



## Article

# *Kaempferia sakolchaii* sp. nov. and *K. phuphanensis* var. *viridans* var. nov. (Zingiberaceae), Two New Taxa from Northeastern Thailand

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**Abstract:** An examination of the diversity of Zingiberaceae in Thailand led to the discovery of two undescribed taxa within the genus *Kaempferia*: *K. sakolchaii* P. Saensouk, Saensouk & Boonma, a newly described species, and *K. phuphanensis* var. *viridans* P. Saensouk, Saensouk & Boonma, a novel variety. Additionally, our investigation encompassed the chromosome numbers, karyotypes, and pollen morphological characteristics of these taxa. Both taxa share a chromosome number of  $2n = 22$ , accompanied by distinctive karyotype patterns. *Kaempferia sakolchaii* displays a karyotype of  $12m + 10sm$  chromosomes, while *K. phuphanensis* var. *viridans* presents a karyotype of  $10m + 10sm + 2st$  chromosomes. The pollen grains of *K. sakolchaii* and *K. phuphanensis* var. *viridans* are monads, spheroidal in shape, radially symmetrical, apolar, inaperturate, and exine sculpturing irregularly arranged elongated ornamentation elements with verrucate features. This comprehensive analysis provides valuable insights into the taxonomic classification of these two taxa, enriching our understanding of Zingiberaceae diversity in Thailand.

**Keywords:** *Kaempferia*; new species; new variety; Sakon Nakhon; taxonomy; Thailand; Zingiberaceae



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## 1. Introduction

*Kaempferia* L. is a genus of terrestrial rhizomatous herbaceous plants within the Zingiberaceae family [1,2]. It encompasses species distributed primarily in tropical and subtropical regions, from India and the Philippines to China and Indonesia [2]. First described by Carl Linnaeus in 1753 [3], this genus is of significant botanical interest due to its diverse morphology and ecological adaptations, with 64 accepted species identified to date [2]. *Kaempferia* represents a notable component of Zingiberaceae, a family renowned for its economic, medicinal, and ornamental significance [4–23].

In particular, Thailand stands out as a hotspot for *Kaempferia* diversity, boasting approximately 46 native species distributed throughout the country, among which, 34 species are endemic [1,7,9,24–37]. This rich species concentration underscores Thailand's importance in the study and for the conservation of *Kaempferia*. Despite extensive research, our understanding of *Kaempferia*'s taxonomic diversity remains incomplete. Subtle morphological

variations have led to challenges in accurately identifying and classifying species within the genus. Moreover, the discovery of new taxa continues to expand our knowledge and challenge existing taxonomic frameworks.

During an exploration of Zingiberaceae diversity in northeastern Thailand, two previously undescribed taxa belonging to the *Kaempferia* subgenus, characterized by their production of terminal inflorescences, were encountered in Sakon Nakhon Province. These taxa exhibit morphological features distinct from those of known species within the genus. The first taxon shares similarities with *K. siamensis* Sirirugsa [9], while the second taxon shows similarities with *K. phuphanensis* Saensouk & P. Saensouk [30]; however, both lack precise matches with any known species. This discrepancy prompted a detailed taxonomic treatment and comparative analysis of all known *Kaempferia* species, revealing the need for further investigation to accurately ascertain their taxonomic statuses. In addition to taxonomic treatment, this study conducts cytological and palynomorphological analyses of both taxa. These analyses will contribute to clarifying the taxonomic positions of the taxa and provide insights into their evolutionary relationships, genetic diversity, and reproductive biology. Such comprehensive investigations are crucial for advancing our understanding of *Kaempferia* diversity and informing conservation efforts in the face of environmental changes.

This work mainly aims to taxonomically characterize and investigate the newly discovered taxa of *Kaempferia* from Sakon Nakhon Province, Thailand.

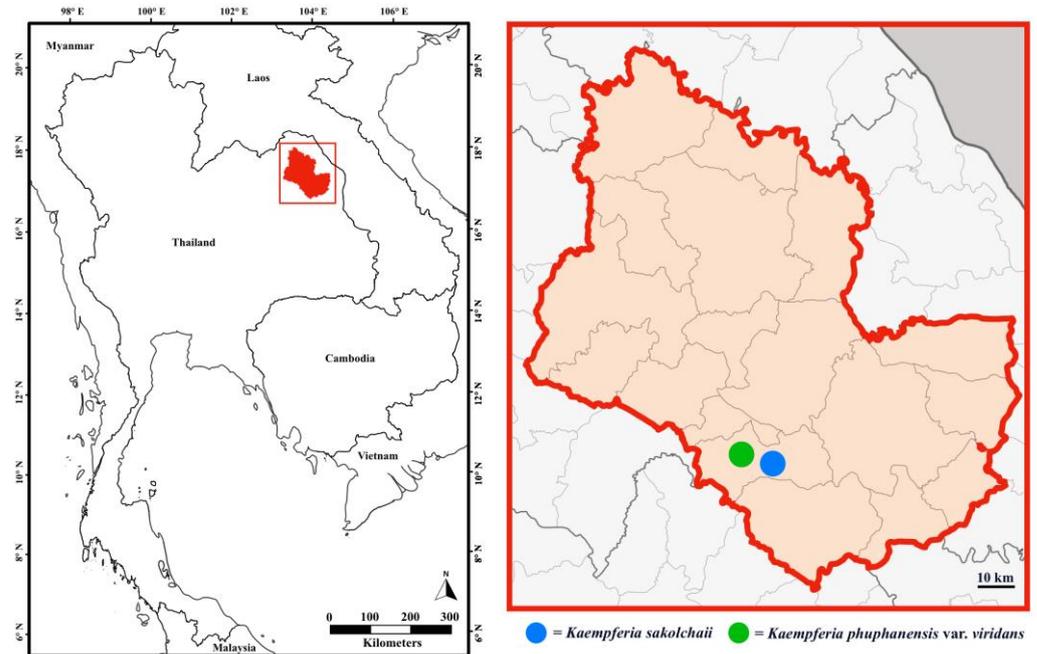
## 2. Materials and Methods

### 2.1. Experimental Materials and Experimental Site

Between 2020 and 2023, plant specimens of the two taxa were obtained from their natural habitat in Sakon Nakhon Province, northeastern Thailand (Figure 1). Both living specimens and specimens preserved in 70% ethyl alcohol were meticulously examined and measured using stereo microscopy. Photographic documentation was carried out for the living specimens in their natural habitat and under cultivation. Holotype specimens were deposited at Khon Kaen University Herbarium (KKU) and isotypes were deposited at Bangkok Herbarium (BK) and The Herbarium of Faculty of Forestry (FOF), while living specimens were housed at the Diversity of Family Zingiberaceae and Vascular Plant for Its Applications Research Unit's Garden in Maha Sarakham Province, Thailand. Detailed descriptions and relevant data for all taxa within the *Kaempferia* genus were systematically compared. Data on their utilization were acquired by local inhabitants near each type's locality. A preliminary conservation assessment was conducted following the IUCN Red List Categories [38]. Elevation data were gathered using the View Ranger application, and line drawings were produced using digital tools on an iPad Air 5 (version 17.2, 2022, Apple Inc., Cupertino, CA, USA) with the Pixelmator Pro Program (version 3.5.6 (Flare), 2023, Pixelmator Team, Vilnius, Lithuania).

### 2.2. Taxonomic Parameters Analysis

A caryological investigation was performed on root tips that underwent pretreatment with 2 mM 8-hydroxyquinoline for 8 h at 4 °C, followed by fixation in ethanol–acetic acid (3:1, *v:v*) for 30 min at room temperature. Subsequently, they were either stored at 4 °C or used immediately. After being washed in distilled water, the samples were hydrolyzed in 1 M HCl for 5 min at 60 °C, followed by another round of washing in distilled water for 5 min. Finally, the root tips were stained with 2% aceto-orcein, squashed, and observed under a microscope. For the chromosome morphology nomenclature, we followed Levan et al. [39]. For the karyotype symmetry degree classification, we adhered to the system established by Stebbins [40]. Diploid chromosome counts for each taxon were determined based on an examination of 20 cells. The karyotype arrangement parameters were derived from metaphase chromosomes, as described by Saenprom et al. [41].



**Figure 1.** Distribution map of *Kaempferia sakolchaii* and *K. phuphanensis* var. *viridans*.

We examined the pollen from the two taxa following Erdtman's method [42]; the samples were dehydrated using an ethanol series of 70%, 80%, 95%, and 100%, with each step lasting for 5 min. Subsequently, the dehydrated pollen was air-dried overnight at room temperature and then mounted onto aluminum panels affixed to stubs using carbon tape; then, the pollen grains were analyzed using a scanning electron microscope (SEM) (Hitachi, TM-4000plus, Hitachi High-Tech, Tokyo, Japan) at Mahasarakham University's Laboratory Equipment Center, Division of Research Facilitation and Dissemination. The details of 20 pollen grains observed under SEM provided insights into the pollen morphology. We adopted Erdtman's pollen shape classification and terminology [42].

### 3. Results

Our taxonomic exploration of Zingiberaceae plants in Thailand revealed interesting additions to the *Kaempferia* genus, including a newly described species and a newly described variety, which are endemic to northeastern Thailand. We list the details of these below.

#### 3.1. *Kaempferia sakolchaii* P. Saensouk, Saensouk & Boonma sp. Nov. (Figures 2 and 3), Subgenus *Kaempferia*

*Kaempferia sakolchaii* is similar to *K. siamensis*, but differs in that it has lamina that are narrowly ovate to broadly ovate, adaxially dark green alternated with silver or white longitudinal stripes, with small black dots along the margin, and abaxially pale green with small black dots (vs. *K. siamensis* having lamina sub-orbicular to orbicular, adaxially plain green, with small black dots only at the distal part, abaxially pale green without small black dots); anther crest c.  $6 \times 3$  mm, bilobed, each lobe apex with four sub-lobes (vs. anther crest c.  $5 \times 2.5$  mm, bilobed, each lobe apex acute); ovary c.  $3 \times 2$  mm (vs. c.  $4 \times 2$ ); and epigynous glands c. 3 mm long (vs. 6 mm long).

Type: Thailand, Northeastern, Sakon Nakhon Province, Saensouk *Kaemp.* 2, 12 July 2020 (holo KKU!, iso BK!, FOF!)

Perennial herb, pseudostem erect, short, 3–4 cm tall. Rhizomes ovoid to subglobose, sympodial, brown externally and yellowish internally, bearing storage roots. Bladeless sheaths 2, 2–4.5 cm long, green with reddish longitudinal tinge, glabrous. One leaf; leaf sheaths up to 4 cm long, green with reddish longitudinal tinge; petiole sessile; ligule inconspicuous; lamina narrowly ovate to broadly ovate, horizontal near the ground, 8–15 × 5–10 cm, apex acute, short mucronate, base attenuate to rounded, adaxially dark green alternated with silver or white longitudinal stripes, margin with small black dots, abaxially pale green with small black dots, glabrous on both surfaces. Inflorescence terminal, blooming one flower daily and lasting a single day; peduncle sessile. Bracts a triangular to lanceolate, with a slight ridge on the outer side, c. 1.5 × 0.3 cm, apex acute, green with reddish longitudinal patches, pubescent. Bracteoles oblong, c. 1.5 × 0.15 cm, glabrous. Flowers white, c. 10 in number, exerted from bracts, staminodes nearly perpendicular to the labellum, not lying in the same plane. Calyx tubular, c. 3 cm long, glabrous. Floral tube 3–3.5 cm long, white with reddish tinge, glabrous; dorsal corolla lobe oblong, 2–2.3 × 0.3 cm, apex acuminate, hooded, white or white with reddish longitudinal tinge, glabrous; lateral corolla lobes oblong, c. 2 × 0.3 cm, apex acuminate, white or white with reddish longitudinal tinge, glabrous. Labellum obovate, c. 2 × 1–1.3 cm, deeply bilobed to half of the labellum length, each lobe obovate, c. 5–7 mm wide, lobes slightly overlapping each other, purple with dark purple patches near sinus and white at base, each lobe apex rounded, margin slightly undulate. Lateral staminodes narrowly obovate, c. 2 × 0.5 cm, white, apex rounded, margin entire. One stamen; filament c. 2 mm, glabrous. Anther c. 3 mm long, c. 2 mm wide, white; anther crest c. 6 × 3 mm, bilobed, with an incision c. 4 mm long, each lobe apex with 4 sub-lobes, white with reddish tinge, glabrous. Ovary c. 3 × 2 mm, glabrous, with reddish longitudinal tinge, glabrous; placentation axile, ovules many; two epigynous glands, c. 3 mm long. Fruit obovoid, 10–15 × 6–7 mm, glabrous, pale green to white at base. Seed ovoid, 4–5 × c. 2 mm, with white arils.

Vernacular name: Proh Ajarn Sumon.

Etymology: The specific epithet "*sakolchai*" was bestowed in honor of Professor Emeritus Dr. Sumon Sakolchai, who has a high level of knowledge and skill in the field of pharmacy. Furthermore, he has made significant contributions to the field of pharmaceutical botany, both in Thailand and across the world. In addition, he makes essential contributions as a behind-the-scenes person, providing consistent assistance to researchers in botany and pharmaceutical sciences. Previously, he was the President of Khon Kaen University in Thailand, the President of the Pharmacy Council of Thailand, and currently acts as the President of Burapha University Council in Thailand.

Distribution: Endemic to Thailand; northeastern, Sakon Nakhon Province (Figure 1).

Ecology: Found in sandy loam soil mixed with rocks, in dipterocarp and deciduous forests, at an altitude ranging from 300 to 400 m above sea level.

Phenology: Flowering from late May to July; anthesis in the morning.

Utilization: This beautiful plant is utilized for ornamental purposes.

Conservation status: This newly discovered species is located exclusively in its type locality, with limited information available regarding its distribution range. The species potentially exists in nearby unexplored areas. Presently classified as Data Deficient (DD) following the IUCN guidelines of 2022 [38], we advise considering it as an endangered taxon unless additional evidence indicates otherwise.

Key to species of solitary leaf *Kaempferia* spp.

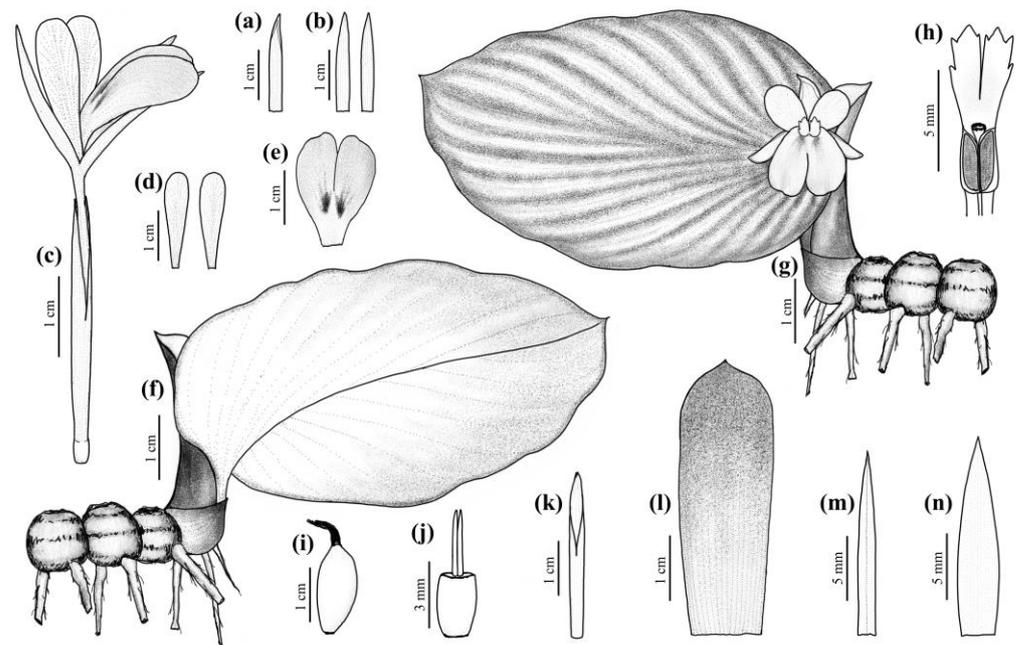
1a. Shoot usually with a solitary leaf, both at anthesis and after anthesis	2
1b. Shoot sometimes with a solitary leaf at anthesis, two or more leaves after anthesis	8
2a. Labellum and staminodes in the same plane	<i>K. picheansoonthonii</i>
2b. Labellum and staminodes not in the same plane	3
3a. Anther crest and ovary white without reddish dots	4
3b. Anther crest and ovary white with reddish dots	7
4a. Lamina adaxially with light grey markings	<i>K. pseudoparviflora</i>
4b. Lamina adaxially without markings	5
5a. Epigynous glands 4 mm long	<i>K. unifolia</i>
5b. Epigynous glands 8 mm long	6
6a. Anther crest bilobed, each lobe apex acute	<i>K. isanensis</i>
6b. Anther crest bilobed, each lobe apex rounded	<i>K. gigantiphylla</i>
7a. Lamina adaxially plain dark green	<i>K. siamensis</i>
7b. Lamina adaxially dark green with silver or white longitudinal stripes	<i>K. sakolchaiti</i>
8a. Rhizome dark purple; labellum and staminodes in the same plane; stigma white	<i>K. parviflora</i>
8b. Rhizome yellowish; labellum and staminodes not in the same plane; stigma red	<i>K. elegans</i>



**Figure 2.** *Kaempferia sakolchaiti* P. Saensouk, Saensouk & Boonma sp. nov.: (a) habit and (b) flower. Photographs by Surapon Saensouk.

### 3.1.1. Chromosomes and Karyotype Formula of *Kaempferia sakolchaiti*

*Kaempferia sakolchaiti* has  $2n = 22$  somatic chromosomes and the Fundamental Number (NF) = 44 (Figure 4a, Table 1). The karyotype has six pairs of metacentric chromosomes and five pairs of submetacentric chromosomes. The karyotype formula of  $12m + 10sm$  chromosomes places it in the symmetrical karyotype group (Figure 4b). The short arm length is from  $0.88 \pm 0.06$  to  $1.78 \pm 0.09 \mu\text{m}$ , the long arm length is from  $1.43 \pm 0.09$  to  $2.77 \pm 0.64 \mu\text{m}$ , and the total chromosomal length is from  $2.44 \pm 0.15$  to  $4.46 \pm 0.73 \mu\text{m}$ . The relative length (RL) ranges from 6.53 to 11.96%, while the centromeric index (CI) ranges from 0.51 to 0.64, as shown in Table 1. Figure 4d shows the idiogram created.



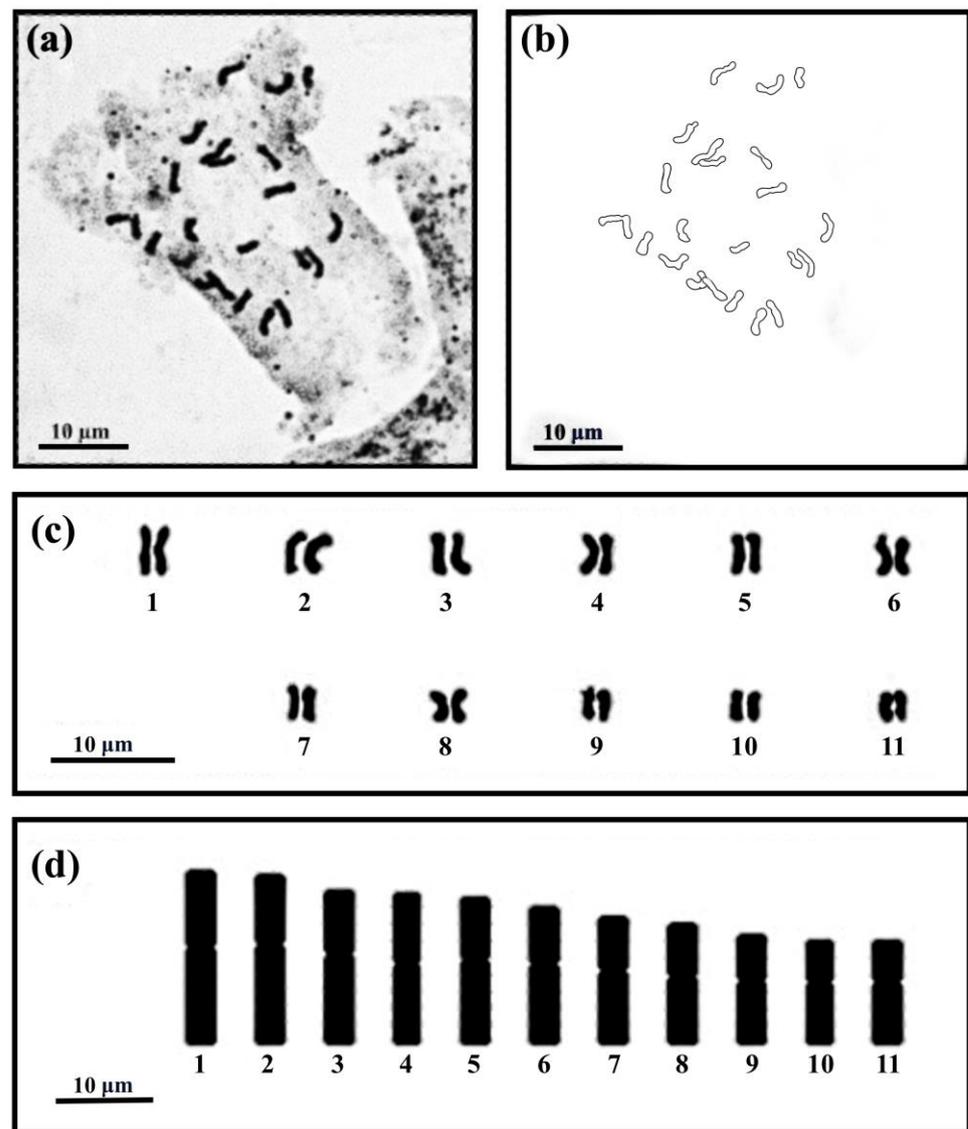
**Figure 3.** *Kaempferia sakolchaitii* P. Saensouk, Saensouk & Boonma sp. nov. (a) Dorsal corolla lobe. (b) Lateral corolla lobes. (c) Side view of flower with calyx and ovary. (d) Staminodes. (e) Labellum. (f) Habit, showing abaxial side of the leaf. (g) Habit with flower, showing adaxial side of leaf. (h) Front view of anther and anther crest. (i) Fruit. (j) Ovary and epigynous glands. (k) Calyx. (l) Bladeless sheath–adaxial surface with black dots. (m) Bracteole. (n) Bract. Drawings by Thawatphong Boonma and Surapon Saensouk.

**Table 1.** Mean length of short arm chromosome (Ls), long arm chromosome (Ll), total arm chromosome (LT), relative length (RL), and centromeric index (CI) of *Kaempferia sakolchaitii*  $2n = 22$ , obtained from 10 metaphase plates. (Standard Deviation = SD).

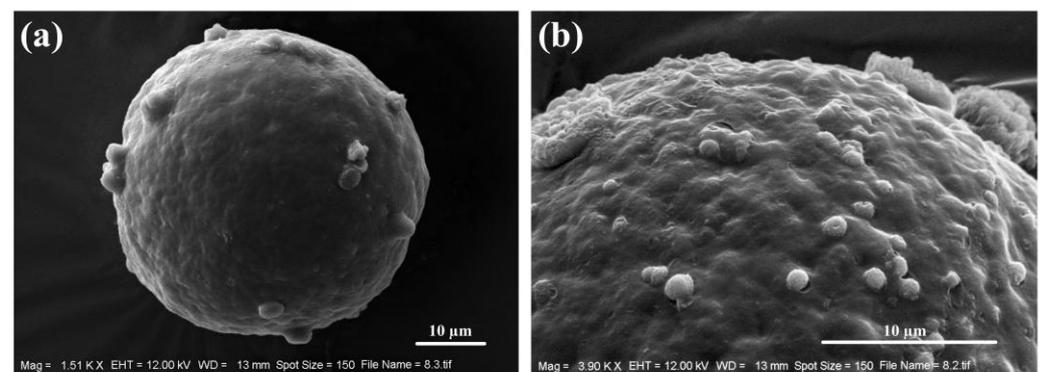
Chro. Pair	Ls $\pm$ SD ( $\mu\text{m}$ )	Ll $\pm$ SD ( $\mu\text{m}$ )	LT $\pm$ SD ( $\mu\text{m}$ )	RL (%)	CI	Chromosome Type
1	1.69 $\pm$ 0.08	2.77 $\pm$ 0.64	4.46 $\pm$ 0.73	11.96	0.62	Submetacentric
2	1.51 $\pm$ 0.09	2.48 $\pm$ 0.14	3.99 $\pm$ 0.23	10.69	0.62	Submetacentric
3	1.78 $\pm$ 0.09	2.08 $\pm$ 0.13	3.86 $\pm$ 0.22	10.35	0.54	Metacentric
4	1.49 $\pm$ 0.09	2.15 $\pm$ 0.13	3.65 $\pm$ 0.22	9.77	0.59	Metacentric
5	1.34 $\pm$ 0.08	2.27 $\pm$ 0.14	3.61 $\pm$ 0.21	9.67	0.63	Submetacentric
6	1.49 $\pm$ 0.09	1.93 $\pm$ 0.11	3.42 $\pm$ 0.20	9.17	0.56	Metacentric
7	1.15 $\pm$ 0.08	1.99 $\pm$ 0.11	3.15 $\pm$ 0.19	8.44	0.63	Submetacentric
8	1.53 $\pm$ 0.08	1.60 $\pm$ 0.11	3.13 $\pm$ 0.19	8.39	0.51	Metacentric
9	1.32 $\pm$ 0.08	1.74 $\pm$ 0.10	3.06 $\pm$ 0.18	8.20	0.57	Metacentric
10	1.13 $\pm$ 0.07	1.43 $\pm$ 0.09	2.55 $\pm$ 0.17	6.84	0.56	Metacentric
11	0.88 $\pm$ 0.06	1.56 $\pm$ 0.09	2.44 $\pm$ 0.15	6.53	0.64	Submetacentric

### 3.1.2. Palynology of *Kaempferia sakolchaitii* (Figure 5)

The pollen grains of *Kaempferia sakolchaitii* are monads, spheroidal in shape,  $47.30 \pm 2.50 \mu\text{m}$  in diameter, radially symmetrical, apolar, inaperturate, and exine sculpturing irregularly arranged elongated ornamentation elements with verrucate features, as shown in Figure 5.



**Figure 4.** Karyology of *Kaempferia sakolchaitii* P. Saensouk, Saensouk & Boonma sp. nov. (by Saensouk and P. Saensouk). (a) Microphotograph of somatic metaphase plate. (b) Explanatory drawing for the microphotograph in (4a). (c) Karyotype. (d) Idiogram.



**Figure 5.** Scanning electron microscopy (SEM) photomicrographs of pollen morphology of *Kaempferia sakolchaitii* P. Saensouk, Saensouk & Boonma sp. nov. (a) Pollen grain. (b) Exine sculpturing with verrucae.

3.2. *Kaempferia phuphanensis* var. *viridans* P. Saensouk, Saensouk & Boonma var. nov.  
(Figures 6–9), Subgenus *Kaempferia*

*Kaempferia phuphanensis* var. *viridans* is similar to *K. phuphanensis*, but differs in having a green lamina adaxial surface with translucent white margins, green abaxial surface (vs. var. *phuphanensis* having green lamina adaxial surface with dark red margins, dark red abaxial surface); bladeless sheaths, bracteoles, and green calyx (vs. dark red); pale green floral tube (vs. reddish).

Type: Thailand, Sakon Nakhon Province, Phu Phan District, Saensouk *Kaemp.* 01, 10 June 2022 (holo KKU!, iso BK!, FOF!)

Perennial herb, pseudostem erect, leaves blade oblique angle to the ground, 5–10 cm (young curling leaf erect, 9–15 cm high from the ground to the tip of the leaf). Rhizomes subglobose, yellowish internally, bearing storage roots. Bladeless sheaths 2–3 in number, up to 2.5 cm long, greenish, pubescent. Two to four leaves; leaf sheaths up to 4 cm long, green, pubescent; petiole concise, c. 0.5 cm long, green, sparsely pubescent; lamina narrowly ovate to elliptic, apex acute, base attenuate to rounded, 7–15 × 3–5 cm, green upper surface with translucent white margins, glabrous, green lower surface and pubescent. Inflorescence terminal, with c. 10 flowers opening once and lasting a single day; peduncle c. 1.5 cm long, green, basal 1/3 enclosed by the innermost leaf sheath. Bract one per flower, green, lanceolate, sparsely pubescent, the outermost 4–5.5 × 2–3 cm, inner ones smaller. Pale green bracteoles, folded, lanceolate, bifid to base, sparsely pubescent, 3–4 × c. 1 cm. Flowers exerted from bracts. Calyx tubular, 2–3.5 cm long, pale green, sparsely pubescent, with unilateral slit c. 1 cm long, apex two-toothed, with a tuft of hairs at apices of teeth. Floral tube 4–5 cm long, white, glabrous; dorsal corolla lobe lanceolate, c. 2 × 0.5 cm, hooded, mucronate at apex, translucent white, glabrous; lateral corolla lobes linear, c. 1.5 × 0.3 cm, translucent white, glabrous, apex rounded. Labellum 3–3.5 × 4–5 cm, deeply bilobed to two-thirds from the tip toward the base; lobes elliptic, overlapping each other, white, sometimes with a darker purplish patch at the inner base of each lobe with a longitudinal white line at the base of lobes. Lateral staminodes obovate, 2–2.5 × 1–1.5 cm, white. Anther c. 4 mm long, white; anther crest c. 6 × 4 mm, apex crenate, white. Stigma subglobose with lateral ciliated ostiole. Ovary cylindrical-obovoid, c. 4 × 2 mm, pubescent, pale green, incompletely three-loculed; placentation axile, ovules many; two epigynous glands, filiform, very slender, c. 7 mm long. Fruit cylindrical-obovoid, 10–18 × 6–7 mm, sparsely hairy, pale green to white at base. Seed ovoid, 4–5 × c. 2 mm, pale brown with dark brown spots, with white arils.

Vernacular name: Proh Tamu-Tami Bai Keaw.

Etymology: The specific epithet ‘*viridans*’ in the variety name ‘*Kaempferia phuphanensis* var. *viridans*’ is derived from the Latin term signifying ‘green’ or ‘of a green color’. The distinguishing feature of this new variety lies in its uniform greenness, which lacks the reddish tinge and red margin observed in the typical variety, *K. phuphanensis*.

Distribution: Endemic to Thailand; northeastern, Sakon Nakhon Province (Figure 1).

Ecology: Found in sandy loam soil mixed with rocks in semi-open deciduous forest areas, at an altitude ranging from 300 to 400 m above sea level.

Phenology: Flowering from June to September; anthesis in the morning.

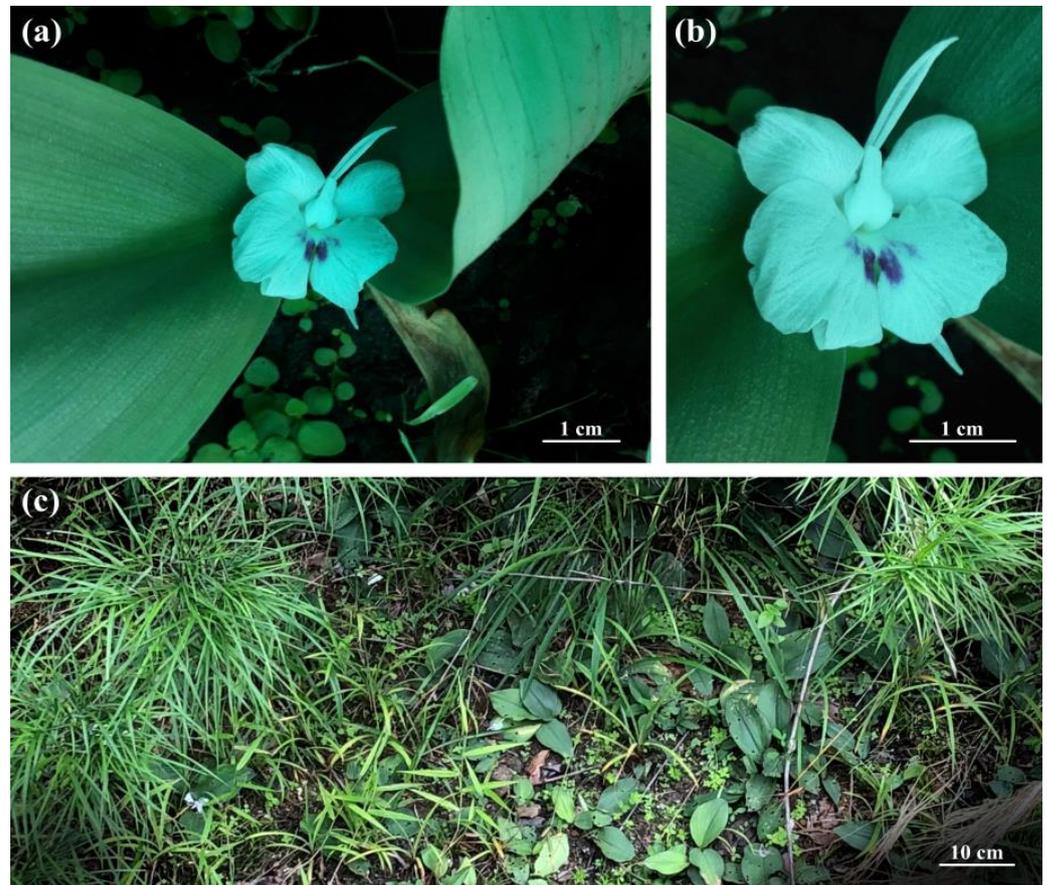
Utilization: This beautiful plant is utilized for ornamental purposes.

Conservation status: After the identification and publishing of the typical species of *Kaempferia phuphanensis* in 2019 [30], the green variety was discovered a year later. Subsequent monitoring revealed that the mature plants in this green population amounted to less than 50, lacking the reddish tinge characteristic of the typical species. Consequently, additional samples were collected on June 10, 2022, for taxonomic treatments, and are described here as a new variety. However, there is insufficient information about its distribution range, and this variety may exist in nearby unexplored locations. Currently categorized as Data

Deficient (DD) following the IUCN guidelines of 2022 [38], we nonetheless recommend considering it as an endangered taxon unless further evidence suggests otherwise.

Key to varieties of *Kaempferia phuphanensis*

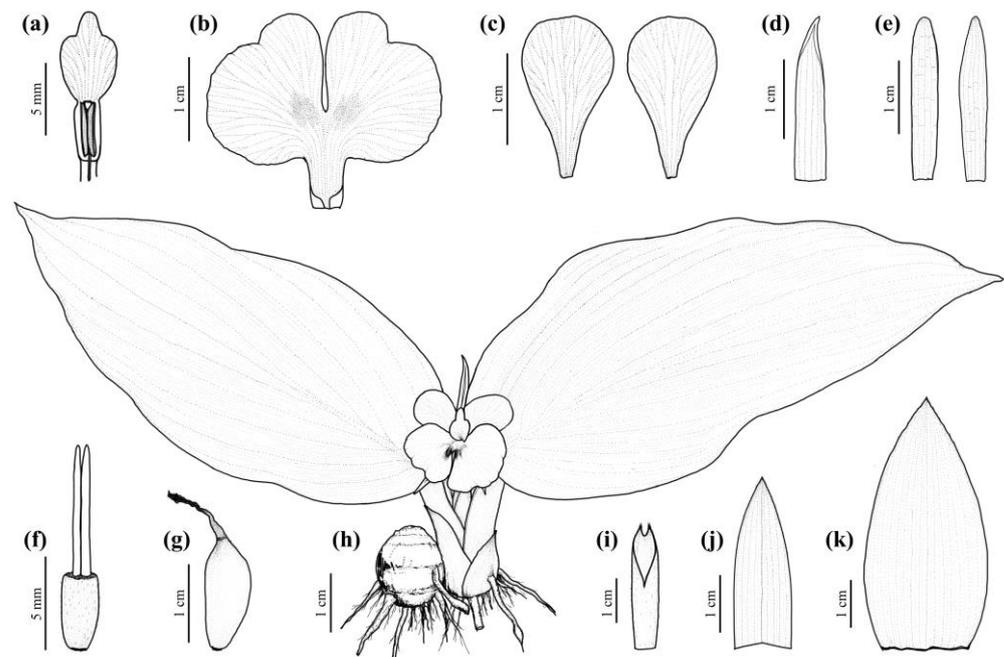
- 1a. Leaves green with dark red margin; abaxially dark red; sheaths dark red      var. *phuphanensis*  
 1b. Leaves green with green margin; abaxially green; sheaths green              var. *viridans*



**Figure 6.** *Kaempferia phuphanensis* var. *viridans* P. Saensouk, Saensouk & Boonma var. nov.: (a) habit from top view, showing the abaxial leaf surface; (b) flower; and (c) natural habitat. Photographs by Surapon Saensouk and Thawatphong Boonma.

### 3.2.1. Chromosomes and Karyotype Formula of *Kaempferia phuphanensis* var. *viridans*

*Kaempferia phuphanensis* var. *viridans* has  $2n = 22$  somatic chromosomes (Figure 8a, Table 2). In the karyotype, there are five pairs of metacentric, five pairs of submetacentric, and one pair of subtelocentric chromosomes. The karyotype formula of  $10m + 10sm + 2st$  chromosomes places it in the asymmetrical karyotype group (Figure 8b). The short arm length is from  $1.01 \pm 0.06$  to  $1.69 \pm 0.09$   $\mu\text{m}$ , the long arm length is from  $1.59 \pm 0.09$  to  $2.53 \pm 0.63$   $\mu\text{m}$ , and the total chromosomal length is from  $2.60 \pm 0.16$  to  $4.07 \pm 0.71$   $\mu\text{m}$ . The relative length (RL) ranges from 6.70 to 10.50%, while the centromeric index (CI) ranges from 0.53 to 0.70, as shown in Table 2.



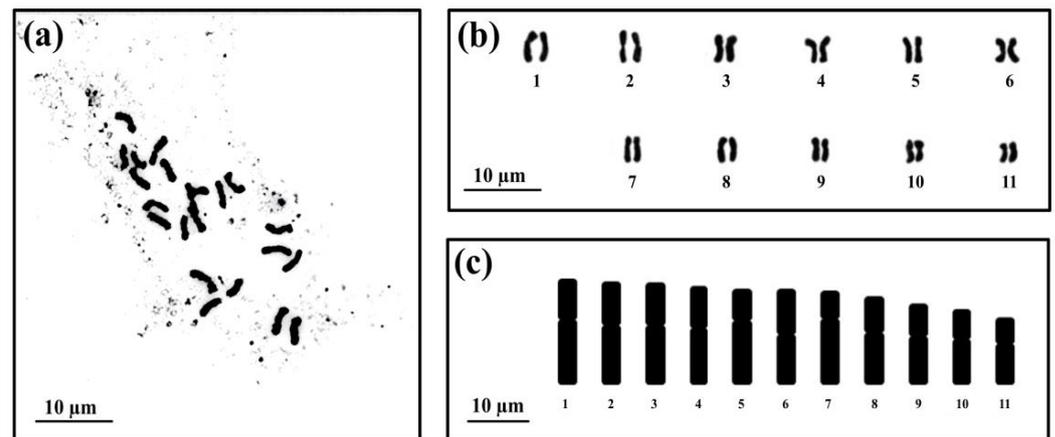
**Figure 7.** *Kaempferia phuphanensis* var. *viridans* P. Saensouk, Saensouk & Boonma var. nov.: (a) front view of anther with anther crest; (b) labellum; (c) stamines; (d) dorsal corolla lobe; (e) lateral corolla lobes; (f) ovary with epigynous glands; (g) fruit; (h) habit; (i) calyx; (j) bracteole; and (k) bract. Drawings by Thawatphong Boonma.

**Table 2.** Mean length of short arm chromosome (Ls), long arm chromosome (Ll), total arm chromosome (LT), relative length (RL), and centromeric index (CI) of *Kaempferia phuphanensis* var. *viridans*  $2n = 22$ , obtained from 10 metaphase plates. (Standard Deviation = SD).

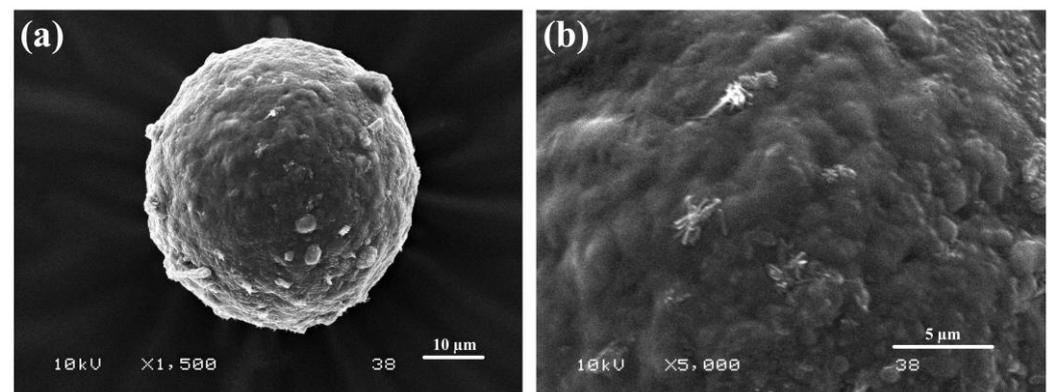
Chro. Pair	Ls $\pm$ SD ( $\mu\text{m}$ )	Ll $\pm$ SD ( $\mu\text{m}$ )	LT $\pm$ SD ( $\mu\text{m}$ )	RL (%)	CI	Chromosome Type
1	1.54 $\pm$ 0.08	2.53 $\pm$ 0.63	4.07 $\pm$ 0.71	10.5	0.62	Submetacentric
2	1.69 $\pm$ 0.09	2.29 $\pm$ 0.14	3.98 $\pm$ 0.23	10.26	0.58	Metacentric
3	1.65 $\pm$ 0.09	2.30 $\pm$ 0.13	3.95 $\pm$ 0.23	10.19	0.58	Metacentric
4	1.59 $\pm$ 0.09	2.20 $\pm$ 0.13	3.79 $\pm$ 0.22	9.77	0.58	Metacentric
5	1.22 $\pm$ 0.07	2.46 $\pm$ 0.14	3.68 $\pm$ 0.21	9.51	0.67	Submetacentric
6	1.71 $\pm$ 0.09	1.96 $\pm$ 0.11	3.67 $\pm$ 0.20	9.46	0.53	Metacentric
7	1.07 $\pm$ 0.08	2.54 $\pm$ 0.12	3.62 $\pm$ 0.20	9.33	0.70	Subtelocentric
8	1.39 $\pm$ 0.08	2.00 $\pm$ 0.12	3.39 $\pm$ 0.19	8.76	0.59	Metacentric
9	1.25 $\pm$ 0.07	1.87 $\pm$ 0.10	3.12 $\pm$ 0.18	8.04	0.60	Submetacentric
10	1.12 $\pm$ 0.07	1.77 $\pm$ 0.10	2.90 $\pm$ 0.17	7.47	0.61	Submetacentric
11	1.01 $\pm$ 0.06	1.59 $\pm$ 0.09	2.60 $\pm$ 0.16	6.70	0.61	Submetacentric

### 3.2.2. Palynology of *Kaempferia phuphanensis* var. *viridans*

The pollen grains of *Kaempferia phuphanensis* var. *viridans* are monads, spheroidal in shape,  $95.20 \pm 2.60 \mu\text{m}$  in diameter, radially symmetrical, apolar, inaperturate, and exine sculpturing irregularly arranged elongated ornamentation elements with verrucae (Figure 9).



**Figure 8.** Karyology of *Kaempferia phuphanensis* var. *viridans* P. Saensouk, Saensouk & Boonma var. nov. (by Saensouk and P. Saensouk): (a) microphotograph of somatic metaphase plate; (b) karyotype; and (c) idiogram.



**Figure 9.** Scanning electron microscopy (SEM) photomicrographs of pollen morphology of *Kaempferia phuphanensis* var. *viridans* P. Saensouk, Saensouk & Boonma var. nov.: (a) pollen grain and (b) exine sculpturing verrucae.

#### 4. Discussion

Due to its production of terminal inflorescence, the new species *Kaempferia sakolchaii* belongs to the *K.* subgenus *Kaempferia*. The group of *Kaempferia* spp., which usually only produce a solitary leaf, includes *K. gigantiphylla* Pichens. & Koonterm [43], *K. isanensis* Saensouk & P. Saensouk [34], *K. pichensoonthonii* Wongsuwan & Phokham [28], *K. pseudoparviflora* Saensouk & P. Saensouk [44], *K. siamensis* Sirirugsa [9], and *K. unifolia* Saensouk & P. Saensouk [34]. *Kaempferia sakolchaii* is notable for its lamina, whose adaxial surface displays a striking pattern of dark green interrupted by alternating silver or white longitudinal stripes. While this leaf pattern may resemble some species in the *Kaempferia* genus, such as *K. attapeuensis* Pichens. & Koonterm [43] and *K. minuta* Jenjitt. & K. Larsen [32], these species produce pinkish flowers with staminodes and labellum on the same plane. In contrast, *K. sakolchaii* produces white–purple flowers, and its staminodes and labellum are not on the same plane. Additionally, *K. jenjittikuliae* Noppornch. [33], a species belonging to another subgenus, *Protanthium* (Horan.) Baker also features leaves patterned with silver or white longitudinal stripes, producing inflorescence before its shoots. However, *K. sakolchaii* differs from these three species—*K. attapeuensis*, *K. minuta*, and *K. jenjittikuliae*—in having a solitary leaf, while the others produce two or more leaves.

The new variety *Kaempferia phuphanensis* var. *viridans* also belongs to the subgenus *Kaempferia*, as it produces terminal inflorescence. This new variety may look superficially similar to *K. champasakensis* Pichens. & Koonterm [45], but it differs in several aspects.

Firstly, it produces a subglobose rhizome, whereas *K. champasakensis* produces a long, creeping rhizome. Additionally, the leaves and leaf sheaths of *K. phuphanensis* var. *viridans* are pubescent, whereas those of *K. champasakensis* are glabrous. Furthermore, the flowers of *K. phuphanensis* var. *viridans* are white–purple, whereas those of *K. champasakensis* are pure white. The staminodes and labellum of *K. phuphanensis* var. *viridans* differ from those of *K. champasakensis* in terms of shape, size, and color.

This study represents the first report on the chromosome numbers of both *Kaempferia sakolchaii* and *K. phuphanensis* var. *viridans*; these numbers are  $2n = 22$ , which is consistent with many species in the *Kaempferia* genus [46,47]. However, they are not consistent with some previously recorded chromosome numbers, such as those in the studies of Omanakumari and Matthew [48,49] and Sharma and Bhattacharyya [50]. These authors reported chromosome numbers of some other *Kaempferia* spp. as  $2n = 36$  and 54.

This study's karyology report on the new species, *Kaempferia sakolchaii*, and the new variety, *K. phuphanensis* var. *viridans*, showed differences in the karyotype formulae with other *Kaempferia* species previously studied by Saenprom et al. [41]. Thus, this study constitutes the first time that the chromosome numbers, karyotypes, karyology information, and idiograms of the newly described taxa have been reported.

The pollen morphologies of the new species, *Kaempferia sakolchaii*, and the new variety, *K. phuphanensis* var. *viridans*, are also reported for the first time. The pollen grains are monads, spheroidal in shape, radially symmetrical, apolar, and inaperturate, which is consistent with previous studies on species within the same genus. This finding is notably consistent with the study by Saensouk and Saensouk [44], who noted that the exine sculpturing of pollen grains of *K. pseudoparviflora* Saensouk & P. Saensouk are verrucate. As recorded by Zou et al., both taxa differ from *K. angustifolia* Roscoe, which has a subspheroidal shape [51].

## 5. Conclusions

The newly described taxa belong to the *Kaempferia* subgenus due to their terminal inflorescence. Both taxa share the same chromosome number and display characteristic pollen grains. The chromosome number, karyotype, karyology information, and idiogram, in addition to the pollen morphology, further confirm their taxonomic placement. This report represents the first documentation of these aspects of both the new species and variety, providing valuable insights in terms of their taxonomic classification. The principal conclusions of our study highlight the distinctiveness of these taxa within the genus; they do not match any known species. Furthermore, our analyses provide valuable insights into their diversity and underscore the importance of continued exploration and research in biodiverse regions for understanding and conserving plant diversity.

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