TAXONOMY AND DNA FINGERPRINTS OF THE GENUS *CAULOKAEMPFERIA* K. LARSEN (ZINGIBERACEAE) IN THAILAND AND LAO PDR

BOONMEE PHOKHAM

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy Program in Health Sciences at Mahasarakham University

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The examining committee has unanimously approved this dissertation, submitted by Mr. Boonmee Phokham, as a partial fulfillment of the requirements for the Doctor of Philosophy in Health Sciences at Mahasarakham University.

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ชื่อเรื่อง อนุกรมวิธานและลายพิมพ์ดีเอ็นเอของพืชสมุนไพรสกุลเปราะต้น (วงศ์ขิง) ที่พบในประเทศไทย และสาธารณรัฐประชาธิปไตยประชาชนลาว
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บทคัดย่อ

พืชสมุนไพรสกุลเปราะต้น (*Caulokaempferia* K. Larsen) เป็นพืชวงศ์ขิง (Zingiberaceae) สกุลหนึ่ง มีสมาชิกราว ๓๐ ชนิด กระจายพันธุ์ตั้งแต่บริเวณเทือกเขาหิมาลัย จนถึงภาคใต้ของจีน ไทย ลาว และเวียดนาม โดยมีศูนย์กลางของความหลากหลายที่ประเทศไทย บางชนิดมีความสำคัญเชิงพฤกษศาสตร์พื้นบ้าน และบางชนิดยังมีข้อถกเถียงในเชิงอนุกรมวิธาน การศึกษานี้มีวัตถุประสงค์เพื่อทบทวนพืชสกุลนี้เชิงอนุกรมวิธาน โดยเฉพาะชนิดที่พบกระจายพันธุ์ในประเทศไทยและสาธารณรัฐประชาธิปไตยประชาชนลาว โดยการศึกษาตัวอย่างพรรณไม้ในถิ่นกำเนิดของพรรณไม้ต้นแบบ และตัวอย่างพรรณไม้แห้ง ผลการศึกษาพบว่าในประเทศไทยมีพืชสกุลนี้ ๑๙ ชนิด ในประเทศลาวมี ๒ ชนิด รวมทั้งสิ้น ๒๑ ชนิด เป็นชนิดใหม่ ๓ ชนิดจากประเทศไทย ได้แก่ *Caulokaempferia pubescens* Picheans & Phokham, *C. picheansoonthonii* Phokham & Prasarn และ *C. kamthorniana* Picheans., Prasarn & Phokham (*sp. nov.*) ในรายงานนี้ได้จัดทำรูปวิธานจำแนกชนิด คำบรรยายลักษณะทางพฤกษศาสตร์ ภาพวาดลายเส้นและภาพถ่ายสีบางชนิด ตลอดจนข้อมูลด้านการกระจายพันธุ์ นิเวศวิทยา และพฤกษศาสตร์พื้นบ้าน ส่วนการศึกษาความสัมพันธ์เชิงวิวัฒนาการชาติพันธุ์ของพืชสกุลนี้ โดยใช้ข้อมูลจากลำดับนิวคลีโอไทด์ในช่วง ITS และ *trn*L-F ด้วยวิธี Maximum Likelihood พบว่าทุกชนิดมาจากสายวิวัฒนาการชาติพันธุ์เดียวกันที่ค่าความเชื่อมั่นระดับสูง (bootstrap = 80-100 %) สามารถแบ่งออกเป็น 4 กลุ่ม ได้แก่ กลุ่มที่ 1 กลุ่มเปราะต้นชยันต์ (*C. chayaniana* group) กลุ่มที่ 2 กลุ่มเปราะต้นพิเชียรสุนทร (*C. picheansoonthonii* group) กลุ่มที่ 3 กลุ่มเปราะต้นจีรวงส์ (*C. jirawongsei* group) และกลุ่มที่ 4 กลุ่มเปราะต้นสตูล (*C. satunensis* group) ตามลำดับ

คำสำคัญ: เปราะต้น, ดีเอ็นเอ, พืชวงศ์ขิง, อนุกรมวิธาน

TITLE Taxonomy and DNA fingerprints of the genus *Caulokaempferia* K. Larsen (Zingiberaceae) in Thailand and Lao PDR

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ABSTRACT

The medicinal plants genus *Caulokaempferia* K. Larsen (Zingiberaceae) comprise about 30 taxa distributed from the Himalaya, south China, Vietnam, Laos, and Thailand, with center of diversity in Thailand. Some species are ethnobotanically important, whereas some taxa are, to date, taxonomically controversial. This study focused mainly in revising taxonomically the members of the plant group distributed in Thailand and Lao PDR. Living specimens of all known species in their type locations and herbarium specimens from major herbaria were thoroughly examined. A total of 21 taxa, 19 from Thailand and 2 from Lao PDR were enumerated, including 3 new taxa from Thailand: *C. pubescens* Picheans. & Phokham, *C. picheansoonthonii* Phokham & Prasarn and *C. kamthorniana* Picheans., Prasarn & Phokham (*sp. nov.*). Key to species, detailed descriptions, distribution, ecology and ethnobotanical information, including line-drawing and photographic illustration of some taxa, were given. From phylogenetic study of these 21 species (32 taxa) based on ITS and *trn*LF sequence data and the Maximum Likelihood analyses indicated that all species are monophyletic. Four clades are established with strongly supported bootstrap value 80-100 %: clade I *C. chayaniana* group, clade II *C. picheansoonthonii* group, clade III *C. jirawongsei* group, and clade IV *C. satunensis* group, respectively, detail of which are discussed.

Key Words: *Caulokaempferia*, DNA, Taxonomy, Zingiberaceae

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List of Abbreviations

A, T, G, C	Nucleotides containing the base adenine, thymine, quinine and cytosine, respectively
BK	Bangkok Herbarium
BKF	Forest Herbarium
bp	Base pairs
°C	Degree Celsius
ca	about
CI	Consistency index
cm	Centimeter
CMU	Chiang Mai University Herbarium
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide triphosphates (dATP, dTTP, dGTP, dCTP)
gDNA	Genomic deoxyribonucleic acid
ITS1	Internal transcribed spacer 1
ITS2	Internal transcribed spacer 2
E	Royal Botanic Garden, Edinburgh
K	Royal Botanic Gardens, Kew Herbarium
$MgCl_2$	Magnesium Chloride
mm	Millimeter
mM	Millimolar
P	Herbier National de Paris
PCR	Polymerase chain reaction
PSU	Prince of Songkla University Herbarium
QBG	Queen Sirikit Botanic Garden Herbarium
RC	Rescaled consistency index
RI	Retention index
SING	Singapore Botanic Gardens Herbarium
µl	Microliter
µM	Micromolar
UV	Ultraviolet

CHAPTER 1

INTRODUCTION

1.1 Background

The Zingiberaceae or ginger family is a monocotyledonae. About 50 genera and *ca.* 1,500 species of Zingiberaceae are represented. So far, 26 genera and *ca.* 300 species, have been recorded from Thailand (Larsen, 2003)

The genus *Caulokaempferia* K. Larsen is taxonomically placed in the family Zingiberaceae. This genus was established by K. Larsen (1964), with 7 species in the Himalaya, China, Vietnam and Thailand. *Caulokaempferia linearis* (Wall.) K. Larsen, one of the species distributed in the Himalayan, was assigned as type for this genus. The genus for Thailand and Lao PDR were reported in 18 species (Chaiyoot, 2007). Some points are contradicted in the genus *Caulokaempferia* (the genus *Jirawongsea*; Picheansoonthon et al., 2008*a*)

Ethnobotanical study in the northeastern Mizoram State of India revealed the use of *C. linearis* (Wall.) K. Larsen by the Chakma tribes for vertigo (Rai & Lalramnghinglova, 2010). Furthermore, Lepcha tribe in India use *C. sikkimensis* (King) K. Larsen for bone dislocation or fracture (Pradhan & Badola, 2008). *C. phutokensis*, *C. phuwoaensis* and *C. phulangkaensis* in northeastern Thailand, are used as medicinal plants (Picheansoonthon & Koonterm, 2008) and *C. chayaniana* in the Shan ethnic group used the whole plants with other herbs for gastric disorders (Tiyaworanant, 2010).

To lay out fundamental knowledge of this genus for future studies of the genus in Thailand and Lao PDR, where the genus is centrally localized, it is an urgent need to fully understand the taxa distributed in Thailand and Lao PDR.

1.2 Research objectives

1.2.1 To revise taxonomically the species diversity of the genus *Caulokaempferi*a K. Larsen in Thailand and Lao PDR

1.2.2 To obtain molecular information on the genus *Caulokaempferi*a K. Larsen in Thailand and Lao PDR

1.3 Scope of research

The scope of this study includes taxonomy and molecular study of the genus *Caulokaempferia* K. Larsen in Thailand and Lao PDR. Plant specimens were be collected from the type location of each species reported in the literature. The specimens were be compared with type specimens and related specimens in major herbaria. Then, an artificial key to the species was constructed. The young leaves of all samples were also collected for molecular study. The DNA (ITS1-ITS2 and trnL-F regions) was extracted, amplified, and sequenced, the results of which were analyzed and the phylogenetic relationship was proposed.

1.4 Research sites

1.4.1 All of herbarium specimens of the genus *Caulokaemferia* K. Larsen available in major Herbaria.

1.4.2 Type locations of the genus focused on taxa in Thailand and Lao PDR.

1.4.3 Faculty of Medicine, Mahasarakham University and Faculty of Sciences, Mahidol University.

1.5 The benefits expected

1.5.1 Taxonomic information of the genus *Caulokaempferia* K. Larsen in Thailand and Lao PDR will be comprehensively documented.

1.5.2 Phylogenetic relationship of all Thai and Lao *Caulokaempferia* is proposed.

CHAPTER 2

LITERATURE REVIEW

2.1 Taxonomy

The Zingiberaceae, or Ginger family, belongs to the order Zingiberales (previously known as Scitamineae) of the class Monocotyledonae. This family was previously divided into 4 tribes: *Globbeae, Zingibereae, Hedychieae* and *Alpinieae* (Smith, 1981). Recently based on molecular studies by Kress and his colleagues (2002), 4 subfamilies were proposed: Zingiberoideae (2 tribes, Zingibereae and Globbeae), Alpinoideae (2 tribes, Alpinieae and Riedelieae), Tamijioidae (only one species, *Tamijia flagellaris*) and Siphonochiloideae (1 genus, *Siphonochilus*). About 50 genera and *ca.* 1,500 species of Zingiberaceae are worldwide represented, and so far *ca.* 300 species (of 26 genera) have been found in Thailand (Larsen, 2003).

In 1820, N. Wallich described 2 new *Kampferia* species: *K. linearis* Wall. and *K. secunda* Wall. and placed them under the group '*caulescent with an entire crest*' (Roxburgh, 1820). He, a decade later, added the third species, *K. elegans* Wall., to this group with an important taxonomic comment that '…*all three ought perhaps to be removed from Kaempferia, and formed into a distinct genus, for which I would propose the name of Monolophus, in allusion to the entire crest of the anther*…' (Wallich, 1830). In this publication, he proposed a provisional generic name, *Monolophus* Wall.

Wallich (1832) used the name, *Monolophus*, in his publication for the above mentioned 3 species: *M. linearis* Wall., *M. secunda* Wall., and *M. elegans* Wall. Since then, the name *Monolophus* Wall. was an except name. After that, the name '*Monolophus*' had been used by several authors: Endlicher (1837), Steudel (1841), and Horaninov (1862). Bentham (1883) treated this plant group in *Kaempferia* L. section *Stachanthesis* Benth. Baker (1894), Schumann (1904) and Loesener (1930) treated *Monolophus* Wall. as a subgenus of the genus *Kaempferia* L.

Larsen (1964) rejected the name *Monolophus* Wall. based on 2 controversial reasons, '*…(1) The type species of* WALLICH*'s Monolophus is a Kaempferia and the description given by* WALLICH *thus does not hold for the group in its sense for today. (2)*

The name refers to a character widely distributed also in neighbouring genera…'. He, therefore, established the new genus, *Caulokaempferia* K. Larsen, and transferred 4 taxa, previously placed either under *Kaempferia* L. or *Monolophus* Wall. to his new genus: 3 Indian [*C. linearis* (Wall.) K. Larsen and *C. secunda* (Wall.) K. Larsen, and *C. sikkimensis* (King) K. Larsen] and 1 Chinese [*C. coenobialis* (Hance) K. Larsen] species. He also added 2 new species (*C. kuapii* K. Larsen and *C. saxicola* K. Larsen) from Thailand and a new taxon (*C. petelotii* K. Larsen) from Vietnam to his account. In this treatment, K. Larsen assigned *C. linearis* (Wall.) K. Larsen as the type specimen for the genus.

Few years later, K. Larsen and his colleague, R.M. Smith, added a new taxon, *C. alba* K. Larsen & R.M. Smith, to the genus *Caulokaempferia* (Larsen & Smith, 1972). They also proposed 2 new sections: section *C. alba* K. Larsen & R.M. Smith (for *C. alba* K. Larsen & R.M. Smith) and section *Pyrgophyllum* [for *C. yunnanensis* (Gagnep.) R.M. Smith, transferred from *Kaemferia yunnanensis* Gagnep]. In 1973, K. Larsen added another 2 new species from Thailand: *C. saksuwaniae* K. Larsen and *C. thailandica* K. Larsen (Larsen, 1973).

T.L. Wu and S. J. Chen (1978) used the generic name *Monolophus* Wall. as an accepted name, and *Caulokaempferia* K. Larsen was placed there under a new synonymous name (*syn. nov.*). He also proposed *M. linearis* Wall. as a lectotype of the genus. However, that year, he did change his mind since he accepted the name *Caulokaempferia* K. Larsen in his account of family Zingiberaceae in 'Flora Reipublicae Popularis Sinica (Chinese edition)' (Wu, 1978). In Flora of Bhutan, the name *Caulokaempferia* K. Larsen was accepted with only 1 species, *C. sikkimensis* (Noltie, 1994). In 'Flora of China (English edition)' the name *Caulokaempferia* was accepted by the Chinese group again, but moved *C. yunnanensis* (Gagnep.) K. Larsen to form a new endemic monotypic genus *Pyrgophyllum* T.L. Wu & Z.Y. Wu. (Wu & Larsen, 2000).

In 2003, K. Larsen and his Thai colleagues reported 3 new species from Thailand, *C. appendiculata* K. Larsen & T. Triboun, *C. bracteata* K. Larsen & S.S. Larsen, and *C. violacea* K. Larsen & S.S. Larsen. It is interesting to note that the type specimen of *C. bracteata* was a purchased specimen from 'Chatuchak Flower Market in Bangkok (brought over from Changwat Nong Khai, a border town to Lao PDR)'

(Larsen, 2003). However, in 2008, Picheansoonthon and his co-workers confirmed that *C. bracteata* K. Larsen & S.S. Larsen is not a Thai species. It was collected from the Laotian site, Phou Khao Khouay National Biodiversity Conservation Area (NBCA) across the Mekhong, and transport to sell in the trans-border market in Changwat Nong Khai of Thailand (Picheansoonthon *et al*, 2008*b*).

Picheansoonthon and Mokkamol added another 4 new species, *C. khaomaenensis* Picheans. & Mokkamul (Picheansoonthon & Mokkamul, 2004*a*), *C. phuluangensis* Picheans. & Mokkamul, *C. jirawongsei* Picheans. & Mokkamul (Picheansoonthon & Mokkamul, 2004*b*) and *C. limiana* Mokkamul & Picheans. (Mokkamul & Picheansoonthon, 2004) from Thailand. In this same year, K. Larsen and Jenjittikul (2004) added a new species, *C .burttii* K. Larsen & Jenjitt., from Lao PDR, based on specimen retrieved from Chatuchak Flower Market in Bangkok (brought over from Champasak Province in southern Lao PDR). Furthermore, Suksathan and Triboun (2004) also reported a new species, *C. larsenii* Suksathan & Triboun from northern Thailand.

K. Larsen and his Thai colleagues, reported another two new taxa, *C. amplexicaulis* Suksuthan and *C. pedemontana* Triboun & K. Larsen from Thailand (Larsen *et al*, 2005). Picheansoonthon and Mokkamul (2006) described a new species, *C. laotica* Picheans. & Mokkamul, from southern Lao PDR. In 2007, Picheansoonthon and his co-workers further added a new species, *C. satunensis* Picheans., from southern Thailand, marked the southernmost distribution limit of the genus. (Picheansoonthon *et al*, 2007).

Picheansoonthon and Koonterm (2008) added *C. bolavenensis* Picheans. & Koonterm from southern Lao PDR. They also reported *C. alba* K. Larsen & R.M. Smith a new record for Lao PDR and confirmed the type locations of *C. bracteata* K. Larsen & S.S. Larsen and *C. burtii* K. Larsen & Jenjitt. in Lao PDR (Picheansoonthon *et al*, 2008*a*). In same year, 3 others new species *C. phuwoaenensis* Picheans. & Koonterm, *C. phulangkaensis* Picheans., and *C. phutokensis* Picheans. from northeastern Thailand were described (Picheansoonthon & Koonterm, 2008).

In 2008, Picheansoonthon and his co-worker established a new genus, *Jirawongsea* Picheans. based on morphological characters and molecular evidence (Picheansoonthon *et al*, 2008*b*). Three non-yellow flowered *Caulokaempferia* species

were transferred to this new genus: *J. alba* K. Larsen & R.M. Smith, *J. burtii* (K. Larsen & Jenjitt.) Picheans. and *J. laotica* (Picheans. & Mokkamul) Picheans. In this publication, the authors also reported *C. thailandica* K. Larsen and *C. violacea* K. Larsen & T. Triboun as a synonymous to *J. alba* (K. Larsen & R.M. Smith) Picheans.

Ngamriabsakul (2009) added *C. sririrugsae* Ngamr., a new species from south Thailand. Tiyaworanant (2010) further reported *C. chayaniana* Tiyaw. as a new taxon from northern Thailand. The genus *Caulokaempferia* K. Larsen in India was revised with more detailed information on all 3 species: *C. linearis* (Wall.) K. Larsen, *C. secunda* (Wall.) K. Larsen, and *C. sikkimensis* (King) K. Larsen (Roy & Barbhuiya, 2013). *C. tamdaoensis* Picheans. & Inthar., a new taxon from Vietnam was also described (Intharapichai *et al*, 2014)**.**

J.D. Mood and J.F. Velkamp (2014) considered the name *Caulokaempferia* K. Larsen superfluous, and restated *Monolophus* Wall. as accepted generic name. Instead of conserving the name, the authors who only seen few specimens of the genus, unnecessarily combined all the previously described *Caulokaempferia* taxa and created more than 20 plant names with long author names, just because of the nomenclatural 'technical problem'. They also combined the genus *Jirawongsea* Picheans. with *Boesenbergia* O. Kuntz based, unfortunately, on molecular information of only 2 species, including 1 probably misidentified (Mood *et al*, 2014).

Phokham and his colleagues (2015) reported 2 new taxa for Thailand, *C. pubescens* Picheans & Phokham (Phokham *et al*, 2015*a*) and *C. picheansoonthonii* Phokham & Prasarn (Phokham *et al*, 2015*b*).

The genus *Caulokaempferia* for Thailand comprises 18 taxa.

1. *Caulokaempferia saxicola* K. Larsen (เปราะหิน)

   (syn. *C. kuapii* K. Larsen)

2. *C. saksuwaniae* K. Larsen (เปราะต้นศักดิ์สุวรรณ)

3. *C. appendiculata* K. Larsen & Triboun (เปราะต้นอ่างขาง)

4. *C. larsenii* Suksathan & Triboun (เปราะต้นผีปันน้ำ)

5. *C. khaomaenensis* Picheans. & Mokkamul (เปราะต้นเขาเหมน)

6. *C. phuluangensis* Picheans. & Mokkamul (เปราะต้นภูหลวง)

7. *C. jirawongsei* Picheans. & Mokkamul (เปราะต้นจีรวงส์)

8. *C. limiana* Picheans. & Mokkamul (เปราะต้นชาติตระการ)

9. *C. amplexicaulis* Suksuthan (เปราะต้นดอยปุย)

10. *C. pedemontana* Triboun & K. Larsen (เปราะต้นนางรอง)

11. *C. satunensis* Picheans. (เปราะต้นสตูล)

12. *C. phuwoaenensis* Picheans. & Koonterm (ว่านเปราะต้น, เปราะต้นภูวัว)

13. *C. phulangkaensis* Picheans. (ว่านเปราะหิน, เปราะหินภูลังกา, เปราะต้นภูลังกา)

14. *C. phutokensis* Picheans. (ว่านเปราะหิน, เปราะต้นภูทอก)

15. *C. sirirugsae* Ngamr. (เปราะต้นศิริรักษ์)

16. *C. chayaniana* Tiyaw. (เปราะต้นชยันต์)

17. *C. pubescens* Picheans. & Phokham (เปราะต้นใบขน)

18. *C. picheansoonthonii* Phokham & Prasarn (เปราะต้นพิเชียรสุนทร)

Two *Caulokaempferia* taxa were reported in Lao PDR

1. *Caulokaempferia bracteata* K. Larsen & S. S. Larsen (เปราะต้นภูเขาควาย)

2. *C. bolavenensis* Picheans. & Koonterm (เปราะต้นบ่อละเวน)

Five *Caulokaempferia* taxa were removed to *Jirawongsea*

1. *Caulokaempferia alba* K. Larsen & M. Smith (เปราะภูดอกขาว)

2. *C .burttii* K. Larsen & Jenjitt. (เปราะภูจำปาสัก)

3. *C. laotica* Picheans. & Mokkamul (เปราะภูประเทศลาว)

4. *C. thailandica* K. Larsen (เปราะภูประเทศไทย)

5. *C. violacea* K. Larsen & S.S. Larsen (เปราะภูดอกม่วง)

2.2 Molecular study

2.2.1 Two conserved regions: ITS and trnL-F

The comparison of nucleotide sequences at specific genomic regions between interested taxa has provided the means for analyzing the phylogenetic relationship within such population. Although the analysis in nucleotide sequences is a powerful tool, selection of appropriate regions for comparison demands several criteria especially the rate of mutation over evolutionary time, polymorphism contents, and the conserved regions for universal primers design. Normally, in most organisms, the regions on the genome which exist as genes encoding for functional proteins would undergo relatively slow mutation, thus, suitable for studying distantly related organisms. However, for the more closely related organisms such as phylogenetic relationship level of plant taxa within the same genus, family, or order, the nucleotide regions with high rate of mutation, e.g., the non-coding sequences, are legitimate. Many molecular phylogenetic studies in plant organisms have focused on the comparison of non-coding nucleotide sequences that resident within or between conserved regions on both nuclear and chloroplast genomes, and revealed for their usefulness in classification and systematics of closely related plant taxa. Despite of redundancy in these potent regions, however, the investigation in phylogenetic relationship of *Caulokaempferia* taxa in this study would be conducted by using two conserved regions, i.e., Internal Transcribed Spacer (ITS) region on the nuclear genome and tRNA-Leucine - tRNA-Phenylalanine (trnL-F) region on the chloroplast genome.

The ITS region has become one of the most popular genomic region used to analyze nucleotide sequences among population based on evolutionary of nuclear genome (Álvarez and Wendel, 2003). The coding and non-coding units within primary transcript 45S ribosomal RNA (pre-rRNA) gene region on the nuclear genome are organized in tandem arrays of up to several thousand copies and widely known to contain the phylogenetic information during the long-time evolutionary of organisms (Rogers and Bendich, 1987). The region contains the coding small subunit (18S), 5.8S and large subunit (26S) rRNA genes separated by two non-coding internal transcribed spacers ‘ITS-1’ and ‘ITS-2’ as shown in Figure 2.1. Due to the fact that this region is evolutionary conserved, the non-coding units of ITS-1 and ITS-2 show high level of

interspecific divergence and have been used frequently in genetic diversity and molecular phylogenetic studies. A pair of universal primers, ITS1 (forward, 5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (reverse, 5′-TCCTCCGCTTATTGATATGC-3′), were previously designed to complementary to the conserved sequences of 18S rRNA gene and 26S rRNA gene, respectively (White *et al*, 1990). The primers are universal among many eukaryotic organisms ranging among fungi, plants, and animals. By using this universal primers pair in amplification from gDNA sample, the nucleotide sequence of partial 18S rRNA gene, complete ITS-1 spacer, complete 5.8S rRNA gene, complete ITS-2 spacer, and partial 26S rRNA gene would be revealed for phylogenetic analysis.

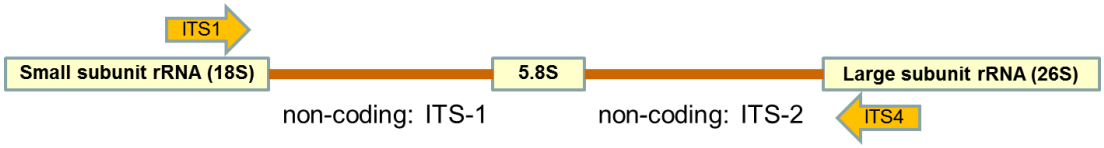

Figure 2.1 The diagram showing the coding and non-coding units of ITS region on the nuclear genome. The figure is not scaled to the actual nucleotide length proportional to each item. The complementary locations of ITS1 and ITS4 primers are roughly indicated.

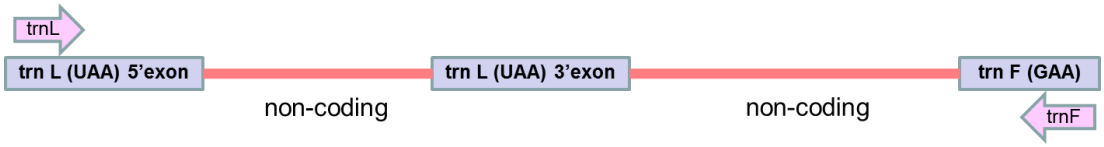

Figure 2.2 The diagram showing the coding and non-coding units of trnL-F region on the chloroplast genome. The figure is not scaled to the actual nucleotide length proportional to each item. The complementary locations of trnL and trnF primers are roughly indicated.

Chloroplast genome is circular double-stranded DNA which contains the size of approximately 100 kb to 200 kb in most plants, and could be greater in some algae. Many chloroplast genomes contain two inverted repeat sections (IRa and IRb), which separate a large single copy section (LSC) from a small single copy section (SSC)

(Shaw *et al*, 2007). Several non-coding regions on these sections are characterized to contain the polymorphism contents and commonly used in the phylogenetic analysis of plant taxa (Shaw *et al*, 2005). Specifically, some tRNA genes are known to localize on the large single copy section and the non-coding sequences of intergenic spacers between them contain the high rate of molecular events during the evolution. Among these, the non-coding sequences between the tRNA-Leucine (*trnL*) and tRNA-Phenylalanine (*trnF*) genes are utilized and widely used for phylogenetic studies of the land plants (Quandt *et al*, 2004). By analyzing the nucleotide sequences of several tRNA genes on chloroplast genome, the structure and location of *trnL* and *trnF* are revealed (Yamada *et al*, 1986). The *trnL* gene possesses the anticodon sequence of UAA and contains intron that separating the gene into 5′-exon and 3′-exon portions. While the *trnF* gene possesses the anticodon sequence of GAA with no intron. Therefore, within the region, two non-coding units are located; one is the intron sequence intercalated between the 5′- and 3′-exons of *trnL* gene and another is spacer between the *trnL* and *trnF* genes as shown in Figure 2.2. A pair of universal primers was previously designed to amplify the entire sequence of these non-coding sequences and universal over algae, terminal and high land plants (Taberlet *et al*, 1991). The forward primer, trnL, 5′- CGAAATCGGTAGACGCTACG-3′, was designed from the conserved sequence of 5′-exon of *trnL* gene. And the reverse primer, trnF, 5′-ATTTGAACTGGTGACACGAG-3′, was designed from the conserved sequence of *trnF* gene. By using this universal primers pair in amplification from gDNA sample, the nucleotide sequence of partial 5′-exon of *trnL* gene, complete intron of *trnL* gene, complete 3′-exon of *trnL* gene, complete intergenic spacer, and partial *trnF* gene would be revealed for phylogenetic analysis.

After the amplification and DNA sequencing using these two conserved universal primers pairs, by comparison the nucleotide sequences of collected *Caulokaempferia* accessions and the relatively related plant species, the phylogenetic analysis would reveal the relationship among them by the mean of evolutionary event over nuclear genome with ITS region, which would be supported by chloroplast genome with *trn*L-F region.

2.2.2 Molecular of the genus *Caulokaempferia* K. Larsen

A. Chaiyoot (2007) has studied on taxonomy and molecular of the genus *Caulokaempferia* in Thailand and Lao PDR and focused on DNA sequence, ITS region of 23 taxa. From molecular studies members of the genus are polyphyletic and can be divided into 2 groups; Group I, non-yellow flowers, inflorescences hidden in the two uppermost leaf sheaths (5 taxa) and Group II, yellow flowers inflorescences not hidden in the two uppermost sheaths (18 taxa).

CHAPTER 3

TAXONOMIC STUDY

3.1 Taxonomic Methodology

This study was based on field plant collections and herbarium specimens.

3.1.1 Intensive fieldworks in the type localities of existing taxa were carried on during June-September of 2011-2014.

3.1.2 Field notes on plant morphology, distribution and ecology studies were prepared. Photographs of plant specimens were taken from the collecting location.

3.1.3 Specimens were prepared in 95% Ethanol and Glycerin (1:1 ratio).

3.1.4 Additional information were retrieved from specimens deposited in major herbaria, e.g. BK, BKF, QBG, K and SING.

3.2 Taxonomic treatment of the genus *Caulokaempferia* K. Larsen Caulokaempferia (nom. cons.)

K. Larsen in Bot. Tidsskr. 60(3): 165–179. 1964; K. Larsen & R.M. Smith in Notes Roy. Bot. Gard. Edinb. 31(2): 287–295. 1972; T.L. Wu & S.J. Chen, Fl. Republicae Popularis Sinicae 16(2): 36–38. 1981; T.L. Wu in F.W. Chen & T.L. Wu, Fl. Guangdong 2: 403. 1991; R.M. Smith in H.J. Noltie, Fl. Bhutan 3(1): 196–198. 1994; S. Kuma in P.K. Hajra & D.M. Verma, Fl. Sikkim 1: 123. 1996; T.L. Wu & K. Larsen in Z.L. Wu & P.H. Raven, Fl. China 24: 377. 2000; D.K. Roy & H.A. Barbhuiya, NeBIO, 4(6): 1. 2013; Intharapichai *et al*, J. Jpn. Bot. 89: 129. 2014.—*Monolophus* Wall., Pl. Asiat. Rar. 1: 24, 1830, nom prov., illeg.; Number. List # 6591–6593, validation; Wall. ex Endl., Gen. Pl. 225. 1837, isonym.; Horan. Monogr. 22. 1862; T.L. Wu & S.J. Chen in zhi wu fen lei xue bao 16(3): 28-29. 1978; J.D. Mood *et al*, Gard. Bull. Sing., 66(2): 215. 2014.—*Kaempferia* L. subgen. *Monolophus* (Wall.) Wall. ex Baker in Hook.f., Fl. Br. Ind. 6: 222. 1892; K. Schum. in Engl. Pflanzenr. 4: 46. 1904; T. Loes. in A. Engl., Das Pflanzenr. 4: 566. 1930.

Perennial herbs, up to 57 cm high; *Pseudostems* slender, with 2-4 bladeless sheaths at base. *Leaves* sessile to petiolate; ligule membranous, apex acute to acuminate. *Inflorescences* terminal; bract(s) 1-10, distichous, lanceolate, 1-4-flowered, margin free to base; bracteoles membranous. *Flowers* purple, white, or yellow; calyx tubular, split on 1 side, apex 2-3-toothed; corolla tube long, narrowly, widened at mouth; 3-lobed, dorsal lobe, hooded, slightly longer and wider than lateral lobes, purple, white, or yellow; lateral staminodes petaloid, broadly elliptic to suborbicular, yellow; labellum broadly elliptic to suborbicular, apex crenate or 3-lobed, yellow; filament very short or absent; anther basifixed; connective forming a conspicuously reflexed crest; ovary 1- or 3-loculed; stylodes 2, short. *Fruits* capsular, oblong. *Seeds* numerous, arillodes.

Flower yellow *Caulokaempferia*, 21 species distributed in Thailand and Lao PDR.

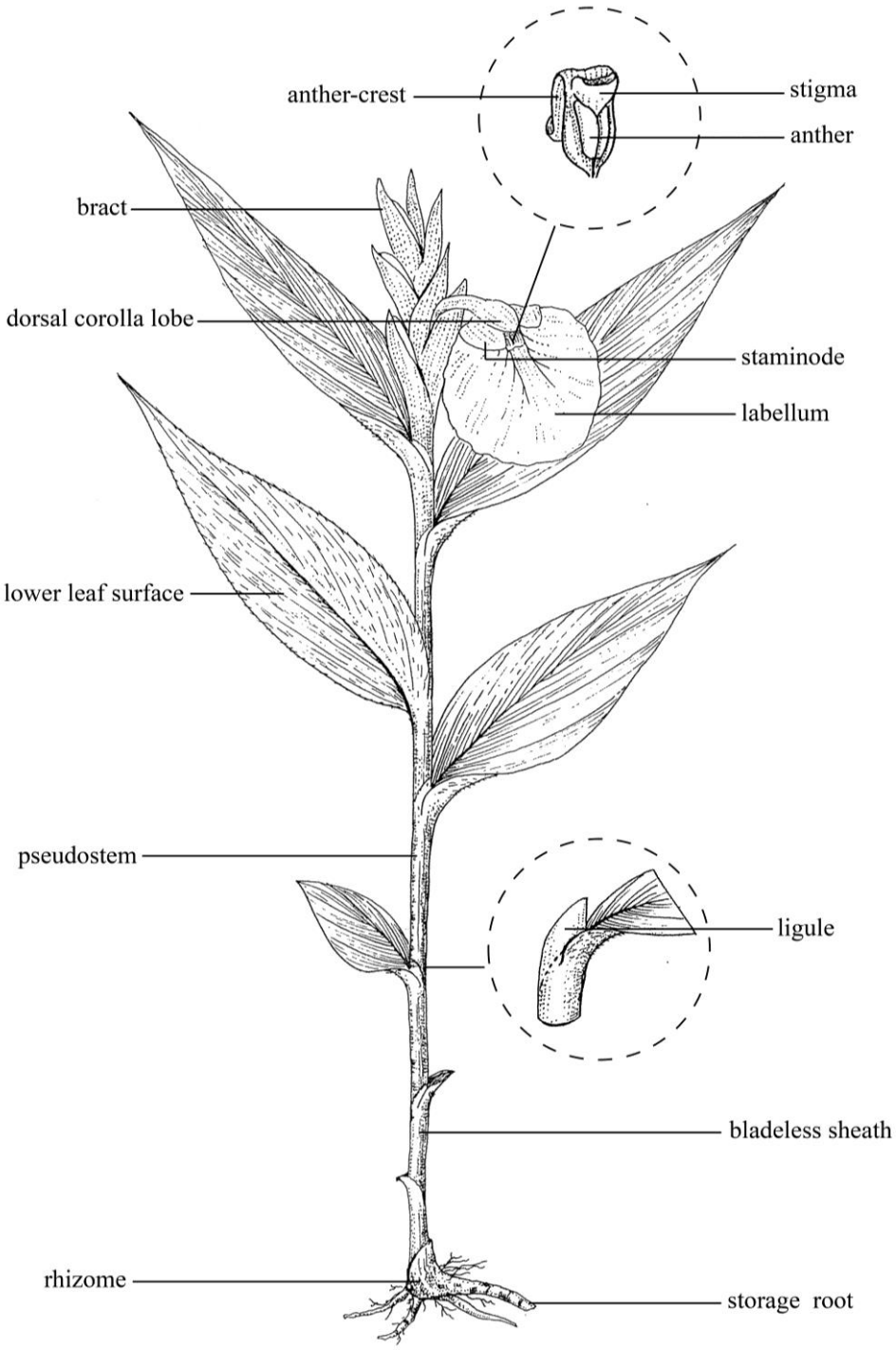

Figure 3.1 Illustrations of *Caulokaempferia* morphology.

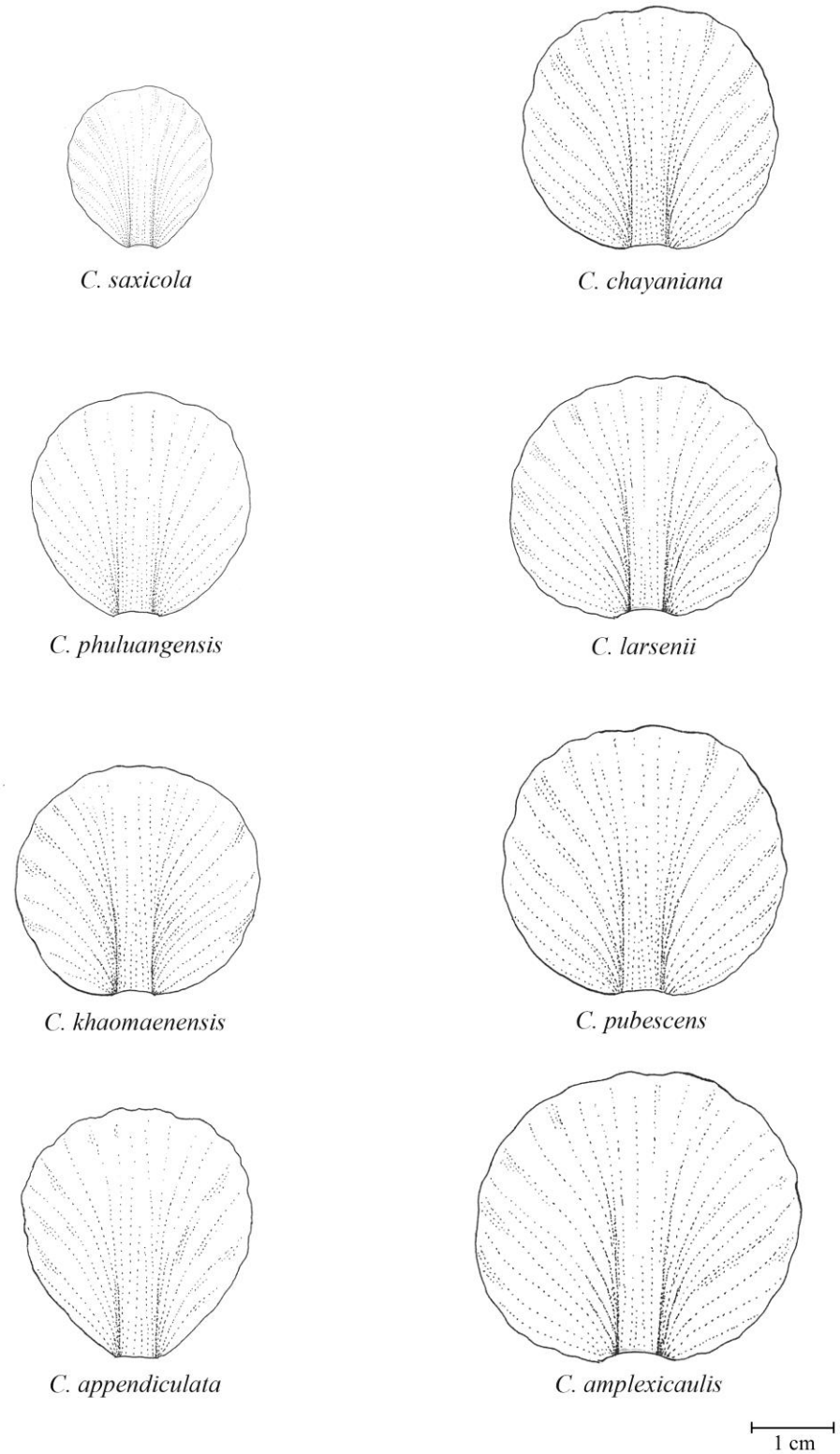

Figure 3.2 Illustrations of *Caulokaempferia* without median lobe labellum.

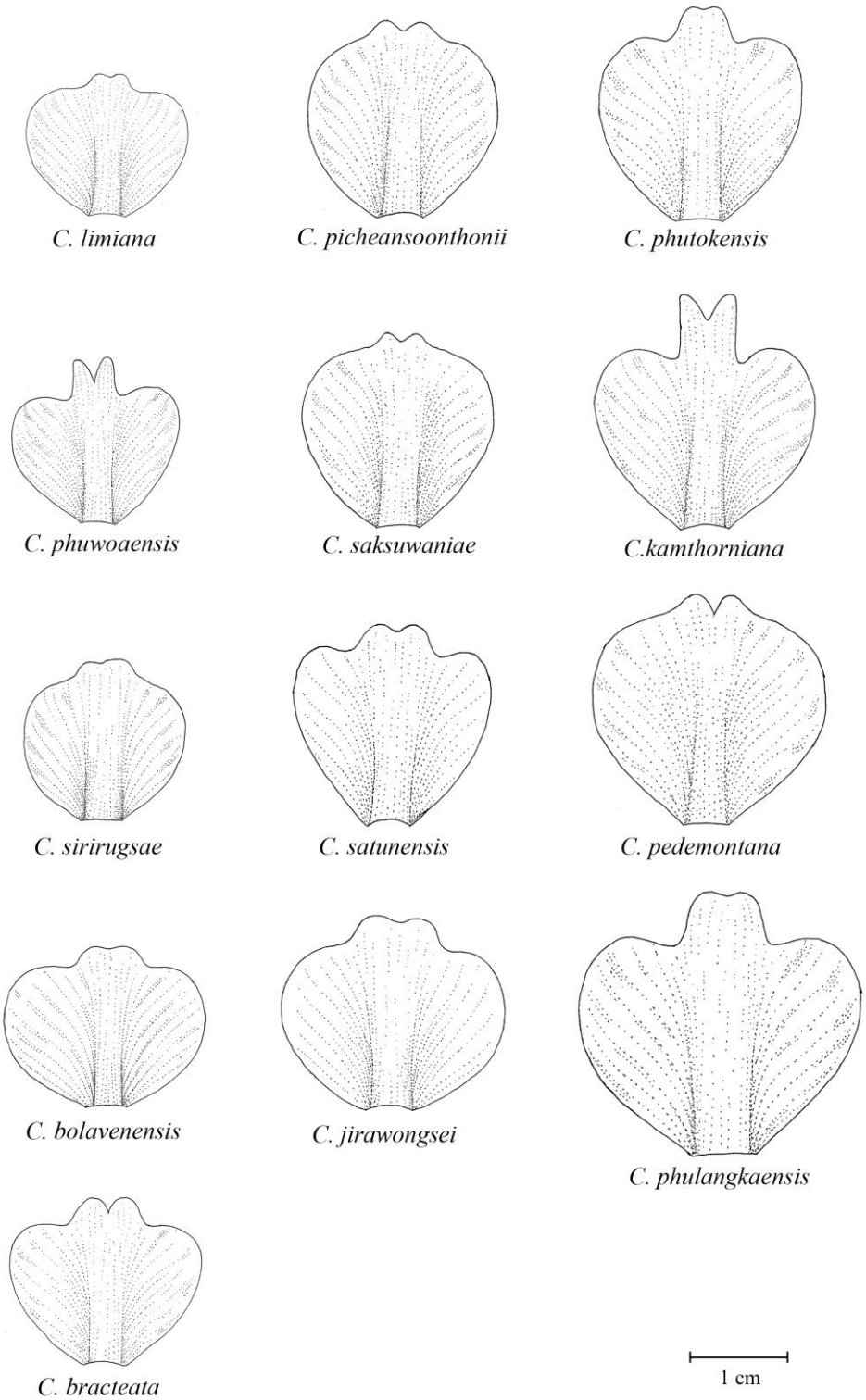

Figure 3.3 Illustrations of *Caulokaempferia* with median lobe labellum.

3.3 Key to *Caulokaempferia* species in Thailand and Lao PDR

1. Labellum broadly suborbicular to orbicular without median lobe, apex rounded..... 2
   2. Bract and axis hairy……..... **20. *C. saxicola***
   2. Bract and axis glabrous……..... 3
      3. Leaf base amplexicaul……..... 4
         4. Bract leaf-liked apices, strictly 1-flowered bract..... **1. *C. amplexicaulis***
         4. Bract acute apices, more than 1-flowered bract..... **5. *C. chayaniana***
      3. Leaf base not amplexicaul…….. 5
         5. Leaf blades pubescence on lower surface..... **17. *C. pubescens***
         5. Leaf blades glabrous on both surface 6
            6. Bract appendix, reddish…… ..... **2. *C. appendiculata***
            6. Bract not appendix, greenish…….. 7
               7. Ligule 1-1.2 cm long, axis with glands ..... **8. *C. khaomaenensis***
               7. Ligule < 1 cm long, axis without glands 8
                  8. Pistil longer than stamen, style inserted between anther sacs.. .....
                  ……...……...…… ..... **13. *C. phuluangensis***
                  8. Pistil shorter than stamen, style not inserted between anther sacs.. ...
                  ……..……...……..…… ..... **9. *C. larsenii***

1. Labellum broadly obovate with median lobe, apex trilobe or bilobe with slightly emarginated…….. 9
   9. Bract none, inflorescence sessile……..... **16. *C. picheansoonthonii***
   9. Bract present, inflorescence pedunculated……..... 10
      10. Bracts imbricate, axis invisible or partially visible in flowering stage…… ... 11
         11. Bracts completely surrounded an inflorescence axis (axis invisible)..... 12
            12. Leaf blades broadly ovate to ovate-elliptic…..... **14. *C. phutokensis***
            12. Leaf blades elliptic to narrowly lanceolate… ..... **7. *C. kamthorniana***
         11. Bracts amplexicaul base, margin free to the base, axis partially visible on one side..... 13
            13. Ligule 6-10 mm long, bract reddish-green and distal recurved….. .....
            ……..... **6. *C. jirawongsei***

13. Ligule up to 5 mm long, bract green and distal not recurved…. 14

14. Bract, leaf sheath and axis with glands… ..... **4. *C. bracteata***

14. Bract, leaf sheath and axis without glands… 15

15. Leaf blades elliptic to ovate, 3-6 leaves…. ..... **15. *C. phuwoaensis***

15. Leaf blades lanceolate, (4-)7-10(-12) leaves… .....

…… .....**12. *C. phulangkaensis***

10. Bracts not imbricate, axis visible in flowering stage… 16

16. Inflorescence enclosed in the two uppermost leaf sheaths, staminodes patent.... 17

17. Seeds with tail…….. ..... **11. *C. pedemontana***

17. Seeds without tail…….. 18

18. 4-7 flowered bract…..……..... **10. *C. limiana***

18. 1-3 flowered bract…….....**3. *C. bolavenensis***

16. Inflorescence produced beyond the two uppermost leaf sheaths, staminodes projecting forward…… 19

19. Ligule reddish, Staminodes narrowly obovate with concave at the base… .....

…..……**21. *C. sirirugsae***

19. Ligule not reddish, Staminodes obovate without concave at the base… 20

20. Bract apex acuminate, 3-5-flowered bract..... **19. *C. satunensis***

20. Bract apex caudate, 1-flowered with 1-abortive flowered bract…… .....

…….…….…….....**18. *C. saksuwaniae***

3.4 Descriptions of *Caulokaempferia* in Thailand and Lao PDR

3.4.1 *Caulokaempferia amplexicaulis* P. Suksathan, Nord. J. Bot. 23(4): 401-406, 2005.

Type: THAILAND, Mae Hong Son, Muang District, Doi Hua Kai-Doi Pui, alt. 1770 m. 25. 07. 2003, *M. Wongnak et al 705* (holotype: QBG, isotype: AAU, BKF, E, K, US).

Perennial herb. *Pseudostem* 8.1 -34.2 cm high, green with 2-3 bladeless sheaths at the base. *Leaves* 4-7, sessile; leaf blade ovate-lanceolate, 3.3-7.5 by 1.4-2.1 cm, base amplexicaul, apex acute to acuminate, margin entire, both surfaces glabrous, green; leaf sheaths completely fused throughout the length, glabrous; ligule membranous, *ca.* 1 mm long, translucent greenish, apex rounded. *Inflorescence* terminal, up to 7 cm long; peduncle glabrous, *ca*. 1.5 cm long. Bract 4-9, distichous, leaf-like, some with infertile bract, ovate-lanceolate to cordate, 3.4-5.2 by 1-1.9 cm, apex acute-acuminate, margin entire, glabrous, greenish, each bract subtends 1 flower; axis visible; bracteoles ovate-lanceolate, 1-1.7 cm by 2-3, membranous, apex acuminate, green; calyx tubular, 1.8-2.3 cm long, green, apex 3-dentate; corolla tube slender, 3-4.6 cm long, yellowish; dorsal corolla lobe oblong-obovate, 1.8-2 by 8-9 mm, apex hooded with thorn like-point, yellow; lateral corolla lobes narrowly oblong-obovate, 1.7-1.9 cm by 6-8 mm, yellow; lateral staminodes, obovate, 1.8-2.2 by 1-1.5 cm, apex round, yellow; labellum suborbicular to orbicular, 3.1-5.1 by 3.2-4.6 cm, apex wavy, yellow; anther-crest, widely ovate to suborbicular, 5-6 by 4-5mm, recurved, apex rounded; stigma funnel-shaped; ovary cylindrical 5-8 mm. long, ovules numerous; stylodes 2, filiform, *ca*. 1 mm. long. *Fruits*, ellipsoid, 1.6-1.8 cm long, yellowish-green. *Seeds* numerous, ovoid, 3-3.5 by *ca.* 1.5 mm.

Thailand: Northern [Mae Hong Son, Doi Hua Kai- Doi Pui].

Phenology: Flowering from July-August, fruiting from September-October.

Distribution: Endemic to Thailand

Ecology: Open, rocky ground on an exposed mountain slope in lower montane oak forest, at the altitude of 1,700-1,800 m.

Use: This species is used as a major ingredient in the treatment of simple goiter by the Karen ethnic group in northern Thailand.

Etymology: The specific epithet is referred to amplexicaul leaf base.

Vernacular name: Proh Ton Doi Pui (เปราะต้นดอยปุย)

Examined material: *Picheansoonthon & Phokham, CP-130713-1* (MSU)

3.4.2 *Caulokaempferia appendiculata* K. Larsen & Triboun, Nord. J. Bot. 22(4): 409-417, 2002.

Type: THAILAND, Chiang Mai, Ang Kang, Kop Dung village, alt. *ca.* 1400 m. 27. 08. 1999, *P. Triboun 617* (holotype: AUU, isotype: BK, BKF).

Lithophytic, perennial herb with short rhizome; root fibrous, some forming a longish storage tuber. *Pseudostem* slender, erect, 14-38.5 cm high, green with 3-4 bladeless sheaths at the base. *Leaves* 4-6, the lowermost 1-2 strongly reduced to bladeless sheath; lamina sessile, narrowly lanceolate, 7.5-11.6 by 1.1-2 cm, base cuneate, apex caudate, margin entire, lower leaf surface sparsely hairy, green; leaf sheath sparsely hairy, brownish; ligule membranous translucent, 0.7-1.2 cm long, apex acute, reddish. *Inflorescence* terminal, up to 9 cm long; peduncle glabrous, 2.8-3 cm long. Bracts 4-6, linear to narrowly lanceolate, 3.1-5.1 cm by 5-7 mm, apex free like an appendages, glabrous, greenish to brownish, each bract enclosing 2-3 flowered cincinnus; bracteoles ovate to lanceolate, *ca.*1 cm by 4-5 mm, margin translucent, apex acute, reddish. *Flowers* yellow; calyx tubular, 1.5- 1.7 cm long, glabrous, lower part green, apex bifid with reddish; corolla tube slender, 2.7-3.2 cm long, yellowish; dorsal corolla lobe lanceolate, 1.4-1.6 cm by 4-5 mm, apex hooded with thorn like-point, yellow; lateral corolla lobes lanceolate, 1.4-1.5 cm by 4-5 mm, yellow; lateral staminodes, broadly elliptic, 1-1.3 cm by 6-7 mm, apex rounded, yellow; labellum suborbicular, 2.4-3.9 by 2-3.1 cm, apex slightly emarginate, yellow; filament 1-2 mm long; anther 2-3 mm long; anther-crest suborbicular, 4-5 by *ca.* 4 mm, recurved, apex rounded, yellow; stigma funnel-shaped; ovary cylindrical 2-3 mm. long, ovules numerous; stylodes 2, filiform, *ca.* 1 mm long. *Fruits* oblong, 0.8-1.2 mm long. *Seeds* numerous.

Thailand: Northern [Chiang Mai, Ang Kang, Kop Dung village].

Distribution: Endemic to Thailand.

Ecology: This species is grown on moist rocks under the shade of lower montane pine-oak forest, at the altitude of 1,400-1,500 m.

Phenology: Flowering from July-August, fruiting from August-September.

Etymology: The specific epithet is referred to appendage bract.

Vernacular name: Proh Ton Ang Kang (เปราะต้นอ่างขาง)

Note: The plant can be easily recognized by its bract with apex free like an appendage.

Examined material: *Picheansoonthon & Phokham, CP-020812-1* (MSU)

3.4.3 *Caulokaempferia bolavenensis* Picheans. & Koonterm, Nat. Hist. Bull. Siam Soc. 56(1): 85-100, 2008.

Type: LAO PDR, Champasak, Pak Song, Tad Kamued Waterfall, the Bolaven Plateau, N 15° 04.330′, E 106° 42.152′, alt. 932 m, *Picheansoonthon & Koonterm 821* (holotype, dried & spirit: BKF; isotype: SING).

Lithophytic, perennial herb with short rhizome; root fibrous, some forming a longish storage tuber. *Pseudostem* slender, erect, 2.7-7.4 cm high, green with 2-3 bladeless sheaths at the base. *Leaves* 5-6, the lowermost 1-2 strongly reduced to bladeless sheath; lamina sessile, upper ones shortly petioles 2-5 mm long, broadly lanceolate, 3.3-7.5 by 1.4-2.1 cm, base cuneate, apex caudate, margin slightly undulate, both surface glabrous, green; ligule membranous, 0.6-1.4 cm long, translucent greenish, apex acute. *Inflorescence* terminal, 2.6-4.2 cm long; peduncle glabrous, 1.1-2.9 cm long. Bracts 1-2, distichous, ovate to lanceolate, 1.8-3.1 by 0.6-1.3 cm, apex acute, margin membranous, glabrous, green, each bract enclosing a 1-3 flowered cincinnus; bracteoles ovate, ca.1 cm by 3 mm, margin membranous, apex acute, green. Flowers yellow; calyx tubular, hidden in the bract, 0.8-1 cm long, apex rounded, glabrous, green; corolla tube slender, 2.4-2.8 cm long, yellow; dorsal corolla lobe ovate-lanceolate, , 0.9-1 cm by 4-5 mm, hooded with thorn like-point yellow; lateral corolla lobes oblong-lanceolate, 0.9-1 cm by 3-4 mm, yellow; lateral staminodes, obovate, 1.2-1.5 by 0.9-1 cm, apex round, yellow; labellum broadly obovate, 1.6-1.8 by 1.8-2.1 cm, trilobed, median lobe extending, apex slightly emarginate, yellow; anther-crest, broadly ovate, 4-7 by 3-4 mm, recurved, apex rounded, yellow; stigma funnel-shaped; ovary cylindrical 2-3 mm. long, ovules numerous; stylodes 2, filiform, ca. 1 mm. long. *Fruits*, oblong, 2.1-2.2 cm long, glabrous, greenish. *Seeds* numerous, ellipsoid, ca. 2 long, apical with long tail-like appendage, hairy, whitish aril.

Lao PDR: Southern [Champasak, Paksong, Tad Kamued Waterfall].

Phenology: Flowering from June-July, fruiting from July-September.

Distribution: This species has so far been found only at the type location.

Ecology: This species is grown on moist sandstone clefts and rocks facing the waterfalls under the shade of moisten evergreen forest.

Etymology: The specific epithet, *bolavenensis* refers to the type locality of this species, the Bolaven Plateau. The Laven, the sub-ethnic group of the Phuthai, is the major ethnic group lived on the plateau.

Vernacular name: Proh Ton Bolaven (เปราะต้นบ่อละเวน)

Examined material: *Picheansoonthon & Phokham, CP-030813-1* (MSU)

3.4.4 *Caulokaempferia bracteata* K. Larsen & S.S. Larsen, Nord. J. Bot. 22(4): 409-417, 2002.

Type: THAILAND, from Chatuchak Market in Bangkok brought in from Nong Khai, *K. Larsen 47337*, 04. 07. 1999. (holotype: AUU, isotype: BKF).

Lithophytic, perennial herb with short rhizome; root fibrous, some forming a longish storage tuber. *Pseudostem* slender, erect, up to 42 cm high, green with 3-4 bladeless sheaths at the base. *Leaves* 6-8, the lowermost 1-2 strongly reduced to bladeless sheath; lamina sessile, lanceolate-ovate, 5.5-13.5 by 1.-2.5 cm, base cuneate-rounded, apex acuminate, margin entire, lower leaf surface glandular, green; leaf sheath glandular, greenish; ligule membranous translucent, 3-5 mm long, apex acute or irregular. *Inflorescence* terminal, up to 8 cm long; peduncle glandular, 1.5-2.2 cm long. *Bracts* (1-)3-5, imbricate, unilateral with amplexicaul base, ovate, 2.2-2.6 by 1.3-1.6 cm, apex acuminate, glabrous, greenish, each bract enclosing 1-2(-3) flowered cincinnus; axis visible on one side; bracteoles ovate to lanceolate, *ca.* 6-7 by 4-5 mm, margin translucent, apex acute. *Flowers* yellow; calyx tubular, 1-1.2 cm long, glabrous, apex rounded with 2 minute teath; corolla tube slender, 2.2-2.4 cm long, yellowish; dorsal corolla lobe oblong, 1-1.2 cm by 4-5 mm, apex hooded with thorn like-point, yellow; lateral corolla lobes oblong, 0.9-1 cm by 3-4 mm, yellow; lateral staminodes, narrowly obovate, 1.3-1.5 by 1-1.1 cm, apex rounded, yellow; labellum broadly obovate, 1.9-2.1 by 2-2.3 cm, strigose at base, with emarginate median lobe, yellow; filament 1-2 mm long; anther 2-3 mm long; anther-crest suborbicular, 4-5 by *ca.* 4 mm,

recurved, apex rounded, yellow; stigma funnel-shaped; ovary cylindrical 2-3 mm. long, ovules numerous; stylodes 2, filiform, *ca.* 1 mm long. *Fruits* ellipsoid, 4-5 by 2-3 mm, glabrous. *Seeds* numerous with whitish aril.

Lao PDR: Central [Phou Khao Khouay National Park, Tad Leuk Waterfalls].

Phenology: Flowering from June-July, fruiting from July-September.

Distribution: This species has so far been found only at the type location.

Ecology: This species is grown on sandstone rocks in the waterfall and in dried evergreen forest, at the altitude of 500 m.

Use: This species is used as an ingredient for external analgesic preparations by traditional healer in Lao PDR.

Etymology: The specific epithet is referred to conspicuous, imbricate bracts.

Vernacular name: Proh Ton Phou Khao Khouay (เปราะต้นภูเขาควาย)

Examined material: *Picheansoonthon & Phokham, CP-290613-1* (MSU)

3.4.5 *Caulokaempferia chayaniana* Tiyaw., Telopea. 12(4): 479-484, 2010.

Type: THAILAND, Mae Hong Son, Pai District, Doi Jik Jong, *C. Picheansoonthon 1017*, alt. c. 900 m. 10. 7. 2008. (holotype: BKF, isotype: BK, SING).

Perennial herb with short rhizome; root fibrous, some forming a longish storage tuber. *Pseudostem* slender, erect, 12.5 -37.4 cm high, green with 2-4 bladeless sheaths at the base. *Leaves* 5-8, the lowermost 2-4 strongly reduced to bladeless sheath; lamina sessile, ovate to ovate-lanceolate, 3.3-7.5 by 2-3.2 cm, with cordate, amplexicaul base, apex acute to acuminate, margin slightly undulate, both surfaces glabrous, green; ligule translucent, 1.5-3 mm long, apex rounded, greenish. *Inflorescence* terminal, 3-5-8.5 cm long; peduncle glabrous, 2.2-5.5 cm long. Bracts 3-8, distichous, ovate to lanceolate, 3.1-4 by 0.5-1.7 cm, apex acute, margin membranous, glabrous, green, each bract enclosing a 1-3 flowered cincinnus; axis visible; bracteoles elliptic, *ca.*1 cm by 5 mm, margin membranous, apex acuminate, greenish. Flowers entirely yellow; calyx tubular, 1.2- 1.5 cm long, hidden in the bract, glabrous, apex 3-4 dentate, greenish; corolla tube slender, 2.1-3.2 cm long, yellowish; dorsal corolla lobe ovate-oblong, 1-1.5 cm by 7-8 mm, hooded with thorn-like point, yellow; lateral corolla lobes ovate-oblong, 1.3-1.7 cm by 5-6 mm, yellow; lateral staminodes, obovate, 1.2-1.5 by 0.8-1.2 cm, apex

emarginate, yellow; labellum suborbicular, 2.7-3.8 by 2.5-3.4 cm, apex wavy, yellow; anther oblong *ca.* 5 mm long; anther-crest, broadly ovate, 4-5 by 5-7 mm, recurved, apex rounded, yellow; stigma funnel-shaped; style inserted between anther sacs; ovary cylindrical 4-5 mm. long, ovules numerous; stylodes 2, filiform, *ca.* 1 mm. long. *Fruits*, oblong, 1.4-2 mm long, greenish. *Seeds* numerous, ellipsoid, *ca.* 2 by 1 mm, brownish.

Thailand: Northern [Mae Hong Son, Pai, Doi Jik Jong].

Phenology: Flowering from June-July, fruiting from July-September.

Distribution: This species has so far been found only at the type location.

Ecology: On moist granite clefts and rocks cleavages under evergreen lower montane scrub at an altitude of 900 m.

Use: The whole plants, with other herbs, are used by the Shan ethnic group for gastric disorders.

Etymology: The specific epithet is named to honor Professor Dr. Chayan Picheansoonthon, Fellow of the Royal Institute of Thailand.

Vernacular name: Proh Ton Chayan (เปราะต้นชยันต์)

Examined material: *Picheansoonthon & Phokham, CP-030813-1* (MSU)

3.4.6 *Caulokaempferia jirawongsei* Picheans. & Mokkamul, Folia Malaysiana. 5(2): 69-80, 2004.

Type: THAILAND, Nong Khai, Phu Woa Wildlife Sanctuary, *Picheansoonthon & Mokkamul 759*, 10. 8. 2002. alt. 297 m. (holotype: BKF, isotype: BK, KEP and SING).

Lithophytic, perennial herb with short rhizome; root fibrous, some forming a longish storage tuber. *Pseudostem* slender, erect, 15.1-31.4 cm high, green with 2-3 bladeless sheaths at the base. *Leaves* 7-8, the lowermost 1-2 strongly reduced to bladeless sheath; lamina petiolate 2-4 mm long, lanceolate, 9.5-12.4 by 1.8-2.3 cm, base cuneate, apex acuminate, margin entire to slightly undulate, glabrous on both sides, green; leaf sheath glabrous, greenish; ligule membranous translucent, 0.6-1 cm long, apex acute. *Inflorescence* terminal, up to 7 cm long; peduncle glabrous, 1.7-2.3 cm long. *Bracts* 1-4(-5), imbricate, unilateral with amplexicaul base, lanceolate, 2.7-3.5 by 1.3-1.6 cm, apex acuminate, margin free to the base, glabrous, greenish, each bract enclosing 5-6 flowered cincinnus; axis visible on one side; bracteoles ovate to

lanceolate, *ca.* 6-7 by 4-5 mm, margin translucent, apex acute. *Flowers* yellow; calyx tubular, 0.8-1 cm long, glabrous, apex rounded, split 2-3 mm down one side; corolla tube slender, 2.5-3 cm long, yellowish; dorsal corolla lobe oblong, 1-1.1 cm by 4-5 mm, apex hooded with thorn like-point *ca*. 5 mm long, yellow; lateral corolla lobes oblong, 0.9-1 cm by 3-4 mm, yellow; lateral staminodes, patent, auricurate, elliptic- obovate, 1.4-1.6 by 0.7-1.1 cm, apex rounded to emarginate, yellow; labellum broadly obovate, 2.2-2.4 by 1.9-2.1 cm, flat and saccate base, distal 3-lobed with emarginate median lobe, yellow; filament 1-2 mm long; anther 2-3 mm long; anther-crest broadly ovate, 4-5 by 5-6 mm, reflexed, apex rounded, yellow; stigma funnel-shaped; ovary cylindrical 2-3 mm. long, ovules numerous; stylodes 2, filiform, *ca.* 1 mm long. *Fruits* fleshy capsule, 0.8-1.5 cm long, glabrous. *Seeds* numerous, ellipsoid, ca. 2 mm long, hairy, light brown with whitish aril.

Thailand: Northeastern [Beung Kan, Phu Wao National Park].

Phenology: Flowering from June-July, fruiting from July-September.

Distribution: This species has so far been found only at the type location.

Ecology: This species is grown on red sandstone rocks at an altitude of 300 m.

Etymology: The specific epithet is named in honor of Professor Dr.Vichiara Jirawongse, Fellow of the Royal Institute of Thailand.

Examined material: *Picheansoonthon & Phokham, CP-300812-1* (MSU)

3.4.7 *Caulokaempferia kamthorniana* Picheans., Prasarn & Phokham *sp. nov.* (Figs. 3.1 & 3.2**)**

TYPE: THAILAND: Beung Kan, Phu Woa Wildlife Sanctury, Si Wilai district [Chet Si Waterfalls], N 18^{o} 09.607’ E 103^{o} 57.016’, altitude 217 m, 11 August 2014, *Picheansoonthon, Prasarn & Phokham 110814-1* (MSU).

Epilithic, perennial herb, slender, with short rhizome; root fibrous, some form longish tuber. Pseudostems, 8.2- 18.2 cm with 2-3 bladeless sheaths and reduced lamina. Leaves (4)5-7, sessile to subsessile, elliptic to narrowly lanceolate, 9.4-11.9 by 1.4-1.5 cm, base cuneate, margin entire, apex acute or acuminate, both sides glabrous; ligule membranous 3-5 mm long, entire, apex acute to acuminate. Inflorescences terminal; peduncle glabrous, 1.2.-2.4 cm long, hidden in the uppermost two leaf sheaths;

flowers entirely yellow; Bracts 1-2, imbricate, bilateral with amplexicaul base, enclosed cincinni, lanceolate, 2.5-4.4 by 0.9-1.4 cm, apex acuminate to caudate up to 5 mm long, margin entrie, glabrous, deeply green, subtends 3-4 flowers, axis visible on one side; bracteoles lanceolate, translucent, 3-4 by *ca*.1 mm. *Flowers* yellow; calyx tubular, 6-8 by 1-2 mm, translucent, split *ca*. 2 mm down one side, apex acute glabrous; corolla tube, 2.4-3.2 cm by 1- 2 mm, glabrous; dorsal corolla lobe oblong, 8-12 by 3-4 mm, hooded, apex acute; lateral corolla lobes oblong, 7-10 by 3-4 mm, apex acute. Staminodes patent, obovate, 1.1-1.6 cm by 7-10 mm, apex emaginate. Labellum with median lobes, broadly obovate, 2.4-2.8 by 1.5-2.5 cm, apex of median lobe 2-lobed, 5-10 by 6-7 mm, divided into 1-4 mm long, apex of lateral lobes entrie; anther, 3-4 mm long; anther crest orbicular, 3-4 by 3–4 mm, apex rounded. Stigma funnel-shaped, inserted between anther sac, ciliate. Ovary oblong, 2-4 by ca. 2 mm, glabrous, ovules numerous; stylodes 2, filiform, *ca*. 2 mm long. *Fruits* capsular, oblong to ovate, 1-1.2 cm by 3-4 mm. *Seeds* numerous.

Thailand: Northern [Beung Kan, Phu Wao Wildlife Sanctury, Chet Si Waterfalls].

Phenology: Flowering from June-July, fruiting from July-September.

Distribution: This species has so far been found only at the type location.

Ecology: This species is grown on moist rocks in waterfalls at the altitude of 200 m.

Etymology: The specific epithet is named to Mr. Kamthorn Intharapichai who verified the genus *Caulokaempferia* in India, China and Vietnam.

Vernacular name: Proh Ton Kamthorn (เปราะต้นกำธร)

Examined material: *Picheansoonthon & Phokham, CP-290812-1* (MSU)

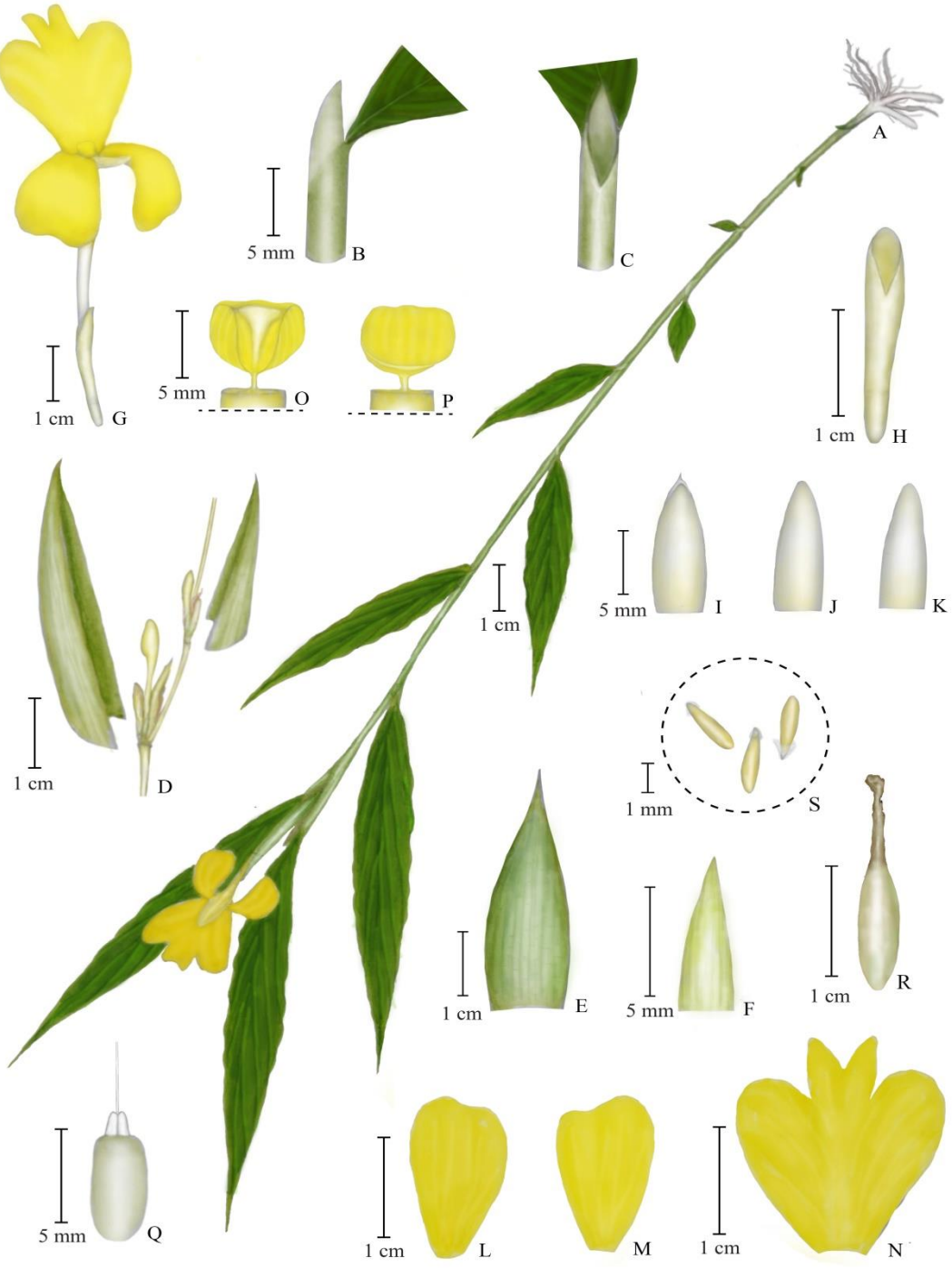

Fig.3.4 Illustrations of *C. kamthorniana* Picheans., Prasarn & Phokham (*sp.nov.*) A. Habit. B.&C. Leaf base, showing ligules (B. lateral view & C. front view). D. Inflorescence. E. Bract. F. Bracteole. G. A flower. H. Calyx tube and ovary. I.&K. Corolla lobes (I. Dorsal, J.&K. Lateral). L.&M. Lateral staminodes. N. Labellum. O.&P. Anthers and anther crests (O. front view, P. rear view). Q. Ovary and stylodes. R. Fruit. S. Seeds. [drawn by Chalermchoke Boonchit]

Fig. 3.5 Photograph illustrations of *C. kamthorniana* Picheans., Prasarn & Phokham (*sp. nov.*) A.&B. Plants in location; C. Oblique view of plant showing leaves and a flower; D. Plants with inflorescences; E. Front view of plant showing leaves and a flower; F. Flowers, showing staminodes, labellum and anther crest.

3.4.8 *Caulokaempferia khaomaenesis* Picheans. & Mokkamul, Folia Malaysiana 5(1): 1-8, 2004.

Type: THAILAND, Nakhon Si Thammarat, near the submit of Khao Maen, N 08° 17.531′, E 99° 39.722′, alt. 1093 m. 13.07.2003, *Mokkamul 130703-01* (holotype: BKF, isotype: BK, KEP, SING).

Perennial herb with short rhizome; root fibrous, some forming a longish storage tuber. *Pseudostem* slender, erect, up to 57.4 cm high, green with 4-6 bladeless sheaths at the base. *Leaves* 6-10, the lowermost 1-2 strongly reduced to bladeless sheath; lamina sessile, lanceolate to ovate-lanceolate, 10.3-16.5 by 2.4-3.1 cm, base rounded, apex acuminate to caudate, margin entire, both surface glabrous, green; ligule membranous up to 1.2 cm long, greenish, apex obtuse. *Inflorescence* terminal, 8-11.5 cm long; axis glandular; peduncle glandular, 3.2-4.5 cm long. *Bracts* (3)4-6, distichous, ovate to lanceolate, 2-3.1 by 0.7-1.1 cm, apex acute, margin membranous, glabrous, green, each bract enclosing a 1-2(3) flowered cincinnus, upper bracts with a flower and an abortive; bracteoles ovate, 5-7 by 4-6 mm, margin membranous translucent, apex acuminate, green, bracteole present every flowers, including the first flower. *Flowers* yellow; calyx tubular, hidden in the bract, 1.2- 1.5 cm long, apex truncate, glabrous, green; corolla tube slender, 3.6-4.2 cm long, yellow; dorsal corolla lobe oblong, 1.6-2.1 cm by 7-8 mm, apex hooded, yellow; lateral corolla lobes oblong, 1.6-2.1 cm by 5-6 mm, yellow; lateral staminodes, broadly elliptic, 1.4-1.8 cm by 8-1.2 cm, apex rounded, yellow; labellum suborbicular to orbicular, 2.5-3 by 2.8-3.6 cm, apex slightly emarginate, yellow; anther-crest suborbicular, 4-5 by 5-6 mm, recurved, apex rounded yellow; stigma funnel-shaped; ovary cylindrical 4-6 mm. long, ovules numerous; stylodes 2, filiform, *ca*. 1 mm. long. *Fruits*, oblong, 8-12 mm long. *Seeds* numerous, ellipsoid, *ca*. 2 by 1 mm.

Thailand: Southern [Nakhon Si Thammarat, Khao Maen].

Phenology: Flowering from June-July, fruiting from July-September.

Distribution: This species has so far been found only at the type location.

Ecology: This species grows in rocky savanna forest at the altitude of 1100 m.

Etymology: The specific epithet is referred to Khao Maen of Nakhon Si Thammarat.

Vernacular name: Proh Ton Khao Maen (เปราะต้นเขาเหมน)

Examined material: *Picheansoonthon & Phokham, CP-150712-1* (MSU)

3.4.9 *Caulokaempferia larsenii* Suksathan & Triboun, Edinburgh J. Bot. 60(3): 513-516, 2004.

Type: THAILAND, Chiang Mai, Doi Phe Pan Nam, alt. 1720 m. 22.07.2002, *Suksathan, Triboun & Wongnak 3429* (holotype: QBG, isotype: AAU, BK, BKF,E, K).

Perennial herb with short rhizome; root fibrous, some forming a longish storage tuber. *Pseudostem* slender, erect, 20.5-39.5 cm high, green with 3-5 bladeless sheaths at the base. *Leaves* 5-9, the lowermost 1-2 strongly reduced to bladeless sheath; lamina sessile, lanceolate, 8-10.6 by 1.5-2.5 cm, base rounded, apex acute to acuminate, margin entire, both surfaces glabrous, green; ligule up to 8 mm long, apex truncate, greenish. *Inflorescence* terminal, 9.7-14.5 cm long; peduncle glabrous, 5.2-6.7 cm long. Bracts 4-8, some lower bract with leaf blade, distichous, ovate to lanceolate, 2.4-4.2 (10.3) by 0.9-1.2 cm, apex acute, margin membranous translucent, glabrous, green, each bract enclosing a 1-3 flowered cincinnus; axis visible; bracteoles elliptic, 0.6-1 cm by 3-5 mm, membranous translucent, apex acute. Flowers entirely yellow; calyx tubular, 1.2-1.5 cm long, translucent, hidden in the bract, glabrous, split down on one side up to 4 mm long, apex bifid; corolla tube slender, 3.1-4.2 cm long, yellowish; dorsal corolla lobe ovate-oblong, 1.1-1.5 cm by 6-7 mm, hooded with thorn-like point, yellow; lateral corolla lobes ovate-oblong, 1.3-1.7 cm by 5-6 mm, yellow; lateral staminodes, obovate, 1.2-1.5 by 0.8-1.1 cm, apex emarginate, yellow; labellum suborbicular, 2.8-3.8 by 3.1-4.4 cm, apex wavy, yellow; anther oblong ca. 5 mm long; anther-crest, suborbicular, 4-5 by 5-6 mm, recurved, apex rounded, yellow; stigma funnel-shaped; style not inserted between anther sacs; ovary cylindrical 5-7 mm. long, ovules numerous; stylodes 2, filiform, ca. 1 mm. long. *Fruits*, ellipsoid, 1.3-1.9 mm long, greenish. *Seeds* numerous, ellipsoid, ca. 2 by 1 mm, brownish.

Thailand: Northern [Chiang Mai, Doi Phe Pan Nam, Khun Chae National Park].

Phenology: Flowering from June-July, fruiting from July-September.

Distribution: This species has so far been found only at the type location.

Ecology: This species grows in lower montane scrub at the altitude of 1800 m.

Etymology: The specific epithet is named in honor of Professor Kai Larsen.

Vernacular name: Proh Ton Phe Pan Nam (เปราะต้นผีปันน้ำ)

Examined material: *Picheansoonthon & Phokham, CP-150713-1* (MSU)

3.4.10 *Caulokaempferia limiana* Mokkamul & Picheans., Folia Malaysiana. 5(3&4): 187-194, 2004.

Type: THAILAND, Phitsanulok, Chat Trakan Waterfalls National Park, N 17° 17.873′, E 100° 40.940′, alt. 230 m. 11.07.2002. *Picheansoonthon & Mokkamul 742* (holotype, dried & spirit: BKF; isotype: BK, KEP, SING).

Lithophytic, perennial herb with short rhizome; root fibrous, some forming a longish storage tuber. *Pseudostem* slender, erect, 12.5 -17.4 cm high, green with 2-3 bladeless sheaths at the base. *Leaves* 7-9, the lowermost 2-3 strongly reduced to bladeless sheath; lamina petiole 4-6 mm, broadly lanceolate, 2.8-3.2 by 10.4-16.1 cm, base cuneate, apex acute to acuminate, margin slightly undulate, both surface glabrous, green; ligule translucent, 5-7 mm long, apex acute. *Inflorescence* terminal, 3.2-3.8 cm long; peduncle 0.9-1.4 cm long, glabrous. Bracts 1-2(-3), tightly imbricate, unilateral, ovate to lanceolate, 2-2.6 by 1-1.1 cm, apex acute to mucunate, margin membranous, glabrous, green, each bract enclosing a 4-7 flowered cincinnus; bracteoles triangular, 5-7 by 2-3 mm, membranous, apex acute. Flowers yellow; calyx tubular, hidden in the bract, 0.8-1 cm long, glabrous, apex rounded, green; corolla tube slender, 1.8-2.1 cm long, yellowish; dorsal corolla lobe triangular, 7-8 by 3-4 mm, hooded with thorn like-point, yellowish; lateral corolla lobes triangular, 6-7 cm by 2-3 mm, yellowish; lateral staminodes, patent, elliptic-obovate, 0.9-1.2 cm by 5-7 mm, apex rounded to slightly emarginated, yellow; labellum broadly obovate, 1.3-1.5 by 1.5-1.8 cm, trilobed, median lobe apex slightly 2-lobed or emarginate; anther-crest, broadly ovate, 4-5 by 3-4 mm, recurved, apex rounded; stigma funnel-shaped; ovary cylindrical 4-5 mm. long, ovules numerous; stylodes 2, filiform, ca. 1 mm. long. *Fruits*, oblong or ellipsoid, 0.8-1.2 cm long. *Seeds* numerous, ellipsoid, ca. 2 by 1 mm, hairy.

Thailand: Northern [Phitsanulok, Chat Trakan National Park].

Phenology: Flowering from June-July, fruiting from July-September.

Distribution: This species has so far been found only at the type location.

Ecology: This species grows on moist rocks in the humid bamboo forest near from waterfalls at the altitude of 1600 m.

Etymology: The specific epithet is named in honor of Datuk Seri Lim Chong Keat.

Vernacular name: Proh Ton Chat Trakan (เปราะต้นชาติตระการ)

Examined material: *Picheansoonthon & Phokham, CP-050812-1* (MSU)

3.4.11 *Caulokaempferia pedemontana* P.Triboun & K. Larsen, Nord. J. Bot. 23(4): 401-406, 2004.

Type: THAILAND, Nakhon Nayok, Nang Rong falls, alt. c. 300 m. 04.08.1992, *K.Larsen, S.S.Larsen, Chr.Tang & C.Niyomdham 43774* (holotype: AAU, isotype: BK).

Lithophytic, perennial herb with short rhizome; root fibrous, some forming a longish storage tuber. *Pseudostem* slender, erect, 12.2-18.3 cm high, green with 2-3 bladeless sheaths at the base. *Leaves* 5-6, the lowermost 1-2 strongly reduced to bladeless sheath; lamina sessile to petiole 3-6 mm long, ovate to ovate-lanceolate, 3.3-7.5 by 1.4-2.1 cm, base cuneate, apex acuminate to caudate, margin entire, both surface glabrous, green; ligule membranous, 4-6 mm long, greenish, apex acute. *Inflorescence* terminal, 3-5.5 cm long; peduncle 2-2.3 cm long, glabrous. Bracts 1-3, distichous, ovate to lanceolate, 2.4-4.9 by 1.5-2.8 cm, apex acute, margin membranous, glabrous, green, each bract enclosing a 1-2(-3) flowered cincinnus; bracteoles lanceolate to broadly ovate, 1-1.3 cm by 4-6 mm, membranous, apex acute. Flowers yellow; calyx tubular, hidden in the bract, 1.2-1.6 cm long, , apex 2-3 dentate, glabrous, green; corolla tube slender, 3.8-4.2 cm long, yellow; dorsal corolla lobe ovate-lanceolate, 0.9-1.1cm by 5-6 mm, hooded, whitish; lateral corolla lobes lanceolate, 1-1.2 cm by 4-5 mm, whitish; lateral staminodes obovate, 1.2-1.9 by 0.8-1 cm, apex rounded to slightly emarginate, yellow; labellum suborbicular, 2.7-3 by 2-2.6 cm, trilobed, median lobe apex emarginate to bi-lobed, yellow; anther-crest, reniform, 4-5 by 3-4 mm, recurved, yellow; stigma funnel-shaped; ovary cylindrical 4-5 mm. long, ovules numerous; stylodes 2, filiform, ca. 1 mm. long. *Fruits*, oblong, 1.4-2.8 cm long. *Seeds* numerous, ellipsoid with tailed, *ca.* 2 by 1 mm, pubescence.

Thailand: Central [Nakhon Nayok, Nang Rong Waterfalls].

Phenology: Flowering from June-July, fruiting from July-September.

Distribution: This species has so far been found only at the type location.

Ecology: This species grows on moist rocks near the waterfalls at the altitude of 350 m.

Etymology: The specific epithet is referred to growing at the base of the hill

Vernacular name: Proh Ton Nang Rong (เปราะต้นนางรอง)

Examined material: *Picheansoonthon & Phokham*, *CP-150812-1* (MSU)

3.4.12 *Caulokaempferia phulangkaensis* Picheans., Taiwania. 53(3): 248-257, 2008.

Type: THAILAND, Nakhon Panom, Ban Paeng, Phu Langka National Park, Tad Kham waterfall, N 17° 57.084′, E 104° 09.436′, alt. 176 m. 21.08.2004, *Picheansoonthon 729* (holotype, dried & spirit: BKF, paratype: BK, SING).

Lithophytic, perennial herb with short rhizome; root fibrous, some forming a longish storage tuber. *Pseudostem* slender, erect, 20-45.5 cm high, green with 2-6 bladeless sheaths or reduced lamina. *Leaves* (4-)7-10(-12); lamina sessile, lanceolate, (5-)7-10.2 by 2-2.6 cm, base cuneate, apex acuminate, margin entire to slightly undulate, lower glabrous on both sides, green; leaf sheath glabrousr, greenish; ligule membranous translucent, 6-9 mm long, apex rounded or apiculate. *Inflorescence* terminal, 5.5-8 cm long; peduncle 1.2-2.5 cm long. *Bracts* (4-)5-8, imbricate, unilateral with amplexicaul base, ovate-oblong, 2.2-3.3 by 0.9-1.3 cm, apex acute to acuminate, glabrous, greenish, each bract enclosing 1-10 flowered cincinnus; axis visible on one side; bracteoles deltoid, 3-4 by 1-2 mm, margin translucent, apex acute. *Flowers* yellow; calyx tubular, 1.5-2 cm by 2-3 mm, apex shallowly bilobed, split down one side to about half way, glabrous; corolla tube slender, 2.8-3.8 cm by ca. 2 mm yellowish; dorsal corolla lobe oblong, 1.3-1.6 cm by 7-8 mm, apex hooded obtuse to apiculate, yellow; lateral corolla lobes oblong, 1.1-1.6 cm by 5-6 mm, yellow; lateral staminodes, projecting forward, not recurved, broadly obovate, slightly angular, 1.2-1.7 by 0.9-1.1 cm, apex rounded, yellow; labellum rotund to broadly depressed obovate, 2.8-3.8 by 3.2-4.1 cm, ciliated at base, margin erose, distal part 3-lobed with median lobe ligulate extending 5-8 mm, apex 2-lobed split 2-4 mm down, apex acute to obtuse, yellow; filament very short 1-2 mm long; anther 4-5 mm long; anther-crest elliptic to

suborbicular, 5.5-6 by 4-5 mm, entire, slightlyreflexed, apex rounded, yellow; stigma shallowly obtriangular, ca. 2.8 mm wide, ciliate, reddish; ovary cylindrical 2-3 mm. long, ovules numerous; stylodes 2, filiform, *ca.* 1 mm long. *Fruits* ellipsoid, 4-5 by 2-3 mm, glabrous, greenish. *Seeds* numerous, hairy with whitish aril.

Thailand: Northeastern [Nakhon Phanom, Phu Langka National Park].

Phenology: Flowering from June-July, fruiting from July-September.

Distribution: This species has so far been found only at the type location.

Ecology: This species grows on moist rocks along the waterfall under the shade of mixed forest at the altitude of 200 m.

Use: Medicinal

Etymology: The specific epithet is referred to the type of this species from Phu Langka National Park.

Vernacular name: Proh Ton Phu Langka (เปราะต้นภูลังกา)

Examined material: *Picheansoonthon & Phokham, CP-310812-1* (MSU)

3.4.13 *Caulokaempferia phuluangensis* Picheans. & Mokkamul, Folia Malaysiana. 5(2): 69-80, 2004.

Type: THAILAND: Loei, Phu Luang, Kok Nok-kraba, alt. 1466 m. 15.05.2002, *Picheansoonthon & Mokkamul 739* (holotype, dried & spirit, slides: BKF, paratype: BK, KEP, SING).

Perennial herb with short rhizome; root fibrous, some forming a longish storage tuber. *Pseudostem* slender, erect, up to 70 cm high, green with 4-5 bladeless sheaths at the base. *Leaves* 5-6, the lowermost 1-2 strongly reduced to bladeless sheath; lamina sessile, lanceolate, 8.1-11.7 by 1.8-2 cm, base rounded, apex acuminate, margin entire, both surface glabrous, green; ligule membranous 1-2 mm long, apex obtuse, greenish. *Inflorescence* terminal, 6-11.5 cm long; axis glabrous; peduncle glabrous, 2.8-4 cm long. Bracts (3)4-6, distichous, lanceolate, 3.2-3.9 cm by 05-9 mm, apex acute-acuminate, glabrous, green, each bract enclosing a 1-2 flowered cincinnus; bracteoles ovate, 5-7 by 4-6 mm, margin membranous translucent, apex acuminate, greenish. Flowers yellow; calyx tubular, hidden in the bract, 1.8- 2 cm long, apex truncate, glabrous, green; corolla tube slender, 3-4.2 cm long, yellow; dorsal corolla lobe oblong, ca. 1.5 cm by 5 mm, apex hooded, yellowish; lateral corolla lobes oblong, 1.7-1.9 cm by

4-5 mm, yellowish; lateral staminodes, obovate, 1.4-1.7 cm by 7-8 mm, apex rounded, yellow; labellum suborbicular, *ca.* 2.9 by 2.8 cm, apex crenulate, yellow; anther-crest suborbicular, 4-5 by 5-6 mm, recurved, apex rounded yellow; stigma funnel-shaped; ovary cylindrical 7-8 mm. long, ovules numerous; stylodes 2, filiform, ca. 1 mm. long. *Fruits*, oblong, 1.6-2.1 cm long, glabrous. *Seeds* numerous, ovate, *ca.* 3 by 1 mm.

Thailand: Northeastern [Loei, Phu Luang Wildlife Sanctury].

Phenology: Flowering from May-June, fruiting from June-August.

Distribution: Endemic to Thailand.

Ecology: This species grows in waterlogged soils near a creek under the shade of lower montane scrub at the altitude of 1450 m.

Etymology: The specific epithet is referred to the type locality.

Vernacular name: Proh Ton Phu Luang (เปราะต้นภูหลวง)

Examined material: *Picheansoonthon & Phokham, CP-280613-1* (MSU)

3.4.14 *Caulokaempferia phutokensis* Picheans., Taiwania. 53(3): 248-257, 2008.

Type: THAILAND, Nongkhai, Si Wilai, Phu Tok Noi, N 18° 08.176′, E 103° 52.731′, alt. 278 m. 21.08.2004, *Picheansoonthon 732* (holotype, dried & spirit: BKF, paratype: BK, SING).

Lithophytic, perennial herb with short rhizome; root fibrous, some forming a longish storage tuber. *Pseudostem* slender, erect, up to 20.5 cm high, green with 2-3 bladeless sheaths at the base. *Leaves* 5-6, the lowermost 1-2 strongly reduced to bladeless sheath, the 2 uppermost ones usually the largest; lamina sessile to ca. 1 cm long, ovate to ovate-elliptic, 9-16.1 by 2.7-6.4 cm, base cuneate-rounded, apex acuminate, margin entire to slightly undulate, both surfaces glabrous, green; leaf sheath glabrous, greenish; ligule membranous translucent, 0.3-1.2 cm long, apex acute or irregular. *Inflorescence* terminal, up to 6 cm long; peduncle glandular, 1.2-1.5 cm long. *Bracts* 1-3, wrapped tightly imbricate around an inflorescence axis, ovate, 2.3-3.5 by 1.5-2.4 cm, apex acuminate, glabrous, greenish, each bract enclosing 3-6 flowered cincinnus; axis invisible in flowering stage but visible on unilateral in fruiting stage; bracteoles ovate to lanceolate, 6-8 by 4-5 mm, margin translucent, apex acute. *Flowers* yellow; calyx tubular, 1.1-1.4 cm long, glabrous, apex obtuse, split down one side;

corolla tube slender, 3-3.4 cm long, yellowish; dorsal corolla lobe oblong to lanceolate, 1-1.2 cm by 5-6 mm, apex hooded with thorn like-point, yellow; lateral corolla lobes oblong to lanceolate, 0.9-1.4 cm by 4-5 mm, place close together below the labellum, yellow; lateral staminodes, patent, auriculate, obovate, 1.6-1.8 by 1-1.1 cm, apex rounded with slightly emarginate, yellow; labellum broadly obovate, 2.4-2.6 by 2.2-2.4 cm, base strigose attenuate into short claw, distal part 3-lobed with median lobe ligulate and emarginated to shallowly bilobe, extending 4-8 mm, yellow; filament sessile; anther 2-3 mm long; anther-crest broadly ovate to suborbicular, 4-5 by *ca.* 3 mm, slightly recurved, apex rounded, yellow; stigma funnel-shaped; ovary cylindrical 2-3 mm. long, ovules numerous; stylodes 2, filiform, *ca.* 1 mm long. *Fruits* ovate-oblong, ovate, 2-4 by 4-5 mm, glabrous. *Seeds* numerous with whitish aril, fusiform 2-3 mm long, hairy, brownish.

Thailand: Northeastern [Bueng Kan, Phu Tok Noi].

Phenology: Flowering from May-June, fruiting from June-August.

Distribution: This species has so far been found only at the type location.

Ecology: This species grows moist red sandstone rocks under the shade of mixed forests at the altitude of 350 m.

Use: This species is used as a major ingredient in the treatment of enlarged prostate gland by the forest monks in northeast Thailand.

Etymology: The specific epithet is referred to the type locality.

Vernacular name: Proh Ton Phu Tok (เปราะต้นภูทอก)

Examined material: *Picheansoonthon & Phokham, CP-300812-1* (MSU)

3.4.15 *Caulokaempferia phuwoaensis* Picheans. & Koonterm, Taiwania. 53(3): 248-257, 2008.

Type: THAILAND: Nongkhai, Bung Khla, Phu Woa Wildlife Sanctuary, N 18° 13.918′, E 103° 57.393′, alt. 225 m. 21.08.2004, *Picheansoonthon 723* (holotype, dried & spirit: BKF, paratype: BK, SING).

Lithophytic, perennial herb with short rhizome; root fibrous, some forming a longish storage tuber. *Pseudostem* slender, erect, up to 27.6 cm high, green with 3-4 bladeless sheaths at the base. *Leaves* 3-6, the lowermost 1-2 strongly reduced to bladeless sheath; lamina subsessile to sessile, the two uppermost ones usually the

largest, elliptic to ovate, 4.6-7.5 by 1.4-2.1 cm, base cuneate-rounded, apex acuminate, margin entire to slightly undulate, both surfaces glabrous, green; leaf sheath glabrous, greenish; ligule membranous translucent, 2-3 mm long, apex bilobe. *Inflorescence* terminal, up to 6.4 cm long; peduncle glabrous, 1.4-1.6 cm long. Bracts 1-3(-4), imbricate, unilateral with amplexicaul base, ovate, 2.1-3.8 by 1-2.1 cm, apex acuminate, glabrous, greenish, each bract enclosing 3-5 flowered cincinnus; axis visible on one side; bracteoles ovate to lanceolate, 6-7 by 3-4 mm, margin translucent, apex acute. *Flowers* yellow; calyx tubular, 1-1.2 cm long, glabrous, apex trilobe; corolla tube slender, 2.1-2.5 cm long, yellowish; dorsal corolla lobe oblong, 0.9-1.1 cm by 4-5 mm, apex hooded with thorn like-point, yellow; lateral corolla lobes oblong, place close together below the labellum, 0.9-1 cm by 3-4 mm, apex acute, yellow; lateral staminodes, patent, auriculate, obovate, 0.9-1 cm by 4-8 mm, apex rounded to emarginate, yellow; labellum broadly obovate, 1.6-1.8 by 1.7-1.8 cm, distal part 3-lobed with median lobe ligulate and emarginate to shallowly bilobe, extending 3-5 mm, yellow; filament 1-2 mm long; anther 2-3 mm long; anther-crest broadly ovate to suborbicular, 4-5 by *ca.* 4 mm, slightly reflexed, apex rounded, yellow; stigma funnel-shaped; ovary cylindrical 2-3 mm. long, ovules numerous; stylodes 2, filiform, *ca.* 1 mm long. *Fruits* ellipsoid, 5-6 by 2-3 mm, glabrous. *Seeds* numerous with whitish aril, oblong to oblanceolate, ca. 2 mm long, hairy.

Thailand: Northeastern [Bueng Kan, Phu Wao Wildlife Sanctury].

Phenology: Flowering from May-June, fruiting from June-August.

Distribution: This species has so far been found only at the type location.

Ecology: This species grows moist sandstone rocks near the waterfall under the shade of mixed forests at the altitude of 300 m.

Use: Medicinal

Etymology: The specific epithet is referred to the type locality.

Vernacular name: Proh Ton Phu Wao (เปราะต้นภูวัว)

Examined material: *Picheansoonthon & Phokham, CP-300812-1* (MSU)

3.4.16 *Caulokaempferia picheansoonthonii* Phokham & Prasarn, J.Jpn.Bot. 90(3): 153-157, 2015.

Type: THAILAND: Kanchanaburi, Amphoe Sangkla Buri, Thung Yai Narasuan Wildlife Sanctury, Kulu Huamee Mountain, near Ro Kee stream, N 15° 12′ 97.4″, E 98° 29′58.2″, alt. 247 m, 16.08.2014, *Phokham, Prasarn & Picheansoonthon, CP160814-1* (holotype: BK).

Epilithic perennial herbs, slender, with short rhizome; root fibrous, some form longish storage tubers. *Pseudostems* 13.5-17.5 cm, with 2 bladeless sheaths and reduced lamina. *Leaves* 7-9, petiole subsessile , upper one shortly petiole 5 mm; ligule membranous, 4-6 mm long, apex acute, not blade opposed; leaf blades lanceolate-oblong, the largest one, 9.5-13 by 1.8-3.2 cm, base cuneate, apex acute-acuminate to caudate up to 2.2 cm long, margin undulate, upper surface greenish, lower surface green-gray, both sides glabrous. *Inflorescence* sessile, hidden in the upper most leaf sheath. Bract none, but with only single bracteole per flower, 2-5-flowered cincinnus. Bracteole lanceolate, 1.2-1.7 cm by 2-3 mm, white to greenish. Calyx tubular, hidden in the bracteole, 1.2-1.5 cm by 2-3 mm, glabrous, greenish, splitted 5-6 mm down on one side, apex acute. Corolla tube 3-3.9 cm by 1-2 mm, yellowish; dorsal corolla lobe oblong, hooded with apex produced into an 1 mm thorn-like point, 8-11 by 3-4 mm, whitish, apex acute; lateral corolla lobes lanceolate, 9-10 by 2-3 mm, whitish, apex acute. Lateral staminodes, patent, obovate, 1.2-1.4 cm by 6-7 mm, yellow, apex obtuse. Labellum broadly obovate, 1.8-2.4 by 1.5-2 cm, yellow, apex emarginated. Filament very short, ca 1 mm; anther 2-3 mm long; anther crest suborbicular, 2-3 by 3-4 mm, yellow. Stylodial glands 2, ca 1 mm long; ovary greenish, oblong, 5-8 by 1-2 mm, glabrous. *Fruit* fleshy capsule, ellipsoid-oblong, greenish, 1.2-1.8 cm by 2-4 mm. *Seeds* numerous, crowned with whitish arillode.

Thailand: Western [Kanchanburi, Sangkla Buri].

Phenology: Flowering July-August, fruiting August-September.

Distribution: Thailand, Changwat Kanchanaburi, Amphoe Sangkhla Buri, Ban Saneh Pawng (Karen Village), Kulu Huamee Mountain, near Ro Kee stream, known only from the type locality.

Ecology: This new species grows on moist ragged limestone cliffs, in shade mixed forest, facing to stream at the altitude of 200 m.

Etymology: The specific epithet is named in honor of Professor Dr.Chayan Picheansoonthon on his 60th birthday in 2015.

Vernacular name: Proh Ton Picheansoonthon (เปราะต้นพิเชียรสุนทร)

Examined material: *Picheansoonthon & Phokham, CP-160814-2* (MSU)

3.4.17 *Caulokaempferia pubescens* Picheans. & Phokham, Taiwania. 60(2): 77-80, 2015.

Type: THAILAND, Mae Hong Son, Amphoe Mueang Mae Hong Son, alt. 1407 m. 13.07.2013, *Picheansoonthon & Phokham*, *CP 130713-3* (holotype: BK).

Perennial herbs, slender, with short rhizome; root fibrous, some form longish tuber. *Pseudostem* erect, 10.5-36.5 cm with 3-7 bladeless sheaths. *Leaves* (3-)4-6(-10), sessile, elliptic to narrowly ovate, 4.5-10.5 x 1.5-4.1 cm, base cuneate to rounded, apex acuminate to caudate to 1.5 cm long, lower surface pubescent, upper surface glabrous; ligule membranous 3-7 mm long, sparsely hairy at base, apex rounded. *Inflorescence* terminal; peduncle, 3.5.-7.5 cm long, hidden in the uppermost two leaf sheaths; flowers entirely yellow. Bracts (3-)4-8(-9), green, broadly elliptic to ovate-oblong, 2.9-3.6 x 1-1.2 cm, apex acute, margin translucent, each subtends 1-3(-4) flowers, some lower bract with leaf-like appendage. Bracteoles membranous, elliptic to ovate-oblong, 5-10x 2.5-5 mm, translucent with prominent greenish midvein, apex acute to obtuse, glabrous. *Flowers* yellow; Calyx tubular, 1.1-1.5 cm x 3-4 mm, translucent, split down one side to about half way, apex bilobed. Corolla tube 3.3-4.2 cm x *ca*. 3 mm, glabrous; dorsal corolla lobe oblong, 1.1-1.7 cm x 5-8 mm, apex obtuse to apiculate, hooded; lateral corolla lobes oblong, 1.2-2 cm x 6-8 mm, apex acute. Staminodes broadly elliptic to obovate, 1.2-1.7 cm x 7-11 mm, apex rounded. Labellum orbicular, 3.4-4.5 x 3.7-4.8 cm, margin undulate, apex rounded; anther, 4-6 mm long; anther crest broadly ovate to orbicular, 5–7 mm diameter, apex rounded. Stigma funnel-shaped, inserted between anther sac, margin raised on both ends, ciliate. Ovary oblong, 6-8 x 2-3 mm, glabrous, 1-locular, ovules numerous; stylodes 2, filiform, *ca*. 1 mm long. *Fruits* fleshy capsule, oblong to elliptic, 1.2-2 cm x 4-5 mm, green, split on one side. Seeds numerous, obovate, *ca.* 2 x 1 mm, crown with whitish arillode.

Thailand: Northern [Mae Hong Son, Amphoe Mueang Mae Hong Son, Doi Pa Yee].

Phenology: Flowering June–August; fruiting July–September.

Distribution: Endemic to Thailand.

Ecology: Lower montane pine-oak forest, at the altitude of 1,350-1,450 m.

Etymology: The specific epithet is referred to lower surface leaf pubescent.

Note: This new species can be easily characterized by its terrestrial habit, short ligule and pubescent lower leaf-surface. The hairiness of the lower leaf-surface has, so far, not been observed in the genus *Caulokaempferia*.

3.4.18 *Caulokaempferia saksuwaniae* K. Larsen, Botaniska Tidsskrift. 68: 157-159, 1973.

Type: THAILAND, Khao Pra Mee, Takuapa District, Phang-Nga province, alt 900 m., *K. Larsen, S. Saksuwan Larsen, I. Nielsen & T. Santisuk 30832*, 10. 7. 1972. (holotype: AUU, isotype: BKF).

Lithophytic, perennial herb with short rhizome; root fibrous, some forming a longish storage tuber. *Pseudostem* slender, erect, 12.6 -17.4 cm high, green with 2-3 bladeless sheaths at the base. *Leaves* 3-9, the lowermost 1-2 strongly reduced to bladeless sheath; lamina subsessile, lanceolate, 6.6-8.3 by 1.1-1.5 cm, base attenuate, apex acuminate, margin entire, both surface glabrous, green; ligule membranous, 3-5 mm long, apex acute. *Inflorescence* terminal, 3-5-6.8 cm long; peduncle glabrous, 2.2-3 cm long. Bracts (1-)5-7, distichous, lanceolate, 3.1-5 cm by 5-7 mm, apex acuminate to caudate, margin membranous, glabrous, green, each bract enclosing 1- flowered with 1-abortive flowered cincinnus; bracteoles ovate, 0.6-1 cm by 3-4 mm, margin membranous, apex acute, greenish. Flowers yellow; calyx tubular, hidden in the bract, *ca*. 1 cm long, apex 3-dented, glabrous, greenish; corolla tube slender, 3.5-4.8 cm long, yellowish; dorsal corolla lobe ovate-lanceolate, 1-1.2 cm by 4-5 mm, apex hooded with thorn like-point, yellowish; lateral corolla lobes lanceolate, 1-1.1 cm by 3-4 mm, yellowish; lateral staminodes elliptic to ovate, 0.9-1.1 cm by 4-5 mm, apex rounded, yellow; labellum broadly obovate to square, 1.8-2.3 by 1.5-2.3 cm, apex slightly emarginate; anther-crest, suborbicular, 4-5 by 3-4 mm, recurved, apex rounded, yellow; stigma funnel-shaped; ovary cylindrical 4-5 mm. long, ovules numerous; stylodes 2,

filiform, *ca*. 1 mm. long. *Fruits*, ovate, 1.4-2.1 cm long, greenish. *Seeds* numerous, ellipsoid, *ca.* 2 by 1 mm, villous.

Thailand: Southern [Phangnga, Takaupa, Khao Pra Mee].

Phenology: Flowering from June-July, fruiting from July-September.

Distribution: This species has so far been found only at the type location.

Ecology: This species grows on moist rocks near the waterfalls at the altitude of 1100 m.

Etymology: The specific epithet is named in honor of S. Saksuwan Larsen.

Vernacular name: Proh Ton Saksuwan (เปราะต้นศักดิ์สุวรรณ)

Examined material: *Picheansoonthon & Phokham, CP-140712-1* (MSU)

3.4.19 *Caulokaempferia satunensis* Picheans**.**, Folia Malaysiana. 8(2): 53-61, 2007.

Type: THAILAND: Changwat Satun, Amphoe Thung Wa, Tan Pliu Waterfall, N 07° 06.809′, E 099° 50.549′, *Picheansoonthon 531*, 08. 07. 2005. alt. 233 m. (holotype, dried & spirit: BKF, paratype: KEP, SING).

Lithophytic, perennial herb with short rhizome; root fibrous, some forming a longish storage tuber. *Pseudostem*, 13.5-17.5 cm high, green with 1-3 bladeless sheaths and reduced lamina. *Leaves* 7-8; lamina sessile, lanceolate, 11.3-13.5 by 1.4-1.7 cm, base cuneate, apex acuminate, margin slightly undulate, glabrous on both sides, green; leaf sheath glabrous, greenish; ligule membranous, 2-6 mm long, apex acute. *Inflorescence* terminal, 4-5.1 cm long; peduncle 1.1-2.3 cm long, enclosed in leaf sheaths of the two uppermost leaves. Bracts (1-)2(-3), to lanceolate, 3.7-5.2 by 1-1.2 cm, apex acute, acuminate to caudate, glabrous, greenish, each bract enclosing 3-5 flowered cincinnus; axis visible on one side; bracteoles deltoid, 0.7-1.5 cm by 2-5 mm, margin translucent, apex acute. *Flowers* yellow; calyx tubular, 1.3-1.8 cm by 2-3 mm, apex acute, split down one side to 3-6 mm long, glabrous; corolla tube slender, 2.8-3.2 cm by *ca.* 2 mm yellowish; dorsal corolla lobe lanceolate, 1-1.2 cm by 3-5 mm, apex hooded with thorn like-point, yellow; lateral corolla lobes lanceolate, place close together below the labellum 1-1.4 cm by 3-4 mm, yellow; lateral staminodes, obovate, 1-1.5 by 0.7-1 cm, apex rounded to slightly emarginate, yellow; labellum broadly depressed obovate, 2.1-2.5 by 1.6-2.1 cm, distal part 3-lobed with median lobe

emarginated to 2-lobed, yellow; filament very short 1-2 mm long; anther *ca.* 3 mm long; anther-crest elliptic to suborbicular, 3-5 by 3-4 mm, entire, slightly reflexed, apex rounded, yellow; stigma shallowly obtriangular, *ca.* 2.8 mm wide, ciliate; ovary cylindrical 2-3 mm. long, ovules numerous, glabrous; stylodes 2, filiform, *ca.* 1 mm long. *Fruits* ellipsoid, *ca.* 1.5 cm long, glabrous, greenish. *Seeds* numerous with whitish aril.

Thailand: Southern [Satun, Thung Wa, Tan Pliu Waterfall].

Phenology: Flowering from June-July, fruiting from July-September.

Distribution: This species has so far been found only at the type location.

Ecology: This species grows on moist rocks in the waterfall, at the altitude of 200 m.

Etymology: The specific epithet is referred to the type locality.

Vernacular name: Proh Ton Satun (เปราะต้นสตูล)

Examined material: *Picheansoonthon & Phokham, CP-120712-3* (MSU)

3.4.20 *Caulokaempferia saxicola* K. Larsen, Botaniska Tidsskrift. 60: 165-179, 1964.

Type: THAILAND: Nakhon Ratchasima-Prachin Buri, Khao Khieo Mountain, Khao Yai National Park, *Smittinand 10160*, 20. 6. 1963. alt. 900-1100 m. (holotype: BKF).

Lithophytic, perennial herb with short rhizome; root fibrous, some forming a longish storage tuber. *Pseudostem* slender, erect, 6.2-19.7 cm high, green with 2-4 bladeless sheaths at the base. *Leaves* 4-7, the lowermost 2-4 strongly reduced to bladeless sheath; lamina sessile, ovate to ovate-lanceolate, 3.9-13.4 by 1.2-1.9 cm, base cuneate, apex acuminate to caudate, margin entire, both surface glabrous, green; ligule membranous, 5-7 mm long, apex rounded, hairy. *Inflorescence* terminal, 3-5.4 cm long; peduncle 1-2.2 cm long, hairy; axis hairy. Bracts 4-9, distichous, narrowly ovate to lanceolate, 2.2-2.4 cm by 3-6 mm, apex acuminate to caudate, hairy, green, each bract enclosing strictly 1-flowered cincinnus; bracteoles elliptic, c.1 by 0.5 cm, margin membranous, apex acuminate, green. *Flowers* yellow; calyx tubular, glabrous, hidden in the bract, 0.6-1.1 cm long, apex 3-dented, hairy at base, green; corolla tube slender, 1.6-2.1 cm long, yellow; dorsal corolla lobe elliptic, 1-1.2 cm by 3-4 mm, apex mucronate,

yellow; lateral corolla lobes lanceolate-elliptic, 0.9-1 cm by 2-3 mm, yellow; lateral staminodes, obovate, 0.9-1.1 cm by 5-6 mm, apex round, yellow; labellum broadly ovate to suborbicular, 2-2.2 by 1.6-2 cm, apex slightly emarginated, yellow; anther-crest broadly ovate, 4-5 by 3-4 mm, recurved, apex rounded, yellow; stigma funnel-shaped; ovary cylindrical 4-5 mm. long, pilose, ovules numerous; stylodes 2, filiform, ca. 1 mm. long. *Fruits*, ovate, 2-2.3 cm by 3-4 mm long. *Seeds* numerous, ellipsoid, *ca*. 2 by 1 mm, hairy.

Thailand: Eastern [Nakhon Ratchasima-Prachin Buri, Khao Yai National Park, Khao Khieo, Pha Deaw Dai; Trat, Khao Kuap], Northern [Mae Hong Son, Doi Pa Yee; Chiang Mai, Khun Chae National Park], Western [Kanchanaburi, Thong Pha Phum National Park].

Phenology: Flowering from June-July, fruiting from July-September.

Distribution: This species has widely distributed in Thailand.

Ecology: This species grows on moist rocks under the shade of rain forest, at the altitude of 1200 m.

Etymology: The specific epithet is referred to epilithic.

Vernacular name: Proh Hin (เปราะหิน)

Examined material: *Picheansoonthon & Phokham, CP-030812-1* (MSU)

3.4.21 *Caulokaempferia sirirugsae* Ngamr., Nordic J. Bot. 26: 1-4, 2008.

Type: THAILAND: Phangnga province, Khao Lumpee waterfall, on rocks near stream, N 08° 46′ 49″. E 98° 28′ 03″, *C Ngamriabsakul 51-01*, 26. 6. 2008. alt. 85 m. (holotype: BKF, isotype:, PSU and Walailak Univ. herbarium).

Epilithic, perennial herb with short rhizome; root fibrous, some forming a longish storage tuber. *Pseudostem* slender, erect, up to 15.8 cm high, green with 3-4 bladeless sheaths at the base. *Leaves* 6-8, the lowermost 1-2 strongly reduced to bladeless sheath; lamina sessile, ovate-lanceolate, 9.3-12.5 by 1.2-1.5 cm, apex acuminate-caudate, margin entire, both sides glabrous, green; ligule membranous, 5-8 mm long, translucent reddish, apex acuminate. *Inflorescence* terminal, 3-5-6.4 cm long; peduncle glabrous, 1-1.5 cm long. *Bracts* 1-2(-3), distichous, oblong-lanceolate, glabrous, green, 2.2-3.8 by 6-8 mm, apex acuminate to shortly caudate, margin membranous, each bract enclosing a 1-3 flowered cincinnus; bracteoles oblong-elliptic,

ca.1 cm by 2 mm, margin membranous, apex acuminate, green. *Flowers* entirely yellow; calyx tubular, hidden in the bract, 1.2- 1.8 cm long, apex 2-lobed, glabrous, green,; corolla tube slender, 2.7-3.4 cm long, yellow; dorsal corolla lobe ovate-lanceolate, hooded, 1-1.1 by 3-4 mm, yellow; lateral corolla lobes lanceolate, 1.-1.1 cm by 2-3 mm, yellow; lateral staminodes narrowly obovate with concave at the base, 1.1-1.5 cm by 4-6 mm, apex round, yellow; labellum obovate, 1.8-2.2 by 1.5-1.8 cm, trilobed, median lobe apex slightly emarginate; filament very short; anther 2-3 mm long; anther-crest, yellow, ovate, 4-5 by 3-4 mm, recurved, apex rounded; stigma funnel-shaped; ovary cylindrical 4-8 mm. long, ovules numerous; stylodes 2, filiform, ca. 1 mm. long. *Fruits*, ellipsoid, 8-12 mm long, glabrous. *Seeds* numerous, ellipsoid, *ca*. 1 mm long, pubescent.

Thailand: Southern [Phangnga, Lumpi waterfall].

Phenology: Flowering from June-July, fruiting from July-September.

Distribution: This species has so far been found only at the type location.

Ecology: This species grows on moist rocks near waterfall, at the altitude of 100 m.

Etymology: The specific epithet is named in honor of Professor Puangpen Sirirugsa.

Vernacular name: Proh Ton Sirirugsa (เปราะต้นศิริรักษ์)

Examined material: *Picheansoonthon & Phokham, CP-140712-2* (MSU)

To distinguish in more details, the morphological study in 21 species of the genus *Caulokaempferia* in Thailand and Lao PDR can be divided into 2 main groups based on labellum characteristics of flowers.

1.) Labellum broadly suborbicular to orbicular without median lobe, apex rounded group: This group is recognized by its labellum without median lobe and apex rounded including; one lithophytic herb with hairy bract, *C.saxicola* and seven terrestrial herbs, *C.amplexicualis*, *C.chayaniana*, *C.pubescens*, *C.appendiculata*, *C.khaomaenensis*, *C.phuluangensis* and *C.larsenii*.

2.) Labellum broadly obovate with median lobe and apex trilobe or bilobe with slightly emarginate group: This group is recognized by its labellum with median lobe including; one none bract and inflorescence sessile herb, *C. picheansoonthonii* and

twelve inflorescence pedunculate with bract herbs which are subdivided into 2 subgroups. The first subgroup is conspicuous by its bract imbricate, axis invisible or partially visible on one side in flowering stage including; *C.phutokensis* and *C.kamthorniana* (*sp. nov.*), are distinguished by their bracts wrapped tightly around an inflorescence axis (axis invisible) in flowering stage and other herbs that their bracts amplexicaul base, margin free to the base, axis partially visible on one side, including; *C.jirawongsei*, *C.bracteata*, *C.phuwoaensis* and *C.phulangkaensis*.

The second subgroup is distinguished by its bract not imbricate, axis visible more than one side in flowering stage including; three species, *C.pedemontana*, *C.limiana* and *C.bolavenensis*, are patent staminodes and inflorescence enclosed in the two uppermost leaf sheaths and three species, *C.satunensis C.sirirugsae* and *C.saksuwaniae*, are distinct on projecting forward staminodes, inflorescence produced beyond the two uppermost leaf sheaths and distributed in peninsular Thailand.

CHAPTER 4

MOLECULAR STUDY

4.1 Methodology and Materials

4.1.1 Plant materials

Totally 31 plant accessions of *Caulokaempferia* species were utilized in phylogenetic analysis, as shown in Table 4.1. Another one accession of *Jirawongsea alba* was also included as one of cladistics. Each accession was identified as isolate 'BMPK' following by the ascending number. For DNA sequencing, the ITS sequences of all 32 isolates were available, however, trnLF sequences of 4 isolates were not available. Two ITS sequences of the other two isolates of *Caulokaempferia* species were retrieved from the GenBank database (as presented with GenBank accession number) to be included in *Caulokaempferia* clade. As well as the ITS and trnLF sequences of 7 species from other 7 genera in family Zingiberaceae, and 3 species from other 3 families in order Zingiberales were also retrieved to be used as cladistics and outgroup in the phylogenetic clustering, respectively.

Table 4.1 Plant accessions and retrieved DNA sequences used in phylogenetic analysis

Isolate	Species	ITS sequence	trnLF sequence	Taxonomic group
BMPK01	*Jirawongsea alba*	available	available	Family Zingiberaceae
BMPK02	*C. amplexicaulis*	available	available	Genus *Caulokaempferia*
BMPK03	*C. appendiculata*	available	available	Genus *Caulokaempferia*
BMPK04	*C. bolavenensis*	available	available	Genus *Caulokaempferia*
BMPK05	*C. bracteata*	available	available	Genus *Caulokaempferia*
BMPK06	*C. chayaniana*	available	available	Genus *Caulokaempferia*
BMPK07	*C. jirawongsei*	available	available	Genus *Caulokaempferia*
BMPK08	*C. saxicola*	available	available	Genus *Caulokaempferia*
BMPK09	*C. khaomaenensis*	available	available	Genus *Caulokaempferia*

Table 4.1 continued.

Isolate	Species	ITS sequence	trnLF sequence	Taxonomic group
BMPK10	*C. phulangkaensis*	available	available	Genus *Caulokaempferia*
BMPK11	*C. kamthorniana*	available	available	Genus *Caulokaempferia*
BMPK12	*C. kamthorniana*	available	available	Genus *Caulokaempferia*
BMPK13	*C. larsenii*	available	available	Genus *Caulokaempferia*
BMPK14	*C. pedemontana*	available	available	Genus *Caulokaempferia*
BMPK15	*C. phuluangensis*	available	available	Genus *Caulokaempferia*
BMPK16	*C. pubescens*	available	available	Genus *Caulokaempferia*
BMPK17	*C. phuwoaensis*	available	available	Genus *Caulokaempferia*
BMPK18	*C. phuwoaensis*	available	not available	Genus *Caulokaempferia*
BMPK19	*C. phuwoaensis*	available	not available	Genus *Caulokaempferia*
BMPK20	*C. picheansoonthonii*	available	available	Genus *Caulokaempferia*
BMPK21	*C. sirirugsae*	available	available	Genus *Caulokaempferia*
BMPK22	*C. saksuwaniae*	available	available	Genus *Caulokaempferia*
BMPK23	*C. satunensis*	available	available	Genus *Caulokaempferia*
BMPK24	*C. saxicola*	available	available	Genus *Caulokaempferia*
BMPK25	*C. phutokensis*	available	available	Genus *Caulokaempferia*
BMPK26	*C. saxicola*	available	available	Genus *Caulokaempferia*
BMPK27	*C. saxicola*	available	not available	Genus *Caulokaempferia*
BMPK28	*C. saxicola*	available	available	Genus *Caulokaempferia*
BMPK29	*C. saxicola*	available	available	Genus *Caulokaempferia*
BMPK30	*C. saxicola*	available	available	Genus *Caulokaempferia*
BMPK31	*C. saxicola*	available	not available	Genus *Caulokaempferia*
BMPK32	*C. limiana*	available	available	Genus *Caulokaempferia*
M3246	*C. limiana*	KF982797	not available	Genus *Caulokaempferia*

Table 4.1 continued.

Isolate	Species	ITS sequence	trnLF sequence	Taxonomic group
M12C193	*C. linearis*	KF982798	not available	Genus *Caulokaempferia*
	Stahlianthus involucratus	AF478796	AY424799	Family Zingiberaceae
	Camptandra parvula	AF478730	AY424780	Family Zingiberaceae
	Pyrgophyllum yunnanense	AF478777	AY424793	Family Zingiberaceae
	Kaempferia elegans	AY424764	AY424790	Family Zingiberaceae
	Boesenbergia aurantiaca	AF202409	AY424778	Family Zingiberaceae
	Cornukaempferia longipetiolata	AY424750	AY424783	Family Zingiberaceae
	Zingiber officinale	KC582866	HM567395	Family Zingiberaceae
	Heliconia stricta	FJ626404	FJ621299	Family Heliconiaceae
	Musa balbisiana	HQ331342	FJ621280	Family Musaceae
	Ravenala madagascariensis	FJ428107	FJ621296	Family Strelitziaceae

4.1.2 Isolation of total genomic DNA samples

Plants in the genus *Caulokaempferia* have been well known to containing high quantity of secondary metabolites which would become impurities and could interfere the downstream molecular biology processes. Thus, the isolation of high quality of gDNA samples from such plants may face difficulty and the common DNA isolation methods may not suitable. Also, when considering on the facts that the dried leaves tissue dehydrated by silica gel were fragile and at ease to homogenize without using the liquid nitrogen that becomes uncomfortable to handle, and this study contained high numbers of plant samples to deal with. A modified DNA isolation method without using of liquid nitrogen and capable of high efficient elimination of impurities and to handle with many plant samples in the short time was required. Therefore, the plant DNA

isolation method by Rotchanapreeda and his colleagues (2015) was applied here as small-scale extraction and used in this study. The protocol demanded the use of micro silica bead as homogenizing agent, introduced the salting out effect of impurities with high concentration of potassium acetate, and could be performed in the short time.

In isolation, two tubes of each plant accession were performed individually. To begin homogenizing, the mortar and pestle were burned with 95% ethanol for sterilization. After cooling down to room temperature, 400 µl of CTAB buffer (2% CTAB, 1.4 M NaCl, 100 mM Tris-HCl pH 8.0, 20 mM EDTA pH 8.0) was loaded to the mortar. Approximately 25 mg of dried leaves blades excluding the petiole was cut into the small pieces into the mortar. One spatula spoon of Silica gel 60 (70 – 230 mesh ASTM, Merck) was put into the mortar and the tissue was grinded until became slurry. Then the mixture was transferred to a 1.5ml microcentrifuge tube. The tube was incubated at 65°C for 15 minutes with the 5-minutes interval vortex. The tube was then cooled down on ice prior to adding of 100 µl of 5 M Potassium acetate to obtain the final concentration at 1 M. For salting out effect, the tube was mixed by vortex and then incubated on ice for 15 minutes (or up to 60 minutes for the highest efficiency). For phase separation, one volume of Chloroform : Isoamyl alcohol (24 : 1, v/v) was added to the tube, mixed by vigorous shaking until forming the emulsion and then centrifuged at 16,000 × g for 15 minutes at room temperature. The upper aqueous phase was transferred to the new 1.5-ml microcentrifuge tube without interrupting the organic phase, and then added with 2 volumes of absolute ethanol at room temperature to precipitate the DNA. The mixture was mixed by gently inversion then the DNA pellet was harvested by centrifugation at 16,000 × g for 15 minutes at room temperature. After centrifugation, the supernatant was carefully discarded and the DNA pellet was washed with 500 µl of 80% ethanol. After the ethanol was discarded, the tube was briefly centrifuged then the residual ethanol was removed by pipetting without interrupting the pellet. The tube was marooned with the opened cap at room temperature until the pellet was dry. Then the pellet was dissolved with 50 µl of TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA pH 8.0). To eliminate RNA contamination, 1 µl of 1 mg/ml RNase A (Sigma-Aldrich, catalogue no. R6513) was added to the mixture (final concentration 20 µg/ml) and the tube was then incubated at 37°C for 30 minutes. Finally, the sample was kept at -20°C for long term storage until further uses.

The isolated gDNA sample contained the total gDNA molecules of nucleus and plastids. Prior to amplification, all isolated gDNA samples were determined for the quantity by spectrophotometry and the quality by gel electrophoresis. For each plant accession, the gDNA sample tube with higher quantity and quality was used in amplification first, and another tube would be kept as a substitution.

4.1.3 PCR amplification of ITS and trnLF regions

In order to obtain the DNA sequences to be used in phylogenetic analysis, the Polymerase Chain Reaction (PCR) technique was performed to amplify two interested genomic regions, i.e., ITS and trnLF, from the nuclear and chloroplast genome, respectively. The forward and reverse primers used in amplification were previously designed from the conserved sequences of such regions and widely used in several plant phylogenetic studies. However, for the ease in sequencing process, the universal primers sequences, pBluescript KS (5′-CTCGAGGTCGACGGTATC-3′) and pBluescript SK (5′-CGCTCTAGAACTAGTGGATC-3′), were 5′-end flanked to either original forward or reverse primers sequences in each pair and newly designed to use in this study. Information of original and newly designed primers are shown in Table 4.2 and 4.3, respectively. The designed primers were oligonucleotide synthesized by 1st BASE company (Malaysia).

Table 4.2 Information of original forward and reverse primers pairs.

Primers name	Type	Primers sequences (5′ to 3′)	Length	References
ITS1	Forward	TCCGTAGGTGAACCTGCGG	19 bp	White et al., 1990
ITS4	Reverse	TCCTCCGCTTATTGATATGC	20 bp	
trnL (UAA) 5′-exon	Forward	CGAAATCGGTAGACGCTACG	20 bp	Taberlet et al., 1991
trnF (GAA)	Reverse	ATTTGAACTGGTGACACGAG	20 bp	

Table 4.3 Information of newly designed forward and reverse primers pairs.

Primers name	Primers sequences (5′ to 3′)	Length	Expected product size
KS_ITS1	CTCGAGGTCGACGGTATCCGTAGGTGAACCTGCGG	35 bp	~700 bp
SK_ITS4	CGCTCTAGAACTAGTGGATCCTCCGCTTATTGATATGC	38 bp	
SK_trnL	CGCTCTAGAACTAGTGGATCCGAAATCGGTAGACGCTACG	40 bp	~1000 bp
KS_trnF	CTCGAGGTCGACGGTATCATTTGAACTGGTGACACGAG	38 bp	

Note: Sequences with single- and double-underlined represented for pBluescript KS and pBluescript SK, respectively.

Prior to amplification, each gDNA sample was diluted with TE buffer to the concentration of 100 ng/µl and then used as template. The PCR reaction was performed in total volume of 100 µl, with two replicates per region of each plant accession. In the reaction, the final concentration of each component was as following; 1X Standard *Taq* Reaction Buffer (containing 1.5 mM $MgCl_2$) (NEB), 0.2 mM dNTPs Mix (0.2 mM each dATP, dCTP, dTTP, dGTP) (Promega), 0.5 µM each forward (KS_ITS1 or SK_trnL) and reverse (SK_ITS4 or KS_trnF) primers, 3 units of *Taq* DNA Polymerase (NEB), and 200 to 500 ng of gDNA sample. However, in some difficult reactions, supplementation of additional $MgCl_2$ (1.5 to 4.5 mM) were required. After that, the reaction mixture was mixed by vortex, briefly spun down, and then put into a programmable DNA thermal cycler (Amplitronyx ATC401, Nyx Technik).

In order to observe the optimal annealing temperature for both ITS and trnLF primers pairs, the thermal gradient PCR reactions were performed. After examine the gradient PCR products with gel electrophoresis, the optimal annealing temperature for both pairs, in the other hand, the PCR program, was equivalent. The PCR program consisted of pre-denaturation step in one cycle at 94°C for 5 minute to completely melt the double-stranded DNA, then two stages of amplification. The first stage contained 15 cycles of denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute, and extension at 72°C for 1 minute, to allowing original primers sequences anneal to gDNA template. Then the second stage contained 25 cycles of denaturation at 94°C for 1 minute, annealing at 65°C for 1 minute, and extension at 72°C for 1 minute, in which, the amplification would occur by using the amplified fragments from the first stage as

template. Finally, final-extension step at 72°C for 10 minutes to fulfill polymerization. At the end of the reaction, the PCR products were stored at 4°C.

After the PCR reaction was completed, 5 µl of each PCR product was determined by gel electrophoresis. For the success amplification, two replicate reactions were combined to obtain the final volume of approximately 200 µl. Then the PCR samples were submitted for DNA sequencing at 1st BASE company (Malaysia) with additional PCR clean-up service to purify DNA sample prior to sequencing. In automated dideoxynucleotide sequencing reaction, the pBluescript KS and pBluescript SK universal primers (offered by the company) would be used in either 5′-end or 3′-end sequencing depending on the primers pairs used in PCR amplification.

4.1.4 Phylogenetic analysis

After the DNA sequencing results were obtained, all DNA sequences needed to be manipulated prior to analysis. In each sample, the DNA sequence obtained from 3′-end sequencing was reverse complemented, and then pairwise aligned with 5′-end sequence using ClustalW2 program (http://www.ebi.ac.uk/Tools/msa/clustalw2) to generate the full-length DNA sequence. The correction of nucleotide bases was revised manually by verifying from the raw chromatogram. After that, the sequences of universal primers pBluescript KS and pBluescript SK were trimmed, and then all DNA sequences were submitted to the GenBank database (http://www.ncbi.nlm.nih.gov) for deposition. For phylogenetic clustering, DNA sequences of both ITS and trnLF regions of appropriate plant species within and outside the family Zingiberaceae were retrieved from GenBank database to be used as cladistics and outgroup, respectively (Table 3.1). For each plant accession, the DNA sequences from ITS and trnLF regions were concatenated, excepting for some accessions that no DNA sequence was available for trnLF region. Then the concatenated sequences were multiple aligned by Multiple Alignment using Fast Fourier Transform program (MAFFT, http://www.ebi.ac.uk/Tools/msa/mafft) with “localpair” FFTS setting. After that, the multiple sequence alignment was manually edited and revised by using the program BioEdit version 7.2.5 (Hall, 1999). The alignment was then tested to find out for a best nucleotide substitution model of Maximum Likelihood method using the MEGA program version 6.06 (Tamura et al, 2013). The best model would be indicated, in

which, model with the lowest Bayesian Information Criterion (BIC) score is the model that displays the maximum posterior probability and considered to describe the substitution pattern the best (Posada and Buckley, 2004). After the best model was indicated, the phylogeny construction was conducted based on Maximum Likelihood statistical method with the following parameters; substitution model of Tamura 3-parameter (Tamura, 1992), rates among sites of gamma distribution with 5 rates of discrete gamma categories, gaps/missing data treatment by using all sites, and testing for reliability by bootstrapping with 1,000 replicates. After the phylogeny clustering was constructed, the phylogram was finalized featured using the MS Powerpoint program.

4.1.5 Quantification of DNA with spectrophotometry

In order to measure for the quantity of isolated gDNA samples, 2 µl of each sample was applied on the pedestal of NanoDrop™ 2000c UV-Vis Spectrophotometer (Thermo Scientific) with using TE buffer as a blank reference. The O.D. at 260 nm would be used to calculate for nucleic acids concentration of the sample, and the ratios of between O.D. 260 nm and 280 nm (260/280 nm) and between O.D. 260 nm and 230 nm (260/230 nm) would be used to indicate for the RNA/proteins and other impurities contamination, respectively.

4.1.6 Qualification of DNA with gel electrophoresis

In order to determine for the quality of isolated gDNA samples and amplified PCR products, the DNA samples were analyzed with gel electrophoresis approach. For gDNA samples, after the concentration was determined, the total mass of 200 ng of each sample was mixed with appropriate volume of TE buffer prior to be loaded. And for PCR products, 5 µl from each reaction would be used. To analyze the DNA, the gDNA or PCR product sample was mixed with 1 µl of 6X Gel Loading Dye, Blue (NEB, catalogue no. B7021S) and then loaded into 1% (w/v) agarose gel, along with 0.25 ng total mass of 2-Log DNA Ladder (NEB, catalogue no. N3200S) as molecular weight standard. The gel was run in 0.5X TAE buffer at 100 V for 25 minutes. After that, the gel was stained in 0.5 µg/ml of ethidium bromide solution for 5 minutes then destained in distilled water for 5 minutes. Finally, DNA fragments on the gel were

visualized and photographed using gel documentation system G:BOX Chemi EF2 (Syngene).

4.2 Results

4.2.1 Quantity and quality of isolated gDNA samples

After the plant leaves samples were isolated for total gDNA using modified CTAB-based DNA isolation method, all gDNA samples were verified for their quantity and quality by spectrophotometry and gel electrophoresis, respectively. For quantity, the spectrophotometry approach was used to measure the O.D. at different wavelengths to determine the numerical presence of various molecules. The absorption at 260 nm reflects the amount of total nucleic acid molecules including of DNA and RNA, while at 280 nm shows the proteins. And the absorption at 230 nm indicates for the impurities majority from salts, carbohydrates, phenol, and peptides. The results obtained from the NanoDrop™ software of some accession samples were showed in Table 4.4.

Table 4.4 Spectrophotometry example results of isolated gDNA samples.

Plant accessions	Nucleic acid concentration	O.D. 260 nm	O.D. 280 nm	260/280 nm	260/230 nm
BMPK32	260 ng/µl	5.218	2.431	2.15	0.94
BMPK33	340 ng/µl	6.814	3.399	2.00	0.79
BMPK10	234 ng/µl	4.688	2.102	2.23	0.76
BMPK69	173 ng/µl	3.460	2.100	1.65	0.68
BMPK70	248 ng/µl	4.960	2.602	1.91	0.69
BMPK83	570 ng/µl	11.415	4.973	2.30	0.75
BMPK84	172 ng/µl	3.442	2.074	1.66	0.51
BMPK24	174 ng/µl	3.492	1.824	1.91	1.28
BMPK88	212 ng/µl	4.256	2.371	1.79	1.19

The absorbance value at 260 nm was used to calculate for total nucleic acid concentration for each gDNA samples. As for nine example accessions showed here, their average concentration was approximately 265 ng/µl. With the 50 µl of TE buffer that dissolving the sample, the average total nucleic acid mass was around 13 µg for each isolated gDNA samples, that equivalent to 26 µg per each plant accession. By treatment of RNase A, the average ratio of 260/280 nm was 1.95, indicating that most of the samples contained low contamination of RNA molecules, excepting for some samples which the ratio was more than 2.00. Also, the ratio of 260/280 nm in all samples was greater than 1.60, indicating for low proteins level. Unfortunately, the average ratio of 260/239 nm was 0.84, which is quite low when considering that the good ratio value should be above 1.80. This low ratio value indicated for high contamination of many impurities such as salts and other molecules which have the maximum absorbance at around 230 nm. The effect of high impurities may be caused by the secondary metabolites presenting at high level in *Caulokaempferia* plants, and other metabolites produced from cell death stasis during long term storage.

And for the quality, the physical properties of isolated gDNA sample and the presence of contaminants were verified by gel electrophoresis. To analyze the samples, the total mass of approximately 300 ng gDNA was loaded on the agarose gel, separated by electrical field, and stained by ethidium bromide solution. The gDNA fragments were visualized and captured by gel document system. The gel photograph of the same example samples presented in spectrophotometry is showed in Figure 4.1.

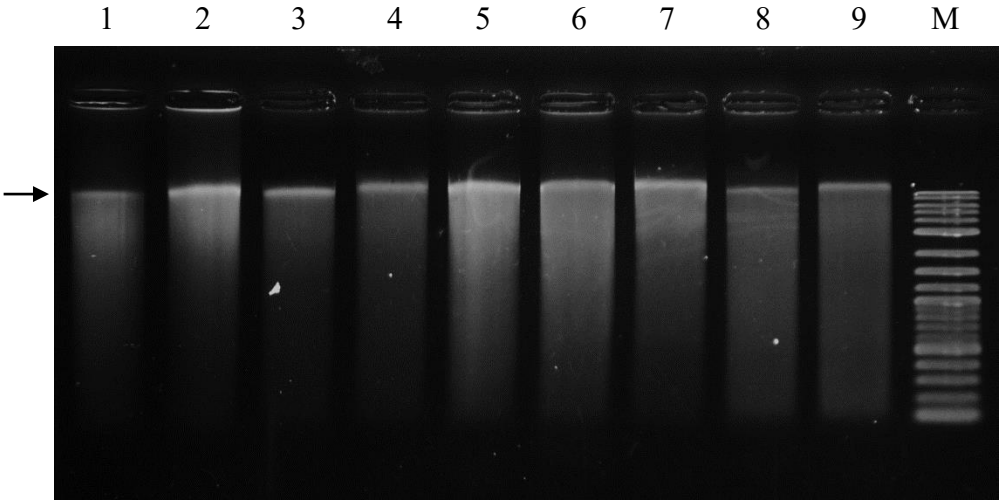

Figure 4.1 The example isolated gDNA samples analyzed by gel electrophoresis. M, 2-Log DNA Ladder (NEB) (total mass of 0.25 µg loaded). Lane 1 to 9 represent for accessions BMPK32, BMPK33, BMPK10, BMPK69, BMPK70, BMPK83, BMPK84, BMPK24, BMPK88, respectively. The arrow indicates the high molecular weight gDNA fragments.

As from the gel photograph, the gDNA samples of all example accessions appeared as the high molecular weight DNA band at above a top-most fragment of ladder, which is 10 kb in length. However, in all samples, the sheer DNA fragments presented beneath the high molecular weight band indicating for the degradation of gDNA molecules, in which, the level of sheerness reflects for the level of degradation. Specifically, the degradation may occur *in situ* during the long term storage in silica gel prior to isolation. When looking at the loaded wells, there were no or very little of remnants remained at the bottom of the wells, which indicating that the impurities detected from spectrophotometry with the ratio value of 260/230 nm were all soluble. Moreover, the absence of any RNA fragments on the gel, which in spite of the ratio of 260/280 nm suggested for low contamination of RNA molecules in some samples, indicated that these RNA molecules could be degraded into very short fragments and incapable to be detected by gel electrophoresis. When combine the results from spectrophotometer and gel electrophoresis, the isolated gDNA samples were appropriate for PCR amplification strategy.

4.2.2 Amplification of PCR products and DNA sequencing of ITS and trnLF regions

Two universal primers pairs, KS_ITS1 / SK_ITS4 and SK_trnL / KS_trnF, were selected to amplify two conserved regions, ITS region on chromosome and trnLF region on chloroplast genome, respectively. After PCR product for each plant accession and region was conducted, all PCR reactions were verified for amplified fragments by gel electrophoresis. The results from PCR amplification generated from ITS and trnLF regions of some example accessions are shown in Figure 4.2 and 4.3, respectively.

From the gel photographs of ITS and trnLF regions, the example PCR products presented at expected size of approximately 700 bp and 1,000 bp, respectively. By the way, when looking at the physical property of PCR products, both for example samples presented here and for overall accessions, the differences between amplification of ITS and trnLF regions were revealed. In ITS reactions, most of the reactions were successfully amplified with none or low amount of additional $MgCl_2$ (1.5 to 2.5 mM), and the PCR fragments analyzed on the agarose gel appeared as single DNA band. However, in the case of trnLF reactions, most of the reactions faced more challenge with the requirement of high additional $MgCl_2$ (3.5 to 4.5 mM) supplementation, thus with such high concentration of $MgCl_2$, the unspecific amplified fragments unfortunately appeared along with expected 1,000-bp PCR fragment. Despite of the present of unspecific bands in trnLF reactions, after the total of 200 µl PCR samples were submitted for purification and DNA sequencing, both ITS and trnLF reaction samples were capable to yield the good DNA sequences, for the example, as shown in Figure 4.4 and 4.5, respectively. The DNA sequencing, only from single direction, usually generates the bad result at the very beginning of the sequence due to limitation of automated sequencing method. However, by perform sequencing from both directions of forward and reverse primers, the reverse-complemented sequence generated from reverse primer direction would cover such bad part of forward primer direction and then allowing the access for full-length sequence of amplified fragment. After the sequences of pBluescript KS and pBluescript SK were trimmed, all sequences were manipulated and then submitted to the GenBank database.

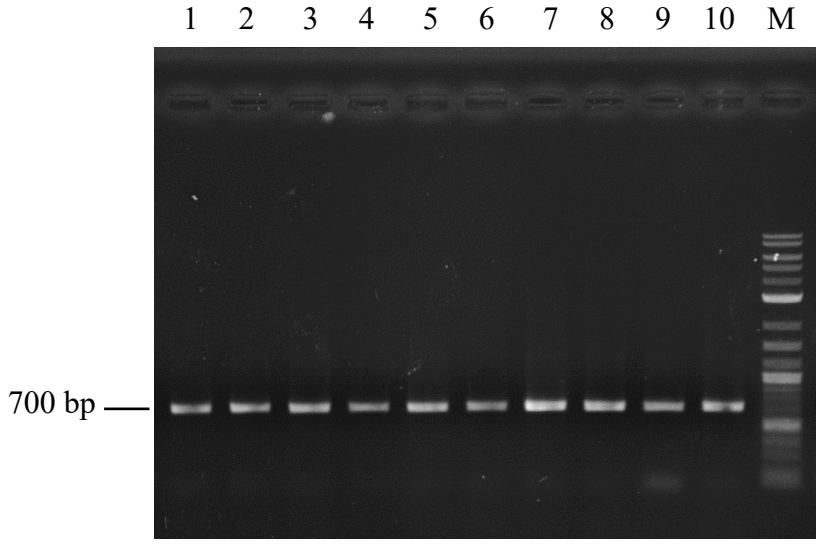

Figure 4.2 The example PCR products from ITS region analyzed by gel electrophoresis. M, 2-Log DNA Ladder (NEB) (total mass of 0.25 µg loaded). Lane 1 to 10 represent for accessions BMPK51, BMPK53, BMPK61, BMPK65, BMPK67, BMPK70, BMPK71, BMPK20, BMPK83, BMPK85, respectively. The expected size of PCR products at approximately 700 bp is indicated.

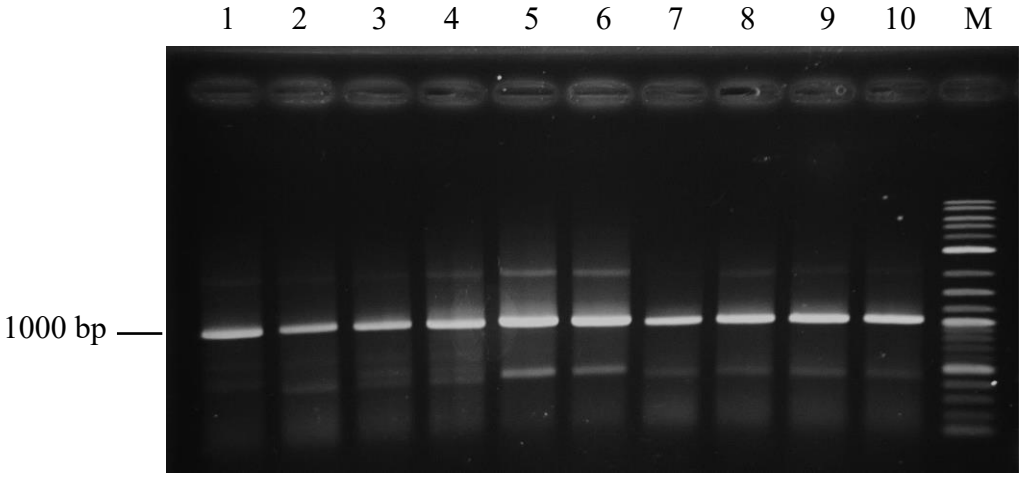

Figure 4.3 The example PCR products from trnLF region analyzed by gel electrophoresis. M, 2-Log DNA Ladder (NEB) (total mass of 0.25 µg loaded). Lane 1 to 10 represent for accessions BMPK61, BMPK65, BMPK67, BMPK71, BMPK81, BMPK82, BMPK83, BMPK84, BMPK85, BMPK86, respectively. The expected size of PCR products at approximately 1,000 bp is indicated.

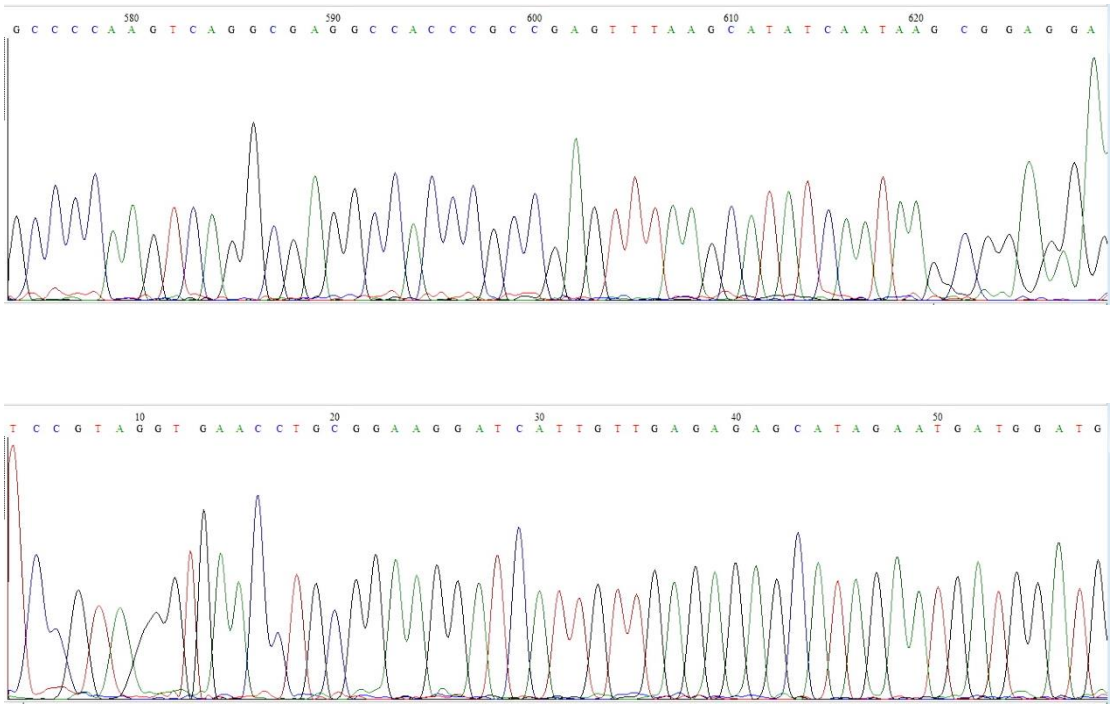

Figure 4.4 The example DNA sequencing result from ITS region. The chromatogram generated from automated Sanger dideoxynucleotide sequencing approach of the accession BMPK12, which sequenced by using pBluescript KS (above) (showing the end of reverse primer) and pBluescript SK (below) (showing the head of forward primer) universal primers. Note that the DNA sequence from pBluescript SK direction is reverse complemented.

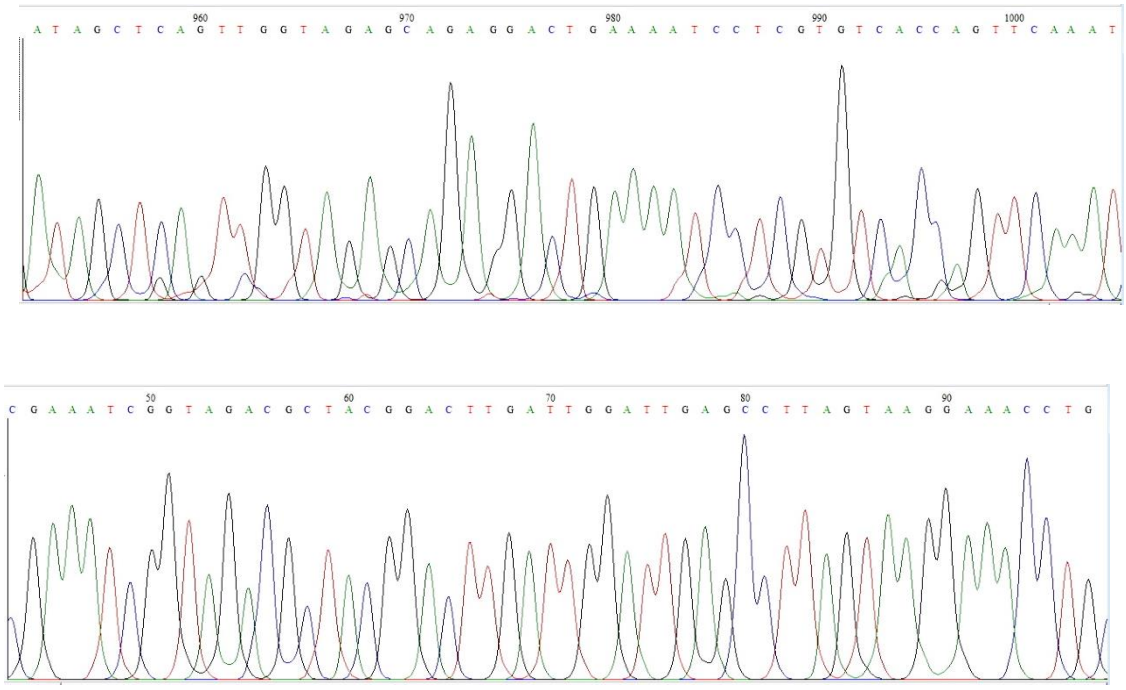

Figure 4.5 The example DNA sequencing result from trnLF region. The chromatogram generated from automated Sanger dideoxynucleotide sequencing approach of the accession BMPK46, which sequenced by using pBluescript SK (above) (showing the end of reverse primer) and pBluescript KS (below) (showing the head of forward primer) universal primers. Note that the DNA sequence from pBluescript KS direction is reverse complemented.

4.2.3 Phylogenetic analysis of *Caulokaempferia* taxa revealed from the DNA sequences

In order to maximize the polymorphic characters, DNA sequences of ITS and trnLF regions in each accessions, if available (Table 4.1), were concatenated into one longer sequence. After multiple aligned to define the gaps, the concatenated sequences generated the total of 1,927 nucleotide positions used for analysis (819 positions for ITS, 1,108 positions for trnLF) (data not shown). Then the multiple alignment was tested to find out a best substitution model to ensure the accurate clustering of the data. The total of 24 nucleotide substitution models were tested using the MEGA program, and the result is shown in Table 4.5.

Table 4.5 Maximum Likelihood fits of 24 different nucleotide substitution models.

Model	BIC value	Model	BIC value	Model	BIC value
T92+G	18999.163	HKY+G+I	19055.757	JC+I	19315.874
TN93+G	19005.330	T92+I	19089.567	JC+G+I	19317.814
T92+G+I	19008.146	K2+I	19098.482	GTR	19754.057
K2+G	19009.495	TN93+I	19100.084	TN93	19754.184
K2+G+I	19026.966	HKY+I	19118.722	T92	19759.071
HKY+G	19028.162	GTR+I	19125.381	HKY	19788.859
GTR+G	19030.137	TN93+G+I	19204.608	K2	19809.388
GTR+G+I	19039.442	JC+G	19233.637	JC	20012.482

For abbreviations. GTR: General Time Reversible; HKY: Hasegawa-Kishino-Yano; TN93: Tamura-Nei; T92: Tamura 3-parameter; K2: Kimura 2-parameter; JC: Jukes-Cantor; G: gamma distribution; I: invariable evolutionary; BIC: Bayesian Information Criterion.

From the result, the model with the lowest BIC value (18999.163) was Tamura 3-parameter with considering of gamma distribution, which used to explain evolutionary rates among sites (T92+G). The model takes into account for the differences in transitional and transversional rates and G+C-content bias. Therefore, this nucleotide substitution model was used to construct phylogeny cluster, and the phylogram is shown in Figure 4.6.

From the figure, the phylogram was presented as the un-rooted tree due to no common ancestral lineage was assumed in the analysis, hence, the tree only specified for relationship among taxa but not evolutionary path. By using Maximum Likelihood statistical method to estimate parameters of selected nucleotide substitution model, the highest likelihood of the observed multiple alignment data was inferred in the clustering, thus, variable branch length was calculated to display quantitative differences between each operational taxon units (OTUs). The reliability of OTUs to be inferred to cluster together was presented by the bootstrap values on each branch node. The values are range from 1 to 100, in which, the higher the value, the more reliable inference.

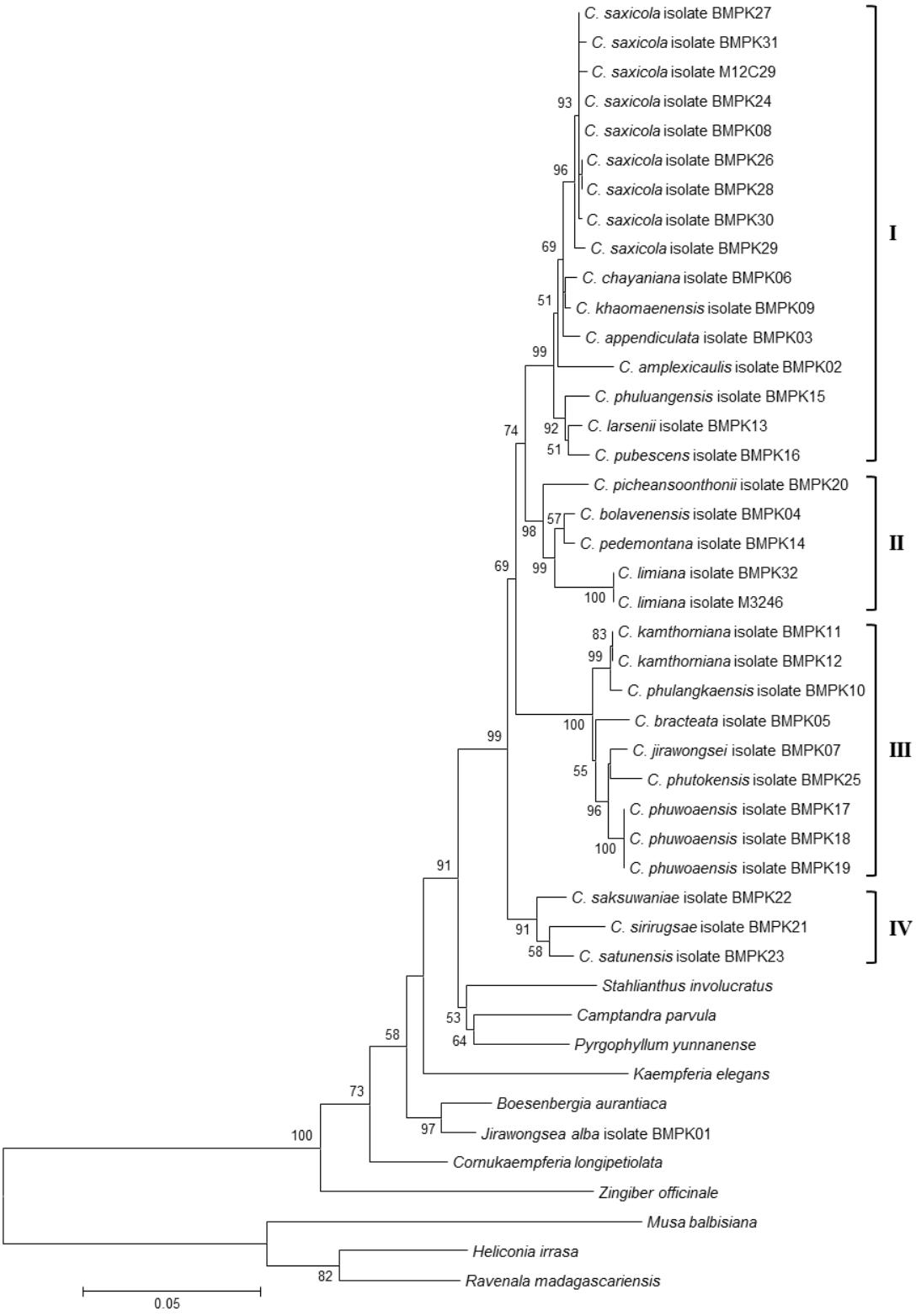

Fig. 4.6 Phylogenetic clustering based on Maximum Likelihood. Only bootstrap values which >50 are indicated on each branch node. The scale bar beneath the tree is proportional to the branch length. Roman numbers represent for sub-clustered clades.

To describe in more details, three species outside the family Zingiberaceae, *Musa balbisiana*, *Heliconia stricta*, and *Ravenala madagascariensis*, were clustered as outgroup. This provided the inferring root to the tree which is virtually located between outgroup and the rest of the taxa. Within the clade of family Zingiberaceae, 8 species from 8 genera, including *Jirawongsea alba* isolate BMPK01, were clustered separately apart from all *Caulokaempferia* taxa as cladistics, in which, *Zingiber officinale* had the least relationship to *Caulokaempferia* clade and the three species of *Stahlianthus involucratus*, *Camptandra parvula*, and *Pyrgophyllum yunnanense* had most such relationship. All 31 accessions of *Caulokaempferia* taxa were clustered together as the major clade. Moreover, the *Caulokaempferia* clade could be sub-clustered into 4 clades based on the remoteness between OTUs measured by branch length. Clade I contained all *Caulokaempferia* species with terrestrial habitat, excepting for *C. saxicola* which is lithophyte as identical to other clades. Clade II composed of species with the unique character of the inflorescence enclosed in leaf sheaths of the two uppermost leaves. Clade III carried species that collected from Northeastern Thailand and Laos border regions. And three species that endemic to Peninsular Thailand were grouped in the Clade IV. The reliability of all abovementioned clusters was supported with high bootstrap values that indicated on each branch node.

CHAPTER 5

DISCUSSION AND CONCLUSION

Twenty one species of the genus *Caulokaempferia* K. Larsen are recognized for Thailand (19 species) and Lao PDR (2 species). Three new species, *Caulokaempferia pubescens* Picheans & Phokham, *C. picheansoonthonii* Phokham & Prasarn and *C. kamthorniana* Picheans, Prasarn & Phokham, *sp. nov.* were revealed for Thailand.

As regards the morphological study the genus *Caulokaempferia* can be divided into 2 main groups based on labellum characteristics of flowers.

1. Labellum broadly suborbicular to orbicular without median lobe, apex rounded group: This group is recognized by its labellum without median lobe and apex rounded including; one lithophytic herb with hairy bract, *C.saxicola* and seven terrestrial herbs, *C.amplexicualis*, *C.chayaniana*, *C.pubescens*, *C.appendiculata*, *C.khaomaenensis*, *C.phuluangensis* and *C.larsenii*.

2. Labellum broadly obovate with median lobe and apex trilobe or bilobe with slightly emarginate group: This group is recognized by its labellum with median lobe including; one none bract and inflorescence sessile herb, *C. picheansoonthonii* and twelve inflorescence pedunculate with bract herbs which are subdivided into 2 subgroups. The first subgroup is conspicuous by its bract imbricate, axis invisible or partially visible on one side in flowering stage including; *C.phutokensis* and *C.kamthorniana* (*sp. nov.*), are distinguished by their bracts wrapped tightly around an inflorescence axis (axis invisible) in flowering stage and other herbs that their bracts amplexicaul base, margin free to the base, axis partially visible on one side, including; *C.jirawongsei*, *C.bracteata*, *C.phuwoaensis* and *C.phulangkaensis*.

The second subgroup is distinguished by its bract not imbricate, axis visible more than one side in flowering stage including; three species, *C.pedemontana*, *C.limiana* and *C.bolavenensis*, are patent staminodes and inflorescence enclosed in the two uppermost leaf sheaths and three species, *C.satunensis C.sirirugsae* and *C.saksuwaniae*, are distinct on projecting forward staminodes, inflorescence produced beyond the two uppermost leaf sheaths and distributed in peninsular Thailand.

As regards the phylogenetic analyses of *Caulokaempferia* using ITS and *trn*LF regions, the phylogenetic tree is shown based on data from Thailand and Lao PDR, GenBank and combined data between both sources. The analysis of combined ITS gene and trnLF sequences in Thai and Laotian *Caulokaempferia* has shown that the genus *Caulokaempferia* is monophyletic and four clades of *Caulokaempferia* species were defined with highly bootstrap supported (bootstrap = 80-100 %).

Clade 1 contains 8 species, *C.chayaniana* group mostly species are terrestrial herbs, including; *C.chyaniana*, *C.appendiculata*, *C.amplexicaulis*, *C.khaomaenensis*, *C.phuluangensis*, *C.larsenii*, and *C.pubescens* except *C.saxicola*, lithophytic. This group is recognized by its labellum without median lobe and apex rounded

Clade 2-4 contains 13 species, this group is recognized by its labellum with median lobe and apex trilobe or bilobe with slightly emarginate.

Clade 2 contains 4 species, *C.picheansoonthonii* group which their inflorescence enclosed in leaf sheaths of the two uppermost leaves, including; *C. picheansoonthonii*, *C.bolavenensis*, *C.pedemontana* and *C.limiana*.

Clade 3 contains 6 species, *C. jirawongsei* group found in Northeastern Thailand and a species in Lao PDR, including*; C.jirawongsei, C.kamthorniana, C.phulangkaensis, C.bracteata, C.phutokensis* and *C.phuwoaensis*.

Clade 4 contains 3 species, *C.satunensis* group found in Peninsular Thailand, including; *C. satunensis*, *C.saksuwaniae* and *C.sirirugsae.*

And the genus *Jirawongsea* representative was separated from the genus *Caulokaempferia* clade.

In conclusion, taxonomic study and phylogenetic analyses are successfully combined for grouping Thai and Laotian *Caulokaempferia* species. The genus can be divided into 4 main groups as follows:

Group 1 contains labellum without median lobe, not imbricate and bilateral bract species and terrestrial herb, *C. chayaniana* group mostly species are terrestrial herbs except *C. saxicola,* lithophytic.

Group 2 contains labellum with median lobe, not imbricate and unilateral bract or none bract species, *C.picheansoonthonii* group which their inflorescence enclosed in leaf sheaths of the two uppermost leaves.

Group 3 contains labellum with median lobe, imbricate and unilateral bract species, *C. jirawongsei* group found in Northeastern Thailand and a species in Lao PDR.

Group 4 contains labellum with median lobe, three species, *C. satunensis* group found in Peninsular Thailand.

From this study, 21 species are enumerated for Thailand (19 species) and Lao PDR (2 species):

The genus *Caulokaempferia* for Thailand comprises 19 taxa.

1. *Caulokaempferia saxicola* K. Larsen (เปราะหิน)
   (syn. *C. kuapii* K. Larsen)
2. *C. saksuwaniae* K. Larsen (เปราะต้นศักดิ์สุวรรณ)
3. *C. appendiculata* K. Larsen & T. Triboun (เปราะต้นอ่างขาง)
4. *C. larsenii* Suksathan & Triboun (เปราะต้นผีปันน้ำ)
5. *C. khaomaenensis* Picheans. & Mokkamul (เปราะต้นเขาเหมน)
6. *C. phuluangensis* Picheans. & Mokkamul (เปราะต้นภูหลวง)
7. *C. jirawongsei* Picheans. & Mokkamul (เปราะต้นจีรวงส์)
8. *C. limiana* Picheans. & Mokkamul (เปราะต้นชาติตระการ)
9. *C. amplexicaulis* Suksuthan (เปราะต้นดอยปุย)
10. *C. pedemontana* Triboun & K. Larsen (เปราะต้นนางรอง)
11. *C. satunensis* Picheans (เปราะต้นสตูล)
12. *C. phuwoaenensis* Picheans & Koonterm (ว่านเปราะต้น, เปราะต้นภูวัว)
13. *C. phulangkaensis* Picheans. (ว่านเปราะหิน, เปราะหินภูลังกา,เปราะต้นภูลังกา)
14. *C. phutokensis* Picheans. (ว่านเปราะหิน, เปราะต้นภูทอก)
15. *C. sirirugsae* Ngamr. (เปราะต้นศิริรักษ์)
16. *C. chayaniana* Tiyaw. (เปราะต้นชยันต์)
17. *C. pubescens* Picheans. & Phokham (เปราะต้นใบขน)
18. *C. picheansoonthonii* Phokham & Prasarn (เปราะต้นพิเชียรสุนทร)

19. *C. kamthorniana* Picheans., Prasarn & Phokham (*sp. nov.*) (เปราะต้นกำธร)

Two *Caulokaempferia* taxa were reported in Lao PDR.

1. *C. bracteata* K. Larsen & S. S. Larsen (เปราะต้นภูเขาควาย)

2. *C. bolavenensis* Picheans. & Koonterm (เปราะต้นบ่อละเวน)

Five non-yellowed *Caulokaempferia* were removed to *Jirawongsea* based on morphological characters and molecular evidence (Picheansoonthon *et al*, 2008)

1. *Caulokaempferia alba* K. Larsen & M. Smith (เปราะภูดอกขาว)

2. *C .burttii* K. Larsen & Jenjitt. (เปราะภูจำปาสัก)

3. *C. laotica* Picheans. & Mokkamul (เปราะภูประเทศลาว)

4. *C. thailandica* K. Larsen (เปราะภูประเทศไทย)

5. *C. violacea* K. Larsen & S.S. Larsen (เปราะภูดอกม่วง)

The genus *Caulokaempferia* has distributed from Himalayan (3 species) (Roy and Barbhuiya, 2013), China (1 species), Vietnam (2 species) (Intharapichai *et al*, 2014), Lao PDR (2 species) and Thailand, the rich area at least 19 species, totally 27 species. All of them are endemic to the type locality except *C. saxicola* which is widely distributed in Thailand.

This study resulted in the better understanding of the genus *Caulokaempferia* K. Larsen distributed in Thailand and Lao PDR. The knowledge gained will lead ones to better understanding all of members of the genus.

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APPENDIX

Research publications

J. Jpn. Bot. **90**: 153–157 (2015)

A New Species of *Caulokaempferia* (*Zingiberaceae*) from Western Thailand

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A new *Caulokaempferia* species (*Zingiberaceae*) from western Thailand, *C. picheansoonthonii* Phokham & Prasarn, is described with full description, ink line-drawing with water-color and photographic illustration. This new species is morphologically closest to *C. kayinensis* Picheans. & Sangnark reported from Kayin State of Myanmar. The sessile inflorescence with strictly 1-cincinnus hidden in the uppermost leaf-sheath is prominent characteristic of this new taxon.

Key words: *Caulokaempferia*, *Caulokaempferia picheansoonthonii*, new species, western Thailand, *Zingiberaceae*.

About 20 species of *Caulokaempferia* K. Larsen (*Zingiberaeae*) were reported for Thailand, some of which are now recognized as conspecific; e.g., *C. kuapii* K. Larsen is synonymous with *C. saxicola* K. Larsen (Chaiyoot 2007). Some members of the genus with non-yellow flowers, trilocular ovaries and hidden inflorescences in the two uppermost leaf sheaths were proposed to transfer to the controversial new genus, *Jirawongsea* Picheans. (Picheansoonthon et al. 2008).

Biogeographically, members of the genus grow on high-altitude mountains and develop endemically. For the yellow-flowered *Caulokaempferia*, to our knowledge, only *C. saxicola* is widely distributed from its type location at Khao Khiew in Khao Yai National Park in central Thailand westward to Kayin State of Myanmar (Sangnark et al. 2015) and northward to Shan State of Myanmar (Benjaphan et al. 2015). Other taxa, however, possess restrict distributions. Only a few species grow in low altitude hills at 100–300 m, i.e., *C. pedemontana* (Larsen et al. 2004) and *C. satunensis* (Picheansoonthon et al. 2007).

In order to complete inventory of the genus for Thailand, the second author has been searching for representatives of the genus in western Thailand, while the third author is working on the genus representation in Kayin and Mon States of Myanmar. The senior author is finishing up his work on molecular studies of the genus covering all taxa reported in India, Myanmar, Thailand, China, Vietnam and Laos.

During our recent survey in western Thailand, we encountered an undescribed entity from Changwat Kanchanaburi. After careful investigation morphologically, we

Fig. 1. *Caulokaempferia picheansoonthonii* Phokham & Prasarn. A. Habit. B, C. Leaf base, showing ligules (lateral view). D. Bracteole. E. A flower. F. Calyx-tube and ovary. G. Dorsal corolla lobe. H, I. Lateral corolla lobes. J, K. Lateral staminodes. L. Labellum. M, N. Anthers and anther crests (M. rear view, N. front view.) O. Ovary and stylodes. P. Infructescence, showing uppermost leaf-sheath with leaf base, bracteole and fruits. Q. Fruit. R. Seeds. Drawn by Chalermchoke Boonchit.

Fig. 2. *Caulokaempferia picheansoonthonii* Phokham & Prasarn. A. Plants in the type locality. B. Plant with inflorescence. C. Detail of inflorescence and infructescence, showing the unique blade-unopposed ligule, a flower and fruits. D, E. Flower showing different view of labellum, lateral staminodes and anther crest. D. Oblique view. E. Front view. F. Plant showing infructescence, fruits, dehiscing fruit and seeds. Photographed by Sittichoke Prasarn (A) and Chayan Picheansoonthon (B–F). Scale: 1 cm.

propose it as a new species and decided to name it *Caulokaempferia picheansoonthonii*. Full description, ink-line drawing with water-color, and photographic illustrations of this new species are given.

Taxonomic treatment

Caulokaempferia picheansoonthonii Phokham & Prasarn, **sp. nov.** [Figs. 1, 2]

This new species is similar to *Caulokaempferia kayinensis* Picheans. & Sangnark, but differs in the following characters: (1) more number leaf blades (7–9 vs. 4–5), (2) longer (4–6 mm vs. 1–2 mm) and leaf-blade unopposed ligules, (3) lanceolate-oblong leaf blades with undulate to wavy margins, (4) strictly 1 cincinnus, and (5)

broadly obovate labellum with emarginate apex.

Type: **THAILAND**. Changwat Kanchanaburi, Amphoe Sangkhla Buri, Thung Yai Narasuan Wildlife Sanctuary, Kulu Huamee Mountain, near Ro Kee stream, 15°12′97.4″N 98°29′58.2″E, alt. 247 m, 16 August 2014, CP 160814-1 (holotype–BK!).

Lithophytic perennial herbs, slender, with short rhizome; root fibrous, some form longish storage tubers. Pseudostems 13.5–17.5 cm, with 2 bladeless sheaths and reduced lamina. Leaves 7–9, petiole subsessile to short petiolate, ca. 5 mm long; ligule membranous, 4–6 mm long, apex acute, blade unopposed; leaf blades lanceolate-oblong, the largest one, 9.5–13 × 1.8–3.2 cm, base cuneate, apex acute-acuminate to caudate up to 2.2 cm long, margin undulate to wavy, upper surface greenish, lower surface green-gray, both sides glabrous. Inflorescence sessile, hidden in the uppermost leaf sheath, strictly 1 cincinnus. Bract none, 2–5-flowered cincinnus. Bracteole lanceolate, 1.3–1.7 cm × 2–3 mm, whitish to greenish. Calyx tubular, hidden in the bracteole, 1.2–1.5 cm × 2–3 mm, glabrous, greenish, splitted 5–6 mm down one side, apex acute. Corolla tube 3.0–3.9 cm × 1–2 mm, yellowish; dorsal corolla lobe oblong, 0.8–1.1 cm × 3–4 mm, whitish, apex hooded and produced into an ca. 1 mm thorn-like point; lateral corolla lobes lanceolate, 0.9–1.0 cm × 2–3 mm, whitish, apex acute. Lateral staminodes, patent, obovate, 1.2–1.4 cm × 6–7 mm, yellow, apex obtuse. Labellum broadly obovate, 1.8–2.4 × 1.5–2 cm, yellow, apex emarginate. Filament very short, ca. 1 mm; anther 2–3 mm long; anther crest suborbicular, 2–3 × 3–4 mm, yellow. Ovary greenish, oblong, 5–8 × 1–2 mm, glabrous; stylodial glands 2, ca. 1 mm long, glabrous; stigma conical, margin ciliate. Fruit fleshy capsule, ellipsoid-oblong, 1.2–1.8 cm × 3–5 mm, greenish. Seed numerous, crowned with whitish arillode. Phenology: Flowering July–August, fruiting August–September.

Distribution: Thailand, Changwat Kanchanaburi in western Thailand, near the Thai-Myanmar border, so far known only from the type locality.

Ecology: This new species grows on moist limestone cliffs facing the stream, under the shade of mixed deciduous forest, at an altitude of about 250 m.

Etymology: We decided to name this new species to honor Professor Dr. Chayan Picheansoonthon, fellow of the Royal Institute of Thailand, on his 60th birthday in 2015. He has been actively engaged in taxonomic research of family *Zingiberaceae*, particularly the genus *Caulokaempferia*. He has described more than 10 taxa of the genus, and much more to come in the near future. His contribution led us to better understanding of the diversity and biogeography of this genus.

Note: This new species can be easily distinguished from other yellow-flowered *Caulokaempferia* by its unique sessile inflorescence with strictly 1 cincinnus and 7–9 leaf blades with undulate to wavy leaf margin. Morphologically, it is closest to *C. kayinensis* Picheans. & Sangnark. The latter, however, possesses 1–3 cincinni of 2–4 flowers each with ebracteate lower 1–2 cincinni hidden in the two uppermost leaf sheaths. The third uppermost cincinnus of *C. kayinensis*, if present, projects out of the two uppermost leaf sheaths and is enclosed in a bract, some of which have a leaf-like appendage (Sangnark et al. 2015).

It is worth noting that the type locality of this new species is less than 40 kilometers from the Thai-Myanmar border and less than 200 kilometers from the type location of *C. kayinensis* in Myanmar's Kayin State. Myanmar's Tanintharyi (Tenasserim) Mountain Range lies between the type locations of two species. We therefore expect to find some more species, and definitely some of which may possess the sessile inflorescences hidden in the leaf-sheath.

Molecular study of all members of the genus by the senior author supports that *C.*

picheansoonthonii is a phylogenetically distinct taxon, the results of which will be published in a forthcoming publication.

Other specimen examined: **THAILAND**. Kanchanaburi, Sangkla Buri, Tambon Laiwo, Ban Saneh Phawnh, 13 August 1993, J. F. Maxwell 93-904 (CMU)

Note on generic nomenclature

When Kai Larsen (1964) established *Caulokaempferia*, it is clear that he regarded *Monolophus elegans* (Wall.) Wall. as the lectotype of the genus *Monolophus* Wall. The name *Caulokaempferia* K. Larsen is, since then, well accepted by leading taxonomists who work on the genus throughout its distribution range. Although realizing that there were some 'technical problems' in K. Larsen's determination, we feel it is inappropriate to reinstate the name *Monolophus* Wall., and make more than 20 species described since then synonymous (Mood et al. 2014). When we revised the genus for Vietnam, we declared the name *Caulokaempferia* K. Larsen as 'nom. cons.' (Intharapichai et al. 2014). Detailed discussion on this determination will be published in a forthcoming publication (Picheansoonthon 2015).

This research is a part of a five-year project ('Studies on fundamental botanical knowledge and DNA fingerprints of the Thai medicinal wan'), financially supported by the Thai Traditional Medical Knowledge Fund of the Ministry of Public Health. The authors would like to thank Professor Dr. Chayan Picheansoonthon for his guidance, advise, and support, also to members of our research group: Mr. Kamthorn Intharapichai, and Mr. Supakorn Thongmeemar for field assistances and Mr. Chalermchoke Boonchit for the beautiful ink line-drawing with water color of this new species.

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B. Phokham[a,b], S. Prasarn[a], S. Sangnark[a], P. Wongsuwan[a]:

タイ西部からのショウガ科 *Caulokaempferia* 属の 1 新種

タイ西部からショウガ科の 1 新種，*Caulokaempferia picheansoonthonii* Phokham & Prasarn を記載した．この新種はミャンマーの Kayin 州から報告された *C. kayinensis* Picheans. & Sangnark に形態的に最も近いが，最上部の葉鞘に完全に包まれた，無柄のサソリ形花序（巻散花序）をもつことがその顕著な特徴である．

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Caulokaempferia pubescens (Zingiberaceae) - A New Species from Northern Thailand

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ABSTRACT: A new species of *Caulokaempferia* K. Larsen (Zingiberaceae), *C. pubescens* Picheans. & Phokham, from Changwat Mae Hong Son in Northern Thailand is reported. Full descriptions, together with ink line–drawing with water color and photographic illustrations are given. Relationship of this new species with their phylogenetically closest related taxa, *C. larsenii* Suksathan & Triboun, is also discussed.

KEY WORDS: *Caulokaempferia*, *Caulokaemferia pubescens*, northern Thailand, Zingiberaceae.

INTRODUCTION

The genus *Caulokaempferia* K. Larsen (Zingiberaceae) was established by K. Larsen (1964). It comprises about 30 species, distributed from the Himalayas (3 species) through south China (2 species), Vietnam (2 species), Laos (5 species) and Thailand, with its centre of diversity in Thailand (Chaiyoot, 2007). So far, however, none has been reported from Myanmar and Cambodia.

A few taxa of the genus were reported to be ethnomedically important. In Thailand, *C. phutokensis* Picheans. & Koonterm is used by Thai forest monks to treat early stage of prostatic hyperplasia (Picheansoonthon & Koonterm, 2008), while *C. linearis* (Wall.) K. Larsen is used by the Chakmas in India and Bangladesh for treating vertigo (Rai & Lalramnghinglova, 2010).

In Thailand, 2 species, *C. saxicola* K. Larsen and *C. kuapii* K. Larsen, were included in K. Larsen's original treatment of the genus (1964). Since then, 16 taxa were further recognized (Chaiyoot, 2007). In the past few years, *C. sirirugsae* Ngamriab. (Ngamriabsakul, 2008) from southern Thailand and *C. chayaniana* Tiyaw. (Tiyaworanant, 2010) from northern Thailand, were further added.

In this paper, a new species from Changwat Mae Hong Son in northern Thailand, *C. pubescens* Picheans. & Phokham, is described with full description and color illustrations. Relationship with its closely related taxon, *C. larsenii* Suksathan & Triboun (Suksathan & Triboun, 2004) will be discussed.

TAXONOMIC TREATMENT

Caulokaempferia pubescens Picheans. & Phokham, ***sp. nov.*** Figures 1,2

Type: **THAILAND**: Changwat Mae Hong Son, Amphoe Mueang Mae Hong Son, altitude 1,407 m, 13 July 2013, *CP 130713-3* (holotype BK).

This new species is similar to *Caulokaempferia larsenii*, but differs in the following characters: shorter and sparsely–hairly ligule, pubescent lower leaf-surface, broadly ovate to orbicular anther crest, style insertion between anther sac, and stigma with raised ends.

Perennial herbs, slender, with short rhizome; root fibrous, some form longish tuber. Pseudostems erect, 10.5–36.5 cm with 3–7 bladeless sheaths. Leaves (3–)4–6(–10), sessile, elliptic to narrowly ovate, 4.5–10.5 by 1.5–4.1 cm, base cuneate to rounded, apex acuminate to caudate to 1.5 cm long, upper surface glabrous, lower surface pubescent; ligule membranous, 3–7 mm long, sparsely hairy at base, apex rounded. Inflorescences terminal; peduncle, 3.5.–7.5 cm long, hidden in the uppermost two leaf sheaths; flowers entirely yellow. Bracts (3–)4–8(–9), green, broadly elliptic to ovate–oblong, 2.9–3.6 by 1–1.2 cm, apex acute to acute–acuminate, margin translucent, each subtends 1–3(–4) flowers, some lower bract with leaf-like appendage. Bracteoles membranous, elliptic to ovate–oblong, 5–10 by 2.5–5 mm, translucent with prominent greenish midvein, apex acute to obtuse, glabrous. Calyx tubular, 1.1–1.5 cm by 3–4 mm, translucent, split down one side to about half way, apex

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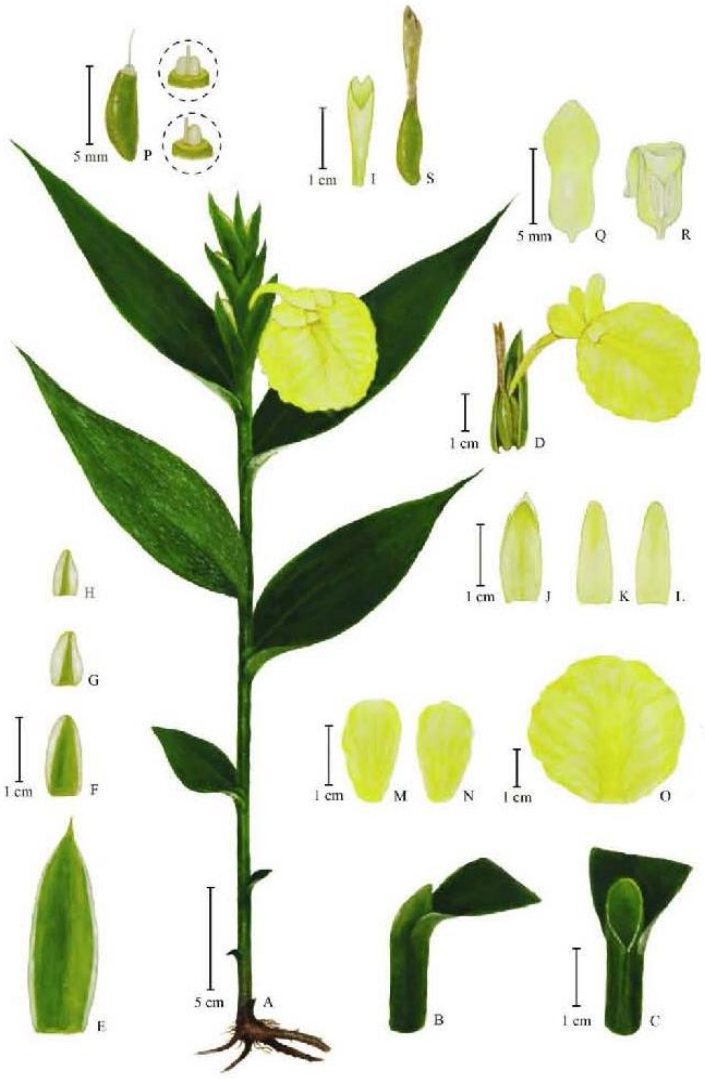

Fig. 1. *Caulokaempferia pubescens* Picheans. & Phokham. A: Habit. B, C: Leaf base, showing ligule (B. side view and C. front view). D: Inflorescence, showing a flower and fruits. E: Bract. F–H: Bracteoles. I: Calyx tube. J–L: Corolla lobes (J. Dorsal, K. and L. Lateral). M, N: Lateral staminodes. O: Labellum. P: Ovary and stylodes with rear view and side view. Q, R: Anthers and anther crests (Q. rear view and R. front view). [drawn by Chalermchoke Boonchit]

bilobed. Corolla tube 3.3–4.2 cm by ca. 3 mm, glabrous; dorsal corolla lobe oblong, 1.1–1.7 cm by 5–8 mm, apex obtuse to apiculate, hooded; lateral corolla lobes oblong, 1.2–2 cm by 6–8 mm, apex acute. Staminodes broadly elliptic to obovate, 1.2–1.7 by 0.7–1.1 cm, apex rounded. Labellum orbicular, 3.4–4.5 by 3.7–4.8 cm, margin undulate, apex rounded; anther, 4–6 mm long; anther crest broadly ovate to orbicular, 5–7 mm diameter, apex rounded, reflexed. Stigma funnel-shaped, inserted between anther sac, margin raised on both ends, ciliate. Ovary oblong, 6–8 by 2–3 mm, glabrous, 1–locular, ovules numerous; stylodes 2, filiform, ca. 1 mm long. Fruits fleshy capsule, oblong to elliptic, 1.2–2 cm by 4–5 mm, green, split on one side. Seeds numerous, obovate, *ca.* 2 by 1 mm, crown with whitish arillode. Flowering June–August; fruiting July–September.

Distribution: Thailand, Mae Hong Son province, Amphoe Mueang Mae Hong Son, Doi Pa Yee, known

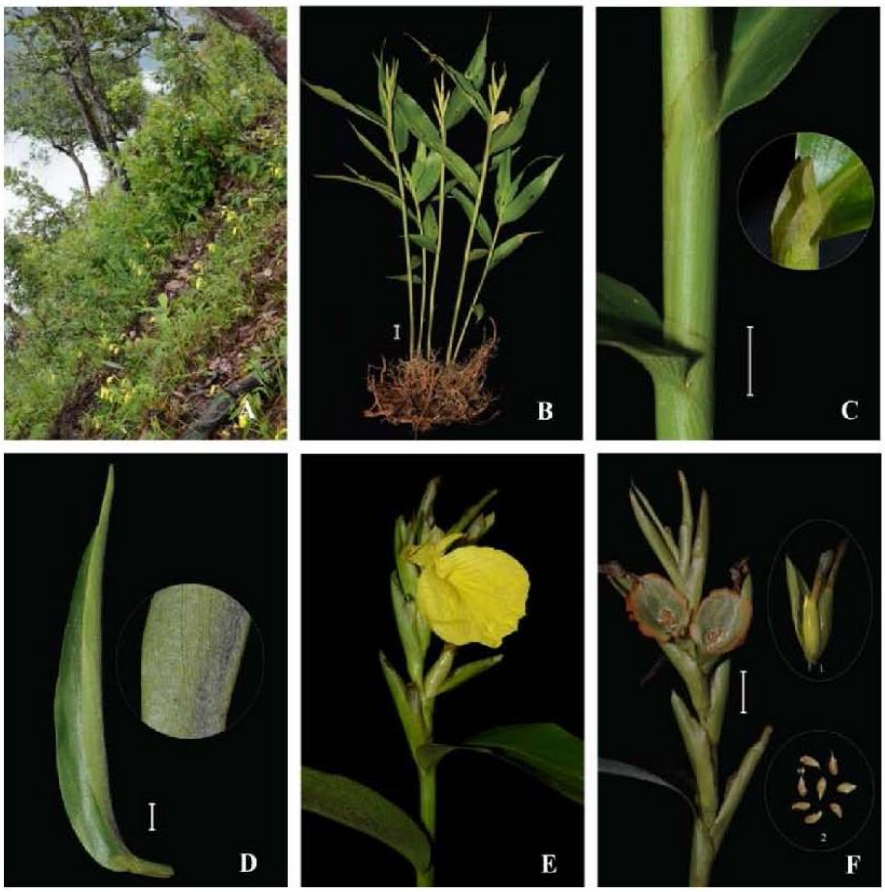

Fig. 2. *Caulokaempferia pubescens* Picheans. & Phokham. A: The plants in its type location. B: Plant habit. C: Part of a pseudostem and lower part of leaves, showing ligules. D: A leaf, showing leaf surfaces (upper surface glabrous and lower surface pubescent). E: An inflorescence, showing detail of a flower and bract arrangement. F: An infructescence, showing dehiscing fruits and seeds, fruits (1) and seeds (2). Scale = 1 cm. [photographed by *Chayan Picheansoonthon*]

only from the type locality.

Ecology: This new species grows on sandstone rocks slopped under the shade of lower montane pine-oak forest, at the altitude of 1,350–1,450 m.

Note: This new species can be easily characterized by its terrestrial habit, short ligule and pubescent lower leaf–surface. The hairiness of the lower leaf-surface has, so far, not been observed in the genus *Caulokaempferia*.

This new taxon is morphologically similar to *Caulokaempferia larsenii*, but differed in several characters as mentioned earlier. From investigation of living specimens of *C. larsenii* and *C. pubescens* in their type locations, both species are morphologically distinct. The pistil of *C. larsenii* is "shorter than the stamen' and 'not inserted between anther sac' (Suksathan & Triboun, 2004). This character is rather unique for the genus. However, the pistil of *C. pubescens*, similar to all other known *Caulokaempferia*s, is longer than the stamen, and the style inserted between the anther sacs.

The type location of this new taxon is in the same mountain range and only less than 20 kilometers away from the type locality of *C. amplexicaulis* Suksathan (Larsen *et al*, 2003). The latter species is, however, morphologically different, particularly in its amplexicaul leaves and bracts with fused leaf-sheaths. It is worth mentioning that in this type locality, a month after this new taxon is in full bloom, *C. saxicola* K. Larsen starts appearing on the moist rock surfaces or

rock crevices facing the valley. This latter species is rather common in high–altitude mountains in Northern Thailand.

Also, on the opposite side of the valley not far away from type locations of both *C. pubescens* and *C. amplexicaulis*, another taxon, *C. chayaniana* Tiyaw., was recently reported. However, *C chayaniana*, in contrast with *C. amplexicaulis*, pocesses amplexicaul leaves, but with open leaf–sheaths (Tiyaworanant, 2010).

Molecular study of all members of the genus which is part of the first author's Ph.D. dissertation, also supports that *C. pubescens* is a phylogenetically distinct taxon, and closest to *C. larsenii*. The result will be published in the forthcoming publication.

Note on generic nomenclature

In 1964, Kai Larsen established the genus *Caulokaempferia*, rejecting *Monolophus* Wall. for both taxonomic and nomenclatural reasons (Larsen, 1964). Since then, all the authors of this plant group (particularly local botanists who worked in the distribution areas from China, Vietnam, Laos, Thailand, Bhutan to India) have accepted his determination, and have used the name (*Caulokaempferia* K. Larsen) in their taxonomic treatments, including the treatments of some 20 new taxa and local floras.

Recently, the name *Caulokaempferia* K. Larsen was proposed to be superfluous, and the name *Monolophus* Wall. was restated based on chronicle literature evaluation (Mood *et al*, 2014). However, their judgement needed to be further debates. Detailed discussion on this nomenclatural controversy was and will be published (Intharapichai, 2015, Picheansoonthon, 2015) in other publications.

Realizing this technical problem, in our treatment of the genus for Vietnam, the name '*Caulokaempferia* K. Larsen' is proposed as conserved name (*nom. cons.*) (Intharapichai *et al*, 2014). Therefore, it would be inappropriate to put the names of more than 20 plant taxa into a synonymous just because of this technical problem. And, it would be practically unacceptable to *place the authorship of the persons who hardly knew, or* seen most members of the genus (Mood *et al*, 2014). We, therefore, treat the genus under the accepted name *Caulokaempferia* K. Larsen.

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This research is a part of a five-year project ('Studies on fundamental botanical knowledge and DNA fingerprints of the Thai medicinal wan'), financially supported by the Thai Traditional Medical Knowledge Fund of the Ministry of Public Health. The authors would like to thank members of our research group: Mr. Supakorn Thongmeemar and Mr. Sittichoke Prasarn for field assistances, and Mr. Chalermchoke Boonchit for the beautiful ink line-drawing with water color.

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BIOGRAPHY

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