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Pollen morphology and leaf anatomy of genus *Globba* in Thailand

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ABSTRACT: Pollen morphology was investigated in 22 taxa of the genus *Globba* (Zingiberaceae) from Thailand by means of light microscopy and scanning electron microscopy. The pollen grains of all taxa examined are monad, radially symmetric, apolar, and inaperturate. The sizes of the pollen grains are medium and large. The pollen grains have various shapes: oblate-spheroidal, spheroidal, and prolate-spheroidal. The exine ornamentation is short-echinate with psilate between the spine, short-echinate with rugulate between the spine, and psilate. The two types of spine apex are blunt and sharp. Palynological data has not been useful at the specific level. The leaf anatomy of 26 taxa was investigated by epidermal peeling and transverse sections of the leaves. The results indicate that the significant leaf anatomical characteristics for species identification are types of stomata, types of trichome, number of rows in intercostal regions, position of hypodermis, shape of midrib, types of vascular system, shape of midrib and leaf margins in transverse sections, and cell inclusion. Based on these anatomical characteristics, an identification key for *Globba* at the species level is given for the first time.

KEYWORDS: Zingiberaceae, palynology, leaf surface, scanning electron microscope

INTRODUCTION

The tribe Globbeae includes four genera, three of which, *Globba*, *Gagnepainia*, and *Hemiorchis*, are native to Thailand, while the fourth genus, *Mantisia*, has not yet been collected in Thailand. With over 100 species, *Globba* L. is the third largest genus of the Zingiberaceae (53 genera, >1200 species)[1], with about 42 species reported in Thailand[2]. The members of the genus are small perennial herbs (< 1 m tall) with terminal inflorescences, although at least one species (*G. racemosa* Sm.)[1] can reach 3 m. The floral features are unique: calyx turbinate to campanulate, 3-lobed to 3-dentate; corolla tube long, slender; labellum 2-lobed or entire, connate to the filament above the staminodes; lateral staminodes petaloid, free from labellum, elliptic, inserted at about same level as petals; and anther with or without lateral appendages. Flowers may be white, yellow, pink, orange or purple[3]. In Thailand, *Globba* is called 'dok khao phansa'. Inflorescence position, fruit shape, stamen length, and lateral staminode position are distinguishing characters for identification. These structures are found in certain seasons and some species are very similar in morphological characteristics. The current classification of the genus recognizes three subgenera, seven sections and two subsections based on the structure of the anther appendage[1]. In Thailand, we found all three subgenera, namely, subgenera *Globba*, *Ceratanthera*, and *Mantisia* (sect. *Globba*, *Nudae*, *Ceratanthera*, and *Haplanthera*, i.e., *G. winitii*, *G. nuda*, *G. pendula*, and *G. racemosa*, respectively). *Globba* species are distributed throughout tropical (and parts of subtropical) Asia, ranging from India to southern China, south and east to the Philippines and New Guinea[4], with the centre of distribution in monsoonal Southeast Asia, especially Thailand[5] and Myanmar[6]. Most of the *Globba* species grow naturally in the different regions and their properties are important in traditional herbal medicine. In Thailand, three species of *Globba* were identified for ethnomedicine such as *G. candida*, *G. obscura*, and *G.* sp. for tuberculosis[7]. Three species were used as ornamental plants such as *G. albiflora* var. *albiflora*, *G. laeta*, *G.* cf. *sherwood-*

iana, *G. winitii*, and *G. williamsiana*. Five species were used as ornamental plants such as *G. albiflora* var. *albiflora*, *G. laeta*, *G.* cf. *sherwoodiana*, *G. winitii*, and *G. williamsiana*. Some *Globba* species were used as ritual plants especially *G. barthei*, *G. laeta*, *G. marantina*, and *G. schomburgkii*[8]. They have cultural importance in Thailand, for example, at the 'Tak Bat Dok Mai' festival in Saraburi Province.

Pollen morphology is important for studying plant taxonomy in the family Zingiberaceae. The pollen morphology of Zingiberaceae has been studied by various researchers. They have used many characteristics of the pollen grains for identifying the species of plants, i.e., shape, size, symmetry, and polar aperture[9–15], and for classification at the generic and sectional levels[16]. The pollen morphology of the genus *Globba* has been studied by various researchers[17–19]. They found that the pollen grains are non-aperturate and exine sculpture is short-spinate. Kaewsri and Paisooksantivatana[20] studied the pollen grains of 14 representatives of Thai *Amomum* using a scanning electron microscope (SEM). The result revealed that pollen grains are spherical to subspherical, inaperturate; exine sculpture is psilate and echinate. Although exine sculpturing can be used to divide the species into two groups, it is less useful for subgeneric classification. Syamsuardi[21] studied the pollen morphology of 9 taxa of *Globba* (*G. leucantha*, *G. patens*, *G. variabilis*, *G. hasseltii*, *G. fecunda*, *G. atrosanguinea*, *G. aurantiaca*, *G. multifolia*, and *G. paniculata*) that were collected from various fields in the regions of West Sumatra. Four diagnostic characteristics (pollen types, shapes, apertures, and ornamentations) were examined and photographed by SEM. Jones and Pearce[22] studied the pollen morphology of *G. propinqua*. The result showed that the pollen grains are inaperturate, spheroidal and the exine sculpture is granulate with gemmae. The size of the pollen grains is 29–42 μm in equatorial view. The pollen grains of Zingiberaceae are generally not resistant to acetolysis. The wall is composed of a very thin exine and a thick intine.

Leaf anatomical characters of some *Globba* species were reported by Tomlinson[23,24], who studied the anatomy of 41 species from 20 genera of Zingiberaceae, i.e., subfamilies Costoideae and Zingiberoideae (tribes Globbeae, Hedychieae, and Alpinieae). The leaf anatomies of two species of *Globba* (*G. bulbifera* and *G. winitii*) were studied. The position of the hypodermis, large solitary bodies of crystalline material occurring in hypodermal cells in the leaf blade (silica sand in the lamina), was confined to the parenchyma of the bundle sheath; trichome presence or absence on the margin of the leaf can be used for species identification. The leaf anatomy of 22 *Alpinia* species from China were studied by Hussin[25]. The results showed that there are interspecific variations in the structure of the midrib and petiole, which can be used for species identification. Lakoet[26] investigated leaf epidermal peels and transverse sections of roots, rhizomes, aerial stems, and leaves that consisted of the blades, margins, midribs, petioles, and leaf sheaths of 4 tribes, 9 genera, and 39 species of Zingiberaceae in Phu Phan National Park, Thailand. The leaf anatomies of 6 species of *Globba* (*G. annamensis*, *G. bartheii*, *G. laeta*, *G. marantina*, *G. panicoides*, and *G. winitii*) were studied. Inclusions in the costal regions on both leaf surfaces, simple unicellular hairs on both leaf surfaces, hypodermis, and stomata on both leaf surfaces, shape in transverse section, and type of cell in the leaf margins, shape and vascular bundle systems in cross section of the midrib, middle parts, and lateral parts of the leaf sheaths can be used for species identification. Saensouk[11] studied the leaf anatomy of 20 taxa of the genus *Alpinia* in Thailand. The leaf anatomy of the genus can be classified using the distribution of stomata, trichomes, shape of subsidiary cells, silica body, silica sand, tannin, and solitary crystals. Saensouk[13] studied the comparative anatomy of three species of *Cornukaempferia* from northeastern Thailand. The presence of stomata, trichome, papillae, hypodermis, shape of leaf margin, and type of vascular system for both species of *Cornukaempferia* were highly similar and cannot be used for species identification, due to the plants in the genus *Globba* being quite similar in morphology. Furthermore, some characters used for species identification are floral parts that cannot be found all the year. Anatomical data are often extremely useful in solving problems of relationships because they can often suggest with greater confidence the homologies of morphological character states, and they can help in the interpretation of evolutionary directionality[27]. For pollen morphology study, it is well known that pollen features have a great taxonomic value, and they have been used in the classification of different genera[28,29] and closely related Zingiberaceae taxa. However, most species of this genus in Thailand have not had their pollen morphology and leaf anatomy studied. The aims of the present paper are to describe the comparative pollen morphology and leaf anatomy of *Globba* in Thailand and to provide useful characteristics in relation to taxonomy.

MATERIALS AND METHODS

Plant collection and plant materials

The plant species were surveyed and specimens were collected from fields in Thailand during May–Aug 2013 and May–Aug 2016. This study is also a review of the literature dealing with *Globba* and related genera distributed in Thailand. The herbarium specimens studied were from BK, BKF, QBG, PSU, and Department of Biology Herbarium Chiang Mai University. Field collections and flowering period observations of *Globba* were made in Thailand. The specimens examined were annotated using taxonomic methods. Specimens were pressed and fixed in 70% ethanol. Living specimens of the genus *Globba* were collected from various locations in Thai forests, then transplanted into pots at Mahasarakham University Nursery. The specimens were collected and deposited as reference specimens in the Mahasarakham University Herbarium. Specimens were studied palynologically and leaf anatomical characters was obtained from spirit specimens.

Palynological studies

The anthers of 22 Thai *Globba* were stored in 70% ethanol. Pollen grains of the genus *Globba* in Thailand were examined by a light microscope (LM) and scanning electron microscope (SEM). 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 per sample were measured for the diameter (μm) of the pollen in the polar axis and equatorial axis. Data analyses were performed using the means and standard errors. Shapes were described according to the P/E ratio (the length of the polar axis to the equatorial axis). The pollen shape and size classification followed Erdtman[30]. Photographs were taken using an Olympus BX50 light microscope. For SEM studies, pollen grains in absolute alcohol were dried on aluminium stubs with double-sided cellophane tape. Samples were sputter-coated with gold-palladium, examined, and then photographs were taken with a JEOL: JSM 8460LV SEM to determine the exine sculpturing and aperture. The terminology adopted to describe the palynological characters was according to Punt[31].

Leaf anatomical studies

The leaves of 26 taxa of *Globba* species were taken from spirit specimens. They were fixed in 70% alcohol or 70% FAA, dehydrated in a tertiary butyl alcohol series, sectioned on a rotary microtome at 5–10 μm thickness and stained in safranin and Fast green. Transverse sections were made from blades, margins, and midribs. For epidermal peeling studies, samples were prepared by mechanical scraping between the midrib and margin of the lamina, stained with 1% safranin in water and then mounted in DePeX[32]. Photographs were taken with the aid of an Olympus BX50 light microscopy. All specimens examined and slide collections are kept at the Mahasarakham University Herbarium.

RESULTS

Pollen morphology

The pollen of 22 taxa of the genus *Globba* in Thailand is monad, radial symmetry, apolar, and inaperturate (Table 1, Figs. 1–3). The pollen shape can be divided into 3 types as follows: oblate-spheroidal, including *G. adhaerens* (violaceus bract), *G. bicolor*, *G. cambodgensis*, *G. laeta*, *G. panicoides*, *G. williamsiana*, and *G. winitii*; spheroidal, including *G. albiflora* var. *albiflora*, *G. annamensis*, *G. marantina*, *G. nuda*, *G. praecox*, *G. racemosa*, *G. sessiliflora*, *G. sherwoodiana*, and *G. xantholeuca*; and prolate-spheroidal, including *G. adhaerens* (albus bract), *G. albiflora* var. *aurea*, *G. globulifera*, *G. pendula*, *G. schomburgkii*, and *G. siamensis*. The pollen grains of most Thai *Globba* are medium sized except in *G. adhaerens* (albus bract), *G. annamensis*, *G. bicolor*, and *G. panicoides*, which are large sized. The wall thickness of the pollen varies from 6.4 ± 2.2 μm (*G. winitii*) to 20.0 ± 7.5 μm (*G. laeta*). The length of the spine varies from 0.4 ± 0.1 μm in *G. bicolor* to 1.8 ± 0.2 μm in *G. racemosa*. The width of the spine base varies from 0.6 ± 0.1 μm in *G. nuda* to 2.8 ± 0.8 μm in *G. laeta*. The spine apex characteristic can be divided into two groups as follows: sharp apex, including *G. adhaerens* (albus bract), *G. albiflora* var. *aurea*, *G. pendula*, *G. praecox*, *G. racemosa*, *G. sessiliflora*, and *G. williamsiana*; and blunt apex, including *G. adhaerens* (violaceus bract) *G. albiflora* var. *albiflora*, *G. annamensis*, *G. bicolor*, *G. cambodgensis*, *G. globulifera*, *G. laeta*, *G. marantina*, *G. nuda*, *G. schomburgkii*, *G. sherwoodiana*, *G. siamensis*, *G. winitii*, and *G. xantholeuca*. The distance between the spines ranges from 0.8 ± 0.2 μm in *G. globulifera* to 4.5 ± 1.7 μm in *G. schomburgkii*. The minimum spine density (0.01 ± 0.00 per μm^2) is observed in *G. laeta* and *G. williamsiana* and the maximum spine density (0.06 ± 0.03 per μm^2) is observed in *G. albiflora* var. *albiflora*. The exine sculpturing can be divided into three types as fol-

Table 1 Pollen morphology data of Thai *Globba*.*

Taxa†	P (μm)	E (μm)	P/E ratio	Shape‡	Size‡	SL (μm)	SW (μm)	SA	WT (μm)	DS (μm)	SD (per μm^2)	ES	Collect No.#
1	43.3±3.8	43.7±3.3	0.99	OS	M	0.9±0.1	1.1±0.1	blunt	16.8±3.4	3.7±1.0	0.02±0.01	SEP	K.41
2	62.9±3.9	61.5±4.2	1.02	PS	L	1.4±0.2	1.6±0.2	sharp	15.3±1.5	4.2±1.2	0.04±0.01	SEP	K.34
3	49.4±4.1	49.0±3.4	1.00	S	M	0.5±0.1	0.6±0.1	blunt	9.5±2.2	1.1±0.2	0.06±0.03	SEP	K.40
4	47.7±2.7	40.4±8.8	1.05	PS	M	0.8±0.1	0.8±0.1	sharp	11.2±3.4	2.5±0.6	0.03±0.02	SER	K.60
5	51.8±2.9	51.5±2.2	1.00	S	L	0.5±0.1	0.9±0.3	blunt	10.4±2.2	3.1±0.6	0.02±0.01	SEP	K.46
6	50.6±3.2	51.3±2.8	0.98	OS	L	0.4±0.1	0.7±0.1	blunt	13.6±2.2	3.2±0.6	0.02±0.00	SER	K.42
7	49.0±2.4	50.6±2.1	0.97	OS	M	1.2±0.2	0.7±0.1	blunt	11.2±1.8	3.4±0.7	0.03±0.00	SEP	K.55
8	9.3±2.2	38.5±1.9	1.02	PS	M	0.5±0.0	0.8±0.2	blunt	11.2±3.4	0.8±0.2	0.04±0.01	SER	K.76
9	46.8±5.7	47.1±5.0	0.99	OS	M	1.6±0.2	2.8±0.8	blunt	20.0±7.5	2.4±0.6	0.01±0.00	SER	K.37
10	49.5±2.1	49.1±2.0	1.00	S	M	1.1±0.1	2.1±0.1	blunt	8.8±3.4	1.0±0.2	0.03±0.01	SEP	K.44
11	37.3±1.8	37.1±1.9	1.00	S	M	0.7±0.1	0.6±0.1	blunt	16.5±3.4	1.8±0.4	0.03±0.01	SER	K.65
12	51.1±4.3	51.9±2.5	0.98	OS	L	–	–	–	7.2±1.8	–	–	psl	K.47
13	46.1±6.7	45.6±3.6	1.01	PS	M	1.2±0.3	1.3±0.2	sharp	12.8±1.5	2.2±0.4	0.04±0.02	SEP	K.68
14	39.4±1.9	39.4±1.9	1.00	S	M	0.5±0.1	0.6±0.2	sharp	9.7±2.2	1.8±0.4	0.02±0.01	SEP	K.80
15	49.3±3.5	49.3±2.9	1.00	S	M	1.8±0.2	2.3±0.3	sharp	10.4±2.2	1.9±0.7	0.02±0.00	SEP	K.90
16	46.8±3.5	46.1±4.3	1.01	PS	M	0.5±0.1	1.2±0.2	blunt	12.8±1.8	4.5±1.7	0.01±0.01	SEP	K.36
17	36.1±2.4	36.0±2.4	1.00	S	M	1.0±0.1	1.2±0.1	sharp	9.8±2.2	4.0±0.9	0.01±0.01	SEP	K.72
18	40.4±8.9	40.1±9.0	1.00	S	M	0.8±0.1	1.2±0.1	blunt	13.6±2.2	2.9±0.7	0.03±0.02	SER	K.85
19	45.2±2.1	44.1±2.3	1.02	PS	M	0.8±0.1	1.8±0.2	blunt	10.4±2.3	3.8±0.3	0.02±0.01	SEP	K.39
20	48.0±2.6	49.2±2.2	0.98	OS	M	1.1±0.1	1.3±0.2	sharp	12.2±2.0	2.3±0.4	0.01±0.00	SEP	K.83
21	46.3±3.5	46.9±3.0	0.98	OS	M	1.3±0.2	1.3±0.1	blunt	6.4±2.2	2.8±0.4	0.03±0.02	SER	K.35
22	47.3±3.4	47.3±3.9	1.00	S	M	0.9±0.1	0.9±0.3	blunt	9.6±2.2	3.1±0.7	0.02±0.01	SER	K.81

* P = polar axis, E = equatorial axis, SL = spine length, SW = spine width, SA = spine apex, WT = wall thickness, DS = distance between the spine, SD = spine density, ES = exine sculpture.

† 1 = *G. adhaerens* Gagnep. (violaceus bract), 2 = *G. adhaerens* Gagnep. (albus bract), 3 = *G. albiflora* var. *albiflora*, 4 = *G. albiflora* var. *aurea*, 5 = *G. annamensis* Gagnep., 6 = *G. bicolor* Gagnep., 7 = *G. cambodgensis* Gagnep., 8 = *G. globulifera* Gagnep., 9 = *G. laeta* K.Larsen., 10 = *G. marantina*, 11 = *G. nuda* K.Larsen., 12 = *G. panicoides* Miq., 13 = *G. pendula* Roxb., 14 = *G. praecox* K.J.Williams & Paisooks., 15 = *G. racemosa* Sm., 16 = *G. schomburgkii* Hook.f., 17 = *G. sessiliflora* Sims, 18 = *G. sherwoodiana* W.J.Kress & V.Gowda, 19 = *G. siamensis* (Hemsl.) Hemsl., 20 = *G. williamsiana* M.F.Newman, 21 = *G. winitii* C.H.Wright, 22 = *G. xantholeuca* Craib.

‡ OS = oblate-spheroidal, PS = prolate-spheroidal, S = spheroidal, L = large, M = medium.

K. = Kajornjit.

lows: short-echinate with psilate between the spine (Fig. 3:a–c, e, g, j, m–q, s, and t), short-echinate with rugulate between the spine (Fig. 3:d, f, h, i, k, r, u, and v), and psilate was found only in *G. panicoides* (Fig. 3l).

Leaf anatomical studies

The results of the leaf anatomy showed some variations and similarities in the leaf anatomical characteristics of the *Globba* species studied. A summary of the leaf anatomical characteristics observed in this study is presented in Tables 2–5.

Leaf surface

The epidermal cells on both surfaces of all the *Globba* species are four to seven-sided (Fig. 4). Most of the stomata on both surfaces are tetracytic (Tables 2 and 3 and Fig. 4a), and guard cells are surrounded by four subsidiary cells except for *G. albiflora* var. *albiflora*, *G. annamensis*, *G. bicolor*, *G. cambodgensis*, *G. laeta*, *G. marantina*, *G. nuda*, *G. purpurascens*, *G. sessiliflora*, *G. sherwoodiana*, *G. siamensis*, *G. williamsiana*, and *G. winitii*, which have a combination of tetracytic and pentacytic stomata on the adaxial surface. On the adaxial surface in *G. praecox* and *G. racemosa* there is a combination of tetracytic, pentacytic (Fig. 4b) and rarely hexacytic (Fig. 4c). In addition, in *G. adhaerens* (albus bract), *G. albiflora* var. *albiflora*, *G. albiflora* var. *aurea*, *G. pendula*, and *G. praecox* there is a combination of tetracytic and pentacytic stomata on the abaxial surface. Stomata are distributed at random on both sides with fewer stomata distributed in rows near veins. The stomata develop on both surfaces but are infrequent on the adaxial surface. The density of stomata for all species is different for every leaf sample in each species (Tables 2 and 3). The width of the epidermal cells on the adaxial side varies from 47.0±4.2 μm in *G. schomburgkii* to 91.5±7.4 μm in *G. sessiliflora* (Table 2), while the width of the epidermal cells on the abaxial side varies from 33.5±2.1 μm in *G. nuda* to 66±12 μm in *G. williamsiana* (Table 3). Epidermal cells in the intercostal regions on the adaxial surface varies from 6 rows (*G. adhaerens* (albus bract), *G. albiflora* var. *aurea*, *G. marantina*, *G. nuda*) to 26 rows

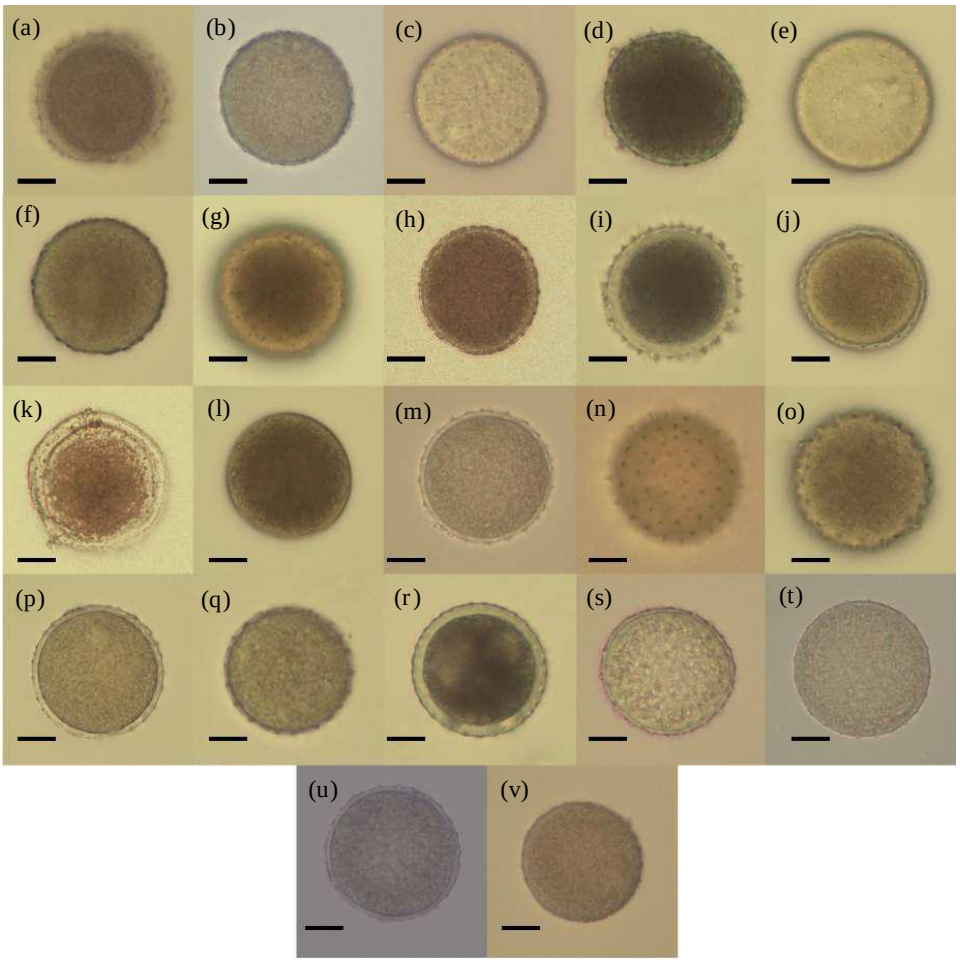

Fig. 1 Light microscopy micrographs of pollen grains. (a) *G. adhaerens* (violaceus bract), (b) *G. adhaerens* (albus bract), (c) *G. albiflora* var. *albiflora*, (d) *G. albiflora* var. *aurea*, (e) *G. annamensis*, (f) *G. bicolor*, (g) *G. cambodgensis*, (h) *G. globulifera*, (i) *G. laeta*, (j) *G. marantina*, (k) *G. nuda*, (l) *G. panicoides*, (m) *G. pendula*, (n) *G. praecox*, (o) *G. racemosa*, (p) *G. schomburgkii*, (q) *G. sessiliflora*, (r) *G. sherwoodiana*, (s) *G. siamensis*, (t) *G. williamsiana*, (u) *G. winitii*, (v) *G. xantholeuca*. Scale bars = 20 μm.

(*G. praecox*), whereas the epidermal cells in the intercostal regions on the abaxial surface are greatest in *G. winitii* (18 rows) and the lowest in *G. albiflora* var. *aurea* and *G. panicoides* (5 rows) (Fig. 4d, e). The greatest length of the guard cell is found in *G. laeta* (39.6 ± 1.7 μm) and the lowest in *G. annamensis* (27.0 ± 2.1 μm) (Table 2). The length of the guard cells on the abaxial surface is greatest in *G. racemosa* (49.5 ± 4.1 μm) and the lowest in *G. praecox* (25.5 ± 2.0 μm) (Table 3). The shape of the subsidiary cells is found to be dome-shaped on both surfaces. The density of the stomata on the adaxial surface ranges from 2 ± 1 per mm^2 in *G. reflexa* to 67 ± 4 per mm^2 in *G. marantina*, while the density of stomata on the abaxial surface varies from 88 ± 8 per mm^2 in *G. racemosa* to 273 ± 15 per mm^2 in *G. reflexa*. Trichomes are present on both surfaces, except in *G. albiflora* var. *albiflora*, *G. albiflora* var. *aurea*, *G. globulifera*, *G. laeta* and *G. nuda*, in which trichomes are not found on both surfaces. Some species have trichomes that are found only on the adaxial surface, such as *G. marantina*, *G. sherwoodiana*, *G. williamsiana*, and *G. xantholeuca*. Most species have trichomes only on the abaxial surface, i.e., *G. cambodgensis*, *G. laeta*, *G. panicoides*, *G. praecox*, *G. purpurascens*, *G. racemosa*, and *G. reflexa*. Four types of trichomes were recorded here.

Type 1: short, simple unicellular trichomes (length of trichomes < 400 μm) are found on adaxial surface, which includes *G. adhaerens* (violaceus bract),

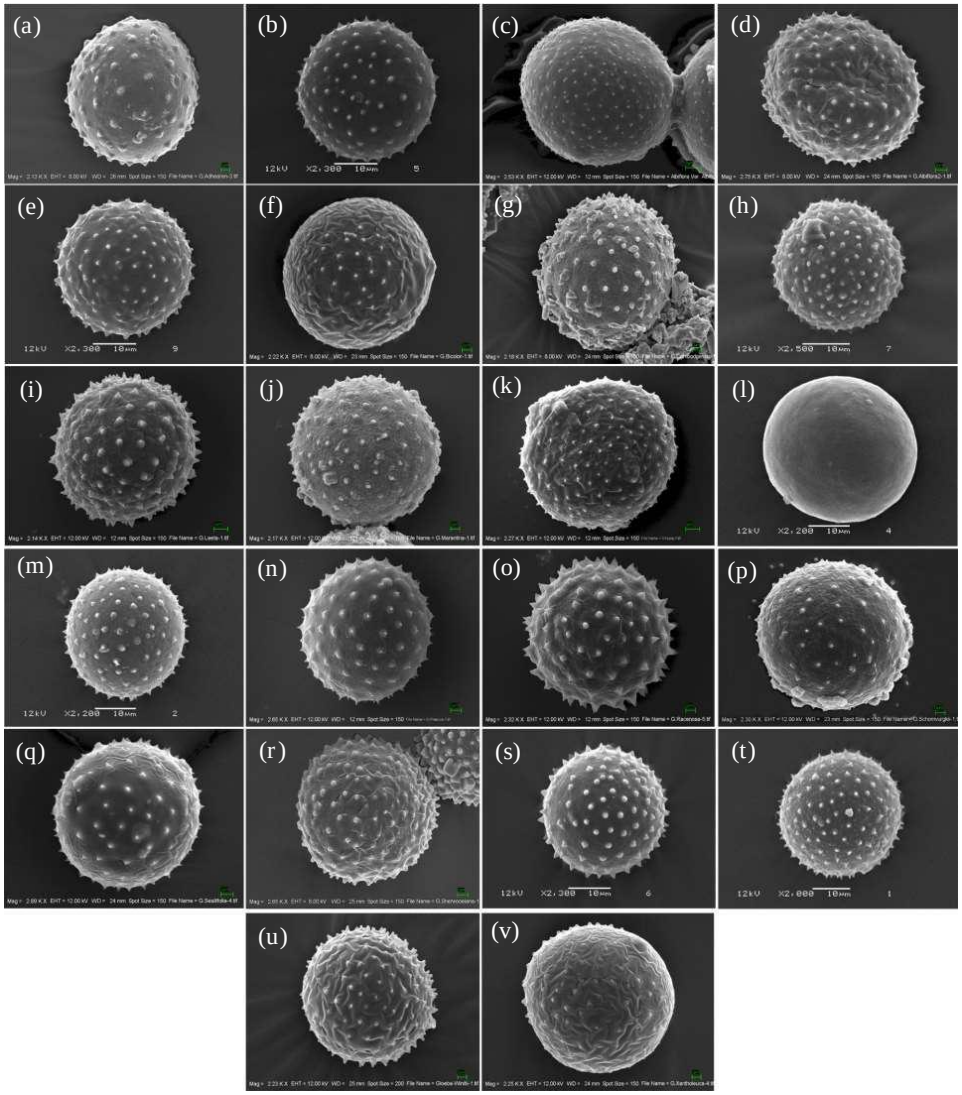

Fig. 2 SEM micrographs of pollen grains. (a) *G. adhaerens* (violaceus bract), (b) *G. adhaerens* (albus bract), (c) *G. albiflora* var. *albiflora*, (d) *G. albiflora* var. *aurea*, (e) *G. annamensis*, (f) *G. bicolor*, (g) *G. cambodgensis*, (h) *G. globulifera*, (i) *G. laeta*, (j) *G. marantina*, (k) *G. nuda*, (l) *G. panicoides*, (m) *G. pendula*, (n) *G. praecox*, (o) *G. racemosa*, (p) *G. schomburgkii*, (q) *G. sessiliflora*, (r) *G. sherwoodiana*, (s) *G. siamensis*, (t) *G. williamsiana*, (u) *G. winitii*, (v) *G. xantholeuca*. (a, d, f, j, n, o–r, u, v) scale bars = 2 μm; (c, g, i, k) scale bars = 3 μm; (b, h) scale bars = 5 μm; (e, l, m, s, t) scale bars = 10 μm.

G. annamensis, *G. candida*, *G. marantina*, *G. pendula*, *G. schomburgkii*, *G. sessiliflora*, and *G. winitii*, while for the abaxial surface they are found in *G. adhaerens* (violaceus bract), *G. bicolor*, *G. cambodgensis*, *G. candida*, *G. laeta*, *G. panicoides*, *G. pendula*, *G. praecox*, *G. racemosa*, *G. schomburgkii*, and *G. sessiliflora* (Fig. 4f).

Type 2: long, simple unicellular trichomes (length of trichomes > 400 μm) are found on adaxial surface, which includes *G.* aff. *winitii*, *G. marantina*, and *G. siamensis*, while for the abaxial surface they are found in *G.* aff. *winitii*, *G. annamensis*, *G. siamensis*, and *G. winitii* (Fig. 4g).

Type 3: long, biseriate trichomes (length of trichomes > 400 μm) are found on adaxial surface, which includes *G. adhaerens* (albus bract) and *G.* aff. *winitii*, while for the abaxial surface they are found in *G. adhaerens* (albus bract), *G.* aff. *winitii*,

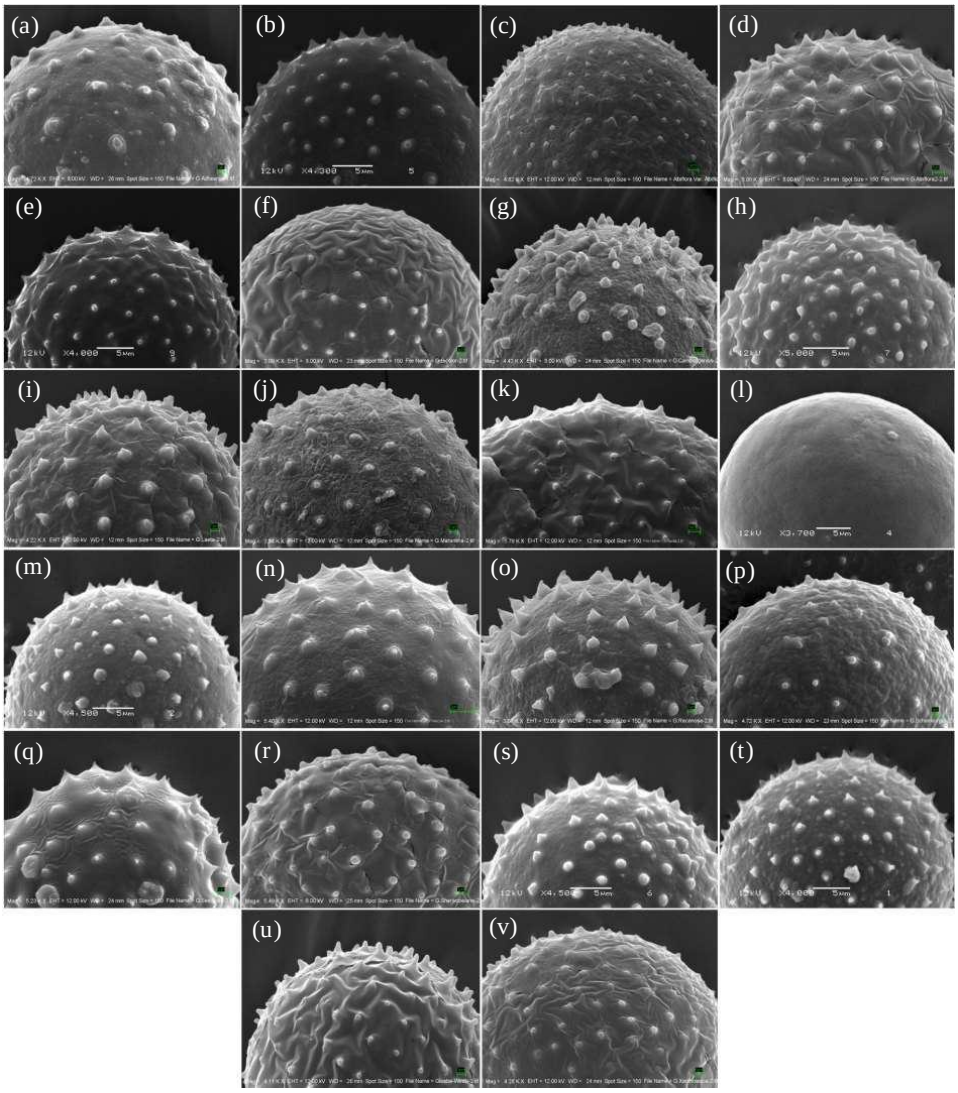

Fig. 3 Exine ornamentation of pollen grains. (a) *G. adhaerens* (violaceus bract), (b) *G. adhaerens* (albus bract), (c) *G. albiflora* var. *albiflora*, (d) *G. albiflora* var. *aurea*, (e) *G. annamensis*, (f) *G. bicolor*, (g) *G. cambodgensis*, (h) *G. globulifera*, (i) *G. laeta*, (j) *G. marantina*, (k) *G. nuda*, (l) *G. panicoides*, (m) *G. pendula*, (n) *G. praecox*, (o) *G. racemosa*, (p) *G. schomburgkii*, (q) *G. sessiliflora*, (r) *G. sherwoodiana*, (s) *G. siamensis*, (t) *G. williamsiana*, (u) *G. winitii*, (v) *G. xantholeuca*. (a, c, d, f, g, i, j, k, o–r, u, v) scale bars = 1 μm; (n) scale bar = 3 μm; (b, e, h, l, m, s, t) scale bars = 5 μm.

G. siamensis, and *G. winitii* (Fig. 4h).

Type 4: glandular trichomes are found on adaxial surface in *G. bicolor* and *G. cambodgensis* while for the abaxial surface they are found in *G. cambodgensis*, *G. candida*, *G. praecox*, *G. purpurascens*, *G. racemosa*, *G. reflexa*, *G. sherwoodiana*, *G. williamsiana*, *G. winitii* and *G. xantholeuca* (Fig. 4b).

The density of trichomes is quite high (219 ± 6 per mm^2) on the abaxial surface when compared to the adaxial surface (54 ± 7 per mm^2) (Tables 2–3).

Oil cells are found on both surfaces in *G. adhaerens* (albus bract), *G. bicolor*, *G. cambodgensis*, *G. globulifera*, *G. marantina*, *G. pendula*, and *G. siamensis*, while in *G. winitii* they are found on the adaxial surface (Fig. 4i). For the abaxial surface oil cells are present in *G. adhaerens* (violaceus bract), *G. albiflora* var. *aurea*, *G. annamensis*, *G. laeta*, *G. reflexa*, and *G. sherwoodiana*. Rhombic crystals are present on both surfaces of all species observed except for *G. adhaerens* (albus bract), *G. albiflora* var. *aurea*, *G. globulifera*, *G. nuda*, *G. pendula*, *G. winitii*,

Table 2 Comparison of characteristics of leaf surface on adaxial side in genus *Globba*.*

Taxa†	St‡	WEP (μm)	WSC (μm)	ICR rows	LGC (μm)	DSt (mm^{-2})	Type of trichome				DT (mm^{-2})	Inc‡	Col. No.
							SU (μm)	LU (μm)	LB (μm)	G (μm)			
1	T	72.8±7.1	20.0±2.9	9	33.4±1.7	14±3	302±45	–	–	–	28±2	–	K.41
2	T	63.0±7.6	19.0±2.7	6	30.8±1.4	34±2	–	–	463±39	–	54±7	O	K.34
3	T	64±11	15.0±2.9	8	31.8±2.3	5±3	–	536±66	454±18	–	49±7	–	K.32
4	T,P	65.8±3.1	25.3±1.8	19	36.5±1.5	3±1	–	–	–	–	–	R	K.40
5	T	74.3±10.5	15.5±3.1	6	39.4±3.2	7±2	–	–	–	–	–	Sq	K.60
6	T,P	63.5±6.3	16.0±2.1	7	27.0±2.1	10±3	293±29	–	–	–	13±3	R	K.46
7	T,P	71.3±6.0	23.5±4.7	7	35.2±1.8	29±4	–	–	–	24±5	–	O,Sq,R,S	K.42
8	T,P	63.5±7.5	21.5±3.8	14	34.3±2.3	22±3	–	–	–	22±3	–	R,O	K.55
9	T	70.5±3.7	13.5±1.3	10	29.5±2.2	23±3	245±46	–	–	–	9±3	R,Rec	K.50
10	T	73.3±5.8	23.5±2.7	7	32.5±2.4	23±4	–	–	–	–	–	O	K.76
11	T,P	70.3±8.2	19.8±1.8	9	39.6±1.7	19±3	–	–	–	–	–	R,S	K.37
12	T,P	60.8±2.4	15.5±2.0	6	32.0±1.9	67±4	243±27	456±47	–	–	14±2	O,R	K.44
13	T,P	61.8±7.1	17.0±2.0	6	34.9±2.1	8±2	–	–	–	–	–	Sq	K.65
14	T	57.0±4.0	13.3±2.4	23	30.0±1.4	12±2	–	–	–	–	–	–	K.47
15	T	66.3±4.4	13.5±2.7	22	30.5±1.5	28±4	80±11	–	–	–	3±1	O,S	K.68
16	T,P,H	64.8±6.8	15.0±2.6	26	29.5±2.0	35±4	–	–	–	–	–	R	K.80
17	T,P	76.5±7.1	26.5±3.8	22	27.5±2.0	21±2	–	–	–	–	–	R,Rec	K.79
18	T,P,H	86.3±5.0	16.5±4.3	22	38.3±2.8	7±2	–	–	–	–	–	Sq,R	K.90
19	T	58.5±3.6	17.0±2.0	10	30.4±1.9	2±1	–	–	–	–	–	R	K.82
20	T	47.0±4.2	17.0±3.5	12	30.0±2.0	5±3	189±40	–	–	–	7±4	–	K.36
21	T,P	91.5±7.4	21.3±3.8	22	39.0±3.0	13±2	244±32	–	–	–	8±3	Sq,R	K.72
22	T,P	58.8±4.9	16.3±3.2	16	28.0±1.9	20±4	–	–	–	–	–	R,S	K.85
23	T,P	57.5±3.9	15.5±3.1	13	32.6±2.1	41±3	–	506±77	–	–	50±3	O,Sq,R	K.39
24	T,P	66.5±10.7	19.8±5.1	12	37.0±1.5	21±2	–	–	–	–	–	R	K.83
25	T,P	74.5±5.0	17.3±3.6	22	30.6±2.3	8±3	316±33	–	–	–	47±6	O,Sq	K.35
26	T	68.3±3.7	13.0±2.0	7	35.3±2.1	11±3	–	–	–	–	–	S	K.81

* St = stomata, WEP = width of epidermal cell, WSC = width of subsidiary cell, ICR = intercostal regions, LGC = length of guard cell, DSt = density of stomata, SU = short, simple unicellular, LU = long, simple unicellular, LB = long, biseriate, G = glandular, DT = density of trichome, Inc = inclusion, Col. No. = collected number, K. = Kajornjit.

† 1 = *G. adhaerens* Gagnep. (violaceus bract), 2 = *G. adhaerens* Gagnep. (albus bract), 3 = *G.* aff. *winitii*, 4 = *G. albiflora* var. *albiflora*, 5 = *G. albiflora* var. *aurea*, 6 = *G. annamensis* Gagnep., 7 = *G. bicolor* Gagnep., 8 = *G. cambodgensis* Gagnep., 9 = *G. candida* Gagnep., 10 = *G. globulifera* Gagnep., 11 = *G. laeta* K.Larsen., 12 = *G. marantina*, 13 = *G. nuda* K.Larsen., 14 = *G. panicoides* Miq., 15 = *G. pendula* Roxb., 16 = *G. praecox* K.J.Williams & Paisooks., 17 = *G. purpurascens* Craib, 18 = *G. racemosa* Sm., 19 = *G. reflexa* Craib, 20 = *G. schomburgkii* Hook.f., 21 = *G. sessiliflora* Sims, 22 = *G. sherwoodiana* W.J.Kress & V.Gowda, 23 = *G. siamensis* (Hemsl.) Hemsl., 24 = *G. williamsiana* M.F.Newman, 25 = *G. winitii* C.H.Wright, 26 = *G. xantholeuca* Craib.

‡ T = tetracytic, P = pentacytic, H = hexacytic, O = oil cell, R = rhombic crystal, Rec = rectangle crystal, S = silica bodies, Sq = square crystal.

and *G. xantholeuca*, where rhombic crystals are not found on both surfaces (Fig. 4j). Square and rectangle crystal are observed on both surfaces in *G. bicolor*, *G. laeta*, *G. nuda*, *G. panicoides*, *G. racemosa*, and *G. purpurascens* (Tables 2–3, Fig. 4k, l). Silica sand (Fig. 4m) and hexagonal crystals are found only on the abaxial surface (Fig. 4n). Silica bodies occur on both surfaces above and below the veins, with one silica body per cell. Silica bodies are present on the adaxial surface in *G. bicolor*, *G. laeta*, *G. pendula*, *G. sherwoodiana*, and *G. xantholeuca*, while for the abaxial surface they are present in *G. globulifera* and *G. panicoides* (Fig. 4o). Our results reveal that they are not found on the adaxial surface in *G. adhaerens* (violaceus bract), *G.* aff. *winitii*, *G. panicoides*, and *G. schomburgkii* or on the abaxial surface in *G. praecox*.

Transverse section of the lamina

In the surface view, the epidermal cells on both surfaces are rectangle to polygonal, unequal in size; anticlinal walls of adaxial and abaxial cells are straight (Fig. 5), adaxial cells larger than abaxial cells. Thin cuticle and stomata are found on both surfaces of all species. Guard cells are located at the same level as the epidermis. Most of the studied species have more stomata on the abaxial surface than the adaxial surface, except in *G. cambodgensis*, *G. candida*, *G. laeta*, *G. marantina*, *G. purpurascens*, *G. winitii*, and *G. xantholeuca*, which have stomata on both surfaces (Table 4). Long, simple unicellular trichomes are present on the abaxial surface, which is found in 15 taxa, except in *G. albiflora* var. *albiflora*, *G. albiflora* var. *aurea*, *G. cambodgensis*, *G. globulifera*, *G. panicoides*, *G. purpurascens*, *G. racemosa*, *G. reflexa*, *G. williamsiana*, *G. wini-*

Table 3 Comparison of characteristics of leaf surface on abaxial side in genus *Globba*.*

Taxa	St	WEP (μm)	WSC (μm)	ICR rows	LGC (μm)	DSt (mm^{-2})	Type of trichome				DT (mm^{-2})	Inclusion‡
							SU (μm)	LU (μm)	LB (μm)	G (μm)		
1	T	39.0±3.8	12.3±1.8	11	33.8±1.3	204±6	369±34	–	–	–	99±5	He,O,R,Ss
2	T,P	38.3±6.0	15.1±2.0	9	33.6±2.2	213±4	–	–	568±44	–	135±3	He,O,R,Ss
3	T	35.8±4.1	10.5±2.0	12	31.5±1.9	202±1	–	612±70	592±42	–	126±9	R,Ss
4	T,P	59.5±6.1	21.3±2.4	10	36.0±1.9	93±6	–	–	–	–	–	He,Sq,R,Rec
5	T,P	35.8±4.4	9.3±3.1	5	38.5±1.9	240±1	–	–	–	–	–	O,R
6	T	44.0±6.6	16.0±1.3	12	27.8±2.1	182±2	–	516±73	–	–	176±3	O,Sq,R
7	T	60.5±7.8	22.8±3.4	11	34.5±2.5	184±12	76±14	–	–	–	8±1	O,Sq,R,Rec
8	T	52±10	15.8±2.9	6	34.8±1.8	219±6	57±8	–		20±5	24±3	Sq,R,Ss
9	T	56±11	14.5±2.0	8	28.5±2.4	221±7	170±24	–	–	18±4	219±6	Sq,R
10	T	50.5±3.7	17.5±3.7	13	35.4±1.9	183±8	–	–	–	–	–	O,Sq,R,S
11	T	45.3±3.6	15.5±3.1	8	36.1±1.9	169±5	298±35	–	–	–	157±4	O,R,Sq,Ss
12	T	44.5±3.5	15.5±2.0	7	32.1±1.9	220±3	–	–	–	–	–	He,O,R,Sq,Ss
13	T	33.5±2.1	8.0±2.0	6	34.8±2.1	215±12	–	–	–	–	–	He,Sq,R,Rec
14	T	55.8±4.4	13.0±2.0	5	32.5±2.4	89±4	100±10	–	–	–	40±8	R,Rec,S
15	T,P	45.3±3.4	11.5±2.7	9	34.3±1.8	149±4	70±8	–	–	–	80±5	O,R
16	T,P	36.0±3.9	13.5±2.7	8	25.5±2.0	89±4	91±17	–	–	23±5	103±7	–
17	T	40.3±2.8	18.0±2.0	14	27.5±2.8	178±4	–	–	–	22±4	–	O,R,Rec
18	T	55.0±5.1	12.5±2.9	15	49.5±4.1	88±8	114±12	–	–	20±2	94±7	Sq,R
19	T	38.5±3.6	13.3±1.2	13	35.5±1.9	273±2	–	–	–	17±5	–	O,Sq,R
20	T	44.0±2.7	10.5±2.0	6	31.8±1.8	218±13	360±31	–	–	–	217±5	R
21	T	45.0±3.7	12.5±1.7	9	49.5±2.5	124±4	164±14	–		–	98±4	R,Ss
22	T	46.3±3.8	17.0±2.6	12	28.8±1.3	215±1	–	–	–	22±4	–	He,O,R,Sq,Rec
23	T	37.0±4.5	13.0±2.0	10	34.9±1.5	224±4	–	542±75	468±43	–	150±3	O,R
24	T	65.5±8.9	19.3±4.1	14	38.3±3.0	93±5	–	–	–	21±6	–	He,Sq,R
25	T	51.8±5.5	14.5±2.0	18	31.4±2.1	150±3	–	521±64	604±38	20±7	125±3	R
26	T	38.5±2.4	10.0±2.4	9	31.0±1.7	216±8	–	–	–	24±5	–	R

* Using the same notation as in Table 2.

‡ He = hexagonal crystal, Ss = silica sand.

tii, and *G. xantholeuca*, which are not found to have long, simple unicellular trichomes. In *G. adhaerens* (albus bract), *G.* aff. *winitii*, *G. pendula*, and *G. siamensis* long, simple unicellular trichomes are present on both surfaces.

Most of the studied species have a hypodermis on the abaxial surface with only one row (Fig. 5a), square or pentagonal shape, the hypodermis is larger than the epidermal cells. Rhombic crystals (Fig. 5a) are present in the hypodermis of 13 taxa, except *G. adhaerens* (violaceus bract), *G. adhaerens* (albus bract), *G. albiflora* var. *albiflora*, *G. albiflora* var. *aurea*, *G. annamensis*, *G. candida*, *G. panicoides*, *G. praecox*, *G. reflexa*, *G. schomburgkii*, *G. sessiliflora*, *G. siamensis*, and *G. winitii*, in which rhombic crystals are absent in the hypodermis. Hexagonal crystals (Fig. 5c) are found on the hypodermis in *G.* aff. *winitii*, *G. bicolor*, *G. cambodgensis*, *G. laeta*, *G. marantina*, *G. purpurascens*, *G. sherwoodiana*, *G. williamsiana*, and *G. xantholeuca*.

The Chlorenchyma tissue occupies the middle region of the leaf blade. The palisade and spongy mesophyll range from one to three layers. The palisade cells are ellipse shaped in periclinal rows, with about one to three cells located under the epidermal cell. The spongy cells next to the palisade cells are hemispherical or hemiellipsoidal on the abaxial side. The width of the mesophyll varies from 51.5±3.8 μm in *G. praecox* to 116.0±9.0 μm in *G. adhaerens* (albus bract). Inclusion, rhombic, square, hexagonal crystals, and silica sand are present in the mesophyll, with most of the studied species having rhombic crystals in the mesophyll. Vascular bundles are collateral with tracheary elements consisting of one to two metaxylem and few protoxylem cells flanked by colourless parenchyma laterally. The bundle sheath extends to both sides of the epidermal cells in all species.

Midrib

The shape of all Thai *Globba* in the transverse section curves to a U-shape on both sides (Fig. 5e–h), except in *G. globulifera* which is slightly flat (Fig. 5d). The epidermal cells on both surfaces are four to seven sided. Most of the species assessed show long, simple unicellular trichomes on the abaxial surface, except in *G. candida* and *G. siamensis*, which has long, simple unicellular trichomes on both surfaces. The vascular system is arranged in several arcs; description follows Tomlinson[22] where the main arc is described as arc I, abaxial arc as arc II, adaxial arc as arc III, and a fourth arc closer to the adaxial surface as arc IV. Four groups in the species are recognized: Arc I (Fig. 5e) found in *G. nuda*,

Table 4 Comparison of characteristics of transverse sections of leaf blades in genus *Globba*.*

Taxa	LU	Stomata	Epidermis width		Hypodermis				Mesophyll				
			AD (μm)	AB (μm)	AD	AB	R	He	Width (μm)	PS	SG	Inc	Crystal
1	AD,AB	AB	55.3 ± 7.5	34.3 ± 4.4	–	–	–	–	99.5 ± 9.9	1	2	–	R,He
2	AB	AB	61.5 ± 7.8	40.0 ± 5.7	–	1	–	–	116.0 ± 9.0	3	2	–	–
3	AD,AB	AB	56.0 ± 6.0	36.5 ± 4.6	–	1	/	/	100.3 ± 7.1	2	2	/	R,Ss,He
4	–	AB	50.8 ± 6.6	51.3 ± 5.3	–	–	–	–	52.3 ± 4.9	1	2	/	R
5	–	AB	63.5 ± 11.9	36.8 ± 3.3	–	–	–	–	70.3 ± 5.5	2	1	–	Sq
6	AB	AB	52.3 ± 9.1	38.5 ± 3.6	–	–	–	–	57.0 ± 5.0	2	2	/	R,Ss
7	AB	AB	53.3 ± 6.0	43.3 ± 5.1	–	1	/	/	77.3 ± 7.9	1	2	–	–
8	–	AD,AB	65.0 ± 7.1	50.3 ± 7.9	–	1	/	/	63.0 ± 5.2	1	2	–	–
9	AB	AD,AB	59.3 ± 7.1	53.5 ± 7.1	–	–	–	–	65.5 ± 6.9	1	3	–	Sq,He
10	–	AB	52.0 ± 11.4	40.3 ± 5.6	–	1	/	–	89.3 ± 7.8	2	2	–	Ss,Sq,He
11	AB	AD,AB	62.3 ± 7.5	51.5 ± 7.5	–	1	/	/	82.3 ± 5.1	2	2	/	–
12	AB	AD,AB	73.3 ± 10.7	52.5 ± 7.0	–	1	/	/	60.8 ± 3.3	2	2	/	R,Ss,Sq,He
13	AB	AB	34.5 ± 4.2	19.5 ± 3.3	–	1	/	–	58.3 ± 4.6	1	2	–	R,Ss,Sq
14	–	AB	52.5 ± 5.2	41.0 ± 7.5	–	–	–	–	40.0 ± 4.9	1	1	–	R
15	AD,AB	AB	53.5 ± 9.2	45.8 ± 5.5	–	1	/	–	65.8 ± 6.4	2	1	/	–
16	AB	AB	44.5 ± 11.5	34.0 ± 5.3	–	1	–	–	51.5 ± 3.8	2	1	–	R
17	–	AD,AB	61.3 ± 7.7	53.8 ± 6.9	–	1	/	/	99.3 ± 5.0	2	3	–	–
18	–	AB	61.3 ± 5.0	49.8 ± 10.6	–	1	/	–	72.8 ± 2.8	2	2	–	R,He
19	–	AB	54.8 ± 6.1	41.5 ± 6.0	–	–	–	–	87.3 ± 11.5	2	3	/	R,Sq,He
20	AB	AB	53.0 ± 8.2	42.8 ± 6.6	–	1	–	–	74.5 ± 7.4	1	3	–	R,Sq,He
21	AB	AB	57.5 ± 5.3	47.5 ± 5.8	–	–	–	–	92.8 ± 8.4	2	3	/	–
22	AB	AB	59.8 ± 8.5	49.5 ± 8.8	–	1	/	/	52.5 ± 6.9	1	2	–	Ss,Sq,He
23	AD,AB	AB	54.0 ± 7.1	39.0 ± 4.1	–	1	–	–	79.0 ± 5.4	1	2	–	R,Sq
24	–	AB	61.3 ± 9.0	58.3 ± 7.0	–	1	/	/	74.5 ± 8.1	2	1	–	Ss
25	–	AD,AB	58.8 ± 5.0	65.0 ± 7.3	–	–	–	–	60.3 ± 7.4	2	2	/	R
26	–	AD,AB	69.5 ± 14.3	54.3 ± 12.1	–	1	/	/	81.3 ± 9.6	2	2	–	Ss,He

* Using the same notation as in Tables 2 and 3, AB = abaxial side, AD = adaxial side, PS = number of palisade layers, SG = number of spongy layers, / = present.

G. pendula, and *G. winitii*; arcs I and II (Fig. 5f) presented in *G. adhaerens* (violaceus bract), *G. adhaerens* (albus bract), *G. albiflora* var. *aurea*, *G. annamensis*, *G. bicolor*, *G. panicoides*, *G. praecox*, and *G. siamensis*; arcs I, II, and III (Fig. 5g) presented in *G.* aff. *winitii*, *G. laeta*, *G. marantina*, *G. racemosa*, *G. sessiliflora*, and *G. sherwoodiana*; arcs I, II, and IV (Fig. 5h) found in *G. albiflora* var. *albiflora*, *G. cambodgensis*, *G. candida*, *G. globulifera*, *G. purpurascens*, *G. reflexa*, *G. williamsiana*, and *G. xantholeuca*; and arcs I, II, III, and IV found only in *G. schomburgkii*. Tannin is present only in *G. laeta*. Rhombic, rectangle, square, hexagonal crystals, and silica sand are present in the midrib of all species of the genus *Globba*. Most of the studied species have silica sand and rhombic crystals. In *G. albiflora* var. *albiflora*, *G. panicoides*, and *G. sessiliflora* there are no crystals in the midrib.

Leaf margin

Three types of leaf margin can be recognized, based on the shape of the margin in transverse section, including taper (Fig. 5i), curved up (Fig. 5j), and curved down (Fig. 5k). The length of the leaf margin (measured from the last group of the vascular bundle to the apex leaf margins) varies from 82.0 ± 7.9 μm in *G. nuda* to 403.0 ± 8.2 μm in *G. williamsiana*.

DISCUSSION

The generalized pollen morphology of all the species in the genus *Globba* are monad, radial symmetry, apolar, and inaperturate, which is similar to that of most genera in Zingiberaceae[9–15,20]. The medium sized pollen grains are the most dominant and are found in 18 taxa of *Globba* in this study. Two pollen sizes, medium and large, are observed in this genus. The latter type has never been recorded for the genus *Globba* in previous work by Mangaly and Nayar[19], which reported that the pollen size of *G. ophioglossa* was the medium size. Hence the large pollen size is reported here for the first time in the genus. Three main types of pollen shape are recognized, oblate-spheroidal, spheroidal, and prolate-spheroidal. Most species have the spheroidal shape for their pollen. The obtained data in the present study are in agreement with Liang[17], Mangaly and Nayar[19], Syamsuardi[21], Jones and Pearce[22], who all reported that the shapes of the pollen grains in the genus *Globba* are spherical and prolate, but in this study we also found oblate-spheroidal and prolate-spheroidal shaped pollen for the *Globba*.

Table 5 Comparison of characteristics of transverse sections of midribs and leaf margins in genus *Globba*.*

Taxa	Midrib						Leaf margins	
	Shape AD	Shape AB	LU	Vascular system	Inc	Crystal	Shape	Length (μm)
1	U	U	AB	Arc I, Arc II	–	R, Rec	taper	135 ± 10
2	U	U	AB	Arc I, Arc II	–	Rec	curved up	259 ± 47
3	U	U	AB	Arc I, Arc II, Arc III	–	Rec, Ss	curved up	163 ± 17
4	U	U	–	Arc I, Arc II, Arc IV	–	–	taper	260 ± 38
5	U	U	–	Arc I, Arc II	–	R, Rec	curved down	193 ± 18
6	U	U	AB	Arc I, Arc II	–	R, Ss	curved up	165 ± 11
7	U	U	–	Arc I, Arc II	–	Ss	taper	226 ± 12
8	U	U	–	Arc I, Arc II, Arc IV	–	R	curved down	203 ± 12
9	U	U	AD, AB	Arc I, Arc II, Arc IV	–	R, Sq	curved down	195 ± 9
10	slightly flat	U	–	Arc I, Arc II, Arc IV	–	Ss	taper	291 ± 17
11	U	U	AB	Arc I, Arc II, Arc III	T	R, Ss	taper	281 ± 26
12	U	U	–	Arc I, Arc II, Arc III	–	Ss	taper	262 ± 8
13	U	U	AB	Arc I	–	Ss	taper	82 ± 8
14	U	U	AB	Arc I, Arc II	–	–	curved down	324 ± 48
15	U	U	–	Arc I	–	R	taper	280 ± 7
16	U	U	–	Arc I, Arc II	–	He	taper	203 ± 7
17	U	U	–	Arc I, Arc II, Arc IV	–	R, Rec	taper	344 ± 14
18	U	U	AB	Arc I, Arc II, Arc III	–	Rec	taper	265 ± 11
19	U	U	–	Arc I, Arc II, Arc IV	–	Ss	curved down	237 ± 12
20	U	U	AB	Arc I, Arc II, Arc III, Arc IV	–	R	curved up	188 ± 10
21	U	U	AB	Arc I, Arc II, Arc III	–	–	curved down	378 ± 11
22	U	U	AB	Arc I, Arc II, Arc III	–	Ss	curved down	323 ± 24
23	U	U	AD, AB	Arc I, Arc II	–	Rec	taper	129 ± 7
24	U	U	–	Arc I, Arc II, Arc IV	–	R, Ss	curved down	403 ± 8
25	U	U	–	Arc I	–	R, Ss	taper	360 ± 8
26	U	U	–	Arc I, Arc II, Arc IV	–	Ss	curved down	298 ± 9

* Using the same notation as in Table 4, Arc I, Arc II, Arc III, Arc IV = vascular systems, T = tannin, U = U-shape.

The P/E ratio shows extensive differences in the shape class of the pollen grains among the studied taxa. The three main types of pollen grain are exine sculpturing as detected, viz., short-echinate with psilate between the spine, short-echinate with rugulate between the spine and psilate. These results agree with Chen[18], Liang[17], Mangaly and Nayar[19], and Syamsuardi[21], who reported that the exine sculpturing of pollen grains of the genus *Globba* are short-echinate and echinate-psilate. However, Jones and Pearce[22] found that the exine sculpturing of the pollen grains of *G. propinqua* is granulate with gemmae. In this study, we found the psilate type of exine ornamentation in the genus *Globba* for the first time. Most Thai *Globba* pollen is primitive because it is short-echinate pollen, except for *G. panicoides* which is advanced because the exine sculpturing is psilate. Our study confirms the pollen of the genus *Globba* are inaperturate, which corresponds to Liang[17], Chen[18], Mangaly and Nayar[19], Syamsuardi[21], Jones and Pearce[22] who reported that the aperture of *Globba* is inaperturate. The spine apex can be divided into two groups, namely, sharp or blunt, and according to Kaewsri and Paisooksantivatana[20] the spine apexes of the genus *Amomum* have sharp or blunt apexes. In addition, the present study reports additional data, i.e., length of spine, width of spine, density of spine, distance between the spines, and wall thickness, which has never been reported in previous studies of the genus *Globba*. Furthermore, using these characters in combination with other characters, such as the internal structure of the pollen grains by transmission electron microscopy and molecular data, may help in clarifying the relationship between the closely related taxa and may help to further explain the taxonomic relationship within the genus.

In the second part of this study, detailed leaf anatomical features of Thai *Globba* were examined. The generalized anatomical characteristics of all species of *Globba* are as follows: epidermal cells on both surfaces are four to seven-sided, stomata on both surfaces are tetracytic, stomata develop on both surfaces but are more frequent on abaxial surface, subsidiary cells are rounded dome-shaped on both surfaces, epidermal cells on both surfaces in transverse section of lamina are rectangle to polygonal and anticlinal walls of adaxial and abaxial cells are straight, thin cuticle, palisade and spongy cells composed of 1–3 layers, vascular bundles are collateral consisting of 1–2 metaxylem and a few protoxylem and bundle sheath extension is found in both surfaces. Nevertheless, there are some characters that may be useful for species identification. Tomlinson[23,24] and Lakoet[26] reported the anatomical characters of some species belonging

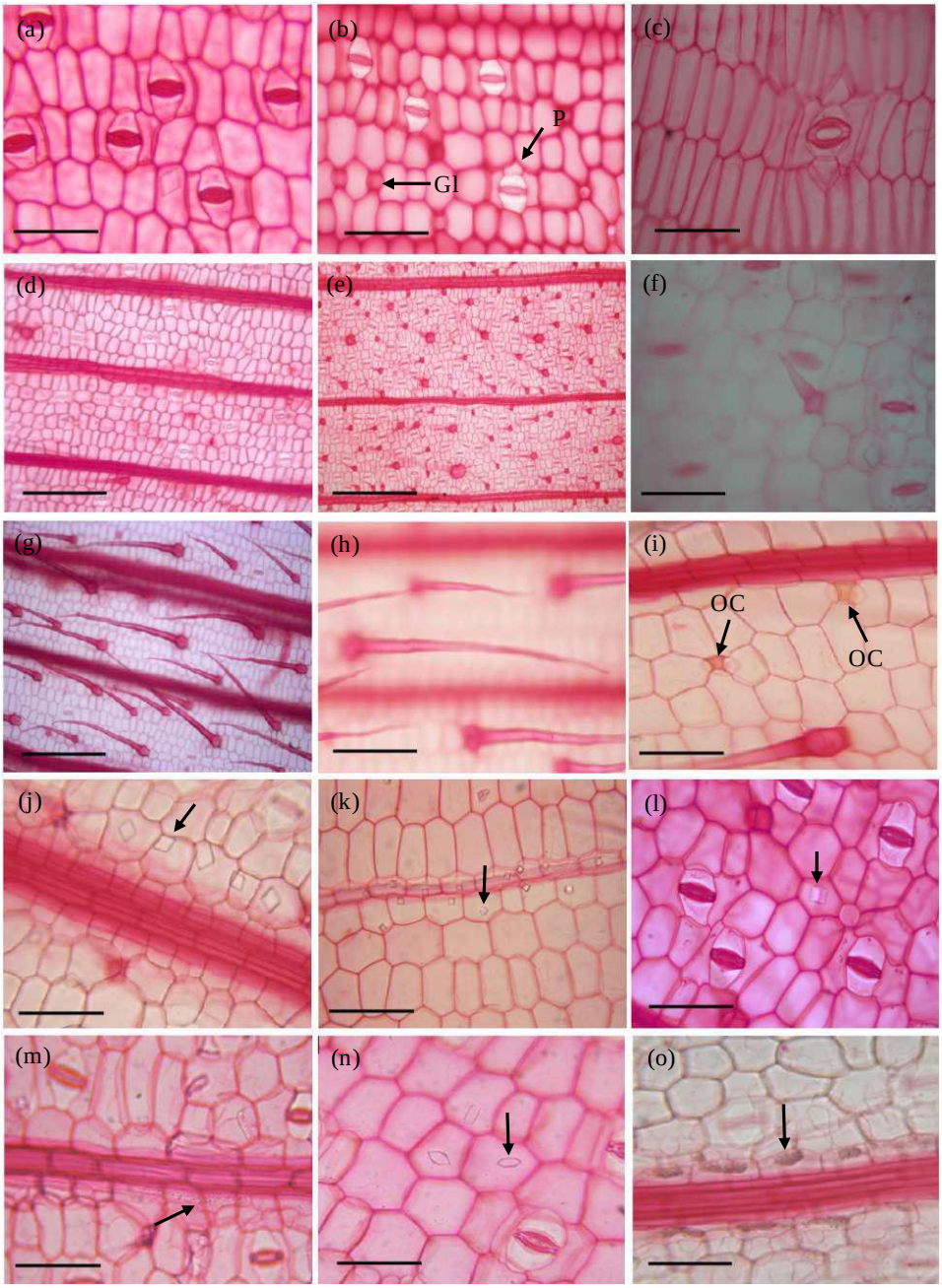

Fig. 4 Leaf epidermal anatomy of the genus *Globba*. (a) Tetracytic stomata on abaxial surface of *G. marantina*. (b) Pentacytic stomata and glandular trichome on abaxial surface of *G. praecox* (arrow). (c) Hexacytic stomata on adaxial surface of *G. racemosa*. (d) Number of rows in intercostal regions on adaxial surface of *G. marantina* as 6 rows. (e) Number of rows in intercostal regions on abaxial surface of *G. annamensis* as 12 rows. (f) Short, simple unicellular trichomes on abaxial surface of *G. bicolor*. (g) Long, simple unicellular trichomes on adaxial surface of *G.* aff. *winitii*. (h) Long, biseriate trichomes on adaxial surface of *G. adhaerens* (albus bract). (i) Oil cell on adaxial surface of *G. adhaerens* (albus bract) (arrow). (j) Rhombic crystal on abaxial surface of *G. marantina* (arrow). (k) Square crystal on adaxial surface of *G. nuda* (arrow). (l) Rectangle crystal on abaxial surface of *G. panicoides* (arrow). (m) Silica sand on abaxial surface of *G. cambodgensis* (arrow). (n) Hexagonal crystal on abaxial surface of *G. albiflora* var. *albiflora* (arrow). (o) Silica bodies on abaxial surface of *G. globulifera* (arrow). (a, b, c, f–o) scale bars = 50 μm; (d) scale bar = 100 μm; and (e) scale bar = 200 μm. Gl = Glandular trichome, OC = Oil cell, P = Pentacytic stomata.

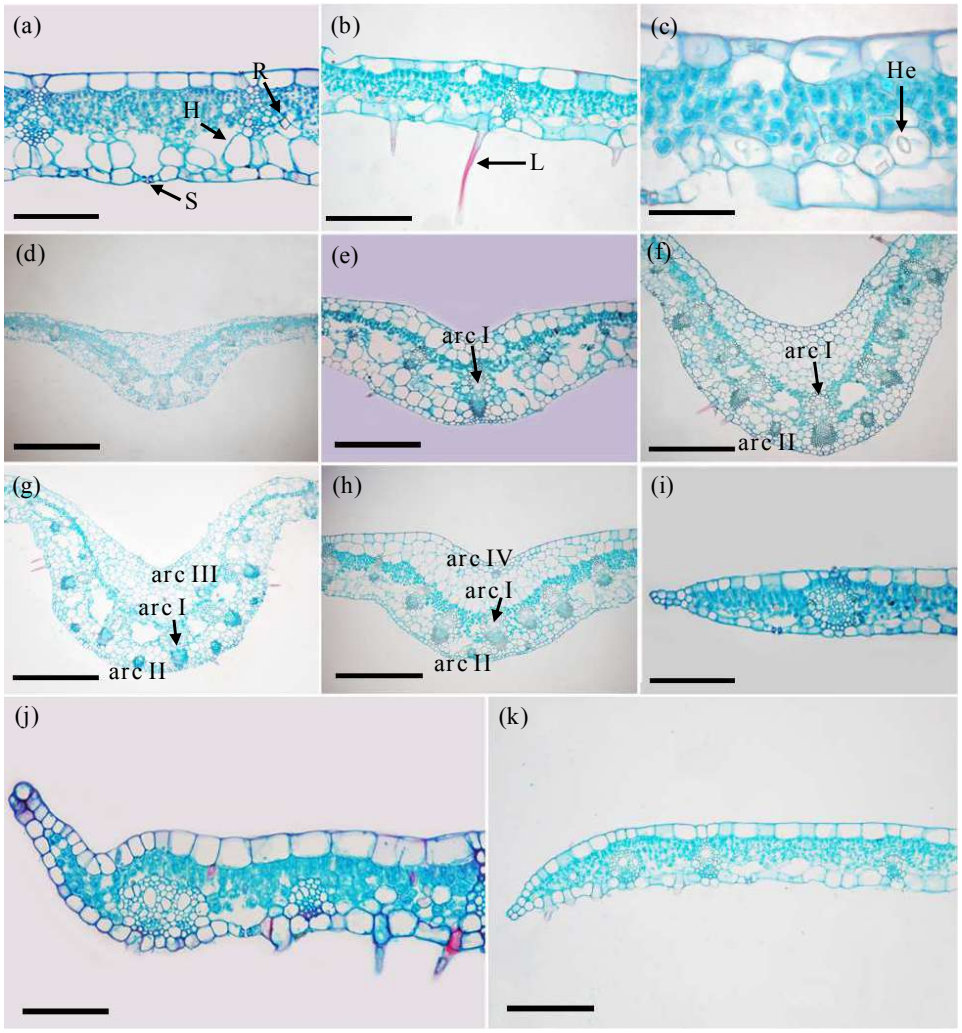

Fig. 5 Transverse sections of (a–c) leaf blades, (d–h) midribs, and (i–k) leaf margins. (a) Stomata, hypodermis, and rhombic crystal in *G. schomburgkii* (arrow). (b) Long, simple unicellular trichomes in *G. candida* (arrow). (c) Hexagonal crystal in *G. xantholeuca* (arrow). (d) Shape of midrib on the adaxial surface is slightly flat in *G. globulifera*. (e) Vascular system arc I in *G. pendula* (arrow). (f) Vascular system arcs I and II in *G. adhaerens* (albus bract) (arrow). (g) Vascular system arcs I, II, and III in *G. racemosa*. (h) Vascular system arcs I, II, and IV in *G. reflexa*. (i) Leaf margins taper in *G. marantina*. (j) Leaf margins curved up in *G. annamensis*. (k) Leaf margins curved down in *G. candida*. (a, e–g, h–k) scale bars = 200 μm; (b) and (d) scale bars = 250 μm; and (c) scale bar = 100 μm. H = Hypodermis, He = Hexagonal crystal, LU = Long, simple unicellular trichomes, R = Rhombic crystal, ST = Stomata, arc I, arc II, arc III, and arc IV = Vascular system.

to this genus (i.e., *G. bulbifera*, *G. winitii*, *G. annamensis*, *G. laeta*, *G. marantina*, and *G. panicoides*), and our results are in accordance with those studies. The epidermal cells on both surfaces of all species are four to seven-sided and the length is more than twice the width, which are consistent with Tomlinson[23,24] and Lakoet[26]. In this study, we did not find cuticle ornamentation on both surfaces, which is different from the study of Lakoet[26] that reported the cuticle ornamentation was streaked and present on both surfaces in *G. laeta*, *G. marantina*, *G. panicoides*, and *G. winitii*. The stomata develop on both surfaces, but only infrequently on the adaxial surface. The stomata of most species are tetracytic, which corresponds to the reports by Tomlinson[23,24] and Lakoet[26], while pentacytic is found in *G. adhaerens* (albus bract), *G. albiflora* var. *albiflora*, *G. annamensis*, *G. bicolor*, *G. cambodgensis*, *G. laeta*, *G. marantina*, *G. nuda*, *G. pendula*, *G. praecox*, *G. purpurascens*, *G. race-*

mosa, *G. sessiliflora*, *G. sherwoodiana*, *G. siamensis*, *G. williamsiana*, and *G. winitii* and hexacytic stomata are found in *G. praecox* and *G. racemosa*, which Tomlinson[23,24] and Lakoet[26] did not report. Stomatal densities in *G. annamensis* (10 ± 3 per mm^2), *G. laeta* (19 ± 3 per mm^2), *G. marantina* (67 ± 4 per mm^2), *G. panicoides* (12 ± 2 per mm^2), and *G. winitii* (8 ± 3 per mm^2), on the adaxial surface, differed from Lakoet[26], who reported the stomata densities found in *G. annamensis* (4.0 ± 1.6 per mm^2), *G. laeta* (25.3 ± 7.9 per mm^2), *G. marantina* (4.6 ± 1.3 per mm^2), *G. panicoides* (50.7 ± 4.7 per mm^2), and *G. winitii* (11.9 ± 5.7 per mm^2) on the adaxial surface. The other characteristic of the leaf epidermis was trichomes that are found to be present on both surfaces, except in *G. albiflora* var. *albiflora*, *G. albiflora* var. *aurea*, *G. globulifera*, and *G. nuda*, in which trichomes are not found on both surfaces. The types of trichomes can be divided into four groups as follows: short, simple unicellular trichomes; long, simple unicellular trichomes; glandular trichomes; and long, biseriate trichomes. The latter type has never been reported for the genus *Globba* in the previous work by Tomlinson[23,24] and Lakoet[26]. Hence long, biseriate trichomes are recorded for the first time in the genus. Most taxa have cell inclusions, such as oil cell, rhombic, square, and rectangle crystals and silica bodies, which are found on both surfaces, whereas silica sand and hexagonal crystal are found only on the abaxial surface. This result differs from Tomlinson[23,24] and Lakoet[26] as they did not find cell inclusions in epidermal cells on both surfaces. Hypodermis in *Globba* is present on the abaxial surface with only one row, in agreement with the studies of Tomlinson[23,24] and Lakoet[26]. Rhombic and hexagonal crystals are present in the hypodermis, which is reported here for the first time in the genus. The mesophyll has a separation that is clear between the palisade and spongy regions; all species have bifacial leaves. Most of the studied species have rhombic crystals in the mesophyll. The presence of crystals in the mesophyll in *Globba* is consistent with Tomlinson[23,24], but differs from Lakoet[26] who did not find crystals in the mesophyll. The vascular bundles are collateral and appear in a single series across the leaf blade. Rhombic, rectangle, square, and hexagonal crystals and silica sand are present on the midrib, which differs from Tomlinson[23,24] and Lakoet[26] as they did not find rhombic, rectangle, square, and hexagonal crystals and silica sand to be present on the midrib. The shape of the leaf margin in the transverse section can be divided into three groups as follows: taper, curved up, and curved down. The latter type has never been reported for the genus *Globba* in the previous work by Tomlinson[23,24] and Lakoet[26]. Hence the shape of leaf margin as being curved up is recorded for the first time in the genus. The outline of the midrib in the transverse section on both surfaces curves to a U-shape, which is consistent with the work of Lakoet[26], while the adaxial surface curves to be slightly flat in *G. globulifera*, which differs from Lakoet[26]. The shape of midrib on adaxial surface of some species are V-wider shaped[26], which differs from the results of this study. Most of the species have long, simple unicellular trichomes on the abaxial surface of the midrib except for *G. candida* and *G. siamensis*, which have long, simple unicellular trichomes on both surfaces of the midrib. The long, simple unicellular trichomes are reported here for the first time in the genus. Based on the types of vascular system in the midrib proposed by Tomlinson[23], the vascular system of the genus *Globba* is composed of arcs I, II, III, and IV. The latter type has never been reported for the genus *Globba* in the previous work by Lakoet[26]. The result of the present investigation shows that leaf anatomical characters could be employed to distinguish between species of *Globba*. In the present study the leaf anatomical features of 21 taxa of Thai *Globba* are recorded for the first time.

CONCLUSIONS

Our findings show that the size, shape, exine sculpturing, and types of spine apex of 22 taxa of Thai *Globba* can be used for grouping in the genus. The pollen characters of all taxa are quite similar in all aspects; hence pollen morphology has low taxonomic value to identify *Globba* taxa. The only qualitative character that can be used to separate *G. panicoides* from other taxa is the exine sculpture. Hence using the pollen characters alone does not help in resolving the classification of the genus as they cannot be used to identify all species, but they provide some information for distinguishing some taxa. Leaf anatomical studies have been used successfully to clarify taxonomic status and help in the identification of taxa. The leaf anatomy of 26 taxa of *Globba* species showed differences in the types of stomata, density of stomata on the abaxial surface, width of subsidiary cell on abaxial surface, types of trichomes, cell inclusion presence or absence in mesophyll, midrib and abaxial surface, number of rows in the intercostal region on abaxial surface, length of guard cell on abaxial surface, hypodermis presence or absence on the abaxial surface of

1. Hypodermis present on abaxial surface in transverse section of leaf blade
   2. Density of stomata on abaxial surface more than 200 per mm^2
      3. Number of rows in intercostal regions on abaxial surface varies from 10–12 rows
         4. Long, biseriate trichomes absent on abaxial surface — *G. sherwoodiana*
         4. Long, biseriate trichomes present on abaxial surface
            5. Rhombic and hexagonal crystals present on hypodermis — *G.* aff. *winitii*
            5. Rhombic and hexagonal crystals absent on hypodermis — *G. siamensis*
      3. Number of rows in intercostal regions on abaxial surface varies from 6–9 rows
         6. Tetracytic stomata present only on adaxial surface
            7. Long, simple unicellular trichomes present on abaxial surface in transverse section of leaf blade — *G. schomburgkii*
            7. Long, simple unicellular trichomes absent on abaxial surface in transverse section of leaf blade — *G. xantholeuca*
         6. Tetracytic and pentacytic stomata present on adaxial surface
            8. Square crystals present on adaxial surface — *G. nuda*
            8. Square crystals absent on adaxial surface
               9. Long and short, simple unicellular trichomes present on adaxial surface — *G. marantina*
               9. Long and short, simple unicellular trichomes absent on adaxial surface — *G. cambodgensis*
   2. Density of stomata on abaxial surface less than 200 per mm^2
      10. Long, simple unicellular trichomes present on midrib
         11. Width of mesophyll more than 90 — *G. adhaerens* (albus bract)
         11. Width of mesophyll less than 90 µm
            12. Rhombic and hexagonal crystals present on mesophyll — *G. racemosa*
            12. Rhombic and hexagonal crystals absent on mesophyll — *G. laeta*
      10. Long, simple unicellular trichomes absent on midrib
         13. Length of guard cell on abaxial surface more than 30 µm
            14. Shape of midrib on adaxial surface curved to slightly flat — *G. globulifera*
            14. Shape of midrib on adaxial surface curved to U-shaped
               15. Length of leaf margin more than 300 — *G. williamsiana*
               15. Length of leaf margin less than 300 µm
                  16. Silica sand present on midrib — *G. bicolor*
                  16. Silica sand absent on midrib — *G. pendula*
         13. Length of guard cell on abaxial surface less than 30 µm
            17. Hexacytic stomata present on abaxial surface — *G. preacox*
            17. Hexacytic stomata absent on abaxial surface — *G. purpurascens*

1. Hypodermis absent on abaxial surface in transverse section of leaf blade
   18. Leaf margin taper
      19. Rhombic crystals present on adaxial surface — *G. albiflora* var. *albiflora*
      19. Rhombic crystals absent on adaxial surface — *G. adhaerens* (violaceus bract)
   18. Leaf margin curved
      20. Leaf margin curved up — *G. annamensis*
      20. Leaf margin curved down
         21. Width of subsidiary cell on abaxial surface longer than adaxial surface — *G. candida*
         21. Width of subsidiary cell on abaxial surface shorter than adaxial surface
            22. Glandular trichomes present on abaxial surface
               23. Short, simple unicellular trichomes present on adaxial surface — *G. winitii*
               23. Short, simple unicellular trichomes absent on adaxial surface — *G. reflexa*
            22. Glandular trichomes absent on abaxial surface
               24. Vascular bundle type arc I, arc II and arc III — *G. sesiliflora*
               24. Vascular bundle type arc I and arc II
                  25. Long, simple unicellular trichomes present on midrib — *G. panicoides*
                  25. Long, simple unicellular trichomes absent on midrib — *G. albiflora* var. *aurea*

Fig. 6 Identification key based on leaf anatomy features for taxa studied.

the lamina, width of the mesophyll layer, shape of midrib, types of vascular system, and shape of leaf margins. Hence a key to the taxa based on leaf anatomy was constructed (Fig. 6).

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REFERENCES

1. Williams KJ, Kress WJ, Manos PS (2004) The phylogeny, evolution, and classification of the genus *Globba* and tribe Globbeae (Zingiberaceae): appendages do matter. *Am J Bot* **91**, 100–14.
2. Larsen K, Larsen SS (2006) *Gingers of Thailand*, Queen Sirikit Botanic Garden, Chiang Mai.
3. Takano A, Okada H (2003) Taxonomy of *Globba* (Zingiberaceae) in Sumatra, Indonesia. *Syst Bot* **28**, 524–46.
4. Schumann K (1904) Zingiberaceae. In: Engler A (ed) *Das Pflanzenreich*, IV. 46, Leipzig, W Engelmann, pp 1–458.
5. Larsen K (1996) A Preliminary checklist of the Zingiberaceae of Thailand. *Thai Forest Bull (Bot)* **24**, 35–49.
6. Kress WJ, DeFilipps RA, Farr E, Kyi DYY (2003) *A Checklist of the Trees, Shrubs, Herbs, and Climbers of Myanmar*, Contributions from the US National Herbarium, Smithsonian Institution Press, DC.
7. Srisanga P, Wongpakam S, Kamkuan W, Pekthong T, Tovaranonte J, Yaso T, Nontachyapoom S (2011) Ethnobotany of Akha in Huay Yuak Pa So village, Mae Fah Luang district and Ban Mai Patthana village, Mae Suai district, Chaing Rai province. *Thai J Bot* **3**, 93–114.
8. Saensouk S, Saensouk P, Pasorn P, Chantaranothaic P (2016) Diversity and uses of Zingiberaceae in Nam Nao National Park, Chaiyaphum and Phetchabun provinces, Thailand, with a new record for Thailand. *Agr Nat Resour* **50**, 445–53.
9. Nairs PKK (1970) *Pollen Morphology of Angiosperms: A Historical and Phylogenetic Study*, Barnes & Noble, New York.
10. Larsen K, Lock JM, Maas H, Maas PJM (1998) Zingiberaceae. In: Kubitzki K (ed) *The Families and Genera of Vascular Plants*, Springer-Verlag, Berlin, pp 474–95.
11. Saensouk S (2006) Taxonomy and biology of the genus Alpinia Robx. (Zingiberaceae) in Thailand. PhD thesis, Khon Kaen Univ.
12. Triboun P (2006) Biogeography and biodiversity of the genus Zingiber in Thailand. PhD thesis, Khon Kaen Univ.
13. Saensouk P, Chahtaranothai P, Theerakulpisut P (2009) Pollen morphology of the genus *Cornukaempferia* (Zingiberaceae) in Thailand. *J Systemat Evol* **47**, 139–43.
14. Chen J, Xia NH (2011) Pollen morphology of Chinese *Curcuma* L. and *Boesenbergia* Kuntz (Zingiberaceae): Taxonomic implications. *Flora* **206**, 458–67.
15. Saensouk P, Theerakulpisut P, Thammathaworn A, Saensouk S, Maknoi C, Kohkaew P (2015) Pollen morphology of the genus *Curcuma* (Zingiberaceae) in Northeastern Thailand. *Sci Asia* **41**, 87–92.
16. Theilade I, Maersk-Moller ML, Theilade J, Larsen K (1993) Pollen morphology and structure of *Zingiber* (Zingiberaceae). *Grana* **32**, 338–42.
17. Liang YH (1988) Pollen morphology of the family Zingiberaceae in China-pollen types and their significance in the taxonomy. *Acta Phytotax Sin* **26**, 265–86 [in Chinese].
18. Chen ZY (1989) Evolutionary patterns in cytology and pollen structure of Asian Zingiberaceae. In: Holm-Nielsen LB, Nielsen IC, Balslev H (eds) *Tropical Forests: Botanical Dynamics, Speciation & Diversity*, Academic Press, pp 185–91.
19. Mangaly JK, Nayar J (1990) Palynology of South Indian Zingiberaceae. *Bot J Linn Soc* **103**, 351–66.
20. Kaewsri W, Paisooksantivatana Y (2007) Morphology and palynology of *Amomum* Roxb. in Thailand. *Gard Bull Singapore* **59**, 105–12.
21. Syamsuardi S, Mansyurdin M, Nurainas N, Susanti T (2010) Variasi morfologi polen genus *Globba* (Zingiberaceae) di Sumatra Barat. *Berk Penel Hayati* **16**, 89–94 [in Indonesian].
22. Jones SE, Pearce KG (2015) A pollen morphology study from the Kelabit Highlands of Sarawak, Malaysian Borneo. *Palynology* **39**, 150–204.
23. Tomlinson PB (1956) Studies in the systematic anatomy of the Zingiberaceae. *Bot J Linn Soc* **55**, 547–92.
24. Tomlinson PB (1969) *Anatomy of the Monocotyledons III Commelinales-Zingiberaceae*, Clarendon Press, Oxford.
25. Hussin KH, Seng TC, Ibrahim H, Gen QW, Ping JL, Nian L (2000) Comparative leaf anatomy of *Alpinia* Roxb. species (Zingiberaceae) from Thailand. *Bot J Linn Soc* **133**, 161–80.
26. Lakoet C (2004) Comparative anatomy of Zingiberaceae in Phu Phan National Park. MSc thesis, Khon Kaen Univ.
27. Stuessy TF (1990) *Plant Taxonomy: the Systematic Evaluation of Comparative Data*, Columbia Univ Press, New York.
28. Troia A, Raimondo FM, Castellano G, Spadaro V (2012) Morphological, karyological and taxonomic remarks on *Ferulago nodosa* (L.) Boiss. (Apiaceae). *Plant Biosyst* **146**, 330–7.
29. Ceter T, Ekici M, Pinar NM, Ozbek F (2013) Pollen morphology of *Astragalus* L. section *Hololeuce* Bunge (Fabaceae) in Turkey. *Acta Bot Gallica* **160**, 43–52.
30. Erdtman G (1969) *Pollen Morphology and Plant Taxonomy: Angiosperms (an Introduction to Palynology I)*, Heffner Publishing, London.
31. Punt W, Hoen PP, Blackmore S, Nilsson S, Le Thomas A (2007) Glossary of pollen and spore terminology. *Rev Palaeobot Palynol* **143**, 1–81.
32. Thammathaworn A (1995) *Handbook for Permanent Slides of Plant Tissue by Paraffin Method*, Department of Biology, Faculty of Science, Khon Kaen Univ [in Thai].