CHAPTER 4

PALYNOLOGY STUDIES OF TRIBE MITREPHOREAE (ANNONACEAE) IN THAILAND

4.1 INTRODUCTION

Annonaceae are a pantropical family of, shrub trees and lianas. The family consists of about 130 genera and 2300 species. The largest number of genera and species are known from Asia (including Australia and the Pacific (Mols & Keßler, 2003). Based on the morphological characters, the infrafamilial classification of the family Annonaceae has been done in different ways by different botanists in the past. They are summarized below.

Bentham and Hooker (1862) classified the Annonaceae into five tribes (Uvarieae, Unonieae, Miliuseae, Mitrephoreae and Xylopieae) based on the aestivation of calyx and corolla and the structure of the stamen connective. In their classification members were group into the tribe Mitrephoreae are *Goniothalmus*, *Mitrephora*, *Pseuduvaria*, *Friesodielsia*, *Orophea*, *Popowia* and *Neo – uvaria* by the character of inner petals curving over the sexual organs forming a dome – shape (mitreform structure).

Ridley (1922) studied Annonaceae in the Malay Peninsular. He placed the genera of Annonaceae into six tribes (Uvarieae, Unonieae, Miliuseae, Mitrephoreae, Annonieae and Xylopieae) and the genera which were classified into the Tribe Mitrephoreae by the character of inner petals arching over the sexual organs and forming a dome, are *Goniothalamus*, *Orophea*, *Oxymitra*, *Mitrephora* and *Popowia*.

Sinclair's (1955) revision of the Malayan Annonaceae, classified them into 6 tribes like Ridley (1922). The members of tribe Mitrephoreae were placed with 2 additional genera, *Pseuduvaria* and *Neo - uvaria* but *Orophea* was transferred into the tribe Miliuseae. It was noted that while *Orophea* fits the circumscription of the tribe Mitrephoreae by virtue of the character of its inner petals, some members of this genus also have unusual stamens that associate it with the tribe Miliuseae. He further noted

that the inner petals of *Mitrephora, Popowia* and *Neo – uvaria* are united only at the beginning of flower development and are separated at anthesis. Only flowers of *Goniothalamus, Oxymitra* and *Pseuduvaria* have true mitreform domes during anthesis.

Fries (1959) modified the Bentham and Hooker classification. He subdivided Bentham and Hooker's tribes into 3 tribes, 14 groups, based on additional characters such as the position of inflorescences, the number and position of ovules, fruit types, the presence of floral bracts and stamens. *Goniothalamus* was considered part of the *Orophea* group, with *Atopostema, Excellia, Oreomitra, Orophea, Petalolophus, Phaeanthus, Platymitra, Pseuduvaria, Richella, Schefferomitra, Trivalvaria* and *Mitrephora.*

The tribe Mitrephoreae which classified by Sinclair (1955) is characterized by sepals valvate. Petals valvate, inner larger or smaller than outer, less often subequal, usually dissimilar, concave, connivent, arching over the sexual organs and forming a dome, if free, their edges at first united for a short time, clawed, the claw often long and narrow or vestigial or absent. Stamen many, flat-topped or convex. In this dissertation a palynological study of the Thai Mitrephoreae pollens of the tribe Mitrephoreae were followed the infrafamilial classification of Sinclair (1955).

There have been few previous studies on the pollen of the genus in the tribe Mitrephoreae, using electron microscopy. Walker (1971) studied pollen characteristics of nine species of *Goniothalamus*. His report indicated that the pollen of *Goniothalamus* consists of tetrahedral to tetragonal tetrads, with heteropolar, bilateral, cataulcerate, disc – like concave – convex grains. The grains are comparatively large to very large, and average 95 µm in diameter. They are microtectate, with no columellae discernible, and with wide pits or otherwise a psilate exine. However, the morphology of pollen and tetrads observed in this study does not offer enough diversity to be useful for classification at the species level. The pollen of *Goniothalamus* is more or less homogenous throughout the genus.

Pollen of the other genus, *Mitrephora*, was studied by Weerasooriya (2001). He reported that the pollen consists of tetrahedral tetrads, with radiosymmetric, inaperturate, globose grains. The exine sculpturing is verrucate. The grain average is

55 µm. However, the morphology of pollen of this genus does not offer enough diversity to be useful for classification at the species level. More recently Su and Saunders (2003) studied the structure of pollen of 42 species of *Pseuduvaria*. The pollen is consistently inaperturate, isopolar and radially symmetrical. Four basic patterns of exine sculpturing are identified: rugulate, verrucate, scabrate and psilate. The exine stratification of one representative species, *P. macrocarpa*, is shown to be entirely ectexinal. The extexine consists of a discontinuous outer tectal layer, a collumellar infratectal layer, and an inner lamellar foliated foot layer; the intine is very thin and fibrillar. The pollen is invariably released as acalymmate tetrads, in which the tectum is absent from the proximal walls. The individual pollen grains within the tetrads are connected by crosswall cohesion, involving both exine and intine. This form of cohesion has not hitherto been reported in the Annonaceae. In addition, pollen grains of neighboring tetrads are connected in two different ways, viz. short exine connections and non – sporopollenin pollen connecting threads. Neither of these cohesion mechanisms has previously been reported for the genus.

So far pollen of Thai Mitrephoreae has not been studied. Therefore, in this dissertation I propose to conduct a palynological study of the Thai Mitrephoreae to test the taxonomic significance of pollen.

4.2 MATERIALS AND METHODS

The pollen members of Mitrephoreae were used from herbarium materials on loan from CMU herbarium or from fresh material in the wild (Table 4.1).

For light microscope (LM) studies, specimens were preserved in formalin–acetic acid–ethyl alcohol (FAA) or 70% alcohol with a small amount of glycerine added. Pollen was acetolysed (Erdtman, 1960) and stained with safranin and examined under an Olympus compound microscope CH–2.

For scanning electron microscope (SEM) studies, pollen grains, if dry were spread on brass stubs covered with double sided adhesive tape. If preserved, the material were dried by the critical point (CPD) method. All specimens were sputter –

coated with a thin layer of gold and viewed with JEOL JSM – 5910LV SEM at 15 – 20 kV.

Pollen terminology follows Erdtman (1972), Walker & Doyle (1975) and Punt *et al.* (1994)

Table 4.1 Taxa and specimens studied of the tribe Mitrephoreae in Thailand except *Mitrephora wangii*, *Mitrephora winitii*, *Pseuduvaria costata*, *Pseuduvaria grandifolia*, *Pseuduvaria hylandii*, *Pseuduvaria mollis*, *Pseuduvaria reticulate* and *Pseuduvaria sessifolia* were studied by Su and Saunders (2003).

Taxon	**Collecter number**	**Locality**
Friesodielsia desmoids (Craib) Steenis	Yuyen 210 (CMU)	Ranong
Goniothalamus sp. Phuye	Yuyen 219 (CMU)	Kanchanaburi
Goniothalamus sp.Chongyen	Chalermglin 6-3-47 (CMU)	Kamphaeng Phet
Goniothalamus tortillipetalus Henderson	Yuyen 234 (CMU)	Kanchanaburi
Goniothalamus tapis Miq.	Yuyen 205 (CMU)	Pattani
Goniothalamus undulatus Ridley	Yuyen 217 (CMU)	Ranong
Mitrephora keithii Ridley	Chalermglin 14-12-1999	Phetchaburi
Mitrephora maingayi Hook. f. et Thomson	Chalermglin 420217	Phayao

4.3 RESULTS

In this study it has not been possible to obtain specimens of *Popowia and Neo – uvaria* in Thailand. Therefore, the pollen morphology of these genera was not reported. All pollen grains studied of tribe Mitrephoreae (*Friesodielsia*, *Goniothalamus*, *Mitrephora* and *Pseuduvaria*) are in tetrads (tetrahedral, tetragonal, decussate and rhomboidal). The pollen sizes are vary from medium to very large. The commonest pollen type is rugulate, then verrucate, then scabrate and then psilate. The aperture type is inaperturate. Pollen morphology of the tribe Mitrephoreae is summarized in table 4.2.

POLLEN MORPHOLOGY OF THE TRIBE MITREPHOREAE

1. *Friesodielsia* Steenis (*c.* 7 *spp*. in Thailand (Bygrave, 1997), 1 *spp.* studied; Figure 4.1: 1 - 2)

Pollen is inaperturate, isopolar, and radially symmetrical. The grains are large *c.* 81 – 83 µm. The pollen remains in permanent acalymmate tetrads at maturity. Tetrads are tetragonal to decussate. Sculpturing is rugulate, with pore – like gaps between rugulae.

2. *Goniothalamus* (Blume) Hook. f. & Thomson (*c.* 21 *spp*. in Thailand (Bygrave, 1997), 5 *spp*. studied; Figure 4.1: 3 – 8 & 4.2: 9 - 12)

Pollen is inaperturate, isopolar, and radially symmetrical. The grains are large *c.* 67 – 109 µm. The pollen is in permanent acalymmate tetrads at maturity. Tetrads are mostly tetrahedral, except *G. tortilipetalus* (Figure 4.1: 7 – 8). It has tetragonal tetrads. All species of *Goniothalamus* are psilate.

3. *Mitrephora* (Blume) Hook. f. & Thomson (*c.* 21 *spp*. in Thailand (Bygrave, 1997), 4 *spp*. studied; Figure 4.2: 13 – 16 & 4.3: 17 - 20)

Pollen is inaperturate, isopolar, and radially symmetrical. The grains are large c. 53 – 88 µm. The pollen is in permanent acalymmate tetrads. Tetrads are mostly tetragonal, but also decussate, with intermediate forms between tetragonal and

decussate. Rhomboidal tetrads occur too. Sculpturing all species is rugulate, with small aereolae, except in *M. winitii* (Figure 4.3: 19 – 20). This species differs from the others in being rugulate, with striae on the rugulae.

4. *Pseuduvaria* Miq. (*c.* 7 *spp.* In Thailand (Bygrave, 1997), 42 *spp*. were studied by Su and Saunders, 2003; Figure 4.4: 21 – 26 & 4.5: 27 - 32)

As it has not been possible to obtain pollen of *Pseuduvaria* the following description is based on Su and Saunders (2003)

Pollen is inaperturate, isopolar, and radially symmetrical. The pollen is asymmetrical and approximately circular in polar view, but with a round distal face and angular proximal faces in equatorial view. The longest pollen grain axis is *c.* (25-) 30 – 50 µm. The pollen is in permanent acalymmate tetrads. Some species possess loosely associated tetrads, however, which can separate during anther dehiscence and consequently sometimes appear as monads. Various types of tetrad occur in the genus; rhomboidal, tetragonal, tetrahedral, and rarely decussate. There are four basic sculpture types in the 42 species of *Pseuduvaria*; the commonest pollen type (17 *spp.*) is rugulate (Figure 4.4: 21 – 24), with variably sized, elongate elements distributed irregularly over the surface. The remaining species (15 *spp.*) have verrucate sculpturing (Figure 4.4: 25 – 26), with small elements. Eight species have scabrate sculpturing (Figure 4.5: 27 – 28), with very fine projections and two species are uncommon in possessing psilate pollen (Figure 4.5: 29 - 32).

Table 4.2 Palynological results of tribe Mitrephoreae.

Taxon	Dispersal unit	Size (µm)	Exine pattern	Aperture type
Genus : *Friesodielsia*				
Friesodielsia desmoids (Craib) Steenis	tetragonal tetrad, decussate tetrad	81-83	rugulate	inaperturate
Genus : *Goniothalamus*				
Goniothalamus sp. Phuye	tetrahedral tetrad	67-81	psilate	inaperturate
Goniothalamus sp.Chongyen	tetrahedral tetrad	98-104	psilate	inaperturate
Goniothalamus tortillipetalus Henderson	tetragonal tetrad	105-109	psilate	inaperturate
Goniothalamus tapis Miq.	tetrahedral tetrad	92-107	psilate	inaperturate
Goniothalamus undulatus Ridley	tetrahedral tetrad	88-95	psilate	inaperturate

Table 4.2 Palynological results of tribe Mitrephoreae (continued).

Taxon	Dispersal unit	Size (μm)	Exine pattern	Aperture type
Genus : *Mitrephora*				
Mitrephora keithii Ridley	tetragonal tetrad, decussate tetrad, rhomboidal tetrad	60-61	rugulate, with large areolae	inaperturate
Mitrephora maingayi Hook. f. et Thomson	tetragonal tetrad, rhomboidal tetrad	53-56	rugulate, with large areolae	inaperturate
Mitrephora wangii Hu	tetragonal tetrad	81-88	rugulate, with large areolae	inaperturate
Mitrephora winitii Craib	tetragonal tetrad, decussate tetrad, rhomboidal tetrad	85-88	rugulate, with striate on regulate	inaperturate
Genus : *Pseuduvaria*				
Pseuduvaria costata (Scheffer) J. Sinclair	rhomboidal tetrad	28-31	scabrate, with small surface elements	inaperturate
Pseuduvaria grandifolia Merr.	tetrahedral tetrad	28-31	scabrate, with small surface elements	inaperturate

Table 4.2 Palynological results of tribe Mitrephoreae (continued).

Taxon	Dispersal unit	Size (μm)	Exine pattern	Aperture type
Pseuduvaria hylandii Jessup	tetrahedral tetrad	39-41	verrucate	inaperturate
Pseuduvaria mollis (Warb.) J. Sinclair	rhomboidal tetrad	29-32	scabrate, with gemmae	inaperturate
Pseuduvaria reticulate (Blume) Miq.	tetrahedral tetrad	31-32	rugulate, with large surface elements	inaperturate
Pseuduvaria sessilifolia J. Sinclair	tetrahedral tetrad	29-33	psilate, with microperforate	inaperturate

* pollen grain size classes (Walker & Doyle, 1975)

very small	< 10	μm
small	10 - 25	μm
medium size	25 - 50	μm
large	50 - 100	μm

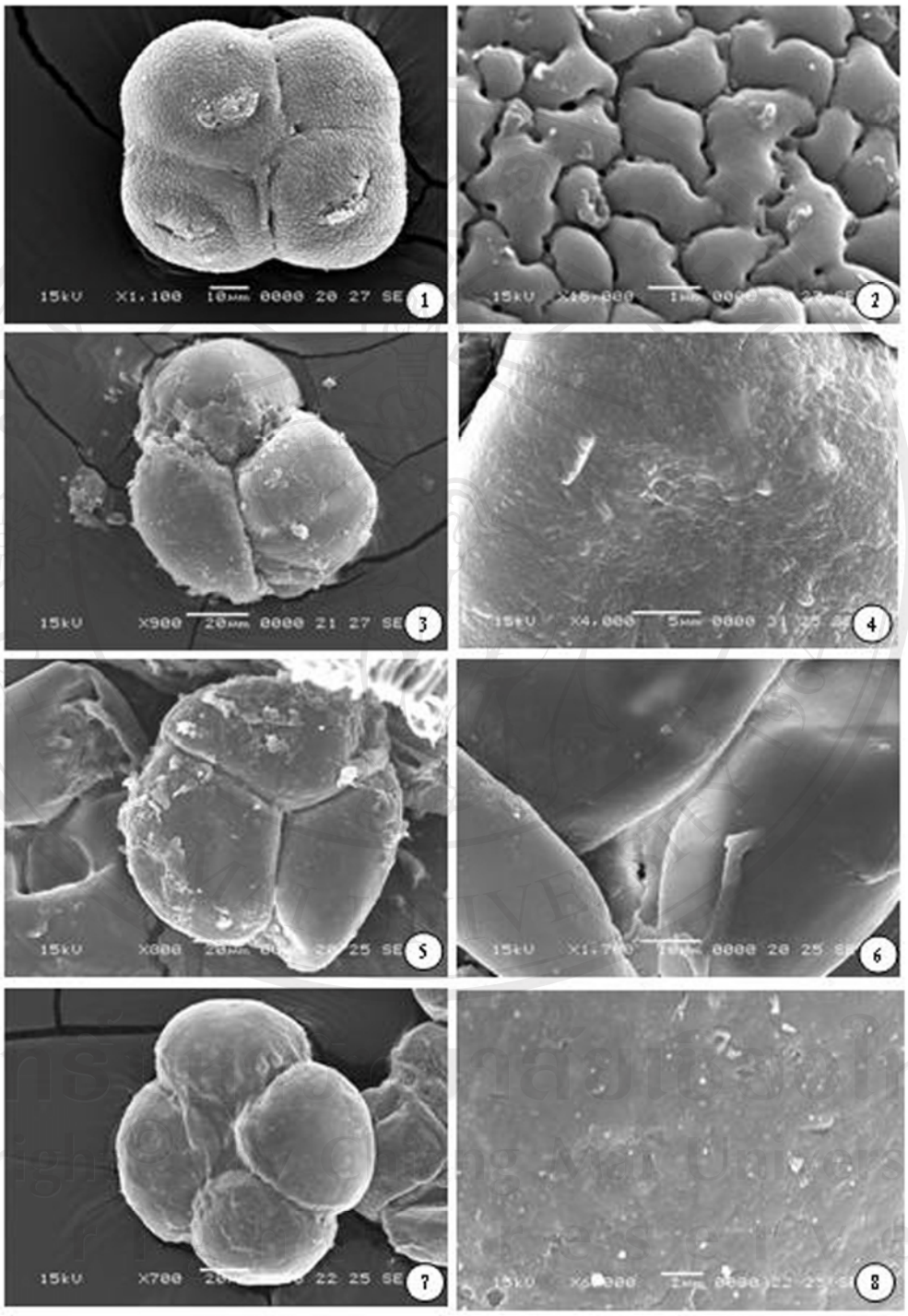

Figure 4.1 SEM micrographs of pollen grains. 1 - 2, *Friesodielsia desmoids*; 3 - 4. *Goniothalamus* sp. Phuye; 5 - 6, *Goniothalamus* sp. Chongyen. 7 – 8, *Goniothalamus tortillipetalus*.

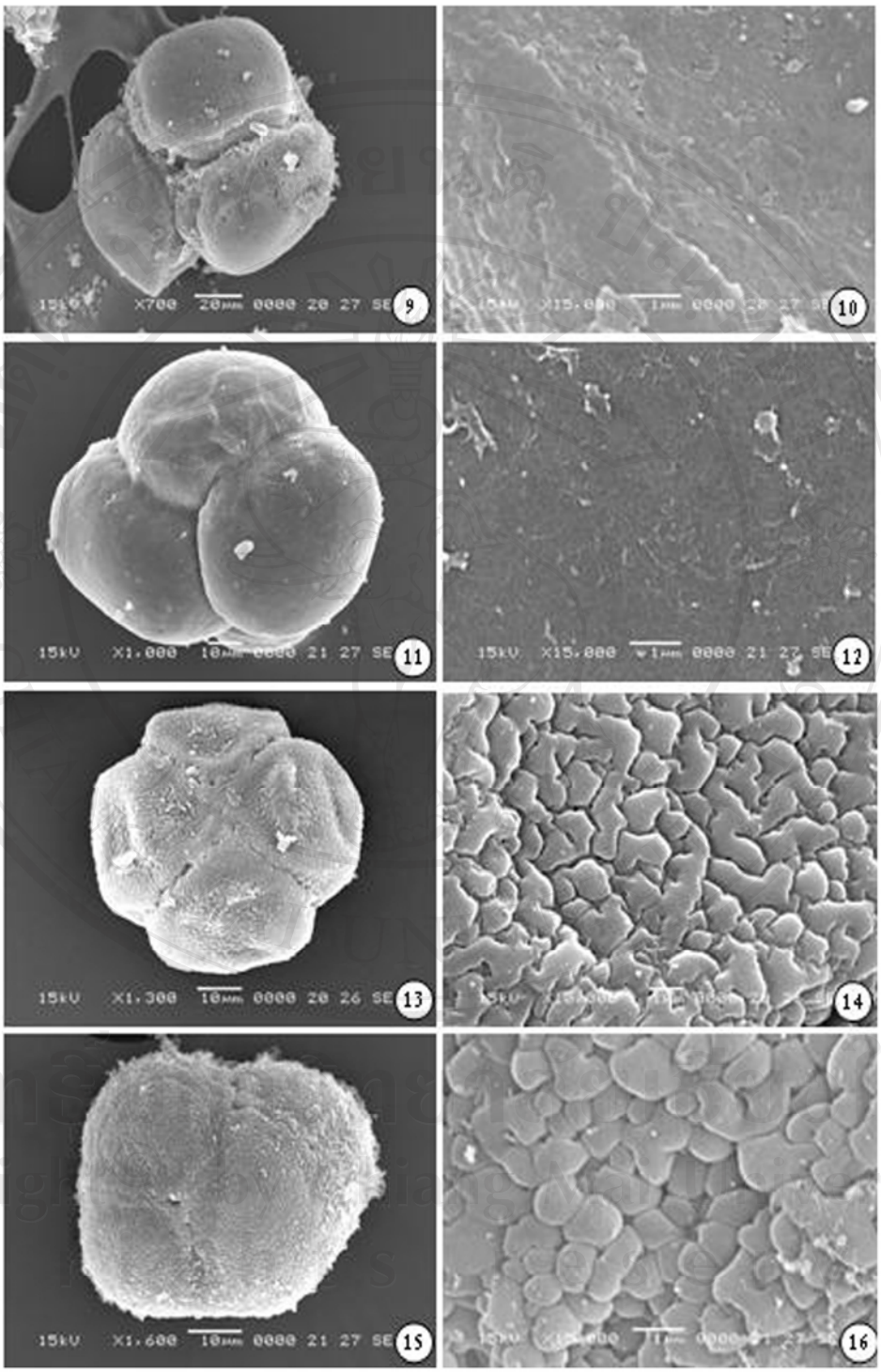

Figure 4.2 SEM micrographs of pollen grains. 9 - 10, *Goniothalamus tapis*; 11 – 12, *Goniothalamus undulatus*; 13 - 14, *Mitrephora keithi*; 15 – 16, *Mitrephora maingayi*.

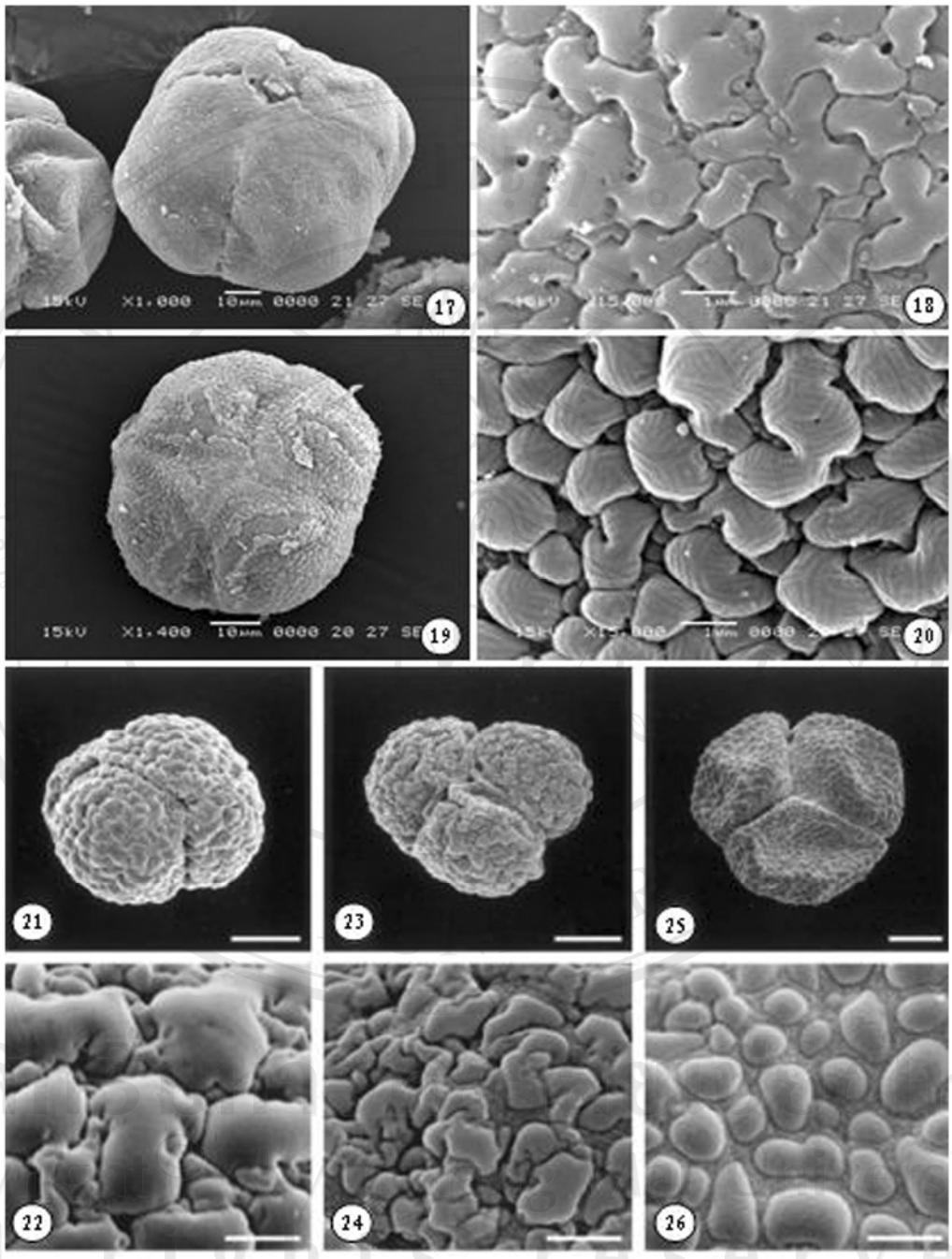

Figure. 4.3 SEM micrographs of pollen grains. 17 - 18, *Mitrephora wangii*; 19 – 20, *Mitrephora winitii*; 21 – 26 (Su and Saunders, 2003). 21 – 22, *Pseuduvaria reticulata*; 23 – 24, *Pseuduvaria grandifolia*; 25 – 26 *Pseuduvaria hylandii*. Scale bars: Figures 21, 23, 25 = 10 µm; Figure 22, 24, 26 = 2 µm.

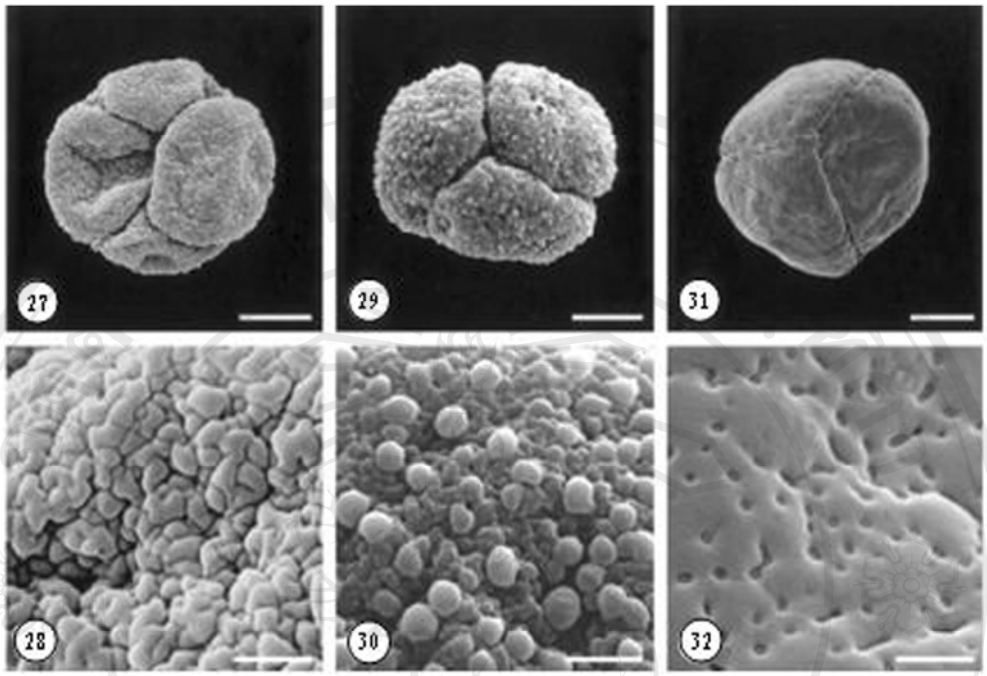

Figure. 4.4 SEM micrographs of pollen (Su and Saunders, 2003). 27 – 28, *Pseuduvaria costata*; 29 – 30, *Pseuduvaria molis*; 25 – 26, *Pseuduvaria sessilifolia.* Scale bars: Figures 27, 29, 31 = 10 µm; Figure 28, 30, 32 = 2 µm;

4.4 DISCUSSION

Table 4.2 summarizes the Palynological features of the tribe Mitrephoreae, examined in the present and the previous studies (Su and Saunders, 2003). The pollen of tribe Mitrephoreae is a little diverse and an invaluable source of taxonomic data, but the morphology of pollen observed in this study does not offer enough diversity to be useful for classification at the generic level of this tribe (the pollen of *Friesodielsia* is homogenous with *Mitrephora*). However, the studies are not completed. Further material will be collected and examined in the future.

THE FORMATION OF POLLEN

The pollen of the tribe Mitrephoreae is in permanent acalymmate tetrads. The formation of pollen in permanent tetrads is common in the Annonaceae, and has been reported in c. 40 genera (Walker, 1971; Le Thomas, 1981). Various types of tetrad occur in the tribe Mitrephoreae; tetrahedral, tetragonal, decussate and rhomboidal. There is usually more than one type of tetrad in the same species (especially *Mitrephora*), some species have three types of tetrad e. g. *Mitrephora keithii* and *M. winitii*; tetragonal, decussate and rhomboidal. From the present study, connections by crosswall cohesion between individual pollen grains was not found in other genera in contrast to the genus *Pseuduvaria* (Su and Saunders, 2003).

POLLEN SIZE

Pollen size of Mitrephoreae are varies from medium to very large. From previous studies of *Pseuduvaria*, average size is medium, *c.* (25) 30 – 50 µm (Su and Saunders, 2003). Moreover, the present investigation supports previous studies of the size of *Goniothalamus* and *Mitrephora*. *Goniothalamus* pollen is large to very large (Walker, 1971)**,** *c.* 67 – 109 µm. and in *Mitrephora*, the grains are large (Weerasooriya, 2001), *c.* 67 – 109 µm. Additionally, *Friesodielsia* has large pollen also, *c*. 81 – 83 µm.

Exine sculpturing

From the previous studies and the present investigation of exine sculpturing of the tribe Mitrephoreae the exine sculpturing is more or less homogenous throughout, except in *Pseuduvaria* which has has a more diverse exine sculpturing. There are four basic sculpture types of *Pseuduvaria* (Su and Saunders, 2003); the commonest pollen type is rugulate, then verrucate, then scabrate and then psilate. All *Goniothalamus* species are psilate. Additionally, *Mitrephora* and *Friesodielsia* have the same exine sculpturing (rugulate). These two genera have pollen which is the same size so they cannot be separated on the basis of their pollen grains.

APERTURE TYPE

The aperture type of all Mitrephoreae is inaperturate. Observations using scanning electron microscopy revealed that emergence of the pollen tube probably begin from thinner regions without thickening which occur on exposed distal surfaces of the grain. As there is only one of these per grain they may represent incipient apertures (Figure. 4.1: 1 & 4.3: 19).

POLLEN RELATIONSHIP WITHIN THE TRIBE

Using exine sculpturing the tribe Mitrephoreae can be divided into two groups. Group I; exine sculpturing is rugulate, (*Mitrephora* and *Friesodielsia*). Group II; exine sculpturing is psilate, (*Goniothalamus*). However, *Pseuduvaria* cannot be classified into these two groups. There are intermediate forms of exine sculpturing; rugulate, verrucate, scabrate and psilate (Su and Saunders, 2003). The commonest pollen type of *Pseuduvaria* is rugulate**;** probably these species are closer to *Mitrephora* and *Friesodielsia* than to *Goniothalamus*.

Sinclair (1955) divided the tribe Mitrephoreae into two groups using inner petals united to form a dome. In group I, the inner petals are united only at the beginning of flower development and are separated at anthesis (*Mitrephora, Popowia* and *Neo – uvaria*). In group II, flowers have true mitreform domes during anthesis (*Goniothalamus, Friesodielsia* and *Pseuduvaria*). Moreover, *Goniothalamus* is closer to *Pseuduvaria* than to *Friesodielsia* in having inner petals with claws, which is absent

in *Friesodielsia*. Therefore, the present investigation supports Sinclair's (1955) classification that *Goniothalamus* is more closely related to *Pseuduvaria* than to *Friesodielsia*. However, this study is still incomplete.

4.5 CONCLUSIONS

The pollen of tribe Mitrephoreae is in permanent acalymmate tetrads. Various types of tetrad occur in the tribe Mitrephoreae; tetrahedral, tetragonal, decussate and rhomboidal. The pollen sizes of Mitrephoreae vary from medium to very large (25) 30 - 109 µm. The commonest pollen type is rugulate, then verrucate, then scabrate and then psilate. All Mitrephoreae are inaperturate. However, from palynological studies of this tribe it is suggested that the pollen of tribe Mitrephoreae is a little diverse and an invaluable source of taxonomic data, but the morphology of pollen observed in this study does not offer enough diversity to be useful for classification at the generic level in this tribe.