Morphology and Palynology of *Amomum* Roxb. in Thailand

W. KAEWSRI [1] AND Y. PAISOOKSANTIVATANA [2]

[1] Department of Biotechnology, Faculty of Science, Mahidol University, Kanchanaburi 71150, Thailand
Email: kawks@mahidol.ac.th
[2] Department of Horticulture, Faculty of Agriculture, Kasetsart University, Chatuchak, Bangkok 10900, Thailand
Email: agryyp@ku.ac.th

Abstract

Morphological characteristics and pollen morphology of Thai *Amomum* Roxb. were studied in order to aid identification and classification. Thirty-one species collected during our field expedition, only 13 species could be identified to species, and 18 species will be proposed as new to science. Investigation of both vegetative and reproductive organs reveals that leaf, flower and fruit are useful for identification/separating the species of *Amomum*. Pollen grains of 14 representatives were examined using a scanning electron microscope (SEM) in order to reveal their morphology and usefulness for infrageneric classification. Two types of exine sculpture, psilate and echinate, were found. Classification by using pollen morphology does not support grouping by the previous authors that emphasized fruit shapes.

Introduction

Amomum Roxb. is one of the largest genera in the ginger family (Zingiberaceae) with about 150-180 species. They are widely distributed in Southeast Asia from the Himalayas to Northern Australia and extend into the central Pacific (Kiew, 1982; Smith, 1985). Sirirugsa (2001) estimated 15-20 *Amomum* species in Thailand. They are generally evergreen herbs inhabiting wet forests, especially in light gaps and at forest margins (Sakai and Nagamasu, 1998). Many species of *Amomum* are used as medicine, spice, condiment or a vegetable. Even though the plants from this genus have been long utilized, the identification is still confusing because of the absence of good specimens in many herbaria. Moreover, there are often misidentified either infragenerically and intergenerically. These lead to

many changes in taxonomic status. For instance, many species of *Amomum* were transferred to the other genera: *Aframomum* K. Schum., *Elettariopsis* Baker, *Alpinia* Roxb., *Etlingera* Giseke and *Hornstedtia* Retz. Besides, there are frequent changes in species identification. These problems need an intensive study for clarification.

Thailand has a very complex biogeography due to its topography and geographic position. Thus the southern Chinese flora reaches its southern limits in Chiang Mai and Nan provinces, while the Malesian flora covers the southern part of peninsular Thailand, the Burmese flora spills over the western limestone mountains, and the central table mountains harbour a rich endemic flora (Larsen, 2003). The diverse ecological habitats also contribute to a rich diversity of plants including species of *Amomum*.

At present, the country forested area decreases rapidly due to deforestation, urbanization and agricultural land expansion. Many plant species in the forest include *Amomum*, are at risk of extinction. In order to set conservation priorities for these potentially endangered species, the aim of the present study is to examine the vegetative and reproductive parts, as well as pollen morphology, useful for classification.

Materials and Methods

Indigenous species of *Amomum* were collected from all parts of Thailand during April 2003-June 2005 (Table. 1). Inflorescences, infructescences, vegetative parts and habitats, were photographed, and field notes were made. Flowers and fruits were preserved in 70% ethanol. The dried vegetative part and inflorescences were deposited at the herbaria BK (Department of Agriculture, Bangkok) and BKF (Royal Forest Department, Bangkok), Thailand. Living specimens are cultivated at the Department of Horticulture, Kasetsart University. Morphological characters were examined either from dry, spirit collections or living plants. Anthers of 14 species of Thai *Amomum* were taken from live plants at mature stage and stored in 70% ethanol. Pollen grains were collected and kept also in 70% ethanol. The samples were passed through an ethanol dehydration starting with 10-15 min in 90% ethanol with three subsequent changes in absolute alcohol at 10 min each. The Critical Point Dryer (BALZERS LINION CPD-020) was used to dry the samples. The pollen were then mounted on SEM stub using double-sided sticky tape and sputter-coated. Photographs were taken with a JSM Jeol 5410LV scanning electron microscope.

Table 1. List of *Amomum* species collected in Thailand during April 2003-June 2005.

No.	Botanical Name	Collector No.	Locality
1.	*Amomum aculeatum* Roxb.	Kaewsri 2, 6, 20, 65, 74	Kanchanaburi
2.	*Amomum biflorum* Jack	Kaewsri 52, 58, 66	Chanthaburi
3.	*Amomum dealbatum* Roxb.	Kaewsri 110	Chiang Mai
4.	*Amomum hastilabium* Ridl.	Kaewsri 37	Ranong
5.	*Amomum koenigii* J.F. Gmel.	Kaewsri 03, 29, 136	Kanchanaburi
6.	*Amomum micranthum* Ridl.	Kaewsri 63, 84	Chanthaburi
7.	*Amomum pierreanum* Gagnep.	Kaewsri 122	Nakhon Nayok
8.	*Amomum repoeense* Pierre ex Gagnep.	Kaewsri 64, 103, 121	Chanthaburi
9.	*Amomum rivale* Ridl.	Kaewsri 04, 23, 142	Kanchanaburi
10.	*Amomum siamense* Craib	Kaewsri 14, 116, 123	Tak
11.	*Amomum testaceum* Ridl.	Kaewsri 15, 16, 96	Tak (Cultivated)
12.	*Amomum uliginosum* König	Kaewsri 30, 32, 33, 92	Nakhon Nayok
13.	*Amomum cf. villosum*	Kaewsri 12, 13	Tak
14.	*Amomum sp.*1	Kaewsri 01, 93	Kanchanaburi
15.	*Amomum sp.*2	Kaewsri 10, 139	Kanchanaburi
16.	*Amomum sp.*3	Kaewsri 19, 75	Prachuap Khiri Khan
17.	*Amomum sp.*4	Kaewsri 22	Kanchanaburi
18.	*Amomum sp.*5	Kaewsri 24, 147	Kanchanaburi
19.	*Amomum sp.*6	Kaewsri 25, 88, 89, 113	Kanchanaburi
20.	*Amomum sp.*7	Kaewsri 27	Uthai Thani
21.	*Amomum sp.*8	Kaewsri 35	Ranong
22.	*Amomum sp.*9	Kaewsri 38	Ranong
23.	*Amomum sp.*10	Kaewsri 50, 54, 104, 105, 107	Sakon Nakhon
24.	*Amomum sp.*11	Kaewsri 68, 79	Chanthaburi
25.	*Amomum sp.*12	Kaewsri 70	Chumphon
26.	*Amomum sp.*13	Kaewsri 81	Ranong
27.	*Amomum sp.*14	Kaewsri 93, 94	Tak
28.	*Amomum sp.*15	Kaewsri 97, 108	Chiang Mai
29.	*Amomum sp.*16	Kaewsri 111, 114, 137	Chiang Mai
30.	*Amomum sp.*17	Kaewsri 134, 135	Nan
31.	*Amomum sp.*18	Kaewsri 151	Kanchanaburi

Results and Discussion

1. Morphological characters of vegetative and reproductive parts

Thirty-one species of *Amomum* collected from all over Thailand including 13 identified species and 18 unidentified species (Table 1) were examined. The sheath surface was either reticulate (Fig. 1A) or triate (Fig. 1B), and the ligule, either bilobed (Fig. 1C) or entire (Fig. 1D). These characters have only limited application in species determination whereas reproductive parts are of more useful to aid identification. The latter includes the differences in shape of the stigma (Fig.1E-G), lateral staminodes (Fig.1H-J), labellum (Fig.1K-N), anther crest (Fig.1O-S), and fruit (Fig.1T-W).

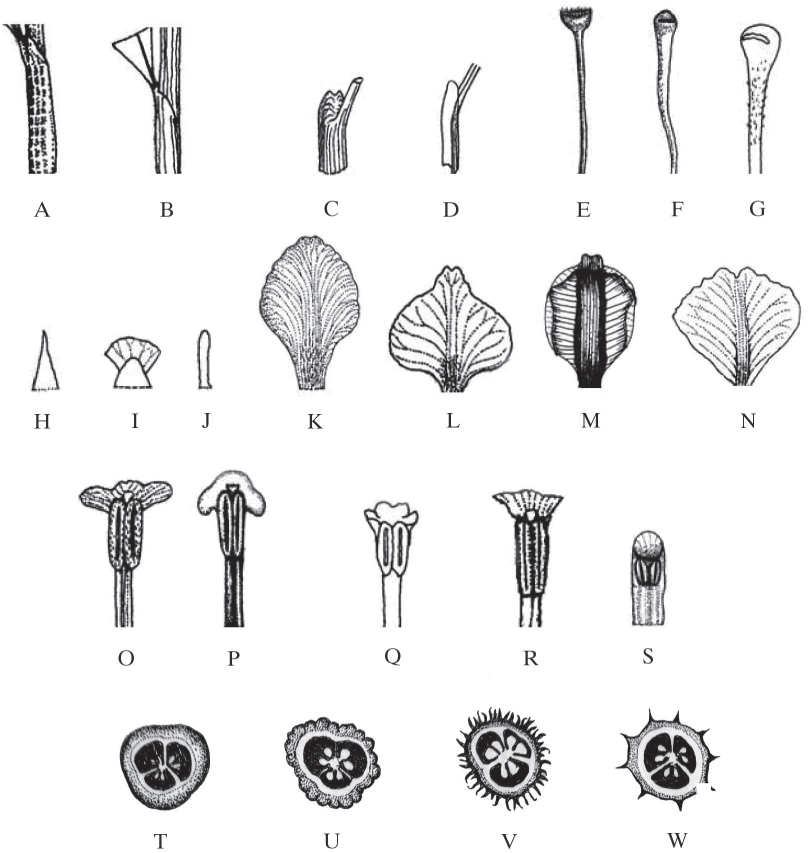

Figure 1. Morphological characteristics of some organs of the genus *Amomum* Roxb.
Leaf sheath: A. reticulate; B. striate. Ligule: C. bilobed; D. rounded. Stigma: E. cup shaped; F. ampullate; G. clavate. Lateral staminode: H. subulate; I. fin-shaped; J. linear. Labellum: K. ovate; L. deltoid; M. orbiculate and hooded; N. flabellate. Anther crest: O. reniform; P. auriculate; Q. trilobed or horn-shaped; R. truncate; S. rounded. X-section of fruit: T. smooth; U. ridged; V. spiny; W. wing.

Due to the closely resemblance in their floral morphology, it is recommended that the fruit types be examined. Three major types of fruits (smooth, hairy and winged) are found among Thai species of *Amomum*. Some variation in the degree of hairiness and smoothness of the fruit are also observed. The fruit shape is highly variable and can be globose, ellipsoid, obovoid, or obconical (Fig. 2A-M).

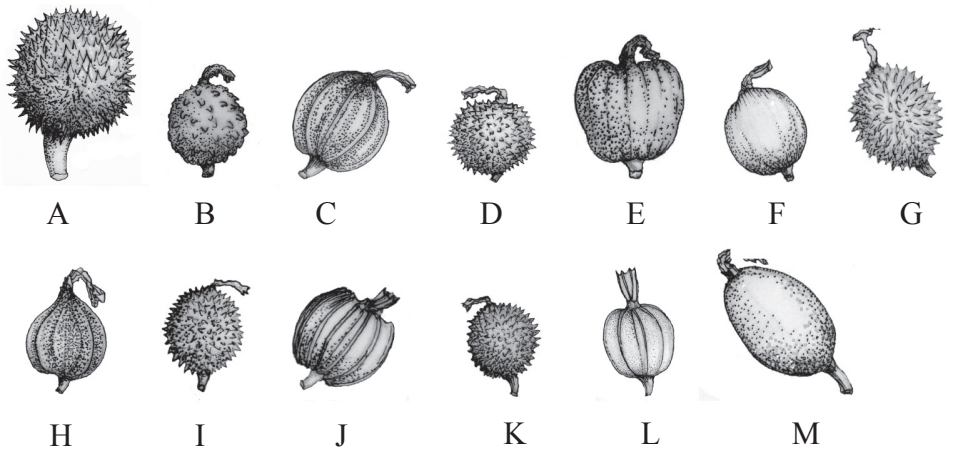

Figure 2. Fruit shapes of *Amomum*: A. *Amomum aculeatum*; B. *A. biflorum*; C. *A. dealbatum*; D. *A. rivale*; E. *A. siamense*; F. *A. testaceum*; G. *A. uliginosum*; H. *Amomum* sp.3; I. *Amomum* sp.4; J. *Amomum* sp.6; K. *Amomum* sp.11; L. *Amomum* sp.12; M. *Amomum* sp.15.

2. Palynological study

The SEM results revealed that the pollen grains of Thai *Amomum* are spherical to sub-spherical, (except for some grains of *A. rivale* Ridl. that are ovoid), 30-70 μm in diameter and the intine layer is 1-7 μm thick. These general results agree with Mangaly and Nayar (1990) which reported that the pollen shape of *Amomum hypoleucum* Thwaites and *A. pterocarpum* Thwaites are sub-spheroidal to ovoid and spheroidal, the diameter being 30-90 and 35-75 μm, respectively and the intine layer 1.25-2.5 μm thick in *Amomum hypoleucum*.

Our results revealed that the pollen can be divided into two groups either by intine thickness (<4 μm; ≥4 μm), and by exine sculpture (echinate or psilate; Table 2, Fig. 3). The results do not coincide with the classification proposed by previous authors, in particular, Schumann (1904) who subdivided the genus (section *Euamomum*) into two series, namely, 1. ser. *Lobulatae* (anther crest with two or three lobes) and 2. ser. *Integrae* (anther crest margin with entire lobe). Our study reveals that most pollen grains are echinate in sculpture, which was consistent in both series and are of less use in classification.

As stated above, the results from palynological study are not useful for classification of Thai species of *Amomum*. Most data from pollen shapes and size characteristics are apparently homoplasious although exine sculpture can divide the species into two groups: echinate and psilate. The echinate group consists of *A. aculeatum, A. biflorum, A. dealbatum, A. siamense, A. uliginosum, Amomum* sp. 3, *Amomum* sp. 4, *Amomum* sp. 6, *Amomum* sp. 11, *Amomum* sp. 12, *Amomum* sp. 15, *Amomum* sp. 16. The psilate group includes only two species: *A. rivale* and *A. testaceum.*

Table 2. Pollen morphology of Thai *Amomum* species.

Species	Shape and size (dia.)	Intine thickness	Exine sculpture
1. A. aculeatum	Spherical, 46-60 µm.	3 µm	Echinate; spine uniformly distributed, sharp apex, having collared base, 1.5-3 µm tall, 2-3 µm thick at base.
2. A. biflorum	Spherical, 35-46 µm.	1-2 µm	Echinate; spine uniformly distributed, usually sharp apex, having collared base, 2.5-3 µm tall, 3-3.5 µm thick at base.
3. A. dealbatum	Spherical, 52-58 µm.	3 µm	Echinate; spine uniformly distributed, blunt and interrupted by sharp apex, 3-3.5 µm tall, 3 µm thick at base.
4. A. rivale	Subspherical or ovoid, ca. 60 µm.	1 µm	Psilate; exine 0.5 µm thick
5. A. siamense	Spherical, 43-53 µm.	5 µm	Echinate; spine uniformly distributed, sharp apex, 2.5-3 µm tall, 3 µm thick at base, surface reticulate.
6. A. testaceum	Spherical to subspherical, 60 µm.	2 µm	Psilate; exine 1 µm thick.
7. A. uliginosum	Spherical, 30-35 µm.	1-2 µm	Echinate; spine irregularly distributed, blunt interupted by sharp apex, having collared base, 1 µm tall, 2 µm thick at base.
8. Amomum sp.3	Spherical, 63-70 µm.	4-5 µm	Echinate; spine uniformly distributed, distributed, sharp and interrupted by blunt apex, 4 µm tall, 6 µm thick at base.

9. Amomum sp.4	Spherical or subspherical, 50-58 µm.	1 µm	Echinate; spine uniformly distributed, sharp or blunt apex, having collared base, ca 2 µm tall, 3 µm thick at base.
10. Amomum sp.6	Spherical to subsperical, 45-50 µm.	4-5 µm	Echinate; spine uniformly distributed, blunt and interrupted by sharp apex, 2-2.5 µm tall,2-3 µm thick at base.
11. Amomum sp.11	Spherical, 56-60 µm.	4-7 µm	Echinate; spine uniformly distributed, sharp apex, having collared base, 3 µm tall, 3 µm thick at base.
12. Amomum sp.12	Spherical, 60-64 µm.	1 µm	Echinate; spine uniformly distributed, sharp apex, 3-4 µm tall, 2-2.3 µm thick at base.
13. Amomum sp.15	Spherical, 50-60 µm.	1 µm	Echinate; spine uniformly distributed, sharp apex, 4 µm tall, 3.5 µm thick at base.
14. Amomum sp.16	Spherical, 60-63 µm.	2-3 µm	Echinate; spine uniformly distributed, sharp apex, 4 µm tall, 4 µm thick at base.

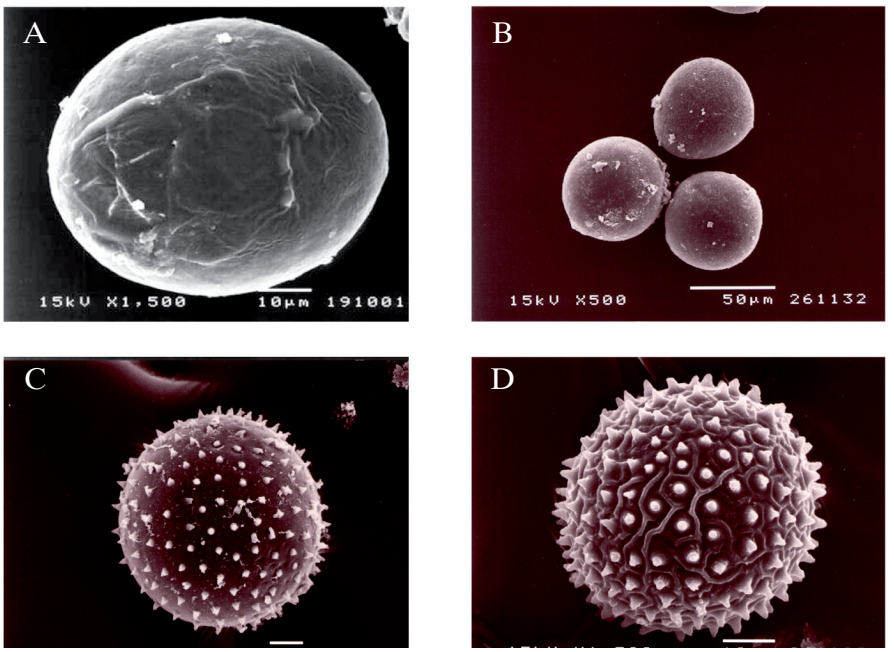

Figure 3. Pollen characteristics of *Amomum*. Psilate type: A. *A. rivale*; B. *A. testaceum.* Echinate type: C. *A. aculeatum.* Echinate-reticulate type: D. *A. siamense.*

Conclusions

1. Morphology of reproductive parts is more useful in aiding precise species identification of *Amomum*, especially the fruit shape.
2. The pollen grains of *Amomum* are spherical to subspherical, inaperturate, and the exine sculpture is either echinate or psilate. Pollen characteristics agree with the previous reports but do not correspond with previous classification based on morphological characteristics. Therefore, the pollen morphology is less useful for subgeneric classification of *Amomum*.

References

Kiew, K. Y. 1982. The genus *Elettariopsis* (Zingiberaceae) in Malaya. *Notes from the Royal Botanical Garden Edinburgh* **42**: 295-314.

Larsen, K. 2003. The Zingiberaceae in Flora of Thailand, pp. 1-5. In: P. Chantaranothai, K. Larsen, P. Sirirugsa and D. Simpson (eds.). *Proceedings of the 3rd Symposium on the Family Zingiberaceae*. Applied Taxonomic Research Center, Department of Biology, Faculty of Science, Khon Kaen University.

Mangaly, J. K. and J. Nayar. 1990. Palynology of south Indian Zingiberaceae. *Botanical Journal of Linnaean Society* **103**: 351-366.

Sakai, S. and H. Nagamasu. 1998. Systematic Studies of Bornean Zingiberaceae I. *Amomum* in Lambir Hills, Sarawak. *Edinburgh Journal of Botany* **55**: 45-64.

Schumann, K.M. 1904. Zingiberaceae. In: A. Engler (ed.), *Das Pflanzenreich IV*, **46**: 1–458.

Sirirugsa, P. 2001**.** Zingiberaceae of Thailand, pp. 63-77. In: V. Baimai and R. Kumhom. *BRT Research Reports 2001*. Biodiversity Research and Training Program. Jirawat Express Co., Ltd., Bangkok. (in Thai)

Smith, R.M. 1985. A review of Bornean Zingiberaceae:1(Alpineae). *Notes from the Royal Botanical Garden Edinburgh* **42**: 295-314.