Thai J. For. 33 (3) : 48-55 (2014)

Original article

Pollen Morphology of some Thai *Artabotrys* R.Br. (Annonaceae)

Wichan Eiadthong* **Thawee Insura**

Faculty of Forestry, Kasetsart University, Chatuchak, Bangkok 10900, Thailand
*Corresponding Author, E-mail: fforwce@ku.ac.th

Received: Jan 30, 2014 Accepted: Mar 10, 2014

ABSTRACT

The pollen morphology of 15 Thai *Artabotrys* species was investigated under light microscope and scanning electron microscope. The results showed that all Thai *Artabotrys* species are monad pollen, inaperture and apolar. The size of pollen grains are medium to large, ranging from 33.91 ± 0.41 to 55.90 ± 3.19 µm. The pollen shape, classified based on P/E, was divided into 2 groups (subprolate and euprolate): 10 species having subprolate, and 5 species having euprolate. The exine thickness ranged from 1.84 ± 0.12 to 2.66 ± 0.16 µm. The exine ornamentations can be classified into 2 types: perforate-fossulate exine sculpture that has 13 species; and rugulate sculpture that has 2 species. The palynological information could be used for species identification; only 1 species, *A. suaveolens*,which is subprolate in shape and has rugulate exine sculpture, while the others have perforate-fossulate exine sculpture. Thus, the pollen morphology of *Artabortys* species is a useful additional character for taxonomic data.

Keywords: Pollen morphology, *Artabotrys*, Thailand, Palynology

INTRODUCTION

The members of the genus *Artabotrys* are distributed widely in tropical Asia and Africa (Kessler, 1993), and it consists of about 100 species. Many of them have been used as ornamental and medicinal plants. The habit of the genus *Artabotrys* species is either woody climber or scandent shrub. Craib (1922, 1925) reported 4 species in Thailand, namely; A. *vanprukii* Craib, A. *spinosus* Craib, A. *brevipes* Craib and A. *oblanceolatus* Craib. The Royal Forest Department (2001) listed an additional 5 species occuring in Thailand, namely,A. *burmanicus* DC., A. *harmandii* Finet & Gagnep., A. *hexapetalus* (L.f.) Bhandari, A. *siamensis* Miq., and A. *suaveolens* Blume. Chalermglin (2001) found A. *grandifolius* King., which was a new recorded species in Thai flora.

Furthermore, Insura (2009) listed additional five species different from the previous list (Craib, 1922;1925, Royal Forest Department, 2001, Chalermglin, 2001, Thongpairoj, 2008),

namely, A. *aereus* Ast, A. *blumei* Benth., A. *havilandii* Ridl., A. *multiflorus* C.E.C.Fisch., and A. *sumatranus* Miq. Seven new species were recorded in Thailand by Eiadthong and Insura (2011), namely, A. *aereus* Ast, A. *blumei* Hook. f. & Thomson, A. *havilandii* Ridl., A. *uniflorus* Craib, A. *oxycarpus* King, A. *lowianus* King and A. *sumatranus* Miq. Eiadthong and Insura (2011) recognized 18 species in Thailand, namely, A. *aereus* Ast, A. *blumei* Hook. f. & Thomson, A. *brevipes* Craib, A. *burmanicus* A.DC., A. *grandifolius* King, A. *harmandii* Finet & Gagnep., A. *havilandii* Ridl., A. *hexapetalus* (L.f.) Bhandari , A. *lowianus* King, A. *multiflorus* C.E.C.Fisch., A. *oblanceolatus* Craib, A. *oxycarpus* King, A. *siamensis* Miq., A. *spinosus* Craib, A. *suaveolens* Blume, A. *sumatranus* Miq. , A. *uniflorus* Craib and A. *vanprukii* Craib.

The morphological characters of the members of the genus *Artabotrys* are affected to obscure because they are mostly closely and difficult to identify. In addition, all documentaries did not solve to identify by using key to species based on macro-morphological data. Thus, it is necessary to use additional data for species identification and increase information about Thai *Artabotrys* in Flora of Thailand. This research studied whether variation in pollen morphology of Thai *Artabotrys* that can be used as a supplement for species identification.

MATERIALS AND METHODS

Pollen samples of 15 *Artabotrys* species were collected in the field, and the voucher specimens from the field work were deposited in the Forest Bangkok Herbarium (BKF). It was not possible to obtain pollen from three *Artabotrys* species; *A. oxycarpus* King, *A. lowianus* King and *A. uniflorus* Craib. Their pollen grains were investigated using light microscope (LM) and scanning electron microscope (SEM), according to the LM & SEM described by van der Ham (1990). The palynological data of *Artabotrys* species were classified into 4 size classes; very small< 10 mm, small 11-25 mm, medium 26-50 mm and large 51–100 mm following by Erdtman (1971). Pollen described terminology following by Punt *et al.* (1994) and Walker and Doyle (1975). The species list of *Artabotrys* were examined and showed in Table 1. The pollen shape classified by a ratio between the polar axis and the equatorial diameter (P/E); 1.33-2.00 = euprolate, 1.14- 1.33 = subprolate (Erdtman, 1952).

For LM, the pollen grains were mounted directly with basic-fuchsine-glycerin jelly. An Olympus microscope with periplan eyepiece was conducted for the examination of polar and equatorial axis length, exine ornamentation and thickness, amb shapes and mesocolpium diameter. Usually 30 pollen grains of each specimen were accounted and measured using 40X objective lens. The morphology and measurement of pollen grains are given in Table 1. The SEM study, the pollen grains were transferred directly to double-sided sticky tape affixed to stubs and were sputter-coat with platinum. Photomicrographs were taken with a JEOL 200 CXII scanning electron microscope at the laboratories of the Section of Scientific Equipment, Central, Bangkhen Campus of Kasetsart University Research and Development Institute.

RESULTS AND DISCUSSION

Based on LM and SEM observations, pollen grains are monads, large, apolar and inaperture. The pollen shape classified using P/E divided the species into 2 groups (Figure1): 1) subprolate; consisting of 10 species, namely; *A. aereus*, *A. blumei*, *A. grandifolius*, *A. harmandii*, *A. havilandii*, *A. oblanceolatus*, *A. siamensis*, *A. spinosus*, *A. suaveolens* and *A. sumatranus;* and 2) euprolate; consisting of 5 species, namely; *A. brevipes*, *A. burmanicus*, *A. hexapetalus*, *A. multiflorus* and *A. vanprukii* (Table 1). The pollen grains are medium to large in size, ranging from 33.91 ± 0.41 to 55.90 ± 3.19 µm, with *A. grandifolius* being the smallest and *A. hexapetalus* the largest (Table 1 and Figure 2). The exine is semi-tectate; and the sculpture is commonly perforate-fossulate (Table 1), and the rugulate sculpture wast found in only one species (*A. suaveolens*). Although, a continuous variation is observed in exine sculpture within perforate-fossulate sculpture type, two sub-types can be distinguished: with 9 species having loosely perforate-fossulate sculpture, namely; *A. brevipes, A. burmanicus*, *A. harmandii*, *A. hexapetalus, A. oblanceolatus*, *A. siamensis*, *A. spinosus*, *A. sumatranus* and *A. vanprukii;* and 5 species having finely perforate-fossulate sculpture, namely; *A. aereus*, *A. blumei*, *A. grandifolius*, *A. havilandii* and *A. multiflorus*. The exine thickness ranged from 1.84 ± 0.12 to 2.66 ± 0.16 µm, with *A. vanprukii* having the thinnest and *A. aereus* having the thickest (Table 1). The results showed that the information on pollen morphology of 15 Thai *Artabotrys* species could be used for species identification clearly of only 1 species (*A. suaveolens* with rugulate exine sculpture), while all the remaining species had perforate-fossulate exine sculpture.

Description of pollen grain of each *Artabotrys* species

A. aereus Ast: pollen grains monad, apolar, radially symmetrical, diameter 36.49 ± 0.68 µm, subprolate, inaperturate. Exine structure semi-tectate, finely perforate-fossulate (Figure 3A), 2.66 ± 0.16 µm thickness.

A. blumei Benth.: pollen grains monads, apolar, radially symmetrical, diameter 36.25 ± 2.5 µm, subprolate, inaperturate. Exine structure semi-tectate, finely perforate-fossulate (Figure 3B), 2.50 ± 0.5 µm thickness.

A. brevipes Craib: pollen grains monads, apolar, radially symmetrical, diameter 38.75 ± 1.25 µm, euprolate, inaperturate. Exine structure semi-tectate, loosely perforate-fossulate (Figure 3C), 2.34 ± 0.16 µm thickness.

A. burmanicus DC.: pollen grains monads, apolar, radially symmetrical, diameter 54.01 ± 2.62 µm, euprolate, inaperturate. Exine structure semi-tectate, loosely perforate-fossulate (Figure 3D), 2.29 ± 0.15 µm thickness.

A. grandifolius King.: pollen grains monads, apolar, radially symmetrical, diameter 33.91 ± 0.41 µm, subprolate, inaperturate. Exine structure semi-tectate, finely perforate-fossulate (Figure 3E), 2.75 ± 0.25 µm thickness.

A. harmandii Finet & Gagnep.: pollen grains monads, apolar, radially symmetrical, diameter 48.31 ± 2.43 µm, subprolate, inaperturate. Exine structure semi-tectate, loosely perforate-fossulate (Figure 3F), 2.11 ± 0.39 µm thickness.

A. havilandii Ridl.: pollen grains monads, apolar, radially symmetrical, diameter 43.25 ± 0.94 µm, euprolate, inaperture. Exine structure semi-tectate, finely perforate-fossulate (Figure 3G), 2.29 ± 0.15 µm thickness.

A. hexapetalus (L.f.) Bhandari: pollen grains monads, apolar, radially symmetrical, diameter 55.90 ± 3.19 µm, euprolate, inaperturate. Exine structure semi-tectate, loosely perforate-fossulate (Figure 3H), 2.29 ± 0.15 µm thickness.

A. multiflorus C.E.C.Fisch.: pollen grains monads, apolar, radially symmetrical, diameter 49.13± 3.19 µm, euprolate , inaperturate. Exine structure semi-tectate, finely perforate-fossulate (Figure 3I), 2.29± 0.15 µm thickness.

A. oblanceolatus Craib: pollen grains monads, apolar, radially symmetrical, diameter 47.81 ± 0.94 µm, subprolate , inaperturate. Exine structure semi-tectate, loosely perforate-fossulate (Figure 3J), 2.34 ± 0.16 µm thickness.

A. siamensis Miq.: pollen grains monads, apolar, radially symmetrical, diameter 52.41 ± 2.41 µm, subprolate inaperturate. Exine structure semi-tectate, loosely perforate-fossulate (Figure 3K), 2.34 ± 0.16 µm thickness.

A. spinosus Craib: pollen grains monads, apolar, radially symmetrical, diameter 44.03 ± 1.92 µm, subprolate, inaperturate. Exine structure semi-tectate, loosely perforate-fossulate (Figure 3L), 2.14 ± 0.16 µm thickness.

A. suaveolens Blume: pollen grains monads, apolar, radially symmetrical, diameter 41.49 ± 3.25 µm, subprolate, inaperturate. Exine structure semi-tectate, regulate (Figure 3M), 1.98 ± 0.26 µm thickness.

A. sumatranus Miq.: pollen grains monads, apolar, radially symmetrical, diameter 47.50 ± 1.07 µm, subprolate, inaperturate. Exine structure semi-tectate, loosely perforate-fossulate (Figure 3N), 2.32 ± 0.18 µm thickness.

A. vanprukii Craib: pollen grains monads, apolar, radially symmetrical, diameter 36.52 ± 0.25 µm, euprolate, inaperturate. Exine structure semi-tectate, loosely perforate-fossulate (Figure 3O), 1.84 ± 0.12 µm thickness.

Table 1 Pollen morphological data of 15 Thai *Artabotrys* species.

Species	Size (µm) $^{n=30}$	Shape	Exine sculpturing[1)]	Exine thickness (µm)
A. aereus	36.49 ± 0.68	subprolate	finely perforate-fossulate	2.66 ± 0.16
A. blumei	36.25 ± 2.50	subprolate	finely perforate-fossulate	2.50 ± 0.25
A. brevipes	38.75 ± 1.25	euprolate	loosely perforate-fossulate	2.34 ± 0.16
A. burmanicus	54.01 ± 2.62	euprolate	loosely perforate-fossulate	2.29 ± 0.15
A. grandifolius	33.91 ± 0.41	subprolate	finely perforate-fossulate	2.75 ± 0.25
A. harmandii	48.31 ± 2.43	subprolate	loosely perforate-fossulate	2.11 ± 0.39
A. havilandii	43.25 ± 0.94	subprolate	finely perforate-fossulate	2.29 ± 0.15
A. hexapetalus	55.90 ± 3.19	euprolate	loosely perforate-fossulate	2.29 ± 0.15
A. multiflorus	49.13 ± 3.19	euprolate	finely perforate-fossulate	2.29 ± 0.15
A. oblanceolatus	47.81 ± 0.94	subprolate	loosely perforate-fossulate	2.34 ± 0.16
A. siamensis	52.41 ± 2.41	subprolate	loosely perforate-fossulate	2.34 ± 0.16
A. spinosus	44.03 ± 1.92	subprolate	loosely perforate-fossulate	2.14 ± 0.16
A. suaveolens	41.49 ± 3.25	subprolate	rugulate	1.98 ± 0.26
A. sumatranus	47.50 ± 1.07	subprolate	loosely perforate-fossulate	2.32 ± 0.18
A. vanprukii	36.52 ± 0.25	euprolate	loosely perforate-fossulate	1.84 ± 0.12

Remark: [1)] see in Figure 3.

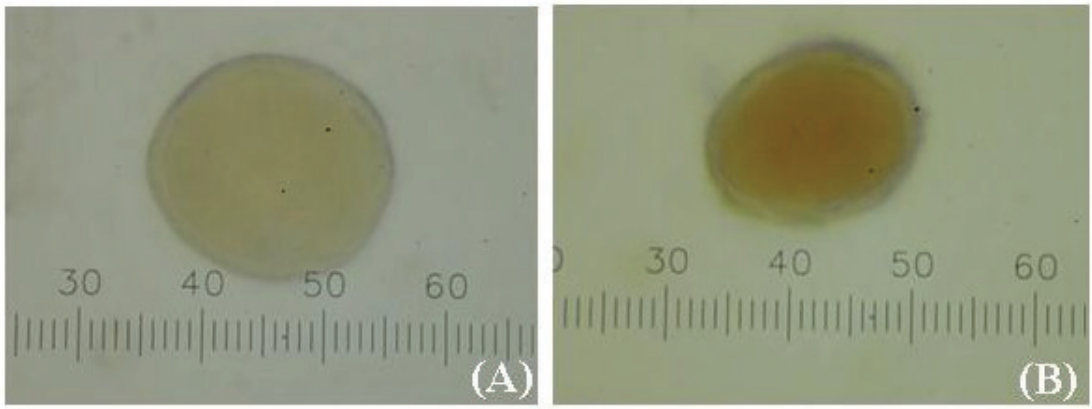

Figure 1 Two pollen shape types of *Artabotrys* pollen grains under LM observation: subprolate (A) and euprolate (B).

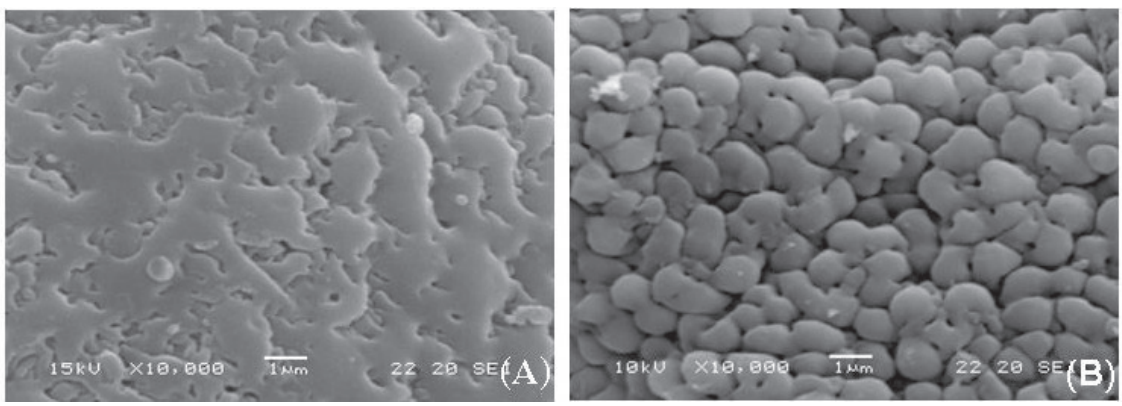

Figure 2 Two exine sculpture types of *Artabotrys* pollen from micrographs under SEM observation: perforate-fossulate (A) and rugulate (B).

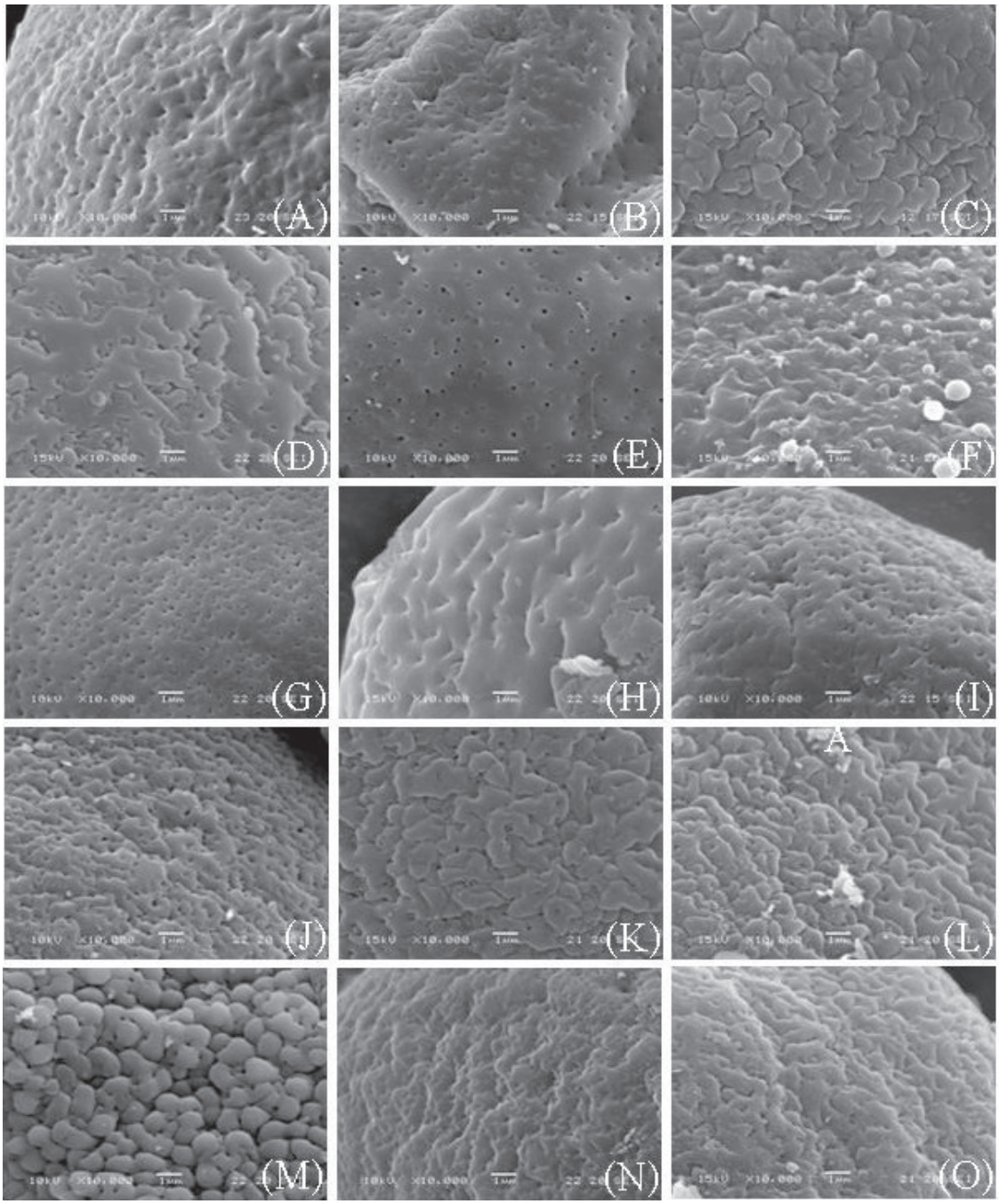

Figure 3 SEM micrographs of the exine sculpture types of some Thai *Artabotrys* species: A) *A. aereus*, B) *A. blumei*, C) *A. brevipes*, D) *A. burmanicus*, E) *A. grandifolius*, F) *A. harmandii*, G) *A. havilandii*, H) *A. hexapetalus*, I) *A. multiflorus*, J) *A. oblanceolatus*, K) *A. siamensis*, L) *A. spinosus*, M) *A. suaveolens*, N) *A. sumatranus*, and O) *A. vanprukii*.

The pollen sample preparation technique for SEM observation involved using the pollens directly from herbarium specimens or fresh samples, then sputter-coat them with platinum, and then observe their palynological characters. The acetolysis method of Erdtman (1960) could not be used to analyze the *Artabotrys* pollen because the acetolysis destroyed the shape and exine structure of the pollen grains.

The results in this study conformed with the palynological information of Thongpairoj (2008), who reported on 11 Thai *Artabotrys* species; namely, *A. brevipes*, *A. burmanicus*, *A. harmandii*, *A. hexapetalus*, *A. multiflorus, A. oblanceolatus, A. suaveolens, A. siamensis, A. spinosus, A. uniflorus* and *A. vanprukii*. In this study, we analyzed more palynological information than Thongpairoj (2008) and increased the number of species of Thai *Artabotrys* species, including, *A. aereus*, *A. blumei, A. grandifolius, A. havilandii,* and *A. sumatranus*.

Thongpairoj (2008) reported 2 species having rugulate exine sculpture (*A. suaveolens* and *A. oblanceoalatus*), while we found only 1 species (*A. suaveolens*) and *A. oblanceoalatus* in our study was loosely perforate-fossulate. Using the palynological terminology of Thongpairoj (2008) used same word among microperforate-fossulate and finely perforate-fossulate in this study. The members of the genus *Artabotrys* are closely macro-morphological characters, that causes unclearly to solve classical taxonomy for species identification.

CONCLUSION

The pollen morphology of 15 Thai *Artabotrys* species was investigated under LM and SEM observations. The results showed that most Thai *Artabotrys* species had monad pollen, subprolate to euprolate shapes, inaperture, and the size class of pollen grain was medium to large ranging from 33.91 ± 0.41 to 55.90 ± 3.19 μm. The pollen shape classified using P/E was divided into 2 groups: 1) subprolate; consisting of 10 species, namely, *A. aereus*, *A. blumei*, *A. grandifolius*, *A. harmandii*, *A. havilandii*, *A. oblanceolatus*, *A. siamensis*, *A. spinosus*, *A. suaveolens* and *A. sumatranus;* and 2) euprolate; consisting of 5 species, namely, *A. brevipes*, *A. burmanicus*, *A. hexapetalus*, *A. multiflorus* and *A. vanprukii* . The exine ornamentation can be classified into 2 types: 1) perforate-fossulate, and 2) rugulate sculpture. Within perforate-fossulate sculpture type, there were 2 subtypes: 9 species having loosely perforate-fossulate sculpture, namely, *A. brevipes, A. burmanicus*, *A. harmandii*, *A. hexapetalus, A. oblanceolatus*, *A. siamensis*, *A. spinosus*, *A. sumatranus* and *A. vanprukii*; and 5 species having finely perforate-fossulate sculpture, namely, *A. aereus*, *A. blumei*, *A. grandifolius*, *A. havilandii* and *A. multiflorus*. Only 1 *Artabotrys* species have rugulate sculpture; *A. suaveolens*. The exine thickness ranged from 1.84 ± 0.12 to 2.66 ± 0.16 μm. The palynological information can be used to identify only 1 species *A. suaveolens* by its subprolate shape and rugulate exine sculpture, while the remaining species have perforate-fossulate exine sculpture. Thus, the pollen morphology of *Artabortys* species can be used as an additional character for taxonomic data.

ACKNOWLEDGEMENTS

This work was partially supported by The TRF/BIOTEC Special Program for Biodiversity Research and the Training Grant BRT T_149013.

REFERENCES

Chalermglin, P. 2001. **Annonaceae**. Publishing House and Garden, Bangkok. (in Thai)

Craib, W.G. 1922. Contribution to the Flora of Siam. Bulletin of Miscellaneous Information, **Kew** 8 (1):7-10.

Craib, W.G. 1925. Contribution to the Flora of Siam. Bulletin of Miscellaneous Information, **Kew** 14 (1):7-10.

Eiadthong, W. and T. Insura. 2011**. Taxonomic note on Thai *Artabotrys* R.Br. (Annonaceae).** Proceeding of 15th Flora of Thailand Meeting, Chiang Mai, Thailand. 7 - 11 November 2011. (Abstract for oral presentation).

Erdtman, G., 1952. **Pollen Morphology and Plant Taxonomy**. Angiosperms. Almqvist and Wiksell, Stockholm.

Erdtman, G., 1960. The acetolysis method, a revised description. **Svensk Bot. Tidskr** 54: 561-564.

Erdtman, G. 1971. **Angiosperm (An introduction to Palynology I).** Pollen Morphology and Plant Taxonomy, 3th ed. Hafner Publishing Co., Ltd., New York.

Insura,T. 2009. **Systematics and some Ecological Characteristics of *Artabotrys* R.Br. (ANNONACEAE) in Thailand.** Master Thesis, Department of Forest Biology, Faculty of Forestry, Kasetsart University (Thai with English abstract).

Kessler, P.J.A. 1993. Annonaceae, pp. 93-129. *In* K. Kubitzki, J.G. Rohwer and V. Bittrich, eds. **The Families and Genera of Vascular Plants. Vol. 2.: Dicotyledons**. Springer –Verlag, Berlin.

Punt, W., S. Blackmore, S. Nilsson, and A. Le Thomas. 1994. **Glossary of pollen and spore Terminology.** LPP Foundation, University of Utrecht, Netherland.

Royal Forest Department, 2001. **Thai Plant Names Tem Smitinand. (revised edition)**. Prachachon Public Co. Ltd., Bangkok.

Thongpairoj, U. 2008. **Taxonomy and molecular phylogeny of *Artabotrys* R. Brown and palynology of tribe unoneae (Annonaceae) in Thailand.** Ph.D. Thesis, Department of Biology, Faculty of Science, Chiang Mai University.

van der Ham, R.W. J. M., 1990. ***Nephelieae* pollen (Sapindaceae): form, function, and evolution.** Ph.D thesis, Leiden University, Netherland.

Walker, J. W. and J. Doyle. 1975. The base of angiosperm phylogeny: paleontology. **Ann. Missouri Bot. Gard** 62: 644-723.