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# Contributions to the Study of Microsporogenesis in *Calamus* L. (Arecaceae)

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**ABSTRACT:** The genus *Calamus* in the Arecaceae has so far remained embryologically unknown. The present work on the development of the microsporangium and male gametophyte in 5 species of *Calamus* (Arecaceae) is the first investigation. The tapetum is of secretory type and its cells become binucleate. The pollen mother cells undergo successive divisions to produce isobilateral and tetrahedral tetrads. The mature pollen grains in *C. nagbettai*, *C. stoloniferus* and *C. travancoricus* are 2-celled at the time of shedding. In *C. gamblei* and *C. rotang* they are, however, 3-celled when shed.

**KEY WORDS:** ***Calamus*, Anther wall, Pollen.**

## INTRODUCTION

Palms are particularly abundant in the Indomalayan region and South America. According to Corner (1966), there are 227 genera and 2613 species in the Arecaceae. In India, there are about 27 genera and 91 species (Ahmedullah and Nayar, 1986). Of the Indian palms, canes are economically considered very important (Lakshmana, 1993). Some of them are endemic and some are endangered (Ahmedullah and Nayar, 1986). More than a million people in India (predominantly tribals and backward classes), depend upon canes and cane crafts for their sustainance. Despite this, it is surprising that canes have not attracted any studies on their reproductive biology. This may be because of their spiny character, inaccessibility and the difficulties in collecting flowers and fruits (Lakshmana, 1993). *Calamus* is a palaeotropical genus represented in India by about 31 species of which 8 are endemic to peninsular India (Ahmedullah and Nayar, 1986). From Karnataka about 14 species of *Calamus* are reported (Lakshmana, 1993).

Earlier literature on their embryology of Arecaceae has been reviewed by Schnarf (1931), Davis (1966) and Johri *et al*., (1992). Palms form a large family of tropical and sub-tropical woody plants, not well studied embryologically (Mahabale and Biradar, 1968). Shirke and Mahabale (1972) also writes that literature on the embryology of palms is rather scanty and they need to be investigated. This is especially so of the canes that have embryologically remained unknown, so far. However, canes have attracted the attention of palynologists early. Sharma (1967) has investigated the pollen of *Calamus rotang* from the herbarium material and has reported 1-2 colpate (2-sincolpate), subspheroidal pollen grains. While, Thanikaimoni (1971) in his monographic studies of the Palmae, has studied the pollen morphology of many species of *Calamus* including *C. huegelianus*, *C. pseudotenuis*, *C. rotang*, *C. thwaitesii* and *C. travancoricus*. Sowunmi (1967, 1968 and 1972) has also studied the pollen morphology of

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the Arecaceae and its bearing on taxonomy. She (Sowunmi, 1972) strongly argues that *Calamus* type of pollen morphology is distinct from the rest of the palm species in having dicolpate pollen grains. Ultrastructural studies have been extended to the pollen grains of canes by Thanikaimoni (1971) and Kedves (1980). Kedves (1980) has investigated *C. andamanicus* and *C. palustris*.

Earlier works on the development of the anther wall in palms is by Juliano and Quisumbing (1931) who studied the anther wall in *Cocos nucifera*. Süssenguth (1921) has reported simultaneous division of the microspore mother cells in 3 species of *Chamaedorea*. The same has been reported by Schnarf (1931) in *Areca triandra* and *Caryota*. Radermacher (1924) has reported successive type of division of microspore mother cells in *Nipa fruticans*. In *Pinanga disticha* also, a similar type of cytokinesis is reported (Davis, 1966). The microspore tetrads are isobilateral, tetrahedral or occasionally T-shaped or linear and the pollen grains are 2-celled when shed (Davis, 1966).

The present work includes the study of microsporangium and the male gametophyte development in 5 species of *Calamus*.

## MATERIALS AND METHODS

Materials for the present investigation included male flowers from different species of *Calamus* that were collected during the period from September 2001 to January 2002 and from different areas in Karnataka (Table 1). Young flower buds were fixed in Formalin-Acetic acid-Alcohol. The floral buds were then dehydrated in a graded ethanol-xylol series. Paraffin infiltration and embedding were done and the sections were cut at 8-10 μm thickness and were stained with Heidenhein's ironalum and Haemetoxylin and were counterstained with erythrosin in clove oil. Pollen bearing materials consisting of anthers were dissected out from male flowers and the pollen preparations were made following the acetolysis method as suggested by Erdtman (1952).

Table 1. Species of *Calamus* collected from different localities in Karnataka.

| Species | Localities | Time of flowering |
|---|---|---|
| *Calamus gamblei* Becc. ex. Becc. & Hk. f. | Evergreen forests of Talacauvery (Coorg district) and moist deciduous forests of Kumrahalli, Sakaleshpur (Hassan district) | November-January |
| *Calamus nagbettai* Fernandez & Dey | Evergreen forests of Gundya & Subramanya (South Canara district) | October-December |
| *Calamus rotang* L. | Lalbagh Botanical Garden, Bangalore district | September-December |
| *Calamus stoloniferus* Renuka | Evergreen forests of Makutta (Coorg district) and moist deciduous forests of Kumrahalli, Sakaleshpur (Hassan district) | November-January |
| *Calamus travancoricus* Bedd. ex. Becc. & Hk. f. | Evergreen forests of Makutta (Coorg district) | November-December |

## RESULTS

The male flowers of the present 5 species of *Calamus* are small and pale yellow in color. There are 6 stamens in a male flower. A transverse section of a male flower shows the 6

anthers and a pistillode (Fig. 1A). The anthers are tetrasporangiate (Fig. 3A). The archesporial cells differentiate early. The primary parietal cells and the primary sporogenous cells are cut off from the archesporium. The primary parietal cells produce a 3-4 layered anther wall which includes an endothecium, 1or 2 middle layers and a glandular tapetum (Fig. 3B). Most of the tapetal cells become binucleate. The behaviour of the tapetum in species of *Calamus* varies. In *C. stoloniferus* they are binucleate as in the other investigated palms (Davis, 1966 and Johri *et al*., 1992). In *C. nagbettai* and *C. rotang* they are 2-nucleate. However in *C. gamblei* the tapetal cells may remain uninucleate or become binucleate. But in *C. travancoricus* they are 2-3 nucleate (Fig. 4A). Although the tapetum is one-layered in most species of *Calamus,* it becomes 2-layered on the side of the connective in *C. rotang.* Similarly, there is an occasional development of 2-layered tapetum in parts, in the microsporangia of *C. gamblei*. In *C. stoloniferus* and *C. travancoricus,* the tapetal cells elongate radially (Fig. 2A) with an increase in their ploidy before they disorganize. In *C. rotang* there is no radial elongation of the tapetal cells. Generally, the disorganization starts even before the commencement of meiosis. They start disorganizing when the dyads and tetrads are formed. In *C. stoloniferus,* the tapetal cells develop prominent vacuoles and there are nuclear fusions when the tapetal cells disorganize. In *C. gamblei* also, polyploidization of the tapetal cells is rarely observed. After providing nutrition to the pollen mother cells and the microspores the tapetal cells completely disorganize in *C. rotang*. But their remains can be observed in the mature microsporangia of *C. nagbettai*, *C. stoloniferus* and *C. travancoricus.*

Generally, there is only one middle layer (as in *C. gamblei, C. stoloniferus* and *C. travancoricus).* In *C. rotang*, however, on the connective side of the microsporangium, there are 2 middle layers. At maturity of the anther, the middle layers are crushed and absorbed.

In the young microsporangium, the epidermal layer is very prominent. During the development, it becomes enucleate (Fig. 6B) and only its remnants are observed in the mature microsporangium.

The endothecial cells are generally uninuclate. In *C. travancoricus,* some of the endothecial cells contain 2 nuclei. At maturity, the cells develop prominent band-like secondary wall thickenings (Fig. 7C). In *C. rotang* and *C. stoloniferus* the endothecial cells become large and vacuolated before they develop thickening. In *C. gamblei,* at the time of dehiscence, in addition to the endothecium, the cells on the connective side also start developing thickenings (Fig. 7A). In this case, the wave of the development of thickenings starts from the connective side and extends to the point of dehiscence where the two adjacent microsporangia coalesce. Generally, a stomium of thick-walled cells is organized between the adjacent microsporangia (Fig. 7B). When the septum becomes disorganized, the two anther loculi coalesce (Fig. 4B). In *C. stoloniferus* each microsporangium organizes its own stomium in the first stage (Fig. 2C) and a general stomium between two adjacent microsporangia is organized at the end.

The primary sporogenous cells produce the sporogenous tissue (Fig. 1B). The sporogenous cells develop in to pollen mother cells (Figs. 3C & 5A). Precocious movement of chromosomes during meiosis-I has been observed in *C. stoloniferus*. Cytokinesis is by successive method and by cell plate formation (Fig. 5B). The first meiotic division is followed by a dyad (Figs. 1C, 1D, 3D & 5C) and the second division results in the formation of a tetrad (Figs. 2A, 2B, 3D & 5D). The tetrads are isobilateral and tetrahedral (Fig. 5D). A newly formed microspore has dense cytoplasm and a centrally placed nucleus (Figs. 6A & 7B ). In *C. nagbettai,* there is some degree of sterility of the microspores and pollen grains. Such a feature has not been observed in any other presently investigated species of *Calamus*.

The division of the microspore nucleus leads to the formation of a small lenticular generative cell and a large vegetative cell. Mature pollen grains in *C. nagbettai*, *C. Stoloniferus* and *C. travancoricus* are 2-celled (Figs. 4C, 8A & 8B). But in *C. gamblei* and *C. rotang* the pollen

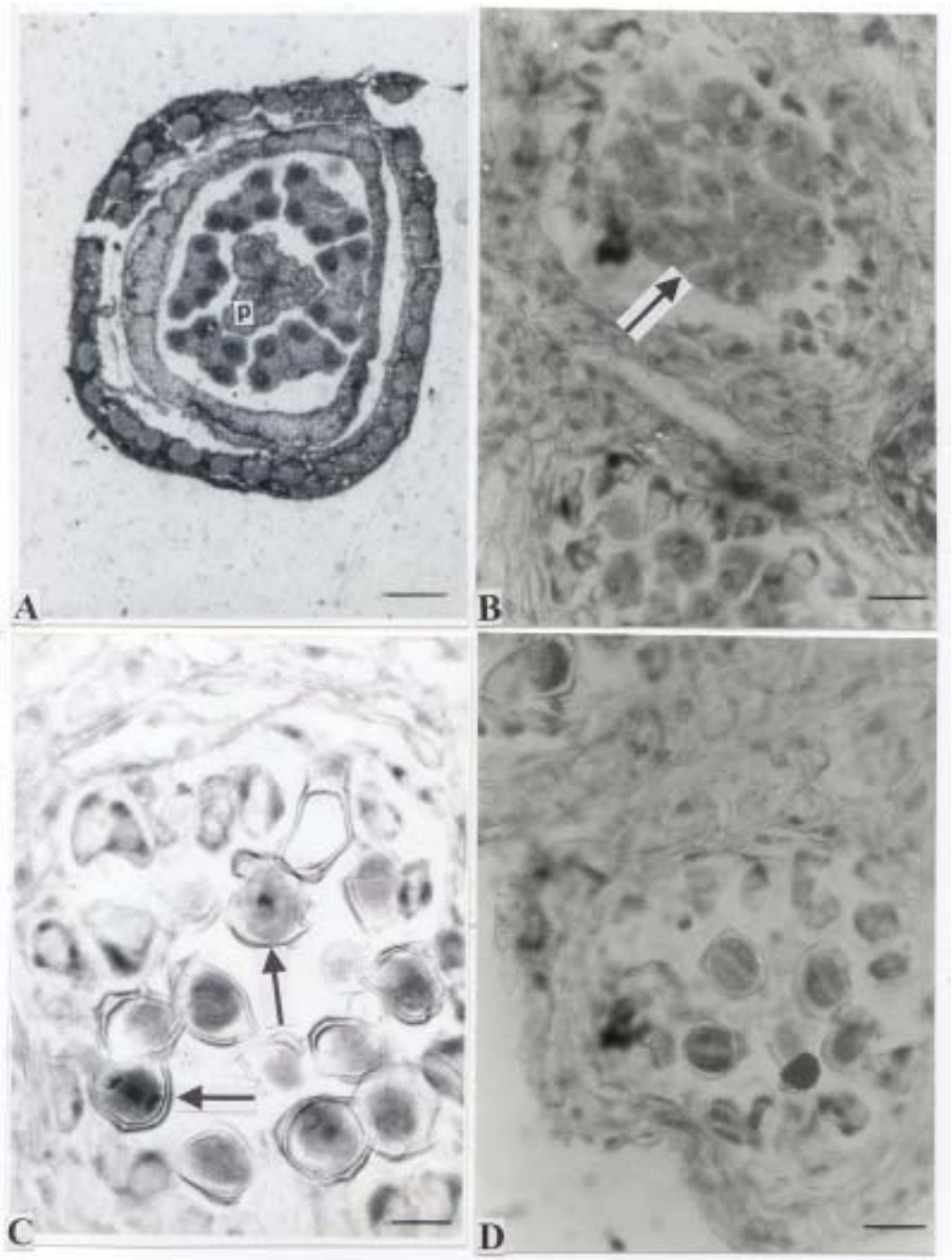


Fig. 1. *Calamus stoloniferus.* A. T. S. of a male flower showing 6 anthers and a pistillode, bar = 0.2 mm. B. Part of T. S. of young microsporangia showing sporogenous tissue (Arrow), bar = 25 μm. C. T. S. of part of microsporangium to show meiosis I in pollen mother cells (Arrows), bar = 25 μm. D. T. S. of microsporangium to show dyads, bar = 25 μm. (p: pistillode)

grains are 3-celled at the time of shedding (Figs. 6C & 7D). In all the investigated species of *Calamus*, the pollen grains are 2-colpate (Fig. 6D). The exine sculpturing is of reticulate type in *C. gamblei*, *C. nagbettai* and *C. rotang*.

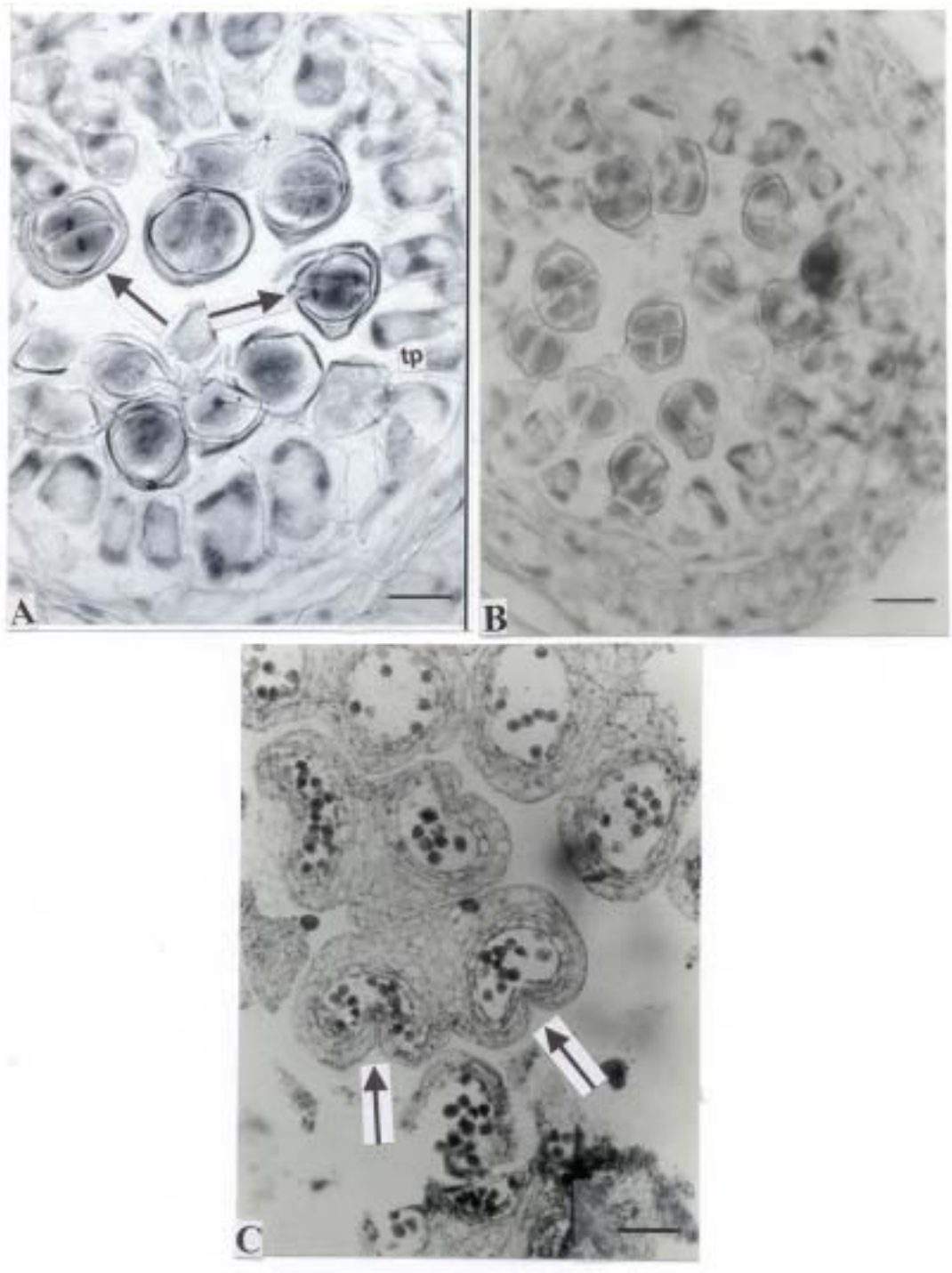


Fig. 2. *Calamus stoloniferus.* A. T. S. of microsporangium to show meiosis II in pollen mother cells (Arrows). Note the tapetal cells are radially elongated, bar = 25 μm. B. T. S. of microsporangium to show tetrads, bar = 25 μm. C. T. S. of an anther to show the organization of stomium in microsporangia (Arrows), bar = 0.1 mm. (tp: tapetum)

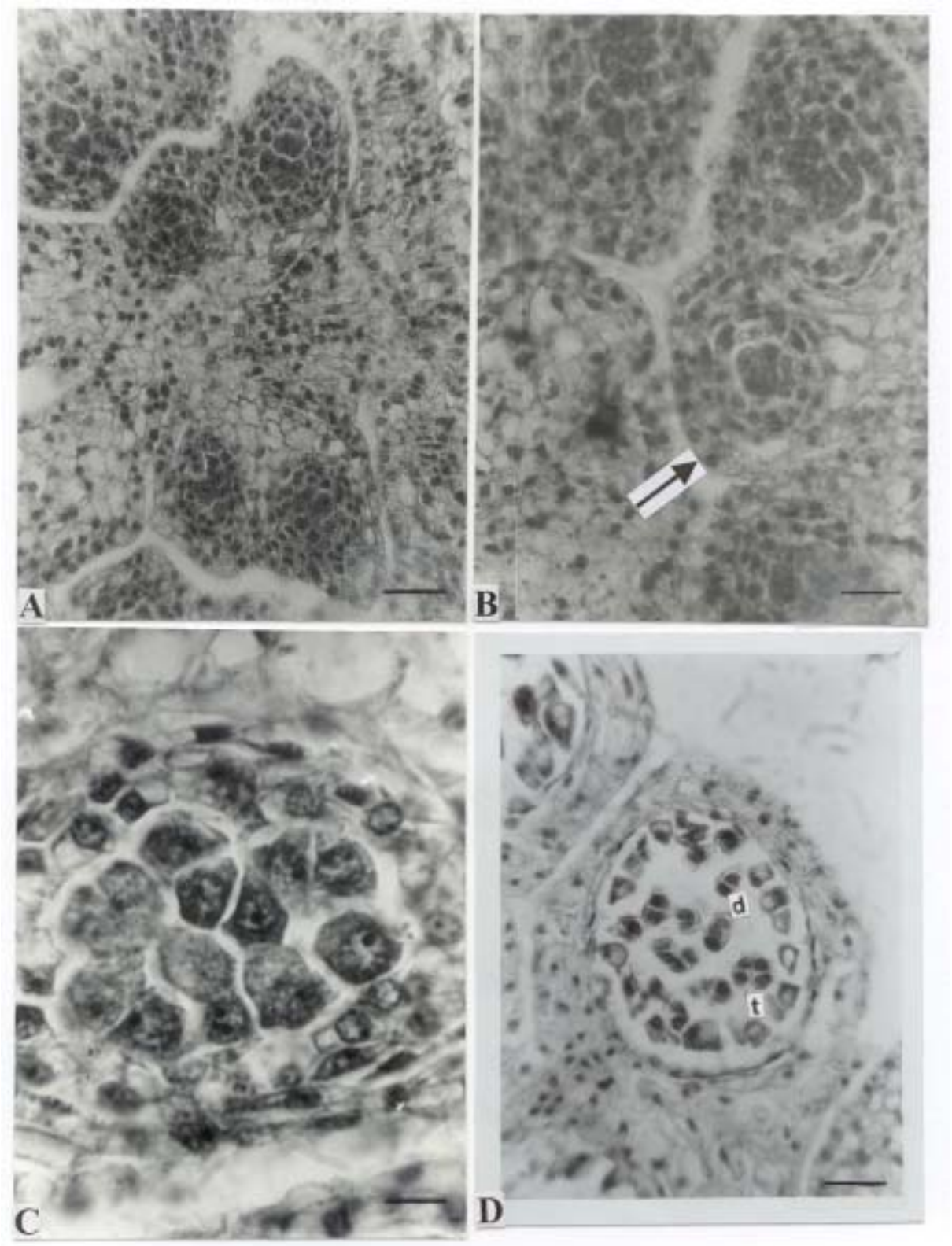


Fig. 3. *Calamus travancoricus.* A. T. S. shows a tetrasporangiate anther, bar = 25 μm. B. T. S. of microsporangium to show the wall layers (Arrow), bar = 25 μm. C. T. S. of a part of microsporangium to show pollen mother cells, bar = 10 μm. D. T. S. of a microsporangium to show dyads and tetrads, bar = 25 μm. (d: dyad, t: tetrad)

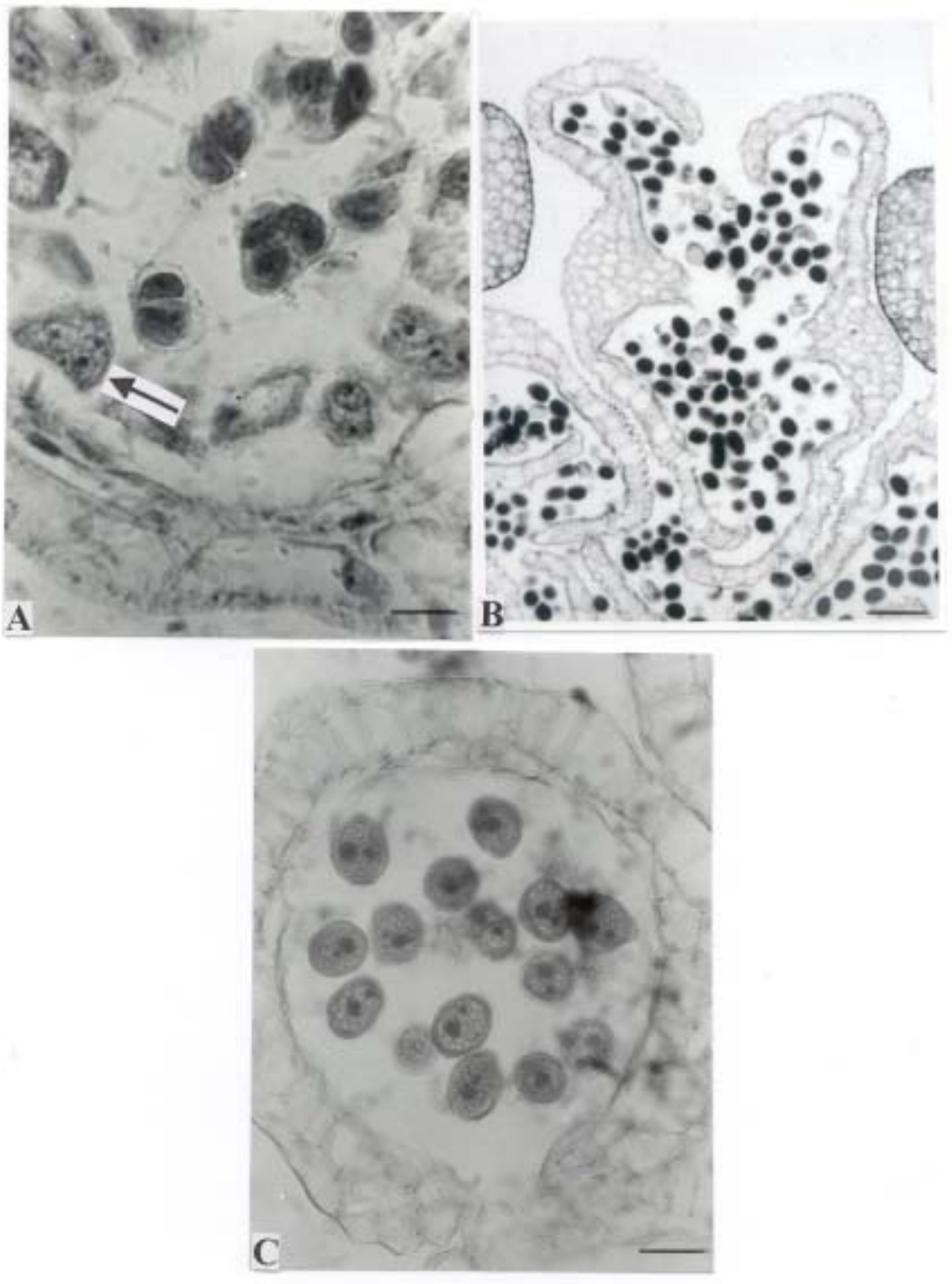


Fig. 4. *Calamus travancoricus.* A. T. S. of part of a microsporangium to show 3-nuceleated tapetal cells (Arrow), bar = 10 μm. B. Shows a mature anther at the time of dehiscence, bar = 50 μm. C. T. S. of microsporangium to show 2-celled pollen grains, bar = 25 μm.

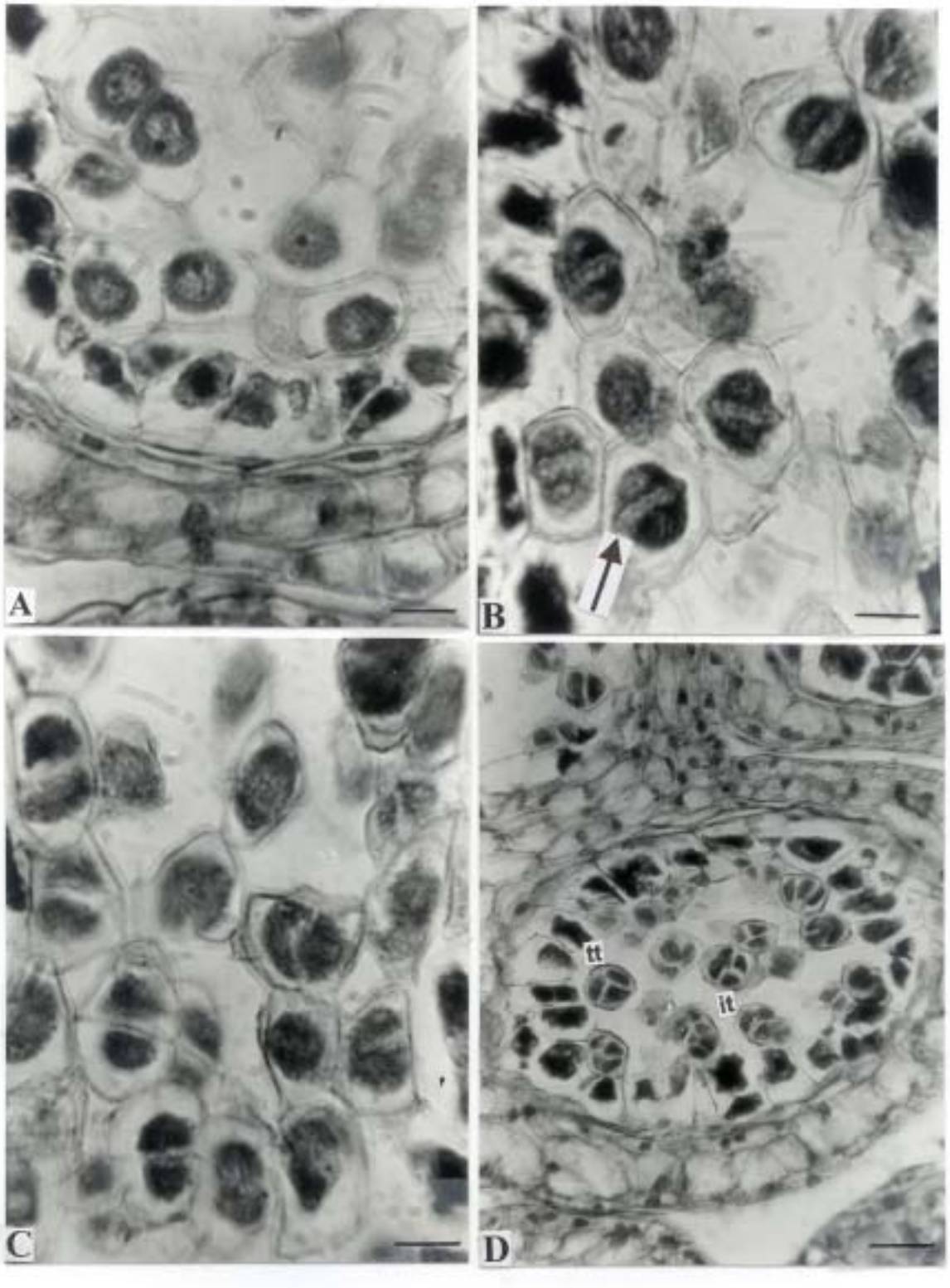


Fig. 5. *Calamus rotang.* A. T. S. of a part of young microsporangium to show pollen mother cells, bar = 10 μm. B. Part of microsporangium to show cell plate formation after meiosis – I in pollen mother cells (Arrow), bar = 10 μm. C. Part of microsporangium to show dyads, bar = 10 μm. D. T. S. of a microsporangium to show tetrads, bar = 25 μm. (it: isobilateral tetrad, tt: tetrahedral tetrad)

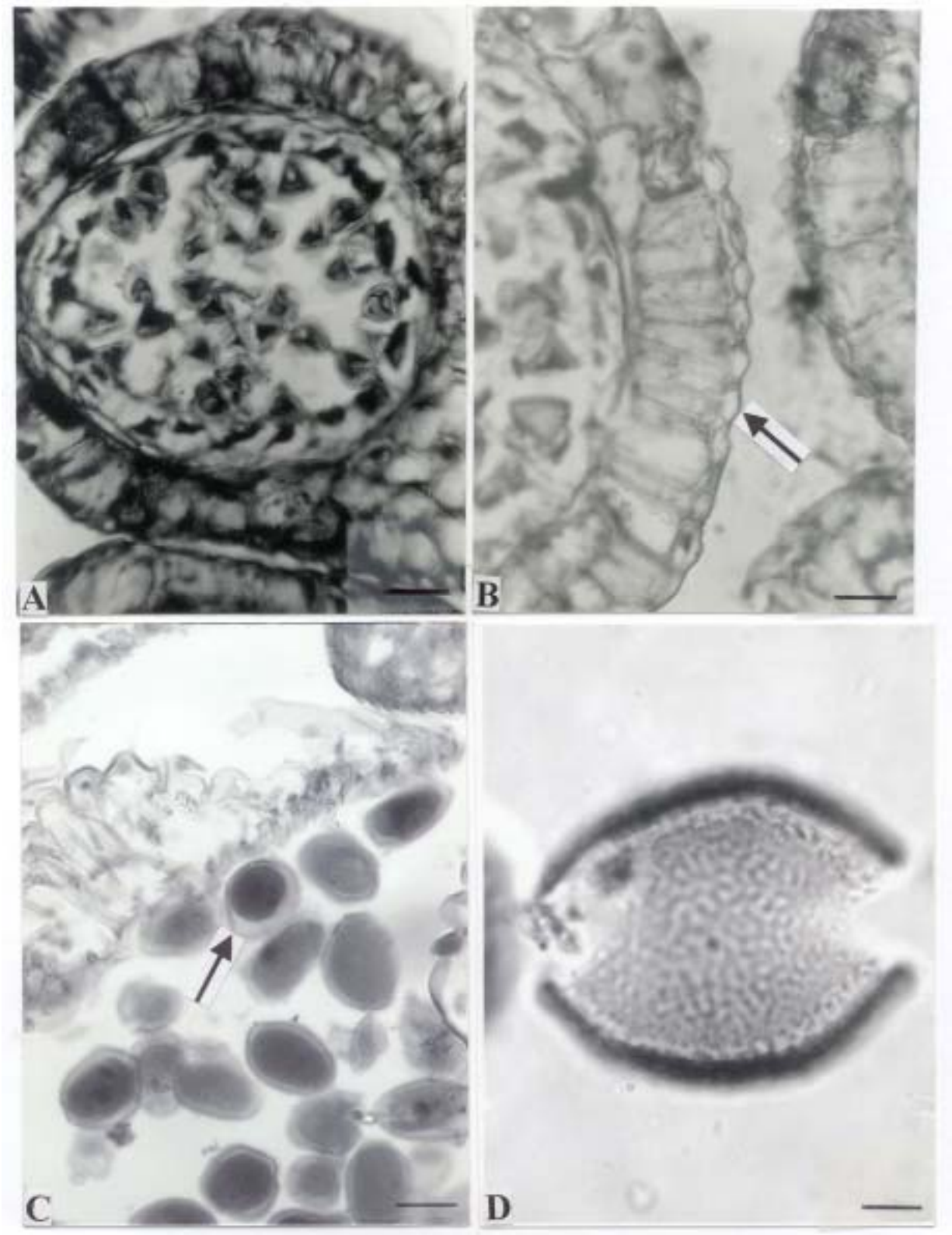


Fig. 6. *Calamus rotang*. A. T. S. of a microsporangium at the microspore stage, bar = 25 μm. B. T. S. of part of a microsporangium to show enucleated epidermis (Arrow), bar = 25 μm. C. Part of T. S. of mature microsprorangium to show 3-celled pollen grain (Arrow), bar = 25 μm. D. An acetolysed pollen grain with 2-colpae, bar = 10 μm.

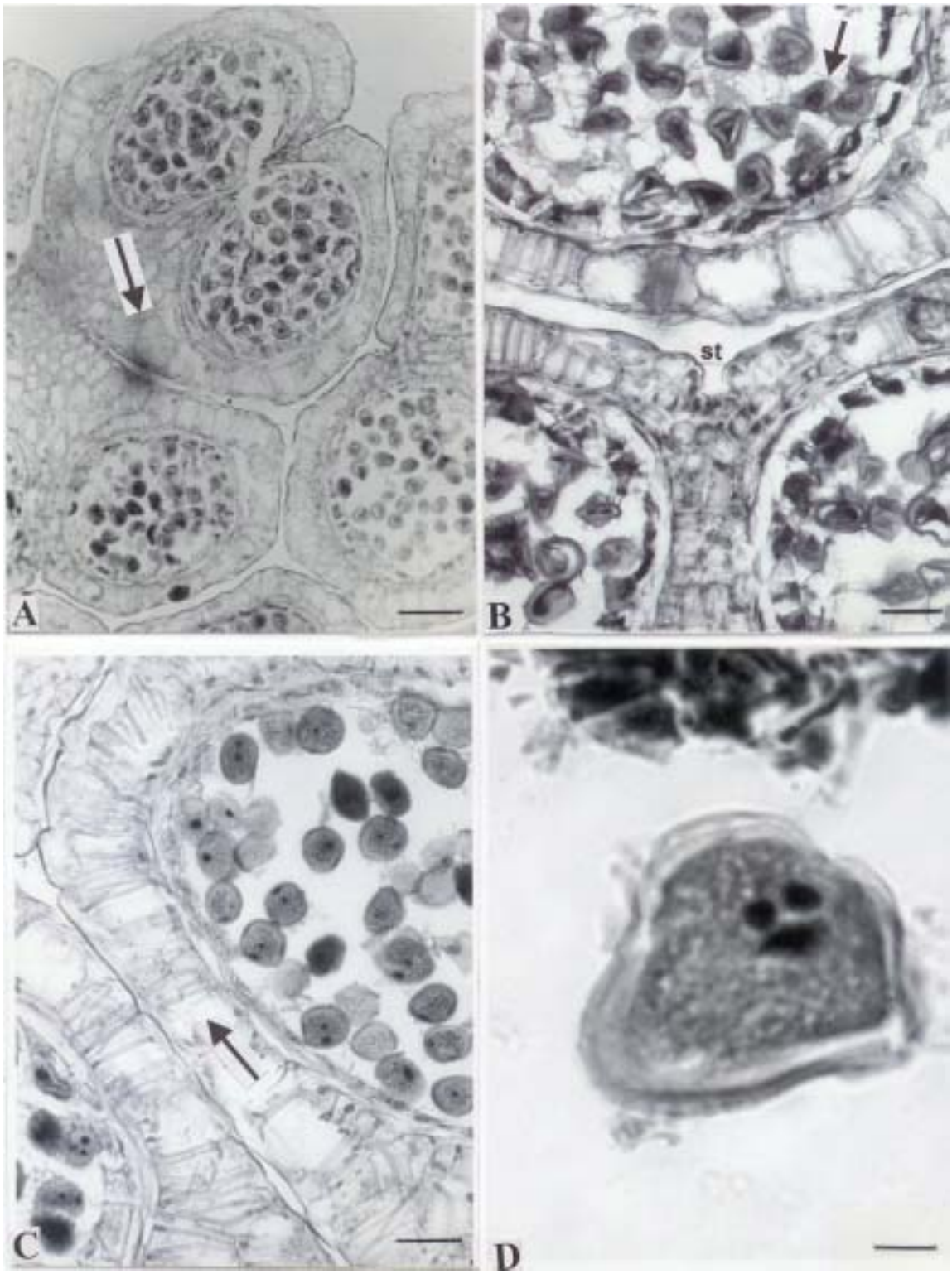


Fig. 7. *Calamus gamblei.* A. T. S. of microsporangia to show secondary wall thickenings on the connective side (Arrow), bar = 0.1 mm. B. Parts of T. S. of microsporangia to show the organization of stomium (st) and a microspore with centrally placed nucleus (Arrow), bar = 25 μm. C. T. S. of part of mature microsporangium to show endothecium with band like secondary wall thickenings (Arrow), bar = 25 μm. D. 3-celled pollen grain, bar = 10 μm.

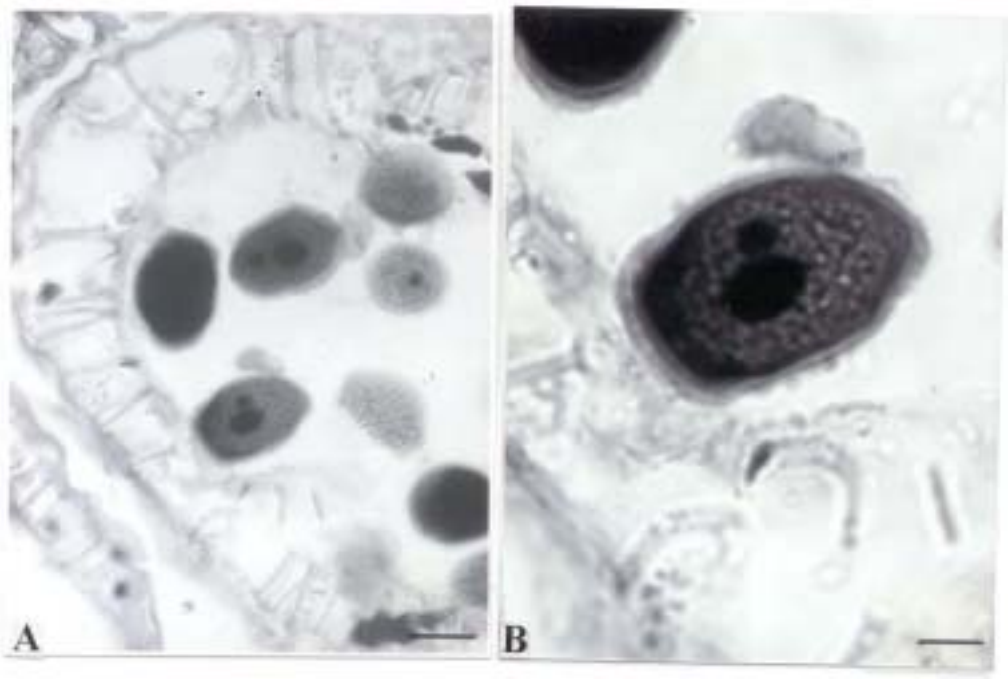


Fig. 8. *Calamus nagbettai.* A. T. S. of microsporangium to show 2-celled pollen grains, bar = 25 μm. B. Enlarged view of a pollen grain, bar = 10 μm.

## DISCUSSION

In all the investigated Arecaceae, the structure of the microsporangium and the development of the male gametophyte is essentially similar (Johri *et al*., 1992). A transverse section of a very young anther is invariably 4-lobed and each lobe lodges the hypodermal archesporium. The periclinal division of the archesporial cells engenders the primary parietal and the primary sporogenous layers. The pattern of development of the microsporangium wall follows the monocotyledonous type (Davis, 1966). The tapetum and the middle layers are the sisters. The tapetum is of the secretory type as in the other investigated species of Palms (Mahabale and Chennaveeraiah, 1957; Mahabale and Biradar, 1968; Biradar, 1968; Biradar and Mahabale, 1968; Kulkarni and Mahabale, 1974.). The parietal cells divide by anticlinal and periclinal divisions to form a wall of 3-4 layers. In the present work also, the species of *Calamus* have a 4-5 layered anther wall. Juliano and Quisumbing (1931) have observed a 6-8 layered anther wall in *Cocos nucifera*. In *Hyphaene indica*, Mahabale and Chennaveeraiah (1957) have found a 5-6 layered anther wall. Such a feature is not noticed in any of the presently investigated species of *Calamus*. In fact, in *C. gamblei*, *C. stoloniferus* and *C. travancoricus*, there is only a 4-layered anther wall with only one middle layer. It is only in *C. rotang* the anther wall is 4-5 layered (with an occasional additional middle layer). The tapetal cells in the present work are uninucleate initially. Subsequently, they become binucleate as in *nagbettai, C. rotang and C. stoloniferus*. This has also been observed in the earlier work on Palms (Mahabale and Chennaveeraiah, 1957; Mahabale and Biradar, 1968; Biradar, 1968; Biradar and Mahabale, 1968; Rao, 1959a & 1959b; Kulkarni and Mahabale, 1974). In *C. gamblei* the tapetal cells may remain uninucleate or binucleate at maturity. Nuclear fusions and polyploidization of tapetal cells is observed in *C. gamblei and C. stoloniferus*. Such a feature has not been observed in the other species.

Generally, the disorganization of the tapetal cells for providing nutrition to the pollen mother cells may start before the commencement of meiosis. The walls of the tapetal cells stretch radially and start breaking down. In *C. rotang*, there is no radial increase of the tapetal cells. The cells start disorganizing only when dyads and tetrads are formed. Such observations on the behaviour of the tapetum have not been made in other investigated Palms (Johri *et al*., 1992). The endothecium develops fibrous secondary wall thickenings as in the other investigated species of Palms (Johri *et al*., 1992). The division of the pollen mother cells is of the successive type as in the majority of investigated Palms (Davis, 1966; Johri *et al*., 1992). Rao (1959b) has reported a simultaneous cell plate formation in *Chrysalidocarpus lutescens*. Such reports (Süssenguth, 1921; Schnarf, 1931; Rao, 1959b) needs a careful reinvestigation.

The microspore tetrads are isobilateral or tetrahedral as in *Phoenix* (Mahabale and Biradar, 1968; Biradar, 1968; Biradar and Mahabale, 1968) and *Caryota urens* (Shirke and Mahabale, 1972). The occurrence of T-shaped tetrads as that has been observed by Mahabale and Chennaveeraiah (1957) in *Hyphaene indica* is not observed in the present work. Rao (1959b) has recorded isobilateral, decussate and tetrahedral tetrads in *Areca catechu* and *Chrysalidocarpus lutescens*. Mature pollen grains are shed at 2-celled stage in *Phoenix sylvestris* and *Caryota urens* as in most investigated species of Palms (Johri *et al*., 1992). In the present work, the pollen grains of *C. nagbettai, C. stoloniferus* and *C. travancoricus* are 2-celled when shed. But in *C. gamblei* and *C. rotang* the pollen grains are 3-celled at the time of shedding. The mature pollen grains in *Phoenix sylvestris* are monocolpate (Mahabale and Biradar, 1968; Shirke and Mahabale, 1972). Thanikaimoni (1971) and Sowunmi (1972) are justified in stating that the pollen morphology of *Calamus* is distinct from all other palm species in having dicolpate pollen grains as in the present work.

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# *Calamus* L. (Arecaceae) 小孢子發育的研究

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## 摘　　要

至今仍未見相關於 *Calamus* 屬 (Arecaceae) 胚胎學的研究，本研究即觀察 *Calamus* 屬五種植物的小孢子囊與雄配子體的發育。結果得知其小孢子囊的營養層為分泌型且細胞具有雙核，花粉母細胞經連續的分裂而形成雙排與四分體型的小孢子。*C. nagbettai*、*C. Stoloniferus* 與 *C. travancoricus* 成熟的花粉粒於藥囊開裂散佈時為二細胞時期，然而 *C. gambleii* 與 *C. rotangs* 則為三細胞時期。

關鍵詞：*Calamus*、花藥壁、花粉。

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