Kaempferia candida (Zingiberaceae): *Curcuma* in disguise

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Results of morphological studies supported by molecular analyses of chloroplast *psbA-trnH* and partial *petA-psbJ* DNA sequences indicate that *Kaempferia candida* cannot be maintained. The species has to be transferred to *Curcuma* and the name needs to be typified.

Figure 1. The original painting of *Kaempferia candida* from Plantae Asiaticae Rariores (1830) (reproduced with permission of Singapore Botanic Gardens) and the Wallich specimen, Wall. Cat. No. 6585, in K-W (reproduced with kind permission of the Trustees of the Royal Botanic Gardens, Kew).

INTRODUCTION

Kaempferia candida Wall. (1830) is a peculiar ginger species described from a material consisting of an inflorescence with flowers only (Fig. 1). Wallich placed this taxon in the genus *Kaempferia*, plausibly based on the lack of anther spurs but presence of well-developed anther crest, inflorescence made of fertile bracts with no coma and unusually big flowers, which appear before the leaves, remotely resembling those of *K. rotunda*. Baker (1890), Schumann (1904) and Gagnepain (1908) with no new material on hand merely adapted the description from the protologue, but did not pay closer attention to this species, placing it unequivocally in *Kaempferia*, subg. *Protanthium* Horan.

The new distributional record of this species by Jenjittikul & Larsen (2000) from Kanchanaburi Province near the border with Myanmar and not far from its type locality (Amherst and Molmyne) included a detailed description, drawing and colour photographs. While the find was indeed a perfect match with Wallich's description and drawing, several morphological features of this unusual species raised our doubts about its generic placement in the genus *Kaempferia* (Fig. 2, Table 2.). All these characters suggest a possible close relationship with several members of *Curcuma*.

Techaprasan et al. (2010) studied 71 accessions of Thai *Kaempferia* and closely related taxa of Zingiberaceae using chloroplast *psbA-trnH* and partial *petA-psbJ* DNA sequences, and concluded that all *Kaempferia* species fall within the same group, except *K. candida*. *Curcuma* species were, however, not included in that study. The original description, type material, other herbarium material (including spirit) and recently collected living material of *K. candida* and *psbA-trnH* and partial *petA-psbJ* DNA sequences of several members of *Curcuma* and *Kaempferia* were therefore analysed in order to assess the generic placement of *K. candida*.

MORPHOLOGICAL & MOLECULAR EVIDENCE

Pairwise nucleotide divergence of the combined data in the *Curcuma* clade ranged between 0.00-0.96% which was lower than in *Kaempferia* (0.00-1.61%). Pairwise nucleotide divergence was particularly low (0.00-0.07%) in *Curcuma* group I (including *K. candida*), suggesting that cpDNA sequences of *psbA-trnH* and *petA-psbJ* in *Curcuma* group I have been conserved. In agreement with a previous study of *Kaempferia* using same markers (Techaprasan et al. 2010), **all *Kaempferia* species were allocated to the same group, except *K. candida*.** In addition, precociously flowering *Kaempferia* species, i.e., *K. rotunda*, *K. grandifolia*, *Kaempferia* sp. 1, *Kaempferia* sp. 2, and *Kaempferia* sp. 3, formed a well-supported group within *Kaempferia*. ***Kaempferia candida* was strongly supported in *Curcuma* clade I** (corresponding to the nominate subgenus *Curcuma*) with 93% bootstrap support. Therefore, based on molecular evidence of maternally inherited cpDNA *psbA-trnH* and partial *petA-psbJ* data, *K. candida* belongs in *Curcuma* (Fig. 3).

Morphological evidence listed in Table 2. also clearly supports inclusion of *Kaempferia candida* into *Curcuma*.

MATERIAL, METHODS & RESULTS

Plant samples for molecular analysis - 42 accessions including 21 *Kaempferia* species, 9 *Curcuma* species and 10 species from the subfamily Zingiberoideae were included in the analysis (Table 1).

DNA extraction, Polymerase Chain Reaction (PCR), and DNA sequencing - The *psbA-trnH* and partial *petA-psbJ* spacer sequences of all *Kaempferia* species and selected Zingiberoideae species were obtained from a previous study by Techaprasan et al. (2006 and 2010). Genomic DNA of all *Curcuma* species, *Boesenbergia rotunda* and *Hedychium longicornutum* was extracted from leaves using the CTAB method with minor modification (Doyle and Doyle 1987). The *psbA-trnH* and *petA-psbJ* regions of each sample were separately amplified and sequenced as described in Techaprasan et al. (2006).

Data analysis - Sequences of *psbA-trnH* and partial *petA-psbJ* spacers were aligned and edited using BioEdit version 7.0.5.2 (Hall 1999). Disregarding indels, nucleotide sequence divergence between pairs of taxa was calculated using a Kimura (1980) 2-parameter model. Maximum parsimony analysis was performed using Phylogenetic Analysis Using Parsimony (PAUP*) version h4.0 Beta 10 (Swofford 2004). Gaps were treated as missing data. The most parsimonious trees were obtained through a heuristic search with tree-bisection-reconnection (TBR) branch swapping option and 10,000 random sequence additions. The Bootstrap method (1,000 replicates) with heuristic search was also performed.

Results - After multiple sequence alignments, the lengths of the nucleotide sequences were 923 bp for *psbA-trnH* and 819 bp for partial *petA-psbJ* spacer. Based on the combined data, 1,742 bp in length were obtained, including 107 and 61 variable parsimoniously uninformative and informative sites, respectively. The pairwise nucleotide divergence of combined data within *Kaempferia*, *Curcuma* and across all investigated samples ranged between 0.00-1.61% (*K. parviflora* and *K. minuta* ined.), 0.00-0.96% (*C. parviflora* and *C. singularis*) and 0.00-2.53% (*K. minuta* ined. and *S. biloba*), repectively. The strict consensus tree of 5,589 most parsimonious trees from the combined data set generated 212 steps with consistency (CI), retention (RI), and homoplasy indices of 0.85, 0.88 and 0.15 respectively (Fig. 3). All *Kaempferia* species in Thailand clustered together in the same clade, except two accessions of *K. candida* which were nested within the *Curcuma* group with strong bootstrap support (93%). The *Curcuma* species were divided into two major groups; group I (*Curcuma* sp., *C. comosa*, *C. latifolia*, *C. rubrobracteata*, and *C. codonantha*) including *K. candida* and group II (*C. harmandii*, *C. rhabdota*, and *C. parviflora*) including *Stahlianthus* sp.

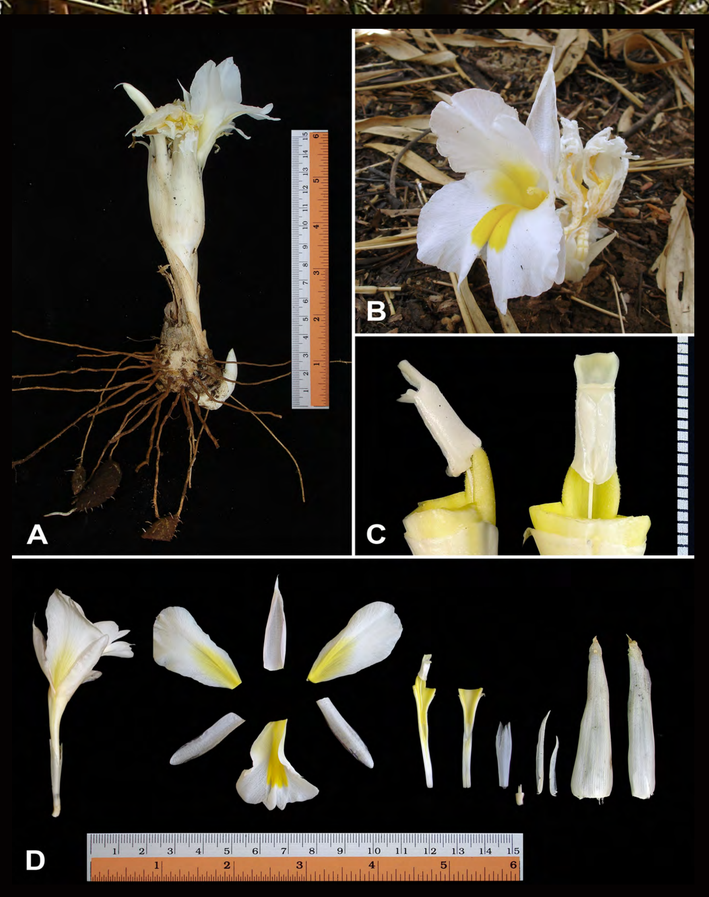

Figure 2. *Curcuma candida*. **A.** Flowering plant with rhizome; **B.** Inflorescence in its natural habitat; **C.** Anther (front and side); **D.** Flower dissection. Based on *JLS-606*, photo by Jana Leong-Škorničková. *Curcuma candida* has unusually large flowers suggesting a large pollinator in order to reach the versatile anther and effect pollination. During our recent field trip, only bees were observed to visit the flowers. They were, however, too small to reach the anther. Jenjittikul & Larsen (2000) mentioned that ants and beetles visit the flowers. While ants were likely to visit simply to steal nectar, beetles might be pollinators given the placement of the flowers at ground level. We have observed that flowers of cultivated plants opened in late evening, close to midnight, and a night pollinator may be a possibility. More observations in the field are needed.

Kaempferia	*Kaempferia candida*	*Curcuma*
usually a very short or poorly developed pseudostem and a few leaves, which are often appressed to the ground	**leafy shoot with a well-developed pseudostem and thin, prominently plicate laminas occasionally with a red patch on the midrib**	**leafy shoot with mostly well-developed pseudostem and thin, prominently plicate laminas occasionally with a red patch on the midrib**
one flower per fertile bract	**cincinnus of 4-6 flowers**	**cincinnus of 2-many flowers, rarely single flower**
short bright yellow patches at the base of the labellum rarely seen in some precocious species, but never across the entire labellum	**a deep yellow median band across the entire labellum**	**a deep yellow median band present in majority of *Curcuma* species**
non versatile anther	**versatile anther, the shape falls comfortably within *Curcuma***	**versatile anther with large spectrum of morphological variability**

Table 2. Morphological comparison of *Kaempferia candida* to the members of the genera *Kaempferia* and *Curcuma*. Shared characters are in bold.

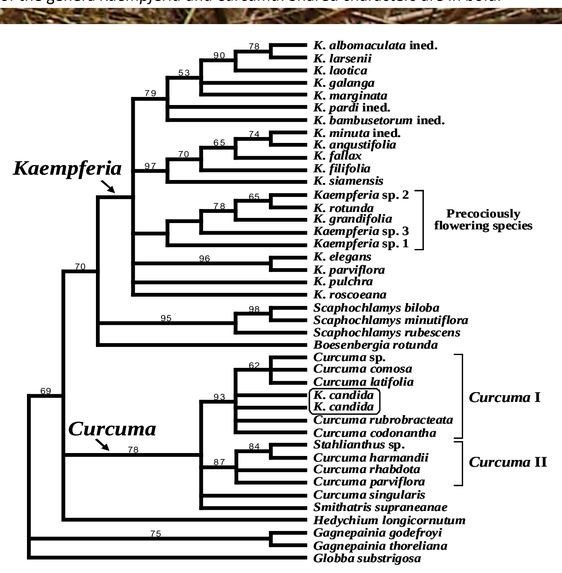

Figure 3. The strict consensus tree of 5,589 most parsimonious trees based on combined chloroplast psbA-trnH and partial petA-psbJ data generated 212 steps with consistency index (CI), retention index (RI), and homoplasy index (HI) of 0.85, 0.88, and 0.15, respectively. Numbers above branches indicate bootstrap values (>50%).

No.	Species	Geographic location	Voucher Coll. No. (Herbarium)	GenBank accession no. *psbA-trnH*	GenBank accession no. *petA-psbJ*	Note
1	*K. albomaculata* ined. Jenjitt. & K. Larsen	Thailand, Lop Buri	TT11560 (SLR)	GQ385994	GQ386077	Techaprasan et al., 2010
2	*K. angustifolia* Roscoe	Thailand, exact locality unknown	JT2005-13 (SLR)	GQ386041	GQ386124	Techaprasan et al., 2010
3	*K. bambusetorum* ined. K. Larsen & Jenjitt.	Thailand, Saraburi, Phra Phutthabat	TT11559-2 (SLR)	GQ385999	GQ386082	Techaprasan et al., 2010
4	*K. candida* Wall.	Thailand, Kanchanaburi, Thong Pha Phum	JT2007-7 (SLR)	GQ386003	GQ386086	Techaprasan et al., 2010
5	*K. candida* Wall.	Thailand, Tak	TT15730 (SLR)	GQ386004	GQ386087	Techaprasan et al., 2010
6	*K. elegans* Wall.	Thailand, Kamphaeng Phet, Mae Wong	JT2007-40 (SLR)	GQ386005	GQ386088	Techaprasan et al., 2010
7	*K. fallax* Gagnep.	Thailand, Ubon Ratchathani, Pha Luang	TT16670 (SING, SLR)	GQ386019	GQ386102	Techaprasan et al., 2010
8	*K. filifolia* K. Larsen	Thailand, Ubon Ratchathani, Pha Luang	TT16669 (SING, SLR)	GQ386023	GQ386106	Techaprasan et al., 2010
9	*K. galanga* L.	Thailand, Tak, Phop Phra	TT16452 (SING, SLR)	GQ385979	GQ386062	Techaprasan et al., 2010
10	*K. grandifolia* S. Saensouk & Jenjitt.	Thailand, Khon Kaen	JT2007-3 (SLR)	GQ386017	GQ386100	Techaprasan et al., 2010
11	*K. laotica* Gagnep.	Thailand, Ubon Ratchathani, Sroi Sawan	TT16535 (SING, SLR)	GQ385987	GQ386070	Techaprasan et al., 2010
12	*K. larsenii* Sirirugsa	Thailand, Ubon Ratchathani	JT2006-5 (SLR)	GQ385990	GQ386073	Techaprasan et al., 2010
13	*K. marginata* Carey ex Roscoe	Thailand, Khon Kaen	JT2007-4 (SLR)	GQ385981	GQ386064	Techaprasan et al., 2010
14	*K. minuta* ined. Jenjitt. & K. Larsen	Thailand, Ubon Ratchathani, Pa Dong Na Tam	TT16550 (SING, SLR)	GQ386043	GQ386126	Techaprasan et al., 2010
15	*K. pardi* ined. K. Larsen & Jenjitt.	Thailand, Phetchabun, Si Thep	JT2006-3 (SLR)	GQ385997	GQ386080	Techaprasan et al., 2010
16	*K. parviflora* Wall. ex Baker	Thailand, Phetchabun, Khao Kho	TT15691 (SING, SLR)	GQ386012	GQ386095	Techaprasan et al., 2010
17	*K. pulchra* Ridl.	Thailand, Prachuap Khiri Khan, Kha On	JT2007-16 (SLR)	GQ386025	GQ386108	Techaprasan et al., 2010
18	*K. roscoeana* Wall.	Thailand, Kanchanaburi, Sai Yok	JT2007-10 (SLR)	GQ386029	GQ386112	Techaprasan et al., 2010
19	*K. rotunda* L.	Thailand, Tak, Ban Rai	JT2007-12 (SLR)	GQ386031	GQ386114	Techaprasan et al., 2010
20	*K. siamensis* Sirirugsa	Thailand, Ubon Ratchathani, Phu Prao	JT2007-25 (SLR)	GQ386039	GQ386122	Techaprasan et al., 2010
21	*Kaempferia* sp. 1 (Proh Mueang Kan)	Thailand, Kanchanaburi, Sai Yok	JT2007-18 (SLR)	GQ386002	GQ386085	Techaprasan et al., 2010
22	*Kaempferia* sp. 2 (Proh Mang Mum)	Thailand, Tak, Pa Hua Khao Din	TT15793 (SLR)	GQ386038	GQ386121	Techaprasan et al., 2010
23	*Kaempferia* sp. 3	Thailand, Saraburi, Muak Lek	JT2006-1 (SLR)	GQ386000	GQ386083	Techaprasan et al., 2010
24	*Boesenbergia rotunda* (L.) Mansf.	Singapore, cultivated	JLS-222 (SING)	HM749001	HM749012	
25	*Curcuma* sp.	Thailand, Nakhon Ratchasima	JT2010-19 (SLR)	HM748993	HM749004	
26	*C. codonantha* Škorničk., M. Sabu & Prasanthk.	India, Andaman Islands	73319 (SING)	HM748999	HM749010	
27	*C. comosa* Roxb.	Thailand, Sakon Nakhon	WICH01 (PBM)	HM748994	HM749005	
28	*C. harmandii* Gagnep.	Thailand, Chachoengsao	73327 (E, SING)	HM748996	HM749007	
29	*C. latifolia* Roscoe	Thailand, Kanchanaburi, Sai Yok	6316 (SLR)	HM748995	HM749006	
30	*C. parviflora* Wall.	Thailand, Saraburi, Muak Lek	JT2006-2 (SLR)	HM748998	HM749009	
31	*C. rhabdota* Sirirugsa & M. F. Newman	Laos, exact locality unknown	73331 (E, SING)	HM748997	HM749008	
32	*C. rubrobracteata* Škorničk., M. Sabu & Prasanthk.	Thailand, Kanchanaburi, Thong Pha Phum	TT18965 (SLR)	HM748992	HM749003	
33	*C. singularis* Gagnep.	Thailand, exact locality unknown	TT16338 (SING, SLR)	HM749000	HM749011	
34	*Gagnepainia godefroyi* (Baill.) K. Schum.	Thailand, Saraburi, Sam Lan	JT2007-14 (SLR)	GQ386050	GQ386133	Techaprasan et al., 2010
35	*Gagnepainia thoreliana* (Baill.) K. Schum.	Thailand, Kanchanaburi Thong Pha Phum	JT2007-6 (SLR)	GQ386051	GQ386134	Techaprasan et al., 2010
36	*Globba substrigosa* King ex Baker	Thailand, Kanchanaburi, Pa Khao Yai	JT2008-6 (SLR)	GQ386048	GQ386131	Techaprasan et al., 2010
37	*Hedychium longicornutum* Griff. ex Baker	Malay Peninsula, exact locality unknown	SBG 20060419 (SING)	HM749002	HM749013	
38	*Scaphochlamys biloba* (Ridl.) Holttum	Thailand, Narathiwat, Su-ngai Padi	JT2007-1 (SLR)	GQ386057	GQ386140	Techaprasan et al., 2010
39	*Scaphochlamys minutiflora* Jenjitt. & K. Larsen	Thailand, Narathiwat, Su-ngai Padi	JT2007-2 (SLR)	GQ386058	GQ386141	Techaprasan et al., 2010
40	*Scaphochlamys rubescens* Jenjitt. & K. Larsen	Thailand, Narathiwat	JT2004-1 (SLR)	DQ408335	DQ104878	Techaprasan et al., 2006
41	*Smithatris supraneanae* W. J. Kress & K. Larsen	Thailand, Lop Buri	TT11561 (SLR)	GQ386055	GQ386138	Techaprasan et al., 2010
42	*Stahlianthus* sp.	Thailand, exact locality unknown	JT2005-15 (SLR)	GQ386047	GQ386130	Techaprasan et al., 2010

Table 1. List of plant samples used in this study. Abbreviation SLR has been adopted here for Suan Luang Rama IX Herbarium.

FORMAL TRANSFER & TYPIFICATION

***Curcuma candida* (Wall.) Techaprasan & Škorničk. comb. nov., in Nord. J. Bot. (in press)**

Basionym: *Kaempferia candida* Wall. Pl. Asiat. Rar. I., part 3, 47, t. 56 (1830)

Typus: *Kaempferia candida*, Moalmyne, 1827, Wallich Cat. No. 6585 (K-W!, proposed lectotype); [icon in] tab 56. in Wallich, Pl. Asiat. Rar. I., part 3, 1830 (proposed epitype).

While in the previous work Jenjittikul & Larsen (2000) indicated that the Wallich specimen, Wall. Cat. No. 6585 at Kew is a holotype, it has to be acknowledged that Wallich did not designate a type specimen nor cite any herbarium material. In conformity with ICBN Art. 9.10., all duplicates of Wallich specimen, Wall. Cat. No. 6585 (Wallich, 1829-1832), as well as the excellent colour painting accompanying Wallich's original description (tab. 56), are considered to be part of the original material suitable for selection as lectotype. Leong-Škorničková et al. (2010) have explained that ideally the specimen and the drawing (if it exists) form material adequate for interpreting historical species names in Zingiberaceae. Therefore, we propose to designate the Wallich specimen, Wall. Cat. No. 6585 at K-W as the lectotype, supported by the colour drawing tab. 56 as its epitype (Fig. 1).

WHAT NEXT?

Due to limited numbers of parsimoniously informative characters, evolutionary relationships within the *Curcuma* clade were unresolved. To determine the closest relative of *Curcuma candida* and to elucidate the evolutionary relationships and possible occurrences of hybridization within the *Curcuma* clade, more samples of *Curcuma* species, and more informative data from other DNA regions are required. *Stahlianthus* sp. and *Smithatris supraneanae* were also nested within the *Curcuma* clade, a result which agrees with previous phylogenetic studies of Zingiberaceae and Zingibereae respectively using both nuclear and chloroplast DNA sequences (Kress et al. 2002, Ngamriabsakul et al. 2004). Work on a new infra-generic classification of the genus *Curcuma* and refinement of the position of *C. candida* is in progress by Záveská et al.

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