

## Pollen morphology of the genus *Curcuma* (Zingiberaceae) in Northeastern Thailand

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**ABSTRACT:** The palynological study of 14 species of *Curcuma*, namely *C. alismatifolia* Gagnep. 1, *C. alismatifolia* Gagnep. 2, *C. alismatifolia* Gagnep. 3, *C. alismatifolia* Gagnep. 4, *C. alismatifolia* Gagnep. 5, *C. alismatifolia* Gagnep. 6, *C. angustifolia* Roxb., *C. gracillima* Gagnep., *C. harmandii* Gagnep., *C. larsenii* Maknoi & Jenjitt, *C. longa* L. 1, *C. longa* L. 2, *C. petiolata* Roxb., *C. parviflora* Wall, *C. cf. parviflora*, *C. pierreana* Gagnep., *C. rhabdota* Sirirugsa & Newman., *C. rubescens* Roxb., *C. singularis* Gagnep., and *C. sparganifolia* Gagnep., was undertaken using a light microscope and scanning electron microscope. The pollen grains were monad, inaperturate, with radial symmetry and large with rugulose exine sculpturing. Pollen grains had various shapes (subspheroidal, prolate spheroidal, spheroidal, subprolate, and prolate). Pollen shape can be used as a supplementary character for identification of the *Curcuma* species. In the present study, the pollen morphology of 13 species of *Curcuma* is recorded for the first time.

**KEYWORDS:** palynology, ginger family, scanning electron microscope

### INTRODUCTION

The Zingiberaceae is a large family including about 50 genera and 1400 species that are distributed mainly in the tropics from India to Malaysia. Kress et al<sup>1</sup> proposed a new classification system for the Zingiberaceae based on molecular phylogeny. They divided Zingiberaceae into 4 subfamilies and 6 tribes: Alpinioideae (Alpinieae & Riedelieae), Siphonochiloideae (Siphonochileae), Tamijioideae (Tamijieae), and Zingiberoideae (Zingibereae & Globbeae). The genus *Curcuma* was placed in the subfamily Zingiberoideae (tribe Zingibereae) which included the genera *Boesenbergia*, *Camptandra*, *Cautleya*, *Cornukaempferia*, *Curcuma*, *Curcumorpha*, *Distichochlamys*, *Haniffia*, *Haplochorema*, *Hedychium*, *Hitchenia*, *Kaempferia*, *Laosanthus*, *Nanochilus*, *Paracautleya*, *Parakaempferia*, *Pommereschea*, *Pyrgophyllum*, *Rhynchanthus*,

*Roscoeia*, *Scaphochlamys*, *Smitharis*, *Stadiochilus*, *Stahlianthus*, and *Zingiber*. In Thailand, the family Zingiberaceae includes about 26 genera and 300 species<sup>2</sup>. The genus *Curcuma* consists of 34 species in Thailand and 60 species worldwide<sup>2</sup>. Maknoi<sup>3</sup> found 8 taxa of the genus *Curcuma* in Northeastern Thailand. The keys to the genus *Curcuma* are short and wide corolla tube short, flowers of various colours, with leaf-blade tapering into petiole<sup>2</sup>. Maknoi<sup>3</sup> divided the plants in the genus *Curcuma* into 5 groups based on morphological characters. Some species of this genus have great ornamental values, e.g., *C. alismatifolia*, *C. petiolata*, *C. pierreana*, *C. rhabdota*, and *C. sparganifolia*. Numerous selected clones are now widely cultivated in Southeast Asia as “Siamese Tulip”. The plants in this genus has been widely cultivated and commercialized in Europe and the USA under various names, e.g., “Pink Curcuma”. The flowers have various

**Table 1** Specimens of the genus *Curcuma* investigated, and their pollen morphology data.

Taxa	Remark	Vouchers	Shape	Size (mean $\pm$ SD $\mu\text{m}$ )	
				Polar axis	Equatorial axis
<i>C. alismatifolia</i> Gagnep. 1	pink bract (cultivated)	P Kohkaew 01/53	prolate spheroidal	70.1 $\pm$ 4.2	66.7 $\pm$ 4.5
<i>C. alismatifolia</i> Gagnep. 2	white with pink bract (cultivated)	P Kohkaew 02/53	subprolate	56.1 $\pm$ 4.2	45.1 $\pm$ 3.7
<i>C. alismatifolia</i> Gagnep. 3	white bract (cultivated)	P Kohkaew 03/53	spheroidal	52.3 $\pm$ 8.1	–
<i>C. alismatifolia</i> Gagnep. 4	purple bract (cultivated)	P Kohkaew 04/53	spheroidal	56.8 $\pm$ 5.0	–
<i>C. alismatifolia</i> Gagnep. 5	pink bract (cultivated)	P Kohkaew 05/53	spheroidal	57.8 $\pm$ 6.9	–
<i>C. alismatifolia</i> Gagnep. 6	pink bract (wild type)	P Kohkaew 06/53	subprolate	53.2 $\pm$ 5.8	40.7 $\pm$ 5.9
<i>C. angustifolia</i> Roxb.	–	P Kohkaew 07/53	subprolate	64.1 $\pm$ 6.0	50.9 $\pm$ 6.9
<i>C. gracillima</i> Gagnep.	–	P Kohkaew 08/53	subprolate	59.2 $\pm$ 6.1	44.7 $\pm$ 5.0
<i>C. harmandii</i> Gagnep.	–	P Kohkaew 09/53	prolate spheroidal	57.6 $\pm$ 4.3	53.5 $\pm$ 4.6
<i>C. larsenii</i> Maknoi & Jenjitt	–	P Kohkaew 10/53	prolate spheroidal	55.8 $\pm$ 4.4	51.2 $\pm$ 4.5
<i>C. longa</i> 1	white bract	P Kohkaew 19/53	subspheroidal	81.2 $\pm$ 7.5	61.8 $\pm$ 6.3
<i>C. longa</i> 2	white with pink bract	P Kohkaew 20/53	prolate	86.9 $\pm$ 7.9	62.6 $\pm$ 7.1
<i>C. petiolata</i> Roxb.	–	P Kohkaew 11/53	subprolate	77.2 $\pm$ 5.7	58.5 $\pm$ 7.3
<i>C. parviflora</i> Wall.	–	P Kohkaew 12/53	prolate spheroidal	50.7 $\pm$ 3.7	47.6 $\pm$ 3.8
<i>C. cf. parviflora</i>	–	P Kohkaew 13/53	prolate spheroidal	50.7 $\pm$ 3.7	46.6 $\pm$ 3.9
<i>C. pierreana</i> Gagnep.	–	P Kohkaew 14/53	prolate	65.1 $\pm$ 5.8	46.9 $\pm$ 5.4
<i>C. rhabdota</i> Siriruga & Newman.	–	P Kohkaew 15/53	prolate spheroidal	58.9 $\pm$ 5.9	55.2 $\pm$ 6.1
<i>C. rubescens</i> Roxb.	–	P Kohkaew 23/53	subspheroidal	77.1 $\pm$ 6.4	65.5 $\pm$ 4.1
<i>C. singularis</i> Gagnep.	–	P Kohkaew 16/53	prolate spheroidal	64.8 $\pm$ 5.8	58.9 $\pm$ 6.0
<i>C. sparganifolia</i> Gagnep.	–	P Kohkaew 17/53	prolate spheroidal	50.5 $\pm$ 3.4	47.7 $\pm$ 3.5

All specimens investigated are deposited at Mahasarakham University.

colours, i.e., white, yellow, red, purple, or bluish<sup>2</sup>. In the family Zingiberaceae, pollen morphology gives important data for their classification and systematics. Pollen morphology of Zingiberaceae has been studied by various researchers<sup>4–9</sup>. They used many characteristics of the pollen grain, i.e., shape, size, symmetry, and polar aperture to identify the species of plants. For the genus *Curcuma*, Chen and Xia<sup>10</sup> have studied the pollen morphology of 14 species of Chinese *Curcuma* by SEM and transmission scanning microscopy (TEM). The present study was carried out in order to provide additional data for biological knowledge, identification and to present some palynological data, which would be useful for taxonomists who study or will study the family. No reports are currently available on pollen morphology for the genus *Curcuma* from Thailand.

## MATERIALS AND METHODS

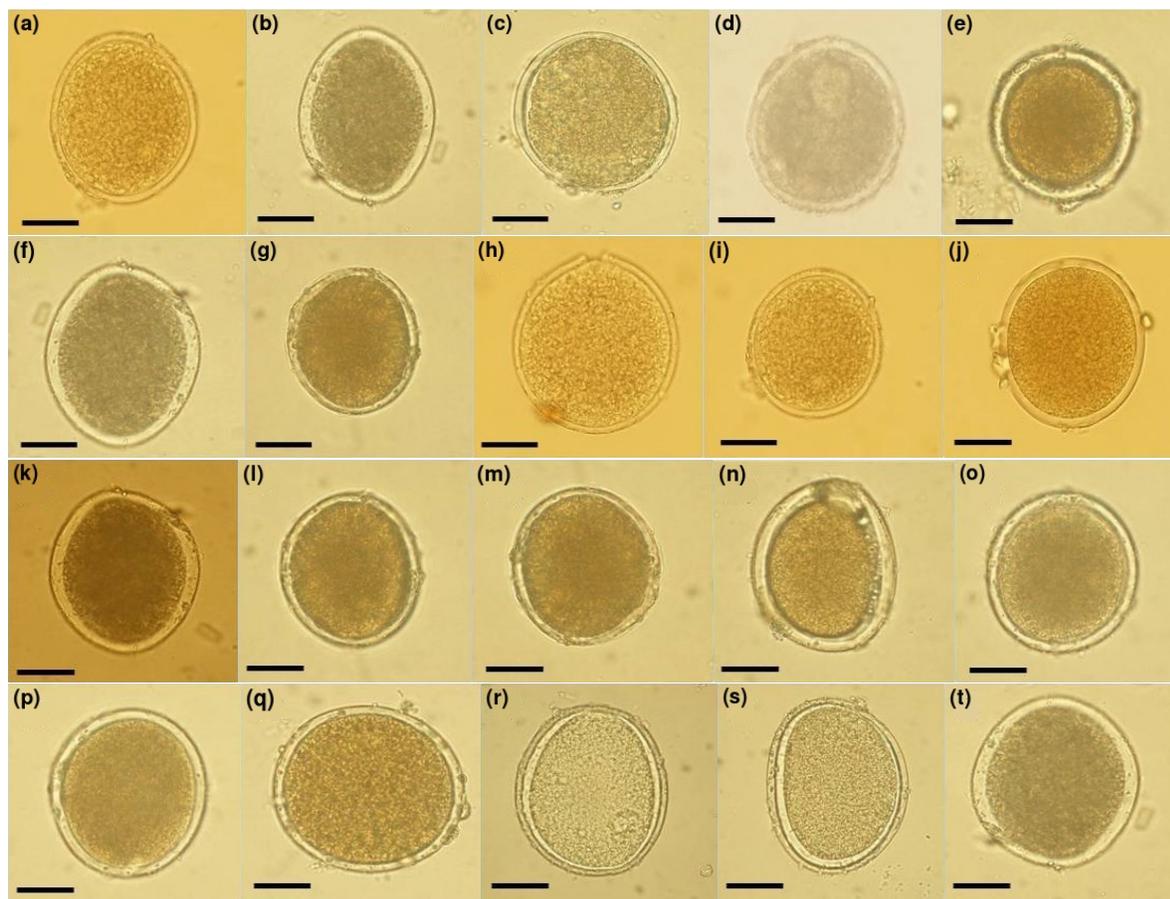
Pollen grains from 20 taxa of the genus *Curcuma* were collected from the fields with soil rich in humus located in mixed deciduous forest in Northeastern Thailand. The pollen grains were examined by light microscopy (LM) and scanning electron microscopy (SEM) (Table 1). The pollen was obtained from alcohol-preserved material. Samples were dehydrated using an alcohol series of 70%, 80%, 95%, and 100%. For LM studies, pollen grains were mounted in silicone oil and sealed with paraffin.

At least 30 pollen grains of each specimen were measured to determine the pollen-diameter ( $\mu\text{m}$ ) and then photographed using the LM (OLYMPUS BX50). For SEM studies, pollen grains in absolute alcohol were dried and affixed to aluminium stubs with double-sided cellophane tape. Samples were sputter-coated with a gold-palladium, examined and then photographed in the SEM (JEOL: JSM 6460 LV). Voucher specimens and all corresponding microscope slides were deposited at the Department of Biology, Faculty of Science, Mahasarakham University, Mahasarakham, Thailand. The terminology used with the pollen follows Punt et al<sup>11</sup>.

## RESULTS

The pollen grains of all species are monad, inaperturate, apolar, with radial symmetry, and rugulose exine sculpturing. The size of the pollen grains varies from 50.5  $\pm$  3.4  $\mu\text{m}$  in *C. sparganifolia* to 86.9  $\pm$  7.9  $\mu\text{m}$  in *C. longa* 2 (Table 1, Figs. 1–3). The pollen grains of some species have aperture-like structures recognized as ridges or furrows such as in *C. alismatifolia* 3 and *C. pierreana*.

The pollen of the genus *Curcuma* in Thailand can be divided into 5 groups based on their shape as follows: Prolate spheroidal, including *C. alismatifolia* 1, *C. harmandii*, *C. larsenii*, *C. parviflora*, *C. cf. parviflora*, *C. rhabdota*, *C. singularis*, and *C. sparganifolia*; subprolate, including *C. alismatifo-*



**Fig. 1** Light micrographs of pollen from the genus *Curcuma*. (a) *C. alismatifolia* 1; (b) *C. alismatifolia* 2; (c) *C. alismatifolia* 3; (d) *C. alismatifolia* 4; (e) *C. alismatifolia* 5; (f) *C. alismatifolia* 6; (g) *C. angustifolia*; (h) *C. gracillima*; (i) *C. harmandii*; (j) *C. larsenii*; (k) *C. longa* 1; (l) *C. longa* 2; (m) *C. petiolata*; (n) *C. parviflora*; (o) *C. cf. parviflora*; (p) *C. pierreana*; (q) *C. rhabdota*; (r) *C. rubescens*; (s) *C. singularis*; (t) *C. sparganifolia*. Scale bars = 20  $\mu\text{m}$ .

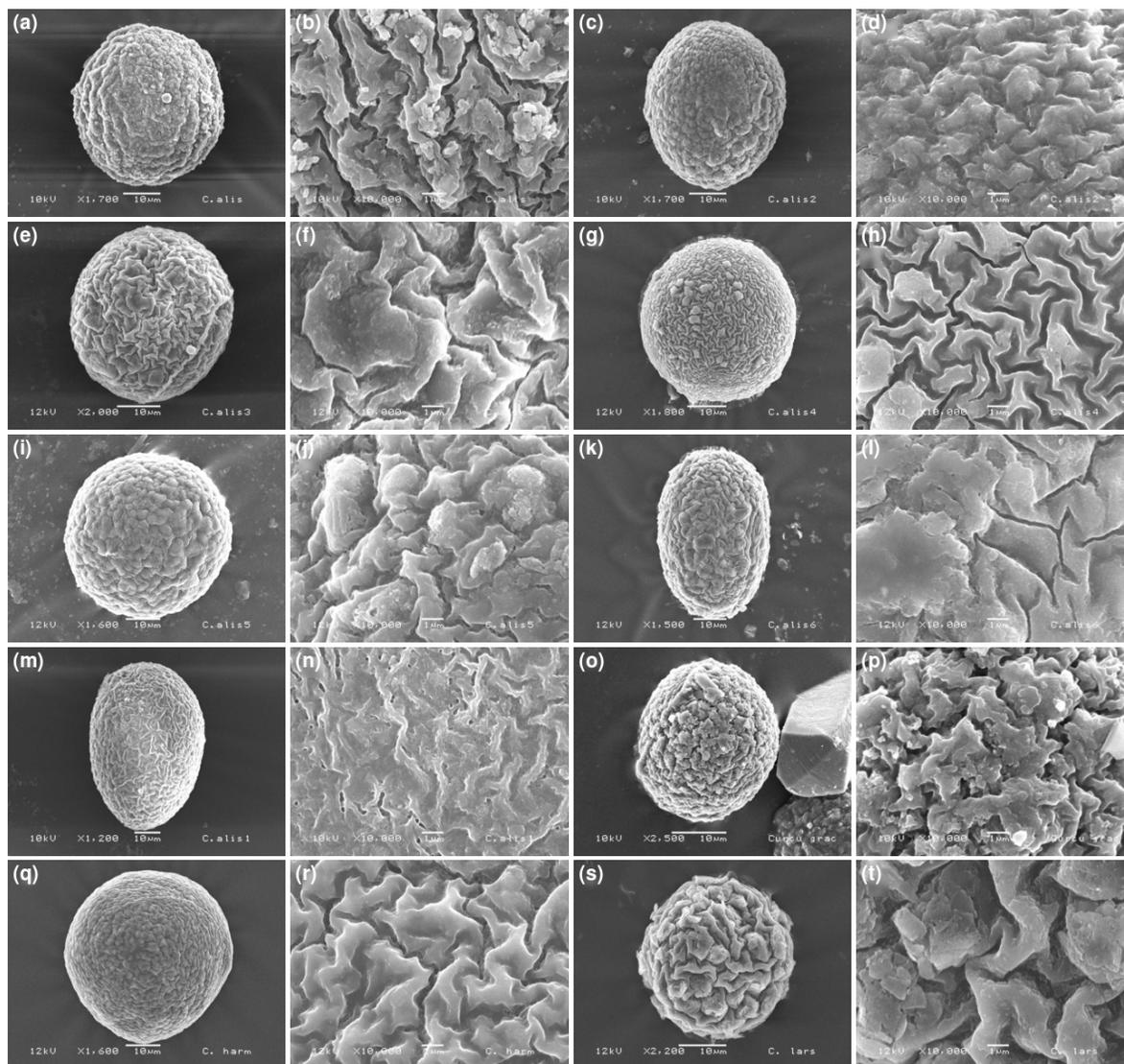
*lia* 2, *C. alismatifolia* 6, *C. angustifolia*, *C. gracillima*, and *C. petiolata*; spheroidal, including *C. alismatifolia* 3, *C. alismatifolia* 4, and *C. alismatifolia* 5; subspheroidal, including *C. longa* 1 and *C. rubescens*; prolate, including *C. longa* 2 and *C. pierreana*.

## DISCUSSION

The pollen wall is composed of thin exine and thick intine, which is similar to that of most genera in Zingiberaceae<sup>4–10,12,13</sup>. The majority of the genus has large sized pollen in the range 50.5–86.9  $\mu\text{m}$ .

These results are in agreement with those of Maknoi<sup>3</sup>, who divided the genus *Curcuma* into 5 groups based on morphological characters: ‘Alismatifolia’, ‘Cochinchinensis’, ‘Ecomata’, ‘Longa’, and ‘Petiolata’ groups. The alismatifolia group is composed of *C. alismatifolia*, *C. gracillima*, *C. harmandii*, *C. parviflora*, *C. rhabdota*, *C. sparganifolia*, and *C. larsenii*. The

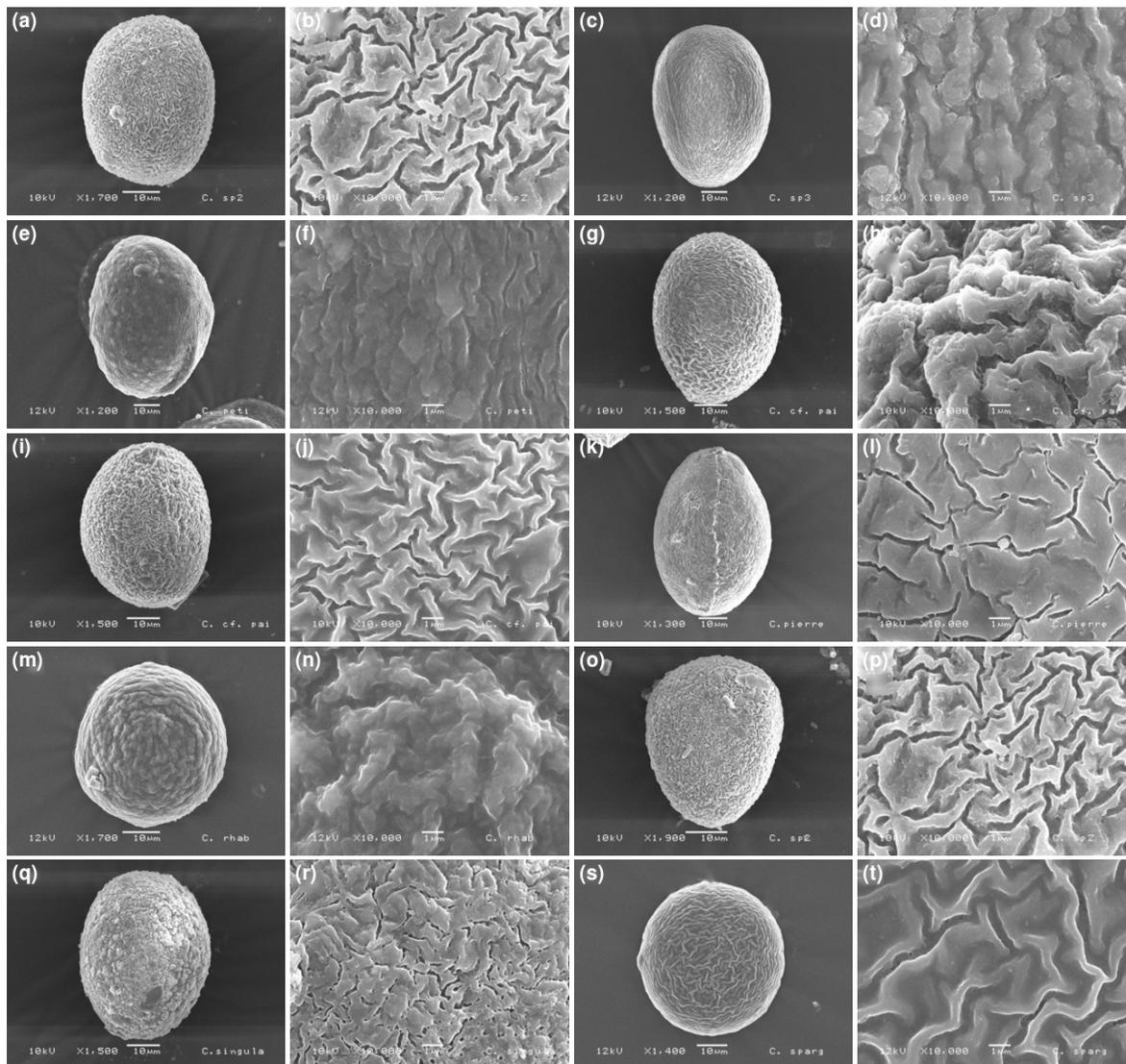
cochinchinensis group includes *C. cochinchinensis* and *C. pierreana*. The Ecomata group is composed of *C. bicolor*, *C. ecomata*, *C. flaviflora*, *C. glans*, *C. singularis*, and *C. stenochila*. The petiolata group includes *C. aurantiaca*, *C. petiolata*, *C. roscoeana*, and *C. rubrobracteata*. The longa group consists of *C. aeruginosa*, *C. amada*, *C. angustifolia*, *C. aromatica*, *C. comosa*, *C. latifolia*, *C. leucorhiza*, *C. longa*, *C. mangga*, *C. rubescens*, *C. viridiflora*, *C. xanthorrhiza*, and *C. zedoaria*. The key characters used in this grouping are the leaf-base, bract apex, bractole, coma bract, flower shape, anther spurs, and stylodes. The most important one is anther spurs, which is unique in each group. The pollen of the wild-type and cultivated *C. alismatifolia* from various locations also exhibit differences in the shape. The pollen morphology of *C. alismatifolia* 1, *C. alismatifolia* 2, *C. alismatifolia* 3, *C. alismatifolia* 4,



**Fig. 2** Scanning electron micrographs of pollen from the genus *Curcuma*. Pollen grain: (a), (c), (e), (g), (i), (k), (m), (o), (q), (s). Exine sculpturing: (b), (d), (f), (h), (j), (l), (n), (p), (r), (t). (a,b) *C. alismatifolia* 1; (c,d) *C. alismatifolia* 2; (e,f) *C. alismatifolia* 3; (g,h) *C. alismatifolia* 4; (i,j) *C. alismatifolia* 5; (k,l) *C. alismatifolia* 6; (m,n) *C. angustifolia*; (o,p) *C. gracillima*; (q,r) *C. harmandii*; (s,t) *C. larsenii*.

*C. alismatifolia* 5, and *C. alismatifolia* 6 are quite similar, except for the pollen shape. Pollen grains of all *C. alismatifolia* are variously shaped, prolate spheroidal, spheroidal, and subprolate. These results may be due to the environmental conditions when the plant was growing in the field. Chen and Xia<sup>10</sup> studied the pollen morphology of 14 species of Chinese *Curcuma* under SEM and TEM. The results showed that pollen grains of all *Curcuma* investigated are ovoid, inaperturate with psilate sculpturing. The pollen wall is composed of a very

thin exine and a thick intine. Chen<sup>14</sup> also reported the exine sculpturing of the pollen of *Curcuma* is psilate. In our study, the exine is rugulose. The results reveal morphological congruence between the pollen grains of species of *Curcuma*, which according to DNA sequence data appears to be a polyphyletic genus. The uniform pollen morphology in *Curcuma* however provides no evidence to divide this genus into separate taxonomic entities. The groups based on palynological characters, however, differ from the groups based on morphological char-



**Fig. 3** Scanning electron micrographs of pollen from the genus *Curcuma*. Pollen grain: (a), (c), (e), (g), (i), (k), (m), (o), (q), (s). Exine sculpturing: (b), (d), (f), (h), (j), (l), (n), (p), (r), (t). (a,b) *C. longa* 1; (c,d) *C. longa* 2; (e,f) *C. petiolata*; (g,h) *C. parviflora*; (i,j) *C. cf. parviflora*; (k,l) *C. pierreana*; (m,n) *C. rhabdota*; (o,p) *C. rubescens*; (q,r) *C. singularis*; (s,t) *C. sparganifolia*.

acters. More research using molecular data and population genetic studies is needed to investigate the relationships in the genus *Curcuma*.

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