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Research Article

Taxonomy, Cytology and Palynology of *Kaempferia pseudoparviflora* (Zingiberaceae), A New and Rare Specie from Northern Thailand

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Abstract

Background and Objective: *Kaempferia pseudoparviflora* is a new species from Thailand. Cytogenetics and palynology of this new species are never reported before. Therefore, this study aimed to study the taxonomical study, karyology and palynology of *Kaempferia pseudoparviflora* sp. nov. from Thailand. **Materials and Methods:** Plant taxonomy study was surveyed to collect specimen, described, measurements reported and illustrated from living specimens and spirit specimens under stereo microscope. Photographed were taken in the field. This specimen was compared with dry specimens at aboard herbaria. The chromosome numbers of this species were studied from root tips and counted from 20 cells. The nomenclature of the chromosome morphology, karyotype symmetry degree. For the arrangement of the karyotypes, the following parameters from metaphase chromosomes were calculated by methods of several scientists. The pollens of this species were analyzed using an alcohol series. The pollen grains morphology were studied using SEM. **Results:** The new species has four distinctive in leaf, pseudostem, peduncle and inflorescence. The morphological characters of *Kaempferia pseudoparviflora* are similar to those of *K. parviflora*. A cytogenetic study of this species was found that chromosome number $2n = 22$ and the karyotype formula to be symmetrical with $8+14m$ and one visible satellite chromosome. The pollen morphology of a new species was monad, spheroidal, $99.37 \pm 3.61 \mu\text{m}$ in diam., large size, radial symmetry, apolar, inaperturate, exine sculpturing verrucate and regulate. **Conclusion:** *Kaempferia pseudoparviflora* was discovered to be a new species and endemic to Thailand.

Key words: *Kaempferia pseudoparviflora*, taxonomy, cytogenetics, pollen, Zingiberaceae

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Zingiberaceae is a family of flowering plants made up of about 50 genera with a total of about 1,600 known species in the world, Thailand is one of the richest ginger floras in the world, 26 genera are found as native within the borders of the Kingdom, about 300 have so far been found in Thailand, the numbers most certainly rise. Genus *Kaempferia* belongs to the tribe Zingibereae, subfamily Zingiberoideae, family Zingiberaceae¹⁻⁴.

The *Kaempferia* L. the genus in the Zingiberaceae family establish by Linnaeus in 1753 to commemorate the German physician and traveller Engelbert Kaempfer (1651-1716), the first westerner who collected plant specimens in Thailand. *Kaempferia* is subdivided into two sub-genera: *K.* subgen. *Kaempferia* and subgen. *Protanthium* (Horan.) Baker base on the position of the inflorescence. *Kaempferia* subgen. *Kaempferia* produces terminal inflorescences while the subgen. *Protanthium* produces the inflorescences directly from the rhizome. About 60 species are found and it is distributed from India to Southeast Asia⁴. Several years ago, Sirirugsa⁵ recognized 15 species of *Kaempferia* from Thailand. This family in Thailand was found the richest biodiversity region with more than 20 extant species⁵⁻¹⁰. Thereafter, many new species of *Kaempferia* genus were found and published continuously up until now. Several *Kaempferia* species in Thailand are well known for their traditional uses by local people such as for food, medicinal plant, ornamental plant and ritual plant⁶⁻¹⁰.

An undescribed species is also in the *Kaempferia* genus, found in the deciduous forest in Umphang District, Tak Province. An undescribed species of *Kaempferia* did not match with any known species and treated it as new species, this species is here described, illustrated and photographed under the name of *Kaempferia pseudoparviflora* sp. nov. Therefore, this study aimed to study the taxonomy, karyology and palynology of *Kaempferia pseudoparviflora* sp. nov. from northern Thailand.

MATERIALS AND METHODS

Material: Plant materials were collected from the field in Tak Province, Northern Thailand in 2020-2021. They were separated to dry specimens, spirit specimens and living specimens.

Plant taxonomy study: The morphology is described and illustrated under stereo microscope. Measurements reported here were taken from living specimens and spirit specimens.

Photographs were taken from living specimens in the field. Type specimens of morphologically similar species were compared with dry specimens which kept at aboard herbaria (AAU, BK, BKF, E, K, P, KKU and QBG), verified directly, living species at Mahasarakham Plant Nursery including digital images available online and all the published studies of species of the genus *Kaempferia*. The traditional use data was taken from interviewed the local people near in type locality area. A preliminary conservation assessment was prepared based on the IUCN Red List categories. Measurement of the elevation from the sea level according to the recorded tracks in the "View-Ranger" Application.

Karyology study: The chromosome numbers study from root tips of *Kaempferia pseudoparviflora* indicated that¹¹, The nomenclature of the chromosome morphology follows the classification of Levan *et al.*¹. The classification of the karyotype symmetry degree is proposed by following Stebbins². The diploid chromosome numbers of each species in this study are counted from 20 cells. For the arrangement of the karyotypes, the following parameters from metaphase chromosomes were calculated¹¹.

Palynology study: The pollens study of *Kaempferia pseudoparviflora* were analyzed using an alcohol series as described by Erdtman³. The pollen was studied using a scanning electron microscope or SEM (Hitachi, TM- 4000plus) at Laboratory Equipment Center, Division of Research facilitation and dissemination, Mahasarakham University. Details of pollen morphology were based on 20 pollen grains observed under SEM analysis. Erdtman³ methodology was used to classify pollen shapes and also provided the terminology used.

RESULTS

Plant taxonomy of new species: The taxonomic treatment, description, flowering period, etymology, distribution, conservation status, ecology, vernacular names, traditional uses, illustration and photographs of *Kaempferia pseudoparviflora* were observed.

Taxonomic treatment

Kaempferia pseudoparviflora sp. Nov

Type: THAILAND. Saensouk 2000, Umphang District, Tak Province, Thailand, 10 June 2018 (holotype KKU!, isotype BK!, BKF!).

Diagnosis: The new species had examined and compared this undescribed species to other similar characteristics species but

Fig. 1(a-b): *Kaempferia pseudoparviflora*

(a) Habitat one leaf flat near the ground (very short pseudostem with inflorescence exerted from pseudostem) and (b) Flower white with labellum (white and purple in the middle)

Table 1: Differences in *Kaempferia pseudoparviflora* and *K. parviflora* species

Characters	<i>K. pseudoparviflora</i>	<i>K. parviflora</i> ⁶⁻⁷
Habit	Pseudostem flat near the ground, 2-3 cm tall (Fig. 1a)	Pseudostem erect and elongate from the ground, tall up to 25 cm
Colour rhizome	Yellowish	Dark blue or dark purple
Number of leaves	1 leaf	4-6 leaves
Leaf shape	Subspheroidal (Fig. 1a)	Elliptic
Upper leaf surface	Dark green with light grey markings (Fig. 1a)	Green
Leaf-sheath	Up to 2 cm long	Up to 7 cm long
Petiole	Up to 0.5 cm long	Up to 5 cm long
Peduncle	Less than 1 cm long	ca. 8 cm long; enclosed by leaf-sheath
Inflorescence	Enclosed by the bladeless sheaths and one leaf-sheath; not exerted from leaf sheath (Fig. 1 and b)	Exerted from the leaf sheath

found that flowers of this species did closely related to flowers of *Kaempferia parviflora* Wall. ex Baker but it differs in colour of inside rhizome, pseudostem, leaf shape, upper leaf surface and inflorescence and treated as a new species under the name of *Kaempferia pseudoparviflora* sp. nov, the epithet name refers to its flowers shown in Fig. 1. Moreover, the difference in *Kaempferia pseudoparviflora* and *K. parviflora* are presented in Table 1.

Perennial herb, short pseudostem with 2-3 cm tall, rhizome yellowish inside, bearing several roots in a fascicle. Bladeless sheaths 2, up to 1.5 cm long, glabrous, reddish. Leaf 1 shown in Fig. 1a, leaf sheath up to 2 cm long, glabrous, reddish, petiole up to 0.5 cm long with glabrous, green and reddish, ligule inconspicuous, blade horizontal near the ground, subspheroidal, 10-12×9-10 cm, both surface glabrous and upper surface dark green with light grey markings and lower surface pale green, margin undulate, apex acute. Inflorescence terminal is shown in Fig. 1a and b enclosed by the bladeless sheaths and one leaf-sheath, peduncle short, less than 1 cm long, white with light pale green at the tip, glabrous. Bract one per flower, lanceolate, the outermost 2-2.2×1-1.3 cm, inner ones lanceolate, smaller than the outer ones, both surfaces glabrous, white towards the base,

greenish towards the apex. Bracteoles folded, lanceolate, ca. 1.8×1 cm, glabrous, translucent white. Flowers 7-10, exerted from bracts. Calyx tubular, 1-1.2 cm long, glabrous, translucent white, unilaterally slit at apex, slit ca. 0.7 cm long. Corolla tube 1.5-2 cm long, glabrous, white, dorsal corolla lobe lanceolate, 2.7-3×0.5-0.6 cm, mucronate at apex, hooded, translucent white, lateral corolla lobes linear, 2.5-2.6×0.4-0.5 cm, glabrous, translucent white. Labellum longer than broad, 3.5-3.7×3-3.2 cm, bilobed with 1/3rds longitudinal cleft from the tip towards the top of dark purple, lobes overlapping, each lobe obovate, apex obtuse to rounded, whitish or pale purple with dark purple patches towards the base and pure white spot at base, further inside purple with sparsely white spots, shown in Fig. 1b. Lateral staminodes obovoid, 3-3.2×1-1.2 cm, white. Anther thecae ca. 5 mm long, parallel, white, anther-crest ca. 8×8 mm, deeply bilobed, white. Stigma was subglobose with lateral ciliated. Ovary cylindrical, ca. 7×3-4 mm, glabrous, creamy white, 3-locule, placentation axile, ovules many, styles 2, filiform, ca. 5 mm long. Fruit and seed didn't observe.

According to Fig. 2 *Kaempferia pseudoparviflora* exhibit, different characters In Fig. 2a the dorsal corolla lobe presented the lanceolate, mucronate at apex, hooded with a translucent

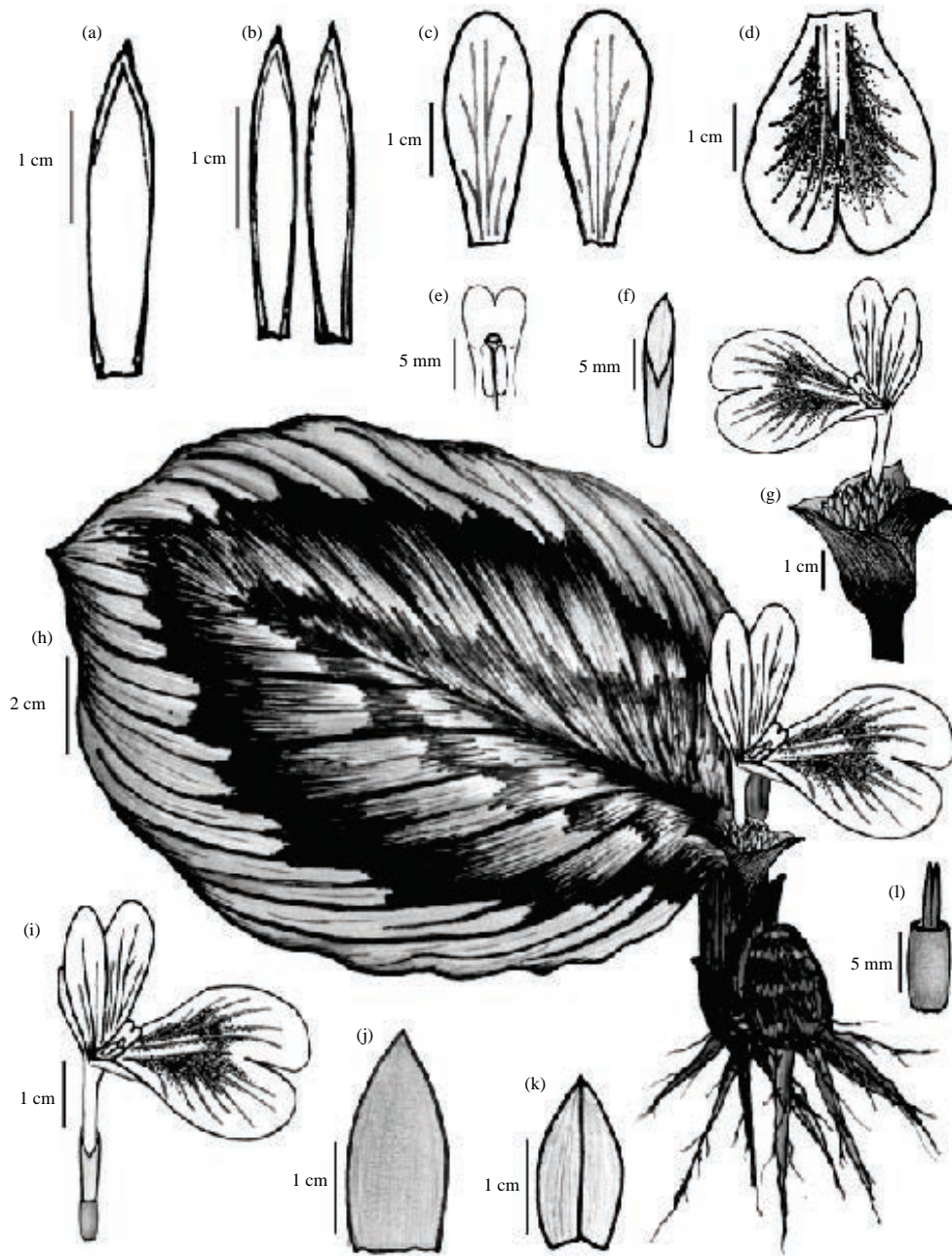


Fig. 2(a-l): *Kaempferia pseudoparviflora*, illustration of habitat, inflorescence and flower details

(a) Dorsal corolla lobe, (b) Lateral corolla lobe, (c) Lateral staminodes, (d) Labellum, (e) Anther parallel, (f) Calyx tubular, (g-h) Habitat, (i) Flower, (j) Bract, (k) Bracteole and (l) Ovary

white. In Fig. 2b representing the lateral corolla lobe explaining the linear structure with translucent white colour, Fig. 2c lateral staminodes presented obovoid and white, Fig. 2d showing the labellum which presented bilobed at apex, each lobe obovate, apex obtuse to rounded, whitish or pale purple with dark purple patches towards the base and pure white spot at base, further inside

purple with sparsely white spots, Fig. 2e anther parallel, white and anther-crest apex deeply bilobed, white, Fig. 2f representing the calyx tubular, glabrous, translucent white, Fig. 2g and h Habitat presented short pseudostem with blade horizontal near the ground, subspheroidal, in Fig. 2i flower exerted from bracts, Fig. 2j bract both surfaces glabrous, white towards the base, greenish towards the apex,

Table 2: Karyotype of *Kaempferia pseudoparviflora* (2n = 22)

Chromosome pair	Ls±SD (µm)	Ll±SD (µm)	LT±SD (µm)	RL (%)	CI	Chromosome type
1	1.47±0.25	3.16±0.26	4.63±0.50	12.00	0.68	Submetacentric
2*	1.53±0.16	2.52±0.25	4.05±0.40	10.49	0.62	Submetacentric
3	1.40±0.13	2.38±0.19	3.78±0.31	9.79	0.63	Submetacentric
4	1.42±0.14	2.30±0.17	3.71±0.31	9.61	0.62	Submetacentric
5	1.64±0.15	2.06±0.16	3.70±0.31	9.58	0.56	Metacentric
6	1.25±0.11	2.20±0.19	3.45±0.29	8.94	0.64	Submetacentric
7	1.45±0.11	1.98±0.17	3.43±0.28	8.89	0.58	Metacentric
8	1.35±0.13	1.90±0.14	3.25±0.27	8.42	0.58	Metacentric
9	1.39±0.13	1.69±0.13	3.09±0.26	7.99	0.55	Metacentric
10	0.94±0.07	2.06±0.16	3.00±0.23	7.77	0.69	Submetacentric
11	0.79±0.08	1.73±0.09	2.52±0.16	6.53	0.69	Submetacentric

*Satellite chromosome, Ls: Length short, Ll: Length long, LT: Length total, RL: Relative length, CI: Centromeric index

Fig. 2k representing the bracteole folded, lanceolate, glabrous, translucent white and Fig. 2l ovary presented cylindrical, glabrous, creamy white.

Flowering period: May-June

Etymology: The specific epithet of the new species is derived from two Latin words, "*pseudo*" means "artificial or not genuine or spurious or sham" and "*parviflora*" means specific epithet of *K. parviflora*.

Distribution: Endemic to Thailand, this new species is not common from the type locality only in Tak Province, Northern, Thailand.

Conservation status: Only a few populations of *Kaempferia pseudoparviflora* has been found, but it is expected to be found in adjacent areas as suitable habitat still seems to exist. Awaiting further field studies and observations, it was suggested to treating this species as Data Deficient (DD).

Ecology: It grows on an open area and under the shade of in the sandy loam soil close to the water stream, in the deciduous forest from northern Thailand. It appears only in the rainy season.

Vernacular Names: Wan-Kra-Chai-Dam-Tiam.

Traditional uses: Ornamental plant.

Karyology of new species: The chromosome numbers study from root tips of *Kaempferia pseudoparviflora* indicated the diploid chromosome numbers of *K. pseudoparviflora* was found 2n = 22 expressed in Fig. 3a and b, Table 2. The karyotype formula was symmetrical with 8m + 14sm and one visible satellite chromosome. The karyotype consisted of four pairs of m-type and seven pairs of sm-type chromosomes. The chromosome length ranged from 2.52±0.16-4.63±0.50 µm.

Palyology of new species: The pollens morphology of *Kaempferia pseudoparviflora* are monad, spheroidal in shape, 99.37±3.61 µm in diameter, radial symmetry, apolar, inaperturate, exine sculpturing verrucate and regulate shown in Fig. 4.

DISCUSSION

Results showed that the undescribed species collect from Umphang District, Tak Province, Thailand is identified from Kress *et al.*⁴, Siriruga⁵ and Larsen and Larsen⁶ belongs to genus *Kaempferia*, tribe Zingibereae, subfamily Zingiberoideae, family Zingiberaceae. It was examined and compared this undescribed species to other similar characteristics species in Siriruga⁵, Larsen and Larsen⁶ Picheansoonthon and Koonterm⁷, Techaprasan *et al.*⁸ Saensouk *et al.*⁹ studies, but found that flowers of this species did closely related to flowers of *Kaempferia parviflora* Wall. ex Baker but it differs in colour of inside rhizome, pseudostem, leaf shape, upper leaf surface and inflorescence (Fig. 1, 2 and Table 1) and treated it as a new species under the name of *Kaempferia pseudoparviflora* sp. nov, the epithet name refers to its flowers (Fig. 1b). This new species has four distinctive characteristics in leaf, pseudostem, peduncle and inflorescence (Table 1). Therefore, the morphological characters of *Kaempferia pseudoparviflora* are similar to those of *K. parviflora*. Finally, *Kaempferia pseudoparviflora* has been found as a new species from northern Thailand. Thus, *Kaempferia* in Thailand has been found continuously to the present and estimated that there are still many species that have not been discovered and explained from Thailand. The local name or vernacular name of this new species is Wan-Kra-Chai-Dam-Tiam.

The cytogenetics of *Kaempferia pseudoparviflora* was studied from root tips which is consistent with the method of Saenprom *et al.*¹¹. The chromosome numbers of *K. pseudoparviflora* were found to be 2n = 22. The karyotype

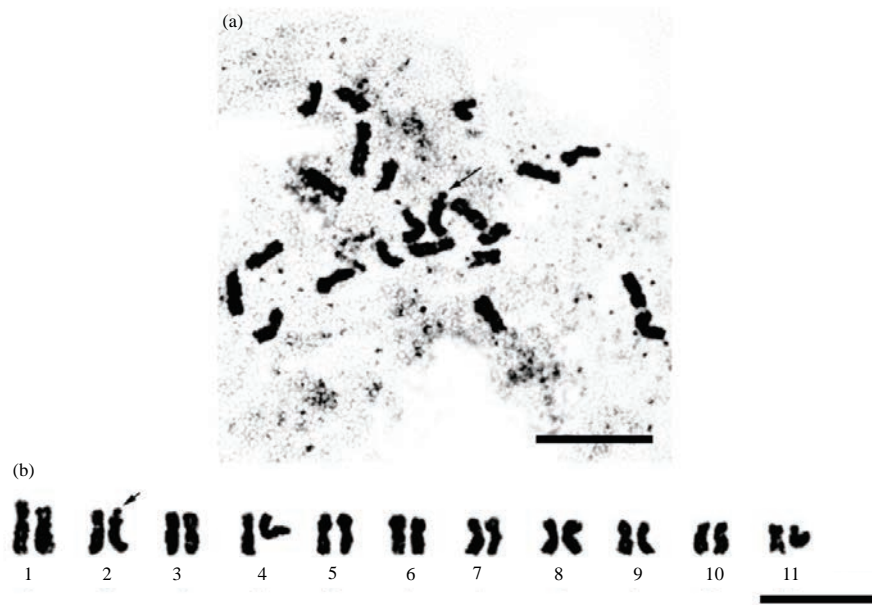


Fig. 3(a-b): Karyology of *Kaempferia pseudoparviflora*

(a) Microphotographs of somatic metaphase plate and (b) Karyotypes by conventional staining with the arrow in pair 2 indicate satellite. Scale bars: 10 μ m

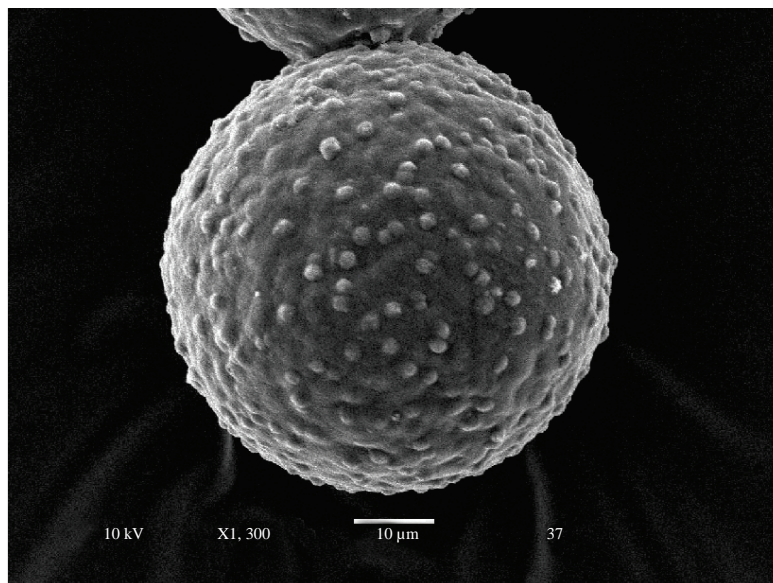


Fig. 4: SEM photomicrographs of pollen morphology of *Kaempferia pseudoparviflora*

(x1,000 and scale bar = 10 μ m)

formula was symmetrical with 8m+14sm and one visible satellite chromosome (Table 2 and Fig. 3) which differs from other *Kaempferia* species by Saenprom *et al.*¹¹. Thus, the chromosome number and karyotype of this new species are the first time reported.

The pollen morphology of *Kaempferia pseudoparviflora* was analyzed using an alcohol series and it was studied under the scanning electron microscope. Therefore, the result found that the pollen morphology of this species is found to be a monad, spheroidal, large pollen, radial symmetry, apolar,

inaperturate, exine sculpturing verrucate and regulate (Fig. 4) which is consistent with Erdtman³. Besides, the pollen of *K. pseudoparviflora* is also the first time studied which is not similar to other literatures³.

This study has limitations in the habitat, it is found that the length of peduncle of this plant vary in size but not too much, depending on the depth of the underground rhizomes and the thickness of the bamboo dry leaves that fall in the deciduous season. To be able to lift the inflorescence and flowers higher than the dry leaves. The length of the peduncle is the most found is very short. Therefore, the suggestion of this study will be to collect specimens in spirit or cultivate them in the nursery for study later.

CONCLUSION

This study discovers a new species in the genus *Kaempferia*, family Zingiberaceae from the deciduous forest, Umphang District, Tak Province, Thailand. The benefits of this species can be used for ornamental plant. The chromosome numbers of *K. pseudoparviflora* from root tips were found $2n = 22$. The karyotype formula was symmetrical with $8m + 14sm$ and one visible satellite chromosome. Pollen morphology is monad, spheroidal, large pollen, radial symmetry, apolar, inaperturate, exine sculpturing verrucate and regulate.

SIGNIFICANCE STATEMENT

This study discovered the *Kaempferia pseudoparviflora* Saensouk and P. Saensouk that can be beneficial for an ornamental plant. This study will help the researchers to uncover the critical areas of *K. pseudoparviflora* Saensouk and P. Saensouk that many researchers were not able to explore. Thus a new theory on *K. pseudoparviflora* Saensouk and P. Saensouk may be arrived at.

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