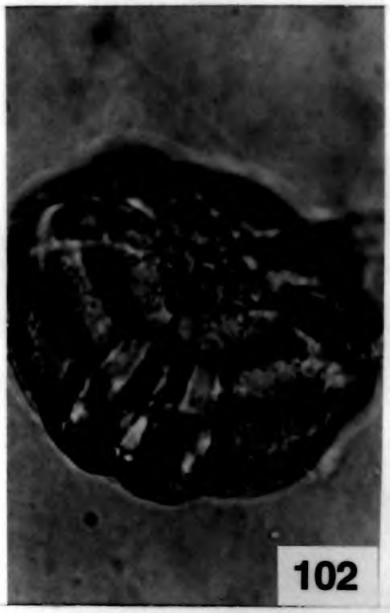
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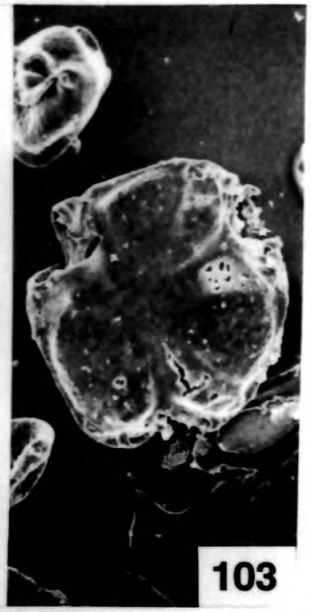
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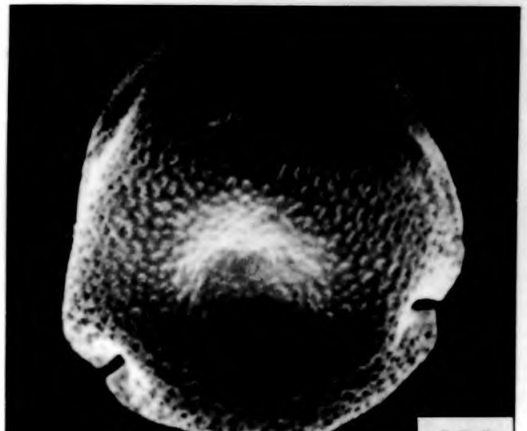

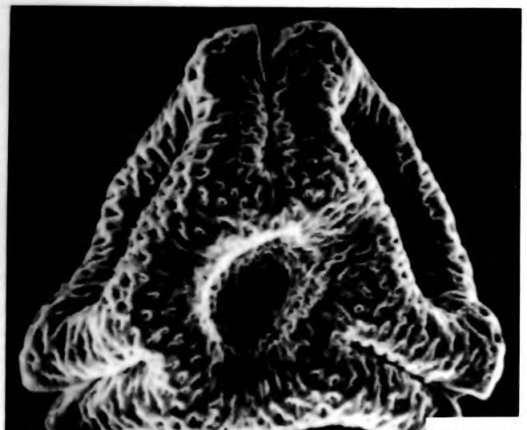

Explanation of Figures

(LM = Light Microscopic photograph; SEM = Scanning Electron microscopic photomicrograph)
(Figs. 108 – 120)

Fig. 108 *Cerbera odollam,* exine surface densely punctate a portion enlarged (SEM, x 2300)

Fig. 109 *Cerbera odollam,* ora lalongate (LM, x 1166)

Fig. 110 *Kopsia fruticosa,* 3-zonocolporate grains (SEM, x 2000)

Fig, 111 *Kopsia fruticosa,* ora lalongate, oblique equatorial view (SEM, x 2100)

Fig. 112 *Kopsia fruticosa,* ora lalongate, a portion enlarged (SEM, x 2300)

Fig. 113 *Kopsia fruticosa,* exine surface reticulate (LM, x = 1166)

Fig. 114 *Catharanthus roseus* (variety 1), 3-zonocolporate grain equatorial view (LM, x 1166)

Fig. 115 *Catharanthus roseus* (variety 1), ora lolongate (LM, x 1166)

Fig. 116 *Catharanthus roseus* (variety 1), exine ornamentation reticulate heterobrochate (LM, x 1166)

Fig. 117 *Catharanthus roseus* (variety 2), polar view showing 3-zonocolporate grain (LM, x 1166)

Fig. 118 *Catharanthus roseus* (variety 2), equatorial view, 3-zonocolporate grain (LM, x 1166)

Fig. 119 *Catharanthus roseus* (variety 2), ora faint with sub colpal bands (margo) on either side (LM, x 1166)

Fig. 120 *Plumeria alba,* 3-zonocolporate grain, oblique equatorial view (SEM, x 2000)


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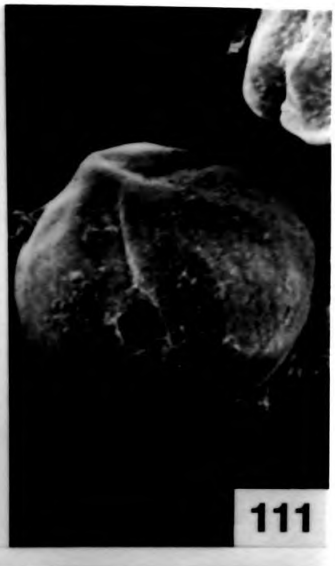
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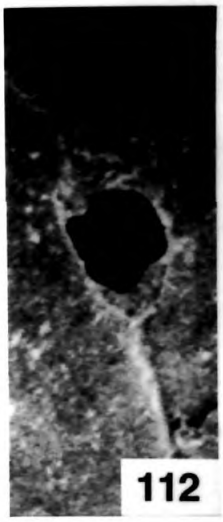
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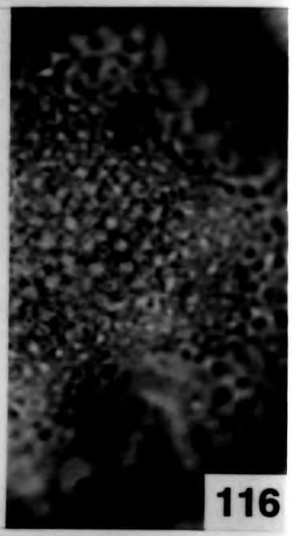
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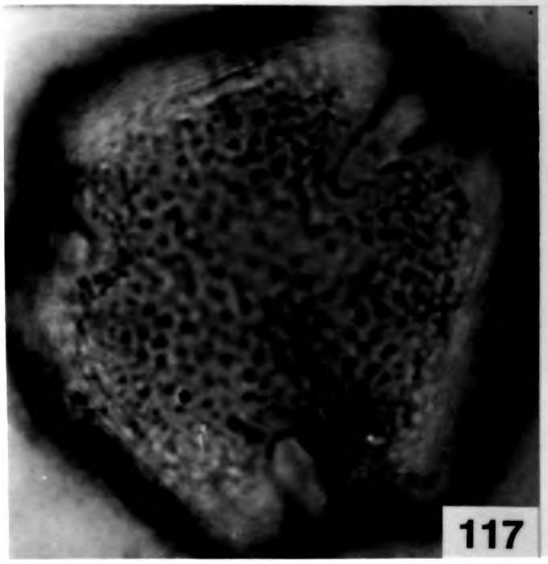
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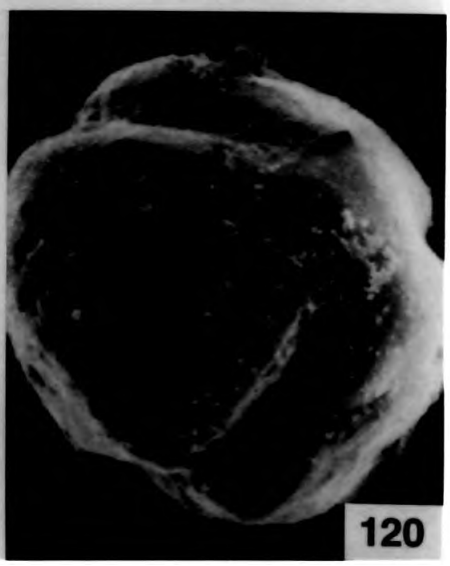
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Explanation of Figures

(LM = Light Microscopic photograph; SEM = Scanning Electron microscopic photomicrograph)
(Figs. 121 – 130)

Fig. 121 *Plumeria alba*, 3-zonocolporate grain, equatorial view (LM, x 1166)

Fig. 122 *Plumeria alba*, exine ornamentation micro reticulate (LM, x 1166)

Fig. 123 *Plumeria rubra*, 3-zonocolporate grain (SEM, x 2000)

Fig. 124 *Plumeria rubra*, exine ornamentation reticulate heterobrochate (LM, x 1166)

Fig. 125 *Plumeria rubra* (variety 1), 3-zonocolporate grain, polar view (LM, x 1166).

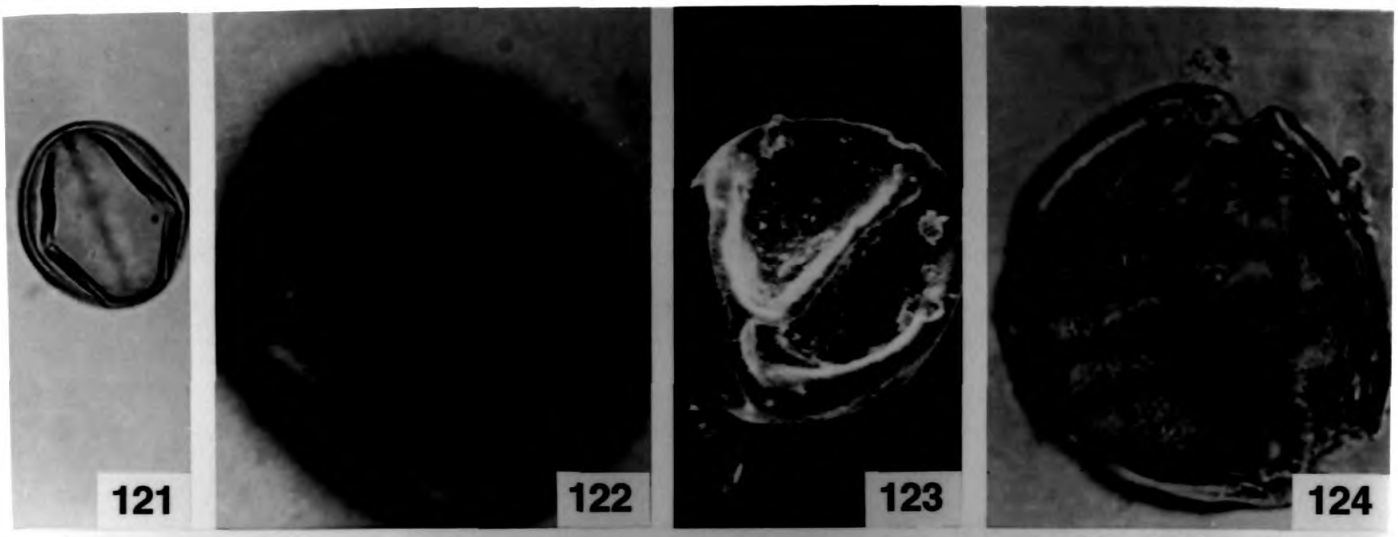
Fig. 126 *Alstonia scholaris*, 3-zonocolporate grain, polar view (SEM, x 2000)

Fig. 127 *Alstonia scholaris*, 3-zonocolporate grain, polar view (LM, x 1166)

Fig. 128 *Alstonia venenata*, 3-zonocolporate grain, polar view (LM, x 1166)

Fig. 129 *Alstonia venenata*, ora lalongate, thickening at the colpus margin (LM, x 1166)

Fig. 130 *Alstonia venenata*, exine ornamentation reticulate heterobrochate (LM, x 1166)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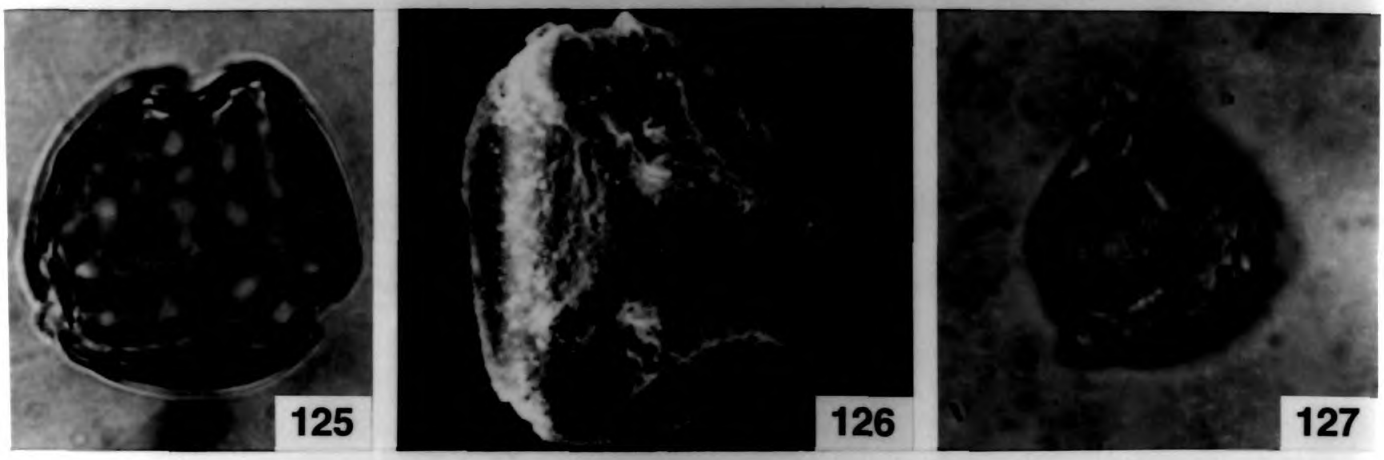
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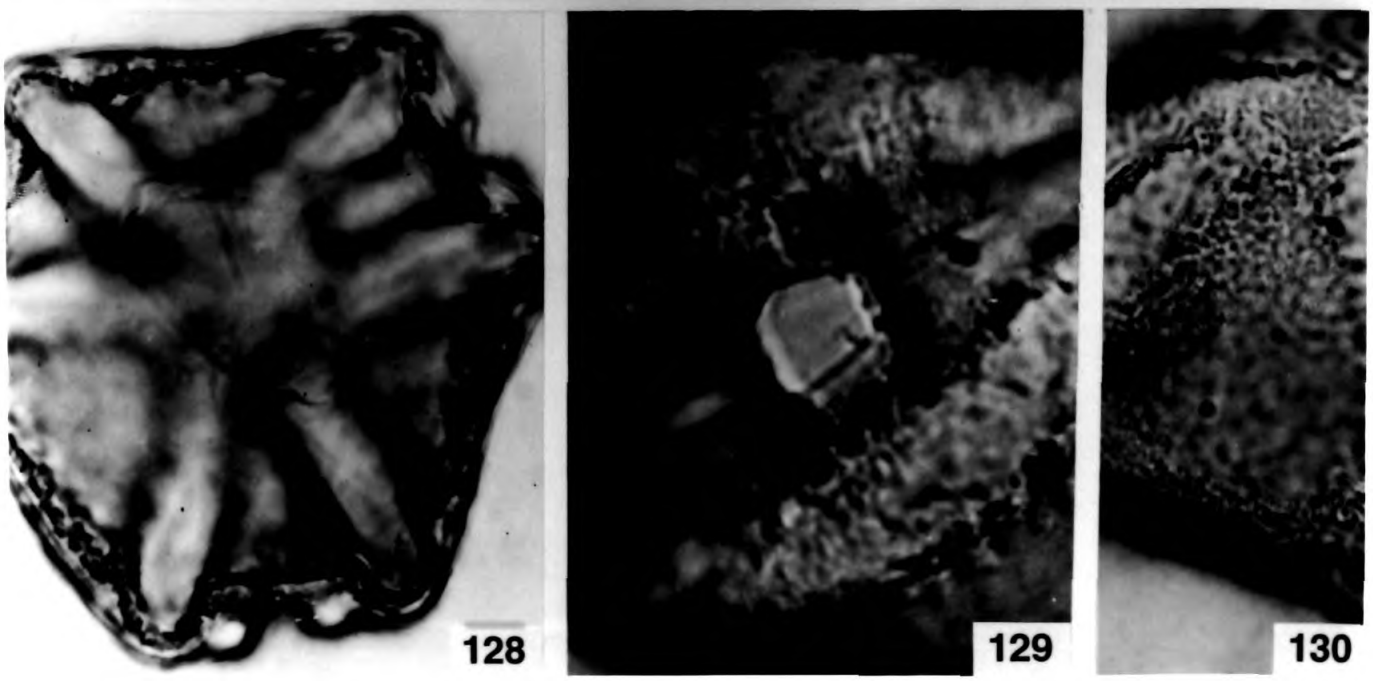
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Explanation of Figures

(LM = Light Microscopic photograph; SEM = Scanning Electron microscopic photomicrograph)
(Figs. 131 – 139)

Fig. 131 *Tabernaemontana dichotoma*, 3-zonocolporate grain, polar view (SEM, x 2000)

Fig. 132 *Tabernaemontana dichotoma*, 3-zonocolporate grain, polar view (LM, x 1166)

Fig. 133 *Tabernaemontana dichotoma*, 4-zonocolporate grain, polar view (LM, x 1166)

Fig. 134 *Tabernaemontana dichotoma*, ora lalongate, equatorial view (LM, x 1166)

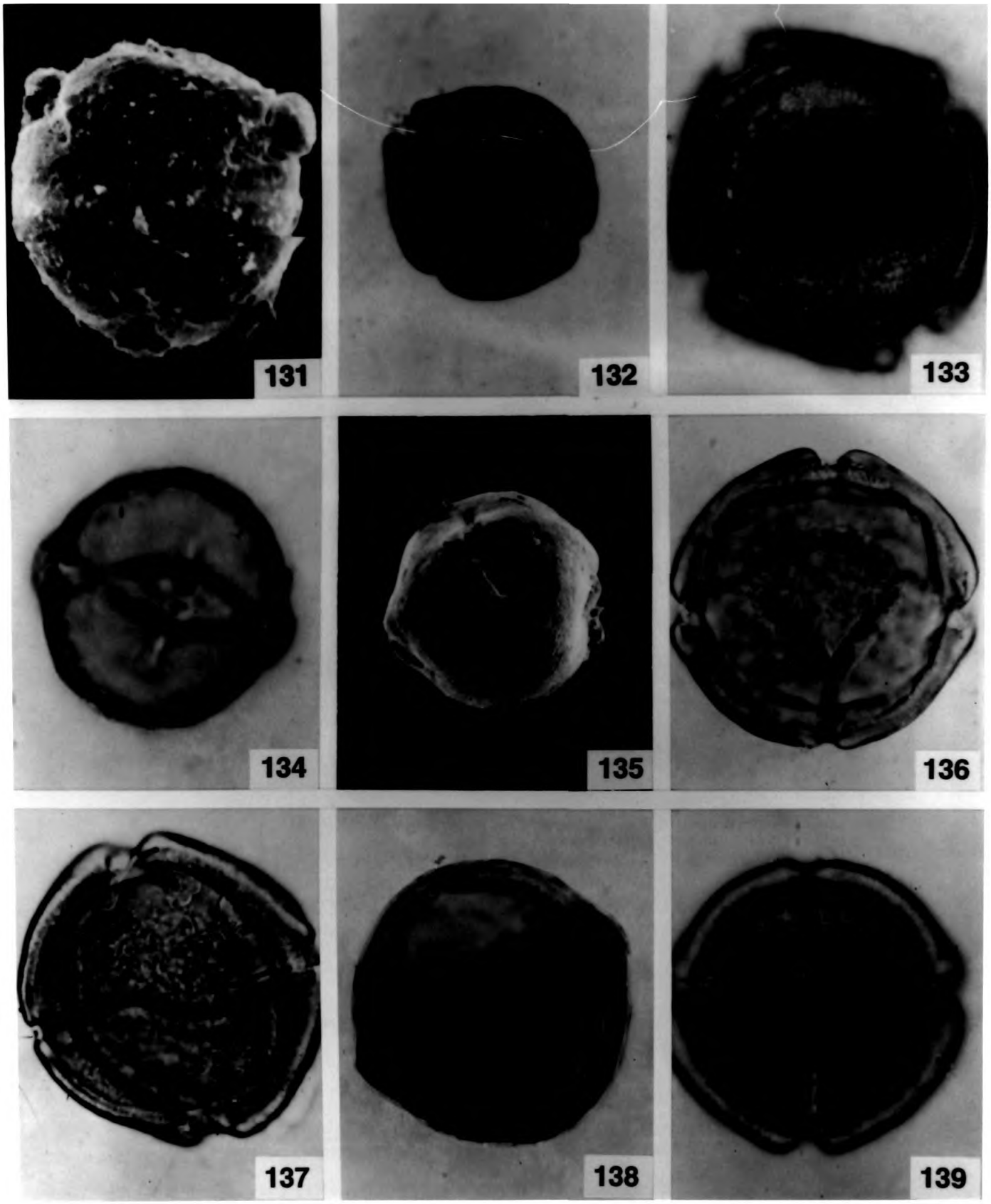
Fig. 135 *Tabernaemontana divaricata* (var.1), polar view (SEM, x 2000)

Fig. 136 *Tabernaemontana divaricata* (var. 1), 4-parasyncolporate grain, polar view (LM, x 1166)

Fig. 137 *Tabernaemontana divaricata* (var. 2), 4-parasyncolporate grain, polar view (LM, x 1166)

Fig. 138 *Tabernaemontana divaricata* (var. 2), equatorial view showing aperture (LM, x 1166)

Fig. 139 *Tabernaemontana divaricata* (var. 3), 4-parasyncolporate grain, exine reticulate heterobrochate, polar view (LM, x 1166).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Explanation of Figures

(LM = Light Microscopic photograph; SEM = Scanning Electron microscopic photomicrograph)
(Figs. 140 – 149)

Fig. 140 *Tabernaemontana divaricata* (var. 3), equatorial view showing aperture (LM, x 1166)

Fig. 141 *Tabernaemontana divaricata* (var. 4), 4-parasyncolporate grain, polar view (LM, x 1166)

Fig. 142 *Holarrhena antidysenterica,* 3-porate grain, polar view (LM, x 1166)

Fig. 143 *Holarrhena antidysenterica,* showing circular pore (LM, x 1166)

Fig. 144 *Holarrhena antidysenterica,* exine ornamentation foveolate (LM, x 2000)

Fig. 145 *Vallaris solanacea,* 4-porate grain, polar view (LM, x 1166)

Fig. 146 *Strophanthus gratus,* 3-porate grain, polar view (SEM, x 2000)

Fig. 147 *Strophanthus gratus,* 3-porate grain, polar view (SEM, x 2000)

Fig. 148 *Strophanthus gratus,* 4-porate grain, polar view (LM, x 1166)

Fig. 149 *Strophanthus gratus,* pore irregular (LM, x 1166)


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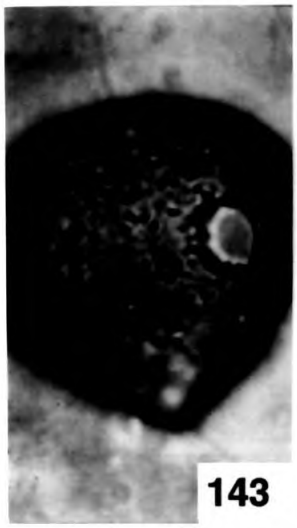
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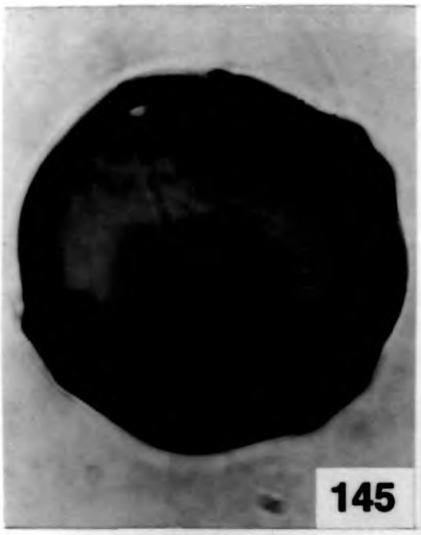
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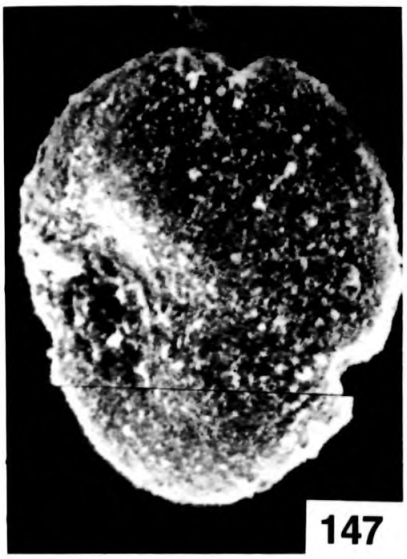
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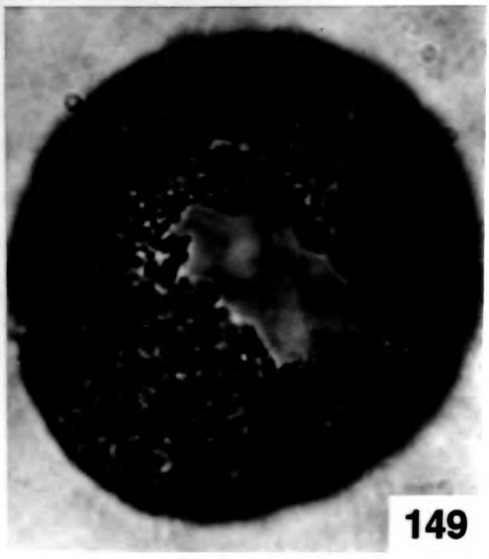
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Explanation of Figures

(LM = Light Microscopic photograph; SEM = Scanning Electron microscopic photomicrograph)
(Figs. 150 – 158)

Fig. 150 *Strophanthus gratus,* exine ornamentation reticulate heterobrochate (LM, x 1166)

Fig. 151 *Strophanthus wightianus,* 3-zonocolporate grain, equatorial view (LM, x 1166)

Fig. 152 *Strophanthus wightianus,* exine ornamentation micro reticulate (LM, x 1166)

Fig. 153 *Ichnocarpus frutescens,* 3-zonocolporate grain, polar view (LM, x 1166)

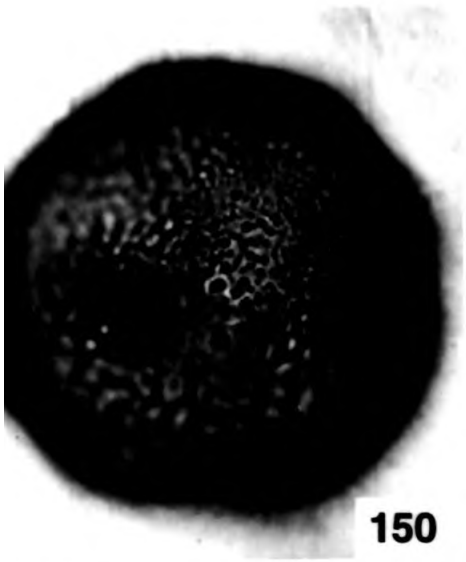
Fig. 154 *Chonemorpha fragrans,* 3-zonocolporate grain, exine ornamentation reticulate (LM, x 1166)

Fig. 155 *Aganosma caryophyllata,* 3-zonocolporate grain, polar view (LM, x 1166)

Fig. 156 *Aganosma caryophyllata,* exine ornamentation reticulate (LM, x 1166)

Fig. 157 *Adenium obesum,* 3-porate grain, polar view (LM, x 1166)

Fig. 158 *Odontadenia grandiflora,* 3-porate grain, pore circular (SEM, x 2000)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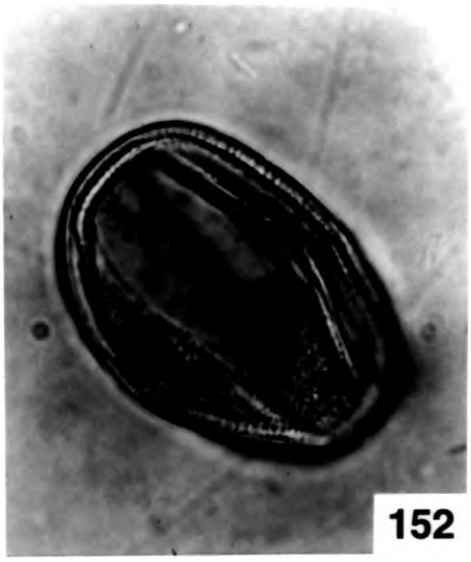
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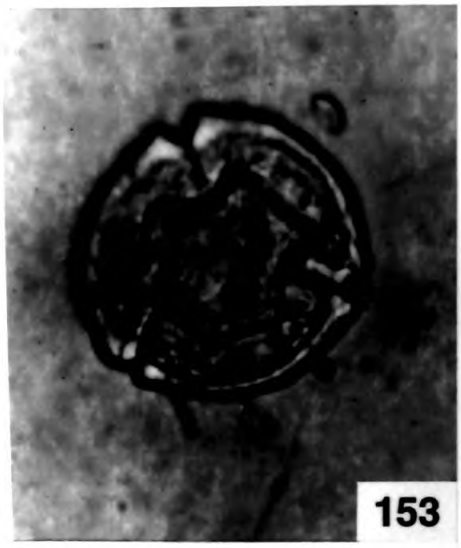
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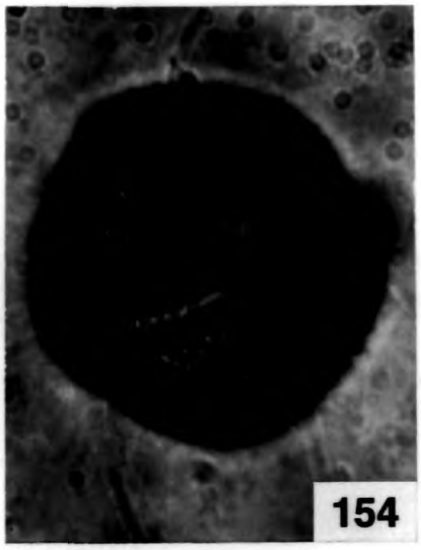
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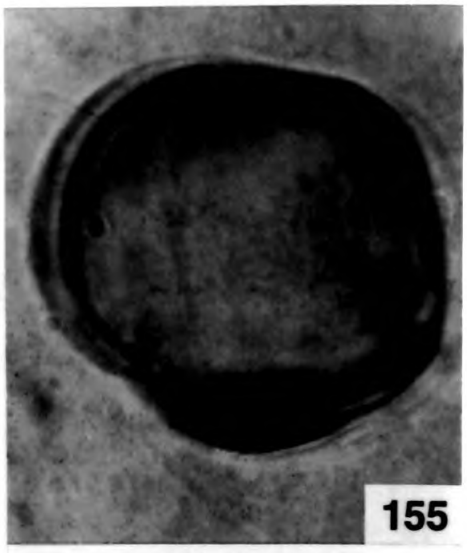
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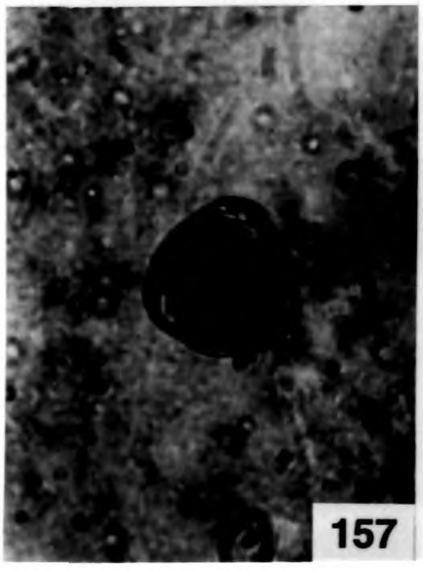
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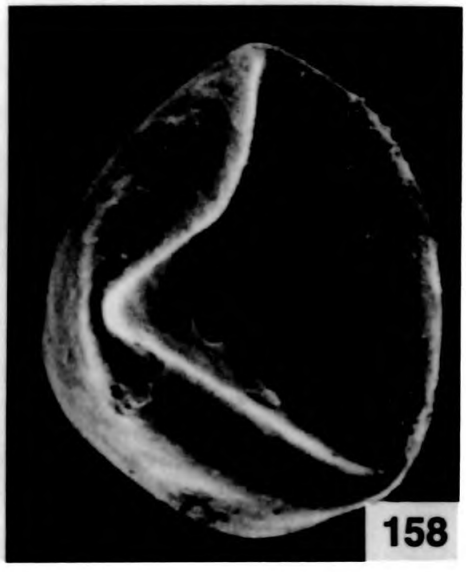
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Explanation of Figures

(*LM* = Light Microscopic photograph; *SEM* = Scanning Electron microscopic photomicrograph)
(Figs. 159 – 164)

Fig. 159 *Odontadenia grandiflora*, exine ornamentation reticulate (LM, x 1166)

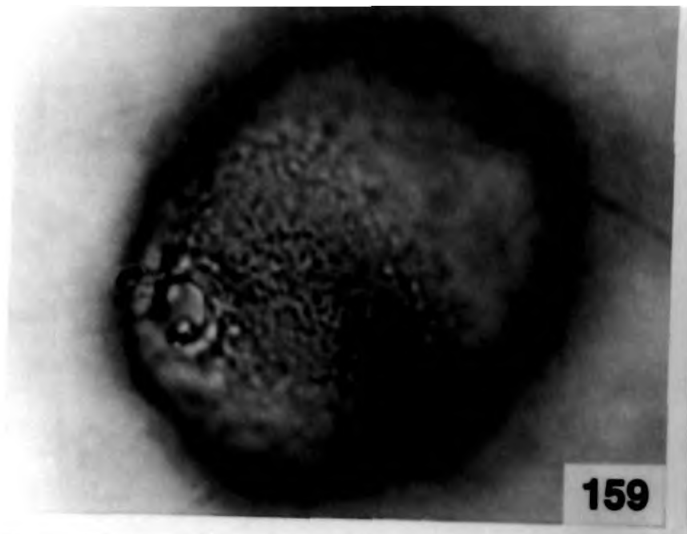
Fig. 160 *Nerium oleander* (variety 1), 4-porate grain (SEM, x 2000)

Fig. 161 *Nerium oleander* (variety 1), exine ornamentation reticulate heterobrochate (LM, x 1166)

Fig. 162 *Nerium oleander* (variety 2), 4-porate grain, polar view (SEM, x 2000)

Fig. 163 *Nerium oleander* (variety 2), exine ornamentation reticulate heterobrochate (LM, x 1166)

Fig. 164 *Nerium oleander* (variety 3), 4-porate grain, polar view (LM, x 2000)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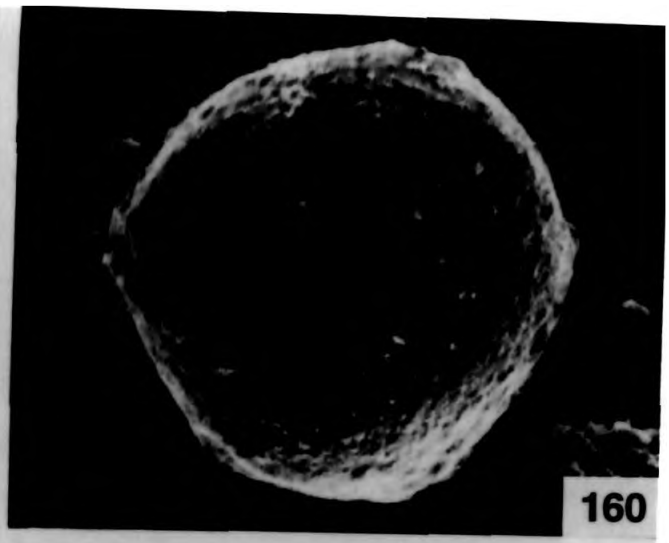
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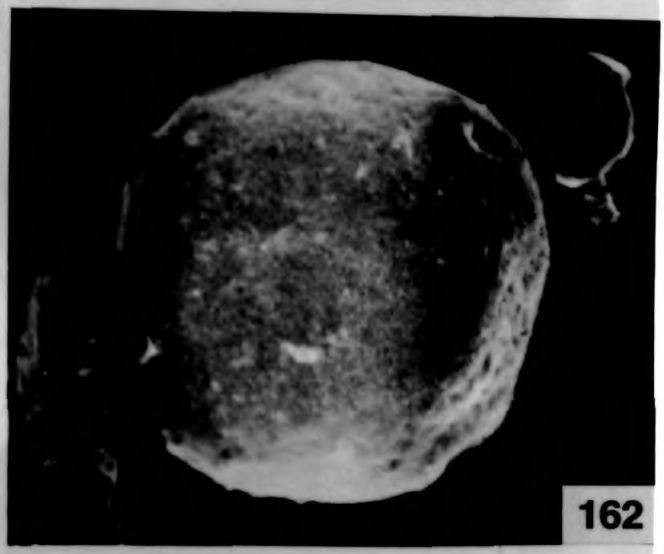
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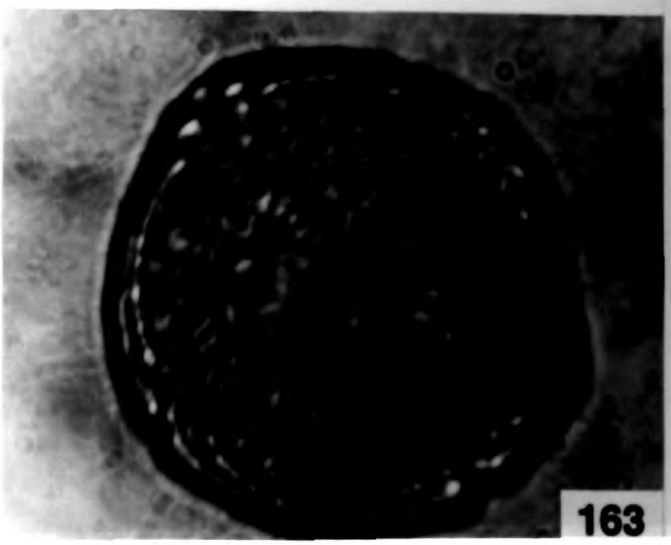
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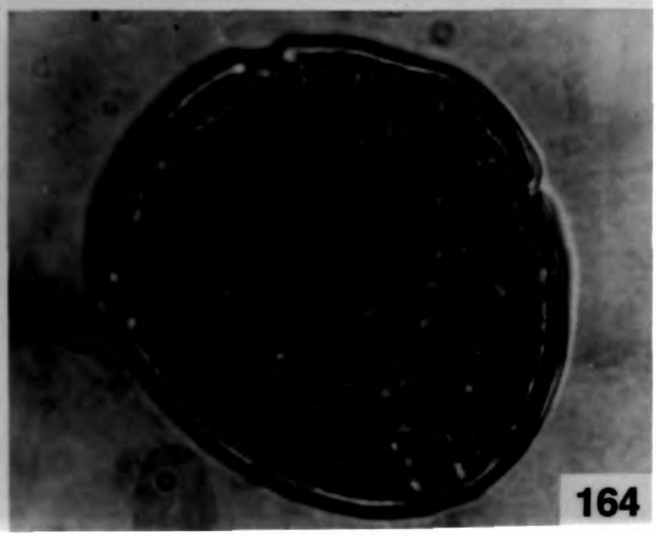
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