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Pollen morphology of subfamily Nelsonioideae (Acanthaceae) in Thailand

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Abstract

The pollen morphology of Thai Acanthaceae Subfamily Nelsonioideae was investigated. The pollen type was tricolpate. Their apertures were longitudinal narrow colpi with acute ends. The polar axis length (P) was 16.3–42.5 µm and equatorial axis length (E) was 17.5–37.5 µm. The largest size was represented in *Staurogyne dispar*, while the smallest showed in *Nelsonia canescens*, *Staurogyne glauca*, and *S. parvicaulis*. The shape varied from suboblate to prolate. Micro-foveolate, foveolate, and rugulate tecta were found in 6, 11, and 7 taxa of *Nelsonia* and *Staurogyne*, respectively. Psilate was only revealed in *Staurogyne macrobotrya*. The wall thickness varied from 1.25–2.5 µm depending on species.

Keywords: *Nelsonia*; *Staurogyne*; Acanthaceae; Plant taxonomy

1. Introduction

Subfamily Nelsonioideae consists of 7 genera (*Anisosepalum*, *Elytraria*, *Gynocraterium*, *Nelsonia*, *Ophiorrhiziphyllon*, *Saintpauliopsis*, and *Staurogyne*) and 170 species of herbs and shrubs that occur primarily in tropical regions of both the Old and New World [7, 13]. There are three genera (*Nelsonia* R. Br., *Ophiorrhiziphyllon* Kurz, and *Staurogyne* Wall.) and ca. 27 species in Thailand. Nelsonioideae lack both cystoliths and retinacula and are recognized as being outside the main group of Acanthaceae, coming fairly close to Scrophulariaceae [5, 8]. However, recently, the genus *Ophiorrhiziphyllon* has been reduced to *Staurogyne* based on the phylogenetic data [3].

In Acanthaceae, there has been disagreement about the relative usefulness of pollen morphology for the delimitation of natural groups. In part, this is due to the sheer magnitude of diversity in pollen morphology of Acanthaceae which has been interpreted in a number of ways [9, 10, 11, 12]. The first palynological study was that of Radlkofer [11], who investigated only a few taxa. Lindau [9, 10] carried out more detailed studies and recognized, for the first time, 11 pollen types within the family. Since then, a number of workers have studied the pollen of various taxa [1, 4, 12]. However, several species remain palynologically unknown, especially in subfamily Nelsonioideae. The purpose of this study was to examine the pollen morphology of Nelsonioideae (*Nelsonia* and *Staurogyne*) in Thailand which may be useful to support the classification or identification of species.

2. Materials and methods

Twenty-five species belonging to the genera *Nelsonia* and *Staurogyne* (Acanthaceae) in Thailand were selected for this study (Table 1). Pollen grains were collected in the field and also from specimens in herbaria. Samples were analyzed using the modified alkaline technique [16]. At least 10 pollen grains from each specimen were investigated to cover their variation. A range of characters was investigated, including shape, size, symmetry, aperture, exine structure, and sculpturing. Observations were made using an Olympus compound light microscope

(LM) and a JEOL JSM-6010LV scanning electron microscope (SEM). The terminology used follows Erdtman [4].

Table 1 Pollen morphology of Thai Nelsonioideae

Taxa	Size (μm)		Shape	Sculpture	Exine Thickness (μm)
	P	E			
N. canescens (Lam.) Spreng.	17.5 – 21.3	17.5 – 20.0	prolate spheroidal	foveolate	1.25
S. argentea Wall.	30.0 – 42.5	25.0 – 30.0	subprolate-prolate	foveolate	1.25 – 2.5
S. concinnula (Hance) Kuntze	27.5 – 32.5	22.5 – 27.5	subprolate	foveolate	1.25 – 2.5
S. cuneata J. B. Imlay	30.0 – 37.5	30.0 – 35.0	prolate spheroidal	micro-foveolate	2.5
S. dispar J. B. Imlay	35.0 – 42.5	27.5 – 37.5	subprolate	foveolate	2.5
S. filipes E. Hossain	22.5 – 27.5	25.0 – 30.0	oblate spheroidal	rugulate	2.5
S. glauca (Nees) Kuntze	17.5 – 20.0	17.5 – 22.5	oblate spheroidal	foveolate	1.25
S. griffithiana (Nees) Kuntze	25.0 – 30.0	25.0 – 27.5	prolate spheroidal	foveolate	2.5
S. helferi (T. Anders.) Kuntze	25.0 – 37.5	30.0 – 32.5	suboblate-oblate spheroidal	foveolate	2.5
S. incana (Blume) Kuntze	27.5 – 32.5	25.0 – 30.0	prolate spheroidal	micro-foveolate	2.5
S. kingiana C. B. Clarke	32.5 – 37.5	22.5 – 30.0	subprolate-prolate	micro-foveolate	2.5
S. lanceolata (Hassk) Kuntze	27.5 – 35.0	25.0 – 35.0	prolate spheroidal	micro-foveolate	2.5
S. lasiobotrys (Nees) Kuntze	25.0 – 30.0	22.5 – 30.0	prolate spheroidal	foveolate	2.5
S. longeciliata Bremek.	20.0 – 32.5	17.5 – 25.0	subprolate	micro-foveolate	2.5
S. macrobotrya (Kurz) T. F. Daniel & McDade	16.3 – 25.0	16.3 – 22.5	prolate spheroidal	psilate	1.25
S. major Benoist	27.5 – 37.5	25.0 – 35.0	prolate spheroidal	rugulate	2.5
S. merguensis (T. Anderson) Kuntze	27.5 – 37.5	25.0 – 32.5	prolate spheroidal-subprolate	rugulate	2.5
S. obtusa (Nees) Kuntze	27.5 – 32.5	25.0 – 30.0	prolate spheroidal	rugulate	2.5
S. parvicaulis B. Hansen	20.0 – 25.0	17.5 – 20.0	subprolate	foveolate	2.5
S. puncata J. B. Imlay	25.0 – 32.5	25.0 – 30.0	prolate spheroidal	micro-foveolate	2.5
S. racemosa (Miq.) Kuntze	32.5 – 37.5	25.0 – 32.5	subprolate	rugulate	2.5
S. setigera (Nees) Kuntze	30.0 – 42.5	25.0 – 30.0	subprolate-prolate	foveolate	2.5
S. singularis Bremek.	25.0 – 30.0	20.0 – 25.0	subprolate	foveolate	2.5
S. spatulata (Blume) Koord.	20.0 – 25.0	20.0 – 22.5	prolate spheroidal	rugulate	2.5
S. tenuispica Bremek.	27.5 – 32.5	25.0 – 30.0	prolate spheroidal	rugulate	2.5

P: polar axis length; E: equatorial axis length

3. Results

The pollen morphology of each taxa is summarized (Table 1). The natural pollen color varied from pale to dark brown, or yellowish brown. Pollen type was tricolpate. Their apertures were a longitudinal narrow colpus with acute ends. Pollen shape varied from suboblate to oblate spheroidal, prolate spheroidal, subprolate, to prolate. The polar axis length (P) varied from 16.3 to 42.5 µm and equatorial axis length (E) varied from 17.5 to 37.5 µm. The largest was found in *Staurogyne dispar* (35.0–42.5 µm × 27.5–37.5 µm), while the smallest was seen in *Nelsonia canescens* (17.5–21.3 µm × 17.5–20.0 µm), *Staurogyne glauca* (17.5–20.0 µm × 17.5–22.5 µm), and *S. parvicaulis* (20.0–25.0 µm × 17.5–20.0 µm). A foveolate tectum was found in 11 taxa, i.e., *N. canescens*, *S. argentea*, *S. concinnula*, *S. dispar*, *S. griffithiana*, *S. glauca*, *S. helferi*, *S. lasiobotrys*, *S. parvicaulis*, *S. setigera*, *S. singularis*. A regulate tectum was represented in 7 taxa, namely *S. filipes*, *S. major*, *S. merguensis*, *S. obtusa*, *S. racemosa*, *S. spatulata*, and *S. tenuispica*. A micro-foveolate tectum occurred in six taxa of *S. cuneata*, *S. kingiana*, *S. punctata*, *S. lanceolata*, *S. longeciliata*, and *S. incana*. A psilate tectum was only revealed in *S. macrobotrya* (Figures 1-5). Wall thickness varied from 1.25–2.5 µm depending on the species.

4. Discussion

As indicated by the results, pollen shape and size were highly variable even within the same genus. The wall ornamentation revealed some differences at the fine level. Usually, pollen shape can be classified into 5 categories: peroblate, oblate, subspheroidal, prolate, and perprolate. However, in the present study, the subspheroidal category was divided into 4 subcategories, i.e., suboblate, oblate-spheroidal, prolate-spheroidal, and subprolate.

In Nelsonioideae, the pollen type is tricolpate, which is distinctive from that of other genera in Acanthaceae except in tribe Acantheae, subfamily Acanthoideae which possessed retinaculate fruits but lacked cystoliths [2, 10, 13]. The presence of tricolpate pollen is a constant feature of all genera within Thai Nelsonioidae and is diagnostic for this group within Acanthaceae. The results of this study conformed to the pollen types of *Nelsonia*, and *Staurogyne* [15] as colpate and of Lindau's classification as "Spaltenpollen" or grains globose or ellipsoid, or almost 3-sided, with three very small grooves, with or without 3 germ-pores [8]. However, it differed from the pollen of *Staurogyne* sp. from China which have been reported as 3-colporate, with the exine ornamentation being psilate [14]. Their apertures were longitudinal narrow colpi with acute ends. The polar axis length (P) was 16.3–42.5 µm and equatorial axis length (E) was 17.5–37.5 µm. The shape varied from suboblate to prolate. The wall thickness did not show much difference in Nelsonioideae. Foveolate, rugulate, and micro-foveolate tectum were found in 11, 7, and 6 taxa of *Nelsonia* and *Staurogyne*, respectively. These types of wall ornamentation, plus the variation of size and shape, are useful for the identification and delimitation of species.

In size, *Staurogyne dispar* represents the largest pollen, while *Nelsonia canescens* showed the smallest. Moreover, *S. glauca* and *S. parvicaulis* revealed the most minute pollen within the genus but were different in shape (oblate-spheroidal vs subprolate, respectively), and also dissimilar in wall thickness. Furthermore, the larger size of pollen conformed to the biggest size of flower and the habit of small shrubs, i.e., *S. argentea*, *S. cuneata*, *S. dispar*, *S. kingiana*, *S. punctata*, *S. major*, and *S. racemosa*.

The sculpturing pattern of the wall is an important factor in the discrimination of species or even genera. Psilate tectum was restricted to *S. macrobotrya*. This conformed to Lindau's pollen type of *Ophiorrhiziphyllon* as "Glatter, runder pollen" or "smooth round pollen" with 3 germ-pores, and smooth exine [8, 10]. It has been used to distinguish *Ophiorrhiziphyllon* from *Staurogyne* together with longer fertile stamens that are exserted with oblong anthers, and a zygomorphic, conspicuously 2-lipped corolla. Even the genus *Ophiorrhiziphyllon* has been reduced to *Staurogyne* in 2014 [3].

Staurogyne dispar differs from other 3 *Staurogyne* species (*S. concinnula*, *S. parvicaulis*, and *S. singularis*) with foveolate ornamental wall and subprolate pollen shape by showing the largest pollen size.

Staurogyne glauca and *S. spatulata* were evaluated as a species complex because of their similarity in inflorescences and flowers, but pollen characteristics can be used to separate them, i.e., pollen sculpture (foveolate vs rugulate), pollen shape (oblate-spheroidal vs prolate-spheroidal), and also a little difference in polar axis length and wall thickness.

Moreover, Hansen [6] reduced *S. longeciliata* to the synonymy of *S. lanceolata* because of the similarity of bract hairs. In this study, *S. lanceolata* and *S. longeciliata* were both found to possess micro-foveolate walls but differed in shape (prolate-spheroidal vs subprolate) with variation in size, especially in equatorial axis length. *S. longeciliata* also had the smallest pollen in the micro-foveolate group. Therefore, they are considered two distinct species in this study.

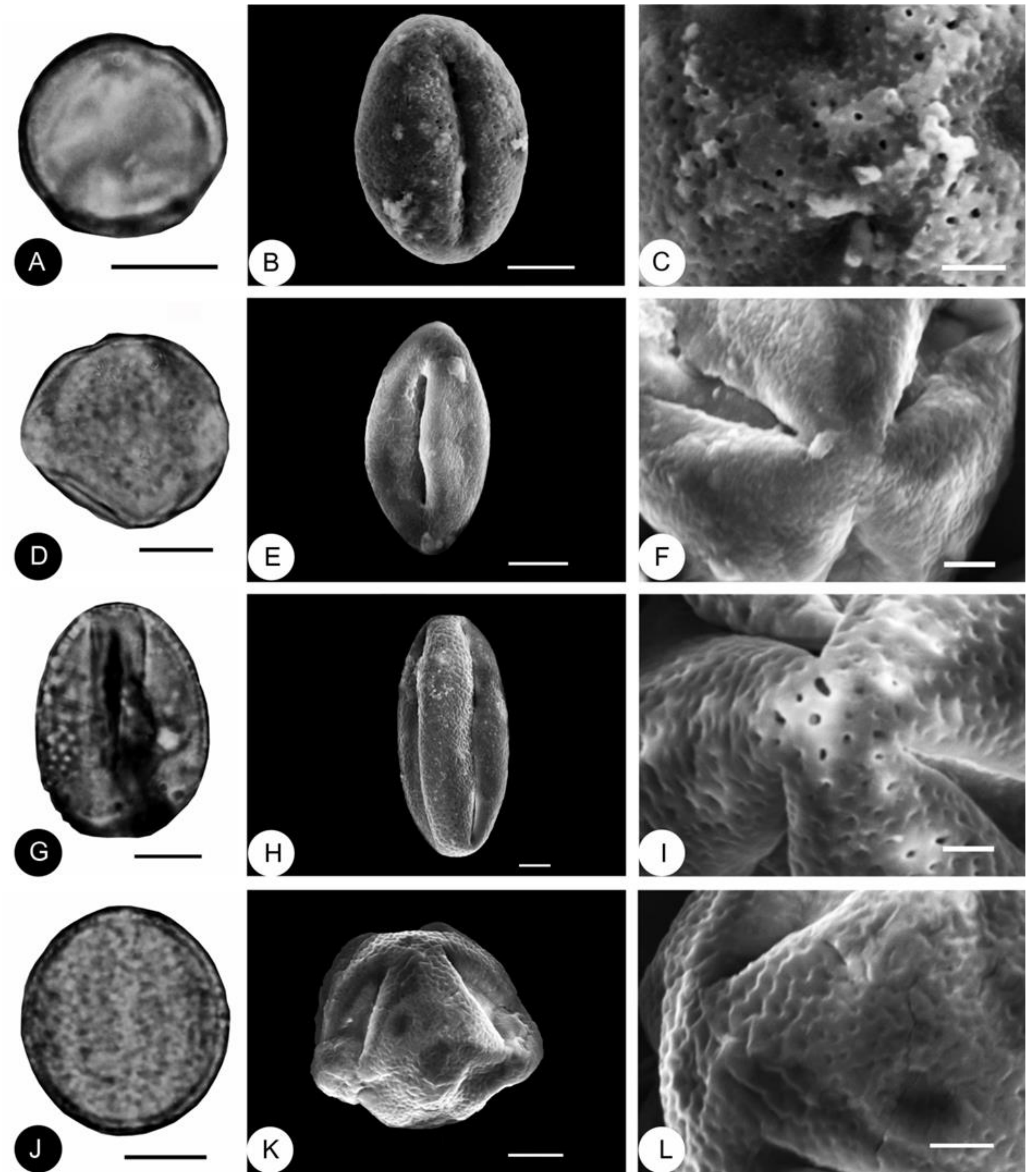

Figure 1 A-C. Pollen grains of *Nelsonia canescens*. **D-F.** Pollen grains of *Staurogyne macrobotry*a. **G-I.** Pollen grains of *Staurogyne argentea*. **J-L.** Pollen grains of *Staurogyne concinnula*. Scale bars 10 µm (A, D, G, J), 5 µm (B, E, H, K), 2 µm (C, F, I, L).

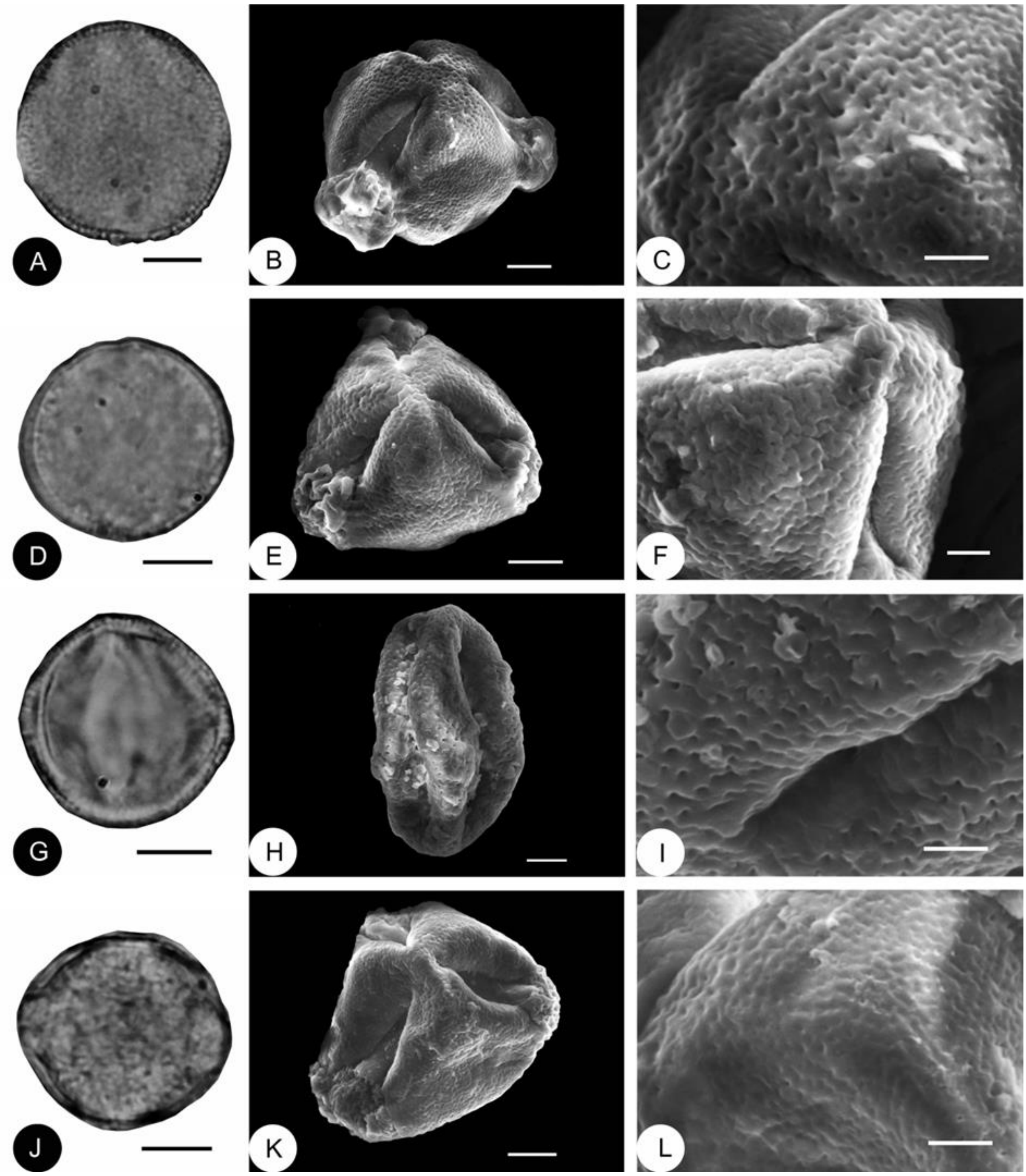

Figure 2 A-C. Pollen grains of *Staurogyne dispar*. **D-F.** Pollen grains of *Staurogyne filipes*. **G-I.** Pollen grains of *Staurogyne griffithiana*. **J-L.** Pollen grains of *Staurogyne incana*. Scale bars 10 µm (A, D, G, J), 5 µm (B, E, H, K), 2 µm (C, F, I, L).

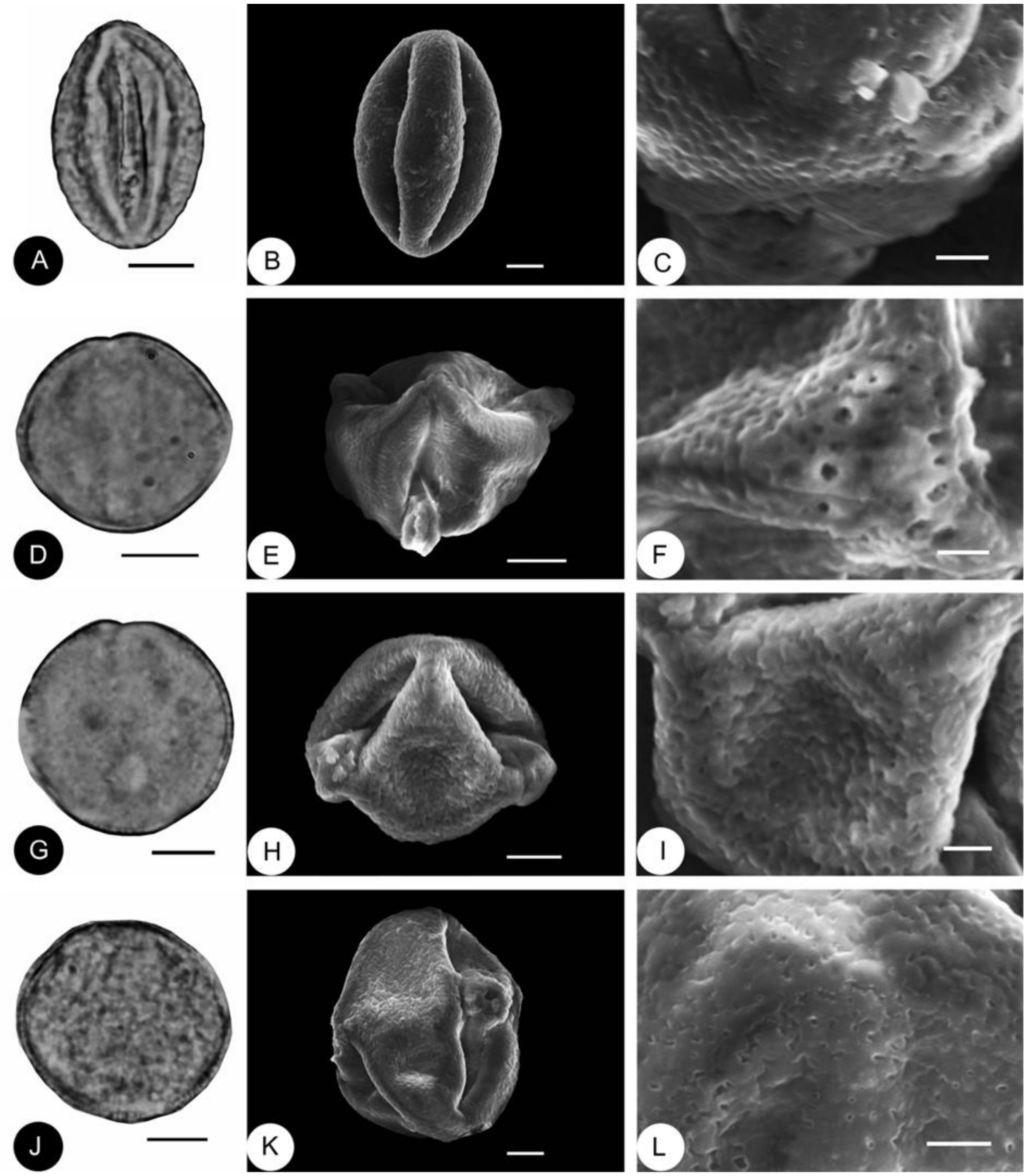

Figure 3 A-C. Pollen grains of *Staurogyne kingiana*. **D-F.** Pollen grains of *Staurogyne lasiobotrys*. **G-I.** Pollen grains of *Staurogyne major*. **J-L.** Pollen grains of *Staurogyne merguensis*. Scale bars 10 µm (A, D, G, J), 5 µm (B, E, H, K), 2 µm (C, F, I, L).

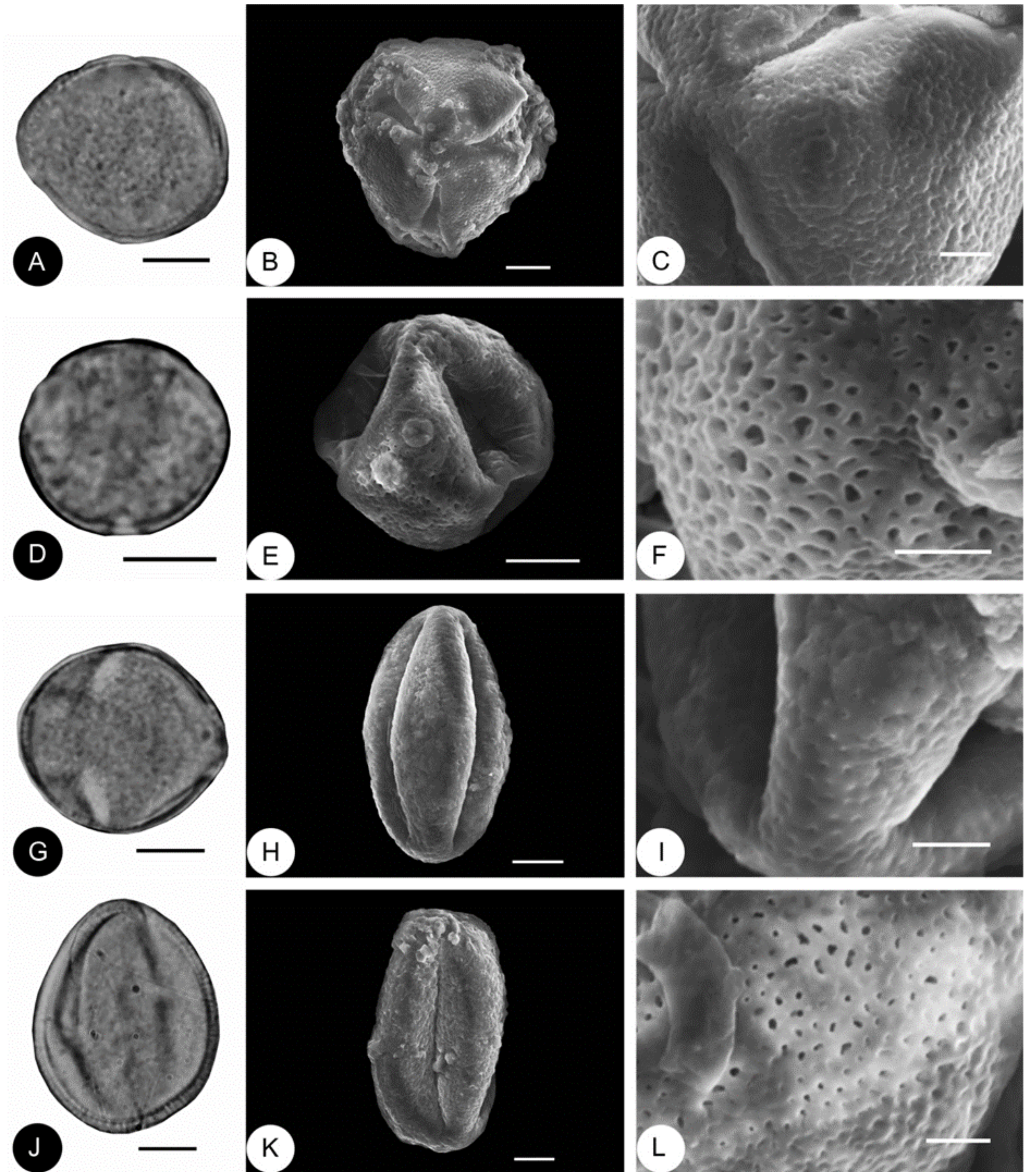

Figure 4 A-C. Pollen grains of *Staurogyne obtusa*. **D-F.** Pollen grains of *Staurogyne parvicaulis*. , **G-I.** Pollen grains of *Staurogyne racemosa*. **J-L.** Pollen grains of *Staurogyne setigera*. Scale bars 10 µm (A, D, G, J), 5 µm (B, E, H, K), 2 µm (C, F, I, L).

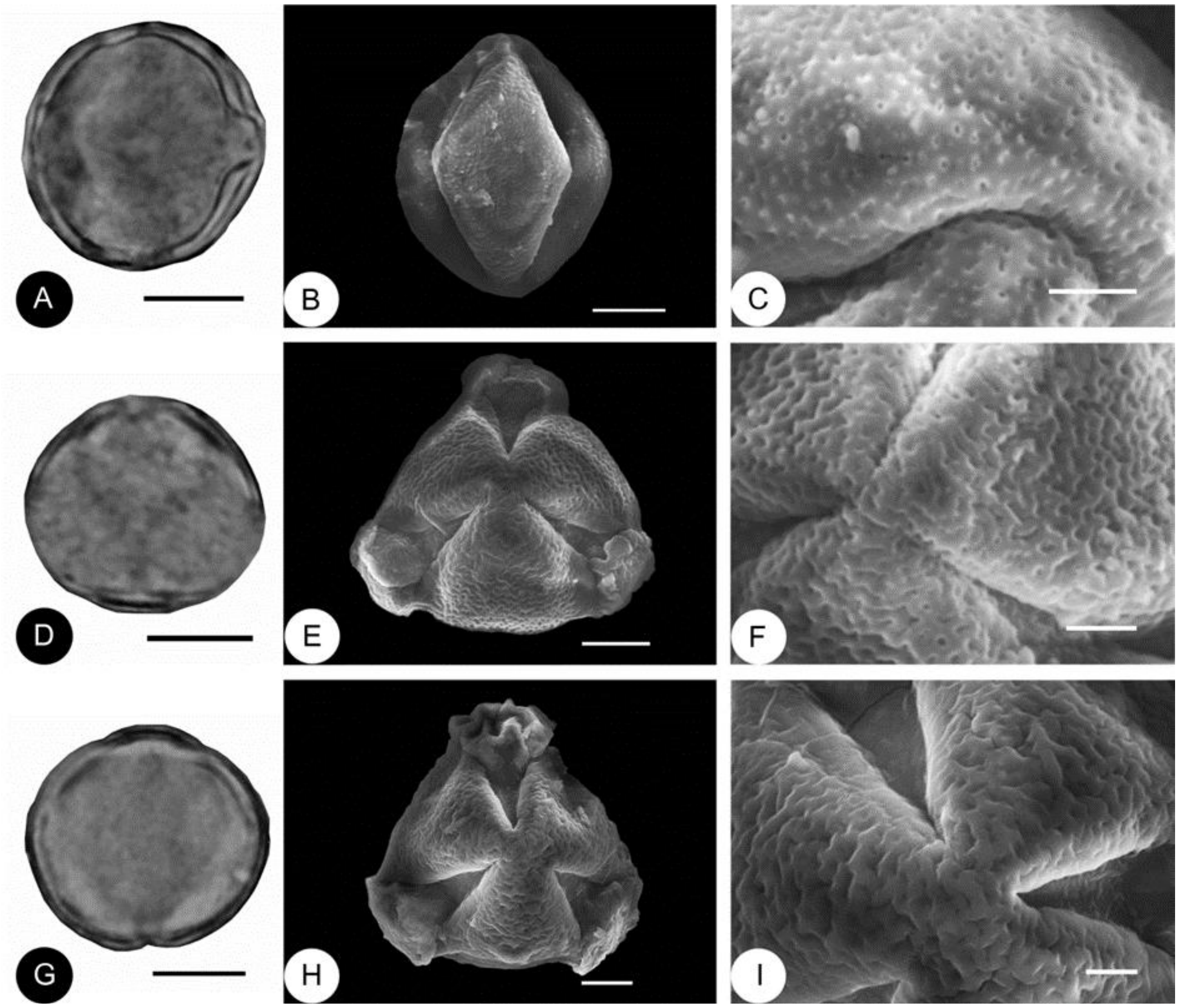

Figure 5 A-C. Pollen grains of *Staurogyne singularis*. **D-F.** Pollen grains of *Staurogyne spatulata*. **G-I.** Pollen grains of *Staurogyne tenuispica*. Scale bars 10 µm (A, D, G), 5 µm (B, E, H), 2 µm (C, F, I).

5. Conclusions

Some of the species, such as *Nelsonia canescens*, *Staurogyne dispar*, *S. glauca*, *S. longeciliata*, and *S. macrobotrya* showed specific characters of pollen, which may be used as taxonomic tools to separate them from other taxa or be used as homologs to develop a phylogeny together with other characters such as morphological and anatomical features. In this research, samples were analyzed using a modified alkaline technique [15]. This method could protect pollen from collapse and could also allow the use of samples in which pollen grains were very few.

Further studies of the pollen morphology of Nelsonioideae are needed. More materials from Thailand and elsewhere would help to confirm identifications and provide useful data in future studies of Acanthaceae classification.

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